JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 973–980

Received December 19, 2000
Accepted April 23, 2001
ISSN 0022-3573

Effect on insulin release of compounds structurally related to the potassium-channel opener 7-chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (BPDZ 73): introduction of heteroatoms on the 3-alkylamino side chain of the benzothiadiazine 1,1-dioxide ring

S. Boverie, M.-H. Antoine, P. de Tullio, F. Somers, B. Becker, S. Sebille, P. Lebrun and B. Pirotte

Abstract

7-Chloro-3-pyridyl(alkyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides and 3-alkylamino-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides containing one or more heteroatoms on the side chain in the 3 position have been synthesized in an attempt to discover new potent K_{ATP}-channel openers. The compounds were tested as putative pancreatic B-cells K_{ATP} channel openers by measuring their inhibitory activity on the insulin releasing process. The influence on the biological activity of the nature of the side chain in the 3 position is discussed.

Laboratoire de Chimie Pharmaceutique, Université de Liège, 1, avenue de l'hôpital, B-4000 Liège, Belgium

S. Boverie, P. de Tullio, F. Somers, S. Sebille, B. Pirotte

Laboratoire de Pharmacodynamie et de Thérapeutique, Université Libre de Bruxelles, 808, route de Lennik, B-1070 Bruxelles, Belgium

M.-H. Antoine, B. Becker, P. Lebrun

Correspondence: S. Boverie, Laboratoire de Chimie Pharmaceutique, Université de Liège, 1, avenue de l'hôpital, B-4000 Liège, Belgium.

Acknowledgements and Funding: This study was supported by grants from the National Fund for Scientific Research (F.N.R.S Belgium) from which P. Lebrun is Research Director and P. de Tullio and B. Pirotte Senior Research Associates. B. Becker was supported by a grant-in-aid from the Université Libre de Bruxelles. The assistance of M. Hermann, D. Dewalque, M. L. Pirard and J. Sergooris is gratefully acknowledged.

Introduction

ATP-sensitive potassium channels (K_{ATP} channels) play a crucial role in controlling cell membrane potential (Cook 1988; Bryan & Aguilar-Bryan 1997) and many cell types link membrane excitability to their metabolic state via K_{ATP} channels. These channels have been characterized in numerous cell types such as cardiac cells (Noma 1983), pancreatic B-cells (Cook & Hales 1984), skeletal muscle cells (Allard & Lazdunski 1993), smooth muscle cells (Standen et al 1989; Quayle et al 1997) and central neurons (Bernardi et al 1988). Over the past few years there has been an increasing interest in drugs (diazoxide, pinacidil, cromakalim) involved in the activation of K_{ATP} channels. Such compounds, named potassium-channel openers, mainly exert their biological effects by promoting membrane hyperpolarisation (Lebrun et al 1988, 1989; Hamilton & Weston 1989). Thus, potassium-channel openers are able to interfere with several physiological processes such as insulin release from pancreatic B-cells (Lebrun et al 1992) and contractile activity from different smooth muscle cells (Cook & Quast 1990). Previous work performed in our laboratories led to the development of original 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides. These compounds could be regarded as hybrid compounds between diazoxide and pinacidil. In this series, BPDZ 44 (3-(3′-methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide) was found to be much more potent than diazoxide at reducing the glucose-induced insulin release

from rat pancreatic islets (Pirotte et al 1993, 1994; de Tullio et al 1996). To discover new K_{ATP}-channel openers, we have prepared and tested benzenic counterparts of BPDZ 44 series (Figure 1). From those, it appeared that BPDZ 73 (7-chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide) was the most potent pancreatic K_{ATP}-channel activator reported to date (Lebrun et al 2000). In the search for original benzothiadiazine dioxides acting as potassium-channel openers, we have now synthesized new 4*H*-1,2,4-benzothiadiazine 1,1-dioxides bearing a chlorine atom in the 7 position and different pyridyl(alkyl)amino- or chemically diverse heteroatom(s)-containing alkylamino- side chains in the 3 position. These original compounds were examined as potassium-channel openers on rat pancreatic islets.

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. The ^{1}H NMR spectra were taken on a Bruker AW-80 (80 MHz) instrument in DMSO-d_6 with HMDS (hexamethyldisiloxone) as internal standard; chemical shifts are reported in δ values (ppm) relative to internal HMDS. In the data below, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. All reactions were routinely checked by TLC on silica gel Merck 60F 254.

