Synthesis and Biological Activity of Spirocyclic Benzopyran Imidazolone Potassium Channel Openers

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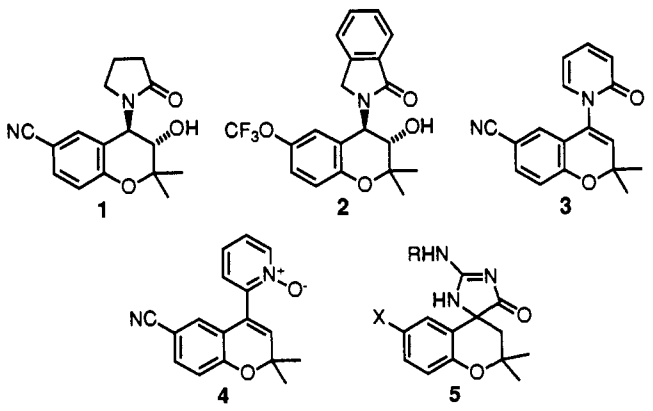
A series of novel spirocyclic benzopyran imidazolones were synthesized as rigid analogues of cromakalim. These compounds cause a dose-dependent membrane hyperpolarization of A10 rat aorta cells. This hyperpolarization was blocked by pretreatment with glyburide, indicating that the spirocyclic benzopyran imidazolones were acting by increasing the open probability of ATP-sensitive potassium channels in A10 cells. Representative compounds also showed potent in vivo activity as hypotensive agents in normotensive rats. Many of the compounds described are much more potent than cromakalim both in vitro and in vivo, with one of the most potent compounds being 2,3-dihydro-2,2-dimethyl-6-nitro-2′-(propylamino)spiro[4*H*-1-benzopyran-4,4′-[4*H*]imidazol]-5′(1′*H*)-one (**5r**). It is concluded that the N1′ nitrogen of the imidazolone is an effective substitute for the carbonyl oxygen of cromakalim. The rigid spirocyclic ring fusion holds this nitrogen in an optimum orientation relative to the benzopyran ring.

Introduction

Since the discovery of cromakalim (racemic **1**) in 1985 as a potent hypotensive agent,[1,2] a large number of related benzopyrans have been reported. Among the most well-known of the lead compounds in this group are celikalim (**2**, WAY-120,491),[3] bimakalim (**3**, EMD-52,692),[4] and Ro 31-6930 (**4**)[5]. All of these benzopyrans exert their hypotensive effect by relaxing smooth muscle via opening of cell membrane ATP-sensitive potassium channels.[6,7] By virtue of this effect, potassium channel openers may have utility for treatment of hypertension, asthma, incontinence, and impotence.[8] In addition, potassium channel openers have been found to be useful for stimulation of hair growth.[9]



1 2 3 4 5

Both X-ray and NMR studies[10,11] indicate that, for steric reasons, the lactam ring of cromakalim prefers an orthogonal relationship with the plane of the benzopyran ring system. The same relative orientation would be expected for the indolone, pyridinone, and pyridine *N*-oxide rings of compounds **2–4**, respectively. Although a large number of benzopyran analogues of cromakalim have appeared in the literature, none have been reported which are conformationally rigid with respect to the heterocycle–benzopyran bond. We describe herein the synthesis and biological activity of a novel series of benzopyran potassium channel openers (**5**) which incorporate a rigid, spirocyclic imidazolone. These compounds have good activity as hypotensive agents and some are considerably more potent than cromakalim.

Chemistry

The spirohydantoins **6a–e** (Scheme I) were chosen as reasonable precursors for the spirocyclic benzopyran imidazolones and were prepared from benzopyranones **7a–e** under standard conditions. Spirohydantoins **6a–c** have been previously reported,[12] while **6d** and **6e** (Table I) are new. Reaction of spirohydantoins **6a–c** with triethyloxonium tetrafluoroborate (Meerwein's reagent) resulted in O-alkylation to afford the 2-ethoxyimidazolones **8a–c** (Table II). Reaction of **8a–c** with various primary alkylamines in refluxing ethanol afforded the 2-(alkylamino)imidazolones **5a–k** (Table II). As described below, the structures of **5a–k** were confirmed by X-ray crystallographic analysis.

In principle, the alkylation of the spirohydantoins **6a–c** with triethyloxonium tetrafluoroborate could have produced four isomeric products: two N-alkylated hydantoins and two O-alkylated imidazolones. Literature precedent[13,14] suggested that only the C2-ethoxy compound should be formed, and indeed, only one compound was isolated in each case. Compounds **8a–c** were assigned as the conjugated tautomers based on their solid state (Nujol mull) IR spectra which showed strong bands ($\nu_{C=O}$ and $\nu_{C=N}$) at 1699–1708 and 1553–1565 cm^{-1}. These absorptions are consistent with the values of 1690 and 1560 cm^{-1} reported[13] for a closely related conjugated 2-ethoxyimidazolone. In addition, they are lower than the bands observed[13] at 1740 and 1655 cm^{-1} for a nonconjugated ethoxyimidazolone. Alkylation of the "urea" carbonyl in preference to the "amide" carbonyl is consistent with its greater electron density.

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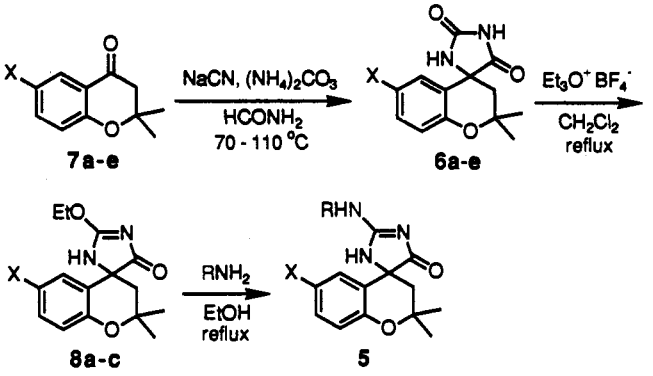
Scheme I



a: X = H; **b**: X = F; **c**: X = Br; **d**: X = CN; **e**: X = NO_2

The spirohydantoins **6d** and **6e** were insoluble in dichloromethane and would not undergo reaction with triethyloxonium tetrafluoroborate in this solvent. Nitromethane (in which the spirohydantoins are soluble) was also examined as a reaction solvent, but again no reaction occurred. A control experiment with spirohydantoin **6a** and triethyloxonium tetrafluoroborate in nitromethane also failed, suggesting that O-ethylation of the spirohydantoins only succeeds in halocarbon solvents. This observation is surprising in view of literature precedent for use of trimethyloxonium tetrafluoroborate in nitromethane.[15]

