Design, Synthesis, and Pharmacological Evaluation of *R/S*-3,4-Dihydro-2,2-dimethyl-6-halo-4-(phenylaminocarbonylamino)-2*H*-1-benzopyrans: Toward Tissue-Selective Pancreatic β-Cell K_{ATP} Channel Openers Structurally Related to (±)-Cromakalim

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In the search of a novel series of benzopyrans structurally related to (±)-cromakalim and acting as pancreatic β-cell potassium channel openers, several *R/S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminocarbonylamino)-2*H*-1-benzopyrans with or without a substituent on the phenyl ring in the 4-position were synthesized. Their activity on rat-insulin-secreting cells and rat aorta rings was compared to that of the K_{ATP} channel activators (±)-cromakalim, diazoxide, (±)-pinacidil, and compound **4**. Structure−activity relationships indicated that the most pronounced inhibitory activity on the pancreatic tissue was obtained by introducing a meta- or para-electron-withdrawing group (a chlorine atom) on the C-4 phenyl ring (drugs **37**−**42**). Such molecules, unlike the parent compound (±)-cromakalim, also exhibited a high selectivity for the pancreatic tissue versus the vascular tissue. Radioisotopic and electrophysiological investigations performed with *R/S*-6-chloro-4-(3-chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**38**) confirmed that the drug activated pancreatic K_{ATP} channels.

Introduction

ATP-sensitive potassium channels (K_{ATP} channels) are mainly regulated by the intracellular [ATP]/[ADP] ratio and couple cell metabolism to cell excitability. They have been identified in a wide range of cell types, including endocrine cells,[1] skeletal and smooth muscle cells,[2−3] cardiac cells,[4] and central neurons.[5] K_{ATP} channels have been shown to be involved in several physiological processes, such as hormone secretion, smooth muscle cell contractile activity, myocardial protection, and neurotransmitter release.[6]

K_{ATP} channels are heteromeric complexes of pore-forming inwardly rectifying potassium channel subunits ($K_{IR}6{\cdot}x$) and regulatory sulfonylurea receptor subunits (SURx) that are assembled as tetramers ($(SURx/K_{ir}6{\cdot}x)_4$) (Figure 1).[7] According to their tissue localization, different isoforms have been identified. For example, the combination of SUR1 and $K_{IR}6.2$ subunits forms the pancreatic (insulin-secreting) β-cell K_{ATP} channel,[8] whereas SUR2B with either $K_{IR}6.1$ or $K_{IR}6.2$ subunits generates the smooth muscle K_{ATP} channel.[9]

The activation of K_{ATP} channels leads to hyperpolarization of the cell membrane, which, in turn, induces tissue-dependent physiological responses. As a result, K_{ATP} channel activation promotes the inhibition of endocrine and/or neurotransmitter release, the relaxation of vascular smooth muscle, and the shortening of cardiac action potentials.[10] Thus, according to their tissue selectivity, PCOs (potassium channel openers) might be expected to become original therapeutic agents for the treatment of diseases, such as arterial hypertension, asthma, urinary incontinence, type 1/type 2 diabetes, obesity and/or hyperinsulinism.[10−16]

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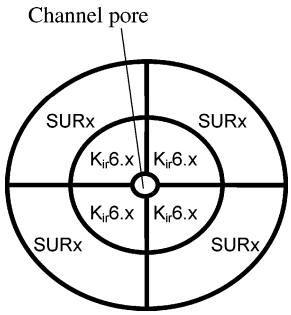

Figure 1. Schematic illustration of SURx and $K_{ir}6{\cdot}x$ subunits assembly into a K_{ATP} channel.

The potassium channel openers comprise a heterogeneous group of chemical families among which are found benzopyrans (i.e., cromakalim (**1**)), benzothiadiazine 1,1-dioxides (i.e., diazoxide (**2**)), and *N*-aryl-*N*′-alkyl-*N*″-cyanoguanidines (i.e., pinacidil (**3**)) (Figure 2).

Cromakalim is the lead compound of the benzopyran PCO family and was found to exert a marked vasorelaxant activity.[17] In contrast to several PCOs belonging to the benzothiadiazine dioxide family such as diazoxide (**2**) and compound **4**, cromakalim was also reported to be poorly active as a pancreatic β-cell K_{ATP} channel opener and as an inhibitor of insulin secretion.[18−20]

Recently, we prepared a series of 4,6-disubstituted 2,2-dimethylchromans structurally related to cromakalim.[21] These molecules were substituted in the 4-position with an alkylurea, an alkylthiourea, an arylsulfonylurea, an alkylcarbamate, or an alkylcarboxamido group, whereas the cyano entity of cromakalim in the 6-position was replaced by a halogen atom. Moreover, the 3-hydroxy group of cromakalim was removed. Pharmacological results indicated that these novel dimethylchromans such as compound **5** (Figure 2) were less effective as myorelaxants and much more effective as inhibitors of insulin

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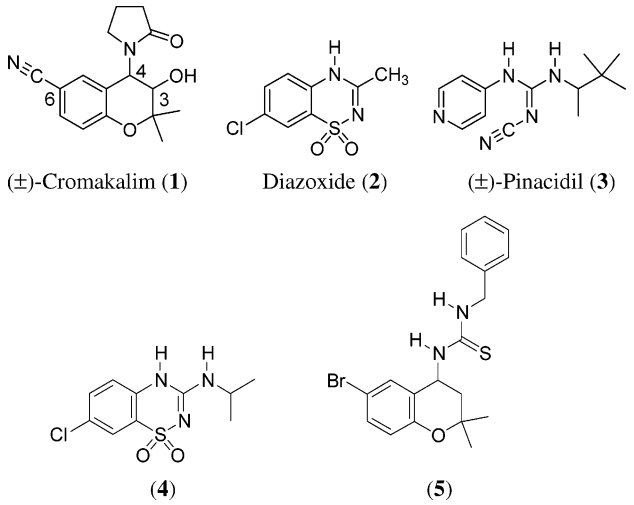


Figure 2. Chemical stucture of (±)-cromakalim, diazoxide, (±)-pinacidil, compound **4**, and compound **5**.

