Accepted Manuscript

4-Phenylureido/thioureido-substituted 2,2-dimethylchroman analogs of cromakalim bearing a bulky 'carbamate' moiety at the 6-position as potent inhibitors of glucose-sensitive insulin secretion

Bernard Pirotte, Xavier Florence, Eric Goffin, Marlen Borges Medeiros, Pascal de Tullio, Philippe Lebrun

PII: S0223-5234(16)30440-8

DOI: 10.1016/j.ejmech.2016.05.042

Reference: EJMECH 8634

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 10 June 2015

Revised Date: 19 May 2016

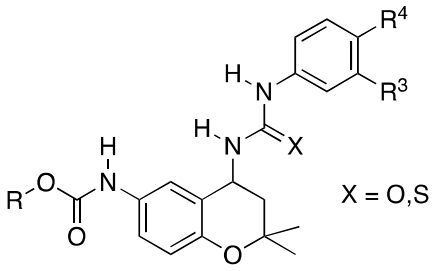
Accepted Date: 20 May 2016

Please cite this article as: B. Pirotte, X. Florence, E. Goffin, M.B. Medeiros, P. de Tullio, P. Lebrun, 4-Phenylureido/thioureido-substituted 2,2-dimethylchroman analogs of cromakalim bearing a bulky 'carbamate' moiety at the 6-position as potent inhibitors of glucose-sensitive insulin secretion, *European Journal of Medicinal Chemistry* (2016), doi: 10.1016/j.ejmech.2016.05.042.

4-Phenylureido/thioureido-substituted 2,2-dimethylchroman analogs of cromakalim bearing a bulky 'carbamate' moiety at the 6-position as potent inhibitors of glucose-sensitive insulin secretion

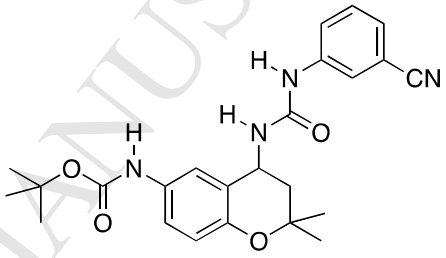
Bernard Pirotte, Xavier Florence, Eric Goffin, Marlen Borges Medeiros, Pascal de Tullio, Philippe Lebrun

Graphical abstract



R = CH_3, CH_2CH_3, $CH(CH_3)_2$, $C(CH_3)_3$

when R^3 = H, R^4 = Cl or CN
when R^4 = H, R^3 = Cl or CN



14o (BPDZ 711)

Inhibition of insulin secretion: IC_{50} = 0.24 μM

4-Phenylureido/thioureido-substituted 2,2-dimethylchroman analogs of cromakalim bearing a bulky 'carbamate' moiety at the 6-position as potent inhibitors of glucose-sensitive insulin secretion

Bernard Pirotte[1]*, Xavier Florence[1,2], Eric Goffin[1], Marlen Borges Medeiros[1], Pascal de Tullio[1], Philippe Lebrun[2]

[1] Laboratoire de Chimie Pharmaceutique, Center for Interdisciplinary Research on Medicines (CIRM), University of Liege, Quartier Hôpital, Avenue Hippocrate 15, B-4000 Liège, Belgium

[2] Laboratoire de Physiologie et Pharmacologie, Université Libre de Bruxelles, Faculté de Médecine, 808 Route de Lennik, B-1070 Bruxelles, Belgium

* Corresponding author

Keywords

2,2-Dimethylchromans; ATP-sensitive potassium channels; potassium channel openers; cromakalim analogs; insulin secretion; smooth muscle contractile activity

Abstract

The synthesis of 2,2-dimethylchromans bearing a 3/4-chloro/cyano-substituted phenylureido or phenylthioureido moiety at the 4-position and an alkoxycarbonylamino ('carbamate') group at the 6-position is described. These new analogs of the potassium channel opener (±)-cromakalim were further tested on rat pancreatic islets as putative inhibitors of insulin release and on rat aorta rings as putative vasorelaxants. All compounds inhibited insulin secretion and induced a myorelaxant activity. Compound **14o** [*R/S*-*N*-3-cyanophenyl-*N'*-(6-*tert*-butoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)urea; BPDZ 711] emerged as the most potent inhibitor of the glucose-sensitive insulin releasing process (IC_{50} = 0.24 µM) and displayed selectivity towards the pancreatic endocrine tissue. Radioisotopic, fluorimetric and pharmacological investigations were performed on rat pancreatic islet and rat vascular smooth muscle cells in order to decipher its mechanism of action. Our findings suggest that the mechanism of action of **14o** is rather unspecific. The compound behaves as a K_{ATP} channel opener, a Ca^{2+} entry blocker, and promotes an intracellular calcium translocation.

Manuscript text

1. Introduction

ATP-sensitive potassium channels (K_{ATP} channels) located at the plasma membrane of a variety of excitable cells are potassium ion channels that link cell metabolism to membrane excitability [1]. An increase in the intracellular concentration ratio of adenosine triphosphate to adenosine diphosphate (ATP/ADP ratio) provokes an inhibition of the channel activity with subsequent membrane depolarization while a decrease of this ratio exerts opposite effects and induces cell membrane hyperpolarization [1-3].

K_{ATP} channels are widely distributed throughout tissues where they are involved in multiple physiological processes, including 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 elucidated as being 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]. Different combinations of the K_{ir}

and SUR subunits lead to tissue-specific K_{ATP} channels; the pancreatic β-cell K_{ATP} channel is known to express a SUR1/K_{ir}6.2 K_{ATP} channel subtype while the SUR2B/K_{ir}6.1 combination is found in vascular smooth muscle cells [8,9].

K_{ATP} channels are recognized as important therapeutic targets and several modulators (openers and/or blockers) are currently used in clinical practice (i.e. hypoglycemic sulfonylureas as oral antidiabetic drugs are pancreatic β-cell K_{ATP} channel blockers) [10]. New modulators of these channels 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. Selective openers of the SUR1/Kir6.2 channel subtype have been proposed for the prevention and/or management of type 1 - type 2 diabetes, congenital hyperinsulinism and insulinoma [11-13]. Diazoxide (**1**) was the first reported reference potassium channel opener (PCO) displaying an activity on the pancreatic (SUR1-type) K_{ATP} channels [14,15] with subsequent inhibition of insulin secretion, while 2,2-dimethylchroman PCOs such as (±)-cromakalim (**2**) were shown to be much more effective on the smooth muscle (SUR2B-type) K_{ATP} channels (inducing a myorelaxant effect) [16]. As a result, diazoxide is currently the therapeutic agent used in medical practice to treat hypoglycemia due to excessive insulin release [17,18].

Recent researches, however, led to the identification of original highly selective pancreatic β-cell (SUR1)-type K_{ATP} channel openers, among which ring-fused 3-alkylamino-substituted 4*H*-1,2,4-thiadiazine 1,1-dioxides (benzo/pyrido/thienothiadiazine dioxides i.e. **3**, **4** and **5**; fig. 1) structurally related to diazoxide [19-21]. More recently, the identification of a xanthine derivative, VU0071063 (**6**), as a selective SUR1/K_{ir}6.2-type channel opener was also reported [22]. By contrast, more restricted investigations have been focused on 2,2-dimethylchromans, with marked pancreatic β-cell selectivity, structurally related to (±)-cromakalim. In this specific field, we reported that, among 2,2-dimethylchroman structures studied as PCOs, 4-phenylureido- and 4-phenylthioureido-substituted 2,2-dimethylchromans diversely substituted at the 6-position exerted a potent inhibitory activity on insulin-secreting cells; an observation attributed to the activation of the pancreatic K_{ATP} channels [23-26]. For a few drugs, such as compounds **7** and **8** (fig. 1), a significant selectivity for the pancreatic tissue versus the vascular smooth muscle tissue was observed [23,25]. Furthermore, a surprising marked activity was disclosed with a series of 4-phenylthioureido-substituted 2,2-dimethylchromans bearing a bulky *tert*-butoxycarbonylamino group at the 6-position of the chroman ring (i.e. compound **9** with an IC_{50} value on pancreatic β-cells close to 1 µM; fig. 1) [26].

According to these observations, we decided to further explore the pharmacological interest of 2,2-dimethylchromans bearing, at the 4-position, the previously selected monosubstituted (3-/4-Cl, 3-/4-CN) phenylureido/thioureido group and, at the 6-position, an alkoxycarbonylamino group ('carbamate' moiety) with a variety of small-sized branched or not branched alkyl groups (see formulae **14** and **15**, scheme 1).

The new compounds were tested as putative inhibitors of insulin release and as putative vasorelaxants. The most active drug was further investigated in radioisotopic, fluorimetric and pharmacological experiments in order to elucidate its mechanism of action.

2. Results and discussion

2.1. Chemistry

The synthesis of the new compounds **14** and **15** is described on scheme 1. The starting intermediate 6-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (**10**) was obtained as previously described [26]. The reaction of **10** with the appropriate alkyl chloroformate (except when R was *tert*-butyl, di-*tert*-butyl dicarbonate was used [26]) gave rise to the 'carbamate' intermediates **11a-d** (6-alkoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-ones). Hydroxylamine hydrochloride in the presence of potassium carbonate converted the ketonic compounds **11a-d** into the corresponding oximes **12a-d**, which were further treated with hydrogen gas in the presence of Raney-Nickel° to provide the corresponding amines **13a-d**. In the last step, the reaction of **13a-d** with the appropriate monosubstituted phenyl isocyanate or isothiocyanate generated the final compounds **14a-p** (*N*,*N*'-disubstituted ureas) and **15a-l** (*N*,*N*'-disubstituted thioureas).

2.2. Biological results

2.2.1. Insulin secretion from rat pancreatic islets

The newly synthesized compounds (compounds **14a-p** and **15a-l**; Table 1) were evaluated as inhibitors of the insulin releasing process on isolated rat pancreatic islets incubated in the presence of an insulinotropic glucose concentration (16.7 mM). An inhibition of the glucose-induced insulin release might reflect the opening of pancreatic β-cells K_{ATP} channels. A residual 5-10% insulin secretion can be considered as reflecting a close to maximal inhibitory

effect according to the glucose-insensitive basal endocrine secretion [27]. (±)-Cromakalim and diazoxide were used as reference PCOs (Table 1). The activity of the new 2,2-dimethylchromans **14a-p** and **15a-l** was also compared to that of the previously described compounds **9**, **16**, **17** and **18** (Table 1) [26].

As previously reported, a 10 µM concentration of (±)-cromakalim was roughly inactive on the pancreatic endocrine tissue (Table 1) [25,26]. Diazoxide at the same concentration (10 µM), however, significantly reduced the insulin secretory rate by about 20% (Table 1) [25,26].

The new 4-phenylureido/thioureido-substituted 2,2-dimethylchromans **14** and **15** exhibited a strong inhibitory activity on the insulin releasing process (Table 1). Among these 2,2-dimethylchromans, the most potent compound was found to be **14o** (BPDZ 711) (Table 1).

The following structure-activity relationships can be deduced from the secretory data. Concerning the substituent on the benzene ring of 4-phenylureido-substituted compounds **14a-p**, the lowest inhibitory activity was systematically observed with the 4-chloro-substituted derivatives (see **14b**, **14f**, **14j** and **14n** at 1 and 10 µM; Table 1). By contrast, the best choice of substituent appeared to be the cyano group at the 3-position (see **14c**, **14g**, **14k** and **14o** at 1 and 10 µM; Table 1), with compound **14o** clearly emerging as the most potent inhibitor of insulin release.

The corresponding thioureas **9, 15a-l, 16-18** also markedly inhibited the glucose-induced insulin release, although less obvious differences were detected according to the nature and the position of the substituent on the phenyl ring (Table 1).

Moreover, by comparing ureas and thioureas bearing similar substituents (R, R^3 or R^4), it was noticed that, in most cases, the thioureas **15** were more potent than their corresponding ureas **14**, except when R^3 corresponded to the cyano group (compare **14c**, **14g**, **14k** and **14o** with **15c**, **15g**, **15k** and **18**; Table 1).

Two structural considerations might explain differences in the biological activity observed between ureas and thioureas. Firstly, the carbon-sulfur interatomic distance is known to be greater than that of carbon-oxygen [for example, the C=S bond length in *N*,*N*'-diphenylthiourea (1.681 Å) [28] is greater than the C=O bond length in *N*,*N*'-diphenylurea (1.233 Å) [29]], presumably causing differences in the establishment of C=S---H or C=O---H hydrogen bonds at the level of the receptor binding site. Secondly, ureas and thioureas, in the solid state and/or in solution, are expected to adopt multiple conformations ensuring optimal electron delocalization in the N-C(=X)-N plane system (see Fig. 2). According to X-ray data previously published for *N*,*N*'-disubstituted ureas and *N*,*N*'-disubstituted thioureas, the A conformation seems to predominate for ureas while thioureas are known to exist in the A as

well as in the B/C conformations (i.e. *N,N'*-diphenylurea was found in the A conformation [29,30], while *N,N'*-diphenylthiourea was found in conformations A [28] and B [31] in the solid state). The D conformation is the less probable due to steric hindrance resulting from the proximity of the two hydrocarbon chains R and R'. It is tempting to speculate that the active conformation assuming optimal interaction with the receptor binding site might be the A conformation.

An additional complication results from the possible presence, in solution, of multiple tautomeric forms corresponding to the equilibrium between ol/one or thiol/thione forms (see conformations A, A' and A", Fig. 2). In the solid state, for *N,N'*-diarylureas/thioureas, the A conformation seems to predominate [28-31]. Moreover, according to the NMR data obtained with the described ureas and thioureas (see Experimental section and "Supplementary data" section), the nitrogen atom linked to the carbon atom at the 4-position (4-C) of the chroman ring undoubtedly bears a hydrogen atom since this proton appears as a doublet due to its coupling with the hydrogen atom attached to the neighbor 4-C carbon atom, thus precluding the A" conformation (see Fig. 2).

The nature of the alkyl chain linked to the 'carbamate' moiety also appeared to affect the biological activity on pancreatic islets. The rank order of potency, both for the 4-phenylureido- and the 4-phenylthioureido-substituted 2,2-dimethylchromans, could be roughly summarized as follows: tert-butyl > isopropyl ≥ ethyl ≥ methyl. Thus, there is a favorable impact, on the biological activity, of the increase of the steric hindrance at the 6-position.

2.2.2. Myorelaxant activity on rat aortic rings

The vasorelaxant activity of the new 2,2-dimethylchromans **14a-p** and **15a-l** was determined on 30 mM K^+-depolarized rat aorta (endothelium-free) rings and was compared to that of (±)-cromakalim, diazoxide and the previously described compounds **9**, **16**, **17**, **18** (Table 1) [26]. As reported, (±)-cromakalim displayed a marked myorelaxant activity on the vascular tissue while diazoxide was less potent (Table 1). Such a vasorelaxant effect of the two reference compounds is known to result from the primary opening of the vascular smooth muscle cells K_{ATP} channels [32,33].

The vasorelaxant activity of compounds **14** and **15** was found to be weaker than that of (±)-cromakalim, although most new drugs were equipotent or more potent than diazoxide (Table

1). The best choice of substituent on the phenyl ring appeared to be the cyano group at the 3-position, as observed for the inhibitory effect on insulin release.

Taken as a whole, these biological data indicated that the new 2,2-dimethylchromans exerted a more pronounced activity on the pancreatic endocrine tissue than on the vascular smooth muscle tissue.

Surprisingly, compound **14o** (BPDZ 711), the most potent inhibitor of insulin secretion (IC_{50} = 0.24 µM, see below), did not come out as the most potent vasodilator. Considering an EC_{50}/IC_{50} ratio equal to 44, a clear selectivity of **14o** for the pancreatic versus the vascular tissue can be deduced.

This compound was selected for further characterization in order to elucidate its mechanism of action.

*2.2.3. Effects of **14o** (BPDZ 711) on insulin release from incubated rat pancreatic islets*

The addition of increasing concentrations of **14o** provoked a concentration-dependent decrease in insulin output from rat pancreatic islets incubated in the presence of 16.7 mM glucose (Fig. 3). After the addition of 0.1 µM, 0.5 µM, 1 µM and 10 µM **14o** to the incubation medium, the residual insulin release averaged 82.0 ± 4.6 % (n = 30); 28.6 ± 1.8 % (n = 21), 15.6 ± 1.2 % (n = 20) and 7.4 ± 1.0 % (n = 25) of the control value, respectively. The IC_{50} value (drug concentration evoking a 50 % reduction of the secretory response to glucose) amounted to 0.24 µM.

In contrast, at the same concentrations, (±)-cromakalim did not affect insulin release from glucose-stimulated pancreatic islets. In the presence of 1 µM and 10 µM (±)-cromakalim in the incubation medium, the insulin output averaged 93.8 ± 4.1 % (n = 16) and 96.4 ± 6.5 % (n = 16) of the control experiments, respectively.

*2.2.4. Effects of **14o** (BPDZ 711) on ^{86}Rb, ^{45}Ca outflow and insulin release from perifused rat pancreatic islets*

Figure 4 clearly reveals that 10 µM **14o** provoked a rapid, marked and sustained increase in the rate of ^{86}Rb (^{42}K substitute) outflow from prelabeled and perifused rat pancreatic islets. Such a massive enhancing effect on ^{86}Rb efflux has never been observed with other reference K_{ATP} channel openers.

The addition of 1 µM **14o** to prelabeled pancreatic islets also induced a sustained, although less pronounced, rise in ^{86}Rb outflow (data not shown). Under the same experimental conditions, 0.1 µM **14o** did not affect the rate of ^{86}Rb outflow (data not shown).