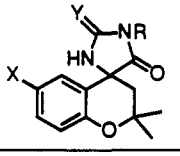
In order to convert spirohydantoins **6d** and **6e** to the desired spirocyclic benzopyran imidazolones, an alternative strategy was employed. As shown in Scheme II, reaction of spirohydantoins **6a–e** with Lawesson's reagent[16] (**9**) afforded the spirothiohydantoins **10a–e** (Table I). The regiochemistry of these compounds was assigned tentatively on the basis of IR data and confirmed by their eventual conversion to 2-(alkylamino)imidazolones (**5**) whose structures were confirmed by X-ray analysis. The spirothiohydantoins show a single carbonyl absorption at 1744–1775 cm^{-1} which is consistent with that reported[17] for a 2-thiohydantoin (1742 cm^{-1}) but not with that observed for the isomeric 4-thiohydantoin (1717 cm^{-1}). As anticipated from literature precedent,[18,19] alkylation of these spirothiohydantoins with alkyl iodides proceeded smoothly to give the 2-(alkylthio)imidazolones **11** (Table II). Except for **11c**, the preferred tautomeric form of these compounds in the solid state appears to be the nonconjugated isomer, based on IR absorptions for νC=O and νC=N (Nujol mull) at 1718–1750 and 1570–1582 cm^{-1}. These bands are in keeping with those reported[20] for a nonconjugated 2-(methylthio)imidazolone (1740 and 1570 cm^{-1}) but are not consistent with the observed absorption bands of the conjugated tautomer (1720 and 1485 cm^{-1}). Interestingly, compound **11c** apparently crystallized as the conjugated tautomer, based on its IR absorptions at 1683 and 1476 cm^{-1}.

Reaction of **11b** with ethylamine gave **5e** which was identical with the material prepared from the 2-ethoxyimidazolone, **8b**. Reaction of **11c** with propylamine gave **5j** which was also identical with the material prepared from **8c**. Having confirmed the feasibility of this approach to 2-(alkylamino)imidazolones, 2-(methylthio)imidazolones **11f** and **11i** were reacted with various primary amines to afford the 2-(alkylamino)imidazolones **5l–r** (Table II).

An X-ray structure of **5n** is shown in Figure 1. Two molecules of **5n** were found in the asymmetric unit, with one having a disordered *N*-propyl group. The X-ray structure of **5n** shows the C=N bond to be endocyclic and in conjugation with the carbonyl in the crystal form. This is supported by the presence of a crystallographically-related intermoleclar hydrogen bond between the propylamine hydrogen and the carbonyl oxygen. A second intermolecular hydrogen bond was seen between the imidazolone C=N nitrogen (N1′) and the imidazolone NH (N3′). Surprisingly, infrared data suggested that compounds **5** existed in the solid state with an exocyclic C=N bond. These compounds show C=O and C=N stretches at 1701–1710 and 1633–1656 cm^{-1}, consistent with spectra of other 2-iminohydantoins reported[18] to have strong bands at 1713 and 1650 cm^{-1}. In contrast, 2-(alkylamino)-imidazolones in which the C=N bond is endocyclic show bands at 1675 and 1603 cm^{-1} for the conjugated tautomer and 1750 and 1650 cm^{-1} for the nonconjugated tautomer.[18,21]

Regardless of the preferred solid-state tautomeric form of the 2-(alkylamino)imidazolones, two or more of the possible tautomers appear to be in equilibrium in solution. The NMR spectra of the (2-alkylamino)imidazolones at room temperature showed two very similar sets of signals which appeared to be due to isomeric (tautomeric) compounds. A spectrum of **5m** obtained at 60 °C showed only one sharp set of signals. We interpret this to mean that two or more tautomers are slowly interconverting at

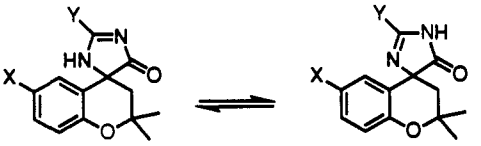
Table I. Data for Spirohydantoins, Spirothiohydantoins, and Spirocyclic 2-(Alkylamino)imidazolones Prepared



compd	X	Y	R	yield (%)	mp (°C)	formula	analysis[a]	IR (cm^{-1})[b]
6d	CN	O	H	39	>300	$C_{14}H_{13}N_3O_3$	C, H, N	1780, 1722
6e	NO_2	O	H	43	>300	$C_{13}H_{13}N_3O_5$	C, H, N	1779, 1720
10a	H	S	H	76	265–266	$C_{13}H_{14}N_2O_2S$	C, H, N, S	1769
10b	F	S	H	87	240–242	$C_{13}H_{13}FN_2O_2S$	C, H, F, N, S	1776
10c	Br	S	H	79	210–212	$C_{13}H_{13}BrN_2O_2S$	C, H, Br, N, S	1773
10d	CN	S	H	69	178–180	$C_{14}H_{13}N_3O_2S$	C, H, N, S	1745
10e	NO_2	S	H	50	238–240	$C_{13}H_{13}N_3O_4S$	C, H, N, S	1753, 1736
12a	F	O	CH_3CH_2	65	155–157	$C_{15}H_{17}FN_2O_3$	C, H, F, N[c]	1774, 1711
12b	Br	O	CH_3CH_2	62	202–204	$C_{15}H_{17}BrN_2O_3$	C, H, Br, N	1772, 1710
12c	CN	O	CH_3CH_2	52	200–202	$C_{16}H_{17}N_3O_3$	C, H, N[d]	1773, 1710

[a] Analytical results are within ±0.4% of the theoretical value unless otherwise indicated. [b] Imidazolone infrared absorptions. [c] Calcd for F: 6.50. Found: 7.16. [d] Calcd for C: 64.20. Found: 63.68.

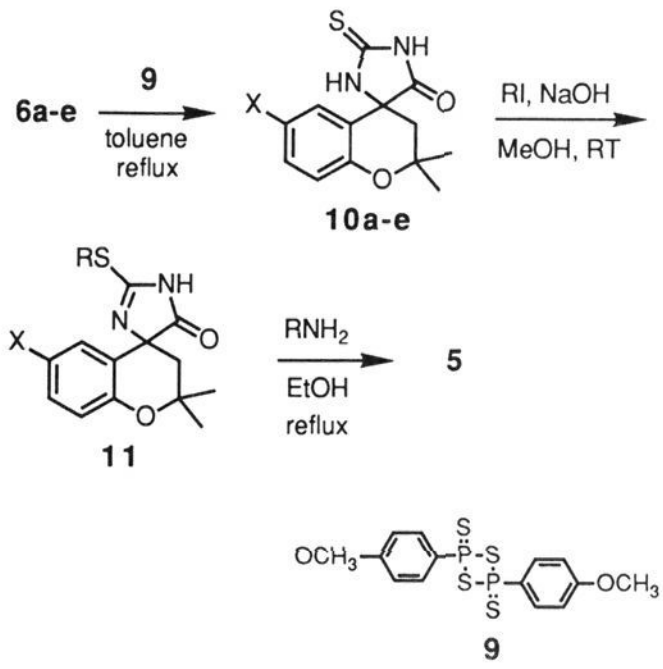
Table II. Data for Spirocyclic 2-Ethoxy-, 2-(Alkylamino)-, and 2-(Alkylthio)imidazolones Prepared



