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Hydroxylated Analogues of ATP-Sensitive Potassium Channel Openers Belonging to the Group of 6- and/or 7-Substituted 3-Isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides: Toward an Improvement in Sulfonylurea Receptor 1 Selectivity and Metabolism Stability

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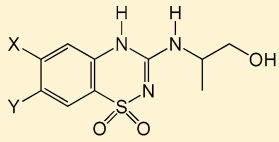
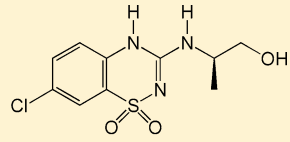
Supporting Information

ABSTRACT: Diversely substituted 3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides are known to be potent K_{ATP} channel openers, with several drugs being selective for the SUR1/Kir6.2 channel subtype. This work examined the biological activity, tissue selectivity, and in vitro metabolic stability of hydroxylated analogues of 3-isopropylaminobenzothiadiazine dioxides. Because of the presence of a chiral center, the *R* and *S* isomers were prepared separately and characterized. *R* isomers were systematically found to be more potent and more selective than *S* isomers on pancreatic tissue (compared to vascular smooth muscle tissue), leading to compounds with an improved sulfonylurea receptor 1 (SUR1) selectivity. An in vitro metabolic study revealed that 7-chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**1a**) was rapidly biotransformed and led in part to a mixture of the corresponding (*R*)- and (*S*)-3-(1-hydroxy-2-propyl)amino-substituted derivatives. Radioisotopic experiments characterized one of the most potent and SUR1-selective enantiomers, (*R*)-7-chloro-3-(1-hydroxy-2-propyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **13a**, as being a K_{ATP} channel opener. Moreover, **13a** exhibited an enhanced metabolic stability. Such a compound can be considered as a new lead candidate displaying improved physicochemical (hydrosolubility) and pharmacological (tissue selectivity) properties as well as improved metabolic stability compared to its nonhydroxylated counterpart, **1a**.



X = H, Cl, Br
Y = H, F, Cl, OCH_3



13a

INTRODUCTION

ATP-sensitive potassium channels (K_{ATP} channels) are transmembrane structures that allow the passive flux of potassium ions through the cell membrane. Regulated by changes in intracellular adenosine triphosphate (ATP) concentrations,[1−4] these channels have been described as complex octameric structures composed of two different subunits: Kir6.*x* (inwardly rectifying potassium channel) and SUR*x* (sulfonylurea receptor), the latter containing the regulatory sites for most K_{ATP} channel modulators.[5] Several isoforms of Kir6.*x* (Kir6.1 and Kir6.2) and SUR*x* (SUR1, SUR2A, and SUR2B) have been reported.[6,7] The combination of these different subunits leads to specific K_{ATP} channel subtypes diversely distributed throughout the different tissues.[8] The SUR1/Kir6.2 K_{ATP} channel subtype is found in the endocrine pancreas and the brain; the SUR2A/Kir6.2 K_{ATP} channel subtype is expressed in the cardiac and skeletal muscles, while the SUR2B/Kir6.1 and SUR2B/Kir6.2 combinations are mainly found in smooth muscles.[9] A mitochondrial K_{ATP} channel has also been described, but the molecular structure of this channel is yet to be clearly established.[10−12] Activation of K_{ATP} channels induces an increase in the outflow of potassium ions through the cytoplasmic membrane and hyperpolarizes the cell membrane. The physiological impact of this hyperpolarization is highly dependent on the tissue localization of the channel. Thus, potassium channel openers (PCOs) can interfere with several physiological processes, such as the release of insulin

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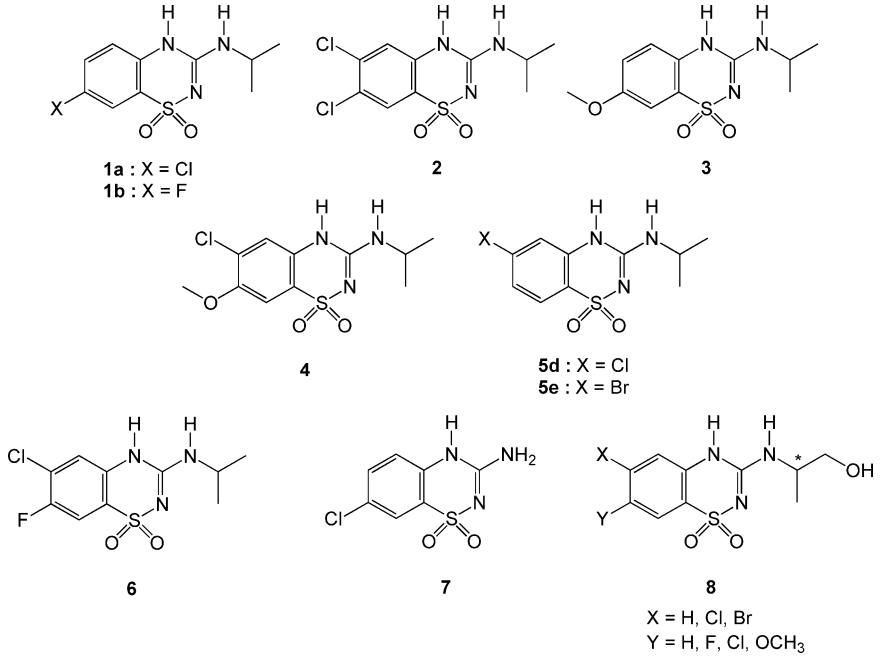

Figure 1. Chemical structure of diversely substituted 3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**1**−**6**) reported as SUR1/Kir6.2-type potassium channel activators. Compound **7** is a metabolite of **1a**, resulting from N-dealkylation. General formula **8** illustrates the newly synthesized hydroxylated analogues of compounds **1**−**6**. The asterisk refers to the presence of a chiral carbon atom.

from pancreatic β-cells and contractile activity in smooth muscle cells.[13,14] For several years, selective activation of pancreatic K_{ATP} channels has been known to be of therapeutic value for the treatment of critical metabolic disorders such as diabetes, obesity, and hyperinsulinemia.[15,16] Taking into account these potential therapeutic benefits, we have developed a series of new compounds belonging to 3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides over the past decade. Among these drugs, compounds **1**−**6** (Figure 1) have been described as potent and selective pancreatic PCOs.[17−20] Structure−activity relationships, assessed from previous studies, indicated that the presence of a small branched alkylamino chain at position 3 (i.e., an isopropylamino chain) as well as the presence of one or two halogen atoms (preferably Cl or F) at position(s) 6 and/or 7 and/or the presence of a small electron-donating group (i.e., OMe) at position 7 were favorable to both in vitro activity and selectivity for pancreatic tissue.[17−21] However, a recent in vitro metabolic study demonstrated the rapid biotransformation of compound **1a**, by hydroxylation, to compound **8a** (X = H, and Y = Cl) or, by dealkylation of the alkylamino side chain, to compound **7** (Figure 1).[22]

Therefore, within the framework of drug discovery and lead optimization processes of this series of compounds, we decided to explore the impact of the introduction of a hydroxy group onto the 3-alkylamino side chain on biological activity, tissue selectivity, and biotransformation. A series of new compounds (see general structure **8** in Figure 1) bearing a hydroxylated alkyl chain at position 3, one or two halogen atoms, and/or a methoxy group at position 6 or 7 were synthesized and evaluated as putative K_{ATP} channel openers on two in vitro pharmacological models. Moreover, and according to the position of the hydroxy group on the alkyl side chain, a stereogenic center was introduced onto the molecule leading to two possible stereoisomers, the *R* and *S* enantiomers. Both enantiomers were then prepared to confirm the influence of stereochemistry on biological activity.

Furthermore, two studies of the metabolism of compound **1a** and its hydroxylated derivative, **13a**, were conducted to identify the major hydroxylated stereoisomer generated during biotransformation of **1a** by hepatic microsomes and to define whether the introduction of a hydroxy group on the alkyl chain at position 3 could improve its metabolic stability.

Lastly, radioisotopic experiments were conducted with **13a** to confirm that the compound behaved as a specific K_{ATP} channel opener on pancreatic β-cells (SUR1/Kir6.2-type K_{ATP} channel opener).

