Novel 1,4-Benzothiazine Derivatives as Large Conductance Ca^{2+}-Activated Potassium Channel Openers

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The design and synthesis of a novel class of 1,4-benzothiazines targeted for the large-conductance calcium-activated potassium channels (BK) are presented. In vitro functional characterization of BK channel opening activity was assessed by measuring the relaxation of isolated rat aortic rings precontracted with KCl 20 mM. The results of this study show that the 1,4-benzothiazine heterocyclic nucleus is a suitable backbone for designing novel BK-openers; indeed, some of these new 1,4-benzothiazine derivatives had a vasorelaxant potency comparable or superior to that of reference BK-activator NS-1619 (**1**).

Introduction

The large-conductance calcium-activated potassium (BK[a] or BK_{Ca} or maxi-K) channels belong to the wide family of voltage-activated potassium channels and exhibit a very high single-channel conductance.[1,2] They are characteristically activated by the concerted influences of membrane depolarization and increases in cytosolic Ca^{2+} levels.[3] These features are determinant for their role as feedback regulators of the activity of voltage-dependent calcium channels. BK channels are present in many cell types, including neurons and smooth muscle cells, where they are involved in a variety of physiological processes.[4] Hence, in the nervous system, BK channels regulate neuronal excitability and neurotransmitter release,[5] while in smooth muscle, they play a key role in setting the contractile tone of vascular,[6,7] bronchotracheal,[7,8] urethral,[9] uterine,[10] or gastrointestinal[11] smooth musculature. These transmembrane proteins exist as a complex of four α-subunits which form the channel and may be coassembled with four β regulatory subunits.[4] The presence of multiple splice variants of α-subunits[12,13] and multiple subtypes of β-subunits (β_1-β_4)[14–17] generates considerable diversity within the BK family that may be tissue- and organ-specific.[4,18] In view of these properties and as a consequence of their central role in regulating cell activity, BK channels are particularly appealing as a therapeutic drug target.[19–23]

In particular, agents that activate BK channels (BK-openers or -activators) stabilize the cell by increasing the efflux of potassium ions, leading to hyperpolarization and thus decrease the cell excitability and/or cause smooth muscle relaxation. Thus, BK channel openers could offer a novel therapeutic approach to several diseases associated with both the central nervous system and smooth muscle such as stroke, epilepsy, bladder overactivity, asthma, and hypertension to mention a few.[24–28]

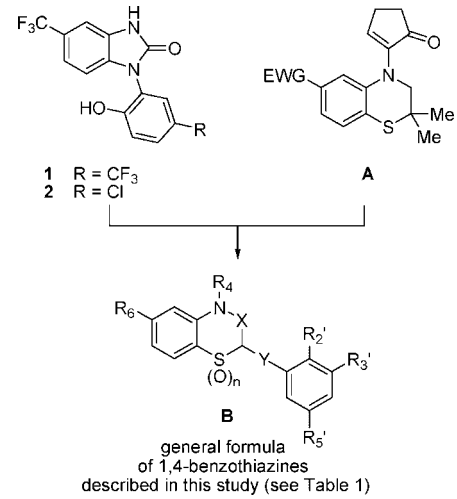

Figure 1. Chemical structures of reference compounds and design concept.

A number of naturally derived compounds[29] or small synthetic molecules[20,21,24,25] have been identified as BK-openers. The benzimidazolone derivatives, typified by NS-1619 (**1**) and NS-004 (**2**),[30] (Figure 1) were the pioneer BK-activators and have been the reference models that have led to the design of several novel synthetic BK-openers. As a consequence, the majority of small synthetic BK-activators have several structural features in common.[20,21,24,25] These include two aromatic rings linked via a spacer unit that may be either a heterocyclic or acyclic moiety, usually a urea function; the heterocyclic spacer is sometimes fused to one of the aromatic rings. In addition, a key feature of many BK-openers is the 5-halo-2-hydroxy or 5-halo-2-methoxy substitution pattern present on one of the aromatic rings; the other aromatic ring often displays electron-withdrawing groups such as a trifluoromethyl group or a chlorine atom.

Recently, we found that the 1,4-benzothiazine nucleus is a versatile skeleton to obtain new potassium channel activators. In fact, 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzothiazine derivatives, suitably functionalized at the N-4 and the C-6 positions as in compounds of type **A** (Figure 1), were identified by us as highly potent ATP-sensitive potassium (K_{ATP}) channel activators

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[a] Abbreviations: BK, large-conductance calcium-activated potassium channel; K_{ATP}, ATP-sensitive potassium channel; LDA, lithium diisopropylamide; TEA, tetraethylammonium chloride; IbTX, iberiotoxin; SAR, structure-activity relationship; ACh, acetylcholine.

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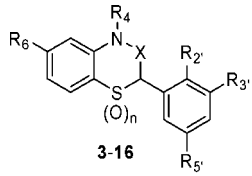
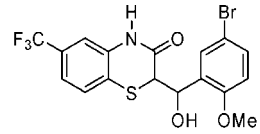
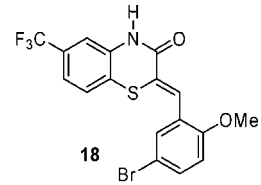
Table 1. Structure, Vasorelaxing Potency and Efficacy of the Compounds Reported in This Study[a]



3-16



17a *threo*
17b *erythro*



18

compd	*n*	R_6	R_4	X	$R_{2'}$	$R_{3'}$	$R_{5'}$	E_{max}[b] ± SEM,[c] %	pIC_{50}[d] ± SEM[c]
3[e]	0	H	H	CO	H	H	H	42 ± 3	NC[f]
4[e]	0	CF_3	H	CO	H	H	H	39 ± 9	NC[f]
5	0	H	H	CO	OMe	H	Br	100	4.80 ± 0.14
6	0	CF_3	H	CO	OMe	H	Br	77 ± 5	7.40 ± 0.12
7	0	CF_3	H	CO	OH	H	Br	44 ± 4	NC[f]
8	0	CF_3	H	CO	OMe	H	Cl	NE[g]	
9	0	CF_3	H	CO	OH	H	Cl	34 ± 4	NC[f]
10	0	CF_3	H	CO	OMe	Br	Br	75 ± 7	4.30 ± 0.07
11	1	CF_3	H	CO	OMe	H	Br	34 ± 10	NC[f]
12	0	CF_3	Me	CO	OMe	H	Br	67 ± 15	6.21 ± 0.18
13	0	CF_3	Me	CO	OH	H	Br	NE[g]	
14	0	CF_3	H	CS	OMe	H	Br	26 ± 3	NC[f]
15	0	CF_3	H	CH_2	OMe	H	Br	<25	NC[f]
16	0	CF_3	H	CH_2	OMe	H	H	100	4.65 ± 0.04
17a								NE[g]	
17b								70	5.20 ± 0.20
18								NE[g]	
1								100	5.30

[a] The synthesized compounds were administered to isolated endothelium-denuded rat aortic rings, precontracted by KCl 20 mM. [b] Maximal vasorelaxing effect expressed as the % of contractile tension. [c] Standard error of a mean 5−10 separate experiments. [d] Vasorelaxant potency expressed as the negative log of the concentration evoking a half-reduction of the contractile tone. [e] The preparation is reported in ref 33. [f] The parameter could not be calculated because of the low efficacy (~ or <50%). [g] The compound was ineffective.

