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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

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Abstract—The preparation and the pharmacological evaluation of the *R*- and *S*-isomers of 3-(2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (BPDZ 42) and 3-(3′-methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (BPDZ 44), two potassium channel openers, is described. Their optical purity was estimated by means of capillary electrophoresis (*R*- and *S*-BPDZ 42) and chiral HPLC (*R*- and *S*-BPDZ 44). The absolute configuration of each isomer of BPDZ 44 was deduced from crystallographic data. Pharmacological assays performed with the *R*- and *S*-isomers of BPDZ 44 revealed only slight differences in their activity on pancreatic B-cells but significant differences in their activity on vascular smooth muscle cells; the *R*-isomer being sixfold more potent than its corresponding *S*-isomer. The *R*-isomer of BPDZ 42 was shown to be more potent than its corresponding *S*-isomer on the endocrine pancreas. *S*-BPDZ 44 as well as *R*- and *S*-BPDZ 42 were found to exhibit tissue selectivity for the pancreatic versus the vascular smooth muscle tissue.

Introduction

ATP-sensitive potassium channels (K_{ATP} channels) are involved in important physiological processes such as regulation of insulin release and the control of muscle tone and contractility.[1–3]

Diazoxide (Fig. 1.) has been known for several years to be a potent inhibitor of insulin release. Such an effect was found to result from the opening of pancreatic B-cells K_{ATP} channels.[4,5] The drug also exhibits significant vasodilator effect according to its opening activity on K_{ATP} channels of vascular smooth muscle cells.[6] By contrast, cromakalim and pinacidil, two other potassium channel openers (PCOs) (Fig. 1), have previously been shown to be more active on the vascular than on the pancreatic K_{ATP} channels.[4–8] As a result, the ability of some PCOs to activate potassium channels may vary significantly according to the tissue localization of K_{ATP} channels.[9]

In previous papers, we reported the synthesis and the pharmacological activity of 3-(alkylamino)-4*H*-pyrido-[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides structurally related to both diazoxide and pinacidil.[10,11] Among those,

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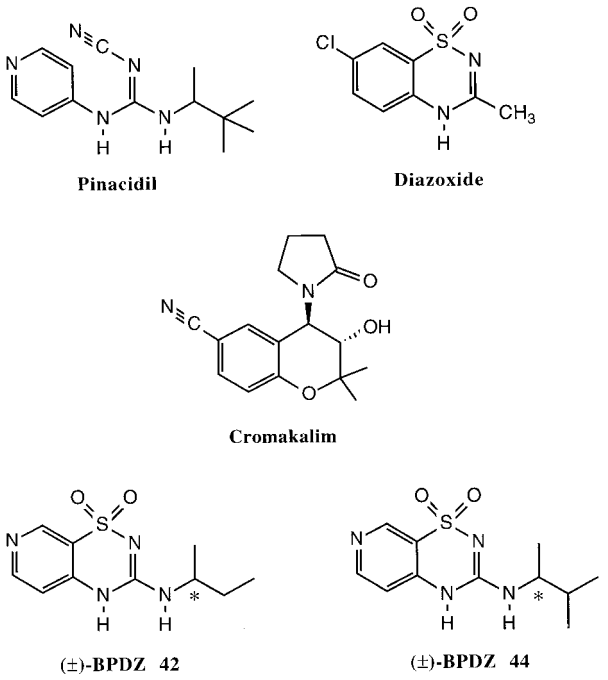


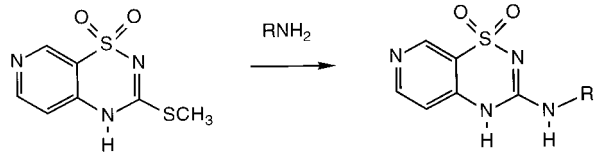
Figure 1. Typical examples of potassium channel openers.

3-(2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (BPDZ 42) (Fig. 1) and 3-(3′-methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (BPDZ 44) (Fig. 1) were selected as the best representatives of this new class of compounds with respect to their efficiency and tissue selectivity. Indeed, the two drugs were found to be more active on pancreatic B-cells than on vascular smooth muscle cells. BPDZ 44, the most powerful inhibitor of insulin release reported to date, was further identified as an activator of the pancreatic K_{ATP} channels.[12] The compounds (BPDZ 42 and BPDZ 44) being racemates, additional investigations were undertaken in order to synthesize and characterize their pure isomers. The present work depicts the influence on biological activity of the stereochemistry associated to the *N*-alkyl side chain of 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-pyridothiadiazine 1,1-dioxides.

Results

Chemistry

The synthesis of BPDZ 42 and BPDZ 44 has already been described (Scheme 1).[10,11] The same method was followed for preparing their two enantiomers. However,



Scheme 1. For (+)- and (−)-BPDZ 42: RNH_2 = (+)- and (−)-$CH_3CH_2CH(CH_3)NH_2$ respectively; for (+)- and (−)-BPDZ 44: RNH_2 = (+)- and (−)-$CH(CH_3)_2CH(CH_3)NH_2$ respectively.

in the last step of the preparation, the pure enantiomeric amines *R*-(−)- and *S*-(+)-2-butylamine, and *R*-(−)- and *S*-(+)-3-methyl-2-butylamine, were used instead of the racemic 2-butylamine and 3-methyl-2-butylamine. Such a process was preferred to the resolution of the final racemic mixture of 3-(alkylamino)-pyridothiadiazine dioxides. Moreover, no epimerization is expected in the reaction conditions used. The synthesis of the two enantiomers of BPDZ 42 was achieved by using the commercially available *R*-(−)-2-butylamine and *S*-(+)-2-butylamine. The optical purities of the *R*- and *S*-enantiomers of BPDZ 42 were determined by means of a single capillary electrophoresis method using a pH 2.2 phosphoric buffer containing 50 mM of dimethyl-β-cyclodextrin. Each enantiomer of BPDZ 42 was obtained with an estimated optical purity of about 95% (90–92% ee).

