3-Alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides Structurally Related to Diazoxide and Pinacidil as Potassium Channel Openers Acting on Vascular Smooth Muscle Cells: Design, Synthesis, and Pharmacological Evaluation

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A series of 3-alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides structurally related to diazoxide and pinacidil were synthesized and tested as possible K_{ATP} channel openers on isolated pancreatic endocrine tissue as well as on isolated vascular, intestinal, and uterine smooth muscle. In contrast to previously described 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides, most of the new compounds were found to be poorly active on B-cells but exhibited clear vasorelaxant properties. 3-(3,3-Dimethyl-2-butylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**4d**) and 7-chloro-3-(3,3-dimethyl-2-butylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**5d**), two compounds bearing the alkyl side chain of pinacidil, were found to be the most active representatives of their respective series on rat aorta rings. 3-Cycloalkylalkylamino- and 3-aralkylamino-7-chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides also expressed myorelaxant activity on electrically stimulated guinea pig ileum and on oxytocin-induced contractions of the rat uterus. Further biological investigations (^{86}Rb efflux measurements, vasodilator potency on 30 and 80 mM KCl-induced contractions in the absence and presence of glibenclamide) revealed that compounds **4d** and **5d**, but not compound **5f**, expressed the pharmacological profile of classical K_{ATP} channel openers. In conclusion, by changing the position of the nitrogen atom in the pyridine ring, we now have obtained a family of drugs expressing an opposite tissue selectivity. Taken as a whole, the present findings also suggest that 3-alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides such as **4c**, **4d**, **5c**, and **5d** may be considered as new examples of K_{ATP} channel openers expressing a pharmacological profile similar to that of pinacidil and diazoxide.

Introduction

Potassium channels regulated by changes in intracellular levels of adenosine triphosphate (ATP-sensitive K^+ channels or K_{ATP} channels) and which link the membrane potential to the metabolic state of the cell have been described in a wide range of cell types including pancreatic B-cells[1] and smooth muscle cells.[2] Their presence was suggested in vascular as well as nonvascular tissues such as gastrointestinal, urinary, and uterine smooth muscles.[3]

Potassium channel openers (PCOs), acting mainly on K_{ATP} channels, may be viewed as a class of pharmacological agents with important therapeutic promise. However, due to the ubiquitous distribution of K_{ATP} channels, the development of novel PCOs should be linked to the expression of a high selectivity for a single channel subtype located on a single target tissue.

We recently reported that 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **3**, structurally related to both diazoxide (**1**) and pinacidil (**2**) (Figure 1), were powerful inhibitors of insulin release from rat pancreatic B-cells.[4,5] From further pharmacological evaluations, 3-(3-methyl-2-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (BPDZ 44) (**3**: R = $CH(CH_3)CH(CH_3)_2$) and 3-(3,3-dimethyl-2-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (BPDZ 62) (**3**: R = $CH(CH_3)C(CH_3)_3$) were identified as pancreatic B-cell K_{ATP} channel activators.[6−9] Moreover, 3-(2-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (BPDZ 42) (**3**: R = $CH(CH_3)CH_2CH_3$) and BPDZ 44 were also found to be less active as vasodilators than diazoxide and their corresponding 3-alkylamino-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide analogues on vascular smooth muscle tissue.[5,10] Thus, the bioisosteric replacement of the chlorobenzenic ring with a pyridinic nucleus led to compounds showing a marked selectivity for the pancreatic endocrine tissue.

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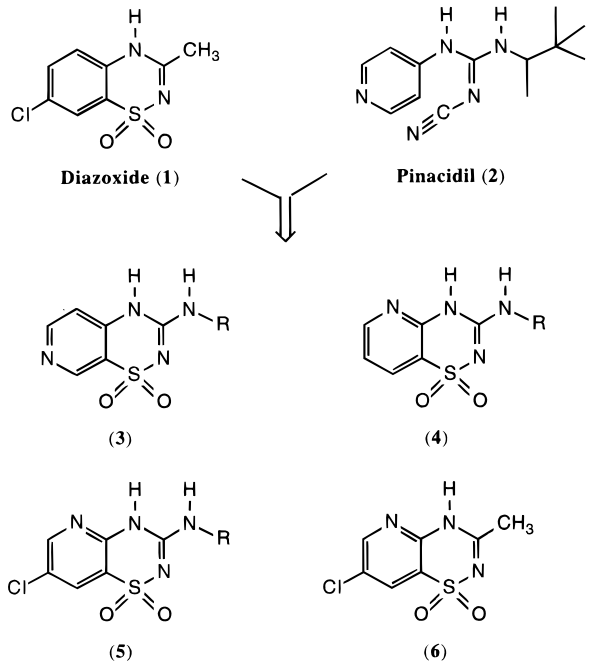


Figure 1. Design of 4*H*-1,2,4-pyridothiadiazine 1,1-dioxides as PCOs related to diazoxide (**1**) and pinacidil (**2**): 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **3**, 3-alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **4**, 3-alkylamino-7-chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **5**, and 7-chloro-3-methyl-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**6**).

In the search for original pyridothiadiazine dioxides acting as PCOs, we examined two novel series of pyridothiadiazine dioxides: namely 3-alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **4** and 3-alkylamino-7-chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **5** (Figure 1). These compounds may be regarded as isomers of 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides ("7-aza" compounds) bearing the nitrogen atom of the pyridine ring in the 5-position of the heterocycle ("5-aza" compounds) instead of the 7-position. Introduction of a chlorine atom in the 7-position of **5** should also confer to such pyridothiadiazines a better structural analogy with the 7-chlorobenzothiadiazine dioxide PCO prototype diazoxide (**1**) (Figure 1). Moreover, particular attention was paid to the possible role on biological efficiency of the absolute geometry associated to the first carbon atom of the hydrocarbon side chain located in the 3-position. 7-Chloro-3-methyl-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**6**), which corresponds to "5-aza"-diazoxide, was also prepared (Figure 1).

The new compounds were examined as putative PCOs on rat pancreatic islets as well as on three different smooth muscle preparations: rat aorta, guinea pig ileum, and rat uterus.

Chemistry

3-Alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **4** were obtained from a nucleophilic substitution reaction conducted with appropriate alkyl/aralkylamines on a 3-(1*H*-imidazol-1-yl)-substituted intermediate **8** (Scheme 1). The latter was obtained from the reaction of 2-aminopyridine-3-sulfonamide (**7**) with thiocarbonyldiimidazole. Such a reaction with *o*-aminoarylsulfonamides and thiocarbonyldiimidazole has already been described and was found to yield 3-thioxo-substituted or 3-(1*H*-imidazol-1-yl)-substituted arylthiadiazine dioxides, according to the starting materials and to the experimental conditions used.[11] In the presence of an excess of the reagent in dioxane−DMF solution, the 3-(1*H*-imidazol-1-yl)-substituted derivative **8** was obtained. Susceptibility of the latter compound toward nucleophilic substitution may be compared to that of a 3-methylsulfanyl-substituted pyridothiadiazine dioxide and was advantageously used in the last step of the synthesis of **4**.

2-Amino-5-chloropyridine-3-sulfonamide (**10**) was obtained from the reaction of 2-amino-5-chloropyridine (**9**) with chlorosulfonic acid, followed by reaction of the sulfonyl chloride intermediate with aqueous ammonia (Scheme 2). Intermediate **10** gave access to 3-alkylamino-7-chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **5** via the formation of the 3-(1*H*-imidazol-1-yl)-substituted intermediate **11** and reaction of the latter with alkyl/aralkylamines, as described in Scheme 1 for 3-alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **4**. 7-Chloro-3-methyl-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**6**) resulted from the ring closure of **10** with acetic anhydride according to a previously reported synthetic procedure (Scheme 2).[5]

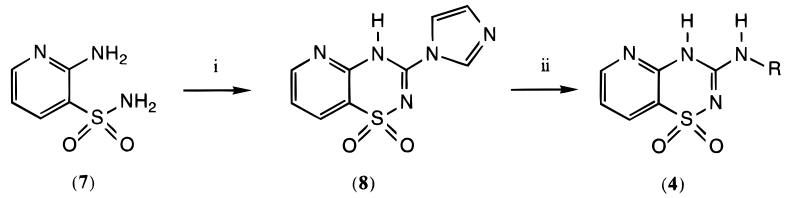
Results and Discussion

The alkylamino side chain introduced in the 3-position of the 4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **4** and **5** was chosen from the best (active on B-cell K_{ATP} channels) hydrocarbon side chains (isopropyl, 2-butyl, 3-methyl-2-butyl, 3,3-dimethyl-2-butyl) deduced from our peliminary investigations with the "7-aza" compounds.[4,5] Moreover, the (*R*)- or (*S*)-1-cyclohexylethyl as well as the (*R*)- or (*S*)-1-phenylethyl moieties were chosen as examples of chiral side chains of known absolute stereochemistry. The cyclohexylmethyl and benzyl moieties are structurally related chains devoid of a chiral center.