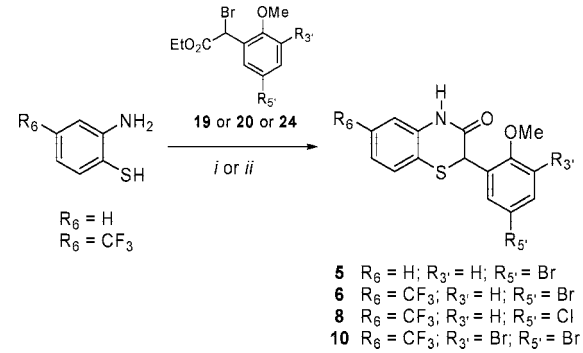
exhibiting vasodilator potency on rat aortic rings in the subnanomolar range.[31,32]

On the basis of this finding and considering the structural features of the known BK-activators, we explored the potential of 1,4-benzothiazine nucleus for designing and synthesizing a new series of type **B** derivatives targeted for BK channels (Figure 1 and Table 1). The rational pathway used to adapt the general pharmacophore model of benzimidazolone series to the 1,4-benzothiazine nucleus began with compound **3**,[33] which has the minimum structural requirements of the BK pharmacophore model, that is, two aromatic areas linked by a 2*H*-1,4-thiazin-3(4*H*)-one spacer. The insertion of the electron−withdrawing trifluoromethyl group at the C-6 position led to derivative **4**.[33] Both of these 2-aryl-1,4-benzothiazine derivatives, as well as their analogues synthesized in this study, generally have at least one stereogenic center at the C-2 position of the benzothiazine nucleus, which was unresolved in the first approach of this pharmacological survey.

Compounds **5**−**9** were then synthesized to evaluate the influence of 5-halo-2-methoxyl or 5-halo-2-hydroxyl substitution pattern at the C-2 phenyl moiety; compound **10**, having an additional bromine atom, was also synthesized.

Recent studies by Li et al.[34,35] demonstrated that the acidic properties of the amide function are an important parameter for the pharmacological activity of BK-openers. Thus, to evaluate if the acidity of the amide function of these 1,4-benzothiazine derivatives is related to their biological activity, it was enhanced by oxidation of the thiazine sulfur atom to sulfoxide as in compound **11**, or suppressed by *N*-methylation as in derivatives **12** and **13**. The importance of the carbonyl moiety as a hydrogen bond site was also investigated through the 3-thioxo derivative **14** and 3-deoxo derivatives **15** and **16**. In addition, the distance between the 1,4-benzothiazine nucleus and the C-2 phenyl group was changed by inserting a hydroxymethylene or a vinyl bridge as in compounds **17** and **18**, respectively. In the case of compound **17**, which has two chiral centers, both *threo* (**17a**) and *erythro* (**17b**) diastereoisomers were obtained, which were separated and then evaluated as enantiomeric mixtures.

Scheme 1[a]



[a] Reagents and conditions: (*i*) DMF; (*ii*) K_2CO_3, DMF, 70−80 °C.

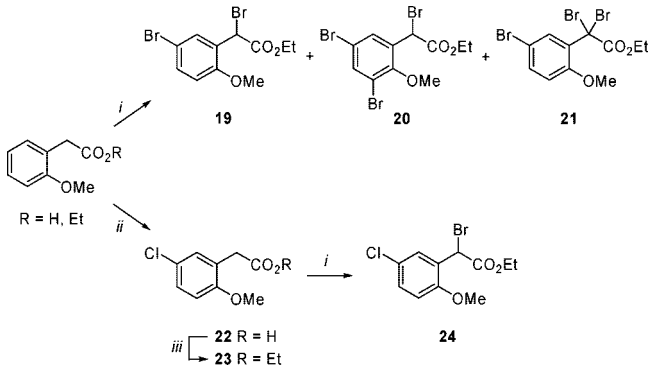
Chemistry

The 2-aryl-2*H*-1,4-benzothiazine-3(4*H*)-one derivatives **5**, **6**, **8**, and **10** were synthesized, analogously to compounds **3**[33] and **4**[33] described in the literature, by reacting 2-aminobenzenethiol or 2-amino-4-(trifluoromethyl)benzenethiol hydrochloride and an appropriate α-bromoarylacetic ester (Scheme 1).

The α-bromoarylacetic esters **19** and **20** were obtained by radical bromination of ethyl (2-methoxyphenyl)acetate[36] together with undesired α,α-dibromo derivative **21** (Scheme 2). The α-bromoarylacetic ester **24** was instead prepared from (2-methoxyphenyl)acetic acid through chlorination, esterification and radical bromination (Scheme 2).

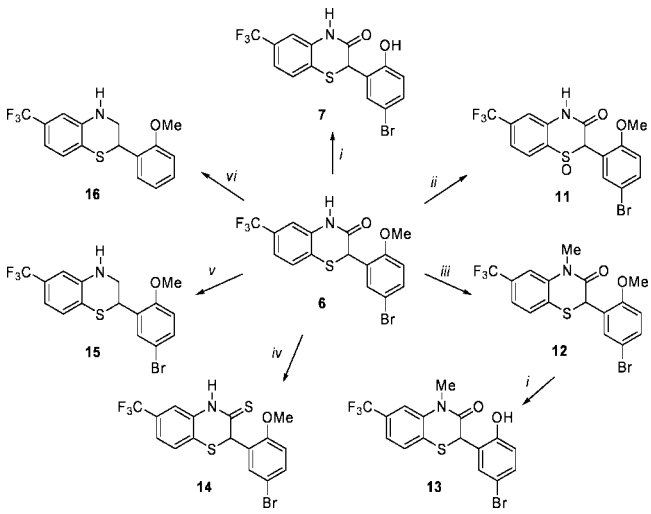
(5-Bromo-2-methoxyphenyl)benzothiazinone derivative **6** was used as starting material to afford different target derivatives **7**, **11**−**16** (Scheme 3). In particular, the de-*O*-methylation of **6** using BBr_3 in CH_2Cl_2 at room temperature afforded phenol derivative **7** almost quantitatively. The same reaction carried

Scheme 2[a]



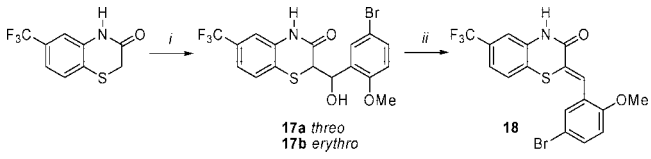
[a] Reagents and conditions: (*i*) Br_2, hν, CCl_4, reflux; (*ii*) SO_2Cl_2, THF, −10 °C; (*iii*) EtI, K_2CO_3, DMF, 50 °C.

Scheme 3[a]



[a] Reagents and conditions: (*i*) BBr_3, CH_2Cl_2; (*ii*) Al_2O_3 (TsOH, H_2O), Oxone 1 mol equiv, $CHCl_3$; (*iii*) MeI, *t*-BuOK, DMF; (*iv*) Lawesson's reagent, MW; (*v*) THF-BH_3 complex, THF, reflux; (*vi*) $LiAlH_4$, THF.