compd	X	Y	yield (%)	mp (°C)	formula	analysis[a]	IR (cm^{-1})[b]	MP assay[c] concn (μM)	MP assay[c] fluorescence decrease (%)
l		cromakalim						1.0	22
								0.1	<5
8a	H	CH_3CH_2O	80	153–155	$C_{15}H_{18}N_2O_3$	C, H, N	1708, 1565	10.0	<5
8b	F	CH_3CH_2O	60	209–211	$C_{15}H_{17}FN_2O_3$	C, H, F, N[d]	1699, 1553	10.0	13
								1.0	<5
8c	Br	CH_3CH_2O	57	182–184	$C_{15}H_{17}BrN_2O_3$	C, H, Br, N[e]	1701, 1555	1.0	20
								0.1	5
5a	H	$CH_3(CH_2)_2NH$	92	205–207	$C_{16}H_{21}N_3O_2$	C, H, N	1705, 1645	1.0	7
5b	H	$(CH_3)_2CHNH$	54	225–227	$C_{16}H_{21}N_3O_2$	C, H, N	1701, 1639	10.0	13
5c	H	$(CH_3)_3CCH_2NH$	55	293–295	$C_{18}H_{25}N_3O_2$	C, H, N	1701, 1640	10.0	7
5d	F	CH_3NH	95	283–285	$C_{14}H_{16}FN_3O_2$	C, H, N, F[f]	1710, 1652	10.0	14
								1.0	11
5e	F	CH_3CH_2NH	77	259–261	$C_{15}H_{18}FN_3O_2$	C, H, N, F	1704, 1643	1.0	24
								0.1	19
5f	F	$CH_3(CH_2)_2NH$	65	216–218	$C_{16}H_{20}FN_3O_2$	C, H, N, F[g]	1703, 1647	1.0	17
								0.1	10
5g	F	$(CH_3)_2CHNH$	60	261–262	$C_{16}H_{20}FN_3O_2$	C, H, N, F[h]	1703, 1640	10.0	22
								1.0	5
5h	Br	CH_3NH	77	>300	$C_{14}H_{16}BrN_3O_2$	C, H, N, Br	1705, 1650	1.0	17
								0.1	22
5i	Br	CH_3CH_2NH	60	277–279	$C_{15}H_{18}BrN_3O_2$	C, H, N, Br[i]	1702, 1656	1.0	19
								0.1	26
5j	Br	$CH_3(CH_2)_2NH$	79	208–210	$C_{16}H_{20}BrN_3O_2$	C, H, N, Br[j]	1704, 1642	0.1	28
								0.01	12
5k	Br	$(CH_3)_2CHNH$	48	235–237	$C_{16}H_{20}BrN_3O_2$	C, H, N, Br	1704, 1633	1.0	22
								0.1	5
5l	CN	CH_3NH	97	296–298	$C_{15}H_{16}N_4O_2$	C, H, N	1710, 1651	1.0	19
								0.1	<5
5m	CN	CH_3CH_2NH	98	256–258	$C_{16}H_{18}N_4O_2$	C, H, N	1706, 1643	1.0	25
								0.1	8
5n	CN	$CH_3(CH_2)_2NH$	64	278–280	$C_{17}H_{20}N_4O_2$	C, H, N	1702, 1640	1.0	27
								0.1	21
								0.01	<5
5o	CN	$(CH_3)_2CHNH$	42	288–290	$C_{17}H_{20}N_4O_2$	C, H, N[k]	1704, 1639	1.0	10
								0.1	<5
5p	NO_2	CH_3NH	98	308–310	$C_{14}H_{16}N_4O_4$	C, H, N	1711, 1652	0.1	38
								0.01	15
								0.001	<5
5q	NO_2	CH_3CH_2NH	83	248–250	$C_{15}H_{18}N_4O_4$	C, H, N	1706, 1641	0.1	28
								0.01	13
								0.001	9
5r	NO_2	$CH_3(CH_2)_2NH$	83	238–240	$C_{16}H_{20}N_4O_4$	C, H, N	1705, 1645	0.1	28
								0.01	19
								0.001	8
11a	H	CH_3S	90	198–200	$C_{14}H_{16}N_2O_2S$	C, H, N, S	1721, 1576	10.0	<5
11b	F	CH_3S	44	192–194	$C_{14}H_{15}FN_2O_2S$	C, H, F, N, S	1718, 1574	10.0	27
								1.0	9
11c	Br	CH_3S	62	213–215	$C_{14}H_{15}BrN_2O_2S$	C, H, Br, N, S	1683, 1476	1.0	27
								0.1	6
11d	Br	CH_3CH_2S	60	156–158	$C_{15}H_{17}BrN_2O_2S$	C, H, Br, N, S	1725, 1580	0.1	18
								0.01	11
								0.001	7
11e	Br	$CH_3(CH_2)_2S$	67	128–130	$C_{16}H_{19}BrN_2O_2S$	C, H, Br, N, S	1726, 1582	1.0	15
								0.1	6
11f	CN	CH_3S	81	205–207	$C_{15}H_{15}N_3O_2S$	C, H, N, S	1722, 1570[l]	10.0	25
								1.0	5
11g	CN	CH_3CH_2S	32	181–183	$C_{16}H_{17}N_3O_2S$	C, H, N, S	1735, 1574[l]	10.0	24
								1.0	6
11h	CN	$CH_3(CH_2)_2S$	45	172–174	$C_{17}H_{19}N_3O_2S$	C, H, N, S	1750, 1570[l]	1.0	17
								0.1	<5
11i	NO_2	CH_3S	74	213–215	$C_{14}H_{15}N_3O_4S$	C, H, N, S	1749, 1578[l]	1.0	16
								0.1	11
								0.01	8

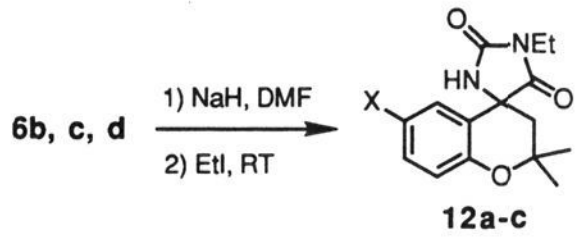
[a] Analytical results are within ±0.4% of the theoretical value unless otherwise indicated. [b] Imidazolone infrared absorptions. [c] Membrane potential assay carried out in A10 cells, $DIBAC_4(3)$ as the indicator. The response was measured from a field of approximately 50 cells. Variation in cellular response is less than 4% within the field. [d] Calcd for F: 6.50. Found: 5.89. [e] Calcd for Br: 22.62. Found: 22.14. [f] Calcd for C: 60.64. Found: 60.14. [g] Calcd for C: 62.77. Found: 62.13. (Calculated value includes 2% ethyl acetate.) [h] Calcd for C: 62.94. Found: 62.48. [i] Calcd for Br: 22.69. Found: 22.11. [j] Calcd for Br: 21.82. Found: 21.20. [k] Calcd for C: 65.37. Found: 64.91. [l] A third band is seen in the carbonyl region at 1615–1607 cm^{-1}.