For the synthesis of the BPDZ 44 enantiomers, preparation of *R*-(−)- and *S*-(+)-3-methyl-2-butylamine from commercially available (±)-3-methyl-2-butylamine was achieved by transforming them into their diastereoisomeric salt with appropriate optically pure carboxylic acids. This process, for practical reasons, was preferred to the multistep enantioselective synthesis of the isomers of (±)-3-methyl-2-butylamine as described by H. C. Brown.[13] Moreover, we have taken advantage from the possibility to access to the absolute configuration of the amine by determining the X-ray structure of the optically pure salts obtained after multiple crystallizations. Thus, (±)-3-methyl-2-butylamine was converted on one hand into its salt with *S*-(+)-naproxen (salt **1**) and, on the other hand, into its salt with *S*-(+)-mandelic acid (salt **2**) in hot ethanol. Optically active acids like tartaric acid, dibenzoyltartaric acid, and 10-camphorsulfonic acid, were tentatively used for the same purpose but did not give satisfactory results. Determination of the optical purity of the amines, from each salt, was deduced from a chromatographic process conducted on their corresponding diastereoisomeric *R*/*S* and *S*/*S* amides with *S*-(+)-naproxen. For that purpose, the hydroxysuccinimidyl ester of *S*-(+)-naproxen was prepared from reaction of the acid with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide (DCC). The hydroxysuccinimidyl ester of *S*-(+)-naproxen was preferred, as chiral acylating agent, to its acid chloride[14] because of its higher stability in the experimental conditions used. Thin layer chromatography (TLC) as well as conventional HPLC were used to evaluate the enantiomeric purity after each crystallization step. The final optical purities of the *R*- and *S*-enantiomers of BPDZ 44 prepared from the pure amines were conclusively determined by using a chiral HPLC analytical assay. The same analytical process applied to the enantiomers of BPDZ 42 did not give satisfactory results. Each enantiomer of BPDZ 44 was obtained with an estimated optical purity of about 93% (86% ee). Further recrystallization of the final compounds did not appreciably improve their enantiomeric purity. The two compounds were found to exhibit sufficient quality for studying the influence of the stereochemistry associated to the short branched alkyl chain on the biological responses.

The absolute configuration of each separated *R*- and *S*-enantiomers of 3-methyl-2-butylamine, and therefore, that of the *R*- and *S*-enantiomers of BPDZ 44, was deduced from X-ray crystallography performed on their *S*-(+)-naproxen and *S*-(+)-mandelic acid salts (Fig. 2). Thus, we found that *R*-(-)-3-methyl-2-butylamine was associated with *S*-(+)-naproxen in salt **1** and *S*-(+)-3-methyl-2-butylamine was associated with *S*-(+)-mandelic acid in salt **2**.

Biological evaluation and discussion

BPDZ 42, BPDZ 44 as well as each optically pure *R*-(−)- and *S*-(+)-isomer of both compounds were evaluated as inhibitors of the insulin release from rat pancreatic islets incubated in the presence of an insulinotropic glucose concentration (16.7 mM). As observed in Table 1, the *R*-(−)- and *S*-(+)-isomers of BPDZ 42 and BPDZ 44 exerted a strong inhibitory activity on insulin secretion. No significant difference was noted between the *R*-(−)- and the *S*-(+)-isomer of BPDZ 44. However, *S*-(+)-BPDZ 42 was found to be less potent than *R*-(−)-BPDZ 42 in inhibiting the insulin releasing process ($p < 0.05$).

Because potassium channels play a key role in the regulation of insulin release, we next explored the effects of the different compounds on ^{86}Rb outflow (^{42}K substitute) from prelabelled and perifused rat pancreatic

