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ROYAL SOCIETY OF CHEMISTRY View Article Online
DOI: 10.1039/C8MD00593A

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RESEARCH ARTICLE

2,2-Dimethyl-3,4-dihydro-2*H*-1,4-benzoxazines as isosteres of 2,2-dimethylchromans acting as inhibitors of insulin release and vascular smooth muscle relaxants

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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The present study describes the synthesis and biological evaluation of 4-phenylureido/thioureido-substituted 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazines as isosteres of corresponding 2,2-dimethylchromans reported to be pancreatic β-cell K_{ATP} channel openers. The benzoxazines were found to be less active as inhibitors of the glucose-induced insulin release than their corresponding chromans, while the myorelaxant activity of some 4-arylureido-substituted benzoxazines was more pronounced than that exhibited by their chroman counterparts. The myorelaxant activity of the most potent benzoxazine **8e** was further characterized on rat aortic rings precontracted by 30 mM KCl in the presence of glibenclamide (10 μM) or precontracted by 80 mM extracellular KCl. Our findings indicate that, on vascular smooth muscle cells, the benzoxazine **8e** mainly behaved as a calcium entry blocker.

1. Introduction

ATP-sensitive potassium channels (K_{ATP} channels) located at the plasma membrane of excitable cells are ion channels linking cell metabolism to membrane excitability.[1-3]

K_{ATP} channels are involved in multiple physiological processes, among which the control of insulin release from pancreatic β-cells and the modulation of vascular smooth muscle tone.[4-6] The structure of those channels at the molecular level has been reported to be octameric complexes of four pore-forming inwardly rectifying K^+ (K_{ir}6.x) channels (K_{ir}6.1 or K_{ir}6.2) and four regulatory sulfonylurea receptor (SURx) subunits (SUR1, SUR2A or SUR2B).[7-9]

Several modulators of the K_{ATP} channels are currently used in clinical practice (i.e. hypoglycemic sulfonylureas as oral antidiabetic drugs) [10] and original K_{ATP} channel openers are expected to become promising therapeutic agents provided that they are able to exert high potency and selectivity for a single K_{ATP} channel subtype.[11] Selective openers of the pancreatic β-cell K_{ATP} channel subtype (SUR1/Kir6.2) have been proposed for the prevention and/or management of type 1 - type 2 diabetes, congenital hyperinsulinism and treatment of insulinoma.[12-14] Likewise, selective openers of the smooth muscle cell K_{ATP} channel subtype (SUR2B/Kir6.1) have been mainly examined as antihypertensive agents or potential bronchodilators.[15]

A typical example of ATP-sensitive potassium channel opener (PCO) is the 2,2-dimethylchroman (±)-cromakalim [reported in Figure 1 as the levorotatory isomer levcromakalim (**1**)], which is known to be much more potent on the smooth muscle SUR2B-type than on the pancreatic endocrine SUR1-type K_{ATP} channel.[16]

Although the configuration of the 3- and 4-positions of the chroman ring of cromakalim were found to be critical for myorelaxant activity (levcromakalim was the most active among four possible isomers) [17], many other examples of PCOs structurally related to cromakalim have been developed; among which compounds devoid of chiral centers at the 3- and 4-positions. The cromakalim analogue Ro31-6930 (**2**, Figure 1) is a PCO devoid of chiral carbon atoms, indicating that a planar sp2 configuration at the 4-position can be tolerated and doesn't negatively impact its pharmacological profile.[18] Moreover, this planar carbon atom at the 4-position can be replaced by a nitrogen atom giving rise to potent PCOs belonging to 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazines, such as YM934 (**3,** Figure 1)[19] tightly related to **2**, and such as compound **4** (Figure 1)[20] bearing an alkylamide residue at the 4-position. This series of heterocyclic compounds may be viewed as isosteres of 2,2-dimethylchromans for which the chiral center at the 4-position has been suppressed. Incidentally, 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzothiazine isosteres (replacement of the oxygen atom at the 1-position with a sulfur atom) such as compound **5** (Figure 1) were reported to be among the most myorelaxant K_{ATP} channel openers ever discovered.[21]

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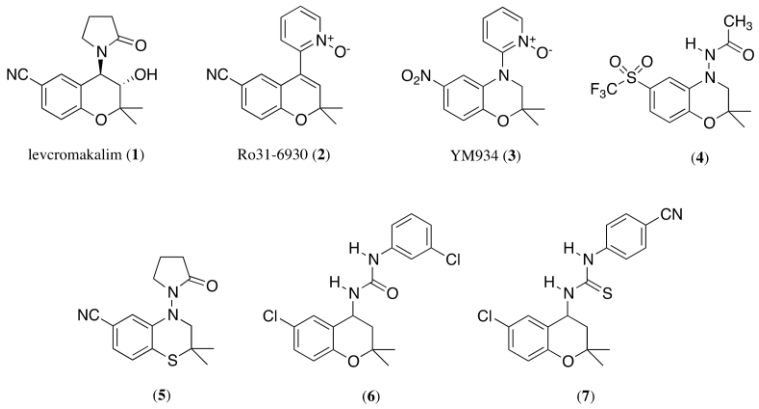

Fig. 1 Chemical structure of K_{ATP} channel openers belonging to 2,2-dimethylchromans (**1**, **6**, **7**), 2,2-dimethylchromens (**2**), 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazines (**3**, **4**) and 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzothiazines (**5**).