Scheme 4[a]



[a] Reagents and conditions: (*i*) LDA, −55 °C/25 °C, 5-bromo-2-methoxybenzaldehyde, THF; (*ii*) (a) MsCl, Et_3N, CH_2Cl_2, (b) 40 °C.

out on compound **8** gave the *para*-chlorophenol derivative **9** (not shown in the Schemes). Sulfoxide **11** was obtained as a diastereoisomeric mixture by oxidizing **6** with oxone in CH_2Cl_2 and in the presence of Al_2O_3. *N*-alkylation of **6** with MeI and *t*-BuOK in DMF yielded derivative **12**, which was further de-*O*-methylated to phenol derivative **13** using BBr_3 in CH_2Cl_2 at room temperature. The reaction of **6** using Lawesson's reagent and catalyzed by domestic microwaves in *neat* condition afforded the thioxo derivative **14**. The reduction of the lactam carbonyl group of **6** by using BH_3−THF complex in THF at reflux afforded the deoxo derivative **15**, while derivative **16** was directly obtained by using $LiAlH_4$ in THF at room temperature.

The *threo* and *erythro* diastereoisomer derivatives, **17a** and **17b** respectively, were prepared (Scheme 4) via an aldol-like condensation of 6-(trifluoromethyl)-2*H*-1,4-benzothiazin-3(4*H*)-one[37] and commercial 5-bromo-2-methoxybenzaldehyde by using lithium diisopropylamide (LDA) prepared in situ; the diastereoisomeric separation was carried out by flash chromatography. The *threo*/*erythro* configuration was assigned on the basis of ^{1}H NMR coupling constant values for the C-2 benzothiazine proton and the benzylic one in agreement with that reported in the literature.[38,39]

Benzylidene derivative **18** was then obtained as *Z*-isomer in a one-pot procedure from the diastereoisomeric mixture **17** (Scheme 4), which was first mesylated at room temperature and then warmed to reflux. No trace of the *E* isomer was found. The *Z* configuration was attributed on the basis of the vinyl proton chemical shift. The *Z*-stereoselectivity is probably due to steric factors as reported in cases of other benzylidene derivatives.[38]

Results and Discussion

For all of the synthesized compounds **3**−**18**, the BK-opening activity was evaluated in vitro as the vasorelaxing effect on endothelium-denuded rat aortic rings precontracted with KCl (20 mM) according to the protocol described in the Experimental Section. The vasorelaxing activity data, expressed as efficacy (%) and potency (pIC_{50}), are reported in Table 1 along with those of benzimidazolone derivative **1** chosen as reference compound.

Although this functional test cannot be considered as an exhaustive pharmacological characterization, it should be viewed as a satisfactory pharmacological tool for a preliminary identification of a BK-mediated mechanism, being that the vasorelaxing activity of BK-openers is one of the best known and characteristic pharmacodynamic features of this class of compounds. In addition, previous studies have demonstrated a good correlation between iberiotoxin (IbTX)-sensitive vasorelaxing effects (recorded by functional tests) and direct activation of vascular BK channels (recorded by patch clamp experiments) and functional studies on vascular smooth muscle preparations continue to be largely used in the pharmacological characterization of BK-activating molecules.[40–43]

The biological data indicate that some of the synthesized compounds had a nearly complete vasorelaxing effect; some had a low efficacy, and only a few showed complete ineffectiveness. With respect to potency, compounds such as **5**, **10**, **16**, and **17b** had pIC_{50} values close to that of reference benzimidazolone **1**, while derivatives **6** and **12** were significantly more potent.

Obviously, this evidence does not exclude a possible contribution of mechanisms of action other than the activation of BK channels. Moreover, the relative similarity of the pore region, observed in many potassium channel subtypes, could be another cause of "nonselectivity" for several potassium channel openers (e.g., pinacidil, cromakalim, etc.). This problem could be satisfactorily resolved by using selective blockers.

On the basis of these considerations, the most potent compounds **6** and **12** were submitted to additional experimental protocols to further investigate their mechanism of action. In particular, the vasorelaxing activities of compounds **6** and **12** were significantly antagonized by tetraethylammonium chloride (TEA) (10 mM; a nonselective blocker of potassium channels). It should be noted that in these experimental procedures, vascular smooth muscle is depolarized by KCl 20 mM causing inward calcium currents, needed to obtain the contractile effect and, consequently, to study a vasorelaxing activity. The presence of TEA can cause a further depolarization which could be a "nonspecific" factor that reduces the activity of the vasodilator agents. However, the presence of such a simple "functional

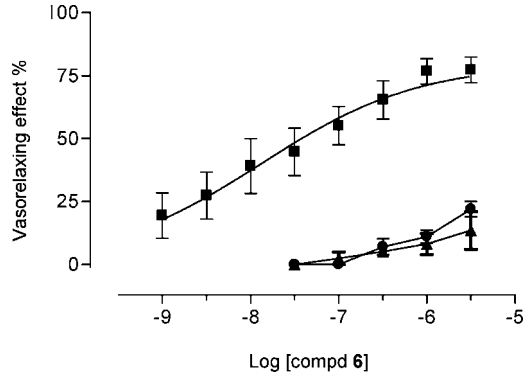


Figure 2. Concentration−vasorelaxing effect curves for compound **6** in control conditions (squares) and in the presence of TEA 10 mM (triangles) or IbTX 100 nM (circles). Vertical bars indicate the standard error.

antagonism" or "nonspecific" interferences can be excluded because in previous studies it was demonstrated that TEA was able to antagonize the effects of some well-known BK-activators while not inhibiting the vasorelaxing effects of vasodilators acting through a different mechanism of action. Moreover, as above indicated, a good correlation was observed between TEA- and IbTX-sensitive vasorelaxing effects (recorded by functional tests) and direct activation of vascular BK potassium channels (recorded by patch clamp experiments).[43] Finally, the specific involvement of BK channels in this pharmacological effect was also investigated by using IbTX, a well-established selective BK blocker. In the presence of this toxin, the vasorelaxing effect of compound **6** was significantly antagonized, suggesting that the activation of BK channel could account for the vasorelaxing properties of these compounds (Figure 2).

These preliminary biological data indicate that the 1,4-benzothiazine nucleus can be considered as a suitable replacement of the benzimidazolone nucleus because some of the 1,4-benzothiazine derivatives reported in this study showed BK-mediated vasorelaxant activities that, in some cases, were higher than that of derivative **1**.

