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

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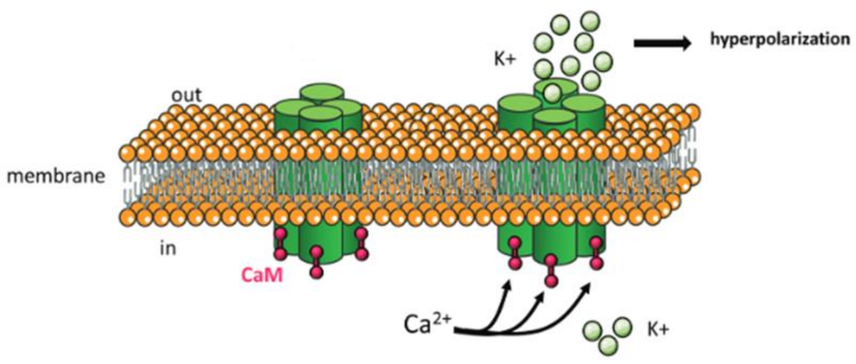
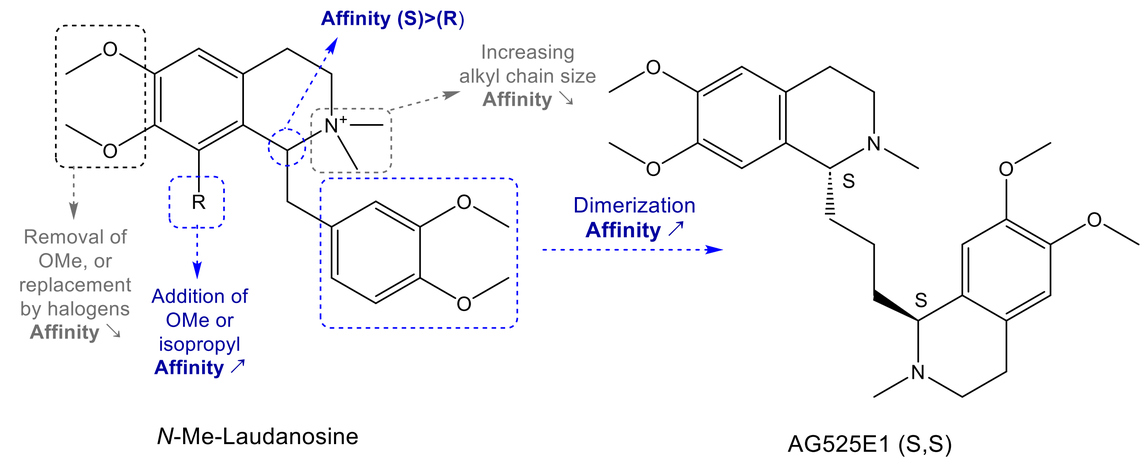
Introduction

SK (KCa2.1-3) channels are small-conductance 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].



Girault, A. et al, *Curr Med Chem* **2012**, *19* (5), 697–713

Background

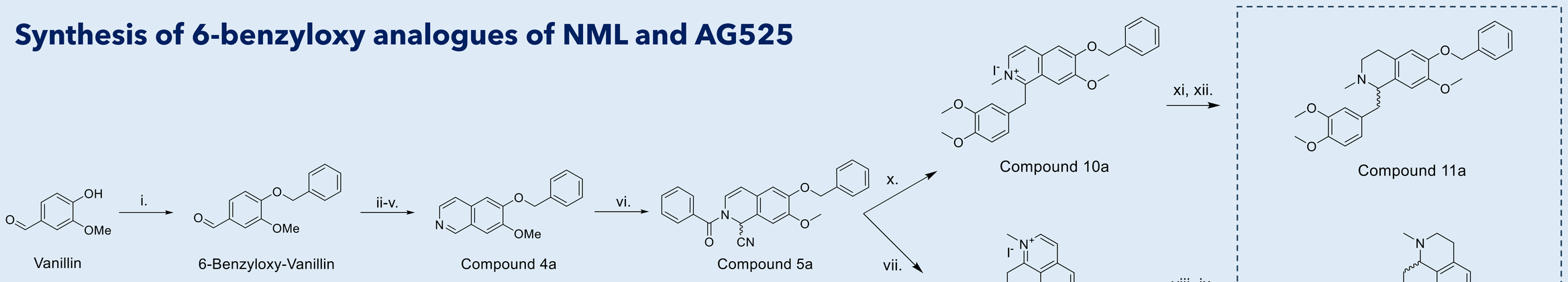


***N*-Methyl-Laudanosine (NML) has been discovered by our group to be a blocker of SK channels with a micromolar inhibition constant (Ki)[4]. During medicinal chemistry studies on this product, the dimerization of the 6,7-dimethoxy-tetrahydroisoquinoline ring has resulted in the synthesis of AG525 stereoisomers. The first eluted stereoisomer in preparative chiral HPLC, AG525E1 (S,S), has shown great promise as an SK channel blocker, particularly for targeting central nervous system SK channels[5].**

