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Synthesis and Biological Effects of New 3-Alkylamino-4*H*-1,2,4-Benzothiadiazine 1,1-Dioxides on Insulin-secreting Cells

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Abstract

3-Alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides with nitro, amino or acetylamino groups in the 7-position have been synthesized in an attempt to discover new tissue-selective K_{ATP}-channel openers.

The compounds were tested as putative pancreatic β-cells K_{ATP}-channel openers by measuring their inhibitory activity on the insulin releasing process. The influence of the substituent in the 7-position on the acidic character (pK_a) and on biological activity is discussed.

The nitrobenzene derivatives were biologically active, but less so than the un-derivatized parent pyridothiadiazine dioxides.

Potassium channels, proteinic trans-membrane structures involved in the movement of potassium ions, are crucially important in controlling cell membrane potential (Cook 1988). Among these, ATP-sensitive potassium channels (or K_{ATP} channels) have been the focus of intensive research in the last decade. They have been identified in a large variety of cells, for example cardiac cells (Noma 1983), pancreatic β-cells (Cook & Hales 1984), skeletal and smooth muscle cells (Standen et al 1989; Allard & Lazdunski 1993) and central neurons (Bernardi et al 1988). Depending on their location, K_{ATP} channels are involved in important physiological processes. In pancreatic β-cells, they have been shown to mediate glucose-induced insulin secretion (Henquin 1990; Lebrun 1993) and in smooth muscle cells they control muscle tone and contractility (Cook & Quast 1990; O'Donnell & Owen 1994; Quayle et al 1997).

Hypoglycaemic sulphonylureas such as tolbutamide or glibenclamide are known to be K_{ATP}-channel blockers (Gylfe et al 1984; Schmid-Antomarchi et al 1987; Boyd 1988). In contrast, compounds such as diazoxide, cromakalim, pinacidil and nicorandil are regarded as K_{ATP}-channel openers (Lebrun et al 1988, 1989; Hamilton & Weston 1989; Antoine et al 1992; Longman & Hamilton 1992).

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Pinacidil and cromakalim have greater activity on smooth muscle cells (relaxation) than on pancreatic β-cells (inhibition of insulin release). Diazoxide, however, is equipotent on both tissues.

We previously synthesized 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides, with different aminoalkyl side-chains in the 3-position (Pirotte et al 1993, 1994; de Tullio et al 1996; Lebrun et al 1996), which can be regarded as hybrid compounds between diazoxide and pinacidil (Figure 1). Pharmacological investigation has recently shown that compounds such as BPDZ 42 (3-(2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide) and BPDZ 44 (3-(3′-methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide) have the pharmacological profiles of K_{ATP}-channel openers (Pirotte et al 1994; Khelili et al 1999). In contrast with diazoxide, which is devoid of tissue selectivity, these two pyridothiadiazine dioxides seemed to be more active on endocrine pancreatic tissue (inhibition of insulin release) than on vascular tissue.

To discover new tissue-selective K_{ATP}-channel openers, we have synthesized and characterized the biological effects of new 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides bearing a nitro group in the 7-position and different short and branched aminoalkyl side chains in the 3-position (Figure 1). Such compounds are expected to be good analogues of the pyridothiadiazine dioxides, because a