Different activity levels were observed in compounds **6**−**9** having a 5-halo-2-hydroxy- or 5-halo-2-methoxy-phenyl ring at the C-2 position. Derivative **6**, in which the second aromatic portion is a 5-bromo-2-methoxyphenyl group, has a high level of vasorelaxing efficacy (77%) and was 100-times more potent than the reference compound **1**. Its 5-bromo-2-hydroxy counterpart **7** was instead inactive, as were the 5-chloro-2-methoxy and 5-chloro-2-hydroxy derivatives **8** and **9**, respectively. These results are quite surprising and difficult to interpret considering that these moieties have generally been associated with a high activity of BK-activators. Compounds **3** and **4**, having a naked phenyl ring at the C-2 position, were also inactive. The introduction of a second bromine atom at the C-3′ position of **6** led to derivative **10**, which was 1000 times less potent than the parent compound. This result is in sharp contrast with the structure−activity relationship (SAR) that was obtained for the bisphenol-based structure BK-openers in which multihalogen substitution such as 2,4-dichloro- or 2,4-dibromo-phenols was pivotal for obtaining highly active compounds.[35] The above observed divergences with respect to the SAR based on benzimidazolones and related compounds led to speculate either the existence of a peculiar binding mode at the same hypothetical receptor site or a different binding site for these benzothiazine derivatives.

On the other hand, the lower activity of C-6 unsubstituted derivative **5** when compared to **6** shows the importance of a CF_3 moiety at the C-6 position, in agreement with the current benzimidazolone SAR.

The role of the amidic function was evaluated by restricting the substitution pattern to a CF_3 at the C-6 position and a 5-bromo-2-methoxphenyl group at the C-2 position, which were found to be optimal in the preliminary set. *N*-methylation of the amide gave derivative **12**, which showed a decreased potency of one logarithmic unit and a slightly decreased efficacy when compared to **6**. The oxidation of the sulfur atom to sulfoxide, in order to increase the acidity of the amidic proton, was detrimental because derivative **11** showed no activity at all. The bioisosteric replacement of the carbonyl function by thiol-carbonyl one gave derivative **14**, which had no activity, and carbonyl reduction to methylene unit gave derivative **15**, which likewise was inactive. These results suggest that, in this case, the amidic function is of primary importance for obtaining active compounds. As reported above, the acidic properties of the amide function are considered as a possible factor that affect the pharmacological activity of some BK-openers. Moreover, it must be pointed out that many BK-activators exhibit a carbonyl moiety in the spacer unit, suggesting that this structural requirement may play a positive role in the interaction with the biological target. In this study, the suppression by methylation of the hydrogen bond donor capacity of the lactam group in the 2*H*-1,4-thiazin-3(4*H*)-one spacer gave the active compound **12**. In contrast, the removal of the carbonyl function seemed to produce a negative impact on the vasorelaxant activity, as shown by the pharmacological profiles exhibited by the couple of analogues **6** and **15**. These results indicate that, at least in the 1,4-benzothiazine BK-openers, the carbonyl function is more important for the activity than the amidic hydrogen.

The introduction of a hydroxymethylene spacer unit at the C-2 position gave rise to the diastereoisomeric derivatives *threo* **17a** and *erythro* **17b**. The *erythro* derivative has a potency intermediate between that of **6** and reference benzimidazolone derivative **1**; the *threo* derivative was instead ineffective. This behavior could be explained by considering that the *threo* derivative can give an intramolecular hydrogen bond[39] between the C-3 carbonyl function and the hydroxyl group that could be detrimental to activity. The introduction of a double bond in C-2 afforded *Z*-benzylidene **18**, which was found to be ineffective, indicating that the presence of a rigid spacer between the two aromatic areas annihilates the activity.

Conclusion

In summary, the main observation that has emerged from this study is that the 1,4-benzothiazine heterocyclic nucleus is a suitable backbone for designing novel BK-activators; indeed, some of these new 1,4-benzothiazine derivatives showed a vasorelaxant potency comparable or superior to that of reference BK-activator **1**. The observed vasorelaxant activity was due to the activation of BK channels because it was antagonized by IbTX, a selective blocker; the highest potency was displayed by the 6-trifluoromethylbenzothiazinone derivative **6** having the 5-bromo-2-methoxyphenyl group linked at the C-2 position of the 1,4-benzothiazine nucleus.

Although many structural characteristics of these benzothiazine derivatives seem to line up with the general pharmacophoric elements typical of most synthetic BK-openers, the preliminary SAR defined for this novel series showed some peculiar divergences with respect to that defined for the benzimidazolone series and related compounds, leading to speculation of a different mode of channel protein recognition. This fact provides an interesting rationale for the further

development of a 1,4-benzothiazine-based series of derivatives, in order to obtain a more detailed understanding of the SAR of this novel class of BK-openers.

Experimental Section

Reagents and solvents were purchased from common commercial suppliers and were used as received. Organic solutions were dried over anhydrous Na_2SO_4 and concentrated with a Büchi rotary evaporator at low pressure. Yields were of purified product. All starting materials were commercially available unless otherwise indicated. All reactions were routinely checked by thin-layer chromatography (TLC) on silica gel 60 F_{254} (Merck) and visualized by using UV. Column chromatography separations were carried out on Merck silica gel 60 (mesh 70−230) and flash chromatography on Merck silica gel 60 (mesh 230−400). Melting points were determined in capillary tubes (Büchi Electrotermal model 9100) and are uncorrected. ^{1}H NMR spectra were recorded on Bruker AC-200 (200 MHz) or Avance DRX 400 (400 MHz) spectrometers in the indicated solvent. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard. The spectral data are consistent with the assigned structures. GC-MS analyses were carried out with an HP 6890 gas chromatograph (25 m dimethyl silicone capillary column) equipped with an HP 5973 mass selective detector. Elemental analyses were performed on a Carlo Erba elemental analyzer, model 1106, and data for C, H, and N are within ± 0.4% of the theoretical values.

2-(5-Bromo-2-methoxyphenyl)-2*H*-1,4-benzothiazin-3(4*H*)-one (5). A solution of α-bromoarylacetic ester **19** (0.704 g, 2.0 mmol) and 2-aminobenzenethiol (0.250 g, 2.0 mmol) in DMF (3 mL) was stirred at room temperature for 30 h. The reaction mixture was then poured into ice−water. The precipitated solid was filtered off and recrystallized from EtOH to afford **5** (0.420 g, 60%) as a white solid; mp 226−228 °C. ^{1}H NMR (200 MHz, DMSO-d_6) 3.78 (s, 3H, OCH_3), 4.94 (s, 1H, SCH), 6.94−7.10 (m, 4H, H−Ar), 7.19−7.27 (m, 2H, H−Ar), 7.46 (dd, 1H, J = 2.5 and 8.8 Hz, H−Ar), 11.0 (bs, 1H, NH). GC-MS *m*/*z* (%): 351 (100) [M^+ + 1], 349 (100), 318 (8), 316 (6), 308 (8), 290 (8), 288 (6), 242 (5), 227 (9), 207 (38), 188 (6), 186 (7), 171 (7), 136 (44), 120 (10), 91 (9).

2-(3,5-Dibromo-2-methoxyphenyl)-6-trifluoromethyl-2*H*-1,4-benzothiazin-3(4*H*)-one (10). The title compound was prepared from 2-amino-4-(trifluoromethyl)benzenethiol hydrochloride and α-bromoarylacetic ester **20** by a procedure similar to that described for the preparation of derivative **5.** The crude product was crystallized from EtOH to yield derivative **10** (46%) as a crystalline whitish solid; mp 193−194 °C. ^{1}H NMR (200 MHz, DMSO-d_6) 3.70 (s, 3H, OCH_3), 5.24 (s, 1H, SCH), 7.25−7.32 (m, 3H, H−Ar), 7.51 (d, 1H, J = 8.4 Hz, H-8), 7.85 (d, 1H, J = 2.4 Hz, H-4′), 11.15 (bs, 1H, NH). GC-MS *m*/*z* (%): 499 (55) [M^+ + 1], 497 (100) [M^+], 495 (52) [M^+ − 1], 455 (10), 454 (19), 453 (10), 375 (9), 373 (8), 293 (13), 291 (19), 289 (9), 266 (17), 249 (6), 204 (50), 207 (33), 184 (6), 75 (10).

