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Modulation of the 6-position of benzopyran derivatives and inhibitory effects on the insulin releasing process

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ABSTRACT

The synthesis of different series of 4- and 6-substituted *R*/*S*-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans is described. All of these new benzopyran derivatives were bearing, at the 4-position, a phenylthiourea moiety substituted on the phenyl ring by a *meta* or a *para*-electron-withdrawing group such as Cl or CN. The study aimed at exploring the influence of the nature of the substituent at the 6-position in order to develop new benzopyran-type K_{ATP} channel activators exhibiting an improved selectivity towards the insulin secreting cells. The original compounds were examined in vitro on rat pancreatic islets (inhibition of insulin release) as well as on rat aorta rings (vasorelaxant effect) and their activity was compared to that of the reference K_{ATP} channel activators (±)-cromakalim, (±)-pinacidil, diazoxide and to previously synthesized cromakalim analogues. Structure–activity relationships indicated that the inhibitory effect on the insulin secreting cells was related to the lipophilicity of the molecules and to the size of the substituent located at the 6-position. A marked inhibitory activity on the insulin secretory process was obtained with molecules bearing a bulky *tert*-butyloxycarbonylamino group at the 6-position (**20**–**23**). The latter compounds were found to have the same efficacy on the pancreatic endocrine tissue than some previously described molecules. Lastly, radioisotopic experiments further identified *R*/*S*-*N*-4-chlorophenyl-*N*′-(6-*tert*-butyloxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (23) as a K_{ATP} channel opener.

1. Introduction

ATP-sensitive potassium (K_{ATP}) channels are transmembrane proteins which have been initially described, more than 25 years ago, by Noma[1] in cardiac cells. Since then, K_{ATP} channels have been identified in many other excitable tissues into which they play a large variety of physiological roles.[2] Such ionic channels have been depicted in pancreatic B-cells[3] and have been shown to be tightly involved in the control of the insulin secretory process.[4,5] In vascular smooth muscle cells, K_{ATP} channels participate in the control of muscle tone.[6]

By coupling their activity to the intracellular concentration of adenosine triphosphate, K_{ATP} channels have been shown to link cell metabolism to membrane excitability.[7]

K_{ATP} channels consist of an octameric complex of two membrane proteins: the inwardly rectifying potassium channel (Kir6.1, Kir6.2) forming the pore of the channel and the sulfonylurea receptor (SUR1, SUR2A, SUR2B) which regulates channel activity.[2,8,9] The combination of the different subunits leads to specific tissue K_{ATP} channels. For instance, the assembly of Kir6.2 and SUR1 generates pancreatic B-cell K_{ATP} channels[10] whereas the combination of Kir6.1 and SUR2B has been found to be expressed in vascular smooth muscles.[11]

According to their wide physiological functions, K_{ATP} channels represent a promising target to develop new therapeutic agents. Such an objective can theoretically be reached through the synthesis of compounds exhibiting a marked specificity and a high selectivity for a single K_{ATP} channel subtype.

The potential and recognized therapeutic indications for potassium channel openers (PCOs) include, among others, the treatment of arterial hypertension,[12,13] angina pectoris,[12,14] cardiac arrhythmias,[12,15] bronchial asthma,[12,16] urinary incontinence[12,17] and androgenic alopecia.[12,18] PCOs have also been proposed for the prevention and/or management of type I, type II diabetes, obesity,[12,13,19,20] nesidioblastosis,[12] insulinomas[12] and polycystic ovary syndrome.[19,21]

PCOs such as diazoxide (**1**), (±)-pinacidil (**2**) and (±)-cromakalim (**3**) exemplify the chemical diversity of this pharmacological family (Fig. 1). (±)-Cromakalim (**3**), leader of the benzopyran derivatives, has been found to exert a marked myorelaxant activity[22,23] although the drug has also been reported to be slightly active as an inhibitor of the insulin secretory process.[24] By contrast,

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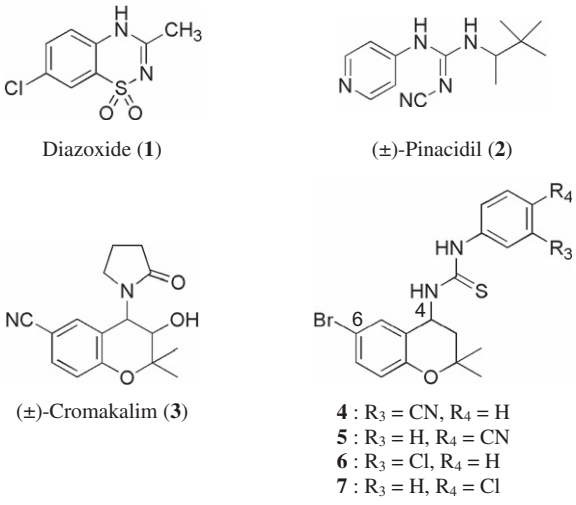


Figure 1. Chemical structure of diazoxide (**1**), (±)-pinacidil (**2**), (±)-cromakalim (**3**) and compounds **4–7**.

diazoxide (**1**) has been reported to be equipotent on the vascular smooth muscle and the insulin secreting-cells.[24,25]

Recently, by exploring a series of *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-2*H*-1-benzopyrans structurally related to cromakalim, we have identified four derivatives (**4–7**) exhibiting a modified tissue selectivity profile.[26,27] These original 6-bromo-substituted *R*/*S*-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans bearing a 3- or 4-substituted phenylthiourea moiety at the 4-position were found to be less effective as vasorelaxants but more potent as inhibitors of the insulin secretory process than the reference molecules diazoxide (**1**) and (±)-cromakalim (**3**).[27] Structure–activity relationships further indicated that the nature of the substituent located at the 4-position on the benzopyran nucleus played a crucial role in the expression of an inhibitory effect on insulin release. Molecules bearing, at the 4-position, a phenylthiourea moiety substituted on the phenyl ring by a *meta*- or a *para*- electron-withdrawing group (a cyano group or a chlorine atom) (Fig 1, compounds **4–7**) were found to be much more potent and selective for the pancreatic B-cells than the reference molecule (±)-cromakalim (**3**).[27]

In the light of such data, and keeping the same substitutions at the 4-position, the present work aimed at exploring the influence of the nature of the substituent at the 6-position in order to develop new 4,6-disubstituted *R*/*S*-2,2-dimethylchromans exhibiting an improved selectivity towards insulin secreting cells. The newly synthesized compounds bearing a 6-amino, a 6-formamido, a 6-acetamido or a 6-*tert*-butyloxycarbonylamino group were examined in vitro as putative potassium channel openers on rat pancreatic islets as well as on rat aorta rings.

2. Chemistry

The key intermediates (**15a–b**) giving access to the target compounds (**16–31**), *R*/*S*-6-acetamido-4-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**15a**) and *R*/*S*-4-amino-6-*tert*-butyloxy carbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**15b**) were, respectively, synthesized in five and seven steps, starting from 4-methoxyaniline (Scheme 1).

The synthesis of the common intermediate, 6-acetamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (**11**), was achieved in three steps. First, 4-methoxyaniline (**8**) was acetylated by acetic anhydride to provide *N*-(4-methoxyphenyl)acetamide (**9**). This reaction was followed by a Friedel–Crafts acylation to give *N*-(3-acetyl-4-hydroxyphenyl)acetamide (**10**). It should be noted that the reaction conditions led to the demethylation of the methoxy group located at the *para*-position of the acetamido moiety. Treatment of compound **10** with acetone, in the presence of pyrrolidine led to chromanone intermediate **11**.

The previously described way of synthesis of the 4-amino-substituted benzopyran key intermediates by the Ritter reaction[28] did not give easy access to the target compounds **15a** and **15b**, due to hydrolysis sensitivity of the molecules. Thus, we have developed an original scheme of synthesis that allowed us to obtain, more rapidly and with an improved yield, the desired compounds.

The acetamido group of intermediate **11** was hydrolyzed in an alcoholic solution of diluted hydrochloric acid. The amine function of the resulting intermediate (**12**) was protected by a *tert*-butyloxycarbonyl group (*t*-BOC) after treatment of compound **12** with di-*tert*-butyl dicarbonate (*t*-BOC anhydride or BOC_2O), providing compound **13**.