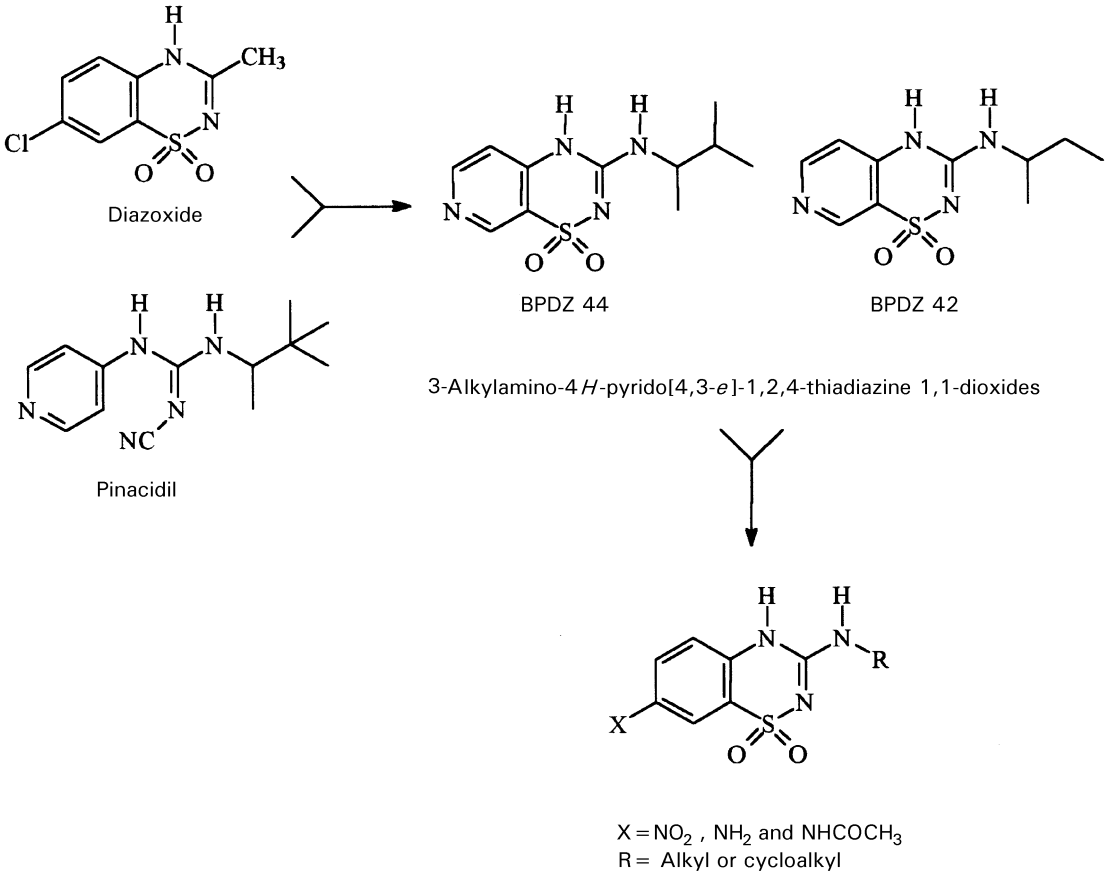


Figure 1. Diazoxide, pinacidil, 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides and 7-nitro-, 7-amino-, 7-acetamido-4*H*-1,2,4-benzothiadiazine 1,1-dioxides related to BPDZ 42 or BPDZ 44.

benzene ring with a nitro group is usually considered to be a classical isostere of the pyridinic ring. Reduction of the nitro group to the amino group, and subsequent acetylation, have also been performed to give the 7-amino- and 7-acetamido-substituted derivatives.

Materials and Methods

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 1000 FT spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AW-80 (80 MHz) using d_6-DMSO as solvent. Chemical shifts (δ ppm) are relative to hexamethyldisiloxane, which was used as internal reference. NH signals appeared as broad singlets exchangeable with D_2O. In the data below, s = singlet, d = doublet, t = triplet, m = multiplet, b = broad.

2-Chloro-5-nitrobenzenesulphonamide

Glacial acetic acid (160 mL) was saturated with gaseous sulphur dioxide and then mixed with an aqueous solution of cupric chloride (7 g/20 mL).

A solution of 2-chloro-5-nitroaniline (10 g) in glacial acetic acid (160 mL) and 12 M HCl (40 mL) was cooled to −5°C and a solution of sodium nitrite (4 g) in water (20 mL) was then added dropwise, with stirring, to the cold solution.

The diazonium salt formed was added, with stirring, to the cold solution of sulphur dioxide in acetic acid prepared previously. After 10 min the mixture was poured on ice (200 g). The resulting precipitate was collected by filtration, washed with water and suspended in 10% aqueous ammonia (200 mL). After stirring at room temperature for 30 min the mixture was concentrated to half the volume by evaporation under reduced pressure. The resulting suspension was adjusted to pH 1 with 12 M HCl. The insoluble material was collected by filtration, washed with water and crystallized from methanol–water (yield 8·8 g; mp 174–176°C).

