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Original article

Influence of the alkylsulfonylamino substituent located at the 6-position of 2,2-dimethylchromans structurally related to cromakalim: From potassium channel openers to calcium entry blockers?

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ABSTRACT

The present study described the synthesis of original *R*/*S*-6-alkylsulfonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans bearing a 3- or 4-substituted phenylthiourea or phenylurea moiety at the 4-position. Their biological effects were evaluated both on insulin-secreting and smooth muscle cells and were compared to those of reference K_{ATP} channel activators such as (±)-cromakalim, diazoxide and previously synthesized cromakalim analogues. The study aimed at exploring the influence of the introduction of an alkylsulfonylamino substituent at the 6-position of 2,2-dimethylchromans in order to improve biological activity, tissue selectivity but also hydrophilicity of dihydrobenzopyran derivatives. Several compounds were found to be equipotent or even more potent than (±)-cromakalim and diazoxide at inhibiting the insulin releasing process. Most of the newly synthesized and more hydrophilic dihydrobenzopyrans also exhibited a marked vasorelaxant activity although they were less potent than (±)-cromakalim. Additional pharmacological and radioisotopic investigations suggested that *R*/*S*-*N*-3-chlorophenyl-*N'*-(3,4-dihydro-6-methylsulfonylamino-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (**21**) did not act as a potassium channel opener but rather as a Ca^{2+} entry blocker.

1. Introduction

Potassium channels represent a wide family of proteins involved in various cellular processes. Such channels are ubiquitously expressed and play a key role in the control of the membrane potential [1]. Among this family of ionic channels, ATP-sensitive potassium channels (K_{ATP} channels) have been shown to link cell metabolism to membrane excitability. Indeed, the activity of K_{ATP} channels is mainly coupled to the intracellular concentration ratio of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). As the ratio increases, the K_{ATP} channel activity is reduced. At the opposite, a decrease of the ATP/ADP ratio activates the K_{ATP} channels [2–4].

K_{ATP} channels are distributed in many tissues where they play a large variety of physiological roles [5]. K_{ATP} channels are involved in the control of the insulin secretory process from pancreatic B-cells [6–8] and have also been depicted as participating in the control of the smooth muscle vascular tone [9].

K_{ATP} channels are octameric complexes comprising four Kir6.x subunits (Kir6.1 or Kir6.2), members of the inwardly rectifying K^+ channel family, and four sulfonylurea receptor (SUR) subunits (SUR1, SUR2A, or SUR2B). The Kir6.0 subunit forms the pore of the K_{ATP} channel complex whereas the SUR subunit acts as a regulator of K_{ATP} channel activity [5]. The combination of the different subunits leads to tissue-specific K_{ATP} channels, potential target sites for drugs. For example, four SUR1 subunits combine with four Kir6.2 subunits to form the SUR1/Kir6.2 K_{ATP} channel subtype as found in the endocrine pancreas and the brain tissue [10] whereas a SUR2A/Kir6.2 channel subtype is expressed in cardiac and skeletal muscle cells [11]. A SUR2B/Kir6.1 combination has been found in vascular smooth muscle cells while a SUR2B/Kir6.2 assembly has been characterized in various smooth muscle cells [11–13].

Abbreviations: Kir, inwardly rectifying potassium channel; SUR, sulfonylurea receptor; K_{ATP} channel, ATP-sensitive potassium channel.

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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 ATP-sensitive potassium channel openers (PCOs) include, among others, the treatment of arterial hypertension [14,15], angina pectoris [14,16], cardiac arrhythmias [14,17], bronchial asthma [14,18], urinary incontinence [14,19] and androgenic alopecia [14,20]. PCOs have also been proposed for the prevention and/or management of type I, type II diabetes, obesity [14,15,21,22], nesidioblastosis [14], insulinomas [14] and polycystic ovary syndrome [21,23].

(±)-Cromakalim (**1**) and diazoxide (**2**) are well known PCOs belonging to different chemical families (Fig. 1). (±)-Cromakalim (**1**), leader of the dihydrobenzopyran-type PCOs, has been found to exert a marked myorelaxant activity [24,25] although the drug has also been reported to be slightly active as inhibitor of the insulin secretory process [26]. By contrast, diazoxide (**2**), leader of the benzothiadiazine 1,1-dioxide-type PCOs, has been reported to be equipotent on the vascular smooth muscle and the insulin secreting-cells [26,27].

We have previously identified original *R*/*S*-3,4-dihydro-2,2-dimethyl-6-halo-2*H*-1-benzopyrans structurally related to (±)-cromakalim, among which four derivatives exhibiting a modified tissue selectivity profile have been characterized [28,29]. 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 (compounds **3**–**6**, Fig. 1) were found to be less effective as vasorelaxants but more potent as inhibitors of the insulin secretory process than the reference molecule (±)-cromakalim (**1**) [28]. Structure–activity relationships further indicated that the nature of the substituent at the 4-position of the benzopyran nucleus played a crucial role in the expression of an inhibitory effect on insulin release. Unfortunately, such compounds exhibited a weak solubility in the physiological medium. Thus, in another study, we kept identical substitutions at the 4-position and explored the influence of the nature of the substituent at the 6-position in order to develop 4,6-disubstituted *R*/*S*-2,2-dimethylchromans displaying improved hydrophilicity and selectivity towards insulin secreting cells. This approach allowed us to synthesize more hydrophilic 4-phenylthiourea-substituted *R*/*S*-6-acetamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans (compounds **7**–**10**, Fig. 1) but the latter compounds were less active on the endocrine pancreatic tissue than previously described derivatives [30].

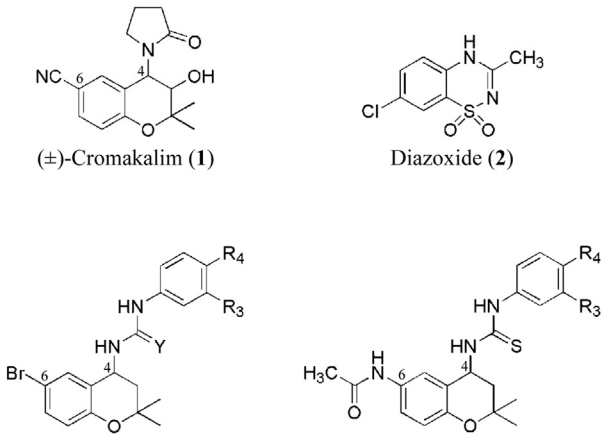


Fig. 1. Chemical structure of (±)-cromakalim (**1**), diazoxide (**2**) and previously described compounds **3**–**10** [30,32].

According to the structure–activity relationships deduced from our previous investigations, the present work aimed at further exploring the influence of the nature of the substituent at the 6-position in order to develop 4,6-disubstituted *R*/*S*-2,2-dimethylchromans displaying a marked inhibitory activity on the insulin secretory process but also an improved hydrophilicity. Therefore, the newly synthesized compounds were bearing, at the 6-position, a sulfonamide function as a bioisosteric group of the amide function characterizing previously described compounds (**7**–**10**) [31].

2. Chemistry

The common intermediate, *R*/*S*-6-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (**15**), giving access to the target compounds (**19**–**34**) was synthesized as previously described [30] in four steps; starting from 4-methoxyaniline (Scheme 1).

Firstly, 4-methoxyaniline (**11**) was acetylated by acetic anhydride to provide *N*-(4-methoxyphenyl)acetamide (**12**). This reaction was followed by a Friedel–Crafts acylation to give *N*-(3-acetyl-4-hydroxyphenyl)acetamide (**13**). 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 **13** with acetone, in the presence of pyrrolidine, led to chromanone intermediate **14**.

The acetamido group of intermediate **14** was hydrolyzed in an alcoholic solution of diluted hydrochloric acid.

The amine function of the resulting intermediate (**15**) was substituted by methanesulfonyl chloride or ethanesulfonyl chloride which provides compound **16a** or **16b**, respectively.

In order to obtain the key intermediates **18a**, **b**, the two ketonic compounds **16a** and **16b** were treated with hydroxylamine hydrochloride and potassium carbonate to give the corresponding oximes **17a**, **b**, which were further hydrogenated in the presence of Raney–Nickel.