Tables 1 and 2 report the biological results obtained with the pyridothiadiazine dioxides in three different in vitro conditions. The first pharmacological model examines the compounds as inhibitors of the insulin releasing process from rat pancreatic islets incubated in the presence of an insulinotropic glucose concentration (16.7 mM). The two other in vitro screening models examine the compounds as myorelaxants on K^+-depolarized rat aorta and on electrically stimulated guinea pig ileum, respectively. The activity of the drugs in the two latter models was expressed as the ED_{50} value which corresponds to the drug concentration giving 50% relaxation of the precontracted smooth muscle preparation.

3-Alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides ("5-aza" compounds) **4** bearing the same aminoalkyl side chains as the best "7-aza" compounds **3** on B-cells (isopropyl, 2-butyl, 3-methyl-2-butyl, 3,3-dimethyl-2-butyl) were examined as putative inhibitors of the insulin releasing process from rat pancreatic islets. As observed in Table 1, none of the "5-aza" compounds was found to express a strong activity on B-cells at a 50 μM concentration. However, these compounds tested on rat aorta rings exhibited a clear vasorelaxant activity. Some

Scheme 1[a]

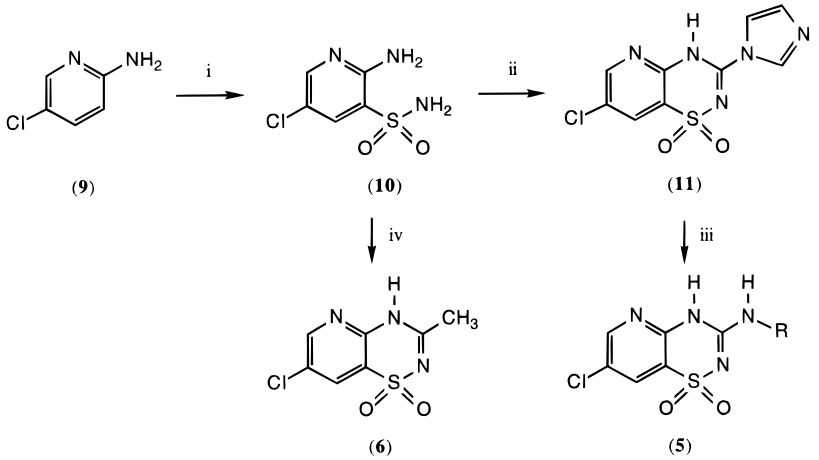


(**7**) (**8**) (**4**)

(**4a**): R = $CH(CH_3)_2$
(**4b**): R = $CH(CH_3)CH_2CH_3$
(**4c**): R = $CH(CH_3)CH(CH_3)_2$
(**4d**): R = $CH(CH_3)C(CH_3)_3$
(**4e**): R = $CH_2C_6H_{11}$
(**4f**): R = (R)-$CH(CH_3)C_6H_{11}$
(**4g**): R = (S)-$CH(CH_3)C_6H_{11}$
(**4h**): R = $CH_2C_6H_5$
(**4i**): R = (R)-$CH(CH_3)C_6H_5$
(**4j**): R = (S)-$CH(CH_3)C_6H_5$

[a] (i) Thiocarbonyldiimidazole, dioxane, DMF, Δ; (ii) R-NH_2, Δ.

Scheme 2[a]



(**9**) (**10**) (**11**) (**6**) (**5**)

(**5a**): R = $CH(CH_3)_2$
(**5b**): R = $CH(CH_3)CH_2CH_3$
(**5c**): R = $CH(CH_3)CH(CH_3)_2$
(**5d**): R = $CH(CH_3)C(CH_3)_3$
(**5e**): R = $CH_2C_6H_{11}$
(**5f**): R = (R)-$CH(CH_3)C_6H_{11}$
(**5g**): R = (S)-$CH(CH_3)C_6H_{11}$
(**5h**): R = $CH_2C_6H_5$
(**5i**): R = (R)-$CH(CH_3)C_6H_5$
(**5j**): R = (S)-$CH(CH_3)C_6H_5$

[a] (i) 1. $ClSO_3H$, 2. NH_4OH; (ii) thiocarbonyldiimidazole, dioxane, DMF, Δ; (iii) R-NH_2, Δ; (iv) $(CH_3CO)_2O$, Δ.

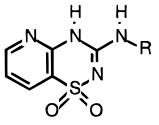
of them were found to be equally active or even more potent than diazoxide but less potent than pinacidil. For compounds **4a**−**4d**, the myorelaxant activity increased with the size and branching of the alkyl chain, the most potent drug being **4d** (BPDZ 79) with an alkyl side chain identical to that of pinacidil. As a result, such an observation is in good accordance with SAR studies previously reported for cyanoguanidines.[12]

By further increasing the size of the alkyl group in the 3-position (see compounds **4e**−**4j**, Table 1), the myorelaxant activity did not increase. It was also found that the *S*-isomer of the 3-(1-phenylethylamino)-substituted compound **4j** was more potent than the corresponding *R*-isomer **4i**. The latter observation is in contradistinction with previous studies on cyanoguanidines showing that the *R*-(−)-isomers (i.e. (*R*)-(−)-pinacidil and its 3-pyridyl analogue) are usually stronger myorelaxants and/or better PCOs than their corresponding *S*-(+)-antipodes.[13−15]

The presence of K_{ATP} channels in other smooth muscle tissues such as the guinea pig ileum has previously been suggested because PCOs were found to exert glibenclamide-sensitive myorelaxant properties.[16,17] As observed with diazoxide, the 3-alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides were also inactive on electrically stimulated contractions of the guinea pig ileum. By contrast, pinacidil was found to be active on guinea pig ileum with an ED_{50} value below 10 μM, albeit its efficacy was about 20 times less pronounced on the ileum than on aorta rings.

From biological results collected with 3-alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides ("5-aza"

Table 1. Effects of 3-Alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets and on Contractile Activity of K^+-Depolarized Rat Aorta Rings and Electrically Stimulated Guinea Pig Ileum Segments



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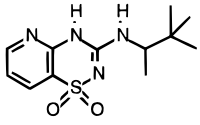
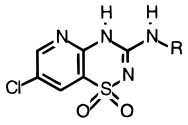


BPDZ 79 (**4d**)

		rat pancreatic B-cells % RIS[a] (mean ± SEM (*n*))		ED_{50} (μM) (mean ± SEM (*n*))		
compd	R	50 μM	10 μM	rat aorta rings[b]	guinea pig ileum[c]	ee[d] (%)
4a	$CH(CH_3)_2$	78.7 ± 5.4 (14)	nd[e]	131.8 ± 42.9 (6)	>300 (2)	
4b	$CH(CH_3)CH_2CH_3$	85.9 ± 4.6 (16)	nd	50.9 ± 24.9 (6)	nd	
4c	$CH(CH_3)CH(CH_3)_2$	87.9 ± 5.4 (16)	nd	27.1 ± 9.1 (10)	>300 (2)	
4d	$CH(CH_3)C(CH_3)_3$	98.9 ± 2.9 (32)	nd	7.5 ± 3.2 (19)	>300 (4)	
4e	$CH_2C_6H_{11}$	94.2 ± 3.5 (16)	nd	45.7 ± 8.9 (6)	145.0 ± 56.0 (4)	
4f	*R*-$CH(CH_3)C_6H_{11}$	79.9 ± 4.1 (16)	nd	29.2 ± 7.8 (6)	>300 (5)	98.6
4g	*S*-$CH(CH_3)C_6H_{11}$	76.2 ± 3.5 (16)	nd	25.2 ± 12.3 (6)	>300 (7)	95.6
4h	$CH_2C_6H_5$	86.2 ± 2.6 (24)	nd	21.9 ± 3.1 (6)	>300 (2)	
4i	*R*-$CH(CH_3)C_6H_5$	71.6 ± 3.3 (23)	nd	65.2 ± 19.4 (10)	>300 (2)	98.9
4j	*S*-$CH(CH_3)C_6H_5$	73.3 ± 3.7 (24)	nd	9.9 ± 3.7 (6)	>300 (2)	96.7
diazoxide		28.8 ± 2.4 (21)[f]	70.0 ± 3.6 (22)[f]	19.3 ± 1.5 (21)	>300 (7)	
pinacidil		92.1 ± 5.5 (21)[g]	96.0 ± 4.2 (20)[g]	0.5 ± 0.1 (28)	9.2 ± 2.2 (18)	
BPDZ 44		7.1 ± 0.6 (14)[h]	26.8 ± 1.8 (21)[h]	154.4 ± 14.5 (8)	>300 (6)	

[a] % RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose. Effects of the drugs at 50 and 10 μM. [b] ED_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings. [c] ED_{50}: drug concentration giving 50% relaxation of the electrically stimulated contraction of guinea pig ileum segments. [d] ee: enantiomeric excess. [e] nd: not determined. Published results: [f] ref 4; [g] ref 8; [h] ref 10.

