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Synthesis and Potassium Channel Opening Activity of Substituted 10*H*-Benzo[4,5]furo[3,2-*b*]indole- and 5,10-Dihydro-indeno[1,2-*b*]indole-1-carboxylic Acids

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Abstract—Compounds in a structurally novel series of substituted 10*H*-benzo[4,5]furo[3,2-*b*]indole-1-carboxylic acids and related 5,10-dihydro-indeno[1,2-*b*]indole-1-carboxylic acids were prepared and shown to possess potent, bladder-selective smooth muscle relaxant properties and thus are potentially useful for the treatment of urge urinary incontinence. Electrophysiological studies using rat detrusor myocytes have demonstrated that prototype compound **7** produces a significant increase in hyperpolarizing current, which is iberiotoxin (IbTx)-reversed, thus consistent with activation of the large-conductance Ca^{2+}-activated potassium channel (BK_{Ca}).

Modulation of potassium channels continues to provide potentially useful approaches to treat pathological conditions involving cell hyperactivity.[1] By virtue of their ability to hyperpolarize cells and bring the membrane potential further from the threshold for activation of voltage-gated Ca^{2+} channels, potassium channel openers (KCOs) reduce excitability and relax smooth muscle cells. The structure–function relationship of physiologically relevant potassium channel subtypes has been reviewed.[2] It has been shown that bladder smooth muscle contains several types of potassium channels and that their activation by a variety of KCOs leads to detrusor muscle relaxation in vitro and in vivo.[3] Tissue-specific agents that selectively activate these bladder channels may show promise as effective therapeutics for the treatment of urge urinary incontinence. We have recently reported on a novel class of 1,2-diaminocyclobutene-3,4-diones as bladder selective K_{ATP} channel openers demonstrating potent in vivo bladder relaxant properties at doses which caused minimal hemodynamic effects.[4] As part of our continuing efforts focused on structural modifications of prototypical K_{ATP} agents, celikalim (**1**), a benzopyran-based antihypertensive K_{ATP} channel opener was chosen as a starting point for new synthesis (Fig. 1). A series of 4-indazolinonyl derivatives (**2**) was prepared and selected compounds were shown to possess potent bladder relaxant properties that were mediated via activation of the K_{ATP} channel.[5] Ring-contracted 2,3-dihydro-benzofuran analogues **3** were subsequently targeted for synthesis.

The initial attempt to reductively cyclize hydrazone **5** (Scheme 1) in the presence of $NaCNBH_3$ and acetic acid afforded only trace amounts of desired dihydro-benzofuran target **6**, but afforded instead the Fischer-indole product 10*H*-benzo[4,5]furo[3,2-*b*]indole (**7**).

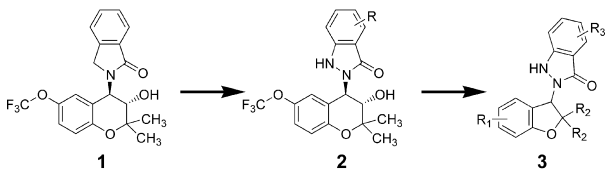

Figure 1. Structural modification of prototype K_{ATP} channel opener celikalim (**1**) afforded indazole leads (**2**). Ring contracted analogues (**3**) were subsequently targeted.

