Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 2495–2501
Bioorganic & Medicinal Chemistry Letters

Synthesis and bladder smooth muscle relaxing properties of substituted 3-amino-4-aryl-(and aralkyl-)cyclobut-3-ene-1,2-diones

John A. Butera,[a,*] Douglas J. Jenkins,[a] Joseph R. Lennox,[a] Jeffrey H. Sheldon,[b] N. Wesley Norton,[b] Dawn Warga[b] and Thomas M. Argentieri[b]

[a]*Chemical and Screening Sciences, Wyeth Research, CN 8000, Princeton, NJ 08543, USA*
[b]*Urologic Diseases, Women's Health Research Institute, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*

Received 10 January 2005; revised 17 March 2005; accepted 17 March 2005
Available online 12 April 2005

Abstract—We have reported on the design, synthesis, and biological characterization of (*R*)-4-[3,4-dioxo-2-(1,2,2-trimethyl-propylamino)-cyclobut-1-enylamino]-3-ethyl-benzonitrile (**1**),[1] a novel, potent, and selective adenosine 5′-triphosphate-sensitive potassium (K_{ATP}) channel opener with potential utility for the treatment of urge urinary incontinence (UUI). Excising the aniline-derived nitrogen atom of **1** or replacing it with an aralkyl group, led to bladder smooth muscle relaxant chemotypes **3** and **4**, respectively. Prototype compounds in these series were found to produce significant increases in an iberiotoxin (IbTx)-sensitive hyperpolarizing current, thus suggesting that these relatively modest structural modifications resulted in a switch in the mechanism of action of these smooth muscle relaxants from K_{ATP} channel openers to activators of the large-conductance Ca^{2+}-activated potassium channel (BK_{Ca}). We report herein the syntheses and biological evaluation of a series of substituted 3-amino-4-aryl-(and aralkyl-)cyclobut-3-ene-1,2-diones.

Transmembrane potassium channels, a diverse family of pore-forming proteins that allow for selective permeation of potassium ions across the cell membrane, continue to provide useful molecular targets for controlling the physiological function of excitable cells. Recent reviews have provided useful perspectives of potassium channel sub-type, structure, function and related medicinal chemistry, and SAR.[2–5] It has been shown that bladder smooth muscle may contain several sub-type families of potassium channels and that modulation of these channels with specific ligands produces significant detrusor muscle relaxation in vitro and in vivo.[6] Potassium channel openers (KCOs) with specificity towards bladder tissue may show promise as potential drugs to treat urge urinary incontinence (UUI), a condition characterized by spontaneously hyperactive bladder smooth muscle.

Subsequent to the discovery and characterization of diaminocyclobutenediones **1a** and **1b** (Fig. 1), two potent and bladder-selective K_{ATP}-channel openers possessing striking oral efficacy in a rat hypertrophied bladder model of UUI,[1,7] metabolic stability studies (in vitro and in vivo) on **1a** and **1b** suggested a rapid turnover to the primary metabolite, a 4-amino-benzonitrile derivative. Concern over potential long-term exposure to these compounds prompted us to focus our synthetic efforts on structural modifications of the aryl vinylogous amide bond. Numerous approaches were implemented and several have been reported: (i) homologation of the anilino-cyclobutenedione linker with concomitant re-optimization of SAR to afford development track compound **2** (KCO-616),[8,9] (ii) acylation of the anilino-nitrogen to afford acyl analogs **5**,[10] which slows but does not prevent formation of the aniline metabolite, and (iii) incorporation of the aryl amine and alkyl amine moieties onto numerous heterocyclic scaffolds (**6**) to circumvent the metabolic cleavage.[11] While approach (i) was successful and resulted in the identification, characterization, and development of a series of nonhydrolyzing benzylamino cyclobutenediones exemplified by KCO-616, concomitant strategies involved the excision of the labile vinylogous amide bond or its replacement with a carbon-based variant such as an olefin, methylene, or substituted methylene unit. Interestingly, preliminary cell-electrophysiological data suggest

Keywords: Bladder instability; Urge urinary incontinence; Potassium channel opener; BK_{Ca} channel opener; Bladder relaxant.
* Corresponding author. Tel.: +1 732 274 4289; fax: +1 732 274 4129; e-mail: buteraj@wyeth.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.03.073

