# 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

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4-*N*-Subsituted and -unsubstituted 3-alkyl- and 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides were synthesized and tested *vs* diazoxide and selected 3-alkyl- and 3-(alkylamino)-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides as potassium channel openers on pancreatic and vascular tissues. Several 4-*N*-unsubstituted 3-(alkylamino)pyridothiadiazines and some 3-(alkylamino)-7-chlorobenzothiadiazines were found to be more potent than diazoxide for the inhibition of the insulin-releasing process. Moreover, the 3-(alkylamino)pyridothiadiazines appeared to be more selective for the pancreatic than for the vascular tissue. By means of the pharmacological results obtained on pancreatic B-cells, structure−activity relationships were deduced and a pharmacophoric model for the interaction of these drugs with their receptor site associated to the pancreatic $K_{ATP}$ channel was proposed. According to their selectivity for the B-cell (endocrine tissue) *vs* the vascular (smooth muscle tissue) ionic channel, selected 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides may serve as pharmacological tools in studying the $K_{ATP}$ channels ("pancreatic-like" $K_{ATP}$ channels) in other tissues.

## Introduction

Potassium channels ($K^+$ channels) play a crucial role in controlling the cellular membrane potential.[1,2] Among those, the ATP-sensitive potassium channels (or $K_{ATP}$ channels) have been characterized in numerous cell types such as cardiac cells, pancreatic B-cells, skeletal and smooth muscle cells, and central neurons.[3,4] These channels appear to be involved in important physiological processes.[4] In pancreatic B-cells, these channels have been shown to mediate the glucose-induced insulin secretion.[5,6] In the vascular tissue, the $K_{ATP}$ channels play an important role in controlling muscle tone and contractility.[7] Hypoglycemic sulfonylureas such as tolbutamide and glibenclamide are generally reported as selective blockers of the $K_{ATP}$ channels.[8] Conversely, several compounds such as cromakalim, minoxidil sulfate, nicorandil, diazoxide (**1**, Figure 1) and pinacidil (**2**) are typical examples of different chemical classes of potassium channel openers (PCOs).[9,10] PCOs exert their biological effects by increasing the $K^+$ channel permeability, a probable result of their opening properties on $K_{ATP}$ channels.[3,11]

The ability of PCOs to activate $K_{ATP}$ channels may vary considerably according to the tissue localization of the channel. The antihypertensive agent diazoxide seems to exert a comparable efficacy in relaxing vascular smooth muscle and in inhibiting insulin secretion.[12,13] These effects of diazoxide result from its ability to activate both the vascular and the pancreatic $K_{ATP}$ channels but without important tissue selectivity. In contrast, pinacidil and cromakalim show a stronger activity on vascular smooth muscle cells than on pancreatic B-cells and exhibit a clear selectivity for the smooth muscle *vs* the endocrine tissue.[12,14,15]

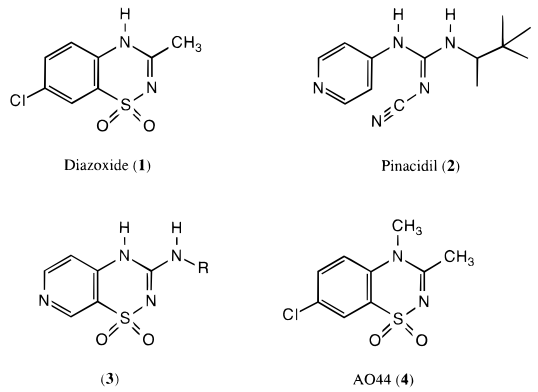


**Figure 1.** Chemical structure of different potassium channel openers (**1**−**3**) and of AO44 (**4**).

Recently, we synthesized novel 4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides (**3**, Figure 1), bearing different aminoalkyl side chains in the 3-position.[16] It was found that some of these compounds were the most powerful inhibitors of insulin secretion reported to date.[16−19] Further pharmacological investigations have shown that the biological target of BPDZ 44 (**3**, R = $CH(CH_3)$-$CH(CH_3)_2$) on pancreatic B-cells, like diazoxide and pinacidil, was the $K_{ATP}$ channel.[17,18] The activity of these new PCOs on other cell types, in particular on vascular smooth muscle cells, remained to be explored.

The present work constitutes an expanded version of our previous Communication to the Editor[16] giving a

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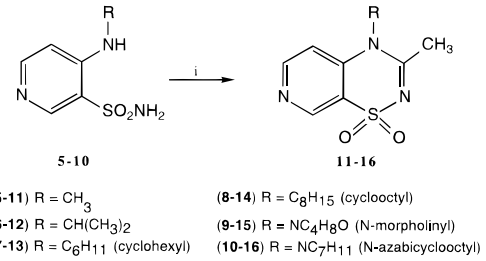
**Scheme 1**[a]




[a] (i) Acetic anhydride.

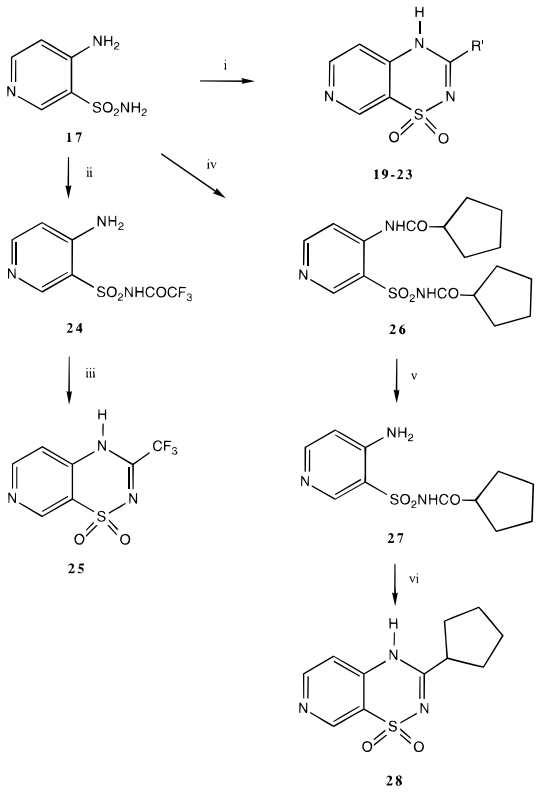
larger chemical and pharmacological exploration of the pyrido- and benzothiadiazinedioxide class of PCOs. Thus, pyridothiadiazinedioxides bearing a variety of 3- or 4-alkyl and 3-aminoalkyl side chains, and 7-chlorobenzothiadiazine dioxides bearing selected 3-alkyl and 3-aminoalkyl side chains were synthesized and evaluated as PCOs on pancreatic B-cells and on isolated rat aorta. The most interesting pyrido- and benzothiadiazinedioxides from different series of compounds were also investigated on guinea pig ileum and on rat heart ventricle. Particular attention was paid to the possible improved selectivity of these pyridothiadiazines and 7-chlorobenzothiadiazines for one of these tissues.

The pharmacological results obtained with the new compounds allowed us to deduce structure−activity relationships that could explain the biological activity of pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides on pancreatic B-cells. Assuming that their activity on insulin secretion results from their $K^+$ channel-opening properties, a pharmacophoric model for their interaction with the pancreatic $K_{ATP}$ channel was proposed.

## Chemistry

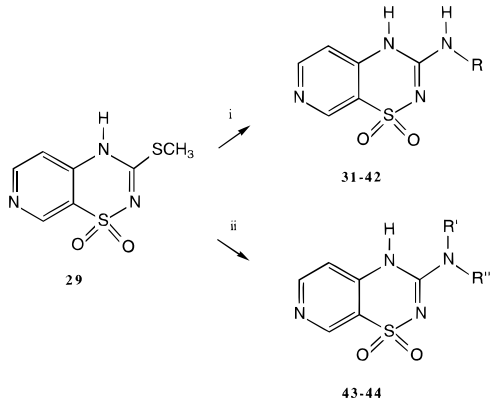
The 4-substituted-3-methyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **11**−**16** were obtained by cyclization of the corresponding (4*N*-substituted-4-aminopyrid-3-yl)sulfonamides[20] **5**−**10** with acetic anhydride (Scheme 1). The synthesis of compounds **19**−**23** was achieved by action of the appropriate anhydride on (4-aminopyrid-3-yl)sulfonamide **17** (Scheme 2). Starting from the same compound **17**, treatment with trifluoroacetic anhydride and cyclization of the monoacylated intermediate **24** by means of $OPCl_3$ led to the pyridothiadiazine **25**. Compound **28** was obtained in three steps as described in Scheme 2 from **17** via a diacylated **26** and a monoacylated **27** intermediate. Hydrolysis of **26** in mild alkaline conditions resulted in a selective deacylation in the 4-position. Such selectivity was explained by the reduction of the electrophilic character of the acylsulfonamide fonction due to its deprotonation in the alkaline medium. The synthesis of the compounds **32**, **39**, **42**, and **44** was achieved by following the experimental conditions previously described for the compounds **31**, **33**−**38**, **40**, **41**, and **43**,[16] starting from the key intermediate **29**, and treating them with an excess of the appropriate amine (Scheme 3). For the 3-(alkylamino)-4-methyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **48**−**51**, the intermediate **47**, obtained from **5** in three steps (Scheme 4), was treated with an excess of the appropriate amine as described above for the 3-aminoalkyl compounds **32**−**43**. However, milder conditions were required for these reactions, probably due to an increasing reactivity of the 4-methyl intermediate **47** compared to the non-4-methyl compound **29**. This higher reactivity of **47** may be explained by the higher electrophilicity of the carbon atom in the 3-position compared to that of compound **29**, since no deprotonation can occur in the 4-position in the presence of the amine.

**Scheme 2**[a]




[a] (i) R′-COOCOR′; (ii) trifluoroacetic anhydride; (iii) $OPCl_3$, Δ; (iv) cyclopentanecarboxylic acid, $OPCl_3$; (v) NaOH, $H_2O$; (vi) Δ.