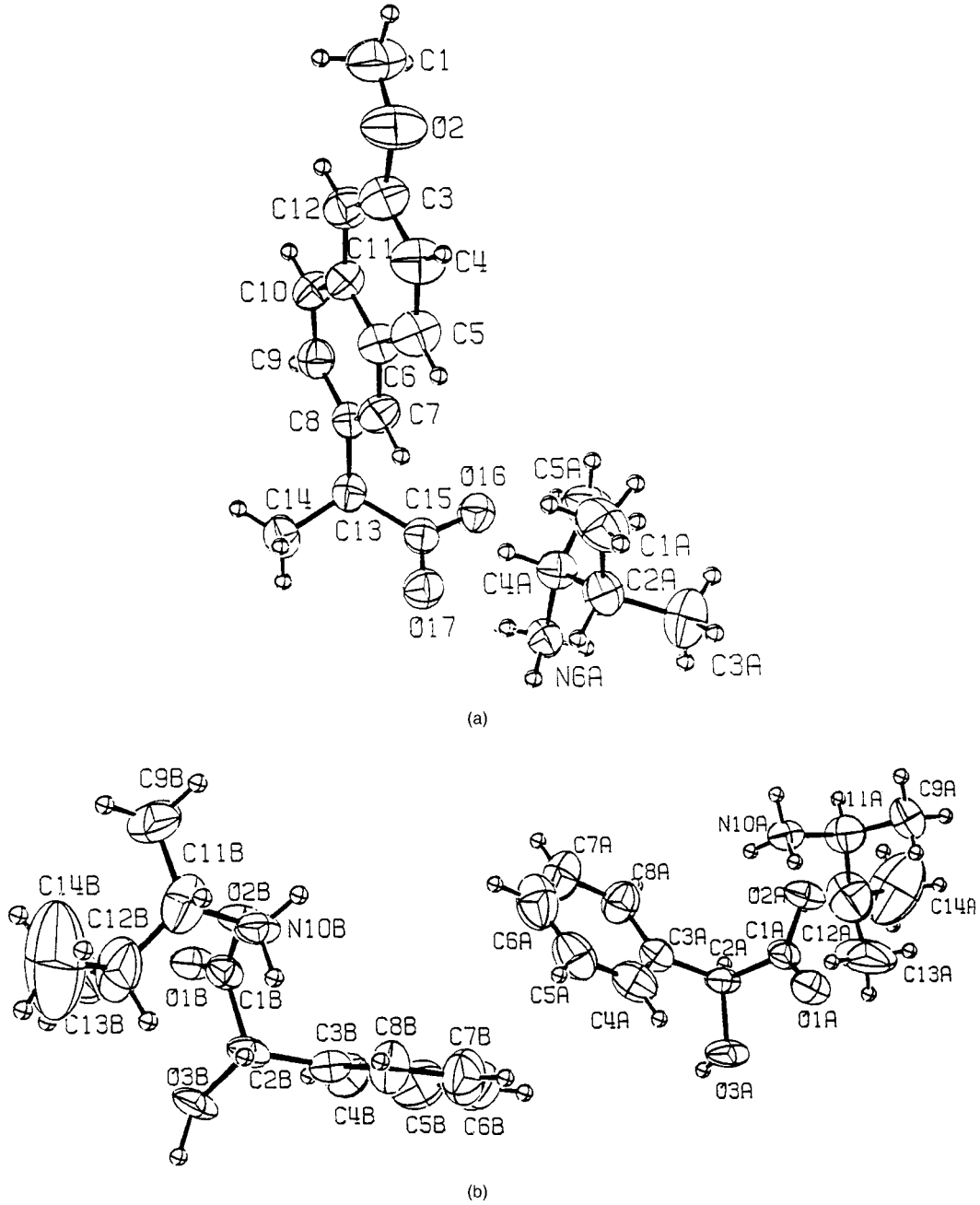


Figure 2. Molecular structures of *R*-3-methyl-2-butylammonium *S*-2-(6-methoxynaphth-2-yl)propionate (salt **1**) and of *S*-3-methyl-2-butylammonium *S*-mandelate (salt **2**) with atomic labeling scheme. Displacement ellipsoids are shown at 50% probability levels. H atoms are drawn as small circles of arbitrary units.

Table 1. Effect of BPDZ 42, BPDZ 44 and their isomers on insulin secretion from rat pancreatic islets and on the contractile activity of K^+-depolarized rat aorta rings

Compound	Residual insulin secretion (%)[a] 50 μM	10 μM	Vasodilator effect ED_{50} (μM)[b]
(±)-BPDZ 42	4.37 ± 2.81 (13)	37.31 ± 3.07(12)	> 300(4)
R-(-)-BPDZ 42	4.50 ± 0.44(11)	39.31 ± 2.88(16)	> 300(5)
S-(+)-BPDZ 42	6.81 ± 0.58(12)	51.93 ± 4.86(12)	> 300(4)
(±)-BPDZ 44	7.08 ± 0.58(14)	26.75 ± 1.83(21)	161.2 ± 9.5(4)
R-(-)BPDZ 44	7.00 ± 0.46(21)	33.92 ± 2.09(47)	35.4 ± 7.4(9)
S-(+)-BPDZ 44	5.68 ± 0.54(13)	31.70 ± 1.80(58)	210.7 ± 10.4(10)
Diazoxide	28.84 ± 2.45(21)[c]	70.02 ± 3.62(22)[c]	19.5 ± 2.8(5)

[a] Insulin release (mean values ± SE) was expressed in percent of the value recorded in control experiments (100%; no added drug and presence of 16.7 mM glucose).
[b] ED_{50}: drug concentration (mean values ± SE) giving 50% relaxation of the KC1-induced contraction. Figures in parentheses refer to number of samples.
[c] Ref. 10.

islets. The racemate BPDZ 42 (50 μM) increased ^{86}Rb outflow from pancreatic islets perifused in the presence of 5.6 mM glucose (data not shown). This stimulatory effect was counteracted by glibenclamide (10 μM; data not shown), a hypoglycaemic sulfonylurea reported to efficiently and selectively close the B-cell K_{ATP} channels. Such data support the view that the racemate BPDZ 42, like the racemate BPDZ 44,[12] activates K_{ATP} channels. *R*-(−)- and *S*-(+)-BPDZ 42 (50 μM) provoked an immediate and sustained increase in ^{86}Rb outflow from pancreatic islets perifused throughout in the presence of 5.6 mM glucose (Fig. 3) The stimulatory effect of *R*-(−)-BPDZ 42 was more marked than that of *S*-(+)-BPDZ 42. Thus, the magnitude of the increment in ^{86}Rb outflow averaged 1.85 ± 0.13%/min after exposure to *R*-(−)-BPDZ 42 and 1.31 ± 0.12%/min after exposure to *S*-(+)-BPDZ 42 ($p < 0.05$).

