Design, Synthesis, and Structure−Activity Relationships of Phthalimide-Phenylpiperazines: A Novel Series of Potent and Selective α_{1a}-Adrenergic Receptor Antagonists

Gee-Hong Kuo,*,† Catherine Prouty,† William V. Murray,† Virginia Pulito,† Linda Jolliffe,† Peter Cheung,† Sally Varga,† Mary Evangelisto,† and Jian Wang‡

Drug Discovery Division, Analytical Development, Global Chemical & Pharmaceutical Development Division, The R.W. Johnson Pharmaceutical Research Institute, 1000 Route 202, P.O. Box 300, Raritan, New Jersey 08869

Received November 30, 1999

Beginning from the screening hit and literature α_1-adrenergic compounds, a hybridized basic skeleton **A** was proposed as the pharmacophore for potent and selective α_{1a}-AR antagonists. Introduction of a hydroxy group to increase the flexibility afforded **B** which served as the screening model and resulted in the identification of the second-generation lead **1**. Using the Topliss approach, a number of potent and selective α_{1a}-AR antagonists were discovered. In all cases, binding affinity and selectivity at the α_{1a}-AR of *S*-hydroxy enantiomers were higher than those of the *R*-hydroxy enantiomers. As compared to the des-hydroxy analogues, the *S*-hydroxy enantiomers had slightly lower binding affinity at α_{1a}-AR but gained more than 2-fold selectivity for α_{1a}-AR over α_{1b}-AR, and 2- to 6-fold selectivity for α_{1a}-AR over α_{1d}-AR. They also had less cross activities against a panel of 25−35 peripheral and CNS receptors. The *S*-hydroxy enantiomers **23** and **24** (K_i = 0.29 nM, 0.33 nM; α_{1b}/α_{1a} >5690, >6060; α_{1d}/α_{1a} = 186, 158, respectively) were slightly less potent but much more selective at α_{1a}-AR than tamsulosin (K_i = 0.13 nM, α_{1b}/α_{1a} = 14.8, α_{1d}/α_{1a} = 1.4). In the functional assay, the *S*-hydroxy enantiomers **20**, **23**, and **24** were less potent than tamsulosin in inhibiting contractions of rat prostate tissue but more selective in the inhibition of tissue contractions of rat prostate versus rat aorta. Compound **24** was selected as the development candidate for the treatment of BPH.

Introduction

The α_1-adrenergic receptors (α_1-AR) are a family of G-protein coupled seven-transmembrane receptors which are involved in the regulation of the cardiovascular and central nervous systems.[1] At least three native α_1-AR subtypes, α_{1A}, α_{1B}, and α_{1D} have been characterized pharmacologically in tissues.[1−3] It has been demonstrated that the α_{1A}-AR mediates the smooth muscle contraction in human prostate.[4,5] Some studies suggest that the α_{1B}-AR mediates contraction of the rat spleen[6] and vasoconstriction of large human arteries.[7] The α_{1D}-AR mediates contraction of the rat aorta.[8] Molecular cloning techniques allowed the preparation of three cloned α_1-AR subtypes—α_{1a}, α_{1b}, and α_{1d}—for a number of species including human.[2,4]

Benign prostatic hyperplasia (BPH) is the most common benign tumor in men. It occurs in over 50% of the male population above age 60, and leads to a variety of urological symptoms including increased frequency in urination, nocturia, and delay in starting the urine flow. Advanced BPH may result in urinary-tract infections, inability to urinate, and kidney damage. Several non subtype selective α_1-AR antagonists such as prazosin, tetrazosin,[9] and doxazosin[10] are being used for the treatment of BPH by relaxing the smooth muscle tone of the prostate. These compounds have been shown to cause side effects such as postural hypotension, dizziness, and syncope that may in part be attributed to a result of their nonselective affinity to α_{1A}-AR subtype.[11] Tamsulosin,[12] the first α_{1A}-AR "selective" antagonist for the treatment of BPH, was approved in the United States in 1997 (Scheme 1). It has been shown to have modest selectivity for α_{1a}-AR over α_{1b}-AR (K_i of α_{1a} = 0.13 nM, K_i of α_{1b} = 1.92 nM, α_{1b}/α_{1a} = 14.8) and almost no selectivity for α_{1a}-AR over α_{1d}-AR (K_i of α_{1d} = 0.18 nM, α_{1d}/α_{1a} = 1.4). Clinical studies indicate that tamsulosin has less effect on blood pressure and causes less symptomatic orthostatic hypotension than previous non subtype selective α_1-AR antagonists.[13a] However, it still shows side effects such as abnormal ejaculation and dizziness.[13b−d] The goal of our research project is to develop a potent and selective α_{1a}-AR antagonist which may be useful for the treatment of BPH with minimal side effects.

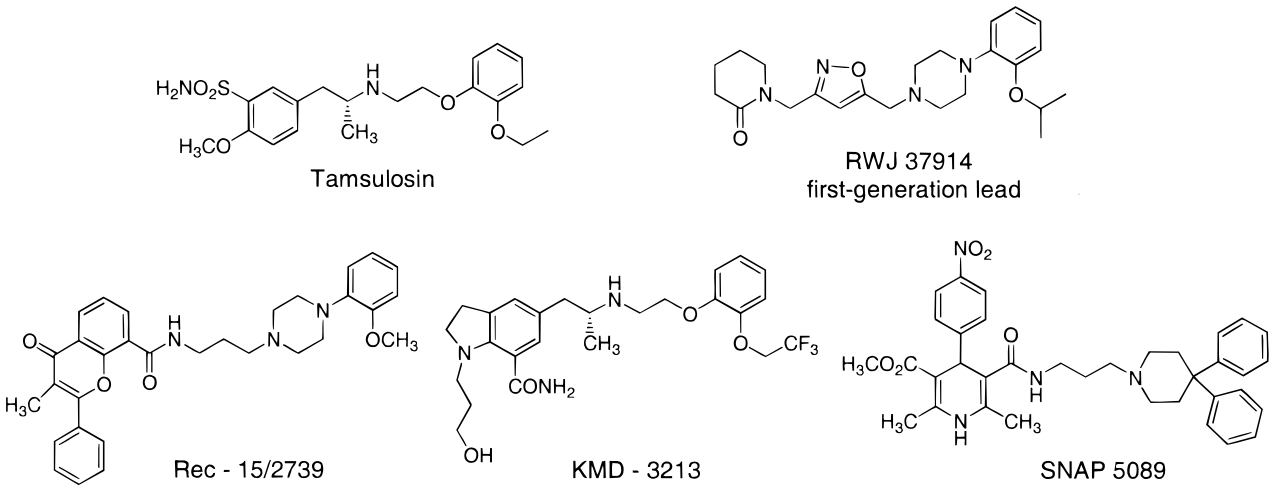
Lead Generation

The R. W. Johnson, PRI library compounds were screened for all three subtypes of α_1-AR binding affinity using ^{125}I-HEAT as the radiolabeled ligand.[14] This study resulted in the discovery of the first-generation lead RWJ 37914, a potent and selective α_{1a}-AR antagonist (K_i of α_{1a} = 16.5 nM, K_i of α_{1b} >2000 nM, and K_i of α_{1d} = 2871 nM) (Scheme 1). Therefore, RWJ 37914 had >120-fold higher affinity for the α_{1a}-AR over α_{1b}-AR and 174-fold higher affinity for the α_{1a}-AR over α_{1d}-AR. In comparison to tamsulosin, RWJ 37914 showed greater selectivity but weaker binding affinity at the α_{1a}-AR.

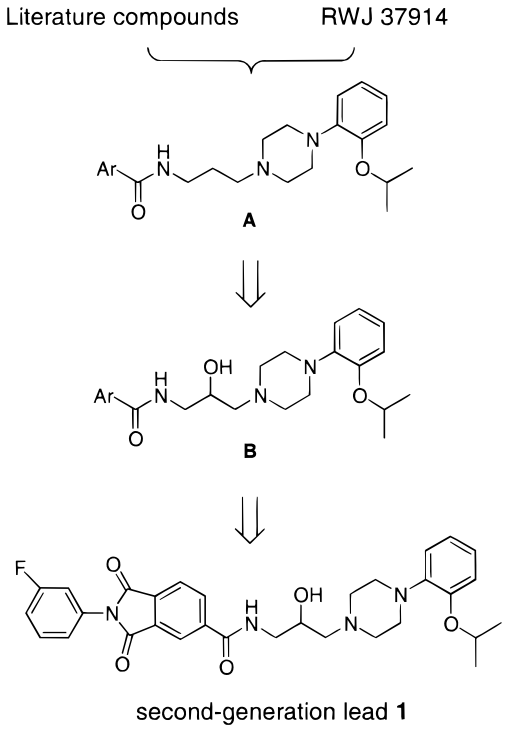
* To whom correspondence should be addressed. Tel: 908-704-4330. Fax: 908-526-6469. E-mail: gkuo@prius.jnj.com.
† Drug Discovery Division.
‡ Analytical Development, Global Chemical & Pharmaceutical Development Division.

10.1021/jm9905918 CCC: $19.00
Published on Web 05/09/2000

Scheme 1



Scheme 2



In an effort to improve the α_{1a}-AR binding affinity, we decided to modify the lead structure based upon other α_{1a}-AR antagonists: tamsulosin, Rec-15/2739,[15] KMD-3213,[16] and SNAP-5089[17] (Scheme 1). Since the positively charged nitrogen atom was suggested to play a major role in the binding to the important aspartic acid residue of α_1-AR,[18,19] we overlapped tamsulosin, Rec-15/2739, KMD-3213, and SNAP-5089 using the basic aliphatic nitrogen as the anchor atom. The study revealed that the degree of structure diversity in the left-hand side of the molecules was greater than that in the right-hand side of the molecules. We therefore proposed a hybridized molecule **A** as the pharmacophore that would allow exploration of the space in the left-hand side of the molecule while maintaining the fixed (*o*-isopropoxyphenyl)piperazine moiety in the right-hand side of the molecule (Scheme 2). We also introduced a hydroxy group (compound **B**), that could serve as a handle to modify the pharmacokinetic properties of the molecule if needed. After a number of analogues of compound **B** were prepared and tested, the second-generation lead **1** (α_{1a} = 1.5 nM, α_{1b} >1835 nM, and α_{1d} = 75 nM) was identified. Compound **1** had >1220-fold higher affinity for the α_{1a}-AR over α_{1b}-AR and 50-fold higher affinity for the α_{1a}-AR over α_{1d}-AR. In comparison to the first-generation lead RWJ 37914, compound **1** showed improved binding affinity (11-fold) and maintained high selectivity at the α_{1a}-AR.

Chemistry

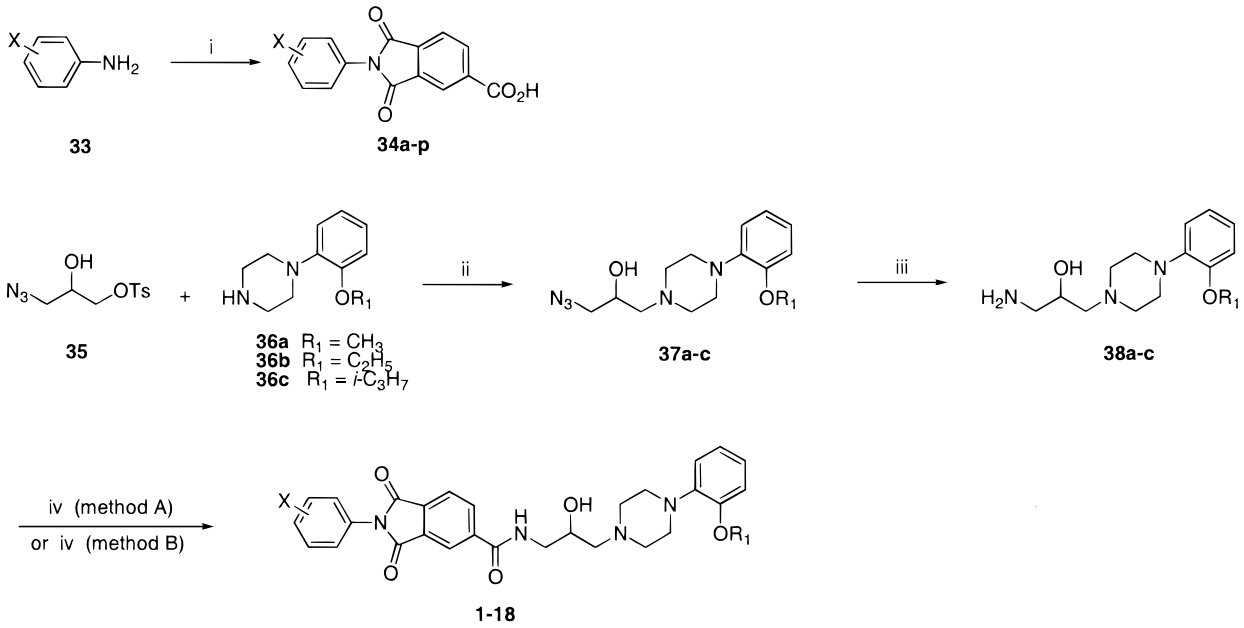
All of the racemic hydroxy compounds **1**−**18** had been synthesized via the route shown in Scheme 3. The molecules were assembled by an amide bond formation of the phthalimide carboxylic acids **34** and the primary amines **38**. Compound **34** (Table 1) were easily prepared by refluxing 1,2,4-benzenetricarboxylic anhydride with the substituted aniline derivatives in glacial acetic acid. The requisite amines **38** were prepared in two steps. Alkylation of either the commercially available alkoxyphenylpiperazines **36a**−**b** or isopropoxyphenylpiperazine **36c**[21] with azido-tosylate **35**[20] gave azido-piperazines **37**. Hydrogenation of **37** gave the amines **38**. The amines **38** were coupled with carboxylic acids **34** using either EDCI, HOBT (method A), or HATU (method B) to give the desired product **1**−**18**. In general, the HATU coupling reagent gave higher yields (Table 2).