Table 2. Effects of 3-Alkylamino-7-chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets and on Contractile Activity of K^+-Depolarized Rat Aorta Rings and Electrically Stimulated Guinea Pig Ileum Segments



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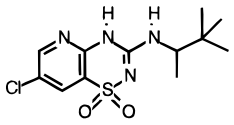
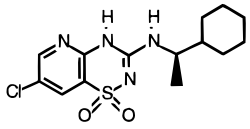
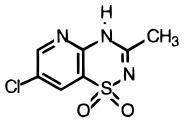


BPDZ 83 (**5d**)



BPDZ 109 (**5f**)



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		rat pancreatic B-cells % RIS[a] (mean ± SEM (*n*))		ED_{50} (μM) (mean ± SEM (*n*))		
compd	R	50 μM	10 μM	rat aorta rings[b]	guinea pig ileum[c]	ee[d] (%)
5a	$CH(CH_3)_2$	74.4 ± 4.6 (16)	nd[e]	14.6 ± 6.4 (6)	>300 (5)	
5b	$CH(CH_3)CH_2CH_3$	62.7 ± 2.0 (16)	nd	20.0 ± 4.3 (11)	nd	
5c	$CH(CH_3)CH(CH_3)_2$	59.3 ± 4.0 (24)	nd	10.6 ± 2.9 (16)	>300 (4)	
5d	$CH(CH_3)C(CH_3)_3$	41.5 ± 2.3 (23)	nd	2.3 ± 0.5 (14)	>300 (4)	
5e	$CH_2C_6H_{11}$	57.7 ± 3.1 (40)	76.8 ± 3.5 (24)	38.2 ± 4.4 (8)	17.4 ± 3.3 (6)	
5f	*R*-$CH(CH_3)C_6H_{11}$	6.7 ± 0.7 (28)	71.3 ± 5.5 (14)	3.5 ± 0.5 (16)	13.6 ± 1.8 (8)	97.0
5g	*S*-$CH(CH_3)C_6H_{11}$	11.8 ± 1.0 (20)	71.9 ± 4.1 (16)	3.8 ± 0.4 (10)	20.4 ± 2.5 (8)	98.0
5h	$CH_2C_6H_5$	74.1 ± 3.1 (15)	nd	43.6 ± 2.8 (10)	nd	
5i	*R*-$CH(CH_3)C_6H_5$	52.3 ± 2.3 (16)	nd	38.8 ± 2.9 (10)	nd	97.7
5j	*S*-$CH(CH_3)C_6H_5$	45.9 ± 3.5 (16)	nd	21.2 ± 1.6 (10)	nd	98.6
6		87.4 ± 4.2 (15)	nd	>300 (4)	nd	
diazoxide		28.8 ± 2.4 (21)	70.0 ± 3.6 (22)	19.3 ± 1.5 (21)	>300 (7)	
pinacidil		92.1 ± 5.5 (21)	96.0 ± 4.2 (20)	0.5 ± 0.1 (28)	9.2 ± 2.2 (18)	
BPDZ 44		7.1 ± 0.6 (14)	26.8 ± 1.8 (21)	154.4 ± 14.5 (8)	>300 (6)	

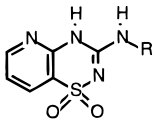
[a–e] See corresponding footnotes to Table 1.

compounds), it can be concluded that this novel series of pyridothiadiazine dioxides developed a biological activity different from that of the previously reported 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides ("7-aza" compounds). Indeed, by changing the position of the nitrogen atom in the pyridine ring, we now have obtained a family of drugs expressing an opposite tissue selectivity.

The introduction of a chlorine atom in the 7-position of 3-alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides (giving the "5-aza-7-chloro" compounds **5a**−**5j**) yielded a general increase in the activity of the drugs on the three in vitro pharmacological models. Thus, a more pronounced activity was observed on pancreatic B-cells. The potency appeared to increase slightly with the size and branching of the alkyl chain (see **5a**−**5d**, Table 2). Longer chains such as a 1-cyclohexylethyl moiety of either the *R*- (**5f**) or *S*-geometry (**5g**) were found to further increase the efficacy of the drugs as inhibitors of the insulin releasing process. In such a case, no marked difference was observed between the two enantiomeric antipodes **5f** and **5g**.

The "7-aza" analogues of compounds **5e**−**5j** were also prepared and were found to be generally less active on B-cells than their corresponding "5-aza-7-chloro" counterparts (data not shown). Such an observation with "7-

Table 3. Effects of Selected 3-Alkylamino-4*H*-1,2,4-pyridothiadiazine 1,1-Dioxides on Oxytocin-Induced Contractions of Rat Uterus



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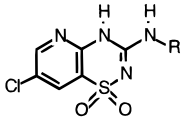

5

		rat uterus % of contraction (mean ± SEM (*n*))[a]		
compd	R	10 μM	50 μM	100 μM
4d	$CH(CH_3)C(CH_3)_3$	93.8 ± 4.9 (8)	68.0 ± 3.5 (8)	57.5 ± 4.9 (8)
5d	$CH(CH_3)C(CH_3)_3$	97.6 ± 3.5 (8)	67.9 ± 2.5 (8)	51.0 ± 2.3 (8)
4j	*S*-$CH(CH_3)C_6H_5$	93.8 ± 3.0 (4)	74.8 ± 0.7 (4)	59.2 ± 1.8 (4)
5e	$CH_2C_6H_{11}$	96.3 ± 3.4 (4)	95.1 ± 6.4 (4)	90.4 ± 5.6 (4)
5f	*R*-$CH(CH_3)C_6H_{11}$	70.6 ± 2.9 (4)	50.9 ± 2.4 (4)	22.9 ± 7.9 (4)
5g	*S*-$CH(CH_3)C_6H_{11}$	84.9 ± 5.0 (4)	71.4 ± 11.0 (4)	43.7 ± 10.9 (4)
5h	$CH_2C_6H_5$	108.2 ± 2.9 (4)	98.9 ± 4.3 (4)	71.9 ± 6.9 (4)
5i	*R*-$CH(CH_3)C_6H_5$	88.4 ± 4.2 (4)	59.4 ± 3.9 (4)	32.6 ± 4.3 (4)
5j	*S*-$CH(CH_3)C_6H_5$	91.0 ± 3.9 (4)	79.3 ± 4.8 (4)	41.1 ± 5.7 (4)
diazoxide		93.8 ± 2.2 (4)	76.3 ± 4.9 (4)	67.7 ± 4.0 (4)
pinacidil		58.1 ± 4.4 (4)	35.5 ± 2.9 (4)	38.1 ± 2.2 (4)
fenoterol		38.4 ± 3.1 (4)	36.3 ± 2.2 (4)	35.5 ± 2.0 (4)

[a] Comparison with diazoxide, pinacidil, and fenoterol. Percentage of contraction of the rat uterine smooth muscle induced by 20 mU oxytocin in the presence of 10, 50, and 100 μM of drugs. The contraction of the muscle in the same experimental conditions but in the absence of drugs is referenced as 100%.

aza" compounds, but not with "5-aza-7-chloro" compounds, is in accordance with previous works indicating that a more bulky substituent was responsible for a loss of activity on the pancreatic tissue.[4]

3-Alkylamino-7-chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides were also found to express a strong myorelaxant activity on vascular smooth muscle cells. As previously observed with "5-aza" compounds devoid of a chlorine atom in the 7-position, the efficacy on rat aorta appeared to increase with the size and branching of the alkyl chain in the 3-position (see **5a**−**5d**, Table 2). The most active compound of this series, BPDZ 83 (**5d**), bears again the alkyl side chain of pinacidil.

The most potent drugs on pancreatic B-cells (**5f** and **5g**) were also found to express a strong myorelaxant activity on K^+-depolarized rat aorta rings, but no difference was observed between the two enantiomers. As already mentioned, such an observation distinguishes this series from the cyanoguanidine series of PCOs (pinacidil-like) for which a stronger activity was usually observed with representatives of the *R*-absolute geometry associated to the alkyl side chain.

