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Chemical modifications of the *N*-methyl-laudanosine scaffold point to new directions for SK channels exploration

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ABSTRACT

An asparagine or a histidine are present in a similar position in the outer pore region of SK2 and SK3 channels, respectively. Therefore, this structural difference was targeted in order to develop selective blockers of SK channel subtypes. Following docking investigations, based on theoretical models of truncated SK2 and SK3 channels, the benzyl side chain of *N*-methyl-laudanosine (NML) was functionalized in order to target this specific amino-acid residues. Chiral butanamide and benzyloxy analogues were prepared, resolved and tested for their affinity for SK2 and SK3 channels. Isoquinolinium (NMIQ) derivatives have a higher affinity for both SK channel subtypes than the corresponding derivative with no functionalized side chain. This trend was observed also for the 1,2,3,4-tetrahydroisoquinoline (THIQ) analogues. A benzyloxy functionalized NML enantiomer has a higher affinity than NML stereoisomers. Otherwise, the conserved affinity of these analogues led to the opportunity to further investigate in terms of possible labeling for in vivo investigations of the role of SK channels.

Small conductance calcium-activated potassium channels or SK ($K_{Ca}2$) channels are interesting and challenging targets in terms of medicinal chemistry developments. Three subtypes have been detected and cloned[1] and are present both in the brain and the periphery.[1,2] By modulating the activity of neurons (firing rate, firing pattern),[3] blockers appear interesting for the treatment of several CNS disorders including cognitive dysfunction, neuronal hyperexcitability or dopamine related disorders.[4] Cardiovascular disorders or cancer are also potential applications for the SK channel modulators.[5–7] Until now, only some peptidic compounds such as Lei-dab[8] or tamapin,[9] have more or less selectivity for SK2 channels. Non-peptidic molecules with high affinity have been obtained[10–13] (Fig. 1) but none possesses a high selectivity for SK2 or SK3 channel subtypes. Indeed, the nanomolar bis-quinolinium cyclophane, UCL1684,[13] is reported to be 34 times selective for SK2 versus SK3,[14] while in our hands, the corresponding ratio is close to 5 as affinity for SK2 and SK3 channels are 0.21 nM and 1.1 nM, respectively. *N*-Methyl-laudanosine (NML), a *N*-methyl-1,2,3,4-tetrahydroisoquinoline (NMTHIQ) derivative, has a

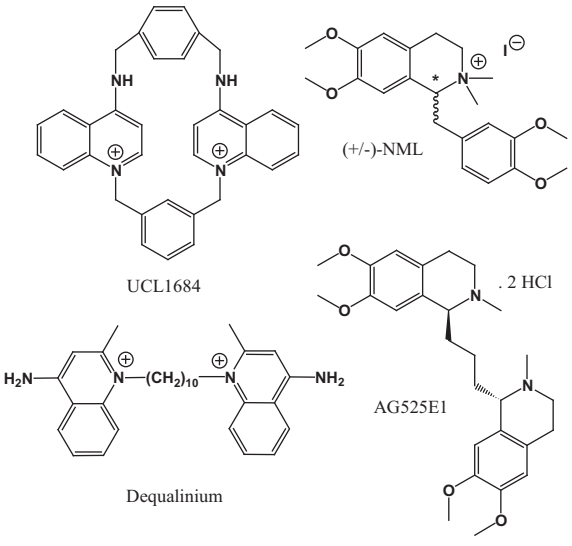

Figure 1. Chemical structure of some SK channel blockers interacting at the apamine binding site.

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micromolar affinity for both SK2 and SK3 channels. It was shown to be a useful tool for studying in vitro neurophysiology of SK channels[15] as a quick reversibility is observed following local application.[16] By comparison, apamin, or other high affinity compounds, block the current for a long time because of their small k_{-1}, which makes their effect essentially irreversible in many systems.[16] It was also reported as radiotracer for biomedical imaging technique positron emission tomography (PET) to image SK_{Ca} channels in the studies of brain, heart and cancer diseases.[17]

Beside SK channel blockers interacting either with the apamine binding site, or TEA binding site, molecules modulating the SK channel gating properties have been described either as positive modulators such as CyPPA, EBIO and NS309 (see Ref. 18 and references associated) or as negative modulators such as NS8593[19] and its related analogues[20] (Fig. 2).

