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New *R*/*S*-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans as K_{ATP} channel openers: Modulation of the 4-position

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ABSTRACT

The present work aimed at exploring a series of diversely 4-arylthiourea-substituted *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-2*H*-1-benzopyrans structurally related to (±)-cromakalim. These new compounds were examined in vitro as putative potassium channel openers (PCOs) on rat pancreatic islets (inhibition of insulin release) as well as on rat aorta rings (relaxation of aorta ring) and their activity was compared to that of the reference K_{ATP} channel activators (±)-cromakalim, (±)-pinacidil, diazoxide and of previously reported cromakalim analogues. Structure–activity relationships indicated that the most pronounced inhibitory activity on the insulin secretory process was obtained with molecules bearing a strong *meta*- or *para*-electron-withdrawing group (CN or NO_2) on the phenyl ring of the arylthiourea moiety at the 4-position of the benzopyran nucleus (compounds **12**–**23**). Among those, *R*/*S*-6-chloro-4-(4-cyanophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**16**) was found to be the most potent benzopyran-type inhibitor of insulin release ever described. Most of these original benzopyran derivatives show increased selectivity for pancreatic versus vascular tissue. Radioisotopic investigations indicated that these new compounds activated pancreatic K_{ATP} channels.

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1. Introduction

ATP-sensitive potassium (K_{ATP}) channels belong to the wide class of potassium channels. The activity of K_{ATP} channels is mainly regulated by changes in the intracellular concentration of adenosine triphosphate, implying that these channels couple cell metabolism to membrane excitability.[1–3] Such channels are distributed in many tissues where they play a large variety of physiological roles. Indeed, K_{ATP} channels are involved in the insulin releasing process,[4–6] in cardioprotection,[7] in the control of the vascular tone,[8] in neuroprotection and in the control of neurotransmitter release.[9,10]

The K_{ATP} channel is an octameric complex of four inwardly rectifying subunits (Kir6.x) forming the channel pore, and four sulfonylurea receptor subunits (SURx) that regulate the channel gating.[11–14] According to their tissue localization, different isoforms of the K_{ATP} channel have been described. In pancreatic β (insulin-secreting)-cells, the K_{ATP} channels consist of an association of Kir6.2 and SUR1[15,16] whereas, in cardiac and skeletal muscle tissue, the channel is composed of Kir6.2 and SUR2A subunits. Kir6.1 and SUR2B assemble to form the K_{ATP} channel detected in vascular muscle cells whilst, in other smooth muscle types, the Kir6.2 and SUR2B subunits combine to shape the channel.[17]

Due to the variety of their physiological functions, targeting the K_{ATP} channels could be an attractive mean to develop new therapeutic agents. Such an objective can theoretically be reached by the synthesis of compounds exhibiting a marked specificity and a high selectivity for a single K_{ATP} channel subtype.

By promoting K_{ATP} channel activity, potassium channel openers (PCOs) reduce the membrane excitability and preserve metabolic expenditure. Thus, and according to their tissue selectivity, PCOs have been proposed to be used as antihypertensive agents,[18] cardioprotectants,[7,18] bronchodilators,[18,19] bladder relaxants[18,20] or hair growth promoters.[21] Moreover, by targeting the pancreatic K_{ATP} channels, PCOs are expected to become new therapeutic agents for the treatment (or management) of endocrine disorders such as type 1/type 2 diabetes, obesity and hyperinsulinism.[22–26]

(±)-Cromakalim (**1**), diazoxide (**2**) and (±)-pinacidil (**3**) are examples of chemically diverse PCOs (Fig. 1). (±)-Cromakalim (**1**), the prototype benzopyran PCO, has been found to exert a marked vasorelaxant activity[27] but is also known, in contrast to diazoxide (**2**), to be only slightly active as an inhibitor of insulin secretion.[28,29]

Recent works aiming at identifying new pancreatic β-cell selective PCOs allowed us to synthesize a series of *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyrans

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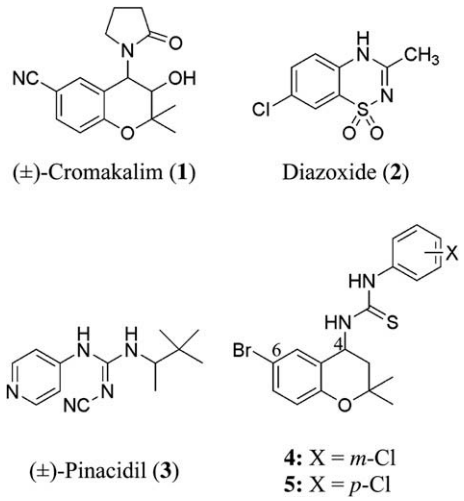


Figure 1. Chemical structure of (±)-cromakalim (**1**), diazoxide (**2**), (±)-pinacidil (**3**), compound **4** and **5**.

structurally related to cromakalim.[25] Several of these original compounds were found to be less effective as vasorelaxants and much more potent as inhibitors of insulin release than the reference molecules diazoxide and cromakalim.[25] Structure–activity relationships further indicated that the nature of the substituent at the 4-position of the benzopyran nucleus played a crucial role in the expression of an inhibitory effect on insulin release. Indeed, molecules bearing a C-4 phenylthiourea moiety substituted on the phenyl ring by a *meta*- or a *para*-electron-withdrawing group (a chlorine atom) (Fig 1, compounds **4** and **5**) were found to be very potent and selective for the pancreatic β-cells.[25]

According to structure–activity relationships deduced from our previous investigations, the present work aims at developing more potent and more insulin-secreting cell-selective dimethylchroman derivatives. We tried to achieve this goal by preparing and testing additional *meta*- or *para*-electron-withdrawing groups, by replacing the phenyl ring with a bioisosteric heteroaromatic ring or by replacing the thiourea function with a bioisosteric cyanoguanidine function.

The new compounds, including the *S*-methylisothiourea intermediates, were examined as putative potassium channel openers on rat pancreatic islets as well as on rat aorta rings. Radioisotopic investigations were performed to confirm the mechanism of action of these novel compounds.

2. Chemistry

The key intermediates (**6a–c**) giving access to the cromakalim analogues (**12–28**) were synthesized in six steps, according to the literature,[30,31] starting from the appropriate *p*-halophenols (Scheme 1).

R/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyrans (**12–23**) and *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(pyridylaminothiocarbonylamino)-2*H*-1-benzopyrans (**24–26**) were obtained from the reaction of amines **6a–c** with the appropriate isothiocyanate (R–N=C=S).

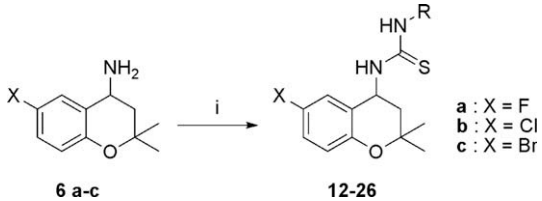
Compounds bearing a thienylaminothiocarbonylamino group (**27–28**) were obtained by a different synthetic pathway (Scheme 2).

Access to compounds **27** and **28** required the synthesis of thien-3-yl isothiocyanate as intermediate for reacting with **6b–c**. This intermediate was synthesized from 3-aminothiophene-2-carboxylic acid methyl ester (**7**) in four steps (Scheme 2).

The ester function of 3-aminothiophene-2-carboxylic acid methyl ester (**7**) was first hydrolyzed by sodium hydroxide and 3-aminothiophene-2-carboxylic acid (**8**) was then decarboxylated by oxalic acid to obtain 3-aminothiophene oxalate (**9**). Compound **9** was treated with an aqueous solution of ammonia to give 3-aminothiophene (**10**). Thien-3-yl isothiocyanate (**11**) was isolated after the reaction of 3-aminothiophene (**10**) with 1,1′-thiocarbonyldiimidazole. Finally, this intermediate was converted to the final *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(3-thienylaminothiocarbonylamino)-2*H*-1-benzopyrans **27** and **28** by reaction with the appropriate *R*/*S*-4-amino-3,4-dihydro-2,2-dimethyl-6-halo-2*H*-1-benzopyran (**6b–c**).

