Synthesis and Radioligand Binding Studies of Bis-isoquinolinium Derivatives as Small Conductance Ca^{2+}-Activated K^+ Channel Blockers

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Starting from the scaffold of *N*-methyllaudanosine and *N*-methylnoscapine, which are known small conductance Ca^{2+}-activated K^+ channel blockers, original bis-isoquinolinium derivatives were synthezised and evaluated using binding studies, electrophysiology, and molecular modeling. These quaternary compounds are powerful blockers, and the most active ones have 10 times more affinity for the channels than dequalinium. The unsubstituted compounds possess a weaker affinity than the analogues having a 6,7-dimethoxy- or a 6,7,8-trimethoxy substitution. The length of the linker has no influence in the alkane derivatives. In relation to the xylene derivatives, the affinities are higher for the ortho and meta isomers. These results are well corroborated by a molecular modeling study. Finally, the most effective compounds have been tested in electrophysiological experiments on midbrain dopaminergic neurons and demonstrate the blocking potential of the apamin-sensitive after-hyperpolarization.

Introduction

Small conductance Ca^{2+}-activated K^+ (SK) channels underlie the prolonged postspike after-hyperpolarization (AHP) that plays an important role in modulating the firing rate and the firing pattern of neurones.[1−2] Three SK channel subunits have been identified by DNA cloning, namely, SK1, SK2, and SK3.[1] The distribution of the subunits has been investigated in the rat by using in situ hybridization and immunohistochemistry. SK1 and SK2 subtypes are mostly expressed in the cortex and hippocampus,[3] while SK3 channel expression is higher in subcortical areas, especially in the monoamine cell regions, e.g., the substantia nigra, dorsal raphe, and locus coeruleus. These features attract great attention to SK channels as putative targets for the treatment of cognitive dysfunction,[4−8] neuronal hyperexcitability,[9] dopamine-related disorders,[10−12] and depression.[7]

So far, the most potent SK channel blocker is apamin. Apamin is an oligopeptide extracted from the honey bee *Apis mellifera* venom. This octadecapeptide possesses two arginine residues in contiguous positions and a rigid cyclic conformation due to two disulfide bridges.[13] Dequalinium, a nonpeptidic ligand, has some SK channel blocking properties.[14] Different chemical modulation of the dequalinium structure led to the discovery of highly potent bis-quinolinium compounds such as UCL1684.[14] Furthermore *N*-methyllaudanosine (NML)[15] and more recently *N*-methylnoscapine (NMN)[16] were reported to block SK channels (Figure 1). Unlike apamin, these two molecules possess medium potency blocking properties and their effects are quickly reversible.[16] In order to improve the affinities of these isoquinolinium blockers, several bis-isoquinolinium derivatives were prepared and evaluated by using an in vitro binding assay and electrophysiological experiments to find compounds with increased affinity and to improve our knowledge of the pharmacophore.

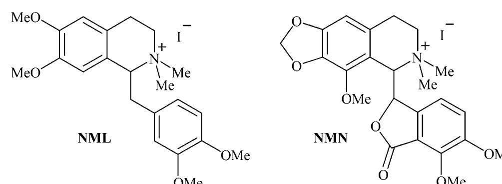

Figure 1. Chemical structures of *N*-methyllaudanosine (NML) and *N*-methylnoscapine (NMN).

Chemistry

The syntheses of methoxyisoquinolines were classically carried out by using a modification of the Pomeranz−Fritsch synthesis.[17] The ensuing bis-alkylation was performed by using the Reissert compound pathway (Scheme 1).[18] The Reissert compounds (**1a**−**c**) were obtained by reaction of the corresponding isoquinoline with benzoyl chloride in the presence of trimethylsilyl cyanide.[19] This reaction was carried out in CH_2Cl_2 and gave the Reissert compounds in good yield.[19] These derivatives were deprotonated by sodium hydride in DMF. The resulting Reissert anions were alkylated by using a half equivalent of the appropriate bis-electrophilic reagent.[20] Then the alkylated Reissert compounds were hydrolyzed to give the bis-isoquinolines (**2a**−**r**). Finally, the bis-isoquinolines were methylated by methyl iodide in DMF under mild warming conditions to obtain the bis-isoquinolinium derivatives (**3a**−**r**).

