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Short communication

Synthesis and pharmacological activity of *N*-(2,2-dimethyl-3,4-dihydro-2*H*-1-benzopyran-4-yl)-4*H*-1,2,4-benzothiadiazine-3-carboxamides 1,1-dioxides on rat uterus, rat aorta and rat pancreatic β -cells

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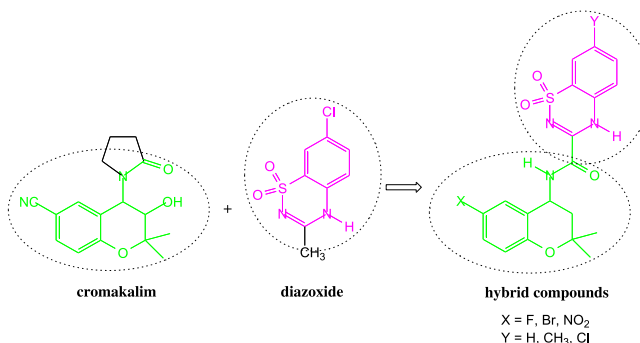
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HIGHLIGHTS

- Hybrid compounds between diazoxide and cromakalim, two potassium channel openers, were synthesized.
- They were evaluated on three K_{ATP} channel-expressing tissues (pancreatic β -cells, aorta, uterus).
- Strong myorelaxant activity on uterus, but not on rat aorta, was found with 6-bromo-substituted dihydrobenzopyran hybrid compounds.
- None of these compounds were found to exert any inhibitory activity on insulin release.

GRAPHICAL ABSTRACT



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ABSTRACT

N-(2,2-Dimethyl-3,4-dihydro-2*H*-1-benzopyran-4-yl)-4*H*-1,2,4-benzothiadiazine-3-carboxamides 1,1-dioxides were prepared and evaluated on rat uterus, rat aortic rings and rat pancreatic β -cells. Pharmacological studies conducted on rat uterus indicated that several of these original hybrid compounds displayed a strong myorelaxant activity. The most active compounds hold a bromine atom at the 6-position of the dihydrobenzopyran ring. Moreover, the compounds failed to display a marked inhibitory effect on insulin secretion and vascular myogenic activity. These features suggest that the 6-bromo compounds could be relatively selective towards the uterine smooth muscle.

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1. Introduction

Chemistry and pharmacology of benzo- or pyridothiadiazine dioxides, as well as of dihydrobenzopyrans, in the field of potassium channel openers, have raised great interest among chemists,

Abbreviations: K_{ATP} channel, ATP-sensitive potassium channel; NMR, nuclear magnetic resonance; DMSO, dimethylsulfoxide; HMDs, hexamethyldisiloxane.

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biologists and pharmacologists [1–8]. Compounds belonging to these classes of molecules are currently used as therapeutic drugs or pharmacological tools (i.e., diazoxide (1) and cromakalim (2); Fig. 1). The physiological interest of such compounds results from their interaction with a particular subtype of potassium channels, namely the ATP-sensitive potassium channel (K_{ATP} channel), onto which the two reference molecules diazoxide and cromakalim exert an opening activity [9,10]. According to such an effect, diazoxide has been reported to provoke an inhibitory activity on the insulin releasing process from pancreatic β -cells [11]. Moreover, this drug, as well as cromakalim, induces vasorelaxant effects as a result of their opening activity on smooth muscle K_{ATP} channels [9,10]. Lastly, cromakalim is also known to provoke the relaxation of other K_{ATP} channel-expressing smooth muscle tissues such as the airway and uterine smooth muscles [12,13].

Within the last decade, we have developed dihydrobenzopyrans and acyclic analogues structurally related to cromakalim, holding an arylsulfonylurea or an arylurea moiety at the 4-position of the heterocycle [14–18] (Fig. 2).

Biological data previously collected revealed that several compounds of general formula 3 ($Z = CH_3$) exhibited a marked myorelaxant activity on vascular smooth muscle (rat aorta) and reduced insulin secretion from rat pancreatic β -cells [15]. So far, no attempt was made to combine the 4*H*-1,2,4-benzothiadiazine 1,1-dioxide core structure (cfr diazoxide) with the dihydrobenzopyran nucleus (cfr cromakalim) in order to generate hybrid compounds between diazoxide and cromakalim. Thus, the present investigation describes the synthesis and the pharmacological evaluation of 4*H*-1,2,4-benzothiadiazine 1,1-dioxides bearing a 2,2-dimethyl-3,4-dihydro-2*H*-1-benzopyran-4-yl moiety at the 3-position of the benzothiadiazine ring (Fig. 3). The different molecules have been tested on rat aortic and rat uterine smooth muscles to measure their myorelaxant properties, and on rat pancreatic islets to detect an effect on insulin secretion.

