JOINT MEETING OF THE AUSTRIAN AND GERMAN PHARMACEUTICAL SOCIETIES

September 20 – 23, 2011
University of Innsbruck, Austria

"Shaping the future – Trends and perspectives in pharmaceutical sciences"

FINAL PROGRAM AND BOOK OF ABSTRACTS

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A-6020 Innsbruck

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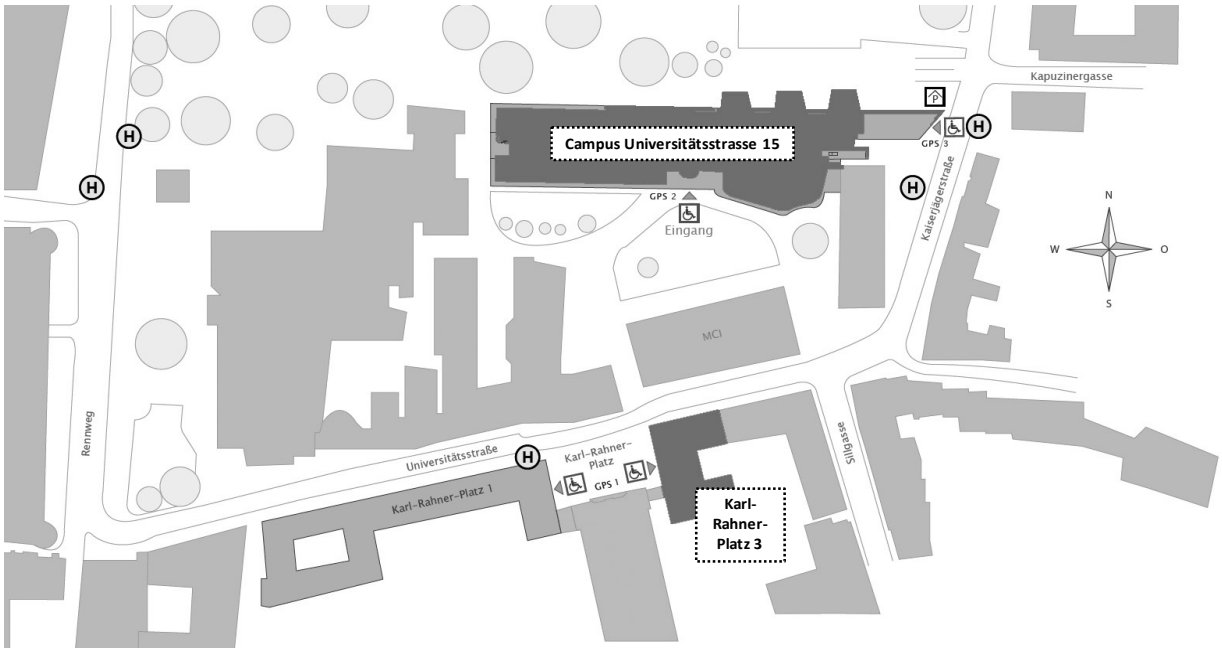
CONFERENCE VENUES

University of Innsbruck
Campus Universitätsstrasse 15 and
Karl-Rahner-Platz 3
6020 Innsbruck, Austria



Campus map of conference venues

REGISTRATION AND OFFICE HOURS

University of Innsbruck
Campus Universitätsstrasse 15
Room O.E-18 (Studierzone)
6020 Innsbruck, Austria

Tuesday, September 20 2011	11:00 – 18:00
Wednesday, September 21 2011	08:00 – 18:00
Thursday, September 22 2011	08:00 – 18:00
Friday, September 23 2011	08:00 – 12:00

WELCOME

Dear Colleagues,

In spite of the increasing expenses for research and development and the growing scientific knowledge, the number of approvals of new drugs has been steadily declining in the last 10 years. The increasing regulatory requirements and ascending expenses for clinical trials are counted among the main reasons for the rising costs.

The pharmaceutical industry tried at first to counterbalance this innovation gap with higher research budgets, then with merging and acquisition of smaller firms and innovative biotechnological companies and finally with reorganization of the R&D processes. For some time now the pharmaceutical industry focuses more and more on strategic co-operations with universities and their pharmaceutical institutes in order to meet its demand for innovation.

Future progress in drug research will depend more and more on the extensive comprehension of the complex causes for diseases and the translation of these insights in clinical trials. New co-operation models are needed in order to incorporate the findings of excellent fundamental research into the development of innovative drugs in Europe.

It is the superior goal of the Joint Meeting of the Austrian and German Pharmaceutical Societies to present outstanding pharmaceutical research key aspects of Austrian and German universities with the aid of thematically focused short presentations and to provide by this way a basis for co-operation. In addition – according to the motto of our meeting – international outstanding scientists will give plenary speeches on trends and perspectives in pharmaceutical sciences.

We thank our co-operation partners, last but not least the Pharmaceutical Society of Japan, for supporting the preparation and conduction of our meeting. We wish all participants of the meeting fruitful scientific discussions that may serve to tighten and expand pharmaceutical networks. Furthermore we wish all participants a pleasant stay in one of the most beautiful cities of the Alps area, may be even the most beautiful!

Prof. Hermann Stuppner
President of the ÖPhG

Prof. Manfred Schubert-Zsilavecz
President of the DPhG

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GENERAL INFORMATIONS

Exhibitions

University of Innsbruck
Campus Universitätsstrasse 15, Foyer
6020 Innsbruck

Oral presentations

PC, beamer and overhead projector are available. Please contact the information desk to deliver your presentations (MS PowerPoint only) at latest in the morning the day your presentation is scheduled.
Plenary Lectures: 45 min (40 + 5 min discussion)
Key Lectures: 25 min (20 + 5 min discussion)
Short lectures 15 min (12 + 3 min discussion)

Posters

Two poster sessions are going to be held:

- Wednesday, September 21 2011, 15.00 – 16.45
- Thursday, September 22 2011, 14.45 – 16.30

Posters will be on display in the Foyer, on the first and on the second floor of the Campus Universitätsstrasse 15.
Please mount the posters for the first session until Wednesday, September 21 2011, 12:00 and for the second session on Thursday, September 22 2011, until 12:00. The necessary material to fix the posters will be provided by the organizers. The poster numbers are indicated in the list of poster presentations in the final program. Presenting authors are asked to be present at their posters during the poster sessions.

Conference dinner

The conference dinner will take place on Thursday evening, September 22 2011 at 19.30 in the restaurant “Stiftskeller”, Stiftgasse 1, 6020 Innsbruck, Austria.

Language

The congress language is English, no simultaneous translation will be provided.

Badges

Badges will be issued to all registered participants and enable access to all scientific sessions. Members of the Organizing Committee wear T-Shirts with the Logo from the University of Innsbruck.

Liability

The Organizers of the Symposium cannot be held responsible for any loss, theft, damage or injury to any person or property during the conference, whatever the cause may be. The liability of persons and enterprises providing means of transportation or other services remains unaffected. Each congress participant and accompanying person takes part in all tours at his/her own risk.

ACKNOWLEDGEMENTS

The members of the Organizing Committee gratefully acknowledge the financial support of the "Joint Meeting of the Austrian and German Pharmaceutical Societies 2011" by following companies and institutions:

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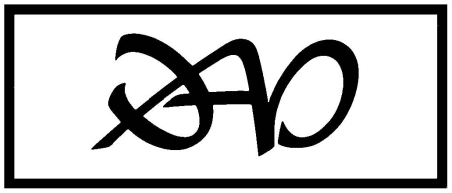


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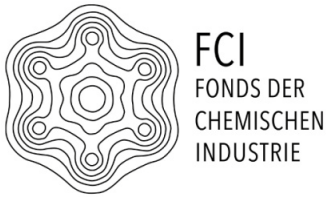
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PRE-SYMPOSIUM
FG ALLGEMEINPHARMAZIE, INDUSTRIEPHARMAZIE UND KLINISCHE PHARMAZIE

Montag, 19.09.2011, 13.00 - 17.30 **Seminarraum VI, Theologische Fakultät**

Kosten-Nutzen-Risiko – Bewertung von Arzneimitteltherapien und Anforderungen an zukünftige Versorgungsstrukturen

Wissenschaftliche Leitung: Dr. M. Hannig (Allgemeinpharmazie), Dr. C. Küster (Industriepharmazie), Prof. T. Bertsche (Klinische Pharmazie)

13.00 – 13.30 **Prof. Dr. Schweim**
Lehrstuhl Drug Regulatory Affairs, Universität Bonn
Entwicklung der Kosten-Nutzen-Bewertung im Verlauf der Jahr(zehnt)e

13.30 – 14.00 **Prof. Dr. Martin Schulz**
Vorsitzender der Arzneimittelkommission der Deutschen Apotheker (AMK), Berlin; Goethe-Universität Frankfurt
Kosten-Nutzen-Risiko: Herausforderungen für die Arzneimittelversorgung aus Sicht der AMK

14.00 – 14.30 **Dorothee Brakmann**
Leiterin "Payer Strategy" bei Janssen-Cilag
Herausforderungen in der Bewertung von Arzneitherapien und Auswirkungen auf Versorgungstrukturen aus Sicht der pharmazeutischen Industrie

14.30 – 15.00 **Kaffeepause**

15.00 – 15.30 **Prof. Dr. Irene Krämer**
Direktorin der Apotheke der Universitätsmedizin Mainz,
Präsidentin des Bundesverbandes Deutscher Krankenhausapotheker, ADKA e.V.
Kosten-Nutzen-Bewertung von Arzneimitteln aus Sicht der Krankenhausapotheke

15.30 – 16.00 **Dr. Ulf Maywald**
Fachbereichsleiter Arzneimittel der AOK Plus
Anforderungen an zukünftige Versorgungsstrukturen und Auswirkungen auf die Arzneitherapie aus Sicht der Krankenkassen

16.00 – 17.00 **Podiumsdiskussion zum Thema**
Moderation: Prof. Dr. Morck
Geschäftsführer der cui bono health-consulting GmbH Berlin und
Chefredakteur i.R. der Pharmazeutischen Zeitung, Eschborn

17.00 **Mitgliederversammlungen der Fachgruppe Klinische Pharmazie**

Wir danken unseren Sponsoren sehr herzlich für Ihre finanzielle Unterstützung.

PRE-SYMPOSIUM
FG ARZNEIMITTELKONTROLLE / PHARMAZEUTISCHE ANALYTIK

Dienstag, 20.09.2011, 09.15 - 17.30 **HS 1, Campus Universitätsstrasse 15**

Analytische Einblicke auf der molekularen Ebene

Moderation: Klaus Raith, Hermann Wätzig

09.15 – 09.30 **Begrüßung**

09.30 – 10.00 **Dr. Matthias Heuermann**
Landesinstitut für Gesundheit und Arbeit NRW, Arzneimitteluntersuchungsstelle, Münster
Nachweis nicht deklarierter Arzneistoffe in Verdachtsproben mittels HPLC/DAD und Absicherung über ESI-MS

10.00 – 10.15 **Dr. Reinhard Bogan**
Zentrales Institut des Sanitätsdienstes der Bundeswehr München
HPLC-DAD / GC-MS Untersuchungen zur Arzneimittelsicherheit von Notfallvorräten – Nachweis, Identifizierung und toxikologische Bewertung eines regulatorisch relevanten Abbauproduktes

10.15 – 10.45 **Dr. Philippe Girard**
Swissmedic Schweizerisches Heilmittelinstitut, OMCL, Bern
NIR und Raman-Spektrometrie für Qualitätskontrolle, PAT und Fälschungsanalytik

10.45 – 11.15 **Kaffeepause**

11.15 – 11.45 **Dr. Andreas Mayrhofer**
AGES PharmMed, Österreichisches Arzneimittelkontrolllabor, Wien
Analyse Illegaler Arzneimittel - eine Herausforderung für OMCLs (und die Massenspektrometrie)

11.45 – 12.15 **Dr. Steven Watt**
A&M Stabtest, Mainz
BioEquality: Eine Plattform zur umfassenden Analytik von Daten aus der Stabilitätsprüfung

12.15 – 12.45 **Dr. Christian Hunzinger**
Merck Serono, Darmstadt
Bioprocess Analytics

12.45 – 14.15 **Mittagspause**

14.15 – 14.45 **Dr. Andreas Körner**
Abbott Products GmbH, Hannover

	How state-of-the-art analytical technologies help to understand solid protein formulation
14.45 – 15.15	**Dr. Isam Rais** *Boehringer Ingelheim, Biberach* **Einsatz der Biacore-Technologie in der Entwicklung und Qualitätskontrolle von Biopharmazeutika**
15.15 – 15.45	**Prof. Dr. Ingo Ott** *Technische Universität Braunschweig* **Herausforderungen und instrumentelle Neuerungen in der (Bio)analytik von Metallkomplexen**
15.45 – 16.15	**Kaffeepause**
16.15 – 16.45	**Prof. Dr. Michael Karas** *J.W. Goethe-Universität Frankfurt/Main* **MALDI for smaller molecules: quantitation and validation**
16.45 – 17.15	**Prof. Dr. Dr. h.c. Hans H. Maurer** *Universität des Saarlandes, Homburg (Saar)* **Current status of hyphenated low and high resolution mass spectrometry in clinical and forensic toxicology as well as in drug metabolism**
17.15 – 17.30	**Pause**
17.30	**Mitgliederversammlung**
20.00	**Gesellschaftsabend im Traditionsrestaurant Weinhaus Happ**

Mittwoch, 21.09.2011, 09.15 - 17.30 **HS 1, Campus Universitätsstrasse 15**

Analytische Einblicke auf der molekularen Ebene

Moderation: Klaus Raith, Hermann Wätzig

09.30 – 10.00	**Prof. Dr. Gérard Hopfgartner** *University of Geneva* **Mass spectrometric QUAL/QUAN approaches to follow pharmaceutical and endogenous metabolite distributions in biological fluids and tissues**
10.00 – 10.30	**Dr. Christian Schmelzer** *Martin-Luther-Universität Halle-Wittenberg* **Molekulare Einblicke in die Alterung elastischer Fasern**
10.30 – 11.00	**Dr. Markus Stöckli** *Novartis AG, Basel* **Über Gewebe, Massenspektrometrie und Bildgebung**
11.00 – 11.30	**Karl Mechtler** *Forschungsinstitut für Molekulare Pathologie (IMP) Wien* **Time Resolved Systems Biology**

11.30	**Abschlussdiskussion, danach Mittagspause**
13.00	**Eröffnungsveranstaltung der Gemeinsamen Jahrestagung der ÖPhG und DPhG**

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PRE-SYMPOSIUM
FG PHARMAZEUTISCHE BIOLOGIE

Mittwoch, 21.09.2011, 09.00 - 12.00 **HS 2, Campus Universitätsstrasse 15**

Naturstoffforschung in Deutschland

Leitung: PD Dr. Werner Knöss (Universität Bonn, Prof. Dr. Thomas Winckler (Universität Jena), Dr. Ilse Zündorf (Universität Frankfurt)

09.00 – 09.10	**Begrüßung**
09.10 – 09.45	**Prof. Dr. Wolfgang Blaschek** *Universität Kiel* **Naturstoffe als Inhibitoren des Natriumabhängigen Glucosetransporters (SGLT)**
09.45 – 10.20	**Prof. Dr. Thomas Schmidt** *Universität Münster* **Naturstoffe gegen vernachlässigte Krankheiten**
10.20 – 10.45	**Pause**
10.45 – 11.20	**Prof. Dr. Heike Brötz-Oesterhelt** *Universität Düsseldorf* **Überaktivierung statt Inhibierung – das neue Wirkprinzip der Acyldepsipeptid-Antibiotika**
11.20 – 11.55	**Prof. Dr. Wolfgang Kreis** *Universität Erlangen* **Substrat-promiske Enzyme und ihre Bedeutung für die Sekundärstoffbildung und Synthesechemie**

PRE-SYMPOSIUM
FG PHARMAKOLOGIE UND TOXIKOLOGIE

Mittwoch, 21.09.2011, 09.00 – 12.00 **HS 3, Campus Universitätsstrasse 15**

Rationale Phytotherapie: Focus ZNS

Chair: W.E. Müller, Frankfurt/Main und K. Leuner, Erlangen

09.00 – 09.30 **J. Klein**
Frankfurt
Neuroprotection by Bilobalide

09.30 – 10.00 **C. Ude**
Frankfurt
Ginkgo biloba Extract EGb 761®, preclinical and clinical evidence for its use in dementia

10.00 – 10.30 **K. Nieber, F. Berger, A. Hensel**
Leipzig
The effect of saffron and trans-crocetin on glutamatergic transmission in the rat cortex

10.30 – 11.00 **K. Leuner**
Erlangen
St. John's Wort, preclinical and clinical evidence for its use in depression

11.00 – 11.30 **W.E. Müller**
Frankfurt
Lavander oil (Silxan), preclinical and clinical evidence for its use in anxiety

11.30 – 12.00 **M. Abdel-Tawab**
Eschborn
Boswellia acids, preclinical and clinical aspects of their use in brain inflammation

PRE-SYMPOSIUM
FG PHARMAZEUTISCHE TECHNOLOGIE

Mittwoch, 21.09.2011, 09.30 - 12.00 **SR 1 / Aula, Campus Universitätsstrasse 15**

Multifunktionelle polymere Hilfsstoffe

Chair: Prof. Dr. Andreas Bernkop-Schnürch

09.30 – 10.00 **Prof. Dr. Andreas Bernkop-Schnürch**
Institute of Pharmacy, Leopold-Franzens-University Innsbruck, Austria
Polymeric excipients exhibiting efflux pump inhibitory properties

10.00 – 10.30 **Dr. Christian Wischke**
Center for Biomaterial Development and Berlin-Brandenburg Center for Regenerative Therapies, Helmholtz-Zentrum Geesthacht, Teltow, Germany
Degradable shape-memory polymer networks as drug carriers

10.30 – 11.00 **Kaffeepause**

11.00 – 11.15 **Stefan Grund**
Institute of Pharmacy, Department of Pharmaceutical Technology, Friedrich-Schiller-University Jena, Germany
The Area Vasculosa of the Chicken Yolk Sac as Tool for Toxicity Testing of Polymers

11.15 – 11.30 **Michael C. Hacker, PhD**
Institute of Pharmacy, Pharmaceutical Technology, Universität Leipzig, Eilenburger Str. 15a, 04317 Leipzig, Germany
Multi-functional macromonomers for biomedical applications

11.30 – 11.50 **Prof. Dr. Claudia Valenta**
Dep.of Pharm.Technol.Biopharm., University of Vienna
Influence of cationic polymers on the physico-chemical parameters of formulations and skin permeation

11.50 – 12.00 **Abschlussdikussion**

PRE-SYMPOSIUM
PHARMAZIEHISTORISCHE VERANSTALTUNG

Mittwoch, 21.09.2011, 09.00 - 12.45 **Madonnensaal, Theologische Fakultät**

Pharmazie in Innsbruck

Diese Veranstaltung ist öffentlich zugänglich und kostenfrei.

Organisation: Prof. Dr. Peter Dilg

09.00 – 09.15	**Prof. Dr. P. Dilg** *Marburg* **Begrüßung und Einführung**
09.15 – 10.00	**Dr. Andreas Winkler** *Innsbruck* **Zur Apothekengeschichte von Innsbruck**
10.00 – 10.45	**Dr. Monika Winkler-Kaufmann** *Innsbruck* **Die Innsbrucker Apothekerfamilie Winkler und die Gründung der Gesellschaft für Geschichte der Pharmazie**
10.45 – 11.15	**Kaffeepause**
11.15 – 12.00	**Prof. Dr. Christa Kletter** *Wien* **Die Entwicklung des Pharmaziestudiums an der Universität Innsbruck**
12.00 – 12.45	**Prof. Dr. Dr. Christa Habrich** *Gießen* **Tiroler Naturalien als Arzneimittel**
12.45	**Ende der Veranstaltung**

PRE-SYMPOSIUM
FG PHARM./MED. CHEMIE

Donnerstag, 22.09.2011, 12.30 - 14.00 **SR 2, Campus Universitätsstrasse 15**

Chair: Prof. Peter Gmeiner

12.30 – 14.00 **Mitgliederversammlung**


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SCIENTIFIC PROGRAM

Joint Meeting of the Austrian and German Pharmaceutical Societies
September 20 - 23, 2011, University of Innsbruck, Austria

Time Schedule

WEDNESDAY, September 21 2011

13.00 - 13.30	**OPENING CEREMONY**				**Aula**
13.30 - 14.15	**PL 1 , Prof. Dr. Gerhard KLEBE (University of Marburg, Germany)** *"Drug discovery stares at binding affinity, but what does this property really mean in detail?"* Chair: Ch. Noe				**Aula HS 1**
14.15 - 15.00	**PL 2, Prof. Dr. Werner SIEGHART (Medical University of Vienna)** *"GABA $_A$ receptor subtypes - exciting targets for the development of clinically important drugs"* Chair: Ch. Noe				**Aula HS 1**
15.00 - 16.45	**Poster presentation and Coffee break**				**Foyer, 1st, 2nd floor**
16.45 - 17.00	**Inflammation** Chairs: R. Bauer / Steinhilber D. **Aula**	Steinhilber Dieter (SL 001) Molecular pharmacology of 5-lipoxygenase and new inhibitors	**G-Proteins** Chairs: U. Holzgrabe / K. Mohr **HS1 / HS2**	Schmidhammer Helmut (SL 009) Advances in the development of zwitterionic opioid receptor agonists and antagonists	
17.00 - 17.15		Maier Thorsten (SL 002) On the role of the pro-inflammatory enzyme 5-lipoxygenase in tumorigenesis		Gmeiner Peter (SL 010) Tailor-made molecular probes for the for the first X-ray crystal structure of an agonist-GPCR complex	
17.15 - 17.30		Laufer Stefan (SL 003) Application of p38 MAP-Kinase-Inhibitors in CNS diseases		Gilsbach Ralf (SL 011) Cell-type specific functions of α2-adrenoceptors	
17.30 - 17.45		Hinz Burkhard (SL 004) Paracetamol and cyclooxygenase inhibition – is there a cause for concern?		Hoffmann Carsten (SL 012) How do orthosteric and allosteric ligands influence the conformational change of the M2 muscarinic acetylcholine receptor differentially?	
17.45 - 18.30		**Coffee break**		**Coffee break**	
18.30 - 18.45		Fürst Robert (SL 005) Endothelial inhibitor of apoptosis proteins (IAPs) – novel promising anti-inflammatory drug targets		Stark Holger (SL 013) Bioisosteric Replacement in Pramipexole-related Dopamine Agonists	
18.45 - 19.00		Kiemer Alexandra (SL 006) Downregulation of Glucocorticoid-induced leucine zipper (GILZ) promotes an inflammatory response in human macrophages		Kassack Matthias (SL 014) The Nucleotide Receptor P2Y11 Plays a Role in Immune System Associated Diseases	
19.00 - 19.15		Burnet Michael (SL 007) Macrocyclic anti-inflammatory compounds		Müller Christa E (SL 015) Anthraquinone Derivatives as Potent and Selective Competitive Antagonists for P2 Receptors	
19.15 - 19.30		Bochkov Valery (SL 008) The Janus face of oxidized phospholipids in inflammation: a story of unmet needs and potential leads		Zlotos Darius (SL 016) Development of Subtype-Selective Ligands for Melatonin Receptors	
19.30 - 20.15	**PL 3, Prof. Dr. Andreas BUSCH (Bayer Pharma AG, Berlin, Germany)** *"Challanges in pharma research and future developments"* Chair: O. Werz				**Aula HS 1**
20.30	**WELCOME EVENING**				**Foyer**

Joint Meeting of the Austrian and German Pharmaceutical Societies
September 20 - 23, 2011, University of Innsbruck, Austria

Time Schedule

THURSDAY, September 22 2011

08.30 - 09.15	**PL 4, Dr. Andreas PREMSTALLER** (Sandoz Biopharmaceuticals, Austria) *"Monoclonal antibodies: The perspective of a developer of biosimilars"* Chair: H. Viernstein						**Aula HS 1**
09.15 - 10.00	**PL 5, Prof. Roberto PELLICIARI (University of Perugia, Italia)** *"Targeting FXR and TGR5 receptor pathways in search for novel treatments for liver and metabolic disorders"* Chair: U. Holzgrabe						**Aula HS 1**
10.00 - 10.30	**Coffee break**						
10.30 - 10.45	**Natural Products** Chairs: H. Stuppner / V. Dirsch **Aula**	Alban Susanne (SL 017) Sulfated polysaccharides of Delesseria sanguinea from the artificial reef Nienhagen	**Transporter** Chairs: G. Ecker, H. Krömer **HS1**	Ecker Gerhard (SL 025) The Medicinal Chemistry of Drug Transport – Ligand-based Design meets Structure-based Design			
10.45 - 11.00		Merfort Irmgard (SL 018) State of the art on the wound healing properties of birch bark		Meyer zu Schwabedissen Henriette E (SL 026) The role of OATP1B transporters in pharmacology and physiology of liver			
11.00 - 11.15		Vollmar Angelika (SL 019) V-ATPase Inhibitors from myxobacteria – promising leads and tools for cancer therapy?		Bauer Stefanie (SL 027) Overcoming ABCG2-mediated drug resistance with new ABCG2 modulators derived from tariquidar			
11.15 - 11.30		Nicoletti Marcello (SL 020) The identification and chemical determination of complex botanical mixtures		Klepsch Freya (SL 028) Analysis of binding modes of propafenones in P-glycoprotein by means of molecular dynamics simulations			
11.30 - 11.45		Werz Oliver (SL 021) Hyperforin: A dual inhibitor of leukotriene and prostaglandin E2 biosynthesis as anti-inflammatory lead from nature	**Nanomedicine** Chair: A. Bernkop-Schnürch **HS3**	Caliceti Paolo (SL 029) Polysaccharide bioconjugates for tumor targeting	**Miscellaneous** Chair: R. Lemmens-Gruber **HS2**	Liebl Johanna (Pharmacology, SL 041) Cyclin dependent kinase 5 (Cdk5) and its function in Hepatocellular carcinoma	
11.45 - 12.00		Bauer Rudolf (SL 022) Investigation of Notopterygium incisum for anti-inflammatory constituents		Schwarz Julia (SL 030) Nanocarriers for dermal drug delivery: multiple W/O/W nanoemulsions for topical application of aciclovir		Rühs Hauke (Clinical Pharm, SL 042) PK/PD model of methotrexate-associated elevation of homocysteine levels	
12.00 -12.15		Heiss Elke (SL 023) Activation of Nrf2 alleviates hyperglycemia and endothelial dysfunction - a new potential target for natural products in the battle against metabolic dysfunction?		Peters Tanja (SL 031) Liposomal drug carriers for neutron capture therapy - Influence of lipid composition on liposome uptake in Glioblastoma cells		Jaffan Linda (Clinical Pharm, SL 043) High susceptibility for prescribing errors of pro re nata (on demand) medication jeopardize children´s medication safety in a hospital setting	
12.15 - 12.30		Pergola Carlo (SL 024) Leukotriene biosynthesis is sex-biased in human monocytes		Pardeike Jana (SL 032) Aspergillosis in falcons – Itraconazole-loaded Nanostructured Lipid Carriers (NLC) for pulmonary application		Niemann Dorothee (Clinical Pharm, SL044) Risk of drug-related problems at the interface of prescription to administration in a paediatric intensive care unit	
12.30-14.00	**Lunch**						

Joint Meeting of the Austrian and German Pharmaceutical Societies
September 20 - 23, 2011, University of Innsbruck, Austria

Time Schedule

THURSDAY, September 22 2011 (Continuation)					
14.00 - 14.45	**PL 6, Prof. Dr. Rainer H. Müller (Freie Universität Berlin, Germany)** *"20 years of nanocrystals - ´state of the art´ and perspectives"* ***Chair: A. Bernkop-Schnürch***				**Aula HS 1**
14.45 - 16.30	**Poster presentation and Coffee break**				**Foyer**
	OMICs and Biopharmaceutics Chair: A. Kungl **HS3**	**Computer Aided Drug Design** Chair: T. Langer **HS1**	**Miscellaneous Topics** Chair: R. Gust **HS2**	**Counterfeit drugs** Chairs: U. Holzgrabe, M. Stein, U. Griesser **Aula**	
16.30 - 16.45	Wätzig Hermann (SL 033) Protein interactions with their surrounding anions and cations precisely detected by Affinity Capillary Electrophoresis	Langer Thierry (SL 037) Hot Topics in Computer-Assisted Drug Discovery	Rademann Jörg (Pharm. Chem, SL 045) Target Validation Using Fragment-Based Protein Ligands	Round Table	
16.45 - 17.00	Huber Lukas (SL 034) Time resolved analyses of signaling/scaffold complexes	Sippl Wolfgang (SL 038) Combining virtual and biological screening for efficient lead structure identification of epigenetic targets	Eichler Jutta (Pharm. Chem, SL 046) Exploring Pathogen - Host Interactions through Synthetic Mimicry of Viral Protein Binding Sites		
17.00 - 17.15	Bonn Günther (SL 0345 Novel Extraction, Enrichment, Separation and Spectroscopic Approaches for Phytomics	Cruciani Gabriele (SL 039) High-throuput, fully automated, specific MetID. A revolution for drug discovery	Culmsee Carsten (Pharmacology, SL 047) Mechanisms of mitochondrial fragmentation as a target for neuroprotective strategies		
17.15 - 17.30	Köberle Andreas (SL 036) Lysophosphatidic acid acyltransferase 3 – a novel candidate enzyme for infertility therapy with dual function in the testis	Sotriffer Christoph (SL 040) Scoring functions for affinity prediction: New routes out of the forest?	Enzenperger Christoph (Pharmacology, SL 048) Fishing for allosteric binding sites at GPCRs with "loop-hooks"? –A way to selectivity?		
17.45 - 18.30	**PL 7, Prof. Dr. Andreas Kungl (University of Graz, Austria)** *"A novel class of biopharmaceuticals based on proteoglycanomic target profiling"* Chair: S. Glasl-Tazreiter				**Aula HS 1**
19.30	**CONFERENCE DINNER (Stiftskeller)**				

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Time Schedule

FRIDAY, September 23 2011		
09.00 - 09.45	**PL 8, Prof. Dr. Heyo KRÖMER (University of Greifswald, Germany)** *"Relevance of drug transport: From micropharmacokinetics to personalized medicine"* Chair: G. Ecker	**Aula HS 1**
10.00 - 10.45	**KL 1, Prof. Masahiro NISHIJIMA, Ph.D., Tokio** *"The molecular basis underlying the low toxicity of a potent adjuvant monophosphoryl lipid A"* **KL 2, Prof. Hideyoshi HARASHIMA, *Hokkaido University*:** *"Multifunctional envelope-type nano device for gene delivery: Concept and application for nanomedicine"* Chair: A. Link	**Aula HS 1**
10.45 - 11.15	**Coffee break**	
11.15 - 12.00	**PL 9, Prof. Dr. Verena DIRSCH (University of Vienna, Austria)** *"Bioactive natural products targeting inflammation"* Chair: S. Alban	**Aula HS 1**
12.00 - 13.00	**AWARDS, HONOURS and CLOSING CEREMONY**	**Aula**
13.00 - 15.30	General assembly DPhG	**Aula**

Wednesday, September 21 2011

13.00 – 13.30 **Aula**

OPENING CEREMONY OF THE JOINT MEETING OF THE AUSTRIAN AND GERMAN PHARMACEUTICAL SOCIETIES

13.30 – 15.00 **Aula / HS 1**

PLENARY LECTURES

13.30 - 14.15 **Plenary Speaker (PL - 001)**
Chair: Noe Ch.

Klebe G
Institute of Pharmaceutical Chemistry, University of Marburg, Germany
DRUG DISCOVERY STARES AT BINDING AFFINITY, BUT WHAT DOES THIS PROPERTY REALLY MEAN IN DETAIL?

14.15 - 15.00 **Plenary Speaker (PL - 002)**
Chair: Noe Ch.

Sieghart W
Center for Brain Research, Department of Biochemistry and Molecular Biology, Medical University Vienna, Austria
GABAA RECEPTOR SUBTYPES – EXCITING TARGETS FOR THE DEVELOPMENT OF CLINICALLY IMPORTANT DRUGS

15.00 - 16.45 **Foyer, 1st and 2nd Floor**

COFFEE BREAK AND POSTER PRESENTATION (PO - 001 TO PO - 129)

16.45 - 17.45 **Aula**

SHORT LECTURES (INFLAMMATION)
Chairs: Bauer R, Steinhilber D

16.45 - 17.00 **Inflammation (SL - 001)**

Steinhilber D, Hofmann B, Wisniewska J, Rödl C, Stark H, Proschak E, Schneider G
Institute of Pharmaceutical Chemistry, Goethe-University, Frankfurt/Main, Germany
MOLECULAR PHARMACOLOGY OF 5-LIPOXYGENASE AND NEW INHIBITORS

17.00 - 17.15 **Inflammation (SL - 002)**

Maier T, Fischer AS, Steinbrink SD, Steinhilber D
Institute of Pharmaceutical Chemistry, Goethe-University, Frankfurt/Main, Germany
ON THE ROLE OF THE PRO-INFLAMMATORY ENZYME 5-LIPOXYGENASE IN TUMORIGENESIS

17.15 - 17.30 **Inflammation (SL - 003)**

Laufer S, Fischer S, Karcher S, Burnet MW
Institute of Pharmacy,Pharm./Med. Chem., University of Tuebingen, Germany
APPLICATION OF P38 MAP-KINASE-INHIBITORS IN CNS DISEASES

17.30 - 17.45 **Inflammation (SL - 004)**

Hinz B
Institute of Toxicology and Pharmacology, University of Rostock, Germany
PARACETAMOL AND CYCLOOXYGENASE INHIBITION – IS THERE A CAUSE FOR CONCERN?

17.45 - 18.30 **Foyer and 1st Floor**

COFFEE BREAK

18.30 - 19.30 **Aula**

SHORT LECTURES (INFLAMMATION)
Chairs: Bauer R, Steinhilber D

18.30 - 18.45 **Inflammation (SL - 005)**

Fürst R, Mayer BA, Rehberg M, Erhardt A, Reichel CA, Krombach F, Tiegs G, Zahler S, Vollmar AM
Center for System-Based Drug Research, Department of Pharmacy, University of Munich, Germany
ENDOTHELIAL INHIBITOR OF APOPTOSIS PROTEINS (IAPS) – NOVEL PROMISING ANTI-INFLAMMATORY DRUG TARGETS

18.45 - 19.00 **Inflammation (SL - 006)**

Kiemer A, Hoppstädter J, Diesel B
Pharmaceutical Biology, Saarland University, Saarbrücken, Germany
DOWNREGULATION OF GLUCOCORTICOID-INDUCED LEUCINE ZIPPER (GILZ) PROMOTES AN INFLAMMATORY RESPONSE IN HUMAN MACROPHAGES

19.00 - 19.15 **Inflammation (SL - 007)**

Burnet M, Guse J, Hahn U, Mencarelli A, Fiorucci S
Synovo GmbH, Tübingen, Germany
MACROCYCLIC ANTI-INFLAMMATORY COMPOUNDS

19.15 - 19.30 **Inflammation (SL - 008)**

Bochkov V
Department of Vascular Biology and Thrombosis Research, Center for Physiology and Pharmacology, Vienna, Austria
THE JANUS FACE OF OXIDIZED PHOSPHOLIPIDS IN INFLAMMATION: A STORY OF UNMET NEEDS AND POTENTIAL LEADS

16.45 - 17.45 **HS1 / HS2**

SHORT LECTURES (G-PROTEINS)
Chairs: Holzgrabe U, Mohr K

16.45 - 17.00 **G-Proteins (SL - 009)**

Schmidhammer H, Spetea M

Department of Pharmaceutical Chemistry, Institute of Pharmacy and Center for Molecular Biosciences, University of Innsbruck, Austria
ADVANCES IN THE DEVELOPMENT OF ZWITTERIONIC OPIOID RECEPTOR AGONISTS AND ANTAGONISTS

17.00 - 17.15 **G-Proteins (SL - 010)**

Gmeiner P
Department of Chemistry and Pharmacy, Emil Fischer Center, Friedrich Alexander University, Erlangen, Germany
TAILOR-MADE MOLECULAR PROBES FOR THE FOR THE FIRST X-RAY CRYSTAL STRUCTURE OF AN AGONIST-GPCR COMPLEX

17.15 - 17.30 **G-Proteins (SL - 011)**

Gilsbach R, Hein L
Institute of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Germany
CELL-TYPE SPECIFIC FUNCTIONS OF A2-ADRENOCEPTORS

17.30 - 17.45 **G-Proteins (SL - 012)**

Hoffmann C, Bätz J, Klöckner J, Ziegler N, Frölich N, Zabel U, Holzgrabe U, Mohr K, Lohse MJ
Institute of Pharmacology and Toxicology, University of Würzburg, Germany
HOW DO ORTHOSTERIC AND ALLOSTERIC LIGANDS INFLUENCE THE CONFORMATIONAL CHANGE OF THE M2 MUSCARINIC ACETYLCHOLINE RECEPTOR DIFFERENTIALLY?

17.45 - 18.30 **Foyer and 1st Floor**

COFFEE BREAK

18.30 - 19.30 **HS1 / HS2**

SHORT LECTURES (G-PROTEINS)
Chairs: Holzgrabe U, Mohr K

18.30 - 18.45 **G-Proteins (SL - 013)**

Stark H, Eichelsbacher EMC, Saur O, Kottke T, Sasse BC, Hill MP, Crossman A, Bezard E
Johann Wolfgang Goethe University, Institute of Pharmaceutical Chemistry, Biocenter, ZAFES/LiFF/CMP/ICNF, Frankfurt, Germany
BIOISOSTERIC REPLACEMENT IN PRAMIPEXOLE-RELATED DOPAMINE AGONISTS

18.45 - 19.00 **G-Proteins (SL - 014)**

Kassack M, Eßer D, Hamacher A
Institute of Pharm. and Med. Chemistry, Heinrich-Heine-University Duesseldorf, Germany
THE NUCLEOTIDE RECEPTOR P2Y11 PLAYS A ROLE IN IMMUNE SYSTEM ASSOCIATED DISEASES

19.00 - 19.15 **G-Proteins (SL - 015)**

Müller C, Baqi Y
PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, Bonn, Germany
ANTHRAQUINONE DERIVATIVES AS POTENT AND SELECTIVE COMPETITIVE ANTAGONISTS FOR P2 RECEPTORS

19.15 - 19.30 **G-Proteins (SL - 016)**

Zlotos D, Attia MI, Markl C, Behnam MAM, Mohsen AMY, Julius J, Heckman D, Clafshenkel WP, Sethi S, Witt-Enderby PA
The German University in Cairo, Dept. of Pharmaceutical Chemistry, New Cairo City, Egypt
DEVELOPMENT OF SUBTYPE-SELECTIVE LIGANDS FOR MELATONIN RECEPTORS

19.30 - 20.15 **Aula / HS1**

PLENARY LECTURE

19.30 - 20.15 **Plenary Speaker (PL - 003)**
Chair: Werz O

Busch A
Bayer HealthCare AG, Head of Global Drug Discovery, Berlin, Germany
CHALLENGES IN PHARMA RESEARCH AND FUTURE DEVELOPMENTS

Thursday, September 22 2011

08.30 - 10.00 **Aula / HS1**

PLENARY LECTURES

08.30 - 09.15 **Plenary Speaker (PL - 004)**
Chair: Viernstein H

Premstaller A
Sandoz GmbH, Austria
MONOCLONAL ANTIBODIES: THE PERSPECTIVE OF A DEVELOPER OF BIOSIMILARS

09.15 - 10.00 **Plenary Speaker (PL - 005)**
Chair: Holzgrabe U

Pelliciari R
Department of Chemistry and Drug Technologies, Università di Perugia, Italy
TARGETING FXR AND TGR5 RECEPTOR PATHWAYS IN SEARCH FOR NOVEL TREATMENTS FOR LIVER AND METABOLIC DISORDERS

10.00 - 10.30 **Foyer and 1st Floor**

COFFEE BREAK

10.30 - 12.30 **Aula**

SHORT LECTURES (NATURAL PRODUCTS)
Chairs: Stuppner H, Dirsch V

10.30 - 10.45 **Natural Products (SL - 017)**

Alban S
Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Germany
SULFATED POLYSACCHARIDES OF DELESSERIA SANGUINEA FROM THE ARTIFICIAL REEF NIENHAGEN

10.45 - 11.00 **Natural Products (SL - 018)**

Merfort I
Inst. Pharm. Science, Dept. Pharm. Biol. Biotechnology, University Freiburg, Germany
STATE OF THE ART ON THE WOUND HEALING PROPERTIES OF BIRCH BARK

11.00 - 11.15 **Natural Products (SL - 019)**

Vollmar A, Schwarzenberg K, Wiedmann R, Liebl J, Efferth T, Trauner D, Wenzel S, Müller R
Center for Drug Research, Department of Pharmacy, University of Munich, Germany
V-ATPASE INHIBITORS FROM MYXOBACTERIA – PROMISING LEADS AND TOOLS FOR CANCER THERAPY?

11.15 - 11.30 **Natural Products (SL - 020)**

Nicoletti M
Department of Enviromental Biology, University Sapienza of Rome, Italy
THE IDENTIFICATION AND CHEMICAL DETERMINATION OF COMPLEX BOTANICAL MIXTURES

11.30 - 11.45 **Natural Products (SL - 021)**

Werz O
Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, University of Jena, Germany
HYPERFORIN: A DUAL INHIBITOR OF LEUKOTRIENE AND PROSTAGLANDIN E2 BIOSYNTHESIS AS ANTI-INFLAMMATORY LEAD FROM NATURE

11.45 - 12.00 **Natural Products (SL - 022)**

Bauer R, Liu X, Blunder M, Schinkovitz A, Kunert O, Atanasov AG, Dirsch VM
Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-Universität Graz, Austria
INVESTIGATION OF NOTOPTERYGIUM INCISUM FOR ANTI-INFLAMMATORY CONSTITUENTS

12.00 - 12.15 **Natural Products (SL - 023)**

Heiss E, Kramer MP, Schachner D, Dirsch VM
Department of Pharmacognosy , University of Vienna, Austria
ACTIVATION OF NRF2 ALLEVIATES HYPERGLYCEMIA AND ENDOTHELIAL DYSFUNCTION - A NEW POTENTIAL TARGET FOR NATURAL PRODUCTS IN THE BATTLE AGAINST METABOLIC DYSFUNCTION?

12.15 - 12.30 **Natural Products (SL - 024)**

Pergola C, Rogge A, Dehm F, Rossi A , Sautebin L, Werz O
Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, University Jena, Germany

LEUKOTRIENE BIOSYNTHESIS IS SEX-BIASED IN HUMAN MONOCYTES

13.00 - 14.00 **HS1 / HS2**

WORKSHOPS FA. DIONEX, WORKSHOP FA. MILESTONES

10.30 - 11.30 **HS1**

SHORT LECTURES (TRANSPORTER)
Chairs: Ecker GF, Krömer H

10.30 - 10.45 **Transporter (SL - 025)**

Ecker GF
University of Vienna, Dept. Medicinal Chemistry, Austria
THE MEDICINAL CHEMISTRY OF DRUG TRANSPORT – LIGAND-BASED DESIGN MEETS STRUCTURE-BASED DESIGN

10.45 - 11.00 **Transporter (SL - 026)**

Meyer zu Schwabedissen H, Ware JA, Schwarz UI, Finkelstein D, Chaudhry AS, Schuetz EG, Tirona RG, Kroemer HK, Kim RB
Department of Pharmacology, Ernst Moritz Arndt University of Greifswald, Germany
THE ROLE OF OATP1B TRANSPORTERS IN PHARMACOLOGY AND PHYSIOLOGY OF LIVER

11.00 - 11.15 **Transporter (SL - 027)**

Bauer S, Ochoa Puentes C, Bernhardt G, König B, Buschauer A
Institute of Pharmacy, University of Regensburg, Germany
OVERCOMING ABCG2-MEDIATED DRUG RESISTANCE WITH NEW ABCG2 MODULATORS DERIVED FROM TARIQUIDAR

11.15 - 11.30 **Transporter (SL - 028)**

Klepsch F, Vosmeer CR, Stockner T, Chiba P, Geerke DP, Ecker GF
University of Vienna, Department of Medicinal Chemistry, Vienna, Austria
ANALYSIS OF BINDING MODES OF PROPAFENONES IN P-GLYCOPROTEIN BY MEANS OF MOLECULAR DYNAMICS SIMULATIONS

11.30 - 12.30 **HS3**

SHORT LECTURES (NANOMEDICINE)
Chair: Bernkop-Schnürch

11.30 - 11.45 **Nanomedicine (SL - 029)**

Caliceti P, Scomparin A, Salmaso S, Satchi-Fainaro R
Dep. of Pharmaceutical Sciences, University of Padua, Italy
POLYSACCHARIDE BIOCONJUGATES FOR TUMOR TARGETING

11.45 - 12.00 **Nanomedicine (SL - 030)**

Schwarz J, Klang V, Karall S, Haberfeld S, Resch GP, Valenta C
University of Vienna, Research Platform "Characterisation of Drug Delivery Systems on Skin and Investigation of Involved Mechanisms", Austria
NANOCARRIERS FOR DERMAL DRUG DELIVERY: MULTIPLE W/O/W NANOEMULSIONS FOR TOPICAL APPLICATION OF ACICLOVIR

12.00 - 12.15 **Nanomedicine (SL - 031)**

Peters T, Grunewald C, Hampel G, Nawroth T, Langguth P
Department of Biopharmaceutics and Pharmaceutical Technology, Johannes Gutenberg-University Mainz, Germany
LIPOSOMAL DRUG CARRIERS FOR NEUTRON CAPTURE THERAPY - INFLUENCE OF LIPID COMPOSITION ON LIPOSOME UPTAKE IN GLIOBLASTOMA CELLS

12.15 - 12.30 **Nanomedicine (SL - 032)**

Pardeike J, Weber S, Zarfl HP, Zimmer A
Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology, Karl-Franzens Universität Graz, Austria
ASPERGILLOSIS IN FALCONS – ITRACONAZOLE-LOADED NANOSTRUCTURED LIPID CARRIERS (NLC) FOR PULMONARY APPLICATION

11.30 - 12.30 **HS2**

SHORT LECTURES (MISCELLANEOUS TOPICS)
Chair: Lemmens-Gruber R

11.30 - 11.45 **Miscellaneous Topics - Pharmacology (SL - 041)**

Liebl J, Stamm S, Günther M, Mayr D, De Toni EN, Vollmar A, Zahler S
Center of Drug Research, Ludwig-Maximilians University, Munich, Germany
CYCLIN DEPENDENT KINASE 5 (CDK5) AND ITS FUNCTION IN HEPATOCELLULAR CARCINOMA

11.45 - 12.00 **Miscellaneous Topics - Clinical Pharmacy (SL - 042)**

Rühs H, Drescher A, Becker A, Panetta JC, Pui CH, Relling MV, Jaehde U
Department of Clinical Pharmacy, University of Bonn, Germany
PK/PD MODEL OF METHOTREXATE-ASSOCIATED ELEVATION OF HOMOCYSTEINE LEVELS

12.00 - 12.15 **Miscellaneous Topics - Clinical Pharmacy (SL - 043)**

Jaffan L, Gorny M, Läer S
Department of Clinical Pharmacy and Pharmacotherapy, Heinrich-Heine-Universität Düsseldorf, Germany
HIGH SUSCEPTIBILITY FOR PRESCRIBING ERRORS OF PRO RE NATA (ON DEMAND) MEDICATION JEOPARDIZE CHILDREN´S MEDICATION SAFETY IN A HOSPITAL SETTING

12.15 - 12.30 **Miscellaneous Topics - Clinical Pharmacy (SL - 044)**

Niemann D, Oelsner S, Ewen AL, Meyrath D, Pickardt B, Henhapl T, Böhm M, Meyburg J, Ruef P, Haefeli WE, Bertsche T
Dept. of Clinical Pharmacy, Institute of Pharmacy, University of Leipzig, Germany
RISK OF DRUG-RELATED PROBLEMS AT THE INTERFACE OF PRESCRIPTION TO ADMINISTRATION IN A PAEDIATRIC INTENSIVE CARE UNIT

13.00 - 14.00 **HS1 / HS2**

WORKSHOPS FA. DIONEX, WORKSHOP FA. MILESTONES

14.00 - 14.45 **Aula / HS1**

PLENARY LECTURE

14.00 - 14.45 **Plenary Speaker (PL - 006)**
Chair: Bernkop-Schnürch A

Müller R
Free University of Berlin, Institute of Pharmacy, Pharmaceutical Technology, Kelchstr. 31, 12169 Berlin, Germany
20 YEARS OF DRUG NANOCRYSTALS – ´STATE OF THE ART´ AND PERSPECTIVES

14.45 - 16.30 **Foyer and 1st Floor**

COFFEE BREAK AND POSTER PRESENTATION (PO - 130 TO PO - 248)

16.30 - 17.30 **HS3**

SHORT LECTURES (OMIC'S AND BIOPHARMACEUTICS)
Chair: Kungl A

16.30 - 16.45 **OMICs and Biopharmaceutics (SL - 033)**

Wätzig H, Redweik S, Xu Y
TU Braunschweig, Institut für Medizinische und Pharmazeutische Chemie, Germany
PROTEIN INTERACTIONS WITH THEIR SURROUNDING ANIONS AND CATIONS PRECISELY DETECTED BY AFFINITY CAPILLARY ELECTROPHORESIS

16.45 - 17.00 **OMICs and Biopharmaceutics (SL - 034)**

Huber L, Stasyk T
Biocenter, Division of Cell Biology, Innsbruck Medical University, Innsbruck, Austria
TIME RESOLVED ANALYSES OF SIGNALING/SCAFFOLD COMPLEXES

17.00 - 17.15 **OMICs and Biopharmaceutics (SL - 035)**

Bonn G, Huck C
Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University Innsbruck, Austria
NOVEL EXTRACTION, ENRICHMENT, SEPARATION AND SPECTROSCOPIC APPROACHES FOR PHYTOMICS

17.15 - 17.30 **OMICs and Biopharmaceutics (SL - 036)**

Köberle A, Shindou H, Harayama T, Yuki K, Shimizu T
Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller-University, Jena, Germany
LYSOPHOSPHATIDIC ACID ACYLTRANSFERASE 3 – A NOVEL CANDIDATE ENZYME FOR INFERTILITY THERAPY WITH DUAL FUNCTION IN THE TESTIS

16.30 - 17.30 **HS1**

SHORT LECTURES (COMPUTER AIDED DRUG DESIGN)
Chair: Langer T.

16.30 - 16.45 **Computer Aided Drug Design (SL - 037)**

Langer T
Prestwick Chemical SAS, Boulevard Gonthier d'Andernach, 67400 Strasbourg-Illkirch, France
HOT TOPICS IN COMPUTER-ASSISTED DRUG DISCOVERY

16.45 - 17.00 **Computer Aided Drug Design (SL - 038)**

Sippl W
Department of Pharmaceutical Chemistry, Martin-Luther-Universität Halle-Wittenberg, Germany
COMBINING VIRTUAL AND BIOLOGICAL SCREENING FOR EFFICIENT LEAD STRUCTURE IDENTIFICATION OF EPIGENETIC TARGETS

17.00 - 17.15 **Computer Aided Drug Design (SL - 039)**

Cruciani G, Zamora I, Fontaine F
University of Perugia, Italy
HIGH-THROUPUT, FULLY AUTOMATED, SPECIFIC METLD. A REVOLUTION FOR DRUG DISCOVERY

17.15 - 17.30 **Computer Aided Drug Design (SL - 040)**

Sotriffer C, Zilian D
Institute of Pharmacy and Food Chemistry, University of Wuerzburg, Germany
SCORING FUNCTIONS FOR AFFINITY PREDICTION: NEW ROUTES OUT OF THE FOREST?

16.30 - 17.30 **HS2**

SHORT LECTURES (MISCELLANEOUS TOPICS)
Chair: Gust R.

16.30 - 16.45 **Miscellaneous Topics - Pharm/Med. Chemistry (SL - 045)**

Rademann J
Institute of Pharmacy, Leipzig University, Germany
TARGET VALIDATION USING FRAGMENT-BASED PROTEIN LIGANDS

16.45 - 17.00 **Miscellaneous Topics - Pharm/Med. Chemistry (SL - 046)**

Eichler J, Berthelmann A, Groß A, Meier J, Möbius K, Mössl M, Schmidt B, Sticht H
Department Medicinal Chemistry, Universität Erlangen-Nürnberg, Erlangen, Germany
EXPLORING PATHOGEN - HOST INTERACTIONS THROUGH SYNTHETIC MIMICRY OF VIRAL PROTEIN BINDING SITES

17.00 - 17.15 **Miscellaneous Topics - Pharmacology (SL - 047)**

Culmsee C, Grohm J, Tobaben S
Institut für Pharmakologie und Klinische Pharmazie, Fachbereich Pharmazie, Philipps-Universität Marburg, Germany
MECHANISMS OF MITOCHONDRIAL FRAGMENTATION AS A TARGET FOR NEUROPROTECTIVE STRATEGIES

17.15 - 17.30 **Miscellaneous Topics - Pharmacology (SL - 048)**

Enzensperger C, Tröger T, Ambrosini D , Lehmann J
Institute for Pharmacy, Department of Medicinal Chemistry, Jena, Germany
FISHING FOR ALOSTERIC BINDING SITES AT GPCRS WITH "LOOP-HOOKS"? –A WAY TO SELECTIVITY?

16.30 - 17.45 **AULA**

COUNTERFEIT DRUGS, ROUND TABLE
Chairs: Holzgrabe U, Griesser U

17.45 - 18.30 **Aula / HS1**

PLENARY LECTURE

17.45 - 18.30 **Plenary Speaker (PL - 007)**
Chair: Glasl-Tazreiter S

Kungl A
ProtAffin Biotechnologie AG, Graz, Austria
A NOVEL CLASS OF BIOPHARMACEUTICALS BASED ON PROTEOGLYCANOMIC TARGET PROFILING

19.30 **Stiftskeller / Stiftgasse 1, 6020 Innsbruck**

CONFERENCE DINNER

Friday, September 23 2011

09.00 - 09.45 **Aula / HS1**

PLENARY LECTURE

09.00 - 09.45 **Plenary Speaker (PL - 008)**
Chair: Ecker GF

Krömer H
Dep. of Pharmacology, Center of Pharmacology and Experimental Therapeutics, Ernst Moritz Arndt University Greifswald, Germany
RELEVANCE OF DRUG TRANSPORT: FROM MICROPHARMACOKINETICS TO PERSONALIZED MEDICINE

10.00 - 10.45 **Aula / HS1**

KEY LECTURES

10.00 - 10.25 **Keylecture (KL - 001)**
Chair: Link A

Nishijima M
President Pharmaceutical Society of Japan, Showa Pharmaceutical University Machida, Tokio, Japan
THE MOLECULAR BASIS UNDERLYING THE LOW TOXICITY OF A POTENT ADJUVANT MONOPHOSPHORYL LIPID A

10.25 - 10.45 **Keylecture (KL - 002)**
Chair: Link A

Harashima H
Laboratory for Molecular Design of Pharmaceutics & Laboratory of Future Medicine, Faculty of Pharmaceutical Sciences, Hokkaido University, Japan
MULTIFUNCTIONAL ENVELOPE-TYPE NANO DEVICE FOR NON-VIRAL GENE DELIVERY: CONCEPT AND APPLICATION FOR NANOMEDICINE

10.45 - 11.15 **Foyer**

COFFEE BREAK

11.15 - 12.00 **Aula / HS1**

PLENARY LECTURE

11.15 - 12.00 **Plenary Speaker (PL - 009)**
Chair: Alban S

Dirsch V
Department of Pharmacognosy, University of Vienna, Austria
BIOACTIVE NATURAL PRODUCTS TARGETING INFLAMMATION

12.00 - 13.00 **Aula**

AWARDS, HONOURS AND CLOSING CEREMONY

13.00 - 15.30 **Aula**

GENERAL ASSEMBLY DPHG

Notes

LIST OF SCIENTIFIC CONTRIBUTIONS

Plenary Lectures

PL - 001 **Drug discovery stares at binding affinity, but what does this property really mean in detail?**
Klebe G

PL - 002 **GABAA receptor subtypes – exciting targets for the development of clinically important drugs**
Sieghart W

PL - 003 **Challenges in pharma research and future developments**
Busch A

PL - 004 **Monoclonal antibodies: The perspective of a developer of biosimilars**
Premstaller A

PL - 005 **Targeting FXR and TGR5 receptor pathways in search for novel treatments for liver and metabolic disorders**
Pellicciari R

PL - 006 **20 years of drug nanocrystals – ´state of the art´ and perspectives**
Müller RH

PL - 007 **A novel class of biopharmaceuticals based on proteoglycanomic target profiling**
Kungl AJ

PL - 008 **Relevance of drug transport: From micropharmacokinetics to personalized medicine**
Kroemer HK

PL - 009 **Bioactive natural products targeting inflammation**
Dirsch VM

Key Lectures

KL - 001 **The molecular basis underlying the low toxicity of a potent adjuvant monophosphoryl lipid A**
Nishijima M

KL - 002 **Multifunctional envelope-type nano device for non-viral gene delivery: concept and application for nanomedicine**
Harashima H

Short Lectures

SL - 001 **Molecular pharmacology of 5-lipoxygenase and new inhibitors**
Steinhilber D, Hofmann B, Wisniewska J, Rödl C, Stark H, Proschak E, Schneider G

SL - 002 **On the role of the pro-inflammatory enzyme 5-lipoxygenase in tumorigenesis**
Maier TJ, Fischer AS, Steinbrink SD, Steinhilber D

SL - 003 **Application of p38 MAP-Kinase-Inhibitors in CNS diseases**
Laufer S, Fischer S, Karcher S, Burnet MW

SL - 004 **Paracetamol and cyclooxygenase inhibition – is there a cause for concern?**
Hinz B

SL - 005 **Endothelial inhibitor of apoptosis proteins (IAPs) – novel promising anti-inflammatory drug targets**
Fürst R, Mayer BA, Rehberg M, Erhardt A, Reichel CA, Krombach F, Tiegs G, Zahler S, Vollmar AM

SL - 006 **Downregulation of Glucocorticoid-induced leucine zipper (GILZ) promotes an inflammatory response in human macrophages**
Kiemer AK, Hoppstädter J, Diesel B

SL - 007 **Macrocyclic anti-inflammatory compounds**
Burnet MW, Guse J, Hahn U, Mencarelli A, Fiorucci S

SL - 008 **The Janus face of oxidized phospholipids in inflammation: a story of unmet needs and potential leads**
Bochkov VN

SL - 009 **Advances in the development of zwitterionic opioid receptor agonists and antagonists**
Schmidhammer H, Spetea M

SL - 010 **Tailor-made molecular probes for the for the first X-ray crystal structure of an agonist-GPCR complex**
Gmeiner P

SL - 011 **Cell-type specific functions of α2-adrenoceptors**
Gilsbach R, Hein L

SL - 012 **How do orthosteric and allosteric ligands influence the conformational change of the M2 muscarinic acetylcholine receptor differentially?**
Hoffmann C, Bätz J, Klöckner J, Ziegler N, Frölich N, Zabel U, Holzgrabe U, Mohr K, Lohse MJ

SL - 013 **Bioisosteric Replacement in Pramipexole-related Dopamine Agonists**
Stark H, Eichelsbacher EMC, Saur O, Kottke T, Sasse BC, Hill MP, Crossman A, Bezard E

SL - 014 **The Nucleotide Receptor P2Y11 Plays a Role in Immune System Associated Diseases**
Kassack MU, Eßer D, Hamacher A

SL - 015 **Anthraquinone Derivatives as Potent and Selective Competitive Antagonists for P2 Receptors**
Müller CE, Baqi Y

SL - 016 **Development of Subtype-Selective Ligands for Melatonin Receptors**
Zlotos DP, Attia MI, Markl C, Behnam MAM, Mohsen AMY, Julius J, Heckman D, Clafshenkel WP, Sethi S, Witt-Enderby PA

SL - 017 **Sulfated polysaccharides of Delesseria sanguinea from the artificial reef Nienhagen**
Alban S

SL - 018 **State of the art on the wound healing properties of birch bark**
Merfort I

SL - 019 **V-ATPase Inhibitors from myxobacteria – promising leads and tools for cancer therapy?**
Vollmar A, Schwarzenberg K, Wiedmann R, Liebl J, Efferth T, Trauner D, Wenzel S, Müller R

SL - 020 **The identification and chemical determination of complex botanical mixtures**
Nicoletti M

SL - 021 **Hyperforin: A dual inhibitor of leukotriene and prostaglandin E2 biosynthesis as anti-inflammatory lead from nature**
Werz O

SL - 022 **Investigation of Notopterygium incisum for anti-inflammatory constituents**
Bauer R, Liu X, Blunder M, Schinkovitz A, Kunert O, Atanasov AG, Dirsch VM

SL - 023 **Activation of Nrf2 alleviates hyperglycemia and endothelial dysfunction - a new potential target for natural products in the battle against metabolic dysfunction?**
Heiss EH, Kramer MP, Schachner D, Dirsch VM

SL - 024 **Leukotriene biosynthesis is sex-biased in human monocytes**
Pergola C, Rogge A, Dehm F, Rossi A , Sautebin L, Werz O

SL - 025 **The Medicinal Chemistry of Drug Transport – Ligand-based Design meets Structure-based Design**
Ecker GF

SL - 026 **The role of OATP1B transporters in pharmacology and physiology of liver**
Meyer zu Schwabedissen HE, Ware JA, Schwarz UI, Finkelstein D, Chaudhry AS, Schuetz EG, Tirona RG, Kroemer HK, Kim RB

SL - 027 **Overcoming ABCG2-mediated drug resistance with new ABCG2 modulators derived from tariquidar**
Bauer S, Ochoa Puentes C, Bernhardt G, König B, Buschauer A

SL - 028 **Analysis of binding modes of propafenones in P-glycoprotein by means of molecular dynamics simulations**
Klepsch F, Vosmeer CR, Stockner T, Chiba P, Geerke DP, Ecker GF

SL - 029 **Polysaccharide bioconjugates for tumor targeting**
Caliceti P, Scomparin A, Salmaso S, Satchi-Fainaro R

SL - 030 **Nanocarriers for dermal drug delivery: multiple W/O/W nanoemulsions for topical application of aciclovir**
Schwarz JC, Klang V, Karall S, Haberfeld S, Resch GP, Valenta C

SL - 031 **Liposomal drug carriers for neutron capture therapy - Influence of lipid composition on liposome uptake in Glioblastoma cells**
Peters T, Grunewald C, Hampel G, Nawroth T, Langguth P

SL - 032 **Aspergillosis in falcons – Itraconazole-loaded Nanostructured Lipid Carriers (NLC) for pulmonary application**
Pardeike J, Weber S, Zarfl HP, Zimmer A

SL - 033 **Protein interactions with their surrounding anions and cations precisely detected by Affinity Capillary Electrophoresis**
Wätzig H, Redweik S, Xu Y

SL - 034 **Time resolved analyses of signaling/scaffold complexes**
Huber LA, Stasyk T

SL - 035 **Novel Extraction, Enrichment, Separation and Spectroscopic Approaches for Phytomics**
Bonn G, Huck C

SL - 036 **Lysophosphatidic acid acyltransferase 3 – a novel candidate enzyme for infertility therapy with dual function in the testis**
Koeberle A, Shindou H, Harayama T, Yuki K, Shimizu T

SL - 037 **Hot Topics in Computer-Assisted Drug Discovery**
Langer T

SL - 038 **Combining virtual and biological screening for efficient lead structure identification of epigenetic targets**
Sippl W

SL - 039 **High-throuput, fully automated, specific MetID. A revolution for drug discovery**
Cruciani G, Zamora I, Fontaine F

SL - 040 **Scoring functions for affinity prediction: New routes out of the forest?**
Sotriffer CA, Zilian D

SL - 041 **Cyclin dependent kinase 5 (Cdk5) and its function in Hepatocellular carcinoma**
Liebl J, Stamm S, Günther M, Mayr D, De Toni EN, Vollmar A, Zahler S

SL - 042 **PK/PD model of methotrexate-associated elevation of homocysteine levels**
Rühs H, Drescher A, Becker A, Panetta JC, Pui CH, Relling MV, Jaehde U

SL - 043 **High susceptibility for prescribing errors of pro re nata (on demand) medication jeopardize children´s medication safety in a hospital setting**
Jaffan L, Gorny M, Läer S

SL - 044 **Risk of drug-related problems at the interface of prescription to administration in a paediatric intensive care unit**
Niemann D, Oelsner S, Ewen AL, Meyrath D, Pickardt B, Henhapl T, Böhm M, Meyburg J, Ruef P, Haefeli WE, Bertsche T

SL - 045 **Target Validation Using Fragment-Based Protein Ligands**
Rademann J

SL - 046 **Exploring Pathogen - Host Interactions through Synthetic Mimicry of Viral Protein Binding Sites**
Eichler J, Berthelmann A, Groß A, Meier J, Möbius K, Mössl M, Schmidt B, Sticht H

SL - 047 **Mechanisms of mitochondrial fragmentation as a target for neuroprotective strategies**
Culmsee C, Grohm J, Tobaben S

SL - 048 **Fishing for alosteric binding sites at GPCRs with "loop-hooks"? –A way to selectivity?**
Enzensperger C, Tröger T, Ambrosini D , Lehmann J

Pre-Symposia

PS - 001 **HPLC-DAD / GC-MS examinations for drug safety of emergency stocks – detection, identification and toxicological qualification of a hitherto new degradation product of pyridostigmine relevant to regulatory legislation**
Bogan R

PS - 002 **Analysis of Proteins with Capillary Gel Electrophoresis – Fabulous Precision**
Cianciulli C, Wätzig H

PS - 003 **NIR and Raman spectroscopy in quality control, PAT and the analytics of counterfeit medicines**
Girard P, Meyer S, Meier R

PS - 004 **Protein Quantitation using various Modes of High Performance Liquid Chromatography**
Grotefend S, Wroblewitz S, Kaminski L, Limberger M, Watt S, El Deeb S, Wätzig H

PS - 005 **Nachweis nicht deklarierter Arzneistoffe in Verdachtsproben mittels HPLC/DAD und Absicherung über ESI-MS**
Heuermann M, Scherges M

PS - 006 **Current status of hyphenated low and high resolution mass spectrometry in clinical and forensic toxicology as well as in drug metabolism**
Maurer Hans H

PS - 007 **Illegal Medicines – a Challenge for OMCLs and Mass Spectrometry**
Mayrhofer A

PS - 008 **A novel Method for Absolute Quantification and Stoichiometry Determination of Protein Complexes**
Mechtler K, Holzmann J, Fuchs J, Petzold G, Pichler P, Peters JM

PS - 009 **Challenges and Innovations in the (Bio)analysis of Metal Complexes**
Ott I

PS - 010 **Molecular insights into the aging of elastic fibers**
Schmelzer CEH, Scharm M, Jung MC, Heinz A, Pankau R, Wohlrab J, Neubert RHH

PS - 011 **About Biological Tissues, Mass Spectrometry and Imaging**
Stoeckli M

PS - 012 **BioEquality: A Platform for the Comprehensive Analysis of Data from Stability Studies and Market Approval of Biosimilars**
Watt SA, Martens L, Ebeling C, Spura J,Rumpl A, Springstubbe S, Wozny M

PS - 013 **The Challenges of Evaluating Drug Therapy and Impact on Health Care Structures as Seen by the Pharmaceutical Industry**

Brakmann D

PS - 014 **Cost-benefit evaluation of medicinal products from a hospital pharmacist´s point of view**
Krämer I

PS - 015 **Needs for future managed care settings and their impact on drug the therapy - from a payers perspective**
Maywald U

PS - 016 **Cost-benefit-risk: challenges for drug therapy – the view of the Drug Commission of German Pharmacists (AMK)**
Schulz M

PS - 017 **Criteria for Quality and Cost Effectiveness in Change…..or "It's the economy, stupid"**
Schweim HG

PS - 018 **Polymeric excipients exhibiting efflux pump inhibitory properties**
Bernkop-Schnürch A

PS - 019 **The Area Vasculosa of the Chicken Yolk Sac as Tool for Toxicity Testing of Polymers**
Grund S, Nowak G, Fischer D

PS - 020 **Multi-functional macromonomers for biomedical applications**
Hacker MC

PS - 021 **Degradable shape-memory polymer networks as drug carriers**
Wischke C, Lendlein A

Posters

PO - 001 **Lanostane glycosides from Chionodoxa sardensis and their in vitro inhibition on COX-1**
Arjune S, Klar F

PO - 002 **Effects of non-thermal atmospheric plasma on intracellular structures and processes of keratinocytes**
Blackert S, Haertel B, Wende K, Woedtke v T, Lindequist U

PO - 003 **Pharmacological activation of KCa2.3 channels reverses microglial activation**
Dolga AM, Letsche T, Gold M, Bacher M, Dodel R, Culmsee C

PO - 004 **Design of dibenzosuberones as highly potent p38α MAP kinase inhibitors: Efficient use of a hydrophobic pocket improve activity**
Fischer S, Karcher S, Dorn A, Laufer S

PO - 005 **6β-Tryptophan substituted 14-O-methyloxymorphone, a potent μ opioid receptor agonist with antinociceptive and immunosuppressive activities**
Follia V, Garczarczyk D, Fink M, Asim MF, Schmidhammer H, Spetea M

PO - 006 **Thiazole substituted pirinixic acid derivatives as dual 5-LO / mPGES-1 inhibitors**
Hanke T, Popella S, Werz O, Schubert-Zsilavecz M

PO - 007 **Synthesis and Structure Activity Relationships of Novel Chemokine Receptor 5 Antagonists**
Junker A, Schepmann D, Wünsch B, Itami K

PO - 008 **Towards selective JNK3 Inhibitors: Introduction of acidic residues improves potency of JNK3 inhibitors**
Klos S, Laufer S

PO - 009 **Synthesis and antiviral activities of potential Picornavirus Capsid Binders**
Krapf K, Pürstinger G, Leyssen P, Neyts J

PO - 010 **Olea europaea L. leaf (Ph.Eur.) extract as well as several of its single phenolics inhibit the gout-related enzyme xanthine oxidase**
Kuchta K, Flemmig J, Arnhold J, Rauwald HW

PO - 011 **Combined efficacy of three novel herbal TCM formulas Kujin-Plus I-III on atopic dermatitis in 94 patients**
Kuchta K, Li S, Wang R, Tamaru N, Miyako J, Iwasaki S, Kobayashi Y, Okunishi H, Kamei T, Rauwald HW

PO - 012 **Physical plasma induced generation of physiologically relevant reactive species in liquids**
Oehmigen K, Weltmann K-D, Woedtke v T

PO - 013 **Development of electrochemical sensors for the quantification of inflammatory disease mediators**
Pekec B, Hauser S, Ortner A, Kalcher K

PO - 014 **Cosmetic's evolution between tradition and innovation: Calendula officinalis L. supercritical CO2 extracts**
Portolan A, Cortese AC, Baratto G, Riva E, Dall'Acqua S, Semenzato A, Rigano L, Meloni M

PO - 015 **Differential eicosanoid biosynthesis during the menstrual cycle**
Rogge A, Pergola C, Werz O

PO - 016 **Effect of Toll-like receptor agonists on tumor-immune cell cross-talk**
Wild CA, Bruderek K, Burgsdorff T, Bergmann C, Lang S, Brandau S

PO - 017 **Polyacetylene derivatives from Notopterygium incisum with PPARg agonistic effect**
Liu X, Blunder M, Fakhrudin N, Kunert O, Schinkovitz A, Heiss EH, Atanasov AG, Dirsch VM, Bauer R

PO - 018 **The cardiac fibroblasts as an inflammatory cell in dilated cardiomyopathy**
Zietsch C, Westermann, D, Lindner D, Schultheiss HP, Tschoepe C

PO - 019 **The development of nanoparticles that bind to the somatostatin receptor**
Abdellatif A, Elbakry A, Pollinger K, Osman S, Zaky A, Liebl R, Brandl F, Breunig M, Tessmar J, Goepferich A

PO - 020 **Adenine receptor: Selective receptor ligands and possible signalling pathways**
Bloßfeld M, Nieber K, Borrmann T, Obst A, Bumnaran B, Müller C, Siegert F

PO - 021 **Synthesis and evaluation of thiazolo[5,4-c]pyridines as adenosine A3 receptor ligands**
Briel D, Kubicová L, Sachse P, Kelm I, Müller CE

PO - 022 **Zwitterionic opioid receptor antagonists: Synthesis and pharmacological evaluation**
Ernst V, Spetea M, Berzetei-Gurske IP, Fink M, Rief SB, Schmidhammer H

PO - 023 **Synthesis and pharmacology of clozapine-derived Histamine H1/H4-receptor-ligands**
Gobleder S, Elz S, Straßer A

PO - 024 **Synthesis and opioid receptor binding profile of novel 6-amino acid substituted 14-alkoxy-N-methylmorphinans**
Guerrieri E, Spetea M, Rief SB, Fink M, Follia V, Asim MF, Schmidhammer H

PO - 025 **New Neurotensin Receptor 2 (NTS2) Selective Peptoid-Peptide Hybrids Synthesis, Receptor Binding and SAR Investigations**
Held C, Hübner H, Gmeiner P

PO - 026 **The role of serotonin 5-HT2A receptors in cabergoline-induced valvular heart disease**
Kekewska A, Pertz HH

PO - 027 **Synthesis of iperoxo-derivatives for receptor activation studies on muscarinic M2 acetylcholine receptors**
Klöckner J, Schrage R, Kaufel D, Mohr K, Holzgrabe U

PO - 028 **Identification of novel allosteric modulators for the G-protein coupled US28 receptor of human cytomegalovirus**
Kralj A, Wetzel A, Mahmoudian S, Stamminger T, Tschammer N, Heinrich MR

PO - 029 **Visualization of the unseen: use of novel radiotracers for imaging the neurotensin 1 receptor (NTS1)**
Lang C, Hübner H, Maschauer S, Prante O, Gmeiner P

PO - 030 **Gene reporter assay for the investigation of human and murine histamine H4 receptor ligands**
Nordemann U, Schnell D, Bernhardt G, Seifert R, Buschauer A

PO - 031 **A molecular mechanism of biased agonism in muscarinic M2 acetylcholine receptors**
Schrage R, Kaufel D, Klöckner J, Schrobang J, Holzgrabe U, Mohr K

PO - 032 **Different signalling properties of human dopamine receptors D2short and D2long compared to full and partial agonists in HEK293 cells**
Schuster S, Gmeiner P

PO - 033 **Dissecting the individual roles of the second extracellular loop of Adenosine A2A and A2B receptors**
Seibt BF, Schiedel AC, Müller CE

PO - 034 **Pharmacological and toxicological properties of a novel selective PDE10A ligand**
Siegert F, Erdmann S, Schwan G, Scholz S, Brust P, Sträter N, Altenburger R, Briel D, Nieber K

PO - 035 **Synthesis and pharmacological characterization of novel thiazolo- and thiazinomorphinans**
Sipos A, Follia V, Berényi S, Antus S, Schmidhammer H, Spetea M

PO - 036 **Fluorophore-labelled EP3 receptor ligands as pharmacological tools**
Tomasch M, Schwed JS, Stark H

PO - 037 **Design and pharmacological characterization of novel FFAR1-(GPR40)-agonists for treatment of type 2 diabetes mellitus**
Urban C, Hamacher A, Ulven T, Gohlke H, Kassack MU

PO - 038 **Evidence for a differential role of the conserved epitope tryptophan 7.35 in different subtypes of muscarinic acetylcholine receptor**
Vogel L, Kaufel D, Janßen N, Mohr-Andrae M, Mohr K

PO - 039 **Mepyramine-JNJ7777120-hybrid compounds show high affinity to hH1R, but low affinity to hH4R**
Wagner E, Wittmann H-J, Elz S, Straßer A

PO - 040 **1,2,3-Triazole Elements in Histamine H3 Receptor Ligands**
Walter M, Kottke T, Weizel L, Schwed JS, Stark H

PO - 041 **Synthesis and Pharmacological Evaluation of Homomelatonin as Ligand for Melatonin Receptors**
Zlotos DP, Behnam MAM, Clafshenkel WP, Witt-Enderby PA

PO - 042 **A novel fluoescence sensor-based microplate assay for determination of heparanase activity**
Alban S, Lühn S, Schiemann S

PO - 043 **Pro-secretory action of individual extracts of STW 5 in human intestine**
Allam S, Krueger D, Kelber O, Demir IE, Ceyhan GO, Zeller F, Schemann M

PO - 044 **Improvement of enzyme activity of recombinant DIP5βR by a rational, bioactivity-guided approach**
Bauer P, Rudolph K, Lanig H, Müller-Uri F, Kreis W

PO - 045 **The synergistic toxicity of saponins and saponin-rich plant-extracts with type-I-RIPs / lectins**
Böttger S, Melzig MF

PO - 046 **Novel derivatives of boswellic acids as inhibitors of cyclooxygenase-1 and platelet-type 12-lipoxygenase**
Dehm F, Siemoneit U, Jauch J, Werz O

PO - 047 **Wound healing effect of birch bark extract and underlying molecular mechanisms**
Ebeling S, Naumann K, Laszczyk M, Scheffler A, Merfort I

PO - 048 **The influence of mannitol solutions on tissue factor activated whole blood coagulation**
Erber M, Schubert A, Lee G

PO - 049 **Research of plant latices of the genus Euphorbia in terms of fibrinolytic activity**
Flemmig M, Melzig MF

PO - 050 **Comparison of vancomycin and structurally related glycopeptides with respect to their simulated interaction with bacterial membranes**
Fuss C, Reder-Christ K, Babunovska S, Falkenstein-Paul H, Stegmann E, Bendas G

PO - 051 **Antileishmanial activity in fractions of chloroformic extract from Valeriana wallichii roots**
Glaser J, Gonzalez-Leal I, Schurigt U, Hazra B, Schirmeister T, Holzgrabe U

PO - 052 **A nuclear receptor as drugable target for natural products: In silico-guided discovery of FXR-agonistic triterpenes from Ganoderma lucidum**
Grienke U, Mihály-Bison J, Schuster D, Afonyushkin T, Binder M, Guan SH, Cheng CR, Wolber G, Stuppner H, Guo DA, Bochkov VN, Rollinger JM

PO - 053 **Putative mycobacterial efflux pump inhibitors isolated from Alpinia katsumadai**
Gröblacher B, Kunert O, Bucar F

PO - 054 **Isolation, structure elucidation and bioactivity of novel cyclic depsipeptides from Xenorhabdus bedingii**
Grundmann F, Bode HB

PO - 055 **The antimycobacterial activity of Euodia rutaecarpa fruits**
Hochfellner C, Kunert O, Bucar F

PO - 056 **Chemical composition, olfactory analysis and antibacterial activity of Thymus vulgaris L. chemotypes geraniol, thujanol-4 / terpinen-4-ol, thymol and linalool cultivated in Southern France**
Höferl M, Schmidt E, Wanner J, Jirovetz L, Buchbauer G, Gochev V, Girova T, Stoyanova A, Geissler M

PO - 057 **Influence of sesquiterpene lactones on gene expression in HaCaT-keratinocytes determined by time dependent microarray analyses – Implications on their potential as contact allergens**
Hoffmann M, Schmidt TJ

PO - 058 **Investigations on the surface activity of saponins**
Hofmann K, Böttger S, Melzig MF

PO - 059 **Contribution of components of STW 5 to its mode of action on intestinal inflammation**
Hoser S, Herr F, Kelber O, Weiser D, Nieber K

PO - 060 **Physicochemical properties and stability of different dosage forms of resveratrol**
Ibold Y, Herbeck M, Ryll M, Lemke S, Kumpugdee-Vollrath M

PO - 061 **Mid-infrared spectroscopy determination of the isoflavone content in nutritional supplements of red clover**
Kasper J, Melzig MF

PO - 062 **Improved method for determining the antioxidant capacity of lipophilic actives**
Keck CM, Peters D

PO - 063 **Influence of STW 5 and its herbal component extracts on neurotransmission in a model of ileal and colonic inflammation**
Kelber O, Sibaev A, Yüce B, Weiser D, Göke B, Storr M

PO - 064 **Quercetin's neuroprotective effects against oxidative stress is demonstrated by an impedance-based assay**
Kling B, Wegener J, Heilmann J, Decker M

PO - 065 **Leonurus cardiaca, L. japonicus, Leonotis leonurus: Simultaneous quantitative HPLC and HPTLC determinations of fourteen flavonoids, phenylethanoids, and phenol carboxylic acids**
Kuchta K, Ortwein J, Savtschenko A, Briel D, Volk RB, Rauwald HW

PO - 066 **Inhibitory effects of lignans from Carthamus tinctorius on indoleamine 2,3-dioxygenase**
Kuehnl S, Schroecksnadel S, Schwaiger S, Rollinger JM, Fuchs D, Stuppner H

PO - 067 **Characterisations of o/w concentrated emulsions with different poorly water-soluble drugs**
Kumpugdee-Vollrath M, Tong L, Krause J-P

PO - 068 **Colon-delivery of resveratrol coated tablets with pH-sensitive polymers**
Lemke S, Tabatabaeifar M, Kumpugdee-Vollrath M

PO - 069 **Influence of Different Drying Air Humidity on the Residual Activity of single droplets of L-Glutamic Dehydrogenase dried in an Ultrasonic Levitator**
Lorenzen E, Lee G

PO - 070 **Biochemistry of Ether Lipid Formation in Myxobacteria**
Lorenzen W, Ring MW, Bode HB

PO - 071 **Extraction of astragalosides from Astragali Radix with different solvents and quantification with HPLC-ELSD**
Monschein M, Rieder J, Ardjomand-Woelkart K, Bauer R

PO - 072 **Content of phenolic compounds in wild populations of Epilobium angustifolium L. at different altitudes**
Monschein M, Jaindl K, Buzimkic S, Dittrich P, Bucar F

PO - 073 **Molecular analysis of prenyltransferases from Hypericum species**
Müller A, Beerhues L

PO - 074 **Honey as a vulnerary – A precious gift to mankind**
Müller J, Anagnostou S, Friedrich C

PO - 075 **Noxa and Mcl-1 play a crucial role in the effectiveness of the proteasome inhibitor MG-132 in combination with different anticancer agents in pancreatic tumour cell lines**
Naumann K, Schmich K, Jaeger C, Kratz F, Merfort I

PO - 076 **PC12-cells as a model for testing natural compounds upon their antioxidant effects**
Ortmann S, Blass S, Zimmer A, Brantner A

PO - 077 **In vitro anti-inflammatory activity of flavonolignans salcolin A and B from Avena sativa**
Pferschy-Wenzig EM, Leliebre-Lara V, Atanasov AG, Binder M, Mihály-Bison J, Kunert O, Heiss EH, Bulusu M, Bochkov VN, Dirsch VM, Bauer R

PO - 078 **Truncation of N-terminal regions of Digitalis lanata progesterone 5b-reductase alters catalytic efficiency and substrate preference**
Rudolph K, Bauer P, Müller-Uri F, Kreis W

PO - 079 **Cardioactive Leonurus cardiaca L. (Ph.Eur.): Ferulic acid acts as a Ca2+-channel antagonist on neonatal rat cardiomyocytes in voltage clamp setup**
Savtschenko A, Kuchta K, Dhein S, Rauwald HW

PO - 080 **Resveratrol reduces hepatic fat accumulation by modulating Farnesoid X receptor signaling**
Scherzberg MC, Steri R, Steinhilber D, Ulrich S

PO - 081 **MHC-II loading enhancement (MLE) - a new immunological activity of natural essential oils and their constituents**
Schnieders A, Günther S, Rötzschke O, Schmidt TJ

PO - 082 **Inhibitory effects of sesquiterpene lactones and further natural products against leukemia-associated transcription factor c-Myb**
Schomburg C, Schühly W, Klempnauer KH, Schmidt TJ

PO - 083 **Activation of PPARa and PPARg by (+)-sesamin**
Schwaiger S, Atanasov AG, Heiss EH, Brandner N, Dirsch VM, Stuppner H

PO - 084 **Biotransformation of cardenolide precursors by transformed Saccharomyces cerevisiae**
Strasser J, Lehmann J, Rieck C, Kreis W

PO - 085 **Phytochemical investigations of Dischidia raffesiana Wall.**
Tran TVA, Schwaiger S, Stuppner H

PO - 086 **Route of administration determines anxiolytic activity of the flavonols kaempferol, quercetin – are they prodrugs?**
Vissiennon C, Kelber O, Butterweck V, Nieber K

PO - 087 **Effects of herbal multi-drug preparations STW 5 and STW 5-II and their main component STW 6 on inflamed rat colon preparations**
Voß U, Kelber O, Okpanyi S, Weiser D, Nieber K

PO - 088 **Sage (Salvia officinalis L.) – medicinal plant or health risk?**
Walch SG, Zimmermann BF, Tinzoh LN, Stühlinger WD, Lachenmeier DW

PO - 089 **Structure-antimycobacterial activity relationship studies of N-alkyl-4(1H)-quinolones bearing hydrophobic moieties at C-2**
Wube A, Hüfner A, Hochfellner C, Bauer R, Bucar F

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Bogum J, Tabor V, Furkert J, Wiesner B, Neuenschwander M, Kries JPv, Rosenthal W, Klussmann E

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Krauß A, Rotmann A, Martiné U, Closs EI, Dannhardt G

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Kreideweiß P, Wölk C, Erdmann N, Dobner B, Lagner A

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Pinto M, Ecker GF

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Prokes K, Klepsch F, Chiba P, Ecker GF

PO - 095 **Specific phospholipids enhance in-vitro absorption of p-glycoprotein substrates**
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Slubowski V, Chiba P, Ecker GF

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Böhme C, Stefan N, Zangemeister-Wittke U, Winkler J

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Erdmann N, Folz M, Drescher S, Dobner B, Langner A

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Müller RH, Shegokar R, Keck CM, Gohla S

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Pardeike J, Strohmeier DM, Zimmer A

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Petschacher C, Barthelmes J, Wagner J, Bernkop-Schnürch A, Zimmer A

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Fuchs JE, Wallnoefer HG, Huber RG, Grafenstein vS, Spitzer GM, Liedl KR

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Kruggel S, Brandt W, Kunick C, Lemcke T

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Lamers C, Steri R, Proschak E, Schubert-Zsilavecz M

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Vosmeer CR, Stee vMF, Perić-Hassler L, Geerke DP

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Waltenberger B, Wiechmann K, Bauer J, Markt P, Noha SM, Wolber G, Rollinger JM, Werz O, Schuster D, Stuppner H

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Mahmoud AM, El-Shabouri SR, Atia NN, El-koussi WM

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Orlovius AK, Parr MK, Guddat S, Gütschow M, Thevis M, Schänzer W

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Taibon J, Pfisterer H, Griesmacher A, Seger C

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Bertsche T, Nachbar M, Fiederling J, Schmitt SPW, Kaltschmidt J, Seidling HM, Haefeli WE

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Borghorst S, Boos J, Hempel G

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Burckhardt BB, Ramusovic S, Jaffan L, Läer S

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Fiss T, Dreier A, Böwing G, Thyrian JR, Hoffmann W

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Birkle S, Dörje F, Schlager H, Lee G, Richter WO

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Müller SM, Jaffan L,Marx S, Kleuber E , Läer S

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Munz M, Wilhelm M, Schlosser D, Birkmann J,Kinzig M, Holzgrabe U, Sörgel F

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Hofer S, Schernthanner B, Schuster D, Odermatt A, Easmon J

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Juli C, Weiwad M, Steinert M, Holzgrabe U

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Keller K, Stolfa DA, Schlimme S, Hauser A-T, Carafa V, Heinke R, Kannan S, Cellamare S, Carotti A, Altucci L, Jung M, Sippl W

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Korpis K, Weber F, Wünsch B, Bednarski PJ

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Ma BN, Gust R

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Oppermann S, Elsaesser K, Schrader F, Glinca S, Klebe G, Schlitzer M, Culmsee C

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Prechter A, Heinrich MR

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Ryczak J, Preu L, Papini M, Jaffe CL, Kunick C

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Schmidt I, Heller E, Holzgrabe U

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Schwan G, Funke U, Deuther-Conrad W, Egerland U, Birkemeyer C, Scheunemann, M, Nieber K, Sträter N, Brust P , Briel D

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Strunz AK, Wünsch B

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Summer D, Matuszczak B

PO - 215 **Design, synthesis and testing of novel small-molecule inhibitors of KasA for treatment of tuberculosis**
Topf C, Kesetovic D, Kisker C, Sotriffer CA, Holzgrabe U

PO - 216 **Synthesis, σ Receptor Affinities and Structure Affinity Relationships of Conformationally Restricted Piperazines and Flexible Analogues**
Weber F, Brune S, Wünsch B

PO - 217 **Synthesis of new selective oxidosqualene cyclase inhibitors**
Wolfgardt A, Müller Ch, Keller M, Bracher F

PO - 218 **Synthesis of inhibitors of the protein kinase DYRK1A based on the alkaloid harmine**
Wurzlbauer A, Sippl W, Walte A, Becker W, Bracher F

PO - 219 **Indinavir as an example of drug induced kidney stone formation**
Adamer V, Prillinger M, Tessadri R, Griesser UJ

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Cwik M, Schubert R

PO - 221 **In vitro and in vivo comparison of Imiquimod containing ointments: Are products from China pharmaceutically equivalent?**
Gogoll K, Stein P, Radsak M, Schild J, Langguth P

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Hintzen F, Laffleur F, Sakloetsakun D, Leithner K, Bernkop-Schnürch A

PO - 223 **Mucoadhesive properties of novel preactivated poly(acrylates)**
Iqbal J, Shahnaz G, Dünnhaupt S, Müller C, Bernkop-Schnürch A

PO - 224 **Development of a Continuous Way for Producing Extrudets**
Kipping Th, Rein H

PO - 225 **High efficiency dry coating of non-subcoated pellets for sustained drug release formulations**
Klar F, Urbanetz NA

PO - 226 **Impact of direct compression excipients on the compaction and tablet properties of high-dose pancreatin formulations**
Klukkert M, Sakmann A, Rades T, Leopold CS

PO - 227 **Influence of compaction force and magnesium stearate content on the degradation of ergocalciferol in tablets**
Krahn V, Sakmann A, Leopold CS

PO - 228 **Enzymatic degradation of thiolated chitosan**
Laffleur F, Hintzen F, Shahnaz G, Bernkop-Schnürch A

PO - 229 **The influence of carrier morphology on the fine particle fraction of dry powder inhaler formulations**
Littringer EM, Mescher A, Schroettner H, Walzel P, Urbanetz NA

PO - 230 **Quantification of α- and β-Mannitol via X-Ray Powder Diffraction**
Muehlenfeld C, De Leersnyder F, Thommes M

PO - 231 **Development of partially oxidized and 2-mercaptoethylamine functionalized chitosan as mucoadhesive and permeation enhancing polymer**
Müller C, Rahmat D, Sarti F, Leithner K, Bernkop-Schnürch A

PO - 232 **Characterization of spray-dried Mannitol particles by Mercury Intrusion Porosimetry**
Noisternig MF, Littringer EM, Urbanetz NA, Griesser UJ

PO - 233 **Effect of surfactant type on physical stability of lycopene-loaded NLC**
Okonogi S, Riangjanapatee P, Keck CM, Müller RH

PO - 234 **Enhanced oral bioavailability of vitamin B12 by thiolated poly(acrylic acid)**
Perera G, Sarti F, Müller C, Iqbal J, Laffleur F, Bernkop-Schnürch A

PO - 235 **Polymorphism of barbiturates — exemplified by pentobarbital**
Rossi D, Gelbrich T, Kahlenberg V, Griesser UJ

PO - 236 **Impact of the lubricant type on compaction and tablet properties of sorbitol formulations**
Saniocki I, Sakmann A, Leopold CS

PO - 237 **Influence of the nozzle type on particle size and its consequence for flowability of spray-dried protein powders**
Schäfer J, Lee G

PO - 238 **Interpretation of FBRM signals by ray tracing**
Scheler S

PO - 239 **In vitro establishment of a new microdialysis based system for determination of drug concentrations in the small intestine**
Schönherr D, Hanke U, Siegmund W, Becker D, Weitschies W

PO - 240 **Small-scale production in twin-screw extrusion: requirements for solid and liquid feed systems**
Thommes M, Muehlenfeld C

PO - 241 **Solubility enhancement of nabilone by complexation with methylated - ß - cyclodextrin**
Viernstein H, Wolschann KP

PO - 242 **Enhancing the performance of dry powder inhalers by tailoring interparticle forces via surface modification of carrier and active**
Zellnitz S, Schroettner H, Urbanetz NA

PO - 243 **Investigation of the σ1 receptor binding site by site directed mutagenesis**
Brune S, Wünsch B

PO - 244 **Biological activity of potentially COS releasing compounds on isolated tissue of guinea pigs**
Hintersteininger M, Brunhofer G, Gabriel M, Erker T, Studenik CR

PO - 245 **The effects of D-camphor- or D,L-camphor with hawthorn berry extract combination on taste sensations, on accuracy in identifying the administered compound, and on blood pressure**
Mauz M, Fuchs A, Braun U, Schandry R

PO - 246 **Investigation on β- and γ-carbolines as putative tools for the development of potential anti‑Alzheimer drugs**
Otto R, Enzensperger C, Appenroth D, Gaube F, Fleck C, Winckler T, Lehmann J

PO - 247 **Inhibition of fibroblast growth factor 2 bioactivity by clodronate**
Rose K, Finger IE

PO - 248 **Modulation of epithelial sodium channel current by TNF-α lectin-like domain derived peptides**
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Notes

PLENARY LECTURES

PL - 001

Drug discovery stares at binding affinity, but what does this property really mean in detail?