release than the reference molecule cromakalim.[21] According to the chemical structure of the drugs, the nature of the substituent in the 4-position appeared to be a key element for the inhibitory effect on insulin release. Compounds with a bulky group in this position (a benzylaminocarbonylamino or a benzylaminothiocarbonylamino group) were found to be more active than the other molecules on rat pancreatic β-cells.[21]

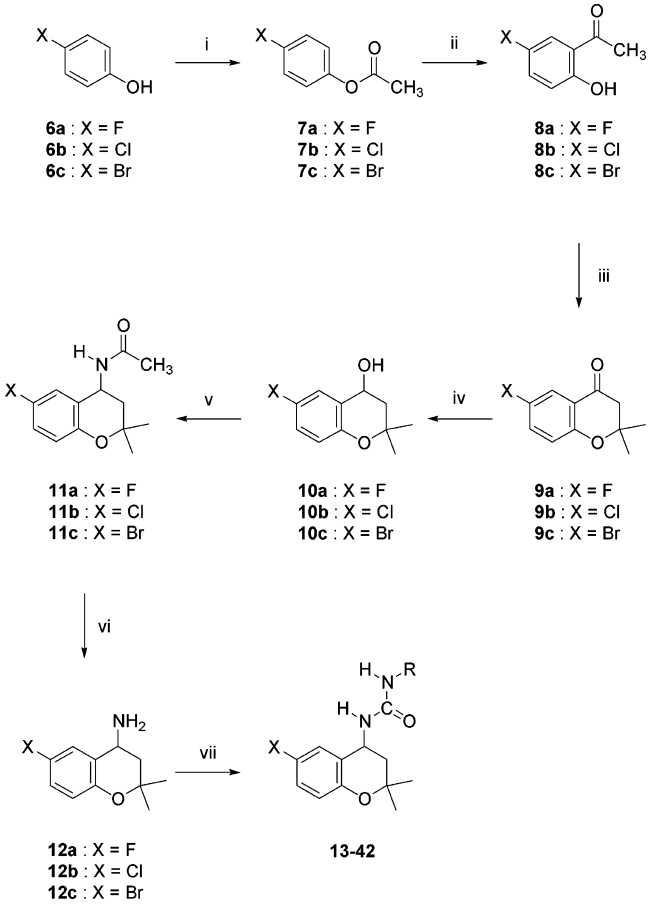
On the basis of these preliminary results, the present work aimed at developing new benzopyrans more active and more selective for the pancreatic tissue bearing a bulky phenylurea substituent at the C-4 position. The new compounds were examined in vitro as putative potassium channel openers on rat pancreatic islets as well as on rat aorta rings. Radioisotopic and electrophysiological investigations were performed to confirm the mechanism of action of these novel compounds.

Chemistry. The key intermediates for the synthesis of compounds **13**−**42**, *R/S*-4-amino-3,4-dihydro-2,2-dimethyl-6-fluoro-2*H*-1-benzopyran (**12a**), *R/S*-4-amino-6-chloro-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**12b**), and *R/S*-4-amino-6-bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**12c**) were obtained, as described previously, in six steps starting from the appropriate *p*-halogenophenols[21,22] (Scheme 1).

In the first step, the esters **7a**−**c** were prepared from *p*-halogenophenols according to a classic esterification reaction catalyzed by concentrated sulfuric acid. The phenolic acetates **7a**−**c** were converted into the corresponding acetophenones **8a**−**c** by the Fries rearrangement[23] in the presence of aluminum chloride at 160 °C. Treatment of acetophenones **8a**−**c** with acetone in the presence of pyrrolidine led to chromanone intermediates **9a**−**c**, which were reduced to chromanols **10a**−**c** with sodium borohydride in methanol. The 4-acetylaminobenzopyrans **11a**−**c** were prepared by the Ritter reaction[22] from chromanols **10a**−**c**. This reaction occurred in acetonitrile supplemented with concentrated sulfuric acid. Finally, the hydrolysis of **11a**−**c** with concentrated hydrochloric acid led to aminochromans **12a**−**c**.

The *R/S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminocarbonylamino)-2*H*-1-benzopyrans (**13**−**42**) were obtained from the reaction of each amine **12a**−**c** with the appropriate isocyanate (RNCO) in methylene chloride at room temperature. Several crystallization steps were needed to obtain the final materials with the required chemical purity.

Scheme 1. Synthesis of Cromakalim Analogues[a]



[a] 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) RNCO, CH_2Cl_2 (**13**−**42**).

Results and Discussion

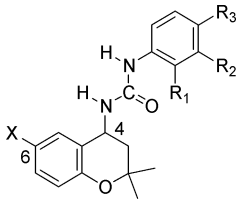
The newly synthesized drugs, reported in Table 1 (compounds **13**−**42**), were tested as inhibitors of insulin release from rat pancreatic islets incubated in the presence of an insulinotropic glucose concentration (16.7 mM). A 90−95% inhibition (5−10% residual insulin secretion) may be considered a full effect relative to the glucose-insensitive basal insulin release.[24] The vasorelaxant activity of the compounds was characterized on 30 mM K^+-depolarized endothelium-free rat aorta.

In the pancreatic model, (±)-cromakalim and (±)-pinacidil were found to be quite inactive at 10 μM (Table 1). However, 10 μM diazoxide and 10 μM compound **4** clearly reduced the glucose-induced insulin output (% of residual insulin secretion = 73.9 ± 4.4% for diazoxide and 4.9 ± 0.4% for compound **4**). Compound **4** was much more active than diazoxide in inhibiting insulin secretion.

As observed in Table 1, most of the new *R/S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(substituted-phenylaminocarbonylamino)-2*H*-1-benzopyrans (**13**−**42**), tested at a 10 μM concentration, were more active on pancreatic β-cells than the reference compound (±)-cromakalim. Many compounds were found to be as potent as diazoxide, whereas some of them were found to be more potent at inhibiting the glucose-induced insulin secretion and exhibited an activity between that of diazoxide and that of compound **4**.

Compounds **13**−**15**, which were devoid of a substituent on the C-4 phenyl ring, were roughly as potent as diazoxide in inhibiting insulin release.