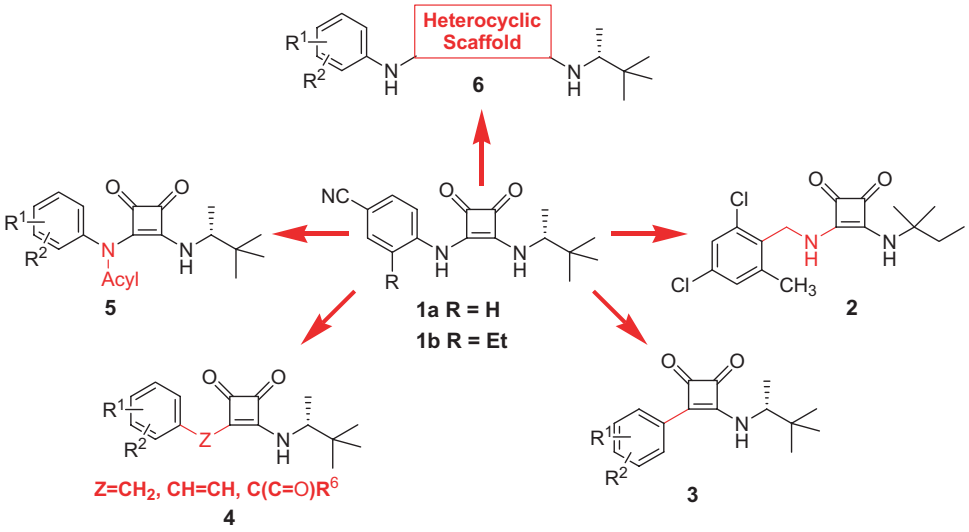


Figure 1. Attempts to modulate the metabolic fate of cyclobutenedione leads (**1a** and **1b**) via structural modification of the aryl vinylogous amide bond led to several novel chemotypes possessing in vitro and in vivo bladder smooth muscle relaxant properties.

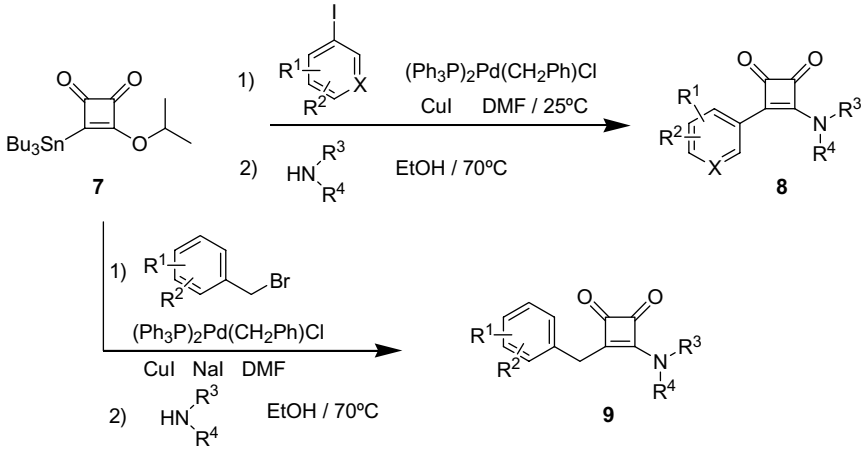
that these seemingly modest structural modifications resulted in a switch in the mechanism of action of these smooth muscle relaxants from K_{ATP} channel openers to activators of the large-conductance Ca^{2+}-activated potassium channel (BK_{Ca}). This report will detail our efforts to synthesize a series of substituted 3-amino-4-aryl, 3-amino-4-aralkyl, and 3-amino-4-vinyl cyclobut-3-ene-1,2-diones, represented by templates **3** and **4**, and their biological evaluation as bladder smooth muscle relaxants.[12]

Cross-coupling reaction conditions (Scheme 1) using 3-isopropoxy-4-tri-*n*-butyl-stannyl)-3-cyclobutene-1,2-dione (**7**)[13] and an appropriate aryl halide (X = CH) or 3-pyridyl halide (X = N) in the presence of benzyl(chloro)bis-(triphenylphosphine)palladium(II) and cuprous iodide afforded the Stille product,[14] which was subsequently treated with a primary or secondary amine in ethanol to give the 3-amino-4-phenyl-(or pyridyl)-cyclobut-3-ene-1,2-dione (**8**).

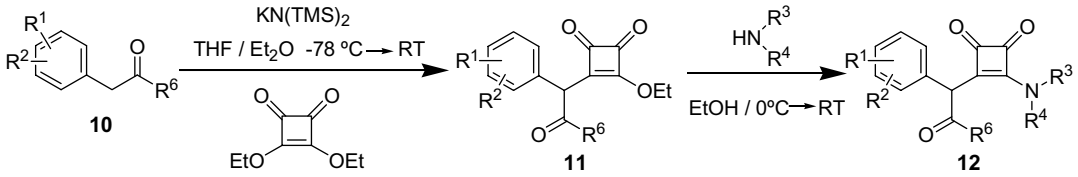
Synthesis of benzyl analogs (**9**) was accomplished similarly by treating stannane **7** with an appropriate benzyl bromide in the presence of benzyl(chloro)bis-(triphenylphosphine)palladium(II), cuprous iodide, under *trans*-halogenation conditions using sodium iodide to afford the benzyl-substituted cyclobutenedione, which was converted to final product **9** by treatment with a primary or secondary amine in ethanol.

Compounds possessing an alkanoyl group appended from the benzylic carbon of chemotype **9** were prepared (Scheme 2) by inverse dropwise addition of the preformed enolate of the appropriate ketone **10** (potassium bis(trimethylsilyl)amide at −78 °C) into a cold solution of diethoxysquaric acid. Adduct **11** was then treated with one equivalent of amine in EtOH at 0 °C to afford compound **12**.

