Journal of Medicinal Chemistry

ARTICLE

pubs.acs.org/jmc

Impact of the Nature of the Substituent at the 3-Position of 4*H*-1,2,4-Benzothiadiazine 1,1-Dioxides on Their Opening Activity toward ATP-Sensitive Potassium Channels

Bernard Pirotte,[⊥,†,*] Pascal de Tullio,[⊥,†] Stéphane Boverie,[†] Catherine Michaux,[‡] and Philippe Lebrun[§]

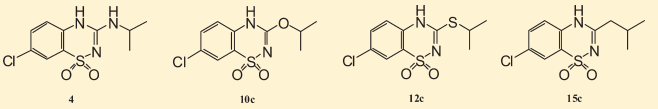
[†]Laboratoire de Chimie Pharmaceutique, Centre Interfacultaire de Recherche du Médicament (Drug Research Center), Université de Liège, C.H.U., 1 Avenue de l'Hôpital, B-4000 Liège, Belgium

[‡]Unité de Chimie Physique Structurale et Théorique, Facultés Universitaires Notre Dame de la Paix, 61, rue de Bruxelles, B-5000 Namur, Belgium

[§]Laboratoire de Pharmacodynamie et de Thérapeutique, Université Libre de Bruxelles, Faculté de Médecine, 808, Route de Lennik, B-1070 Bruxelles, Belgium

Supporting Information

ABSTRACT: The synthesis of diversely substituted 3-isopropoxy-, 3-isopropylsulfanyl-, 3-isopropylsulfinyl-, and 3-isobutyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides is described. Their activity on pancreatic β-cells (inhibitory effect on the insulin releasing process) and on vascular and uterine smooth muscle tissues (myorelaxant effects) was compared to that of previously reported K_{ATP} channel openers belonging to 3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides. The present study aimed at evaluating the impact on biological activity of the isosteric replacement of the NH group of 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides by a O, S, S(=O), or CH_2 group. By comparing compounds bearing identical substituents, the following rank order of potency on pancreatic β-cells was observed: 3-isopropylamino > 3-isobutyl > 3-isopropoxy > 3-isopropylsulfanyl > 3-isopropylsulfinyl-substituted 4*H*-1,2,4-benzothiadiazine 1,1-dioxides (NH > CH_2 > O > S > S(=O)). A molecular modeling study revealed that 3-isopropoxy-, 3-isopropylsulfanyl-, and 3-isopropylamino-substituted compounds adopted a similar low-energy conformation (preferred orientation of the isopropyl chain). Moreover, no direct relationship was detected between the conformational freedom of the different classes of benzothiadiazines (from the most to the lowest conformationally constrained compounds: NH > O > S > CH_2) and their biological activity on insulin-secreting cells. Therefore, the present study confirmed the critical role of the NH group at the 3-position for the establishment of a strong hydrogen bond responsible for optimal activity expressed by 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides on insulin-secreting cells. Radioisotopic and fluorimetric experiments conducted with 7-chloro-3-isopropoxy-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **10c** demonstrated that such a compound, bearing a short branched *O*-alkyl group instead of the *N*H-alkyl group at the 3-position, also behaved as a specific K_{ATP} channel opener. Lastly, the present work further identified 3-(alkyl/aralkyl)sulfanyl-substituted 7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides as a class of promising myorelaxant drugs acting on uterine smooth muscles, at least in part, through the activation of K_{ATP} channels.



INTRODUCTION

Among the wide variety of potassium channels, the ATP-sensitive potassium channels (K_{ATP} channels) represent a particular type for which opening and closing processes are mainly linked to changes in intracellular levels of adenine nucleotides (ADP, ATP).[1,2] K_{ATP} channels are also known to be complex octameric structures combining two kinds of transmembrane proteins, the 'sulfonylurea receptor' (SURx) subunit and the 'inwardly rectifying potassium channel' (Kir6.x) subunit. The assembly of the Kir6.x (Kir6.1 and Kir6.2) and the SURx (SUR1, SUR2A, and SUR2B) subunits in multiple combinations led to the existence of different isoforms of K_{ATP} channels diversely distributed throughout tissues.[2] For example, four SUR1 subunits combine with four Kir6.2 subunits to form the SUR1/Kir6.2 K_{ATP} channel subtype as found in the endocrine pancreas and the brain, whereas a SUR2A/Kir6.2 channel subtype is expressed in the cardiac and the skeletal muscle cells, and a SUR2B/Kir6.1 or a SUR2B/Kir6.2 channel subtype is found in smooth muscle cells.[3] A putative mitochondrial K_{ATP} channel (mitoK_{ATP} channel) has also been reported in the literature, but the exact identity of the pore-forming subunits still remains controversial.[4−6] The latter channel is expected to be involved in myocardial preconditioning and cytoprotection in different tissues.[4−6]

'Potassium channel openers' (PCOs) represent a pharmacological class of drugs able to activate the K_{ATP} channels. (−)-Cromakalim (**1**), (±)-pinacidil (**2**), and diazoxide (**3**) (Figure 1) are typical examples of such compounds.[7,8] Among these reference PCOs, diazoxide remains an important pharmacological tool and is currently used by some research groups as a 'selective' opener of the cardiac mitoK_{ATP} channels.[9] This compound is known to activate the SUR1- and SUR2B-type K_{ATP} channels but appears to be only weakly active on the SUR2A/Kir6.2 channels.[3,10] Thus, at least in

Received: October 21, 2010
Published: March 24, 2011

dx.doi.org/10.1021/jm200100c | *J. Med. Chem.* 2011, 54, 3188–3199

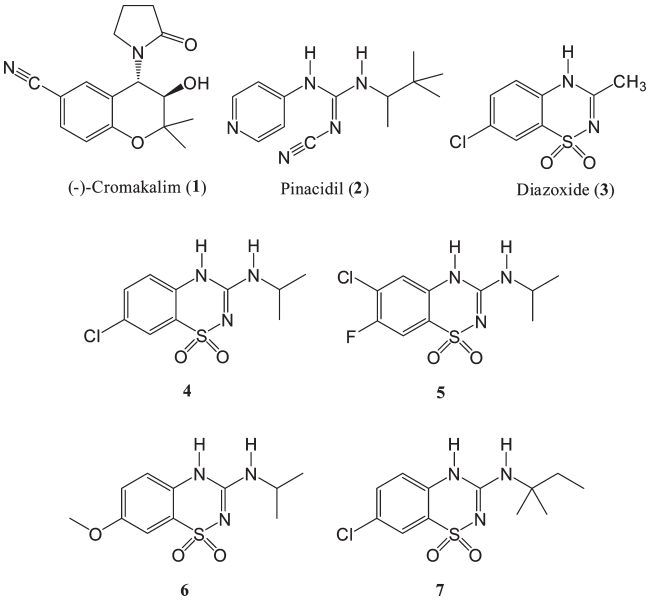


Figure 1. Typical examples of reference PCOs (**1**−**3**) and recently described benzothiadiazine-type analogues of diazoxide (**4**−**7**).

cardiomyocytes into which SUR2A/Kir6.2 K_{ATP} channels are expressed at the plasma membrane, the cell protective activity of diazoxide could be linked to the opening of the mitoK_{ATP} channels.[11]

In our efforts to develop SUR1-specific PCOs, we previously prepared several series of original diazoxide analogues characterized by the introduction of an alkylamino side chain at the 3-position of the benzothiadiazine ring.[12−15] Compared to diazoxide, such a chemical modification induced an improvement of the K_{ATP} channel opening activity.[12−14] Moreover, according to the nature of the alkyl chain at the 3-position and the nature of the substituents on the aromatic ring, these original series of drugs provided either potent and selective activators of the endocrine pancreatic K_{ATP} channels (i.e., **4**, **5**, **6**; Figure 1) or potent myorelaxant drugs devoid of activity on pancreatic β-cells (i.e., **7**; Figure 1).[12−14] These previous works on benzothiadiazine dioxides also highlighted the fact that the presence of a very short branched alkylamino chain at the 3-position (preferably an isopropylamino chain) was responsible for the selectivity toward SUR1-type channels.[12−15]

The aim of the present study was to assess the biological impact of the isosteric replacement, by an isopropoxy, an isopropylsulfanyl, an isopropylsulfinyl, or an isobutyl chain, of the isopropylamino side chain at the 3-position (concretely the replacement of the NH group by a O, S, S(=O), or CH_2 group) on the putative inhibitory effect of these diazoxide analogues on the insulin releasing process and the smooth muscle contractile activity.

The mechanism of action of active representatives from different series was also determined. Moreover, a molecular modeling approach was used to highlight the differences between these drugs, especially regarding the conformational space and the most preferable conformations adopted by typical examples of compounds. Finally, the pK_a values of representative drugs were determined to predict their ionization state at physiological pH.

CHEMISTRY

Access to 6- and/or 7-substituted 3-isopropoxy-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **10** and 3-isopropylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **12** is described in Scheme 1.