*7-Chloro-3-(2′,2′,2′-trifluoroethyl)amino-4*H-*1,2,4-benzothiadiazine 1,1-dioxide (**7**)*

A mixture of 7-chloro-3-(methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**4**) (Di Bella et al 1972) (0.5 g, 2.02 mmol) and 5 mL of 2,2,2-trifluoroethylamine was heated in a sealed vessel at 160°C for 8 h. The excess of 2,2,2-trifluoroethylamine was removed under reduced pressure and the 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 3–4 with 6 M HCl. The precipitate was collected by filtration, washed with water and recrystallized from methanol–water (50:50) (yield 60%). Mp 302–304°C. IR (KBr): 3283, 3186, 1627, 1582, 1483, 1265, 1161 cm^{-1}. ^{1}H NMR (DMSO-d_6, HMDS; δ ppm): 4.00 (q, 2H, NHCH_2), 7.20 (d, 1H, 5-*H*), 7.60 (d + s, 2H, 6-*H* + 8-*H*), 11.00 (bs, 1H, N*H*).

*3-(2-Aminoethyl)amino-7-chloro-4*H-*1,2,4-benzothiadiazine 1,1-dioxide monohydrate (**8**)*

A mixture of 7-chloro-3-(methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**4**) (0.5 g, 2.02 mmol) and 5 mL of ethylenediamine was heated at 160°C until completion of the reaction as monitored by TLC. The excess of amine was removed by distillation under reduced pressure and the residue was dissolved in methanol and then supplemented with ether. The precipitate was collected by filtration and dissolved in 40 mL of hot water. The solution was treated with charcoal. After filtration, the solution was concentrated by distillation under reduced pressure and cooled at 4°C for 4 h. The title compound was collected by filtration, washed with cold water and dried (yield 77%). Mp 200–204°C. IR (KBr): 3498, 1637, 1560, 1481, 1264, 1163 cm^{-1}. ^{1}H NMR (DMSO-d_6, HMDS; δ ppm): 2.70 (t, 2H, $CH_2CH_2NH_2$), 3.20 (t, 2H, $NHCH_2CH_2NH_2$), 5.25 (b, 6H, 2 x $NH + H_2O + NH_2$), 6.95 (d, 1H, 5-*H*), 7.30 (d, 1H, 6-*H*), 7.45 (s, 1H, 8-*H*).

*7-Chloro-3-(2-formylaminoethyl)amino-4*H-*1,2,4-benzothiadiazine 1,1-dioxide (**15**)*

Formic acid (1 mL) and acetic anhydride (2 mL) were heated at 50°C for 20 min. After cooling, 3-(2-aminoethyl)amino-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide monohydrate (**8**) (0.5 g, 1.8 mmol) was added to the solution and stirred for 2 h at room temperature. After addition of water (20 mL) and stirring for 15 min, the crude precipitate was collected by filtration, washed with water and dried (yield 89%). Mp 245–247°C. IR (KBr): 3374, 1662, 1646, 1571, 1485, 1274, 1178 cm^{-1}. ^{1}H NMR (DMSO-d_6, HMDS; δ ppm): 3.10–3.60 (b, 4H, NH(CH_2)$_2$, 7.25 (d, 2H, 5-*H* + N*H*CH_2CH_2), 7.60 (dd, 1H, 6-*H*), 7.90 (d, 1H, 8-*H*), 8.05 (s, 1H, NHCO*H*), 10.90 (bs, 1H, N*H*).

*7-Chloro-3-(2-acetylaminoethyl)amino-4*H-*1,2,4-benzothiadiazine 1,1-dioxide (**16**)*

The title compound was obtained from 3-(2-aminoethyl)amino-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide monohydrate (**8**) (0.5 g, 1.8 mmol) and acetic anhydride (3 mL) by following the experimental conditions described for 7-chloro-3-(2-formylaminoethyl)-amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**15**) (yield 87%). Mp 295–297°C. IR (KBr): 3333, 1663, 1625, 1576, 1483, 1273, 1180 cm^{-1}. ^{1}H NMR (DMSO-d_6, HMDS; δ ppm): 1.70 (s, 3H, NHCOCH_3), 3.05–3.40 (b, 4H, NH(CH_2)$_2$NH), 7.15 (d, 2H, 5-*H* + N*H*CH_2CH_2), 7.50 (dd, 1H, 6-*H*), 7.60 (d, 1H, 8-*H*), 7.90 (bs, 1H, N*H*COCH_3), 10.80 (s, 1H, N*H*).

