Synthesis and Structure−Activity Relationship Studies of 2-(N-Substituted)-aminobenzimidazoles as Potent Negative Gating Modulators of Small Conductance Ca^{2+}-Activated K^+ Channels

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Small conductance Ca^{2+}-activated K^+ channels (SK channels) participate in the control of neuronal excitability, in the shaping of action potential firing patterns, and in the regulation of synaptic transmission. SK channel inhibitors have the potential of becoming new drugs for treatment of various psychiatric and neurological diseases such as depression, cognition impairment, and Parkinson's disease. In the present study we describe the structure−activity relationship (SAR) of a class of 2-(N-substituted)-2-aminobenzimidazoles that constitute a novel class of selective SK channel inhibitors that, in contrast to classical SK inhibitors, do not block the pore of the channel. The pore blocker apamin is not displaced by these compounds in binding studies, and they still inhibit SK channels in which the apamin binding site has been abolished by point mutations. These novel SK inhibitors shift the concentration−response curve for Ca^{2+} toward higher values and represent the first example of negative gating modulation as a mode-of-action for inhibition of SK channels. The first described compound in this class is NS8593 (**14**), and the most potent analogue identified in this study is the racemic compound **39** (NS11757), which reversibly inhibits SK3-mediated currents with a K_d value of 9 nM.

Introduction

The excitability and the patterns of action potential firing of neurons as well as the function and plasticity of synapses are balanced by the activity of many ion channels, including small conductance Ca^{2+}-activated K^+ channels (SK[a] or $K_{Ca}2$ channels).[1−3] SK channels belong to the class of Ca^{2+}-activated potassium channels (K_{Ca}) that are subdivided into three types: big conductance K_{Ca} (BK), intermediate conductance K_{Ca} (IK) (also named SK4), and SK channels. Whereas BK channels are also gated by voltage, IK and SK channels are entirely activated via binding of Ca^{2+} to the protein calmodulin (CaM), which is constitutively attached to the C-terminus of each of the four SK channel subunits.[4] A recent study has demonstrated that the concentration−response relationship for Ca^{2+} is influenced by the phosphorylation state of CaM, where the protein kinase CK2 and the protein phosphatase 2A respectively decrease or increase the apparent Ca^{2+}-sensitivity by phosphorylation and dephosphorylation of CaM in the SK-CaM complex.[5]

During a neuronal action potential, calcium ions enter the cell via voltage-gated Ca^{2+} channels and activate SK channels that then mediate a subsequent afterhyperpolarization (AHP). During the AHP, the membrane potential is moved further away from the action potential threshold and the excitability of the neuron is consequently reduced. This important physiological role of SK channels is the background for research aimed at identifying compounds that are able to inhibit their function. Such compounds should in principle increase neuronal excitability and therefore hold a therapeutic potential for diseases like depression,[6] Parkinson's disease, and cognitive and memory disorders.[1,2,7]

Three highly homologous SK channel subunits have been cloned: SK1, SK2, and SK3.[8] In the central nervous system (CNS), the two former types are mostly expressed in the cortex and the hippocampus whereas SK3 channels to a larger extent are confined to the subcortical areas, in particular in the monoaminergic regions like substantia nigra pars compacta, dorsal raphe, and locus coeruleus.[9] The relatively high abundance of SK3 channels in the substantia nigra is the basis for the hypothesized symptomatic effect of SK blockers in Parkinson's disease.[1,2,7]

The most potent, and highly selective, SK channel pore blocker known is the octadecapeptide apamin, isolated from venom of the honey bee *Apis mellifera*. Apamin contains two disulfide bridges and two basic arginine residues (Arg^{13} and Arg^{14}) which have been identified as an essential requirement for its effect on the SK channel, probably by interacting with negative amino acids located in the outer pore mouth of the channel.[10] Similarly, the scorpion venom toxin scyllatoxin (leiurotoxin I) contains a disulfide bridge and two basic arginine moieties and it has, based on binding studies using [^{125}I]apamin, been found to interact with the apamin binding site. The structural elements of these SK-selective neurotoxins are mimicked in a number of small-molecule SK blockers such as atracurium (**1**), tubocurarine (**2**), and pancuronium (**3**), as well as in dequalinium (**4**) and the cyclic bis-quinolinium cyclophanes UCL1684 (**5**) and UCL1848 (**6**)[11,12] (Figure 1). These compounds all displace [^{125}I]apamin binding and are thus considered as pore blockers acting at the apamin binding site. In compounds **5** and **6**, the cyclic structure of apamin, formed by the disulfide

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[a] Abbreviations: SK, small conductance Ca^{2+}-activated K^+; BK, big conductance Ca^{2+}-activated K^+; IK, intermediate conductance Ca^{2+}-activated K^+; CaM, calmodulin; CK2, casein kinase 2; AHP, afterhyperpolarization; CNS, central nervous system; SAR, structure−activity relationship; EDCI, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide; MW, microwave; HEK293, human embryonic kidney 293; TM, transmembrane; n_H, Hill coefficient; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran; TEA, triethylamine.

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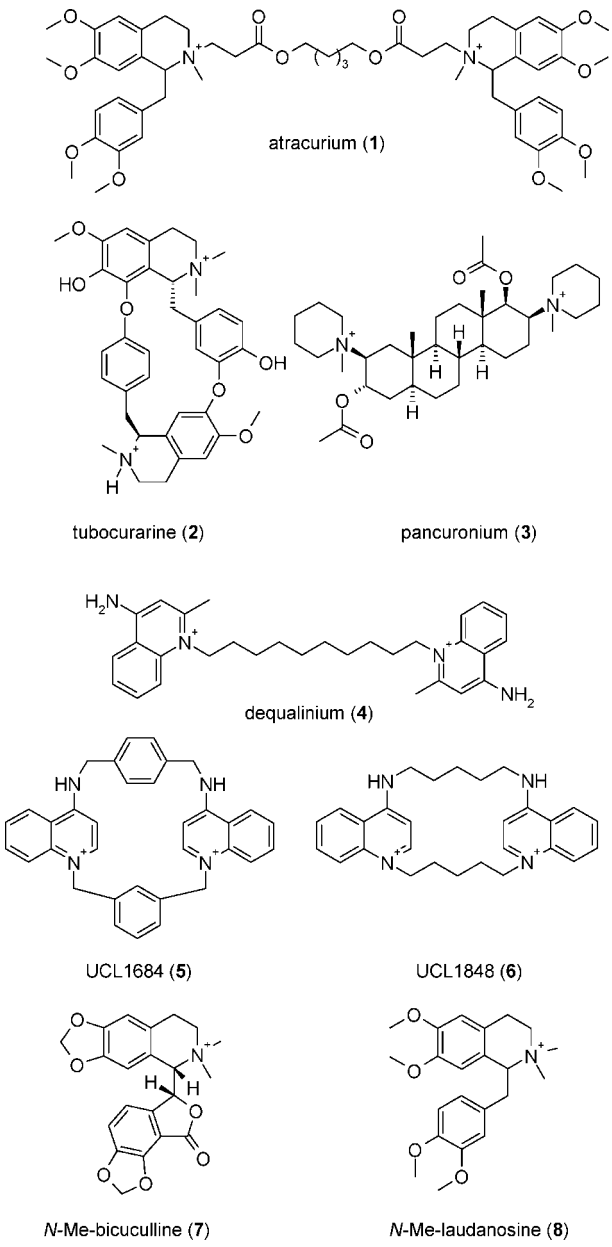


Figure 1. Examples of known SK channel pore blockers.

