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

Pascal de Tullio,[†] Bénédicte Becker,[#] Stéphane Boverie,[†] Michael Dabrowski,[‡] Philip Wahl,[§] Marie-Hélène Antoine,[#] Fabian Somers,[†] Sophie Sebille,[†] Raogo Ouedraogo,[#] John Bondo Hansen,[§] Philippe Lebrun,[#] and Bernard Pirotte*[,†]

Centre de Recherche en Pharmacochimie des Substances Naturelles et Synthétiques, Laboratoire de Chimie Pharmaceutique, Université de Liège, 1, Avenue de l'Hôpital, CHU, Tour 4, B-4000 Liège, Belgium, University Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, U.K., Laboratoire de Pharmacodynamie et de Thérapeutique, Université Libre de Bruxelles, 808, Route de Lennik, B-1070 Bruxelles, Belgium, and Discovery, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Malov, Denmark

Received December 16, 2002

3-(Alkylamino)-7-halo-4*H*-1,2,4-benzothiadiazine 1,1-dioxides were synthesized, and their activity on rat-insulin-secreting cells and rat aorta rings was compared to that of the K_{ATP} channel activators diazoxide and pinacidil. Structure−activity relationships indicated that an improved potency and selectivity for the pancreatic tissue was obtained by introducing a fluorine atom in the 7-position and a short linear (preferably ethyl) or cyclic (preferably cyclobutyl) hydrocarbon chain on the nitrogen atom in the 3-position. By contrast, strong myorelaxant activity was gained by the introduction of a halogen atom different from the fluorine atom in the 7-position and a bulky branched alkylamino chain in the 3-position. Thus, 3-(ethylamino)-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**11**) expressed a marked inhibitory activity on pancreatic B-cells ($IC_{50} = 1\ \mu M$) associated with a weak vasorelaxant effect ($ED_{50} > 300\ \mu M$), whereas 7-chloro-3-(1,1-dimethylpropyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**27**), which was only slightly active on insulin-secreting cells ($IC_{50} > 10\ \mu M$), was found to be very potent on vascular smooth muscle cells ($ED_{50} = 0.29\ \mu M$). Radioisotopic and electrophysiological investigations performed with 7-chlorinated, 7-iodinated, and 7-fluorinated 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides confirmed that the drugs activated K_{ATP} channels. The present data revealed that subtle structural modifications of 3-(alkylamino)-7-halo-4*H*-1,2,4-benzothiadiazine 1,1-dioxides can generate original compounds activating K_{ATP} channels and exhibiting different in vitro tissue selectivity profiles.

Introduction

Potassium channels sensitive to intracellular levels of adenosine triphosphate (ATP-sensitive K^+ channels or K_{ATP} channels), which link the membrane potential to the metabolic state of the cell, have been described in a wide range of cell types including pancreatic B-cells[1] and smooth muscle cells.[2] In pancreatic B-cells (insulin-secreting cells), these channels have been shown to be involved in the insulin secretory process.[3,4] In the vascular tissue, the K_{ATP} channels play an important role in controlling muscle tone and contractility.[5]

Recent progress in molecular biology indicated that K_{ATP} channels consist of octameric complexes of two unrelated subunits: a pore-forming subunit (Kir6.*x*) and a regulatory subunit, the sulfonylurea receptor (SUR*x*).[6] According to their tissue localization, K_{ATP} channels exist in different isoforms resulting from the assembly of the SUR and Kir subunits in multiple combinations. The combination of the SUR1 with the Kir6.2 subunits (SUR1/Kir6.2) forms the pancreatic B-cell type K_{ATP} channels, whereas the SUR2A/Kir6.2 and the SUR2B/Kir6.2 or Kir6.1 combinations form the cardiac type and the smooth muscle type K_{ATP} channels, respectively.[6]

A variety of compounds have been reported to activate K_{ATP} channels. Those drugs, named potassium channel openers/activators (or PCOs), are potentially of great value as new therapeutic agents.[7] Indeed, pancreatic B-cells K_{ATP} channel activators could find a considerable therapeutic interest in the prevention or treatment of several diseased states involving altered endocrine pancreatic function, such as type 1 and type 2 diabetes.[8−10] However, because of the ubiquitous distribution of K_{ATP} channels, and in order to reduce the side effects of PCOs, the development of novel compounds should be linked to a high selectivity for a single channel subtype. Although the K_{ATP} channel activator diazoxide was found to preserve endogenous insulin production in patients with newly manifested autoimmune diabetes[8] and was found to reduce body fat in hyperinsulinaemic obese adults,[10] the drug was also responsible for marked side effects such as hypertrichosis, oedema, headache, and hypotension as a result of its lack of tissue selectivity. Those side effects considerably limit the interest of diazoxide in clinical practice and encourage the development of pancreatic B-cells selective PCOs for such therapeutic indications.

We recently reported that 3-(alkylamino)-4*H*-pyrido-[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides such as BPDZ 44

* To whom correspondence should be addressed. Phone: +32 4 366 43 65. Fax: +32 4 366 43 62. E-mail: B.Pirotte@ulg.ac.be.
[†] Université de Liège.
[#] Université Libre de Bruxelles.
[‡] University Laboratory of Physiology.
[§] Novo Nordisk A/S.

10.1021/jm021117w CCC: $25.00
Published on Web 06/19/2003

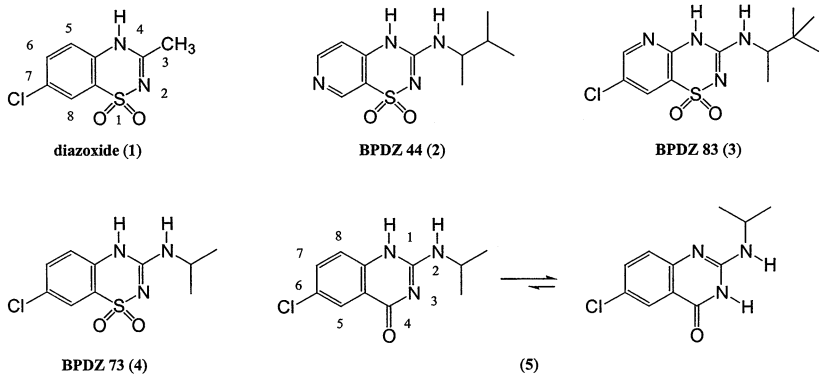


Figure 1. Chemical structure of diazoxide and several analogues described as potassium channel openers: BPDZ 44, BPDZ 83, BPDZ 73 and its quinazolinone isostere (**5**) in two possible tautomeric forms.

(**2**), structurally related to the K_{ATP} channel activator diazoxide (**1**) (Figure 1), were powerful inhibitors of insulin release from rat pancreatic B-cells.[11,12] Compound **2** was identified as a pancreatic B-cell K_{ATP} channel activator [13,14] and was also found to be far less potent than diazoxide at relaxing smooth muscle tissue.[12,15]

In the search for a novel series of pyridothiadiazine dioxides acting as PCOs, we also developed 3-alkylamino-7-chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides such as BPDZ 83 (**3**) [16] (Figure 1). These compounds may be regarded as isomers of 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides ("7-aza" compounds) bearing the nitrogen atom of the pyridine ring in the 5- instead of the 7-position of the heterocycle ("5-aza" compounds) and bearing a chlorine atom in the 7-position such as the PCO prototype diazoxide (7-chlorobenzothiadiazine dioxide) (**1**, Figure 1). Compound **3** was found to be much more potent as a vasodilator than as an inhibitor of insulin secretion, revealing that this "5-aza" (**3**) and related compounds exhibited an opposite tissue selectivity.[16] Moreover, further investigations indicated that compound **3** expressed, on the vascular tissue, the pharmacological profile of a typical K_{ATP} channel activator.[16]

Recently, we have prepared 7-chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (BPDZ 73) (**4**) and its 7-methoxy analogue, 7-methoxy-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (BPDZ 216), which were revealed to be potent and quite tissue-selective pancreatic B-cell K_{ATP} channel activators.[8,17] In addition, we also synthesized quinazolinone isosteres of BPDZ 73 (for example, compound **5**). The latter compounds lacked tissue selectivity and were active on pancreatic and vascular tissues.[18]

The present work aimed at developing new analogues of BPDZ 73 (**4**) by varying the nature of the halogen atom in the 7-position and the size as well as the branching of the alkylamino chain in the 3-position. Particular attention was paid to the identification of structural requirements leading to improvement of potency and pancreatic B-cell selectivity. The new compounds were examined as putative potassium channel openers on rat pancreatic islets (inhibition of glucose-induced insulin release) as well as on rat aorta (myorelaxant effect on 30 mM KCl-precontracted aorta rings). From the biological data collected, tissue selectivity linked to structure−activity relationships was discussed. Finally, radioisotopic and electrophysiological investigations were performed in order to elucidate the mechanism of action of the newly synthesized compounds.

Chemistry

3-(Alkylamino)-7-halo-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**10**−**25**, **28**−**46**) were obtained in four steps starting from the appropriate para halo-substituted aniline (Scheme 1). The first step was the synthesis of 7-halo-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides (**7a**−**d**) from the reaction of different anilines (**6a**−**d**) with chlorosulfonyl isocyanate, according to a previously described procedure.[19] The 3-oxo compounds (**7a**−**d**) were converted into the corresponding 3-thioxo derivatives (**8a**−**d**) by means of phosphorus pentasulfide in pyridine, as previously described.[8] 7-Halo-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides (**8a**−**d**) reacted with methyl iodide in a hydromethanolic solution of sodium bicarbonate to give the corresponding 7-halo-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**9a**−**d**). The 3-methylsulfanyl-substituted intermediates (**9a**−**d**) were heated in a sealed vessel with an excess of the appropriate alkyl/cycloalkylamine to give the desired 3-(alkylamino)-7-halo-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**10**−**25**, **28**−**46**).

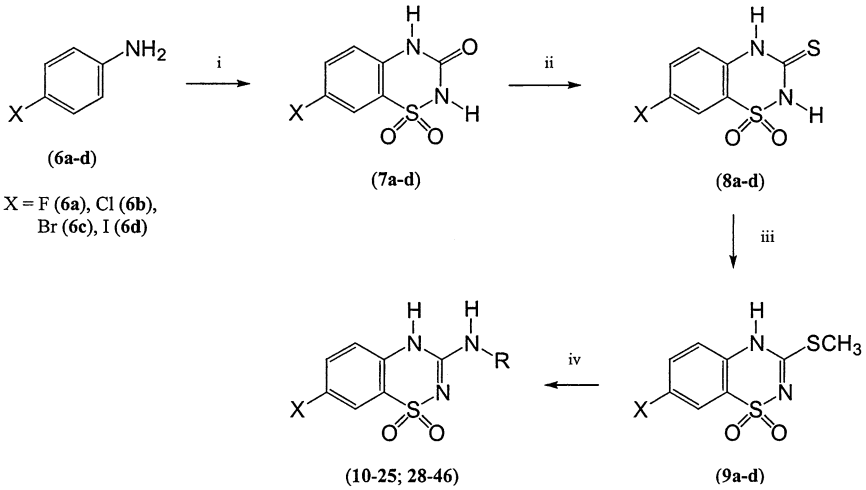
Compounds with a more bulky alkylamino group in the 3-position, such as 7-chloro-3-(*tert*-butylamino)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**26**) and 7-chloro-3-(1,1-dimethylpropylamino)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**27**), could not be obtained from the reaction between **9b** and the amine because of the steric hindrance of the alkyl moiety and because of the poor reactivity of the 3-methylsulfanyl intermediate. Reactivity toward nucleophilic substitution was improved by converting 7-chloro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**9b**) into the corresponding 3-methylsulfinyl intermediate (**49**),[20] which reacted with the bulky alkylamines to give the expected final compounds (**26**, **27**) (Scheme 2).

Last, 7-chloro-3-(isopropylamino)-4-methyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**48**) (or "4-methyl-substituted BPDZ 73") was obtained from the reaction of the 4-methyl-substituted 3-methylsulfanyl intermediate (**47**) with isopropylamine.

