Design and SAR of Novel Potassium Channel Openers Targeted for Urge Urinary Incontinence. 2. Selective and Potent Benzylamino Cyclobutenediones

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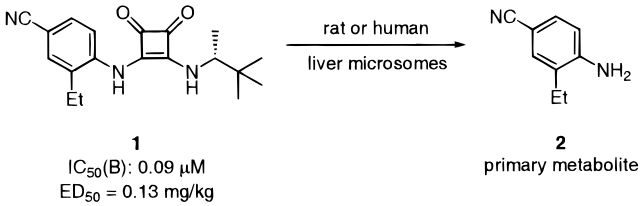
A novel series of benzylamine, potassium channel openers (KCOs) is presented as part of our program toward designing new, bladder-selective compounds for the treatment of urge urinary incontinence (UUI). We have found that the in vitro potency of (*R*)-4-[3,4-dioxo-2-(1,2,2-trimethyl-propylamino)-cyclobut-1-enylamino]-3-ethyl-benzonitrile **1** in the relaxation of pre-contracted rat detrusor strips can also be obtained with cyanobenzylamine derivative **4** (IC_{50} = 0.29 μM) (Figure 3). Addition of a 2-Cl substituted benzylamine moiety and changing the alkylamino substituent of **4** to a *t*-Bu amine gives **31** (IC_{50} = 0.14 μM)—a compound with similar in vitro potency as **4** as well as relaxant activity on bladder smooth muscle in vivo when administered orally (**31**, ED_{50} = 3 mg/kg) in a rodent model of bladder instability. Further modifications, particularly the replacement of the *t*-Bu amino substituent with a *tert*-amylamine, gave a similarly active compound **60** (IC_{50} = 0.10 μM) which shows excellent in vivo efficacy (ED_{50} = 0.6 mg/kg). Moreover, **60**, 3-(2,4-dichloro-6-methyl-benzylamino)-4-(1,1-dimethyl-propylamino)-cyclobut-3-ene-1,2-dione (WAY-151616), shows excellent tissue selectivity for bladder K channels over arterial tissue (**60**, MAP ED_{20} = 100 mg/kg; selectivity: MAP ED_{20}/bladder ED_{50} = 166). Other manipulations of the benzylamino cyclobutenediones, acylation of the benzylamine, conversion of the benzylamine substituent to a benzamide, homologation of the benzylamine to a phenethylamine, and incorporation of a methyl group at the benzyl carbon, all led to substantial loss of in vitro activity, although some in vivo activity was maintained in the acylated analogues. Compound **60** represents an attractive candidate for development in the treatment of UUI.

Introduction

The previous paper in this issue from our group describes (*R*)-4-[3,4-dioxo-2-(1,2,2-trimethyl-propylamino)-cyclobut-1-enylamino]-3-ethyl-benzonitrile **1**, a potent and orally active K_{ATP} channel opener (KCO) that effectively relaxes isolated rat detrusor strips in vitro (IC_{50} = 0.09 μM), is effective in a rat hypertrophied model of bladder instability (ED_{50} = 0.13 mg/kg) without significant effects on mean arterial blood pressure (MAP) and heart rate, and represents an attractive developmental candidate for the treatment of urge urinary incontinence (UUI).[1,2]

Metabolism studies of **1** using preparations of rat or human liver microsomes produces phenylamine **2** as the primary metabolite. At 10 μM concentrations of substrate and 1 mg/mL concentrations of microsomes, the apparent rate of metabolism (from 0 to 10 min) of **1** was 0.52 nmol/min/mg and 0.64 nmol/min/mg in rat and human liver microsomes, respectively.

Although **1** and primary metabolite **2** were found to be Ames negative and clean in Nova Screen receptor binding profile, concern over long-term patient exposure to **2** prompted us to search for biological leads with different metabolic profiles. Various manipulations were performed on **1**: (i) introduction of bulky flanking groups on the phenyl ring of **1** failed to block or slow metabolic cleavage,[3] (ii) modification of the electronics of the phenyl ring (primarily by changing the *p*-substituent) resulted in the loss of in vitro activity,[1] (iii) acylation of the phenylamino nitrogen in **1** failed to block production of **2** (compounds deacylate and then release **2** upon exposure to rat liver microsomes),[3] and (iv) a large number of cyclobutenedione mimetics were synthesized (heterocyclic, carbocyclic and acyclic templates); however, no suitable replacement could be found which possessed the potency of the cyclobutenedione molecules in vitro nor their in vivo efficacy (Figure 2).[4]



1
IC_{50}(B): 0.09 μM
ED_{50} = 0.13 mg/kg

2
primary metabolite

Figure 1. Rat liver microsome metabolism of **1** produces aniline **2** as the major metabolite.

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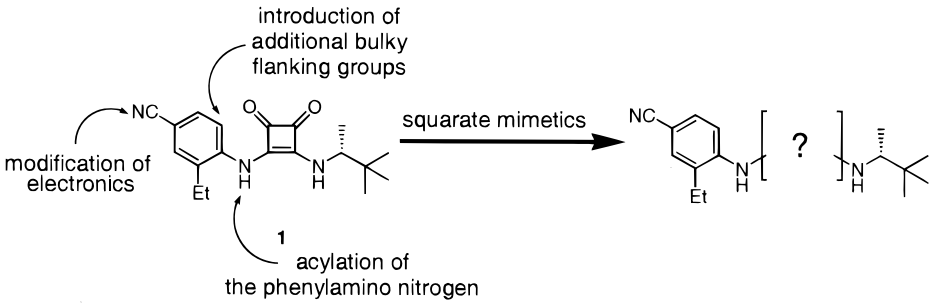


Figure 2. Attempts to block metabolism of cyclobutenedione **1** to prevent liberation of phenylamine **2**.

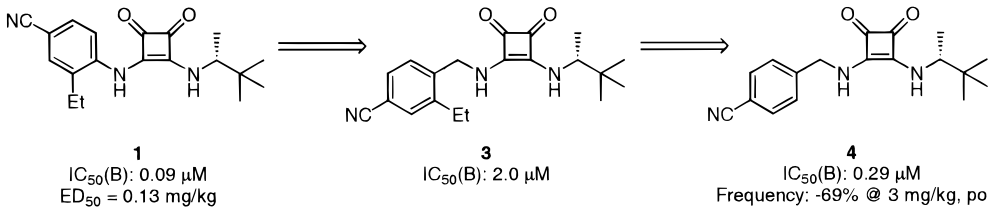


Figure 3. Progression from aryl cyclobutenedione **1** to benzylamino cyclobutenedione **4**.

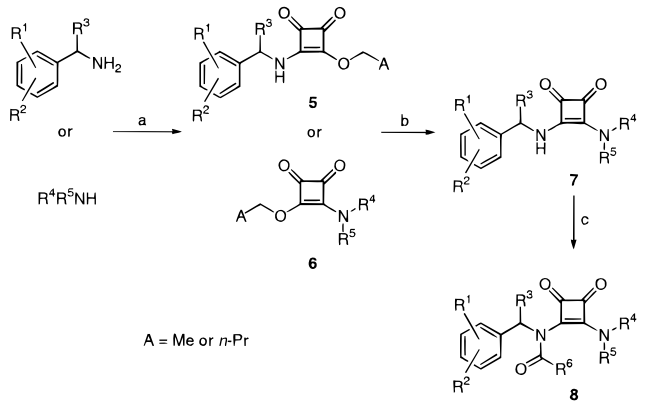
A potentially more promising idea would be to homologate the phenylamine moiety in **1** to a benzylamine—thereby eliminating the possible concern of long-term exposure to a metabolically liberated aniline. Direct homologation of aniline **1** to benzylamine **3** results in a substantial loss of in vitro potency. Removal of the *o*-Et group produces **4**, a compound with a similar in vitro potency to **3** but much weaker in vivo efficacy (Figure 3). Structure−activity relationship efforts were undertaken toward improving the in vivo profile of benzylamino analogue **4** with the view of maintaining the potency and selectivity of **1**.

In this article, we report our study of a series of novel benzylamino cyclobutenedione derivatives. Extensive manipulation of the substituents on both the benzylamino and alkyl amino groups produces selective benzylamino cyclobutenedione bladder agonists of the K_{ATP} channel which upon oral administration in rats causes significant bladder effects and minimal effects on MAP that are similar to biological lead **1**.

Chemistry

Benzylamino cyclobutenediones were synthesized using the procedure shown in Scheme 1. The reaction of a benzylamine ($(R^1R^2)PhCH(R^3)NH_2$) or an alkylamine (R^4R^5NH) with 1 to 1.2 equiv of 3,4-diethoxy-3-cyclobutene-1,2-dione or 3,4-di-*n*-butoxy-3-cyclobutene-1,2-dione in THF or EtOH at 23 °C gives monosubstituted benzylamino cyclobutenediones **5** or monosubstituted alkylamine cyclobutenediones **6**. Performing these reactions in refluxing THF or EtOH gives symmetrically disubstituted amino cyclobutenediones in addition to the desired monosubstituted compounds. Reacting **5** or **6** with the other amine component at 25 °C or in refluxing THF or EtOH gives diamino cyclobutenediones **7**. In contrast to the synthesis of the phenylamino cyclobutenediones, the order of addition of the amines to the 3,4-dialkoxy-3-cyclobutene-1,2-diones is not important. Both monosubstituted cyclobutenediones **5** and **6** are sufficiently reactive to combine with benzylamines and alkylamines to produce **7**. Selective acylation of the benzylamine component can be accomplished by first deprotonating **7** with NaH in DMF and then reacting the resulting anion with an acid anhydride to give **8**.

Scheme 1[a]



[a] (a) 3,4-Diethoxy-3-cyclobutene-1,2-dione or 3,4-di-*n*-butoxy-3-cyclobutene-1,2-dione, THF or EtOH, 23 °C; (b) R^4R^5NH or $(R^1R^2)PhCH(R^3)NH_2$, THF or EtOH, 23, 66, or 78 °C; (c) (1) NaH, THF/DMF, 23 °C, (2) $(R^6CO)_2O$, 23 °C.

Pharmacology

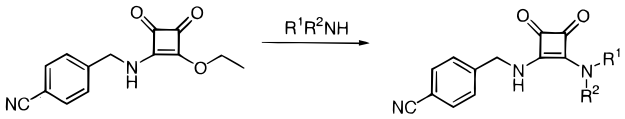
Compounds were first evaluated in vitro for their ability to relax KCl precontracted rat detrusor muscle strips. Following stabilization, increasing concentrations of test compounds were superfused and isometric force was measured. Glyburide, a specific blocker of the K_{ATP} channel, was added at the end of each experiment to look for the recovery of contractile activity.

Selected compounds were evaluated for in vivo efficacy in rat hypertrophied model of bladder instability previously described by Malmgren and co-workers.[5,6] In vivo active compounds were selected for hemodynamic assessment in a separate group of animals. Effect on mean arterial pressure (MAP) was recorded after oral administration of drug.