In previous works, we described novel series of cromakalim analogues as potential K_{ATP} channel openers belonging to 2,2-dimethylchromans.[22-30] The introduction of a phenylurea or a phenylthiourea residue at the 4-position of the chroman ring led to compounds such as **6** and **7** (Figure 1), which, in contrast to cromakalim, were found to exert a strong opening activity on the pancreatic SUR1-type K_{ATP} channels. [22,26]

The present study was an attempt to examine the impact of the introduction of such phenylurea/thiourea moieties, at the 4-position of 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazines (Figure 2), on insulin secreting cells activity and vascular smooth muscle tone. Both biological responses could reflect a potential interaction with K_{ATP} channels or voltage-sensitive calcium channels.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds (**8a-h**) is described in scheme 1. Starting from 2-amino-4-chlorophenol (**9**), ring closure reaction with 2-bromo-2-methylpropionyl bromide led to the 2,2-dimethylbenzoxazine-3-one (**10**). The amide function of **10** was reduced by means of borane-tetrahydrofuran complex to provide the corresponding secondary amine (**11**), which reacted with nitrous acid to be converted into the 4-nitroso-substituted 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazine (**12**). Reduction of the nitroso group with zinc metal in acetic acid gave access to the 4-amino-substituted benzoxazine (**13**). The latter reacted with the appropriate phenyl isocyanate or phenyl isothiocyanate to provide the corresponding 4-phenyl(thio)ureido-substituted 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazines (**8a-h**).

An interesting observation was conducted with the ^{1}H-NMR data obtained with the 4-arylurea-substituted compounds **8a-d** compared to their 4-arylthiourea-substituted counterparts **8e-h** (see experimental section 3.1). Although the signals attributed to the two methylenic protons at the 2-position of the benzoxazine ring and to the six protons of the two methyl groups linked at the 2-position for the arylurea compounds **8a-d** logically appeared as singlets (around 3.27 ppm for $-CH_2-$ and around 1.37 ppm for the two $-CH_3$), the picture was clearly different for their arylthiourea counterparts **8e-h**. In the latter case, the two methylenic protons at the 3-position were differencied and a coupling was observed (doublets at around 3.15 ppm and 3.38 ppm). Moreover, the two methyl groups at the 2-position appeared at two different chemical shifts (singlets around 1.34 and 1.48). It is tempting to speculate that, for compounds **8e-h**, the thiourea group adopted a permanent position in the space that differently influenced the electronic environment of the two methylenic protons and the two methyl groups. The higher length of the C=S bound, compared to the C=O, bound should be responsible for a lack of free rotation around the N-N single bound.

2.2. Biological results

The biological effects of the newly synthesized 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazines **8a-h**, of the corresponding 2,2-dimethylchromans **6**, **7**, **14a-d** and the reference compounds (±)-cromakalim and diazoxide, on the glucose-induced insulin release from rat pancreatic islets and on the contractile activity of K^+-depolarized rat aorta rings are reported in Table 1.

The benzoxazines were systematically found to be less active than their corresponding chromans on the glucose-induced insulin release, while the myorelaxant activity on rat aorta was found to be more pronounced with the 4-arylurea-substituted benzoxazines than with the corresponding 4-arylurea-substituted chromans (see **8g** and **8h** versus **6** and **14d**). None of them, however, expressed the myorelaxant properties of the 2,2-dimethylchroman reference compound (±)-cromakalim (Table 1).

Looking at the activity on insulin secretion (10 µM), it was observed that, for thioureas **8a** and **8b**, the para position for the CN substituent was preferred to the meta position, while the situation was found to be different in the urea series (see **8e** and **8f**).

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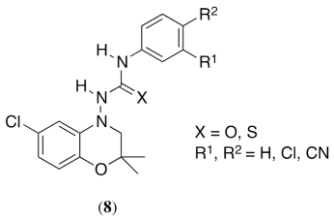

(8)

Fig. 2 General formula of the newly synthesized 4-arylureido/thioureido-substituted 6-chloro-2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazines (**8**).

By comparing the myorelaxant activity of 4-arylurea-substituted benzoxazines **8e-h** with their 4-arylthiourea-substituted counterparts **8a-d**, it was clearly observed that the arylureas were more potent than the corresponding arylthioureas. Such a comparison was less obvious on the pancreatic endocrine tissue.

The most active 2,2-dimethylbenzoxazine on rat pancreatic islets was the 4-cyanophenylthiourea-substituted compound **8b**, which was the isosteric analogue of the most active 4-cyanophenylthiourea-substituted 2,2-dimethylchroman **7** (Table 1). Both compounds were clearly more potent on the insulin releasing process than the reference compound diazoxide (Table 1).

Although most 2,2-dimethylchromans reported in table 1 expressed some tissue selectivity, being more potent on the endocrine tissue than on the smooth muscle tissue, this feature did not apply for the majority of corresponding 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazines (Table 1). The 4-arylthiourea-substituted benzoxazines **8a** and **8b** expressed some pancreatic endocrine tissue selectivity, while the corresponding 4-arylurea-substituted analogues **8e** and **8f** were found to be active on both tissues in the same range of concentrations (Table 1). Compounds **8g** and **8h** were also equipotent on pancreatic β-cells and vascular cells.

A critical information can be deduced from the virtual logP value ('average' logP value) calculated for each compound (Table 1). As expected, the isosteric replacement of a –CH- moiety by a –N- atom was responsible for an increase in the hydrophilicity. Likewise, the thiourea derivatives were found to be more lipophilic than the corresponding urea derivatives in both series of compounds.

