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Chloro-Substituted 3-Alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides as ATP-Sensitive Potassium Channel Activators: Impact of the Position of the Chlorine Atom on the Aromatic Ring on Activity and Tissue Selectivity

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The synthesis of 5-chloro-, 6-chloro-, and 8-chloro-substituted 3-alkylamino/cycloalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides is described. Their inhibitory effect on the insulin releasing process and their vasorelaxant activity was compared to that of previously reported 7-chloro-3-alkylamino/cycloalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides. "5-Chloro" compounds were found to be essentially inactive on both the insulin-secreting and the smooth muscle cells. By contrast, "8-chloro" and "6-chloro" compounds were found to be active on insulin-secreting cells, with the "6-chloro" derivatives emerging as the most potent drugs. Moreover, the "6-chloro" analogues exhibited less myorelaxant activity than their "7-chloro" counterparts. 8-Chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**25b**) and 6-chloro-3-cyclobutylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**19e**) were further identified as K_{ATP} channel openers by radioisotopic measurements conducted on insulin-secreting cells. Likewise, current recordings on HEK293 cells expressing human SUR1/Kir6.2 channels confirmed the highly potent activity of **19e** (EC_{50} = 80 nM) on such types of K_{ATP} channels. The present work indicates that 6-chloro-3-alkylamino/cycloalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides appear to be more attractive than their previously described 7-chloro-substituted analogues as original drugs activating the SUR1/Kir6.2 K_{ATP} channels.

Introduction

ATP-sensitive potassium channels (K_{ATP} channels[a]) are found in a wide variety of excitable cells.[1] Opening and closure of this channel are linked to changes in intracellular levels of adenine nucleotides, more specifically ATP and ADP. Thus, K_{ATP} channels couple membrane potential to the metabolic state of the cell.[1]

K_{ATP} channels are octameric complexes combining four sulfonylurea receptor (SURx) subunits to four inwardly rectifying potassium channel (Kir6.x) subunits. The channel exists in different isoforms resulting from the assembly of the Kir6.x (Kir6.1 and Kir6.2) and the SURx (SUR1, SUR2A, and SUR2B) subunits in multiple combinations.[2] For example, SUR1 combines with Kir6.2 to form a SUR1/Kir6.2 K_{ATP} channel subtype as found in the pancreas and the brain, whereas the cardiac and the skeletal muscle rather express a SUR2A/Kir6.2 channel subtype and smooth muscles a SUR2B/Kir6.1 or a SUR2B/Kir6.2 channel subtype.[3] Considerable attention was also recently focused on a putative mitochondrial K_{ATP} channel (mitoK_{ATP} channel) suspected to be involved in myocardial preconditioning and cytoprotection in different tissues, but for which the identity of the pore-forming units remains unclear.[4−6]

Given their many physiological functions, K_{ATP} channels represent promising drug targets. Therefore, one main challenge in the development of new K_{ATP} channel modulators as therapeutic agents is the discovery of compounds with the highest selectivity for a single K_{ATP} channel subtype.

A variety of compounds named "potassium channel openers" (PCOs) have been reported to activate K_{ATP} channels. Recent works have revealed that the binding sites for such drugs are located on the SUR subunit.[7−9] The most well-known prototypic examples of PCOs are **1** [(−)-cromakalim], **2** [(±)-pinacidil], and **3** (diazoxide) (Figure 1). **1**, a benzopyran-type PCO, is reported to preferentially activate the SUR2A- and the SUR2B-based K_{ATP} channels but is rather ineffective as an activator of the SUR1-based K_{ATP} channels.[9] The benzothiadiazine-type PCO **3** is known to open SUR1- and SUR2B-containing K_{ATP} channels and to activate the cardiac mitoK_{ATP} channels but is only weakly active on

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[a] Abbreviations: K_{ATP} channel, ATP-sensitive potassium channel; ATP, adenosine triphosphate; ADP, adenosine diphosphate; HEK293 cells, human embryonic kKidney 293 cells; SUR, sulfonylurea receptor; Kir, inwardly rectifying potassium channel; PCO, potassium channel opener; IC_{50}, half maximal inhibitory concentration; EC_{50}, half maximal effective concentration; FTIR, Fourier transform infrared; NMR, nuclear magnetic resonance; HMDS, hexamethyldisiloxane; TMS, tetramethylsilane; TLC, thin layer chromatography; SEM, standard error of the mean; EGTA, ethyleneglycol tetraacetic acid; FOR, fractional outflow rate; mitoK_{ATP} channel, mitochondrial ATP-sensitive potassium channel.

