***In Vitro* Blood-Brain Barrier Permeability Predictions for GABAA Receptor Modulating Piperine Analogs**

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**Introduction:** The alkaloid piperine from black pepper was recently identified as a positive allosteric modulator of γ-aminobutyric acid type A (GABAA) receptors interacting at a benzodiazepine-independent binding site [1, 2]. Subsequently, three generations of piperine analogs with improved pharmacological properties were synthesized. In order to reach the CNS, these compounds need to enter the brain by crossing the blood-brain barrier (BBB).

**Aims:** We here evaluated the BBB permeability of piperine and five selected analogs (SCT-66, SCT-64, SCT-29, LAU397, and LAU399) in three *in vitro* BBB models: a recently validated human model with immortalized hBMEC cells, a stem cell-derived human brain-like endothelial cells (BLEC) model, and a primary animal (bovine endothelial/rat astrocytes co-culture) model.

**Methods:** For each compound, reliable quantitative UHPLC-MS/MS methods in the range of 5–500 ng/mL in the corresponding matrix were developed, and permeability coefficients in the three BBB models were determined.

**Results:** *In vitro* predictions from the two human BBB models were in good agreement, while permeability data from the animal model differed to some extent, possibly due to protein binding of the screened compounds. In all three BBB models, piperine and SCT-64 displayed the highest BBB permeation potential. This was corroborated by data from *in silico* prediction. For the other piperine analogs (SCT-66, SCT-29, LAU397, and LAU399), BBB permeability was low to moderate in the two human BBB models, and moderate to high in the animal BBB model [3].

**Conclusions:** These results serve for selecting the most promising candidate molecule for the next cycle of medicinal chemistry optimization.

**Keywords:** GABAA receptor, human stem cells, immortalized cell line, *in vitro* blood–brain barrier (BBB) model, primary cells.

**References:**

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[2] Khom S et al., Biochem Pharmacol 2013; 85: 1827-1836.

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