When the basal medium contained 10 µM of the hypoglycemic sulfonylurea glibenclamide, a recognized K_{ATP} channel blocker [34], the stimulatory effect of 10 µM **14o** was abolished (Fig. 4).

These radioisotopic data indirectly suggest that **14o** increases the membrane K^+ permeability and activates K_{ATP} channels in islets cells. Such an activation of K_{ATP} channels should hyperpolarize the insulin-secreting cells, reduce Ca^{2+} inflow through voltage-sensitive Ca^{2+} channels, decrease the cytosolic calcium concentration and, consequently, inhibit insulin secretion.

To corroborate this hypothesis, additional experiments have been conducted. Figure 5 (upper panel) shows that, in islets exposed throughout to an insulinotropic glucose concentration (16.7 mM) and extracellular Ca^{2+}, the addition of 10 µM **14o** elicited an immediate and pronounced inhibition of ^{45}Ca outflow from prelabeled and perifused rat pancreatic islets.

Under such experimental conditions, a decrease in the ^{45}Ca fractional outflow rate is known to reflect a reduction in $^{40}Ca^{2+}$ entry into the islets cells [27,35].

Moreover, the simultaneous measurement of the glucose-sensitive secretory process further indicated that 10 µM **14o** provoked an inhibition of the insulin secretory rate displaying a time course identical to that of the ^{45}Ca response (Fig. 5, lower panel).

To further characterize the effects of **14o** on ^{45}Ca movements, similar experiments were conducted in the continuous presence of 16.7 mM glucose but absence of extracellular Ca^{2+} in the perifusing medium. In islets exposed to Ca^{2+}-depleted media, the basal rate of ^{45}Ca outflow (min 40-44) was lower but the addition of 10 µM **14o** induced a clear-cut increase in ^{45}Ca efflux (Fig. 5, upper panel).

14o (10 µM) also provoked a sustained increase in ^{45}Ca outflow from islets perifused in the presence of a non-insulinotropic glucose concentration (2.8 mM), whether the physiological medium contained or was deprived of extracellular Ca^{2+} (data not shown). Under such experimental conditions, Ca^{2+} entry is impaired and the isotopic exchange between influent ^{40}Ca and effluent ^{45}Ca inhibited.

Thus, the **14o**-induced increases in ^{45}Ca outflow from islets perifused either in the absence of extracellular Ca^{2+} or in the presence of extracellular Ca^{2+} but at a non-stimulatory glucose concentration might result from an intracellular calcium redistribution with subsequent changes in ^{45}Ca outflow [36,37].

In the last series of radioisotopic experiments, we determined the effects of **14o** on the ^{45}Ca response elicited by a rise in the extracellular K^+ concentration from islets perifused in the presence of a non-insulinotropic glucose concentration (2.8 mM). A sudden increase in the extracellular concentration of K^+ from 5 to 50 mM provoked a rapid, sustained and reversible enhancement in ^{45}Ca outflow (Fig. 6).

When the same experiment was repeated in the continuous presence of **14o** (10 µM) in the perifusate, the basal rate of ^{45}Ca outflow was higher. Such an observation, detected in islets exposed throughout to 2.8 mM glucose, corroborates previous findings suggesting that the drug might interfere with an intracellular target site.

Moreover, the continuous presence of **14o** (10 µM) in the perifusing medium counteracted the ^{45}Ca response evoked by 50 mM extracellular K^+.

These experimental data indirectly suggest that **14o** also inhibits Ca^{2+} entry by acting at the level of the voltage-sensitive Ca^{2+} channels. Indeed, the ^{45}Ca response to high K^+ concentrations, which is mediated by the opening of voltage-sensitive Ca^{2+} channels, is known to be sensitive to Ca^{2+} entry blockers but rather resistant to "pure" K^+ channel openers [27,35,38].

*2.2.5. Effects of **14o** (BPDZ 711) on the fura-2 fluorescence from single rat pancreatic islet cells*

A rise in the extracellular glucose concentration from 2.8 to 20 mM provoked, as expected, an initial decrease rapidly followed by a biphasic and sustained rise in cytosolic Ca^{2+} concentration (fluorescence ratio 340:380 nm) (Fig. 7, upper panel) [27]. The addition of **14o** (10 µM) during the sustained phase induced a slow but continuous reduction in the cytosolic Ca^{2+} concentration (Fig. 7, upper panel).

When the same experiment was performed with a physiological medium deprived of external calcium, an experimental condition into which Ca^{2+} influx was prevented, 20 mM glucose induced a slight reduction in cytosolic calcium with no secondary rise. Under such experimental conditions, the subsequent addition of **14o** (10 µM) provoked a slow but sustained increase in the intracellular Ca^{2+} concentration (Fig. 7, lower panel).

Additional experiments indicated that, whether the physiological medium contained or was deprived of extracellular calcium, the addition of 10 µM **14o** increased the fluorescence intensity of fura 2-loaded islets cells exposed to a non-insulinotropic glucose concentration (2.8 mM). (data not shown).

These calcium fluorimetry experiments indicate that, in islets cells stimulated by an insulinotropic glucose concentration (20 mM), a reduction in the cytosolic Ca^{2+} concentration as mediated by **14o** evidences a decrease in Ca^{2+} entry.

Moreover, the fluorimetric data further support the hypothesis that **14o** could promote an intracellular calcium redistribution with subsequent changes in the cytosolic calcium concentration.

*2.2.6. Effects of **14o** (BPDZ 711) on the contractile activity of rat aortic rings*

In rat aortic rings, a sudden rise in the extracellular K^+ concentration provoked an increase in muscle tension, which rapidly reached a sustained plateau phase. The cumulative application of **14o** (0.1 µM to 200 µM) to aortic rings continuously exposed to 30 mM K^+ induced concentration-dependent relaxations.

The vasorelaxant potency of **14o**, as calculated from the EC_{50} value (drug concentration giving 50% relaxation), was roughly 50 fold lower than that of the reference compound cromakalim (Table 2).

The concomitant presence of glibenclamide (1 µM or 10 µM) in the bathing solution failed to affect the myorelaxant properties of **14o** (Table 2). By contrast, the K_{ATP} channel blocker glibenclamide induced a dose-dependent reduction in the vasorelaxant response to cromakalim (Table 2).

Incubation of aortic rings with glibenclamide (1 µM or 10 µM) did not impair the myorelaxant capacity of the Ca^{2+} entry blocker verapamil (Table 2).

The myorelaxant activity of **14o** was further characterized on rat aortic rings precontracted by 80 mM extracellular K^+. Table 2 indicates that, under the latter experimental condition, the vasorelaxant potency of **14o** was maintained. Under identical experimental conditions, the myorelaxant effects of the K_{ATP} channel opener cromakalim were drastically blunted whilst those of the Ca^{2+} entry blocker verapamil were unaffected (Table 2).

These findings indicate that, on vascular smooth muscle cells, **14o** mainly behaved as a Ca^{2+} entry blocker rather than as a K^+ channel opener.

3. Conclusions

We reported here the synthesis of original 2,2-dimethylchromans bearing a phenylureido or a phenylthioureido moiety at the 4-position and an alkoxycarbonylamino group at the 6-position. The new compounds are structurally related to the reference potassium channel opener (±)-cromakalim. Most compounds exerted a marked inhibitory activity on the insulin releasing process and were found to be much more active than (±)-cromakalim and diazoxide. The thioureas were commonly more potent than their corresponding ureas, except when bearing a cyano group at the 3-position. Compared with (±)-cromakalim, the new 4-phenylureido/thioureido-substituted 2,2-dimethylchromans displayed a weaker vasorelaxant activity. *R/S*-*N*-3-Cyanophenyl-*N'*-(6-*tert*-butoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)urea (**14o**) was the most active compound on pancreatic islets and also exhibited a pancreatic tissue selectivity. This drug was selected to be further investigated on rat pancreatic islet cells and rat vascular smooth muscle cells in order to characterize its mechanism of action. Altogether, our radiosiotopic, fluorimetric and pharmacological observations suggest that the mechanism of action of **14o** is rather "unspecific". The compound activates K_{ATP} channels, expresses Ca^{2+} entry blocker properties and promotes a calcium translocation by acting at the level of intracellular organelles.

4. Experimental section

4.1. 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 target 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).

The synthesis of compounds **10**, **11d**, **12d**, **13d** and **15a-d** has been previously described [26].

4.1.1. General synthetic pathway to 6-alkoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-ones (**11a-c**)

The solution of 6-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (**10**) [26] (7 g, 36.6 mmol) in acetonitrile (100 mL) was supplemented with potassium carbonate (30.36 g, 219.6 mmol, 6 equiv.), then with the appropriate alkyl chloroformate (51.2 mmol, 1.4 equiv.), and stirred for 30 min. at room temperature. The reaction medium was treated with water (200 mL) to solubilize potassium carbonate. The resulting suspension was extracted three times with ethyl acetate (3 x 200 mL). The organic layers were collected, dried over magnesium sulfate, and filtered. The filtrate was concentrated to dryness under reduced pressure and the solid residue was dissolved in the minimum volume of methanol. The addition of two volumes of water under stirring provoked the precipitation of the title compound, which was collected by filtration, washed with water and dried. The crude compound was used in the next step without further purification (yields: 85-95%).

4.1.2. 3,4-Dihydro-2,2-dimethyl-6-methoxycarbonylamino-2*H*-1-benzopyran-4-one (**11a**): IR (KBr) : υ : 3343 (N-H), 1724, 1682 (C=O) cm^{-1}; RMN ^1H (DMSO-d_6, 500 MHz) : 1.37 (s, 6H, CH_3), 2.76 (s, 2H, CH_2), 3.66 (s, 3H, CH_3), 6.93 (d, 1H, 8-*H*), 7.58 (dd, 1H, 7-*H*), 7.84 (s, 1H, 5-*H*), 9.57 (s, 1H, -N*H*).

4.1.3. 3,4-Dihydro-2,2-dimethyl-6-ethoxycarbonylamino-2*H*-1-benzopyran-4-one (**11b**): IR (KBr) : υ : 3343 (N-H), 1721, 1671 (C=O) cm^{-1}; RMN ^1H (DMSO-d_6, 500 MHz) : 1.24 (t, 3H, CH_3-CH_2), 1.37 (s, 6H, CH_3), 2.76 (s, 2H, CH_2), 4.11 (q, 2H, CH_3-CH_2), 6.93 (d, 1H, 8-*H*), 7.57 (dd, 1H, 7-*H*), 7.86 (s, 1H, 5-*H*), 9.60 (s, 1H, -N*H*).

4.1.4. 3,4-Dihydro-2,2-dimethyl-6-isopropoxycarbonylamino-2*H*-1-benzopyran-4-one (**11c**) : IR (KBr) : υ : 3322 (N-H), 1726, 1682 (C=O) cm^{-1}; RMN 1H (DMSO-d_6, 500 MHz) : 1.24 (d, 6H, (CH_3)$_2$-CH), 1.37 (s, 6H, CH_3), 2.76 (s, 2H, CH_2), 4.88 (m, 1H, (CH_3)$_2$-C*H*), 6.92 (d, 1H, 8-*H*), 7.56 (dd, 1H, 7-*H*), 7.88 (s, 1H, 5-*H*), 9.54 (s, 1H, -N*H*).

4.1.5. General synthetic pathway to 6-alkoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-hydroxyimines (**12a-c**)

The appropriate 6-alkoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (**11a-d**) (21.44 mmol) was solubilized in ethanol (65 mL) and supplemented with potassium carbonate (42.88 mmol, 2 equiv.) and hydroxylamine hydrochloride (42.88 mmol, 2 equiv.). The resulting suspension was refluxed for 3 h, after which the reaction medium was poured on ice and water. The resulting precipitate of the title compound was collected by filtration, washed with water and dried. The crude compound was used in the next step without further purification (yields: 80-95%).

4.1.6. 3,4-Dihydro-2,2-dimethyl-6-methoxycarbonylamino-2*H*-1-benzopyran-4-hydroxyimine (**12a**): IR (KBr) : υ : 3427 (N-H), 1743 (C=O) cm^{-1}; RMN ^1H (DMSO-d_6, 500 MHz) : 1.28 (s, 6H, C*H*$_3$), 2.74 (s, 2H, C*H*$_2$), 3.64 (s, 3H, C*H*$_3$), 6.77 (d, 1H, 8-*H*), 7.32 (dd, 1H, 7-*H*), 7.89 (s, 1H, 5-*H*), 9.48 (s, 1H, -N*H*), 11.23 (s, 1H, =NO*H*).

4.1.7. 3,4-Dihydro-2,2-dimethyl-6-ethoxycarbonylamino-2*H*-1-benzopyran-4-hydroxyimine (**12b**): IR (KBr) : υ : 3295 (N-H), 1697 (C=O) cm^{-1}; RMN ^1H (DMSO-d_6, 500 MHz) : 1.24 (t, 3H, C*H*$_3$-CH$_2$), 1.28 (s, 6H, C*H*$_3$), 2.73 (s, 2H, C*H*$_2$), 4.10 (q, 2H, CH$_3$-C*H*$_2$), 6.75 (d, 1H, 8-*H*), 7.31 (dd, 1H, 7-*H*), 7.90 (s, 1H, 5-*H*), 9.45 (s, 1H, -N*H*), 11.22 (s, 1H, =N-O*H*).

4.1.8. 3,4-Dihydro-2,2-dimethyl-6-isopropoxycarbonylamino-2*H*-1-benzopyran-4-hydroxyimine (**12c**): IR (KBr) : υ : 3348 (N-H), 1699 (C=O) cm^{-1}; RMN ^1H (DMSO-d_6, 500 MHz) : 1.24 (d, 6H, (C*H*$_3$)$_2$-CH), 1.28 (s, 6H, C*H*$_3$), 2.73 (s, 2H, C*H*$_2$), 4.86 (m, 1H, (CH$_3$)$_2$-C*H*), 6.75 (d, 1H, 8-*H*), 7.30 (dd, 1H, 7-*H*), 7.92 (s, 1H, 5-*H*), 9.39 (s, 1H, -N*H*), 11.22 (s, 1H, =N-O*H*).

4.1.9. General synthetic pathway to *R/S*-6-alkoxycarbonylamino-4-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans (**13a-c**)

Raney-Nickel° (3 g) was suspended in a solution of the appropriate 6-alkoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-hydroxyimine (**12a-d**) (2.5 g, 8-9 mmol) in

methanol (50 mL). The reaction mixture was stirred for 3-4 h in a sealed hydrogenator under a hydrogen pressure of 5 bars. The catalyst was filtered off and the filtrate was evaporated to dryness under reduced pressure. The crude product was dissolved in the minimum methanol and the resulting solution was mixed with two volumes of water. The precipitate of the title compound was collected by filtration, washed with water and dried. The crude compound was used in the next step without further purification (yields: 80-95%).

4.1.10. *R/S*-4-Amino-3,4-dihydro-2,2-dimethyl-6-methoxycarbonylamino-2*H*-1-benzopyran (**13a**): IR (KBr) : υ : 3365 (N-H), 1719 (C=O) cm^{-1}; RMN ^1H (DMSO-d_6, 500 MHz) : 1.17 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.51 (t, 1H, CH_2), 1.80 (s, 2H, NH_2), 1.99 (m, 1H, CH_2), 3.62 (s, 3H, CH_3), 3.79 (m, 1H, CH-NH_2), 6.58 (d, 1H, 5-*H*), 7.12 (d, 1H, 7-*H*), 7.57 (s, 1H, 8-*H*), 9,29 (s, 3H, N*H*).

4.1.11. *R/S*-4-Amino-3,4-dihydro-2,2-dimethyl-6-ethoxycarbonylamino-2*H*-1-benzopyran (**13b**): IR (KBr) : υ : 3352 (N-H), 1723 (C=O) cm^{-1}; RMN ^1H (DMSO-d_6, 500 MHz) : 1.17 (s, 3H, CH_3), 1.22 (t, 3H, CH_3-CH_2), 1.32 (s, 3H, CH_3), 1.51 (t, 1H, CH_2), 1.79 (s, 2H, NH_2), 1.99 (m, 1H, CH_2), 3.79 (m, 1H, C*H*), 4.08 (q, 2H, CH_3-CH_2), 6.57 (d, 1H, 8-*H*), 7.11 (d, 1H, 7-*H*), 7.59 (s, 1H, 5-*H*), 9.26 (s, 1H, -N*H*).

4.1.12. *R/S*-4-Amino-3,4-dihydro-2,2-dimethyl-6-isopropoxycarbonylamino-2*H*-1-benzopyran (**13c**): IR (KBr) : υ : 3361 (N-H), 1720 (C=O) cm^{-1}; RMN ^1H (DMSO-d_6, 500 MHz) : 1.17 (s, 3H, CH_3), 1.23 (d, 6H, (CH_3)$_2$CH), 1.32 (s, 3H, CH_3), 1.51 (t, 1H, CH_2), 1.77 (s, 2H, NH_2), 1.99 (m, 1H, CH_2), 3.79 (m, 1H, CH-NH_2), 4.84 (m, 1H, (CH_3)$_2$C*H*), 6.56 (d, 1H, 5-*H*), 7.09 (d, 1H, 7-*H*), 7.61 (s, 1H, 8-*H*), 9.17 (s, 3H, N*H*).