Finally, regarding the perspective of development of new therapeutic drugs, the newly synthesized benzoxazines appeared to exhibit a more favorable hydrophilic/lipophilic balance compared to the previously described chromans. Indeed, the 2,2-dimethylchromans previously synthesized exhibited an estimated average logP higher than 4, and sometimes close to 5, being at the limit of the criterion defined by the Lipinski's "rule of five" for acceptable oral bioavailability.[31]

In order to decipher the mechanism of action of the most potent myorelaxant benzoxazine **8e**, its vasorelaxant activity was further characterized on rat aortic rings precontracted by 30 mM KCl in the presence of glibenclamide (10 µM) or precontracted by 80 mM extracellular KCl.

The concomitant presence of the K_{ATP} channel blocker glibenclamide (10 µM) in the bathing solution failed to affect the myorelaxant properties of **8e** ($P > 0.05$, Table 2); as it can be observed with calcium entry blockers such as verapamil.[29,37] By contrast, the K_{ATP} channel blocker glibenclamide induced a marked reduction in the vasorelaxant response to the potassium channel opener (±)-cromakalim (Table 2).

When the aorta rings were precontracted by 80 mM KCl, the vasorelaxant potency of compound **8e** was not significantly affected ($P > 0.05$); as previously reported for the calcium entry blocker verapamil.[29,37] By contrast, and under the same experimental conditions, the myorelaxant effect of the potassium channel opener (±)-cromakalim was drastically reduced (Table 2). On the whole, these findings indicate that, on vascular smooth muscle cells, **8e** mainly behaved as a calcium entry blocker.

3. Experimental section

3.1. Chemistry

All commercial chemicals (Sigma-Aldrich, Belgium; Appolo Scientific, United Kingdom and Fluorochem, United Kingdom) and solvents were reagent grade and used without further purification. Melting points were determined on a Stuart SMP3 apparatus in open capillary tubes and are uncorrected. NMR spectra were recorded on a Bruker Avance 500 spectrometer (^{1}H: 500 MHz; ^{13}C: 125 MHz) using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard; chemical shifts are reported in δ values (ppm) relative to internal TMS. The abbreviation s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet and bs = broad signal are used throughout. Elemental analyses (C, H, N, S) were carried out on a Thermo Flash EA 1112 series elemental analyzer and were within ± 0.4% of the theoretical values. This analytical process ensured, for each final compound, a purity equal or greater than 95 %. All reactions were followed by TLC (silica gel $60F_{254}$ Merck) and visualization was accomplished with UV light (254 or 366 nm).

3.1.1. 6-Chloro-2,2-dimethyl-2*H*-benzoxazin-3(4*H*)-one (10)

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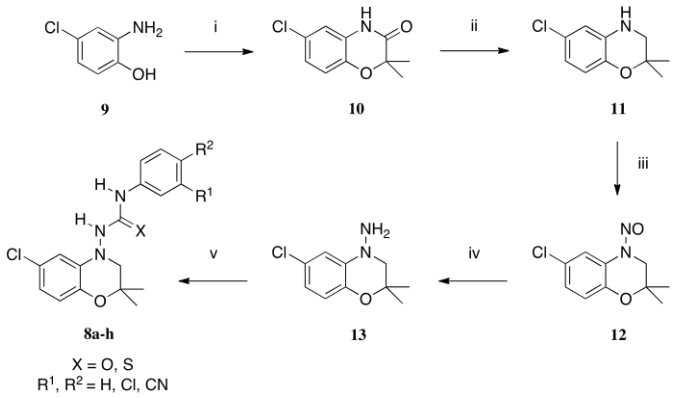

Scheme 1: Synthetic pathway to 4-phenyl(thio)ureido-substituted 6-chloro-2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazines **8**. Reagents: i: 1. Br-CO-C(CH_3)$_2$-Br, Na_2CO_3, $CHCl_3$, H_2O; 2. K_2CO_3, DMF, 80°C; ii : BH_3, THF; iii $NaNO_2$, AcOH; iv : Zn, AcOH; v : 3-/4-chloro-/ 3-/4-cyanophenyl iso(thio)cyanate, CH_2Cl_2.

The title compound was obtained in two steps. Firstly, 2-amino-4-chlorophenol (**9**) (14.36 g, 0.1 mol) was suspended in a mixture of $CHCl_3$ (600 mL) in a saturated solution of sodium carbonate (350 mL). The medium was then cooled to 0 °C and 2-bromo-2-methylpropionyl bromide (18.54 mL, 0.15 mol) was added dropwise. The mixture was stirred 4 hours at room temperature. The organic layer was decanted, washed with water, dried over $MgSO_4$ and evaporated under reduced pressure.

Secondly, the crude product was solubilized in DMF (250 mL) and K_2CO_3 (13.82 g, 0.1 mol) was added. The mixture was then stirred at 80 °C during 3 hours. At the end of the reaction, water (250 mL) was added. The resulting precipitate was collected by filtration and the filtrate was extracted by $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The precipitate and the crude material were crystallized in boiling ether. The title compound was collected by filtration, washed by ether and dried (16.93g, 80 %): mp: 164-166 °C; IR (KBr) υ: 1687 (C=O), 3132 (N-H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.40 (s, 6H, CH_3), 6.89 (s, 1H, 7-*H*), 6.96 (s, 2H, 5-*H* et 8-*H*), 10.73 (s, 1H, -NH_2). Anal. ($C_{10}H_{10}ClNO_2$) theoretical: C, 56.75; H, 4.76; N, 6.62. Found: C, 56.94; H, 4.68; N, 6.58.