In the present draft, we describe to our knowledge the first report of a rational design based on the structure of the target. Indeed, in our research program, we have reported additional informations that indicate that apamin does not act as a pore blocker, contrary to TEA but more likely binds in the outer pore region according to recent reports.[21–25] The sequence of SK2 and SK3 channels presents two different residues in this region. In the SK2 protein, an asparagine (N) is present in position 368 while in the SK3 subtype, the amino acid is replaced by a histidine (H) in the equivalent position 522 (Fig. 3). This could be a potential opportunity for developing more selective non-peptidic molecules. Previous docking studies of apamin and NML revealed that NML occupies a part of the binding site of apamin.[26] An example of docking results of *R*-enantiomer is illustrated in Figure 3. The binding site of NML is very close to the discriminant residues N 368 for SK2 or H 522 for SK3 (Fig. 3). Therefore, we intended to modulate NML in order to selectively target one SK channel subtype. Additional docking studies allowed identifying different functional groups able to specifically interact with the discriminant residues. Thus, according to the best scoring results, for a putative compound interacting with the N residue, an amide group was chosen while for the compound interacting with the H residue, a phenyl moiety because of its potential ability to interact with the imidazole moiety of H 522 was used.

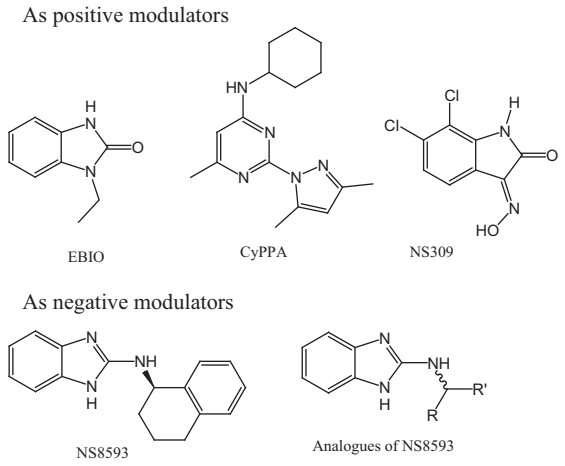


Figure 2. Chemical structure of some molecules modulating SK channel gating properties.

As an exploratory investigation, two groups of molecules were synthesized with different intermediates (NMIQ, THIQ and NMTHIQ) to be tested for their affinity for SK channels (Fig. 4). The racemic mixtures of THIQ analogues were resolved by chiral HPLC. These compounds were tested for their capacity to compete with iodinated apamin on cloned SK2 and SK3 channels according to the procedure previously reported.[24–27]

Targeted compounds were prepared according to previously used synthetic pathways[27,28] that involved Reissert's compounds as key intermediates. Both side chains were obtained from vanilline following two different chemical pathways (Scheme 1A and B).

In order to prepare the amide side chain, vanilline reacted by an O-alkylation with ethyl 4-bromobutanoate (Scheme 1A). The aldehyde group was then reduced with sodium borohydride. The halogenomethyl analogue (**1**) was prepared by the treatment of the corresponding alcohol with thionyl chloride and used without further purification.

For the preparation of the benzyloxy side chain, vanilline was O-alkylated on the phenol group using benzyl chloride (Scheme 1B). The aldehyde moiety was then reduced with sodium borohydride. The halogenomethyl analogue (**2**) was prepared by reacting the corresponding alcohol with thionyl chloride and used without further purification.

