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Potassium Channel Activators Based on the Benzopyran Substructure: Synthesis and Activity of the C-8 Substituent

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Abstract—The synthesis of a series of methoxy bearing 2,2-dimethyl-2*H*-1-benzopyrans have been achieved for testing as potassium channel activators. The synthesis involves formation of 6-cyano-8-methoxy-2,2-dimethyl-2*H*-1-benzopyran from vanillin, epoxidation, then ring opening of the epoxide with nitrogen nucleophiles to produce the new benzopyrans. Biological testing showed a dramatic decrease in activity thus revealing an important site of activity in this class of compounds.

Introduction

The benzopyran ring system **1** is found in over 4000 natural and designed products, exhibiting an extensive range of biological activities.[1] It also serves as the foundation of a range of tannins, which can be found in teas, red wines and fruits all which are important for their health-related effects.[2] Modifications of the pyran olefin adds a further dimension to the biological properties often not observed with the olefinic complement. For example, 4-benzylpiperazinobenzopyran (**2**) has been shown to modulate multidrug resistance activity, while benzopyran **3** has been shown to act as a highly selective inhibitor of phosphodiesterase IV (Fig. 1).[3,4] Cromakalin[5] (**4**) and bimakalim[6] (**5**) are potent activators of ATP-sensitive potassium channels. Also in this class of potassium channel activators (KCAs), is pinacidil (**6**) which, like **4** and **5**, is a smooth muscle relaxant used intermittently in the treatment of hypertension and asthma.[7] The action of KCAs has been shown to be through interaction with the ß-subunit of K_{ATP} channels[8,9] thus increasing the outward movement of potassium ions through vascular smooth muscle membrane channels. This movement hyperpolarizes the cell membrane, closing voltage-dependent calcium channels, and relaxes the smooth muscle.

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These modified benzopyrans are the most extensively studied chemical structures associated with the opening of K_{ATP} channels,[10] and structure–activity studies of benzopyrans have focused mainly on modifications of the 4- and 6-positions.[11] Small and highly electronegative moieties at the 6-position have shown to be most effective, while lactam groups appear to provide the most effective substituents at the 4-position. The contribution of the 6-position to receptor affinity has been postulated to involve two possible mechanisms. One mechanism involves a withdrawing of electrons from the benzene moiety of the benzopyran nucleus thus increasing charge-transfer interactions of the aromatic moiety with the receptor, or alternatively, these substituents can contribute by direct interaction with the receptor site.

Although numerous studies on singly substituted benzopyrans have been reported, the synthesis and biological effects of doubly substituted benzopyrans have been largely ignored. To our knowledge, only the disubstituted benzopyran **7**, which combines the electron-withdrawing cyano group at the 6-position with an electron-donating amino function at C-7, has been reported, and also revealed to give enhanced potency.[12] We have therefore synthesized a new series of doubly substituted benzopyrans supporting an electron-donating group at the 8-position and evaluated the relaxant activity of these compounds on the rat aorta and portal vein. The starting material for the synthesis is vanillin, which also has the added benefit of being obtained from renewable biomass.[13]

