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Bioorganic & Medicinal Chemistry 16 (2008) 6124–6130
Bioorganic & Medicinal Chemistry

# Synthesis and activity on rat aorta rings and rat pancreatic β-cells of ring-opened analogues of benzopyran-type potassium channel activators

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Received 27 March 2008; accepted 16 April 2008
Available online 25 April 2008

**Abstract**—Ring-opened analogues of dihydrobenzopyran potassium channel openers (PCOs) were prepared and evaluated as putative PCOs on rat aorta rings (myorelaxant effect) and rat pancreatic β-cells (inhibition of insulin secretion). These derivatives are characterized by the presence of a sulfonylurea, a urea or an amide function. Some compounds bearing an arylurea moiety provoked vasorelaxant effects and a marked inhibition of insulin release. Derivatives bearing a sulfonylurea or an amide function were, however, poorly active on both tissues. Structure–activity relationships and apparent tissue selectivity are discussed.


## 1. Introduction

ATP-sensitive potassium channel openers consist of a class of compounds with a wide range of chemical structures, among which the most known belong to benzopyrans (i.e., cromakalim), cyanoguanidines (i.e., pinacidil) and arylthiadiazine dioxides (i.e., diazoxide).[1] Cromakalim derivatives bearing amide, urea, thiourea, sulfonylurea and carbamate moieties have been recently described (Fig. 1).[2–5] Some of these compounds provoked a marked inhibition of insulin secretion from rat pancreatic β-cells, and/or exhibited smooth muscle myorelaxant activity on rat aorta and rat uterus.[2–5]

In particular, amides with a short R alkyl chain (see Fig. 1) were found to be more active than diazoxide as myorelaxants.[3] On the other hand, ureas with R' corresponding to a *m*- or a *p*-chlorophenyl moiety (see Fig. 1), were identified as potent inhibitors of insulin release.[5] The biological effects of these drugs were related to the activation of ATP-sensitive potassium channels.[3,5]

In order to develop new drugs, we undertook the synthesis and pharmacological evaluation of some simplified compounds resulting from both the opening of the pyran ring of dihydrobenzopyrans and the introduction of amide, urea or sulfonylurea moieties (Fig. 2). These original drugs should be more flexible, which might lead to additional pharmacological properties.

Moreover, benzopyran-type arylureas devoid of a substituent at the 6-position of the heterocycle were also synthesized. These compounds constitute the link between the ring-opened drugs and previously described 6-halo-substituted benzopyran-type arylureas.

*Keywords*: Ring-opened cromakalim analogues; Inhibition of insulin secretion; Vasorelaxant effect; Potassium channel opener.
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doi:10.1016/j.bmc.2008.04.043

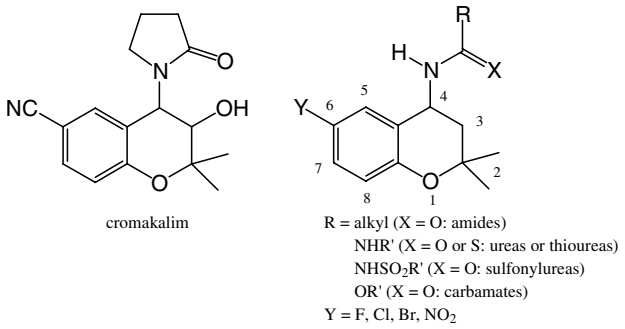



**Figure 1.** Structure of cromakalim and of some recently described dihydrobenzopyans bearing miscellaneous functional groups at the 4-position.