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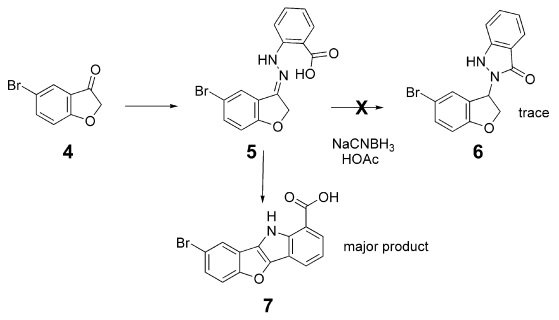
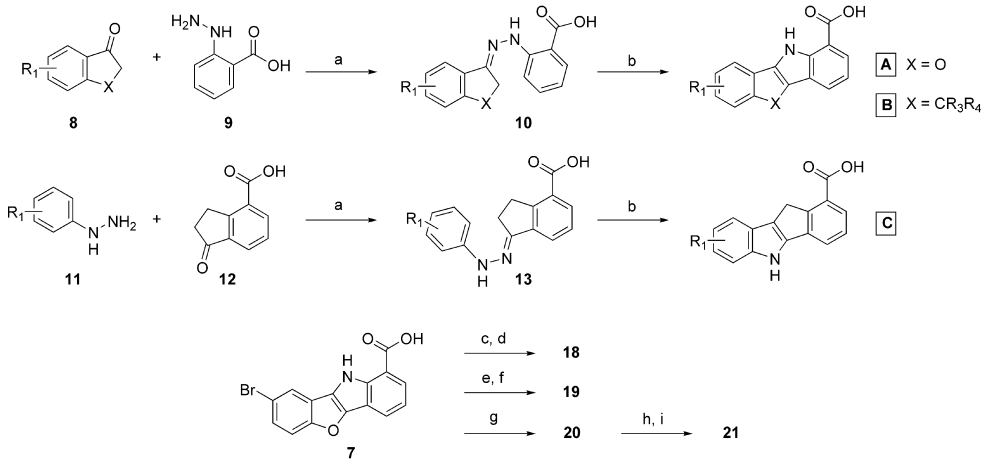


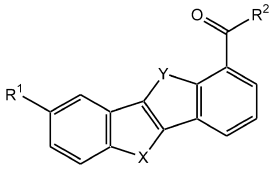
Scheme 1. An attempt to reductively cyclize hydrazone **5** to produce 2-benzofuranyl-indazolone **6** gave instead Fischer-indole product **7**, which was evaluated as a bladder relaxant.

The compound was evaluated for its relaxant properties in both rat detrusor tissue and thoracic aortic rings and was found to be a highly selective bladder relaxant ($IC_{50}=15$ μM) in vitro (IC_{50} ratio aorta/bladder = 8). While relaxation of bladder tissue associated with our earlier K_{ATP} channel activator leads was readily reversed by addition of glyburide (a selective K_{ATP} channel blocker), relaxation induced by **7** was only reversed by addition of iberiotoxin, a selective blocker of the large conductance, Ca^{2+}-activated potassium channel (BK_{Ca}). Due to its novel structure and unique in vitro profile, a series of benzofuro-indoles and indeno-indoles related to **7** was generated and the compounds were evaluated for their smooth muscle relaxant properties.



Scheme 2. Synthesis of 10*H*-benzo[4,5]-furo[3,2-*b*]indoles (template A) and 5,10-dihydro-indeno[1,2-*b*]indoles (templates B and C): (a) H_2O, ethanol, 20 °C; (b) HCO_2H, microwave irradiation; (c) DMF, NaH, MeI, −5 to 20 °C; (d) THF, H_2O, LiOH, 20 °C; (e) Et_2O, PCl_5, 20 °C; (f) Et_2O, NH_3; (g) H_2SO_4, CH_3OH, 100 °C; (h) THF, $LiAlH_4$, 20 °C; (i) CH_3CN, Dess–Martin periodinane, 20 °C.

Table 1. In vitro effects of tetracycles **A**, **B**, and **C** on precontracted rat bladder smooth muscle strips and rat aortic rings (IC_{50}, μM)