Compounds **29** and **30** were obtained by treatment of the *N*-(6-bromo-2,2-dimethyl-3,4-dihydro-2*H*-chromen-4-yl)-*N*′-phenylthioureas **14** and **5** with iodomethane (Scheme 3). Lastly, compounds **31** and **32** were synthesized by reaction between, respectively, compound **29** or **30** with cyanamide (Scheme 3).

All these derivatives (**12–32**) were crystallized from appropriate solvents and characterized by IR, ^{1}H NMR and elemental analyses to obtain the final materials with the chemical purity required prior to pharmacological evaluations.



Scheme 1. Synthesis of cromakalim analogues **12–26**. Reagents: (i) RNCS, CH_2Cl_2.

3. Results and discussion

The ability of the newly synthesized cromakalim analogues (Table 1, compounds **12–32**) to inhibit the insulin releasing process was evaluated on isolated rat pancreatic islets incubated in the presence of an insulinotropic glucose concentration (16.7 mM). Moreover, the vasorelaxant activity of the compounds was determined on 30 mM K^+-depolarized rat aorta (endothelium-free) rings.

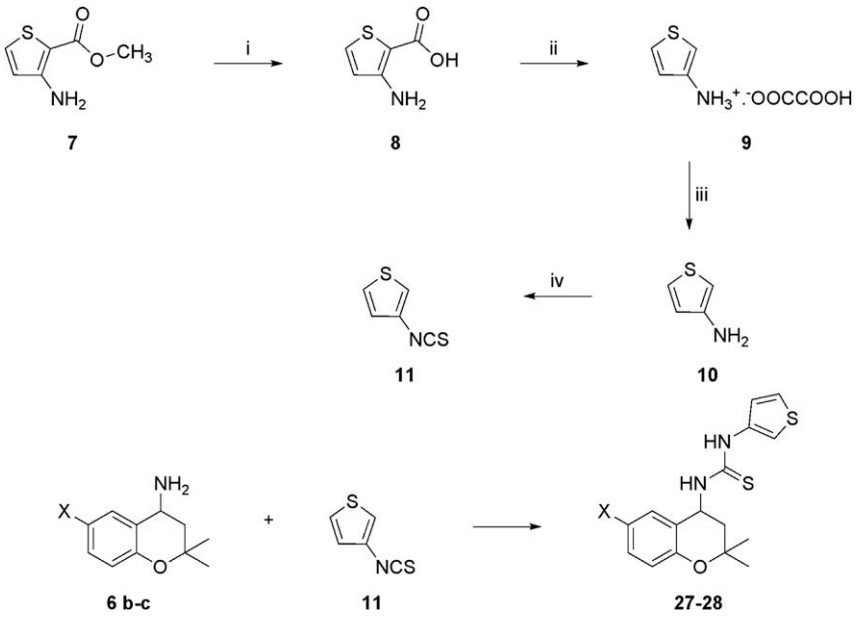
(±)-Cromakalim (**1**), diazoxide (**2**), (±)-pinacidil (**3**), compound **4** and **5** were used as reference PCOs (Fig. 1).

On the pancreatic model, (±)-cromakalim (**1**) and (±)-pinacidil (**3**) have previously been shown to be barely active at a 10 μM concentration. Diazoxide (**2**), however, as well as compounds **4** and **5**, clearly reduced the glucose-induced insulin release (Table 1). Compounds **4** and **5** were much more potent than diazoxide at inhibiting the secretory process.

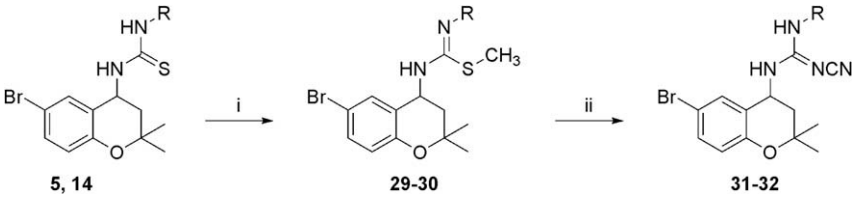
Our secretory data further revealed that the new benzopyran derivatives (**12–32**) were more potent on pancreatic β-cells than the reference compounds (±)-cromakalim, (±)-pinacidil and diazoxide (Table 1).

Several compounds (**12–23**), tested at a 10 μM concentration, provoked a 85–90% inhibition of the glucose-induced insulin release; a result which can be considered as a near to maximal effect considering the glucose-insensitive secretory process.

Our previous work[25] indicated that an electron-withdrawing group, such as a chlorine atom, in the *meta* or *para* position of the phenyl ring of *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyrans enhanced the inhibitory activity on the pancreatic tissue. As expected, compounds **12–23**, bearing a strong electron-withdrawing group in the *meta* or *para*-phenyl position (CN, NO_2), exhibited a marked inhibitory effect on the glucose-induced insulin secretion (Table 1). Compared with the reference molecules, compounds **12–23** were found to have an



Scheme 2. Synthetic pathway to *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(3-thienylaminothiocarbonylamino)-2*H*-1-benzopyrans **27** and **28**. Reagents: (i) NaOH 2 M; (ii) oxalic acid; (iii) NH_3, H_2O; (iv) 1,1′-thiocarbonyldiimidazole.



Scheme 3. Synthetic pathway to *R*/*S*-*N*-(6-bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-*N*′-(3- or 4-substituted phenyl)-*S*-methylisothioureas **29** and **30** and to *R*/*S*-*N*-(6-bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-*N*′-(3- or 4-substituted phenyl)-*N*″-cyanoguanidines (**31–32**). Reagents: (i) CH_3I; (ii) H_2NCN, 1,4-diazabicyclo[2.2.2]octane.

identical activity at 10 μM (p >0.05) than compound **5** but a stronger activity than compound **4** (p <0.05). At 1 μM, these compounds (**12**–**23**) displayed a similar activity than compounds **4** and **5**, except for **16** which was found to express a stronger inhibitory activity than references **4** and **5** (p <0.05) and for **17** which was more potent than the reference compound **5** (p <0.05). The nature of the halogen atom at the 6-position of the benzopyran nucleus did not markedly affect the activity of compounds **12–23** on the pancreatic tissue. At 1 μM, however, compounds **13** (6-Cl) and **14** (6-Br) were found to be somewhat more potent than compound **12** (6-F) (p <0.05), **16** (6-Cl) expressed a stronger inhibitory activity than **15** (6-F) (p <0.05), as observed for compounds **19** (6-Cl) and **20** (6-Br) versus **18** (6-F) (p <0.05) and **23** (6-Br) versus **21** (6-F) (p <0.05). The position of the electron withdrawing moiety on the C-4 phenyl ring (compounds **12**–**23**) had no marked influence on the pancreatic activity, except for compound **13** versus **16** (p <0.05 at 10 μM and 1 μM). According to these data, compound **16** can be considered as the most potent molecule of this series.

Compounds resulting from the introduction of a pyridinyl moiety on the thiourea chain (**24**–**26**) were also more potent than (±)-cromakalim, (±)-pinacidil and diazoxide at inhibiting insulin secretion but less potent than the reference compounds **4** and **5**. When comparing the pyridinyl compounds **24**–**26** with their corresponding unsubstituted phenyl analogues previously described,[25] the isosteric replacement of the phenyl ring with the pyridin-3-yl nucleus gave compounds expressing essentially the same activity on pancreatic β-cells. The pyridin-3-yl derivatives **24**–**26**, however, were found to be less potent on β-cells than their corresponding cyano- or nitro-substituted phenyl analogues **12**–**23** (Table 1). The nature of the halogen atom at the 6-position of the benzopyran nucleus affected the activity on the pancreatic tissue (p <0.05) (except: **24** vs **25** and **26**, p >0.05), the 6-fluoro compound **24** being the less potent and the 6-bromo compound **26** the most potent.

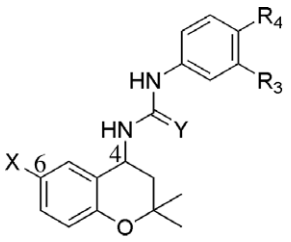
Compounds bearing a thienyl moiety on the thiourea chain (**27**–**28**) were found to exert, on the pancreatic β-cells, a more pronounced inhibitory activity than (±)-cromakalim, (±)-pinacidil and diazoxide (p <0.05), and were less effective than compounds **4** and **5** (p <0.05). The nature of the halogen atom at the 6-position of the benzopyran nucleus did not significantly affect the activity on the pancreatic tissue (p >0.05).