Table 1. Inhibition of Apamin Binding in Striatal Membranes and of Whole-Cell rSK3 Currents Induced by Reference Compounds[a]

compd	apamin binding, K_i (μM)	rSK3 currents, K_d (μM)
1	5.9 ± 0.2	6.4 ± 0.4
2	9.2 ± 1.3	16 ± 1
3	16 ± 4	21 ± 2
4	0.14 ± 0.02	0.18 ± 0.03
5	0.0018 ± 0.0003	0.0027 ± 0.0004
6	0.0014 ± 0.0001	0.00043 ± 0.00009
7	4.4 ± 0.8	7.0[b]
8	1.5 ± 0.2	2.6 ± 0.3
9	>50	positive modulator
10	>50	positive modulator
11	>50	positive modulator
14	>50	0.077 ± 0.007

[a] All data are generated at NeuroSearch as described in the Experimental Section. [b] IC_{50} value (n_H = 0.76).

bridging, was mimicked in a macrocyclic structure, resulting in the most potent synthetic blockers known (Table 1). In recent years, other classes of pore blockers have been described and examples of two relatively weak but often used compounds for

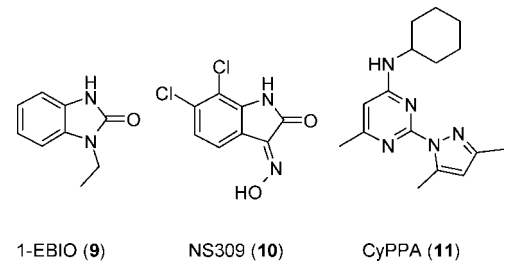


Figure 2. Examples of known positive modulators of SK channels.

biological investigations are *N*-methylbicuculline (**7**) and, in particular, *N*-methyllaudanosine (**8**), which is without activity on GABA-A receptors.

In summary, compounds of several structural classes are capable of blocking the SK channels and until recently all SK blockers described contained highly basic or permanently charged groups that interact with the apamin binding site. This has been the basis for several studies aiming to identify a pharmacophore model for compounds acting at this binding site.[13−15]

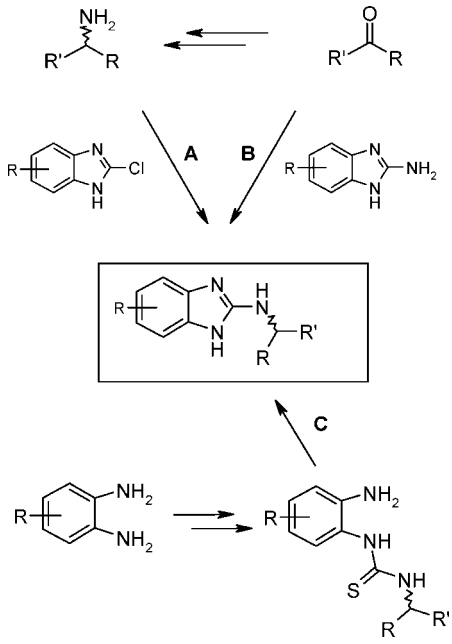
Regarding compounds acting instead by modulation of the SK channel gating properties, there are several examples of positive modulators such as 1-EBIO (**9**), NS309 (**10**), and the SK3/SK2 subtype selective compound CyPPA (**11**)[16] (Figure 2). These compounds exert their positive modulatory effect via a concentration-dependent leftward shift of the concentration−response curve for Ca^{2+}. The compound (1*H*-benzoimidazol-2-yl)-(*R*)-1,2,3,4-tetrahydronaphthalen-1-ylamine (NS8593, **14**) was recently published as the first example of a negative modulator of SK channels,[17] and in the present paper we describe the SAR of this new class of potent and selective SK inhibitors.[18] They are all 2-aminobenzimidazoles and are structurally distant from previously known SK channel blockers by being "druglike" small molecules that do not carry permanent charges.

Chemistry

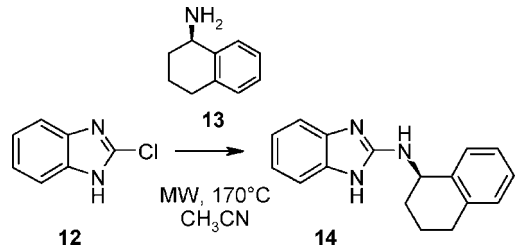
The compounds described in this study are all 2-(N-substituted)-aminobenzimidazoles. Such 2-aminobenzimidazoles can be prepared by several methods of which the most frequently used is the condensation of an N-substituted amine derivative and a 2-chlorobenzimidazole (method A, Scheme 1). This transformation has been carried out either by thermal[19−23] or high-pressure[24] reactions or by using a CuI[20] or palladium catalyzed amination of the 2-chlorobenzimidazole.[23,25−27] Similar displacement reactions have been used, though much less regularly, on the corresponding 2-bromo-[28,29] and 2-fluorobenzimidazoles[30] as well as on the related 2-sulfonic acid[31] and 2-sulfone.[29,32] Other commonly used strategies for the formation of 2-(N-substituted)-aminobenzimidazoles are the reductive amination of an aldehyde or ketone with a 2-aminobenzimidazole[33,34] (method B, Scheme 1) or, alternatively, via an *o*-phenylenediamine derivative followed by desulfurization and cyclization of the corresponding *N*-(2-aminoaryl)-*N*′-substituted thiourea (method C, Scheme 1). This cyclization has been promoted by use of HgO,[35−42] MeI,[43] or peptide coupling reagents such as DCC,[43] diisopropyl carbodiimide,[44] or EDCI (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide).[45−47]

In the present study, we initially used the direct reaction of commercially available amines with 2-chlorobenzimidazole (**12**) (Scheme 2). An example is the synthesis of **14**, which was prepared from **12** and commercially available (*R*)-1,2,3,4-tetrahydro-1-naphthylamine (**13**) (Scheme 2). The reaction was

Scheme 1. General Procedures for the Preparation of 2-(N-Substituted)-aminobenzimidazoles



