SYNTHESIS AND CHIRAL SEPARATION OF NEW BENZYLOXY ANALOGUES OF TWO SK CHANNEL-BLOCKING ALKALOIDS: NML AND AG525

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SK (KCa2.1-3) channels are potassium channels opened by an intracellular increase of calcium and are voltage insensitive. Three subtypes, SK1, SK2, and SK3, are expressed in humans. These channels are known to be involved in the medium-duration afterhyperpolarization of the neuronal membrane which follows the action potential, and they contribute to the repolarization of the action potential in atrial cardiomyocytes[1]. Blocking these channels could be useful in treating various pathologies like depression or atrial fibrillation[2,3].

In the previous work, our team demonstrated that *N*-methyl-laudanosine (NML) is a blocker of SK channels with a micromolar inhibition constant (Ki)[4]. The dimerization of the 6,7-dimethoxy-tetrahydroisoquinoline ring has resulted in the synthesis of 1,3-bis(6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)propane diastereoisomeric mixture (AG525). The first eluted stereoisomer in preparative chiral HPLC, AG525E1, has shown great promise as an SK channel blocker, particularly for targeting central nervous system SK channels[5]. In these studies, a part of the chemical space focused on adding an aromatic group as a substituent in positions 6,7, and 8 of the tetrahydroisoquinoline ring remains unexplored. This work aims to create similar compounds to NML and AG525 by replacing one methoxy group with a benzyloxy one to evaluate the potential of these compounds as ligands for SK channels.

In this study, we synthesized 6-benzyloxy-7-methoxy analogues of these two alkaloids. For this purpose, 6-benzyloxy-7-methoxy-isoquinoline was prepared to get the corresponding Reissert compound, used for synthesizing alkylated isoquinolines. After methylation and reduction of alkylated isoquinolines, the stereoisomers were isolated using a semi-preparative HPLC technique. The absolute configuration of the stereoisomers was subsequently determined by X-ray crystallography.

A biological screening was performed to test affinities of the synthetised compounds on SK2 and SK3 channels using [^{125}I]-radiolabelled apamin in a competition binding assay. Interestingly, in preliminary results, (*S,S*)-1,3-bis(6-(benzyloxy)-7-(methoxy)-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)propane displaces 34% or 65% of the radioligand at a concentration of 1 µM on SK2 and SK3 channels respectively, which is in a similar range than the reference compound (AG525E1).

Shortly, the other benzyloxy analogues will be synthetised and extensive *in vitro* binding and patch-clamp experiments will be conducted on our new synthetized compounds to further characterize their binding affinities and blocking potencies on SK2 and SK3 channels.

References

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