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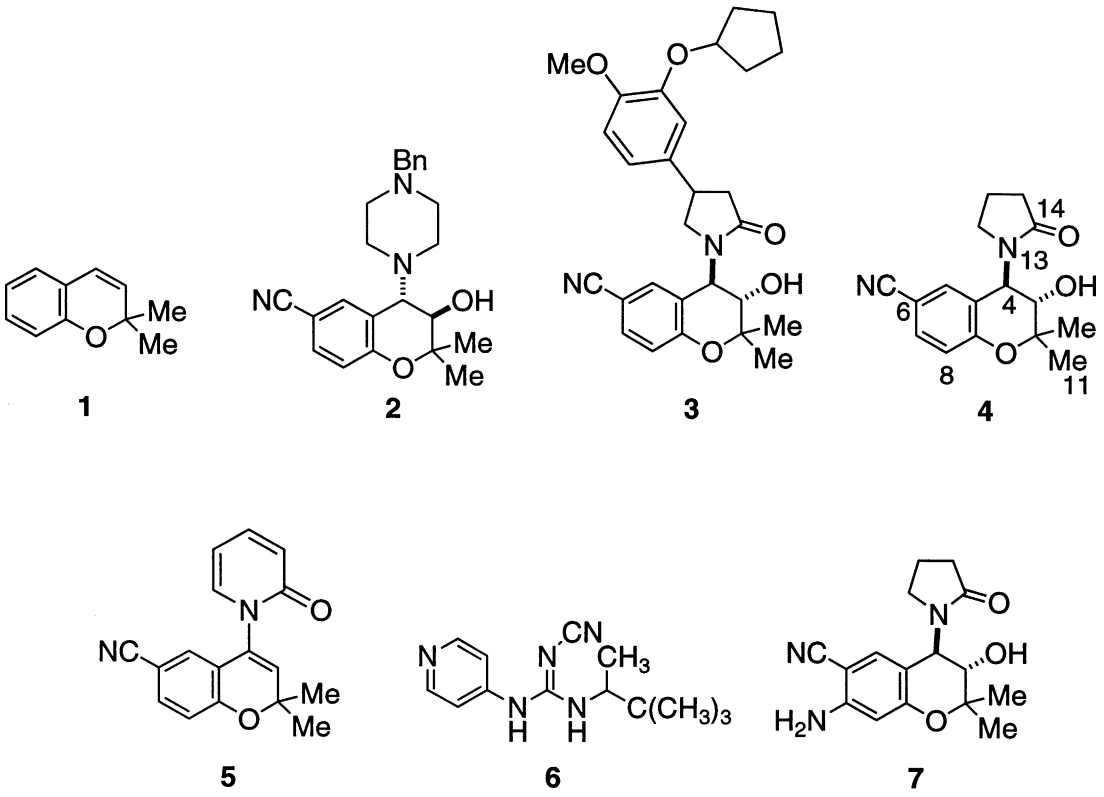


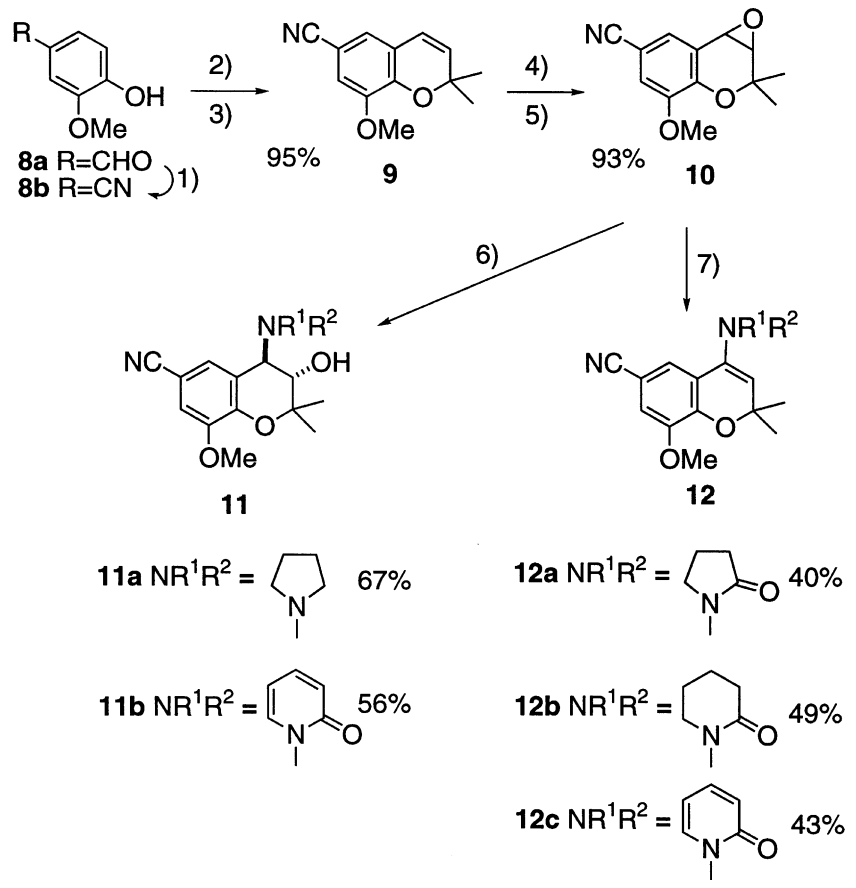
Figure 1. Benzopyrans.