Scheme 2



first performed in refluxing toluene or acetonitrile which, however, after several days of heating only gave a small amount of product (LC−MS) together with unconverted starting materials. Instead we found that heating in acetonitrile to 170 °C by means of microwave (MW) irradiation in a sealed vial gave almost complete conversion to the desired 2-aminobenzimidazole after a reaction time of 40 min. For compounds where the required amine was not commercially available, we did in some cases prepare the amine from the corresponding tetralone (**19**) by conversion into the *O*-methyloxime (**20**) followed by reduction to the primary amine (Scheme 3).[48] In compounds **17** and **18**, we prepared 2-phenylpyrrolidine (**15**) and 2-phenylpiperidine (**16**) from piperidine and pyrrolidine, respectively, by conversion into the corresponding dihydropyrrole or tetrahydropyridine, followed by reaction with phenyllithium (Scheme 3).[49]

The direct condensation of 2-aminobenzimidazole (**21**) and tetralone using standard reaction conditions, tetrahydrofuran as the solvent, a catalytic amount of acetic acid, and molecular sieves gave low conversion to the desired imine intermediate (according to LC−MS), and upon addition of sodium triacetoxyborohydride only trace amounts of the corresponding amine was formed. Imine formation had apparently not taken place, and an alternative procedure was therefore investigated. Moreover, titanium(IV) isopropoxide, which has been used as an efficient and mild reagent in reductive aminations,[50] indeed proved very efficient in yielding the desired imines and subsequently the amines in high isolated yields after treatment with sodium triacetoxyborohydride (Scheme 3). The mechanistic role of titanium(IV) isopropoxide is not fully understood, but it can possibly act both as a Lewis acid catalyst and as a water scavenger. An amount of 2 equiv of titanium(IV) isopropoxide was applied, and LC−MS analysis of the reaction mixtures showed clean and almost complete conversion into the imine intermediate. Subsequent reaction with sodium triacetoxyborohydride at room temperature gave the 2-(N-substituted)-aminobenzimidazoles as racemic mixtures in good to excellent yield (Table 4). This condensation reaction between **21** and the carbonyl compound could in principle lead to more than one product because of the presence of both an exocyclic and endocyclic nitrogens in **21**. However, we observed clean conversion and only identified the product resulting from imine formation at the exocyclic 2-amino group. This structure was confirmed for **27** by comparing the products formed either via reductive amination with 2-tetralone or via the condensation of tetrahydro-1-naphthylamine and 2-chlorbenzimidazole (**12**), which for both methods gave identical ^{1}H NMR spectra.

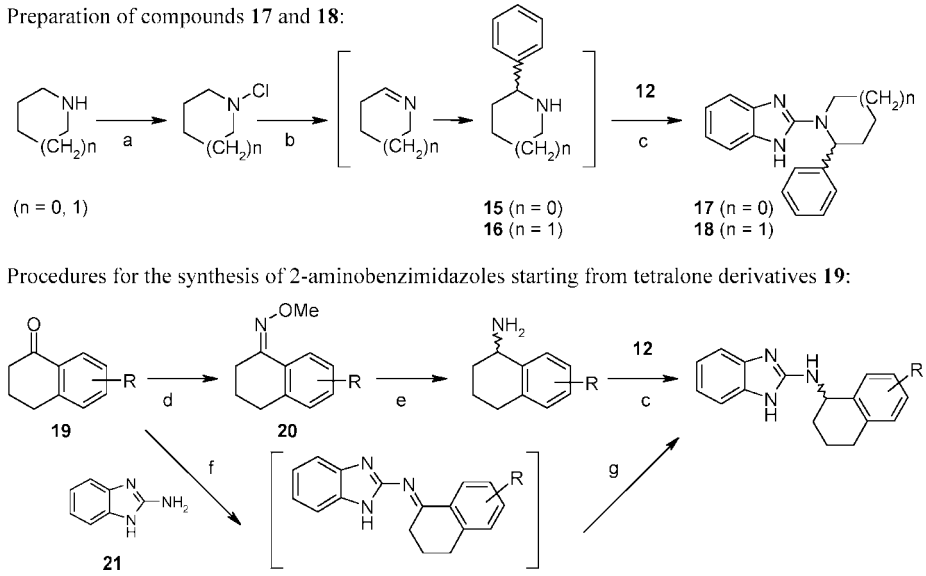
For the synthesis of compound **40** we had to prepare the required tetralone, 7-methyl-3,4-dihydro-2*H*-naphthalen-1-one. This intermediate was synthesized from 4-(*p*-tolyl)butyric acid by conversion in neat thionyl chloride into the corresponding acid chloride followed by an intramolecular Friedel−Crafts acylation reaction using aluminum chloride in toluene to give the 7-methyltetralone in 84% yield (Scheme 4). Furthermore, structural modification of one of the synthesized 2-(N-substituted)-benzimidazoles was carried out for the bromo-derivative **38**, which was reacted in a Suzuki−Miyaura cross-coupling reaction with phenylboronic acid in the presence of a catalytic amount of bis(triphenylphosphine)palladium(II) chloride and using MW irradiation as heat source to give **48** in 79% isolated yield (Scheme 5).

Pharmacology

The medicinal chemistry described herein is based on selected hits from a SK3 high-throughput screening campaign conducted at NeuroSearch based on the membrane potential sensitive dye $DiBAC_4(3)$. We initially identified the 2-aminobenzimidazole **22** as a weak SK3 inhibitor with a K_d value of 2.7 μM determined in a whole-cell patch clamp electrophysiology experiment using HEK293 cells stably expressing rat SK3 channels (Table 2). Further elaboration on the structure of **22** led to the corresponding 4-chlorophenyl- and 3,4-dichlorophenyl derivatives **23** and **24**, respectively, demonstrating up to 13-fold higher potency (Table 2).

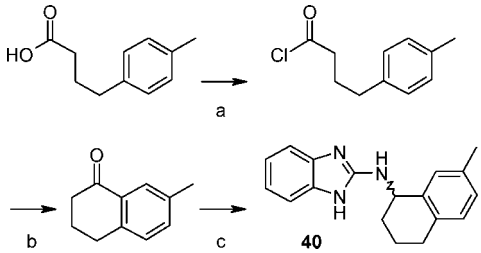
Compounds **22**−**24** possess a high structural flexibility due to the three rotational bonds linking the benzimidazole and the phenyl group. A series of conformationally constrained compounds was synthesized to obtain knowledge on the optimal relative positioning of the benzimidazole ring system and the phenyl ring. In compounds **17**, **18**, and **25**, the benzimidazole and the phenyl moieties were connected by an additional ring system that also contained the 2-amino group (Table 3), and these compounds therefore do not have the hydrogen donating properties of the 2-amino substituent. Both compounds **17** and **18** are nevertheless more potent than the noncyclized analogue **22**, and a hydrogen bond donor in this position is therefore not essential for inhibition of the SK3 channel. In comparison, the *N*-(2-benzimidazolyl)-1,2,3,4-tetrahydroisoquinoline **25** is a very weak inhibitor. This difference in biological activity could be explained by **25** having a higher degree of conformational constraint. Thus, the tetrahydroisoquinoline part of **25** can be regarded as a linear and relatively inflexible extension of the benzimidazole moiety, whereas the phenyl group in **17** and **18**

