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MEETING ABSTRACT

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A pharmacological and computational study on sewarine, a naturally derived alkaloid, as a new ligand interacting with the κ opioid receptor

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Background: The kappa opioid receptor (KOR), a member of the opioid receptors family, has received extensive attention in recent years, and nowadays it emerges as a potential target for the treatment of a variety of human disorders, including pain, affective disorders, drug addiction, and psychotic disorders. The KOR is distributed throughout the brain, the spinal cord and various peripheral tissues. The structure of the KOR was elucidated by X-ray crystallography, giving insights into the binding pocket of the KOR. Applying a pharmacophore-based virtual screening strategy, we have recently reported on a novel KOR ligand, sewarine. It is a naturally derived alkaloid from the plant *Rhazya stricta*, used in traditional medicine against human diseases such as cancer, rheumatism, skin diseases, or pain. Herein we present a comparative pharmacological study on the interaction and signaling of sewarine at the KOR from guinea-pig and human origin. In addition, the binding mode of sewarine to the crystal structure of the human KOR is described.

Methods: Binding and activity at the KOR were determined using radioligand binding, [^{35}S]GTPγS functional and forskolin-induced cAMP accumulation assays. Molecular docking in the human KOR crystal structure was performed using GOLD 5.1 software.

Results: In *in vitro* binding studies, sewarine showed high KOR selectivity with similar binding affinities to the KOR in the guinea-pig brain and CHO cells expressing the human KOR. While in guinea-pig brain, sewarine displayed KOR antagonism, in CHO-hKOR cells it acted as a KOR partial agonist. The relatively low stimulatory effect of sewarine at the human KOR was fully reversed by the selective KOR antagonist nor-BNI. The apoptotic effect of sewarine in human leukemia CEM-C7H2 cells was also demonstrated to involve the KOR, based on the significant antagonism of nor-BNI. The structural features that promote binding of sewarine to the human KOR were investigated by molecular docking studies. Similar to well-known KOR ligands, the salt bridge between the protonable nitrogen in sewarine and Asp138 was maintained, and hydrophobic contacts were established with Val108, a residue responsible for KOR selectivity.

Discussion: Through combination of biochemical, pharmacological and computational approaches, we highlight the outcomes on the selective interaction and signaling of sewarine via the KOR in neuronal and cellular systems expressing KOR. The present findings provide insights into the binding mode and signaling at the KOR of sewarine as a novel KOR ligand of plant origin, which may represent a promising lead molecule for optimization towards superior probes targeting the KOR, and ultimately for the development of new therapeutics for human neurological and other disorders.

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