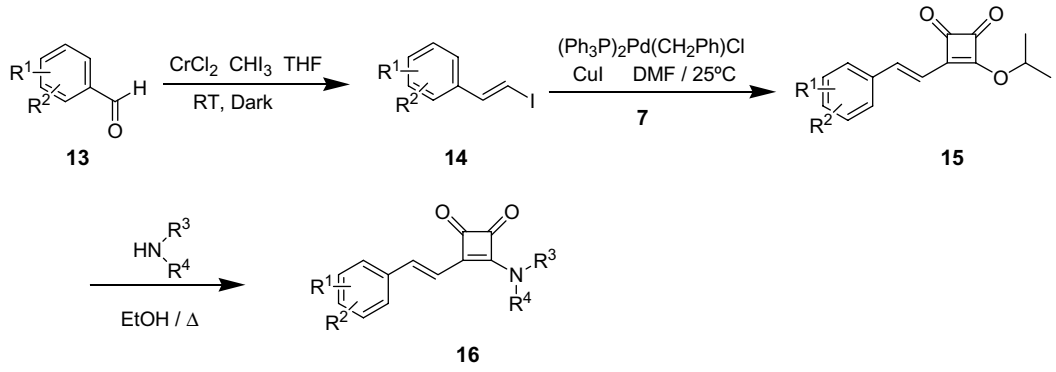
A third linker was explored where the aniline nitrogen of **1** was replaced with a C=C double bond. Thus, iodo-



Scheme 1. Synthesis of 3-amino-4-phenyl- and 3-amino-4-pyridyl-cyclobut-3-ene-1,2-diones **8** and benzyl analogs **9**.



Scheme 2. Synthesis of acyl benzyl analogs **12**.



Scheme 3. Synthesis of styrenylcyclobutenedione analogs **16**.

methylation of aldehyde **13** (Scheme 3) in the presence of iodoform and chromium(II) chloride at rt (in the absence of light)[15] gave the corresponding vinyl iodide **14**, which was reacted with stannane **7** in the presence of benzyl(chloro)bis-(triphenylphosphine)-palladium(II) and cuprous iodide in DMF to afford styrenylcyclobutenedione derivative **15**. Displacement of the isopropoxy group with an amine in EtOH at reflux gave vinylogous amide **16**.

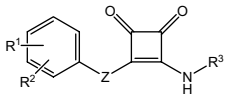
Our initial biological evaluation utilized a functional assay, which measured the compound's ability to relax bladder smooth muscle. In vitro bladder smooth muscle relaxant properties were determined for all test compounds in tissue baths utilizing KCl pre-contracted rat detrusor muscle strips.[17] The data for prototype compounds examining linker variants (Z = direct bond, CH_2, $CHCOCH_3$, and CH=CH) are shown in Table 1.

We began our assessment of potential linker replacements for the anilino NH of squarate leads **1** by holding the alkyl amino group constant as the 1,2,2-trimethylpropyl moiety, the optimal substituent identified from our previously reported SAR optimization effort in this series.[1] Direct excision of the anilino NH in **1a**, resulted in a drastic reduction (~100-fold) in smooth muscle relaxant properties as seen when comparing **1a** with **8.1**. Interestingly, in contrast with diaminocyclobutenediones **1a**, **1b**, and **2**, the relaxation resulting from treatment with phenyl cyclobutenedione **8.1** *was not* reversed by the sulfonylurea glyburide, a specific blocker of the K_{ATP} channel. This suggested that the smooth muscle relaxation was probably due to an alternative mechanism of action. Replacement of the 4-cyano moiety of **8.1** with a 4-methoxy group (**8.2**) reinstated smooth muscle relaxant properties although the compound was still ~25-fold less potent than **1a**. The in vitro relaxant effects of both **8.1** and **8.2** were found to be reversed by iberiotoxin, a selective blocker of the large conductance, Ca^{2+}-activated potassium channel (BK_{Ca}). Another distinguishing feature of compound **8.2** was its ~2.6-fold selectivity for bladder tissue versus aortic tissue in contrast to the diaminocyclobutenediones, which trended toward aortic selectivity in vitro.

Our initial survey of linker replacements for the anilino NH of **1** was widened to examine CH_2 (**9.1** and **9.2**), $CH(COCH_3)$ (**12.1** and **12.2**), and a *trans*-olefin (**16.1** and **16.2**). These changes resulted in loss of activity when compared to **1a** except in the case of **16.1** (IC_{50} = 2.94 μM). Due to their interesting smooth muscle relaxant properties, their unique structure, and their amenability to synthesis, we chose to focus our efforts on generating a series of substituted phenyl cyclobutenediones and evaluating them as bladder smooth muscle relaxants. The data are shown in Table 2.

Studying the effects of α-*gem*-disubstitution on the alkylamino side, the 1,2,2-trimethylpropyl-amino group in **8.2** was replaced with a 1,1-dimethyl-2-phenyl ethyl moiety to afford **8.3**, a compound with 8-fold improvement in bladder relaxant properties, which still maintains its ~2.6-fold selectivity toward bladder tissue versus aortic rings. Compound **8.10**, another *gem*-disubstituted analog, was found to exhibit the most selectivity for bladder tissue with an IC_{50} ratio of ~15. Again probing the effects of exchanging the 4-methoxy group, the 4-CF_3 and 4-Br analogs (**8.4** and **8.5**, respectively) were found to be much less potent than **8.2**, **8.3**, and **8.10**. Moving the methoxy substituent from the 4- to the 3-position (**8.9** and **8.11**) afforded molecules devoid of bladder relaxant properties. In fact, compound **8.9** further

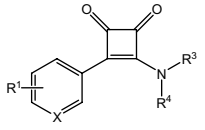
Table 1. In vitro effects of linker (Z) variants of K_{ATP} channel openers **1** and **2** on pre-contracted rat bladder detrusor muscle strips and aortic rings (IC_{50}, μM)



