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1,4,2-Benzo/pyridodithiazine 1,1-Dioxides Structurally Related to the ATP-Sensitive Potassium Channel Openers 1,2,4-Benzo/pyridothiadiazine 1,1-Dioxides Exert a Myorelaxant Activity Linked to a Distinct Mechanism of Action

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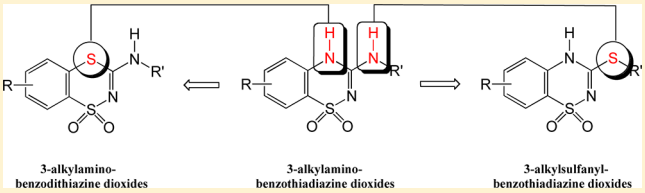
Supporting Information

ABSTRACT: The synthesis of diversely substituted 3-alkyl/aralkyl/arylamino-1,4,2-benzodithiazine 1,1-dioxides and 3-alkylaminopyrido[4,3-*e*]-1,4,2-dithiazine 1,1-dioxides is described. Their biological activities on pancreatic β-cells and on smooth muscle cells were compared to those of the reference ATP-sensitive potassium channel (K_{ATP} channel) openers diazoxide and 7-chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide. The aim was to assess the impact on biological activities of the replacement of the 1,2,4-thiadiazine ring by an isosteric 1,4,2-dithiazine ring. Most of the dithiazine analogues were found to be inactive on the pancreatic tissue, although some compounds bearing a 1-phenylethylamino side chain at the 3-position exerted a marked myorelaxant activity. Such an effect did not appear to be related to the opening of K_{ATP} channels but rather reflected a mechanism of action similar to that of calcium channel blockers. Tightly related 3-(1-phenylethyl)sulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides were also found to exert a pronounced myorelaxant activity, resulting from both a K_{ATP} channel activation and a calcium channel blocker mechanism. The present work highlights the critical importance of an intracyclic NH group at the 4-position, as well as an exocyclic NH group linked to the 3-position of the benzo- and pyridothiadiazine dioxides, for activity on K_{ATP} channels.



INTRODUCTION

ATP-sensitive potassium channels (K_{ATP} channels) are known to be complex octameric structures combining two kinds of transmembrane proteins, the "sulfonylurea receptor" (SUR*x*) subunit and the "inwardly rectifying potassium channel" (Kir6.*x*) subunit.[1] The assembly of the Kir6.*x* (Kir6.1 and/or Kir6.2) and the SUR*x* (SUR1, SUR2A, and/or SUR2B) subunits in multiple combinations gives rise to different isoforms of K_{ATP} channels that are diversely distributed throughout the body.[1−5] These K_{ATP} channels are also known to be the target for clinically used compounds such as the hypoglycemic sulfonylurea glibenclamide **1** (a K_{ATP} channel blocker) or the antihypertensive drug diazoxide **2** (a K_{ATP} channel opener belonging to the benzothiadiazine dioxide chemical class) (Figure 1).[6]

Our previous work on diazoxide analogues, i.e., the 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides (**3**, Figure 1) and the 4*H*-1,2,4-benzothiadiazine 1,1-dioxides diversely substituted at the 3-position of the heterocycle (3-alkyl, 3-alkylamino, 3-alkoxy, 3-alkylsulfanyl groups) (**4**, Figure 1) as well as on the benzene ring fused to the thiadiazine nucleus (5-, 6-, 7-, or 8-position of the heterocycle), highlighted the possibility to identify K_{ATP} channel openers expressing a marked channel subtype selectivity (SUR1 versus SUR2A and/

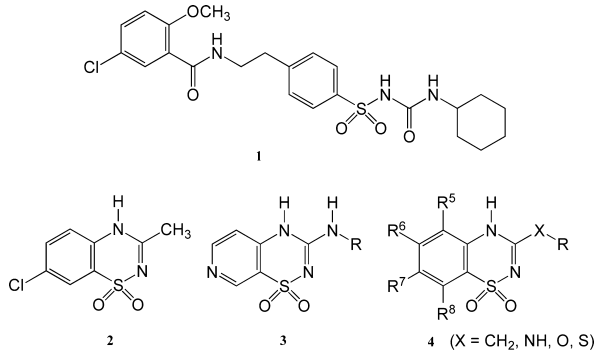


Figure 1. Chemical structures of glibenclamide (**1**) and diazoxide (**2**) and general formulas of 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides (**3**) and diversely 3-substituted 4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**4**) reported as potassium channel openers.

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or SUR2B) according to the nature of the substituents introduced at diverse critical positions.[7−17] Pharmacophoric models generated from our previous experimental data are summarized in Figure 2.

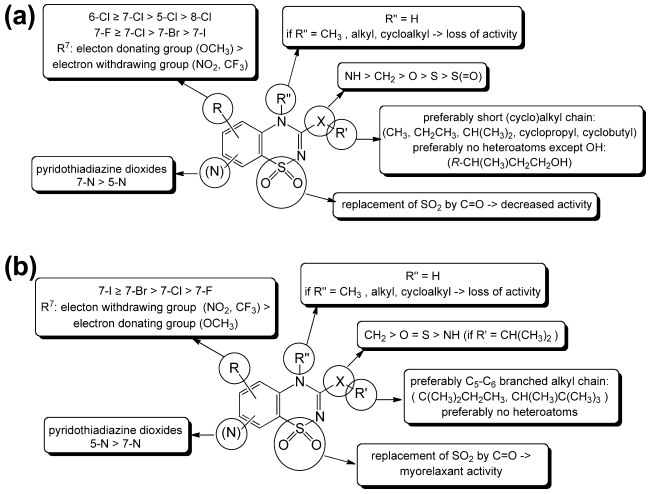

Figure 2. Pharmacophore model summarizing the structural elements responsible for the activity of benzo/pyridothiadiazine dioxides on (a) insulin-secreting pancreatic β-cells and (b) vascular smooth muscle cells.

Briefly, a strong activity on pancreatic β-cells (inhibition of insulin release) was observed with benzothiadiazine dioxides bearing a short branched alkylamino side chain at the 3-position [X = NH; R′ = short (cyclo)alkyl chain] and a halogen atom (preferably F or Cl) at the 6- and/or the 7-position(s) (R = 6-Cl and/or 7-Cl/7-F) (Figure 2a).[11−13,15] The 4-position needed only to bear a hydrogen atom (R″ = H).[9] The replacement of the sulfonyl group (SO_2) by a carbonyl group (C═O), which provides quinazolinones, led to a remarkable decrease of activity on the pancreatic endocrine tissue.[10] For 3-alkylamino-substituted pyridothiadiazine dioxides, the "7-aza" compounds (pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides) appeared to be much more potent on the pancreatic insulin secreting cells than the "5-aza" compounds (pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides).[7−9,14]