The previously reported 6- and/or 7-substituted 3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **8** were used as starting materials.[12−14] Usually, direct alkylation of 3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides preferentially occurred on the nitrogen atom at the 2-position, and poor yields of the product from O-alkylation were obtained. We have observed that O-alkylation can be favored if the steric hindrance of the alkylating agent was increased. When the bulky isopropyl iodide was used, a reasonably good yield of the product of O-alkylation was obtained. To separate the latter from the product of N-alkylation, the mixture of compounds **9** and **10** was suspended in an aqueous solution of sodium hydroxide. Compounds of general formula **10** were solubilized by forming a sodium salt (by deprotonation at the 4-position), while compounds of general formula **9** remained insoluble. After separation of the insoluble material by filtration, the filtrate regenerated the desired compound as a precipitate after acidification.

For the synthesis of the 3-isopropylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **12**, the '3-oxo' derivatives **8** were converted into the corresponding 3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **11** by means of phosphorus pentasulfide.[12,13] Alkylation always occurred on the sulfur atom, generating the desired compounds **12** in good yields.

The synthesis of 3-isobutyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **15** was performed according to the process described for the 3-cyclopentyl-substituted analogue of diazoxide (Scheme 2).[16] The selective acylation of the primary amine function was accomplished by using 1 equiv of the acyl chloride in the presence of pyridine at low temperature. Ring closure occurred by heating the intermediates **14** in an aqueous alkaline medium. After acidification, the expected compounds **15** precipitated and were collected by filtration.

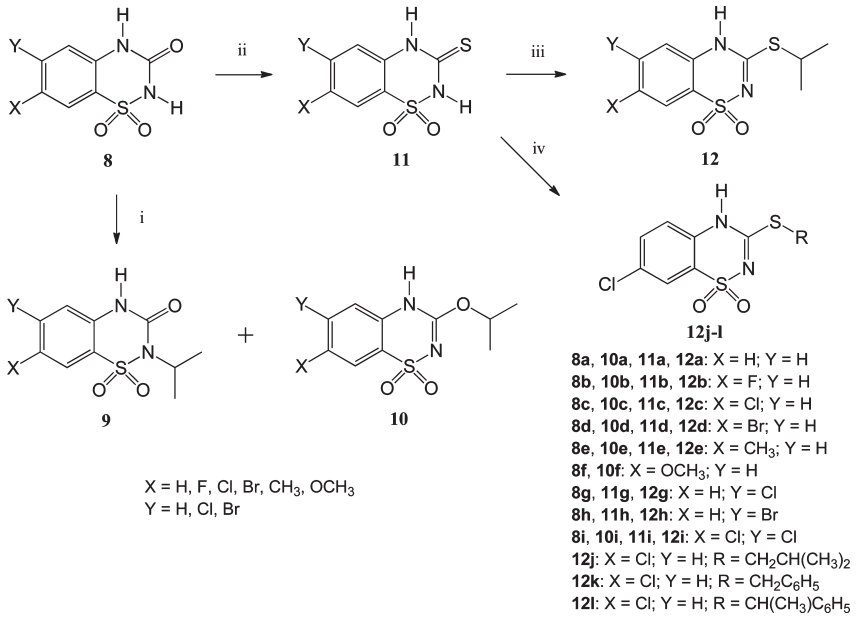
The sulfoxide derivative **16** was obtained by treating an alkaline solution of compound **12c** with bromine, which, under these conditions, was converted into sodium hypobromite by dismutation (Scheme 3).

RESULTS AND DISCUSSION

Activity on Pancreatic β-Cells. 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 diazoxide and the previously described 6- or 7-substituted 3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1- dioxides.[12−15]

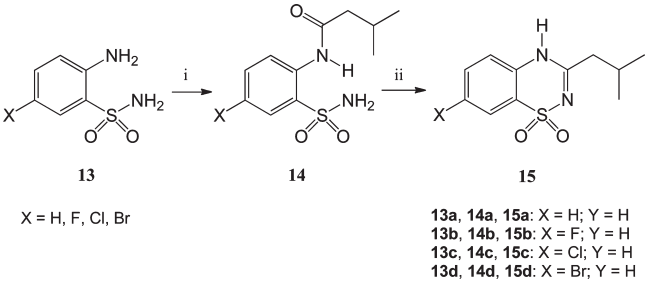
The 3-isopropylamino-substituted benzothiadiazine 1,1-dioxides **17** clearly behaved as the most potent compounds inhibiting the insulin secretory process. Among this series, the 6-chloro-substituted compound **17g** was the most active. The 3-isobutyl-substituted benzothiadiazine 1,1-dioxides **15** expressed a moderate activity, roughly similar to that of diazoxide, another example of 3-alkyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide, except for **15c**, which was somewhat more potent than the reference compound at inhibiting insulin release (diazoxide: IC_{50} = 18.4 μM;[17] **15c**: estimated IC_{50} = 5.8 μM; see Table 2). 3-Isopropoxy-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **10** were found to be equipotent or even slightly less potent than the 3-alkyl-substituted benzothiadiazine 1,1-dioxides **15**. Finally, examination of the results obtained at 10 μM and 50 μM (when available) showed that 3-isopropylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides **12** expressed an activity on pancreatic β-cells somewhat less pronounced than their 'isopropoxy-substituted' counterparts **10**. By

Scheme 1[a]



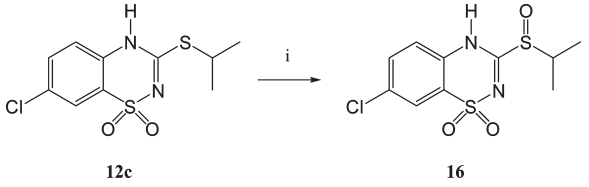
[a] Reagents: (i) NaOH, $ICH(CH_3)_2$, DMF, Δ; (ii) P_2S_5, pyridine, Δ; (iii) NaOH, $ICH(CH_3)_2$, $CH_3CH_2NO_2$, DMF, Δ; (iv) NaOH, R-Br, $CH_3CH_2NO_2$, DMF, Δ.

Scheme 2[a]



[a] Reagents: (i) $(CH_3)_2CHCH_2COCl$, pyridine, dioxane; (ii) NaOH 1% in H_2O, Δ.

Scheme 3[a]



[a] Reagent: (i) Na_2CO_3, NaOH, Br_2.

oxidizing the sulfur atom of **12c** to give the corresponding sulfoxide **16**, no gain of activity was observed.

As a first conclusion, and when comparing the activity of the 7-chloro-substituted compound in each series of benzothiadiazine dioxides (compounds **4**, **10c**, **12c**, **15c**, and **16**), we can deduce the following rank order of potency: 3-$NHCH(CH_3)_2$ (**4**) > 3-$CH_2CH(CH_3)_2$ (**15c**) > 3-$OCH(CH_3)_2$ (**10c**) > 3-$SCH(CH_3)_2$ (**12c**) ≥ 3-S(=O)$CH(CH_3)_2$ (**16**).

Concerning the nature of the substituent introduced on the benzene ring, we observe, for the 3-isobutyl-substituted benzothiadiazine dioxides **15**, that the best choice of substituent at the 7-position was the chlorine atom (Cl > Br > F > H), although, in the 3-alkylamino series **17**, there was no major differences between the activity of the 7-chloro and the 7-bromo compounds (compare **4** and **17d**). In the 3-isopropoxy and the 3-isopropylsulfanyl series **10** and **12**, the 7-bromo compounds became the most effective at inhibiting the insulin releasing process (Br > Cl > F), although differences were small and sometimes not significant. Interestingly, in the 3-isopropylsulfanyl series **12**, the 6-bromo and 6-chloro compounds appeared to exert a more pronounced activity than their corresponding 7-bromo and 7-chloro counterparts (compare **12h** with **12d** and **12g** with **12c**). The 6,7-dichloro substitution provided the most potent compound in the 3-isopropylsulfanyl series (see compound **12i**), but such a disubstitution has a lower impact in the 3-isopropoxy (**10i**) and 3-isopropylamino (**17h**) series. Compared to the 7-halo-substituted compounds, the presence of a methyl or a methoxy group at the 7-position did not improve the activity on pancreatic β-cells.

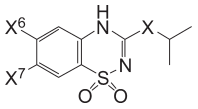
Determination of the Acidic Character. In order to explain the differences in activity as a result of the replacement of the amino group (NH) at the 3-position by a methylene group (CH_2), an oxygen atom (O), or a sulfur atom (S), we expected that the acidic character of the molecules, linked to the presence of a labile proton at the 4-position, varied in accordance with the nature of the 'bridge' at this 3-position.

Thus, we have determined the pK_a value of a representative of each series of compounds, namely the 7-chloro-substituted compounds **4**, **10c**, **12c**, and **15c** (Table 2) and observed that the acidic character decreased as follows (from the most to the less acidic compound): S (**12c**: 7.01) > O (**10c**: 8.00) > CH_2 (**15c**: 8.52) > NH (**4**: 9.51).[12] By comparison, the pK_a value of diazoxide has been reported to be 8.62,[12] close to the pK_a value of its 3-isobutyl-substituted analogue **15c**.