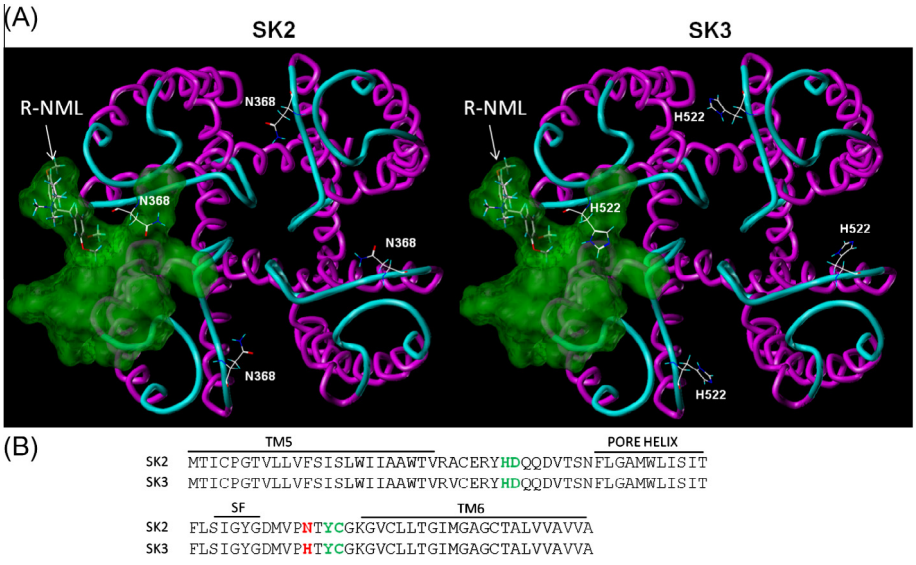


Figure 3. (A) Extracellular view of docking results of *R*-NML into the pore region of SK2 and SK3 channels. *R*-NML is shown to interact with a part of the binding site of apamin highlighted green. (B) Sequence alignment of the pore region of SK2 and SK3 channels. The discriminant residues N368 and H522 are highlighted red. The residues involved in the interaction with NML are highlighted green.

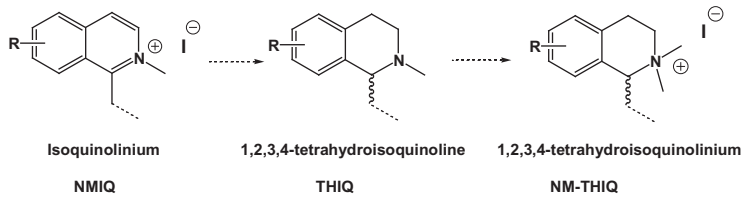
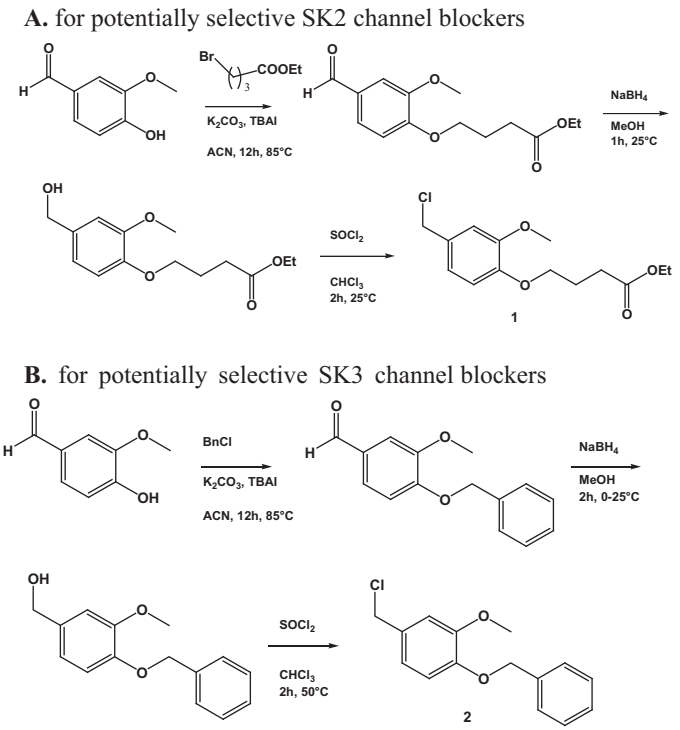


Figure 4. Chemical entities to target SK channels from the isoquinoline scaffold.

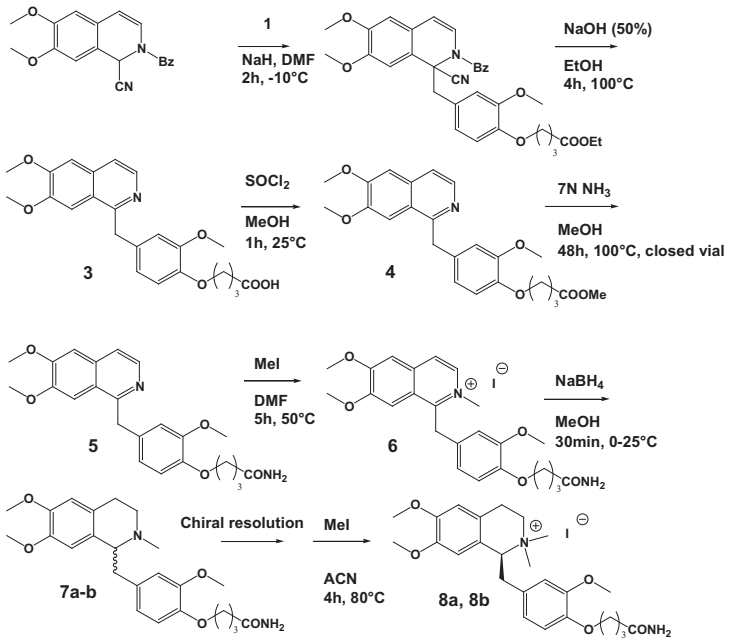


Scheme 1. Synthesis of intermediates in order to functionalize side chains.