Results

The in vitro binding data are summarized in Tables 1 and 2. First, the compounds were screened at 10 μM. Because all compounds displaced ^{125}I-apamin by more than 58%, the K_i values were determined for all drugs.

In our binding conditions, (±)-NML and dequalinium (DQ+) have an affinity (K_i) for the apamin-sensitive sites of ∼1300 and 220 nM, respectively.

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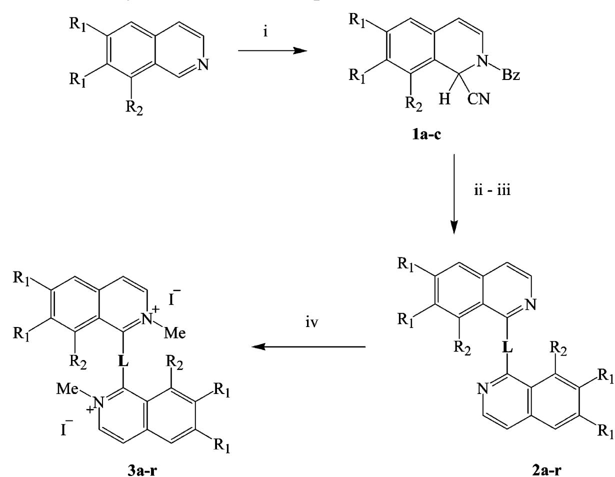
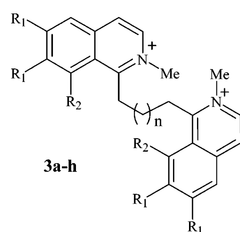
Scheme 1. Synthesis of Bis-isoquinolinium Derivatives[a]



[a] R_1 and R_2 = H or R_1 = OMe and R_2 = H or R_1 and R_2 = OMe, L = $(CH_2)_n$ with n = 3−5, *o*-xylyl, *m*-xylyl, *p*-xylyl; (i) Me_3SiCN, BzCl, $AlCl_3$, CH_2Cl_2, room temp; (ii) X−L−X (X = Br or I), NaH, DMF, −10 °C; (iii) NaOH, EtOH/ H_2O, reflux; (iv) MeI, DMF, Δt.

Table 1. Binding Affinities of *N*-Methyllaudanosine (NML), Dequalinium (DQ+), and Bis-isoquinolinium Alkanes (**3a−h**) for Rat Cortical Apamin-Sensitive Sites



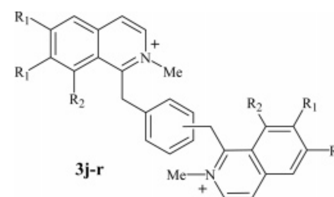
compd	R_1	R_2	n	K_i (nM)
NML				1295 ± 15
DQ+				221 ± 11
3a	H	H	1	1687 ± 203
3b	H	H	2	1962 ± 315
3c	H	H	3	1465 ± 35
3d	OMe	H	1	196 ± 19
3e	OMe	H	2	164 ± 18
3f	OMe	H	3	150 ± 6
3g	OMe	OMe	1	90 ± 13
3h	OMe	OMe	2	42 ± 8

The affinities of compounds without substitution on the isoquinoline nucleus and with an alkane linker (**3a−c**) are equal to 1687 ± 203, 1962 ± 315, and 1465 ± 35 nM, respectively, for propane (**3a**), butane (**3b**), and pentane (**3c**) derivatives. With a 6,7-dimethoxy substitution (**3d−f**), the affinities of the compounds are 196 ± 19, 164 ± 18, and 150 ± 6 nM, respectively, for propane (**3d**), butane (**3e**), and pentane (**3f**) derivatives. Finally, the compounds with the 6,7,8-trimethoxy substitution (**3g**,**h**) have an affinity of 90 ± 13 and 42 ± 8 nM, respectively, for propane (**3g**) and butane (**3h**) derivatives.