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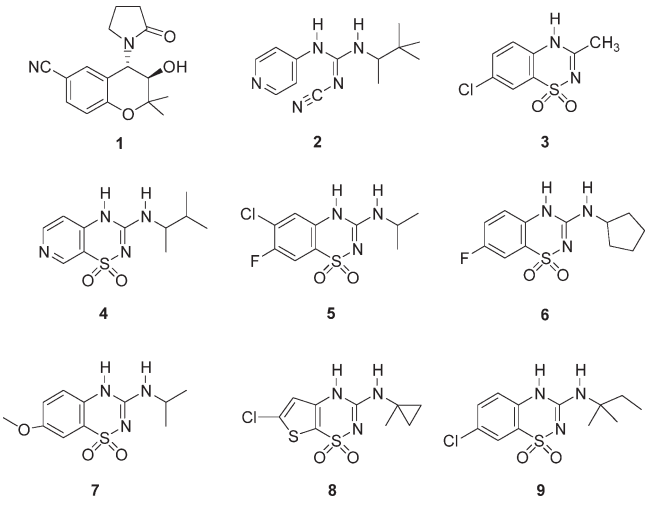
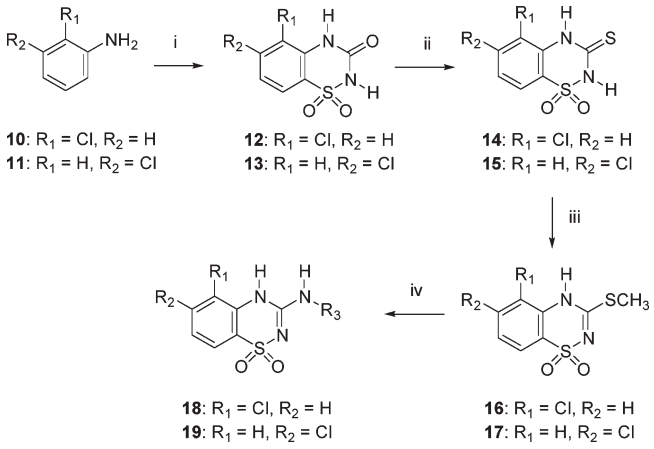


Figure 1. Chemical structure of reference compounds and recently reported pyrido-, benzo-, and thienothiadiazine 1,1-dioxides potassium channel openers: **1**, (−)-cromakalim; **2**, (±)-pinacidil; **3**, diazoxide; **4**, 3-(1,2-dimethylpropyl)amino-4*H*-pyrido[4,3-*e*]-1.2.4-thiadiazine 1.1-dioxide (BPDZ 44[11]); **5**, 6-chloro-7-fluoro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (BPDZ 259[13]); **6**, 3-cyclopentylamino-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (BPDZ 176[12]); **7**, 3-isopropylamino-7-methoxy-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (BPDZ 216[16]); **8**, 6-chloro-3-(1-methylcyclopropyl)amino-4*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (NN414[14]); **9**, 7-chloro-3-(1,1-dimethylpropyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide.

SUR2A/Kir6.2 channels.[3,9] Therefore, at least in cardiomyocytes, **3** is usually recognized as a "selective" opener of the mitoK_{ATP} channel versus the sarcolemmal (SUR2A/Kir6.2) K_{ATP} channel.[10]

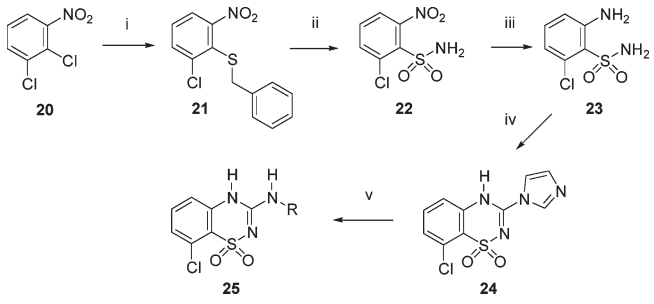
In the search of new potent and selective K_{ATP} channel activators, we recently developed several series of original analogues of **3** with the aim at identifying SUR1-specific PCOs.[11−14] Such compounds expressing selectivity for the pancreatic tissue have been proposed to be of clinical value in the prevention and/or treatment of several metabolic disorders[15] (i.e., hyperinsulinism, diabetes and obesity). Compounds with a high selectivity for the pancreatic versus the vascular tissue such as the pyridothiadiazine dioxide **4**, the benzothiadiazine dioxides **5**, **6**, and **7**, and the thienothiadiazine dioxide **8** emerged from these previous studies (Figure 1). The 7-methoxy-substituted benzothiadiazine dioxide **7** as well as compounds **6** and **8** were further found to be selective for the SUR1/Kir6.2 versus SUR2A/Kir6.2 or SUR2B/Kir6.2 K_{ATP} channels expressed in *Xenopus laevis* oocytes.[12,16,17] By contrast, the 7-chloro-substituted benzothiadiazine dioxide **9** was shown to exert, in vitro, a potent vasorelaxant effect and a negligible activity on pancreatic β-cells,[12] supporting the view that this compound expressed a SUR2B selectivity. The latter result indicates that discrete chemical modifications of the structure of 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides can markedly influence the tissue selectivity of these drugs.[12] As a result, and to complete our knowledge of the structure−activity relationships, we decided to synthesize and evaluate novel examples of 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides bearing a chlorine atom at all possible positions of the benzene ring of benzothiadiazine dioxides (5-, 6-, 7-, and 8-chloro-substituted 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides). The chlorine atom was chosen as a substituent because 7-chloro-substituted compounds have previously been extensively studied as reference PCOs

Scheme 1[a]



[a] Reagents: (i) (1) $ClSO_2NCO$, CH_3NO_2; (2) $AlCl_3$; (ii) P_2S_5, pyridine; (iii) CH_3I, $NaHCO_3$, CH_3OH/H_2O; (iv) R_3NH_2.

Scheme 2[a]



[a] Reagents: (i) (1) $C_6H_5CH_2SH$; (ii) Cl_2, HOAc, H_2O; (2) NH_4OH; (iii) Fe, NH_4Cl; (iv) 1,1-thiocarbonydiimidazole; (v) RNH_2.