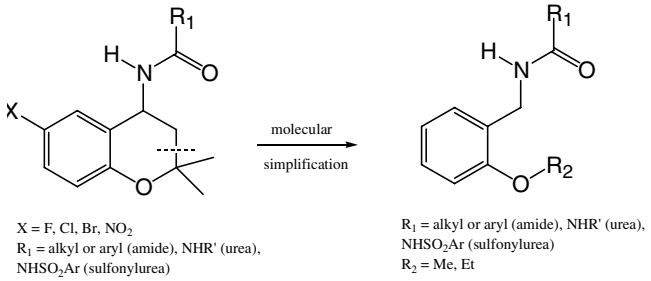



**Figure 2.** General structure of the target compounds after molecular simplification of dihydrobenzopyrans.

## 2. Results

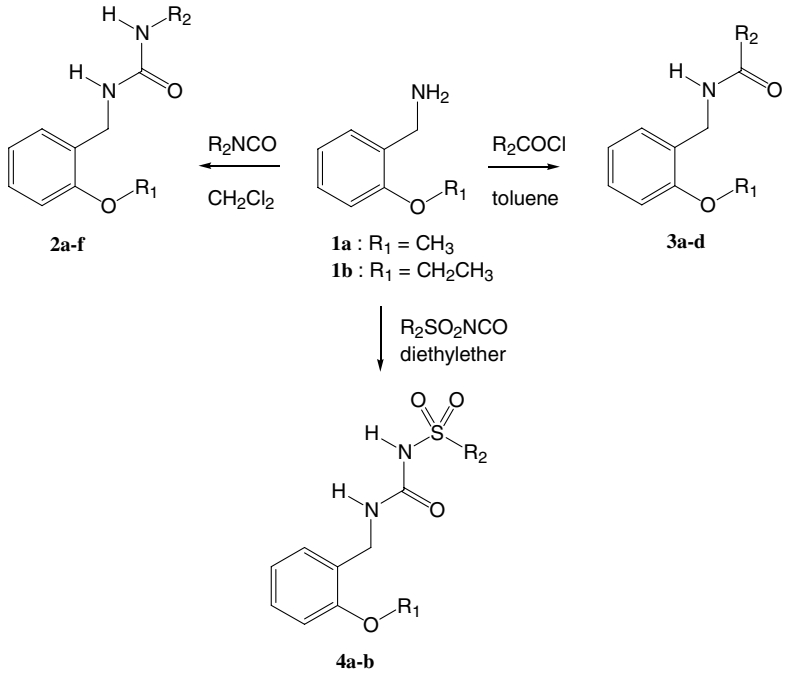
### 2.1. Synthesis

Scheme 1 illustrates the synthetic route used to prepare the target compounds **2a–f**, **3a–d** and **4a–b** from the *ortho*-alkoxybenzylamines **1a** and **1b**, by reacting the latter intermediates with alkyl or aryl isocyanates, acyl chlorides and arylsulfonyl isocyanates, respectively. The resulting compounds were isolated in good yields after vacuum filtration.

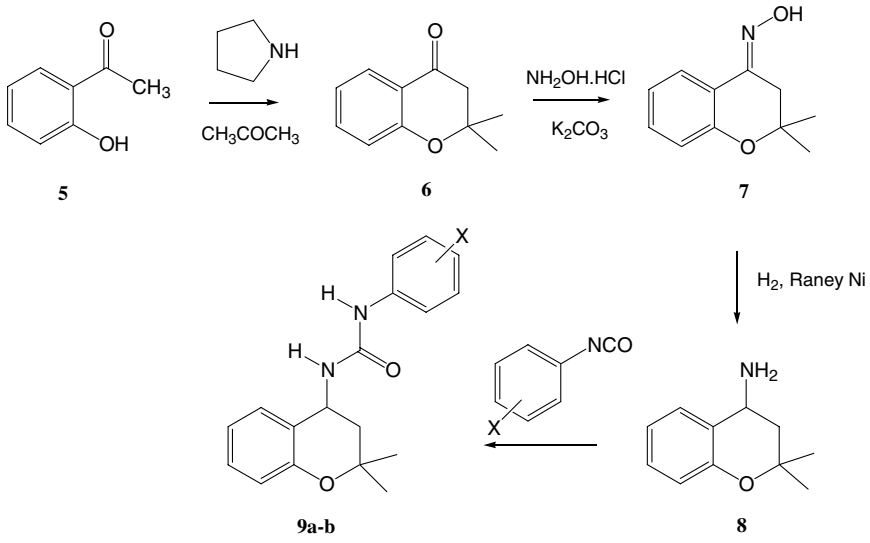
The synthesis of the non-substituted benzopyran-type arylureas **9a–b** is depicted in Scheme 2. Starting from 2-hydroxyacetophenone **5**, the classical ring closure reaction using acetone in the presence of pyrrolidine led to the expected 2,2-dimethylchroman-4-one **6**. Access to 4-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran **8** was achieved, in excellent yields, by means of an unusual route consisting in the reduction of the oxime intermediate **7** by hydrogenation in presence of Raney Nickel.[8] The phenylureas **9a–b** were finally obtained after the reaction of the primary amine **8** with the appropriate chloro-substituted phenyl isocyanate.

### 2.2. Biological evaluation

**2.2.1. Inhibitory activity on insulin secretion from rat pancreatic islets.** Compounds **2a–f**, **3a–d**, **4a–b** and **9a–b** were evaluated as inhibitors of insulin secretion from rat pancreatic islets incubated in the presence of an insulinotropic glucose concentration (16.7 mM). As observed in Table 1, urea derivatives **2c–f** for which $R_2$ corresponded to *p*-$ClC_6H_5$ or *m*-$ClC_6H_5$ exhibited a marked inhibitory effect on the insulin releasing process. Such arylurea derivatives were much more potent than cromakalim and pinacidil at reducing the glucose-induced insulin release. Most drugs were found to be equipotent or even more potent than the $K_{ATP}$ channel opener diazoxide. Thus, the estimated $IC_{50}$ value (drug concentration inducing a 50% reduction in the insulin secretory rate) amounted to 18.9 μM for compound **2d**, 22.0 μM




**Scheme 1.** Synthetic route to the target compounds **2a–f**, **3a–d** and **4a–b**.




**Scheme 2.** Synthetic route to the target compounds **9a**–**b**.

**Table 1.** Effects of compounds **2a**–**f**, **3a**–**d**, **4a**–**b**, **9a**–**b** and **10a**–**b** as well as several reference PCOs on insulin secretion from rat pancreatic islets and on the contractile activity of $K^+$-depolarized rat aorta rings (results expressed as means ± SEM (*n*))

