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New *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyrans structurally related to (±)-cromakalim as tissue-selective pancreatic β-cell K_{ATP} channel openers

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Abstract—The present work was aimed at exploring a series of *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyrans structurally related to (±)-cromakalim and differently substituted at the 4- and 6-positions. The biological effects of these putative activators of ATP-sensitive potassium channels (K_{ATP}) were characterized in vitro on the pancreatic endocrine tissue (inhibition of insulin release) and on the vascular smooth muscle tissue (relaxation of aorta rings). The biological activity of these new dimethylchroman derivatives was further compared to that of (±)-cromakalim, (±)-pinacidil, diazoxide and BPDZ 73. Structure–activity relationships indicated that an improved potency for the pancreatic tissue was obtained by introducing a *meta*- or a *para*-electron-withdrawing group such as a chlorine atom on the C-4 phenyl ring, independently of the nature of the halogen atom at the 6-position of the benzopyran nucleus. Most original dimethylchroman thioureas were more potent than their 'urea' homologues and even more potent than diazoxide at inhibiting insulin release. Moreover, and unlike (±)-cromakalim or (±)-pinacidil, such compounds appeared to be highly selective towards the pancreatic tissue. Radioisotopic and fluorimetric investigations indicated that the new drugs activated pancreatic K_{ATP} channels. Lastly, conformational studies suggested that the urea/thiourea dimethylchromans can be regarded as hybrid compounds between cromakalim and pinacidil.

1. Introduction

ATP-sensitive potassium channels (K_{ATP} channels) represent an important class of ionic channels whose function is mainly regulated by changes in the intracellular levels of adenosine triphosphate. These channels are closed when intracellular ATP levels are elevated and opened when intracellular ATP declines whereas intracellular ADP concentration increases, thus linking membrane potential to the metabolic state of the cell.[1]

K_{ATP} channels have been identified in a wide range of cell types. They have initially been described in cardiac myocytes[2] and later found in endocrine cells,[3] skeletal and smooth muscle cells[4,5] and central neurons.[6] Such channels have been shown to be involved in major physiological processes such as hormone secretion, smooth muscle contractile activity and neurotransmitter release.[7]

The K_{ATP} channel is an octameric complex of a sulfonylurea receptor (SURx) and a pore-forming inwardly rectifying potassium channel (Kir6.×) in a 4 + 4 stoichiometry.[8] According to their tissue localization,

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K_{ATP} channels exist in different isoforms resulting from the assembly, in multiple combinations, of the Kir6.x (Kir6.1 and Kir6.2) and the SUR× (SUR1, SUR2A and SUR2B) subunits. For example, SUR1 combines with Kir6.2 to form the pancreatic (insulin-secreting)-β cell K_{ATP} channels.[9] The cardiac and skeletal muscle types consist of SUR2A and Kir6.2 subunits whereas the smooth muscle K_{ATP} channels are composed of SUR2B and Kir6.1 or Kir6.2 subunits.[10]

Given their numerous physiological functions, K_{ATP} channels represent promising drug targets. Therefore, one main challenge in the development of new K_{ATP} channel modulators as therapeutic agents is the discovery of compounds exhibiting the highest selectivity for a single K_{ATP} channel subtype.

A variety of compounds, named 'potassium channel openers' (or PCOs), have been reported to activate K_{ATP} channels. The opening of these K^+ channels leads to plasma membrane hyperpolarization and subsequent reduction in cell excitability. As a result, the activation of K_{ATP} channels has been shown to inhibit endocrine and/or neurotransmitter release, to relax vascular and non-vascular smooth muscle and to shorten cardiac action potentials.[11] Thus, and according to their tissue selectivity, PCOs may be expected to become new therapeutic agents for diseases such as type 1/type 2 diabetes, obesity, hyperinsulinism, arterial hypertension, angina pectoris, bronchial asthma or urinary incontinence.[11–16]

Potassium channel openers consist of a group of compounds with a wide range of chemical structures such as (−)-cromakalim (**1**), (±)-pinacidil (**2**) and diazoxide (**3**) (Fig. 1). (−)-Cromakalim (**1**) is the prototype of the benzopyran potassium channel openers. This drug has been found to exert a marked myorelaxant activity[17] but cromakalim is also known to be only slightly active as an inhibitor of insulin secretion, in contrast to other PCOs like diazoxide (**3**) or BPDZ 73 (**4**) (Fig. 1).[18–20]

In the search for new pancreatic β-cell selective PCOs, we have recently prepared a series of *R*/*S*-4,6-disubstituted 2,2-dimethylchromans structurally related to cromakalim.[21,22] Some of these original drugs were found to be less effective as myorelaxants and much more active as inhibitors of insulin release than the reference molecule cromakalim. These cromakalim analogues can be considered as the first series of benzopyrans that markedly activate the pancreatic β-cell K_{ATP} channels.[21,22] Structure–activity relationships further indicated that the nature of the substituent at the 4-position of the benzopyran nucleus played a crucial role for the development of an inhibitory effect on insulin release. Indeed, a C-4 phenylurea moiety (Fig. 1, compound **5**) was preferred to a C-4 alkylurea, aralkylurea, alkylthiourea, aralkylthiourea, arylsulfonylurea, alkylcarbamate or alkylcarboxamide group.[21,22] Moreover, the introduction of a *meta*- or a *para*-electron-withdrawing group on the phenyl ring

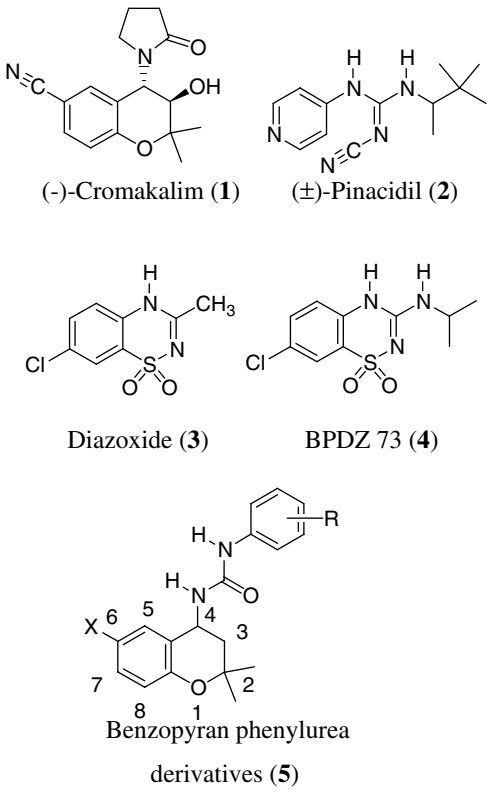


Figure 1. Chemical structure of (−)-cromakalim (**1**), (±)-pinacidil (**2**), diazoxide (**3**), BPDZ 73 (**4**) and benzopyran phenylurea derivatives (**5**).

(R, Fig. 1, compound **5**) was also shown to enhance the pancreatic activity and selectivity of these original *R*/*S*-4,6-disubstituted 2,2-dimethylchromans.[22]

According to these structure–activity relationships deduced from our previous investigations, the present work aimed at developing more active and more insulin-secreting cell selective dimethylchroman derivatives by replacing the C-4 phenylurea moiety by a bioisosteric phenylthiourea group.

The new compounds were examined as putative potassium channel openers on rat pancreatic islets (inhibition of glucose-induced insulin release) and on rat aorta rings (myorelaxant effect on KCl-precontracted aorta rings) in order to evaluate their potency and tissue selectivity. The biological effects of the dimethylchroman thioureas were also compared with those of their urea analogues and the effects of compounds **40** and **42** were further characterized on pancreatic transmembrane cationic movements.

It should be noted that, due to the existence of a possible isosterism between arylurea/arylthiourea and arylcyanoguanidine moieties, such thiourea analogues as well as their C-4 phenylurea counterparts[22] might be regarded as hybrid compounds between cromakalim and pinacidil (Fig. 2). Thus, a conformational study was conducted to judge putative similarities between these dimethylchroman ureas/thioureas and the prototype compounds cromakalim and pinacidil.

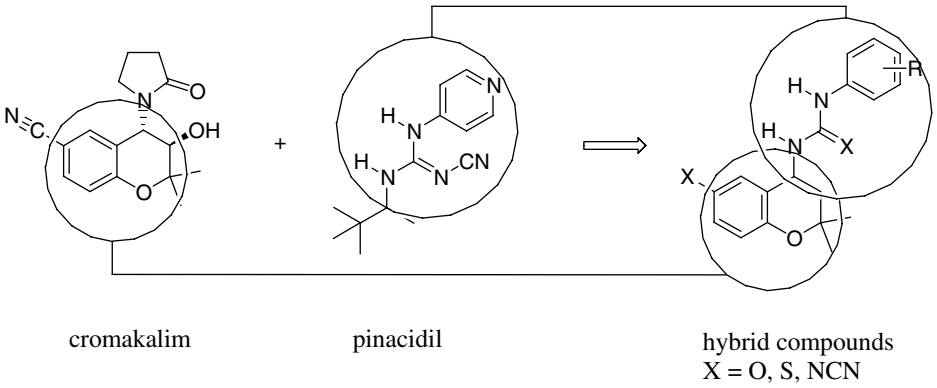
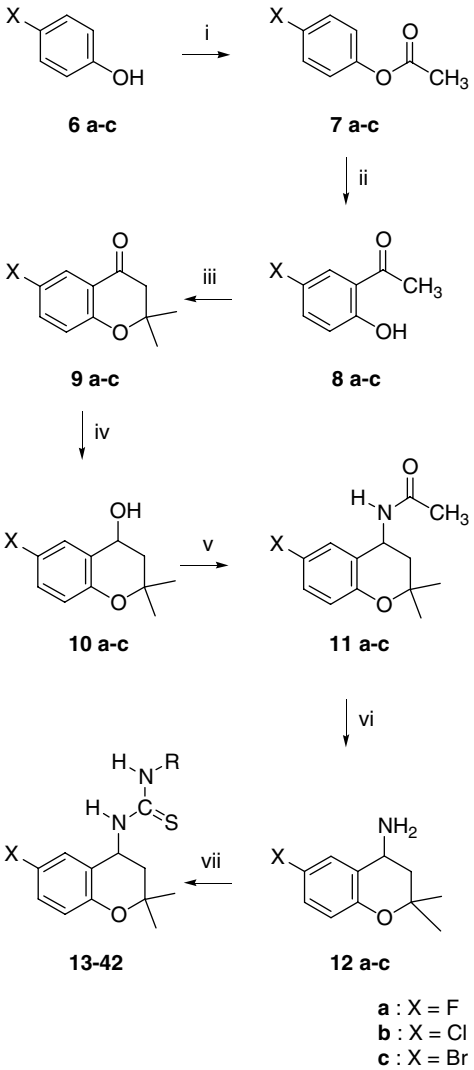


Figure 2. Benzopyran phenylurea and phenylthiourea derivatives as hybrid compounds between cromakalim and pinacidil.

2. Chemistry

Access to *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyrans (**13**–**42**) is described on Scheme 1.[21,23] Such compounds were obtained from the appropriate *para*-halogenophenols in seven steps.