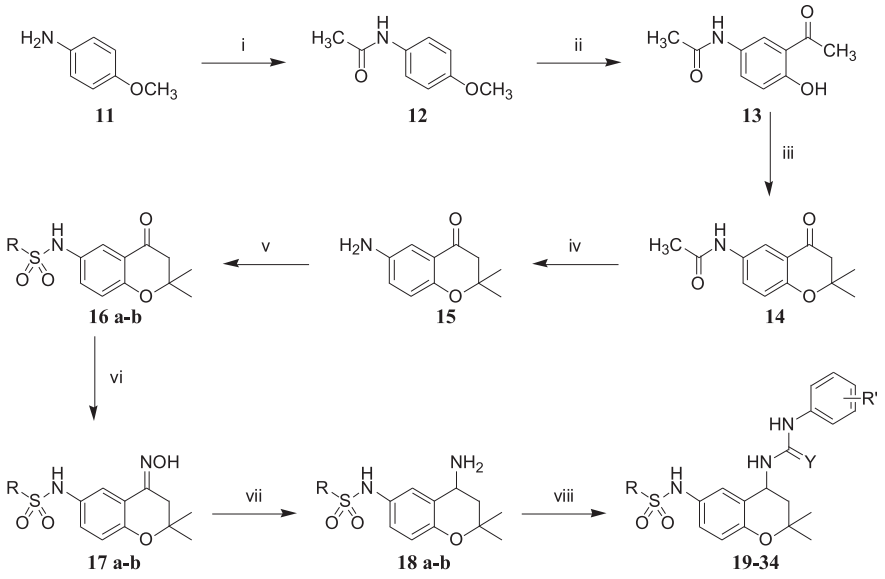
R/*S*-*N*-(*m*/*p*-substituted)phenyl-*N*′-(6-methylsulfonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thioureas (**19**–**22**) or ureas (**23**–**26**) as well as *R*/*S*-*N*-(*m*/*p*-substituted)phenyl-*N*′-(6-ethylsulfonlamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thioureas (**27**–**30**) or ureas (**31**–**34**) were obtained from the reaction of the amines 18a–b with the appropriate *m*/*p*-cyano/chlorophenyl isothiocyanate (R–N=C=S) or isocyanate (R–N=C=O) (Scheme 1).

3. Results and discussion

3.1. Insulin secretion

The ability of the newly synthesized compounds (Table 1, compounds **19**–**34**) 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).

(±)-Cromakalim and diazoxide were used as reference PCOs (Table 1). The biological activity of the original 2,2-dimethylchroman derivatives was also compared to that of previously described molecules (**7**–**10** and **3**–**6**) (Table 1) [28–30].



-R = -CH_3 (**16a**, **17a**, **18a**) or -CH_2CH_3 (**16b**, **17b**, **18b**)

Reagents: (i) Ac_2O, HOAc; (ii) AcCl, $AlCl_3$; (iii) acetone, pyrrolidine, methanol; (iv) HCl 5 N in ethanol; (v) RSO_2Cl, pyridine, CH_2Cl_2; (vi) $NH_2OH.HCl$, K_2CO_3, ethanol; (vii) H_2, Raney-Nickel®, ethanol ; (viii) 3- or 4-chloro/3- or 4-cyanophenyl isothiocyanate (R'–N=C=S), CH_2Cl_2.

Scheme 1. Synthetic pathway to (±)-cromakalim analogues **19**–**34**.

Biological data obtained with (±)-cromakalim, tested at a 10 μM concentration, indicated that this reference PCO was roughly inactive on the pancreatic endocrine tissue (Table 1). Diazoxide (10 μM), however, provoked a ±20% reduction in the insulin secretory rate (Table 1).

By contrast, previously synthesized 2,2-dimethylchromans (**7**–**10** and **3**–**6**), although being only slightly water soluble, were much more potent than (±)-cromakalim and diazoxide at inhibiting the glucose-induced insulin release ($p < 0.05$) (Table 1). Structure–activity relationships suggested that the presence of an electron-withdrawing 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 improved the inhibitory effect on the insulin releasing process [28,29]. Therefore, an electron-withdrawing group, such as a chlorine atom or a cyano group, was held at the 3- or 4-position and new moieties located at the 6-position were explored in order to develop original dimethylchroman-type analogues with an enhanced hydrophilicity.

The secretory data showed that the new chroman derivatives (**20**–**34**, except **19** and **25**), by contrast to (±)-cromakalim, inhibited the insulin-releasing process. Among these newly synthesized analogues, some compounds were found to be equipotent ($p > 0.05$) (**20**, **23**, **24**, **26**, **31**–**34**) or even more potent than diazoxide (**21**, **22**, **27**–**30**) at reducing the glucose-induced insulin response ($p < 0.05$) (Table 1).

6-Methylsulfonylamino-substituted benzopyran derivatives bearing a chlorine atom on the phenyl ring linked to the thiourea function (**21**, **22**) were more potent than the corresponding compounds bearing a cyano group (**19**, **20**) ($p < 0.05$). Drugs **21**, **22** were even more potent than diazoxide at inhibiting the insulin secretory process ($p < 0.05$). The inhibitory effect on the insulin releasing process was more marked for the phenylthiourea derivatives bearing a chlorine atom than for the corresponding chloro-substituted phenylurea compounds (**21**, **22** vs **25**, **26**, $p < 0.05$). However, the isosteric replacement of the 6-acetamido group (**7**–**10**) by the 6-methylsulfonylamino group (**19**–**22**) did not improve the inhibitory activity on the pancreatic endocrine tissue. Compounds **19** and **20** were found to be less potent than **7** and **8** ($p < 0.05$) and compounds **21** and **22** were equipotent to **9** and **10** ($p > 0.05$).

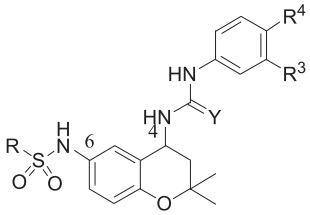
Among the compounds bearing a 6-ethylsulfonylamino group (**27**–**34**), the thiourea derivatives (**27**–**30**) were more potent than the corresponding urea derivatives (**31**–**34**, $p < 0.05$) and than diazoxide at inhibiting the insulin releasing process ($p < 0.05$). Drugs bearing a chlorine atom (**29** and **30**) at the meta or para position of the phenyl group of the thiourea chain were again more potent than those bearing a cyano group (**27** and **28**, $p < 0.05$). Table 1 indicated that several compounds bearing a 6-ethylsulfonylamino moiety (**27**–**34**) were more active ($p < 0.05$) than those bearing a 6-methylsulfonylamino group (**19**–**26**) (except **23** vs **31** and **24** vs **32**).

Unfortunately, all these newly synthesized 6-methyl- and 6-ethylsulfonylamino-substituted 2,2-dimethylchromans remained less active at reducing the insulin secretory rate than the previously described 6-bromo-substituted analogues (**3**–**6**, $p < 0.05$).

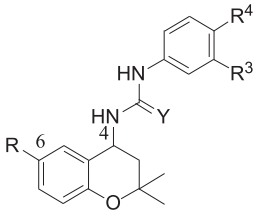
3.2. Myogenic activity

The potential vasorelaxant activity of the original derivatives was determined on 30 mM K^+-depolarized rat aorta (endothelium-free) rings and was compared to that of (±)-cromakalim and

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.



19-34



3-10

Compounds	*Y*	*R*	R^3	R^4	AC log *P*[a]	Residual insulin secretion (%)[b]	Myorelaxant activity
						10 μM	EC_{50} (μM)[c]
19	S	CH_3–	CN		2.91	91.7 ± 4.8 (29)	9.8 ± 2.4 (4)
20				CN	2.91	81.7 ± 3.3 (30)	11.6 ± 3.1 (4)
21			Cl		3.71	69.2 ± 2.9 (24)	9.8 ± 1.6 (11)
22				Cl	3.71	63.9 ± 3.8 (22)	11.6 ± 1.7 (4)
23	O	CH_3–	CN		2.61	84.4 ± 4.6 (30)	9.3 ± 1.5 (4)
24				CN	2.61	81.6 ± 4.0 (29)	19.8 ± 3.1 (5)
25			Cl		3.41	94.5 ± 4.3 (23)	15.2 ± 1.1 (4)
26				Cl	3.41	88.5 ± 4.0 (23)	>30.0 (8)
27	S	CH_3–CH_2–	CN		3.42	64.7 ± 2.4 (16)	12.1 ± 4.3 (6)
28				CN	3.42	57.9 ± 2.1 (16)	12.0 ± 2.1 (6)
29			Cl		4.22	54.3 ± 2.3 (24)	9.8 ± 2.1 (4)
30				Cl	4.22	49.1 ± 2.2 (23)	5.1 ± 0.4 (3)
31	O	CH_3–CH_2-	CN		3.12	75.6 ± 3.2 (24)	9.5 ± 2.1 (5)
32				CN	3.12	73.6 ± 4.3 (24)	11.8 ± 3.2 (6)
33			Cl		3.92	72.1 ± 3.6 (31)	17.2 ± 1.6 (4)
34				Cl	3.92	78.1 ± 3.0 (30)	>10.0 (8)
7	S	CH_3–CO–NH–	CN		3.54	60.9 ± 3.3 (21)[d]	11.9 ± 1.4 (4)[d]
8				CN	3.54	55.8 ± 3.4 (21)[d]	>10.0 (4)[d]
9	S	CH_3–CO–NH–	Cl		4.34	63.1 ± 2.9 (21)[d]	>10.0 (6)[d]
10				Cl	4.34	60.4 ± 3.3 (21)[d]	8.3 ± 1.7 (4)[d]
3	S	Br–	Cl		5.43	23.0 ± 2.4 (31)[d]	>10 (5)[d]
4				Cl	5.43	12.2 ± 1.2 (20)[d]	>10 (4)[d]
5	O	Br–	Cl		5.14	28.6 ± 1.7 (22)[e]	>30 (4)[e]
6				Cl	5.14	25.7 ± 1.5 (23)[e]	>300 (4)[e]
(±)-Cromakalim					1.82	94.4 ± 4.1 (32)[d]	0.13 ± 0.01 (7)[d]
Diazoxide					n.d.	80.8 ± 3.7 (32)	26.1 ± 2.9 (4)

[a] Ref [33].
[b] Percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)).
[c] EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aorta rings (mean ± SEM (*n*)).
[d] Published compound and results (Ref [30]).
[e] Published compound and results (Ref [32]).

diazoxide. The myorelaxant properties of the newly synthesized compounds were also compared with those of previously described molecules (**7**–**10** and **3**–**6**) [28–30].