2. Results

2.1. Synthesis

Scheme 1 shows the synthetic route used to prepare novel *N*-(2,2-dimethyl-3,4-dihydro-2*H*-1-benzopyran-4-yl)-4*H*-1,2,4-benzothiadiazine-3-carboxamides 1,1-dioxides (7a–g). Compound 9 was prepared according to a previously described method [19] from 4-substituted anilines 8 and chlorosulphonyl isocyanate in nitromethane. Such a procedure further comprised a ring closure reaction of a chlorosulphonylurea intermediate in the presence of aluminium chloride. Acidic hydrolysis of 9 yielded the *o*-amino-benzenesulfonamides 10 which reacted with ethyl oxalyl chloride to give compounds 11 [20]. Ring closure of compounds 11 with sodium ethanolate in anhydrous ethanol provided compounds 12 [20]. The latter compounds 12 yielded the target molecules (7a–g) after reaction with several 6-substituted 4-amino-2*H*-dihydrobenzopyrans, which were synthesized as previously described [14].

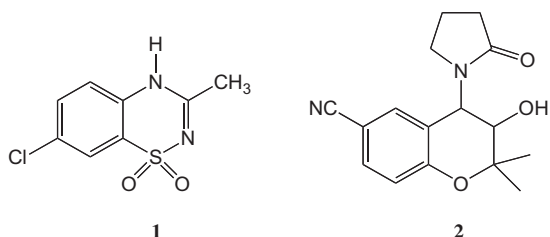


Fig. 1. Chemical structure of diazoxide (1) and cromakalim (2).

2.2. Biological assays

2.2.1. Inhibitory activity on insulin secretion from rat pancreatic islets

Compounds 7a–g were evaluated as inhibitors of insulin secretion from rat pancreatic islets incubated in the presence of an insulinotropic glucose concentration (16.7 mM). As observed in Table 1, all compounds, tested at 50 μ M, were less active than diazoxide [21] at inhibiting insulin release. Their effects were close to that of cromakalim or pinacidil.

2.2.2. Myorelaxant effect on rat aorta rings

Table 1 also reports the myorelaxant effects of compounds 7a–g and of reference drugs [21] on rat aorta rings pre-contracted with a hyperpotassic 30 mM KCl solution. None of these new compounds exhibited a marked myorelaxant effect.

2.2.3. Myorelaxant effect on rat uterus

As shown in Table 2, at the concentration of 10 μ M, compounds 7c and 7d were more active than diazoxide [22] but equi or slightly less potent than pinacidil [22] at relaxing rat uterus contracted by oxytocin. The other analogues (7a–7b, 7e–7g), tested at the same concentration, failed to inhibit the oxytocin-induced contractile activity.

At a 50 μ M concentration, four compounds elicited obvious activity. Compounds 7c and 7d were again markedly more potent than diazoxide, with both drugs being equipotent to pinacidil. Compounds 7e and 7f exhibited an inhibitory effect equivalent to that of diazoxide but were less potent than pinacidil.

At a 100 μ M concentration, pinacidil expressed the same activity than at a 50 μ M concentration, reflecting a “ceiling effect”, while compounds 7c and 7d nearly completely suppressed the uterine contractile activity induced by oxytocin. Under the same experimental conditions, 7e and 7f were as potent as pinacidil in reducing the oxytocin-induced uterine contractions. Altogether, the data indicated that 7d behaved as the most active uterine myorelaxant.

3. Discussion and conclusion

The data reported revealed that the introduction of a dihydrobenzopyran moiety at the 3-position of the benzothiadiazine dioxide core structure provided a new class of hybrid molecules (general formula 7) expressing myorelaxant properties on rat uterus. Some of these new compounds, especially compounds bearing a bromine atom at the 6-position of the dihydrobenzopyran ring, were even more potent than the reference compound pinacidil. Moreover, the drugs were barely active as vascular myorelaxants and did not markedly affect the glucose-induced insulin releasing process. Such features highlight tissue selectivity towards the uterine smooth muscle.