Results and Discussion

Biological results revealed that most compounds were powerful inhibitors of the glucose-induced insulin re-

Scheme 1[a]



(6a-d)

X = F (**6a**), Cl (**6b**), Br (**6c**), I (**6d**)

(7a-d)

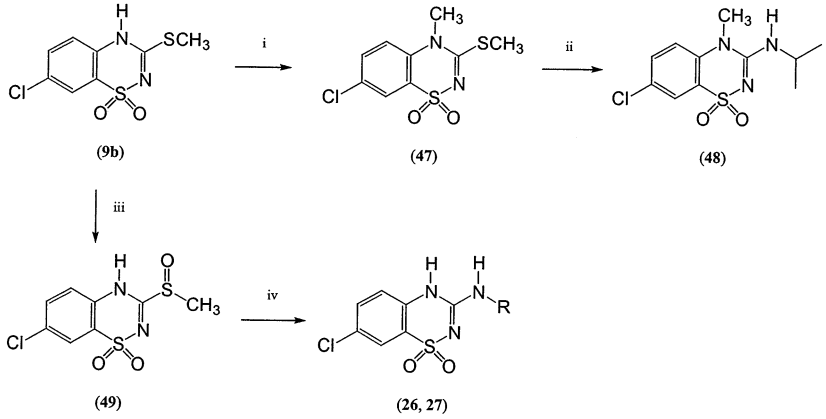
(8a-d)

(9a-d)

(10-25; 28-46)

[a] (i) $ClSO_2NCO$, CH_3NO_2, $AlCl_3$; (ii) P_2S_5, pyridine, Δ; (iii) $NaHCO_3$, CH_3I, CH_3OH/H_2O; (iv) RNH_2, Δ.

Scheme 2[a]



(9b)

(47)

(48)

(49)

(26, 27)

[a] (i) K_2CO_3, CH_3I, CH_3CN/DMF; (ii) $(CH_3)_2CHNH_2$, Δ; (iii) Na_2CO_3, Br_2, H_2O; (iv) RNH_2, Δ.

lease from incubated rat pancreatic islets (see Tables 1−4), some of them (**22**, **34**, **36**) being 25−50 times more potent than the reference compound diazoxide. Except for the 7-fluoro-substituted compounds (Table 1), the size and the branching of the alkyl chain introduced on the exocyclic nitrogen atom in the 3-position markedly affected the activity on insulin-secreting cells. Thus, for the 7-chloro- (Table 2), 7-bromo- (Table 3), and 7-iodo-substituted (Table 4) benzothiadiazine dioxides, the inhibitory activity on the insulin-releasing process decreased with an increase in the size of the hydrocarbon chain. More precisely, the rank order of potency appeared to be the following: methyl < ethyl ≤ isopropyl > propyl ≥ *sec*-butyl > 1,2-dimethylpropyl. The most potent drugs had a short linear or branched hydrocarbon chain such as ethyl or isopropyl (see **22**, **4, 34**, **36**, and **42**). For the 7-fluoro-substituted derivatives, the influence of the size and the branching of the alkyl chain was less pronounced (see **10**−**15**) although the best choice for the hydrocarbon moiety was again ethyl or isopropyl (see **11** and **13**). Bulky hydrocarbon chains such as *tert*-butyl and 1,1-dimethylpropyl (quaternary carbon atoms) were responsible for a marked decrease of activity on B-cells (compare **26** with **4** and **27** with **24**).

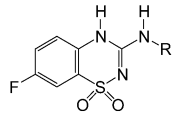
When cycloalkyl groups were present, the most potent compounds were obtained with the cyclobutyl rather than with the cyclopropyl moiety (compare **17**, **30**, and **40** with **16**, **29**, and **39**). The activity of the 3-cyclobutylamino-substituted derivatives was close to that of the ethylamino- and isopropylamino-substituted compounds in their respective series of 7-halobenzothiadiazine dioxides.

The nature of the halogen atom in the 7-position was found to have little influence on the activity on B-cells, although 7-iodo-substituted compounds were less potent than the 7-fluoro-, 7-chloro-, and 7-bromo-substituted drugs.

The myorelaxant activity of the benzothiadiazine dioxides was affected by the nature of the hydrocarbon chain on the exocyclic nitrogen atom in the 3-position but also by the nature of the halogen atom in the 7-position.

In marked contrast to the biological effects on B-cells, a more pronounced myorelaxant activity was observed when the size and the branching of the hydrocarbon chain in the 3-position were increased (see compounds **10**−**15** in Table 1, compounds **21**−**27** in Table 2, compounds **33**−**38** in Table 3, and compounds **42**−**44** in Table 4). Thus, whatever the nature of the halogen atom in the 7-position, the weaker vasorelaxing effect was obtained in each series with a methylamino substituent in the 3-position (see **10**, **21**, and **33**), whereas the stronger myorelaxant potency was observed with a

Table 1. Effects of 3-Alkylamino-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets and on Contractile Activity of K^+-Depolarized Rat Aorta Rings

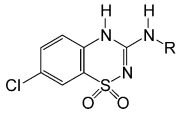


10-20

		Rat pancreatic B-cells					Rat aorta rings	
		% RIS [a]				IC_{50} [b] (μM)	ED_{50} [c] (μM)	ED_{50}/IC_{50} [d]
Cpd	R	50 μM	10 μM	1 μM	0.1 μM			
10	CH_3	5.7 ± 0.9 (20)	7.7 ± 0.6 (15)	76.1 ± 5.6 (21)	n.d.	2.2	> 300 (4)	> 136
11	CH_2CH_3	6.3 ± 0.5 (14)	8.6 ± 1.1 (18)	50.8 ± 3.9 (14)	n.d.	1.0	> 300 (4)	> 300
12	$CH_2CH_2CH_3$	7.7 ± 0.7 (16)	15.1 ± 1.5 (19)	69.2 ± 3.7 (24)	n.d.	2.0	166 ± 15 (4)	83
13	$CH(CH_3)_2$	3.3 ± 0.7 (13)	3.7 ± 0.6 (13)	47.3 ± 3.7 (23)	96.9 ± 4.9 (16)	0.8	43.1 ± 10.7 (5)	54
14	$CH(CH_3)CH_2CH_3$	5.9 ± 0.5 (15)	6.9 ± 0.6 (15)	64.6 ± 3.6 (13)	n.d.	1.6	39.2 ± 6.1 (4)	25
15	$CH(CH_3)CH(CH_3)_2$	7.9 ± 0.6 (15)	10.6 ± 1.4 (15)	87.7 ± 5.2 (16)	n.d.	2.8	26.4 ± 4.3 (5)	9
16	$CH(CH_2)_2$ [e]	7.5 ± 0.7 (22)	32.5 ± 2.3 (15)	104.5 ± 7.3 (14)	n.d.	5.1	> 300 (4)	> 58
17	$CH(CH_2)_3$ [f]	5.9 ± 0.5 (15)	7.8 ± 1.0 (21)	58.2 ± 3.9 (16)	n.d.	1.3	71.8 ± 19.3 (9)	55
18	$CH(CH_2)_4$ [g]	5.4 ± 0.4 (15)	6.6 ± 0.6 (19)	68.8 ± 3.5 (20)	n.d.	1.8	58.0 ± 10.0 (5)	32
19	$CH_2CH(CH_2)_2$ [h]	6.4 ± 0.3 (15)	39.8 ± 4.1 (21)	n.d. [i]	n.d.	<10	146 ± 6 (4)	> 15
20	$CH_2CH{=}CH_2$	4.1 ± 1.1 (15)	12.8 ± 1.1 (11)	84.1 ± 5.2 (15)	n.d.	2.7	> 300 (4)	> 111
Diazoxide		26.7 ± 1.6 (16)	73.9 ± 4.4 (16)	87.5 ± 5.0 (15)	n.d.	22.6	22.4 ± 2.1 (11)	1.0
Pinacidil		88.8 ± 4.5 (13)	92.1 ± 3.9 (13)	97.7 ± 6.7 (19)	n.d.	>100	0.35 ± 0.02 (11)	< 0.0035

[a] % RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)). [b] IC_{50}: drug concentration giving 50% inhibition of insulin release (estimated value). [c] ED_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (*n*)). [d] ED_{50}/IC_{50}: estimated selectivity ratio. [e] Cyclopropyl. [f] Cyclobutyl. [g] Cyclopentyl. [h] Cyclopropylmethyl. [i] Not determined.

Table 2. Effects of 3-Alkylamino-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets and on Contractile Activity of K^+-Depolarized Rat Aorta Rings



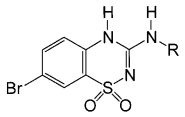
21-32

		Rat pancreatic B-cells					Rat aorta rings	
		% RIS [a]				IC_{50} [b] (μM)	ED_{50} [c] (μM)	ED_{50}/IC_{50} [d]
Cpd	R	50 μM	10 μM	1 μM	0.1 μM			
21	CH_3	2.4 ± 0.5 (10)	4.4 ± 0.6 (12)	55.1 ± 4.7 (14)	n.d.	1.1	142 ± 15 (4)	129
22	CH_2CH_3	2.1 ± 0.4 (11)	10.7 ± 1.1 (12)	29.2 ± 3.5 (13)	n.d.	< 1.0	39.9± 6.3 (4)	> 40
23	$CH_2CH_2CH_3$	3.6 ± 0.8 (14)	14.2 ± 1.1 (11)	83.4 ± 3.6 (15)	n.d.	2.7	55.5 ± 3.8 (5)	21
4	$CH(CH_3)_2$	5.7 ± 0.5 (35) [h]	4.9 ± 0.4 (32) [h]	36.2 ± 2.4 (31) [h]	90.4 ± 3.5 (23) [h]	0.7 [h]	36.3 ± 2.2 (6) [h]	52
24	$CH(CH_3)CH_2CH_3$	5.5 ± 0.4 (16) [h]	19.9 ± 1.4 (14)	85.4 ± 3.7 (15)	n.d.	3.1	15.5 ± 1.7 (6)	5
25	$CH(CH_3)CH(CH_3)_2$	11.0 ± 0.8 (16)	62.7 ± 3.6 (15)	88.1 ± 4.8 (21)	n.d.	>10	1.2 ± 0.2 (6)	< 0.12
26	$C(CH_3)_3$	n.d. [i]	64.4 ± 4.4 (26)	n.d.	n.d.	>10	1.4 ± 0.2 (4)	< 0.14
27	$C(CH_3)_2CH_2CH_3$	n.d.	83.2 ± 4.4 (16)	n.d.	n.d.	>10	0.29 ± 0.05 (5)	< 0.029
28	$CH(CH_2CH_3)_2$	8.1 ± 1.0 (12)	31.7 ± 1.8 (28)	93.7 ± 4.2 (16)	n.d.	4.5	93.6 ± 26.0 (6)	21
29	$CH(CH_2)_2$ [e]	5.8 ± 0.9 (13)	26.6 ± 3.0 (18)	84.0 ± 4.6 (16)	n.d.	3.4	190 ± 19 (5)	56
30	$CH(CH_2)_3$ [f]	8.1 ± 1.7 (11)	6.4 ± 0.3 (14)	60.2 ± 5.5 (11)	n.d.	1.3	34.9 ± 4.4 (5)	27
31	$CH_2CH(CH_2)_2$ [g]	15.2 ± 1.3 (14)	65.9 ± 3.9 (23)	n.d.	n.d.	>10	41.2 ± 12.2 (5)	< 4
32	$CH_2CH{=}CH_2$	7.8 ± 0.6 (10)	17.3 ± 1.3 (21)	87.9 ± 3.8 (24)	n.d.	3.1	28.9 ± 5.5 (4)	9
Diazoxide		26.7 ± 1.6 (16)	73.9 ± 4.4 (16)	87.5 ± 5.0 (15)	n.d.	22.6	22.4 ± 2.1 (11)	1.0
Pinacidil		88.8 ± 4.5 (13)	92.1 ± 3.9 (13)	97.7 ± 6.7 (19)	n.d.	>100	0.35 ± 0.02 (11)	< 0.0035

[a] % RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)). [b] IC_{50}: drug concentration giving 50% inhibition of insulin release (estimated value). [c] ED_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (*n*)). [d] ED_{50}/IC_{50}: estimated selectivity ratio. [e] Cyclopropyl. [f] Cyclobutyl. [g] Cyclopropylmethyl. [h] Published results (ref 8). [i] Not determined.