Optically active *S*-hydroxy compounds (**20**, **22**−**25**) were prepared as shown in Scheme 4 starting from the optically active *S*-azido-tosylate *S*-**35**.[22] The *R*-hydroxy enantiomers (**19** and **21**) were prepared from the corresponding *R*-azido-tosylate (Table 3).

Preparation of the des-hydroxy compounds (**26**−**30**) is shown in Scheme 5. Alkylation of piperazine **36c** with N-Boc-protected 3-bromopropylamine gave the piperazine derivative **40**. Removal of the protecting group, followed by the coupling reaction with carboxylic acid **34**, gave the des-hydroxy compounds (Table 3).

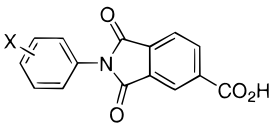
The preparation of the O-methylated compound **31** was shown in Scheme 6. Methylation of the hydroxy-azido-piperazine **37c** gave compound **41**. Hydrogenation of **41** followed by amide bond formation with carboxylic acid **34a** gave the O-methylated compound **31** (Table 3).

Scheme 3[a]



[a] (i) 1,2,4-Benzenetricarboxylic anhydride, glacial acetic acid, 130 °C; (ii) NMP, 100 °C; (iii) 10% Pd/C, H_2, CH_3OH; (iv) method A: **34**, EDCl, HOBT, DMAP, CH_2Cl_2; method B: **34**, HATU, CH_2Cl_2.

Table 1. Characterization of Phthalimide Carboxylic Acid



34	X	yield (%)	MS[a]
a	3-F	77	284
b	H	84	266
c	4-Cl	92	300
d	3,4-Cl_2	91	334
e	4-OCH_3	87	296
f	4-CH_3	89	280
g	3-Cl	91	300
h	4-$N(CH_3)_2$	71	309
i	4-NO_2	97	311
j	4-$C(CH_3)_3$	89	322
k	3-CH_3	*b*	280
l	4-OH	85	282
m	2-OCH_3	77	296
n	4-F	*b*	284
o	3,4-$(OCH_3)_2$	91	326
p	3,4,5-$(OCH_3)_3$	97	356

[a] Electrospray mass spectra, *m*/*z* $M-H^+$. [b] Commercially available from Salor.

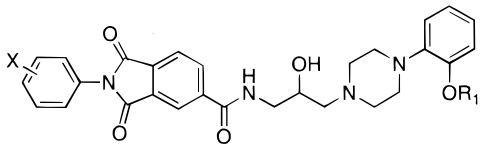
Results and Discussion

Lead Optimization. With the second-generation lead **1** in hand, we briefly reexamined the structure−activity relationships (SAR) of the alkoxy-substituted phenylpiperazines at the R_1 site of compounds **1**−**3** (Table 4). Indeed, compound **1** which has the sterically bulkier *o*-isopropoxy moiety adopted from RWJ 37914 was superior to the less bulky *o*-ethoxy **2** or *o*-methoxy **3** for both α_{1a}-AR binding affinity (K_i = 1.5 nM versus 4.25 nM or 22 nM) and selectivity (α_{1b}/α_{1a} >1220-fold versus >470-fold or >90-fold; α_{1d}/α_{1a} = 50-fold versus 32-fold or 25-fold, respectively).

Therefore we focused our efforts optimizing at the X site employing the Topliss approach[23,24] while maintaining the optimal isopropyl group at R_1 (Table 4). The Topliss approach took into account the electronic, lipophilic, and steric factors for substitution on a phenyl ring. We first prepared the unsubstituted-phenyl **4**, 4-chlorophenyl **5**, 3,4-dichlorophenyl **6**, and 4-methoxyphenyl **7** analogues. All four compounds had comparable α_{1a}-AR potency, with compound **7** being the most potent one (K_i = 0.77 nM). As compared to 3-fluorophenyl **1**, 4-methoxyphenyl **7** showed further improvement at α_{1a}-AR affinity (∼2-fold) and maintained the high selectivity for α_{1a}-AR over either α_{1b}-AR or α_{1d}-AR. These initial studies suggested that compounds with electron-withdrawing substituents (**1** and **6**) had lower α_{1a}-AR binding affinity. Therefore, we proceeded to synthesize analogues under the branch of 4-chlorophenyl **5** and 4-methoxyphenyl **7** of the Topliss tree. Compounds **8**, **9**, and **10** all possessed similar high α_{1a}-AR potency (K_i = 0.66 nM, 0.76 nM, and 0.80 nM, respectively) and high selectivity as compared to that of **7** except 3-chlorophenyl **9** which showed relatively lower selectivity for α_{1a}-AR over α_{1b}-AR (800-fold versus >2600-fold). Replacement of the 4-methyl group with a bulky 4-*tert*-butyl group (compound **11**) led to a significant decrease of α_{1a}-AR affinity (K_i = 3.05 nM). Since the 4-dimethylaminophenyl **10** was equipotent to 4-methoxyphenyl **7**, we chose to synthesize another polar analogue, 4-hydroxyphenyl **12**. Compound **12** also possessed α_{1a}-AR potency and selectivity comparable to that of compound **7**.

To ensure that we did not miss the optimal substituents at the X site, we also prepared 3-methylphenyl **13**, 2-methoxyphenyl **14**, and 4-fluorophenyl **15** under the branch of 3-chlorophenyl **9** as well as 4-nitrophenyl **16** under the branch of 3,4-dichlorophenyl **6** of the Topliss tree. Indeed, none of them was more potent than compound **7** although the majority still maintained the high selectivity at α_{1a}-AR. Comparing 2-methoxyphenyl **14** (K_i = 1.47 nM) and 4-methoxyphenyl **7**, the ortho-substitution suffered about a 2-fold decrease in α_{1a}-AR binding affinity. The lower potency of 4-fluorophenyl **15**

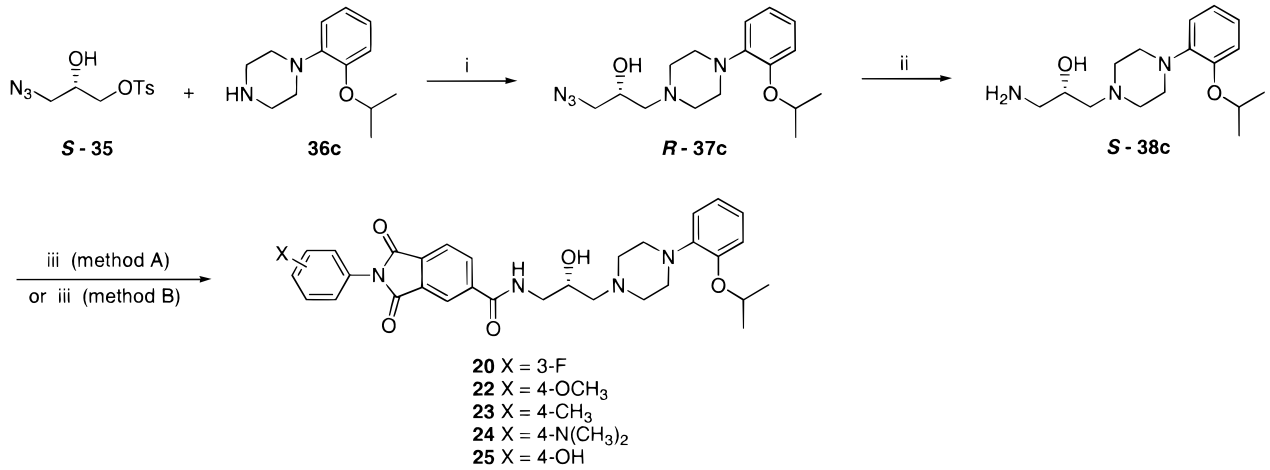
Table 2. Characterization of Phthalimido Phenylpiperazine



compd	R_1	X	yield (%)	method	MS[a]	formula	analysis
1	*i*-C_3H_7	3-F	23	A	561	$C_{31}H_{33}FN_4O_5$	C, H, N
2	C_2H_5	3-F	20	A	547	$C_{30}H_{31}FN_4O_5 \cdot 0.3H_2O$	C, H, N
3	CH_3	3-F	47	B	533	$C_{29}H_{29}FN_4O_5$	C, H, N
4	*i*-C_3H_7	H	26	A	543	$C_{31}H_{34}N_4O_5 \cdot 0.5H_2O$	C, H, N
5	*i*-C_3H_7	4-Cl	15	A	577	$C_{31}H_{33}ClN_4O_5 \cdot 0.2H_2O$	C, H, N
6	*i*-C_3H_7	3,4-Cl_2	6	A	611	$C_{31}H_{32}Cl_2N_4O_5$	C, H, N
			32	B			
7	*i*-C_3H_7	4-OCH_3	27	A	573	$C_{32}H_{36}N_4O_6$	C, H, N
8	*i*-C_3H_7	4-CH_3	27	A	557	$C_{32}H_{36}N_4O_5$	C, H, N
9	*i*-C_3H_7	3-Cl	13	A	577	$C_{31}H_{33}ClN_4O_5 \cdot 0.3H_2O$	C, H, N
10	*i*-C_3H_7	4-$N(CH_3)_2$	26	A	586	$C_{33}H_{39}N_5O_5$	C, H, N
11	*i*-C_3H_7	4-$C(CH_3)_3$	46	B	599	$C_{35}H_{42}N_4O_5$	*b*
12	*i*-C_3H_7	4-OH	23	A	559	$C_{31}H_{34}N_4O_6 \cdot 0.5H_2O$	C, H, N
13	*i*-C_3H_7	3-CH_3	30	A	557	$C_{32}H_{36}N_4O_5 \cdot 1.6H_2O$	C, H, N
14	*i*-C_3H_7	2-OCH_3	26	A	573	$C_{32}H_{36}N_4O_6$	C, H, N
15	*i*-C_3H_7	4-F	43	B	561	$C_{31}H_{33}FN_4O_5 \cdot 0.2H_2O$	C, H, N
16	*i*-C_3H_7	4-NO_2	0	A	588	$C_{31}H_{33}N_5O_7 \cdot 0.3H_2O$	C, H, N
			13	B			
17	*i*-C_3H_7	3,4-$(OCH_3)_2$	28	B	603	$C_{33}H_{38}N_4O_7 \cdot 0.2H_2O$	C, H, N
18	*i*-C_3H_7	3,4,5-$(OCH_3)_3$	10	B	633	$C_{34}H_{40}N_4O_8 \cdot 0.5H_2O$	C, H, N

[a] Electrospray mass spectra, *m/z* M + H^+. [b] HRMS, calcd 599.3233, found 599.3232.

Scheme 4[a]

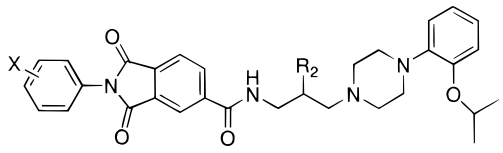


[a] (i) NMP, 100 °C; (ii) 10% Pd/C, H_2, CH_3OH; (iii) method A: **34**, EDCl, HOBT, DMAP, CH_2Cl_2; method B: **34**, HATU, CH_2Cl_2.

(K_i = 1.50 nM) and 4-nitrophenyl **16** (K_i = 1.80 nM) was consistent with the earlier observation that electron-withdrawing substituents seemed slightly detrimental to the α_{1a}-AR affinity. We thus decided to prepare two more analogues, 3,4-dimethoxyphenyl **17** and 3,4,5-trimethoxyphenyl **18**. The additional electron-donating groups in the phenyl ring, however, gave no improvement at the α_{1a}-AR potency (K_i = 0.98 nM and 1.22 nM). Up to this point, the SAR studies at the X site via the Topliss approach led to several α_{1a}-AR potent and selective compounds. For example, compounds **4**, **7**−**10**, **12**, and **13** were highly potent (K_i <1 nM) and selective for α_{1a}-AR over either α_{1b}-AR (majority being >1000-fold) or α_{1d}-AR (majority being >50-fold).

On the basis of the potency, selectivity, hydrophobic π parameters, and electronic σ values, compounds **1**, **7**, **8**, **10**, and **12** were selected for further evaluation at the R_2 site (Table 5). Both enantiomers of compounds **1** and **7** were synthesized. For compound **1**, the *S*-enantiomer **20** was about 8-fold more potent than the *R*-enantiomer **19** at α_{1a}-AR (K_i = 1.02 nM versus 8.0 nM), highly selective for α_{1a}-AR over α_{1b}-AR (>1960 versus >250), and around 23-fold more selective for α_{1a}-AR over α_{1d}-AR (186 versus 8). It is interesting to note that the same trend was also observed for compound **7**. The *S*-enantiomer **22** was about 9-fold more potent than the *R*-enantiomer **21** at α_{1a}-AR (K_i = 0.23 nM versus 2.09 nM), highly selective for α_{1a}-AR over α_{1b}-AR (>7610 versus >960), and around 22-fold more selective for α_{1a}-AR over α_{1d}-AR (539 versus 25). It is not uncommon that the binding of the ligand to the receptor is highly stereospecific because the interacting proteins are also chiral in nature. We were pleased to see that both the binding affinity and selectivity at α_{1a}-AR of *S*-enantiomer **20** and **22** were improved over the corresponding racemates **1** and **7**. The *S*-enantiomers **23**−**25** were also