In islets exposed throughout to 5.6 mM glucose, the addition of *R*-(−)- or *S*-(+)-BPDZ 44 (50 μM) also provoked a marked increase in ^{86}Rb fractional outflow rate (Fig. 4). The capacity of BPDZ 44 to stimulate ^{86}Rb outflow was slightly, although not significantly, more pronounced with the *R*-(−)-isomer. Indeed, the integrated outflow of ^{86}Rb observed during stimulation averaged 1.70 ± 0.15%/min after addition of *R*-(−)-BPDZ 44 and 1.60 ± 0.19%/min after addition of *S*-(+)-BPDZ 44 ($p > 0.05$).

The present pharmacological results clearly show that BPDZ 42 and BPDZ 44 isomers provoke a marked rise in ^{86}Rb outflow from prelabelled and perifused rat pancreatic islets. The BPDZ-induced increases in ^{86}Rb outflow were not due to any direct toxicity of the drugs since the cationic responses to the different compounds

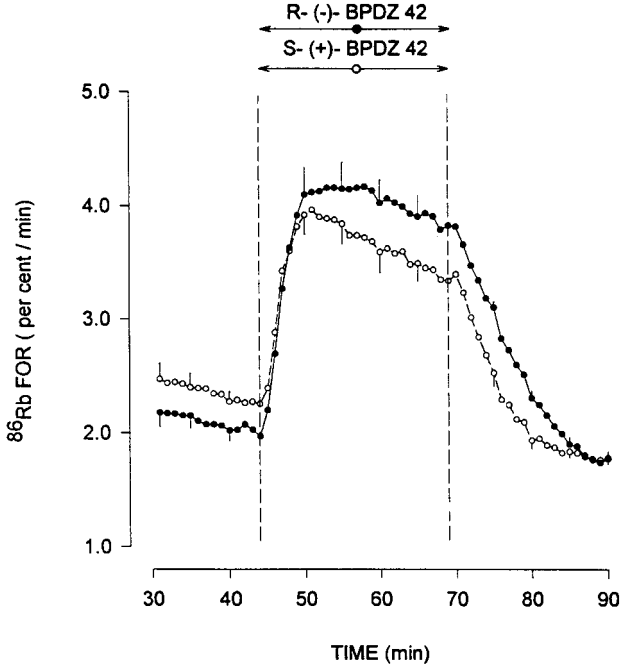


Figure 3. Effect of *R*-(−)-BPDZ 42 (●; 50 μM) and *S*-(+)-BPDZ 42 (○; 50 μM) on ^{86}Rb outflow from islets perifused throughout in the presence of 5.6 mM glucose. Mean values (± SE) refer to eight individual experiments.

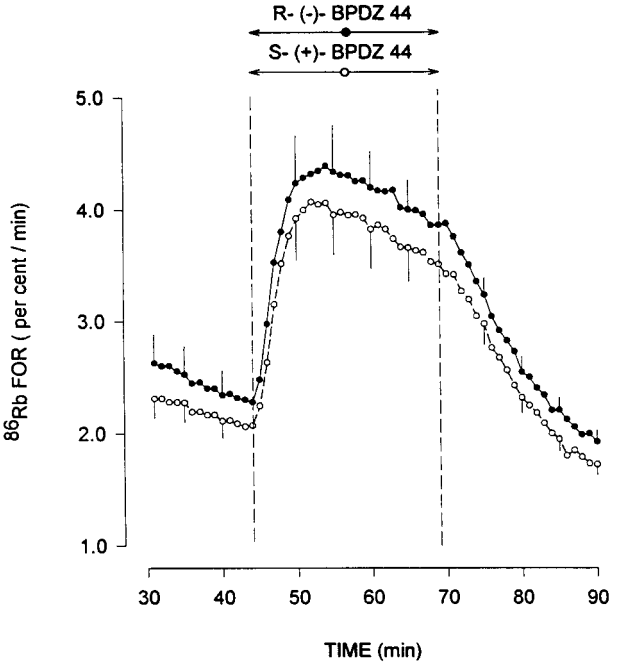


Figure 4. Effect of *R*-(−)-BPDZ 44 (●; 50 μM) and *S*-(+)-BPDZ 44 (○; 50 μM) on ^{86}Rb outflow from islets perifused throughout in the presence of 5.6 mM glucose. Mean values (± SE) refer to 8 individual experiments.

were always clearly reversible. Although the measurement of ^{86}Rb outflow rate underestimates the real changes in K^+ fluxes,[5,15] the present findings indicate that the BPDZ 42 and BPDZ 44 isomers also increase the K^+ permeability of the insulin secreting pancreatic B-cell. Our data further reveal that the *R*-(−)-BPDZ 42 isomer was more potent than the *S*-(+)-BPDZ 42 isomer at increasing the membrane permeability of potassium ions. These findings are compatible with the view that the inhibitory effect of *R*-(−)-BPDZ 42 on the insulin releasing process was more marked than that of *S*-(+)-BPDZ 42.