2-Amino-5-nitrobenzenesulphonamide
A suspension of 2-chloro-5-nitrobenzenesulphonamide (8 g) in concentrated aqueous ammonia (80 mL) was saturated with ammonia just before its introduction to a sealed vessel. It was then placed in an autoclave and heated at 120°C for 5 h. The reaction mixture was then concentrated to half its volume by evaporation under reduced pressure and the resulting precipitate was collected by filtration, washed with water and dried (yield 6·1 g; mp 202–204°C).

*3-(1*H*-Imidazol-1-yl)-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide imidazolium salt*
Thiocarbonyldiimidazole (14 g) was added to a hot solution of 2-amino-5-nitrobenzenesulphonamide (5 g) in dioxane (150 mL) and the mixture was heated under reflux for 5 h. After cooling, the precipitate of the title compound was collected by filtration, washed with dioxane and dried (yield 5·7 g; mp 246–248°C).

*General procedure for preparation of 3-alkylamino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides*
A mixture of 3-(1*H*-imidazol-1-yl)-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (0·5 g) and the appropriate alkylamine (5 mL) was heated in a sealed vessel at 150°C for 5 to 6 h until completion of the reaction as monitored by thin-layer chromatography (TLC). Excess amine was removed by distillation under reduced pressure and the oily residue was dispersed in water. NaOH (2·5 M) was added to the suspension until dissolution was complete. The resulting solution was treated with charcoal, filtered, and the filtrate was adjusted to pH 5–6 with 6 M HCl. The precipitate was collected by filtration, washed with water and recrystallized from methanol–water (yield 60–70%).

*3-Isopropylamino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**5**)*
mp 311–313°C. IR (KBr): 3363, 3221, 2980, 1657, 1646, 1615, 1601, 1572, 1532, 1494, 1339, 1284, 1267, 1155, 1106 cm^{-1}. ^{1}H NMR (DMSO-d_6, HMDS; δ ppm): 1·10 (d, 6H, 2 × CH_3), 3·90 (m, 1H, NH-C*H*), 7·35 (m, 2H, 5-H + N*H*-CH), 8·35 (m, 2H, 6-H + 8-H), 10·90 (bs, 1H, NH).

*3-(2-Butyl)amino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**6**)*
mp 287–288°C. IR (KBr): 3354, 3223, 3111, 2969, 2935, 2877, 2853, 2361, 1601, 1573, 1529, 1489, 1452, 1401, 1383, 1280, 1245, 1178, 1130, 1104, 1056 cm^{-1}. ^{1}H NMR (DMSO-d_6, HMDS; δ ppm): 0·80 (t, 3H, CH_2-CH_3), 1·10 (d, 3H, CH-CH_3), 1·50 (m, 2H, C$H_2$$CH_3$), 3·70 (m, 1H, NH-C*H*), 7·30 (m, 2H, 5-H + N*H*-CH), 8·30 (m, 2H, 6-H + 8-H), 10·90 (bs, 1H, NH).

*3-(3-Methyl-2-butyl)amino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**7**)*
mp 308–311°C. IR (KBr): 3294, 3199, 3101, 2965, 1636, 1600, 1566, 1538, 1497, 1485, 1349, 1252, 1166, 1153, 1107 cm^{-1}. ^{1}H NMR (DMSO-d_6, HMDS; δ ppm): 0·80 (d, 6H, CH-(CH_3)$_2$), 1·10 (d, 3H, CH-CH_3), 1·70 (m, 1H, C*H*-(CH_3)$_2$), 3·65 (m, 1H, NH-C*H*), 7·20 (m, 2H, 5-H + N*H*-CH), 8·30 (m, 2H, 6-H + 8-H), 10·80 (bs, 1H, NH).