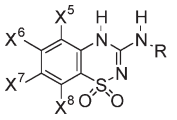
(i.e., **3** and 7-chloro-3-isopropylamino-4*H*-1.2.4-benzothiadiazine 1.1-dioxide **26c** (BPDZ 73[12]). The alkylamino/cycloalkylamino chains introduced at the 3-position were short chains known to be responsible for high potency and selectivity for the pancreatic (SUR1/Kir6.2) K_{ATP} channel.[12−14]

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 rings (myorelaxant effect on 30 mM KCl-precontracted aorta rings). In addition, radioisotopic experiments were performed on rat pancreatic islets with compounds **19e** and **25b** in order to elucidate the mechanism of action of the drugs. Finally, compound **19e** was further evaluated on HEK293 cells stably expressing human SUR1/Kir6.2 channels.

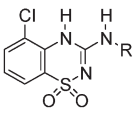
Chemistry

Access to 5-chloro-substituted (**18a−d**) and 6-chloro-substituted (**19a−e**) 3-alkylamino/cycloalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides is described on Scheme 1. The appropriate aniline **10** or **11** was transformed after reaction with chlorosulfonyl isocyanate into the corresponding 5-chloro-substituted or 6-chloro-substituted 3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**12** or **13**). In the case of the 6-chloro-substituted derivative, the latter reaction also generated the formation of 8-chloro-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide as a minor contaminant (30% 8-chloro compound versus 70% 6-chloro compound), which was eliminated from **13** after crystallization in hot water. Subsequent thionation of the oxo derivatives **12** and

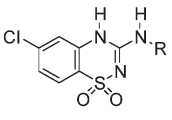
Table 1. Effects of Chloro-Substituted 3-Alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets and Contractile Activity of K^+-Depolarized Rat Aorta Rings



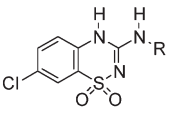
18a-d



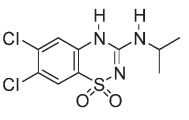
19a-e



26a-e



25a-c



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compd	X^5	X^6	X^7	X^8	R	RIS[a](50 μM)	RIS[a](10 μM)	RIS[a](1 μM)	RIS[a](0.1 μM)	EC_{50} (μM)[b]
18a	Cl	H	H	H	ethyl	80.40 ± 4.36 (15)				90.4 ± 9.0 (4)
18b	Cl	H	H	H	propyl	94.69 ± 4.70 (16)				179.5 ± 14.2 (4)
18c	Cl	H	H	H	isopropyl	96.88 ± 5.32 (16)				> 300 (3)
18d	Cl	H	H	H	cyclobutyl	102.55 ± 3.74 (22)				125.5 ± 14.7 (5)
19a	H	Cl	H	H	ethyl		9.55 ± 0.55 (30)	21.70 ± 1.97 (15)		54.8 ± 5.1 (4)
19b	H	Cl	H	H	propyl	8.74 ± 0.62 (14)	14.45 ± 0.65 (15)	71.50 ± 3.84 (30)		73.2 ± 7.2 (4)
19c	H	Cl	H	H	isopropyl	6.38 ± 0.70 (16)	7.51 ± 0.82 (15)	13.20 ± 1.20 (16)	76.72 ± 4.34 (15)	53.4 ± 3.6 (4)
19d	H	Cl	H	H	cyclopropyl	7.97 ± 0.42 (11)	12.74 ± 1.95 (14)	93.02 ± 4.36 (22)		155.8 ± 5.7 (4)
19e	H	Cl	H	H	cyclobutyl	9.58 ± 0.42 (22)	11.27 ± 0.65 (23)	66.17 ± 4.31 (15)		> 300 (7)
26a[c]	H	H	Cl	H	ethyl	2.09 ± 0.35 (11)	10.65 ± 1.10 (12)	40.79 ± 2.38 (35)		39.9 ± 6.3 (4)
26b[c]	H	H	Cl	H	propyl	3.56 ± 0.76 (14)	14.24 ± 1.05 (11)	83.41 ± 3.64 (15)		55.5 ± 3.8 (5)
26c[c]	H	H	Cl	H	isopropyl	5.66 ± 0.53 (35)	4.84 ± 0.35 (32)	36.19 ± 2.38 (31)	90.41 ± 3.48 (23)	36.3 ± 2.2 (6)
26d[c]	H	H	Cl	H	cyclopropyl	5.82 ± 0.87 (13)	26.56 ± 3.03 (18)	84.00 ± 4.55 (16)		190.5 ± 19.1 (5)
26e[c]	H	H	Cl	H	cyclobutyl	8.11 ± 1.72 (11)	6.44 ± 0.34 (14)	60.19 ± 5.50 (11)		34.9 ± 4.4 (5)
25a	H	H	H	Cl	ethyl		37.55 ± 1.44 (21)	95.72 ± 4.65 (24)		> 300 (8)
25b	H	H	H	Cl	isopropyl		33.19 ± 1.52 (23)	95.18 ± 4.12 (23)		> 300 (6)
25c	H	H	H	Cl	cyclobutyl		30.23 ± 1.63 (22)	77.56 ± 3.93 (24)		> 30 (6)
27[c]	H	Cl	Cl	H	isopropyl	4.96 ± 0.41 (12)	6.29 ± 0.67 (12)	13.19 ± 0.98 (26)	84.90 ± 4.50 (21)	2.3 ± 0.2 (8)

[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 aorta rings (mean ± SEM (*n*)). [c] Published results (ref 12 and 13).

13 with phosphorus pentasulfide in pyridine led to the corresponding 3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **14** and **15**. In the next step, the synthesis of 3-(methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **16** and **17** was achieved by reaction of the thioxo derivatives **14** and **15** with methyl iodide in the presence of sodium hydrogenocarbonate. Finally, the different 5-chloro-substituted (**18a**−**d**) and 6-chloro-substituted (**19a**−**e**) 3-alkylamino/cycloalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides were obtained, according to the literature,[18] by heating the 3-methylsulfanyl intermediates **16** and **17** in a sealed vessel with an excess of the appropriate alkylamine or cycloalkylamine.