4.1.13. General synthetic pathway to *R/S*-*N*-(3/4-monosubstituted (Cl/CN)-phenyl)-*N'*-(6-alkoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)ureas (**14**) **and *R/S*-*N*-(3/4-monosubstituted (Cl/CN)-phenyl)-*N'*-(6-alkoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thioureas** (**15**)

The appropriate *R/S*-6-alkoxycarbonylamino-4-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**13**) (0.5 g) was dissolved in methylene chloride (8 mL) and then supplemented with the appropriate 3- or 4-monosubstituted (Cl, CN)-phenyl isocyanate (1.2 equiv.) or the

appropriate 3- or 4-monosubstituted (Cl, CN)-phenyl isothiocyanate (1.2 equiv.). The reaction mixture was stirred during 30 min., after which the resulting precipitate of the title compound was collected by filtration, washed with methylene chloride and dried (yields: 85-95%). The title compound can further be purified by DCVC chromatography.

4.1.14. *R/S*-*N*-3-Chlorophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-methoxycarbonylamino-2*H*-1-benzopyran-4-yl)urea (**14a**): m.p. : 217-219°C; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.73 (dd, J=12.9 Hz/11.2 Hz, 1H, 3-*H*), 2.08 (dd, J=13.2 Hz/6.2 Hz, 1H, 3-*H*), 3.60 (s, 3H, NHCOOCH_3), 4.93 (dd, J=15.8 Hz/10.0 Hz, 1H, 4-*H*), 6,61 (d, J=8.8 Hz, 1H, CHN*H*CONHAr), 6.67 (d, J=8.8 Hz, 1H, 8-*H*), 6.97 (ddd, J=7.7 Hz/2 Hz/1.4 Hz, 1H, 6'-*H*), 7.22-7.28 (m, 3H, 4'-*H*/5'-*H*/7-*H*), 7.35 (s, 1H, 5-*H*), 7.75 (t, J=2 Hz, 1H, 2'-*H*), 8.75 (s, 1H, CHNHCON*H*Ar), 9.40 (s, 1H, N*H*COOCH$_3$); ^{13}C NMR (DMSO-d_6) δ 24.4 (CH$_3$), 29.1 (CH$_3$), 40.0 (C-3), 42.3 (C-4), 51.4 (OCH$_3$), 74.9 (C-2), 116.1 (C-6'), 116.8 (C-8), 117.1 (C-2'), 117.6 (C-5), 119.4 (C-7), 120.8 (C-4'), 123.1 (C-4a), 130.3 (C-5'), 131.6 (C-6), 133.1 (C-3'), 141.9 (C-1'), 148.8 (C-8a), 154.1 (NH*C*OOCH$_3$), 154.9 (CHNH*C*ONHAr). Anal. ($C_{20}H_{22}ClN_3O_4$) theoretical: C, 59.48; H, 5.49; N, 10.40. Found: C, 59.10; H, 5.54; N, 10.04.

4.1.15. *R/S*-*N*-4-Chlorophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-methoxycarbonylamino-2*H*-1-benzopyran-4-yl)urea (**14b**): m.p. : 191-193°C; ^{1}H NMR (DMSO-d_6) d 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.71 (dd, J=13 Hz/11.2 Hz, 1H, 3-*H*), 2.08 (dd, J=13.2 Hz/6.2 Hz, 1H, 3-*H*), 3.60 (s, 3H, NHCOOCH_3), 4.93 (dd, J=15.9 Hz/9.9 Hz, 1H, 4-*H*), 6.56 (d, J=8.8 Hz, 1H, CHN*H*CONHAr), 6,66 (d, J=8.8 Hz, 1H, 8-*H*), 7.23 (d, J=7.8 Hz, 1H, 7-*H*), 7.29 (d, J=8.9 Hz , 2H, 3'-*H*/5'-*H*), 7.35 (s, 1H, 5-*H*), 7.48 (d, J=8.9 Hz, 1H, 2'-*H*/6'-*H*), 8.68 (s, 1H, CHNHCON*H*Ar), 9.40 (s, 1H, N*H*COOCH$_3$); ^{13}C NMR (DMSO-d_6) δ 24.4 (CH$_3$), 29.1 (CH$_3$), 40.2 (C-3), 42.2 (C-4), 51.4 (OCH$_3$), 74.9 (C-2), 116.8 (C-8), 117.7 (C-5), 119.2 (C-2'/C-6'), 119.4 (C-7), 123.2 (C-4a), 124.6 (C-4'), 128.5 (C-3'/C-5'), 131.6 (C-6), 139.4 (C-1'), 148.8 (C-8a), 154.1 (NH*C*OOCH$_3$), 154.9 (CHNH*C*ONHAr). Anal. ($C_{20}H_{22}ClN_3O_4$) theoretical: C, 59.48; H, 5.49; N, 10.40. Found: C, 59.84; H, 5.43; N, 10.66.

4.1.16. *R/S*-*N*-3-Cyanophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-methoxycarbonylamino-2*H*-1-benzopyran-4-yl)urea (**14c**) : m.p. 127-129°C; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.74 (t, J=12 Hz, 1H, 3-*H*), 2.08 (dd, J=13 Hz/6 Hz, 3-*H*), 3.60 (s, 3H, NHCOOCH_3), 4.95 (dd, J=16 Hz/9.5 Hz, 1H, 4-*H*), 6.67 (d, J=8.8 Hz, 1H, 8-*H*), 6,76 (d,

J=8.6 Hz, 1H, CHN*H*CONHAr), 7.23 (d, J=8.2 Hz, 1H, 7-*H*), 7.36 (s, 1H, 5-*H*), 7.37 (d, J=7.3 Hz, 1H, 4'-*H*), 7.46 (t, J=8 Hz, 1H, 5'-*H*), 7.65 (d, J=8.3 Hz, 1H, 6'-*H*), 8.01 (s, 1H, 2'-*H*), 8.94 (s, 1H, CHNHCON*H*Ar), 9.37 (s, 1H, N*H*COOCH$_3$); ^{13}C NMR (DMSO-d_6) δ 24.4 (CH$_3$), 29.1 (CH$_3$), 40.0 (C-3), 42.4 (C-4), 51.4 (NHCOO*C*H$_3$), 74.9 (C-2), 111.5 (C-3'), 116.8 (C-8), 117.6 (C-5), 119.0 (CN), 119.5 (C-7), 120.4 (C-2'), 122.4 (C-6'), 123.1 (C-4a), 124.6 (C-4'), 130.1 (C-5'), 131.6 (C-6), 141.3 (C-1'), 148.8 (C-8a), 154.1 (NH*C*OOCH$_3$), 154.9 (CHNH*C*ONHAr). Anal. ($C_{21}H_{22}N_4O_4$) theoretical: C, 63.95; H, 5.62; N, 14.20. Found: C, 63.61; H, 5.51; N, 14.39.

4.1.17. *R/S*-*N*-4-Cyanophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-methoxycarbonylamino-2*H*-1-benzopyran-4-yl)urea (**14d**) : m.p. : 221-223°C; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 3H, C*H*$_3$), 1.36 (s, 3H, C*H*$_3$), 1.74 (dd, J=13 Hz/11.2 Hz, 1H, 3-*H*), 2.09 (dd, J=13.2 Hz/6.3 Hz, 1H, 3-*H*), 3.59 (s, 3H, NHCOOC*H*$_3$), 4.94 (dd, J=15.9 Hz/9.9 Hz, 1H, 4-*H*), 6.67 (d, J=8.8 Hz, 1H, 8-*H*), 6,76 (d, J=8.7 Hz, 1H, CHN*H*CONHAr), 7.24 (d, J=8 Hz, 1H, 7-*H*), 7.35 (s, 1H, 5-*H*), 7.64 (d, J=8.9 Hz , 2H, 3'-*H*/5'-*H*), 7.70 (d, J=8.9 Hz, 1H, 2'-*H*/6'-*H*), 9.08 (s, 1H, CHNHCON*H*Ar), 9.40 (s, 1H, N*H*COOCH$_3$); ^{13}C NMR (DMSO-d_6) δ 24.4 (CH$_3$), 29.0 (CH$_3$), 39.8 (C-3), 42.4 (C-4), 51.4 (OCH$_3$), 74.9 (C-2), 102.5 (C-4'), 116.8 (C-8), 117.6 (C-5/C-3'/C-5'), 119.4 (CN), 119.8 (C-7), 122.9 (C-4a), 131.7 (C-6), 133.2 (C-2'/C-6'), 144.8 (C-1'), 148.8 (C-8a), 154.1 (NH*C*OOCH$_3$), 154.6 (CHNH*C*ONHAr). Anal. ($C_{21}H_{22}N_4O_4$) theoretical: C, 63.95; H, 5.62; N, 14.20. Found: C, 63.90; H, 5.62; N, 13.91.

4.1.18. *R/S*-*N*-3-Chlorophenyl-*N'*-(6-ethoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)urea (**14e**): m.p. : 197-199°C; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J=7.1 Hz, 3H, CH$_2$C*H*$_3$), 1.25 (s, 3H, C*H*$_3$), 1.36 (s, 3H, C*H*$_3$), 1.72 (dd, J=12.9 Hz/11.3 Hz, 1H, 3-*H*), 2.08 (dd, J=13.2 Hz/6.2 Hz, 1H, 3-*H*), 4.06 (q, J=7.1 Hz, 2H, C*H*$_2$CH$_3$), 4.94 (dd, J=15.9 Hz/9.9 Hz, 1H, 4-*H*), 6,61 (d, J=8.7 Hz, 1H, CHN*H*CONHAr), 6.66 (d, J=8.8 Hz, 1H, 8-*H*), 6.96 (ddd, J=7.7 Hz/2 Hz/1.4 Hz, 1H, 6'-*H*), 7.22-7.28 (m, 3H, 4'-*H*/5'-*H*/7-*H*), 7.36 (s, 1H, 5-*H*), 7.75 (t, J=2 Hz, 1H, 2'-*H*), 8.75 (s, 1H, CHNHCON*H*Ar), 9.37 (s, 1H, N*H*COOCH$_2$CH$_3$); ^{13}C NMR (DMSO-d_6) δ 14.5 (CH$_2$*C*H$_3$), 24.4 (CH$_3$), 29.1 (CH$_3$), 40.0 (C-3), 42.3 (C-4), 59.9 (*C*H$_2$CH$_3$), 74.9 (C-2), 116.1 (C-6'), 116.7 (C-8), 117.2 (C-2'), 117.6 (C-5), 119.5 (C-7), 120.8 (C-4'), 123.1 (C-4a), 130.3 (C-5'), 131.7 (C-6), 133.1 (C-3'), 141.9 (C-1'), 148.7 (C-8a), 153.7 (NH*C*OOCH$_2$CH$_3$), 154.9 (CHNH*C*ONHAr). Anal. ($C_{21}H_{24}ClN_3O_4$) theoretical: C, 60.36; H, 5.79; N, 10.06. Found: C, 60.25; H, 5.81; N, 9.78.

4.1.19. *R/S*-*N*-4-Chlorophenyl-*N'*-(6-ethoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)urea (**14f**): m.p. : 211-213°C; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J=7.1 Hz, 3H, CH_2CH_3), 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.71 (dd, J=12.9 Hz/11.2 Hz, 1H, 3-*H*), 2.08 (dd, J=13.2 Hz/6.2 Hz, 1H, 3-*H*), 4.05 (q, J=7.1 Hz, 2H, CH_2CH_3), 4.93 (dd, J=15.9 Hz/9.8 Hz, 1H, 4-*H*), 6.53 (d, J=8.8 Hz, 1H, CHN*H*CONHAr), 6,66 (d, J=8.8 Hz, 1H, 8-*H*), 7.21 (d, J=7.7 Hz, 1H, 7-*H*), 7.29 (d, J=8.9 Hz, 2H, 3'-*H*/5'-*H*), 7.38 (s, 1H, 5-*H*), 7.48 (d, J=8.9 Hz, 2H, 2'-*H*/6'-*H*), 8.66 (s, 1H, CHNHCON*H*Ar), 9.36 (s, 1H, N*H*$COOCH_2CH_3$); ^{13}C NMR (DMSO-d_6) δ 14.5 (CH_2CH_3), 24.4 (CH_3), 29.1 (CH_3), 40.2 (C-3), 42.3 (C-4), 59.9 (CH_2CH_3), 74.9 (C-2), 116.7 (C-8), 117.7 (C-5), 119.2 (C-2'/C-6'), 119.3 (C-7), 123.2 (C-4a), 124.6 (C-4'), 128.5 (C-3'/C-5'), 131.7 (C-6), 139.4 (C-1'), 148.8 (C-8a), 153.7 (NH*C*$OOCH_2CH_3$), 154.9 (CHNH*C*ONHAr). Anal. ($C_{21}H_{24}ClN_3O_4$) theoretical: C, 60.36; H, 5.79; N, 10.06. Found: C, 60.55; H, 5.83; N, 9.88.

4.1.20. *R/S*-*N*-3-Cyanophenyl-*N'*-(6-ethoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)urea (**14g**): m.p. 167-169°C; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J=7 Hz, 3H, $NHCOOCH_2CH_3$), 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.74 (t, J=12 Hz, 1H, 3-*H*), 2.08 (dd, J=13 Hz/6 Hz, 3-*H*), 4.06 (q, J=7 Hz, 2H, $NHCOOCH_2CH_3$), 4.94 (dd, J=16 Hz/9.5 Hz, 1H, 4-*H*), 6.66 (d, J=8.8 Hz, 1H, 8-*H*), 6,75 (d, J=8.7 Hz, 1H, CHN*H*CONHAr), 7.21 (d, J=7.8 Hz, 1H, 7-*H*), 7.36-7.39 (m, 2H, 5-*H*/4'-*H*), 7.46 (t, J=8 Hz, 1H, 5'-*H*), 7.65 (d, J=8.3 Hz, 1H, 6'-*H*), 8.01 (s, 1H, 2'-*H*), 8.93 (s, 1H, CHNHCON*H*Ar), 9.34 (s, 1H, N*H*$COOCH_2CH_3$); ^{13}C NMR (DMSO-d_6) δ □□□□□□□NHCOOCH$_2$*C*H$_3$□, 24.9 (CH_3), 29.6 (CH_3), 40.5 (C-3), 42.9 (C-4), 60.3 (NHCOO*C*H_2CH_3), 75.3 (C-2), 112.0 (C-3'), 117.2 (C-8), 118.1 (C-5), 119.5 (CN), 120.0 (C-7), 120.9 (C-2'), 122.9 (C-6'), 123.5 (C-4a), 125.1 (C-4'), 130.5 (C-5'), 132.2 (C-6), 141.8 (C-1'), 149.2 (C-8a), 154.1 (NH*C*$OOCH_2CH_3$), 155.4 (CHNH*C*ONHAr). Anal. ($C_{22}H_{24}N_4O_4$) theoretical: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.70; H, 6.04; N, 13.63.

4.1.21. *R/S*-*N*-4-Cyanophenyl-*N'*-(6-ethoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)urea (**14h**): m.p. : 208-210°C; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J=7.1 Hz, 3H, CH_2CH_3), 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.74 (dd, J=12.9 Hz/11.3 Hz, 1H, 3-*H*), 2.08 (dd, J=13.2 Hz/6.2 Hz, 1H, 3-*H*), 4.05 (q, J=7.1 Hz, 2H, CH_2CH_3), 4.94 (dd, J=15.9 Hz/9.8 Hz, 1H, 4-*H*), 6.66 (d, J=8.8 Hz, 1H, 8-*H*), 6,77 (d, J=8.7 Hz, 1H, CHN*H*CONHAr), 7.22 (d, J=7.6 Hz, 1H, 7-*H*), 7.37 (s, 1H, 5-*H*), 7.64 (d, J=8.8 Hz, 2H, 3'-*H*/5'-*H*), 7.70 (d,

J=8.8 Hz, 2H, 2'-*H*/6'-*H*), 9.09 (s, 1H, CHNHCON*H*Ar), 9.36 (s, 1H, N*H*COOCH$_2$CH$_3$); ^{13}C NMR (DMSO-d_6) δ 14.5 (CH$_2$*C*H$_3$), 24.4 (CH$_3$), 29.0 (CH$_3$), 39.8 (C-3), 42.4 (C-4), 59.9 (*C*H$_2$CH$_3$), 74.9 (C-2), 102.5 (C-4'), 116.8 (C-8), 117.6 (C-5/C-3'/C-5'), 119.4 (CN), 119.6 (C-7), 122.9 (C-4a), 131.7 (C-6), 133.2 (C-2'/C-6'), 144.8 (C-1'), 148.7 (C-8a), 153.7 (NH*C*OOCH$_2$CH$_3$), 154.6 (CHNH*C*ONHAr). Anal. ($C_{22}H_{24}N_4O_4$) theoretical: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.70; H, 5.94; N, 13.45.