2-(5-Bromo-2-methoxyphenyl)-6-trifluoromethyl-2*H*-1,4-benzothiazin-3(4*H*)-one (6). A solution of 2-amino-4-(trifluoromethyl)benzenethiol hydrochloride (2.30 g, 10 mmol) in DMF (5 mL) was added dropwise to a suspension of α-bromoarylacetic ester **19** (3.52 g, 10 mmol) and K_2CO_3 (2.76 g, 20 mmol) in DMF (5 mL), and the resulting mixture was stirred at 70−80 °C for 4.5 h. After cooling, the mixture was poured into ice−water and the precipitate solid was filtered off, dried, and recrystallized from MeOH to afford **6** (3.43 g, 82%) as a white solid; mp 184−187 °C. ^{1}H NMR (200 MHz, acetone-d_6) 3.90 (s, 3H, OCH_3), 5.15 (s, 1H, SCH), 7.05 (d, 1H, J = 8.8 Hz, H-3′), 7.32 (d, 1H, J = 2.5 Hz, H-6′), 7.34−7.41 (m, 1H, H-7), 7.47−7.56 (m, 3H, H−Ar), 10.25 (bs, 1H, NH). GC-MS *m*/*z* (%): 419 (100) [M^+], 417 (99), 404 (6), 402 (7), 400 (8), 376 (15), 374 (15), 295 (10), 204 (25), 188 (8), 186 (6), 157 (6), 118 (6).

2-(5-Chloro-2-methoxyphenyl)-6-trifluoromethyl-2*H*-1,4-benzothiazin-3(4*H*)-one (8). The title compound was prepared following the same procedure used to preparation of **6** except α-bromoarylacetic ester **24** was used instead of **19**. The crude product was crystallized from EtOH to yield derivative **8** (45%) as white needles; mp 169−170 °C. ^{1}H NMR (200 MHz, $CDCl_3$) 3.86 (s, 3H, OCH_3), 5.18 (s, 1H, SCH), 6.86 (d, 1H, J = 8.8 Hz, H-3′), 7.15 (d, 1H, J = 2.5 Hz, H-6′), 7.17−7.22 (m, 1H, H-5), 7.23−7.29 (m, 2H, H−Ar), 7.38 (d, 1H, J = 8.1 Hz, H-8), 9.72 (bs, 1H, NH). GC-MS *m*/*z* (%): 375 (40) [M^+ + 1], 373 (100) [M^+ − 1], 354 (6), 340 (11), 330 (19), 312 (10), 204 (31), 167 (9), 155 (11), 142 (15), 125 (9), 111 (4), 91 (5).

2-(5-Bromo-2-hydroxyphenyl)-6-trifluoromethyl-2*H*-1,4-benzothiazin-3(4*H*)-one (7). Compound **6** (0.500 g, 1.2 mmol) was added in small portions under nitrogen atmosphere to a 1 M solution of BBr_3 in CH_2Cl_2 (15 mL, 15 mmol). The reaction was stirred for 4 h, quenched with water and then $CHCl_3$ (20 mL) was added. The organic layer was washed with water and brine, dried, and evaporated to dryness. The residue was crystallized from cyclohexane/EtOAc (8:2) to yield derivative **7** (0.422 g, 87%) as a whitish solid; mp 242−243 °C. ^{1}H NMR (200 MHz, acetone-d_6) 5.16 (s, 1H, SCH), 6.92 (d, 1H, J = 8.5 Hz, H-3′), 7.20−7.40 (m, 3H, H−Ar), 7.50−7.60 (m, 2H, H−Ar), 9.30 (bs, 1H, OH), 10.17 (bs, 1H, NH). GC-MS *m*/*z* (%): 404 (8) [M^+], 403 (46) [M^+ − 1], 401 (45) [M^+ − 2], 375 (16), 373 (15), 267 (17), 266 (100), 201 (28), 199 (30), 173 (10), 175 (8), 145 (8), 143 (10), 63 (10).

2-(5-Chloro-2-hydroxyphenyl)-6-trifluoromethyl-2*H*-1,4-benzothiazin-3(4*H*)-one (9). It was prepared following the same procedure used to prepare **7** starting from derivative **8**. The crude product was crystallized from cyclohexane/EtOAc (8:2) to yield derivative **9** (75%) as a whitish solid; mp 237−239 °C. ^{1}H NMR (200 MHz, acetone-d_6) 5.18 (s, 1H, SCH), 6.98 (d, 1H, J = 8.6 Hz, H-3′), 7.14 (d, 1H, J = 2.5 Hz, H-6′), 7.19 (dd, 1H, J = 2.5 and 8.6 Hz, H-4′), 7.32−7.37 (m, 1H, H-7), 7.51−7.56 (m, 2H, H−Ar), 9.26 (s, 1H, OH), 10.13 (bs, 1H, NH). GC-MS *m*/*z* (%): 361(20) [M^+], 359 (48), 330 (9), 326 (10), 204 (24), 193 (13), 192 (100), 167 (19), 161 (40), 141 (13), 112 (11), 77 (13), 75 (11), 51 (7).

2-(5-Bromo-2-hydroxyphenyl)-4-methyl-6-trifluoromethyl-2*H*-1,4-benzothiazin-3(4*H*)-one (13). It was prepared following the same procedure employed for the preparation of **7** starting from derivative **12**. The crude product was crystallized from cyclohexane/EtOAc (8:2) to yield the derivative **13** (56%) as a cream-colored solid; mp 167−168 °C. ^{1}H NMR (200 MHz, $CDCl_3$) 3.55 (s, 3H, NCH_3), 4.98 (s, 1H, SCH), 6.75 (d, 1H, J = 8.5 Hz, H-3′), 7.20−7.30 (m, 3H, H−Ar), 7.40−7.47 (m, 1H, H−Ar), 7.65 (d, 1H, J = 8.1 Hz, H-8), 7.95 (bs, 1H, OH). GC-MS *m*/*z* (%): 419 (48) [M^+ + 1], 417 (47) [M^+ − 1], 386 (22), 384 (22), 218 (43), 215 (11), 214 (20), 208 (10), 207 (17), 206 (100), 204 (17), 186 (14), 184 (12), 175 (22), 162 (26), 157 (11), 77 (16).