Finally, the 3-pyridinyl- (**24**–**26**) and the 3-thienyl-substituted (**27**–**28**) compounds appeared to exert close to similar inhibitory effects on the insulin releasing process and the pyridine or the thiophene ring can be considered as less appropriate than the benzene ring substituted with a *meta*- or *para*-electron-withdrawing group.

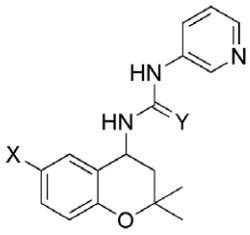
Molecules bearing a phenylisothiourea group (**29**–**30**) were also more potent than (±)-cromakalim, (±)-pinacidil and diazoxide (p <0.05), and less potent than the reference compounds **4** and **5** (p <0.05). As a result, and compared to the non *S*-methylated derivatives **14** and **5**, the alkylation of the sulfur atom of the thiourea function appeared to be responsible for a loss of activity on pancreatic β-cells (p <0.05).

Compounds **31** and **32**, bearing a cyanoguanidine group, were found to be slightly less potent at reducing the glucose-dependent

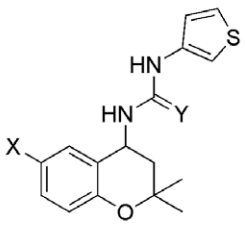
Table 1
Residual insulin secretion and myorelaxant activity of original 4-substituted *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-2*H*-1-benzopyrans compared to (±)-cromakalim, (±)-pinacidil, diazoxide, compound **4** and **5**



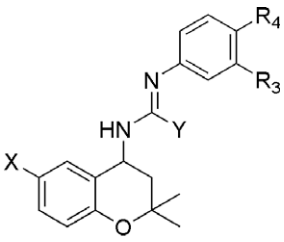
12-23 and **31-32**



24-26



27 and **28**



29 and **30**

Compounds	X	Y	R_3	R_4	% Residual insulin secretion[a]		Myorelaxant activity EC_{50}[b] (μM)
					10 μM	1 μM	
12	F	S	CN		16.1 ± 1.5 (21)	80.0 ± 4.4 (30)	2.6 ± 0.5 (4)
13	Cl	S	CN		15.8 ± 0.9 (18)	66.2 ± 2.5 (31)	0.60 ± 0.06 (4)
14	Br	S	CN		14.2 ± 1.3 (21)	65.5 ± 3.6 (30)	0.64 ± 0.05 (5)
15	F	S		CN	12.0 ± 0.8 (15)	64.7 ± 4.0 (19)	>3.0 (5)
16	Cl	S		CN	10.6 ± 0.7 (24)	47.8 ± 2.8 (29)	>10.0 (4)
17	Br	S		CN	11.7 ± 0.7 (24)	57.5 ± 3.0 (29)	>10.0 (6)
18	F	S	NO_2		11.5 ± 0.9 (15)	76.1 ± 4.3 (15)	>3.0 (4)
19	Cl	S	NO_2		14.4 ± 0.6 (22)	64.3 ± 3.4 (23)	>3.0 (4)
20	Br	S	NO_2		13.5 ± 0.7 (23)	64.3 ± 2.2 (24)	>10.0 (5)
21	F	S		NO_2	10.7 ± 1.1 (13)	74.3 ± 3.8 (34)	>3.0 (4)
22	Cl	S		NO_2	10.3 ± 0.7 (13)	67.3 ± 3.6 (19)	>10.0 (4)
23	Br	S		NO_2	13.5 ± 1.0 (28)	61.3 ± 3.4 (31)	>10.0 (4)
24	F	S			62.1 ± 3.6 (15)	—	6.6 ± 2.0 (4)
25	Cl	S			50.2 ± 2.7 (24)	—	2.8 ± 0.4 (6)
26	Br	S			44.3 ± 2.8 (23)	—	3.7 ± 1.0 (4)
27	Cl	S			51.9 ± 2.8 (32)	—	>3.0 (4)
28	Br	S			44.0 ± 2.9 (35)	—	>10.0 (4)
29	Br	S–CH_3	CN		37.7 ± 2.7 (16)	93.7 ± 5.5 (15)	>30.0 (4)
30	Br	S–CH_3		Cl	40.8 ± 2.9 (13)	89.4 ± 6.0 (15)	>10.0 (4)
31	Br	N–CN	CN		45.3 ± 1.7 (15)	90.5 ± 5.6 (13)	5.2 ± 0.6 (5)
32	Br	N–CN		Cl	42.7 ± 2.7 (14)	95.8 ± 5.3 (14)	11.4 ± 3.5 (3)
(±)-Cromakalim					94.4 ± 4.1 (32)[c]	95.3 ± 3.8 (31)[c]	0.13 ± 0.01 (7)[c]
(±)-Pinacidil					92.1 ± 3.9 (13)[d]	97.7 ± 6.7 (19)[d]	0.35 ± 0.02 (11)[d]
Diazoxide					73.9 ± 4.4 (16)[d]	87.5 ± 5.0 (15)[d]	22.4 ± 2.1 (11)[d]
4	Br	S	Cl		23.0 ± 2.4 (31)[e]	74.6 ± 4.0 (21)[e]	>10.0 (5)[e]
5	Br	S		Cl	12.2 ± 1.2 (20)[e]	75.7 ± 3.2 (24)[e]	>10.0 (4)[e]

[a] Percentage of residual insulin release 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 aorta rings (mean ± SEM (*n*)).
[c] Published results: Ref. 33.
[d] Published results: Ref. 32.
[e] Published results: Ref. 25.

secretory rate than the corresponding isothiourea compounds **29** and **30** (p >0.05) and less potent than the corresponding non *S*-alkylated compounds **14** and **5** (p <0.05). However, these compounds (**31**–**32**) remained more potent than (±)-cromakalim, (±)-pinacidil and diazoxide (p <0.05). Thus, the isosteric replacement of the thiourea function of **14** or **5** by a cyanoguanidine group appeared to worsen the activity on the pancreatic tissue.

On the vascular tissue, and as previously reported, diazoxide exhibited a moderate myorelaxant activity (EC_{50} = 22.4 ± 2.1 μM) while (±)-cromakalim and (±)-pinacidil exhibited marked vasorelaxant properties (EC_{50} = 0.13 ± 0.01 μM and 0.35 ± 0.02 μM, respectively).[25,32,33] Our two reference compounds **4** and **5** were less potent than cromakalim and pinacidil.

Compounds **12**, **13**, **14**, **24**, **25**, **26** and **31** were more potent on the vascular tissue than compounds **4**, **5** and diazoxide (p <0.05). The myorelaxant activity of **13** and **14** was in the same range, although less potent (p <0.05), than (±)-pinacidil. It should be noted that, for the cyano-substituted compounds **12**–**17**, the *meta*-position of the electron-withdrawing group was clearly more favorable than the *para*-position for myorelaxant activity (compare compounds **12**, **13**, **14** with **15**, **16**, **17**).

Unfortunately, and as previously observed with **4** and **5**, most compounds precipitated in the bathing medium before reaching their maximal activity, making it difficult to accurately determine their respective EC_{50} values. However, it can be concluded that these compounds were less potent on the vascular model than (±)-cromakalim and (±)-pinacidil.

Due to its profile, additional radioisotopic experiments were performed with **23** in order to determine if this compound was a pancreatic ATP-sensitive potassium channel opener.