**Scheme 3**[a]

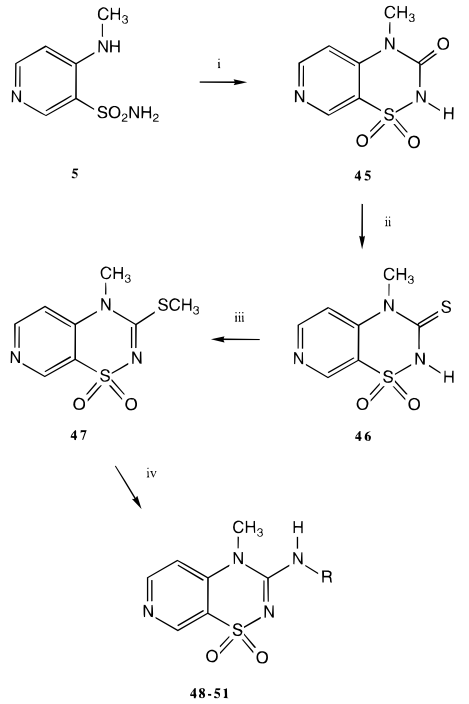



[a] (i) $RNH_2$; (ii) R′-NHR″.

Compound AO44 was prepared from the reaction of diazoxide and methyl iodide in alcoholic alcaline solution as reported in the litterature.[21]

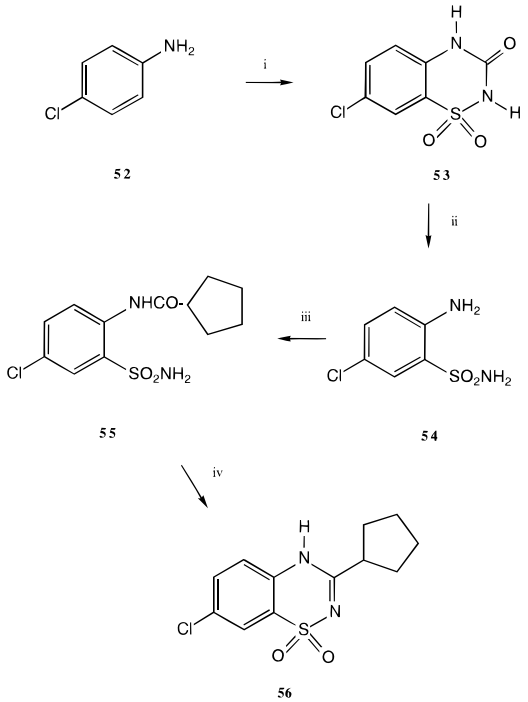
Compound **56** was obtained in four steps (Scheme 5) starting from the reaction of *p*-chloroaniline **52** with chlorosulfonyl isocyanate according to a previously described process leading to the corresponding 3-oxobenzothiadiazine dioxide **53**.[22] Hydrolysis of **53** gave the corresponding aminobenzenesulfonamide **54**[22] which was acylated in the presence of cyclopentanecarboxylic acid chloride to give the monoacylated intermediate **55**.

**Scheme 4**[a]



[a] (i) $NH_2CONH_2$, Δ; (ii) $P_2S_5$, pyridine; (iii) $NaHCO_3$, $CH_3I$; (iv) $RNH_2$.

**Scheme 5**[a]

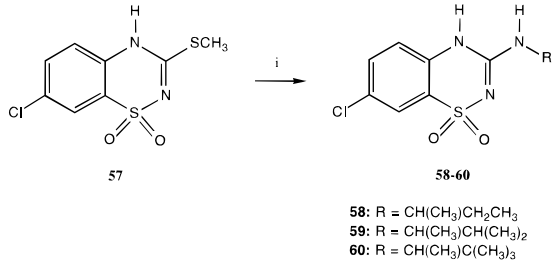


[a] (i) $ClSO_2NCO$, $CH_3NO_2$; $AlCl_3$; (ii) $H_2SO_4$ 50%; Δ; (iii) $C_5H_9COCl$, $C_5H_5N$; (iv) NaOH 1%, Δ.

Cyclization of the former into the benzothiadiazine dioxide **56** was achieved in aqueous alkaline medium.

Compounds **58**, **59**,[16] and **60** were obtained from the reaction of the 3-(methylthio)benzothiadiazine intermediate **57** with an excess of the appropriate amine according to a previously described process (Scheme 6).[16]

**Scheme 6**[a]



[a] (i) $RNH_2$.

## Results and Discussion

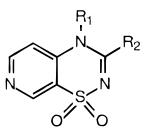
The different compounds reported in Tables 1−3 were tested as inhibitors of the insulin release from rat pancreatic islets incubated in the presence of an insulinotropic glucose concentration (16.7 mM). A 90% inhibition (10% residual insulin secretion) may be considered as a full effect relative to the glucose-insensitive basal insulin release. The same compounds were also investigated for their vasorelaxant activity on $K^+$-depolarized rat aorta without endothelium.

As observed in Table 1, 3-alkyl- and 4-alkyl-3-methyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides were less active than diazoxide and 7-chloro-3-cyclopentyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**56**) on pancreatic B-cells and on rat aorta. Compounds **14**, **23**, and **28** exhibited a poor vasorelaxant activity with $ED_{50}$ values (∼250 μM) higher than that of diazoxide (21 μM) and **56** (2.8 μM). Interestingly, compound **56** (BPDZ 84), which is the 3-cyclopentyl analog of diazoxide and is also the chlorobenzenic analog of **28**, was found to be of comparable potency than the reference compound diazoxide on pancreatic B-cells but was clearly more potent than the former on vascular tissue. The present *in vitro* results on isolated rat aorta corroborate previous observations reported with several diazoxide analogs. In a series of 3-alkyl-4*H*(2*H*)-1,2,4-benzothiadiazine 1,1-dioxides, an increasing blocking activity on the norepinephrine-induced contractile response of the rat aorta was observed when the size of the alkyl substituent in the 3-position was increased.[23]

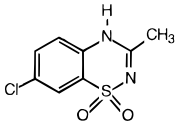
The pharmacological results obtained with 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides and 3-(alkylamino)-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides on the pancreatic and vascular tissues are shown in Table 2. Some results on the B-cell secretory process have been previously reported.[16] Compound **31**, which is devoided of an alkyl substituent on the exocyclic amino group, and the morpholinyl derivative **42** were found to be essentially inactive on the two tissues. However, the pyridinic and the chlorobenzenic monoalkylamine derivatives (**32**−**41** and **58**−**60**) showed a strong inhibitory activity on the insulin secretion. The pyridinic but not the chlorobenzenic compounds clearly exhibited a lower vasorelaxant activity than that of diazoxide.

Cromakalim, which is a strong vasorelaxant agent, was reported to be ineffective as a PCO in exerting an agonistic activity on the pancreatic $K_{ATP}$ channel.[24] On the other hand, the antihypertensive agent pinacidil (**2**) is known to exert a stronger activity on the vascular than on the pancreatic tissue onto which this drug was found to be 10 times less active than diazoxide in inhibiting insulin release.[12,14] Interestingly, the pyrido compounds **36**, **38**, and **39**, bearing a short branched alkyl chain closely related (**36**, **38**) to or identical (**39**) to that of pinacidil, were the most efficacious pyrido-

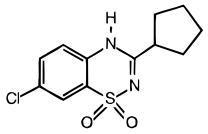
**Table 1.** Percentage of Residual Insulin Secretion and Contractile Activity of 4-Alkyl-3-methyl- and 3-Alkyl-4*H*-pyrido-[4,3-*c*]-1,2,4-thiadiazine 1,1-Dioxides Compared to Diazoxide and 7-Chloro-3-cyclopentyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide **56**



**11-16, 18-23, 25, 28**



**diazoxide**

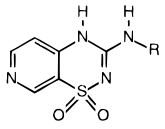


**56**

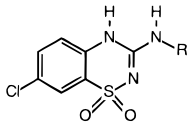
| compd | $R_1$ | $R_2$ | % residual insulin secretion ± SEM ($n$)[a] 50 $\mu$M | 10 $\mu$M | % residual contractile activity ± SEM ($n$)[b] 500 $\mu$M[c] | $ED_{50}$ ($\mu$M)[d] |
|---|---|---|---|---|---|---|
| **11**[c] | $CH_3$ | $CH_3$ | 108.8 ± 4.7 (8) | | 94 ± 1 (3) | |
| **12** | $CH(CH_3)_2$ | $CH_3$ | 68.1 ± 8.3 (8) | | 86 ± 3 (3) | |
| **13** | $C_6H_{11}$ (cyclohexyl) | $CH_3$ | 87.9 ± 3.8 (14) | | 52 ± 4 (5) | |
| **14** | $C_8H_{15}$ (cyclooctyl) | $CH_3$ | 90.6 ± 6.9 (8) | | +++[f] | 248 ± 57 (5) |
| **15** | $NC_4H_8O$ (*N*-morpholinyl) | $CH_3$ | 93.5 ± 6.7 (8) | | 89 ± 9 (3) | |
| **16** | $NC_7H_{12}$ (azabicyclooctyl) | $CH_3$ | 71.9 ± 4.7 (8) | | 53 ± 15 (4) | |
| **18**[e] | H | H | 99.5 ± 6.2 (8) | | 94 ± 6 (3) | |
| **19**[e] | H | $CH_3$ | 100.4 ± 6.8 (16) | | 92 ± 2 (5) | |
| **20** | H | $CH_2CH_3$ | 90.2 ± 5.9 (16) | | 74 ± 8 (6) | |
| **21** | H | $CH_2CH_2CH_3$ | 85.1 ± 6.2 (16) | | 77 ± 4 (4) | |
| **22** | H | $CH(CH_3)_2$ | 79.7 ± 6.4 (16) | | 71 ± 7 (6) | |
| **23** | H | $C(CH_3)_3$ | 81.1 ± 5.0 (13) | | +++ | 258 ± 78 (6) |
| **25** | H | $CF_3$ | 90.4 ± 6.3 (23) | | 96 ± 4 (3) | |
| **28** | H | $C_5H_9$ (cyclopentyl) | 99.3 ± 4.6 (32) | | +++ | 257 ± 44 (5) |
| diazoxide | | | 28.8 ± 2.5 (22) | 70.0 ± 3.6 (22) | +++ | 21 ± 7 (6) |
| **56** | | | 14.1 ± 0.9 (15) | 85.7 ± 2.8 (16) | +++ | 2.8 ± 0.7 (5) |

[a] Percentage of residual insulin release from rat pancreatic islets incubated in presence of 16.7 mM glucose. [b] Activity measured on $K^+$-depolarized rat aorta. [c] Percentage of residual contractile response to 30 mM KCl in isolated rat aorta. [d] $ED_{50}$: drug concentration giving 50% relaxation of the KCl-induced contraction. [e] For the synthesis see ref 25. [g] +++ indicates that a maximal vasorelaxant activity was observed at 500 $\mu$M.