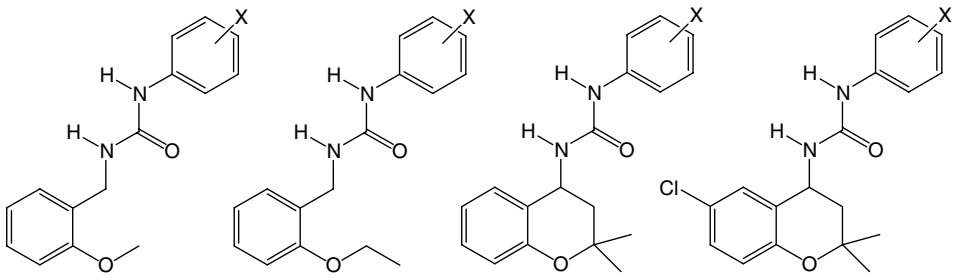
| No | $R_1$ | $R_2$ | Residual insulin secretion % | | Myorelaxant activity $ED_{50}$ (μM) |
|---|---|---|---|---|---|
| | | | (**50** μM) | (**10** μM) | |
| **2a** | $CH_3$ | $CH_2CH_3$ | 97.0 ± 5.4 (24) | nd | >300 (4) |
| **2b** | $CH_2CH_3$ | $CH_2CH_3$ | 95.7 ± 4.2 (16) | nd | >300 (4) |
| **2c** | $CH_3$ | $p-ClC_6H_5$ | 38.8 ± 2.2 (16) | 90.8 ± 3.8 (15) | >30 (4)[b] |
| **2d** | $CH_2CH_3$ | $p-ClC_6H_5$ | 23.7 ± 1.9 (15) | 72.6 ± 4.1 (16) | >30 (4)[b] |
| **2e** | $CH_3$ | $m-ClC_6H_5$ | 25.9 ± 2.3 (22) | 79.5 ± 3.3 (16) | 10.3 ± 1.4 (4) |
| **2f** | $CH_2CH_3$ | $m-ClC_6H_5$ | 12.7 ± 0.9 (15) | 64.1 ± 3.2 (15) | 7.8 ± 0.8 (4) |
| **3a** | $CH_3$ | $p-NitroC_6H_5$ | 86.6 ± 5.8 (15) | nd | >30 (4)[b] |
| **3b** | $CH_2CH_3$ | $p-NitroC_6H_5$ | 86.5 ± 8.0 (16) | nd | >30 (4)[b] |
| **3c** | $CH_3$ | 2-Thienyl | 83.3 ± 5.8 (14) | nd | >30 (4)[b] |
| **3d** | $CH_2CH_3$ | 2-Thienyl | 93.3 ± 6.6 (15) | nd | >30 (4)[b] |
| **4a** | $CH_3$ | $p-ClC_6H_5$ | 112.6 ± 5.9 (15) | nd | >30 (4)[b] |
| **4b** | $CH_2CH_3$ | $p-ClC_6H_5$ | 107.7 ± 3.1 (16) | nd | >30 (4)[b] |
| **9a** | — | — | nd | 63.6 ± 4.6 (15) | 12.0 ± 1.7 (4) |
| **9b** | — | — | nd | 45.5 ± 2.5 (16) | 10.0 ± 2.1 (5) |
| **10a** | — | — | nd | 34.6 ± 1.9 (21)[a] | > 300 (4)[a] |
| **10b** | — | — | nd | 25.0 ± 1.3 (24)[a] | >30 (4)[a] |
| Diazoxide | — | — | 27.9 ± 1.5 (37)[a] | 71.7 ± 2.8 (38)[a] | 23.8 ± 2.4 (10)[a] |
| Pinacidil | — | — | 92.1± 5.5 (21)[a] | 96.0 ± 4.2 (20)[a] | 0.35 ± 0.02 (11)[a] |
| Cromakalim | — | — | 77.2 ± 4.3 (22)[a] | 94.7 ± 4.3 (24)[a] | 0.13 ± 0.01 (7)[a] |

[a] Published results: Refs. [3–7].
[b] Precipitates at $10^{-4}$ M.

for compound **2e**, 14.1 μM for compound **2f** and 19.7 μM for diazoxide, respectively.

Ureas **2a** and **2b** with an ethyl group at $R_2$ were inactive, indicating that an aromatic group with an electron-withdrawing substituent is more suitable than an aliphatic group to promote a reduction of the insulin secretory rate. It can also be observed that ureas with $R_1$ = ethyl were more potent than ureas bearing a methyl group at $R_1$. Lastly, and in contrast to most of their ring-closed analogues,[3,4] amide and sulfonylurea derivatives, **3a**–**d** and **4a**–**b**, respectively, were poorly active (Table 1).

Looking at the effect of compounds **9a**–**b** on the insulin releasing process, their potency was intermediate between that of the ring-opened arylureas **2c**–**f** and the previously described ring-closed 6-halo-substituted arylureas **10a**–**b**.[5] Interestingly, there was a progressive improvement of the inhibitory activity on pancreatic β-cells from the simplest methoxy compounds **2c** and **2e** to the ethoxy derivatives **2d** and **2f**, followed by the non-substituted ring-closed compounds **9a**–**b**, and finally the 6-chloro-substituted ring-closed compounds **10a**–**b** (Fig. 3 and Table 1). In any case, the *meta*-chlorophenylureas were found to be slightly more potent than the




**2c**: X = p-Cl
**2e**: X = m-Cl

**2d**: X = p-Cl
**2f**: X = m-Cl

**9a**: X = p-Cl
**9b**: X = m-Cl

**10a**: X = p-Cl
**10b**: X = m-Cl

**Figure 3.** From ring-opened to ring-closed benzopyran-type arylureas.

corresponding *para*-chlorophenylureas (Table 1). These results further highlight the critical importance of a substituent (i.e., a halogen atom) at the 6-position of the benzopyran ring.

**2.2.2. Myorelaxant effects on precontracted rat aorta rings.** The myorelaxant effects of compounds **2a**–**f**, **3a**–**d**, **4a**–**b** and **9a**–**b** were examined on rat aorta rings precontracted by 30 mM KCl (Table 1). The new ring-opened cromakalim analogues were less potent than cromakalim and pinacidil at reducing the vascular tone. Except for the *m*-chlorophenyl compounds **2e** and **2f** and for compounds **9a**–**b**, the vasorelaxant activity of these new analogues was also less pronounced than that of diazoxide. By comparing compounds **9a**–**b** with compounds **10a**–**b**, it clearly appeared that the introduction of a halogen atom at the 6-position of the benzopyran ring decreased the myorelaxant activity and enhanced the inhibitory effect on the insulin releasing process.