3.1.2. 6-Chloro-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (11)

A borane tetrahydrofuran complex solution 1M (106 mL, 106 mmol) was added to a ice-cooled solution of 6-chloro-2,2-dimethyl-2*H*-benzoxazin-3(4*H*)-one (**10**) (15 g, 47.24 mmol) in anhydrous tetrahydrofuran. The mixture was stirred overnight at room temperature. Water was then added to quench the reaction. The title compound was extracted by ethyl acetate. The organic layer was dried over $MgSO_4$ and was evaporated under reduced pressure. The crude product was used in the next step without further purification (10.93 g, 78 %): IR (KBr) υ: 3385 (N-H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.22 (s, 6H, CH_3), 3.00 (s, 2H, CH_2), 6.47 (dd, 1H, 7-*H*), 6.59 – 6.61 (m, 2H, 5-*H* et 8-*H*), 7.06 (bs, 1H, -NH).

3.1.3. 6-Chloro-3,4-dihydro-2,2-dimethyl-4-nitroso-2*H*-benzoxazine (12)

6-Chloro-2,2-dimethyl-3,4-dihydro-2*H*-benzoxazine (**11**) (5.79 g, 9,29 mmol) was dissolved in methanol and acetic acid (3.62 mL, 63.22 mmol) was added. The solution was cooled to 0 °C and an aqueous solution of $NaNO_2$ (3.51 g, 50.82 mmol) was cautiously added. The mixture was stirred overnight at room temperature. After completion of the reaction, the mixture was adjusted to pH=10 with sodium hydroxide 10%. The title compound was precipitated by water, collected by filtration, washed by water and dried (5.98 g, 78 %): mp: 65-67 °C; IR (KBr) υ: 1494 (N=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.27 (s, 6H, CH_3), 3.95 (s, 2H, CH_2), 7.08 (d, 1H, 8-*H*), 7.32 (dd, 1H, 7-*H*), 7.97 (d, 1H, 5-*H*). Anal. ($C_{10}H_{11}ClN_2O_2$) theoretical: C, 52.99; H, 4.89; N, 12.36. Found: C, 52.82; H, 4.89; N, 12.29.

3.1.4. 4-Amino-6-chloro-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (13)

6-Chloro-2,2-dimethyl-4-nitroso-3,4-dihydro-2*H*-benzoxazine (**12**) (5 g, 22,06 mmol) was dissolved in ice-cooled methanol (140 mL). Acetic acid (24 mL, 419 mmol) and zinc powder (7.07 g, 108 mmol) were added. The solution was stirred 15 minutes at room temperature. When reaction ended, the mixture was adjusted to pH=10 with concentrated ammonia. The title compound was extracted by ethyl acetate. The organic layers were pooled, dried over MgSO4 and evaporated under reduced pressure. The crude product was purified by chromatography on a silica gel 60 column eluted by methylene chloride. Pure fractions were pooled and the solvent was evaporated under reduced pressure (3.57 g, 76 %): mp: 79-81 °C; IR (KBr) υ: 3347 (N-H_2) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.27 (s, 6H, CH_3), 3.08 (s, 2H, CH_2), 4.46 (s, 2H, NH_2), 6.55-6.61 (m, 2H, 7-*H* et 8-*H*), 7.97 (d, 1H, 5-*H*). Anal. ($C_{10}H_{13}ClN_2O$) theoretical: C, 56.48; H, 6.16; N, 13.17. Found: C, 56.55; H, 6.02; N, 12.92.

3.1.5. 6-Chloro-4-(3-cyanophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (8a)

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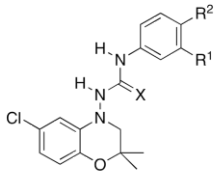
Table 1: Estimated logP (Average logP) values and effects of original 4-phenyl(thio)ureido-substituted 6-chloro-2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazines**,** 4-phenyl(thio)ureido-substituted 6-chloro-2,2-dimethyl-3,4-dihydro-2*H*-chromans, (±)-cromakalim and diazoxide on the glucose-induced insulin release from rat pancreatic islets and on the contractile activity of K^+ depolarized rat aorta rings.



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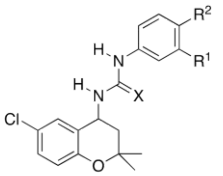

6, 7, 14a-d

Compounds	X	R^1	R^2	Average log *P*[a]	Residual insulin secretion (%)[b] 10 µM	Residual insulin secretion (%)[b] 1 µM	Myorelaxant activity EC_{50} (µM)[c]
8a	S	CN		3.83	27.0 ± 1.3 (31)	83.1 ± 4.8 (23)	>30 (4)
8b	S		CN	3.84	10.8 ± 0.6 (23)	73.3 ± 3.0 (22)	>30 (4)
8c	S	Cl		4.71	71.8 ± 3.8 (31)	-	>30 (6)
8d	S		Cl	4.71	81.4 ± 4.7 (30)	-	>30 (6)
8e	O	CN		3.42	35.7 ± 2.5 (14)	79.5 ± 4.6 (15)	1.9 ± 0.6 (5)
8f	O		CN	3.43	43.1 ± 2.8 (16)	79.0 ± 5.4 (32)	3.7 ± 1.0 (4)
8g	O	Cl		4.31	46.0 ± 3.0 (15)	92.5 ± 3.7 (23)	9.9 ± 2.7 (7)
8h	O		Cl	4.32	56.2 ± 3.5 (16)	89.5 ± 5.1 (21)	13.3 ± 2.6 (4)
14a	S	CN		4.04	15.8 ± 0.9 (18)[d]	66.2 ± 2.5 (31)[d]	0.60 ± 0.06 (4)[d]
7	S		CN	4.04	10.6 ± 0.7 (24)[d]	47.8 ± 2.8 (29)[d]	>10.0 (4)[d]
14b	S	Cl		4.90	16.2 ± 1.6 (22)[e]	89.5 ± 3.7 (23)[e]	>10.0 (4)[e]
14c	S		Cl	4.90	8.9 ± 0.7 (15)[e]	87.9 ± 3.0 (16)[e]	>10.0 (5)[e]
6	O	Cl		4.55	25.0 ± 1.3 (24)[f]	81.0 ± 4.0 (23)[f]	>30 (4)[f]
14d	O		Cl	4.55	34.6 ± 1.9 (21)[f]	85.4 ± 5.4 (22)[f]	>300 (4)[f]
(±)-cromakalim				n.d.	96.4 ± 6.5 (16)[g]	-	0.13 ± 0.01 (7)[g]
diazoxide				n.d.	80.8 ± 3.7 (32)[g]	-	26.1 ± 2.9 (4)[g]