8-Chloro-3-alkylamino/cycloalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **25a**−**c** were obtained in five steps starting from 1,2-dichloro-3-nitrobenzene **20** (Scheme 2). Benzylmercaptan, obtained from the reaction of benzyl chloride with thiourea followed by treatment with sodium hydroxide, reacted with **20** to give 2-(benzylsulfanyl)-1-chloro-3-nitrobenzene (**21**) as a result of the nucleophilic substitution of the most reactive chlorine atom of **20** located in the ortho position of the nitro group. Subsequent treatment of **21** in acetic acid with gaseous chlorine generated 6-chloro-2-nitrobenzenesulfonyl chloride, which reacted with aqueous ammonia to provide the corresponding benzenesulfonamide **22**. Reduction of the nitro group of **22** into an amino group by means of iron in the presence of ammonium chloride provided 2-amino-6-chlorobenzenesulfonamide **23**, which gave access to 8-chloro-3-(1*H*-imidazol-1-yl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **24** after a ring closure reaction in the presence of *N*,*N*′-thiocarbonyldiimidazole. The 3-imidazolyl-substituted compound **24** reacted with the appropriate alkylamine or cycloalkylamine to provide the expected 3-alkylamino/cycloalkylamino-8-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **25a**−**c**.

Results and Discussion

The ability of the newly synthesized compounds to inhibit the glucose-induced insulin secretion from isolated rat pancreatic islets is reported in Table 1. The in vitro data are expressed as the percentage of residual insulin release recorded at different drug concentrations and are compared to the results obtained with the previously described 7-chloro-substituted 3-alkylamino/cycloalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **26a**−**e**.[12,13]

The position of the chlorine atom on the benzene ring strongly affected the activity on insulin-secreting cells. Compounds **18a**−**d**, bearing the chlorine atom at the 5-position of the aromatic ring, were found to be almost inactive at a 50 μM concentration, whatever the nature of the alkylamino side chain at the 3-position. The 8-chloro-substituted compounds **25a**−**c**, however, were active at 10 μM (60−70% inhibition of insulin release) but barely effective at a 1 μM concentration (except the 3-cyclobutylamino-substituted compound **25c**, which exhibited a weak inhibitory activity around 20%). Thus, looking at the effects of 50, 10, and 1 μM, the previously described 7-chloro-substituted compounds **26a**, **26c**, and **26e** were systematically more potent on rat pancreatic islets than their corresponding 5-chloro- and 8-chloro-substituted analogues on rat pancreatic islets. Interestingly, 6-chloro-substituted 3-alkylamino/cycloalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides, and

in particular **19a**, **19c**, and **19e**, were at least equipotent to their corresponding 7-chloro-substituted analogues. Among the "6-chloro" derivatives, 6-chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**19c**) was the most active at 1 μM. When tested at 0.1 μM, the latter drug was even more potent than the corresponding 7-chloro-3-isopropylamino-substituted compound **26c** and as potent as the 6,7-dichloro-3-isopropylamino-substituted compound **27**.[13] Taken as a whole, the rank order of potency of 3-isopropylamino-substituted compounds on pancreatic β-cells was found to be 6-chloro = 6,7-dichloro > 7-chloro > 8-chloro > 5-chloro.

The myorelaxant activity of the newly synthesized compounds was evaluated on K^+-depolarized rat aorta rings (Table 1). The vasorelaxant effect of the drugs was again affected by the position of the chlorine atom on the benzene ring. It should be noted that the "8-chloro" compounds **25a**−**c** were inactive as myorelaxants, indicating that such compounds exhibit a clear-cut selectivity for the pancreatic versus the smooth muscle tissue. Moreover, most of the 5-chloro-substituted compounds (**18a**−**d**) failed to express an obvious vasorelaxant effect. The "6-chloro" analogues **19a**−**e** exhibited a weak myorelaxant activity, which was less pronounced than that evoked by the previously reported "7-chloro" compounds **26a**−**e**. It should be noticed that 6-chloro-3-cyclobutylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**19e**) was totally inactive up to a 300 μM concentration. As a result, this compound, being a strong inhibitor of insulin release, appears to be one of the most potent and selective drugs affecting the pancreatic endocrine tissue. Lastly, the 3-isopropylamino-substituted compound **19c**, which is the most active "6-chloro" derivative on pancreatic islets (extrapolated IC_{50} value of 0.23 μM) and a poor vascular smooth muscle relaxant (EC_{50} value of 53 μM), showed a selectivity ratio (EC_{50} vasc/IC_{50} pancr) around 230 while the corresponding "7-chloro" compound **26c** with an extrapolated IC_{50} value of 0.48 μM and an EC_{50} value of 36 μM clearly expressed a lower selectivity ratio of 75. The latter results indicate that selected 6-chloro compounds are more attractive than their corresponding 7-chloro analogues as selective SUR1/Kir6.2 K_{ATP} channel openers. However, the weak in vitro myorelaxant activity of 6-chloro compounds cannot imply a lack of cardiovascular effects in vivo. Several compounds of this class have previously been reported to express, through an unknown mechanism, a hypotensive effect in rats.[18]

Using the UV spectrophotometry method, we have determined and compared the pK_a values of 3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides bearing the chlorine atom at the 5-, 6-, 7-, and 8-position (compounds **18c**, **19c**, **26c**, and **25b**). The weak acidic character of 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides is known to be linked to the lability of the hydrogen atom at the 4-position of the thiadiazine ring.[12,19] Our data indicate that the position of the chlorine atom on the benzene ring affected the pK_a value (**18c**, 9.11; **19c**, 9.35; **26c**, 9.51; **25b**, 9.54). Thus, the rank order of acidic character was found to be 5-chloro > 6-chloro > 7-chloro > 8-chloro, as expected by the inductive effect of the halogen atom. There was, however, no correlation between the acidic character of the compounds and their pharmacological profile.