Compound Number	Z	R_1	R_2	R_3	IC_{50} bladder (μM)[a]	n[b]	% Reversed by glyburide[c]	IC_{50} aorta (μM)[d]	n[b]
1a	NH	4-CN	H	(*R*) 1,2,2-Trimethylpropyl	0.37 ± 0.07	4	110	0.064 ± 0.015	3
1b	NH	4-CN	2-Et	(*R*) 1,2,2-Trimethylpropyl	0.09 ± 0.02	5	77	0.017 ± 0.004	2
2	CH_2–NH	2,4-Di-Cl	6-Me	1,1-Dimethyl-1-propyl	0.1 ± 0.03	4	100	0.042 ± 0.014	3
8.1	—[f]	4-CN	H	(*R,S*) 1,2,2-Trimethylpropyl	>30	4	0	NT[e]	—
8.2	—	4-OCH_3	H	(*R,S*) 1,2,2-Trimethylpropyl	9.5 ± 1.8	4	0	24.3 ± 6.3	5
9.1	CH_2	4-CN	H	(*R,S*) 1,2,2-Trimethylpropyl	>30	3	0	NT	—
9.2	CH_2	4-OCH_3	H	(*R,S*) 1,2,2-Trimethylpropyl	>30	2	0	NT	—
12.1	CH($COCH_3$)	4-OCH_3	H	(*R,S*) 1,2,2-Trimethylpropyl	>30	2	0	NT	—
12.2	CH($COCH_3$)	4-CF_3	H	(*R,S*) 1,2,2-Trimethylpropyl	16.2 ± 6.82	3	0	NT	—
16.1	CH=CH	4-CN	H	(*R,S*) 1,2,2-Trimethylpropyl	2.94 ± 0.62	5	0	NT	—
16.2	CH=CH	4-Br	H	(*R,S*) 1,2,2-Trimethylpropyl	>30	3	25	NT	—

[a] Drug concentration ± SE (μM) that relaxed KCl-induced contractions in rat detrusor strips by 50%.
[b] Number of experiments.
[c] Percent of the observed relaxation that was reversed by the addition of the glyburide, a selective K_{ATP} channel blocker.
[d] Drug concentrations ± SE (μM) that relaxed KCl-induced contractions in rat aortic rings by 50%.
[e] Not tested.
[f] Direct bond.

Table 2. In vitro effects of phenyl cyclobutenedione derivatives **8** on pre-contracted rat bladder detrusor muscle strips and aortic rings (IC_{50}, μM)



Compound number	X	R_1	R_3	R_4	IC_{50} bladder (μM)[a]	n[b]	% Reversal by glyburide[c]	IC_{50} aorta (μM)[d]	n[b]
8.1	CH	4-CN	(*R,S*) 1,2,2-Trimethylpropyl	H	>30	4	0	NT[e]	—
8.2	CH	4-OCH_3	(*R,S*) 1,2,2-Trimethylpropyl	H	9.5 ± 1.8	4	0	24.3 ± 6.3	5
8.3	CH	4-OCH_3	1,1-Dimethyl-2-phenyl-ethyl	H	1.2 ± 0.03	2	0	3.25 ± 0.35	2
8.4	CH	4-CF_3	(*R,S*) 1,2,2-Trimethylpropyl	H	>30	7	0	NT	—
8.5	CH	4-Br	(*R,S*) 1,2,2-Trimethylpropyl	H	25.7 ± 1.1	3	0	NT	—
8.6	N	H	1,1-Dimethyl-1-propyl	H	>30	2	0	NT	—
8.7	CH	4-OCH_3	(*R*)-1-Phenyl-ethyl	H	27 ± 0.3	2	0	NT	—
8.8	CH	4-OCH_3	2-(3-Trifluoromethyl-phenyl)-ethyl	H	>30	4	0	NT	—
8.9	CH	3-OCH_3	1,1-Dimethyl-1-propyl	H	Contracted[f]	2	—	NT	—
8.10	CH	4-OCH_3	1,1-Dimethyl-1-propyl	H	2.5 ± 0.49	4	0	37.65 ± 6.05	2
8.11	CH	3-OCH_3	1,1-Dimethyl-1-propyl	H	>30	2	0	NT	—
8.12	CH	4-OCH_3	2-Phenyl-1-propyl	H	>30	2	0	NT	—
8.13	CH	4-(3-Phenylpropoxy)	1,1-Dimethyl-1-propyl	H	Contracted[f]	2	—	NT	—
8.14	CH	4-OCH_3	2-Propyl	CH_3	11.99 ± 2.4	4	0	46.2 ± 7.8	3

[a] Drug concentration ± SE (μM) that relaxed KCl-induced contractions in rat detrusor strips by 50%.
[b] Number of experiments.
[c] Percent of the observed relaxation that was reversed by the addition of the glyburide, a selective K_{ATP} channel blocker.
[d] Drug concentrations ± SE (μM) that relaxed KCl-induced contractions in rat aortic rings by 50%.
[e] Not tested.
[f] Drug caused an additional contraction of rat detrusor strips beyond that seen after treatment with KCl.

contracted the bladder strips. The underlying mechanism for this observation is not well understood; however the data could suggest that **8.9** is a potassium channel blocker. Replacement of the substituted phenyl group with a 3-pyridyl moiety (**8.6**) also resulted in loss of in vitro bladder relaxant activity.