First, *para*-halogenophenols **6a**–**c** were acetylated to provide 4-halogenophenyl acetates **7a**–**c**. Fries rearrangement of the esters was carried out using aluminium chloride at 160 °C to give hydroxyacetophenones **8a**–**c**. The acetophenone intermediates were then involved in a ring closure reaction in the presence of acetone and pyrrolidine and the resulting ketones **9a**–**c** were reduced to alcohols **10a**–**c** with sodium borohydride. The 4-acetylaminobenzopyrans **11a**–**c** were prepared by the Ritter reaction from chromanols **10a**–**c**. This reaction occurred in acetonitrile supplemented with concentrated sulfuric acid. The subsequent hydrolysis of **11a**–**c** with concentrated hydrochloric acid led to the aminochromans **12a**–**c**. Finally, these intermediates were converted to the final *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyrans (**13**–**42**) by reaction with the appropriate isothiocyanate (R–N=C=S). All these derivatives (**13**–**42**) were crystallised from appropriate solvents and characterized by IR, ^{1}H NMR and elemental analyses to obtain the final materials with the chemical purity required before pharmacological evaluations.



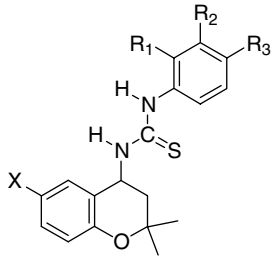
Scheme 1. Synthesis of *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyrans. Reagents: (i) $(CH_3CO)_2O$, H_2SO_4; (ii) $AlCl_3$; (iii) acetone, pyrrolidine; (iv) $NaBH_4$, CH_3OH; (v) CH_3CN, H_2SO_4; (vi) HCl 37%; (vii) RNCS, CH_2Cl_2 (**13**–**42**).

3. Results and discussion

The ability of the newly synthesized dimethylchromans (compounds **13**–**42**) to inhibit the glucose-induced insulin secretion was evaluated on isolated rat pancreatic islets and the myorelaxant activity of the compounds was determined on K^+-depolarized rat aorta rings. (±)-Cromakalim, (±)-pinacidil, diazoxide and BPDZ 73 were used as reference PCOs (Table 1).

(±)-Cromakalim and (±)-pinacidil have previously been shown to be quite inactive at inhibiting insulin release whilst diazoxide and BPDZ 73 reduced the glucose-induced insulin output (Table 1). BPDZ 73 was found to be much more active than diazoxide (% of residual insulin secretion at 10 μM = 73.9 ± 4.4% for diazoxide and 4.9 ± 0.4% for BPDZ 73).

Table 1. Residual insulin secretion and myorelaxant activity of original dimethylchroman thioureas compared to (±)-cromakalim, diazoxide, (±)-pinacidil and BPDZ 73



Compounds	X	R_1	R_2	R_3	% Residual insulin secretion[a] (10 μM)	Myorelaxant activity EC_{50} (μM)[b]
13	F	H	H	H	63.2 ± 4.4 (22)	7.6 ± 0.6 (4)
14	Cl	H	H	H	46.6 ± 2.3 (31)	11.1 ± 2.2 (4)
15	Br	H	H	H	32.8 ± 2.0 (23)	51.5 ± 19.4 (8)
16	F	OCH_3	H	H	82.9 ± 3.5 (23)	>10 (4)
17	Cl	OCH_3	H	H	76.8 ± 5.4 (20)	5.5 ± 1.2 (4)
18	Br	OCH_3	H	H	65.1 ± 3.1 (20)	>10 (6)
19	F	H	OCH_3	H	52.0 ± 2.3 (24)	6.2 ± 0.6 (4)
20	Cl	H	OCH_3	H	34.3 ± 2.3 (21)	2.9 ± 0.5 (4)
21	Br	H	OCH_3	H	36.6 ± 3.1 (19)	>10 (5)
22	F	H	H	OCH_3	73.8 ± 3.2 (24)	21.6 ± 3.4 (4)
23	Cl	H	H	OCH_3	50.4 ± 2.2 (24)	12.7 ± 4.3 (5)
24	Br	H	H	OCH_3	53.1 ± 2.7 (23)	>10 (4)
25	F	CH_3	H	H	69.8 ± 4.2 (16)	11.1 ± 0.8 (4)
26	Cl	CH_3	H	H	69.0 ± 3.0 (24)	>30 (4)
27	Br	CH_3	H	H	57.0 ± 2.5 (29)	>30 (4)
28	F	H	CH_3	H	53.1 ± 3.6 (24)	7.3 ± 0.8 (4)
29	Cl	H	CH_3	H	43.0 ± 3.2 (16)	137.3 ± 10.3 (4)
30	Br	H	CH_3	H	37.2 ± 2.3 (22)	>30 (4)
31	F	H	H	CH_3	60.2 ± 3.0 (32)	>30 (4)
32	Cl	H	H	CH_3	32.8 ± 1.9 (16)	>30 (4)
33	Br	H	H	CH_3	52.2 ± 3.1 (15)	>30 (4)
34	F	Cl	H	H	81.3 ± 2.2 (24)	24.4 ± 1.3 (4)
35	Cl	Cl	H	H	68.2 ± 3.2 (19)	3.2 ± 0.6 (4)
36	Br	Cl	H	H	57.5 ± 3.3 (26)	6.5 ± 0.4 (5)
37	F	H	Cl	H	18.0 ± 1.3 (22)	>10 (4)
38	Cl	H	Cl	H	16.2 ± 1.6 (22)	>10 (4)
39	Br	H	Cl	H	23.0 ± 2.4 (31)	>10 (5)
40	F	H	H	Cl	10.5 ± 1.1 (15)	>10 (4)
41	Cl	H	H	Cl	8.9 ± 0.7 (15)	>10 (5)
42	Br	H	H	Cl	12.2 ± 1.2 (20)	>10 (4)
(±)-Cromakalim	—	—	—	—	94.4 ± 4.1 (32)[c]	0.13 ± 0.01 (7)[c]
(±)-Pinacidil	—	—	—	—	92.1 ± 3.9 (13)[d]	0.35 ± 0.02 (11)[d]
Diazoxide	—	—	—	—	73.9 ± 4.4 (16)[d]	22.4 ± 2.1 (11)[d]
BPDZ 73	—	—	—	—	4.9 ± 0.4 (32)[d]	36.3 ± 2.2 (6)[d]

[a] Percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (means ± SEM (*n*)).

[b] EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aorta rings (means ± SEM (*n*)). *n* refers to the number of samples.

[c] Published results: Ref. 22.

[d] Published results: Ref. 24.

The present biological data revealed that the new *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyrans (**13**–**42**), tested at a 10 μM concentration, were more active on pancreatic β-cells than the reference compounds (±)-cromakalim and (±)-pinacidil. Moreover, most compounds were even found to be more potent than diazoxide. A few drugs provoked a 90% inhibition of glucose-induced insulin release, which can be considered as a close to maximal effect relative to the glucose-insensitive basal insulin release (5–10% residual insulin secretion).

Compounds **13**, **14** and **15**, devoid of substituent on the C-4 phenyl ring, exhibited a pancreatic effect between that of diazoxide and that of BPDZ 73. The nature of the 6-substituent affected the activity and the rank order of potency was 6-Br (**15**) > 6-Cl (**14**) > 6-F (**13**) ($p < 0.05$).

The introduction of an electron-donating group on the C-4 phenyl ring [methoxy (compounds **16**–**24**) or methyl (compounds **25**–**33**)] did not improve the activity on pancreatic β-cells. Most of these compounds were, again, more potent than diazoxide and less potent than

BPDZ 73 at inhibiting the glucose-induced insulin release. Biological results obtained with methoxy drugs (**16**–**24**) indicated that the nature of the halogen atom at the 6-position of the benzopyran nucleus affected the pancreatic activity. Indeed, 6-Cl and 6-Br derivatives appeared to be significantly more potent than their 6-F homologues (compare the activity of **16** vs **18** ($p < 0.05$), **19** vs **20** and **21** ($p < 0.05$), **22** vs **23** and **24** ($p < 0.05$)). Furthermore, *meta*-methoxy and *para*-methoxy drugs were shown to be more active than their *ortho*-methoxy counterparts (compare the activity of **16** vs **19** ($p < 0.05$), **17** vs **20** and **23** ($p < 0.05$), **18** vs **21** and **24** ($p < 0.05$)). Such structure–activity relationships were less obvious for methyl derivatives **25**–**33**.

By contrast, and compared to the unsubstituted derivatives **13**–**15**, the introduction of a weak electron-withdrawing group (a chlorine atom) on the C-4 phenyl ring (compounds **34**–**42**) generally enhanced the activity on the pancreatic tissue. Thus, except for *ortho*-chloro compounds **34**, **35** and **36**, *meta*- (**37**–**39**) and *para*- (**40**–**42**) chloro products provoked a notable inhibitory effect on the glucose-induced insulin secretion ($p < 0.05$). At a 10 μM concentration, these compounds (**37**–**42**) induced an 80–90% inhibition of the insulin secretory rate. The nature of the halogen atom at the 6-position of the benzopyran nucleus of these compounds did not affect the activity on the pancreatic tissue ($p > 0.05$, NS). It is noteworthy that the *m*-chloro- and *p*-chloro-substituted phenylureas counterparts were also the most potent at inhibiting insulin release.[22]

On the vascular model, and as previously reported,[21,24,25] diazoxide and BPDZ 73 provoked a moderate myorelaxant activity ($EC_{50} = 22.4 \pm 2.1$ μM and 36.3 ± 2.2 μM, respectively), while (±)-cromakalim and (±)-pinacidil exhibited marked vasorelaxant properties ($EC_{50} = 0.13 \pm 0.01$ μM and 0.35 ± 0.02 μM, respectively) (Table 1).

The unsubstituted compounds **13**, **14** and **15** also induced vasorelaxant effects although they were less potent than (±)-cromakalim and (±)-pinacidil ($p < 0.05$). Unfortunately, most methoxy-, methyl- and chlorophenylaminothiocarbonylamino drugs (compounds **16**–**24**, **25**–**33** and **34**–**42**) precipitated in the medium before reaching their maximal activity, making difficult to determine their respective EC_{50} values. Nevertheless, all drugs were found to be less active on the vascular tissue than (±)-cromakalim and (±)-pinacidil. Some compounds were even less potent than diazoxide and BPDZ 73 (Table 1).

According to these in vitro data, *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(3- or 4-chlorophenylaminothiocarbonylamino)-2*H*-1-benzopyrans (**37**–**42**) have particularly drawn our attention. Indeed, these compounds were potent inhibitors of the insulin-releasing process (all drugs, tested at a 10 μM concentration, were much more active than diazoxide) while displaying a weak myorelaxant activity (all drugs were less potent than (±)-cromakalim and (±)-pinacidil). Thus, IC_{50} pancreatic value and IC_{50}/EC_{50} ratio were evaluated in order to assess insulin-secreting cell potency and tissue selectivity (pancreatic vs vascular tissue) (Table 2).

The IC_{50} values of the original dimethylchroman thioureas **37**, **38**, **39**, **40**, **41** and **42**, although being higher than that of BPDZ 73, were much lower than those of (±)-cromakalim, (±)-pinacidil and even diazoxide (Table 2). Moreover, and compared to their parent compound (±)-cromakalim, the IC_{50}/EC_{50} ratios indicated that these new drugs exhibited a clear-cut inversion of tissue selectivity (Table 2). Incidentally, and because such new derivatives present a stereogenic centre at the C-4 position of the benzopyran nucleus, the tissue selectivity of the individual optical isomers remains to be determined.