In accordance with such pK_a values, we may predict that the 3-isopropylsulfanyl-substituted compounds **12** are essentially ionized at the physiological pH of 7.4. Considering that the nonionic form of the molecule could be the active form, it is

dx.doi.org/10.1021/jm200100c |J. Med. Chem. 2011, 54, 3188–3199

Table 1. Effects of Diversely Substituted 4*H*-1,2,4-Benzothiadiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets



no.	X^6	X^7	X	RIS[a] (50 μM)	RIS[a] (10 μM)	RIS[a] (1 μM)	RIS[a] (0.1 μM)
10a	H	H	O	–	90.4 ± 3.1 (23)	–	–
10b	H	F	O	44.1 ± 3.3 (15)	87.5 ± 4.3 (23)	–	–
10c	H	Cl	O	17.5 ± 2.0 (11)	77.3 ± 4.6 (14)	–	–
10d	H	Br	O	–	62.5 ± 3.2 (16)	–	–
10e	H	CH_3	O	–	79.5 ± 2.8 (15)	–	–
10f	H	OCH_3	O	–	80.8 ± 4.4 (15)	–	–
10i	Cl	Cl	O	–	73.7 ± 4.3 (15)	–	–
12a	H	H	S	–	93.9 ± 4.2 (15)	–	–
12b	H	F	S	55.1 ± 1.8 (15)	88.7 ± 5.4 (21)	–	–
12c	H	Cl	S	51.3 ± 3.9 (19)	104.0 ± 5.8 (23)	–	–
12d	H	Br	S	47.3 ± 2.6 (14)	90.3 ± 3.8 (24)	–	–
12e	H	CH_3	S	–	77.0 ± 4.7 (16)	–	–
12g	Cl	H	S	44.0 ± 3.5 (16)	80.2 ± 4.4 (23)	–	–
12h	Br	H	S	33.8 ± 2.5 (14)	87.0 ± 5.1 (23)	–	–
12i	Cl	Cl	S	12.9 ± 1.1 (13)	70.0 ± 3.7 (16)	–	–
15a	H	H	CH_2	–	85.2 ± 4.1 (16)	–	–
15b	H	F	CH_2	–	75.3 ± 5.6 (15)	–	–
15c	H	Cl	CH_2	21.0 ± 1.1 (22)	43.2 ± 2.2 (15)	85.8 ± 4.6 (16)	–
15d	H	Br	CH_2	–	61.8 ± 3.7 (15)	–	–
16	H	Cl	S=O	89.8 ± 4.2 (21)	92.3 ± 3.6 (15)	–	–
17a[b]	H	H	NH	–	34.9 ± 2.0 (16)	73.7 ± 5.2 (15)	–
17b[b]	H	F	NH	3.3 ± 0.7 (13)	3.7 ± 0.6 (13)	47.3 ± 3.7 (23)	96.9 ± 4.9 (16)
4[b]	H	Cl	NH	5.7 ± 0.5 (35)	4.8 ± 0.4 (32)	36.2 ± 2.4 (31)	90.4 ± 3.5 (23)
17d[b]	H	Br	NH	6.7 ± 1.7 (12)	8.1 ± 0.8 (12)	34.9 ± 2.8 (12)	91.0 ± 5.3 (13)
17e[b]	H	CH_3	NH	3.5 ± 0.3 (13)	8.5 ± 0.7 (14)	71.3 ± 3.5 (15)	–
17f[b]	H	OCH_3	NH	4.4 ± 0.7 (12)	8.5 ± 0.9 (24)	67.6 ± 4.3 (20)	–
17g[b]	Cl	H	NH	6.4 ± 0.7 (16)	7.5 ± 0.8 (15)	13.2 ± 1.2 (16)	76.7 ± 4.3 (15)
17h[b]	Cl	Cl	NH	5.0 ± 0.4 (12)	6.3 ± 0.7 (12)	13.2 ± 1.0 (26)	84.9 ± 4.5 (21)
diazoxide[b]				26.7 ± 1.6 (16)	73.9 ± 4.4 (16)	87.5 ± 5.0 (15)	–

[a] RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)). [b] Published results (refs [12−15]).

tempting to speculate that the marked ionization of the 3-alkylsulfanyl-substituted derivatives could justify their poor activity on the pancreatic tissue. Moreover, considering that the binding sites of K_{ATP} channel modulators (activators and blockers) have been reported to be located at the cytoplasmic face of the cell membranes,[18,19] the ionization state of the molecules could have a critical impact on the capacity of the drugs to permeate the biological membranes and to reach the binding sites.

Previous works with two other series of potent SUR1-selective K_{ATP} channel activators, i.e., 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides (pK_a values between 8.1 and 8.2[16]) and 3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides (pK_a values between 8.2 and 8.5[20]), reinforce the assumption that a pK_a value higher than 8 warrants a sufficient fraction of nonionized molecules at physiological pH that can cross the cell membranes and exert their biological activity.

Molecular Modeling. The modification of the nature of the 'bridge' introduced at the 3-position might also have an impact on the conformational space of the molecules and on the freedom of rotation of the bond linking the substituent at the 3-position, thus influencing the spatial orientation of the alkyl (isopropyl) chain.

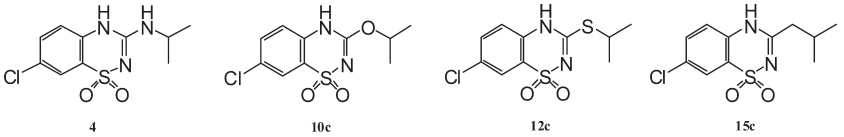
Considering that the 'active' conformation of 3-substituted 1,2,4-benzothiadiazine 1,1-dioxides is provided by the most potent series of compounds, namely the 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides, we hypothesized that the isopropyl chain had to be firmly blocked in the spatial orientation, as found with compound **4**. For such a compound as well as for the other 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides, crystal structures indicated that the 'guanidine' moiety was always found to be in the plane (assuming optimal electron delocalization between the three nitrogen atoms) with the two NH groups systematically oriented in parallel.[21,22]

In addition, the crystallographic data obtained with **4**,[22] **10c**,[23] and **12c**[24] indicated that the three compounds adopted the 4*H*-tautomeric form in the solid state. Indeed, the bond length between C(3) and N(4) was always greater than the bond length between N(2) and C(3) [C(3)−N(4) for **4**, **10c**, and **12c** = 1.352 Å, 1.339 Å, and 1.360 Å, respectively; N(2)−C(3) for **4**, **10c**, and **12c** = 1.330 Å, 1.289 Å, and 1.310 Å, respectively],[22−24] assuming that the double bond in the thiadiazine ring is located at N(2)−C(3) rather than at C(3)−N(4). Therefore, the hydrogen atom must be found at the N(4) position (4*H*-tautomerism).

We have used these crystallographic data as the starting low energy conformation (for **15c**, no X-ray data were available because no convenient monocrystal was obtained), and we further explored the conformational space resulting from the rotation of the bond linking the substituent at the 3-position

dx.doi.org/10.1021/jm200100c |*J. Med. Chem.* 2011, 54, 3188–3199

Table 2. Effects of Selected 4*H*-1,2,4-Benzothiadiazine 1,1-Dioxides, Diazoxide, and Pinacidil on Insulin Release, on Contractile Activity of K^+-Depolarized Rat Aorta Rings, and on Oxytocin-Induced Contraction of Rat Uterus (bolus of oxytocin)



			rat uterus % residual contraction[c]			
no.	pancreatic islets IC_{50} (μM)[a]	rat aorta rings EC_{50} (μM)[b]	100 μM	50 μM	10 μM	pK_a value
4	0.55 ± 0.10 (3)[d]	36.3 ± 2.2 (6)[d]	75.6 ± 5.1 (4)	95.0 ± 1.7 (4)	94.4 ± 4.9 (4)	9.51[d]
10c	19.1[e]	22.7 ± 1.9 (7)	68.7 ± 10.6 (4)	85.8 ± 6.2 (4)	88.0 ± 6.0 (4)	8.00
12c	~50[e]	23.0 ± 3.6 (6)	2.3 ± 1.9 (4)	54.7 ± 14.8 (4)	98.8 ± 11.4 (4)	7.01
15c	5.8[e]	5.8 ± 1.5 (4)	55.0 ± 4.3 (4)	64.7 ± 2.8 (4)	73.9 ± 6.3 (4)	8.52
diazoxide	18.4 ± 2.2 (5)[d]	22.4 ± 2.1 (11)[d]	67.7 ± 4.0 (4)	76.3 ± 4.9 (4)	93.8 ± 2.2 (4)	8.62[d]
pinacidil	202.3 ± 28.3 (3)[d]	0.62 ± 0.17 (15)[d]	38.1 ± 2.2 (6)	42.7 ± 2.4 (6)	44.9 ± 5.1 (6)	–

[a] IC_{50}: drug concentration giving 50% inhibition of insulin release (mean ± SEM (n)). [b] EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (n)). [c] % residual contraction: percentage of residual contraction of rat uterus induced by 20 mU oxytocin injected as a bolus in the superfusion system (mean ± SEM (n)). [d] Published results (refs 12 and 17). [e] Estimated IC_{50} value.