4.1.22. *R/S*-*N*-3-Chlorophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-isopropoxycarbonylamino-2*H*-1-benzopyran-4-yl)urea (**14i**): m.p. : 105-107°C; ^{1}H NMR (DMSO-d_6) δ 1.20 (dd, J=6.2 Hz/1 Hz, 6H, CH(C*H*$_3$)$_2$), 1.25 (s, 3H, C*H*$_3$), 1.36 (s, 3H, C*H*$_3$), 1.72 (dd, J=12.9 Hz/11.2 Hz, 1H, 3-*H*), 2.08 (dd, J=13.2 Hz/6.2 Hz, 1H, 3-*H*), 4.82 (hept, J=6.2 Hz, 1H, C*H*(CH$_3$)$_2$), 4.92 (dd, J=15.8 Hz/9.9 Hz, 1H, 4-*H*), 6,59 (d, J=8.7 Hz, 1H, CHN*H*CONHAr), 6.65 (d, J=8.8 Hz, 1H, 8-*H*), 6.96 (ddd, J=7.7 Hz/2 Hz/1.3 Hz, 1H, 6'-*H*), 7.23-7.26 (m, 3H, 4'-*H*/5'-*H*/7-*H*), 7.40 (s, 1H, 5-*H*), 7.76 (t, J=2 Hz, 1H, 2'-*H*), 8.74 (s, 1H, CHNHCON*H*Ar), 9.31 (s, 1H, N*H*COOCH(CH$_3$)$_2$); ^{13}C NMR (DMSO-d_6) δ 21.2 (CH(*C*H$_3$)$_2$), 24.4 (CH$_3$), 29.1 (CH$_3$), 40.0 (C-3), 42.3 (C-4), 67.1 (*C*H(CH$_3$)$_2$), 74.8 (C-2), 116.1 (C-6'), 116.7 (C-8), 117.2 (C-2'), 117.6 (C-5), 119.5 (C-7), 120.8 (C-4'), 123.0 (C-4a), 130.3 (C-5'), 131.8 (C-6), 133.1 (C-3'), 141.9 (C-1'), 148.7 (C-8a), 153.3 (NH*C*OOCH(CH$_3$)$_2$), 154.9 (CHNH*C*ONHAr). Anal. ($C_{22}H_{26}ClN_3O_4$) theoretical: C, 61.18; H, 6.07; N, 9.73. Found: C, 60.91; H, 6.23; N, 9.62.

4.1.23. *R/S*-*N*-4-Chlorophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-isopropoxycarbonylamino-2*H*-1-benzopyran-4-yl)urea (**14j**): m.p. : 195-197°C; ^{1}H NMR (DMSO-d_6) δ 1.20 (d, J=6.2 Hz, 6H, CH(C*H*$_3$)$_2$), 1.24 (s, 3H, C*H*$_3$), 1.36 (s, 3H, C*H*$_3$), 1.71 (dd, J=13 Hz/11.2 Hz, 1H, 3-*H*), 2.08 (dd, J=13.2 Hz/6.2 Hz, 1H, 3-*H*), 4.82 (hept, J=6.3 Hz, 1H, C*H*(CH$_3$)$_2$), 4.92 (dd, J=15.8 Hz/9.8 Hz, 1H, 4-*H*), 6.53 (d, J=8.8 Hz, 1H, CHN*H*CONHAr), 6,65 (d, J=8.8 Hz, 1H, 8-*H*), 7.19 (d, J=6.6 Hz, 1H, 7-*H*), 7.29 (d, J=8.9 Hz, 2H, 3'-*H*/5'-*H*), 7.40 (s, 1H, 5-*H*), 7.48 (d, J=8.9 Hz, 2H, 2'-*H*/6'-*H*), 8.66 (s, 1H, CHNHCON*H*Ar), 9.30 (s, 1H, N*H*COOCH(CH$_3$)$_2$); ^{13}C NMR (DMSO-d_6) δ 21.2 (CH(*C*H$_3$)$_2$), 24.4 (CH$_3$), 29.1 (CH$_3$), 40.2 (C-3), 42.3 (C-4), 67.0 (*C*H(CH$_3$)$_2$), 74.8 (C-2), 116.7 (C-8), 117.6 (C-5), 119.2 (C-2'/C-6'), 119.5 (C-7), 123.1 (C-4a), 124.6 (C-4'), 128.5 (C-3'/C-5'), 131.8 (C-6), 139.4 (C-1'), 148.7 (C-8a), 153.3 (NH*C*OOCH(CH$_3$)$_2$), 154.9

(CHNH*C*ONHAr). Anal. ($C_{22}H_{26}ClN_3O_4$) theoretical: C, 61.18; H, 6.07; N, 9.73. Found: C, 61.11; H, 6.08; N, 9.50.

4.1.24. *R/S*-*N*-3-Cyanophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-isopropoxycarbonylamino-2*H*-1-benzopyran-4-yl)urea (**14k**): m.p. : 105-107°C; ^{1}H NMR (DMSO-d_6) □ 1.20 (t, J=7 Hz, 6H, NHCOOCH(CH_3)$_2$), 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.74 (t, J=12 Hz, 1H, 3-*H*), 2.08 (dd, J=13 Hz/6 Hz, 3-*H*), 4.82 (dt, J=12 Hz/6 Hz, 1H, NHCOOC*H*(CH$_3$)$_2$), 4.94 (dd, J=16.2 Hz/9.5 Hz, 1H, 4-*H*), 6.65 (d, J=8.8 Hz, 1H, 8-*H*), 6,74 (d, J=8.7 Hz, 1H, CHN*H*CONHAr), 7.20 (d, J=7.2 Hz, 1H, 7-*H*), 7.37 (d, J=7.5 Hz, 1H, 4'-*H*), 7.40 (m, 2H, 5-*H*), 7.46 (t, J=7.9 Hz, 1H, 5'-*H*), 7.65 (d, J=8.3 Hz, 1H, 6'-*H*), 8.01 (s, 1H, 2'-*H*), 8.93 (s, 1H, CHNHCON*H*Ar), 9.27 (s, 1H, N*H*COOCH(CH$_3$)$_2$); ^{13}C NMR (DMSO-d_6) δ □□□□□□NHCOOCH(*C*H$_3$□□□, 24.9 (CH$_3$), 29.6 (CH$_3$), 40.2 (C-3), 42.9 (C-4), 67.6 (NHCOO*C*H(CH$_3$)$_2$), 75.3 (C-2), 112.0 (C-3'), 117.2 (C-8), 118.1 (C-5), 119.5 (CN), 120.0 (C-7), 120.9 (C-2'), 122.9 (C-6'), 123.5 (C-4a), 125.1 (C-4'), 130.5 (C-5'), 132.3 (C-6), 141.8 (C-1'), 149.2 (C-8a), 153.8 (NH*C*OOCH(CH$_3$)$_2$), 155.4 (CHNH*C*ONHAr). Anal. ($C_{23}H_{26}N_4O_4$) theoretical: C, 65.39; H, 6.20; N, 13.26. Found: C, 65.23; H, 6.07; N, 13.36.

4.1.25. *R/S*-*N*-4-Cyanophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-isopropoxycarbonylamino-2*H*-1-benzopyran-4-yl)urea (**14l**): m.p. : 210-212°C; ^{1}H NMR (DMSO-d_6) δ 1.20 (d, J=6.2 Hz, 6H, CH(CH_3)$_2$), 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.73 (dd, J=12.9 Hz/11.2 Hz, 1H, 3-*H*), 2.08 (dd, J=13.2 Hz/6.3 Hz, 1H, 3-*H*), 4.82 (hept, J=6.2 Hz, 1H, C*H*(CH$_3$)$_2$), 4.94 (dd, J=15.9 Hz/9.8 Hz, 1H, 4-*H*), 6.66 (d, J=8.8 Hz, 1H, 8-*H*), 6,73 (d, J=8.7 Hz, 1H, CHN*H*CONHAr), 7.20 (d, J=6.9 Hz, 1H, 7-*H*), 7.39 (s, 1H, 5-*H*), 7.64 (d, J=8.9 Hz , 2H, 3'-*H*/5'-*H*), 7.70 (d, J=8.9 Hz, 2H, 2'-*H*/6'-*H*), 9.06 (s, 1H, CHNHCON*H*Ar), 9.30 (s, 1H, N*H*COOCH(CH$_3$)$_2$); ^{13}C NMR (DMSO-d_6) δ 22.0 (CH(*C*H$_3$)$_2$), 24.4 (CH$_3$), 29.0 (CH$_3$), 39.8 (C-3), 42.4 (C-4), 67.1 (*C*H(CH$_3$)$_2$), 74.8 (C-2), 102.5 (C-4'), 116.8 (C-8), 117.6 (C-3'/C-5'), 118.3 (C-5), 119.4 (CN), 119.6 (C-7), 122.8 (C-4a), 131.8 (C-6), 133.2 (C-2'/C-6'), 144.8 (C-1'), 148.7 (C-8a), 153.3 (NH*C*OOCH(CH$_3$)$_2$), 154.5 (CHNH*C*ONHAr). Anal. ($C_{23}H_{26}N_4O_4$) theoretical: C, 65.39; H, 6.20; N, 13.26. Found: C, 65.46; H, 6.15; N, 13.22.

4.1.26. *R/S*-*N*-3-Chlorophenyl-*N'*-(6-*tert*-butoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)urea (**14m**): m.p. 113°C (dec); ^{1}H NMR (DMSO-d_6) □ 1.24 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.42 (s, 9H, NHCOOC(CH_3)$_3$), 1.71 (dd, J=13 Hz/11

Hz, 1H, 3-*H*), 2.09 (dd, J=13 Hz/6 Hz, 3-*H*), 4.92 (dd, J=16 Hz/10 Hz, 1H, 4-*H*), 6.55 (d, J=8.7 Hz, 1H, CHN*H*CONHAr), 6,63 (d, J=8.8 Hz, 1H, 8-*H*), 6.96 (ddd, J=1.2 Hz/2 Hz/8.7 Hz, 1H, 4'-*H*), 7.17 (d, J=7.1 Hz, 1H, 7-*H*), 7.22 (d, J=8.6 Hz, 1H, 6'-*H*), 7.26 (t, J=8 Hz, 1H, 5'-*H*), 7.40 (s, 1H, 5-*H*), 7.75 (t, J=2 Hz, 1H, 2'-*H*), 8.70 (s, 1H, CHNHCON*H*Ar), 9.08 (s, 1H, N*H*COOC(CH_3)$_3$); ^{13}C NMR (DMSO-d_6) δ 24.4 (CH_3), 28.1 (C(*C*H_3)$_3$), 29.0 (CH_3), 40.1 (C-3), 42.4 (C-4), 74.8 (C-2), 78.6 (*C*(CH$_3$)$_3$), 116.2 (C-6'), 116.7 (C-8), 117.2 (C-2'), 117.6 (C-5), 119.6 (C-7), 120.8 (C-4'), 122.9 (C-4a), 130.2 (C-5'), 132.0 (C-6), 133.1 (C-3'), 141.9 (C-1'), 148.6 (C-8a), 153.0 (NH*C*OOC(CH$_3$)$_3$), 154.9 (CHNH*C*ONHAr). Anal. ($C_{23}H_{28}ClN_3O_4$) theoretical: C, 61.95; H, 6.33; N, 9.42. Found: C, 61.60; H, 6.36; N, 9.46.

4.1.27. *R/S*-*N*-4-Chlorophenyl-*N'*-(6-*tert*-butoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)urea (**14n**): m.p.: 113°C (dec); ^{1}H NMR (DMSO-d_6) □ 1.24 (s, 3H, C*H*$_3$), 1.35 (s, 3H, C*H*$_3$), 1.42 (s, 9H, NHCOOC(C*H*$_3$)$_3$), 1.70 (dd, J=13 Hz/11 Hz, 1H, 3-*H*), 2.08 (dd, J=13 Hz/6 Hz, 3-*H*), 4.91 (dd, J=16 Hz/9 Hz, 1H, 4-*H*), 6.50 (d, J=8.7 Hz, 1H, CHN*H*CONHAr), 6,63 (d, J=8.8 Hz, 1H, 8-*H*), 7.17 (d, J=6.7 Hz, 1H, 7-*H*), 7.29 (d, J=8.7 Hz , 2H, 3'-*H*/5'-*H*), 7.40 (s, 1H, 5-*H*), 7.48 (d, J=8.6 Hz, 1H, 2'-*H*/6'-*H*), 8.64 (s, 1H, CHNHCON*H*Ar), 9.08 (s, 1H, N*H*COOC(CH$_3$)$_3$). ^{13}C NMR (DMSO-d_6) δ 24.5 (CH_3), 28.1 (C(*C*H$_3$)$_3$), 29.0 (CH_3), 40.5 (C-3), 42.4 (C-4), 74.8 (C-2), 78.5 (*C*(CH$_3$)$_3$), 116.6 (C-8), 117.6 (C-5), 119.3 (C-2'/C-6'), 119.6 (C-7), 122.9 (C-4a), 124.6 (C-4'), 128.5 (C-3'/C-5'), 132.0 (C-6), 139.4 (C-1'), 148.6 (C-8a), 153.0 (NH*C*OOC(CH$_3$)$_3$), 154.9 (CHNH*C*ONHAr). Anal. ($C_{23}H_{28}ClN_3O_4$) theoretical: C, 61.95; H, 6.33; N, 9.42. Found: C, 62.12; H, 6.39; N, 9.82.

4.1.28. *R/S*-*N*-3-Cyanophenyl-*N'*-(6-*tert*-butoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)urea (**14o**): m.p.: 189-191°C; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 3H, C*H*$_3$), 1.36 (s, 3H, C*H*$_3$), 1.42 (s, 9H, NHCOOC(C*H*$_3$)$_3$), 1.73 (dd, J=13 Hz/11 Hz, 1H, 3-*H*), 2.08 (dd, J=13 Hz/6 Hz, 3-*H*), 4.93 (dd, J=11 Hz/6 Hz, 1H, 4-*H*), 6.64 (d, J=8.8 Hz, 1H, 8-*H*), 6,70 (d, J=8.8 Hz, 1H, CHN*H*CONHAr), 7.17 (d, J=8.7 Hz, 1H, 7-*H*), 7.37 (d, J=8 Hz, 1H, 4'-*H*), 7.41 (bs, 1H, 5-*H*), 7.46 (t, J=8 Hz, 1H, 5'-*H*), 7.64 (d, J=8 Hz, 1H, 6'-*H*), 8.01 (s, 1H, 2'-*H*), 8.89 (s, 1H, CHNHCON*H*Ar), 9.09 (s, 1H, N*H*COOC(CH$_3$)$_3$); ^{13}C NMR (DMSO-d_6) δ 24.4 (CH_3), 28.1 (C(*C*H$_3$)$_3$), 29.0 (CH_3), 40.3 (C-3), 42.5 (C-4), 74.8 (C-2), 78.6 (*C*(CH$_3$)$_3$), 111.5 (C-3'), 116.6 (C-8), 117.6 (C-5), 119.0 (CN), 119.6 (C-7), 120.4 (C-2'), 122.5 (C-6'), 122.8 (C-4a), 124.7 (C-4'), 130.1 (C-5'), 132.0 (C-6), 141.3 (C-1'), 148.5 (C-8a), 152.9

(NH*C*OOC(CH_3)$_3$), 154.9 (CHNH*C*ONHAr). Anal. ($C_{24}H_{28}N_4O_4$) theoretical: C, 66.04; H, 6.47; N, 12.84. Found: C, 66.20; H, 6.53; N, 12.95.

4.1.29. *R/S*-*N*-4-Cyanophenyl-*N'*-(6-*tert*-butoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)urea (**14p**): m.p.: 231-233°C; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.42 (s, 9H, NHCOOC(CH_3)$_3$), 1.74 (t, J=12 Hz, 1H, 3-*H*), 2.08 (dd, J=12 Hz/6 Hz, 3-*H*), 4.93 (dd, J=16 Hz/8 Hz, 1H, 4-*H*), 6.64 (d, J=7.7 Hz, 1H, 8-*H*), 6,72 (d, J=8.3 Hz, 1H, CHN*H*CONHAr), 7.18 (d, J=6.5 Hz, 1H, 7-*H*), 7.39 (s, 1H, 5-*H*), 7.63 (d, J=7 Hz , 2H, 3'-*H*/5'-*H*), 7.70 (d, J=6.9 Hz, 1H, 2'-*H*/6'-*H*), 9.05 (s, 1H, CHNHCON*H*Ar), 9.08 (s, 1H, N*H*COOC(CH_3)$_3$); ^{13}C NMR (DMSO-d_6) δ 24.4 (CH_3), 28.1 (C(*C*H_3)$_3$), 29.0 (CH_3), 39.8 (C-3), 42.4 (C-4), 74.8 (C-2), 78.6 (*C*(CH_3)$_3$), 102.5 (C-4'), 116.7 (C-8), 117.6 (C-5/C-3'/C-5'), 119.4 (CN), 119.6 (C-7), 122.6 (C-4a), 132.0 (C-6), 133.2 (C-2'/C-6'), 144.8 (C-1'), 148.5 (C-8a), 153.0 (NH*C*OOC(CH_3)$_3$), 154.6 (CHNH*C*ONHAr). Anal. ($C_{24}H_{28}N_4O_4$) theoretical: C, 66.04; H, 6.47; N, 12.84. Found: C, 65.94; H, 6.35; N, 12.45.

4.1.30. *R/S*-*N*-3-Chlorophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-methoxycarbonylamino-2*H*-1-benzopyran-4-yl)thiourea (**15a**): m.p. : 171-173°C; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.77 (t, J=12 Hz, 1H, 3-*H*), 2.16 (dd, J=13 Hz/6.3 Hz, 1H, 3-*H*), 3.63 (s, 3H, NHCOOCH_3), 5.77 (dd, J=16.5 Hz/7.4 Hz, 1H, 4-*H*), 6,69 (d, J=8.8 Hz, 1H, 8-*H*), 7.16 (d, J=7.2 Hz, 1H, 6'-*H*), 7.23 (d, J=8.2 Hz, 1H, 7-*H*), 7.32-7.37 (m, 2H, 4'-*H*/5'-*H*), 7.40 (s, 1H, 2'-*H*), 7.74 (s, 1H, 5-*H*), 8.25 (d, J=7.7 Hz, 1H, CHN*H*CSNHAr), 9.44 (s, 1H, N*H*COOCH$_3$), 9.72 (s, 1H, CHNHCSN*H*Ar); ^{13}C NMR (DMSO-d_6) δ 24.2 (CH_3), 29.2 (CH_3), 38.1 (C-3), 47.2 (C-4), 51.5 (OCH$_3$), 75.0 (C-2), 116.8 (C-8), 117.4 (C-2'), 119.5 (C-7), 121.6 (C-4'), 122.0 (C-4a), 122.6 (C-5), 123.8 (C-6'), 130.2 (C-5'), 131.7 (C-6), 132.7 (C-3'), 140.7 (C-1'), 148.8 (C-8a), 154.1 (NH*C*OOCH$_3$), 180.5 (CHNH*C*SNHAr). Anal. ($C_{20}H_{22}ClN_3O_3S$) theoretical: C, 57.20; H, 5.28; N, 10.01; S, 7.64. Found: C, 57.39; H, 5.28; N, 10.19; S, 7.63.

