Synthesis and Radioligand Binding Studies of Methoxylated 1,2,3,4-Tetrahydroisoquinolinium Derivatives as Ligands of the Apamin-Sensitive Ca^{2+}-Activated K^+ Channels

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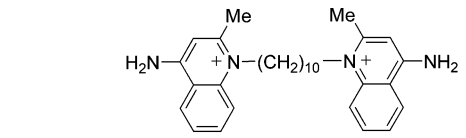
Several methoxylated 1,2,3,4-tetrahydroisoquinoliniums derived from *N*-methyl-laudanosine and *N*-methyl-noscapine were synthesized and evaluated for their affinity for apamin-sensitive binding sites. The quaternary ammonium derivatives have a higher affinity with regard to the tertiary amines. 6,7-Dimethoxy analogues possess a higher affinity than the 6,8- and 7,8-dimethoxy isomers. A 3,4-dimethoxybenzyl or a 2-naphthylmethyl moiety in C-1 position are more favorable than a 3,4-dimethoxyphenethyl group. Smaller groups such as propyl or isobutyl are unfavorable. In 6,7-dimethoxy analogues, increasing the size and lipophilicity with a naphthyl group in the C-1 position leads to a slight increase of affinity, while the same group in the 6,7,8-trimethoxy series is less favorable. The 6,7,8-trimethoxy derivative **3f** is the first tertiary amine in the series to possess an affinity close to that of *N*-methyl-laudanosine and *N*-methyl-noscapine. Moreover, electrophysiological studies show that the most effective compound **4f** blocks the apamin-sensitive afterhyperpolarization in rat dopaminergic neurons.

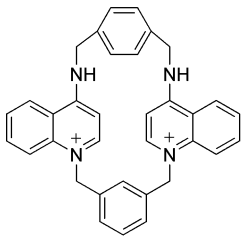
Introduction

Ion channels have been shown to play a role in many functions, including neuronal communication and behavioral plasticity, secretion, and cell proliferation. Therefore, ion channels represent promising targets for the development of new CNS drugs.[1] In addition to their role in regulating cell excitability, the channels themselves can be modulated in a cell-specific manner through second messengers, hormones, and neurotransmitters. Calcium-activated potassium channels are separated in three families: BK, IK, and SK, according to their large, intermediate, and small conductance, respectively.[2,3] Small conductance Ca^{2+}-activated K^+ (SK) channels are voltage-insensitive and are activated by an increase in the intracellular calcium concentration. They play an important role in modulating the firing rate and the firing pattern of several types of neurons, because their activation induces a prolonged postspike afterhyperpolarization (AHP).[4−6] Three SK channel subunits have been identified by DNA cloning, namely, SK1, SK2, and SK3.[4] The distribution of the SK channel subunits has been investigated in the rat by using in situ hybridization and immunohistochemistry. These experiments revealed that SK1 and SK2 subunits are mostly expressed in the cortex and hippocampus,[7] while SK3 channel expression is higher in subcortical areas, especially in the monoamine cell regions. According to the literature, SK channel modulation could have therapeutic effects in various diseases such as cognitive dysfunction,[8−12] neuronal hyperexcitability,[13] dopamine related disorders,[14−16] and depression.[11]

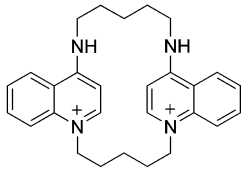
Nature is frequently the source of potent ionotropic agents. Indeed, venoms of arthropods or insects contain natural peptides, which are high affinity blockers of SK channels. So far, the most potent SK channel blocker is apamin, a toxin with an octadecapeptide structure isolated from honey bee (*Apis mellifera*) venom, which possesses an affinity in the picomolar range.[17] Leiurotoxin I, another peptide isolated from the scorpion *Androctonus mauretanicus mauretanicus*, blocks human SK2 and SK3, but not SK1 channels.[18] Recently isolated from the venom of the scorpion *Mesobuthus tamulus*, tamapin represents a novel, promising pharmacological tool, as it blocks SK2 channels ∼1750- and ∼70-fold more potently than SK1 and SK3 channels, respectively, making it the most potent and selective SK2 channel natural toxin characterized so far.[19] Besides these toxins, a small number of nonpeptidic blockers have been discovered. Dequalinium has an affinity of 245 nM for rat cortical apamin-sensitive sites.[20] Extensive structure−activity relationship studies on dequalinium (Figure 1) led to a family of dimeric compounds, including UCL1684 and UCL1848 (Figure 1), with a low nanomolar affinity.[21] On the other hand, *N*-methyl-bicuculline (Figure 2) was shown to potentiate burst firing in dopaminergic neurons by blocking the apamin-sensitive Ca^{2+}-activated K^+ current.[22,23] Unfortunately, this compound also possesses an unwanted $GABA_A$ receptor antagonist effect.



Dequalinium



UCL1684



UCL1848

Figure 1. Chemical structure of non-peptidic SK channel blockers.

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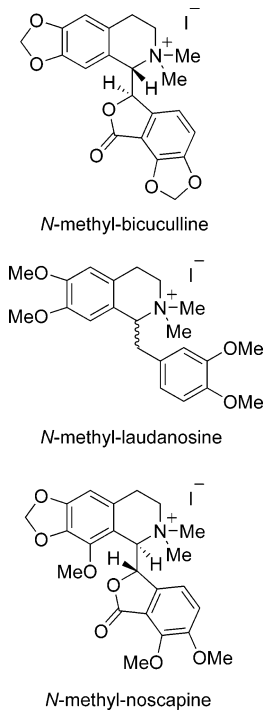


Figure 2. Chemical structure of benzyltetrahydroisoquinoline derivatives interacting with SK channels.