Finally, some 3-alkylamino-7-chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides were also identified as strong myorelaxants in the guinea pig ileum. Thus, compared to "5-aza" compounds **4**, the introduction of a chlorine atom in the 7-position generally improved the efficacy of the drugs on the smooth muscle preparations. However, the most active compound on rat aorta rings, compound **5d**, was devoid of activity on electrically stimulated contractions of the guinea pig ileum.

Compound **6**, the "5-aza" analogue of diazoxide, was found to be inactive on rat pancreatic B-cells and rat aorta rings. One explanation could be that, in contrast to diazoxide, the "5-aza" compound **6** could exist, at least in part, as an ionized species at the physiological pH of 7.4. Indeed, the pK_a values of **4a**, **5a**, **6**, and diazoxide determined by UV spectrophotometry amounted to 8.62, 8.03, 7.58, and 8.62,[18] respectively. Thus, it can be expected that **4a** and **5a**, taken as representatives of 3-alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides with or without a chlorine atom in the 7-position, as well as diazoxide should exist in aqueous solution at pH 7.4 essentially as nonionized species. By contrast, the "5-aza" analogue of diazoxide **6** should exist in part as an anionic species as a result of the partial deprotonation of the hydrogen atom in the 4-position. Thus, it could be concluded that the replacement in the 3-position of an alkyl moiety with an aminoalkyl side chain was responsible for an increase of the pK_a value as a probable result of the nitrogen atom lone pair delocalization into the guanidinic system.

As previously described with 3-alkylamino-4*H*-pyrido-[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides,[5] the present data also indicate that the presence of a hydrogen atom in the 4-position is required for biological activity.

The most active compounds on the vascular and intestinal smooth muscle tissues were further examined on rat uterus as possible inhibitors of the contractions elicited by oxytocin (Table 3). In previous reports, PCOs such as cromakalim and pinacidil have been described as active myorelaxants on the oxytocin-induced contraction of rat uterus.[19,20] The effects of the latter drugs were partially antagonized by glibenclamide, suggesting that the myorelaxant activity of the drugs reflected their K_{ATP} channel opening properties.[20]

In the experimental conditions used for testing our drugs, the reference compound fenoterol (β-adrenomimetic drug used as a tocolytic agent) was found to inhibit oxytocin-induced (20 mU) contractions of the rat uterus up to a maximum of about 65% (Table 3). Thus, the drug was unable to completely suppress the uterine contractions even at the higher concentration (100 μM). Pinacidil was found to dose-dependently counteract the contractile effect of 20 mU oxytocin but also failed to completely suppress the contractile activity. Diazoxide was less active as a myorelaxant on rat uterus.

As shown in Table 3, the most active compounds on rat aorta rings from each series of pyridothiadiazine dioxides, bearing the 3,3-dimethyl-2-butyl side chain of

Table 4. Effects of Selected 3-Alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides on Contractile Activity of 80 and 30 mM K^+-Depolarized Rat Aorta Incubated in the Absence or Presence of 1 and 10 μM Glibenclamide

compd	rat aorta rings 80 mM KCl ED_{50} (μM) (mean ± SEM (*n*))	rat aorta rings 30 mM KCl ED_{50} (μM) (mean ± SEM (*n*))		
		0 μM glib	1 μM glib	10 μM glib
4c	>300 (4)	27.1 ± 9.1 (10)	nd[a]	nd
4d	>300 (6)	10.1 ± 7.3 (6)	65.2 ± 16.6 (6)	107.0 ± 16.0 (6)
5c	49.3 ± 9.5 (6)	8.2 ± 3.9 (6)	34.1 ± 11.7 (6)	45.1 ± 7.8 (6)
5d	33.0 ± 1.5 (6)	1.6 ± 0.4 (6)	22.6 ± 1.3 (6)	33.9 ± 1.8 (6)
5f	12.3 ± 2.5 (10)	3.7 ± 0.6 (12)	5.0 ± 0.7 (11)	8.6 ± 1.0 (12)
diazoxide	>300 (6)	19.5 ± 2.7 (6)	85.8 ± 22.2 (6)	163.4 ± 41.2 (6)
pinacidil	16.0 ± 1.4 (8)	0.2 ± 0.1 (8)	10.4 ± 1.1 (8)	42.5 ± 2.8 (8)

[a] nd: not determined; glib: glibenclamide.

pinacidil (compounds **4d** and **5d**), were found to exert poor myorelaxant properties on rat uterus. Compound **4j**, another "5-aza" derivative, also moderately reduced the ocytocin-induced contractile activity (Table 3).

The most active pyridothiadiazine dioxides on guinea pig ileum, i.e., the "5-aza-7-chloro" compounds with cycloalkylalkyl and aralkyl side chains (**5e**−**5j**), were also investigated on the uterine smooth muscle tissue. Among those, compound **5f**, active on both vascular and intestinal smooth muscle tissues, was also found to be efficacious in inhibiting the oxytocin-induced uterine contraction (about 80% inhibition at 100 μM). Three other compounds, **5g**, **5i**, and **5j**, also developed a noticeable activity.

To determine the mechanism of action of the active compounds on the vascular smooth muscle, we investigated a selection of pyridothiadiazine dioxides in three additional pharmacological conditions. First, the myorelaxant activity of **4c**, **4d**, **5c**, **5d**, and **5f** was examined versus diazoxide and pinacidil on rat aorta rings precontracted with a 80 mM KCl concentration (Table 4). Under such experimental conditions, the vasodilator efficiency of pure K^+ channel openers must theoretically be suppressed, or at least significantly reduced, compared to their activity on 30 mM KCl-induced contractions.[21] Conversely, drugs interfering directly at the level of the L-type Ca^{2+} channels, such as Ca^{2+} entry blockers, are expected to express the same myorelaxant efficacy on 30 and 80 mM KCl-precontracted aorta rings. As shown in Table 4, the myorelaxant activity of the selected compounds (expressed as the ED_{50} value) was strongly reduced in aorta rings contracted with 80 mM KCl, as expected for K^+ channel openers.[21] Pinacidil and diazoxide also exhibited a less pronounced myorelaxant activity on 80 mM KCl-depolarized rat aorta rings. Compound **5f** was found to be only 3 times less active under these experimental conditions.

Second, the compounds were also tested on 30 mM K^+-depolarized rat aorta rings incubated in the absence and presence of 1 or 10 μM glibenclamide, a blocker of K_{ATP} channels. As shown in Table 4, a dose-dependent decrease of the myorelaxant efficiency was observed when increasing the glibenclamide concentration in the medium. This pattern was noticed with the reference compounds and the selected pyridothiadiazine dioxides, except with **5f**. Such findings are consistent with the pharmacological profile of K_{ATP} channel openers. The particular behavior of **5f**, however, remains to be elucidated by further pharmacological investigations. It could be speculated that **5f** acts partly as a L-type Ca^{2+} channel blocker and partly as a K_{ATP} channel opener.

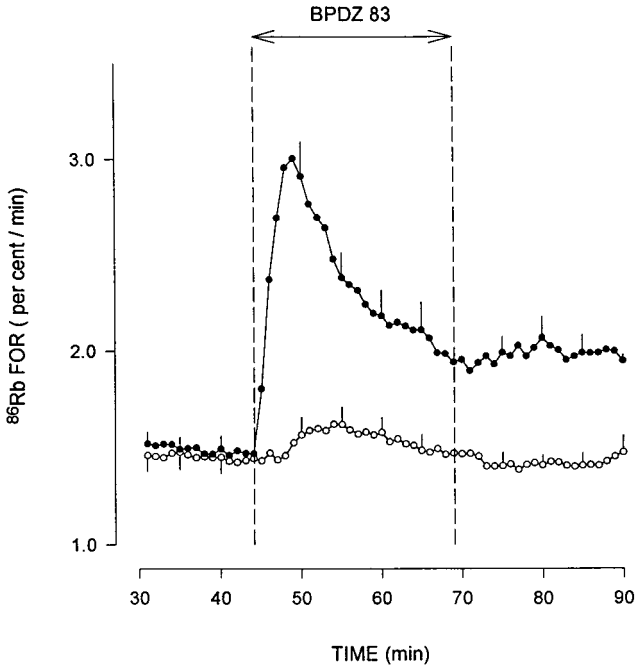

Figure 2. Effect of BPDZ 83 (**5d**) (100 μM) on ^{86}Rb outflow from aortic rings perifused in the absence (●) and presence (○) of glibenclamide (10 μM) throughout. The perifusate contained 25 mM K^+. Mean values (±SEM) refer to 4 individual experiments.