Table 2. Effects of *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(3- or 4-chlorophenylaminothiocarbonylamino)-2*H*-1-benzopyrans, (±)-cromakalim, diazoxide, (±)-pinacidil and BPDZ 73 on insulin secretion from rat pancreatic islets and on the KCl-induced contractions of rat aorta rings

Compounds	Rat pancreatic β-cells		Rat aorta rings		
	% Residual insulin secretion[a]		IC_{50} (μM)[b]	EC_{50} (μM)[c]	IC_{50}/EC_{50}[d]
	10 μM	1 μM			
37	18.0 ± 1.3 (22)	90.2 ± 4.6 (38)	3.25	>10 (4)	<0.32
38	16.2 ± 1.6 (22)	89.5 ± 3.7 (23)	3.12	>10 (4)	<0.31
39	23.0 ± 2.4 (31)	74.6 ± 4.0 (21)	2.59	>10 (5)	<0.25
40	10.5 ± 1.1 (15)	77.5 ± 4.0 (15)	2.30	>10 (4)	<0.23
41	8.9 ± 0.7 (15)	87.9 ± 3.0 (16)	2.75	>10 (5)	<0.27
42	12.2 ± 1.2 (20)	75.7 ± 3.2 (24)	2.26	>10 (4)	<0.22
(±)-Cromakalim	94.4 ± 4.1 (32)[e]	95.3 ± 3.8 (31)[e]	>100[e]	0.13 ± 0.01 (7)[e]	>770
(±)-Pinacidil	92.1 ± 3.9 (13)[f]	97.7 ± 6.7 (19)[f]	>100[f]	0.35 ± 0.02 (11)[f]	>285
Diazoxide	73.9 ± 4.4 (16)[f]	87.5 ± 5.0 (15)[f]	22.6[f]	22.4 ± 2.1 (11)[f]	1.01
BPDZ 73	4.9 ± 0.4 (32)[f]	36.2 ± 2.4 (31)[f]	0.73[f]	36.3 ± 2.2 (6)[f]	0.02

[a] Percentage of residual insulin release from rat pancreatic islets incubated in presence of 16.7 mM glucose (means ± SEM (*n*)).
[b] IC_{50}: drug concentration giving 50% inhibition of insulin release (estimated value).
[c] EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (means ± SEM (*n*)).
[d] Estimated selectivity ratio: pancreatic versus vascular tissue.
[e] Published results: Ref. 22.
[f] Published results: Ref. 24.

Among these new drugs, compounds **40** and **42** were shown to be quite selective for the pancreatic tissue and the most potent at inhibiting the glucose-induced insulin secretion from incubated rat pancreatic islets. Therefore, the next objective of the present study was to determine, by using radioisotopic and fluorimetric approaches, whether the inhibitory effects of such compounds were related to the activation of K_{ATP} channels.

Figure 3 illustrates the effects of compound **40** (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 (•), **40** provoked a rapid, sustained and rapidly reversible increase in the rate of ^{86}Rb outflow. The presence in the perifusate of the hypoglycemic sulfonylurea glibenclamide (10 μM, ○), a pharmacological tool known to block the K_{ATP} channels,[26,27] completely abolished the stimulatory effect of compound **40** on ^{86}Rb outflow. Identical results were obtained when testing, under the same experimental conditions, the effects of compound **42** (data not shown). Such data support the view that compounds **40** and **42** increased the pancreatic β-cell membrane K^+ permeability through the activation of ATP-sensitive potassium channels.[20,28–30]

Because the activation of K_{ATP} channels should hyperpolarize the insulin-secreting cells and restrict Ca^{2+} inflow, we further examined the effects of compounds **40** (10 μM) and **42** (10 μM) on ^{45}Ca FOR and insulin release from islets exposed throughout to insulinotropic glucose concentrations (16.7 mM). In the presence of extracellular Ca^{2+} in the perifusing medium, the addition of compound **40** (10 μM) provoked an immediate, pronounced and reversible inhibition of both ^{45}Ca FOR (Fig. 4, upper panel, •) and insulin release (Fig. 4, lower panel, •). Under such experimental conditions, a decrease in ^{45}Ca FOR is known to result from a reduction in $^{40}Ca^{2+}$ entry through voltage-sensitive Ca^{2+} channels [31]. Thus, the inhibitory effect of **40** on ^{45}Ca outflow can be viewed as the result of a reduction in $^{40}Ca^{2+}$ entry with subsequent reduction in the insulin secretory rate. Such a proposal is further substantiated by the lack of effect of compound **40** (10 μM) on ^{45}Ca outflow from islets perifused throughout in the absence of extracellular Ca^{2+} (Fig. 4, upper panel, ○).

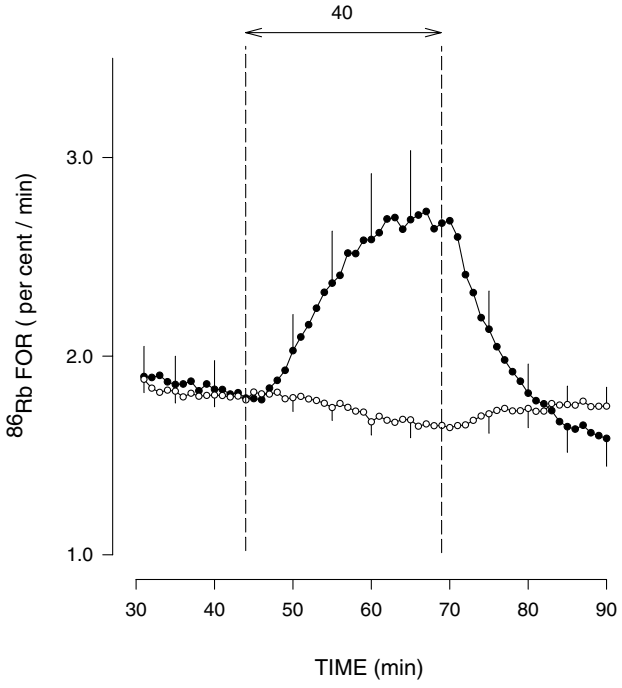


Figure 3. Effect of **40** (10 μM) on ^{86}Rb outflow from rat pancreatic islets perifused throughout in the absence (•) or presence (○) of glibenclamide (10 μM). Basal media contained 5.6 mmol/L glucose and extracellular Ca^{2+}. Mean values (±SEM) refer to six individual experiments.

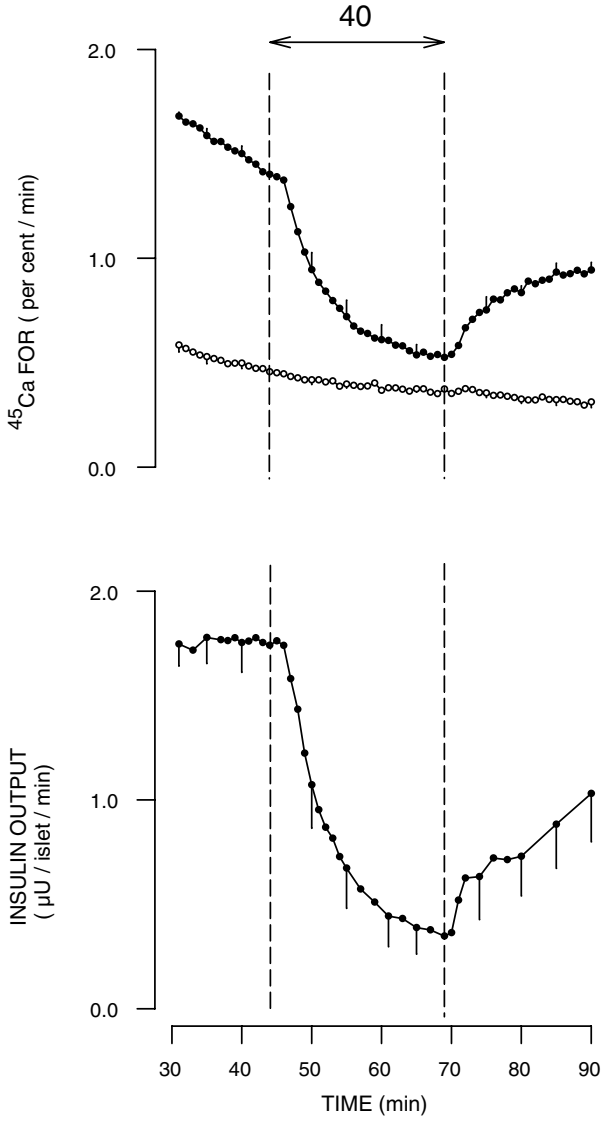


Figure 4. Effect of **40** (10 μM) on ^{45}Ca outflow (upper panel) and insulin release (lower panel) from rat pancreatic islets perifused throughout in the presence of 16.7 mM glucose. Basal media contained extracellular Ca^{2+} (•) or were deprived of Ca^{2+} and enriched with EGTA (○). Mean values (±SEM) refer to four individual experiments.

Experiments conducted with compound **42** gave essentially the same results (data not shown).

Calcium fluorimetry experiments were performed to confirm the ability of compounds **40** and **42** to reduce the pancreatic β-cell cytosolic Ca^{2+} concentration, as a result of their capacity to activate the plasma membrane K_{ATP} channels.

Figure 5 clearly shows that a rise in the extracellular concentration of glucose from 2.8 to 20 mM (upper panel) or in the extracellular concentration of K^+ from 5 to 50 mM (lower panel) provoked a marked increase in $[Ca^{2+}]_i$. The subsequent addition of drug **40** (10 μM) or **42** (10 μM, data not shown) dramatically reduced the glucose-induced rise in cytosolic free Ca^{2+} concentration (Fig. 5, upper panel), indicating that these drugs were able to counteract the increase in $[Ca^{2+}]_i$ provoked by an insulinotropic glucose concentration. On the other hand, compounds **40** (10 μM) and **42** (10 μM; data not shown) barely affected the KCl-induced rise in $[Ca^{2+}]_i$ (Fig. 5, lower panel), demonstrating that the main action of the molecules was accounted for by their capacity to raise K^+ permeability. Indeed, physiological responses to high K^+ concentrations are known to be sensitive to Ca^{2+} channel blockers but resistant to PCOs.[17,20,28,31]

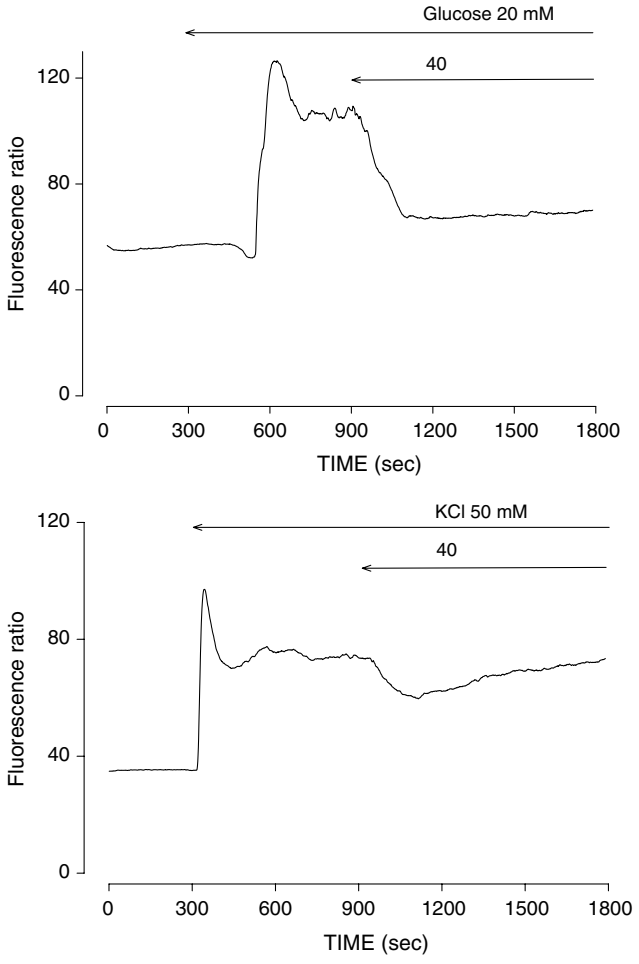


Figure 5. Effect of 10 μM **40** on glucose (20 mM; upper panel)- and KCl (50 mM; lower panel)-induced increase in $[Ca^{2+}]_i$. Basal media contained 2.8 mM glucose and extracellular Ca^{2+}. Each graph is a representative experiment conducted on a single rat pancreatic β-cell.