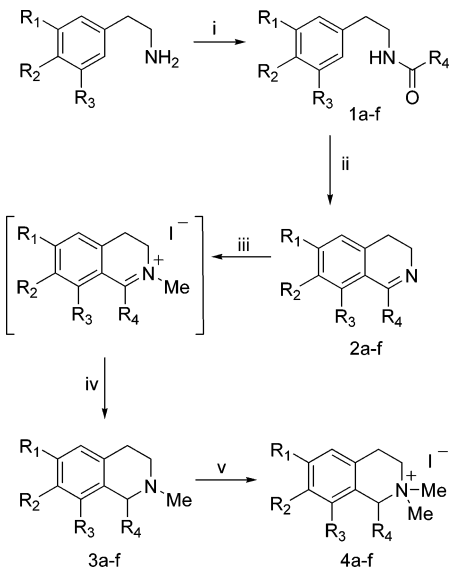
Therefore, different molecules structurally close to *N*-methyl-bicuculline were evaluated as potential specific SK channel blockers. A first series of studies started with *N*-methyl-laudanosine (NML;[24−26] Figure 2) and more recently with *N*-methyl-noscapine (NMN;[26,27] Figure 2). These two molecules block the AHP with a medium potency and are quickly reversible.[27] Different modifications of these compounds have been carried out, such as the size and the substitution of the side chain in the C-1 position,[25] the absence of substituents in the C-6 and C-7 positions,[25] the nature of the fourth group on the nitrogen,[25] and also the presence of a halogen, an alkyl, or a methoxy group in the C-8 position[26] and the presence of a halogen in the C-5 position.[26] Because NML is chiral, both enantiomers have been separated and tested.[25] As no difference was found between these two stereoisomers, all compounds were tested as racemate.

In the present paper, we focus on the synthesis and biological evaluation of new NML and NMN derivatives obtained by changing the position of the methoxy groups on the tetrahydroisoquinoline ring and the nature of the substituent in the C-1 position.

Chemistry

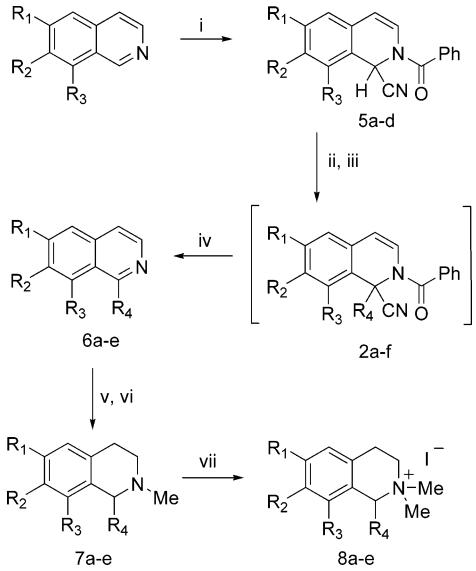
Two procedures are used depending on the benzene substitution of the tetrahydroisoquinoline ring. Indeed, some compounds are prepared from a Bischler−Napieralski procedure (Scheme 1). The appropriate acid anhydride or acid chloride, the latter being commercially available or directly prepared by reaction of the corresponding carboxylic acid with thionyl chloride and used without further purification, reacts with the adequate amine to obtain the amide (**1a**−**f**). In the next step, the amide is cyclodehydrated by heating to reflux with phosphorus pentoxide in toluene to afford the 3,4-dihydroisoquinoline derivative (**2a**−**f**).[28] Subsequently, the 3,4-dihydroisoquinoline is methylated by methyl iodide in refluxing MeCN. The resulting 2-methyl-3,4-dihydroisoquinolinium analogue is immediately reduced by sodium borohydride in MeOH to give the tertiary amine (**3a**−**f**). By methylation of the 1,2,3,4-tetrahydroisoquinoline derivative (**3a**−**f**) with methyl iodide, the 2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium derivative is obtained (**4a**−**f**).

Scheme 1. Synthesis of 1-Substituted Di- and Trimethoxylated 2,2-Dimethyl-1,2,3,4-tetrahydroisoquinolinium Derivatives by the Bischler−Napieralski Procedure[a]



[a] Key: (i) R_4COCl, NEt_3, MeCOOEt, rt; (ii) P_2O_5, ArMe, reflux; (iii) MeI, MeCN, reflux; (iv) $NaBH_4$, MeOH, rt; (v) MeI, MeCN, reflux. R_1, R_2, R_3 = H or OMe. R_4 = *n*-propyl (a), isobutyl (b), 2-(3,4-dimethoxyphenyl)-ethyl (c), 3,4-dimethoxybenzyl (d,f), 2-naphthylmethyl (e).

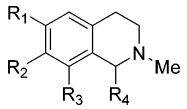
Scheme 2. Synthesis of 1-Substituted Di- and Trimethoxylated 2,2-Dimethyl-1,2,3,4-tetrahydroisoquinolinium Derivatives from Reissert Compounds[a]



[a] Key : (i) $(Me)_3SiCN$, $AlCl_3$, BzCl; (ii) NaH, DMF, −10 °C; (iii) R_4X, DMF, −10 °C; (iv) 50% NaOH, EtOH−H_2O, reflux; (v) MeI, MeCN, reflux; (vi) $NaBH_4$, MeOH, rt; (vii) MeI, MeCN, reflux. R_1, R_2, R_3 = H or OMe. R_4X = 3,4-dimethoxybenzyl chloride (b), 2-naphthylmethyl bromide (a,c,d,e).

The other procedure follows Reissert compound pathway (Scheme 2) for the alkylation of the isoquinoline nucleus[29] in the C-1 position. These intermediates are usually synthesized from the appropriate nitrogen heterocycles and acyl chlorides in the presence of a cyanide source.[30] Indeed, the isoquinolines react with benzoyl chloride, and the resulting acyliminium undergoes a nucleophilic addition with cyanide to afford the Reissert compounds (**5a**−**d**).[31] This reaction is carried out with

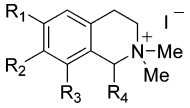
Table 1. Tertiary Analogues (**3a**−**f**, **7a**−**e**): Physical Data and Preliminary Screening Results for Binding on Rat Cortical Apamin-Sensitive Sites