On the vascular tissue, (±)-cromakalim displayed a marked myorelaxant activity while diazoxide exhibited moderate vasorelaxant properties (Table 1). Several previously synthesized 6-acetamido-substituted and 6-bromo-substituted 2,2-dimethylchromans (**7**–**10** and **3**–**6**) also produced a vasorelaxant effect but such compounds were less potent than (±)-cromakalim (Table 1). It should be noticed that compounds **8**, **9** and **3**–**6** precipitated in the bathing medium before reaching their maximal myorelaxant activity [30,32], making it difficult to accurately determine their EC_{50} values (Table 1).

The new 6-methyl- and ethylsulfonylamino-substituted 2,2-dimethylchroman analogues (**19**–**34**), which did not precipitate into the experimental medium (except for compounds **26** and **34**) and displayed accurately quantifiable EC_{50} values, exhibited a weaker vasorelaxant activity than (±)-cromakalim. However, most of these original compounds exerted a more pronounced myorelaxant activity than diazoxide ($p < 0.05$). Compound **24** was found to be equipotent to diazoxide ($p > 0.05$).

In most cases, the thiourea derivatives (**19**–**22** and **27**–**30**) appeared to elicit a slightly more potent myorelaxant effect than their corresponding urea analogues (**23**–**26** and **31**–**34**). Moreover, the nature and the position of the substituent on the phenyl ring did not markedly affect the myorelaxant activity of the alkanesulfonamides (**19**–**34**) ($p > 0.05$). Compound **30** was found to be the most potent 6-alkanesulfonamide derivative.

The biological data reported on Table 1 further suggest that the myorelaxant capacity of the newly synthesized alkanesulfonamide analogues was more pronounced than that of the reference 2,2-dimethylchroman compounds, namely the acetamide analogues (**7**–**10**) and the 6-bromo-substituted 2,2-dimethylchromans (**3**–**6**).

In accordance with the predicted AC log *P* values [33], the new molecules were also found to be more hydrophilic and therefore should be more soluble in the physiological medium than the previously described 6-bromo-substituted and 6-acetamido-substituted 2,2-dimethylchromans (Table 1) [30].

3.3. *Effects of **21** on the contractile activity of rat aorta rings incubated in the presence of different glibenclamide or KCl concentrations*

Compound **21**, a methanesulfonamide derivative more active on the smooth muscle than on the insulin-secreting cells, was selected

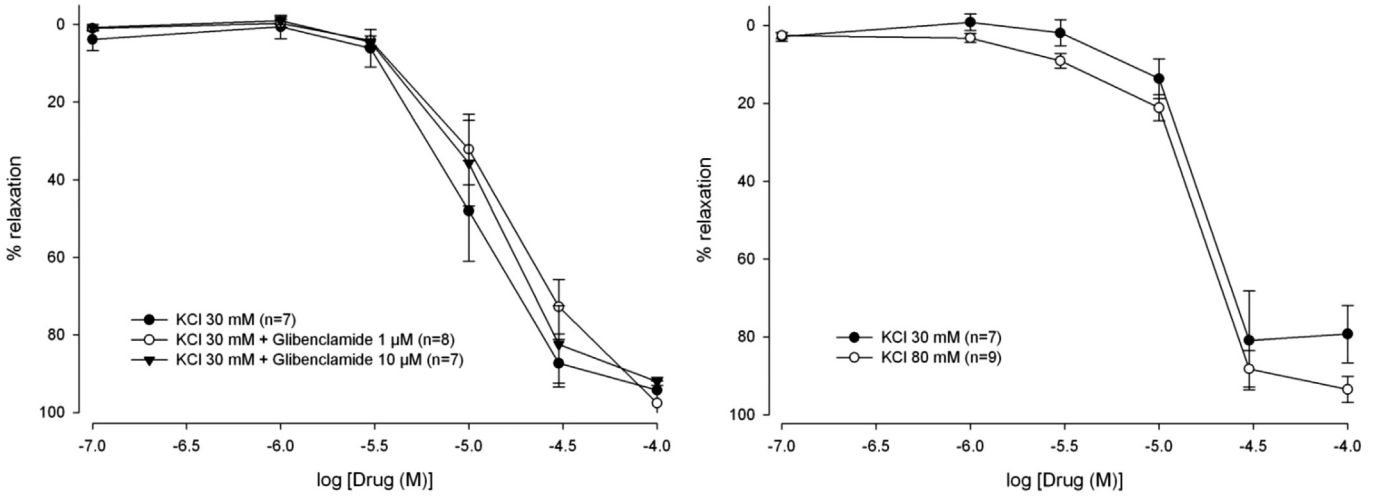


Fig. 2. Left panel: concentration–response curves for the myorelaxant effect of compound **21** on KCl (30 mM)-induced contraction of rat aorta rings incubated in the absence or presence of different glibenclamide concentrations. Mean values ± S.E.M refer 7–8 individual experiments. Right panel: concentration–response curves for the myorelaxant effect of compound **21** on KCl (30 mM or 80 mM)-induced contraction of rat aorta rings. Mean values ± S.E.M refer to 7–9 individual experiments.

to perform additional experiments aiming at characterizing the mechanism of action of this original compound.

In rat aortic rings exposed to 30 mM K^+, the cumulative application of **21** induced concentration-dependent relaxations (Fig. 2). When the same experiment was conducted on rat aorta rings exposed throughout to either 1 or 10 μM of the K_{ATP} channel blocker glibenclamide [5,34], the vasorelaxant capacity of **21** was unaffected ($p > 0.05$, Table 2 and Fig. 2 left panel). By contrast, the presence of glibenclamide in the physiological medium provoked a concentration-dependent rightward shift of the dose–response curves for the reference K_{ATP} channel openers diazoxide and/or (±)-cromakalim (Table 2). Indeed, diazoxide was found to be 4.4 fold less potent in the presence of 1 μM glibenclamide ($p < 0.05$) and 8.0 fold less potent in the presence of 10 μM glibenclamide ($p < 0.05$) in the physiological medium (Table 2). Likewise, 1 μM glibenclamide provoked a 10 fold reduction ($p < 0.05$) whilst 10 μM glibenclamide induced a 130 fold reduction ($p < 0.05$) in the (±)-cromakalim response (Table 2).

In the next series of experiments, contractile activity was elicited by high concentrations of extracellular K^+ (80 mM). Under such experimental conditions, the vasorelaxant properties of **21** remained unchanged ($p > 0.05$, Table 2 and Fig. 2 right panel) whereas the myorelaxant effects of diazoxide and (±)-cromakalim were dramatically reduced ($p < 0.05$, Table 2).

It should be stressed that the Ca^{2+} entry blocker verapamil [35] also provoked concentration-dependent relaxations of the myogenic responses induced by 30 mM extracellular K^+ (Table 2).

As indicated in Table 2, the myorelaxant effects of verapamil were unaffected by the presence of 1 μM ($p > 0.05$) or 10 μM ($p > 0.05$) glibenclamide in the bathing medium and persisted in 80 mM K^+ media ($p > 0.05$, Table 2).

Altogether, these data reveal that compound **21** displays a pharmacological profile different from that of K_{ATP} channel openers and similar to that of the Ca^{2+} entry blocker verapamil.

3.4. Effects of **21** on ^{86}Rb outflow and on KCl-induced changes in ^{45}Ca outflow from perifused rat aortic rings

Radioisotopic experiments were further conducted to characterize, indirectly, the effects of compound **21** on transmembrane ionic movements.

First, the effect of the methanesulfonamide derivative **21** was evaluated on ^{86}Rb (^{42}K substitute) fractional outflow rate (FOR) from prelabeled and perifused rat aorta rings exposed throughout to 30 mM extracellular K^+. Under such experimental conditions, which mimicked the conditions used to measure muscle tension, the addition of 25 or 100 μM **21** to the perifusing medium failed to affect ^{86}Rb FOR (data not shown), suggesting that the drug did not interfere with the membrane permeability to K^+.

In another series of experiments, we quantified the effects of **21** on ^{45}Ca outflow from prelabelled and perifused rat aortic rings.

As previously described, a rise in the extracellular concentration of K^+ from 5 to 80 mM provoked a rapid and marked increase in ^{45}Ca FOR (Fig. 3) [36]. This cationic response to high K^+ is known to reflect a process of ^{40}Ca–^{45}Ca exchange into which ^{40}Ca influent, flowing through activated voltage-sensitive Ca^{2+} channels, displaces ^{45}Ca from intracellular binding sites [36,37].