Results

Scheme 1 outlines our synthesis starting with the conversion of vanillin (**8a**) into 4-hydroxy-3-methoxybenzonitrile (**8b**) in 91% yield.[14] Etherification with 3-chloro-3-methyl-1-butyne and cyclization of the crude material by Claisen rearrangement in boiling xylenes produces chromene **9** in 95% yield for the two steps. Epoxidation of **9** was attempted using a variety of conditions. We initially attempted to use Jacobsen's catalyst[15] and NaOCl as this would lead to the optically active epoxide, however only trace amounts of **10** were formed. Alternatively, the use of catalytic amounts of methyltrioxorhenium[16] (MTO) and hydrogen peroxide also failed to provide significant amounts of the epoxide. Epoxidation using mCPBA does produce **10**, but isolation of pure product is difficult. We therefore used a hydrobromination–epoxidation strategy to give racemic epoxide **10** in 93% yield. Final transformation into the required racemic benzopyrans was accomplished with several strategies. Formation of **11a** was accomplished in 67% yield by reaction of **10** with pyrrolidine in boiling ethanol. Similarly, **11b** was formed in 56% yield, only using 2-pyridinol and toluene as the solvent. Attempted synthesis of **12a** and **12b** by heating in a variety of solvents failed to produce any of the desired benzopyrans. We therefore heated epoxide **10** with 2-pyrrolidinone or 2-piperidone in the presence of NaH thus producing **12a** and **12b** in 40 and 49%, respectively. Finally, **12c** was obtained in 43% yield by treating **10** with 2-pyridinol followed by elimination with $KHSO_4$ in THF.

The biological activity of **11a**–**12c** have been evaluated and compared to (±)-cromakalim and (±)-pinacidil (Fig. 2). In tests with the isolated spontaneously contracting rat portal vein, cromakalim at 3×10^{-8}–3×10^{-6} M, pinacidil at 10^{-7}–3×10^{-6} M and **11a** at 10^{-5}–10^{-4} M relaxed the vein. In relaxing the rat portal vein, **11a** [$pIC_{50} = 4.11 \pm 0.11$, number of determinations (nd = 6)] was about 1000 times less potent than (±)-cromakalim ($pIC_{50} = 6.99 \pm 0.07$, nd = 10) or (±)-pinacidil ($pIC_{50} = 6.73 \pm 0.03$, nd = 11). The incorporation of a pyrrolidinone moiety, as in cromakalim, as compared to the pyrrolidine moiety in **11a**, has been shown to result in a 3-fold increase in potency.[17] However, this alone does not explain the dramatic decrease in activity for **11a**. We therefore tested the remaining compounds (**11b**, **12a**, **12b**, and **12c**), and as can be seen these gave comparable results or were even less active than **11a**, with **12b** being the least potent.

Given this surprising result, we next tested **11a** with the isolated aorta from 18-month-old spontaneously hypertensive rat (SHR). For vasodilators to be useful in the treatment of hypertension, they must be effective in the presence of hypertension-associated hypertrophy of blood vessels, and the aorta of 18-month-old SHR have hypertension-associated hypertrophy.[18] This test would give more revealing insight to the effectiveness and usefullness of one of our more active compounds. In tests with the isolated aorta, **11a** at 10^{-8}–10^{-4} M had no effect on the quiescent tissue ($n = 4$), although at 3×10^{-6}–10^{-4} M it produced relaxation of the KCl-contracted



Scheme 1. Synthesis of disubstituted KCAs: (1) NH_2OH HCl, AcOH; (2) $ClMe_2CC{\equiv}CH$, KI, K_2CO_3, MeCN; (3) boiling xylenes; (4) NBS, H_2O; (5) NaH; (6) **11a** = pyrrolidine, EtOH; **11b** = 2-pyridinol, toluene; (7) **12a** = 2-pyrrolidinone, NaH, DMF; **12b** = 2-piperidone, NaH, DMF; **12c** = 2-pyridinol, NaH, DMF then $KHSO_4$, THF.