These radioisotopic and fluorimetric data suggest that *R*/*S*-4-(4-chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-6-fluoro-2*H*-1-benzopyran **40** and *R*/*S*-6-bromo-4-(4-chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran **42** activate the pancreatic β-cell ATP-sensitive potassium channels. Such an event, in turn, decreases Ca^{2+} inflow, reduces the cytosolic Ca^{2+} concentration and, ultimately, inhibits the insulin secretory rate.

From a structural point of view, the present dimethylchroman thioureas, as well as their previously described 'urea' homologues,[22] might be regarded as hybrid compounds between cromakalim and pinacidil (Fig. 2). Indeed, such dimethylchroman derivatives bear the benzopyran nucleus of cromakalim onto which an arylurea/arylthiourea moiety was introduced at the 4-position, the latter moieties being recognized as isosteric substitutes of arylcyanoguanidines (cfr pinacidil; Fig. 2). In order to substantiate this assumption, conformational studies were conducted with a double objective.

The first objective was to investigate the flexibility of the phenyl moiety located on the 'pinacidil-like' part of ureas/thioureas and to compare it with the flexibility of the pyridine ring of pinacidil. Such an analysis should indicate if arylureas/thioureas are able to adopt an energetically accessible conformation close to the preferred conformation of pinacidil.

The second objective was to appreciate the fit of the ureas/thioureas with cromakalim when their benzopyran nucleuses were superimposed. The critical position taken by the exocyclic carbonyl group of cromakalim was then compared to the position adopted by the carbonyl/thiocarbonyl group of the ureas/thioureas.

Geometry optimization first revealed that the preferred conformation adopted by arylureas, arylthioureas and pinacidil confers the same parallel orientation to the two N–H groups of the urea/thiourea/cyanoguanidine function.

A conformational scan was then performed by varying the so-called T1 dihedral angle (1–2–3–4; see Fig. 6) from 0° to 360° by steps of 15° (calculations from simplified structures in the R configuration).

The conformation, stabilized by a CH_{arom}...O bond, into which the urea and the phenyl groups were planar (T1 = 0°) correspond to the most stable conformation for urea derivatives (Fig. 6A). However, as the energetic barriers to the internal rotation T1 were quite low (around 6 kcal/mol), all conformations could be observed.

The conformational space of arylthiourea was quite different (Fig. 6B). The most stable conformation (T1 = 45°) did not favour neither CH_{arom}...S bond

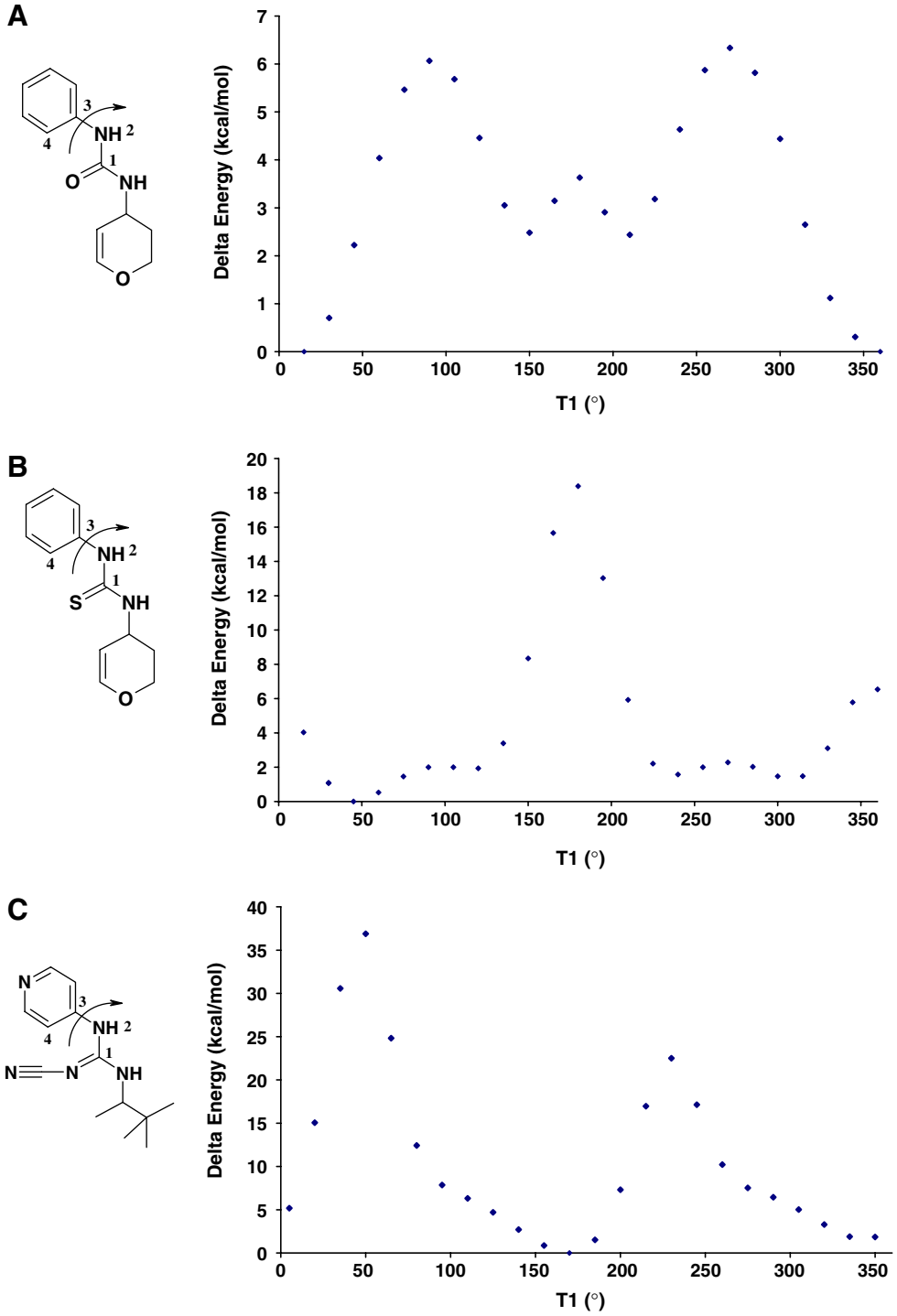


Figure 6. Conformational scan around T1 angle of urea derivative (A), thiourea derivative (B) and pinacidil (C).

nor electronic delocalization between the phenyl and the thiourea groups, though the energetic barrier to reach planarity was very low. Conformations with T1's ranging between 150° and 200° were quite unstable ($\Delta E > 10$ kcal/mol).

Finally, the conformational space of pinacidil was found to be relatively restrained (Fig. 6C). Conformations with T1 = 0° and 180°, where the pyridine and NH moiety are coplanar, were the most stable. In such a case, however, the CN moiety is not located in the vicinity of the hydrogen atom at the 3-position of the pyridine ring. Conformations with the CN moiety in close contact with such a hydrogen atom are not energetically favourable.

The conformations with T1 close to 0°, energetically accessible for the three families of compounds (arylureas, arylthioureas and arylcyanoguanidines), are therefore expected to be the bioactive conformations.

The conformation of the urea/thiourea dimethylchromans was also compared with that of cromakalim, by analysing the position and orientation of the carbonyl (or thiocarbonyl) group considered as being potentially significant for biological activity. Another conformational scan was performed by varying the so-called dihedral T2 angle (5–6–7–8; see Fig. 7A) (calculations from simplified structures in the R configuration).

The conformational space of thiourea, urea and '*des-cyano*'-cromakalim was found to be identical (Fig. 7A, B and C) with two energy minima corresponding to T2 values around 60° and 250°. Therefore, we can conclude that the bioactive conformation of all these compounds is quite similar.

Taken as a whole, the present conformational study reveals that the new urea and thiourea dimethylchromans