Figure 2 illustrates the effects of compound **23** (10 μM) on ^{86}Rb fractional outflow rate (FOR) from prelabeled and perifused rat pancreatic islets exposed throughout to 5.6 mM glucose and extracellular Ca^{2+}. In the absence of glibenclamide in the perifusing medium (○), **23** provoked a rapid, marked and reversible increase in the rate of ^{86}Rb outflow. The presence in the perifusate of the

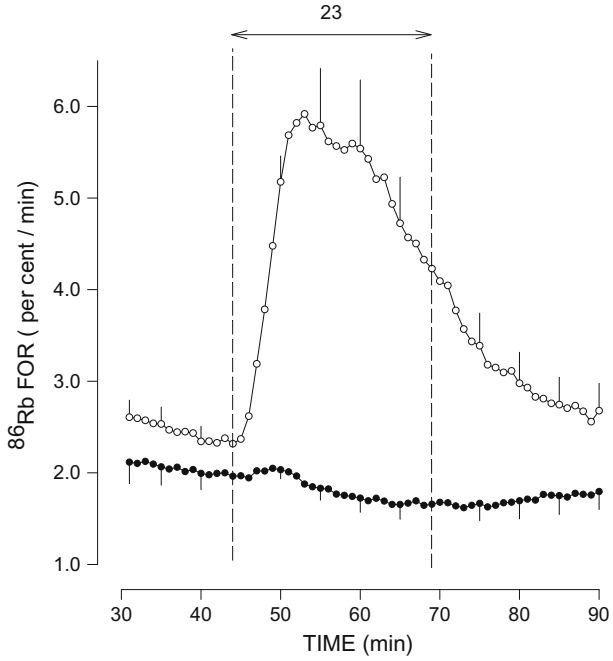


Figure 2. Effect of **23** (10 μM) on ^{86}Rb outflow from rat pancreatic islets perifused throughout in the absence (○) or presence (●) of glibenclamide (10 μM). Basal media contained 5.6 mM glucose and extracellular Ca^{2+}. Mean values (±SEM) refer to 5–6 individual experiments.

hypoglycemic sulfonylurea glibenclamide (10 μM, ●), a pharmacological tool known to block the K_{ATP} channels,[34,35] abolished the stimulatory effect of compound **23** on ^{86}Rb outflow.

Taken as a whole, these radioisotopic data suggest that, in insulin-secreting cells, compound **23** activated K_{ATP} channels.[29,36–38]

4. Conclusion

In the present study, we have synthesized and characterized the biological activity of several novel *R*/*S*-4-arylthiourea-substituted 3,4-dihydro-2,2-dimethyl-6-halo-2*H*-1-benzopyrans structurally related to the potassium channel opener (±)-cromakalim. Some of these new dimethylchroman derivatives included, at the 4-position, a phenylthiourea moiety substituted on the phenyl ring by a *meta*- or *para*-electron-withdrawing group such as NO_2 or CN. Compounds bearing an arylthiourea group with either a 3-pyridinyl or a 3-thienyl moiety replacing the phenyl ring were also synthesized. Moreover, the thiourea function was further replaced by a *S*-methylisothiourea group or a bioisosteric cyanoguanidine function. The biological activity of these new compounds was compared with that of (±)-cromakalim, (±)-pinacidil, diazoxide and compounds **4** and **5**, used as reference PCOs.

All these new dimethylchromans (**12**–**32**) were, however, more potent than the reference molecules (±)-cromakalim, (±)-pinacidil and diazoxide at inhibiting the insulin releasing process. Vascular data further revealed that the new compounds were less potent than (±)-cromakalim and (±)-pinacidil as vasorelaxant agents. Biological results also indicated that compounds bearing a strong *meta*- or *para*-electron-withdrawing group (**12**–**23**) on the C-4 phenyl ring of the thiourea moiety were potent inhibitors of insulin release. Compound **16** was found to be the most potent benzopyran derivative tested on the present pancreatic β-cell 'in vitro' model.

Among these newly synthesized dimethylchromans, molecules **15**–**23** can be regarded as the most promising compounds due to their marked inhibitory effect on the insulin releasing process and their weak myorelaxant activity. Radioisotopic experiments confirmed that the inhibition of insulin release was mediated by the activation of pancreatic β-cell ATP-sensitive potassium channels.

Additional pharmacomodulations, and more specifically the substitution of the halogen atom at the 6-position by other groups, are planned in order to discriminate more potent and more selective dimethylchroman derivatives inhibiting the insulin releasing process.

5. Experimental section

5.1. Chemistry

Melting points were determined on a Stuart SMP3 capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin–Elmer 1000 FTIR spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker Avance (500 MHz) instrument using DMSO-d_6 as the solvent with TMS as an internal standard; chemical shifts are reported in δ values (ppm) relative to that of internal TMS. The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and br = broad are used throughout. Elemental analyses (C, H, N, S) were realized on a Thermo Scientific FlashEA 1112-elemental analyzer and were within ±0.4% of the theoretical values. All reactions were routinely checked by TLC on silica gel Merck 60 F_{254}.

5.1.1. *R*/*S*-4-Amino-3,4-dihydro-2,2-dimethyl-6-halo-2*H*-1-benzopyrans (6a–c)

These compounds were prepared from the appropriate parahalophenols (**6a**–**c**), according to previously described synthetic procedures.[30,31]

5.1.2. *R*/*S*-4-(3-Cyanophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-6-fluoro-2*H*-1-benzopyran (12)

3-Cyanophenyl isothiocyanate (0.38 g, 2.46 mmol) was added to a solution of **6a**[30] (0.4 g, 2.05 mmol) in methylene chloride (5 mL). After 30 min, the solvent was removed under vacuum, and the crude product was triturated with ethyl acetate. The insoluble material was collected by filtration, and petroleum ether was added to the filtrate. The resulting precipitate was collected by filtration, washed with petroleum ether, and dried. The product was then crystallized in a mixture of methanol/water. The final precipitate was collected by filtration, washed with water, and dried (0.20 g, 28%): mp: 168–169 °C; IR (KBr) *v*: 3227 (N–H), 3041 (C–H aromatic), 2980, 2927 (C–H aliphatic), 2236 (C≡N), 1199 (C=S) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.27 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.80 (m, 1H, H_A of CH_2), 2.22 (m, 1H, H_B of CH_2), 5.78 (m, 1H, C*H*), 6.78 (dd, *J* = 4.75, 8.83 Hz, 1H, 8-*H*), 7.01 (m, 1H, 7-*H*), 7.07 (d, *J* = 8.40 Hz, 1H, 5-*H*), 7.51–7.57 (m, 2H, 4′-*H*, 5′-*H*), 7.76 (d, *J* = 7.60 Hz, 1H, 6′-*H*), 8.05 (s, 1H, 2′-*H*), 8.37 (d, *J* = 7.55 Hz, 1H, N*H*(CH)), 9.82 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{18}N_3OSF$) theoretical: C, 64.21; H, 5.10; N, 11.82; S, 9.02. Found: C, 63.85; H, 5.36; N, 11.66; S, 8.75.

5.1.3. *R*/*S*-6-Chloro-4-(3-cyanophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (13)

The title compound was obtained as described for **12**, starting from **6b**[31] (0.4 g, 1.89 mmol) and 3-cyanophenyl isothiocyanate (363 mg, 2.27 mmol) (0.44 g, 62%): mp: 185–186 °C; IR (KBr): *v*: 3338, 3275, 3212 (N–H), 3056 (C–H aromatic), 2976, 2932 (C–H aliphatic), 2237 (C≡N), 1202 (C=S) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.28 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.81 (m, 1H, H_A of CH_2), 2.23 (m, 1H, H_B of CH_2), 5.80 (m, 1H, C*H*), 6.79 (d, *J* = 8.70 Hz, 1H, 8-*H*), 7.19 (d, *J* = 8.65 Hz, 1H, 7-*H*), 7.27 (s, 1H, 5-*H*), 7.52–7.57 (m, 2H, 4′-*H*, 5′-*H*), 7.77 (d, *J* = 7.65 Hz, 1H, 6′-*H*), 8.05 (s, 1H, 2′-*H*), 8.40 (br d, 1H, N*H*(CH)), 9.84 (s, 1H, N*H*(C_6H_4)).

Anal. ($C_{19}H_{18}N_3OSCl$) theoretical: C, 61.36; H, 4.88; N, 11.30; S, 8.62. Found: C, 61.16; H, 5.07; N, 11.32; S, 8.95.