In order to obtain the key intermediates **15a–b**, the two ketonic compounds **11** or **13** were treated with hydroxylamine hydrochloride and potassium carbonate to give the oxime intermediates **14a–b** which were further hydrogenated in the presence of Raney-Nickel®.

R/*S*-*N*-(*m*/*p*-substituted)phenyl-*N*′-(6-acetamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thioureas (**16–19**) and *R*/*S*-*N*-(*m*/*p*-substituted)phenyl-*N*′-(6-*tert*-butyloxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thioureas (**20–23**) were obtained from the reaction of the amines **15a–b** with the appropriate phenyl isothiocyanate (R–N=C=S) (Scheme 2).

To prepare compounds **24–27**, the *t*-BOC protective group of compounds **20–23** was removed by treatment with diluted hydrochloric acid in ethanol.

Finally, the reaction of compounds **24–27** with acetic formic anhydride, generated in situ by the reaction of formic acid with acetic anhydride, provided compounds **28–31**.

3. Results and discussion

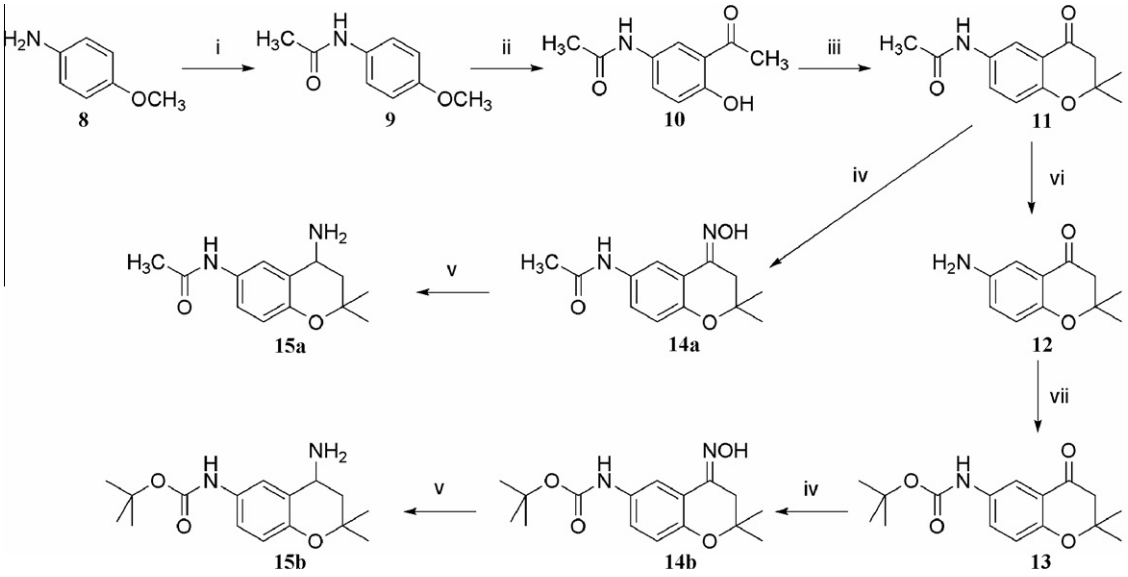
The ability of the original compounds (Table 1, compounds **16–31**) to inhibit the insulin releasing process was evaluated on isolated rat pancreatic islets incubated in the presence of an insulinotropic glucose concentration (16.7 mM). The vasorelaxant activity of the compounds was determined on 30 mM K^+-depolarized rat aorta (endothelium-free) rings.

(±)-Cromakalim (**1**), diazoxide (**2**) and (±)-pinacidil (**3**) were used as reference PCOs (Fig. 1). The activity of the original chroman derivatives was also compared to that of previously described molecules (**4–7**)[26,27] (Fig 1).

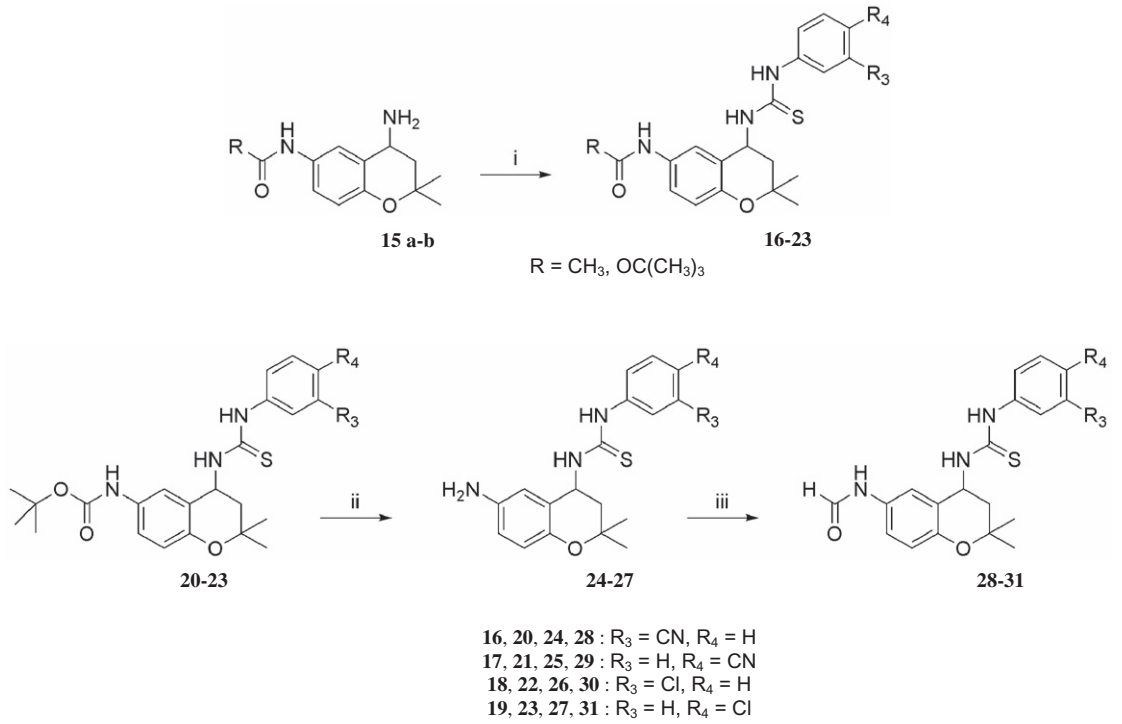
Biological results obtained with classical K_{ATP} channel openers indicated that (±)-cromakalim and (±)-pinacidil, at a 10 μM concentration, were roughly inactive on the pancreatic endocrine tissue (percentage of residual insulin secretion: 94.4 ± 4.1% and 92.1 ± 3.9%, respectively) (Table 1). Diazoxide (10 μM), however, provoked a ±25% reduction in the insulin secretory rate (Table 1). By contrast, previously synthesized benzopyrans (**4–7**) were found to be much more potent than diazoxide at inhibiting the glucose-induced insulin release (Table 1).

Our previous investigations indicated that an electron-withdrawing group, such as a chlorine atom or a cyano group at the *meta* or *para* position of the phenyl ring of *R*/*S*-*N*-phenyl-*N*′-(6-bromo-3,4-dihydro-2,2-dimethyl-4-2*H*-1-benzopyran-4-yl)thioureas, enhanced the inhibitory activity on the insulin secretory rate.[26,27] Therefore, we decided to keep such an electron-withdrawing group at the 3- or 4-position and further explored new moieties located at the 6-position.

The current secretory data revealed that the new benzopyran derivatives (**16–31,** except **24–27**) were more active on pancreatic B-cells than the reference compounds (±)-cromakalim and (±)-pinacidil (Table 1). Numerous cromakalim analogues were even



Scheme 1. Synthesis of key intermediates **15a–b**. Reagents: (i) Ac_2O, HOAc, NaOAc; (ii) AcCl, $AlCl_3$; (iii) acetone, pyrrolidine; (iv) $NH_2OH{\cdot}HCl$, K_2CO_3, ethanol; (v) H_2, Raney-Nickel®, ethanol; (vi) HCl 5 N in ethanol; (vii) BOC_2O, K_2CO_3.



Scheme 2. Synthesis of cromakalim analogues **16–31**. Reagents: (i) 3- or 4-chloro/3- or 4-cyanophenyl isothiocyanate (R–N=C=S), CH_2Cl_2; (ii) HCl 5 N in ethanol; (iii) HCOOH, Ac_2O, THF.