Scheme 3[a]



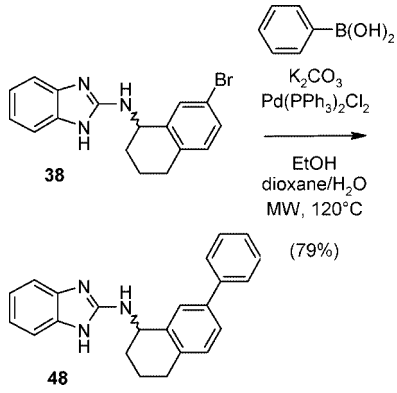
[a] (a) *N*-chlorosuccinimide, Et_2O, room temp; (b) phenyllithium (2.5 equiv, 1.9 M in *t*-Bu_2O), Et_2O; (c) CH_3CN, 170 °C (MW heating); (d) $NH_2OMe \cdot HCl$, NaOAc, MeOH, room temp; (e) $BH_3 \cdot THF$, THF, 0−60 °C; (f) titanium(IV) isopropoxide, THF, room temp; (g) $NaB(O_2CCH_3)_3H$, room temp.

Scheme 4[a]



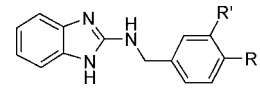
[a] (a) $SOCl_2$, cat. *N,N*-dimethylformamide; (b) $AlCl_3$, toluene, 0 °C; (c) (1) titanium(IV) isopropoxide, THF, room temp; (2) $NaB(O_2CCH_3)_3H$, room temp.

Scheme 5



can cover a wider space relative to the benzimidazole. On the basis of these findings, we prepared the series of compounds **26**−**28** containing instead two rotational bonds between the benzimidazole moiety and the phenyl ring. In these structures, the 2-amino group is not included in the carbocyclic skeleton and the phenyl group is instead annulated by an aliphatic carbocyclic ring of varying size. When comparing the activities of **26**−**28**, we found that the six-membered ring system in **27** resulted in the most potent compound with a K_d value of 0.13 μM. In addition, the point of attachment between the 2-amino group and the new bicyclic ring is of importance, as we found

Table 2. Functional Data on rSK3 Channels for Aminobenzimidazoles **22**−**24**

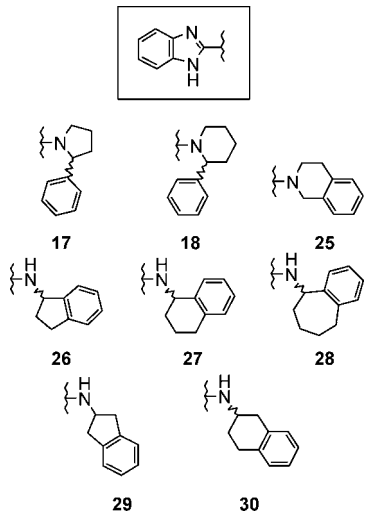


compd	R	R′	K_d (rSK3, μM)	*n*
22	H	H	2.7 ± 0.1	3
23	Cl	H	0.64 ± 0.06	3
24	Cl	Cl	0.20 ± 0.05	4

that the 2-substituted derivatives **29** and **30** were both less potent than the corresponding 1-substituted analogues **26** and **27**, respectively. On the basis of the observations described above (Table 3), it is evident that optimization of the relative positioning of the benzimidazole and the phenyl rings is important for the SK inhibitory properties of the compounds. The tetrahydronaphthyl derivative **27** turned out to be the most potent compound with a 20-fold increase in the SK3 inhibition compared to compound **22**, and although this increase in potency could simply reflect a positive correlation between binding site interactions and the introduction of additional lipophilicity in the form of aliphatic carbon skeleton, it also shows that the structural elements of **27** are locked in a more optimal and planar conformation.

On the basis of compound **27**, we subsequently prepared a series of close analogues having different substitution pattern of the tetrahydronaphthyl group (Table 4). First, the influence of methyl substitution on the aliphatic part of the tetrahydronaphthyl ring was explored, and it was found that the 2-substituted analogue **31** had a potency comparable to that of compound **27**. Thus, although a 2-methyl substituent introduces a degree of sterical hindrance between the tetrahydronaphthyl group and the benzimidazole moiety, the relative positioning of these two groups that leads to SK channel activity seems nevertheless not compromised when tested as a mixture of the diastereomers. In comparison, analogue **32** contains a methyl group in the 4-position, and here, the substituent does not influence the free rotation of the molecule and in fact results in an improved K_d value of 0.032 μM. Despite this increase in potency, we did not further pursue compounds having this 4-substitution, as it does introduce an additional center of

Table 3. Functional Data on rSK3 Channels for Aminobenzimidazoles



compd	method	yield (%)	K_d (rSK3, μM)	n
17	A	38	1.6 ± 0.2	3
18	A	10	0.75 ± 0.03	3
25	A	94	14 ± 5	3
26	A	32	0.79 ± 0.09	5
27	A	61	0.13 ± 0.02	7
28	B	56	0.59 ± 0.03	3
29	A	30	1.7 ± 0.3	3
30	B	98	2.0 ± 0.4	3