**Table 2.** Percentage of Residual Insulin Secretion and Contractile Activity of 3-(Alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides and 3-(Alkylamino)-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides



**31-42**



**58-60**

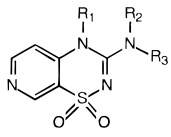
| compd | R | % residual insulin secretion ± SEM ($n$)[a] 50 $\mu$M | 10 $\mu$M | 1 $\mu$M | % residual contractile activity ± SEM ($n$)[b] 500 $\mu$M[c] | $ED_{50}$ ($\mu$M)[d] |
|---|---|---|---|---|---|---|
| **31**[c] | H | 104.1 ± 6.5 (14) | | | 77 ± 13 (3) | |
| **32** | $CH_2CH_3$ | 59.2 ± 3.6 (14) | | | 87 ± 2 (5) | |
| **33**[c] | $(CH_2)_2CH_3$ | 57.7 ± 3.8 (14) | 87.3 ± 6.7 (15) | 97.1 ± 5.9 (8) | 88 ± 7 (4) | |
| **34**[c] | $CH(CH_3)_2$ | 42.6 ± 6.9 (8) | 55.3 ± 8.1 (8) | 110.3 ± 11.3 (6) | 89 ± 2 (4) | |
| **35**[c] | $(CH_2)_3CH_3$ | 38.6 ± 5.6 (7) | 82.0 ± 6.2 (8) | 83.7 ± 6.2 (8) | 74 ± 3 (4) | |
| **36**[c] | $CH(CH_3)CH_2CH_3$ | 9.4 ± 0.9 (8) | 49.0 ± 5.4 (7) | 97.8 ± 9.1 (8) | 34 ± 10 (6) | |
| **37**[c] | $CH_2CH(CH_3)_2$ | 23.4 ± 3.1 (8) | 71.8 ± 7.2 (16) | 84.2 ± 11.0 (8) | +++[f] | 205 ± 53 (12) |
| **38**[c] | $CH(CH_3)CH(CH_3)_2$ | 8.6 ± 0.9 (23) | 13.7 ± 1.2 (23) | 70.5 ± 4.4 (19) | +++ | 302 ± 68 (8) |
| **39** | $CH(CH_3)C(CH_3)_3$ | 10.3 ± 0.8 (20) | 41.0 ± 2.2 (19) | 70.0 ± 3.4 (24) | +++ | 104 ± 80 (7) |
| **40**[c] | $C_5H_9$ (cyclopentyl) | 31.6 ± 4.0 (7) | 71.1 ± 11.2 (7) | 82.8 ± 9.7 (8) | 60 ± 4 (4) | |
| **41**[c] | $C_6H_{11}$ (cyclohexyl) | 40.5 ± 4.3 (8) | 64.4 ± 9.5 (7) | 74.4 ± 4.5 (8) | 40 ± 12 (4) | |
| **42**[c] | $NC_4H_8O$ (*N*-morpholinyl) | 98.8 ± 3.9 (16) | | | 93 ± 6 (6) | |
| **58** | $CH(CH_3)CH_2CH_3$ | 5.5 ± 0.4 (16) | 19.9 ± 1.4 (14) | 85.4 ± 3.7 (15) | +++ | 25 ± 8 (11) |
| **59**[c] | $CH(CH_3)CH(CH_3)_2$ | 11.0 ± 0.8 (16) | 62.7 ± 3.6 (15) | 88.9 ± 6.0 (15) | +++ | 32 ± 15 (5) |
| **60** | $CH(CH_3)C(CH_3)_3$ | 9.3 ± 1.0 (30) | 46.9 ± 4.1 (16) | 87.7 ± 4.5 (16) | +++ | 10 ± 3 (15) |
| diazoxide | | 28.8 ± 2.5 (21) | 70.0 ± 3.6 (22) | 78.9 ± 3.8 (21) | +++ | 21 ± 7 (6) |

[a] Percentage of residual insulin release from rat pancreatic islets incubated in presence of 16.7 mM glucose. [b] Activity measured on $K^+$-depolarized rat aorta. [c] Percentage of the contractile response to 30 mM KCl in isolated rat aorta. [d] $ED_{50}$: drug concentration giving 50% relaxation of the KCl-induced contraction. [e] For the synthesis see ref 16. [f] +++ indicates that a maximal vasorelaxant activity was observed at 500 $\mu$M.

thiadiazines tested on B-cells. They were more active than diazoxide at a 50 and at a 10 $\mu$M concentration. Compound **38** (BPDZ 44) exhibited an $IC_{50}$ value of 3.80 ± 0.46 $\mu$M. When introduced on the 7-chlorobenzothiadiazine structure, these three selected aminoalkyl side chains led to the compounds **58**-**60**, exhibiting a potent inhibitory activity on the insulin-releasing process which was of the same order of magnitude to that expressed by their pyridinic counterparts. Thus, they were equal or superior to diazoxide as putative PCOs acting on the pancreatic tissue and seemed to confirm the favorable influence of an aminoalkyl chain in the 3-position in targeting the pancreatic receptor.

On isolated rat aorta, compound **39** (BPDZ 62) was found to be the most active pyridinic derivative ($ED_{50}$ = 104 $\mu$M), but remained less potent than diazoxide ($ED_{50}$ = 21 $\mu$M) or the other 7-chloro-(3-alkyl/3-alkyl-

**Table 3.** Percentage of Residual Insulin Secretion and Contractile Activity of 3-(Dialkylamino)- and 3-(Alkylamino)-4-methyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides Compared to Diazoxide and AO44



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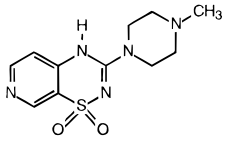

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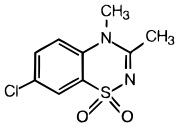


AO44

| | | | | % residual insulin secretion ± SEM (*n*)[a] | | % residual contractile activity ± SEM (*n*)[b] | |
|---|---|---|---|---|---|---|---|
| compd | $R_1$ | $R_2$ | $R_3$ | 50 $\mu$M | 10 $\mu$M | 500 $\mu$M[c] | $ED_{50}$ ($\mu$M)[d] |
| **43** | H | $CH_3$ | $(CH_2)_3CH_3$ | 76.1 ± 4.3 (7) | | 47 ± 8 (3) | |
| **44** | H | $C_4H_8NCH_3$ | (*N*-methylpiperazinyl) | 90.6 ± 7.6 (15) | | +++[e] | 125 ± 30 (11) |
| **48** | $CH_3$ | H | $CH(CH_3)_2$ | 79.4 ± 5.9 (15) | | 84 ± 7 (3) | |
| **49** | $CH_3$ | H | $CH(CH_3)CH_2CH_3$ | 81.0 ± 7.1 (13) | | 61 ± 15 (3) | |
| **50** | $CH_3$ | H | $CH(CH_3)CH(CH_3)_2$ | 95.4 ± 5.3 (14) | | 69 ± 12 (3) | |
| **51** | $CH_3$ | H | $CH(CH_3)C(CH_3)_3$ | 88.5 ± 8.2 (12) | | 74 ± 13 (3) | |
| AO44 | | | | 83.9 ± 5.4 (23) | | | |
| diazoxide | | | | 28.8 ± 2.5 (21) | 70.0 ± 3.6 (22) | +++ | 21 ± 7 (6) |

[a] Percentage of residual insulin release from rat pancreatic islets incubated in presence of 16.7 mM glucose. [b] Activity measured on $K^+$-depolarized rat aorta. [c] Percentage of the contractile response to 30 mM KCl in isoalted rat aorta. [d] $ED_{50}$: drug concentration giving 50% relaxation of the KCl-induced contraction. [e] +++ indicates that a maximal vasorelaxant activity was observed at 500 $\mu$M.

amino)benzothiadiazine derivatives, and markedly less potent than pinacidil ($ED_{50}$ = 0.55 $\mu$M).[15]

A very interesting observation in this series of compounds was that the 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides (i.e. **34**, **36**, **38**, **39**) were clearly more selective for the pancreatic tissue than for the vascular tissue. By contrast, the 3-alkyl- and 3-(aminoalkyl)-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides studied (diazoxide, **56**, **58**, **59**, **60**) were less selective. The most potent drug on insulin secretion, and also the most selective for the pancreatic tissue, was the compound **38** with an $ED_{50}$ (vasorelaxant activity)/$IC_{50}$ (insulin release inhibition) ratio of 302 $\mu$M/3.8 $\mu$M = ~80. The $ED_{50}/IC_{50}$ ratio of diazoxide (21 $\mu$M/24.8 $\mu$M), which is close to the unity, clearly indicates a poor tissue selectivity for the reference compound.

Some 3-*N*,*N*-disubstituted aminoalkyl compounds were also prepared and evaluated on the pancreatic and vascular tissue (Table 3). In particular, compound **44** is the pyridinic analog of 7-chloro-3-(*N*-methylpiperazinyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide, a diazoxide analog reported as an antihypertensive agent.[25] It appeared that the absence of a hydrogen atom on the exocyclic nitrogen atom (compounds **43** and **44**) led to a decrease of the activity on pancreatic B-cells. However, compound **44** exhibited a poor but measurable efficacy in relaxing $K^+$-depolarized rat aorta.

Since the 4-methyl derivative of diazoxide AO44 was reported to exhibit a stronger *in vivo* hyperglycemic activity than diazoxide,[20] this compound and the 3-(alkylamino)-4-methyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **48**−**51** were synthesized and tested on pancreatic B-cells. These compounds were found to be less active than their non-methyl counterparts (**34**, **36**, **38**, and **39**) at a 50 $\mu$M concentration. Moreover, at this concentration, AO44 did not show any significant *in vitro* activity, in apparent contradistinction to its previously reported *in vivo* effect.[20]

Selected compounds i.e. **28**, **38**, **39**, **56**, and **59** (Figure 3), which are representative of the different classes of pyrido- and benzothiadiazinedioxides tested on the pancreatic and the vascular tissues, were also examined *vs* diazoxide and pinacidil on the guinea pig ileum and on the rat ventricle.