Regarding the vascular smooth muscle cells, a myorelaxant effect was observed with benzothiadiazine dioxides bearing an alkyl/cycloalkyl or a C_5−C_6 branched alkylamino side chain at the 3-position (thus exhibiting an increased steric hindrance compared to the alkylamino side chains required for activity on pancreatic β-cells) (Figure 2b).[8,11] Moreover, the presence of an electron withdrawing group (typically NO_2 or CF_3) at the 7-position was more appropriate than an electron donating group (i.e., OCH_3), while such requirement was inverted to develop an inhibitory activity on pancreatic β-cells.[12] Another difference concerned the nature of the halogen atom at the 7-position. While an iodine or a bromine atom was more suitable than a chlorine or a fluorine atom for developing a strong myorelaxant activity, the opposite situation prevailed for disclosing an inhibitory activity on the pancreatic endocrine tissue.[11] Finally, the last difference concerned the pyridothiadiazine dioxides, since the "5-aza" compounds (pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides) were found to express a more marked myorelaxant activity than their "7-aza" counterparts (pyrido-[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides).[9]

In our efforts to provide additional information on the structure−activity relationships explaining both the interaction of benzo- and pyridothiadiazine dioxides with K_{ATP} channels and their selectivity toward a specific K_{ATP} channel subtype, we decided to study the impact of the isosteric replacement of the intracyclic NH group at the 4-position of 3-alkylamino-4*H*-1,2,4-benzo/pyridothiadiazine 1,1-dioxides (**5** and **7**) by a sulfur atom leading to 3-alkylamino-1,4,2-benzo/pyridodithiazine 1,1-dioxides analogues (**6** and **8**) (Figure 3). The new compounds were tested on rat pancreatic β-cells (SUR1-expressing tissue) and rat aorta rings (SUR2B-expressing tissue) to characterize the tissue selectivity.

Moreover, a new series of 3-(1-phenylethyl)sulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**10**) was also prepared with

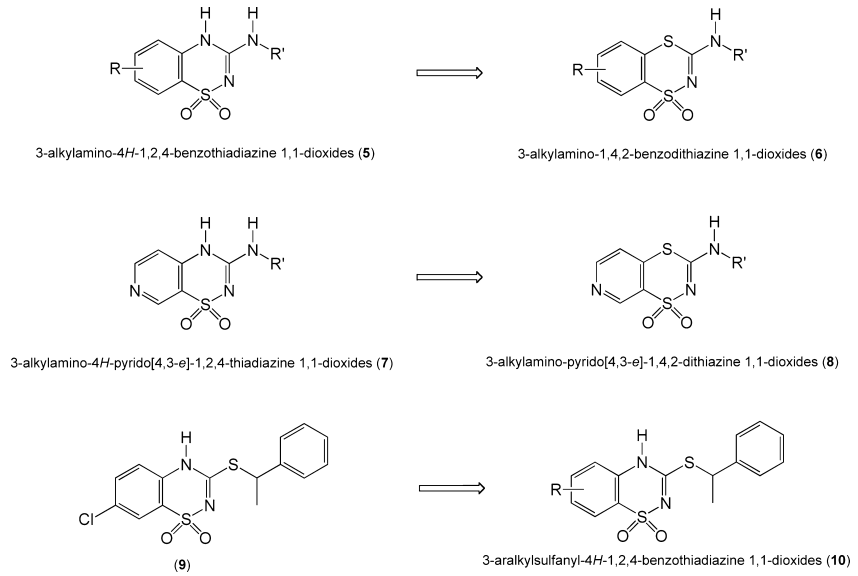

Figure 3. Design of benzo- (**6**) and pyridodithiazine dioxides (**8**) as structural analogues of benzo- (**5**) and pyridothiadiazine dioxides (**7**) and design of novel 3-aralkylsulfanyl-substituted benzothiadiazine dioxides (**10**) as structural analogues of the myorelaxant compound *R*/*S*-7-chloro-3-(1-phenylethyl)sulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**9**).

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the aim of comparing the activity of such compounds with 3-(1-phenylethyl)amino-1,4,2-benzodithiazine 1,1-dioxides [**6**, R′ = $CH(CH_3)C_6H_5$] and with the previously described 7-chloro-3-(1-phenylethyl)sulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**9**) known to express a marked myorelaxant activity (Figure 3).[16]

The mechanism of action of active representatives from the different series was determined.

CHEMISTRY

Access to diversely substituted 3-alkylamino-4*H*-1,4,2-benzodithiazine 1,1-dioxides **6** is described in Scheme 1. The previously reported[12,18] or commercially available 2-chlorobenzenesulfonamides **12** were used as starting materials. The isopropylamino side chain located at the 3-position was considered as the most accurate chain to compare the new compounds with reference compound **22**.

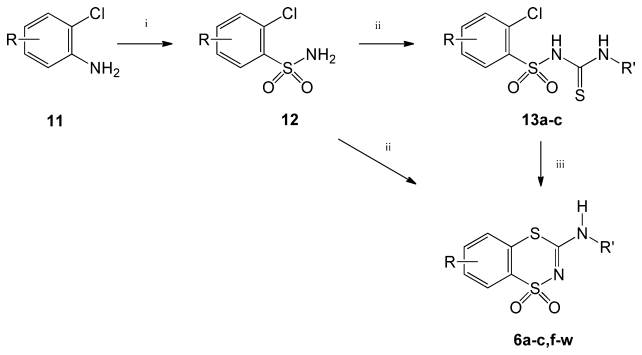
The reaction of the appropriate isothiocyanate with **12** provided two kinds of compounds. When the substituent at the 5-position of 2-chlorobenzenesulfonamides **12** was not a strong electron withdrawing group (typically a chlorine atom at the 3-, 4-, or 5-position of **12**), the sulfonylthiourea intermediates **13a–c** were yielded (Scheme 1). Such intermediates gave access to the final compounds **6a–c** after ring closure in hot DMF/CH_3CN, resulting from the intracyclic nucleophilic attack of the sulfur atom on the carbon bearing the chlorine atom. By contrast, when the substituent at the 5-position of **12** was a strong electron withdrawing group (typically a nitro, a trifluoromethyl, a cyano, or a methylsulfonyl group), the ring-opened intermediates **13** were not isolated and ring closure spontaneously occurred giving rise to the expected benzodithiazine dioxides **6**. Such a reaction could be explained by the activation of the chlorine atom at the 2-position of **12** by the strong electron withdrawing groups at the ortho (SO_2NH_2) and para positions.

For obtaining the 7-cyano-substituted compound **6d**, the synthesis of 4-chloro-3-sulfamoylbenzonitrile **16** was required. The latter compound was obtained in two steps from 4-chloro-3-sulfamoylbenzoic acid **14**, as described in Scheme 2. Alkaline hydrolysis of **6d** gave rise to the corresponding 7-COOH-substituted benzodithiazine dioxide **6e** (Scheme 2).

3-Isopropylaminopyrido[4,3-*e*]-1,4,2-dithiazine 1,1-dioxide **19** was obtained from 4-chloropyridine-3-sulfonamide **17**,[7] according to the same synthetic pathway (Scheme 3) as that reported in Scheme 1. Access to the *N*-oxide counterpart **20** required a preliminary conversion of **17** into the corresponding 4-chloropyridine-3-sulfonamide *N*-oxide **18** following reaction of **17** with meta-chloroperbenzoic acid (mCPBA) (Scheme 3).