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In a drug development program a given lead structure is chemically optimized with respect to its potency to interfere with the function of a target protein under investigation. Usually binding with high affinity to the target protein is of utmost importance. This "high affinity" is measured in a functional assay for example by how much the catalytic turn-over of a target enzyme is blocked or how efficiently the basal activity of a receptor in signal transduction is up or down regulated. But what is this target value "affinity" really and how should it be optimized? Usually, conditions of equilibrium thermodynamics are assumed and the affinity is related to the equilibrium constant of the law-of-mass applied to the formation of a protein-ligand complex. Structurally it is argued that a ligand should exhibit optimal complementarity in shape and interaction properties with the binding site of the protein. From a thermodynamic point of view the affinity is a Gibbs free energy value and it can be decomposed into an enthalpic and entropic contribution. Do we have to consider both if we optimize a ligand with respect to its binding site? Can we optimize enthalpy and entropy independently from each other? Finally, what type of binding should be preferred, an enthalpy- or entropy-driven one and does this depend on the target protein?
The mentioned structural complementary hypothesis is used as a kind of dogma in text books to explain phenomena of protein-ligand binding. However, often residual mobility is given at the binding site, and either ligands or protein residues are distributed over several conformational states. Complementarity is also often completed by the involvement of water molecules in binding. These aspects are important for the resulting thermodynamic profile of ligand binding. Furthermore, the efficiency of binding is further determined by the kinetics determining how slow or fast a ligand binds to a protein, how long it resides at the binding site and how slow or fast it is released from the protein again. Some dramatic differences of the binding efficacy of drug molecules showing deviating interaction kinetics has been reported during clinical applications. As a result it appears increasingly important to record during lead optimization and characterization of binding either structural, thermodynamic or interaction kinetic data. At present concepts to establish clear-cut structure-thermodynamics or structure-binding kinetics relationship for ligand optimization in a drug development program are still in their infancy.

PL - 002

$GABA_A$ receptor subtypes – exciting targets for the development of clinically important drugs

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$GABA_A$ receptors are the major inhibitory transmitter receptors in the central nervous system and are the site of action of a variety of pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, neuroactive steroids, anesthetics and convulsants. They are composed of five subunits that can belong to different subunit classes and form the central chloride ion channel. The existence of 19 subunits from 8 subunit classes gives rise to a large variety of different $GABA_A$ receptor subtypes with distinct cellular distribution and pharmacology. Most of the drugs currently available cannot differentiate between different receptor subtypes. Studies with mice carrying a point mutation in a $GABA_A$ receptor subunit however indicated that individual receptor subtypes mediate specific drug actions. This conclusion was confirmed by benzodiazepine site ligands that selectively modulate certain $GABA_A$ receptor subtypes and elicit quite specific behavioural effects. Using crystal structures of remote homologues of $GABA_A$ receptors as templates, we recently generated multiple homology models of the extracellular domains of $GABA_A$ receptors. By using an unbiased docking protocol, we identified a binding hypothesis for the diazepam-bound structure of the benzodiazepine site, which is confirmed by vast experimental evidence. Moreover, two independent virtual screening approaches based on this structure identified known benzodiazepine site ligands from different structural classes and predicted potential new ligands for this site. These predictions were then confirmed by experimental evidence, thus supporting the validity of our diazepam-bound structure of the benzodiazepine binding pocket and demonstrating its suitability for drug discovery and structure-based drug design. In another study, we recently identified ligands for a novel drug binding site at $GABA_A$ receptors. Drugs interacting with this site cannot directly activate receptors but only modulate ongoing GABAergic activity, similar to benzodiazepines, but address receptors completely different from that addressed by benzodiazepines. The development of drugs interacting with this novel binding site of $GABA_A$ receptors will thus increase the spectrum of $GABA_A$ receptor subtypes that can be addressed experimentally and clinically and will thus have a substantial therapeutic potential.

PL - 003

Challenges in pharma research and future developments

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Innovation driven pharmaceutical companies face severe challenges: The costs for innovation are rising and there is an escalating demand on product value by regulators and payers. At the same time significant investments in Emerging Markets are required. The presentation will focus on the Bayer strategy to address these issues.

PL - 004

Monoclonal antibodies: The perspective of a developer of biosimilars

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Biologic drugs offer new therapeutic alternatives for many unmet needs, particularly complex diseases. Monoclonal antibodies are uniquely suited to selectively target diseases. A number of therapies from this class of molecules targeting cancer or autoimmune diseases have been successfully developed and approved in the past two decades.
Patient access to this potent class of drugs is increasingly limited by high costs and rapidly growing global demand at a time of increasing pressure on healthcare budgets, making access an unmet medical need. High-quality, clinically-proven biosimilars are making biologics more affordable and maximize access and benefits for patients. To successfully develop biosimilars, Sandoz applies a targeted development strategy encompassing three phases, namely the target definition phase, the targeted process development and the final confirmation of biosimilarity by appropriate analytical, biological, preclinical and clinical studies similar to the comparability exercise after process changes. A complete array of analytical tools is of key importance to enable and support the target directed development and the final comparability exercise. A highly comparable product profile of the biosimilar product to the reference product is achieved during process development, starting with the selection of expression system and clone. Adjustments of the bioprocess and the design of the purification process enable a fine-tuning of the quality characteristics. We will discuss specific challenges in and present case studies from biosimilar development, showing how the manufacturing process is developed and adjusted to meet the predefined criteria and achieve a high quality product qualifying as biosimilar.

PL - 005

Targeting FXR and TGR5 receptor pathways in search for novel treatments for liver and metabolic disorders

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Recent years are seeing an ever growing interest in bile acids (BAs) as versatile signaling molecules endowed with systemic endocrine functions. Indeed, beside being endogenous ligands of nuclear receptors, such as Farnesoid X receptor (FXR), they are also modulators of G-protein-coupled receptors, such as TGR5. Through the activation of these different receptors, BAs regulate diverse signaling pathways of metabolic functions, including BA synthesis, triglyceride, cholesterol, energy and glucose homeostasis. As a consequence, a number of studies have dealt with the development of natural and synthetic modulators of BA receptors as a way to provide novel drug candidates to treat common metabolic diseases. In this framework, we have previously reported the design and synthesis of INT-747 and INT-777 as potent and selective ligands of FXR and TGR5, respectively. These compounds are on track for preclinical and clinical assessments in a number of metabolic disorders such as primary biliary cirrhosis (PBC), obesity and type 2 diabetes, with INT-747 having successfully reached phase III of clinical studies in PBC. In this presentation, further developments in the chemistry and biology of BA derivatives will be reported. Moreover, the results of advanced computational studies on the hitherto hidden conformational aspects of BAs will be presented and discussed.

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PL - 006

20 years of drug nanocrystals – ´state of the art´ and perspectives

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The year 1991 can be seen as the invention of the pharmaceutical drug nanocrystals described in the patent application by G. Liversidge. Drug nanocrystals are a formulation approach for poorly soluble drugs to increase their bioavailability or to make them i.v. injectable. Transfer of material from the normal size (µm) to the nanodimension changes its physico-chemical properties. Nanocrystals possess an increased saturation solubility and dissolution velocity. In less than 10 years the first nanocrystal product appeared on the pharmaceutical market (Rapamune). Nanocrystals can be considered as one of the most successful pharmaceutical nanotechnologies, including block buster products (Tricor). By now development focussed on oral products, meanwhile the first injectable appeared on the market (Invega Sustenna). Identical to the liposomes, the nanocrystals are meanwhile also on the cosmetic market (e.g. platinum rare / la prairie). However, the dermal pharmaceutical market has been neglected.
The main established production technologies (1st generation) are bead milling (most products) and high pressure homogenization (HPH). Meanwhile more sophisticated 2nd generation production technologies were developed, in general combination technologies (e.g. bead mill plus HPH, H42, H69 etc.). They allow to produce nanocrystals faster, or to make accessible smaller sizes (e.g. < 100 nm). The future nanocrystal products will be “smarter”, main focuses will be very small sizes (e.g. 20-50 nm) and polymer coated, surface modified nanocrystals. The small nanocrystals will be able to mimick the pharmacokinetic of injected solutions (e.g. paclitaxel). Surface-modification will lead to targeted i.v. nanocrystals, e.g. for brain delivery. The use might extend from oral and injectable to topical administration routes.

Reference:
Müller, R. H., Gohla, S., Keck, C. M., State of the Art of Nanocrystals - special features, production, nanotoxicology aspects & intracellular delivery, Eur. J. Pharm. Biopharm. 78 (1), 1-9, doi:10.1016/j.ejpb.2011.01.007), 2011

PL - 007

A novel class of biopharmaceuticals based on proteoglycanomic target profiling

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Rationale: Glycosaminoglycans (GAGs) are integral/covalent part of a class of membrane and membrane-associated proteins, the so-called proteoglycans (PGs). GAGs have been identified as interaction partners of a large number of proteins (e.g. chemokines, growth factors, metalloproteinases, etc.), whereby they modulate the biological activity of these interacting proteins significantly. Especially in the context of inflammation, GAGs and PGs play a very important role in activating pro-inflammatory chemokines, such as CXCL8 and CCL2. As such, GAGs are becoming increasingly ever more accepted as therapeutic targets in acute and chronic inflammatory diseases [1].
Methods: We have engineered higher affinity for GAG binding into human CXCL8 and CCL2 while simultaneously knocking out their GPCR activity using our CellJammer® platform technology, by which we obtained decoy proteins which interfere with the CXCL8/GAG and CCL2/GAG interactions. These protein mutants have been characterised in vitro and in vivo where they have shown their potent anti-inflammatory characteristics.
Results: biophysical investigations using IFT, ITC and SPR have revealed a >20-fold improvement in GAG-binding affinity for the decoy proteins. Chemotactic cell migration experiments have shown the impaired GPCR activity of the CXCL8 and CCL2 mutant proteins. In vivo, the CXCL8 decoy protein exhibited convincing biological activity in many animal models [2] including LPS- and smoke-induced lung inflammation (murine model for COPD), whereas the CCL2-based mutants showed highly potent activity in the EAE model (for multiple sclerosis) as well as in murine myocardial infarct and restenosis models [3].
Conclusions: The data obtained in vitro and in vivo for our new class of biopharmaceuticals show their strong activity on a molecular as well as on a whole-animal level. Therefore, our CXCL8- and CCL2-based decoy proteins represent a class of new promising biologic therapeutics with a novel mechanism of action for interfering with acute and chronic inflammation.

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PL - 008

Relevance of drug transport: From micropharmacokinetics to personalized medicine

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In order to reach their therapeutic targets (eg, heart, blood vessels or peripheral cells) drugs have to pass multiple biomembranes. Successful therapy by the oral route, for example, requires uptake into the enterocyte, transfer to the vascular system and subsequent uptake into target tissue. Within the last decade, a significant number of in vitro experiments, animal studies and clinical trials pointed out that drug-transporting proteins are important modifiers along the entire process of a drug reaching its site of action.
Following oral administration drugs reach the intestinal wall as its first absorption barrier. The process of drug absorption is rather complex and modulated by active and passive uptake processes, mucosal metabolism and efflux transport.
Peripheral blood cells express various transport proteins and may hence constitute individual pharmacokinetic microcompartments [1]. Jedlitschky et al identified MRP4 in human platelets by immuno blotting and immuno fluorescence [2]. These data indicate a function of MRP4 in platelet mediator storage giving rise to various forms of MRP4 associated platelet dysfunctions [3]. Subsequent studies suggested platelet MRP4 to play a major role in aspirin resistance [4].
An SLC transporter expressed in human platelets is OATP2B1 and expression is mainly restricted to the plasma membrane [5]. Regulation of OATP2B1 is in part driven by PKC-mediated internalization [6]. Drug transporters expressed in platelets can modulate intracellular concentrations and hence actions of drugs. These proteins may contribute to interindividual variability in drug action and may constitute a confounding factor in Personalized Medicine [7].

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PL - 009

Bioactive natural products targeting inflammation

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Although the impact of natural products (NPs) in the field of drug discovery during the last century was substantial, interest of the pharmaceutical industry in NPs declined due to many challenges that are connected to NP-based lead discovery [1]. Thus, smart approaches are required to effectively identify new bioactive lead compounds from natural sources. Two approaches that help to increase effectivity are the pre-selection of extracts/compounds using ethnopharmacologial or ethnobotanical data [1,2] or to screen natural product databases against a set of pharmacophore models [1,3].
Both strategies are used in the multi-disciplinary Austrian research network "Drugs from Nature Targeting Inflammation (DNTI)". Aim of this network is the identification and the chemical/pharmacological characterisation of compounds that are able to interfere with inflammatory processes, specifically in the cardiovascular system. Two examples of this consortium will be presented:
Pharmacophore modelling and natural product database screening led to the identification of the peroxisome proliferator-activated receptor (PPAR)γ agonists magnolol, dieugenol and tetrahydrodieugenol [4].
One important contribution to cardiovascular diseases such as atherosclerosis and restenosis is the migration and proliferation of vascular smooth muscle cells (VSMC). Indirubin-3'-monoxime (I3MO) was identified as an inhibitor of platelet-derived growth factor (PDGF)-induced proliferation of vascular smooth muscle cells by blocking STAT3 signaling and neointima formation in vivo [5]. Since inhibition of STAT3 signaling is a rather unexpected strategy to interfere with VSMC growth, further cell signaling studies are performed to characterize the molecular mechanism of action of I3MO.

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KEY LECTURES

KL - 001

The molecular basis underlying the low toxicity of a potent adjuvant monophosphoryl lipid A

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Lipopolysaccharide (LPS) from Gram-negative bacteria triggers various pathophysiological responses via TLR-4. For example, LPS stimulates macrophages, resulting in the production of pro-inflammatory cytokines. Excess production of these cytokines induced by LPS causes endotoxin shock. On the other hand, the ability of LPS to induce these immune responses has been suggested to make it useful as a potent adjuvant; however, the margin between its clinical benefit and unacceptable toxicity is exceedingly narrow. The activation of caspase-1 has been shown to be essential for the induction of endotoxin shock. Recently, it has been reported that activation of caspase-1 occured upon the assembly of an intracellular complex, designated as the inflammasome. The inflammasome includes caspase-1, caspase-11, NALPs, and ASC. Lipid A, the membrane anchor portion of LPS, is responsible for the endotoxin activity of LPS and induces many inflammatory responses in macrophages. Monophosphoryl lipid A (MPL), a lipid A derivative lacking a phosphate residue, is well known for its low toxicity and high potency as adjuvant. We have recently shown that MPL is capable of activating the TLR-4/MyD88-dependent pathway, but incapable of activating caspase-1. In contrast, lipid A is capable of activating caspase-1. Thus, we suggest that the difference in the ability to activate caspase-1 between lipid A and MPL is related to the difference in their toxicity. Furthermore, we showed that lipid A induced the binding of a mouse NALP and ASC in RAW264.7 cells. On the other hand, the MPL stimulus did not induce the binding.

KL - 002

Multifunctional envelope-type nano device for non-viral gene delivery: concept and application for nanomedicine

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Development of MEND: Recently, we developed a multifunctional envelope-type nano device (MEND) as a novel non-viral gene delivery system based on a new packaging concept termed "Programmed Packaging". Programmed Packaging was proposed to develop a rational non-viral gene delivery system equipped with various functional devices, including ligands for specific receptors, pH-sensitive fusogenic peptides for endosomal escape, and a nuclear localization signal (NLS) for enhanced nuclear delivery, to overcome several barriers in the process of gene delivery to the nucleus of target cells [1].
siRNA delivery: R8-MEND can be applied for the delivery of siRNA. We succeeded to develop how to package siRNA into R8-MEND, although there is some difference in packaging method between pDNA and siRNA. Endosomal escap was enhaced by introducing GALA peptide, which is known as a pH-sensitive, membrane fusogenic peptide, in the form of cholesteryl-GALA. We have found an importance to control the number of membrane layer, since excess layers covering siRNA core in cytosol can be a rate limiting step. Di-lamellar MEND (D-MEND), which has two membrane layers can induce remarkable enhanced silencing effect in the HeLa cells stably expressing luciferase. The optimized R8/GALA-D-MEND was applied to knockdown SOCS1 gene in dendritic cells (DC) to enhance immune response. Significant antitumor effects were observed by administering DC which knocking down SOCS1 gene ex vivo [2].
In vivo application of MEND: In order to apply MEND to tumor tissue via a systemic administration, it is essential to endow a long circulation property, however, PEG coating significantly inhibited transfection activities. Most of the current cleavable PEG devices have been designed to be cleaved in response to some feature of the intracellular microenvironment (i.e. low pH of the endsome/lysosome and a reducing environment in the cytoplasm). Therefore, a cleavable PEG-lipid triggered in a tumor specific manner would be favorable for tumor gene delivery. To realize a tumor-specific cleavable PEG system, we focused on the enzyme, matrix metalloproteinase (MMP), which is involved in angiogenesis, invasion and metastasis of malignant tumors due to its ability to degrade the extracellular matrix (ECM). The MMP substrate peptide was inserted between the PEG and DOPE as a linker, and the resulting conjugated PEG-peptide-DOPE ternary conjugate is referred to as PPD. A PPD-MEND encapsulating siRNA with a diameter of 100-200 nm exhibited a higher in vivo gene silencing than a conventional (non-cleavable) PEG-lipid modified MEND (PEG-MEND) depending on the level of expression of MMP [3].

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Notes

ABSTRACTS OF SHORT LECTURES

Inflammation

SL - 001

Molecular pharmacology of 5-lipoxygenase and new inhibitors

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5-Lipoxygenase (5-LO) catalyzes the key reaction in the generation of leukotrienes from arachidonic acid. Leukotrienes are mediators of inflammatory and allergic responses and play a role in host defence reactions. 5-LO inhibitors are supposed to be of therapeutic value for the treatment of asthma, allergic rhinitis, atherosclerosis and certain types of cancer. The inhibitory potency of many nonredox-type 5-LO inhibitors depends on the cellular peroxide concentration and the mechanism of 5-LO enzyme activation. Therefore, there is a need for the development of new inhibitors with a different pharmacological profile which potently inhibit the enzyme activity regardless of the mode of enzyme activation. By ligand-based virtual screening we could identify new scaffolds for 5-LO inhibition such as thiazolinone-based compounds with low micromolar 5-LO inhibitory activity. One of the most potent structures (Z)-5-(4-methoxybenzylidene)-2-(p-tolyl)-5H-thiazol-4-one (compound C06) is a highly selective 5-LO inhibitor over other arachidonic acid binding proteins, namely the peroxisome proliferator-activated receptor (PPAR) subtypes, cytosolic phospholipase A_2, 12- and 15-LO as well as cyclooxygenase-2 (COX-2) [1]. Furthermore, our experimental results suggest an allosteric binding distinct from the C2-like domain of 5-LO. A second promising 5-LO inhibitor developed from virtual screening hits is EP6 [2]. It suppresses 5-LO activity in leukocytes with an IC_{50} = 0.16 µM and exhibits full inhibitory potency in cell free assays (IC_{50} = 0.05 µM for purified 5-LO). EP6 provides distinctive properties, including no mutagenic and cytotoxic potential, but promising antiproliferative effects, and lacks disadvantages of nonredox-type inhibitors. Thus, the drug may possess therapeutic potential for intervention with inflammatory diseases and certain types of cancer including leukemia.

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SL - 002

On the role of the pro-inflammatory enzyme 5-lipoxygenase in tumorigenesis

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Leukotrienes (LTs) are bioactive lipids generated by the 5-lipoxygenase (5-LO) pathway. An increasing body of evidence accounts for a crucial role of 5-LO products already during the earliest stages of pancreatic, prostate and colorectal carcinogenesis. In the present lecture we present several experimental data which form the basis for this hypothesis and which suggest a correlation between 5-LO expression and tumor cell viability. First, several independent studies reported an overexpression of 5-LO in primary tumor cells as well as in established cancer cell lines [1]. Second, addition of 5-LO products to cultured tumor cells also led to increased cell proliferation and activation of anti-apoptotic signaling pathways [2]. 5-LO antisense technology approaches demonstrated impaired tumor cell growth due to reduction of 5-LO expression [3]. Lastly, treatment of tumor cells with 5-LO inhibitors potently suppressed cell growth by inducing cell cycle arrest and triggering cell death via the intrinsic apoptotic pathway [4]. One the other hand, these data are contrasted with own studies demonstrating

strong cytotoxic off-target effects of 5-LO inhibitors, which question the relationship between 5-LO products and tumorigenesis [5]. Furthermore, recent clinical trials with patients suffering from advanced adenocarcinoma of the pancreas did not reveal any therapeutic benefit of treatment with LT antagonists [6]. At the end of the lecture we provide several approaches to explain the various controversial studies on the role of 5-LO in tumorigenesis.

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SL - 003

Application of p38 MAP-Kinase-Inhibitors in CNS diseases

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Proteinkinase inhibitors are validated small molecule anti cytokine drug candidates. Elevated levels of proinflammatory cytokines as IL-1ß and TNFα are associated with many inflammatory or autoimmune diseases (e.g RA, COPD, IBD, Psoriasis). Increasing amount of evidence indicate that inflammatory processes are involved in neurotoxicity of AD [1]. Based on positive results in clinical studies following perispinal application of anti-TNFa antibody etanercept [2], there is a strong evidence for TNFα modulation in the treatment of AD [3]. p38a MAP-Kinase is a prominent target as it controls TNFa release on a transcriptional and translational level. However both highly selective and CNS permeable candidates would be necessary.
Given the potential to treat CNS pathology with modulators of p38α, we screened for analogs with the potential to permeate the CNS using classical criteria (LogD, PSA, molecular weight). Appropriate compounds were clustered and the pharmacokinetics of representative compounds was studied to determine the plasma, CNS and major organ exposure. Various compounds with high apparent brain concentrations following application to WT C57Blk6 mice were further investigated for their oral bioavailability in simple experimental formulations in transgenic mice with severe cerebral amyloidosis (APPPS mice) [4]. Using doses of 10 µmol/kg p.o., partition to the brain organs, eyes, spinal cord and major organs was determined 3h after application. Compounds were contrasted with GW-856553, a GSK p38 inhibitor currently in trials for CNS indications, notably depression. The GW compound exhibited an average plasma concentration of ca. 8.5 µM over 3 hours and reached 340 nM in the brain. A series of linear p38 inhibitors, developed from triciyclic leads [5] were, in contrast, found between 0.9 and 3.9 µM in the brain vs. levels of ca. 1.4 to 1.9 µM in plasma. Levels of the same substances in the eye were ca. 17 µM. Based on these data, we undertook a study of the effect of these compounds on the inflammatory pathology secondary to amyloidosis in mice with elevated blood glucose, a condition known to exacerbate Alzheimer's pathology in man. Provision of centrally acting p38 inhibitors at a dose of 2 mg/kg/d for up to 6 months resulted in a reduction in mortality from approx. 90% in vehicle to 15-36% in treated groups. Functional MRI and PET suggested that the effects were not related to amyloid load, and there was no apparent effect on blood glucose. Histological examination for inflammatory markers is on-going.

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SL - 004

Paracetamol and cyclooxygenase inhibition – is there a cause for concern?

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Paracetamol is recommended as first-line therapy for pain associated with osteoarthrosis and is one of the most widely used over-the-counter analgesic drugs worldwide. Despite its extensive use, its mode of action is still unclear. Although it is commonly stated that paracetamol acts centrally, recent data imply a likewise inhibitory effect on the activity of peripheral prostaglandin-synthesizing cyclooxygenase (COX) enzymes. In this context paracetamol has been suggested to inhibit both isoforms in tissues with low levels of peroxide by reducing the higher oxidation state of the COX enzymes. In addition, two recent proband studies have demonstrated a preferential COX-2 inhibition by paracetamol under different clinically relevant conditions. This short lecture attempts to relate the data on paracetamol´s inhibitory action on peripheral COX enzymes to the published literature on its hitherto underestimated side effects elicited by COX inhibition. In conclusion of these considerations, a profound COX-2 inhibition by paracetamol is expected to occur in the endothelium, possibly explaining its pronounced cardiovascular risk in epidemiological studies. In addition to this epidemiological data, a recently published randomized, double-blind, placebo-controlled, crossover study demonstrated that paracetamol induces a significant increase in ambulatory blood pressure in patients with coronary artery disease. A careful analysis of paracetamol´s cardiovascular side effects in further randomized studies is therefore strongly advised. Moreover, on the basis of epidemiological data showing an increased gastrointestinal risk of paracetamol at high doses or when coadministered with classical COX inhibitors, paracetamol´s long-term gastrointestinal impact should eventually be investigated in randomized trials. Finally, paracetamol´s fast elimination and consequent short-lived COX-2 inhibition requires repetitive dosing. To avoid overdosage leading to hepatotoxicity this fact has strictly to be considered when using this analgesic.

SL - 005

Endothelial inhibitor of apoptosis proteins (IAPs) – novel promising anti-inflammatory drug targets

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Inflammatory processes are involved in the pathogenesis and progression of many severe disorders, such as arthritis, sepsis, atherosclerosis, and even cancer. Today's anti-inflammatory pharmacological strategies are often insufficient or associated with intolerable side effects. Thus, there is still a great need to identify novel anti-inflammatory drugs and, in particular, the respective drug targets.
Inhibitor of apoptosis proteins (IAPs), such as XIAP, cIAP1, and cIAP2, are important regulators of apoptosis and IAP antagonists are currently investigated in clinical trials as anti-cancer agents. Beyond apoptosis regulation, IAPs are associated with the TNF receptor signaling complex and the NF-κB signaling. Surprisingly, however, their precise role in inflammatory processes, especially in endothelial cells, has not been analyzed to date.
In mice, the experimental IAP inhibitor ABT (A-4.10099.1) blocked antigen-induced arthritis and concanavalin A-evoked liver injury by suppressing leukocyte extravasation. *In vitro*, ABT reduced leukocyte-endothelial cell interaction and diminished ICAM-1 expression, but did not increase endothelial apoptosis rate. Interestingly, ABT did not interfere with the NF-κB signaling, but decreased the activation of the kinases TAK1, p38 MAPK, and JNK. By silencing of XIAP, cIAP1, and cIAP2, we found that only cIAP1/2 are responsible for the action of ABT.

Moreover, we could show that ABT induces a proteasomal degradation of cIAP1/2, which goes along with an altered ratio of the levels of the TNF receptor-associated factors TRAF2 and 5.
Taken together, we could for the first time identify IAPs as crucial players in the inflammation-induced activation of endothelial cells. Most importantly, we could disclose IAP inhibition as novel pharmacological approach against inflammatory processes.

Acknowledgements: ABT (A-4.10099.1) was kindly provided by Abbott Research Corp. Worcester, MA, USA.

SL - 006

Downregulation of Glucocorticoid-induced leucine zipper (GILZ) promotes an inflammatory response in human macrophages

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The activation of a group of innate immune receptors, i.e. Toll-like receptors (TLR), has been shown to contribute to both infectious as well as sterile inflammatory processes [1]. Alveolar macrophages govern inflammatory diseases of the lung, such as COPD [2]. Besides the increased production of inflammatory mediators, also the downregulation of anti-inflammatory regulators can determine disease progression. Glucocorticoid-induced leucine zipper (GILZ, TSC22D3) is an anti-inflammatory, glucocorticoid-inducible protein that has been shown to interact with the inflammatory transcription factor NF-κB [3].
TLR activation rapidly reduced mRNA and protein expression of GILZ both in primary human alveolar macrophages as well as *in vivo* in mice. Studies addressing the mechanisms that determine GILZ downregulation showed that the effect is strictly dependent on the TLR adapter molecule MyD88. GILZ disappeared upon a TLR-facilitated reduction of GILZ mRNA half life, which is dependent on the 3'-untranslated region of GILZ mRNA. The mRNA binding protein tristetraprolin (TTP) was found to be involved in the modulation of GILZ expression.
Investigations on the functional significance of GILZ downregulation revealed the promotion of an inflammatory response. This is why prevention of GILZ downregulation or induction of GILZ is suggested a promising therapeutic target. We show for the first time that the plant compound curcumin induces GILZ in macrophages. The anti-inflammatory action of curcumin in mouse macrophages, exhibited by reduced NF-κB activity and iNOS inhibition, is dependent on GILZ induction, representing a novel mechanism of action.
Our findings make clear that the abrogation of GILZ decay under inflammatory conditions represents an interesting pharmacological target. Interaction with responsible molecular players might help to control inflammatory responses, which are resistant towards glucocorticoids (as found in COPD) and open therapeutic opportunities with less side effects omitting steroids.

Acknowledgements: Goethe University Frankfurt, Lisa K. Eifler, Dr. Tobias Schmid, Prof. Dr. Bernhard Brüne.
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SL - 007

Macrocyclic anti-inflammatory compounds

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The macrocyclic antibiotics based on erythromycin also exert mild anti-inflammatory action in various clinical settings. In certain instances (e.g. cystic fibrosis, atherosclerosis), this effect has been attributed to antibacterial or anti-chlamydial action, albeit without conclusive clinical evidence of this pharmacological mode of action. The use of these substances as anti-inflammatory compounds is, however, hindered by their potential to select for generalised anti-biotic resistance via multi-resistant plasmids. We set out, therefore, to discover non-anti-bacterial analogs that retained anti-inflammatory activity. Structural studies suggest that the desosamine sugar of the macrolides is the primary point of interaction with the ribosome, however, derivatisation at this position with sterically bulky groups only reduced the activity in the range of 10-fold vs. key Gram positive organisms. Modification of the macro-lactone, however, resulted, in specific cases, of reduction in the order of 20-100-fold relative to well known standards such as erythromycin and azithromycin. Certain of these analogs also retained inhibition of interferon gamma production in stimulated spleenocytes and were further characterised for their anti-inflammatory effects. Active compounds typically exhibited IC50s for nitric oxide, IL-1 and IL-2 production in LPS stimulated spleen derived cells in the range of 0.1 to 1 μM. Application of the substances in the DSS or TNBS models of inflammatory bowel disease resulted in significant disease suppression using any of body weight, stool or histological markers as metrics. Doses in the range of 12.5 to 60 μmol/kg p.o were effective in these models. Azithromycin influences the monocyte inflammatory phenotype toward the M2 form[1], and we also observed that macrolide treated cells harvested from the Lamina propria of treated animals are modified in production of inflammatory markers notably TNFalpha, eicosanoids and myeloperoxidase.

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SL - 008

The Janus face of oxidized phospholipids in inflammation: a story of unmet needs and potential leads

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Oxidized phospholipids (OxPLs) that are generated by oxidation of esterified fatty acids demonstrate a variety of biological activities potentially relevant to human pathology. In particular, OxPLs were suggested to be culprits of inflammation in atherosclerosis. Interestingly, the mechanisms of OxPL-induced inflammation seem to be different from the inflammation induced by classical agonists such as tumor necrosis factor or lipopolysaccharide thus raising a possibility for selective modulation of proinflammatory action of OxPLs without impairing general immune defense. Previous in vitro studies identified several druggable targets mediating effects of OxPLs such as protein kinase CK2, transcription factor NFAT and unfolded protein response that can be regulated by chemical chaperones.

Paradoxically, under different biological conditions OxPLs can inhibit inflammation. OxPLs inhibit recognition of bacterial products by several TLRs and protect animals from acute sepsis. This effect is mediated via different mechanisms and is observed at lower concentrations as compared to the proinflammatory action. We hypothesize that at low concentrations OxPLs are anti-inflammatory preventing undue reactions to bacterial products, while local accumulation of high concentrations, e.g. in atheroma can promote inflammation.

In summary, OxPLs presents new challenges for pharmacologists. On the one hand, there is an unmet need for selective inhibition of proinflammatory action of OxPLs in atherosclerotic disease; on the other hand OxPLs represent a potential molecular scaffold for design of new inhibitors of TLRs with modified selectivity.

G-Proteins

SL - 009

Tailor-made molecular probes for the for the first X-ray crystal structure of an agonist-GPCR complex

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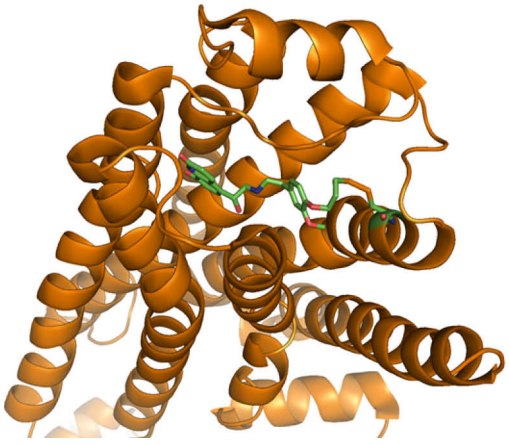
Although G-protein coupled receptors (GPCRs) have been studied extensively in the past, our understanding of their function at the molecular level is still incomplete. It has been shown that GPCRs mediate diverse signal-transduction pathways by similar mechanisms by binding a variety of ligands and G-proteins. Their interaction with agonists leads to a low-affinity conformation of the active state that is thought to facilitate G-protein binding. Only in the presence of both agonist and G-protein is the high-affinity receptor state formed, which promotes signal transduction.

The β2-adrenergic receptor (β2AR), which represents an important target for cardiac and asthma drugs, is an extensively studied model system for the large superfamily of G-protein coupled receptors (GPCRs). In 2007, the receptor was crystallized in its inactive conformation bound to the partial inverse agonist carazolol, **1** [1-3].

To overcome relatively rapid association and dissociation rates of commercial β2AR agonists, our strategy depended on a combination on a covalent β2AR agonist incorporating a β2-adrenergic agonist core and a reactive chemical group that could be targeted to a specific residue on the receptor. Employing the structure of the carazolol-bound β2AR as a template, a flexible linker was added to bridge these two components. Thus, the covalent attachment would not inhibit binding of the agonist core or conformational flexibility of the transmembrane helices. The covalent b2AR-agonist complex formed efficiently, and was able to activate a heterotrimeric G protein. A covalent agonist-bound b2AR–T4L fusion protein could be crystallized and determined for its structure at 3.5A˚ resolution [4].



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SL - 010

Cell-type specific functions of α_2-adrenoceptors

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α_2-adrenoceptors mediate diverse physiological functions of the sympathetic system and are targets for pharmacological therapy. However the therapeutic potential of these receptors is limited by diverse side effects. Knowledge about the contribution of the three α_2-adrenoceptorsubtypes to pharmacological and physiological actions was derived from gene-targeting in mice. These studies indicate that most pharmacologically relevant functions rely on α_{2A}-adrenoceptors. This subtype is known to inhibit exocytosis of norepinephrine from central and peripheral adrenergic neurons. In addition α_{2A}-adrenoceptors have been identified in several neuronal and non-neuronal cell-types. However the involved cell-types and underlying intracellular signaling events of pharmacological and physiological functions have been largely unknown until recently.
An important step was the generation of a new transgenic mouse model to discriminate α_{2A}-adrenoceptors on adrenergic and non-adrenergic cells. Results from this model indicate that only a few physiological functions previously ascribed to α_{2A}-adrenoceptors were mediated by receptors on adrenergic neurons, including spontaneous locomotor activity and feedback inhibition of norepinephrine release from sympathetic nerves. The majority of agonist effects including sedative and analgesic responses as well as the major part of hypotensive and bradycardic agonist effects were elicited by α_{2A}-adrenoceptors in non-adrenergic cells. Remarkably, in vivo data demonstrate desensitization of α_{2A}-adrenoceptors in sympathetic neurons in situations of chronically elevated sympathetic tone. These findings were supported by in vitro data showing α_{2A}-adrenoceptor internalization after agonist stimulation in primary cultured sympathetic neurons. Interestingly, the degree of internalization differed between α_2-agonists, suggesting agonist specific efficacy of β-arrestin activation. Future transgenic mouse models are required to further characterize the involved cell types and to define the role of β-arrestin signaling in α_{2A}-adrenoceptor pharmacology. This knowledge will potentially provide new avenues for future drug development.

SL - 011

How do orthosteric and allosteric ligands influence the conformational change of the M2 muscarinic acetylcholine receptor differentially?

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Recent findings show that upon binding, different orthosteric ligands can induce distinct changes in the conformation of G protein-coupled receptors (GPCRs). We investigated this phenomenon using the M_2 muscarinic acetylcholine receptor (AChR). With this receptor being a well studied model as far as allosteric modulation is concerned, we also studied the effect of allosteric ligands on the change of receptor-conformation. We generated two FRET-sensors that both had the cyan fluorescent protein fused to their C-terminus and the FlAsH-binding domain introduced within the third intracellular loop beneath transmembrane domain (TM) 5 or beneath TM6, respectively. These constructs were stably expressed in HEK293 cells. We tested commonly known orthosteric ligands and a set of allosteric ligands with regard to their effect on the change in conformation of the M_2AChR using fluorescence resonance energy transfer (FRET)-microscopy. A comparison between the FRET-signals of the TM5- and TM6-constructs evoked by the orthosteric ligands showed no ligand-specific difference in the conformational change of the M_2AChR. However, the results obtained for some of the allosteric

ligands suggest that the receptor takes on a different conformation than with the endogenous ligand acetylcholine alone.

SL - 012

Bioisosteric Replacement in Pramipexole-related Dopamine Agonists

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Pramipexole has been established as therapeutic for Parkinson´s disease for a long time due to its high agonist affinity at dopamine D_2 and D_3 receptor subtypes. The 2-aminothiazole moiety of its 4,5,6,7-tetrahydrobenzthiazol-2,6-diamine core element has been generally taken as catechol bioisoster [1].
A novel series of 2-deaminated derivatives and other heteroaromatic five-membered ring variations with different substituents on the 6-amino functionality have been prepared and tested for their affinities at D_2-like receptors [2]. With a reduction of hydrogen bond donators and an increase in lipophilicity these compounds keep high affinity at dopamine D_2 and even preferred at dopamine D_3 receptor subtypes. Some of these derivatives display up 400fold preference for D_3 over D_2 receptors on binding properties whereas in a functional assay on [^{35}S]GTPγS binding the preference was less pronounced.
On a 6-OH-DOPA-Parkinsonian model on mice selected compounds showed high agonist activity with intraperitonial and some also on oral application route. Especially 2-chloro-*N*-propyl-4,5,6,7-tetrahydrobenzthiazole-6-amine and *N*^{6}-ethyl-4,5,6,7-tetrahydrobenzoselenazole-2,6-diamine proved to be highly effective with long duration. Structure-activity relationships will be discussed.

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SL - 013

The Nucleotide Receptor P2Y11 Plays a Role in Immune System Associated Diseases

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Nucleotides are well recognized physiological ligands of ion channels (P2X receptors) and GPCRs (P2Y receptors). The use of nucleotide receptors as molecular drug targets is long established as can be seen by the introduction of the P2Y12 antagonist clopidogrel. However for other P2 receptors, a therapeutic use was delayed due to the lack of selective and potent ligands to explore nucleotide receptor physiology. In the meantime, clinical studies with selective ligands for both P2X and P2Y receptors have been launched. The P2Y11R has so far played a niche role, attributed again to a lack of selective ligands and furthermore a lack of knockout animals to study the physiological importance of this receptor. The P2Y11R is activated by ATP and dually coupled to Gq and Gs proteins. P2Y11R plays roles in the immune system and is mainly expressed in the brain, spleen, lymphocytes, dendritic cells and neutrophils.

Our group has provided the first potent and selective antagonists and the first non-nucleotide agonist for P2Y11R [1, 2]. Systematic variation has led to the nanomolar potency antagonist NF340 (apparent Ki of 19.2 nM) [2]. NF340 turned out very useful for *in vitro* and *ex vivo* tests [2,3] but cannot be used for *in vivo* experiments due to its polyanionic character. In a first attempt to develop bioavailable ligands, carboxylic acid derivatives were synthesized and tested for inhibition using a functional calcium assay in 1321N1 P2Y11 astrocytoma cells. Nanomolar potency antagonists were discovered among a series of di- and tetracarboxylic acid compounds. Structure activity relationships will be discussed. Potent ligands are currently being used to explore the role of P2Y11R in various inflammatory diseases. The increased lifespan of neutrophils in vascular inflammation can be reduced to normal by P2Y11R antagonists. This opens therapeutic options for P2Y11R antagonists in vascular inflammatory disease. Stimulation by the selective agonist NF546 of P2Y11R reduces ATP-induced cell death in T lymphocytes and NK cells containing a SNP in the 3´ untranslated region of P2Y11R. This SNP (rs2305795 A allele) shows significant association with narcolepsy and leads to reduced P2Y11R expression. P2Y11R agonists may thus be useful in the treatment of the autoimmune disease narcolepsy.

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SL - 014

Anthraquinone Derivatives as Potent and Selective Competitive Antagonists for P2 Receptors

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Membrane receptors activated by purine and/or pyrimidine nucleotides ("P2 receptors", subdivided into G protein-coupled P2Y receptors, and ligand-gated ion channel or P2X receptors) are widely distributed and constitute novel drug targets. The $P2Y_{12}$ receptor, an ADP receptor expressed on blood platelets, is the target of the antithrombotic drugs clopidogrel and prasugrel. Both drugs have to be enzymatically activated and their metabolites lead to irreversible inhibition of the platelet $P2Y_{12}$ receptor by binding to an allosteric site. Recently, the first reversible, competitive $P2Y_{12}$ antagonist, ticagrelor (Brilique®) has been approved for marketing. Reactive Blue 2 (RB-2), a sulfonated anthraquinone derivative is a moderately potent, non-selective antagonist at P2 receptors. RB-2 is a negatively charged molecule of high molecular weight (>800 g/mol), which does not exhibit drug-like properties. Development of new synthetic methods [1,2] allowed for the preparation of a library of more drug-like analogs of RB-2 with modifications in the 2- and 4-position of the anthraquinone core. Recently we also developed a simple method to obtain 1-deaminated derivatives of RB-2 analogs. The library was screened at various P2Y and P2X receptor subtypes [3-8]. Careful analysis of structure-activity relationships at each target and systematic optimization led to new biological tools for several targets: (i) we developed very potent and selective $P2Y_{12}$ receptor antagonists [2,6]; (ii) the first potent and selective P2X2 receptor antagonists were obtained [8], and (iii) potent and selective inhibitors of ecto-5'-nucleotidase were prepared [3].

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SL - 015

Development of Subtype-Selective Ligands for Melatonin Receptors

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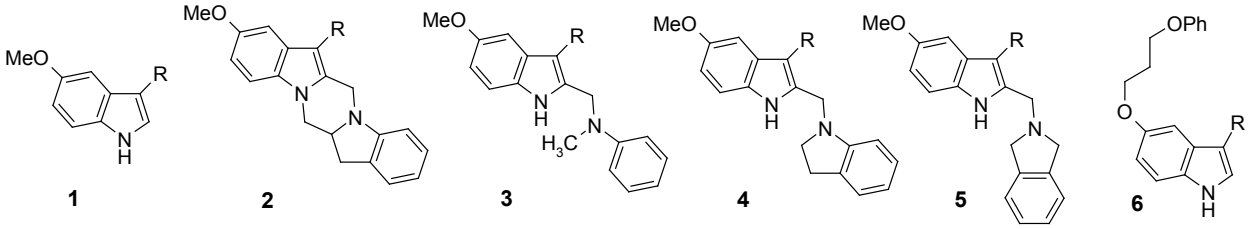
Melatonin (**1**) exerts its diverse physiological actions mostly through activation of the two G-protein-coupled MT_1 and MT_2-receptors. An accurate characterisation of melatonin receptor mediated functions requires MT_1 and MT_2-selective agents. While pronounced MT_1-selectivity is still a challenge, many MT_2-selective ligands have been reported in the last decade. Our recent contributions to the field including the moderately MT_2-selective agents **2** [1] and **3** [2], the high-affinity MT_2-selective antagonists **4** [3] and **5**, and the MT_1-selective agonist **6** [4] are discussed.



R = $-CH_2-CH_2-NHAc$

Acknowledgements: Institute of Pharmacy and Food Chemistry, Würzburg University, U. Holzgrabe

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SL - 016

Advances in the development of zwitterionic opioid receptor agonists and antagonists

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Opioid receptors, μ (MOP), κ (KOP) and δ (DOP), are members of the rhodopsin family of G-protein coupled receptors (GPCRs), which are today the major targets for drug discovery. Besides the well-characterized actions of endogenous and exogenous opioid ligands in the central nervous system (CNS), activation of opioid receptors in the periphery (i.e. sensory neurons, gut, immune cells, viscera) is associated with important physiological responses including pain inhibition (analgesia), regulation of immune functions (anti-inflammatory effects) or a modulatory role of intestinal functions such as gastrointestinal motility and secretion (i.e. anti-diarrheal/constipative effects). As a result, medicinal chemistry and opioid research focuses increasingly on exploring the therapeutic potential of peripheral opioid receptors, aiming for the development of peripherally acting opioid compounds as novel therapies for pain, inflammatory diseases and bowel dysfunction. Combining

synthetical and pharmacological methodologies, opioids with hydrophilic groups attached to the C-6 position of the morphinan skeleton were targeted in an effort to obtain peripherally acting MOP agonists and antagonists. Ionizable 6-amino acid conjugates of 14-*O*-methyloxymorphone and 14-*O*-phenylpropyloxymorphone were developed in an effort to obtain potent MOP opioid agonists with limited access to the CNS. These compounds show in vivo potent antinociception via interaction with peripheral MOP and immunosuppressive activities in vitro. On the basis of our observations in the agonist series, we introduced amino acid residues at C-6 of the opioid antagonist 14-*O*-methylnaltrexone. These zwitterionic morphinans exhibited high affinity and antagonist potency at MOP. A comprehensive SAR analysis on several analogues was pursued and primary chemical features responsible for MOP and peripheral activity have been identified. Such approach of peripheralization of opioids represents a new direction in opioid drug development for improved and innovative therapy of pain and gastrointestinal disorders.

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Natural Products

SL - 017

Sulfated polysaccharides of *Delesseria sanguinea* from the artificial reef Nienhagen

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In 2003, a large-scale artificial reef was established in the Baltic Sea close to Nienhagen (Germany) in order to increase the local fishery resources. In addition to an increase of the fishing value, the reef structures turned out to be abundantly colonized by algae, predominantly by the perennial red macroalga *Delesseria sanguinea* (D.s.). Since algae-based products are of growing interest, the sub-project 'Evaluation of the economic applicability of the Baltic Sea red alga Delesseria sanguinea' was initiated.
D.s. turned out to contain substantial amounts of sulfated polysaccharides (D.s.-SP), which consist of a homogenous fraction of branched sulfated xylogalactans (gal:xyl ~5.4) and exhibit a pharmacological profile indicating anti-inflammatory and anti-skin aging potencies [1,2]. Compared with heparin, D.s.-SP revealed stronger inhibitory effects on the enzymes elastase, hyaluronidase, heparanase, collagenase (MMP-1) as well as on alternative and classical complement activation, cell adhesion to P-selectin and cytokine release from LPS-activated monocytes, but have only moderate anticoagulant activity and no cytotoxic effects.
Crucial for an economic use is the availability of adequate amounts of D.s.-SP with reproducible high quality. For evaluation and optimization, 30 D.s. batches were harvested and extracted since 2005 resulting in almost 200 D.s.-SP batches. The analytical and pharmacological results impressively demonstrated that "the process defines the product" as known from plant extracts. But by applying the established procedure, a scaling-up experiment (100 kg fresh D.s.) led to D.s.-SP of identical quality as obtained in our lab.

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SL - 018

State of the art on the wound healing properties of birch bark

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Delayed wound healing and chronic wounds are still severe problems in medicine today and a challenging task for the treating physicians. Skin-wound healing is a biological complex process divided into three phases: inflammation, proliferation and tissue remodelling. Besides of the conventional remedies phytomedicines turned out to be an interesting alternative to beneficially influence these phases. Here extracts from birch bark (*Betula pendula*) have gained more and more interest.
Birch bark has a long lasting history as a traditional medicinal remedy. Recently, efficacy was also proven clinically. Thus, a case report was published on the successful treatment of necrotising herpes zoster when using a birch bark emulsion (1) and another one on the successful treatment of a burning (2). What are the effective compounds and how can the beneficial effects be explained?
It is generally accepted that pentacyclic triterpenes are responsible for these effects. An n-heptane extract from birch bark contains 95% triterpenes from the lupane type with about 80% of betulin, besides lupeol, betulinic acid, oleanolic acid, and erythrodiol. A broad variety of biological activities have been proven for these triterpenes among which the anti-inflammatory activity plays an important role in the wound healing effect (3). In our group we are focussing on the inflammation phase. Here a variety of proinflammatory molecules like cytokines, chemokines and prostaglandins play a significant role and their deficiency impair wound healing. Results are given, that birch bark extract and betulin elevate levels of some of these mediators.
Altogether, the talk summarizes current knowledge on the effective compounds, their biological activities and clinical studies with preparations of birch bark focussing on wound healing.

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SL - 019

V-ATPase Inhibitors from myxobacteria – promising leads and tools for cancer therapy?

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Myxobacterial compounds have great potential in modern drug discovery strategies due to their enormous chemical diversity as well as their biotechnological access. The *DFG-Research Group 1406* specifically aims at the exploitation of myxobacterial compounds as potential anti-tumor agents. Archazolid B is a potent inhibitor of V-ATPase and originates from the myxobacterium *Archangium gephyra (Sasse et al. J Antibiot, 2003)* but has also been synthesized by the Menche andTrauner group (*Menche et al. J Org Chem 2009*; *Roethle et al. J Am Chem Soc, 2007*). The work presents archazolid being a potent compound to fight highly invasive tumor cells as well as a helpful chemical tool to decipher the role of V-ATPase in tumor growth and metastasis. In detail, gene array analysis of highly invasive mammary and pancreatic tumor cells exposed to archazolid revealed among others a distinct upregulation of BNIP-3 protein, a proapoptotic BH3 protein which has not been studied in detail yet. We show that archazolid increases mitochondrial localization of BNIP-3 which is able to scavenge the antiapoptotic Bclx-protein. Consecutely activation of proapoptotic Bax, disruption of mitochondrial membrane potential, cytochrome C release and caspase-9,-8 and -3 has been monitored which leads to apoptosis of cells

after 48 h. Archazolid also kills 3D mammospheres and significantly affects long term survival of tumor cells as monitored by colony formation assays. Interestingly, non-apoptotic archazolid dosis and exposure time (16h) results in a significant inhibition of migration of invasive tumor cells as analyzed by the scratch- and the boyden chamber assay. Life cell imaging of motitily of tumor cells toward an EGF gradient revealed that archazolid inhibits the directed movement of invasive tumor cells. First experiments aiming at characterization of the underlying mechanism point to the small GTP-ase Rac playing a pivotal role in motility of cells. Archazolid significantly reduces the number of actin ruffles which is indicates an involvement of Rac. In fact, shown by Rac pull down experiments as well as by confocal microscopy of migrating cells archazolid is able to inhibit EGF-induced Rac activation. Finally genetic deletion of the 16kDa c-subunit of V-ATPase which is the binding site for archazolid also leads to inhibition of migration and points to V-ATPase as a promising target in fighting off highly invasive tumor entities.

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SL - 020

The identification and chemical determination of complex botanical mixtures

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The discovery, development and marketing of food supplements, nutraceuticals and related products, generally named botanicals for their plant origins, are currently one of the fastest growing segments of the food industry. Functional foods can be considered part or borderline to these products and may be defined as foods or food ingredients that have additional health or physiological benefits over and above the normal nutritional value they provide. The market of these products, after the first period of enthusiastic explosive emerging, is entering into the maturation period, with three important arguments to face: a) security in composition, production and selling, avoiding easy conversion and new approaches and favouring competence and quality b) definition of influence in metabolic aspects, including the scientific validation c) regulatory aspects, eg. the claims definition and relative influences. However, they urgently need adequate analytical controls, since their nowadays success could continue and be insured only by quality assessments, first by the exact determination of the composition. Therefore, the availability of analytical tools specially tailored to face the complexity of natural products mixtures is crucial. Controls should be based on simple, viable, comprehensible and low cost methods.

The complexity of composition of these multiingredient products requires a multidevice approach, in order to obtain a great quantity of data in accordance with the different types of natural products present. Total information derives from the complementary use of many analytical tools. like HPTLC for general composition, NMR for structural determination, HPLC for quantitative determination.

Recent applications of this analytical approach are reported, including the analysis of aerial parts of the horse tail, *Equisetum arvense*, presenting a fingerprint very different from those of other *Equisetum* spp. also utilized, like *E. maximum*, characterized by the presence of several colored spots absent in the other species [1]; the comparison between feverfew, *Tanacetum parthenium* L. (= *Chrysanthemum parthenium* (L.) Pers.), and the Mexican feverfew, *Chrysanthellum indicum* Turner (= *Chrysanthellum americanum* L.), both present in marketed products; difference between the European bearberry's leaves, *Arctostaphylos uva-ursi folia*, and those of the North-American *Arctostaphylos pungens* lower in cost and easier to be found [2]; presence of synthetic substances in botanicals added to improve activity.

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SL - 021

Hyperforin: A dual inhibitor of leukotriene and prostaglandin E_2 biosynthesis as anti-inflammatory lead from nature

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Leukotrienes (LTs) and prostaglandins (PGs), produced by the 5-lipoxygenase (5-LO) and cyclooxygenase (COX) pathway, are involved in inflammatory processes. These lipid mediators, in particular PGE_2, contribute to fever, pain, inflammation, allergy, cardiovascular diseases, and various types of cancer. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit COX enzymes and are, besides glucocorticoids, the major class of drugs to treat inflammatory diseases but their use is associated with severe side effects. The acylphloroglucinol hyperforin (Hyp) from St. John's wort was reported to possess anti-inflammatory and anti-carcinogenic properties. Regarding the biochemical basis of the anti-inflammatory effects, we previously showed that Hyp inhibits the activity of 5-LO and COX-1 [1], and others observed inhibition of PGE_2 release from activated macrophages. Here, we present detailed mechanistic insights into the molecular interaction of Hyp with 5-LO and with the PGE_2 biosynthetic pathway. Our data imply that hyperforin is a novel type of 5-LO inhibitor (IC_{50} = 0.09 µM) that affects LT biosynthesis by functional interference with the putative phospholipid-binding site of the C2-like domain of 5-LO, thus interrupting the stimulatory interaction of 5-LO with coactosin-like protein and with phospholipid membranes [2]. Moreover, we reveal Hyp as a potent inhibitor of the microsomal PGE_2 synthase (mPGES)-1 (IC_{50} = 1 µM) [3]. Hyp suppressed PGE_2 formation in whole blood assays (0.03 to 1 µM), whereas the concomitant generation of other COX-derived prostanoids was not significantly inhibited. Finally, we demonstrate that Hyp exhibits remarkable efficacy in animal models of inflammation *in vivo,* connected to reduced levels of LTs and PGE_2. Taken together, Hyp represents an interesting lead from a natural source acting as a potent dual inhibitor of 5-LO and mPGES-1, with excellent *in vivo* efficacy and low potential for gastrointestinal and cardiovascular side effects.

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SL - 022

Investigation of *Notopterygium incisum* for anti-inflammatory constituents

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The roots and rhizomes of *Notopterygium incisium* Ting ex H. T. Chang (*Qiang Huo*) have been used in traditional Chinese medicine for the treatment of rheumatism, headache and common cold. In a screening for anti-inflammatory effects, the dichloromethane extract showed various activities. By activity guided isolation, we

could identify six polyacetylenes, 8-acetoxyfalcarinol, falcarindiol, 9-epoxy-falcarindiol, crithmumdiol, 9-heptadecene-4,6-diyn-1-ol and 2*Z*,9*Z*)-2,9-heptadecadiene-4,6-diyn-1-ol, which showed inhibitory activity on NO production in RAW 264.7 macrophages.[1] The polyacetylenes exhibited also potency as PPARγ agonists selectively activating PPARγ without affecting the other two PPAR subtypes.[2] In addition, we have isolated eight new polyacetylene derivatives, which are fused with a sesquiterpenoid moiety,[3] and three polyacetylene ferulic acid conjugates[4]: a new falcarindiol 8-O-ferulic acid ester and two polyacetylene ferulic acid fusion molecules with a new skeleton which may be formed by Diels-Alder reaction followed by reduction. Some of the new compounds also possess PPARγ agonistic effects.

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SL - 023

Activation of Nrf2 alleviates hyperglycemia and endothelial dysfunction - a new potential target for natural products in the battle against metabolic dysfunction?

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Hyperglycemia as seen in insulin-resistant and diabetic patients can be associated with severe micro-and macrovascular complications, at least partly due to the pro-oxidant and pro-inflammatory signals initiated by aberrantly high glucose levels. Nuclear factor 2- related factor 2 (Nrf2) is a transcription factor commonly known for triggering the cellular defense towards oxidant and xenobiotic insults. Activation of Nrf2 is a feature of many phytochemicals and is regarded as a mean how natural products exert a beneficial influence during inflammation or cancer initiation and promotion [1,2].

In this study we investigated the role of activated Nrf2 in the protection against hyperglycemia and hyperglycemia-induced endothelial dysfunction, respectively. For this, we tested a derivative of oleanolic acid, CDDO-IM, known as selective Nrf2 activator [3], in cell-based models for glucose uptake and endothelial release of nitric oxide (NO). Nrf2 dependency of our observations was confirmed by siRNA-mediated knockdown and subsequent abrogation of the CDDO-IM-elicited effects.

Treatment of glucose-stressed primary human endothelial cells with CDDO-IM restored impaired bioavailability of NO (as assessed by DAF-2 fluorescence) in a Nrf2-dependent fashion, and could be pinned down to increased expression of hemeoxygenase-1, reduced levels of reactive oxygen species (ROS) and transiently decreased levels of endothelial NO synthase (eNOS) ensuring eNOS coupling during situations of limited amounts of reduced tetrahydrobiopterin, a pivotal eNOS cofactor. Administration of CDDO-IM to normal and insulin-resistant murine adipocytes led to increased basal and insulin-dependent glucose uptake, again in an Nrf2-dependent manner. Insulin sensitization may be explained by the observed reduction of ROS levels and pro-inflammatory signals upon Nrf2 activation. The molecular effectors mediating the basal hypoglycemic effect still remain elusive.

Overall, we provide evidence that activation of Nrf2 may help to counteract both hyperglycemia and subsequent endothelial dysfunction. Thus, we (i) encourage investigations of known Nrf2 activators from nature in systems related to hyperglycemia and vascular dysfunction, and (ii) propose that Nrf2 activation may underlie some of the so far unresolved beneficial effects of multiple phytochemicals in the context of metabolic disorders.

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SL - 024

Leukotriene biosynthesis is sex-biased in human monocytes

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Leukotrienes (LTs) are lipid mediators of inflammation and allergy, produced by the enzyme 5-lipoxygenase (5-LO) [1]. We have previously observed sex-disparities in LT biosynthesis in vitro, in stimulated human whole blood and neutrophils [2]. Moreover, using an experimental model of inflammation in rats, we show that sex-differences in LT synthesis occur also in vivo. Here, we investigated the influence of sex on the activity of 5-LO in human peripheral monocytes, which represent another major source of LTs besides neutrophils. After stimulation, cells from females produced higher amounts of 5-LO products than cells from males. Male plasma and 5α-dihydrotestosterone (5α-DHT) down-regulated 5-LO product formation in female monocytes, while estradiol had no significant effects and progesterone caused only a slight inhibition. In female monocytes, 5α-DHT caused activation of extracellular signal-regulated kinases (ERK) and reduction of phospholipase D (PLD) activity and of diacylglyceride (DAG) levels, which are required for full 5-LO activity. Accordingly, PLD activity and DAG formation were lower in male than in female cells, connected to increased ERK phosphorylation states. Our data indicate that ERK activation by androgens in monocytes reduces 5-LO product formation due to lack of activating DAGs and can add to the understanding of sex differences in pathologies related to LTs. Together, such sex differences in LT biosynthesis call for consideration of a gender-tailored therapy in LT-related diseases.

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Transporter

SL - 025

The Medicinal Chemistry of Drug Transport – Ligand-based Design meets Structure-based Design

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Drug transporters play a major role in the uptake, disposition, efficacy and safety of drugs and drug candidates. Up to now more than 400 membrane transporters have been identified in the human genome. P-glycoprotein (P-gp), the paradigm transporter in the field, has been discovered more than 30 years ago as being responsible for multiple drug resistance in tumor cells. Although hundreds of compounds have been identified as inhibitors of P-gp and more than 20 entered clinical studies, none of them has been approved so far. This questions the drugability of P-gp and related ABC-transporters.
With an increasing understanding of the function and physiological role of ABC-transporters, their major contribution to bioavailability, brain permeation and clearance of drug candidates became evident. Thus, designing in and designing out substrate properties of potential drug candidates comprises a hot topic and – due

to the polyspecificity of the transporters – also a major challenge. Within the talk an overview on our ligand- and structure-based approaches for modulating drug/transporter interaction will be presented. Special emphasis will be given on knowledge-driven concepts such as experimental data guided ligand docking and on the application of machine learning for classification of substrates and non-substrates.

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SL - 026

The role of OATP1B transporters in pharmacology and physiology of liver

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Organic anion transporting polypeptides (OATPs), particularly the members of the OATP1B subfamily, are assumed to mediate hepatocellular uptake of several endogenous and exogenous compounds. Particularly OATP1B1 has been shown to influence pharmacokinetics of drugs in clinical use. Using a mouse model lacking Slco1b2, the murine ortholog of the OATP1B subfamily, we confirmed the pharmacological phenotype resulting in reduced hepatic clearance capacity for substrate drugs. In order to obtain insights into the physiological phenotype we studied the influence of the Oatp1b2 deletion on bile acid (BA) metabolism. Taken together we report reduced expression of Cyp7a1, the key enzyme of BA synthesis from cholesterol. In accordance knockout mice exhibited elevated cholesterol levels after high dietary fat challenge. Furthermore, Slco1b2$^{-/-}$ mice exhibited delayed clearance after oral glucose challenge resulting from reduced hepatic glucose uptake. We showed that this phenotype is due to the loss of liver-specific Oatp1b2-mediated hepatocellular thyroid hormone entry, which then leads to reduced transcriptional activation of target genes of hepatic thyroid hormone receptor (TR), and results in reduced hepatic expression of genes involved in cholesterol and glucose homeostasis. Importantly, human relevance of this finding was assessed using a cohort of archived human livers in which OATP1B1 expression was noted to be highly associated with TR target genes, especially for glucose facilitating transporter 2 (GLUT2). In addition, GLUT2 expression was significantly decreased in livers harbouring a common genetic polymorphism in SLCO1B1. Taken together OATP1B transporters mediate hepatic thyroid hormone entry and are therefore key determinants of cholesterol and glucose homeostasis.

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SL - 027

Overcoming ABCG2-mediated drug resistance with new ABCG2 modulators derived from tariquidar

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ABCG2 (BCRP), an ATP-driven drug efflux transporter, is among other transporters like ABCB1 and ABCC2 responsible for multidrug resistance (MDR) in cancer [1]. Furthermore, as a critical constituent of the blood-brain barrier, ABCG2 limits the efficacy of different potent cytostatic drugs (e.g. topotecan) in the treatment of malignant tumors of the CNS, as these compounds are substrates of the efflux pump. Consequently, by analogy with an approach described for ABCB1 [2], co-administration of ABCG2 modulators is an attractive strategy to treat MDR tumors and to improve chemotherapy in the CNS by increasing drug levels in the brain [3,4].
Recently, we synthesized a series of new tariquidar analogs, which were surprisingly identified as potent and selective ABCG2 modulators [5]. Aiming at increased solubility compounds bearing triethylenglycol groups were synthesized [6]. With regard to future in vivo studies, three of the most promising new tariquidar-like ABCG2 modulators, UR-COP77, UR-COP78 and UR-COP134, were selected and the effect on the proliferation of U-118 MG glioblastoma cells and ABCG2-overexpressing MCF-7 breast cancer cells (MCF-7/Topo cells) was investigated using a kinetic chemosensitivity assay.

ABCG2-overexpressing MCF-7/Topo cells show resistance against the cytostatic drug topotecan, which is a known BCRP substrate, up to concentrations as high as 500 nM [5]. The cells were incubated with topotecan alone and in combination with 3 different concentrations of the tariquidar analogs. To consider that a possible cytotoxic effect results from ABCG2 inhibition, the effect of the tariquidar analogs alone on the cell proliferation was tested as well. None of the compounds showed a cytotoxic effect on MCF-7/Topo cells concentrations as high as 1 µM and 5 µM, respectively.
The combination of UR-COP78 at a concentration of 100 nM with topotecan at a nontoxic concentration of 100 nM resulted in a strong cytostatic effect. The cytostatic effect was affected only to a very moderate extent by an increased inhibitor concentration of 500 nM. UR-COP77 showed similar results. Effective inhibition of ABCG2 led to a total reversal of the ABCG2-mediated topotecan resistance. The combination with UR-COP134 was less effective, only resulting in a weak cytostatic effect at a concentration of 500 nM.
Compounds UR-COP77 and UR-COP78 are highly potent ABCG2 modulators which may have the potential to increase drug levels in the CNS. The increased solubility and ABCG2 inhibitory efficacy of these new substances compared to the previously described parent compounds [5] is very promising with respect to in vivo studies using a refined tumor model in nude mice.

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SL - 028

Analysis of binding modes of propafenones in P-glycoprotein by means of molecular dynamics simulations

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The membrane-bound ABC-transporter P-glycoprotein (P-gp, ABCB1, MDR1) is responsible for the export of xenotoxic compounds out of the cell. However, due to its chemically diverse substrate profile, overexpression of P-gp is highly related to the acquisition of multidrug resistance (MDR), one major reason for the failure of antibiotic and antitumor chemotherapy. A promising concept for overcoming these obstacles would be the identification of potential P-gp inhibitors. Propafenone analogs are known for their P-gp modulating activity and several *in silico* studies could identify a clear structure-activity relationship between this class of molecules and P-gp [1]. Nevertheless, the absence of structural information of the protein hindered the identification of the concrete binding mode.
Using homology modeling the putative structure of human P-gp in two different conformations, representing the high- and low-affinity state of the protein, has been determined. Additionally, the application of a knowledge-based docking protocol retrieved a small number of possible binding modes, which suggested important interactions on the molecular level [2]. As docking only represents a snapshot of the protein-ligand interaction, the binding modes were further investigated by means of molecular dynamics computer simulations. By including a phospholipid bilayer in the simulations, not only the flexibility of the complex but also the effect of the membrane could be considered in the calculations. Post MD-analysis tools were applied to compare the stability of the system and of the different binding modes.
In agreement with the 'classical' interpretation of the catalytic cycle of P-gp, the MD simulations suggest that propafenone binding poses in the high-affinity state may be more stable than in the low-affinity conformation. The high-affinity state poses were characterized by hydrogen bonds that were either formed directly with the protein or mediated by a water molecule. The functional groups of the ligand contributing to hydrogen bonding have been previously shown to be important for P-gp activity. On the protein side the amino acid residues Tyr307 and Tyr310 were found to be mainly involved in hydrogen bond interactions. The docking poses in the low-affinity structures primarily formed interactions with water molecules in the central pore, which may result in weakened protein-ligand interactions. These findings form the basis for future simulation studies that could help in elucidating the differences in activity between propafenone analogs.

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Nanomedicine

SL - 029

Polysaccharide bioconjugates for tumor targeting

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Polymer bioconjugation is a promising tool for anticancer chemotherapy as it can enhance the biopharmaceutical performance of molecules with poor physicochemical properties and a low therapeutic index. According to Ehrlich's "magic bullet" concept, Ringsdorf's macromolecular model and the enhanced permeability and retention (EPR) effect described by Maeda and Matsumura, a variety of polymer therapeutics have been designed to ameliorate the pharmacokinetic behaviour of anticancer drugs, favour the tumour targeting either by passive or active mechanisms, promote the cellular uptake into tumours and tumour endothelial cells and finally provide for intra- or extra-cellular drug release depending on the polymer-linker-drug design.

So far, several polymers have been investigated to produce macromolecular bioconjugates for passive and active anticancer drug delivery, which include polyacrylates, polyoxyalkanes, polyaminoacids and polysaccharides. These macromolecules are characterised by high biocompatibility and solubility, easy clearance from circulation and multiple conjugation points, which make them suitable for construction of multifunctional supramolecular derivatives.
Among natural and semi-synthetic polysaccharides, hyaluronic acid, chitosan and dextran have been investigated for drug bioconjugation as the therapeutic properties of anticancer drugs can be combined with the biological properties of these polymers. Tumour targeting and selective anti-cancer drug delivery can in fact be accomplished by receptor-mediated uptake or passive fluid-phase endocytosis of polysaccharide prodrugs while a few polysaccharides play a role in cancer biology, and may be exploited for tumour cell targeting or to inhibit tumor angiogenesis and metastasis.
Pullulan is a polysaccharide with excellent biological and physicochemical properties for drug delivery, namely biodegradability, low immunogenicity and polyfunctionality, fair solubility in aqueous and few organic solvents. Accordingly, pullulan has been explored as a platform to construct colloidal drug delivery formulations such as pH-sensitive nanoparticles, assemblies and bioconjugates for anticancer drug delivery
Novel pullulan derivatives have been designed for active and passive tumour targeting. Oxireductive chemical protocols that allow for the introduction of diverse functional moieties have been properly exploited to functionalise the polysaccharide backbone with different chemical functions. This chemical approach yielded suparmolecular bioconjugates bearing targeting agents, modifiers and drugs that have been conjugated to the polymer via stimuli-sensitive or stable bonds.
Supramolecular bioconjugates obtained by pullulan derivatisation with doxorubicin via hydrazone bond have been found to possess suitable physicochemical properties for passive tumor targeting via EPR, prolonged body exposure and pH dependent drug release. The introduction of a targeting agent, namely folic acid, was found to bestow active targeting properties.
In vitro studies showed that pullulan derivatised with doxorubicin an doxorubicin/PEG is internalizated into FR(+) human cervical cancer HeLa cells within 5 and 30 min, respectively, as followed by flow cytometry. Our targeted conjugate inhibited the proliferation of FR(+) KB human nasopharyngeal carcinoma cells and of human umbilical vein endothelial cells (HUVEC) exhibiting an IC_{50} of 4 □M and 0,07 □M respectively. The targeted conjugate showed higher affinity and anticancer effect for the FR overexpressing cell lines, compared to the non-targeted one. The increased efficacy of our system towards HUVEC opens up potential application for further investigations on its anti-angiogenetic properties.

SL - 030

Nanocarriers for dermal drug delivery: multiple W/O/W nanoemulsions for topical application of aciclovir

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Nanoemulsions are of great interest in nanomedicine, drug delivery and cosmetics due to their excellent long-term stability. Moreover, by employing low-energy emulsification techniques such as the phase inversion temperature (PIT) method the incorporated drug is not exposed to the stress of high energy input (e.g. high-pressure homogenisation). The PIT method is a highly convenient and rapid technique to produce nano-sized O/W emulsion systems [1]. Recently, the PIT technique was employed to create novel W/O/W nanoemulsion systems for the incorporation of hydrophilic materials [2]. Unlike conventional O/W nanoemulsions, these systems contain an additional surfactant to solubilise hydrophilic substances in reverse micelles distributed homogenously within the oil phase. However, the potential of these vehicles as drug delivery systems used for dermal application has not been investigated so far. Especially antiviral and antimycotic drugs are of great practical

importance for topical administration as side-effects can be reduced significantly compared to other routes. Thus, the present study focuses on the development of novel W/O/W nanoemulsion systems for the dermal delivery of aciclovir. Initially, experiments were executed in order to optimise the physicochemical formulation properties while the amount of surfactant was held as low as possible. The minimum required content of hydrophilic surfactant was found to be 5% (w/w). Subsequently, the influence of two different lipophilic surfactants on the drug incorporation in reverse micelles was investigated. Moreover, the mean particle sizes of the developed W/O/W nanoemulsions were compared to the results obtained with conventional O/W nanoemulsions in which the hydrophilic drugs were likewise incorporated. Stability studies revealed that in O/W formulations, aciclovir tends to precipitate during storage, particularly under refrigerated conditions. This can be avoided by the newly developed W/O/W systems. Furthermore, in vitro skin permeation studies were performed using Franz-type diffusion cells and additional tape stripping experiments. The W/O/W nanoemulsions showed satisfying skin permeation, being clearly superior to the permeation of aciclovir from an aqueous solution. In addition, cryoTEM images of the W/O/W systems [Fig.1] were recorded. They confirmed the particle size analysis by dynamic light scattering as well as the homogeneity of the formulations. The formulations' homogeneity, which is also expressed in the polydispersity index, was found to be significantly lower and more stable for the W/O/W nanoemulsions.

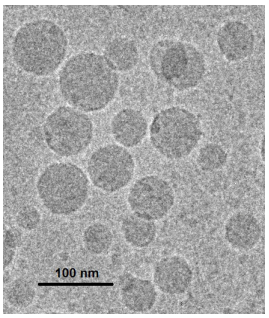

Fig. 1

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SL - 031

Liposomal drug carriers for neutron capture therapy - Influence of lipid composition on liposome uptake in Glioblastoma cells

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Neutron capture therapy relies on local enrichment of neutron capture agents such as gadolinium in tumor cells. Our aim is the development of liposomal nanocarriers for improved transport and targeting of NCT agent gadolinium in order to optimize the NCT agent uptake in tumor cells and thus, enhance effectiveness of radiation therapy.
Since lipid composition has proven to be a crucial factor for delivery and bioavailability of liposome contents at the target site, the influence of different lipid formulations on uptake of liposomes in two glioblastoma cells lines (F98,

LN229) was investigated. Analyzed lipid compositions included cationic DOTAP, anionic cardiolipin and PEGylated lipids (PEG-PE, folate-PEG-PE) which were added to a DOPC – cholesterol mixture of defined proportions. Introduction of fluorophore NBD-PE allowed quantitative determination of liposome transported.
Inclusion of charged lipids into liposome bilayer leads to considerably higher liposome uptake than employment of neutral lipids. Cationic lipid DOTAP yields high absorption for both cell lines used (>0,4µg lipid/µg protein), whereas anionic cardiolipin shows only in F98 cells considerable quantities of lipid. PEG – coated formulations showed no significant accumulation of lipid (<0,05µg lipid/µg protein). However, application of folate-PEG-PE liposomes achieved a high amount of lipid taken up in both cell lines (>0,4µg lipid/µg protein). These findings are notable, since only LN229 cells were considered to overexpress folate receptor. Hence, further studies need to clarify if there exist other uptake mechanisms than receptor - mediated uptake for folate-PEG-coated nanocarriers. Investigations will also include studies on time and concentration- dependence of endocytosis.

Acknowledgements: We thank Lipoid GmbH for generously providing lipids (DOTAP, DOPE and PEG-DOPE), and the BMBF, 05KS7UMA.
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SL - 032

Aspergillosis in falcons – Itraconazole-loaded Nanostructured Lipid Carriers (NLC) for pulmonary application

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Aspergillosis is the most common and most lethal disease in captive falcons leading to a loss of 40% in falconry. Standard treatment, systemic application of Itraconazole, is limited due to hepatotoxicity. NLC are a nanoparticulated delivery system with a particle matrix composed of a blend of a solid lipid and a liquid lipid being solid at body temperature. In this study isotonic, sterile and physically long term stable Itraconazole-loaded NLC were developed, nebulized with a new Nanonebulizer based on jet-stream nebulisation and their stability investigated. Furthermore, the deposition of the aerosol in the respiratory tract of a falcon suffering from Aspergillosis was investigated by gamma-scintigraphy.
NLC composed of Itraconazole, Precirol ATO 5, oleic acid, Tween 20, Glycerol and MilliQ water were prepared by hot high pressure homogenization and subjected to stem sterilization. The sterile formulation had an average particle size of 106±5 nm. No particles larger than 250 nm were detected in the formulation by laser diffraction. The particle size as well as the entrapment efficiency (i.e. 99.98%) stayed unchanged over an observation period of 3 month. Nebulizing the formulation with a new Nanonebulizer resulted in an aerosol with an average particle size below 250 nm. Furthermore, it could be shown, that the particle size of Itraconazole-loaded NLC was not affected by nebulization, i.e. an average particle size of 102±6 nm was measured after nebulisation and collection of the aerosol mist. By gamma-scintigraphy in one patient it could be shown, that radiolabeled Itraconazole-loaded NLC deposit primarily in both lung lobes and the caudal air sacs. These regions are the areas first ventilated during respiration and the major locations of Aspergillosis infection in falcons.
This study showed that isotonic and sterile Itraconazole-loaded NLC with an high entrapment efficiency could be developed. The nanoparticulate carrier system is physical stable during storage and nebulisation. Furthermore, nebulizing Itraconazole-loaded NLC with the new Nanonebulizer resulted in a very fine aerosol mist which was able to penetrate deeply into the respiratory tract of a falcon suffering from Aspergillosis, being a precondition for the treatment of the mycotic infection.

OMICs and Biopharmaceutics

SL - 033

Protein interactions with their surrounding anions and cations precisely detected by Affinity Capillary Electrophoresis

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Investigations of protein properties and functionality require precise analytical tools and a thorough understanding of protein physicochemistry. The influence of ions in the surrounding solution on proteins is thus investigated by Affinity Capillary Electrophoresis (ACE). The interactions with proteins are detected by the mobility changes which occur in the presence of various ligands [1]. ACE is able to explore changes in the conformation as well as charge changes of proteins. Unspecific and specific interactions on proteins can be measured without special reagents or kits. In order to compensate for changes on the migration time, which are not due to ligand binding, the mobility ratio of an EOF-marker and the protein is used [2]. Six repeats were done with a very good precision due to the use of a special rinsing procedure [3]. The RSDs [%] of the migration times and the mobility ratios were typically below 2%, very often below 0.2%. The influence of various charged species e.g. metal ions, such as Cu^{2+}, Mn^{2+} and Mg^{2+}, pharmaceutical cations as ephedrine hydrochloride and pirenzepine dihydrochloride or anions such as glutamic acid and succinic acid was tested on the migration behavior of BSA, ß-lactoglobulin and ovalbumin. Organic cations and metal ions showed clearly different interactions with the proteins. In most cases significant interactions were found, which differed markedly from protein to protein for different ligands. Thus selective binding mechanisms are likely. This offers very valuable insights into biological functions of protein-ligand interactions in aqueous media. Specific interactions of anions instead of the global behaviour according to Hoffmeister can explain some effects on formulations and stability of proteins which are not well understood yet.

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SL - 034

Time resolved analyses of signaling/scaffold complexes

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Protein signaling complexes, which are organized by scaffold and adaptor proteins, in specific subcellular locations, coordinate cellular functions such as proliferation, differentiation, apoptosis and migration. We have shown previously that the Mitogen-activated protein kinase (MAPK) scaffold protein 1 (MP1) is localized to late endosomes by the adaptor protein p14 [1-5]. The other major scaffold is KSR1, regulating the formation of a MAPK signaling unit at the plasma membrane. We used tandem affinity purification (SH-TAP) coupled to mass spectrometry to identify the major scaffold signaling complexes along the EGFR/MAPK pathway, in order to obtain an interaction atlas of protein partners associating with these complexes.

All known and several novel p14/MP1 interacting proteins, including proteins of unknown function, have been identified, suggesting new functions of these proteins. We have also performed time resolved analysis of signaling/scaffold complexes upon Epidermal Growth Factor (EGF) stimulation. About ten proteins consisted p14/MP1 core interactome, several proteins were interacting with scaffold proteins in signal dependent manner. Relatively small interactome of p14/MP1 proteins suggested more specific function of the scaffold complex on late endosomes, which is opposite to rather large signaling/scaffold complexes organized by KSR1.

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SL - 035

Novel Extraction, Enrichment, Separation and Spectroscopic Approaches for Phytomics

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It is commonly believed that herbal products are safe as they are naturally occurring; however this is a misconception [1]. Additionally, active pharmacological ingredients (API´s) are often present in small quantities within a highly complex matrix. Therefore, it is our aim to establish novel analytical tools enabling quality control of a broad range of substance classes beginning with raw plant material, continuing during extraction, production and finally of metabolites in human body fluids for pharmacokinetic studies.

The analytical strategy depicted in Figure 1 is based on new extraction, enrichment, separation and spectroscopic techniques for improved, characterization and profiling. Special emphasis is put on the development of novel extraction protocols enabling an increase in anti-oxidative, -microbial and –inflammatory properties. For the selective enrichment of highly potent molecular candidates stationary phases for solid-phase extraction (SPE) are designed, which are implemented in a house-made automated robotic system for high-throughput analysis. Micro-liquid chromatographic (µ-LC) systems employing a tailored poly(p-methylstyrene-co-1,2-bis(p-vinylphenyl)ethane) monolithic stationary phase enables analysis and structural elucidation by an improved coupling to mass spectrometry via nano electrospray ionization (nano-ESI/MS/MS) followed by a database search [2]. Novel carriers for screening of low-molecular weight analytes employing matrix-free laser desorption ionization time of flight mass spectrometry (mf-LDI-MS), and of high-molecular weight ingredients using material enhanced laser desorption ionization mass spectrometry (MELDI-MS) are of high interest. Near-infrared spectroscopy (NIRS) offers an attractive, fast, non-invasive fingerprint technique for on-, in- and off-line qualitative and quantitative analytical approaches. Mid-infrared (MIR) and NIR imaging and mapping techniques combined with attenuated total reflection (ATR) allows to get knowledge upon the active ingredient distribution within e.g. a special plant compartment at a resolution as low as 1.5 µm [3].

Merging these techniques in a synergistic manner, this analytical strategy allows not only to enhance the efficiency of analytical investigations but also to get completely new insights. The application and validation of this strategy is discussed in detail.

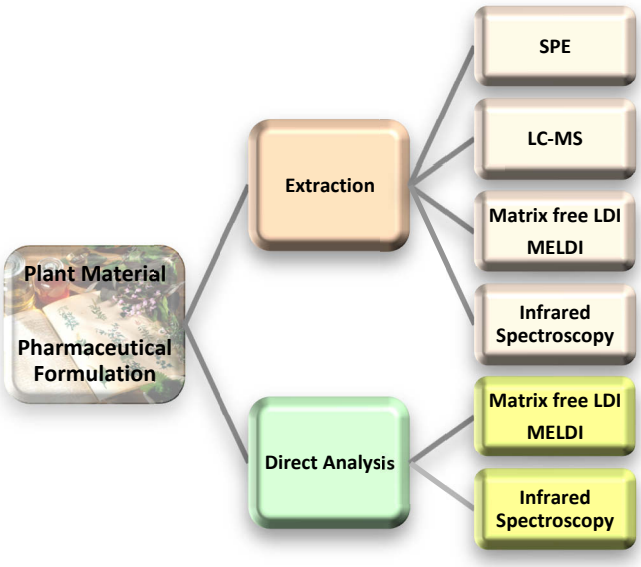

Figure 1. Analytical strategy

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SL - 036

Lysophosphatidic acid acyltransferase 3 – a novel candidate enzyme for infertility therapy with dual function in the testis

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Membrane lipids comprise hundreds of different species whose dysregulation correlates with diverse pathologies including infertility. Male fertility essentially depends on long-chain polyunsaturated fatty acids (PUFAs) which accumulate in mammalian testis during puberty (1). Highest levels of PUFAs among testicular cell-types (Leydig, Sertoli and germ cells) were found for germ cells which lack the enzymatic requirements for an efficient PUFA biosynthesis and thus are dependent on their uptake (2). To investigate whether lysophospholipid acyltransferases determine the PUFA composition of testicular phospholipids during murine pubertal development, we compared their mRNA expression, *in vitro* activity and specificity with the lipidomic profile of major phospholipids. The accumulation of PUFAs in phosphatidylcholine, -ethanolamine and -serine correlated with an induced lysophosphatidic acid acyltransferase (LPAAT)3 mRNA expression, increased microsomal LPAAT3 activity and shift of LPAAT specificity to PUFA-coenzyme (Co)A. LPAAT3 catalyses the conversion of lysophosphatidic acid to phosphatidic acid – the precursor of glycerophospholipids - preferring docosahexaenoyl-, arachidonoyl- and less pronounced linoleoyl-CoA as acyl-donor substrates (3, 4). LPAAT3 is expressed in all testicular cell-types and was strongly induced during germ cell maturation as shown by immunofluorescence microscopy. Accordingly, differentiation of mouse GC-2spd(ts) spermatocytes into spermatides upregulated LPAAT3 mRNA, increased the amount of polyunsaturated phospholipids and shifted the specificity for the incorporation of deuterium-labelled docosahexaenoic acid towards phosphatidylcholine and -ethanolamine. Stable knockdown of LPAAT3 in GC-2spd(ts) and mouse TM4 Sertoli cells significantly decreased microsomal LPAAT3 activity (4),

reduced levels of polyunsaturated phosphatidylcholine, -ethanolamine, -serine and/or -glycerol species in both cell lines and of free PUFAs in Sertoli cells (4) and impaired cell proliferation/survival of GC-2spd(ts) spermatocytes during geneticin selection. In conclusion, Sertoli cells efficiently synthesize PUFAs, incorporate them into phosphatidic acid by LPAAT3 and generate pools of polyunsaturated membrane phospholipids whose size correlates with the availability of free PUFAs. Maturating germ cells, being attached to nursing Sertoli cells, take up PUFAs and incorporate them into phospholipids again via LPAAT3 affecting sperm cell production. Thus, our data propose LPAAT3 as promising target for pharmacological intervention related to male infertility.

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Computer Aided Drug Design

SL - 037

Hot Topics in Computer-Assisted Drug Discovery

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Today, a plethora of concepts, tools, and software solutions are available for computer-assisted molecular design and prediction of molecular properties. Increasing pressure for enhancing efficiency in the drug discovery process has largely contributed to this development, creating new interfaces between scientists involved in the chemistry and in the biology of early phase drug discovery.
In this short introduction to the session, some hot topics will be highlighted, including elaboration of widely applicable and more accurate scoring functions for evaluating virtual screening experiments, high throughput prediction and characterization of metabolic profiles of early candidates, as well as linking in silico and in vitro techniques for superior performance in difficult target areas.

SL - 038

Combining virtual and biological screening for efficient lead structure identification of epigenetic targets

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A variety of structure- and ligand-based techniques for automated virtual library screening have been developed over the last ten years. Pharmacophore and docking tools are often applied in a hierarchical manner in order to reduce the number of potential hits from millions of compounds to a small subset of manageable size. The growing number of successful virtual screening studies have shown the potential of this approach. If the three-dimensional structure of the biological target is not available, protein models represent an alternative for ligand docking. It is widely accepted that docking to homology models is more challenging than docking to X-ray structures of proteins.
Chromatin modifications have emerged as new fundamental regulatory mechanisms for the control of gene transcription and are associated with many cellular processes. It is increasingly recognized that epigenetic

modifications constitute important regulatory mechanisms for the pathogenesis of malignant transformations. The present talk will highlight the results obtained for several epigenetic targets where we used a combination of homology modelling and virtual screening methods in order to find novel lead structures. For the analyzed protein targets HAT[1], PRMT1[2,3] and Sirt2[4,5], we were able to identify novel bioactive molecules. Good results were obtained applying a multi step approach including pharmacophore search, structure-based virtual screening and free energy calculations with subsequent biological evaluation of selected compounds.

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SL - 039

High-throuput, fully automated, specific MetID. A revolution for drug discovery

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Introduction
Studies to identify the site of metabolism (SoM) are conducted in Drug Discovery to aid design of metabolically stable molecules. High resolution chromatography / mass spectrometry in combination with accurate mass and mass defect filtering have largely facilitated metabolite identification at the level of finding drug related material (DRM). However, spectral interpretation leading to definitive structural assignments of metabolites is the limiting (and expensive) factor for increased throughput.
Here we report on the use of a new tool, called MassMetaSite, for the automated identification of DRM and reporting of metabolite structures in batch mode. MassMetaSite uniquely combines prediction of SoM and processing of mass spectra for the automated assignment of metabolite structures directly from the acquired MSE or data dependent MS2 raw data files.
MassMetSite was validated against proprietary data on more than 300 compounds from the Human Cythocrome Consortium source on different CYPs and HLM, and on public metabolism data for 20 commercially available compounds in human liver microsomes (HLM). MassMetaSite performed very well and the correct assigned structure, compared to manual inspection of the data (made by experts), picking in the first rank in approx. 85% of the cases with 95% of success with the first three shots. The MSMS spectra included information that was missed with MSE only for 12 compounds. It is important to mention that all of these compounds had relatively good ionization properties which probably contributed to the good results since one of the main reasons when the use of MSE-acquired data fails is low sensitivity.
Further analyses showed that matrix components did not impact DRM identification of major metabolites but had some effect on identification of DRM for minor components. Plasma and urine showed little impact of matrix components on identification of DRM whereas bile components resulted in generally lower (15-20%) metabolites detected as DRM due to ion suppression. However for the most abundant metabolites representing >10% of DRM this effect was minimal. Matrix component from other tissue did not significantly increase the number of false positive results that was generally below 2% of all metabolites detected.
In conclusion, MassMetaSite can strongly alleviate the huge work of a biotransformation scientist and decrease the workload by assigning the structure for a totality of major metabolites and for a majority of the minor metabolites.
The full metabolic profile of a compound can be made in few minutes instead of several days of work. Full kinetic analysis is also possible as showed with a practical example in this paper.

SL - 040

Scoring functions for affinity prediction: New routes out of the forest?

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Scoring protein-ligand interactions is a fundamental prerequisite for any computational method dedicated to the identification, investigation, or optimization of ligands binding to a protein. Scoring functions are, thus, an essential component of structure-based drug design and virtual screening [1]. In general, good results can be obtained in docking applications with respect to the prediction of near-native binding modes. Also the ability to distinguish active ligands from unlikely binders appears at least to be sufficient to make virtual screening a practically useful endeavour. However, the correlation with the experimental binding free energy and the possibility to quantitate the effects of small structural changes on the ligand affinity are in many cases still disappointing. Large test data sets have unveiled these shortcomings. On the other hand, these data sets also provide opportunities for improvement. Most promising results have recently been obtained with machine-learning methods, in particular with the Random Forests approach [2]. Here, $SFCscore^{RF}$ is presented as an example of the possible improvements, illustrating that the currently best functions are getting into a practically useful predictivity range, despite the remaining limitations.