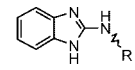
chirality resulting in compounds that are mixtures of the diastereomers. A 4-chloro-substitution on the phenyl ring of **22** had, as described above, resulted in the more potent SK inhibitor **23**, and when preparing the corresponding chloro-substituted tetrahydronaphthyl analogues, we obtained **33**−**36**, of which compound **34**, being substituted in the 7-position, was the most potent with a K_d value of 0.017 μM. Replacement of the chlorine atom with a fluorine gave **37** with a K_d value of 0.034 μM, whereas the larger bromo-substituent in **38** improved the K_d further to 0.011 μM. Preparing the 6,7-dichloro substituted derivative **39** confirmed the effect of increased bulk and lipophilicity in this part of the molecule, as **39** (NS11757), with a K_d value of 0.0091 μM, together with **38** turned out to be the most potent SK3 inhibitors found within this study. Electron-withdrawing substituents such as chlorine and bromine clearly enhance potency, but also electron-donating groups such as methyl or methoxy can be equally well accommodated. Thus, compounds **40** and **41** with either 7-methyl or 5,7-dimethyl substitution are both active as SK inhibitors, again with the disubstituted analogue **41** as the more potent (K_d = 0.033 μM). Also, introduction of the more polar methoxy group in the 7-position gave **42** with a potency of 0.061 μM. Regarding the consequences of adding polarity in the molecules, we made the finding that changing the tetrahydronaphthyl group into a thiochromanyl moiety as in compound **44** had no effect on the activity whereas the oxygen containing chromanyl group in **43** was detrimental to SK activity. This clearly shows that the polar chromanyl group cannot be tolerated, whereas the corresponding thiochromanyl analogue **44** is equally potent as the physicochemically similar compound **27** that contains a carbon atom of almost identical electronegativity as that of sulfur. On the basis of these results, it could therefore be speculated that the activity of these compounds is partly determined by the ability of the tetrahydronaphthyl group to bind in a hydrophobic region of the binding site. This hypothesis was supported when testing the corresponding pyridyl analogue **45**, which turned out to be only a weak inhibitor (K_d = 6.5 μM). Considering that substituents in the 7-position of the tetrahydronaphthyl moiety had been well tolerated, we were interested in studying if this could be extended to an additional phenyl ring. This modification was first made in the ring-fused 1,2,3,4-tetrahydrophenanthren-4-yl derivative **47**, which in fact showed a 4-fold increase in potency compared to **27**. In contrast, a phenyl group connected via a rotational bond in compound **48** gave a substantial decrease in activity (K_d = 0.60 μM). Finally, structural modification into the 4,5,6,7-tetrahydrobenzo[*b*]thiophenyl derivative **46** gave, compared to the six-membered carbocyclic group in **27**, a significant decrease in activity (K_d = 0.54 μM). In conclusion, structural modification of **27** in the phenyl ring of the tetrahydronaphthyl moiety with preferably nonpolar and large groups leads to increased SK channel inhibition. The incorporation of polar heteroatoms as in **43** and **45** was detrimental to activity, and despite that there could in principle be a reduced ability of these compounds to cross the cell membrane, it supports the hypothesis that the compounds are in general interacting with a mainly lipophilic region of the binding site. Also, a limit for the space available in this region was seen for compound **48** in which a phenyl group extending out from the 7-position resulted in a decrease in activity.

2-Tetrahydronaphthylamines are chiral compounds, and in order to study the pharmacology of the stereoisomers, we had used **27** as an example and prepared both enantiomers from reaction of 2-chlorobenzimidazole (**12**) with either of the stereochemically pure (*R*)- or (*S*)-tetrahydronaphthylamines.[17] As can be seen in Table 5, there is a 6-fold difference in the activity of the (*R*)-enantiomer (**14**, K_d = 0.077 μM) and the (*S*)-enantiomer (**49**, K_d = 0.45 μM). However, both compounds are still relatively potent inhibitors, and since the difference in activity is relatively small, we decided that for the subsequent SAR study we would base our general conclusions on the pharmacological data obtained from the tested racemates.

Generally, the 2-aminobenzimidazole moiety has basic properties (pK_a = 7.54 for 2-aminobenzimidazole compared to 5.53 for benzimidazole)[51] and has for this reason been used regularly as a guanidine mimetic. Hence, there have been several applications of 2-aminobenzimidazoles within medicinal chemistry, recently in a variety of areas such as antibiotics,[22,46] melanin-concentrating hormone antagonists,[44] histamine antagonists,[19,31] integrin $\alpha_V\beta_3$ antagonists,[37−39] vanilloid receptor-1 antagonists,[20,21] RNA cleavers,[35] and kinase inhibitors.[28,43,52] To examine whether a basic 2-aminobenzimidazole group is essential for effect in our class of SK inhibitors, the two nonbasic benzimidazole analogues **50** and **51** were tested (Figure 3). Compound **50** is an analogue of **22** in which the 2-amino group has been replaced by a methylene group, whereas **51** is the corresponding amide analogue of **23**. These modifications lead to compounds without basic properties, and in fact both compounds turned out to be inactive (K_d >10 μM). Hence, activity of this class of compounds seems to require an interaction with a basic and possibly protonated nitrogen atom on the 2-aminobenzimidazole moiety.

Compound **14** has as a representative of the tetrahydronaphthyl compound class been characterized in more detail with respect to mode-of-action and pharmacology on other potassium channels.[17] Most interestingly, when tested in excised patches, it was found that the inhibition by **14** decreased as the intracellular [Ca^{2+}] was increased and that the compound was equipotent when applied from either the intracellular or the extracellular side of the cell membrane. This reflected that the concentration−response curve for Ca^{2+} was right-shifted in

Table 4. Functional Data on rSK3 Channels for the Racemic Aminobenzimidazoles Derived from **27**



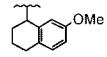
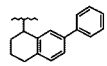
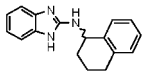
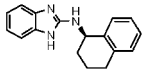
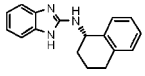
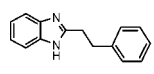
	R	Method	Yield (%)	K_d (rSK3, μM)	(n)		R	Method	Yield (%)	K_d (rSK3, μM)	(n)
27		A	61	0.13 ± 0.02	(7)	**40**		B	81	0.020 ± 0.003	(3)
31		B	78	0.17 ± 0.06	(4)	**41**		B	90	0.033 ± 0.005	(4)
32		B	60	0.032 ± 0.012	(6)	**42**		B	62	0.061 ± 0.005	(4)
33		B	67	0.027 ± 0.020	(3)	**43**		B	89	>10	(3)
34		B	68	0.017 ± 0.007	(3)	**44**		B	74	0.13 ± 0.02	(3)
35		B	93	0.12 ± 0.02	(3)	**45**		B	57	6.5 ± 1.5	(3)
36		B	57	0.044 ± 0.011	(3)	**46**		B	76	0.54 ± 0.08	(4)
37		A	44	0.034 ± 0.004	(3)	**47**		B	7	0.031 ± 0.005	(3)
38		B	91	0.011 ± 0.004	(3)	**48**		-	79	0.60 ± 0.08	(3)
39		B	87	0.0091 ± 0.0011	(3)						

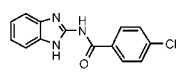
Table 5. Functional Data on rSK3 Channels for the Enantiomers of **27**