CHEMISTRY

The synthetic pathways giving access to the 6- and/or 7-substituted 3-hydroxyalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **8**, **13**, and **14** are described in Scheme 1. The key intermediates for the synthesis of the 6- and 7-substituted compounds were the previously reported or newly synthesized 3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **12**.[17,19,20] Such intermediates were obtained from the corresponding anilines **9** in three steps (Scheme 1). The first step is the well-known Girard reaction, which allows ring closure through Friedel−Craft conditions. The cyclic sulfonylureas (**10**) obtained were then transformed into their sulfonylthiourea analogues (**11**) by the action of phosphorus pentasulfide in pyridine. In the next step, the 3-thioxo-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **11** were alkylated with methyl iodide to give the corresponding 3-methylsulfanyl-substituted key intermediates **12**. These compounds were then heated for several hours with the appropriate racemic or optically pure 1-hydroxy-2-propylamine at 150 °C, leading to the expected racemic mixtures **8**, *R* stereoisomers **13**, and *S* stereoisomers **14**.

The key intermediate for the synthesis of the 6,7-dichloro-substituted compounds was the previously described 6,7-dichloro-3-(1*H*-imidazol-1-yl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **11c**.[18] This intermediate was obtained from 2-amino-

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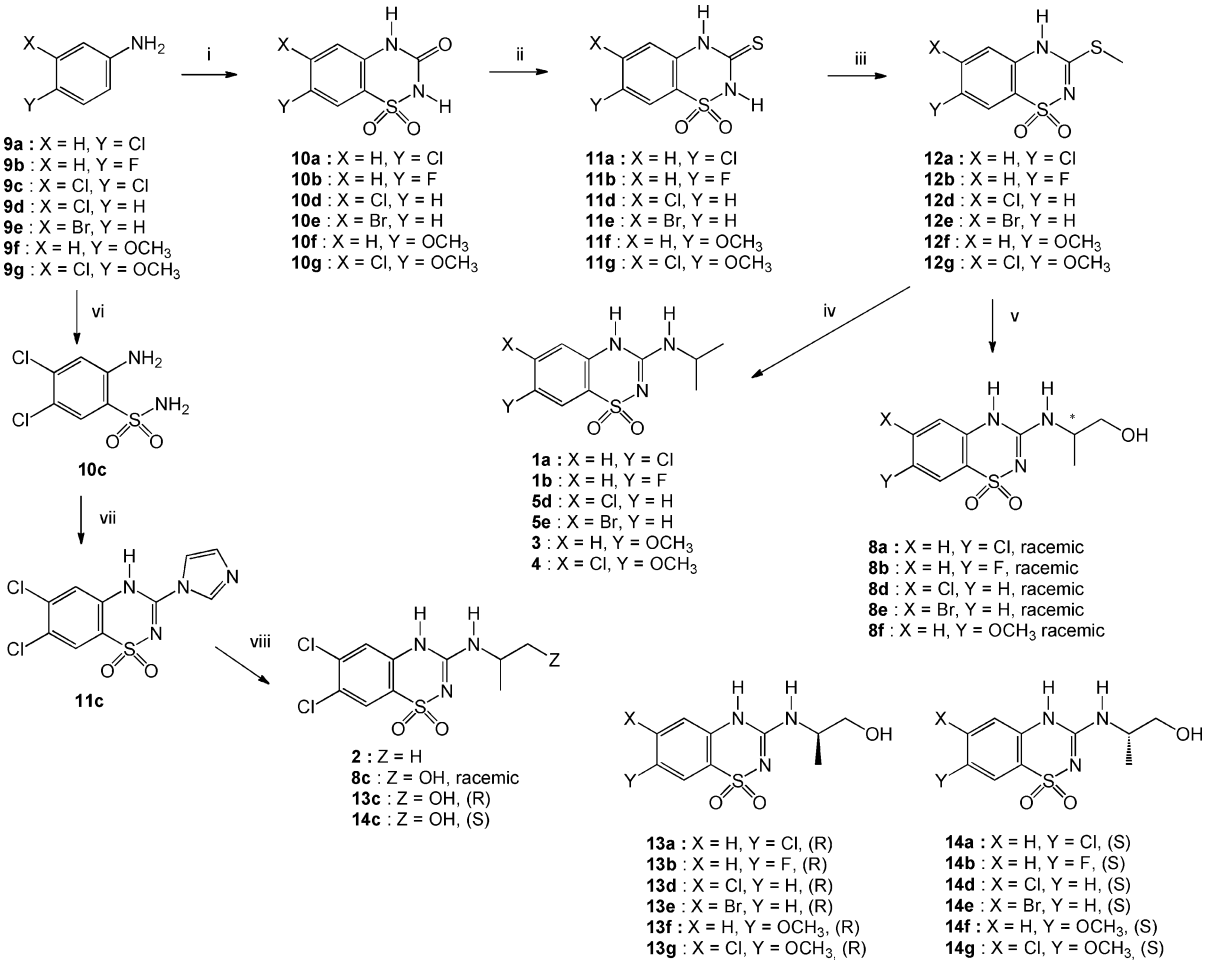
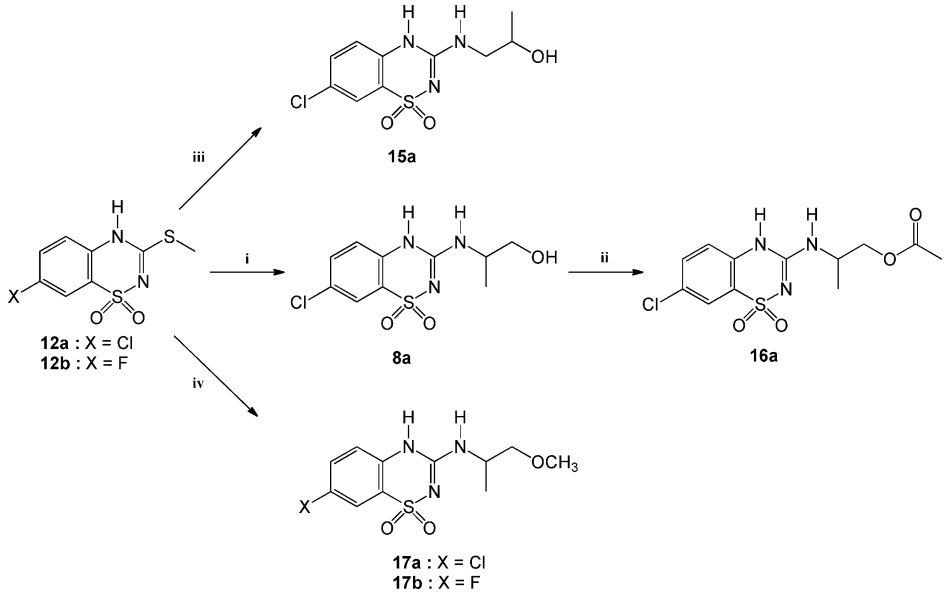
Scheme 1. [a]



[a](i) (1) $ClSO_2NCO$, CH_3NO_2; (2) $AlCl_3$; (ii) P_2S_5, pyridine; (iii) CH_3I, $NaHCO_3$, CH_3OH/H_2O; (iv) isopropylamine, Δ; (v) 1-hydroxy-2-propylamine, Δ; (vi) (1) $ClSO_3H$; (2) NH_4OH; (vii) 1,1′-thiocarbonyldiimidazole; (viii) isopropylamine or 1-hydroxy-2-propylamine, Δ.

Scheme 2. [a]



[a](i) (*R*,*S*)-1-hydroxy-2-propylamine, Δ; (ii) Ac_2O; (iii) (*R*,*S*)-2-hydroxypropylamine, Δ; (iv) (*R*,*S*)-1-methoxy-2-propylamine, Δ.