Compounds bearing a phenethyl group, which lack the α-*gem* disubstitution (i.e., **8.8** and **8.12**) and those bearing an α-methyl benzyl group (**8.7**) demonstrated a loss of activity. Also, as suggested by **8.14**, methylation of the vinylogous amide N is not tolerated. Finally, homologation of the 4-methoxy group of **8.10** to a larger

substituent such as a 3-phenyl-propoxy moiety (**8.13**) also converts the compound into a smooth muscle contractor.

The compound with the highest measured intrinsic selectivity for bladder tissue, 3-(1,1-dimethyl-propylamino)-4-(4-methoxy-phenyl)-cyclobut-3-ene-1,2-dione (**8.10**), was selected for further evaluation in electrophysiological studies to confirm the underlying mechanism of action of its observed smooth muscle relaxing properties. Voltage-clamp studies (Fig. 2) on isolated human bronchial smooth muscle cells[18] showed that compound **8.10** at a concentration of 1 μM increased outward current above +40 mV. This increase in outward current was reversed back to control levels by addition of the selective BK_{Ca} channel blocker, iberiotoxin.

The intrinsic in vitro selectivity for bladder tissue observed with this novel class of KCOs provided a compelling reason to expand the SAR. A more focused electrophysiological evaluation of these compounds on various isolated smooth muscle cell types may be required to better understand this measured selectivity.

In conclusion, numerous strategies were implemented with the goal of attenuating the metabolic instability of the vinylogous amide bond in the potent diaminocyclobutenedione K_{ATP} channel openers **1a** and **1b**. Replacement of the labile NH linker with carbon-based linkers (i.e., methylene, substituted methylene, olefin) was not tolerated. Excision of the NH linker to afford a directly bound aryl-cyclobutenedione generated a novel scaffold (**8**), which possessed smooth muscle relaxing properties that were not glyburide sensitive. Optimization of the alkylamine substituent led to compound **8.10**. Voltage-clamp studies on isolated human bronchial smooth muscle cells suggested that compound **8.10** caused an iberiotoxin-sensitive increase in hyperpolarizing current consistent with activation of the large conductance Ca^{2+}-activated potassium channel (BK_{Ca}). Additional studies to better understand the intrinsic bladder selectivity of these derivatives and to more fully evaluate their potential for use in the treatment of urge urinary incontinence are underway.

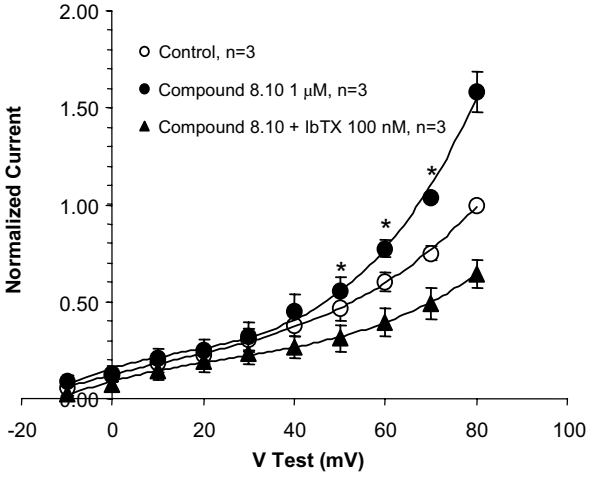


Figure 2. Current–voltage relationship for outward current before (control, ○, $n = 3$) and after compound **8.10** (1 μM, ●, $n = 3$). This increase was reversed by the BK_{Ca} channel blocker iberiotoxin (100 nM, ▲, $n = 3$). Compound **8.10** was associated with a significant increase in outward current above +40 mV ($*p \leqslant 0.05$). Data were normalized to peak control current.