Compd	Template	X	Y	R^1	R^2	IC_{50} bladder[a]	n[b]	IC_{50} aorta[c]	n[b]
7	A	O	NH	Br	OH	15.1±4.7	8	118± 21.8	9
14	A	O	NH	I	OH	6.1±3.0	8	128±16.9	3
15	A	O	NH	Cl	OH	5.8±1.5	6	268±82.3	4
16	A	O	NH	NO_2	OH	6.3±2.6	8	125±13.8	3
17	A	O	NH	H	OH	30±6.5	4	NT[d]	—
18	A	O	NCH_3	Br	OH	>30	2	NT	—
19	A	O	NH	Br	NH_2	>30	2	NT	—
20	A	O	NH	Br	OCH_3	>30	4	NT	—
21	A	O	NH	Br	H	>30	2	NT	—
22	B	CH_2	NH	Br	OH	8.6±3.0	7	210±45.0	4
23	B	$C(CH_3)_2$	NH	NO_2	OH	5.2±1.8	4	17.2±2.3	4
24	C	NH	CH_2	Br	OH	4.4±0.95	8	109±12.4	6
25	C	NH	CH_2	I	OH	10.1±3.2	5	NT	—
26	C	NH	CH_2	Cl	OH	22.6±1.8	5	NT	—
27	C	NH	CH_2	SO_2NH_2	OH	>30	2	NT	—
28	C	NH	CH_2	OCF_3	OH	15.4±4.8	4	NT	—

[a]Drug concentration ± SE (μM) that relaxed KCl-induced contractions in rat detrusor strips by 50%.
[b]Number of experiments.
[c]Drug concentrations ± SE (μM) that relaxed KCl-induced contractions in rat aortic rings by 50%.
[d]Not tested.

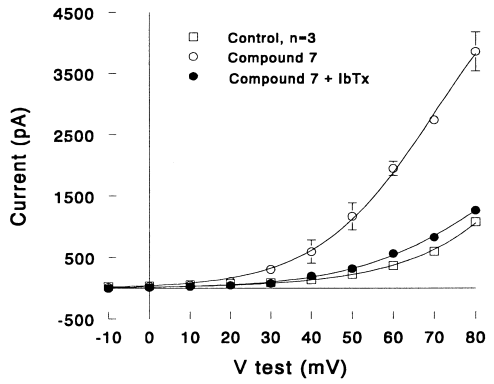


Figure 2. Current voltage relationship calculated from measured current from rat bladder smooth muscle cells. Compound **7** (10 μM, $n=3$) was associated with an increase in outward current above +30 mV. A 3.5× increase in outward current was observed at +80 mV. These increases were reversed by iberiotoxin (100 nM).

Treatment (Scheme 2) of an appropriately substituted benzofuranone (**8**, X = O) or indanone (**8**, X = C) with 2-hydrazinobenzoic acid in aqueous media afforded phenyl hydrazones **10**, which were subjected to microwave-facilitated[6,7] Fischer-indole cyclization in formic acid to afford a series of 10*H*-benzo[4,5]-furo[3,2-*b*]indoles (template A) or 5,10-dihydro-indeno[1,2-*b*]indoles (template B). Regioisomeric indeno-indoles (template C) were generated by condensing substituted phenylhydrazines (**11**) with indanone carboxylic acids (**12**) followed by microwave-facilitated Fischer-indole cyclization of **13**. The *N*-methylindole analogue **18** and carboxylic acid analogues **19**–**21** were prepared from **7** using standard transformations described in Scheme 2. All target compounds were evaluated in vitro for their ability to relax KCl precontracted rat detrusor muscle strips.[8] To ascertain intrinsic tissue selectivity, selected compounds were screened in similar tissue baths using rat aortic rings. The data are shown in Table 1.

The effect of the 8-bromo substituent was studied first. While removal of the bromine atom (**17**) resulted in 2-fold loss of in vitro activity, replacement with I, Cl, or NO_2 (**14**, **15**, and **16**, respectively) resulted in over 2-fold increase in bladder relaxant potency. Of these five analogues, 8-Cl derivative **15** was the most bladder selective smooth muscle relaxant (ratio = 46). The free indole N–H and carboxylic acid proton are critical for in vitro bladder relaxing properties as demonstrated by the loss of activity associated with *N*-methylation (**18**) and conversion of the acid to a primary amide (**19**), ester (**20**), or aldehyde (**21**). The oxygen atom of the benzofuran portion of the tetracycle could be replaced by carbon (**22** and **23**) with retention of both bladder relaxant potency and selectivity over aortic tissue. Regioisomeric 5,10-dihydro-indeno[1,2-*b*]indoles **24**–**26** and **28** retained in vitro bladder relaxant activity. In general, the same types of substituents are tolerated in place of bromine. A direct comparison of regioisomers **22** and **24** reveals about a 2-fold preference for the 8-bromo-5,10-dihydro-indeno[1,2-*b*]indole-1-carboxylic acid versus the 3-bromo-5,10-dihydro-indeno[1,2-*b*]indole-6-carboxylic acid with surprisingly similar tissue selectivities for both compounds.