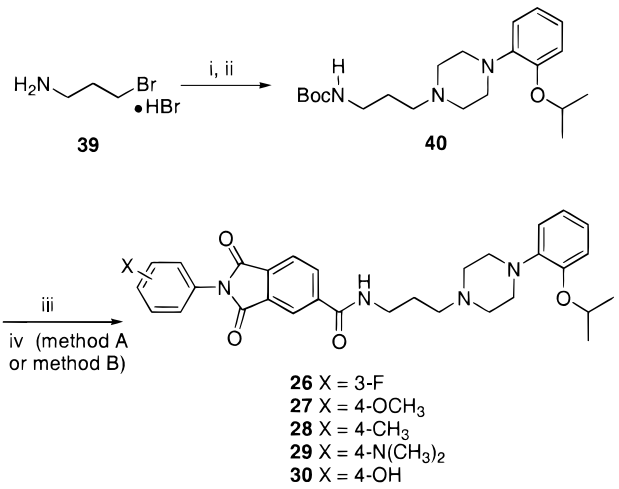
Table 3. Characterization of Phthalimido Phenylpiperazine



compd	R_2	X	yield (%)	method	MS[a]	formula	analysis
19	*R*-OH	3-F	15	A	561	$C_{31}H_{33}FN_4O_5$	C, H, N
20	*S*-OH	3-F	43	B	561	$C_{31}H_{33}FN_4O_5$	C, H, N
21	*R*-OH	4-OCH_3	17	A	573	$C_{32}H_{36}N_4O_6 \cdot H_2O$	C, H, N
22	*S*-OH	4-OCH_3	27	A	573	$C_{32}H_{36}N_4O_6 \cdot 0.5H_2O$	C, H, N
23	*S*-OH	4-CH_3	52	B	557	$C_{32}H_{36}N_4O_5$	C, H, N
24	*S*-OH	4-$N(CH_3)_2$	41	B	586	$C_{33}H_{39}N_5O_5 \cdot 0.3H_2O$	C, H, N
25	*S*-OH	4-OH	17	B	559	$C_{31}H_{34}N_4O_6 \cdot 0.5H_2O$	C, H, N
26	H	3-F	23	A	545	$C_{31}H_{33}FN_4O_4$	C, H, N
27	H	4-OCH_3	41	B	557	$C_{32}H_{36}N_4O_5 \cdot H_2O$	C, H, N
28	H	4-CH_3	26	B	541	$C_{32}H_{36}N_4O_4 \cdot 0.2H_2O$	C, H, N
29	H	4-$N(CH_3)_2$	23	B	570	$C_{33}H_{39}N_5O_4 \cdot 0.7H_2O$	C, H, N
30	H	4-OH	34	A	543	$C_{31}H_{34}N_4O_5 \cdot 0.3H_2O$	C, H, N
31	OCH_3	3-F	29	B	575	$C_{32}H_{35}FN_4O_5$	*b*

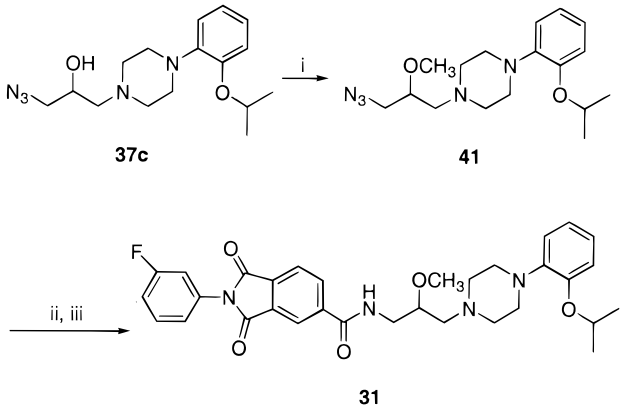
[a] Electrospray mass spectra, *m/z* M + H^+. [b] HRMS, calcd 575.2670, found 575.2672.

Scheme 5[a]



[a] (i) $(Boc)_2O$, CH_2Cl_2; (ii) **36c**, Cs_2CO_3, CH_3CN, reflux; (iii) 25% TFA/CH_2Cl_2; (iv) method A: **34**, EDCl, HOBT, DMAP, CH_2Cl_2; method B: **34**, HATU, CH_2Cl_2.

Scheme 6[a]



[a] (i) NaH, CH_3I, THF; (ii) 10% Pd/C, H_2, CH_3OH; (iii) **34a**, HATU, CH_2Cl_2.

prepared and evaluated. In all three cases, the *S*-enantiomers were about 2-fold (K_i = 0.29 −0.46 nM versus 0.66−0.88 nM) more potent at α_{1a}-AR, maintained at least the same high selectivity for α_{1a}-AR over α_{1b}-AR (at least >4350 versus >2000), and were about 2−9-fold (186−298 versus 94−32) more selective for α_{1a}-AR over α_{1d}-AR as compared to the corresponding racemates **8**, **10**, and **12**.

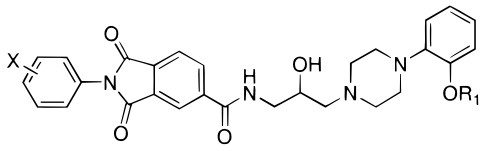
To examine the influence of the hydroxy group on the potency and selectivity at α_{1a}-AR, the des-hydroxy compounds **26**−**30** were prepared. In comparison to the des-hydroxy compounds **26**, **27**, **29**, and **30** (except 4-methylphenyl **28**), the *S*-hydroxy enantiomers (**20**, **22**, **24**, and **25**) sacrificed about 2-fold binding affinity at α_{1a}-AR but gained more than 2-fold selectivity for α_{1a}-AR over α_{1b}-AR, and increased about 2−6-fold selectivity for α_{1a}-AR over α_{1d}-AR. Conversion of hydroxy compound **1** to the corresponding methoxy compound **31** led to a 5-fold loss of potency (K_i = 1.5 nM versus 6.9 nM) at α_{1a}-AR.

The optimization at the R_1, X, and R_2 position of **1** resulted in the identification of a number of equal or slightly less potent but much more selective compounds than tamsulosin at α_{1a}-AR.

Lead Selection

Three *S*-hydroxy compounds (**20**, **23**, and **24**), three des-hydroxy compounds (**26**, **27**, and **28**), and tamsulosin were chosen for receptor selectivity screen against a panel of 25−35 peripheral and central nervous system receptor binding assays (MDS Panlabs). Only those assays with significant response (higher than 50% inhibition at 1 μM concentration) were summarized in Table 6. It was very interesting to note that, in general, all three *S*-hydroxy compounds had less cross activities than those three des-hydroxy compounds. For comparison purpose, K_i values of **24** and tamsulosin were listed in Table 7. Both **24** and tamsulosin showed significant binding affinities to dopamine and serotonin receptors. These results were not too surprising due to the fact that α-AR subtypes share about 45% identity with serotoninergic and dopaminergic receptors.[25] However, compound **24** had at least a 30-fold difference between α_{1a}-AR and all tested receptors while tamsulosin had less than 10-fold selectivity between α_{1a}-AR and dopamine D_3 (2.2-fold) or serotonin 5-HT_{1A} (6.1-fold).

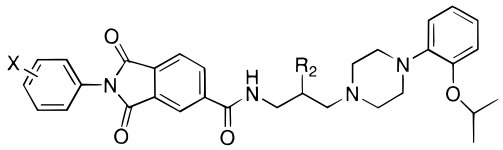
Table 4. Binding Affinities at Cloned Human α_1-AR Subtypes[a]



				$K_i \pm$ SEM (nM)[b]		K_i ratio	
compd	R_1	X	α_{1a}	α_{1b}	α_{1d}	α_{1b}/α_{1a}	α_{1d}/α_{1a}
1	*i*-C_3H_7	3-F	1.50 ± 0.30	>2000, 1835	75 ± 1	>1220	50
2	C_2H_5	3-F	4.25 ± 1.15	>2000, >2000	137 ± 1	>470	32
3	CH_3	3-F	22.00 ± 4.00	>2000, >2000	540 ± 152	>90	25
4	*i*-C_3H_7	H	0.9 ± 0.01	>2000, 1640	65 ± 4	>1820	72
5	*i*-C_3H_7	4-Cl	0.95 ± 0.03	606 ± 137	55 ± 14	640	58
6	*i*-C_3H_7	3,4-Cl_2	1.19 ± 0.03	1225 ± 45	51 ± 1	1030	43
7	*i*-C_3H_7	4-OCH_3	0.77 ± 0.12	>2000, >2000	51 ± 8	>2600	66
8	*i*-C_3H_7	4-CH_3	0.66 ± 0.07	>2000, >2000	62 ± 3	>3030	94
9	*i*-C_3H_7	3-Cl	0.76 ± 0.11	605 ± 64	41 ± 4	800	54
10	*i*-C_3H_7	4-$N(CH_3)_2$	0.80 ± 0.15	>2000, 1600	40 ± 4	>2000	50
11	*i*-C_3H_7	4-$C(CH_3)_3$	3.05 ± 0.45	>2000, >2000	75 ± 14	>650	25
12	*i*-C_3H_7	4-OH	0.88 ± 0.08	>2000, >2000	28 ± 4	>2270	32
13	*i*-C_3H_7	3-CH_3	0.9 ± 0.00	>2000, >2000	73 ± 16	>2220	81
14	*i*-C_3H_7	2-OCH_3	1.47 ± 0.22	1639 ± 111	72 ± 2	1110	49
15	*i*-C_3H_7	4-F	1.50 ± 0.10	1658, >2000	111 ± 6	>1100	74
16	*i*-C_3H_7	4-NO_2	1.80 ± 0.20	>2000, >2000	38 ± 5	>1110	21
17	*i*-C_3H_7	3,4-$(OCH_3)_2$	0.98 ± 0.23	>2000, >2000	56 ± 3	>2040	57
18	*i*-C_3H_7	3,4,5-$(OCH_3)_3$	1.22 ± 0.28	>2000, >2000	152 ± 32	>1640	125

[a] Displacement of ^{125}I-HEAT from α_1-AR subtypes as described in the Experimental Section. [b] SEM: standard error mean.

Table 5. Binding Affinities at Cloned Human α_1-AR Subtypes[a]



				$K_i \pm$ SEM (nM)[b]		K_i ratio	
compd	R_2	X	α_{1a}	α_{1b}	α_{1d}	α_{1b}/α_{1a}	α_{1d}/α_{1a}
19	*R*-OH	3-F	8.00 ± 0.87	>2000, >2000	63 ± 4	>250	8
20	*S*-OH	3-F	1.02 ± 0.08	>2000, >2000	190 ± 35	>1960	186
21	*R*-OH	4-OCH_3	2.09 ± 0.11	>2000, >2000	52 ± 4	>960	25
22	*S*-OH	4-OCH_3	0.23 ± 0.00	1750, >2000	124 ± 5	>7610	539
23	*S*-OH	4-CH_3	0.29 ± 0.01	1650, >2000	54 ± 11	>5690	186
24	*S*-OH	4-$N(CH_3)_2$	0.33 ± 0.03	>2000, >2000	52 ± 5	>6060	158
25	*S*-OH	4-OH	0.46 ± 0.07	>2000, >2000	137 ± 13	>4350	298
26	H	3-F	0.41 ± 0.03	482 ± 51	22 ± 2	1180	54
27	H	4-OCH_3	0.11 ± 0.01	361 ± 103	16 ± 1	3280	145
28	H	4-CH_3	0.16 ± 0.06	>2000, >2000	37 ± 0	>12500	231
29	H	4-$N(CH_3)_2$	0.18 ± 0.01	465 ± 131	15 ± 1	2580	83
30	H	4-OH	0.20 ± 0.04	255 ± 16	10 ± 3	1270	50
31	OCH_3	3-F	6.90 ± 0.10	>2000, >2000	407 ± 71	>290	59
32, tamsulosin			0.13 ± 0.02	1.92 ± 0.17	0.18 ± 0.02	14.8	1.4

[a] Displacement of ^{125}I-HEAT from α_1-AR subtypes as described in the Experimental Section. [b] SEM: standard error mean.

Since these *S*-hydroxy compounds (**20**, **23**, and **24**) were found to be more selective than those des-hydroxy compounds (**26**, **27**, and **28**) for α_{1a}-AR over 25−35 receptors, they were further examined in the functional assay. Antagonist activity was assessed by inhibition of (±)-norepinephrine-induced contractions in isolated rat prostate which predominantly express the α_{1A}-AR subtype and aorta tissues which predominantly express the α_{1D}-AR subtype (MDS Panlabs). Because most compounds showed nonparallel shifts of dose−response curves with either tissue, affinity were reported as pK_B values in Table 8.[26] Although all three antagonists (**20**, **23**, and **24**) were less potent than tamsulosin in their ability to inhibit rat prostate contractions (pK_B = 7.81, 6.78, and 7.39, respectively, versus 9.33), they displayed greater tissue selectivity for inhibition of rat prostate contractions over rat aorta contractions (ratio = 22.6, 7.6, and 35.2, respectively, versus (26.9)). Tamsulosin actually showed a reversed selectivity (26.9-fold) with higher potency to inhibit rat aorta contraction over rat prostate contraction (pK_B = 10.76 versus 9.33). Currently, the apparent discrepancies between the binding affinity at α_{1a}-AR and functional potency in the rat prostate tissues of **20**, **23**, and **24** are not well understood. However, there are two possible explanations. First, the difference might be explained by the fact that these antagonists are inverse agonists (negative antagonists), and hence their affinity is system-dependent.[27a] Second, the difference might suggest the existence of the putative α_{1L}-AR subtype.[27b]

Table 6. Percent Inhibition (±SEM) of Selected Receptors at 1 μM Concentration (Panlabs)

	20	**23**	**24**	**26**	**27**	**28**	**32**, tamsulosin
α_{2A}	29 ± 5	19 ± 3	18 ± 3	78 ± 3	80 ± 5	87 ± 2	82 ± 1
α_{2B}	70 ± 3	75 ± 3	73 ± 7	78 ± 2	79 ± 3	66 ± 4	86 ± 1
α_{2C}	8 ± 5	14 ± 6	11 ± 6	86 ± 3	76 ± 2	83 ± 2	41 ± 4
dopamine D_{2L}	55 ± 4	68 ± 2	71 ± 2	89 ± 1	83 ± 2	83 ± 1	53 ± 2
dopamine D_{2S}	51 ± 3	70 ± 3	71 ± 2	90 ± 2	87 ± 2	94 ± 1	73 ± 1
dopamine D_3	46 ± 3	38 ± 3	47 ± 4	97 ± 1	83 ± 3	86 ± 2	109 ± 1
dopamine $D_{4.7}$	43 ± 1	50 ± 4	50 ± 3	69 ± 4	32 ± 5	20 ± 7	21 ± 2
Ca^{+2} channel, dihydropyridine	67 ± 3	NT[a]	53[b]	76 ± 3	NT	53 ± 3	22 ± 7
Ca^{2+} channel, phenylalkylamine	81 ± 7	NT	57 ± 2	72 ± 11	NT	69 ± 2	20 ± 5
histamine H_1, central	95 ± 3	93 ± 1	88 ± 3	87 ± 4	97 ± 1	97 ± 1	18 ± 5
histamine H_1, peripheral	69 ± 4	NT	NT	75 ± 7	NT	NT	7 ± 3
imidazoline I_2, central	−12 ± 6	NT	NT	66 ± 4	NT	NT	64 ± 2
serotonin 5-HT_1	49 ± 3	64 ± 5	66 ± 3	88 ± 3	90 ± 3	69 ± 3	62 ± 2
serotonin 5-HT_{1A}	98 ± 3	92 ± 2	92 ± 4	95 ± 1	104 ± 2	90 ± 3	115 ± 1
serotonin 5-HT_7	95 ± 4	90 ± 1	95 ± 2	92 ± 4	92 ± 3	95 ± 3	74 ± 2

[a] Percent inhibition not tested. [b] Percent inhibition tested at 10 μM concentration.