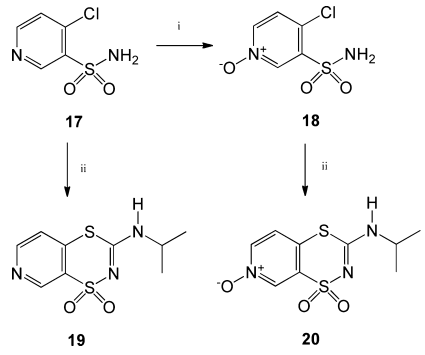
Because (*R*)-1-phenylethyl isocyanate and (*S*)-1-phenylethyl isocyanate were commercially available, the synthesis of (*R*)-(**23**) and (*S*)-3-(1-phenylethylamino)-7-trifluoromethyl-1,4,2-benzodithiazine 1,1-dioxide (**24**) was performed in order to examine the impact of the optical geometry of the first carbon atom of the aralkyl side chain on the biological activity.

Scheme 1[a]



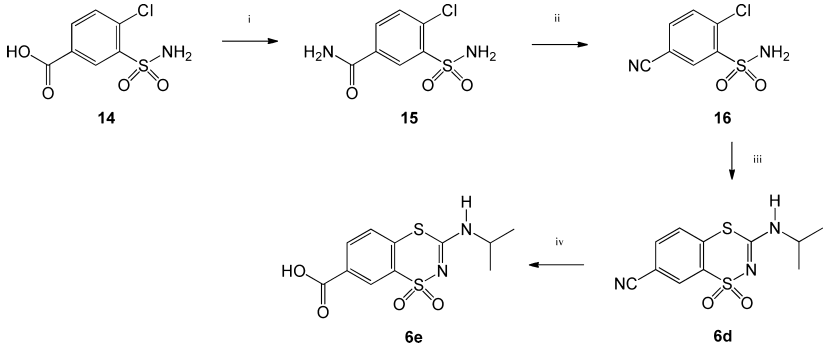
[a]Reagents: (i) (1) $NaNO_2$, HCl; SO_2, HOAc, $CuCl_2$; (2) NH_3; (ii) R′N=C=S, CH_3COCH_3, K_2CO_3; (iii) Δ, CH_3CN, DMF.

Scheme 3[a]



[a]Reagent: (i) mCPBA, CH_3COCH_3; (ii) $(CH_3)_2CHN=C=S$, CH_3COCH_3, K_2CO_3.

Scheme 2[a]



[a]Reagents: (i) (1) $SOCl_2$; (2) NH_3, dioxane; (ii) THF, $N(Et)_3$, $(CF_3CO)_2O$; 2 N NaOH; (iii) $(CH_3)_2CHN=C=S$, CH_3COCH_3, K_2CO_3; (iv) NaOH, HOH.

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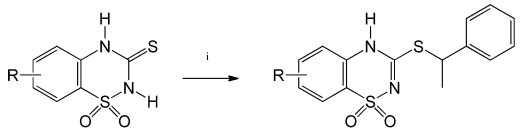
3-Alkylamino-1,4,2-benzo/pyridodithiazine 1,1-dioxides are weak acids (linked to the lability of the exocyclic NH proton). Thus, the purification process at the end of the reaction generally consisted of the dissolution of the final product in strong aqueous alkaline medium, treatment with charcoal, and reacidification of the filtrate to precipitate the final compound.

Finally, 3-(1-phenylethyl)sulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **10** resulted from alkylation of the corresponding 3,4-dihydro-3-thioxo-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **21**[11,15] with 1-phenethyl bromide (Scheme 4).

Scheme 4[a]



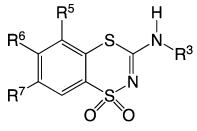
[a]Reagent: (i) $C_6H_5CH(CH_3)Br$, NaOH, DMF.

RESULTS AND DISCUSSION

Up to now, only little information has been published about 1,4,2-benzodithiazine 1,1-dioxides and pyrido[4,3-*e*]-1,4,2-dithiazine 1,1-dioxides. Significant contribution in this field was essentially provided by Brzozowski and co-workers who described synthetic pathways to prepare such heterocycles and who reported potential therapeutic properties for 3-alkyl/arylamino-substituted benzo/pyridodithiazine dioxides (antiproliferative, antiretroviral, or diuretic activity).[19−22] However, none of these investigations were devoted to the study of such compounds as isosteric analogues of diazoxide or 3-alkylamino-substituted pyrido/benzothiadiazine dioxides acting as potassium channel openers on K_{ATP}-channel-expressing tissues.

In the present study, we have synthesized original 3-alkyl/arylamino-substituted benzo/pyridodithiazine dioxides with the aim of evaluating their activity as putative potassium channel openers structurally related to 3-alkylamino-substituted benzo/pyridothiadiazine dioxides. The ability of the newly synthesized compounds to inhibit the glucose (16.7 mM) induced insulin secretion from isolated rat pancreatic islets and to relax precontracted (30 mM KCl) rat aorta rings is reported in Table

Table 1. Effects of Diversely Substituted 1,4,2-Benzodithiazine 1,1-Dioxides and Pyrido[4,3-*e*]-1,4,2-dithiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets and on the Contractile Activity of K^+-Depolarized Rat Aorta Rings



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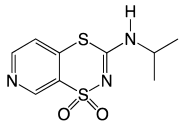

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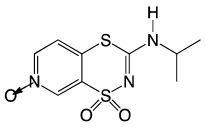

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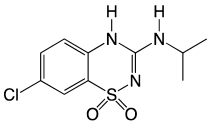

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compd	R^5	R^6	R^7	R^3	RIS[a] (10 μM)	EC_{50} [b] (μM)
6a	H	H	Cl	$CH(CH_3)_2$	92.2 ± 5.4 (23)	>100 (4)
6b	H	Cl	H	$CH(CH_3)_2$	78.9 ± 4.3 (15)	>30 (4)
6c	Cl	H	H	$CH(CH_3)_2$	82.4 ± 5.5 (15)	>30 (4)
6d	H	H	CN	$CH(CH_3)_2$	86.2 ± 4.0 (24)	152.4 ± 11.3 (5)
6e	H	H	COOH	$CH(CH_3)_2$	68.5 ± 3.4 (24)	>200 (4)
19	H	H	(N)	$CH(CH_3)_2$	90.6 ± 3.7 (23)	189.8 ± 7.8 (5)
20	H	H	(N−O)	$CH(CH_3)_2$	76.7 ± 5.0 (20)	>200 (4)
6f	H	H	NO_2	CH_2CH_3	100.4 ± 5.4 (14)	>30 (6)
6g	H	H	NO_2	$CH(CH_3)_2$	97.7 ± 3.8 (16)	175.8 ± 14.8 (4)
6h	H	H	NO_2	$CH(CH_2)_2$	88.2 ± 3.7 (15)	>200 (6)
6i	H	H	NO_2	$CH(CH_3)CH_2CH_3$	110.4 ± 5.4 (16)	59.9 ± 7.3 (5)
6j	H	H	NO_2	$CH_2CH(CH_3)_2$	110.0 ± 7.3 (16)	69.1 ± 3.1 (5)
6k	H	H	NO_2	$CH_2CH(CH_2)_2$	90.2 ± 3.7 (14)	61.1 ± 7.9 (5)
6l	H	H	NO_2	$CH(CH_2)_4$	100.4 ± 5,6 (15)	42.6 ± 4.5 (4)
6m	H	H	NO_2	C_6H_5	89.5 ± 4.3 (14)	>200 (4)
6n	H	H	NO_2	$CH_2C_6H_5$	77.7 ± 3.6 (15)	>30 (4)
6o	H	H	NO_2	$CH(CH_3)C_6H_5$	91.5 ± 5.9 (15)	15.9 ± 0.6 (5)
6p	H	H	CF_3	$CH(CH_3)_2$	107.6 ± 5.2 (23)	53.7 ± 3.3 (4)
6q	H	H	CF_3	$CH(CH_3)CH_2CH_3$	109.6 ± 5.2 (24)	38.8 ± 4.1 (6)
6r	H	H	CF_3	$CH(CH_2)_4$	89.5 ± 5.1 (13)	48.7 ± 5.5 (5)
6s	H	H	CF_3	C_6H_5	67.8 ± 3.2 (14)	13.7 ± 2.2 (5)
6t	H	H	CF_3	$CH(CH_3)C_6H_5$	97.0 ± 5.6 (15)	11.6 ± 1.7 (5)
6u	H	H	SO_2CH_3	$CH(CH_3)_2$	76.3 ± 4.3 (16)	>200 (4)
6v	H	H	SO_2CH_3	C_6H_5	92.4 ± 5.1 (15)	>30 (4)
6w	H	H	SO_2CH_3	$CH(CH_3)C_6H_5$	83.4 ± 6.8 (13)	>30 (4)
diazoxide (**2**)[c]					73.9 ± 4.4 (16)[c]	26.2 ± 4.4 (5)
22[c]					4.8 ± 0.4 (32)[c]	31.8 ± 3.9 (4)