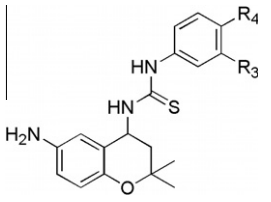
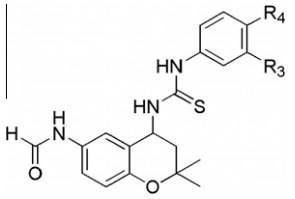
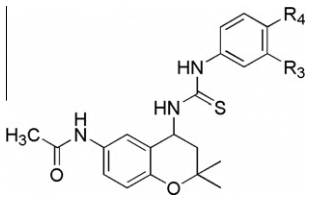
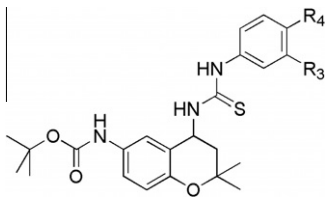
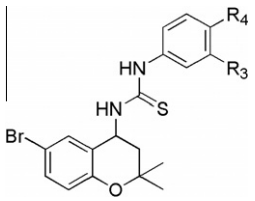
more potent than diazoxide at reducing the glucose-induced insulin response (Table 1).

Pharmacological results obtained on pancreatic endocrine tissue further indicated that drugs bearing an amine group (**24–27**) at the 6 position barely affected the insulin secretory process. At a 10 μM concentration, their activity was equivalent to that of (±)-cromakalim (p >0.05) and (±)-pinacidil (p >0.05). Compared to diazoxide, compound **24**, **25** and **27** were less effective (p <0.05) whilst compound **26** was equipotent (p >0.05) at reducing the insulin secretory rate.

The nature and the position of the electron-withdrawing group on the phenyl ring of the phenylthiourea chain did not markedly affect the activity of compounds **24–27** on the pancreatic tissue (p >0.05).

Drugs bearing a formamide group (–NHCHO) (**28–31**) at the 6 position exhibited a more pronounced inhibitory activity than (±)-cromakalim (p <0.05) and (±)-pinacidil (p <0.05) on the glucose-induced insulin release. The inhibitory activity of compounds 28–31 was comparable to that of diazoxide (p >0.05). The efficacy of such compounds was also more marked than that of molecules

Table 1
AC log *P* estimated values and effects of original dimethylchromans on insulin secretion from rat pancreatic islets and on the contractile activity of rat aorta rings

Compounds		R_3	R_4	AC log *P*	Residual insulin secretion[a] (%)		Myorelaxant activity
					10 μM	1 μM	EC_{50}[b] (μM)
	**24**	CN		3.21	96.9 ± 3.4 (23)	—	>30.0 (4)
	25		CN	3.21	87.0 ± 4.1 (24)	—	>10.0 (6)
	26	Cl		4.01	84.6 ± 4.1 (22)	—	24.6 ± 6.7 (5)
	27		Cl	4.01	85.0 ± 3.2 (23)	—	15.6 ± 0.2 (3)
	**28**	CN		3.27	70.6 ± 3.1 (30)	—	>30.0 (4)
	29		CN	3.27	71.3 ± 3.3 (31)	—	>30.0 (4)
	30	Cl		4.07	68.9 ± 3.1 (23)	—	>30.0 (3)
	31		Cl	4.07	69.6 ± 2.9 (30)	—	>30.0 (4)
	**16**	CN		3.54	60.9 ± 3.3 (21)	—	11.9 ± 1.4 (4)
	17		CN	3.54	55.8 ± 3.4 (21)	—	>10.0 (4)
	18	Cl		4.34	63.1 ± 2.9 (21)	—	>10.0 (6)
	19		Cl	4.34	60.4 ± 3.3 (21)	—	8.3 ± 1.7 (4)
	**20**	CN		4.79	13.3 ± 1.8 (19)	72.2 ± 4.3 (24)	8.5 ± 1.0 (7)
	21		CN	4.79	12.9 ± 1.7 (19)	51.6 ± 2.9 (21)	>3.0 (4)
	22	Cl		5.59	17.1 ± 1.5 (23)	69.0 ± 2.5 (22)	>3.0 (4)
	23		Cl	5.59	15.5 ± 1.4 (18)	76.4 ± 3.3 (23)	>30.0 (5)
(±)-Cromakalim				1.82	94.4 ± 4.1 (32)[c]	95.3 ± 3.8 (31)[c]	0.13 ± 0.01 (7)[c]
(±)-Pinacidil				nd	92.1 ± 3.9 (13)[c]	97.7 ± 6.7 (19)[c]	0.35 ± 0.02 (11)[c]
Diazoxide				nd	73.9 ± 4.4 (16)[c]	87.5 ± 5.0 (15)[c]	22.4 ± 2.1 (11)[c]
	**4**	CN		4.63	14.2 ± 1.3 (21)[c]	65.5 ± 3.6 (30)[c]	0.64 ± 0.05 (5)[c]
	5		CN	4.63	11.7 ± 0.7 (24)[c]	57.5 ± 3.0 (29)[c]	>10.0 (6)[c]
	6	Cl		5.43	23.0 ± 2.4 (31)[d]	74.6 ± 4.0 (21)[d]	>10.0 (5)[d]
	7		Cl	5.43	12.2 ± 1.2 (20)[d]	75.7 ± 3.2 (24)[d]	>10.0 (4)[d]

[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. 27.
[d] Published results: Ref. 26.

24–27 (p <0.05) but less pronounced than the reference 4-substituted *R*/*S*-6-bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran derivatives (**4–7**).

Molecules substituted by an acetamido moiety (–$NHCOCH_3$) (**16–19**) at the 6-position provoked a marked inhibition of the insulin secretory process. Such molecules were more active than (±)-cromakalim (p <0.05), (±)-pinacidil (p <0.05) and even diazoxide (p <0.05). Moreover, these original compounds were more effective than molecules **24–27** and **28–31** (p <0.05) [except **18** vs **30** (p >0.05)]. Nevertheless, the efficacy of the benzopyran derivatives **16–19** remained less pronounced than that of the previously described reference molecules **4–7**.

As depicted above, the nature of the substituent on the phenyl ring of the phenylthiourea chain did not affect the activity of compounds **28–31** and **16–19** (p >0.05).

Compounds substituted with a *tert*-butyloxycarbonylamino bulky group (**20–23**) at the 6 position were found to exert a pronounced inhibitory effect on the insulin secretory rate. These benzopyran derivatives were much more potent than (±)-cromakalim (p <0.05), (±)-pinacidil (p <0.05) and diazoxide (p <0.05). Compounds (**20–23**), tested at a 10 μM concentration, provoked a ±85% inhibition of the glucose-induced insulin release; an effect which can be considered as near to maximal according to the glucose-insensitive secretory rate.[25,29] The inhibitory activity of compounds **20–23**, tested at 10 and 1 μM, was equivalent to that exhibited by their brominated reference analogues (**4–7**) (p >0.05) (Table 1).

As previously reported for compounds **4–7**,[26,27] the most effective molecule of the 6-*tert*-butyloxycarbonylamino series was bearing a 4-cyanophenylthiourea chain (**21**) at the 4 position.

An important aspect to consider for the establishment of structure–activity relationships is the electronic impact of the substituent at the 6-position. Thus, the replacement of the weak electron-withdrawing bromine atom (compounds **4**–**7**) by an electron-donating group such as NH_2 (compounds **24**–**27**) resulted in a marked decrease of the biological activity on insulin secreting cells. By acylating the amine function (formylation for compounds **28**–**31** and acetylation for compounds **16**–**19**), which moderately increased the electron-withdrawing impact of the substituent at the 6-position, the activity on the pancreatic tissue was slightly improved. However, only the concomitant increase in both the size and the lipophilicity of the molecule, by the introduction of a *tert*-butoxycarbonylamino moiety (compounds **20**–**23**), was responsible for a marked improvement of the inhibitory effect on the insulin releasing process. The predicted log *P* values (expressed as the AC log *P* values[30]) reported in Table 1 were in accordance with the impact on lipophilicity of the substituent at the 6-position and of the substituent on the phenyl ring. Thus, the replacement of the cyano group by a chlorine atom on the phenyl ring at the 4-position provoked an increase of the AC log *P* value of about 0.8 log *P* units. Regarding the nature of the substituent at the 6-position, an increasing lipophilicity (as well as an increasing activity on pancreatic B-cells) was observed with the following order: $-NH_2$ < –NHCOH < $-NHCOCH_3$ < $-NHCOOC(CH_3)_3$. With the *tert*-butoxycarbonylamino group, the lipophilicity of the compounds reached that of the brominated derivatives.