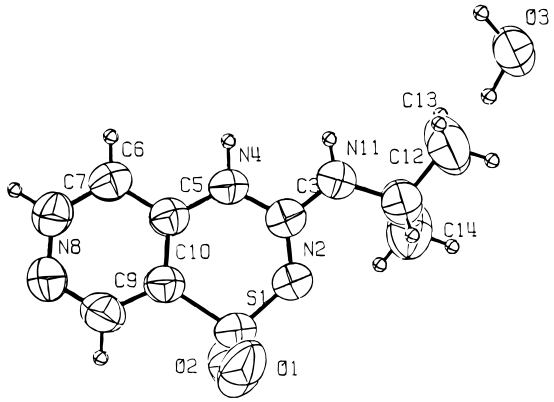



**Figure 2.** Molecular structure of compound **34** with atom-labeling scheme. Displacement ellipsoids are shown at 50% probability levels. H atoms are drawn as small circles of arbitrary units.

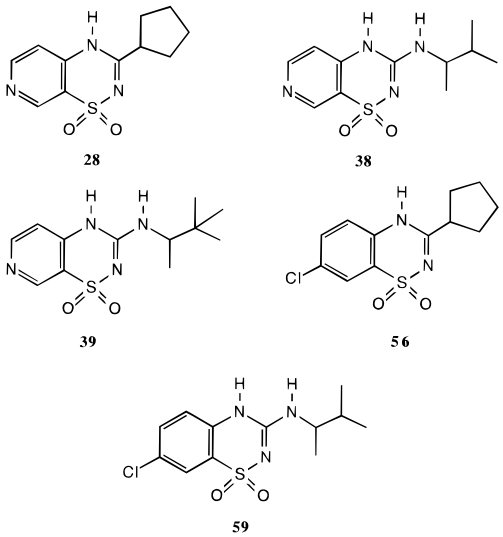



**Figure 3.** Chemical structure of selected compounds representative of the different classes of pyrido- and benzothiadiazinedioxide PCOs.

PCOs i.e. cromakalim and pinacidil have been found to exert interesting biological activities on a variety of gastrointestinal tissue preparations such as inhibition of contractions evoked by electrical field stimulation or

by high $K^+$ (10−30 mM KCl) solutions.[10] Since glibenclamide antagonized the relaxant effects of the two PCOs on the gastrointestinal smooth muscle cells, the presence of putative $K_{ATP}$ channels was suggested.[10]

The inhibitory effect of diazoxide, pinacidil, and the selected drugs **28**, **38**, **39**, **56**, and **59** on the electrically induced contractions (coaxial stimulation) of the guinea pig ileum was measured. Pinacidil was found to be the most potent drug with an $ED_{50}$ of 18.0 ± 6.6 $\mu$M ($n = 5$) (~75% inhibition at a $1 \times 10^{-4}$ M concentration of drug). None of the other drugs, including diazoxide, were found to exert a strong inhibitory activity on the ileum contractions (<20% inhibition at $1 \times 10^{-4}$ M; $n = 5$) except compound **59** which gave ~40% inhibition at $1 \times 10^{-4}$ M ($n = 5$). Thus the most active compound on vascular tissue (compound **56**) was found to be clearly less active than the compound **59**, indicating different possible responses of PCOs when tested on different smooth muscle cell types.

In addition to shortening the cardiac action potential duration, PCOs such as cromakalim have displayed negative inotropic activity in isolated cardiac tissue. However, the effects of PCOs on this tissue were mainly observed at considerably higher concentrations than those required for effective vasorelaxation.[10]

The contractile activity of right ventricular strips from rat heart was measured in the absence and the presence of the selected pyrido- and benzothiadiazines and of the two reference compounds diazoxide and pinacidil. The negative inotropic effect of the two references and the five selected compounds at a $1 \times 10^{-4}$ M concentration of drug ($n = 4$) never exceeded 15% decrease in the systolic tension of rat ventricles.

These results on two other muscle cell types seem to indicate that the selected pyrido- and benzothiadiazinedioxide PCOs examined in the present work are not expected to exert a significant activity on these tissues at concentrations for which a biological response was observed on the vascular and/or the pancreatic endocrine tissues.

From pharmacological results reported on pancreatic B-cells, structure−activity relationships for the inhibitory activity of the new drugs can be deduced. 3-(Alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides (compounds **32**−**41**) and 3-(alkylamino)-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (compounds **58**−**60**) were active drugs on insulin secretion but the pyridinic derivatives are the most selective for the pancreatic tissue. The activity of the pyridothiadiazines increased with the enhancement of the size and the ramification of the alkylamino chain in the 3-position. A limit of steric hindrance for the alkylamino substituent in this position should correspond to the side chain of compounds **38** and **39** since a decreasing activity was observed with more bulky substituents such as cycloalkylamino side chains (see compounds **40** and **41**). Interestingly, one of the best alkylamino substituents for the pyrido- and benzothiadiazines is the (1,2,2-trimethylpropyl)amino chain also encountered in the chemical structure of pinacidil.

The presence of an hydrogen atom on the 4-N nitrogen atom and on the exocyclic nitrogen atom seemed to be required to maintain a strong inhibitory activity on insulin secretion. Indeed, methylation of these two positions considerably reduced the biological activity. An electron-deficient hydrogen atom in the 4-position is also expected to help the establishment of a strong hydrogen bond interaction with the receptor site.

Because the acidic character of pyrido- and benzothiadiazinedioxides is related to the lability of the former 4-H atom, it was of considerable interest to determine the $pK_a$ values of some representative drugs. The ionization constant was determined for selected 3-alkyl- (**19**, $pK_a = 7.65$; **28**, $pK_a = 7.95$) and 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides (**32**, $pK_a = 8.09$; **39**, $pK_a = 8.15$). The 3-alkyl derivatives presented a lower $pK_a$ value compared to that of the 3-aminoalkyl ones, the latter drugs having also a lower $pK_a$ value than that of diazoxide ($pK_a = 8.62$[26]). In accordance with their $pK_a$ values, diazoxide as well as compounds **32** and **39** are mainly present in solution as nonionic species at physiological pH. Thus, it is concluded that the neutral form of the drug, which warrants the hydrogen atom in the 4-position, is required for optimal interaction with the binding site.

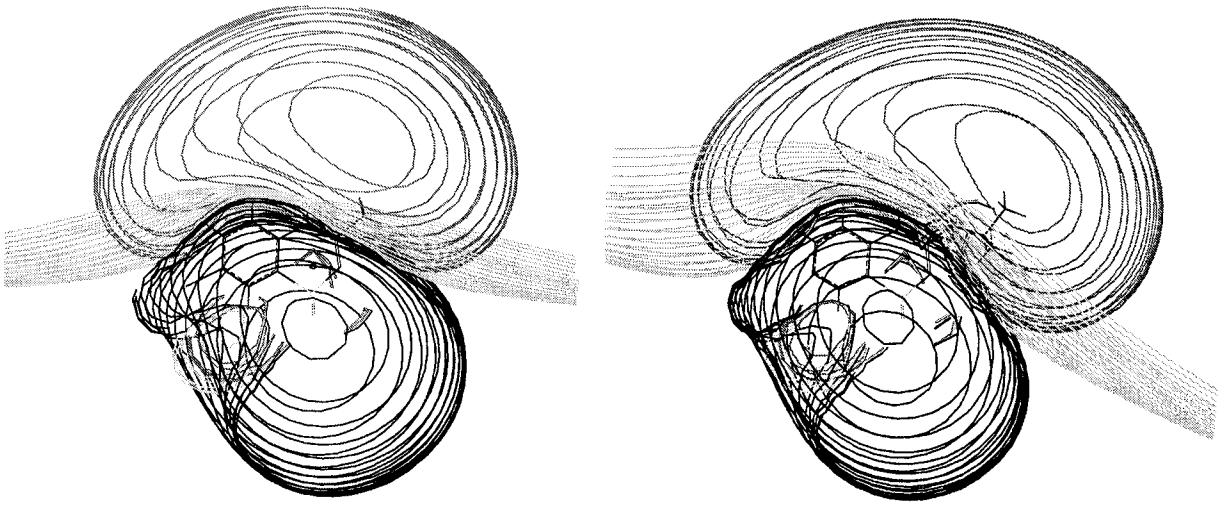
Assuming that crystallographic informations on 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides could also help our knowledge of the structural requirements responsible for optimal activity on the pancreatic tissue, we tried to determine the X-ray structure of the most active and most representative compound **38**. However, due to some difficulties in obtaining suitable crystals of **38**, its isopropyl homolog **34** was ultimately and successfully selected for this purpose.

It was found that the N(2)−C(3) length (1.315 Å) of compound **34** was shorter than its C(3)−N(4) length (1.366 Å), supporting the view that the C═N double bond of the thiadiazine ring is preferentially located in the 2,3-positions corresponding for **34** to the adoption of a 4*H*-tautomeric form. Such a preferred conformation has already been observed with diazoxide[27] and with previously reported 3-alkylpyridothiadiazines[26,28,29] in the solid state.

Interestingly, the displacement-ellipsoid representation of compound **34** (Figure 2) has clearly shown that the two N−H hydrogen atoms located either on the exocyclic or on the 4-N nitrogen atom adopt a spatial orientation which is quite similar to that taken by the H atoms of the two cyanoguanidine N−H groups of pinacidil in the solid state.[30] (See Figure 1 for the crystal conformation of pinacidil.) It is tempting to speculate that such a similar conformation of the "guanidinic" moiety will be that preferentially adopted by both molecules in solution and maybe during their binding site interaction with their pancreatic B-cell receptors.

From X-ray data of diazoxide[27] and compound **34**, their isopotential map was performed using SYBIL 6.1 software package (atomic charge distribution calculated with MOPAC AM1). The comparison between these two stereoelectronical representations did not show marked differences (Figure 4). The present result confirms, in this particular case, the excellent analogy between a chlorobenzenic and a pyridinic moiety for which the heterocyclic nitrogen atom is located at the same position as that of the halogeno-substituted carbon atom.

By means of the physicochemical and pharmacological results discussed, structure−activity relationships were deduced and a pharmacophoric model for the interaction



**Diazoxide** **Compound 34**

**Figure 4.** Electrostatic isopotential maps of diazoxide and of compound **34**. Negative fields (−1 kcal/mol) are presented in bold black (inferior contour), neutral fields (0 kcal/mol) in light gray, and positive fields (+1 kcal/mol) in dark gray (superior contour).