In the xylene series, the unsubstituted compounds (**3j−l**) have an affinity of 438 ± 83, 132 ± 17, and 1103 ± 117 nM for the ortho (**3j**), meta (**3k**), and para (**3l**) isomers, respectively. The affinities of the compounds possessing a 6,7-dimethoxy substitution (**3 m−o**) are equal to 33 ± 1, 106 ± 12, and 163 ± 30 nM for the ortho (**3m**), meta (**3n**), and para (**3o**) isomers, respectively. In the presence of three methoxy groups (**3p−r**), the affinity is 35 ± 1, 23 ± 5, and 280 ± 10 nM for the ortho (**3p**), meta (**3q**), and para (**3r**) isomers, respectively.

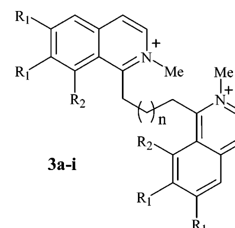
In electrophysiological experiments, compounds **3h**, **3m**, **3p**, and **3q** block the apamin-sensitive after-hyperpolarization in dopaminergic neurons with an IC_{50} of 4.4 ± 1.2 μM (n = 3), 3.8 ± 0.7 μM (n = 3), 4.1 ± 1.4 μM (n = 3), and 2.4 ± 0.3 μM (n = 3), respectively.

Table 2. Binding Affinities of *N*-Methyllaudanosine (NML), Dequalinium (DQ+), and Bis-isoquinolinium Xylenes (**3j−r**) for Rat Cortical Apamin Sensitive Sites



compd	R_1	R_2	isomer	K_i (nM)
NML				1295 ± 15
DQ+				221 ± 11
3j	H	H	ortho	438 ± 83
3k	H	H	meta	132 ± 17
3l	H	H	para	1103 ± 117
3m	OMe	H	ortho	33 ± 1
3n	OMe	H	meta	106 ± 12
3o	OMe	H	para	163 ± 30
3p	OMe	OMe	ortho	35 ± 1
3q	OMe	OMe	meta	23 ± 5
3r	OMe	OMe	para	280 ± 10

Table 3. Results of the Molecular Modeling Study for Bis-isoquinolinium Alkane Derivatives



compd	R_1	R_2	n	distance *A* [a] (Å)	distance *B* [b] (Å)	ΔE [c] (kcal/mol)
3a	H	H	1	5.59	6.90	2.32
3b	H	H	2	5.76	5.93	5.83
3c	H	H	3	5.60	9.11	7.26
3d	OMe	H	1	5.65	6.09	4.47
3e	OMe	H	2	5.59	6.58	4.50
3f	OMe	H	3	5.61	8.10	1.04
3g	OMe	OMe	1	5.64	6.35	4.52
3h	OMe	OMe	2	5.65	7.51	2.79
3i	OMe	OMe	3	5.72	9.41	6.65

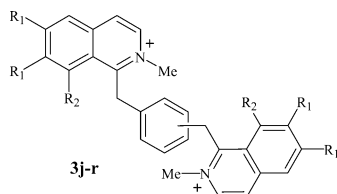
[a] N^+-N^+ distance of the conformer closest to the pharmacophoric distance (5.6 Å) previously described.[21] [b] N^+-N^+ distance of the minimum energy conformer. [c] ΔE between the conformer closest to the pharmacophore and the minimum energy conformer.

A molecular modeling study was performed in order to determine the N^+-N^+ distance of the conformers closest to the pharmacophore previously described,[21] the N^+-N^+ distance of the minimum energy conformers, and the ΔE between the two conformers. The results of the molecular modeling study are summarized in Tables 3 and 4.