Additional experiments were conducted on rat aorta rings in order to detect and to quantify the potential vasorelaxant properties of the newly synthesized compounds.

On the vascular tissue, diazoxide displayed a moderate myorelaxant activity while (±)-pinacidil and (±)-cromakalim were at least 50 to 150-fold more potent at inducing a vasorelaxant effect (Table 1).[27,29,31] Reference compounds **4–7** were found to be less potent than (±)-pinacidil and (±)-cromakalim, although compound **4** was found to exert a marked vasorelaxant activity (EC_{50} = 0.64 μM) (Table 1).[27]

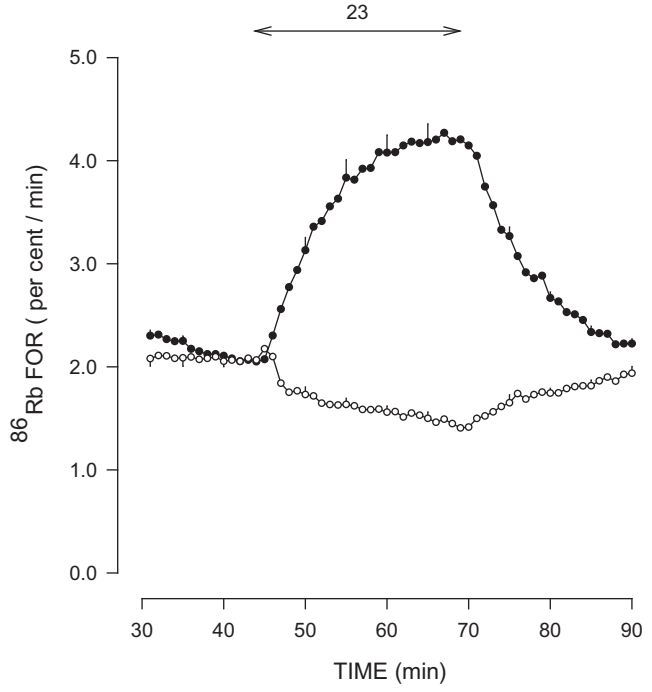


Figure 2. Effect of **23** (10 μM) on ^{86}Rb outflow from rat pancreatic islets perifused throughout in the absence (●) or presence (○) of glibenclamide (10 μM). Basal media contained 5.6 mM glucose and extracellular Ca^{2+}. Mean values (±SEM) refer to 5–6 individual experiments.

Unfortunately, and as previously observed with **5–7**,[27] most original dimethylchromans derivatives precipitated in the bathing medium before reaching their maximal myorelaxant activity, making it difficult to accurately determine their respective EC_{50} values.

However, and despite the poor solubility of several compounds, all new benzopyran analogues (**16–31**) exhibited a weaker vasorelaxant activity than (±)-pinacidil and (±)-cromakalim (p <0.05). When accurately quantifiable, the EC_{50} values indicated that the vasorelaxant potency of cromakalim was at least 60 times higher than that of the newly synthesized compounds. The latter molecules also appeared to be less potent on the vascular tissue than the reference 4-substituted *R*/*S*-6-bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran derivative **4**.

Compound **23** was selected to perform additional radioisotopic experiments in order to determine whether such a compound behaved as a pancreatic ATP-sensitive potassium channel opener.

Figure 2 illustrates the effects of compound **23**, tested at a 10 μM concentration, on the ^{86}Rb fractional outflow rate (FOR) from prelabeled and perifused rat pancreatic islets exposed throughout to 5.6 mM glucose and extracellular Ca^{2+}. The addition of compound **23** provoked a marked, sustained and reversible increase in the rate of ^{86}Rb outflow (●). When the perifusate was enriched with the hypoglycemic sulfonylurea glibenclamide (○, 10 μM), a pharmacological tool known to block the K_{ATP} channels,[24,25,27] the stimulatory effect of compound **23** was completely abolished (Fig. 2).

Such radioisotopic data suggest that compound **23** activates K_{ATP} channels in islet cells.[25]

4. Conclusion

In the present work, 16 original 4,6-disubstituted *R*/*S*-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans (**16–31**) structurally related to previously described 4-substituted *R*/*S*-6-bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans (**4–7**) were synthesized and their biological activity evaluated. All of these new benzopyran derivatives comprised, at the 4-position, a phenylthiourea moiety substituted on the phenyl ring by a *meta* or a *para*-electron-withdrawing group such as Cl or CN. Such compounds (**16–31**) were also bearing, at the 6-position, either an amino, a formamido, an acetamido or a *tert*-butyloxycarbonylamino moiety instead of a bromine atom (compounds **4–7**). The biological activity of these original compounds was compared to that of (±)-cromakalim, (±)-pinacidil, diazoxide and previously reported compounds **4–7**, used as reference PCOs.

Some of the new dimethylchromans were found to be barely active (**24–27**) whilst the others were more active than (±)-cromakalim and (±)-pinacidil at inhibiting the insulin releasing process. Among the latter compounds, drugs bearing at the 6-position an acetamido group (**16–19**) or a *tert*-butyloxycarbonylamino moiety (**20–23**) were even more active than diazoxide.

All these new benzopyrans were found to be less active than (±)-cromakalim and (±)-pinacidil as vasorelaxant agents.

Biological results clearly indicated that the inhibitory activity of the new drugs on the insulin secretory process raised with increasing the size of the substituent located at the 6-position and with increasing the lipophilicity of the molecules. Indeed, molecules bearing a bulky group such as a *tert*-butyloxycarbonylamino moiety on the 6-position (**20–23**) provoked a marked inhibition of the insulin secretory rate. These compounds **20–23** were more active than drugs bearing an acetamido group (**16–19**), themselves more efficient than drugs bearing the less bulky formamido group (**28–31**). The less active compounds (**24–27**) were those only bearing the amino function at the 6-position. Compounds **20–23** were also found to be equipotent to compounds **4–7** at inhibiting the insulin secretory rate but less active on the vascular tissue than

the reference *R*/*S*-6-bromo-4-(3-cyanophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**4**). Therefore, molecules **20–23** can be regarded as the most promising compounds, being active on the insulin secretory cells and exhibiting a pharmacological profile clearly different from that of the parent molecule (±)-cromakalim.

Lastly, radioisotopic experiments performed on prelabeled and perifused rat pancreatic islet indicated that the inhibitory effect on the insulin releasing process of the new benzopyran derivatives was mediated by the activation of pancreatic B-cell ATP-sensitive potassium channels.

Additional pharmacomodulations, and more specifically the exploration of the requirement and the steric hindrance of the carbamate function, are planned in order to further characterize original benzopyran derivatives specifically activating the insulin secreting-cell K_{ATP} channels.

5. Experimental section

5.1. Chemistry

Reagents and solvents were purchased from usual commercial suppliers and were used without further purification. Yields reported refer to purified products. All reactions were routinely checked by thin-layer chromatography (TLC) on Silica Gel 60 F_{254} (Merck) and visualization was performed with UV light (254 nm). Melting points were determined on a Stuart SMP3 apparatus in open capillary tubes and were 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 solvent and tetramethylsilane (TMS) as internal standard; chemical shifts were reported in δ values (ppm) relative to internal TMS. The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and b = broad signal, were used throughout. Elemental analyses (C, H, N, S) were determined on a Thermo Flash EA 1112 series elemental analyzer and were within ±0.4% of the theoretical values.