*7-Chloro-3-(pyrid-2-yl)methylamino-4*H-*1,2,4-benzothiadiazine 1,1-dioxide (**11**)*

A mixture of 7-chloro-3-(methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**4**) (0.5 g, 2.02 mmol) and 4 mL of (pyrid-2-yl)methylamine was heated at 140°C for a few hours. After addition of 20 mL of water, the suspension was adjusted to pH 4 with formic acid and the resulting precipitate was collected by filtration, washed and dried. The precipitate was dissolved in hot methanol and the solution was discoloured with charcoal, filtered and cooled. The title compound, which precipitated, was collected by filtration, washed and dried (yield 45%). Mp 244–262°C. (KBr): 3317, 1634, 1530, 1481, 1265, 1163 cm^{-1}. ^1H NMR (DMSO-d_6, HMDS; δ ppm): 4.50 (d, 2H, NHCH_2), 6.95–7.80 (m, 7H, N*H*CH_2, 5-*H*, 6-*H*, 8-*H* benzene + 3-*H*, 4-*H*, 5-*H* pyridine), 8.50 (d, 1H, 6-*H* pyridine), 10.95 (bs, 1H, N*H*).

*7-Chloro-3-(pyrid-3-yl)methylamino-4*H-*1,2,4-benzothiadiazine 1,1-dioxide (**12**)*

The title compound was obtained from 7-chloro-3-(methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**4**) (0.5 g, 2.02 mmol) and 4 mL of (pyrid-3-yl)-methylamine by following the experimental conditions described for the synthesis of 7-chloro-3-(pyrid-2-yl)-methylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**11**) (yield 49%). Mp 260–266°C. IR (KBr): 3362, 1647, 1547, 1478, 1288, 1163 cm^{-1}. ^1H NMR (DMSO-d_6, HMDS; δ ppm): 4.40 (bs, 2H, NHCH_2), 7.00–7.80 (m, 6H, N*H*CH_2 + 5-*H*, 6-*H*, 8-*H* benzene + 3-*H*, 4-*H* pyridine), 8.40 (d, 1H, 6-*H* pyridine), 8.50 (bs, 1H, 2-*H* pyridine).

*7-Chloro-3-(pyrid-4-yl)methylamino-4*H-*1,2,4-benzothiadiazine 1,1-dioxide (**13**)*

The title compound was obtained from 7-chloro-3-(methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**4**) (0.5 g, 2.02 mmol) and 4 mL of (pyrid-4-yl)-methylamine by following the experimental conditions described for the synthesis of 7-chloro-3-(pyrid-2-yl)methylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**11**) (yield 40%). Mp 274–279°C. IR (KBr): 3435, 3293, 1627, 1571, 1474, 1250, 1159 cm^{-1}. ^1H NMR (DMSO-d_6, HMDS; δ ppm): 4.40 (bd, 2H, NHCH_2), 7.00–7.80 (m, 6H, N*H*CH_2 + 5-*H*, 6-*H*, 8-*H* benzene + 3-*H*, 5-*H* pyridine), 8.40 (d, 2H, 2-*H* + 6-*H* pyridine), 10.90 (bs, 1H, N*H*).

*7-Chloro-3-[2-(pyrid-2-yl)ethyl]amino-4*H-*1,2,4-benzothiadiazine 1,1-dioxide monohydrate (**14**)*

The title compound was obtained from 7-chloro-3-(methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**4**) (0.5 g, 2.02 mmol) and 4 mL of 2-(pyrid-2-yl)-ethylamine by following the experimental conditions described for the synthesis of 7-chloro-3-(pyrid-2-yl)-methylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**11**) (yield 51%). Mp 180–182°C. IR (KBr) 3507, 3363, 1637, 1587, 1476, 1266, 1168 cm^{-1}. ^1H NMR (DMSO-d_6, HMDS; δ ppm): 2.80–3.70 (m, 6H, NHCH_2CH_2 + H_2O), 6.90–7.80 (m, 7H, N*H*CH_2 + 5-*H*, 6-*H*, 8-*H* benzene + 3-*H*, 4-*H*, 5-*H* pyridine), 8.45 (d, 1H, 6-*H* pyridine), 10.70 (bs, 1H, N*H*).