5.1.4. *R/S*-6-Bromo-4-(3-cyanophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (14)

The title compound was obtained as described for **12**, starting from **6c**[30] (0.4 g, 1.56 mmol) and 3-cyanophenyl isothiocyanate (300 mg, 1.87 mmol) (0.34 g, 53%): mp: 181–182 °C; IR (KBr): *v*: 3329, 3162 (N–H), 3006 (C–H aromatic), 2984, 2930 (C–H aliphatic), 2230 (C≡N), 1200 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.28 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.81 (m, 1H, H_A of CH_2), 2.23 (m, 1H, H_B of CH_2), 5.80 (m, 1H, C*H*), 6.74 (d, *J* = 8.70 Hz, 1H, 8-*H*), 7.31 (d, *J* = 8.65 Hz, 1H, 7-*H*), 7.39 (s, 1H, 5-*H*), 7.52–7.57 (m, 2H, 4′-*H*, 5′-*H*), 7.77 (d, *J* = 7.70 Hz, 1H, 6′-*H*), 8.04 (s, 1H, 2′-*H*), 8.40 (br d, 1H, N*H*(CH)), 9.84 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{18}N_3OSBr$) theoretical: C, 54.81; H, 4.36; N, 10.09; S, 7.70. Found: C, 54.57; H, 4.25; N, 10.13; S, 8.13.

5.1.5. *R/S*-4-(4-Cyanophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-6-fluoro-2*H*-1-benzopyran (15)

The title compound was obtained as described for **12**, starting from **6a**[30] (0.4 g, 2.05 mmol) and 4-cyanophenyl isothiocyanate (394 mg, 2.46 mmol) (0.34 g, 47%): mp: 202–203 °C; IR (KBr): *v*: 3248 (N–H), 3096, 3055 (C–H aromatic), 2989, 2974, 2950, 2933 (C–H aliphatic), 2227 (C≡N), 1197 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.27 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.79 (m, 1H, H_A of CH_2), 2.25 (m, 1H, H_B of CH_2), 5.77 (m, 1H, C*H*), 6.78 (dd, *J* = 4.75, 8.80 Hz, 1H, 8-*H*), 7.01 (m, 1H, 7-*H*), 7.07 (d, *J* = 8.80 Hz, 1H, 5-*H*), 7.76 (d, *J* = 8.60 Hz, 2H, 2′-*H*, 6′-*H*), 7.80 (d, *J* = 8.55 Hz, 2H, 3′-*H*, 5′-*H*), 8.51 (br d, 1H, N*H*(CH)), 10.02 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{18}N_2OSF$) theoretical: C, 64.21; H, 5.10; N, 11.82; S, 9.02. Found: C, 63.86; H, 5.51; N, 11.52; S, 8.81.

5.1.6. *R/S*-6-Chloro-4-(4-cyanophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (16)

The title compound was obtained as described for **12**, starting from **6b**[31] (0.4 g, 1.89 mmol) and 4-cyanophenyl isothiocyanate (363 mg, 2.27 mmol) (0.44 g, 63%): mp: 216–217 °C; IR (KBr): *v*: 3243 (N–H), 3092, 3039 (C–H aromatic), 2989, 2974, 2954, 2933 (C–H aliphatic), 2226 (C≡N), 1197 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.28 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.80 (m, 1H, H_A of CH_2), 2.25 (m, 1H, H_B of CH_2), 5.78 (m, 1H, C*H*), 6.80 (d, *J* = 8.75 Hz, 1H, 8-*H*), 7.20 (d, *J* = 8.68 Hz, 1H, 7-*H*), 7.27 (s, 1H, 5-*H*), 7.76 (d, *J* = 8.65 Hz, 2H, 2′-*H*, 6′-*H*), 7.80 (d, *J* = 8.55 Hz, 2H, 3′-*H*, 5′-*H*), 8.51 (br d, 1H, N*H*(CH)), 10.01 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{18}N_3OSCl$) theoretical: C, 61.36; H, 4.88; N, 11.30; S, 8.62. Found: C, 61.39; H, 4.73; N, 11.28; S, 9.00.

5.1.7. *R/S*-6-Bromo-4-(4-cyanophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (17)

The title compound was obtained as described for **12**, starting from **6c**[30] (0.4 g, 1.56 mmol) and 4-cyanophenyl isothiocyanate (300 mg, 1.87 mmol) (0.50 g, 77%): mp: 207–208 °C; IR (KBr): *v*: 3248 (N–H), 3035 (C–H aromatic), 2988, 2934 (C–H aliphatic), 2226 (C≡N), 1197 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.28 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.80 (m, 1H, H_A of CH_2), 2.25 (m, 1H, H_B of CH_2), 5.78 (m, 1H, C*H*), 6.75 (d, *J* = 8.60 Hz, 1H, 8-*H*), 7.32 (d, *J* = 8.55 Hz, 1H, 7-*H*), 7.40 (s, 1H, 5-*H*), 7.76–7.81 (m, 4H, 2′-*H*, 3′-*H*, 5′-*H*, 6′-*H*), 8.58 (br s, 1H, N*H*(CH)), 10.06 (br s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{18}N_3OSBr$) theoretical: C, 54.81; H, 4.36; N, 10.09; S, 7.70. Found: C, 55.09; H, 4.62; N, 10.27; S, 7.73.

5.1.8. *R/S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(3-nitrophenylaminothiocarbonylamino)-2*H*-1-benzopyran (18)

The title compound was obtained as described for **12**, starting from **6a**[30] (0.4 g, 2.05 mmol) and 3-nitrophenyl isothiocyanate (443 mg, 2.46 mmol) (0.36 g, 47%): mp: 175–176 °C; IR (KBr): *v*: 3244 (N–H), 3098, 3035 (C–H aromatic), 2976, 2930 (C–H aliphatic), 1528, 1350 (N=O), 1197 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.28 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.80 (m, 1H, H_A of CH_2), 2.24 (m, 1H, H_B of CH_2), 5.79 (m, 1H, C*H*), 6.78 (dd, *J* = 4.80, 8.90 Hz, 1H, 8-*H*), 7.01 (m, 1H, 7-*H*), 7.08 (d, *J* = 8.95 Hz, 1H, 5-*H*), 7.60 (m, 1H, 5′-*H*), 7.86 (d, *J* = 7.95 Hz, 1H, 4′-*H*), 7.94 (d, *J* = 8.05 Hz, 1H, 6′-*H*), 8.44–8.60 (m, 2H, 2′-*H*, N*H*(CH)), 9.97 (br s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}N_3O_3SF$) theoretical: C, 57.59; H, 4.83; N, 11.19; S, 8.54. Found: C, 57.24; H, 4.86; N, 11.16; S, 8.14.

5.1.9. *R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(3-nitrophenylaminothiocarbonylamino)-2*H*-1-benzopyran (19)

The title compound was obtained as described for **12**, starting from **6b**[31] (0.4 g, 1.89 mmol) and 3-nitrophenyl isothiocyanate (408 mg, 2.27 mmol) (0.60 g, 81%): mp: 188–189 °C; IR (KBr): *v*: 3183 (N–H), 3097, 3024 (C–H aromatic), 2980, 2928, 2950 (C–H aliphatic), 1530, 1345 (N=O), 1198 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.29 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.84 (m, 1H, H_A of CH_2), 2.25 (m, 1H, H_B of CH_2), 5.80 (m, 1H, C*H*), 6.80 (d, *J* = 8.70 Hz, 1H, 8-*H*), 7.20 (d, *J* = 8.65 Hz, 1H, 7-*H*), 7.29 (s, 1H, 5-*H*), 7.60 (m, 1H, 5′-*H*), 7.87 (d, *J* = 7.80 Hz, 1H, 4′-*H*), 7.92 (d, *J* = 7.85 Hz, 1H, 6′-*H*), 8.45–8.61 (m, 2H, 2′-*H*, N*H*(CH)), 9.95 (br s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}N_3O_3SCl$) theoretical: C, 55.17; H, 4.63; N, 10.72; S, 8.18. Found: C, 54.98; H, 4.83; N, 10.78; S, 7.92.

5.1.10. *R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(3-nitrophenylaminothiocarbonylamino)-2*H*-1-benzopyran (20)

The title compound was obtained as described for **12**, starting from **6c**[30] (0.4 g, 1.56 mmol) and 3-nitrophenyl isothiocyanate (338 mg, 1.87 mmol) (0.64 g, 94%): mp: 186–187 °C; IR (KBr): *v*: 3227 (N–H), 3092, 3026 (C–H aromatic), 2981, 2926, 2949 (C–H aliphatic), 1530, 1349 (N=O), 1198 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.29 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.81 (m, 1H, H_A of CH_2), 2.25 (m, 1H, H_B of CH_2), 5.81 (m, 1H, C*H*), 6.75 (d, *J* = 8.70 Hz, 1H, 8-*H*), 7.32 (d, *J* = 8.68 Hz, 1H, 7-*H*), 7.41 (s, 1H, 5-*H*), 7.60 (m, 1H, 5′-*H*), 7.87 (d, *J* = 7.85 Hz, 1H, 4′-*H*), 7.94 (d, *J* = 7.95 Hz, 1H, 6′-*H*), 8.47–8.61 (m, 2H, 2′-*H*, N*H*(CH)), 9.97 (br s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}N_3O_3SBr$) theoretical: C, 49.55; H, 4.16; N, 9.63; S, 7.35. Found: C, 49.28; H, 4.36; N, 9.69; S, 7.09.