The vasodilator effect of BPDZ 42, BPDZ 44 and their *R*- and *S*-isomers was also examined on rat aorta rings precontracted by 30 mM KCl (Table 1). The experiments revealed that BPDZ 42 and its two isomers were less active than BPDZ 44 and its two isomers on vascular smooth muscle cells. Moreover, BPDZ 42 and its isomers exhibited a significant tissue selectivity for the endocrine versus the smooth muscle tissue. Indeed, the ED_{50} values for the inhibitory effect of BPDZ 42 and its isomers on insulin release was clearly lower ($\leq 10\,\mu M$) than the ED_{50} values for the inhibitory effect of the compounds on the contractile activity of vascular smooth muscle ($> 300\,\mu M$) (see Table 1). By contrast, *R*-(−)-BPDZ 44 was found to be more potent than *S*-(+)-BPDZ 44 and the racemate as a vasodilator. As a result, according to the estimated ED_{50} values on pancreatic B-cells ($< 10\,\mu M$ for BPDZ 44, *R*-(−)-BPDZ 44 and *S*-(+)-BPDZ 44) and on smooth muscle cells (see Table 1), *S*-(+)-BPDZ 44 expressed stronger endocrine pancreatic tissue selectivity than its corresponding *R*-isomer.

Conclusion

Previous works on a large variety of 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides revealed that the presence of a methyl radical on the first carbon atom of the exocyclic *N*-alkyl side chain was responsible for an important increase of their biological efficiency.[10,11] However, such chemical modification led to the introduction of a chiral carbon atom generating racemic compounds.

Our data indicate that the isomers, like the racemic compounds, markedly increase the potassium permeability of insulin secreting cells.

The present work also led to the conclusion that, on pancreatic B-cells, only slight differences in biological efficiency were associated with the stereochemistry of the *N*-alkyl side chain of the 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides. Slight but significant differences were essentially noted between the *R*-(−)- and the *S*-(+)-isomers of the 2-butylamino-substituted pyridothiadiazine BPDZ 42. BPDZ 42 and its isomers, however, exhibited a marked tissue selectivity for the pancreatic endocrine tissue versus the vascular smooth muscle tissue. Noticeable differences were observed between *R*-(−)- and *S*-(+)-BPDZ 44 on vascular smooth muscle cells but not on pancreatic B-cells; the *R*-(−)-isomer being significantly more potent than its corresponding *S*-(+)-isomer in relaxing KCl-precontracted rat aorta. As a result, *S*-(+)-BPDZ 44 was found to express more endocrine tissue selectivity than the *R*-(−)-isomer.

In conclusion, the present work gives new insights in the search for more tissue selective potassium channel openers structurally related to 3-(alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides.

Experimental

Melting points were determined on a Bûchi–Tottoli capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin–Elmer 1750 FT spectrophotometer. The ^1H NMR spectra were taken on a Bruker AW-80 (80 MHz) instrument in DMSO-d_6 or in $CDCl_3$ with hexamethyldisiloxane (HMDS) as an internal standard; chemical shifts are reported in d 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. The conventional and the chiral HPLC analyses were performed with a Merck–Hitachi apparatus and capillary electrophoresis was performed on a Spectra phoresis 1000 CE instrument (Spectraphysics, San Jose, CA, USA). The following experimental protocols are available as 'supplementary material' under request to the corresponding author: (1) analysis of salts **1** and **2** by TLC; (2) analysis of amides **3**–**8** by conventional HPLC; (3) analysis of (±)-BPDZ 42, *R*-(−)-BPDZ 42 and *S*-(+)-BPDZ 42 by capillary electrophoresis; (4) analysis of (±)-BPDZ 44, *R*-(−)-BPDZ 44 and *S*-(+)-BPDZ 44 by chiral HPLC; (5) X-ray crystallography: table of crystal data for salts **1** and **2**, table of atomic coordinates, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates, isotropic displacement parameters and structure factors.

Chemistry

Resolution of *R*-(−)-3-methyl-2-butylamine. *S*-(+)-naproxen (20 g, 86.9 mmol) was dissolved in ethanol (240 mL) and heated at 55°C. (±)-3-Methyl-2-butylamine (10 mL, 86.8 mmol) diluted in ethanol (40 mL) was added to the previous solution. After 24 h at room temperature, the resulting precipitate of 3-methyl-2-butylammonium (6-methoxynaphth-2-yl)propionate (salt **1**) was collected by filtration, washed with cold ethanol and dried. Recrystallization of the salt in ethanol was repeated three to four times (1g/10–15 mL ethanol). One hundred milligrams of the salt of each step was kept for ulterior analysis. Final compound: mp = 150–152°C. Anal. calcd for $C_{19}H_{27}NO_3$: C, 71.89; H, 8.57; N, 4.41. Found: C, 71.65; H, 8.96; N, 4.59.

Solid sodium hydroxide (8.3 g, 207.5 mmol) was added to the solution of the salt (5.95 g, 18.7 mmol) in distilled water (200 mL). *R*-(−)-3-Methyl-2-butylamine ($[\alpha]_D$-2.31°, 20°C; methanol) was distilled from the previous solution at 86–90°C and 760 mm Hg. The yield of the distillation was 75% (3.45 mL).