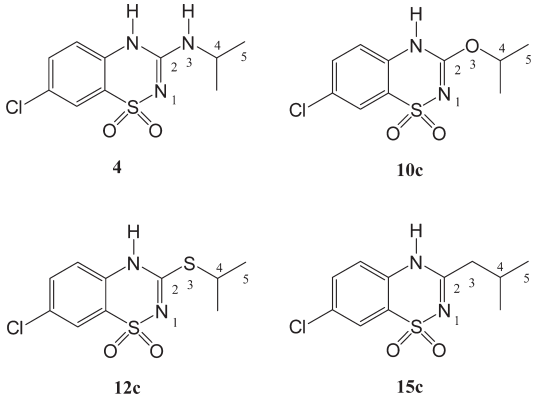


Figure 2. Chemical structure of 3-isopropylamino- (**4**), 3-isopropoxy- (**10c**), 3-isopropylsulfanyl- (**12c**), and 3-isobutyl- (**15c**) substituted 7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide with indication of the torsion angles T1 (atoms 1−2−3−4) and T2 (atoms 2−3−4−5).

(torsion angle T1 defined by atoms 1−2−3−4 in Figure 2) and also resulting from the rotation of the bond linking the 'isopropyl' moiety to the 'bridge' at the 3-position (torsion angle T2 defined by atoms 2−3−4−5 in Figure 2). Particular attention was paid to the preferred orientation adopted by the isopropyl chain, in each case.

As shown in Figure 3, the greatest freedom of rotation of the bond linking the substituent at the 3-position (defined by the T1 angle) was observed with the compound possessing a 'methylene' (CH_2) bridge. The following rank order of conformational freedom is given as follows: CH_2 > S > O > NH.

Regarding the 3-alkylamino-substituted derivatives, free rotation was excluded because the optimal delocalization of the lone pairs of the nitrogen atoms in the guanidine moiety was only achieved when the system was coplanar. Two situations may warrant such optimal delocalization (see conformations A and B, Figure 4), but for steric reasons, the first conformation A was preferred. Such a feature was confirmed by the present molecular modeling study and by previously published X-ray data for diversely substituted 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides.[21,22]

The oxygen atom and the sulfur atom also possess electronic lone pairs and the possibility for delocalization of the lone pairs in the 'amidinic' (N═C−NH) moiety of the thiadiazine ring could also favor the planar conformation. In fact, the lowest energy conformer in each series has been found to be the one with the torsion angle T1 near 0° (see Figure 5). Moreover, in both series, and probably for the same steric reasons, the preferred orientation of the isopropyl chain was similar to that found with the 3-isopropylamino-substituted compound.

Our molecular modeling approach further revealed that the 3-alkoxy- and 3-alkylsulfanyl-substituted 1,2,4-benzothiadiazine 1,1-dioxides preferentially adopt a conformation similar to that observed with the most potent (biologically efficient on the insulin-releasing process) 3-alkylamino-substituted compounds. Such a feature, however, was not sufficient to ensure a marked biological activity. These data rather confirm the critical role of the NH group at the 3-position of 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides for the establishment of a strong hydrogen bond with the receptor binding site responsible for optimal activity on pancreatic β-cells.

Pharmacological Evaluation on Smooth Muscles. Further pharmacological investigations were conducted with the 7-chloro-substituted benzothiadiazine dioxides **10c**, **12c**, and **15c** on two types of smooth muscle cells. The three compounds were compared to the previously studied compound **4**, to diazoxide, and to pinacidil for their myorelaxant activity on rat aortic rings precontracted with 30 mM KCl (Table 2). The following EC_{50} values (drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings) were obtained: **4** (NH): EC_{50} = 36.3 μM;[12] **10c** (O): EC_{50} = 22.7 μM; **12c** (S): EC_{50} = 23.0 μM; **15c** (CH_2): EC_{50} = 5.8 μM.

As a result, the compound exhibiting the most potent vascular myorelaxant activity was the 3-alkyl-substituted benzothiadiazine dioxide **15c**, being also poorly tissue selective. Indeed, its EC_{50} value on the vascular smooth muscle cells (EC_{50} = 5.8 μM) was

dx.doi.org/10.1021/jm200100c |*J. Med. Chem.* 2011, 54, 3188–3199

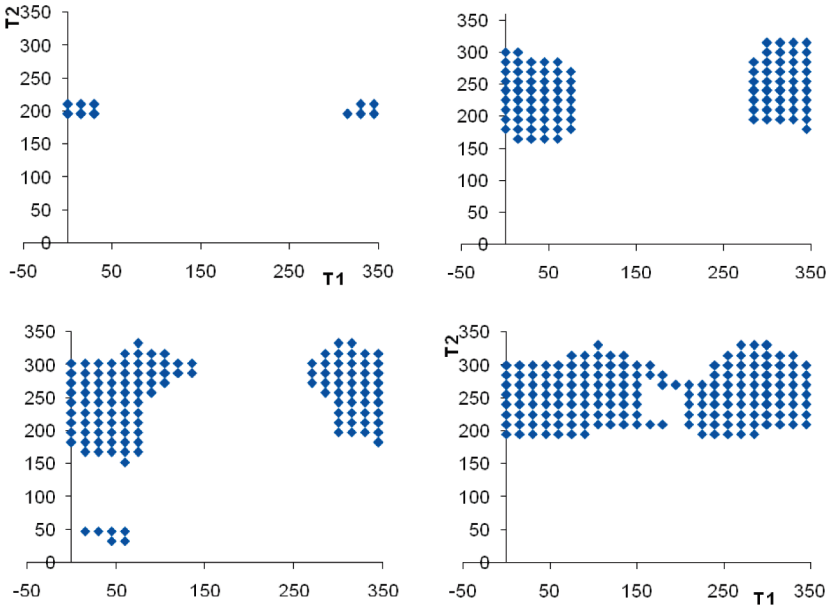


Figure 3. 2D conformational scan (T1, T2) of compounds **4** (left upper panel), **10c** (right upper panel), **12c** (left lower panel), and **15c** (right lower panel). The pictured conformations are within 10 kcal/mol of energy.

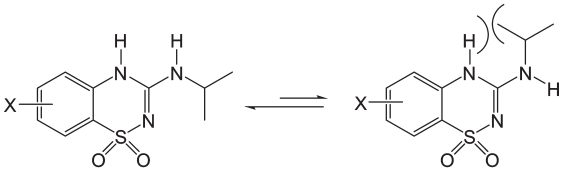


Figure 4. Two possible conformations adopted by 3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides, assuming optimal delocalization of the electron lone pairs of the nitrogen atoms in the guanidine moiety.

found to be similar to the estimated IC_{50} value on pancreatic β-cells (IC_{50} = 5.8 μM) (Table 2). Such a feature was previously noticed with the reference compound diazoxide, another 3-alkyl-substituted 1,2,4-benzothiadiazine 1,1-dioxide (EC_{50} = 22.4 μM; IC_{50} = 18.4 μM; Table 2).[16] Thus, for this class of compounds, it was observed that the bulky isobutyl chain induced a more marked effect on vascular smooth muscles and on pancreatic β-cells than the methyl chain (e.g.: diazoxide). However, and according to recent published data obtained with a series of 7-chloro-3-cycloalkyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides,[25] it was expected that a substituent more bulky than an isobutyl chain (i.e., a cyclohexyl chain) at the 3-position should induce a loss of activity on SUR1-type K_{ATP} channels (pancreatic β-cell type channels), while keeping a high level of activity on the SUR2B-type K_{ATP} channels (smooth muscle cell type channels).[25]

The less vasorelaxant compound among the selected benzothiadiazines (see Table 2) was the 3-alkylamino-substituted benzothiadiazine dioxide **4**, being also the most selective toward the pancreatic tissue.

When the data obtained for rat uterus were examined, another smooth muscle tissue expressing K_{ATP} channels, it was found that the 3-alkylsulfanyl-substituted compound **12c** was the most powerful myorelaxant (inhibition of the contraction induced by 20 mU oxytocin injected as a bolus in the superfusion system) although none of the tested compounds exhibited a strong inhibitory effect (Table 2). The rank order of potency on the uterine smooth muscle was 3-alkylsulfanyl (S) > 3-alkyl (CH_2) > 3-alkoxy (O) > 3-alkylamino (NH). On the rat uterine tissue, the reference compound diazoxide was found to be less potent than the other 3-alkyl-substituted benzothiadiazine dioxide **15c**. Among the compounds tested on the different tissues (aortic, uterine, pancreatic), the 3-alkylamino-substituted compound **4** remained the most selective toward the endocrine pancreas versus smooth muscle tissues.

Because the reference SUR2B-type PCO pinacidil was unable to completely suppress the uterine contractions induced by a bolus of oxytocin (about 40% residual contraction in the presence of 100 μM pinacidil), we decided to explore other experimental conditions in order to have access to EC_{50} values. When oxytocin was continuously superfused (50 mU/L) on the muscle preparation, a regular set of reproducible small contractions was observed. Under such experimental conditions, pinacidil completely suppressed the oxytocin-induced contractions, and concentration−response curves led us to calculate EC_{50} values.

The EC_{50} value of pinacidil (EC_{50} = 1.3 μM) was found to be shifted to higher values in the continuous presence of 1 μM (EC_{50} = 8.1 μM) or 10 μM (EC_{50} = 28.3 μM) of the K_{ATP} channel blocker glibenclamide[26] in the physiological medium (Table 3). This finding confirms the involvement of K_{ATP} channels in the myorelaxant effect of pinacidil on rat uterus.