In conclusion, we succeeded to develop novel compounds, hybrid structures between cromakalim and diazoxide, that were found to exert a myorelaxant activity on rat uterus and a marked uterine tissue selectivity. The development of this type of compounds needs to be pursued and the relationships between their biological activity and the modulation of K_{ATP} channels should be explored.

4. Experimental

Melting points were determined on a Büchi–Tottoli capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin–Elmer 1750 FT spectrophotometer. The 1H NMR spectra were taken on a Bruker (500 MHz) instrument in $DMSO-d_6$ with tetramethylsilane (TMS) as an internal standard. Chemical

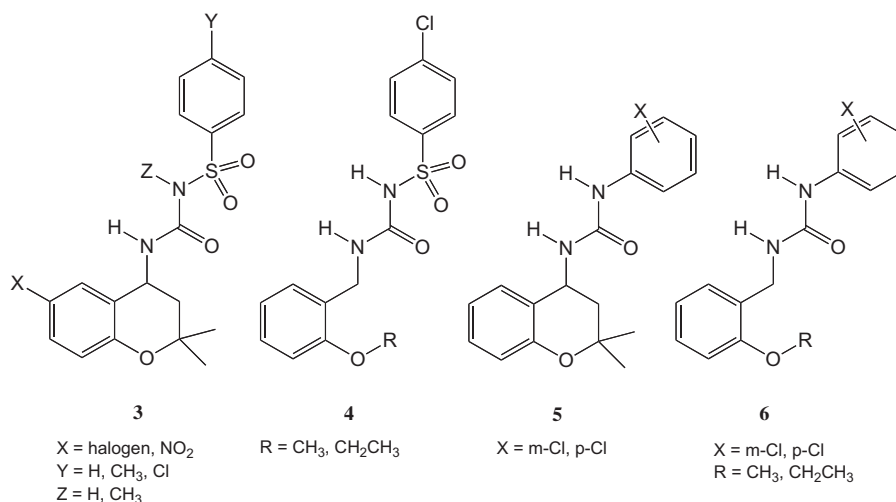


Fig. 2. Dihydrobenzopyrans and acyclic analogues, structurally related to cromakalim, holding arylsulfonylurea and arylurea moieties.

shifts are reported in δ values (ppm) relative to internal TMS. The abbreviation s = singlet, d = doublet, t = triplet, m = multiplet, CH_{arom.} = aromatic CH 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 $\pm 0.4\%$ of theoretical values. All reactions were routinely checked by TLC on silica gel Merck 60F 254.

4.1. Chemistry, general procedure

The appropriate compound **12** (2.4 mmol, 1 eq.) was added to the solution of the appropriate 4-amino-2,2-dimethyl-2H-1-benzopyran (2.4 mmol, 1 eq.) in anhydrous dichloromethane (20 mL). After refluxing the mixture for 2 h, the precipitate formed was collected by filtration, washed with petroleum ether 40/65, dried and recrystallized in methanol.

4.1.1. N-(2,2-Dimethyl-6-fluoro-3,4-dihydro-2H-1-benzopyran-4-yl)-4H-1,2,4-benzothiadiazine-3-carboxamide 1,1-dioxide (**7a**)

White powder (90%); mp 304–305 °C; ¹H NMR (500 MHz, d₆-DMSO) δ : 1.26 (3H, s, CH₃), 1.39 (3H, s, CH₃), 2.01 (1H, m, H_A of CH₂), 2.15 (1H, m, H_B of CH₂), 5.21 (1H, m, CH), 6.77 (1H, m, 8-H), 7.00 (2H, m, 5-H + 7-H), 7.52 (1H, t, 7'-H), 7.74 (1H, t, 6'-H), 7.85 (2H, m, 5'-H + 8'-H), 9.55 (1H, d, CONH), 12.65 (1H, bs, NH); ¹³C NMR (125 MHz, d₆-DMSO) δ : 23.78, 29.42, 37.14, 42.90, 75.36, 113.20, 115.33, 117.98, 118.04, 119.33, 121.63, 123.16, 123.22, 127.17, 133.27, 146.94, 149.61, 159.08. Anal. (C₁₉H₁₈N₃O₆S) C, H, N, S.