		K_d (rSK3, μM)	(n)
27		0.13 ± 0.02	(7)
14		0.077 ± 0.007	(25)
49		0.45 ± 0.09	(5)

the presence of compound, a negative gating mode-of-action that had not been described previously. In contrast to the pore blockers (Figure 1), compound **14** does not displace [^{125}I]apamin in binding studies when tested in rat striatal membranes (K_i >50 μM) as well as in HEK293 cells expressing hSK3.[17] To further support this finding, we have tested **14** on an hSK3 channel that was mutated in the apamin binding site[10] and found that **14** is in fact able to modulate this apamin-insensitive channel. As shown in Figure 4 (upper left panel), a HEK293 cell transiently transfected with hSK3 channels was inhibited by 80% upon application of 100 nM apamin and by 75% after application of 300 nM **14**. The mutated hSK3 channel with Gln493Ala and Asp495Ala mutations between transmembrane segment 5 and the pore loop (Figure 4, upper right panel) displayed a current−voltage relationship similar to the wild-type hSK3 channel but was insensitive to 100 nM apamin. In contrast, **14** inhibited the mutated channel by 45% at 300 nM. Compound **14** thus remained active on the apamin-insensitive SK3 channel, although the K_d value of 0.43 ± 0.11 μM (n = 10) was 4-fold higher than found for a wild-type hSK3 channel (K_d = 0.10 ± 0.01 μM, n = 12). As the potency of **14** is dependent on the degree of SK3 channel activation, the decreased potency could thus reflect an increased apparent Ca^{2+}-sensitivity of the mutated channels. However, when characterized in inside-out patches, the mutated hSK3 channel was found to have an activation curve for Ca^{2+} (Figure 4, middle and lower panels) similar to that of a wild-type hSK3 channel ($EC_{50}(Ca^{2+})$ = 0.44 and 0.40 μM, respectively). Similar to the whole-cell experiments, the potency of **14** was reduced 3-fold from 0.67 ± 0.1 μM (n = 8) to 2.2 ± 0.4 μM (n = 5) when tested at a Ca^{2+} concentration of 500 nM. Apamin is only active when applied from the extracellular side of the membrane and was therefore not tested on the inside-out patches. The slightly compromised effect of **14** on SK channels devoid of the apamin binding site could indicate that



50: K_d >10 μM (rSK3, n = 3)



51: K_d >10 μM (rSK3, n = 3)

Figure 3. Functional data on rSK3 channels for two nonbasic benzimidazole derivatives **50** and **51**.

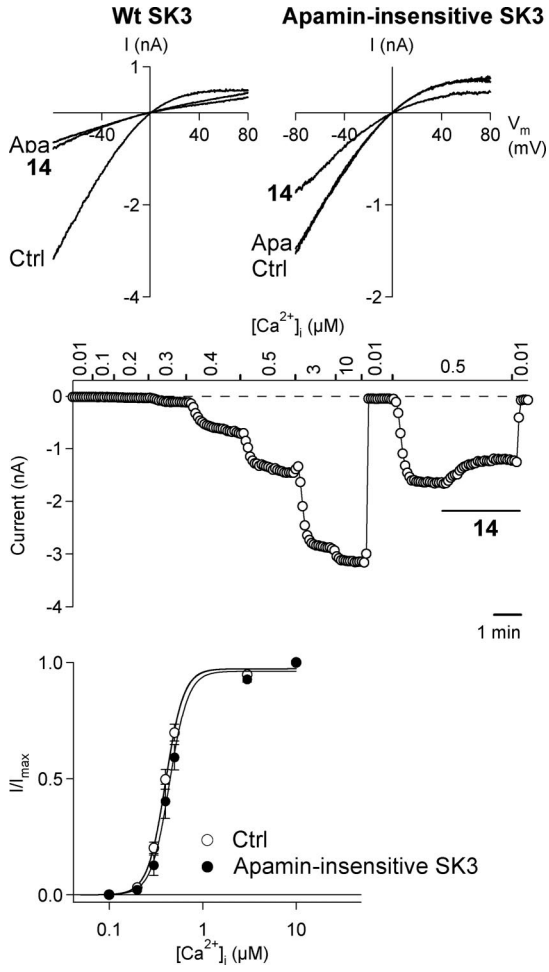


Figure 4. Compound **14** inhibits an apamin-insensitive hSK3 channel. Upper panels show whole-cell current−voltage curves obtained from HEK293 cells transiently transfected with hSK3 (left panel) or the apamin-insensitive hSK3(Q493A;D495A) (right panel). Each panel shows the curves before (Ctrl) as well as during application of 300 nM **14** and during application of 100 nM apamin (Apa). The middle panel shows Ca^{2+}-induced activation of SK channels and potency of **14** when tested on an inside-out patch pulled from HEK293 cells transiently transfected with the apamin-insensitive hSK3(Q493A;D495A). During the first part of the experiment, the Ca^{2+}-sensitivity was measured by exposing the patch to the various Ca^{2+}-buffered solutions indicated at the *x*-axis. In the second part of the experiment, the potency of **14** was determined by application of 300 nM compound during the period indicated by the bar (K_d in this patch = 2.3 μM). The lower panel depicts the Ca^{2+} concentration−response relationships of the wild-type channels (open symbols, $n = 9$) and of the SK3(Q493A;D495A) (filled symbols, $n = 4$). The currents were normalized to the currents induced by 10 μM Ca^{2+} and represent the mean ± SEM. The solid lines show the fitted Hill curves yielding EC_{50} values and n_H of 0.40 μM and 4.5, as well as 0.44 μM and 4.4 for wild-type and SK3(Q493A;D495A), respectively.

amino acids close to the pore loop are involved in mediating the negative gating modulation.

In the present study, none of the novel 2-aminobenzimidazole derivatives **17**, **18**, and **22**−**48** presented displaced [^{125}I]apamin binding to rat striatal membranes (all showing $K_i > 50$ μM) and all the active compounds are therefore considered as being gating modulators. However, the racemic compound **39**, characterized by having a more than 8-fold higher potency than **14** ($K_d = 0.0091$ μM versus 0.077 μM), was characterized with respect to mode-of-action as well as selectivity. Figure 5 (upper panel) shows the time course for inhibition induced by 100 nM **39**. The whole cell SK current slowly returns toward control

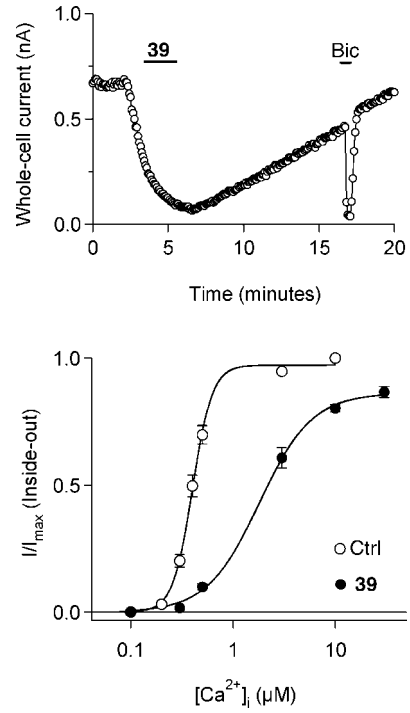


Figure 5. Inhibition of hSK3 induced by **39** is due to a shift in the Ca^{2+} response curve. The upper panel shows the time course of a whole-cell experiment where **39** (100 nM) was applied during the period indicated by the bar. The inhibition reached steady state after approximately 5 min and was reversible upon wash. The reference blocker bicuculline methobromide (Bic, 100 μM) was applied for 20 s during the washing period. The current was measured every 5 s at 0 mV upon application of voltage ramps (−120 to +30 mV). The K_d value in this experiment was fitted to 7.8 nM. The lower panel shows the Ca^{2+} response curves for hSK3 obtained from inside-out patches in the absence (open circles) and in the presence (filled circles) of **39** (0.3 μM). The symbols are the average currents (relative to the maximal current in the patch) from five to nine experiments, and the solid lines represent the best fit to the Hill equation. In the absence of compound an EC_{50} value of 0.40 μM and Hill coefficient of 4.5 was obtained, whereas the values were 1.81 μM and 1.7 in the presence of **39**.