Table 7. Receptor Selectivity Studies (Panlabs)

	24		**32**, tamsulosin	
assay	K_i ± SEM (nM)	K_i ratio	K_i ± SEM (nM)	K_i ratio
α_{1a}	0.33 ± 0.05	1	0.13 ± 0.05	1
α_{2A}	ND[a]	ND	63 ± 7	490
α_{2B}	169 ± 18	512	80 ± 15	620
D_{2L}	23 ± 7	70	13 ± 2	100
D_{2S}	25 ± 5	76	78 ± 13	600
D_3	62 ± 6	188	0.28 ± 0.08	2.2
Ca^{+2}(phe)	760 ± 117	2303	ND	ND
H_1(cen)	38 ± 7	115	ND	ND
5-HT_1	773 ± 300	2342	944 ± 386	7260
5-HT_{1A}	15 ± 5	45	0.79 ± 0.11	6.1
5-HT_7	10 ± 2	30	84 ± 5	650

[a] K_i values not determined because low binding activity was seen in the initial screen.

Table 8. Rat Prostate and Aorta Tissue Contraction Studies (Panlabs)

	pK_B ± SEM[a]		
compd	rat aorta	rat prostate	ratio[b]
20	6.47 ± 0.10	7.81 ± 0.29	22.6
23	5.90 ± 0.15	6.78 ± 0.06	7.6
24	5.84 ± 0.37	7.39 ± 0.70	35.2
tamsulosin	10.76 ± 0.12	9.33 ± 0.43	(26.9)[c]

[a] Antagonist dissociation equilibrium constants (pK_B) were calculated as described in the Experimental Section. [b] The ratio of rat aorta K_B/rat prostate K_B. [c] The ratio of rat prostate K_B/rat aorta K_B.

The high potency and selectivity at α_{1a}-AR in the binding assay and the good tissue selectivity observed in the functional assay strongly supported that these *S*-hydroxy phthalimide-phenylpiperazines be further evaluated in pharmacokinetic studies, in vivo intraurethral pressure versus mean arterial pressure studies. As a result,[28] compound **24** which had the best overall profile was selected as the development candidate for the treatment of BPH.

Conclusion

Starting from the library screening hit RWJ 37914 and literature candidates, compound **B** was used as the screening model and resulted in the identification of the second-generation lead **1**. Using the Topliss approach, a number of potent and selective α_{1a}-AR antagonists were developed. As compared to the des-hydroxy compounds, in general, the *S*-hydroxy enantiomers were less potent but more selective at α_{1a}-AR against a panel of 25−35 receptors. In comparison to tamsulosin, the *S*-hydroxy enantiomers (**23** and **24**) were slightly less potent but much more selective at α_{1a}-AR. In the functional assay, the *S*-hydroxy enantiomers were less potent than tamsulosin in inhibiting rat prostate contractions but more selective in inhibiting rat prostate contractions over rat aorta contractions.

Experimental Section

Chemistry. ^{1}H NMR spectra were measured on a Bruker AC-300 (300 MHz) spectrometer using tetramethylsilane as an internal standard. Elemental analyses were obtained by Quantitative Technologies Inc. (Whitehouse, NJ), and the results were within 0.4% of the calculated values unless otherwise mentioned. Melting points were determined in open capillary tubes with a Thomas-Hoover apparatus and were uncorrected. The optical rotation was measured at 25 °C with an Autopol III polarimeter. Electrospray mass spectra (MS-ES) were recorded on a Hewlett-Packard 59987A spectrometer. High-resolution mass spectra (HRMS) were obtained on a Micromass Autospec. E. spectrometer. The term "DMAP" refers to (dimethylamino)pyridine, "TFA" refers to trifluoroacetic acid, "EDCI" refers to 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, "HOBT" refers to hydroxybenzotriazole hydrate, "HATU" refers to *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, "NMP" refers to 1-methyl-2-pyrrolidinone.

General Procedure for the Synthesis of 34a−p. 2-(3-Fluorophenyl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34a). A mixture of the 1,2,4-benzenetricarboxylic anhydride (10 g, 52 mmol) and 3-fluoroaniline (5.77 g, 52 mmol) in glacial acetic acid (200 mL) was stirred at 130 °C for 16 h. The light-brown solution was cooled to 20 °C to give a yellow solid precipitate. The yellow solid was collected via filtration and was washed thoroughly with water to remove the trace amount of acetic acid. The product was dried at 60 °C for 36 h under vacuum to give 11.41 g (77%) of **34a** as a yellow solid: mp 268−269 °C; ^{1}H NMR (DMSO-d_6) δ 8.42 (d, J = 8.1 Hz, 1 H), 8.31 (s, 1 H), 8.09 (d, J = 7.8 Hz, 1 H), 7.60 (m, 1 H), 7.36 (m, 3 H).

2-Phenyl-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34b): mp 257−259 °C; ^{1}H NMR (DMSO-d_6) δ 13.75 (brs, 1 H), 8.42 (d, J = 7.9 Hz, 1 H), 8.31 (s, 1 H), 8.09 (d, J = 7.7 Hz, 1 H), 7.52 (m, 5 H).

2-(4-Chlorophenyl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34c): mp 293−294 °C; ^{1}H NMR (DMSO-d_6) δ 13.75 (brs, 1 H), 8.42 (d, J = 8.1 Hz, 1 H), 8.32 (s, 1 H), 8.09 (d, J = 7.7 Hz, 1 H), 7.62 (d, J = 8.7 Hz, 2 H), 7.51 (d, J = 8.7 Hz, 2 H).

[2-(3,4-Dichloro)phenyl]-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34d): mp 290−292 °C; ^{1}H NMR (DMSO-d_6) δ 13.80 (brs, 1 H), 8.43 (d, J = 8.5 Hz, 1 H), 8.32 (s, 1 H), 8.11 (d, J = 7.7 Hz, 1 H), 7.84 (m, 2 H), 7.53 (dd, J = 8.7, 2.3 Hz, 1 H).

2-(4-Methoxyphenyl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34e): mp 260−261 °C; ^{1}H NMR (DMSO-d_6) δ 8.41 (d, J = 8.5 Hz, 1 H), 8.30 (s, 1 H), 8.06 (d, J = 7.7 Hz, 1 H), 7.36 (d, J = 8.8 Hz, 2 H), 7.08 (d, J = 8.9 Hz, 2 H), 3.81 (s, 3 H).

2-(4-Methylphenyl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34f): mp 258−259 °C; ^{1}H NMR (DMSO-d_6) δ 8.41 (d, J = 7.4 Hz, 1 H), 8.30 (s, 1 H), 8.07 (d, J = 7.8 Hz, 1 H), 7.33 (s, 4 H), 2.38 (s, 3 H).

2-(3-Chlorophenyl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34g): mp 283−284 °C; ^{1}H NMR (DMSO-d_6) δ 8.42 (d, J = 8.0 Hz, 1 H), 8.32 (s, 1 H), 8.10 (d, J = 7.8 Hz, 1 H), 7.54 (m, 4 H).

[2-(4-Dimethylamino)phenyl]-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34h): mp 273−274 °C; ^{1}H NMR (DMSO-d_6) δ 8.40 (d, J = 8.5 Hz, 1 H), 8.28 (s, 1 H), 8.05 (d, J = 7.8 Hz, 1 H), 7.22 (d, J = 8.9 Hz, 2 H), 6.82 (d, J = 8.9 Hz, 2 H), 2.96 (s, 6 H).

2-(4-Nitrophenyl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34i): mp 287−288 °C; ^{1}H NMR (DMSO-d_6) δ 8.43 (m, 3 H), 8.34 (s, 1 H), 8.13 (d, J = 7.7 Hz, 1 H), 7.80 (d, J = 8.7 Hz, 2 H).

[2-(4-*tert*-Butyl)phenyl]-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34j): mp 282−283 °C; ^{1}H NMR (DMSO-d_6) δ 8.42 (d, J = 7.3 Hz, 1 H), 8.31 (s, 1 H), 8.09 (d, J = 7.7 Hz, 1 H), 7.56 (d, J = 8.6 Hz, 2 H), 7.38 (d, J = 8.6 Hz, 2 H), 1.34 (s, 9 H).

2-(4-Hydroxyphenyl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34l): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 13.75 (brs, 1 H), 9.80 (s, 1 H), 8.41 (d, J = 7.5 Hz, 1 H), 8.28 (s, 1 H), 8.05 (d, J = 7.7 Hz, 1 H), 7.22 (d, J = 8.6 Hz, 2 H), 6.88 (d, J = 8.7 Hz, 2 H).

2-(2-Methoxyphenyl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34m): mp 239−240 °C; ^{1}H NMR (DMSO-d_6) δ 8.44 (d, J = 7.9 Hz, 1 H), 8.33 (s, 1 H), 8.09 (d, J = 7.8 Hz, 1 H), 7.51 (t, J = 7.6 Hz, 1 H), 7.40 (d, J = 7.3 Hz, 1 H), 7.23 (d, J = 8.2 Hz, 1 H), 7.10 (t, J = 7.5 Hz, 1 H), 3.76 (s, 3 H).

[2-(3,4-Dimethoxy)phenyl]-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34o): mp 271−272 °C; ^{1}H NMR (DMSO-d_6) δ 8.41 (d, J = 7.5 Hz, 1 H), 8.30 (s, 1 H), 8.06 (d, J = 7.8 Hz, 1 H), 7.09 (m, 2 H), 6.97 (dd, J = 8.6, 2.1 Hz, 1 H), 3.82 (s, 3 H), 3.74 (s, 3 H).

[2-(3,4,5-Trimethoxy)phenyl]-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic Acid (34p): mp 269−270 °C; ^{1}H NMR (DMSO-d_6) δ 8.43 (d, J = 8.1 Hz, 1 H), 8.31 (s, 1 H), 8.08 (d, J = 7.8 Hz, 1 H), 6.83 (s, 2 H), 3.77 (s, 6 H), 3.73 (s, 3 H).

General Procedure for the Synthesis of 37a−c. α-(Azidomethyl)-4-[2-(1-methylethoxy)phenyl]-1-piperazineethanol (37c). The fumarate salt of 1-(2-isopropoxyphenyl)-piperazine **36c** (3.91 g, 12 mmol) was basified with 20% NaOH (aq) (100 mL) and extracted with methylene chloride. The combined organic layers were dried (Na_2SO_4) and concentrated to give 2.74 g of oil. A mixture of the oil and 1-azido-3-(*p*-toluenesulfonyloxy)propan-2-ol **35** (3.25 g, 12 mmol) in NMP was stirred at 100 °C for 36 h. The cooled mixture was diluted with water and extracted with ether, dried (Na_2SO_4), and concentrated. The product was purified by column chromatography (SiO_2) to give 2.92 g (76%) of **37c** as a light-brown oily solid: ^{1}H NMR ($CDCl_3$) δ 6.91 (m, 4 H), 4.59 (m, 1 H), 3.93 (m, 1 H), 3.67 (brs, 1 H), 3.42 (dd, J = 12.6, 3.8 Hz, 1 H), 3.23 (dd, J = 12.6, 5.4 Hz, 1 H), 3.12 (m, 4 H), 2.83 (m, 2 H), 2.53 (m, 3 H), 2.42 (dd, J = 12.2, 3.8 Hz, 1 H), 1.34 (d, J = 6.0 Hz, 6 H); MS (ES) m/z 320 (M + H$^+$). Anal. ($C_{16}H_{25}N_5O_2$) C, H, N.

α-(Azidomethyl)-4-[2-(1-methoxy)phenyl]-1-piperazineethanol (37a): brown oil (89%); ^{1}H NMR ($CDCl_3$) δ 6.85−7.04 (m, 4 H), 3.94 (m, 1 H), 3.87 (s, 3 H), 3.42 (dd, J = 12.7, 3.8 Hz, 1 H), 3.23 (dd, J = 12.7, 5.4 Hz, 1 H), 3.09 (brs, 4 H), 2.87 (m, 2 H), 2.40−2.71 (m, 4 H); MS (ES) m/z 292 (M + H$^+$). Anal. ($C_{14}H_{21}N_5O_2$) C, H, N.

α-(Azidomethyl)-4-[2-(1-ethoxy)phenyl]-1-piperazineethanol (37b): white oil (66%); ^{1}H NMR ($CDCl_3$) δ 6.92 (m, 4 H), 4.06 (q, J = 7.0 Hz, 2 H), 3.94 (m, 1 H), 3.42 (dd, J = 12.8, 3.8 Hz, 1 H), 3.24 (dd, J = 12.6, 5.3 Hz, 1 H), 3.12 (m, 4 H), 2.93 (m, 2 H), 2.59 (m, 3 H), 2.43 (dd, J = 12.2, 3.7 Hz, 1 H), 1.45 (t, J = 6.9 Hz, 3 H); FAB−HRMS (M + H$^+$) calcd 306.1941, found 306.1941.