H + 8'-H), 9.55 (1H, d, CONH), 12.65 (1H, bs, NH); ¹³C NMR (125 MHz, d₆-DMSO) δ : 23.78, 29.42, 37.14, 42.90, 75.36, 113.20, 115.33, 117.98, 118.04, 119.33, 121.63, 123.16, 123.22, 127.17, 133.27, 146.94, 149.61, 159.08. Anal. (C₁₉H₁₈N₃O₆S) C, H, N, S.

4.1.2. N-(2,2-Dimethyl-6-fluoro-3,4-dihydro-2H-1-benzopyran-4-yl)-7-methyl-4H-1,2,4-benzothiadiazine-3-carboxamide 1,1-dioxide (**7b**)

White powder (95%); mp 272–273 °C; ¹H NMR (500 MHz, d₆-DMSO) δ : 1.27 (3H, s, CH₃), 1.40 (3H, s, CH₃), 2.01 (1H, m, H_A of CH₂), 2.14 (1H, m, H_B of CH₂), 2.41 (3H, s, CH₃), 5.20 (1H, m, CH), 6.78 (1H, m, 8-H), 7.00 (2H, m, 5-H + 7-H), 7.56 (1H, d, 5'-H), 7.67 (1H, s, 8'-H), 7.73 (1H, d, 6'-H), 9.53 (1H, d, CONH), 12.61 (1H, bs, NH); ¹³C NMR (125 MHz, d₆-DMSO) δ : 20.52, 23.77, 29.41, 37.13, 42.90, 75.36, 113.18, 115.25, 117.98, 118.04, 119.19, 121.50, 122.58, 123.17, 134.23, 137.21, 146.56, 149.62, 159.09. Anal. (C₂₀H₂₀N₃O₄S) C, H, N, S.

4.1.3. N-(6-Bromo-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran-4-yl)-4H-1,2,4-benzothiadiazine-3-carboxamide 1,1-dioxide (**7c**)

White powder (90%); mp 225–226 °C; ¹H NMR (500 MHz, d₆-DMSO) δ : 1.28 (3H, s, CH₃), 1.39 (3H, s, CH₃), 2.02 (1H, m, H_A of CH₂), 2.13 (1H, m, H_B of CH₂), 5.21 (1H, m, CH), 6.74 (1H, m, 8-H), 7.31 (2H, m, 5-H + 7-H), 7.54 (1H, t, 7'-H), 7.75 (1H, t, 6'-H), 7.86 (2H, m, 5'-H + 8'-H), 9.59 (1H, d, CONH), 12.65 (1H, bs, NH); ¹³C NMR

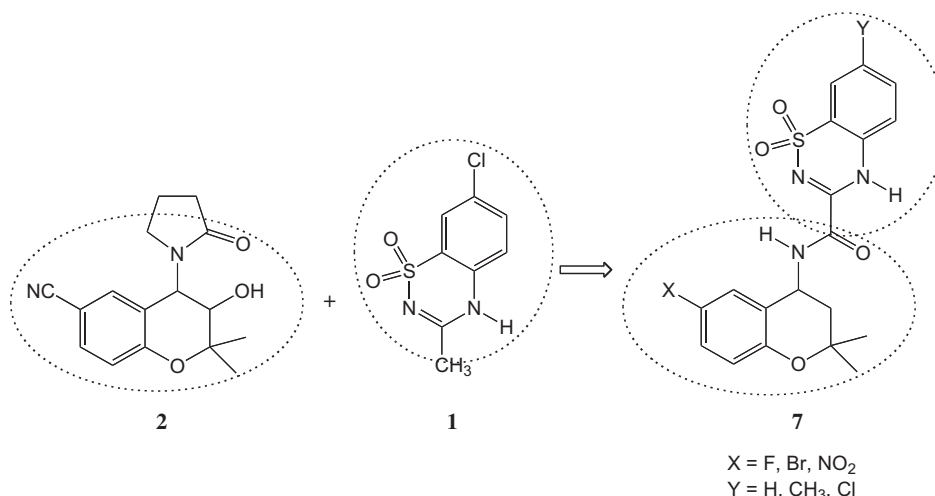
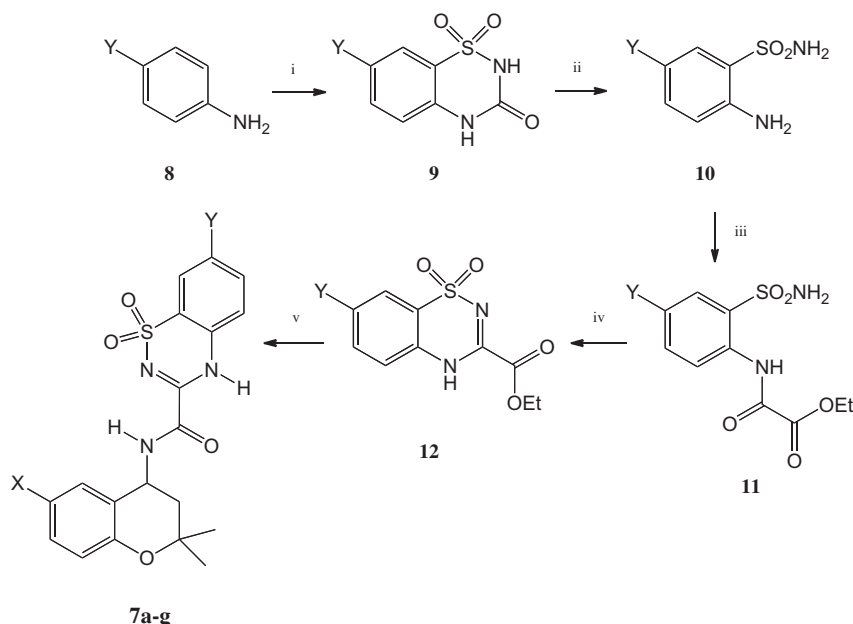