When compound **21** (30 μM) was present in the perifusing medium, the ^{45}Ca response to 80 mM K^+ was reduced (Fig. 3). Thus, the peak ^{45}Ca outflow averaged 6.06 ± 0.36% per min. ($n = 5$) in the absence and 3.68 ± 0.43% per min. ($n = 6$) in the presence of 30 μM **21** ($p < 0.05$). Such an inhibitory effect of **21** on the ^{45}Ca response to 80 mM K^+ was reminiscent of that provoked by the Ca^{2+} entry blocker verapamil [37].

These radioisotopic data indirectly suggest that the effects of **21** result from a direct inhibition of Ca^{2+} entry into smooth muscle cells and that **21** behaves as a Ca^{2+} entry blocker.

4. Conclusion

In search of more hydrosoluble 4,6-disubstituted *R*/*S*-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans acting as pancreatic PCOs, we have synthesized 16 original 4-substituted *R*/*S*-6-alkylsulfonylamino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans (**19**–**34**) structurally related to *R*/*S*-6-acetamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans bearing a substituted phenylthiourea moiety at the 4-position (**7**–**10**). All these new dihydrobenzopyran derivatives were bearing, at the 4-position, a phenylthiourea (**19**–**22** and **27**–**30**) or a phenylurea chain (**23**–**26** and **31**–**34**) substituted on the phenyl ring by a *meta* or a *para* electron withdrawing group such as a cyano group or a chlorine atom. Some of these new 2,2-dimethylchromans included, at the 6-position, a methylsulfonylamino (**19**–**26**) or an ethylsulfonylamino group

Table 2
Myorelaxant effects of compound **21**, diazoxide, (±)-cromakalim and verapamil on 30 mM and 80 mM KCl-precontracted rat aorta rings incubated in the absence or presence of glibenclamide.

Compounds	Myorelaxant activity			
	EC_{50} (μM)[a]			
	KCl 30 mM	KCl 30 mM + 1 μM Gliben.[b]	KCl 30 mM + 10 μM Gliben.[b]	KCl 80 mM
21	12.0 ± 2.0 (7)	16.6 ± 3.3 (8)	15.7 ± 2.9 (7)	
	15.8 ± 0.7 (7)			16.8 ± 1.2 (9)
(±)-Cromakalim	0.22 ± 0.07 (6)	2.5 ± 0.7 (9)	28.8 ± 6.0 (5)	
	0.17 ± 0.01 (4)			137.7 ± 10.4 (10)
Diazoxide	34.8 ± 2.7 (6)	153.7 ± 8.0 (7)	279.8 ± 11.8 (4)	
	23.8 ± 5.0 (6)			>300 (8)
Verapamil	0.031 ± 0.004 (4)	0.038 ± 0.007 (6)	0.035 ± 0.003 (5)	
	0.045 ± 0.004 (5)			0.052 ± 0.006 (7)

[a] EC_{50}: drug concentration giving 50% relaxation (mean ± SEM (*n*)).
[b] Gliben.: glibenclamide.

(**27**–**34**). The *in vitro* biological activity (inhibition of insulin release from pancreatic B-cells and myorelaxant effect on vascular smooth muscle) of these original compounds was compared to that of (±)-cromakalim, diazoxide and previously reported compounds **3**–**10**.

The huge majority of the new 6-alkylsulfonylamino-substituted dihydrobenzopyran derivatives was more potent than the reference compound (±)-cromakalim at inhibiting the insulin releasing process. Some compounds were found to be equipotent or even more potent than diazoxide.

The newly synthesized dihydrobenzopyrans, although being less potent than (±)-cromakalim, exhibited marked vasorelaxant properties. The myorelaxant activity of most original alkanesulfonamide derivatives was more pronounced than that of diazoxide and than that of previously described 6-bromo- or 6-acetamido-substituted 2,2-dimethylchromans.

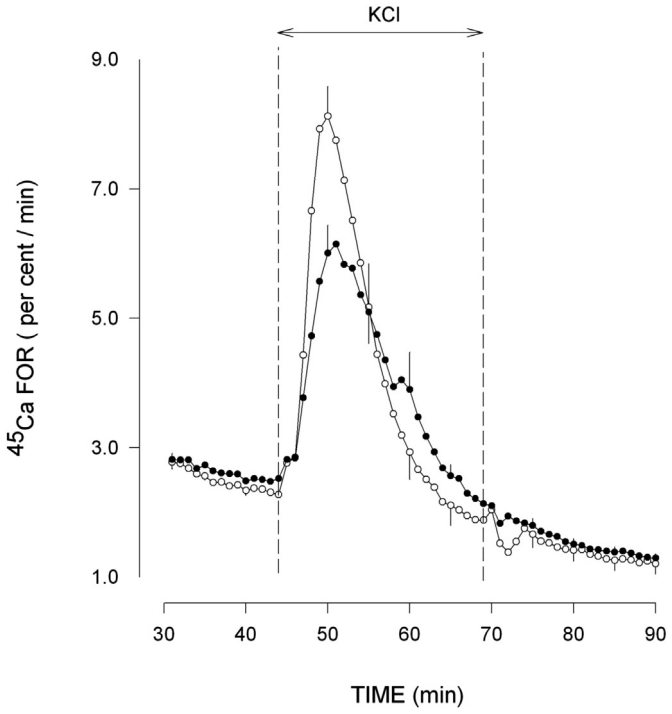


Fig. 3. Effects of KCl 80 mM on ^{45}Ca outflow from rat aorta rings perifused throughout in the absence (○) or presence of compound **21** (●; 30 μM). Mean values ± S.E.M refer to 5–6 individual experiments.

Whether on the glucose-stimulated insulin releasing process or the KCl-induced myogenic activity, the phenylthiourea derivatives appeared to be more active than their corresponding phenylurea analogues.

Biological results further indicated that the bioisosteric replacement of the acetamide group at the 6-position by an alkylsulfonylamino substituent allowed to identify compounds rather acting on the vascular smooth muscle tissue. Moreover, it should also be pointed out that, compared to the previously described *R*/*S*-6-bromo- and *R*/*S*-6-acetamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans, the hydrophilicity of the new compounds was notably increased.

Additional pharmacological and radioisotopic experiments conducted with *R*/*S*-*N*-3-chlorophenyl-*N*'-(3,4-dihydro-6-methylsulfonylamino-2,2-dimethyl-2*H*-1-benzopyran-4-yl)thiourea (**21**) suggested that the myorelaxant properties of the 6-alkylsulfonylamino-substituted dihydrobenzopyran mainly resulted from a direct Ca^{2+} entry blockade. In such a way, compound **21** behaved as a Ca^{2+} entry blocker rather than as a specific potassium channel opener.

5. Experimental

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 by 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 ^1H NMR spectra were recorded on a Bruker Avance 500 instrument (^1H: 500 MHz; ^{13}C 125 MHz) 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 abbreviation 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.

N-(4-Methoxyphenyl)acetamide (**12**), *N*-(3-Acetyl-4-hydroxyphenyl)acetamide (**13**), 6-Acetamido-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (**14**) and 6-Amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (**15**) were obtained according to previously described procedures [30].

*5.1.1. 3,4-Dihydro-2,2-dimethyl-6-methylsulfonylamino-2H-1-benzopyran-4-one (**16a**)*

6-Amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (**15**) (8 g, 41.83 mmol) was dissolved in methylene chloride (80 mL). Pyridine (5.08 mL, 62.75 mmol) and methanesulfonyl chloride (4.86 mL, 62.75 mmol) were added to the solution which was stirred at 25 °C. After completion of the reaction, the solvent was evaporated under reduced pressure. The crude product was triturated with a hot sodium hydroxide solution (10% NaOH w/w). The insoluble material was collected by filtration and concentrated hydrochloric acid was added to the filtrate until pH 1. The resulting precipitate was collected by filtration, washed with water and dried (10.92 g, 97%): mp: 195–197 °C; IR (KBr) υ: 1155 and 1336 (S=O), 1682 (C=O), 3446–3246 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.39 (s, 6H, CH_3), 2.79 (s, 2H, CH_2), 2.93 (s, 3H, –NHSO$_2$CH_3), 6.99 (d, 1H, 8-*H*), 7.40 (dd, 1H, 7-*H*), 7.56 (d, 1H, 5-*H*), 9.60 (s, 1H, –N*H*SO$_2$CH$_3$); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 191.83, 156.45, 131.23, 130.02, 119.81, 119.24, 117.57, 79.41, 47.78, 38.81, 25.96. The product was used in the next step without further purification.