5.1.1. *N*-(4-Methoxyphenyl)acetamide (9)

4-Methoxyaniline (50 g, 0.406 mol) was dissolved in a solution of sodium acetate (10.24 g, 0.125 mol) in glacial acetic acid (41 ml, 0.716 mol), the mixture was then cooled to 0 °C and acetic anhydride (45.05 ml, 0.477 mol) was added dropwise. At the end of the reaction, water (100 ml) was added to the mixture. The resulting precipitate was collected by filtration. The product was then crystallized in boiling water. The final precipitate was collected by filtration, washed with water and dried (61.70 g, 92%): mp: 126–127 °C; IR (KBr) *v*: 3068 (C–H aromatic), 2934 (C–H aliphatic), 1647 (C=O), 1245 (C–O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 2.00 (s, 3H, –NHCOC*H*$_3$), 3.75 (s, 3H, –OC*H*$_3$), 6.85 (d, 2H, 3-*H* and 5-*H*), 7.47 (d, 2H, 2-*H* and 6-*H*), 9.74 (s, 1H, –N*H*COCH$_3$). Anal. ($C_9H_{11}NO_2$) theoretical: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.68; H, 6.82; N, 8.32.

5.1.2. *N*-(3-Acetyl-4-hydroxyphenyl)acetamide (10)

Acetyl chloride (25 ml, 0.352 mol) was added to a stirred suspension of **9** (20 g, 0.121 mol) in carbon disulfide (50 ml). Aluminum chloride (55 g, 0.412 mol) was added step by step. The mixture was then heated at 80–90 °C. After 90 min, the solvent was evaporated under vacuum. Crushed ice and water was added cautiously to the residue. The resulting precipitate was collected by filtration and washed with water. The crude product was then dissolved in an aqueous solution of sodium hydroxide 5% (w/v) and filtered through Celite®. The filtrate was then acidified to pH 1 by addition of concentrated hydrochloric acid. The title compound was collected by filtration, washed with water and dried (21.05 g, 90%): mp: 162–164 °C; IR (KBr) *v*: 3452 cm^{-1} (O–H), 1657 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 2.02 (s, 3H, –NHCOC*H*$_3$), 2.58 (s, 3H, –COC*H*$_3$), 6.90 (d, 1H, 5-*H*), 7.65 (dd, 1H, 6-*H*), 8.06 (s, 1H, 2-*H*), 9.89 (s, 1H, –N*H*COCH$_3$), 11.54 (s, 1H, –O*H*). Anal. ($C_{10}H_{11}NO_3$) theoretical: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.35; H, 5.82; N, 7.19.

5.1.3. 6-Acetamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (11)

A solution of **10** (25 g, 0.129 mol), acetone (32 ml, 0.434 mol), and pyrrolidine (15 ml, 0.182 mol) in methanol (575 ml) was stirred at 25 °C overnight. On the next day, the solvents were removed under reduced pressure. Water was added to the residue and the mixture was adjusted to pH 1 with concentrated hydrochloric acid. The resulting precipitate was collected by filtration. The crude product was then dissolved in methanol. The solution was treated with charcoal, filtered and water was added to the filtrate. The title compound was collected by filtration, washed with water and dried (27.47 g, 91%): mp: 164–165 °C; IR (KBr) *v*: 1664 and 1693 (C=O), 3313 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.38 (s, 6H, C*H*$_3$), 2.01 (s, 3H, –NHCOC*H*$_3$), 2.76 (s, 2H, C*H*$_2$), 6.92 (d, 1H, 8-*H*), 7.66 (dd, 1H, 7-*H*), 7.99 (s, 1H, 5-*H*), 9.91 (s, 1H, –N*H*COCH$_3$). Anal. ($C_{13}H_{15}NO_3$) theoretical: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.79; H, 6.59; N, 5.96.

5.1.4. 6-Amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (12)

Compound **11** (20 g, 85.74 mmol) was dissolved in an ethanolic solution (250 ml) of hydrochloric acid 5 N. The solution was then refluxed for 3 h. When the reaction was ended, the mixture was poured in water (500 ml). The title compound was precipitated by addition of a solution of sodium hydroxide 40% until pH 10. The final product was collected by filtration, washed with water and dried (16.23 g, 99%): mp: 148–149 °C; IR (KBr) *v*: 1667 (C=O), 3446–3246 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.36 (s, 6H, C*H*$_3$), 2.66 (s, 2H, C*H*$_2$), 4.85 (s, 2H, –N*H*$_2$), 6.68 (d, 1H, 8-*H*), 6.82 (dd, 1H, 7-*H*), 6.90 (d, 1H, 5-*H*). Anal. ($C_{11}H_{13}NO_2$) theoretical: C, 69.09; H, 6.85; N, 7.32. Found: C, 69.35; H, 6.96; N, 7.34.

5.1.5. 6-*tert*-Butyloxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (13)

Compound **12** (15 g, 78.44 mmol) was dissolved in a mixture (385 ml) of tetrahydrofuran and water (v/v 1:1). Potassium carbonate (21.9 g, 0.158 mol) and di-*tert*-butyl dicarbonate (20.1 g, 92.10 mmol) were added to the solution. The reaction mixture was stirred at 25 °C for 4 days. The organic layer was isolated and the water layer was extracted three times with ethyl acetate. Organic layers were pooled together and then dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was dissolved in methanol and water was added. The title compound was collected by filtration, washed with water and dried (22.40 g, 98%): mp: 168–170 °C; IR (KBr) *v*: 1684 and 1719 (C=O), 3360 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.36 (s, 6H, C*H*$_3$), 1.47 (s, 9H, –NHCOOC(C*H*$_3$)$_3$), 2.75 (s, 2H, C*H*$_2$), 6.88 (d, 1H, 8-*H*), 7.50 (dd, 1H, 7-*H*), 7.89 (s, 1H, 5-*H*), 9.30 (s, 1H, –N*H*COOC(CH$_3$)$_3$). Anal. ($C_{16}H_{21}NO_4$) theoretical: C, 65.96; H, 7.27; N, 4.81. Found: C, 65.87; H, 7.16; N, 4.84.

5.1.6. 6-Acetamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-hydroxyimine (14a)

Potassium carbonate (5.93 g, 42.87 mmol) and hydroxylamine hydrochloride (2.98 g, 42.87 mmol) were added to a stirred solution of **11** (5 g, 21.44 mmol) in ethanol (65 ml). The suspension was refluxed for 3 h. The reaction was then poured onto ice and, after complete melting of ice, water was added to obtain a final

volume of 195 ml. The title compound was collected by filtration, washed with water and dried (5.06 g, 95%): mp: 203–204 °C; IR (KBr) *v*: 1634 (C=O), 3229 (N–H) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.28 (s, 6H, CH_3), 2.00 (s, 3H, –NHCOCH_3), 2.74 (s, 2H, CH_2), 6.75 (d, 1H, 8-*H*), 7.39 (dd, 1H, 7-*H*), 8.06 (s, 1H, 5-*H*), 9.77 (s, 1H, –N*H*COCH$_3$), 11.19 (s, 1H, =N–O*H*). Anal. ($C_{13}H_{16}N_2O_3$) theoretical: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.57; H, 6.78; N, 11.63.

5.1.7. *R*/*S*-6-Acetamido-4-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (15a)

Raney-Nickel (3 g) was added to a stirred solution of **14a** (2.5 g, 10.07 mmol) in methanol. The solution was stirred in a sealed hydrogenator under a hydrogen pressure of 5 bars. When the reaction was ended (3–4 h), the catalyst was filtered off and the filtrate was evaporated under reduced pressure. The crude product was dissolved in methanol and water was added. The title compound was collected by filtration, washed with water and dried (2.03 g, 86%): mp: 103–104 °C; IR (KBr) *v*: 1658 (C=O), 3308–3468 (N–H2) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.17 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.51 (t, 1H, 3-*H*), 1.79 (br s, 2H, –CH–NH_2), 1.99 (m, 4H, –NHCOCH_3 and 3-*H*), 3.79 (m, 1H, –C*H*–NH$_2$), 6.57 (d, 1H, 8-*H*), 7.29 (d, 1H, 7-*H*), 7.64 (s, 1H, 5-*H*), 9.69 (s, 1H, –N*H*COCH$_3$). Anal. ($C_{13}H_{18}N_2O_2$) theoretical: C, 66.64; H, 7.74; N, 11.96. Found: C, 66.84; H, 7.45; N, 11.81.