Fig. 3. Design of hybrid compounds (**7**) with structural features of both cromakalim (**2**) and diazoxide (**1**).

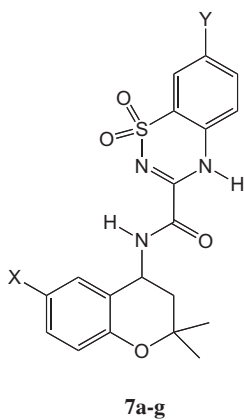


Scheme 1. i: 1. ClSO₂NCO/nitromethane; 2. AlCl₃; ii: H₂SO₄/H₂O; iii: ClCOCO₂Et/Et₃N; iv NaOEt/EtOH; v: 4-amino-6-X-3,4-dihydro-2H-1-benzopyran ²²/CH₂Cl₂ (X = F, Br, NO₂).

(125 MHz, d₆-DMSO) δ : 23.89, 29.33, 37.15, 42.74, 75.77, 111.20, 119.17, 119.24, 121.59, 123.33, 124.39, 127.26, 129.63, 131.25, 133.33, 134.72, 146.81, 152.76, 159.02. Anal. (C₁₉H₁₈BrN₃O₄S) C, H, N, S.

Table 1

Effects of compounds **7a–g** on insulin secretion from rat pancreatic islets and on the contractile activity of rat aorta rings.



7a–g

Compound	X	Y	Residual insulin secretion ^a (%) Effect at 50 μ M	Myorelaxant activity rat aorta EC ₅₀ ^b (μ M)
7a	F	H	77.3 \pm 3.8 (13)	>30.0 (4)
7b	F	CH ₃	96.9 \pm 5.2 (14)	>30.0 (4)
7c	Br	H	86.7 \pm 5.4 (25)	>30.0 (4)
7d	Br	CH ₃	91.4 \pm 4.5 (24)	>30.0 (4)
7e	NO ₂	H	77.9 \pm 4.6 (13)	>30.0 (4)
7f	NO ₂	Cl	77.0 \pm 4.7 (21)	>30.0 (4)
7g	NO ₂	CH ₃	75.7 \pm 4.0 (21)	>30.0 (3)
Diazoxide	–	–	28.8 \pm 2.4 (21) ^c	22.4 \pm 2.1 (11) ^c
Pinacidil	–	–	92.1 \pm 5.5 (21) ^c	0.35 \pm 0.02 (11) ^c
Cromakalim	–	–	77.2 \pm 4.3 (22) ^c	0.13 \pm 0.01 (7) ^c

^a Percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean \pm SEM (n)).

^b EC₅₀: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aorta rings (mean \pm SEM (n)).