Results/Discussion

All of the benzylamino cyclobutenediones below are presented as K_{ATP} channel openers. The bladder relaxant activity for all of these compounds is reversed by glyburide (specific data not presented). In addition, the

Table 1. In Vitro Effects of Alkylamine Variation in **4** on Precontracted Rat Bladder Smooth Muscle Strips (IC_{50}, μM)[a]



cmpd	R^1	R^2	% yield	mp (°C)	formula[b]	anal.[c]	IC_{50} (μM)[a]	n[d]
4	(*R*)−3,3-dimethyl-2-butyl	H	93	288−291 (dec)	$C_{18}H_{21}N_3O_2$	C, H, N	0.29 ± 0.04	2
9	H	H	64	>260	$C_{12}H_9N_3O_2$	C, H, N[e]	>30	3
10	Me	H	89	302−306 (dec)	$C_{13}H_{11}N_3O_2$	C, H, N[f]	>30	4
11	Me	Me	68	244−246	$C_{14}H_{13}N_3O_2$	C, H, N	>30	2
12	*n*-Pr	H	68	241−245	$C_{15}H_{15}N_3O_2$	C, H, N	21.9 ± 7.2	3
13	*i*-Pr	H	86	276−278	$C_{15}H_{15}N_3O_2$	C, H, N[g]	14.0 ± 4.7	7
14	*n*-Bu	H	79	250−252	$C_{16}H_{17}N_3O_2$	C, H, N	>30	4
15	*t*-Bu	H	95	283−287 (dec)	$C_{16}H_{17}N_3O_2$	C, H, N	0.27 ± 0.04	4
16[h]	*t*-Bu	Me	58	224−226 (dec)	$C_{17}H_{19}N_3O_2$	C, H, N[i]	18.3 ± 2.8	4
17	(*R*)−2-butyl	H	64	254−257	$C_{16}H_{17}N_3O_2$	C, H, N	4.0 ± 1.1	4
18	(*S*)−2-butyl	H	64	253−256	$C_{16}H_{17}N_3O_2$	C, H, N	2.2 ± 0.5	2
19	2-*i*-Bu	H	83	255−257	$C_{16}H_{17}N_3O_2$	C, H, N[j]	10.2 ± 2.2	3
20	3-pentyl	H	88	265−268 (dec)	$C_{17}H_{19}N_3O_2$	C, H, N	1.3 ± 0.2	2
21	*tert*-amyl	H	81	257−260	$C_{17}H_{19}N_3O_2$	C, H, N[k]	1.1 ± 0.4	3
22	2-hydroxy-1,1-dimethyl-ethyl	H	44	253−257	$C_{16}H_{17}N_3O_3$	C, H, N	21.3 ± 7.2	4
23	cyclopentyl	H	86	283−286 (dec)	$C_{17}H_{17}N_3O_2$	C, H, N[l]	9.4 ± 2.7	4
24	2,2,3,3,3-pentafluoropropyl	H	31	252−256	$C_{15}H_{10}F_5N_3O_2$	C, H, N	14.4	1
25[m]	4-$CNC_6H_4CH_2$	H	4	>260	$C_{20}H_{14}N_4O_2$	C, H, N[n]	>30	4

[a] IC_{50}: drug concentration that relaxed KCl-induced contractions in rat detrusor strips by 50%. The bladder relaxant activity could be reversed by the addition of glyburide. [b] Structures of compounds confirmed by ^{1}H NMR, IR, and MS. [c] Analytical results are within ±0.4% of the theoretical value unless otherwise noted. [d] Number of experiments. [e] C: calcd, 63.43; found, 62.93. [f] C: calcd, 64.72; found, 64.19. [g] C: calcd, 66.90; found, 66.24. [h] Compound **16** was synthesized from 3-methoxy-4-(*N*-*tert*-butyl-*N*-methylamino)-cyclobut-3-ene-1,2-dione and 4-cyanobenzylamine. [i] C: calcd, 68.67; N: 14.13; found, C: 67.17; N: 13.69. [j] C: calcd, 67.83; found, 67.32. [k] C: calcd, 68.67; found, 68.10. [l] C: calcd, 69.14; found, 68.61. [m] Compound **25** was isolated as a minor impurity in the synthesis of 3-ethoxy-4-(4-cyano-benzylamino)-cyclobut-3-ene-1,2-dione. [n] C: calcd, 70.17; found, 69.57.

closely related phenylamino analogues such as **1** have been established as K_{ATP} channel openers by cellular electrophysilogy.[1,2]

Although potent in the KCl-contracted rat detrusor muscle strip assay, compound **4** lacked comparable in vivo efficacy compared to **1**. Initial SAR investigations focused on manipulation of the alkylamine portion of **4** (Table 1). Primary amino (**9**), monomethylamino (**10**), and dimethylamino (**11**) analogues have extremely weak in vitro activity. Short-chained alkyl substituents (*n*-Pr, *i*-Pr, and *n*-Bu) in **12**−**14** also show weak inhibitory activity although some modest activity is observed with branched *i*-Pr compound **13**. The *t*-Bu compound **15** (with a highly branched alkyl group) shows activity (IC_{50} = 0.27 μM) which is comparable to **4**. Methylation of the *t*-Bu amine in **15** gives **16**, a compound with reduced activity. A similar trend was observed for the phenylamino compounds.[1]

Both the (*R*) **17** and (*S*) **18** enantiomers of the 2-butyl alkyl compounds showed similar activity in the rat detrusor muscle strip assay. A compound that lacks the α branching, the 2-*i*-Bu compound **19**, shows comparatively weaker inhibition, thus following similar trends seen in the phenyl series.[1] The 3-pentyl and 1,2-dimethyl-propyl compounds **20** and **21** have inhibitory activity that is comparable to **17** and **18**, but they are not as potent as **4** or **15**. That the area of space probed by the alkyl amino substituent must be hydrophobic is suggested by the reduced activity of **22**, the hydroxylated analogue of **15**. Cycloalkyl groups appear to be poor choices as **23** is only moderately active. A pentafluoro-*n*-propyl analogue **24** is slightly more potent than the corresponding *n*-propyl analogue **12** but not as potent as the more highly branched alkyls. Finally a *p*-CN benzylamino substituent was incorporated, but the resulting cyclobutenedione **25** had an IC_{50} > 30 μM.

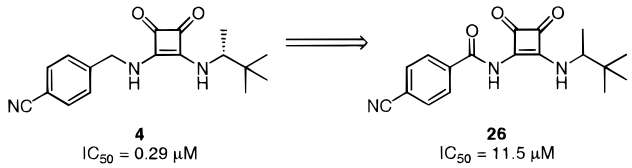


Figure 4. Comparison of benzylamino cyclobutenedione **4** with its corresponding vinylogous imide **26**.

Overall, highly branched alkyl chains were optimal ((*R*)-3,3-dimethyl-2-butyl **4** and *t*-Bu **15**) with good activity seen in compounds with branching in the carbon α to the amine (**4**, **15**, **17**, **18**, **20**, and **21**). In addition, the nitrogen bearing the alkyl group must have a hydrogen as shown by the N(H)*t*-Bu compound **15** (IC_{50} = 0.27 μM) which is almost 68 times more potent than its N(Me)*t*-Bu analogue **16** (IC_{50} = 18.3 μM).

Structurally modifying the benzylamine moiety of **4** to the corresponding racemic benzamide **26** resulted in a loss of activity (**26**, IC_{50} = 11.5 μM) (Figure 4). It is not clear whether this loss of activity is due to a conformational change and/or modulation in pK_a of the benzylamine proton. It is believed that this effect is not due to **4** containing the enantiomerically pure (*R*)-3,3-dimethyl-2-butylamino moiety while **26** contains the racemic 3,3-dimethyl-2-butylamino group.[7]

Attempts were also made to improve activity by homologating the benzylamine substituent to a phenethylamino amino group. However, this transformation also led to a marked loss in activity. Potent (4-CN) benzylamine **4** loses most of its in vitro activity when homologated to the phenethylamino (4-CN) analogue **27** (IC_{50} > 30 μM). Similarly the (4-CN, *t*-Bu) derivative **15** also undergoes a loss of in vitro activity when homologated to **28** (IC_{50} > 30 μM) (Figure 5).

The effect of adding a methyl group to the benzylic carbon was also studied. The (4-CNPhC(Me)H) cy-

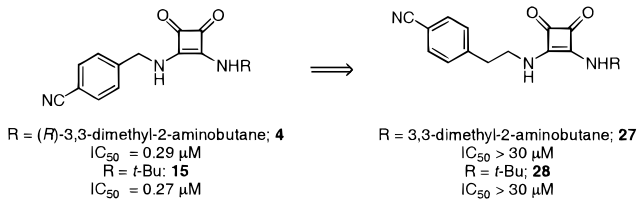


Figure 5. Comparison of benzylamino cyclobutenediones **4** and **15** with their homobenzylic analogues **27** and **28**.

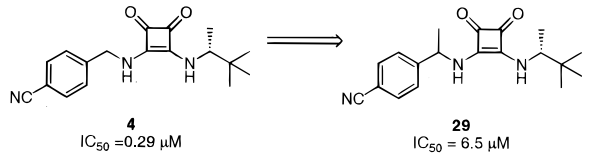


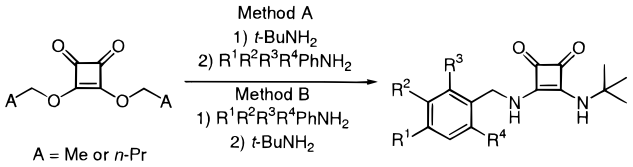
Figure 6. Comparison of benzylamino cyclobutenedione **4** with its α-methyl benzyl analogue **29**.

clobutenedione **29** was prepared, and a noticeable loss in activity is seen compared to benzylamine **4** (**29**, IC_{50} = 6.5 μM; **4**, IC_{50} = 0.29 μM) (Figure 6).

Compound **15** was next used for an SAR optimization study where substituents on the benzylamine were varied and the *t*-Bu amino group was held constant (Table 2). The electron-withdrawing 4-CN group in **15** is important for the molecule's potency as seen in the unsubstituted **30**—although this molecule still possesses inhibitory activity. Introduction of a 2-Cl substituent in **15** produces **31** (IC_{50} = 0.14 μM), a compound with similar activity to **15** although the SEM value of this compound is large. Changing the 2-Cl group in **31** to a Et group (**32**) also gives a compound with good potency. One should note that this 4-CN, 2-Et substitution pattern is identical to the phenyl substitution pattern in **1**.[1] The 2,4 relationship of the Cl and Et substitution in **31** and **32** appears to be optimal; movement of the Cl and Et groups from the 2-position to the 3-position (**33** and **34**) results in a loss of activity.

It is possible to substitute a pyridine ring for the phenyl group as shown for 4-pyridyl analogue **35**, although a 10-fold loss in activity is seen compared to **15**. The 3-pyridyl compound **36** is equipotent to **35** although the 2-pyridyl **37** is less potent. It appears that, for the *t*-Bu benzylamino cyclobutenediones, the pyridyl ring does not give an increase in activity as shown for the phenylamino compounds;[1] **35** and **36** have comparable potency to the unsubstituted benzyl compound **30**. The 4-OMe analogue **38** also has similar potency to **30**, but the 4-F analogue **39** appears to be slightly better. Ortho substituted analogues of **39**, (2,4-F_2) **40**, and (2-Cl, 4-F) **41** fail to give increases in activity. The 2-Cl moiety in **41** fails to give a similar increase in activity as seen going from the 4-CN compound **15** to the (2-Cl, 4-CN) derivative. Movement of the F to the 3-position (**42**) or the 2-position (**43**) gives compounds of similar potency. The (2,5-F_2) analogue **43** has similar activity compared to the (2,4-F_2) analogue **40** while the (2,6-F_2) compound **45** shows a slightly higher IC_{50} value.

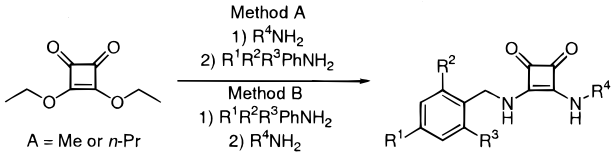
Table 2. In Vitro Effects of Alkylamine Variation in **15** on Precontracted Rat Bladder Smooth Muscle Strips (IC_{50}, μM)[a]



cmpd	R^1	R^2	R^3	R^4	% yield step 1 (method[b])	% yield step 2	mp (°C)	formula[c]	anal.[d]	IC_{50} (μM)[a]	n[e]
15	CN	H	H	H	95 (B)	95	283−287 (dec)	$C_{16}H_{17}N_3O_2$	C, H, N	0.27 ± 0.04	4
30	H	H	H	H	87 (A)	84	306−307 (dec)	$C_{15}H_{18}N_2O_2$	C, H, N	3.4 ± 0.6	6
31	CN	H	Cl	H	87 (A)	50	243−245	$C_{16}H_{16}ClN_3O_2$	C, H, N	0.14 ± 0.34	4
32	CN	H	Et	H	87 (B)	37	224−228	$C_{18}H_{21}N_3O_2$	C, H, N	0.24 ± 0.03	4
33	CN	Cl	H	H	81 (B)	30	276−278 (dec)	$C_{16}H_{16}ClN_3O_2$	C, H, N	1.1 ± 0.1	4
34	CN	Et	H	H	45 (B)	52	233−235	$C_{18}H_{21}N_3O_2$	C, H, N[f]	6.1 ± 3.8	4
35			4-pyridyl		87 (A)	76	271 (dec)	$C_{14}H_{17}N_3O_2$	C, H, N	3.0 ± 0.2	6
36			3-pyridyl		87 (A)	66	296 (dec)	$C_{14}H_{17}N_3O_2$	C, H, N	2.6 ± 0.2	4
37			2-pyridyl		87 (A)	94	236 (dec)	$C_{14}H_{17}N_3O_2$	C, H, N	11.5 ± 4.3	4
38	OMe	H	H	H	87 (A)	74	278−279 (dec)	$C_{16}H_{20}N_2O_3$	C, H, N	4.8 ± 2.1	4
39	F	H	H	H	87 (A)	86	296−298 (dec)	$C_{15}H_{17}FN_2O_2$	C, H, N	1.5 ± 0.3	4
40	F	H	F	H	87 (A)	18	227−228 (dec)	$C_{15}H_{16}F_2N_2O_2$	C, H, N	2.1 ± 0.9	4
41	F	H	Cl	H	87 (A)	59	292−294	$C_{15}H_{16}ClFN_2O_2$	C, H, N	1.4 ± 0.4	4
42	H	F	H	H	87 (A)	75	295−297 (dec)	$C_{15}H_{17}FN_2O_2$	C, H, N	2.4 ± 0.4	3
43	H	F	H	F	87 (A)	68	273−274 (dec)	$C_{15}H_{16}F_2N_2O_2$	C, H, N	3.9 ± 1.1	4
44	H	H	F	H	87 (A)	73	260−262 (dec)	$C_{15}H_{17}FN_2O_2$	C, H, N	2.1 ± 0.3	4
45	H	H	F	F	87 (A)	56	269 (dec)	$C_{15}H_{16}F_2N_2O_2$	C, H, N	7.5 ± 4.5	4
46	Cl	H	H	H	87 (A)	69	308−309 (dec)	$C_{15}H_{17}ClN_2O_2$	C, H, N	1.7 ± 1.0	2
47	Cl	H	Cl	H	87 (A)	74	229−230 (dec)	$C_{15}H_{16}Cl_2N_2O_2$	C, H, N	1.3 ± 0.6	4
48	Cl	H	Cl	Me	87 (A)	74	246−250 (dec)	$C_{18}H_{23}BrN_2O_2$	C, H, N[g]	0.22 ± 0.08	2
49	H	H	Cl	Cl	87 (A)	38	265−266 (dec)	$C_{15}H_{16}N_2O_2$	C, H, N	1.1 ± 0.4	4
50	Br	H	Et	H	44 (B)	37	228−232	$C_{17}H_{21}BrN_2O_2$	C, H, N	0.27 ± 0.08	3
51	Br	H	Me	Me	87 (A)	62	267−271 (dec)	$C_{17}H_{21}BrN_2O_2$	C, H, N	0.29 ± 0.02	2
52	Me	H	Me	H	87 (A)	47	228−229	$C_{17}H_{22}N_2O_2$	C, H, N	0.63 ± 0.15	4