Aim of the work

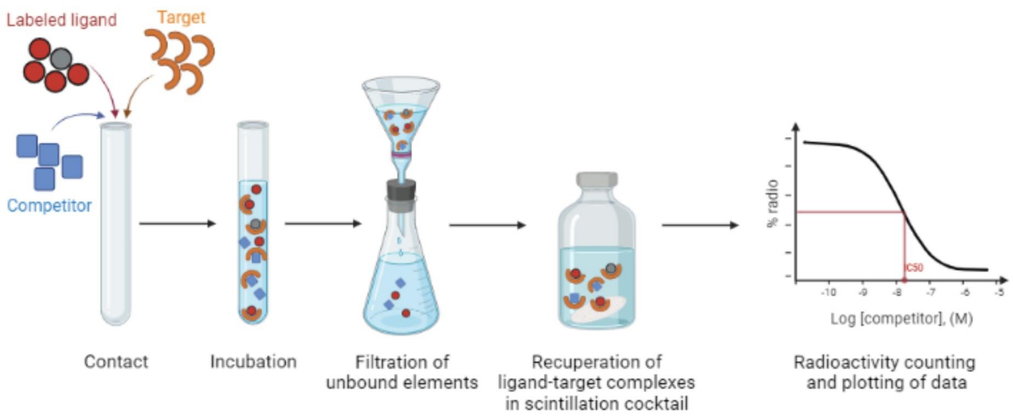
Docking models for these alkaloids showed that addition of an aromatic group in one of the positions 6,7, or 8 of the tetrahydroisoquinoline ring could be of interest to increase the affinity of these compounds for the target (details regarding models are confidential). This work aims to create analogs of NML and AG525 by replacing one of the methoxy groups by a benzyloxy group to evaluate the potential of these compounds as ligands for SK channels.

Synthesis of 6-benzyloxy analogues of NML and AG525



i. (bromomethyl)benzene, acetonitrile, K_2CO_3, reflux 4h, R=84%. ii. 2,2-dimethoxyethan-1-amine, toluene, reflux, 3h R=84% (crude). iii. $NaBH_4$, methanol, RT, 2h, R=81% (crude) . iv. Tosylchloride, CH_2Cl_2, $N(Et)_3$, RT, 1h, R=84% (crude). v. HCl 6N, 1,4-dioxanne, reflux, 2h, R=66%. vi. Me_3SiCN, Benzyle chloride, $AlCl_3$, CH_2Cl_2, reflux, 2h, R=79%. vii. 1) 1,3-diiodopropane, NaH, DMF, 1h. 2) NaOH, EtOH/H_2O, 1h, R=68%. viii. CH_3I, DMF, 80°C, 2h, R=63%. ix. $NaBH_4$, DMF, RT, 2h, R=88%. x. 1) 4-(chloromethyl)-1,2-dimethoxybenzene, NaH, DMF, 1h 2) NaOH, EtOH/H_2O, R=84%. xi. CH_3I, ACN, reflux, 2h, R=85%. xii. $NaBH_4$, MeOH, RT, R=91%. xiii. Semi-preparative HPLC, Chiralpak® IC (250x20mm, 5µm), ACN + 0,05% DEA, 10mL/min. λ= 280 nm. xiv. Semi-preparative HPLC, Lux® Cellulose-4 (250x30mm, 5µm), IPA/Hexanes 3/7 + 0,05% DEA, 40mL/min. λ=230 nm.

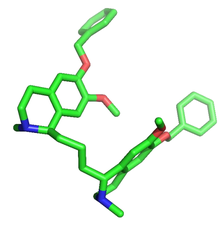
In vitro binding assay



	% [I^{125}]-Apamin displaced on hSK2		% [I^{125}]-Apamin displaced on hSK3	
	1µM	10µM	1µM	10µM
7a	56,6	97,2	70,0	98,6
8a E1 (S,S)	34,0	93,6	64,5	98,2
8a E2 (R,R)	12,8	86,8	25,1	94,2
8a meso (S,R)	7,5	75,5	12,2	90,0
10 a	1,0	36,5	13,9	66,8
11a E1 (S)	3,4	17,6	4,36	37,5
AG525E1 (S,S)	38,4	87,3	57,4	92,2

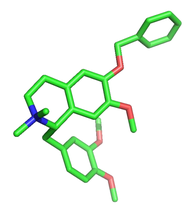
xiii. xiv. **Chiral resolution, Methylation (11a) X-ray crystallography**

Compound 8aE2



↪ E2=(R,R), E1=(S,S) and meso=(S,R)

Compound N-Me-11a E1



↪ E1=(S), E2=(R)

Absolute configuration was determined using X-ray crystallography, structures are shown here above.

Discussion and perspectives

Interestingly, in preliminary experiments, (S,S)-1,3-bis(6-(benzyloxy)-7-(methoxy)-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)propane (8aE1) displaced 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). Also, the effect of stereochemistry on the biological activity seems to be important, as it was observed on AG525 stereoisomers.

To further investigate the effect of a benzyloxy substituent on the biological properties of NML and AG525, the other benzyloxy analogues will be synthetized 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.

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