*7-Chloro-3-((R,S)-1′-methoxy-2′-propyl)amino-4*H-*1,2,4-benzothiadiazine 1,1-dioxide (**9**)*

A mixture of 7-chloro-3-(methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**4**) (0.5 g, 2.02 mmol) and 5 mL of (*R*,*S*)-1-methoxy-2-propylamine was heated at 150°C for a few hours. After addition of 20 mL of water, the suspension was adjusted to pH 9–10 with NaOH (5%). The resulting solution was treated with charcoal, filtered and the filtrate was adjusted to pH 2 with 6 M HCl. The precipitate was collected by filtration, washed and dried (yield 70%). Mp 150–153°C. IR (KBr) 3294, 1631, 1582, 1480, 1250, 1162 cm^{-1}. ^1H NMR (DMSO-d_6, HMDS; δ ppm): 1.05 (d, 3H, CH(CH_3)CH_2OCH_3), 3.00–3.40 (m, 5H, CH(CH_3)CH_2OCH_3 + OCH_3), 3.90 (m, 1H, NHC*H*), 6.95 (b, 1H, N*H*CH), 7.10 (d, 1H, 5-*H*), 7.50 (d + s, 2H, 6-*H* + 8-*H*), 10.45 (s, 1H, N*H*).

*7-Chloro-3-(pyrid-2-yl)amino-4*H-*1,2,4-benzothiadiazine 1,1-dioxide (**6**)*

A mixture of 7-chloro-3-(methylsulfinyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**5**) (Wollweber et al 1981) (0.5 g, 1.96 mmol) and *o*-aminopyridine (0.5 g, 5.32 mmol) in 5 mL of 3-chlorotoluene was heated at 150°C. The precipitate was collected by filtration and dissolved in aqueous NaOH (5%). The solution was treated with charcoal and filtered. The filtrate was adjusted to pH 4 with 6 M HCl, and the precipitate of crude **6** was collected by filtration, washed with water and dried. Recrystallization occurred from hot methanol (yield 45%). Mp > 300°C. IR (KBr): 3285, 3216, 3087, 1631, 1570, 1538, 1460, 1286, 1163, 1107 cm^{-1}. ^1H NMR (DMSO-d_6, HMDS; δ ppm): 7.10–8.00 (m, 7H, NH + 5-H, 6-H, 8-H benzene + 3-H, 4-H, 5-H pyridine) 8.40 (m, 1H, 6-H pyridine), 11.00 (b, 1H, NH).

*7-Chloro-3-(2′,2′-diethoxyethyl)amino-4*H-*1,2,4-benzothiadiazine 1,1-dioxide (**10**)*

A mixture of 7-chloro-3-(methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**4**) (0.5 g, 2.02 mmol) and 5 mL of 2-aminoacetaldehyde diethyl acetal was heated

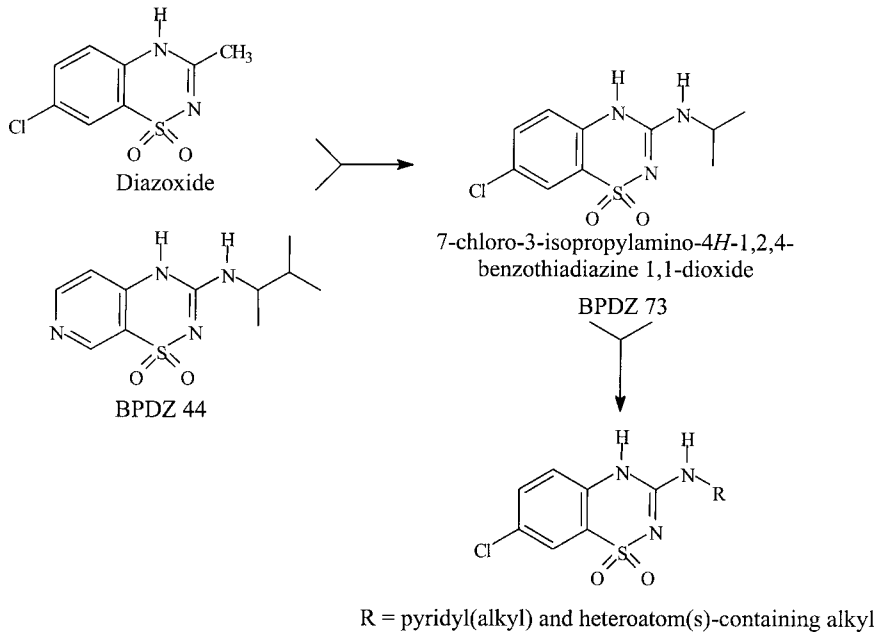


Figure 1 Diazoxide, BPDZ 44, BPDZ 73, 7-chloro-3-pyridyl(alkyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides and 3-alkylamino-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides containing one or more heteroatoms on the side chain in the 3 position.