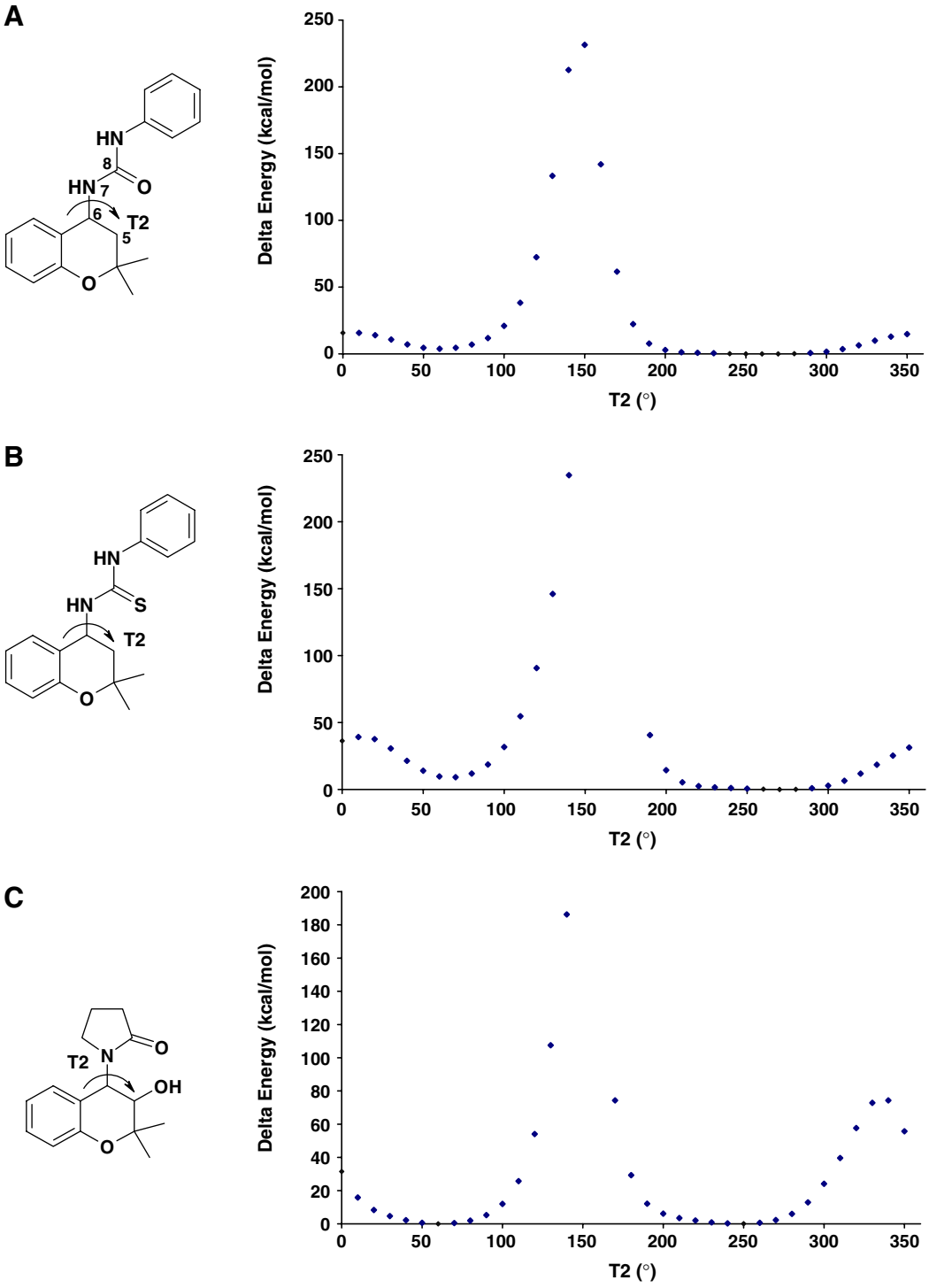


Figure 7. Conformational scan around T2 angle of urea derivative (A), thiourea derivative (B) and '*des-cyano*'-cromakalim (C).

display structural similarities with cromakalim as well as with pinacidil and may thus be considered as hybrid compounds.

4. Conclusion

In the search for new pancreatic selective PCOs, we have synthesized and examined in vitro, on two different models (rat insulin-secreting cells and rat aorta rings), a series of *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyrans structurally related to (±)-cromakalim. These 6-halo compounds (6-F, 6-Cl or 6-Br) were devoid of a substituent, substituted by an electron-donating group (a methoxy or a methyl moiety) or by a weak electron-withdrawing group (a chlorine atom) on the C-4 phenyl ring. Their biological activity was compared to that of (±)-cromakalim, (±)-pinacidil, diazoxide and BPDZ 73, used as reference PCOs.

The new drugs were found to be less active than (±)-pinacidil and (±)-cromakalim as vasorelaxant agents.

On the other hand, data obtained on rat insulin-secreting cells indicated that the new compounds, tested at a 10 μM concentration, were more active than (±)-pinacidil or their parent molecule (±)-cromakalim at inhibiting insulin release. Most drugs were even more effective than diazoxide and compounds **40**–**42** exhibited an IC_{50} value as low as 2–3 μM. Except for compounds **34**–**36**, these new dimethylchroman thioureas were more potent than their 'urea' homologues at reducing the insulin secretory rate.[22]

The highest pancreatic activity was obtained with derivatives bearing a weak electron-withdrawing group on the C-4 phenyl ring, such as a chlorine atom in the *meta*- or *para*-position, independently of the nature of the halogen atom in the 6-position of the benzopyran nucleus. Furthermore, the dimethylchroman thioureas displayed an inverted tissue selectivity (pancreatic tissue vs vascular smooth muscle) compared to that observed with the reference molecule (±)-cromakalim ($IC_{50}/EC_{50} \leqslant 0.32$ and >770, respectively).

Combined radioisotopic and fluorimetric data indicated that the drug-induced inhibition of insulin release was mediated by the activation of pancreatic β-cell ATP-sensitive K^+ channels leading to a decrease in Ca^{2+} influx and a subsequent reduction in cytosolic free Ca^{2+} concentration.

Lastly, conformational studies revealed that the new dimethylchroman thioureas and their previously reported urea counterparts may be considered as 'cromakalim' as well as 'pinacidil' analogues. Such hybrid compounds, however, exhibit a pharmacological profile different from that of the two reference PCOs.

5. Experimental

5.1. Chemistry

Melting points were determined on a Büchi 530 capillary apparatus and were uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1000 FTIR spectrophotometer. The ^1H NMR spectra were recorded on a Bruker Avance (500 MHz) instrument using DMSO-d_6 as solvent with TMS as an internal standard; chemical shifts are reported in δ values (ppm) relative to internal TMS. The abbreviation s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet and b = broad are used throughout. Elemental analyses (C, H, N, S) were realized on a Carlo-Erba EA 1108-elemental analyser 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. Starting materials (12a–c). Starting materials for the synthesis of the original *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyrans (**13**–**42**) are *R*/*S*-4-amino-3,4-dihydro-2,2-dimethyl-6-halo-2*H*-1-benzopyrans (**12a**–**c**). These compounds were prepared from the appropriate *para*-halogenophenols **6a**–**c** according to previously described synthetic procedures.[21,23]

5.1.2. *R*/*S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyran (13). Phenyl isothiocyanate (0.29 mL, 2.4 mmol) was added to a solution of **12a**[23] (0.4 g, 2 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 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 (0.32 g, 47%): mp 164–165 °C; IR (KBr) υ 3357, 3190 (N–H), 3036 (C–H aromatic), 2976, 2929 (C–H aliphatic), 1195 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.26 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.81 (m, 1H, H_A of CH_2), 2.19 (m, 1H, H_B of CH_2), 5.81 (m, 1H, C*H*), 6.76 (dd, 1H, 8-*H*), 6.99 (m, 1H, 7-*H*), 7.05 (d, 1H, 5-*H*), 7.12 (t, 1H, 4′-*H*), 7.33 (m, 2H, 3′-*H*, 5′-*H*), 7.46 (d, 2H, 2′-*H*, 6′-*H*), 8.07 (d, 1H, N*H*(CH)), 9.61 (s, 1H, N*H*(C_6H_5)). Anal. ($C_{18}H_{19}FN_2OS$) theoretical: 65.43% C, 5.80% H, 8.48% N, 9.70% S; found: 65.09% C, 5.76% H, 8.56% N, 9.55% S.

5.1.3. *R*/*S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyran (14). The title compound was obtained as described for **13** starting from **12b**[21] (0.4 g, 1.9 mmol) and phenyl isothiocyanate (0.27 mL, 2.3 mmol) (0.36 g, 55%): mp 172.5–173 °C; IR (KBr) υ 3368, 3192 (N–H), 2971 (C–H aliphatic), 1194 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.27 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.82 (m, 1H, H_A of CH_2), 2.19 (m, 1H, H_B of CH_2), 5.82 (m, 1H, C*H*), 6.78 (d, 1H, 8-*H*), 7.13 (t, 1H, 4′-*H*), 7.18 (d, 1H, 7-*H*), 7.27 (s, 1H, 5-*H*), 7.33 (m, 2H, 3′-*H*, 5′-*H*), 7.47 (d, 2H, 2′-*H*, 6′-*H*), 8.10 (bd, 1H, N*H*(CH)), 9.62 (s, 1H, N*H*(C_6H_5)). Anal. ($C_{18}H_{19}ClN_2OS$) theoretical:

62.33% C, 5.52% H, 8.08% N, 9.24% S; found: 62.57% C, 5.61% H, 8.25% N, 9.05% S.

5.1.4. *R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(phenylaminothiocarbonylamino)-2*H*-1-benzopyran (15). The title compound was obtained as described for **13** starting from **12c**[21] (0.4 g, 1.6 mmol) and phenyl isothiocyanate (0.23 mL, 1.9 mmol) (0.40 g, 65%): mp 172–174 °C; IR (KBr) υ 3363, 3189 (N–H), 3017 (C–H aromatic), 2974 (C–H aliphatic), 1194 (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), 2.19 (m, 1H, H_B of CH_2), 5.82 (m, 1H, C*H*), 6.72 (d, 1H, 8-*H*), 7.13 (t, 1H, 4′-*H*), 7.29–7.35 (m, 3H, 7-*H*, 3′-*H*, 5′-*H*), 7.39 (s, 1H, 5-*H*), 7.46 (d, 2H, 2′-*H*, 6′-*H*), 8.10 (d, 1H, N*H*(CH)), 9.62 (s, 1H, N*H*(C_6H_5)). Anal. ($C_{18}H_{19}BrN_2OS$) theoretical: 55.25% C, 4.89% H, 7.16% N, 8.19% S; found: 55.07% C, 4.89% H, 7.24% N, 8.04% S.

5.1.5. *R/S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(2-methoxyphenylaminothiocarbonylamino)-2*H*-1-benzopyran (16). The title compound was obtained as described for **13** starting from **12a**[23] (0.4 g, 2 mmol) and 2-methoxyphenyl isothiocyanate (0.32 mL, 2.4 mmol) (0.29 g, 40%): mp 132–134 °C; IR (KBr) υ 3246 (N–H), 3029 (C–H aromatic), 2978, 2924 (C–H aliphatic), 1254 (CH_3–O–R), 1196 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.26 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.78 (m, 1H, H_A of CH_2), 2.12 (m, 1H, H_B of CH_2), 3.82 (s, 3H, CH_3O), 5.83 (m, 1H, C*H*), 6.75 (dd, 1H, 8-*H*), 6.92 (m, 1H, 5′-*H*), 6.98 (m, 1H, 7-*H*), 7.00–7.05 (m, 2H, 5-*H*, 3′-*H*), 7.17 (m, 1H, 4′-*H*), 7.77 (d, 1H, 6′-*H*), 8.11 (d, 1H, N*H*(CH)), 9.08 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}FN_2O_2S$) theoretical: 63.31% C, 5.87% H, 7.77% N, 8.90% S; found: 63.04% C, 6.25% H, 7.89% N, 8.50% S.

5.1.6. *R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(2-methoxyphenylaminothiocarbonylamino)-2*H*-1-benzopyran (17). The title compound was obtained as described for **13** starting from **12b**[21] (0.4 g, 1.9 mmol) and 2-methoxyphenyl isothiocyanate (0.31 mL, 2.3 mmol) (0.39 g, 54%): mp 140–141 °C; IR (KBr) υ 3369, 3181 (N–H), 2976, 2951 (C–H aliphatic), 1260 (CH_3–O–R), 1195 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.26 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.78 (m, 1H, H_A of CH_2), 2.12 (m, 1H, H_B of CH_2), 3.83 (s, 3H, CH_3O), 5.84 (m, 1H, C*H*), 6.76 (d, 1H, 8-*H*), 6.92 (m, 1H, 5′-*H*), 7.05 (d, 1H, 3′-*H*), 7.16–7.19 (m, 2H, 7-*H*, 4′-*H*), 7.24 (s, 1H, 5-*H*), 7.75 (d, J = 7.05 Hz, 1H, 6′-*H*), 8.10 (d, 1H, N*H*(CH)), 9.10 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}ClN_2O_2S$) theoretical: 60.55% C, 5.62% H, 7.43% N, 8.51% S; found: 60.59% C, 6.00% H, 7.58% N, 8.16% S.