No	R_1	R_2	R_3	R_4	yield (%)	mp (°C)	formula[a]	%[b]
3a	OMe	OMe	H	*n*-C_3H_7	90	183−184	$C_{15}H_{23}NO_2$·HCl	0
3b	OMe	OMe	H	*i*-C_4H_9	94	148−150	$C_{16}H_{25}NO_2$·HCl·0.5H_2O	0
3c	OMe	OMe	H	3,4-$(MeO)_2C_6H_3CH_2CH_2$	85	74−76	$C_{22}H_{29}NO_4$	11
3d	OMe	H	OMe	3,4-$(MeO)_2C_6H_3CH_2$	65	99−101	$C_{21}H_{27}NO_4$·$C_2H_2O_4$·0.75H_2O	0
3e	OMe	H	OMe	2-$(C_{10}H_7)CH_2$	81	137−138	$C_{23}H_{25}NO_2$	0
3f	OMe	OMe	OMe	3,4-$(MeO)_2C_6H_3CH_2$	75	94−95	$C_{22}H_{29}NO_5$·$C_4H_4O_4$·H_2O	27
7a	OMe	OMe	OMe	2-$(C_{10}H_7)CH_2$	27	86−88	$C_{24}H_{27}NO_3$	0
7b	H	OMe	OMe	3,4-$(MeO)_2C_6H_3CH_2$	59	159−161	$C_{21}H_{27}NO_4$·HCl	3
7c	H	OMe	OMe	2-$(C_{10}H_7)CH_2$	30	108−110	$C_{23}H_{25}NO_2$·$C_2H_2O_4$	0
7d	OMe	OMe	H	2-$(C_{10}H_7)CH_2$	47	212−214	$C_{23}H_{25}NO_2$·HCl	6
7e	H	H	H	2-$(C_{10}H_7)CH_2$	53	149−151	$C_{21}H_{21}N$·HCl·0.75H_2O	0

[a] According to CHN analysis. [b] The % of ^{125}I-apamin displaced at 10 μM.

Table 2. Quaternary Analogues (**4a**−**f**, **8a**−**e**): Physical Data and Preliminary Screening Results for Binding on Rat Cortical Apamin-Sensitive Sites



No	R_1	R_2	R_3	R_4	yield (%)	mp (°C)	formula[a]	%[b]
4a	OMe	OMe	H	*n*-C_3H_7	85	228−229	$C_{16}H_{26}NO_2I$	6
4b	OMe	OMe	H	*i*-C_4H_9	93	189−191	$C_{17}H_{28}NO_2I$	0
4c	OMe	OMe	H	3,4-$(MeO)_2C_6H_3CH_2CH_2$	78	165−166	$C_{23}H_{32}NO_4I$·0.5H_2O	32
4d	OMe	H	OMe	3,4-$(MeO)_2C_6H_3CH_2$	77	151−152	$C_{22}H_{30}NO_4I$·0.5H_2O	20
4e	OMe	H	OMe	2-$(C_{10}H_7)CH_2$	83	140−142	$C_{24}H_{28}NO_2I$·0.25H_2O	2
4f	OMe	OMe	OMe	3,4-$(MeO)_2C_6H_3CH_2$	88	103−104	$C_{23}H_{32}NO_5I$·0.25H_2O	72
8a	OMe	OMe	OMe	2-$(C_{10}H_7)CH_2$	57	133−135	$C_{25}H_{30}NO_3I$·0.5H_2O	53
8b	H	OMe	OMe	3,4-$(MeO)_2C_6H_3CH_2$	67	204−206	$C_{22}H_{30}NO_4I$	24
8c	H	OMe	OMe	2-$(C_{10}H_7)CH_2$	58	121−122	$C_{24}H_{28}NO_2I$·0.5H_2O	18
8d	OMe	OMe	H	2-$(C_{10}H_7)CH_2$	75	212−214	$C_{24}H_{28}NO_2I$·0.5H_2O	70
8e	H	H	H	2-$(C_{10}H_7)CH_2$	75	219−221	$C_{22}H_{24}NI$	1

[a] According to CHN analysis. [b] The % of ^{125}I-apamin displaced at 10 μM.

trimethylsilyl cyanide in anhydrous CH_2Cl_2 in good yields. All Reissert compounds (**5a**−**d**) are then deprotonated by sodium hydride in DMF. The resulting Reissert anions are alkylated by using 3,4-dimethoxybenzyl chloride or 2-(bromomethyl)-naphthalene. Then, the alkylated Reissert compounds are hydrolyzed to 1-(3,4-dimethoxybenzyl)-isoquinolines and 1-(2-naphthylmethyl)-isoquinolines (**6a**−**e**). Compounds **6a**−**e** are methylated by methyl iodide in refluxing MeCN. Due to their chemical lability, the resulting *N*-methylisoquinoliniums are directly reduced to *N*-methyl-1,2,3,4-tetrahydroisoquinolines (**7a**−**e**) by using an excess of sodium borohydride in MeOH. A further methylation by using methyl iodide in refluxing MeCN gives the quaternary ammoniums (**8a**−**e**).

Results

Biological data from binding experiments on rat cortical apamin-sensitive sites are summarized in Tables 1−3. A preliminary screening is performed at a concentration of 10 μM (Tables 1 and 2), then drugs that displace at least 25% of ^{125}I-apamin are further tested to determine their affinity (Table 3).

In our experimental conditions, ^{125}I-apamin has a K_d of 2.02 ± 0.31 pM, while the unlabeled apamin has an affinity of 3.8 ± 1.1 pM.

The tertiary compound **3f** displaces 27% of radioligand at 10 μM and has a K_i of 5.8 μM for the apamin-sensitive binding sites.

The quaternary ammonium compounds **4c**, **4f**, **8a**, and **8d** displace 32, 72, 53, and 70% of the radioligand at 10 μM, respectively. The affinities (K_i) for the apamin-sensitive binding sites of the rat cortex preparation are 5.4, 0.73, 2.2, and 0.91 μM for **4c**, **4f**, **8a**, and **8d**, respectively.

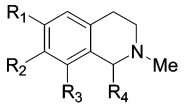
Compounds **3a**−**e**, **4a**, **4b**, **4d**, **4e**, **7a**−**e**, **8b**, **8c**, and **8e** have no significant affinity, because a negligible fraction of radioligand is displaced.