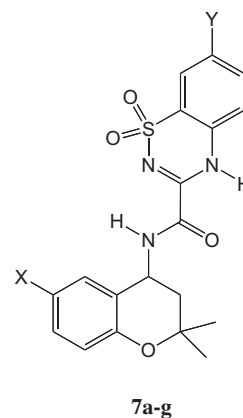
^c Published results: Ref. [21].

4.1.4. N-(6-Bromo-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran-4-yl)-7-methyl-4H-1,2,4-benzothiadiazine-3-carboxamide 1,1-dioxide (**7d**)

White powder (95%); mp 265–266 °C; ¹H NMR (500 MHz, d₆-DMSO) δ : 1.28 (3H, s, CH₃), 1.40 (3H, s, CH₃), 2.02 (1H, m, H_A of CH₂), 2.13 (1H, m, H_B of CH₂), 2.41 (3H, s, CH₃), 5.21 (1H, m, CH), 6.73 (1H, m, 8-H), 7.31 (2H, m, 5-H + 7-H), 7.57 (1H, d, 5'-H), 7.67 (1H, s, 8'-H), 7.73 (1H, d, 6'-H), 9.57 (1H, d, CONH), 12.61 (1H, bs, NH); ¹³C NMR

Table 2

Effects of compounds **7a–g** on the contractile activity of rat uterus.



7a–g

Compound	X	Y	Residual contractile activity of rat uterus (% \pm SEM (n)) ^a		
			10 μ M	50 μ M	100 μ M
7a	F	H	123.7 \pm 5.1 (4)	97.9 \pm 4.7 (4)	76.8 \pm 4.3 (4)
7b	F	CH ₃	116.6 \pm 5.7 (4)	132.4 \pm 8.5 (4)	124.4 \pm 8.5 (4)
7c	Br	H	60.1 \pm 4.5 (4)	44.3 \pm 6.9 (4)	19.9 \pm 4.9 (4)
7d	Br	CH ₃	76.8 \pm 2.8 (4)	44.5 \pm 5.4 (4)	6.6 \pm 1.7 (4)
7e	NO ₂	H	96.8 \pm 3.5 (4)	62.4 \pm 4.4 (4)	34.7 \pm 4.5 (4)
7f	NO ₂	Cl	97.9 \pm 9.0 (4)	70.9 \pm 4.7 (4)	34.7 \pm 2.8 (4)
7g	NO ₂	CH ₃	103.9 \pm 7.4 (4)	94.9 \pm 6.5 (4)	90.6 \pm 4.1 (4)
Diazoxide	–	–	93.8 \pm 2.2 (4) ^b	76.3 \pm 4.9 (4) ^b	67.7 \pm 4.0 (4) ^b
Pinacidil	–	–	58.1 \pm 4.4 (4) ^b	35.5 \pm 2.9 (4) ^b	38.1 \pm 2.2 (4) ^b

^a Residual contractile activity of rat uterus (%) contracted by oxytocin (20 mU) injected in the superfusion circuit.

^b Published results: Ref. [22].

(125 MHz, d_6 -DMSO) δ : 23.89, 29.32, 37.11, 42.73, 75.77, 111.19, 119.18, 121.48, 122.59, 124.38, 124.41, 129.61, 131.24, 134.26, 137.27, 146.41, 152.76, 159.00. Anal. ($C_{20}H_{20}N_3O_4S$) C, H, N, S.

4.1.5. *N*-(2,2-Dimethyl-6-nitro-3,4-dihydro-2H-1-benzopyran-4-yl)-4H-1,2,4-benzothiadiazine-3-carboxamide 1,1-dioxide (**7e**)

White powder (90%); mp 272–273 °C; 1H NMR (500 MHz, d_6 -DMSO) δ : 1.35 (3H, s, CH_3), 1.47 (3H, s, CH_3), 2.17 (2H, m, CH_2), 5.30 (1H, m, CH), 6.99 (d, 1H, 8-H), 7.54 (1H, t, 7'-H), 7.74 (t, 1H, 6'-H), 7.87 (2H, m, 5'-H + 8'-H), 8.07 (2H, m, 7-H + 5-H), 9.69 (1H, d, CONH), 12.73 (1H, bs, NH); ^{13}C NMR (125 MHz, d_6 -DMSO) δ : 24.16, 29.11, 36.76, 42.62, 77.78, 117.97, 119.28, 121.60, 122.92, 123.34, 123.70, 124.54, 127.33, 133.37, 134.70, 140.34, 146.65, 159.28. Anal. ($C_{19}H_{18}N_4O_6S$) C, H, N, S.