4.1.31. *R/S*-*N*-4-Chlorophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-methoxycarbonylamino-2*H*-1-benzopyran-4-yl)thiourea (**15b**): m.p. : 202-203°C; ^{1}H NMR (DMSO-d_6) δ 1.24 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.75 (t, J=12 Hz, 1H, 3-*H*), 2.15 (dd, J=13 Hz/6 Hz, 1H, 3-*H*), 3.63 (s, 3H, NHCOOCH_3), 5.76 (bs, 1H, 4-*H*), 6,68 (d, J=8.8 Hz, 1H, 8-*H*), 7.23 (d, J=7.9 Hz, 1H, 7-*H*), 7.37 (d, J=8.8 Hz , 2H, 2'-*H*/6'-*H*), 7.39 (s, 1H, 5-*H*), 7.52 (d, J=8.8 Hz, 1H, 3'-*H*/5'-

H), 8.18 (d, J=6.3 Hz, 1H, CHN*H*CSNHAr), 9.44 (s, 1H, N*H*COOCH$_3$) , 9.69 (s, 1H, CHNHCSN*H*Ar); ^{13}C NMR (DMSO-d_6) δ 24.2 (CH$_3$), 29.2 (CH$_3$), 38.1 (C-3), 47.2 (C-4), 51.5 (OCH$_3$), 75.0 (C-2), 116.8 (C-8), 117.4 (C-5), 119.5 (C-7), 122.1 (C-4a), 125.0 (C-3'/C-5'), 128.0 (C-4'), 128.4 (C-2'/C-6'), 131.6 (C-6), 138.1 (C-1'), 148.8 (C-8a), 154.2 (NH*C*OOCH$_3$), 180.5 (CHNH*C*SNHAr). Anal. ($C_{20}H_{22}ClN_3O_3S$) theoretical: C, 57.20; H, 5.28; N, 10.01; S, 7.64. Found: C, 57.30; H, 5.23; N, 10.31; S, 7.77.

4.1.32. *R/S*-*N*-3-Cyanophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-methoxycarbonylamino-2*H*-1-benzopyran-4-yl)thiourea (**15c**) : m.p. 182-184°C; ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 3H, C*H*$_3$), 1.37 (s, 3H, C*H*$_3$), 1.77 (t, J=12 Hz, 1H, 3-*H*), 2.16 (dd, J=13 Hz/6 Hz, 3-*H*), 3.63 (s, 3H, NHCOOC*H*$_3$), 5.77 (bs, 1H, 4-*H*), 6.69 (d, J=8.8 Hz, 1H, 8-*H*), 7.23 (d, J=8.4 Hz, 1H, 7-*H*), 7.43 (s, 1H, 5-*H*), 7.51-7.56 (m, 2H, 4'-*H*/5'-*H*), 7.75 (d, J=7.7 Hz, 1H, 6'-*H*), 8.06 (s, 1H, 2'-*H*), 8,40 (d, J=5.4 Hz, 1H, CHN*H*CSNHAr), 9.42 (s, 1H, N*H*COOCH$_3$), 9.87 (s, 1H, CHNHCSN*H*Ar); ^{13}C NMR (DMSO-d_6) δ 24.2 (CH$_3$), 29.2 (CH$_3$), 38.1 (C-3), 47.3 (C-4), 51.4 (NHCOO*C*H$_3$), 75.0 (C-2), 111.2 (C-3'), 116.9 (C-8), 117.4 (C-5), 118.6 (CN), 119.5 (C-7), 121.9 (C-4a), 126.1 (C-2'), 127.6 (C-4'), 127.9 (C-6'), 129.9 (C-5'), 131.7 (C-6), 140.2 (C-1'), 148.8 (C-8a), 154.1 (NH*C*OOCH$_3$), 180.7 (CHNH*C*SNHAr). Anal. ($C_{21}H_{22}N_4O_3S$) theoretical: C, 61.44; H, 5.40; N, 13.65; S, 7.81. Found: C, 61.33; H, 5.34; N, 13.69; S, 7.72.

4.1.33. *R/S*-*N*-4-Cyanophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-methoxycarbonylamino-2*H*-1-benzopyran-4-yl)thiourea (**15d**) : m.p. : 216-217°C; ^{1}H NMR (DMSO-d_6) δ 1,26 (s, 3H, C*H*$_3$), 1,37 (s, 3H, C*H*$_3$), 1,76 (m, 1H, 3-*H*), 2,20 (dd, J=13.1 Hz/6.3 Hz, 1H, 3-*H*), 3,62 (s, 3H, NHCOOC*H*$_3$), 5,75 (dd, 1H, J=17.3 Hz/7.9 Hz, 4-*H*), 6,70 (d, J=8.8 Hz, 1H, 8-*H*), 7,24 (d, J=8.2 Hz, 1H, 7-*H*), 7,39 (s, 1H, 5-*H*), 7,76 (d, J=8.9 Hz, 2H, 3'-*H* et 5'-*H*), 7,80 (d, J=8.9 Hz, 2H, 2'-*H* et 6'-*H*), 8,50 (d, J=8.3 Hz, 1H, CHN*H*CSNHAr), 9,44 (s, 1H, N*H*COOCH$_3$), 10,00 (s, 1H, CHNHCSN*H*Ar); ^{13}C NMR (DMSO-d_6) δ 24.2 (CH$_3$), 29.1 (CH$_3$), 38.0 (C-3), 47.1 (C-4), 51.5 (OCH$_3$), 75.0 (C-2), 104.9 (C-4'), 116.9 (C-8), 117.4 (C-5), 119.1 (CN), 119.7 (C-7), 121.6 (C-4a), 121.7 (C-3'/C-5'), 131.7 (C-6), 132.8 (C-2'/C-6'), 143.9 (C-1'), 148.8 (C-8a), 154.2 (NH*C*OOCH$_3$), 180.2 (CHNH*C*SNHAr). Anal. ($C_{21}H_{22}N_4O_3S$) theoretical: C, 61.44; H, 5.40; N, 13.65; S, 7.81. Found: C, 61.37; H, 5.33; N, 13.63; S, 7.42.

4.1.34. *R/S*-*N*-3-Chlorophenyl-*N'*-(6-ethoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-

1-benzopyran-4-yl)thiourea (**15e**): m.p. : 180-182°C; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, J=7.1 Hz, 3H, CH_2CH_3), 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.76 (t, J=12.1 Hz, 1H, 3-*H*), 2.15 (dd, J=13 Hz/6.3 Hz, 1H, 3-*H*), 4.09 (q, J=7.1 Hz, 2H, CH_2CH_3), 5.76 (dd, J=16 Hz/7 Hz, 1H, 4-*H*), 6,68 (d, J=8.8 Hz, 1H, 8-*H*), 7.16 (d, J=7.3 Hz, 1H, 6'-*H*), 7.21 (d, J=8 Hz, 1H, 7-*H*), 7.32-7.35 (m, 2H, 4'-*H*/5'-*H*), 7.43 (s, 1H, 2'-*H*), 7.74 (s, 1H, 5-*H*), 8.25 (d, J=7.2 Hz, 1H, CHN*H*CSNHAr), 9.41 (s, 1H, N*H*COOCH$_2$CH$_3$), 9.73 (s, 1H, CHNHCSN*H*Ar); ^{13}C NMR (DMSO-d_6) δ 14.6 (CH_2CH_3), 24.2 (CH_3), 29.2 (CH_3), 38.1 (C-3), 47.2 (C-4), 59.9 (CH_2CH_3), 75.0 (C-2), 116.8 (C-8), 117.5 (C-2'), 119.6 (C-7), 121.6 (C-4'), 122.0 (C-4a), 122.6 (C-5), 123.8 (C-6'), 130.2 (C-5'), 131.8 (C-6), 132.7 (C-3'), 140.7 (C-1'), 148.7 (C-8a), 153.7 (NH*C*OOCH$_2$CH$_3$), 180.5 (CHNH*C*SNHAr). Anal. ($C_{21}H_{24}ClN_3O_3S$) theoretical: C, 58.12; H, 5.57; N, 9.68; S, 7.39. Found: C, 58.07; H, 5.55; N, 9.98; S, 7.22.

4.1.35. *R/S*-*N*-4-Chlorophenyl-*N'*-(6-ethoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (**15f**): m.p. : 171-173°C; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, J=7.1 Hz, 3H, CH_2CH_3), 1.24 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.75 (t, J=12.1 Hz, 1H, 3-*H*), 2.15 (dd, J=13 Hz/6.2 Hz, 1H, 3-*H*), 4.09 (q, J=7.1 Hz, 2H, CH_2CH_3), 5.76 (dd, J=16.4 Hz/7.5 Hz, 1H, 4-*H*), 6,67 (d, J=8.8 Hz, 1H, 8-*H*), 7.21 (d, J=7.4 Hz, 1H, 7-*H*), 7.36 (d, J=8.8 Hz, 2H, 2'-*H*/6'-*H*), 7.40 (s, 1H, 5-*H*), 7.53 (d, J=8.8 Hz, 2H, 3'-*H*/5'-*H*), 8.21 (d, J=7.6 Hz, 1H, CHN*H*CSNHAr), 9.41 (s, 1H, N*H*COOCH$_2$CH$_3$), 9.74 (s, 1H, CHNHCSN*H*Ar); ^{13}C NMR (DMSO-d_6) δ□14.6 (CH_2CH_3), 24.2 (CH_3), 29.2 (CH_3), 38.1 (C-3), 47.2 (C-4), 59.9 (CH_2CH_3), 75.0 (C-2), 116.8 (C-8), 117.4 (C-5), 119.5 (C-7), 122.1 (C-4a), 125.0 (C-3'/C-5'), 128.0 (C-4'), 128.4 (C-2'/C-6'), 131.7 (C-6), 138.1 (C-1'), 148.7 (C-8a), 153.7 (NH*C*OOCH$_2$CH$_3$), 180.5 (CHNH*C*SNHAr). Anal. ($C_{21}H_{24}ClN_3O_3S$) theoretical: C, 58.12; H, 5.57; N, 9.68; S, 7.39. Found: C, 58.49; H, 5.62; N, 9.93; S, 7.40.

4.1.36. *R/S*-*N*-3-Cyanophenyl-*N'*-(6-ethoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (**15g**): m.p. 192-194°C; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, J=7 Hz, 3H, NHCOOCH$_2$C*H*$_3$), 1.25 (s, 3H, C*H*$_3$), 1.37 (s, 3H, C*H*$_3$), 1.77 (t, J=12 Hz, 1H, 3-*H*), 2.16 (dd, J=13 Hz/6 Hz, 3-*H*), 4.09 (q, J=7 Hz, 2H, NHCOOC*H*$_2$CH$_3$), 5.77 (bs, 1H, 4-*H*), 6.68 (d, J=8.7 Hz, 1H, 8-*H*), 7.21 (d, J=8.2 Hz, 1H, 7-*H*), 7.45 (s, 1H, 5-*H*), 7.51-7.56 (m, 2H, 4'-*H*/5'-*H*), 7.75 (d, J=7.6 Hz, 1H, 6'-*H*), 8.06 (s, 1H, 2'-*H*), 8,40 (d, J=7.2 Hz, 1H, CHN*H*CSNHAr), 9.39 (s, 1H, N*H*COOCH$_2$CH$_3$), 9.86 (s, 1H, CHNHCSN*H*Ar); ^{13}C NMR (DMSO-d_6) δ □□□□□□ NHCOOCH$_2$*C*H$_3$□□□24.7 (CH_3), 29.7 (CH_3), 38.6 (C-3), 47.8 (C-4), 60.4

(NHCOO*C*H_2CH$_3$), 75.5 (C-2), 111.7 (C-3'), 117.3 (C-8), 117.9 (C-5), 119.1 (CN), 120.0 (C-7), 122.4 (C-4a), 126.6 (C-2'), 128.0 (C-4'), 128.4 (C-6'), 130.4 (C-5'), 132.3 (C-6), 140.7 (C-1'), 149.2 (C-8a), 154.2 (NH*C*OOCH$_2$CH$_3$), 181.2 (CHNH*C*SNHAr). Anal. ($C_{22}H_{24}N_4O_3S$) theoretical: C, 62.24; H, 5.70; N, 13.20; S, 7.55. Found: C, 62.05; H, 5.66; N, 13.33; S, 7.42.

4.1.37. *R/S*-*N*-4-Cyanophenyl-*N'*-(6-ethoxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (**15h**): m.p. : 201-202°C; ^{1}H NMR (DMSO-d_6) δ 1.21 (t, J=7.1 Hz, 3H, CH$_2$C*H*$_3$), 1,26 (s, 3H, C*H*$_3$), 1,37 (s, 3H, C*H*$_3$), 1,76 (m, 1H, 3-*H*), 2,20 (dd, J=13.1 Hz/6.3 Hz, 1H, 3-*H*), 4.08 (q, J=7.1 Hz, 2H, C*H*$_2$CH$_3$), 5.74 (dd, J=16.4 Hz/7.5 Hz, 1H, 4-*H*), 6,69 (d, J=8.8 Hz, 1H, 8-*H*), 7,22 (d, J=8.1 Hz, 1H, 7-*H*), 7,41 (s, 1H, 5-*H*), 7,76 (d, J=8.9 Hz, 2H, 3'-*H* et 5'-*H*), 7,80 (d, J=8.9 Hz, 2H, 2'-*H* et 6'-*H*), 8,49 (d, J=8.3 Hz, 1H, CHN*H*CSNHAr), 9,41 (s, 1H, N*H*COOCH$_2$CH$_3$), 10,00 (s, 1H, CHNHCSN*H*Ar); ^{13}C NMR (DMSO-d_6) δ 14.5 (CH$_2$*C*H$_3$), 24.2 (CH$_3$), 29.1 (CH$_3$), 38.0 (C-3), 47.2 (C-4), 59.9 (*C*H$_2$CH$_3$), 75.0 (C-2), 104.9 (C-4'), 116.9 (C-8), 117.3 (C-5), 119.1 (CN), 119.7 (C-7), 121.6 (C-4a), 121.7 (C-3'/C-5'), 131.8 (C-6), 132.8 (C-2'/C-6'), 143.9 (C-1'), 148.8 (C-8a), 153.7 (NH*C*OOCH$_2$CH$_3$), 180.2 (CHNH*C*SNHAr). Anal. ($C_{22}H_{24}N_4O_3S$) theoretical: C, 62.24; H, 5.70; N, 13.20; S, 7.55. Found: C, 62.03; H, 5.64; N, 13.26; S, 7.36.

4.1.38. *R/S*-*N*-3-Chlorophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-isopropoxycarbonylamino-2*H*-1-benzopyran-4-yl)thiourea (**15i**): m.p. : 163-165°C; ^{1}H NMR (DMSO-d_6) δ 1.23 (dd, J=6.2 Hz/2 Hz, 6H, CH(C*H*$_3$)$_2$), 1.25 (s, 3H, C*H*$_3$), 1.36 (s, 3H, C*H*$_3$), 1.76 (t, J=12.1 Hz, 1H, 3-*H*), 2.15 (dd, J=13 Hz/6.3 Hz, 1H, 3-*H*), 4.85 (hept, J=6.2 Hz, 1H, C*H*(CH$_3$)$_2$), 5.76 (dd, J=15.7 Hz/8.5 Hz, 1H, 4-*H*), 6,67 (d, J=8.8 Hz, 1H, 8-*H*), 7.16 (d, J=7.3 Hz, 1H, 6'-*H*), 7.19 (d, J=7 Hz, 1H, 7-*H*), 7.32-7.35 (m, 2H, 4'-*H*/5'-*H*), 7.45 (s, 1H, 2'-*H*), 7.74 (s, 1H, 5-*H*), 8.24 (d, J=7.8 Hz, 1H, CHN*H*CSNHAr), 9.35 (s, 1H, N*H*COOCH(CH$_3$)$_2$), 9.73 (s, 1H, CHNHCSN*H*Ar); ^{13}C NMR (DMSO-d_6) δ 22.0 (CH(*C*H$_3$)$_2$), 24.2 (CH$_3$), 29.2 (CH$_3$), 38.1 (C-3), 47.3 (C-4), 67.1 (*C*H(CH$_3$)$_2$), 75.0 (C-2), 116.8 (C-8), 117.3 (C-2'), 119.5 (C-7), 121.6 (C-4'), 121.9 (C-4a), 122.6 (C-5), 123.8 (C-6'), 130.2 (C-5'), 131.8 (C-6), 132.7 (C-3'), 140.7 (C-1'), 148.7 (C-8a), 153.3 (NH*C*OOCH(CH$_3$)$_2$), 180.5 (CHNH*C*SNHAr). Anal. ($C_{22}H_{26}ClN_3O_3S$) theoretical: C, 58.98; H, 5.85; N, 9.38; S, 7.16. Found: C, 58.97; H, 5.78; N, 9.52; S, 6.81.