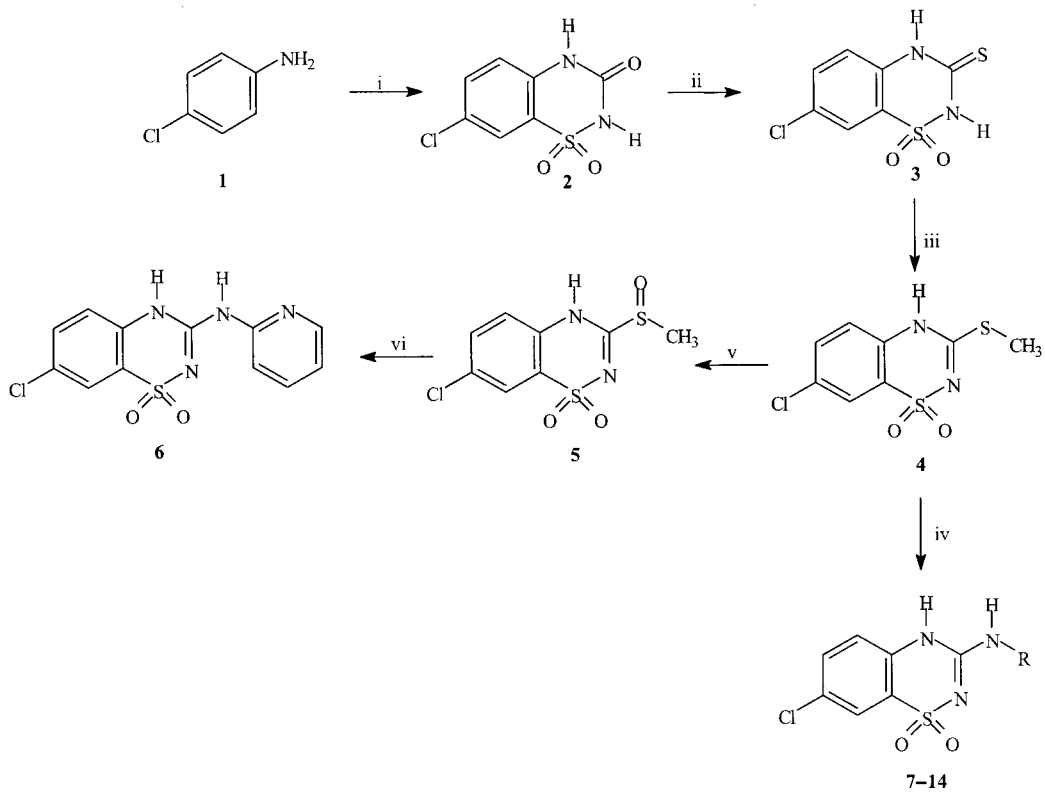


Figure 2 Synthesis of 7-chloro-3-pyridyl(alkyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**6**, **11**–**14**) and 3-alkylamino-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxides containing heteroatoms on the side chain (**7**–**10**). Reagents: i, $ClSO_2NCO$, $AlCl_3$; ii, P_2S_5, pyridine; iii, CH_3I, $NaHCO_3$, H_2O, CH_3OH; iv, NH_2-R; v, Br_2, Na_2CO_3; vi, *o*-aminopyridine.