current level upon wash-out of the compound. In order to definitively identify **39** as a negative gating modifier, Ca^{2+} response curves were performed using inside-out patches in the absence and presence of 0.3 μM **39**, respectively. Figure 5 (lower panel) shows that the Ca^{2+} response curve is right-shifted ($EC_{50}(Ca^{2+}) = 1.8$ μM; $n_H = 1.7$) compared to the control curve ($EC_{50}(Ca^{2+}) = 0.4$ μM; $n_H = 4.5$), thus confirming the modulator mode-of-action of this series of compounds.

Compound **39** was furthermore tested for SK subtype selectivity. However, using inside-out patches, the compound inhibited the human isoforms hSK1, hSK2, and hSK3 with K_d values ($n = 5$) of 0.054 ± 0.012, 0.047 ± 0.013, and 0.060 ± 0.012 μM, respectively. Thus, although this compound, in comparison to **14**, was improved with respect to potency, it did not confer subtype selectivity.

In conclusion, we have identified a class of potent and selective SK channel inhibitors that act through a novel mechanism. These compounds inhibit SK currents by shifting the concentration−response curve for Ca^{2+} toward higher Ca^{2+} concentrations rather than blocking the pore, and they are therefore the first negative modulators of SK channels described. They were found to inhibit SK channels in which the apamin binding site was abolished by point mutations, confirming that these novel compounds do not interact with the apamin binding site. Also, they are structurally distant from all previously

described small-molecule blockers of SK channels. They do not contain permanent charges and can be described as "druglike" small molecules with physiochemical properties that favor penetration of the blood−brain barrier. Indeed, compound **14** (3 and 10 mg/kg intravenously) is able to affect firing rate and firing pattern of dopaminergic neurons *in vivo*[53] in C57Bl/6 mice, and we are currently studying further the potential of this class of compounds as a tool for identifying CNS effects of SK inhibition.

We have described the SAR leading from (1*H*-benzoimidazol-2-yl)benzylamine (**22**) to the conformationally restricted 1,2,3,4-tetrahydronaphthylamine **27**. Further increase in biological activity was obtained by modification of **27** with large and preferably nonpolar groups on the phenyl ring of the tetrahydronaphthyl moiety. In contrast, the introduction of the polar heteroatoms nitrogen or oxygen in compounds **43** and **45** was detrimental to activity. Adding an additional fused phenyl ring in **47** was well tolerated, whereas the phenyl group extending out from the 7-position in **48** resulted in a loss of activity. Finally, we have found that inhibition of the SK channel activity by this class of compounds requires that the basic properties of the 2-aminobenzimidazole moiety are maintained. Among the compounds tested, the racemic 1,2,3,4-tetrahydronaphthylamine analogues **38** and **39** were identified as the most potent SK inhibitors with K_d values of 0.011 and 0.0091 μM, respectively.

Experimental Section

General Experimental Information. Chemistry. Reagents and solvents, as well as test compounds **50** and **51**, were purchased from commercial sources and used without further purification. Melting points were determined in an open capillary and are uncorrected. MW irradiation was performed using an Emrys Optimizer EXP from Biotage. Proton and carbon NMR spectra were recorded on a 500 MHz instrument at 500 and 125 MHz, respectively. Elemental analyses were performed at the Department of Chemistry, University of Copenhagen, and are within ±0.4 ppm unless indicated otherwise. Crude compounds were purified by preparative reversed-phase HPLC using a Waters autopurification system with a Waters XTerra Prep MS C8 OBD column (30 mm × 100 mm, 5 μm). The column was equilibrated at a flow rate of 43 mL/min with a mobile phase containing 80% solvent A (10 mM aqueous NH_4HCO_3) and 20% solvent B (CH_3CN). Purification was done using a linear gradient from 20% to 95% of solvent B (CH_3CN) over 10 min for the elution of pure compounds. Analytical HPLC analyses were performed on the same autopurification system using the similar Waters XTerra analytical column.

General Procedure for the Preparation of N-Substituted 2-Aminobenzimidazoles from the Corresponding Amines and 2-Chlorobenzimidazole (12) (Method A). Synthesis of (1*H*-Benzoimidazol-2-yl)-(*R*)-1,2,3,4-tetrahydronaphthalen-1-ylamine (14, NS8593). 2-Chlorobenzimidazole (**12**, 5.0 g, 32.8 mmol) and 1.2 equiv of (*R*)-1,2,3,4-tetrahydro-1-naphthylamine (5.8 g, 39.3 mmol) were suspended in acetonitrile (5 mL) in a closed vial and heated to 170 °C for 40 min by use of MW irradiation. After the mixture was cooled to room temperature the precipitated solid was filtered off and washed with acetonitrile to give the title compound as the HCl salt (white solid, 6.42 g, 65%): mp 263−265 °C; MS(ES^+) *m*/*z* 264 ($[M + 1]^+$, 100); optical rotation 58.7° (MeOH, 25 °C); ^{1}H NMR (DMSO-d_6) δ 1.75−1.85 (m, 1H), 1.88−1.97 (m, 2H), 2.06−2.13 (m, 1H), 2.72−2.88 (m, 2H), 5.01−5.07 (m, 1H), 7.16−7.28 (m, 5H), 7.34−7.43 (m, 3H), 9.48 (m, 1H), 12.8 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 19.7, 28.8, 30.0, 51.9, 111.7, 123.2, 126.4, 127.9, 128.8, 129.4, 130.4, 135.5, 137.6, 150.0. Anal. ($C_{17}H_{17}N_3 \cdot$HCl) C, H, N.

2-(2-Phenylpyrrolidin-1-yl)-1*H*-benzoimidazole (17). Method A was used (2-phenylpyrrolidin was prepared from literature[49] procedure). Purification was by aqueous basic workup, and recrystallization was from CH_3CN/H_2O: 150 mg (solid, 38%); mp 226−230 °C; MS (ES^+) *m*/*z* 264 ($[M + 1]^+$, 100); ^{1}H NMR (DMSO-d_6) δ 1.80−2.04 (m, 3H), 2.34−2.46 (m, 1H), 3.55−3.64 (m, 1H), 3.81−3.90 (m, 1H), 5.12−5.18 (m, 1H), 6.78−6.92 (m, 2H), 7.07−7.13 (m, 2H), 7.18−7.34 (m, 5H), 11.1 (s, 1H). Anal. ($C_{17}H_{17}N_3$) C, H, N.