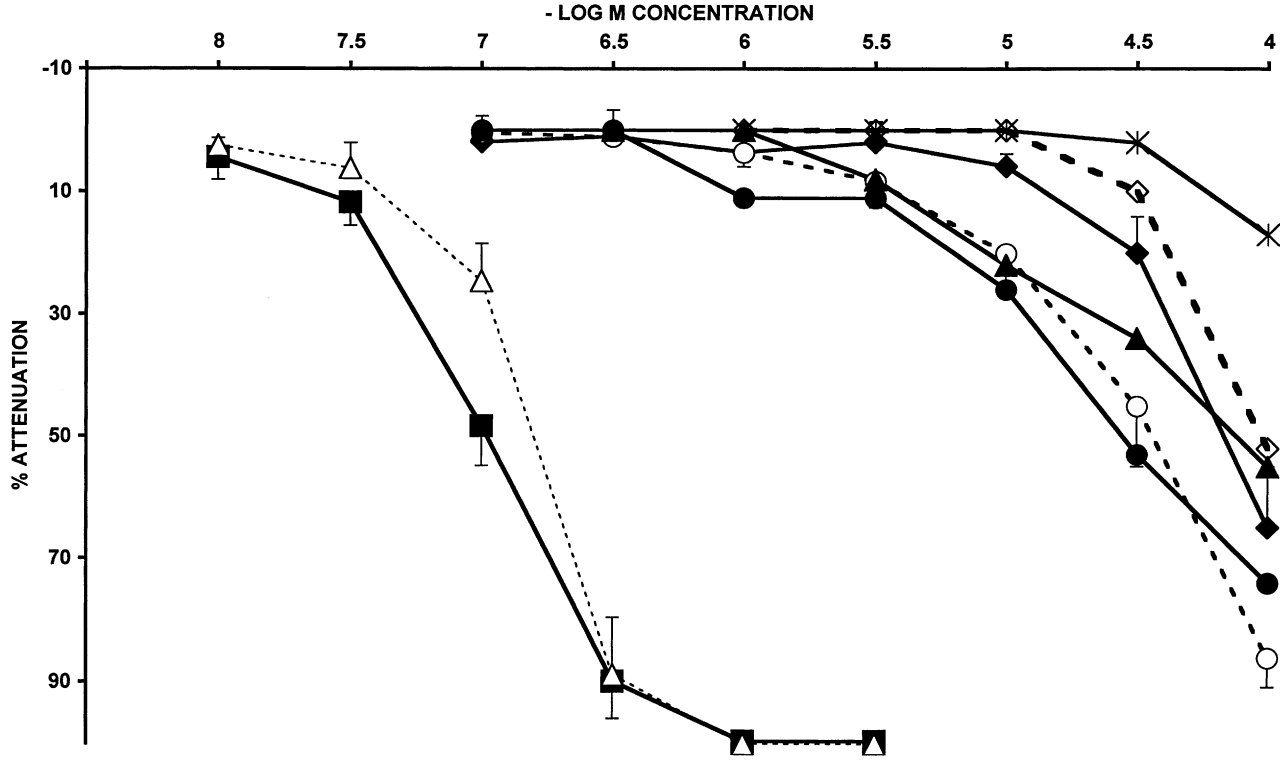


Figure 2. Activity tests on the spontaneous contractions of the isolated portal vein: cromakalim (filled squares), pinacidil (open triangles), **11a** (filled diamonds), **11b** (star), **12a** (filled triangle), **12b** (filled circle) and **12c** (empty diamond). Each value is the mean ± SEM of 6–11 determinations. Activity using KCl-contracted rat aorta: **11a** (open circles).

aorta with a $pIC_{50} = 4.54 \pm 0.11$, nd = 7 (Fig. 2). Therefore, **11a** remains only slightly effective in tissue from hypertensive animals.