[a]RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)). [b]EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (*n*)). [c]Published compounds and results (refs 17, 28).

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1. The in vitro data on pancreatic islets are expressed as a percentage of residual insulin release recorded at 10 μM drug and are compared to the results obtained with diazoxide (**2**) and 7-chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**22**, BPDZ 73), a previously described compound expressing a marked inhibitory activity on the insulin releasing process.[23] Experimental data on rat aorta rings are expressed as the EC_{50} value, which corresponds to the concentration of drug giving 50% relaxation of the KCl-induced contraction of aortic rings.

Results reported in Table 1 indicate that none of the newly synthesized benzo- and pyridodithiazine dioxides exerted, at 10 μM, a marked inhibitory effect on the insulin releasing process. Compound **6a**, the most tightly related isoster of the reference compound **22** (exclusively resulting from the replacement at the 4-position of the intracyclic NH group by S), was inactive at 10 μM (>90% residual insulin release), while **22**, at the same concentration, was responsible for an almost complete inhibition of insulin secretion (5% residual insulin release). Several benzo- and pyridodithiazine dioxides (**6e**, **6n**, **6s**, **6u**, **20**), however, revealed a moderate activity on β-cells close to that of the reference drug diazoxide (20−30% inhibition of insulin secretion at 10 μM).

The myorelaxant activity of benzodithiazine dioxides bearing a short branched alkylamino chain (i.e., isopropylamino chain) at the 3-position was weak or even almost absent. However, several benzodithiazine dioxides bearing a strong electron withdrawing group at the 7-position, such as NO_2 and CF_3 (i.e., **6o**, **6s**, **6t**), exerted a pronounced myorelaxant activity similar to or even more marked than that of diazoxide. Interestingly, two vasorelaxant compounds (**6o** and **6t**) exhibited a *R*/*S*-1-phenylethylamino side chain at the 3-position that was similar to the *R*/*S*-1-phenylethylsulfanyl side chain of compound **9**, a 3-aralkylsulfanyl-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide previously reported as a strong myorelaxant drug.[16]

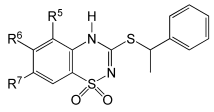
According to such results, we synthesized and examined the myorelaxant properties of new examples of 3-(1-phenylethyl)-sulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides bearing a halogen atom at the 5-, 6-, or 7-position (compounds **10a**−**e**). These compounds may also be regarded as the result of a permutation of the sulfur atom at the intracyclic 4-position with the NH group at the exocyclic 3-position of 3-(1-phenylethyl)-amino-1,4,2-benzodithiazine 1,1-dioxides. Their myorelaxant activity was compared with that of compound **9** and with the most potent 3-(1-phenylethyl)amino-1,4,2-benzodithiazine 1,1-dioxide **6t** (Table 2).

Except for compound **10d**, all these new compounds expressed marked myorelaxant activity (EC_{50} values between 5 and 15 μM), while their inhibitory activity on the insulin releasing process was found to be minor (Table 2).

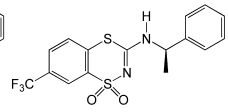
Of the pure (*R*)- and (*S*)-enantiomers of (*RS*)-3-(1-phenylethyl)amino-7-trifluoromethyl-1,4,2-benzodithiazine 1,1-dioxide (**6t**), it was observed that the (*S*)-isomer (**24**) was more potent than the (*R*)-isomer (**23**) as a myorelaxant drug, a result that corroborated previous observations made with tightly related (*R*)- and the (*S*)-enantiomers of (*RS*)-3-(1-phenylethyl)amino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides.[9]

Compound **6t**, the most potent 3-arylamino-1,4,2-benzodithiazine 1,1-dioxide, and compound **10a**, the most water-soluble 3-arylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide, were selected for further pharmacological investigations in order to determine their mechanism of action. Table 3 reports

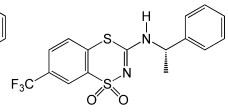
Table 2. Effects of Diversely Substituted 3-(1-Phenylethyl)sulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets and on the Contractile Activity of K^+-Depolarized Rat Aorta Rings



10a-e



23



24

compd	R^5	R^6	R^7	RIS[a] (10 μM)	EC_{50}[b] (μM)
10a	H	H	F	95.5 ± 5.2 (16)	15.0 ± 0.6 (4)
9[c]	H	H	Cl	71.1 ± 4.2 (24)	5.4 ± 0.1 (4)[c]
10b	H	H	Br	91.7 ± 4.0 (16)	11.9 ± 1.9 (6)
10c	H	H	I	81.4 ± 4.3 (30)	5.4 ± 0.1 (4)
10d	Cl	H	H	79.2 ± 3.9 (15)	>30 (4)
10e	H	Cl	H	76.3 ± 4.1 (16)	5.7 ± 0.2 (4)
6t (*R*/*S*)				97.0 ± 5.6 (15)	11.6 ± 1.7 (5)
23 (*R*)				nd[d]	>30 (9)
24 (*S*)				nd[d]	11.3 ± 2.0 (11)

[a]RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)). [b]EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (*n*)). [c]Published compound and results (ref 16). [d]nd: not determined.

the myorelaxant effects of compounds **6t** and **10a** on the contractile activity of 30 and 80 mM KCl-precontracted rat aorta rings incubated in the absence or presence of the K_{ATP} channel blocker glibenclamide.[4,6,14] Diazoxide was used as a reference potassium channel opener.