1,2-dimethylpropylamino (see **15**, **25**, **38**, and **44**), a *tert*-butylamino (see **26**), or a 1,1-dimethylpropylamino (see **27**) group. As a result, compound **27** with an ED_{50} value below 0.5 μM was found to be as potent as the reference PCO pinacidil. Such an observation is in accordance with the structure−activity relationships previously

Table 3. Effects of 3-Alkylamino-7-bromo-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets and on Contractile Activity of K^+-Depolarized Rat Aorta Rings

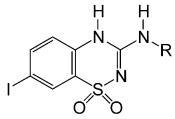


33-41

		Rat pancreatic B-cells					Rat aorta rings	
		% RIS [a]				IC_{50} [b] (μM)	ED_{50} [c] (μM)	ED_{50}/IC_{50} [d]
Cpd	R	50 μM	10 μM	1 μM	0.1 μM			
33	CH_3	6.4 ± 1.1 (13)	7.3 ± 0.6 (16)	69.9 ± 4.0 (15)	n.d.	1.8	31.0 ± 4.6 (4)	17
34	CH_2CH_3	4.6 ± 0.6 (14)	6.5 ± 0.4 (15)	38.1 ± 2.9 (14)	n.d.	< 1.0	6.2 ± 0.4 (5)	> 6
35	$CH_2CH_2CH_3$	7.1 ± 0.7 (15)	34.7 ± 1.6 (16)	n.d. [g]	n.d.	< 10	5.9 ± 0.9 (4)	> 0.6
36	$CH(CH_3)_2$	6.7 ± 1.7 (12)	8.1 ± 0.8 (12)	34.9 ± 2.8 (12)	91.0 ± 5.3 (13)	0.5	4.8 ± 0.7 (5)	9.6
37	$CH(CH_3)CH_2CH_3$	4.0 ± 0.3 (15)	26.8 ± 2.7 (14)	92.5 ± 3.8 (16)	n.d.	4.0	5.6 ± 0.8 (6)	1.4
38	$CH(CH_3)CH(CH_3)_2$	21.4 ± 1.3 (16)	98.4 ± 4.2 (15)	97.2 ± 4.3 (15)	n.d.	>10	2.6 ± 0.8 (4)	< 0.26
39	$CH(CH_2)_2$ [e]	4.6 ± 1.1 (14)	21.1 ± 1.5 (16)	85.1 ± 5.0 (15)	n.d.	3.1	89.4 ± 13.1 (5)	29
40	$CH(CH_2)_3$ [f]	6.1 ± 1.2 (15)	9.6 ± 0.6 (15)	54.8 ± 2.3 (14)	n.d.	1.1	21.7 ± 4.3 (4)	20
41	$CH_2CH{=}CH_2$	4.8 ± 0.5 (13)	23.7 ± 1.6 (13)	77.2 ± 4.4 (15)	n.d.	2.8	3.0 ± 0.4 (4)	1.1
Diazoxide		26.7 ± 1.6 (16)	73.9 ± 4.4 (16)	87.5 ± 5.0 (15)	n.d.	22.6	22.4 ± 2.1 (11)	1.0
Pinacidil		88.8 ± 4.5 (13)	92.1 ± 3.9 (13)	97.7 ± 6.7 (19)	n.d.	>100	0.35 ± 0.02 (11)	< 0.0035

[a] % RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)). [b] IC_{50}: drug concentration giving 50% inhibition of insulin release (estimated value). [c] ED_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (*n*)). [d] ED_{50}/IC_{50}: estimated selectivity ratio. [e] Cyclopropyl. [f] Cyclobutyl. [g] Not determined.

Table 4. Effects of 3-Alkylamino-7-iodo-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets and on Contractile Activity of K^+-Depolarized Rat Aorta Rings



42-46

		Rat pancreatic B-cells					Rat aorta rings	
		% RIS [a]				IC_{50} [b] (μM)	ED_{50} [c] (μM)	ED_{50}/IC_{50} [d]
Cpd	R	50 μM	10 μM	1 μM	0.1 μM			
42	$CH(CH_3)_2$	5.6 ± 0.3 (15)	9.6 ± 0.8 (31)	74.8 ± 3.2 (31)	99.2 ± 5.3 (15)	2.1	7.7 ± 1.6 (4)	3.7
43	$CH(CH_3)CH_2CH_3$	22.5 ± 1.3 (14)	64.8 ± 4.2 (16)	84.2 ± 3.7 (15)	n.d.	>10	1.3 ± 0.2 (6)	< 0.13
44	$CH(CH_3)CH(CH_3)_2$	51.7 ± 3.0 (16)	86.2 ± 3.8 (16)	91.9 ± 3.9 (8)	n.d.	>50	0.94 ± 0.41 (4)	< 0.02
45	$CH(CH_2CH_3)_2$	17.0 ± 1.4 (15)	67.0 ± 4.1 (14)	n.d. [f]	n.d.	>10	79.1 ± 20.6 (6)	< 7.9
46	$CH(CH_2)_3$ [e]	n.d.	30.6 ± 1.7 (15)	n.d.	n.d.	<10	22.2 ± 4.4 (4)	> 2.2
Diazoxide		26.7 ± 1.6 (16)	73.9 ± 4.4 (16)	87.5 ± 5.0 (15)	n.d.	22.6	22.4 ± 2.1 (11)	1.0
Pinacidil		88.8 ± 4.5 (13)	92.1 ± 3.9 (13)	97.7 ± 6.7 (19)	n.d.	>100	0.35 ± 0.02 (11)	< 0.0035

[a] % RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)). [b] IC_{50}: drug concentration giving 50% inhibition of insulin release (estimated value). [c] ED_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (*n*)). [d] ED_{50}/IC_{50}: estimated selectivity ratio. [e] Cyclobutyl. [f] Not determined.

established for pinacidil analogues for which the 1,2,2-trimethylpropyl and the 1,1-dimethylpropyl side chains were recognized as the most interesting hydrocarbon chains for improving myorelaxant activity.[21,22]

The introduction of a short cycloalkylamino group in the 3-position provoked a marked decrease of activity in rat aorta rings. The cyclopropyl derivatives (see **16**, **29**, and **39**) were systematically found to be less active than their corresponding cyclobutyl analogues (see **17**, **30**, and **40**).

The nature of the halogen atom in the 7-position also strongly affected the activity on the vascular tissue. The introduction of a fluorine atom in the 7-position decreased the myorelaxant activity of benzothiadiazine dioxides. The rank order of potency was defined as follows: 7-fluoro compounds < 7-chloro compounds ≤ 7-bromo compounds ≤ 7-iodo compounds.

An ED_{50}/IC_{50} ratio can be calculated in order to assess the tissue selectivity (vascular versus pancreatic tissue) of the new compounds (see Tables 1−4). Diazoxide,

which is known to be poorly selective,[12] was found to exhibit a selectivity ratio of about 1 (see Tables 1-4), indicating that the compound was almost equipotent on both tissues. Not surprisingly, pinacidil was found to be clearly selective for the vascular tissue with a selectivity ratio lower than 0.005 (see Tables 1−4).

The 7-fluoro-substituted benzothiadiazine dioxides (see Table 1) may be regarded as the most interesting series of drugs in terms of tissue selectivity. These compounds were very potent as inhibitors of the insulin-releasing process (all drugs were more potent than diazoxide), while their myorelaxant activity was weak (all drugs were less potent than diazoxide). In the linear hydrocarbon side chain series, the 7-methylamino- and the 7-(ethylamino)-substituted derivatives **10** and **11** markedly inhibited insulin release (IC_{50} = 2.2 and 1.0 μM, respectively) and were found to be essentially inactive as myorelaxants (ED_{50} > 300 μM). As a result, these drugs were at least 135- to 300-fold more potent on B-cells than on vascular smooth muscle cells. Likewise, the cyclobutyl derivative **17** exhibited a pronounced activity on pancreatic B-cells (IC_{50} = 1.3 μM) associated with a weak myorelaxant activity on vascular smooth muscle cells (ED_{50} = 72 μM). Although the allyl derivative **20** also expressed a marked tissue selectivity, its efficiency on the pancreatic tissue was less pronounced (IC_{50} = 2.7 μM).

As expected, the selectivity of the 7-fluoro-substituted compounds for the pancreatic versus the vascular smooth muscle tissue decreased with an increase in the size and the branching of the hydrocarbon side chain in the 3-position (see selectivity ratio in Table 1). Such an observation can also be made for the other series of 7-halo-substituted benzothiadiazine dioxides (see Tables 2−4). The selectivity ratio of the 7-chloro-substituted compounds was between 129 for the methylamino derivative **21** and <0.03 for the 1,1-dimethylpropylamino derivative **27**. With a selectivity ratio greater than 40 and an IC_{50} value on pancreatic B-cells near or below 1 μM, compounds **21** (methyl), **22** (ethyl), and **4** (isopropyl) are examples of 7-chloro compounds expressing an attractive pancreatic B-cell selectivity and potency. In this series of chlorinated derivatives, compound **27**, with a bulky quaternary carbon atom located on the 3-alkylamino side chain, was the most potent and vascular smooth muscle selective drug.

In the series of 7-bromo- and 7-iodo-substituted benzothiadiazine dioxides, as a result of their more pronounced myorelaxant activity, only a few drugs expressed selectivity for the pancreatic tissue (see Tables 3 and 4). However, the cyclobutylamino side chain in the 3-position again conferred a marked effect on B-cells associated with a weak activity on aorta rings (see compound **40**).

The introduction of a methyl group in the 4-position of compound **4** (leading to compound **48**) was responsible for a complete loss of activity on pancreatic B-cells and on vascular smooth muscle cells [% RIS at 50 μM is 96.5 ± 5.1% (n = 14); ED_{50} on aorta rings is >100 μM (n = 4)]. Such a feature is in accordance with our previous data reporting the effect of 3-(alkylamino)-4-methyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides[12] and reflects the critical importance, for biological activity, of the presence of a hydrogen atom in the 4-position of the heterocycle.

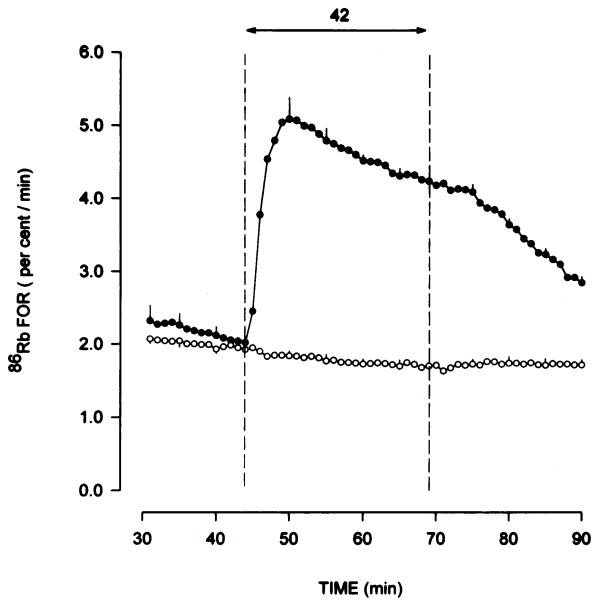


Figure 2. Effect of compound **42** (50 μM) on ^{86}Rb outflow from rat pancreatic islets perifused in the absence (●) and presence (○) of glibenclamide (10 μM) throughout. Mean values (±SEM) refer to four individual experiments.

Taken as a whole, the present pharmacological data give important insights into the structural requirements leading to potency and tissue selectivity (pancreatic versus vascular smooth muscle). Looking at the nature of the halogen atom in the 7-position and the alkylamino side chain in the 3-position, selectivity for the pancreatic tissue is obtained by introducing a fluorine atom in the 7-position and a short linear (preferably ethyl) or cyclic (preferably cyclobutyl) hydrocarbon chain on the nitrogen atom in the 3-position. An improvement in myorelaxant activity is gained by the introduction of a halogen atom different from the fluorine atom in the 7-position and a bulky branched alkylamino chain in the 3-position. The presence of a hydrogen atom in the 4-position is also required for biological activity.

Chlorinated, iodinated, and fluorinated compounds exhibiting a marked inhibitory effect on the insulin secretory rate were selected for further pharmacological investigations. The aim of these studies was to ascertain that the biological effects of the drugs (and their analogues) on pancreatic B-cells were related to K_{ATP} channel activation.