Table 1. Residual Insulin Secretion (%) and Myorelaxant Activity of Original Benzopyrans Compared to (±)-Cromakalim, Diazoxide, (±)-Pinacidil and Compound **4**



compds	X	R_1	R_2	R_3	% residual insulin secretion[a] (10 μM)	myorelaxant activity EC_{50} (μM)[b]
13	F	H	H	H	83.8 ± 3.5 (31)	17.1 ± 3.0 (6)
14	Cl	H	H	H	87.7 ± 3.5 (30)	7.8 ± 0.5 (4)
15	Br	H	H	H	75.9 ± 4.0 (37)	2.3 ± 0.2 (8)
16	F	OCH_3	H	H	84.4 ± 4.8 (24)	>10 (4)
17	Cl	OCH_3	H	H	94.6 ± 3.7 (23)	>10 (4)
18	Br	OCH_3	H	H	72.8 ± 5.5 (12)	>10 (4)
19	F	H	OCH_3	H	66.6 ± 3.3 (23)	>10 (5)
20	Cl	H	OCH_3	H	84.0 ± 4.8 (22)	>10 (4)
21	Br	H	OCH_3	H	73.5 ± 4.0 (15)	>10 (6)
22	F	H	H	OCH_3	89.0 ± 3.9 (30)	15.5 ± 3.4 (5)
23	Cl	H	H	OCH_3	79.3 ± 4.6 (23)	>30 (4)
24	Br	H	H	OCH_3	79.2 ± 3.7 (15)	>10 (4)
25	F	CH_3	H	H	79.0 ± 3.3 (29)	>30 (5)
26	Cl	CH_3	H	H	85.0 ± 3.1 (24)	>10 (4)
27	Br	CH_3	H	H	79.5 ± 3.5 (30)	>30 (4)
28	F	H	CH_3	H	75.2 ± 2.7 (29)	>30 (3)
29	Cl	H	CH_3	H	65.8 ± 4.4 (23)	>10 (4)
30	Br	H	CH_3	H	63.4 ± 3.1 (23)	>10 (4)
31	F	H	H	CH_3	70.6 ± 3.8 (28)	>30 (4)
32	Cl	H	H	CH_3	69.5 ± 3.4 (23)	>30 (4)
33	Br	H	H	CH_3	72.9 ± 4.0 (16)	>30 (4)
34	F	Cl	H	H	51.4 ± 3.1 (31)	>30 (5)
35	Cl	Cl	H	H	40.3 ± 1.5 (23)	>300 (5)
36	Br	Cl	H	H	41.8 ± 3.5 (24)	>30 (3)
37	F	H	Cl	H	28.0 ± 1.5 (23)	>300 (4)
38	Cl	H	Cl	H	25.0 ± 1.3 (24)	>30 (4)
39	Br	H	Cl	H	28.6 ± 1.7 (22)	>30 (4)
40	F	H	H	Cl	22.8 ± 1.2 (32)	> 30 (6)
41	Cl	H	H	Cl	34.6 ± 1.9 (21)	>300 (4)
42	Br	H	H	Cl	25.7 ± 1.5 (23)	>300 (4)
(±)-cromakalim					94.4 ± 4.1 (32)	0.13 ± 0.01 (7)[c]
diazoxide					73.9 ± 4.4 (16)[d]	22.4 ± 2.1 (11)[d]
(±)-pinacidil					92.1 ± 3.9 (13)[d]	0.35 ± 0.02 (11)[d]
4					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 (mean ± SEM(*n*)). [b] EC_{50}: Drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aorta rings (mean ± SEM(*n*)). *n* refers to the number of samples. [c] Published results: ref 21. [d] Published results: ref 25.

Compared with the unsubsituted derivatives **13**−**15**, the introduction of an electron-donating group on the C-4 phenyl ring (methoxy or methyl) (compounds **16**−**24** and **25**−**33**) induced a slight but not uniform increase in activity. The position of the electron-donating substituent on the C-4 phenyl ring (ortho, meta, or para) affected the inhibitory effect on the glucose-induced insulin release. Indeed, several meta and para derivatives appeared to be more active than the ortho compounds (compare the activity of **19** vs that of **16** (p <0.05), **23** vs that of **17** (p <0.05), **29** and **32** vs that of **26** (p <0.05), and **30** vs that of **27** (p <0.05)).

The introduction of a weak electron-withdrawing group (a chlorine atom) on the C-4 phenyl ring (compounds **34**−**42**) significantly increased the activity on the pancreatic tissue compared with that of the unsubsituted derivatives **13**−**15** (p <0.05). All of these compounds (**34**−**42**) exhibited an activity on pancreatic β-cells halfway between the activity of diazoxide and compound **4**, the two reference compounds. Furthermore, *meta*-chloro and *para*-chloro drugs were shown to be more potent than their *ortho*-chloro counterparts (compare the activity of **37** and **40** vs that of **34** (p <0.05), **38** and **41** vs that of **35** (p <0.05), **39** and **42** vs that of **36** (p <0.05)).

On the vascular model, diazoxide and compound **4** exhibited a moderate myorelaxant activity (EC_{50} = 22.4 ± 2.1 μM for diazoxide and 36.3 ± 2.2 μM for compound **4**), compared with that of (±)-cromakalim (EC_{50} = 0.13 ± 0.01 μM) and (±)-pinacidil (0.35 ± 0.02 μM) (Table 1).

Compounds **13**, **14**, and **15** induced vasorelaxant responses. They were more potent than diazoxide (EC_{50} = 22.4 μM) (p <0.05, except for compound **13** (p >0.05)) and compound **4** (EC_{50} = 36.3 μM) (p <0.05) on precontracted rat aorta rings but less active than (±)-cromakalim (EC_{50} = 0.13 μM) and (±)-pinacidil (EC_{50} = 0.35 μM) (p <0.05). In contrast to their effects on the pancreatic tissue, the myorelaxant activity of these drugs was strongly affected by the nature of the halogen atom in the 6-position of the benzopyran nucleus. The 6-bromo was more active than the 6-chloro (p <0.05), itself more potent than the 6-fluoro analogue (p <0.05).