Scheme II



room temperature and are both "visible" to the NMR spectrometer. At 60 °C, the rate of tautomerization is fast and the spectrometer sees only one "average" compound. Similar behavior was seen in the NMR spectra of the 2-ethoxyimidazolones (8) and the 2-(alkylthio)imidazolones (11). Similar tautomeric interconversions of 2-(methylthio)imidazolones in solution have been reported.[20,22]

Several N-ethylated spirohydantoins were also prepared for comparison of their spectral data and biological activity with the 2-(alkylamino)imidazolones. As shown in eq 1,



(1)

deprotonation of the spirohydantoins **6b–d** with sodium hydride in DMF and alkylation with ethyl iodide afforded the *N*-ethyl spirohydantoins **12a–c** (Table I). The structural assignments of these spirohydantoins as N3-alkylated rather than N1-alkylated or O-alkylated are made on the basis of ample literature precedent for the N-alkylation of hydantoins.[23,24]

Biological Activity

The spirocyclic benzopyran imidazolones were tested in vitro in a membrane potential assay using the A10 cell line derived from embryonic rat aorta.[25] In this assay, potassium channel openers cause cell membrane hyperpolarization which can be detected by a decrease in fluorescence intensity of a voltage-sensitive dye. The results from this assay for compounds **8a–c**, **5a–r**, and **11a–i** are shown in Table II along with cromakalim for comparison. Many of these compounds cause membrane hyperpolarization as would be expected of ATP-sensitive potassium channel openers. The spirohydantoins **6a–e** and **12b–d** as well as the spirothiohydantoins **10a–e** were inactive in this assay.

It was found that the hyperpolarization induced by **5n** (0.1 μM) could be blocked by pretreatment with glyburide (1 μM), a well-known blocker of ATP-sensitive potassium channels. This confirms that the spirocyclic benzopyran imidazolones, like cromakalim and its analogues, indeed

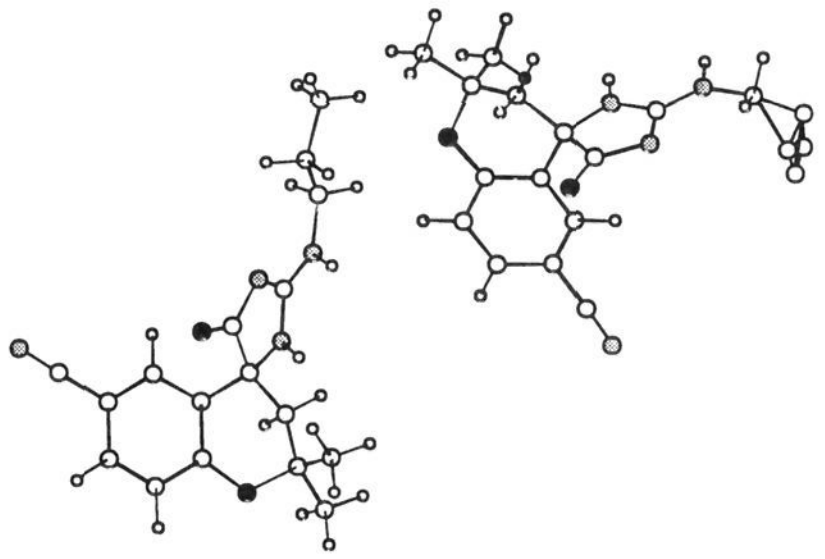


Figure 1. X-ray structure of compound **5n**. Two molecules were present in the asymmetric unit; one showed a disordered *n*-propyl group. The large open circles are carbon atoms, the small open circles are hydrogen atoms, the dark, shaded circles are oxygen atoms, and the light, shaded circles are nitrogen atoms.

Table III. Cardiovascular Data for Selected Spirocyclic Benzopyran Imidazolones

compd	dose (mg/kg)	cum dose (mg/kg)[a]	ΔMAP ± SEM (mmHg)[b]	ΔHR ± SEM (bpm)[c]
1	0.01	0.01	−14.7 ± 5.7	+33.8 ± 11.8
	0.03	0.04	−22.6 ± 5.2	+53.4 ± 9.2
	0.10	0.14	−36.1 ± 4.0	+65.6 ± 14.6
	0.30	0.44	−52.7 ± 5.4	+63.0 ± 25.8
5e	0.01	0.01	−7.1 ± 2.7	−0.4 ± 10.3
	0.03	0.04	−10.7 ± 3.4	+1.7 ± 10.4
	0.10	0.14	−28.0 ± 3.2	+38.0 ± 21.9
	0.30	0.44	−44.6 ± 3.2	+45.6 ± 26.0
5j	0.01	0.01	−25.6 ± 5.4	+44.9 ± 32.0
	0.03	0.04	−56.7 ± 2.2	+25.6 ± 25.7
	0.10	0.14	−70.1 ± 2.6	+46.3 ± 25.7
	0.30	0.44	−70.6 ± 3.0	+92.8 ± 20.0
5n	0.01	0.01	−23.6 ± 6.5	+13.8 ± 7.6
	0.03	0.04	−50.8 ± 6.4	+19.2 ± 6.5
	0.10	0.14	−66.2 ± 5.9	+29.8 ± 19.6
	0.30	0.44	−70.0 ± 5.8	+39.2 ± 21.9
5r	0.001	0.001	−19.8 ± 5.3	+6.8 ± 4.7
	0.003	0.004	−51.3 ± 8.4	+3.3 ± 6.4
	0.010	0.014	−66.0 ± 10.0	+3.8 ± 15.2
	0.030	0.044	−72.0 ± 10.0	+31.8 ± 32.2
11d	0.01	0.01	−2.5 ± 3.0	+15.5 ± 15.5
	0.03	0.04	−16.3 ± 7.0	+29.0 ± 14.6
	0.10	0.14	−21.5 ± 8.0	+16.5 ± 10.9
	0.30	0.44	−25.0 ± 6.6	+17.5 ± 12.7
vehicle control[d]	0.0	0.0	−1.1 ± 1.4	+11.8 ± 1.6
	0.0	0.0	−5.5 ± 1.1	+24.1 ± 3.1
	0.0	0.0	−5.5 ± 0.2	+30.4 ± 2.0
	0.0	0.0	−5.2 ± 0.2	+29.7 ± 2.4

[a] Rats were step-dosed. This value indicates the cumulative dose. [b] Change in mean arterial pressure. [c] Change in heart rate in beats per minute. [d] 20% DMA/saline.

function by opening cell membrane ATP-sensitive potassium channels.

