SYNTHESIS AND RADIOLIGAND BINDING ASSAYS OF 6, 7 OR 8 BENZYLOXY ANALOGS OF 1-(3,4-DIMETHOXYBENZYL) - 6,7-DIMETHOXY-2-METHYLISOQUINOLINIUM AND 1,1'-(PROPANE-1,3-DIYL)BIS(6,7-DIMETHOXY-2-METHYLISOQUINOLINIUM)

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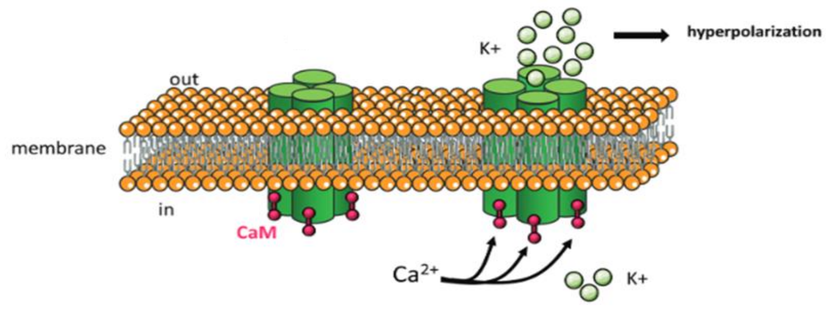
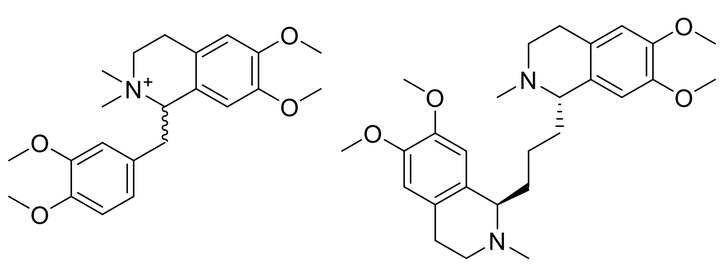
Introduction

Small-conductance calcium-activated potassium channels (SK channels) open in response to an intracellular calcium increase and are voltage-insensitive. Three subtypes are expressed in humans, namely SK1, SK2, and SK3. These channels contribute to medium-duration afterhyperpolarization of the neuronal membrane and the repolarization of the action potential in atrial cardiomyocytes[1]. Blocking these channels could be beneficial in treating various pathologies, such as depression or atrial fibrillation[2,3].