[a] IC_{50}: drug concentration that relaxed KCl-induced contractions in rat detrusor strips by 50%. The bladder relaxant activity could be reversed by the addition of glyburide. [b] Method A: (1) 3,4-alkoxy-3-cyclobutene-1,2-dione, *t*-$BuNH_2$; (2) $R^1R^2R^3R^4PhNH_2$, Method B: (1) 3,4-alkoxy-3-cyclobutene-1,2-dione, $R^1R^2R^3R^4PhNH_2$; (2) *t*-$BuNH_2$. [c] Structures of compounds confirmed by ^1H NMR, IR, and MS. [d] Analytical results are within ±0.4% of the theoretical value unless otherwise noted. [e] Number of experiments. [f] C: calcd, 69.43; found, 68.64. [g] C: calcd, 57.00; found, 56.55.

Table 3. In Vitro Effects of Alkylamine/Benzylamine Variation in **27** on Precontracted Rat Bladder Smooth Muscle Strips (IC_{50}, μM)[a]



cmpd	R^1	R^2	R^3	R^4	% yield step 1 (method[b])	% yield step 2	mp (°C)	formula[c]	anal.[d]	IC_{50} (μM)[a]	n[e]
31	CN	Cl	H	*t*-Bu	87 (A)	50	243−245	$C_{16}H_{16}ClN_3O_2$	C, H, N	0.14 ± 0.34	4
53	CN	Cl	H	(*R*)−3,3-dimethyl-2-butyl	95 (B)	52	298−300	$C_{18}H_{20}ClN_3O_2$	C, H, N	0.18 ± 0.33	4
54	Cl	Cl	H	(*R*)−3,3-dimethyl-2-butyl	95 (A)	29	235−239	$C_{17}H_{20}Cl_2N_2O_3$	C, H, N[f]	0.34 ± 0.23	3
55	CN	Cl	Me	(*R*)−3,3-dimethyl-2-butyl	10 (B)	67	>300	$C_{19}H_{22}ClN_3O_2$ 0.04 CH_2Cl_2	C, H, N	1.9 ± 1.1	4
56	Cl	Cl	Me	(*R*)−3,3-dimethyl-2-butyl	95 (A)	63	>300	$C_{18}H_{22}Cl_2N_2O_2$	C, H, N	9.8 ± 5.1	4
57	CN	Cl	H	*tert*-amyl	86 (A)	65	216−220	$C_{12}H_{18}ClN_3O_2$	C, H, N[g]	0.13 ± 0.03	6
58	CN	Cl	Me	*tert*-amyl	10 (B)	62	258−262 (dec)	$C_{18}H_{20}ClN_3O_2$	C, H, N	0.16 ± 0.01	2
59	Cl	Cl	H	*tert*-amyl	86 (A)	40	196−197	$C_{16}H_{18}Cl_2N_2O_2$	C, H, N	0.21 ± 0.04	4
60	Cl	Cl	Me	*tert*-amyl	86 (A)	92	247−248	$C_{17}H_{20}Cl_2N_2O_2$	C, H, N	0.10 ± 0.03	4
61	H	Cl	Me	*tert*-amyl	78 (B)	59	224−226	$C_{17}H_{21}ClN_2O_2$	C, H, N	4.4 ± 4.1	4
62	H	Cl	Cl	*tert*-amyl	86 (A)	44	239−241 (dec)	$C_{16}H_{18}Cl_2N_2O_2$	C, H, N	0.28 ± 0.03	3
63	CN	Et	H	*tert*-amyl	86 (A)	12	183−186	$C_{19}H_{23}N_3O_2$	C, H, N	0.11 ± 0.01	2

[a] IC_{50}: drug concentration that relaxed KCl-induced contractions in rat detrusor strips by 50%. The bladder relaxant activity could be reversed by the addition of glyburide. [b] Method A: (1) 3,4-alkoxy-3-cyclobutene-1,2-dione, R^4NH_2; (2) $R^1R^2R^3PhNH_2$, Method B: (1) 3,4-alkoxy-3-cyclobutene-1,2-dione, $R^1R^2R^3PhNH_2$; (2) R^4NH_2 [c] Structures of compounds confirmed by ^1H NMR, IR, and MS. [d] Analytical results are within ±0.4% of the theoretical value unless otherwise noted. [e] Number of experiments. [f] C: calcd, 57.48; found, 57.96. [g] C: calcd, 61.54; found, 60.81.

The 4-Cl compound **46** has a similar IC_{50} in the rat detrusor muscle strip assay as the 4-F derivative **39**. The addition of a 2-Cl substituent (**47**) fails to increase activity, but the corresponding (2,4-Cl_2, 6-Me) analogue **48** is noticeably more potent (IC_{50} = 0.22 μM). The (2,6-Cl_2) derivative **49** loses this extra potency—showing that the 4-substituent is important for submicromolar activity in this series. 4-Bromine substituents give potent compounds when combined with 2 or 2,6 substitution: (4-Br, 2-Et) analogue **50** (IC_{50} = 0.27 μM), (4-Br, 2,6-Me_2) analogue **51** (IC_{50} = 0.29 μM). Sub micromolar activity is also produced with a 2,4-methyl substitution pattern on the benzylamine **52** (IC_{50} = 0.63 μM).

Two of the *t*-Bu benzylamino cyclobutenediones were tested in the rat bladder instability model (Table 5). Rats were dosed orally, and the reduction in the frequency of spontaneous bladder contractions was measured. Compound **15** causes a 52% reduction in the frequency of bladder contractions at 3 mg/kg, results similar to that seen with the equipotent analogue **4** (**15**, ED_{50} = 3 mg/kg; **4** frequency, −69% reduction at 3 mg/kg). The (4-CN, 2-Cl) benzylamine cyclobutenedione **31** showed in vivo results similar to those of **4** and **15** (**31**, ED_{50} of 3.0 mg/kg).

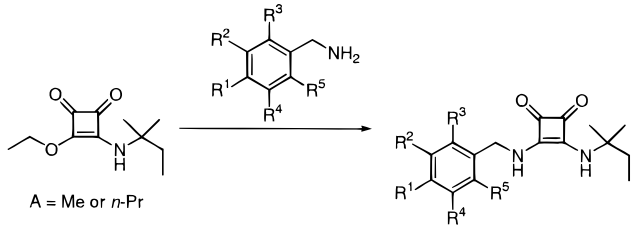
It was next decided to vary both the alkyl and the benzylamine moieties in compounds similar to **31** in hopes of increasing in vitro activity and in vivo efficacy. It is known from the SAR of compounds in Tables 1 and 2 that excellent in vitro potency can be obtained in benzylamine cyclobutenediones with 2,4 disubstituted or 2,4,6 trisubstituted benzylamines. In addition, the nature the alkylamine moiety has a large effect. This led to another phase of our SAR study with the view of combining previously studied (see above) benzylamines/alkylamines that would lead to increased potency.

As stated above, these new elaborations began with **31** (Table 3). Replacement of the *t*-Bu substituent with a (*R*)-3,3-dimethyl-2-butyl group gives equipotent analogue **53**. Changing the 4-CN group in **31** to a 4-Cl group produces the (2,4-Cl) derivative **54**, a compound with similar potency to **31**. Addition of a 6-Me group to **53** to give **55** causes a loss in activity. The (2,4-Cl_2, 6-Me) pattern (**56**) slightly reduces the activity of **55**. This is quite surprising since the addition of a 6-Me group to **47** in the *t*-Bu series (Table 2) gives a noticeable increase in activity.

Introduction of a *tert*-amylamine group within this subset of leads proved important as will be shown below. The *tert*-amyl analogue of **31** and **53** was prepared (**57**), and this gave an equipotent compound (IC_{50} = 0.13 μM). This result was surprising since in the 4-CN benzyl series (Table 1) the *tert*-amyl derivative **21** has reduced activity compared to the (*R*)-3,3-dimethyl-2-butyl **4** and *t*-Bu **15** analogues. In general the addition of ortho substituents to the para substituted benzyl ring in the *tert*-amyl series gives potent compounds as previously documented in the *t*-Bu series. The (2-Cl, 4-CN, 6-Me) analogue **58** (IC_{50} = 0.16 μM) as well as the (2,4-Cl) derivative **59** (IC_{50} = 0.21 μM) possess good relaxant activity. From the *t*-Bu series (Table 2), it was known that adding a 6-Me group to the (2,4-Cl) derivative increased potency; this was also confirmed for the *tert*-amyl series as the (2,4-Cl, 6-Me) analogue **60** (WAY-151616) has an IC_{50} as potent as any of the best benzylamino cyclobutenediones (**60**, IC_{50} = 0.10 μM). The importance of the 4-Cl substituent in **60** can be seen in the (2-Cl, 6-Me) analogue **61** which loses activity. However, changing the 6-Me group in **61** to a 6-Cl gives a potent compound (**62**, IC_{50} = 0.28 μM). Finally, (2-Et, 4-CN) derivative **63** is a potent compound—note again that the (2-Et, 4-CN) was the same substitution pattern used in the potent and efficacious phenylamino cyclobutenedione **1**.