According to the initial results on rat uterus reported in Table 2, 3-alkylsulfanyl-substituted compounds can be expected to represent a promising series of myorelaxant drugs. As a result, we decided to examine, in the new experimental test conditions, compound **12c** and three other of its analogues bearing a different hydrocarbon chain linked to the sulfur atom at the 3-position (see compounds **12j**−**l**; Scheme 2). Table 3 reports the results obtained with these compounds as well as with diazoxide and pinacidil on rat uterus and rat aorta rings.

dx.doi.org/10.1021/jm200100c |*J. Med. Chem.* 2011, 54, 3188–3199

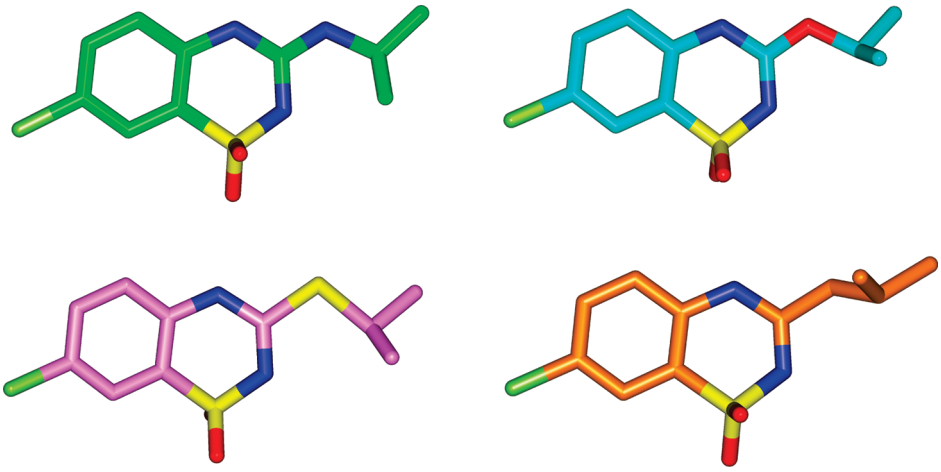
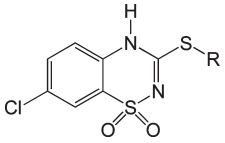


Figure 5. The most stable conformation of **4** (green), **10c** (blue), **12c** (pink), and **15c** (orange).

Table 3. Effects of Selected 3-Alkylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides, Diazoxide, and Pinacidil on the Contractile Activity of K^+-Depolarized Rat Aorta Rings and on Oxytocin-Induced Contraction of Rat Uterus (continuous superfusion of oxytocin)



			rat uterus EC_{50} (μM)[b]		
no.	R	rat aorta rings EC_{50} (μM)[a]	no glibenclamide	+ 1 μM glibenclamide	+ 10 μM glibenclamide
12c	$CH(CH_3)_2$	23.0 ± 3.6 (6)	>30	nd[d]	nd
12j	$CH_2CH(CH_3)_2$	6.2 ± 0.9 (4)	13.5 ± 1.8 (4)	nd	nd
12k	$CH_2C_6H_5$	5.4 ± 0.1 (4)	11.7 ± 0.5 (4)	nd	nd
12l	$CH(CH_3)C_6H_5$	5.4 ± 0.1 (4)	4.4 ± 0.3 (12)	6.8 ± 0.9 (4)	24.2 ± 2.4 (12)
diazoxide	–	22.4 ± 2.1 (11)[c]	>30	nd	nd
pinacidil	–	0.62 ± 0.17 (15)[c]	1.3 ± 0.3 (18)	8.1 ± 0.8 (16)	28.3 ± 3.3 (16)

[a] EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (n)). [b] EC_{50}: drug concentration giving 50% relaxation of the oxytocin-induced contraction of rat uterus continuously superfused with 50 mU/L oxytocin in the absence or presence of glibenclamide (mean ± SEM (n)). [c] Published results (refs 12 and 17). [d] Not determined.

Compound **12c** exhibited an EC_{50} above 30 μM. The three analogues **12j**, **12k**, and **12l** were found to be more potent than **12c** with EC_{50} values of 13.5 μM, 11.7 μM, and 4.4 μM, respectively. The marked myorelaxant effects of **12j**, **12k**, and **12l** was confirmed on vascular smooth muscle cells (EC_{50} = 6.2 μM for **12j**; EC_{50} = 5.4 μM for **12k** and **12l**). Moreover, the EC_{50} value of the most potent compound **12l** on rat uterus was shifted to higher values when the experiment was repeated in the continuous presence of glibenclamide in the physiological medium (Table 3), indicating that the myorelaxant effect of the drug was, at least in part, mediated by the activation of K_{ATP} channels. However, due to the assumption that access and binding to K_{ATP} channels should be mediated by nonionized drugs and according to the ionization state of 3-alkylsulfanyl-substituted benzothiadiazine dioxides at physiological pH, the unexpected marked activity of these compounds on rat uterus probably involve additional myorelaxant mechanisms. Further chemical and pharmacological developments are required to elucidate the whole mechanism of action and to confirm the possible interest of 3-alkylsulfanyl-substituted 4*H*-1,2,4-benzothiadiazine 1,1-dioxides as a new class of tocolytic agents.

Radioisotopic and Fluorimetric Experiments. Additional in vitro experiments have been performed to specify the mechanism of action of the new benzothiadiazine dioxides. In the first series of experiments, we characterized the effects of selected compounds on ^{86}Rb (^{42}K substitute), ^{45}Ca outflow, and insulin release from perifused rat pancreatic islets.

The addition of the chloro-substituted 3-isopropoxy-4*H*-1,2,4-benzothiadiazine 1,1 dioxide **10c** to prelabeled pancreatic islets exposed throughout to 5.6 mM glucose and extracellular Ca^{2+} provoked a concentration-dependent increase in the rate of ^{86}Rb outflow (Figure 6). Thus, the magnitude of the increase in ^{86}Rb outflow observed during exposure to **10c** averaged 0.23 ± 0.05%/min after the addition of 10 μM and 1.32 ± 0.30%/min after the addition of 50 μM **10c**, respectively ($P < 0.05$). The withdrawal of the drug from the perifusate was followed by a decrease in ^{86}Rb outflow (Figure 6).

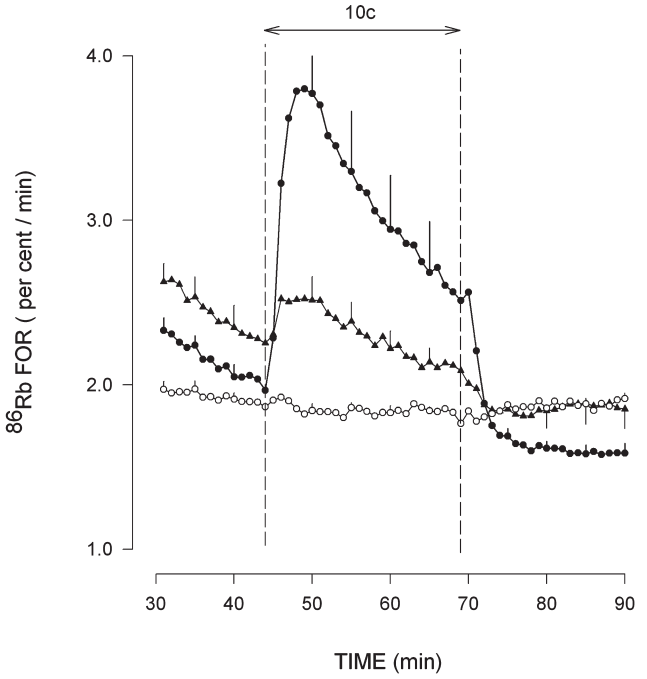


Figure 6. Effects of **10c** (10 μM, ▲; 50 μM, ●) and **10c** (50 μM) in the continuous presence of glibenclamide (10 μM, ○) on ^{86}Rb outflow from prelabeled and perifused rat pancreatic islets. Basal media contained 5.6 mM glucose and extracellular Ca^{2+}. Mean values (±SEM) refer to six individual experiments.

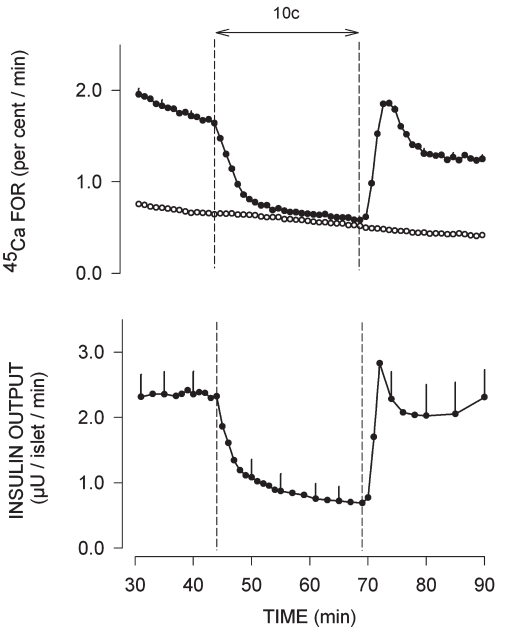


Figure 7. Effects of **10c** (50 μM) on ^{45}Ca outflow (upper panel) and insulin output (lower panel) from prelabeled rat pancreatic islets perifused throughout in the presence of 16.7 mM glucose. Basal media contained extracellular Ca^{2+} (●) or were deprived of Ca^{2+} (○). Mean values refer to four to six individual experiments.