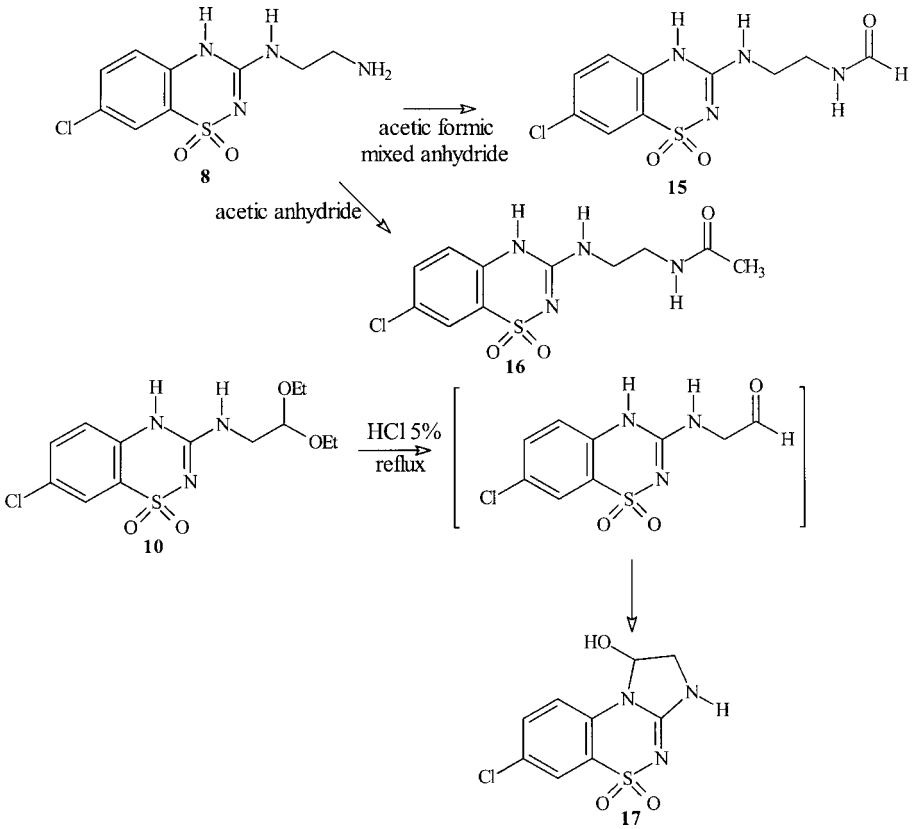


Figure 3 Synthesis of the 3-(2-formylaminoethyl)- (**15**), 3-(2-acetylaminoethyl)-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**16**) and 7-chloro-1-hydroxy-2,3-dihydro-1*H*-imidazo[2,1-*c*]-1,2,4-benzothiadiazine 5,5-dioxide (**17**).

at 150°C for 40 min. After addition of 20 mL of water, the precipitate was collected by filtration, washed with water and dried (yield 75%). Mp 219–220°C. IR (KBr): 3367, 3194, 3092, 2978, 1627, 1611, 1585, 1570, 1486, 1251, 1169, 1135, 1105, 1066 cm^{-1}. ^1H NMR (DMSO-d_6, HMDS; δ ppm): 1.05 (t, 6H, 2 x OCH_2CH_3), 3.25 (bm, 2H, $NHCH_2CH$), 3.55 (q, 4H, 2 x OCH_2CH_3), 4.55 (t, 1H, $NHCH_2CH$), 7.00 (b, 1H, $NHCH_2CH$), 7.10 (d, 1H, 5-H), 7.55 (d + s, 2H, 6-H, 8-H), 10.65 (b, 1H, NH).

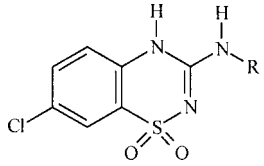
(R/S)-*7-Chloro-1-hydroxy-2,3-dihydro-1*H-*imidazo[2,1-c]-1,2,4-benzothiadiazine 5,5-dioxide (**17**)*
A mixture of 7-chloro-3-(2′,2′-diethoxyethyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**10**) (0.54 g, 1.97 mmol) and 10 mL 1 M HCl was heated under reflux for 45 min. The solution was concentrated under reduced pressure and the crude product which precipitated was collected by filtration, washed with water and dried. The compound was recrystallized from chloroform–methanol (70:30) (yield 48%). Mp 258°C (dec). IR (KBr): 3430, 1626, 1473, 1453, 1272, 1161, 1121, 1092 cm^{-1}. ^1H NMR (DMSO-d_6, HMDS; δ ppm): 3.30 (dd*, 1H, 4-H_a imidazole), 3.85 (dd, 1H, 4-H_b imidazole), 6.05 (dd*, 1H, 5-H imidazole), 7.10 (b, 1H, NH), 7.30 (d, 1H, 5-H), 7.60 (d + s, 2H, 6-H + 8-H), 8.60 (bs, 1H, OH). (*After addition of CF_3COOD).