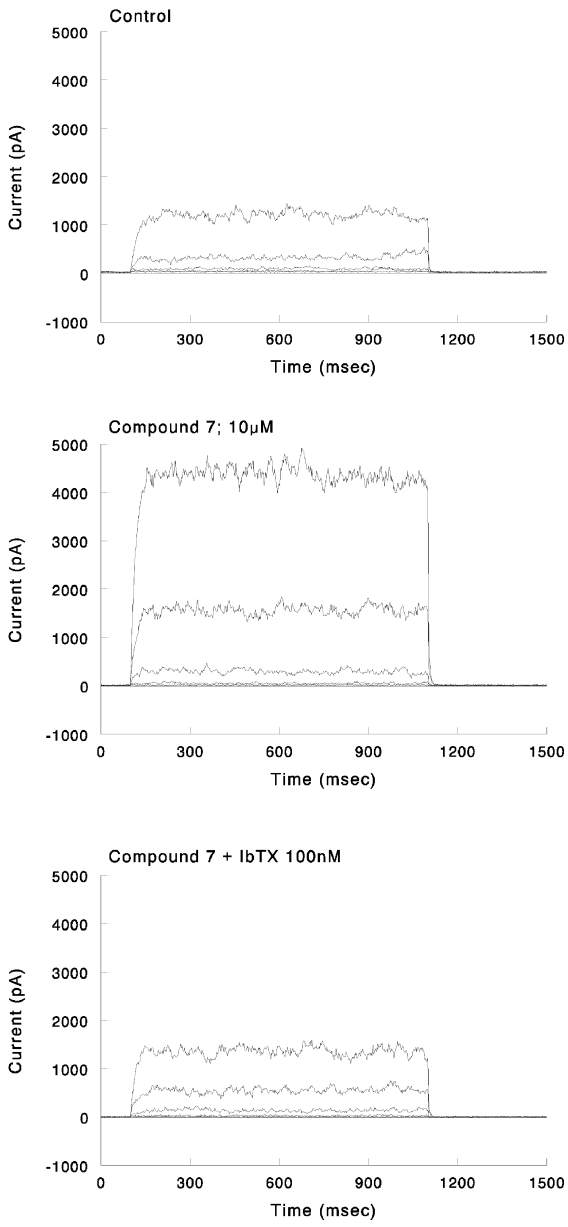


Figure 3. Representative tracings recorded from rat bladder smooth muscle cells. Cells were held at −10 mV and stepped to +80 mV in 10 mV increments for 1000 ms. The top panel shows control currents. Compound **7** (10 μM, $n=3$) increased outward currents as illustrated in the center panel. After exposure to iberiotoxin (100 nM), recorded currents returned to control levels (lower panel). Steps at 0, 20, 40, 60, and 80 mV are displayed.

To investigate the underlying mechanism of action of this novel series of selective bladder smooth muscle relaxants, several cell electrophysiological studies were undertaken with prototype compound **7**. Voltage clamp studies[9] on isolated rat detrusor myocytes (control resting membrane potential = −45 ± 5 mV) showed that exposure to **7** (10 μM) increased outward current above +30 mV test potentials (Fig. 2). A 3.5-fold increase in outward current was observed at +80 mV. Concurrent exposure with iberiotoxin, a selective BK_{Ca} channel blocker, reversed these increases back to control level currents. Representative whole cell voltage clamp tracings are shown in Figure 3.