Unfortunately, the vast majority of methoxy, methyl, and chlorophenylaminocarbonylamino drugs (compounds **16**−**24**,

Table 2. Effects of *R/S*-3,4-Dihydro-2,2-dimethyl-6-halo-4-(3- or 4-chlorophenylaminocarbonylamino)-2*H*-1-benzopyrans, (±)-Cromakalim, Diazoxide, (±)-Pinacidil, and Compound **4** on Insulin Secretion from Rat Pancreatic Islets and on the KCl-Induced Contractions of Rat Aorta Rings

	rat pancreatic β-cells			rat aorta rings	
	% residual insulin secretion[a]				
compds	10 μM	1 μM	IC_{50} (μM)[b]	EC_{50} (μM)[c]	IC_{50}/EC_{50}[d]
37	28.0 ± 1.5 (23)	86.0 ± 4.9 (23)	3.67	>300 (4)	<0.01
38	25.0 ± 1.3 (24)	81.0 ± 4.0 (23)	3.13	>30 (4)	<0.10
39	28.6 ± 1.7 (22)	91.5 ± 3.7 (23)	4.06	>30 (4)	<0.14
40	22.8 ± 1.2 (32)	91.4 ± 5.4 (23)	3.60	>30 (6)	<0.12
41	34.6 ± 1.9 (21)	85.4 ± 5.4 (22)	4.29	>300 (4)	<0.01
42	25.7 ± 1.5 (23)	92.2 ± 4.7 (24)	3.85	>300 (4)	<0.01
(±)-cromakalim	94.4 ± 4.1 (32)	95.3 ± 3.8 (31)	>100	0.13 ± 0.01 (7)[e]	>769.23
diazoxide	73.9 ± 4.4 (16)[f]	87.5 ± 5.0 (15)[f]	22.6[f]	22.4 ± 2.1 (11)[f]	1.00[f]
(±)-pinacidil	92.1 ± 3.9 (13)[f]	97.7 ± 6.7 (19)[f]	>100[f]	0.35 ± 0.02 (11)[f]	>285.71[f]
4	4.9 ± 0.4 (32)[f]	36.2 ± 2.4 (31)[f]	0.73[f]	36.3 ± 2.2 (6)[f]	0.02[f]

[a] Percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)). [b] IC_{50}: drug concentration giving 50% inhibition of insulin release. [c] EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aorta rings (mean ± SEM (*n*)). *n* refers to the number of samples. [d] Estimated selectivity ratio: pancreatic versus vascular tissue. [e] Published results: ref 21. [f] Published results: ref 25.

25−**33**, and **34**−**42**) precipitated in the bathing medium before reaching their maximal activity, making the determination of their EC_{50} values difficult. Nevertheless, all drugs were found to be less active on the vascular tissue than (±)-cromakalim or (±)-pinacidil (Table 1).

On the basis of these biological data, the *R/S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(3- or 4-chlorophenylaminocarbonylamino)-2*H*-1-benzopyrans (**37**−**42**) can be regarded as the most promising drugs. Such compounds, at a 10 μM concentration, were potent inhibitors of the insulin-releasing process (all drugs were more active than diazoxide) and displayed a weak myorelaxant activity (all drugs were less potent than (±)-cromakalim and (±)-pinacidil).

The IC_{50} values (inhibition of insulin release) and the IC_{50}/EC_{50} ratio were determined to assess the apparent tissue selectivity (pancreatic vs vascular) of the different compounds (Table 2).

As expected, (±)-cromakalim and (±)-pinacidil were found to be clearly selective for the vascular tissue with a selectivity ratio higher than 769 and 285, respectively (Table 2). However, with a selectivity ratio of 0.02 and an IC_{50} value below 1 μM, compound **4** appeared rather selective for the pancreatic tissue.[20] Diazoxide, a K_{ATP} channel opener,[24] exhibited a selectivity ratio of 1, indicating that the compound was equipotent on both tissues.

The data further indicated that the original *R/S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(3- or 4-chlorophenylaminocarbonylamino)-2*H*-1-benzopyrans (**37**−**42**) displayed selectivity for the pancreatic versus the vascular tissue (see IC_{50}/EC_{50} ratio (Table 2)). The EC_{50} values of the different molecules (**37**−**42**) were higher than those observed for the reference compounds (±)-cromakalim, diazoxide, and (±)-pinacidil, whereas their IC_{50} values, although being above that of compound **4**, were lower than that of diazoxide. Therefore, compared with the reference benzopyran (±)-cromakalim, the new drugs clearly exhibited an inverted tissue selectivity.

Because these new compounds (**37**−**42**) present a stereogenic center at the C-4 position of the benzopyran nucleus, the tissue selectivity (pancreatic versus vascular) of the individual optical isomers remains to be determined.

Taken as a whole, the present biological data indicate that the newly synthesized dimethylchromans are less effective as vasorelaxants and much more active as inhibitors of insulin secretion than the reference molecule (±)-cromakalim. Numerous derivatives were even more potent at inhibiting the insulin-secretory process than diazoxide and our initially developed (±)-cromakalim analogues.[21] Looking at the nature and position of the substituent on the C-4 phenyl ring, the most marked activity on insulin-secreting β-cells was obtained by introducing a meta or para electron-withdrawing group (a chlorine atom) on the C-4 phenyl ring (compounds **37**−**42**). These molecules also exhibited a pronounced selectivity for pancreatic versus vascular tissue.

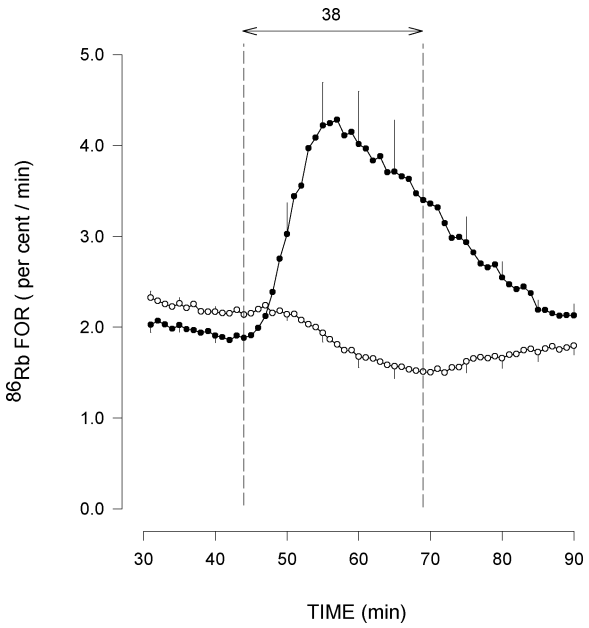


Figure 3. Effect of **38** (50 μM) on ^{86}Rb outflow from pancreatic islets perifused throughout in the absence (●) or presence (○) of glibenclamide (10 μM). Basal media contained 5.6 mmol/L glucose. Mean values (±SEM) refer to six individual experiments.