When the physiological medium was enriched with the hypoglycemic sulfonylurea glibenclamide, a K_{ATP} channel blocker,[26] the enhancing effect of 10 μM (data not shown) or 50 μM **10c** was completely suppressed (Figure 6). Experiments performed with compound **17f** indicated that, under the same experimental conditions, the drug also elicited a reversible and glibenclamide-sensitive increase in ^{86}Rb outflow from prelabeled and perifused rat pancreatic islets (data not shown). These findings indirectly indicate that 3-alkoxy-substituted benzothiadiazine 1,1-dioxides (such as **10c**) and 3-alkylamino-substituted compounds (such as **17f**) provoke an increase in membrane K^+ permeability and further suggest that the K^+ permeability changes are mediated by the activation of ATP-sensitive K^+ channels.[14,26]

An increase in K_{ATP} channel activity might be expected to shift the membrane potential below the threshold required for the opening of voltage-sensitive Ca^{2+} channels, thereby reducing the Ca^{2+} entry, the cytosolic Ca^{2+} concentration, and the secretory process. Such a cascade of events is corroborated by additional radioisotopic and fluorimetric experiments.

First, compound **10c** (Figure 7, upper panel), as well as compound **17f** (data not shown), reduced ^{45}Ca outflow from prelabeled rat pancreatic islets exposed throughout to 16.7 mM glucose and extracellular Ca^{2+}. Under such experimental conditions, namely in the presence of an insulinotropic glucose concentration and extracellular Ca^{2+} in the physiological medium, a decrease in the ^{45}Ca outflow rate reflects a reduction in $^{40}Ca^{2+}$ entry into the islets cells.[14,26] The lack of effect of compound **10c** (Figure 7, upper panel) and **17f** (data not shown) on ^{45}Ca outflow from pancreatic islets exposed to Ca^{2+}-free media corroborates this interpretation. Second, calcium fluorimetry experiments conducted on isolated pancreatic islet cells clearly revealed the capacity of compound **10c** to counteract the rise in cytosolic Ca^{2+} concentration mediated by an insulinotropic glucose concentration (data not shown). Third, the simultaneous measurement of insulin release from pancreatic islets perifused throughout in the presence of 16.7 mM glucose and extracellular Ca^{2+} further revealed an inhibitory effect, displaying a time course identical to that of the ^{45}Ca outflow responses, of compounds **10c** (Figure 7, lower panel) and **17f** (data not shown) on the insulin secretory rate.

In islets exposed throughout to 2.8 mM glucose and extracellular Ca^{2+}, the ^{45}Ca response to a sudden rise in the extracellular concentration of K^+ (5 to 50 mM) was unaffected by the presence of **10c** (50 μM) in the basal medium (data not shown). Thus, the magnitude of the increase in ^{45}Ca outflow evoked by 50 mM K^+ averaged 1.24 ± 0.05%/min in the absence and 1.12 ± 0.07%/min in the presence of 50 μM **10c** ($P > 0.05$). Such a finding further indicates that **10c** fails to interact directly at the level of the voltage-sensitive Ca^{2+} channels.[26]

Altogether, these experimental data indicate that the 3-alkoxy-substituted benzothiadiazine **10c**, as well as the 3-alkylamino-substituted benzothiadiazine **17f**, activates the plasma membrane K_{ATP} channels and ultimately inhibits the insulin releasing process through a reduction in Ca^{2+} entry. Incidentally, the ionic and secretory responses to compounds **10c** (see Figures 6 and 7) and **17f** (data not shown) were always rapidly reversible, implying a lack of damaging effect of such compounds to the insulin-secreting cells.

In the last series of radioisotopic experiments, we determined the effects of the 3-alkyl-substituted benzothiadiazine **15c**, an original compound exhibiting a marked myorelaxant activity, on the rate of ^{86}Rb outflow from prelabeled and perifused rat aortic rings. Figure 8 clearly shows that **15c** (100 μM) induced a fast, sustained, and reversible increase in ^{86}Rb outflow. The presence of the K_{ATP} channel blocker glibenclamide[26] in the perifusing medium strongly reduced the stimulatory effect of **15c**. Such observations further suggest that the vasorelaxant effect of **15c**

dx.doi.org/10.1021/jm200100c |J. Med. Chem. 2011, 54, 3188–3199

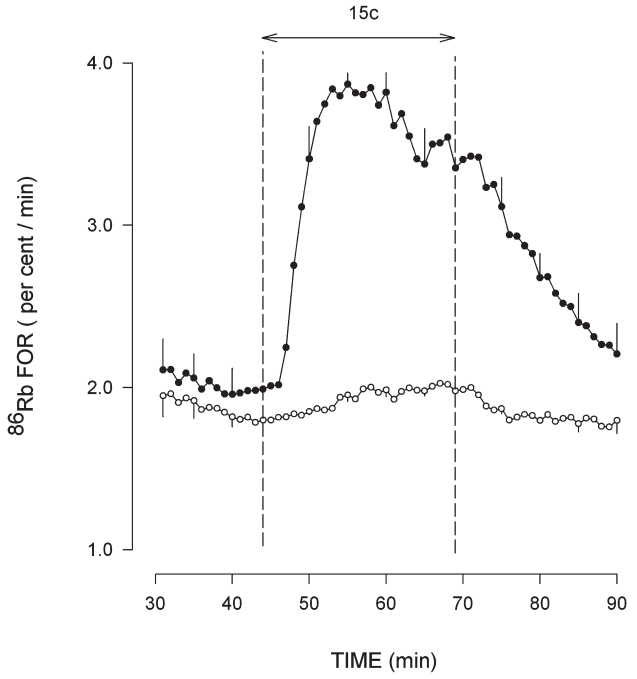


Figure 8. Effect of **15c** (100 μM) on ^{86}Rb outflow from prelabeled rat aortic rings perifused throughout in the absence (●) or presence (○) of glibenclamide (10 μM). Mean values (±SEM) refer to four to five individual experiments.

results from the activation of K_{ATP} channels, as already reported for the reference compound diazoxide, another 3-alkyl-substituted benzothiadiazine dioxide.[27]

CONCLUSION

Diversely substituted 3-isopropoxy-, 3-isopropylsulfanyl-, 3-isopropylsulfinyl-, and 3-isobutyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides were synthesized and their activity on pancreatic β-cells (inhibition of insulin release) and vascular and uterine smooth muscle tissues (myorelaxant effects) was compared to that of previously reported K_{ATP} channel openers belonging to the 3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide series. The impact on biological activity of the isosteric replacement of the NH group of 3-alkylamino-substituted benzothiadiazines by a O, S, S(=O), or CH_2 group was examined. The following rank order of potency on insulin-secreting cells was observed: 3-isopropylamino- (NH) > 3-isobutyl- (CH_2) > 3-isopropoxy- (O) > 3-isopropylsulfanyl- (S) > 3-isopropylsulfinyl- (S(=O)) substituted 4*H*-1,2,4-benzothiadiazine 1,1-dioxides.

The present study also confirmed the critical role of the NH group at the 3-position for the establishment of a strong hydrogen bond responsible for optimal activity expressed by 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides on the insulin-secreting cells. Compared to the three other series of drugs, the latter series of compounds also expressed the highest selectivity for the pancreatic tissue versus smooth muscle tissues (aorta and uterus).

Interestingly, 3-(alkyl/aralkyl)sulfanyl-substituted 7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides were identified as potent myorelaxant drugs acting on uterine smooth muscles. The most potent compound, *R*/*S*-7-chloro-3-(1-phenylethyl)sulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**12l**), was found to exert its biological activity, at least in part, through the activation of K_{ATP} channels.

Further radioisotopic and fluorimetric experiments conducted with 7-chloro-3-isopropoxy-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **10c** demonstrated that such a compound bearing a short branched *O*-alkyl group instead of an *N*H-alkyl or an alkyl group at the 3-position also behaved as a specific opener of the ATP-sensitive potassium channels. This study is the first report on the identification of a 3-alkoxy-4*H*-1,2,4-benzothiadiazine 1,1-dioxide as a K_{ATP} channel opener.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined on a Stuart SMP3 capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1000 FTIR spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker Avance (500 MHz) instrument using DMSO-d_6 as the solvent with TMS as an internal standard; chemical shifts are reported in δ values (ppm) relative to that of internal TMS. The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and b = broad are used throughout. Elemental analyses (C, H, N, S) were realized on a Thermo Scientific FlashEA 1112-elemental analyzer and were within ±0.4% of the theoretical values. This analytical method certified a purity ≥95% for each tested compound. All reactions were routinely checked by TLC on silica gel Merck 60 F_{254}. The synthesis of compounds **10a,b**, **10d**−**f**, **10i**, **12a,b**, **12d,e**, **12g**−**k**, **14a**, **b**, **14d**, **15a,b**, and **15d** is detailed in the Supporting Information. The synthesis of **10a** and **12a** has been reported previously.[28,29]