2-(2-Phenylpiperidin-1-yl)-1*H*-benzoimidazole (18). Method A was used (2-phenylpiperidin was prepared from literature[49] procedure) and purification was by preparative LC−MS to give the title compound as the free base: 80 mg (solid, 10%); mp 198−200 °C; MS (ES^+) *m*/*z* 278 ($[M + 1]^+$, 100); ^{1}H NMR (DMSO-d_6) δ 1.26−1.41 (m, 1H), 1.50−1.71 (m, 3H), 1.89−2.03 (m, 1H), 2.32−2.42 (m, 1H), 3.06−3.17 (m, 1H), 4.07−4.16 (m, 1H), 5.48−5.55 (m, 1H), 6.81−6.97 (m, 2H), 7.08−7.19 (m, 2H), 7.19−7.39 (m, 5H), 11.3 (s, 1H). Anal. ($C_{18}H_{19}N_3$) H, N. C: calcd, 77.95; found, 77.50.

General Procedure for the Preparation of N-Substituted 2-Aminobenzimidazoles by Reductive Amination (Method B). Synthesis of (1*H*-Benzoimidazol-2-yl)-(1,2,3,4-tetrahydronaphthalen-2-yl)amine (30). To a solution of 2-aminobenzimidazole (**21**, 0.50 g, 3.76 mmol) in dry THF (10 mL) (under N_2 atmosphere) was added 1.3 equiv of β-tetralone (0.71 g, 4.88 mmol) followed by the addition of 1.5 equiv of titanium(IV) isopropoxide (1.60 g, 5.63 mmol). The reaction mixture was stirred for 3.5 h at room temperature followed by the addition of 2 equiv of sodium triacetoxyborohydride (1.58 g, 7.51 mmol). After the mixture was stirred overnight at room temperature, aqueous saturated $NaHCO_3$ was added and the layer was extracted with EtOAc. The combined organic phases were dried ($MgSO_4$), filtered, and evaporated to dryness to give the crude product which was purified by flash chromatography (50−100% EtOAc/TEA (99:1) in hexane) to give the title compound in 98% isolated yield (solid, 966 mg): mp 211−214 °C; MS(ES^+) *m*/*z* 264 ($[M + 1]^+$, 100); ^{1}H NMR (DMSO-d_6) δ 1.71−1.85 (m, 1H), 2.09−2.18 (m, 1H), 2.80 (dd, 1H, J = 9.1 and 16.3 Hz), 2.85−2.93 (m, 2H), 3.14 (dd, 1H, J = 4.8 and 16.3 Hz), 3.96−4.07 (m, 1H), 6.61−6.66 (m, 1H), 6.86 (br s, 2H), 7.07−7.19 (m, 6H), 10.6 (br s, 1H). Anal. ($C_{17}H_{17}N_3$) C, H, N.

(1*H*-Benzoimidazol-2-yl)(7-phenyl-1,2,3,4-tetrahydronaphthalen-1-yl)amine (48). Compound **38** (0.50 g, 1.46 mmol) was dissolved in a mixture of dioxane (5 mL) and water (0.5 mL). Then 1.3 equiv of phenylboronic acid (0.23 g, 1.90 mmol), ethanol (5 mL), and 3 equiv of potassium carbonate (0.61 g, 4.3 mmol) were added to the mixture, which was degassed with nitrogen, and 0.01 equiv of bis(triphenylphosphine)palladium(II) chloride (11 mg, 0.015 mmol) was added. The reaction mixture was heated to 120 °C in the MW oven for 100 min and cooled to room temperature. Saturated aqueous $NaHCO_3$ was added, and the layer was extracted with EtOAc. The organic phases were dried ($MgSO_4$), filtered, and evaporated to dryness to give the crude product which was purified by flash chromatography (10−75% EtOAc in hexane, 5%TEA) to give **48** in 79% yield (390 mg) as a white solid: mp >250 °C; MS (ES^+) *m*/*z* 340 ($[M + 1]^+$, 100); ^{1}H NMR (DMSO-d_6) δ 1.75−2.14 (m, 4H), 2.72−2.93 (m, 2H), 5.05−5.15 (m, 1H), 6.81−6.94 (m, 2H), 7.02−7.08 (m, 1H), 7.11−7.20 (m, 2H), 7.21−7.26 (m, 1H), 7.28−7.33 (m, 1H), 7.37−7.43 (m, 2H), 7.46−7.50 (m, 1H), 7.53−7.58 (m, 2H), 7.62−7.65 (m, 1H), 10.6 (s, 1H). Anal. ($C_{23}H_{21}N_3$) H, N. C: calcd, 81.39; found, 80.58.

Pharmacology. Electrophysiological experiments and [^{125}I]-apamin binding to striatal membranes were carried out according to previously described procedures.[17] In short, the potency of inhibition is given as K_i values when obtained from displacement of radioactive apamin in equilibrium binding experiments. Three independent concentration−response experiments were performed using 3−10 concentrations. In patch clamp experiments, K_d values obtained by single concentration kinetics experiments are used (with n equaling the number of independent experiments) except for bicuculline methbromide, where the potency is given as an IC_{50} value obtained from an equilibrium concentration response (fast inhibition kinetics of this compound prevents accurate measurement of blocker kinetics). Data are given as mean ± SEM. All electrophysiological data were, unless specifically stated, obtained

with rat SK3 channels and using an extracellular solution containing 4 mM K^+. In all whole-cell experiments the intracellular/pipette solution contained Ca^{2+} buffered at 400 nM.

Molecular Biology. hSK3 was cloned from total human skeletal muscle RNA as previously described.[54] The hSK3 cDNA was subcloned into the pNS3h vector. Mutated hSK3 was generated using uracilated plasmid as template in a mutagenesis reaction in which mutagenic oligonucleotides and T7 DNA polymerase were used to introduce the mutations. An aliquot of the mutagenesis reaction was transformed into *E. coli* XL1-Blue cells, and mutated hSK3 were identified by the elimination of an Acc65I restriction site. The sequence of the mutagenic oligonucleotide was as follows: hSK3-AA, gtgaaaggtatcatgacgcgcaggccgtaactagtaacttt. The fidelity of the construct was verified by sequencing. HEK293 cells were transiently transfected using lipofectamin and standard trasnsfection methods. Electrophysiological recordings were made 2−4 days after transfection.

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Supporting Information Available: Experimental details, ^1H NMR data, and melting points for the compounds **22**−**29** and **31**−**47**; table of combustion analysis data for all novel compounds, **14**, **17**, **18**, **24**−**26**, and **28**−**48**. This material is available free of charge via the Internet at http://pubs.acs.org.

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