$IC_{50}/ED_{50}$ ratios have been determined to assess the apparent tissue selectivity (pancreatic endocrine versus vascular tissue) of the most active ring-opened cromakalim analogues. The $IC_{50}/ED_{50}$ ratio was found to be >0.63 for **2d**, 2.14 for **2e** and 1.80 for **2f**, respectively. Previous investigations indicated that cromakalim exhibited an $IC_{50}/ED_{50}$ selectivity ratio above 769, pinacidil above 285 and diazoxide around 1.[5]

## 3. Discussion and conclusion

The present results clearly revealed that the molecular simplification of dihydrobenzopyrans, by opening the pyran ring, removing the substituent on the benzene ring and introducing arylurea moieties led to a new class of compounds expressing identical biological effects than their ring-closed analogues.[2–4] The introduction on the ring-opened analogues of alkylurea, arylamide or arylsulfonylurea moieties failed to generate biologically active compounds.

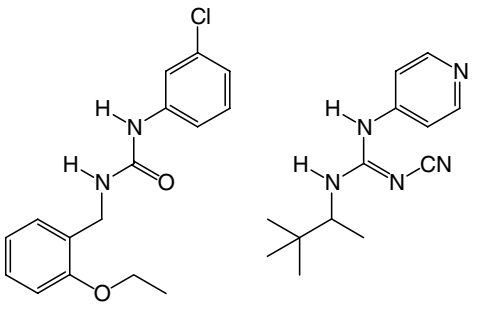
Arylurea derivatives bearing an aromatic group with an electron-withdrawing substituent at the *m*- or *p*-position were shown to be much more potent than cromakalim and pinacidil at reducing the insulin secretory rate whilst displaying weaker vasorelaxant activities than the reference molecules.

However, and in contrast to the reference compounds cromakalim and pinacidil, the biologically active ring-opened arylurea derivatives did not express a clear-cut tissue selectivity (pancreatic endocrine versus vascular tissue). As a result, these new compounds are rather comparable to the $K_{ATP}$ channel opener diazoxide.

Moreover, the biological results obtained with several ring-closed analogues devoid of any substituent at the 6-position of the benzopyran ring also highlight the critical importance of the presence of such a substituent (i.e., a halogen atom) for activity and selectivity on pancreatic β-cells.

Since the chemical structure of arylureas such as **2f** is closely related to that of pinacidil (Fig. 4), another $K_{ATP}$ channel opener, these new compounds may also be regarded as structural analogues of *N*-alkyl-*N*′-aryl-*N*″-cyanoguanidine $K_{ATP}$ channel openers. It is tempting to speculate that the new arylureas, like their 'isostere' pinacidil, could act as $K_{ATP}$ channel openers, but with a different affinity for the target tissues. The latter assumption remains to be demonstrated by further pharmacological investigations.

In conclusion, we succeeded to develop novel ring-opened analogues of cromakalim that are much more




**2f** pinacidil

**Figure 4.** Chemical structure of **2f** and pinacidil.

active on the pancreatic insulin-secreting tissue than the reference molecules. Such data call for further structural modification to identify original ring-opened analogues of cromakalim with improved insulin-secreting cell selectivity. Additional investigations are also required to ascertain that the biological effects of these cromakalim (or pinacidil)-derived compounds are related to the activation of ATP-sensitive $K^+$ channels.

## 4. Experimental

### 4.1. Chemistry, general procedures

Melting points were determined on a Büchi–Tottoli capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1750 FT spectrophotometer. The $^1$H NMR spectra were taken on a Bruker (500 MHz) instrument in DMSO-$d_6$ or in $CDCl_3$ with hexamethyldisiloxane (HMDS) as an internal standard. Chemical shifts are reported in δ values (ppm) relative to internal HMDS. The abbreviation s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, $CH_{arom}$ = aromatic CH, $CH_{aliph}$ = aliphatic CH, and b = broad are used throughout. Elemental analyses (C, H, N, S) were realized on a Carlo-Erba EA 1108-elemental analyser and were within ±0.4% of theoretical values. All reactions were routinely checked by TLC on silica gel Merck 60F 254.

**4.1.1. General procedure for compounds 2a–f.** An appropriate alkyl or aryl isocyanate (11 mmol; 1.1 equiv) was added to a solution of **1a** or **1b** (10 mmol; 1 equiv) in dichloromethane (20 mL). The mixture was stirred at room temperature for two hours. The resulting white precipitate was then filtered under vacuum and washed with a small amount of diethyl ether. In the absence of precipitate, the solvent was evaporated under reduced pressure and the residue was washed with a small amount of diethyl ether and dried.

**4.1.2. General procedure for compounds 3a–d.** An appropriate acyl chloride (11 mmol; 1.1 equiv) was added to a solution of **1a** or **1b** (10 mmol; 1 equiv) in toluene (20 mL). The mixture was refluxed for two hours and then cooled. The white precipitate was filtered under vacuum, washed with a small amount of diethyl ether and dried.

**4.1.3. General procedure for compounds 4a and 4b.** An appropriate arylsulfonyl isocyanate (11 mmol; 1.1 equiv) was added to a solution of **1a** or **1b** (10 mmol; 1 equiv) in diethyl ether (20 mL). The mixture was stirred at room temperature for one hour. The resulting white precipitate was then filtered under vacuum, washed with a small amount of diethyl ether and dried.