In accordance with their high tissue selectivity and activity on pancreatic β-cells, compound **19e**, as a representative of the "6-chloro" derivatives, and compound **25b**, as a representative of the "8-chloro" derivatives, were selected for further pharmacological evaluation.

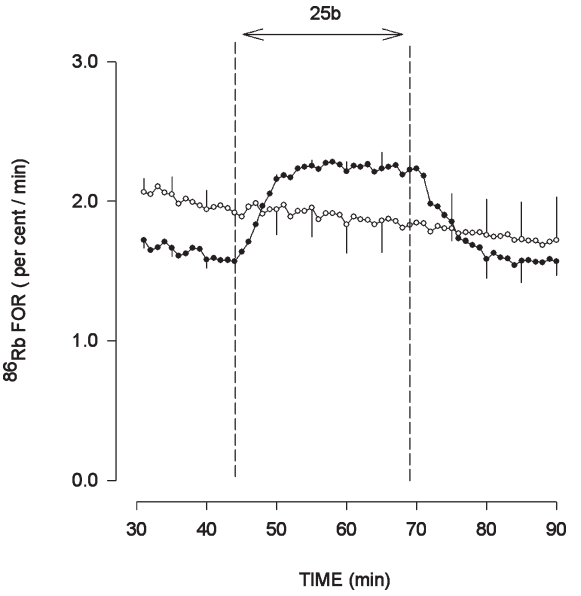

Figure 2. Effect of 10 μM **25b** on ^{86}Rb outflow from rat pancreatic islets perifused throughout in the absence (●) or presence (○) of glibenclamide (10 μM). Basal media contained extracellular Ca^{2+} and 5.6 mM glucose. Mean values (±SEM) refer to four individual experiments.

In the next series of experiments, we characterized the effects of the selected chloro-substituted 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides on ^{86}Rb, ^{45}Ca outflow and insulin release from prelabeled and perifused rat pancreatic islets.

The addition of 10 μM **25b** to islets perifused in the presence of extracellular calcium and 5.6 mM glucose provoked a rapid, sustained, and reversible increase in ^{86}Rb (^{42}K substitute) outflow (Figure 2).

When 10 μM glibenclamide, a K_{ATP} channel blocker,[13,20] was added to the basal medium, the stimulatory effect of **25b** was completely abolished (Figure 2).

Experiments conducted with 10 μM **19e**, instead of compound **25b**, gave essentially the same results, although **19e** exhibited a more pronounced stimulatory effect (Figure 3). Thus, the integrated outflow of ^{86}Rb observed during exposure to the drugs averaged 0.57 ± 0.09%/min after addition of **25b** (10 μM) and 1.45 ± 0.02%/min after addition of **19e** (10 μM).

The finding that chloro-substituted 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides induce a glibenclamide-sensitive increment in ^{86}Rb outflow indirectly suggests that such compounds activate ATP-sensitive K^+ channels.[13,20,21]

Activation of K_{ATP} channels should hyperpolarize the insulin-secreting cells and reduce Ca^{2+} inflow through voltage-sensitive Ca^{2+} channels. This hypothesis is attested by the effect of 10 μM **25b** on ^{45}Ca outflow from rat pancreatic islets exposed to 16.7 mM glucose (Figure 4 upper panel). Because the inhibitory effect of compound **25b** was dependent on the presence of extracellular Ca^{2+}, the decrease in ^{45}Ca outflow mediated by **25b** can be viewed as the result of a reduction in Ca^{2+} entry.[13,20,21]

Moreover, in the presence of 16.7 mM glucose and extracellular calcium in the basal medium, 10 μM **25b** elicited modifications in insulin output displaying a time course similar to that of the ^{45}Ca outflow response (Figure 4, lower panel).

Such radioisotopic and secretory data are reminiscent of those recorded with other PCOs.[20,21,25,26]

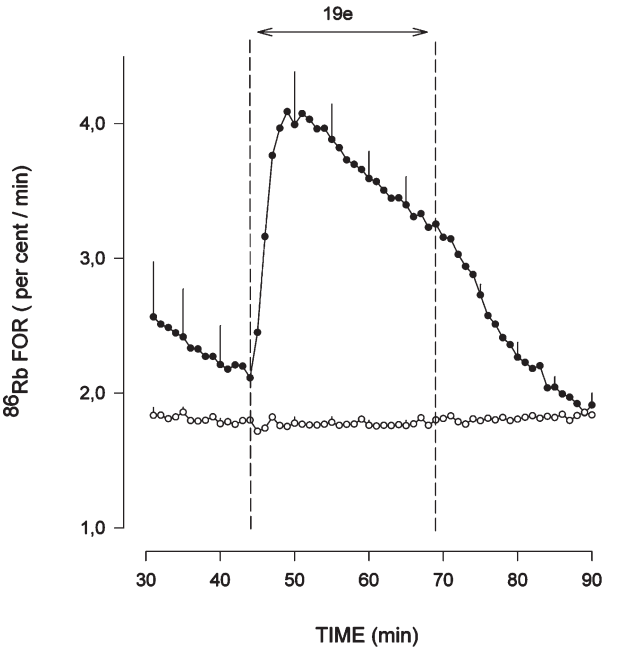

Figure 3. Effect of 10 μM **19e** on ^{86}Rb outflow from rat pancreatic islets perifused throughout in the absence (●) or presence (○) of glibenclamide (10 μM). Basal media contained extracellular Ca^{2+} and 5.6 mM glucose. Mean values (±SEM) refer to 3−4 individual experiments.