5.1.7. *R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(2-methoxyphenylaminothiocarbonylamino)-2*H*-1-benzopyran (18). The title compound was obtained as described for **13** starting from **12c**[21] (0.4 g, 1.6 mmol) and 2-methoxyphenyl isothiocyanate (0.26 mL, 1.9 mmol) (0.41 g, 63%): mp 154–155 °C; IR (KBr) υ 3364, 3185 (N–H), 2976, 2950 (C–H aliphatic), 1258 (CH_3–O–R), 1195 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.26 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.78 (m, 1H, H_A of CH_2), 2.12 (m, 1H, H_B of CH_2), 3.84 (s, 3H, CH_3O), 5.85 (m, 1H, C*H*), 6.71 (d, 1H, 8-*H*), 6.92 (m, 1H, 5′-*H*), 7.05 (d, 1H, 3′-*H*), 7.17 (m, 1H, 4′-*H*), 7.29 (d, 1H, 7-*H*), 7.36 (s, 1H, 5-*H*), 7.75 (d, 1H, 6′-*H*), 8.11 (d, 1H, N*H*(CH)), 9.10 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}BrN_2O_2S$) theoretical: 54.16% C, 5.02% H, 6.65% N, 7.61% S; found: 54.25% C, 5.32% H, 6.77% N, 7.34% S.

5.1.8. *R/S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(3-methoxyphenylaminothiocarbonylamino)-2*H*-1-benzopyran (19). The title compound was obtained as described for **13** starting from **12a**[23] (0.4 g, 2 mmol) and 3-methoxyphenyl isothiocyanate (0.34 mL, 2.4 mmol) (0.36 g, 48%): mp 147–148.5 °C; IR (KBr) υ 3181 (N–H), 3028 (C–H aromatic), 2978, 2926 (C–H aliphatic), 1260 (CH_3–O–R), 1194 (C=S) 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, H_A of CH_2), 2.19 (m, 1H, H_B of CH_2), 3.74 (s, 3H, CH_3O), 5.81 (m, 1H, C*H*), 6.69 (d, 1H, 4′-*H*), 6.76 (dd, 1H, 8-*H*), 6.95–7.01 (m, 2H, 7-*H*, 6′-*H*), 7.06 (d, 1H, 5-*H*), 7.16 (s, 1H, 2′-*H*), 7.22 (m, 1H, 5′-*H*), 8.09 (d, 1H, N*H*(CH)), 9.63 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}FN_2O_2S$) theoretical: 63.31% C, 5.87% H, 7.77% N, 8.90% S; found: 63.43% C, 6.17% H, 7.92% N, 8.68% S.

5.1.9. *R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(3-methoxyphenylaminothiocarbonylamino)-2*H*-1-benzopyran (20). The title compound was obtained as described for **13** starting from **12b**[21] (0.4 g, 1.9 mmol) and 3-methoxyphenyl isothiocyanate (0.32 mL, 2.3 mmol) (0.40 g, 56%): mp 152–154 °C; IR (KBr) υ 3308, 3167 (N–H), 2976, 2930 (C–H aliphatic), 1262 (CH_3–O–R), 1201 (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), 2.19 (m, 1H, H_B of CH_2), 3.74 (s, 3H, CH_3O), 5.82 (m, 1H, C*H*), 6.70 (d, 1H, 4′-*H*), 6.77 (d, 1H, 8-*H*), 6.96 (d, 1H, 6′-*H*), 7.15–7.24 (m, 3H, 5-*H*, 7-*H*, 5′-*H*), 7.27 (s, 1H, 2′-*H*), 8.11 (d, 1H, N*H*(CH)), 9.64 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}ClN_2O_2S$) theoretical: 60.55% C, 5.62% H, 7.43% N, 8.51% S; found: 60.77% C, 6.00% H, 7.56% N, 8.27% S.

5.1.10. *R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(3-methoxyphenylaminothiocarbonylamino)-2*H*-1-benzopyran (21). The title compound was obtained as described for **13** starting from **12c**[21] (0.4 g, 1.6 mmol) and 3-methoxyphenyl isothiocyanate (0.27 mL, 1.9 mmol) (0.40 g, 61%): mp 157–158 °C; IR (KBr) υ 3309, 3166 (N–H), 2974, 2930 (C–H aliphatic), 1262 (CH_3–O–R), 1201 (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), 3.74 (s, 3H, CH_3O), 5.83 (m, 1H, C*H*), 6.69–6.73 (m, 2H, 8-*H*, 4′-*H*), 6.95 (d, 1H, 6′-*H*), 7.15 (s, 1H, 2′-*H*), 7.22 (m, 1H, 5′-*H*), 7.29 (d, 1H, 7-*H*), 7.39 (s, 1H, 5-*H*), 8.11 (d, 1H, N*H*(CH)), 9.64 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}BrN_2O_2S$) theoretical: 54.16% C, 5.02% H, 6.65% N, 7.61% S; found: 54.35% C, 5.33% H, 6.79% N, 7.43% S.

5.1.11. *R*/*S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(4-methoxyphenylaminothiocarbonylamino)-2*H*-1-benzopyran (22). The title compound was obtained as described for **13** starting from **12a**[23] (0.4 g, 2 mmol) and 4-methoxyphenyl isothiocyanate (0.32 mL, 2.4 mmol) (0.37 g, 51%): mp 158–159.5 °C; IR (KBr) υ 3182 (N–H), 3031 (C–H aromatic), 2972, 2930 (C–H aliphatic), 1253 (CH_3–O–R), 1196 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.25 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.81 (m, 1H, H_A of CH_2), 2.13 (m, 1H, H_B of CH_2), 3.74 (s, 3H, CH_3O), 5.81 (m, 1H, C*H*), 6.75 (dd, 1H, 8-*H*), 6.90 (d, 2H, 3′-*H*, 5′-*H*), 6.97 (m, 1H, 7-*H*), 7.02 (d, 1H, 5-*H*), 7.28 (d, 2H, 2′-*H*, 6′-*H*), 7.85 (bd, 1H, N*H*(CH)), 9.43 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}FN_2O_2S$) theoretical: 63.31% C, 5.87% H, 7.77% N, 8.90% S; found: 63.46% C, 6.26% H, 7.91% N, 8.68% S.

5.1.12. *R*/*S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(4-methoxyphenylaminothiocarbonylamino)-2*H*-1-benzopyran (23). The title compound was obtained as described for **13** starting from **12b**[21] (0.4 g, 1.9 mmol) and 4-methoxyphenyl isothiocyanate (0.31 mL, 2.3 mmol) (0.35 g, 48%): mp 158–160 °C; IR (KBr) υ 3192 (N–H), 3036 (C–H aromatic), 2974, 2929 (C–H aliphatic), 1249 (CH_3–O–R), 1199 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.26 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.82 (m, 1H, H_A of CH_2), 2.14 (m, 1H, H_B of CH_2), 3.74 (s, 3H, CH_3O), 5.82 (m, 1H, C*H*), 6.76 (d, 1H, 8-*H*), 6.90 (d, 2H, 3′-*H*, 5′-*H*), 7.16 (d, 1H, 7-*H*), 7.24 (s, 1H, 5-*H*), 7.28 (d, 2H, 2′-*H*, 6′-*H*), 7.87 (d, 1H, N*H*(CH)), 9.44 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}ClN_2O_2S$) theoretical: 60.55% C, 5.62% H, 7.43% N, 8.51% S; found: 60.64% C, 5.95% H, 7.58% N, 8.44% S.

5.1.13. *R*/*S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(4-methoxyphenylaminothiocarbonylamino)-2*H*-1-benzopyran (24). The title compound was obtained as described for **13** starting from **12c**[21] (0.4 g, 1.6 mmol) and 4-methoxyphenyl isothiocyanate (0.26 mL, 1.9 mmol) (0.33 g, 51%): mp 153.5–155 °C; IR (KBr) υ 3239 (N–H), 3039 (C–H aromatic), 2975, 2924 (C–H aliphatic), 1251 (CH_3–O–R), 1195 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.26 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.82 (m, 1H, H_A of CH_2), 2.13 (m, 1H, H_B of CH_2), 3.74 (s, 3H, CH_3O), 5.83 (m, 1H, C*H*), 6.71 (d, 1H, 8-*H*), 6.91 (d, 2H, 3′-*H*, 5′-*H*), 7.28 (m, 3H, 7-*H*, 2′-*H*, 6′-*H*), 7.36 (s, 1H, 5-*H*), 7.88 (bd, 1H, N*H*(CH)), 9.44 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}BrN_2O_2S$) theoretical: 54.16% C, 5.02% H, 6.65% N, 7.61% S; found: 54.10% C, 5.32% H, 6.76% N, 7.27% S.

5.1.14. *R*/*S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(2-methylphenylaminothiocarbonylamino)-2*H*-1-benzopyran (25). The title compound was obtained as described for **13** starting from **12a**[23] (0.4 g, 2 mmol) and 2-methylphenyl isothiocyanate (0.32 mL, 2.4 mmol) (0.40 g, 57%): mp 163.5–165 °C; IR (KBr) υ 3340, 3130 (N–H), 2972 (C–H aliphatic), 1191 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.25 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.83 (m, 1H, H_A of CH_2), 2.08 (m, 1H, H_B of CH_2), 2.22 (s, 3H, 2′-CH_3), 5.84 (m, 1H, C*H*), 6.74 (dd, 1H, 8-*H*), 6.95–7.01 (m, 2H, 5-*H*, 7-*H*), 7.15–7.25 (m, 4H, 3′-*H*, 4′-*H*, 5′-*H*, 6′-*H*), 7.81 (bd, 1H, N*H*(CH)), 9.22 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}FN_2OS$) theoretical: 66.25% C, 6.14% H, 8.13% N, 9.31% S; found: 66.39% C, 6.36% H, 8.27% N, 9.00% S.

5.1.15. *R*/*S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(2-methylphenylaminothiocarbonylamino)-2*H*-1-benzopyran (26). The title compound was obtained as described for **13** starting from **12b**[21] (0.4 g, 1.9 mmol) and 2-methylphenyl isothiocyanate (0.31 mL, 2.3 mmol) (0.35 g, 51%): mp 156.5–157.5 °C; IR (KBr) υ 3345, 3130 (N–H), 2974 (C–H aliphatic), 1199 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.25 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.84 (m, 1H, H_A of CH_2), 2.08 (m, 1H, H_B ofCH_2), 2.23 (s, 3H, 2′-CH_3), 5.86 (m, 1H, C*H*), 6.75 (d, 1H, 8-*H*), 7.15–7.25 (m, 6H, 5-*H*, 7-*H*, 3′-*H*, 4′-*H*, 5′-*H*, 6′-*H*), 7.83 (bd, 1H, N*H*(CH)), 9.25 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}ClN_2OS$) theoretical: 63.23% C, 5.86% H, 7.76% N, 8.88% S; found: 63.42% C, 6.12% H, 7.93% N, 8.65% S.