Table 3 clearly shows that diazoxide expressed the classical profile of a K_{ATP} channel opener because the EC_{50} value for its myorelaxant effect on 30 mM KCl-precontracted rat aorta rings increased with the concentration of glibenclamide in the incubation medium (EC_{50} of 35 μM in the absence of glibenclamide, 154 μM in the presence of 1 μM glibenclamide, and 280 μM in the presence of 10 μM glibenclamide).[14] Moreover, the myorelaxant activity of diazoxide was dramatically reduced (EC_{50} > 300 μM) in 80 mM K^+-depolarized rat aortic rings (Table 3).[14]

By contrast, the results obtained with compound **6t** distinguish a compound that does not behave as a potassium channel opener (Table 3). Indeed, the EC_{50} value of **6t** on the 30 mM KCl-precontracted rat aorta rings was not affected by the K_{ATP} channel blocker glibenclamide [EC_{50} of 18 μM (no glibenclamide), 17 μM (1 μM glibenclamide), 16 μM (10 μM glibenclamide)], and the myorelaxant potency of **6t** was identical on 30 and 80 mM KCl-depolarized aortic rings (EC_{50} of 16 and 15 μM, respectively). It must be noted that a similar profile has already been observed for 7-chloro-3-(1-phenylethyl)amino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides tightly related to compound **6t**.[9,24]

To further determine the pharmacological profile of the original benzodithiazine 1,1-dioxide **6t**, we tested, under identical experimental conditions, the effects of the Ca^{2+} entry blocker verapamil on the myogenic activity of K^+-depolarized rat aorta rings (Table 3). Whether in the absence or presence of glibenclamide in the bathing medium, the cumulative application of the calcium channel blocker verapamil induced concentration-dependent relaxations of 30 or 80 mM K^+-depolarized rat aorta rings (Table 3). The

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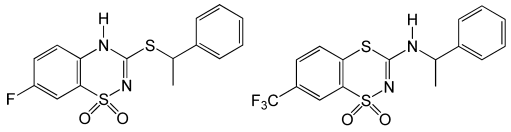
Table 3. Myorelaxant Effects (EC_{50}) of *R*/*S*-7-Fluoro-3-(1-phenylethyl)sulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (10a), *R*/*S*-3-(1-Phenylethyl)amino-7-trifluoromethyl-1,4,2-benzodithiazine 1,1-Dioxide (6t), and Diazoxide on 30 and 80 mM K^+-Induced Contractions of Rat Aorta Rings Incubated in the Absence or Presence of Glibenclamide



10a **6t**

	EC_{50} (μM)[a]			
compd	30 mM KCl	30 mM KCl + 1 μM GLB	30 mM KCl + 10 μM GLB	80 mM KCl
10a	19.9 ± 2.9 (10)	33.3 ± 4.2 (7)	36.2 ± 3.9 (8)	
	13.2 ± 1.6 (10)			27.5 ± 4.1 (11)
6t	18.4 ± 1.4 (4)	17.3 ± 1.5 (6)	15.5 ± 1.1 (5)	
	16.2 ± 4.5 (5)			14.9 ± 0.6 (6)
diazoxide	34.8 ± 2.7 (6)	153.7 ± 8.0 (7)	279.8 ± 11.8 (4)	
	23.8 ± 5.0 (6)			>300 (8)
verapamil	0.031 ± 0.004 (4)	0.038 ± 0.007 (6)	0.035 ± 0.003 (5)	
	0.045 ± 0.004 (5)			0.052 ± 0.006 (7)

[a] EC_{50} (μM): drug concentration (μM) giving 50% relaxation of the 30 or 80 mM KCl-induced contraction (mean ± SEM (*n*)). GLB: glibenclamide.

sensitivity toward verapamil of the 80 mM K^+-induced myogenic activity and the lack of effects of the hypoglycaemic sulfonylurea glibenclamide toward the vasorelaxant effect of verapamil indicated myorelaxant properties resulting from a direct inhibition of voltage-sensitive Ca^{2+} channels.[25]

Altogether, these biological data clearly indicate that compound **6t** displays a pharmacological profile similar to that of the voltage-sensitive Ca^{2+} channel blocker verapamil.[14,24,25]

The situation appeared to be more complex for compound **10a** for which the myorelaxant activity was slightly but not markedly blunted by the presence of increasing concentrations of glibenclamide in the incubation medium [EC_{50} of 20 μM (no glibenclamide), 33 μM (1 μM glibenclamide), 36 μM (10 μM glibenclamide)] (Table 3). Likewise, in the presence of 80 mM KCl in the bathing medium, **10a** still provoked a pronounced myorelaxant effect although the concentration response curve was displaced 2-fold to the right [EC_{50} of 13 μM (30 mM KCl), 28 μM (80 mM KCl)] (Table 3). These results suggest that compound **10a** exhibited a mixed pharmacological profile, acting partly as a potassium channel opener and partly as a calcium channel blocker.

From a structural point of view, the isothiourea planar system of 3-alkylamino-1,4,2-benzodithiazine 1,1-dioxides, assuming optimal electron delocalization between the three heteroatoms, can theoretically generate two different tautomeric forms, each form adopting two conformations resulting from two possible orientations of the alkyl chain at the 3-position (namely, A, B, C, or D; Figure 4). Crystallographic data obtained with compound **6g**[26] indicated that this drug, and probably also the other 3-alkylamino-1,4,2-benzodithiazine 1,1-dioxides, preferentially adopted the A conformation (Figure 4) with the alkyl side chain orientation similar to those found for 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides and 3-alkylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides.[16] Atomic distances in **6g** were consistent with a delocalization of the double bond between the two nitrogen atoms (N_2–C_3 = 1.315 Å; C_3–$N_{exocycl}$ = 1.318 Å). However, in the crystal, the hydrogen atom was found to be located on the exocyclic nitrogen atom because the former appeared to be involved in the establishement of a hydrogen bond with the oxygen atom of the sulfonyl group of a

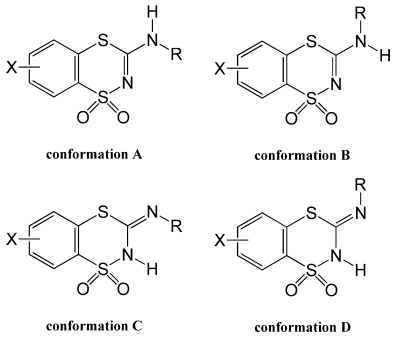


Figure 4. Different conformations of the tautomeric forms adopted by 3-alkylamino-1,4,2-benzodithiazine 1,1-dioxides.

second molecule.[26] These results suggest that 3-alkylamino-1,4,2-benzodithiazine 1,1-dioxides adopt a conformation close to that of the K_{ATP} channel openers 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (such as **22**) with the double bond located between N_2 and C_3 and with a similar orientation of the exocyclic NH group and of the alkyl side chain at the 3-position. However, even if most structural elements are present, the absence for 3-alkylamino-1,4,2-benzodithiazine 1,1-dioxides of the critical NH group at the 4-position could justify the lack of activity of these compounds as K_{ATP} channel openers.