Several of the more potent spirocyclic benzopyran imidazolones were also tested for hypotensive activity in anesthetized rats. Compounds were administered iv in a stepped dosing fashion. The changes in mean arterial pressure and heart rate induced by these compounds are shown in Table III.

Discussion

A substantial number of potassium channel openers based on the benzopyran ring system have been reported.[26] In general, the SAR for this group of compounds dictates that there must be an electron-rich hydrogen bond accepting group present within a set distance from the C4

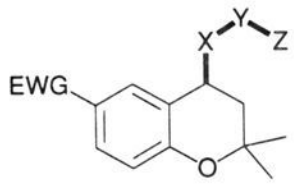


Figure 2. Depiction of structural requirements of the benzopyran class of potassium channel openers. EWG is an electron-withdrawing group, X and Y are typically part of a heterocyclic ring, and Z is a hydrogen-bond acceptor.

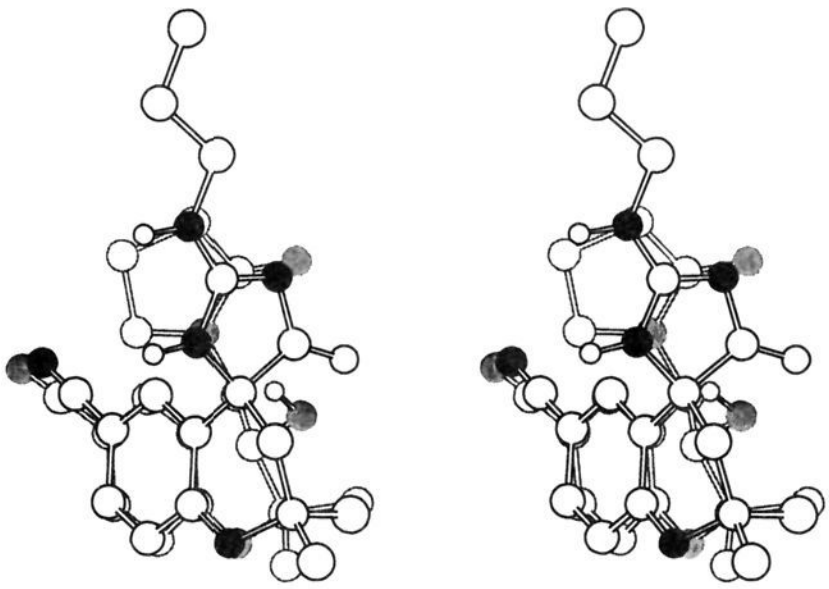


Figure 3. Relaxed stereoview of a computer-generated overlap drawing of **1** and **5n**. The gray structure is **1** and the black structure is **5n**. For the sake of clarity, all hydrogen atoms attached to carbons have been deleted and the two structures have been shifted slightly relative to one another along the *Z*-axis. Large open circles represent carbon atoms, small open circles represent hydrogen atoms on heteroatoms, and filled circles represent heteroatoms (gray for **1** and black for **5n**).

of the benzopyran ring. Most often this distance requirement is met by a three–bond connection at C4 as shown in Figure 2.[27] In this model, Z is the hydrogen-bond accepting group and X and Y are typically part of a ring system such that Z and the benzopyran are held cisoid. Furthermore, the plane of the X–Y–Z system prefers an orthogonal relationship with the plane of the benzopyran for steric reasons and this orthogonal relationship appears to be necessary for good activity.[28] Z can be either an electron-rich oxygen atom (as in compounds **1**–**4** and many of their analogues) or a nitrogen atom.[29]

It was anticipated that a spirocyclic heterocycle would mimic the geometrically constrained hydrogen-bond accepting groups of **1**–**4**. The spirocyclic ring fusion forces the plane of the heterocycle to be orthogonal to that of the benzopyran ring and holds a hydrogen-bond accepting group at the correct distance from the benzopyran C4. Since the C3 hydroxyl found in **1** is not necessarily required for activity,[30] it was not incorporated into the target molecules. The excellent activity of the spirocyclic benzopyran imidazolones confirms that these changes are well-tolerated in the benzopyran class of potassium channel openers.

The relationship between the imidazolone ring of the spirocyclic benzopyran imidazolones and the lactam ring of **1** is illustrated by the overlap drawing in Figure 3 of compound **5n** and the X-ray structure (inverted) of **1**.[11] Compound **5n** can exist in two half-chair conformations which are calculated to differ by 1.2 kcal/mol. Although in the crystal (Figure 1) compound **5n** is in the lower energy half-chair conformation, the slightly higher energy conformer is used in the overlap drawing so as to better match cromakalim. The calculated energy difference between the two conformers is small and may not reflect conformational preferences in aqueous solution.

As shown in Figure 3, the imidazolone C=N nitrogen (N1′) of compound **5n** occupies the same space relative to the benzopyran ring system as the carbonyl oxygen of **1**. This imidazolone nitrogen presumably serves as the required electron-rich, hydrogen bond accepting group. This assumption is supported by the observation that this nitrogen was seen to be a hydrogen bond acceptor in the crystal structure of **5n**. In addition, the activity of the spirocyclic imidazolones parallels the electron density of the N1′ nitrogen. The 2-(alkylamino)imidazolones were generally more active than either the 2-ethoxy- or 2-(alkylthio)imidazolones.

The nature of the C6 electron-withdrawing group also affected the activity of these compounds, with the 6-bromo- and 6-nitrobenzopyrans having the greatest activity. In particular, **5j**, **p**–**r** and **11d** were found to be the most active compounds. The requirement for an electron-withdrawing substituent at the C6 position of the benzopyran is in keeping with the known SAR of **1** and its analogues.[31]

Finally, the excellent activity with these compounds confirms that a C3 hydroxyl is not essential. The C3 hydroxyl of **1** may simply serve to hold the lactam and pyran rings in optimum conformations for interaction with the channel or associated proteins.

In summary, a series of novel spirocyclic benzopyran imidazolones related to **1** have been prepared. In these compounds, the C4 heterocycle typically present in potassium channel openers of the benzopyran class has been replaced by a rigid spirocyclic ring, and the C3 hydroxyl has been deleted. Activity has been retained and even enhanced. These compounds cause membrane hyperpolarization in smooth muscle A10 cells and cause a reduction in mean arterial pressure in normotensive rats. The spirocyclic benzopyran imidazolones are a novel addition to the family of benzopyran openers of ATP-sensitive potassium channels and serve to further illustrate and extend the structure–activity relationships of this family.