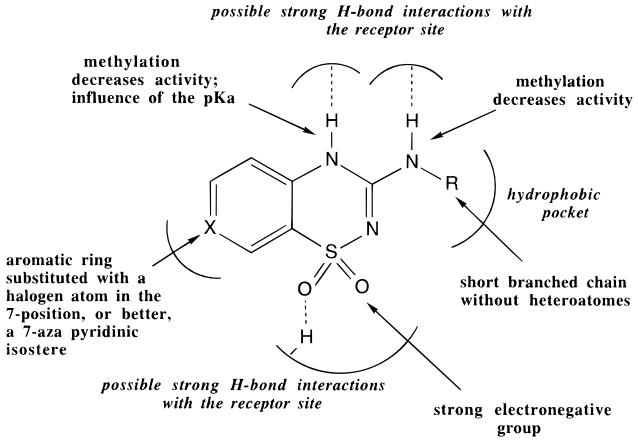



**Figure 5.** Pharmacophoric model for an agonistic activity on the pancreatic $K_{ATP}$ channel.

of the active compounds with the pancreatic $K_{ATP}$ channel was proposed (Figure 5).

Critical structural requirements influence the agonistic activity on the B-cell $K_{ATP}$ channel. Firstly, a short branched lipophilic chain on the nitrogen atom in the 3-position should be required for an optimal interaction with an hydrophobic pocket of a limited size at the receptor site. Up to now, the side chain of compound **38** constitutes the best choice of size and ramification. But since the compound is a racemate, the stereochemistry of the best alkyl chain remains to be determined.

Secondly, strong hydrogen bond interactions are probably established between the receptor site and one or better two N−H groups. Indeed, methylation of these two former positions decreased the activity. The presence of a sulfonyl group should be required in order to impose a particular tautomeric form to the molecules that adequately orientates the two "guanidinic" N−H groups. A strong electron-withdrawing functional group at the 1-position is also able to establish strong hydrogen bond interactions with the receptor site, but such a mechanism remains to be proved i.e. by comparing the biological response of compounds devoid of a sulfonyl group in this position. Nevertheless, the presence of the former group could explain the electron-deficient character of the hydrogen atom in the 4-position, a possible favorable requirement for an optimal activity.

Finally, in the series of 3-aminoalkyl derivatives, an aromatic ring with an halogen atom at the 7-position, or better, a 7-aza pyridinic ring isostere, is favorable to an optimal binding site interaction. More examples of compounds remains to be synthesized and tested in order to define the exact nature of the substituent in the 7-position.

Interestingly, the structure−activity relationships found for an agonistic activity on the *pancreatic* $K_{ATP}$ channel ressembles, at least in part, those suggested for benzopyrans (i.e. cromakalim) on the *smooth muscle* $K_{ATP}$ channel.[31] Indeed, the presence of either an aromatic ring with adequate substituents at the 6-position or (6-N)-pyridinic isosteres, a strong electronegative moiety as hydrogen bond acceptor site (i.e. the pyrolidinone carboxamide group) and a hydrophobic (di)alkyl group (i.e. the dimethyl group) are all structural requirements that could be satisfactorily superimposed to those suggested for the pyrido- and benzothiadiazine dioxide PCOs. However, the present pharmacophore is concerned with an endocrine type of $K_{ATP}$ channels (pancreatic $K_{ATP}$ channels) onto which benzopyran PCOs such as cromakalim are devoid of strong agonistic activity.

In summary, we have synthesized pyridothiadiazines bearing a variety of 3- or 4-alkyl and 3-aminoalkyl side chains. The synthesis of the 3-cyclopentyl analog of diazoxide and of selected 3-(alkylamino)-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides was also made and these benzothiadiazines as well as the pyridothiadiazines were tested *vs* diazoxide as inhibitors of insulin release on pancreatic B-cells and as vasorelaxants on isolated rat aorta. Three 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides (compounds **36**, **38**, and **39**) appeared to be powerful inhibitors of insulin release and are clearly more selective than their chlorobenzenic homologs (compounds **58**−**60**) or than diazoxide for the pancreatic tissue *vs* the vascular tissue. The pharmacophoric model proposed for their interaction with the pancreatic $K_{ATP}$ channel could help the design of new therapeutic agents acting on specific tissues. Moreover, pyridothiadiazine PCOs may serve as interesting phar-

macological tools in studying the $K_{ATP}$ channel ("pancreatic-like" $K_{ATP}$ channels) in numerous tissues.

Lastly, potent inhibitors of the insulin secretion with possible lower effects than those of diazoxide on blood pressure can be expected to become excellent substitutes for diazoxide in the treatment of pancreatic disorders associated to an excess of insulin release.

## 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 $^1$H NMR spectra were taken on a Brucker AW-80 (80 MHz) instrument in DMSO-$d_6$ with HMDS as an internal standard; chemical shifts are reported in $\delta$ values (ppm) relative to internal HMDS. The abbreviation s = singlet, d = doublet, t = triplet, m = multiplet, and b = broad are used throughout. Elemental analyses (C, H, N, S) were realized on a Carlo-Erba EA 1108-elemental analyser and were within ±0.4% of the theoretical values. All reactions were routinely checked by TLC on silica gel Merck 60F 254.

**4-Isopropyl-3-methyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (12).** A mixture of 4-(isopropylamino)-pyridine-3-sulfonamide (**6**,[20] 1.0 g, 4.65 mmol) and acetic anhydride (10 mL) was heated under reflux for 4 h. After cooling, an equal volume of diethyl ether was added under stirring. The resulting precipitate was collected by filtration, washed with diethyl ether, and dried. Recrystallization from methanol and water gave **12** as a white crystalline compound (0.78 g, 80%): mp 196−197 °C; IR (KBr) 1657, 1596, 1568, 1535 (C═N, C═C), 1315, 1163 (S═O) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.55 (d, 6H, 4-CH(C$H_3$)$_2$), 2.5 (s, 3H, 3-C$H_3$), 4.8 (m, 1H, 4-C$H$(CH$_3$)$_2$), 7.65 (d, 1H, 5-H), 8.65 (d, 1H, 6-H), 8.9 (s, 1H, 8-H). Anal. ($C_{10}H_{13}N_3O_2S$) C, H, N, S.

**4-Cyclohexyl-3-methyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (13).** The title compound was obtained from 4-(cyclohexylamino)pyridine-3-sulfonamide (**7**, 1.0 g, 3.9 mmol) by following the experimental conditions described for **12** (0.71 g, 65%), mp 224−226 °C; IR (KBr) 2943, 2860 (C−H), 1596, 1566, 1535 (C═N, C═C), 1312, 1174 (S═O) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.3−2.2 (bm, 10H, 4-CHC$_5H_{10}$), 2.5 (s, 3H, 3-C$H_3$), 4.2 (m, 1H, 4-C$H$C$_5$H$_{10}$), 7.75 (d, 1H, 5-H), 8.7 (d, 1H, 6-H), 8.9 (s, 1H, 8-H). Anal. ($C_{13}H_{17}N_3O_2S$) C, H, N, S.

**4-Cyclooctyl-3-methyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (14).** The title compound was obtained from 4-(cyclooctylamino)pyridine-3-sulfonamide (**8**, 1.0 g, 3.53 mmol) by following the experimental conditions described for **12** (0.70 g, 65%): mp 207−210 °C; IR (KBr) 2924, 2854 (C−H), 1601, 1567, 1542 (C═N, C═C), 1313, 1172 (S═O) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.3−2.2 (bm, 14H, 4-CHC$_7H_{14}$), 2.5 (s, 3H, 3-C$H_3$), 4.5 (m, 1H, 4-C$H$C$_7$H$_{14}$), 7.45 (d, 1H, 5-H), 8.65 (d, 1H, 6-H), 8.9 (s, 1H, 8-H). Anal. ($C_{15}H_{21}N_3O_2S$) C, H, N, S.

**4-*N*-Morpholinyl-3-methyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (15).** The title compound was obtained from 4-(*N*-morpholinylamino)pyridine-3-sulfonamide (**9**, 1.0 g, 3.87 mmol) by following the experimental conditions described for **12** (0.76 g, 70%): mp 285−288 °C; IR (KBr) 2966, 2927, 2893, 2869 (C−H), 1609, 1576, 1553 (C═N, C═C), 1304, 1165 (S═O) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 80 MHz) $\delta$ 2.5 (s, 3H, 3-C$H_3$), 3.1−3.9 (bm, 8H, 4-*N*-morpholinyl), 7.65 (d, 1H, 5-H), 8.7 (d, 1H, 6-H), 8.9 (s, 1H, 8-H). Anal. ($C_{11}H_{14}N_4O_3S$) C, H, N, S.

**4-*N*-Azabicyclo[3.3.0]octyl-3-methyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (16).** The title compound was obtained from 4-(*N*-azabicyclo[3.3.0]octylamino)pyridine-3-sulfonamide (**10**, 1.0 g, 3.5 mmol) by following the experimental conditions described for **12** (0.64 g, 60%): mp 219−222 °C; IR (KBr) 2953, 2867 (C−H), 1610, 1575, 1553 (C═N, C═C), 1303, 1167 (S═O) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.2−1.9 (bm, 6H, 3′,4′,5′-C$H_2$ azabicyclo), 2.5 (s, 3H, 3-C$H_3$), 2.8−3.5 (bm, 6H, azabicyclo), 7.25 (d, 1H, 5-H), 8.7 (d, 1H, 6-H), 8.9 (s, 1H, 8-H). Anal. ($C_{14}H_{18}N_4O_2S$) C, H, N, S.

**3-Ethyl-4*H*-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide monohydrate (20).** A mixture of 4-aminopyridine-3-sulfonamide (**17**,[26] 1.0 g, 5.8 mmol) and propionic anhydride (10 mL) was heated at 150 °C for 8 h. After cooling, an equal volume of diethyl ether was added to the solution. The white precipitate of crude **20** was collected by filtration, washed with diethyl ether, dried, and recrystallized from hot water (0.67 g, 50%): mp 220−223 °C; IR (KBr) 3488 (N−H), 1637, 1614, 1577, 1510 (C═N, C═C, N−H), 1295, 1167 (S═O) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.15 (t, 3H, 3-CH$_2$C$H_3$), 2.55 (q, 2H, 3-C$H_2$CH$_3$), 7.15 (d, 1H, 5-H), 8.6 (d, 1H, 6-H), 8.9 (s, 1H, 8-H), 12.0 (bs, 1H, N−H). Anal. ($C_8H_9N_3O_2S\cdot H_2O$) C, H, N, S.