General Procedure for the Synthesis of 38a−c. α-(Aminomethyl)-4-[2-(1-methylethoxy)phenyl]-1-piperazineethanol (38c). A 10% HCl (6 mL) solution was added to a mixture of **37c** (2.43 g, 7.6 mmol) and 10% Pd/C (1.22 g) in MeOH (60 mL), and the mixture was hydrogenated under H_2 (50 psi) in a Parr shaker for 16 h at 20 °C. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was basified with 20% NaOH and extracted with methylene chloride. The combined organic layers were dried (Na_2SO_4) and concentrated to give 2.2 g (95%) of **38c** as a yellowish oil which was stored under nitrogen in the freezer and used without further purification: ^{1}H NMR ($CDCl_3$) δ 6.91 (m, 4 H), 4.59 (m, 1 H), 3.76 (m, 1 H), 3.12 (m, 4 H), 2.83 (dd, J = 12.7, 3.7 Hz, 2 H), 2.82 (m, 1 H), 2.25−2.68 (m, 8 H), 1.34 (d, J = 6.1 Hz, 6 H); MS (ES) m/z 294 (M + H$^+$).

α-(Aminomethyl)-4-[2-(1-methoxy)phenyl]-1-piperazineethanol (38a): white solid (93%); ^{1}H NMR ($CDCl_3$) δ 6.95 (m, 4 H), 3.87 (s, 3 H), 3.66 (m, 1 H), 3.10 (brs, 4 H), 2.85 (m, 3 H), 2.59 (m, 3 H), 2.44 (m, 2 H); MS (ES) m/z 266 (M + H$^+$).

α-(Aminomethyl)-4-[2-(1-ethoxy)phenyl]-1-piperazineethanol (38b): yellowish oil (87%); ^{1}H NMR ($CDCl_3$) δ 6.92 (m, 4 H), 4.06 (q, J = 7.1 Hz, 2 H), 3.73 (m, 1 H), 3.12 (brs, 4 H), 2.83 (m, 3 H), 2.65 (m, 3 H), 2.43 (m, 2 H), 1.46 (t, J = 7.1 Hz, 3 H); MS (ES) m/z 280 (M + H$^+$).

General Procedure for the Synthesis of 1−18 (Method A). 2-(3-Fluorophenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (1). A mixture of the amine **38c** (226 mg, 0.77 mmol), carboxylic acid **34a** (220 mg, 0.77 mmol), EDCI (151 mg, 0.78 mmol), HOBT (105 mg, 0.78 mmol), DMAP (cat.), and *N*,*N*-diisopropylethylamine (0.52 mL) in methylene chloride (6 mL) was stirred at 20 °C for 3 h under nitrogen. The mixture was concentrated, diluted with 5% K_2CO_3 (aq), and extracted with EtOAc. The combined organic layer was dried (Na_2SO_4) and concentrated. The product was purified by column chromatography (SiO_2) to give 101 mg (23%) of **1** as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.34 (s, 1 H), 8.30 (d, J = 7.8 Hz, 1 H), 8.04 (d, J = 7.8 Hz, 1 H), 7.48 (m, 1 H), 7.26 (m, 1 H), 7.14 (m, 1 H), 6.91 (m, 6 H), 4.59 (m, 1 H), 4.02 (m, 1 H), 3.79 (m, 1 H), 3.45 (m, 1 H), 3.13 (m, 4 H), 2.87 (m, 2 H), 2.62 (m, 2 H), 2.50 (m, 2 H), 1.34 (d, J = 6.0 Hz, 6 H).

2-(3-Fluorophenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-(2-ethoxyphenyl)-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (2). Replacing **38c** with **38b** and following the same procedure as in the preparation of **1** gave **2**: ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1 H), 8.30 (d, J = 7.6 Hz, 1 H), 8.03 (d, J = 7.7 Hz, 1 H), 7.48 (m, 1 H), 7.26 (m, 1 H), 7.13 (m, 1 H), 6.93 (m, 6 H), 4.05 (m, 3 H), 3.79 (m, 1 H), 3.43 (m, 1 H), 3.13 (m, 4 H), 2.87 (m, 2 H), 2.62 (m, 2 H), 2.54 (m, 2 H), 1.45 (t, J = 6.9 Hz, 3 H).

General Procedure for the Synthesis of 1−18 (Method B). 2-(3-Fluorophenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (3). The primary amine **38a** (150 mg, 0.57 mmol) was dissolved in a mixture of *N*,*N*-diisopropylethylamine (290 mg, 2.26 mmol) and methylene chloride (3 mL). To this solution was added carboxylic acid **34a** (160 mg, 0.57 mmol) and HATU (220 mg, 0.57 mmol). The mixture was stirred at 20 °C for 18 h under N_2. The mixture was concentrated, diluted with 5% K_2CO_3 (aq), extracted with ether, dried (Na_2SO_4), and concentrated. The product was purified by column chromatography (SiO_2) to give 140 mg (47%) of **3** as a oily solid: ^{1}H NMR ($CDCl_3$) δ 8.34 (s, 1 H), 8.31 (d, J = 7.6 Hz, 1 H), 8.03 (d, J = 7.8 Hz, 1 H), 7.48 (m, 1 H), 7.26 (m, 2 H), 7.13 (m, 2 H), 7.02 (m, 1 H), 6.92 (m, 3 H), 4.02 (m, 1 H), 3.86 (s, 3 H), 3.79 (m, 1 H), 3.42 (m, 1 H), 3.10 (m, 4 H), 2.89 (m, 2 H), 2.55 (m, 4 H).

2-Phenyl-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (4): light-tan solid; ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1 H), 8.30 (d, J = 7.6 Hz, 1 H), 8.04 (d, J = 7.7 Hz, 1 H), 7.53 (m, 2 H), 7.44 (d, 3 H), 6.90 (m, 5 H), 4.59 (m, 1 H), 4.00 (m, 1 H), 3.77 (m, 1 H), 3.44 (m, 1 H), 3.12 (m, 4 H), 2.85 (m, 2 H), 2.52 (m, 4 H), 1.35 (d, J = 6.1 Hz, 6 H).

2-(4-Chlorophenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (5): off-white solid; ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1 H), 8.30 (d, J = 8.0 Hz, 1 H), 8.03 (d, J = 7.7 Hz, 1 H), 7.50 (d, J = 8.8 Hz, 2 H), 7.41 (d, J = 8.8 Hz, 2 H), 6.91 (m, 5 H), 4.59 (m, 1 H), 4.00 (m, 1 H), 3.79 (m, 1 H), 3.43 (m, 1 H), 3.12 (m, 4 H), 2.85 (m, 2 H), 2.52 (m, 4 H), 1.35 (d, J = 6.1 Hz, 6 H).

[2-(3,4-Dichloro)phenyl]-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (6): light-tan solid; ^{1}H NMR ($CDCl_3$) δ 8.34 (s, 1 H), 8.30 (d, J = 7.9 Hz, 1 H), 8.03 (d, J = 7.7 Hz, 1 H), 7.63 (d, J = 2.2 Hz, 1 H), 7.58 (d, J = 8.7 Hz, 1 H), 7.37 (dd, J = 8.5, 2.3 Hz, 1 H), 7.09 (m, 1 H), 6.90 (m, 4 H), 4.59 (m, 1 H), 4.02 (m, 1 H), 3.79 (m, 1 H), 3.43 (m, 1 H), 3.13 (m, 4 H), 2.86 (m, 2 H), 2.54 (m, 4 H), 1.35 (d, J = 6.1 Hz, 6 H).

2-(4-Methoxyphenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (7): off-white solid; ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1 H), 8.29 (d, J = 7.5 Hz, 1 H), 8.02 (d, J = 7.7 Hz, 1 H), 7.33 (d, J = 8.9 Hz, 2 H), 7.02 (d, J = 9.0 Hz, 2 H), 6.94 (m, 5 H), 4.59 (m, 1 H), 4.01 (m, 1 H), 3.85 (s, 3 H), 3.79 (m, 1 H), 3.42 (m, 1 H), 3.12 (m, 4 H), 2.85 (m, 2 H), 2.52 (m, 4 H), 1.35 (d, J = 6.0 Hz, 6 H).

2-(4-Methylphenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (8): light-tan solid; ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1 H), 8.28 (d, J = 7.6 Hz, 1 H), 8.02 (d, J = 7.7 Hz, 1 H), 7.31 (m, 4 H), 6.92 (m, 5 H), 4.59 (m, 1 H), 4.00 (m, 1 H), 3.79 (m, 1 H), 3.43 (m, 1 H), 3.12 (m, 4 H), 2.85 (m, 2 H), 2.55 (m, 4 H), 2.42 (s, 3 H), 1.34 (d, J = 6.0 Hz, 6 H).

2-(3-Chlorophenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (9): light-tan solid; ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1 H), 8.30 (d, J = 7.8 Hz, 1 H), 8.04 (d, J = 7.7 Hz, 1 H), 7.43 (m, 3 H), 6.92 (m, 6 H), 4.58 (m, 1 H), 4.02 (m, 1 H), 3.79 (m, 1 H), 3.46 (m, 1 H), 3.12 (m, 4 H), 2.87 (m, 2 H), 2.55 (m, 4 H), 1.35 (d, J = 5.9 Hz, 6 H).

2-[4-(Dimethylamino)phenyl]-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (10): light brown solid; ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 1 H), 8.27 (d, J = 8.0 Hz, 1 H), 8.01 (d, J = 7.8 Hz, 1 H), 7.23 (s, 2 H), 6.90 (m, 5 H), 6.80 (d, J = 8.9 Hz, 2 H), 4.60 (m, 1 H), 4.00 (m, 1 H), 3.76 (m, 1 H), 3.44 (m, 1 H), 3.12 (m, 4 H), 3.00 (s, 6 H), 2.85 (m, 2 H), 2.52 (m, 4 H), 1.35 (d, J = 6.3 Hz, 6 H).

[2-(4-*tert*-Butyl)phenyl]-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (11): light-tan solid; ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1 H), 8.28 (d, J = 7.7 Hz, 1 H), 8.02 (d, J = 7.8 Hz, 1 H), 7.53 (d, J = 8.5 Hz, 2 H), 7.35 (d, J = 8.5 Hz, 2 H), 6.93 (m, 5 H), 4.59 (m, 1 H), 4.03 (m, 1 H), 3.79 (m, 1 H), 3.43 (m, 1 H), 3.13 (m, 4 H), 2.89 (m, 2 H), 2.55 (m, 4 H), 1.34 (d, J = 6.5 Hz, 15 H).

2-(4-Hydroxyphenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (12): off-white solid; ^{1}H NMR ($CDCl_3$) δ 8.26 (m, 2 H), 7.98 (d, J = 8.1 Hz, 1 H), 7.24 (s, 2 H), 7.04 (m, 1 H), 6.90 (m, 6 H), 4.59 (m, 1 H), 4.03 (m, 1 H), 3.78 (m, 1 H), 3.45 (m, 1 H), 3.13 (m, 4 H), 2.87 (m, 2 H), 2.55 (m, 4 H), 1.35 (d, J = 6.0 Hz, 6 H).

2-(3-Methylphenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (13): light-tan solid; ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1 H), 8.29 (d, J = 7.6 Hz, 1 H), 8.02 (d, J = 7.7 Hz, 1 H), 7.37 (m, 1 H), 7.23 (m, 2 H), 6.92 (m, 6 H), 4.59 (m, 1 H), 4.01 (m, 1 H), 3.79 (m, 1 H), 3.43 (m, 1 H), 3.12 (m, 4 H), 2.85 (m, 2 H), 2.54 (m, 4 H), 2.43 (s, 3 H), 1.35 (d, J = 6.0 Hz, 6 H).

2-(2-Methoxyphenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (14): off-white solid; ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1 H), 8.27 (d, J = 8.4 Hz, 1 H), 8.02 (d, J = 7.9 Hz, 1 H), 7.45 (brt, J = 7.2 Hz, 1 H), 7.08 (m, 2 H), 6.88 (m, 6 H), 4.61 (m, 1 H), 4.00 (m, 1 H), 3.80 (m, 4 H), 3.46 (m, 1 H), 3.13 (m, 4 H), 2.88 (m, 2 H), 2.55 (m, 4 H), 1.35 (d, J = 6.0 Hz, 6 H).

2-(4-Fluorophenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (15): light-tan solid; ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1 H), 8.28 (d, J = 7.8 Hz, 1 H), 8.03 (d, J = 7.7 Hz, 1 H), 7.43 (m, 2 H), 7.23 (t, J = 8.7 Hz, 2 H), 7.04 (m, 1 H), 6.91 (m, 4 H), 4.59 (m, 1 H), 4.00 (m, 1 H), 3.80 (m, 1 H), 3.42 (m, 1 H), 3.12 (m, 4 H), 2.86 (m, 2 H), 2.53 (m, 4 H), 1.35 (d, J = 6.0 Hz, 6 H).

2-(4-Nitrophenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (16): light-tan solid; ^{1}H NMR ($CDCl_3$) δ 8.36 (m, 3 H), 8.07 (d, J = 7.8 Hz, 1 H), 7.77 (d, J = 8.9 Hz, 2 H), 7.09 (m, 1 H), 6.90 (m, 5 H), 4.59 (m, 1 H), 4.02 (m, 1 H), 3.76 (m, 1 H), 3.43 (m, 1 H), 3.13 (m, 4 H), 2.86 (m, 2 H), 2.56 (m, 4 H), 1.35 (d, J = 6.0 Hz, 6 H).

[2-(3,4-Dimethoxy)phenyl]-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (17): light-tan solid; ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1 H), 8.28 (d, J = 8.0 Hz, 1 H), 8.02 (d, J = 7.7 Hz, 1 H), 6.92 (m, 8 H), 4.59 (m, 1 H), 4.01 (m, 1 H), 3.93 (s, 3 H), 3.90 (s, 3 H), 3.80 (m, 1 H), 3.44 (m, 1 H), 3.12 (m, 4 H), 2.85 (m, 2 H), 2.53 (m, 4 H), 1.35 (d, J = 6.0 Hz, 6 H).

[2-(3,4,5-Trimethoxy)phenyl]-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (18): off-white solid; ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1 H), 8.29 (d, J = 8.0 Hz, 1 H), 8.02 (d, J = 7.6 Hz, 1 H), 6.92 (m, 5 H), 6.65 (s, 2 H), 4.59 (m, 1 H), 4.01 (m, 1 H), 3.89 (s, 3 H), 3.88 (s, 6 H), 3.76 (m, 1 H), 3.43 (m, 1 H), 3.12 (m, 4 H), 2.85 (m, 2 H), 2.56 (m, 4 H), 1.35 (d, J = 6.0 Hz, 6 H).