Experimental Section

General. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Only the strongest or most significant peaks in each IR and mass spectrum are listed. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra were recorded at 75 MHz. All chemical shifts are reported downfield from an internal Me_4Si standard. IR spectra, mass spectra, and elemental analyses were determined by the Upjohn Physical and Analytical Chemistry Unit. Flash chromatography was carried out using Merck Kieselgel 60, 230–400 mesh. All compounds are named using current Chemical Abstracts Service nomenclature. Unless otherwise indicated, all starting materials were purchased from the Aldrich Chemical Co.

General Procedure for the Synthesis of Spirohydantoins 6a–e. A glass pressure tube was charged with a mixture of the benzopyranone (20 mmol), KCN (40 mmol), and $(NH_4)_2CO_3$ (146 mmol). Enough formamide was added to fill the pressure tube nearly completely. The mixture was heated at 70 °C for 24 h and then at 110 °C for another 48 h. The reaction mixture was then cooled and poured over ice. Acidification with concentrated HCl gave a precipitate which was filtered, washed twice with water, and then dried overnight in a vacuum oven at 60 °C and approximately 30 Torr. The product was then purified by recrystallization from CH_3OH/CH_2Cl_2. Spirohydantoins **6a**–**c** have already been reported in the literature.[12] Spectral data for

6d are representative and are given below. Data for 6e can be found in the supplementary material.

6-Cyano-2,3-dihydro-2,2-dimethylspiro[4*H*-1-benzopyran-4,4'-imidazolidine]-2',5'-dione (6d) was prepared from 3,4-dihydro-2,2-dimethyl-4-oxo-2*H*-1-benzopyran-6-carbonitrile[32,33] (**7d**): IR (mull) 3407, 3282, 3059, 2954, 2925, 2227, 1780, 1722, 1612, 1491, 1462, 1424, 1174 cm^{-1}; ^1H NMR (DMSO) δ 11.15 (1 H, bs), 8.71 (1 H, s), 7.65 (2 H, m), 6.97 (1 H, d, J = 8 Hz), 2.33 (1 H, HA of AB, J_{AB} = 14 Hz), 2.22 (1 H, HB of AB, J_{AB} = 14 Hz), 1.43 (3 H, s), 1.27 (3 H, s); ^{13}C NMR (DMSO) δ 176.7, 157.4, 156.9, 133.9, 132.8, 121.5, 119.5, 118.5, 103.5, 75.4, 59.4, 43.2, 29.0, 24.4; MS (70 eV, EI) m/z 271 (parent), 215 (base), 185, 144, 116, 40.

General Procedure for the Synthesis of Spirocyclic Benzopyran 2-Ethoxyimidazolones 8a–c. A solution of spirohydantoin (5 mmol) and triethyloxonium tetrafluoroborate (1 M solution in CH_2Cl_2, 10 mL, 10 mmol) in 50 mL of CH_2Cl_2 was heated to reflux for 24 h. After cooling the mixture to room temperature, it was diluted with 50 mL of CH_2Cl_2. This solution was washed sequentially with 10% $NaHCO_3$ and water, dried (Na_2SO_4), filtered, and then concentrated. The residue was flash chromatographed on silica gel using 4% MeOH/$CHCl_3$ as eluent to afford the product. Spectral data for **8a** are representative and are given below. Data for **8b–c** can be found in the supplementary material.

2'-Ethoxy-2,3-dihydro-2,2-dimethylspiro[4*H*-1-benzopyran-4,4'-[4*H*]imidazol]-5'(1'*H*)-one (8a): IR (mull) 3275, 2954, 2925, 1708, 1584, 1565, 1554, 1494, 1486, 1457, 1333, 1252 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.22 (1 H, m), 6.87 (3 H, m), 5.25 (1 H, bs), 4.63 (2 H, q, J = 7 Hz), 2.60 (1 H, d, J = 7 Hz), 1.98 (1 H, d, J = 7 Hz), 1.49 (3 H, s), 1.45 (3 H, t, J = 7 Hz), 1.30 (3 H, s); ^{13}C NMR ($CDCl_3$) δ 190.7, 176.8, 157.3, 153.6, 130.6, 127.0, 121.3, 118.5, 73.1, 68.2, 61.1, 43.7, 29.4, 24.0, 14.4; MS (70 eV, EI) m/z 274 (parent), 259, 245 (base), 231, 219, 202, 191, 174, 160, 147, 120, 91, 70.

General Procedure for the Synthesis of Spirothiohydantoins 10a–e. A suspension of spirohydantoin (6.7 mmol) and Lawesson's reagent (2.71 g, 6.7 mmol) in 30 mL of dry dioxane was refluxed for 24 h. The mixture was then concentrated, and the residue was flashed chromatographed on silica gel using 2% MeOH/$CHCl_3$ as eluent to afford the product. Spectra data for **10a** are representative and are given below. Data for **10b–e** can be found in the supplementary material.

2,3-Dihydro-2,2-dimethyl-2'-thioxospiro[4*H*-1-benzopyran-4,4'-imidazolidin]-5'-one (10a): IR (mull) 3518, 3219, 2954, 2925, 2407, 1768, 1586, 1520, 1498, 1490, 1387, 1298, 1253, 1225, 1196, 1183 cm^{-1}; ^1H NMR (CD_3OD) δ 7.15 (1 H, td, J = 8, 1 Hz), 6.87 (1 H, dd, J = 7, 1 Hz), 6.83 (1 H, t, J = 8 Hz), 6.73 (1 H, d, J = 8 Hz), 2.40 (1 H, HA of AB, J_{AB} = 14 Hz), 2.20 (1 H, HB of AB, J_{AB} = 14 Hz), 1.38 (3 H, s), 1.25 (3 H, s); ^{13}C NMR (CD_3OD) δ 184, 179, 155, 131, 128, 122, 119.88, 119.79, 74.7, 65.3, 44.6, 29.8, 25.9; MS (70 eV, EI) m/z 262 (parent, base), 207, 120, 102, 91.

General Procedure for the Synthesis of Spirocyclic Benzopyran 2-(Alkylthio)imidazolones 11a–i. To a solution of spirothiohydantoin (1.5 mmol) and sodium hydroxide (0.06 g, 1.5 mmol) in 5 mL of CH_3OH and 0.5 mL of water was added the appropriate alkyl iodide (1.5 mmol), and resulting mixture was stirred at room temperature for 18 h (for CH_3I) or at reflux for 24 h (for other alkyl iodides). The mixture was concentrated, and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with H_2O and saturated NaCl solution and then dried (Na_2SO_4), filtered, and concentrated to afford an oil. This oil was flash chromatographed on silica gel using 20–30% ethyl acetate/hexane as a eluent to afford the product. Spectral data for **11a** are representative and are given below. Data for **11b–i** can be found in the supplementary material.