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Miscellaneous Topics

SL - 041

Cyclin dependent kinase 5 (Cdk5) and its function in Hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide. It represents the third leading cause of cancer-related death due to a high chemoresistance and poor prognosis. Unfortunately, at this time, no curative systemic therapy exists. Therefore, the identification and characterization of novel drugable targets is of paramount clinical importance.
Cyclin-dependent kinase 5 (Cdk5) is essential for CNS development and function. During recent years, functions of Cdk5 in non-neuronal systems have been elucidated, few studies indicate a role of Cdk5 in tumor cell survival, and we discovered that Cdk5 regulates angiogenesis. Since HCC is a highly vascularized tumor and anti-angiogenic treatment (Sorafenib) has shown some therapeutic benefit, we hypothesize that Cdk5 might be an interesting target for HCC therapy. Here, we show first results elucidating the function of Cdk5 in HCC.
Histology of tissue micro arrays and westernblot analysis indicate an increased expression of Cdk5 in human HCC tissue in comparison to the respective healthy liver tissue of the same patient. In HCC cells (Huh7 and HepG2), pharmacological inhibition of Cdk5 with the small molecule roscovitine (R-Roscovitine, Seliciclib) reduces proliferation and clonogenic survival, induces G2/M cell cycle arrest and apoptosis, and reduces motility and adhesion. Specific downregulation of Cdk5 with siRNA also reduces clonogenic survival as well as migration of HUH7 cells. In a subcutaneous HCC xenograft model, treatment with the Cdk5 inhibitor roscovitine reduced tumor growth and angiogenesis, indicated by decreased tumor weight and volume, and reduced vessel density.

Moreover, cotreatment of HCC cells with roscovitine and tumor necrosis factor related apoptosis inducing ligand (TRAIL) has a more than additive effect on the induction of apoptosis. This coincides with a strongly reduced phosphorylation and thereby activity of the anti-apoptotic transcription factor Stat3 at both phosphorylation sites, Ser727 that is directly phosphorylated by Cdk5, and Tyr705. In line with this, the expression of the anti-apoptotic protein Mcl-1 is reduced by inhibition of Cdk5.
Our results give first insights into the role of Cdk5 in HCC, suggesting Cdk5 as an interesting pharmacologically druggable target for HCC therapy.

SL - 042

PK/PD model of methotrexate-associated elevation of homocysteine levels

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Objectives: Elevated homocysteine concentrations have been associated with methotrexate (MTX)-induced neurotoxicity. The aim of this investigation was to develop a PK/PD model based on plasma MTX and homocysteine (HCY) concentrations measured in patients treated for acute lymphoblastic leukemia (ALL) and to simulate the effect of folinate (leucovorin) on homocysteine concentrations.
Patients and Methods: Based on methotrexate and homocysteine plasma concentrations of 391 children with ALL from the TOTAL XV study [1], a PK-PD model was built with NONMEM 7.1.2 using the FOCE interaction method. Several compartmental and indirect response models [2] were investigated to describe the PK/PD relationship. Body size, age, sex and renal function were investigated as potential covariates in the model.
Results: The pharmacokinetics of MTX was best described by a two-compartmental model. HCY concentrations were included by an indirect response model where the HCY elimination rate was inhibited by MTX described by an Emax model [2]. The HCY baseline level was found to be age-dependent. Simulations revealed that folinate rescue therapy only affected peak concentrations of HCY to a negligible extent.
Conclusions: Our semi-mechanistic PK-PD model describes the increase of methotrexate-induced homocysteine concentrations with satisfactory precision and indicates no major impact of folinate rescue on homocysteine parameters.

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SL - 043

High susceptibility for prescribing errors of pro re nata (on demand) medication jeopardize children´s medication safety in a hospital setting

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Background The prescribing process of drugs in children is more complex than in adults because of individual dose calculations based on weight or body-surface area. Paediatric drug prescriptions are therefore more susceptible to errors than adult drug prescriptions. Besides regular prescriptions, prescriptions of pro re nata (PRN) medications (on demand) are an essential part of paediatric pharmacotherapy. They are, however, potentially prescribed with less attention than regular prescriptions and often administered outside the routine working hours by personal being unfamiliar with the patients´ disease and thus jeopardize children´s medication

safety. It is therefore mandatory to investigate whether prescriptions of PRN medications are more often involved in prescribing errors than regular prescriptions.
Methods During a 12-month period (Jan 2009-Dez 2009) a clinical pharmacist reviewed medication records on a paediatric ward for cardiology and pneumology at the university hospital of Düsseldorf. Prescriptions and prescribing errors were collected. A prescribing error was defined as an incomplete prescription where at least one part of the prescription was missing either drug, single dose, dosing frequency or route of administration. Prescriptions were classified into regular prescriptions which are part of the obligatory therapy and pro re nata (PRN) prescriptions which are only given on demand. Drugs were coded by means of the Anatomical Chemical Classification and patients according a modified WHO age classification. Primary endpoint was the number and percentage of prescriptions implied at least one prescribing error. Secondary endpoints were different types of errors due to missing part of the prescription, drugs involved in prescribing errors and differences in the age classes.
Results The clinical pharmacist analysed 413 patient charts including 2323 prescriptions representing 214 different drugs. Out of all, 83.4% of the reviewed prescriptions were classified into regular prescriptions, 16.6% to PRN prescriptions. In total, 237 incomplete prescriptions involving 41 drugs were identified giving a total prescribing error rate of 10.2%. Prescribing errors in prescriptions of PRN medication were found in 82.7% compared to 17.3% in regular prescriptions ($p<0.05$) resulting in a fifty times higher prescribing error rate. Analgesics and antipyretics were the two drug classes most involved in prescribing errors and they belong to the drug classes which were mostly prescribed as PRN medication. Prescribing errors of Paracetamol, Ibuprofen and Metamizole prescribed on a PRN basis account for 62.9% of all prescribing errors. Missing information on dosing frequency occurred in 85.7% of the prescribing errors and was in 80.6% associated with prescriptions of PRN medication. No significant differences were found in prescribing errors according to age classes of paediatric patients.
Conclusion PRN medications are highly susceptible to prescribing errors in paediatric patients with a fifty times higher prescribing error rate than in regular medications. To avoid these errors, technical support by an electronic prescribing system and further special training for physicians is mandatory to ensure proper attention to all types of prescriptions and consecutive medication safety for children.

SL - 044

Risk of drug-related problems at the interface of prescription to administration in a paediatric intensive care unit

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Introduction: Children suffer three times more often from drug-related problems than adults and particularly high rates of medication errors are reported during drug prescription and administration. Information on the translation of prescriptions into administration in children is sparse. We therefore aimed to investigate the prevalence of deviations between prescription and administration. **Methods:** We performed an observational study in a 10-bed paediatric intensive care unit (PICU). Trained clinical pharmacists (n=4) observed nurses while administering medications in the morning hours in four independent monitoring phases. Administered drugs were compared to physicians' prescriptions. Detected deviations were classified into four categories: (i) risk of potential overdosage, (ii) overdose administered, (iii) underdose administered, (iv) and other relevant deviation from prescription. **Results:** We analysed 1002 drug administrations in total during 75 days conducted by 39 nurses in 78 patients (median 0.80 years Q25/Q75 0.09/7.20) including premature infants and multimorbid patients in a PICU. (i) 240/1002 (24%) administrations bore a risk for potential overdosage, (ii) 63/1002 (6%) administrations were indeed overdosed, (iii) 58/1002 (6%) were underdosed, and (iv) 29/1002 (3%) administrations deviated in another relevant aspect. The drugs most frequently involved in dosing deviations were analgesics (78% of administrations involving analgesics), CNS drugs (73%), antiinfective drugs (52%), and caffeine used as a respiratory stimulant

(50%). Severe adverse events at the patient level resulting from dosing deviations were not identified. **Conclusion:** The prevalence of drug- and dosing-related problems is unexpectedly frequent at the interface of prescription to administration and overdoses were indeed administered in 6% of all drug administrations. The high risk of dosing problems stresses the need for effective prevention strategies.

SL - 045

Target validation using fragment-based protein ligands

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Fragment-based methods for drug discovery are increasingly popular because they provide drug leads with greater ligand efficiency than conventional high-throughput screening. Most methods for the detection of low-affinity fragments lack a practical solution of the linkage problem [1]. Therefore, we have developed a set of assays which combine the identification of active fragment combinations with reversible, i.e. dynamic ligation of fragments [2,3]. In these ligation assays, active fragment condensations could be detected either by competition with a fluorogenic substrate [2] or in binding assays using fluorescence polarization for detection [3]. Currently, the concept is extended towards fragment-based substrate enhancement. In this approach, pre-substrates are turned into active enzyme substrates by a reversible fragment ligation step. Activation of the presubstrate can be detected easily by observing the enzymatic turnover. The system has been applied to several different ligation reactions and allowed us to investigate the scope of reaction partners suitable for ligation.
The contribution will discuss what we can learn about fragment recognition using fragment ligation methods on various protein targets including proteases, protein tyrosine phosphatases, and protein-protein interactions and how such approaches can contribute to inhibitor development and target validation [4].

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SL - 046

Exploring Pathogen - Host Interactions through Synthetic Mimicry of Viral Protein Binding Sites

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Assembled and scaffolded synthetic peptides that mimic the binding sites of viral and bacterial surface proteins for their host cell receptors, are promising immunogen candidates to elicit neutralizing antibodies, as well as tools to explore the molecular mechanisms of host-pathogen interactions [1]. We have previously presented a synthetic mimetic of the discontinuous binding site of HIV gp120 for its cellular receptor CD4 (CD4bs) [2]. This peptide was found to compete with gp120 for binding to CD4, as well as broadly neutralizing antibodies mAbs b12 [3] and VRC01 [4], whose epitopes overlap the CD4bs, establishing the CD4bs as a prime HIV neutralizing epitope. Furthermore, antibodies raised against the CD4bs mimetic peptide recognize gp120 with a specificity related to that of mAb b12.

In recent and ongoing studies, we have generated and used synthetic protein binding site mimetics to dissect differences in binding specificities of CD4bs antibodies, and to explore the species selectivity of the gp120-CD4 interaction, as well as the structural basis of the functional mimicry of the CD4bs by synthetic peptides. The results of these studies will guide the design of improved synthetic CD4bs mimetics as immunogen candidates for the elicitation of broadly neutralizing anti-HIV antibodies.

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SL - 047

Mechanisms of mitochondrial fragmentation as a target for neuroprotective strategies

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Impaired regulation of mitochondrial dynamics shifting the balance towards fission is associated with neuronal death in model systems of neurodegenerative diseases and acute brain injury. Here, we investigated the roles of dynamin-related protein 1 (Drp1), one of the key regulators of mitochondrial fission, and the pro-apoptotic Bcl2-family protein Bid in mitochondrial pathways of cell death induced by glutamate toxicity in immortalized HT-22 hippocampal neurons. Exposure of HT-22 cells to glutamate or increased expression of tBid induced pronounced mitochondrial fission, relocation of the fragmented organelles around the nuclei of damaged cells, and finally cell death as determined by MTT assays or impedance-based real time measurements. Mitochondrial fission and loss of mitochondrial membrane potential were preceded by translocation of Drp1 and Bid to mitochondria. Drp1 siRNA and small molecule inhibitors of Drp1 prevented mitochondrial fission, loss of mitochondrial membrane potential, and cell death induced by glutamate or tBid over-expression. Interestingly, Drp1 inhibitors and the Bid inhibitor BI6c9 prevented Drp1 translocation and subsequent mitochondrial demise in glutamate-damaged HT-22 cells. These results showed that mitochondrial translocation of both, Drp1 and activated Bid contributed to detrimental mitochondrial fission as a key feature preceding the loss of mitochondrial membrane potential and cell death. Further, our data indicate an interaction of Drp1 and Bid with the Bcl-2 protein Bax at the site of mitochondria during glutamate-induced cell death. In conclusion, inhibition of Drp1-Bid interactions at the site of mitochondria is proposed as an efficient strategy for neuroprotection.

SL - 048

Fishing for alosteric binding sites at GPCRs with “loop-hooks”? –A way to selectivity?

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Receptor-subtype selectivity is an important issue in medicinal chemistry and can be very difficult if the actual binding pockets are highly conserved. Some receptors tolerate larger residues of the ligands without a complete loss in affinity. In such cases functionalized linkers could stick out of the binding pocket to interact with loop regions, which are usually very variable even within one receptor family.

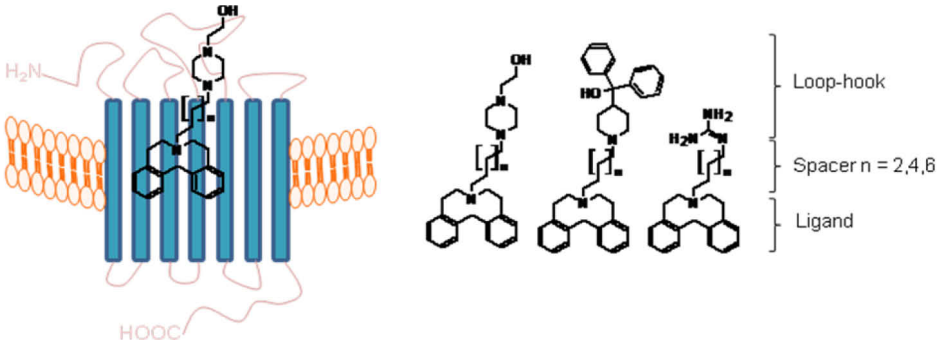

Figure 1: Orthosteric ligand hooking the loop-region

The dopamine D1 receptor family (D1 and D5) is highly conserved within the binding pockets, but show high differences in the first and second extracellular loop. So we decided to build heterobivalent ligands consisting of a known dibenzazecine as ligand for the orthosteric binding pocket, spacers with different length and a “loop-hook” comprising of H donors, H acceptors or hydrophobic groups (see Figure 1).
The high affinity of these new ligands proofs that the loop-hooks address additional binding sites outside the orthosteric binding pocket and the concept should be further investigated.

ABSTRACTS OF PRE-SYMPOSIA

FG Arzneimittelkontrolle Pharmazeutische Analytik

PS - 001

HPLC-DAD / GC-MS examinations for drug safety of emergency stocks – detection, identification and toxicological qualification of a hitherto new degradation product of pyridostigmine relevant to regulatory legislation

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During the analysis of stockpiled pyridostigmine bromide tablets, an unknown substance was detected after long-time storage. Subsequent investigations showed that the latter is a degradation product of the active pharmaceutical ingredient (API). The percentage share of this degradation product exceeds the threshold values permitted by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) for new medicinal drugs.
In order to learn about the origin of the unknown substance, stress experiments were conducted with pyridostigmine bromide tablets as well as with the active pharmaceutical ingredient and the matrix. Investigations on the identity and content of the degradation product were made by means of HPLC with diode array detection (HPLC-DAD) and gas chromatography – mass spectrometry (GC-MS).
The structure of the unknown substance was elucidated and confirmed by means of two independent procedures. The substance identified is the degradation product tetramethylurea (TMU, C5H12N2O, CAS No. 632-22-4), which has not been described so far. TMU may develop from pyrodistigmine bromide as a result of hydrolysis, decarboxylation and subsequent formation of acid amide. During the investigations conducted, the TMU content per tablet was found to be between 230 µg and 391 µg, which corresponds to a daily dose of TMU between 690 µg and 1173 µg, with 30 mg of pyridostigmine bromide being administered three times. Current data do not suggest an acute toxic effect in humans for this dose range. Animal studies have shown that in higher doses, TMU exhibits teratogenic properties after oral application. Since in the field of reproduction toxicology, stochastic effects must also be considered, the risk of a teratogenic effect in the dose range relevant for human beings can not be excluded.
A risk-oriented investigation of emergency stocks gives insights on degradation paths and degradation products after long time storage, about which comparable findings are frequently lacking. It reveals data for a scientific based risk estimation. This serves to improve drug safety in the event of a crisis.

PS - 002

Analysis of Proteins with Capillary Gel Electrophoresis – Fabulous Precision

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Capillary Gel Electrophoresis (CGE), also known as Capillary Electrophoresis-Sodium Dodecyl Sulfate (CE-SDS), is established in the pharmaceutical industry replacing SDS-PAGE gel electrophoresis, for example for the purity control of monoclonal antibodies. The method of an application note from Agilent [1] was used and optimized with a protein standard containing myoglobin, carbonic anhydrase, ovalbumin and bovine serum albumin (1-1.5 mg/ml). Separation voltage was -16.5 kV (-30 µA) for 30 min to analyse proteins with a maximal molecular weight (MW) of 70 kDa. It is beneficial to inject hydrodynamically instead of electrokinetically which was employed in earlier works [1-3]. Both techniques were compared in long series runs (n=48). Furthermore, the use of an internal standard was investigated. The RSD% of the migration time was reduced from 0.9% to 0.25%. The RSD% of peak area was improved as well by the use of an internal standard. However, the evaluation by the 100% method avoiding the computation of the injection error into the results demonstrated RSD% for the peak

areas typically between 1 and 2%. It was crucial to obtain signal-to-noise ratios greater than 100 for optimum precision. Further, the use of CISS integration software proved to be favourable. The optimized method was used to investigate about its purity a model antibody and the MW of different reduced fragments.

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PS - 003

NIR and Raman spectroscopy in quality control, PAT and the analytics of counterfeit medicines

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The use of NIR[1] and Raman spectroscopy is increasing over the last years. The spectrometric methods, combined with chemometric approaches (i.e. PCA[2]), are used to solve a variety of analytical tasks throughout the pharmaceutical world.
The basics of the different techniques as well as a generic statistical approach (PCA) are explained. Several examples of applications for NIR and Raman are presented and advantages, disadvantages and limitations are discussed. The advantages include easy, rapid, non-invasive measurement even directly through primary packaging, the disadvantages lie in the demanding and time consuming development of the methods/chemometric models in order to be able to interpret and use the readily obtained measurement results. Qualitative and quantitative determinations are possible. To be able to quantify an API[3], tablets with different concentration have to be available to generate data for the statistical modelling. Limitations consist in detection limitation for low concentrations of contaminations and ingredient as well as interferences of fluorescence signals with the Raman spectra.
As a conclusion it can be stated that renaissance of NIR and Raman spectroscopy due to miniaturisation and the availability of sufficient computing power to use chemometric methods have opened interesting and promising possibilities to reduce measurement time, sample preparation and therefore costs for large panel of analytical tasks. Identification of incoming components using NIR or Raman is increasingly popular in quality control. The methods also provide new possibilities for novel approaches like PAT[3] to better understand pharmaceutical processes and help tackling recent issues as the detection and identification of counterfeit medicines.

References:
[1] Near Infrared
[2] Principal component analyses, statistical approach to reduce the number of parameter
[3] Active product ingredients
[4] Process analytical technology, approach to design, analyze, and control pharmaceutical manufacturing processes in a more efficient manner

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PS - 004

Protein Quantitation using various Modes of High Performance Liquid Chromatography

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Pharmaceuticals based on proteins (biologicals), such as monoclonal antibodies (mAb), attain more and more relevance since they were established as potent drugs in anticancer therapy or for treatment of autoimmune based diseases. Due to their high efficiency it is essential to have accurate and precise methods for protein quantitation and the detection of protein aggregates, which may lead to adverse effects after application [1]. In order to improve selectivity as well as precision compared to classic protein quantification methods such as the Bradford assay or SDS-PAGE, which do not achieve the necessary specifications of quality control (QC) purposes, High Performance Size Exclusion (HP-SEC) and Anion Exchange Chromatography (SAX) were already introduced as high selective and precise methods (e.g. SEC: < 1.9% and SAX: < 5% RSD % for peak areas inter-day) with low quantitation limits for the model proteins Ovalbumin, Myoglobin and Bovine Serum Albumin [2]. The weak Cation Exchange- (WCX) and the RP-HPLC, both already successfully applied in protein analysis, will be presented as two further possible alternatives for the QC of proteins. Both methods also provide data of high precision (RSD % peak area day-to-day < 2% for RP and < 3.5% for WCX) and low quantitation limits (< 10 µg/ml). Consecutively, the four separation modes will be compared in terms of precision, selectivity, analysis time, effort of sample and mobile phase preparation as well as separating capacity. Moreover the analysis of a monoclonal antibody is included in this study.

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PS - 005

Nachweis nicht deklarierter Arzneistoffe in Verdachtsproben mittels HPLC/DAD und Absicherung über ESI-MS

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Die Arzneimitteluntersuchungsstelle im LIGA.NRW untersucht im behördlichen Auftrag Arzneimittelproben des legalen und illegalen Arzneimittelhandels. Dem OMCL (Official Medicines Control Laboratory) werden von Zoll-, Polizei- und Arzneimittelüberwachungsbehörden illegal gehandelte Arzneimittel zur Untersuchung als Verdachtsproben vorgelegt. Darunter befinden sich häufig Doping-, Potenz und Schlankheitsmittel, Haarwuchspräparate und als rein pflanzlich deklarierte Nahrungsergänzungsmittel verschiedenster Indikationen). Zur rechtlichen Einstufung der Präparate und zur ggf. erforderlichen gerichtlichen Verfolgung von Straftaten sind der analytische Nachweis der verwendeten Wirkstoffe und meistens auch eine Gehaltsbestimmung erforderlich. Erste Hinweise auf mögliche pharmakologisch aktive Substanzen ergeben sich nach Einsatz einer HPLC/DAD Sceeningmethode. Die eindeutige Identifizierung, Absicherung oder Strukturaufklärung erfolgt dann mittels HLPC-MS.

PS - 006

Current status of hyphenated low and high resolution mass spectrometry in clinical and forensic toxicology as well as in drug metabolism

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The reliability of analytical methods helps to ensure the quality of analytical data needed for correct interpretation of analytical findings in clinical and forensic toxicology thus helping to avoid wrong treatment of the patient or

analytical data being contested in court. The analytical strategy mostly includes screening, confirmation and identification followed by quantification of relevant compounds and pharmacokinetics-based interpretation of the results. Mass spectrometry coupled to gas chromatography (GC-MS) or liquid chromatography (LC-MS) is the gold standard in this field because of its universality, reliability, high sensitivity and specificity. Besides GC-MS more and more LC-MS techniques are used for target and comprehensive screening, library-assisted identification, and validated quantification of drugs, poisons and their metabolites in blood, urine or alternative matrices. Concepts and procedures using LC-MS techniques in the areas of toxicology and drug monitoring with special focus on multi-analyte procedures as well as in studying the metabolism of drugs of abuse will be presented and discussed [1-6]. The presentation will close with a short discussion of the potential of high resolution mass analyzers and future perspectives of MS in these fields.

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PS - 007

Illegal Medicines – a Challenge for OMCLs and Mass Spectrometry

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Medicines are counterfeited much more than all other goods - approx. 15% of world market versus 7%. This criminal activity is quickened by the free global trade and especially by the misuse of internet. This presentation shows the role and function of an OMCL for protecting public health from the serious risks of organized pharma crime. Key aspects will be the used analytical technologies, especially mass spectroscopy coupled with chromatographic separation techniques and some case studies on actual illegal medicines and counterfeits.

PS - 008

A novel Method for Absolute Quantification and Stoichiometry Determination of Protein Complexes

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Novel Aspect: SRM-based absolute quantification allows determination of stoichiometry and cell copy numbers of major protein complexes.

In eukaryotes, a cascade of events during cell division ensures the correct segregation of chromosomes to prevent aneuploidy. First, duplicated chromosomes are physically tethered together by a multi-subunit complex

called cohesin. This process referred to as sister chromatid cohesion is established during replication in S phase and is maintained until mitosis.
We use SRM based absolute quantification to determine copy numbers and stoichiometries of these complexes throughout the cell cycle. Using our previously described **EtEP** method we generated an equimolar mixture of more than 100 isotopically labeled reference peptides to monitor about 30 proteins. Complex stoichiometries are determined from affinity purified protein complexes and copy numbers are measured from total cell extracts, both obtained from HeLa cells enriched in different cell cycle stages by double thymidine block and release experiments.

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PS - 009

Challenges and Innovations in the (Bio)analysis of Metal Complexes

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The quantitative determination of metal trace impurities in pharmaceutical compositions is an important issue in quality control during drug manufacturing in industry. Also the sensitive detection of metals in probes of biological origin is of high relevance in research and development of metal based drugs (e.g. cisplatin) as this allows to draw conclusions on the biodistribution of the agents on the cellular and subcellular level. Metallomics is a new emerging interdisciplinary science (complementary to genomics or proteomics) dealing with the investigation of trace metals in biology.
Traditionally instrumental analytical techniques such as atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectroscopy (ICP-MS) have been routinely used for quantification purposes based on their high selectivity and impressively low detection limits. However, for the analysis of metals in complex biological materials matrix effects (e.g. due to high salt concentrations) or incomplete analyte recovery are common problems often preventing the use of simple standard calibration procedures or lengthening sample preparation procedures.
Over the last years several technical innovations have been made in the field of qualitative and quantitative metal bioanalysis. These include high resolution atomic absorption spectroscopy (HRCS-AAS) as an AAS method with improved (simultaneous) background correction or total reflection X-ray fluorescence spectrometry (TXRF), which offers options for a simultaneous quantitative multielement analysis.
The presentation will give a non comprehensive overview of the topic of metal (bio)analysis and will highlight recent developments with a focus on HRCS-AAS and TXRF.

PS - 010

Molecular insights into the aging of elastic fibers

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Depending on its physiological function and the anatomical requirements of vital tissue, elastin forms different types of structures with a characteristic organization of cross-linked elastic fibers inside the extracellular matrix. Damaged fibers, which may occur as a consequence of processes such as enzyme dysregulation, pathological conditions and aging, result in a loss of elasticity. Furthermore, the release of elastin-derived peptides with biological effects may influence various cell activities including cell adhesion, proliferation and apoptosis. To understand the structural changes of elastin during these processes, it is necessary to gain insight into the morphological and molecular constitution of the native protein.
Due to its insolubility, mature elastin is hardly accessible for most studies. To date, various procedures for extracting matrix compounds from tissue and subsequently obtaining elastin have been described, of which most use relatively harsh experimental conditions. Elastin isolated by such methods is pre-damaged and thus unsuitable for further investigations.
We developed a method that facilitates the isolation of highly purified and intact elastin from small single punch biopsies. These biopsies were derived from cartilage, skin, foreskin and aorta of individuals aged between 4 and 90 years. Furthermore, few samples were obtained from the skin of patients having a genetic disorder called Williams syndrome. All elastin samples were then hydrolyzed using an elastase and the solubilized peptides were analyzed using a highly reproducible analytical approach based on nanoflow liquid chromatography coupled on-line to a high resolution mass spectrometer with nanoelectrospray ionization. The LC/MS data was then processed using a sophisticated software tool developed in-house for the extraction and normalization of all molecular species from the 3-D dataset. Comparisons were carried out by subsequent multivariate statistical analyses such as PCA.
Results of this study show clear tissue-, age- and disease-related differences in the peptide patterns of elastins. Such differences occur as complete absence of certain peptide species as well as distinct quantitative variations. For identification purposes of such peptides selected samples were subjected to tandem mass spectrometric measurements and the resulting fragment spectra were processed using a combined de novo and database sequencing approach. The identification of such markers enables the investigation of elastinolytic abilities of proteases to degrade elastin at particular residues and, thus, allow conclusions to be drawn about molecular characteristics of different elastins. Moreover, it makes it possible to identify potential changes and modifications of elastin, for instance in aged or UV-exposed skin or in tissue affected by diseases of the ECM.

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PS - 011

About Biological Tissues, Mass Spectrometry and Imaging

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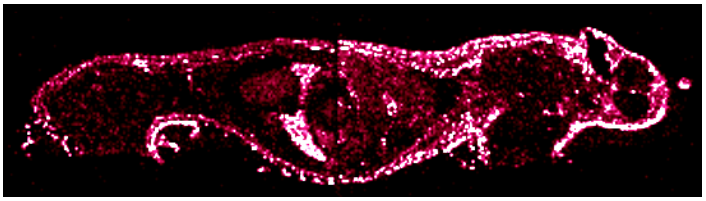
When mass spectrometric imaging started emerging from academic labs, the pharmaceutical research community rapidly developed interest in this promising new technology. Expectations ranged from discovery of new drug targets, evaluation of compound metabolism to finding of biomarkers. During the development of the technology, many aspects were tested in a number of different labs and impressive results were reported in literature. This lecture discusses the successful (and failed) applications, their value to pharmaceutical research and currently unmet needs.



This examples shows the compound distribution in a dosed rat, measured label-free by MALDI mass spectrometric imaging.

Acknowledgements go to Dieter Staab, Brendan Prideaux, Nicole Ehrhard and Gregory Morandi who provided data shown in this presentation.

The web site http://maldi.ms maintained by MS imaging experts contains background information relevant to this topic.

PS - 012

BioEquality: A Platform for the Comprehensive Analysis of Data from Stability Studies and Market Approval of Biosimilars

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Since the first approval and release of a biotechnologically produced recombinant protein in the nineteen eighties it is now estimated, that by the year 2016 eight of the top ten drugs marketed world wide will be a Biopharmaceutical [1]. Adding to this trend is the fact that by the end of 2015 Biopharmaceuticals with a revenue of 64 billion US Dollars will lose their patent protection, paving the way for generic Biopharmaceuticals the so-called Biosimilars [2].

The complexity of protein structures poses new challenges for scientists involved in early development through to production and market approval. Smallest modifications in this structure caused by slightest changes in production or during the shelf life of the product, can lead to a loss of efficacy or serious immunogenic reactions in patients. In addition to the classical analytical methods, which monitor the overall quality of the drug product, new analytical tools need to be employed, which are able to monitor modifications in the primary structure of a Biopharmaceutical.

High resolution mass spectrometry has proven to be such a tool, which delivers information about the primary structure of a protein including all its wanted and unwanted modifications. The highly complex mass spectrometric data can be analysed by specialised software solutions, which give an indication of the protein structure. One software package purpose-built for this task is the MassMap software designed by Prof. Dr. Wozny.

However the comparison of data obtained from proteins of different production batches or even a Biosimilar to the Originator is still very complex and hence time consuming.

The software designed in the BioEquality project will provide a platform for the storage and comparison of data sets created by MassMap. This will enable an analyst to monitor the primary structures of proteins during development, manufacturing and large scale stability studies. The BioEquality software is the first software package which offers its user a complete history of the Biopharmaceutical from early development right through to QC samples from market batches.

The BioEquality software will have an even greater impact on the analysis of Biosimilars. The regulatory agencies demand that the manufacturer displays the equivalence of Biosimilar to the Originator before granting market approval [3,4]. The developed software is designed to aid this process helping to reduce the high development costs for Biosimilars.

In addition to the mass spectral data the software will also integrate information from other methods, e.g., chromatographic LC and CE data, water content, osmolality ect. With this additional information researchers can correlate changes in the primary protein structure to, e.g, an altered water content in the drug product. In this way BioEquality will aid researchers to efficiently pinpoint factors that influence the efficacy of the drug product.

The BioEquality software is the first software solution that integrates structural information of the pharmaceutical active protein from mass spectrometry with test results from other methods that describe the overall quality of the drug product.
This integrative software tool will help to develop safer Biopharmaceuticals and Biosimilars, while at the same time reducing the overall development costs.

Acknowledgements: Prof. Dr. Wozny MassMap GmbH & Co. KG, Bundesministerium für Wirtschaft und Technologie für die Förderung des BioEquality Projects im Rahmen des Zentralen Innovationsprogramm Mittelstand.
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FG Klinische Pharmazie Industriepharmazie Allgemeinpharmazie

PS - 013

The Challenges of Evaluating Drug Therapy and Impact on Health Care Structures as Seen by the Pharmaceutical Industry

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The German health system bears considerable potential in terms of both quality and economic efficiency. With new regulations imminent, the primary focus lies on pharmaceuticals, which is a consequence of rising cost pressures in the health system.
Pharmaceutical companies confront the resulting challenges by having their drugs evaluated early and by taking responsibility for adequately proving the efficacy and efficiency of their drugs in clinical trials. By so doing they make their research transparent and facilitate verification of the research results and outcomes.
Plus, rising drug expenses do not automatically imply inefficient health care. It is necessary to look at the whole picture and check methods to identify economic efficiency reserves. Looking at drugs when isolated and detached from their context in the health care process takes us only half the way to the finish line. Even when trials deliver clear results, physicians, patients and any other parties involved in the therapeutic process need to work together to obtain optimum results in the daily health care processes. After all, the method in which a drug is used in the daily health care setting is crucial [for a successful therapy outcome].
A specific and integrated health care management can be an important contribution to shaping a health system that will be sustainable in the future. Approaches focussing on the health care process itself to achieve maximum efficiency and alignment are worthy of being supported. The Integrated Schizophrenia Care Scheme in Lower Saxony is a good example.
Although all stakeholders in the German health system widely agree that more cross-sector and cross-occupational integrated health care schemes are needed, these scheme do not yet see nationwide application in 2011. What is needed is a cross-sector and interdisciplinary health care management that systematically aligns ambulatory care, hospital care and rehabilitation therapy for patients to achieve a long-lasting improvement of the treatment process. A better coordination of health care and follow-up care can mean facilitation of targeted treatment, saving of costs and faster and easier recovery for patients. This is the reason why the pharmaceutical industry is committed to reviewing all relevant health care processes including the applied means in the health system as a whole instead of evaluating only isolated parts of it.

PS - 014

Cost-benefit evaluation of medicinal products from a hospital pharmacist´s point of view

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The multiplicity of medicinal products available and the complexities surrounding their safe and effective use makes a sound evaluation of medications to be used in the hospital necessary. The evaluation is a major task of the pharmacy and therapeutics (P&T) committee, where the chief pharmacist, physicians and other health care professional are voting members. Based on the evaluation results, medicinal products are included in the formulary. Pharmacists play a primary role in assessing the relative safety and efficacy of medicinal products to be added or deleted from the formulary. They evaluate information on clinical, quality of life, safety and pharmacoeconomic outcomes of drug entities and present the results to the P&T committee. Points to consider in the hospital are known or suspected adverse effects or interactions, unusual drug administration or monitoring requirements. Pharmacists are responsible for drug product selection considering the principles of generic substitution and therapeutic interchange. The selection includes evaluation and assessment of pharmacokinetic and bioequivalence data, storage, dispensing, administration characteristics and costs. The assessment of biological products such as blood products, vaccines, proteins and antibodies requires special attention [1]. Moreover a medication-use evaluation is necessary with the goal to achieve optimal patient outcomes. Outcomes are to be classified as clinical (mortality, morbidity, readmissions), social (quality of life, quality adjusted life years) and economic outcomes. In cost-benefit analysis alternative medication or interventions are compared in terms of their inputs and outputs (= outcomes). An important consideration in economic analysis is the perspective taken. Hospital pharmacists and the P&T committee evaluate cost-cost, cost-effectiveness and cost-utility from the hospital?s standpoint and the DRG based reimbursement system. Results of the evaluation may differ from results taken from another perspective, such as the ambulatory setting. Medicines budget management in the hospital relies on the results of cost-benefit analysis of drug therapy. More and more the question of affordability is also to be evaluated because of limited resources.
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PS - 015

Needs for future managed care settings and their impact on drug the therapy - from a payers perspective

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With the introduction of the new risk structure compensation scheme in 2009 the financing of the staturory health insurance in Germany was completely redesigned. From this time, managed care is an essential field of business even in the staturory health insurance. Analysing the reality of care and, consecutively, improving process quality by managed care contrcts, should and will improve health outcomes.

One example is the rheumatoid arthritis (RA). The number of rheumatologists in Germany is not sufficient. But, even stable patients have at least one visit per quarter by the specialist only because it is possible to charge the visit at the SHI. On the other side, many patients with possible RA don’t get a visit at the specialist and therefore no sufficient diagnosis. This results in unnecessary drug expenditures, because if the diagnosis comes too late, an initial therapy with anti-TNF-agents is often necessary. If the diagnosis comes early enough, a relatively cheap DMARD-therapy is normally sufficient for many years. If the managed care contract leds to more new and less stable patients (which can be adequately treated at the GP) at the specialist this will also have an impact on drug expeditures (sould led to savings).

Besides this example there are many settings in different indications, where the quality of care could be improved without additional drug costs at all. The prerequisites as human resources for analytics at the SHI, willingness to be managed (at patients and physicians side), and a sufficient IT-solution between SHI and physician must be created.

PS - 016

Cost-benefit-risk: challenges for drug therapy – the view of the Drug Commission of German Pharmacists (AMK)

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Effective January 1, 2011, the act for restructuring the drug market (AMNOG) introduced for the very first time an (early) assessment of the additional benefits of (new) drug products coming on the market in Germany. Consequently in the future, reimbursement of a new drug is to be oriented to its therapeutic value compared to existing therapies. For all new drugs, the manufacturer is required to provide appropriate evidence of any additional benefits. For market introduction or for approval of new application areas, a dossier must be submitted that demonstrates any specific, additional benefits. Only then, negotiations about higher reimbursement become possible. All other new products are reimbursed at the level of comparable products. In addition, different types of rebate contracts both for patented and generic drugs were and are to be closed between individual pharmaceutical companies and statutory health insurance funds (SHI). These include an increasing number of risk-/cost-share contracts, also for drugs were the benefit assessment has been judged negative by the G-BA eventually. The AMNOG provides additional opportunities for the pharmaceutical industry to become engaged in integrated care approaches (§140b) and for SHI closing selective contracts for product(s) even with selected physicians (§130c). In consequence, it may be assumed that a patient shall be treated according to his type of SHIs' "positive/ reimbursement list" and a GP is required to follow these lists depending on his involvement in specific contracts and/or integrated care approaches. The AMK questions whether this will be in the best interest of patients at any time. Effectiveness and drug safety are better assured being independent of selective contracts. Prescribing drug substances instead of brand products based on a broad but evidence-based disease-specific catalogue is encouraged. Independent of the type of SHI and to improve medication adherence as well as drug safety, patients with polypharmacotherapy (≥4 me ds) shall be eligible for a physician/pharmacist-based medicines management.

PS - 017

Criteria for Quality and Cost Effectiveness in Change…..or "It's the economy, stupid "

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The beginning
Up to 1962 there was no explicit Drug Law in Germany and Cost Effectiveness was no topic on the agenda of politicians. By order of the „Roman Treaties" it became mandatory even for Germany. The first Drug Law was only "registration", no substantial examination. The "back-ground" was "Wellfare of the Pharmaceutical Industry" no focus on the consumer. The first "real" Drug Law, issued 1976, implemented 1978, 15 times amended since, had as "core" Pharmacovigilance by Approval Procedures including quality, efficacy and safety. At that time quality and efficacy was only used in medical, not oeconomical manner.

Openess
1976 also was the year of the missed opportunities. Since the “Rentenlüge” the real problems of ageing society were obvious but ignored by the politicians. This continues in principle in all political parties up today. Honestly it should have communicated to the public, the social systems are no longer able to pay anything for everybody. It’s time to find a national consensus for rationing.
“Health reforms”
This is the German „nickname” for austerity mesures. The payers are not involved in the de-cissions. Politicians make “active job policy”, not “health policy”, with the payers money, in-cluding action against existing law. An awful example: The eradication of non-prescription drugs from the reembursement by the sickness funds. Prescription or non-prescripting is a security issue, not an oeconomic one. Since 1976 we had 15 health care reforms, two of them (2003, 2006) were called “The Reform of the Century”.
Institute for Quality and Efficiency in Health Care
Proof of Efficiency is common since the late ’80th in various countries. Disclosingly in Ger-many it started in 2004, said to improve quality, from my opinion to save money.
The latest “instrument of torture” is the AMNOG since 2011
Some examples, why it’s impossible to make serious early statements, will be presented.
Conclusio:
Politicians are “running” in the wrong direction. The measures must be patient-oriented, not money-saving-oriented. Open discussions are essential, what can be payed by sickness funds, what out of the poket by patients. To pay all for everyone is impossible. Rationating is there, clandestine. An open dialog within the whole society has to be started on this topic.

1) was a phrase in American politics widely used during Bill Clinton's successful 1992 presidential campaign

Pre-Symposium - FG Pharmazeutische Technologie

PS - 018

Polymeric excipients exhibiting efflux pump inhibitory properties

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Drug efflux pump transporters of the ATP-binding cassette family such as multidrug resistance proteins 1 and 2 (MRP 1 and MRP 2) and P-glycoprotein are believed to confer multidrug resistance to tumor cells and affect overall absorption, distribution, metabolism, and elimination of non-invasively administered clinically important therapeutic agents. By inhibiting these efflux transporters, an extrusion of the drugs back to the mucosal site can be diminished and consequently systemic exposure to non-invasively administered drugs can be significantly improved. A number of auxiliary agents have been described which inhibit functioning of efflux pump transporters. Apart from the low-molecular-mass efflux pump inhibitors frequently undergoing interactions with co-administered active pharmaceutical ingredients and leading to systemic adverse effects, various polymers have recently been found to exhibit an inhibitory effect. Due to their high molecular mass, polymeric efflux pump inhibitors offer the advantage of not being absorbed from mucosal membranes and subsequently systemic toxic side effects can be excluded. Results of various *in vitro* and *in vivo* studies confirmed the efficacy of polymeric excipients as efflux pump inhibitors in particular in oral drug delivery. The use of polyoxylates, pluronic block copolymers, dendrimers, polysaccharides, and thiolated polymers (thiomers) in formulations appears to be a promising strategy.

PS - 019

The Area Vasculosa of the Chicken Yolk Sac as Tool for Toxicity Testing of Polymers

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The development of non-animal methods for testing the irritancy or toxic potential of substances became more and more important in the past and will remain essential in the future. As an already existing Draize-test alternative the Hen's egg test on chorioallantoic membrane (HET-CAM) [1] is used. As a disadvantage, polymer testing with HET-CAM according to official guidelines gives no satisfactory results. For toxic polymers no effects can be seen with the HET-CAM. Here we present a toxicity test on the chick yolk sac area vasculosa (CAV) of fertilized white leghorn chicken eggs which could be used as an alternative to HET-CAM [2,3].
Fertilized chicken eggs were incubated, and afterwards was explanted shell less into a petri dish. Test substances were applied on the CAV (Fig.1) and the appearance of different effects (vascular lysis, haemorrhage, aggregation of blood components, lethality) was determined by light microscopy. For comparison of CAV test to HET-CAM, experiments on the CAV were performed and evaluated according to HET-CAM guideline from the Interagency Coordination Committee on the Validation of Alternative Methods (ICCVAM). This means an investigation for 300 s and calculation of an irritation score (IS) according to a given formula. On the other hand an evaluation of effects over a period of 48 h was performed. Different polymers like poly(ethylene glycol) (PEG; neutral), poly(ethylene imine) (PEI; cationic) and dextran sulphate (DS; anionic), as well as guideline-conform negative (0.9 % NaCl) and positive controls (1 % sodium dodecyl sulphate (SDS) and 0.1 N NaOH) were investigated with both tests and compared to each other.

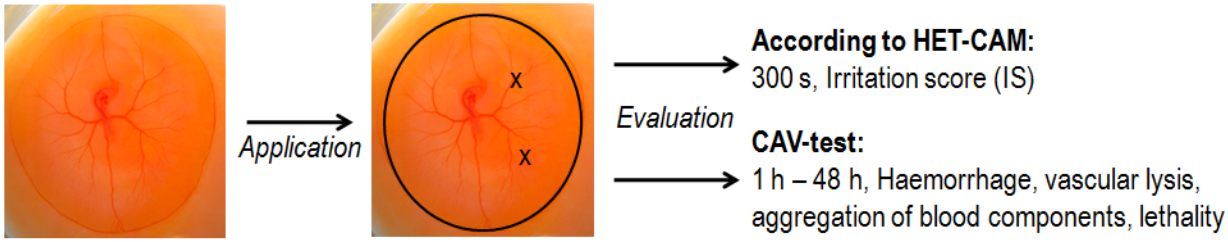

Figure 1: Scheme of experimental design. Framed = CAV, x = application points.

For the CAV test we received comparable results to HET-CAM for the guideline-conform negative and positive controls. However all tested polymers showed no effects after 300 s of investigation. With longer incubation times (1 h – 48 h), effects such as vessel lysis and blood component aggregation could be detected. Additionally to HET-CAM, lethality as well as recovery of the CAV could be observed. Differences between neutral, positively and negatively charged polymers were obtained and a time and concentration dependency could be shown. PEI showed strong vessel lysis and aggregation of blood components whereas DS and PEG showed none of these effects. Lethality was found to increase from PEG < DS < PEI and is concentration and time dependent. The results demonstrate that differences, regarding the toxicity of the used polymers, can be shown with this test. The findings in the CAV test can be correlated with already existing data as well.
In summary, the CAV test provides same data (testing control substances) and more information (recovery and lethality) compared to HET-CAM and could be a suitable model for toxicity testing of polymers.

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PS - 020

Multi-functional macromonomers for biomedical applications

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Oligo- or polymeric molecules that contain functional groups that can take part in further polymerization reaction, so called macromonomers, play an important role in a variety of biomaterial applications ranging from drug delivery to cell delivery vehicles for regenerative medicine. In order to control mechanical properties, degradation behaviour and physical gelation properties as well as to introduce bioactive molecules in these biomaterial systems, such macromonomers have to be versatile in their chemical design and comprise different physico-chemical functionalities. In this work, several amphiphilic, poly(*N*-isopropylacrylamide) (PNiPAAm)-based macromonomers with different additional functionalities were developed. The amphiphilic structure of the macromers was obtained through the use of pentaerythritol diacrylate monostearate (PEDAS), a bifunctional building block that can impart hydrolytic degradability to the macromonomers and contains stearic acid as lipophilic domain. The lipophilic domain has been demonstrated to support gel stability upon thermal gelation due to increased disperse interactions. Lipophilicity has also been described as an important general design criterion for cell carrier materials.
The first set of macromonomers containing PEDAS and NiPAAm as comonomers was designed to be water-soluble and form physical hydrogels in response to a temperature increase to physiological conditions. To synthesize these thermoresponsive macromonomers, acrylamide and hydroxyethyl acrylate were employed as additional comonomers to adapt the hydrophilic-lipophilic balance of the macromonomers and to introduce free hydroxyl groups that can be derivatised with (meth)acrylate moieties, respectively [1]. Correlations between comonomer composition, gelation temperature and hydrogel stability were established. The macromonomers were demonstrated to be sufficiently biocompatible to allow for the direct encapsulation and cultivation of bone marrow-derived stromal cells [2].
The second set of macromonomers has been designed to be able to interact with divalent cations, such as calcium. The functional comonomer used for the synthesis of these calcium-binding macromers was vinylphosphonic acid (VPA), which could be incorporated by the free radical polymerization protocol developed for the thermogelling macromers. Interactions of these macromers with calcium ions where shown to be dependent on the VPA content of the macromers, and molecular interactions of the macromers, such as supramolecular aggregation and thermogelation, were altered in the presence of calcium ions [3].
Recently, maleic anhydride (MA) was implemented as a further functional comonomer for the synthesis of NiPAAm-based macromonomers. Chemical integration of MA was shown by NMR spectrometry and acidimetric titration techniques allowed for the quantification of intact and hydrolysed MA. The resulting macromers were soluble in organic solvents and showed instant reactivity with oligo- and polymeric amines. The reactivity was analysed with different polyetheramines by oscillation rheometry and macromer reactivity was found to be dependent on MA content and molecular weight. The thermogelation properties of the hydrolyzed macromers were dependent on MA content and sensitive to pH and concentration of divalent ions. Reactive MA-containing macromonomers were also used to cross-link gelatine and form transparent hydrogels of variable physico-chemical properties depending on hydrogel composition and chemistry and content of the macromonomer. The cytocompatibility of the resulting hydrogels was demonstrated with L929 fibroblasts and results of a cell culture study with mesenchymal stromal cells will be presented.
A series of novel biocompatible, multi-functional macromonomers is presented that provide a platform for the design of hydrogels for diverse biomedical applications.

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PS - 021

Degradable shape-memory polymer networks as drug carriers

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Pre-defined movements of medical implants such as for anchoring can be realized by shape-memory polymers (SMPs) as stimuli-sensitive materials. This functionality of thermosensitive SMPs bases on their network architecture, which defines the permanent shape of a sample, and a thermomechanical process to create a temporary shape (programming). Upon exposure to a suitable stimulus, e.g., by transferring it into physiological environment, the recovery of the permanent shape is initiated.
Important goals associated with the future usage of SMPs as biomaterials in humans and/or animals are their capability to combine several functionalities, namely shape-memory for anchoring an implant in the tissue, biodegradability to avoid surgical intervention for explantation, and controlled drug release for local treatment. An evaluation strategy to transfer SMPs into Pharmaceutical Sciences has been reported and applied on totally amorphous as well as semi-crystalline SMPs. For instance, a family of semi-crystalline SMPs were synthesized from dimethacrylate-functionalized copolyesters such as oligo[(ε-caprolactone)-*co*-glycolide]dimethacrylate (oCG-DMA) by altering the number average molecular weight (M_n) and glycolide molar content (χ_G) of the network precursors. In some cases, 60 wt.% *n*-butylacrylate has been introduced as comonomer to obtain AB networks [AB-CG(χ_G)-M_n]. The comonomer ratio and the precursor chain length allowed to adjust the switching temperature T_{sw} in the range of 23 °C to 53 °C. Different drug loading techniques for the networks with ethacridine lactate as a model drug, namely swelling or embedding before crosslinking were explored and generally did not effect T_{sw} (Fig. 1A). Furthermore, the network composition was useful to control drug release rates (Fig. 1B). A timely separation of drug release and subsequent slow degradation were observed, which was similar in vivo compared to in vitro for selected materials.

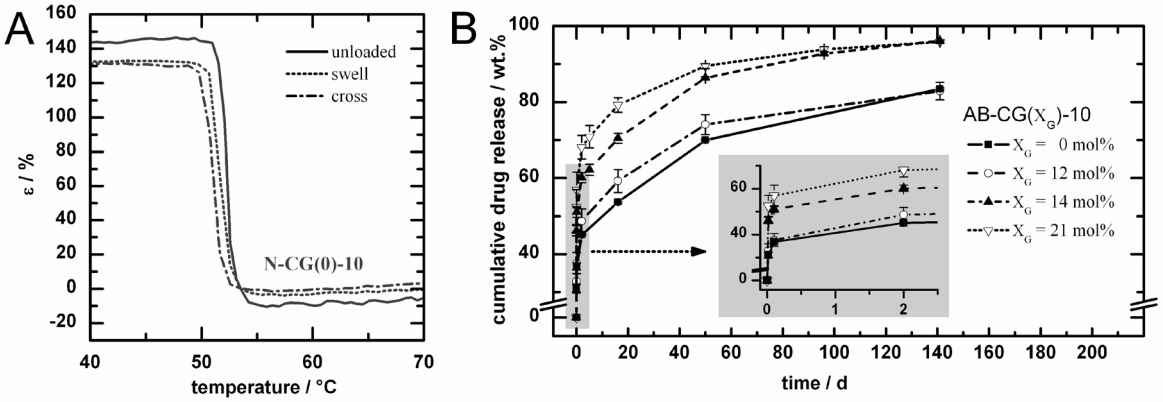

Fig. 1: Shape-recovery (A) and ethacridine lactate drug release (B) from SMP networks; figures modified from Eur. J. Pharm. Sci 2010 (A) and Macromol. Biosci. 2010 (B), reprinted with permission.

References:
J. Contr. Rel. (2009) 138: 243 - 250; Mat. Res. Soc. Proc. (2009) 1190: 131 - 136; Pharm Res. (2010) 27: 527 - 529; Adv. Polym. Sci. (2010) 226: 177 - 205; Eur. J. Pharm. Sci. (2010) 62: 274 - 147; Macromol. Biosci. (2010) 10: 1063 - 1072; Exp. Rev. Med. Dev. (2010) 7: 357 - 379.

Notes

WORKSHOPS

Workshop - 001

Simultaneous Analysis of API and Counter Ions by HPLC with Charged Aerosol Detection

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Salt formation is important in drug development to improve biopharmaceutical and physicochemical properties of the drug. In fact, approximately 50% of all drug molecules are administered as salts.
Sodium and chloride are most commonly used pharmaceutical counterions. For the assay of counterions by liquid chromatography, anions and cations need to be analyzed separately using different methods, different separation columns, and very often, different instruments. It is also true for API and counterion analysis. Similarly, in pharmaceutical analysis, both API and the counterion are often analyzed using different methods, different separation columns, and different instruments. Moreover, many medicines contain neutral drugs as well as acidic and basic ones with respective counterions. Therefore, simultaneous determination of all APIs and counterions would be even more challenging.
Clearly, it is highly desirable to separate and detect both anions and cations, and both API and the counterion on the same column and within a single analysis. To do so, the stationary phase must provide anion-exchange, cation-exchange and reversed-phase functionalities.
Mixed-Mode chromatography is a separation mode that utilizes both RP and IEX interactions. In other words, mixed-mode stationary phases possess both RP and IEX moieties. The biggest benefit of mixed-mode columns is that the selectivity can be optimized by adjusting mobile phase ionic strength, pH and/or organic solvent. As the result, not only are these columns complementary to RP columns, but also complementary to themselves under different conditions. The presence of both RP and IEX functionalities requires no ion-pairing agents in the mobile phase to separate highly hydrophilic charged analytes, which simplifies the mobile phase and is compatible with MS or other evaporative detectors. With adjustable selectivity, it is also possible to separate analytes with dramatically different hydrophobicity and charge state, such as simultaneous separation of pharmaceutical API and counterion, in a single analysis.
In general, Mixed-mode packing materials are classified into three categories: AEX/RP, CEX/RP and AEX/CEX/RP. In this presentation, we will focus on a newly developed AEX/CEX/RP trimode phase.
While most of the API can be detected by spectroscopic or spectrometric detection techniques, the counter ion detection could be a challenge, especially if they have no chromophoric structure elements. The Corona Charged Aerosol Detector (CAD) is a novel, mass-sensitive, universal detector for the routine determination of any nonvolatile and many semivolatile chemical species.

Workshop - 002

Real time observation of microwave-enhanced reactions via fast FTIR spectroscopy and UV/Vis spectroscopy in the reaction solution

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Microwave-enhanced reactions are very fast in comparison to thermal reactions. The determination of optimal end point often fails, because conventional analytical methods (e.g. TLC, NMR or HPLC) are too slow. This often causes decomposition of the product and sophisticated purification steps are necessary. Therefore two fast methods using FTIR spectroscopy and UV/Vis spectroscopy in the reaction solution were established. The results of the reaction control analysis are obtained within less than 1 minute. Both methods can be used in open vessels and in special sealed reactors.

For the FTIR method a sample is taken out of the reaction mixture, the solvent is vaporized and a spectrum is measured immediately. This method is useful for every reaction, except for those with high boiling solvents (e.g. DMF, DMSO, and NMP).
The UV/Vis method can be used for every reaction where the absorption maxima of starting materials and product are higher than of the solvent. The difference of the absorption maxima between reactants and product should be greater than 20 nm better is 50 nm.
The reaction times were shortened and the yields often increased in comparison to reactions carried out in the classical way and with microwave-enhanced reactions run without fast reaction control.

Notes

ABSTRACTS OF POSTERS

Inflammation

PO - 001

Lanostane glycosides from *Chionodoxa sardensis* and their *in vitro* inhibition on COX-1

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Chionodoxa is a small genus within the Asparagaceae (former Hyacinthaceae). With the exception of *Ch. luciliae* which is rich in homoisoflavones and triterpenoids [1], natural products of these species have not yet been investigated intensively. Hydrophilic bulb extracts from different Chionodoxa species, including *Ch. sardensis* Whittal ex Barr & Sugdenshow, show moderate inhibition on cyclooxidase-1 (COX-1) activity *in vitro* [2]. Due to the fact that homoisoflavones have no known inhibitory activity on cyclooxidases, we isolated tripernenoids from *Ch. sardensis* and tested its potential for COX-1 inhibition.
Substances were isolated from fresh bulbs via extraction with ethanol and partitioned between n-butanol and water. Separation of compounds was carried out via column chromatography on silica gel and Sephadex® LH20. The substances were identified using spectroscopic methods primary FT-IR and NMR. Lanostane tripernoids and lanostane triterpene glycosides could be detected. Several structures, known from bulbs of *Ch. luciliae* and *Muscari paradoxum* were found [3]. Furthermore, two new lanostane triterpene glycosides could be purified and its structures were clarified.
Biological activity of the new compounds and three known compounds were tested using a COX-1 catalysed prostaglandin biosynthesis assay *in vitro* and moderate inhibition could be confirmed.

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PO - 002

Effects of non-thermal atmospheric plasma on intracellular structures and processes of keratinocytes

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The application of non-thermal atmospheric pressure plasma in medicine, especially in dermatology, is a very promising approach to improve the treatment of chronic wounds. This application has to be effective as well as safe. That is why it is important to understand the effects of plasma on living cells and its genetic information.
Subject of this study was to define effects of surface dielectric barrier discharge (DBD) plasma (working gas: air) on human HaCaT keratinocytes, a well established cell culture model for human keratinocytes. Adherent HaCaT cells were plasma-treated for 2- 20 min. DNA damage was measured subsequently as well as 24 h after treatment using Alkaline Single-Cell Gel Electrophoresis. Further, cell number and induction of reactive oxygen species (ROS) were determined. H_2DCFDA was used to indicate intracellular oxidative stress. It is able to pass cell membranes and is oxidized to fluorescent DCF in the presence of ROS.

Immediately after plasma treatment a correlation between duration of plasma treatment and DNA damage level was observed. 24 h after plasma exposition for 2 or for 5 min no DNA damage was detected (showed the same level as that of control cells), although the number of adherent cells decreased. In opposite, plasma treatment for 20 min resulted in a permanent DNA damage. Changing cell culture medium subsequently after treatment reduced effects on DNA. Since oxidative stress as well as inflammation causes DNA damages, it was of importance to measure the induction of ROS by plasma. Indeed, plasma treatment caused a time dependent increase of intracellular ROS, which was less pronounced after changing the medium than without medium change. Induction of ROS correlated well with the observed DNA damages and the reduced number of viable HaCaT cells.
In conclusion, effects of plasma on DNA depend on duration of plasma treatment, plasma composition and other parameters. They are related to an increase of ROS inside the cells. Exchange of cell culture medium resulted in minor effects of plasma. In dependence of treatment and regeneration time one could speculate that HaCaT cells are able to repair plasma caused defects of DNA. Possibly also other components of plasma (e.g. RNS, UV light) contribute to the effects on DNA damage. So there is still a need for further investigations to establish a safe regimen for the use of non-thermal atmospheric pressure plasma in wound healing.

* Work supported by the *German Federal Ministry of Education and Research* (grant no. 13N9774 and 13N9779)

PO - 003

Pharmacological activation of $K_{Ca}2.3$ channels reverses microglial activation

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In cortical and hippocampal neurons, small-conductance calcium activated potassium (KCNN/SK/$K_{Ca}2$) channels shape synaptic functions, maintain calcium homeostasis after NMDA receptor activation and prevent excitotoxic neuronal death. However, little is known about their function in non-neuronal cells, such as microglial cells. The aim of this study was to investigate the potential $K_{Ca}2$ channel function in microglia under physiological and pathophysiological conditions.
Stimulation of cultured microglial cells with bacterial lipopolysaccharide (LPS) induced morphological reorganization, microglial activation and proliferation. These effects were evaluated by the xCELLigence impedance-based system and MTT assays. In addition, microglial immunogenic activation was determined by measuring cytokine production and nitric oxide release. Pharmacological modulators, such as CyPPA and apamin, and specific inhibitory peptides allowed distinguishing effects mediated by the $K_{Ca}2$ channel subtypes.
Real-time measurements with the xCELLigence system and MTT assays demonstrated that LPS induced microglial proliferation and morphological reorganization. These structural changes were also detected by microglial immunostainings with CD11b and F4/80 antibodies. The $K_{Ca}2.2$ and $K_{Ca}2.3$ channel activator, CyPPA reduced LPS-induced microglial proliferation in a dose-dependent manner, whereas the general $K_{Ca}2$ channel blocker apamin did not affect LPS-induced microglial proliferation. Specific inhibition of $K_{Ca}2.3$ channels, but not $K_{Ca}2.2$ inhibition, reversed the CyPPA-effects on LPS-induced microglial proliferation. Moreover, CyPPA alone did not alter the production of TNF-α or IL-6 cytokines, but strongly reduced the LPS-dependent TNF-α and IL-6 production.
In summary, these results indicate that $K_{Ca}2.3$ channel stimulation can reverse microglial activation and proliferation. Thus, $K_{Ca}2.3$ channels may serve as a therapeutic target for reducing microglia activity and related inflammatory responses in the nervous system.

PO - 004

Design of dibenzosuberones as highly potent p38α MAP kinase inhibitors: Efficient use of a hydrophobic pocket to improve activity

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Dysregulation of inflammatory cellular signal transduction cascades is known to be involved in the development of many diseases including chronic inflammatory and autoimmune diseases like rheumatoid arthritis (RA) and inflammatory bowel disease (IBD). p38α MAP kinase is a key enzyme in regulation of the pro-inflammatory cytokines TNF-α and IL-1β and is therefore a promising target for the treatment of many diseases.[1,2]
By now, successful development of p38α MAP kinase inhibitors as drugs has been compromised by poor selectivity and / or high toxicity.[3] The ATP-binding pocket of kinases is highly conserved, offering nearly no possibilities to achieve selectivity. Therefore our efforts concentrated on exploiting unique features of the enzyme. One of them is the ability to perform the so called "glycine-flip", which is already enforced by the suberone template. Another one is the "selectivity-pocket". This region is located adjacent to the ATP-binding-pocket. In contrast to other kinases the "selectivity-pocket" is accessible because of the small gatekeeper residue Thr 106. As it is mostly surrounded by lipophilic residues, we had to design moieties fitting exactly in this location. By extending these moieties towards the deep pocket, we were able to achieve high potency in the p38α MAP kinase enzyme assay down to an IC_{50} of 1nM.

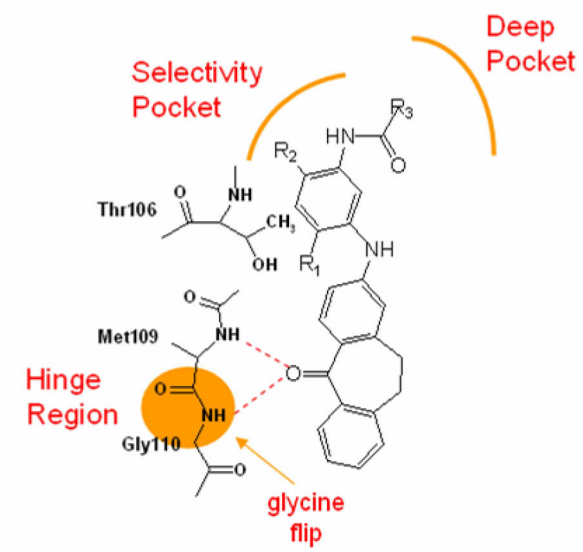

Figure 1: Proposed binding mode of dibenzosuberones and essential interaction sites (Hinge Region, Selectivity Pocket, Deep Pocket)

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PO - 005

6β-Tryptophan substituted 14-*O*-methyloxymorphone, a potent μ opioid receptor agonist with antinociceptive and immunosuppressive activities

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Inflammatory bowel disease (IBD) is a family of chronic and relapsing inflammatory diseases of the gastrointestinal tract characterized by intestinal inflammation, mucosal damage, diarrhea and severe pain that is still inadequately managed. Several studies indicate that activation of peripheral μ opioid receptors (MOP) produces antinociceptive and anti-inflammatory effects. Medicinal chemistry and opioid pharmacology focuses increasingly on exploring the therapeutic potential of peripheral MOP, targeting the development of peripheral opioids as novel therapies not only for pain and but also for inflammatory conditions. Strategies to reduce the access of opioids to the central nervous system include chemical modifications that increase hydrophilicity. We aim for identification of opioids with zwitterionic moieties (i.e. amino acid) attached to the C-6 position of the morphinan skeleton as novel therapeutic molecules for IBD. Herein, we describe the pharmacological and immunological investigations on the 6β-Tryptophan (Trp) substituted derivative of 14-*O*-methyloxymorphone (14-OMO). In in vitro binding assays, the novel opioid morphinan exhibited affinity in the subnanomolar range to the MOP, while in functional studies it showed full agonist activity towards the MOP. The 6β-Trp conjugate of 14-OMO produced a significant inhibition of the nuclear transcription factor kappaB (NF-κB) activation in tumor necrosis factor-α (TNF-α)- and lipopolysaccharide (LPS)-stimulated human monocytic leukemia THP- 1 Blue cells using the Quanti-blue enzyme assay. *In vivo*, this derivative produced dose-dependent and pronounced antinociceptive effects after subcutaneous administration in a mouse model of visceral pain (acetic acid-induced writhing test). Novel MOP agonists acting in the periphery with combined immunosuppressive and antinociceptive properties may provide a new approach for the treatment of IBD.

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PO - 006

Thiazole substituted pirinixic acid derivatives as dual 5-LO / mPGES-1 inhibitors

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Imbalances in the lipid signaling network contribute to the pathogenesis of a large number of human diseases. The metabolic fate of arachidonic acid (AA) plays a crucial role within this network and is associated with pathophysiological conditions such as inflammation, analgesia, asthma and cancer.
The metabolic pathway of AA can be divided into two different ways: The formation of prostaglandins (PGs) by cyclooxygenases (COXs) and the biosynthesis of leukotrienes (LTs) by 5-lipoxygenase (5-LO). Non steroidal anti-inflammatory drugs (NSAIDs) and COX-2 selective inhibitors (coxibs) are the most wide-spread drugs in the anti-inflammatory therapy. However, especially in long-term therapy their use is closely related to severe side effects such as gastrointestinal and renal complications (NSAIDs) or an increased cardiovascular risk (coxibs) due to the suppression of physiological relevant prostaglandins [1]. Consequently, new pharmacological strategies for anti-inflammatory therapy are urgently needed. One promising approach is the selective inhibition of downstream-acting enzymes such as the microsomal prostaglandin E_2 synthase-1 (mPGES-1), which catalyzes the formation of PGE_2 from PGH_2. PGE_2 is the most prominent mediator in inflammatory pain. On the other hand, LTs produced by 5-LO are important inflammatory mediators which act as bronchoconstrictors and increase vascular

permeability. The dual inhibition of 5-LO and mPGES-1 is considered as a novel strategy to avoid COX-related side effects such as the analgesic asthma syndrome and to maintain the physiological prostaglandin levels.
The structural basis of the presented compounds is pirinixic acid, which is inactive on both, mPGES-1 and 5-LO. Especially the introduction of *n*-alkyl chains in α-position led to potent dual 5-LO / mPGES-1 inhibitors. Furthermore, a broad modification of the lipophilic backbone is possible with an equal or increased activity [2]. Herein we present a novel class of pirinixic acid derivatives featuring a thiazole-scaffold at the lipophilic backbone. The resulting thiazole substituted derivatives show balanced dual inhibition of mPGES-1 and 5-LO with IC_{50} values from the high nM to low µM range.

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PO - 007

Synthesis and Structure Activity Relationships of Novel Chemokine Receptor 5 Antagonists

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The chemotactic cytokine receptor 5 (CCR5) is a G-protein coupled, β-chemokine binding receptor, which was found to play a key role in the development of different kinds of inflammatory diseases like rheumatoid arthritis, asthma, allograft rejection and atherosclerosis.[1] Furthermore the CCR5 receptor is known to act as the second co-receptor for the HIV1 cell entry into the target cells.[2] About 1 % of Caucasians are homozygous for a polymorphism at the CC5 receptor gene, this polymorphism encodes truncated CCR5 receptors through a 32-base pair deletion (Δ32-CCR5). Those phenotypically healthy individuals are not only highly resistant to HIV infection[2], they also suffer less from myocardial infarction and show a highly prolonged renal-transplant survival.[3,4]
On account of this, the development of new CCR5 antagonists became increasingly attractive. One of the first highly potent CCR5 antagonists was TAK-779[5] (IC_{50} = 1.4nM) (Figure 1), developed by Takeda Chemical Ind. in 1999 as a potential anti-HIV-1-agent, but failed due to poor oral bioavailability.
The aim of this work is the synthesis of novel CCR5 antagonists and the investigation of their structure activity relationships (SAR) using [^{3}H]-labeled TAK-779 as radioligand.
Blocking or modulating the activity of CCR5 receptors may be an important step in the control of many inflammatory processes. The application of novel [^{18}F]-labeled CCR5 antagonists in PET (positron emission tomography) studies will allow to investigate the possible up- and down-regulation of CCR5 receptors in physiological and pathophysiological situations.

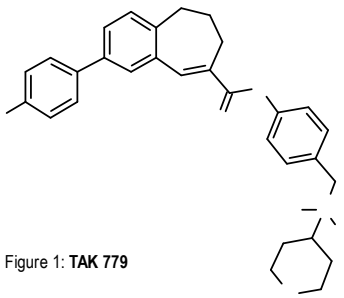

Figure 1: **TAK 779**

Acknowledgements: Financial support by the IRTG Münster – Nagoya (DFG) is gratefully acknowledged.
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PO - 008

Towards selective JNK3 Inhibitors: Introduction of acidic residues improves potency of JNK3 inhibitors

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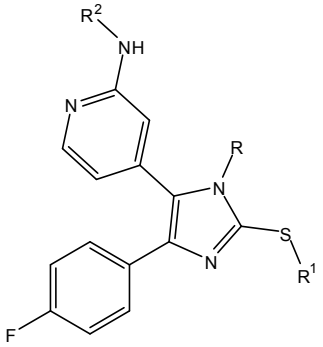
As member of mitogen activated protein kinases (MAPK), JNK3 (c-Jun-NH_2-terminal kinase) is mainly activated in response to inflammation, endotoxins and environmental stress. Once activated the Serine/Threonine-kinase phosphorylates the N-terminal transactivation domain of c-Jun, resulting in enhancement of c-Jun dependent transcriptional events (e.g. proinflammatory gene expression). Mice lacking the JNK3 gene have been shown to be resistant to kainic acid induced excitotoxicity and associated apoptotic cell death. Thus inhibiting JNK3 is a promising therapeutic target for neurodegenerative diseases such as Parkinson´s disease, Alzheimer´s disease and other CNS disorders.
Within the MAPK family p38α is a closely related family member differing at the ATP-binding site in only one amino acid at the gatekeeper region (Thr 106 (p38α) vs. Met 146 (JNK3)), which makes it difficult to develop selective JNK3 inhibitors. Pyridinyl imidazole compounds are known as highly potent p38α inhibitors, often cross-inhibiting JNK3. Formerly we could show that by introduction of carboxylic acid moieties, targeting the hydrophobic region II, it is possible to obtain potent inhibitors of JNK3 with high selectivity over p38α, probably by negative rejections of acidic amino acids (Asp112) located in the hydrophobic region of p38α.
Motivated by these findings, we tried to address other acidic amino acid-residues by permutating acidic groups at R, R^1 and R^2 of the aminopyridine template.
Within a series of 12 compounds, nanomolar JNK3 inhibitors with some selectivity over p38α could be obtained.



PO - 009

Synthesis and antiviral activities of potential Picornavirus Capsid Binders

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The goal of the EU - Project SILVER - '**S**mall-molecule **I**nhibitor **L**eads **V**ersus **E**merging and neglected **R**NA viruses' - is to identify the most promising viral protein targets and antiviral compounds for selected clinically important RNA viruses.
Starting with a compound known as enterovirus capsid inhibitor[1+2] a wide range of differently substituted bis(phenoxymethyl)benzene derivates was synthesized and tested in cell culture against Picornaviruses, focused on Coxsackievirus B3, Echovirus 11, Poliovirus 1, Rhinovirus 2 + 14 and Enterovirus 71. The antiviral and cytotoxic activities of the compounds were determined by means of a cell protection assay.
For Poliovirus 1 and Echovirus 11 some of the tested compounds showed activity in the low nanomolar range. The ones with interesting activity had substituents, like halogens, methyl or methoxygroups, in the 2- and 4-position. Some hits against Rhinovirus 2 were achieved in the micromolar range. Against Coxsackievirus B3, Rhinovirus 14 and Enterovirus 71 the bis(phenoxymethyl)benzene derivates tested so far did not show significant activity.

Acknowledgements: The Project SILVER is financially supported by the European Union.
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PO - 010

Combined efficacy of three novel herbal TCM formulas Kujin-Plus I-III on atopic dermatitis in 94 patients

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TCM offers interesting novel treatment options for atopic dermatitis (AD). In the present open-label clinical trial, the efficacy and safety of the multi component therapy approach Kujin-Plus was investigated. Therefore, 94 AD patients received the formula Kujin-Plus-I orally, combined with both the skin lotion Kujin-Plus-II, and the ointment Kujin-Plus-III. Each of the below mentioned plant drugs was extracted with boiling water for 5 h, concentrated and reworked into the respective formulation. Standardised scores were used for evaluating the severity of the disease (clinical score; 0-4) and the severity of pruritus (pruritus score; 0-4) with both scores having significantly improved at the end of treatment ($P<0.01$; nonparametric test) after twelve months. Blood eosinophil ratio and serum IgE levels, which were elevated in AD patients, were significantly reduced at the end of the therapy ($P<0.001$). Of 94 AD patients treated with Kujin-Plus, 32 were markedly improved, 59 were improved, 3 were slightly improved with no case of ineffective treatment. There was no hint of renal or hepatic toxicity or of any other adverse effects. Thus, the present study confirms that the Kujin-Plus treatment is clinically efficacious on AD, accomplishing a significant reduction in both blood eosinophil ratio and serum IgE level. The study at hand provides extensive evidence supporting the efficacy of Kujin-Plus in the therapy of intractable AD.

Kujin-Plus-I:
Sophora flavescens root, *Polygonum cuspidatum* rhizome, *Isatis tinctoria* leaves, *Smilax glabra* rhizome, *Angelica dahurica* root, *Glycyrrhiza uralensis* rhizome, *Scutellaria baicalensis* root, *Chrysanthemum indicum* flowers, *Prunella vulgaris* entire plant, *Oldenlandia diffusa* entire plant

Kujin-Plus-II:
Sophora flavescens root, *Coptis chinensis* root, *Scutellaria baicalensis* root, *Stemona sessilifolia* root, *Punica granatum* pericarp, *Chrysanthemum indicum* flowers, *Paris polyphylla* root

Kujin-Plus-III:
Sophora flavescens root, *Polygonum cuspidatum* rhizome, *Isatis tinctoria* leaves, *Angelica dahurica* root, *Glycyrrhiza uralensis* rhizome, *Scutellaria baicalensis* root, *Citrus* × *limon* pericarp, *Dryobalanops aromatica* resin

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PO - 011

Olea europaea L. leaf (Ph.Eur.) extract as well as several of its single phenolics inhibit the gout-related enzyme xanthine oxidase

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In Mediterranean folk medicine, olive leaf extract (*Olea oleuropaea* L.) is a common remedy for gout [1-3]. Therefore, in this *in-vitro* study kinetic measurements were performed to investigate its possible inhibitory effects on xanthine oxidase (XO), an enzyme well known to contribute to this pathological process [4]. In these experiments, both the whole leaf extract of *O. europaea* [5] as well as many of its characteristic phenolic constituents significantly inhibited the XO activity. Dixon and Lineweaver-Burk plot analyses were used to determine K_i values and inhibition modes for the single substances, the HPLC determination of which we discussed in an earlier study [5]. Among these, the flavone aglycone apigenin, which constitutes 0.033% of the extract, exhibited by far the strongest effect on XO with a K_i of 0.52µM. In comparison, the phenolic secoiridoid oleuropein the main ingredient of the extract (24.8%) had a considerable higher K_i (53.0µM). However, it still displayed a significant inhibition of XO. Caffeic acid (K_i11.5µM/1.89%), luteolin-7-glucoside (K_i15.0µM/0.86%) and luteolin (K_i2.9µM/0.086%) also contributed to the XO inhibiting effect of *O. europaea* whole leaf extract. For oleuropein, a competitive mode of inhibition was found, while all other active substances displayed a mixed mode of inhibition. Tyrosol, hydroxytyrosol, verbascosid, and apigenin-7-glucoside were inactive in all tested concentrations but one has to take into consideration that apigenin-7-glucoside, which makes up for 0.3% of the extract, is transformed into active apigenin in the mammalian body [6], thus also contributing substantially to the anti-gout activity of olive leaf extract.

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PO - 012

Physical plasma induced generation of physiologically relevant reactive species in liquids

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A lot of physiological reactions are mediated by reactive nitrogen and oxygen species (RNS, ROS). $NO^{\bullet}$, $NO_2^{\bullet}$, $ONOO^-$ and $O_2^{\bullet -}$ are involved in many body processes, e.g. inflammation and oxidative modification of biomolecules. Such oxidative changes may be a future target for pharmacological interventions.[1] One innovative method is the use of physical plasma to generate such species in liquids and to influence living cells. This is one important aim of the new research field called plasma medicine. However, because of their short life times the

detection of RNS and ROS is very difficult. To get more insight into mechanisms of change of liquid composition by plasma treatment, theoretical reactions in water have been assumed which may lead to generation of protons, nitrate, nitrite and hydrogen peroxide.[2, 3] Using different techniques like photometric methods, pH measurement and ion chromatography, these low-molecular inorganic species could be measured in plasma-treated water. Furthermore, plasma-induced inactivation of bacteria in aqueous liquids was found just as influences on different characteristics of mammalian cells.[2-4] Consequently, it was possible to reduce the manipulation of cellular functions by plasma treatment at least partially to the generation of low-molecular inorganic species in the vital environment of cells. Further investigations have to be done to confirm the generation of specific RNS and ROS and eventually to provide possibilities of specific therapeutic applications of physical plasma.

Acknowledgement: This study was realized within the joint research project "Campus PlasmaMed" supported by the German Federal Ministry of Education and Research (grants no. 13N9779 and 13N11188).

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PO - 013

Development of electrochemical sensors for the quantification of inflammatory disease mediators

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Inflammation is a reaction of the body as a consequence of different stimuli (mechanical, chemical, thermal or toxic injuries) as well as of pathogens (microorganism or parasites). These noxa cause a release of different mediators that induce inflammation with its typical symptoms such as swelling, reddening, heat or fever, pain and decreased mobility. Inflammatory mediators play a key role in the evolution of diseases like allergy, arteriosclerosis and arthritis. They are body-own paracrine hormones histamine, serotonine and kinines but also substances of the arachidon acid cascade like prostaglandin, prostacyclin, thromboxan, leucotriene and lipoxin. An interaction of these mediators with the cells of the immune system leads to inflammation [1].
The aim of this work is to design electrochemical sensing systems for the determination of inflammatory mediators in organic matrix (e.g. blood, plasma and tissue). Especially histamine lies in our focus. Nowadays histamine is usually determined using HPLC or capillary electrophoresis [2]. Also analysis using gas chromatography is possible [3]. These methods require time consuming sample preparation or in the case of gas chromatography need pre- or post column derivatisation. Electrochemical sensors would be a rapid, simple and cheap possibility for the diagnostic of inflammation diseases in a symptom-free stadium as well as they would offer an elegant way to keep chronic forms under supervision.
Another key aspect of this work is the development of an electrochemical method for the quantification of the so called "total antioxidant capacity" of food samples. Antioxidants are substances that reduce the reactive oxygen species (ROS) and therefore have anti-inflammatory activity [4]. ROS are high reactive products, formed when arachidonic acid is catabolised by lipoxygenase. They are strong oxidants and cause damage on cell-own material. Knowing the concentration of antioxidants in food samples is therefore an important part of this study. For the basic experiments we picked the most common basic-structures of the substances with antioxidative power (e.g. resorcinol, catechol, hydroquinol).
In this poster we present the development results of an electrochemical sensing system for the quantification of histamine as well as for the determination of "total antioxidative capacity" of food samples. In both cases we have chosen a carbon-paste working electrode. While the antioxidants showed reproducible oxidation peaks at +0.4 respectively +0.7 V vs. saturated calomel electrode (SCE) in 0.1 M phosphate buffer pH 7, histamine showed its signal at +1.2 V at same conditions. To cause a peak shift of the histamine signal to lower potentials different

modification experiments were performed. Especially the modification using copper-decorated nanotubes showed promising results and is therefore presented in this poster.

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PO - 014

Cosmetic's evolution between tradition and innovation: Calendula officinalis L. supercritical CO2 extracts

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Calendula officinalis L. is a well known medicinal plant which is used in the folk medicine from ancient times for different purposes. The most important part expecially for topical uses are flowers that are used for their antiinflammatory properties. The phytochemical composition is complex: carotenoids, triterpenes glycosides, flavonoid glycosides and triterpene esters with fatty acids are the most important classes of secondary metabolites.
Extracts from Calendula has been used as a medicinal and a cosmetic since ancient times, as many others plants. Starting from this origins, many studies have been performed to better understand wich are the main active constituents that give plants their properties and effects. In the Third millennium cosmetic science, the importance of what is "green" or from natural origin has a strong impact both on research and development and in marketing aspects. In particular are growing lot of kind of organic products. This study is a resume of an experience of applied research done in cooperation between industry and university. The work started with a well known in cosmetic use bothanical species: Calendula officinalis L., trying to obtain some high quality innovative extracts and strongly concentrated in some active ingredients, extracted by supercritical CO_2 that was the best way to separate lipophilic compounds from the raw plant. Target constituents where Faradiol derivatives, in particular Faradiol esters, identified as a strongly active compounds involved in antiinflammatory effect of Calendula extract. The single extract constituents have been identified by HLPC-MS and HLPC-ELSD method, and lately from the extract have been separated and purified target compounds, that have been identified by NMR techniques. They became the standard reference for the analitical cheques. The obtained extracts have been used to realise some cosmetics products that must to combine ease of use and pleasant feeling, with carrier effect and promoting efficacy for the extract. The obtained extracts where significantly different for organoleptic aspect and quantity of active constituents has shown by analitical spectra and efficacy in-vitro (FT-skin) and in-vivo studies (on human volunteers). These patterns where significantly different and higher that traditional Calendula extracts and oils. Looking at the green aspect, all the step, starting from plants cultivation ending by the extract, where performer with a "green and sustainable" feeling. Finally the cosmetic bases where effective too on carrying and promoting extract's effect.

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7. Primary Flavonoids in Marigold Dye: Extraction, Structure and Involvement in the Dyeing ProcessPAULINE GUINOT,1 ANNICK GARGADENNEC,1 GILLES VALETTE,2 ALAIN FRUCHIER3 and CLAUDE ANDARY1*1Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, UMR 5175 (CEFE), 15 avenue Charles Flahault, 34093 Montpellier Cedex 5, France2Laboratoire de Mesures Physiques, Université de Montpellier 2, cc 010, place Eugène Bataillon, 34095 Montpellier Cedex 5, France 3Ecole Nationale Supérieure de Chimie, UMR 5076, 8 rue de l'Ecole Normale, 34 296 Montpellier Cedex 5, France

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PO - 015

Differential eicosanoid biosynthesis during the menstrual cycle

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During female menstrual cycle hormonal changes occur, which have major influences on the immune system as well as on the course of autoimmune diseases [1]. Leukotrienes (LT) and prostaglandins belong to the eicosanoids that are pivotal lipid mediators with major roles in inflammation and innate immunity. Eicosanoids are reported to play an important role in the reproductive tract during female cycle. For example, LTs are known to be increased in serum of female patients suffering from dysmenorrhea which do not respond to anti-prostaglandin therapy. Furthermore, premenstrual asthma has been described which can be successfully treated with LT receptor antagonists. Here, we investigated whether or not eicosanoid biosynthesis depends on the menstrual cycle. We show that LT formation in stimulated blood (Ca^{2+}-ionophore A23187 or LPS/fMLP) is higher (2 to 3-fold) during the luteal phase (day 15-28) compared to the follicular phase (day 1-14). In contrast, LT formation in isolated neutrophils (the major source of LTs in blood) is not affected by the cycle. Also the number of neutrophils is equal. On the other hand, formation of the prostanoid 12-hydroxyheptadecatrienoic acid (12-HHT) in stimulated blood is lower (2-fold) as well as the 12-lipoxygenase product 12-hydroxyeicosatetraenoic acid (12-HETE) during the luteal phase compared to the follicular phase. This could be correlated to the higher number of 12-HHT/12-HETE-forming platelets (1.4-fold) during the follicular phase. These results add impact on the topic of gender pharmacology and the differential treatment of diseases in females which are influenced by the menstrual cycle.

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PO - 016

Effect of Toll-like receptor agonists on tumor-immune cell cross-talk

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Objective: Nucleic acid-based Immune enhancers gain more and more interest in cancer therapy. The Toll-like receptor (TLR-) ligands Imiquimod and Poly(I:C) are such candidates. In addition to immune cells, also tumor cells themselves express TLRs. In this study we investigated the effect and the consequences of tumor cell stimulation by nucleid-acid based immunotherapeutic agents Poly (I:C) and Imiquimod.

Methods & Results: We analyzed the cytokine release and viability of human and mouse head and neck squamous cell carcinoma cell lines (HNSCC). Poly(I:C) and Imiquimod increased G-CSF, IL-6 and IL-8 release of the tumor cells, all three enhancers of neutrophilic activity. Regarding cell viability, the way of delivery and the concentration played a substantial role for the outcome. Imiquimod for instances, strongly reduced human and mouse cancer cell viability in a dose dependent manner. Interestingly, Poly(I:C) slightly promoted cell viability of human HNSCC, when the substance was simply co-cultured with the cells consequently. In contrast, when Poly(I:C) was delivered directly intracellular by electroporation, the molecule strongly reduced cell viability.
Imiquimod could directly enhance immunity by increasing release of MIP-1β by neutrophils and Type I IFN by peripheral blood mononuclear cells (PBMCs). Imiquimod treated PBMCs enhanced their cytolytic activity towards HNSCC as measured in a chromium-release assay. In current studies we analyze the mode of tumor cell death, which was induced by Imiquimod and the impact on human PBMCs and neutrophils.
Conclusion: Our data suggest that the way of drug delivery and the mode of tumor cell death may determine successful therapy and thus represents a huge challenge for the galenic and clinical development. A careful analysis of TLR-mediated signaling in the tumor-microenvironment is crucial for the optimization of immune therapies.

PO - 017

Polyacetylene derivatives from *Notopterygium incisum* with PPARγ agonistic effect

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Notopterygium incisium Ting ex H. T. Chang (*Qiang Huo*) is used in Traditional Chinese Medicine for the treatment of rheumatism, headache and common cold. The dichloromethane extract of dried underground parts exhibited significant activation of Peroxisome Proliferator Activated Receptor gamma (PPARγ). The latter is involved in many inflammatory processes, and has become an important pharmacological target. After finding a series of regular polyacetylenes possessing PPARγ agonistic activity, we have now isolated two new compounds. Their structures have been elucidated by NMR and MS as polyacetylene adducts of ferulic acid. Compound **2** represents a new skeleton. Pharmacological evaluation revealed that **1** and **2** exhibited strong PPARγ activation (ca. 200 % and 150 % stimulation at 10 μM, respectively).

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PO - 018

The cardiac fibroblasts as an inflammatory cell in dilated cardiomyopathy

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Background:
Dilated cardiomyopathy (DCM) is a common form of cardiomyopathy. In DCM the left ventricle dilates. This is known to be associated with increased wall stress, as one mechanical stressor to all cardiac cells. This is also associated with alterations in the extracellular matrix (ECM). In this disease, in which no inflammatory stimulus like a virus is presents inflammatory cells play an important role. The crosstalk between ECM regulation and inflammation is not well understood. The most abundant cell type in the myocardium is the cardiac fibroblast, which is responsible for the synthesis of the ECM.
We wanted to assess the influence of stretch on the fibroblasts in regard to the DCM phenotype. Therefore, we established an in vitro system to stretch fibroblasts and to investigate differences in ECM formation and therefore a possible influence on cardiac inflammation.
Methods and Results:
Primary cardiac fibroblasts were obtained by outgrowth from hearts from C57BL/6j mice. The fibroblasts were stimulated by sinusoidal cyclic deformation with different stretch intensity (2.5%, 5%, 7.5%, and 10%) on the Flexercell ® FX-4000 system for 24 and 96 hours. For investigating the transdifferentiation of fibroblasts we determined the protein expression. The protein–expression (96 hours) of collagen 1 but not of α-smooth-muscle actin was increase 2.fold after 10% elongations compared to 2.5% elongation. Total RNA of 24 hours was extracted from the cells and transcribed to cDNA, before analyzing the gene expression versus TaqMan. Interestingly, MCP-1 as well as the MCP-3-mRNA expression and the protein-expression were increased dependently from stretch intensity.
Conclusion:
We here demonstrate that strain intensification results in higher expression of collagen in a dose dependent manner, there we have shown that mechanical stretch leads to differentiation of fibroblast to myofibroblasts. Moreover, these pathological activated fibroblasts produce chemokines, such as MCP-1 and MCP-3, which may explain in part the cardiac inflammation found in DCM hearts. Therefore, we conclude that the myofibroblast is next to its known role as an ECM producer but also to an inflammatory cell type.

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G-Proteins

PO - 019

The development of nanoparticles that bind to the somatostatin receptor

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Somatostatin receptors are expressed in numerous healthy, but also tumor cells which makes them a promising target for anti-tumor therapy using ligand decorated nanoparticles. In a model study we tried to attach two different peptides (somatostatin (SST) and octreotide (OC)) to gold nanoparticles and poly (ethylene glycol) (PEG) as a surrogate for PEGylated nanoparticles respectively using two different techniques. We coated gold nanoparticles (AuNPs) with SST (AuNP-SST) using layer-by-layer technique reported previously [1-2] taking

advantage of the positive net charge of somatostatin at neutral pH. OC was intended to be covalently linked to nanoparticles carrying carboxylate groups for the future. Since it has 2 potential binding sites (the amine of the N-terminus and another one in the side chain of a lysine molecule), we established a reaction using a model PEG at low pH to react it selectively with its N-terminus [3]. To this end we conjugated a carboxylated methoxy-poly(ethylene glycol) (Me-PEG-COOH) with OC using 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) as a water-soluble cross linker. The gold particles were characterized by UV–Vis spectroscopy, HPLC, and SDS page. The size average and zeta-potential of the AuNP-SST were analyzed using dynamic light scattering. The PEG-conjugated OC was characterized by ^{1} HNMR, and MALDI-ToF mass spectrometry.
The results indicate a successful coating of AuNPs with SST with an average size of 20±0.3nm and 27±0.3nm for AuNP and AuNP-SST respectively. The UV absorbance spectra showed a red shift in the surface plasmon resonance peak of AuNP-SST compared with the AuNPs. Furthermore, HPLC and SDS page confirmed the coating of AuNPs with SST. The covalent attachment of OC to Me-PEG-COOH was confirmed by the increase of molecular weight from 5000 Da to 6131.1 Da as indicated by mass spectroscopy. ^{1}H NMR showed that the product was obtained with 71% conversion. Both products will allow investigation of the binding behavior of nanoparticles to somatostatin-receptor positive cells. While the gold particles can be used to track interactions by transmission electron microscopy, the PEGylated octreotide can be used to investigate the impact of the PEG tether on the affinity of the ligand to its receptor.