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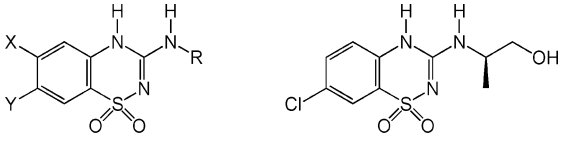
Table 1. Effects of Diversely Substituted 4*H*-1,2,4-Benzothiadiazine 1,1-Dioxides on Secretion of Insulin from Rat Pancreatic Islets and on the Contractile Activity of K^+-Depolarized Rat Aorta Rings



13a

	X	Y	R	RIS[a] (10 μM)	RIS[a] (1 μM)	RIS[a] (0.1 μM)	IC_{50}[b] (μM)	EC_{50}[c] (μM)	SI[d]
15a	H	Cl	$CH_2CH(CH_3)OH$	59.2 ± 2.6 (21) at 50 μM		nd[f]	>50	>300 (4)	nd[f]
8a	H	Cl	$CH(CH_3)CH_2OH$	9.4 ± 0.7 (22)	94.0 ± 4.7 (23)	nd[f]	3.03	230 ± 27 (6)	76
17a	H	Cl	$CH(CH_3)CH_2OCH_3$	95.0 ± 5.1 (24)	nd[f]	nd[f]	38.9	nd[f]	nd[f]
16a	H	Cl	$CH(CH_3)CH_2OCOCH_3$	27.0 ± 2.1 (10)	85.9 ± 4.8 (24)	nd[f]	3.58	nd[f]	nd[f]
13a	H	Cl	(*R*)-$CH(CH_3)CH_2OH$	8.5 ± 0.6 (13)	41.7 ± 2.8 (14)	97.2 ± 4.9 (22)	0.62	189 ± 17 (10)	305
14a	H	Cl	(*S*)-$CH(CH_3)CH_2OH$	77.3 ± 4.7 (24)	nd[f]	nd[f]	37.5	124 ± 11 (7)	3.3
1a[e]	H	Cl	$CH(CH_3)_2$	4.8 ± 0.4 (32)	36.2 ± 2.4 (31)	90.4 ± 3.5 (23)	0.48	31.8 ± 3.9 (4)	66
8b	H	F	$CH(CH_3)CH_2OH$	32.4 ± 2.0 (12)	81.7 ± 4.0 (23)	nd[f]	3.78	>300 (4)	>79
17b	H	F	$CH(CH_3)CH_2OCH_3$	82.6 ± 4.7 (21)	nd[f]	nd[f]	26.5	nd[f]	nd[f]
13b	H	F	(*R*)-$CH(CH_3)CH_2OH$	12.4 ± 1.3 (12)	71.8 ± 5.7 (16)	nd[f]	2.05	>300 (5)	>146
14b	H	F	(*S*)-$CH(CH_3)CH_2OH$	70.8 ± 4.6 (15)	nd[f]	nd[f]	>10	nd[f]	nd[f]
1b	H	F	$CH(CH_3)_2$	3.7 ± 0.6 (13)	47.3 ± 3.7 (23)	96.9 ± 4.9 (16)	0.76	43.3 ± 10.7 (5)	57
8c	Cl	Cl	$CH(CH_3)CH_2OH$	16.0 ± 1.1 (11)	59.8 ± 4.7 (14)	nd[f]	1.41	17.6 ± 0.7 (5)	12.5
13c	Cl	Cl	(*R*)-$CH(CH_3)CH_2OH$	5.1 ± 0.4 (15)	21.1 ± 1.8 (16)	92.5 ± 5.4 (24)	0.35	7.5 ± 0.2 (4)	21.4
14c	Cl	Cl	(*S*)-$CH(CH_3)CH_2OH$	51.0 ± 2.5 (15)	99.3 ± 5.6 (22)	nd[f]	8.67	17.4 ± 2.9 (6)	2.0
2[e]	Cl	Cl	$CH(CH_3)_2$	6.3 ± 0.7 (12)	13.2 ± 1.0 (26)	84.9 ± 4.5 (21)	0.28	2.3 ± 0.2 (8)	8.2
8d	Cl	H	$CH(CH_3)CH_2OH$	13.4 ± 1.4 (13)	67.0 ± 5.0 (14)	nd[f]	1.81	>200 (5)	>110
13d	Cl	H	(*R*)-$CH(CH_3)CH_2OH$	5.0 ± 0,3 (13)	35.6 ± 2.6 (14)	97.3 ± 5.6 (23)	0.52	>200 (6)	>385
14d	Cl	H	(*S*)-$CH(CH_3)CH_2OH$	99.1 ± 5,9 (16)	nd[f]	nd[f]	>10	>200 (3)	nd[f]
5d[e]	Cl	H	$CH(CH_3)_2$	7.5 ± 0.8 (15)	13.2 ± 1.2 (16)	76.7 ± 4.3 (15)	0.23	53.4 ± 3.6 (4)	232
8e	Br	H	$CH(CH_3)CH_2OH$	10.0 ± 0.7 (16)	66.8 ± 3.8 (13)	nd[f]	1.73	nd[f]	nd[f]
13e	Br	H	(*R*)-$CH(CH_3)CH_2OH$	9.7 ± 0.9 (15)	65.8 ± 3.1 (21)	nd[f]	1.67	241 ± 22 (6)	144
14e	Br	H	(*S*)-$CH(CH_3)CH_2OH$	77.6 ± 6.7 (22)	nd[f]	nd[f]	>10	nd[f]	nd[f]
5e	Br	H	$CH(CH_3)_2$	4.1 ± 0.3 (14)	18.2 ± 1.4 (14)	68.9 ± 3.8 (16)	0.20	55.7 ± 2.0 (6)	279
8f	H	OCH_3	$CH(CH_3)CH_2OH$	42.1 ± 4.6 (21)	97.9 ± 5.0 (23)	nd[f]	6.31	nd[f]	nd[f]
13f	H	OCH_3	(*R*)-$CH(CH_3)CH_2OH$	32.8 ± 1,9 (16)	99.3 ± 5.4 (20)	nd[f]	4.93	>300 (5)	>61
14f	H	OCH_3	(*S*)-$CH(CH_3)CH_2OH$	84.1 ± 4,9 (15)	nd[f]	nd[f]	>10	nd[f]	nd[f]
3[e]	H	OCH_3	$CH(CH_3)_2$	8.5 ± 0.9 (24)	67.6 ± 4.3 (20)	nd[f]	1.75	274 ± 19 (5)	157
13g	Cl	OCH_3	(*R*)-$CH(CH_3)CH_2OH$	22.4 ± 1.6 (14)	81.0 ± 3.9 (21)	nd[f]	2.98	53.1 ± 2.0 (4)	17.8
14g	Cl	OCH_3	(*S*)-$CH(CH_3)CH_2OH$	88.6 ± 6.2 (20)	nd[f]	nd[f]	>10	nd[f]	nd[f]
4[e]	Cl	OCH_3	$CH(CH_3)_2$	7.7 ± 0.7 (15)	20.6 ± 1.9 (13)	73.8 ± 4.1 (19)	0.24	37.0 ± 2.7 (6)	154

[a]RIS is the percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose [mean ± SEM (*n*)]. [b]Estimated IC_{50} is the drug concentration giving 50% inhibition of insulin release. [c]EC_{50} is the drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings [mean ± SEM (*n*)]. [d]SI is the selectivity index (=EC_{50}/IC_{50}). [e]Published compounds and results (refs [17−19]). [f]Not determined.

4,5-dichlorobenzenesulfonamide **10c** by a ring closure reaction using 1,1′-thiocarbonyldiimidazole (Scheme 1). Intermediate **10c** was synthesized by a classical chlorosulfonation reaction on 3,4-dichloroaniline **9c** followed by treatment with aqueous ammonia.[18]

Compound **15a**, the position isomer of **8a**, was prepared by replacement of 1-hydroxy-2-propylamine with racemic 2-hydroxypropylamine in the last step (Scheme 2).

Compound **16a**, bearing a racemic 1-acetoxy-2-propylamino chain at position 3, was synthesized by reacting acetic anhydride on (*R*,*S*)-7-chloro-3-(1-hydroxy-2-propyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**8a**) (Scheme 2).

The 3-[(1-methoxy)-2-propyl]amino-substituted compounds **17a** and **17b** were obtained by means of the use of 1-methoxy-2-propylamine on intermediates **12a** and **12b**, respectively (Scheme 2).

RESULTS AND DISCUSSION

Two in vitro models were used to assess the biological activity of the original drugs on two different tissues expressing K_{ATP} channels. The first one determined the ability of the newly prepared compounds to inhibit the glucose-induced secretion of insulin from rat pancreatic islets. Data collected with this model were expressed as the percentage of residual insulin release recorded at different drug concentrations and, for several compounds, as the estimated IC_{50} values, expressing the drug concentration leading to 50% inhibition of insulin release. The results obtained within each series of compounds were compared to previously reported data[17−19] obtained with the nonhydroxylated 3-isopropylamino-substituted counterparts **1a**, **1b**, **2**−**4**, **5d**, and **5e** (Figure 1).