Additional *tert*-amyl cyclobutenedione analogues are presented in Table 4. Overall, the 2,4 disubstitution and 2,4,6 trisubstitution patterns on the phenyl ring generally produces the most potent compounds. While these new *tert*-amyl compounds were very potent, no com-

Table 4. In Vitro Effects of Benzylamine Variation for *tert*-Amyl Cyclobutenediones on Precontracted Rat Bladder Smooth Muscle Strips (IC_{50}, μM)[a]



cmpd	R^1	R^2	R^3	R^4	R^5	% yield	mp (°C)	formula[b]	anal.[c]	IC_{50} (μM)[a]	n[d]
60	Cl	H	Cl	H	Me	92	247−248	$C_{17}H_{20}Cl_2N_2O_2$	C, H, N	0.10 ± 0.03	4
64	F	H	H	H	H	72	255−257	$C_{16}H_{19}FN_2O_2$	C, H, N	0.36 ± 0.14	4
65	F	H	F	H	H	80	242−244	$C_{16}H_{18}F_2N_2O_2$	C, H, N	0.22 ± 0.04	2
66	F	H	Cl	H	H	60	201−203	$C_{16}H_{18}ClFN_2O_2$	C, H, N	1.5 ± 1.0	3
67	F	F	H	H	H	62	273−275	$C_{16}H_{18}F_2N_2O_2$	C, H, N	1.6 ± 0.8	2
68	H	F	H	F	H	80	242−244	$C_{16}H_{18}F_2N_2O_2$	C, H, N	0.75 ± 0.45	2
69	F	Cl	H	H	H	65	242−244	$C_{16}H_{18}ClFN_2O_2$	C, H, N	0.87 ± 0.42	2
70	CN	Cl	H	H	H	32	267−271	$C_{17}H_{18}ClN_3O_2$	C, H, N	0.26 ± 0.02	2
71	Me	H	Me	H	H	45	177−178	$C_{18}H_{24}N_2O_2$	C, H, N	0.53 ± 0.23	3
72	Me	H	Me	H	Me	42	284−285 (dec)	$C_{19}H_{26}N_2O_2$	C, H, N	0.31 ± 0.08	3
73	Me	H	Me	H	Cl	26	265−271 (dec)	$C_{18}H_{23}ClN_2O_2$	C, H, N[e]	0.20 ± 0.08	2
74	H	H	Me	H	Cl	59	224−226	$C_{17}H_{21}ClN_2O_2$	C, H, N	4.4 ± 4.1	4
75	H	H	Me	H	Me	45	227−231	$C_{18}H_{24}N_2O_2$	C, H, N	0.83 ± 0.66	2
76	H	Me	H	Me	H	54	222−224	$C_{18}H_{24}N_2O_2$	C, H, N	>30	2
77	Me	Me	H	H	H	30	248	$C_{18}H_{24}N_2O_2$	C, H, N	>30	2
78	MeS	H	H	H	H	23	280−281 (dec)	$C_{17}H_{22}N_2O_2S$	C, H, N	5.6	1
79	Br	H	Me	H	Me	71	246−250 (dec)	$C_{18}H_{23}BrN_2O_2$	C, H, N[f]	1.2 ± 1.1	3

[a] IC_{50}: drug concentration that relaxed KCl-induced contractions in rat detrusor strips by 50%. The bladder relaxant activity could be reversed by the addition of glyburide. [b] Structures of compounds confirmed by ^{1}H NMR, IR, and MS. [c] Analytical results are within ±0.4% of the theoretical value unless otherwise noted. [d] Number of experiments. [e] C: calcd, 64.57; found, 63.81. [f] C: calcd, 57.00; found, 56.55.

Table 5. Comparison of in Vivo Effects of Benzylamine Cyclobutenediones **4**, **15**, **31**, and **60** on Frequency of Spontaneous Bladder Contractions in the Rat Hypertrophic Bladder Model (percent change from pre-drug value, X ± SE)

	spontaneous bladder contractions				blood pressure[a]		
cmpd	dose (mg/kg) po	n[b]	frequency (% change)[c]	ED_{50} (mg/kg)[d]	dose (mg/kg) po	n	maximum % change
4	3	5	−69 ± 16	NT	10	4	+7 ± 3
15	3	4	−52 ± 8	3.0	NT		
31	3	6	−49 ± 8	3.0	NT		
60	3	4	−82 ± 5	0.6	10	6	−7 ± 3

[a] Initial BP values ranged from 105 ± 2 to 116 ± 5 mmHg. [b] Number of experiments. [c] Vehicle (PEG-200) effects: −2 ± 11. [d] ED_{50}: drug dose (po) that caused a 50% reduction in the frequency of spontaneous bladder contractions in a rat hypertrophied model of bladder instability. NT = not tested.

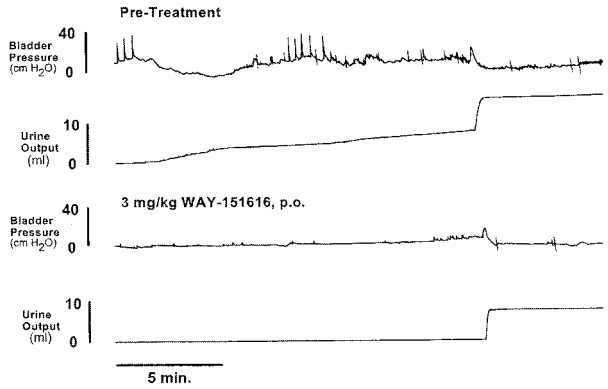


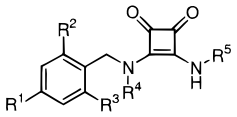
Figure 7. Rat hypertrophied bladder model: cystometry plot of **60** (WAY-151616). Spontaneous spikes during the bladder filling phase are observed, indicating the presence of bladder instability prior to the micturition contraction (upper panel). After treatment with 3 mg/kg (po) of **60**, the frequency of spontaneous bladder contractions is reduced and a normal micturition response is observed (lower panel).

pound from this series is more potent than compounds such as **31** and **60**.

Compound **60**, one of the most potent benzylamines in the in vitro rat detrusor muscle strip assay, was tested in the rat hypertrophied model of bladder instability (Table 5). Gratifyingly, **60** shows an excellent 82% reduction in the frequency of spontaneous bladder contractions at 3 mg/kg (po). A dose−response study demonstrates that **60** possesses a reduced ED_{50} versus compounds **15** and **31** (**60**, ED_{50} = 0.6 mg/kg). The effect of **60** on MAP in normotensive rats was also tested. At 10 mg/kg, compound **60** only decreased blood pressure 7%. A dose−response study with **60** shows that the ED_{20} for blood pressure lowering was greater than 100 mg/kg.

A rat hypertrophied bladder cystometry plot for one bladder filling/emptying cycle before and after treatment with **60** is presented in Figure 7. The pretreatment plot (upper panel) shows spontaneous spikes which develop during the bladder filling phase. These spontaneous contractions lead to concomitant urine leakage as demonstrated by increases in urine volume output. At the end of bladder filling (~10 min), a micturition contraction is observed, and the bladder empties. The lower panel shows the cystometry after oral administration of compound **60** at 3 mg/kg. The spontaneous bladder contractions observed during the filling phase have been significantly reduced. In addition, no urine leakage is observed as urine output remains at zero during the bladder filling phase. After bladder filling (~10 min), a normal micturition response is observed, and urine output increases. The onset of action for **60** at this dose is 1 h, and the duration of action is 3.7 h.

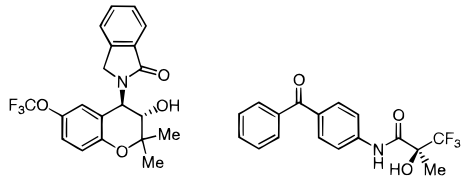
Table 6. Comparison of Benzylamine Cyclobutenediones **4**, **80**, **60**, and **81** on in Vitro Precontracted Rat Bladder Smooth Muscle Strips and the in Vivo Frequency of Spontaneous Bladder Contractions in the Rat Hypertrophic Bladder Model (in vitro data: IC_{50}, μM);[a] in vivo data: reported as ED_{50}[d])



cmpd	R^1	R^2	R^3	R^4	R^5	mp (°C)	formula[b]	anal.[c]	IC_{50} (μM)[a]	ED_{50} (mg/kg; po)[d]
4	CN	H	H	H	(*R*)−3,3-dimethyl-2-butyl	288−291 (dec)	$C_{18}H_{21}N_3O_2$	C, H, N	0.27 ± 0.04	*F*: −69% at 3 mg/kg, po[e]
80	CN	H	H	*n*-PrCO	(*R*)−3,3-dimethyl-2-butyl	101−103	$C_{22}H_{27}N_3O_3$	C, H, N	12.2 ± 10.2	8.0 ± 4.4
60	Cl	Cl	Me	H	*tert*-amyl	247−248	$C_{17}H_{20}Cl_2N_2O_2$	C, H, N	0.10 ± 0.10	0.6
81	Cl	Cl	Me	*n*-PrCO	*tert*-amyl	117−118	$C_{21}H_{26}Cl_2N_2O_3$	C, H, N	*C* = 245 ± 15%[f]	3.9 ± 1.4

[a] IC_{50}: Drug concentration that relaxed KCl-induced contractions in rat detrusor strips by 50%. The bladder relaxant activity could be reversed by the addition of glyburide. [b] Structures of compounds confirmed by ^1H NMR, IR, and MS. [c] Analytical results are within ±0.4% of the theoretical value unless otherwise noted. [d] ED_{50}: Drug dose (po) that caused a 50% reduction in the frequency of spontaneous bladder contractions in the rat hypertrophied model of bladder instability. Vehicle (PEG-200) effects: −2 ± 11. [e] F: decrease in frequency of bladder contractions in the rat hypertrophied model of bladder instability. A dose−response study was not performed on this compound to determine an ED_{50}. [f] *C* = 245 ± 15%: a 245% additional contraction of a KCl precontracted rat bladder strip at 30 μM.

Chart 1



82 Celikalim **83** ZD-6169

Thus **60** prevents the spontaneous contractions in the hypertrophied rat bladder during filling and prevents urine leakage while still allowing normal urination after bladder filling is complete.

In an attempt to further modify solubility and absorption properties, putative prodrugs of compounds **4** and **60** were prepared by deprotonating benzylamine nitrogen with NaH and trapping the resulting anion with *n*-PrCOCl (Scheme 1). Acylation of the aniline nitrogen in the previously described phenylamino cyclobutenediones was shown to have small effects on in vitro potency and in vivo efficacy,[3] but in the benzylamine series, acylation attenuates in vitro potency while the compounds maintain some in vivo activity (Table 6). The (4-CNbenzyl, (*R*)-3,3-dimethyl-2-butyl) compound **4** is moderately active in vivo (frequency, −69% at 3 mg/kg) despite its potent IC_{50} (IC_{50} = 0.27 μM). The corresponding *n*-butylamide **80** is 42 times less potent in vitro but maintains some in vivo efficacy when administered po (ED_{50} = 8 mg/kg). The (2,4-Cl, 6-Me, *tert*-amyl) analogue **60** has good in vitro (IC_{50} = 0.10 μM) and very good in vivo activity (ED_{50} = 0.6 mg/kg). The corresponding *n*-butylamide **81** is a bladder smooth muscle contractor in vitro[8] and also maintains some in vivo relaxant activity (ED_{50} = 3.9 mg/kg; po). These data suggest that *n*-butylamide analogues **80** and **81** may be acting as prodrugs, either by liberating parent benzylamines or a comparably active metabolite in vivo.

An assessment of the efficacy and bladder selectivity of novel KCOs **1** and **60** was performed and compared to celikalim **82**, an antihypertensive KCO, and to ZD-6169 **83**, an anilide tertiary carbinol KCO currently in Phase II clinical trials for the treatment of UUI (Chart 1). Celikalim **82** shows both bladder and hemodynamic effects in our models and could not be used selectively as a bladder relaxant agent (ratio MAP ED_{20}/bladder ED_{50} = 0.7) (Table 7).[2] ZD-6169 **83** exhibits weaker activity in vitro than **82** as well as being weaker in vivo as a bladder relaxant in our models. However, **83** shows selectivity in the rat hypertrophied model of bladder instability over MAP (ratio MAP ED_{20}/bladder ED_{50} = 2.9).[2] Diamino cyclobutenedione **1** shows excellent in vitro potency in the precontracted rat bladder strip model as well as potent in vivo bladder activity. Importantly, the hemodynamic effects of **1** are much less potent than its observed bladder effects (ratio MAP ED_{20}/bladder ED_{50} = 17.7).[2] Compound **60** shows even greater promise as a selective agent for UUI. While not as active in the rat hypertrophied bladder model as **1** (**60**, ED_{50} = 0.6 mg/kg; **1**, ED_{50} = 0.13 mg/kg), its ED_{20} for blood pressure lowering is 100 mg/kg. Thus **60** is 166-fold more selective for bladder effects versus hemodynamic effects and shows great promise as a selective agent.

Table 7. Comparison of in Vitro and in Vivo Effects of Celikalim **82**, ZD-6169 **83**, Compound **1**, and Compound **60**

	bladder effects		hemodynamic effects	selectivity
compd	in vitro IC_{50} (μM)[a]	in vivo ED_{50} (mg/kg)[b]	in vivo ED_{20} (mg/kg)[c]	ratio (MAP ED_{20}/ bladder ED_{50}
82[d]	0.03	0.3	0.2	0.7
83[d]	0.93	2.4	6.96	2.9
1[d]	0.09	0.13	2.3	17.7
60	0.10	0.6	100	166

[a] IC_{50}: drug concentration that relaxed KCl-induced contractions in rat detrusor strips by 50%. [b] ED_{50}: drug dose (po) that caused a 50% reduction in the frequency of spontaneous bladder contractions in the rat hypertrophied model of bladder instability. Vehicle (PEG-200) effects: −2 ± 11. [c] ED_{20}: drug dose (po) that caused a 20% drop in MAP in normotensive rats. Initial BP values ranged from 105 ± 2 to 116 ± 5 mmHg. [d] Reference 2.