Finally, to complete the comparison between 3-alkylamino-1,4,2-benzodithiazine 1,1-dioxides and 3-alkylamino/alkylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides, we also determined the acidic character of a representative compound belonging to the new benzodithiazine series, i.e., compound **6a** bearing a chlorine atom at the 7-position such as compounds **22** and **25** (Figure 5). The acidic character of 3-alkylaminobenzodithiazine dioxides is linked to the lability of the hydrogen atom located on the exocyclic NH group. We found a pK_a of 9.76 for **6a**, while the pK_a values of its 3-alkylaminobenzothiadiazine counterpart **22** and its 3-alkylsulfanylbenzothiadiazine counterpart **25** were previously established at 9.51 and 7.01, respectively (Figure 5).[16] Thus, 3-alkylaminobenzodithiazine dioxides should exist at physiological pH as un-ionized drugs bearing an exocyclic NH group available for the establishement of a hydrogen bond with the

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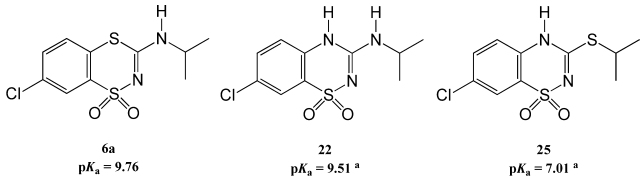

Figure 5. Chemical structures of compounds **6a**, **22**, and **25** with indication of their ionization constant (pK_a value) in water at 25 °C ([a]ref 16).

receptor binding site. As mentioned above, however, this structural element appears to be insufficient to provide marked activity on the K_{ATP} channels.

CONCLUSION

We described diversely substituted 3-alkyl/aralkyl/arylamino-1,4,2-benzodithiazine 1,1-dioxides and 3-alkylamino-pyrido[4,3-*e*]-1,4,2-dithiazine 1,1-dioxides structurally related to the K_{ATP} channel openers 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides and 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides. The aim of the present study was to evaluate the impact, on the biological activity, of the isosteric replacement of the NH group by a sulfur atom at the 4-position of 3-alkylamino-4*H*-1,2,4-benzo/pyridothiadiazine 1,1-dioxides.

Benzo- and pyridodithiazine dioxides bearing a short branched alkylamino chain at the 3-position (i.e., an isopropylamino chain), and whatever the substituent at the 5-, 6-, or 7-position, were found to be inactive or only slightly active as inhibitors of insulin secretion or as vasorelaxants. However, the presence of a bulky aralkylamino chain at the 3-position, in particular a (1-phenylethyl)amino chain, disclosed marked vasorelaxant activity. Such a myorelaxant effect was also observed with benzothiadiazine dioxides bearing a (1-phenylethyl)sulfanyl chain at the 3-position (permutation of the intracyclic sulfur atom at the 3-position of benzodithiazine dioxides with the exocyclic NH group at the 4-position).

The myorelaxant properties of 3-(1-phenylethyl)amino-1,4,2-benzodithiazine 1,1-dioxides did not appear to be linked to the opening of K_{ATP} channels but rather reflected a mechanism reminiscent of that evoked by calcium channel blockers. Tightly related 3-(1-phenylethyl)sulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides, however, exhibited a vasorelaxant activity resulting from both a K_{ATP} channel activation and a direct calcium entry blockade.

This study allows completion of our knowledge of the critical structural elements required for a K_{ATP} channel opening activity on pancreatic β-cells and on vascular smooth muscle cells. The presence of a nitrogen atom bearing a hydrogen atom at the 4-position of benzo/pyridothiadiazine dioxides appears to be crucial to the design of compounds acting as K_{ATP} channel openers on the SUR1-expressing pancreatic tissue and on the SUR2B-expressing vascular tissue.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined on a Büchi Tottoli capillary apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Bruker AW-80 (80 MHz) or a Bruker Avance (500 MHz) instrument using deuterated dimethylsulfoxide (DMSO-d_6) as the solvent with hexamethyldisiloxane (HMDS) (80 MHz) or tetramethylsilane (TMS) (500 MHz) as an internal standard. Chemical shifts are reported in δ values (ppm) relative to that of internal HMDS or TMS. The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and b = broad are used throughout. Elemental analyses (C, H, N, S) were realized on a Thermo Scientific Flash EA 1112 elemental analyzer, and the results were within ±0.4% of the theoretical values. This analytical method certified a purity of ≥95% for each tested compound. All reactions were routinely checked by TLC on Merck 60 F_{254} silica gel.

Most of the 2-chlorobenzenesulfonamides **12** were commercially available or were obtained from the corresponding 2-chloroaniline **11** according to a previously described process consisting of the Meerwein variation of the Sandmeyer diazotization reaction.[18] 2-Chloro-5-trifluoromethylbenzenesulfonamide (**12e**) and 2-chloro-5-methylsulfonylbenzenesulfonamide (**12f**) were obtained as previously described,[12] while 4-chloropyridine-3-sulfonamide (**17**) was synthesized from 4-hydroxypyridine.[7] The halo-substituted 3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides (**21a–e**) were synthesized according to the literature.[11,15]

General Procedures for the Synthesis of 3-(Alkyl/aralkyl/aryl)amino-1,4,2-benzodithiazine 1,1-Dioxides (6). *Method A.* Potassium carbonate (0.54 g) and the appropriate alkyl isothiocyanate (3.85 mmol) were added to a solution of the appropriate 2-chlorobenzenesulfonamide **12** (3.25 mmol) in acetone (20 mL). The reaction mixture was heated under reflux until completion of the reaction as monitored by TLC. Then the solvent was removed by distillation under reduced pressure. The residue, mainly consisting of the *N*-alkyl-*N*′-(benzenesulfonyl)thiourea intermediate **13**, was dissolved in a 1:1 mixture of acetonitrile–DMF (20 mL) and heated under reflux until completion of the ring closure reaction monitored by TLC. The solvents were removed by distillation under reduced pressure, and the residue of the title compound was suspended in water (20 mL). The insoluble material was dissolved by addition of an aqueous 10% NaOH solution. The alkaline solution was clarified by treatment with charcoal, and after filtration, the clear solution was adjusted to pH 1 by means of 6 N HCl. The resulting precipitate of the title compound was collected by filtration, washed with water, and dried (yield, 70–80%).

Method B. Potassium carbonate (0.54 g) and the appropriate alkyl/aralkyl/aryl isothiocyanate (4.45 mmol) were added to a solution of the appropriate 2-chlorobenzenesulfonamide **12** (3.25 mmol) in acetone (20 mL). The reaction mixture was heated under reflux until completion of the reaction as monitored by TLC. Then the solvent was removed by distillation under reduced pressure and the residue of the title compound was suspended in water (20 mL). The insoluble material was dissolved by addition of an aqueous 10% NaOH solution. The alkaline solution was clarified by treatment with charcoal, and after filtration, the clear solution was adjusted to pH 1 by means of 6 N HCl. The resulting precipitate of the title compound was collected by filtration, washed with water, and dried (yield, 80–90%).

7-Chloro-3-isopropylamino-1,4,2-benzodithiazine 1,1-Dioxide (6a). Method A. White solid: mp 214–216 °C; ^1H NMR (80 MHz, DMSO-d_6) δ 1.10 (d, 6H, $CH(CH_3)_2$), 4.05 (m, 1H, $CH(CH_3)_2$), 7.65 (s, 2H, 5-*H* + 6-*H*), 7.85 (s, 1H, 8-*H*), 9.55 (s, 1H, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.5, 46.1, 123.8, 127.8, 130.3, 132.1, 133.3, 134.4, 161.2. Anal. ($C_{10}H_{11}ClN_2O_2S_2$) theoretical: C, 41.30; H, 3.81; N, 9.63; S, 22.05. Found: C, 41.18; H, 3.85; N, 9.75; S, 22.04.