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

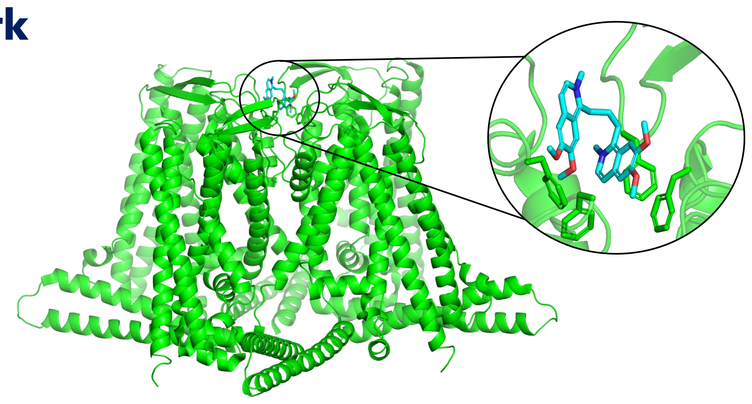
Background



(+/-)-*N*-Me-Laudanosine AG525E1

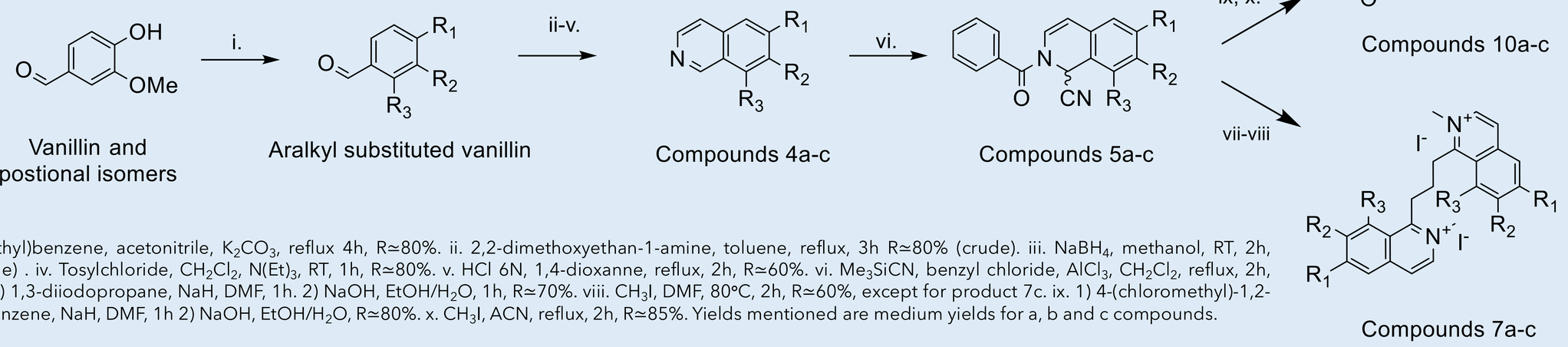
N-Methyl-Laudanosine (NML) and AG525E1(*S,S*) have been identified as SK channel ligands with micromolar and sub-micromolar inhibition constants (Ki), respectively[4-5]. Previous studies also showed that their isoquinolinium precursors are biologically active against SK channels[4-6]. As achiral and synthetically accessible intermediates, these precursors offer a valuable platform to evaluate the impact of modifications on *in vitro* biological properties prior to selecting candidates for the synthesis of chirally pure *N,N*-dimethyl-1,2,3,4-tetrahydroisoquinoline analogues.

Aim of the work



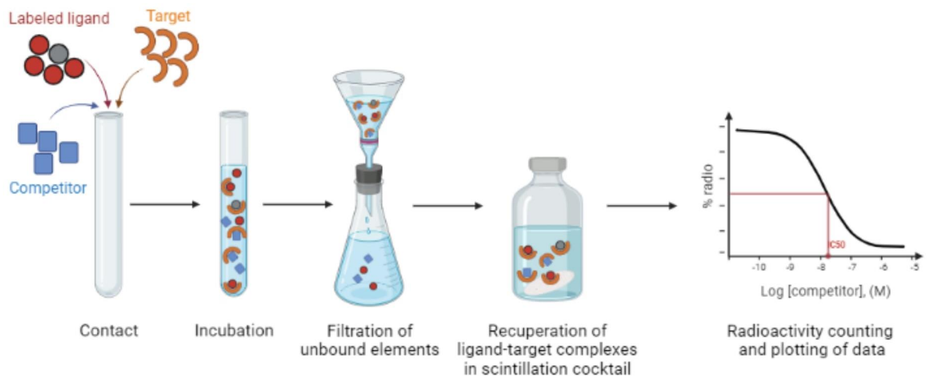
Recent Cryo-EM structures[7] of the SK2 channel revealed phenylalanine residues near the binding site of AG525E1 and NML isoquinolinium precursors. Docking studies indicated that benzyloxy substitutions could enhance binding via π-stacking with these residues. Accordingly, 6-, 7-, and 8-benzyloxy isoquinolinium analogs were synthesized. Depending on their activity, further synthesis of chiral *N,N*-dimethylated tetrahydroisoquinoline (THIQ) derivatives may follow. This stepwise approach allows early SAR evaluation before complex chiral resolution.