*7-Chloro-3-isopropoxy-4H-1,2,4-benzothiadiazine 1,1-dioxide Monohydrate (**10c**).* 7-Chloro-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**8c**)[12] (4.3 mmol) was solubilized in methanol (10 mL) by adding, under stirring, 1 equiv of NaOH (4.3 mmol). The resulting solution was evaporated to dryness under reduced pressure, and the residue was dissolved in DMF (20 mL). Isopropyl iodide (6.45 mmol) was added to the mixture, and the solution was heated at 70−80 °C during 4 to 6 h. The reaction gave rise to the formation of two major compounds: 7-chloro-2-isopropyl-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**9c**) and 7-chloro-3-isopropoxy-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**10c**). The solvent was eliminated by distillation under reduced pressure, and the residue was suspended in water (30 mL). The mixture was alkalinized under stirring by adding a 5% m/v aqueous solution of NaOH until pH 14. The insoluble material was eliminated by filtration, and the filtrate was treated with charcoal. After filtration, the filtrate was acidified to pH 10 by means of 12 N HCl. The precipitate was eliminated by filtration, and the filtrate was supplemented with 12 N HCl until pH 1. The resulting precipitate of the title compound was collected by filtration, washed with water, and dried (yields: 40%); mp: 227−229 °C; IR (KBr): 3582, 3522, 2986, 1613, 1580, 1523, 1482, 1329, 1312, 1288, 1255, 1172 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.35 (d, 6H, 2 × CH_3), 5.15 (m, 1H, C*H*), 7.29 (d, 1H, 5-*H*), 7.70 (d, 1H, 6-*H*), 7.79 (s, 1H, 8-*H*), 12.22 (bs, 1H, N-*H*). Anal. ($C_{10}H_{11}ClN_2O_3S.H_2O$) C, H, N, S.

General Synthetic Pathway to 7-Substituted 3-Isopropylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides (12). The appropriate 6/7-substituted 3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**11**)[12,13,15] (2.0 mmol) was dissolved in methanol (10 mL) supplemented with NaOH (2.0 mmol). The solvent was evaporated under reduced pressure, and the residue was solubilized in nitroethane−DMF 5:1. Isopropyl iodide (2.2 mmol) was added to the solution, and the mixture was heated for 3 h at 80 °C. Then, the solvents were removed by distillation under reduced pressure, and the residue was dissolved in a 10% w/v aqueous solution of NaOH. The resulting solution was treated with charcoal and filtered, and the filtrate was treated with 12 N HCl until pH 1. The resulting precipitate was collected by filtration, washed with water, and dried; yields: 40−60%.

*7-Chloro-3-isopropylsulfanyl-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**12c**).* The title compound was obtained according to the general synthetic pathway starting from 7-chloro-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**11c**); mp: 220−226 °C; IR (KBr): 3228, 3179, 3077, 1604, 1553, 1506, 1475, 1305, 1194, 1157, 1135, 1109 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.41 (d, 6H, 2 × C*H*$_3$), 3.85 (m, 1H, C*H*), 7.30 (d, 1H, 5-*H*), 7.73 (d, 1H, 6-*H*), 7.84 (s, 1H, 8-*H*), 12.58 (bs, 1H, N-*H*). Anal. ($C_{10}H_{11}ClN_2O_2S_2$) C, H, N, S.

*R/S-7-Chloro-3-(1-phenylethyl)sulfanyl-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**12l**).* The title compound was obtained according to the general synthetic pathway starting from 7-chloro-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**11c**) and 1-phenylethyl bromide instead of isopropyl iodide; mp: 216−218 °C; IR (KBr): 3250, 1599, 1547, 1508, 1479, 1298, 1159 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.75 (d, 3H, C*H*$_3$), 5.00 (q, 1H, C*H*), 7.25−7.50 (m, 6H, C$_6$*H*$_5$ + 5-*H*), 7.75 (dd, 1H, 6-*H*), 7.85 (d, 1H, 8-*H*), 12.55 (s, 1H, N-*H*). Anal. ($C_{15}H_{13}ClN_2O_2S_2$) C, H, N, S.

General Synthetic Pathway to 5-Substituted 2-(3-Methylbutyrylamino)benzenesulfonamides (14). The appropriate 5-substituted aminobenzenesulfonamide (**13**) (3.2 mmol) was dissolved in dioxane (12 mL) and supplemented with pyridine (3.2 mmol) and 2-methylbutyryl chloride (3.8 mmol). The mixture was stirred at room temperature for 1 h. The solvent was removed by distillation under reduced pressure, and the residue was solubilized with a 5% w/v aqueous solution of NaOH. The resulting solution was adjusted to pH 6−7 by means of 1 N HCl, and the resulting precipitate was collected by filtration, washed with water, and dried; yields = 70−80%.

*5-Chloro-2-(3-methylbutyrylamino)benzenesulfonamide (**14c**).* Starting from 2-amino-5-chlorobenzenesulfonamide (**13c**); mp: 175−177 °C. Anal. ($C_{11}H_{15}ClN_2O_3S$) C, H, N, S.

*7-Chloro-3-isobutyl-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**15c**).* The solution of 5-chloro-2-(3-methylbutyrylamino)benzenesulfonamide (**14c**) (2.2 mmol) in a 1% w/v aqueous solution of NaOH (32 mL) was refluxed for 30 min. After cooling, the solution was adjusted to pH 6−7 by means of 1 N HCl. The resulting precipitate was collected by filtration, washed with water, and dried (yields: 85%); mp: 235−238 °C; IR (KBr): 3283, 3191, 3118, 2959, 1622, 1610, 1580, 1525, 1483, 1289, 1157, 1143, 1109 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.96 (d, 6H, 2 × C*H*$_3$), 2.13 (m, 1H, C*H*), 2.41 (m, 2H, C*H*$_2$), 7.37 (d, 1H, 5-*H*), 7.73 (d, 1H, 6-*H*), 7.84 (s, 1H, 8-*H*), 12.12 (bs, 1H, N-*H*). Anal. ($C_{11}H_{13}ClN_2O_2S$) C, H, N, S.

*R/S-7-Chloro-3-isopropylsulfinyl-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**16**).* The suspension of 7-chloro-3-isopropylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**12c**) (0.5 g, 1.72 mmol) in an aqueous solution of sodium carbonate (0.22 g/25 mL) was supplemented under stirring with 2 N NaOH until complete dissolution. The alkaline solution was then supplemented, under stirring at room temperature, with bromine (0.1 mL). After 10 min, the mixture was adjusted to pH 2 by means of 12 N HCl, and the resulting precipitate was collected by filtration, washed with water, and suspended in methanol (15 mL) under stirring during 1 h. The insoluble material was collected by filtration, washed with methanol, and dried (0.40 g, 76%); mp: 257−260 °C; IR (KBr): 3146, 2977, 1606, 1594, 1570, 1507, 1476, 1326, 1172, 1060, 1027 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.19 (d, 3H, C*H*$_3$-A), 1.38 (d, 3H, C*H*$_3$-B) 3.40 (m, 1H, C*H*), 7.83−7.95 (m, 3H, 5-*H*, 6-*H*, 8-*H*), 12.70 (bs, 1H, N-*H*). Anal. ($C_{10}H_{11}ClN_2O_3S$) C, H, N, S.

Conformational Studies. Quantum mechanical calculations at the HF/6-31G* level have been used to characterize the intrinsic conformational preferences of **10c**, **12c**, **4**, and **15c** in the gas phase. All calculations have been performed with the Gaussian03 program. Starting from the crystal structure of **10c**,[23] **12c**,[24] and **4**,[22] a conformational scan was performed by varying both so-called dihedral angles T1 and T2 from 0 to 360°. In the case of **15c**, a QM-minimized structure was first calculated as a starting point, because no crystal structure was available.

Measurements of Insulin Release from Incubated Rat Pancreatic Islets. The method used to measure insulin release from incubated rat pancreatic islets was previously described.[14,17,26,30]

Measurement of the Contractile Activity in Rat Aorta. The method used to measure the myorelaxant effect of the drugs on 30 mM KCl-precontracted rat aortic rings was previously described.[14,17,26,30]

Measurement of the Myorelaxant Activity on Rat Uterus. *First Model: Contractions Induced by Bolus of Oxytocin Injected in the Superfusion System.* Fed Wistar rats (150−200 g) were treated the day before killing with diethylstilboestrol diproprionate [i.m. injection of 0.1 mL/100 g of a 1 mg/mL oily solution of diethylstilboestrol diproprionate (Sigma)]. The rats were anaesthetized and then sacrificed. The two uterine horns were removed, cleared of adhering fat and connective tissue, and separated. Each horn was superfused with a Tyrode solution (in mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, glucose 5.6) bubbled continuously with a mixture of O_2 (95%) and CO_2 (5%). The superfusate was maintained at 37 °C. After a stabilization period of 30 min, injection of 20 mU oxytocin (200 μL of a 0.1 U/mL solution of the hormone in 9 ‰ NaCl) in the superfusion channel was repeated at 10 min intervals until the recorded contractions (AUC) were constant. The mean of the three last injections gave the 100% of the contractile response to oxytocin. For each drug concentration added in the medium (10, 50, and 100 μM), injection of 20 mU oxytocin was repeated at least three times. The contractile responses recorded in the presence of different drug concentrations added in the superfusate medium (mean of the three AUC) were expressed as a percentage of the reference value (contractile response to oxytocin in the absence of drug).