Conclusions

We have shown that the novel phenylamino cyclobutendione KCO **1** can be successfully modified into a series of related benzylamino cyclobutenediones. Conversion of the anline portion of **1** to a benzylamine in conjunction with changing the alkylamine portion of **1** from a (*R*)-3,3-dimethyl-2-butyl moiety to a *t*-Bu group gives a series of compounds with potent in vitro potency in the KCl-contracted rat bladder strip assay. Ortho and

para substituents on the benzyl moiety improve activity. Electron-withdrawing groups (CN, F, Cl, Br) in the para position appear to be optimal, although in some cases activity is maintained with electron-donating groups. Ortho substituents appear to improve in vitro activity. Unfortunately, the *N*-benzyl-*N*′-*t*-Bu cyclobutenediones have weaker in vivo activity than their *N*-phenyl-*N*′-alkyl counterparts.

Replacement of the *t*-Bu group with a *tert*-amyl moiety increases the in vitro potency of the benzylamino cyclobutenediones—many of the *tert*-amyl compounds have in vitro IC_{50}s below 1.0 μM. As in the case of the *t*-Bu compounds, para substituents on the phenyl ring give optimal activity; ortho substituents can further improve activity. The potent *tert*-amyl compound **60** was tested in vivo and showed excellent efficacy (**60**, ED_{50} = 0.6 mg/kg, po). Most importantly, **60** represents a series of novel KCOs with in vivo selectivity for bladder relaxant effects over hemodynamic effects (ratio MAP ED_{20}/bladder ED_{50} = 166). Stability assessment studies using rat and human liver microsomes show that **60** remians intact—no benzylamine is produced as a metabolite. Compound **60** was also found to be clean in a NOVA Screen receptor binding profile and AMES negative at all concentrations tested (up to 5000 μg/plate). In addition, a 10 day pilot toxicological study in the rat at doses up to 200 mg/kg produced no drug related abnormalities. 3-(2,4-Dichloro-6-methyl-benzylamino)-4-(1,1-dimethyl-propylamino)-cyclobut-3-ene-1,2-dione **60** represents an attractive developmental candidate for the treatment of UUI.

Experimental Section

Melting points were determined on a Thomas-Hoover Meltemp apparatus and are uncorrected. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz on a Bruker DPX-300 spectrometer using tetramethylsilane (δ 0.0) as an internal standard. Infrared (IR) spectra were obtained as KBr pellets. Combustion analyses were obtained using a Perkin-Elmer Series II 2400 CHNS/O analyzer. Mass spectra were obtained using a Micromass Platform Electrospray Ionization Quadrapole mass spectrometer. Flash chromatography was performed using EM Science 230−400 mesh silica gel. Thin-layer chromatography (TLC) was performed on Analtech silica gel GHLF 250 μM prescored plates.

Structures of all tested compounds have been confirmed by ^{1}H NMR, IR, MS, and combustion analysis. Yields, melting points, and analytical results are tabulated in Tables 1−4 and 6. The syntheses and spectral data for representative compounds from these tables are shown below. The term "rotamers" which appears as a comment in some of the ^{1}H NMR data is defined as conformational isomers due to restricted rotation about the vinylogous amide bond.

(*R*)-3-Ethoxy-4-(1,2,2-trimethyl-propylamino)-cyclobut-3-ene-1,2-dione. Tetrahydrofuran (15 mL), 50 mL (10 mmol) of a 0.2 M solution of (*R*)-2-amino-3,3-dimethylbutane[9] in absolute EtOH, and 2.26 g (10 mmol) of 3,4-dibutoxy-3-cyclobutene-1,2-dione were stirred together for approximately 65 h at room temperature. The waxy solid remaining after removal of solvent was dissolved in chloroform (15 mL) and flash chromatographed on SiO_2 gel (hexanes/EtOAc). The appropriate fractions were freed of solvent to yield 2.41 g (9.53 mmol, a 95% yield) of a cream-colored waxy solid: mp 90−99 °C. Two recrystallizations of 1.1 g of this material from hexanes provided 833 mg of the title compound as a white solid: mp 90−93 °C; ^{1}H NMR (DMSO-d_6) δ 8.73 and 8.50 (two br d, 1H, rotamers), 4.64 (m, 2H), 3.92 and 3.41 (two m, 1H, rotamers), 1.71 (m, 2H), 1.38 (m, 2H), 1.11 (m, 3H), 0.91 (t, 3H), 0.84 (m, 9H); IR (KBr) 3135, 1800, 1690 cm^{-1}; MS 253 (M^+). Anal. $C_{14}H_{23}NO_3$: C, H, N.

4-{[3,4-Dioxo-2-(1,2,2-trimethyl-propylamino)-cyclobut-1-enylamino]-methyl}-3-ethyl-benzonitrile (3). 4-Cyano-2-ethylbenzaldehyde oxime[10] (2.9 g, 17 mmol) and 142 mL of 2 N HCl were stirred at room temperature. Acetone (100 mL) was added to produce a homogeneous solution. After 5 days the reaction mixture was diluted with 500 mL of EtOAc, and solid NaCl was added until the aqueous layer was saturated. After the mixture was stirred at room temperature for 1 h, the EtOAc layer was separated and dried over Na_2SO_4. Concentration under reduced pressure yielded 7.0 g of a solid which was chromatographed (SiO_2 gel) with hexanes and then hexanes:EtOAc (8:1) to give 2.0 g (12.6 mmol, a 75% yield) of 4-cyano-2-ethylbenzaldehyde, which was used without further purification.

4-Cyano-2-ethylbenzaldehyde (2.0 g, 13 mmol) in 63 mL of MeOH was cooled to 0 °C. Solid $NaBH_4$ (480 mg, 13 mmol) was added. The reaction mixture was stirred at 0 °C for 1 h. A second portion of $NaBH_4$ (0.48 g, 13 mmol) was added, and stirring was continued at 0 °C for 40 min. A third portion of $NaBH_4$ (0.48 g, 13 mmol) was added, and the reaction mixture was stirred at 0 °C for 20 min. The ice bath was then removed, and stirring was continued at room temperature for 1 h. Water (28 mL) was then added, and the reaction mixture was stirred at room temperature for 24 h. The solution was then diluted with EtOAc (100 mL) and divided into two portions, which were each extracted with EtOAc (300 mL) and brine (40 mL). The combined EtOAc extracts were concentrated under reduced pressure, and the resulting residue was chromatographed (SiO_2 gel) with hexanes:EtOAc (7:1) and then hexane:EtOAc (3:1) to yield 1.53 g (9.5 mmol, a 75% yield) of 4-cyano-2-ethylbenzyl alcohol a white solid: ^{1}H NMR (DMSO-d_6) δ 7.70−7.50 (m, 3H), 5.39 (br m, 1H), 4.59 (s, 2H), 2.60 (q, 2H), 1.18 (t, 3H).

4-Cyano-2-ethylbenzyl alcohol (950 mg, 5.9 mmol), 1.05 g (7.1 mmol) of phthalimide, 1.85 g (7.1 mmol) of Ph_3P, and 39 mL of THF were mixed and cooled to 0 °C in an ice bath. Diethylazodicarboxylate (1.09 mL, 6.9 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature as the ice bath melted. After 24 h the reaction mixture was concentrated under reduced pressure, and the resulting residue was chromatographed (SiO_2 gel) with hexanes/EtOAc (5/1), hexanes/EtOAc (3/1), and then hexanes/EtOAc (2/1) to yield 1.85 g (6.37 mmol, a 108% yield) of a white solid: ^{1}H NMR (DMSO-d_6) δ 8.00−7.80 (m, 4H), 7.70 (s, 1H), 7.59 (d, 1H), 7.30 (d, 1H), 4.86 (s, 2H), 2.81 (q, 2H), 1.22 (t, 3H).

N-(4-Cyano-2-ethylbenzyl)phthalimide (1.73 g, 6.0 mmol), 1.07 mL of 35% N_2H_4 (12 mmol), and 105 mL of absolute EtOH were heated at 65 °C under argon for 3 h and then at 85 °C for 5 h. The reaction mixture was concentrated under reduced pressure, resuspended in 35 mL of absolute EtOH, filtered, and rinsed with absolute EtOH (2 × 30 mL). Concentration under reduced pressure yielded a solid, which was suspended in 100 mL of EtOAc and filtered. The filtrate was concentrated under reduced pressure to give 770 mg (4.81 mmol, an 81% yield) of 4-cyano-2-ethylbenzylamine as a moist solid: ^{1}H NMR ($CDCl_3$) δ 7.50 (m, 3H), 3.94 (s, 2H), 2.70 (m, 2H), 1.43 (br m, 2H), 1.21 (t, 3H).

4-Cyano-2-ethylbenzylamine (0.21 g, 1.3 mmol) was placed in 5.5 mL of absolute EtOH. (*R*)-3-Ethoxy-4-(1,2,2-trimethyl-propylamino)-cyclobut-3-ene-1,2-dione (300 mg, 1.3 mmol) was added, followed by 5 mL of CH_2Cl_2. The clear solution was stirred at room temperature for 5 days. The reaction mixture was concentrated under reduced pressure, and the resulting solid was chromatographed (SiO_2 gel) with 1% MeOH in CH_2Cl_2, then 3% MeOH in CH_2Cl_2 to give 190 mg (0.56 mmol, a 43% yield) of the (*R*) isomer of the **3** as a light tan solid: mp 208−212 °C; $[\alpha]^{25}_D$ 13.4° (*c* 0.0086 g/mL, DMSO); ^{1}H NMR (DMSO-d_6) δ 7.71 (m, 2H), 7.60 (br m, 1H), 7.48 (br d, 1H), 7.27 (br d, 1H), 4.85 (m, 2H), 3.90 (m, 1H), 2.69 (q, 2H), 1.17 (t, 3H), 1.10 (d, 3H), 0.86 (s, 9H); IR (KBr) 3200, 2970, 2230, 1800, 1650 cm^{-1}; MS 339 (M^+). Anal. $C_{20}H_{25}N_3O_2$ Calcd: C, 70.77; H, 7.42; N, 12.38. Found: C, 70.05; H, 7.29; N, 12.13.

3-Ethoxy-4-(4-cyano-benzylamino)-cyclobut-3-ene-1,2-dione. To 1.2 g of 4-cyanobenzylamine (9.1 mmol) in 40 mL of absolute EtOH was added 1.6 g (9.4 mmol) of 3,4-diethoxy-3-cyclobutene-1,2-dione. The reaction mixture was stirred at room temperature for 5 days. The resulting white suspension was filtered and dried under vacuum at 0.4 mm and 65 °C to give 1.07 g (4.16 mmol, a 46% yield) of the title compound as a white solid: ^{1}H NMR (DMSO-d_6) δ 9.29 and 9.08 (two br m, 1H, rotamers), 7.83 (d, 2H), 7.49 (d, 2H), 4.80−4.50 (m, 4H), 1.36 and 1.28 (two t, 3H, rotamers); MS 257 (MH^+).

(*R*)-4-{[3,4-Dioxo-2-(1,2,2-trimethyl-propylamino)-cyclobut-1-enylamino]-methyl}-benzonitrile (4). 3-Ethoxy-4-(4-cyano-benzylamino)-cyclobut-3-ene-1,2-dione (400 mg, 1.56 mmol) in 15 mL of absolute EtOH and 11.7 mL of a 0.2 M solution of (*R*)-2-amino-3,3-dimethylbutane in absolute EtOH (2.3 mmol) were heated at reflux for 17 h. The reaction mixture was cooled to room temperature, and the resulting white suspension was filtered, rinsed with absolute EtOH (2 × 10 mL), and dried under vacuum (0.4 mm, 85 °C) to give 450 mg (1.45 mmol, a 93% yield) of **4** as a white solid: mp 288−291 °C (dec); $[\alpha]^{25}_D = +28.2°$ (9.7 mg/mL, DMSO); ^{1}H NMR (DMSO-d_6) δ 7.85 (d, 2H), 7.70 (br m, 1H), 7.52 (d, 2H), 7.29 (br d, 1H), 4.84 (m, 2H), 3.91 (br m, 1H), 1.10 (d, 3H), 0.85 (s, 9H); IR (KBr) 3200, 2960, 2250, 1800, 1650 cm^{-1}; MS 312 (MH^+). Anal. $C_{18}H_{21}N_3O_3$: C, H, N.

4-[(2-Amino-3,4-dioxo-cyclobut-1-enylamino)-methyl]-benzonitrile (9). 3-Ethoxy-4-(4-cyano-benzylamino)-cyclobut-3-ene-1,2-dione (250 mg, 0.98 mmol) and 4.8 mL of NH_3(g) saturated CH_3CN were mixed in 4.8 mL absolute ethanol at 23 °C. After standing for several hours, the resulting white precipitate was filtered, rinsed with EtOAc, and dried under vacuum to give 140 mg (0.62 mmol, a 64% yield) of **9** as a white solid: mp > 260 °C; ^{1}H NMR (DMSO-d_6) δ 8.00−7.20 (br s, 1H), 7.83 (d, 2H), 7.50 (d, 2H), one N−H proton not seen; IR (KBr) 3420, 3100, 1800, 1650, cm^{-1}; MS 227 (M^+). Anal. $C_{12}H_9N_3O_2$: C, H, N.