**3-Propyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide Monohydrate (21).** A mixture of 4-aminopyridine-3-sulfonamide (**17**, 1.0 g, 5.8 mmol) and butyric anhydride (10 mL) was heated at 180 °C for 18 h. After cooling, the precipitate of crude **21** was collected by filtration, washed with butyric anhydride and diethyl ether, dried, and recrystallized from hot water (0.78 g, 55%): mp 210−212 °C; IR (KBr) 3511 (N−H), 1648, 1614, 1578, 1509 (C═N, C═C, N−H), 1295, 1167 (S═O) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 80 MHz) $\delta$ 0.85 (t, 3H, 3-CH$_2$-CH$_2$C$H_3$), 1.6 (m, 2H, 3-CH$_2$C$H_2$CH$_3$), 2.5 (t, 2H, 3-C$H_2$CH$_2$-CH$_3$), 7.15 (d, 1H, 5-H), 8.6 (d, 1H, 6-H), 8.9 (s, 1H, 8-H), 12.2 (bs, 1H, N−H). Anal. ($C_9H_{11}N_3O_2S\cdot H_2O$) C, H, N, S.

**3-Isopropyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (22).** A mixture of 4-aminopyridine-3-sulfonamide (**17**, 1.0 g, 5.8 mmol) and isobutyric anhydride (10 mL) was heated at 170 °C for 72 h. After cooling, the precipitate of crude **22** was collected by filtration, washed with diethyl ether, dried, and dissolved in hot methanol. This solution was discolored with charcoal, and the filtrate was supplemented with 3 volumes of water. After one night at 4 °C, the crystalline **22** was collected, washed with water, and dried (0.59 g, 45%): mp 248−250 °C; IR (KBr) 3291 (N−H), 1613, 1573, 1510 (C═N, C═C, N−H), 1288, 1154 (S═O) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.15 (d, 6H, 3-CH(C$H_3$)$_2$), 2.8 (m, 1H, 3-C$H$(CH$_3$)$_2$), 7.2 (d, 1H, 5-H), 8.7 (d, 1H, 6-H), 8.9 (s, 1H, 8-H), 12.1 (bs, 1H, N−H). Anal. ($C_9H_{11}N_3O_2S$) C, H, N, S.

**3-*tert*-Butyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (23).** The title compound was obtained as described for compound **22**, by starting from 4-aminopyridine-3-sulfonamide (**17**, 1.0 g, 5.8 mmol) and trimethylacetic anhydride (10 mL) (0.63 g, 45%): mp 290−293 °C; IR (KBr) 3303 (N−H), 1614, 1569, 1500 (C═N, C═C, N−H), 1291, 1164 (S═O) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.2 (s, 9H, 3-C(C$H_3$)$_3$), 7.5 (d, 1H, 5-H), 8.65 (d, 1H, 6-H), 8.9 (s, 1H, 8-H), 11.4 (bs, 1H, N−H). Anal. ($C_{10}H_{13}N_3O_2S$) C, H, N, S.

***N*-[(4-Aminopyrid-3-yl)sulfonyl]trifluoroacetamide (24).** A mixture of 4-aminopyridine-3-sulfonamide (**17**, 1.0 g, 5.8 mmol) and trifluoroacetic anhydride (7.5 mL) was heated under reflux for 4 h. After cooling, the precipitate was collected by filtration, washed with diethyl ether, dried, and recrystallized from hot methanol (1.33 g, 85%): mp 262−266 °C; IR (KBr) 3390, 3312, 3219 (N−H), 2688 (N$^+$−H zwitterionic form), 1647, 1520 (C═N, C═C, N−H), 1311, 1194 (S═O) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 80 MHz) $\delta$ 6.9 (d, 1H, 5-H), 7.4 (bs, 2H, NH$_2$), 8.0 (d, 1H, 6-H), 8.45 (s, 1H, 2-H), 13.0 (bs, 1H, SO$_2$N$H$CO). Anal. ($C_7H_6N_3O_3SF_3$) C, H, N, S.

**3-(Trifluoromethyl)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide Monohydrate (25).** *N*-[(4-Aminopyrid-3-yl)-sulfonyl]trifluoroacetamide (**24**, 1.0 g, 3.7 mmol) was heated under reflux in phosphorus oxychloride (20 mL) for 18 h. Most of the excess reactant was eliminated by distillation under reduced pressure, and the oily residue obtained was triturated with cold water (5 mL). The precipitate was collected by filtration, washed with water, and dried. Recrystallization from methanol, diethyl ether, and petroleum ether (40−60 °C) 1:1:3 gave **25** (0.61 g, 65%): mp 236−240 °C; IR (KBr) 2623, 2144 (N$^+$−H zwitterionic form), 1646, 1617, 1535, 1503 (C═N, C═C), 1302, 1168 (S═O), 1317, 1183, 1143 (CF$_3$) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 80 MHz) $\delta$ 6.9 (brs, H$_2$O + NH), 7.45 (d, 1H, 5-H), 8.5 (d, 1H, 6-H), 9.2 (s, 1H, 8-H). Anal. ($C_7H_4N_3O_2SF_3\cdot H_2O$) C, H, N, S.

***N*-[4-(Cyclopentanecarboxamidopyrid-3-yl)sulfonyl]-cyclopentanecarboxamide (26).** A mixture of 4-aminopyridine-3-sulfonamide (**17**, 1.0 g, 5.8 mmol), cyclopentanecarboxylic acid (2 mL), and phosphorus oxychloride (8 mL) was

heated under reflux for 10 min. Most of the excess reactant was eliminated by distillation under reduced pressure, and the resulting oily residue was triturated with cold water (100 mL), giving a white precipitate of crude **26**. The product was collected by filtration, washed with water, and dried. Recrystallization from methanol and water 1:2 gave **26** (1.8 g, 85%): mp 219−222 °C; IR (KBr) 3370 (N−H amide), 2958, 2870 (C−H aliphatic), 2738, 2602 ($N^+$−H zwitterionic form), 1708 (C=O, amide), 1583, 1499 (C=N, C=C), 1320, 1154 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.2−2 (m, 16H, 2 × $(CH_2)_4$, cyclopentyl), 2.75 (bs, 2H, 2 × COC*H*$C_4H_8$), 8.4 (d, 1H, 5-H), 8.7 (d, 1H, 6-H), 8.95 (s, 1H, 2-H), 9.7 (s, 1H, CN*H*CO-cyclopentyl). Anal. ($C_{17}H_{23}N_3O_4S$) C, H, N, S.

***N*-[(4-Aminopyrid-3-yl)sulfonyl]cyclopentanecarboxamide (27).** The solution of *N*-[(4-cyclopentanecarboxamidopyrid-3-yl)sulfonyl]cyclopentanecarboxamide (**26**, 1.0 g, 2.8 mmol) in an aqueous solution of NaOH (0.22 g of NaOH in 20 mL water) was refluxed for 2 h. After treatment with charcoal and cooling, the solution was adjusted to pH 2 with 0.1 N HCl. The resulting precipitate was collected by filtration, washed with water, and dried (0.64 g, 85%): mp 218−220 °C; IR (KBr) 3388, 3310, 3212 (N−H), 2958, 2939, 2867 (C−H aliphatic), 2656, 2047 ($N^+$−H zwitterionic form), 1653 (C=O), 1605, 1516 (C=N, C=C), 1287, 1154 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.5 (bs, 8H, CO-CHC$_4H_8$), 2.5 (bs, DMSO + COC*H*$C_4H_8$), 6.75 (d, 1H, 5-H), 7.15 (bs, 2H, N$H_2$), 8.05 (d, 1H, 6-H), 8.4 (s, 1H, 2-H). Anal. ($C_{11}H_{15}N_3O_3S$) C, H, N, S.

**3-Cyclopentyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (28).** *N*-[(4-Aminopyrid-3-yl)sulfonyl]cyclopentanecarboxamide (**27**, 0.7 g, 2.6 mmol) was heated at 220 °C (fusion) for 10 min. The residue was dissolved in hot methanol, discolored with charcoal and cooled. The crude **28** which precipitates was collected by filtration (0.47 g, 72%): mp 298−302 °C; IR (KBr) 3288 (N−H), 2952, 2870 (C−H aliphatic), 1618, 1571, 1506 (C=N, C=C, N−H), 1293, 1155 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.55 (bs, 8H, CHC$_4H_8$), 3.0 (bs, 1H, C*H*$C_4H_8$), 7.2 (d, 1H, 5-H), 8.65 (d, 1H, 6-H), 8.9 (s, 1H, 8-H), 12.1 (bs, 1H, N−*H*). Anal. ($C_{11}H_{13}N_3O_2S$) C, H, N, S.

**1,2,2-Trimethylpropanal Oxime (30a).** An aqueous solution of $Na_2CO_3$ (2.6 g in 10 mL of water) was added dropwise with vigorous stirring to a suspension of pinacolone (5.0 g, 50 mmol) in an aqueous solution of hydroxylamine hydrochloride (3.5 g/50 mmol in 7 mL of water). After 3 h, the crystalline oxime **30a** was collected by filtration, washed with water, and dried (4.72 g, 82%): mp 73−75 °C; IR (KBr) 3284 (O−H), 1663 (C=N) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.0 (s, 9H, (C$H_3$)$_3$), 1.65 (s, 3H, 1-C$H_3$), 10.1 (s, 1H, NO*H*).

**1,2,2-Trimethylpropylamine Hydrochloride (30b).** Sodium metal (3.0 g, 0.13 mol) was added slowly to a solution of 1,2,2-trimethylpropanal oxime (**30a**, 2.0 g, 17.4 mmol) in absolute ethanol (10 mL). After complete addition, the mixture was refluxed until total dissolution of sodium was achieved. The solution was cooled in an ice bath, mixed with brine (50 mL), and extracted with diethyl ether (100 mL). The organic layer was washed with water (20 mL) and extracted with 6 N HCl (30 mL). The aqueous solution was concentrated to dryness under reduced pressure. The crude hydrochloride was dissolved in the minimum ethanol, filtered, and mixed with 5 volumes of diethyl ether to give the title compound (1.2 g, 68%): mp 289−292 °C; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 0.9 (s, 9H, (C$H_3$)$_3$), 1.1 (d, 3H, CHC$H_3$), 2.9 (m, 1H, C*H*CH$_3$). Anal. ($C_6H_{16}NCl$) C, H, N.