2-(5-Bromo-2-methoxyphenyl)-6-trifluoromethyl-2*H*-1,4-benzothiazin-3(4*H*)-one 1-oxide (11). Six g of Al_2O_3 (type 507 C neutral, Fluka) were equilibrated with the atmosphere into a 150 mL round-bottomed flask at 120 °C for at least 48 h; it was then cooled to 25 °C and a solution of *p*-toluensulfonic acid (0.08 mmol/g) in CH_2Cl_2 (20 mL) was added. After evaporation of the solvent at reduced pressure, water (0.5 mL) was added and the adsorbent was tumbled on a rotary evaporator at atmospheric pressure until uniformly free-flowing. A solution of **6** (1.00 g, 2.4 mmol) in $CHCl_3$ (100 mL) was added under stirring followed by Oxone (2.95 g, 4.8 mmol). The slurry was stirred at 25 °C for 48 h. The adsorbent was then filtered off by washing with EtOAc. The organic layer was washed with saturated aqueous solution of $FeSO_4$, dried, and evaporated to dryness. The residue was purified by flash chromatography using a step gradient of cyclohexane/EtOAc (8:2 to 4:6) as eluant to afford **11** (0.33 g, 32%) as a *diastereoisomeric mixture* in about 60/40 ratio. ^{1}H NMR (400 MHz, acetone-d_6) 3.86 (s, 1.2H, OCH_3), 3.96 (s, 1.8H, OCH_3), 5.52 (s, 0.6H, SCH), 5.77 (s, 0.4H, SCH), 7.13 (d, 0.4H, J = 8.8 Hz, H-3′), 7.15 (d, 0.6H, J = 8.8 Hz, H-3′), 7.31 (d, 0.6H, J = 2.4 Hz, H−Ar), 7.55−7.68 (m, 3H, H−Ar), 7.80 (d, 0.4H, J = 2.5 Hz, H−Ar), 7.91 (d, 0.6H, J = 7.7 Hz, H−Ar), 8.11 (d, 0.4H, J = 6.9 Hz, H−Ar), 10.50 (bs, 1H, NH). Anal. ($C_{16}H_{11}BrF_3NO_3S$) C, H, N.

2-(5-Bromo-2-methoxyphenyl)-4-methyl-6-trifluoromethyl-2*H*-1,4-benzothiazin-3(4*H*)-one (12). A mixture of derivative **6** (0.100 g, 0.24 mmol) and *t*-BuOK (0.030 g, 0.27 mmol) in dry DMF (5 mL) was stirred for 15 min. A solution of MeI (0.02 mL, 0.26 mmol) in dry DMF (5 mL) was then added dropwise. After 8 h, the mixture was poured into ice−water, acidified to pH ≈ 1 with 1 N HCl, and then extracted with EtOAc. The combined organic layers were washed with brine, dried, and evaporated to dryness. Crystallization from cyclohexane/EtOAc (8:2) of the crude product afforded **12** as a white solid (0.093 g, 90%); mp 145−146 °C. ^{1}H NMR (200 MHz, $CDCl_3$) 3.52 (s, 3H, NCH_3), 3.77 (s, 3H, OCH_3), 4.97 (s, 1H, SCH), 6.72 (d, 1H, J = 8.8 Hz, H-3′), 7.10 (d, 1H, J = 2.3 Hz, H-6′), 7.20−7.44 (m, 4H, H−Ar). GC-MS *m*/*z* (%): 434 (30) [M^+ + 2], 433 (100) [M^+ + 1], 432 (28) [M^+], 430 (91) [M^+ − 1], 400 (25), 398 (21), 387 (10), 291 (6), 218 (56), 215 (13), 214 (29), 212 (12), 210 (12), 207 (53), 204 (23), 202 (12), 188 (33), 186 (35), 157 (8), 91 (11).

2-(5-Bromo-2-methoxyphenyl)-6-trifluoromethyl-2*H*-1,4-benzothiazine-3(4*H*)-thione (14). Compound **6** (0.301 g, 0.72 mmol) and Lawesson's reagent (0.291 g, 0.72 mmol) were mixed in a glass tube. The glass tube was then placed in an alumina bath inside a domestic microwave oven (350 W) and irradiated for 1 min. The mixture was chromatographed on silica flash eluting with cyclohexane/EtOAc (8:2) to yield the thioxo derivative **14** as a yellowish solid (0.162 g, 52%); mp 212 dec °C. ^{1}H NMR (400 MHz, acetone-d_6) 3.95 (s, 3H, OCH_3), 5.58 (s, 1H, SCH), 7.05 (d, 1H, J = 8.8 Hz, H-3′), 7.10 (d, 1H, J = 2.4 Hz, H-6′), 7.41−7.44 (m, 2H, H−Ar), 7.50 (d, 1H, J = 8.2 Hz, H-8), 7.78−7.83 (m, 1H, H-5), 12.01 (bs, 1H, NH). Anal. ($C_{16}H_{11}BrF_3NOS_2$) C, H, N.

2-(5-Bromo-2-methoxyphenyl)-6-trifluoromethyl-3,4-dihydro-2*H*-1,4-benzothiazine (15). A 1 M solution of THF−BH_3 complex (0.90 mL, 0.90 mmol) was added dropwise to a solution of derivative **6** (0.205 g, 0.49 mmol) in dry THF (10 mL) at 0 °C. The solution was refluxed for 2 h, cooled, carefully diluted with MeOH (15 mL), and again refluxed for an additional 30 min. After cooling, the reaction was acidified with 2 N HCl refluxed for 1 h, cooled, basified with 10% NaOH, and finally extracted with EtOAc. The combined organic layers were washed with brine, dried, and evaporated to dryness to give **15** (0.186 g, 94%) as a semisolid. ^{1}H NMR (200 MHz, $CDCl_3$) 3.50−3.60 and 3.65−3.70 (each m, 1H, CH_2), 3.85 (s, 3H, OCH_3), 4.31 (bs, 1H, NH), 4.82 (dd, 1H, J = 3.0 and 7.8 Hz, SCH), 6.74−6.81 (m, 2H, H-5 and H-3′), 6.85−6.92 (m, 1H, H-7), 7.14 (d, 1H, J = 8.1 Hz, H-8), 7.40 (dd, 1H, J = 2.3 and 8.6 Hz, H-4′), 7.45 (d, 1H, J = 2.3 Hz, H-6′). GC-MS *m*/*z* (%): 406 (16) [M^+ + 2], 405 (83) [M^+ + 1], 404 (17) [M^+], 403 (82) [M^+ − 1], 385 (5), 291 (4), 25 (11), 204 (100), 201 (56), 184 (9), 171 (14), 118 (8), 90 (6).

2-(2-Methoxyphenyl)-6-trifluoromethyl-3,4-dihydro-2*H*-1,4-benzothiazine (16). A solution of derivative **6** (0.400 g, 0.96 mmol) in dry THF (10 mL) was added dropwise to a suspension of $LiAlH_4$ (0.181 g, 4.78 mmol) in dry THF (15 mL). The mixture was stirred for 4 h, and then EtOAc (15 mL) was carefully added. After 10 min, the mixture was filtered through a pad of celite and the filter washed with EtOAc (50 mL). The filtrate was evaporated under reduced pressure, and the resulting solid was stirred with aqueous HCl 0.2 N (40 mL) and then extracted with EtOAc (60 mL). The organic layer was separated and washed consecutively with water and brine, dried, and the solvent removed under reduced pressure. The crude product was purified by flash chromatography eluting with cyclohexane/EtOAc (9:1) to yield the desired compound **16** as a yellow solid (0.065 g, 21%); mp 88−89 °C. ^{1}H NMR (200 MHz, methanol-d_4) 3.45 (dd, 1H, J = 8.2 and 12.2 Hz, C*H*αHβ), 3.63 (dd, 1H, J = 12.2 and 3.1 Hz, CHα*H*β), 3.78 (s, 3H, OCH_3), 4.66 (s, 1H, NH), 4.73 (dd, 1H, J = 3.1 and 8.2 Hz, SCH), 6.69−6.79 (m, 4H, H−Ar), 7.03 (d, 1H, J = 8.0 Hz, H-8), 7.18−7.26 (m, 2H, H−Ar). GC-MS *m*/*z* (%): 325 (94) [M^+], 306 (5), 292 (6), 216 (7), 204 (100), 184 (7), 121 (53), 107 (5), 91 (24), 77 (5).