2,3-Dihydro-2,2-dimethyl-2'-(methylthio)spiro[4*H*-1-benzopyran-4,4'-[4*H*]imidazol]-5'(1'*H*)-one (11a): IR (mull) 3178, 3083, 2954, 2924, 1721, 1576, 1487, 1401, 1204, 1159, 1152, 929 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.20 (1 H, s), 7.20 (1 H, td, J = 8, 1 Hz), 6.67 (2 H, m), 6.77 (1 H, dd, J = 8, 1 Hz), 2.49 (3 H, s), 2.42 (1 H, d, J = 14 Hz), 1.87 (1 H, d, J = 14 Hz), 1.50 (3 H, s), 1.49 (3 H, s); ^{13}C NMR ($CDCl_3$) δ 186.2, 158.5, 153.8, 129.8, 127.1, 120.7, 118.8, 118.4, 73.9, 70.8, 42.4, 30.0, 25.2, 12.5; MS (70 eV, EI) m/z 276 (parent), 261, 221, 218, 201 (base), 160, 120, 91.

General Procedure for the Synthesis of Spirocyclic Benzopyran 2-(Alkylamino)imidazolones 5a–k from Spirocyclic Benzopyran 2-Ethoxyimidazolones 8a–c. A solution of a spirocyclic benzopyran 2-ethoxyimidazolone (4.4 mmol) and an alkylamine (8.8 mmol) in 15 mL of absolute ethanol was heated to reflux for 18 h. The mixture was then concentrated and treated with anhydrous ether to afford the product as a solid. The product normally was recrystallized from MeOH/EtOAc. Spectral data for **5a** are representative and are given below. Data for **5b–k** can be found in the supplementary material.

2,3-Dihydro-2,2-dimethyl-2'-(propylamino)spiro[4*H*-1-benzopyran-4,4'-[4*H*]imidazol]-5'(1'*H*)-one (5a): IR (mull) 3250, 3185, 3064, 3041, 2954, 2925, 1731, 1705, 1645, 1582, 1524, 1486, 1431, 1307, 1297, 1256, 1243, 1202, 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.13 (1 H, m), 6.83 (3 H, m), 3.24 (2 H, t, J = 7 Hz), 2.45 (1 H, d, J = 14 Hz), 1.88 (1 H, d, J = 14 Hz), 1.55 (2 H, q, J = 7 Hz), 1.44 (3 H, s), 1.23 (3 H, s), 0.91 (3 H, t, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 192.1, 170.5, 158.4, 129.3, 126.9, 120.5, 120.0, 117.9, 73.0, 62.3, 43.8, 43.6, 29.4, 23.8, 22.7, 10.9; MS (70 eV, EI) m/z 287 (parent, base), 258, 244, 232, 216, 202, 187, 176, 160, 145, 126, 91, 69, 42.

General Procedure for the Synthesis of Spirocyclic Benzopyran 2-(Alkylamino)imidazolones 5e,j,l–r from Spirocyclic Benzopyran 2-(Methylthio)imidazolones 11b,c,f,i. A solution of the appropriate spirocyclic benzopyran 2-(methylthio)imidazolone (2 mmol) and an alkylamine (4 mmol) in 10 mL of absolute ethanol was heated in a sealed tube at 70 °C for 24 h. This mixture, on cooling, normally gave a crystalline product which was filtered off. In cases where the product did not crystallize immediately, the mixture was concentrated and the residue was treated with anhydrous ether to afford a crystalline product. Spectral data for **5a** (above) are representative of these compounds. Data for **5l–r** can be found in the supplementary material.

General Procedure for the Synthesis of Spirohydantoins 12a–c. To a suspension of NaH (96 mg, 4 mmol) in 5 mL of DMF was added dropwise a solution of the appropriate spirohydantoin (2 mmol) in 5 mL of DMF. After complete addition, the mixture was stirred at room temperature for 1 h. Ethyl iodide (0.312 g, 0.160 mL, 2 mmol) was added to the mixture and stirred at room temperature for 18 h. The mixture was poured onto ice and extracted with $CHCl_3$ (2×, 25 mL). The $CHCl_3$ extract was washed with saturated NaCl solution, dried (Na_2SO_4), filtered, and concentrated. The residue, on trituration with anhydrous ether, afforded crystalline product. Spectral data for **12a** are representative and are given below. Data for **12b–c** can be found in the supplementary material.

1'-Ethyl-6-fluoro-2,3-dihydro-2,2-dimethylspiro[4*H*-1-benzopyran-4,4'-imidazolidine]-2',5'-dione (12a): IR (mull) 3312, 2956, 2925, 1774, 1711, 1493, 1450, 1426, 1266, 1205, 1145 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.92 (1 H, td, J = 8, 5 Hz), 6.68 (1 H, dd, J = 9, 5 Hz), 6.47 (1 H, dd, J = 9, 3 Hz), 3.48 (2 H, q, J = 7 Hz), 2.46 (1 H, d, J = 14 Hz), 1.88 (1 H, d, J = 14 Hz), 1.37 (3 H, s), 1.19 (3 H, s), 1.12 (3 H, t, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 174.6, 158.4, 156.7 (d, J_{CF} = 236 Hz), 149.8, 120.0 (d, J_{CF} = 8 Hz), 119.3 (d, J_{CF} = 8 Hz), 118.3 (d, J_{CF} = 23 Hz), 112.5 (d, J_{CF} = 23 Hz), 73.3, 59.4, 43.4, 34.0, 29.3, 23.9, 13.3; MS (70 eV, EI) m/z 292 (parent), 236 (base), 206, 192, 178, 166, 137, 109.