In electrophysiological experiments, compound **4f** blocks the apamin-sensitive AHP in dopaminergic neurons with an IC_{50} of 9.0 ± 0.7 μM (n = 3), while compound **3f** blocks 51.9 ± 1.2% of the AHP at 100 μM (n = 3).

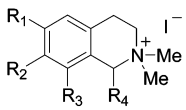
Discussion

The first benzylisoquinoline alkaloid reported to be active on apamin-sensitive binding sites was the *N*-methyl derivative of bicuculline (Figure 2). Unfortunately, this drug is not an optimal tool because it presents a $GABA_A$ receptor antagonist activity and a poor stability due to its lactone function in physiological conditions.[23] Then NML, a compound structurally related to the bicuculline template, was prepared and tested to determine its potency to block the apamin-sensitive AHP. This compound revealed an interesting binding profile[24] and a very quickly reversible blocking effect on the apamin-sensitive AHP, with no effect on $GABA_A$ transmission.[27] However, after extensive biological evaluation, a non-SK mediated effect on the serotonergic neurons of the dorsal raphe has been detected.[27] Structurally close to NML and *N*-methyl-bicuculline, NMN was also prepared and evaluated in in vitro binding and electrophysiological experiments. It was found that this drug has a

Table 3. Affinity of *N*-Methyl-laudanosine (NML), *N*-Methyl-noscapine (NMN), and Methoxylated Analogues for Binding on Rat Cortical Apamin-Sensitive Sites



3f



4e, 4f, 8a, 8d

compd	R_1	R_2	R_3	R_4	%[a]	$K_i \pm$ SD[b] (μM)
NML	OMe	OMe	H	3,4-$(MeO)_2C_6H_3CH_2$	57	1.3 ± 0.02
NMN					27	3.6 ± 1.3
3f	OMe	OMe	OMe	3,4-$(MeO)_2C_6H_3CH_2$	27	5.8 ± 0.4
4c	OMe	OMe	H	3,4-$(MeO)_2C_6H_3CH_2CH_2$	32	5.4 ± 0.6
4f	OMe	OMe	OMe	3,4-$(MeO)_2C_6H_3CH_2$	72	0.73 ± 0.11
8a	OMe	OMe	OMe	2-$(C_{10}H_7)CH_2$	53	2.2 ± 0.4
8d	OMe	OMe	H	2-$(C_{10}H_7)CH_2$	70	0.91 ± 0.11
apamin						3.8 ± 1.1 pM

[a] The % of ^{125}I-apamin displaced at 10 μM. [b] Only compounds displacing more than 25% of ^{125}I-apamin at 10 μM were further evaluated; $n = 3$ except for NML ($n = 8$).

quickly reversible effect on SK channels without affecting serotonergic neurons but possesses a lower affinity than NML.[27] Like NML, NMN is devoid of $GABA_A$ receptor antagonist effects.[27] From the structural point of view, the substitution of the isoquinoline nucleus of NMN differs from that of NML by a methoxy group in the C-8 position and by an unstable lactone in the side chain in the C-1 position (Figure 2). Following these observations, different compounds structurally close to the NML and NMN templates were synthesized and evaluated by radioligand binding studies to extend our medicinal chemistry program.[24−26]

In our binding conditions, binding parameters of ^{125}I-apamin and apamin are in the same range than those previously reported.[20,32]

In vitro binding results show that the quaternary compounds (**4a**−**f**, **8a**−**e**; Table 2) have a higher affinity than the tertiary derivatives (**3a**−**f**, **7a**−**e**; Table 1) for apamin-sensitive binding sites. However, the tertiary amine **3f** has an affinity of 5.8 μM (Table 3). This is the first basic compound in this series with a significant affinity for examined sites. Presently, all known SK channel blockers have at least one ionized function. Indeed, animal toxins like apamin, leiurotoxin, or tityus κ possess one or more arginine residues.[18,33−35] Synthetic blockers such as dequalinium and UCL compounds (Figure 1) possess two charged centers, namely, two quinolinium nuclei.[20,21,33]

When comparing the affinities of C-1-substituted compounds, it is clear that the presence of a small alkyl group in this position (**4a**,**b**) is deleterious for the affinity for rat brain apamin-sensitive sites (Table 2). These data are in accordance with the inefficiency of C-1-unsubstituted, methylated, and unsubstituted benzylated derivatives previously reported.[25] Otherwise, the homologous derivative of NML **4c**, possessing a 3,4-dimethoxyphenethyl group, shows a lower affinity ($K_i = 5.4 \pm 0.6$ μM, $n = 3$) than NML ($K_i = 1.3 \pm 0.02$ μM, $n = 8$; Table 3). Thus, the presence of a 3,4-dimethoxybenzyl or a 2-naphthylmethyl group in the C-1 position represents so far the most effective substitution in these series. Moreover, these side chains have a different impact according to the nature of the 1,2,3,4-tetrahydroisoquinoline nucleus. Indeed, in the 6,7-dimethoxy series, **8d** with a naphthylmethyl group has an affinity inferior to 1 μM. Thus, increasing lipophilicity of this group would appear favorable in this series. 6,7,8-Trimethoxy analogue **4f** with a 3,4-dimethoxybenzyl moiety in the C-1 position is two times more effective than NML with an affinity of 0.73 μM, while the 2-naphthylmethyl analogue **8a** has an affinity of 2.2 μM (Table 3). In this case, lipophilicity of the naphthyl group does not appear favorable with such a substitution on the main ring unless steric hindrance by the presence of the group in the C-8 position would be critical. This can be illustrated by the fact that 6,8- (**4d**,**e**) and 7,8-dimethoxy (**8b**,**c**) substitution are unfavorable (Table 2). Nevertheless, other parameters could be involved since we previously reported that the compound possessing only a methoxy group in the C-8 position does not interact with the SK channel.[26] This observation is interesting and challenging because the 8-isopropyl derivative previously described has an affinity similar to that of NML.[26] The presence of an electronically rich atom could be responsible for this absence of affinity as we also reported that a chlorine or a bromine in the C-8 position is not favorable.[26]

To demonstrate the blocking potential of the drugs on SK channels, the most effective compound of this series, compound **4f**, was tested in electrophysiological experiments performed in rat brain slices. In these experiments, compound **4f** blocks the AHP recorded in midbrain dopaminergic neurons with an IC_{50} of 9.0 ± 0.7 μM, meaning that this compound is twice more active than NML ($IC_{50} = 15$ μM, $n = 3$).[24] These data are in good agreement with in vitro binding data in which the affinity of compound **4f** ($IC_{50} = 0.73 \pm 0.11$ μM, $n = 3$) is also almost twice superior to that of NML ($IC_{50} = 1.3 \pm 0.02$ μM, $n = 8$; Table 3). The lower activity of both drugs when evaluated in brain slices may be due in part to a more difficult access to the targets in such a preparation. Compound **3f**, having an affinity ~5 times weaker than NML, induces a 50% inhibition of the AHP at 100 μM. As a result of the low potency of this compound further evaluations on selectivity were not performed in this study.

