Application of Chemical Function Based Pharmacophore Modelling to Protein Kinase C Inhibitory Peptides

K. Poptodorov[1], J. Soder[1], R. D. Hoffmann[2], J. Hofmann[3], C. Schächtele[4] and T. Langer[1]

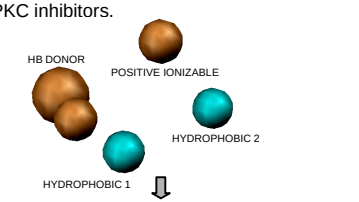
[1]*Department of Pharmaceutical Chemistry, University of Innsbruck, Innrain 52a, A-6020 Innsbruck*
[2]*Molecular Simulations SARL, Parc Club Orsay Université, 20, rue Jean Rostand, F-91893 Orsay*
[3]*Institute of Medical Chemistry and Biochemistry, University of Innsbruck, Fritz-Pregl-Str. 3, A-6020 Innsbruck*
[4]*Institute of Molecular Medicine, Tumor Biology Center Freiburg, Breisacher Str. 117, D-79106 Freiburg*

INTRODUCTION

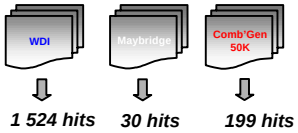
The development of inhibitors for the protein kinase C isozyme (PKC) family still remains in the focus of drug discovery research. Several potent and among them some isozyme selective PKC inhibitors are available up to date, mostly competing with the ATP binding site [1]. A number of relatively short peptides that resemble PKC substrate/pseudosubstrate sequences or mimic PKC substrates has been reported to be able to bind to PKC, however the possibility to develop nonpeptidic substrate binding site directed inhibitors has not been considered yet.

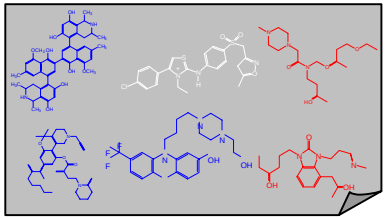
RESULTS

Only two four-feature hypotheses with identical coordinates, the second rank hypothesis containing HBA instead of HBD, were obtained. The first hypothesis was used as a query for searching 3D structure databases in order to identify compounds that potentially bind to PKC. Moreover, the model was also used to search large virtual combinatorial libraries generated with the *Comb'Gen* software package [4] in order to define novel lead structures of PKC inhibitors.

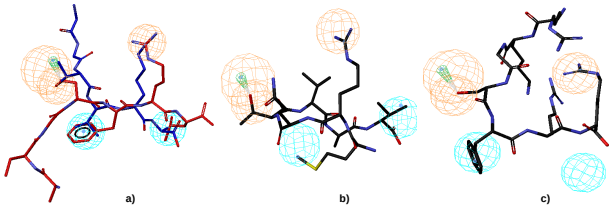


50 000 compound 3D structure database search

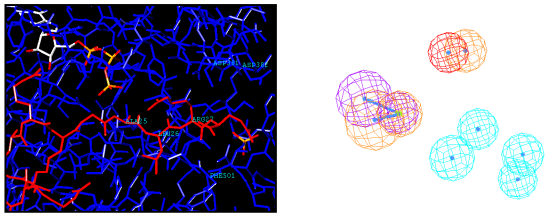




Examples of promising structures retrieved



Mappings of selected members of the training set showing different binding possibilities: a) annexin (blue, principle compound) and histone (red), b) MRQTVAV (C-N orientation) and c) RKGSFRR (N-C orientation)



A model of PKC βII by Orr and Newton [5] based on homology to PKA (left) and a comparison of the common features hypothesis generated from the current training set to a hypothesis derived from the model (right) showing good correlation

METHODS

Training Set: eleven heptapeptides containing residues -3 to +3 around the phosphorylable serine or threonine were synthesized and tested in a single PKC enzyme assay at 10 μg/ml with histone IIIS as substrate.

Peptide	Effect on PKC isozymes									
	α	βI	βII	γ	δ	ε	ζ	η	μ	ι	θ
KRFSFKK	•	•	•	•	•	○	•	•	○	○	●
RKGSFRR	•	•	•	•	○	•	•	•	○	○	•
IQASFRG	•	•	•	•	○	○	•	•	○	○	•
RRLSSLR	•	•	•	•	•	○	•	•	•	○	•
RKRTLRR	•	•	•	•	○	○	○	•	○	○	•
MRQTVAV	○	•	•	•	○	•	•	○	•	○	•
RKGSFFY	○	•	•	•	•	•	•	•	○	○	•
KRPSQRS	○	•	•	•	•	•	•	•	•	○	•
PKGSFFY	○	•	•	○	•	○	•	•	○	•	•
RVTSAAR	•	•	•	•	○	○	•	•	•	•	•
AARRSYV	•	•	•	•	○	○	•	•	•	•	•

• inhibition ○ activation • no effect

Although considerable variations in the inhibitory activity towards the different isozymes could be observed (up to 54%), the obtained data was not suitable for generation of an activity based pharmacophore model. Therefore the ***HipHop* algorithm** implemented in the Catalyst™ software [2] was used to generate sets of *Common Features Hypotheses* without taking biological data into account. The sequences RKGSLRQ and KRPSQRS were additionally included in the training set, as well as two crystal structure fragments of natural PKC substrates: annexin IV (1ANN, ^{6}GGTVKA^{11}) and histone H5 (1HST, chain B, ^{89}ASGSFRL^{96}) from the Protein Data Bank™ [3]. Annexin was set as reference compound.

Functions: *H-Bond Acceptor, H-Bond Donor, Hydrophobic* and *Positive Ionizable*. HBA, HBD and Positive Ionizable were customized to exclude peptide backbone elements. Only ideal H-Bond geometry was used.

CONCLUSIONS

The chemical function based pharmacophore model presented here gives an example of utilizing the structure-activity information delivered by peptides to find novel lead structures for PKC inhibitors. The problems caused by the flexibility and large number of features in heptapeptides can be overcome to a great extent by integrating crystal structure conformations into the training set and by customizing the Catalyst™ functions. The best hypothesis contains features known to be important for the binding to the enzyme and retrieves at least one compound class known to inhibit PKC from the WDI [6]. Thus the described approach appears to be useful as starting point in developing inhibitors for protein kinase C.

REFERENCES

1. P. Goekjian, M. Jirousek, *Curr. Med. Chem.*, **6**, 877-903 (1999)
2. Molecular Simulations Inc., San Diego, CA, USA (1997)
3. http://www.rcsb.org/pdb/
4. G. Wolber, T. Langer (2000)
5. J. W. Orr, A. Newton, *J. Biol. Chem*., **269**(11), 8383-8387 (1994)
6. E. L. White et al., *Arch. Biochem. Biophys*., **365**(1), 25-30, (1999)

konstantin.poptodorov@uibk.ac.at