Discussion

Pharmacologically, *N*-methyllaudanosine and *N*-methylnoscapine are two interesting reversible blockers of SK channels, but their affinities for apamin-sensitive sites are relatively weak.[16] The main difference between these *N*-methyl alkaloids and the more potent SK channel blockers[14] is the presence of two positively charged centers in the latter.[14,22] In order to obtain more potent blockers, bis-isoquinoliniums were therefore syn-

Table 4. Results of the Molecular Modeling Study for Bis-isoquinolinium Xylene Derivatives



compd	R_1	R_2	isomer	distance A [a] (Å)	distance B [b] (Å)	ΔE [c] (kcal/mol)
3j	H	H	ortho	5.67	8.14	4.39
3k	H	H	meta	5.12	5.11	0.41
3l	H	H	para	7.62	8.44	4.87
3m	OMe	H	ortho	5.64	8.15	4.34
3n	OMe	H	meta	5.53	5.48	5.65
3o	OMe	H	para	7.88	9.25	11.46
3p	OMe	OMe	ortho	5.77	8.04	6.36
3q	OMe	OMe	meta	5.63	8.21	3.25
3r	OMe	OMe	para	8.42	8.49	4.53

[a] N^+-N^+ distance of the conformer closest to the pharmacophoric distance (5.6 Å) previously described.[21] [b] N^+-N^+ distance of the minimum energy conformer. [c] ΔE between the conformer closest to the pharmacophore and the minimum energy conformer.

thesized. Three types of substitutions of the isoquinoliniums were used, namely, unsubstituted, 6,7-dimethoxyisoquinoline, and 6,7,8-trimethoxyisoquinoline. The trimethoxylated derivatives were prepared because the tetrahydroisoquinolinium, 8-methoxy-NML, was shown to have an affinity superior to that of NML (K_i = 730 and 1295 nM, respectively).[23] In a previous study, it had also been shown that 8-isopropyl substitution influenced the affinity as the 6,7-dimethoxy substitution, but the cost and difficulties to synthesize the compound are serious drawbacks for further development.[24] A methyl group was used for the N-alkylation because it is the most effective substituent among ethyl, benzyl, and butyl groups as previously reported.[25]

These bis-isoquinoliniums were then tested by in vitro binding in order to determine their affinities for apamin-sensitive sites. A pharmacophoric study examining only UCL compounds (bis-quinoliniums) has shown that the distance between the centroids of the pyridinium rings of the quinolinium groups must be close to 5.8 Å.[26] Another study taking into account apamin and UCL structures shows that a distance of ~5.6 Å is the optimal distance between the two ionic centers.[21] So for all compounds tested, the N^+-N^+ distance of the minimum energy conformers and the ΔE between the minimum energy conformer and the conformer closest to the pharmacophore (5.6 Å) were determined (Figure 2).

In the alkane series (**3a**−**i**), the affinity increased clearly with the presence of methoxy substituents on the isoquinolinium nucleus. Thus, in the propane series, **3d** (6,7-dimethoxy-substituted compound, K_i = 196 nM) and **3g** (6,7,8-trimethoxy-substituted compound, K_i = 90 nM) have higher affinities than the unsubstituted compound **3a** (K_i = 1687 nM). Moreover, the same observations could be made for the butane analogues (1962, 164, and 42 nM, respectively, for unsubstituted (**3b**), 6,7-dimethoxy-substituted (**3e**), and 6,7,8-trimethoxy-substituted (**3h**) compounds) and for the pentane analogues (1465 and 150 nM, respectively, for unsubstituted (**3c**) and 6,7-dimethoxy-substituted (**3f**) compounds). The methoxy substitution therefore appears to be very important in this series. Interestingly, the length of the linker is not an essential parameter, and this finding is well correlated with the results of the molecular modeling study. Indeed, almost all compounds could adopt with good probability (ΔE < 5 kcal/mol) a configuration in which both

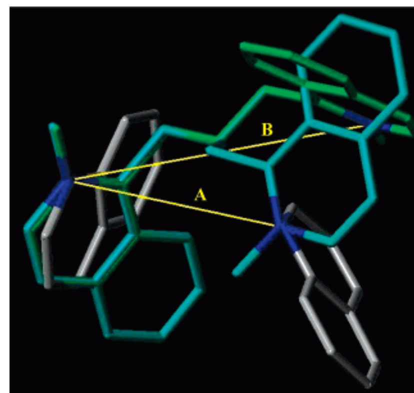


Figure 2. Superimposition of the pharmacophore (colors by atom type), the lowest energy conformer of compound **3a** (green), and the best fitting conformer (green-blue): (A) N^+-N^+ distance of the conformer closest to the pharmacophoric distance (5.6 Å); (B) N^+-N^+ distance of the minimum energy conformer.