*3-(Cyclopropylmethyl)amino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**8**)*
mp 276–278°C. IR (KBr): 3290, 3193, 3104, 2994, 1635, 1603, 1564, 1539, 1499, 1486, 1347, 1276, 1251, 1153, 1111 cm^{-1}. ^{1}H NMR (DMSO-d_6, HMDS; δ ppm): 0·50–1·30 (bm, 5H, 2 × CH_2 + CH), 3·10 (t, 2H, NH-CH_2-CH), 7·35 (m, 2H, 5-H + N*H*-CH), 8·30 (m, 2H, 6-H + 8-H), 11·15 (bs, 1H, NH).

*7-Nitro-3-propylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**9**)*
mp 268–270°C. IR (KBr): 3307, 3192, 3166, 3086, 3018, 2971, 2882, 1642, 1600, 1561, 1533, 1498, 1486, 1399, 1339, 1280, 1245, 1167, 1111 cm^{-1}. ^{1}H NMR (DMSO-d_6, HMDS; δ ppm): 0·85 (t, 3H, CH_3-CH_2), 1·50 (m, 2H, CH_2-CH_3), 3·10 (m, 2H, NH-CH_2), 7·35 (m, 2H, 5-H + N*H*-CH), 8·30 (m, 2H, 6-H + 8-H), 11·10 (bs, 1H, NH).

*3-Cyclobutylamino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**10**)*
mp 298–301°C. IR (KBr): 3305, 3192, 3087, 2998, 1640, 1598, 1567, 1532, 1497, 1484, 1400, 1341, 1283, 1249, 1163, 1113 cm^{-1}. ^{1}H NMR (DMSO-d_6, HMDS; δ ppm): 1·30–2·60 (bm, 6H, 3 × CH_2), 4·20 (m, 1H, NH-C*H*), 7·35 (d, 1H, 5-H), 7·70 (bd, 1H, N*H*-CH), 8·30 (m, 2H, 6-H + 8-H), 10·95 (bs, 1H, NH).

*3-Alkylamino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**11**)*
mp 233–236°C. IR (KBr): 3308, 3186, 3092, 2992, 1636, 1599, 1562, 1533, 1497, 1484, 1426, 1397, 1340, 1280, 1246, 1162, 1112 cm^{-1}. ^{1}H NMR (DMSO-d_6, HMDS; δ ppm): 3·90 (bt, 2H, NH-CH_2), 5·15 (m, 2H, CH = CH_2), 5·60–6·10 (m, 1H,

C*H*=CH_2), 7·30 (d, 1H, 5-H), 7·60 (m, 1H, N*H*-CH_2), 8·30 (m, 2H, 6-H + 8-H), 11·20 (bs, 1H, NH).

*7-Amino-3-isopropylamino-4*H-*1,2,4-benzothiadiazine 1,1-dioxide (**12**)*

Pd/C (10%, 0·06 g) was added to a solution of 3-isopropylamino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **5** (0·6 g) in hot methanol (25 mL) and the mixture was treated with hydrogen gas under pressure (4 bar) for 1 h at 40°C. Insoluble material was removed by filtration and the filtrate was concentrated to dryness by evaporation under reduced pressure. The residue of the crude title compound was recrystallized from methanol–water (yield 0·45 g).

mp 278–283°C. IR (KBr): 3455, 3363, 3216, 2972, 1618, 1575, 1510, 1298, 1255, 1174, 1146, 1113 cm^{-1}. ^1H NMR (DMSO-d_6, HMDS; δ ppm): 1·10 (d, 6H, 2 × CH_3), 3·85 (m, 1H, NH-C*H*), 5·15 (bs, 2H, NH_2), 6·50–6·95 (bm, 4H, 5-H + 6-H + 8-H + N*H*-CH), 9·75 (bs, 1H, NH).