4.1.39. *R/S*-*N*-4-Chlorophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-

isopropoxycarbonylamino-2*H*-1-benzopyran-4-yl)thiourea (**15j**): m.p. : 184-186°C; ^{1}H NMR (DMSO-d_6) δ 1.23 (dd, J=6.3 Hz/2.3 Hz, 6H, CH(CH_3)$_2$), 1.24 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.74 (t, J=12.1 Hz, 1H, 3-*H*), 2.15 (dd, J=13 Hz/6.2 Hz, 1H, 3-*H*), 4.85 (hept, J=6.2 Hz, 1H, C*H*(CH$_3$)$_2$), 5.75 (dd, J=15.9 Hz/8.8 Hz, 1H, 4-*H*), 6,67 (d, J=8.8 Hz, 1H, 8-*H*), 7.19 (d, J=7 Hz, 1H, 7-*H*), 7.36 (d, J=8.8 Hz , 2H, 2'-*H*/6'-*H*), 7.42 (s, 1H, 5-*H*), 7.52 (d, J=8.8 Hz, 2H, 3'-*H*/5'-*H*), 8.15 (d, J=8.2 Hz, 1H, CHN*H*CSNHAr), 9.35 (s, 1H, N*H*COOCH(CH$_3$)$_2$) , 9.68 (s, 1H, CHNHCSN*H*Ar); ^{13}C NMR (DMSO-d_6) δ 22.0 (CH(*C*H$_3$)$_2$), 24.2 (CH$_3$), 29.2 (CH$_3$), 38.1 (C-3), 47.3 (C-4), 67.1 (*C*H(CH$_3$)$_2$), 75.0 (C-2), 116.8 (C-8), 117.4 (C-5), 119.5 (C-7), 122.0 (C-4a), 125.0 (C-3'/C-5'), 128.0 (C-4'), 128.4 (C-2'/C-6'), 131.8 (C-6), 138.1 (C-1'), 148.7 (C-8a), 153.3 (NH*C*OOCH(CH$_3$)$_2$), 180.5 (CHNH*C*SNHAr). Anal. ($C_{22}H_{26}ClN_3O_3S$) theoretical: C, 58.98; H, 5.85; N, 9.38; S, 7.16. Found: C, 58.83; H, 5.79; N, 9.58; S, 6.85.

4.1.40. *R/S*-*N*-3-Cyanophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-isopropoxycarbonylamino-2*H*-1-benzopyran-4-yl)thiourea (**15k**): m.p.: 170-172°C; ^{1}H NMR (DMSO-d_6) δ 1.23 (d, J=6.2 Hz, 6H, NHCOOCH(CH_3)$_2$), 1.25 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.77 (t, J=12 Hz, 1H, 3-*H*), 2.16 (dd, J=13 Hz/6 Hz, 3-*H*), 4.86 (dt, J=12.3 Hz/6 Hz, 1H, NHCOOC*H*(CH$_3$)$_2$), 5.77 (bs, 1H, 4-*H*), 6.67 (d, J=8.7 Hz, 1H, 8-*H*), 7.19 (d, J=8.2 Hz, 1H, 7-*H*), 7.47 (s, 1H, 5-*H*), 7.50-7.56 (m, 2H, 4'-*H*/5'-*H*), 7.75 (d, J=7.7 Hz, 1H, 6'-*H*), 8.06 (s, 1H, 2'-*H*), 8,39 (d, J=6.6 Hz, 1H, CHN*H*CSNHAr), 9.32 (s, 1H, N*H*COOCH(CH$_3$)$_2$), 9.87 (s, 1H, CHNHCSN*H*Ar); ^{13}C NMR (DMSO-d_6) δ □□□□□□NHCOOCH(*C*H$_3$□□□, 24.7 (CH$_3$), 29.7 (CH$_3$), 38.6 (C-3), 47.8 (C-4), 67.6 (NHCOO*C*H(CH$_3$)$_2$), 75.5 (C-2), 111.7 (C-3'), 117.3 (C-8), 117.8 (C-5), 119.1 (CN), 120.1 (C-7), 122.3 (C-4a), 126.6 (C-2'), 128.0 (C-4'), 128.4 (C-6'), 130.4 (C-5'), 132.4 (C-6), 140.7 (C-1'), 149.1 (C-8a), 153.8 (NH*C*OOCH(CH$_3$)$_2$), 181.2 (CHNH*C*SNHAr). Anal. ($C_{23}H_{26}N_4O_3S$) theoretical: C, 62.99; H, 5.98; N, 12.78; S, 7.31. Found: C, 62.73; H, 5.98; N, 12.87; S, 7.38.

4.1.41. *R/S*-*N*-4-Cyanophenyl-*N'*-(3,4-dihydro-2,2-dimethyl-6-isopropoxycarbonylamino-2*H*-1-benzopyran-4-yl)thiourea (**15l**): m.p. : 200-202°C; ^{1}H NMR (DMSO-d_6) δ 1.22 (dd, J=6.2 Hz/2.5 Hz, 6H, CH(CH_3)$_2$), 1,25 (s, 3H, CH_3), 1,37 (s, 3H, CH_3), 1,75 (m, 1H, 3-*H*), 2,20 (dd, J=13.0 Hz/6.3 Hz, 1H, 3-*H*), 4.85 (hept, J=6.3 Hz, 1H, C*H*(CH$_3$)$_2$), 5.74 (dd, J=16.9 Hz/8.1 Hz, 1H, 4-*H*), 6,69 (d, J=8.8 Hz, 1H, 8-*H*), 7,20 (d, J=7.5 Hz, 1H, 7-*H*), 7,43 (s, 1H, 5-*H*), 7,76 (d, J=8.8 Hz, 2H, 3'-*H* et 5'-*H*), 7,80 (d, J=8.8 Hz, 2H, 2'-*H* et 6'-*H*), 8,49 (d, J=8.2

Hz, 1H, CHN*H*CSNHAr), 9,35 (s, 1H, N*H*COOCH(CH_3)$_2$), 10,00 (s, 1H, CHNHCSN*H*Ar); ^{13}C NMR (DMSO-d_6) δ 22.0 (CH(*C*H_3)$_2$), 24.2 (CH_3), 29.1 (CH_3), 38.0 (C-3), 47.2 (C-4), 67.1 (*C*H(CH_3)$_2$), 74.9 (C-2), 104.9 (C-4'), 116.9 (C-8), 117.5 (C-5), 119.1 (CN), 119.8 (C-7), 121.6 (C-4a), 121.7 (C-3'/C-5'), 131.8 (C-6), 132.8 (C-2'/C-6'), 144.0 (C-1'), 148.7 (C-8a), 153.3 (NH*C*OOCH(CH_3)$_2$), 180.2 (CHNH*C*SNHAr). Anal. ($C_{23}H_{26}N_4O_3S$) theoretical: C, 62.99; H, 5.98; N, 12.78; S, 7.31. Found: C, 63.07; H, 5.93; N, 12.84; S, 6.97.

4.2. Biological assays

(±)-Cromakalim (Tocris, UK), diazoxide (Sigma Chemical Co, USA) and verapamil (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 laboratory animal care was approved by the local ethic's committee of the Université Libre de Bruxelles.

4.2.1. Measurement of insulin secretion from incubated rat pancreatic islets

The method used to measure insulin release from incubated rat pancreatic islets was previously described [27,34,40].

4.2.2. Measurement of myorelaxant activity on rat aorta rings

The method used to measure the myorelaxant effect of drugs on KCl-precontracted rat aortic rings was previously described [34,38,40].

4.2.3. Measurements of ^{86}Rb, ^{45}Ca outflow and insulin release from perifused rat pancreatic islets

The experimental conditions previously reported for measuring ^{86}Rb, ^{45}Ca outflow and insulin release from perifused rat pancreatic islets were applied to compound **14o** [27,40].

4.2.4. Measurement of cytosolic Ca^{2+} concentration from isolated rat pancreatic islet cells

The experimental conditions previously reported for measuring the cytosolic Ca^{2+} concentration (fura-2 fluorescence) from single rat pancreatic islet cells were applied to compound **14o** [27,40].

Supplementary data

Examples of ^{1}H and ^{13}C NMR spectra of target compounds from the two series (ureas - thioureas) and bearing the same substituents (**14g**, **14h** versus **15g**, **15h**; **14o**, **14p** versus **18**, **9**).

Author information

Corresponding Author

*Phone: + 32 4 366 43 65. Fax : + 32 4 366 43 62. E-mail : b.pirotte@ulg.ac.be

Acknowledgements

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

Abbreviations used

ADP: adenosine diphosphate; ATP: adenosine triphosphate; DCVC: dry column vacuum chromatography; DMSO: dimethyl sulfoxide; EC_{50}: half maximal effective concentration; IC_{50}: half maximal inhibitory concentration; K_{ATP} channel: ATP-sensitive potassium channel; Kir: inwardly-rectifying potassium channel; NMR: nuclear magnetic resonance; PCO: potassium channel opener; SUR: sulfonylurea receptor; TLC: thin layer chromatography; TMS: tetramethylsilane.

References

[1] F.M. Ashcroft, Adenosine 5'-triphosphate-sensitive potassium channels, Annu. Rev. Neurosci. 11 (1988) 97-118.

[2] M.A. Burke, R.K. Mutharasan, H. Ardehali, The sulfonylurea receptor, an atypical ATP-binding cassette protein, and its regulation of the K_{ATP} channel, Circ. Res. 102 (2008) 164-176.

[3] C.G. Nichols, K_{ATP} channels as molecular sensors of cellular metabolism. Nature 440 (2006) 470-476.

[4] D.L. Cook, C.N. Hales, Intracellular ATP directly blocks K^+ channels in pancreatic B-cells, Nature 311 (1984) 271-273.

[5] N. Inagaki, S. Seino, ATP-sensitive potassium channels: structures, functions, and pathophysiology, Jpn. J. Physiol. 48 (1998) 397-412.

[6] A. Tinker, Q. Aziz, A. Thomas, The role of ATP-sensitive potassium channels in cellular function and protection in the cardiovascular system, Br. J. Pharmacol. 171 (2014) 12-23.

[7] S. Seino, ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies, Annu. Rev. Physiol. 61 (1999) 337-362.

[8] D. Tricarico, A. Mele, A.L. Lundquist, R.R. Desai, A.L. George, D.C. Camerino, Hybrid assemblies of ATP-sensitive K^+ channels determine their muscle-type-dependent biophysical and pharmacological properties, Proc. Natl. Acad. Sci. USA 103 (2006) 1118-1123.

[9] N.-Q. Shi, B. Ye, J.C. Makielski, Function and distribution of the SUR isoforms and splice variants. J. Mol. Cell. Cardiol. 39 (2005) 51-60.

[10] S. Seino, H. Takahashi, T. Takahashi, T. Shibasaki, Treating diabetes today: a matter of selectivity of sulphonylureas, Diabetes Obes. Metab. 14 (2012) 9-13.

[11] A. Jahangir, A. Terzic, K_{ATP} channel therapeutics at the bedside, J. Mol. Cell. Cardiol. 39 (2005) 99-112.

[12] P. De Tullio, P. Lebrun, X. Florence, P. Francotte, B. Pirotte, Selective pancreatic ATP-sensitive potassium channel openers for the treatment of glucose homeostasis disorders, Drug Future 31 (2006) 991-1001.

[13] S. Seino, T. Miki, Physiological and pathophysiological roles of ATP-sensitive K^+ channels, Prog. Biophys. Mol. Biol. 81 (2003) 133-176.

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

[15] F.M. Gribble, S.J. Tucker, F.M. Ashcroft, The essential role of the walker a motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxide, EMBO J. 16 (1997) 1145-1152.

[16] K. Masuzawa, M. Asano, T. Matsuda, Y. Imaizumi, M. Watanabe, Comparison of effects of cromakalim and pinacidil on mechanical activity and ^{86}Rb efflux in dog coronary arteries, J. Pharmacol. Exp. Ther. 253 (1990) 586-593.

[17] A. Tabarin, B. Goichot, Treatment: symptomatic treatment of hypoglycaemia, Ann. Endocrinol. (Paris) 74 (2013) 196-199.

[18] T. Yorifuji, Congenital hyperinsulinism: current status and future perspectives, Ann. Pediatr. Endocrinol. Metab. 19 (2014) 57-68.

[19] M. Dabrowski, F.M. Ashcroft, R. Ashfield, P. Lebrun, B. Pirotte, J. Egebjerg, J. Bondo Hansen, P. Wahl, The novel diazoxide analog 3-isopropylamino-7-methoxy-4*H*-1,2,4-benzothiadiazine 1,1-dioxide is a selective Kir6.2/SUR1 channel opener, Diabetes 51 (2002) 1896-1906.

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

[21] R.D. Carr, C.L. Brand, T.B. Bodvarsdottir, J.B. Hansen, J. Sturis, NN414, a SUR1/Kir6.2-selective potassium channel opener, reduces blood glucose and improves glucose tolerance in the VDF zucker rat, Diabetes 52 (2003) 2513-2518.

[22] R. Raphemot, D.R. Swale, P.K. Dadi, D.A. Jacobson, P. Cooper, A.P. Wojtovich, S. Banerjee, C.G. Nichols, J.S. Denton, Direct activation of β-cell K_{ATP} channels with a novel xanthine derivative, Mol. Pharmacol. 85 (2014) 858-865.

[23] S. Sebille, D. Gall, P. de Tullio, X. Florence, P. Lebrun, B. Pirotte, Design, synthesis and pharmacological evaluation of *R/S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminocarbonylamino)-2*H*-1-benzopyrans: towards tissue-selective pancreatic B-cells

K_{ATP} channel openers structurally related to (±)-cromakalim, J. Med. Chem. 49 (2006) 4690-4697.

[24] S. Sebille, P. de Tullio, X. Florence, B. Becker, M.-H. Antoine, C. Michaux, J. Wouters, B. Pirotte, P. Lebrun, New R/S-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyrans structurally related to (+/-)-cromakalim as tissue-selective pancreatic beta-cell K_{ATP} channel openers, Bioorg. Med. Chem. 16 (2008) 5704-5719.

[25] X. Florence, S. Sebille, P. de Tullio, P. Lebrun, B. Pirotte, New R/S-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans as K_{ATP} channel openers: modulation of the 4-Position, Bioorg. Med. Chem. 17 (2009) 7723-7731.

[26] X. Florence, S. Dilly, P. de Tullio, B. Pirotte, P. Lebrun, Modulation of the 6-position of benzopyran derivatives and inhibitory effects on the insulin releasing process, Bioorg. Med. Chem. 19 (2011) 3919-3928.

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

[28] A. Ramnathan, K. Sivakumar, K. Subramanian, N. Janarthanan, K. Ramadas, H.-K. Fun, Symmetrically substituted thiourea derivatives, Acta Cryst. C51 (1995) 2446-2450.

[29] Rajnikant, Dinesh, M.B. Deshmukh, Kamni, Synthesis, X-ray structure and N-H…O interactions in 1,3-diphenyl-urea, Bull. Mater. Sci. 29 (2006) 239-242.

[30] L. Checinska, W. Morgenroth, C. Paulmann, D. Jayatilaka, B. Dittrich, A comparison of electron density from Hirshfeld-atom refinement, X-ray wavefunction refinement and multipole refinement on three urea derivatives, CrysEngComm 15 (2013) 2084-2090.

[31] A. Okuniewski, J. Chojnacki, B. Becker, *N,N'*-Diphenylthiourea acetone monosolvate, Acta Cryst. E67 (2011) o55.

[32] U. Quast, N.S. Cook, In vitro and in vivo comparison of two K^+ channel openers, diazoxide and cromakalim, and their inhibition by glibenclamide, J. Pharmacol. Exp. Ther. 250 (1989) 261-271.

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

[34] P. Lebrun, B. Becker, N. Morel, P. Ghisdal, M.-H. Antoine, P. de Tullio, B. Pirotte, K_{ATP} channel openers: tissue selectivity of original 3-alkylaminopyrido- and 3-alkylaminobenzothiadiazine 1,1-dioxides, Biochem. Pharmacol. 75 (2008) 468-475.

[35] B. Pirotte, P. de Tullio, S. Boverie, C. Michaux, P. Lebrun, Impact of the nature of the substituent at the 3-position of 4*H*-1,2,4-benzothiadiazine 1,1-dioxides on their opening activity toward ATP-sensitive potassium channels, J. Med. Chem. 54 (2011) 3188-3199.

[36] M.-H. Antoine, M. Hermann, A. Herchuelz, P. Lebrun, Ionic and secretory response of pancreatic islet cells to minoxidil sulfate, J. Pharmacol. Exp. Ther. 258 (1991) 286-291.

[37] T. Tagmose, S.C. Schou, J.P. Mogensen, F.E. Nielsen, P.O.G. Arkhammar, P. Wahl, B.S. Hansen, A. Worsaae, H.C.M. Boonen, M.-H. Antoine, P. Lebrun, J. Bondo Hansen, Arylcyanoguanidines as activators of Kir6.2/SUR1 K_{ATP} channels and inhibitors of insulin release, J. Med. Chem. 47 (2004) 3202-3211.

[38] B. Becker, M.-H. Antoine, Q.-A. Nguyen, B. Rigo, K.E. Cosgrove, P.D. Barnes, M.J. Dunne, B. Pirotte, P. Lebrun, Synthesis and characterization of a quinolinonic compound activating ATP-sensitive K^+ channels in endocrine and smooth muscle tissues, Br. J. Pharmacol. 134 (2001) 375-385.

[39] X. Florence, V. Desvaux, E. Goffin, P. de Tullio, B. Pirotte, P. Lebrun, Influence of the alkylsulfonylamino substituent located at the 6-position of 2,2-dimethylchromans structurally related to cromakalim: from potassium channel openers to calcium entry blockers? Eur. J. Med. Chem. 80 (2014) 36-46.