**4.1.3.1. 1-(2-Methoxybenzyl)-3-ethylurea (2a).** White powder (92%); mp 138–140 °C; IR (KBr) ν: 3340, 3320, 3120, 1525 (NH); 1635 (CO); 3010 ($CH_{arom}$); 2840 ($CH_{aliph}$) cm$^{-1}$. $^1$H NMR ($d_6$-DMSO) δ (ppm): 0.99 (t, 3H, NHCH$_2$*CH$_3$*), 3.02 (q, 2H, NH*CH$_2$*CH$_3$), 3.79 (s, 3H, OCH$_3$), 4.15 (d, 2H, C$_6$H$_5$*CH$_2$*NH), 5.89 (t, 1H, *NH*CH$_2$CH$_3$), 6.05 (t, 1H, C$_6$H$_5$CH$_2$*NH*), 6.89 (t, 1H, $CH_{arom}$), 6.96 (d, 1H, $CH_{arom}$), 7.16 (d, 1H, $CH_{arom}$), 7.21 (t, 1H, $CH_{arom}$). Calculated (%) for $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.74; N, 13.45. Found (%): C, 63.42; H, 7.73; N, 13.44.

**4.1.3.2. 1-(2-Ethoxybenzyl)-3-ethylurea (2b).** White powder (93%); mp 130–131 °C; IR (KBr) ν: 3320, 3120, 1540 (NH); 1635 (CO); 3040 ($CH_{arom}$); 2970 ($CH_{aliph}$) cm$^{-1}$. $^1$H NMR ($d_6$-DMSO) δ (ppm): 0.99 (t, 3H, NHCH$_2$*CH$_3$*), 1,35 (t, 3H, OCH$_2$*CH$_3$*), 3.02 (q, 2H, NH*CH$_2$*CH$_3$), 4.03 (q, 2H, O*CH$_2$*CH$_3$), 4.15 (d, 2H, C$_6$H$_5$*CH$_2$*NH), 5.93 (t, 1H, *NH*CH$_2$CH$_3$), 6.02 (t, 1H, C$_6$H$_5$CH$_2$*NH*), 6.88 (t, 1H, $CH_{arom}$), 6.93 (d, 1H, $CH_{arom}$), 7.14 (d, 1H, $CH_{arom}$), 7.18 (t, 1H, $CH_{arom}$). Calculated (%) for $C_{12}H_{18}N_2O_2$: C, 64.84; H, 8.16; N, 12.60. Found (%): C, 64.85; H, 8.15; N, 12.59.

**4.1.3.3. 1-(2-Methoxybenzyl)-3-(4-chlorophenyl)urea (2c).** White powder (93%); mp 202–203 °C; IR (KBr) ν: 3350, 3280, 3200, 3170, 1540 (NH); 1640 (CO); 3025 ($CH_{arom}$) cm$^{-1}$; 2835 ($CH_{aliph}$). $^1$H NMR ($d_6$-DMSO) δ (ppm): 3.83(s, 3H, OCH$_3$), 4.24 (d, 2H, C$_6$H$_5$C*H$_2$*NH), 6.46 (t, 1H, C$_6$H$_5$CH$_2$*NH*), 6.91 (t, 1H, $CH_{arom}$), 6.98 (d, 1H, $CH_{arom}$), 7.22 (m, 2H, $CH_{arom}$), 7.25 (d, 2H, $CH_{arom}$), 7.41 (d, 2H, $CH_{arom}$), 8.71 (s, 1H, *p*-ClC$_6$H$_5$*NH*). Calculated (%) for $C_{15}H_{15}ClN_2O_2$: C, 61.97; H, 5.20; N, 9.64. Found (%): C, 62.00; H, 5.21; N, 9.61.

**4.1.3.4. 1-(2-Ethoxybenzyl)-3-(4-chlorophenyl)urea (2d).** White powder (92%); mp 196–197 °C; IR (KBr) ν: 3330, 3160, 1520 (NH); 1635 (CO); 3040 ($CH_{arom}$) cm$^{-1}$; 2960 ($CH_{aliph}$). $^1$H NMR ($d_6$-DMSO) δ (ppm):1,33 (t, 3H, OCH$_2$*CH$_3$*), 4.03 (q, 2H, O*CH$_2$*CH$_3$), 4.42 (d, 2H, C$_6$H$_5$*CH$_2$*NH), 6.46 (t, 1H, C$_6$H$_5$CH$_2$*NH*), 6.90 (t, 1H, $CH_{arom}$), 6.97 (d, 1H, $CH_{arom}$), 7.21 (m, 2H, $CH_{arom}$), 7.26 (d, 2H, $CH_{arom}$), 7.42 (d, 2H, $CH_{arom}$), 8.90 (s, 1H, *p*-ClC$_6$H$_5$*NH*). Calculated (%) for $C_{16}H_{17}ClN_2O_2$: C, 63.05; H, 5.62; N, 9.19. Found (%): C, 63.02; H, 5.59; N, 9.18.

**4.1.3.5. 1-(2-Methoxybenzyl)-3-(3-chlorophenyl)urea (2e).** White powder (94%); mp 188–189 °C; IR (KBr) ν: 3330, 3280, 3160, 3120, 1535 (NH); 1640 (CO); 3015 ($CH_{arom}$); 2835 ($CH_{aliph}$) cm$^{-1}$. $^1$H NMR ($d_6$-DMSO) δ (ppm): 3.73 (s, 3H, OCH$_3$), 4.42 (d, 2H, C$_6$H$_5$C*H$_2$*NH), 6.44 (t, 1H, C$_6$H$_5$CH$_2$*NH*), 6.90 (t, 1H, $CH_{arom}$), 6.96 (d, 1H, $CH_{arom}$), 7.21 (m, 2H, $CH_{arom}$), 7.26 (d, 2H, $CH_{arom}$), 7.42 (d, 1H, $CH_{arom}$), 8.70 (s, 1H, *p*-ClC$_6$H$_5$*NH*). Calculated (%) for $C_{15}H_{15}ClN_2O_2$: C, 61.97; H, 5.20; N, 9.64. Found (%): C, 62.01; H, 5.19; N, 9.62.