[a] Average logP values calculated according to the ALOGPS 2.1 program (ref. 25). [b] Percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (n)). [c] EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aorta rings (mean ± SEM (n)). [d] Published compounds and results (ref. 22). [e] Published compounds and results (ref. 27). [f] Published compounds and results (ref. 26). [g] Published results (ref. 37).

3-Cyanophenyl isothiocyanate (415 mg, 2.59 mmol) was added to a solution of 4-amino-6-chloro-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (**13**) (500 mg, 2.35 mmol) in methylene chloride (5 mL). The solution was stirred 24 hours at room temperature. The product was collected by filtration, washed with *n*-hexane an dried (560 mg, 64 %): mp: 202-204 °C; IR (KBr) υ: 1543 (C=S), 2228 (C≡N), 3300 (N-H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.34 (s, 3H, CH_3), 1.48 (s, CH_3), 3.16 (d, J = 11.0 Hz, 1H, CH_2), 3.39 (d, J = 10.9 Hz, 1H, CH_2), 6.77 (d, J = 1.3 Hz, 1H, 5-*H*), 6.79 (d, J = 8.6 Hz, 1H, 8-*H*), 6.86 (d, J = 7.6 Hz, 1H, 7-*H*), 7.55 (t, J = 7.9 Hz, 1H, 5'-*H*), 7.63 (d, J = 7.6 Hz, 1H, 6'-*H*), 7.94 (d, J = 7.8 Hz, 1H, 4'-*H*), 8.13 (s, 1H, 2'-*H*), 9.84 (s, 1H, N*H*), 10.14 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6, 125 MHz): δ: 24.3 (*C*H$_3$), 26.0 (*C*H$_3$), 57.8 (*C*H$_2$), 75.2 (C-2), 110.7 (C-3'), 114.3 (C-5), 118.0 (C-8), 118.5 (CN), 121.3 (C-7), 124.0 (C-6), 128.2 (C-2'), 128.6 (C-6'), 129.4 (C-5'), 130.0 (C-4'), 134.8 (C-4a), 139.9 (C-1'), 142.6 (C-8a), 180.3 (CS). Anal. ($C_{18}H_{17}ClN_4OS$) theoretical: C, 57.98; H, 4.60; N, 15.03; S, 8.60. Found: C, 57.86; H, 4.53; N, 15.15; S, 8.56.

3.1.6. 6-Chloro-4-(4-cyanophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (8b)

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Table 2. Myorelaxant effects of **8e** and (±)-cromakalim on 30 mM or 80 mM KCl-precontracted rat aorta rings incubated in the absence or presence of glibenclamide.

Compounds	Myorelaxant activity EC_{50} (μM)[a]		
	KCl 30 mM	KCl 30 mM + 10 μM Gliben.[b]	KCl 80 mM
8e	3.0 ± 0.7 (4) 3.1 ± 0.5 (6)	3.9 ± 0.5 (8)	5.0 ± 1.2 (6)
(±)-Cromakalim	0.22 ± 0.07 (6)[c] 0.17 ± 0.01 (4)[c]	28.8 ± 6.0 (5)[c]	137.7 ± 10.4 (10)[c]

[a] EC_{50}: drug concentration giving 50% relaxation (mean ± SEM (n)). [b] Gliben.: glibenclamide. [c] Published results (ref. 37).

4-Cyanophenyl isothiocyanate (415 mg, 2.59 mmol) was added to a solution of 4-amino-6-chloro-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (**13**) (500 mg, 2.35 mmol) in methylene chloride (5 mL). The solution was refluxed for 1 hour. Thereafter, the mixture was cooled to room temperature. The resulting precipitate was collected by filtration, washed with *n*-hexane and dried (641 mg, 73 %): mp: 212-214 °C; IR (KBr) υ: 1541 (C=S), 2228 (C≡N), 3296 (N-H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.33 (s, 3H, CH_3), 1.48 (s, CH_3), 3.15 (d, J=11.0 Hz, 1H, CH_2), 3.38 (d, J = 10.7 Hz, 1H, CH_2), 6.75 (s, 1H, 5-*H*), 6.79 (d, J = 8.4 Hz, 1H, 8-*H*), 6.87 (d, J = 7.7 Hz, 1H, 7-*H*), 7.80 (d, J = 8.7 Hz, 2H, 3'-*H*/5'-*H*), 7.93 (d, J = 7.9 Hz, 2H, 2'-*H*/6'-*H*), 9.91 (s, 1H, N*H*), 10.20 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6, 125 MHz): δ: 24.2 (*C*H_3), 26.0 (*C*H_3), 57.7 (*C*H_2), 75.2 (C-2), 106.6 (C-4'), 114.3 (C-5), 118.0 (C-8), 118.9 (CN), 121.4 (C-7), 124.0 (C-6), 124.2 (C-2'/C-6'), 132.3 (C-3'/C-5'), 134.8 (C-4a), 142.7 (C-8a), 143.3 (C-1'), 179.8 (CS). Anal. ($C_{18}H_{17}ClN_4OS$) theoretical: C, 57.98; H, 4.60; N, 15.03; S, 8.60. Found: C, 57.87; H, 4.53; N, 15.16; S, 8.56.