The nature of the side chain in the 3-position (bulky long substituent instead of short branched chain) could play a role in such a modification.

Third, for compound **5d** (BPDZ 83), the most powerful agent on vascular smooth muscle, the effect of a 100 μM concentration was characterized on the rate of ^{86}Rb outflow from rat aortic rings perfused in the absence and presence of glibenclamide (10 μM). As shown in Figure 2, BPDZ 83 (**5d**) (100 μM) provoked a marked and sustained increase in ^{86}Rb outflow from prelabeled and perfused rat aortic rings. When the same experiment was repeated in the presence of 10 μM glibenclamide throughout, the cationic response to BPDZ 83 (**5d**) represented only 10.9 ± 5.0% of that recorded in control conditions.

Experiments conducted with compound **4d** (BPDZ 79) (data not shown), pinacidil,[22] and diazoxide[21] gave essentially the same results.

Taken as a whole, the present findings suggest that 3-alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides such as **4c**, **4d**, **5c,** and **5d** may be considered as new examples of K_{ATP} channel openers expressing a pharmacological profile similar to that of pinacidil and

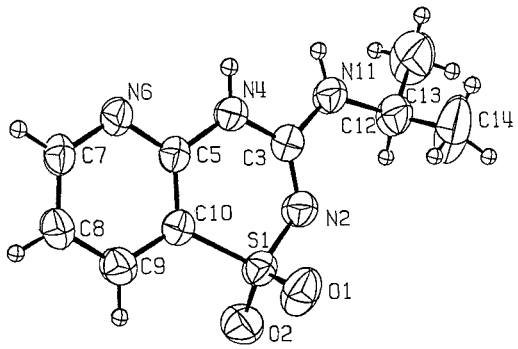

Figure 3. Molecular structure of **4a** with atom-labeling scheme. Thermal ellipsoids are shown at 50% probability levels. H atoms are drawn as small circles of arbitrary units.

diazoxide. The potent compounds **4d** and **5d**, representative of this new pyridothiadiazine dioxide series, are examples of drugs bearing the same alkyl side chain as pinacidil, supporting the view that such a short branched hydrocarbon chain may constitute the best choice for optimal binding site interactions.

Moreover, 3-alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides such as **4d** and **5d** express an interesting tissue selectivity for the vascular smooth muscle cells. Other compounds such as **5f** and **5g** are less selective since these drugs expressed a marked biological activity on endocrine cells as well as on the different smooth muscle preparations.

Previously described 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides ("7-aza" compounds) are known to be tissue selective for the endocrine pancreatic B-cells. We have characterized in the present study another class of pyridothiadiazine dioxides, namely 3-alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides ("5-aza" compounds) with or without a chlorine atom in the 7-position, expressing tissue selectivity and targeting the vascular smooth muscle cells. Thus, the position of the nitrogen atom in the pyridine ring may have important consequences upon the pharmacological profile of the drug. However, such a structural modification in the heterocyclic ring system did not appear to induce dramatic consequences on the conformational behavior of the new molecules. Indeed, we have determined the X-ray structure of **4a** (see Figure 3) and compared the crystallographic data with those previously obtained with 3-(isopropylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**3**: R = $CH(CH_3)_2$).[5] It was found that the N(2)−C(3) length of compound **4a** (1.312 Å) was shorter than its C(3)−N(4) length (1.361 Å), supporting the view that, as previously reported for 3-(isopropylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide,[5] the C=N double bond of the thiadiazine ring was located in the 2,3-positions. Thus, compound **4a** and its "7-aza" analogue appeared to adopt the same preferred 4*H*-tautomeric conformation.

The displacement-ellipsoid representation (Figure 3) also showed that, in the solid state, the two N-H groups of the guanidinic moiety of **4a** were in the same spatial configuration as the guanidinic N-H groups of 3-(isopropylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide **5** and pinacidil.[23] Such an observation suggests for the "5-aza" compound **4a**, as already pointed out for the corresponding "7-aza" compound, an evident structural analogy with pinacidil.

Conclusions

3-Alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides with or without a chlorine atom in the 7-position were found to express vasodilator properties. The presence of a chlorine atom usually improved the myorelaxant effect of the drugs. 3-Cycloalkylalkylamino- and 3-aralkylamino-7-chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides also expressed a marked myorelaxant activity on electrically stimulated guinea pig ileum and an effect on oxytocin-contracted rat uterus.

The most active compound on the vascular tissue, compound **5d**, which exhibits the hydrocarbon side chain of pinacidil, was further identified as a PCO. The effects of the drug on $^{86}Rb^+$ outflow and on 30 or 80 mM KCl-induced contractile activity, as well as the effects of glibenclamide on the **5d**-induced responses, revealed that the compound expressed the mechanism of action of classical K_{ATP} channel openers.

By contrast, compound **5f**, which has marked activity on the different tissues, revealed less sensitivity to the antagonistic effect of glibenclamide on 30 mM KCl-precontracted aorta rings and maintained a clear vasodilator effect in the presence of 80 mM extracellular potassium. Such findings could indicate another pharmacological profile than that of a pure K_{ATP} channel opener.

Except for the particular behavior of some cycloalkylalkylamino-substituted compounds such as **5f**, the present work further reports that most 3-alkylamino-4*H*-1,2,4-pyridothiadiazine 1,1-dioxides can be considered as K_{ATP} channel openers and that they express different tissue selectivity according to the position of the nitrogen atom in the pyridinic ring.

Recent evidence reported that K_{ATP} channels result from the association of two distinct transmembrane subunits: a sulfonylurea receptor (SUR) associated to an inward rectifier K^+ channel (Kir6.x) in a 4:4 stoichiometry.[24] It has been suggested that, depending on its tissue localization, the channel is expressed in distinct isoforms according to the nature of the SUR subunit associated to the Kir6.x channel (Kir6.2/SUR1 in the endocrine pancreas and Kir6.2/SUR2B in the vascular smooth muscle).[24] Therefore, since the SUR subunit was proposed as the receptor protein for PCOs, small differences in the nature of the receptor binding site could explain subtle differences in the recognition process of tightly related molecules. The present results obtained with pyridothiadiazine dioxides expressing distinct tissue selectivity in regard to subtle structural modifications might contribute to the understanding of the differences among channel subtypes.

Experimental Section

Chemistry. Melting points were determined on a Büchi-Tottoli capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1750 FT-spectrophotometer. The ^1H NMR spectra were taken on a Bruker AW-80 (80 MHz) instrument in DMSO-d_6 with HMDS as an internal standard; chemical shifts are reported in δ values (ppm) relative to internal HMDS. The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, quint = quintuplet, m = multiplet and b = broad are used throughout. Elemental analyses (C, H, N, S) were realized on a Carlo-Erba EA 1108-elemental analyzer and were within ±0.4% of the theoretical values. All reactions were routinely checked by TLC on silica gel Merck 60F 254.

3-(1*H*-Imidazol-1-yl)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (8). The mixture of 2-aminopyridine-3-sulfonamide (**7**)[18] (5 g, 28.9 mmol) and thiocarbonyldiimidazole (15.5 g, 87 mmol) in dioxane (62.5 mL) and DMF (12.5 mL) was refluxed for 1 h. The solvent was removed by distillation under reduced pressure and the residue was suspended in distilled water (100 mL). A solution of NaOH (2.5 g) in water (20 mL) was added and the solution was stirred at room temperature for 10 min. The alkaline solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 4 with formic acid. The crude **8** which precipitates was collected by filtration, washed with water, and purified by dissolution in a mixture of methanol (30 mL) and 1 N NaOH in water (30 mL). The alkaline solution was treated with charcoal and filtered, and the filtrate was acidified with formic acid until pH 4. The resulting precipitate was collected by filtration, washed with water and dried (4.81 g, 67%): mp >300 °C; IR (KBr) 3165 (N−H), 2377, 1968 (N^+−H), 1598, 1520 (C=N, C=C), 1289, 1163 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 7.25 (m, 1H, 7-H), 7.45 (bs, 1H, 5′-H imidazole), 8.00 (bs, 1H, 4′-H imidazole), 8.20 (bd, 1H, 6-H), 8.55 (bd, 1H, 8-H), 9.30 (s, 1H, 2′-H imidazole). Anal. ($C_9H_7N_5O_2S$) C, H, N, S.