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PO - 020

Adenine receptor: Selective receptor ligands and possible signalling pathways

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Adenine has recently been identified as an endogenous ligand of a novel G-protein coupled receptor in rats. Radioligand binding studies using membrane preparations provide evidence for expression of adenine receptors in rat neuronal tissue. The aim of the present study was to detect the adenine receptor on rat neuroblastoma cells (B104) and to study the signalling pathways using novel selective receptor ligands. Additionally, intracellular recordings were made on cortical pyramidal cells in rat brain slices. The adenine receptor mRNA was detected using qualitative RT-PCR. Adenine (10µM to 1mM) as well as the receptor agonist TB-74 (1µM to 100µM) depressed concentration-dependently the electrical evoked synaptic potentials in rat brain slices and inhibited the ATP-induced increase of intracellular Ca^{2+} concentration in B104 cells. The potential antagonist PSB-08162 (10µM to 500µM) showed in our experiments partial agonistic effects. It responded comparable to adenine with a lower extend. An effect of adenine was not detectable after preincubation of cells with PSB-08162 (100µM). Our results indicate that the adenine receptor is expressed on B104 cells as well as on rat cortical pyramidal cells. Our results suggest that TB-74 is more potent than adenine. Additionally, in rat neuronal cells PSB-08162 interacts as a partial agonist. Inhibition of signalling pathway steps indicates that the adenine receptor couples to a G_q protein in rat neuronal cells. These findings provide evidence for the existence of different subtypes of the adenine receptor in rats.

PO - 021

Synthesis and evaluation of thiazolo[5,4-*c*]pyridines as adenosine A_3 receptor ligands

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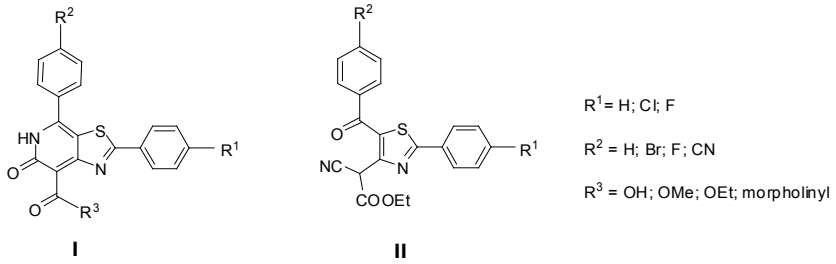
Adenosine receptors A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R are cell surface receptors belonging to G protein-coupled receptors (GPCRs). Recently, they have been intensively explored as possible therapeutic targets for neurodegenerative and other diseases. Synthetic adenosine agonists and antagonists are developed as therapeutic or diagnostic agents [1]. The aim of this study was to synthesize a set of substituted thiazolo[5,4-*c*]pyridines (**I**) and to evaluate their binding activity toward A_3R using radioligand binding assays. Some intermediates were also tested.
A set of thiazolo[5,4-*c*]pyridines **I** was prepared by a synthetic way according to Briel [2], with some modifications. Starting from thiobenzamides and methylthioacrylonitrile derivatives, 2-aryl-6-imino-*6H*-1,3-thiazinium tetrafluoroborates were synthesized and treated with phenacyl bromides to give thiazoles (**II**). The final thiazolo[5,4-*c*]pyridines **I** were obtained via ring transformation by a heating of **II** with acetic acid. Thiazolo[5,4-*c*]pyridines **I** and some of the intermediates **II** were evaluated for their binding activity to human A_3 receptor using methods according [3]. Potent and selective A_3 antagonists have been identified.



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PO - 022

Zwitterionic opioid receptor antagonists: Synthesis and pharmacological evaluation

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μ Opioid receptors (MOP) are expressed in the central nervous system (CNS) and in peripheral tissues including the gut. Experimental and clinical work showed that MOP antagonists (e.g. alvimopan, methylnaltrexone), which inactivate peripheral MOP, can stimulate gut motility and secretion without interfering with opioid-induced analgesia. Alvimopan is clinically used only for short term therapy of post-operative ileus (POI) following bowel resection in hospitalized adults due to a number of severe side effects in long term treatment. Methylnaltrexone is approved for the treatment of opioid-induced bowel dysfunction (OBD), but not for POI since the effectiveness of this quaternary derivative is rather low due to its moderate binding to MOP. Within a new drug discovery program, we directed our research towards identification of novel peripherally active MOP antagonists with improved

benefit/risk ratio as new therapeutic molecules in debilitating conditions associated with bowel motility. On the basis of our observations that 6-amino acid derivatives of 14-*O*-methyloxymorphone represent novel opioid agonists with high antinociceptive potency while exhibiting limited access to the CNS [1,2], we introduced amino acid residues at C-6 of the opioid antagonist 14-*O*-methylnaltrexone [3]. Synthesis of the novel zwitterionic morphinans was accomplished by multi-step syntheses. Following synthesis, the new compounds underwent pharmacological evaluations. In binding assays, the novel zwitterionic opioid morphinans exhibited high affinity to MOP, while the ligand-stimulated functional studies revealed their high MOP antagonist potency in the subnanomolar range. These MOP antagonists may represent new therapeutic molecules in debilitating conditions associated with bowel motility, such as POI and OBD.

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PO - 023

Synthesis and pharmacology of clozapine-derived Histamine H_1/H_4-receptor-ligands

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The histamine H_4-receptor is discussed to be involved in inflammation and regulation of the immune system [1]. Together with the histamine H_1-receptor it is responsible for type-I-allergic diseases, such as rhinitis and conjunctivitis. The design of new drugs including one common H_1-/H_4-pharmacophore could result in a new therapy-option of these diseases, because the combined application of mepyramine, a H_1R antagonist and JNJ 777120, a H_4R ligand, lead to a synergistic effect in the acute murine asthma model [2]. Clozapine (**1)** is known to be – among others - a ligand to the H_1- and the H_4- receptors, that has a high affinity to the H_1- and a moderate affinity to the H_4-receptor [3]. Soft structure-modifications **(2),(3)** lead to an increase of affinity to the H_4-receptor under constant high affinity to the H_1-receptor [4].

	pK_i hH_1R	pK_i hH_4R
4	6.2	5.1
5	4.5	5.3
6	6.2	4.7
7	5.9	5.5
9	3.9	4.3

The aim is to obtain compounds with high affinity to both receptors by slicing the diazepine-ring of clozapine. The resulting compound with an "open" clozapine-structure **(4)** was further modified by I) varying the position of the chloro-substituent and by II) inserting other substituents in para-position. Examples of the synthesized compounds according to I) are shown in **(5)**,**(6)** and **(7),** whereas **(8)** and **(9)** represent compounds that correspond to strategy II).
First pharmacological data revealed that the new compounds show, compared to clozapine, a decreased affinity to hH_1R and hH_4R. However, these new compounds will give more insight onto structure-activity relationships at hH_1R and hH_4R

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PO - 024

Synthesis and opioid receptor binding profile of novel 6-amino acid substituted 14-alkoxy-*N*-methylmorphinans

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Traditionally, analgesic effects of opioids have been associated with stimulation of opioid receptors in the central nervous system (CNS). A number of preclinical and clinical studies have demonstrated the existence of opioid receptors outside the CNS and the generation of analgesia via peripheral opioid mechanisms by activation of receptors localized on sensory neurons. Activation of peripheral μ opioid receptors (MOP) was also reported to produce anti-inflammatory effects. These findings have stimulated the development of a new generation of opioids acting in the periphery without unwanted CNS side effects. Approaches to limit the access to the CNS include the incorporation of highly polar hydrophilic substituents. Recently, ionizable 6-amino acid conjugates (Gly, Ala, Phe) of 14-*O*-methyloxymorphone (14-OMO) [1] and 6-Gly substituted 14-*O*-phenylpropyloxymorphone [2] were developed in an effort to obtain potent opioid agonists with limited access to the CNS. These compounds show high antinociceptive potency by interacting with peripheral MOP. In order to further reduce the CNS penetration, 6-amino acid derivatives (e.g. Asp, Glu, Lys) of 14-OMO with favorable physicochemical properties compared to the respective Gly conjugates were synthesized. In opioid receptor binding assays, all new compounds interacted with high affinity with MOP, being comparable to the 6-Gly analogues. Based on the calculated log*P* and log*D* values, an increase in hydrophilicity, and thus peripheral selectivity can be achieved by inclusion of amino acid residues at C-6 of the morphinan skeleton. Such novel zwitterionic morphinans may represent important opioid agonists for the treatment of chronic pain and inflammatory conditions without the adverse actions of centrally-acting opioids.

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PO - 025

New Neurotensin Receptor 2 (NTS2) Selective Peptoid-Peptide Hybrids Synthesis, Receptor Binding and SAR Investigations

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The human neurotensin receptor subtype 2 (NTS2), a G-protein coupled receptor, is activated by the endogenous peptide neurotensin.
The C-terminal fragment NT(8-13) (H-Arg-Arg-Pro-Tyr-Ile-Leu-OH) was identified to be the pharmacologically active motif.[1] As NTS2 is involved in the modulation of chronic pain sensitivity [2], it is regarded as a highly attractive target. Moreover, antipsychotic-like activity for a metabolically stabilized ligand with NTS2 preference was reported very recently.[3] The investigation of NTS2 selective ligands is becoming more and more interesting and a fairly selective ligand with the sequence NMe-Arg-Arg-Pro-D-1-Nal-*t*-Leu-Leu-OH has already been published in literature.[4]
Very recently, we found a new peptoid-peptide hybrid that displays single-digit nanomolar NTS2 binding and more than 1000 fold selectivity over NTS1. A crucial structural modification of this NT (8-13) derivative is the replacement of tyrosine in position 11 by a homo-tyrosine derived peptoid moiety**.** [5]
Based on the above mentioned NTS2 ligand, this poster describes synthesis, receptor binding studies and SAR investigations on peptoid-peptide analogs with focus on the basic amino acids as well as the proline and the homo-tyrosine derived peptoid moiety. It will be shown that small modifications can still enhance the affinity for NTS2.

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PO - 026

The role of serotonin 5-HT_{2A} receptors in cabergoline-induced valvular heart disease

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Ergoline-derived drugs such as pergolide and cabergoline are dopamine receptor agonists used for the treatment of Parkinson's disease (PD). A severe side effect of both drugs is heart valve fibrosis that may lead to valvular regurgitation and stenosis. A 5 to 6-fold higher risk for valvular heart disease was observed in PD patients treated with pergolide or cabergoline compared to non-parkinsonian controls [1]. The same side effect was also reported in patients taking appetite suppressants such as fenfluramine [2]. In addition, fibrotic heart valve disease was observed in carcinoid syndrome, a disorder causing high serotonin plasma levels [3]. This and the fact that 5-HT receptors are expressed in human heart valves suggest the involvement of serotonergic activity in this disease [4]. The predominant cell type in heart valves consists of valvular interstitial cells (VICs) which are responsible for the synthesis and turnover of the extracellular matrix (ECM). In fibrotic disorders an overexpression of the ECM components (collagen and glucosaminoglycan) is a characteristic histopathological indicator [2].
In previous studies we used functional assays to demonstrate the agonist activity of cabergoline at 5-HT_{2A}- and 5-HT_{2B} receptors and to identify the molecular fragment responsible for agonism of this drug [5]. In order to examine the effect of cabergoline on VICs, we isolated primary culture from aortic and mitral porcine VICs. We incubated the cells for 48 h in the presence of either tritium labelled proline or glucosamine, which were used as markers for

newly synthesized collagen or glucosaminoglycan, respectively. Aiming to reveal the receptor subtypes responsible for the synthesis of the above mentioned ECM components, the experiments were conducted in the presence of selective antagonists for 5-$HT_{1B/D}$, 5-HT_{2A} and 5-HT_{2B} subtypes. Our results showed that both 5-HT and cabergoline increased the incorporation of [^{3}H]-proline and [^{3}H]-glucosamine. The increase was inhibited in the presence of the selective 5-HT_{2A} receptor antagonist MDL100907. In contrast, SB 204741 (5-HT_{2B} receptor antagonist) and GR127935 (5-$HT_{1B/1D}$ receptor antagonist) showed no inhibitory effect on either collagen or glucosaminoglycan synthesis.
Although other studies have shown that the 5-HT_{2B} receptor subtype is the mediator of drug-induced fibrotic effects on heart valves, our findings suggest that the 2A subtype receptor might also play an important role in this pathological condition.

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PO - 027

Synthesis of iperoxo-derivatives for receptor activation studies on muscarinic M_2 acetylcholine receptors

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Muscarinic receptors belong to the superfamily of G-protein-coupled receptors. They are classified in 5 subtypes (M_1-M_5), which differ in tissue distribution and G-protein-coupling specificity. Concerning muscarinic M_2 receptors the endogenous activator acetylcholine as well as other agonists preferably activate G_i proteins but may also activate G_s proteins [1]. The epitope M_2-tyrosine $104^{3.33}$ plays an important role for binding affinities and potencies of conventional muscarinic agonists and inverse agonists. This epitope is located in the third transmembrane domain and next to aspartate $103^{3.32}$ which is thought to strongly interact with the positively charged nitrogen of common muscarinic agonists. In order to determine the influence of the substitution pattern on the nitrogen, a systematically varied series of derivatives of the muscarinic super agonist iperoxo were synthesized.
First of all the key precursor iperoxo base was built in a three step synthesis, starting with the conversion of isopentyl nitrite with 1-bromo-3-chloropropane in the presence of $NaNO_2$. The obtained 3-nitro-Δ^2-isoxazoline was combined with 4-dimethylaminobut-2-yn-1-ol, which was formed by means of a Mannich reaction using 2-propyn-1-ol, dimethylammonium hydrochloride and aqueous formaldehyde solution in the presence of copper sulfate. Subsequently the resulting compound, iperoxo base, was treated with various bromo- or iodoalkanes, respectively [2]. Therefore the two compounds were heated in a pressure tube in chloroform or acetonitrile with catalytic amounts of KI/K_2CO_3. The reaction time varies from 1 day to several weeks.

Agonist binding affinities at the muscarinic M_2 wild type receptor and the M_2^{Y104A} mutant stably expressed in CHO cells were investigated applying radioligand binding assays using [^{3}H]N-methylscopolamine ([^{3}H]NMS) as a radioligand. Furthermore the agonist-induced receptor activation was studied on G protein and whole cell level by [^{35}S]GTPγS binding experiments in receptor-containing CHO cell membranes and by measuring dynamic mass

redistribution in living cells, e.g. the N-butyl-iperoxo shows a biased mechanism of receptor activation as a full agonist for the activation of G_i-proteins and being only a feeble partial agonist for G_s-activation.

Acknowledgements: Thanks are due to the Deutsche Forschungsgemeinschaft (DFG HO 1368-12/1) for financial support.
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PO - 028

Identification of novel allosteric modulators for the G-protein coupled US28 receptor of human cytomegalovirus

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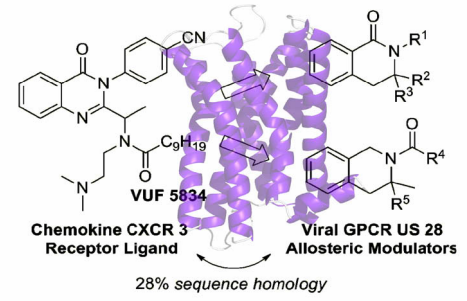
Due to its implication on viral dissemination, cardiovascular diseases and tumorigenesis the highly constitutive active G-protein coupled receptor US28 of human cytomegalovirus (HCMV) is an interesting pharmacological target.[1] Applying radical carboamination reactions [2] followed by non-radical transformations we rapidly synthesized tetrahydroisoquinolinone and tetrahydroisoquinoline scaffolds,[3] which were identified as promising lead structures for novel US28 allosteric inverse agonists.[4]



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PO - 029

Visualization of the unseen: use of novel radiotracers for imaging the neurotensin 1 receptor (NTS1)

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Neurotensin receptors (NTS) are involved in a wide range of pharmacological effects, e.g. dopamine transmission, analgesia, and hypothermia [1]. Due to this different attributes and its appearance in brain and periphery, including certain tumors [2], there is keen interest to study the physiological and pathophysiological distribution of the receptor subtypes NTS 1 and NTS 2 in living subjects with molecular imaging techniques, such as positron emission tomography (PET). Recently, we have reported on the design of a radiolabeled derivative of NT 8-13, the active fragment of the endogenous peptidic agonist neurotensin [3]. As an extension of this research, we herein report the synthesis of a ^{18}F-labeled non-peptidic NTS 1 selective ligand (^{18}F-LC40), derived from the potent NTS antagonist SR142948A by taking advantage of our click chemistry based ligation of 2-deoxy-2-[^{18}F]fluoroglucosyl azide (azido-[^{18}F]FDG) to the alkyne functionalized analog of the lead structure. Receptor binding experiments using NTS 1 expressing CHO cells indicated a K_i value for F-LC40 of 0.5 nM. The precursor for ^{18}F-labeling was obtained by a palladium-catalyzed aminocarbonylation [4] of the respective bromoarene derivative with an alkynylamine using $Mo(CO)_6$ as carbon monoxide source. The CuAAC using azido-[^{18}F]FDG afforded the glycosyl ligand ^{18}F-LC40 in a radiochemical yield of 20% in a total synthesis time of 75 min. ^{18}F-LC40 showed a $logD_{7.4}$ value of -0.24 (clogP = 1.9). In vitro autoradiography of rat brain slices showed specific binding of ^{18}F-LC40 to NTS 1-rich brain regions, as demonstrated by displacement with NT(8-13) and SR142948A. Currently, biodistribution studies with ^{18}F-LC40 are performed.

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PO - 030

Gene reporter assay for the investigation of human and murine histamine H_4 receptor ligands

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Histamine exerts its effects via four receptor subtypes, referred to as H_1, H_2, H_3 and H_4 receptors (H_1R-H_4R), all belonging to class A of G-protein coupled receptors. The H_4R is considered a promising target for pharmacotherapy of inflammatory diseases [1] such as allergic asthma. However, the investigation of the (patho)physiological role of the H_4R and its validation as a possible drug target using antagonists and agonists in translational animal models are seriously hampered by pronounced species-dependent discrepancies regarding potencies and receptor selectivities of the pharmacological tools [2-4].
Aiming at a more distal read-out compared to radioligand binding and GTPase or GTPγS assays a cAMP gene reporter (luciferase) assay was established using the cAMP responsive element (CRE) in HEK293 cells, stably expressing the human (hH_4R) or the mouse H_4R (mH_4R). In addition to radioligand binding the genetically engineered cells were used for the functional investigation of H_4R agonists and antagonists. Most ligands displayed lower affinity and activity at the mH_4R compared to the hH_4R, for example, [^{3}H]UR-PI294 [5] binds with high affinity to the hH_4R (K_D = 7.5 nM), whereas the affinity for the mH_4R was tenfold lower. The endogenous ligand histamine acted as full agonist at both receptors, but with considerably higher potency at the hH_4R (EC_{50}: 12 nM vs. 79 nM). The H_4R inverse agonist thioperamide increased the forskolin-stimulated luciferase activity indicating constitutive activity of both the mH_4R and hH_4R. The established cellular test systems are complementary to functional assay on membrane preparations of Sf9 cells expressing the receptor of interest.

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PO - 031

A molecular mechanism of biased agonism in muscarinic M_2 acetylcholine receptors

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Introduction
Muscarinic acetylcholine receptors belong to the superfamily of G protein-coupled receptors and are able to activate different types of G proteins. In muscarinic M_2 receptors the endogenous activator acetylcholine and other conventional agonists induce preferential activation of G_i proteins, but in addition these agonists may also activate G_s proteins [1].
Binding affinities and potencies of common muscarinic agonists and inverse agonists are highly dependent on the epitope M_2-tyrosine $104^{3.33}$ (Ballesteros and Weinstein numbers in superscript) in the third transmembrane domain [2]. This epitope is located next to aspartate $103^{3.32}$ which is thought to strongly interact with the positively charged nitrogen of conventional muscarinic agonists. In order to gain more insight into the role of the ligand-receptor-interaction in this receptor area, we studied the consequences for binding affinity and signaling of both a more space filling substitution at the agonist's quaternary nitrogen and a mutation of tyrosine 104 to alanine.
Results
Binding experiments. We investigated agonist binding affinities at the muscarinic M_2 wild type receptor and the M_2^{Y104A} mutant stably expressed in CHO cells applying radioligand binding assays using [^{3}H]N-methylscopolamine ([^{3}H]NMS) as a radioligand (10mM HEPES, 10 mM $MgCl_2$, 100 mM NaCl, pH 7.4, 30°C).
In the M_2^{Y104A} mutant, the highly potent agonist iperoxo [3] as well as the endogenous ligand acetylcholine displayed a massive loss of affinity amounting to a factor of 1000. Substitution of a methyl-group by an n-butyl-residue in the quaternary nitrogen of iperoxo yielding "butyliperoxo" reduced the affinity for the wild type M_2-receptor by a factor of 1000, suggesting a major reorientation of butyliperoxo's quaternary nitrogen in the M_2 receptor's ligand binding pocket.
Remarkably, the binding affinity of N-butyliperoxo was slightly increased in the M_2^{Y104A} mutant in comparison to the wild type receptor.
Receptor activation experiments. Agonist-induced receptor activation with respect to potency and efficacy was studied on G protein and whole cell level by [^{35}S]GTPγS binding experiments in receptor-containing CHO cell membranes (10mM HEPES, 10 mM $MgCl_2$, 100 mM NaCl, 10µM GDP, pH 7.4, 30°C) and by measuring dynamic mass redistribution (DMR) in living CHO cells (EPIC®-system; Hanks' balanced salt solution with 20 mM HEPES, pH 7.4, 28°C), respectively. In both functional readouts, the *potency* of iperoxo, acetylcholine and other conventional muscarinic agonists was dramatically reduced in the M_2^{Y104A} mutant relative to wild-type M_2, whereas the potency of butyliperoxo did not change in both assays. These findings correspond with the observations made in the binding studies. In the mutant receptor M_2^{Y104A}, the intrinsic *efficacy* was clearly diminished for acetylcholine, but not for iperoxo and butyliperoxo, being superagonists under these conditions.
Signaling pathway selectivity. After inhibition of G_i proteins with pertussis toxin, DMR experiments revealed that butyliperoxo activates G_s proteins with small intrinsic efficacy (Mean±S.E.: 28±2%) at M_2 wild type receptors relative to acetylcholine and iperoxo (G_s response set to 100%). In the M_2^{Y104A} mutant, neither butyliperoxo nor acetylcholine and iperoxo induced a clear G_s-response.
Conclusion
Taken together, the epitope M_2-tyrosine 104 is essential for the receptor to fully exploit its signalling repertoire. In line with this, N-butyliperoxo, the receptor interaction of which hardly depends on M_2-tyrosine 104, shows a biased mechanism of receptor activation, being a full agonist for G_i activation and only a weak partial agonist for the activation of G_s proteins.

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PO - 032

Different signalling properties of human dopamine receptors D_{2short} and D_{2long} compared to full and partial agonists in HEK293 cells

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Dopamine receptors are important for central nervous diseases like schizophrenia, Parkinson's disease, drug addiction and erectile dysfunction. Considering the great homology of the dopamine receptors, they still have different binding and signalling profiles. Taking a closer look at D_{2long} and D_{2short}, which differ only by an insert of 29 amino acids in the third intracellular loop, differences in phosphorylation, G protein binding and signalling appear [1]. According to literature D_{2long} prefers the postsynaptic side of the receptor whereas D_{2short} favours the presynaptic side [2]. With the [^{35}S]GTPyS assay we are able to measure preferred binding of certain receptors or ligands to defined G proteins very early in the signalling cascade, bypassing complexities inherent in other downstream assays[3].
In our testing system we used transient cotransfections in HEK293 cells of either D_{2long} and D_{2short} with pertussis toxin insensitive $G_{\alpha o/i2}$. We tested known agonists and partial agonists. To compare their intrinsic efficacy curves were normalized to their specific basal level.
Dopamine, quinpirole, 7OH-DPAT and 5OH-DPAT produced a higher signal at D_{2long}+$G_{\alpha o}$ than D_{2short}+$G_{\alpha o}$. This picture was inverted with $G_{\alpha i2}$. From our in house library we tested partial agonists 1,4-disubstituted phenylpiperazines FAUC321, FAUC335 and FAUC350, latter exhibits functional selectivity [4] . Interestingly D_{2short}+$G_{\alpha o}$ shows a higher intrinsic efficacy than D_{2long}+$G_{\alpha o}$. Both cotransfections with $G_{\alpha i2}$ exhibit only a weak activation with the phenylpiperazines.
Overall the D2 splice variants differ in their ability to couple with varying G proteins, also partial and full agonists seem to react differently. Considering these findings they will eventually enlighten the influence of both D2 receptors in vivo.

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PO - 033

Dissecting the individual roles of the second extracellular loop of Adenosine A_{2A} and A_{2B} receptors

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The human adenosine A_{2B} receptor, which belongs to the familiy of class A G protein-coupled receptors (GPCRs), appears to play an important role in inflammatory processes. In the respiratory system A_{2B} receptor activation results in proinflammatory effects. In contrast, the closely related A_{2A} receptor subtype mediates antiinflammatory and immunosuppressive effects. Adenosine and most of its derivatives, e.g. NECA and CGS-21680, show considerably higher affinity for the A_{2A} than for the A_{2B} receptor, despite high sequence identity of 56 % and a similarity of 70 % (for the human receptors). In many GPCRs the second extracellular loop (EL2) is known to participate in ligand binding. In the present study the complete EL2 of the A_{2B} receptor was replaced by the EL2 of the A_{2A} receptor by overlap extension mutagenesis. Furthermore single amino acid residues Asp148, Ser149, Thr151, Glu164 and Ser165 in the EL2 were exchanged for alanine by site-directed mutagenesis. The resulting

mutant receptors were stably expressed in CHO cells using a retroviral expression system, and characterized by radioligand binding studies using [^{3}H]PSB-603 as a radioligand, and by cAMP accumulation assays. All agonists investigated (adenosine, NECA, and the non-nucleosidic A_{2B} agonist BAY60-6583) showed increased efficacy at the mutant A_{2B}(EL2-A_{2A}) receptor as compared to the wildtype (wt) A_{2B} receptor (using forskolin as a control). In contrast to the wt A_{2B} receptor, at which CGS-21680 is inactive, the A_{2B}(EL2-A_{2A}) mutant could be activated by the A_{2A}-selective agonist CGS-21680 exhibiting an EC_{50} value of 47 µM. Interestingly, the agonists were more potent at the E164A mutant than at the wildtype receptor indicating that this amino acid might contribute to the relatively low affinity of agonists at the A_{2B} receptor subtype. The EL2 of the adenosine A_2 receptor subtypes appears to play an important role in receptor activation.

PO - 034

Pharmacological and toxicological properties of a novel selective PDE10A ligand

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The phosphodiesterase 10A (PDE10A) has an important role in neurotransmission regulating the intracellular cyclic nucleotides especially in dopaminergic neurons. The PDE10A is a promising candidate for drug development and inhibiton of the PDE10A could be an interesting therapeutic stategy for treatment of brain dysfunctions, such as schizophrenia. The present study was designed to examine pharmacological and toxicological properties of a potent and selective brain permeable inhibitor of PDE10A (K_i = 31.9 nM) as non-radioactive derivative for the development of a radiotracer for positron emission tomography (PET).
The lead compound (3006) and the fluoric substituted derivative with prolonged alkyl chain by one methylen group (3039) had no effect on basal intracellular calcium concentration $[Ca^+]_i$ in human neuroblastoma cells (SH-SY5Y). High concentrations (100 µM) of 3039 but not 3006 increased potassium-induced calcium mobilisation. Electrophysiological investigations on rat brain slices indicated no effect of 3039 or 3006 on postsynaptic membrane parameters and synaptic transmission up to 100 µM. After long-term incubation (48 h) 3039 and 3006 enhanced metabolic activity and reduced LDH-release of SH-SY5Y cells up to 1 µM whereas at high concentration (100 µM) metabolic activity was decreased due to slightly increased cell damage. Using the zebrafish embryo toxicity test mortality was observed at concentration of 100 µM for 3039 and $\geq$ 1µM for 3006 after incubation of 48 h.
The results suggest a different pharmacological profile of 3039 in comparison to its lead compound 3006 possibly by distinct binding characteristics to the PDE10A enzyme. Both compounds had no toxic effects in concentrations relevant for PET ligands. 3039 seems to be an appropriate candidate for developing a PET probe for studying distribution of PDE10A *in vivo*.

PO - 035

Synthesis and pharmacological characterization of novel thiazolo- and thiazinomorphinans

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The need for opioid analgesics with reduced undesirable side-effects has initiated a vast amount of scientific efforts, which have led to a number of new opioid ligands and significant expansion of knowledge in opioid pharmacology [1]. We established a procedure for the synthesis of thiazolo- and (benzo)thiazinomorphinans exploiting the known ability of 14β-bromocodeinone to react like an α-bromoketone [2,3]. However, this first set of heteroring fused morphinans were derived with the pharmacologically less favourable 3-methoxy template, and it is known that the *O*-demethylation of morphinans having a conjugated diene in ring C is especially challenging due to their (acid) sensitivity. For this reason, a new synthetic route to the pharmacologically more promising 3-hydroxymorphinan congeners starting from 14β-bromomorphinone was designed. Binding affinities of the newly synthesized compounds and the intermediate 14β-bromomorphinans to opioid receptors were determined by in vitro competition binding assays using rodent brain membranes. These studies revealed remarkable results for two compounds: 14β-bromodihydromorphinone showed high affinity and selectivity to the μ opioid receptor, while the benzothiazinomorphinan derivative was found to have high affinity to μ and δ sites and moderate affinity towards κ receptors. In ligand-stimulated [^{35}S]GTPγS binding to membranes from Chinese hamster ovary (CHO) cells stably transfected with human opioid receptors, both compounds were potent full agonists at the μ receptor and the benzothiazinomorphinan derivative also at the δ receptor.

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PO - 036

Fluorophore-labelled EP_3 receptor ligands as pharmacological tools

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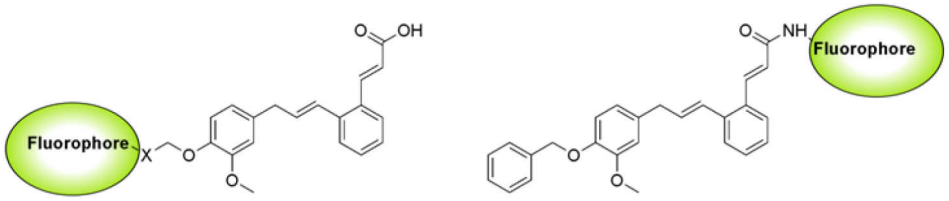
Prostanoid receptors are G-protein-coupled receptors which are activated by cyclooxygenase metabolites of arachidonic acid. Prostaglandin E_2 (PGE_2) acts at four G-protein-coupled prostanoid receptors EP_1, EP_2, EP_3, and EP_4 [1]. The EP_3 receptor plays a crucial role in fever generation, hyperalgesia, uterine contraction, gastric acid secretion, smooth muscle contraction of the gastrointestinal tract, neurotransmitter release, sodium/water reabsorption in kidney tubules, platelet aggregation, and thrombosis [2,3]. Due to its divers physiological and pathophysiological effects it is of special interest to detect and localise the EP_3 receptors under certain conditions and stimuli.

Based on recently disclosed lead structures [4,5] *ortho*-substituted cinnamic acids and their acylsulfonamides were labelled with different fluorescent moieties *via* Heck reaction, acylsulfonamide coupling with EDC and other coupling reactions. Affinities of the synthesized compounds were determined by radioligand displacement assay using [^{3}H]-PGE_2 at recombinant human EP_3 prostanoid receptors (ChemiSCREEN™ membrane preparation) showing affinities in the nanomolar concentration range for most of the derivatives.

Affine fluorophore-labelled compounds were used as marker for detection and visualisation of EP_3 receptors on human colon adenocarcinoma cells (HT-29 cells - grade II cell line). EP_3 receptor localisations and fluorescence intensities on HT29 cells could be measured by a confocal laser scanning microscopy. Resulting data confirmed the potential of the novel fluorescent-labelled EP_3 receptor ligands as pharmacological tools for the characterisation of the biological role of the EP_3 receptor.



Acknowledgements: This work has been kindly supported by the LOEWE programs OSF and NeFF.
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PO - 037

Design and pharmacological characterization of novel FFAR1-(GPR40)-agonists for treatment of type 2 diabetes mellitus

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The G-protein coupled receptor FFAR1 (free fatty acid receptor 1, formerly known as GPR40) is mainly expressed in pancreatic β-cells. Its stimulation by free fatty acids enhances glucose-stimulated insulin secretion (GSIS). FFAR1 is thus thought to be a novel target for treatment of Type 2 Diabetes mellitus [1,2]. Over 200 compounds were synthesized and functionally characterized at recombinantly expressed FFAR1 receptors. Assays included changes in intracellular calcium and cAMP concentrations. Potent ligands were investigated for their ability to induce GSIS in the rat insulinoma cell line INS1-E [3,4]. TUG 469 obtained a pEC_{50} of 7.78 ± 0.03 (calcium mobilization), about 240-fold more potent than the physiological ligand linoleic acid and 2-fold more potent than the well-known and potent FFAR1 agonist GW9508 [5] [Fig. 1]. TUG 469 was further explored in New Zealand Obese (NZO) mice demonstrating its *in vivo* effect on glucose tolerance.

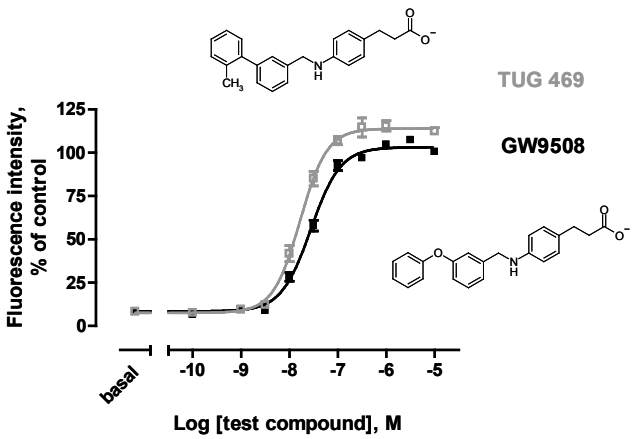

Figure 1: Chemical structure and functional potency of TUG 469 (grey line) and GW9508 (black line) using Ca^{2+} mobilization assay; pEC_{50} [TUG 469]: 7.78 ± 0.03; pEC_{50} [GW9508]: 7.56 ± 0.02.

In order to understand the structure-activity relationship of the compounds, the pharmacological data set from FFAR1 agonist characterization (about 170 compounds) was used for receptormodel-based 3D-QSAR analysis (AFMoC). The model shows a good predictive power (q^2 = 0.59; s_{PRESS} = 0.49 log units) and was validated with internal and external data sets. The model was used to design potential FFAR1 agonists with further improved potency. These compounds are currently being synthesized and tested. In summary, this study led to the development of FFAR1 agonists with nanomolar potency. *In vivo* activity in improving glucose tolerance was demonstrated in NZO mice for the highly potent compound TUG 469. In addition, receptor model-based 3D-QSAR was used to gain insight into receptor-ligand interactions at a molecular level and to design improved ligands in regard to their functional potency.

Acknowledgements:
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Klinik für Endokrinologie, Rheumatologie und Diabetologie, Universitaetsklinikum Duesseldorf, Dr. S. Schinner.
Dr. Hilmer-Foundation for financial support.

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PO - 038

Evidence for a differential role of the conserved epitope tryptophan 7.35 in different subtypes of muscarinic acetylcholine receptor

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Within the superfamily of G protein-coupled receptors the class A GPCR including muscarinic acetylcholine receptors play a prominent role as drug targets. Whereas the five muscarinic receptor subtypes are highly homologous in their orthosteric acetylcholine binding site, the less conserved allosteric site proved to be a promising target for the development of subtype selective ligands.
With respect to allosteric epitopes, the amino acid Trp 7.35 at the beginning of TM7 has particular functional properties: In inactive muscarinic receptors, this epitope provides subtype independent baseline affinity [1], whereas in the active M_2 receptor this epitope contributes to the binding affinity of the full agonist acetylcholine and the intrinsic efficacy of the partial agonist pilocarpine [2]. In the present study we checked whether the role of Trp 7.35 for ligand binding is related to the G protein-coupling preference of the receptor subtype. The even-numbered subtypes M_2 and M_4 prefer coupling to G_i whereas the odd-numbered subtypes such as M_3 prefer coupling to G_q.
Using tritiated N-methylscopolamine ([^{3}H]NMS) as a probe, binding experiments were carried out in membrane homogenates of CHO Flp-In™ cells overexpressing either the wild-type of the human muscarinic receptor M_2, M_3 and M_4 or the respective Trp→Ala mutant receptor (10mM Hepes, 10mM $MgCl_2$, 100mM NaCl, pH 7.4, 30°C). Next to the orthosteric acetylcholine and pilocarpine the dualsteric (orthosteric/allosteric) agonist “hybrid 1” was included [3]. The incubation time for equilibrium binding conditions was chosen considering the half-life time of [^{3}H]NMS-dissociation ($t_{1/2}$ diss) and, in case of hybrid 1, an allosteric prolongation of $t_{1/2}$ diss. The affinity constants (pK) are listed in table 1.

Receptor	pK acetylcholine means ±S.E.M. (n)	pK pilocarpine means ±S.E.M. (n)	pK hybrid 1 means ±S.E.M. (n)
M_2-wt	6.30±0.07 (4)	5.68±0.15 (4)	7.04±0.10 (4)
M_2^{422}Trp→Ala ΔpK	5.75±0.09 (6)* 0.55	5.72±0.13 (4)	7.12±0.05 (3)
M_4-wt	6.35±0.11 (7)	5.26±0.09 (4)	5.74±0.07 (4)

M_4^{435}Trp→Ala ΔpK	5.37±0.13 (5)* 0.98	5.33±0.07 (4)	5.42±0.17 (4)
M_3-wt	5.09±0.13 (7)	5.54±0.16 (4)	4.64±0.10 (4)
M_3^{526}Trp→Ala ΔpK	4.42±0.18 (7)* 0.67	4.73±0.03 (6)* 0.81	4.08±0.04 (4)* 0.56

Table 1: Consequences of the mutation Trp 7.35→Ala in M_2, M_4 and M_3 on affinity constants
* significant difference to respective wild-type receptor

Whereas the consequences of the mutation were similar in the M_2 and M_4 receptor (pronounced loss of affinity of acetylcholine, unchanged affinity of pilocarpine and hybrid 1), the replacement of Trp 7.35 in the M_3 receptor induced a loss of affinity for all applied agonists.
These results provide first evidence that the role of Trp 7.35 for receptor function is not conserved among the muscarinic receptor subtypes, but may be related to the specificity of G protein-coupling.

Acknowledgements: Prof. Dr. U. Holzgrabe, Institute of Pharmaceutical Chemistry, University of Würzburg, Germany, Prof. Dr. M. De Amici, Department of Pharmaceutical Sciences, University of Milan, Italy.
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PO - 039

Mepyramine-JNJ7777120-hybrid compounds show high affinity to hH_1R, but low affinity to hH_4R

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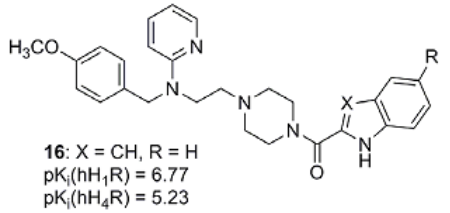
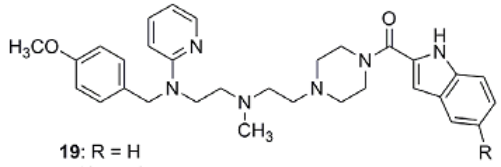
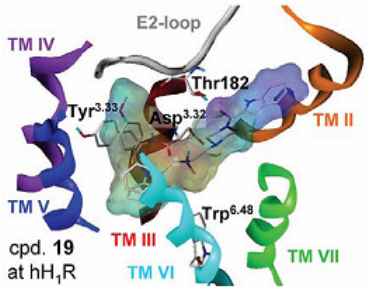
In literature, a synergism between histamine H_1 and H_4 receptor is discussed [1]. Furthermore, it was shown, that the combined application of mepyramine, a H_1 antagonist and JNJ7777120, a H_4 receptor ligand leads to a synergistic effect in the acute murine asthma model [2]. Thus, the aim of this study was to develop new hybrid ligands, containing one H_1 and one H_4 pharmacophor, connected by an appropriate spacer, in order to address both, H_1R and H_4R. A similar approach was already applied successfully for H_1R and H_2R [3]. Within this study, we synthesized nine hybrid compounds, which were pharmacologically characterized at hH_1R and hH_4R. The new compounds revealed (high) affinity to hH_1R, but showed only low affinity to hH_4R. Additionally, we performed molecular dynamic studies for some selected compounds at hH_1R, in order to obtain information about the binding mode of these compounds on molecular level and to explain the pharmacological data.

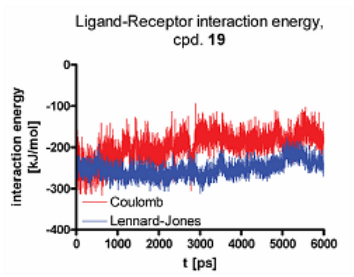


16: X = CH, R = H
$pK_i(hH_1R) = 6.77$
$pK_i(hH_4R) = 5.23$



19: R = H
$pK_i(hH_1R) = 8.15$
$pK_i(hH_4R) = 5.05$





In general, this study revealed important insights onto structure-activity relationships at hH_1R and hH_4R. However, further molecular modelling studies at hH_4R have to be performed in order to explain the pharmacological data of these compounds at hH_4R. Additionally, other combinations of H_1/H_4-pharmacophors will be designed and pharmacologically characterized in order to analyze, if affinity to both receptors depends on pharmacophor combinations.

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PO - 040

1,2,3-Triazole Elements in Histamine H_3 Receptor Ligands

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In the central nervous system (CNS) the human histamine H_3 receptor (hH_3R) controls the histamine mediated neurotransmission. Its autoreceptor function and cross-linking to other neurotransmitter systems *via* heteroreceptors play a central role in maintaining the neurotransmitter balance. Due to the resulting modulation of numerous (patho)physiological brain functions the H_3R might be an attractive target for a multitude of CNS disorders [1].
The common pharmacophore blueprint of H_3R antagonists/inverse agonists offers an amine moiety in the receptor-targeting western part of the molecule linked by an alkyl spacer to a mostly lipophilic central core structure, which can be substituted by a broad range of structural elements, defining the activity of the compound [2]. We identified 1,2,3-triazole moieties as suitable structural elements on the eastern part of the H_3R antagonist construction pattern [3]. In this study we investigated the influence of different polar substituents of the 1,2,3-triazole structure on the binding behaviour towards the receptor, which was easily evaluated by radioligand displacement assay. The triazole moiety was eligibly achieved by a Huisgen 1,3-dipolar cycloaddition of azides and alkynes under copper-(I)-catalysis [4].

The click chemistry approach offered an advantageous synthetic way for the incorporation of 1,2,3-triazoles in the H_3R pharmacophore. Whereas lipophilic alkynes were preferred in 1,3-dipolar cycloreactions, we successfully could transfer the synthetic procedure to polar structures, leading to high affine H_3R antagonists/inverse agonists.

Acknowledgements: This work was kindly supported by the Else Kröner-Fresenius-Stiftung, Hesse LOEWE Schwerpunkt NeFF and EU COST Action BM0806.

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PO - 041

Synthesis and Pharmacological Evaluation of Homomelatonin as Ligand for Melatonin Receptors

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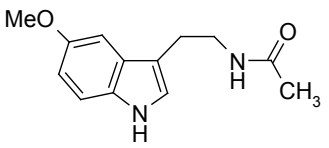
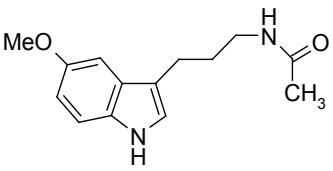
The neurohormone melatonin (MLT) exerts its diverse physiological actions mostly through activation of the two G-protein-coupled MT_1 and MT_2 receptors. The influence of numerous structure modifications of MLT on binding affinity and subtype selectivity has been extensively investigated [1]. However, the effect of the elongation of the ethylamide side chain has not been explored yet. In order to examine how the latter structure modification affects the affinity for the MT_1 and MT_2 receptors, homomelatonin (HMLT), formally obtained by insertion of one methylene group into the side chain of MLT, has been prepared and pharmacologically evaluated at human MT_1 and MT_2 receptors



MLT

HMLT



The four-steps synthesis of the novel target compound involved Vilsmeier formylation of 5-methoxyindole, Horner-Wadsworth-Emmons olefination, $NaBH_4/NiCl_2$ reduction of the resulting unsaturated nitrile, and final acetylation of the propylamine group to the corresponding acetamide.
In contrast to MLT, which is a high-affinity ligand at both receptor subtypes, HMLT displayed considerably lower affinities for both MT_1 and MT_2 receptors showing a slight preference towards the MT_2 subtype.

Acknowledgements: Institute of Pharmacy and Food Chemistry, Würzburg University, U Holzgrabe, Ch. Markl

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Natural Products

PO - 042

A novel fluoescence sensor-based microplate assay for determination of heparanase activity

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Since tumors and other diseases are characterized by increased heparanase (HEP) levels, HEP is considered to be both a diagnostic marker and a promising target for antitumor therapy. Therefore, methods are needed to determine increased HEP concentrations and to examine potential inhibitors. Based on previous findings that (1) HEP degrades not only heparan sulfate, but also the also the synthetic highly sulfated pentasaccharide fondaparinux (Arixtra®, FPX) [1] and that (2) sulfated glycans can be quantified by its effect on the fluorescence intensity (FI) of the sensor molecule Polymer-H [2], we aimed to develop a HEP activity assay using FXP as substrate and detecting its degradation by fluorimetry.
Initial experiments proved that FXP concentration-dependently increases the FI of Polymer-H, whereas the di- and trisaccharide resulting from degradation of FXP by HEP have no effect on the FI. Thus, the prerequisite for the assay concept was given. Evaluation and optimization of various assay parameters led to the following two-step procedure: (1) Incubation of a HEP containing solution with FPX. (2) Dilution of the samples and detection of the remaining FPX by adding Polymer-H (7.5 µg/mL) and measuring the FI (λem 330nm, λex 510nm). The degradation of FXP showed to increase with both the HEP conc. and the incubation time. By varying incubation time and FPX conc., the LOD can be considerably decreased. Testing of several known HEP inhibitors and sulfated glycans revealed concentration- and structure-dependent inhibition of HEP, so that the assay is suitable for inhibitor screening.
In conclusion, a rapid, simple and robust microplate HEP activity assay was developed. A major advantage is the use of FPX as substrate. In contrast to heparan sulfate, it is chemically defined, well available and has not to be labeled with radioactive or other markers for detection. Another advantage is the detection of FPX by fluorescence sensor technology, which is much more convenient than its quantification by its anti-Factor Xa activity [2] or HPLC-MS.

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PO - 043

Pro-secretory action of individual extracts of STW 5 in human intestine

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Background and Aims: The herbal medicine STW 5 (Iberogast®) consisting of nine hydroethanolic extracts (bitter candy tuft, chamomile flower, peppermint leaves, caraway fruit, liquorice root, lemon balm leaves, angelica root,

greater celandine herbs, and milk thistle fruit) is successfully used to treat functional gut diseases such as functional dyspepsia or irritable bowel syndrome (IBS) [1]. We recently reported that STW 5 increased chloride secretion in the human intestine and proposed that this pro-secretory action may be involved in its clinical efficacy. However, it is not known which of the individual components are responsible for this secretory action. It was therefore aim of this study to investigate the secretory effects of all nine individual components on human gut epithelium.
Methods: We used the Ussing chamber voltage clamp method to measure the short circuit current (Isc) which reflects mucosal ion secretion. Studies were performed in vitro on 368 human small or large intestinal samples from 46 patients and the human epithelial cell line T84 (84 wells). For measurements the surgical resections were dissected to obtain mucosa/submucosa preparations.
Results: We first prepared a combination of the individual extracts (sSTW 5) at a concentration which corresponded to their concentrations in 512µg/ml STW 5, a concentration that evoked reliable prosecretory actions [2]. We confirmed that this mixture had similar pro-secretory actions (sSTW 5: 19.7±5.2 µA/cm^2, STW 5: 21±7.2 µA/cm^2). However, none of the individual extracts given at the concentrations present in 512µg/ml STW 5 had any pro-secretory actions. At ten fold higher concentration, which is still below the clinically used dose, three of the nine extracts significantly increased epithelial ion secretion. Application of the extracts of peppermint leaves (37.2 µg/ml), angelica root (89.5 µg/ml) and lemon balm leaf (57.9 µg/ml) induced an increase in Isc in human small or large intestine and in T84 cells (peppermint leaves: 18.9±9.6 or 8.2±2.7 and 4.9±1.9 µA/cm^2; angelica root: 21.2±5.69 or 12.1±5 and 5.5±1.24 µA/cm^2; lemon balm leaf: 21.7±8.8 or 9.7±4.2 and 4.35±1.6 µA/cm^2). The extract of chamomile flower (114.3 µg/ml) showed only a weak, non-significant effect in human small or large intestine (5.6±3.5 or -1.9±6.8, µA/cm^2) but had a strong effect in T84 cells (26.9±5.7 µA/cm^2). The pro-secretory effect of peppermint leaves, angelica root and lemon balm leaves extracts was additive, because a combination of these three extracts increased the Isc to 33.6±5.5 µA/cm^2 in human tissue and to 15.9±4.6 µA/cm² in T84 cells. Addition of chamomile flower extract further increased the Isc in T84 cells to 47.5±3.4 µA/cm².
Summary and Conclusion: The results show that a combination of hydroethanolic extracts from STW 5 exerted pro-secretory actions similar in magnitude to the mother compound. In addition, we demonstrated that the pro-secretory action of STW 5 is due to the additive effects of the four components peppermint leaves, angelica root, chamomile flower and lemon balm leaf. Their effects seem to depend on direct activation of epithelial cells.

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PO - 044

Improvement of enzyme activity of recombinant *Dl*P5βR by a rational, bioactivity-guided approach

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One of the most pressing tasks of biotechnological research is the modulation and adaptation of the properties of recombinant enzymes in order to generate new or better enzymes for biosynthetic or industrial applications (synthetic biology, white chemistry). We here report the rational enzyme design of a recombinant progesterone-5β-reductase (P5βR) from *D. lanata* (r*Dl*P5βR), an enzyme supposed to be involved in the biosynthesis of the 5β-cardenolides used to treat cardiac insufficiency. The enzyme catalyses the stereo-specific 1,4-reduction of progesterone and other enones [1,2]. Orthologous genes/enzymes have also been found in cardenolide-free plant species including *A. thaliana* [3,4]. The *A thaliana* P5βR (r*At*P5βR) is about 10-50 times more efficient in the reduction of progesterone, than the r*Dl*P5βR. The about 10 P5βRs cloned and overexpressed so far can be divided into two clusters according to their activity levels. Considering sequence and activity data, we were able to define a set of amino acids crucial for variations in enzyme activity. Our predictions were tested by site-directed

mutagenesis (SDM) of tyrosine-156, asparagine-205 and serine-248 in r*Dl*P5βR. These residues were individually replaced with lipophilic amino acids found in the r*At*StβR in the corresponding sites. The efficiency in reducing progesterone to 5β-pregnane-3,20-dione could be improved by a factor of 2 to 3 (in the Y156V mutant) and 6 in the double mutants N205A and N205M.

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PO - 045

The synergistic toxicity of saponins and saponin-rich plant-extracts with type-I-RIPs / lectins

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The synergistic cytotoxicity between saponins and lectins, especially the naturally very low cytotoxic activity showing type-I-RIPs (ribosome-inactivating protein type I) / lectins is known for years by now [1]. It has become a promising strategy in anti-cancer research [2]. While the pre-appliance of certain saponins can drastically amplify the cytotoxicity of the type-I-RIPs [3, 4], it may also minimize the required effective dose of these very expensive (especially when linked to human antibodies) and time-consuming to purify / to create substances [5].
In our work we searched for new saponins and saponin-rich plant-extracts capable of increasing the cytotoxicity of the naturally very low cytotoxic activity showing lectin saporin, considered as a standard type-I-RIP. The spotlight of our research was put on the plant-family of Caryophyllaceae, but saponins and saponin-rich plant-extracts from other plant-families were also tested when fulfilling certain structural conditions. All tests were performed in a cell culture model using ECV-304 cells. The cytotoxicity was measured by MTT assay and DNA quantification.

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PO - 046

Novel derivatives of boswellic acids as inhibitors of cyclooxygenase-1 and platelet-type 12-lipoxygenase

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Extracts of the gum resins of *Boswellia* species are traditionally applied in folk medicine to treat various chronic inflammatory diseases such as rheumatoid arthritis and asthma. Boswellic acids (BAs) are assumed as the anti-inflammatory principles [1]. Previously, we showed that BAs interfere with platelet-type 12-lipoxygenase (p12-LO) [2] and cyclooxygenase-1 (COX-1) in human platelets, where 3-*O*-acetyl-11-keto-β-BA (AKBA) inhibited COX-1

with an IC_{50} of 6 µM [3]. We used the triterpenic acid structure of BAs as template for novel inhibitors of COX-1 and 12-LO. We find that related triterpenic acids from *Boswellia* species inhibit 12-LO and COX as well (IC_{50} values in the range of 0.9 to 5.9 µM), and that structural modifications in C(3) position, yielding compounds with an additional carboxy moiety, leads to potent inhibitors of COX-1 and p12-LO in platelets. In particular, 3-*O*-glutaryl-β-boswellic acid (GluBA) is a highly efficient inhibitor of COX-1 and p12-LO with IC_{50} values of 0.9 µM and 1.3 µM, respectively). GluBA also suppresses the activity of isolated COX-1, whereas the activity of COX-2 was neither influenced in cell-based nor in cell-free assays. In summary, we present triterpenic acids and novel semisynthetic BAs that are potent inhibitors of key enzymes within the arachidonic acid cascade.

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PO - 047

Wound healing effect of birch bark extract and underlying molecular mechanisms

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Birch bark extract (TE), which consists mainly of different triterpenes such as betulin, lupeol and betulinic acid, was shown to exert promising wound healing effects in patients and in an in vitro model of wounding, the scratch assay. In this study we investigated the underlying molecular mechanisms of this wound healing activity.
During the inflammatory phase of wound healing, a variety of proinflammatory molecules like cytokines and prostaglandins play crucial roles in cell migration, proliferation and angiogenesis. Hence, it was shown that deficiency of these mediators remarkably impaired wound healing [1,2,3]. We were able to demonstrate that TE, as well as the single compound betulin, increased the amount of cyclooxygenase-2 mRNA and prostaglandin E_2 in primary human keratinocytes. We could provide evidence that TE and betulin considerably extend the mRNA half life of COX-2 mRNA, a process in which the mitogen-activated protein kinase p38 is essentially involved.
Moreover, TE and betulin increased the amount of IL-6 at the mRNA and protein level. This could be the reason for an observed activation of the transcription factor STAT3, which is involved in cell proliferation [4]. Additionally, IL-8, which is a strong chemoattractant, was shown to be increased by TE and betulin treatment at the mRNA and protein level.
Further studies are in progress to elucidate the complex wound healing mechanism of the birch bark extract.

Acknowledgement: Financial support from the Federal Ministry of Economics and Technology is gratefully acknowledged.
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PO - 048

The influence of mannitol solutions on tissue factor activated whole blood coagulation

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The purpose of this study is to determine the influence of different concentrated mannitol solutions on whole blood coagulation activated by adding tissue factor after recalcification. The effect of mannitol on blood coagulation has been reported before for a specific concentration of 15% (m/V) mannitol in water to compare the effect of hypertonic mannitol solution with hypertonic saline on whole blood coagulation since these are used to reduce intracranial pressure in traumatic brain injury and craniotomy [1]. The mannitol solutions investigated in this study cover a range from 2 %(m/V) up to 20 % (m/V) to generate a scientific basis for further analyses.
The coagulation profile was observed using a ROTEM® device. The ROTEM® system is based on Thromboelastometry (TEM). In addition to the clotting time (CT), which correlates with the protrombin time (PT) in a standard coagulation assay and the Clot Formation Time (CFT) , another ROTEM parameter which represents the time until a stable clot is formed after the CT, the ROTEM® generates different data over a long period of time. Therefore it is possible to observe the whole procedure of blood clotting including clot formation, clot stabilization and finally the destabilization of the clot by fibrinolysis by using a ROTEM®.
The CT was only slightly influenced by the different mannitol concentrations [Figure 1] and no trend was observed up to 20 % (m/V). Therefore the time until clotting starts is not influenced by the mannitol solutions.
The CFT increased constantly with the increasing mannitol concentration of the added solution [Figure 1]. The CFT of the references was about 109.67s ± 27.26s. The CFT of the blood after addition of 50µL 20% (m/V) mannitol solution was 212.43s ± 50.67s. So the 20%(m/V) mannitol solution lead to a mean increase in CFT of 93.70% in comparison to the references. The α – angle was also decreased with increasing mannitol concentrations [Figure 2].
The ability of blood to initiate clotting is therefore not influenced by mannitol contents up to 2.56% (m/V) (50 µL of 20% (m/V) solution) in the blood.
The activation of the clotting cascade via TF remains independent of the mannitol content in the blood. The stabilization and its speed, represented by the α – angle, of the formed clot is altered depending on the content of mannitol in the investigated sample. Mannitol seems to hinder the formation of a stable clot when the concentration is higher than 1,026 %(m/V)(50µL of 8% (m/V) solution) in whole blood. Since platelets are a crucial part of the coagulation the most probable explanation is that mannitol destabilizes platelets due to the high osmotic pressure formed on membranes. This theory has to be verified by further investigations.

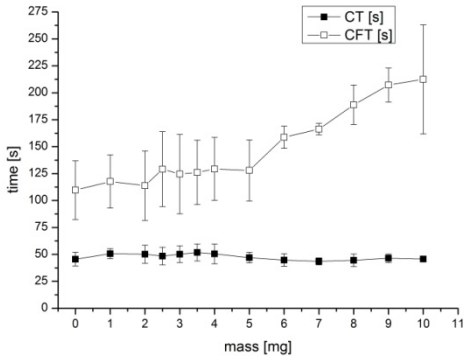

Figure 1: Comparison between CFT and CT depending on mannitol mass per 50 µL

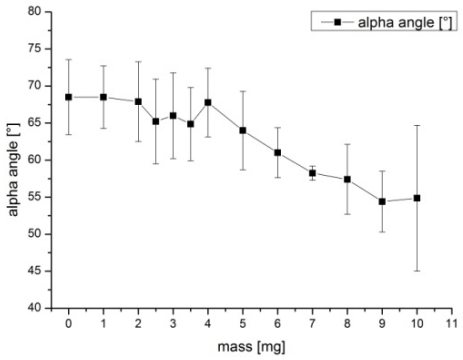

Figure 2: Decreasing alpha - angle depending on mannitol mass per 50µL

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PO - 049

Research of plant latices of the genus *Euphorbia* in terms of fibrinolytic activity

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Myocardial infarction and ischemic stroke are two examples for life-threatening diseases, because in consequence of a blood clot in the vessel, the surrounding tissue is undersupplied of oxygen. A fast fibrinolytic therapy should redissolve the blood clot and reduce the degradation of the tissue. T-PA and u-PA are the natural occurring fibrinolytic agents, which are also used in therapy. Modified variants of the human fibrinolytic agents and similar substances isolated from animals and microorganisms are partly available or under investigation. Our interests are focussed on fibrinolytic enzymes, which are located in plants. All proteases which are part of the human blood coagulation and fibrinolytic system belong to the serine protease family. Proteases found in plant latices belong mainly to the cysteine and serine protease family [1]. We have analysed if there are latices of the genus *Euphorbia* with fibrinolytic activity.

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PO - 050

Comparison of vancomycin and structurally related glycopeptides with respect to their simulated interaction with bacterial membranes

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The antibiotic vancomycin has been approved as last-line antibiotic in 1958 for the treatment of infections caused by Gram-positive bacteria. Vancomycin inhibits the synthesis of bacterial cell wall by binding to the cell wall precursor lipid II. However, increasing resistance to vancomycin has been developed in many different strains (e.g. VRE, VISA, VRSA, VR *C. difficile*) thus often making this peptide ineffective in the antibiotic therapy. To overcome this resistance, telavancin as a semisynthetic derivative of vancomycin has been developed and was approved by FDA in 2009. This peptide additionally displays a hydrophobic side chain which allows for non-targeted peptide-membrane interactions. The combined activity of targeting lipid II and the non-targeted membrane interaction makes telavancin as effective as vancomycin but possesses a more rapid bactericidal activity. It raises the question whether further minor structural changes of vancomycin are suitable to improve its activity and membrane affinity.
In the present study the interaction of glycopeptides with bacterial membranes as target is simulated by biosensor tools (QCM, SAW, and CV) and ITC. Vancomycin was used as reference for the closely related derivatives telavancin and balhimycin (Fig. 1). All peptides were analyzed regarding to their binding kinetics to the cell wall precursor lipid II and compared to their affinity for target free model membranes.

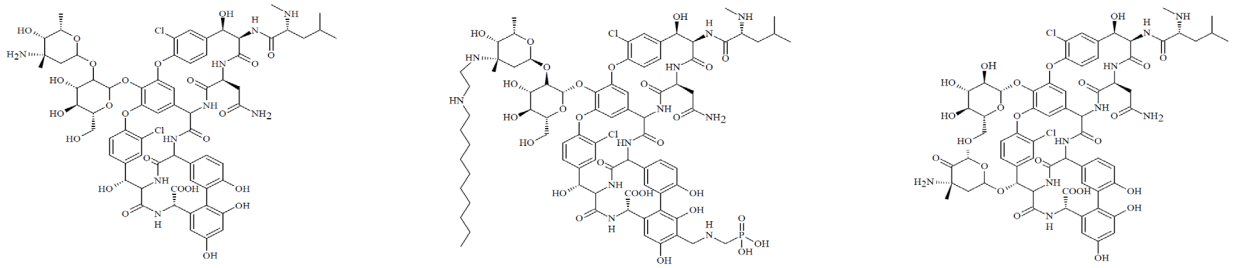

Fig. 1: *Structures of vancomycin (left), telavancin (middle) & balhimycin (right). Compared to vancomycin, telavancin contains a hydrophobic decylaminoethyl side chain and a hydrophilic (phosphonomethyl)aminoethyl group. Balhimycin structure contains no vancosamine, but dehydrovancosamine.*

Besides the binding of lipid II as a common feature for all three peptides, telavancin showed the strongest interaction with target-free membranes which is due to its fatty acid residue (Fig. 2).

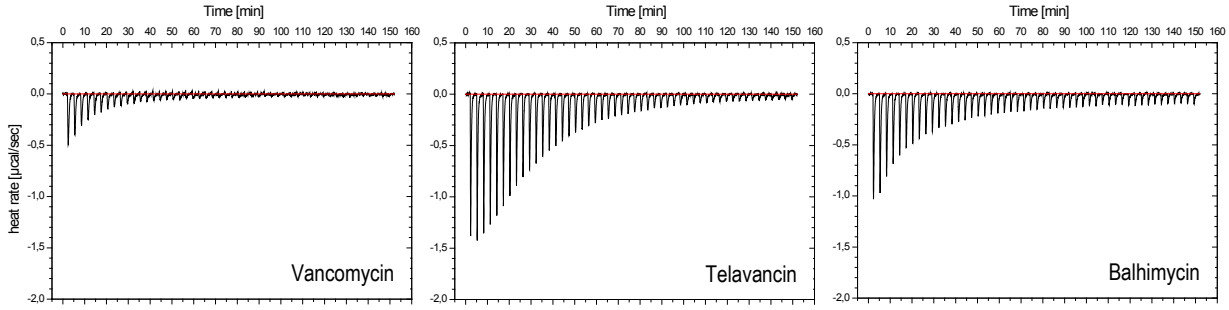

Fig. 2: *Membrane insertion of vancomycin, telavancin and balhimycin, detected by ITC.*

Surprisingly, a comparable membrane insertion was observed for balhimycin which might be related to its dehydrovancosamine sugar as partially hydrophobic moiety. To verify this hypothesis, a number of balhimycin derivatives were analyzed. The structural modifications were correlated to the amount of membrane insertion as well as to the ability of binding the cell wall precursor lipid II.
The present results give an example on the impact of minor structural changes of natural occurring antimicrobial compounds on their bactericidal activity and the contribution of different modes of action.

PO - 051

Antileishmanial activity in fractions of chloroformic extract from *Valeriana wallichii* roots

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Leishmaniasis is the most common parasitic disease after malaria and affects more than 12 million people worldwide. Every year some 2 million people get infected mostly in parts of the world with low socio-economic standards like India and Africa [1]. Growing resistance against widely used antileishmanial agents and the significant toxicity of the drugs in use make it important to look for new antileishmanial substances. In traditional Indian folk medicine *Valeriana wallichii* is well known and used e.g. in the treatment of insomnia, epilepsy or anxiety [2]. Recently Ghosh et al. [3] reported the discovery of activity against *Leishmania major* promastigotes in a fraction of a chloroformic extract of *V. wallichii* roots. For further investigation chloroformic root extracts of *V. wallichii* were obtained from Banasri Hazra (Kolkata, India) and fractionated by HPLC using a ZORBAX Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm) with a gradient of Methanol/H_2O as mobile phase. First testings of the

obtained 12 fractions against *L. major* promastigotes using an Alamar Blue assay resulted indeed in promising IC_{50}-values in the range of 2 – 10 µg/ml in certain fractions. Protease LmCPB2.8 inhibition tests with some fractions still containing a mixture of compounds prepared by column chromatography on silica gel with $CHCl_3$/MeOH (4.8:0.2) showed an up to 90 % inhibition in one fraction (IC_{50}: ~3.7 µg/ml). The isolation and identification of the compound(s) responsible for this activity is still ongoing.

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PO - 052

A nuclear receptor as drugable target for natural products: *In silico*-guided discovery of FXR-agonistic triterpenes from *Ganoderma lucidum*

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Farnesoid X receptor (FXR) is a ligand-dependent transcription activator, which is responsible for bile acid homeostasis [1]. Available structural information of the ligand-binding domain and its relevance in the regulation of glucose and lipid metabolism render this nuclear receptor attractive for computer-assisted drug discovery approaches. By virtual screening experiments of in-house databases applying structure-based pharmacophore models [2], lanostane-type triterpenes were identified as putative FXR-ligands [3]. A rich source of this compound class was found in the fruit body of the famous TCM fungus *Ganoderma lucidum* Karst..

Ganoderma extracts and 25 isolated compounds were experimentally investigated in a reporter gene assay. Five lanostane triterpenes, i.e. ergosterol peroxide, lucidumol A, ganoderic acid TR, ganodermanontriol, and ganoderiol F, dose-dependently induce FXR with EC_{50} values between 1 and 20 µM [3]. Down-regulation of *CYP7A1* corroborates FXR-agonism of these five constituents. Interestingly, a significant inhibition of TNF or LPS induced expression of IL-8 and E-selectin by the *Ganoderma* lanostanes indicates an involvement of FXR in the mechanism of inflammatory regulation [3]. In addition, first structure activity relationships could be derived from molecular docking studies [3].

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PO - 053

Isolation, structure elucidation and bioactivity of novel cyclic depsipeptides from *Xenorhabdus bedingii*

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Many pharmacophores have their origin in natural products. Therefore it is likely that new mechanisms of action through natural products may be found and *Xenorhabdus* has been shown to be a rich source for such compounds [1]. *Xenorhabus* is entomopathogenic and has a complex life cycle due to its symbiosis with nematodes[2]. After *Xenorhabdus* kills the insect it has to defend the insect cadaver against other food competitors like soil living bacteria or fungi. This and the richness of the genome with polyketide synthase (PKS), non-ribosomal peptide synthethase (NRPS) and PKS-NRPS hybrid gene clusters for secondary metabolite biosynthesis makes bacteria of the genus *Xenorhabdus* a good candidate for the isolation of natural products which could turn out to be new anti-infectives[1].
After the cultivation of *X. bedingii,* it was possible to isolate three lipophilic cyclic depsitripeptide derivatives. The isolation is performed by extraction with ethylacetate and preparative reversed phase high performance liquid chromatography (RP-HPLC). The sum formula was determined from feeding experiments in ^{13}C and ^{15}N enriched media and high resolution mass spectrometry data (HRMS).The structure was elucidated by one and two dimensional nuclear magnetic resonance (NMR) experiments (COSY, TOCSY, HSQC, HMBC) and iontrap mass spectrometry fragmentation. The absolute configuration of the building blocks was determined using the advanced Marfey's method. Additional derivates of this novel class of compounds could be detected in minute amounts whose structure was elucidated by detailed labeling experiments followed by MS experiments.

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PO - 054

Putative mycobacterial efflux pump inhibitors isolated from *Alpinia katsumadai*

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Recently the emergence of antibiotic resistance has received specific attention in antimicrobial therapy including infectious diseases like tuberculosis.[1,2] In mycobacteria the hydrophobic cell wall and multidrug efflux pumps are regarded to be the two main mechanisms that are involved in drug resistance.[3] For this reason the identification of new efflux pump inhibitors (EPIs) derived from natural sources represents an appropriate target to reduce the development of antibiotic resistance.[4,5] Four compounds isolated from the hexane extract of *Alpinia katsumadai*, *Zingiberaceae*, were assessed for their antimycobacterial activity and synergistic activities with different antibiotics against *M. smegmatis* mc^2 155. Additionally an ethidium bromide (EtBr) accumulation and efflux assay were used to evaluate their potential as efflux pump inhibitors. Considering the fact that these compounds revealed weak antimycobacterial activities (MIC ≥ 64 mg/L), they could modulate the MIC of EtBr and rifampicin at least by a factor of four. Especially compound 1, a diarylheptanoid, achieved considerable results as a modulator (MF 4 against Etbr and 8 against rifampicin) as well as a decrease of the Etbr efflux in *M. smegmatis* mc^2 155 comparable to known EPIs.

Acknowledgements: The 'Office of Research Management and Service' of the Karl-Franzens-University of Graz is thanked for a studentship of B. Gröblacher.
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PO - 055

The antimycobacterial activity of *Euodia rutaecarpa* fruits

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The increasing occurrence of extensively drug resistant tuberculosis (XDR/CDR-TB) demands the development of new drugs with new mechanisms of action. Former studies revealed the antimycobacterial properties of 1-methyl-2-alkyl-4(1*H*)-quinolones isolated from the fruits of *Euodia rutaecarpa* Hook f. & Thomson.(Rutaceae) with MIC values ranging from 2 – 8 mg/L against the fast growing mycobacterial strain *M. smegmatis* ATTC 14468 using a microbroth dilution assay. [1]
The present study should show if, aside from the fraction of quinolone alkaloids, other compounds contribute to the the significant antimycobacterial activity of the crude extract. HPLC-PDA and LC-MS analysis showed that the main constituents of the ethanolic extract of *Evodiae fructus* are the two indolequinazoline alkaloids evodiamine and rutaecarpine. The ethanolic extract was fractionated by SPE and evodiamine and rutaecarpine, as the main compounds, were isolated using semi-preparative HPLC. Tested against *M. smegmatis* the pure compounds exposed only weak antimycobacterial activity (MIC values = <128 mg/l). A comparison of different fractions indicated that the activity against fast growing mycobacteria is only due to the content of 1-methyl-2-alkyl-4(1*H*)-quinolones, especially to evocarpine. *Euodia rutaecarpa* with the major bioactive compounds evodiamine, dihydroevodiamine and rutaecarpine is also known to posses for example anti-inflammatory activity by inhibiting NO production, COX-2 induction and NF-kappa B activation as well as antioxidative or antihypertensive properties [2].

Acknowledgements: This work is financially supported by the Austrian Science Fund (FWF), project no. P21152-B18.
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PO - 056

Chemical composition, olfactory analysis and antibacterial activity of *Thymus vulgaris* L. chemotypes geraniol, thujanol-4 / terpinen-4-ol, thymol and linalool cultivated in Southern France

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In the present study, the essential oils of four chemotypes [1] of *Thymus vulgaris* L. (Lamiaceae) were analyzed concerning composition and antibacterial activity to point out their different properties. GC-MS and GC-FID analyses revealed that the essentials oils could be attributed to the chemotypes thymol (41.0 % thymol), geraniol (26.4 % geraniol), linalool (72.5 % linalool) and thujanol-4/terpinen-4-ol (42.2 % cis- and 7.3 % trans-sabinene hydrate); an olfactory examination confirmed the explicit differences between the chemotypes. Furthermore, antibacterial activity was investigated against several strains of two Gram-positive (*Brochothrix thermosphacta* and *Staphylococcus aureus*) and four Gram-negative food-borne bacteria (*Escherichia coli*, *Salmonella abony*, *Pseudomonas aeruginosa* and *Pseudomonas fragi*). All essential oil samples demonstrated to be highly effective against Gram-positive strains, whereas the impact on Gram-negative microorganisms was significantly smaller but yet considerable. The results obtained indicate that – despite their different properties – the essential oils of selected T. vulgaris chemotypes are potent antimicrobials to be employed as useful additives in food products [2] as well as for phytotherapeutic applications

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PO - 057

Influence of sesquiterpene lactones on gene expression in HaCaT-keratinocytes determined by time dependent microarray analyses – Implications on their potential as contact allergens

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In allergic contact dermatitis (ACD), keratinocytes (KCs) are the first and largest population of cells to get in contact with the allergenic agent. They form a matrix for cells usually considered key players in this type of allergic reaction such as Langerhans cells, dendritic cells and T-lymphocytes. It has also been reported that KCs contribute directly to the inflammatory response of the skin by secreting proinflammatory cytokines in response to hapten exposure [1]. Thus, we have previously shown that Interleukin (IL)-6 levels in cell culture supernatants of HaCaT KCs are increased after exposure to sensitizing agents [2].
Sesquiterpene lactones (STLs) as constituents of plants, especially of Asteraceae, are well known to produce allergic contact dermatitis [3] by forming covalent adducts with proteins of the skin, ultimately functioning as antigens. However, the majority of biological effects described for STLs is rather of anti-inflammatory quality. Much less is known about their potential to produce the pro-inflammatory signals essentially required, besides the mere presence of an antigen, to elicit an allergic immune reaction, e.g. by activating antigen presenting cells.
To obtain insights into the effects that STLs elicit at the transcriptional level in KCs, mRNA microarray analyses with focus on immunological processes were performed. To this end, KCs (HaCaT cell line) were incubated with helenalin (a constituent e.g. of *Arnica* flowers and reportedly a strong sensitizer [3]) at a subtoxic concentration of 3 µM for 4, 8 and 24 hours. PIQOR microarray analyses [4] were performed and compared with the mRNA expression pattern of untreated control cells.
The expression of 105 of the total 1070 investigated genes was significantly up- or downregulated by helenalin. The regulated genes spread over a variety of cellular functions ranging from transcription factors via genes related to coagulation to constituents of the cytoskeleton. Thus, e.g. the gene for transcription factor c-Fos was most strongly down regulated at every measured time point. C-Fos is thought to be involved in cell differentiation, proliferation and signal transduction [5]. Quite interestingly, after 24h of incubation, the mRNA levels for the pro-inflammatory cytokine Interleukin 8 (IL-8) and and for Prostaglandin G/H synthase 2 (PGH2, or COX2) were significantly elevated. For IL-8 this result could already be confirmed on protein levels by ELISA measurements.
For a more comprehensive overview, a gene annotation cluster analysis using the webtool DAVID 6.7 [6] was performed. The five most significantly enriched clusters contained genes related to (1) regulation of transcription,

(2) protein modification including ubiquitination, (3) biopolymer methylation, (4) stress response and (5) response to exogenous stimuli. Overall, a considerable predominance of mechanisms associated with the inhibition of proliferation, induction of apoptosis and pro-inflammatory signalling was observed in these clusters.
Helenalin, despite its proven anti-inflammatory activity, is also known to be cytotoxic [7] and pro-apoptotic [8]. Our present data for keratinocytes show that it induces cellular stress affecting multiple targets which may ultimately cause cellular damage accompanied by inflammatory signals contributing to the onset of contact allergy.
In addition to the data for helenalin, those obtained with another STL, parthenolide, and with the synthetic chemical dinitrochlorobenzene DNCB, known as a strong sensitizer, will be presented. Commonalities and differences between the three compounds will be discussed.
These results will help to understand the complex processes involved in the genesis of ACD after skin contact to plants of the Asteraceae family.

Acknowledgements: Financial support from brial allergen GmbH, Greven, is gratefully acknowledged.
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PO - 058

Investigations on the surface activity of saponins

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The amphiphilic character and the property of saponins to lower surface tension as well as their capability to interact with biological membranes is well known. We investigated the surface activity of several saponins as well as their capability to enhance the cytotoxity of the naturally only very low toxic activity showing toxic type-I-RIP (ribosome-inactivating protein type I) lectin saporin using cell culture experiments in order to analyse a potential correlation.
Several triterpen and steroidal saponins as well as some aglycons that differ in their aglygone strukture, side chain and acidic properties have been measured by using the Wilhelmy plate method with the K 10 tensiometer by Krüss. For these mesurements mainly pure compounds and some isolation products were used. The mesurements were made in different 0,01 M saline buffered aqueous solutions over a pH range from 5 to 9 in order to investigate the pH dependent surface activitiy. Were solubility of the saponins made it possible the critical micelle concentration (CMC) has been determined and compared.
In this study we try to get informations to which extent physico-chemical properties of the surface active compounds influence the magnitude of the enhancement of cytotoxic synergism. This might also lead to new ideas concering the not yet completly understood mechanism of this enhancement.

PO - 059

Contribution of components of STW 5 to its mode of action on intestinal inflammation

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STW 5 (Iberogast®), an established gastrointestinal phytotherapeutic medication, consists of nine individual ethanolic plant extracts. It is successfully used in the therapy of irritable bowel syndrome (IBS). Studies prove that STW 5 acts on disturbed motility and inflammatory processes which are postulated in IBS. The contribution of each component to the effect of STW 5 still remains unclear. Therefore, we examined the effects of STW 5 and its components on acetylcholine-induced contraction, TNF-α release and cell death using the lactate dehydrogenase (LDH) test.
ACh (10 µM)-induced contractions were measured isometrically in rat colon preparations after incubation with 2,4,6-trinitrobenzene sulfonic acid (TNBS, 0.01 M) for 30 minutes. TNF-α release was examined in LPS (100 ng/ml)-stimulated human monocytes using a commercially available ELISA kit. The LDH cytotoxicity assay was performed in supernatants of differentiated THP-1 cells after incubation with TNBS (100 µM). Methotrexate (MTX, 10 nM) was used as reference compound.
STW 5 (62.7-500.5 µg/ml) reduced ACh-induced contractions in a concentration-dependent manner. Peppermint leaf (*Mentha piperita* L.) and chamomile flower (*Matricaria recutita* L.) induced a less pronounced reduction, whereas the other components revealed no effect on ACh-induced contractions. STW 5 (500.5 µg/ml) inhibited TNF-α release by 87 %. Bitter candytuft (*Iberis amara* L.), peppermint leaf (*Mentha piperita* L.), chamomile flower (*Matricaria recutita* L.), liquorice root (*Glycyrrhiza glabra* L.) and angelica root (*Angelica archangelica* L.) in concentrations equivalent to those in STW 5 reduced TNF-α release though less pronounced as compared to STW 5. Caraway fruit (*Carum carvi* L.), milk thistle fruit (*Silybum marianum* L.), lemon balm leaf (*Melissa officinalis*) and greater celandine herb (*Chelidonium majus* L.) were without effects. STW 5 (500.5 µg/ml) inhibited TNBS-induced cell death significantly by 43.8 %. All components were equipotent to STW 5 to reduce TNBS-induced LDH activity. Our results indicate that the herbal components contribute differently to the effects of STW 5. The effects of STW 5 result from the combination of these nine extracts.

PO - 060

Physicochemical properties and stability of different dosage forms of resveratrol

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Resveratrol (trans-3,5,4'-trihydroxystilbene) is a polyphenolic secondary natural substance with a relative molecular weight of 228.25 g/mol. Resveratrol was first isolated from leafs of the white lily (*Lilium candidum*). Resveratrol (res) can be used as anti-inflammatory, anti-aging, anti-heart disease, and anti-cancer agent. However, resveratrol has low water solubility (30 mg/l) which causes in low bioavailability in human body [1-3]. It is well known that the solubility of a drug can be increased by a complexation with cyclodextrins [4-5] or by a solid dispersion with surfactants [2]. The suitable type of cyclodextrin (cyd) or surfactant should be however selected. Therefore in our project we have prepared different cyd-complexes and solid dispersions with res. The stability of res is important for both the preparation of a dosage form and for characterization; therefore we have systematically studied the stability of res-solution in order to better understand the protection of degradation under the storage and production.
In our project, the complexes of different cyclodextrins (alpha, beta, grammar, 2-hydroxypropyl-beta (2-HP-beta), dimethyl-beta (DM-beta) and resveratrol were prepared at molar ratios of 1:1, 1:3, and 1:5 by mixing with small amount of ethanol and afterward dried in a vacuum hot air oven at room temperature (RT, 25°C). The solid dispersions of different surfactants (Imwitor 742, Imwitor 928, PEG 6000, Span 40, Span 60, Solutol HS15) and res were prepared at 5%w/w of res by melting at 60°C and afterward cooling to RT. The solubility of the mixtures was determined by UV-VIS spectroscopy at the wavelength of 305 nm. The complexation formation was confirmed by DSC. The stability of resveratrol solutions was monitored by storage the solution in different temperatures (RT, 80°C) and containers (clear or amber glass) and the concentration of trans-resveratrol was determined by the UV-spectroscopy [6]. Comparison between the cyd-complexes at different ratios, the complex of res with 2-HP-beta-cyd (1:3) showed the highest water solubility. Surprisingly grammar-cyd decreased the

water solubility of res to lower than 30 mg/l. On the other hand, comparing between solid dispersions studied, we have found that the solid dispersion from res and Solutol HS 15 showed the highest water solubility. The concentration of res in clear glass has a reduction of about 11% after one day storage at RT. On the other hand, the concentration in amber glass kept almost constant during this period. The stress test at 80°C for 6h did not reduce the concentration of trans-res, but the concentration of res was significantly reduced after the storage under UV-light for only 3h. Our study showed that the solubility of res in water can be significantly increased after preparation of cyd-complexes or solid dispersions. The preparation and characterization of res-dosage forms should be carefully performed under protection from UV-light. Heat up to 80°C for 6h did not affect the stability of res-solution. The solid and liquid dosage forms can be further prepared from the cyd-complex or solid dispersion but they should be better kept in amber container to avoid degradation. The results will be a basis for better formulation of resveratrol with higher bioavailability.

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PO - 061

Mid-infrared spectroscopy determination of the isoflavone content in nutritional supplements of red clover

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Botanical preparations of red clover (*Trifolium pratense* L.) have gained interest as an alternative treatment for menopausal problems such as hot flushes. This shows the need for simple and rapid analysis methods. In the present study, FTIR-ATR spectroscopy has been applied for the characterization and identification of the active compounds of red clover belonging to the class of isoflavones, e.g. formononetin and biochanin A. Information about functional groups and chemical composition could be obtained, making FTIR a powerful tool for a fast and non-destructive quality control. Moreover, using chemometrics such as principal component analysis (PCA) calibration models were employed. The results of the multivariate calibration demonstrated the potential of this method to estimate the amount of isoflavones in red clover.

PO - 062

Improved method for determining the antioxidant capacity of lipophilic actives

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The human body possesses a lot of enzymatic and non-enzymatic reduction mechanisms, e.g. the thioredoxin reductase system, glutathione peroxidase or vitamin E. Free radicals, which possess at least one unpaired electron, can be formed in different ways, e.g. air pollution, smoking, inflammation or UV light. If the human body is not able to scavenge these radicals, "oxidative stress" can be created. In consequence of oxidative stress serious diseases like atherosclerosis or even cancer can occur. Healthy nutrition and nutritional supplements can help to scavenge this radical overkill. Therefore, it is of interest to determine their antioxidant capacity (AOC) and to identify their benefit to a healthy nutrition. A variety of test systems are already established which possess different advantages and disadvantages [1]. The aim of our study was to develop a new test system for determining the AOC, which is easy to use, low cost, fast, robust and highly reproducible.
The novel TEMPO test is based on the UV-reduction of the stable free nitroxide TEMPO by antioxidants. The UV-active radical will be dissolved in medium chain triglycerides. After adding the antioxidative sample, the decreasing UV-absorption can be determined at 470 nm. Through the very good shielding of the radical solution is stable for at least one week, in contrast to other established radicals like DPPH solution with a loss of 25% absorbance within 2 hours. Depending on the sample concentration it is possible to receive reliable AOC data in between 2 hours or one week, enabling not only the determination of the AOC, but also the determination of the AOC reaction kinetics. The TEMPO test proved to work for the 7 investigated oils (Kukui, Sacha Inchi, hemp oil, soy oil, black seed oil, $\wedge$–3 fish oil and jojoba oil), spices (e.g. piper nigrum, capsicum anuum, carum carvi) as well as food (tomato puree, green tea) and even for aqueous nanocrystal dispersions. All results were compared to the conventional DPPH test.
The TEMPO test was predominant compared to the conventional DPPH test. It has a greater number of possible applications, is more robust, low cost, easy to use and giving reliable predictions for the AOC of all investigated samples. Moreover, it allows a reliable determination of the AOC kinetics.

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PO - 063

Influence of STW 5 and its herbal component extracts on neurotransmission in a model of ileal and colonic inflammation

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Background and aims: The question was raised, whether the herbal medicine STW 5 (Iberogast®) and its component extracts act on contraction after electrical field stimulation (EFS), on intestinal slow wave activity and on neuromuscular signalling in small and large intestine of mice in an *in vitro* model of lipopolysacharide (LPS) triggered inflammation.
Methods: In the organ bath, segments of distal ileum of BalbC mice were used for registration of spontaneous and EFS stimulated contractions. In the electrophysiological studies, segments of distal ileum or proximal colon were used for intracellular recording, after removal of mucosa and submucosa.
STW 5 and the individual herbal fluid extracts were tested in a cumulative setting, in dilutions of 1:1000, 1:500, 1:250, 1:125, 1:50, after incubation of the segments with LPS (20 µg/ml) for 90 min.
Results: LPS induced a slight, non significant enhancement of contractility. This effect was obviously not neuronally induced, as no significant effects on intracellular recordings of resting membrane potential (RMP), slow wave amplitude or excitatory or inhibitory junction potentials (EJP, sIJP, fIJP) were observed. In the ileum, STW 5 and the extracts reduced EFS induced contractility. Electrophysiological parameters were not influenced significantly in LPS pretreated or control preparations.

Summary and conclusions: Stimulation with LPS results in inflammatory changes of motility, which are significantly influenced by STW 5. The underlying mechanisms may be of relevance in the treatment of functional gastrointestinal diseases, as these are discussed to be influenced by subclinical inflammatory changes.

PO - 064

Quercetin's neuroprotective effects against oxidative stress is demonstrated by an impedance-based assay

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Oxidative stress occurs due to overproduction of reactive oxygen species (ROS) and is considered to play a key role in the pathogenic mechanisms of neurodegenerative disorders such as Alzheimer's disease (AD). In AD, amyloid beta is both, source and result of ROS formation [1].

Cytotoxicity screenings can be performed by various assays. It is often measured by colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) tests using cell death as end point. Electric cell-substrate impedance sensing (ECIS) is a label-free and time-resolved alternative to monitor cytotoxicity non-invasively. In ECIS adherent cells are grown on the surface of planar gold film electrodes which are deposited on the bottom of a regular cell culture dish. Cell culture medium serves as the bulk electrolyte providing a closed electric circuit for AC current flow. The AC impedance of the cell-covered electrode is measured as function of time. Specific alterations in the impedance signal are caused by cell shape changes due to external stimuli like toxic substances [2]. MTT assays use death and metabolic activity of cells as cytotoxicity indicator, whereas ECIS reads minute changes in cell shape to monitor any impact on the cells' viability in real-time.
To get data with physiological relevance in the field of neuroprotection the neuronal cell line HT-22 was chosen as a model system. HT-22 cells are a glutamate sensitive sub clone of HT-4 cells and are derived from murine hippocampal tissue [3].
A variety of vegetables and fruits contain the flavonoid quercetin (3,3,4',5,7 -pentahydroxyflavone). Quercetin was described as cytoprotective agent against oxidative stress in vitro [4] and in vivo [5]. In this work, the impedance-based assay was applied to evaluate the protective potentials of quercetin towards oxidative stress in neurons caused by *tert*-butyl hydroperoxide (tBOOH). The neuroprotective effect of quercetin is demonstrated to be dose dependent. In pilot studies searching for inducers of oxidative stress, we used the MTT test to demonstrate neurotoxicity for three different oxidative stress generating stimuli: tBOOH, monosodium-L-glutamate and amyloid ß, respectively. All showed toxic effects in a dose dependent manner. ECIS experiments upon tBOOH stimulus confirmed the dose dependent neurotoxicity of tBOOH by showing specific alterations in the impedance signal due to cell shape changes. ECIS and MTT data correlate on toxic tBOOH effect, but ECIS provides an earlier monitoring of the toxic effects due to time-resolved curves instead of MTT's endpoint data. ECIS is therefore considered to be the more informative assay when screening for neurotoxic and neuroprotective effects.
In summary, both neurotoxic and neuroprotective effects can be measured using the neuronal cell line HT-22 together with impedance monitoring. Evaluation of further natural and synthetic compounds for their putative neuroprotective effect will be pursued in onward investigations.

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PO - 065

Leonurus cardiaca, *L. japonicus*, *Leonotis leonurus*: Simultaneous quantitative HPLC and HPTLC determinations of fourteen flavonoids, phenylethanoids, and phenol carboxylic acids

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Leonurus cardiaca L. [1,2], *Leonurus japonicus* Houtt. [3,4], and the related *Leonotis leonurus* (L.)R.Br. [5-7] are traditionally used against cardiovascular and gynaecological ailments in Western herbalism [1,2], TCM [3], Japanese Kampo [4], and South-African Muti [5-7], respectively. Although phenolic constituents of *Leonurus* species have been demonstrated to contribute to their pharmacological activities [1], till now only one HPLC analytical study of these compounds, solely for *L. cardiaca*, has been reported from our lab [8]. In the present study, a novel RP-HPLC method was developed and used for monitoring the content of twelve phenolics – chlorogenic acid, caffeic acid, ferulic acid, cichoric acid, lavandulifolioside, verbascoside, hyperoside, isoquercitrin, rutoside, rosmarinic acid, apigenin-7-O-D-glucoside, quercitrin – in eighteen herbal and seed samples of the three species as well as in a newly developed *L. cardiaca* refined extract [1]. The theorised presence of leonoside A and B was refuted beforehand via HPTLC. Only ferulic acid was found in every sample, whereas rosmarinic acid and apigenin-7-O-D-glucoside were not detected in any sample. Chlorogenic acid, caffeic acid, cichoric acid and rutoside were found in the vast majority of samples regardless of species. Interestingly, the phenylethanoids lavandulifolioside and verbascoside were not present in any sample of *L. japonicus*, but in every sample of the aerial parts of *L. cardiaca*. Lavandulifolioside was additionally observed for the first time in the aerial parts of *L. leonurus*, for which no earlier HPLC analysis on phenolics has been reported. Hyperoside was not found in *L. cardiaca* but in both *L. japonicus* and *L. leonurus*, whereas isoquercitrin was detected in most samples of *L. cardiaca* and *L. leonurus* but not in *L. japonicus*. These results facilitate straightforward identification and quality control even of powdered drug samples via HPLC / HPTLC fingerprint determination of the individual phenolics.