Resolution of *S*-(+)-3-methyl-2-butylamine. *S*-(+)-mandelic acid (26.4 g, 173.6 mmol) was dissolved in ethanol (160 mL) and heated at 55°C. (±)-3-Methyl-2-butylamine (20 mL, 173.6 mmol) diluted in ethanol (28 mL) was added to the previous solution. After 24 h at room temperature, the resulting precipitate of 3-methyl-2-butylammonium mandelate (salt **2**) was collected by filtration, washed with cold ethanol and dried. Recrystallization of the salt in ethanol was repeated three to four times (1g/7 mL ethanol). One hundred milligrams of the salt of each step was kept for ulterior analysis. Final compound: mp 148–150°C. Anal. calcd for $C_{13}H_{21}NO_3$: C, 65.25; H, 8.84; N, 5.85. Found: C, 65.34; H, 8.89; N, 6.06. Solid sodium hydroxide (8.3 g, 207.5 mmol) was added to the solution of the salt (12.5 g, 51.9 mmol) in distilled water (200 mL). *S*-(+)-3-Methyl-2-butylamine ($[\alpha]_D$ + 2.71°; 20°C; methanol) was distilled from the previous solution at 86–90°C and 760 mm Hg. The yield of the distillation was 80% (4.45 mL).

Succinimidyl (*S*)-2-(6-methoxynaphth-2-yl)propionate (2). To a solution of *S*-(+)-naproxen (1 g, 4.3 mmol) and *N*-hydroxysuccinimide (0.46 g, 4 mmol) in dioxane (20 mL), was added dropwise a solution of DCC (0.83 g, 4 mmol) in dioxane (20 mL). The mixture was stirred at room temperature for 16 h. After filtration of the formed dicyclohexylurea and elimination of dioxane under reduced pressure, the residue was dissolved in ethyl acetate (15 mL) and the suspension was filtered. The filtrate was washed with a saturated solution of sodium bicarbonate, then with water, and was dried over anhydrous $MgSO_4$. Addition of petroleum ether (40–60°C) gave rise to the precipitation of succinimidyl (*S*)-(6-methoxynapht-2-yl)propionate which was collected by filtration, washed with petroleum ether (40–60°C) and dried (95%), mp = 117–118°C. ^{1}H NMR: δ 1.55 (d, 3H, CH(C*H*$_3$)), 2.7 (s, 4H, C*H*$_2$-C*H*$_2$), 3.8 (s, 3H, OC*H*$_3$), 4.3 (q, 1H, C*H*(CH$_3$)), 7.0–8.0 (m, 6H, $C_{10}H_6$). Anal. calcd for $C_{18}H_{17}NO_5$: C, 66.03; H, 5.24; N, 4.28. Found: C, 66.15; H, 5.35; N, 4.37.

***N*-[(*R*)-2-butyl]-(*S*)-2-(6-methoxynaphth-2-yl)propionamide (3).** Succinimidyl (*S*)-(6-methoxynapht-2-yl)propionate (50 mg, 0.15 mmol) and *R*-(−)-2-butylamine (15 μL, 0.16 mmol) were dissolved in dichloromethane (5 mL), and stirred at room temperature for 1 h. The solution was washed with saturated sodium bicarbonate, then with 1 N HCl and water, and dried over magnesium sulfate. Elimination of the solvent gave the pure amide (90%), mp 134–135°C. IR (KBr): 3306, 3058, 2970, 2934, 1642, 1607, 1535, 1486 cm^{-1}. ^{1}H NMR: δ 0.6–1.1 (t + d, 6H, CH$_2$-C*H*$_3$ + NH-CH(C*H*$_3$)), 1.15–1.5 (m + d, 5H, C*H*$_2$-CH$_3$ + CH(C*H*$_3$)CO), 3.4–3.9 (m, 2H, C*H*(CH$_3$)CO + NH-C*H*(CH$_3$)), 3.8 (s, 3H, OC*H*$_3$), 6.9–7.9 (m, 7H, N*H* + $C_{10}H_6$). Data from conventional HPLC: RT [(*R,S*) isomer] = 8.59 min; % (*R,S*) = 97.6; % (*S,S*) = 2.4. Anal. calcd for $C_{18}H_{23}NO_2$: C, 75.74; H, 8.13; N, 4.91. Found: C, 75.40; H, 8.54; N, 5.15.

***N*-[(*S*)-2-butyl]-(*S*)-2-(6-methoxynaphth-2-yl)propionamide (4).** The synthetic process used was identical to that reported for **3** starting from *S*-(+)-2-butylamine (90%), mp 126–127°C. IR (KBr): 3350, 2970, 2936, 1642, 1606, 1524 cm^{-1}. ^{1}H NMR: δ 0.6 (t, 3H, CH$_2$-C*H*$_3$), 0.95 (d, 3H, NH-CH(C*H*$_3$)), 1.1–1.5 (m + d, 5H, C*H*$_2$-CH$_3$ + CH(C*H*$_3$)CO), 3.4–3.9 (m, 2H, C*H*(CH$_3$)CO + NH-C*H*(CH$_3$)), 3.8 (s, 3H, OC*H*$_3$), 6.9–7.9 (m, 7H, N*H* + $C_{10}H_6$). Data from conventional HPLC: RT [(*S,S*) isomer] = 10.06 min; % (*R,S*) = 3.3; % (*S,S*) = 96.7. Anal. calcd for $C_{18}H_{23}NO_2$: C, 75.74; H, 8.13; N, 4.91. Found: C, 75.62; H, 8.37; N, 5.09.