5.1.11. *R/S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(4-nitrophenylaminothiocarbonylamino)-2*H*-1-benzopyran (21)

The title compound was obtained as described for **12**, starting from **6a**[30] (0.4 g, 2.05 mmol) and 4-nitrophenyl isothiocyanate (443 mg, 2.46 mmol) (0.56 g, 73%): mp: 192–194 °C; IR (KBr): *v*: 3221 (N–H), 3040 (C–H aromatic), 2980, 2933 (C–H aliphatic), 1522, 1346 (N=O), 1196 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.28 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.80 (m, 1H, H_A of CH_2), 2.26 (m, 1H, H_B of CH_2), 5.76 (m, 1H, C*H*), 6.79 (br s, 1H, 8-*H*), 7.02–7.09 (m, 2H, 5-*H*, 7-*H*), 7.88 (d, *J* = 7.00 Hz, 2H, 2′-*H*, 6′-*H*), 8.20 (d, *J* = 7.25 Hz, 2H, 3′-*H*, 5′-*H*), 8.63 (br s, 1H, N*H*(CH)), 10.20 (br s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}N_3O_3SF$) theoretical: C, 57.59; H, 4.83; N, 11.19; S, 8.54. Found: C, 57.37; H, 4.82; N, 11.21; S, 8.17.

5.1.12. *R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(4-nitrophenylaminothiocarbonylamino)-2*H*-1-benzopyran (22)

The title compound was obtained as described for **12**, starting from **6b**[31] (0.4 g, 1.89 mmol) and 4-nitrophenyl isothiocyanate (408 mg, 2.27 mmol) (0.62 g, 84%): mp: 194–196 °C; IR (KBr): *v*: 3226 (N–H), 3059 (C–H aromatic), 2986, 2932 (C–H aliphatic), 1518, 1335 (N=O), 1198 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.29 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.81 (m, 1H, H_A of CH_2), 2.27 (m, 1H, H_B of CH_2), 5.78 (m, 1H, C*H*), 6.81 (d, *J* = 8.75 Hz, 1H, 8-*H*), 7.21 (d, *J* = 8.70 Hz, 1H, 7-*H*), 7.29 (s, 1H,

5-*H*), 7.89 (d, *J* = 8.95 Hz, 2H, 2′-*H*, 6′-*H*), 8.20 (d, *J* = 9.05 Hz, 2H, 3′-*H*, 5′-*H*), 8.64 (br s, 1H, N*H*(CH)), 10.20 (br s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}N_3O_3SCl$) theoretical: C, 55.17; H, 4.63; N, 10.72; S, 8.18. Found: C, 55.30; H, 4.79; N, 10.81; S, 8.45.

5.1.13. *R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(4-nitrophenylaminothiocarbonylamino)-2*H*-1-benzopyran (23)

The title compound was obtained as described for **12**, starting from **6c**[30] (0.4 g, 1.56 mmol) and 4-nitrophenyl isothiocyanate (338 mg, 1.87 mmol) (0.54 g, 80%): mp: 201–202 °C; IR (KBr): *ν*: 3246 (N–H), 3050 (C–H aromatic), 2986, 2932 (C–H aliphatic), 1518, 1334 (N=O), 1199 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): *δ*: 1.29 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.81 (m, 1H, H_A of CH_2), 2.27 (m, 1H, H_B of CH_2), 5.78 (m, 1H, C*H*), 6.75 (d, *J* = 8.70 Hz, 1H, 8-*H*), 7.32 (d, *J* = 8.65 Hz, 1H, 7-*H*), 7.41 (s, 1H, 5-*H*), 7.88 (d, *J* = 8.85 Hz, 2H, 2′-*H*, 6′-*H*), 8.20 (d, *J* = 9.10 Hz, 2H, 3′-*H*, 5′-*H*), 8.64 (br s, 1H, N*H*(CH)), 10.19 (br s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}N_3O_3SBr$) theoretical: C, 49.55; H, 4.16; N, 9.63; S, 7.35. Found: C, 49.75; H, 4.20; N, 9.71; S, 7.32.

5.1.14. *R/S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(3-pyridylaminothiocarbonylamino)-2*H*-1-benzopyran (24)

3-Pyridyl isothiocyanate (274 μL, 2.46 mmol) was added to a solution of **6a**[30] (0.4 g, 2.05 mmol) in methylene chloride (5 mL). After 30 min, the solvent was removed under vacuum and the crude product was triturated with methanol. The insoluble material was collected by filtration, and water was added to the filtrate. The resulting precipitate was collected by filtration, washed with water, and dried (0.38 g, 56%): mp: 161–162 °C; IR (KBr): *ν*: 3174 (N–H), 2989 (C–H aliphatic), 1197 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): *δ*: 1.27 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.80 (m, 1H, H_A of CH_2), 2.20 (m, 1H, H_B of CH_2), 5.78 (m, 1H, C*H*), 6.78 (dd, *J* = 4.80, 8.80 Hz, 1H, 8-*H*), 7.00 (m, 1H, 7-*H*), 7.07 (d, *J* = 8.05 Hz, 1H, 5-*H*), 7.36 (m, 1H, 5′-*H*), 7.96 (d, *J* = 7.75 Hz, 1H, 4′-*H*), 8.32 (m, 2H, 6′-*H*, N*H*(CH)), 8.60 (s, 1H, 2′-*H*), 9.68 (s, 1H, N*H*(C_5H_4N)). Anal. ($C_{17}H_{18}N_3OSF$) theoretical: C, 61.61; H, 5.47; N, 12.68; S, 9.67. Found: C, 61.41; H, 5.54; N, 11.77; S, 9.36.

5.1.15. *R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(3-pyridylaminothiocarbonylamino)-2*H*-1-benzopyran (25)

The title compound was obtained as described for **24**, starting from **6b**[31] (0.4 g, 1.89 mmol) and 3-pyridyl isothiocyanate (253 μL, 2.27 mmol) (0.50 g, 76%): mp: 144–145 °C; IR (KBr): *ν*: 3166 (N–H), 2986 (C–H aliphatic), 1198 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): *δ*: 1.28 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.81 (m, 1H, H_A of CH_2), 2.22 (m, 1H, H_B of CH_2), 5.80 (m, 1H, C*H*), 6.79 (d, *J* = 8.70 Hz, 1H, 8-*H*), 7.19 (d, *J* = 8.65 Hz, 1H, 7-*H*), 7.27 (s, 1H, 5-*H*), 7.36 (m, 1H, 5′-*H*), 7.97 (d, *J* = 7.90 Hz, 1H, 4′-*H*), 8.31–8.35 (m, 2H, 6′-*H*, N*H*(CH)), 8.60 (s, 1H, 2′-*H*), 9.70 (s, 1H, N*H*(C_5H_4N)). Anal. ($C_{17}H_{18}N_3OSCl$) theoretical: C, 58.70; H 5.22; N, 12.08; S, 9.22. Found: C, 58.59; H, 5.17; N, 11.82; S, 9.57.