N^+ are close to the pharmacophoric distance (5.6 Å between both N^+) with the exception of three compounds that probably adopt the lowest energy conformation (**3b**, ΔE = 5.8 kcal/mol; **3c**, ΔE = 7.3 kcal/mol; **3i**, ΔE = 6.7 kcal/mol).

In the xylene series, the presence of methoxy substituents on the isoquinolinium also appears to be essential, but the gap of affinity between unsubstituted and methoxylated compounds is less important than that in some of the alkane derivatives. Indeed, the affinities of methoxylated derivatives (**3m**, K_i = 33 nM; **3p**, K_i = 35 nM) in the ortho series are only 10 times higher than that of the unsubstituted compound (**3j**, K_i = 438 nM) rather than 50 times as found for alkane derivatives **3b** and **3h**, for example. The para-linker series also displays a significant difference between unsubstituted (**3p**, K_i = 1103 nM) and substituted (**3o**, K_i = 163 nM; **3q**, K_i = 280 nM) compounds. With the *m*-xylene linker, the difference of affinity is not well marked between unsubstituted and methoxylated analogs (**3k**, K_i = 132 nM; **3n**, K_i = 106 nM; **3q**, K_i = 23 nM) because the trimethoxylated analogue is 4−5 times more potent than the two other analogues. Both binding and molecular modeling results show clearly that the ortho and meta linkers lead to more favorable conformations than the para linker. In fact, ortho and meta derivatives have always higher affinities than the corresponding para derivatives. This is particularly clear with the 6,7,8-trimethoxy compounds (*o*-**3p**, K_i = 35 nM; *m*-**3q**, K_i = 23 nM; *p*-**3r**, K_i = 280 nM) for which the affinity of the para isomer is 10 times lower than that of the meta and ortho isomers. The molecular modeling study explains this observation by the fact that the para derivative is unable to adopt a conformation in accordance with the determined pharmacophore. Indeed, for the para derivatives, the conformers close to the pharmacophore have a N^+-N^+ distance larger than 7.6 Å (**3l**, 7.6 Å; **3o**, 7.9 Å; **3r**, 8.4 Å). Moreover, for compound **3o**, the ΔE between the minimum energy conformer and the pharmacophoric conformer is particularly high (ΔE = 11.5 kcal/mol).

In order to confirm a channel blockade, the four most effective compounds (**3h**, **3m**, **3p**, and **3q**) were evaluated in electrophysiological experiments performed in rat brain slices. In these electrophysiological experiences, all compounds block the apamin-sensitive AHP recorded in midbrain dopaminergic neurons with an IC_{50} between 2.4 and 4.4 μM. Thus, these compounds are 3−7 times more potent than NML (IC_{50} = 15 μM, n = 3) in these experiments.

In conclusion, this study shows that bis-isoquinolinium derivatives are potential pharmacological tools and reveals four

derivatives having an affinity nearly 50 times higher than the affinity of NML and shows the importance of the pharmacophoric distance between the two nitrogens. This distance factor is not the only obvious condition for an optimal interaction with the SK channels because the substitution of the aromatic nucleus could also greatly influence the affinity for the apamin-sensitive sites, as shown with methoxy substituents.