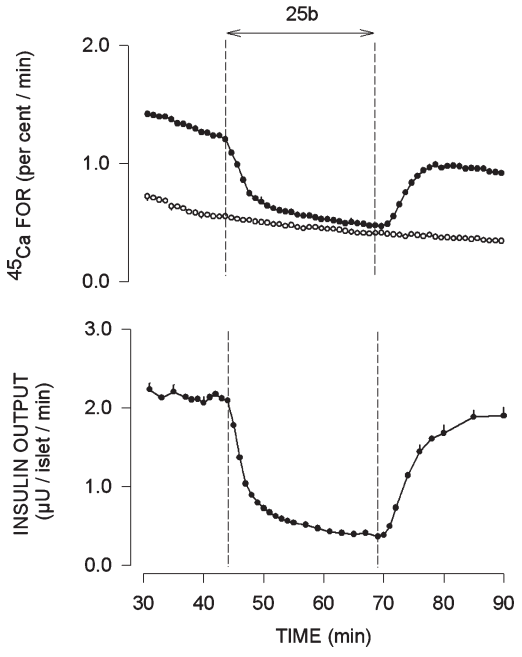

Figure 4. Effect of 10 μM **25b** on ^{45}Ca outflow (upper panel) and insulin release (lower panel) from rat pancreatic islets perifused throughout in the presence of 16.7 mM glucose. Basal media contained extracellular Ca^{2+} (●) or were deprived of Ca^{2+} and enriched with EGTA (○). Mean values (±SEM) refer to 4−6 individual experiments.

Together, these findings indicate that the 8-chloro-substituted benzothiadiazine 1,1-dioxide-induced inhibition of insulin release was correlated with the activation of K_{ATP} channels, leading to a reduction in Ca^{2+} inflow.

Last, we characterized the effect of **19e** on human SUR1/Kir6.2 channels expressed in HEK 293 cells by using the patch clamp technique in the whole cell configuration (Figure 5).

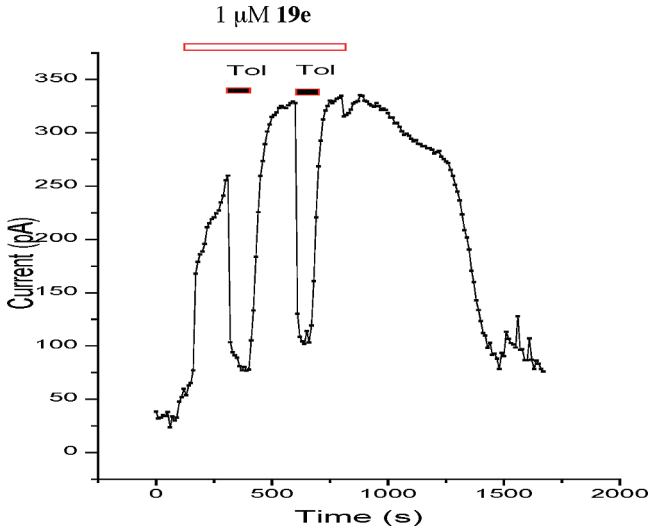

Figure 5. Whole-cell currents through SUR1/Kir6.2 channels expressed in HEK293 cells. Each data point represents the current amplitude in response to a 10 mV depolarizing voltage sted from a holding potential of −80 mV. Application of **19e** (1 μM) and tolbutamide (250 μM) is indicated by solid horizontal lines.

Compound **19e** (1 μM) induced a marked and reversible increase in the ionic current flowing through the recombinant SUR1/Kir6.2 channels.

The EC_{50} value for compound **19e** averaged 0.08 ± 0.01 μM (n = 5) while, under the same experimental conditions, the EC_{50} value for **3** amounted to 23.5 ± 2.7 μM (n = 3).

The subsequent and repetitive addition of 250 μM tolbutamide, a K_{ATP} channel blocker,[13,20] provoked a reversible inhibition of the ion current activated by 1 μM **19e** (Figure 5).

Such patch clamp data confirm the K_{ATP} channel opening properties of chloro-substituted benzothiadiazine 1,1-dioxides and further highlight the efficacy of compound **19e**.

Conclusion

This work highlights the feature that, to achieve a high potency and selectivity toward pancreatic-type K_{ATP} channels, the best position for a chlorine atom on the aromatic ring of 3-alkylamino/cycloalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides is the 6- rather than the 7-position of the heterocycle. 6-Chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**19c**) and 6-chloro-3-cyclobutylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**19e**) emerged as potent and highly selective pancreatic β-cell K_{ATP} channel openers. Patch clamp experiments further revealed that **19e** was 300-fold more potent than the reference PCO **3** at activating the whole cell ionic current through human recombinant SUR1/Kir6.2 channels.

Experimental Section

Chemistry. Melting points were determined on a Büchi 530 capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1000 FTIR spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker AW-80 (80 MHz) or a Bruker Avance (500 MHz) instrument using DMSO-d_6 as solvent with HMDS (80 MHz) or TMS (500 MHz) as an internal standard; chemical shifts are reported in δ values (ppm) relative to internal TMS. The abbreviation s = singulet, d = doublet, t = triplet, q = quadruplet, 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 60 F_{254}.