5.1.8. 6-*tert*-Butyloxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-hydroxyimine (14b)

The title compound was obtained as described for **14a**, starting from **13** (20 g, 68.65 mmol), K_2CO_3 (18.98 g, 137.3 mmol) and hydroxylamine hydrochloride (9.54 g, 137.3 mmol) (19.77 g, 94%): mp: 162–163 °C; IR (KBr) *v*: 1705 (C=O), 3369 (N–H) cm $^{-1}$; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.27 (s, 6H, CH_3), 1.49 (s, 9H, –NHCOOC(CH_3)$_3$), 2.72 (s, 2H, CH_2), 6.71 (d, 1H, 8-*H*), 7.23 (dd, 1H, 7-*H*), 7.95 (s, 1H, 5-*H*), 9.13 (s, 1H, –N*H*COOC(CH$_3$)$_3$), 11.16 (s, 1H, =N–O*H*). Anal. ($C_{16}H_{22}N_2O_4$) theoretical: C, 62.73; H, 7.24; N, 9.14. Found: C, 62.64; H, 7.05; N, 8.88.

5.1.9. *R*/*S*-4-Amino-6-*tert*-butyloxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (15b)

The title compound was obtained as described for **15a**, starting from **14b** (6.25 g, 20.41 mmol) (5.25 g, 88%): mp: 144–146 °C; IR (KBr) *v*: 1710 (C=O), 3366 (N–H) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.17 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.45 (m, 10H, –NHCOOC(CH_3)$_3$ and 3-*H*), 1.79 (s, 2H, NH_2), 1.98 (dd, 1H, 3-*H*), 3.78 (dd, 1H, –C*H*–NH$_2$), 6.54 (d, 1H, 8-*H*), 7.04 (d, 1H, 7-*H*), 7.62 (s, 1H, 5-*H*), 8.94 (s, 1H, –N*H*COOC(CH$_3$)$_3$). Anal. ($C_{16}H_{24}N_2O_3$) theoretical: C, 65.73; H, 8.27; N, 9.58. Found: C, 65.94; H, 8.17; N, 9.59.

5.1.10. *R*/*S*-*N*-3-Cyanophenyl-*N*′-(6-acetamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (16)

3-Cyanophenyl isothiocyanate (410.3 mg, 2.56 mmol) was added to a solution of **15a** (0.5 g, 2.13 mmol) in methylene chloride (10 ml). After 30 min, the resulting precipitate was collected by filtration, washed with methylene chloride, and dried (0.63 g, 75%): mp: 147–149 °C; IR (KBr) *v*: 1655 (C=O), 2231 (C≡N), 3335 (N–H) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.26 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.77 (t, 1H, 3-*H*), 2.02 (s, 3H, –NHCOCH_3), 2.17 (m, 1H, 3-*H*), 5.78 (m, 1H, 4-*H*), 6.69 (d, 1H, 8-*H*), 7.41 (d, 1H, 7-*H*), 7.46–7.55 (m, 3H, 5-*H* and 4′-*H* and 5′-*H*), 7.75 (d, 1H, 6′-*H*), 8.06 (s, 1H, 2′-*H*), 8.35 (br s, 1H, –N*H*CSNH–C$_7$H$_4$N), 9.76 (br s, 2H, –N*H*COCH$_3$ and –NHCSN*H*–C$_7$H$_4$N). Anal. ($C_{21}H_{22}N_4O_2S$) theoretical: C, 63.94; H, 5.92; N, 14.20; S, 8.13. Found: C, 63.65; H, 5.54; N, 14.27; S, 8.26.

5.1.11. *R*/*S*-*N*-4-Cyanophenyl-*N*′-(6-acetamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (17)

The title compound was obtained as described for **16**, starting from **15a** (0.5 g, 2.13 mmol) and 4-cyanophenyl isothiocyanate (410.3 mg, 2.56 mmol) (0.63 g, 75%): mp: 172–174 °C; IR (KBr) *v*: 1652 (C=O), 2226 (C≡N), 3323 (N–H) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.26 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.77 (t, 1H, 3-*H*), 1.98 (s, 3H, –NHCOCH_3), 2.20 (m, 1H, 3-*H*), 5.77 (m, 1H, 4-*H*), 6.69 (d, 1H, 8-*H*), 7.43 (d, 2H, 7-*H*, 5-*H*), 7.74–7.82 (dd, 4H, 2′-*H*, 6′-*H*, 3′-*H* and 5′-*H*), 8.43 (br s, 1H, –N*H*CSNH–C$_7$H$_4$N). Anal. ($C_{21}H_{22}N_4O_2S$) theoretical: C, 63.94; H, 5.92; N, 14.20; S, 8.13. Found: C, 63.68; H, 5.77; N, 14.23; S, 8.12.

5.1.12. *R*/*S*-*N*-3-Chlorophenyl-*N*′-(6-acetamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (18)

The title compound was obtained as described for **16**, starting from **15a** (0.5 g, 2.13 mmol) and 3-chlorophenyl isothiocyanate (336 μl, 2.56 mmol) (0.70 g, 81%): mp: 170–172 °C; IR (KBr) *v*: 1653 (C=O), 3257 (N–H) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.25 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.77 (t, 1H, 3-*H*), 1.98 (s, 3H, –NHCOCH_3), 2.16 (m, 1H, 3-*H*), 5.78 (br s, 1H, 4-*H*), 6.68 (d, 1H, 8-*H*), 7.14–7.44 (br m, 5H, 5-*H*, 7-*H*, 2′-*H*, 3′-*H* and 4′-*H*), 7.76 (s, 1H, 6′-*H*), 8.21 (br s, 1H, –N*H*CSNH–C$_6$H$_4$Cl), 9.69 (br s, 1H, –NHCSN*H*–C$_6$H$_4$Cl), 9.76 (s, 1H, –N*H*COCH$_3$). Anal. ($C_{20}H_{22}ClN_3O_2S$) theoretical: C, 59.47; H, 5.49; N, 10.40; S, 7.94. Found: C, 59.42; H, 5.66; N, 10.55; S, 7.98.

5.1.13. *R*/*S*-*N*-4-Chlorophenyl-*N*′-(6-acetamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (19)

The title compound was obtained as described for **16**, starting from **15a** (0.5 g, 2.13 mmol) and 4-chlorophenyl isothiocyanate (434.3 mg, 2.56 mmol) (0.71 g, 82%): mp: 151–154 °C; IR (KBr) *v*: 1657 (C=O), 3264 (N–H) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.76 (t, 1H, 3-*H*), 1.99 (s, 3H, –NHCOCH_3), 2.15 (m, 1H, 3-*H*), 5.78 (br s, 1H, 4-*H*), 6.67 (d, 1H, 8-*H*), 7.36 (d, 2H, 3′-*H* and 5′-*H*), 7.41 (s, 2H, 7-*H*, 5-*H*), 7.53 (d, 2H, 2′-*H*, 6′-*H*), 8.12 (br s, 1H, –N*H*CSNH–C$_6$H$_4$Cl), 9.64 (br s, 1H, –NHCSN*H*–C$_6$H$_4$Cl), 9.76 (s, 1H, –N*H*COCH$_3$). Anal. ($C_{20}H_{22}ClN_3O_2S$) theoretical: C, 59.47; H, 5.49; N, 10.40; S, 7.94. Found: C, 59.46; H, 5.56; N, 10.32; S, 7.98.

5.1.14. *R*/*S*-*N*-3-Cyanophenyl-*N*′-(6-*tert*-butyloxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (20)

3-Cyanophenyl isothiocyanate (789 mg, 4.92 mmol) was added to a solution of **15b** (1.20 g, 4.10 mmol) in methylene chloride (5 ml). After 30 min, the solvent was removed under vacuum and the crude product was dissolved in ethyl acetate. The final product was crystallized by addition of *n*-hexane, collected by filtration, washed with *n*-hexane, and dried (1.75 g, 94%): mp: 121–124 °C; IR (KBr) *v*: 1691 (C=O), 2231 (C≡N), 3339 (N–H) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.25 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.45 (s, 9H, –NHCOOC(CH_3)$_3$), 1.76 (t, 1H, 3-*H*), 2.16 (m, 1H, 3-*H*), 5.75 (br s, 1H, 4-*H*), 6.65 (d, 1H, 8-*H*), 7.17 (d, 1H, 7-*H*), 7.45–7.56 (m, 3H, 5-*H* and 4′-*H* and 5′-*H*), 7.76 (d, 1H, 6′-*H*), 8.04 (s, 1H, 2′-*H*), 8.30 (d, 1H, –N*H*CSNH–C$_7$H$_4$N), 9.10 (s, 1H, –NHCSN*H*–C$_7$H$_4$N), 9.78 (s, 1H, –N*H*COOC(CH$_3$)$_3$). Anal. ($C_{24}H_{28}N_4O_3S$) theoretical: C, 63.69; H, 6.24; N, 12.38; S, 7.08. Found: C, 63.66; H, 5.97; N, 12.58; S, 6.86.