Experimental Section

1. Chemistry. Melting points were determined on a Büchi-Tottoli capillary melting point apparatus in open capillary and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 500 (500 and 125 MHz) spectrometer. IR spectra were performed on a Perkin-Elmer FTIR-1750 spectrometer. IR spectra were measured using KBr discs. Only significant bands from the IR are reported. Elemental analyses were determined using a Carlo-Erba elemental analyzer CHNSO model EA1108, and the results are within 0.4% of the theoretical values. All starting materials and reagents were obtained from Aldrich Chemical Co. and Acros Chemical Co. and were used without further purification. Separations by column chromatography were carried out using Merck Kieselgel 60 (230−400 mesh). Concentration and evaporation refer to removal of volatile materials under reduced pressure (10−15 mmHg at 30−50 °C) on a Büchi rotavapor.

The methoxylated isoquinolines were prepared by the Jackson modification of the Pomeranz−Fritsch synthesis.[17] Compound **1a** was obtained from isoquinoline by the procedure described by Uff et al.[27]

2-Benzoyl-1-cyano-6,7-dimethoxy-1,2-dihydroisoquinoline 1b. Anhydrous aluminum chloride (10 mg) was added to a stirred solution of 6,7-dimethoxyisoquinoline (2.97 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 added dropwise to the stirred solution over the course of 5 min. The mixture was warmed to 30 °C if no exothermic reaction has begun after the addition of benzoyl chloride. After the mixture was stirred for a further 3 h, water (50 mL) was added and stirring 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. The resulting oil crystallized by trituration with Et_2O (20 mL). The crystals were collected, washed with small volumes of Et_2O, and dried (3.5 g): yield, 70%; mp 165−166 °C. IR (KBr) 1661, 1631, 1345 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3H), 3.94 (s, 3H), 5.99 (br d, 1H, J = 6.6 Hz), 6.51 (br s, 2H), 6.72 (s, 1H), 6.85 (br s, 1H), 7.47 (t, 2H, J = 7.4 Hz), 7.55 (t, 1H, J = 7.4 Hz) 7.60 (d, 2H, J = 7.4 Hz). Anal. ($C_{19}H_{16}N_2O_3$) C, H, N.

1,1′-(Propane-1,3-diyl)bis-isoquinoline 2a. A solution of 2-benzoyl-1-cyano-1,2-dihydroisoquinoline **1a** (5.0 g, 19.2 mmol) and 1,3-diiodopropane (1.1 mL, 9.6 mmol) in DMF (15 mL) was added dropwise to a stirred suspension of sodium hydride (0.46 g, 19.2 mmol) in DMF (30 mL) at −10 °C. The mixture was stirred for 4 h and poured into ice-cold water (200 mL). The creamy solid was filtered off. After the mixture was dried, the solid was dissolved in EtOH (50 mL) and was then hydrolyzed by treatment with a 50% aqueous NaOH (20 mL) at reflux. After removal of EtOH, the crude residue was dissolved in toluene (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 white solid, which recrystallized from petroleum ether 100−140 °C (2.5 g): yield, 40%; mp 96−97 °C. IR (KBr) 1560, 1386, 826, 820 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.49 (pentuplet, 2H, J = 7.7 Hz), 3.50 (t, 4H, J = 7.7 Hz), 7.51 (d, 2H, J = 5.7 Hz), 7.56 (t, 2H, J = 7.5 Hz), 7.65 (t, 2H, J = 7.5 Hz), 7.80 (d, 2H, J = 7.5 Hz), 8.18 (d, 2H, J = 7.5 Hz), 8.44 (d, 2H, J = 5.7 Hz). Anal. ($C_{21}H_{18}N_2$) C, H, N.

1,1′-(Propane-1,3-diyl)bis(2-methylisoquinolinium) Diiodide 3a. Compound **2a** (1.3 g, 4.4 mmol) in DMF (10 mL) was heated until dissolution with an excess of methyl iodide (1.0 mL, 16 mmol). After 2 h, Et_2O (100 mL) was added, resulting in a rapid crystallization to give a yellow solid. The precipitate was filtered off, washed with Et_2O (2 × 10 mL), and dried (2.3 g): yield, 89%; mp 237−238 °C, dec. IR (KBr) 1634, 1399, 821 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 2.11 (br m, 2H), 4.08 (t, 4H, J = 8.3 Hz), 4.54 (s, 6H), 8.12 (t, 2H, J = 7.7 Hz), 8.23 (t, 2H, J = 7.7 Hz), 8.31 (d, 2H, J = 8.1 Hz), 8.43 (d, 2H, J = 6.9 Hz), 8.71 (d, 2H, J = 6.9 Hz), 9.00 (d, 2H, J = 8.6 Hz). ^{13}C NMR (DMSO-d_6) δ 26.6 (CH_2), 28.9 (CH_2), 46.5 (CH_3), 124.1 (CH), 128.1 (CH), 128.2 (CH), 131.3 (CH), 135.9 (CH), 137.1 (CH). Anal. ($C_{23}H_{24}N_2I_2 \cdot {}^1/_4H_2O$) C, H, N.