[40] B. Pirotte, P. de Tullio, S. Boverie, C. Michaux, P. Lebrun, Impact of the nature of the substituent at the 3-position of 4*H*-1,2,4-benzothiadiazine 1,1-dioxides on their opening activity toward ATP-sensitive potassium channels, J. Med. Chem. 54 (2011) 3188-3199.

Legends for figures

Figure 1. Chemical structure of reference K_{ATP} channel openers and previously described 2,2-dimethylchromans acting on insulin secreting cells (**1**: diazoxide; **2**: (±)-cromakalim; **3**: BPDZ 216; **4**: BPDZ 44; **5**: NN414; **6**: VU0071063; **7-9**: 4-phenylureido/thioureido-substituted 2,2-dimethylchromans reported to be potent inhibitors of insulin release) [19-26].

Figure 2. Multiple conformations adopted by *N,N'*-disubstituted ureas (X = O) and *N,N'*-disubstituted thioureas (X = S) in solution and/or in the solid state (conformations A, B, C and D). For each conformation, ureas and thioureas are expected to exist in different tautomeric forms due to the ol/one or thiol/thione equilibrium (i.e. conformations A' and A" from conformation A). For the ureas and thioureas depicted in this work, R corresponds to the chroman moiety and R' to the monosubstituted phenyl ring.

Figure 3. Effect of increasing concentrations of **14o** (BPDZ 711) on insulin release from rat pancreatic islets incubated in the presence of an insulinotropic glucose concentration (16.7 mM). Insulin release (mean values ± SEM) was expressed in percentage of the value recorded in control experiments (100 %; no added drug and presence of 16.7 mM glucose). Figures in parentheses refer to number of samples.

Figure 4. Effect of **14o** (BPDZ 711, 10 µM) on ^{86}Rb outflow from rat pancreatic islets perifused throughout in the absence (●) or presence (○) of the K_{ATP} channel blocker glibenclamide (10 µM). Basal media contained 5.6 mM glucose and extracellular Ca^{2+}. Mean values (± SEM) refer to 6 individual experiments.

Figure 5. Effect of **14o** (BPDZ 711, 10 µM) on ^{45}Ca outflow (upper panel) and insulin release (lower panel) from rat pancreatic islets perifused throughout in the presence of an insulinotropic glucose concentration (16.7 mM). Basal media contained extracellular Ca^{2+}(●) or were deprived of Ca^{2+} and enriched with EGTA (0.5 mM; ○). Mean values (± SEM) refer to 5-6 individual experiments.

Figure 6. Effect of a rise in the extracellular K^+ concentration from 5 to 50 mM on ^{45}Ca outflow from rat pancreatic islets perifused throughout in the absence (○) or presence (●) of **14o** (BPDZ 711, 10 µM). Basal media contained 2.8 mM glucose and extracellular Ca^{2+}. Mean values (± SEM) refer to 10 individual experiments.

Figure 7. Effects of **14o** (BPDZ 711) on the fura-2 fluorescence from single rat pancreatic islet cells. Upper panel: the extracellular glucose concentration was raised from 2.8 to 20 mM with subsequent addition of **14o** (BPDZ 711, 10 µM). Lower panel: same experiment performed with a physiological medium deprived of extracellular Ca^{2+} and enriched with EGTA (0.5 mM).

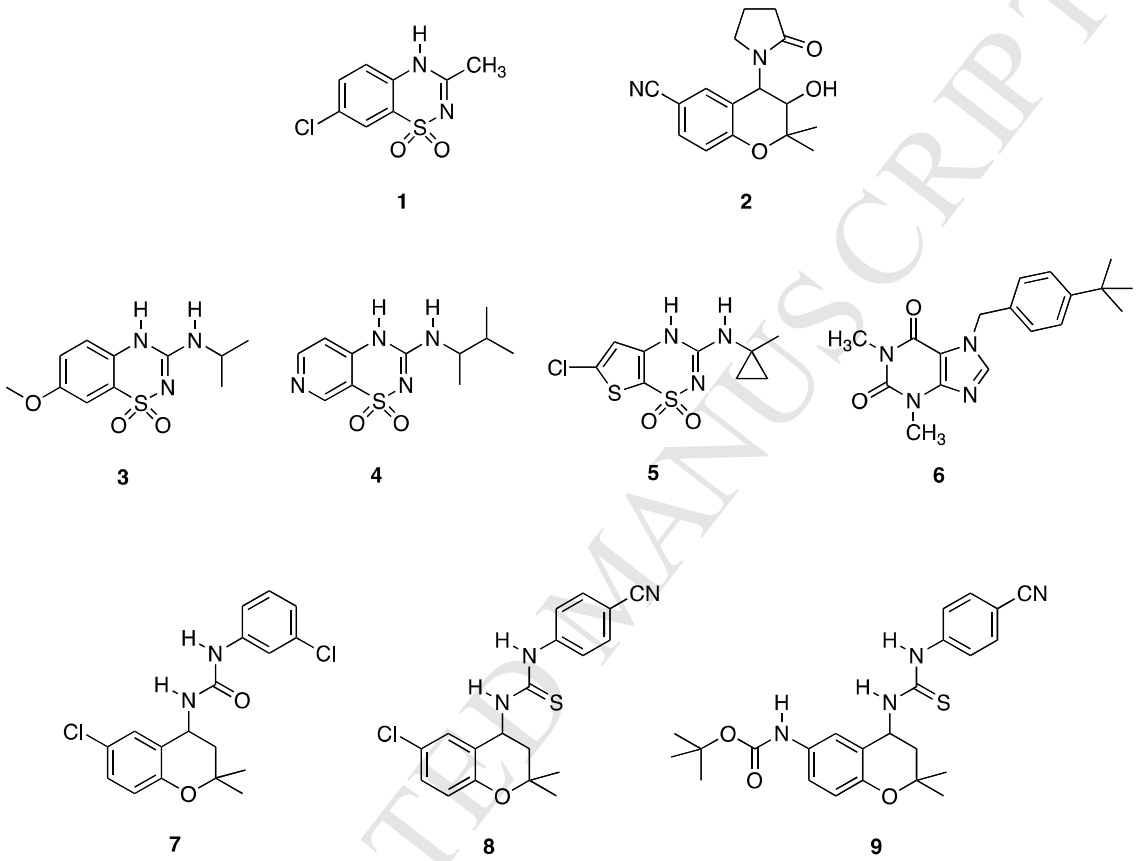


Figure 1

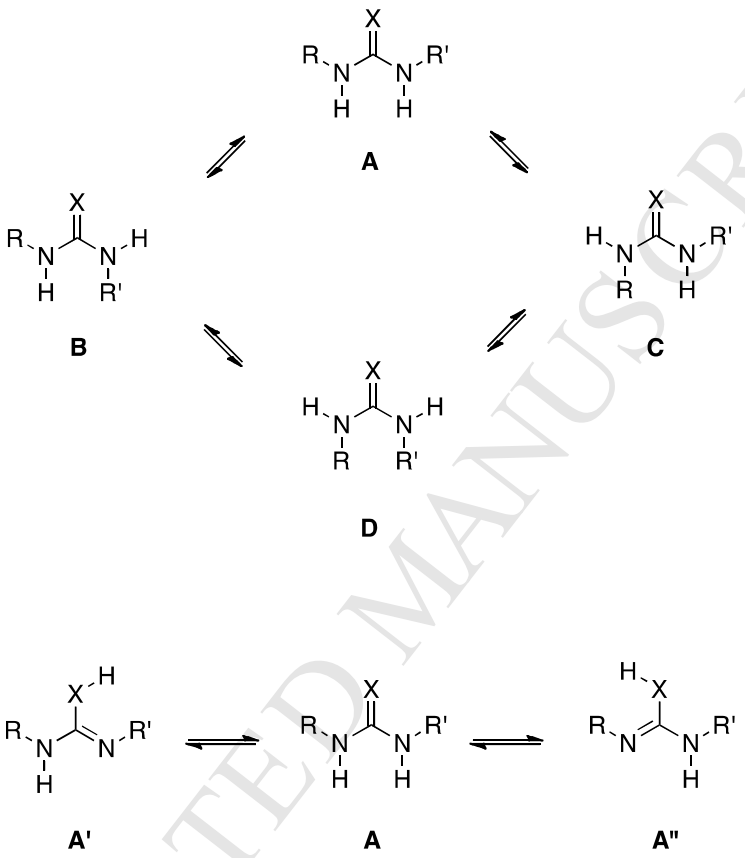


Figure 2

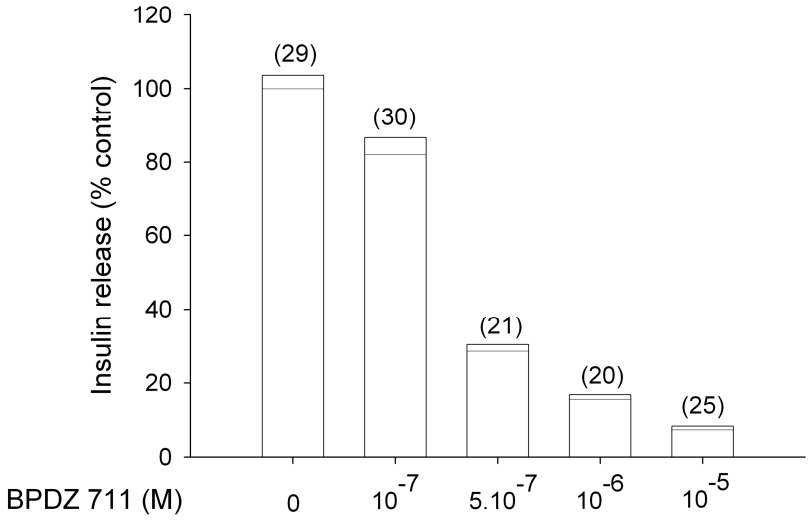


Figure 3

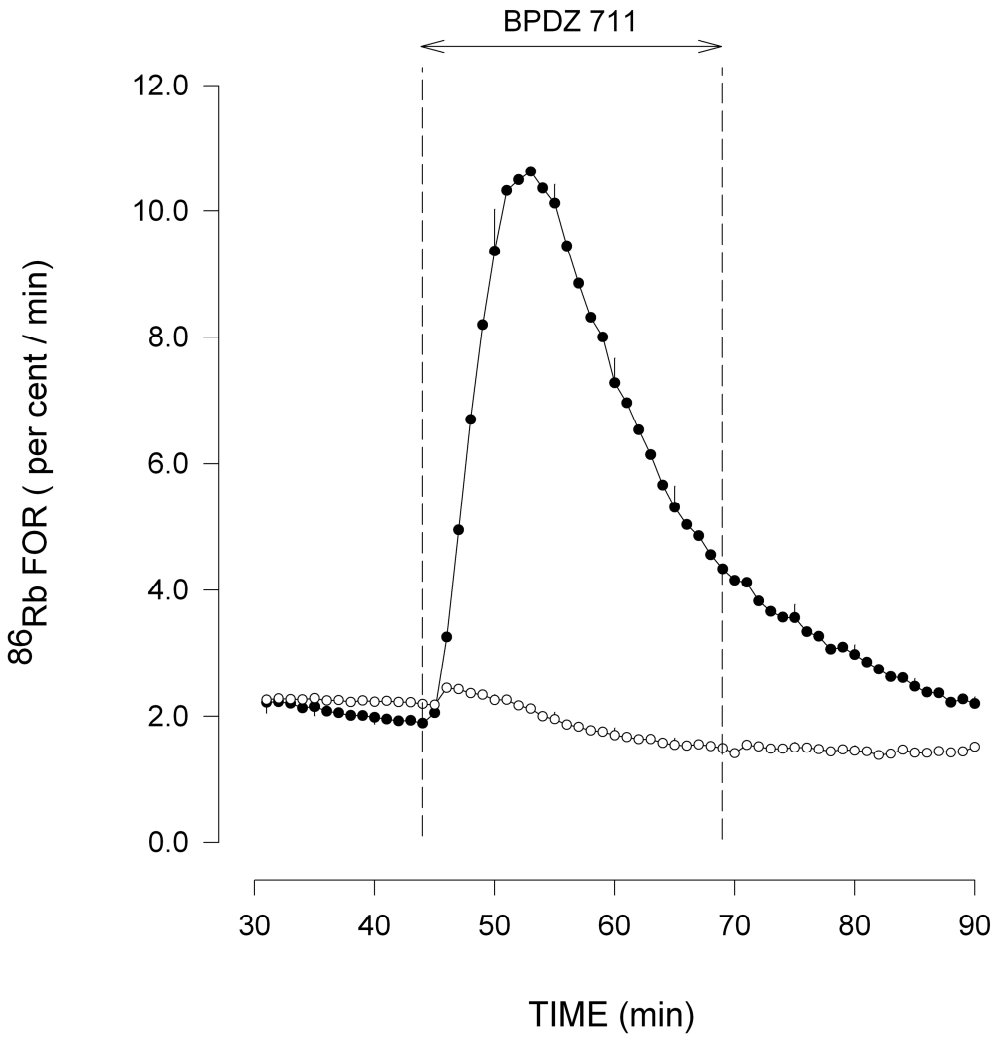

BPDZ 711
12.0
10.0
8.0
6.0
4.0
2.0
0.0
86Rb FOR (per cent / min)
30
40
50
60
70
80
90
TIME (min)

Figure 4

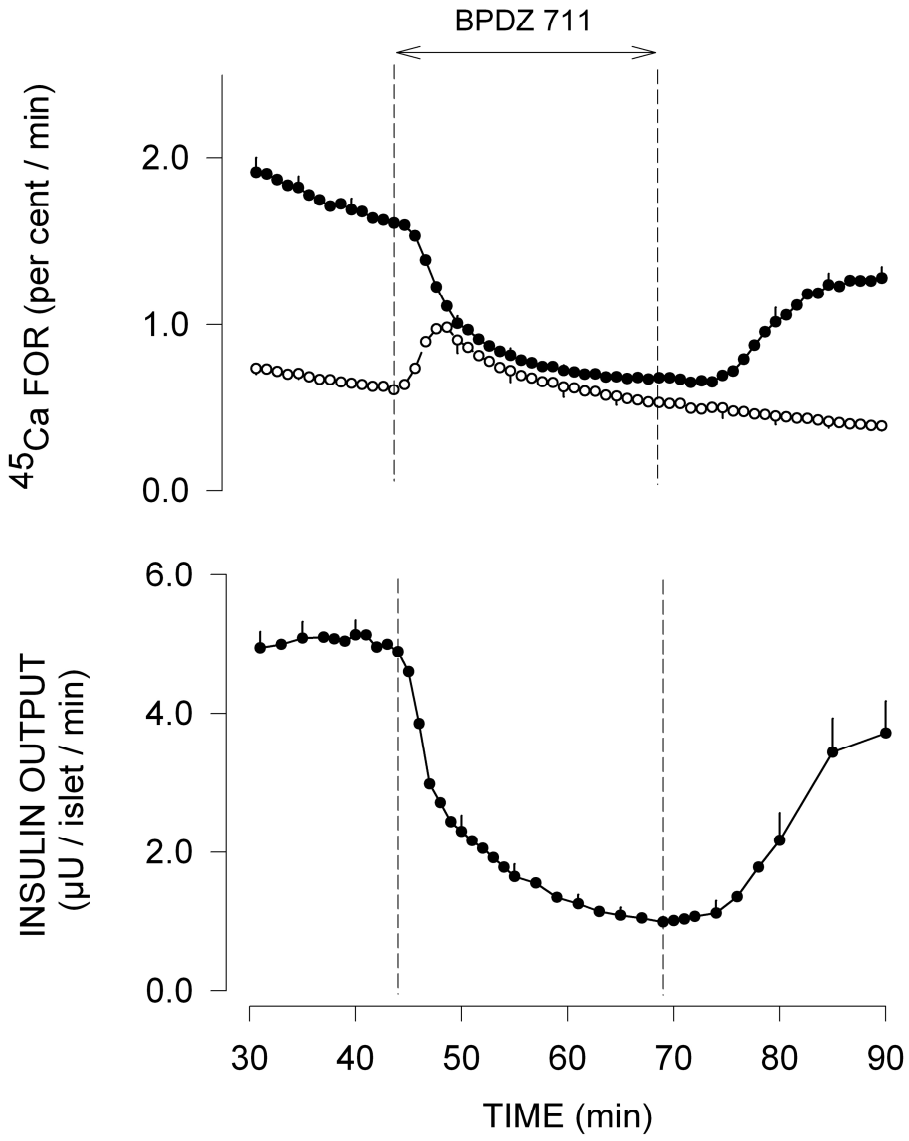

BPDZ 711
^{45}Ca FOR (per cent / min)
2.0
1.0
0.0
INSULIN OUTPUT
(µU / islet / min)
6.0
4.0
2.0
0.0
30
40
50
60
70
80
90
TIME (min)