5.1.16. *R*/*S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(2-methylphenylaminothiocarbonylamino)-2*H*-1-benzopyran (27). The title compound was obtained as described for **13** starting from **12c**[21] (0.4 g, 1.6 mmol) and 2-methylphenyl isothiocyanate (0.26 mL, 1.9 mmol) (0.35 g, 55%): mp 162.5–163 °C; IR (KBr) υ 3347, 3168 (N–H), 2972 (C–H aliphatic), 1199 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.25 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.84 (m, 1H, H_A of CH_2), 2.08 (m, 1H, H_B of CH_2), 2.23 (s, 3H, 2′-CH_3), 5.86 (m, 1H, C*H*), 6.70 (d, 1H, 8-*H*), 7.16–7.28 (m, 5H, 7-*H*, 3′-*H*, 4′-*H*, 5′-*H*, 6′-*H*), 7.35 (s, 1H, 5-*H*), 7.82 (bd, 1H, N*H*(CH)), 9.25 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}BrN_2OS$) Theoretical: 56.30% C, 5.22% H, 6.91% N, 7.91% S; found: 56.42% C, 5.29% H, 7.06% N; 7.59% S.

5.1.17. *R*/*S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(3-methylphenylaminothiocarbonylamino)-2*H*-1-benzopyran (28). The title compound was obtained as described for **13** starting from **12a**[23] (0.4 g, 2 mmol) and 3-methylphenyl isothiocyanate (0.32 mL, 2.4 mmol) (0.45 g, 64%): mp 137–139 °C; IR (KBr) υ 3376, 3171 (N–H), 3010 (C–H aromatic), 2977, 2922 (C–H aliphatic), 1197 (C=S) 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, H_A of CH_2), 2.18 (m, 1H, H_B of CH_2), 2.29 (s, 3H, 3′-CH_3), 5.80 (m, 1H, C*H*), 6.76 (dd, 1H, 8-*H*), 6.94–7.06 (m, 3H, 5-*H*, 7-*H*, 4′-*H*), 7.19–7.26 (m, 3H, 2′-*H*, 5′-*H*, 6′-*H*), 8.02 (d, 1H, N*H*(CH)), 9.55 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}FN_2OS$) theoretical: 66.25% C, 6.14% H, 8.13% N, 9.31% S; found: 66.55% C, 6.45% H, 8.28% N, 8.99% S.

5.1.18. *R*/*S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(3-methylphenylaminothiocarbonylamino)-2*H*-1-benzopyran (29). The title compound was obtained as described for **13** starting from **12b**[21] (0.4 g, 1.9 mmol) and 3-methylphenyl isothiocyanate (0.31 mL, 2.3 mmol) (0.41 g, 60%): mp 151–152 °C; IR (KBr) υ 3367, 3164 (N–H), 2976 (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), 2.19 (m, 1H, H_B of CH_2), 2.29 (s, 3H, 3′-CH_3), 5.81 (m, 1H, C*H*), 6.77 (d, 1H, 8-*H*), 6.95 (d, 1H, 4′-*H*), 7.17–7.26 (m, 5H, 5-*H*, 7-*H*, 2′-*H*, 5′-*H*, 6′-*H*), 8.07 (bd, 1H, N*H*(CH)), 9.58 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}ClN_2OS$) theoretical: 63.23% C, 5.86% H, 7.76% N, 8.88% S; found: 62.94% C, 5.94% H, 7.83% N, 8.83% S.

5.1.19. *R*/*S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(3-methylphenylaminothiocarbonylamino)-2*H*-1-benzopyran (30). The title compound was obtained as described for **13** starting from **12c**[21] (0.4 g, 1.6 mmol) and 3-methylphenyl isothiocyanate (0.26 mL, 1.9 mmol) (0.29 g, 46%): mp 160–161 °C; IR (KBr) υ 3363, 3163 (N–H), 2976 (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), 1.80 (m, 1H, H_A of CH_2), 2.18 (m, 1H, H_B of CH_2), 2.30 (s, 3H, 3′-CH_3), 5.82 (m, 1H, C*H*), 6.72 (d, 1H, 8-*H*), 6.95 (d, 1H, 4′-*H*), 7.20–7.31 (m, 4H, 7-*H*, 2′-*H*, 5′-*H*, 6′-*H*), 7.38 (s, 1H, 5-*H*), 8.05 (bd, 1H, N*H*(CH)), 9.56 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}BrN_2OS$) theoretical: 56.30% C, 5.22% H, 6.91% N, 7.91% S; found: 56.53% C, 5.30% H, 7.06% N, 7.64% S.

5.1.20. *R*/*S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(4-methylphenylaminothiocarbonylamino)-2*H*-1-benzopyran (31). The title compound was obtained as described for **13** starting from **12a**[23] (0.4 g, 2 mmol) and 4-methylphenyl isothiocyanate (0.35 mL, 2.4 mmol). The product was then crystallised in a mixture of ethyl acetate/petroleum ether 40:60 (1:3) (0.40 g, 57%): mp 170–170.5 °C; IR (KBr) υ 3181 (N–H), 3031 (C–H aromatic), 2977, 2926 (C–H aliphatic), 1195 (C=S) 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, H_A of CH_2), 2.16 (m, 1H, H_B of CH_2), 2.27 (s, 3H, 4′-CH_3), 5.80 (m, 1H, C*H*), 6.75 (dd, 1H, 8-*H*), 6.96–7.05 (m, 2H, 5-*H*, 7-*H*), 7.13 (d, 2H, 3′-*H*, 5′-*H*), 7.30 (d, 2H, 2′-*H*, 6′-*H*), 7.96 (bd, 1H, N*H*(CH)), 9.51 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}FN_2OS$) theoretical: 66.25% C, 6.14% H, 8.13% N, 9.31% S; found: 65.95% C, 6.11% H, 8.17% N, 9.09% S.

5.1.21. *R*/*S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(4-methylphenylaminothiocarbonylamino)-2*H*-1-benzopyran (32). The title compound was obtained as described for **13** starting from **12b**[21] (0.4 g, 1.9 mmol) and 4-methylphenyl isothiocyanate (0.34 mL, 2.3 mmol) (0.41 g, 60%): mp 172–173.5 °C; IR (KBr) υ 3376, 3191 (N–H), 3035 (C–H aromatic), 2975, 2924 (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), 2.17 (m, 1H, H_B of CH_2), 2.27 (s, 3H, 4′-CH_3), 5.82 (m, 1H, C*H*), 6.77 (d, 1H, 8-*H*), 7.13–7.18 (m, 3H, 7-*H*, 3′-*H*, 5′-*H*), 7.25 (s, 1H, 5-*H*), 7.30 (d, 2H, 2′-*H*, 6′-*H*), 7.98 (bd, 1H, N*H*(CH)), 9.53 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}ClN_2OS$) theoretical: 63.23% C, 5.86% H, 7.76% N, 8.88% S; found: 62.91% C, 6.07% H, 7.79% N, 8.78% S.

5.1.22. *R*/*S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(4-methylphenylaminothiocarbonylamino)-2*H*-1-benzopyran (33). The title compound was obtained as described for **13** starting from **12c**[21] (0.4 g, 1.6 mmol) and 4-methylphenyl isothiocyanate (0.28 mL, 1.9 mmol) (0.29 g, 46%): mp 176–176.5 °C; IR (KBr) υ 3373, 3189 (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), 1.39 (s, 3H, CH_3), 1.81 (m, 1H, H_A of CH_2), 2.16 (m, 1H, H_B of CH_2), 2.28 (s, 3H, 4′-CH_3), 5.82 (m, 1H, C*H*), 6.72 (d, 1H, 8-*H*), 7.14 (d, 2H, 3′-*H*, 5′-*H*), 7.28–7.31 (m, 3H, 7-*H*, 2′-*H*, 6′-*H*), 7.37 (s, 1H, 5-*H*), 7.98 (bd, 1H, N*H*(CH)), 9.52 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{19}H_{21}BrN_2OS$) theoretical: 56.30% C, 5.22% H, 6.91% N, 7.91% S; found: 56.02% C, 5.43% H, 6.96% N, 7.70% S.

5.1.23. *R*/*S*-4-(2-Chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-6-fluoro-2*H*-1-benzopyran (34). The title compound was obtained as described for **13** starting from **12a**[23] (0.4 g, 2 mmol) and 2-chlorophenyl isothiocyanate (0.31 mL, 2.4 mmol) (0.43 g, 57%): mp 160–162 °C; IR (KBr) υ 3379, 3219 (N–H), 3025 (C–H aromatic), 2978, 2928 (C–H aliphatic), 1196 (C=S) 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, H_A of CH_2), 2.14 (m, 1H, H_B of CH_2), 5.80 (m, 1H, C*H*), 6.76 (dd, 1H, 8-*H*), 6.99 (m, 1H, 7-*H*), 7.05 (d, 1H, 5-*H*), 7.26 (m, 1H, 4′-*H*), 7.34 (m, 1H, 5′-*H*), 7.50 (d, 1H, 3′-*H*), 7.64 (d, 1H, 6′-*H*), 8.26 (d, 1H, N*H*(CH)), 9.31 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}ClFN_2OS$) theoretical: 59.25% C, 4.97% H, 7.68% N, 8.79% S; found: 59.04% C, 5.04% H, 7.74% N, 8.61% S.

5.1.24. *R*/*S*-6-Chloro-4-(2-chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (35). The title compound was obtained as described for **31** starting from **12b**[21] (0.4 g, 1.9 mmol) and 2-chlorophenyl isothiocyanate (0.30 mL, 2.3 mmol) (0.31 g, 42%): mp 158–159.5 °C; IR (KBr) υ 3360, 3164 (N–H), 2975 (C–H aliphatic), 1195 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.27 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.82 (m, 1H, H_A of CH_2), 2.14 (m, 1H, H_B of CH_2), 5.82 (m, 1H, C*H*), 6.77 (d, 1H, 8-*H*), 7.17 (d, 1H, 7-*H*), 7.26–7.36 (m, 3H, 5-*H*, 4′-*H*, 5′-*H*), 7.51 (d, 1H, 3′-*H*), 7.62 (d, 1H, 6′-*H*), 8.26 (bd, 1H, N*H*(CH)), 9.33 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}Cl_2N_2OS$) theoretical: 56.70% C, 4.76% H, 7.35% N, 8.41% S; found: 56.39% C, 4.77% H, 7.37% N, 8.29% S.

5.1.25. *R*/*S*-6-Bromo-4-(2-chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (36). The title compound was obtained as described for **13** starting from **12c**[21] (0.4 g, 1.6 mmol) and 2-chlorophenyl isothiocyanate (0.24 mL, 1.9 mmol) (0.36 g, 54%): mp 162–163 °C; IR (KBr) υ 3368, 3164 (N–H), 2977, 2949, 2926 (C–H aliphatic), 1196 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.27 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.82 (m, 1H, H_A of CH_2), 2.13 (m, 1H, H_B of CH_2), 5.83 (m, 1H, C*H*), 6.72 (d, 1H, 8-*H*), 7.26–7.30 (m, 2H, 7-*H*, 4′-*H*), 7.35 (m, 1H, 5′-*H*), 7.42 (s, 1H, 5-*H*), 7.51 (d, 1H, 3′-*H*), 7.60 (d, 1H, 6′-*H*), 8.25 (bd, 1H, N*H*(CH)), 9.33 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}BrClN_2OS$) theoretical: 50.78% C, 4.26% H, 6.56% N, 7.53% S; found: 50.40% C, 4.24% H, 6.58% N, 7.34% S.