**3-(Ethylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (32).** A solution of 3-(methylthio)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide monohydrate (**29**, 0.5 g, 2.02 mmol) in a 70% w/v aqueous solution of ethylamine (10 mL) was heated in a hermetically closed autoclave at 120 °C during 6 h. The excess of ethylamine was removed under reduced pressure and the residue was dissolved in water. The solution was adjusted to pH 6 with 1 N HCl and the title product which precipitates was collected by filtration, washed with water, and dried (0.33 g, 72%): mp 246−248 °C; IR (KBr) 3383, 3288, 3166 (N−H), 1651, 1604, 1582, 1551 (C=N, C=C, N−H), 1278, 1173 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.0 (t, 3H, N-CH$_2$C$H_3$), 3.2 (q, 2H, NC$H_2$CH$_3$), 7.05 (d, 1H, 5-H), 7.25 (bs, 1H, 3-N*H*CH$_2$CH$_3$), 8.4 (d, 1H, 6-H), 8.6 (s, 1H, 8-H), 12.1 (bs, 1H, N−H). Anal. ($C_8H_{10}N_4O_2S$) C, H, N, S.

**3-[(1′,2′,2′-Trimethylpropyl)amino]-4H-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide Monohydrate (39).** 1,2,2-Trimethylpropylamine was obtained from the hydrochloride **30b** (5.0 g) after dissolution of the former in the minimum amount of water (3 mL) and addition of a solution of NaOH (2.2 g) in water (3 mL). The sparingly water-soluble amine which separates was decanted, filtered, and rectified by distillation (boiling point 85 °C at 760 mmHg). A solution of 3-(methylthio)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide monohydrate (**29**, 0.5 g, 2.02 mmol) in 1,2,2-trimethylpropylamine (5 mL) was heated under reflux during 2 days. The solution was concentrated under reduced pressure, and the residue was dissolved in aqueous NaOH (0.1 N). The solution was treated with charcoal and filtered. The filtrate was adjusted to pH 5 with formic acid, and the precipitate of crude **39** was collected by filtration, washed with water, and dried. Recrystallization from methanol and water 1:2 gave **39** (0.4 g, 66%): mp 257−260 °C, IR (KBr) 3297 (N−H), 1631, 1581 (C=N, C=C, N−H), 1281, 1162 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 0.8 (s, 9H, NCH(CH$_3$)C(C$H_3$)$_3$), 1.0 (d, 3H, NCH-(C$H_3$)C(CH$_3$)$_3$), 3.8 (m, 1H, NC*H*(CH$_3$)C(CH$_3$)$_3$), 7.0 (d, 1H, 5-H), 7.25 (bs, 1H, 3-N*H*CH(CH$_3$)), 8.45 (d, 1H, 6-H), 8.7 (s, 1H, 8-H), 12.1 (bs, 1H, N−H). Anal. ($C_{12}H_{18}N_4O_2S\cdot H_2O$) C, H, N, S.

**3-(Methylbutylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (43).** 3-(Methylthio)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide monohydrate (**29**, 0.5 g, 2.02 mmol) was heated under reflux with *N*-methylbutylamine (5 mL) for 24 h. Most of the excess of *N*-methylbutylamine was removed by distillation under reduced pressure. The residue was dissolved in water (10 mL), and the solution was adjusted to pH 5 with formic acid. The precipitate of the crude **43** was collected by filtration, washed with water, dried, and recrystallized from methanol and water 1:1 (0.39 g, 66%): mp 201−204 °C; IR (KBr) 3287, 3202 (N−H), 1613, 1598, 1580 (C=N, C=C, N−H), 1283, 1154 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 0.9 (t, 3H, 3-N(CH$_2$)$_3$C$H_3$), 1−1.8 (m, 4H, 3-NCH$_2$(C$H_2$)$_2$-CH$_3$), 3.05 (s, 3H, 3-NCH$_3$), 3.5 (t, 2H, 3-NC$H_2$(CH$_2$)$_2$CH$_3$) 7.35 (d, 1H, 5-H), 8.5 (d, 1H, 6-H), 8.7 (s, 1H, 8-H), 10.6 (bs, 1H, N−*H*). Anal. ($C_{11}H_{16}N_4O_2S$) C, H, N, S.

**3-(4′-Methyl-1′-piperazinyl)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (44).** 3-(Methylthio)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide monohydrate (**29**, 0.6 g, 2.43 mmol) was heated under reflux with *N*-methylpiperazine (3 mL) for 30 min. After cooling, diethyl ether (60 mL) was added to the mixture, and an oily product was isolated by decantation. The oil was dissolved in a small volume of methanol, after which diethyl ether was added until precipitation of the crude **44** occurred. After cooling, the precipitate was collected by filtration, washed with diethyl ether, and dried. Recrystallization from methanol and diethyl ether 1:4 gave **44** (0.55 g, 80%): mp 257−260 °C; IR (KBr) 3250, 3162 (N−H), 1607, 1570, 1521 (C=N, C=C, N−H), 1290, 1158 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 2.25 (s, 3H, 4′-NC$H_3$), 2.4 (t, 4H, 3′,5′-C$H_2$), 3.6 (t, 4H, 2′,6′-C$H_2$), 7.05 (d, 1H, 5-H), 8.3 (d, 1H, 6-H), 8.6 (s, 1H, 8-H), 12.1 (bs, 1H, NH). Anal. ($C_{11}H_{15}N_5O_2S$) C, H, N, S.

**4-Methyl-3-oxo-2,3-dihydro-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (45).** A mixture of 4-(methylamino)-pyridine-3-sulfonamide (**5**, 9.4 g, 50.2 mmol) and urea (6.1 g, 100.4 mmol) was heated at 200 °C (fusion). After cooling, the residue was dissolved in 1 N NaOH and the solution was adjusted to pH 5 with formic acid. The precipitate of **45** was collected by filtration, washed with water, and dried (6.75 g, 63%): mp 273-275 °C; IR (KBr) 3363 (N−H), 1637, 1568, 1511 (C=N, C=C, N−H, C=O), 1291, 1174 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 3.35 (s, 3H, 4-N-C$H_3$), 6.1 (bs, $H_2O$ + NH), 7.5 (d, 1H, 5-H), 8.55 (d, 1H, 6-H), 8.9 (s, 1H, 8-H). Anal. ($C_7H_7N_3O_3S$) C, H, N, S.

**4-Methyl-3-thioxo-2,3-dihydro-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (46).** The mixture of 4-methyl-3-oxo-2,3-dihydro-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**45**, 6.0 g, 28.1 mmol) and phosphorus pentasulfide (12.0 g, 27 mmol) in anhydrous pyridine was heated under reflux for

2 days. The resulting suspension was concentrated under reduced pressure, and the residue was dissolved in the minimum 2 N NaOH. This solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 2 with 1 N HCl. The yellow precipitate was collected by filtration, washed with water, and dissolved in a saturated aqueous solution of $NaHCO_3$ (150 mL). The insoluble material was filtered off, and the filtrate was adjusted again to pH 2 with 1 N HCl. The crystalline **46** was collected by filtration, washed with water, and dried (4.0 g, 62%): mp 243−245 °C; IR (KBr) 3495 (N−H), 2581 (S−H tautomeric form) 1633, 1539, 1490 (C=N, C=C, N−H), 1289, 1166 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 4.0 (s, 3H, 4-N-C$H_3$), 5.95 (bs, 4H, NH + $H_2O$), 7.55 (d, 1H, 5-H), 8.65 (d, 1H, 6-H), 8.95 (s, 1H, 8-H). Anal. ($C_7H_7N_3O_2S_2$) C, H, N, S.

**4-Methyl-3-(methylthio)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (47).** 4-Methyl-3-thioxo-2,3-dihydro-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**46**, 3.2 g, 14 mmol) was dissolved in a solution of NaHCO3 (2.4 g) in water (106 mL). Under stirring, methanol (80 mL) and methyl iodide (10.2 mL, 164 mmol) were successively added to this solution. After 30 min under agitation at room temperature, the mixture was adjusted to pH 3 with 1N HCl, and the methanol was removed under reduced pressure. After cooling, the title product which precipitated, was collected by filtration, washed with water, and dried (2.65 g, 78%): mp 208−210 °C; IR (KBr) 1591, 1564, 1520 (C=N, C=C), 1306, 1173 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 2.5 (s, 3H, 3-SC$H_3$), 3.65 (s, 3H, 4-NC$H_3$), 7.45 (d, 1H, 5-H), 8.7 (d, 1H, 6-H), 8.9 (s, 1H, 8-H). Anal. ($C_8H_9N_3O_2S_2$) C, H, N, S.

**3-(Isopropylamino)-4-methyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (48).** A solution of 4-methyl-3-(methylthio)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**47**, 0.5 g, 2.05 mmol) in isopropylamine (5 mL) was heated under reflux for 1 h. The excess of amine was removed under reduced pressure, and the residue was triturated with water. The title compound was collected by filtration, washed with water, and recrystallized from methanol and water 1:3 (0.35 g, 69%): mp 272−274 °C; IR (KBr) 3299 (N−H), 1598, 1554 (C=N, C=C, N−H), 1285, 1158 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.1 (d, 6H, 3-NCH(C$H_3$)$_2$), 3.4 (s, 3H, 4-NC$H_3$), 4.05 (m, 1H, 3-NC$H$(CH$_3$)$_2$), 7.45 (d, 1H, 5-H), 7.65 (bs, 1H, N−H), 8.65 (d, 1H, 6-H), 8.8 (s, 1H, 8-H). Anal. ($C_{10}H_{14}N_4O_2S$) C, H, N, S.

**4-Methyl-3-[(1′-methylpropyl)amino]-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (49).** The title compound was obtained as described for the compound **48**, by starting from the intermediate **47** (0.5 g, 2.05 mmol) and *sec*-butylamine (5 mL) (0.36 g, 67%): mp 215−217 °C; IR (KBr) 3312 (N−H), 1599, 1554 (C=N, C=C, N−H), 1285, 1156 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 0.75 (t, 3H, 3-N-CH(CH$_3$)-CH$_2$C$H_3$), 1.1 (d, 3H, 3-NCH(C$H_3$)CH$_2$CH$_3$), 1.5 (m, 2H, 3-NCH(CH$_3$)C$H_2$CH$_3$), 3.4 (s, 3H, 4-NC$H_3$), 3.75 (m, 1H, 3-NC$H$(CH$_3$)CH$_2$CH$_3$), 7.35 (d, 1H, 5-H), 7.45 (bs, 1H, N−H), 8.65 (d, 1H, 6-H), 8.75 (s, 1H, 8-H). Anal. ($C_{11}H_{16}N_4O_2S$) C, H, N, S.