Figure 5

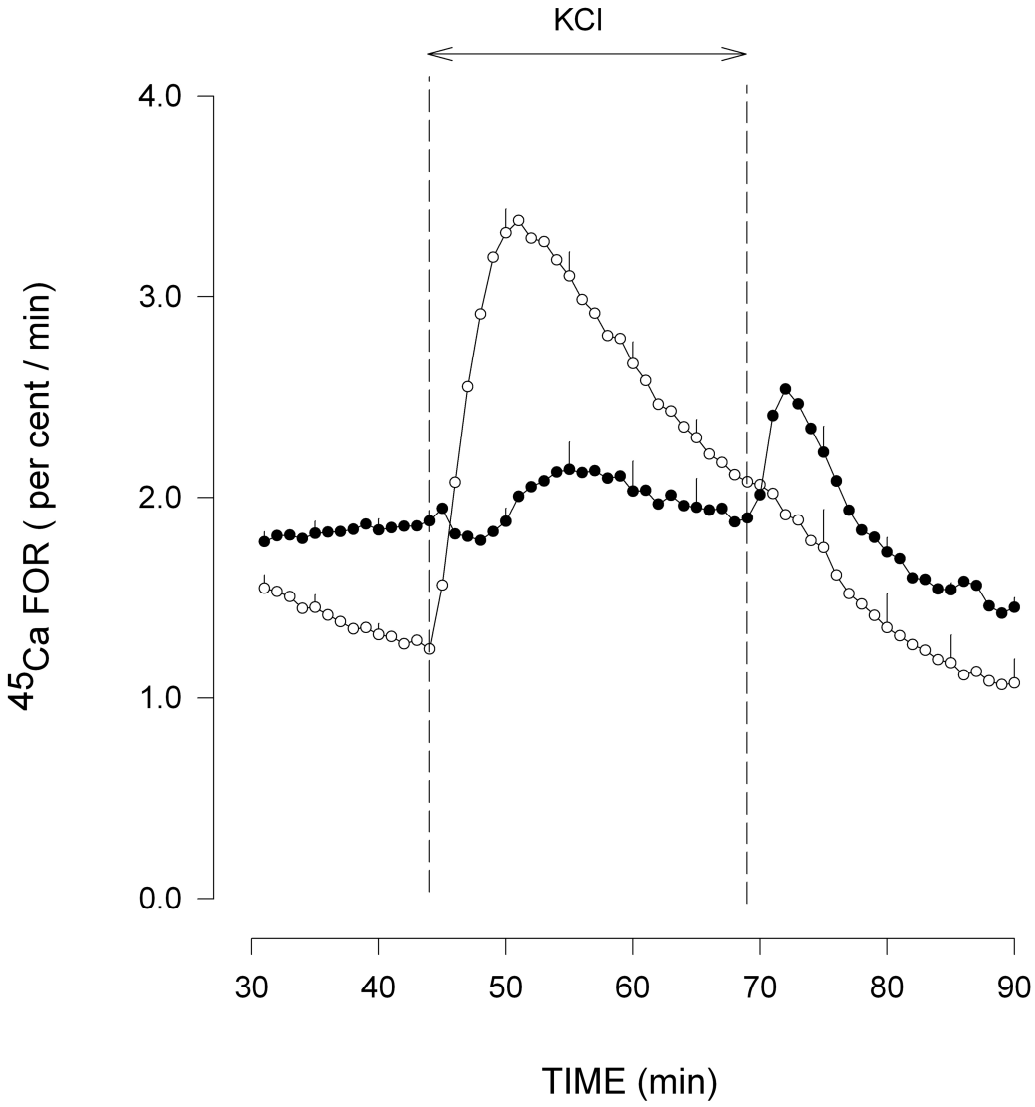

KCl
4.0
3.0
2.0
1.0
0.0
45Ca FOR (per cent / min)
30
40
50
60
70
80
90
TIME (min)

Figure 6

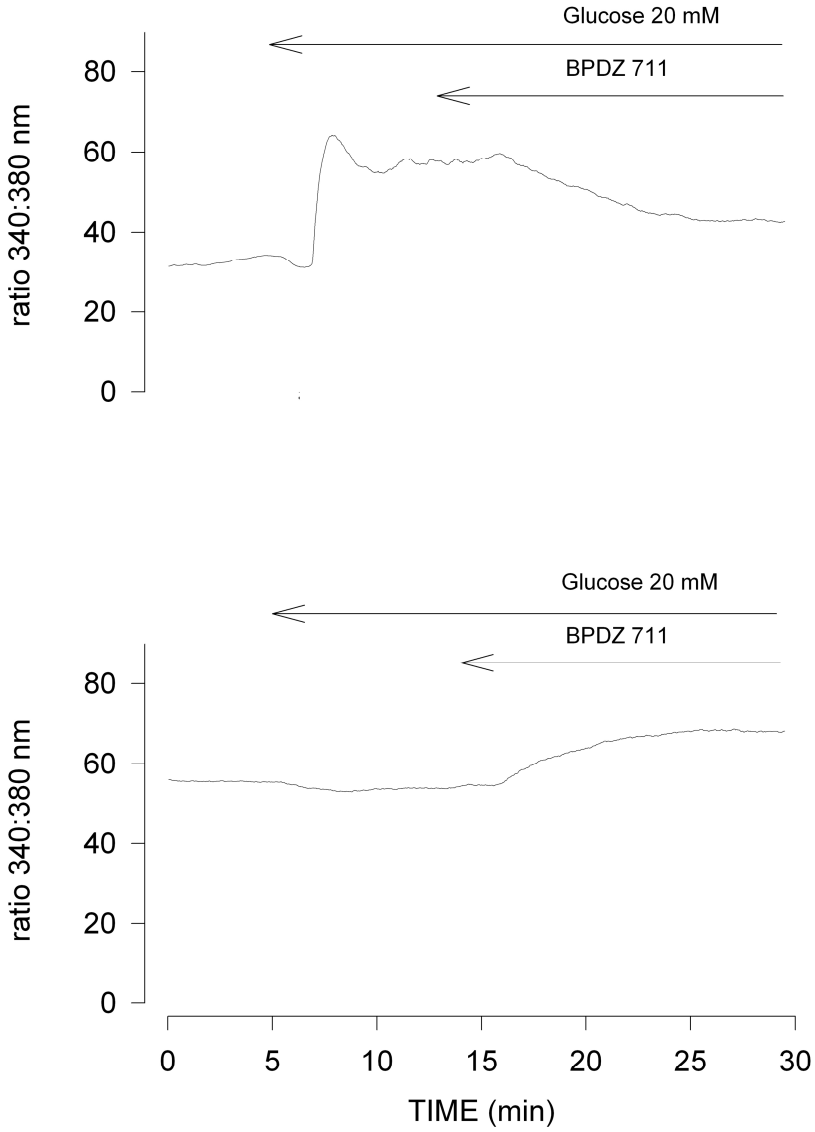
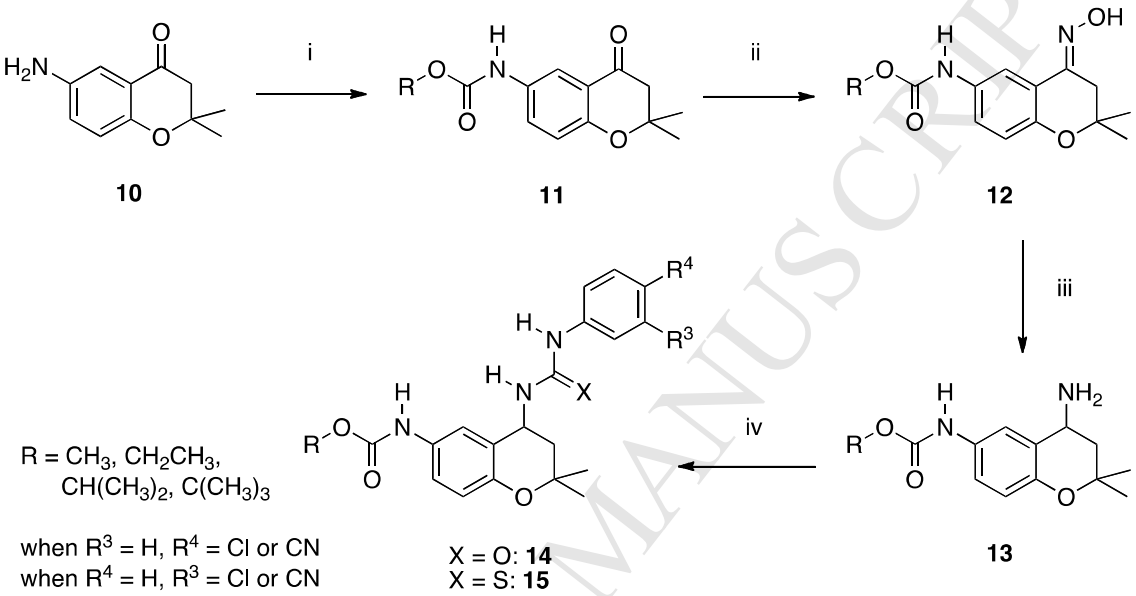

Glucose 20 mM
BPDZ 711
ratio 340:380 nm
0
20
40
60
80
Glucose 20 mM
BPDZ 711
ratio 340:380 nm
0
20
40
60
80
0
5
10
15
20
25
30
TIME (min)

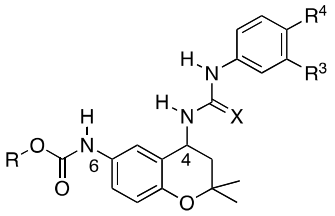
Figure 7



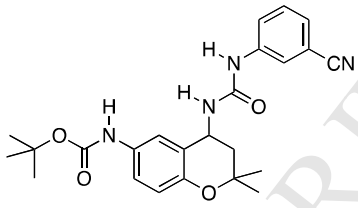
Reagents: i: ROCOCl, K_2CO_3, CH_3CN or Boc_2O, K_2CO_3, THF/H_2O; ii: $NH_2OH.HCl$, K_2CO_3, EtOH; iii: Raney-Nickel°, H_2 5 bars, CH_3OH; iv: R-N=C=O, CH_2Cl_2 for **14**; R-N=C=S, CH_2Cl_2 for **15**.

Scheme 1

Table 1. Effects of 4-phenylureido-substituted (14) and 4-phenylthioureido-substituted (15) 4-alkoxycarbonylamino-2,2-dimethylchromans on insulin secretion from rat pancreatic islets and on the contractile activity of K^+-depolarized rat aorta rings



14a-p, **15a-l**



14o (BPDZ 711)

Cpd	R	X	R^3	R^4	RIS[a] 10 µM	RIS[a] 1 µM	EC_{50}[b]
14a	CH_3	O	Cl	H	35.7 ± 1.7 (16)	92.0 ± 4.9 (23)	> 30.0 (4)
14b	CH_3	O	H	Cl	53.7 ± 2.2 (16)	92.7 ± 3.5 (23)	> 30.0 (3)
14c	CH_3	O	CN	H	9.4 ± 0.5 (12)	88.3 ± 3.8 (30)	16.9 ± 4.3 (5)
14d	CH_3	O	H	CN	36.9 ± 2.0 (23)	89.7 ± 4.9 (22)	> 30.0 (6)
15a	CH_3	S	Cl	H	26.1 ± 1.9 (15)	84.2 ± 4.7 (24)	15.4 ± 1.5 (3)
15b	CH_3	S	H	Cl	22.8 ± 1.6 (16)	84.9 ± 3.8 (31)	24.4 ± 7.0 (6)
15c	CH_3	S	CN	H	29.3 ± 1.4 (16)	85.1 ± 4.3 (32)	4.8 ± 0.1 (3)
15d	CH_3	S	H	CN	27.8 ± 1.8 (14)	93.8 ± 5.8 (23)	> 30.0 (4)
14e	CH_2CH_3	O	Cl	H	36.1 ± 2.3 (28)	82.8 ± 5.0 (21)	13.1 ± 3.2 (4)
14f	CH_2CH_3	O	H	Cl	49.9 ± 3.0 (30)	92.1 ± 4.4 (24)	21.2 ± 3.5 (4)
14g	CH_2CH_3	O	CN	H	5.9 ± 0.8 (23)	81.0 ± 4.0 (23)	14.2 ± 1.8 (5)
14h	CH_2CH_3	O	H	CN	17.4 ± 1.0 (14)	79.5 ± 3.5 (15)	> 30.0 (9)
15e	CH_2CH_3	S	Cl	H	21.5 ± 2.0 (29)	73.7 ± 3.1 (16)	22.7 ± 2.5 (4)
15f	CH_2CH_3	S	H	Cl	22.0 ± 2.0 (29)	78.4 ± 3.9 (16)	8.9 ± 1.7 (4)
15g	CH_2CH_3	S	CN	H	14.8 ± 1.0 (15)	77.6 ± 3.7 (24)	12.3 ± 2.0 (4)
15h	CH_2CH_3	S	H	CN	10.2 ± 0.9 (16)	80.2 ± 3.8 (24)	19.4 ± 4.4 (6)
14i	$CH(CH_3)_2$	O	Cl	H	23.7 ± 1.6 (21)	92.0 ± 4.4 (21)	12.6 ± 2.6 (5)
14j	$CH(CH_3)_2$	O	H	Cl	48.7 ± 2.6 (15)	92.3 ± 4.3 (24)	46.2 ± 9.2 (4)
14k	$CH(CH_3)_2$	O	CN	H	12.7 ± 1.2 (18)	50.5 ± 2.8 (45)	12.1 ± 3.6 (5)
14l	$CH(CH_3)_2$	O	H	CN	14.1 ± 1.0 (20)	80.7 ± 3.8 (32)	17.7 ± 1.5 (3)
15i	$CH(CH_3)_2$	S	Cl	H	19.3 ± 1.6 (20)	81.5 ± 4.0 (23)	11.0 ± 2.9 (6)
15j	$CH(CH_3)_2$	S	H	Cl	14.9 ± 1.0 (23)	81.2 ± 2.6 (23)	10.4 ± 2.1 (7)
15k	$CH(CH_3)_2$	S	CN	H	14.2 ± 1.0 (15)	79.3 ± 3.6 (23)	5.6 ± 0.9 (4)
15l	$CH(CH_3)_2$	S	H	CN	14.8 ± 1.2 (12)	86.0 ± 4.1 (24)	7.2 ± 0.9 (4)
14m	$C(CH_3)_3$	O	Cl	H	11.0 ± 0.8 (20)	86.4 ± 5.0 (22)	18.7 ± 2.8 (4)
14n	$C(CH_3)_3$	O	H	Cl	36.4 ± 2.7 (23)	86.1 ± 4.0 (24)	9.4 ± 2.5 (5)
14o	$C(CH_3)_3$	O	CN	H	7.4 ± 1.0 (25)	15.6 ± 1.2 (20)	10.5 ± 1.8 (4)
14p	$C(CH_3)_3$	O	H	CN	5.8 ± 0.7 (23)	53.4 ± 3.0 (22)	6.5 ± 0.6 (4)
16[c]	$C(CH_3)_3$	S	Cl	H	17.1 ± 1.5 (23)[c]	69.0 ± 2.5 (22)[c]	> 3.0 (4)[c]
17[c]	$C(CH_3)_3$	S	H	Cl	15.5 ± 1.4 (18)[c]	76.4 ± 3.3 (23)[c]	> 30.0 (5)[c]
18[c]	$C(CH_3)_3$	S	CN	H	13.3 ± 1.8 (19)[c]	72.2 ± 4.3 (24)[c]	8.5 ± 1.0 (7)[c]
9[c]	$C(CH_3)_3$	S	H	CN	12.9 ± 1.7 (19)[c]	51.6 ± 2.9 (21)[c]	> 3.0 (4)[c]
1	diazoxide	-	-	-	80.8 ± 3.7 (32)[c]	91.0 ± 4.7 (24)	23.8 ± 5.0 (6)[c]
2	(±)-cromakalim	-	-	-	94.4 ± 4.1 (32)[c]	95.3 ± 3.8 (31)[c]	0.13 ± 0.01 (7)[c]

[a] RIS: percentage of residual insulin secretion from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (n)). [b] EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (n)). [c] Published compounds and results (ref. 26 and 39).

Table 2. Myorelaxant effects of 14o (BPDZ 711), (±)-cromakalim and verapamil on 30 and 80 mM K^+-induced contractions of rat aorta rings incubated in the absence or presence of the K_{ATP} channel blocker glibenclamide

	EC_{50} (µM)[a]			
Compounds	**30 mM KCl**	**30 mM KCl + 1 µM Gliben.[b]**	**30 mM KCl + 10 µM Gliben.[b]**	**80 mM KCl**
14o	10.1 ± 1.5 (9) 10.9 ± 1.9 (7)	11.6 ± 2.5 (12)	9.4 ± 1.8 (11)	12.2 ± 1.4 (10)
(±)-Cromakalim[c]	0.22 ± 0.07 (6) 0.17 ± 0.01 (4)	2.5 ± 0.7 (9)	28.8 ± 6.0 (5)	137.7 ± 10.4 (10)
Verapamil[c]	0.031 ± 0.004 (4) 0.045 ± 0.004 (5)	0.038 ± 0.007 (6)	0.035 ± 0.003 (5)	0.052 ± 0.006 (7)

[a] EC_{50}: drug concentration provoking a 50% relaxation (mean ± SEM (n)); [b] Gliben.: glibenclamide; [c] published data (ref. 39).

4-Phenylureido/thioureido-substituted 2,2-dimethylchroman analogs of cromakalim bearing a bulky 'carbamate' moiety at the 6-position as potent inhibitors of glucose-sensitive insulin secretion

Bernard Pirotte, Xavier Florence, Eric Goffin, Marlen Borges Medeiros, Pascal de Tullio, Philippe Lebrun

Highlights

1) 2,2-Dimethylchromans bearing a carbamate group at the 6-position are described.
2) All compounds inhibited insulin secretion and induced a myorelaxant activity.
3) The most potent inhibitor (**14o**) displayed selectivity for the pancreatic tissue.
4) Compound **14o** behaved as a K_{ATP} channel opener and a Ca^{2+} entry blocker.
5) Moreover, **14o** promoted an intracellular calcium translocation.