In the 7-chloro series, four different alkylamino chains were introduced at position 3: the (*R*,*S*)-(2-hydroxypropyl)amino (compound **15a**), the (*R*,*S*)-(1-hydroxy-2-propyl)amino (com-

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pound **8a**), the (*R*,*S*)-(1-methoxy-2-propyl)amino (compound **17a**), and the (*R*,*S*)-(1-acetoxy-2-propyl)amino (compound **16a**) chains. The comparison between the inhibitory effects on insulin secretion of the latter drugs with that of compound **1a** (Table 1) indicated that the presence of an (*R*,*S*)-(1-hydroxy-2-propyl)amino chain, which is structurally close to the isopropylamino chain, led to a potent inhibitor of the insulin-releasing process (compound **8a**). Alkylation and, to a lesser extent, acylation of the hydroxy group of **8a** induced a decrease in the inhibitory activity on the pancreatic endocrine tissue (see **17a** and **16a** in Table 1). According to these results, the (*R*,*S*)-(1-hydroxy-2-propyl)amino chain was selected to be introduced into the other series of compounds. This modulation led to compounds **8b**−**8f** that had a high efficacy but were less potent in inhibiting insulin release than their 3-isopropylamino counterparts (compounds **1b**, **2**, **5d**, **5e**, and **3**, respectively). As previously observed,[18] the 6,7-dichloro substitution appeared to be favorable for activity (**8c**). The rank order of potency on pancreatic β-cells was as follows: 6,7-dichloro > 6-chloro = 6-bromo > 7-chloro ≥ 7-fluoro > 7-methoxy.

As expected, the introduction of a hydroxy group onto the first carbon atom of the 2-isopropylamino chain was responsible for the formation of a stereogenic center. Therefore, in each series, the two enantiomers were prepared and tested to characterize the influence of stereochemistry on biological activity. Looking at the inhibitory effect of the *R* and *S* enantiomers on the insulin-releasing process, we noticed that, whatever the nature of the substituent on the aromatic ring, the *R* isomer was systematically found to be more potent than the *S* isomer and the racemate (except for **13e** vs racemate **8e**) (Table 1). The three most potent *R* isomers exhibited estimated IC_{50} values below the micromolar range (0.35 μM for **13c**, 0.52 μM for **13d**, and 0.62 μM for **13a**).

The myorelaxant activity of most of the newly synthesized compounds was evaluated on K^+-depolarized (30 mM KCl) rat aorta rings, and the results are reported as EC_{50} values (Table 1). In all cases, the introduction of a (1-hydroxy-2-propyl)-amino chain at position 3 subsequently decreased the vasorelaxant effect of the hydroxylated derivatives, in comparison with the effects of their nonhydroxylated 3-isopropylamino-substituted counterparts [see, for example, racemic compounds **8a**−**8d** vs nonhydroxylated compounds **1a**, **1b**, **2**, and **5d**, respectively (Table 1)]. As expected from previous investigations with the nonhydroxylated drugs,[18] the most potent hydroxylated compounds on rat aorta rings belonged to the 6,7-dichloro series (**8c**, **13c**, and **14c**), followed by compound **13g**, belonging to the 6-chloro-7-methoxy series. Hydroxylated compounds with other substituents on the benzene ring failed to express a relevant myorelaxant activity. It is interesting to note that, in this pharmacological model, the stereochemistry of the side chain at position 3 exerted a weak effect [see, for example, **13a** vs **14a** and **13c** vs **14c** (Table 1)].

The selectivity indexes [SI = EC_{50}/IC_{50} (see Table 1)] were calculated to compare the apparent tissue selectivity (vascular vs pancreatic tissue) of the drugs. All nonhydroxylated drugs (**1a**, **1b**, **3**, **4**, **5d**, and **5e**), as previously reported in the literature[17−20] (except for **5e**), were found to be more selective for the pancreatic than for the aortic tissue. The rank order of selectivity was as follows: 6-Br > 6-Cl > 7-methoxy = 6-Cl and 7-methoxy > 7-Cl = 7-F > 6,7-diCl. The presence of a hydroxy group on the alkylamino chain induced various effects on the selectivity ratio of the drugs. Indeed, while the selectivity appeared to be weakly affected for the racemic compounds (see, for example, **8a** vs **1a** and **8c** vs **2**) or negatively affected for the *S* enantiomers (see, for example, **14a** vs **1a** and **14c** vs **2**), the inversion of chirality led to compounds (*R* enantiomers) expressing, in most cases, an improved selectivity toward the endocrine tissue (see **13a**−**13d** vs **1a**, **1b**, **2**, and **5d**, respectively). The 6-bromo-substituted compound **13e** may constitute an exception to this rule because its analogue, **5e**, a powerful inhibitor of the insulin-releasing process (IC_{50} = 0.20 μM), is already the most tissue-selective nonhydroxylated drug (SI = 279).

The 7-chloro-substituted and the 6-chloro-substituted *R* enantiomers **13a** and **13d**, respectively, appeared to be the most promising optically pure compounds in terms of activity and tissue selectivity. These drugs exhibited a very potent activity on pancreatic β-cells (IC_{50} < 1 μM) together with a high selectivity ratio (EC_{50}/IC_{50} > 300). By contrast, 6,7-disubstituted hydroxylated *R* enantiomers such as compound **13c** did not express a marked level of tissue selectivity. Such a feature is in accordance with structure−activity relationships deduced from previous works on 3-alkylamino-substituted benzothiadiazine 1,1-dioxides.[17−21]

A previous metabolic study highlighted the rapid biotranformation of **1a** into a major and nondesired N-dealkylated derivative **7** and into a minor metabolite found to be hydroxylated derivative **8a** (X = H, and Y = Cl).[22] In this study, the determination of the exact stereochemistry of the formed metabolites appeared to be critical to improve extrapolation of the in vivo activity of **1a** as well as of several related 3-alkylaminobenzothiadiazine dioxides. For this purpose, the resulting metabolites obtained with **1a** after rat liver microsome biotransformation were analyzed by capillary electrophoresis using cyclodextrin for chiral separations. The results clearly demonstrate that there is a nonstereoselective hydroxylation of the alkylamino side chain. Indeed, both enantiomers of 7-chloro-3-(1-hydroxy-2-propyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide were formed in the same ratio during in vitro biotransformation (Figure 2). The in vitro

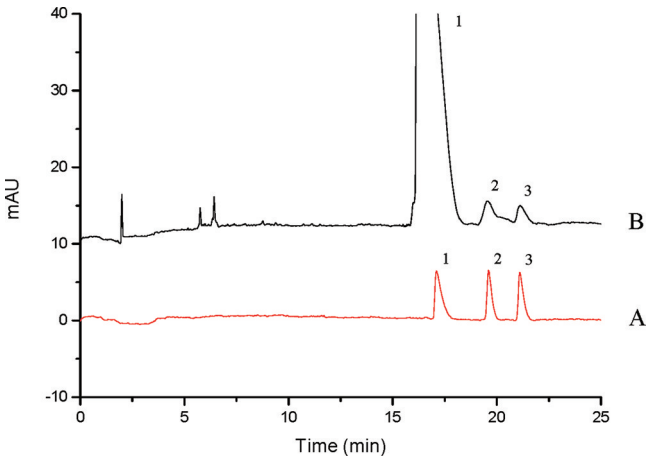

Figure 2. (A) Electropherogram of a standard solution of compounds **1a** (62.5 μg/mL) and **8a** (62.5 μg/mL) in an ACN/H_2O mixture (50:50, v/v). (B) Electropherogram resulting from in vitro metabolism of compound **1a**. Peaks: 1, compound **1a**; 2, *R* enantiomer **13a**; 3, *S* enantiomer **14a**.

metabolic stability of the most interesting isomer, (*R*)-7-chloro-3-(1-hydroxy-2-propyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13a**), was further evaluated using phenobarbital-induced

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rat liver microsomes. The use of this type of microsome is a well-established method both for producing expected in vivo phase I metabolites and for determining the metabolic weakness of drugs. The residue of biotransformation of compound **13a** was analyzed using the slightly modified described liquid chromatography (LC) conditions.[22] As shown in Figure 3, the parent product **13a** was eluted at a