Thus, among the chemical modifications performed on NML and NMN structures, it appears that some limited changes increase the interaction with the apamin-sensitive sites. The presence of a 6,7,8-trimethoxy substitution on the 1,2,3,4-tetrahydroisoquinoline nucleus is the most effective, followed by 6,7-dimethoxy (NML) and 8-alkyl, while 6,8- and 7,8-dimethoxy, 8-methoxy, 5-halogeno, and 8-halogeno substitution are less interesting.[25,26] Concerning the group in the C-1 position of the 1,2,3,4-tetrahydroisoquinoline ring, a 3,4-dimethoxybenzyl (NML, **4f**) or a 2-naphthylmethyl (**8a**, **8d**) moiety appears to be the most efficient to increase the affinity in these series of compounds, while a small alkyl group must be avoided. The most interesting combinations found are, on the one hand, a 6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline with a 3,4-

dimethoxybenzyl in the C-1 position (**4f**), and, on the other hand, a 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline with a 2-naphthylmethyl in the C-1 position (**8d**). In the first case, it is interesting to mention that such a combination leads for the first time to a tertiary amine (**3f**) with a significant affinity for apamin-sensitive binding sites. Although its affinity remains in the micromolar range, compound **3f** with its nonpermanent ionized function may be useful for the development of more potent lipophilic blockers, able to cross the blood brain barrier. Indeed, a critical step in the evaluation of the consequences of SK channel modulation in the CNS is the development of drugs that can be administrated systemically. When such a compound will be developed, its putative selectivity for SK channel subtypes and other targets will be investigated.

Experimental Section

Chemistry. Melting points were determined on a Büchi−Tottoli capillary melting point apparatus in open capillary and are uncorrected. NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500 MHz. IR spectra were performed on a Perkin-Elmer FTIR-1750 spectrometer. IR spectra were measured using KBr discs. Only significant bands from IR are reported. Elemental analyses were determined using a Carlo−Erba elemental analyzer CHNS-O model EA1108, and the results are within 0.4% of the theoretical values. All starting materials and reagents were obtained from Aldrich Chemical Co. or from Acros Organics Co. and were used without further purification. 6,7- and 7,8-dimethoxy-isoquinoline and 6,7,8,-trimethoxy-isoquinoline are prepared according to the procedure previously described.[29] Concentration and evaporation refer to removal of volatile materials under reduced pressure (10−15 mmHg at 30−50 °C) on a Buchi rotavapor.

General Preparation of Amides (1a−f): *N*-[2-(3,4-Dimethoxyphenyl)-ethyl]-butanamide (1a). Butyric anhydride (3.6 mL; 23.5 mmol) was added dropwise to a solution of 2-(3,4-dimethoxyphenyl)-ethylamine (4.0 mL; 23.5 mmol) and of an excess of triethylamine (5 mL) in MeCOOEt (30 mL) at room temperature. After 1 h, the organic medium was washed with 5% aqueous Na_2CO_3 (2 × 50 mL) and then with 1 N aqueous HCl until the washing phase became acidic. The organic layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was recrystallized from petroleum ether 100−140 °C to afford **1a** as a white solid (4.7 g); yield, 79%; mp 47−48 °C. Anal. ($C_{14}H_{21}NO_3$) C, H, N.

General Procedure for Preparing 3,4-Dihydroisoquinoline Derivatives (2a−f): 6,7-Dimethoxy-1-propyl-3,4-dihydroisoquinoline Hydrochloride (2a). A solution of compound **1a** (1.5 g; 6 mmol) in refluxing ArMe (50 mL) was treated with phosphorus pentoxide (5 g) added during 15 min. After the mixture had refluxed 30 min, ArMe was decanted and the sticky residue was dissolved in water and washed with Et_2O (2 × 30 mL). The aqueous solution was made alkaline with NH_4OH and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude oil was treated with a saturated etherous HCl solution to isolate **2a** as a hydrochloride salt. Recrystallization from MeCN/Et_2O gave **2a** as a white solid; yield, 81%; mp, 175−176 °C. Anal. ($C_{14}H_{19}NO_2$. HCl) C, H, N.

General Procedure for Preparing 1,2,3,4-Tetrahydroisoquinoline Analogues (3a−f, 7a−e): 6,7-Dimethoxy-2-methyl-1-propyl-1,2,3,4-tetrahydroisoquinoline Hydrochloride (3a). A solution of 6,7-dimethoxy-1-propyl-3,4-dihydroisoquinoline (0.69 g; 2.9 mmol) in MeCN (10 mL) was refluxed with an excess of methyl iodide (1.0 mL; 16 mmol). After 2 h, Et_2O was added, resulting in a rapid crystallization of yellow solid. The precipitate was filtered off, washed with Et_2O (2 × 10 mL), dried, and used without further purification. Under an inert atmosphere, $NaBH_4$ (0.82 g; 21.6 mmol) was added to a solution of the corresponding isoquinolinium compound (1 g; 2.7 mmol) in MeOH (50 mL) at room temperature. After 15 min, MeOH was removed under reduced pressure, and the crude residue was dissolved in a 1 N aqueous HCl (100 mL). The acidic layer was washed with Et_2O (3 × 20 mL) and then basified with NH_4OH. The suspension was extracted with CH_2Cl_2 (3 × 30 mL). The organic layers were collected, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to afford colorless oil, which was isolated as hydrochloride salt. Further recrystallization from MeCN/Et_2O gave compound **3a** as a white solid (0.74 g); yield, 90%; mp, 183−184 °C. Anal. ($C_{15}H_{23}NO_2$·HCl) C, H, N.