General Procedure for the Synthesis of 3-(1-Phenylethyl)-sulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides (10). NaOH (0.08 g, 2 mmol) was added, under stirring, to a solution of the appropriate 3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **21** (2 mmol) in methanol (10 mL). The solvent was removed by distillation under reduced pressure, and the residue of the sodium salt of **21** was dissolved in a 5:1 mixture of nitroethane–DMF and heated at 80 °C. 1-Phenylethyl bromide (0.41 g, 2.2 mmol) was added dropwise, and the reaction mixture was heated at 80 °C until completion of the reaction as monitored by TLC. The solvents were removed by distillation under reduced pressure, and the residue was dissolved in a 10% aqueous solution of NaOH. The alkaline solution was clarified after treatment with charcoal, and the filtrate was adjusted to pH 1 by means of 12 N HCl. The precipitate of the title compound

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was collected by filtration, washed with water, and dried (yield, 40–70%).

(*RS*)-7-Fluoro-3-(1-phenylethyl)sulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Monohydrate (10a). White solid: mp 196–198 °C; ^{1}H NMR (80 MHz, DMSO-d_6) δ 1.75 (d, 3H, CH(CH_3)C_6H_5), 5.00 (q, 1H, CH(CH$_3$)C_6H_5), 7.50 (m, 6H, C_6H_5 + 5-*H*), 7.75 (m, 1H, 6-*H*), 7.85 (m, 1H, 8-*H*), 12.65 (s, 1H, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.9, 45.0, 109.5, 120.0, 121.4, 122.7, 127.5, 127.8, 128.7, 132.3, 140.9, 157.7, 159.7. Anal. ($C_{15}H_{13}FN_2O_2S_2\cdot H_2O$) theoretical: C, 50.83; H, 4.27; N, 7.90; S, 18.09. Found: C, 50.69; H, 3.98; N, 8.08; S, 18.01.

4-Chloro-3-sulfamoylbenzamide (15). The mixture of 4-chloro-3-sulfamoylbenzoic acid (**14**) (23.5 g, 0.1 mol) and thionyl chloride (200 mL) was refluxed for 3 h. The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in dioxane (100 mL). The resulting solution was added dropwise under stirring to a 7% aqueous solution of ammonia (375 mL). After 30 min, the excess ammonia was removed under reduced pressure by concentration of the solution to a third of its volume. The resulting precipitate of the title compound was collected by filtration, washed with water, and dried (11.7 g, 50%): mp 220–222 °C (lit. 223–225 °C).[27]

4-Chloro-3-sulfamoylbenzonitrile (16). Triethylamine (11.5 mL) and trifluoroacetic anhydride (10 mL) were added to a cold suspension of 4-chloro-3-sulfaloylbenzamide (15) (6.25 g, 26.6 mmol) in anhydrous THF (40 mL). After completion of the reaction as monitored by TLC, the reaction mixture was concentrated under reduced pressure. The residue was taken off with water (50 mL), and the resulting precipitate was collected by filtration, washed with water, and dissolved in a 2 N aqueous solution of NaOH (35 mL). After 30 min at room temperature, the solution was clarified by treatment with charcoal, and the filtrate was adjusted to pH 1 by means of 2 N HCl. The resulting precipitate was collected by filtration, washed with water, and dried (4.04 g, 70%): mp 199–201 °C (lit. 197–199 °C).[27]

7-Cyano-3-isopropylamino-1,4,2-benzodithiazine 1,1-Dioxide (6d). Potassium carbonate (0.54 g) and isopropyl isothiocyanate (0.39 g, 3.85 mmol) were added to a solution of 4-chloro-3-sulfamoylbenzonitrile (**16**) (0.70 g, 3.23 mmol) in acetone (20 mL). The reaction mixture was heated under reflux until completion of the reaction as monitored by TLC. Then the solvent was removed by distillation under reduced pressure and the residue of the title compound was suspended in water (20 mL). The insoluble material was dissolved by addition of an aqueous 2% NaOH solution. The alkaline solution was clarified by treatment with charcoal, and after filtration, the clear solution was adjusted to pH 2 by means of 2 N HCl. The resulting precipitate of the title compound was collected by filtration, washed with water, and dried (0.64 g, 70%): mp 243–244 °C; ^{1}H NMR (80 MHz, DMSO-d_6) δ 1.10 (d, 6H, CH(CH_3)$_2$), 4.05 (m, 1H, CH(CH$_3$)$_2$), 7.80 (bd, 1H, 5-*H*), 8.05 (bd, 1H, 6-*H*), 8.30 (bs, 1H, 8-*H*), 9.70 (bd, 1H, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.4, 46.2, 111.6, 117.2, 128.0, 129.6, 133.6, 134.9, 135.1, 160.5. Anal. ($C_{11}H_{11}N_3O_2S_2$) theoretical: C, 46.96; H, 3.94; N, 14.93; S, 22.79. Found: C, 46.31; H, 4.11; N, 14.70; S, 22.45.

3-Isopropylamino-1,4,2-benzodithiazine-7-carboxylic Acid 1,1-Dioxide Monohydrate (6e). A solution of 7-cyano-3-isopropylamino-1,4,2-benzodithiazine 1,1-dioxide (**6d**) (0.4 g, 1.42 mmol) in a 15% aqueous solution of NaOH (30 mL) was stirred at room temperature for 15 h. Then the alkaline solution was clarified by treatment with charcoal and the filtrate was adjusted to pH 1 by means of 2 N HCl. The resulting precipitate of the title compound was collected by filtration, washed with water, and dried (0.43 g, 95%): mp >300 °C; ^{1}H NMR (80 MHz, DMSO-d_6) δ 1.15 (d, 6H, CH(CH_3)$_2$), 4.00 (m, 1H, CH(CH$_3$)$_2$), 7.75 (d, 1H, 5-*H*), 8.05 (bd, 1H, 6-*H*), 8.30 (s, 1H, 8-*H*), 9.60 (bd, 1H, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.4, 46.0, 124.5, 128.9, 131.3, 132.1, 132.9, 133.8, 160.8, 165.6. Anal. ($C_{11}H_{12}N_2O_4S_2\cdot H_2O$) theoretical: C, 41.50; H, 4.43; N, 8.80; S, 20.14. Found: C, 41.13; H, 4.28; N, 8.68; S, 20.46.

4-Chloropyridine-3-sulfonamide *N*-Oxide (18). A solution of 4-chloropyridine-3-sulfonamide (**17**) (5 g, 26 mmol) and 3-chloroperbenzoic acid (17.89 g, 104 mmol) in acetone (40 mL) was heated under reflux until completion of the reaction as monitored by TLC. The title compound, which precipitated in the medium, was collected by filtration, washed with acetone, and dried (4.87 g, 90%): mp 94–96 °C. The compound was used in the next step without further purification.