Because compound **38** was the most potent at inhibiting the insulin secretory process (Table 2), additional radioisotopic and electrophysiologic experiments were performed to ascertain that the drug acted through the activation of pancreatic ATP-sensitive potassium channels.

In the first series of experiments, using the efflux of ^{86}Rb as an index of K^+ permeability,[15,19,20,25] we have characterized the effect of compound **38** on the ^{86}Rb fractional outflow (FOR) rate from prelabeled and perifused rat pancreatic islets (Figure 3). The addition of **38** (50 μM) provoked a rapid, pronounced, and sustained increase in the rate of ^{86}Rb outflow. When the same experiment was repeated in the presence of glibenclamide (10 μM) in the perifusing medium, a hypoglycemic sulfonylurea

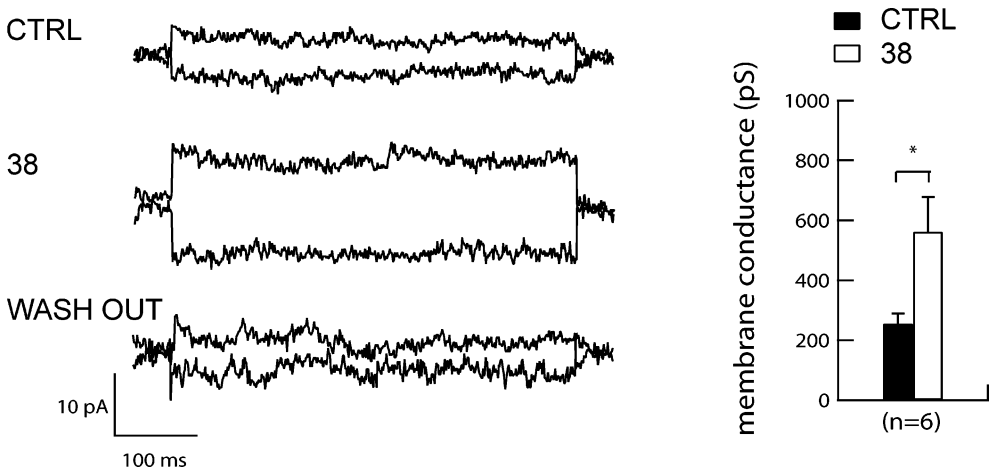


Figure 4. Effect of compound **38** on whole-cell K currents in a single rat pancreatic β-cell. Left panel: whole-cell K current resulting from voltage stimulation going from −70 to −60 or −80 mV, in the absence (CTRL = control, upper recording) and in the presence of 50 μM of compound **38** (middle recording) and after the removal of compound **38** (wash-out, lower recording). Right panel: histogram reports the corresponding increase in membrane conductance (CTRL = control). Mean values (±SEM) refer to six individual experiments.

known to block K_{ATP} channels,[15,20] the capacity of **38** to stimulate ^{86}Rb FOR was completely abolished (Figure 3).

In the second series of experiments performed on single rat pancreatic β-cells, we recorded whole-cell current in the presence of 3 mM ATP in the pipet solution to inhibit the K_{ATP} channel activity. The K current was monitored in voltage clamp by successively holding the membrane potential at −60 and −80 mV during 500 ms from an initial potential of −70 mV. This protocol allowed us to evaluate the resting membrane resistance in the absence and presence of drug **38**. In the presence of 50 μM of drug **38**, the membrane current more than doubled (Figure 4). This enhancing effect of compound **38** was reversible. Figure 4 (right panel) also indicates that the corresponding membrane conductance increased from 256 ± 33 pS, in the control condition, to 563 ± 115 pS in the presence of drug **38** ($n = 6$, $p < 0.05$).

All together, these radioisotopic and electrophysiological data suggest that in insulin-secreting cells compound **38** activated K_{ATP} channels.

Conclusion

In the present study, we have synthesized and examined the biological activity of several novel *R/S*-4,6-disubstituted 2,2-dimethylchromans, structurally related to the potassium channel opener (±)-cromakalim, bearing diverse phenylurea groups at the 4-position of the benzopyran nucleus.

Biological results indicated that compounds devoid of a substituent (**13**−**15**) or substituted by an electron-donating group (**16**−**33**) on the C-4 phenyl ring inhibited glucose-induced insulin secretion. An electron-withdrawing group substitution such as a chlorine atom on the C-4 phenyl ring (**34**−**42**) markedly enhanced the inhibitory effect on insulin release. In such a series, the *meta*- and *para*-chloro derivatives (**37**−**42**) were found to be more active than the *ortho*-chloro compounds (**34**−**36**). Most of these new dimethylchromans were more potent than the reference molecule (±)-cromakalim at reducing the insulin-releasing process. Vascular data further indicated that the new drugs were less active than (±)-cromakalim as vasorelaxant agents.

Among the newly synthesized dimethylchromans, drugs **37**−**42** (*meta*- and *para*-chloro derivatives) can be regarded as the most promising compounds. Indeed, they were potent inhibitors of the glucose-induced insulin release (IC_{50} = 3.67, 3.13, 4.06, 3.60, 4.29, and 3.85 μM, respectively), but displayed a weak myorelaxant activity (EC_{50} >300, >30, >30, >30, >300, and > 300 μM, respectively). Therefore, in contrast to the reference molecule cromakalim (IC_{50}/EC_{50} >770), novel compounds **37**−**42** exhibited an apparent selectivity for the endocrine pancreatic tissue (IC_{50}/EC_{50} <0.14).

In conclusion, the present dimethylchromans can be considered as the first examples of cromakalim analogues exhibiting a marked selectivity for pancreatic vs vascular tissue.