5.1.16. *R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(3-pyridylaminothiocarbonylamino)-2*H*-1-benzopyran (26)

The title compound was obtained as described for **24**, starting from **6c**[30] (0.4 g, 1.56 mmol) and 4-nitrophenyl isothiocyanate (255 μL, 1.87 mmol) (0.44 g, 72%): mp: 146–148 °C; IR (KBr): *ν*: 3183 (N–H), 3033 (C–H aromatic), 2975, 2923 (C–H aliphatic), 1198 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): *δ*: 1.27 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.80 (m, 1H, H_A of CH_2), 2.21 (m, 1H, H_B of CH_2), 5.80 (m, 1H, C*H*), 6.74 (d, *J* = 8.65 Hz, 1H, 8-*H*), 7.32 (d, *J* = 8.30 Hz, 1H, 7-*H*), 7.36–7.40 (m, 2H, 5-*H*, 5′-*H*), 7.96 (d, *J* = 7.35 Hz, 1H, 4′-*H*), 8.32–8.40 (m, 2H, 6′-*H*, N*H*(CH)), 8.60 (s, 1H, 2′-*H*), 9.75 (s, 1H, N*H*(C_5H_4N)). Anal. ($C_{17}H_{18}N_3OSBr$) theoretical: C, 52.05; H, 4.62; N, 10.71; S, 8.17. Found: C, 51.92; H, 4.61; N, 10.46; S, 8.46.

5.1.17. 3-Aminothiophene oxalate salt (9)

Methyl 3-amino-2-thiophenecarboxylate (15 g, 95.4 mmol) in 2 M aqueous sodium hydroxide was refluxed for 30 min. The mixture was then cooled to 0 °C and the solution was adjusted to pH 1 with concentrated hydrochloric acid. The resulting precipitate was collected by filtration. The solution of the product in acetone (150 mL) was dried over magnesium sulfate, filtered and evaporated under reduced pressure. Oxalic acid dihydrate (13.2 g, 104.7 mmol) was added to the solution of the crude product in isopropanol (60 mL). The solution was stirred at 38 °C for one hour and was then cooled to 0 °C. Diethyl ether (180 mL) was added to the solution. The final precipitate was collected by filtration, washed with diethyl ether, and dried (13.2 g, 56%): mp: 139–142 °C.

5.1.18. 3-Aminothiophene (10)

Ammonium hydroxide solution was added to a suspension of **9** (1 g, 5.29 mmol) in water (35 mL) until the solution was adjusted to pH 14. The product was extracted with methylene chloride. The organic layer was dried over magnesium sulfate, filtered and evaporated under reduced pressure. The resulting oil (0.52 g, 100%) was used directly in the next step without further purification.

5.1.19. 3-Thienyl isothiocyanate (11)

1,1′-Thiocarbonyldiimidazole (1.01 g, 5.67 mmol) was added to a solution of **10** (0.55 g, 5.55 mmol) in dry 1,4-dioxane (15 mL). The mixture was refluxed under nitrogen. After 20 min, the solvent was removed under vacuum and diethyl ether was added to the oily residue. The organic layer was washed with 0.1 M hydrochloric acid, and evaporated under reduced pressure. The resulting oil (0.51 g, 79%) was used directly in the next step without further purification.

5.1.20. *R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(3-thienylaminothiocarbonylamino)-2*H*-1-benzopyran (27)

3-Thienyl isothiocyanate (**11**) (0.16 g, 1.13 mmol) was added to a solution of *R/S*-4-amino-6-chloro-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**6b**[31]) (0.2 g, 0.95 mmol) in methylene chloride (5 mL). After 30 min, the resulting precipitate was collected by filtration, washed with methylene chloride, and dried (0.24 g, 69%): mp: 196–197 °C; IR (KBr): *ν*: 3340, 3160 (N–H), 3087 (C–H aromatic), 2978, 2933 (C–H aliphatic), 1199 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): *δ*: 1.27 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.81 (m, 1H, H_A of CH_2), 2.18 (m, 1H, H_B of CH_2), 5.77 (m, 1H, C*H*), 6.78 (d, *J* = 8.70 Hz, 1H, 8-*H*), 7.11 (d, *J* = 4.75 Hz, 1H, 4′-*H*), 7.18 (d, *J* = 8.63 Hz, 1H, 7-*H*), 7.24 (s, 1H, 5-*H*), 7.46 (m, 1H, 5′-*H*), 7.64 (s, 1H, 2′-*H*), 8.02 (br s, 1H, N*H*(CH)), 9.79 (br s, 1H, N*H*(C_4H_3S)). Anal. ($C_{16}H_{17}N_2OS_2Cl$) theoretical: C, 54.45; H, 4.86; N, 7.94; S, 18.17. Found: C, 54.48; H, 5.01; N, 7.74; S, 18.43.

5.1.21. *R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(3-thienylaminothiocarbonylamino)-2*H*-1-benzopyran (28)

3-Thienyl isothiocyanate (**11**) (0.07 g, 0.5 mmol) was added to a solution of *R/S*-4-amino-6-bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**6c**[30]) (0.1 g, 0.39 mmol) in methylene chloride (5 mL). After 30 min, the solvent was removed under vacuum, the crude product was solubilised with ethyl acetate and *n*-hexane was added to the solution. The resulting precipitate was collected by filtration, washed with *n*-hexane, and dried (0.10 g, 65%): mp: 181–182 °C; IR (KBr): *ν*: 3331, 3161 (N–H), 2971, 2930 (C–H aliphatic), 1198 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): *δ*: 1.27 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.81 (m, 1H, H_A of CH_2), 2.18 (m, 1H, H_B of CH_2), 5.79 (m, 1H, C*H*), 6.73 (d, *J* = 8.65 Hz, 1H, 8-*H*), 7.11 (d, *J* = 4.65 Hz, 1H, 4′-*H*), 7.29 (d, *J* = 8.62 Hz, 1H, 7-*H*), 7.36 (s, 1H, 5-*H*), 7.46 (m, 1H, 5′-*H*), 7.64 (s, 1H, 2′-*H*), 7.99 (br d, 1H,

N*H*(CH)), 9.78 (s, 1H, N*H*(C_4H_3S)). Anal. ($C_{16}H_{17}N_2OS_2Br$) theoretical: C, 48.36; H, 4.31; N, 7.05; S, 16.14. Found: C, 48.19; H, 4.14; N, 7.79; S, 15.96.

5.1.22. *R/S-N*-(6-Bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-*N′*-(3-cyanophenyl)-*S*-methylisothiourea (29)

Iodomethane (179 μL, 2.88 mmol) was added to a solution of de *R/S*-6-bromo-4-(3-cyanophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**14**) (0.4 g, 0.96 mmol) in acetone (1.5 mL) and the solution was stirred overnight at rt. The next day, *R/S-N*-(6-bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-*N′*-(3-cyanophenyl)-*S*-methylisothiouronium hydroiodide was collected by filtration, washed with *n*-hexane, and dried (0.36 g, 67%): mp: 128–130 °C; IR (KBr): *ν*: 3435 (N–H), 2975, 2928 (C–H aliph.), 2233 (C≡N), 1578 (C=N) cm^{-1}. Anal. ($C_{20}H_{21}BrN_3OSI$) theoretical: C, 43.03; H, 3.79; N, 7.53; S, 5.74. Found: C, 42.95; H, 4.00; N, 7.29; S, 5.99.

After purification by column chromatography (ethyl acetate–petroleum 1:4) on silica gel, the title compound was isolated as the corresponding base (0.041 g, 15%): mp: 155–156 °C; IR (KBr): *ν*: 3375 (N–H), 2972, 2930 (C–H aliphatic), 2226 (C≡N), 1586 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): *δ*: 1.25 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.89 (m, 1H, H_A of CH_2), 2.20 (m, 1H, H_B of CH_2), 2.38 (s, 3H, S(CH_3)), 5.32 (m, 1H, C*H*), 6.73 (d, *J* = 8.65 Hz, 1H, 8-*H*), 7.13–7.20 (m, 3H, 2′-*H*, 6′-*H*, N*H*(CH)), 7.30 (d, *J* = 8.63 Hz, 1H, 7-*H*), 7.39–7.46 (m, 2H, 4′-*H*, 5′-*H*), 7.51 (s, 1H, 5-*H*). Anal. ($C_{20}H_{20}N_3OSBr$) theoretical: C, 55.82; H, 4.68; N, 9.76; S, 7.45. Found: C, 55.88; H, 4.37; N, 9.69; S, 7.77.

5.1.23. *R/S-N*-(6-Bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-*N′*-(4-chlorophenyl)-*S*-methylisothiourea (30)

The title compound, as the hydroiodide, salt was obtained as described for **29**, starting from **5** (0.4 g, 0.94 mmol) and iodomethane (175 μL, 2.82 mmol) and further converted to the corresponding base.