3.1.7. 6-Chloro-4-(3-chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (8c)

The title compound was obtained as described for **8b**, starting from **13** (500 mg, 2.35 mmol) and 3-chlorophenyl isothiocyanate (340 μL, 2.59 mmol) (467 mg, 52 %): mp: 207-209 °C; IR (KBr) υ: 1540 (C=S), 3297 (N-H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.33 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 3.15 (d, 1H, CH_2), 3.38 (d, 1H, CH_2), 6.75-6.85 (m, 3H, 5-*H*, 7-*H* et 8-*H*), 7.23 (d, 1H, 4'-*H*), 7.36 (t, 1H, 5'-*H*), 7.56 (d, 1H, 6'-*H*), 7.81 (s, 1H, 2'-*H*), 9.73 (s, 1H, N*H*), 10.04 (s, 1H, N*H*).
^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.33 (s, 3H, CH_3), 1.47 (s, CH_3), 3.15 (d, J = 11.0 Hz, 1H, CH_2), 3.38 (d, J = 10.7 Hz, 1H, CH_2), 6.75 (s, 1H, 5-*H*), 6.78 (d, J = 8.5 Hz, 1H, 8-*H*), 6.86 (d, J = 6.9 Hz, 1H, 7-*H*), 7.23 (d, J = 7.0 Hz, 1H, 6'-*H*), 7.36 (t, J = 8.1 Hz, 1H, 5'-*H*), 7.57 (d, J = 7.6 Hz, 1H, 4'-*H*), 7.81 (s, 1H, 2'-*H*), 9.73 (s, 1H, N*H*), 10.04 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6, 125 MHz): δ: 24.3 (*C*H_3), 26.0 (*C*H_3), 57.7 (*C*H_2), 75.2 (C-2), 114.2 (C-5), 118.0 (C-8), 121.2 (C-7), 123.5 (C-6'), 124.0 (C-6), 124.5 (C-2'), 124.8 (C-4'), 129.6 (C-5'), 132.1 (C-3'), 134.9 (C-4a), 140.4 (C-1'), 142.6 (C-8a), 180.1 (CS). Anal. ($C_{17}H_{17}Cl_2N_3OS$) theoretical: C, 67.63; H, 5.68; N, 12.45; S, 9.50. Found: C, 67.41; H, 5.69; N, 12.36; S, 9.69.

3.1.8. 6-Chloro-4-(4-chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (8d)

The title compound was obtained as described for **14**, starting from **13** (500 mg, 2.35 mmol) and 4-chlorophenyl isothiocyanate (439 mg, 2.59 mmol) (530 mg, 59 %): mp: 205-207 °C; IR (KBr) υ: 1533 (C=S), 3268 (N-H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.33 (s, 3H, CH_3), 1.47 (s, CH_3), 3.16 (d, J = 11.0 Hz, 1H, CH_2), 3.38 (d, J = 10.3 Hz, 1H, CH_2), 6.75 (s, 1H, 5-*H*), 6.78 (d, J = 8.5 Hz, 1H, 8-*H*), 6.85 (d, J = 6.6 Hz, 1H, 7-*H*), 7.40 (d, J = 8.8 Hz, 2H, 3'-*H*/5'-*H*), 7.62 (d, J = 8.7 Hz, 2H, 2'-*H*/6'-*H*), 9.71 (s, 1H, N*H*), 9.99 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6, 125 MHz): δ: 24.3 (*C*H_3), 26.0 (*C*H_3), 57.7 (*C*H_2), 75.1 (C-2), 114.1 (C-5), 118.0 (C-8), 121.2 (C-7), 124.0 (C-6), 126.8 (C-2'/C-6'), 127.9 (C-3'/C-5'), 129.1 (C-4'), 134.9 (C-4a), 137.9 (C-1'), 142.6 (C-8a), 180.2 (CS). Anal. ($C_{17}H_{17}Cl_2N_3OS$) theoretical: C, 67.63; H, 5.68; N, 12.45; S, 9.50. Found: C, 67.40; H, 5.67; N, 12.37; S, 9.65.