4-[(2-Methylamino-3,4-dioxo-cyclobut-1-enylamino)-methyl]-benzonitrile (10). 3-Ethoxy-4-(4-cyano-benzylamino)-cyclobut-3-ene-1,2-dione (250 mg, 0.98 mmol), 0.12 mL (0.98 mmol) of an 8 M solution of $MeNH_2$ in EtOH, and 19.5 mL of absolute ethanol were allowed to stand at room temperature for 3 days. The resulting white precipitate was filtered, rinsed with EtOAc, and dried under vacuum to give 210 mg (0.87 mmol, an 89% yield) of **10** as a white solid: mp 302−306 °C (dec); ^{1}H NMR (DMSO-d_6) δ 7.90 (br m, 1H), 7.84 (d, 2H), 7.50 (d, 2H), 7.30 (br m, 1H), 4.77 (d, 2H), 3.11 (br s, 3H); IR (KBr) 3180, 2980, 2250, 1800, 1650 cm^{-1}; MS 241 (M^+). Anal. $C_{13}H_{11}N_3O_2$ Calcd: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.19; H, 4.44; 17.10.

4-[(2,2-Dimethylamino-3,4-dioxo-cyclobut-1-enylamino)-methyl]-benzonitrile (11). 3-Ethoxy-4-(4-cyano-benzylamino)-cyclobut-3-ene-1,2-dione (250 mg, 0.98 mmol) was dissolved in 4.8 mL of CH_2Cl_2 and 4.8 mL of absolute EtOH to give a clear solution. *N*,*N*-Diisopropylethylamine (0.17 mL, 126 mg, 0.98 mmol) was added followed by 79.6 mg (0.98 mmol) of *N*,*N*-dimethylamine hydrochloride, and the resulting mixture was stirred at 23 °C. The resulting white precipitate was filtered, rinsed with EtOAc, and dried under vacuum to give 170 mg (0.67 mmol, a 68% yield) of **11** as a white solid: mp 244−246 °C; ^{1}H NMR (DMSO-d_6) δ 8.21 (t, J = 6.4 Hz, 1H), 7.82 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 4.82 (d, J = 6.4 Hz, 2H), 3.16 (s, 6H); IR (KBr) 3190, 2200, 1760, 1730 cm^{-1}; MS 255 (M^+). Anal. $C_{14}H_{13}N_3O_2$: C, H, N.

4-[(2-*n*-Propylamino-3,4-dioxo-cyclobut-1-enylamino)-methyl]-benzonitrile (12). The title compound was prepared according to the procedure of compound **10** using 250 mg (0.98 mmol) of 3-ethoxy-4-(4-cyano-benzylamino)-cyclobut-3-ene-1,2-dione and 0.08 mL (58 mg, 0.98 mmol) of *n*-$PrNH_2$: yield 180 mg (0.67 mmol, a 68% yield); mp 241−245 °C; ^{1}H NMR (DMSO-d_6) δ 7.84 (d, 2H), 7.80 (br m, 1H), 7.50 (d, 2H), 7.45 (br m, 1H), 4.78 (d, 2H), 3.44 (m, 2H), 1.50 (m, 2H), 0.86 (t, 3H); IR (KBr) 3170, 2980, 2250, 1800, 1660 cm^{-1}; MS 269 (M^+). Anal. $C_{15}H_{15}N_3O_2$: C, H, N.

4-[(2-*iso*-Propylamino-3,4-dioxo-cyclobut-1-enylamino)-methyl]-benzonitrile (13). The title compound was prepared according to the procedure of compound **10** using 1.0 g (3.9 mmol) of 3-ethoxy-4-(4-cyano-benzylamino)-cyclobut-3-ene-1,2-dione and 1.2 mL (828 mg, 14 mmol) of *i*-$PrNH_2$: yield 900 mg (3.35 mmol, an 86% yield); mp 276−278 °C; ^{1}H NMR (DMSO-d_6) δ 7.84 (d, 2H), 7.70 (br m, 1H), 7.51 (d, 2H), 7.40 (br m, 1H), 4.79 (d, 2H), 4.09 (br m, 1H), 1.18 (d, 6H); IR (KBr) 3150, 2980, 2250, 1800, 1660 cm^{-1}; MS 270 (MH^+). Anal. $C_{15}H_{15}N_3O_2$ Calcd: C, 66.90; H, 5.61, N, 15.60. Found: C, 66.24; H, 5.45, N, 15.39.

4-[(2-*n*-Butylamino-3,4-dioxo-cyclobut-1-enylamino)-methyl]-benzonitrile (14). The title compound was prepared according to the procedure of compound **10** using 250 mg (0.98 mmol) of 3-ethoxy-4-(4-cyano-benzylamino)-cyclobut-3-ene-1,2-dione and 0.096 mL (72 mg, 0.98 mmol) of *n*-$BuNH_2$: yield 220 mg (0.78 mmol, a 79% yield); mp 250−252 °C; ^{1}H NMR (DMSO-d_6) δ 7.84 (d, 2H), 7.80 (br m, 1H), 7.50 (d, 2H), 7.40 (br m, 1H), 4.78 (d, 2H), 3.49 (br m, 2H), 1.47 (m, 2H), 1.29 (m, 2H), 0.87 (t, 3H); IR (KBr) 3160, 2950, 2250, 1810, 1640 cm^{-1}; MS 283 (M^+). Anal. $C_{16}H_{17}N_3O_2$: C, H, N.

4-[(2-*tert*-Butylamino-3,4-dioxo-cyclobut-1-enylamino)-methyl]-benzonitrile (15). The title compound was prepared according to the procedure of compound **10** using 1.5 g (5.9 mmol) of 3-ethoxy-4-(4-cyano-benzylamino)-cyclobut-3-ene-1,2-dione and 0.62 mL (432 mg, 5.9 mmol) of *t*-$BuNH_2$: yield 1.59 g (5.62 mmol, a 95% yield); mp 283−287 °C (dec); ^{1}H NMR (DMSO-d_6) δ 7.85 (d, 2H), 7.84 (br t, 1H), 7.56 (br s, 1H), 7.52 (d, 2H), 4.81 (d, 2H), 1.35 (s, 9H); IR (KBr) 3150, 2980, 2250, 1800, 1660 cm^{-1}; MS 284 (MH^+). Anal. $C_{16}H_{17}N_3O_2$: C, H, N.

4-Cyano-*N*-[3,4-dioxo-2-(1,2,2-trimethyl-propylamino)-cyclobut-1-enyl]-benzamide (26). To a −10 °C solution of 3,4-diethoxy-3-cyclobutene-1,2-dione (0.51 mL, 582 mg, 3.42 mmol), 500 mg (3.42 mmol) of 4-cyanobenzamide, and 35 mL of DMF was added 205 mg (6.84 mmol) of an 80% NaH/mineral oil dispersion. The mixture was allowed to stir at 0 °C for 2 h. After pouring into 100 mL of brine/5 mL of 1 N HCl(aq), the resulting mixture was extracted with EtOAc (3 × 50 mL). The combined organics were dried over $MgSO_4$, filtered, and evaporated with 10 g of SiO_2 gel. The resulting powder was placed on top of a SiO_2 gel flash column and eluted with hexanes/EtOAc (1.5/1 to 1/1) to produce 260 mg (0.96 mmol, a 28% yield) of 4-cyano-*N*-[3,4-dioxo-2-ethoxy-cyclobut-1-enyl]-benzamide.

To a −10 °C solution of 260 mg (0.96 mmol) of 4-cyano-*N*-[3,4-dioxo-2-ethoxy-cyclobut-1-enyl]-benzamide and 10 mL of MeCN was added 0.195 mL (147 mg, 1.44 mmol) of 2-amino-3,3-dimethylbutane. After the mixture was stirred at −10 °C for 2 h, the solvent was evaporated, and the resulting residue was triturated with CH_2Cl_2/petroleum ether to give 300 mg (0.92 mmol, a 96% yield) of **26** as a white solid: mp 265−266 °C; ^{1}H NMR (DMSO-d_6) δ 8.15 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.4 Hz, 2H), 7.68 (br d, 1H), 4.05−4.09 (m, 1H), 1.17 (d, J = 6.8 Hz, 3H), 0.91 (s, 9H); MS 325 (M^+). Anal. $C_{18}H_{19}N_3O_3$: C, H, N.

4-{2-[3,4-Dioxo-2-(1,2,2-trimethyl-propylamino)-cyclobut-1-enylamino]-ethyl}-benzonitrile (27). To a solution of 913 mg (5.0 mmol) of 2-(aminoethyl)benzonitirle hydrochloride,[11] 1.27 g (5.0 mmol) of 3-butoxy-4-(1,2,2-trimethyl-propylamino)-cyclobut-3-ene-1,2-dione, and 10 mL of pyridine was added 0.71 mL (506 mg, 5.0 mmol) of Et_3N. After being stirred at room temperature for 70 h, the mixture was evaporated to an off-white solid, triturated with 3 × 25 mL of H_2O, washed with 3 × 25 mL of Et_2O, and dried under vacuum to produce 1.39 g (4.27 mmol, an 85% yield) of **27** as a white solid: mp 273−275 °C (dec); ^{1}H NMR (DMSO-d_6) δ 7.76 (d, J = 7.3 Hz, 2H), 7.44 (d, J = 6.1 Hz, 2H), 7.30−7.22 (brm, 1H), 7.22−7.15 (brm, 1H), 3.92−3.84 (brm, 1H), 3.72−3.82 (m, 2H), 2.94 (t, J = 5.3 Hz, 2H), 1.08 (d, J = 5.2 Hz, 3H), 0.83 (s, 9H); IR (KBr) 3300, 3150, 2960, 1780, 1600 cm^{-1}; MS 325 (M^+). Anal. $C_{19}H_{23}N_3O_2$: C, H, N.

4-[2-(2-*tert*-Butylamino-3,4-dioxo-cyclobut-1-enylamino)-ethyl]-benzonitrile (28). To a solution of 913 mg (5.0 mmol) of 2-(aminoethyl)benzonitrile hydrochloride,[11] 1.12 g (5.0 mmol)

of 3-butoxy-4-(*tert*-butylamino)-cyclobut-3-ene-1,2-dione, and 10 mL of pyridine was added 0.71 mL (506 mg, 5.0 mmol) of Et_3N. After being stirred at room temperature for 70 h, the mixture was evaporated to an off-white solid, triturated with 3 × 25 mL of H_2O, washed with 3 × 25 mL of Et_2O, and dried under vacuum to produce 767 mg (2.58 mmol, a 52% yield) of **28** as an off-white solid: mp 307−308 °C (dec); ^{1}H NMR (DMSO-d_6) δ 7.77 (d, J = 7.3 Hz, 2H); 7.45 (d, J = 6.1 Hz, 2H); 7.39 (m, 2H), 3.79 (q, J = 4.9 Hz, 2H), 2.94 (t, J = 5.3 Hz, 2H), 1.33 (s, 9H); IR (KBr) 3250, 3200, 2960, 2400, 1780, 1660 cm $^{-1}$; MS 297 (M^+). Anal. $C_{17}H_{19}N_3O_2$: C, H, N.

4-{1-[3,4-Dioxo-2-(1,2,2-trimethyl-propylamino)-cyclobut-1-enylamino]-ethyl}-benzonitrile (29). To (*R*)-3-ethoxy-4-(1,2,2-trimethyl-propylamino)-cyclobut-3-ene-1,2-dione (200 mg, 0.89 mmol) and 4.4 mL of absolute EtOH was added 130 mg (0.89 mmol) of 4-[(±)-1-aminoethyl]benzonitrile, and the resulting solution was stirred at 23 °C. After 15 h, the reaction mixture was diluted with 6 mL of MeCN, and the resulting precipitate was filtered and set aside (fraction #1). To the filtrate of this reaction was added 130 mg (0.89 mmol) of 4-[(±)-1-aminoethyl]benzonitrile, and the resulting mixture was heated to 85 °C. After 8 h, an additional portion of 260 mg (1.78 mmol) of 4-[(±)-1-aminoethyl]benzonitrile was added. After a total of 32 h, the reaction mixture was cooled to 23 °C, and the resulting precipitate was isolated by filtration and washed with 2 × 5 mL of CH_3CN. The resulting solid was set aside (fraction #2). The filtrate was evaporated to one-third of its volume which produces additional precipitate (fraction #3). All three fractions are combined to give 150 mg (0.46 mmol, 52% yield) of **29** as a white solid: mp 262−264 °C; ^{1}H NMR (DMSO-d_6) δ 7.85 (d, J = 8.4 Hz, 2H), 7.75 (br m, 1H), 7.55 (d, J = 7.9 Hz, 2H), 7.21 (br m, 1H), 5.27−5.25 (br m, 1H), 3.96−3.94 (br m, 1H), 1.54 (d, J = 7.0 Hz, 3), 1.11 (d, J = 6.8 Hz, 2H), 0.86 (s, 9H); IR (KBr) 3200, 2950, 1800, 1670 cm^{-1}; MS 326 $(M)^+$. Anal. $C_{19}H_{23}N_3O_2$ Calcd: C, 70.13; H, 7.13; N, 12.91. Found: C, 69.46; H, 6.99; 12.96.