Synthesis of 6,7 or 8-benzyloxy analogs of Isoquinoliniums and bis-Isoquinoliniums

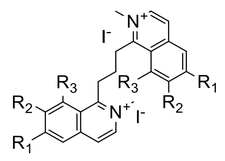
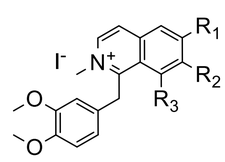


i. (bromomethyl)benzene, acetonitrile, K_2CO_3, reflux 4h, R≃80%. ii. 2,2-dimethoxyethan-1-amine, toluene, reflux, 3h R≃80% (crude). iii. $NaBH_4$, methanol, RT, 2h, R≃80% (crude) . iv. Tosylchloride, CH_2Cl_2, $N(Et)_3$, RT, 1h, R≃80%. v. HCl 6N, 1,4-dioxanne, reflux, 2h, R≃60%. vi. Me_3SiCN, benzyl chloride, $AlCl_3$, CH_2Cl_2, reflux, 2h, R≃75%. vii. 1) 1,3-diiodopropane, NaH, DMF, 1h. 2) NaOH, EtOH/H_2O, 1h, R≃70%. viii. CH_3I, DMF, 80°C, 2h, R≃60%, except for product 7c. ix. 1) 4-(chloromethyl)-1,2-dimethoxybenzene, NaH, DMF, 1h 2) NaOH, EtOH/H_2O, R≃80%. x. CH_3I, ACN, reflux, 2h, R≃85%. Yields mentioned are medium yields for a, b and c compounds.

In vitro binding assay protocol and results



Affinities for hSK2/hSK3 channels were assessed via in vitro [^{125}I]-apamin binding competition assay. Initial screening (10^{-5} and 10^{-6} M) identified active compounds, followed by complete competition assays (8 concentrations, n=3). Non-specific binding was defined with apamin. Radioactivity was measured by liquid scintillation (Ecolite Plus, TriCarb 2910).

	Structure	R_1	R_2	R_3	K_i (µM) ± SD SK2	K_i (µM) ± SD SK3	LE*[2]
Ref		OMe	OMe	H	0,6 ± 0,2[8]	0,21 ± 0,06[8]	0,27
7a		OBn	OMe	H	0,16 ± 0,05	0,33 ± 0,05	0,20
7b		OMe	OBn	H	0,12 ± 0,02	0,20 ± 0,03	0,21
Ref		OMe	OMe	H	/	/	/
10a		OBn	OMe	H	3,61 ± 0,68	4,34 ± 0,65	0,23
10b		OMe	OBn	H	3,61 ± 0,90	9,81 ± 1,23	0,23
10c		H	OMe	OBn	2,27 ± 0,45	3,16 ± 0,05	0,23

Discussion and perspectives

Interestingly, introducing a benzyloxy group at positions 6, 7, or 8 did not affect the affinity for the target significantly. While this modification preserves binding, the associated drop in ligand efficiency limits its value for further optimization. Nevertheless, the maintained affinity leaves room for exploring alternative substituents with distinct steric, electronic, or polarity profiles in position 6, 7 or 8 to improve binding efficiency on human SK channels.

*[1] Docking studies were performed using Autodock Vina 1.2.5. Ligands were drawn and exported to mol2 format prior to docking using Marvin® from ChemAxon®. Binding pose was shown using PyMol® 3.1

*[2] Ligand efficiencies (LE) were nearly equal on both subtypes of channels. For this reason, only one value is reported on the poster.

References : 1) Brown, B. M et al, Annu Rev Pharmacol Toxicol **2020**, 60, 219–240. 2) Rouchet, N et al, Eur J Neurosci **2008**, 28, 1108–1115 3) Diness, J. G et al, Circ Arrhythm Electrophysiol **2010**, 3, 380–390. 4) Graulich et al. Bioorg Med Chem **2005**, 13, 1201–1209, 5) Graulich et al. Bioorg Med Chem Lett **2008**, 18, 3440–3445, 6) Graulich et al. J Med Chem **2007**, 50, 5070–5075. 7) Nam, YW et al.. Nat Commun **2025**, 16, 3690. 8) Badarau et al, Bioorg Med Chem **2011**, 21, 6756-6759.

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