4.1.6. 7-Chloro-*N*-(2,2-dimethyl-6-nitro-3,4-dihydro-2H-1-benzopyran-4-yl)-4H-1,2,4-benzothiadiazine-3-carboxamide 1,1-dioxide (**7f**)

White powder (95%); mp 284–287 °C; 1H NMR (d_6 -DMSO) δ : 1.35 (3H, s, CH_3), 1.50 (3H, s, CH_3), 2.20 (2H, m, CH), 5.30 (1H, m, CH), 7.35 (1H, m, CH_{arom}), 7.75 (2H, m, CH_{arom}), 7.80 (1H, s, CH_{arom}), 8.10 (2H, m, CH_{arom}), 9.65 (1H, s, CONH). Anal. ($C_{19}H_{17}ClN_4O_6S$) C, H, N, S.

4.1.7. *N*-(2,2-Dimethyl-6-nitro-3,4-dihydro-2H-1-benzopyran-4-yl)-7-methyl-4H-1,2,4-benzothiadiazine-3-carboxamide 1,1-dioxide (**7g**)

White powder (90%); mp 310–312 °C; 1H NMR (500 MHz, d_6 -DMSO) δ : 1.35 (3H, s, CH_3), 1.47 (3H, s, CH_3), 2.15 (2H, m, CH_2), 5.12 (1H, m, CH), 6.83 (1H, d, 8-H), 7.40 (1H, d, 5'-H), 7.51 (1H, s, 8'-H), 7.58 (1H, d, 6'-H), 7.90 (2H, m, 5-H + 7-H), 9.49 (1H, d, CONH), 12.51 (1H, bs, NH); ^{13}C NMR (125 MHz, d_6 -DMSO) δ : 20.53, 24.16, 29.12, 36.74, 42.70, 77.79, 117.97, 119.17, 121.48, 122.61, 122.94, 123.69, 124.54, 132.29, 134.33, 137.39, 140.33, 146.28, 159.28. Anal. ($C_{19}H_{18}N_4O_6S$) C, H, N, S.

4.2. Rat pancreatic islets

Pancreatic islets were isolated by the collagenase method from fed Wistar rats (180–220 g). Groups of 10 islets, each derived from the same batch of islets, were preincubated for 30 min at 37 °C in 1 mL of a physiological salt medium (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24) supplemented with 2.8 mM glucose, 0.5% (w/v) 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 a further 90 min in 1 mL of the same medium containing 16.7 mM glucose and, in addition, the reference compound or the required cromakalim derivative. The release of insulin was measured radio-immunologically using rat insulin as a standard [23]. Residual insulin release was expressed as a percentage of the value recorded in control experiments (100%), i.e., in the absence of drug and presence of 16.7 mM glucose.

4.3. Rat aorta rings

All experiments were performed on aorta collected from fed Wistar rats (180–220 g), as previously described [23]. The organ bath solution contained (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5. The physiological solution was maintained at 37 °C and bubbled with O_2 (95%) and CO_2 (5%). The ED_{50} value was assessed, for each dose–response curve, as the concentration producing 50% inhibition of the plateau phase induced by KCl 30 mM. Results are expressed as mean values (\pm SEM).

4.4. Rat uterus

Fed wistar rats (150–200 g) were treated the day before killing with diethylstilboestrol dipropionate [i.m. injection of 0.1 mL/100 g

of a 1 mg/mL oily solution of diethylstilboestrol dipropionate (Sigma)]. The rats were anesthetized and then sacrificed. The two uterine horns were removed, cleared of adhering fat and connective tissue, and separated. Each horn was superfused with a Tyrode solution (in mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, glucose 5.6) bubbled continuously with a mixture of O_2 (95%) and CO_2 (5%). The superfusate was maintained at 37 °C. Injections of 20 mU oxytocin (200 μ L of a 0.1 U/mL solution of the hormone in 0.9% NaCl) in the superfusion channel were repeated at 10-min intervals until the recorded contractions (AUC) were constant. The mean of the three last injections gave the 100% of the contractile response to oxytocin. For each drug concentration added in the medium, injection of 20 mU oxytocin was repeated at least three times. The contractile responses recorded (mean of three AUC) were expressed as a percent of the reference value (contractile response to oxytocin in the absence of drug) [22].

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2012.05.011.

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