**3-[(1′,2′-Dimethylpropyl)amino]-4-methyl-4*H*-pyrido-[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (50).** The title compound was obtained as described for **48** starting from **47** (0.5 g, 2.05 mmol) and 1,2-dimethylpropylamine (5 mL) (0.48 g, 85%): mp 160−162 °C; IR (KBr) 3339 (N−H), 1595, 1549 (C=N, C=C, N−H), 1289, 1158 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 0.85 (d, 6H, 3-NCH(CH$_3$)CH(C$H_3$)$_2$), 1.1 (d, 3H, 3-NCH(C$H_3$)CH(CH$_3$)$_2$), 1.75 (m, 1H, 3-NCH(CH$_3$)C$H$(CH$_3$)$_2$), 3.42 (s, 3H, 4-NC$H_3$), 3.75 (m, 1H, 3-NC$H$(CH$_3$)CH(CH$_3$)$_2$), 7.35 (d, 1H, 5-H), 7.5 (bs, 1H, NH), 8.65 (d, 1H, 6-H), 8.75 (s, 1H, 8-H). Anal. ($C_{12}H_{18}N_4O_2S$) C, H, N, S.

**4-Methyl-3-[(1′,2′,2′-trimethylpropyl)amino]-4H-pyrido-[4,3-e]-1,2,4-thiadiazine 1,1-Dioxide (51).** The title compound was obtained as described for the compound **48** starting from the compound **47** (0.5 g, 2.05 mmol) and 1,2,2-trimethylpropylamine (5 mL) (0.32 g, 54%): mp 253−256 °C; IR (KBr) 3325 (N−H), 1597, 1555 (C=N, C=C, N−H), 1303, 1163 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO, 80 MHz) $\delta$ 0.9 (s, 9H, 3-NCH(CH$_3$)C-(C$H_3$)$_3$), 1.1 (d, 3H, 3-NCH(C$H_3$)C(CH$_3$)$_3$), 3.6 (s, 3H, 4-NC$H_3$), 4.1 (m, 1H, 3-NC$H$(CH$_3$)C(CH$_3$)$_3$), 7.2 (bs, 1H, NH), 7.45 (d, 1H, 5-H), 8.65 (d, 1H, 6-H), 8.75 (s, 1H, 8-H). Anal. ($C_{13}H_{20}N_4O_2S$) C, H, N, S.

**5-Chloro-2-cyclopentanecarboxamidobenzenesulfonamide (55).** A solution of 2-amino-5-chlorobenzenesulfonamide (**54**,[23] 1.0 g, 4.8 mmol), pyridine (0.5 mL), and cyclopentanecarboxylic acid chloride (0.7 g, 5.3 mmol) in dioxane (15 mL) was stirred at room temperature for 1 h. The solvent was removed by distillation under reduced pressure, and the residue was triturated with water (50 mL). The resulting insoluble material was collected by filtration, washed with water and recrystallized from methanol/water 1:1 (1.26 g, 86%): mp 165−168 °C; IR (KBr) 3377, 3236 (N−H), 2955, 2868 (C−H aliphatic), 1636 (C=O), 1564, 1521 (C=N, C=C), 1344, 1157 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.2−2.0 (bm, 8H, COCHC$_4H_8$), 2.7 (m, 1H, COC$H$C$_4$H$_8$), 7.55 (d, 1H, 4-H), 7.8 (bs, 3H, 6-H + $SO_2NH_2$), 8.2 (d, 1H, 3-H), 9.3 (s, 1H, CON$H$). Anal. ($C_{12}H_{15}N_2O_3SCl$) C, H, N, S.

**7-Chloro-3-cyclopentyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (56).** The solution of 2-cyclopentanecarboxamido-5-chlorobenzenesulfonamide (**55**, 0.64 g, 2.12 mmol) in NaOH 1% w/v (32 mL) was refluxed for 30 min. The solution was adjusted to pH 7 with 1 N HCl, and the resulting precipitate was collected by filtration, washed with water, and dried. Recrystallization from acetone/petroleum ether 1:3 gave the title compound (0.38 g, 63%): mp 278−280 °C; IR (KBr) 3285 (N−H), 2957, 2870 (C−H aliphatic), 1618, 1578, 1525, 1482 (C=N, C=C), 1288, 1157 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 1.2−2.0 (bm, 8H, COCHC$_4H_8$), 2.9 (m, 1H, COC$H$C$_4$H$_8$), 7.30 (d, 1H, 5-H), 7.65 (d, 1H, 6-H), 7.7 (s, 1H, 8-H). Anal. ($C_{12}H_{13}N_2O_2SCl$) C, H, N, S.

**7-Chloro-3-[(1′-methylpropyl)amino]-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (58).** The title compound was obtained as previously described for the compound **59**[16] by starting from the intermediate **57**[16] (0.5 g, 1.90 mmol) and *sec*-butylamine (5 mL) (0.49 g, 89%): mp 240−242 °C; IR (KBr) 3296, 3182 (N−H), 2970, 2934 (C−H aliphatic), 1630, 1583, 1480 (C=N, C=C, N−H), 1250, 1157 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 0.8 (t, 3H, 3-NCH(CH$_3$)CH$_2$C$H_3$), 1.05 (d, 3H, 3-NCH(C$H_3$)CH$_2$CH$_3$), 1.4 (m, 2H, 3-NCH(CH$_3$)C$H_2$CH$_3$), 3.7 (m, 1H, 3-NC$H$(CH$_3$)CH$_2$CH$_3$), 6.95 (bd, 1H, 3-N$H$), 7.10 (d, 1H, 5-H), 7.5 (d, 1H, 6-H), 7.55 (s, 1H, 8-H), 10.3 (s, 1H, 4-N$H$). Anal. ($C_{11}H_{14}N_3O_2SCl$) C, H, N, S.

**7-Chloro-3-[(1′,2′,2′-trimethylpropyl)amino]-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (60).** The title compound was obtained as described for the compound **59**[16] by starting from the intermediate **57**[16] (0.5 g, 1.90 mmol) and 1,2,2-trimethylpropylamine (5 mL) (0.44 g, 73%): mp 288−290 °C; IR (KBr) 3301, 3184 (N−H), 2967 (C−H aliphatic), 1630, 1582, 1480 (C=N, C=C, N−H), 1250, 1161 (S=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 80 MHz) $\delta$ 0.85 (s, 9H, 3-NCH(CH$_3$)C(C$H_3$)$_3$), 1.0 (d, 3H, 3-NCH(C$H_3$)C(CH$_3$)$_3$), 3.7 (m, 1H, 3-NC$H$(CH$_3$)C(CH$_3$)$_3$), 6.8 (bd, 1H, 3-N$H$), 7.10 (d, 1H, 5-H), 7.5 (d, 1H, 6-H), 7.55 (s, 1H, 8-H), 10.2 (s, 1H, 4-N$H$). Anal. ($C_{13}H_{18}N_3O_2SCl$) C, H, N, S.

**Ionization Constants.** The p$K_a$ value of the compounds **19**, **28**, **32**, and **39** was determined spectroscopically by means of 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 p$K_a$ values were calculated by the Debye-Hükkel equation at the maximum basic form absorbance.[32]

**Calculations and Molecular Graphics.** The displacement-ellipsoid view of compound **34** was undertaken with the program ORTEP-II.[33] The molecular design and the isopotential map were performed using SYBIL 6.1 software package[34] on a Silicon Graphics Personal IRIS Indigo Elan workstation. The geometries of diazoxide[27] and of the compound **34** were taken from the X-ray crystallographic structure. The crystallographic models were submitted to AM1 calculations (MOPAC version 6.0)[35] to determine the atomic charge distributions.

**X-ray Crystallography.** A crystal of compound **34** was grown by slow evaporation from a methanol−water solution. Diffraction data were measured at room temperature using a Stoe-Siemens AED four-circle diffractometer. Final cell dimensions of crystal **34** were obtained by a least-squares fit to

the automatically centered setting for 22 reflexions ($70.51 < 2\theta < 78.61°$). Two reference reflexions monitored during each experiment showed no significant variation. Intensity data were corrected for Lorentz polarization effects and for absorption (semiempirical method, $\psi$ scan). 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 86[36]). The least-square refinement (SHELXL 93[37]) included independent position parameters and anisotropic displacement coefficients for all non-hydrogen atoms, and two global isotropic thermal parameters for hydrogen atoms (one for nonmethyl and one for methyl atoms). During refinement, H atoms have been included as riding atoms at calculated positions, except H4(N4) and H(water) in **34**, whose positions obtained from Fourier-difference map were kept fixed. For both experiments, the final difference Fourier map had no significant features. Atomic scattering factors were taken from ref 38.

**Biological Assays. Measurements of Insulin Release from Incubated Islets.** 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, either diazoxide, AO44, or the pyridothiadiazine derivatives. The release of insulin was measured radioimmunologically using rat insulin as a standard.[39] $IC_{50}$ values were graphically assessed.

**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 36 ± 1 °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 Grass force-displacement transducer. After 60 min of equilibration, the rings were exposed to 30 mM KCl. When the tension had stabilized (plateau tension between 1 and 2 g), the agonist was added to the bath at increasing concentrations until maximal relaxation (or until 1 mM). 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. Results were expressed as the mean (± sem) of 3−12 experiments.

**Experiments on Guinea Pig Ileum.** Adult guinea pigs of either sex (body weight: 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 (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), maintained at 37 °C and gassed with a mixture of 5% $CO_2$ and 95% $O_2$. Coaxial stimulation was carried out as described by Paton[40] with rectangular pulses of 0.5 ms duration, 0.1 Hz, 5−25 V delivered and measured by a Grass stimulator. Muscle contractions were recorded isometrically by a transducer.

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).

**Studies on Rat Ventricle.** The contractile activity of right ventricular strips (10 mm length) from adult male Wistar rats (200−250 g) was recorded as described by Finet et al.[41] Tissues were incubated at 30 °C in oxygenated (95% $O_2$−5% $CO_2$) Tyrode's solution (composition (in mM): NaCl 137; KCl 6; $CaCl_2$ 1.8; $MgCl_2$ 1; $NaH_2PO_4$ 0.42; $NaHCO_3$ 11.5; glucose 5.5) under an initial tension of 0.5 g and were stimulated with rectangular 10 ms pulses at a frequency of 1 Hz and 1−10 V. Drugs were added after an initial equilibration period of 90 min and systolic tension was followed for 35−40 min after their addition. Tension in the presence of drugs was expressed as percentage of initial value.

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**Supporting Information Available:** Crystal data for compound **34** (7 pages); observed and calculated structure factors for **34** (4 pages). Ordering information is given on any current masthead page.

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