3-(Alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides as Powerful Inhibitors of Insulin Release from Rat Pancreatic B-Cells: A New Class of Potassium Channel Openers?

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Potassium channels, which are regulated by changes in intracellular levels of adenosine triphosphate (ATP-sensitive K^+ channels or K_{ATP} channels), have been identified in cardiac cells,[1] pancreatic B-cells,[2] skeletal muscle,[3] smooth muscle,[4] central neurons,[5] and adenohypophysis cells.[6] It has been suggested that the so-called potassium channel openers (PCO's), a new class of compounds that specifically enhance K^+ channel permeability of cell membranes, may open the K_{ATP} channel in a number of tissues.[7-10] However, the unique implication of this channel in the mode of action of PCO's still remains controversial in some instances.[10-12] Moreover, since the ability of PCO's to activate—and of antidiabetic sulfonylureas to block—the K_{ATP} channel may vary considerably with tissue localization, a heterogeneous population of the channel has been evoked.[10,13] In particular, PCO's such as cromakalim (1) and pinacidil (2) show stronger activity than diazoxide (3) in vascular tissues,[7,14] whereas diazoxide is much more potent than the former two in opening pancreatic K_{ATP} channel.[15-18] The role of K_{ATP} channels is probably best understood in pancreatic B-cells, where these channels have been shown to mediate the glucose-induced insulin secretion.[19-22] The antidiabetic sulfonylureas are specific blockers of this channel, and therefore induce insulin secretion.[23-25] In contrast, diazoxide is known to decrease insulin release[17,24,26] and to induce hyperglycemia *in vivo*.[27,28] A few examples of compounds structurally related to diazoxide have also been reported to exert hyperglycemic activity [AO 44 (4),[29] LN 5330 (5),[30,31] and some 3-(alkylamino)benzothiadiazines such as 6 initially developed as antihypertensives[32] (see Figure 1)]. Pinacidil was recently investigated as a K^+ channel activator in insulin-secreting cells[16,33,34] and was found to effectively inhibit insulin release, albeit at higher doses than does diazoxide.

It is tempting to speculate that powerful activators of the pancreatic K_{ATP} channel, with an improved tissue selectivity compared to diazoxide, could help further pharmacological and physiological investigations on K_{ATP} channels in various tissues. We have synthesized novel pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides bearing different aminoalkyl side chains in the 3-position (Table I). The pyridine ring may be considered as a bioisostere of a benzene ring bearing an electron-withdrawing substituent (halogeno, nitro, cyano). In particular, the nitrogen atom in the 7-position of the selected pyridothiadiazines is topologically superimposable with the 7-chloro substituent of diazoxide and its analogues. These compounds may also be regarded as sulfonylguanidines, bioisosteres of cyanoguanidines, and, therefore, are structurally related to pinacidil. Moreover, structure–activity relationships of K^+ channel openers have shown that, in the benzopyran series (i.e. cromakalim), the phenyl nucleus bearing an electron-withdrawing group (typically CN) at C6 can be replaced with an N6-pyridyl ring without loss of biological activity.[8,9,35] Inversely, in the pyridylalkylcyanoguanidine series (i.e. pinacidil), the N4-pyridyl nucleus can be replaced with a 4-cyanophenyl moiety with retention of biological activity.[8]

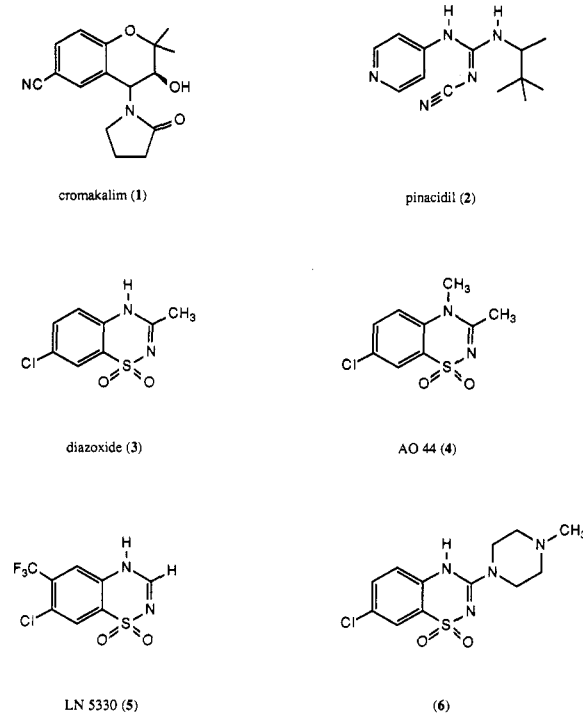

Figure 1. Chemical structure of different potassium channel openers (1–3) and of diazoxide analogues reported to exhibit an hyperglycemic activity (4–6).