In the first series of experiments, using the efflux of ^{86}Rb (^{42}K substitute) as an index of K^+ permeability,[8,13,14,23] we have characterized the effects of compounds **4**, **24**, and **42** on ^{86}Rb outflow from prelabeled and perifused rat pancreatic islets. Figure 2 illustrates the effects of compound **42**, but all three drugs provoked a pronounced and sustained increase in the rate of ^{86}Rb outflow. When the same experiments were repeated in the presence of the K_{ATP} channel blocker glibenclamide in the perifusing medium, the stimulatory effects of compound **4**, **24**, and **42** were completely abolished (Figure 2 and data not shown). Such data suggest that the drugs activated K_{ATP} channels in rat islet cells.

In a second set of experiments, we investigated the effect of **18** (BPDZ 176) on K_{ATP} channels heterologously

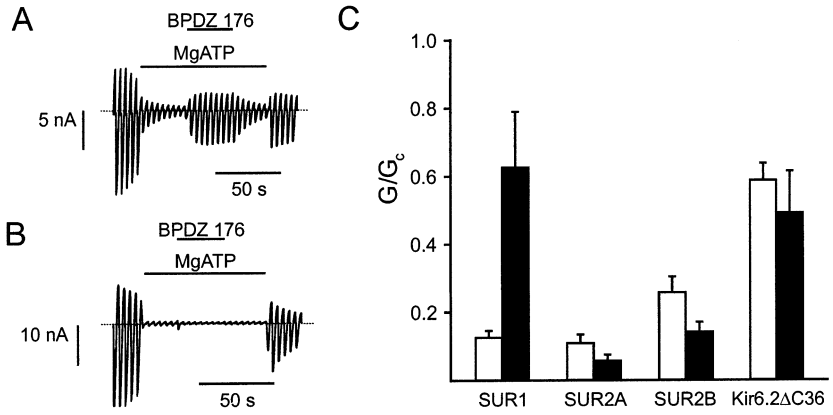


Figure 3. Effects of BPDZ 176 (**18**) on SUR1/Kir6.2, SUR2A/Kir6.2, SUR2B/Kir6.2 or Kir6.2ΔC36 currents. Macroscopic currents were recorded from inside-out patches in response to a series of voltage ramps from −110 to +100 mV from oocytes coexpressing Kir6.2 and either SUR1 (A) or SUR2A (B). 100 μM MgATP and 100 μM BPDZ 176 were added to the intracellular solution as indicated by the bars. (C) Mean macroscopic slope conductances (G) in the presence of 100 μM MgATP (white columns) or 100 μM BPDZ 176 and 100 μM MgATP (black columns) in excised patches from oocytes expressing either Kir6.2 and SUR1, SUR2A, SUR2B, or Kir6.2ΔC36 alone. Data are expressed as a fraction of the mean slope conductance in control solution (no additions) (G_C). The vertical bars indicate 1 SEM (n = 3−6 in each column).

expressed in *Xenopus* oocytes. Figure 3 shows that the compound activated the pancreatic B-cell type K_{ATP} channel (Kir6.2/SUR1) but not the cardiac (Kir6.2/SUR2A) or the smooth muscle (Kir6.2/SUR2B) type. The mean data are presented in Figure 3C showing that, in the presence of 100 μM MgATP, 100 μM BPDZ 176 only activated SUR1 comprising K_{ATP} channels. A small inhibition on SUR2A or SUR2B comprising K_{ATP} channels was also observed, suggesting that the compound interacted with SUR2A and SUR2B without activating any currents. Moreover, the effects seen on the K_{ATP} channels expressed in oocytes were likely to be mediated by the SUR rather than the Kir6.2 subunits because compound **18** had no effect on Kir6.2 expressed in the absence of SUR (Figure 3C).

As performed with 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides such as **2**[12] and 3-(alkylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides such as **3**,[16] the pK_a value of representative 7-halo-substituted 3-(alkylamino)-4*H*-1,2,4-benzothiadiazine 1,1-dioxides was determined in order to predict the ionization state of the drugs at the physiological pH of 7.4. By analogy with pyridothiadiazine dioxides, the acidic character of the benzothiadiazine dioxides should also be linked to the lability of the hydrogen atom in the 4-position of the heterocycle. Previous studies on pyridothiadiazine dioxides indicated that the active form of the drugs in aqueous solution was the nonionized species and that a partial ionization of the drugs at physiological pH could be responsible for a loss of biological activity.[12,16]

The pK_a values of **13**, **4**, **36**, and **42** (the 3-isopropylamino-substituted derivatives in each series of 7-halobenzothiadiazine dioxides) determined by UV spectrophotometry amounted to 9.63, 9.51, 9.41, and 9.28, respectively. By comparison, the pK_a value of diazoxide has been reported to be 8.62.[24] As observed with pyridothiadiazine dioxides,[12,16] the replacement of the alkyl chain in the 3-position with an alkylamino chain (compare diazoxide and **4**) provoked an increase in the pK_a value. Such an effect probably results from the lone pair delocalization of the exocyclic nitrogen atom into the guanidinic system. Incidentally, the acidic character of 7-halobenzothiadiazine dioxides appears to be correlated with the electronic impact of the substituent in the 7-position (para position relative to the nitrogen atom bearing the labile proton), as reflected by its Hammett σ_p constant (σ_p = 0.15, 0.24, 0.26, and 0.28 for the fluorine, the chlorine, the bromine, and the iodine atoms, respectively).[25] Taking into account their pK_a values, the 7-halo-substituted benzothiadiazine dioxides are expected to be present as nonionized species in aqueous solution at physiological pH.

Crystallographic studies conducted with typical examples of 3-alkylaminopyridothiadiazine dioxides[12,16,26] led to the conclusion that those compounds, whatever the position of the nitrogen atom in the pyridine ring, adopted in the solid state a 4H-tautomeric form. In the latter form, the two N−H groups of the "guanidine" moiety were in the same spatial configuration as the guanidinic N−H groups of pinacidil. X-ray data previously obtained with the 3-isopropylamino-substituted benzothiadiazine **42**[27] suggested that in the solid state 3-(alkylamino)-7-halo-4*H*-1,2,4-benzothiadiazine 1,1-dioxides also adopted the same 4H-tautomeric conformation and the same spatial disposition of the guanidinic N−H groups as 3-(alkylamino)-4*H*-1,2,4-pyridothiadiazine 1,1-dioxides.

Conclusions

Several 3-(alkylamino)-7-halo-4*H*-1,2,4-benzothiadiazine 1,1-dioxides were synthesized and examined as putative PCOs on two different tissues: rat pancreatic B-cells and rat aorta rings. The nature of the alkylamino side chain in the 3-position and the nature of the halogen atom in the 7-position were found to considerably affect both the potency and the in vitro tissue selectivity. Selectivity for the pancreatic tissue was obtained by introducing a fluorine atom in the 7-position and a short linear (preferably ethyl) or cyclic (preferably cyclobutyl) hydrocarbon chain on the nitrogen atom in the 3-position. By contrast, strong myorelaxant activity was reached after the introduction of a halogen atom different from the fluorine atom in the 7-position and the addition of a bulky branched alkylamino chain in the 3-position.

Radioisotopic and electrophysiological investigations performed with different 3-(alkylamino)-7-halo-4*H*-1,2,4-benzothiadiazine 1,1-dioxides indicated that the drugs activated K_{ATP} channels and further revealed the channel subtype selectivity of the fluorinated compound **18** (Kir6.2/SUR1 versus Kir6.2/SUR2A and KIR6.2/SUR2B).

Pancreatic B-cells selective K_{ATP} channel activators could be of high therapeutic value for the prevention and/or treatment of type 1 and type 2 diabetes. Thus, the present data contribute to the identification of new compounds expected to become appropiate substitutes for diazoxide in the treatment of glucose homeostasis disorders.

Experimental Section

Chemistry. Melting points were determined on a Büchi-Tottoli capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a FTIR Perkin-Elmer Spectrum 1000 spectrophotometer. UV data and spectra were obtained with a Hitachi U 3010 spectrophotometer. The ^{1}H NMR spectra were taken on a Bruker AW-80 (80 MHz) instrument in DMSO-d_6 with HMDS as an internal standard; chemical shifts are reported in δ values (ppm) relative to internal HMDS. The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, quint. = quintuplet, m = multiplet, and b = broad are used throughout. Elemental analyses (C, H, N, S) were realized on a Carlo-Erba EA 1108 elemental analyzer and were within ±0.4% of the theoretical values. All reactions were routinely checked by TLC on silica gel Merck 60F 254.

7-Fluoro-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (7a). Chlorosulfonyl isocyanate (16.8 mL, 0.193 mol) and nitromethane (160 mL) were mixed together in a closed dried vessel. The mixture was cooled at −5 °C (ice and salt bath) and protected from moisture during the slow addition, under vigorous stirring, of 4-fluoroaniline (17.8 g, 0.16 mol) dissolved in nitromethane (50 mL). At the end of addition (∼15 min), anhydrous $AlCl_3$ (28.0 g, 0.21 mol) was added to the resulting suspension and the mixture was refluxed for 30−45 min. The hot solution was poored onto ice (800 g), and after stirring and complete melting of ice, the resulting precipitate was collected by filtration and washed twice with water (75 mL). The insoluble crude material was suspended in an aqueous solution of sodium bicarbonate (10 g/200 mL) and heated until most of the precipitate was solubilized. The suspension was treated with charcoal and was filtered, and the filtrate was adjusted to pH 1 by means of 12 N HCl. The white product, which precipitated, was collected by filtration, washed with water, and dried (21.8 g, 63%): mp >300 °C; ^{1}H NMR (DMSO-d_6, 80 MHz) δ 7.10−7.70 (m, 3H, 5-H + 6-H + 8-H), 9.55 (b, 1H, N−H), 11.25 (bs, 1H, SO_2NH). Anal. ($C_7H_5FN_2O_3S$) C, H, N, S.

7-Chloro-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (7b). The title compound was obtained from 4-chloroaniline (20.4 g, 0.16 mol) by following the experimental conditions described for **7a** (24.2 g, 65%): mp >300 °C (lit., 316−318 °C[19]).

7-Bromo-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (7c). The title compound was obtained from 4-bromoaniline (27.7 g, 0.16 mol) by following the experimental conditions described for **7a** except that the crude material was dissolved in a hydromethanolic 1:1 solution of sodium bicarbonate (10 g/400 mL) instead of an aqueous solution (31.0 g, 70%): mp >300 °C (lit., 335 °C [28]).

7-Iodo-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (7d). The title compound was obtained from 4-iodoaniline (35.3 g, 0.16 mol) by following the experimental conditions described for **7c** (29.6 g, 57%): mp >300 °C (lit., 311−313 °C[29]).

7-Fluoro-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (8a). A mixture of 7-fluoro-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**7a**, 20.3 g, 95 mmol) and phosphorus pentasulfide (40 g) in anhydrous pyridine (250 mL) was refluxed for 4 h. The solvent was removed under reduced pressure, and the residue was dissolved in an aqueous solution of NaOH (50 g/500 mL). The alkaline solution was treated with charcoal and was filtered, and the filtrate was adjusted to pH 1 by means of 12 N HCl. The compound, which precipitated, was collected by filtration, washed with water, and suspended in an aqueous solution of sodium bicarbonate (20 g/400 mL). The suspension was heated until most of the insoluble material dissolved and was then treated with charcoal and filtered. The filtrate was adjusted to pH 1 with 12 N HCl, and the white precipitate that appeared was collected by filtration, washed with water, and dried (16.4 g, 75%): mp 193−195 °C; ^{1}H NMR (DMSO-d_6, 80 MHz) δ 7.25−7.80 (m, 3H, 5-H + 6-H + 8-H). Anal. ($C_7H_5FN_2O_2S_2$) C, H, N, S.

7-Chloro-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (8b). The title compound was obtained from 7-chloro-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**7b**, 22.0 g, 95 mmol) by following the experimental conditions described for **8a** (17.7 g, 75%): mp 229−232 °C (lit., 217−218 °C[30]).