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PO - 066

Inhibitory effects of lignans from *Carthamus tinctorius* on indoleamine 2,3-dioxygenase

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Carthamus tinctorius (Asteraceae) has been used in ethnopharmacology against cancer and depression [1,2]. As indoleamine 2,3-dioxygenase (IDO) catalyses tryptophan degradation, this enzyme is involved in both of these diseases and is a possible target to explain the traditional use of *C. tinctorius*. In neurological disorders,

degradation of tryptophan can reduce serotonin synthesis, which is related to major depression [3]. In cancer cells, the expression of IDO leads to a local suppression of T-cell responses and promotes immune tolerance [4]. Therefore, three lignans, trachelogenin (**1**), arctigenin (**2**), and matairesinol (**3**), isolated from cold pressed seeds of *C. tinctorius* were investigated for their inhibitory activity against IDO in peripheral blood mononuclear cells (PBMCs). Interestingly, the three isolates showed different effects on IDO, though their structures are closely related. While **1** obtained an IC_{50} value of 57.35 µM, the IC_{50} of **2** reached 26.49 µM whereas **3** showed a significant activity at a concentration of 192.5 µM.

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PO - 067

Characterisations of o/w concentrated emulsions with different poorly water-soluble drugs

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Emulsions are dispersed systems containing two phases, i.e. hydrophilic and lipophilic phases. They are thermodynamic instable, therefore they must be stabilized by additional substances which are surface active e.g. surfactant, polymer, protein. These substances are called emulsifiers. Emulsions can be prepared by different techniques e.g. simple mixing, high pressure homogenizer, ultrasonic probe, etc. The resulted emulsions can be simple emulsions, multiple emulsions, concentrated emulsions, mini-emulsions or nanoemulsions with different size ranges (nanometres to micrometres). However, we are interested in the special emulsions which are called concentrated emulsions (CEs). CE is also known as gel-like emulsion, emulsion-gel, viscous emulsion, biliquid foam, aphron, and high internal phase emulsion [1]. CEs differ from the conventional emulsions mainly because of the volume fraction. CEs can contain the dispersed phase greater than 0.74. In some case, the volume fraction of 0.99 can be reached because of the deformation of the droplets. CEs have fewer problems concerning phase separation based on coalescence. CEs were already applied in different fields e.g. food, pharmaceutical, polymer and chemical industry [1,2]. Because of these advantages we have prepared oil-in-water (o/w) CEs with different poorly water-soluble drugs (i.e. lidocaine base and resveratrol). The stabilization was performed by using amphiphilic substances and they were characterized with various techniques. The light scattering technique (LST) (Mastersizer, Model S, Malvern Instruments Ltd, UK) was used to determine the particle size distribution of CEs. The CE was diluted with 10 wt% SDS (sodium dodecyl sulfate) aqueous solution at the ratio of 1:100 prior to the measurement. The particle size distribution of CE with lidocaine base showed a monomodal distribution with more narrow size than the CEs with resveratrol. Their average diameters D [3,2] based on the surface distribution were smaller than that by the volume distribution D [v]. The morphological properties and the droplet size of the CEs were observed using a light microscope (LM). The droplets of CE without dilution were un-deformed, it means that, these particles had a dense packing after a high centrifugation (> 5400 rpm). In order to compare the results, the CEs were diluted at the same condition as the measurement by LST. The results showed that the average droplet size were between 2.4 to 4.2 µm. The results from both technique (LST and LM) are in agreement with each other. Moreover, the CEs were determined by the X-ray scattering (SAXS and WAXS). For this purpose the samples were radiated with X-ray radiation from the synchrotron source (B1, DESY-Hamburg). The SAXS scattering patterns (0-1 Å) were acquired using a large area pixel detector (PILATUS 1M, Dectris, Switzerland). The WAXS (1-4 Å) was measured simultaneously using a Mythen strip detector (Dectris, Switzerland) and the X-ray energy was set at 14 keV. The raw scattering data were background corrected, integrated and calibrated using a MATLAB-based analysis suite, which is available at the beamline. Peak

positions were determined using Origin-Program by fitting a Gaussian equation to the data. All emulsions demonstrated two broad peaks at the scattering vectors (Q) of 0.37 and 1.37 $Å^{-1}$. The repeat distances (d) were calculated by using the modified Bragg equation [3]. The results showed that the addition of lidocaine base had a high effect on Q at 0.37 but less on 1.37 $Å^{-1}$. On the other hand, resveratrol has an effect on both regions. The repeat distances shift to the lower Q which means higher repeat distances. The results showed that different lipophilic drugs seemed to affect the CEs in different way. The droplet size increased and there was a change in the SAXS-WAXS signals. The possibility of using CEs as a drug carrier was demonstrated.

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PO - 068

Colon-delivery of resveratrol coated tablets with pH-sensitive polymers

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Resveratrol is a polyphenolic secondary natural substance and can be used against different diseases e.g. inflammatory, cancer, gastrointestinal (GI) tract and heart disease. However, resveratrol tablets have a low solubility, very low oral bioavailability and short half-life. Resveratrol can be rapidly absorbed through the GI tract as well as fast metabolized in the upper part of GI and the liver. Therefore our project focuses on increasing the bioavailability by preparation of the colon delivery system (CDS). This dosage form can avoid the rapid absorption and metabolism resulting in increasing the bioavailability. The CDS can be performed in different ways e.g. i) coating with polymer such as pH-sensitive polymer; ii) covalent linkage of drug with a carrier such as cyclodextrin, azo or dextran; or iii) embedding in matrices such as biopolymer. In our project we have used all the techniques mentioned above. The coating process was performed with a fluidized bed apparatus and pH-sensitive polymer Kollicoat® MAE 30 DP for preparation of the coated tablets. Kollicoat® MAE 30 DP is the aqueous dispersion of the copolymer of methacrylic acid and ethylacrylate and can dissolve at the pH > 5.5. The core containing resveratrol and different excipients e.g. surfactant or cyclodextrins will be applied. The coating process parameter, the release profiles of resveratrol in different media as well as the morphology of the coating film will be presented. The results of the tablets only with matrices will be compared with the tablets coated with polymers.

Acknowledgements:
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PO - 069

Influence of Different Drying Air Humidity on the Residual Activity of single droplets of L-Glutamic Dehydrogenase dried in an Ultrasonic Levitator

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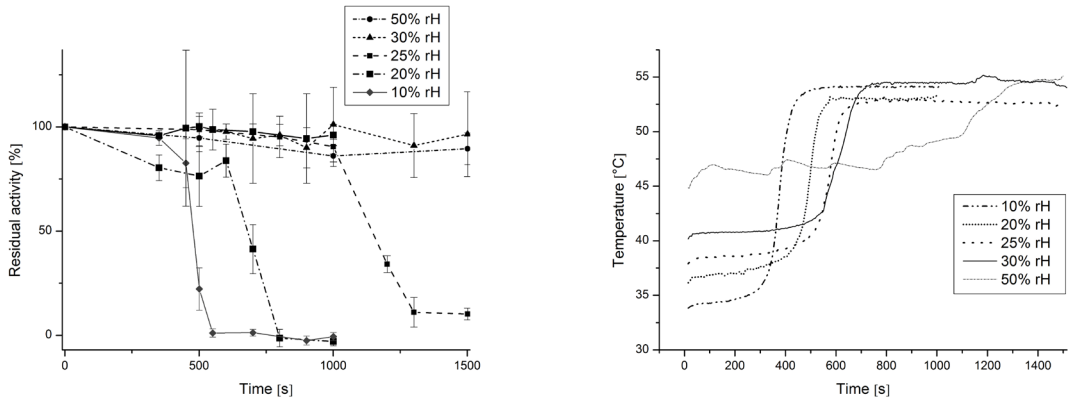
Introduction
The inactivation of proteins during spray drying caused by thermal stress is a known problem [1]. To investigate the influence of different drying conditions on protein stability, a single droplet ultrasonic levitator is a helpful tool [2]. By drying droplets under defined conditions and analysing the droplets / particles at different drying times, one can get a good insight into the time dependence of the inactivation process. The aim of our investigations was to examine the influence of the relative humidity of the drying air on the inactivation of L-Glutamic Dehydrogenase during drying in an ultrasonic levitator.

Materials and methods
Droplets of protein solution containing 0,7-1,2% L-Glutamic Dehydrogenase were dried at 60°C and a relative humidity ranging from 10-50% in a 58kHz ultrasonic levitator. The levitator is encased by an acrylic chamber, keeping a constant temperature and relative humidity. A controlled evaporation mixer provides drying air of controlled temperature and humidity directly into the chamber. The drying process is observed by an optical CCD – Camera. Droplet / particle temperature is constantly measured using an infrared camera (setting as described in [3] and [4]).
Activity measurements were carried out at different time intervals during the drying process. The samples were dissolved immediately in 100mM triethanolamine-hcl buffer pH 7.4 and the residual activity was assessed by an UV-spectrometer using α-ketoglutarate as substrate.

Results and discussion
After the critical point (CP) a decrease in enzyme activity can be observed. The time to CP increases with mounting relative humidity, also the time between CP and 50% activity loss increases from 110sec (10%rH) to 560s (25%rH). No activity loss was measured for 30%rH and above over the total drying time of 1500sec.
Temperature profiles of the droplets show a higher surface temperature with higher rH. These results are in good agreement with the calculated wet bulb temperatures . A sharp increase in droplet / particle temperature can be seen at the CP, therefore particles dried at higher rH need more time to reach the final temperature, which is with 53-55°C nearly equal for all rH.

Conclusion
We found that a drying air of high relative humidity (30% and more) stabilises L-Glutamic Dehydrogenase during drying in an ultrasonic levitator. Apparently drying is less aggressive which can be seen in the increase in time to the CP. We also suggest that a thin layer of water molecules at the droplet / particle surface interacts with the hydrophilic groups of the protein, thereby stabilising its native form. SEM pictures of dried particles support this conclusion, showing soft, sticky particles when dried at 50%rH.



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PO - 070

Biochemistry of Ether Lipid Formation in Myxobacteria

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Myxobacteria are gram-negative, motile, soil-dwelling δ-proteobacteria known for their complex life cycle including the formation of complex spore-containing fruiting bodies as well as for their richness in diverse secondary metabolites formed by partly unique biosynthetic pathways [1,2]. Many of these compounds exhibit antibacterial, antifungal or cytotoxic biological activities, which make them interesting as drug candidates [3]. Non-ribosomal peptide synthethases (NRPS) and polyketide synthases (PKS) as well as NRPS/PKS hybrids are prevalently involved in the synthesis of many of these secondary metabolites.
A detailed analysis of *Myxococcus xanthus* cells exposed to various stresses showed that the formation of lipid bodies is a common response of those cells towards environmental adversities [4]. These starvation induced lipid bodies proved to contain substantial amounts of unusual branched-chain fatty-acid-derived ether lipids. Those ether lipids make up at least one third of all lipids found in mature myxospores [5].
When investigating the biosynthesis of these ether lipids we discovered a gene encoding a multifunctional PKS/NRPS-like enzyme, designated as *elbD,* which is part of a five gene operon. This operon is present in all myxobacteria sequenced so far. *In-silico* analysis of the respective domains of this enzyme indicated its involvement in the primary lipid metabolism. After its inactivation, a strong reduction in ether lipid formation in *M. xanthus* and a complete loss of ether lipid formation in *S. aurantiaca* under vegetative growth and starvation conditions was observed. Additionally, the speed of fruiting body formation is being affected in the respective mutants.
Therefore we cloned, heterologously expressed and purified ElbD in order to investigate the function of this protein using *in-vitro* assays and mass spectroscopy. The results indicate that this protein catalyses the first steps in myxobacterial ether lipid biosynthesis.

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PO - 071

Extraction of astragalosides from Astragali Radix with different solvents and quantification with HPLC-ELSD

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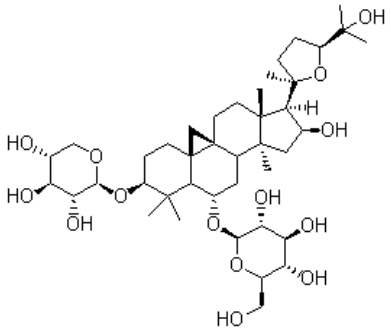
Astragaloside IV (AGS-IV), a cycloartane-type triterpene glycoside, is used as an active marker for the quality control of Astragali Radix (Huangqi; *Astragalus mongholicus* Bunge, Leguminosae) in Chinese Medicine [1, 2]. An HPLC-ELSD (HPLC-Evaporative light scattering detection) quantification method has been used to determine astragaloside content in Astragali radix using different extraction solvents of different polarity. The highest yield of astragalosides was obtained with acetonitrile-water (84:16). Yields of AGS-IV ranged from 0 to 0.0043 %. Therefore, the limit of 0.04 % AGS-IV, which is required by the pharmacopoeias, has by far not been achieved without hydrolysis. Therefore, AGS-IV seems not to be a genuine major constituent in Astragali Radix. Consequently the method used in the pharmacopoeia is a convention method. For best assay results methanol as extraction solvent, as used in the pharmacopoeias should be exchanged into acetonitrile-water (84:16).



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PO - 072

Content of phenolic compounds in wild populations of *Epilobium angustifolium* L. at different altitudes

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The aim of this study was to evaluate the influence of altitudinal variation on the content of phenolic compounds in *Epilobium angustifolium* L. (Onagraceae). Aerial, herbaceous plant material has been collected at three different altitudes (800m, 1000m and 1500m) at the Zirbitzkogel (Styria, Austria) during two growing periods. Plant samples (eleven to thirteen samples per altitude and year) were extracted with methanol using accelerated solvent extraction (ASE). Identification and quantification of the constituents were achieved by chromatographic means of HPLC-PDA and LC-PDA-MS (ESI, negative ion mode) analysis with external standards using a reversed-phase column (Phenomenex Synergi Hydro-RP) as a stationary phase and a gradient of water containing 0.5% acetic acid and acetonitrile as mobile phase [1]. Rising concentrations of flavonol-3-*O*-glycosides could be detected with increasing altitude. The content of the major compound quercetin 3-*O*-glucuronide ranged from 0.44 ± 0.205% (800m) to 0.49 ± 0.103% (1000m) up to 0.66 ± 0.114% (1500m). The total amount of flavonol 3-*O*-glycosides in 73 analyzed samples ranged from 2.16 ± 0.132% at 800m to 2.84 ±0.164% at 1000m and up to 3.29 ± 0.190% at 1500m. The methanolic plant extracts showed considerable radical scavenging effects (IC_{50} = 3.38 ± 1.094 µg/mL) with IC_{50} values ranging from 3.38 ± 0.427µg/ml (1500m) to 4.77 ± 1.012µg/ml (800m) in the DPPH assay. The present studies confirm that environmental factors at higher altitude result in elevated levels of flavonols in aboveground plant tissues. Which specific factors could influence the polyphenol content and the antioxidant activity [2, 3] has to be clarified in further studies.

Acknowledgements: Financial support by the Office of Research Management and Service, University of Graz, and the Dr. Heinrich Joerg-Stiftung, is gratefully acknowledged.
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PO - 073

Molecular analysis of prenyltransferases from *Hypericum* species

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The "Prozac of herbs" is a name given to St. John's wort (*Hypericum perforatum* L.) because of the wide use of its extracts in the treatment of mild to moderate depression (1). Many clinical trials confirmed the effectiveness and tolerability of St. John's wort extracts. Hyperforin, a polyprenylated phloroglucinol derivative, is one of the major compounds responsible for the antidepressant effect (2). Neither prenylating enzymes involved in hyperforin biosynthesis nor prenylating enzymes contributing to secondary metabolism of other *Hypericum* species have so far been characterised at the molecular level. However, these prenyltransferases are highly interesting because they catalyze formation of a broad spectrum of pharmacologically active prenylated secondary metabolites in a number of more than 400 *Hypericum* species (3).
We have discovered four different fragments of putative prenyltransferase cDNAs in a subtractive cDNA library of H. calycinum cell cultures, in which the first step of hyperforin biosynthesis has been biochemically demonstrated and formation of prenylated xanthones is inducible upon elicitor treatment. Two of the fragments have been completed toward the 5' and 3' ends to give full-length cDNAs, named HcPT-2 and HcPT-3. These two open reading frames consisting of 1143 and 1191 bp share 34 and 36%, respectively, amino acid sequence similarities with prenyltransferases of Arabidopsis lyrata. Heterologous expression of the membrane-bound enzymes in yeast cells and subsequent functional analysis are in progress. With detailed knowledge of the present sequences at hand we hope to isolate further prenyltransferase cDNAs from species of the genus Hypericum, especially those responsible for hyperforin biosynthesis in H. perforatum.

Acknowledgements: We thank the DFG and the Dr. Hilmer-Stiftung for funding this project.
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PO - 074

Honey as a vulnerary – A precious gift to mankind

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Honey is one of the oldest wound healing agents known today. For a long time it was the only sweetener and was therefore highly appreciated by the hunters and food-gatherers of the Stone Age. This has been documented by cave-paintings. It is the only natural food that never decays. Also the power of honey to conserve other foods was discovered soon by mankind, even the primitive man used it to preserve fruits and meat. It was only a small step to recognize the analogy of preventing foods and lesions from rotting by covering it with the yellow bees' product. The Edwin Smith papyrus (about 1550 BC), the world's oldest surviving document on trauma surgery, lists various prescriptions containing honey and proves the use of honey in the wound therapy even in Ancient Egypt. Because of its recent rediscovery as a treatment for chronic, infected wounds and wounds of immunodeficient

patients, it seems to be essential to trace back its medical-pharmaceutical tradition and to investigate in how far it can explain and support present applications.

PO - 075

Noxa and Mcl-1 play a crucial role in the effectiveness of the proteasome inhibitor MG-132 in combination with different anticancer agents in pancreatic tumour cell lines

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Pancreatic cancer progresses aggressively and due to chemoresistance responds poorly to chemotherapy. Thus, there is an urgent need to understand the mechanisms of cancer cell resistance in order to generate effective strategies to circumvent intrinsic chemoresistance in this tumour indication. In this work, three pancreatic cancer cell lines, MIA PaCa-2, MDA Panc-3, and AsPC-1, were treated with the proteasome inhibitor MG-132 together with camptothecin, doxorubicin or paclitaxel. The combination of MG-132 and camptothecin in a ratio of 5:1 gave the most promising results and enhanced cytotoxicity compared to the single compounds in MIA PaCa-2 cells. The increase is shown to be due to an enhanced caspase-3 activity resulting in apoptosis. Moreover, this combination upregulated the levels of the pro-apoptotic protein Noxa and reduced the levels of the anti-apoptotic protein Mcl-1. In contrast, the combination of MG-132 with doxorubicin only resulted in an increased cytotoxic, but also in a decreased apoptotic effect. The lack of the enhanced apoptosis induction could be correlated with high levels of Mcl-1 in response to the combined treatment with MG-132 and doxorubicin.
Thus, the results indicate that regulation of the anti- and pro-apoptotic Bcl-2 family members Noxa and Mcl-1 predicts the effectiveness of the combination of MG-132 with different anticancer agents on apoptosis induction in pancreatic cancer cells.

Acknowledgements: We gratefully acknowledge the financial support of the Dr. Mildred Scheel Stiftung of the Deutsche Krebshilfe (German Cancer Aid).

PO - 076

PC12-cells as a model for testing natural compounds upon their antioxidant effects

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PC12-cells have been established as an in vitro model system for neurobiological and neurochemical studies [1]. Therefore this cell-line was used to design a procedure for testing natural products upon their antioxidant effects. The natural products tested were baicalein a flavonoid derived from the roots of *Scutellaria baicalensis* G., a plant used in the Traditional Chinese Medicine and galanthamine an alkaloid, originally derived from various kinds of narcissus species. The cells were incubated with hydrogen peroxide for a defined period of time to simulate oxidative stress. Subsequently the cells were incubated with different concentrations of baicalein or galanthamine. The viability of the cells was determined via MTS-assay and neutral red assay.
The LDH-assay was applied to investigate the cytoprotective effect of baicalein in terms of peroxide damage to the cell membrane.
The main issue was to define the optimal experimental parameters concerning the concentration as well as the incubation time with hydrogen peroxide in order to obtain quantitative results. Best results were achieved with

600µM concentration of hydrogen peroxide and 40 minutes time of incubation. In general, the incubation with galanthamine showed a slight but not significant increase of the viability on the neutral red assay. However, baicalein didn't show any positive effects on the viability of the cells for all concentrations applied.
In conclusion, the set-up of such in vitro cytotoxicity test systems is rather difficult, especially the incubation time and concentration of all the substances tested in these experiments have to be optimized and investigated more closely. Up to now it is not clear if the common procedure of the PC12 cell assay will be able to screen the biological effects of baicalein.

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PO - 077

In vitro anti-inflammatory activity of flavonolignans salcolin A and B from *Avena sativa*

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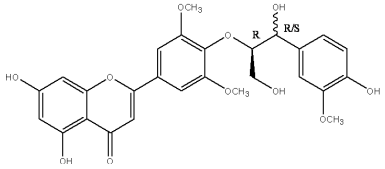
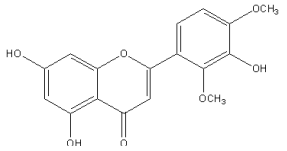
As part of our ongoing phytochemical and pharmacological studies on *Avena sativa* L. (oat), we investigated the two diastereomeric flavonolignans salcolin A and B as well as their common flavonoid moiety tricin for anti-inflammatory activity *in vitro*.
At a concentration of 30 µM, the two flavonolignans clearly inhibited LPS-induced IL-8 expression in HUVECtert cells, with salcolin B being slightly more active than salcolin A (60.9±8.2 vs. 44.5±12.7% inhibition).
The two compounds also showed pronounced inhibition of NF-κB activity in TNF-α-stimulated HEK293 cells stably transfected with an NF-κB-driven luciferase reporter gene (70.5±3.5 % for salcolin A and 87.5±4.9 % for salcolin B at 30 µM). Therefore, the observed IL-8 expression inhibition is supposed to be due to inhibition of the NF-κB signalling pathway.
Tricin, the flavonoid moiety of the two compounds, did not show any activity in these assays.



Salcolin A:Tricin 4'-O-erythro-β-guaiacylglycerylether
Salcolin B: Tricin-4´-O-4'-O-threo-β-guaiacylglycerylether



Tricin

Acknowledgements: This project is supported by the FWF (Project Nr. S10705-B13)

PO - 078

Truncation of N-terminal regions of *Digitalis lanata* progesterone 5β-reductase alters catalytic efficiency and substrate preference

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The short-chain dehydrogenase/reductase (SDR) superfamily of proteins is a large and diverse group of enzymes with members found in Eucaryota, Bacteria and Archaea [1]. Progesterone 5β-reductases (P5βR, P5βR2), that have been identified previously as members of the SDR superfamily, are supposed to be involved in 5β-cardenolide biosynthesis, an important group of plant natural products [2]. P5βRs catalyze the enantio-selective reduction of the C=C bond of progesterone to 5β-pregnane-3.20-dion and other enones, including 1-cyclohexen-2-one [2,3].
We here designed N-terminal truncated forms of P5βR taking a *Digitalis lanata* progesterone 5β-reductase c-DNA with a hexahistidine tag attached at the C-terminus (termed r*Dl*P5βRc) as the starting point. Four derivatives truncated in the N-terminal region, termed r*Dl*P5βRcn-10, r*Dl*P5βRcn-20, r*Dl*P5βRcn-30 and r*Dl*P5βRcn-40 were obtained by deletion mutagenesis.
The full-length r*Dl*P5βRc as well as two of the truncation-mutant enzymes (r*Dl*P5βRcn-10, r*Dl*P5βRcn-20) were catalytically active. In all enzymes NADPH was the required co-substrate which could not be replaced by NADH. Active enzymes were characterized with respect to their kinetic parameters and substrate preferences. All of them converted progesterone and 1-cyclohexen-2-one. Among the enzymes tested the full-length r*Dl*P5βRc had the highest affinity for progesterone (K_M = 31.5 µM). 1-cyclohexen-2-one, a small progesterone mimic, was converted about 50 to 100 times faster than progesterone by all of the enzymes created here.

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PO - 079

Cardioactive *Leonurus cardiaca* L. (Ph.Eur.): Ferulic acid acts as a Ca^{2+}-channel antagonist on neonatal rat cardiomyocytes in voltage clamp setup

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Just recently, aqueous preparations of *Leonurus cardiaca* – such as a newly developed refined extract – were reported to exert a series of effects on cardiac electrophysiology, such as $I_{Ca.L}$ blockade, a reduction of the repolarising current $I_{K.r}$, as well as the prolongation of both AP-duration and the activation time constant of the I_f current, while I_{Na} was strikingly unaffected [1]. This synergistic action on multiple electrophysiological targets may limit potential adverse effects, especially the risk of proarrhythmia, and is thus in accordance with the traditional use of this medicinal plant as an antianginal in European herbalism [2]. Several extract samples were therefore examined by RP-HPLC for phenolic substances potentially contributing to these pharmacological activities. In this screening procedure, ferulic acid (4-hydroxy-3-methoxy-trans-cinnamic-acid) was detected in every single sample with contents between 0.001 and 0.008% in the crude drug. The content in the refined extract, which was prepared from a sample containing 0.001%, was enriched to 0.008%. Although a variety of pharmacological actions on the cardiovascular system have been reported for this caffeic acid derivative [3], specific molecular pharmacological actions on cardiac ion channels – as observed for the *L. cardiaca* refined extract [1] – have not been reported for the isolated compound up to now. In subsequent voltage clamp experiments, carried out according to a protocol previously developed in our group [4], ferulic acid at concentration of 3 µM influences neonatal rat ventricular cardiomyocytes by $I_{Ca.L}$ blockade while I_{Na} was not affected, thus mimicking parts of the effect of the previously described *L. cardiaca* refined extract. However, the measured concentrations of ferulic acid are too low to serve as the singular explanation of its pharmacological activity, thus exhibiting a 'multi-component / multi-target' active principle [1].

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PO - 080

Resveratrol reduces hepatic fat accumulation by modulating Farnesoid X receptor signaling

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Introduction: Resveratrol (Res), a diet-derived polyphenol, was recently found to be protective in the development of non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome. This seems to be mediated by induction of NAD^+-dependent deacetylase SIRT1, thus mimicking calorie restriction, at present the only available therapy option in the treatment of NAFLD [1-3]. However, detailed modes of Res action still remain to be specified. Farnesoid X Receptor (FXR) is a nuclear receptor which regulates the expression of a large number of genes involved in bile acid metabolism as well as lipid and glucose homeostasis, thus presenting a promising target for NAFLD therapy [4].
This work aims to characterize molecular mechanisms of Res-activity in the context of experimental NAFLD with a special regard to FXR mediated signaling.
Methods: HepG2 cells were treated with increasing concentrations of Res [50-100µM] for 24h. Intracellular fat accumulation was induced either by mixtures of fatty acids [1mM] or monosaccharides [100mM] and detected by Nile red staining. SIRT1 activation was detected by a commercially available assay. mRNA levels were quantified by real time qPCR and intracellular protein levels were determined by Western Blot analysis.
Results: Our results show impressively, that Res significantly counteracts fatty acid- and carbohydrate-induced fat accumulation in HepG2 cells [**p<0.01]. This strictly correlates with a dose-dependent induction of SIRT-1 activity (~100%) as well as an induction of intracellular FXR receptor activity after 24h of incubation. Moreover, resveratrol is also capable of modulating the expression of FXR target gene SHP on mRNA [**p<0.01] and protein levels in a dose-dependent manner. Res further shows the tendency to counteract fatty-acid induced up-regulation of the metabolic target genes SREBP-1, FAS, PEPCK and L-FABP.
Conclusion: These data suggest that resveratrol attenuates severity of non-alcoholic liver disease by induction of transcription factor FXR receptor activity, thereby modulating the expression of disease-relevant target genes.

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PO - 081

MHC-II loading enhancement (MLE) - a new immunological activity of natural essential oils and their constituents

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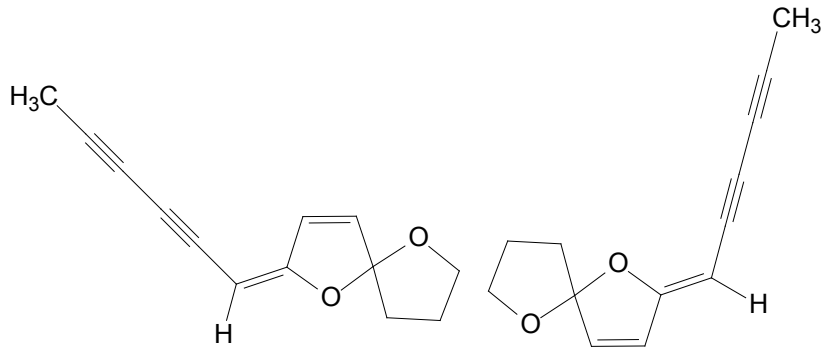
Enhancement of MHC-II peptide loading by low-molecular weight chemicals is of twofold interest in immunological research. Compounds that elicit an increased loading of MHC-II molecules with immunogenic peptides (MHC loading enhancers, MLEs) may be involved in the pathophysiology of autoimmune diseases. On the other hand, such compounds might be of potential use to enhance the activity of vaccines and of antitumor immunotherapies [1].
We have now discovered that some natural essential oils are able to increase the loading of MHC-II allele HLA DR1 to a very significant extent. In a screening based on Dissociation-Enhanced Lanthanide Fluorescent Immuno-assay (DELFIA) [2], we found that a variety of essential oil as well as isolated constituents could increase the spontaneous loading of soluble HLA DR1 [3] with a influenza A haemagglutinin peptide (HA 306-318, [4]).
Quite interestingly, structurally simple and widespread monoterpenes (citronellol, geraniol) showed the strongest activity among >40 pure compounds tested.
Of 28 essential oils tested so far, chamomile oil (*Matricaria recutita* L.) showed the strongest effect, comparable with the reference compound, adamantylethanol, a potent MLE [1].
Activity-directed isolation led to the identification of E-ene-yne-dicycloether **1** as the strongest MLE compound, about 3 times stronger than the Z-isomer **2**. Bisabolol oxides A and B were also significantly active but much less potent than the E-Spiroether.



1 **2**

These findings indicate that MHC-II loading enhancement might be involved in the immunological activities of essential oils and may also open new perspective with respect to potential applications.

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PO - 082

Inhibitory effects of sesquiterpene lactones and further natural products against leukemia-associated transcription factor c-Myb

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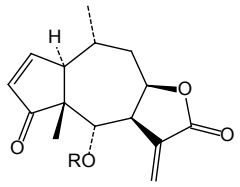
The transcription factor c-Myb plays an important role during haematopoiesis. The highest level and function is found in immature myeloid and lymphoid progenitor cells. If c-Myb is not down regulated during the differentiation,

as it usually happens in haematopoiesis, the differentiation of the myeloid and lymphoid progenitor cells is impaired. Acute myeloid leukemia and chronic myeloid leukemia cells require c-Myb for their continued proliferation and they seem to be more sensitive to inhibition of c-Myb function than their normal counterparts [1]. This makes c-Myb an interesting therapeutic target for the treatment of leukemia. However, no low molecular weight inhibitor suitable as drug or lead structure is known so far.

Recently we reported for the first time that sesquiterpene lactones (STLs), which are known for their wide variety of biological activities, are inhibitors of c-Myb activity [2]. Using a reporter gene assay that reflects a direct correlation between fluorescence activity and c-Myb activity, we could demonstrate inhibitory activity for a variety of currently 50 different sesquiterpene lactones of various skeletal subtypes. Helenalin acetate **2** is one of the most active STLs tested so far, with an IC_{50} value below 1 µM.



	R	IC_{50} (µM)
1	H	2.5
2	acetate	<1
3	isobutyrate	2.5
4	isovalerate	7.0

From the different levels of activity observed among the tested STLs, we can draw first conclusions with respect to structure-activity-relationships. The presence of more than one potential Michael acceptor structure leads to the highest level of activity. Alkylation is thus the likely mechanism of action. However, we could demonstrate that the terpenoid scaffold is also of crucial impact on inhibitory activity. Thus, e.g., the activity of helenalin **1** and its ester derivatives (acetate **2**, isobutyrate **3**, isovalerate **4**) is strongest in the acetate and decreases in higher esters in the order of size/lipophilicity.

In addition to these studies with STLs, we have also widened our search for potential c-Myb inhibitors to other classes of natural products and found several compounds which c-Myb activity at a promisingly low concentration level, i.e. with IC_{50} <1 µM.

On the whole, we have discovered 11 out of 70 tested compounds having an IC_{50} value less than 3 µM. For these most active compounds, in parallel to the c-Myb assay, MTS-assays measuring mitochondrial function, and thus cell viability, were conducted. In most cases, these compounds exhibited impairment of cell viability only at much higher concentration levels, so that a general cytotoxic effect can be exluded as mere cause for the observed activity.

We therefore believe that antileukemic agents directed towards c-Myb activity can be retrieved and developed from the mentioned or further natural products yet under investigation. First experiments for selectivity towards leukemic bone marrow cells are in progress.

Acknowledgements: Financial Support from the José Carreras Leukämie-Stiftung is most gratefully acknowledged.

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PO - 083

Activation of PPARα and PPARγ by (+)-sesamin

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Beside 40 to 60% triglycerides the seeds of *Sesamum indicum* L. contain also around 1% of different lignans, which contribute to many health benefits of sesame seeds. Especially the major lignan derivative (+)-sesamin shows several pharmacological activities associated with lipid metabolism including an increase of hepatic fatty acid β-oxidation, a reduction of serum and liver cholesterol, a decrease of hepatic lipogenesis and others [1]. Up to now it remained unclear whether (+)-sesamin exerts these activities also by activation of peroxisome proliferator activated receptors (PPAR). The aim of the performed study was therefore focused on the isolation of

(+)-sesamin from sesame seeds and its pharmacological analysis in PPARα and PPARγ luciferase reporter gene assays in HEK 293 cells. Unpeeled sesame seeds (1.00 kg) were defatted with petroleum ether and extracted with methanol. The obtained extract was separated by liquid-liquid extraction with water and solvents of different polarity (diethyl ether, ethylacetate, and *n*-butanol). The major compound of the diethyl ether soluble part, isolated by different chromatographic techniques and identified by 1- and 2D-NMR and HPLC-MS, was identified as the desired (+)-sesamin. Pharmacological investigations of the starting methanolic extract exerted a selective but weak activation of PPARα by 40% at a concentration of 10 µg/ml while the isolated compound (+)-sesamin showed no activity up to a tested concentration of 30 µM. These findings indicate that the described effects of (+)-sesamin are not mediated by PPARα or PPARγ agonism but give evidence for (an) up to now unknown PPARα activating constituent(s) in the MeOH extract.

Acknowledgements: This work was granted by the Austrian Science Foundation (FWF; DNTI S10703).
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PO - 084

Biotransformation of cardenolide precursors by transformed *Saccharomyces cerevisiae*

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Cardenolides, pharmacologically active compounds used in the therapy of cardiac insufficiency in humans, are still isolated from *Digitalis* plants. Therefore we aim at the reconstruction of several steps of the putative cardenolide pathway in *Saccharomyces cerevisiae* to produce commercially not available intermediates from simple sterol or pregnane precursors. About 20 enzymes which might be involved in cardiac glycoside biosynthesis have been described “downstream” of cholesterol [1]. Some of these enzymes have already been isolated and characterized. We cloned three genes encoding enzymes that are supposed to transform pregnanes during cardenolide formation: Δ5-3β-hydroxysteroid dehydrogenase (3β-HSD, EC 1.1.1.51) from *Digitalis lanata*, which converts pregnenolone to isoprogesterone [2], *Arabidopsis thaliana* Δ4,5-steroid 5β-reductase (5β-StR, EC 1.1.1.145/1.3.1.23) which reduces progesterone stereoselectively to 5β-pregnane-3,20-dione [3] and steroid 21-hydroxylase (Cyp21a1, EC 1.14.99.10) from *Mus musculus* which is able to hydroxylate pregnanes in position 21. Up to now no plant enzymes catalyzing the 21-hydroxylation of pregnanes are known. Therefore, we also try to identify plant cytochrome P450 monooxygenases involved in miscellaneous metabolic pathways which might be able to hydroxylate intermediates of the cardenolide pathway as well. For this purpose we selected suitable cytochrome P450 enzymes from *Arabidopsis thaliana*: CYP90A1 (steroid C-23 hydroxylase) and CYP90B1 (steroid C-22 hydroxylase), both involved in brassinosteroid biosynthesis and CYP714A2, a steviol synthetase capable of hydroxylating, *e. g.*, gibberellins at C-13. All of these genes were cloned into the Gateway® pYES-DEST52 vector system and transformed into *Saccharomyces cerevisiae* strains. Our data so far indicate, that the Cyp21a1 and the 3β-HSD genes are functionally expressed in the recombinant yeast strain. Product formation was monitored and demonstrated by GC-MS and TLC. The heterologous expression of 5β-StR, CYP90A1 and CYP90B1 was also demonstrated. Ongoing studies will show the functional expression of the individual genes in yeast. The next step will be cotransformation and coexpression of more than one gene/enzyme.

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PO - 085

Phytochemical investigations of *Dischidia raffesiana* Wall.

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Dischidia raffesiana Wall. (Asclepiaceae), distributed in the south of Vietnam, is an epiphytic vine with modified leaves in the shape of a pitcher. The leaves are normally indwelt by different ant species. Alcoholic extract of leaves are used in Vietnamese folk medicine for the treatment of rheumatism [1]. In order to rationalize the use of this plant, a methanolic extract of the leaves was prepared, which was further phytochemically investigated.
The methanolic extract was separated by liquid-liquid extraction with water and solvents of different polarity (petroleum ether, diethyl ether, ethylacetate, and *n*-butanol) resulting in a large total yield (68.14 w% of the starting extract) of the two most lipophilic fractions. The three major compounds of the petroleum ether soluble part, isolated by different chromatographic techniques and identified by 1- and 2D-NMR and HPLC-MS, were identified as the known triterpens β-amyrine, friedelin, and 3β-friedelinol. Since the remaining water fraction (10.48 w%) showed only one prominent spot in TLC analysis (pink colour with vanillin sulphuric acid reagent) the responsible compound was also isolated. Structure elucidation by 1- and 2D-NMR and HPLC-MS revealed the compound as the known cyclitol conduriol A. The mixture of α-amyrine and β-amyrine has been reported to suppress local inflammatory cytokines and COX-2 expression, possibly via inhibition of NF-κB pathway [2]. Friedelin possesses potent anti-inflammatory, analgesic and antipyretic activities *in vivo* [3]. Therefore, the found triterpenes as well as their remaining derivatives, present in a high concentration in the investigated extract, might explain the traditional use of this plant.

Acknowledgements: This work was granted by the OeAD and the Austria Science Foundation (FWF; DNTI S 10703).
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PO - 086

Route of administration determines anxiolytic activity of the flavonols kaempferol, quercetin – are they prodrugs?

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In vivo and in vitro studies have confirmed that flavonols are metabolized by the intestinal microflora to their corresponding hydroxyphenylacetic acids. It is known that the flavonoids kaempferol and quercetin exhibit anxiolytic activity after peroral administration using the elevated plus maze. The aim of this study was to examine the influence of the administration route on anxiolytic activity representing central effects and thus the question if flavonoids are prodrugs that need to be transformed by the intestinal microflora. Therefore the anxiolytic activity of the flavonols kaempferol and quercetin after peroral (PO) and intraperitoneal (IP) administration to mice in a dose range of 0.1 to 2.0 mg/kg was detected using the elevated plus maze. In addition, their corresponding metabolites p-hydroxyphenylacetic acid (pHPAA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were tested after IP administration. No anxiolytic effects were observed when kaempferol and quercetin were given via the IP administration route. The corresponding hydroxyphenylacetic metabolites pHPAA and DOPAC showed anxiolytic effects after IP application. In order to further test the hypothesis that flavonoids are possible prodrugs which require activation by intestinal bacteria, gut sterilization was performed using pretreatment with the antibiotic

enrofloxacin (7.5 mg/day, PO, for 4 days). After inactivation of bacterial microflora the anxiolytic effect of kaempferol and quercetin disappeared, whereas it was still present for the positive control diazepam. In summary the findings support the hypothesis that flavonoids act as prodrugs and have to be transformed into their active form by the intestinal microflora. It was pointed out that the anxiolytic activity of kaempferol and quercetin depends on oral administration and an intact bacterial microflora, which leads to the conclusion that these flavonols are activated by colonic bacteria. Since administration of p-HPAA and DOPAC revealed similar anxiolytic activities it is likely that these metabolites act as active agents.

PO - 087

Effects of herbal multi-drug preparations STW 5 and STW 5-II and their main component STW 6 on inflamed rat colon preparations

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Iberogast® (STW 5) is a herbal multi-drug preparation used in the treatment of gastrointestinal disorders. Pharmacological studies revealed a multi-target effect which may be involved in its clinically proven efficacy in IBS.
We compare the effects of STW 5, containing nine extracts, STW 5-II, containing six extracts, and their main component STW 6 (*Iberis amara*) on rat colonic preparations before and after induction of inflammation. The experiments were performed in conformity with the Guiding Principles in the Care and Use of Animals. The inflammation was induced by intraluminal instillation of 2,4,6-trinitrobenzene sulfonic acid (TNBS, 0.01M/ 0.1M, 30min). Contractions were measured isometrically. Both, STW 5 (64-512 µg/ml) and STW 5-II (66.7-533.2 µg/ml) shifted the concentration-response-relationship of acetylcholine (ACh)-induced contractions (0.01-1000µM) to the right with EC_{50} values of 0.28 µM and 0.77 µM, respectively in untreated preparations. STW 6 in equivalent concentrations (3-24.1 µg/ml) did not influence the ACh-induced contractions. Preincubation of the preparations with TNBS resulted in an inhibition of the ACh-induced contractions. Coincubation of STW 5 (256 and 512 µg/ml) or STW 5-II (266.6 and 533.3 µg/ml) with TNBS did not prevented this effect whereas STW 6 in an equivalent concentration (24.1 µg/ml) did. Van Giesson Staining indicated that TNBS induced morphological disturbances of smooth muscular layers and mucosa, which were less distinct after coincubation with STW 6. Both, STW 5 and STW 5-II did not alter the TNBS-induced morphological damages.
Our study suggests that STW 6 differs from STW 5 and STW 5-II in its pharmacological profile. Therefore the results confirm that the components play a distinct role in the effect of the two combinations.

PO - 088

Sage (*Salvia officinalis L.*) – medicinal plant or health risk?

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The sage plant Salvia officinalis L. is used as ingredient in foods and beverages as well as in herbal medicinal products. A major use is in the form of aqueous infusions as sage tea, which is legal to be sold as either food or

medicine. Sage may contain two health relevant substances, thujone and camphor. A GC/MS procedure was applied for the analysis of α- and β-thujone and camphor with cyclodecanone as internal standard. The precision was between 0.8 and 12.6%, linearity was obtained from 0.1 - 80 mg/L. The recoveries of spiked samples were between 93.7 and 104.0% (average 99.1%). The time of infusion had a considerable influence on the content of analytes found in the teas. The average thujone and camphor contents were 4.4 mg/L and 16.7 mg/L in food tea infusions and 11.3 mg/L and 25.4 mg/L in medicinal tea infusions.
Several in vivo and in vitro studies point to sage polyphenols as active principles that may inhibit lipid peroxidation and improve antioxidant defences. This study describes an UHPLC methodology with UV and MS/MS detection, which allows the separation, identification and quantification of the major phenolic constituents in sage tea within 34 min. This method was used to characterize 16 commercial brands of sage tea. The quantitatively dominating compounds were either rosmarinic acid (12.2 to 296 mg/L) or luteolin-7-O-glucoside (37.9 to 166 mg/L). The ORAC antioxidant capacity and Folin-Ciocalteu index of the samples show correlation with rosmarinic acid and its derivatives.
The current results suggest that on average between 3 and 6 cups of sage tea could be daily consumed without reaching toxicological thresholds. In general, considerable differences in polyphenolic composition and antioxidant capacity between the commercial brands were detected leading to the demand for quality standardization and control especially if the teas are used for therapeutic purposes.

PO - 089

Structure-antimycobacterial activity relationship studies of N-alkyl-4(1H)-quinolones bearing hydrophobic moieties at C-2

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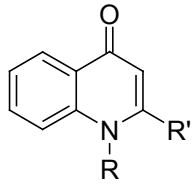
The identification of nalidixic acid as a synthesis by-product of chloroquine in the 1960s pave the way for the discovery of a wide varieties of quinolone therapeutic agents which showed amazing broad spectrum antibiotic activities. Despite the emergence of resistance to quinolones mainly caused by their wide spread overuses and misuses, they remain the subject of considerable interest. Our investigations into the design and synthesis of this new class of antimycobacterial, *N*-alkyl-4(1*H*)-quinolones, started with the initial discovery of the potent antimycobacterial properties of quinolone alkaloids isolated from the Chinese medicinal plant *Euodia rutaecarpa* [1, 2].
A series of 4(1*H*)-quinolones having alkyls, alkenyls and alkynyls were synthesized as analogues of evocarpine and tested *in vitro* against fast growing strains of mycobacteria. Results of our studies revealed that compounds with C12-C14 aliphatic groups with either double or triple bond exerted potent inhibition of the growth of *Mycobacterium smegmatis* compared to their saturated analogues [3, 4].



R = methyl, ethyl, propyl, prop-2-enyl, prop-2-ynyl, butyl, pentyl,
R' = alkyl, alkenyl, and alkynyl

The observed SAR manifested dependences of activity on two important molecular features of the hydrophobic moieties at position 2, i.e., chain unsaturation and total number of carbon atoms in the aliphatic chains.

Acknowledgements: This research work is funded by the Austrian Science Fund (FWF) project no P21152-B18 and is gratefully acknowledged.
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Transporter

PO - 090

Understanding mechanisms underlying the aquaporin-2 redistribution in renal principal cells by using small molecule inhibitors

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The water channel aquaporin-2 is expressed in renal collecting duct principal cells. Arginine-vasopressin regulates AQP2-mediated water reabsorption from primary urine. It binds to the vasopressin V2 receptor on the surface of the principal cells and thereby increases cAMP and activates PKA [1]. PKA phosphorylates AQP2 at the C-terminal Serine256 residue [2, 3]. This, in turn, leads to a redistribution of AQP2 from intracellular vesicles into the plasma membrane and facilitates water reabsorption from the collecting duct.
To date, only a few proteins and stimuli are known that can modulate this process [4] and might serve as targets to pharmacological approaches to treat diseases associated with excessive water reabsorption, such as chronic heart failure (CHF), liver cirrhosis or the syndrome of inappropriate antidiuretic hormone hyper secretion (SIADH).
In a cell-based screening approach we identified two small molecules inhibiting the cAMP-dependent AQP2 redistribution by two different modes of action.
Through detailed characterization of effects of the small molecules and identification of their targets we aim to elucidate mechanisms underlying AQP2 translocation.

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PO - 091

The potential for crossing physiological barriers – Studies in a series of benzimidazol-2-yl-amino-substituted (L)-amino acids designed as NMDA receptor glycine$_B$ site antagonists

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Improving the blood-brain barrier permeation of CNS effective drugs, especially of NMDA receptor antagonists with their carboxylic acid function being crucial for activity at the glycine binding site, remains a challenging task [1]. With this in mind, a series of benzimidazol-2-yl-amino-substituted (L)-amino acids, varied in the aromatic

substitution pattern or in the chain length of the amino acid part, had been synthesized. Importantly, amino acids can be both subject to active transport and to passive diffusion, the extent depending on their physicochemical properties [2]. Furthermore, an amphiphilic or (poly)cationic character of compounds may facilitate the permeation of the blood-brain barrier [3].
In this series, measurement of pk_a- and log D values by a semi-automatic potentiometric titration method revealed a favourable lipophilicity profile especially for the dibromo derivative AKD 112 (log D_{Oct} = 1,4 at pH = 7,3 = IP).
Competition of compounds with [^{3}H]-L-phenylalanine uptake in C6 rat glioblastoma cells showed a clear dependency on pK_a of the guanidino-type substructure (ranging from 5,0 to 7,3) and on lipophilicity (ClogP), which would be consistent with the substrate profile of system L amino acid transporters. The best K_m value of 90,0 µM was found for AKD 112, hence being in the range of known drugs transported by LAT1 [4]. This large neutral amino acid transporter has a high transport capacity and is widely expressed, also in the luminal membrane of the BBB [5].

Acknowledgements: Sirius Analytical Instruments Ltd. (Forest Row, East Sussex, UK) for help with the refinement of zwitterion log P data
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PO - 092

Cationic lipids for liposomal gen transfer

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The process of introducing nucleic acids into eukaryotic cells by different methods is defined as gene-transfection. In addition to vector-mediated, non vector-mediated gene transfer by microinjection or electroporation represents another possibility. Despite the simplicity of use and lack of toxicity, non vector-mediated transfer systems suffering from less satisfying results for gene expression based on fast DNA degradation and the restriction of application for certain tissues. In regard to vector-mediated delivery two different systems have been established. Viruses as vectors have been explored as one alternative method to deliver genes into cells. Because of their biological properties they are able to achieve high levels of gene transfer and long-term expression. But, low packaging capacity, and the potential for activation of latent diseases are only some drawbacks for these methods.
Non-viral gene transfer, for example with cationic liposomes, is a promising way to deliver genetic material with low toxicity, lack of immune response and high DNA-loading capacity. However, lower transfer and gen-expression levels compared to virus-mediated transfection are major challenges in this range.
We synthesized new cationic lipids with different head-, backbone-, and alkylic-groups. These cationic lipids were combined with different ratios of helper lipid like dioleoylphosphatidylethanolamine (DOPE) or cholesterol and constant amount of plasmid DNA to form lipoplexes able to surpass the reference Lipofectamine™. Liposomes and lipoplexes were characterized per photo correlation spectroscopy (PCS). Cytotoxicity was analyzed with MTT-test, in which only living cells are able to transform the yellow dye in a purpur ones, being detected photometrically. Transfection efficiencies were detected with ortho-nitrophenyl-β-galactoside (ONPG-assay) measuring the galactosidase activity after incorpoation of the Lac-Z-gene containing plasmid DNA.

PO - 093

In silico prediction of ABCC2 substrate specificity

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The ATP-binding cassette (ABC) proteins represent a large family of transmembrane proteins that use the energy of ATP hydrolysis to transport a wide variety of physiological substrates across biological membranes [1]. Of them, particular attention has been focused in the last years on the role of the ABCC2 transporter in drug clearance and disposition.
The ABCC2 transporter is a transmembrane protein expressed in the apical cell membrane of hepatocytes and epithelial cells of small intestine and kidney, where it is involved in the elimination of many endogenous and exogenous substrates from the cell, including compounds clinically relevant [2]. Alteration in the disposition and elimination of these compounds can modify their pharmacokinetic and pharmalogical profiles, leading to reduced efficacy or increased toxicity.
In this scenario, the aim of the present work was the development of an *in silico* model based on the Gottesman database [3] able to predict if certain compounds of interest are ABCC2 substrates or not. To this end, several machine learning methods were explored using the data analysis platform KNIME [4]. Molecules were represented by 2D and VolSurf descriptors calculated with the MOE software [5]. Feature selection was used to improve the efficiency of the data mining algorithms and identify the contribution of different features. According to our results, naive Bayesian updatable (NBU) and instance-based learner (IB1) had the highest performance, with an overall prediction accuracy of 74,5% and a Matthew's correlation coefficient of 0.49. Furthermore, sensitivity and specificity values were significantly improved with values of 79% and 70%, respectively.

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PO - 094

Machine Learning and Pharmacophore-Based Models as an Efficient Virtual Screening Tool for Identification of P-Glycoprotein Inhibitors

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P-Glycorpotein (ABCB1 or P-gp) is a member of the ABC-transporter family, which actively exports chemically diverse molecules (including drugs and toxins) across the cellular membranes. A vast number of currently marketed anticancer drugs are substrates of P-gp, leading to poor pharmacokinetics profile, and failure of chemotherapy, and drug-drug interactions (DDI). Therefore, it is highly relevant to have simple and efficient virtual screening models to identify potential P-gp inhibitors. In the present study we developed virtual screening models using machine-learning methods (such as Decision Tree, Random Forest, Self-Organizing Map) and Pharmacophore modelling. Models were constructed based on a set of about 1300 P-gp inhibitors and non-inhibitors using Random Forest and Self-Organising Map. The best ones obtained correctly predict >85% of the inhibitors and 80% of the non-inhibitors in an external test sets (≈700). In addition to this, a simple "drug-like" descriptors based model was developed using a decision tree, which correctly predicts more than 75% compounds of the test set.
In addition to machine learning models, a four-point pharmacophoric feature (Ph4-hypothesis 1) model was

identified from a set of potent P-gp inhibitors. In combination with machine leaning models, Ph4-hyphothesis 1 can be used as an additional filter in the virtual screening process. All the developed models are simple and fast and allow to efficiently identify P-gp inhibitors.

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PO - 095

Development of in silico models for identification of new ligands acting as pharmacochaperones for P-glycoprotein

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P-glycoprotein is an ATP depended drug efflux pump belonging to the MDR/TAP subfamily characterized by a broad substrate specificity. Overexpression of p-glycoprotein is a major reason for multidrug resistance (MDR) and thus responsible for the failure of antibiotic and cancer therapies. Therefore, inhibitors of p-glycoprotein are promising candidates for overcoming the problem of MDR. Defective folding in Pgp prevents maturation of the protein. The pharmacological chaperone GPV0062 may repair the folding defects either by promoting dimerization of the two nucleotide binding domains (NBDs) or by promoting correct folding of the transmembrane domains (TMDs). The aim of our studies was to develop *in silico* models, which can be used for the identification of new inhibitors as well as pharmacochaperones for P-glycoprotein.
Two different computational approaches were used in order to build up screening models. Pharmacophore screening as well as similarity screening were performed. The LigandScout program package of Inte:Ligand GmbH was used for the construction of a structure-based pharmacophore model, taking a complex of GPV0062 bound to P-glycoprotein [1] as a starting point, whereas the similarity screens were performed according to SHED fingerprint similarity [2], using a script embedded in the MOE molecular modeling program package.
In order to validate the method, the pharmacophore model was tested against a database consisting of 2150 compounds classified as active and inactive according to the literature. The Precision as well as the Specificity values of 0.82 and 0.98, respectively, demonstrated the reliability of the model.
The next step was the screening of the Life Chemicals Database containing more than 300.000 compounds against the pharmacophore model, resulting in the identification of 364 hits. In parallel we performed a SHED similiarity fingerprint screen at a threshold of 82%. This led to the acquisition of 410 additional hits that were further reduced by comparing their pharmacophoric fits, thus identifying 18 compounds matching 6 of 8 features of the pharmacophore.

Acknowledgements: We acknowledge financial support by the Austrian Science Fund, grant F03502
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PO - 096

Specific phospholipids enhance in-vitro absorption of p-glycoprotein substrates

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Several pharmaceutically active substances underlie cellular efflux processes within the human organism. If the efflux transporter is located in the membrane of the enterocytes, as for the ATP-dependent P-glycoprotein (P-gp), the drug's intestinal absorption is diminished and its systemic bioavailability significantly decreased. Therefore, a specific inhibition of this transporter poses a promising approach in oral drug therapy with P-gp substrates by enhancing their systemic therapeutic efficacy enabling a reduction of their dose and side effects.
Previous studies indicated distinct P-gp-inhibitory potential of particular phospholipids (PL)[1], which we have reproducibly confirmed for Di-8:0 – and Di-10:0 phosphatidylcholine (PC). The results were obtained by transport experiments across Caco2- and MDCK II mdr1 cell monolayers in the 2-chamber Transwell® system measuring the flux of the model substrate digoxin. Another cellular method, the calcein accumulation assay (CAA), quantified the P-gp dependent efflux of the fluorescent dye calcein, whereas P-gp ATPase activity measurements determined the release of inorganic phosphate in artificial, P-gp containing membranes depending on the degree of enzyme stimulation or inhibition.
The current focus lies on the elucidation of the PL's binding pattern within the transmembrane regions by means of in-silico molecular modeling.
Moreover, the identified PL shall be formulated with a popular P-gp substrate to investigate its in-vitro permeability behaviour compared to one of the commercially available product.

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PO - 097

Comparison of human, rat and mouse ABC-transporters on basis of their substrate and inhibitor profiles

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P-glycoprotein (P-gp) is an ABC-transporter of the MDR/TAP subfamily which is extensively expressed in the intestinal epithelium, hepatocytes, renal proximal tubular cells, adrenal gland, capillary endothelial cells and blood brain barrier. In humans it is encoded by the MDR1/MDR3 gene, in rats by pgp1/pgp2/pgp3 and in mice by mdr1/mdr2/mdr3. The protein is an ATP-dependant efflux pump for xenobiotic compounds with a broad substrate and inhibitor specificity. Therefore it plays a major role in multidrug resistance and for the bioavailability of drug candidates. In the drug development process, the pharmacokinetic profile as well as the toxicity of a drug candidate is determined in animal (usually mouse or rat) models. Thus, besides establishing predictive *in silico* models for identification of ligands for human P-gp it is also important to develop predictive models for mouse and rat P-gp.
Recently a crystal structure of mouse P-gp was determined and provides new possibilities for structure-based drug design approaches [1]. The high sequence identity between rat and mouse P-gp (92%) and the importance of rats in animal ADME models motivated us to create a homology model of rat P-gp taking the crystallized mouse P-gp as a template. A multiple sequence alignment was performed using ClustalW2 among different species (dog, frog, hamster, human, mouse, rabbit, rat and sheep) and the resulting alignment was then used for model building with MODELLER.
Subsequently the docking software GOLD was used to dock 6 PGP inhibitors [2] with known IC50 values for rat P-gp into the rat homology model. Frequently, interactions were observed with residues F335 located in TM helix 6 and F983 in TM helix 12. Also residue T306 was involved, whose human analogue T307 was shown to be important in ligand interactions [3]. Comparison of the rankings obtained with GOLDScore as scoring function and experimental activity was quite promising. The docking was able to correctly assign the ranking for all but one of the experimentally tested compounds, only ranks 3 and 4 were switched.

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PO - 098

Pharmacoinformatic approaches to predict substrates and inhibitors of ABCB11/BSEP

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There is increasing evidence that cholestatic forms of drug-induced liver damage result from a drug- or metabolite-mediated inhibition of hepatobiliary transporter systems, such as ABCB1, ABCB4, ABCB11, and ABCG2. In addition to their key role in determining hepatic drug exposure and clearance, the coordinated action of these ABC-transporters is essential for bile formation and the biliary secretion of xenobiotics. Any drug-mediated functional disturbance of these processes can lead to an intracellular accumulation of potentially harmful bile constituents and result in the development of cholestatic liver cell damage [1].
More than 30 years ago ABCB1/P-glycoprotein was discovered to be responsible for multidrug resistance (MDR) in tumor cells [2].
In 1995, ABCB11/BSEP/Spgp was originally discovered as a sister gene of P-glycoprotein. Further studies showed that ABCB11 transports various bile salts and that disruption of the ABCB11 gene in mice caused persistent intrahepatic cholestasis [3]. Because inhibition of BSEP or transportation of drugs have severe consequences such as cholestasis or drug resistance, we aim at developing in silico models for prediction of substrates and inhibitors of ABCB11.
Some substances have already been tested *in vitro* by Wang et al. using fluorescent substrates of BSEP as markers [4]. In addition to several compounds asserted to be inhibitors, the compounds suggested to be ABCB11 substrates might also inhibit ABCB11 competitively. Hirano et al. developed in vitro high-speed screening and quantitative structure-activity relationship (QSAR) analysis methods to investigate the interaction with a variety of drugs [3]. QSAR analysis has been performed using chemical fragmentation codes generated by the Markush TOPFRAG program. However, both data sets are quite small and thus cover only a limited chemical space. Therefore, we used the data set published by Szakács et al. which comprises substrate/non-substrate data for a set of 1.400 compounds from the NCI60 screening panel [5].
The inherent promiscuity of ABC-transporters caused by multiple separate or overlapping binding sites renders it difficult to apply traditional molecular modelling methods. In addition, the available data sets generally are highly imbalanced with respect to class population. Thus, we applied cost sensitive bagging combined with random forest, decision tree, and k-nearest neighbour as classifier. All methods showed acceptable performance with the decision tree being slightly superior to the other two methods (overall accuracy 0.71, MCC-value 0.44, sensitivity 0.75 and specificity 0.69). This model will serve as basis for extending our studies towards the large NCI60 data set, which comprises more than 33.000 compounds.

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PO - 099

Applying molecular dynamics simulations in order to elucidate the molecular basis of subtype selectivity of the Gamma-Aminobutyric Acid Transporter (GAT)

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The human transporters for the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) hGAT-1, 2, and 3, and hBGT-1 belong to the neurotransmitter-sodium symporter (NSS) family of membrane transport proteins. hGAT-1 has been a target for the design of antiepileptic therapeutics [1], with Tiagabine (Gabitril®) being the only GAT inhibitor on the market. The lack of specific inhibitors for the other hGAT subtypes, results from a still missing detailed understanding of the molecular basis of drug-transporter interactions of the respective subtypes.
With an integrated approach - molecular modelling and data retrieval from literature – we aim at elucidating plausible binding modes for ligands (in the occluded as well as in the outward-facing conformation) of each subtype, respectively. In short terms, we built up homology models for hGAT-1 in the occluded and outward-facing conformation based on the respective high resolution structures of the leucine transporter of Aquifex aeolicus (LeuT) (pdb-codes: 2A65 and 3F3A). Afterwards, the natural substrate GABA was docked into the occluded state model and Tiagabine into the outward-facing model by making use of the Induced Fit Docking module of Schrödinger, LLC. Both models were further subject to extensive Molecular Dynamics (MD) simulation studies, which in a first step served us to evaluate the quality and stability of the obtained complexes. For this purpose, the complexes were inserted into a preequilibrated and solvated POPC membrane by making use of the g_membed routine (implemented into GROMACS) and ions were added to the solute to neutralize excess charges and adjust the final ion concentration of 150 mM. For all the following minimization, equilibration and production runs the GROMACS software package Version 4.5.3 was used and the Gromos96 53A6 united-atom force field and periodic boundary conditions were applied.
The complexes reached their equilibrium after 70 and 80ns, respectively (according to protein backbone RMSD- and ligand RMSD curves). The resulting binding modes of GABA and Tiagabine are in good accordance with the ones described in literature [2]. The availability of a stable trajectory for both the occluded (~130ns) and the open-to-out state of hGAT1 (~120ns) now discloses new possibilities of extracting representative structures to sample the whole conformational space. First results from Molecular Docking into those snapshots revealed their ability to reproduce the binding modes shown by MD. Subsequently, ligands retrieved from literature (with an experimentally measured binding affinity) will be docked into the equilibrated structures from MD simulations, with the final aim to disclose their (common) binding mode(s). Additionally, our studies will be extended to the other hGAT subtypes so as to elucidate the secret of subtype selectivity of GABA Transporters.

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Nanomedicine

PO - 100

Development of coenzyme Q10 loaded ultra-small nanostructured lipid carriers (NLC)

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Coenzyme Q10 (Q10) is a vitamin like, poorly water soluble compound with strong antioxidant capacity, making it an interesting drug for treating oxidative stress. However, the bioavailability upon oral administration is very limited. Lipid nanoparticles, e.g. solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) can be exploited to increase the bioavailability of lipophilic, poorly water soluble drug [1]. These particles are typically produced by hot high pressure homogenization, leading to particle size in the range from about 150 to 300 nm. For both SLN and NLC, it is known that the particle size is directly related to their bioactivity, i.e. the smaller size of the nanoparticles, the bioavailability is higher. Preferentially particles should be < 100 nm [2]. Therefore, the aim of this study was to develop a method to produce Q10 loaded lipid nanoparticles with sizes well below 100 nm.
For this investigation, the method of the determination of required HLB value, known from the development of emulsions was exploited. In addition the production parameters e.g. number of homogenisation cycles; homogenization pressure, concentration of lipid phase and concentration of stabilizer were varied to identify the optimal production conditions, leading to ultra-small sized and physically stable particles.
Required HLB values were obtained using Davies' equation and Griffin's method. To obtain surfactant solutions with these HLB values, mixtures of Span 20 and Tween 80 were prepared and used to produce NLC (without Q10). The size of these NLC was 53.98±0.589 nm (Davies equation) and 45.12±0.757 nm (Griffin`s method) and the narrow polydispersity index were 0.157±0.032 and 0.167±0.040, respectively. The best production conditions were found to be 800 bar homogenization pressure and three homogenization cycles at 75°C. The obtained ultra-small NLCs were stable for at least 3 month. These optimized conditions were used to produce 0.5% Q10 loaded NLC. The Q10 loaded NLC possessed a particle size (49.97±1.091nm) and a polydispersity index (0.167±0.039) similar to the unloaded NLC.
In conclusion, using the required HLB method it was possible to develop ultra-small sized lipid nanoparticles with a size well below 100 nm. Drug loading was possible and did not alter the size of the particles, when compared to the non-loaded NLC. The production conditions are very similar to the conventional production methods, thus large scale production will be possible.

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PO - 101

Development of a mucoadhesive drug delivery system for a targeted drug release in the bladder

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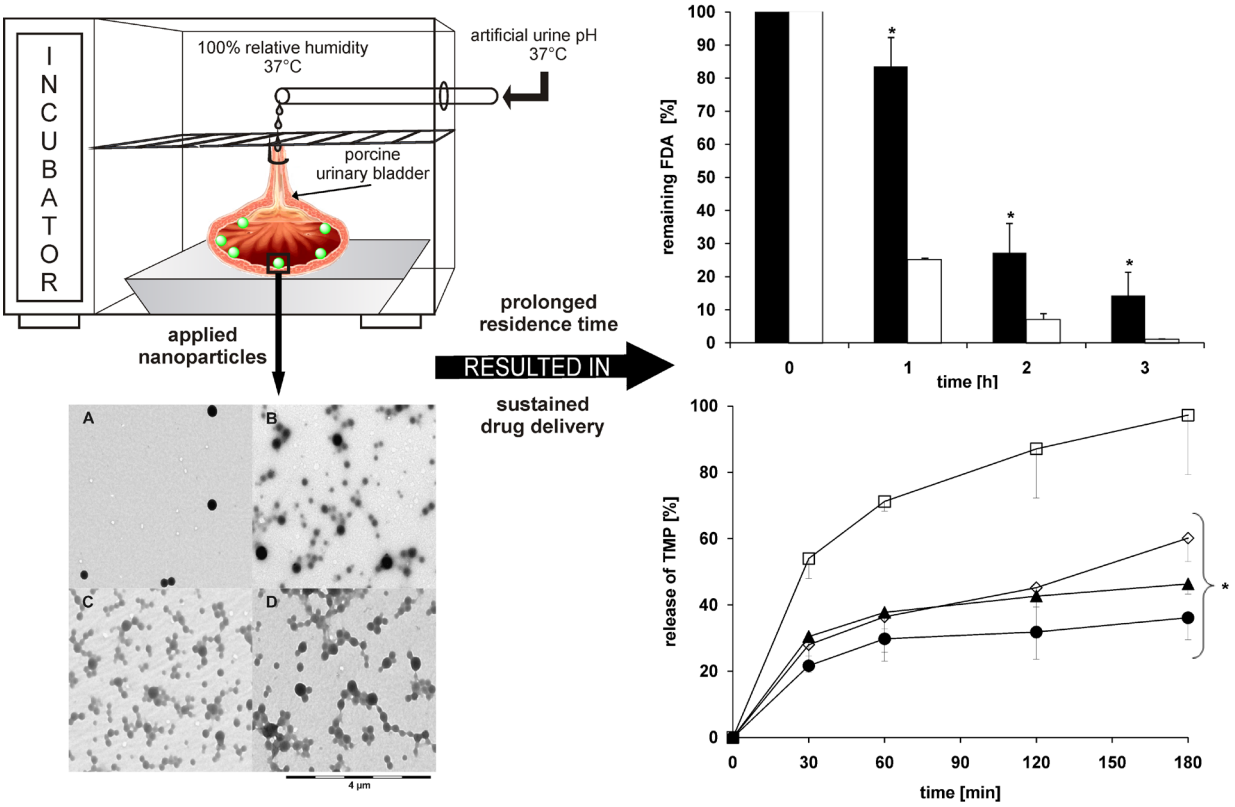
Purpose of the present study was the development of a mucoadhesive nanoparticulate drug delivery system for local use in intravesical therapy of interstitial cystitis, since only a small fraction of drug actually reaches the affected site by conventional treatment of bladder diseases via systemic administration.
Methods. Chitosan-thioglycolic acid (chitosan-TGA) nanoparticles (NP) and unmodified chitosan NP were formed via ionic gelation with tripolyphosphate (TPP). Trimethoprim (TMP) was incorporated during the preparation process of NP. Thereafter, the mucoadhesive properties of NP were determined in porcine urinary bladders and the release of TMP among simulated conditions with artificial urine was evaluated.
Results. The particles size ranged from 183 nm to 266 nm with a positive zeta potential of +7 to +13 mV. Under optimized conditions the encapsulation efficiency of TMP was 37%. The adhesion of prehydrated chitosan-TGA NP on the urinary bladder mucosa under continuous urine voiding was 14-fold higher in comparison to unmodified chitosan NP. Release studies indicated a more sustained TMP release from covalently cross linked particles in comparison to unmodified chitosan-TPP NP over a period of 3 h in artificial urine at 37°C.
Conclusion. Utilizing the method described here, chitosan-TGA NP might be a useful tool for local intravesical drug delivery in the urinary bladder. In the near future in vivo studies will be performed to demonstrate the whole efficiency of the drug delivery system.



Graphical Abstract

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PO - 102

Influence of the molecular weights of linear poly(ethylene glycol)-poly(ethylene imine)–copolymers on the delivery of nucleic acids

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The delivery of nucleic acids with polymers has become a promising tool for the modulation of gene expression inside cells. Branched poly(ethylene imine) (bPEI) is still the gold standard, but also shows a significant toxicity to cells. In order to overcome this problem derivatives of this polymer were proposed, especially block copolymers from linear PEIs of low molecular weight and poly(ethylene glycol) (PEG). While all other approaches created branched, graft copolymers, we propose strictly linear PEG-PEI block copolymers. We hypothesize that this strategy is more favourable for the formation of well-defined and better shielded nanocarriers because the sterically isolated PEG does not interfere with the interaction between PEI and the nucleic acid. To proof our hypothesis we synthesized a library of PEG-PEI-copolymers and varied the molecular weight of the PEI part as well as the weight of the PEG part. We formed polyplexes (complexes of nucleic acids and polymers) with these copolymers and tested their stability by gel retardation and competition assays. Furthermore, we determined their size and zeta potential to see if the polyplexes are small and nearly uncharged which indicates well shielded stable particles. Afterwards we had a look at the polyplex uptake, transfection efficiency and toxicity in cell culture.

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Designed Ankyrin Repeat Protein (DARPin) fusion proteins with basic peptides for targeted delivery of siRNA to tumor cells

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DARPins (Designed Ankyrin Repeat Proteins) are a novel class of non Ig-G binding proteins derived from natural ankyrin repeat proteins consisting of repetitive structural units [1]. Their architecture displays a large interaction surface for target binding. The high stability, small size and favourable biophysical properties of DARPins result in high expression yields of soluble active proteins in *E. coli* and facilitate engineering technologies and the introduction of site-specific modifications and conjugations [2].
In a proof of concept study, a fusion protein of an EpCAM specific DARPin and a highly basic peptide was generated as a nanoscale delivery system capable of complexing several siRNA molecules by charge interaction without losing binding activity and specificity for EpCAM. The generated delivery system specifically and effectively delivered the siRNA cargo to antigen positive cells and resulted in knockdown of the targeted mRNA [3].
In order to improve delivery efficiency, both antigen affinity and siRNA loading capacity are being optimized. Using combinatorial ankyrin repeat protein libraries and ribosome display, novel DARPin binders were engineered against the extracellular domain of the carcinoma-associated epithelial cell adhesion molecule (EpCAM). The new binder Ec1 with high antigen specificity and excellent affinity in the low nanomolar range was selected for further development.
Several fusion proteins with varying number and density of basic amino acids (arginine and/ or lysines) were engineered and expressed in high yields in *E. coli*. In order to remove bacterial nucleic acids persistently sticking to the oligonucleotide binding motive, the fusion proteins were purified under denaturing conditions with

subsequent renaturation to yield their native form. siRNA oligonucleotides against the well established cancer-associated-gene *bcl-2* and *galectin-1*, which has been implicated in carcinogenesis and tumor progression, were designed and synthesized.
Since the loading capacity of the fusion protein for its cargo is expected to be the critical parameter for sufficient cellular uptake and target down-regulation, different protein (N) / siRNA (P) complex ratios were investigated. All Ec1 fusion proteins resulted in efficient complexation by electrostatic interaction between the negatively charged siRNA and the positively charged amino acids at low N / P charge ratios. Peptides with higher charge density complexed siRNA oligonucleotides more efficiently.
In vitro analyses in EpCAM positive cells were performed using a luciferase reporter assay to investigate the influence of complexation efficiency and the effects of N / P ratio on target down-regulation. The results underline the suitability of DARPin fusion proteins for targeted siRNA delivery and highlight the important properties essential for targeted delivery systems.

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PO - 104

Exchange of temoporfin between different liposomal formulations and human plasma lipoproteins

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Photodynamic therapy (PDT) is one of a multitude of concepts in the field of anticancer drugs and therapy gaining in importance since cancer is still the second most frequent cause of death in Germany today. PDT is based on the co-action of a non-darktoxic photosensitiser, red visible light and oxygen. Temoporfin (mTHPC) is a highly potent second-generation photosensitiser, which has been granted an European approval for palliative treatment of patients with advanced head and neck cancer. Liposomes as colloidal carrier mTHPC formulation was shown to be superior to the commercial formulation Foscan® containing the drug dissolved in a mixture of ethanol and propylene glycol[1]. In the study presented here the interactions of temoporfin-loaded liposomes with human plasma lipoproteins were analysed employing fast protein liquid chromatography (FPLC).
The liposomes were prepared by thin lipid film hydration with isotonic glucose solution (5 % w/w) followed by extrusion through polycarbonate membranes with a nominal pore size of 200 nm. For liposome preparation lipids with different phase transition temperature as well as different functional lipids for surface modification of the liposomes were used. Particle size was determined by photon correlation spectroscopy. All liposomes used for these experiments were labelled by [1a,2a (n)^{3}H]-cholesteryloleyl-ether and a mixture of [5,10,15,20-^{14}C] temoporfin and non-labeled temoporfin was used. Due to this double label it was possible to determine both the content of the liposomes and the incorporated drug in each separated lipoprotein fraction. The plasma was obtained by blood donation of human fasting females and centrifugation at 4500 g on an Eppendorf 5804 R centrifuge at 25°C for ten minutes. Aliquots were frozen to –20°C and thawed on the day they were used. The experimental set-up[2,3] was composed of a peristaltic pump (Pharmacia P1), which was linked to a Sepharose CL 6B (1.4 cm diameter x 45 cm length) column, an injection valve (V7, Pharmacia), to which a sample loop of 500 µl volume had been applied, and a fraction collector (Pharmacia LKB RediFrac). The running buffer (mobile phase) consisted of 150 mM NaCl/10 mM Tris, preserved with 0.03 % sodium azide and adjusted to pH 7.4. In order to investigate the exchange of temoporfin between liposomes and plasma lipoproteins 250 µl plasma and 37.5 µl liposomal formulation (corresponding to 1 µmol lipid) were mixed together and adjusted to a final volume of 500 µl by the addition of running buffer. After incubation at 37°C for 30 min separation was performed at room temperature. The flow rate was set to 12 ml/h resulting in a total run time of 6 h. Fractions of 1.5 ml were

collected and absorption at 280 nm was measured in order to determine the lipoprotein content. Additionally the concentration of the liposomes as well as temoporfin was calculated for each fraction with the help of radioactivity measurements (Tricarb 2100 TR, Perkin Elmer).
In earlier in vivo experiments the circulation profile of liposomal temoporfin was found to be dependent on the membrane fluidity as well as the functionality of the membrane lipids. As one possible reason we assumed that temoporfin was – at least partly – transferred to lipoproteins. To which extend this transfer effectively takes place and the potential influence of the lipid composition on the transfer was investigated in this study.

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PO - 105

Combining two technologies: Multifunctional polymers and self-nanoemulsifying drug delivery systems (SNEDDSs)

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To enhance the bioavailability of insulin, a self-nanoemulsifying drug delivery system (SNEDDS) based on thiolated chitosan (chitosan-TGA) with incorporated insulin was developed. Series of SNEDDSs were prepared and their emulsification behaviors were visually observed. All SNEDDS formulations were characterized by particle size, self-emulsifying time, entrapment efficiency and drug release. Pseudo-ternary phase diagrams were constructed in the absence or presence of insulin and polymer to identify the self-nanoemulsion regions and to optimize a ratio between oil and surfactant mixture in the SNEDDSs. The optimized SNEDDS consists of 65% miglyol 840, 25% cremophor EL and 10% co-solvents (a mixture of DMSO and glycerol). SNEDDS in the presence or absence of insulin were spherical with a size range between 91 – 119 nm (Fig.1) and the self-emulsifying time was observed within 30 sec. As illustrated in Table 1, entrapment efficiency of insulin increased to 98% when thiolated chitosan was employed in comparison to the insulin control with 89% encapsulated insulin. The release of insulin from SNEDDSs was measured in phosphate buffer, pH 6.8. At the beginning, release of insulin from insulin/chitosan-TGA system was slow, but markedly increased as a function of time to 95% of the total insulin amount. By contrary, an initial rapid rate of release from insulin SNEDDS was observed followed by a period of slower release rate (30 – 240 min) equivalent to 60% of the initial value. Within 3 days of stability studies, the droplet size of particles in buffer, pH 6.8 did not alter. In addition, viability of Caco-2 cells incubated with SNEDDSs containing thiolated and unmodified chitosan assessed by resazurin assay was not influenced. More than 80% of cells were viable after 6 h incubation. In vivo studies confirmed the potential of these thiolated SNEDDS by a 3.3-fold increased relative bioavailability of insulin compared to insulin solution and a 1.7-fold improvement in comparison to a chitosan-TGA solution. Accordingly to these results, it seems that a combination of multifunctional polymers and SNEDDSs can succeed in the generation of effective drug delivery systems for oral administration. So far, to our knowledge, studies combining these two technologies have not been undertaken.

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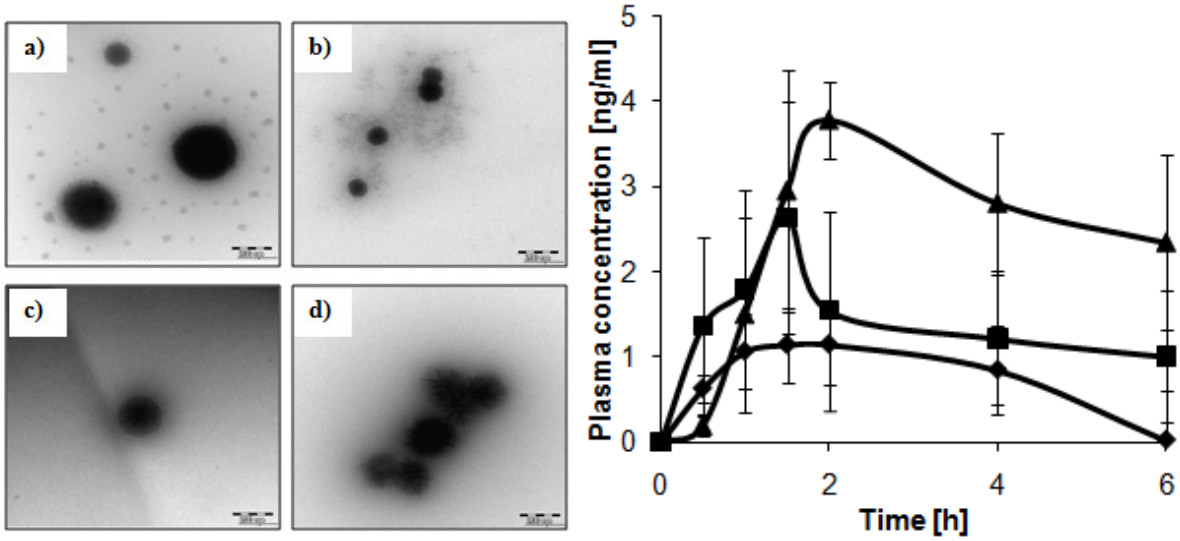

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Time dependent fluorescence studies of novel cationic liposomes as gene delivery systems in COS-7 cells

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Gene transfection means the incorporation of genetic material – e.g. plasmid DNA, oligonucleodides, siRNA – into eukaryotic cells. The aim is the treatment of inherited and acquired diseases by substitution, inhibition, or enhancement of gene functions. For delivering genetic material into host cells a vector is required which can be classified into viral and non-viral systems. Non-viral vehicles – such as cationic liposomes – have several advantages compared with their viral counterparts, including low immune response, the ability to transfer large DNA molecules, and the reproducible production in large scales of constant quality. However, the lower efficiency in comparison to the viral vectors is still the major disadvantage.
To improve the efficiency of cationic liposome mediated gene transfection it is necessary to investigate the interaction of these vesicles with cells and the intracellular mechanism of these systems. Especially the cellular uptake of complexes composed of cationic liposomes and polyanionic DNA (lipoplex) is an essential step for gene transfection. Furthermore, the incorporation and the intracellular transport along with the release of the genetic material from the vesicle have to be investigated. Therefore, novel cationic liposomes – synthesised in our research group – were labelled using the fluorescence dye ATTORho6G® and were mixed with fluorescence labelled plasmid DNA after forming liposomes. The incubation of these lipoplexes on COS-7 cells was stopped at different times in order to study the fluorescence intensity via laser scanning microscopy.
With this strategy we might have the chance to visualize the pathway of the lipoplexes within the cell after transfection and to show the location of the cationic lipids as well as the DNA in a time-resolve manner.

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Small scale preparation of nanoemulsions by extrusion through polycarbonate membranes

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Nanoemulsions are transparent or transluscent systems typically in the range of 20-200 nm [1]. The O/W nanoemulsions are designed for the incorporation of poorly water soluble substances [2]. Unlike microemulsions, they are not thermodynamically stable but are only kinetically stable. Being non-equilibrium systems, they cannot be formed spontaneously instead requires high energy input, which can be achieved by using high-shear stirrers, high pressure homogenizers or ultrasound generators. The high energy input leads to deforming forces that are able to break the droplets into smaller ones, provided the Laplace pressure is overcome [1]. However, these methods allow the preparation of nanoemulsion at a minimum volume of about 10 ml, which makes the cellular studies using radioactive or fluorescence labelled nanoemulsions relatively expensive.
Detergent removal method has been used successfully to prepare unilamellar liposomes. Detergents like bile salts as physiological detergents are non-toxic even for parenteral application at low concentration. Also, they are inexpensive and can be used to prepare unilamellar liposomes [3]. In our study, a combination of hand extrusion and detergent removal methods were adapted to prepare nanoemulsions.
The main objective of this study is to prepare and compare nanoemulsions in small (for cellular studies) and large (high pressure homogenizer) scales. Nanomemulsions are characterized by measuring their size and zeta-potential using Photon Correlation Spectroscopy and Cryo-TEM pictures. The stability of the nanoemulsions is also studied on the basis of change in the size and zeta-potential during the storage period under different conditions.
In future, different ligands or antibodies will be used to study their cellular uptake by different cell lines. Upon successful uptake of drug loaded nanoemulsions, they will be finally targeted to the cells of interest, i.e., immune cells or tumour cells to study the anticancer activity of the loaded drug.

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PO - 108

In-vitro investigation of nanosuspension delivery out of micro-osmotic pumps: Parameters influencing release behaviour

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Since there are approximately 40% of newly developed active pharmaceutical ingredients poorly soluble, nano-suspensions have emerged as an important tool in drug delivery in the past decades to overcome solubility problems[1,2]. Alzet® pumps are widely used in experimental pharmacology and present an interesting tool for the sustained and chronical intraperitoneal or subcutaneous release of nanosuspensions[3]. In this work we elucidated the influence of the physicochemical parameters (1) formulation viscosity and (2) pump orifice position on the release of fenofibrate nanosuspensions from Alzet® pumps by a simple *in-vitro* test method. Pumps containing different formulations were placed with varying positions in beaker glasses and the amount of substance released was determined by HPLC/UV analysis. Nanosuspension particles showed burst release out of the pumps, which

was diminished by increasing the viscosity of the formulation. Also the positioning of the pump orifice exhibited a strong influence on the release behaviour. The results exhibit the need of *in-vitro*-experiments for determining the release profiles of osmotic pumps. The relevance of the findings for in-vivo studies will be further investigated.

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PO - 109

Effect of γ-Cyclodextrin on In Vitro skin permeation of steroidal drugs from nanoemulsions: Impact of experimental setup

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In the field of topical formulation development, cyclodextrins (CDs) are mainly used to complex lipophilic compounds to improve their aqueous solubility or to stabilise them against chemical degradation. However, CDs may likewise be employed to influence the skin permeation of lipophilic actives [1]. Recently, the in vitro skin permeation of steroidal drugs from fluid nanoemulsions through porcine skin was found to be remarkably enhanced by incorporation of γ-CD [2,3]. As a next step, it should be clarified whether this effect can be reproduced under different experimental in vitro setups or whether it was caused by the specific conditions of the performed studies, i.e. infinite dose application, occlusion of the skin and aqueous saline as a receptor medium. To this end, extensive in vitro skin permeation studies were performed with corresponding formulations containing fludrocortisone acetate and variable amounts of γ-CD. Experimental conditions such as the dose of application, occlusion conditions, pre-treatment of the skin and the nature of the receptor medium were systematically modified. In addition, comparative tape stripping experiments were performed in order to provide a more realistic setup and to avoid hydration effects caused by the prolonged exposure of the excised skin to the aqueous receptor medium. The results showed that a positive effect of γ-CD was indeed noticeable in the diffusion cell studies which was strongly magnified by infinite dose-application. It may be assumed that these results represent an overestimation of the enhancement potential of the CD in everyday application of topical formulations. In vitro tape stripping confirmed the observed tendencies, but neither the effect of 0.5% nor 1% (w/w) of γ-CD reached statistically significant values. It remains to be clarified whether the enhancement potential of CDs can be employed for infinite dose-application systems such as transdermal patches.

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PO - 110

Analysis of crystalline state of lipid nanoparticles: Influence of water content on results

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Thermal analyses by Differential Scanning Calorimetry (DSC) are used to investigate the crystallinity of lipid nanoparticles and obtain the information about lipid matrix in early stage research. The amount of sample analysed should be standardized to contain 1 to 2 mg lipid nanoparticles. Hence, the samples with low NLC content contained more water than the samples with high NLC content. The aim of this study was to investigate the influence of water content of nanostructured lipid carrier (NLC) dispersions on DSC results.
Dynasan (D) and Softisan (S) NLC formulations containing 20% (w/w) lipid phase were prepared via hot high-pressure homogenization. NLC dispersions with 5 and 10% (w/w) lipid phase were obtained by diluting these original samples with different amounts of water. Thus, the size and the composition of the particles were similar for each formulation. The amount of sample analysed was standardized to contain 1.5 mg lipid nanoparticles (i.e. 7.5, 15 and 30 mg for 20, 10 and 5% NLC dispersions, respectively).
For both types of NLCs a decrease in the melting enthalpy was observed with increasing the water content. Another observation was the broadening of the peaks with an increasing in water content. The width of the melting peak at 63°C was 4.0, 4.1 and 4.9°C for 20, 10 and 5% D NLC. The decrease of enthalpies and expansions of peaks caused the total disappearing of some thermal events and thus would lead to misleading interpretation of the data. The melting peak at 39.5C°, which had an enthalpy of 0.67 J/g for 20% D NLC, was undetectable for 5 and 10% dispersions. Additionally, no melting event was found for 5% S NLC, although 20% dispersion showed two melting peaks at 38.7 and 52.2°C, which had 2.43 and 1.26 J/g as enthalpy values, respectively.
As conclusion, the water content of the sample can influence the DSC result. For a correct comparison of the results, analysed dispersions should have a similar water content. In addition, to detect the thermal events for small quantities, water content should be reduced or other methods should be used instead.

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Nanoparticulate drug delivery systems in native mucus: improvement of penetration behaviour

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Gastrointestinal mucus is the first barrier through which orally delivered drug must diffuse. Mucus clearance mechanisms display a critical obstacle to drugs delivered to mucosal surfaces. Therefore, drug carriers must be capable of penetrating and crossing mucus barriers designed to prevent the transport of foreign pathogens (1). Referring to Olmsted et al. small capsid viruses are capable of diffusing through the protective mucus due to their dense cover with both positive and negative charges (2). Therefore, the aim of this study was to evaluate the influence of surface chemistry on particle transport rates through native intestinal porcine mucus using chitosan (CHIT), polyacrylic acid (PAA) and chitosan-polyacrylic acid (CHIT-PAA) nanoparticles.
Polyacrylic acid and chitosan nanoparticles were obtained by ionic gelation with calcium-ions and tripolyphosphate, respectively. CHIT-PAA particles pere prepared on the basis of ionic interactions between the two polymers. Afterwards all particles were fluorescence labelled by fluorescein diacetate (FDA). Nanoparticles were characterized regarding size, zetapotential and drug load. Additionally surface hydrophilicity was examined using rose bengal dye.
For diffusion studies ussing type chambers were modified by snapwell inserts to maintain a vertical mucus layer between polycarbonate filters. One percent particle suspension were placed into the donor chamber and samples were withdrawn from the acceptor compartments every 60 min over an observation period of 5 hours.
Using the preparation methods described above, PAA, CHIT and PAA-CHIT nanoparticles (mean diameter between 158 and 292 nm) can be generated. The surface charge of PAA particles was determined to be negative, CHIT nanoparticles were positively charged and PAA-CHIT nanoparticles have shown a neutral surface charge. Determination of rose bengal binding constant K have shown that PAA-CHIT nanoparticles display the lowest hydrophilicity (K = 0.35) compared to PAA and CHIT nanoparticles which provide a more hydrophilic surface. Diffusion studies indicate a strong influence of surface charge on particle mobility in native intestinal porcine mucus. The neutral CHIT-PAA nanoparticles have shown highest transport rates (3 ± 0.3 %) compared to stronger surface charged nanoparticles. All results are summarized in Table 1.

Although particle size is reported as a crucial factor concerning penetration properties (3), for PAA-CHIT nanoparticles, displaying 1.8-fold bigger size than PAA particles, highest diffusion properties could be observed. In conclusion, the surface chemistry is probably the most important physicochemical characteristic influencing the diffusion rate of nanoparticles. The presence of both positive and negative charges on PAA-CHIT nanoparticle surface might minimize affinity bonds to mucins.