*7-Acetylamino-3-isopropylamino-4*H-*1,2,4-benzothiadiazine 1,1-dioxide (**13**)*

A mixture of 3-isopropylamino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **5** (0·18 g) and acetic anhydride (0·6 mL) in dioxane (2 mL) was stirred at room temperature. The mixture was then mixed with water (5 mL) and stirred for 1 h. The solvents were removed by distillation under reduced pressure and the residue of the crude title compound was recrystallized from methanol–water (yield 0·16 g).

mp 260–262°C. IR (KBr): 3353, 3316, 3240, 3101, 3069, 2974, 1672, 1624, 1608, 1581, 1546, 1497, 1467, 1391, 1279, 1159, 1142, 1123, 1104 cm^{-1}. ^1H NMR (DMSO-d_6, HMDS; δ ppm): 1·10 (d, 6H, 2 × CH_3), 2·00 (s, 3H, COCH_3), 3·90 (m, 1H, NH-C*H*), 6·85 (bd, 1H, N*H*-CH), 7·05 (d, 1H, 5-H), 7·60 (bd, 1H, 6-H), 8·00 (bs, 1H, 8-H), 10·00, (bs, 1H, CONH), 10·10 (bs, 1H, NH).

Results and Discussion

Chemistry

2-Amino-5-nitrobenzenesulphonamide, a key intermediate in the preparation of the final products, was obtained in a two-step reaction starting from 2-chloro-5-nitroaniline (**1**) (Figure 2). The aniline (**1**) was transformed by diazotization into the corresponding 2-chloro-5-nitrobenzenesulphonamide (**2**) and subsequent substitution of the chlorine atom in the 2-position, with concentrated ammonia under pressure at 150°C, led to the key intermediate (**3**). The synthesis of the 3-(1*H*-imidazol-1-yl)-substituted intermediate (**4**) was achieved by reaction of 2-amino-5-nitrobenzenesulphonamide (**3**) with excess 1,1-thiocarbonyldiimidazole in dioxane.

The 3-alkylamino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**5**–**11**) were obtained by reaction of 3-(1*H*-imidazol-1-yl)-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**4**) with excess of the appropriate alkylamine (Figure 2). The reaction occurs with good yields in an hermetically closed autoclave heated at 150°C for 4 to 6 h.

The 7-amino-substituted derivative (**12**) was prepared from **5** by reduction of the nitro function under hydrogen gas, with palladium on activated carbon as catalyst (Figure 3). Subsequent acetylation of **12** with acetic anhydride led to the corre-

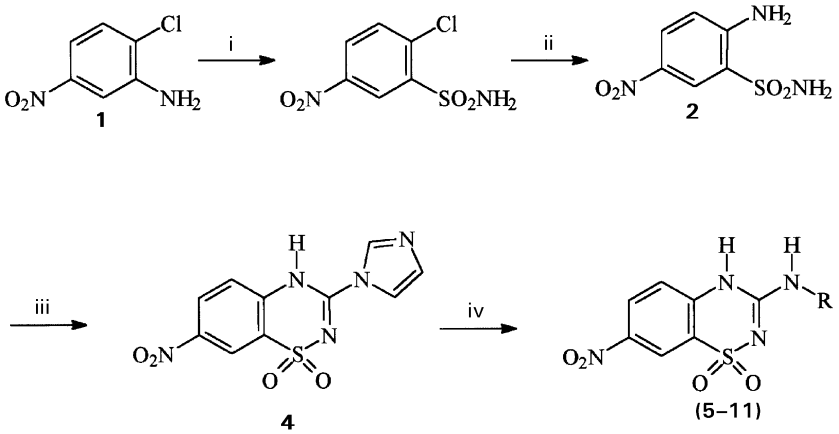


Figure 2. Synthesis of 3-aminoalkyl-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1 dioxides. i. $NaNO_2$, HCl; CH_3COOH, SO_2, $CuCl_2$, NH_4OH dil.; ii. NH_4OH conc., 150°C; iii. 1,1-thiocarbonyldiimidazole; dioxane; iv. RNH_2.

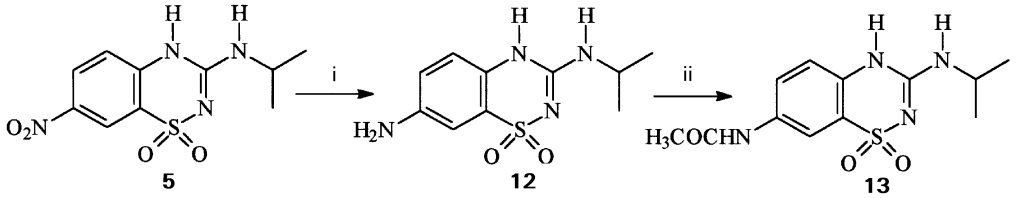


Figure 3. Synthesis of the 7-amino- and 7-acetamido-substituted derivatives. i. H_2, 4 bar, palladium; ii. $(CH_3CO)_2O$.

sponding 7-acetamido-substituted compound (**13**) (Figure 3).