**4.1.3.6. 1-(2-Ethoxybenzyl)-3-(3-chlorophenyl)urea (2f).** White powder (92%); mp 174–178 °C; IR (KBr) ν: 3330, 1520 (NH); 1640 (CO); 3040 ($CH_{arom}$); 2970 ($CH_{aliph}$) cm$^{-1}$. $^1$H NMR ($d_6$-DMSO) δ (ppm): 1.33 (t, 3H, OCH$_2$*CH$_3$*), 4.07 (q, 2H, O*CH$_2$*CH$_3$), 4.26 (d, 2H, C$_6$H$_5$C*H$_2$*NH), 6.45 (t, 1H, C$_6$H$_5$CH$_2$*NH*), 6.89 (t, 1H, $CH_{arom}$), 6.92 (d, 1H, $CH_{arom}$), 6.97 (d, 1H, $CH_{arom}$), 7.17 (d, 1H, $CH_{arom}$), 7.22 (m, 4H, $CH_{arom}$), 8.78 (s, 1H, *m*-ClC$_6$H$_5$*NH*). Calculated (%) for $C_{16}H_{17}ClN_2O_2$:

C, 63.05; H, 5.62; N, 9.19. Found (%): C, 63.01; H, 5.60; N, 9.20.

**4.1.3.7. *N*-(2-Methoxybenzyl)-4-nitrobenzamide (3a).** White powder (94%); mp 135–136 °C; IR (KBr) ν: 3349 (NH); 1650 (CO); 3084 ($CH_{arom}$); 2830 ($CH_{aliph}$), 1450 ($NO_2$) $cm^{-1}$. $^1H$ NMR ($d_6$-DMSO) δ (ppm): 3.65 (s, 3H, $OCH_3$), 4.48 (d, 2H, $C_6H_5CH_2NH$), 6.91 (t, 1H, $CH_{arom}$), 7.08 (d, 1H, $CH_{arom}$), 7.21 (d, 1H, $CH_{arom}$), 7.25 (t, 1H, $CH_{arom}$), 8.14 (d, 2H, $CH_{arom}$), 8.32 (d, 2H, $CH_{arom}$), 9.18 (t, 1H, $C_6H_5CH_2NH$). Calculated (%) for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Found (%): C, 62.90; H, 4.92; N, 9.80.

**4.1.3.8. *N*-(2-Ethoxybenzyl)-4-nitrobenzamide (3b).** White powder (95%); mp 130–132 °C; IR (KBr) ν: 3330 (NH); 1645 (CO); 3083 ($CH_{arom}$); 2886 ($CH_{aliph}$), 1430 ($NO_2$) $cm^{-1}$. $^1H$ NMR ($d_6$-DMSO) δ (ppm): 1.35 (t, 3H, $OCH_2CH_3$), 4.15 (q, 2H, $OCH_2CH_3$), 4.45 (d, $C_6H_5CH_2NH$), 6.89 (t, 1H, $CH_{arom}$), 7.09 (d, 1H, $CH_{arom}$), 7.22 (d, 1H, $CH_{arom}$), 7.27 (t, 1H, $CH_{arom}$), 8.13 (d, 2H, $CH_{arom}$), 8.3O (d, 2H, $CH_{arom}$), 9.20 (t, 1H, $C_6H_5CH_2NH$). Calculated (%) for $C_{16}H_{16}N_2O_4$: C, 63.99; H, 5.37; N, 9.33. Found (%): C, 64.00; H, 5.38; N, 9.30.

**4.1.3.9. *N*-(2-Methoxybenzyl)thiophene-2-carboxamide (3c).** White powder (91%); mp 157–1159 °C; IR (KBr) ν: 3320 (NH); 1651 (CO); 3093 ($CH_{arom}$); 2830 ($CH_{aliph}$) $cm^{-1}$. $^1H$ NMR ($d_6$-DMSO) δ (ppm): 3.81 (s, 3H, $OCH_3$), 4.42 (d, $C_6H_5CH_2NH$), 6.91(t, 1H, $CH_{arom}$), 6.99 (d, 1H, $CH_{arom}$), 7.17 (m, 2H, $CH_{arom}$), 7.24 (t, 1H, $CH_{arom}$), 7.76 (d, 1H, $CH_{arom}$), 7.85 (d, 1H, $CH_{arom}$), 8.86 (t, 1H, $C_6H_5CH_2NH$). Calculated (%) for $C_{13}H_{13}NO_2S$: C, 63.13; H, 5.30; N, 5.66; S, 12.97. Found (%): C, 63.10; H, 5.29; N, 5.65; S, 12.98.

**4.1.3.10. *N*-(2-Ethoxybenzyl)thiophene-2-carboxamide (3d).** White powder (95%); mp 133–135 °C; IR (KBr) ν: 3323 (NH); 1667 (CO); 3098 ($CH_{arom}$); 2892 ($CH_{aliph}$), 1450 ($NO_2$) $cm^{-1}$. $^1H$ NMR ($d_6$-DMSO) δ (ppm): 1.35 (t, 3H, $OCH_2CH_3$), 4.05(q, 2H, $OCH_2CH_3$), 4.43 (d, $C_6H_5CH_2NH$), 6.89 (t, 1H, $CH_{arom}$), 6.97 (d, 1H, $CH_{arom}$), 7.16 (m, 2H, $CH_{arom}$), 7.25 (t, 1H, $CH_{arom}$), 7.75 (d, 1H, $CH_{arom}$), 7.87 (d, 1H, $CH_{arom}$), 8.92 (t, 1H, $C_6H_5CH_2NH$). Calculated (%) for $C_{14}H_{15}NO_2S$: C, 64.34; H, 5.79; N, 5.36; S, 12.27. Found (%): C, 64.35; H, 5.80; N, 9.37; S, 12.28.