3-Butoxy-4-*tert*-butylamino-cyclobut-3-ene-1,2-dione. A solution of 3,4-dibutoxy-3-cyclobutene-1,2-dione (11.31 g, 50 mmol) and 3.66 g (50 mmol) of *t*-$BuNH_2$ in 80 mL of THF was stirred at room temperature for 71 h. The solvent was removed, and a solution of the residue in chloroform was washed with water and dried over Na_2SO_4. Removal of the solvent and gravity chromatography on 350 g of neutral activity III SiO_2 gel (eluting with hexanes/$CHCl_3$) provided 9.83 g (43.69 mmol, an 87% yield) of 3-butoxy-4-*tert*-butylamino-cyclobut-3-ene-1,2-dione as white solid: mp 67.0−68.5 °C. Two recrystallizations of 800 mg of this product afforded 551 mg of the title compound as a white solid: mp 68−69 °C; ^{1}H NMR (DMSO-d_6) δ 8.75 and 8.59 (two br s, 1H, rotamers), 4.66 (br m, 2H), 1.72 (m, 2H), 1.40 (m, 2H), 1.31 (m, 9H), 0.91 (t, 3H); IR (KBr) 3140, 1780, 1700 cm^{-1}; MS 225 (M^+). Anal. $C_{12}H_{19}NO_3$: C, H, N.

3-Benzylamino-4-(*tert*-butylamino)-cyclobut-3-ene-1,2-dione (30). The title compound was prepared according to the procedure of compound **10** using 0.65 mL (638 mg, 6.0 mmol) of benzylamine and 1.13 g (5.0 mmol) of 3-butoxy-4-*tert*-butylamino-cyclobut-3-ene-1,2-dione: yield 1.08 g (4.18 mmol, an 84% yield); mp 306−307 °C (dec); ^{1}H NMR (DMSO-d_6) δ 7.74 (br s, 1H), 7.49 (br s, 1H), 7.28−7.40 (complex m, 5H), 4.71 (d, J = 6.2 Hz, 2H), 1.35 (s, 9H); IR (KBr) 3200, 1780, 1700 cm^{-1}; MS 258 (M^+). Anal. $C_{15}H_{18}N_2O_2$: C, H, N.

4-[(2-*tert*-Butylamino-3,4-dioxo-cyclobut-1-enylamino)-methyl]-3-chloro-benzonitrile (31). A mixture of 3-chloro-4-methylbenzonitrile (22.74 g, 150 mmol), 32.04 g (180 mmol) of NBS, and 2.46 g (15 mmol) of 2,2′-azobis-2-methylpropionitrile in 120 mL of CCl_4 was *carefully* warmed to reflux temperature whereupon a *moderate exotherm* occurred and refluxing proceeded for approximately 10 min without external heating. Heating was then resumed, and refluxing continued for 26 h. The hot reaction mixture was suction filtered, and the insolubles were rinsed with CCl_4 (3 × 25 mL). The combined CCl_4 fractions were washed with H_2O and dried over Na_2SO_4. Removal of solvent gave a yellow mush which was crystallized from hexane (using decolorizing charcoal). The product again was recrystallized from hexane to yield 20.44 g (88.68 mmol, a 59% yield) of 2-chloro-4-cyanobenzyl bromide as a white solid: mp 81−84 °C; ^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1H), 7.82 (m, 2H), 4.69 (s, 2H). IR (KBr) 2220 cm^{-1}.

A mixture of 2-chloro-4-cyanobenzyl bromide (20.29 g, 88.0 mmol) and 17.92 g (96.8 mmol) of potassium phthalimide in 200 mL of DMF was stirred at room temperature, and the reaction temperature rose to approximately 36 °C after approximately 5 min with formation of a tan suspension. The temperature then receded, and stirring was continued for 2 h at room temperature. After removal of solvent, the residue was triturated thoroughly with H_2O and dried. The resulting buff solid was treated with approximately 500 mL of boiling EtOAc, gravity filtered to remove a small amount of white insoluble material, heated to boiling, treated with charcoal, and filtered. Concentration and cooling of the filtrate precipitates 20.26 g (68.3 mmol, a 78% yield) of the *N*-(2-chloro-4-cyanobenzyl)-phthalimide as a white solid: mp 172.5−173.0 °C; ^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1H), 7.90 (m, 4H), 7.75 (dd, 1H), 7.52 (d, 1H), 4.88 (s, 2H); IR (KBr) 2220, 1770, 1715 cm^{-1}.

A mechanically stirred suspension of *N*-(2-chloro-4-cyanobenzyl)phthalimide (18.99 g, 64 mmol) in 150 mL of absolute EtOH was treated with 6.41 g (128 mmol) of $N_2H_4 \cdot H_2O$, and the resulting mixture was refluxed for 1 h and then allowed to stand at room temperature for approximately 16.5 h. The reaction mixture was stirred at room temperature, 90 mL of 2 N HCl was added slowly, and the mixture was stirred for 10 min. The mixture was filtered, and the insolubles were triturated thoroughly with absolute EtOH and then with H_2O. The combined filtrates and triturates were evaporated, and resulting solids were added to 250 mL of ice−H_2O and basified with 90 mL of 2.5 N NaOH. The resulting mass was extracted thoroughly with $CHCl_3$, and the extracts were washed with H_2O and brine and dried (anhydrous Na_2SO_4). Removal of solvent gave a cream-colored solid which was recrystallized from hexane to provide 6.85 g (41.11 mmol, a 64% yield) of 2-chloro-4-cyanobenzylamine as a white solid: mp 85.0−87.0 °C; ^{1}H NMR (DMSO-d_6) δ 7.96 (d, 1H), 7.82 (dd, 1H), 7.77 (m, 1H), 3.82 (s, 2H), 2.12 (br m, 2H); IR (KBr, cm^{-1}) 3380, 3320, 2230.

Tetrahydrofuran (50 mL), 6.76 g (30 mmol) of 3-butoxy-4-*tert*-butylamino-cyclobut-3-ene-1,2-dione, and 5.0 g (30 mmol) of 2-chloro-4-cyanobenzylamine were refluxed for 6 h and then allowed to stand at room temperature for 16 h. Following removal of solvent from the reaction mixture, the residue was triturated thoroughly with Et_2O and dried to give a buff solid. This material in approximately 1.4 L of boiling acetone was filtered to remove a small amount of white solid. The hot filtrate was treated with charcoal, filtered, concentrated, and cooled to afford 6.52 g (20.52 mmol, a 68% yield) of **31** as a cream-colored solid. Two additional recrystallizations of this material from acetone gave 4.78 g (15.03 mmol, a 50% yield) of the title compound as a white solid: mp 243.5−245 °C; ^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1H), 7.88 (dd, 1H), 7.82 (m, 1H), 7.66 (br s, 1H), 7.61 (d, 1H), 4.88 (d, 2H), 1.34 (s, 9H); IR (KBr) 3320, 3230, 2240, 1780, 1665 cm^{-1}; MS 317/319 (M^+). Anal. $C_{16}H_{16}ClN_3O_2$: C, H, N, Cl.

3-*tert*-Butylamino-4-[(pyridin-4-ylmethyl)-amino]-cyclobut-3-ene-1,2-dione (35). To a solution of 1.13 g (5.0 mmol) of 3-butoxy-4-(4-cyano-benzylamino)-cyclobut-3-ene-1,2-dione and 5 mL of pyridine was added 1.0 mL (1.08 g, 10 mmol) of 4-(aminomethyl)pyridine, and the resulting solution was stirred at 23 °C for 8 h. Evaporation of solvent leaves a white solid. Ether (25 mL) is added, and the resulting slurry was stirred at 23 °C for 1 h. Filtration and washing of the collected solid with 3 × 10 mL of Et_2O yields 990 mg (3.82 mmol, a 76% yield) of the title compound as a white solid: mp 271 °C (dec); ^{1}H NMR (DMSO-d_6) δ 8.55 (dd, J = −4.3, 1.1 Hz, 2H), 7.81 (brt, 1H), 7.58 (brs, 1H), 7.32 (dd, J = 4.3, 1.1 Hz, 2H), 4.76 (d, J = 4.8 Hz, 2H), 1.36 (s, 9H); IR (KBr) 3320, 3230, 1780, 1660 cm $^{-1}$; MS 259 (M^+). Anal. $C_{14}H_{17}N_3O_2$: C, H, N.

3-*tert*-Butylamino-4-(2,4-dichloro-6-methyl-benzylamino)-cyclobut-3-ene-1,2-dione (48). The title compound was prepared according to the procedure for compound **10** using 3-ethoxy-4-*tert*-butylamino-cyclobut-3-ene-1,2-dione (0.22 g, 1.1

mmol) and 2,4-dichloro-6-methylbenzylamine (0.22 g, 1.2 mmol, containing approximately 5% of a compound which is regioisomeric with respect to the substitution on the aryl ring): yield 340 mg (0.99 mmol, an 89% yield) of a white solid which contains approximately 5% of a compound which is regioisomeric with respect to substitution on the aryl ring: mp 264−268 °C; ^{1}H NMR (DMSO-d_6) δ 7.54 (d, 1H), 7.46 (s, 1H), 7.43 (br t, 1H), 7.39 (d, 1H), 4.89 (d, 2H), 4.72 (d, minor isomer), 2.40 (s, 3H), 2.31 (s, minor isomer), 1.34 (s, 9H); IR (KBr, cm^{-1}) 3200, 2950, 1800, 1650; MS 340/342/344 (M^+). Anal. $C_{16}H_{18}Cl_2N_2O_2$: C, H, N.

3-Chloro-4-{[3,4-dioxo-2-(1,2,2-trimethyl-propylamino)-cyclobut-1-enylamino]-methyl}-5-methyl-benzonitrile (55). To 2-chloro-4-cyano-6-methylbenzaldehyde oxime (1.2 g, 6.2 mmol) in 12.3 mL of glacial HOAc was added 1.6 g (24 mmol) of Zn powder. The slurry was heated to reflux. When the bubbling subsided, a second portion of zinc powder (1.6 g, 24 mmol) was added, and the slurry was heated to boiling. When the reaction had cooled to room temperature, it was diluted with absolute EtOH, filtered through Celite, rinsed with absolute EtOH, and concentrated under reduced pressure. The resulting residue was mixed with 3,4-diethoxy-3-cyclobutene-1,2-dione (0.91 mL, 6.2 mmol) and 5 mL of absolute EtOH and then allowed to stand at room temperature for 18 h. The solid precipitate which forms was filtered and rinsed with EtOAc to give 510 mg of a white solid. This solid was dissolved in CH_2Cl_2, filtered, and rinsed with CH_2Cl_2, and the filtrate was concentrated under reduced pressure to give 190 mg (0.62 mmol, a 10% yield) of 3-(2-chloro-4-cyano-6-methyl-benzylamino)-4-ethoxy-cyclobut-3-ene-1,2-dione as a white solid: ^{1}H NMR (DMSO-d_6) δ 8.97 and 8.74 (br m, 1H, rotamers), 7.92 (s, 1H), 7.74 (s, 1H), 4.93 (br m, 1H), 4.78−4.60 (br m, 3H), 2.43 (s, 3H), 1.36 (br m, 3H); MS 304/306 (M^+).

3-(2-Chloro-4-cyano-6-methyl-benzylamino)-4-ethoxy-cyclobut-3-ene-1,2-dione (0.19 g, 0.62 mmol) and 4.7 mL (0.94 mmol) of a 0.2 M solution of (*R*)-2-amino-3,3-dimethylbutane in absolute EtOH were stirred at room temperature for 2 d. The slurry was filtered, rinsed with MeCN (3 × 3 mL), and dried to give 150 mg (0.42 mmol, a 67% yield) of **55** as a white solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 7.98 (s, 1H), 7.77 (s, 1H), 7.38 (br m, 1H), 7.18 (br d, 1H), 4.97 (m, 2H), 3.90 (m, 1H), 2.46 (s, 3H), 1.08 (d, 3H), 0.84 (s, 9H); IR (KBr) 3180, 2980, 2250, 1800, 1640 cm^{-1}; MS 359/361 (M^+). Anal. $C_{19}H_{22}ClN_3O_2 \cdot 0.04\ CH_2Cl_2$: C, H, N (the presence of 0.04 mol of CH_2Cl_2 confirmed by ^{1}H NMR analysis).