*5.1.2. 3,4-Dihydro-2,2-dimethyl-6-ethylsulfonylamino-2H-1-benzopyran-4-one (**16b**)*

Compound **16b** was prepared following the same procedure as that described for compound **16a**, starting from compound **15** (8 g, 41.83 mmol) and ethanesulfonyl chloride (5.95 mL, 62.75 mmol) (11.38 g, 96%): mp: 151–153 °C; IR (KBr) υ: 1147 and 1335 (S=O), 1687 (C=O), 3253 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.19 (*t*, 3H, –NHSO$_2$CH$_2$CH_3), 1.38 (s, 6H, CH_3), 2.79 (s, 2H, CH_2), 3.02 (*q*, 2H, –NHSO$_2$CH_2CH$_3$), 6.99 (d, 1H, 8-*H*), 7.40 (dd, 1H, 7-*H*), 7.56 (d, 1H, 5-*H*), 9.69 (s, 1H, –N*H*SO$_2$CH$_2$CH$_3$); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 191.83, 156.23, 131.25, 129.54, 119.81, 119.27, 117.03, 79.38, 47.79, 44.79, 25.97, 7.96. The product was used in the next step without further purification.

*5.1.3. 3,4-Dihydro-2,2-dimethyl-6-methylsulfonylamino-2H-1-benzopyran-4-hydroxyimine (**17a**)*

Potassium carbonate (7.18 g, 51.98 mmol) and hydroxylamine hydrochloride (3.61 g, 51.98 mmol) were added to a stirring solution of **16a** (7 g, 25.99 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 (6.95 g, 94%): mp: 221–223 °C; IR (KBr) υ: 1154 and 1322 (S=O), 3241 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.29 (s, 6H, CH_3), 2.75 (s, 2H, CH_2), 2.91 (s, 3H, –NHSO$_2$CH_3), 6.82 (d, 1H, 8-*H*), 7.11 (dd, 1H, 7-*H*), 7.66 (d, 1H, 5-*H*), 9.45 (s, 1H, –N*H*SO$_2$CH$_3$), 11.32 (s, 1H, =N–O*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 150.89, 146.75, 130.95, 124.31, 118.48, 118.37, 115.51, 75.35, 38.60, 33.16, 26.35. The product was used in the next step without further purification.

*5.1.4. 3,4-Dihydro-2,2-dimethyl-6-ethylsulfonylamino-2H-1-benzopyran-4-hydroxyimine (**17b**)*

Compound **17b** was prepared following the same procedure as that described for compound **17a**, starting from compound **16b** (7 g, 24.71 mmol), potassium carbonate (6.83 g, 49.42 mmol) and hydroxylamine hydrochloride (3.43 g, 49.42 mmol) (7.22 g, 98%): mp: 207–209 °C; IR (KBr) υ: 1153 and 1321 (S=O), 3251 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.20 (*t*, 3H, –NHSO$_2$CH$_2$CH_3), 1.29 (s, 6H, CH_3), 2.75 (s, 2H, CH_2), 2.99 (*q*, 2H, –NHSO$_2$CH_2CH$_3$), 6.81 (d, 1H, 8-*H*), 7.11 (dd, 1H, 7-*H*), 7.66 (d, 1H, 5-*H*), 9.53 (s, 1H, –N*H*SO$_2$CH$_2$CH$_3$), 11.32 (s, 1H, =N–O*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 150.70, 146.75, 130.95, 123.99, 118.50, 118.36, 115.09, 75.32, 44.49, 33.17, 26.34, 7.96. The product was used in the next step without further purification.

*5.1.5. R/S-4-Amino-3,4-dihydro-2,2-dimethyl-6-methylsulfonylamino-2H-1-benzopyran (**18a**)*

Raney-Nickel (2 g) was added to a stirred solution of **17a** (1.7 g, 5.98 mmol) in methanol (80 mL). The solution was stirred in a sealed hydrogenator under a hydrogen pressure of 5 bars. When the reaction was completed (3–4 h), the catalyst was removed by filtration 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 (1.36 g, 84%): mp: 159–161 °C; IR (KBr) υ: 1153 and 1315 (S=O), 3340 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.19 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.52 (*t*, 1H, CH_2), 2.00 (m, 1H, CH_2), 2.88 (s, 3H, –NHSO$_2$CH_3), 3.80 (m, 1H, C*H*–NH$_2$), 6.63 (d, 1H, 8-*H*), 6.93 (dd, 1H, 7-*H*), 7.43 (d, 1H, 5-*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 150.46, 129.85, 127.92, 122.60, 122.04, 116.61, 75.14, 43.84, 43.62, 38.64, 29.68, 24.62. The product was used in the next step without further purification.

*5.1.6. R/S-4-Amino-3,4-dihydro-2,2-dimethyl-6-ethylsulfonylamino-2H-1-benzopyran (**18b**)*

Following the same procedure than that described for compound **18a**, the catalytic hydrogenation of compound **17b** generated product **18b** (1.44 g, 89%): mp: 148–150 °C; IR (KBr) υ: 1143 and 1320 (S=O), 3360 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.20 (m, 6H, –NHSO$_2$CH$_2$CH_3 and CH_3), 1.33 (s, 3H, CH_3), 1.52 (*t*, 1H, CH_2), 2.00 (m, 1H, CH_2), 2.97 (m, 2H, –NHSO$_2$CH_2CH$_3$), 3.80 (m, 1H, C*H*–NH$_2$), 6.62 (d, 1H, 8-*H*), 6.93 (dd, 1H, 7-*H*), 7.42 (d, 1H, 5-*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 150.23, 129.82, 127.90, 122.08, 121.49, 116.61, 75.11, 44.41, 43.88, 43.61, 29.68, 24.62, 7.99. Anal. ($C_{13}H_{20}N_2O_3S$) theoretical: C, 54.92; H, 7.09; N, 9.85; S, 11.28. Found: C, 54.92; H, 7.11; N, 9.80; S, 10.92.

*5.1.7. General procedure for the preparation of compounds **19**–**34***

The appropriate phenyl isothiocyanate or phenyl isocyanate was added to a boiling solution of *R*/*S*-4-amino-3,4-dihydro-2,2-dimethyl-6-methylsulfonylamino-2*H*-1-benzopyran (**18a**) (0.5 g, 1.85 mmol) or *R*/*S*-4-amino-3,4-dihydro-2,2-dimethyl-6-ethylsulfonylamino-2*H*-1-benzopyran (**18b**) (0.5 g, 1.76 mmol) in methylene chloride (20 mL). The solution was refluxed for 30 min. Thereafter, the mixture was cooled to 0 °C. The resulting precipitate was collected by filtration, washed with cold methylene chloride and dried.

*5.1.7.1. R/S-N-3-Cyanophenyl-N′-(3,4-dihydro-6-methylsulfonylamino-2,2-dimethyl-2H-1-benzopyran-4-yl)thiourea (**19**).* Was obtained from **18a** (0.5 g, 1.85 mmol) and 3-cyanophenyl isothiocyanate (356 mg, 2.22 mmol): yield: 0.772 g, 97%; mp: 198–200 °C; IR (KBr) υ: 1156 and 1320 (S=O), 1519 (C=S), 2231 (C≡N), 3313 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.27 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.80 (*t*, 1H, CH_2), 2.16 (m, 1H, CH_2), 2.91 (s, 3H, –NHSO$_2$CH_3), 5.81 (m, 1H, C*H*), 6.75 (d, 1H, 8-*H*), 7.04 (dd, 1H, 7-*H*), 7.18 (d, 1H, 5-*H*), 7.51–7.58 (m, 2H, 4′-*H* and 5′-*H*), 7.73 (d, 1H, 6′-*H*), 7.98 (s, 1H, 2′-*H*), 8.34 (d, 1H, CH–N*H*–CS–NH–), 9.36 (s, 1H, N*H*), 9.85 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ:180.80, 150.50, 139.94, 130.43, 129.99, 128.23, 127.81, 126.36, 122.60, 122.48, 120.70, 118.65, 117.41, 111.30, 75.38, 47.23, 38.67, 37.77, 29.22, 24.17. Anal. ($C_{20}H_{22}N_4O_3S_2$) theoretical: C, 55.79; H, 5.15; N, 13.01; S, 14.89. Found: C, 55.57; H, 5.08; N, 13.20; S, 14.64.

*5.1.7.2. R/S-N-4-Cyanophenyl-N′-(3,4-dihydro-6-methylsulfonylamino-2,2-dimethyl-2H-1-benzopyran-4-yl)thiourea (**20**).* Was obtained from **18a** (0.5 g, 1.85 mmol) and 4-cyanophenyl

isothiocyanate (356 mg, 2.22 mmol): yield: 0.677 g, 85%; mp: 154–156 °C; IR (KBr) υ: 1149 and 1317 (S=O), 1537 (C=S), 2228 (C≡N), 3254 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.27 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.79 (*t*, 1H, CH_2), 2.20 (m, 1H, CH_2), 2.92 (s, 3H, –NHSO$_2$CH_3), 5.78 (m, 1H, C*H*), 6.75 (d, 1H, 8-*H*), 7.05 (dd, 1H, 7-*H*), 7.18 (d, 1H, 5-*H*), 7.75 (m, 4H, 2′-*H*, 6′-*H*, 3′-*H* and 5′-*H*), 8.49 (d, 1H, CH–N*H*–CS–NH–), 9.35 (s, 1H, N*H*), 10.02 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 180.35, 150.58, 143.76, 132.87, 130.38, 122.71, 122.35, 121.96, 120.82, 119.07, 117.47, 105.08, 75.36, 47.12, 38.71, 37.71, 29.17, 24.19. Anal. ($C_{20}H_{22}N_4O_3S_2$) theoretical: C, 55.79; H, 5.15; N, 13.01; S, 14.89. Found: C, 55.82; H, 5.11; N, 12.99; S, 15.22.