Conclusion

In considering these results, it appears that the placement of an electron-donating group at the C-8 position of the benzopyran can have a significant effect on the activity of this class of substituted benzopyrans. In line with the postulated mechanisms, charge-transfer interactions of the aromatic moiety with the receptor could be affected by the inclusion of the methoxy unit. Alternatively, direct interaction of the methoxy group with the receptor could also be occurring. In view of benzopyran **7** exhibiting increased activity, a molecule that also contains an electron donating group on the aromatic ring, we believe that latter explanation is more likely. Since only high concentrations of **11a** relaxed blood vessels, the mechanism underlying these relaxant effects was not further investigated. However, these results show that C-8 substitution represents part of the benzopyran pharmacophore and this may enable the activity to be further tuned. We are therefore currently undertaking studies on an array of substitution at C-8 and these results will be reported in due course.

Experimental

General

^1H and ^{13}C NMR spectra were recorded on a Varian spectrometer at 300 and 75 MHz with chemical shifts reported relative to $CDCl_3$ (7.23 and 77.0, respectively). IR spectra were measured on a FT-IR spectrometer. Elemental analyses were obtained from Huffman Laboratories, Inc., Golden, CO and HR-MS was obtained using a Mariner TOF spectrometer. All solvents were distilled from appropriate drying agents prior to use. Standard syringe techniques were employed for handling air-sensitive reagents and all reactions were carried out under argon.

4-Hydroxy-3-methoxybenzonitrile 8. A mixture of vanillin **8a** (3.04 g, 20.0 mmol) and NH_2OH HCl (2.09 g, 30.0 mmol) in acetic acid (16 mL) was refluxed for 1 h. The solution was cooled, poured into Et_2O (50 mL) and washed once with H_2O (25 mL) and twice with 5% NaOH (25 mL). The combined aqueous layers were extracted once with Et_2O (25 mL) and the ether layers dried over $MgSO_4$. Recrystallization from toluene gave 2.70 g of **8b** as a white solid (91% yield).

6-Cyano-8-methoxy-2,2-dimethyl-2*H*-1-benzopyran 9. To a 100 mL flask equipped with reflux condenser and side-arm was added 4-hydroxy-3-methoxybenzonitrile **8b** (2.60 g, 17.4 mmol), K_2CO_3 (2.89 g, 20.9 mmol) and KI (4.63 g, 27.9 mmol). The flask was purged with argon then MeCN (40 mL) and 3-chloro-3-methyl-1-butyne (5.37 g, 5.88 mL, 52.4 mmol) were added. The mixture was refluxed for 16 h, cooled and filtered, washing the solids with acetone. The solvents were removed, xylenes (30 mL) were added and the mixture refluxed overnight (~16 h). The reaction mixture was concentrated in vacuo and the residue purified by flash chromatography hexanes/EtOAc (1:1) (95% yield) or by recrystallization using hexanes/Et_2O (86% yield) to give benzopyran **9**: ^1H NMR (300 MHz, $CDCl_3$) δ 6.97 (s, 1H, H-7), 6.95 (s, 1H, H-5), 6.27 (d, $J = 10.0$ Hz, 1H, H-4), 5.70 (d, $J = 10.0$ Hz, 1H, H-3), 3.87 (s, 3H, MeO), 1.50 (s, 6H, gem-Me_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.4, 146.1, 132.0, 123.3, 122.1, 120.8, 119.3, 114.9, 103.1, 78.0, 56.3, 28.1, 28.1. IR (neat) 2977, 2225, 1477, 1148, 1088 cm^{-1}; MS *m/z* calcd for $C_{13}H_{13}NO_2$ (M + 1) 216.10191, found 216.10160.

3,4-Epoxy-3,4-dihydro-2,2-dimethyl-6-cyano-8-methoxy-2*H*-1-benzopyran 10. To **9** (420 mg, 1.92 mmol) in THF (6 mL) and H_2O (4 mL) at 0 °C was added solid NBS (480, 2.69 mmol) over 10 min. The mixture was stirred for 5 h at room temperature in the dark and then concentrated in vacuo. The residual solution was extracted twice with ether (10 mL) and dried over $MgSO_4$.