7-Bromo-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (8c). The title compound was obtained from 7-bromo-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**7c**, 26.0 g, 95 mmol) by following the experimental conditions described for **8a**. After acidification of the alkaline solution, the insoluble material was suspended in a hydromethanolic 1:1 solution of sodium bicarbonate (10 g/400 mL) and the mixture was heated until most of the insoluble material dissolved. The suspension was treated with charcoal and filtered. The filtrate was adjusted to pH 0−1 with 12 N HCl, and the white precipitate that appeared was collected by filtration, washed with water, and dried (18.9 g, 68%): mp 209−211 °C (lit., 223−225 °C[31]).

7-Iodo-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (8d). The title compound was obtained from 7-iodo-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**7d**, 30.8 g, 95 mmol) by following the experimental conditions described for **8c** (25.5 g, 79%): mp 219−222 °C (lit., 217−218 °C[30]).

7-Fluoro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Monohydrate (9a). 7-Fluoro-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**8a**, 12 g, 52 mmol) was dissolved in a 1:1 hydromethanolic solution of sodium bicarbonate (7 g/280 mL), and an excess of methyl iodide (12 mL) was added. After the mixture was stirred for 30 min, the resulting suspension was adjusted to pH 5−6 by means of 6 N HCl. The suspension was concentrated under reduced pressure to half of the volume, and the white precipitate was collected by filtration, washed with water, and dried (12.2 g, 89%): mp 265−268 °C; ^{1}H NMR (DMSO-d_6, 80 MHz) δ 2.45 (s, 3H, SCH_3), 7.10−7.50 (m, 2H, 5-H + 6-H), 7.60 (d, 1H, 8-H), 12.50 (bs, 1H, N−H). Anal. ($C_8H_7FN_2O_2S_2 \cdot H_2O$) C, H, N, S.

7-Chloro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (9b). The title compound was obtained from 7-chloro-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**8b**, 12 g, 48 mmol) by following the experimental conditions described for **9a** (12.6 g, 85%): mp 287−290 °C (lit., 290−291 °C[30]).

7-Bromo-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (9c). The title compound was obtained from 7-bromo-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**8c**, 12 g, 41 mmol) by following the experimental conditions described for **9a** (12.6 g, 82%): mp 282−284 °C; ^{1}H NMR (DMSO-d_6, 80 MHz) δ 2.45 (s, 3H, SCH_3), 7.15 (d, 1H, 5-H), 7.75 (d, 1H, 6-H), 7.85 (s, 1H, 8-H), 12.55 (bs, 1H, N−H).

7-Iodo-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (9d). The title compound was obtained from 7-iodo-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**8d**, 12 g, 35 mmol) by following the experimental conditions described for **9a** (12.4 g, 79%): mp 271−273 °C; ^{1}H NMR (DMSO-d_6, 80 MHz) δ 2.45 (s, 3H, SCH_3), 7.00 (d, 1H, 5-H),

7.90 (d + s, 2H, 6-H + 8-H), 12.50 (bs, 1H, N−H). Anal. ($C_8H_7IN_2O_2S_2$) C, H, N, S.

General Procedures for the Synthesis of 3-(Alkylamino)-7-halo-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides (10−25, 28−46). Method A. A mixture of the appropriate 7-halo-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**9a**−**d**) (0.5 g) and the appropriate alkylamine (or if not commercially available, a concentrated aqueous solution of the amine) (5 mL) was heated in a sealed vessel for 4 h at 140 °C. The excess of amine was eliminated by distillation under reduced pressure, and the residue was dissolved in an aqueous 2% w/v solution of NaOH (20 mL). The alkaline solution was treated with charcoal and was filtered, and the filtrate was adjusted to pH 4−5 with 6 N HCl. The precipitate was collected by filtration, washed with water, and dried. The compound was recrystallized in methanol/water.

Method B. A mixture of the appropriate 7-halo-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**9a**−**d**) (0.5 g), the appropriate alkylamine (1 mL), and dioxane (5 mL) was heated in a sealed vessel for 4 h at 140 °C. After removal of the solvent and the excess of amine under reduced pressure, the compound was isolated and purified as described in method A.

Method C. A mixture of the appropriate 7-halo-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**9a**−**d**) (0.5 g) and the appropriate alkylamine (5−10 mL) was refluxed for 72 h. After removal of the excess of amine under reduced pressure, the compound was isolated and purified as described in method A.

The following compounds were obtained according to the synthetic procedures described above.

7-Fluoro-3-methylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Monohydrate (10). Method A Using a 40% w/v Aqueous Solution of Methylamine. Yield 0.35 g (75%); mp 275−277 °C; IR (KBr) 3324 (N−H), 1637, 1597, 1494 (C=C, C=N, N−H), 1271, 1148 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 2.75 (d, 3H, NHCH_3), 7.10 (m, 2H, 5-*H* + N*H*CH$_3$), 7.30 (dd, 1H, 6-*H*), 7.45 (d, 1H, 8-*H*), 9.65 (s, 1H, N*H*). Anal. ($C_8H_8FN_3O_2S\cdot H_2O$) C, H, N, S.

7-Fluoro-3-(ethylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (11). Method A Using a 70% w/v Aqueous Solution of Ethylamine. Yield 0.36 g (79%); mp 235−237 °C; IR (KBr) 3289 (N−H), 1636, 1593, 1491 (C=C, C=N, N−H), 1260, 1144 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.05 (t, 3H, CH_2CH_3), 3.15 (m, 2H, NHCH_2CH$_3$), 6.80−7.55 (m, 4H, 5-*H* + 6-*H* + 8-*H* + N*H*CH$_2$). Anal. ($C_9H_{10}FN_3O_2S$) C, H, N, S.

7-Fluoro-3-propylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (12). Method A. Yield 0.39 g (80%); mp 199−202 °C; IR (KBr) 3304 (N−H), 1636, 1595, 1494 (C=C, C=N, N−H), 1257, 1144 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.80 (t, 3H, $CH_2CH_2CH_3$), 1.50 (m, 2H, $CH_2CH_2CH_3$), 3.15 (m, 2H, NHCH_2CH$_2$CH$_3$), 6.80−7.55 (m, 4H, 5-*H* + 6-*H* + 8-*H* + N*H*CH$_2$), 10.55 (bs, 1H, N*H*). Anal. ($C_{10}H_{12}FN_3O_2S$) C, H, N, S.

7-Fluoro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (13). Method A. Yield 0.36 g (74%); mp 242−244 °C; IR (KBr) 3304 (N−H), 1639, 1568, 1509, 1493 (C=C, C=N, N−H), 1267, 1148 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.10 (d, 6H, CH(CH_3)$_2$), 3.90 (m, 1H, NHC*H*(CH$_3$)$_2$), 7.00 (bs, 1H, N*H*CH(CH$_3$)$_2$), 7.10−7.50 (m, 3H, 5-*H* + 6-*H* + 8-*H*), 10.25 (bs, 1H, N*H*). Anal. ($C_{10}H_{12}FN_3O_2S$) C, H, N, S.

***R,S*-3-(2-Butylamino)-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Monohydrate (14). Method A.** Yield 0.41 g (75%); mp 203−205 °C; IR (KBr) 3492 (N−H), 1651, 1595, 1568, 1498 (C=C, C=N, N−H), 1267, 1159 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.80 (t, 3H, CH(CH$_3$)CH$_2$CH_3), 1.05 (d, 3H, CH(CH_3)CH$_2$CH$_3$), 1.40 (m, 2H, CH(CH$_3$)CH_2CH$_3$), 3.60 (m, 1H, NHC*H*), 7.00 (bd, 1H, N*H*CH), 7.10 (d, 1H, 5-*H*), 7.30 (d, 1H, 6-*H*), 7.40 (s, 1H, 8-*H*), 10.35 (s, 1H, N*H*). Anal. ($C_{11}H_{14}FN_3O_2S\cdot H_2O$) C, H, N, S.

***R,S*-7-Fluoro-3-(3-methyl-2-butylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (15). Method C.** Yield 0.38 g (70%); mp 193−198 °C; IR (KBr) 3298 (N−H), 1636, 1593, 1504, 1494 (C=C, C=N, N−H), 1258, 1168 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.80 (d, 6H, CH(CH$_3$)CH(CH_3)$_2$), 1.00 (d, 3H, CH(CH_3)CH(CH$_3$)$_2$), 1.65 (m, 1H, CH(CH$_3$)-C*H*(CH$_3$)$_2$), 3.60 (m, 1H, NHC*H*), 6.90 (bd, 1H, N*H*CH), 7.15 (d, 1H, 5-*H*), 7.30 (d, 1H, 6-*H*), 7.45 (s, 1H, 8-*H*), 10.15 (s, 1H, N*H*). Anal. ($C_{12}H_{16}FN_3O_2S$) C, H, N, S.

3-Cyclopropylamino-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (16). Method A. Yield 0.34 g (70%); mp 242−245 °C; IR (KBr) 3274 (N−H), 1620, 1530, 1483 (C=C, C=N, N−H), 1245, 1158 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.40−1.00 (m, 4H, CH(CH_2)$_2$), 2.60 (m, 1H, NHC*H*), 7.00 (b, 1H, N*H*CH), 7.25 (d, 1H, 5-*H*), 7.40 (d, 1H, 6-*H*), 7.70 (s, 1H, 8-*H*), 10.50 (s, 1H, N*H*). Anal. ($C_{10}H_{10}FN_3O_2S$) C, H, N, S.

3-Cyclobutylamino-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (17). Method B. Yield 0.36 g (70%); mp 252−253 °C; IR (KBr) 3288 (N−H), 1631, 1580, 1557, 1474 (C=C, C=N, N−H), 1258, 1153 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.00−2.35 (bm, 6H, CH(CH_2)$_3$), 4.15 (m, 1H, NHC*H*), 6.95−7.80 (m, 4H, 5-*H* + 6-*H* + 8-*H* + N*H*CH), 10.40 (bs, 1H, N*H*). Anal. ($C_{11}H_{12}FN_3O_2S$) C, H, N, S.

3-Cyclopentylamino-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (18). Method B. Yield 0.41 g (76%); mp 237−239 °C; IR (KBr) 3299 (N−H), 1639, 1594, 1568, 1492 (C=C, C=N, N−H), 1257, 1161 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.00−2.15 (bm, 8H, CH(CH_2)$_4$), 4.00 (bm, 1H, NHC*H*), 6.95−7.55 (m, 4H, 5-*H* + 6-*H* + 8-*H* + N*H*CH), 10.15 (bs, 1H, N*H*). Anal. ($C_{12}H_{14}FN_3O_2S$) C, H, N, S.

3-Cyclopropylmethylamino-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (19). Method A. Yield 0.31 g (60%); mp 221−222 °C; IR (KBr) 3295 (N−H), 1641, 1592, 1495 (C=C, C=N, N−H), 1240, 1155 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.30−1.30 (m, 5H, CH(CH_2)$_2$ + C*H*(CH$_2$)$_2$), 3.05 (t, 2H, NHCH_2), 7.00−7.60 (m, 4H, N*H* + 5-*H* + 6-*H* + 8-*H*), 10.50 (bs, 1H, N*H*). Anal. ($C_{11}H_{12}FN_3O_2S$) C, H, N, S.

3-Allylamino-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (20). Method A. Yield 0.30 g (62%); mp 209−210 °C; IR (KBr) 3353 (N−H), 1615, 1595, 1570, 1493 (C=C, C=N, N−H), 1259, 1164 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 3.80 (bm, 2H, CH_2CH=CH$_2$), 4.90−5.30 (m, 2H, CH$_2$-CH=CH_2), 5.55−6.05 (m, 1H, CH$_2$C*H* = CH$_2$), 6.90−7.60 (m, 4H, N*H* + 5-*H* + 6-*H* + 8-*H*), 10.60 (s, 1H, N*H*). Anal. ($C_{10}H_{10}FN_3O_2S$) C, H, N, S.

7-Chloro-3-methylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (21). Method A Using a 40% w/v Aqueous Solution of Methylamine. Yield 0.30 g (65%); mp >300 °C (lit., 318−320 °C[32]); IR (KBr) 3311 (N−H), 1636, 1596, 1477 (C=C, C=N, N−H), 1260, 1138 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 2.55 (d, 3H, NHCH_3), 7.55 (bd, 1H, 5-*H*), 7.60 (d, 1H, 6-*H*), 7.65 (s, 1H, 8-*H*), 8.45 (bm, 1H, N*H*CH$_3$), 10.55 (s, 1H, N*H*). Anal. ($C_8H_8ClN_3O_2S$) C, H, N, S.