*5.1.7.3. R/S-N-3-Chlorophenyl-N′-(3,4-dihydro-6-methylsulfonylamino-2,2-dimethyl-2H-1-benzopyran-4-yl)thiourea (**21**).* Was obtained from **18a** (0.5 g, 1.85 mmol) and 3-chlorophenyl isothiocyanate (291 μl, 377 mg, 2.22 mmol): yield: 0.618 g, 76%; mp: 184–185 °C; IR (KBr) υ: 1148 and 1317 (S=O), 1532 (C=S), 3252 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.26 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.80 (*t*, 1H, CH_2), 2.16 (m, 1H, CH_2), 2.91 (s, 3H, –NHSO$_2$CH_3), 5.80 (m, 1H, C*H*), 6.74 (d, 1H, 8-*H*), 7.04 (dd, 1H, 7-*H*), 7.17 (s, 2H, 5-*H* and 4′-*H*), 7.34 (d, 2H, 5′-*H* and 6′-*H*), 7.67 (s, 1H, 2′-*H*), 8.22 (d, 1H, –CH-N*H*-CS-NH-), 9.35 (s, 1H, N*H*), 9.73 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 180.63, 150.53, 140.51, 132.69, 130.37, 130.22, 124.02, 122.87, 122.71, 122.51, 121.85, 120.71, 117.38, 75.38, 47.16, 38.68, 37.79, 29.22, 24.19. Anal. ($C_{19}H_{22}ClN_3O_3S_2$) theoretical: C, 51.87; H, 5.04; N, 9.55; S, 14.57. Found: C, 51.88; H, 4.99; N, 9.72; S, 14.44.

*5.1.7.4. R/S-N-4-Chlorophenyl-N′-(3,4-dihydro-6-methylsulfonylamino-2,2-dimethyl-2H-1-benzopyran-4-yl)thiourea (**22**).* Was obtained from **18a** (0.5 g, 1.85 mmol) and 4-chlorophenyl isothiocyanate (377 mg, 2.22 mmol): yield: 0.740 g, 91%; mp: 165–167 °C; IR (KBr) υ: 1147 and 1317 (S=O), 1539 (C=S), 3254 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.26 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.78 (*t*, 1H, CH_2), 2.14 (m, 1H, CH_2), 2.91 (s, 3H, –NHSO$_2$CH_3), 5.80 (m, 1H, C*H*), 6.73 (d, 1H, 8-*H*), 7.03 (dd, 1H, 7-*H*), 7.17 (d, 1H, 5-*H*), 7.37 (d, 2H, 3′-*H* and 5′-*H*), 7.46 (d, 2H, 2′-*H* and 6′-*H*), 8.12 (d, 1H, –CH–N*H*–CS–NH–), 9.34 (s, 1H, N*H*), 9.69 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 180.67, 150.54, 137.81, 130.36, 128.54, 128.34, 125.34, 122.82, 122.58, 120.76, 117.35, 75.39, 47.23, 38.65, 37.81, 29.26, 24.15. Anal. ($C_{19}H_{22}ClN_3O_3S_2$) theoretical: C, 51.87; H, 5.04; N, 9.55; S, 14.57. Found: C, 51.53; H, 4.97; N, 9.62; S, 14.26.

*5.1.7.5. R/S-N-3-Cyanophenyl-N′-(3,4-dihydro-6-methylsulfonylamino-2,2-dimethyl-2H-1-benzopyran-4-yl)urea (**23**).* was obtained from **18a** (0.5 g, 1.85 mmol) and 3-cyanophenyl isocyanate (320 mg, 2.22 mmol): yield: 0.728 g, 95%; mp: 209–211 °C; IR (KBr) υ: 1154 and 1311 (S=O), 1683 (C=O), 2233 (C≡N), 3350 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.27 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.77 (*t*, 1H, CH_2), 2.09 (m, 1H, CH_2), 2.88 (s, 3H, –NHSO$_2$CH_3), 4.96 (m, 1H, C*H*), 6.73 (d, 2H, 8-*H* and CH– N*H*CONH-), 7.03 (dd, 1H, 7-*H*), 7.15 (d, 1H, 5-*H*), 7.37 (d, 1H, 4′-*H*), 7.46 (*t*, 1H, 5′-*H*), 7.65 (d, 1H, 6′-*H*), 8.00 (s, 1H, 2′-*H*), 8.90 (s, 1H, N*H*), 9.31 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 155.46, 151.02, 141.70, 130.91, 130.58, 125.23, 124.25, 122.97, 122.89, 121.40, 120.88, 119.45, 117.81, 111.95, 75.74, 42.79, 39.77, 39.12, 29.60, 24.89. Anal. ($C_{20}H_{22}N_4O_4S_2$) theoretical: C, 57.96; H, 5.35; N, 13.52; S, 7.73. Found: C, 57.76; H, 5.31; N, 13.51; S, 7.63.

*5.1.7.6. R/S-N-4-Cyanophenyl-N′-(3,4-dihydro-6-methylsulfonylamino-2,2-dimethyl-2H-1-benzopyran-4-yl)urea (**24**).* Was obtained from **18a** (0.5 g, 1.85 mmol) and 4-cyanophenyl isocyanate (320 mg, 2.22 mmol): yield: 0.759 g, 99%; mp: 253–255 °C; IR (KBr) υ: 1151 and 1323 (S=O), 1685 (C=O), 3332 (C≡N), 3375 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.27 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.76 (*t*, 1H, CH_2), 2.10 (m, 1H, CH_2), 2.88 (s, 3H, –NHSO$_2$CH_3), 4.96 (m, 1H, C*H*), 6.73 (d, 1H, 8-*H*), 6.75 (d, 1H, CH–N*H*CONH-), 7.03 (dd, 1H, 7-*H*), 7.14 (d, 1H, 5-*H*), 7.63 (d, 2H, 3′-*H* and 5′-*H*), 7.70 (d, 2H, 2′-*H* and 6′-*H*), 9.08 (s, 1H, N*H*), 9.31 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 154.63, 150.54, 144.76, 133.22, 130.40, 123.62, 122.44, 120.91, 119.41, 117.67, 117.35, 102.61, 75.24, 42.30, 40.23, 38.65, 29.06, 24.43. Anal. ($C_{20}H_{22}N_4O_3S_2$) theoretical: C, 57.96; H, 5.35; N, 13.52; S, 7.73. Found: C, 58.16; H, 5.35; N, 13.59; S, 7.33.

*5.1.7.7. R/S-N-3-Chlorophenyl-N′-(3,4-dihydro-6-methylsulfonylamino-2,2-dimethyl-2H-1-benzopyran-4-yl)urea (**25**).* Was obtained from **18a** (0.5 g, 1.85 mmol) and 3-chlorophenyl isocyanate (371 μl, 341 mg, 2.22 mmol): yield: 0.753 g, 96%; mp: 189–191 °C; IR (KBr) υ: 1152 and 1313 (S=O), 1680 (C=O), 3381 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.27 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.75 (*t*, 1H, CH_2), 2.10 (m, 1H, CH_2), 2.88 (s, 3H, –NHSO$_2$CH_3), 4.95 (m, 1H, C*H*), 6.61 (d, 1H, CH–N*H*CONH-), 6.72 (d, 1H, 8-*H*), 6.97 (d, 1H, 4′-*H*), 7.03 (dd, 1H, 7-*H*), 7.15 (d, 1H, 5-*H*), 7.22–7.28 (m, 2H, 5′-*H* and 6′-*H*), 7.74 (s, 1H, 2′-*H*), 8.74 (s, 1H, N*H*), 9.32 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 154.94, 150.55, 141.85, 133.11, 130.37, 130.27, 123.83, 122.42, 120.94, 120.85, 117.31, 117.22, 116.21, 75.26, 42.24, 40.32, 38.64, 29.09, 24.42. Anal. ($C_{19}H_{22}ClN_3O_4S$) theoretical: C, 53.83; H, 5.23; N, 9.91; S, 7.56. Found: C, 53.91; H, 5.24; N, 10.08; S, 7.21.