3.1.9. 6-Chloro-4-(3-cyanophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (8e)

3-Cyanophenyl isocyanate (373 mg, 2.35 mmol) was added to a solution of 4-amino-6-chloro-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (**13**) (500 mg, 2.35 mmol) in methylene chloride (5 mL). The solution was stirred 30 minutes at room temperature. The resulting precipitated product was collected by filtration, washed with *n*-hexane an dried (822 mg, 98 %): mp: 194-196 °C; IR (KBr) υ: 1698 (C=O), 2227 (C≡N), 3363 (N-H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.37 (s, 6H, CH_3), 3.28 (s, 2H, CH_2), 6.75 (s, 2H, 7-*H*/8-*H*),

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6.85 (s, 1H, 5-*H*), 7.42 (d, J = 7.7 Hz, 1H, 4'-*H*), 7.48 (t, J = 7.9 Hz, 1H, 5'-*H*), 7.81 (d, J = 7.9 Hz, 1H, 6'-*H*), 8.03 (s, 1H, 2'-*H*), 8.72 (s, 1H, N*H*), 9.09 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6) δ 25.1 (*C*H$_3$), 58.0 (*C*H$_2$), 74.7 (C-2), 111.3 (C-3'), 113.0 (C-5), 117.7 (C-8), 118.8 (CN), 120.3 (C-7), 121.4 (C-2'), 123.5 (C-6'), 124.2 (C-6), 125.5 (C-4'), 130.0 (C-5'), 135.8 (C-4a), 139.7 (C-1'), 140.9 (C-8a), 155.1 (CO). Anal. ($C_{18}H_{17}ClN_4O_2$) theoretical: C, 60.59; H, 4.80; N, 15.70. Found: C, 60.67; H, 4.65; N, 15.74.

3.1.10. 6-Chloro-4-(4-cyanophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (8f)

The title compound was obtained as described for **8e**, starting from **13** (500 mg, 2.35 mmol) and 4-cyanophenyl isocyanate (373 mg, 2.59 mmol) (721 mg, 86 %): mp: 195-197 °C; IR (KBr) υ: 1693 (C=O), 2219 (C≡N), 3370 (N-H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.37 (s, 6H, C*H*$_3$), 3.28 (s, 2H, C*H*$_2$), 6.74 (s, 2H, 7-*H*/8-*H*), 6.84 (s, 1H, 5-*H*), 7.72 (s, 4H, 2'-*H*/3'-*H*/5'-*H*/6'-*H*), 8.76 (s, 1H, N*H*), 9.25 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6, 125 MHz): *δ*: 25.1 (*C*H$_3$), 57.9 (*C*H$_2$), 74.7 (C-2), 103.5 (C-4'), 113.0 (C-5), 117.7 (C-8), 118.6 (C-2'/C-6'), 119.3 (CN), 120.0 (C-7), 124.2 (C-6), 133.1 (C-3'/C-5'), 135.8 (C-4a), 142.1 (C-8a), 144.0 (C-1'), 154.7 (CO). Anal. ($C_{18}H_{17}ClN_4O_2$) theoretical: C, 60.59; H, 4.80; N, 15.70. Found: C, 60.35; H, 4.73; N, 15.34.

3.1.11. 6-Chloro-4-(3-chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (8g)

The title compound was obtained as described for **8e**, starting from **13** (500 mg, 2.35 mmol) and 3-chlorophenyl isocyanate (316 µL, 2.59 mmol) (757 mg, 88 %): mp: 181-183 °C; IR (KBr) υ: 1692 (C=O), 3371 (N-H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.37 (s, 6H, C*H*$_3$), 3.27 (s, 2H, C*H*$_2$), 6.74 (s, 2H, 7-*H*/8-*H*), 6.83 (s, 1H, 5-*H*), 6.86 (d, J = 6.9 Hz, 1H, 7-*H*), 7.02 (dd, J = 7.9 Hz/1.5 Hz, 1H, 4'-*H*), 7.28 (t, J = 8.1 Hz, 1H, 5'-*H*), 7.43 (d, J = 8.0 Hz, 1H, 6'-*H*), 7.74 (d, J = 1.8 Hz, 1H, 2'-*H*), 8.62 (s, 1H, N*H*), 8.94 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6) δ 25.0 (*C*H$_3$), 57.9 (*C*H$_2$), 74.7 (C-2), 113.0 (C-5), 117.2 (C-6'), 117.6 (C-2'), 118.2 (C-8), 119.6 (C-7), 121.7 (C-4'), 124.2 (C-6), 130.2 (C-5'), 133.0 (C-3'), 135.9 (C-4a), 141.1 (C-1'), 141.7 (C-8a), 155.0 (CO). Anal. ($C_{17}H_{17}Cl_2N_3O_2$) theoretical: C, 55.75; H, 4.68; N, 11.47. Found: C, 55.84; H, 4.56; N, 11.45.

3.1.12. 6-chloro-4-(4-chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-benzoxazine (8h)

The title compound was obtained as described for **8e**, starting from **13** (500 mg, 2.35 mmol) and 4-chlorophenyl isocyanate (331 µL, 2.59 mmol) (663 mg, 77 %): mp: 180-182 °C; IR (KBr) υ: 1690 (C=O), 3362 (N-H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.37 (s, 6H, C*H*$_3$), 3.27 (s, 2H, C*H*$_2$), 6.73 (s, 2H, 7-*H*/8-*H*), 6.83 (s, 1H, 5-*H*), 7.30 (d, J = 8.9 Hz, 2H, 3'-*H*/5'-*H*), 7.56 (d, J = 8.9 Hz, 2H, 2'-*H*/6'-*H*), 8.56 (s, 1H, N*H*), 8.87 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6, 125 MHz): *δ*: 25.5 (*C*H$_3$), 58.4 (*C*H$_2$), 75.1 (C-2), 113.3 (C-5), 118.1 (C-8), 120.0 (C-7), 120.8 (C-2'/C-6'), 124.6 (C-6), 126.1 (C-4'), 128.9 (C-3'/C-5'), 136.4 (C-4a), 139.0 (C-1'), 142.2 (C-8a), 155.5 (CO). Anal. ($C_{17}H_{17}Cl_2N_3O_2$) theoretical: C, 55.75; H, 4.68; N, 11.47. Found: C, 56.06; H, 4.62; N, 11.11.