2-Amino-5-chloropyridine-3-sulfonamide (10). Chlorosulfonic acid (200 mL) was cooled on an ice−salt bath and then carefully supplemented with 2-amino-5-chloropyridine (**9**) (40.0 g, 0.31 mol). The mixture was heated under reflux for 3 h. After cooling, the reaction mixture was poured on ice (500 g) under stirring, and the resulting white precipitate was collected by filtration and washed with cold water. The solid was suspended under stirring in a 10% w/v aqueous solution of ammonia (1000 mL). After 30 min, the alkaline suspension was concentrated under reduced pressure up to a volume of 300 mL. The precipitate was collected by filtration, washed with water and dissolved in a 10% w/v aqueous solution of NaOH (200 mL). The alkaline solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 7 with 12 N HCl. The crystalline precipitate was collected by filtration, washed with water and dried (34.6 g, 54%): mp 215−220 °C; IR (KBr) 3416, 3377, 3279, 3153 (N−H), 1645, 1588, 1546 (N−H, C=N, C=C), 1328, 1160 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 5.60 (b, $SO_2NH_2 + H_2O$), 7.45 (bs, 2H, NH_2), 7.75 (d, 1H, 6-H), 8.05 (d, 1H, 4-H). Anal. ($C_5H_6ClN_3O_2S$) C, H, N, S.

7-Chloro-3-(1*H*-imidazol-1-yl)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (11). The solution of 2-amino-5-chloropyridine-3-sulfonamide (**10**) (8.0 g, 39 mmol) and thiocarbonyldiimidazole (20.5 g, 115 mmol) in dioxane (80 mL) and DMF (20 mL) was refluxed for 3 h. The solvents were removed by distillation under reduced pressure and the residue was dissolved in an aqueous solution of NaOH (4 g in 150 mL water). The solution turned rapidly to a suspension of the sodium salt of **11**. The salt was collected by filtration and washed with a small volume of water. The salt was dissolved in hot water (200 mL), treated with charcoal, and filtered and the filtrate was adjusted to pH 5−6 with 1 N HCl. The precipitate was collected by filtration, washed with water and dried (8.8 g, 80%): mp 330−331 °C; IR (KBr) 3212, 3179, 3149 (N−H), 2850−2300 (N^+−H), 1589, 1573, 1543, 1526 (N−H, C=N, C=C), 1389, 1142 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 7.65 (s, 1H, 5′-H imidazole), 8.15 (bd, 2H, 6-H + 4′-H imidazole), 8.55 (d, 1H, 8-H), 9.70 (s, 1H, 2′-H imidazole). Anal. ($C_9H_6ClN_5O_2S$) C, H, N, S.

General Procedure for the Synthesis of 3-Alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides 4a−4d and 3-Alkylamino-7-chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides 5a−5d. The mixture of 3-(1*H*-imidazol-1-yl)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**8**) (0.5 g, 2 mmol) or 7-chloro-3-(1*H*-imidazol-1-yl)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**11**) (0.5 g, 1.76 mmol) and the appropriate alkylamine (10 mL) was heated in a sealed vessel for 3−4 h at 180 °C. The excess of the amine was eliminated by distillation under reduced pressure, and the residue was dissolved in an aqueous 5% w/v solution of NaOH (20 mL). The alkaline solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 6 with formic acid. The precipitate was collected by filtration, washed with water and dried.

The following compounds were obtained according to this synthetic procedure.

3-(Isopropylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (4a): yield 0.31 g (65%); mp 269−270 °C; IR (KBr) 3318 (N−H), 2972 (C−H aliph), 1628, 1603, 1574, 1557 (N−H, C=N, C=C), 1287, 1155 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.05 (d, 6H, 2 × CH_3), 3.80 (m, 1H, C*H*), 6.90 (bd, 1H, N−H exocycl), 7.20 (dd, 1H, 7-H), 8.05 (d, 1H, 6-H), 8.45 (d, 1H, 8-H), 10.60 (bs, 1H, N−H intracycl). Anal. ($C_9H_{12}N_4O_2S$) C, H, N, S.

3(*R/S*)-(2-Butylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (4b): yield 0.41 g (81%); mp 242−246 °C; IR (KBr) 3317 (N−H), 2970 (C−H aliph), 1626, 1601, 1571, 1556 (N−H, C=N, C=C), 1287, 1160 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.80 (t, 3H, $CH(CH_3)CH_2CH_3$), 1.05 (d, 3H, $CH(CH_3)CH_2CH_3$), 1.45 (quint, 2H, $CH(CH_3)CH_2CH_3$), 3.70 (m, 1H, $CH(CH_3)CH_2CH_3$), 6.90 (bd, 1H, N−H exocycl), 7.25 (dd, 1H, 7-H), 8.05 (d, 1H, 6-H), 8.45 (d, 1H, 8-H), 10.60 (bs, 1H, N−H intracycl). Anal. ($C_{10}H_{14}N_4O_2S$) C, H, N, S.

3(*R/S*)-(3-Methyl-2-butylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (4c): yield 0.37 g (69%); mp 246−249 °C; IR (KBr) 3302 (N−H), 2968 (C−H aliph), 1621, 1571 (N−H, C=N, C=C), 1284, 1167 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.80 (d, 6H, $CH(CH_3)CH(CH_3)_2$), 1.00 (d, 3H, $CH(CH_3)CH(CH_3)_2$), 1.65 (m, 1H, $CH(CH_3)CH(CH_3)_2$), 3.65 (m, 1H, $CH(CH_3)CH(CH_3)_2$), 6.85 (bd, 1H, N−H exocycl), 7.25 (dd, 1H, 7-H), 8.05 (d, 1H, 6-H), 8.45 (d, 1H, 8-H), 10.60 (bs, 1H, N−H intracycl). Anal. ($C_{11}H_{16}N_4O_2S$) C, H, N, S.

3(*R/S*)-(3,3-Dimethyl-2-butylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (4d): yield 0.25 g (44%); mp 292−293 °C; IR (KBr) 3349 (N−H), 2966 (C−H aliph), 1619, 1566 (N−H, C=N, C=C), 1275, 1162 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.85 (s, 9H, $CH(CH_3)C(CH_3)_3$), 1.00 (d, 3H, $CH(CH_3)C(CH_3)_3$), 3.65 (m, 1H, $CH(CH_3)C(CH_3)_3$), 6.85 (bd, 1H, N−H exocycl), 7.25 (dd, 1H, 7-H), 8.05 (d, 1H, 6-H), 8.45 (d, 1H, 8-H), 10.55 (bs, 1H, N−H intracycl). Anal. ($C_{12}H_{18}N_4O_2S$) C, H, N, S.

7-Chloro-3-(isopropylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (5a): yield 0.35 g (72%); mp 262−264 °C; IR (KBr) 3362 (N−H), 1619, 1572 (N−H, C=N, C=C), 1275, 1154 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.10 (d, 6H, 2 × CH_3), 3.85 (m, 1H, C*H*), 7.00 (bd, 1H, N−H exocycl), 8.20 (d, 1H, 6-H), 8.50 (d, 1H, 8-H), 10.90 (bs, 1H, N−H intracycl). Anal. ($C_9H_{11}ClN_4O_2S$) C, H, N, S.

3(*R/S*)-(2-Butylamino)-7-chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (5b): yield 0.44 g (87%); mp 240−241 °C; IR (KBr) 3382, 3175 (N−H), 2970 (C−H aliph), 1624, 1570 (N−H, C=N, C=C), 1275, 1180, 1150, 1115 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.80 (t, 3H, $CH(CH_3)CH_2CH_3$), 1.05 (d, 3H, $CH(CH_3)CH_2CH_3$), 1.40 (quint, 2H, $CH(CH_3)CH_2CH_3$), 3.70 (m, 1H, $CH(CH_3)CH_2CH_3$), 7.05 (bd, 1H, N−H exocycl), 8.20 (d, 1H, 6-H), 8.50 (d, 1H, 8-H), 10.90 (bs, 1H, N−H intracycl). Anal. ($C_{10}H_{13}ClN_4O_2S$) C, H, N, S.

7-Chloro-3(*R/S*)-(3-methyl-2-butylamino)-4*H*-pyrido-[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (5c): yield 0.46 g (86%); mp 284−286 °C; IR (KBr) 3363 (N−H), 2964 (C−H aliph), 1619, 1571 (N−H, C=N, C=C), 1283, 1171 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.80 (d, 6H, $CH(CH_3)CH(CH_3)_2$), 1.00 (d, 3H, $CH(CH_3)CH(CH_3)_2$), 1.65 (m, 1H, $CH(CH_3)CH(CH_3)_2$), 3.70 (m, 1H, $CH(CH_3)CH(CH_3)_2$), 6.90 (b, 1H, N−H exocycl), 8.20 (d, 1H, 6-H), 8.50 (d, 1H, 8-H), 10.80 (bs, 1H, N−H intracycl). Anal. ($C_{11}H_{15}ClN_4O_2S$) C, H, N, S.