General Preparation of Quaternary Ammonium Derivatives (4a−f, 8a−e): 6,7-Dimethoxy-2,2-dimethyl-1-propyl-1,2,3,4-tetrahydroisoquinolinium Iodide (4a). A solution of compound **3a** (0.25 g; 1.0 mmol) in MeCN (10 mL) was refluxed with an excess of methyl iodide (0.5 mL; 8 mmol). After 4 h, the solvent was removed under reduced pressure. The white residue (0.33 g) recrystallized from MeCN gave **4a** as a white solid; yield, 85%; mp, 228−229 °C. Anal. ($C_{16}H_{26}NO_2I$) C, H, N.

General Procedure of Reissert Compounds (5a−c): 2-Benzoyl-1-cyano-6,7,8-trimethoxy-1,2-dihydroisoquinoline (5a). Anhydrous aluminum chloride (10 mg) was added to a stirred solution of 6,7,8-trimethoxyisoquinoline (3.44 g; 15.7 mmol) and trimethylsilyl cyanide (3.9 mL; 31.4 mmol) in anhydrous CH_2Cl_2 (50 mL) at room temperature. Then benzoyl chloride (3.6 mL; 31.4 mmol) was dropwise added to the stirred solution over a course of 5 min. The mixture was warmed to 30 °C if no exotherm had begun after the addition of benzoyl chloride. After stirring for an additional 3 h period, water (50 mL) was added and stirring was continued for 30 min. The organic layer was collected and washed successively with 1 N aqueous HCl (2 × 50 mL), water (50 mL), 1 N aqueous NaOH (2 × 50 mL), and finally water (50 mL). The organic solution was dried over anhydrous $MgSO_4$ and evapored under reduced pressure to give an oil that was triturated with Et_2O (20 mL), resulting in crystallization. The solid was collected, washed with small volumes of Et_2O, and dried (3.3 g) to give **5a** as a white crystal; yield, 60%; mp, 155−157 °C. Anal. ($C_{20}H_{18}N_2O_4$) C, H, N.

2-Benzoyl-1-cyano-1,2-dihydroisoquinoline **(5d)** is prepared according to a previous report.[36]

General Procedure for Preparing 1-Substituted Isoquinolines (6a−e): 6,7,8-Trimethoxy-1-(2-naphthylmethyl)-isoquinoline (6a). A solution of 2-benzoyl-1-cyano-6,7,8-trimethoxy-1,2-dihydroisoquinoline **5a** (2.41 g; 6.89 mmol) and of 2-(bromomethyl)-naphthalene (1.3 g; 6.89 mmol) in DMF (15 mL) was dropwise added to a stirred suspension of sodium hydride (0.2 g; 8.33 mmol) in DMF (30 mL) at −10 °C. The medium was stirred for 4 h and poured into ice-cold water (200 mL). The creamy solid was filtered off. After drying, the solid was hydrolyzed by treatment with 50% NaOH in a 1:1 EtOH−water solution at reflux. After removal of EtOH, the crude residue was dissolved in ArMe (50 mL) and water (50 mL). The organic layer was collected, washed with water (50 mL), and then extracted with 1 N aqueous HCl (2 × 50 mL). The acidic layers were basified with concentrated NH_4OH and finally extracted with CH_2Cl_2 (3 × 30 mL). The organic layers were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to afford a solid that was purified by flash chromatography (Me_2CO). Finally the compound was recrystallized from petroleum ether 100−140 °C to give **6a** as a cream solid; yield, 90%; mp, 184−185 °C dec. Anal. ($C_{23}H_{21}NO_3$) C, H, N.

Radioligand Binding Studies and Data Analysis. Synaptosomes Preparation. Rats (male Wistar, ±250 g) were killed by decapitation, and the brains were quickly removed and kept on ice during dissection. Crude cortex was dispersed in 0.32 M sucrose by using a Potter homogenizer. After a first centrifugation at 1500 × g for 10 min, the supernatant was centrifuged at 25 000 × g for 10 min. The resulting pellet was dispersed in 5 mL of 0.32 M sucrose to be aliquoted. Protein concentration was determined by the method of Hartree with bovine serum albumin as a standard.[37]

Binding Experiments. The incubation buffer consisted of a 10 mM Tris−HCl (pH 7.5) solution containing 5.4 mM KCl and 0.1% bovine serum albumin. The radioligand was ^{125}I-apamin (Perkin-Elmer, specific activity 81.4 TBq mmol^{-1}). Glass fiber filters (Whatman GF/C) used in these experiments were coated for 1 h in

0.5% polyethylenimine and then washed with 2.5 mL of the ice-cold buffer just before use. Binding experiments were always terminated as follows. Aliquots were filtered under reduced pressure through Whatman filters. Filters were rapidly washed twice with 2.5 mL of buffer. The radioactivity remaining on the filter was evaluated with a Packard Tri-Carb 1600TR liquid scintillation analyzer with an efficacy of 69%. ^{125}I-apamin binding to the filters was also estimated in the absence of synaptosomes. This binding was also subtracted from the total binding. Curve fitting was carried out using GraphPad Prism.

Saturation Binding Experiments. Synaptosomes (0.2 mg of protein/mL) were incubated with increasing concentrations of ^{125}I-apamin (25 μL) with 975 μL of incubation buffer for 1 h at 0 °C. Samples were then filtered on Whatman GF/C filter, and the radioactivity was measured as described above. Nonspecific binding was determined in parallel experiments in the presence of an excess of unlabeled apamin (0.1 μM) and subtracted from the total binding to obtain the specific binding.

Competition Experiments between ^{125}I-Apamin and Drugs. Synaptosomes (0.2 mg of protein/mL) were incubated for 1 h at 0 °C with ±10 pM of ^{125}I-apamin (25 μL) and nine concentrations of drugs (10^{-4} to 10^{-7} M). Nonspecific binding was determined in the presence of an excess of unlabeled apamin (0.1 μM). Samples were then filtered on Whatman filter and the radioactivity was measured as described above.