Second Model: Contractions Induced by a Continuous Superfusion of Oxytocin. After a stabilization period of 30 min, each horn was superfused with Tyrode solution containing oxytocin at a low concentration (50 mU/L). After a period of several minutes, the uterine contractions were recorded during 15 min. The mean of three successive contractions (AUC: area under the curve) provided 100% of the contractile response to oxytocin. This sequence of events was repeated with a superfusate solution containing oxytocin (50 mU/L) and the tested drug at increasing concentrations. The contractile responses recorded in the presence of different drug concentrations (mean of three AUC) were expressed as a percentage of the reference value (contractile response to oxytocin in the absence of drug). For several drugs, tested at increasing concentrations, the experiment was repeated in the continuous presence of 1 or 10 μM glibenclamide. Results were expressed as the percentage of residual contraction, and an EC_{50} value was calculated corresponding to the drug concentration giving 50% residual contraction induced by oxytocin.

Measurements of ^{86}Rb Outflow from Rat Perifused Pancreatic Islets and Rat Aortic Rings. The methods used for measuring ^{86}Rb (^{42}K substitute) outflow from prelabeled and perifused rat pancreatic islets or from prelabeled and perifused rat aortic rings were previously described.[17,26,30]

Measurements of ^{45}Ca Outflow and Insulin Release from Perifused Rat Pancreatic Islets. The methods used for simultaneously measuring ^{45}Ca outflow and insulin release from prelabeled and perifused rat pancreatic islets were previously described.[26,30]

Measurements of Cytosolic Ca^{2+} Concentration from Isolated Rat Pancreatic Islets Cells. The method used for measuring the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) from single islet cells was previously described.[26,30]

Ionization Constants. The pK_a values of the compounds were determined by means of UV 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.[31]

■ ASSOCIATED CONTENT

Supporting Information. General synthetic pathways to 7-substituted 3-isopropoxy-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**10**) and 7-substituted 3-isobutyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**15**); synthesis of compounds **10a**,**b**, **10d**−**f**, **10i**, **12a**,**b**, **12d**,**e**, **12g**−**k**, **14a**,**b**, **14d**, **15a**,**b**, and **15d**; elemental analysis results for the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 32-4-3664365. Fax: 32-4-3664362. E-mail: B.Pirotte@ulg.ac.be.

Author Contributions

⊥These authors contributed equally to the work.

■ 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 and P. Lebrun is a Research Director. The authors gratefully acknowledge the technical assistance of S. Counerotte, Y. Abrassart, F. Leleux, A.-M. Vanbellinghen, and A. Van Praet.

■ ABBREVIATIONS

K_{ATP} channel, ATP-sensitive potassium channel; Kir, inwardly rectifying potassium channel; PCO, potassium channel opener; SUR, sulfonylurea receptor

■ REFERENCES

(1) Burke, M. A.; Mutharasan, R. K.; Ardehali, H. The Sulfonylurea Receptor, An Atypical ATP-binding Cassette Protein, and Its Regulation of the K_{ATP} Channel. *Circ. Res.* **2008**, *102*, 164–176.

(2) Seino, S. ATP-sensitive Potassium Channels: a Model of Heteromultimeric Potassium Channel/Receptor Assemblies. *Annu. Rev. Physiol.* **1999**, *61*, 337–362.

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

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

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

(6) Ye, B.; Kroboth, S. L.; Pu, J. L.; Sims, J. J.; Aggarwal, N. T.; McNally, E. M.; Makielski, J. C.; Shi, N. Q. Molecular Identification and Functional Characterization of a Mitochondrial Sulfonylurea Receptor 2 Splice Variant Generated by Intraexonic Splicing. *Circ. Res.* **2009**, *105*, 1083–1093.

(7) Sebille, S.; de Tullio, P.; Boverie, S.; Antoine, M. H.; Lebrun, P.; Pirotte, B. Recent Developments in the Chemistry of Potassium Channel Activators: the Cromakalim Analogs. *Curr. Med. Chem.* **2004**, *11*, 1213–1222.

(8) Mannhold, R. K_{ATP} Channel Openers: Structure-activity Relationships and Therapeutic Potential. *Med. Res. Rev.* **2004**, *24*, 213–266.

(9) Garlid, K. D.; Paucek, P.; Yarov-Yarovoy, V.; Murray, H. N.; Darbenzio, R. B.; D'Alonzo, A. J.; Lodge, N. J.; Smith, M. A.; Grover, G. J. Cardioprotective Effect of Diazoxide and Its Interaction With Mitochondrial ATP-sensitive K^+ Channels. Possible Mechanism of Cardioprotection. *Circ. Res.* **1997**, 1072–1082.

(10) Moreau, C.; Prost, A. L.; Dérand, R.; Vivaudou, M. SUR, ABC Proteins Targeted by K_{ATP} Channel Openers. *J. Mol. Cell. Cardiol.* **2005**, *38*, 951–963.

(11) Abdallah, Y.; Wolf, C.; Meuter, K.; Piper, H. M.; Reusch, H. P.; Ladilov, Y. Preconditioning with Diazoxide Prevents Reoxygenation-induced Rigor-type Hypercontracture. *J. Mol. Cell Cardiol.* **2010**, *48*, 270–276.

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

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

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

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

(16) 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.

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

(18) Schwanstecher, M.; Schwanstecher, C.; Dickel, C.; Chudziak, F.; Moshiri, A.; Panten, U. Location of the Sulphonylurea Receptor at the Cytoplasmic Face of the Beta-cell Membrane. *Br. J. Pharmacol.* **1994**, *113*, 903–911.

(19) Stephan, D.; Salamon, E.; Weber, H.; Russ, U.; Lemoine, H.; Quast, U. K_{ATP} Channel Openers of the Benzopyran Type Reach their Binding Site via the Cytosol. *Br. J. Pharmacol.* **2006**, *149*, 199–205.

(20) Nielsen, F. E.; Bodvarsdottir, T. B.; Worsaae, A.; MacKay, P.; Stidsen, C. E.; Boonen, H. C.; Pridal, L.; Arkhammar, P. O.; Wahl, P.; Ynddal, L.; Junager, F.; Dragsted, N.; Tagmose, T. M.; Mogensen, J. P.; Koch, A.; Treppendahl, S. P.; Hansen, J. B. 6-Chloro-3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide Derivatives Potently and Selectively Activate ATP Sensitive Potassium Channels of Pancreatic β-Cells. *J. Med. Chem.* **2002**, *45*, 4171–4187.

(21) Dupont, L.; Pirotte, B.; de Tullio, P. 7-Iodo-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1999**, *C55*, 1152–1154.

(22) Dupont, L; Pirotte, B .; de Tullio, P. Crystal Structure of 7-Chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide, $C_{10}H_{12}ClN_3O_2S$. *Z. Kristallogr. NCS 220* **2005**, 353–354.

(23) Dupont, L .; Boverie, S.; Pirotte, B.; de Tullio, P. Crystal Structure of 7-Chloro-3-isopropoxy-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Monohydrate, $C_{10}H_{13}ClN_2O_4S \cdot H_2O$. *Z. Kristallogr., New Cryst. Struct.* **2005**, *220*, 565–566.

(24) Dupont, L.; Boverie, S.; de Tullio, P.; Pirotte, B. Crystal Structure of 7-Chloro-3-isopropylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide, $C_{10}H_{11}ClN_2O_2S_2$. *Z. Kristallogr., New Cryst. Struct.* **2005**, *220*, 467–468.

(25) Lachenicht, S.; Fischer, A.; Schmidt, C.; Winkler, M.; Rood, A.; Lemoine, H.; Braun, M. Synthesis of Modified 4*H*-1,2,4-Benzothiadiazine-1,1-dioxides and Determination of their Affinity and Selectivity for Different Types of K_{ATP} Channels. *Chem. Med. Chem.* **2009**, *4*, 1850–1858.

(26) 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.

(27) Antoine, M. H.; Berkenboom, G.; Fang, Z. Y.; Fontaine, J.; Herchuelz, A.; Lebrun, P. Mechanical and Ionic Response of Rat Aorta to Diazoxide. *Eur. J. Pharmacol.* **1992**, *216*, 299–306.

(28) Pecorari, P.; Albasini, A.; Raffa, L. 1,2,4-Benzothiadiazines. XLVIII. Heat-induced Transformations of 3-Alkoxy Derivatives of 1,2,4-Benzothiadiazine-1,1-dioxides. *Il Farmaco, Ed. Sci.* **1973**, *28*, 203–213.

(29) Raffa, L.; Di Bella, M.; Melegari, M.; Vampa, G. 1,2,4-Benzothiadiazine Derivatives. XX. Synthesis of 3-Thio-1,2,4-benzothiadiazine 1,1-Dioxide and its Derivatives. *Il Farmaco, Ed. Sci.* **1962**, *17*, 320–330.

(30) Becker, B.; Antoine, M. H.; Nguyen, Q. A.; Rigo, B.; Cosgrove, K. E.; Barnes, P. D.; Dunne, M. J.; Pirotte, B.; Lebrun, P. Synthesis and Characterization of a Quinolinonic Compound Activating ATP-sensitive K^+ Channels in Endocrine and Smooth Muscle Tissues. *Br. J. Pharmacol.* **2001**, *134*, 375–385.

(31) Albert, A.; Serjeant, E. P. *The Determination of Ionization Constants*; Chapman & Hall: London, 1971; pp 44−64.