7-Chloro-3(*R/S*)-(3,3-dimethyl-2-butylamino)-4*H*-pyrido-[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (5d): yield 0.17 g (30%): mp >300 °C; IR (KBr) 3356 (N−H), 2965 (C−H aliph), 1617, 1570 (N−H, C=N, C=C), 1278, 1166 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.85 (s, 9H, $CH(CH_3)C(CH_3)_3$), 1.00 (d, 3H, $CH(CH_3)C(CH_3)_3$), 3.65 (m, 1H, $CH(CH_3)C(CH_3)_3$), 6.90 (b, 1H, N−H exocycl), 8.20 (d, 1H, 6-H), 8.50 (d, 1H, 8-H), 10.75 (bs, 1H, N−H intracycl). Anal. ($C_{12}H_{17}ClN_4O_2S$) C, H, N, S.

General Procedure for the Synthesis of 3-Cycloalkylalkyl/aralkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides 4e−4j and 7-Chloro-3-cycloalkylalkyl/aralkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides 5e−5j. The solution of 3-(1*H*-imidazol-1-yl)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**8**) (0.5 g, 2 mmol) or 7-chloro-3-(1*H*-imidazol-1-yl)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**11**) (0.5 g, 1.76 mmol) in the appropriate cycloalkylalkylamine or aralkylamine (5 mL for compounds **4e**−**4j**; 2.5 mL for compounds **5e**−**5j**) was heated under reflux for 0.5−1 h (until completion of the reaction checked by TLC). After cooling, the reaction mixture was introduced in a separatory funnel and distributed between diethyl ether (100−200 mL) and an aqueous solution of NaOH (1 g in 200 mL). The separated aqueous layer was treated with charcoal and filtered and the clear solution was adjusted to pH 6 with formic acid. The precipitate was collected by filtration, washed with water, and dried.

The following compounds were obtained according to this synthetic procedure.

3-(Cyclohexylmethylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (4e): yield 0.46 g (78%); mp 252−254 °C; IR (KBr) 3372, 3264, 3188 (N−H), 2931, 2852 (C−H aliph), 1619, 1563 (N−H, C=N, C=C), 1280, 1168 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.65−1.85 (bm, 11H, C_6H_{11}), 3.25 (m, 2H, CH_2), 7.05 (bt, 1H, N−H exocycl), 7.25 (dd, 1H, 7-H), 8.05 (d, 1H, 6-H), 8.45 (d, 1H, 8-H), 10.70 (bs, 1H, N−H intracycl). Anal. ($C_{13}H_{18}N_4O_2S$) C, H, N, S.

3(*R*)-(1-Cyclohexylethylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide hemihydrate (4f): yield 0.47 g (74%); mp 257−260 °C; IR (KBr) 3337 (N−H), 2929, 2852 (C−H aliph), 1613, 1578 (N−H, C=N, C=C), 1288, 1161 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.70−1.85 (bm, 11H, C_6H_{11}), 1.00 (d, 3H, CH_3), 3.70 (m, 1H, C*H*), 6.90 (bd, 1H, N−H exocycl), 7.25 (dd, 1H, 7-H), 8.05 (d, 1H, 6-H), 8.45 (d, 1H, 8-H), 10.60 (bs, 1H, N−H intracycl). Anal. ($C_{14}H_{20}N_4O_2S\cdot0.5H_2O$) C, H, N, S.

3(*S*)-(1-Cyclohexylethylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide hemihydrate (4g): yield 0.46 g (73%); mp 258−261 °C; IR (KBr) 3340 (N−H), 2929, 2853 (C−H aliph), 1614, 1578 (N−H, C=N, C=C), 1288, 1161 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.70−1.85 (bm, 11H, C_6H_{11}), 1.00 (d, 3H, CH_3), 3.70 (m, 1H, C*H*), 6.90 (bd, 1H, N−H exocycl), 7.25 (dd, 1H, 7-H), 8.05 (d, 1H, 6-H), 8.45 (d, 1H, 8-H), 10.60 (bs, 1H, N−H intracycl). Anal. ($C_{14}H_{20}N_4O_2S\cdot0.5H_2O$) C, H, N, S.

3-(Benzylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (4h): yield 0.50 g (87%); mp 232−233 °C; IR (KBr) 3377, 3295 (N−H), 1625, 1581, 1568 (N−H, C=N, C=C), 1279, 1161 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 4.40 (bd, 2H, CH_2), 7.20−7.25 (bm, 6H, 7-H + C_6H_5), 7.45 (bt, 1H, N−H exocycl), 8.10 (d, 1H, 6-H), 8.50 (d, 1H, 8-H), 11.10 (b, 1H, N−H intracycl). Anal. ($C_{13}H_{12}N_4O_2S$) C, H, N, S.

3(*R*)-(1-Phenylethylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (4i): yield 0.50 g (83%); mp 207−210 °C; IR (KBr) 3354, 3317 (N−H), 1613, 1577 (N−H, C=N, C=C), 1278, 1169 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.40 (d, 3H, CH_3), 4.95 (m, 1H, C*H*), 7.10−7.65 (bm, 7H, 7-H + C_6H_5 + N−H exocycl), 8.05 (d, 1H, 6-H), 8.45 (d, 1H, 8-H), 10.70 (bs, 1H, N−H intracycl). Anal. ($C_{14}H_{14}N_4O_2S$) C, H, N, S.

3(S)-(1-Phenylethylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (4j): yield 0.48 g (79%); mp 213−214 °C; IR (KBr) 3354, 3316 (N−H), 1613, 1576 (N−H, C=N, C=C), 1276, 1169 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.40 (d, 3H, CH_3), 4.95 (m, 1H, C*H*), 7.10−7.65 (bm, 7H, 7-H + C_6H_5 + N−H exocycl), 8.05 (d, 1H, 6-H), 8.45 (d, 1H, 8-H), 10.70 (bs, 1H, N−H intracycl). Anal. ($C_{14}H_{14}N_4O_2S$) C, H, N, S.

7-Chloro-3-(cyclohexylmethylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (5e): yield 0.47 g (81%); mp 282−283 °C; IR (KBr) 3366, 3165 (N−H), 2930, 2850 (C−H aliph), 1623, 1596, 1563 (N−H, C=N, C=C), 1281, 1168 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.60−1.80 (bm, 11H, C_6H_{11}), 3.05 (b, 2H, CH_2), 7.10 (b, 1H, N−H exocycl), 8.20 (d, 1H, 6-H), 8.50 (d, 1H, 8-H), 11.05 (bs, 1H, N−H intracycl). Anal. ($C_{13}H_{17}ClN_4O_2S$) C, H, N, S.

7-Chloro-3(*R*)-(1-cyclohexylethylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (5f): yield 0.42 g (70%); mp 301−302 °C; IR (KBr) 3344, 3186 (N−H), 2928, 2852 (C−H aliph), 1617, 1573 (N−H, C=N, C=C), 1277, 1168 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.60−1.80 (bm, 11H, C_6H_{11}), 1.00 (d, 3H, CH_3), 3.60 (b, 1H, C*H*), 6.90 (bd, 1H, N−H exocycl), 8.20 (d, 1H, 6-H), 8.50 (d, 1H, 8-H), 10.80 (b, 1H, N−H intracycl). Anal. ($C_{14}H_{19}ClN_4O_2S$) C, H, N, S.

7-Chloro-3(*S*)-(1-cyclohexylethylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (5g): yield 0.47 g (78%); mp 303−304 °C; IR (KBr) 3344, 3186 (N−H), 2927, 2853 (C−H aliph), 1618, 1571 (N−H, C=N, C=C), 1277, 1167 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 0.60−1.80 (bm, 11H, C_6H_{11}), 1.00 (d, 3H, CH_3), 3.60 (b, 1H, C*H*), 6.90 (b, 1H, N−H exocycl), 8.20 (b, 1H, 6-H), 8.50 (bd, 1H, 8-H), 10.80 (b, 1H, N−H intracycl). Anal. ($C_{14}H_{19}ClN_4O_2S$) C, H, N, S.

3-(Benzylamino)-7-Chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (5h): yield 0.49 g (86%); mp 244−245 °C; IR (KBr) 3370, 3295 (N−H), 3058 (C−H arom.), 1624, 1572 (N−H, C=N, C=C), 1278, 1165 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 4.40 (d, 2H, CH_2), 7.30 (bs, 5H, C_6H_5), 7.50 (bm, 1H, N−H exocycl), 8.20 (d, 1H, 6-H), 8.50 (d, 1H, 8-H), 11.30 (bs, 1H, N−H intracycl). Anal. ($C_{13}H_{11}ClN_4O_2S$) C, H, N, S.