Table 1. Characterization and transport rate through native porcine mucus of generated polyacrylic acid, chitosan and polyacrylic acid-chitosan nanoparticles.

nanoparticles	size [nm]	zetapotential [mV]	drug load FDA (%)	rose bengal constant K [ml/µg]	transport rate (%) after 5h
PAA	158 ± 22	-15 ± 2	8.4	0.03	1.4 ± 0.2
CHIT	276 ± 12	22 ± 2	7.7	0.14	1.0 ± 0.2
PAA-CHIT	292 ± 17	0.8 ± 0.2	8.1	0.35	3.0 ± 0.3

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PO - 112

Simple screening method of surfactants/stabilizers to optimize nanosuspension formulation

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The role of surfactant or stabilizer is a very important basis in formulation of long-term stable nanosuspension. Up to this day, screening procedure of surfactant or stabilizer to be applied in the formulation is mostly based on formulator's experience, relatively empirical and also time consuming. To overcome this problem, a systematically and widely applicable screening procedure for wet bead milling based on design of experiment (DoE) was developed. In this study, production of small scale nanosuspension was prepared using low energy wet bead milling of ytrrium stabilized milling beads (diameter 0.5 mm) with electric stirring system at 900 rpm. Milling was carried out up to 72h and samples were drawn every 24h to evaluate nanosuspension formed. Various surfactants and stabilizers at 3 different concentrations (low, medium, high) were examined using ibuprofen and resveratrol as model compound. Nanosuspensions were characterized using photon correlation spectroscopy (PCS), laser diffractometry (LD), light polarized microscopy and laser doppler anemometry (zeta potential). Short term stability was observed at room temperature and 8°C for 2 weeks after milling. Results shows that best particle size obtained for ibuprofen was 300 nm and 400 with PVA and SDS,respectively. In contrast, all of the surfactant/stabilizer used with resveratrol resulted particle size below 200nm for the same treatment. Electrostatic stabilization of SDS led to better short term stability of ibuprofen compared to the weak electrosteric stabilization of the PVA/ibuprofen system. In the case of resveratrol, both SDS and PVA were able to retain particle size of the nanosuspension. In conclusion, this relatively simple but effective screening method can be used to identify optimal surfactant/stabilizer for the production of nanosuspension. In addition, applying the DoE principles helped to reduce number of experiments performed in order to identify the best surfactant/stabilizer.

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A simple but effective formulation for the treatment of inflammatory ophthalmic diseases

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The delivery of poorly water soluble drugs like Cyclosporine A or several corticoids to the anterior eye is a major challenge in the treatment of inflammatory diseases like the dry eye syndrome. Even though these drugs are known to be highly efficient, they, unfortunately, suffer from their low solubility in aqueous media [1]. Oily eye drops, ointments, suspension or emulsions are typical ways of alternative administration, but they all suffer from problems like low stability, complex manufacturing or low bioavailability [2]. We hypothesise that by using a suitable non-ionic surfactant, a lipophilic drug can easily be processed to a transparent colloidal dispersion that does not suffer from these problems.
For our investigations we selected three different types of non ionic surfactants, Solutol® HS15, a Macrogol-15-hydroxystearate, Sympatens AS, a Macrogol-20-stearylether, and Sympatens ACS, a mixture of Macrogol-20-stearylether and macrogol-20-cetylether. Cyclosporine A and Budesonid were used as drug substances. Using high performance liquid chromatography we observed outstanding solubility of Cyclosporine A in water with all three surfactants. In a first stability study the systems with Sympatens AS and Sympatens ACS proved to be stable. Using dynamic light scattering we could show that the size of the micellar structures is about 10nm with a polydispersity index – a gauge for the width of a particle distribution – below 0,1.
In-vitro cell experiments with primary human epithelial cornea cells showed excellent tolerability of a 0,05% Budesonid solution with 10% Solutol compared to a reference solution (Na-hyaluronate eye drops).
Overall we could show that suitable non-ionic solubilizers are ideal for the manufacturing of simple, novel aqueous formulations for lipophilic drugs and a promising approach towards the causal therapy of inflammatory ophthalmic diseases.

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PO - 114

Nanocrystals in dermal formulations - from the academic idea to the market

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Nanocrystals are a well known formulation approach for poorly soluble drugs [1]. Several oral products are already in the pharmaceutical market since 2000. However, the dermal application route was completely overseen. The first cosmetic nanocrystal products were launched in 2007, in the line Juvedical by Juvena Switzerland, and by la prairie group in 2009 (e.g. platinum rare, cream and serum). The idea behind was to use the increased saturation solubility of nanocrystals for better penetration into the skin. The cosmetic products launched are based on the poorly soluble plant antioxidants rutin (Juvedical) and hesperidin (platinum rare). The antioxidative effect of nanocrystals and their water soluble glycoside active was studied in human by measuring not skin concentrations but the biological effects, i.e. the sun protection factor (SPF) and fibroblast condition. Rutin and hesperidin nanocrystals increased the SPF (59% and 36%, resp.), and were superior to the

synthesized water soluble rutin glycoside despite having a 500 times lower dissolved concentration. The increase in SPF compared to the water-soluble rutin derivative was 2 times higher, that means the nanocrystals of the poorly soluble molecule can be considered – simplified – 2x500=1,000 fold more active than the hydrophilic water-soluble derivative. This confirmed that chemical derivatization is not superior for better performance. The volunteer's skin biopsies showed that the fibroblasts were protected against UV damage when nanocrystal formulations were applied. Meanwhile apigenin and hesperetin nanocrystals (300 nm) were developed which doubled the in vitro antioxidant activity compared to the coarse powder. The nanocrystal technology allows to use new groups of plant molecules which were not dermally applicable before due to their low solubility, cosmetically but also pharmaceutically. After the successful oral products, pharmaceutical dermal products are predicted to come to the market.

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PO - 115

Target-nanoparticles – enhancer-drug carriers for local radiotherapy of cancer

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Enhancers, e.g. heavy metal chelates, increase the therapeutic effect of radiation and decrease side effects by formation of secondary radiation products upon fluorescence-like excitation by a therapy beam. The effects can be further enforced by bio-nanoparticles as enhancer-drug carriers to the effect location inside the cancer cells (DNA).
Our therapeutic nanoparticles for enhanced radiotherapy are combined from lipids, bio-polymers and magnetic iron oxides [1,2]. The particles of typically 100 nm size carry an enhancer load of lanthanide chelates or boron as radiation absorption target and chemotherapeutics, e.g. Gd-DTPA or cis-Platin derivatives, as proliferation inhibitors.
A large part of the development is done with target liposomes [1,3], which contain surface signals for cellular uptake, and the drug load as entrapped solution. A premature uptake of the therapeutic liposomes is avoided by novel stealth lipids bearing a polyglycerol head [4]. Further target nanoparticles contain polymer entities and poly-ferrofluids for magnetic drug targeting MDT [1,2]. The nanoparticles carry up to 10^7 molecules of the enhancer per particle. They are characterized by DLS, EM, neutron and x-ray scattering (SANS, SAXS, ASAXS) and nano-dissolution for drug release. A specific cancer cell targeting is enforced by ligand-lipid signalling with a combination of lipid rafts (Cholesterol-Sphingomyelin) and signal-lipids. Therapy tests are done with cell cultures as tumor models and animal tests with neutrons, but mostly with photons at the ESRF synchrotron Grenoble and the radiotherapy clinics Mainz.

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Folic acid nanosuspension – Influence of particle size reduction on the solubility and dissolution velocity

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In the present study a folic acid nanosuspension was prepared by high pressure homogenization and the influence of particle size reduction on the saturation solubility and the dissolution velocity evaluated.
Folic acid suspension and nanosuspension were composed of 10% folic acid (DSM, The Nederland's), 3% Tween 20 (Cognis, Germany) and 87% MilliQ water. The particle size of the formulations was measured using a Mastersizer 2000 (Malvern, UK). The influence of modification changes of folic acid by high pressure homogenization was evaluated by x-ray diffraction. The saturation solubility and dissolution velocity of folic acid from suspension and nanospension were compared.
By high pressure homogenization a significant particle size reduction of folic acid was possible. By laser diffraction an LD 50 of 2.830 µm, LD 90 of 6.658 µm and an LD 95 of 8.476 µm were measured for a suspension of folic acid. For the nanosuspension an LD 50 of 0.418 µm, LD 90 of 0.906 µm and an LD 95 of 1.087 µm were obtained. By x-ray diffraction it could be confirmed that no polymorphic changes occurred reducing the particle size of folic acid by high pressure homogenization. Therefore, an influence polymorphic transition on the saturation solubility and dissolution velocity can be excluded. Reducing the particle size of folic acid from the micrometer range to the nanometer range led to an increase of the saturation solubility by 53.7%. The dissolution velocity of folic acid nanosuspension was significantly enhanced compared to folic acid suspension, i.e. after 5 min 78.6% of folic acid was dissolved from the nanosuspension but only 6.2% from the suspension.
Therefore, it can be concluded, that size reduction of folic acid leads to a significant increase in saturation solubility and dissolution rate being advantages for the application and bioavailability of the poorly soluble drug.

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Scale-up of thiomer-protamine nanoparticle production with a continuously operating microreactor

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Introduction: A challenging task for pharmaceutical nanotechnology is the scale-up of production processes. Because of changing parameters at larger volumes in batch reactors the achievement of appropriate nanoparticles turns out to be difficult or even impossible. This also applies for biodegradable, self-assembled nanoparticles consisting of protamine and polyacrylic acid-cysteine (= thiomer), which are designed for drug application on mucus covered parts of the body. As the introduction of a continuously operating microreactor has been shown to have many advantages for the scale-up of nanoparticle formation processes [1], this approach is investigated for the production of thiomer-protamine nanoparticles. After the development and characterization of these particles at the milliliter scale, the results of the particle characterization will be compared with those achieved from first scale-up experiments with a continuously operating microreactor.
Materials & Methods: The characterization of thiomer-protamine nanoparticles included size and zeta potential measurements, performed by Dynamic Light Scattering, Scanning Electron Microscopy and Laser Doppler

Velocimetry. The binding efficiency of the components was determined by fluorimetric and photometric experiments using OPA (= o-phthaldialdehyde) and 4-DPS (= 4,4'-dithiodipyridine).
Results: After defining the mass ratio thiomer:protamine 1:3 as most promising, narrow distributed particles of about 150 nm in diameter were produced at the milliliter scale. The zeta potential of these particles showed values around +30 mV indicating a stable nanosuspension. Indeed a suspension stability of at least 9 months was proven by long term studies. The investigation of the binding efficiency of the two components resulted in 40% (= 120 µg/ml) protamine and 96% (= 96 µg/ml) thiomer bound within the particles. Subsequent to their successful development and characterization at the milliliter scale, first scale-up experiments with a continuously operating microreactor were conducted. Depending on the applied flow rate, nanoparticles comparable to those produced at the milliliter scale were achieved, showing a size below 130 nm and a zeta potential around +40 mV.
Conclusion: Highlights of this presentation are the successful results concerning the production scale-up of thiomer-protamine nanoparticles achieved by a continuously operating microreactor.

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PO - 118

Receptor mediated targeting of nanoparticles to kidney podocytes

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It is a well-accepted fact that nanoparticles such as quantum dots (Qdots) can be eliminated from the body by renal excretion [1,2]. This passage of the renal filter allows for targeting kidney specific cell types such as podocytes. Renal podocytes are a highly attractive target for nanoparticles, since there are various inherited and acquired diseases that lead to podocyte dysfunction and subsequent renal failure. Nanoparticles can serve here as specifically directed drug delivery devices.
We investigated, if it was possible to bind nanoparticles to podocytes. In doing so, Qdots, as model nanoparticles, were modified with cyclo(RGDfC) allowing for the specific binding of nanoparticles to the αvβ3 integrin on podocytes.
Flow cytometry (FACS) experiments with isolated podocytes revealed that the cyclo(RGDfC) modified Qdots show a strong binding to the cells. In competitive displacement experiments using a surplus of free cyclo(RGDfC) nanoparticle binding to the cells was confirmed to be receptor-mediated. Confocal laser scanning microscopy (CLSM) further indicated that the nanoparticles were distributed in vesicular structures throughout the cytosol. This distribution pattern suggests an endocytosis of nanoparticles by podocytes, which is a prerequisite regarding an intracellular drug delivery. Further binding experiments with whole glomeruli maintaining podocytes in their natural 3-D environment confirmed these results.
In conclusion we could show that cyclo(RGDfC) coupled nanoparticles show a high potential for a receptor mediated binding to podocytes, which opens the possibility for a receptor specific nanoparticle targeting within the kidney.

Acknowledgements: This work was supported by DFG, Graduiertenkolleg 760 and DFG grant No GO565/17-1.
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PO - 119

Modulation of disulfide bonds for design of a mucoadhesive nanoparticulate peptide/protein drugs delivery system

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Within this study, nanoparticles were generated via ionic gelation using HEC-cysteamine with free thiol groups content of 1743 µmol/g. Tripolyphosphate (TPP) 0.2% (m/v) solution was directly added to the polymer solutions at the concentration of 1, 1.5 and 2% (m/v). Thereafter, HEC-cysteamine nanoparticles were covalently crosslinked via disulfide bonds by oxidation using varying concentration of H_2O_2 in the range of 21.5–645 µmol. Mucoadhesion studies were carried out using porcine intestinal mucosa whereas permeation studies were performed across Caco-2 cell monolayers using fluoreceinisothiocyanate-dextran 4 (FD4) as a fluorescence marker. Cellulase from *Trichoderma viride* (EC 3.2.1.4) was used for biodegradation studies. The mean diameter of the resulting nanoparticles was in the range of 262.4 ± 45.7–716 ± 162.5 nm. Due to the removal of TPP, the zeta potential of the nanoparticles was raised from -9.36 ± - 2.39 up to 9.71 ± 1.07. Since free thiol groups still remained on the oxidized nanoparticles in the range of 218–1074 µmol/g, their mucoadhesive properties still existed within 3 h of experimental period. The addition of the nanoparticles without oxidation resulted in the highest improvement with a 3.19-fold increase in FD4 transport as compared to buffer only. Generally, the more cross-linked the nanoparticles were, the higher was their stability. Accordingly, this nanoparticulate system leads to a promising tool for delivery of peptide/protein drugs targeted to the colon mucosa.

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Comparison of two novel, combinative particle size reduction methods for the production of ultrasmall drug nanocrystals

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The purpose of this study was to analyze systematically the parameters influencing the reduction effectiveness of a novel particle size reduction method which employs spray drying (SD) as bottom-up step for solvent evaporation and structure modification followed by high pressure homogenization (HPH) as top-down process. This reduction effectiveness was compared to the results of a prior study employing the freeze drying (FD)-HPH combinative technology. The poorly water soluble compound glibenclamide was used as a model compound. The influence of drug and surfactant concentrations during SD on the particle size reduction effectiveness of the HPH was studied. The drug was dissolved in ethanol to make a solution concentration of 1, 2 or 3% (w/w). The amounts of docusate sodium salt (DSS) in the ethanolic solution were 0%, 0.1% or 0.2% w/w. Then the ethanolic solution was spray dried using a Mini Spray Dryer B-290 coupled to an Inert Loop B-295 (Büchi Labortechnik AG, Switzerland). Morphology and solid state of the spray dried powders were analyzed by SEM, DSC and XRPD. Suspensions were produced dispersing 1% (w/w) glibenclamide (modified/unmodified) in

demineralized water. In the case of the spray dried powders without surfactant, docusate sodium salt (DSS) 0.2% (w/w) was added to make the drug dispersion before further processing. These suspensions were pre-homogenized for one minute at 9000 rpm using an Ultra-Turrax and then homogenized at high pressure (1500 bar, 20 cycles) using a Micron LAB 40 homogenizer (APV Systems, Germany). The particle size of the nanosuspensions was measured using both photon correlation spectroscopy (PCS) and laser diffractometry (LD). It was established that high API concentrations and high DSS concentrations resulted in more porous and flowable powders after SD, which resulted smallest particle size. It was found that the drug modification by means of SD can significantly improve the particle size reduction effectiveness of HPH. The nanosuspensions produced with the spray dried powders (high API, high surfactant concentrations) showed a very small particle size and a narrow size distribution. Both combinative technologies can be successfully implemented for the production of very small nanocrystals. The surfactant and drug concentration used for the SD process need to be selected carefully to obtain smaller nanocrystal after combinative approach.

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Selective siRNA delivery to Rhabdomyosarcoma by co-modified liposomes

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Rhabdomyosarcoma is the most frequent malignant soft tissue tumor in childhood. Especially the more aggressive subtype named Alveolar RMS (ARMS) is highly resistant to all forms of therapeutical treatment that are currently available [1]. RNA interference offers a new promising approach for the treatment of ARMS but lacks an effective way to deliver the siRNA to the target site.
This study aims to develop a liposomal formulation which is able to protect the siRNA from degradation and can lead to specific interaction and successful uptake into the cancer cells. For this purpose a co-modified delivery system was designed carrying the RGD and the TAT peptide as targeting moieties on its surface. Dual Asymmetric Centrifugation (DAC) was used for liposome preparation as this novel technique enables high encapsulation efficiencies [2]. Furthermore sterile as well as RNase-free conditions can be easily established. The targeting moieties were coupled to the liposomal surface subsequent to liposome preparation using the Sterol-based Post-Insertion Technique (SPIT) [3]. The RGD peptide is intended to selectively interact with rhabdomyosarcoma cells whereas effective uptake should be mediated by the cell-penetrating peptide TAT.
Experimental results revealed enhanced uptake of co-modified liposomes. Future studies are intended to confirm efficient siRNA knockdown.

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PO - 122

Application of high pressure to control particle size of drug nanocrystals in solvent-antisolvent precipitation process

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Solvent-antisolvent precipitation process is considered as a simple and cost effective process among various bottom-up approaches of preparing drug nanocrystal. This process has the potential to produce small size nanocrystal. Various experimental parameters such as solvent(S)-antisolvent (AS) ratio, mixing efficiency and stabilizer selection were varied. The influence of high pressure application along with the precipitation step, in reduction of the final particle size, was also studied. Ibuprofen (IBP) was selected as a model compound because of its poor water solubility and good solubility in organic solvent. IBP was solubilized in isopropanol (IPA) and the solution was added in an aqueous solution to form a ternary phase. The states of the mixtures after mixing of three phases were plotted in a ternary diagram. In the ternary diagram, IBP concentration was varied from 2.5 % (w/v) to 80% (w/v) and volume ratio of IPA-water was varied from 0:10 to 10:0. Different zones were identified from the ternary diagram. Few points were selected from ternary diagram representing various zones. Stabilizer (SDS/HPMC) was added in AS phase (aqueous). AS phase was mixed with S phase using a stirrer and immediately homogenized in a Micron LAB 40 high pressure homogenizer for 5 cycles. The same formulation was also processed using an Avestin C5 homogenizer to minimize the time difference between solvent-antisolvent mixing and homogenization. Particle size was analyzed by laser diffractometry (LD) and photon correlation spectroscopy (PCS).
Depending on the state of the system after mixing S and AS phase, four distinct zones were identified in the ternary diagram i.e. suspension, precipitation, biphasic/metastable and solution zone. Biphasic and precipitation zones were unstable zone and found suitable to precipitate drug particles. Precipitation without high pressure led to bigger size particle (d(0.5)~10 µm). Application of high pressure using Micron LAB 40 immediately after precipitation process was effective to reduce the particle size to a great extent (d(0.5)~1.5 µm, d(0.1)~0.11 µm) after 5 pass. Superior particle size reduction was achieved using Avestin C5. Particle size reached to submicron level (d (0.5)~ 0.5 µm) only after 1 pass in Avestin C5. Better particle size reduction was achieved using Avestin C5 operated at a much lower pressure compared to Micron LAB 40. SDS was found to be a better stabilizer than HPMC in this study. Ternary phase diagram can be leveraged to select optimum solvent-antisolvent ratio to carry out precipitation study. High pressure can be used along with precipitation step to reduce the particle size of the final nanocrystal product. Superior result from Avestin C5 was mainly due to better mixing of solvent and antisolvent in the high pressure zone and reduction of time difference between precipitation and of high pressure application.

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Synthesis of phenylene-modified single-chain bolaamphiphiles

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Certain species of archaebacteria are able to survive under extreme conditions, like high temperatures or low pH-values. Therefore, the microorganism forms specific, bipolar substances in the cell membranes consisting of two hydrophilic headgroups connected by one or two membrane-spanning alkyl chains. Due to the membrane-

stabilizing properties of these bipolar lipids (bolalipids, BLs) they are attractive candidates in vesicular drug delivery systems.
The complex structure of the BLs complicates the synthesis and resulted in previous studies in the simplification of the BL-structure. In consequence, the synthesis of symmetric polymethylene-1,ω-bis(phosphocholines) with chain lengths (n) from 22 to 32 carbon atoms (Figure 1) was established via cooper catalysed GRIGNARD coupling and subsequent phosphorylation and quarternisation[1]. However, the mixing behaviour with conventional phospholipids (DPPC, DMPC) is limited due to packing problems. The BLs prefer to self-assemble into fibres caused by the larger space requirement of the PC headgroup of PC-C32-PC compared to the small cross-sectional area of the alkyl chain[2]. The idea arose if an additional phenyl ring or biphenyl rings within the alkyl chain have the ability to expand the cross section of the lipophilic chain and, hence, enhance the miscibility with phospholipids.
In the presented work we describe the results of the synthetic approaches for the preparation of phenylene and biphenylene modified BLs by copper-catalysed GRIGNARD reaction on the one hand by simultaneous coupling of the two modified alkyl chains on the phenyl/biphenyl ring and on the other hand by stepwise coupling of the modified alkyl chains sequentially.

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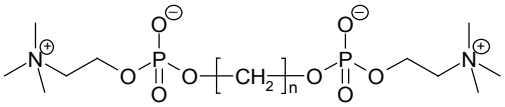

Figure 2: Chemical structure of PC-Cn-PC with n=22-32.

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Transport studies of neutrally charged polystyrene nanoparticles across the buccal mucosa

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External, also called epithelial barriers protect the body against uncontrolled uptake of a variety of substances from outside. These barriers consist of cellular (epithelium) and acellular parts (dead cells, mucus). However, nanoparticles are small enough to overcome these barrier functions. They have to pass the acellular layer with potential change of the particles by this passage and to permeate/penetrate the epithelial layer either in a destructive or in a non-destructive manner [1]. This study was conducted to investigate the transport/permeability of nanoparticles across/into the buccal mucosa. A physiological ex-vivo/in-vitro system was used [2]. Since recent studies demonstrate that viruses are able to infiltrate mucosal tissues due to their specific neutral surface, which is equally coated with positive and negative charges [3], neutrally charged polystyrene particles (PP) were used as model particles.
PP particles were used in sizes of 25 and 200 nm and the physicochemical properties in biological media (saliva) were determined. The permeability through excised porcine buccal mucosa was carried out at 37 °C and 4 °C with Franz diffusion cells. Before every experiment the viability and structural integrity of the tissue was assessed. Additionally, in-vitro permeability tests were performed using transwell® systems cultured with a buccal human squamous epithelial cell line (TR 146 cells). Particle uptake into oral TR 146 cells was recorded with fluorescence microscopy and cell damage was measured.
The results demonstrate that all particles increased in size due to aggregation and the formation of a protein corona. PP particles penetrated into deeper regions of the buccal tissue. The calculated uptake rate for 25 nm PP was 11.57% ± 1.39% SD and for 200 nm PP 9.25% ± 2.64% SD. By decreasing the temperature to 4°C the 200nm PP particles showed a minor uptake rate, whereas no penetration of the 25 nm particles could be detected.
It can be concluded that the transport mechanism for 25 nm particles is mainly governed by the endocytotic pathway. However, it seems that 200 nm neutrally charged particles can penetrate into the mucosa via two routes, passive diffusion and endocytotic mechanisms.

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PO - 125

MRI molecular imaging with targeted albumin-based nanoparticles: conceptual design strategies to create the Magic Bullet

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Introduction: Paul Ehrlich in 1900 [1] described the idea of a targeted vehicle carrying a drug. His notion of a „Magic Bullet" changed the whole concept of medical treatment. Richard Feynmann in 1959 [2] challenged scientists to create nanomachines. Since then Nanomedicine has promised much improved delivery of drugs, with reduced dosages and side-effects realizing the dream of personalized medicine. Why has Nanomedicine not delivered on this promise? Two major challenges hinder realisation of this dream: 1. identification of suitable biomarkers; 2. poor availability of nanoparticles capable of homing to biomarker molecules present behind (intact) tissue barriers. We will review the reasons for these difficulties and delineate some approaches with potential to overcome them.
Materials and Methods: Albumin-based nanoparticles bearing gadolinium were developed and extensively characterised. Tomato lectin was attached to the particles to mediate specific adhesion to oligolactosamines. MR Imaging was carried out on living rats and both imaging and quantitative approaches were applied. In studies coordinated with the MRI, chemical and immunohistochemical analyses tracked the component parts of the nanoparticles in longitudinal time series after injection, from 15 minutes to 6 weeks obtaining large runs of quantitative data.
Results: Our nanoparticles showed sizes ~ 30 nm diameter with polydispersity indices as high as 1.7. They were pure, containing no starting materials and displayed good imaging properties with relaxivities ~ $1 \cdot 10^7$ 1/Ms, they showed internal stability in various *in vitro* testings. *In vitro* haemagglutination tests showed positive agglutination of red blood cells, when intravenously injected into living rats MR high-resolution imaging of the vascular wall was obtained for longer than two hours. Declining concentrations of gadolinium in the blood caused fading of the MR images and were due to uptake primarily into the liver. The numbers of (lectin) targeting groups required for successful molecular imaging and the numbers of chelated gadolinium ions per nanoparticle necessary for high-resolution imaging were ascertained and related to particle size and type of crosslinking.
Discussion: The critical parameters for MR Imaging and for MR Molecular Imaging by use of nanoparticles were determined, and useful sizes of protein-based nanoparticles were identified. These particles require a second type of targeting group in order to migrate across the vascular walls and access the subendothelial interstitial compartment, in order to provide Molecular Imaging and Molecular Targeting of disease sites behind intact tissue barriers. The concept of multiple targeting is new in Nanomedicine and represents the hurdle that limits present-day techniques [3]. On the assumption that the quantitative aspects of targeting will be similar for each of the multiple targeting groups, we already know how to design nanoparticles for targeting of both drugs and contrast agents to disease lesions hiding behind blood-tissue barriers. Multiply-targeted nanoparticles are Ehrlich's "magic bullets".

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Development and characterization of Dexamethasone-loaded Nanostructured Lipid Carriers (NLC) for pulmonary application

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The pulmonary application is taken more and more into account due to some advantageous aspects like reaching the lung epithelium directly and therefore the side of action and avoiding systemic toxicity. NLC are a nanosized carrier system which possess several advantages for the pulmonary application like deep lung deposition, accumulation and retention in the lung, good tolerability and controlled release of the drug. However, formulation development for pulmonary application is quite challenging since the NLC formulation has to be sterile, isotonic and show a pH value between 3 and 8.5.
In this study Dexamethasone-loaded NLC composed of Dexamethasone, Precirol ATO 5, castor oil, Tween 20 and MilliQ water were prepared by hot high pressure homogenization and characterized (size, polydispersity index (PI), laser diffraction parameters LD50 and LD99, zeta potential, tonicity, pH value). Three isotonisation agents (glucose, glycerol 85% and saccharose) were investigated. The used sterilization method was steam sterilization. The following table summarizes the results of our investigations.

	NLC without isotonisation agent	NLC with glucose as isotonisation agent	NLC with glycerol as isotonisation agent	NLC with saccharose as isotonisation agent
size [nm]	157 ± 0.702	163 ± 2.211	155 ± 1.320	158 ± 1.401
PI	0.231 ± 0.012	0.228 ± 0.009	0.217 ± 0.008	0.291 ± 0.018
LD50 [nm]	139 ± 0.577	144 ± 2.309	144 ± 2.082	159 ± 0.577
LD99 [nm]	340 ± 11.547	303 ± 5.774	290 ± 10.000	4240 ± 28.868
zeta potential [mV]	-24.4 ± 0.436	-22.7 ± 1.179	-20.1 ± 0.666	-26.2 ± 0.153
tonicity [mosmol/kg]	30	292	275	274
pH value	5.2	5.3	5.3	5.4
Autoclaving possible	yes	yes	yes	yes

Adding glucose or glycerol did not lead to a change of the investigated characteristics of Dexamethasone-loaded NLC whereas laser diffraction measurements showed bigger particles for the formulation with saccharose.
Altogether it was possible to develop isotonic, sterile Dexamethasone-loaded NLC with a pH value well in the required pH range using glucose and glycerol as isotonisation agents being a precondition for pulmonary application.

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Synthesis of cationic lipids with peptide-like malonic acid diamide backbone

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Gene therapy, which uses polynucleotides (DNA, siRNA) as drugs, is a promising method for the treatment of genetic disorders and numerous acquired diseases. To realise the transfer of polynucleotides into cells special delivery systems (vectors) are used. At present viral gene delivery systems dominate in clinical trials but, due to

the drawbacks using viral vectors, which are not negligible, the development of non-viral gene delivery systems is a promising alternative. Non-viral vectors are less immunogenic than the viral ones and do not induce cancer. However, the toxicity and the low transfection efficiency of these systems are still problematical and require new developments and new substances (e.g. cationic lipids or cationic polymers) in this field.
One promising field of non-viral gene transfer is the lipofection using cationic lipids (cytofectines) as vectors. Following the structural pattern of potent lipids with malonic acid diamide structure published recently [1,2], ongoing research focuses on the synthesis of new compounds with a peptide-like backbone consisting of lysine and malonic acid. This results in a novel backbone generating lipids with two head groups (see figure 1).
The presented work deals with the stepwise synthesis of these novel compounds, which is performed under mild conditions and easy in handling. The strategy of the synthesis allows a simple alkyl chain variation of the lipophilic part of the lipid (C14, C16, and C18) enabling the investigation of structure-function-relationships. Furthermore, we vary the head group structure using different spacers (e.g. tris(aminoethyl)amine or ethylene diamine) and we diversify the numbers of lysines (from 1 to 3), resulting in cytofectines with different numbers of protonable amino groups in the head group region.

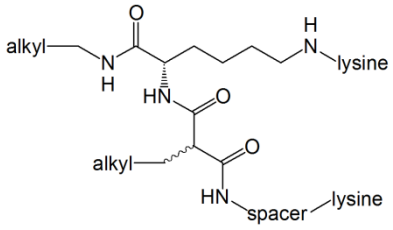

Figure 1: novel lipid backbone

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PO - 128

Layer-by-Layer assembled core-shell nanoparticles for the delivery of nucleic acids

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Nanoparticles are a versatile tool for the delivery of nucleic acids to mammalian cells. However, a big challenge in nanoparticle research is the tailoring of the specific size and surface charge, as the major factors for cellular uptake, of the carrier systems. We use a layer-by-layer approach on gold nanoparticles to overcome this problem [1].
The layer-by-layer principle is based on the alternating deposition of oppositely charged polymers on a flat or curved template. In our case we used gold nanoparticles as a core template which is covered with nucleic acids. Gold nanoparticles are widely used in biomedical applications because of their inertness, easy synthesis and visibility in transmission electron microscopy. Those particles are stabilized by mercaptoundecanoic acid by a thiol bonding to the gold surface which also conserves a negative charge on the particle surface. Polyethylenimine (PEI) is known for its transfection capacity and its ability to disrupt the endosome. As a positively charged polymer, PEI forms the first layer of the core shell particles, followed by a layer of negatively charged nucleic acids, such as DNA or siRNA. Finally the assembled particles are coated with a last layer of PEI to avoid degradation of the nucleic acids. This layer-by-layer approach led to a size and charge specific surface modification of nanoparticles which enables systematic studies on the cellular uptake.
Here we present the layer-by-layer build-up of our core-shell nanoparticles and their characterization by dynamic light scattering, zeta-potential and Vis-spectrometry. The size of the nanoparticles increases about 10nm for each polymer layer and the characteristic plasmon peak of the gold colloid is redshifted of about 2nm. Additionally we show the cellular uptake of mammalian cells by transmission electron microscopy.

Acknowledgements: This project is funded by DFG-grant BR 3566/2-1
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PO - 129

Preparation of below 100 nm gelatin nanoparticles – influence of production parameters

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Particulate nanocarriers with particle size around/below 50 nm can increase transdermal absorption, e.g. inter-dermal uptake by the hydrophilic channels. The present study aims to develop gelatin nanoparticles in this size range suitable for dermal application of hydrophilic actives.
Gelatin nanoparticles were produced by a modified "Two-Step-Desolvation" method as reported before[1]. Three important factors (gelatin concentration, volume of acetone and pH value) and their impact on the resulted gelatin nanoparticles were investigated. Gelatin nanoparticles were characterized for particle size, polydispersity index and zeta potential by photon correlation spectroscopy (PCS) (Zetasizer Nano ZS, Malvern, UK). Light microscopy (Orthoplan, Zeiss, Germany) was applied for detection of aggregates. The gelatin nanoparticles possessed a mean particle size of 56 nm and a polydispersity index of 0.019, and no aggregates or large particles was observed by light microscopy. The zeta potential was 19.4 mV when measured in conductivity adjusted water (50 µS/cm). Gelatin concentration had significant impact on the particle size and the size distribution. Higher gelatin concentration led not only increase of particle size but also aggregation and flocculation. The size and concentration of gelatin nanoparticles remained stable when the volume of acetone increased from 100 to 120 ml, indicating that all the dissolved gelatin was formulated into gelatin particles. Within the pH region we investigated, increase of acidic conditions correlated with decrease of the particle size at low pH values. Intense intermolecular electrostatic repulsion forces seemed to be responsible for the reduction of particle size. Gelatin nanoparticles below 100 nm could be prepared by this special modified method. Selection of suitable gelatin concentration and optimum pH value of redissolved gelatin solution were crucial to obtain smaller gelatin nanoparticles with good physical stability.

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OMICs and Biopharmaceutics

PO - 130

Evaluation of the effect of pH, buffer and extremolytes on thermostability of granulocyte colony stimulating factor using differential scanning fluorimetry and design of experiments methodology

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Design of experiments (DOEs) is a powerful tool to identify and optimize stable protein formulations in a rapid and efficient way. In this study, we have screened thermal stability of granulocyte colony stimulating factor (G-CSF) in various buffers, at different pH values and in the presence of 8 naturally occurring extremolytes (trehalose, sucrose, firoin from rhodothermus marinus, ectoine, hydroxyectoine, taurine, sorbite, mannite). For the thermostability screening we applied the method differential scanning fluorimetry (DSF) in combination with design of experiments (DoE) methodology. At a primary buffer screening the two most stabilizing buffers, sodium acetate and sodium glutamate, were determined for further investigations. The highest thermostability could be achieved with a low pH and a low buffer concentration for both buffers. Based on the results from the primary buffer screen the effect of extremolytes and the influence of critical buffer, buffer concentration and pH on G-CSF were tested using a central composite circumscribed design. The experimental results showed a significant stabilizing effect of trehalose, sucrose, sorbite, mannite, taurine and hydroxyectoine, whereas firoin and ectoine had a destabilizing effect. The thermal protective effect of the extremolytes was observed to be concentration dependent. pH was the most influencing factor regarding thermal stability of G-CSF. Stabilization of G-CSF was improved by decreasing pH in a quadratic manner. Interactive effects were seen between pH and buffer concentration and between some extremolytes and pH.

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Mass spectrometry of glycans derivatized with isoniazid

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Glycoproteins are involved in a wide range of biological processes, physiological as well as pathological. For this reason, they can be applied as diagnostic markers and for medical treatment. In order to gain deeper insight into a function of an involved glycan, its structural and functional elucidation is of great importance.
A number of derivatization procedures have already been applied to mass spectrometric analysis of glycans[1]. Charged or hydrophobic groups with chromo- or fluorophores attached to the glycans can improve their separation and detection after chromatography. In mass spectrometry - a powerful tool for glycan analysis - derivatization of glycans offers advantages in terms of the enhancement of ionization efficiency and detection sensitivity[2].
However, oligosaccharide analysis is still a laborious and time-consuming task, and simple and efficient ways of derivatization are in great demand.
Here, a new derivatization reagent for carbohydrate analysis is presented. With its hydrazide group, isoniazide can easily and specifically be coupled to the reducing end of glycans in an one-step reaction. The label was linked to different monosaccharides, standard glycans and glycans released from several glycoproteins. These carbohydrates were analyzed using liquid chromatography and mass spectrometry (ESI and MALDI). The results indicated that isoniazid favourably increases detection sensitivity of glycans. Hence, it can be considered for further mass-spectrometric studies in order to perform structural characterization of the corresponding glycan derivatives.

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PO - 132

IVIVC for fenofibrate immediate release tablets using solubility and permeability as *in vitro* predictors for pharmacokinetics

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[Purpose] The goal of this study was to investigate the *in vitro-in vivo* correlation (IVIVC) for fenofibrate immediate release tablets based on Melt Dose®-technique.
[Methods] The human study was conducted with six solid dosage forms (Tab.1) [1]. Bioequivalence with Tricor® (formulation B) in fasted conditions should be demonstrated in this randomized cross-over study. *In vitro* solubility and permeation studies were conducted in medium simulating the fasted state conditions in the upper jejunum, $FaSSIF_{mod}$ (modified fasted state simulated intestinal fluid) [2], containing the surfactant compositions of the six formulations at different concentrations.
[Results and Discussion] The obtained solubility and permeation data were combined and compared with the C_{max} values for the fenofibrate formulations, assuming a 50 ml *in vivo* dissolution volume. A good IVIVC was observed for five fenofibrate formulations which were based on the same manufacturing technique (R^2 = 0.94). The *in vitro* studies revealed that the formulation compositions containing sodium dodecyl sulfate (SDS) interfered with the vesicular drug solubilizing system of the biorelevant medium (Fig.1) and antagonized its solubilization capacity.
[Conclusions] Interactions between naturally occurring phospholipid solubilizers and SDS may decrease rather than increase solubility. Thus, SDS must be avoided in formulations with the intention to raise fenofibrate's solubility and thus dissolution rate in vivo. Solubility and permeability of the solubilized drug may be used to predict the in vivo performance of the dosage forms.

Tab.1: Mean C_{max} and $AUC_{0\text{-}inf}$ (relative to formulation B, n ≥ 12) following oral administration of formulations containing 145 mg fenofibrate. Surfactant composition of the tablet formulations. S1 and S2 are surfactants from the class of poloxamers. S3 is a surfactant from the class of polyethoxylated castor oils.

	C_{max} [%]	$AUC_{0\text{-}inf}$ [%]	Surfactant (1)	Surfactant (2)	Surfactant (2) amount [mg/tablet]
Formulation A	123.4	107.5	S1	-	-
Formulation B	100	100	Docusate-Na	SDS	10.2
Formulation C	95.7	103.9	S2	S3	n/a
Formulation D	86.3	102.1	S2	SDS	19.3
Formulation E	73.9	99.9	S2	SDS	41.9
Formulation F	72.4	100.1	S2	SDS	40.2

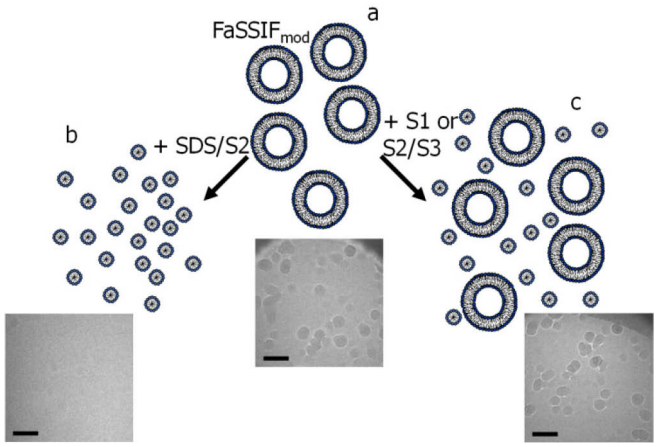

Fig.1: Illustration and cryo-TEM micrographs (the scale bars represent 100 nm) of the structural changes when the surfactant compositions of formulations D-F (SDS/S2) (b) and the surfactant compositions of formulations A or C (S1 and S2/3) (c) are dissolved in $FaSSIF_{mod}$. In the first case the surfactants interfere with the vesicular system of $FaSSIF_{mod}$ (a) and finally micelles are formed (b). In the second case vesicles and micelles coexist (c).

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Characterization of the immunoprecipitated EGFR adaptor protein complex by LC-ESI-tandem mass spectrometry after EGF stimulation

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Background and aim: The epidermal growth factor receptor (EGFR) is one part of the promiscuous signaling network that regulates proliferation, growth and differentiation of mammalian cells. Its overexpression, aberrant or enhanced activation are frequent events in human cancers and are presumably involved in the development of drug resistance and adverse effects of cancer therapies. EGFR signaling is realized by recruiting specific adaptor proteins to the activated intracellular receptor tyrosine kinase domain. We hypothesize that the pattern of recruited adaptor proteins is characteristic for respective EGFR related cellular responses. Accordingly, we intended to develop a method to decode interactions between different mitogens and the EGFR with special interest on their impact on the EGFR adaptor protein pattern.
Methods: A431 cells were stimulated with 100 ng/ml EGF for 30 minutes and the EGFR-adaptor protein-complex was isolated by immunoprecipitation after gentle lysis using the specific EGFR antibody cetuximab biotinylated and bound to magnetic beads. Controls were precipitated from lysates of unstimulated A431 cells. After pre-fractionation by 1-D-SDS-PAGE and in gel digestion, protein complexes were characterized by LC-ESI-tandem mass spectrometry (LC-MS/MS) using fourier transform ion cyclotron resonance (FTICR), Orbitrap or Orbitrap Velos detection.
Results: LC-MS/MS analyses reproducibly identified 68 proteins in three replicates of precipitates from lysates of unstimulated A431 cells, independently from the MS type. 63 of these proteins were assigned to gene ontology (GO) annotations and significantly enriched domains included intracellular localisation and protein transport proteins (29 proteins) and the EGFR signalling pathway (5 proteins). The first domain contained structural proteins such as actin and tubulin as well as importins, exportins and transportins. Proteins within the EGFR signaling cathegory were growth factor receptor bound protein 2 (Grb2) and signal transducer and activator of transcription 3 (STAT3). As detection with Orbitrap MS occurred to be most sensitive, we used this method to compare the EGFR-adaptor protein complex after stimulating cells with EGF with unstimulated cells. 58 proteins were detected that were uniquely present or at least fivefold enriched upon EGF stimulation. Go annotations assigned 26 proteins to be significantly enriched in the categories DNA replication and chromosomal structure proteins (11 proteins) such as retinoblastoma binding protein 4 (RBB4) and minichoromosome maintenance complex components (MCM), EGFR signaling pathway (7 proteins) including a catalytic subunit of the PI3-kinase, son of sevenless homolog 2, Grb-7, as well as Cbl-proteins and subunits of the adaptor-related protein complex, the latter of which are additionally assigned to the process of endocytosis (7 proteins). Further proteins that were not assigned to a GO category but are also associated with receptor endocytosis and degradation were identified including protein amplified in osteosarcoma (OS-9), ubiquitin-associated and SH3-domain containing protein B (UBS3B) and casein kinase.
Conclusions: We were able to identify proteins which are part of the dynamic processes of the EGFR signalling pathway and also detected specification and modification in protein abundance by EGF stimulation. Due to separately sample preparation, the detected changes are not sufficiently reliable. Therefore, stable isotope labeling of amino acids in cell culture (SILAC) will enable to quantify these changes in protein pattern. These further insights into mechanisms of EGFR signal activation may reveal mechanism of events in cancer and therapies that are drawbacks for therapeutic response.

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Membrane-buffer partition coefficients of ß-Blockers determined via Laurdan-labelled liposomes

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Membrane permeability and distribution of drugs are key parameters for their pharmacokinetic behaviour. Traditionally the octanol/water system has been used to describe drug partitioning between non-miscible lipophilic and hydrophilic phases. Due to the lack of the octanol/water system to reflect physiological conditions in biological membranes, liposome/buffer systems have been developed to mimic biomembranes more realistic [1,2].
In this project an indirect screening method to investigate the liposome/buffer distribution coefficient of drugs using the membrane-anchored fluorescence dye 6-Lauryl-2-dimethylaminonaphtalene (Laurdan) was established. Laurdan possesses a distinctive emission spectrum which is characterized by a shoulder at 434 nm and a maximum at 486 nm. The ratio of the fluorescence intensities at these two wavelengths is termed as the membrane state parameter (MSP). MSP values depend on the polarity of the dye's environment. After insertion of drug molecules into the liposomal membrane, the MSP value decreases as a function of the amount of drug inserted. The calculation of the MSP for different drug concentrations and various lipid amounts enables the determination of the drug's membrane/buffer partition coefficient [3]. This indirect method offers the advantage of analyzing the distribution behaviour of different drug classes without requirement of a separation step and/or drug-specific analytics.
In an initial study we determined the partitioning of bile salts as amphiphilic model drugs [4]. Now the Laurdan method has also been applied to the ß-blockers as additional substance class and the attained results are comparable to literature [5]. Since liposomes promote ionic interactions, charged compounds as the ß-blockers display remarkably increased distribution coefficients in comparison to the octanol/water system.

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PO - 135

Protein and monoclonal antibody analysis using various modes of High Performance Liquid Chromatography

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Pharmaceuticals based on proteins (biologicals), such as monoclonal antibodies (mAb), attain more and more relevance since they were established as potent drugs in anticancer therapy or for treatment of autoimmune based diseases. Due to their high efficiency it is unavoidable to have unfailing methods for protein quantification and the detection of protein aggregates, which may lead to adverse effects after application [1]. In order to improve selectivity as well as precision compared to classic protein quantification methods such as the Bradford assay or SDS-PAGE, which do not achieve the necessary specifications of quality control (QC) purposes, High Performance Size Exclusion (HP-SEC) and Anion Exchange Chromatography (SAX) were already introduced as high selective and precise methods (e.g. SEC: < 1.9% and SAX: < 5% RSD % for peak areas inter-day) with low quantitation limits for the model proteins Ovalbumin, Myoglobin and Bovine Serum Albumin [2]. The weak Cation

Exchange- (WCX) and the RP-HPLC, both already successfully applied in protein analysis, will be presented as two further possible alternatives for QC purposes of proteins. Both methods also reach data of high precision (RSD % peak area day-to-day < 2% for RP and < 3.5% for WCX) and low quantitation limits (< 10 µg/ml). Consecutively, the four separation modes will be compared in terms of precision, peak capacity, analysis time, practicability and overall results. Moreover the analysis of the monoclonal antibody Matuzumab is included in this study.

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Increasing precision of two-dimensional gel electrophoresis (2-D-GE) by optimizing the dimension transfer

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Two-dimensional gel electrophoresis (2-D-GE) is one of the most appropriate techniques in proteomics to separate complex protein mixtures. Due to its excellent selectivity several thousand different proteins can be separated within one single gel. However, the precision and reproducibility still need to be improved. By enhancing the precision of 2-D-GE the capabilities of this technique can be extended to e.g. the analysis of biologicals in pharmaceutical routine quality control. Thus we investigated the process of dimension transfer, which is crucial for precision in 2-D-GE, by varying the equilibration step. In this study bovine serum albumin (BSA), ovalbumin, ß-lactoglobulin, myoglobin and the monoclonal antibody Matuzumab served as model proteins. Different times of equilibration and varying constitutions of the equilibration solutions were tested. Simultaneously the focussing step in the first dimension was optimized using different Volthours (Vhs) in the range of 30 to 120 Vhs.

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FDA labled biomarkers in the personalized medicine of drug prescriptions on a pediatric ward

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Background: Personalized medicine (PM) has been proven to be of value for a safer and more effective drug therapy in adult patients with individual differences in their genetic make-up. The “Food and Drug Administration” (FDA) has established a drug list with pharmacogenomic information in their labels for adults. The genetic differences include gene variants, functional deficiencies, expression changes, chromosomal abnormalities, and others. Since pediatric patients might also benefit from PM, we investigated whether the drugs from the FDA list can also be identified in pediatric prescriptions.
Methods: In the university clinic of Düsseldorf, 1350 prescriptions of a pediatric ward for cardiology and pneumology have been collected in a retrospective, observational study from January 2010 to March 2011 from the web-based electronic prescription data base KinderDosierung.de. The FDA listed drugs with pharmacogenomic information in drug labels (63 drugs listed, dated March 2011) have been compared with the drugs prescribed in KinderDosierung.de to find matches. The data were analysed with the statistic program SPSS, 19.0.

Results: In these prescriptions, 13 drugs matched with the 63 drugs in the FDA list. These thirteen drugs have been prescribed 64 times out of 1350 prescriptions in Kinderdosierung.de. Six out of 13 were cardiovascular drugs, 2 of 13 were psychiatric drugs, 2 of 13 were anti-infectives and 1 out of 13 belonged to rheumatologic, analgesic or gastroenterologic drugs. The number one prescribed drug was omeprazole with 30 out of 64 prescriptions, the number two drug was propafenone with 6 and number three drug was tramadol with 5 prescriptions. Ten out of 13 drugs have biomarkers of the CYP-system, in 50% of these CYP2D6 as well as CYP2C19 were identified and involved. ***Conclusion:*** The results pointed out, that parts of the drugs listed by the FDA with pharmacogenomic labels for adults are used in pediatric drug prescriptions. Since the maturation of the metabolizing enzymes involved in the pharmacogenomics of identified PM drugs are well known in the pediatric population, further evaluation of their ontogeny are important to target the PM´s drug information for the pediatric age groups.

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Self-medication in Germany: The new role of pharmacists to contribute to pharmacovigilance

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Background: In Europe, Germany has gained a leading role in the marketing of "over the counter" (OTC)-drugs due to a politically driven demand for self-responsiveness in patients´ health and economic status during the last decade. For example drugs such as ibuprofen, diclofenac, naproxen and naratriptan have been switched from prescription to non-prescription drugs to offer patients a differentiated pain treatment due to their own decision. Thus, German pharmacists now play a key role in informing patients about the safe and effective use of such drugs and thus fill out a new role in pharmacovigilance. Therefore, we conducted an analysis about key aspects of self-medication counselling interviews in German community pharmacies for future improvement in this area. ***Methods:*** In the first phase, we generated a questionnaire with 11 crucial points of pharmaceutical counselling based on the counselling guideline of the German "Bundesapothekerkammer" for self-medication in German community pharmacies. We tested 12 different indications in 71 public pharmacies in North Rhine-Westphalia, Germany. The data were analysed using a pre-determined score and were tested by one-sided binomial test. In the second phase, we optimized the questionnaire by standardizing three different pharmaceutical counselling interviews for headache using the drugs paracetamol, acetylic acid and ibuprofen. So far, 20 community pharmacies had been tested. ***Results:*** In phase 1, 3 out of 11 key aspects for counselling interviews have been advised significantly over 50% in 71 pharmacies in the questionnaire as clarification to "who is the patient", "information about dosing" and "application of the drug". Five of 11 key aspects have been advised significantly fewer than in 50% of the counselling interviews such as "environment", "already taken drugs", "drug interaction", and "limits of self-medication". In phase 2, preliminary data analysis indicated insufficient pharmaceutical guidance due to all three standardized counselling scenarios. ***Conclusion:*** The results pointed out, however, that major aspects in pharmaceutical counselling have to be improved to make sure that the pharmacist can fill out his new role in pharmacovigilance as a key player in informing patients how to best treat themselves.

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Preparation of artificial stratum corneum lipid films and matrices and determination of their lamellarities

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Introduction

The outermost layer of the epidermis, the stratum corneum (SC) provides protection for the body against endogenous substances and loss of water. These functions are guaranteed due to the unique structure of the SC. Overlapping corneocytes are surrounded by lipid matrix of ordered lamellae. Artificial matrices of SC lipids can be used as model to mimic "mortar" representing the skin barrier. Aim of the present study is to produce SC lipid films utilising a new spraying procedure, modified from Bouwstra. [1] The spraying of lipidic emulsions results in the same lamellarity as found with the traditional centrifugation process.

Materials and Methods

Two types of matrices were used. The first consisted only of free fatty acids (FFA) (see [2]), the second one was composed of FFA and NaOH in same ratio, cholesterol 26.2mol%, ceramides 27.4mol% and water 32% (w/w). For centrifuged matrices all contents were weighed into centrifuge tubes which were constricted to 7mm and centrifuged through the capillary until homogenous. For sprayed films FFA or FFA, ceramides and cholesterol were dissolved in chloroform/methanol (2:1). FFA lipid composition was also emulsified with water and NaOH. These different mixtures were sprayed onto microscopic slides for later polarized light microscopy (PLM) or on a XRD sample carrier to perform small angle X-ray diffraction (SAXD). Matrices were observed with an Olympus microscope IMT-2 using crossed polarizers at room temperature. Pictures were taken with a Pixelink IL-A662 camera. SAXD-meaurements were carried out with an X-Pert X-ray diffractometer (Phillips, Kassel, Germany) with 40kV at λ=0.1542nm from 25°C to 75°C in increments of 10°C.

Results

Interlamellar distances were calculated with Bragg's law. The distances determined were: 41.88Å for the centrifuged FFA matrix, 36.32Å for the sprayed FFA solution (waterfree) and 38.89Å for the sprayed emulsion (Figure 1). PLM showed similar pattern for all samples, oily streaks can be observed. PLM with sprayed FFA emulsion additionally showed droplets. (Figure 2) For centrifuged matrix consisting of FFA, cholesterol and ceramides an interlamellar distance of 42.65Å was measured. For the sprayed film of this type it was difficult to find high lamellar fraction, but a distance of 37.59Å could be determined.

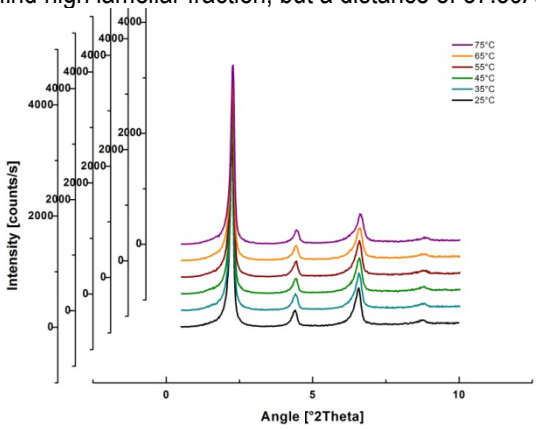

Figure 1: Diffractogram of sprayed FFA emulsion

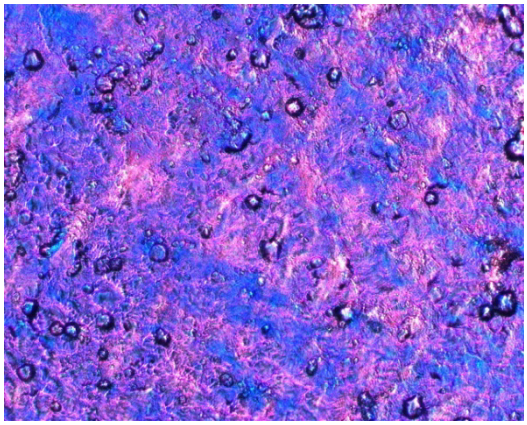

Figure 2: PLM of sprayed FFA emulsion

Conclusion

With the used solutions and preparations it is possible to obtain a lamellar SC matrix. It is evident that the interlamellar distance is depended on the water content. The largest distance is found in the centrifuged matrices. By spraying the emulsion water could evaporate or escape the lamellar structure, so the distance is reduced. The smallest lamellar distance was found in the sprayed solutions where no water was present to incorporate into the structure. The emulsion method avoids layer breakup observed on hydration of waterfree films [1].

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New approaches in characterisation of herbal preparations

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PCR-based methods and NMR-fingerprint analysis in combination with principal component analysis have recently been demonstrated to be useful to retrace and characterise medicinal plants and herbal substances [1, 2]. A usual approach in natural product research has been the bio-assay-guided fractionation to search for target structures in drug-development. By using recently available holistic techniques a different approach is to correlate biological activity with fingerprint profiling. This strategy should provide new insights in the profiles of multi-component mixtures with respect to their safety and efficacy. A model object is *Chelidonium majus* L.. Extracts with solvents of different polarity like ethanol, ethanol 50% (V/V), dichloromethane and water were analysed with TLC, HPLC and ^{1}H-NMR-fingerprinting. Cytotoxic effects of the different extracts were investigated after application to a human liver cell culture (HepG2) in a real time cellular monitoring system (xCELLigence, Roche). For ethanolic extracts and 50% (V/V) ethanolic extracts cytotoxic effects were observed. Only minor cytotoxic effects could be monitored after application of aqueous and dichloromethane extracts. In order to investigate possible effects of Chelidonium extracts on liver cells microarray technology is used for expression profiling.

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Using High Performance Size Exclusion Chromatography to investigate the role of sample pretreatment in protein analysis

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Related to the stability of proteins it is a serious issue that they tend to form aggregates. These aggregates can also lead to adverse effects, like an immune response after application [1]. Since the number of protein based Pharmaceuticals, such as monoclonal antibodies (mAb), increases continuously, their analysis is becoming more and more important. A major point in protein analysis is the sample preparation, due to the fact that e.g. the application of ultrasonification for better solubility can lead to structural and functional changes in proteins [2]. A precise and selective Size Exclusion method (HP-SEC) analyzing the model proteins Myoglobin and Ovalbumin [3, 4] is used to observe changes in retention times, selectivity, peak areas and potential appearing aggregates and splitting products of the proteins due to different sample pretreatment. According and modified to [1] various terms (e.g. storage, ultrasonification, temperature) will be changed during sample preparation. Furthermore, it will be shown, that the non-ionic detergent Brij 35 leads to better protein solubility, without influencing retention times and selectivity in contrary to SDS, which leads to protein denaturation and consequently to different peak forms as well as the emersion of other peaks.

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Protein interaction with ions in their surrounding solution detected by Affinity Capillary Electrophoresis

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The influence of ions in the surrounding solution of proteins is investigated by Affinity Capillary Electrophoresis (ACE). ACE is able to explore changes in the conformation as well as charge changes of proteins which can occur under the influence of a ligand. Unspecific and specific interactions on proteins can be measured without special reagents or kits.
The interactions on proteins are detected by the mobility changes which occur using various ligands. Therefore the changes of the protein migration times are measured due to a ligand influence [1]. In order to compensate for changes on the migration time, which are not caused by binding, the mobility ratio of an EOF-marker and the protein is used [2]. Six repeats were done with a very good precision due to the use of a special rinsing procedure [3]. The RSDs [%] of the migration times and the mobility ratios were typically below 2%, very often below 0.2%. The influence of various charged species e.g. metal ions, such as Cu^{2+}, Mn^{2+} and Mg^{2+}, pharmaceutical cations as ephedrine hydrochloride and pirenzepine dihydrochloride or anions such as glutamic acid and succinic acid was tested on the migration behavior of BSA, ß-lactoglobulin and ovalbumin. Organic cations and metal ions showed clearly different interactions with the proteins.

Acknowledgements: This work was financially supported by the Alexander von Humboldt-Foundation.
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Formation of piracetam-tartaric acid cocrystals during high shear granulation

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Cocrystals are defined as stoichiometric multiple component crystals, in which at least two compounds are solid under ambient conditions. They are of increasing interest for the pharmaceutical community because cocrystals differ in their physicochemical properties, such as solubility and bioavailability, as it was shown for example for carbamazepine [3]. Cocrystal formation is known to occur during various pharmaceutical processes, such as during spray-drying, dry-grinding, and liquid-assisted grinding.
In this study the formation of a known piracetam-tartaric acid cocrystal [1] during high shear granulation was investigated. Piracetam and tartaric acid at a 1:1 molar ratio were granulated in a high shear mixer using small amounts of water as granulation liquid. After 30 min of granulation, the product was examined by X-ray powder diffractometry (XRPD). Peaks characteristic for the cocrystal showed up in the pattern at 15.2, 23.6, and 28.6 °2θ. However, several peaks of the physical mixture remained in the diffractogram at 11.7, 21.7, 29.7 °2 θ, indicating incomplete cocrystal formation during high shear granulation. The XRPD results were confirmed by differential scanning calorimetry and Raman spectroscopy. The formation mechanism is suggested to be comparable to that observed during liquid-assisted grinding, namely enhancement of the molecular mobility by partially dissolving the API and the cocrystal former, respectively, and mechanical activation [2].
This observation is of major importance regarding product safety, as inadvertent cocrystallization during granulation can lead to unexpected changes in bioavailability [3].

Acknowledgements: Joachim Ludwig, Department of Mineralogy, for the XRPD measurements.
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Novel insight into the CCN1-pathway activating integrin function in tumour cell metastasis and interference with heparin

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Background: The integrin VLA-4 (very late activation antigen-4) on the human melanoma cell line MV3 is crucial for cell adhesion in course of haematogenous metastasis. With respect to therapeutic interference in metastasis, heparin was found to inhibit the VLA-4 mediated binding of MV3 cells to VCAM-1 *in vitro* and structural requirements of heparin have been reported [1]. Recently, the activation of other integrins than VLA-4 on tumour cells was shown by binding to secreted cysteine-rich protein 61 (Cyr61/CCN1) as a kind of autocrine stimulation. Since Cyr61 has binding ability to heparin, an indirect activity of heparin on VLA-4 via Cyr61 can also be assumed to partly be responsible for attenuated cell adhesion.
Aim/objectives and methods: To confirm direct binding of heparin to VLA-4, SAW biosensor studies were performed using a series of modified heparins and a VLA-4 containing MV3 membrane preparation providing kinetic binding data. Furthermore, the same technique was applied to characterize the binding of heparin to Cyr61. To further focus on the Cyr61/VLA-4 binding pathway and potential interference by heparin, the effects of exogenous added Cyr61 and the downregulation of Cyr61 in MV3 melanoma cells by shRNA technology were analysed by microscopically detection of cell adhesion.
Results: The kinetic binding data suggest a direct interaction between heparin and VLA-4. Binding affinities of fractionated heparin in the low micromolar range were attenuated by N-acetylation or size fractionation of heparin. Other modifications, such as partial desulfation or ring opening of the saccharides affected the affinity only slightly. Binding affinities of heparin to Cyr61 were matching to data collected by adhesion assay. Preliminary data suggest a reduced binding of Cyr61-knockdown cells. The hypothesis of heparin interference on this pathway can be assumed and will be the matter of further investigations.
Conclusions: Our data provide evidence for a direct interference of heparin with VLA-4 mediated melanoma cell binding. Aside this findings there is a first indication for the Cyr61 pathway on the activation of VLA-4 and its repression by heparin as an indirect interference. This sheds light on the use of heparin in antimetastatic approaches.

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Sustained parenteral peptide delivery providing extended plasma half life

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It was the aim of this study to design a sustained parenteral peptide delivery system for systemic circulation to provide an extended plasma half life. Consequently, a sustained parenteral peptide delivery system was developed by immobilizing a model peptide drug (DALCE) to thiolated carboxymethyl dextran-cysteine (CMD-Cys) [1] via disulfide bond formation. The resulting CMD-Cys–DALCE conjugate displayed a payload of 22.6 ± 7.9 % (wt/wt) of DALCE (mean ± S.D.; *n*=3). The conjugation of DALCE with CMD-Cys was confirmed by FTIR-ATR spectroscopy. In vitro release studies of the conjugate CMD-Cys–DALCE in the presence of 2 µM/ml reduced glutathione (GSH) being also available in the plasma showed a sustained peptide release over a time period of 8 h, because of thiol/disulfide exchange reactions. For in vivo pharmacokinetic studies, DALCE and CMD-Cys–DALCE were administered intravenously to male Sprague-Dawley rats at a dose of 1 mg/kg. The AUC_{0-8} (ng.min/ml) was determined to be 268848 ± 924 and 40019 ± 495 for CMD-Cys–DALCE and DALCE, respectively. The mean residence time (MRT) was determined to be 256 ± 8 and 53.1 ± 9.5 min for CMD-Cys–DALCE and for DALCE, respectively. CMD-Cys–DALCE showed a greater than 5-fold increased elimination half-life ($p < 0.01$), a 3-fold decreased volume of distribution ($p < 0.01$) and a 6.7-fold decreased plasma clearance rate ($p < 0.01$) compared to DALCE. According to these findings, CMD-Cys–DALCE seems to act as prodrug by improving half life and decreasing plasma clearance.

Acknowledgements: Higher Education Commission Pakistan (HEC), Austrian Agency for International Cooperation in Education and Research (ÖAD)

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Technique to determine kinetics of shrinkage and cracking of amorphous cakes during freeze-drying

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Introduction

Lyophilization of amorphous materials with a low collapse temperature leads to a product that does not fill the entire interior of the vial, but leaves a gap between the inner wall of the vial and the cake, which becomes manifest in shrinkage. This phenomenon can affect not only the product elegance but also can produce a serious heterogeneity with respect to the residual water content, which can damage proteins [1]. Furthermore freeze drying of these materials may cause cracks during drying by removal of unfrozen water, which accelerates the lyophilization process [2,3]. These cracks impair also the product elegance.

For these reasons cracking and shrinkage during lyophilization should be avoided. To achieve this, the investigation of the formation of shrinkage and cracking during the freeze drying process in dependence of the process parameters is imperative. The challenge is to establish the time point of the lyophilization process where shrinkage and cracking occur for the first time, to estimate the rate of increase of both phenomena and to determine the point of time where no further changes in the cake structure take place. Therefore the whole lyophilization process has to be monitored regarding the progress of cracking and shrinkage, the shelf temperature, the product temperature, the current sublimation rate and the cumulative water loss. Based on these data the kinetics of shrinkage and cracking can be formulated as a function of lyophilization time. The adaption of the freeze drying process in order to minimize shrinkage and cracking should then be better understood.

Materials and methods

To measure the progress of cracking and shrinkage the vials (2R) were cut below the neck, filled with a trehalose solution (30%) and freeze-dried with Martin Christ Delta 1-24 KD on the top shelf in a hexagonal position around a microbalance (Martin Christ, CWS-40 2nd edition), which was used to obtain the cumulative water loss and the current sublimation rate. It weighs one of the cut vials in predefined intervals during lyophilization, by lifting it with a lifting arm. Every weighing step can be monitored online by a process control system (PC and the software system WZ-KO_4-06) and can be transferred to spreadsheet software. Based on this the cumulative water loss

and the momentary drying rate were determined. With an automatically controlled camera (Canon EOS 60D, controlled by EOS Utilities 2.9) with a macro lens (Canon macro lens EF 100mm f/2.8 USM) pictures were taken every 5 minutes of a vial in a center position through the transparent top cover of the freeze dryer. The camera was fixed on a tripod to ensure that always the same vial is photographed. In this way it was possible to discover the progress of cracking and shrinkage during lyophilization. On the basis of these pictures the degree of shrinkage in % relative to a reference inner surface and of cracking in % relative to the cake surface was evaluated during the whole process via Axio Vision by Carl Zeiss (Release 4.8.2; 06/2010) and Matlab. The shelf temperature and the product temperature were measured by thermocouples every 5 minutes and observed by a data logging system (OMEGA 4 Channel Thermocouple Temperature Recorder QM-CP-QUADTEMP. To obtain the kinetics of shrinkage and cracking all data were plotted against the total process time.

Results

At the beginning of primary drying no cracks and no shrinkage has yet appeared (fig.1). At the change to secondary drying, some cracks already occurred (fig. 2). During the first 2.5 hours of secondary drying most of the development of the cracks took place (fig.3). In the next 2.5 hours only small changes appeared (fig.4) No shrinkage occurred in this example of 30% trehalose.

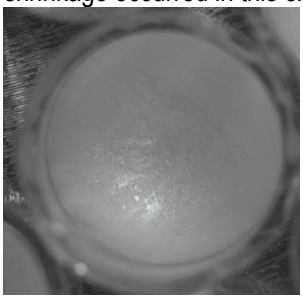
Fig. 1: Start Primary Drying

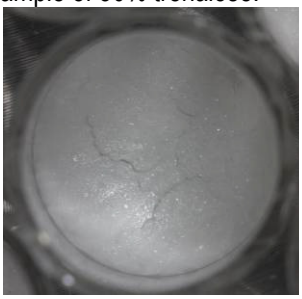
Fig. 2: Start Secondary Drying

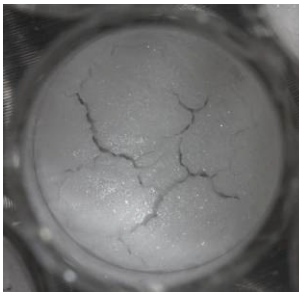
Fig. 3: At 2.5h Secondary Drying

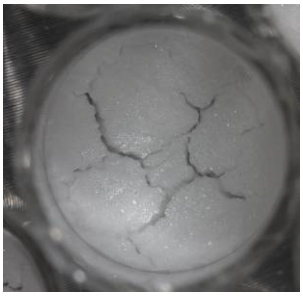
Fig. 4: At 5h Secondary Drying

Conclusion

This method can be used to investigate the formation of cracking during the entire lyophilization process in order to determine the kinetics in dependence of the freeze drying parameters.

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Computer Aided Drug Design

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Classification of an Imbalanced Dataset of P-Glycoprotein Substrates and Non-substrates using Cost-Sensitive Machine Learning Methods

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P-glycoprotein (P-gp/ ABCB1) is expressed in membrane barriers and harnesses the energy of ATP hydrolysis to drive the unidirectional transport of harmful xenobiotics from the cytoplasm to the extracellular space. In the context of cancer cells, this protective mechanism becomes destructive, leading to the expulsion of a wide variety of structurally and functionally diverse cytotoxic drugs from tumor cells. Substrates of P-gp are associated with drug-drug interactions, low blood-brain-barrier permeability and poor pharmacokinetic profiles. Therefore, it is relevant to have models which can predict whether a NME is P-gp substrate or not. However, the highly imbalanced nature of real-life datasets rules out the use of standard classification algorithms.

In the present study, we explored the applicability of various cost-sensitive supervised machine learning approaches to a set of 32944 P-gp substrates and non-substrates retrieved from the National Cancer Institute (NCI). The impact of various attribute selection techniques was examined using various physicochemical properties, fingerprints and ADMET-properties. Best models were obtained using random forest and support vector machine in combination with descriptors retrieved from the BestFirst feature selection method. Overall accuracy of the best models was >75% for the test set. The models generated using cost-sensitive bagging approach perform significantly better than the standard machine learning algorithms, demonstrating that this classification procedure effectively handles highly imbalanced datasets.

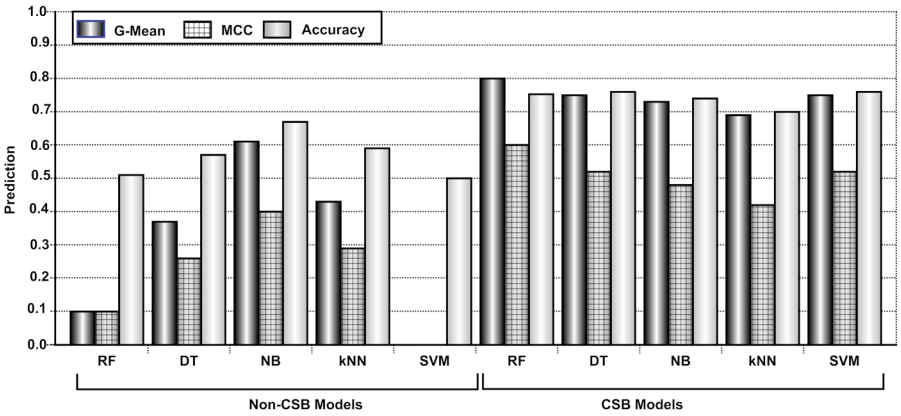

Figure: Comparison of Cost-sensitive bagging (CSB) models with non-CSB models for test set

In addition, models were validated with a set of drug compounds and compared to previously developed models. Applicability domain experiments were performed to explore the possibilities of our models towards prediction of new compounds. Models developed in this study are relatively simple and precise enough to be applicable for virtual screening of large chemical libraries for early identification of compounds which are being transported by P-glycoprotein.

Acknowledgements: We acknowledge financial support provided the Innovative Medicines Initiative Joint Undertaking, project eTOX (115002).

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Computer-aided discovery of sewarine, the first κ opioid receptor antagonist of plant origin

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The search for new active ligands utilizing computational approaches became an important tool in drug discovery. Over the past years, it became increasingly evident that the **κ** opioid receptor (KOP) plays a significant role in a broad range of physiological functions and there is evidence that receptor blockade might be useful for the treatment of depression, anxiety, addiction, psychosis, and eating disorders. The pharmacology of currently available KOP antagonists shows a delay in onset of action and an extremely long duration of action in vivo, which might limit their therapeutic potential. In the absence of direct structural information of the KOP, a

systematic study was initiated to develop a pharmacophore model that may serve as a powerful search tool to identify new chemical entities as potential KOP ligands. We present the application of a computer-aided drug design approach to discover new molecular scaffolds as novel KOP antagonists. A merged feature 3D ligand-based pharmacophore model for the KOP was generated and validated using the LigandScout and Catalyst software packages, respectively. The integrated computational screening strategy has led to the discovery of sewarine [curan-17-oic acid, 2,16,19,20-tetradehydro-10-hydroxy-, methyl ester, (19E)- (9CI)] as KOP ligand. This phenolic alkaloid from the plant *Rhazya stricta* binds with high selectivity to the KOP and shows antagonist activity towards the KOP. A comprehensive SAR analysis on several analogues was pursued and primary chemical features responsible for KOP activity have been identified. A homology model for the KOP was developed and docking studies of sewarine and other KOP antagonists have been pursued. This study uncovers a new class of ligands interacting with KOP and sharpens the understanding of ligand-receptor interactions, thus increasing the chance of developing useful clinical agents among KOP ligands.

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A PBPK-modeling approach to elucidate the Impact of CYP2D6 polymorphisms on Tamoxifen pharmacokinetics in female patients

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Physiologically-based pharmacokinetic (PBPK)-modeling comprises substance-specific parameters and sophisticated knowledge of anatomical and physiological species-specific parameters within the mechanistic framework of a generic model structure. This mechanistic framework enables the prediction of absorption, distribution, metabolization and excretion (ADME) processes in laboratory animals and humans following the administration of a substance [1,2]. The model structure of a whole body PBPK model is thereby built by compartments that represent physiological organs as well as sub-structures of organs such as the vascular, interstitial and intracellular space [2].
Tamoxifen is a first-line endocrine agent in the adjuvant treatment of estrogen receptor positive mammary carcinoma. Endoxifen is an important metabolite considered to mainly contribute to the anti-tumoral activity of the parent compound. It is formed from tamoxifen via the polymorphic cytochrome P450 (CYP) 2D6. Recently, contradicting results have been published concerning the question whether or not CYP2D6 polymorphisms may affect tamoxifen treatment outcome in a clinically significant way [3].
We have developed a coupled PBPK model of tamoxifen and its three main metabolites N-desmethyltamoxifen, 4-hydroxytamoxifen and endoxifen that is able to reflect the influence of CYP2D6 extensive (EM) and poor metabolizer (PM) phenotypes on 4-hydroxytamoxifen and endoxifen steady-state plasma concentrations. As no human plasma concentration-time data following intravenous (i.v.) administration was available, a PBPK model was developed for i.v. administration of tamoxifen to rats in order to describe the disposition kinetics. Subsequently, the rat model was extrapolated to humans accounting for human physiology. The human tamoxifen PBPK model served as a template for PBPK-models of N-desmethyltamoxifen, 4-hydroxytamoxifen and endoxifen, whereby altered physicochemical properties of the metabolites were taken into consideration. The coupled PBPK model of tamoxifen and its three metabolites was adjacently used to simulate plasma concentration-time profiles for the four substances following tamoxifen single and multiple oral administrations to a female patient of CYP2D6 EM and PM phenotype, respectively. Population simulations were then carried out for a CYP2D6 EM population of 1000 female patients and a CYP2D6 PM population of 1000 female patients accounting for their particular enzyme activities [4,5,6] and physiological variability.

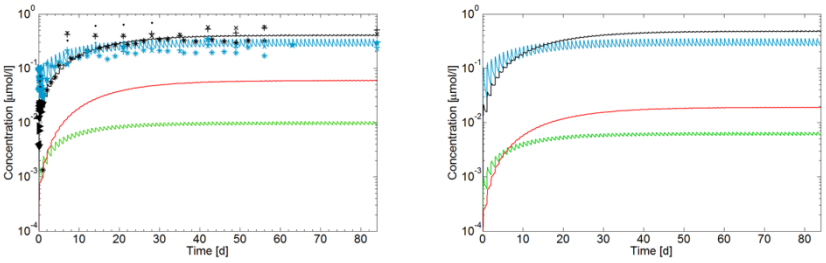

Figure 1: Steady-state plasma concentrations of tamoxifen (blue), N-desmethyltamoxifen (black), 4-hydroxytamoxifen (green) and endoxifen (red) in a female patient of CYP2D6 EM [left] and PM phenotype [right] during three months of 20 mg tamoxifen once daily.

Tamoxifen oral PK could be described in humans assuming absorption of tamoxifen from proximal as well as distal segments of the intestine. Possible important factors may be enterohepatic circulation followed by reabsorption or flip-flop kinetics due to poor intestinal solubility as tamoxifen is a Biopharmaceutics Classification System Class II compound and hence poorly soluble. The model is able to reflect the influence of CYP2D6 PM phenotype on endoxifen steady-state plasma concentrations showing that 4-hydroxytamoxifen steady-state plasma concentrations do not change in the same magnitude in CYP2D6 PMs as endoxifen steady-state plasma concentrations do [Fig. 1].
This PBPK model can be extended in order to simulate tumor response with respect to tamoxifen and endoxifen plasma and tissue concentrations influenced by CYP2D6 activity in different patient populations as outlined in [7].

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Ligand based screening for insulin mimetic compounds

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Diabetes mellitus is a chronic disease which is characterized by the lack of or by resistance to insulin action and subsequently elevated blood glucose levels. Current treatments of type 2 diabetes mellitus show sometimes severe side-effects. A key step in the insulin signalling pathway is the binding of insulin to its receptor. Small molecules activating the insulin receptor and thereby mimicking the effect of insulin might be a possible treatment for both type 1 and type 2 diabetes mellitus. A small molecule (L-783,281, demethylasterriquinone B-1 or DMAQ-B1) from a fungal extract was discovered in 1999[1], which activates the human insulin receptor by binding directly to its intracellular kinase domain. In the following years, many derivatives of this molecule were reported in the literature. The aim of our studies was to develop computational models on basis of these molecules, which can be used to screen for new insulin-mimetic compounds. As most of the currently published derivatives of DMAQ-B1 contain the original quinone ring, the main focus was to perform scaffold hopping in order to overcome potential toxicity problems.
Three different computational approaches were used to build up screening models: Self-organizing maps (using 2D autocorrelation vectors as well as VSA descriptors), fingerprint similarity, and shape similarity. Screening a large vendor library of more than 600 000 compounds with the *in silico* models led to the identification of 367 potential hits. The molecules were clustered according to their scaffolds, and selected compounds from well represented scaffolds were chosen for biological evaluation. The hits were evaluated by measuring the phosphorylation of Akt (a downstream target of the activated insulin receptor) in mouse embryonic fibroblasts using Western blot analysis. Ten compounds were tested in the first pass, with three of them showing Akt Ser473 phosphorylation at a concentration of 30 µM. One of these compounds, which also increased the glucose uptake

rate in myocytes, was selected for hit follow-up by testing additionally purchased derivatives. Here, 8 of 13 tested derivatives were activating Akt, one of them showing comparable activity to DMAQ-B1 at 10 µM.
Conclusively, our computational models were able to find compounds with a different type of scaffold compared to the intial structures. Especially the self-organizing maps performed well in identifying novel bio-active molecules.

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Novel hydroxamic acid derivatives as potential antitumor agents: Synthesis, biological and antiproliferative studies

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Histone Deacetylases (HDACs) are zinc-dependent hydrolases that mediate chromatin remodelling and gene expression by removal of acetyl groups from histone lysine residues. In cancer cells this results in cell cycle arrest, differentiation and the induction of apoptosis. Currently there are several HDAC inhibitors (HDACi) in clinical trials. Based on the proposed pharmacophore model, [1] we have synthesized novel hydroxamic acid based HDACi where the cap consists of a benzazole ring and the spacer derived from a benzenesulfonylpiperazine moiety. Out of this series, the 4-phenylhydroxamic acid derivatives inhibit mouse HDAC 1 with IC_{50} ~ 5 µM whereas the 3-phenylhydroxamic acids analogues show an IC_{50} > 100 µM. In a cell based assay, the 4-phenylhydroxamic acids have the ability to reactivate a silenced GFP construct, demonstrating that these compounds can modulate gene expression in cancer cells. In the NCI-60 cell line *in vitro* assay, the active compounds showed selectivity to leukemia, colon and CNS cancers as well as melanoma. The differential biological activities obtained could be explained by docking of the various isomeric compounds into the active centre of HDAC1.

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PO - 152

Local dynamics in serine protease recognition

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Proteases catalyze cleavage of peptide bonds and are vitally important in a wide range of fundamental cellular processes. Far more than 500 proteases have been identified in the human genome individually tied to a unique cleavage pattern. These patterns reach from specificity for a single peptide to broad spectra of cleaved peptides.
To analyze the impact of local dynamics on protease specificity, subpocket-wise protease specificity scores are presented for a series of homologous chymotrypsin-like serine proteases based on cleavage data from the MEROPS database [1]. These specificity scores appear to be linked to local flexibility of the binding site region

[2]. Consequently, B-factors from X-ray structures as well as all-atom 100ns molecular dynamics trajectories using the AMBER package [3] are compared in respect to specificity.
Analyses of specificity and flexibility patterns reveal a correlation of binding site rigidity and specificity. As increased flexibility is paralleled by a broader conformational space, a mechanism of conformational selection [4] in the binding process of serine proteases is proposed.

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PO - 153

Synthesis and in vitro characterization of Imatinib derivatives as new Farnesoid X Receptor (FXR) modulators

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Farnesoid X Receptor (FXR) is a member of the nuclear receptor superfamily and acts as a ligand-activated transcription factor [1]. It is highly expressed in liver, intestine and kidney and binds to its DNA response elements as heterodimer with Retinoid X Receptor (RXR). FXR regulates a large number of target genes which are involved in bile acid metabolism as well as in lipid and glucose homeostasis. Bile acids (most active: chenodeoxycholic acid), their metabolites and polyunsaturated fatty acids are known as natural ligands for FXR.
During the last years, FXR became a promising target for the treatment of several diseases associated with metabolic disorders, such as dyslipidemia, diabetes and atherosclerosis. Additionally, recent studies revealed its beneficial effects in inflammatory bowel disease (IBD) [2].
Synthetic ligands, such as GW4064 (a non-steroidal isoxazole derivative) or 6-ECDCA (6α-ethylchenodeoxycholic acid), were developed and led to an increased knowledge of function and role of FXR in metabolic regulation as well as in inflammatory pathways within intestine. 6-ECDCA (also called INT-747) is currently in clinical phase II for the treatment of non-alcoholic fatty liver disease (NAFLD) and primary biliary cirrhosis (PBC), but no further compounds have reached later stages of drug development so far. Nevertheless, FXR activation by synthetic ligands turned out to reduce plasma triglycerides, cholesterol and atherosclerotic lesions accompanied by improved glucose homeostasis in animal models.[3, 4] Furthermore, it was recently discovered that FXR activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease (IBD) in a mouse model [2].
This work contains the development and synthesis of new FXR modulators based on the lead structure of Imatinib. Imatinib was invented as cytostatic drug as a specific inhibitor of the tyrosine kinases abl, c-kit and PDGF-R. During a dual ligand based in silico/in vitro DrugBank screening, Imatinib was identified as a FXR agonist. The in vitro characterization of FXR activity was performed by a luciferase-based reporter gene assay established in our group utilizing human full-length FXR. Our aim was to develop derivatives of Imatinib for SAR studies that reveal the molecule properties responsible for an enhanced FXR agonistic activity as well as those for a reduced efficacy as kinase inhibitors. Our data are supported by docking experiments that show specific interactions of the ligands within the ligand binding pocket of FXR.

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PO - 154

Impact of tetramerization on neuraminidase dynamics and binding site conformations

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Influenza neuraminidase (NA) is a tetrameric surface protein of the influenza virus and the target for antiviral drugs e.g., oseltamivir and zanamivir. The conformational diversity of the 150-loop was revealed by crystal structures of the group 1 NAs [1] and investigated by molecular dynamics (MD) simulations [2, 3]. The open state conformation shows an additional sub-pocket (150-cavity) exploitable for drug design [1].
We present a systematic analysis of three NAs (avian 2005, pandemic 1918, pandemic 2009) with all-atom, explicit solvent MD simulations applying the Amber forcefield ff99SB. Comparative simulations of monomeric, dimeric and tetrameric systems show that the sampled conformational phase space for the tetramer is distinct from the monomer simulations. We demonstrate that interactions with adjacent NA subunits alter the dynamics of the 150-loop.
These results underline the importance of protein-protein-interactions in the NA tetramer for the examination of molecular flexibility and consequently for drug development.

Acknowledgements : Supported by the Austrian Research Fund (P23051 to KRL), University of Innsbruck (SvG).
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PO - 155

Galaxy drug discovery pipelines

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Introduction: A variety of software tools and components exists for compound analyses and drug discovery research, including tools for ligand- and structure-based in-silico screenings. Typically, certain processes have to be executed in predefined orders (pipelines) on sets of compounds differing from a few to several millions. At the interfaces of the tools reformatting of data is frequently inevitable. In large projects, certain processes need reviews of different researchers, requiring appropriate software for collaboration management. Furthermore, workflows have to be reproducible in terms of repeatability and traceability and should be executable without further programming or computer knowledge.
Thus, a well-established workflow management system was used to integrate a toolbox for pharmaceutical researchers with predefined software components allowing for the use of ready-to-use pipelines as well as the creation of new pipelines for drug discovery.
Methods & Results: Based on the Galaxy workflow managment system [1], several existing and newly developed tools were combined in a toolbox allowing for various applications:

- import, export, and reformatting interfaces from and to different formats (e.g. .pdb, .mol/.sdf) and IDs (PubChem ID, CAS RN, etc.) for small and large datasets,
- calculation of compound attributes (weight, number of rings, LogP, etc.) and searches or filters for compounds with certain attributes,

- similarity searches with different optimised fingerprints depending on calculated attributes or molecular structure,
- compound literature research supported by text-mining software (e.g. CIL [2]),
- prediction of pharmacokinetic properties (e.g. Cytochrome P450 metabolising or toxicity) with machine learning approaches such as artificial neural networks, support vector machines, and decision tree approaches, and
- report display and print of calculation results.

The system is accessible via internet browser. All calculations are carried out on a central server at the University of Freiburg.

Availability and Future Prospects: The Galaxy Drug Discovery Pipelines toolbox is available via internet for third parties on request. The newly developed software as well as the pipelines can be shared with other Galaxy providers if desired.

In the near future further software components for instance, ligand-docking applications are planned to include.

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PO - 156

SAR, ligand efficiency (LE) and lipophilic efficiency (LipE) studies of a series of benzophenone-type inhibitors of the multidrug transporter P-glycoprotein

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The role of the ATP-binding cassette transporter P-glycoprotein (P-gp) in mediating the resistance to a broad spectrum of antitumor agents in cancer therapy is well recognized [1]. As the interaction of ligands with the tranporter is supposed to take place in the membrane bilayer, ligand efficiency (LE) [2] and lipophilic efficiency (LipE) [3] of the inhibitors and substrates of ABC transporters should provide insights for the design of new ligands. The parameters normalise biological activity towards size and logP, thus helping to identify the derivatives with the best activity/logP (or size) ratio.

Series of benzophenones were synthesized and pharmacologically tested for their ability to inhibit P-glycoprotein mediated daunomycin efflux in multidrug resistant CCRF-CEM vcr 1000 cells [4]. Some of the benzophenones showed ligand efficiency and lipophilic efficiency behavior comparable with the compounds in different stages of clinical investigations. Interestingly, although P-gp inhibitors are highly lipophilic, they showed LipE values below the threshold considered to be necessary for promising drug candidates. This might be due to the fact that the ligand-protein interaction takes place directly in the membrane bilayer. Docking studies in a homology model of P-glycorptoein suggest that benzophenones selected on the bases of their ligand efficiency and lipophilic efficiency profiles mainly interact with TM5, 6, 7, 8 and 12. Whereas the common scaffold of the structures interact with Phe343 and Phe303 near the entry gate, the lipophilic substitution in the vicinity of the basic nitrogen atom in the molecules is surrounded by hydrophobic amino acid residues Leu724, Ile720, Val981, Ala727, Ile765 and Ala761. Benzophenone poses bridge the two binding sites for enantiomers of benzopyrano[3,4-b][1,4]oxazines [5]

Acknowledgements: This work was supported by the Austrian Science Fund (grant F03502) and Higher Education Commission (HEC) Pakistan.

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PO - 157

Pharmacophore modeling of neuropeptide S receptor ligands

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Neuropeptide S (NPS) represents the latest identified neuropeptide. Animal studies in rats and mice showed that the intracerebrally administration of NPS leads to an anxiolytic effect with a simultaneous increase of wakefulness [1]. This unusual activity profile of NPS differs from classical anxiolytic drugs which cause beside the desired effects also dizziness, drowsiness, ataxia or language disorders [2]. It is therefore of high interest to investigate ligands of the NPS-receptor (NPSR) for their anxiolytic properties and evaluate their potential use as anxiolytic drugs. Up to now, only a few non-peptidic compounds are known to interfere with the NPS-receptor causing an urgent need for the discovery of novel lead structures. The search for novel bioactive compounds and scaffolds can be achieved by several methods. One promising strategy is the use of virtual screening tools, which can effectively reduce cost and time efforts [3]. This approach resulted in the first pharmacophore modeling study of NPSR ligands. Since the 3D structure of the G-protein coupled NPSR is not known, ligand-based pharmacophore models were generated. The physicochemical information of five different compound scaffolds was used as the basis for model generation. Theoretically evaluation by two test sets containing non-peptidic active compounds and decoys revealed enrichment factors and ROC curves, which indicate a good predictive power of the generated models and will contribute to the rationalized search for novel NPSR ligands.

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PO - 158

3D-QSAR of thieno[2,3-*b*]pyridines as inhibitors of *Pf*GSK-3

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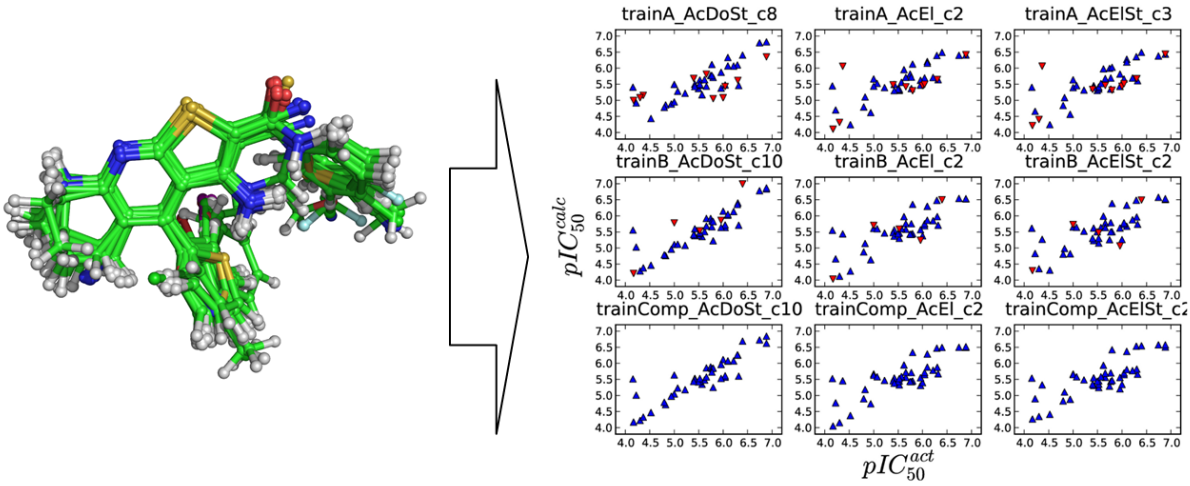
Malaria remains a global emergency with estimated 225 million cases and 781000 deaths wordwide in 2009 [1]. New options in the treatment are becoming absolutely essential because of the alarming development of resistance against the actual WHO-recommended first-line-therapy artemisinin and its derivatives [1-3]. Inhibitors of glycogen synthase kinase-3 of *Plasmodium falciparum* (*Pf*GSK-3), the causative agent of malaria tropica, have been shown to be potential leads in the development of new antimalarial drugs [4,5]. Because the crystal structure of *Pf*GSK-3 has not been published yet, we developed homology models before [6] and proposed several binding modes based on docking experiments.
These proposed binding modes served as starting points for 3D-QSAR calculations presented here. Based on alignments representing the different potential binding modes, CoMSIA-models [7] were calculated. The models were analysed with respect to their statistical performance and by visualization of the corresponding contour maps, respectively.