References and notes

1. Butera, J. A.; Antane, M. M.; Antane, S. A.; Argentieri, T. M.; Freeden, C.; Graceffa, R. F.; Hirth, B. H.; Jenkins, D.; Lennox, J. R.; Matelan, E.; Norton, N. W.; Quagliato, D.; Sheldon, J. H.; Spinelli, W.; Warga, D.; Wojdan, A.; Woods, M. *J. Med. Chem.* **2000**, *43*(6), 1187.
2. Coghlan, W. J.; Caroll, W. A.; Gopalakrishnan, M. *J. Med. Chem.* **2001**, *44*(11), 1627.
3. Gribkoff, V. K.; Starrett, J. E. In *Ann. Rep. Med. Chem.*; Doherty, A. M., Ed.; 2002; Vol. 37, pp 237–246.
4. Aguilar-Bryan, L.; Clement, J. P.; Gonzalez, G.; Kunjilwar, K.; Babenko, A.; Bryan, J. *Physiol. Rev.* **1998**, *78*, 227.
5. Bryan, J.; Aguilar-Bryan, L. *Curr. Opin. Cell. Biol.* **1997**, *9*, 553.
6. (a) Zografos, P.; Li, J. H.; Kau, S. T. *Pharmacology (Basel)* **1992**, *45*, 216; (b) Bonev, A. D.; Nelson, M. T. *Am. J. Physiol.* **1993**, *264*, C1190; (c) Malmgren, A.; Andersson, K. E.; Sjogren, C.; Andersson, P. O. *J. Urol.* **1989**, *142*, 1134; (d) Nurse, D. E.; Restorick, J. M.; Mundy, A. R. *Br. J. Urol.* **1991**, *68*, 27; (e) Howe, B. B.; Halterman, T. J.; Yochim, C. L.; Do, M. L.; Pettinger, S. J.; Stow, R. B.; Ohnmacht, C. J.; Russell, K.; Empfield, J. R.; Trainor, D. A.; Brown, F. J.; Kau, S. T. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 884.
7. Wojdan, A.; Freeden, C.; Woods, M.; Oshiro, G.; Spinelli, W.; Colatsky, T. J.; Sheldon, J.; Norton, N. W.; Warga, D.; Antane, M.; Antane, A.; Butera, J. A.; Argentieri, T. M. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 1410.
8. Gilbert, A. M.; Antane, M. M.; Argentieri, T. M.; Butera, J. A.; Francisco, G. D.; Freeden, C.; Gundersen, E. G.; Graceffa, R. F.; Herbst, D.; Hirth, B. H.; Lennox, J. R.; McFarlane, G.; Norton, N. W.; Quagliato, D.; Sheldon, J. H.; Warga, D.; Wojdan, A.; Woods, M. *J. Med. Chem.* **2000**, *43*, 1203.
9. Butera, J. A.; Argentieri, T. M. *Drugs Fut.* **2000**, *25*, 239.
10. Antane, M. M.; Antane, S. A.; Argentieri, T. A.; Butera, J. A.; Francisco, G. P.; Freeden, C.; Gilbert, A. M.; Graceffa, R. F.; Gundersen, E. G.; Herbst, D. R.; Hirth, B. H.; Ho, D. M.; Jenkins, D.; Lennox, J. R.; Matelan, E.; McFarlane, G. R.; Norton, N. W.; Oshiro, G.; Primeau, J. L.; Quagliato, D. A.; Sheldon, J. H.; Spinelli, W.; Warga, D.; Wojdan, A.; Woods, M. Abstract of Papers, 218th National Meeting of the American Chemical Society, New Orleans, LA; American Chemical Society: Washington DC, 1999; Abstract MEDI 17.
11. Data not shown in this manuscript.
12. All compounds gave satisfactory spectral data. The preparations of compounds **8.3**, **9.2**, **12.2**, and **16.2** will serve as sample experimentals for the syntheses of directly bound phenyl squarates (**8**), benzyl squarates (**9**), alkanoyl-substituted benzyl squarates (**12**), and styrenyl squarates (**16**), respectively. Compound **8.3**: To a cooled (0 °C) solution of 3-isopropoxy-4-(tri-*n*-butylstannyl)-3-cyclobutene-1,2-dione[13] (8.00 g, 18.65 mmol) in DMF (40 mL) was added 4-iodoanisole (4.85 g, 20.72 mmol). Benzylchlorobis-(triphenylphosphine)palladium(II) (0.942 g, 1.24 mmol) and cuprous iodide (0.355 g, 1.87 mmol)