3-Butoxy-4-(1,1-dimethyl-propylamino)-cyclobut-3-ene-1,2-dione. A solution of 3,4-dibutoxy-3-cyclobutene-1,2-dione (4.53 g, 20 mmol) and 1.74 g (20 mmol) of 1,1-dimethylpropylamine in 20 mL of THF was stirred at room temperature for approximately 19.5 h. The solvent was removed, and the residue was chromatographed by gravity ($CHCl_3$/hexane) on neutral, activity III silica (150 g). The white solid isolated from the appropriate eluates was recrystallized from hexane to give 4.105 g (17.18 mmol, an 86% yield) of the title compound as a white solid: mp 56.5−57.5 °C. One gram (4.18 mmol) of this material was recrystallized twice from hexane to provide 794 mg (3.32 mmol) of the title compound as a white solid: mp 56−57 °C; ^{1}H NMR (DMSO-d_6) δ 8.63 and 8.48 (two br s, 1H, rotamers), 4.67 (m, br, 2H), 1.67 (m, br, 4H), 1.39 (m, 2H), 1.26 (m, br, 6H), 0.91 (t, 3H), 0.78 (t, 3H); IR (KBr) 3170, 1790, 1700 cm^{-1}; MS: 239 (M^+). Anal. $C_{13}H_{21}NO_3$: C, H, N.

3-Chloro-4-{[2-(1,1-dimethyl-propylamino)-3,4-dioxo-cyclobut-1-enylamino]-methyl}-benzonitrile (57). 3-Butoxy-4-(1,1-dimethylpropylamino)-cyclobut-3-ene-1,2-dione (957 mg, 4 mmol), 666 mg (4 mmol) of 2-chloro-4-cyanobenzylamine, and 10 mL of THF were stirred together at room temperature for 138 h. Removal of solvent, trituration of the residue with Et_2O, and drying provided a white solid. Recrystallization of this solid from MeCN (charcoal) followed by a second recrystallization from CH_3CN gave 863 mg (2.60 mmol, a 65% yield) of **57** as a white solid: mp 215.5−219.5 °C; ^{1}H NMR (DMSO-d_6) δ 8.11 (d, 1H), 7.89 (m, 1H), 7.86 (m, 1H), 7.61 (d, 1H), 7.53 (s, 1H), 4.90 (d, 2H), 1.68 (q, 2H), 1.30 (s, 6H), 0.82 (t, 3H); IR (KBr) 3300, 2230, 1790, 1660 cm^{-1}; MS 331/333 (M^+). Anal. $C_{12}H_{18}ClN_3O_2$ Calcd: C, 61.54; H, 5.47; N, 12.66. Found: 60.81; H, 5.40; N, 12.52.

3-Chloro-4-{[2-(1,1-dimethyl-propylamino)-3,4-dioxo-cyclobut-1-enylamino]-methyl}-5-methyl-benzonitrile (58). To 3-(2-chloro-4-cyano-6-methyl-benzylamino)-4-ethoxy-cyclobut-3-ene-1,2-dione (0.24 g, 0.79 mmol) prepared according to the procedure for compound **55** were added 5 mL (3.7 g, 42.8 mmol) of 1,1-dimethylpropylamine and 3 mL of CH_2Cl_2, and the resulting mixture was heated at reflux for 8 h. The solvent was removed under reduced pressure to give a solid, which was triturated with EtOAc, filtered, rinsed with additional EtOAc, and dried in vacuo (0.4 mm, 70 °C) to give 170 mg (0.49 mmol, a 62% yield) of **58** as a white solid: mp 258−262 °C (dec); ^{1}H NMR (DMSO-d_6) δ 7.99 (s, 1H), 7.78 (s, 1H), 7.51 (br t, 1H), 7.35 (s, 1H), 4.98 (d, 2H), 2.46 (s, 3H), 1.66 (q, 2H), 1.29 (s, 6H), 0.80 (t, 3H); IR (KBr) 3200, 2980, 2200, 1800, 1650 cm^{-1}; MS 345/347 (M^+). Anal. $C_{18}H_{20}ClN_3O_2$: C, H, N.

3-(2,4-Dichlorobenzylamino)-4-(1,1-dimethylpropylamino)-cyclobut-3-ene-1,2-dione (60). The title compound was prepared according to the procedure of compound **10** using 16.67 g (79.0 mmol) of 3-ethoxy-4-(1,1-dimethyl-propylamino)-cyclobut-3-ene-1,2-dione and 15.02 g (79.0 mmol) of 2,4-dichloro-6-methylbenzylamine: yield 25.7 g (72.3 mmol, a 92% yield); mp 247.1−248.3 °C; ^{1}H NMR (DMSO-d_6) δ 7.54 (d, 1H), 7.44 (br t, 1H), 7.39 (d, 1H), 7.31 (s, 1H), 4.90 (d, 2H), 2.40 (s, 3H), 1.66 (q, 2H), 1.28 (s, 6H), 0.80 (t, 3H); IR (KBr) 3200, 2980, 1800, 1650 cm^{-1}; MS 354/356/358 (M^+). Anal. $C_{17}H_{20}Cl_2N_2O_2$: C, H, N.

***N*-(4-Cyano-benzyl)-*N*-[3,4-dioxo-2-(1,2,2-trimethyl-propylamino)-cyclobut-1-enyl]-butyramide (80).** Compound **4** (500 mg, 1.6 mmol) in 20 mL of THF was placed under Ar at room temperature. Solid NaH (77 mg of a 60% dispersion in mineral oil, 1.9 mmol) was added, and the reaction mixture was stirred at room temperature for 20 min. Butyric acid anhydride (0.79 mL, 4.8 mmol) was then added, and the solution was stirred at room temperature for 3 h and then refluxed for 8 h. The reaction mixture was loaded onto a plug of silica gel and eluted with hexanes:EtOAc (1:1) to give a residue, which was triturated repeatedly with hexanes to give 280 mg (0.73 mmol, a 45% yield) of **80** as a pale yellow solid: mp 101−103 °C; $[\alpha]^{25}_D$ −101.9° (*c* 0.01, DMSO); ^{1}H NMR (DMSO-d_6) δ 8.05 (br m, 1H), 7.83 (d, 2H), 7.45 (d, 2H), 5.22 (br m, 2H), 4.07 (m, 1H) 2.50−2.30 (m, 2H), 1.52 (m, 2H), 1.16 (d, 3H), 0.87 (s, 9H), 0.82 (t, 3H). IR (KBr) 3450, 3350, 2980, 2250, 1800, 1730 cm^{-1}; MS 382 $(MH)^+$. Anal. $C_{22}H_{27}N_3O_3$: C, H, N.

***N*-(2,4-Dichloro-6-methyl-benzyl)-*N*-[2-(1,1-dimethyl-propylamino)-3,4-dioxo-cyclobut-1-enyl]-butyramide (81).** To 3-(2,4-dichloro-6-methyl-benzylamino)-4-(1,1-dimethyl-propylamino)-cyclobut-3-ene-1,2-dione (500 mg, 1.41 mmol) in 2 mL of DMF and 8 mL of THF was added NaH (62 mg of a 60% dispersion in mineral oil, 1.54 mmol) at 0 °C. The frothy suspension was stirred for 1 h as the mixture was warmed to 23 °C. After cooling to 0 °C, 240 mg (1.54 mmol) of butyric acid anhydride was added, and the reaction mixture was stirred at 0 °C for 15 min and then allowed to warm to room temperature. After being stirred for a total of 12 h, the reaction mixture was poured into brine (50 mL) and extracted with EtOAc (3 × 50 mL). The organic layer was dried over $MgSO_4$ and decolorized (charcoal). The solvent was removed in vacuo, and the remaining oil was triturated with Et_2O/petroleum ether to yield 310 mg (0.73 mmol, a 53% yield) of **77** as a white solid: mp 117.2−118.4 °C; ^{1}H NMR (DMSO-d_6) δ 8.80 (br s, 1H), 7.39 (d, 1H), 7.28 (d, 1H), 5.06 (s, 2H), 2.34 (s, 3H), 2.29 (t, 2H), 1.67 (q, 2H), 1.51 (q, 2H), 1.30 (s, 6H), 0.82 (q, 6H); IR (KBr) 3230, 2950, 1800, 1744, 1700, 1570 cm^{-1}; MS 424 $(M)^+$. Anal. $C_{21}H_{26}Cl_2N_2O_3$: C, H, N.

Pharmacological Methods. All animal studies were approved by the Wyeth-Ayerst Institutional Animal Care and Use Committee and were performed in accordance with the guidelines of the Animal Welfare Act and the American Association for Accreditation of Laboratory Animal Care.

In Vitro Studies: A. Isolated Bladder Strip Contraction Studies. Female Sprague−Dawley rats (Charles River,

Wilmington, MA; 250 to 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). Tissues were pretensioned (0.25 to 0.5 g) and allowed to equilibrate for 30 min. Strips were then 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 were allowed to reach steady state. Signals were digitized (486 based personal computer, 12 bit resolution, 1 s sampling interval, custom software) for online 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.

In Vivo Studies: A. Rat Hypertrophied Bladder Model. The method for producing hypertrophied, unstable bladders was modified from that reported by Malmgren et al.[5] Briefly, female Sprague−Dawley rats (Charles River, Wilmington, MA; 190−210 g) were anesthetized with isoflurane, and the bladder and urethra were exposed through a 2 cm midline incision. A 4-0 silk ligature was tied around the proximal urethra in the presence of a stainless steel rod (1 mm diameter). The rod was then removed thus resulting in a partial premeasured occlusion. The abdominal musculature was closed using 3-0 silk, and the skin was closed with surgical staples. Each rat received 150 000 units (im) of bicilin C-R (Wyeth Laboratories, Philadelphia, PA). During the following 6 week period, the increased urethral outlet resistance from the partial occlusion caused the bladders to hypertrophy and become unstable.

After 6 weeks, the ligature was removed under isoflurane anesthesia, and a flared catheter (PE60) was placed in the dome of the bladder and secured with a purse-string suture. The catheter was exteriorized under the skin and through an opening in the back of the neck. The abdominal incision was sutured and the free end of the catheter sealed. Following surgery, animals were given bicilin C-R (150 000 units/rat, im).

Two days after catheter implantation, the animals were used for cystometric evaluation. The night before testing, the animals were placed into metabolic cages. The catheter was connected to a Harvard infusion pump, and bladders were perfused overnight with saline at a rate of 2 mL/h. The next morning a Statham pressure transducer (model P23Db) was positioned in line with the Harvard infusion pump (using a "T" connector) to record bladder pressure. A plastic beaker attached to a force displacement transducer (Grass FTO3) was placed under the metabolic cage to collect and record urine volume. The cystometric evaluation of bladder function was started by infusing saline (20 mL/h), and after the first micturition the infusion was maintained for 20 min. Two hours after the first cystometry period, the rats were dosed orally with the test compound, and a second cystometry was approximately 1 h after administration of test compound. Vehicle (poly(ethylene glycol) 200) was similarly administered to groups of rats that served as controls.

B. Hemodynamic Assessment. Male Sprague−Dawley rats (Charles River, Wilmington, MA; 315−410 g) were anesthetized with isofluorane. A femoral artery and vein were cannulated with polyethylene tubing (PE50). The rats were placed in Bollman cages, and the tail along with two cannulas was extended through a hole at one end of the cage. The animal was further immobilized by securely taping the tail to the benchtop. Arterial blood pressure (BP) was obtained from the cannulated femoral artery by means of a Statham pressure transducer (model P23Db). Transducer signals were recorded on a Grass (model 7) polygraph. Heart rate (HR) was calculated manually from the BP traces.

Supporting Information Available: Experimental procedures for compounds **16**−**24, 32**−**34**, **36**−**47**, **50**−**54**, **56**, **59**, and **61**−**79**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) That the loss in the activity going from **4** to **26** is due to conversion of the benzylamine of **4** to the benzamide of **26** and not that **4** is a single enantiomer while **26** is a racemate is suggested by analysis of chiral and racemic 3,3-dimethyl-2-butylamino analogues presented in the phenylamino cyclobutenedione series (i.e., compare compounds **34** and **42** in ref 1).[1]

(8) $C = 245 \pm 15\%$ at 30 μM (administration of compound **77** to a KCl-contracted rat bladder strip additionally contracts this tissue $245 \pm 15\%$ at 30 μM).

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