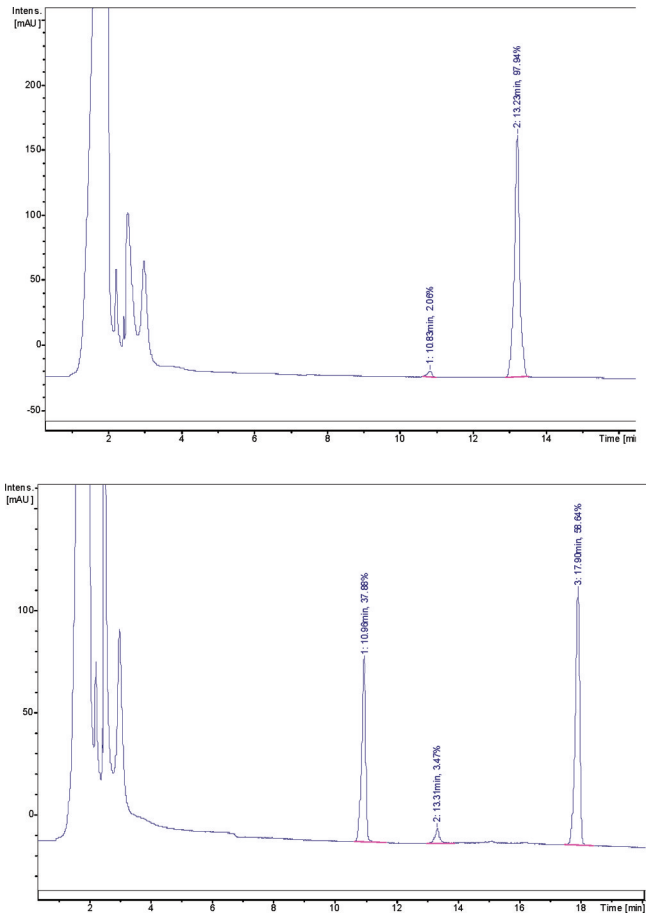

Figure 3. Metabolic profiles of compounds **13a** (top) and **1a** (down). Retention times of parent peaks: 13.23 min for **13a** and 17.90 min for **1a**. Retention time of compound **7**: 10.83 or 10.96 min.

retention time of approximately 13 min. Examination of the chromatogram of the sample that had undergone P-450-mediated biotransformation for an incubation period of 1 h highlighted the presence, besides the parent compound, of only one metabolite in a negligible quantity (≈2%). Comparison of this chromatogram with the metabolic profile of compound **1a** obtained under identical experimental conditions (Figure 3) clearly revealed the marked enhancement in metabolic stability due to the introduction of a hydroxy group on the alkylamino side chain at position 3. The metabolite detected at a retention time of 10.83 min was probably the N-dealkylated derivative [**7** (Figure 1)].

An improvement in the hydrosolubility could be expected with the introduction of a hydroxy group onto the alkyl side chain of previously described 3-alkylamino-substituted benzothiadiazine dioxides. To verify such an assumption, we have compared the water solubility at room temperature of compound **13a** [(*R*)-7-chloro-3-(1-hydroxy-2-propyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide] with that of its non-hydroxylated counterpart, **1a** (7-chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide), by examining the concentration of the two drugs in saturated aqueous solutions. The fraction of the molecule in solution was determined by high-performance liquid chromatography using the same experimental conditions that were used for the biotransformation study. The experimental results confirmed the expected increase in water solubility due to the introduction of a hydroxy group onto the isopropyl side chain, with solubility values of 1.71 mg/mL for compound **13a** and 0.04 mg/mL for compound **1a** (>40-fold increase).

In the last series of experiments, radioisotopic measurements were conducted on whole pancreatic islets to confirm the mechanism of action of the newly synthesized compounds.

Figure 4 clearly shows that the addition of the selected compound **13a** provoked a rapid, pronounced, and sustained

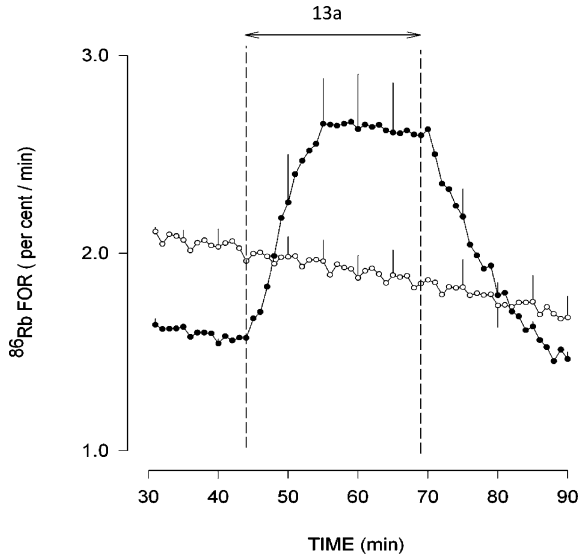

Figure 4. Effect of compound **13a** (10 μM) on the outflow of ^{86}Rb from rat pancreatic islets perifused throughout in the absence (●) or presence (○) of glibenclamide (10 μM). Basal medium contained glucose (5.6 mM) and extracellular Ca^{2+} (2.56 mM). Mean values (±SEM) refer to four individual experiments.

increase in the rate of outflow of ^{86}Rb (^{42}K substitute) from prelabeled and perifused rat pancreatic islets. In islets exposed throughout to the K_{ATP} channel blocker glibenclamide,[18,24] the stimulatory effect of **13a** was completely abolished (Figure 4).

These findings indicate that **13a** activates K_{ATP} channels in islet cells.

The enhancement of membrane K^+ permeability induced by the activation of K_{ATP} channels should hyperpolarize the insulin-secreting cells, restrict the activity of voltage-dependent Ca^{2+} channels, decrease the rate of Ca^{2+} entry, and, ultimately, inhibit the secretory process.

Such a cascade of events is indirectly revealed by the effects of **13a** on the outflow of ^{45}Ca from rat pancreatic islets perifused in the presence of an insulinotropic glucose concentration (Figure 5).

Thus, **13a** inhibited the fractional rate of outflow of ^{45}Ca from islets exposed throughout to 16.7 mM glucose and extracellular Ca^{2+} but failed to affect the outflow of ^{45}Ca from islets perifused throughout in the presence of 16.7 mM glucose but in the absence of extracellular Ca^{2+} (Figure 5, top panel). In

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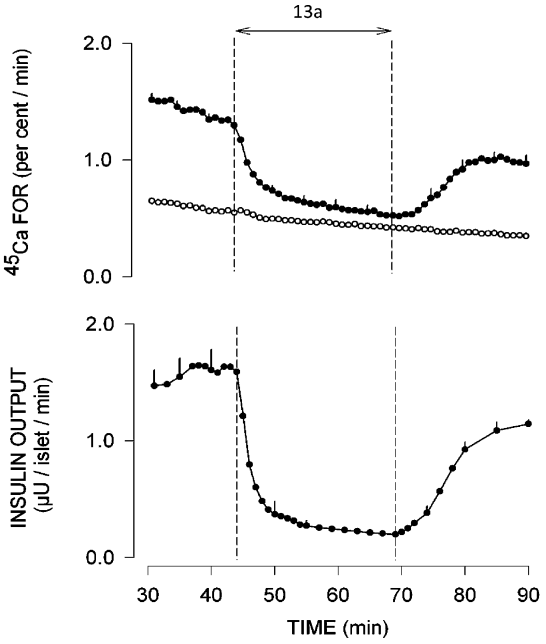

Figure 5. Effect of compound **13a** (10 μM) on the outflow of ^{45}Ca and release of insulin from perifused rat pancreatic islets. The top panel shows the effect of **13a** on the outflow of ^{45}Ca from pancreatic islets perifused throughout in the presence of an insulinotropic glucose concentration (16.7 mM). Basal medium contained extracellular Ca^{2+} (●; 2.56 mM) or were deprived of Ca^{2+} and enriched with EGTA (○; 0.5 mM). The bottom panel shows the effect of **13a** on the release of insulin from pancreatic islets perifused throughout in the presence of an insulinotropic glucose concentration (16.7 mM). Basal medium contained extracellular Ca^{2+} (●; 2.56 mM). Mean values (±SEM) refer to four to six individual experiments.

islets exposed to an insulinotropic glucose concentration and extracellular Ca^{2+}, a decrease in the rate of ^{45}Ca outflow is known to reflect a reduction in the rate of Ca^{2+} entry.[18,24]

Figure 5 (bottom panel) also reveals that **13a** provoked modifications in the insulin secretory rate displaying a time course parallel to that of the ^{45}Ca outflow response.