(*R*)-α-(Azidomethyl)-4-[2-(1-methylethoxy)phenyl]-1-piperazineethanol (*R*-37c). The fumarate salt of 1-(2-isopropoxyphenyl)-piperazine **36c** (112.5 g, 345 mmol) was basified with 20% NaOH (aq) (500 mL) and extracted with methylene chloride (3×). The combined organic extracts were dried (Na_2SO_4) and concentrated to give about 70 g of oil. A mixture of the oil and (2*S*)-3-azido-2-hydroxypropyl *p*-toluenesulfonate *S*-**35**[22] (91 g, 335 mmol) was stirred at 100 °C in NMP in the presence of triethylamine (70 g, 690 mmol) for 30 h. The cooled mixture was diluted with water and extracted with ether (3 × 500 mL), back-washed once with NaCl (sat.) (100 mL), dried (Na_2SO_4), and concentrated. The product was purified by column chromatography (SiO_2) and recrystallized from methylene chloride/hexane to give 70.6 g (66%) (98.8% ee assay by reverse phase chiralcel AD column, 4.6 × 150 mm, mobile phase: 95/5/0.1 of hexane/ethanol/diethylamine, retention time = 9.217 min) of *R*-**37c** as a off-white oily solid: $[\alpha]_D^{25}$ = −3.6° (c = 1, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 6.91 (m, 4 H), 4.59 (m, 1 H), 3.93 (m, 1 H), 3.67 (brs, 1 H), 3.42 (dd, J = 12.6, 3.8 Hz, 1 H), 3.23 (dd, J = 12.6, 5.4 Hz, 1 H), 3.12 (m, 4 H), 2.83 (m, 2 H), 2.53 (m, 3 H), 2.42 (dd, J = 12.2, 3.8 Hz, 1 H), 1.34 (d, J = 6.0 Hz, 6 H); MS (ES) m/z 320 (M + H$^+$). Anal. ($C_{16}H_{25}N_5O_2$) C, H, N.

(*S*)-α-(Aminomethyl)-4-[2-(1-methylethoxy)phenyl]-1-piperazineethanol (*S*-38c). Replacing **37c** with *R*-**37c** and following the same procedure as in the preparation of **38c** gave *S*-**38c** (96%) as a yellowish oil which was stored under nitrogen in the freezer and used without further purification: $[\alpha]_D^{25}$ = +23.6° (c = 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.91 (m, 4 H), 4.59 (m, 1 H), 3.76 (m, 1 H), 3.12 (m, 4 H), 2.83 (dd, J = 12.7, 3.7 Hz, 2 H), 2.82 (m, 1 H), 2.24−2.69 (m, 8 H), 1.34 (d, J = 6.1 Hz, 6 H); MS (ES) m/z 294 (M + H$^+$).

(*S*)-2-(3-Fluorophenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (20). Replacing **38a** with *S*-**38c** and following the procedure as in the preparation of **3** (method B) gave **20** (43%) as a light-tan solid: $[\alpha]_D^{25} = +8.3°$ ($c = 1$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.34 (s, 1 H), 8.30 (d, J = 7.8 Hz, 1 H), 8.04 (d, J = 7.8 Hz, 1 H), 7.48 (m, 1 H), 7.26 (m, 1 H), 7.14 (m, 1 H), 6.91 (m, 6 H), 4.59 (m, 1 H), 4.02 (m, 1 H), 3.79 (m, 1 H), 3.45 (m, 1 H), 3.13 (m, 4 H), 2.87 (m, 2 H), 2.62 (m, 2 H), 2.50 (m, 2 H), 1.34 (d, J = 6.0 Hz, 6 H).

(*S*)-2-(4-Methoxyphenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (22). Replacing **38c**, **34a** with *S*-**38c**, **34e** and following the procedure as in the preparation of **1** (method A) gave **22** (27%) as a light-tan solid; $[\alpha]_D^{25} = +9.2°$ ($c = 1$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.31 (s, 1 H), 8.28 (d, J = 7.5 Hz, 1 H), 8.02 (d, J = 7.7 Hz, 1 H), 7.34 (d, J = 8.8 Hz, 2 H), 7.03 (d, J = 9.0 Hz, 2 H), 6.90 (m, 5 H), 4.59 (m, 1 H), 4.00 (m, 1 H), 3.86 (s, 3 H), 3.80 (m, 1 H), 3.43 (m, 1 H), 3.12 (m, 4 H), 2.86 (m, 2 H), 2.52 (m, 4 H), 1.35 (d, J = 6.2 Hz, 6 H).

(*S*)-2-(4-Methylphenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (23): off-white solid; $[\alpha]_D^{25} = +11.7°$ ($c = 0.2$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.32 (s, 1 H), 8.28 (d, J = 7.8 Hz, 1 H), 8.01 (d, J = 7.8 Hz, 1 H), 7.31 (m, 4 H), 7.02 (m, 1 H), 6.90 (m, 4 H), 4.59 (m, 1 H), 4.01 (m, 1 H), 3.79 (m, 1 H), 3.43 (m, 1 H), 3.12 (m, 4 H), 2.85 (m, 2 H), 2.55 (m, 4 H), 2.42 (s, 3 H), 1.35 (d, J = 6.0 Hz, 6 H).

(*S*)-2-[4-(Dimethylamino)phenyl]-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (24): light-tan solid; $[\alpha]_D^{25} = +9.6°$ ($c = 0.2$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.29 (s, 1 H), 8.27 (d, J = 8.0 Hz, 1 H), 8.00 (d, J = 7.7 Hz, 1 H), 7.23 (s, 2 H), 6.90 (m, 5 H), 6.80 (d, J = 8.9 Hz, 2 H), 4.59 (m, 1 H), 4.00 (m, 1 H), 3.78 (m, 1 H), 3.44 (m, 1 H), 3.12 (m, 4 H), 3.00 (s, 6 H), 2.85 (m, 2 H), 2.52 (m, 4 H), 1.35 (d, J = 6.1 Hz, 6 H).

(*S*)-2-(4-Hydroxyphenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (25): off-white solid; $[\alpha]_D^{25} = +8.2°$ ($c = 0.2$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.26 (m, 2 H), 7.98 (d, J = 8.1 Hz, 1 H), 7.24 (s, 2 H), 7.05 (m, 1 H), 6.90 (m, 6 H), 4.59 (m, 1 H), 4.03 (m, 1 H), 3.78 (m, 1 H), 3.44 (m, 1 H), 3.13 (m, 4 H), 2.85 (m, 2 H), 2.55 (m, 4 H), 1.35 (d, J = 6.0 Hz, 6 H).

(*S*)-α-(Azidomethyl)-4-[2-(1-methylethoxy)phenyl]-1-piperazineethanol (*S*-37c). Replacing (2*S*)-3-azido-2-hydroxypropyl *p*-toluenesulfonate *S*-**35** with (2*R*)-3-azido-2-hydroxypropyl *p*-toluenesulfonate *R*-**35** and following the same procedure as in the preparation of *R*-**37c** gave *S*-**37c** (50%) (99.3% ee assay by reverse phase chiralcel AD column, 4.6 × 150 mm, mobile phase: 95/5/0.1 of hexane/ethanol/diethylamine, retention time = 8.383 min) as a off-white oily solid: $[\alpha]_D^{25} = +3.4°$ ($c = 1$, CH_3OH); ^1H NMR ($CDCl_3$) δ 6.92 (m, 4 H), 4.59 (m, 1 H), 3.92 (m, 1 H), 3.67 (brs, 1 H), 3.42 (dd, J = 12.6, 3.8 Hz, 1 H), 3.23 (dd, J = 12.6, 5.4 Hz, 1 H), 3.12 (m, 4 H), 2.83 (m, 2 H), 2.53 (m, 3 H), 2.42 (dd, J = 12.2, 3.8 Hz, 1 H), 1.34 (d, J = 6.0 Hz, 6 H); MS (ES) m/z 320 ($M + H^+$).

(*R*)-α-(Aminomethyl)-4-[2-(1-methylethoxy)phenyl]-1-piperazineethanol (*R*-38c). Replacing **37c** with *S*-**37c** and following the same procedure as in the preparation of **38c** gave *R*-**38c** (90%) as a yellowish oil which was stored under nitrogen in the freezer and used without further purification: $[\alpha]_D^{25} = -22.5°$ ($c = 1$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.91 (m, 4 H), 4.59 (m, 1 H), 3.76 (m, 1 H), 3.12 (m, 4 H), 2.83 (dd, J = 12.7, 3.7 Hz, 2 H), 2.82 (m, 1 H), 2.25−2.68 (m, 8 H), 1.34 (d, J = 6.1 Hz, 6 H); MS (ES) m/z 294 ($M + H^+$).

(*R*)-2-(3-Fluorophenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (19): light-tan solid; $[\alpha]_D^{25} = -8.4°$ ($c = 1$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.35 (s, 1 H), 8.30 (d, J = 7.8 Hz, 1 H), 8.04 (d, J = 7.8 Hz, 1 H), 7.48 (m, 1 H), 7.26 (m, 1 H), 7.15 (m, 1 H), 6.92 (m, 6 H), 4.59 (m, 1 H), 4.03 (m, 1 H), 3.79 (m, 1 H), 3.46 (m, 1 H), 3.13 (m, 4 H), 2.88 (m, 2 H), 2.63 (m, 2 H), 2.51 (m, 2 H), 1.34 (d, J = 6.0 Hz, 6 H).

(*R*)-2-(4-Methoxyphenyl)-2,3-dihydro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (21): off-white solid; $[\alpha]_D^{25} = -9.3°$ ($c = 1$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.32 (s, 1 H), 8.28 (d, J = 7.5 Hz, 1 H), 8.03 (d, J = 7.7 Hz, 1 H), 7.34 (d, J = 8.8 Hz, 2 H), 7.04 (d, J = 9.0 Hz, 2 H), 6.91 (m, 5 H), 4.59 (m, 1 H), 4.01 (m, 1 H), 3.86 (s, 3 H), 3.81 (m, 1 H), 3.43 (m, 1 H), 3.13 (m, 4 H), 2.86 (m, 2 H), 2.53 (m, 4 H), 1.35 (d, J = 6.2 Hz, 6 H).

[3-[4-[2-(1-Methylethoxy)phenyl]-1-piperazinyl]propyl-carbamic Acid, 1,1-Dimethylethyl Ester (40). 3-Bromopropylamine hydrobromide **39** (5 g, 22.8 mmol) was dissolved in 10% NaOH (50 mL) and extracted with methylene chloride. The combined extracts were dried (Na_2SO_4) and concentrated. To the free base in methylene chloride was added $(Boc)_2O$ (5.23 g, 23.9 mmol), and this mixture was stirred at 20 °C for 4 h. The methylene chloride reaction mixture was washed with water, diluted citric acid (6%), $NaHCO_3$ (aq), and NaCl (aq), dried (Na_2SO_4), and concentrated. The product was purified by column chromatography (SiO_2) to yield the Boc-protected amine (4.84 g, 89%). The fumarate salt of 1-(2-isopropoxyphenyl)-piperazine **36c** (5.1 g, 15 mmol) was basified with 20% NaOH (aq) (100 mL), extracted with methylene chloride, dried (Na_2SO_4), and concentrated to give a yellow oil (3.15 g). A mixture of the yellow oil, the Boc-protected amine (3.42 g, 14.3 mmol), and Cs_2CO_3 (4.66 g, 14.3 mmol) in CH_3CN (50 mL) was heated at reflux overnight. The solid was filtered off, and the filtrate was concentrated. The product was purified by column chromatography (SiO_2) to give 4.4 g (81%) of **40** as a thick oil: MS (ES) m/z 378 ($M + H^+$). Anal. ($C_{21}H_{35}N_3O_3$) C, H, N.

General Procedure for the Synthesis of 26−30 (Method A). 2-(3-Fluorophenyl)-2,3-dihydro-*N*-[3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (26). The Boc-protected amine **40** (3.97 g, 10.5 mmol) was dissolved in 25% TFA/methylene chloride (50 mL) and stirred at 20 °C for 5 h. The solvent was evaporated, and the residue was dissolved in 20% NaOH (aq) (100 mL) and stirred for 40 min. The free base was extracted into methylene chloride (3×), and the solvent was evaporated to give 3.0 g (95%) of the free amine as a light yellow oil. A solution of this free amine (3.0 g, 10.8 mmol) and *N*,*N*-diisopropylethylamine (5.6 g, 43.3 mmol) in methylene chloride (80 mL) was added to a mixture containing EDCI (2.08 g, 10.8 mmol), HOBT (1.46 g, 10.8 mmol), a catalytic amount of DMAP (5 mol %), and carboxylic acid **34a** (3.09 g, 10.8 mmol) and stirred overnight at 20 °C under N_2. The reaction mixture was washed with 5% K_2CO_3 (aq), NaCl (aq), dried (Na_2SO_4), and concentrated. The product was purified by column chromatography (SiO_2) to give 1.34 g (23%) of **26** as a light yellow solid: ^1H NMR ($CDCl_3$) δ 9.22 (brs, 1 H), 8.34 (m, 2 H), 7.99 (d, J = 8.1 Hz, 1 H), 7.46 (m, 1 H), 7.18 (m, 3 H), 6.89 (m, 4 H), 4.57 (m, 1 H), 3.67 (m, 2 H), 3.09 (m, 4 H), 2.71 (m, 6 H), 1.87 (m, 2 H), 1.33 (d, J = 6.1 Hz, 6 H).