5.1.15. *R*/*S*-*N*-4-Cyanophenyl-*N*′-(6-*tert*-butyloxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (21)

The title compound was obtained as described for **20**, starting from **15b** (1.2 g, 4.10 mmol) and 4-cyanophenyl isothiocyanate (789 mg, 4.92 mmol) (1.71 g, 92%): mp: 188–189 °C; IR (KBr) *v*: 1703 (C=O), 2224 (C≡N), 3309–3369 (N–H) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.23 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.45

(s, 9H, –NHCOOC(CH_3)$_3$), 1.75 (t, 1H, 3-*H*), 2.21 (m, 1H, 3-*H*), 5.73 (br s, 1H, 4-*H*), 6.66 (d, 1H, 8-*H*), 7.18 (s, 1H, 7-*H*), 7.42 (s, 1H, 5-*H*), 7.74 (d, 2H, 2′-*H* and 6′-*H*), 7.80 (d, 2H, 3′-*H* and 5′-*H*), 8.45 (br s, 1H, –N*H*CSNH–C_7H_4N), 9.11 (br s, 1H, –NHCSN*H*–C_7H_4N), 9.97 (s, 1H, –N*H*COOC(CH_3)$_3$). Anal. ($C_{24}H_{28}N_4O_3S$) theoretical: C, 63.69; H, 6.24; N, 12.38; S, 7.08. Found: C, 63.35; H, 6.54; N, 12.38; S, 7.05.

5.1.16. *R*/*S*-*N*-3-Chlorophenyl-*N*′-(6-*tert*-butyloxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (22)

The title compound was obtained as described for **20**, starting from **15b** (1.2 g, 4.10 mmol) and 3-chlorophenyl isothiocyanate (646 μl, 4.92 mmol) (1.67 g, 88%): mp: 117–120 °C; IR (KBr) *v*: 1689 (C=O), 3338 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.24 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.47 (s, 9H, –NHCOOC(CH_3)$_3$), 1.75 (t, 1H, 3-*H*), 2.16 (m, 1H, 3-*H*), 5.74 (br s, 1H, 4-*H*), 6.65 (d, 1H, 8-*H*), 7.16 (m, 2H, 2′-*H* and 6′-*H*), 7.34 (m, 2H, 3′-*H* and 5′-*H*), 7.44 (s, 1H, 7-*H*), 7.74 (s, 1H, 5-*H*), 8.18 (d, 1H, –N*H*CSNH–C_6H_4Cl), 9.10 (s, 1H, –NHCSN*H*–C_6H_4Cl), 9.67 (s, 1H, –N*H*COOC(CH_3)$_3$). Anal. ($C_{23}H_{28}ClN_3O_3S$) theoretical: C, 59.79; H, 6.11; N, 9.10; S, 6.94. Found: C, 59.67; H, 6.45; N, 9.08; S, 7.03.

5.1.17. *R*/*S*-*N*-4-Chlorophenyl-*N*′-(6-*tert*-butyloxycarbonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (23)

The title compound was obtained as described for **20**, starting from **15b** (1.2 g, 4.10 mmol) and 4-chlorophenyl isothiocyanate (835 mg, 4.92 mmol) (1.86 g, 98%): mp: 176–178 °C; IR (KBr) *v*: 1689 (C≡O), 3336 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.24 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.46 (s, 9H, –NHCOOC(CH_3)$_3$), 1.73 (t, 1H, 3-*H*), 2.16 (m, 1H, 3-*H*), 5.73 (br s, 1H, 4-*H*), 6.64 (d, 1H, 8-*H*), 7.16 (br s, 1H, 7-*H*), 7.35 (d, 2H, 2′-*H* and 6′-*H*), 7.43 (s, 1H, 5-*H*), 7.54 (d, 2H, 3′-*H* and 5′-*H*), 8.09 (br s, 1H, –N*H*CSNH–C_6H_4Cl), 9.11 (br s, 1H, –NHCSN*H*–C_6H_4Cl), 9.62 (s, 1H, –N*H*COOC(CH_3)$_3$). Anal. ($C_{23}H_{28}ClN_3O_3S$) theoretical: C, 59.79; H, 6.11; N, 9.10; S, 6.94. Found: C, 60.03; H, 6.42; N, 9.18; S, 7.00.

5.1.18. *R*/*S*-*N*-3-Cyanophenyl-*N*′-(6-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (24)

A suspension of compound **20** (600 mg, 1.3 mmol) in an ethanolic solution (20 ml) of hydrochloric acid 5 N was heated for 5 min. The mixture was then poured into water (40 ml). The title compound was precipitated by addition of an aqueous solution of sodium hydroxide 40% until pH=10. The final product was collected by filtration, washed with water and dried (360 mg, 77%): mp: 156–158 °C; IR (KBr) *v*: 2229 (C≡N), 3373 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.22 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.71 (t, 1H, 3-*H*), 2.13 (m, 1H, 3-*H*), 4.67 (br s, 2H, –NH_2), 5.66 (br s, 1H, 4-*H*), 6.41–6.52 (m, 3H, 5-*H*, 7-*H*, 8-*H*), 7.51 (m, 2H, 4′-*H* and 5′-*H*), 7.77 (d, 1H, 6′-*H*), 8.11 (s, 1H, 2′-*H*), 8.29 (d, 1H, –N*H*CSNH–C_7H_4N), 9.75 (s, 1H, –NHCSN*H*–C_7H_4N). Anal. ($C_{19}H_{20}N_4OS$) theoretical: C, 64.75; H, 5.72; N, 15.90; S, 9.10. Found: C, 64.46; H, 5.79; N, 15.77; S, 8.73.

5.1.19. *R*/*S*-*N*-4-Cyanophenyl-*N*′-(6-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (25)

The title compound was obtained as described for **24**, starting from **21** (600 mg, 1.3 mmol) (458 mg, 98%): mp: 171–174 °C; IR (KBr) *v*: 2229 (C≡N), 3320–3386 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.22 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.70 (t, 1H, 3-*H*), 2.16 (m, 1H, 3-*H*), 4.59 (s, 2H, –NH_2), 5.64 (br s, 1H, 4-*H*), 6.41–6.50 (m, 3H, 5-*H*, 7-*H*, 8-*H*), 7.75 (d, 2H, 2′-*H*, 6′-*H*), 7.84 (d, 2H, 3′-*H*, 5′-*H*), 8.37 (br s, 1H, –N*H*CSNH–C_7H_4N), 9.91 (br s, 1H, –NHCSN*H*–C_7H_4N). Anal. ($C_{19}H_{20}N_4OS$) theoretical: C, 64.75; H, 5.72; N, 15.90; S, 9.10. Found: C, 64.51; H, 6.06; N, 15.53; S, 8.76.

5.1.20. *R*/*S*-*N*-3-Chlorophenyl-*N*′-(6-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (26)

The title compound was obtained as described for **24**, starting from **22** (600 mg, 1.3 mmol) (418 mg, 89%): mp: 161–163 °C; IR (KBr) *v*: 3214–3338 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.21 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.70 (t, 1H, 3-*H*), 2.13 (m, 1H, 3-*H*), 4.59 (s, 2H, –NH_2), 5.65 (br s, 1H, 4-*H*), 6.41–6.51 (m, 3H, 5-*H*, 7-*H*, 8-*H*), 7.14 (d, 1H, 4′-*H*), 7.31–7.37 (m, 2H, 5′-*H* and 6′-*H*), 7.83 (s, 1H, 2′-*H*), 8.13 (br s, 1H, –N*H*CSNH–C_6H_4Cl), 9.62 (s, 1H, –NHCSN*H*–C_6H_4Cl). Anal. ($C_{18}H_{20}ClN_3OS$) theoretical: C, 59.74; H, 5.57; N, 11.61; S, 8.86. Found: C, 59.75; H, 5.80; N, 11.74; S, 8.48.