The electrophysiological data indicate that furano-indole **7** can activate a hyperpolarizing current, consistent with the large-conductance calcium-dependent potassium channel, in isolated rat bladder cells. The resulting transmembrane hyperpolarization would be expected to underlie, in part, the bladder smooth muscle relaxing properties of this compound. Of major interest is the apparent selectivity for the bladder versus aortic smooth muscle. Other investigators have demonstrated that vascular smooth muscle cells contain BK_{Ca} channels; therefore the bladder selectivity cannot simply be attributed to the lack of BK_{Ca} channels in the aortic tissues. Evaluation of the electrophysiological effects of these compounds on isolated vascular smooth muscle cells will be required to understand the difference in potency between bladder and aortic smooth muscle.

In conclusion, an initial attempt to prepare a structurally modified analogue of benzopyranyl-based K_{ATP} channel opener **2** afforded the unexpected Fischer-indole product **7**. Assessment of its biological properties revealed a unique bladder selective in vitro smooth muscle relaxing profile consistent with activation of the BK_{Ca} channel. A series of related 10*H*-benzo[4,5]-furo[3,2-*b*]indoles and 5,10-dihydro-indeno[1,2-*b*]indoles was generated utilizing a microwave-induced Fischer-indole cyclization as the key step. Compounds **7** and **14**–**28** were evaluated for their ability to relax KCl pre-contracted rat detrusor strips and thoracic aortic rings. Test compounds were found to be highly bladder selective with aorta/bladder IC_{50} ratios ranging from 8 to 46-fold. Voltage clamp studies on isolated rat bladder myocytes suggest that prototype furano-indole **7** causes an iberiotoxin-sensitive increase in hyperpolarizing current consistent with activation of the BK_{Ca} channel. Studies are in progress to further evaluate this series for potential use in the treatment of urge urinary incontinence.

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7. All compounds gave satisfactory spectral data. The preparations of compounds **7**, **22**, and **24** serve as sample experimentals for the syntheses of templates **A**, **B**, and **C**, respectively. To a solution of 5-bromo-3(2*H*)-benzofuranone[10] (3.10 g, 14.6 mmol) in ethanol (100 mL) was added a solution of 2-hydrazinobenzoic acid hydrochloride (5.49 g, 29.1 mmol) in deionized water (200 mL). The mixture was stirred for 1 h at 20 °C and then cooled to 0 °C. Vacuum filtration and drying in vacuo afforded 3.65 g (72%) of *o*-[(2,3-dihydro-5-bromobenzofuran-3-ylidene)hydrazino]-benzoic acid as a brown solid: mp 195 °C (dec) which was used without further purification. The hydrazone (0.500 g, 1.51 mmol) was suspended in formic acid (2 mL, 96%) and was irradiated for 2 min in a closed cap Teflon vessel in a microwave oven (700 W). The mixture was vacuum filtered hot and the solid was dried in vacuo to yield 0.271 g (57%) of **7** as a yellow solid: mp 312–313 °C; ^{1}H NMR (DMSO-d_6) δ 13.36 (s, 1H), 11.64 (s, 1H), 8.27 (d, J = 2.1 Hz, 1H), 8.07 (d, J = 7.9 Hz, 1H), 7.92 (dd, J_1 = 7.5 Hz, J_2 = 1.0 Hz, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.50 (dd, J_1 = 8.7 Hz, J_2 = 2.1 Hz, 1H), 7.27 (dd, J_1 = 7.9 Hz, J_2 = 7.5 Hz, 1H); IR (KBr): 3420, 1685 cm^{-1}; MS (*m*/*z*) 329 (M^+). Anal. calcd for $C_{15}H_8BrNO_3$: C, 54.57; H, 2.44; N, 4.24; found: C, 54.22; H, 2.32; N, 4.30. To a solution of 6-bromoindanone[11] (0.447 g, 2.12 mmol) in ethanol (100 mL) was added a solution of 2-hydrazinobenzoic acid hydrochloride (0.800 g, 4.24 mmol) in deionized water (50 mL). The mixture was stirred for 1 h then cooled to 0 °C. The precipitated *o*-[(2,3-dihydro-6-bromoinden-3-ylidene)hydrazino]-benzoic acid was vacuum filtered and dried in vacuo, yield: 0.628 g (86%) of a yellow solid: mp 186 °C (dec) which was used as is. The hydrazone (0.620 g, 1.80 mmol) in formic acid (2 mL, 96%) was irradiated for 2 min in a closed cap Teflon vessel of a microwave oven (700 W). The mixture was vacuum filtered hot and the solid was dried in vacuo to yield 0.360 g (61%) of **22** as a yellow solid: mp 245–247 °C (dec); ^{1}H NMR (DMSO-d_6) δ 13.43 (s, 1H), 11.73 (s, 1H), 8.32 (d, J = 2.0 Hz, 1H), 7.86 (d, J = 7.9 Hz, 1H), 7.78 (dd, J_1 = 7.6 Hz, J_2 = 0.8 Hz, 1H), 7.49 (d, J = 8 Hz, 1H), 7.36 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H), 7.16 (dd, J_1 = 7.9 Hz, J_2 = 7.6 Hz, 1H), 3.71 (s, 2H); IR (KBr): 3460, 1650 cm^{-1}; MS (*m*/*z*) 327 (M^+). Anal. calcd for $C_{16}H_{10}BrNO_2$: C, 58.56; H, 3.07; N, 4.27; found: C, 58.62; H, 2.83; N, 4.22. 1-Oxo-4-indancarboxylic acid[12] (0.528 g, 2.98 mmol) and 4-bromophenylhydrazine (0.566 g, 3.00 mmol) in formic acid (2 mL, 96%) were irradiated for 2 min in a closed cap Teflon vessel of a microwave oven (700 W). The mixture was vacuum filtered. The crude product was dissolved in acetone/diethyl ether (1:1) and treated with decolorizing carbon, filtered, concentrated and dried in vacuo to yield 0.530 g (54%) of **24** as a white solid: mp 328–330 °C (dec); ^{1}H NMR (DMSO-d_6) δ 13.43