X-ray Structure Determination for 5n: $C_{17}H_{18}N_4O_2$, M_r = 310.35, triclinic, $P\bar{1}$, a = 9.924(2) Å, b = 10.154(1) Å, c = 18.186(3) Å, α = 93.36(2)°, β = 96.79(1)°, γ = 108.57(2)°, V = 1715.9(6) Å^3, Z = 4, D_c = 1.20 g cm^{-3}, graphite monochromatized radiation λ(Cu Kα) = 1.5418 Å, μ(Cu Kα) = 5.87 cm^{-1}, T = 135 K, R = 0.061 for 5651 unique reflections. A clear, chunky plate of dimensions 0.11 × 0.41 × 0.52 mm was used for intensity measurements on a Siemens $P2_1$ diffractometer controlled by a Harris computer. Cu Kα radiation and a graphite monochromator were used for intensity measurement. The step-scan technique was used with a scan rate of 4 deg/min, a scan width of 3.4°, and a $2\theta_{max}$ = 136°. Ten reflections periodically monitored showed no loss of intensity during the data collections. Of the 5651 unique reflections measured, 4865 had intensities > 3σ. Standard deviations in the

intensities were approximated by the equation:

$$\sigma^2(I) = \sigma^2(I)_{\text{counting statistics}} + 0.0201(I^2)$$

where the coefficient of I was calculated from the variations in intensities of the monitored reflections. Unit cell parameters were determined accurately by least squares fit of Cu $K\alpha_1$ 2θ values (λ(Cu $K\alpha_1$) = 1.5402) for 25 high 2θ reflections.[34] A polarization correction appropriate for a monochromator with 50% perfect character was applied, but there was no correction for absorption. The structure was solved by direct methods using MULTAN.[35] For one of the molecules, two alternate positions were discovered from a difference Fourier map for C(17) and C(18) of the propylamine moiety; occupancies of 0.50 and isotropic temperature factors were assigned to each of the four atoms. Hydrogen atoms were clearly found in a difference map except for the C(17) and C(18) atoms of the disordered molecule. The structures were refined by least squares with the coordinates and anisotropic thermal parameters for nonhydrogen atoms included in the refinement. Isotropic thermal parameters for hydrogen atoms were set $^1/_2$ unit higher than the isotropic equivalent of the thermal parameters of the attached heavier atom. The function minimized in the refinement was $\sum w(F_o^2 - F_c^2)^2$, where weights w were $1/\sigma^2(F_o^2)$. Atomic form factors were from Doyle and Turner,[36] except for hydrogens which were from Stewart, Davidson, and Simpson.[37] In the final refinement cycle, all shifts were 0.54σ. The final R was 0.061, and the standard deviation of fit was 4.34. A final difference map showed no peaks >0.86 e Å^{-3}. The CRYM system of computer programs was used.[38]

Cell Culture. The A10 cell line derived from embryonic rat aorta[28] was obtained from the American Type Culture Collection (CRL 1476). The cell line was cultured at 37 °C in 6.0% CO_2 in Dulbecco's Modified Eagle's Medium supplemented with 15% (v/v) fetal bovine serum. For cell imaging assays, the A10 cells were subcultured on Labtech glass coverslips and used one to two days postconfluent. Fresh medium was provided to the cells one day prior to being assayed. The A10 cells were not used past passage 20.

Fluorescence Membrane Potential Assay. The membrane potential of A10 cells was measured by a modification of the procedure described by Brauner.[39] Immediately prior to analysis, postconfluent A10 cells were washed with 5 μM $DiBAC_4(3)$[40] in EBSS containing 20 mM HEPES, pH 7.3. The cultures were then incubated in 5 μM $DiBAC_4(3)$ in EBSS–HEPES for a minimum of 15 min at 37 °C to permit equilibration of the dye across the plasma membrane. $DiBAC_4(3)$ fluorescence was monitored at 37 °C using the ACAS 570 laser-based imaging cytometer (Meridian Instruments, Okemos, MI). The ACAS 570 was configured to excite the cells at 488 nm using an argon ion laser while monitoring the fluorescent emissions at 525 nm. Data was collected every 60 s from a 400-μm^2 area of cells, with each field containing approximately 50 cells using the Kinetics Program on the ACAS. The raw fluorescence values in each experiment were normalized to allow comparison between individual experiments. Compounds were added to the cells from 1000X stocks prepared in either DMSO or H_2O. Prior experiments established that up to 0.5% DMSO had no effect on the fluorescence membrane potential assay. Control experiments also established that 3–5 μM $DiBAC_4(3)$ was the optimal concentration of dye for this assay. Historical data with a standard potassium channel opener (P-1075, *N*-cyano-*N*′-(1,1-dimethylpropyl)-*N*″-3-pyridinylguanidine) showed that over 36 experiments, the average change in fluorescence was 24.95 ± 0.12% (SEM) at 1 μM.

Blockade of 5n with Glyburide. A10 cells were pretreated for 15 min with glyburide which was introduced as sufficient 1000X stock in DMSO to make the final cell culture 1 μM in glyburide. Under the conditions of the membrane potential assay described above, the cells were challenged with compound **5n**. Compound **5n** was introduced as a 1000X stock solution in DMSO in sufficient quantity to make the final cell culture 100 nM in **5n**. The decrease in fluorescence under these conditions was only 7%—marginally above baseline. Treatment of cells from the same culture with 100 nM **5n** alone caused a 29% decrease in fluorescence.

CUP-Anesthetized Rat Test for Hypotensive Activity. Fasted female Sprague-Dawley rats (Harlan Labs) weighing 200–250 g were anesthetized with an 8 mL/kg kiv tail vein injection of 40 mg/kg α-chloralose (Fisher), 20 mg/kg urethane (Aldrich), and 20 mg/kg Na^+ pentobarbital (Fort Dodge Labs).[41] The rats were placed on a heated surgery rack, and 30 min later a femoral artery and vein were each cannulated with PE-50 catheters to monitor phasic and mean arterial pressure (MAP) and administer drug, respectively. Mean arterial pressure (MAP) was recorded continuously with a Statham transducer and a Grass polygraph, and heart rate (HR) was recorded with a Grass 7P4 tachograph triggered by the systolic arterial pulse wave. Rectal temperature was monitored with a YSI Tele-thermometer and was maintained between 36 and 38 °C with a heat lamp. A 30-min postsurgical equilibration period was then allowed prior to iv drug injection.

Test agents were dissolved in dimethylacetamide (DMA; Aldrich) and then serially diluted with isotonic saline to achieve drug concentrations permitting single iv doses of <1 mL/kg per bolus dose. For each drug, a step dose protocol was followed, with an initial dose followed by three progressively higher doses administered at intervals of 20 min. Control rats (pooled $n = 6$) were similarly prepared and treated with repetitive injections of the same DMA/saline vehicle (DMA concentration = 20%). At the end of the experiments the rats were sacrificed with an iv overdose of Na^+ pentobarbital and saturated KCl. The MAP and HR traces were read and averaged for four to five rats per drug treatment group, and the data were expressed as the mean change in MAP and HR from pretreatment (ΔMAP and ΔHR, respectively) as a function of both the individual and cumulative dosage.

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Supplementary Material Available: A listing of complete crystal data, including atomic fractional coordinates, bond lengths, angles, torsional angles, close intermolecular contacts between non-hydrogen atoms, hydrogen bonds, anisotropic thermal parameters, a line drawing with the atom numbering scheme, variable-temperature NMR data for **5m**, and full spectral data for **6e**, **8b–c**, **10b–e**, **11b–i**, **5b–r**, and **12b–c** (18 pages); observed and calculated structure factors (30 pages). Ordering information is given on any current masthead page.

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