Electrophysiological Experiments. The procedure is largely described in previous papers.[24,25] Briefly, male Wistar rats (150−200 g) were anaesthetized with chloral hydrate (400 mg/kg IP) and decapitated. The brain was excised quickly and placed in cold (~4 °C) artificial cerebro-spinal fluid (ACSF) of the following composition (in mM) : NaCl, 126; KCl, 2.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.2; $CaCl_2$, 2.4; glucose, 11; $NaHCO_3$, 18; saturated with 95% O_2 and 5% CO_2 (pH 7.4). A block of tissue containing the midbrain was cut in horizontal slices (thickness 350 μm) in a Vibratome (Lancer). The slice containing the region of interest was placed on a nylon mesh in a recording chamber (volume 500 μL). The tissue was completely immersed in a continuously flowing (~2 mL/min) ACSF and heated at 35 °C. Most recordings were made from dopaminergic neurons located in the substantia nigra pars compacta. Intracellular recordings were performed using glass microelectrodes filled with KCl 2 M (resistance 70 to 150 MΩ). All recordings were made in the bridge balance mode, using a npi SEC1L amplifier (Tamm, Germany). The accuracy of the bridge was checked throughout the experiment. Membrane potentials and injected currents were recorded on a Gould TA240 chart recorder and on a Fluke Combiscope oscilloscope. The Flukeview software was used for off-line analysis. Drug effects on the prominent apamin-sensitive AHP in dopaminergic neurons were quantified as the percent reduction of the surface area of the AHP (in mVs), which was blocked by a maximally active concentration of apamin (300 nM). Drugs were applied by superfusion; complete exchange of the bath solution occurred within 2−3 min. Curve fitting was carried out using GraphPad Prism and the standard equation: $E = E_{max}/[1 + (IC_{50}/x)^h]$, where x is the concentration of the drug and h is the Hill coefficient. Numerical values are expressed as means ± SEM. Apamin (Sigma) and tested drugs were dissolved in water.

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Supporting Information Available: Routine experimental, spectroscopic, and elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Coghlan, M. J.; Gopalakrishnan, M.; Carroll, W. A. Potassium channel modulators for the treatment of CNS disorders. *Annu. Rep. Med. Chem.* **2001**, *36*, 11−20.
(2) Sah, P. Ca^{2+}-activated K^+ current in neurons: types, physiological roles and modulation. *Trends Neurosci.* **1996**, *19*, 150−154.
(3) Vergara, C.; Latorre, R.; Marrion, N. V.; Adelman, J. P. Calcium-activated potassium channels. *Curr. Opin. Neurobiol.* **1998**, *8*, 321−329.
(4) Kolher, M.; Hirschberg, B.; Bond, C. T.; Kinzie, J. M.; Marrion, N. V.; Maylie, J.; Adelman, J. P. Small-conductance, calcium-activated potassium channels from mammalian brain. *Science* **1996**, *273*, 1709−1714.
(5) Stocker, M.; Krause, M.; Pedarzani, P. An apamin-sensitive Ca^{2+}-activated K^+ current in hippocampal pyramidal neurons. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4662−4667.
(6) Waroux, O.; Massotte, L.; Alleva, L.; Graulich, A.; Thomas, E.; Liégeois, J.-F.; Scuvée-Moreau, J.; Seutin, V. SK channels control the firing pattern of midbrain dopaminergic neurons in vivo. *Eur. J. Neurosci.* **2005**, *22*, 3111−3121.
(7) Stocker, M.; Pedarzani, P. Differential distribution of three Ca^{2+}-activated K^+ channel subunits SK(1−3) in the adult rat central nervous system. *Mol. Cell. Neurosci.* **2000**, *15*, 476−493.
(8) Messier, C.; Mourre, C.; Bontempi, B.; Sif, J.; Lazdunski, M.; Destrade, C. Effect of apamin, a toxin that inhibits calcium-dependent potassium channels, on learning and memory processes. *Brain Res.* **1991**, *551*, 322−326.
(9) Deschaux, O.; Bizot, J. C.; Goyffon, M. Apamin improves learning in an object recognition task in rats. *Neurosci. Lett.* **1997**, *222*, 159−162.
(10) Ikonen, S.; Riekkinen, P., Jr. Effect of apamin on memory processing of hippocampal-lesioned mice. *Eur. J. Pharmacol.* **1999**, *382*, 151−156.
(11) Van der Staay, F. J.; Fanelli, R. J.; Blokland, A.; Schmidt, B. H. Behavioral effects of apamin, a selective inhibitor of the SK_{Ca} channel, in mice and rats. *Neurosci. Biobehav. Rev.* **1999**, *23*, 1087−1110.
(12) Fournier, C.; Kourrich, S.; Soumireu-Mourat, B.; Mourre, C. Apamin improves reference memory but not procedural memory in rats by blocking small conductance Ca^{2+}-activated K^+channels in an olfactory discrimination task. *Behav. Brain Res.* **2001**, *121*, 81−93.
(13) Pedarzani, P.; Mosbacher, J.; Rivard, A.; Cingolani, L. A.; Oliver, D.; Stocker, M.; Adelman, J. P.; Fakler, B. Control of electrical activity in central neurons by modulating the gating of small conductance Ca^{2+}-activated K^+ channels. *J. Biol. Chem.* **2001**, *276*, 9762−9769.
(14) Steketee, J. D.; Kalavas, P. W. Effect of microinjections of apamin into the A10 dopamine region of rats: a behavioural and neurochemical analysis. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 711−719.
(15) Shepard, P. D.; Bunney, B. S. Repetitive firing properties of putative dopamine-containing neurons in vitro: regulation by an apamin-sensitive Ca^{2+}-activated K^+ conductance. *Exp. Brain Res.* **1991**, *86*, 141−150.
(16) Seutin, V.; Johnson, S. V.; North, R. A. Apamin increases NMDA-induced burst firing of rat mesencephalic dopamine neurons. *Brain Res.* **1993**, *630*, 341−344.
(17) Vincent, J. P.; Schweitz, H.; Lazdunski, M. Structure−function relationships and site of action of apamin, a neurotoxic polypeptide of bee venom with an action on the central nervous system. *Biochemistry* **1975**, *14*, 2521−2525.
(18) Zerrouk, H.; Lavaba-Djerbari, F.; Fremont, V.; Meki, A.; Darbon, H.; Mansuelle, P.; Oughuideni, R.; Van Rietschoten, J.; Rochat, H.; Martin-Eauclaire, M. F. Characterization of PO1, a new peptide ligand of the apamin-sensitive Ca^{2+}-activated K^+ channel. *Int. J. Pept. Protein Res.* **1996**, *48*, 514−521.
(19) Pedarzani, P.; D'hoedt, D.; Doorty, K. B.; Wadsworth, J. D.; Joseph, J. S.; Jeyaseelan, K.; Kini, R. M.; Gadre, S. V.; Sapatnekar, S. M.; Stocker, M.; Strong, P. N. Tamapin, a venom peptide from the Indian red scorpion (*Mesobuthus tamulus*) that targets small conductance Ca^{2+}-activated K^+ channels and afterhyperpolarization currents in central neurons. *J. Biol. Chem.* **2002**, *277*, 46101−46109.
(20) Finlayson, K.; McLuckie, J.; Hern, J.; Aramori, I.; Olverman, H. J.; Kelly, J. S. Characterization of [^{125}I]-apamin binding sites in rat brain membranes and HEK293 cells transfected with SK channel subtypes. *Neuropharmacology* **2001**, *41*, 341−350.