General Procedure for the Synthesis of 26−30 (Method B). 2-(4-Methoxyphenyl)-2,3-dihydro-*N*-[3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (27). The free amine of **40** (1 equiv) obtained as above (method A) was stirred with carboxylic acid **34e** (1 equiv) and HATU (1 equiv) in methylene chloride overnight and worked up as above to give **27** as an off-white solid: ^1H NMR ($CDCl_3$) δ 9.11 (brs, 1 H), 8.30 (m, 2 H), 7.97 (d, J = 8.2 Hz, 1 H), 7.28 (m, 2 H), 7.01 (d, J = 7.0 Hz, 2 H), 6.90 (m, 4 H), 4.57 (m, 1 H), 3.86 (s, 3 H), 3.67 (m, 1 H), 3.09 (m, 4 H), 2.70 (m, 6 H), 1.86 (m, 2 H), 1.33 (d, J = 6.0 Hz, 6 H).

2-(4-Methylphenyl)-2,3-dihydro-*N*-[3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (28): light-tan solid; ^1H NMR ($CDCl_3$) δ 9.12 (brs, 1 H), 8.31 (m, 2 H), 7.98 (d, J = 8.2 Hz, 1 H), 7.28 (m, 4 H), 6.90 (m, 4 H), 4.57 (m, 1 H), 3.66 (m, 2 H), 3.08 (m, 4 H), 2.80 (m, 6 H), 2.42 (m, 3 H), 1.86 (m, 2 H), 1.33 (d, J = 6.0 Hz, 6 H).

[2-(4-Dimethylamino)phenyl]-2,3-dihydro-*N*-[3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (29): light brown solid; ^{1}H NMR ($CDCl_3$) δ 9.04 (brs, 1 H), 8.28 (m, 2 H), 7.96 (d, J = 7.6 Hz, 1 H), 7.20 (d, J = 8.9 Hz, 2 H), 6.86 (m, 6 H), 4.57 (m, 1 H), 3.66 (m, 2 H), 3.08 (m, 4 H), 3.01 (s, 6 H), 2.75 (m, 6 H), 1.86 (m, 2 H), 1.33 (d, J = 6.0 Hz, 6 H).

2-(4-Hydroxyphenyl)-2,3-dihydro-*N*-[3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (30): light-tan solid; ^{1}H NMR ($CDCl_3$) δ 8.96 (brs, 1 H), 8.29 (m, 2 H), 7.95 (d, J = 7.9 Hz, 1 H), 7.18 (d, J = 8.4 Hz, 2 H), 6.86 (m, 6 H), 4.56 (m, 1 H), 3.64 (m, 2 H), 3.10 (m, 4 H), 2.71 (m, 6 H), 1.87 (m, 2 H), 1.33 (d, J = 6.0 Hz, 6 H).

1-(3-Azido-2-methoxypropyl)-4-[2-(1-methylethoxy)-phenyl]piperazine (41). Compound **37c** (0.8 g, 2.5 mmol) was dissolved in anhydrous THF (50 mL) and cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 0.2 g, 5.0 mmol) was added, the solution was stirred for 10 min, and CH_3I (0.53 g, 3.8 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h. The second portions of sodium hydride (0.1 g, 2.5 mmol) and CH_3I (0.15 mL) were added, and this mixture was stirred for another 2 h at 0 °C. The reaction was quenched with saturated NH_4Cl (aq), the organic solvent was evaporated, and the aqueous layer was extracted with methylene chloride (3×), dried (Na_2SO_4), and concentrated. The product was purified by column chromatography (SiO_2) to give 0.69 g (83%) of **41** as oily solid: ^{1}H NMR ($CDCl_3$) δ 6.90 (m, 4 H), 4.60 (m, 1 H), 3.50 (m, 5 H), 3.35 (m, 1 H), 3.18 (m, 4 H), 2.55 (m, 6 H), 1.32 (d, J = 6.1 Hz, 6 H); MS (ES) m/z 334 (M + H$^+$).

2-(3-Fluorophenyl)-2,3-dihydro-*N*-[2-methoxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1*H*-isoindole-5-carboxamide (31). A 10% HCl solution (0.3 mL) was added to a mixture of **41** (0.64 g, 1.9 mmol) and 10% Pd/C (0.13 g) in MeOH (5 mL). This mixture was hydrogenated under H_2 (50 psi) in a Parr shaker overnight and filtered through Celite, and the filtrate was concentrated. The residue was basified with 20% NaOH (aq), extracted with methylene chloride (3×), dried (Na_2SO_4), and concentrated to give the free amine (~100%) as a yellow oil. The amine was used directly without further purification: MS (ES) m/z 308 (M + H$^+$).

The amine (0.15 g, 0.49 mmol) was dissolved in a mixture of methylene chloride (4 mL) and *N*,*N*-diisopropylethylamine (0.25 g,1.95 mmol). To this solution was added a mixture of HATU (0.185 g, 0.49 mmol) and carboxylic acid **34a** (0.14 g, 0.49 mmol), and the reaction was stirred overnight under N_2 at 20 °C. The solvent was evaporated, and the product was purified by flash chromatography (SiO_2) to give 0.08 g (29%) of **31** as a light yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.36 (m, 2 H), 8.18 (brs, 1 H), 8.02 (d, J = 7.7 Hz, 1 H), 7.47 (m, 1 H), 7.22 (m, 3 H), 7.00 (m, 1 H), 6.87 (m, 3 H), 4.57 (m, 1 H), 3.76 (m, 3 H), 3.50 (s, 3 H), 3.22 (m, 10 H), 1.35 (d, J = 6.0 Hz, 6 H).

Biology. Receptor Binding Assays. DNA Cloning, COS Cell Transfection, and Membrane Preparation. The cDNA clones encoding the three human α_1-AR subtypes were obtained by reverse transcription-polymerase chain reaction amplification from human hippocampus and prostate polyA+ RNA libraries (Clontech, Palo Alto, CA). cDNA clones were verified by sequence analysis, and any deviation from published sequence was corrected by site-directed mutagenesis. cDNAs were subcloned into the pcDNA3 mammalian expression vector (Invitrogen Corp, Carlsbad, CA). COS-1 cells were transfected by the standard DEAE-dextran method with chloroquine shock.[29] Each tissue culture dish was inoculated with 3.5 × 106 cells and transfected with 10 μg of DNA. At 72 h post-transfection, the cells were scraped into TE buffer (50 mM Tris-HCl, 5 mM EDTA, pH 7.4). The cell suspension was disrupted with a Brinkman Polytron, setting 8, for 10 s. The disrupted cells were centrifuged at 1000*g* for 10 min at 4 °C. Supernatants were centrifuged at 34500*g* for 20 min at 4 °C. The membrane pellets were suspended in TNE buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, pH 7.4). The protein concentration was determined with the Bio-Rad DC Protein Assay kit (Hercules, CA) following membrane solubilization with Triton X-100.

Competitive Binding Assays. Assays were done in 96-well plates with a 200 μL final volume per well. Test compound concentrations for competition curves ranged from 0.1 pM to 10 μM in half-log increments. Next, 0.1 μg of α_{1a}- or α_{1b}-AR-expressing membrane protein, or 2.6 μg of α_{1d}-AR-expressing membrane protein, were added to TNE buffer with a final concentration of 50 pM [^{125}I] HEAT (2200Ci/mmol) and the appropriate concentration of test compound. Following a 25 °C incubation for 1 h, the plates were filtered onto GF/C filterplates (Packard Instruments Co., Meriden, CT) and washed with ice cold saline and 0.05% Tween-20. Levels of radioactivity were determined using a Packard TopCount liquid scintillation counter. Competition curves were analyzed with the use of the curve-fitting capabilities of GraphPad Prism software (GraphPad Software, Inc., San Diego, CA). The concentration of antagonist needed to inhibit specific binding by 50% (IC_{50}) was used to calculate K_i values according to the relationship $K_i = IC_{50}/(1 + [\text{radioligand}]/K_d)$, where the K_d is the dissociation constant of the radioligand at the receptor.[30]

Radioligand Binding Studies (Panlabs). The percent inhibition of radioligand binding activity in the presence of 1 μM of each tested compound was determined in duplicate. Radioligand binding assays for each of the monoamine receptors and calcium channels examined were performed in a 50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, pH 7.4 buffer. The method for each receptor or Ca^{2+} channel is summarized here and was performed essentially as described in the references noted. The recombinant human α_{2a}- and α_{2c}-adrenergic, dopaminergic D_{2L}, D_{2S}, D_3, $D_{4.7}$, and serotonergic 5-HT_{1A} and 5-HT_7 receptors were expressed in CHO cells, from which membranes were prepared as described for the α_1-adrenoceptors. Competition binding assays to the α_{2a}- and α_{2c}-adrenergic receptors were performed using the [^{3}H]MK912 radioligand and an incubation period of 60 min at 25 °C.[31] WB4101 (10 μM) was used to determine the level of nonspecific binding. Rat kidney cortical membranes were the source of the α_{2b}-adrenergic receptors, and [^{3}H]yohimbine at 25 °C for 30 min was used to determine the level of binding activity.[32] Phentolamine (10 μM) was used to measure nonspecific binding. For D_{2L} and $D_{4.7}$ the level of binding of [^{3}H]spiperone was determined following a 25 °C incubation for 120 min, and a 37 °C incubation for D_{2S} and D_3 for 120 min.[33−39] Haloperidol (10 μM) was used to determine nonspecific binding levels of the D_{2L}, D_{2S}, and $D_{4.7}$ receptors; and 25 μM S(−)sulpiride was used to determine the nonspecific binding for the D_3 receptor. The level of binding of [^{3}H]8-OH-DPAT at the 5-HT_{1A} receptor following a 60 min incubation at 25 °C was determined.[40] Metergoline (10 μM) was used to measure nonspecific binding. Binding of [^{3}H]LSD to the 5-HT_7 receptor was measured following a 120 min incubation at 37 °C, with 5 μM 5-HT used to determine nonspecific binding.[41,42] Membranes from rat cerebral cortex were used as the source of serotonin 5-HT_1 receptors, dihydropyridine-sensitive Ca^{2+} L channels, and imidazoline I_2 receptors. [^{3}H]5-HT was used in the binding studies for the 5-HT_1 receptor, with a 10 min incubation at 37 °C, and 10 μM 5-HT for determination of nonspecific binding.[43] Binding to the dihydropyridine-sensitive Ca^{2+} L channels was measured with the radioligand [^{3}H]nitrendipine in a 25 °C incubation for 90 min.[44,45] Nifedipine (1 μM) was used to determine the degree of nonspecific binding. Binding at 25 °C for 30 min of the radioligand [^{3}H]idazoxan was used to measure the activity at the imidazoline I_2 receptor.[46,47] Nonspecific binding was determined in the presence of 1 μM idazoxan. Guinea pig brain and guinea pig lung were the sources of central and peripheral histamine H_1 receptors, respectively. The radioligand used to assay binding activity at the histamine receptors was [^{3}H]-pyrilamine with a 25 °C incubation for 60 min for the central receptors and 30 min for the peripheral receptors.[48,49] Pyrilamine (1 μM) and 1 μM mepyramine were used to determine nonspecific binding levels for the central and peripheral H_1 receptors, respectively. Following the incubation period, the membranes were filtered and washed three to four times prior

to determining the level of radioligand binding by liquid scintillation counting.

K_i values are reported as the mean ± SEM of three independent experiments. Competition curves were fitted, and IC_{50} values were estimated, by nonlinear least squares regression analysis using GraphPad Prism Software (GraphPad, San Diego, CA). K_i values were derived from the IC_{50} values by the Cheng−Prusoff equation.[30]

Functional Assays. Rat Isolated Prostate and Aorta Tissue Assays (Panlabs). Long Evans male rats weighing 275 ± 25 g were killed by cervical dislocation, and the abdominal aorta and prostate gland were removed. Aortic strips 3−4 mm wide were prepared and placed in 10 mL isolated tissue baths under a resting tension of 2 g. Prostate strips measuring 8−10 mm in length and 1−2 mm in width were placed under a resting tension of 2 g. Tissues were bathed in modified Kreb's solution of the following composition (g/L): NaCl 6.9, KCl 0.35, KH_2PO_4 0.16, $NaHCO_3$ 2.1, $CaCl_2$ 0.28, $MgSO_4 \cdot 7H_2O$ 0.29, (+)-glucose 1.0. Baths were maintained at 37 °C and constantly bubbled with 95% oxygen and 5% CO_2, pH 7.4, with the solution being changed at frequent intervals throughout the 60 min equilibration period. Tissue strips were connected to isometric transducers connected to a strip chart recorder. Prior to beginning concentration−response curves, tissues were exposed to (±)-norepinephrine (NE) at a concentration of 1.0 μM. A response of 0.5 g tension was required for the tissue to be used for concentration−response curves. Following a 90 min wash period, a cumulative concentration−response curve was obtained to NE concentrations of 0.001−100 μM in half log increments. After completion of the concentration−response curve, the tissue was washed for 90 min, and one of three concentrations of antagonist was added and incubated for 5 min before a second cumulative concentration curve was obtained. In a number of cases, Schild analysis could not be performed as a result of depression of the maximal response by high antagonist concentrations and the resulting nonparallel slopes of the concentration−response curves. Estimates of affinity were obtained by the receptor dissociation constant, K_B, from the concentration−response curves, and represented as the negative logarithm, pK_B. Each NE concentration−response curve was analyzed using GraphPad Prism software to estimate midpoint location (EC_{50}). EC_{50} values were obtained in the presence and absence of antagonist and used to calculate the antagonist dissociation equilibrium constants according to the relationship $K_B = [B]/(CR - 1)$, where [B] is the antagonist concentration at which a concentration ratio could be accurately determined and CR is the concentration ratio.

Acknowledgment. We thank Linda Mulcahy and Deborah Loughney for helpful discussions.

Supporting Information Available: Elemental analyses data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bylund, D. B.; Eikenberg, D. C.; Hieble, J. P.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Molinoff, P. B.; Ruffolo, R. R., Jr.; Trendelenburg, U. IV. International union of pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.* **1994**, *46*, 121−136.

(2) Hieble, J. P.; Bylund, D. B.; Clarke, D. E.; Eikenburg, D. C.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Ruffolo, R. R. International union of pharmacology X. Recommendation for nomenclature of α_1-adrenoceptors: consensus update. *Pharmacol. Rev.* **1995**, *47*, 267−270.