Measurements of insulin release from incubated pancreatic islets

Pancreatic islets were isolated by the collagenase method from fed female Wistar rats, 180–220 g (Pirotte et al 1993, de Tullio et al 1996, Lebrun et al 2000). Groups of 10 islets, each derived from the same batch of islets, were pre-incubated for 30 min at 37°C in 1 mL of a physiological salt medium containing (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) dialysed 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

Table 1 Effects of functionalized 3-alkyl- and pyridyl(alkyl)amino-7-chloro-4*H*-1,2,4 benzothiadiazine 1,1-dioxide, diazoxide, BPDZ 44 and BPDZ 73 on insulin release from pancreatic B-cells.



Compound	R	Residual insulin secretion (%)[a]	
		50 μM	10 μM
6	Pyrid-2-yl	107.4 ± 4.8 (16)	ND[b]
7	CH_2CF_3	14.3 ± 1.9 (22)	74.1 ± 5.6 (12)
8	$CH_2CH_2NH_2$	108.7 ± 6.0 (16)	ND
9	$CH(CH_3)CH_2OCH_3$	49.1 ± 3.3 (15)	ND
10	$CH_2CH(OC_2H_5)_2$	76.7 ± 5.7 (15)	ND
11	CH_2-pyrid-2-yl	104.4 ± 5.8 (15)	ND
12	CH_2-pyrid-3-yl	98.2 ± 7.1 (15)	ND
13	CH_2-pyrid-4-yl	95.1 ± 3.9 (16)	ND
14	CH_2CH_2-pyrid-2-yl	81.3 ± 5.7 (23)	ND
15	CH_2CH_2NHCOH	94.3 ± 4.6 (22)	ND
16	$CH_2CH_2NHCOCH_3$	98.6 ± 5.4 (23)	ND
17	Cyclic imidazolidine	94.7 ± 5.6 (22)	ND
Diazoxide	–	28.8 ± 2.5 (21)	70.0 ± 3.6 (22)
BPDZ 44	–	7.1 ± 0.6 (14)	26.8 ± 1.8 (21)
BPDZ 73	–	5.7 ± 0.5 (35)	4.8 ± 0.4 (32)

[a]Percentage of residual insulin secretion from rat pancreatic islets incubated in the presence of 16.7 mM glucose; values are expressed as means ± s.e.m (n). [b]Not determined.

and, in addition, the reference compound or the benzothiadiazine derivative. The release of insulin was measured radioimmunologically using rat insulin as a standard (Leclercq-Meyer et al 1985).

Results and Discussion

Chemistry

3-(Methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**4**), the key intermediate in the preparation of the final products, was obtained in a three-step reaction starting from 4-chloroaniline (**1**; Figure 2) (Di Bella et al 1972; Pirotte et al 1993). The aniline was converted with chlorosulfonyl isocyanate into the corresponding 7-chloro-2,3-dihydro-3-oxo-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**2**) (Girard et al 1979). Subsequent thionation with P_2S_5 in pyridine of the oxo-derivative (**2**) led to 7-chloro-2,3-dihydro-3-thioxo-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**3**). The synthesis of 7-chloro-3-(methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide was achieved by reaction of the thioxo-derivative with methyl iodide in a water–methanol solution containing $NaHCO_3$.

The 7-chloro-3-alkyl- and pyridylalkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**7**–**14**) were obtained by reaction of the key intermediate (**4**) with an excess of the appropriate amine (Figure 2). Compound **6** was obtained by action of *o*-aminopyridine on the 7-chloro-3-(methylsulfinyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**5**) intermediate (Wollweber et al 1981). The former was prepared by oxidation of 7-chloro-3-(methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**4**) with bromine (Figure 2). Compounds **15** and **16** were synthesized, respectively, by formylation or acylation of 3-(2-aminoethyl)amino-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**8**) (Figure 3). Surprisingly, acid hydrolysis of compound **10** led not to the expected aldehydic derivative, but to a compound identified as being 7-chloro-1-hydroxy-2,3-dihydro-1*H*-imidazo[2,1-*c*]-1,2,4-benzothiadiazine 5,5-dioxide (**17**) (Figure 3). The IR spectrum of compound **17** did not exhibit an absorption band in the range of 1700 cm^{-1} and clearly showed the presence of a O-H vibration

band (3430 cm^{-1}). The NMR data of compound **17** indicated that the isolated structure was the cyclic form, as suggested by the presence of a chemical shift corresponding to the O-H proton (8.60 ppm, exchangeable with CF_3COOD) and the lack of the peak corresponding to the N_4-H proton (near 10.5–11.0 ppm).

Pharmacological evaluation

The 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 50 μM concentration and the most active compound was also examined at 10 μM. Diazoxide, BPDZ 44 and BPDZ 73 were used as reference drugs.