To a solution of NaH (69.1 mg, 2.88 mmol) in DMF (1 mL) at 0 °C was added the bromohydrin in DMF (2 mL) drop wise over 5 min, complete transfer of the bromohydrin was ensured by washing the flask with an additional 1 mL of DMF. The mixture was allowed to stir for 1 h at room temperature and then poured into ether. The ether solution was washed twice with saturated $NaHCO_3$ (10 mL) once with water (10 mL) and dried ($MgSO_4$). Recrystallization from toluene/hexanes gave **10** as a creme colored solid in 93% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 7.30 (d, $J = 1.9$ Hz, 1H, H-7), 7.06 (d, $J = 1.9$ Hz, 1H, H-5), 3.88 (d, $J = 4.4$ Hz, 1H, H-4), 3.83 (s, 3H, MeO), 3.51 (d, $J = 4.4$ Hz, 1H, H-3), 1.65 (s, 3H, gem-Me), 1.30 (s, 3H, gem-Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.3, 146.0, 126.1, 121.4, 118.7, 115.6, 103.6, 74.8, 62.3, 56.2, 49.7, 25.4, 22.7. IR (neat) 2982, 2223, 1587, 1498, 1367, 1293, 1132 cm^{-1}.

***trans*-3,4-Dihydro-6-cyano-3-hydroxy-8-methoxy-4-pyrrolidine-2,2-dimethyl-2*H*-1-benzopyran 11a.** To epoxide **10** (242 mg, 1.046 mmol) in EtOH (3 mL) was added pyrrolidine (0.184 mL, 2.20 mmol) and the mixture was brought to a boil. After refluxing for 24 h, the solution was cooled, concentrated and the residue purified by flash chromatography with hexanes/ethyl acetate (1:1) to give 210 mg (67%) of **11a** as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.22 (s, 1H, H-7), 6.95 (s, 1H, H-5), 3.97 (d, $J = 10.0$ Hz, 1H, H-4), 3.86 (s, 3H, MeO), 3.60 (d, $J = 10.0$ Hz, 1H, H-3), 3.10 (brs, 1H, OH), 3.01 (m, 2H, NCH_2), 2.89 (m, 2H, NCH_2), 1.89 (m, 4H, NCH_2CH_2), 1.58 (s, 3H, gem-Me), 1.26 (s, 3H, gem-Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.2, 147.5, 124.7, 123.6, 119.5, 112.7, 102.4, 79.7, 70.5, 58.2, 56.1, 48.5, 26.8, 24.6, 18.6. IR (neat) 3479, 2972, 2225, 1477, 1142, 1094 cm^{-1}; elemental analysis calcd for $C_{17}H_{22}N_2O_3$ (302.37): C 67.53, H 7.33; found: C 67.70, H 7.68.

***trans*-3,4-Dihydro-6-cyano-4-(1,2-dihydro-2-oxo-1-pyridyl)-3-hydroxy-8-methoxy-2,2-dimethyl-2*H*-1-benzopyran 11b.** To epoxide **10** (218 mg, 0.931 mmol) in toluene

(5 mL) was added 2-pyridinol (133 mg, 1.40 mmol) and K_2CO_3 (515 mg, 3.72 mmol) and the mixture was brought to a boil. After refluxing for 3 h, the solution was cooled and filtered with the aid of EtOAc. Recrystallization (two crops) from EtOAc gave 210 mg (68%) of **11b** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (t, J = 8.4 Hz, 1H, H-16), 6.90 (s, 1H, H-7), 6.84 (d, J = 6.9 Hz, 1H, H-18), 6.70 (s, 1H, H-5), 6.66 (d, J = 9.3 Hz, 1H, H-15), 6.32 (d, J = 9.8 Hz, 1H, H-4), 6.24 (t, J = 6.9 Hz, 1H, H-17), 4.18 (brs, 1H, OH), 3.88 (s, 3H, MeO), 3.85 (d, J = 10.3 Hz, 1H, H-3), 1.60 (s, 3H, gem-Me), 1.36 (s, 3H, gem-Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.2, 149.9, 147.7, 140.1, 133.9, 124.4, 121.4, 120.3, 118.6, 113.6, 108.2, 104.3, 81.6, 75.5, 56.3, 55.3, 26.2, 18.2. IR (neat) 3328, 2224, 1661, 1580, 1488, 1096 cm^{-1}; MS m/z calcd for $C_{18}H_{17}N_2O_4$ (M-1): 325.11938, found 325.11900.