**4.1.3.11. 1-(2-Methoxybenzyl)-3-(4-chlorophenyl)sulfonylurea (4a).** White powder (90%); mp 163–164 °C; ν (NH) = 3340, 3310, 3180, 3120, 1555; ν (CO) =ł1655, 1700; ν ($SO_2$-N) = 1345; ν ($SO_2$) = 1160, 1245, 1280 $cm^{-1}$. $^1H$ NMR ($d_6$-DMSO) δ (ppm): 3,75 (s, 3H, $OCH_3$); 4,1 (s, 2H, $C_6H_5CH_2NH$); 7 (m, 4H, $CH_{arom}$); 7,8 (m, 4H, $CH_{arom}$); 10,75 (s, 1H, $SO_2NH$). Calculated (%) for $C_{15}H_{15}ClN_2O_4S$: C, 50.78; H, 4.26; N, 7.90; S, 9.04. Found (%): C, 50,75; H, 4,25; N, 7,92; S, 9,07.

**4.1.3.12. 1-(2-Ethoxybenzyl)-3-(4-chlorophenyl)sulfonylurea (4b).** White powder (90%); mp 142–143 °C; ν (NH) = 3340, 3310, 3110, 1550; ν (CO) = 1665, 1700; ν ($SO_2$-N) = 1345; ν ($SO_2$) = 1160, 1240, 1285 $cm^{-1}$. $^1H$ NMR ($d_6$-DMSO) δ (ppm): 1,35 (t, 3H, $OCH_2CH_3$); 4 (q, 2H, $OCH_2CH_3$); 4,15 (s, 2H, $C_6H_5CH_2NH$); 7 (m, 4H, $CH_{arom}$); 7,7 (d, 2H, $CH_{arom}$); 7,9 (d, 2H, $CH_{arom}$); 10,85 (s, 1H, $SO_2NH$). Calculated (%) for $C_{16}H_{17}ClN_2O_4S$: C, 52.10; H, 4.65; N, 7.60; S, 8.69. Found (%): C, 52,07; H, 4,63; N, 7,55; S, 8,73.

### 4.1.4. Synthesis of the ring-closed analogues 9a and 9b

**4.1.4.1. 3,4-Dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (6).** A solution of 2-hydroxyacetophenone (1.76 mL, 14.7 mmol), acetone (3.62 mL, 49 mmol) and pyrrolidine (1.72 mL, 21 mmol) in methanol (20 mL) was stirred at 25 °C overnight. The mixture was then concentrated to dryness by evaporation of the solvents under reduced pressure. Water was added to the residue, and the mixture was adjusted to pH 1 with concentrated hydrochloric acid. The insoluble material was collected by filtration and dissolved in a small volume of methanol. The solution was treated with activated charcoal and the filtrate was supplemented with water until precipitation of the title compound. The precipitate was collected by filtration, washed with water and dried. The product was purified by chromatography on silica gel eluted with chloroform to give the title compound: pale yellow powder (54%); mp 85–86 °C; IR (KBr) *ν*: 2977, 2934 ($CH_{aliph}$), 1687 (CO) $cm^{-1}$. $^1H$ NMR ($d_6$-DMSO) *δ* (ppm) : 1.39 (s, 6H, CH3), 2.79 (s, 2H, CH2), 7.00 (m, 2H, $CH_{arom}$), 7.55 (dd, 1H, $CH_{arom}$), 7.72 (d, 1H, $CH_{arom}$). Calculated ((%): C, 74.67; H, 6.96.

**4.1.4.2. 3,4-Dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-hydroxyimine (7).** A mixture of 3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-one (**6**) (1 g, 5.68 mmol), hydroxylamine hydrochloride (0.7934 g, 11.42 mmol), potassium carbonate (1.587 g 11.48 mmol) and ethanol (40 mL) was refluxed for 3 h. After completion of the reaction, the mixture was poured onto crushed ice. The precipitate was collected by filtration, washed with water, and dried: white powder (92%); mp 123–125 °C (litt. 121–123 °C[8]); IR (KBr) *υ*: 3264 (NH), 2977, 2935 ($CH_{aliph}$), 1650 (CN) $cm^{-1}$. $^1H$ NMR ($d_6$-DMSO) δ (ppm): 1.30 (s, 6H, $CH_3$), 2.77 (s, 2H, $CH_2$), 6.82 (d, 1H, $CH_{arom}$), 6.89 (dd, 1H, $CH_{arom}$), 7.24 (dd, 1H, $CH_{arom}$), 7.74 (d, 1H, $CH_{arom}$), 11.19 (s, 1H, =N–OH). Calculated (%) for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.32. Found (%): C, 69.40; H, 6.77; N, 7.34.