As shown in Table 1, none of the 7-chloro-3-pyridyl(alkyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**6, 11**–**14**) were found to express a marked activity as inhibitors of insulin release. Except for compounds **7** and **9**, none of the drugs bearing a heteroatom(s)-containing alkylamino chain in the 3 position (**8**, **10**, **15**–**17**) exhibited a clear inhibitory activity on pancreatic B-cells. 7-Chloro-3-(2′,2′,2′-trifluoroethyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**7**) was found to be as potent as diazoxide but less potent than BPDZ 44 or BPDZ 73. Compared with BPDZ 73, the presence of a strong electron-withdrawing group (CF_3) on the side chain strongly influenced the electronic distribution. This feature should be responsible for a loss of biological activity.

By examining the 3-pyridyl(alkyl)amino-substituted compounds (**6**, **11**–**14**), no improvement on biological activity was observed when the distance separating the pyridine ring from the thiadiazine ring or the position of the nitrogen atom in the pyridine ring was varied.

Compared with reference drugs, the enhancement of the steric hindrance in the 3 position could also explain in part the loss of activity.

One may expect the basic NH_2 group on the side chain of **8** to be protonated at physiological pH (7.4) and such an event could explain the lack of biological activity observed with **8**. However, even if this NH_2 function was acylated, and therefore not ionized at pH 7.4, no activity was recovered (see compounds **15** and **16**).

Conclusion

From these results, it clearly appears that the nature of the side chain in the 3 position of the benzothiadiazine ring affects the biological activity on pancreatic B-cells. The introduction of a pyridyl-, a pyridylmethyl- or a pyridylethylamino group in the 3 position generates compounds inactive or poorly active on the insulin-releasing process. Except for compounds **7** and **9**, the introduction of heteroatoms on the side chain in the 3 position also induced a marked decrease in biological activity.

However, these two compounds (**7** and **9**) were found to be less active than the reference molecule BPDZ 73.

Beside the possible impact of heteroatom(s) on electronic distribution, the enhancement of the steric hindrance by bulky groups in the 3 position could explain in part the lack of measurable biological activity with some of the new compounds.

It appears that, to develop compounds acting as inhibitors of insulin release, a short branched alkylamino side chain in the 3 position of the benzothiadiazine ring (like for BPDZ 73) constitutes, in this series, the best choice of substituent.

References

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

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

Bryan, J., Aguilar-Bryan, L. (1997) The ABC of ATP-sensitive potassium channels: more pieces of the puzzle. *Curr. Opin. Cell. Biol.* **9**: 553–559

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

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

Cook, N. S., Quast, U. (1990) Potassium channel 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**: 937–948

Di Bella, M., Rinaldi, M., Fabio, U., Manicardi, G. (1972) Azione antimicrobica di derivati dell' 1,2,4-benzothiadiazine-1,1-diossido. *Il Farmaco*. **27**: 990–998

Girard, Y., Atkinson, J. G., Rokach, J. (1979) A new synthesis of 1,2,4-benzothiadiazine and selective preparation of *o*-aminobenzenesulphonamides. *J. Chem. Soc. Perkin* **I**: 1043–1047

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

Lebrun, P., Devreux, V., Hermann, M., Herchuelz, A. (1988) Pinacidil inhibits insulin release by increasing K^+outflow from pancreatic B-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., Herchuelz A. (1992) Minireview: K^+ channel openers and insulin release. *Life Sci.* **51**: 795–806

Lebrun, P., Arkhammar, P., Antoine, M.-H., Nguyen, Q.-A., Bondo Hansen, J., Pirotte, B. (2000) A potent diazoxide analogue activating ATP-sensitive K^+ channels and inhibiting insulin release. *Diabetologia* **43**: 723–732

Leclerq-Meyer, V., Marchand, J., Woussen-Colle M. C., Giroix, M. H., Malaisse W. J. (1985) Multiple effects of leucine on glucagon, insulin and somatostatin secretion from the perfused rat pancreas. *Endocrinology* **116**: 1168–1174

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

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 B-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

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

Wollweber, H., Horstmann, H., Stoepel, K., Garthoff, B., Puls, H., Krause, H. P., Thomas, G. (1981) 3-Amino-2H-benzothiadiazin-1,1-dioxide mit antihypertensiver und potentieller diabetogener Wirkung. *Arzneim. Forsch.* **31**: 279–288