Alignment and corresponding residual plots of some calculated CoMSIA models.

Although statistical values were moderate (r^2, q^2 and F-value up to 0.786, 0.491 and 35.181) the models were useful in interpreting the contour maps in combination with the binding site of *Pf*GSK-3 and by this means combining ligand based and structure based information. The results confirmed one common binding mode of the thieno[2,3-*b*]pyridines similar to an unpublished X-ray structure of the human glycogen synthase kinase-3β in complex with a compound of the data set.

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PO - 159

Docking based development of new synthetic ligands for Farnesoid X Receptor

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Farnesoid X Receptor (FXR) is a member of the nuclear receptor superfamily and acts as a ligand-activated transcription factor [1]. It is highly expressed in liver, intestine and kidney and binds to its DNA response elements as a heterodimer with Retinoid X Receptor (RXR). FXR regulates a large number of target genes which are involved in bile acid metabolism, lipid and glucose homeostasis. Bile acids (most active: chenodeoxycholic acid) as well as their metabolites and polyunsaturated fatty acids are known as natural ligands for FXR.
FXR became a promising target for the treatment of several diseases like non-alcoholic fatty liver disease (NAFLD) and primary biliary cirrhosis (PBC). Synthetic ligands, such as GW4064 (a non-steroidal isoxazole derivative) or 6-ECDCA (6α-ethylchenodeoxycholic acid) were developed and led to an increased knowledge of function and role of FXR in metabolic regulation of bile acids and cholesterol as well as in inflammatory pathways within intestine. 6-ECDCA, also called INT-747, is actually in phase II of clinical trials for treatment of PBC and NAFLD. FXR activation by synthetic ligands turned out to

reduce plasma triglycerides, cholesterol and atherosclerotic lesions accompanied by improved glucose homeostasis in animal models [2,3].
Furthermore, it was recently discovered that FXR activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease (IBD) in a mouse model [4].
This work contains the development of potential FXR modulators. Docking studies of two substances previously developed in our workgroup implied a binding to the ligand binding domain of FXR. To identify novel lead structures for FXR ligands, we synthesized various derivatives of one hit compound. Enlargement of the lipophilic backbone of this hit compound should improve activity with regard to the large lipophilic moiety of bile acids as natural ligands. EC_{50} values were determined with a human full-length FXR transactivation assay. Further development based on the presented compounds is outlined.

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PO - 160

Investigation of the ATP-binding sites of IR and IGF-1R kinases with the GRID/GOLPE approach

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Over the last decade, teceptor tyrosine kinases (RTKs) have emerged as promising targets in cancer therapy. They transform extracellular signals from growth hormones into cellular responses - modulating growth, differentiation and cell survival. Increased expression of certain RTKs has been linked to various human tumor conditions.[1] The ATP-binding site of their intracellular kinase-domain has been extensively investigated and has been found to be a viable molecular target for small-molecule ATP-competitive inhibition. There are, however, some 500 human protein kinases that share a highly conserved active site making the design of selective inhibitors extremely difficult.[2, 3]
Disrupting the Insulin-like Growth Factor Receptor 1 (IGF-1R) signaling axis – either via monoclonal antibody or small molecule inhibition – has been proven to be a promising approach for susceptible tumor conditions. The IGF-1R has been intensively targeted for this reason; However, its ATP-binding site is almost identical to its highly homologous neighbor – the Insulin Receptor (IR).[4, 5] In order to avoid off-target activity and predicted unwanted side-effects, IR inhibition is an undesired property of novel compounds. Though dual inhibition might be beneficial in special cases [6], inhibitors that are selective for the IGF-1R are needed. In the absence of sequence differences, conformational variations between both binding sites have to be taken into account. Employing the GRID/GOLPE approach [7-9], we combine the description of the binding sites by molecular interaction fields (MIFs) with statistical analysis on a set of thirty kinase domains. The distinct phosphorylation states of these domains are considered separately by dividing the set in active and inactive structures or open and closed, respectively, and are examined with different GRID probes representing interactions with hydrophilic and hydrophobic, as well as hydrogen-bonding moieties. Protein flexibility is accounted for both by using multiple structures in a consensus principal component analysis (CPCA) and by incorporating a second examination with flexible instead of rigid amino-acid side chains.
A representative CPCA score plot is depicted in Figure 1: The squares represent the examined protein structures and form two separate clusters that can be used to distinguish IGF-1R from IR by the first principal component (PC1).
Interestingly, distinct conformational states of a conserved residue in close proximity to the DFG-motif (the conserved amino acid triplet Asp-Phe-Gly at the beginning of the highly flexible activation loop) may be used to differentiate the IGF-1R from the IR active site for example. Revealing these individual and – possibly – kinase

dependent properties, the results may eventually aid in the challenging task of structure-based design of selective IGF-1R inhibitors.

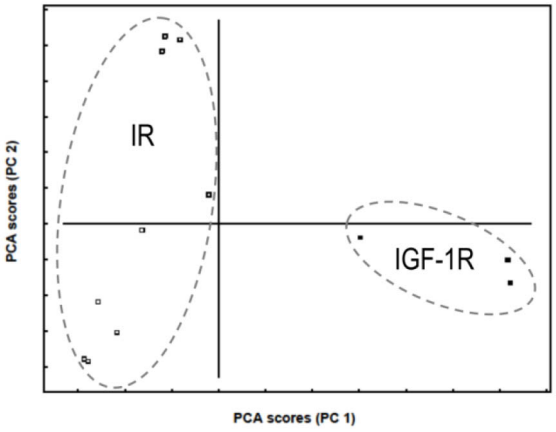

Figure 1: CPCA Score Plot

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PO - 161

Development of novel PPARγ agonists based on the molecular modeling of Telmisartan analogues

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Telmisartan, a well-known angiotensin II receptor antagonist used in the treatment of hypertension, revealed to be a partial agonist of the peroxisome proliferator-activated receptor γ (PPARγ), which is a pharmaceutical target of high interest due to its potential in the treatment of diabetes type 2 and other related diseases [1]. Clinically used PPARγ agonists, such as members of the thiazolidindiones family (glitazones), show extensive side effects like water re-tention leading to edema and an increased risk of cardiovascular complications [2], while telmisartan is well-tolerated [3]. Therefore, telmisartan is a promising starting point for the development of new PPARγ agonists with a more advantageous pharmacological profile. Previous studies on telmisartan analogues determined a 4'-[(2-propyl-1*H*-benzimidazole-1-yl)methyl]biphenyl-2-carboxylic acid core as the minimum requirement for activity on PPARγ [4]. This lead structure was extended by creating a virtual database of multiple structural derivates using ilib:diverse software [5]. Based on X-ray crystal structures of the PPARγ binding pocket with synthetic ligands, a pharmacophore model was developed and used for screening the virtual database employing LigandScout [6]. Potential PPARγ agonists that returned as virtual hits are currently synthesized and biologically evaluated.

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PO - 162

Discovery of a novel Granzyme B inhibitor by pharmacophore-based and docking-based virtual screening

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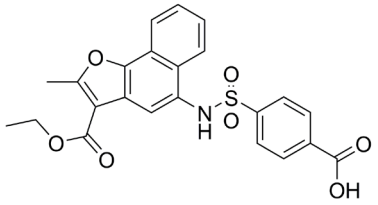
Cytotoxic T lymphocytes (CTL) and natural killer (NK) cells eliminate virus-infected or tumor cells by means of introduction of various proteases, referred to as granzymes, to the target cell cytosol. Granzyme B (GrB), for example, enters the target cell by a perforin-dependent mechanism and induces apoptosis, representing a form of cell death that is usually immunologically silent [1]. However, the study by Metkar et al., which showed a profound resistance to LPS-induced shock in GrA and GrB-deficient mice, indicated that these important enzymes have a distinct role as instigators in inflammation [2]. This hypothesis is supported by a recent study that showed a role of GrB in the conversion of the precursor of IL-18 to its pharmacologically active form [3].
In the present study, the development of a 3D pharmacophore model based on the investigation of the active site for potentially interacting regions, referred to as hot spots, was conducted [4]. The developed 3D model was used to virtually screen the Specs database (www.specs.net). The focused hit list was submitted to molecular docking in order to rescore the retrieved molecules. Biological evaluation of prioritized molecules in an *in vitro* assay against GrB was conducted, yielded one novel compound **1** (see Figure) with potency against GrB (IC_{50} =12.1 ± 1.2µM; t=30min).



1
IC_{50} =12.1 ± 1.2µM
2D representation of novel bioactive compound **1**

Acknowledgments: We thank the DFG for financial support of this project.
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PO - 163

MM/PBSA and MM/GBSA calculation for estimating the free energy of binding of Glycogen Synthase Kinase-3 inhibitors

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Glycogen Synthase Kinase-3 (GSK-3) is a serine/threonine protein kinase involved in many cellular processes. In higher eurcaryotes, GSK-3 plays a role in the Wnt and insulin signalling pathway, glycogen and protein synthesis, cell proliferation and adhesion, tumorgenesis, neuronal death, apoptosis, etc.[1,2]. This wide influence has GSK-3 turned into an interesting target for cancer, diabetes mellitus and Alzheimer therapy.
Over the past few years the three-dimensional structure of GSK-3 in complex with different inhibitors has been determined by several work groups offering promising starting points for structure based design of new potent and selective GSK-3 inhibitors. One of the crucial aspects of structure based design is the identification of the correct binding geometry of putative inhibitors by predicting binding free energies via molecular dynamics (MD) simulation and MM/PBSA/GBSA calculations [3].
Seven crystal structures of GSK-3 inhibitor complexes were selected from Protein Database (PDB) and IC_{50} values for these complexes, determined with identical ATP concentration, were taken from the literature [4]. We studied the effects of parameter settings in MD simulations and MM/PBSA/GBSA calculations (simulation time, step size, snapshot range, dielectric constant) in order to find optimized program conditions [5]. Correlation coefficients between the free energy of binding estimated by MM/PBSA/GBSA and pIC_{50} were in the range of 0-0.7, depending on the chosen parameters [6,7].

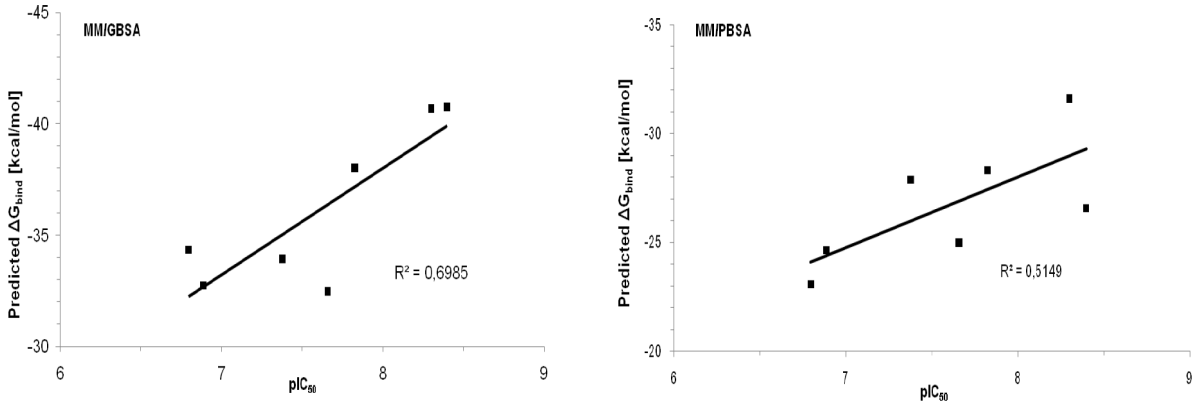

Figure 1: Calculated binding free energies plotted against experimental pIC_{50} value for optimized calculation settings.

The results of our systematic investigation will be discussed. The optimized setup was finally used for the prioritization of docking poses from a redocking experiment of a GSK-3*β* inhibitor-complex crystal structure.[8]

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PO - 164

Alzheimer's Disease: New promising compounds with multiple effects and blood-brain-mobility

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Based on DUO compounds, which are potent ditopic inhibitors of acetylcholin-esterase (AChE) [1], but too long for the catalytic gorge, new compounds have been developed. Using roughly only one part of the molecule led to permanently positively charged molecules of high AChE inhibitory activity. However, they are not able to pass the blood-brain barrier (BBB) anymore [2]. Therefore the pyridinium ring was replaced by a piperidine ring whose nitrogen can be protonated or non-protonated. Modeling studies revealed that the observed loss of activity is due to the non-aromatic piperidine ring. Thus a "flat" pyridine hydrazone ring system was employed. These new lead compounds show satisfying inhibitory effects on AChE, as well as an activity on butyrylcholinesterase (BuChE) and show inhibitory effects in ROS tests. Furthermore they inhibit the amyloid β-fibril formation and lead to a disaggregation of preformed amyloid β-fibrils [3]. Even though the inhibitory AChE-activity of these compounds was diminished, they clearly show the possibility to develop multitarget molecules. New modeling studies pointed to raised inhibitory effect on AChE elongating the hydrazone part or extending the conjugated system. Derivatives with an elongated hydrazine part (**1**) exhibited a better inhibitory potency (AChE) whereas compounds with an unsaturated linker attached to the dihydropyridine nitrogen (**2**) show lower activity. Both compounds were able to pass the blood-brain-barrier and thus ideal leads for further drug development.

1
(IC50 AChE 0.063±0.009 µM)

2
(IC50 AChE 0.94±0.07µM)

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PO - 165

Modeling and synthesis of new aromatic sulfonamides with IOP lowering effect

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Compounds bearing sulfonamide groups have long been known to be potent inhibitors of the carbonic anhydrases (CA) [1]. Various substituted aromatic and heterocyclic sulfonamides have been synthesized and evaluated for possible therapeutic use as antiglaucoma agents. It is well established [1, 2] that a water-soluble sulfonamide, also possessing relatively balanced lipid solubility, would be an effective antiglaucoma drug via the

topical route. In this work we report the design and synthesis of a novel drug-like aromatic sulfonamides (e. g. 4-sulfamoyl-N-(3-morpholinopropyl) benzamide, N-(3-morpholinopropyl)benzene-1,4-disulfonamide and their hydrochloride salts) with favorable biological, structural, physicochemical and some pharmacokinetic properties comparable to those obtained for therapeutically useful acetazolamide, dorzolamide and brinzolamide [3-5]. The solid-state structure of novel aromatic sulfonamides has been examined by X-ray crystallography. Methods of theoretical medicinal chemistry were applied for structural characterization of these compounds in the gas phase and water solution. Of particular interest are the molecular geometries of neutral and anionic species, acidities, and lipophilicities. The results of theoretical studies of sulfonamides were compared with the available experimental data and discussed with the present theories of action of these inhibitors of carbonic anhydrase.

Acknowledgements: The authors thank the Slovak Ministry of Education (Slovak Grant Agency VEGA contract No 1/0084/11).
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PO - 166

The human PKMYT1 as a target in G2/M transition: Discovery of new lead structures

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The membrane-associated Myt1 kinase (PKMYT1) plays a significant role in the cell cycle. It acts as a negative regulator for entry into mitosis by phosphorylating the Cyclin-dependent-kinase 1 (CDK1) in a dual specific manner on threonine-14 as well as tyrosine-15. These phosphorylations have an inhibitory effect on the kinase activity of CDK1-Cyclin complexes and therefore prevent the cell from passing the G2/M transition [1]. PKMYT1 has been associated with various pathological alterations including different carcinomas (e.g. UV-A induced skin cancer [2]). Abrogation of the G2/M checkpoint by inhibition of the CDK1(Tyr-15) specific Wee1 kinase results in an increased anti-cancer activity of DNA-damaging agents in p53-deficient tumor cells [3]. With regard to this finding, the discovery of selective inhibitors for PKMYT1 as potential drug candidates could be a valuable addition to conventional chemotherapy in order to help overcome resistances.
Since the nature of PKMYT1 specificity is not fully understood yet and the literature is contradictory, fluorescence polarisation immunoassays and western blotting techniques were used to elucidate the acceptance of different substrates. Unspecific polypeptide substrates, specific CDK1-derived peptides as well as the whole CDK1 protein and CDK1/CycB1 complex were used as substrates to determine the minimal structure needed. In addition, a PKMYT1 variant consisting of the kinase domain only was investigated in terms of substrate acceptance. This will provide the means to establish an assay platform in order to determine the effects of potential inhibitors on the human PKMYT1. Computer aided designed ATP-competitive compounds as well as glycoglycerolipids derived from a marine algae natural product were synthesized to develop new lead structures for Wee kinase family.

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PO - 167

Pharmacophore-based discovery of natural products as protein tyrosine phosphatase 1B (PTP1B) inhibitors

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Protein tyrosine phosphatases (PTPs) represent important regulators of intracellular signaling, immune response, cell proliferation, differentiation, migration, gene transcription, metabolism, cell-cell communication, ion channel activity, and others. Thus they are associated with several human diseases including obesity, diabetes, cancer, inflammation, and neurodegenerative diseases [1,2]. On the molecular level, PTPs catalyze dephosphorylation reactions, which can result in stimulation as well as inhibition of specific pathways [1-3].
PTP1B is widely expressed and consists of 435 amino acids. It is located on the cytosolic surface of the endoplasmic reticulum fixed via its 35 amino acid C-terminal sequence [4]. The enzyme acts as a negative key regulator of the metabolically essential insulin and leptin signaling pathways [5]. Thus it represents an interesting potential target for the therapy of diabetes and obesity [2,6].
For the discovery of novel bioactive compounds, virtual screening has evolved as a valuable and efficient strategy to focus experimental efforts on the most promising compounds [7]. To identify new potential lead structures, we built structure-based pharmacophore models for PTP1B inhibitors. After theoretical model validation, the best-performing models were selected to screen in-house natural products databases (the DIOS [8] and the natural products database [9]). Consensus hits from several searches and identified prominent natural compounds were submitted to biological testing using a colorimetric in vitro enzyme assay with human recombinant PTP1B. Out of eight evaluated compounds, four, namely octadecanedioic acid, docosanedioic acid, riboflavin-5'-phosphate, and folic acid, showed dose-dependent PTP1B inhibition with IC_{50} values of 12.9 µM, 1.2 µM, 6.9 µM, and 79.6 µM, respectively.

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PO - 168

Structural insights onto the histamine H_1-receptor: Crystal structures, molecular dynamics and semiempirical calculations

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G protein coupled receptors represent important targets for several drugs [1]. Experimental studies, like site directed mutagenesis, coupled with pharmacological assays can be performed in order to get knowledge about

amino acids of GPCRs, which have influence onto pharmacology. But in addition to these experimental studies, molecular modelling studies are important in order to get more detailed insights onto ligand-receptor interactions on molecular level for efficient development of new drugs. In general, models of GPCRs are generated by homology modelling, based on an appropriate crystal structure.
Since up to now, no crystal structure of hH_1R was available, we used the $h\beta_2R$ or opsin crystal structure as template for modelling hH_1R. A large number of pharmacological data could be explained well with these models. Recently, the crystal structure of a hH_1R-doxepine-complex (3RZE.pdb) was published [2]. A comparison of our models with the crystal structure revealed a significant difference with regard to $Trp^{4.56}$. Furthermore, subsequent molecular dynamic studies of our $h\beta_2R$-based hH_1R-model and the hH_1R crystal structure revealed large differences in stable hydrogen bond networks as well as in conformation of distinct amino acid side chains, like $Trp^{4.56}$ and $Trp^{6.48}$. The latter is discussed to be involved in a rotamer toggle switch during receptor activation.
With force-field based molecular dynamic simulations it is not possible to observe different protonation states of acidic and basic amino acid side chains in the receptor. In order to study, if different protonation states of amino acid side chains are responsible for differences in hydrogen-bond networking and ligand-binding, semiempirical calculations of the whole receptor including water molecules were performed additionally. These studies revealed some energetically preferred, water mediated proton transfers in the intracellular part of hH_1R. Since conserved amino acids are predicted to be involved in protone transfers, these results might be also important for other biogenic amine receptors.

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PO - 169

Finding and analyzing the binding mode of new cyclooxygenase inhibitors with pharmacophore modeling

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Cyclooxygenase (COX) 1 and 2 are important factors within the inflammatory cascade and their inhibition and regulation is a very interesting goal in drug design [1]. Many of the currently used COX inhibitors cause severe side effects, especially during long term treatment, so the search for new COX inhibitors with more advantageous adverse effect profiles continues. A COX 1 and 2 pharmacophore model collection was developed on the basis of the existing COX crystal structures in the PDB database [2] and enhanced with ligand based models to find a wide variety of active scaffolds. The optimized model collection was used to screen large compound databases and several new compounds were found and considered for biological testing. In addition a collection of natural quinonic COX inhibitors was screened [3]. While most of the inhibitors were found to follow a radicalic redox mechanism targeting the heme group on the surface of the enzyme, a few displayed activity that could be only explained by a traditional NSAID binding mechanism. These ligands (primin, alkannin, diospyrin, and embelin) were found by our model collection and possible binding modes were proposed.

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Classification models of TRPV1 antagonists

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Transient receptor potential vanilloid 1 (TRPV1) belongs to the family of transient receptor potentials located principally in the nociceptive neurons of the peripheral nervous system. It is a tetrameric non-selective cation channel, each domain containing six-transmembrane subunits. Its' activation by protons, heat, or natural agonists such as capsaicin or resinferatoxin, leads to excitation of primary sensory neurons and consequently to the sharp und burning pain perception. Development of TRPV1 antagonists is thus one of the recent potential research trends for treating diverse pain conditions [1].
In order to create classification models for active/inactive compounds we built binary classification models based on PEOE, VSA and VSURF descriptors as implemented in MOE [2] for a set of 739 compounds filtered from the TRPV1 antagonists available in CHEMBL DB [3]. The set was divided equally into active/inactive with a threshold value of 6.65 for the pIC_{50}.
Adequate models were obtained with R^2 ranging from 0.749 to 0.794. The best models were obtained with VSA (VDW surface area) descriptors with accuracy on prediction equal to 0.785 (prediction of active compounds in training set - 0.789, inactive - 0.781). With respect to descriptor performance, the VSA descriptors performed slightly better than the VSURF.

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In silico prediction of ligand affinities of propanolol derivates to cytochrome P450 2D6

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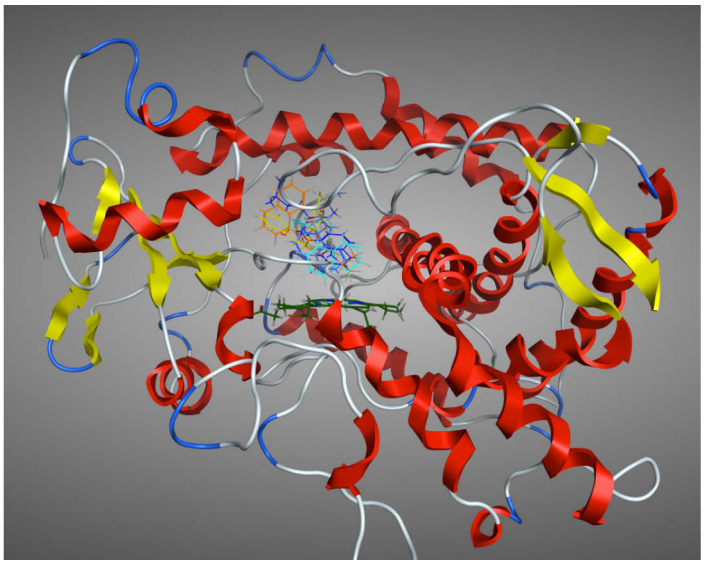
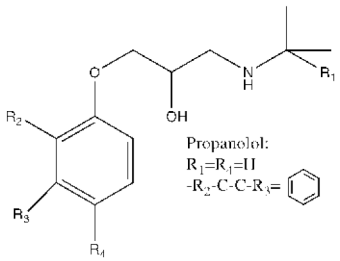
The accurate prediction of ligand-receptor binding affinities remains one of the true challenges in the field of computer-aided drug design. The flexibility and plasticity of proteins such as Cytochrome P450 (CYP) 2D6 makes complete sampling of its chemical space difficult and time-consuming. Our research aims on establishing an effective predictive model for ligand binding to CYP2D6 that fully takes into account the ligand flexibility and protein plasticity.
The Linear Interaction Energy (LIE) method[1] offers an efficient manner to predict protein-ligand binding affinities in terms of free energies of binding. Based on molecular dynamics (MD) simulations of a set of ligands for which binding affinities are experimentally known, the LIE electrostatic and van der Waals parameters can be calibrated. Subsequently, MD simulations can be used for affinity predictions.
As a powerful extension of the LIE method, Stjernschantz and Oostenbrink recently introduced a method to take into account various starting protein-ligand conformations, based on a Boltzmann weighting scheme.[2] This

extension enables inclusion of a variety of relevant ligand binding orientations and protein conformations into LIE binding-affinity predictions, as well as a weighting to the various conformations. We applied this approach to a set of propranolol analogues, with experimental IC_{50} values ranging between 0,03 and 100 µM.[3] Three protein conformations were selected to dock the ligands into, based on the work of Hritz *et al.*[4] Starting poses for the ligands were selected to be as diverse as possible.



Left: Cytochrome P450 2D6 with five propanolol poses docked inside

Below: Propanolol backbone



Compared to models based on single protein-ligand complex simulations, the LIE model obtained by including the various starting poses of the ligands as well as the three protein conformations is found to yield a better fit of calculated binding free energies to experimental values. Our study also shows that the best-weighted starting poses often do not correspond to the best-scored docking pose.

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Pharmacophore models for 11β-hydroxysteroid dehydrogenases, pre-receptor regulators of local glucocorticoid concentrations

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11β-Hydroxysteroid dehydrogenases (11β-HSDs) are responsible for the local concentration of cortisone and cortisol in human tissues: 11β-HSD1 converts the inactive cortisone to active cortisol and 11β-HSD2 catalyzes the opposite reaction [1]. Selective 11β-HSD1 inhibitors could be used in the treatment of metabolic syndrome and type 2 diabetes, while inhibition of 11β-HSD2 causes hypokalemia and hypertension [2-3]. Despite of the antitarget nature of 11β-HSD2, inhibition of 11β-HSD2 serves as a way to increase the cortisol levels. In addition, the more is known about the structure of 11β-HSD2, the easier it is to avoid unwanted (side) effects arisen from 11β-HSD2 inhibition. During the 11β-HSD selectivity studies, we created a homology model of 11β-HSD2 [4]. Comparison of the crystal structure of 11β-HSD1 and the homology model of 11β-HSD2 revealed the putative differences between the binding sites of these enzymes, leading to a hypothesis of the selectivity. Using this

hypothesis and six selective 11β-HSD2 glycyrrhetinic acid derivatives, we derived two pharmacophore models of 11β-HSD2 inhibitors. These models were successfully used in the screening of the Innhouse, Specs, and Maybridge databases. From these databases 25 compounds were selected and biologically tested against 11β-HSD activity. Six of them selectively inhibited 11β-HSD2 in a micromolar range (Figure 1).

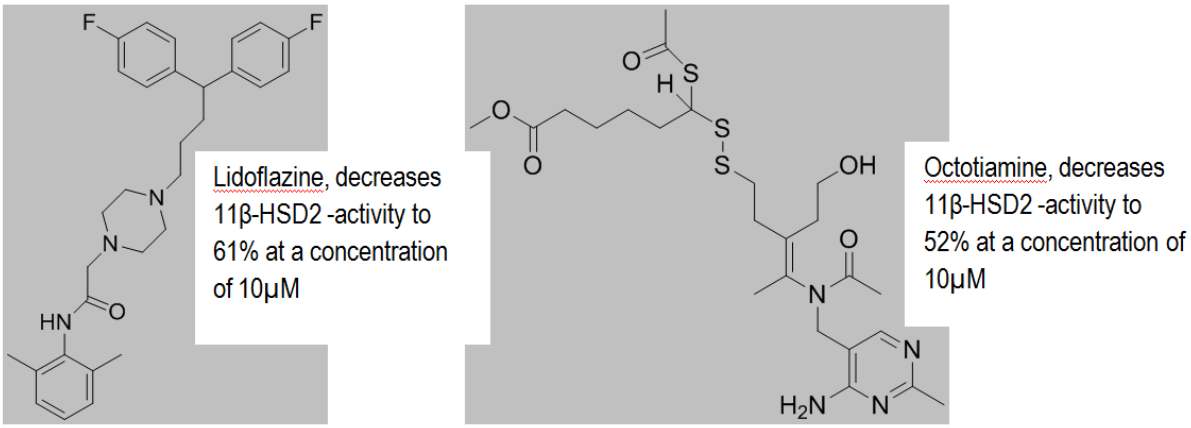

Figure 1: Two examples of 11β-HSD2 inhibitors found during this study

Acknowledgements: A.V. and D.S. thank the University of Innsbruck, Nachwuchsförderung, for supporting this work.
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Microsomal prostaglandin E_2 synthase-1 (mPGES-1): Pharmacophore modeling and virtual screening leading to novel acidic inhibitors

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Microsomal prostaglandin E_2 synthase 1 (mPGES-1) is a key enzyme in the prostaglandin (PG) E_2 biosynthesis pathway [1]. Its expression is increased in response to pro-inflammatory stimuli [2]. Specific inhibition of mPGES-1 is expected to leave the physiologic PGE_2 synthesis as well as other COX-derived prostanoids unaffected [3,4]. Hence, there is an increasing interest in this potential therapeutic strategy as an alternative to presently available anti-inflammatory drugs.
This study aimed at the identification of novel mPGES-1 inhibitors. Therefore, two pharmacophore models for acidic inhibitors of mPGES-1 were developed and theoretically validated. The chemical databases supplied from the National Cancer Institute (NCI) and the Specs were virtually screened using the models as search query. Biological investigation of 29 selected virtual hits led to the identification of nine chemically diverse compounds which concentration-dependently inhibited mPGES-1 in a cell-free assay with IC_{50} values between 0.4 and 7.9

μM. Most of these nine compounds also inhibited 5-lipoxygenase (5-LO) in a cell-free assay and in intact polymorphonuclear leucocytes (PMNL) with IC_{50} values in the low micromolar range. Such dual inhibitors are advantageous since simultaneous inhibition of several physiologically related targets should provide benefits in pharmacotherapy in respect of synergistic therapeutic effects as well as reduction of the incidence of side effects. In summary, pharmacophore modeling and virtual screening led to the identification of nine novel chemical scaffolds potently inhibiting mPGES-1, a promising anti-inflammatory target [5].

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Combining SFCscore with Random Forests leads to improved affinity prediction for protein-ligand complexes

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SFCscore is a collection of empirical scoring functions derived from a set of over 60 descriptors for protein-ligand complexes of known structure [1]. By the time of their derivation, SFCscore functions were the best-performing scoring functions tested on large heterogeneous data sets, but the overall correlation was still not within the desired range. Similarly, despite the ever increasing amount of structure and affinity data, the general advancements in the development of empirical scoring functions have been rather moderate over the past years. However, more recently, Ballester et al. [2] published a function that outperformed current state-of-the-art scoring functions when tested against the PDBbind benchmark set [3]. This function uses relatively simple atom contact counts as descriptors and is derived by the Random Forest algorithm. Here, we present a study in which we used Random Forests to derive a new SFCscore function based on the SFCscore descriptors as input data. Although this is not a fully non-parametric approach, the descriptors are supposed to capture more accurately the physically relevant interactions. We tested the new function against the PDBbind benchmark set and, in addition, performed the Leave-Cluster-Out validation as proposed by Kramer and Gedeck [4]. The results suggest that the new function significantly improves the predictive power of SFCscore, as it increases the correlation between predicted and experimentally determined affinities of the PDBbind benchmark set from $r^2 = 0.42$ (best previous SFCscore function) to $r^2 = 0.62$ ($SFCscore^{RF}$).

Acknowledgements: Financial support by the DFG (KFO216) is gratefully acknowledged.
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Counterfeit Drugs

PO - 175

Influence of injection parameters on detection and quantification limit in capillary zone electrophoresis

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The European Pharmacopoeia (Ph.Eur.) gives detailed information about varying parameters of analytical techniques such as HPLC, thin layer chromatography and gas chromatography in order to fulfil system suitability criteria. However, the general chapter about capillary electrophoresis is lacking such instructions. Since even small variations in one of the multitude of parameters in capillary electrophoresis such as pH value of the running buffer, applied voltage or temperature can have a great impact on resolution, migration time and even migration order of analytes [1], these correlations have to be investigated.
Therefore, the aim of this work was to analyse the influence of injection parameters of hydrodynamic injection and internal diameter of the capillary on limit of detection (LOD) and quantification (LOQ). This was achieved by altering injection pressure and time of a validated method [2] for quantifying lidocaine by capillary zone electrophoresis and determining LOD and LOQ based on the signal-to-noise ratios of 3:1 and 10:1, respectively. The LOD of 1,3 µM at validated conditions ranged from 0,4 to 30 µM when changing parameters.
However, a decreased detection limit by simply changing injection parameters may result in lower resolution or a loss of baseline separation through band broadening.

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PO - 176

Seizure of unapproved drug named “1-Androsterone”

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New analogues of androgens that had never been available as approved drugs are have been recently available in unapproved pharmaceuticals, and are often marketed as “dietary supplements”. They are mainly advertised to promote muscle mass and are considered by the governmental authorities in various countries, as well as by the World Anti-doping Agency for sport, as being pharmacologically and/or chemically related to anabolic steroids.
In the present study, we report the detection of a steroid in a product seized by the State Bureau of Criminal Investigation Schleswig-Holstein, Germany. The product “1-Androsterone” of the brand name “Advanced Muscle Science” was labeled to contain 100 mg of “1-Androstene-3b-ol,17-one” per capsule.
The product extract was analyzed underivatized and as bis-TMS derivative by GC–MS and by nuclear magnetic resonance. Semi-quantitation revealed an amount of 3β-hydroxy-5α-androst-1-en-17-one in the capsules as labeled. Following oral administration to a male volunteer, the main urinary metabolites were monitored by GC-MS.

1-Testosterone (17β-hydroxy-5α-androst-1-en-3-one), 1-androstenedione (5α-androst-1-ene-3,17-dione), 3α-hydroxy-5α-androst-1-en-17-one, 5α-androst-1-ene-3α,17β-diol, and 5α-androst-1-ene-3β,17β-diol were detected besides the parent compound and two more metabolites (up to now not finally identified but most likely C-18 and C-19 hydroxylated 5α-androst-1-ene-3,17-diones). The steroids were identified by comparison of the GC-MS properties with those of reference steroids. As most of the "designer steroids" and their metabolites are not commercially available, in house syntheses of 3β-hydroxy-5α-androst-1-en-17-one and the 5α-androst-1-ene-3ξ,17β-diols were performed by reduction of 5α-androst-1-ene-3,17-dione with LS-Selectride or K-Selectride, while 3α-hydroxy-5α-androst-1-en-17-one was derived by selective opening of the intermediate epoxide of 5α-androst-2-ene-17-one.

Acknowledgements: Manfred Donike Institut für Doping Analytik e.V., Cologne, Federal Ministry for the Interior, Berlin, and NADA, Bonn
Reference:
Parr MK et al. (2011) Steroids 76: 540-547

Miscellaneous - Analytics

PO - 177

Formation of host-guest complexes with calixarenes as detected by circular dichroism

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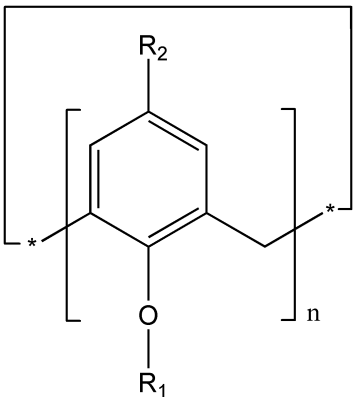
Calixarenes (CAs) are a class of cyclooligomers formed via a phenol-formaldehyde condensation [1]. Due to their cage-like shape with a hydrophobic cavity, calixarenes are one of the best known host molecules along with cyclodextrins, and crown ethers. They have the ability to form host-guest complexes with a variety of organic or inorganic compounds. However, these compounds have been less studied compared to cyclodextrins [2-5]. This work is devoted to the evaluation of the ability of eleven different calixarenes to form host-guest complexes with eleven chiral active pharmaceutical ingredients (APIs) in different solutions (acetonitrile, methanol and water), including comparisons between water-soluble calixarenes and three pharmaceutically relevant cyclodextrins (α-, β- and γ- cyclodextrins) by means of circular dichroism spectroscopy (CD). The obtained CD spectra provided the absolute configuration of the chiral APIs, as well as of the interactions with host-molecules. An attempt to understand the complexation mechanism of calixarenes was undertaken based on the CD spectra of the drugs with different host macrocycles. These results indicate that calixarenes could serve as candidate host molecules in the pharmaceutical researches due to their versatility and the ease of adding different moieties to their upper and/or lower rim, which makes it easier to change the affinity of these cyclooligomers towards target molecules and/or increase the solubility of the calixarenes.

calixarenes	CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8	CA9	CA10	CA11
n	4	4	4	4	4	6	6	6	4	6	8
R2	H	*tert.*-butyl	*tert.*-butyl	*tert.*-octyl	*tert.*-octyl	H	*tert.*-butyl	*tert.*-butyl	SO_3H	SO_3H	SO_3H
R1	CH_2-COOH	CH_2-COOH	H	CH_2-COOH	CH_2-$COOC_2H_5$	CH_2-COOH	H	CH_2-COOH	H	H	H



Acknowledgements: C. Chamseddin has a PhD scholarship from the DAAD and the Syrian ministry of high education.
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PO - 178

Qualification in Capillary Electrophoresis

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Capillary electrophoresis (CE) is a well established and frequently used analytical technique in drug development and quality control. Regulatory authorities such as the European Medicines Agency (EMEA) and the Federal Drug Administration (FDA), require a qualification for analytical instruments like CE systems. The European Pharmacopeia, for example, provides some fundamental information about the requirements [1]. Moreover, a concept for Analytical Instrument Qualification (AIQ) was included to the US Pharmacopeia as the General Chapter <1058>. Two parts of the AIQ, operational qualification (OQ) and performance qualification (PQ) are of particular interest in daily laboratory routine.

Due to these guidelines, a concept for OQ and PQ was developed to assure the specifications of a CE system. For every important parameter, a qualification method is presented with acceptance criteria. In particular, it should be tested if the temperature adjusted by the cooling system and the voltage supply operate accurate and precise. Aside of that the detector should be analysed about its noise and drift, its wavelength accuracy and its linearity. The last step should be the qualification of the injection linearity, accuracy and precision. An important factor is also the sequence of the different qualification methods. Especially in the last part of the qualification, previously tested parameters, e. g. voltage precision and detector linearity, influence the result of the injection testing.

Testing some parameters holistically, the breakdown of an instrument should only last a few hours, so that the PQ can be performed very economically.

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PO - 179

Novel simple HPTLC approach to avert plasma sample preparation: Application to the determination of gemifloxacin mesylate using fluorescence detection

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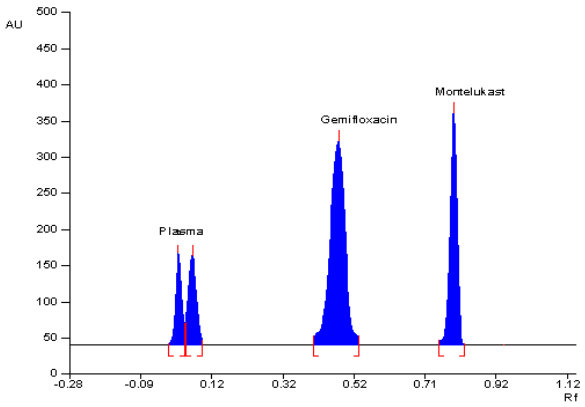
Novel, simple, selective and sensitive high performance thin-layer chromatography (HPTLC) approach with fluorescence detection has been successfully developed and validated to avert plasma sample preparation. The developed assay employed silica gel as a polar stationary phase. In this approach, the analyte-containing plasma sample was directly applied onto HPTLC plate. The optimum conditions for the developed assay were carefully studied and applied to the direct determination of gemifloxacin mesylate (GFX), fluoroquinolone antibiotic, in plasma. Montelukast (MK), 150 ng/band, was used as internal standard. GFX and MK in plasma samples were separated using a mobile phase consisting of a mixture of ethyl acetate: methanol: 25% ammonia, (8: 4.5:3, v/v/v). The emission intensity was measured using optical filter K400 after excitation at 342nm. The R_f values for GFX and MK were 0.45±.0.03 and 0.79± 0.02 respectively. Under the optimum conditions, a linear relationship with good correlation coefficient (r = 0.9965, n = 6) was found between the peak area ratio of GFX to MK and GFX concentrations in the range of 1.5-200 ng/band. The LOD and LOQ of the proposed method were 0.45 and 1.5 ng/band, respectively. The intra and inter-assay precisions were satisfactory; the relative standard deviations did not exceed ±3.52%. The accuracy of the method was proven; the recovery of GFX from spiked human plasma was 94.24–101.85 ± 1.52-3.51%. The assay results correlated well with those obtained by high-performance liquid chromatography (r = 0.992). The method had higher throughput and short run-time (<10 min). Moreover, the proposed method was successfully applied for determination of GFX in its combined dosage form and in presence of its photo-degradants (as stability-indicating assay). The approach described herein was validated for GFX only, but also, is anticipated that the same methodology can be applied for determination of similar analytes.



Chromatographic peak of human plasma spiked with gemifloxacin mesylate (60ng/band) and montelukast (IS) 150ng/band.

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PO - 180

Sulfoconjugated metabolites for doping control analysis

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Several doping relevant substances are excreted mainly sulfonconjugated or are sulfoconjugated after phase-I metabolism in urine. Inhere we focus on conjugates of stimulants like ephedrine, etilefrine, amphetamine, octopamine and β2-agonists like terbutaline and salbutamol which can be misused in sports as doping substances and are listed on the 2011 prohibited list of the World Anti-Doping Agency.
To screen for those mentioned substances or their parent compound, up to now chemical hydrolysis is performed in sample preparation of urine specimen. To avoid time-consuming sample preparation of urine samples and with the perspective to combine screening procedures (does not sound good, please modify), reference substances of the sulfoconjugates and their characterisation are necessary.
As these reference compounds are not commercially available, synthesis of them was performed in house. Since all molecules have a phenylethylamine moiety, the aglycons are treated similarly for the synthesis of the corresponding sulfoconjugates: Stirring with sulfur trioxide pyridine complex yield different mono, di, and trisulfated conjugates. After elaborate clean up, characterisation, mainly for the mono-sulfated compounds, was performed with LC/(ESI)-MS/MS, ESI-high resolution/high accuracy Orbitrap mass spectrometry and NMR.
Application to excretion studies has proven the occurrence of phenolic mono-sulfoconjugated metabolites of the substances or their phase-I metabolites, respectively. Other possible positions in the molecule were proven not to be sulfoconjugated. This has been presumed in literature but was not proven up to now.

Acknowledgements: World Anti-Doping Agency (reference numbers 071007WS and 05D11WS)
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PO - 181

Immunosuppressant therapeutic drug monitoring with HPLC-MS/MS – are all methods equal?

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HPLC-MS/MS is often considered as golden standard method in clinical biochemistry. Its selectivity is praised, its accuracy, precision and sensitivity is considered to be “equal or better” than clinical chemistry routine methodologies in endocrinology or therapeutic drug monitoring (TDM); especially compared to immunoassays. On the other side however, it has been shown in immunosuppressant drug TDM proficiency testing (PT) schemes, that most often locally designed (“*homebrewn*”) LC-MS/MS platforms show a higher degree of inter-laboratory variability than automated commercial immunoassays [1]. To allow a deeper insight into the factors contributing to the observed imprecision of LC-MS/MS assays, we combined widely used sample preparation protocols [2,3,4,5,6,7,8] with the online-SPE-LC-MS/MS immunosuppressant drug TDM assay designed at our institute [9].
Both qualitative and quantitative experiments were performed; anonymized leftover routine whole blood samples were used. Extraction yields were evaluated by peak area comparisons for cyclosporine A, tacrolimus, everolimus and sirolimus. The corrective action of the routinely used internal standards (IS) in a quantitative assay setup was investigated for cyclosporine A (IS cyclosporine D) and tacrolimus (IS ascomycin) using identical calibrator materials. The peak area comparison experiment did clearly show that several protocols [5,6,7,8] had significantly

lower analyte yields. Comparison of extraction yields from calibrator and patient sample materials did unveil, that only two protocols [2,9] did not discriminate these materials. Quantitative results comparison to the ZIMCL method did show significant deviations (±10% to ±30%) for some but not all of the sample preparation protocols. It can be concluded from our data, that the increased inter-laboratory variability observed in PT schemes stems most likely from different extraction yields in the whole blood sample preparation and not from an inter-laboratory calibration bias.

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Miscellaneous - Clinical Pharmacy

PO - 182

Prospective investigation of a computerised decision support system in community pharmacies

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Introduction: To prevent medication errors in self medication, community pharmacies should offer a high counselling quality. For physicians, computerized decision support improved guideline adherence and patient-related outcomes. We newly developed a computerised pharmacy decision support system (PDSS) for allergic rhinitis and conjunctivitis and assessed its effects on patient counselling in a prospective two-phase intervention study. **Methods:** Community pharmacists were invited to participate in a prospective two-phase intervention study. In a first interview (pre-intervention), pharmacists counselled a virtual patient (portrayed by trained pharmacy students) for allergic rhinitis and conjunctivitis. Immediately thereafter, pharmacists were trained to operate the PDSS, which they applied in a second patient interview immediately following the instruction (post-intervention). Primary outcome was the completeness of mandatory questions asked during counselling of an allergy patient as defined by national guidelines. McNemar test or Wilcoxon-Signed-Rank test were used for statistical analysis as appropriate. **Results:** The median age of participating pharmacists (n=50, 78% female) was 34 years (interquartile range: 27-40), they had 8 (2-14.5) years of practical experience, and 32% were specialized in community pharmacy. While using the PDSS, pharmacists more frequently asked mandatory questions to confirm appropriateness of self-medication with 7 (5.25-9; 78%) from 9 questions compared to 2 (1-3; 22%) without additional support (median; $P<0.001$). With the PDSS they also asked more questions (9/12, 6.25-10; 75%) relevant for appropriate drug selection than without (4/12, 3-5; 33%; $P<0.001$). The mean time per interview increased from 3.6 (95% confidence interval: 3.2-4.1) to 5.1 minutes (4.6-5.5; $P<0.001$). **Conclusion:** Community pharmacists frequently omitted questions mandatory to assess whether self-medication is appropriate. By using a newly developed PDSS, the number of mandatory questions that were indeed asked more than doubled. The results suggest that the PDSS is ready for evaluation of its impact in real patients.

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Age-dependent volume of distribution for pegylated asparaginase (Oncaspar®)

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Asparaginase is a key component in the treatment of acute lymphoblastic leukaemia (ALL) in children and adults. Today there are three different formulations available derived from either Escherichia Coli (native E.coli asparaginase, Asparaginase medac®) or Erwinia chrysanthemi (Erwinase®). Furthermore, a pegylated form of native E.coli asparaginase is available (PEG-asparaginase, Oncaspar®). Dosing and schedule of asparaginase is specified in multi-agent treatment protocols developed for the paediatric as well as the adult population.
A higher volume of distribution normalized to body surface area (V/BSA) was reported for PEG-asparaginase in adults[1]. A Population pharmacokinetic (PopPK) analysis for PEG-asparaginase in children also identified a trend towards higher V/BSA with increasing age[2]. Therefore, we analysed serum activities from both children and adults to get a better insight into possible age dependent pharmacokinetics of PEG-asparaginase.
We analysed 2089 serum activity measurements in 449 patients aged 0.8 to 80.6 years (median age 27.1) from the paediatric ALL/NHL-BFM 95 and ALL/NHL-BFM REZ protocol as well as the adult GMALL 07/03 and GMALL Elderly 1/2003 protocol using nonlinear mixed effect modelling (NONMEM Vers. VI). Paediatric patients received 500, 750, 1,000 or 2,500 U/m^2 PEG-asparaginase (Oncaspar^{TM}) during induction and relapse treatment, adult dosage ranged from 500 to 2,000 U/m^2.
A one-compartment model with BSA as covariate for clearance (Cl) and volume of distribution (V) as well as Cl increasing with time best described the pharmacokinetics of PEG-asparaginase in children and adults. Age was included as covariate on V according to the formula: $V = V_i * AGE^{0.395}$ where V_i is the initial volume of distribution. Parameters found were: V_i= 2.08 l ± 30.5% per 1.73m^2 and Cl= 6.83 ml/h ± 56.7% per 1.73m^2 (mean ± interindividual variability).
Children and adolescents younger than 18 years of age exhibit a lower volume of distribution normalized to BSA when compared to adults (1.13 vs 2.82 l/m^2). The influence of age on dosing and schedule of PEG-asparaginase will be analysed in future studies.

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PO - 184

Identification of children at risk for high blood pressure out of routine blood pressure monitoring data

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Background The incidence of high blood pressure in the paediatric population is increasing due to overweight and obesity. Therefore, a better identification of children at risk for hypertension is highly warranted. According to the first guideline of the European Society of Hypertension[1] for children and adolescence in 2009, normal values for the classification of paediatric blood pressure values in children were firstly presented. The determination of the systolic and diastolic value, however, follow a complicated and time consuming procedure by means of percentiles of height, age and sex. Therefore, we integrated the normal values for height, age and blood pressure as interactive percentiles into a web-based clinical decision support system "KinderDosierung.de" to foster the identification of children at risk for high blood pressure.

Methods Routine blood pressure measurements of children and adolescence determined by physicians and nurses of the paediatric cardiology and pneumology ward of the university hospital Düsseldorf, Germany, between October 2010 and June 2011 were examined by retrospective data analysis. A pharmacist reviewed the patient's records and integrated demographic, diagnosis, laboratory values, medical prescriptions and blood pressure values into the clinical decision support system "KinderDosierung.de". Only patient's records with at least information about sex, age, height and associated blood pressure value were included to the analysis. In line with the current recommendations of the European Society of Hypertension[4], blood pressure (BP) values between 90. and 95. percentile were assessed as high-normal, between 95.-99. percentile plus 5mmHg as hypertensive (stage 1) and above 99. percentile plus 5mmHg as hypertensive (stage 2). These criteria were used for systole and diastole. This classification according to the BP percentiles conducted by "KinderDosierung.de" has been checked in parallel by hand. The patient collective represented an age range of 0-18 years and was classified by age according to WHO-criteria.
Results In total 1144 paediatric patients were admitted to the ward during this time frame. In total, 152 records of 146 paediatric patients fulfilled the necessary requirements and had been recorded in "KinderDosierung.de". Out of these, 90 cases indicated at least high-normal values ($\geq$ 90. percentile) by minimum one-time measurement. Fifty of them showed a systolic or diastolic value between 95.-99. plus 5 mmHg and the remaining 27 children and adolescence had blood pressure values above 99. percentile plus 5 mmHg. The following diseases were diagnosed in the patients: congenital heart failures (29), tachycardia (5), suspected cases of hypertension (4), infections (e.g. pneumonia) (44), dizziness (2), abdominal pain (3), asthma (2), cystic fibrosis (5), oncological diseases (6), HIV (1), others (e.g. accident) (34) and no diagnose was available in 11 cases.
Conclusion "KinderDosierung.de" helped to identify children at risk for hypertension out of routine monitoring blood pressure data in a fast and precise way. According to the guidelines, these children need to be double checked with BP measurements before starting further diagnostic procedures.

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J Hypertens 2009, 27: 1719-1742

PO - 185

Detection of drug related problems in patients with dementia – study design of the medication management in the cluster-randomized study DelpHi-MV study

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Introduction: Drug related problems (DRP) are prevalent in the pharmacotherapy of dementia patients. Potentially inappropriate medications (PIM) are associated with a higher risk of falls, affect cognition negatively and may increase mortality. Instruments to detect and solve DRP need to be evaluated for this specific application.
Aim: Development and testing of medication management tools for the population based, cluster-randomized intervention study DelpHi-MV (dementia: life and patient centred help in Mecklenburg Western Pomerania).
Methods: We have conducted a non-systematic literature review to identify relevant DRP for patients with dementia. Assessment tools for the home medication review (HMR) were modified according to our experiences with the AGnES-studies [1]. The Beliefs about Medicine Questionnaire (BMQ) [2] was added for the computer assisted personal interview (CAPI). A MySQL-based database was developed for documentation of drugs. The HMR will be conducted by specially qualified Dementia Care Manager [3]. 25 patients were included in the feasibility study.
Results: The following DRP were identified as relevant for dementia patients: intake of PIM, adherence, clinically relevant drug-drug-interaction, and adverse drug reaction. The PRISCUS list was implemented in the study to detect PIM. For improvement of adherence we have developed a reminder system including the MEMS-system.

The BMQ was proven as too confusing for dementia patients. The local pharmacy is responsible for medication management.
Discussion: Medication management becomes increasingly important in Germany. We have developed an innovative system under participation of the GP, the local pharmacy, and patient's care giver. Over a period of 3 years approximately 1000 dementia patients will receive HMR whereof 500 patients will be allocated to medication management. Results of the first feasibility study will be presented.

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PO - 186

Reduction of Cardiovascular Risk Factors by Preventive Care Services provided by Community Pharmacies for People aged 50 - 70 Years – Results of a Pilot Evaluation

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Background: The Northeast of Bavaria, Germany, is a region with a higher prevalence of coronary heart disease than other areas of Bavaria [1]. **Objective:** To reduce cardiovascular risk in this high-risk region by preventive care services in community pharmacies. **Methods:** People aged 50 to 70 years were invited to a screening assessment including blood pressure, body mass index, lipid values (e.g. total cholesterol, HDL cholesterol, LDL cholesterol, small dense LDL, lipoprotein (a), VLDL-composition) and other cardiovascular risk factors. Preventive care services like dietary counselling were offered in thirteen participating community pharmacies for a 12 month period. Subsequently all parameters were assessed in a final screening visit. **Results:** A total of 1930 patients were enrolled in this project. The first pharmacy, including 124 participants, completed the active preventive care period in April 2011. Modifiable risk factors were detected in 87% of the participants (n=108). Considering the participants (n=86) that were assessed at the follow-up, 55% (n=47) reduced their risk. 10 of them achieved the therapeutic goal, 17 reached a previously defined lower risk category and 20 showed only a minor improvement. **Conclusion:** Cardiovascular risk screening and preventive care services seem to be feasible services in community pharmacies. In this pilot evaluation a positive effect was obtained in about half of the participants of the first pharmacy study site. If these preliminary results can be confirmed by the other 12 participating pharmacies, this project has a promising potential to be expanded to other community pharmacies.

Acknowledgement: Dr. August und Dr. Anni Lesmüller-Stiftung
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Identification of critical drugs for application via enteral feeding tubes

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Introduction: Drug administration by nurses on paediatric wards is an error-prone process [1]. The appropriate administration of oral drugs via enteral feeding tube presents a particular challenge. Administration errors or the choice of the wrong dosage form can result in tube occlusion, altered bioavailability and an increase of preventable adverse events or therapy failure [2,3]. Therefore, we aimed to study the prevalence of drugs that are not appropriate for administration by feeding tube including an assessment of alternatives. **Methods:** By analysing the prescription data of a paediatric ward, inappropriate oral drugs for administration via tube were identified on a general paediatric ward. We assessed appropriateness for tube administration according to the summary of product characteristics (SPC), databases (e.g. Pharmatrix.de, own databases), inquiries to pharmaceutical companies, and literature. **Results:** Altogether, 163 different drugs for oral administration prescribed between January and May 2011 were assessed for their appropriateness for tube administration. From those, 140 (86%) were classified as appropriate for administration by feeding tube. Of 18 (11%) drugs not classified as appropriate for tube administration, 14 could be substituted by a brand containing the same active ingredient in a different preparation. In three cases of sustained release preparations the dosing interval had to be adjusted. In 4 (3%) of prescribed drugs, an appropriate drug would not be available on the German market and, therefore, a change of therapy should be recommended to the responsible physician. For 5 drugs not any information on tube administration was available in the contacted sources of information. **Conclusion:** For most drugs, actually prescribed on paediatric wards, administration via enteral feeding tube would be appropriate or an appropriate alternative is available. There is, however, a limited number of drugs for which special administration technique is required or data is missing.

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Evaluation of the benefits of pharmaceutical care in a department for haematology and oncology by a clinical pharmacist

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Background: Cancer patients are at high risk for adverse drug reactions and drug-drug interactions. Crucial factors are the frequent polypharmacy and multimorbidity with concomitant renal and / or hepatic insufficiency.
Objective: To evaluate the benefits of pharmaceutical care by a clinical pharmacist with regard to critical points of pharmacotherapeutic treatment in cancer patients.
Methods: In the context of pharmaceutical care we collected and reviewed 589 medication plans of 385 patients and patient related data of the clinical laboratory of cancer patients. We assessed the data with respect to possible adverse events due to drug-drug interactions and renal or hepatic insufficiency. Drug-drug interactions were categorized with respect to possible harm and probability of occurrence.
Results: Among the 385 patients studied, we found 59 patients with moderate (eGFR=30-60ml/min) and 27 patients with severe renal insufficiency (eGFR<30ml/min). Moreover we assigned 49 patients as liver insufficient (serum bilirubin$_{total}$>1,2mg/dL). 17 patients suffered from both, renal and hepatic insufficiency. Assessing the corresponding medication yielded 32 patients and 11 different medications where dose adjustments were to be taken into consideration. Possible drug-drug interactions were discussed with the attending physician and resulted in the generation of a synoptic table of relevant interactions, which was incorporated in the intranet of the clinic.
Discussion: Dose adjustments related to insufficient organ function as well as the assessment of interactions in view of clinical relevance are fields, where pharmaceutical care can, and as we propose, should result in multidisciplinary rounds of physicians and clinical pharmacists. Benefits are the improvement of pharmacotherapy resulting in prevention of failures and adverse events, which will be presented by us in a variety of examples.

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Dextromethorphan use and abuse in Germanadolescents: Do we need to change its market access?

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Background Dextromethorphan hydrobromide (DXM) is a widely used antitussive in many over-the-counter (OTC) cough and cold preparations.Known side effects of DXM are psychotropic effects, e.g. euphoria, hallucinations but also disorientation, depersonalisation, confusion or somnolence.Emergency room and poison control data from the United States had suggested that abuse of DXM put adolescents at risk of severe side effects or even death. In Germany, however, poison control centres reported about one death in the context of DXM abuse and websites in the internet indicate an intense exchange among abusers about tips and tricks how to organize and use DXM. Since the pharmacist has responsibilities for the adequate use of DXM in the population, we investigated pattern of DXM use in community pharmacies and in the OTC market, and also abuse statistics from the German drug regulatory office.
Methods A pilot survey questionnaire in patients from community pharmacies in North Rhine Westphalia for a period of 6-month were performed to document the request of DXM preparations concerning age above (adults) or below 18 years (mostly adolescents), gender, migration background, number of packages, specific DXM product and counselling requirements. From the German drug regulatory office BfArM (Bundesinstitut für Arzneimittel und Medizinprodukte) a list of abuse cases between 2006 and 2010 were analysed concerning age, gender and specific DXM products.At least, the annual number of DXM products from the IMS® OTC report 2010 was extracted and the internet was screened for more insights into specific product recommendations for abuse purposes.
Results In total, 140 questionnaires from 30 community pharmacies were received. In adolescents significant differences compared to adult patients were noted for gender, counselling and the specific drug product used: Below 18 years of age, 77% of patients weremale compared to 50% in adults ($p<0.05$). Counselling was appreciated less in adolescents than in adults (32% versus 67%, $p<0.05$). Number one DXM product in adolescents was Hustenstiller ratiopharm®with 59% (12% in adults) and number one product in adults was WickMediNait® with 32% (5% in adolescents). Statistical analysis of BfArMabuser cases showed an increase in the number of potential DXM intoxications from 2 in 2006 to 4 (2007), 10 (2008), 19 (2009) and 25 in 2010. The number of adolescents and male DXM abusers was higher than in adults and adolescents were asking preferentially for Hustenstiller ratiopharm®. The 2010 annual sales statistic from the IMS® OTC report identified Wick MediNait® as the product with highest sales numbers.Internet websites showed well-directed instructions for DXM abuse for Hustenstiller ratiopharm®.
Conclusion There is an ongoing alarming increase in reported DXM abuse cases in Germany. An overlapping pattern of male gender and product specific DXM use of Hustenstiller ratiopharm® in adolescents from survey data and BfArM statistics indicate that pharmacists should discuss restriction of DXM sales to adults and a prescription requirement of DXM to adolescents.

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Assessment of drug prescribing on a general paediatric ward - an explorative study

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Introduction: Presumably up to 100,000 people in the US die each year due to medical errors including a high fraction caused by drug-related problems (DRPs) [1]. Frequently, DRPs lead to potential adverse drug events (ADEs). In children, the rate of potential ADEs is 3 times higher than in adults with 79% occurring at the stage of prescribing [2]. In Germany, data on DRPs in prescribing for children are scarce. Therefore, we aimed to assess the nature and prevalence of DRPs. **Methods:** In a prospective explorative study of 4 weeks (including weekends) we assessed prescriptions taken from the prescription sheets of consecutive patients on a paediatric general ward. We evaluated formal criteria and dosing considering standards from Summary of Product Characteristics, ABDA-database and BNF for Children using an adjusted classification based on Ghaleb et.al. [3]. Severe DRPs included e.g. under-/overdosing of > 25%. All daily prescribed drugs were assessed but emergency therapy and drugs constantly adjusted to laboratory data. We identified DRPs related to the number of (i) patients, (ii) prescriptions, (iii) drugs and (iv) days of treatment. **Results:** In 1,328 prescriptions of 82 patients 3,985 DRPs were found. In those prescriptions we detected at least one severe DRP in (i) 77 from 82 patients, (ii) 747 from 1,328 prescriptions, (iii) 69 from 103 drugs and (iv) 276 in 292 days of treatment. Due to missing information, dosing was not completely analysable in at least one prescription in (i) 63 from 82 patients, (ii) 415 from 1,328 prescriptions, (iii) 58 from 103 drugs and (iv) 181 from 292 days of treatment. Regarding the remaining 913 prescriptions, under-/overdosing of > 25% was one of the most frequent deviations with each single and daily dose deviations in 274 (30.0%) prescriptions. Analgesics, antiasthmatics and expectorants/antitussives were the drug classes most frequently involved in DRPs. **Conclusion:** Prescribing is an error-prone process in children stressing the need for strategies supporting physicians in the prescribing process at the point of care.

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Shock-waves influence mechanism in fibroblasts that improve wound healing and skin remodelling

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Introduction: Starting with poor healing wounds [1] the meaning of extracorporeal shock-wave therapy (ESWT) for skin lesions extended to indications like necrosis [2] and burns [3] during the last years. Fibroblasts are important cells in connective tissue which is responsible for the appearance and integrity of skin. Examining the influence of shock-waves on fibroblasts may help to understand the underlying mechanism of ESWT.
Methods: Normal human dermal fibroblasts were treated with shock waves. Viability and proliferation were determined using MTT and BCA protein assays as well as coulter counter. For observing cell migration wound and heal assay was carried out. Isolated mRNA was used for studying genetic expression of collagen, elastin, fibronectin and hyaluronan synthase performing qRT-PCR. To verify these results the secreted collagen, elastin and hyaluronan was measured by staining or ELISA.
Results: Migration is visibly enhanced after shock wave application. Cell viability decreased slightly caused by treatment, but surviving cells showed slightly higher proliferation rate after recovering time. However MTT and BCA protein assay showed higher results for treated cells than cell counting the first days after treatment. Genetic expression in the named pathways was influenced, too. Height and time of increase differs depending on the substance. Results were verified by the amount of collagen, elastin and hyaluronan in the supernatant.
Conclusion: Enhanced migration is one of the most notable early effects of shock wave treatment. After recovering time proliferation is enhanced, whereas metabolism shown in MTT and BCA protein assay increases previous. Synthesis and secretion of important substances of the skin are also influenced.

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A prospective clinical study to evaluate pain assessment on an orthopaedic ward

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Introduction: Overall, 86% of surgery inpatients suffer from postoperative pain [1]. An appropriate pain assessment, however, is indispensable in those patients for individualised pain treatment. We therefore aimed to evaluate the status quo of pain assessment in routine care on daily ward rounds. **Methods:** In a prospective clinical study at a tertiary teaching hospital, a clinical pharmacist acted as an independent monitor on an orthopaedic ward. All patient interviews by physicians on daily ward rounds were assessed for pain-related comments. We analysed the prevalence of interviews in which pain was addressed and identified by whom. Additionally we classified pain-related comments in different subcategories and evaluated patient's satisfaction with pain therapy. **Results:** We documented 574 interviews with 105 orthopaedic patients (among these 49 with hip- and 24 with knee-replacement) by 7 physicians on 33 days. Pain or pain medication was addressed in 227 interviews (40%), initiated by patients in 121 and by physicians in 102 interviews (in 4 interviews by others or not assessable). In 116 interviews (51% of 227 interviews) pain-related questions were asked by the physician during the interview. The most frequently asked questions (with multiple categories possible) were existence of pain in 56 (48%) interviews, location of pain in 36 (31%) and intensity of pain in 19 (16%). Prevalence of interviews with pain-related questions ranged from 5-100% depending on the physician and from 0-71% depending on the ward round. Patients were dissatisfied mainly with the level or the duration of pain relief (addressed in 20 interviews) and with the delay in time of administration (13 interviews). **Conclusions:** We identified pain assessment as a process frequently not standardised and depending on the individual situation of the health care professional. These findings, expected to be highly generalizable, facilitate systematic improvement of pain management.

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Difficulties swallowing solid oral drugs: frequency and subsequent dosage form modifications in a general practice population

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Introduction - It is well known that patients with dysphagia may have difficulties swallowing food or liquids and solid oral dosage forms, which may tempt them to modify their drugs thus impairing safety and effectiveness of drug treatment. We aimed to quantify frequency and relevance of problems with swallowing solid dosage forms in a general practice population and to evaluate subsequent drug modifications by these patients.
Methods – After obtaining approval by the Ethics Committee of the Medical Faculty of the University of Heidelberg a questionnaire survey was conducted from November 2010 to February 2011 at 11 general practices

(registration number: DRKS00000607). Consecutive adult patients taking at least one solid oral drug ≥4 for weeks were included.

Results – 1051 patients completed the questionnaire (mean age: 61.8±15.6 years; 55.9% female; mean number of drugs: 3.4±2.5). Of all patients 37.4% reported having problems with swallowing solid medicines. Of the affected patients 24.2% experienced such difficulties always, daily or often and 75.8% sometimes or rarely. More than one third classified (39.4%) their problems as very severe or severe and 48.1% as not particularly severe. For this reason, 58.8% of the affected patients had already modified their medication (22.1% always or often), 49.4% of them not being aware that modifying dosage forms is not always allowed and can cause severe health problems; 32.0% modified despite being aware. Modifications included e.g. opening capsules, splitting, or crushing tablets. Additionally, 9.4% of the affected patients were non-adherent due to their swallowing difficulties.

Conclusion - One in eleven general practice patients expressed frequent problems with swallowing medicines and a substantial fraction of these patients modified their drugs in a way that may alter pharmacokinetics and thus safety and efficacy. Therefore, such difficulties should be addressed during the prescription process and the pharmaceutical industry should focus more on the development of alternative dosage forms as fast dissolving or liquid medicines.

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Overton`s rule helps to estimate the penetration of antiinfectives into the cerebrospinal fluid in patients

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In 1900, Ernst Overton found that the entry of anilin dyes through the cell membrane of living cells depended on the lipophilicity of the dyes. The brain is surrounded by the blood-CSF and blood-brain barrier consisting of lipid layers, however, hosting several inward and outward active transport systems. In the absence of meningeal inflammation, the CSF penetration of antiinfectives in humans as estimated by the ratios of the areas of the concentration-time curves in CSF and serum (AUC_{CSF}/AUC_S) correlated positively with the lipid-water partition coefficient at pH 7.0 (log D) (Spearman`s rank correlation coefficient $r_S = 0.40$, $p = 0.01$) and negatively with the molecular mass (MM) (r_S= -0.33, $p = 0.04$). The ratio AUC_{CSF} divided by the fraction unbound of AUC_S ($AUC_{CSF}/AUC_{S\ free}$) strongly correlated with log D ($r_S = 0.67$, $p < 0.0001$).

In the presence of meningeal inflammation, AUC_{CSF}/AUC_S also correlated positively with log D ($r_S = 0.46$, $p = 0.002$) and negatively with the molecular mass (MM) ($r_S = -0.37$, $p = 0.01$). The correlation of $AUC_{CSF}/AUC_{S\ free}$ with log D ($r_S = 0.66$, $p < 0.0001$) was as strong as in the absence of meningeal inflammation.

Despite these clear correlations, Overton`s rule was able to explain only part of the differences of CSF penetration of the individual compounds. The site of CSF withdrawal (lumbar versus ventricular CSF), the age of the patients, the underlying diseases, and active transport also appeared to strongly influence the CSF penetration of the drugs studied. For these reasons, we were unable to construct a nomogram to predict the entry of antiinfectives into the CSF based on their physicochemical properties.

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Reduction of medication regimen complexity at discharge from internal medicine

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BACKGROUND: Numerous properties may add to the complexity of a medication regimen (MR) and can thus reduce patient adherence to drug therapy. At the same time, nonadherence negatively correlates with clinical outcomes of drug therapy suggesting that complexity should be minimized.
OBJECTIVE: We assessed the prevalence of characteristics that are known to reduce patient adherence to drug therapy and investigated options for simplification of complex MR.
METHODS: We retrospectively evaluated MR of 500 consecutive patients for whom drugs with defined dosage regimen were prescribed in an electronic prescribing system (AiD*Klinik*). Each MR was checked for the following characteristics: (1) $\geq$12 single drug administrations per day, (2) $\geq$1 drug with multiple doses per day, (3) $\geq$3 drugs with different dosing intervals, (4) $\geq$1 drug with different dosages depending on daytimes, (5) tablet splitting, (6) inhalative drugs, and (7) necessity to take $\geq$1 drug with food. Following strategies were identified to simplify MR: substitution by a drug with lower strength to avoid tablet splitting, adjusting prescribed dosing frequency to the frequency specified in the Summary of Product Characteristics or switching to long-acting drugs, and switching to fixed-dose combinations to reduce multiple dosing, number of different dosing intervals, and number of individual administrations.
RESULTS: On average, every MR had 2.9 ±1.7 characteristics (range 0-7) known to impair patient adherence. The proposed simplification strategies reduced MR complexity to a mean value of 2.7 (±1.8, range 0-7, $p < 0.001$), and thus increased the number of patients without any characteristic defined as complex from 37 to 54.
CONCLUSION: This study showed that complexity of MR can be reduced by simple modifications of the medication scheme and might thus facilitate drug management and administration processes of the patient.

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Collaboration between community pharmacists and practitioners in Eastern Germany- A survey

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Objective: This pilot survey of a pharmaceutical-care-study intended to determine and compare attitudes of community pharmacists and general practitioners in Mecklenburg-Vorpommern, Eastern Germany, towards each other.
Method: 749 general practitioners as well as practitioners specialized in diabetes and 344 community pharmacists in Eastern Germany each received a six-paged 38-item-survey which they were asked to fill in and return to the study centre. Topics addressed by these items were reliability, functions, frequency and helpfulness of contact, cooperation in promoting medication adherence and quality of communication and were extracted from studies that have been conducted to investigate this issue in other countries before.[1-4] This enabled the comparison in an international context. For comparative analyses two-tailed-Mann-Whitney-U-Test, Chi-Square-Test and T-Test were used.
Results: The response rate of practitioners was 19.4% (n=145) and of pharmacists 24.4% (n=84). 76.6±14.5% of the practitioners (n=142) and 71.5±13.8% of the pharmacists (n=78) strongly trust the other health-care-providers´ statements and expertise (p=0.0076). Practitioner-pharmacists interactions are on average rather infrequent but estimated as helpful, if perceived. Item means on a scale ranging from -2 (very infrequently) to +2

(very frequently) for "discussing adverse effects" were -0.35 from practitioners´ views and +0.27 from pharmacists´ views (p=0.001), for "cost-related factors" item means were -0.80 and -0.23 (p=0.018), "asking or being asked for input on selection of an appropriate drug therapy" was judged at -1.10 and -1.13 (p=0.694), in the case of "overuse" item means were -0.76 and -0.26 (p=0.001), and for "discussing OTC-drugs" means were found -1.04 and -0.95 (p=0.458) for practitioners' and pharmacists' views, respectively. The main part (62.4%, n=83) of practitioners says that the extent to which pharmacists should be responsible for supporting patient adherence to chronic medications could be 25% whereas 27.8% (n=37) say that this extent should be 0%. The majority of pharmacists (58.8%, n=47) suggests an equal ratio. Pharmacists´ current influence on patients´ adherence to chronic medication is judged by 6.8% of the practitioners to be strongly positive, 59.8% regard the influence as somewhat positive, 33.3% as minimal, and 0% as negative. On the contrary 74.7% of pharmacists regard their influence as strongly positive.

Conclusions: Pharmacists-practitioners-interactions are occurring infrequently but generally assessed to be helpful, if perceived. Practitioners concede less responsibility in advancing patient adherence to pharmacists than pharmacists demand. Both still have different role perceptions. Similarly, cooperation has still to be improved in many fields, especially in discussing OTC-drugs.

Acknowledgements: We thank all practitioners and community pharmacists taking part in this survey.

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Miscellaneous – Pharm./ Med. Chemistry

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Novel substituted quinoxaline derivatives as potential correctors of the F508del-CFTR trafficking defect

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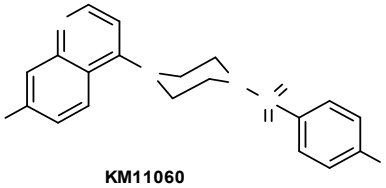
Cystic fibrosis (CF) – a common lethal genetic disease – is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). [1] Whereas several mutations have been documented in the CFTR gene, the most common by far is a phenylalanine deletion (F508del) in the first nucleotide binding domain. [2] Recently, structural analogs of sildenafil which are characterized by a phenylsulfonylpiperazino subunit were identified as novel correctors of the F508del-CFTR trafficking defect, out of this series, KM11060 (*i.e.* 7-chloro-4-{4-[(4-chlorophenyl)sulfonyl]piperazino}quinoline) was found to be the most potent derivative. [3]

In the course of our studies on the development of bioactive compounds with heterocyclic core, quinoxalines of type **I** became an object of our interest. Here we will present the multistep synthesis of these novel substituted bicyclic compounds, the results of screening as CFTR corrector, and preliminary structure-activity relationships.



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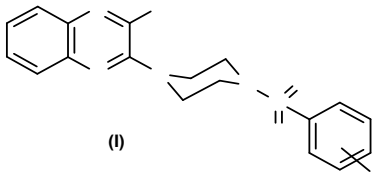

(I)

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Benzoic Acid Hydrazides as Novel Dual IGF-1R/SRC Inhibitors

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Since overexpression of the insuline-like growth factor receptor-1 tyrosine kinase (IGF-1R) and its physiological ligands have been observed in several solid human tumors, small molecules inhibiting IGF-1R have been suggested as potential anticancer drugs [1,2]. SRC is a nonreceptor tyrosine kinase mediating mitogenic signals between IGF-1R and downstream signalling cascades. A variety of investigations show that SRC plays a prominent role in cancer cell proliferation, invasion, and motility [3]. A dual inhibition of IGF-1R and SRC by one inhibitor could offer the advantage of interfering with two successive events of the same cancer-relevant signaling axis, constituting a therapeutic concept potentially lowering the risk for resistance development. We here disclose the syntheses and IGF-1R/SRC inhibitory activities of distinct N' acylated benzohydrazides **1**.

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Development of novel pharmacological inhibitors of the heat shock transcription factor 1 being crucial for the regulation of apoptosis in multiple myeloma

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Multiple myeloma (MM) is a malignant disorder of plasma cells within the bone marrow. Despite some progress in treating patients in the past, the median time of survival could be enhanced to 4-5 years only and MM remains still incurable [1]. For that reason the development of novel drugs is urgently needed.
A promising approach is provided by recent observations on the role of Heat Shock Proteins (HSP) in MM [2]. Since HSP90 and HSP70 are frequently overexpressed, the knockdown either genetically or by pharmacological inhibitors induces apoptosis of the malignant cells. Furthermore, it was observed that the knockdown of the heat

shock transcription factor 1 (HSF-1), which possesses a key role in controlling the up-regulation of HSPs during cellular stress, induces apoptosis in a similar manner. Although there are few inhibitors of HSP90, efficient and selective inhibitors of HSF-1 or HSP70 are still not available.
To fill this gap, several substituted quinolines and isoquinolines were synthesized and screened for their ability to inhibit the HSF-mediated HSP70 induction. Based on the observation that HSP72 is HSF-1-dependently up-regulated after inhibition of HSP90, INA-6 and MM-1s cells were pre-incubated with the potential anti-HSF-1 drugs before treating with a HSP90 inhibitor. The subsequently measured HSP72 protein levels were associated with the effectiveness of the tested compounds. First results revealed the tetrahydroisoquinolinone skeleton to be a promising lead structure. To prove this concept and gain access to structure-activity relationship analysis, a library of highly diverse tetrahydroisoquinolinones have been synthesized and screened for HSF-1 inhibition.

Acknowledgement: Thanks are due to the Clinical Research Unit 216 funded by DFG.
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PO - 200

7-Morpholino-4-quinolone-3-carboxamide 1 as new lead structure against *Trypanosoma brucei* [1]

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Human African trypanosomiasis (HAT) is one of Africa's old plagues and still a very serious disease in sub-Saharan Africa with 50,000 – 70,000 new cases occurring annually [2]. To combat the infection, which is caused by the two subspecies *T. brucei* (*T. b. gambiense* and *T. b. rhodesiense*), only a few drugs are commercially available. All treatment regimes were established during the last 20 years and some suffer from severe, sometimes life-threatening side-effects. Moreover, drug resistance is spread in Africa; thus the current drugs are rapidly losing their effectiveness. Hence the search for new, easy accessible active compounds is inescapable. Recently, we showed the enhanced trypanocidal activity of 7-amino-4-quinolone-3-carboxamide derivatives which were build up by microwave assisted Gould-Jacobs synthesis followed by nucleophilic substitution with cyclic or acyclic amines in position 7 and amidation in position 3. Structure-activity relationships were established to identify essential structural elements and finally led to the promising structure **1** with an IC_{50} value of 47 nM after 48 h of incubation and low cytotoxicity against macrophages J774.1. For identifying the target, compound **1** was subjected to a microscopy based fluorescence screen and exhibited changed morphology of the mitochondrion. Following cell cycle analysis suggested that compound **1** interferes with correct segregation of the kinetoplast resulting in a segregation defect which was not observed by knocking down the mitochondrial topoisomerase II ($TbTopoII_{mt}$). This formally excludes the possibility that 4-quinolone-3-carboxamide **1** just targets $TbTopoII_{mt}$ alone like ciprofloxacin, but suggests the interaction with other proteins involved in the *T. brucei* kinetoplast segregation. By means of x-ray structure analysis we found that 4-quinolone-3-carboxamide derivatives crystallize in layer lattice which might be the reason for their low water solubility. Thus, we are modifying the 4-quinolone skeleton of compound **1** to increase the water solubility for further investigations.

Acknowledgements: Financial support of DFG (SFB630)
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PO - 201

New subtype-selective inhibitors of the *cdc2-like kinases* (CLK)

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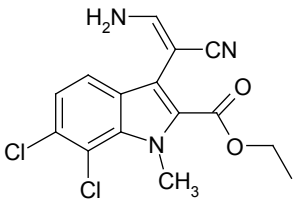
The dual specificity protein kinases of the CLK family (*cdc2-like kinases)*, comprising the subtypes CLK1 to 4, play a crucial role within the regulation of alternative splicing and therefore can take influence on a variety of physiological and pathophysiological events such as glucose metabolism and viral infections. As many aspects of the biology of CLK subtypes still remain unknown, there is a need for highly subtype-selective small molecule CLK inhibitors.

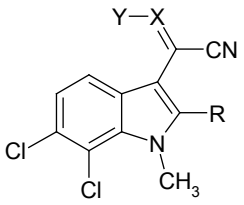
In previous investigations we found the 6,7-dichloroindolyl enaminonitrile KH-CB19 (**1**) to be a very potent inhibitor of both CLK1 and 4 isoforms, as well as of the closely related DYRK enzymes [1]. The novel chemotype of compound **1** allows kinase inhibition in a non-ATP-mimetic manner, with the halogen substituents and the nitrile group being essential for interaction with the active site of the target.

Based on data from a co-crystal structure of KH-CB19 (**1**) with CLK1 [2], we managed to increase both affinity and subtype selectivity by altering substitution patterns at ring positions 2 and 3 of the lead structure.

Some of the resulting compounds of type **2**, bearing either an enaminonitrile moiety or bioisosteric groups, proved to be very advantageous regarding affinity and subtype selectivity on CLK and several kinases from other families as well.



KH-CB 19 (**1**)



2 R = H, ester, aryl, halogen
X = CH, N
Y = NH_2, OH, CH_3

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PO - 202

Benzazol-2-yl piperazine derivatives as 11β-hydroxysteroid dehydrogenase 1 inhibitors: synthesis and biological studies

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Glucocorticoid hormones play a key role in the development of diabetes and metabolic syndrome. The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyzes the conversion of inactive cortisone to active cortisol. Inhibition of 11β-HSD1 is an attractive strategy to treat metabolic diseases [1].
Using a molecular modeling approach we screened our database for small organic molecules, applying a previously built pharmacophore model for 11β-HSD1 [2]. It suggested that compound 2-[4-(4-*tert*-butyl-phenylsulfonyl)-piperazine-1-yl]-benzothiazole (SH007C) is a possible inhibitor of 11β-HSD1. Subsequently it was shown that SH007C inhibited 11β-HSD1 activity with 89% at 20μM *in vitro.* Based on these results we synthesized a broad series of *N*-(benzazolyl) arylsulfonamides. All compounds were tested for 11β-HSD1-inhibitory activity *in vitro*. It turned out that the compound 1-methyl-2-[4-toluene-4-sulfonyl)-piperazine-1-yl]-1benzimidazole (SH034C) was the most active, with an IC_{50} of 0.95μM. The molecular modeling, the synthesis and the biological activity of these compounds will be presented.

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PO - 203

Development of novel inhibitors of MIP, a target of *Legionella pneumophila*

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Legionellosis may occur as two distinct progressive forms: Legionnaires' disease, a severe form of pneumonia with mortality rates of up to 20 % [1] and Pontiac fever, a milder respiratory disease with symptoms resembling acute influenza. The common causative agent which occurs in fresh water is the Gram-negative pathogen *Legionella pneumophila* presenting the protein MIP (macrophage infectivity potentiator) on the surface of the bacterium. MIP being the major virulence factor of *L. pneumophila* shows peptidyl prolyl *cis/trans* isomerase (PPIase) activity and is enabled to bind to collagen IV in the human lungs. Based on these attributes the pathogen is able to cross epithelial cells and extracellular matrix (ECM) of the lung tissue and to affect alveolar macrophages.
The surface protein belonging to the FKBP family is a receptor of the immunosuppressive drugs FK 506 and rapamycin which both inhibit the enzymatic function of MIP [2]. Because of the immunosuppressive effect Legionellosis can not be treated with these drugs. Therefore, novel lead structures with an effect on MIP had to be explored. By means of computer-aided design, subsequent syntheses and biological screening a novel small molecule (A) of the pipecolic acid type with an IC_{50} of 6 μM was found [3]. A small substance library of structure B-type was synthesized and subjected to a protease coupled PPIase assay, recently. On the basis of these results, the SAR will be established followed by an extension of the substance library.
To verify the activity against the MIP protein not only in the enzyme assay, cell-based measurements were performed to examine the influence of substance A on invasion and intracellular replication in human macrophage-like cells. Since no influence on the replication was found it can be concluded that the MIP protein,

but not its PPIase activity is required for the infection. Thus, it will be interesting to identify the unknown target of the MIP mediated PPIase activity and to analyze the effect of substance A in guinea pigs [3].

Acknowledgement: Financial support DFG (SFB 630)
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PO - 204

Carbamates as a new prodrug concept for HDACs

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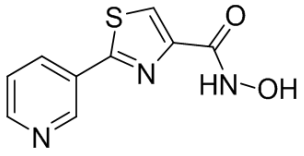
Epigenetics describes heritable changes in gene expression without changes in the genome. Biochemical modifications like DNA methylation and histone modifications serve as epigenetic markers that establish and regulate these changes. Dysfunction in epigenetic regulation is able to cause diseases like cancer. Therefore, drugs that target these dysfunctions are very interesting for the treatment of cancer. One of the modifications that is implicated in transcriptional activation is the acetylation of histone proteins. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are responsible for maintaining the acetylation equilibrium. HDACs are amidohydrolases that cleave the acetamide bond by a zinc-dependent mechanism. Inhibitors of HDACs have shown great promise in the development as new anticancer agents. Hence, a prodrug principle for HDAC inhibitors is a valuable concept to optimize cellular activity.
Our experimental results validate the general applicability of the carbamate group as a prodrug for hydroxamates, as compound **2** is inducing cellular hyperacetylation in a similar range of concentration as compared to the parent hydroxamate **1**. These results demonstrate that the introduction of a carbamate group is a valuable concept for the design of new HDAC inhibitors with improved pharmacokinetic properties.
Introducing a carbamate group on the hydroxamic acid function of SAHA (suberoylanilide hydroxamic acid), a drug already approved as a HDAC inhibitor, led to further prodrugs, such as compound **3.** We synthesized further carbamates from SAHA with different moieties to further investigate stability, cellular activity and to study the impact of different substitution patterns by considering pharmacokinetic aspects like solubility.



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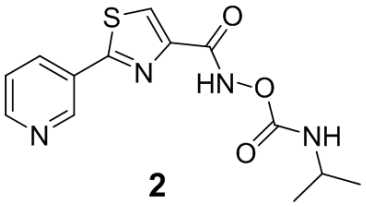

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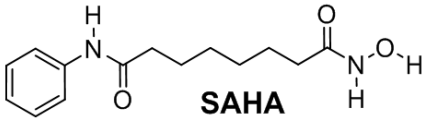
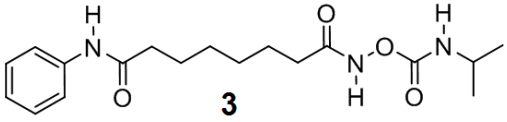


SAHA



3

Acknowledgements : We thank the Deutsche Forschungsgemeinschaft (SPP 1463) for financing.
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PO - 205

New heterocyclic sigma-receptor ligands and their testing as potential anticancer drugs

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Sigma receptors are found in peripheral organs and in the central nervous system. They are classified into two distinct subtypes: σ_1 and σ_2. The σ_1 receptor has been recently classified as a receptor chaperone at the endoplasmic reticulum membrane. Ligands to this receptor display neuroprotective and neuroregulative functions and in particular the σ_1 receptor is under evaluation for the treatment of a number of neurological disorders such as depression or schizophrenia. In addition to the relevance of these receptors in neurological disorders both subtypes are overexpressed in a wide variety of human tumor cell lines in which σ_1 receptor antagonists and σ_2 receptor agonists exert antiproliferative actions through different apoptotic pathways.[1] Therefore synthetic ligands to this receptor could play an important role in cancer diagnosis and therapy.
The aim of this project is to determine the cytotoxic potential of new developed sigma receptor ligands and to investigate their mechanism of antiproliferative action.

WMS 34-14

We are studying the cytotoxic effects of new synthesized heterocycles and their enantiomers in cultures of human cancer cell lines with either a microtiter assay based on staining adherent cancer cells lines with crystal violet or the MTT method for determining of suspension cell lines. Potency was characterized by the IC_{50} value. The tested compounds show promising cytotoxic activity with IC_{50} values around 10 µM for selected cell lines. We hypothesized that the inhibition of cell survival was caused by cell apoptosis. Therefore flow cytometry with Annexin V and propidium iodide was performed to observe cellular alterations due to apoptosis. Apoptotic

measurements of cancer cells treated with compound WMS 34-14 showed apoptosis levels increasing from 1% in the control to 23% in the treated cells. Currently, another apoptosis assay is being used where we measure the activation of caspase 3 to identify that apoptosis is the real mechanism of action.
In the development of new therapeutics it is important to integrate metabolism studies in an early phase of the drug development project. Thus selected compounds were characterized in metabolism studies by using Sprague-Dawley rat liver microsomes. Various P450 isoforms were induced in rats by treatment with specific inducing agents, i.e., phenobarbital and dexamethasone. Control experiments with microsomes from untreated rats were also done.[2] Microsomal incubations indicate that CYP is actively involved in the metabolism of the compounds. Moreover, the CYP 2B1 isoform appears to be most important in the compound turnover. Currently, LC/MS analysis of reaction mixtures is being used to identify the oxidative metabolites, the results of which will be presented.

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PO - 206

2-Arylpaullones as potential anti-leishmanial agents

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Leishmaniasis is a major protozoan parasitic disease found in tropical and subtropical regions worldwide. It is actually a spectrum of diseases, caused by about 21 species and subspecies belonging to genus *Leishmania* [1]. About 2 million cases appear every year with an annual death burden of about 60.000 people [2]. The disease is transmitted through the bite of *Phlebotomine* sandflies. Three main forms of leishmaniasis exist in humans: visceral, cutaneous and mucocutaneous. The disease causing stage of the parasite is primarily the intracellular amastigote existing in macrophages of the mammalian host [1]. Analysis of the *Leishmania* genome suggests that parasite protein kinases might be targets for new drugs [3].
Several structures of paullones have already been synthesized and tested against *Leishmania* parasites. A series of 9-*tert*-butylpaullone derivatives **2** showed good anti-leishmanial activity on axenic amastigotes and infected macrophages, while being nontoxic to a human macrophage cell line [4]. Modifications of **2** by exchange of the α,β-unsaturated ketone for a rigid aromatic ring were carried out in order to enhance the anti-leishmanial activity.

The resulting 2-arylpaullones **3** were synthesized via Suzuki-Miyaura cross-coupling reaction using a mono-mode microwave device by coupling 2-iodo-9-*tert*-butylpaullone **1** [4] with several substituted phenylboronic acids. Synthetic procedures and preliminary results regarding the antileishmanial activity of **3** will be presented in the poster.

Acknowledgements: This joint research project was financially supported by the State of Lower-Saxony, Hannover, Germany.
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PO - 207

The activity of metallo salophene complexes against tumor cells

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Metallo salophene complexes have outstanding biological effects in various types of tumor cells. The influence of the central atom and the role of the ligand on cytotoxic activity were examined in previous studies wherein iron and nickel containing complexes showed the highest impact.[1,2,3,4] Iron as central atom is important for growth inhibitory effects. Those complexes induce the generation of free radicals within tumor cells which implicates the release of superoxide dismutase and induction of apoptosis via the mitochondrial pathway.[1] On the other hand, nickel complexes induce apoptosis via CD-95/Fas signalling.[3] Furthermore, different salophene complexes of both transition metals show the ability to overcome drug resistance in daunorubicin, doxorubicin and vincristin resistant cancer cell lines.[4] Contrariwise, healthy leukocytes seem to be spared by salophene based chelates. In-vitro structure-activity-relationship studies of iron-(III) complexes in different types of humane carcinoma cell lines demonstrated the variability of antitumor activity in dependence of the position of electron donating groups (-OMe) in the salicylic moiety. This effect was also confirmed for nickel salophenes.[2,3]
Our current research involves synthesis and exploration of various new complexes based on the salophene matrix, for example the modification and enlargement of aromatic parts of the chelating agent in order to obtain complexes with improved cytotoxicity or the opportunity for coupling with polymers. Another aim is the development of in-vitro methods to evaluate cytotoxic activity of those complexes under magnetic influence.