7-Chloro-3(*R*)-(1-phenylethylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (5i): yield 0.54 g (91%); mp 261−262 °C; IR (KBr) 3360 (N−H), 3055 (C−H arom.), 1615, 1575, 1549 (N−H, C=N, C=C), 1275, 1170 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.40 (d, 3H, CH_3), 4.95 (m, 1H, C*H*), 7.30 (bs, 5H, C_6H_5), 7.60 (b, 1H, N−H exocycl), 8.20 (d, 1H, 6-H), 8.50 (d, 1H, 8-H), 10.85 (b, 1H, N−H intracycl). Anal. ($C_{14}H_{13}ClN_4O_2S$) C, H, N, S.

7-Chloro-3(*S*)-(1-phenylethylamino)-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (5j): 0.48 g (81%); mp 262−265 °C; IR (KBr) 3361 (N−H), 3054 (C−H arom.), 1615, 1575, 1549 (N−H, C=N, C=C), 1274, 1170 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 1.40 (d, 3H, CH_3), 4.95 (m, 1H, C*H*), 7.25 (bs, 5H, C_6H_5), 7.55 (bm, 1H, N−H exocycl), 8.20 (d, 1H, 6-H), 8.50 (d, 1H, 8-H), 10.90 (b, 1H, N−H intracycl). Anal. ($C_{14}H_{13}ClN_4O_2S$) C, H, N, S.

7-Chloro-3-methyl-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (6). The mixture of 2-amino-5-chloropyridine-3-sulfonamide (**10**) (1.0 g, 4.8 mmol) and acetic anhydride (10 mL) was heated under reflux for 6 h. After cooling, the crystalline precipitate was collected by filtration, washed with a small volume of acetic anhydride then with diethyl ether, and dried. The solid was dissolved in 0.1 N NaOH (50 mL). The alkaline solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 4 with formic acid. The precipitate was collected by filtration, washed with water and dried (0.72 g, 65%): mp >300 °C; IR (KBr) 3231, 3150 (N−H), 1590, 1566, 1511 (N−H, C=N, C=C), 1298, 1155 (S=O) cm^{-1}; ^1H NMR (DMSO-d_6, 80 MHz) δ 2.25 (s, 3H, CH_3), 8.40 (bd, 1H, 6-H), 8.70 (bd, 1H, 8-H), 12.80 (bs, 1H, N−H). Anal. ($C_7H_6ClN_3O_2S$) C, H, N, S.

Ionization Constants. The pK_a value of compounds **4a**, **5a** and **6** was determined spectroscopically by means of a Perkin-Elmer UV/Vis 554 spectrophotometer at 20 °C. UV spectra of compounds were taken in different aqueous buffers of pH ranking from 3 to 12. The pK_a values were calculated by the Debye−Hükkel equation at the maximum basic form absorbance.[25]

Determination of Optical Purity. The optical purity of the *R*- and *S*-isomers of 3-(1-cyclohexylethyl)- and 3-(1-phenylethyl)-substituted pyridothiadiazines was determined by high-pressure liquid chromatography on a Merck-Hitachi apparatus (L6000 pump, L4000 UV detector and T2000 integrator) equipped with a CHIRALCEL OD-R column. Chromatographic conditions were as follows: mobile phase, 50 mM KPF_6 in water/acetonitrile (in %: 63/37 for **4f**, **4g**, **4i** and **4j**; 55/45 for **5f**, **5g**, **5i** and **5j**); flow rate, 0.5 mL/min; temperature, 25 °C; sample concentration, 0.2 mg/mL; injection volume, 5.0 μL; detection, UV at 225 nm. The enantiomeric

excesses were calculated from the following equation: for the *R*-isomer: ee% = [(peak surface of *R* − peak surface of *S*)/(peak surface of *R* + peak surface of *S*)] × 100; for the *S*-isomer: ee% = [(peak surface of *S* − peak surface of *R*)/(peak surface of *S* + peak surface of *R*)] × 100. The reported values in Tables 1 and 2 are the means of two or three individual determinations.

X-ray Crystallography. Crystal of compound **4a** was grown by slow evaporation from a methanol solution. Diffraction data were measured at room temperature using a Stoe-Siemens AED four-circle diffractometer. Final cell dimensions were obtained by a least-squares fit to the automatically centered settings for 22 reflections ($69.11° < 2\theta < 74.51°$). Two reference reflections monitored during each experiment showed no significant variation. Intensity data were corrected for Lorentz polarization effects, and for absorption (semiempirical method, ψ scan). Crystal data are listed in Table 1. Space group assignments were suggested by cell geometry and average values of normalized structure factors; choices were confirmed by successful refinement.

The structure was solved by direct methods (SHELXS-97[26]). The least-squares refinement (SHELXL-97[27]) included independent position parameters and anisotropic thermal coefficients for all non-hydrogen atoms and two global isotropic thermal parameters for hydrogen atoms (one for non-methyl and one for methyl atoms). During refinement, H atoms have been included as riding atoms at calculated positions. The final difference Fourier map had no significant features. Atomic scattering factors were taken from ref 28. The thermal ellipsoid views of **4a** were undertaken with the program ORTEP-III.[29]

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

The islets were then incubated at 37 °C for 90 min in 1 mL of the same medium containing 16.7 mM glucose and, in addition, the reference compound or the pyridothiadiazine derivative. The release of insulin was measured radioimmunologically using rat insulin as a standard.[30]

2. Measurements of Tension in Rat Aorta. All experiments were performed in aortae removed from fed Wistar rats (250−300 g). A section of the aorta was cleared of adhering fat and connective tissue and was cut into transverse rings (3−4 mm long). The endothelium was removed by rubbing the intimal surface with forceps. The segments were suspended under 1 g tension by means of steel hooks in an organ bath containing 20 mL of a Krebs-bicarbonate-buffered solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5. The physiological solutions were maintained at 37 °C and bubbled continuously with a mixture of O_2 (95%) and CO_2 (5%). The isometric contractions of the aortic rings were measured with a force-displacement transducer. After 60 min of equilibration, the rings were exposed to 30 or 80 mM KCl. When the tension had stabilized (plateau tension between 1 and 2 g), the drugs were added to the bath at increasing concentrations until maximal relaxation (or until 1 mM). Same experiments were repeated in the presence of glibenclamide throughout (1 and 10 μM). The relaxation response was expressed as the percentage of the contractile response to KCl. The ED_{50} value was graphically assessed for each dose−response curve as the concentration evoking 50% inhibition of the plateau induced by KCl.

3. Measurements on Guinea Pig Ileum. Adult guinea pigs (300−400 g) were stunned and bled. Segments of the ileum (4 cm long) were removed at least 10 cm from the cecum. They were set up under an initial load of 1 g in a Krebs-bicarbonate-buffered solution (see above), maintained at 37 °C and gassed with a mixture of 5% CO_2 and 95% O_2. Coaxial stimulation was carried out as previously described[31] with rectangular pulses of 0.5-ms duration, 0.1 Hz, 5−25 V. Muscle contractions were recorded isometrically.

The inhibitory effects of drugs were assessed on electrically induced contractions by adding increasing concentrations to the bath until maximal effect. Results were expressed as percentages of control responses (measured during 5 min before adding the drug). ED_{50} values (means ± SEM) were graphically assessed.

4. Measurements on Rat Uterus. Fed Wistar rats (150−200 g) were treated the day before killing with diethylstilboestrol dipropionate [im injection of 0.1 mL/100 g of a 1 mg/mL oily solution of diethylstilboestrol dipropionate (Sigma)]. The rats were anesthetized and then sacrified. 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) and bubbled continuously with a mixture of O_2 (95%) and CO_2 (5%). The superfusate was maintained at 37 °C. Injections of 20 mU oxytocin (200 μL of a 0.1 U/mL solution of the hormone in 9‰ NaCl) in the superfusion channel were 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 reponse to oxytocin. For each drug concentration added in the medium, injection of 20 mU oxytocin was repeated at least three times. The contractile responses recorded (mean of three AUC) were expressed as a percent of the reference value (contractile response to oxytocin in the absence of the drug).

5. Measurements of ^{86}Rb Outflow from Rat Aorta. The method used for measuring ^{86}Rb (^{42}K substitute) outflow from rat aorta segments was the same as that previously described.[21,22] The compounds were tested at a 100 μM concentration and the experiments were conducted in the absence and presence of 10 μM glibenclamide throughout.

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Supporting Information Available: Tables of atomic coordinates, thermal parameters, bond distances, and bond and torsional angles for **4a**; also elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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