3-Isopropylaminopyrido[4,3-*e*]-1,4,2-dithiazine 1,1-Dioxide (19). Potassium carbonate (0.54 g) and isopropyl isothiocyanate (0.45 g, 4.45 mmol) were added to a solution of 4-chloropyridine-3-sulfonamide (**17**) (0.70 g, 3.63 mmol) in acetone (20 mL). The reaction mixture was heated under reflux until completion of the reaction as monitored by TLC. Then the solvent was removed by distillation under reduced pressure and the residue of the title compound was suspended in water (20 mL). The insoluble material was dissolved by addition of an aqueous 10% NaOH solution. The alkaline solution was clarified by treatment with charcoal, and after filtration, the clear solution was adjusted to pH 4–5 by means of 2 N HCl. The resulting precipitate of the title compound was collected by filtration, washed with water, and dried (0.78 g, 83%): mp 137–138 °C; ^{1}H NMR (80 MHz, DMSO-d_6) δ 1.10 (d, 6H, CH(CH_3)$_2$), 4.05 (m, 1H, CH(CH$_3$)$_2$), 7.70 (bd, 1H, 5-*H*), 8.65 (bd, 1H, 6-*H*), 8.95 (bs, 1H, 8-*H*), 9.70 (bd, 1H, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.4, 46.1, 122.5, 128.3, 140.0, 144.2, 151.5, 159.5. Anal. ($C_9H_{11}N_3O_2S_2$) theoretical: C, 42.01; H, 4.31; N, 16.33; S, 24.92. Found: C, 42.19; H, 4.25; N, 16.32; S, 25.01.

3-Isopropylaminopyrido[4,3-*e*]-1,4,2-dithiazine 1,1,7-Trioxide (20). The title compound was obtained as described for **19**, starting from 4-chloropyridine-3-sulfonamide *N*-oxide (**18**) (0.75 g, 3.60 mmol) and isopropyl isothiocyanate (0.45 g, 4.45 mmol) (0.72 g, 73%): mp 255–256 °C; ^{1}H NMR (80 MHz, DMSO-d_6) δ 1.10 (d, 6H, CH(CH_3)$_2$), 4.05 (m, 1H, CH(CH$_3$)$_2$), 7.70 (d, 1H, 5-*H*), 8.30 (bd, 1H, 6-*H*), 8.45 (s, 1H, 8-*H*), 9.80 (bs, 1H, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.4, 46.4, 125.1, 126.0, 131.4, 134.0, 141.5, 160.6. Anal. ($C_9H_{11}N_3O_3S_2$) theoretical: C, 39.55; H, 4.06; N, 15.37; S, 23.46. Found: C, 39.29; H, 3.89; N, 15.14; S, 23.35.

(*R*)-3-(1-Phenylethyl)amino-7-trifluoromethyl-1,4,2-benzodithiazine 1,1-Dioxide (23). A solution of 2-chloro-5-trifluoromethylbenzenesulfonamide (**12e**) (0.3 g, 1.16 mmol) in a 1:1 mixture of acetone and DMF (10 mL) was supplemented with potassium carbonate (0.23 g, 1.66 mmol) and (*R*)-1-phenylethyl isothiocyanate (0.2 g, 1.23 mmol). The suspension was refluxed for 5 h and then concentrated under reduced pressure. The residue was picked up with water (20 mL), and the resulting suspension was alkalinized with a 10% w/v aqueous solution of NaOH until solubilization of the title compound. After treatment with charcoal and filtration, the filtrate was acidified with 6 N HCl. The resulting precipitate was collected by filtration, washed with water, and dried. The compound was crystallized in ethyl acetate–hexane (0.25 g, 56%): mp 214–216 °C; enantiomeric purity (by chiral HPLC), 99.8%; ^{1}H NMR (500 MHz, DMSO-d_6) 1.50 (d, 3H, CH(CH_3)C_6H_5), 5.23 (q, 1H, CH(CH$_3$)-C_6H_5), 7.28 (dd, 1H, 4′-*H*), 7.36 (d, 4H, 2′-*H* + 3′-*H* + 5′-*H* + 6′-*H*), 8.00 (d, 1H, 5-*H*), 8.06 (dd, 1H, 6-*H*), 8.15 (s, 1H, 8-*H*), 10.29 (s, 1H, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.6, 53.0, 120.8, 122.0, 124.2, 126.1, 127.4, 128.5, 128.6, 129.0, 129.3, 130.0, 133.4, 134.2, 142.0, 161.2. Anal. ($C_{16}H_{13}F_3N_2O_2S_2$) theoretical: C, 49.73; H, 3.39; N, 7.25; S, 16.60. Found: C, 49.54; H, 3.62; N, 7.16; S, 16.49.

(*S*)-3-(1-Phenylethyl)amino-7-trifluoromethyl-1,4,2-benzodithiazine 1,1-Dioxide (24). The title compound was obtained as described for **23**, starting from 2-chloro-5-trifluoromethylbenzenesulfonamide (**12e**) (0.3 g, 1.16 mmol) and (*S*)-1-phenylethyl isothiocyanate (0.2 g, 1.23 mmol). The compound was crystallized in ethyl acetate–hexane (0.27 g, 60%): mp 214–216 °C; enantiomeric purity (by chiral HPLC), 99.9%; ^{1}H NMR (500 MHz, DMSO-d_6) 1.50 (d, 3H, CH(CH_3)C_6H_5), 5.23 (q, 1H, CH(CH$_3$)C_6H_5), 7.28 (dd, 1H, 4′-*H*), 7.36 (d, 4H, 2′-*H* + 3′-*H* + 5′-*H* + 6′-*H*), 8.00 (d, 1H, 5-*H*), 8.06 (dd, 1H, 6-*H*), 8.15 (s, 1H, 8-*H*), 10.29 (s, 1H, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.7, 53.0, 120.8, 122.0, 124.2, 126.1, 127.4, 128.5, 128.6, 129.0, 129.2, 130.0, 133.4, 134.2, 142.1, 161.1. Anal. ($C_{16}H_{13}F_3N_2O_2S_2$) theoretical: C, 49.73; H, 3.39; N, 7.25; S, 16.60. Found: C, 49.32; H, 3.57; N, 7.13; S, 16.22.

Measurements of Insulin Release from Incubated Rat Pancreatic Islets. The method used to measure insulin release from incubated rat pancreatic islets was previously described.[14,23]

Measurements of the Contractile Activity in Rat Aorta. The method used to measure the myorelaxant effect of the drugs on 30 or 80 mM KCl-precontracted rat aortic rings was previously described.[14,23]

Ionization Constants. The pK_a value of the compound **6a** was determined by means of UV spectrophotometry using a Perkin-Elmer UV/vis 554 spectrophotometer at 25 °C. UV spectra of the compound were taken in different aqueous buffers of pH ranging from 7 to 11. The pK_a value was calculated by the Debye−Hückel equation at the wavelength giving the maximum absorbance of the ionized form.[29]

Determination of the Enantiomeric Purity. Chiral HPLC was performed to determine the enantiomeric purity of compounds **23** and **24**. The following experimental conditions were used: column, Chiralcel OD-H (0.46 cm × 25 cm); mobile phase, isopropanol/hexane (25:75); flow rate, 1 mL/min. The following retention times were obtained: 6.80 min for compound **23** [(*R*)-isomer] and 9.43 min for compound **24** [(*S*)-isomer].

■ ASSOCIATED CONTENT

Supporting Information

Physicochemical data (mp, ^{1}H NMR, ^{13}C NMR, elemental analysis) of compounds **6b−w** and **10b−e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

Kir, inwardly rectifying potassium channel; SUR, sulfonylurea receptor; K_{ATP} channel, ATP-sensitive potassium channel; DMF, dimethylformamide; DMSO-d_6, deuterated dimethyl sulfoxide; HMDS, hexamethyldisiloxane; mCPBA, meta-chloroperbenzoic acid; TMS, tetramethylsilane; NMR, nuclear magnetic resonance; TLC, thin layer chromatography

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