7-Chloro-3-(ethylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (22). Method A Using a 70% w/v Aqueous Solution of Ethylamine. Yield 0.30 g (60%); mp 252−253 °C (lit., 246−250 °C[32]); IR (KBr) 3306 (N−H), 1635, 1585, 1483 (C=C, C=N, N−H), 1267, 1170 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.05 (t, 3H, CH_2CH_3), 3.15 (m, 2H, NHCH_2), 7.15 (d, 2H, 5-*H* + N*H*CH$_2$), 7.50 (d + s, 2H, 6-*H* + 8-*H*), 10.60 (bs, 1H, N*H*). Anal. ($C_9H_{10}ClN_3O_2S$) C, H, N, S.

7-Chloro-3-propylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (23). Method A. Yield 0.36 g (70%); mp 211−218 °C (lit., 215−216 °C[32]); IR (KBr) 3296 (N−H), 1631, 1584, 1482 (C=C, C=N, N−H), 1262, 1166 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.80 (t, 3H, $CH_2CH_2CH_3$), 1.45 (m, 2H, CH_2CH_2-CH_3), 3.10 (m, 2H, NHCH_2CH$_2$CH$_3$), 7.10 (d, 2H, N*H*CH$_2$ + 5-*H*), 7.50 (d + s, 2H, 6-*H* + 8-*H*), 10.55 (bs, 1H, N*H*). Anal. ($C_{10}H_{12}ClN_3O_2S$) C, H, N, S.

7-Chloro-3-(3-pentylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (28). Method C. Yield 0.34 g (60%); mp 233−236 °C; IR (KBr) 3290 (N−H), 1627, 1582, 1482 (C=C, C=N, N−H), 1242, 1158 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.75 (t, 6H, CH(CH$_2$CH_3)$_2$), 1.40 (m, 4H,CH(CH_2CH$_3$)$_2$), 3.55 (m, 1H,NHC*H*), 6.95 (bd, 1H, N*H*CH), 7.10 (d, 1H, 5-*H*),

7.50 (d, 1H, 6-*H*), 7.55 (s, 1H, 8-*H*), 10.30 (bs, 1H, N*H*). Anal. ($C_{12}H_{16}ClN_3O_2S$) C, H, N, S.

7-Chloro-3-cyclopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (29). Method A. Yield 0.36 g (69%); mp 261−262 °C (lit., 246−249 °C[32]); IR (KBr) 3307 (N−H), 1616, 1590, 1521, 1484 (C=C, C=N, N−H), 1263, 1144 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.40−0.90 (m, 4H, CH(CH_2)$_2$), 2.60 (m, 1H, NHCH(CH$_2$)$_2$), 7.30 (d, 2H, NHCH(CH$_2$)$_2$ + 5-H), 7.50 (d + s, 2H, 6-H + 8-H), 10.50 (bs, 1H, NH). Anal. ($C_{10}H_{10}ClN_3O_2S$) C, H, N, S.

7-Chloro-3-cyclobutylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (30). Method B. Yield 0.35 g (65%); mp 275−278 °C; IR (KBr) 3284 (N−H), 1633, 1575, 1479 (C=C, C=N, N−H), 1238, 1155 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.10−2.35 (bm, 6H, CH(CH_2)$_3$), 4.15 (m, 1H, NHCH), 7.15 (d, 1H, 5-H), 7.30−7.65 (m, 3H, 6-H + 8-H + NHCH), 10.25 (bs, 1H, NH). Anal. ($C_{11}H_{12}ClN_3O_2S$) C, H, N, S.

7-Chloro-3-cyclopropylmethylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (31). Method A. Yield 0.34 g (62%); mp 288−290 °C; IR (KBr) 3298 (N−H), 1635, 1582, 1483 (C=C, C=N, N−H), 1267, 1160 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.30−1.30 (m, 5H, CH(CH_2)$_2$ + CH(CH$_2$)$_2$), 3.05 (m, 2H, NHCH_2), 7.05 (s, 1H, NH), 7.15 (d, 1H, 5-H), 7.50 (d, 1H, 6-H), 7.60 (s, 1H, 8-H). Anal. ($C_{11}H_{12}ClN_3O_2S$) C, H, N, S.

3-Allylamino-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (32). Method A. Yield 0.32 g (62%); mp 220−222 °C; IR (KBr) 3310 (N−H), 1651, 1630, 1584, 1483 (C=C, C=N, N−H), 1239, 1164 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 3.80 (bm, 2H, CH_2CH=CH$_2$), 4.95−5.25 (m, 2H, CH$_2$-CH=CH_2), 5.55−6.05 (m, 1H, CH$_2$CH=CH$_2$), 7.15 (d, 1H, 5-H), 7.30 (b, 1H, NH), 7.50 (d, 1H, 6-H), 7.60 (s, 1H, 8-H), 10.65 (s, 1H, NH). Anal. ($C_{10}H_{10}ClN_3O_2S$) C, H, N, S.

7-Bromo-3-methylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Monohydrate (33). Method A Using a 40% w/v Aqueous Solution of Methylamine. Yield 0.35 g (70%); mp 305−307 °C; IR (KBr) 3355 (N−H), 1641, 1581, 1547, 1477 (C=C, C=N, N−H), 1263, 1145 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 2.70 (d, 3H, NHCH_3), 7.10 (d, 2H, NHCH$_3$ + 5-H), 7.50−7.80 (m, 2H, 6-H + 8-H), 9.70 (s, 1H, NH). Anal. ($C_8H_8BrN_3O_2S\cdot H_2O$) C, H, N, S.

7-Bromo-3-(ethylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (34). Method A Using a 70% w/v Aqueous Solution of Ethylamine. Yield 0.31 g (63%); mp 267−268 °C; IR (KBr) 3305, 3189 (N−H), 1630, 1583, 1478 (C=C, C=N, N−H), 1249, 1159 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.00 (t, 3H, CH$_2$CH_3), 3.10 (m, 2H, NHCH_2CH$_3$), 7.15 (m, 2H, NHCH$_2$CH$_3$ + 5-H), 7.60 (d, 1H, 6-H), 7.70 (s, 1H, 8-H), 10.60 (bs, 1H, NH). Anal. ($C_9H_{10}ClN_3O_2S$) C, H, N, S.

7-Bromo-3-propylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (35). Method A. Yield 0.39 g (75%); mp 234−235 °C; IR (KBr) 3295 (N−H), 1631, 1580, 1567, 1479 (C=C, C=N, N−H), 1260, 1164 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.85 (t, 3H, CH$_2$CH$_2$CH_3), 1.50 (m, 2H, CH$_2$CH_2CH$_3$), 3.15 (m, 2H, NHCH_2CH$_2$CH$_3$), 7.00 (s, 1H, NHCH$_2$), 7.15 (s, 1H, 5-H), 7.55 (s, 1H, 6-H), 7.70 (s, 1H, 8-H), 10.60 (bs, 1H, NH). Anal. ($C_{10}H_{12}BrN_3O_2S$) C, H, N, S.

7-Bromo-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (36). Method A. Yield 0.21 g (40%); mp 220−221 °C; IR (KBr) 3293 (N−H), 1619, 1578, 1480 (C=C, C=N, N−H), 1276, 1146 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.20 (d, 6H, CH(CH_3)$_2$), 3.95 (m, 1H, NHCH), 7.15 (bd, 1H, NHCH), 7.20 (d, 1H, 5-H), 7.75 (m, 2H, 6-H + 8-H), 10.45 (bs, 1H, NH)). Anal. ($C_{10}H_{12}BrN_3O_2S$) C, H, N, S.

***R,S*-7-Bromo-3-(2-butylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (37). Method A.** Yield 0.30 g (56%); mp 224−226 °C; IR (KBr) 3320 (N−H), 1622, 1579, 1480 (C=C, C=N, N−H), 1277, 1158 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.80 (t, 3H, CH(CH$_3$)CH$_2$CH_3), 1.10 (d, 3H, CH-(CH_3)CH$_2$CH$_3$), 1.40 (m, 2H, CH(CH$_3$)CH_2CH$_3$), 3.70 (m, 1H, NHCH), 6.95 (s, 1H, NHCH), 7.10 (d, 1H, 5-H), 7.60 (d, 1H, 6-H), 7.65 (s, 1H, 8-H), 10.35 (s, 1H, NH). Anal. ($C_{11}H_{14}BrN_3O_2S$) C, H, N, S.

***R,S*-7-Bromo-3-(3-methyl-2-butylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (38). Method C.** Yield 0.39 g (69%); mp 254−256 °C; IR (KBr) 3319 (N−H), 1618, 1578, 1468 (C=C, C=N, N−H), 1252, 1151 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.90 (d, 6H, CH(CH$_3$)CH(CH_3)$_2$), 1.00 (d, 3H, CH(CH_3)CH(CH$_3$)$_2$), 1.65 (m, 1H, CH(CH$_3$)CH(CH$_3$)$_2$), 3.60 (m, 1H, NHCH), 6.95 (bs, 1H, NHCH), 7.10 (d, 1H, 5-H), 7.65 (d, 1H, 6-H), 7.70 (s, 1H, 8-H), 10.30 (bs, 1H, NH). Anal. ($C_{12}H_{16}BrN_3O_2S$) C, H, N, S.

7-Bromo-3-cyclopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (39). Method A. Yield 0.36 g (70%); mp 248−253 °C; IR (KBr) 3274 (N−H), 1621, 1528, 1483 (C=C, C=N, N−H), 1251, 1158 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.35−0.95 (m, 4H, CH(CH_2)$_2$), 3.60 (bs, 1H, NHCH), 7.20 (d, 1H, 5-H), 7.65 (m, 2H, 6-H + 8-H), 10.50 (bs, 1H, NH). Anal. ($C_{10}H_{10}BrN_3O_2S$) C, H, N, S.

7-Bromo-3-cyclobutylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (40). Method B. Yield 0.34 g (63%); mp 279−281 °C; IR (KBr) 3288 (N−H), 1631, 1580, 1563, 1480 (C=C, C=N, N−H), 1258, 1153 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.00−2.35 (bm, 6H, CH(CH_2)$_3$), 4.15 (m, 1H, NHCH), 7.10 (d, 1H, 5-H), 7.55 (dd, 1H, 6-H), 6.90−7.80 (b, NHCH), 8.70 (d, 1H, 8-H), 10.50 (bs, 1H, NH). Anal. ($C_{11}H_{12}BrN_3O_2S$) C, H, N, S.

3-Allylamino-7-bromo-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (41). Method A. Yield 0.33 g (65%); mp 243−245 °C; IR (KBr) 3361 (N−H), 1631, 1582, 1477 (C=C, C=N, N−H), 1252, 1162 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 3.80 (bm, 2H, CH_2CH=CH$_2$), 4.95−5.25 (m, 2H, CH$_2$CH=CH_2), 5.55−6.05 (m, 1H, CH$_2$CH=CH$_2$), 7.10 (d, 1H, 5-H), 7.30 (bs, 1H, NH), 7.65 (m, 2H, 6-H + 8-H), 10.70 (s, 1H, NH). Anal. ($C_{10}H_{10}BrN_3O_2S$) C, H, N, S.

7-Iodo-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (42). Method A. Yield 0.34 g (67%); mp 306−308 °C; IR (KBr) 3286 (N−H), 1617, 1571, 1476 (C=N, C=C, N−H), 1245, 1174 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.15 (d, 6H, CH(CH_3)$_2$), 3.90 (m, 1H, NHCH), 7.00 (d, 1H, 5-H), 7.15 (bd, 1H, NHCH), 7.75 (d, 1H, 6-H), 7.85 (s, 1H, 8-H), 10.45 (bs, 1H, NH)). Anal. ($C_{10}H_{12}IN_3O_2S$) C, H, N, S.