*5.1.7.8. R/S-N-4-Chlorophenyl-N′-(3,4-dihydro-6-methylsulfonylamino-2,2-dimethyl-2H-1-benzopyran-4-yl)urea (**26**).* Was obtained from **18a** (0.5 g, 1.85 mmol) and 4-chlorophenyl isocyanate (284 μl, 341 mg, 2.22 mmol): yield: 0.768 g, 98%; mp: 248–250 °C; IR (KBr) υ: 1148 and 1318 (S=O), 1678 (C=O), 3382 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.27 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.74 (*t*, 1H, CH_2), 2.09 (m, 1H, CH_2), 2.88 (s, 3H, –NHSO$_2$CH_3), 4.95 (m, 1H, C*H*), 6.55 (d, 1H, CH–N*H*CONH-), 6.72 (d, 1H, 8-*H*), 7.03 (d, 1H, 7-*H*), 7.15 (s, 1H, 5-*H*), 7.29 (d, 2H, 3′-*H* and 5′-*H*), 7.48 (d, 2H, 2′-*H* and 6′-*H*), 8.66 (s, 1H, N*H*), 9.31 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 155.01, 150.56, 139.30, 130.36, 128.50, 124.70, 123.91, 122.45, 121.00, 119.33, 117.30, 75.26, 42.20, 40.44, 38.64, 29.08, 24.45. Anal. ($C_{19}H_{22}ClN_3O_3S$) theoretical: C, 53.83; H, 5.23; N, 9.91; S, 7.56. Found: C, 53.71; H, 5.20; N, 9.95; S, 7.52.

*5.1.7.9. R/S-N-3-Cyanophenyl-N′-(6-ethylsulfonylamino-3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-4-yl)thiourea (**27**).* Was obtained from **18b** (0.5 g, 1.76 mmol) and 3-cyanophenyl isothiocyanate (338 mg, 2.11 mmol): yield: 0.594 g, 76%; mp: 115–117 °C; IR (KBr) υ: 1142 and 1314 (S=O), 1531 (C=S), 2232 (C≡N), 3306 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.22 (*t*, 3H, –NHSO$_2$CH$_2$CH_3), 1.26 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.81 (*t*, 1H, CH_2), 2.15 (m, 1H, CH_2), 3.00 (*q*, 2H, –NHSO$_2$CH_2CH$_3$), 5.80 (m, 1H, C*H*), 6.73 (d, 1H, 8-*H*), 7.03 (dd, 1H, 7-*H*), 7.18 (d, 1H, 5-*H*), 7.55 (m, 2H, 4′-*H* and 5′-*H*), 7.73 (d, 1H, 6′-*H*), 7.97 (s, 1H, 2′-*H*), 8.32 (d, 1H, CH–N*H*CSNH-), 9.44 (s, 1H, N*H*), 9.84 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 180.87, 150.27, 139.94, 130.35, 129.99, 128.26, 127.83, 126.40, 122.64, 122.12, 120.31, 118.63, 117.39, 111.29, 75.36, 47.21, 44.51, 37.74, 29.23, 24.18, 7.98. Anal. ($C_{21}H_{24}N_4O_3S_2$) theoretical: C, 56.74; H, 5.44; N, 12.60; S, 14.42. Found: C, 56.48; H, 5.52; N, 12.65; S, 14.03.

*5.1.7.10. R/S-N-4-Cyanophenyl-N′-(6-ethylsulfonylamino-3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-4-yl)thiourea (**28**).* Was obtained from **18b** (0.5 g, 1.76 mmol) and 4-cyanophenyl isothiocyanate (338 mg, 2.11 mmol): yield: 0.571 g, 73%; mp: 193–195 °C; IR (KBr) υ: 1140 and 1318 (S=O), 1546 (C=S), 2233 (C≡N), 3300 (N–H) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ: 1.21 (*t*, 3H, –NHSO$_2$CH$_2$CH_3), 1.27 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.79 (*t*, 1H, CH_2),

2.19 (m, 1H, CH_2), 3.00 (*q*, 2H, −NHSO$_2$CH_2CH$_3$), 5.78 (m, 1H, C*H*), 6.74 (d, 1H, 8-*H*), 7.03 (dd, 1H, 7-*H*), 7.18 (d, 1H, 5-*H*), 7.75 (m, 4H, 2′-*H*, 3′-*H*, 5′-*H* and 6′-*H*), 8.47 (d, 1H, CH−N*H*CSNH-), 9.43 (s, 1H, N*H*), 10.02 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 180.40, 150.35, 143.76, 132.87, 130.33, 122.39, 122.32, 121.98, 120.42, 119.07, 117.46, 105.10, 75.33, 47.10, 44.53, 37.70, 29.17, 24.19, 7.99. Anal. ($C_{21}H_{24}N_4O_3S_2$) theoretical: C, 56.74; H, 5.44; N, 12.60; S, 14.42. Found: C, 57.13; H, 5.55; N, 12.76; S, 14.23.

*5.1.7.11. R/S-N-3-Chlorophenyl-N′-(6-ethylsulfonylamino-3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-4-yl)thiourea (**29**).* Was obtained from **18b** (0.5 g, 1.76 mmol) and 3-chlorophenyl isothiocyanate (277 μl, 358 mg, 2.11 mmol): yield: 0.575 g, 72%; mp: 177–179 °C; IR (KBr) υ: 1140 and 1315 (S=O), 1533 (C=O), 3293 (N–H) cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.20 (*t*, 3H, −NHSO$_2$CH$_2$CH_3), 1.26 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.80 (*t*, 1H, CH_2), 2.14 (m, 1H, CH_2), 3.00 (*q*, 2H, −NHSO$_2$CH_2CH$_3$), 5.80 (m, 1H, C*H*), 6.72 (d, 1H, 8-*H*), 7.02 (dd, 1H, 7-*H*), 7.17 (s, 2H, 5-*H* and 4′-*H*), 7.34 (d, 2H, 5′-*H* and 6′-*H*), 7.67 (s, 1H, 2′-*H*), 8.21 (d, 1H, CH−N*H*CSNH−), 9.43 (s, 1H, N*H*), 9.73 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 180.68, 150.29, 140.50, 132.70, 130.32, 130.22, 124.04, 122.91, 122.75, 122.11, 121.87, 120.31, 117.36, 75.36, 47.14, 44.51, 37.77, 29.23, 24.18, 7.99. Anal. ($C_{20}H_{24}ClN_3O_3S_2$) theoretical: C, 52.91; H, 5.33; N, 9.26; S, 14.12. Found: C, 52.96; H, 5.30; N, 9.30; S, 13.79.

*5.1.7.12. R/S-N-4-Chlorophenyl-N′-(6-ethylsulfonylamino-3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-4-yl)thiourea (**30**).* Was obtained from **18b** (0.5 g, 1.76 mmol) and 4-chlorophenyl isothiocyanate (358 mg, 2.11 mmol): yield: 0.79 g, 99%; mp: 166–168 °C; IR (KBr) υ: 1130 and 1310 (S=O), 1531 (C=O), 3309 (N–H) cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.20 (*t*, 3H, −NHSO$_2$CH$_2$CH_3), 1.25 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.78 (*t*, 1H, CH_2), 2.14 (m, 1H, CH_2), 3.00 (*q*, 2H, −NHSO$_2$CH_2CH$_3$), 5.79 (m, 1H, C*H*), 6.72 (d, 1H, 8-*H*), 7.02 (dd, 1H, 7-*H*), 7.17 (d, 1H, 5-*H*), 7.37 (d, 2H, 3′-*H* and 5′-*H*), 7.46 (d, 2H, 2′-*H* and 6′-*H*), 8.10 (d, 1H, CH−N*H*CSNH-), 9.43 (s, 1H, N*H*), 9.68 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 180.71, 150.32, 137.79, 130.29, 128.54, 128.37, 125.38, 122.86, 122.14, 120.33, 117.34, 75.37, 47.23, 44.46, 37.79, 29.27, 24.15, 8.00. Anal. ($C_{20}H_{24}ClN_3O_3S_2$) theoretical: C, 52.91; H, 5.33; N, 9.26; S, 14.12. Found: C, 53.10; H, 5.55; N, 9.00; S, 13.74.

*5.1.7.13. R/S-N-3-Cyanophenyl-N′-(6-ethylsulfonylamino-3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-4-yl)urea (**31**).* Was obtained from **18b** (0.5 g, 1.76 mmol) and 3-cyanophenyl isocyanate (304 mg, 2.11 mmol): yield: 0.663 g, 88%; mp: 161–163 °C; IR (KBr) υ: 1142 and 1316 (S=O), 1689 (C=O), 2241 (C≡N), 3358 (N–H) cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.18 (*t*, 3H, −NHSO$_2$CH$_2$CH_3), 1.26 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.77 (*t*, 1H, CH_2), 2.08 (m, 1H, CH_2), 2.97 (*q*, 2H, −NHSO$_2$CH_2CH$_3$), 4.95 (m, 1H, C*H*), 6.70–6.73 (m, 2H, 8-*H* and CH−N*H*CONH-), 7.01 (dd, 1H, 7-*H*), 7.16 (d, 1H, 5-*H*), 7.37 (d, 1H, 4′-*H*), 7.46 (*t*, 1H, 5′-*H*), 7.65 (d, 1H, 6′-*H*), 8.00 (s, 1H, 2′-*H*), 8.90 (s, 1H, N*H*), 9.38 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 154.99, 150.32, 141.21, 130.32, 130.08, 124.74, 123.83, 122.48, 122.07, 120.53, 120.41, 118.96, 117.31, 111.46, 75.23, 44.49, 42.32, 39.22, 29.11, 24.38, 7.93. Anal. ($C_{21}H_{24}N_4O_4S$) theoretical: C, 58.86; H, 5.65; N, 13.07; S, 7.48. Found: C, 58.58; H, 5.62; N, 13.14; S, 7.12.