Together, these data indicate that the inhibitory effect of **13a** on the insulin-releasing process is mediated by a reduction in the rate of Ca^{2+} entry resulting from the activation of K_{ATP} channels.

CONCLUSION

This work reveals that the introduction of a hydroxy group onto the alkylamino side chain at position 3 of well-known benzothiadiazine 1,1-dioxide K_{ATP} channel openers could lead to an improvement in their pharmacodynamic profile. The presence of an (*R*)-1-hydroxy-2-propylamino chain at position 3 of the benzothiadiazine ring increased in most cases selectivity for the pancreatic tissue. Such a pharmacomodulation leads to compounds exhibiting an activity on the endocrine tissue below the micromolar concentration range and a selectivity ratio (EC_{50} vascular/IC_{50} pancreatic) of >300 for the 7-chloro- and 6-chloro-substituted derivatives **13a** and **13d**, respectively. Moreover, from a physicochemical point of view, and according to the results obtained with **13a**, the presence of a hydroxy group on the exocyclic alkyl chain of 3-alkylaminobenzothiadiazine dioxides can be expected to lead to an enhancement of the in vitro metabolic stability and to a marked increase in water solubility. Radioisotopic experiments conducted with **13a** further indicated that the compound affected the pancreatic endocrine tissue through the activation of K_{ATP} channels. Altogether, these data indicate that (*R*)-7-chloro-3-(1-hydroxy-2-propyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13a**) can be considered as a very promising lead compound belonging to the group of benzothiadiazine-type SUR1-selective K_{ATP} channel openers.

MATERIALS AND METHODS

Chemistry. Melting points were determined on a Stuart SMP3 capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1000 FTIR spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker AW-80 (80 MHz) or a Bruker Avance (500 MHz) instrument using d_6-DMSO as the solvent with TMS as an internal standard; chemical shifts are reported as δ values (parts per million) relative to that of the internal reference. The abbreviations s (singlet), d (doublet), m (multiplet), and b (broad) are used throughout. Elemental analyses (C, H, N, S) were used to confirm the purity of all the compounds (>95%) and were conducted with a Thermo Scientific FlashEA 1112-elemental analyzer (results within ±0.4% of the theoretical values). All reactions were routinely checked by TLC on silica gel Merck 60 F_{254}.

*6-Bromo-3-isopropylamino-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**5e**).* The title compound was obtained according to a method previously described for the corresponding 6-chloro-substituted analogue **5d** starting from 3-bromoaniline instead of 3-chloroaniline.[19] The intermediate 6-bromo-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**12e**) obtained in three steps reacted with isopropylamine according to ref 19 to give the title compound: mp 252−259 °C; IR (KBr) 3309, 3115, 2971, 1625, 1576, 1465, 1388, 1275, 1239, 1162, 1140, 1119, 1074, 1059 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.16 [d, 6H, CH(C*H*$_3$)$_2$], 3.92 (m, 1H, NHC*H*), 7.24 (bs, 1H, N*H*CH), 7.42 (m, 2H, 5-*H* + 7-*H*), 7.59 (d, 1H, 8-*H*), 10.35 (s, 1H, N*H*). Anal. ($C_{10}H_{12}BrN_3O_2S$) C, H, N, S.

General Synthetic Pathway to 3-Hydroxyalkyl/3-Methoxyalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides (8a, 8b, 8d−8f, 13a, 13b, 13d−13g, 14a, 14b, 14d−14g, 15a, 17a, and 17b) (method A). The appropriate 3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**12a−12g**)[17−19] (0.5 g) and the appropriate hydroxyalkylamine/methoxyalkylamine (5 mL) were stirred and heated at 150 °C for several hours. After cooling, the solution was supplemented with water (20 mL), and the pH was adjusted to 12 with an aqueous solution of NaOH (5%). After treatment with charchoal, the filtrate was acidified with 6 N HCl and the resulting precipitate was collected by filtration, washed with water, and dried. The isolated solid was recrystallized in a MeOH/water mixture (yields of 50−75%).

All details relative to compounds **8b**, **8d−8f**, **13b**, **13d−13g**, **14b**, **14d−14g**, **15a**, and **17b** are reported in the Supporting Information.

General Synthetic Pathway to 6,7-Dichloro-3-hydroxyalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides 8c, 13c, and 14c (method B). The synthetic pathway described above was used to obtain compounds **8c**, **13c**, and **14c**, except that intermediates **12a−12g** were replaced by 6,7-dichloro-3-(1*H*-imidazol-1-yl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **11c**[18] in the reaction with the appropriate hydroxyalkylamine. The isolated solid was recrystallized in a MeOH/water mixture (yields of 50−60%).

All details relative to compounds **13c** and **14c** are reported in the Supporting Information.

*(R,S)-7-Chloro-3-(1-hydroxy-2-propyl)amino-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**8a**).* The title compound was obtained according to method A starting from 7-chloro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**12a**)[17] and (*R,S*)-1-hydroxy-2-propylamine: mp 216−217 °C; IR (KBr) 3429, 3285, 3102, 1626, 1582, 1482, 1272, 1162, 1122, 1105 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.05 [d, 3H, CH(C*H*$_3$)CH$_2$OH], 3.30 [bd, 2H, CH(CH$_3$)C*H*$_2$OH], 3.80 [m, 1H, C*H*(CH$_3$)CH$_2$OH], 4.85 [bs, 1H, CH(CH$_3$)CH$_2$O*H*], 6.90 (bd, 1H, N*H*CH), 7.10 (d, 1H, 5-*H*), 7.50 (d, 1H, 6-*H*), 7.55 (s, 1H, 8-*H*), 10.50 (bs, 1H, N*H*). Anal. ($C_{10}H_{12}ClN_3O_3S$) C, H, N, S.

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*(R,S)-6,7-Dichloro-3-(1-hydroxy-2-propyl)amino-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**8c**).* The title compound was obtained according to method B starting from 6,7-dichloro-3-(1*H*-imidazol-1-yl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **14**[18] and (*R,S*)-1-hydroxy-2-propylamine: mp 222−227 °C; IR (KBr) 3436, 1640, 1577, 1461, 1372, 1276, 1149, 1077, 1048 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.13 [d, 3H, CH(CH_3)CH_2OH], 3.42 [m, 2H, $CH(CH_3)CH_2OH$], 3.85 [m, 1H, CH(CH_3)CH_2OH], 4.97 [bs, 1H, $CH(CH_3)CH_2OH$], 7.23 (bs, 1H, NHCH), 7.47 (bs, 1H, 5-*H*), 7.87 (s, 1H, 8-*H*), 10.55 (s, 1H, N*H*). Anal. ($C_{10}H_{11}Cl_2N_3O_3S$) C, H, N, S.

*(R)-7-Chloro-3-(1-hydroxy-2-propyl)amino-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**13a**).* The title compound was obtained according to method A starting from 7-chloro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**12a**)[17] and (*R*)-1-hydroxy-2-propylamine: mp 228−230 °C; IR (KBr) 3469, 3307, 2978, 1626, 1581, 1481, 1279, 1248, 1153, 1123, 1095, 1047 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.10 [d, 3H, CH(CH_3)CH_2OH], 3.30 [bd, 2H, $CH(CH_3)CH_2OH$], 3.80 [m, 1H, CH(CH_3)CH_2OH], 4.90 [b, 1H, $CH(CH_3)CH_2OH$], 6.90 (bd, 1H, NHCH), 7.10 (d, 1H, 5-*H*), 7.50 (d, 1H, 6-*H*), 7.55 (s, 1H, 8-*H*), 10.50 (bs, 1H, N*H*). Anal. ($C_{10}H_{12}ClN_3O_3S$) C, H, N, S.