2. Radioligand Binding Studies and Data Analysis. 2.1. 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 25000*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.[28]

2.2. Binding Experiments. The 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 of 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%. Curve fitting was carried out using GraphPad Prism.

2.2.1. 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 substracted from the total binding to obtain the specific binding.

2.2.2. 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 eight concentrations of drugs ($10^{-4.5}$−10^{-8} 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.

2.3. Electrophysiological Experiments. The procedure is largely described in a previous paper.[15] Briefly, male Wistar rats (150−200 g) were anesthetized with chloral hydrate (400 mg/kg ip) and decapitated. The brain was excised quickly and placed in cold (~4 °C) artificial cerebrospinal 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 into horizontal slices (thickness of 350 μm) in a Vibratome (Lancer). The slice containing the region of interest was placed on a nylon mesh in a recording chamber (volume of 500 μL). The tissue was completely immersed in a continuously flowing (~2 mL/min) ACSF, heated at 35 °C. Most recordings were made from dopaminergic neurons located in the substantia nigra pars compacta. Identification of dopaminergic cells was performed as described previously.[15] Intracellular recordings were performed using glass microelectrodes filled with 2 M KCl (resistance of 70−150 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 in most cases. Drug effects on the prominent apamin-sensitive AHP in dopaminergic neurones were quantified as the percent reduction of the surface area of the AHP (in mV s), which was blocked by a maximally active concentration of apamin (300 nM).[15] Averages of four sweeps were considered in all cases. The spontaneous firing of the neurons was usually reduced by constant current injection (−20 to −100 pA) in order to increase the amplitude of the AHP. Because the amplitude of the AHP is very sensitive to the firing rate, care was taken to compare all AHPs of one cell at the same firing rate. All 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 the Hill coefficient. Numerical values are expressed as the mean ± SD. Apamin (Sigma) was dissolved in water. Compounds **3h**, **3m**, **3p**, and **3q** were first dissolved in dimethyl sulfoxide (2×10^{-2} M) and then in water to reach the appropriate concentration.

3. Molecular Modeling. Models of the compounds were built under the Sybyl 6.91 molecular modeling package (SYBYL 6.9, 2001, Tripos Inc., 1699 South Hanley Road, St. Louis, MO 63144-2913) running on Silicon Graphics Octane 2 workstations using standard fragments library. Their geometry was then optimized roughly by the method of Powell available in the Maximin2 procedure before generating conformational spaces by the CONFEX method[29] based on the distance geometry program DGEOM.[30] These databases were minimized with the Maximin2 method and reduced to their most representative conformations. The heads of the various conformational families were compared to a pharmacophoric model of the SK channels blockers developed previously at the laboratory. This comparison was carried out by an in-house macrocommand written in Sybyl Programming Language (SPL), which also calculated the energy of the conformers using the Tripos force field[31] including the electrostatic term calculated from Del Re atomic charges. Later, the energy of the entire conformational space for each compound was calculated following the same method to find out the lowest energy conformation. The lowest energy conformer and the best fitting conformation of compound **3a** were aligned manually on the pharmacophore to superimpose the nitrogens without modifying their geometry.

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Supporting Information Available: Routine experimental procedures (compounds **1c**, **2b**−**r**, and **3b**−**r**), spectroscopic data (compounds **1c**, **2b**−**r**, and **3b**−**r**), and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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