6-Cyano-8-methoxy-4-(pyrrolidin-2-one)-2,2-dimethyl-2*H*-1-benzopyran 12a. To NaH (60 mg, 2.50 mmol, washed with hexanes and dried), was added DMF (1.0 mL). A mixture of 2-pyrrolidinone (173 mg, 2.03 mmol) and epoxide **10** (238 mg, 1.02 mmol) in DMF (2 mL) was canulated into the NaH mixture at room temperature, washing the flask with additional DMF (1 mL). The mixture was stirred 0.5 h at room temperature then heated to 75 °C for 4 h. The mixture was cooled and poured into H_2O (5 mL), then extracted three times with Et_2O (20 mL) and dried ($MgSO_4$). Flash chromatography with EtOAc/hexanes (3:1) gave 121 mg (40%) of **12a** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 1H, H-7), 6.87 (s, 1H, H-5), 5.65 (s, 1H, H-3), 3.82 (s, 3H, MeO), 3.56 (t, J = 7.0 Hz, 2H, H-17), 2.52 (t, J = 8.0 Hz, 2H, H-15), 2.17 (p, 2H, H-16), 1.49 (s, 6H, gem-Me2); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 174.9, 148.9, 146.8, 130.0, 127.8, 119.8, 119.6, 119.0, 115.2, 103.1, 78.4, 56.3, 49.8, 31.0, 27.7 (2C), 18.7. IR (neat) 2970, 2226, 1698, 1478, 1380 cm^{-1}. MS m/z calcd for $C_{17}H_{19}N_2O_3$ (M + 1): 299.13902, found 299.14022.

6-Cyano-8-methoxy-4-(piperidin-2-one)-2,2-dimethyl-2*H*-1-benzopyran 12b. To NaH (24 mg, 1.00 mmol, washed with hexanes and dried), was added DMF (1.0 mL). A mixture of 2-piperidone (68 mg, 0.683 mmol) and epoxide **10** (80 mg, 0.342 mmol) in DMF (2 mL) was canulated into the NaH mixture at room temperature, washing the flask with additional DMF (1 mL). The mixture was stirred 0.5 h at room temperature then heated to 85 °C for 5 h. The mixture was cooled and poured into H_2O (5 mL), then extracted three times with Et_2O (20 mL) and dried ($MgSO_4$). Recrystallization with Et_2O gave 52 mg (49%) of **12b** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (d, J = 1.7 Hz, 1H, H-7), 6.81 (d, J = 1.7 Hz, 1H, H-5), 5.64 (s, 1H, H-3), 3.84 (s, 3H, MeO), 3.41 (m, 2H, H-18), 2.52 (m, 2H, H-15), 1.92 (m, 4H, H-16, H-17), 1.54 (s, 3H, gem-Me), 1.51 (s, 3H, gem-Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 148.9, 147.0, 134.6, 128.4, 119.7, 119.3, 119.2, 115.3, 103.3, 78.6, 56.4, 50.7, 32.4, 28.2, 27.7, 23.2, 21.3. IR (neat) 2952, 2224, 1651, 1466, 1376, 1285 cm^{-1}. MS m/z calcd for $C_{18}H_{21}N_2O_3$ (M + 1): 313.15467, found 313.15568.