Radioisotopic and electrophysiological experiments performed with the most potent drug inhibiting the insulin secretory rate, namely, compound **38** (*R/S*-6-chloro-4-(3-chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran), confirmed that the drug activated the pancreatic K_{ATP} channels.

Thus, these novel molecules might serve as new leads for further developments of K_{ATP} channel openers targeting the insulin secreting cell.

Additional pharmacomodulations, and particularly the substitution of the urea moiety by a bioisosteric thiourea group, are planned to characterize more active and more selective chroman derivatives inhibiting the insulin releasing process.

Experimental Section

Chemistry. Melting points were determined on a Büchi 530 capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1000 FTIR spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker Avance (500 MHz) instrument using d_6-DMSO as the solvent with TMS as an internal standard; chemical shifts are reported in δ values (ppm) relative to that of internal TMS. The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and b = broad are used throughout. Elemental analyses (C, H, N, S) were realized on a Carlo-Erba EA 1108-elemental analyzer and were within ±0.4% of the theoretical values. All reactions were routinely checked by TLC on silica gel Merck 60 F_{254}.

Starting Materials. Convenient starting materials for the synthesis of the original *R/S*-3,4-dihydro-2,2-dimethyl-6-halo-4-(phenylaminocarbonylamino)-2*H*-1-benzopyrans (**13**−**42**) are *R/S*-4-amino-3,4-dihydro-2,2-dimethyl-6-fluoro-2*H*-1-benzopyran (**12a**), *R/S*-4-amino-6-chloro-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**12b**), and *R/S*-4-amino-6-bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**12c**). These compounds were prepared according to the methods previously reported.[21,22]

***R/S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(phenylaminocarbonylamino)-2*H*-1-benzopyran (13).** Phenyl isocyanate (0.26 mL, 2.4 mmol) was added to a solution of **12a**[22] (0.4 g, 2 mmol) in

methylene chloride (5 mL). After 20 min at room temperature, the resulting precipitate was collected by filtration, washed with petroleum ether 40/60 and dried (0.55 g, 85%): mp 191−193 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{18}H_{19}FN_2O_2$) C, H, N.

***R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(phenylaminocarbonylamino)-2*H*-1-benzopyran (14).** Phenyl isocyanate (0.25 mL, 2.3 mmol) was added to a solution of **12b**[21] (0.4 g, 1.9 mmol) in methylene chloride (5 mL). After 20 min at room temperature, the resulting precipitate was collected by filtration, washed with petroleum ether 40/60 and dried. The crude product was triturated with ethyl acetate. The insoluble material was eliminated by filtration and petroleum ether 40/60 was added to the filtrate. The resulting precipitate was collected by filtration, washed with petroleum ether 40/60, and dried (0.33 g, 53%): mp 194−196 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{18}H_{19}ClN_2O_2$) C, H, N.

***R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(phenylaminocarbonylamino)-2*H*-1-benzopyran (15).** The title compound was obtained as described for **14**, starting from **12c**[21] (0.4 g, 1.6 mmol) and phenyl isocyanate (0.21 mL, 1.9 mmol) (0.33 g, 57%): mp 193−195 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz. Anal. ($C_{18}H_{19}BrN_2O_2$) C, H, N.

***R/S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(2-methoxyphenylaminocarbonylamino)-2*H*-1-benzopyran (16).** 2-Methoxyphenyl isocyanate (0.32 mL, 2.4 mmol) was added to a solution of **12a**[22] (0.4 g, 2 mmol) in methylene chloride (5 mL). After 30 min at room temperature, the solvent was removed under reduced pressure, and the crude product was triturated with ethyl acetate. The insoluble material was eliminated by filtration, and petroleum ether 40/60 was added to the filtrate. The resulting precipitate was collected by filtration, washed with petroleum ether 40/60, and dried. The product was then crystallized in a mixture of methanol/water. The resulting precipitate was collected by filtration, washed with water, and dried. The crude product was triturated with ethyl acetate. The insoluble material was eliminated by filtration, and petroleum ether 40/60 was added to the filtrate. The resulting precipitate was collected by filtration, washed with petroleum ether 40/60, and dried (0.08 g, 11%): mp 172−175 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}FN_2O_3$) C, H, N.

***R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(2-methoxyphenylaminocarbonylamino)-2*H*-1-benzopyran (17).** The title compound was obtained as described for **14**, starting from **12b**[21] (0.4 g, 1.9 mmol) and 2-methoxyphenyl isocyanate (0.30 mL, 2.3 mmol) (0.41 g, 60%): mp 180−181 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}ClN_2O_3$) C, H, N.

***R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(2-methoxyphenylaminocarbonylamino)-2*H*-1-benzopyran (18).** The title compound was obtained as described for **14**, starting from **12c**[21] (0.4 g, 1.6 mmol) and 2-methoxyphenyl isocyanate (0.25 mL, 1.9 mmol) (0.37 g, 58%): mp 191−193 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}BrN_2O_3$) C, H, N.

***R/S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(3-methoxyphenylaminocarbonylamino)-2*H*-1-benzopyran (19).** 3-Methoxyphenyl isocyanate (0.31 mL, 2.4 mmol) was added to a solution of **12a**[22] (0.4 g, 2 mmol) in methylene chloride (5 mL). After 20 min at room temperature, the resulting precipitate was collected by filtration, washed with petroleum ether 40/60, and dried. The crude product was triturated with ethyl acetate. The insoluble material was eliminated by filtration, and petroleum ether 40/60 was added to the filtrate. The resulting precipitate was collected by filtration, washed with petroleum ether 40/60, and dried. The product was then crystallized in a mixture of methanol/water. The resulting precipitate was collected by filtration, washed with water, and dried. The crude product was dissolved in a minimum of hot methanol, and the insoluble material was eliminated by filtration. After cooling, the resulting precipitate was collected by filtration and dried (0.17 g, 25%): mp 189.5−190.5 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}FN_2O_3$) C, H, N.

***R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(3-methoxyphenylaminocarbonylamino)-2*H*-1-benzopyran (20).** The title compound was obtained as described for **14**, starting from **12b**[21] (0.4 g, 1.9 mmol) and 3-methoxyphenyl isocyanate (0.30 mL, 2.3 mmol) (0.45 g, 66%): mp 164−167 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}ClN_2O_3$) C, H, N.