(21) Campos Rosa, J.; Galanakis, D.; Piergentili, A.; Bhandari, K.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. Synthesis, molecular modeling, and pharmacological testing of bis-quinolinium cyclophanes: potent, non-peptidic blockers of the apamin-sensitive Ca^{2+}-activated K^+ channel. *J. Med. Chem.* **2000**, *43*, 420−431.
(22) Johnson, S. W.; Seutin, V. Bicuculline methiodide potentiates NMDA-dependent burst firing in rat dopamine neurons by blocking apamin-sensitive Ca^{2+}-activated K^+ currents. *Neurosci. Lett.* **1997**, *231*, 13−16.
(23) Seutin, V.; Johnson, S. W. Recent advances in the pharmacology of quaternary salts of bicuculline. *Trends Pharmacol. Sci.* **1999**, *20*, 268−270.
(24) Scuvée-Moreau, J.; Liégeois, J.-F.; Massotte, L.; Seutin, V. Methyl-laudanosine: a new pharmacological tool to investigate the function of small-conductance Ca^{2+}-activated K^+ channels. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 1176−1183.
(25) Graulich, A.; Scuvée-Moreau, J.; Seutin, V.; Liégeois, J.-F. Synthesis and biological evaluation of *N*-methyl-laudanosine iodide analogues as potential SK channel blockers. *Bioorg. Med. Chem.* **2005**, *13*, 1201−1209.
(26) Graulich, A.; Scuvée-Moreau, J.; Seutin, V.; Liégeois, J.-F. Synthesis and radioligand binding studies of C-5- and C-8-substituted 1-(3,4-dimethoxybenzyl)-2,2-dimethyl-1,2,3,4-tetrahydroisoquinoliniums as SK channel blockers related to *N*-methyl-laudanosine and *N*-methyl-noscapine. *J. Med. Chem.* **2005**, *48*, 4972−4982.
(27) Scuvée-Moreau, J.; Boland, A.; Graulich, A.; Van Overmeire, L.; D'hoedt, D.; Graulich-Lorge, F.; Thomas, E.; Abras, A.; Stocker, M.; Liégeois, J.-F.; Seutin, V. Electrophysiological characterization of the SK channel blockers methyl-laudanosine and methyl-noscapine in cell lines and rat brain slices. *Br. J. Pharmacol.* **2004**, *143*, 753−764.
(28) Whaley, W.; Govindachari, T. The preparation of 3,4-dihydroisoquinolines and related compounds by the Bischler−Napieralski reaction. *Org. React.* **1951**, *6*, 74−150.
(29) Birch, A.; Jackson, A.; Shannon, P. A new modification of the Pomeranz−Fritsch isoquinoline synthesis. *J. Chem. Soc., Perkin Trans. 1* **1974**, *1*, 2185−2190.
(30) Popp, F. D. Developments in the chemistry of Reissert compounds (1968−1978.). *Adv. Heterocycl. Chem.* **1979**, *24*, 187−214.
(31) Ruchirawat, S.; Phadungkul, N.; Chuankamnerdkarn, M.; Thebtaranonth, C. A versatile synthesis of Reissert compounds. *Heterocycles* **1977**, *6*, 43−46.
(32) Wadsworth, J. D. F.; Doorty, K. B.; Strong, P. N. Comparable 30-kDa apamin binding polypeptides may fulfill equivalent roles within putative subtypes of small conductance Ca^{2+}-activated K^+ channels *J. Biol. Chem.* **1994**, *269*, 18053−18061.
(33) Liégeois, J.-F.; Mercier, F.; Graulich, A.; Graulich-Lorge, F.; Scuvée-Moreau, J.; Seutin, V. Modulation of small conductance calcium-activated potassium (SK) channels: a new challenge in medicinal chemistry. *Curr. Med. Chem.* **2003**, *10*, 1241−1253.
(34) Granier, C.; Pedroso Muller, E.; Van Rietschoten, J. Use of synthetic analogs for a study on the structure−activity relationship of apamin. *Eur. J. Biochem.* **1978**, *82*, 293−299.
(35) Habermann, E. Apamin. *Pharmacol. Ther.* **1984**, *25*, 255−270.
(36) Uff, B. C.; Kershaw, J. R.; Neumeyer, J. L. Alkylation of isoquinolines via 2-benzoyl-1,2-dihydroisoquinaldonitriles: 1-benzylisoquinoline. *Org. Synth.* **1977**, *56*, 19−25.
(37) Hartree, E. F. Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.* **1972**, *48*, 422−427.

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