(3) Muramatsu, I.; Ohmura, T.; Kigoshi, S.; Hashimoto, S.; Oshita, M. Pharmocological subclassification of α_1-adrenoceptors in vascular smooth muscle. *Br. J. Pharmacol.* **1990**, *99*, 197−201.

(4) Forray, C.; Bard, J. A.; Wetzel, J. M.; Chiu, G.; Shapiro, E.; Tang, R.; Lepor, H.; Hartig, P. R.; Weinshank, R. L.; Branchek, T. A.; Gluchowski, C. The α_1-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human α_{1c} subtype. *Mol. Pharmacol.* **1994**, *45*, 703−708.

(5) Marshall, I.; Burt, R. P.; Chapple, C. R. Noradrenaline contractions of human prostate mediated by α_{1A}-(α_{1c}-)adrenoceptor subtype. *Br. J. Pharmacol.* **1995**, *115*, 781−786.

(6) Burt, R. P.; Chapple, C. R.; Marshall, I. The role of capacitative Ca^{+2} influx in the α_{1B}-adrenoceptor-mediated contraction to phenylephrine of the rat spleen. *Br. J. Pharmacol.* **1995**, *116*, 2327−2333.

(7) Hatano, A.; Takahashi, H.; Tamaki, M.; Komeyama, T.; Koizumi, T.; Takeda, M. Pharmacological evidence of distinct α_1-adrenoceptor subtypes mediating the contraction of human prostatic urethra and peripheral artery. *Br. J. Pharmacol.* **1994**, *113*, 723−728.

(8) (a) Kenny, B. A.; Chalmers, D. H.; Philpott, P. C.; Naylor, A. M. Characterization of an α_{1D}-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. *Br. J. Pharmacol.* **1995**, *115*, 981−986. (b) Aboud, R.; Shafii, M.; Docherty, J. R. Investigation of the subtypes of α_1-adrenoceptor mediating contractions of rat aorta, vas deferens and spleen. *Br. J. Pharmacol.* **1993**, *109*, 80−87.

(9) Lepor, H.; Henry, D.; Laddu, A. R. The efficacy and safety of terazosin in the treatment of BPH. *Prostate* **1991**, *18*, 345−355.

(10) Holme, J. B.; Christensen, M. M.; Rasmussen, P. C.; Jacobsen, F.; Nielsen, J.; Norgaard, J. P.; Olesen, S.; Noev, I.; Wolf, H.; Husted, S. E. 29-Week doxazosin treatment in patients with symptomatic benign prostatic hyperplasia. *Scand. J. Urol. Nephrol.* **1994**, *28*, 77−82.

(11) Lepor, H. The emerging role of alpha antagonists in the therapy of benign prostatic hyperplasia. *J. Androl.* **1991**, *12*, 389−394.

(12) (a) Kawabe, K.; Ueno, A.; Takimoto, Y.; Aso, Y.; Kato, H. Use of an α_1-blocker, YM 617, in the treatment of benign prostatic hypertrophy. *J. Urol.* **1990**, *144*, 908−912. (b) Rabasseda, X.; Fitzpatrick, J. M. Tamsulosin, the first prostate-selective α_{1A}-adrenoceptor antagonist for the treatment of symptomatic benign prostatic hyperplasia. *Drugs Today* **1996**, *32* (Suppl. C), 1−12.

(13) (a) Djavan, B.; Marberger, M. A meta-analysis on the efficacy and tolerability of α_1-adrenoceptor antagonists in patients with lower urinary tract symptoms suggestive of benign prostatic obstruction. *Eur. Urol.* **1999**, *36*, 1−13. (b) Lowe, F. Alpha-1-adrenoceptor blockade in the treatment of benign prostatic hyperplasia. Prostate Cancer Prostatic Dis. **1999**, *2*, 110−119. (c) Schulman, C. C.; Cortvriend, J.; Jonas, U.; Lock, T. M. T. W.; Vaage, S.; Speakman, M. J. Tamsulosin, the first prostate-selective alpha-1A-adrenoceptor antagonist. *Eur. Urol.* **1996**, *29*, 145−154. (d) Jardin, A.; Andersson, K. E.; Caine, M.; et al. Alpha-blockers in the treatment of BPH. In Denis, L.; Griffiths, K.; Khoury, S.; Cockett, A. T. K. McConnell, J.; Chatelain, C.; Murphy, G.; Yoshida, O. *The fourth international consultation on benign prostatic hyperplasia (BPH)*; Health Publication Ltd: Plymouth, United Kingdom, 1998; pp 599−632.

(14) [^{125}I] HEAT: ((±)-β-([^{125}I] Iodo-4-hydroxyphenyl)-1-ethyl-aminoethyl-tetralone) was obtained from NEN™ Life Science Products (Boston, MA).

(15) Testa, R.; Guarneri, L.; Taddei, C.; Poggesi, E.; Angelico, P.; Sartani, A.; Leonardi, A.; Gofrit, O. N.; Meretyk, S.; Caine, M. Functional antagonistic activity of Rec 15/2739, a novel alpha 1 antagonist selective for the lower urinary tract, on noradrenaline-induced contraction of human prostate and mesenteric artery. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 1237−1246.

(16) Shibata, K.; Foglar, R.; Horie, K.; Obika, K.; Sakamoto, A.; Ogawa, S.; Tsujimoto, G. KMD-3213, a novel, potent, α_{1a}-adrenoceptor-selective antagonist: characterisation using recombinant human α_1-adrenoceptors and native tissues. *Mol. Pharmacol.* **1995**, *48*, 250−258.

(17) Wetzel, J. M.; Miao, S. W.; Forray, C.; Borden, L. A.; Branchek, T. A.; Gluchowski, C. Discovery of α_{1a}-adrenergic receptor antagonists based on the L-type Ca^{+2} channel antagonist niguldipine. *J. Med. Chem.* **1995**, *38*, 1579−1581.

(18) Cui, W.; Nakanishi, H.; Wetzel, J. M.; Gluchowski, C. A molecular docking study of the binding of the dihydropyridine α_{1a} antagonist SNAP 5089 to the human α_{1a}-adrenergic receptor. *Abstracts of Papers*; 210th National Meeting of the American Chemical Society, Chicago, IL, August 20−24 1995, American Chemical Society: Washington, DC, 1995; Issue Pt. 2, MEDI-045.

(19) Hamaguchi, N.; True, T. A.; Saussy, D. L., Jr.; Jeffs, P. W. Phenylalanine in the second membrane-spanning domain of α_{1A}-adrenergic receptor determines subtype selectivity of dihydropyridine antagonists. *Biochemistry* **1996**, *35*, 14312−14317.

(20) Holy, A. *Collect. Czech. Chem. Commun.* **1989**, *54*, 446−454.

(21) Martin, G. E.; Elgin, R. J., Jr.; Mathiasen, J. R.; Davis, C. B.; Kesslick, J. M.; Baldy, W. J.; Shank, R. P.; DiStefano, D. L.; Fedde, C. L.; Scott, M. K. Activity of aromatic substituted phenylpiperazines lacking affinity for dopamine binding sites in a preclinical test of antipsychotic efficacy. *J. Med. Chem.* **1989**, *32*, 1052−1056.

(22) Juricova, K.; Smrckova, S.; Holy, A. *Collect. Czech. Chem. Commun.* **1995**, *60*, 237−250.

(23) Topliss, J. G. Utilization of operational schemes for analogue synthesis in drug design. *J. Med. Chem.* **1972**, *15*, 1006−1011.

(24) Topliss, J. G. A manual method for applying the Hansch approach to drug design. *J. Med. Chem.* **1977**, *20*, 463−469.
(25) Trumpp-Kallmeyer, S.; Hoflack, J.; Bruinvels, A.; Hibert, M. Modeling of G-protein-coupled receptors: application to dopamine, acetylcholine and mammalian opsin receptors. *J. Med. Chem.* **1992**, *35*, 3448−3462.
(26) Van Rossum, J. M. Cumulative dose−response curves II. Technique for the making of dose−response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn.* **1963**, *143*, 299−330.
(27) (a) Leff, P. The two-state model of receptor activation. *Trends Pharmacol. Sci.* **1995**, *16*, 89−97. (b) Ford, A. P. D. W.; Arredondo, N. F.; Blue, D. R.; Bonhaus, D. W.; Jasper, J.; Kava, M. S.; Lesnick, J.; Pfister, J. R.; Shieh, I. A.; Vimont, R. L.; Williams, T. J.; McNeal, J. E.; Stamey, T. A.; Clarke, D. E. RS 17053 (N-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine hydrochloride), a selective α_{1A}-adrenoceptor antagonist, displays low affinity for functinal α_1-adrenoceptors in human prostate: implications for adrenoceptor classification. *Mol. Pharmacol.* **1996**, *49*, 209−215.
(28) Detailed results of pharmacokinetic studies and in vivo animal studies will be published elsewhere.
(29) (a) McCutchan, J. H.; Pagano, J. S. Enhancement of the infectivity of simian virus 40 deoxyribonucleic acid with diethyl aminoethyl-dextran. *J. Natl. Cancer Inst.* **1968**, *41*, 351−356. (b) Luthman, H.; Magnusson, G. High efficiency polyoma DNA transfection of chloroquine treated cells. *Nucleic Acids Res.* **1983**, *11*, 1295−1308.
(30) Cheng, Y.-C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(31) Uhlen, S.; Porter, A. C.; Neubig, R. R. The novel alpha-2 adrenergic radioligand [^{3}H]MK912 is alpha-2C selective among human alpha-2A, alpha-2B and alpha-2C adrenoceptors. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1558−1565.
(32) Connaughton, S.; Docherty, R. Functional evidence for heterogeneity of peripheral prejunctional α_2-adrenoceptors. *Br. J. Pharmacol.* **1990**, *101*, 285−290.
(33) Grandy, D. K.; Marchionni, M. A.; Makam, H.; Stofko, R. E.; Alfano, M.; Frothingham, L.; Fischer, J. B.; Bruke-Howie, K. J.; Bunzow, J. R.; Seiver, A. C.; Civelli, O. Cloning of the cDNA and gene for a human D_2 dopamine receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9762−9766.
(34) Bunzow, J. R.; Van Tol, H. H. M.; Grandy, D. K.; Albert, P.; Salon, J.; Christie, M.; Machida, C. A.; Neve, K. A.; Civelli, O. Cloning and expression of rat D_2 dopamine receptor cDNA. *Nature* **1988**, *336*, 783−787.
(35) Hayes, G.; Biden, T. J.; Selbie, L. A.; Shine, J. Structural subtypes of the dopamine D_2 receptor are functionally distinct: Expression of the clone D_{2A} and D_{2B} subtypes in a heterologous cell line. *Mol. Endocrin.* **1992**, *6*, 920−926.
(36) Sokoloff, P.; Giros, B.; Martres, M. P.; Bouthenet, M. L.; Schwartz, J. C. Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. *Nature* **1990**, *347*, 146−151.
(37) Sibley, D. R.; Monsma, F. J. Molecular biology of dopamine receptors. *Trends Pharmacol. Sci.* **1992**, *13*, 60−69.
(38) Van Tol, H. H. M.; Wu, C. M.; Guan, H. C.; O'Hara, K.; Bunzow, J. R.; Civelli, O.; Kennedy, J.; Seeman, P.; Niznik, H. B.; Jovanovic, V. Multiple dopamine D_4 receptor variants in the human population. *Nature* **1992**, *358*, 149−152.
(39) Van Tol, H. H. M.; Bunzow, J. R.; Guan, H. C.; Sunahara, R. K.; Seeman, P.; Niznik, H. B.; Civelli, O. Cloning of the gene for a human dopamine D_4 receptor with high affinity for the antipsychotic clozapine. *Nature* **1991**, *350*, 610−614.
(40) Martin, G. R.; Humphrey, P. P. A. Receptor for 5-hydroxytryptamine: current perspectives on classification and nomenclature. *Neuropharmacology* **1994**, *33*, 261−273.
(41) Shen, Y.; Monsma, F. J., Jr.; Metcalf, M. A.; Jose, P. A.; Hamblin, M. W.; Sibley, D. R. Molecular cloning and expression of a 5-hydroxytryptamine 7 serotonin receptor subtype. *J. Biol. Chem.* **1993**, *268*, 18200−18204.
(42) Roth, B. L.; Craigo, S. C.; Choudhary, M. S.; Uluer, S.; Monsma, F. J., Jr.; Shen, Y.; Meltzer, H. Y.; Sibley, D. R. Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 1403−1410.
(43) Middlemiss, D. N. Stereoselective blockade at [^{3}H]5-HT binding sites and at the 5-HT autoreceptor by propranolol. *Eur. J. Pharmacol.* **1984**, *101*, 289−293.
(44) Gould, R. J.; Murphy, K. M. M.; Snyder, S. H. [^{3}H]nitrendipine-labeled calcium channels discriminate inorganic calcium agonists and antagonists. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 3650−3656.
(45) Ehlert, F. J.; Roeske, W. R.; Itoga, E.; Yamamura, H. I. The binding of [^{3}H]nitrendipine to receptors for calcium channel antagonists in the heart, cerebral cortex and ileum of rats. *Life Sci.* **1982**, *30*, 2191−2202.
(46) Brown, C. M.; Mackinnon, A. C.; McGrath, J. C.; Spedding, M.; Kilpatrick, A. T. α_2-Adrenoceptor subtypes and imidazoline-like binding in the rat brain. *Br. J. Pharmacol.* **1990**, *99*, 803−809.
(47) Michel, M. C.; Ernsberger, P. Keeping and eye on the I site: imidazoline-preferring receptor. *Trends. Pharmacol. Sci.* **1992**, *13*, 369−370.
(48) Hill, S. J.; Emson, P. C.; Young, J. M. The binding of tritiated mephyramine to histamine H1 receptors in guinea pig brain. *J. Neurochem.* **1978**, *31*, 997−1004.
(49) Dini, S.; Caselli, G. F.; Ferrari, M. P.; Giani, R.; Clavenna, G. Heterogeneity of [^{3}H]-mepyramine binding sites in guinea pig cerebellum and lung. *Agents Actions* **1991**, *33*, 181−184.

JM9905918