3.2. Biological assays

(±)-Cromakalim (Tocris, United Kingdom) and diazoxide ((Sigma Chemical Co, USA) were used as reference compounds. All experiments were performed with aortae or pancreatic islets isolated from adult fed Wistar Rats (Charles River Laboratories, Belgium). The experimental procedure using animals was approved by the ethical and animal welfare committee of the Université libre de Bruxelles (CEBEA n° 493N) in accordance with the Belgian legislation for the animals protection and welfare (Royal Order of the 15th May 2001 modified by the Royal Order of the 29th May 2013).

3.2.1. Measurement of insulin secretion from incubated rat pancreatic islets.

Pancreatic islets were isolated by the collagenase method and freshly isolated islets were used for measurements of insulin secretion. 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 (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1, $NaHCO_3$ 24) supplemented with 2.8 mM glucose, 0.5% (w/v) albumin (RIA grade) 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, either the reference compounds or the required chroman derivative. The release of insulin was measured radioimmunologically using rat insulin as a standard. Residual insulin secretion was expressed as a percentage of the value recorded in control experiments (100%); that is in the absence of drug and presence of 16.7 mM glucose.[32-34]

3.2.2. Measurement of myorelaxant activity on rat aorta rings.

The rat thoracic aorta was removed, cut into transverse rings (3-4 mm), and adhering fat and connective tissue was detached. After removal of the endothelium, the segments were suspended under 1.5 g tension in an organ bath containing 20 ml of a buffered physiological solution (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 solution was maintained at 37 °C and continuously oxygenated with a mixture of 95% O_2 and 5% CO_2. After equilibration for 60 min, isometric contractions were measured with a force-displacement transducer. Contractile activity was induced by increasing the extracellular concentration of K^+ (30 mM or 80 mM KCl). When the tension was stabilized, drugs were added cumulatively until maximal relaxation or until a maximum concentration of 200 µM. Some experiments were repeated in the continuous presence of 10 µM glibenclamide (Sigma Chemical Co, USA) in the physiological medium. The contractile responses were expressed as the percentage of the contractile response to KCl (100 %). The EC_{50} values (concentration evoking 50% inhibition of the plateau phase induced by KCl) were assessed from concentration–response curves using Datanalyst software (EMKA Technologies, France).[33-35]

3.3. Statistical evaluation

The statistical significance of differences between mean data was assessed by using the non-paired Student's *t*-test. The biological results were considered as statistically different when *p* value was <0.05.

3.4. Predicted partition coefficients

The program ALOGPS 2.1 (VCCLAB, Virtual Computational Chemistry Laboratory, http://www.vcclab.org, 2005) was used for the calculation of Average log P values.[36] Such a program is available at the Virtual Computational Chemistry Laboratory and used algorithms have been previously described in the literature.[36]

Conclusions

The synthesis of 4-phenylureido/thioureido-substituted 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazines as isosteres of the corresponding 4-phenylureido/thioureido-substituted 2,2-dimethylchromans was described and the impact of such structural modification on the pharmacological profile of the new drugs reported. More specifically, the inhibitory activity on the insulin-releasing process and the vasorelaxant properties of the original compounds were measured; both biological responses reflecting a potential interaction with K_{ATP} channels or voltage-sensitive calcium channels.

The benzoxazines were found to be less active than their corresponding chromans on the insulin secreting cells, but some 4-arylureido-substituted benzoxazines appeared to be more potent as vasorelaxants than their chroman counterparts.

The most active myorelaxant compound, i.e. the 4-arylureido-subsituted benzoxazine **8e**, was selected for further characterization of its mechanism of action. Altogether, our pharmacological observations suggest that **8e** behaved as a calcium entry blocker rather than as a K_{ATP} channel opener.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This study was supported in part by grants from the "National Fund for Scientific Research" (F.N.R.S., Belgium) from which P. Lebrun is a Research Director. The authors gratefully acknowledge the technical assistance of S. Counerotte, F. Leleux, and A.-M. Vanbellinghen.

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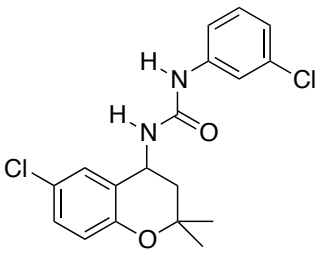

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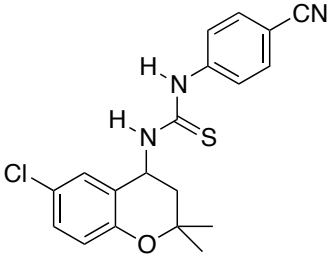

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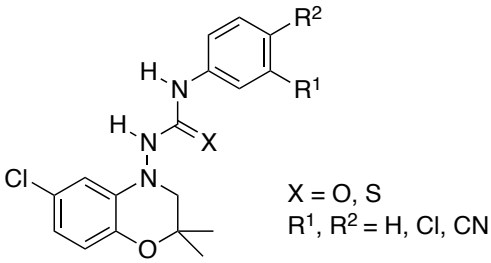

(**8**)

Pancreatic β-cell K_{ATP} channel openers belonging to 2,2-dimethylchromans

2,2-dimethyl-3,4-dihydro-2*H*-benzoxazines resulting from –CH-/-N- isosteric replacement