Pharmacological evaluation

The different compounds reported in Table 1 were tested as inhibitors of insulin release from rat pancreatic islets incubated in the presence of an insulinotropic glucose concentration (16·7 mM). The drugs were tested at a concentration of 50 μM and the most active compounds were also examined at 10 μM. Results were compared with those obtained by use of three reference drugs—pinacidil, diazoxide and BPDZ 44 (a pyridothiadiazine dioxide).

It is apparent from Table 1 that with the exception of compound **5** the activity of the newly synthesized 3-alkylamino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides on pancreatic β-cells was moderate or poor. Two compounds, **6** and **10**, were more potent than pinacidil but less potent than diazoxide and BPDZ 44. Comparison of the 7-nitro-substituted derivative (**5**) with its 7-amino- and 7-acetamido-substituted counterparts (**12** and **13**) showed the biological activity of **12** and **13** to be substantially lower. Thus, reduction of the nitro group does not seem to be a suitable means of maintaining or increasing their activity as inhibitors of insulin release.

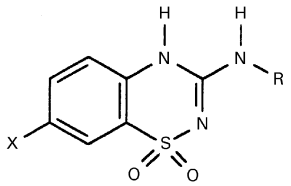
The most potent compound of the series, the 3-isopropylamino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide derivative (**5**), markedly inhibited insulin release. At a concentration of 10 μM, however, the drug was less potent than BPDZ 44.

It can be concluded that an isopropylamino side-chain in the 3-position of the benzothiadiazine ring constitutes, in this series, one of the best choices of lateral substituent.

Determination of pK_a

The hydrogen atom in the N-4 position of the thiadiazine ring is expected to be labile and can be

Table 1. Effects of 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides, diazoxide, pinacidil and BPDZ 44 on insulin release from pancreatic β-cells, and pK_a values of selected compounds.



Compound	X	R	% RIS ± s.e.m. (n)		pK_a
			50 μM	10 μM	
5	NO_2	$CH(CH_3)_2$	4·8 ± 0·6 (14)	64·0 ± 3·8 (37)	7·91
6	NO_2	$CH(CH_3)CH_2CH_3$	63·3 ± 3·8 (18)	ND	ND
7	NO_2	$CH(CH_3)CH(CH_3)_2$	90·4 ± 5·2 (16)	ND	ND
8	NO_2	CH_2-cyclopropyl	95·7 ± 6·0 (16)	ND	ND
9	NO_2	$CH_2CH_2CH_3$	88·9 ± 4·6 (16)	ND	ND
10	NO_2	Cyclobutyl	59·6 ± 4·9 (13)	ND	ND
11	NO_2	$CH_2CH=CH_2$	82·7 ± 5·8 (15)	ND	ND
12	NH_2	$CH(CH_3)_2$	85·8 ± 4·3 (15)	ND	10·49
13	$NHCOCH_3$	$CH(CH_3)_2$	89·3 ± 5·8 (16)	ND	9·80
Diazoxide	–	–	28·8 ± 2·5 (21)[a]	70·0 ± 3·6 (22)[a]	8·62[a]
Pinacidil	–	–	92·7 ± 8·0 (14)[a]	99·3 ± 8·9 (13)[a]	ND
BPDZ 44	–	–	7·1 ± 0·6 (14)[a]	26·7 ± 1·8 (21)[a]	≈ 8·1

RIS is the percentage residual insulin secretion from rat pancreatic islets incubated in the presence of 16·7 mM glucose. ND, not determined. [a]Published results.

removed by aqueous alkali. The pK_a of 1,2,4-benzothiadiazine 1,1-dioxides is modulated by the nature and position of the substituent on the benzene ring. Electron-withdrawing groups should increase acidity whereas electron-donor groups should reduce it.