Chemistry. Starting from (4-hydroxypyrid-3-yl)sulfonic acid (7),[36] the corresponding 4-chloropyrid-3-yl sulfonamide (10) and -sulfonylguanidine (8) were obtained *via* a 4-chloropyridine-3-sulfonyl chloride intermediate. Direct intramolecular cyclization of 8 gave compound 9. For the other pyridothiadiazines, the key intermediate 14, obtained from 10 in four steps (see Scheme I), was treated with an excess of the appropriate amine to give the respective 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides 15–23 (Table I). Compound 25, which is the 7-chlorobenzothiadiazine analogue of 20, was obtained from the methylthio derivative 24[37] as described above for the pyrido compounds (Scheme II).

Pharmacological Results. The different compounds reported in Table I were tested versus diazoxide as inhibitors of the insulin release from rat pancreatic islets incubated in the presence of glucose (16.7 mM). A 90%

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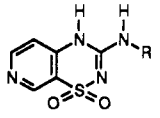
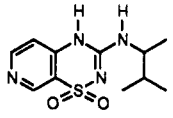
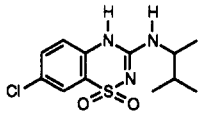
Table I. Percent Residual Insulin Secretion (16.7 mM Glucose)





BPDZ 44

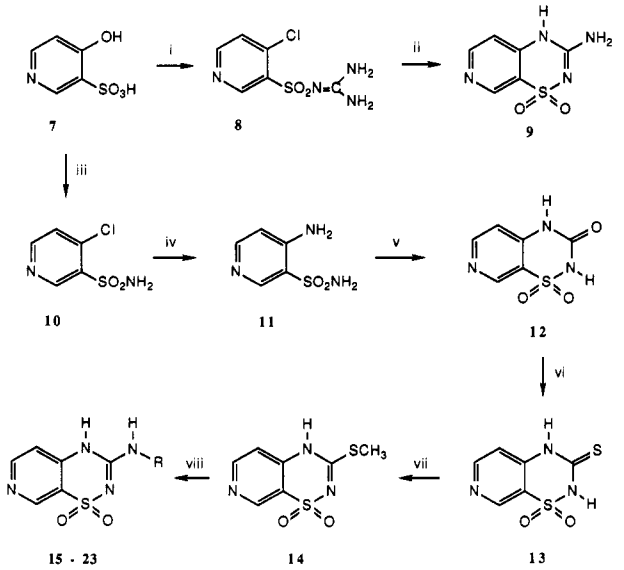


BPDZ 49

compd	int no.[a]	substituent (R)	mp, °C	% residual insulin secretion, at various concentrations (μM) of compound, ±SEM (n)				
				500	100	50	10	1
9	BPDZ 29	H	327–330			104.1 ± 6.5 (14)		
15	BPDZ 39	$(CH_2)_2CH_3$	194–197	9.0 ± 1.0 (15)	21.9 ± 3.4 (14)	57.7 ± 3.8 (14)	87.3 ± 6.7 (15)	97.1 ± 5.9 (8)
16	BPDZ 40	$CH(CH_3)_2$	197–200	8.8 ± 0.9 (8)	18.4 ± 3.9 (8)	42.5 ± 6.9 (8)	55.3 ± 8.1 (8)	110.3 ± 11.3 (6)
17	BPDZ 41	$CH_2(CH_2)_2CH_3$	167–170	9.0 ± 2.0 (8)	32.5 ± 5.0 (8)	38.6 ± 5.6 (7)	82.0 ± 6.2 (8)	83.7 ± 6.2 (8)
18	BPDZ 42	$CH(CH_3)CH_2CH_3$	212–215	9.4 ± 2.1 (7)	7.1 ± 0.6 (8)	9.4 ± 0.9 (8)	49.0 ± 5.4 (7)	97.8 ± 9.1 (8)
19	BPDZ 43	$CH_2CH(CH_3)_2$	221–224	9.7 ± 0.8 (8)	10.2 ± 0.4 (8)	23.4 ± 3.1 (8)	71.8 ± 7.2 (16)	84.2 ± 11.0 (8)
20	BPDZ 44	$CH(CH_3)CH(CH_3)_2$	199–202	8.4 ± 0.9 (24)	8.8 ± 0.9 (24)	8.6 ± 0.9 (23)	13.7 ± 1.2 (23)	70.5 ± 4.4 (19)
21	BPDZ 45	C_5H_9 (cyclopentyl)	237–240	4.4 ± 0.7 (8)	9.2 ± 0.4 (8)	31.5 ± 4.0 (7)	71.1 ± 11.2 (7)	82.8 ± 9.7 (8)
22	BPDZ 46	C_6H_{11} (cyclohexyl)	134–137	6.2 ± 0.5 (8)	18.4 ± 3.5 (8)	40.5 ± 4.3 (8)	64.4 ± 9.5 (7)	74.4 ± 4.5 (8)
23	BPDZ 47	NC_4H_8O (*N*-morpholinyl)	291–293			94.8 ± 6.4 (8)		
25	BPDZ 49	(see above)	238–241	4.6 ± 0.8 (8)	6.5 ± 0.8 (16)	11.0 ± 0.8 (16)	62.7 ± 3.6 (15)	88.9 ± 6.0 (15)
diazoxide[b]				8.5 ● 0.7 (21)	14.0 ± 1.4 (21)	28.8 ± 2.4 (21)	70.0 ± 3.6 (22)	78.9 ± 3.8 (21)