2-[(5-Bromo-2-methoxyphenyl)hydroxymethyl]-6-trifluoromethyl-2*H*-1,4-benzothiazin-3(4*H*)-one (17a, 17b). A nitrogen-flushed solution of freshly distilled diisopropylamine (0.951 g, 1.32 mL, 9.4 mmol) in dry THF (20 mL) was cooled to −78 °C and a 1.6 M *n*-BuLi solution in hexane (5.9 mL, 9.4 mmol) was added dropwise. The mixture was stirred for 30 min and then a solution 6-(trifluoromethyl)-2*H*-1,4-benzothiazin-3(4*H*)-one[37] (1.00 g, 4.3 mmol) in dry THF (20 mL) was added. The resulting dark-red mixture was warmed to −55 °C, and a solution of 5-bromo-2-methoxybenzaldehyde (0.925 g, 4.3 mmol) in dry THF (20 mL) was added dropwise. During the addition, the solution color changed from dark-red to orange to yellow. The mixture was stirred at −55 °C for 30 min, and then at room temperature for an additional 3 h. Quenching with aqueous saturated solution of NH_4Cl (60 mL), extraction with Et_2O, drying, and solvent evaporation to dryness gave a mixture of the diastereomeric aldols, which were separated by flash chromatography eluting with benzene/EtOAc (9:1).

Fractions containing the faster moving compound, upon evaporation, gave *threo-isomer* derivative **17a** (0.344 g, 18%) as white needles; mp 224−225 °C. ^{1}H NMR (400 MHz, $CDCl_3$) 3.38 (d, 1H, J = 5.0 Hz, CHO*H*), 3.79 (s, 3H, OCH_3), 4.03 (d, 1H, J = 4.5 Hz, SCH), 5.56 (dd, 1H, J = 4.5 and 5.0 Hz, C*H*OH), 6.71 (d, 1H, J = 8.8 Hz, H-3′), 7.08 (bs, 1H, H−Ar), 7.30−7.50 (m, 2H, H−Ar), 7.60−7.61 (m, 1H, H−Ar), 9.01 (bs, 1H, NH). Anal. ($C_{17}H_{13}BrF_3NO_3S$) C, H, N. The evaporation of fractions containing the later isomer gave *erythro-isomer* **17b** (0.792 g, 36%) as a white solid; mp 199−200 °C. ^{1}H NMR (400 MHz, $CDCl_3$) 3.84 (s, 3H, OCH_3), 3.86 (d, 1H, J = 7.5 Hz, CHO*H*), 3.92 (d, 1H, J = 8.5 Hz, SCH), 5.02 (dd, 1H, J = 7.5 and 8.5 Hz, C*H*OH), 6.77 (d, 1H, J = 8.7 Hz, H-3′), 7.10 (bs, 1H, H−Ar), 7.26−7.28 (m, 1H, H−Ar), 7.34−7.39 (m, 3H, H−Ar), 9.10 (bs, 1H, NH). Anal. ($C_{17}H_{13}BrF_3NO_3S$) C, H, N.

(2*Z*)-2-(5-Bromo-2-methoxybenzylidene)-6-trifluoromethyl-2*H*-1,4-benzothiazin-3(4*H*)-one (18). A solution of MsCl (0.046 g, 0.40 mmol) in CH_2Cl_2 (15 mL) and then Et_3N (0.044 g, 0.44 mmol) in CH_2Cl_2 (15 mL) were added at room temperature to a solution of diastereoisomeric mixture **17** (0.180 g, 0.40 mmol) in CH_2Cl_2 (50 mL). The reaction mixture was stirred for 15 min, then warmed to 40 °C for 1 h. After cooling, the mixture was washed with water, dried, and evaporated to dryness. The residue was purified by crystallization from CH_2Cl_2 to afford the derivative **18** as a pale-yellow solid (0,115 g; 67%); mp 255−256 °C. ^{1}H NMR (200 MHz, DMSO-d_6) 3.83 (s, 3H, OCH_3), 7.09 (d, 1H, J = 8.8 Hz, H-3′), 7.32 (d, 1H, J = 8.5 Hz, H-5′), 7.56−7.67 (m, 3H, H-6′, H-7, H-8), 7.85 (s, 1H, H-vinyl), 11.3 (bs, 1H, NH). Anal. ($C_{17}H_{11}BrF_3NO_2S$) C, H, N.

Ethyl Bromo-(5-bromo-2-methoxyphenyl)acetate (19), Ethyl Bromo-(3,5-dibromo-2-methoxyphenyl)acetate (20) and Ethyl Dibromo-(5-bromo-2-methoxyphenyl)acetate (21). A solution of Br_2 (0.32 mL, 6.3 mmol) in CCl_4 (20 mL) was added dropwise at room temperature to a solution of ethyl (2-methoxyphenyl)acetate[36] (0.408 g, 2.1 mmol) in CCl_4 (20 mL). The reaction mixture was irradiated with a 300 W lamp and refluxed for 2 h. After cooling, the mixture was quenched with an aqueous solution of $Na_2S_2O_3$, then the organic layer was separated and evaporated to dryness. The obtained oil residue was dissolved in EtOAc and sequentially washed with aqueous saturated solution of $NaHCO_3$ and brine. The organic layer was then dried and evaporated to dryness, and the residue was subjected to flash chromatography eluting with cyclohexane/EtOAc (95:5) to afford the fastest moving tribromo derivative **20**, followed by the intermediate moving dibromo derivative **19** and the slowest moving one **21**.

Compound **19**, white solid (0.318 g, 43%); mp 52−53 °C. ^{1}H NMR (200 MHz, $CDCl_3$) 1.30 (t, 3H, J = 7.1 Hz, CH_2CH_3), 3.89 (s, 3H, OCH_3), 4.29 (q, 2H, J = 7.1 Hz, CH_2CH_3), 5.80 (s, 1H, CHCO), 6.80 (d, 1H, J = 8.8 Hz, H-3), 7.45 (dd, 1H, J = 2.4 and 8.8 Hz, H-4), 7.79 (d, 1H, J = 2.4 Hz, H-6).

Compound **20**, semisolid (0.150 g, 17%). ^{1}H NMR (200 MHz, $CDCl_3$) 1.28 (t, 3H, J = 7.1 Hz, CH_2CH_3), 3.91 (s, 3H, OCH_3), 4.23 (q, 2H, J = 7.1 Hz, CH_2CH_3), 5.73 (s, 1H, CHCO), 7.67 and 7.77 (each d, 1H, J = 2.4 Hz, H−Ar).