5.1.26. *R/S*-4-(3-Chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-6-fluoro-2*H*-1-benzopyran (37). The title compound was obtained as described for **31** starting from **12a**[23] (0.4 g, 2 mmol) and 3-chlorophenyl isothiocyanate (0.31 mL, 2.4 mmol) (0.36 g, 48%): mp 171–172 °C; IR (KBr) υ 3380, 3221 (N–H), 3043 (C–H aromatic), 2977, 2931 (C–H aliphatic), 1196 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.27 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.80 (m, 1H, H_A of CH_2), 2.21 (m, 1H, H_B of CH_2), 5.78 (m, 1H, C*H*), 6.77 (dd, 1H, 8-*H*), 7.00 (m, 1H, 7-*H*), 7.05 (d, 1H, 5-*H*), 7.16 (d, 1H, 4′-*H*), 7.33–7.37 (m, 2H, 5′-*H*, 6′-*H*), 7.73 (s, 1H, 2′-*H*), 8.27 (bd, 1H, N*H*(CH)), 9.72 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}ClFN_2OS$) theoretical: 59.25% C, 4.97% H, 7.68% N, 8.79% S; found: 58.87% C, 5.03% H, 7.69% N, 8.47% S.

5.1.27. *R/S*-6-Chloro-4-(3-chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (38). The title compound was obtained as described for **13** starting from **12b**[21] (0.4 g, 1.9 mmol) and 3-chlorophenyl isothiocyanate (0.30 mL, 2.3 mmol) (0.41 g, 57%): mp 161.5–163 °C; IR (KBr) υ 3339, 3159 (N–H), 3006 (C–H aromatic), 2976, 2925 (C–H aliphatic), 1199 (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), 2.22 (m, 1H, H_B of CH_2), 5.79 (m, 1H, C*H*), 6.79 (d, 1H, 8-*H*), 7.16–7.20 (m, 2H, 7-*H*, 4′-*H*), 7.27 (s, 1H, 5-*H*), 7.33–7.38 (m, 2H, 5′-*H*, 6′-*H*), 7.73 (s, 1H, 2′-*H*), 8.30 (bd, 1H, N*H*(CH)), 9.74 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}Cl_2N_2OS$) theoretical: 56.70% C, 4.76% H, 7.35% N, 8.41% S; found: 56.68% C, 4.73% H, 7.40% N, 8.25% S.

5.1.28. *R/S*-6-Bromo-4-(3-chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (39). The title compound was obtained as described for **13** starting from **12c**[21] (0.4 g, 1.6 mmol) and 3-chlorophenyl isothiocyanate (0.24 mL, 1.9 mmol) (0.45 g, 68%): mp 162–163 °C; IR (KBr) υ 3337, 3155 (N–H), 3001 (C–H aromatic), 2975, 2924 (C–H aliphatic), 1199 (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), 2.21 (m, 1H, H_B of CH_2), 5.80 (m, 1H, C*H*), 6.73 (d, 1H, 8-*H*), 7.16 (d, 1H, 4′-H), 7.30–7.39 (m, 4H, 5-*H*, 7-*H*, 5′-*H*, 6′-*H*), 7.73 (s, 1H, 2′-*H*), 8.30 (d, 1H, N*H*(CH)), 9.74 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}BrClN_2OS$) theoretical: 50.78% C, 4.26% H, 6.58% N, 7.53% S; found: 50.81% C, 4.34% H, 6.62% N, 7.25% S.

5.1.29. *R/S*-4-(4-Chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-6-fluoro-2H 1-benzopyran (40). 4-Chlorophenyl isothiocyanate (0.40 mL, 2.4 mmol) was added to a solution of **12a**[23] (0.4 g, 2 mmol) in methylene chloride (5 mL). After 30 min, the resulting precipitate was collected by filtration, washed with petroleum ether and dried. The crude product was triturated with ethyl acetate. The insoluble 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 recrystallised in ethyl acetate/petroleum ether 40:60 (1:3) (0.35 g, 46%): mp 193–194 °C; IR (KBr) υ 3237 (N–H), 3041 (C–H aromatic), 2978, 2929 (C–H aliphatic), 1197 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.26 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.80 (m, 1H, H_A of CH_2), 2.19 (m, 1H, H_B of CH_2), 5.78 (m, 1H, C*H*), 6.77 (dd, 1H, 8-*H*), 6.99 (m, 1H, 7-*H*), 7.05 (d, 1H, 5-*H*), 7.37 (d, 2H, 3′-*H*, 5′-*H*), 7.51 (d, 2H, 2′-*H*, 6′-*H*), 8.18 (bs, 1H, N*H*(CH)), 9.68 (bs, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}ClFN_2OS$) theoretical: 59.25% C, 4.97% H, 7.68% N, 8.79% S; found: 58.86% C, 5.00% H, 7.65% N, 8.50% S.

5.1.30. *R/S*-6-Chloro-4-(4-chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (41). 4-Chlorophenyl isothiocyanate (0.39 mL, 2.3 mmol) was added to a solution of **12b**[21] (0.4 g, 1.9 mmol) in methylene chloride (5 mL). After 30 min, the resulting precipitate was collected by filtration, washed with petroleum ether and dried. The crude product was triturated with ethyl acetate. The insoluble 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 (0.33 g, 46%): mp 186–187.5 °C; IR (KBr) υ 3240 (N–H), 3042 (C–H aromatic), 2983, 2933 (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), 2.20 (m, 1H, H_B of CH_2), 5.80 (m, 1H, C*H*), 6.78 (d, 1H, 8-*H*), 7.18 (d, 1H, 7-*H*), 7.26 (s, 1H, 5-*H*), 7.37 (d, 2H, 3′-*H*, 5′-*H*), 7.51 (d, 2H, 2′-*H*, 6′-*H*), 8.19 (bd, 1H, N*H*(CH)), 9.68 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}Cl_2N_2OS$) theoretical: 56.70% C, 4.76% H, 7.35% N, 8.41% S; found: 56.35% C, 4.78% H, 7.37% N, 8.40% S.

5.1.31. *R/S*-6-Bromo-4-(4-chlorophenylaminothiocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (42). The title compound was obtained as described for **13** starting from **12c**[21] (0.4 g, 1.6 mmol) and 4-chlorophenyl isothiocyanate (0.32 mL, 1.9 mmol) (0.40 g, 60%): mp 173.5–174.5 °C; IR (KBr): υ: 3239 (N–H), 3088, 3041 (C–H aromatic), 2984, 2933 (C–H aliphatic), 1197 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 1.27 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.80 (m, 1H, H_A of CH_2), 2.19 (m, 1H, H_B of CH_2), 5.80 (m, 1H, C*H*), 6.73 (d, 1H, 8-*H*), 7.30 (d, 1H, 7-*H*), 7.37–7.39 (m, 3H, 5-*H*, 3′-*H*, 5′-*H*), 7.51 (d, 2H, 2′-*H*, 6′-*H*), 8.19 (bd, 1H, N*H*(CH)), 9.68 (s, 1H, N*H*(C_6H_4)). Anal. ($C_{18}H_{18}BrClN_2OS$) theoretical: 50.78% C, 4.26% H, 6.58% N, 7.53% S; found: 50.87% C, 4.21% H, 6.63% N, 7.44% S.

5.2. Biological assays

(±)-Cromakalim (Beecham Pharmaceutical, UK), diazoxide (Sigma Chemical, USA), BPDZ 73 and (±)-pinacidil (Laboratory of Medicinal Chemistry, ULg, Belgium) were tested as reference compounds.

5.2.1. 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) dialysed 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.[18]

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.

5.2.2. Measurement of tension in rat aorta rings. Experiments were performed with aortae 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).[32]

5.2.3. Measurements of ^{86}Rb, ^{45}Ca outflow and insulin release from perifused rat pancreatic islets. Experiments were performed with pancreatic islets isolated from adult fed Wistar rats (Charles River Laboratories, Belgium).

The media used for incubating, washing and perifusing the islets consisted of a physiological salt medium (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.56, $NaHCO_3$ 24, $MgCl_2$ 1) supplemented with 0.5% (w/v) dialysed albumin (fraction V, Sigma) and gassed with O_2 (95%) and CO_2 (5%).

The methods used to measure ^{86}Rb outflow, ^{45}Ca outflow and insulin release from perifused pancreatic islets have been described previously.[28,31] Briefly, groups of 100 islets were incubated for 60 min at 37 °C in the physiological medium containing 16.7 mM glucose and either ^{86}Rb (0.15–0.25 mmol/L:50 μCi/mL) or ^{45}Ca (0.02–0.04 mmol/L:100 μCi/mL). The validity of ^{86}Rb as a tracer for the study of K^+ handling in the islets has been assessed previously.[33] After incubation, the islets were washed three times with non-radioactive medium and then placed in a perfusion chamber. The perifusate 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 (0.6 mL) was used for scintillation counting while the remainder was stored at −20 °C for insulin radioimmunoassay. At the end of the perifusion, the radioactive content of the islets was also determined. The outflow of ^{86}Rb or ^{45}Ca (cpm/min) was expressed as a fractional outflow rate (% of instantaneous islet content per min; FOR).

Some media contained no $CaCl_2$ and were enriched with 0.5 mM EGTA (Sigma).

Results are expressed as means (±SEM) together with the number of individual experiments (n).

5.2.4. Measurement of cytosolic Ca^{2+} concentration from single rat pancreatic islet cells. Pancreatic islets were disrupted in a Ca^{2+}-deprived medium and then centrifuged through an albumin solution to remove debris and dead cells. Cells were seeded onto glass coverslips and maintained in tissue culture for 72 h before use. The cells were then incubated with fura-2 AM (2 μmol/L) (Molecular Probes) for 1 h. The medium used to perifuse the cells contained (in mM) NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24, glucose 2.8 and was gassed with O_2 (95%) and CO_2 (5%). When high concentrations (50 mM) of extracellular K^+ were used, the concentration of extracellular NaCl was lowered to keep the osmolarity constant. Fura-2 fluorescence of single-loaded cells was measured by use of dual-excitation microfluorimetry with a Carl Zeiss ratio imaging system (Carl Zeiss Belgium). The excitation and emission wavelengths were set at 340/380 and 510 nm, respectively. Data are presented as ratio signals (F340/F380). The experiments were repeated on different cell populations.

5.3. Statistical evaluation

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

5.4. Conformational studies

Quantum mechanical calculations using Density Functional Theory at the PBE0/6-31G(d) level have been used to investigate the intrinsic conformational preferences of urea and thiourea compounds in the gas phase in comparison with pinacidil and cromakalim. All calculations have been performed with the Gaussian03 program.

A conformational scan was first performed by varying the so-called T1 dihedral angle (1–2–3–4; see Fig. 6) from 0 to 360°. The crystal structure of pinacidil[34] and *R*/*S*-6-bromo-4-(4-chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran was used as a starting point. A QM-minimized structure was first calculated for thiourea because the crystal structure was not available. In order to simplify the calculations, only one part of molecules, in the R configuration, was used.

In the second set of calculations, the crystal structure of cromakalim[35] was used as a starting point but the cyano group at the 6-position was removed in order to simplify the calculations. The halo substituents on the aromatic rings were also removed from urea and thiourea dimethylchromans. The three structures were in the R configuration.

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