were added, and the mixture was stirred at rt overnight. Dilution with diethyl ether (200 mL) followed by aqueous work up with NH_4Cl, 10% KF, and brine afforded crude product, which was dissolved in hot ethyl acetate, decolorized (charcoal), and filtered. The filtrate was treated with hexane and allowed to cool. 3-Isopropoxy-4-(4-methoxyphenyl)-cyclobut-3-ene-1,2-dione precipitated as a light yellow solid (2.46 g, 54%): ^{1}H NMR (DMSO-d_6): δ 7.89 (d, 2H), 7.15 (d, 2H), 5.45 (hept, 1H), 3.82 (s, 3H), 1.48 (d, 6H). To a solution of the intermediate (0.150 g, 0.609 mmol) in ethanol (3 mL) was added 1,1-dimethyl-2-phenyl-ethylamine (0.182 g, 1.22 mmol). The mixture was stirred at 70 °C overnight, then filtered hot through a pad of silica gel. The filtrate was concentrated and the resulting residue was recrystallized from ethyl acetate/hexanes to give 0.170 g (83%) of 3-(1,1-dimethyl-2-phenyl-ethylamino)-4-(4-methoxy-phenyl)-cyclobut-3-ene-1,2-dione (**8.3**) as a tan solid: mp 150–151 °C; ^{1}H NMR (DMSO-d_6): δ 8.28 (s, 1H), 8.00 (m, 2H), 7.28 (m, 2H), 7.26 (m, 1H), 7.13 (m, 2H), 7.09 (m, 2H), 3.82 (s, 3H), 3.10 (s, 2H), 1.42 (s, 6H); MS (*m*/*z*) 335 [M$^+$]. Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.31; N, 4.18. Found: C, 74.35; H, 6.41; N, 3.90. Compound **9.2**: To a heterogeneous mixture of CuI (178 mg, 0.932 mmol) and NaI (2.09 g, 14.0 mmol) in anhydrous DMF (5.0 mL) was added 4-methoxybenzyl chloride. The reaction mixture, was stirred for 30 min at rt, whereupon a solution of 3-isopropoxy-4-(tri-*n*-butyl-stannyl)-3-cyclobut-3-ene-1,2-dione (4.00 g, 9.32 mmol) in DMF (5.0 mL) was added, followed by addition of the benzylchlorobis-(triphenylphosphine)palladium(II) catalyst (530 mg, 0.699 mmol). Upon stirring at rt for 2 h, the mixture was diluted with ethyl acetate (250 mL), and subjected to aqueous work up with saturated NH_4Cl, 10% KF and brine (100 mL). The organic phase was dried over Na_2SO_4/charcoal and submitted to flash chromatography (40% ether–petroleum ether) affording 3-(4-methoxy-benzyl)-4-isopropoxy-cyclobut-3-ene-1,2-dione as golden brown oil (1.04 g, 43%): ^{1}H NMR ($CDCl_3$): δ 7.20 (ABq, 2H), 6.85 (ABq, 2H), 5.38 (hept, 1H), 3.83 (s, 2H), 3.79 (s, 3H), 1.43 (d, 6H). In a manner similar to compound **8.3**, the intermediate (350 mg, 1.34 mmol) was dissolved in anhydrous isopropyl alcohol (7.0 mL) and was treated with 2-amino-3,3-dimethylbutane (272 mg, 2.69 mmol) at rt, affording 0.124 g (31%) of 3-(4-methoxy-benzyl)-4-(1,2,2-trimethyl-propylamino)cyclobut-3-ene-1,2-dione (**9.2**) one-quarter hydrate (^{1}H NMR in DMSO-d_6 suggested the presence of amide rotamers in a ratio of approximately 3:1): mp 137.4–138.1 °C; ^{1}H NMR (DMSO-d_6): δ 8.63 (d, 1H), 7.16 (ABq, 2H), 6.87 (ABq, 2H), 3.90 (m, 1H), 3.70 (s, 2H), 1.15 (d, 3H), 0.84 (s, 9H); MS (*m*/*z*) 301 [M+]. Anal. Calcd for $C_{18}H_{23}NO_3 \cdot 0.25\ H_2O$: C, 70.68; H, 7.74; N, 4.58. Found: C, 70.43; H, 7.77; N, 4.55. Compound **12.2**: A solution of 1-[(4-trifluoromethyl)phenyl]-2-propanone[16] (1.86 g, 9.208 mmol) in THF (10 mL) was added dropwise (under N_2) to a cooled (−78 °C) solution of potassium bis(trimethylsilyl)amide (19.3 mL; 0.5 M in toluene, 9.67 mmol) in THF/diethyl ether (1:1 ratio, 80 mL). The mixture stirred at −78 °C for 15 min and was then stirred at rt for 2.5 h. The enolate solution was cooled to −78 °C and added by cannula to a cooled (−78 °C) flask containing diethyl squarate (1.50 mL, 10.13 mmol) in THF/diethyl ether (1:1 ratio, 20 mL). The reaction was stirred for 15 min at −78 °C and was then allowed to warm to rt over a 1 h. The reaction was concentrated to give a residue, which was partitioned between 0.1 N HCl and ethyl acetate. The organic phase was washed with brine, dried ($MgSO_4$), and concentrated to give crude product. Purification by flash column chromatography (2:1 hexanes/ethyl acetate) followed by trituration with petroleum ether afforded 1.18 g (39%) of 3-[2-oxo-1-(4-trifluoromethyl-phenyl)-propyl]-4-ethoxy-cyclobut-3-ene-1,2-dione as a light yellow solid: ^{1}H NMR (DMSO-d_6): δ 11.51 (s, 1H), 7.60 (ABq, 2H), 7.24 (ABq, 2H), 4.59 (q, 2H), 1.89 (s, 3H), 1.19 (t, 3H). This intermediate (0.350 g, 1.07 mmol) and 2-amino-3,3-dimethylbutane (0.13 mL, 0.998 mmol) were stirred together in ethanol (6 mL) at rt overnight. Diethyl ether (25 mL) was added and the precipitated product was collected by filtration. It was stirred in diethyl ether/petroleum ether overnight, filtered, and dried in vacuo to afford 0.15 g (37%) of 3-[2-oxo-1-(4-trifluoromethyl-phenyl)-propyl]-4-(1,1,1-trimethyl-propylamino)-cyclobut-3-ene-1,2-dione (**12.2**) as an off-white solid (^{1}H NMR in $CDCl_3$ suggested the presence of both the keto and enol forms in about an 8:1 ratio): mp 178.2–179.8 °C; ^{1}H NMR ($CDCl_3$): δ 11.74 (s, 1H), 7.73 (ABq, 2H), 7.39 (ABq, 2H), 3.92 (m, 1H), 1.86 (s, 3H), 0.93 (d, 3H), 0.69 (s, 9H); MS (*m*/*z*) 381 [M$^+$]. Anal. Calcd for $C_{20}H_{22}NO_3F_3$: C, 62.98; H, 5.81; N, 3.67. Found: C, 62.67; H, 5.72; N, 3.56. Compound **16.2**: To a heterogeneous mixture of $CrCl_2$ (3.98 g, 32.4 mmol) in THF (40 mL) at 0 °C was added via syringe pump over 1 h (in the total absence of light) a solution of 4-bromobenzaldehyde (1.00 g, 5.40 mmol) and CHI_3 (4.25 g, 10.8 mmol) in THF (20 mL). The cold bath was removed, and the mixture stirred at rt for 1 h. The reaction mixture was diluted with 1:1 ether–hexanes (200 mL), then filtered through a short pad of SiO_2, and concentrated to give crude (*E*)-1-iodo-2-(4-bromophenyl)ethylene. The compound (5.4 mmol) was used as is and treated with CuI (0.103 g, 0.54 mmol), benzylchlorobis-(triphenylphosphine)palladium(II) (0.307 g, 0.42 mmol), and 3-isopropoxy-4-(tri-*n*-butylstannyl)-3-cyclobutene-1,2-dione (3.11 mL, 8.27 mmol) in DMF (18 mL) at rt (in the dark). An aqueous workup and filtration through a plug of silica gel afforded 0.298 g (17%) of 4-isopropoxy-3-[(*E*)-2-(4-bromophenyl)-vinyl]-cyclobut-3-ene-1,2-dione as a dark oil, which was used directly in the next step. To this intermediate (0.288 g, 0.898 mmol) in ethanol (3.5 mL) was added 2-amino-3,3-dimethylbutane (241 μL, 1.80 mmol). The mixture was stirred at rt overnight, diluted with methanol and filtered. The solid was washed with cold methanol, and dried in vacuo at 60 °C to afford 3-[(*E*)-2-(4-bromo-phenyl)-vinyl]-4-(1,2,2-trimethyl-propylamino)-cyclobut-3-ene-1,2-dione (127 mg, 39%) (**16.2**) as a hygroscopic yellow solid (^{1}H NMR in DMSO-d_6 suggested the presence of amide rotamers in a ratio of approximately 4:1): mp 192.3–192.9 °C; ^{1}H NMR (DMSO-d_6): δ 8.87 (d, 1H), 7.71 (m, 5H), 7.46 (d, 1H), 4.02 (dq, 1H), 1.19 (d, 3H), 0.90 (s, 9H); IR (KBr) 3310, 2970, 1760, 1705, 1575, 1480, 1430, 1300, 1220, 1160, 1070, 1005, 975, 875, 820, 800 cm^{-1}; MS (*m*/*z*) 361/363 [M$^+$]. Anal. Calcd for $C_{18}H_{20}BrNO_2$: C, 59.68; H, 5.56; N, 3.87. Found: C, 59.55; H, 5.38; N, 3.72.