*(S)-7-Chloro-3-(1-hydroxy-2-propyl)amino-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**14a**).* The title compound was obtained according to method A starting from 7-chloro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**12a**)[17] and (*S*)-1-hydroxy-2-propylamine: mp 224−228 °C; IR (KBr) 3462, 3308, 2978, 1626, 1581, 1481, 1279, 1248, 1161, 1123, 1096, 1045 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.10 [d, 3H, CH(CH_3)CH_2OH], 3.30 [bd, 2H, $CH(CH_3)CH_2OH$], 3.80 [m, 1H, CH(CH_3)CH_2OH], 4.85 [bs, 1H, $CH(CH_3)CH_2OH$], 6.90 (bd, 1H, NHCH), 7.10 (d, 1H, 5-*H*), 7.50 (d, 1H, 6-*H*), 7.55 (s, 1H, 8-*H*), 10.50 (bs, 1H, N*H*). Anal. ($C_{10}H_{12}ClN_3O_3S$) C, H, N, S.

*(R,S)-7-Chloro-3-(1-acetoxy-2-propyl)amino-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**16a**).* The mixture of (*R,S*)-7-chloro-3-(1-hydroxy-2-propyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**8a**) (0.5 g, 1,72 mmol) and acetic anhydride (3 mL) was stirred at room temperature for 2 h. The reaction mixture was then supplemented with water (20 mL) and stirred for 20 min. The resulting precipitate was collected by filtration, washed with water, and dried: mp 205−206 °C; IR (KBr) 3299, 3181, 3084, 1739, 1631, 1579, 1480, 1244, 1160, 1103 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.17 [d, 3H, $CH_2CH(CH_3)OAc$], 2.02 (s, 3H, $COCH_3$), 3.99 [m, 1H, CH(CH_3)-CH_2OH], 4.11 [m, 2H, $CH(CH_3)CH_2OAc$], 7.24 (bm, 2H, NHCH + 5-*H*), 7.61 (m, 1H, 6-*H*), 7.67 (s, 1H, 8-*H*), 10.62 (bs, 1H, N*H*). Anal. ($C_{12}H_{14}ClN_3O_4S$) C, H, N, S.

*(R,S)-7-Chloro-3-(1-methoxy-2-propyl)amino-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**17a**).* The title compound was obtained according to method A starting from 7-chloro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**12a**)[17] and (*R,S*)-1-methoxy-2-propylamine: mp 150−153 °C; IR (KBr) 3294, 3186, 3118, 3084, 2983, 2932, 2880, 1631, 1582, 1480, 1250, 1162, 1105 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.05 [d, 3H, $CH(CH_3)CH_2OCH_3$], 3.00−3.40 [m, 5H, $CH(CH_3)CH_2OCH_3$ + OCH_3], 3.90 [m, 1H, CH(CH_3)CH_2OCH_3], 6.95 (bs, 1H, NHCH), 7.10 (d, 1H, 5-*H*), 7.50 (d + s, 2H, 6-*H* + 8-*H*), 10.45 (bs, 1H, N*H*). Anal. ($C_{11}H_{14}ClN_3O_3S$) C, H, N, S.

Metabolism. The in vitro biological test system selected to metabolize the parent compounds was the phenobarbital (PB)-induced male rat liver microsome system.[22] The parent compounds were dissolved in methanol and added directly to the incubation medium, yielding a final substrate concentration of 200 μM and a final percentage in methanol of <1%. The incubations were performed at 37 °C in a water shacking bath with a final protein content of 1 mg/mL in a total volume of 1 mL. The reactions were initiated by addition of a NADPH regenerating system. The reactions were stopped after an incubation time of 60 min by addition of 1 mL of methanol and 2 mL of acetonitrile and by a subsequent vortexing step. Samples were further centrifuged at 2000*g* for 5 min. The supernatant was further decanted into a glass tube, and organic solvents were evaporated under an inert nitrogen flux to concentrate the samples.

Capillary Electrophoresis Experiments. *Instrumentation.* Capillary electrophoresis (CE) experiments were conducted on a $HP^{3D}CE$ system (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler, an on-column diode array detector, and a temperature control system (15−60 ± 0.1 °C). A CE Chemstation (Hewlett-Packard, Palo Alto, CA) was used for instrument control, data acquisition, and data handling. Fused-silica capillaries were provided by ThermoSeparation Products (San Jose, CA).

Electrophoretic Technique. Electrophoretic separations were conducted with uncoated fused-silica capillaries having a 50 μm internal diameter and a 48.5 cm length (40 cm to the detector). At the beginning of each working day, the capillary was washed with 1 N NaOH, water, and the background electrolyte containing the cyclodextrin (BGE-CD) for 10 min. Before each injection, the capillary was washed successively with 1 N NaOH for 7 min and water for 3 min and then equilibrated with the BGE-CD for 10 min. The applied voltage was 25 kV in the negative polarity mode, and UV detection was set at 210 nm. Injections were made by applying a pressure of 50 mbar for a period of 4 s, and the capillary was thermostated at 15 °C. The BGE-CD used for electrophoretic experiments consisted of 10 mM octakis-6-*O*-sulfo-γ-CD in 100 mM phosphoric acid adjusted to pH 3 with triethanolamine. Octakis-6-*O*-sulfo-γ-CD was a gift from G. Vigh (Texas A&M University, College Station, TX).

The residue from the in vitro biological metabolization test was redissolved in 500 μL of ACN, vigorously stirred for 5 min, and then gently evaporated to dryness under a nitrogen flux. The residue was finally redissolved in 100 μL of an ACN/H_2O mixture (50:50) and vigorously stirred for 3 min. The solution was finally centrifuged at 13600 rpm for 5 min and injected into the CE system.

LC Conditions for Biotransformation and Solubility Studies. The LC separations were conducted on an Agilent 1100 series LC system equipped with a quaternary pump, a column thermostat, an autosampler, and a diode array detector. The analyte separations were performed on an Alltech Hypersil BDS C18 column (15 mm × 4.6 mm, inside diameter; particle size of 3 μm) from Alltech (Breda, The Netherlands) using mobile phase A (water) and mobile phase B (ACN) with a flow rate of 0.8 mL/min and the following linear gradient: 10% ACN at 0 min, 40% ACN at 24 min, 60% ACN at 27 min, and 10% ACN at 30 min. The column temperature was set at 40 °C.

The residue from the in vitro biological metabolization test was redissolved in 500 μL of ACN, the solution vigorously stirred for 5 min, and then the residue gently evaporated to dryness under a nitrogen flux. The residue was finally redissolved in 100 μL of an ACN/H_2O mixture (50:50) and the solution vigorously stirred for 3 min. The solution was finally centrifuged at 13600 rpm for 5 min and injected into the LC system.

Solubility Studies. A suspension of 5−10 mg of compound was stirred in 5 mL of distilled water at room temperature for 1 h. After decantation for 30 min, 1 mL was collected and centrifuged at 4000*g* for 5 min. The supernatant was decanted and used as an injection solution.

A calibration curve for each derivative was determined using reference solutions at different concentrations under the same LC conditions. These curves were used to calculate the water solubility of the two molecules.

Measurements of Release of Insulin from Incubated Rat Pancreatic Islets. The method used to measure the release of insulin from incubated rat pancreatic islets was previously described.[23,24]

Measurement of the Contractile Activity in Rat Aorta. The method used to measure the myorelaxant effect of the drugs on 30 mM KCl-precontracted rat aortic rings was previously described.[23,24]

Measurements of Outflow of ^{86}Rb, Outflow of ^{45}Ca, and Release of Insulin from Perifused Rat Pancreatic Islets. The methods used for measuring outflow of ^{86}Rb (^{42}K substitute), outflow of ^{45}Ca, and release of insulin from prelabeled and perifused rat pancreatic islets were previously described.[19,24]

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■ ASSOCIATED CONTENT

Supporting Information

Synthesis of compounds **8b**, **8d**−**8f**, **13b**−**13g**, **14b**−**14g**, **15a**, and **17b** and elemental analysis of compounds **5e**, **8a**−**8f**, **13a**−**13g**, **14a**−**14g**, **15a**, **16a**, **17a**, and **17b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

P.L. and B.P. equally supervised this work.

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■ ABBREVIATIONS

K_{ATP} channel, ATP-sensitive potassium channel; SUR, sulfonylurea receptor; Kir, inwardly rectifying potassium channel; PCO, potassium channel opener; RIS, residual insulin release percentage; IC_{50}, half-maximal inhibitory concentration; EC_{50}, half-maximal effective concentration; FTIR, Fourier transform infrared; NMR, nuclear magnetic resonance; TMS, tetramethylsilane; TLC, thin layer chromatography; SEM, standard error of the mean; EGTA, ethylene glycol tetraacetic acid; FOR, fractional outflow rate; NADPH, nicotinamide adenine dinucleotide phosphate; CE, capillary electrophoresis; BGE-CD, background electrolyte containing the cyclodextrin

■ REFERENCES

(1) Miki, T.; Nagashima, K.; Seino, S. The Structure and Function of the ATP-sensitive K^+ Channel in Insulin-secreting Pancreatic β-cells. *J. Mol. Endocrinol.* **1999**, *22*, 113−123.