***N*-[(*R*/*S*)-2-butyl]-(*S*)-2-(6-methoxynaphth-2-yl)propionamide (5).** The synthetic process used was identical to that reported for **3** starting from *R*/*S*-2-butylamine. (90%), mp = 118–120°C. IR (KBr): 3307, 3058, 2968, 2935, 1641, 1607, 1539, 1504, 1486, 1453 cm^{-1}. ^{1}H NMR: δ 0.4–1.6 (m, 11H, CH$_2$-C*H*$_3$ + NH-CH(C*H*$_3$) + C*H*$_2$-CH$_3$ + CH(C*H*$_3$)CO), 3.4–3.9 (m + s, 5H, C*H*(CH$_3$)CO + NH-C*H*(CH$_3$) + OC*H*$_3$), 6.9–7.9 (m, 7H, NH + $C_{10}H_6$). Anal. calcd for $C_{18}H_{23}NO_2$: C, 75.74; H, 8.13; N, 4.91. Found: C, 75.59; H, 8.46; N, 5.01.

***N*-[(*R*)-3-methyl-2-butyl]-(*S*)-2-(6-methoxynaphth-2-yl)-propionamide (6).** The synthetic process used was identical to that reported for **3** starting from *R*-(−)-3-methyl-2-butylamine (85%), mp = 119–120°C. IR (KBr): 3301, 3058, 2974, 2934, 2876, 1641, 1608, 1544, 1504, 1486, 1453 cm^{-1}. ^{1}H NMR: δ 0.7 (d, 6H, CH(C*H*$_3$)$_2$), 0.85 (d, 3H, NH-CH(C*H*$_3$)), 1.35 (d, 3H, CH(C*H*$_3$)CO), 1.55 (m, 1H, C*H*(CH$_3$)$_2$), 3.4-3.9 (m, 2H, C*H*(CH$_3$)CO + NH-C*H*(CH$_3$)), 3.8 (s, 3H, OC*H*$_3$), 6.95–7.9 (m, 7H, N*H* + $C_{10}H_6$). Data from conventional HPLC: RT [(*R,S*) isomer] = 7.13 min; % (*R,S*) = 91.2; % (*S,S*) = 8.8. Anal. calcd for $C_{19}H_{25}NO_2$: C, 76.22; H, 8.42; N, 4.68. Found: C, 76.02; H, 8.30; N, 4.50.

***N*-[(*S*)-3-methyl-2-butyl]-(*S*)-2-(6-methoxynaphth-2-yl)-propionamide (7).** The synthetic process used was identical to that reported for **3** starting from *S*-(+)-3-methyl-2-butylamine (90%), mp 134–136°C. IR (KBr): 3293, 3057, 2960, 1639, 1608, 1545, 1504, 1485, 1449 cm^{-1}. ^{1}H NMR: δ 0.55 (d, 6H, CH(C*H*$_3$)$_2$), 0.9 (d, 3H, NH-CH(C*H*$_3$)), 1.35 (d, 3H, CH(C*H*$_3$)CO), 1.55 (m, 1H, C*H*(CH$_3$)$_2$), 3.3–3.9 (m, 2H, C*H*(CH$_3$)CO + NH-C*H*(CH$_3$)), 3.8 (s, 3H, OC*H*$_3$), 6.95–7.9 (m, 7H, N*H* + $C_{10}H_6$). Data from conventional HPLC: RT [(*S,S*) isomer] = 8.83 min; % (*R,S*) = 6.2; % (*S,S*) = 93.8. Anal. calcd for $C_{19}H_{25}NO_2$: C, 76.22; H, 8.42; N, 4.68. Found: C, 76.16; H, 8.35; N, 4.55.

***N*-[(*R*/*S*)-3-methyl-2-butyl]-(*S*)-2-(6-methoxynaphth-2-yl)-propionamide (8).** The synthetic process used was identical to that reported for **3** starting from *R*/*S*-3-methyl-2-butylamine (90%), mp 110–111°C. IR (KBr): 3301, 3058, 2962, 2934, 2874, 1641, 1608, 1544, 1504, 1485, 1453 cm^{-1}. ^{1}H NMR: δ 0.45–1.1 (m, 9H, CH(C*H*$_3$)$_2$ + NH-CH(C*H*$_3$)), 1.35 (d, 3H, CH(C*H*$_3$)CO), 1.55 (m,

1H, C*H*(CH_3)$_2$), 3.3–3.9 (m, 2H, C*H*(CH_3)CO + NH-C*H*(CH_3)), 3.8 (s, 3H, OC*H*$_3$), 6.95–7.9 (m, 7H, N*H* + $C_{10}H_6$). Anal. calcd for $C_{19}H_{25}NO_2$: C, 76.22; H, 8.42; N, 4.68. Found: C, 76.10; H, 8.25; N, 4.45.

***R*-(−)-3-(2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide monohydrate (9).** To the solution of *R*-(−)-2-butylamine (1 mL, 11 mmol) in dioxane (3 mL) was added 3-methylsulfanyl-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide monohydrate **1** (0.3 g, 1.21 mmol).[10] The solution was heated at 150°C in a sealed tube for 1–2 h. After elimination of the solvent, the residue was dissolved in alkaline water (0.16 g NaOH/10 mL H_2O), treated with charcoal and filtered. The filtrate was acidified with formic acid to pH 6 and the precipitate was washed with water, then recrystallized from methanol (80%), mp 89–95°C (dec.: loss of water; solid.; mp 206–209°C), $[\alpha]_D$ −31.84°; 20°C; methanol (10 mg/mL). IR (KBr): 3470, 3336, 2971, 2939, 2881, 1649, 1557, 1283, 1172. ^{1}H NMR (DMSO-d_6): δ 0.80 (t, 3H, CH_2CH_3), 1.10 (d, 3H, $CHCH_3$), 1.45 (m, 2H, CH_2), 3.70 (m, 1H, CH), 7.05 (d, 1H, 5-H), 7.15 (bs, 1H, N*H*CH), 8.45 (d, 1H, 6-H), 8.65 (s, 1H, 8-H), 10.55 (bs, 1H, 4-NH). Anal. calcd for $C_{10}H_{14}N_4SO_2$, H_2O: C, 44.10; H, 5.92; N, 20.57; S, 11.78. Found: C, 44.16; H, 6.21; N, 20.80; S, 11.92.