R/S-N-(6-bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-*N′*-(3-cyanophenyl)-*S*-methylisothiouronium hydroiodide: Yield: 0.44 g, 83%; IR (KBr): *ν*: 3396 (N–H), 2976, 2928 (C–H aliph.), 1584 (C=N) cm^{-1}.

R/S-N-(6-Bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-*N′*-(3-cyanophenyl)-*S*-methylisothiourea: Yield: 0.096 g, 28%; mp: 113 °C; IR (KBr): *ν*: 3396 (N–H), 2976, 2928 (C–H aliphatic), 1584 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): *δ*: 1.24 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.89 (m, 1H, H_A of CH_2), 2.18 (m, 1H, H_B of CH_2), 2.35 (s, 3H, S(CH_3)), 5.31 (m, 1H, C*H*), 6.72 (d, *J* = 8.70 Hz, 1H, 8-*H*), 6.79 (d, *J* = 8.50 Hz, 2H, 3′-*H*, 5′-*H*), 6.97 (d, *J* = 8.30 Hz, 1H, N*H*(CH)), 7.26–7.30 (m, 3H, 7-*H*, 2′-*H*, 6′-*H*), 7.50 (d, 1H, 5-*H*). Anal. ($C_{19}H_{20}N_2OSBrCl$) theoretical: C, 51.89; H, 4.58; N, 6.37; S, 7.29. Found: C, 51.75; H, 4.62; N, 6.53; S, 7.45.

5.1.24. *R/S-N*-(6-Bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-*N′*-(3-cyanophenyl)-*N″*-cyanoguanidine (31)

Cyanamide (0.06 g, 1.4 mmol) and 1,4-diazabicyclo[2.2.2]octane (0.0071 g, 0.063 mmol) were added to a solution of *R/S-N*-(6-bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-*N′*-(3-cyanophenyl)-*S*-methylisothiouronium hydriodide (0.25 g, 0.45 mmol) in butanol (1.7 mL) and the solution was refluxed for 5 h. The solvent was removed under vacuum. Water was added to the residue and the product was extracted with methylene chloride. The organic layer was evaporated under reduced pressure. The crude product was solubilised in methanol and 3 volumes of water were added. The resulting precipitate was collected by filtration, washed with water and dried. The final product was purified on silica gel eluted with ethyl acetate/petroleum ether (1:1 v/v) to give the title compound (28 mg, 15%): mp: 218–219 °C; IR (KBr): *ν*: 3352, 3240 (N–H), 3153 (C–H aromatic), 2978, 2934 (C–H aliphatic), 2161 (C≡N), 1568 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): *δ*: 1.26 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.80 (m, 1H, CH_2), 2.18 (m, 1H, CH_2), 5.13 (m, 1H, C*H*), 6.71 (d, *J* = 8.70 Hz, 1H, 8-*H*), 7.30 (d, *J* = 8.65 Hz, 1H, 7-*H*), 7.40 (s, 1H, 5-*H*), 7.53–7.60 (m, 3H, 4′-*H*, 5′-*H*, 6′-*H*), 7.70 (s, 1H, 2′-*H*), 7.84 (d, *J* = 8.35 Hz, 1H, N*H*(CH)), 9.54 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{20}H_{18}N_5OBr$) theoretical: C, 56.61; H, 4.28; N, 16.51. Found: C, 56.35; H, 4.32; N, 16.12.

5.1.25. *R/S-N*-(6-Bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-*N′*-(4-chlorophenyl)-*N″*-cyanoguanidine (32)

The title compound was obtained as described for **31**, starting from **30** (0.4 g, 0.71 mmol), cyanamide (0.089 g, 2.1 mmol) and 1,4-diazabicyclo[2.2.2]octane (0.0112 g, 0.1 mmol) (46 mg, 15%): mp: 187–189 °C; IR (KBr): *ν*: 3373 (N–H), 3059 (C–H aromatic), 2977, 2942 (C–H aliphatic), 2186 (C≡N), 1577 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): *δ*: 1.25 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.82 (m, 1H, CH_2), 2.14 (m, 1H, CH_2), 5.13 (m, 1H, C*H*), 6.70 (d, *J* = 8.68 Hz, 1H, 8-*H*), 7.26–7.31 (m, 3H, 7-*H*, 3′-*H*, 5′-*H*), 7.35 (s, 1H, 5-*H*), 7.40 (d, *J* = 8.80 Hz, 2H, 2′-*H*, 6′-*H*), 7.64 (d, *J* = 8.28 Hz, 1H, N*H*(CH)), 9.37 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{17}H_{18}N_3OSBr$) theoretical: C, 52.61; H, 4.18; N, 12.92. Found: C, 52.74; H, 4.32; N, 12.81.

5.2. Biological assays

(±)-Cromakalim (Tocris, UK), diazoxide (Sigma Chemical, USA) and (±)-pinacidil (Laboratory of Medicinal Chemistry, ULg, Belgium) were tested as reference compounds.

5.3. Measurement of insulin release from incubated rat pancreatic islets

Experiments were performed with pancreatic islets isolated from adult fed Wistar rats (Charles River Laboratories, Belgium).

Groups of 10 islets, each derived from the same batch of islets, were preincubated for 30 min at 37 °C in 1 mL of a physiological salt medium (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24) supplemented with 2.8 mM glucose, 0.5% (w/v) dialyzed albumin (fraction V, Sigma) and equilibrated against a mixture of O_2 (95%) and CO_2 (5%). The islets were then incubated at 37 °C for 90 min in 1 mL of the same medium containing 16.7 mM glucose and, in addition, the reference compound or the required chroman derivative. The release of insulin was measured radioimmunologically using rat insulin as a standard.[28] 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.

5.4. Measurement of tension in rat aorta rings

Experiments were performed with aortas removed from adult fed Wistar rats (Charles River Laboratories, Belgium).

A section of the thoracic aorta was cleared of adhering fat and connective tissue and was cut into transverse rings (3–4 mm long). The endothelium was removed by rubbing the intimal surface with forceps. The segments were suspended under 1.5 g tension by means of steel hooks in an organ bath containing 20 mL of a 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 physiological solution was maintained at 37 °C and continuously bubbled with a mixture of O_2 (95%) and CO_2 (5%). Isometric contractions of the aortic rings were measured with a force–displacement transducer. After 60 min of equilibration, the rings were exposed to KCl (30 mM). When the tension had stabilized, the drug was added to the bath at increasing concentrations until maximal relaxation (or until 300 μM). The relaxation response was expressed as the percentage of the contractile response to KCl. The EC_{50} values (concentration evoking

50% inhibition of the plateau phase induced by KCl) were assessed from dose–response curves using Datanalyst software (EMKA Technologies, France).[39]

5.5. Measurements of ^{86}Rb outflow from perifused rat pancreatic islets

Experiments were performed with pancreatic islets isolated from adult fed Wistar rats (Charles River Laboratories, Belgium).

The methods used to measure ^{86}Rb (^{42}K substitute) outflow from perifused rat pancreatic islets have been described previously.[28,29,38] Groups of 100 islets were incubated for 60 min at 37 °C in a bicarbonate-buffered medium (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24) supplemented with 0.5% (w/v) dialyzed albumin (fraction V, Sigma) and containing 16.7 mM glucose and ^{86}Rb (0.15–0.25 mmol/L:50 μCi/mL). The incubation medium was gassed with O_2 (95%) and CO_2 (5%). After incubation, the islets were washed three times with a nonradioactive medium and then placed in a perifusion chamber. The perifusate [bicarbonate-buffered medium supplemented with 0.5% (w/v) dialyzed albumin (fraction V, Sigma) and gassed with O_2 (95%)/CO_2 (5%)] was delivered at a constant rate (1.0 mL/min). From the 31st to the 90th min, the effluent was continuously collected over successive periods of 1 min each. An aliquot of the effluent (0.5 mL) was used for scintillation counting. At the end of the perifusion, the radioactive content of the islets was also determined. The outflow of ^{86}Rb (cpm/min) was expressed as a fractional outflow rate (FOR, % of instantaneous islet content per min).[28,29,38]

5.6. Statistical calculation

The statistical significance of difference between mean data was assessed by using the Student's *t*-test or by an analysis of variance followed for multiple comparisons by a Bonferroni test procedure. The biological results were considered as statistically different when p was <0.05.

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