***R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(3-methoxyphenylaminocarbonylamino)-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 isocyanate (0.25 mL, 1.9 mmol) (0.53 g, 84%): mp 172−174 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}BrN_2O_3$) C, H, N.

***R/S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(4-methoxyphenylaminocarbonylamino)-2*H*-1-benzopyran (22).** The title compound was obtained as described for **14**, starting from **12a**[22] (0.4 g, 2 mmol) and 4-methoxyphenyl isocyanate (0.31 mL, 2.4 mmol) (0.21 g, 30%): mp 158−177 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}FN_2O_3$) C, H, N.

***R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(4-methoxyphenylaminocarbonylamino)-2*H*-1-benzopyran (23).** 4-Methoxyphenyl isocyanate (0.30 mL, 2.3 mmol) was added to a solution of **12b**[21] (0.4 g, 1.9 mmol) in methylene chloride (5 mL). After 20 min at room temperature, the resulting precipitate was collected by filtration, washed with petroleum ether 40/60, and dried. The crude product was triturated with ethyl acetate. The insoluble material was eliminated by filtration, and petroleum ether 40/60 was added to the filtrate. The resulting precipitate was collected by filtration, washed with petroleum ether 40/60, and dried. The crude product was dissolved in a minimum of hot methanol, and the insoluble material was eliminated by filtration. After cooling, the resulting precipitate was collected by filtration and dried (0.35 g, 51%): mp 159−161 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}ClN_2O_3$) C, H, N.

***R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(4-methoxyphenylaminocarbonylamino)-2*H*-1-benzopyran (24).** The title compound was obtained as described for **23**, starting from **12c**[21] (0.4 g, 1.6 mmol) and 4-methoxyphenyl isocyanate (0.25 mL, 1.9 mmol) (0.24 g, 38%): mp 182−185 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}BrN_2O_3$) C, H, N.

***R/S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(2-methylphenylaminocarbonylamino)-2*H*-1-benzopyran (25).** The title compound was obtained as described for **14**, starting from **12a**[22] (0.4 g, 2.1 mmol) and 2-methylphenyl isocyanate (0.31 mL, 2.4 mmol) (0.51 g, 75%): mp 197−198 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}FN_2O_2$) C, H, N.

***R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(2-methylphenylaminocarbonylamino)-2*H*-1-benzopyran (26).** The title compound was obtained as described for **14**, starting from **12b**[21] (0.4 g, 1.9 mmol) and 2-methylphenyl isocyanate (0.28 mL, 2.3 mmol) (0.40 g, 61%): mp 190.5−191.5 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}ClN_2O_2$) C, H, N.

***R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(2-methylphenylaminocarbonylamino)-2*H*-1-benzopyran (27).** The title compound was obtained as described for **14**, starting from **12c**[21] (0.4 g, 1.6 mmol) and 2-methylphenyl isocyanate (0.23 mL, 1.9 mmol) (0.43 g, 70%): mp 198−199 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}BrN_2O_2$) C, H, N.

***R/S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(3-methylphenylaminocarbonylamino)-2*H*-1-benzopyran (28).** The title compound was obtained as described for **14**, starting from **12a**[22] (0.4 g, 2.1 mmol) and 3-methylphenyl isocyanate (0.31 mL, 2.4 mmol) (0.51 g, 75%): mp 193−194 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}FN_2O_2$) C, H, N.

***R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(3-methylphenylaminocarbonylamino)-2*H*-1-benzopyran (29).** The title compound was obtained as described for **23**, starting from **12b**[21] (0.4 g, 1.9 mmol) and 3-methylphenyl isocyanate (0.29 mL, 2.3 mmol) (0.15 g, 22%): mp 191−192 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}ClN_2O_2$) C, H, N.

***R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(3-methylphenylaminocarbonylamino)-2*H*-1-benzopyran (30).** The title compound was obtained as described for **23**, starting from **12c**[21] (0.4 g, 1.6 mmol) and 3-methylphenyl isocyanate (0.24 mL, 1.9 mmol) (0.20 g, 33%): mp 197−198 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}BrN_2O_2$) C, H, N.

***R/S*-3,4-Dihydro-2,2-dimethyl-6-fluoro-4-(4-methylphenylaminocarbonylamino)-2*H*-1-benzopyran (31).** The title compound was obtained as described for **13**, starting from **12a**[22] (0.4 g, 2.1 mmol) and 4-methylphenyl isocyanate (0.31 mL, 2.4 mmol) (0.53 g, 79%): mp 185−186 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}FN_2O_2$) C, H, N.

***R/S*-6-Chloro-3,4-dihydro-2,2-dimethyl-4-(4-methylphenylaminocarbonylamino)-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 isocyanate (0.29 mL, 2.3 mmol) (0.55 g, 84%): mp 193−194 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}ClN_2O_2$) C, H, N.

***R/S*-6-Bromo-3,4-dihydro-2,2-dimethyl-4-(4-methylphenylaminocarbonylamino)-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 isocyanate (0.24 mL, 1.9 mmol) (0.52 g, 85%): mp 192−193 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{19}H_{21}BrN_2O_2$) C, H, N.

***R/S*-4-(2-Chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-6-fluoro-2*H*-1-benzopyran (34).** The title compound was obtained as described for **14**, starting from **12a**[22] (0.4 g, 2.1 mmol) and 2-chlorophenyl isocyanate (0.30 mL, 2.4 mmol) (0.39 g, 54%): mp 181−183 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{18}H_{18}ClFN_2O_2$) C, H, N.

***R/S*-6-Chloro-4-(2-chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (35).** 2-Chlorophenyl isocyanate (0.27 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 at room temperature, the solvent was removed under reduced pressure, and the crude product was triturated with ethyl acetate. The insoluble material was eliminated by filtration, and petroleum ether 40/60 was added to the filtrate. The resulting precipitate was collected by filtration, washed with petroleum ether 40/60, and dried (0.44, 64%): mp 195−195.5 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{18}H_{18}Cl_2N_2O_2$) C, H, N.