***S*-(+)-3-(2′-Butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide monohydrate (10).** The procedure described for **9** but using *S*-(+)-2-butylamine was applied to have **10** (65%), mp 90–96°C (dec: loss of water; solid.; mp 206–209°C, $[\alpha]_D$ +31.34°; 20°C; methanol (10 mg/mL). IR (KBr): 3470, 3335, 2971, 2939, 2881, 1649, 1557, 1283, 1172. ^{1}H NMR (DMSO-d_6): δ 0.80 (t, 3H, CH_2CH_3), 1.10 (d, 3H, $CHCH_3$), 1.45 (m, 2H, CH_2), 3.70 (m, 1H, CH), 7.05 (d, 1H, 5-H), 7.15 (bs, 1H, N*H*CH), 8.45 (d, 1H, 6-H), 8.65 (s, 1H, 8-H), 10.55 (bs, 1H, 4-NH). Anal. calcd for $C_{10}H_{14}N_4SO_2$, H_2O: C, 44.10; H, 5.92; N, 20.57; S, 11.78. Found: C, 44.23; H, 6.05; N, 20.76; S, 12.07.

***R*-(−)-3-(3′-Methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide monohydrate (11).** The procedure described for **9** but using *R*-(−)-3-methyl-2-butylamine was applied to have **11** (70%), mp 93–95°C (dec.: loss of water; solid.; mp 200–204°C), $[\alpha]_D$ −31.0°; 20°C, methanol (10 mg/mL). IR (KBr): 3480, 3329, 2966, 1651, 1564, 1283, 1171. ^{1}H NMR (DMSO-d_6): δ 0.80 (d, 6H, $CH(CH_3)_2$), 1.00 (d, 3H, CH_3), 1.70 (m, 1H, C*H*(CH_3)$_2$), 3.65 (m, 1H, C*H*NH), 7.00 (bs, 1H, N*H*CH), 7.05 (d, 1H, 5-H), 8.45 (d, 1H, 6-H), 8.65 (s, 1H, 8-H), 10.45 (bs, 1H, 4-NH). Data from chiral HPLC: RT = 15.63 min; % (*R*) = 92.63; % (*S*) = 7.37. Anal. calcd for $C_{11}H_{16}N_4SO_2$, H_2O: C, 46.14; H, 6.34; N, 19.57; S, 11.20. Found: C, 45.95; H, 6.33; N, 19.55; S, 10.81.

***S*-(+)-3-(3′-Methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide monohydrate (12).** The procedure described for **9** but using *S*-(+)-3-methyl-2-butylamine was applied to have **12** (75%), mp 92–95°C (dec.: loss of water; solid.; mp 200–204°C), $[\alpha]_D$ +34.14°; 20°C; methanol (10 mg/mL). IR (KBr): 3480, 3329, 2966, 1648, 1562, 1283, 1171. ^{1}H NMR (DMSO-d_6): δ 0.80 (d, 6H, $CH(CH_3)_2$), 1.00 (d, 3H, CH_3), 1.70 (m, 1H, C*H*(CH_3)$_2$), 3.65 (m, 1H, C*H*NH), 7.00 (bs, 1H, N*H*CH), 7.05 (d, 1H, 5-H), 8.45 (d, 1H, 6-H), 8.65 (s, 1H, 8-H), 10.45 (bs, 1H, 4-NH). Data from chiral HPLC: RT = 17.22 min; % (*S*) = 92.43; % (*R*) = 7.57. Anal. calcd for $C_{11}H_{16}N_4SO_2$, H_2O: C, 46.14; H, 6.34; N, 19.57; S, 11.20. Found: C, 45.48; H, 6.40; N, 19.75; S, 11.02.

Biological assays

Rat pancreatic B-cells. All experiments were performed with islets isolated from the pancreas of fed Wistar rats. The methods used to measure insulin release from incubated islets and to measure ^{86}Rb (^{42}K substitute) efflux from perifused pancreatic islets were previously described.[11,12,15] The outflow of ^{86}Rb (counts/min/min) was expressed as a fractional outflow rate (FOR; percentage of instantaneous islet content per minute). Results are expressed as mean values (± SE). The magnitude of the increase in ^{86}Rb outflow was estimated in each individual experiment from the integrated outflow of ^{86}Rb observed during stimulation (45–68th min) after correction for basal value (40–44th min). The statistical significance of differences between mean data was assessed by use of Student's *t*-test.

Rat aorta rings. All experiments were performed on aortae removed from fed Wistar rats, as previously described.[11] The ED_{50} was graphically assessed for each dose–response curve as the concentration evoking 50% inhibition of the plateau induced by KCl 30 mM. Results are expressed as mean values (± SE).

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