6-Cyano-8-methoxy-4-(1,2-dihydro-2-oxo-1-pyridyl)-2,2-dimethyl-2*H*-1-benzopyran 12c. To NaH (29 mg, 1.20 mmol, washed with hexanes and dried), was added DMF (1.0 mL). A mixture of 2-pyridinol (85.3 mg, 0.896 mmol) and epoxide **10** (140 mg, 0.598 mmol) in DMF (2 mL) was canulated into the NaH mixture at room temperature, washing the flask with additional DMF (1 mL). The mixture was stirred 0.5 h at room temperature then heated to 80 °C for 4 h. The mixture was cooled and poured into H_2O (5 mL), then extracted three times with Et_2O (20 mL) and dried ($MgSO_4$). To the white solid was added THF (3 mL) and $KHSO_4$ (150 mg) and the mixture heated at reflux for 12 h. The solution was poured into EtOAc (20 mL), washed twice with H_2O (5 mL) and dried ($MgSO_4$). Recrystallization with EtOAc gave 80 mg (43%) of **12c** as a white solid.: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (t, J = 9.0 Hz, 1H, H-16), 7.14 (d, J = 6.8 Hz, 1H, H-18), 7.04 (s, 1H, H-7), 6.66 (d, J = 8.0 Hz, 1H, H-17), 6.65 (s, 1H, H-5), 6.27 (t, J = 6.8 Hz, 1H, H-15), 5.81 (s, 1H, H-3), 3.90 (s, 3H, MeO), 1.61 (s, 3H, gem-Me), 1.58 (s, 3H, gem-Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.6, 148.8, 146.3, 140.6, 137.5, 133.6, 129.4, 121.5, 119.5, 119.2, 118.7, 115.6, 106.5, 103.4, 78.6, 56.3, 27.8, 27.7. IR (neat) 2985, 2230, 1671, 1588, 1470, 1380, 1277, 1148 cm^{-1}. MS m/e calcd for $C_{18}H_{17}N_2O_3$ (M + 1): 309.12337, found 309.12427.

Biological methods: relaxant responses in rat portal vein and aorta

A comparison of the effects of **11a**, (±)-cromakalim and (±)-pinacidil on the spontaneous contractile activity of the portal vein 20-week-old Wistar rats was made. The effects of **11a** on the quiescent and KCl-contracted aorta of 18-month-old Spontaneously Hypertensive rats (SHRs) were determined. **11a** at 5 × 10^{-2} M was dissolved in absolute ethanol, (±)-cromakalim and (±)-pinacidil at 10^{-2} M were dissolved in 70% ethanol. Dilutions were made in distilled water. Parallel experiments showed that the ethanol vehicle has no effect alone on the portal veins or aortae.

Rats were stunned and exsanguinated. The portal vein or aorta was removed and placed in Krebs solution saturated with 5% carbon dioxide in oxygen. All experiments were performed in the presence of a modified Krebs solution (composition in mM: NaCl, 116; KCl, 5.4: $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 22.0; D-glucose, 11.2) that was being bubbled with 5% CO_2 in O_2 at 37 °C. An unstretched 10–12 mm length of portal vein was mounted longitudinally in a 5 mL organ bath under 10 mN tension. Each endothelium-intact thoracic aorta of about 3 mm in length was suspended in an organ bath under 15 mN tension. Contractile responses were measured isometrically with force displacement transducers (Grass model FTO3.C) and displayed on a polygraph (Grass model 79B). Portal veins and aortae were equilibrated for 60 min during which 250 mL Krebs superfused the tissues. Superfusion of the portal veins was stopped, and the contractions were allowed to stabilize over 20–30 min. Cumulative challenges to **11a**, (±)-cromakalim and (±)-pinacidil on 6 min cycle, or until a maximum response was obtained,

were determined. Superfusion of aortic rings was stopped, and some rings remained quiescent whereas others were contracted by the addition of 15 mM KCl. When a plateau response to KCl had been reached, quiescent and contracted aortae were challenged with **11a** on 6 min cycle, or until a maximum response was obtained.

The amplitudes of the final three contractions of the portal veins before the addition of each concentration of **11a**, (±)-cromakalim and (±)-pinacidil were measured and averaged. The contraction amplitude induced by KCl on the aorta prior to the addition of **11a** was measured. Responses were calculated as% attenuation of the spontaneous contractile activity of the portal vein or the KCl contraction of the aorta. pIC_{50} values (the negative logarithm of the molar concentration that inhibits the contraction by 50%) were calculated for individual curves by linear regression over the steepest part of the curve.

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