*5.1.7.14. R/S-N-4-Cyanophenyl-N′-(6-ethylsulfonylamino-3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-4-yl)urea (**32**).* Was obtained from **18b** (0.5 g, 1.76 mmol) and 4-cyanophenyl isocyanate (304 mg, 2.11 mmol): yield: 0.746 g, 99%; mp: 239–241 °C; IR (KBr) υ: 1148 and 1318 (S=O), 1682 (C=O), 2226 (C≡N), 3376 (N–H) cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.18 (*t*, 3H, −NHSO$_2$CH$_2$CH_3), 1.26 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.77 (*t*, 1H, CH_2), 2.09 (m, 1H, CH_2), 2.96 (*q*, 2H, −NHSO$_2$CH_2CH$_3$), 4.95 (m, 1H, C*H*), 6.71 (d, 1H, 8-*H*), 6.75 (d, 1H, CH−N*H*CONH-), 7.02 (dd, 1H, 7-*H*), 7.15 (d, 1H, 5-*H*), 7.63 (d, 2H, 3′-*H* and 5′-*H*), 7.70 (d, 2H, 2′-*H* and 6′-*H*), 9.08 (s, 1H, N*H*), 9.38 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 154.65, 150.30, 144.77, 133.21, 130.38, 123.68, 122.10, 120.52, 119.41, 117.67, 117.33, 102.61, 75.21, 44.50, 42.32, 39.18, 29.07, 24.41, 7.94. Anal. ($C_{21}H_{24}N_4O_4S$) theoretical: C, 58.86; H, 5.65; N, 13.07; S, 7.48. Found: C, 58.50; H, 5.59; N, 13.15; S, 7.23.

*5.1.7.15. R/S-N-3-Chlorophenyl-N′-(6-ethylsulfonylamino-3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-4-yl)urea (**33**).* Was obtained from **18b** (0.5 g, 1.76 mmol) and 3-chlorophenyl isocyanate (257 μl, 324 mg, 2.11 mmol): yield: 0.554 g, 72%; mp: 214–216 °C; IR (KBr) υ: 1143 and 1318 (S=O), 1686 (C=O), 3381 (N–H) cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: 1.17 (*t*. 3H, −NHSO$_2$CH$_2$CH_3), 1.26 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.75 (*t*, 1H, CH_2), 2.08 (m, 1H, CH_2), 2.97 (*q*, 2H, −NHSO$_2$CH_2CH$_3$), 4.94 (m, 1H, C*H*), 6.60 (d, 1H, CH−N*H*CONH-), 6.70 (d, 1H, 8-*H*), 6.97 (d, 1H, 4′-*H*), 7.01 (dd, 1H, 7-*H*), 7.15 (d, 1H, 5-*H*), 7.25 (m, 2H, 5′-*H* and 6′-*H*), 7.74 (s, 1H, 2′-*H*), 8.75 (s, 1H, N*H*), 9.38 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 154.97, 150.33, 141.85, 133.11, 130.32, 130.26, 123.90, 122.11, 120.85, 120.57, 117.30, 117.23, 116.22, 75.23, 44.49, 42.26, 39.37, 29.10, 24.40, 7.93. Anal. ($C_{20}H_{24}ClN_3O_4S$) theoretical: C, 54.85; H, 5.52; N, 9.59; S, 7.32. Found: C, 55.06; H, 5.58; N, 9.84; S, 7.02.

*5.1.7.16. R/S-N-4-Chlorophenyl-N′-(6-ethylsulfonylamino-3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-4-yl)urea (**34**).* Was obtained from **18b** (0.5 g, 1.76 mmol) and 4-chlorophenyl isocyanate (270 μl, 324 mg, 2.11 mmol): yield: 0.762 g, 99%; mp: 234–236 °C; IR (KBr) υ: 1142 and 1312 (S=O), 1679 (C=O), 3379 (N–H) cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz): *δ*: RMN 1H (DMSO-d6, 500 MHz): 1.18 (*t*, 3H, −NHSO$_2$CH$_2$CH_3), 1.26 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.74 (*t*, 1H, CH_2), 2.08 (m, 1H, CH_2), 2.96 (*q*, 2H, −NHSO$_2$CH_2CH$_3$), 4.94 (m, 1H, C*H*), 6.54 (d, 1H, CH−N*H*CONH-), 6.70 (d, 1H, 8-*H*), 7.01 (dd, 1H, 7-*H*), 7.15 (d, 1H, 5-*H*), 7.29 (d, 2H, 3′-*H* and 5′-*H*), 7.47 (d, 2H, 2′-*H* and 6′-*H*), 8.66 (s, 1H, N*H*), 9.38 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 155.03, 150.33, 139.31, 130.34, 128.49, 124.70, 123.96, 122.11, 120.62, 119.33, 117.28, 75.23, 44.49, 42.21, 39.44, 29.09, 24.44, 7.94. Anal. ($C_{20}H_{24}ClN_3O_4S$) theoretical: C, 54.85; H, 5.52; N, 9.59; S, 7.32. Found: C, 55.08; H, 5.61; N, 9.81; S, 7.04.

5.2. Biological assays

(±)-Cromakalim (Tocris, UK), diazoxide (Sigma Chemical Co, USA) and verapamil (Sigma Chemical Co, USA) were used as reference compounds. All experiments were performed with aortae or pancreatic islets isolated from adult fed Wistar Rats (Charles River Laboratories, Belgium). The laboratory animal care was approved by the local ethic's committee of the Université Libre de Bruxelles.

5.2.1. Measurement of insulin secretion from incubated rat pancreatic islets

Pancreatic islets were isolated by the collagenase method and freshly isolated islets were used for measurements of insulin secretion [27,34].

Groups of 10 islets, each derived from the same batch of islets, were pre-incubated for 30 min at 37 °C in 1 mL of a physiological salt medium (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1, $NaHCO_3$ 24) supplemented with 2.8 mM glucose, 0.5% (w/v) albumin (RIA grade) and equilibrated against a mixture of O_2 (95%) and CO_2 (5%). The islets were then incubated at 37 °C for a further 90 min in 1 mL of the same medium containing 16.7 mM glucose and, in addition, either the reference compounds or the required chroman derivative. The release of insulin was measured radioimmunologically

using rat insulin as a standard. Residual insulin secretion was expressed as a percentage of the value recorded in control experiments (100%); that is in the absence of drug and presence of 16.7 mM glucose [27,34].

5.2.2. Measurement of myorelaxant activity on rat aorta rings

The rat thoracic aorta was removed, cut into transverse rings (3–4 mm), and adhering fat and connective tissue was detached [34,37,38]. After removal of the endothelium by rubbing the intimal surface with a cotton wad, the segments were suspended under 1.5 g tension in an organ bath containing 20 mL of a buffered physiological solution (in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5). The solution was maintained at 37 °C and continuously oxygenated with a mixture of 95% O_2 and 5% CO_2. After equilibration for 60 min, isometric contractions were measured with a force-displacement transducer. Contractile activity was induced by increasing the extracellular concentration of K^+ (30 or 80 mM KCl). When the tension was stabilized, drugs were added cumulatively until maximal relaxation or until a maximum concentration of 300 μM. Some experiments were repeated in the continuous presence of 1 or 10 μM glibenclamide (Sigma Chemical Co) in the physiological medium. The contractile responses were expressed as the percentage of the contractile response to KCl (100%). The EC_{50} values (concentration evoking 50% inhibition of the plateau phase induced by KCl) were assessed from concentration–response curves using Datanalyst software (EMKA Technologies, France) [34,37,38].

5.2.3. Measurement of ^{86}Rb and ^{45}Ca outflow from perifused rat aorta rings

Groups of 2 aorta rings (2 mm long) were preincubated for 30 min at 37 °C in a physiological solution (NaCl 115 mM, KCl 5 mM, $CaCl_2$ 2.5 mM, $MgCl_2$ 1 mM, $NaHCO_3$ 24 mM) equilibrated against a mixture of O_2 (95%) and CO_2 (5%) [34,37,38]. The segments were then incubated for a further 60 min in the same medium to which ^{86}Rb (0.15–0.25 mM:50 μCi/mL) or ^{45}Ca (0.02–0.04 mM:100 μCi/ml) had been added. After incubation, the aorta rings were washed four times with a non-radioactive medium and placed in a perifusion chamber. The perifusate (physiological solution) was delivered at a constant flow 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 aorta rings was determined. The outflow of ^{86}Rb (^{42}K substitute) or ^{45}Ca (cpm/min) was expressed as a fractional outflow rate (FOR, % of instantaneous aorta content per min). Peak ^{45}Ca outflow was estimated from the difference in ^{45}Ca outflow between the highest value recorded during stimulation and the mean basal value found within the same experiment between the 40th and the 44th min of perifusion [34,37,38].

5.2.4. Statistical evaluation

The statistical significance of differences between mean data was assessed by using the 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 value was <0.05.

5.2.5. 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 [33]. Such a program is available at the Virtual Computational Chemistry Laboratory and used algorithms have been previously described in the literature [33].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.024.

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