The pK_a values of compounds **5**, **12** and **13** were determined by UV spectrophotometry, using the Debye–Hückel (Albert & Serjeant 1971) relationship linking pK_a with the experimental optical density. The results are reported in Table 1. As expected, the pK_a values correlated directly with the electron-withdrawing character of the substituent in the 7-position. The 7-nitro-substituted derivative (**5**), representative of the nitrobenzene isosteres, was found to be more acidic (pK_a 7·91) than diazoxide (pK_a 8·62). Compared with 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides (pK_a values $\approx$ 8·1) (de Tullio et al 1996), at physiological pH (7·4) the nitrobenzene isosteres (**5**–**11**) are probably present in solution to a greater extent as ionic species. Such species are expected to be ineffective because it can be predicted that only the non-ionic form of the drug should be able to interact favourably with the receptor binding site (de Tullio et al 1996). Thus, except for the 3-isopropylamino-substituted compound (**5**), the poor biological activity of the 3-alkylamino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**6**–**11**) could be explained, at least in part, by their more pronounced acidicity than their pyridothiadiazinic isosteres.

The 7-amino-substituted compound (**12**) had the highest pK_a (10·49). Thus, reduction of the nitro group to the amino electron-donor group reduces the acidic character of the molecule. Finally, the 7-acetamido-substituted derivative (**13**) with intermediate electron-withdrawing character was found to have an intermediate pK_a (9·80). Although these two compounds (**12**, **13**) would exist in solution mainly as non-ionized species, they are poorly active in contrast with their 7-nitro-substituted counterpart (**5**). Thus **12** and **13** are not acceptable bioisosteres of the active pyridothiadiazines.

Conclusions

Some of the newly synthesized 3-alkylamino-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides inhibit insulin release and could thus be considered as putative K_{ATP}-channel openers. Their biological activity is modulated by the nature of the aminoalkyl side chain. Short branched chains result in the maximum effect (isopropyl > cyclobutyl $\approx$ 2-butyl). Biological activity is also affected by the nature of the substituent in the 7-position. Indeed, an electron-withdrawing group seems to promote activity.

In conclusion, although the nitrobenzene analogues of the active 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides are acceptable bioisosteres, their biological activity on β-cells was less pronounced.

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References

Albert, A., Serjeant, E. P. (1971) Determination of ionization constants by spectrometry. In: The Determination of Ionization Constants. Chapman & Hall, London, 44–64

Allard, B., Lazdunski, M. (1993) Pharmacological properties of ATP-sensitive K^+ channels in mammalian skeletal muscle cells. Eur. J. Pharmacol. 236: 419–426

Antoine, M.-H., Berkenboom, G., Fang, Z. -Y., Fontaine, J., Herchuelz, A., Lebrun, P. (1992) Mechanical and ionic response of rat aorta to diazoxide. Eur. J. Pharmacol. 216: 299–306

Bernardi, H., Fosset, M., Lazdunski, M. (1988) Purification and affinity labelling of brain (^{3}H) glibenclamide binding protein, a putative neuronal ATP-regulated K^+ channel. Proc. Natl Acad. Sci. USA 85: 9816–9820

Boyd, A. E. (1988) Sulfonylurea receptors, ion channels and fruit flies. Diabetes 37: 847–850

Cook, D. L., Hales, C. N. (1984) Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. Nature 311: 271–273

Cook, N. S. (1988) The pharmacology of potassium channels and their therapeutic potential. Trends Pharmacol. Sci. 9: 21–28

Cook, N. S., Quast, U. (1990) Potassium channels pharmacology.In: Cook, N. S. (ed.) Potassium Channels, Structure, Classification, Function and Therapeutic Potential, Ellis Horwood, Chichester, UK, pp 181–255

de Tullio, P., Pirotte, B., Lebrun, P., Fontaine, J., Dupont, L., Antoine, M. H., Ouedraogo, R., Khelili, S., Maggetto, C., Masereel, B., Diouf, O., Podona, T., Delarge, J. (1996) 3- and 4-Substituted 4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides as potassium-channel openers: synthesis, pharmacological evaluation and structure-activity relationships. J. Med. Chem. 39: 937–947