**4.1.4.3. *R*/*S*-4-Amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (8).** A mixture of 3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-hydroxyimine (**7**) (10 g, 52.29 mmol), Raney Ni (2 g) and methanol (130 mL) was stirred under hydrogen pressure (5bar) at 25 °C overnight. The catalyst was filtered off, washed with methanol and the filtrate was evaporated under reduced pressure. The oily residue was purified by chromatography on silica gel eluted with chloroform and ethyl acetate (15:5 v/v) to give the title compound as a colourless oil (90%). For analytical purposes, the hydrochloride was obtained as a white powder; mp 268 °C (dec); IR (KBr) *υ*: 3044, 2978, 2894 ($NH^+$) $cm^{-1}$. $^1H$ NMR ($d_6$-DMSO) δ (ppm): 1.21 (s, 3H, $CH_3$), 1.42 (s, 3H, $CH_3$), 1.81 (t, 1H, $CH_2$), 2.32 (m, 1H, $CH_2$), 4.49 (m, 1H, 4-*H*), 6.80 (d, 1H, $CH_{arom}$ ), 6.96 (dd,

1H, $CH_{arom}$), 7.24 (dd, 1H, $CH_{arom}$), 7.69 (d, 1H, $CH_{arom}$), 8.80 (bs, ~3H, $-NH_3^+$). Calculated (%) for $C_{11}H_{15}NO.HCl$: C, 61.82; H, 7.55; N, 6.55. Found (%): C, 61.52; H, 7.59; N, 6.63.

**4.1.4.4. *R*/*S*-4-(4-Chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (9a).** 4-Chlorophenyl isocyanate (413 μL, 3.38 mmol) was added to a solution of **8** (500 mg, 2.82 mmol) in methylene chloride (5 mL). After completion of the reaction, the resulting precipitate was collected by filtration, washed with *n*-hexane, and dried: white powder (93%); mp 214–216 °C; IR (KBr) υ: 3328 (NH), 2978, 2929 ($CH_{aliph}$), 1633 (CO) $cm^{-1}$. $^1H$ NMR ($d_6$-DMSO) δ (ppm): 1.27 (s, 3H, $CH_3$), 1.38 (s, 3H, $CH_3$), 1.73 (t, 1H, $CH_2$), 2.11 (m, 1H, $CH_2$), 4.96 (m, 1H, 4-*H*), 6.53 (d, 1H, –N*H*–CO–NH–$C_6H_4Cl$), 6.73 (d, 1H, $CH_{arom}$), 6.87 (dd, 1H, $CH_{arom}$), 7.13 (dd, 1H, $CH_{arom}$), 7.25 (d, 1H, $CH_{arom}$), 7.28 (d, 2H, $CH_{arom}$), 7.46 (d, 2H, $CH_{arom}$), 8.63 (s, 1H, –NH–CO–N*H*–$C_6H_4Cl$). Calculated (%) for $C_{18}H_{19}ClN_2O_2$: C, 65.35; H, 5.79; N, 8.47. Found (%): C, 65.13; H, 5.76; N, 8.43.

**4.1.4.5. *R*/*S*-4-(3-Chlorophenylaminocarbonylamino)-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (9b).** The title compound was obtained as described for **9a** starting from **8** (500 mg, 2.82 mmol) and 3-cyanophenyl isocyanate (413 μl, 3.38 mmol): white powder (92%); mp 193–195 °C; IR (KBr) υ: 3322 (NH), 2980, 2928 ($CH_{aliph}$), 1631 (CO) $cm^{-1}$. $^1H$ NMR ($d_6$-DMSO) δ (ppm): 1.27 (s, 3H, $CH_3$), 1.38 (s, 3H, $CH_3$), 1.75 (t, 1H, $CH_2$), 2.12 (m, 1H, $CH_2$), 4.96 (m, 1H, 4-*H*), 6.59 (d, 1H, –N*H*–CO–NH–$C_6H_4Cl$), 6.73 (d, 1H, $CH_{arom}$), 6.87 (dd, 1H, $CH_{arom}$), 6.96 (d, 1H, $CH_{arom}$), 7.13 (dd, 1H, $CH_{arom}$), 7.24 (m, 3H, $CH_{arom}$), 7.73 (s, 1H, $CH_{arom}$), 8.71 (s, 1H, –NH–CO–N*H*–$C_6H_4Cl$). Calculated (%) for $C_{18}H_{19}ClN_2O_2$: C, 65.35; H, 5.79; N, 8.47. Found (%): C, 65.39; H, 5.89; N, 8.53.

### 4.2. Rat pancreatic β-cells

Pancreatic islets were isolated by collagenase method from fed Wistar rats (180–220 g). Groups of 10 islets, each derived from the same batch of islets, were preincubated for 30 min at 37 °C in 1 mL of a physiological salt medium (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24) supplemented with 2.8 mM glucose, 0.5% (w/v) dialyzed albumin (Sigma) and equilibrated against a mixture of $O_2$ (95%) and $CO_2$ (5%). The islets were then incubated at 37 °C for 90 min in 1 mL of the same medium containing 16.7 mM glucose and, in addition, the reference compound or the ring-opened cromakalim derivative. The release of insulin was measured radioimmunologically using rat insulin as a standard[9]. Residual insulin release was expressed as a percentage of the value recorded in control experiment (100%), that is, in the absence of drug and presence of 16.7 mM glucose.

### 4.3. Rat aorta rings

All experiments were performed on aorta removed from fed Wistar rats (180–220 g), as previously described.[2,6,9] The $ED_{50}$ was assessed for each dose–response curve as the concentration evoking 50% inhibition of the plateau phase induced by KCl 30 mM. Results are expressed as mean values (±SEM).

## Acknowledgments

This work was supported in part by 'Le Ministère de l'Enseignement Supérieur et de la Recherche Scientifique' of Algeria and by the National Fund for Scientific Research (Belgium) from which P.L. is a Research Director, P. de T. a Senior Research Associate and X.F. a F.R.I.A. Researcher. The technical assistance of S. Counerotte, F. Leleux, A.M. Vanbellinghen and A. Van Praet is gratefully acknowledged.

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