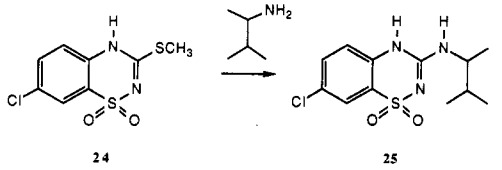
[a] Internal number and denomination. [b] The effect of 500 μM pinacidil was identical to that obtained with 50 μM diazoxide, indicating for pinacidil a 10-fold lower potency on pancreatic B-cells.[16,33]

Scheme I[a]



[a] (i) (1) $OPCl_3$, PCl_5, (2) $(NH_2)_2C{=}NH{\cdot}H_2CO_3$, NaOH; (ii) K_2CO_3, DMF; (iii) (1) $OPCl_3$, PCl_5, (2) NH_4OH; (iv) NH_4OH, Δ; (v) $(NH_2)_2C{=}O$; (vi) P_2S_5, pyridine; (vii) $NaHCO_3$, CH_3I; (viii) RNH_2.

Scheme II



inhibition may be considered as a full effect relative to the glucose-insensitive basal insulin release. As observed in Table I, except for **9**, which is devoid of alkyl substituent on the exocyclic amino group, and for the morpholinyl derivative **23**, the other compounds have shown a strong inhibitory activity on insulin secretion. Some of them, such as **18** (BPDZ 42), **20** (BPDZ 44), and **25** (BPDZ 49), were found to be more active at 10 and 50 μM than diazoxide. The most efficaceous tested is the pyridothiadiazine derivative **20**, which carries a short branched alkyl chain closely related to that of pinacidil. Of particular interest is the improved activity of the pyridothiadiazine **20** compared with the structurally associated benzothiadiazine homologue **25**. As a result, the bioisosteric replacement applied in this particular case was found to be favorable for activity on pancreatic B-cells.

The three best compounds, **18**, **20** and **25**, giving a maximal inhibition of insulin release at 50 μM, were also investigated at the same concentration for their vasorelaxant activity on K^+-depolarized rat aorta. The percentage of decrease in the contractile response to 30 mM KCl (means ± SEM) of the isolated rat aorta were found to be the following (%): 11.0 ± 5.9 (n = 5) for **18**; 31.0 ± 11.7 (n = 5) for **20**; 74.5 ± 13.1 (n = 6) for **25**. Under the same experimental conditions, diazoxide at 50 μM gives 82.7 ± 11.4% relaxation (n = 4), whereas, at the same concentration, pinacidil completely abolishes the response to KCl. These results are in accordance with previous reports describing the effects of these two potassium channel openers in rat aorta.[38,39]

Thus we can observe an improved selectivity of the pyridyl compounds **18** and **20** for the pancreatic tissue compared to the chlorophenyl derivatives diazoxide and **25** since compounds **18** and **20** clearly show a stronger activity on pancreatic B-cells.

BPDZ 44 has been selected for further pharmacological investigations on B-cells. It was found that, like diazoxide, the target of this new compound appears to be the pancreatic K_{ATP} channel.[40]

Since BPDZ 44 is a racemic compound, resolution of the racemate may lead to the isolation of a more efficient K^+ channel opener.

In conclusion, 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides appear to be the most powerful inhibitors of insulin release reported to date. These compounds should be useful tools in further studies of the structure and functions of the ATP-sensitive potassium channel. Ultimately, the better knowledge of structural requirements for channel selectivity may lead to the design of new therapeutic agents acting on specific tissues.

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Supplementary Material Available: Full details on synthetic procedures for the preparation of the different compounds reported, including IR and NMR data, and description of the methodology employed for the two biological assays (9 pages). Ordering information is given on any current masthead page.

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