Synthesis of compounds **12**, **14**, **16**, **18a**−**d**, **19a**−**d**, **25a**,**c** is described in the Supporting Information. Spectroscopic details of compounds **21**−**24** are also in the Supporting Information.

6-Chloro-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (13). The mixture of chlorosulfonyl isocyanate (16.8 mL, 193 mmol) and nitromethane (160 mL) in a dried vessel was placed on an ice bath after which the solution of 3-chloroaniline (**11**) (20.8 g, 163 mmol) in nitromethane (50 mL) was added under stirring. At the end of addition, the resulting suspension was supplemented with anhydrous aluminum chloride (28 g, 210 mmol) and the mixture was refluxed for 30 min. The warm solution was poured onto ice (800 g) under vigorous stirring and, after complete melting of ice, the resulting precipitate was collected by filtration and washed with water. The crude product was suspended in an aqueous solution of sodium hydrogenocarbonate (5 g/100 mL) and heated until dissolution of most of the insoluble material. After treatment with charcoal, the filtrate was adjusted to pH 1 by addition of 12N HCl. The white precipitate collected at pH 1 (mixture of 6- and 8-chloro-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide) was crystallized three times in hot water (600 mL) to give **13** as a pure compound (20.9 g, 55%); mp 298−301 °C (lit.[22] 301 °C).

6-Chloro-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (15). The mixture of 6-chloro-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13**) (20 g, 86 mmol) and phosphorus pentasulfide (40 g, 180 mmol) in anhydrous pyridine (250 mL) was heated under reflux for 3 h. The resulting suspension was concentrated under reduced pressure, and the residue was dissolved in 2N NaOH (600 mL). This solution was treated with charcoal, filtered, and the filtrate was adjusted to pH 1 with 12N HCl. The crystalline solid was collected by filtration, washed with water, and dried. The compound was used in the next step without further purification (16.5 g, 77%); mp 215−217 °C (lit.[23] 215−217 °C).

6-Chloro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Monohydrate (17). 6-Chloro-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**15**) (12 g, 48 mmol) was dissolved in an aqueous solution of $NaHCO_3$ (7 g/280 mL). Methanol (280 mL) and methyl iodide (12 mL, 192 mmol) were successively added under stirring. After 30 min at room temperature, the mixture was adjusted to pH 3 with 1 N HCl and the organic solvent was removed under reduced pressure. After cooling, the title product was collected by filtration, washed with water, and dried (11.4 g, 90%); mp 275−278 °C (lit.[23] 288−289 °C). Anal. ($C_8H_7ClN_2O_2S_2 \cdot H_2O$) C, H, N, S.

6-Chloro-3-cyclobutylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (19e). The mixture of 6-chloro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide monohydrate **17** (0.5 g, 1.9 mmol) and a solution of cyclobutylamine (1 mL) in dioxane (5 mL) was heated in a sealed vessel for 4−5 h at 140 °C. The excess of amine was eliminated by distillation under reduced pressure, and the residue was suspended in water (20 mL). An aqueous solution of NaOH (5% w/v) was added dropwise until dissolution of the residue. The alkaline solution was treated with charcoal and filtered. The filtrate was adjusted to pH 4−5 with 6N HCl. The precipitate was collected by filtration, washed with water, and dried. The compound was recrystallized in methanol−water. Yield: 0.37 g (69%); mp 306−307 °C. IR (KBr) 3285 (N−H), 1631, 1583, 1549, 1470 (N−H, C=N, C=C), 1245, 1167 (S=O) cm^{-1}. ^1H NMR (DMSO-d_6, 80 MHz) δ: 1.30−2.40 (m, 6H, $CH(CH_2)_3$), 4.15 (m, 1H, NHC*H*), 7.20 (m, 3H, N*H*CH + 5-*H* + 7-*H*), 7.55 (d, 1H, 8-*H*), 10.00−10.50 (b, 1H, N*H*). Anal. ($C_{11}H_{12}ClN_3O_2S$) C, H, N, S.

2-(Benzylsulfanyl)-1-chloro-3-nitrobenzene (21). The solution of benzyl chloride (5.7 mL, 49.8 mmol) and thiourea (3.8 g, 49.9 mmol) in a mixture of ethanol and water (1:1) supplemented with a few drops of ammonia was refluxed for 3 h. A solution of 1,2-chloro-3-nitrobenzene (9.6 g, 50 mmol) in ethanol (20 mL) was added, under stirring, to this solution and then, dropwise, an aqueous solution of KOH (7 g/50 mL). The mixture was refluxed for 2 h. After cooling on an ice bath, the resulting precipitate was collected by filtration, washed with water, and dried. The crude compound was used in the next step without further purification; yield: 11.6 g (83%); mp 56−58 °C.

6-Chloro-2-nitrobenzenesulfonamide (22). Gaseous chlorine, obtained from the reaction of concentrated hydrochloric acid on potassium permanganate, was introduced under stirring into a solution of 2-(benzylsulfanyl)-1-chloro-3-nitrobenzene (3 g, 10.7 mmol) (**21**) in acetic acid (75 mL) and water (1.5 mL) during 30 min. The mixture was poured onto ice and extracted twice with diethyl ether (150 mL). The organic layer was washed with water (100 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting oily residue was dissolved in dioxane (30 mL) and added dropwise to a 30% aqueous solution of ammonia (60 mL). After 30 min stirring, the mixture was concentrated under reduced pressure to a volume of 40 mL, giving rise to the formation of a precipitate. The aqueous suspension was alkalinized with a 10% m/v aqueous solution of NaOH until complete dissolution of the insoluble material. The alkaline solution was treated with charcoal, filtered, and adjusted to pH 3 with 6N HCl. The resulting precipitate was collected by filtration, washed with water, and dried. The crude compound was used in the next step without further purification; yield: 2.0 g (79%); mp 128−132 °C.