Compound **21**, whitish solid; (0.190, 21%); mp 92−94 °C. ^{1}H NMR (200 MHz, $CDCl_3$) 1.31 (t, 3H, J = 7.1 Hz, CH_2CH_3), 3.88 (s, 3H, OCH_3), 4.34 (q, 2H, J = 7.1 Hz, CH_2CH_3), 6.79 (d, 1H, J

= 8.7 Hz, H-3), 7.52 (dd, 1H, J = 2.4 and 8.7 Hz, H-4), 8.14 (d, 1H, J = 2.4 Hz, H-6).

Ethyl Bromo-(5-chloro-2-methoxyphenyl)acetate (24). Sulfuryl chloride (5.7 mL, 0.70 mmol) was added dropwise over 1 h to a stirred solution of (2-methoxyphenyl)acetic acid (8.30 g, 50 mmol) in dry THF (50 mL) cooled to −10 °C. After the complete addition, the reaction mixture was poured into ice−water and the obtained slurry was stirred for 3 h. The obtained white precipitate was collected by filtration, washed with water and dried to afford *(5-chloro-2-methoxyphenyl)acetic acid* **22**[44] (9.33 g, 93%) as an off-white solid; mp 125−127 °C.

A solution of **22** (8.22 g, 41 mmol) in dry DMF (20 mL) was added dropwise to a stirred suspension of anhydrous K_2CO_3 (11.3 g, 82 mmol) and dry DMF (30 mL). EtI (35 mL, 44 mmol) was then added dropwise. The reaction mixture was warmed to 50 °C for 3 h and then poured into water and extracted with CH_2Cl_2. The combined organic layers were sequentially washed with 0.1 N HCl and brine, then dried and evaporated to dryness. The residue was purified by vacuum distillation (90 °C, 25 mmHg) to yield *ethyl (5-chloro-2-methoxyphenyl)acetate* **23** as dark-brown viscous oil (8.34 g, 89%) suitable for the next step without any further purification. ^{1}H NMR (200 MHz, $CDCl_3$) 1.25 (t, 3H, J = 7.1 Hz, CH_2CH_3), 3.57 (s, 2H, CH_2CO), 3.79 (s, 3H, OCH_3), 4.15 (q, 2H, J = 7.1 Hz, CH_2CH_3), 6.81 (d, 1H, J = 8.5 Hz, H-3), 7.13−7.23 (m, 2H, H−Ar).

Compound **23** (7.32 g, 32 mmol) was converted to the title compound by using the same procedure as for **19**, except that a longer reaction time (10 h) was required. The obtained crude residue was purified by flash chromatography eluting with cyclohexane/CH_2Cl_2 (1:1) to afford the bromoderivative **24** (4.13 g, 42%) as a white solid; mp 48−49 °C. ^{1}H NMR (200 MHz, $CDCl_3$) 1.23 (t, 3H, J = 7.1 Hz, CH_2CH_3), 3.85 (s, 3H, OCH_3), 4.20 (q, 2H, J = 7.1 Hz, CH_2CH_3), 5.71 (s, 1H, CHCO), 6.74 (d, 1H, J = 8.8 Hz, H-3), 7.21 (dd, 1H, J = 2.6 and 8.8 Hz, H-4), 7.56 (d, 1H, J = 2.6 Hz, H-6).

Vasorelaxant Activity. All the experimental procedures were carried out following the guidelines of the European Community Council Directive 86-609. To determine a possible vasodilator mechanism of action, the compounds were tested on isolated thoracic aortic rings of male normotensive Wistar rats (250−350 g). The rats were sacrificed by cervical dislocation under light ether anesthesia and bled. The aortae were immediately excised and freed of extraneous tissues. The endothelial layer was removed by gently rubbing the intimae surface of the vessels with a hypodermic needle. Five mm wide aortic rings were suspended, under a preload of 2 g, in 20 mL organ baths, containing Tyrode solution (composition of saline in mM: NaCl 136.8; KCl 2.95; $CaCl_2$ 1.80; $MgSO_4$ $7H_2O$ 1.05; NaH_2PO_4 0.41; $NaHCO_3$ 11.9; glucose 5.5), thermostatted at 37 °C and continuously gassed with a mixture of O_2 (95%) and CO_2 (5%). Changes in tension were recorded by means of an isometric transducer (Basile model 7005), connected to a unirecord microdynamometer (Basile model 7050). After an equilibration period of 60 min, the endothelial removal was confirmed by administering acetylcholine (ACh) (10 μM) to KCl (20 mM)-precontracted vascular rings. A <10% relaxation of the KCl-induced contraction was indicative of an acceptable lack of the endothelial layer, while the organs, showing a relaxation ≥10% (i.e., significant presence of the endothelium), were discarded. Thirty to forty min after the confirmation of the endothelium removal, the aortic preparations were contracted by treatment with a single concentration of KCl (20 mM). When the contraction reached a stable plateau, 3-fold increased concentrations of the tested compounds or of the reference compound **1** were added cumulatively. Preliminary experiments showed that both the KCl (20 and 60 mM)-induced contractions remained in a stable tonic state for at least 40 min. In other sets of experiments, the nonselective potassium channel blocker TEA (10 mM) or the BK-selective blockers IbTX (100 nM) were added, after the KCl (20 mM)-induced contraction, followed by the administration of selected compounds.

The reference drug **1** (Sigma) was dissolved (10 mM) in EtOH 95% and further diluted in Tyrode solution. ACh chloride (Sigma) was dissolved (100 mM) in EtOH 95% and further diluted in bidistilled water, KCl was dissolved (2 M) in Tyrode solution, TEA (Sigma) was dissolved (10 mM) in Tyrode solution, IbTX (Sigma) was dissolved (100 nM) in bidistilled water. All the synthesized derivatives **3**−**18** were dissolved (10 mM) in DMSO and further diluted in bidistilled water. All the solutions were freshly prepared immediately before the pharmacological experimental procedures. Previous experiments showed a complete ineffectiveness of the administration of the vehicle.

The vasorelaxing efficacy was evaluated as the maximal vasorelaxing response, expressed as the percentage (%) of the contractile tone induced by KCl 20 mM. When the limit concentration of 10 μM (the highest concentration that could be administered) of the tested compounds did not reach the maximal effect, the efficacy parameter represented the vasorelaxing response, expressed as the percentage of the contractile tone induced by KCl 20 mM, evoked by this limit concentration. The potency parameter was expressed as pIC_{50}, which was calculated as the negative logarithm of the molar concentration of the test compounds, evoking a half-reduction of the contractile tone induced by 20 mM KCl. The pIC_{50} could not be calculated for those compounds that had an efficacy parameter close to or less than 50%. The efficacy and potency parameters were expressed as the mean ± standard error for 5−10 experiments. The student t-test was selected for the statistical analysis; P < 0.05 was considered a significant statistical difference. Experimental data were analyzed by a computer fitting procedure (software Graph Pad Prism 3.0).

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Supporting Information Available: A Table of combustion analysis data for target compounds **11**, **14**, **17a**−**b**, and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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