Gylfe, E., Hellman, B., Sehlin, J., Täljedal, I. B. (1984) Interaction of sulfonylureas with the pancreatic β-cell. Experientia 40: 1126–1135

Hamilton, T. C., Weston, A. H. (1989) Cromakalim, nicorandil and pinacidil: novel drugs which open potassium channels in smooth muscle. Gen. Pharmacol. 20: 1–9

Henquin, J. C. (1990) Les mécanismes cellulaires du contrôle de la sécrétion d'insuline. Arch. Int. Physiol. Biochim. 98: A61–A81

Khelili, S., de Tullio, P., Antoine, M.-H., Ouedraogo, R., Dupont, L., Fontaine, J., Felekidis, A., Leclerc, G., Delarge, J., Pirotte, B. (1999) Preparation and pharmacological evaluation of the *R* and *S* enantiomers of 3-(2′-butylamino)-4*H*- and 3-(3′-methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, two tissue-selective ATP-sensitive potassium channel openers. Bioorg. Med. Chem. 7: 1513–1520

Lebrun, P. (1993) Flux cationiques dans les cellules B des îlots pancréatiques et investigations pharmacologiques. Rev. Franç. Endocrinol. Clin. 34: 241–254

Lebrun, P., Devreux, V., Hermann, M., Herchuelz, A. (1988) Pinacidil inhibits insulin release by increasing K^+ outflow from pancreatic β-cells. Eur. J. Pharmacol. 156: 283–286

Lebrun, P., Devreux, V., Hermann, M., Herchuelz, A. (1989) Similarities between the effects of pinacidil and diazoxide on ionic and secretory events in rat pancreatic islets. J. Pharmacol. Exp. Ther. 250: 1011–1018

Lebrun, P., Antoine, M.-H., Ouedraogo, G., Dunne, M., Kane, C., Hermann, M., Herchuelz, A., Masereel, B., Delarge, J., de Tullio, P., Pirotte, B. (1996) Activation of ATP-dependent K^+ channels and inhibition of insulin release: effect of BPDZ 62. J. Pharmacol. Exp. Ther. 277: 156–162

Longman, S. D., Hamilton, T. C. (1992) Potassium channel activator drugs: mechanism of action, pharmacological properties, and therapeutic potential. Med. Res. Rev. 12: 73–148

Noma, A. (1983) ATP-regulated K^+ channels in cardiac muscle. Nature 305: 147–148

O'Donnell, M. E., Owen, N. E. (1994) Regulation of ion pumps and carriers in vascular smooth muscle. Physiol. Rev. 74: 683–721

Pirotte, B., de Tullio, P., Lebrun, P., Antoine, M. H., Fontaine, J., Masereel, B., Schynts, M., Dupont, L., Herchuelz, A., Delarge, J. (1993) 3-(Alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides as powerful inhibitors of insulin release from rat pancreatic β-cells: a new class of potassium channel openers? J. Med. Chem. 36: 3211–3213

Pirotte, B., Antoine, M. H., de Tullio, P., Hermann, M., Herchuelz, A., Delarge, J., Lebrun, P. (1994) A pyridothiadiazine (BPDZ 44) as a new and potent activator of ATP-sensitive K^+ channels. Biochem. Pharmacol. 47: 1381–1386

Quayle, J. M., Nelson, M. T., Standen, N. B. (1997) ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. Physiol. Rev. 77: 1165–1232

Schmid-Antomarchi, H., de Weille, J., Fosset, M., Lazdunski, M. (1987) The receptor for antidiabetic sulfonylureas controls the activity of the ATP-modulated K^+ channel in insulin-secreting cells. J. Biol. Chem. 262: 15840–15844

Standen, N. B., Quayle, J. M., Davies, N. W., Brayden, J. E., Huang, Y., Nelson, M. T. (1989) Hyperpolarising vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. Science 245: 177–180