***R,S*-7-Iodo-3-(2-butylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (43). Method A.** Yield 0.37 g (70%); mp 253−254 °C; IR (KBr) 3318 (N−H), 1618, 1573, 1476 (C=N, C=C, N−H), 1247, 1149 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.80 (t, 3H, CH(CH$_3$)CH$_2$CH_3), 1.10 (d, 3H, CH-(CH_3)CH$_2$CH$_3$), 1.40 (m, 2H, CH(CH$_3$)CH_2CH$_3$), 3.60 (m, 1H, NHCH), 6.80−7.10 (m, 2H, 5-H + NHCH), 7.60−7.90 (m, 2H, 6-H + 8-H), 10.30 (bs, 1H, NH). Anal. ($C_{11}H_{14}IN_3O_2S$) C, H, N, S.

***R,S*-7-Iodo-3-*R,S*-(3-methyl-2-butylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (44). Method C.** Yield 0.33 g (60%); mp 267−270 °C; IR (KBr) 3317 (N−H), 1616, 1573, 1475 (C=N, C=C, N−H), 1248, 1156 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.75 (d, 6H, CH(CH$_3$)CH(CH_3)$_2$), 1.00 (d, 3H, CH(CH_3)CH(CH$_3$)$_2$), 1.65 (m,1H, CH(CH$_3$)CH(CH$_3$)$_2$), 3.60 (m,-1H, NHCH), 6.80−7.05 (bd, 2H, 5-H + NHCH), 7.60−7.90 (bd, 2H, 6-H + 8-H), 10.25 (bs, 1H, NH). Anal. ($C_{12}H_{16}IN_3O_2S$) C, H, N, S.

7-Iodo-3-(3-pentylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (45). Method C. Yield 0.33 g (60%); mp 210−220 °C; IR (KBr) 3314 (N−H), 1617, 1574, 1475 (C=C, C=N, N−H), 1277, 1151 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.75 (t, 6H, CH(CH$_2$CH_3)$_2$), 1.40 (m, 4H,CH(CH_2CH$_3$)$_2$), 3.60 (m, 1H,NHCH), 6.95 (bd, 2H, NHCH + 5-H), 7.75 (d, 1H, 6-H), 7.85 (s, 1H, 8-H), 10.25 (bs, 1H, NH). Anal. ($C_{12}H_{16}IN_3O_2S$) C, H, N, S.

7-Iodo-3-cyclobutylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (46). Method B. Yield 0.32 g (60%); mp 295−297 °C; IR (KBr) 3297 (N−H), 1633, 1556, 1476 (C=C, C=N, N−H), 1241, 1148 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.30−2.30 (m, 6H, CH(CH_2)$_3$), 4.10 (m, 1H, NHCH), 6.95 (d, 1H, 5-H), 7.50 (d, 1H, NHCH), 7.75 (d, 2H, 6-H + 8-H), 10.35 (bs, 1H, NH). Anal. ($C_{11}H_{12}IN_3O_2S$) C, H, N, S.

7-Chloro-4-methyl-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (47). K_2CO_3 (0.96 g, 6.9 mmol) and an excess of methyl iodide (5 mL) were added to a solution of 7-chloro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-diox-

ide (**9b**, 2.0 g, 7.6 mmol) in acetonitrile/DMF, 4:1 (25 mL). The suspension was stirred for 10 h at room temperature. The solvent was removed by distillation under reduced pressure, and the solid residue was taken up by water (40 mL). The resulting aqueous suspension was adjusted to pH 2 by means of formic acid, and the solid was collected by filtration and washed with water. The crude compound was recrystallized in methanol/water (1.87 g, 89%): mp 211−213 °C (lit., 219−220 °C).[33] Anal. ($C_9H_9ClN_2O_2S_2$) C, H, N, S.

7-Chloro-3-isopropylamino-4-methyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Hemihydrate (48). A mixture of 7-chloro-4-methyl-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**47**, 0.4 g, 1.45 mmol) in isopropylamine (4 mL) was refluxed for 90 min. The amine was removed by distillation under reduced pressure, and the residue was suspended in water (20 mL). After the mixture was stirred for 1 h at room temperature, the precipitate was collected by filtration, washed with water, and recrystallized in methanol/water (0.27 g, 63%): mp 176−183 °C; IR (KBr) 3321 (N−H), 1603, 1554, 1470 (C═N, C═C, N−H), 1283, 1171 (S═O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 80 MHz) δ 1.10 (d, 6H, CH(CH_3)$_2$), 3.20 (s, H_2O), 3.35 (s, 3H, N−CH_3), 3.95 (m, 1H, NHC*H*), 7.30−7.70 (m, 4H, N*H* + 5-*H* + 6-*H* + 8-*H*). Anal. ($C_{11}H_{14}ClN_3O_2S\cdot 0.5H_2O$) C, H, N, S.

7-Chloro-3-methylsulfinyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (49). The suspension of 7-chloro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**9b**, 5.0 g, 19 mmol) in an aqueous solution of Na_2CO_3 (2.2 g/25 mL) was supplemented with 2 N NaOH until complete dissolution. Bromine (1.0 mL) was added under stirring at room temperature, and after the mixture was vigorously stirred for 10 min, the resulting suspension was adjusted to pH 2−3 by means of 6 N HCl. The insoluble portion was collected by filtration, washed with water, and suspended under stirring in methanol (20 mL). The precipitate was collected by filtration, washed with methanol, and dried (4.5 g, 85%): mp 260−261 °C; ^{1}H NMR (DMSO-d_6, 80 MHz) δ 2.95 (s, 3H, SOCH_3), 7.80−8.00 (m, 3H, 5-H + 6-H + 8-H). Anal. ($C_8H_7ClN_2O_3S_2$) C, H, N, S.

7-Chloro-3-*tert*-butylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (26). A mixture of 7-chloro-3-methylsulfinyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**49**, 0.5 g, 1.79 mmol) and *tert*-butylamine (8 mL) was heated in a sealed vessel for 2 h at 160 °C. The excess of amine was eliminated by distillation under reduced pressure, and the residue was dissolved in a hydromethanolic (1:1) 2% w/v solution of NaOH (20 mL). The alkaline solution was treated with charcoal and was filtered, and the filtrate was adjusted to pH 4−5 with 6 N HCl. The precipitate was collected by filtration, washed with water, and dried. The compound was recrystallized in methanol/water (0.28 g, 55%): mp 264−266 °C; IR (KBr) 3328 (N−H), 1627, 1576, 1481 (C═N, C═C, N−H), 1280, 1141 (S═O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 80 MHz) δ 1.30 (s, 9H, C(CH_3)$_3$), 6.75 (bs, 1H, N*H* C(CH$_3$)$_3$), 7.05 (d, 1H, 5-*H*), 7.50 (d, 1H, 6-*H*), 7.60 (s, 1H, 8-*H*), 10.20 (bs, 1H, N*H*). Anal. ($C_{11}H_{14}ClN_3O_2S$) C, H, N, S.

7-Chloro-3-(1,1-dimethylpropylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (27). The compound was obtained as described for **26**, starting from 7-chloro-3-methylsulfinyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**49**, 0.5 g, 1.79 mmol) and using 1,1-dimethylpropylamine (8 mL) instead of *tert*-butylamine (0.22 g, 40%): mp 258−259 °C; IR (KBr) 3305 (N−H), 1629, 1566, 1479 (C═N, C═C, N−H), 1250, 1149 (S═O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 80 MHz) δ 0.75 (t, 3H, C(CH$_3$)$_2$-CH$_2$CH_3), 1.20 (s, 6H, C(CH_3)$_2$CH$_2$CH$_3$), 1.70 (q, 2H, C(CH$_3$)$_2$-CH_2CH$_3$), 6.65 (bs, 1H, N*H* C(CH$_3$)$_2$CH$_2$CH$_3$), 7.05 (d, 1H, 5-*H*), 7.50 (d, 1H, 6-*H*), 7.60 (s, 1H, 8-*H*), 10.20 (bs, 1H, N*H*). Anal. ($C_{12}H_{16}ClN_3O_2S$) C, H, N, S.

Ionization Constants. The pK_a values of compounds **4**, **13**, **36**, and **42** were determined spectroscopically by means of a Perkin-Elmer UV/vis 554 spectrophotometer at 20 °C. UV spectra were taken in different aqueous buffers of pH ranging from 3 to 12. The pK_a values were calculated by the Debye−Hückel equation at the maximum basic form absorbance.[16]

Biological Assays. Measurements of Insulin Release from Incubated Pancreatic Islets. Pancreatic islets were isolated by the collagenase method from fed Wistar rats (180−220 g). Groups of 10 islets, each derived from the same batch of islets, were preincubated for 30 min at 37 °C in 1 mL of a physiological salt medium (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24) supplemented with 2.8 mM glucose and 0.5% (w:v) dialyzed albumin (Sigma) and equilibrated against a mixture of O_2 (95%) and CO_2 (5%).

The islets were then incubated at 37 °C for 90 min in 1 mL of the same medium containing 16.7 mM glucose and, in addition, the reference compound or the benzothiadiazine derivative. The release of insulin was measured radioimmunologically using rat insulin as a standard.[34]

Residual insulin secretion was expressed as a percentage of the value recorded in control experiments (100%), i.e., in the absence of drug and presence of 16.7 mM glucose.

Measurements of Contractile Activity in Rat Aorta. All experiments were performed with aortae removed from fed Wistar rats (180−220 g). A section of the aorta was cleared of adhering fat and connective tissue and was cut into transverse rings (3−4 mm long). The endothelium was removed by rubbing the intimal surface with forceps. The segments were suspended under 1.5 g of tension by means of steel hooks in an organ bath containing 20 mL of a Krebs bicarbonate buffered solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5. The physiological solutions were maintained at 37 °C and bubbled continuously with a mixture of O_2 (95%) and CO_2 (5%). The isometric contractions of the aortic rings were measured with a force−displacement transducer. After 60 min of equilibration, the rings were exposed to 30 mM KCl. When the tension had stabilized, the drugs were added to the bath at increasing concentrations until maximal relaxation (or until 0.3 mM). The relaxation response was expressed as the percentage of the contractile response to KCl. The ED_{50} values (drug concentration evoking 50% inhibition of the plateau phase induced by KCl) were assessed from dose−response curves using Datanalyst software (EMKA Technologies, France).

Measurements of ^{86}Rb Outflow from Rat Pancreatic Islets. The method used for measuring ^{86}Rb (^{42}K substitute) outflow from prelabeled and perifused rat pancreatic islets was previously decribed in detail.[8,13,23] Experiments were conducted in the absence and presence of glibenclamide in the basal medium.

Electrophysiology. Mouse Kir6.2 (Genbank D50581), rat SUR1 (Genbank L40624), SUR2A (Genbank D83598), and SUR2B (Genbank D86038) cDNAs were cloned in the pBF vector. A truncated form of Kir6.2 (Kir6.2ΔC36), which lacks the C-terminal 36 amino acids and forms functional channels in the absence of SUR, was prepared as described previously.[35] Capped mRNA was prepared using the mMESSAGE large scale in vitro transcription kit (Ambion, Austin, TX), as previously described.[36] Female *Xenopus laevis* were anaesthetized with MS222 (2 g/L added to the water). One ovary was removed via a minilaparotomy, the incision was sutured, and the animal was allowed to recover. Once the wound had completely healed, the second ovary was removed in a similar operation and the animal was then killed by decapitation while under anaesthesia. Immature stages V−VI oocytes were incubated for 60 min with 1.0 mg/mL collagenase (Sigma, type V) and manually defolliculated. Oocytes were either injected with ∼1 ng of Kir6.2ΔC36 mRNA or co-injected with ∼0.1 ng Kir6.2 mRNA and ∼2 ng of mRNA encoding wild-type SUR. The final injection volume was 50 nL/oocyte. Isolated oocytes were maintained in Barth's solution and studied 1−5 days after injection. Patch pipets were pulled from thick-walled borosilicate glass and had resistances of 250−500 kΩ when filled with pipet solution. Macroscopic currents were recorded from giant excised inside-out patches at a holding potential of 0 mV and at 20−24 °C. Currents were evoked by repetitive 3 s voltage ramps from −110 to +100 mV and recorded using an EPC7 patch−clamp amplifier (List Electronik, Darmstadt, Germany). They were filtered at 0.2 kHz, digitized at 0.4 kHz

using a Digidata 1200 interface, and analyzed using pClamp software (Axon Instruments Inc., Foster City, CA). The pipet (external) solution contained the following (mM): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES (pH 7.4 with KOH). The intracellular (bath) solution contained the following (mM): 107 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES (pH 7.2 with KOH; final $[K^+] \approx 140$ mM). Compound **18** (BPDZ 176) was prepared as a 100 mM stock solution in DMSO. In control experiments, the maximal DMSO concentration applied (0.1%) had no effect on the K_{ATP} current. Solutions containing ATP were made up fresh each day.