***R/S*-6-Bromo-4-(2-chlorophenylaminocarbonylamino)-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 isocyanate (0.23 mL, 1.9 mmol) (0.39 g, 60%): mp 184−185.5 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{18}H_{18}BrClN_2O_2$) C, H, N.

***R/S*-4-(3-Chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-6-fluoro-2*H*-1-benzopyran (37).** The title compound was obtained as described for **14**, starting from **12a**[22] (0.4 g, 2.1 mmol) and 3-chlorophenyl isocyanate (0.30 mL, 2.4 mmol) (0.40 g, 56%): mp 214−215 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{18}H_{18}ClFN_2O_2$) C, H, N.

***R/S*-6-Chloro-4-(3-chlorophenylaminocarbonylamino)-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 isocyanate (0.28 mL, 2.3 mmol) (0.61 g, 89%): mp 190−192 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{18}H_{18}Cl_2N_2O_2$) C, H, N.

***R/S*-6-Bromo-4-(3-chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (39).** The title compound was obtained as described for **14**, starting from **12c**[21] (0.4 g, 1.6 mmol) and 3-chlorophenyl isocyanate (0.23 mL, 1.9 mmol) (0.41 g, 64%): mp 196−198 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{18}H_{18}BrClN_2O_2$) C, H, N.

***R/S*-4-(4-Chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-6-fluoro-2*H*-1-benzopyran (40).** 4-Chlorophenyl isocyanate (0.38 g, 2.4 mmol) was added to a solution of **12a**[22] (0.4 g, 2 mmol) in methylene chloride (5 mL). After 20 min at room temperature, the resulting precipitate was collected by filtration, washed with petroleum ether 40/60, and dried. The product was then crystallized in a mixture of methanol/water. The resulting precipitate was collected by filtration, washed with water, and dried. The crude product was triturated with ethyl acetate. The insoluble material was eliminated by filtration, and petroleum ether 40/60 was added to the filtrate. The resulting precipitate was collected by filtration, washed with petroleum ether 40/60, and dried (0.25 g, 35%): mp 188−189 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{18}H_{18}ClFN_2O_2$) C, H, N.

***R/S*-6-Chloro-4-(4-chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (41).** The title compound was obtained as described for **40**, starting from **12b**[21] (0.4 g, 1.9 mmol) and 4-chlorophenyl isocyanate (0.35 g, 2.3 mmol) (0.31 g, 44%): mp 205−211 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{18}H_{18}Cl_2N_2O_2$) C, H, N.

***R/S*-6-Bromo-4-(4-chlorophenylaminocarbonylamino)-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 isocyanate (0.29 g, 1.9 mmol) (0.56 g, 87%): mp 211−212 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{18}H_{18}BrClN_2O_2$) C, H, N.

Biological Assays. (±)-Cromakalim (Beecham Pharmaceutical, U.K.), diazoxide (Sigma Chemical, U.S.A.), BPDZ 73, and (±)-pinacidil (Natural and Synthetic Drugs Research Center, Laboratoire de Chimie Pharmaceutique, ULg, Belgium) were tested as reference compounds. All tested compounds were dissolved in dimethyl sulfoxide, which was added to both control and test media at final concentrations not exceeding 0.1% (v/v).

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 and 0.5% (w/v) dialyzed albumin (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 required chroman derivative.

The release of insulin was measured radioimmunologically using rat insulin as a standard.[20]

Residual insulin secretion was expressed as a percentage of the value recorded in control experiments (100%), that is, in the absence of the drug and in the presence of 16.7 mM glucose.

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 and the segments suspended under 1.5 g tension 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 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 chroman derivative 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).

Electrophysiological Measurements. Rat pancreatic islets were isolated from adult fed Wistar rats (Charles River Laboratories, Belgium).

Single cells were prepared by shaking in low calcium media as previously described.[26] Isolated cells were plated on glass cover slips and kept in tissue culture for up to 2 days in RPMI 1640

culture medium (Invitrogen, Merelbeke, Belgium) supplemented with 5 mM glucose, 10% v/v newborn calf serum, 100 μg/mL of streptomycin, and 100 IU/mL of penicillin.

Whole-cell K currents were recorded in the whole-cell configuration of the patch-clamp technique.[27] Ionic currents were recorded using an EPC-8 amplifier (List Electronic, Darmstadt, Germany) and stored on a computer. The Pulse software (HEKA, Lambrecht/Pfalz, Germany) and an ITC-16 AD/DA converter (Instrutech, New York, USA) were used to control the experiments. The current signals were digitized at 1 kHz. Prior to digitizing, the signals were filtered at 500 Hz using an 8-pole Bessel filter (Frequency Devices, Haverhill, MA). All experiments were carried out at room temperature. The standard extracellular solution was composed of (in mM) NaCl 138, KCl 5.6, $CaCl_2$ 2.6, $MgCl_2$ 1.2, glucose 5, and HEPES 5 (pH 7.40 with NaOH). The pipet solution consisted of (in mM) KCl 140, NaCl 10, $MgCl_2$ 1, EGTA 0.05, HEPES 5, and Mg-ATP 3 (pH 7.15 with KOH).

Measurements of ^{86}Rb Outflow from Perifused Rat Pancreatic Islets. Experiments were performed with pancreatic islets isolated from adult fed Wistar rats (Charles River Laboratories, Belgium).

Groups of 100 islets were incubated for 60 min at 37 °C in 120 μL of a bicarbonate-buffered medium (in mM: NaCl 115, KCl 5, $CaCl_2 \cdot 2H_2O$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24, dialyzed albumin 0.5% w/v) containing 16.7 mM glucose and ^{86}Rb (^{42}K substitute) (0.15−0.25 mmol/L:50 μCi/mL). After incubation, the islets were washed four times with a nonradioactive medium and then placed in a perifusion chamber. The perifusate was delivered at a constant rate (1.0 mL/min). From the 31st to the 90th min, and 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. At the end of the perifusion, the radioactive content of the islets was also determined.

The outflow of ^{86}Rb (cpm/min) was expressed as an FOR (% of instantaneous islet content per min).[19,20]

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

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Supporting Information Available: Spectroscopic data (IR and NMR) and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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