2-Amino-6-chlorobenzenesulfonamide (23). 6-Chloro-2-nitrobenzenesulfonamide (**22**) (1.8 g, 7.6 mmol) was dissolved in a hot mixture of ethanol and water 1.1 (20 mL) and then supplemented with ammonium chloride (0.8 g, 15 mmol) and iron powder (1.6 g, 28.7 mmol). The suspension was refluxed for 45 min. The reaction medium was filtered, and the filtrate was concentrated under reduced pressure to half of the volume and then extracted three times with dichloromethane (100 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure to a small volume. After addition of hexane (15 mL), the resulting precipitate was collected by filtration, washed with hexane, and dried. Yield: 0.97 g (62%); mp 130−135 °C. Anal. ($C_6H_7ClN_2O_2S$) C, H, N, S.

8-Chloro-3-(1*H*-imidazol-1-yl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (24). A solution of 2-amino-6-chlorobenzenesulfonamide (**23**) (0.7 g, 3.4 mmol) and *N*,*N*′-thiocarbonyl-diimidazole (1.4 g, 7.8 mmol) in dioxane (10 mL) was refluxed for 2 h. The solvent was removed by distillation under reduced pressure, and the residue was suspended in water (15 mL). A solution of NaOH in water (0.25 g/2 mL) was added, and the solution was stirred at room temperature for 10 min. The alkaline solution was treated with charcoal and filtered. The filtrate was adjusted to pH 2 with 6N HCl. The precipitate was collected by filtration, washed with water, and crystallized in methanol. Yield: 0.65 g (68%); mp 230−235 °C. Anal. ($C_{10}H_7ClN_4O_2S$) C, H, N, S.

8-Chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (25b). The mixture of 8-chloro-3-(1*H*-imidazol-1-yl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**24**) (0.5 g, 1.77 mmol) and isopropylamine (5 mL) was heated in a hermetically closed autoclave at 140 °C for 4−5 h. After cooling, the excess of amine was removed by distillation under reduced pressure and the residue was suspended in water (20 mL). An aqueous solution of NaOH (5% w/v) was added dropwise until dissolution of the residue. The alkaline solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 4−5 with 6N HCl. The precipitate was collected by filtration, washed with water, and dried. The compound was purified by crystallization in methanol−water. Yield: 0.16 g (34%); mp 292−294 °C. IR (KBr) 3288 (N−H), 1633, 1559, 1464, 1412 (N−H, C=N, C=C), 1248, 1140 (S=O) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz) δ: 1.17 (d, 6H, $CH(CH_3)_2$), 3.91 (m, 1H, NHC*H*), 6.91 (bs, 1H, N*H*CH_2), 7.12 (d, 1H, 5-*H*), 7.27 (d, 1H, 7-*H*), 7.49

(m, 1H, 6-*H*), 10.43 (s, 1H, N*H*). Anal. ($C_{10}H_{12}ClN_3O_2S$) C, H, N, S.

Ionization Constants. The pK_a values of the compounds were determined by means of U.V. spectrophotometry using a Perkin-Elmer UV/vis 554 spectrophotometer at 25 °C. UV spectra of compounds were taken in different aqueous buffers of pH ranking from 5 to 12. The pK_a values were calculated by the Debye−Hückel equation at the wavelength giving the maximum absorbance of the ionized form.[24]

Biological Assays. Measurements of Insulin Release from Incubated Rat Pancreatic Islets. The method used to measure insulin release from incubated rat pancreatic islets was previously described in detail.[13,20,25]

Measurement of the Contractile Activity in Rat Aorta. The methods used to measure the myorelaxant effect of the drugs on 30 mM KCl-precontracted rat aorta rings was previously described in detail.[13,25]

Measurement 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 described in detail.[20,26]

Measurements of ^{45}Ca Outflow and Insulin Release from Perifused Islets. The method used for measuring ^{45}Ca outflow from prelabeled and perifused rat pancreatic islets was previously described in detail.[20,26]

Electrophysiology. HEK293 cells stably expressing human SUR1 and human Kir6.2 channel were used in this study. Whole-cell currents were recorded at 20−22 °C using an EPC9 patch-clamp amplifier and Pulse+PulseFit v8.07 software. The extracellular bath solution contained (in mM): 140 NaCl, 5 KCl, 10 Hepes, 1.8 $CaCl_2$, and 20 mannitol (pH 7.4 with NaOH). Cells were dialyzed with intracellular solution containing (in mM): 120 KCl, 1 $MgCl_2$, 5 EGTA, 2 $CaCl_2$, 20 Hepes (pH 7.3 with KOH), 3.0 K_2-ATP, and 0.3 K_2-ADP. Cells were clamped at −80 mV and currents were evoked by repetitive 200 ms, 10 mV depolarizing voltage steps. Signals were sampled at 20 kHz and filtered at 5 kHz.

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Supporting Information Available: Synthesis of compounds **12**, **14**, **16**, **18a−d**, **19a−d**, **25a**,**c**, all spectral data except for **19e** and **25b**, as well as elemental analyses of compounds **16**, **17**, **18a−d**, **19a−e**, **23**, **24**, and **25a−c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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