5.1.21. *R*/*S*-*N*-4-Chlorophenyl-*N*′-(6-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (27)

The title compound was obtained as described for **24**, starting from **23** (600 mg, 1.3 mmol) (428 mg, 91%): mp: 173–174 °C; IR (KBr) *v*: 3240 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.21 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.69 (t, 1H, 3-*H*), 2.12 (m, 1H, 3-*H*), 4.59 (s, 2H, –NH_2), 5.65 (br s, 1H, 4-*H*), 6.40–6.51 (m, 3H, 5-*H*, 7-*H*, 8-*H*), 7.36 (d, 2H, 2′-*H*, 6′-*H*), 7.55 (d, 2H, 3′-*H*, 5′-*H*), 8.04 (br s, 1H, –N*H*CSNH–C_6H_4Cl), 9.56 (br s, 1H, –NHCSN*H*–C_6H_4Cl). Anal. ($C_{18}H_{20}ClN_3OS$) theoretical: C, 59.74; H, 5.57; N, 11.61; S, 8.86. Found: C, 59.75; H, 5.84; N, 11.74; S, 8.48.

5.1.22. *R*/*S*-*N*-3-Cyanophenyl-*N*′-(6-formamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (28)

A mixture of acetic anhydride (2 ml, 21.16 mmol) and formic acid (1 ml, 26.50 mmol) was heated at 55 °C for 2 h. The mixture was then cooled to 0 °C and anhydrous tetrahydrofuran (2 ml) was added. A solution of **24** (300 mg, 0.851 mmol) in anhydrous tetrahydrofuran was then added dropwise. At the end of the reaction, water (15 ml) was added and the resulting precipitate was collected by filtration. The crude product was then purified by column chromatography on silica gel using a chloroform/methanol solution (20:1) as eluent. The purified product was then crystallized in methanol/water mixture, the resulting precipitate collected by filtration, washed with water and dried (139 mg, 43%): mp: 197–198 °C; IR (KBr) *v*: 1661 (C=O), 2232 (C≡N), 3314 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.26 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.78 (t, 1H, 3-*H*), 2.18 (m, 1H, 3-*H*), 5.78 (br s, 1H, 4-*H*), 6.72 (d, 1H, 8-*H*), 7.42 (d, 1H, 7-*H*), 7.51–7.56 (m, 3H, 5-*H*, 4′-*H* and 5′-*H*), 7.75 (d, 1H, 6′-*H*), 8.03 (s, 1H, 2′-*H*), 8.18 (s, 1H, –N*H*CHO), 8.35 (br s, 1H, –N*H*CSNH–C_7H_4N), 9.81 (s, 1H, –NHCSN*H*–C_7H_4N), 10.03 (m, 1H, –NHC*H*O). Anal. ($C_{20}H_{20}N_4O_2S$) theoretical: C, 63.14; H, 5.30; N, 14.73; S, 8.43. Found: C, 62.97; H, 5.41; N, 14.75; S, 8.63.

5.1.23. *R*/*S*-*N*-4-Cyanophenyl-*N*′-(6-formamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (29)

The title compound was obtained as described for **28**, starting from **25** (300 mg, 0.851 mmol) (282 mg, 87%): mp: 180–181 °C; IR (KBr) *v*: 1659 (C=O), 2228 (C≡N), 3308 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.27 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.77 (t, 1H, 3-*H*), 2.22 (m, 1H, 3-*H*), 5.77 (br s, 1H, 4-*H*), 6.73 (d, 1H, 8-*H*), 7.43 (d, 1H, 7-*H*), 7.50 (s, 1H, 5-*H*), 7.75–7.80 (dd, 4H, 2′-*H*, 3′-*H*, 5′-*H* and 6′-*H*), 8.18 (s, 1H, –N*H*CHO), 8.47 (br s, 1H, –N*H*CSNH–C_7H_4N), 9.98–10.01 (m, 2H, –NHCSN*H*–C_7H_4N and –NHC*H*O). Anal. ($C_{20}H_{20}N_4O_2S$) theoretical C, 63.14; H, 5.30; N, 14.73; S, 8.43. Found: C, 62.75; H, 5.43; N, 14.65; S, 8.12.

5.1.24. *R*/*S*-*N*-3-Chlorophenyl-*N*′-(6-formamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (30)

The title compound was obtained as described for **28**, starting from **26** (300 mg, 0.829 mmol) (307 mg, 95%): mp: 171–173 °C; IR (KBr) *v*: 1666 (C=O), 3233 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6,

500 MHz): δ: 1.26 (s, 3H, C*H*$_3$), 1.37 (s, 3H, C*H*$_3$), 1.78 (t, 1H, 3-*H*), 2.17 (m, 1H, 3-*H*), 5.78 (br s, 1H, 4-*H*), 6.71 (d, 1H, 8-*H*), 7.15 (d, 1H, 5′-*H*), 7.32–7.37 (br m, 2H, 2′-*H*′ and 4′-*H*), 7,43 (d, 1H, 7-*H*), 7,49 (s, 1H, 5-*H*), 7,73 (s, 1H, 6′-*H*), 8,18–8,23 (m, 2H, –N*H*CSNH–C_6H_4Cl and –N*H*CHO), 9.69 (br s, 1H, –NHCSN*H*–C_6H_4Cl), 10.01 (s, 1H, –NHC*H*O). Anal. ($C_{19}H_{20}ClN_3O_2S$) theoretical C, 58.53; H, 5.17; N, 10.78; S, 8.22. Found: C, 58.90; H, 5.23; N, 10.56; S, 7.87.

5.1.25. *R*/*S*-*N*-4-Chlorophenyl-*N*′-(6-formamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (31)

The title compound was obtained as described for **28**, starting from **27** (300 mg, 0.829 mmol) (268 mg, 95%): mp: 197–198 °C; IR (KBr) *ν*: 1663 (C=O), 3311 (N–H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ: 1.25 (s, 3H, C*H*$_3$), 1.37 (s, 3H, C*H*$_3$), 1.76 (t, 1H, 3-*H*), 2.16 (m, 1H, 3-*H*), 5.76 (br s, 1H, 4-*H*), 6.70 (d, 1H, 8-*H*), 7.36 (d, 2H, 2′-*H*, 6′-*H*), 7.41 (d, 1H, 7-*H*), 7.51 (m, 3H, 5-*H*, 3′-*H* and 5′-*H*), 8.13 (br s, 1H, –N*H*CSNH–C_6H_4Cl), 8.18 (s, 1H, –N*H*CHO), 9.64 (br s, 1H, –NHCSN*H*–C_6H_4Cl), 10.01 (s, 1H, –NHC*H*O). Anal. ($C_{19}H_{20}ClN_3O_2S$) theoretical C, 58.53; H, 5.17; N, 10.78; S, 8.22. Found: C, 58.30; H, 5.36; N, 10.70; S, 7.93.

5.2. Biological assays

(±)-Cromakalim (Tocris, UK), diazoxide (Sigma Chemical, USA) and (±)-pinacidil (Sigma Chemical, USA) 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 a further 90 min in 1 ml of the same medium containing 16.7 mM glucose and, in addition, the reference compound or the required chroman derivative. The release of insulin was measured radioimmunologically using rat insulin as a standard.[24,25] 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 200 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).[32]

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.[24,25,27] Briefly, groups of 100 rat pancreatic 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) albumin (fraction V, Sigma) and containing 16.7 mM glucose and ^{86}Rb (0.15–0.25 mmol/L:50 μCi/ml). After incubation, the islets were washed three times with a nonradioactive medium and then placed in a perifusion chamber. The perifusate [in mM: NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24 supplemented with 0.5% (w/v) albumin] 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 and, at the end of the experiment, the radioactive content of the islets was determined. The outflow of ^{86}Rb (cpm/min) was expressed as a fractional outflow rate (FOR, % of instantaneous islet content per min).[24,25,27]

5.6. Statistical evaluation

The statistical significance of differences 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 value was <0.05.

5.7. Predicted partition coefficients

The program ALOGPS 2.1 (VCCLAB, Virtual Computational Chemistry Laboratory, http://www.vcclab.org, 2005) was used for the calculation of AC log *P* values.[30] Such a program is available at the Virtual Computational Chemistry Laboratory and used algorithms have been previously described in the literature.[30]

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