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PO - 208

Development of novel Bid inhibitors for the treatment of neurodegenerative diseases

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Mitochondrial pathways of apoptosis are major features of neuronal death after acute brain damage and in neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. The Bcl-2 protein Bid is a

key player in these mechanisms, causing mitochondrial dysfunction and detrimental release of pro-apoptotic proteins into the cytosol. Previous studies showed that small molecule Bid inhibitors such as BI6c9 effectively prevent neuronal cell death in vitro. In vivo, however, the available Bid-inhibitors failed to protect brain tissue likely because the compounds were not bioavailable and did not cross the blood brain barrier. Therefore, the aim of the present study is the development of novel, potent Bid inhibitors which are neuroprotective and available for applications in model systems of brain damage in vivo. In a first approach, chemical modifications of BI-6c9 were performed resulting in modified structures and new molecules with different pharmacophors.

The first screening of these 50 compounds addressed their ability to prevent glutamate induced cell death compared to BI-6c9 in immortalized mouse hippocampal neurons (HT-22 cells). In this model system, glutamate induces a decrease of intracellular glutathione levels resulting in lipoxygenase activity and enhanced formation of toxic reactive oxygen species (ROS). Bid siRNA and small molecule inhibitors of Bid prevented the following mitochondrial damage and cell death, suggesting that Bid was a key player in the cascade of cell death signaling in this model system.

We applied the xCELLigence System, which allows continuous real-time monitoring of cell viability to determine neuroprotective potency of the newly synthesized structures. In this system seven of the tested compounds significantly attenuated cell death by glutamate. In addition, we used the MTT- assay to confirm the protective effects of the protective compounds.

In conclusion, we were able to identify seven new molecules with different structures that provided protective effects in the model of glutamate toxicity in HT-22 cells. Next steps of the project include further optimization of the identified structures and analyses of their binding to the pro-apoptotic protein Bid. Therefore recombinant Bid and t-Bid are expressed and purified from a pET 15b plasmid construct for co-crystallization of available and novel structures with the Bid protein as a basis for improved design of neuroprotective Bid inhibitors for therapy studies in models of neurodegenerative diseases.

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New synthetic approaches to amino acids and other pharmaceutically relevant substances by radical and enzymatic chemistry

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In recent years, interest in radical reactions as well as biocatalytic transformations has been continuously growing. Based on these general developments, we aimed at new synthetic strategies featuring a combination of radical and enzymatic methods. Arylalkylazo compounds **1** are readily accessible by radical carboamination reactions developed in our group (from compound **A**) [1]. Using an enzymatic resolution as a further step, these can be converted to enantiomerically enriched quaternary amino alcohols **2** [2]. In addition, we recently developed a method comparable to the Beckmann rearrangement to obtain compounds of type **1** from cyclic hydroperoxides (from compound **B**) [3].The products can serve as versatile intermediates for the preparation of a variety of different compounds such as indoles **3**, cyclic amides **4** or long-chain unnatural amino acids **5** [2a,3].

Since radical reactions provide new pathways to a wide range of pharmaceutically relevant substance classes, further research is directed towards combining these methodologies with biocatalytic procedures.

Acknowledgements: The Universität Bayern e.V. is gratefully acknowledged for a "Bayerische Elitefördung" fellowship.
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PO - 210

Synthesis and in vitro antiprotozoal activities of novel cinnamamide paullone hybrids

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Tropical diseases such as leishmaniasis and trypanosomiasis cause high mortality and morbidity in the developing countries. Drug research for these neglected diseases is needed due to the lack of safe, effective, affordable treatments. Chalcone paullone hybrid molecules **1** have recently been reported as new antileishmanial lead structures [1]. A series of 11 new paullone derivatives **2** incorporating a cinnamamide substructure [Figure 1] was prepared via a six-step synthetic route [Figure 1]. In vitro antiprotozoal activities against two parasites (*Trypanosoma brucei rhodesiense* trypomastigotes and *Leishmania donovani* axenic amastigotes) and toxicity on human cells were determined by using the alamarBlue viability assay [2]. Cinnamamide paullone hybrids display antileishmanial and antitrypanosomal activity but are toxic to THP-1 cells.

Figure 1. Structure of chalcone paullone hybrid molecules **1** and cinnamamide paullone hybrid molecules **2**.

Acknowledgement: Funding of the project by the Deutsche Forschungsgemeinschaft (to J.R. and C.K.) is gratefully acknowledged.
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PO - 211

Novel active compounds against Leishmania, Plasmodia and Trypanosoma - Bistacrine derivatives

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Leishmaniasis, Malaria and the African sleeping sickness are tropical diseases caused by protozoa. Left untreated, these diseases can be fatal. The currently available drugs are showing increasing resistance, being often toxic and sometimes too expensive. Thus, the development of new and cheap drugs is urgently needed.
Tacrine (1) is a reversible AChE inhibitor and was used for treating Alzheimer´s disease. Because of its hepatotoxicity it was withdrawn from the German market [1]. However it shows activity against Leishmania, Trypanosoma and Plasmodia. Its homodimeric hexyl-linked derivative, Bistacrine, was found to have even better activity against Leishmania in micromolar range of concentration and against Plasmodia and Trypanosoma actually in nanomolar range of concentration. In order to reduce the cytotoxicity structural variations were performed resulting in Oxotacrin (2), Hydroxytacrine (3), Dehydrotacrine (4) and its hexyl-linked dimers. They show similar antiprotozoal activity, but increased cyctotoxicity. Our future work is focused on the synthesis and elucidation of activity and toxicology to achieve antiprotozoal derivatives of low toxicity.

Acknowledgements: Financial support of DFG (SFB 630).
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PO - 212

Development of a PET ligand for imaging PDE10A in brain - synthesis, potency, metabolism and radiochemistry of a 7-(2-fluoroethoxy)-6-methoxy-quinazoline derivative

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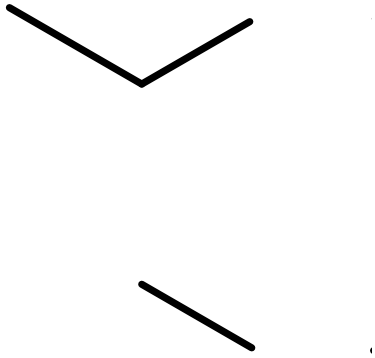
The phosphodiesterase (PDE) 10A plays an important role in neurotransmission by regulating intracellular levels of the cyclic nucleotides cAMP and cGMP in dopaminergic neurons. In consequence, PDE10A is associated with dopamine-related central nervous diseases such as Huntington's disease and schizophrenia. Thus, PDE10A is a promising candidate for drug development with a variety of selective PDE10A inhibitors published during the last decade [1, 2]. The aim of the presented work is the development of a positron emission tomography (PET) radiotracer for imaging of PDE10A *in vivo*.
Based on a lead structure ($IC_{50PDE10A}$ = 8 nM), published for therapeutic applications [3], three nonradioactive fluoroalkoxy derivatives (**1**, **2**, **3**) were enantioselectively synthesized over 11-14 steps and characterized

regarding their potency and selectivity to inhibit PDE10A in a cAMP competition assay. Prolongation of the alkyl chain from **1** to **3** by one methylene group each resulted in decreased inhibitory potency from IC_{50} = 24 nM over 106 nM to 144 nM. Metabolic stability of **2** was determined in comparison to the lead compound in an *in-vitro* metabolism assay using rat liver S9-fractions. Metabolites were structurally characterized using ESI-MS-MS coupling techniques.
With regard to radiochemical accessibility, derivative **2** appeared as the most promising candidate for radioligand investigation. Initially, a two-step synthesis of [^{18}F]**2,** consisting of ^{18}F-labelling of 1,3-bistosyloxyethane and following coupling with phenolic precursor **4,** was carried out. For the ^{18}F-fluoroalkylation step labeling yields (LY) of 30-45% were achieved. Consequently, in a one-step procedure the tosylethoxy precursor **5** was used for ^{18}F-labelling, improving LY up to 42-72%. Biodistribution studies in female CD-1 mice revealed high initial brain uptake of [^{18}F]**2**. However, it was not significantly inhibited by competition with **2** or by pre-treatment with MP-10, a high PDE10A specific inhibitor, indicating lack of specificity in vivo. In conclusion, these results motivate for further structural variation of the lead compound to make it suitable for neuroimaging of PDE10A with PET.



Acknowledgements: We would like to thank J. Ortwein (Institute of Pharmacy, University of Leipzig) and the team of L. Hennig (Institute for Analytical Chemistry, University of Leipzig) for their analytical support. This project was financed by resources of the European Fond for Regional Development (EFRE) and the Free State of Saxony.
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PO - 213

Development of CCR2 antagonists for PET diagnosis in atherosclerosis

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The chemokine system is a complex network of small soluble proteins and corresponding receptors.
Effects on the cellular signalling pathway are mediated by G-protein coupled receptors (GPCRs) expressed on immune cells and endothelial cells. The chemokine receptor 2 (CCR2) plays a crucial role in inflammatory processes. CCR2 is expressed on a number of immune cells such as inflammatory monocytes and mediates both cellular movement and activation. In the chronic inflammatory process of atherosclerosis the interaction of CCR2 and its endogenous ligand MCP-1 (monocyte chemoattractant protein 1) leads to mobilization of monocytes from

the blood vessel into the arterial wall. This is an important early key step for the development of artherosclerotic plaques [1,2].
As a result selective CCR2 antagonists can inhibit both the start and the progression of atherosclerosis.
The final introduction of [^{18}F]fluoride will lead to PET tracers for imaging of CCR2 receptors in vivo for diagnosis and therapy .
Derived from chemical leads described in literature[3,4] we designed new CCR2 receptor antagonists of general structures **1**. The potential CCR2 antagonists consist of four building blocks (A-D). These building blocks can be modified and combined in different ways.

Key step of the synthesis is the Oxa-Pictet-Spengler reaction of phenylethanol derivatives (A) and N-acylated piperidones (B) providing a spirocyclic piperidine. The butyramide substructure is prepared by acylation of benzylamines (D) with butyric acid derivatives (C).

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PO - 214

Novel potential bioactive 4-alkoxy-[1,2,4]triazolo[4,3-*a*]quinoxalines: Synthetic strategies and unexpected results

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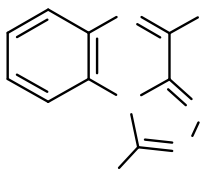
Heterocyclic compounds, especially nitrogen-based ones, represent essential subunits of a wide variety of bioactive compounds and modifications at or of the heterocyclic moiety represent important strategies in the development and for the optimization of potential drugs.
Our previous studies have demonstrated that several substituted [1,2,4]triazolo[4,3-*a*]quinoxalines are potent ligands at A_1, A_{2A}, and A_3 adenosine receptors, respectively (up to nanomolar).[1] Based on these results we became interested in further derivatives. Here we will present strategies to synthesize compounds of type I bearing an alkoxy substituent in position 4 of the tricyclic core (*i.e.* R' = OR"). Moreover, unexpected results (split off of R' during the formation of the tricyclic system) found in the course of these studies will be shown and discussed.



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PO - 215

Design, synthesis and testing of novel small-molecule inhibitors of KasA for treatment of tuberculosis

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Mycolic acids, the main component of the mycobacterial cell wall, are synthesized in mycobacteria by a series of proteins which are part of the type II fatty acid synthase (FAS) system. As the difference between the type II FAS and the multifunctional type I synthase found in eukaryotes is significant, there is a possibility to design inhibitors of the FAS II system being effective and selective antibiotics against mycobacteria. The enzyme of interest in our work is the β-keto-acyl ACP synthase (KasA), an elongating enzyme in the FAS II system of *Mycobacterium tuberculosis*. The recently solved crystal structure of KasA in complex with the well-known inhibitor thiolactomycin (TLM) [1] provides important information about the binding pocket, essential protein-ligand interactions and the mechanism of inhibition.
To identify novel lead structures a set of compounds has been retrieved by virtual screening of databases of commercially available compounds with a pharmacophore model based on the TLM binding mode. Subsequently, these compounds were docked into the KasA binding pocket to inspect the predicted binding modes, and the chemical accessibility of possible modifications was checked. Structures requiring time-consuming synthesis were purchased, while more readily accessible substances from different chemical classes were synthesized and varied by altering the substitution pattern. The inhibitory activity of new inhibitors was determined via a fluorescence assay: The inhibitor-induced decrease of the intrinsic fluorescence of KasA was measured in a time-dependent manner in order to calculate K_d values. First results revealed several structures as inhibitors of KasA in a lower micromolar K_d range, among these chromone-, nitroisatine and uracil-derivatives. Testing of the compounds in a whole-cell mycobacterial assay will reveal the most promising candidates for further development.

Acknowledgements: Financial support of DFG (SFB630)
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PO - 216

Synthesis, σ Receptor Affinities and Structure Affinity Relationships of Conformationally Restricted Piperazines and Flexible Analogues

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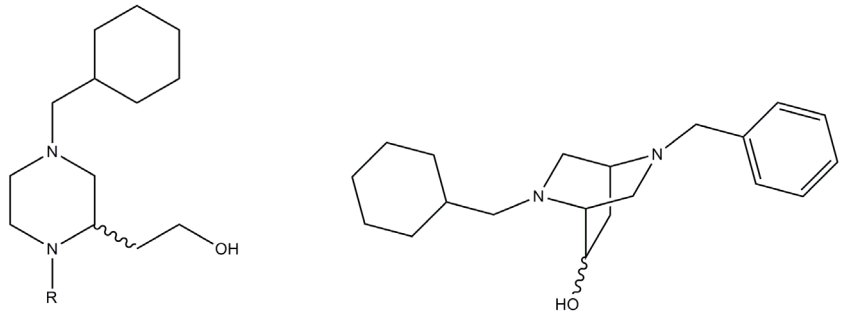
σ Receptors represent an own class of receptors including two subtypes, which are termed σ_1 and σ_2 receptor [1]. They vary in their molecular weight, distribution and ligand binding profile.
σ Receptors are widely distributed in central and peripheral tissues of the human body. It is known, that certain human tumor cell lines (kidney, colon, breast cancer) overexpress both σ_1 and σ_2 receptors [2]. Especially in proliferating tumor cells the expression of σ receptors is 10-100 times higher than in silent tumor cells.

Due to this knowledge, σ receptor ligands seem to be useful for diagnostic imaging and for the treatment of cancer.
The synthesis and σ receptor affinities of monocyclic piperazines of type **1** and of diastereomeric bridged piperazines **2** will be presented on this poster. Results of a human binding assay will be compared to those from an assay based on animal tissues. Structure affinity relationships will be derived from the determined receptor binding data.



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PO - 217

Synthesis of new selective oxidosqualene cyclase inhibitors

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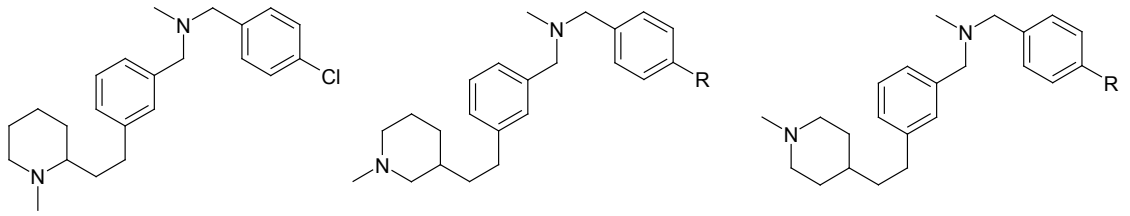
Oxidosqualene cyclase (OSC) is a key enzyme within the sterol biosynthetic pathway. It catalyzes the cyclisation of the open-chain triterpene oxidosqualene through a number of carbocationic intermediates into various sterols, the precursors of phytosterols, cholesterol and ergosterol [1].
In previous investigations we developed selective inhibitors of human oxidosqualene cyclase, e.g. compound **1**. The rationale of these investigations was to mimic two of the cationic high energy intermediates (HEI) of the cyclisation cascade by introducing sp^3-hybridized, protonable nitrogen atoms at pertinent positions.
Based on this lead structure and the already reported crystal structure of the enzyme OSC [2], we undertook some modifications of the piperidine moiety of inhibitor **1**, which imitates the first HEI in the cascade, located in ring A of the emerging sterol ring system. We present synthetic approaches to structural isomers of **1**. Biological characterisation in an assay based on the GC-MS detection of accumulated oxidosqualene in treated human cell lines showed that selected isomers of **1** exhibit significantly improved enzyme inhibition.



1 **2** **3**

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PO - 218

Synthesis of inhibitors of the protein kinase DYRK1A based on the alkaloid harmine

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Screening of the β-carboline alkaloid harmine (**1**), isolated from various plant species like *Peganum harmala* and *Banisteriopsis caapi*, on a broad panel of protein kinases revealed that this alkaloid is an inhibitor with excellent specificity for DYRK1A (Dual-specificity tyrosine (Y) phosphorylation-Regulated Kinase 1A). The activity of this kinase is associated with neurodegenerative diseases like Down syndrome and Alzheimer´s disease. But harmine is not applicable as a drug due to its additional activity as a potent inhibitor of the enzyme monoamine oxidase A (MAO A) [1]. Our project is aimed at the development of analogues of harmine that should be consistently good or even better inhibitors of DYRK1A, but show significantly decreased MAO A inhibitory potency.
Based on the known crystal structure of DYRK1A and on a model of the DYRK1A/harmine complex, it was suggested that the accessible volume of the ATP binding pocket of the kinase can accommodate additional substituents at the β-carboline nucleus [1]. On the other hand, simulations suggested that substituents at the indole nitrogen (N-9) of harmine (**1**) should reduce the affinity to MAO A. In continuation of our research on bioactive β-carboline alkaloids [2, 3] we present here synthetic approaches to the target compounds **2**, containing additional substituents at different ring positions. We also show the results of the screenings for inhibition of DYRK1A, monoamine oxidase A, and for general cytotoxicity.

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Miscellaneous - Pharmaceutical Technology

PO - 219

Indinavir as an example of drug induced kidney stone formation

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Indinavir belongs to a limited number of drugs that may directly crystallize in the kidney causing urolithiasis. The calculi can be composed of the drug itself or one of its metabolites or the medication causes a supersaturation of badly water soluble components in the urine that consequently crystallize [1]. For the protease inhibitor indinavir it has been reported that up to 50% of the patients treated with this HIV drug suffer from kidney stones [2]. It is formulated as the sulphate salt in capsules for oral administration (Crixivan®, Merck Inc.). However, as was

observed with powder X-ray diffraction (PXRD) [3], Fourier transform infrared spectroscopy [4, 5] and mass spectrometry [4] of isolated urinary stones, the pure hydrate of indinavir base crystallizes in the neutral to weakly alkaline environment of the urine. Since the solubility of indinavir is strongly pH-dependent [4], a large (~ 5000 fold) decrease in solubility occurs between the application site (acidic stomach fluid) and the urine (pH 6.7 to 7.4).
The present study aimed at a more detailed characterization of the solid state properties of indinavir base. The monohydrate was precipitated from an alkaline (pH 8-9) aqueous solution of indinavir sulphate which resulted in colourless flat prisms after recrystallization from aqueous ethanol (40%). Three other forms of indinavir base were found in a polymorph screening program. An amorphous form was obtained by evaporating a solution of the hydrate in 1-propanol with a rotavapor. Annealing of the amorphous form at 130°C resulted in a mixture of two polymorphs (modifications I° and II). Mod. II melts at 155 °C and mod. I° at 167 °C. A phase pure mod. II can be prepared by drying the hydrate at 140 °C. From the heats of fusion (differential scanning calorimetry) and the order of the melting points a monotropic relation between mod. I° and II was derived, which means that mod. I° is the stable polymorph in the entire temperature range. The monohydrate melts at 136 °C in a saturated water vapor atmosphere. However, in a dry atmosphere the hydrate dehydrates already at 25 °C forming an isomorphic dehydrate. Moisture sorption experiments show that the dried hydrate reabsorbs water already at 10% relative humidity. The PXRD patterns indicate that the structures of the hydrate and dehydrate are strongly related which explains the missing hysteresis between the moisture sorption and desorption process.
Though the hydrate cannot be regarded as extraordinary stable (referred to temperature and low humidity conditions) in comparison with other drug hydrates, the existence of this water adduct must be regarded as one of the most critical issues for the kidney stone formation. Hydrates in general exhibit the lowest water solubility of all solid state forms in an aqueous medium and it is very striking that the majority of the drug compounds that have been observed in kidney stones form such water adducts.

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PO - 220

Powder Layering in Fluid Bed: Comparison of Pellet Characteristics

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Powder layering techniques have been well established in fluid bed rotary granulator and centrifugal granulator [2,3,5]. Former investigations have proved that drug powder layered pellets can successfully be prepared using a modified fluid bed processor Ventilus 25 (Innojet Herbert Hüttlin, Steinen, Germany), too. The powder layering process was developed using a freely water soluble model drug. Efforts have been made to reduce the size of starter cores, because for further processing the pellet size is a key parameter. Due to high drying efficiency inherent in fluid bed processor very small sugar spheres (250 – 355 µm) which tend to agglomerate could be favourably layered [1].
In this study, additionally, paracetamol as a slightly water soluble drug was used for powder layering. Coating efficiency and pellet characteristics are presented and compared to results obtained with the freely water soluble model drug.
Drug powder was mixed with talcum (H & B, Neuenburg, Germany) as a flow aid. Polyvinylpyrrolidone (Plasdone K-29/32, ISP Tech., Texas City, USA) dissolved in water was used as binder liquid. Drug powder was dosed with a conventional screw feeder and dispersed by pressurised air. Fluidised powder was tackified by nebulised binder liquid and layered on sugar spheres (Suglets®, 250 - 355 µm, NP Pharm, Bazainville, France).
Pellet size distribution was determined by image analysis (Camsizer, Retsch Technology GmbH, Haan, Germany). The sample size was 5.0 g of pellets. Pellets were compared regarding mean size and width of the size distribution.

The sphericity of pellets, as a parameter of particle shape, was calculated on particle projections according to the following equation [4]:

$$Sphericity = \frac{4 \cdot \pi \cdot area}{perimeter^2}$$

Measurements were performed in triplicate.
Pellet surface properties were evaluated by stereomicroscope (Leica M 205 C, Leica Micro-systems GmbH, Wetzlar, Germany).
Drug release rate was measured corresponding to Ph Eur.
Powder layered pellets could successfully be produced using small sugar spheres as starter cores and paracetamol as model drug. In comparison to the former used freely water soluble drug process parameters had to be slightly adapted when using paracetamol. Coating efficiency is high (≥ 90 %) and comparable for the freely and the slightly water soluble model drug. Additionally, drug release was fast for both model drugs.

Acknowledgement: We thank Acino Pharma AG (Basel, Switzerland) for generous financial support.
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PO - 221

In vitro and in vivo comparison of Imiquimod containing ointments: Are products from China pharmaceutically equivalent?

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Imiquimod is an immune response modifier with agonistic properties at Toll-like receptor 7 that induces the formation of interferon and other cytokines through the innate immune system and stimulates cell-mediated immunity through T-cells [1]. Since 1997 it is approved by the FDA for treating external genital and perianal warts [2], unlicensed used for numerous skin diseases [3], [4].
For development of 5% Imiquimod containing ointments several challenges need to be taken into consideration.
Imiquimod is practically insoluble in water and sparingly soluble in other common pharmaceutical solvents with the exceptions of Isostearic acid, oleic acid and linoleic acid [5].
In the present work our investigations focus on four medical products from People's Republic of China in comparison with commonly used Aldara™ 5% ointment.
Light microscopic and subsequent modified franz-diffusion cell model investigations with synthetic membrane (MW-cutoff 10000DA) and rodent skin showed significant differences (p=0.05) between Aldara™ and the Chinese ointments "Nan Bo", "You Bi Qing", "Med Shine Li Di" and "Li Ke Ji" (figure 1) with an approximate drug content range between 4.6% (Med-Shine Li Di) to 5.3% (Nan Bo) [6].
In images 1-6 different Imiquimod containing formulations are depicted with crossed polarized filters to determine crystal structures. (from left to right: Imiquimod crystals, Aldara™, Nan Bo, You Bi Qing, Med Shine Li Di, Li Ke Ji.)

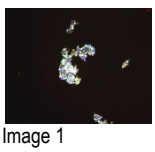
Image 1


Image 2


Image 3

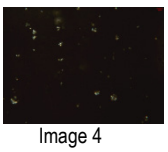
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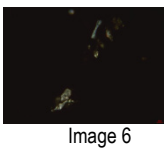
Image 5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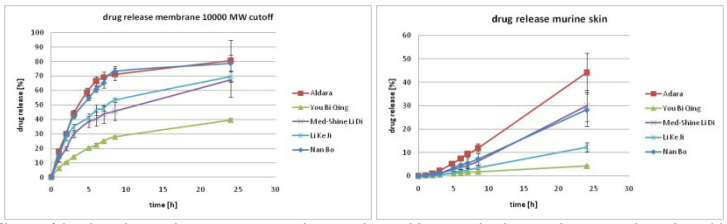

Figure 1 in vitro drug release concentration vs. time with a synthetic membrane and murine skin

Subsequent data collected using murine skin showed that crystal structures or solved state of drug is off importance for drug release and penetration but not the only relevant parameter to predict drug release and in vivo effects.
Drug release and permeation levels of Imiquimod from Aldara™ were significantly higher in comparison with Far East preparations.

Acknowledgements: we wish to thank the following scientists: Gordon Amidon and Hai Wai for their support.
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In situ gelling properties of anionic thiomers

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The aim of this study was to investigate in situ crosslinking systems of anionic thiolated polymers. In order to accelerate the increase in dynamic viscosity of thiolated polymers (thiomers), they were combined with hydrogen peroxide, carbamide peroxide and ammonium persulfate [1]. Thiomers (pectin-cysteine (Pec-Cys), sodium carboxymethylcellulose-cysteine (NaCMC-Cys) and poly(acrylic acid)-cysteine (PAA-Cys)) were synthesized via amide bond formation between the carboxylic acid group of polymers and the primary amino group of L-cysteine [2,3]. The rheological properties of 1% (m/v) thiomer solutions were compared by adding different amounts of oxidizing agents. Pec-Cys and NaCMC-Cys with hydrogen- and carbamide peroxide showed a sol-gel phase transition within a few minutes and scored up to 13,000-fold increase of dynamic viscosity. Furthermore, significant increase in viscosity was achieved with polymers exhibiting a polysaccharide backbone (Pec-Cys and NaCMC-Cys) ($p < 0.05$). In contrast, carbohydrate thiomers in combination with ammonium persulfate showed an initial increase in viscosity. Afterwards a decrease in viscosity was observed by reason of chain scission. According to these results, carbohydrate thiomer / oxidizing agent systems might be usable for various pharmaceutical applications such as for in situ gelling liquid / semisolid formulations or tissue engineering [4].

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PO - 223

Mucoadhesive properties of novel preactivated poly(acrylates)

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The aim of this study was to improve the mucoadhesive and cohesive properties of poly(acrylic acid)-cysteine (PAA–cys) conjugates. This was achieved by the oxidative S-S coupling of PAA-cys with 2-mercaptonicotinic acid (2MNA) [1]. Unmodified PAAs, PAAs-cys (thiomers) and novel PAA-cys-2MNA (100-, 250- and 450 kDa) conjugates were compressed into tablets to perform disintegration tests, mucoadhesion studies and rheological measurements [2]. Cytotoxicty was determined using Caco-2 cells. The resulting PAA–cys–2MNA (100-, 250- and 450 kDa) conjugates displayed 113.5 ± 12.7, 122.7 ± 12.2 and 117.3 ± 4.6 µmol/g of 2-mercaptonicotinic acid, respectively. Due to the immobilization of 2MNA, the novel PAA-cys-2MNA (preactivated thiomers) conjugates exhibit comparatively higher swelling properties and disintegration time to the corresponding unmodified and thiolated polymers. On the rotating cylinder, tablets based on PAA-cys-2MNA (100-, 250- and 450 kDa) conjugates displayed 5.0-, 5.4- and 960-fold improved mucoadhesion time in comparison to the corresponding unmodified PAAs. The apparent viscosity of PAA–cys–2MNA (100-, 250- and 450 kDa) conjugates was improved 1.6-, 2.5- and 206.2-fold, respectively, in comparison to the corresponding unmodified PAAs. Moreover, preactivated thiomers/mucin mixtures showed a time dependent increase in viscosity up to 24 hours, leading to 7.0-, 18.9- and 2678-fold increased viscosity in comparison to unmodified PAAs (100-, 250- and 450 kDa), respectively. All polymers were found non-toxic over Caco-2 cells. Thus, on the basis of achieved results the novel preactivated thiomers seem to represent a promising novel generation of mucoadhesive polymers which are safe to use for prolonged residence time of drug delivery systems to target various mucosa.

Acknowledgements: Higher Education Commission Pakistan (HEC), Austrian Agency for International Cooperation in Education and Research (ÖAD).
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PO - 224

Development of a Continuous Way for Producing Extrudets

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Right now hot-melt extrusion is gaining more and more interest in pharmaceutical industry. One of the main advantages, apart from improving the bioavailability of an API, is the possibility to establish a continuous production line. The major problem right now is giving shape to the final products. There are several different approaches for manufacturing solid dosage forms by extrusion. Some of them are already successfully established on the market, like for example calendaring or pelletizing. The main aspect of this work involves the implementation of a dynamic fly-knife cutting machine type Dynamat 60 from Metzner (Neu Ulm, Germany) into the production line. This sort of machine is widely used in plastics industry for cutting of cables and wires. A co-rotating twin-screw extruder type ZSE 27 HPPH from Leistritz (Nürnberg, Germany) was used for melting the material. Five different types of starch were used as basic substances. Phenazone and lidocaine were chosen as model substances. The melt was shaped by a round nozzle with an internal diameter of 5 mm into endless strands. After a short period of cooling in a slope, the strands enter a belt feeder, which transports the material to the desired position.
Mainly there are two different configurations for influencing the cutting process: Continuous mode and stop-and-go cut. The continuous mode provides a high throughput of material (up to 2000 cuts per minute) but cannot

reach the high accuracy of the stop-and-go cut concerning cutting edge and cutting angle. For our tested materials it could be shown, that this process is also suitable for long-term operation. Blunting and clogging effects turned out to be much lower than expected. This new technique opens up a vast field of application. Apart from manufacturing extrudets it is also possible to produce thin-layer muco-adhesive films, or implants. Extrudates were analyzed concerning uniformity of mass, tensile strength, glass transition temperature and crystallinity.

Acknowledgements: Leistritz Extrusionstechnik, Metzner Maschinenbau, Roquette.

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High efficiency dry coating of non-subcoated pellets for sustained drug release formulations

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Dry coating is method for production of filmed dosage forms without usage of water or solvents. Some techniques need a subcoat on the cores to be coated, which is usually achieved by a conventional coating method, to increase film-former's adhesion on the core surface. Previous studies showed that usage of a rotary fluid bed was more effective, whereby subcoating is not required for this procedure [1]. Nevertheless, coating efficiencies for some coating polymers are relatively low. However addition of capillary force promoters (CFP) to the liquid plasticizer was found to overcome this drawback [2]. In this work we present dry coating for sustained drug release formulations using ethylcellulose (EC) or amino methacrylate copolymers (AMCs) as film-formers. The latter show pronounced plasticization with triethyl citrate as plasticizer, in which a mixture with isopropyl stearate (IPS) facilitates coating efficiencies (CE) up to 86%. IPS does not have an appreciable plasticizing effect on AMCs, however it efficiently reduces the glass transition temperature (Tg) of EC, and thereby permits the abstinence of other plasticizers in EC formulations. As the CE is only 80%, a search for other substances as CFP for EC was conducted. Floramac® 10, a blend of ethyl esters of different fatty acids from macadamia nut oil, was found to upgrade the CE to 87% besides acting as efficient plasticizer to EC. Originally Floramac® 10 is used as emollient for topical formulations, in which its spreadability is near to IPS and other isopropyl esters. Poloxamer 407 (Lutrol® micro 127) was added to EC by mixing to modify the drug release. DSC measurements showed, that this additive has no influence on the EC's T_g and its plasticization.

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PO - 226

Impact of direct compression excipients on the compaction and tablet properties of high-dose pancreatin formulations

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Direct compression is a commonly used technique for the manufacture of pancreatin tablets because of its comparably small effect on product stability. The degree of plastic behaviour and elastic deformation of tablets effects the extent of inactivation of pressure-sensitive drugs such as pancreatin during compaction. The aim of the present study is to investigate the influence of various direct compression excipients on the compaction and tablet properties of formulations containing either 70 % or 90 % pancreatin and 2 % magnesium stearate. These properties are expected to allow an estimation of pancreatin inactivation. Compaction is performed with an instrumented single-punch eccentric press equipped with flat-faced punches of 10 mm diameter applying three levels of compaction force (3, 7, 11 kN). The plastic deformation properties (Mean Yield Pressure) and the degree of elastic deformation of powder blends containing the excipients microcrystalline cellulose (MCC), silicified microcrystalline cellulose (SMCC), and magnesium aluminometasilicate (Neusilin®), respectively, are characterized by analysis of the Heckel Plot. With MCC and SMCC formulations, a primarily plastic deformation is observed (Mean Yield Pressures: 63.4 – 76.0 MPa) while the Mean Yield Pressure of tablets containing Neusilin® is 101.2 MPa. The rank order of elastic recovery of the tablets derived from the Heckel Plot is as follows: Neusilin® < MCC < SMCC. All tablets show an increase in elastic recovery with increasing excipient fraction. From 7 kN on all tablet formulations exhibit an acceptable tensile strength. Interestingly, the fraction of excipient does not influence tablet hardness. In conclusion, MCC, SMCC and Neusilin® are suitable as direct compression excipients for pancreatin formulations with regard to compaction and tablet properties. Particularly, SMCC appears to be a suitable excipient for pancreatin tablet formulation as is leads to tablets with a high degree of elastic recovery and still sufficient hardness.

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Influence of compaction force and magnesium stearate content on the degradation of ergocalciferol in tablets

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Ergocalciferol, also known as Vitamin D_2, belongs to the group of D vitamins (calciferols). Because of its triene structure, it is sensitive to light and oxygen. Chemical degradation of ergocalciferol can also be induced by mechanical stress, as could be shown by Hüttenrauch et al. with powder mixtures containing lactose monohydrate, an excipient which is known for its brittle fracture behavior during tableting [1, 2]. In the present study, ergocalciferol is mixed with the commonly used tableting excipient microcrystalline cellulose, which shows a predominantly plastic deformation behavior. The powder mixtures are compacted with an excentric press equipped with flat-faced punches of 10 mm diameter to tablets of 245 mg weight. Three different levels of compaction force are applied (4, 9, 16 kN). The uncompacted powder and the tablets are stored at 21°C, 45% RH and light protection. At predefined intervals, the drug content in the tablets and powder mixtures is determined with UV spectroscopy at 265 nm after tablet disintegration in absolute ethanol. The ergocalciferol content is monitored over the storage time period. A measurable degradation of the vitamin starts after two days of storage of the tablets. The uncompressed powder does not show significant changes in the ergocalciferol content during the time course of the study. In the tablets, drug degradation clearly depends on the applied compaction force. As expected, an increase of the compaction force leads to an increase of the interparticular friction and thus mechanical activation, which ultimately results in an enhanced degradation of ergocalciferol. An increase of the magnesium stearate concentration of up to 1.5% not only reduces the friction between particles and die wall and punches, but also the interparticular friction and thus the mechanical stress, resulting in a less pronounced degradation of ergocalciferol.

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Enzymatic degradation of thiolated chitosan

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The objective of this study was to evaluate the biodegradability of thiolated chitosans in comparison to unmodified chitosan. Mediated by carbodiimide, thioglycolic acid (TGA) and mercaptonicotinic acid (MNA) were covalently attached to chitosan via formation an amide bond. Applying two different concentrations of carbodiimide 50 mM and 100 mM, two chitosan conjugates (TGA A and TGA B) were obtained. Both chitosan (3% m/v) and thiomer (3% m/v) solutions were prepared and chitosanolytic enzyme solutions were added. Lysozyme, pectinase and cellulose were examined in chitosan degrading activity. The enzymatic degradability of these thiomers was investigated by viscosity measurements with a plate-plate viscometer. The obtained TGA conjugate A displayed 267.7 µmol and the TGA conjugate B displayed 116.3 µmol of immobilized thiol groups. With 325.4 µmol immobilized thiol groups, chitosan MNA conjugate displayed the highest content of thiol groups. In rheological studies subsequently the modification proved that chitosan TGA conjugates with a higher coupling rate of thiol groups were not only degraded to a lesser extent by 20.9% to 26.4% but also more slowly than chitosan. Chitosan mercaptonicotinic acid was degraded by 31.4% to 50.1% and even faster than unmodified chitosan depending on the investigated enzyme.
According to these results the biodegradability can be influenced by various modifications of the polymer which showed in particular that the rate of biodegradation is increased when MNA is the ligand, whereas the degradation is hampered when TGA is used as ligand for chitosan.

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The influence of carrier morphology on the fine particle fraction of dry powder inhaler formulations

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The most important requirement when administering active pharmaceutical ingredient (API) powders to the deep lung is an aerodynamic diameter of 1 µm to 5 µm. Larger particles tend to fly straight ahead due to their inertness instead of following the airstream into the branching alveolar tree during inhalation and impact mainly on the mucosa of the upper airways. In contrast, smaller particles, which depend on deposition by diffusion, often do not have enough time to diffuse to the alveolic tissue and are exhaled again [1].
Powders of the required particle size (1 µm to 5 µm) are rather cohesive and exhibit poor flowing properties. However dosing which is done volumetrically relies on good flowability. Therefore the API particles are adhered via a mixing procedure to coarser carrier particles which show sufficient flowability. Upon inhalation, the API particles attached to the carrier surface have to be detached again in order not to be impacted together with the carrier on the upper airways. Therefore, an important characteristic of such interactive mixtures are the interparticle forces between API and carrier particles that have to be large enough to guarantee mixing uniformity and stability during storage and transport and low enough to allow maximal API detachment upon inhalation. Due to the fact that the contact area between API and carrier particles and consequently surface morphology of the carrier are crucial to interparticle forces the variation of these parameters may lead to optimized dry powder

inhaler (DPI) formulations. Maas [2] showed that spray drying mannitol samples at different outlet temperatures at lab scale leads to varying morphologies, however these particles were too small as to be used as carriers in DPIs.
The aim of this work is to study the influence of carrier particle morphology on the respirable fraction of the API in DPI formulations. Therefore mannitol powders of different morphology and sufficient size were prepared by variation of the spray drying exhaust air temperature on a pilot scale spray-dryer. Subsequently interactive mixtures of the spray-dried carrier particles with salbutamol sulphate as model API were made and the respirable fraction was determined according to the European Pharmacopoea using the next generation impactor (NGI).
It could be shown that the respirable fraction significantly depends on the shape as well as on the roughness of the spray-dried carrier particles. The highest fraction was achieved for round and rough carrier particles (Fig. 1).

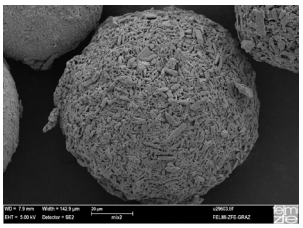

Fig. 1: SEM micrograph of an interactive mixture of spray-dried mannitol carrier particles and micronized salbutamol sulphate.

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Quantification of α- and β-Mannitol via X-Ray Powder Diffraction

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Many pharmaceutical solids exhibit polymorphism. During the production, manufacturing and storage of pharmaceutical solids, a polymorphic change might occur. The aim of this study was to quantify the ratio of different modifications of pharmaceutical solids using X-ray powder diffraction (XRPD). A method for quantification was developed and refined for routine use.
Mannitol was chosen as model substance, because it is a commonly used excipient in the pharmaceutical industry and the different polymorphs are not distinguishable using the common used DSC methods. At least three different crystalline forms of mannitol exist: α-, β- and δ-mannitol. The β-form is the most stable among these; therefore it is usually used in pharmaceutical products [1]. The delivered β-mannitol (E10DC, Roquette, Lestrem, France) was used as received. α-mannitol was prepared from ß-mannitol.
Measurements were performed by X-Ray diffraction (X'Pert Pro MPD, Panalytical, Almelo, Netherlands). A Cu Kα radiation point source (λ = 1.5406Å) was operated at 40 kV and 40 mA. The samples were manually powdered using mortar and pestle and placed in back-loaded holders with a defined compression force and time (3000kg during 10s) by using a hydraulic press (Hydraulic Laboratory Press, Perkin-Elmer, Waltham, USA). Measurements were taken in the reflection mode from 10° to 50° 2θ.
In order to determine concentrations, a calibration curve from mixtures with known concentrations was done. Four mixtures of α- and β-Mannitol were made: 20%, 40%, 60% and 80% α-mannitol. Together with the pure α-mannitol and pure β-mannitol, six different powders are measured by XRPD. Data pre-treatment was performed using different methods. After correcting the shift in 2θ direction and normalizing the intensity values in order to

negate fluctuations. Afterwards, a first method for linear regression analysis is used. For each 2θ value, a linear function is calculated from the intensity in function of the concentration. 68% of the information is chosen to use in further calculations to get a robust model. Due to a scaling of the intensity all data will be comparable and will not depend anymore on the 2θ value. This relative intensity will be close to zero when the mixtures do not contain α-mannitol. Similarly, the value will be close to one when a 100% α-mannitol mixture is measured and analyzed. An efficient method was developed to characterize different modifications of α-mannitol in a quantitatively way using a calibration curve. Based on the data analysis including MLR, it is possible to calculate the concentrations of unknown samples containing α- and β-mannitol.

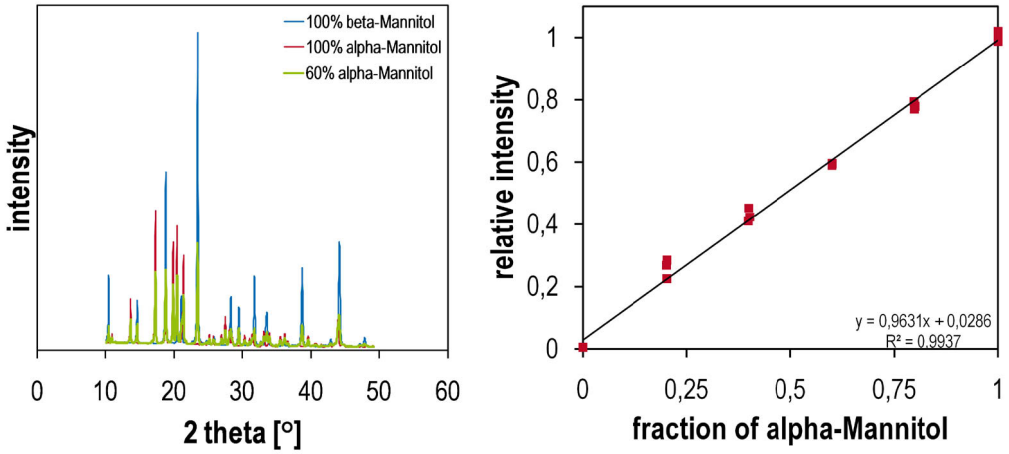

Fig. 1 (left). X-ray pattern of pure α- and β-mannitol as well as a mixture of 60% α- and 40% β-mannitol.
Fig.2 (right). Calibration curve of different mixtures after data pre-treatment.

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Development of partially oxidized and 2-mercaptoethylamine functionalized chitosan as mucoadhesive and permeation enhancing polymer

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Thiolated chitosans are mucoadhesive and biocompatible polymers [1] that are able to increase the paracellular permeability and improve the oral bioavailability of hydrophilic and macromolecular drugs such as peptides and proteins [2]. Due to low hydratability of most conjugates at pH values below 6.5, it was the aim of this study to develop a thiomer as permeation enhancer for body compartments where pH is raised. For this purpose chitosan was oxidized by means of sodium periodate under cleavage of the polysaccharide structure. The intermediate polymer was coupled with 2-mercaptoethylamine and reduced with sodium cyanoborohydride to form the secondary amine moiety. The 2-mercaptoethylamine grafted chitosan (chitosan-2-MEA) was characterized regarding water absorbing capacity, mucoadhesive properties, cytotoxicity and permeation enhancing effect of the model drug FD4. Ellman's assay displayed a total amount of sulfhydryl groups up to 2476±184 µmol per gram polymer. By fundamental alteration of chitosans backbone and following thiol immobilization, the paracellular transport of FD4 across rat intestinal mucosa was 5.1-fold improved compared with control (buffer). Mucoadhesion could be 20-fold improved based on increased chain flexibility and disulfide formation between chitosan-2-MEA and glycoproteins in mucosa. The novel thiomer showed excellent hydratability in aqueous

media, and was proven to be non-toxic by resazurin and LDH assay. According to these results, chitosan-2-MEA seems to be a promising pharmaceutical carrier for sufficient absorption of hydrophilic therapeutic agents.

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Characterization of spray-dried Mannitol particles by Mercury Intrusion Porosimetry

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Mercury Intrusion Porosimetry (MIP) is a common method to characterize the micro-structural features of porous materials. The principle of MIP is the detection of pores that can be intruded by mercury within a controlled pressure range. In contrast to other methods, such as the nitrogen adsorption method (BET), MIP requires only small sample amounts (a few 100 milligrams), which is one of the key benefits of the method, whereas the drawback is that the samples must be disposed after the measurement. However, our engagement in MIP applications revealed that the method can be used to generate valuable information about key characteristics of powdered materials that goes far beyond the common applications of MIP. With only two MIP measurement cycles several important powder and particle parameters can be generated, which usually requires a series of different methods. This includes specific surface area, particle size distribution and bulk densities at various pressures, besides the more conventional quantities such as porosity, pore size and pore size distribution. In the present contribution the applicability of the MIP method in the assessment of different particle and powder properties is demonstrated for spray-dried mannitol particles.
Three different spray-drying production parameters (outlet temperature at 67 °C, 84 °C and 102 °C using an aqueous mannitol solution) were selected to prepare mannitol particles with different surface roughness named as M67, M84 and M102. The main fraction (63 µm to 160 µm) of the spray-dried product was used for further investigations. SEM pictures, in particular cross sectional views, show different forms (perfect spheres, single or multiple indented shells), which indicates unequal drying mechanisms at the individual outlet temperatures, resulting in different form stability of the shell. The particles are hollow and exhibit a dense shell. The outer surface shows small mannitol crystals whereas at the inside wall surface large mannitol crystals can be identified (see scheme below).

The MIP experiments allow a clear differentiation between the intra- and interparticular pore volume of the samples, which enables an enhanced particle and powder characterization with this technique. The intraparticular pore volume is measured at higher pressures (1 bar to 2000 bar) and identifies the ”real pores” (7.5 nm to 15 µm diameter) of the single particles. The interparticular pore volume is recorded at lower pressures and allows the assessment of bulk powder properties [1] and the estimation of the particle size [2, 3]. In order to distinguish between “real pores“ of the shell and the hollow space (>15 µm) of the spheres, an additional MIP run (pressure increase and decrease) was performed. In contrast to “real pores”, ink-bottle shaped pores and pores larger than 15 µm in diameter remain filled after the first MIP run and are thus not detectable in the second MIP run. By subtracting the pore volume measured in the second run from the pore volume detected in the first run, the hollow space volume is obtained, which is clearly larger in the M67 particles than in those of M84. For M102 the smallest volume was obtained. M102 shows also the smallest “real pore” volume and the smallest pore size and thus the

closest packing of primary mannitol particles. For the sample M67 quite the opposite was observed. Moreover, a so called “breakthrough pressure” was determined, which is indirect proportional to the particle size. Mayer and Stowe [2] developed this very special particle sizing method forty years ago and recently [3] they critically discussed the applicability of the method. However, for the investigated mannitol samples we found a good agreement between the particle size obtained with MIP and the laser light diffraction method.
The present study demonstrates that MIP is a very useful method in the characterization of particle and powder properties, which is particularly true for more complex shaped particles and for cases where only small sample amounts are available.

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Effect of surfactant type on physical stability of lycopene-loaded NLC

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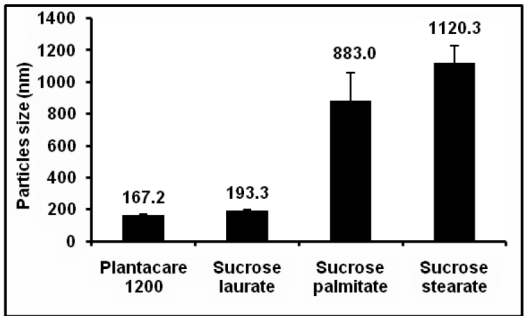
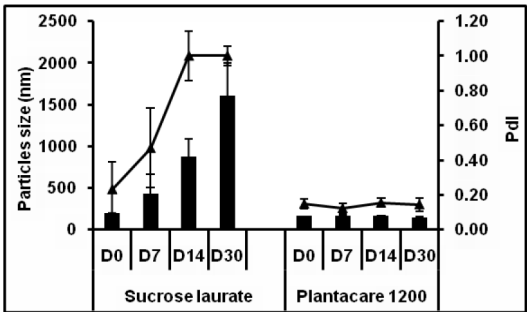
Nanostructured lipid carriers (NLC) have been attracted increasing scientific and commercial attention during the last few years. The application of NLC has been expanded in both pharmaceutical and cosmetic fields. NLC can enhance stability of many active substances from environmental stress. The extremely small size of NLC plays an important role on skin penetration. The unchanged size of NLC upon storage indicates its stability. The aim of this study was to investigate the effect of surfactant type on physical stability of lycopene-loaded NLC. The preparation of NLC was achieved by using high pressure homogenization. The results indicated that different type of surfactant yielded the NLC with different particle size and zeta potential. It was found that two surfactants, plantacare 1200 and sucrose laurate, with smaller contact angle gave the NLC with smaller size. Plantacare 1200 showed the most suitable for lycopene-loaded NLC. It yielded the NLC with the smallest size. In comparison with sucrose laurate, plantacare 1200 gave the NLC with higher zeta potential. The particle size of Lycopene-loaded NLC prepared with plantacare 1200 was unchanged during 30 days of storage. It was concluded the surfactant influenced the size and zeta potential of lycopene-loaded NLC. The result obtained could be used to predict at an early state of stability of lycopene-loaded NLC.





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PO - 234

Enhanced oral bioavailability of vitamin B12 by thiolated poly(acrylic acid)

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Patients with pernicious anemia and other intestinal disorders develop vitamin B12 deficiency since they cannot absorb the tiny amount of vitamin B12 present in food. Thus, they require lifelong intramuscular injections or high oral doses in order to cover their needs (1).
Thiolated poly(acrylates) have been shown to exhibit very useful properties for oral drug delivery such as strong permeation enhancing and efflux pump inhibitory effects as well as strong mucoadhesion (2). In order to develop an efficient oral delivery system for vitamin B12, it was the aim of this study to investigate the potential of thiolated poly(acrylic acid) (PAA) as a modulator of vitamin B12 absorption after oral application. Preliminary studies revealed that PAA-cysteine conjugates with a molecular mass of 250 kDa (PAA_{250}) are the most promising modulators of vitamin B12 transport. Hence, permeation studies across freshly excised rat intestine in Ussing-type chambers, release studies from minitablets and in vivo bioavailability studies in rats have been performed with this kind of excipient.
Within permeation studies it could be demonstrated that vitamin B12 transport can be improved almost 4-fold by addition of 0.5 % of PAA_{250}-cysteine conjugate to the donor chamber. A further slight improvement was observed in the presence of 0.5 % reduced glutathione. Based on this promising observation, minitablets containing vitamin B12 were prepared on the basis of PAA_{250}-cysteine. Release studies with such minitablets revealed that thiolation of the polymer leads to a significantly delayed liberation of vitamin B12. Release from thiolated minitablets was complete after four hours whereas unmodified minitablets needed approximately two hours to release vitamin B12 completely. The oral bioavailability of such minitablets was investigated in rats in comparison to a solution of vitamin B12 and unmodified PAA minitablets. Vitamin B12 was also administered intravenously in order to determine the absolute bioavailability. Within these studies, it was observed that t_{max} was significantly delayed when vitamin B12 was administered in PAA_{250}-cysteine minitablets. However, the peak plasma concentration was significantly increased while the absolute bioavailability was almost 3-fold improved. These data correspond well with results from in vitro permeation studies. A summary of the pharmacokinetic parameters is provided in Table 1.
Previous permeation studies on rat intestine have indicated that vitamin B12 is subject to an efflux mechanism (3). Moreover, it has been shown several times that thiolated polyacrylates exhibit remarkable permeation enhancing properties (4). Hence, it is assumed that permeation enhancement by tight junction modulation and efflux pump inhibition support an enhanced transport across intestinal membranes and therefore an increased oral bioavailability. Even though the oral bioavailability does not exceed 1 % so far, these results are promising for future developments of further vitamin B12 delivery systems on the basis of thiolated polyacrylates and other thiolated polymers.

Table 1. Main pharmacokinetic parameters calculated after oral administration of solution, PAA minitablets, PAA-cysteine minitablets and intravenous injection of vitamin B12 to rats (means ± SD, *n* = 5)

	oral solution	PAA minitablets	PAA-cys minitablets	i.v. injection
AUC_{0-8} (µg/mL/h)	1.51 ± 1.06	2.63 ± 1.83	4.41 ± 3.27	49.12 ± 37.8
c_{max} (µg/mL)	0.016	0.022	0.053	4.03
t_{max} (h)	0.5	2	2	-
F_{abs} (%)	0.30	0.52	0.89	-

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PO - 235

Polymorphism of barbiturates — exemplified by pentobarbital

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The interest in polymorphism has grown strongly in the last decade. This is not least due to some well-publicised incidents such as the appearance of a lower-energy, more stable and low-soluble polymorph of the drug Ritonavir® two years after its launch, which resulted in drug formulations with insufficient oral bioavailability [1]. Detailed knowledge about the occurrence of polymorphs is mandatory in the production of solid pharmaceuticals and it is also of increasing interest to the understanding of the principles of supramolecular aggregation in the solid state. A survey of the Pharmacopoeia Europea 5.8 revealed that about 40% of all solid organic drug compounds are polymorphic [2]. Moreover, one third of them form hydrates and 18% form solvates. This means that more than 64% of the official drugs can exist in multiple crystal forms. An analogous survey was performed on a group of 110 barbiturates (18 salts, 92 neutral), showing that 42% of these barbiturates are described as being polymorphic, whereas the number of known hydrates (14%) and solvates (9%) is comparatively low in this set. We assume that these numbers would increase significantly as a result of further studies.
We are interested in barbiturates (5,5-disubstituted barbituric acid derivatives) because of their specific hydrogen-bond donor and acceptor functionalities in connection with their rigid molecular geometry. These characteristics result in just a limited number of feasible H-bonded motifs. This enables a meaningful classification of the resulting crystal structures [3]. Key methods for the investigation of the solid state characteristics of barbiturates are thermal analysis/calorimetry and X-ray crystallography. A general aim of this study was it to provide reliable information for the development of ab initio crystal structure prediction tools.
Previously, barbiturates have been used in the treatment of insomnia, anxiety, stress and as short-acting anaesthetics. Based on their selective anticonvulsant properties, barbiturates are today mainly indicated for the treatment of specific forms of epilepsy. Probably the most important example is phenobarbital (Luminal®) which in 2009 had a world production of 386 tons [4] and is listed in the WHO Essential Medicines Library. 19 barbiturates are officinal in the present editions of the European- and United States Pharmacopoeias.
As part of our comprehenisve investigation of barbiturates we have studied pentobarbital (5-ethyl-5-(1-methylbutyl)-barbituric acid), which is still in medical use. Four polymorphic modifications had been claimed in earlier reports [5,6]. Our investigation confirmed the existence of these forms. Comprehensive solvent screening did not reveal the existence of any solvated forms. The polymorphs were analyzed by X-ray diffraction, thermal analysis, solution calorimetry, polarized-light microscopy and vibrational spectroscopy (IR and Raman). Forms I and II were found to have very similar physical properties, indicating very close structural similarity, and they show small, but significant differences in their melting points and IR-spectra. The crystal structures of polymorphs I, III and IV were determined for the first time. The results of this study allow us to draw a clear picture of the thermodynamic relationships between the polymorphs and to understand the structural basis of the polymorphism in this system. The obtained IR and crystal structure data are also consistent with a previously established general correlation scheme [4] between the IR spectra and H-bond motifs of 5,5-substituted barbituric acid derivatives. This scheme allows the identification of the type of extended hydrogen bonded structure that is present in a barbiturate simply on the basis of certain features of its infrared spectrum without prior knowledge of the crystal structure.

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Impact of the lubricant type on compaction and tablet properties of sorbitol formulations

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Sorbitol is a commonly used tablet excipient suitable for direct compression and plays a major role in the manufacture of chewable and sublingual tablets. One limitation of the use of sorbitol is that its hygroscopic nature may cause sticking of tablets to the punch surfaces. The aim of the present study is to evaluate the suitability of various lubricants for reduction of friction and for prevention of sticking during direct compression of Neosorb® P60W formulations. The lubricant effect of the most commonly used lubricant magnesium stearate is compared to that of sodium stearyl fumarate (Pruv®), micropilled poloxamer 407 (Lutrol® micro 127), and PEG 4000. In the first part of the study MCC formulations with a Neosorb® content of 25 %, 50 % and 75 %, respectively, and a lubricant content of 4 % each, are compacted using an instrumented eccentric tablet press. The rank order of lubricant effect of the formulations is characterized by friction coefficients ($\text{Force}_{\text{max lower punch}}/\text{Force}_{\text{max upper punch}}$) as well as ejection forces measured during compaction. The following order is found: Pruv® ≥ magnesium stearate > Lutrol® > PEG 4000, independent of the Neosorb® content in the formulations. However, during compaction of formulations lubricated with Lutrol® and PEG 4000 sticking to the punch surfaces is observed even at a low Neosorb® content. Thus, the antiadherent effect of the lubricants Lutrol® and PEG 4000 is not sufficient for compaction of Neosorb® formulations. In the second part of the study, tableting of formulations lubricated with either Pruv® or magnesium stearate is performed with an instrumented rotary die tablet press. Again, the lubricant effect of Pruv® is found to be only slightly better with regard to ejection forces than that of magnesium stearate. However, determination of the properties of the resulting tablets revealed that formulations lubricated with Pruv® lead to tablets with sufficient tablet hardness and immediate disintegration. In contrast, tableting of formulations lubricated with magnesium stearate lead to rather soft tablets exhibiting prolonged disintegration times due to the lipophilic nature of the lubricant. Furthermore, the influence of the compression speed on tablet properties turned out to be less pronounced if Pruv® is used as lubricant. In conclusion, with regard to compaction as well as tablet properties lubrication of Neosorb® formulations with Pruv® is preferable.

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Interpretation of FBRM signals by ray tracing

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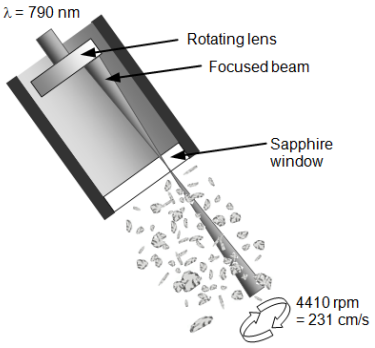
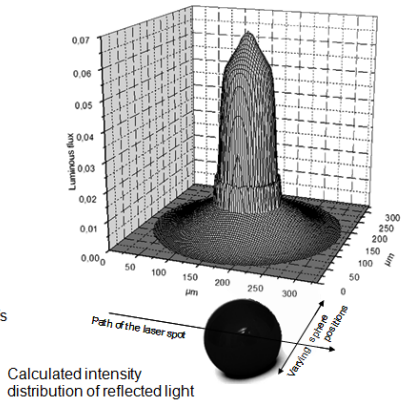
Focused beam reflectance measurement (FBRM) has become a well established method for inprocess particle size monitoring. It has found widespread use in laboratory scale development, in upscaling and in continuous tracking of production processes. As an extremely versatile in-line particle characterization technique FBRM has also emerged as one of the most often used tools in process analytical technology (PAT). Typical fields of application cover all steps from API synthesis to formulation and quality control of finished dosage forms like crystallization, wet milling, high shear and fluid bed granulation, pellet layering/coating, microparticle production and disintegration testing [1]. The method works with a fast rotating laser beam emitted by a dip probe. Every time the focused beam passes a particle a light impulse is reflected back to probe window where the duration of the signal is registered by a detector. The impulse length is the time span which the focus point needs to pass from one edge of the particle to the other and thus characterizes a chord length of the particle. The thus obtained chord length distribution (CLD) of a particle collective differs considerably from its particle size distribution (PSD) because each single particle produces a set of different chord lengths if it is several times randomly swept by the beam. Hence, the interpretation of CLDs is difficult and even further complicated by the strong impact of reflection phenomena caused by the surface morphology and the optical properties of the particles. None of the

mathematical models so far developed for conversion of CLDs into PSDs does account for these optical effects [2,3]. Many users of FBRM tend to cast doubts on the validity of the measured results for the often complex distribution curves are frequently contradictory to any expected pattern and apparently beyond any reasonable explanation. In this work for the first time, based on the ray tracing technique, a calculation method was developed which is able to fully interpret FBRM signals of spherical particles by considering also their reflective behavior.

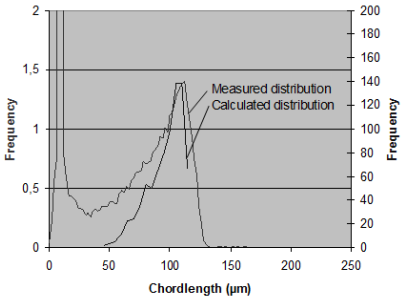
CLDs of colored and transparent monodisperse polystyrene spheres with different surface roughness were measured by FBRM. A mathematical model was developed which is able to calculate the resulting CLDs of spherical particles considering their reflective behavior as well as instrumental parameters of the FBRM device. Calculation is based on tracking the light rays directed to each of 7200 imaginary equally sized surface elements of a particle's upper hemisphere and considering also every possible spatial position of the particle. The calculated curves were fitted to the measured distributions to obtain valuable information on the particles' matrix and surface properties.



Functional principle of FBRM



Calculated intensity distribution of reflected light



Lambertian scattering: 60%
Specular reflection: 0%
Subsurface scattering 40%

Measured and simulated size distributions of 100 µm polystyrene spheres

The simulation reveals that CLDs measured with FBRM devices, are strongly skewed by optical effects due to the particles' opacity, roughness or light absorption. Because of the extreme complexity of signal generation and processing it is hardly possible to correlate intuitively certain maxima or other characteristics of the CLD curve with specific particle properties. The new method forms the basis to deduce conventional PSDs from in-line data measured by FBRM without any bias from the particles' optical properties. Because the calculation is based on spherical objects it is an ideal method for monitoring the preparation of microspheres. These processes usually involve phase transitions which are accompanied by changes in optical behavior. Thus, the determination of absolute particle sizes is only possible if a method is available to correct the data for reflection phenomena. Conversely, by a deep understanding of the FBRM signal, the method can also be used to detect structural changes of fixed-sized particles.

Acknowledgements: The authors gratefully acknowledges the assistance received from Mrs. K. Vay, Sandoz GmbH, Kundl, Austria
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PO - 238

Influence of the nozzle type on particle size and its consequence for flowability of spray-dried protein powders

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Purpose

This work shall investigate the influence of different nozzle types on the particle morphology of spray dried protein powders. The flowability is to be characterized.

Materials and methods

For the drying experiments a lab-scale spray dryer from Pro-C-ept (Zelzate, Belgium) is used. Its special design is able to produce large droplets in spite of a small sample volume and liquid feed. A two-part spray tower with an overall length of 140 cm was assembled. A standard two-fluid nozzle (cap-orifice diameter 1.2 mm, atomizing air pressure 20.0 kPa) and two ultrasonic nozzles (25 kHz; 60 kHz; Sono-Tek Corp., Milton, USA) were used to generate an atomized spray containing droplets as large and consistent as possible. For the liquid feed a peristaltic pump with 10 compression rolls was used. Catalase from Bovine liver and Trizma hydrochloride were purchased from Sigma-Aldrich, Germany and used as obtained.

In order to characterize flowability a vibrating spatula (Gro-Mor Inc., Massachusetts, USA) was applied. The device was connected to an analytical balance (Sartorius LA 120S; accuracy: 0.1 mg) [1].

Results

A solution of Catalase (0.1 g/ml) in 0.5M Trizma-buffer was atomized and dried with an inlet temperature of 130°C. With the two-fluid nozzle particle sizes range from 1 to 20 µm with an irregular gauged particle surface (fig. 1). The 60 kHz ultrasonic nozzle generates particles with a similar size but less fines (fig. 2). Particles dried with the 25 kHz nozzle are larger with sizes ranging from 15 to 60 µm. The surface seems a little bit smoother (fig. 3). For flowability measurement the vibrating spatula was loaded with 160.0 mg of each spray dried powder and turned on for 3 minutes. The mass flow was recorded so that it can be expressed as a function of time. Figure 4 shows the difference in flowing behaviour. Mass flow can be observed with both ultrasonic nozzles, whereas the 25 kHz nozzle shows the best result.

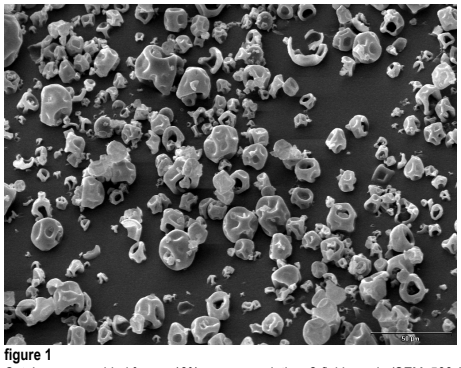

figure 1
Catalase, spray dried from a 10% aqueous solution, 2-fluid nozzle (SEM, 500x)

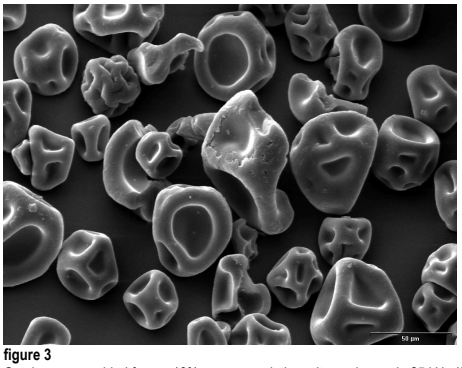

figure 3
Catalase, spray dried from a 10% aqueous solution, ultrasonic nozzle 25 kHz (SEM, 500x)

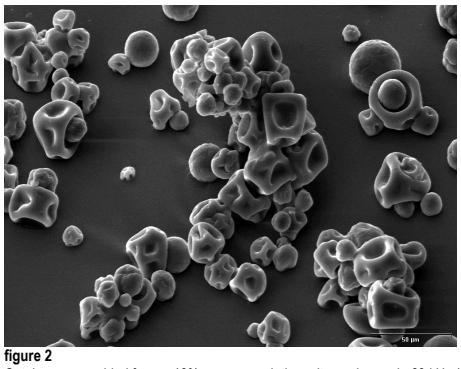

figure 2
Catalase, spray dried from a 10% aqueous solution, ultrasonic nozzle 60 kHz (SEM, 500x)

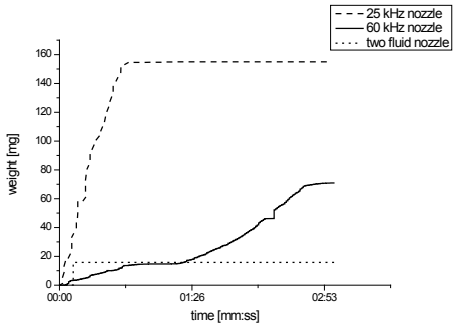

figure 4
Mass flow of spray-dried powders from a vibrating spatula

Conclusion

Atomizing nozzle types have an influence on the size and the morphology of the resulting dried particles. Flowability of the powder differs from each other. Ultrasonic atomization and spray drying could lead to flowable powders. It seems that atomization with the 25 kHz nozzle results in the largest particles and the best flowability.

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PO - 239

In vitro establishment of a new microdialysis based system for determination of drug concentrations in the small intestine

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A novel microdialysis system has been developed that consists of a minimodule and a tubing system. The minimodule is made up of 30 polysulfone hollow fiber capillaries (Fresenius®, inner diameter 200 µm, molecular weight cut off 6000 da). Paracetamol, amoxicillin, and valsartan were chosen as model substances.
The minimodule was inserted into a chamber filled with phosphate buffer USP 34 pH 6.8. Phosphate buffer USP 34 pH 6.8 served as dialysate. For the investigations an equilibration time of one minute was used as established in previous experiments. Two pumps were used to transport the fractions (volume 300 µl) after the intended equilibration period and to produce air bubbles separating the fractions. Concentrations in the separated fractions were determined via UV spectroscopy. After preincubation a solution of the model substance in phosphate buffer was added to the chamber and the concentration in dialysate fractions was determined (resulting theoretical concentration 20 mg/l for paracetamol and amoxicillin or 1.6 mg/l for valsartan, respectively, first concentration leap). After the intended incubation period phosphate buffer was added to the chamber to reduce the concentration (resulting theoretical concentration 10 mg/l for paracetamol and amoxicillin or 0.8 mg/l for valsartan, respectively, second concentration leap). Concentrations in the fractions were determined as described above. The sensitivity of the microdialysis system towards concentration leaps was determined in order to evaluate the system.
The experiments showed a sufficient detection of concentration leaps within one minute of equilibration time. Paracetamol concentrations from 17.0 to 18.1 mg/l for the first concentration leap and 9.0 to 9.7 mg/l for the second concentration leap were determined. Experiments with amoxicillin and valsartan are in progress.
In conclusion, model substance concentrations and concentration changes over time were successfully monitored.

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Small-scale production in twin-screw extrusion: requirements for solid and liquid feed systems

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Regarding the requirements of the development of new formulations, there is a need for extruders with low material throughput for small-scale production. A major obstacle for developing miniaturized extruders is the adjustment of crucial parts of the equipment when downsizing. Many investigations have been done with regard to scale-down of the process section, consisting of the barrel, screw and die, but the limitations imposed by the feeding systems has not been given adequate consideration. Using a lab-scale twin-screw extruder (figure), the feeding systems were investigated in order to determine the limitations of the material feeding, which was identified as crucial. Wet extrusion process was selected to perform the experiments because both solid and liquid feed systems have to be considered.
First of all, the accuracy and uniformity of the feeding systems were tested independently of the extrusion process. Based on these results the accuracy of both feeders was considered as adequate and disregarded in further investigations. In contrast to the liquid feed rate uniformity, the uniformity of the powder feed rate showed fluctuations based on the accumulation of powder inside the coupling to the extruder barrel. Thus randomly slipping off powder lumps from the inside wall of the coupling led to a non-uniform feeding. A shorter coupling

with an asymmetric funnel shape was designed to improve the uniformity. After modification of the powder feeder, both feeders were investigated in conjunction with extrusion.
After validating both feeders independently of the extrusion system, further investigations in conjunction with extrusion were required. Even if the liquid feeder showed a very uniform flow rate independently of the extrusion process, there were also remarkable fluctuations in the liquid feed rate when the process was running. These fluctuations were correlated with pressure oscillations and were attributed to an inadequate design of the liquid nozzle. An optimization of the geometry of the nozzle was done by reducing the dead volume in front of the nozzle. The inner diameter was also reduced to increase the pressure of the liquid feeder. Due to the two modifications to the nozzle the uniformity of the liquid feed rate as well as the moisture of the extrudates was improved.
The powder and the liquid feed rate were identified as crucial parameters in small-scale extrusion. Both feeder modifications reduced the variability of the moisture content in the extrudates by one order of magnitude. This led to a reliable small-scale extrusion process. Without both modifications the wet extrusion process had failed.

Fig. Lab-scale twin-screw extruder (ZSE 18GL-40D, Leistritz Extrusion Technology. Nuremberg, Germany)

Acknowledgements: Leistritz Extrusion Technology (Nuremberg, Germany), Pharmatrans SANAQ (Basel, Switzerland), Meggle (Wasserburg, Germany).

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Solubility enhancement of nabilone by complexation with methylated - ß - cyclodextrin

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Nabilone is a synthetic cannabinoid and its structure is similar to active incredients of Cannabis sativa. This compound can be used as analgesic for neuropathic pain. Unfortunately, this class of compounds exhibits a very low solubility in aqueous solution and this leads to a significantly reduced bioavailability. As cyclodextrins are important excipients to increase the solubility of many low soluble drugs, the complexation with nabilone has been studied experimentally using thermodynamic investigations, and by molecular calculations.
The complexation of nabilone with β-cyclodextrin, measured by the solubility method, results in a slight increase of the solubility of nabilone only. In contrary, the complexation with 2,6-dimethyl-β-cyclodextrin (or with the technologically more often used RAMEB) leads to an unexpectedly high increase of the solubility and an anormal temperature dependence of the complexation constant. In previous investigations it has been found that the thermodynamics of the complexation by various cyclodextrins is different [1], and in particular the contribution from the reaction entropy is important.
To understand the molecular interactions which are essential for the complexation of nabilone with the interior of the various cyclodextrins, molecular calculations have been performed on nabilone and the corresponding

complexes, using M06-2X with various basis sets. Such calculations are feasible nowadays as modern computer technology provides sufficient amounts of computer resources [2]. The detailed structure analyses of the complexes provide information about the high affinity of nabilone particular with 2,6-dimethyl-β-cyclodextrin. These results will be correlated with the experimental data.

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PO - 242

Enhancing the performance of dry powder inhalers by tailoring interparticle forces via surface modification of carrier and active

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The aim of this work is to improve the performance of carrier based dry powder inhalers (DPI) via the modification of interparticle interactions between the drug and the carrier. In order to reach the deep lung active pharmaceutical ingredient (API) particles must have an aerodynamic diameter of 1 µm to 5 µm. Particles of this size range are very cohesive and exhibit rather poor flow properties [1] what makes volumetrically dosing very difficult. To overcome this problem and to improve flowability the API is attached to larger carrier particles (50 µm – 200 µm). Drug detachment from the carrier during inhalation is essential to ensure that the drug particles reach their targeted site, the deep lung. Otherwise they will impact together with the coarse carrier on the upper airways. Thus interparticle interactions play a crucial role in carrier based formulations. It is important, that they are on the one hand high enough that uniform dosing is possible and on the other hand low enough that drug detachment during inhalation is guaranteed.
In the present study interparticle interactions were altered by the surface modification of glass beads, used as model carrier because of their ideal geometry and the various options to modify their surface chemically as well as physically, without affecting other factors that also influence interparticle forces like particle shape and size. Furthermore interparticle forces were modified by spray drying of the API. Salbutamol sulphate and salbutamol base, used as model drug, were spray dried using different solvents, different concentrations and spray drying conditions. The sphericity and cristallinity of the generated particles was analyzed dependent on these parameters. The challenge is to generate particles of appropriate size (1 µm – 5 µm) which are spherical and crystalline. Experiments of Chawla [2] showed that spray drying of salbutamol sulphate produces spherically shaped particles with a mass median diameter of 4.5 µm. But compared to micronized salbutamol sulphate the spray dried powder tends to be less crystalline [2]. In contrast our first experiments have shown that spray drying of ethanolic salbutamol base solutions seems to be most promising to create crystalline particles with the requested size. Figure 1 shows a scanning electron micrograph of a salbutamol base particle spray dried at 100 °C from ethanol.

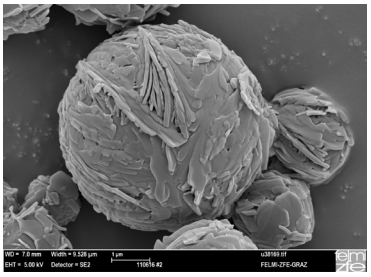

Fig.1: SEM of salbutamol base, spray dried at 100 °C from ethanol

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Miscellaneous - Pharmacology

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Investigation of the σ_1 receptor binding site by site directed mutagenesis

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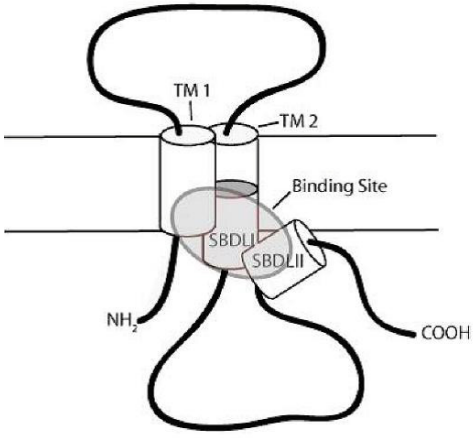
σ Receptors, first described in 1976, are specified as an unique receptor class consisting of two subtypes, σ_1 and σ_2 receptors. They differ in their molecular weight, distribution as well as in their ligand binding profile. High density of σ_1 receptors was found in the central nervous system but they are also present in some peripheral organs. Moreover, several tumor cell lines show overexpression of σ_1 and σ_2 receptors. σ_1 Receptors modulate some other receptor proteins and ion channels. Possible therapeutic applications for σ_1 receptor ligands are psychiatric disorders, Alzheimer's disease, drug abuse and pain. Furthermore, the high expression of σ_1 receptors in a variety of tumor cell lines indicates potential of σ_1 receptor ligands in diagnostic tumor imaging and tumor therapy [1].
The σ_1 receptor has several hydrophobic regions, which are shown in Figure 1: two transmembrane domains (TM 1, TM 2) and two regions, which are called 'steroid binding domain like' 1 and 2 (SBDL 1, SBDL 2). SBDL 1 and 2 were found to be in juxtaposition [2]. The ligand binding site of the σ_1 receptor is not characterized exactly because the protein has not been crystallized yet. Mutagenesis and photoaffinity experiments led to the assumption that the ligand binding site consists of TM 1, SBDL 1 and SBDL 2 (see Figure) [3]. Furthermore it is known from mutagenesis experiments that the two anionic amino acids Aspartate 126 and Glutamate 172 outside of SBDL 1 and 2 are obligatory for haloperidol binding [4].
On this poster, site directed mutagenesis experiments combined with radioligand receptor binding studies are presented, which are directed with the aim to characterize the ligand binding site of the σ_1 receptor.



Putative model of the σ_1 receptor with the proposed receptor binding site [3]

Acknowledgements: Deutsche Forschungsgemeinschaft, Prof. Dr. Karl-Heinz Klempnauer (Institut für Biochemie, WWU Münster)

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PO - 244

Biological activity of potentially COS releasing compounds on isolated tissue of guinea pigs

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The aim of this study was to investigate the efficacy and the pharmacodynamics of three compounds focusing on their inotropic, chronotropic, vasodilating and spasmolytic action. Compound **1** is a 3,3'-thiobis-(2-nitro)-thiophen, compound **2** a 1,1'thiocarbonylimidazole and compound **3** an ethyl(2-(dimethylamino)propoxy)methanthioat. Force of contraction (f_c) on electrically stimulated papillary muscles (1Hz), spontaneously beating right atria, aortic-rings, arteria pulmonalis-rings (precrontracted with 90 mM KCl) and terminal ilea (precrontracted with 60 mM KCl) of guinea pigs was measured using the method described by Reiter[1]. The effects of compound 1 and its EC_{50} values on vascular smooth muscles (aorta and arteria pulmonalis) and terminal ilea are presented in figure 1.

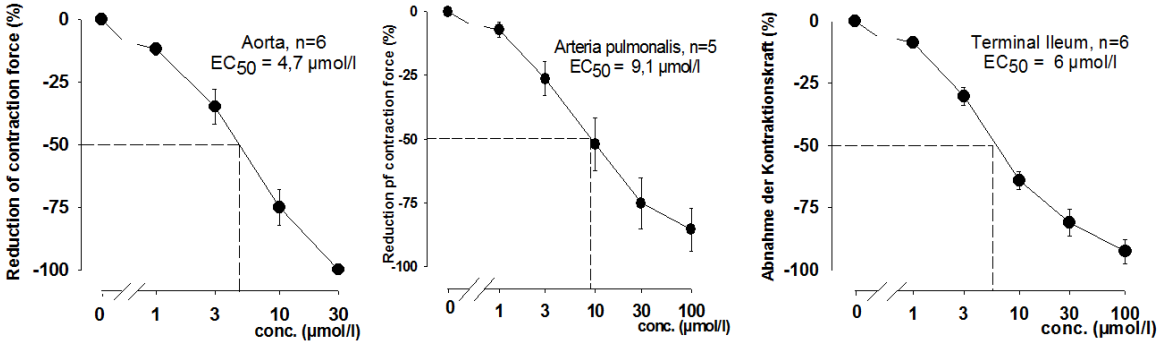

Fig. 1

All three compounds did not show any effect on heart muscle preparations such as papillary muscles and right atria. The effect of compound **2** with an imidazole moiety on smooth muscle was less potent with an EC_{50} of 41.7 µmol/l (aorta), 12.7 µmol/l (terminal ilea) and > 100 µmol/l (arteria pulmonalis). Compound **3,** a dithiocarbonic acid derivative had no effect on vascular smooth muscles and terminal ilea. To elucidate the possible mode of action compound **1** was studied in presence of glibenclamide, a K_{ATP}-channel inhibitor, nitro-L-arginine, an inhibitor of the endothelial nitric oxide (NO) synthase and phenylephrine hydrochloride in aortic rings.

Our results suggest that compound **1** induces relaxation in guinea pig smooth muscle preparations partly by opening $K_{(ATP)}$ channels possibly mediated through carbonylsulfide (COS), but other mechanisms may play a role as well.

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PO - 245

The effects of D-camphor- or D,L-camphor with hawthorn berry extract combination on taste sensations, on accuracy in identifying the administered compound, and on blood pressure

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The study investigated on the basis of a randomized, placebo-controlled, double-blind design the effects of D-camphor (Korodin Herz-Kreislauf-Tropfen®) or D,L-camphor with hawthorn berry and l-Menthol extract combination on taste sensations, on accuracy in identifying the administered compound, and on blood pressure among young females.
The main questions of the study were: (1) Is there a difference in subjective taste experience after administration of the two verum liquids and placebo (60% ethanol, brown coloured)? (2) Can our results from previous studies be correlated, pertaining to the effects on blood pressure, tolerability, and safety of a D-camphor- with hawthorn berry extract combination [1,2,3,4]?
Seventy-two female participants were enrolled and randomly assigned to one of the three study groups (N = 24 per group, two groups receiving active compounds, one placebo group). Mean age of the participants was 23 years. Mean resting diastolic blood pressure of the total sample was 68.7 mmHg, mean resting systolic blood pressure was systolic 100.3 mmHg. Thus, blood pressure ranged close to hypotension (i.e. per WHO definition less than 100 mmHg systolic).
Taste sensations were assessed immediately after administration of the 25 drops of verum or placebo. The scale comprised eight different taste qualities being rated on a visual analogue scale. Blood pressure was measured by the Riva-Rocci (RR) method and additionally in a continuous manner by the *Finapres* technique. RR-readings were taken after a resting phase and after administration of 25 drops of liquid at a time schedule of 1, 6, 11 und 16 minutes. Continuous measurement started one minute before administration and lasted for 15 minutes.
After administration of the D-camphor compound as well as after D,L-camphor compound significant blood pressure elevations occurred, ranging from 5 to 7 mmHg for systolic and diastolic blood pressure. In this respect, no difference between the two substances was observed. After placebo administration no comparable effect was seen.
Medical exploration and documentation revealed no drop-outs and no unexpected events. Participants' guesses about having received verum or placebo did not exceed chance level.
The present data on the blood pressure responses correspond to our previous findings [3,4] of a fast blood pressure increase administration of Korodin Herz-Kreislauf-Tropfen®. Since the medical documentation did not reveal any unexpected events, the active substances can be rated as of excellent tolerability and safety.

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PO - 246

Investigation on β- and γ-carbolines as putative tools for the development of potential anti-Alzheimer drugs

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Several analogous β- and γ-carbolines were synthesized as potential nootropic compounds. In previous studies we modified the structure of the neuroprotective and nootropic 9-methyl-β-carboline to incorporate structural elements of the potential antialzheimer drug dimebone and the MAO-inhibitor rasagiline. Further, we prepared analogous γ-carbolines to evaluate the effect of the carboline-scaffold besides the influence of the substitution. The antialzheimer potential of the prepared derivatives was evaluated by in vitro determination of their inhibitory effect on cholinesterases and NMDA receptors. In vivo studies in a scopolamine rat model were also carried out to evaluate their antidementive potential.
Now, further modifications on the carboline scaffold have been done and more in vitro assays performed to investigate structure-activity relations. Former performed in vivo studies showed an interesting superiority of all γ-carbolines compared to their β-carboline counterparts. By doing additional investigation we want to find a simple in vitro method correlating to the previous findings.

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Inhibition of fibroblast growth factor 2 bioactivity by clodronate

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Fibroblast growth factor 2 (FGF2) is one of the best characterised member of the family of heparin-binding growth factors. FGF2 is important for cell proliferation and angiogenic processes and is overexpressed in a large number of different tumors. Recently, we have demonstrated that ATP can bind to FGF2 and stabilises the growth factor against proteolytic cleavage and thermal degradation [Rose et al., 2010]. Here we present data that some non-nitrogen containing bisphosphonates, in particular clodronate, also interact with FGF2, protect it from tryptic digestion and compete with ATP for FGF2-binding [Rose et al., 2011]. Clodronate reduces the FGF2-induced proliferation of human umbilical vein endothelial cells (HUVECs) in a concentration-dependent way. Furthermore, clodronate reduces FGF2-induced intracellular signaling in HUVECs via AKT and ERK1/2 pathways. The putative usefulness of non-nitrogen-containing bisphosphonates in reducing angiogenic processes induced by FGF2 will be discussed.

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PO - 248

Modulation of epithelial sodium channel current by TNF-α lectin-like domain derived peptides

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The amiloride-sensitive epithelial sodium channel (ENaC) plays a prominent role in sodium uptake from alveolar fluid and is the major component in alveolar fluid clearance in normal and diseased lungs. The mechanism underlying the modulation of ENaC current by TNF-α and TNF-α lectin-like domain derived (TIP) peptides was investigated using the patch-clamp technique. TIP peptides caused a substantial increase in sodium current through ENaC in human alveolar epithelial lung cancer cells (A549) in both whole cell as well as single channel patch clamp configurations. The EC_{50} value of the lead TIP peptide AP301 (54.3±0.8 nM, n=5) was higher than

that of TNF-α (8.2±0.1 nM, n=5) but still in the nanomolar range. In contrast, neither TNF-α nor AP301 had any effect on the sodium current through ENaC in human nasal epithelial carcinoma (RPMI 2650) cells (at concentrations up to 10 μM). The effect of AP301 was also investigated on primary rat type II alveolar epithelial cells, in which AP301 increased sodium current through ENaC from 10.7± 3.8 to 66.2±5.3 pA (n=3). Earlier work had shown that TNF-α exerts a pH-dependent and TNF-receptor-independent increase in membrane conductance in primary murine lung microvascular endothelial cells and macrophages (1). We observed that a change in pH resulted in a small difference in the current-modulating activity of AP301 in A549 cells at a holding potential of -100 mV: the sodium current enhancing ability of AP301 at pH 6.0 was found to be approximately 90% of that observed at the physiological pH of 7.4. Prostasin cleaves ENaC thereby activating it. Nafamostat mesylate was applied to inhibit prostasin and to investigate the activity of AP301 in presence of the prostasin inhibitor. ENaC current was blocked by nafamostat and was unaffected when TIP was applied in addition to nafamostat. Our results further characterise the modulation of ENaC current by TIP peptides.

Acknowledgments: W.S and P.H. received financial support by APEPTICO GmbH and the Austrian Research Promotion Agency (FFG).
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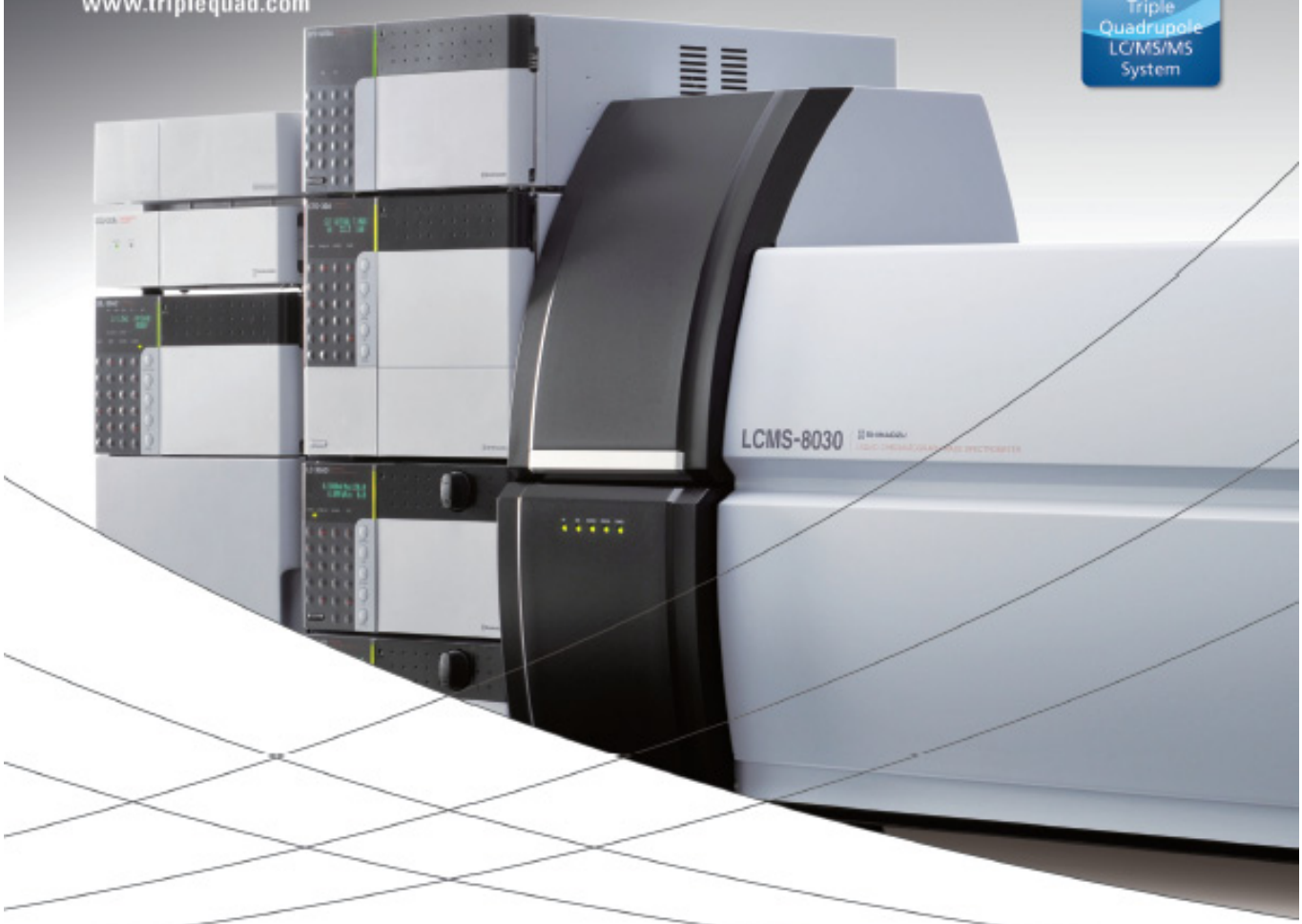

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