(s, 1H), 11.85 (s, 1H), 7.78–7.83 (m, 3H), 7.50 (dd, $J_1 = 7.89$ Hz, $J_2 = 7.47$ Hz, 1H), 7.43 (d, $J = 8.3$ Hz, 1H), 7.16 (dd, $J_1 = 8.5$ Hz, $J_2 = 2.1$ Hz, 1H), 4.00 (s, 2H); IR (KBr): 3440, 1690 cm^{-1}; MS (*m*/*z*) 327 (M^+). Anal. calcd for $C_{16}H_{10}BrNO_2$: C, 58.56; H, 3.07; N, 4.27; found: C, 58.57; H, 2.88; N, 4.30.

8. In vitro studies protocol as described in ref 4a. Briefly: female Sprague–Dawley rats (Charles River, Wilmington, MA, USA; 250–350 g) were rendered unconscious via inhalation of CO_2 and exsanguinated. The entire bladder was removed and placed into room temperature PSS of the following composition (mM): NaCl (118.4), KCl (4.7), $CaCl_2$ (2.5), $MgSO_4$ (1.2), KH_2PO_4 (1.2), $NaHCO_3$ (24.9) and D-glucose (11.1) gassed with O_2–CO_2, 95%/5% to achieve a pH of 7.4. The dome of the bladder was isolated from the trigone region and the mucosa was removed. The detrusor was then cut into strips 4–5 mm wide by 10 mm long. One end was secured to the bottom of a 10 mL tissue bath and the other to a Grass isometric force transducer (Grass Instruments, Quincy, MA, USA). Tissues were pretensioned (0.25–0.5 g) and allowed to equilibrate for 30 min. After which, strips were contracted with an additional 15 mM KCl and again allowed to equilibrate for approximately 90 min. Compounds were administered directly into the tissue baths as cumulative concentrations and responses allowed to reach steady state. Signals were digitized (486 based personal computer, 12-bit resolution, 1 s sampling interval, custom software) for on-line analysis. Since isolated bladder strips contract with irregular frequency and amplitude, a 5-min area-under-the-contraction curve was used to assess contractility after achieving steady state for each concentration. Aortic ring studies were performed in a similar matter.

9. Cell electrophysiological studies protocol as described in ref 4d.

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