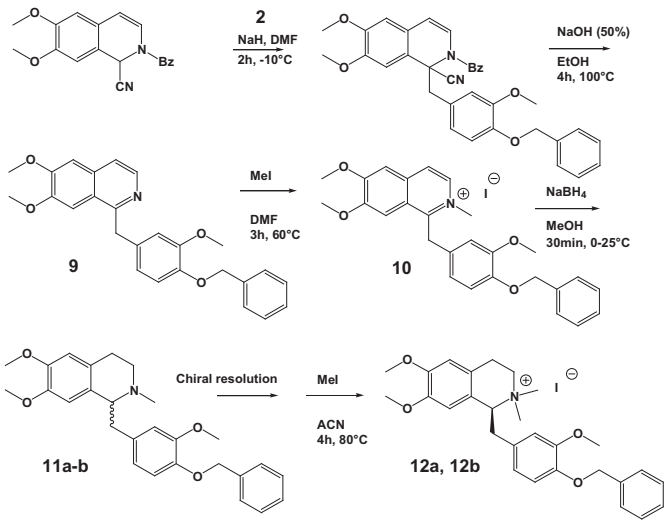
The previously obtained chlorides were used for the alkylation of the Reissert's derivatives in anhydrous DMF (Schemes 2 and 3). Then, the alkylated products were subsequently hydrolyzed in a 50% aqueous NaOH/EtOH solution. The aromatic analogue were methylated with methyl iodide before reducing the isoquinolinium (NMIQ) derivative to a 1,2,3,4-tetrahydroisoquinoline (THIQ) by using sodium borohydride. For the amide side chain (Scheme 2), the acid derivative was firstly esterified to synthesize the amide group in a second time before the treatment with methyl iodide. The rest of the sequence was the same than that for the benzyloxy analogue (Scheme 3).

The racemic mixtures of THIQ derivatives (**7a**–**b**, **11a**–**b**) were resolved by a preparative HPLC method with a chiral stationary phase to get both enantiomers. Due to limited solubility, the amide analogue was resolved in more polar conditions than those previously used.[27] Subsequent treatment of THIQ enantiomers (**7a**, **7b**, **11a**, **11b**) with methyl iodide gave the NMTHIQ derivatives (**8a**, **8b**, **12a**, **12b**). The absolute configuration was determined for one enantiomer of each series by X-ray analysis. For compound **8a** and **12b**, the crystal structure has been deposited at the Cambridge Crystallographic Data Centre and the CCDC deposition number is 979360 and 979359, respectively.

Regarding the affinity for SK2 and SK3 channels, in the series of NML, experimental data are reported in Table 1. Some data were reported previously in the literature but they concern affinity on rat brain preparations.[28–31] The NMIQ analogue, *N*-methyl-papaverine, and the THIQ enantiomers (laudanosine enantiomers) present a low affinity as determined during the screening evaluation. Among this series of compounds, the enantiomers of NML have

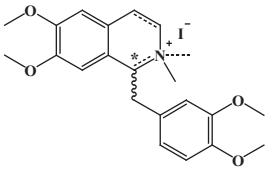


Scheme 2. Synthesis of target compounds aimed at interacting with SK2 channels.



Scheme 3. Synthesis of target compounds aimed at interacting with SK3 channels.

Table 1
Affinity of NML derivatives for SK2 and SK3 channels (K_i in μM or % of displacement at 10 μM)



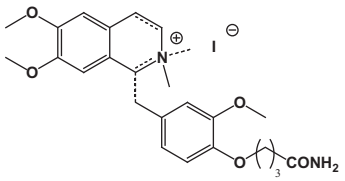
Compounds	Chemical entity	SK2	SK3
N-Methylpapaverine	NMIQ	10%	NT
(*S*)-Laudanosine	THIQ	13%	35%
(*R*)-Laudanosine	THIQ	−2%	35%
(*S*)-NML	NMTHIQ	0.941 ± 0.169	1.886 ± 0.344
(*R*)-NML	NMTHIQ	1.391 ± 0.120	1.715 ± 0.125

the highest affinity in the μM range. The *S*-enantiomer shows a slightly higher affinity for SK2 than SK3 channels.