Acknowledgment. This study was supported by grants from the National Fund for Scientific Research (F.N.R.S., Belgium) from which P. de Tullio is a Research Associate, B. Pirotte is a Senior Research Associate, and P. Lebrun is a Research Director. The technical assistance of S. Counerotte, D. Dewalque, P. Neven, M. L. Pirard, A.-M. Vanbellinghen, and A. Van Praet is gratefully acknowledged.

References

(1) Cook, D. L.; Hales, C. N. Intracellular ATP Directly Blocks K^+ Channels in Pancreatic B Cells. *Nature* **1984**, *311*, 271−273.
(2) Standen, N. B.; Quayle, J. M.; Davies, N. W.; Brayden, J. E.; Huang, Y.; Nelson, M. T. Hyperpolarizing Vasodilators Activate ATP-Sensitive K^+ Channels in Arterial Smooth Muscle. *Science* **1989**, *245*, 177−180.
(3) Ashcroft, F. M. Adenosine 5′-Triphosphate-Sensitive Potassium Channels. *Annu. Rev. Neurosci.* **1988**, *11*, 97−118.
(4) Petersen, O. H.; Dunne, M. J. Regulation of K^+ Channels Plays a Crucial Role in the Control of Insulin Secretion. *Pfluegers Arch.* **1989**, *414*, S115−S120.
(5) Kolb, H. A. Potassium Channels in Excitable and Non-Excitable Cells. *Rev. Physiol. Biochem. Pharmacol.* **1990**, *15*, 51−79.
(6) Babenko, A. P.; Aguilar-Bryan, L.; Bryan, J. A View of SUR/Kir 6.X K_{ATP} Channels. *Annu. Rev. Physiol.* **1998**, *60*, 667−687.
(7) Robertson, D. W.; Steinberg, M. I. Potassium Channel Modulators: Scientific Applications and Therapeutic Promise. *J. Med. Chem.* **1990**, *33*, 1529−1541.
(8) 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.
(9) Björk, E.; Berne, C.; Kämpe, O.; Wibell, P.; Oskarsson, P.; Karlsson, F. A. Diazoxide Treatment at Onset Preserves Residual Insulin Secretion in Adults with Autoimmune Diabetes. *Diabetes* **1996**, *45*, 1427−1430.
(10) Alemzadeh, R.; Langley, G.; Upchurch, L.; Smith, P.; Slonim, A. E. Beneficial Effect of Diazoxide in Obese Hyperinsulinemic Adults. *J. Clin. Endocrinol. Metab.* **1998**, *83*, 1911−1915.
(11) Pirotte, B.; de Tullio, P.; Lebrun, P.; Antoine, M.-H.; Fontaine, J.; Masereel, B.; Schynts, M.; Dupont, L.; Herchuelz, A.; Delarge, J. 3-(Alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides as Powerful Inhibitors of Insulin Release from Rat Pancreatic B-Cells: A New Class of Potassium Channel Openers? *J. Med. Chem.* **1993**, *36*, 3211−3213.
(12) de Tullio, P.; Pirotte, B.; Lebrun, P.; Fontaine, J.; Dupont, L.; Antoine, M.-H.; Ouedraogo, R.; Khelili, S.; Maggetto, C.; Masereel, B.; Diouf, O.; Podona, T.; Delarge, J. 3- and 4-Substituted 4*H*-Pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides as Potassium Channel Openers: Synthesis, Pharmacological Evaluation, and Structure−Activity Relationships. *J. Med. Chem.* **1996**, *39*, 937−948.
(13) Pirotte, B.; Antoine, M.-H.; de Tullio, P.; Hermann, M.; Herchuelz, A.; Delarge, J.; Lebrun, P. A Pyridothiadiazine (BPDZ 44) as a New and Potent Activator of ATP-Sensitive K^+ Channels. *Biochem. Pharmacol.* **1994**, *47*, 1381−1386.
(14) Antoine, M.-H.; Pirotte, B.; Hermann, M.; de Tullio, P.; Delarge, J.; Herchuelz, A.; Lebrun, P. Cationic and Secretory Effects of BPDZ 44 and Diazoxide in Rat Pancreatic Islets. *Experientia* **1994**, *50*, 830−832.
(15) Khelili, S.; de Tullio, P.; Lebrun, P.; Fillet, M.; Antoine, M.-H.; Ouedraogo, R.; Dupont, L.; Fontaine, J.; Felekidis, A.; Leclerc, G.; Delarge, J.; Pirotte, B. Preparation and Pharmacological Evaluation of the *R*- and *S*-Enantiomers of 3-(2′-Butylamino)-4*H*- and 3-(3′-Methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide, Two Tissue Selective ATP-Sensitive Potassium Channel Openers. *Bioorg. Med. Chem.* **1999**, *7*, 1513−1520.
(16) Pirotte, B.; Ouedraogo, R.; de Tullio, P.; Khelili, S.; Somers, F.; Boverie, S.; Dupont, L.; Fontaine, J.; Damas, J.; Lebrun, P. 3-Alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides Structurally Related to Diazoxide and Pinacidil as Potassium Channel Openers Acting on Vascular Smooth Muscle Cells: Design, Synthesis and Pharmacological Evaluation. *J. Med. Chem.* **2000**, *43*, 1456−1466.
(17) Dabrowski, M.; Ashcroft, F. M.; Ashfield, R.; Lebrun, P.; Pirotte, B.; Egebjerg, J.; Hansen, J. B.; Wahl, P. The novel diazoxide analog 3-isopropylamino-7-methoxy-4*H*-1,2,4-benzothiadiazine 1,1-dioxide is a selective Kir6.2/SUR1 channel opener. *Diabetes* **2002**, *51*, 1896−1906.
(18) Somers, F.; Ouedraogo, R.; Antoine, M.-H.; de Tullio, P.; Becker, B.; Fontaine, J.; Damas, J.; Dupont, L.; Rigo, B.; Delarge, J.; Lebrun, P.; Pirotte, B. Original 2-Alkylamino-6-halogenoquinazolin-4(3*H*)-ones and K_{ATP} Channel Activity. *J. Med. Chem.* **2001**, *44*, 16, 2575−2585.
(19) Girard, Y.; Atkinson, J. G.; Rokach, J. A New Synthesis of 1,2,4-Benzothiadiazines and a Selective Preparation of *o*-Aminobenzenesulphonamides. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1043−1047.
(20) Wollweber, H.; Horstmann, H.; Stoepel, K.; Garthoff, B.; Puls, W.; Krause, H. P.; Thomas, G. 3-Amino-2*H*-benzothiadiazine-1,1-dioxide mit Antihypertensiver und Potentieller Diabetogener Wirkung (3-Amino-2*H*-benzothiadiazine 1,1-dioxide with antihypertensive and potential diabetogenic effect). *Arzneim.-Forsch.* **1981**, *31*, 279−288.
(21) Manley, P. W.; Quast, U. Structure−Activity Studies of Potassium Channel Opening in Pinacidil-Type Cyanoguanidines, Nitroethenediamines, Thioureas, and Ureas. *J. Med. Chem.* **1992**, *35*, 2327−2340.
(22) Quast, U.; Bray, K. M.; Andres, H.; Manley, P. W.; Baumlin, Y.; Dosogne, J. Binding of the K^+ Channel Opener [^{3}H]P1075 in Rat Isolated Aorta: Relationship to Functional Effects of Openers and Blockers. *Mol. Pharmacol.* **1993**, *43*, 474−481.
(23) Lebrun, P.; Antoine, M.-H.; Ouedraogo, R.; Dunne, M.; Kane, C.; Hermann, M.; Herchuelz, A.; Masereel, B.; Delarge, J.; de Tullio, P.; Pirotte, B. Activation of ATP-Dependent K^+ Channels and Inhibition of Insulin Release: Effect of BPDZ 62. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 156−162.
(24) de Tullio, P.; Pirotte, B.; Dupont, L.; Masereel, B.; Laeckmann, D.; Podona, T.; Diouf, O.; Lebrun, P.; Delarge, J. Synthesis and Structural Studies of a New Class of Heterocyclic Compounds: 1,2,4-Pyridothiadiazine 1,1-Dioxides, Pyridyl Analogues of 1,2,4-Benzothiadiazine 1,1-Dioxides. *Tetrahedron* **1995**, *51*, 3221−3234.
(25) March, J. *Advanced Organic Chemistry*, 3rd ed.; Wiley: New York, 1985.
(26) Dupont, L.; de Tullio, P.; Tinant, B.; Pirotte, B. (*R*/*S*)-3-(3-Methyl-2-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide Monohydrate. *Acta Crystallogr.* **2001**, *E57*, o1050−o1051.
(27) Dupont, L.; Pirotte, B.; de Tullio, P. 7-Iodo-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide. *Acta Crystallogr.* **1999**, *C55*, 1152−1154.
(28) Di Bella, M.; Monzani, A.; Albasini, A.; Conti, G. Ricerche Nel Campo della 1,2,4-Benzotiadiazina (Searches in the Field of 1,2,4-Benzothiadiazines). *Farmaco* **1966**, *12*, 829−838.
(29) Di Bella, M.; Rinaldi, M.; Coppi, G.; Fabio, U.; Manicardi, G. Azione Antimicrobica di Derivati dell' 1,2,4-Benzotiadiazin-1,1-diossido (Antimicrobial action of derivatives of 1,2,4-benzothiadiazine 1,1-dioxide). *Farmaco* **1974**, *29*, 915−922.
(30) Di Bella, M.; Rinaldi, M.; Fabio, U.; Manicardi, G. Azione Antimicrobica di Derivati dell'1,2,4-Benzotiadiazin-1,1-diossido (Antimicrobial action of derivatives of 1,2,4-benzothiadiazine 1,1-dioxide). *Farmaco* **1972**, *27*, 990−998.
(31) Parenti, C.; Constantino, L.; Di Bella, M.; Raffa, L.; Baggio, G. G.; Rossi, T. Heteroaryl-alkanoic Acids with Possible Anti-inflammatory Activities IV. *Boll. Chim. Farm.* **1987**, *126*, 239−243.
(32) Raffa, L.; Di Bella, M.; Ferrari, P.; Rinaldi, M.; Ferrari, N. Azione Cardiovasculare di Derivati dell'1,2,4-Benzothiadiazin-1,1-diossido. Nota IV (Cardiovascular Action of Derivatives of 1,2,4-Benzothiadiazine 1,1-Dioxide. Part IV). *Farmaco, Ed. Sci.* **1974**, *29*, 411−423.
(33) Di Bella, M.; Rinaldi, M.; Fabio, U.; Manicardi, G. Azione Antimicrobica di Derivati dell'1,2,4-Benzotiadiazin-1,1-diossido (Antimicrobial Action of Derivatives of 1,2,4-Benzothiadiazine 1,1-Dioxide). *Farmaco* **1973**, *28*, 411−417.
(34) Leclercq-Meyer, V.; Marchand, J.; Woussen-Colle, M. C.; Giroix, M.-H.; Malaisse, W. J. Multiple Effects of Leucine on Glucagon, Insulin, and Somatostatin Secretion from the Perfused Rat Pancreas. *Endocrinology* **1985**, *116*, 1168−1174.
(35) Tucker, S. J.; Gribble, F. M.; Zhao, C.; Trapp, S.; Ashcroft, F. M. Truncation of Kir6.2 Produces ATP-sensitive K-channels in the Absence of the Sulphonylurea Receptor. *Nature* **1997**, *387*, 179−181.
(36) Gribble, F. M.; Ashfield, R.; Ämmälä, C.; Ashcroft, F. M. Properties of Cloned ATP-Sensitive K-Currents Expressed in Xenopus Oocytes. *J. Physiol.* **1997**, *498*, 87−98.

JM021117W