(2) Ballanyi, K. Protective Role of Neuronal K_{ATP} Channels in Brain Hypoxia. *J. Exp. Biol.* **2004**, *207*, 3201−3212.

(3) Ardehali, H.; O'Rourke, B.; Marban, E. Cardioprotective Role of the Mitochondrial ATP-Binding Cassette Protein 1. *Circ. Res.* **2005**, *97*, 740−742.

(4) Ko, E. A.; Han, J.; Jung, I. D.; Park, W. S. Physiological Roles of K^+ Channels in Vascular Smooth Muscle Cells. *J. Smooth Muscle Res.* **2008**, *44*, 65−81.

(5) Babenko, A. P.; Aguilar-Bryan, L.; Bryan, J. A View of SUR/KIR6.X, K_{ATP} Channels. *Annu. Rev. Physiol.* **1998**, *60*, 667−687.

(6) Inagaki, N.; Gonoi, T.; Clement, J. P. IV; Namba, N.; Inazawa, J.; Gonzalez, G.; Aguilar-Bryan, L.; Seino, S.; Bryan, J. Reconstitution of IK_{ATP}: An Inward Rectifier Subunit plus the Sulfonylurea Receptor. *Science* **1995**, *270*, 1166−1170.

(7) Hambrock, A.; Loffler-Walz, C.; Kloor, D.; Delabar, U.; Horio, Y.; Kurachi, Y.; Quast, U. ATP-Sensitive K^+ Channel Modulator Binding to Sulfonylurea Receptors SUR2A and SUR2B: Opposite Effects of MgADP. *Mol. Pharmacol.* **1999**, *55*, 832−840.

(8) Seino, S. ATP-Sensitive Potassium Channels: A Model of Heteromultimeric Potassium Channel/Receptor Assemblies. *Annu. Rev. Physiol.* **1999**, *61*, 337−362.

(9) Shi, N. Q.; Ye, B.; Makielski, J. C. Function and Distribution of the SUR Isoforms and Splice Variants. *J. Mol. Cell. Cardiol.* **2005**, *39*, 51−60.

(10) Szewczyk, A.; Skalska, J.; Glab, M.; Kulawiak, B.; Malinska, D.; Koszela-Piotrowska, I.; Kunz, W. S. Mitochondrial Potassium Channels: From Pharmacology to Function. *Biochim. Biophys. Acta* **2006**, *1757*, 715−720.

(11) Ardehali, H.; O'Rourke, B. Mitochondrial K_{ATP} Channels in Cell Survival and Death. *J. Mol. Cell. Cardiol.* **2005**, *39*, 7−16.

(12) Sandler, S.; Andersson, A. K.; Larsson, J.; Makeeva, N.; Olsen, T.; Arkhammar, P. O.; Hansen, J. B.; Karlsson, F. A.; Welsh, N. Possible Role of an Ischemic Preconditioning-like Response Mechanism in K_{ATP} Channel Opener-mediated Protection Against Streptozotocin-induced Suppression of Rat Pancreatic Islet Function. *Biochem. Pharmacol.* **2008**, *76*, 1748−1756.

(13) Lawson, K. Potassium Channel Activation: A Potential Therapeutic Approach? *Pharmacol. Ther.* **1996**, *70*, 39−63.

(14) Lebrun, P.; Antoine, M. H.; Ouedraogo, R.; Herchuelz, A.; de Tullio, P.; Delarge, J.; Pirotte, B. Pyridothiadiazines as Potent Inhibitors of Glucose-induced Insulin Release. *Adv. Exp. Med. Biol.* **1997**, *426*, 145−148.

(15) Rasmussen, S. B.; Sorensen, T. S.; Hansen, J. B.; Mandrup-Poulsen, T.; Hornum, L.; Markholst, H. Functional Rest Through Intensive Treatment with Insulin and Potassium Channel Openers Preserves Residual β-cell Function and Mass in Acutely Diabetic BB Rats. *Horm. Metab. Res.* **2000**, *32*, 294−300.

(16) Hansen, J. B. Towards Selective Kir6.2/SUR1 Potassium Channel Openers, Medicinal Chemistry and Therapeutic Perspectives. *Curr. Med. Chem.* **2006**, *13*, 361−376.

(17) de Tullio, P.; Becker, B.; Boverie, S.; Dabrowski, M.; Wahl, P.; Antoine, M. H.; Somers, F.; Sebille, S.; Ouedraogo, R.; Hansen, J. B.; Lebrun, P.; Pirotte, B. Toward Tissue-Selective Pancreatic B-Cells K_{ATP} Channel Openers Belonging to 3-Alkylamino-7-halo-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides. *J. Med. Chem.* **2003**, *46*, 3342−3353.

(18) de Tullio, P.; Boverie, S.; Becker, B.; Antoine, M. H.; Nguyen, Q. A.; Francotte, P.; Counerotte, S.; Sebille, S.; Pirotte, B.; Lebrun, P. 3-Alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides as ATP-Sensitive Potassium Channel Openers: Effect of 6,7-Disubstitution on Potency and Tissue Selectivity. *J. Med. Chem.* **2005**, *48*, 4990−5000.

(19) Pirotte, B.; de Tullio, P.; Nguyen, Q. A.; Somers, F.; Fraikin, P.; Florence, X.; Wahl, P.; Hansen, J. B.; Lebrun, P. Chloro-Substituted 3-Alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides as ATP-Sensitive Potassium Channel Activators: Impact of the Position of the Chlorine Atom on the Aromatic Ring on Activity and Tissue Selectivity. *J. Med. Chem.* **2010**, *53*, 147−154.

(20) Boverie, S.; Antoine, M. H.; Somers, F.; Becker, B.; Sebille, S.; Ouedraogo, R.; Counerotte, S.; Pirotte, B.; Lebrun, P.; de Tullio, P. Effect on K_{ATP} Channel Activation Properties and Tissue Selectivity of the Nature of the Substituent in the 7- and the 3-Position of 4*H*-1,2,4-Benzothiadiazine 1,1-Dioxides. *J. Med. Chem.* **2005**, *48*, 3492−3503.

(21) de Tullio, P.; Dupont, L.; Francotte, P.; Counerotte, S.; Lebrun, P.; Pirotte, B. Three-Dimensional Quantitative Structure-Activity Relationships of ATP-Sensitive Potassium (K_{ATP}) Channel Openers Belonging to the 3-Alkylamino-4*H*-1,2,4-benzo- and 3-Alkylamino-4*H*-1,2,4-pyridothiadiazine 1,1-Dioxide Families. *J. Med. Chem.* **2006**, *49*, 6779−6788.

(22) Gillotin, F.; Chiap, P.; Frederich, M.; Van Heugen, J. C.; Francotte, P.; Lebrun, P.; Pirotte, B.; de Tullio, P. Coupling of Liquid Chromatography/Tandem Mass Spectrometry and Liquid Chromatography/Solid-Phase Extraction/NMR Techniques for the Structural Identification of Metabolites Following in Vitro Biotransformation of SUR1-Selective ATP-Sensitive Potassium Channel Openers. *Drug Metab. Dispos.* **2010**, *38*, 232−240.

(23) Lebrun, P.; Becker, B.; Morel, N.; Ghisdal, P.; Antoine, M. H.; de Tullio, P.; Pirotte, B. K_{ATP} Channel Openers: Tissue Selectivity of Original 3-Alkylaminopyrido- and 3-Alkylaminobenzothiadiazine 1,1-Dioxides. *Biochem. Pharmacol.* **2008**, *75*, 468−475.

(24) Lebrun, P.; Arkhammar, P.; Antoine, M.-H.; Nguyen, Q.-A.; Hansen, J. B.; Pirotte, B. A. Potent Diazoxide Analogue Activating ATP-sensitive K^+ Channels and Inhibiting Insulin Release. *Diabetologia* **2000**, *43*, 723−732.