In the series of butanamide analogues (Table 2), the NMIQ analogue **6** has an affinity close to 10 μM thus higher than that observed for the corresponding analogue in the NML series. No SK2 versus SK3 selectivity is observed. For the THIQ enantiomers **7a** and **7b**, the affinity of both compounds is lower than that of the NMIQ analogue **6** and they interact almost equally on both channel subtypes. For the NMTHIQ enantiomers **8a** and **8b**, the affinity is significantly increased in comparison with the NMIQ analogue **6**. Both enantiomers interact almost similarly on both channel subtypes except **8a** which have a slightly higher affinity for SK2 channels. The affinity of the enantiomers is quite similar to that of NML enantiomers.

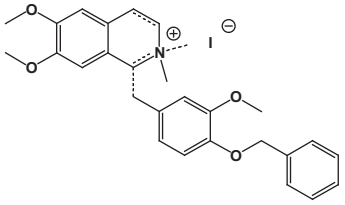
In the series of benzyloxy analogues (Table 3), the affinity of the NMIQ analogue **10** is ~7.9 and 5.9 μM for SK2 and SK3 channels. This is higher than the value observed for the corresponding analogue in the NML series and also slightly higher than that of compound **6**. For the THIQ enantiomers **11a** and **11b**, the affinity is lower than that of NMIQ analogue **10** and in a similar range than enantiomers **7a** and **7b**. For the NMTHIQ enantiomers **12a** and **12b**, the affinity is significantly increased in comparison with the NMIQ analogue **10**. Both enantiomers interact almost similarly on both channel subtypes except **12b** which has a slightly higher affinity for SK2 channels even higher than that of the corresponding NML enantiomer.

Table 2
Affinity of butanamideoxy derivatives for SK2 and SK3 channels (K_i in μM; mean ± SD)



Compounds	Chemical entity	SK2	SK3
6	NMIQ	11.515 ± 5.872	11.167 ± 1.993
7a	THIQ E1	26.843 ± 4.017	23.300 ± 16.994
7b	THIQ E2	32.690 ± 9.014	20.442 ± 16.552
8a	NMTHIQ E1	1.044 ± 0.154	1.726 ± 0.182
8b	NMTHIQ E2	1.115 ± 0.138	1.210 ± 0.045

Table 3
Affinity of benzyloxy derivatives for SK2 and SK3 channels (K_i in μM; mean ± SD)



Compounds	Chemical entity	SK2	SK3
10	NMIQ	7.905 ± 0.780	5.824 ± 2.768
11a	THIQ E1	19.957 ± 9.845	29.118 ± 5.046
11b	THIQ E2	18.630 ± 1.998	15.746 ± 8.971
12a	NMTHIQ E1	0.897 ± 0.148	0.967 ± 0.100
12b	NMTHIQ E2	0.772 ± 0.022	1.426 ± 0.396

Following these chemical modifications of NML structure, different informations can be put forward. For the NMIQ analogues (**6**, **10**), it appears that the presence of both functionalized arms has a favourable impact in terms of affinity for SK channels. The THIQ stereoisomers (**7a**–**b**, **11a**–**b**) have a weak affinity that limits further experiments for exploring CNS targets. Among the NMTHIQ enantiomers (**8a**, **8b**, **12a**, **12b**), globally the presence of the additional side chain does not modify significantly the affinity for both channels in comparison with enantiomers of NML except the benzyloxy enantiomer (**12b**) which present a higher affinity for SK2. In previous works,[29] we had shown the superiority in terms of interaction of 6,7-dimethoxy analogues in comparison with the unsubstituted derivatives and therefore, we were focused on these types of molecules for further developments. In this work, the unsubstituted analogue of **10** was prepared and tested. This compound has an affinity of ~4.7 and 3.1 μM for SK2 and SK3 channels, respectively. This is interesting and could mean that a possible competition between the 6,7-dimethoxy moiety and the functionalized arm would appear during the interaction with the target. In the unsubstituted analogue, the absence of 6,7-dimethoxy would be compensated by the interaction of the arm and the protein. Currently, these results are not explained by further docking experiments. This point was also recently reported in a separate work[32] and this might be related to the limited size of the pore region examined in the theoretical model. In silico models will be strongly improved when more complete structure of the target will be available. Indeed, an X-ray crystal structure of the channel or at least a larger part of the pore region should facilitate the design of new molecules and this represents a fascinating challenge.

Thus, selective SK channel modulators remain a challenge. Otherwise, the current chemical modifications have permitted to keep the affinity for SK channels and in some cases to increase it. As a consequence, this work raises the opportunity to further functionalized NML structure to prepare labeled compounds such as fluorescent probes for in vitro cellular investigations of the role of SK channels.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.083.

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