13. Liebeskind, L. S.; Fengl, R. W. *J. Org. Chem.* **1990**, *55*, 5359.
14. Goure, W. F.; Wright, M. E.; Davis, P. D.; Labadie, S. S.; Stille, J. K. *J. Am. Chem. Soc.* **1984**, *106*, 6417.
15. Takai, K.; Nitta, K.; Utimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 7408.
16. Saari, W. S.; Williams, J.; Britcher, S. F.; Wolf, D. E.; Kuehl, F. A. *J. Med. Chem.* **1967**, *10*, 1008.
17. Protocol for determination of in vitro relaxant properties in bladder and aortic tissue reported in: Butera, J. A.; Antane, S. A.; Hirth, B. H.; Lennox, J. R.; Sheldon, J. H.; Norton, N. W.; Warga, D.; Argentieri, T. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2093.
18. Brief cell EP protocol: Human bronchial smooth muscle cells were acquired from Clonetics (Walkersville, MD) and

cultured in house. Cells were removed from the culture dishes by brief exposure to trypsin and then placed directly into the tissue bath for study. Cells were studied using standard voltage clamp technique[19] in 32 °C physiological salt solution (PSS) that contained (mM): NaCl (118.4), KCl (5.0), $CaCl_2$ (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 pipette solution contained (mM): KCl (126.0), $MgCl_2{\cdot}6H_2O$ (4.5), ATP Mg salt (4.0), GTP tris salt (0.3), creatine PO_4 (14.0), D-glucose (9.0), EGTA (9.0), HEPES (9.0). The pH was adjusted to 7.4 with KOH. Electrodes had tip resistances of 2–4 MΩ. Whole cell recordings were made using an Axon Instruments 200A patch clamp amplifier (3 kHz high frequency cut-off) and pClamp software (Axon Instruments, Foster City, CA). Currents were evoked from a holding potential of −50 mV and then ramping the membrane voltage from −100 to +80 mV at a rate of 3.4 mV/s. Stable baseline recordings were made for a minimum of 5 min after which time drug was added to the superperfusate. Currents were again given a minimum of 5 min to stabilize before measurements were made. After drug administration, iberiotoxin was also added to the superperfusate to reverse any effects on BK_{Ca} current.[20] Data were analyzed using a 586-based personal computer and pClamp (Axon Instruments, Foster City, CA) software. Measured currents were normalized to peak control current and comparisons were made between control and treatment for each cell. Data are represented as mean ± SEM for each treatment group and analyzed for statistical significance utilizing the paired Student's *t*-test at the $p \leqslant 0.05$ level of significance.

19. Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. *Pflug. Arch.—Eur. J. Physiol.* **1981**, *391*, 85.
20. Galves, A.; Gimenez-Gallego, G.; Reuben, J. P.; Roy-Contancin, L.; Feigenbaum, P.; Kaczorowski, G. J.; Garcia, M. L. *J. Biol. Chem.* **1990**, *265*, 11083.