Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 2263–2275

BIOORGANIC & MEDICINAL CHEMISTRY

Design, Synthesis and Biological Evaluation of Pyridine-Phenylpiperazines: A Novel Series of Potent and Selective α_{1a}-Adrenergic Receptor Antagonist

Gee-Hong Kuo,[a,*] Catherine Prouty,[a] William V. Murray,[a] Virginia Pulito,[a] Linda Jolliffe,[a] Peter Cheung,[a] Sally Varga,[a] Mary Evangelisto[a] and Charles Shaw[b]

[a]*Drug Discovery Division, The R.W. Johnson Pharmaceutical Research Institute, 1000 Route 202, PO Box 300, Raritan, NJ 08869, USA*

[b]*Analytical Development Global Chemical & Pharmaceutical Development Division, The R.W. Johnson Pharmaceutical Research Institute, 1000 Route 202, PO Box 300, Raritan, NJ 08869, USA*

Received 24 February 2000; accepted 16 May 2000

Abstract—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 the *R*-hydroxy enantiomers. As compared to the des-hydroxy analogues, the ***S***-hydroxy enantiomers displayed comparable potency and better selectivity at α_{1a}-AR. The *S*-hydroxy enantiomer **17** ($K_i = 0.79$ nM; $\alpha_{1b}/\alpha_{1a} = 800$; $\alpha_{1d}/\alpha_{1a} = 104$) was slightly less potent but much more selective at α_{1a}-AR than tamsulosin ($K_i = 0.13$ nM, $\alpha_{1b}/\alpha_{1a} = 15$, $\alpha_{1d}/\alpha_{1a} = 1.4$). Compound **17** displayed higher selectivity in inhibiting rat prostate contraction over rat aorta contraction and also exhibited a higher degree of uroselectivity than tamsulosin in the anesthetized dog model.

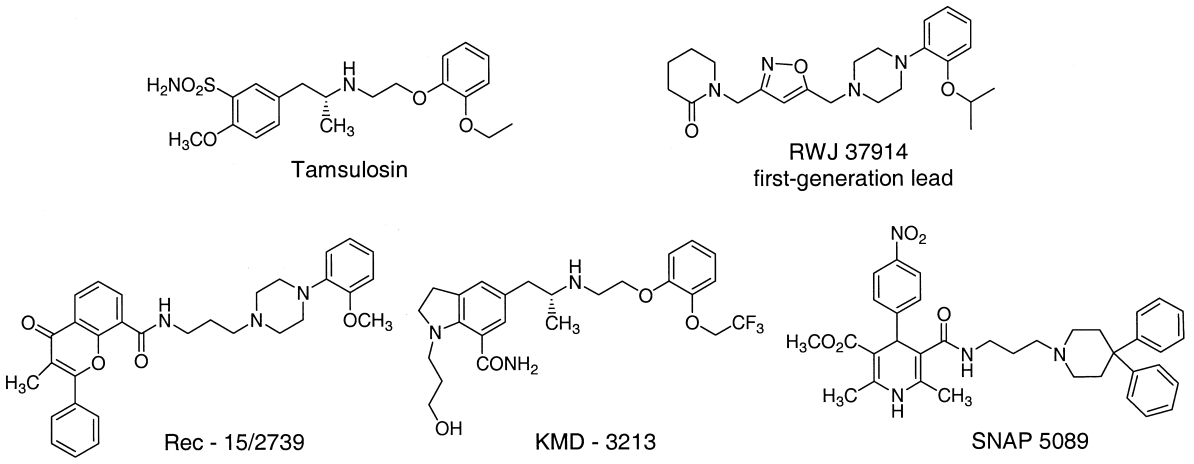
Introduction

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 system.[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 be in part attributed to their non-selective 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 US in 1997 (Scheme 1). It has been shown to have modest selectivity for α_{1a}-AR over α_{1b}-AR (K_i of $\alpha_{1a} = 0.13$ nM, K_i of $\alpha_{1b} = 1.92$ nM, $\alpha_{1b}/\alpha_{1a} = 15$) and almost no selectivity for α_{1a}-AR over α_{1d}-AR (K_i of $\alpha_{1d} = 0.18$ nM, $\alpha_{1d}/\alpha_{1a} = 1.4$). 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.

*Corresponding author. Tel.: +1-908-704-4330; fax: +1-908-526-6469; e-mail: gkuo@prius.jnj.com

0968-0896/00/$ - see front matter
PII: S0968-0896(00)00151-6

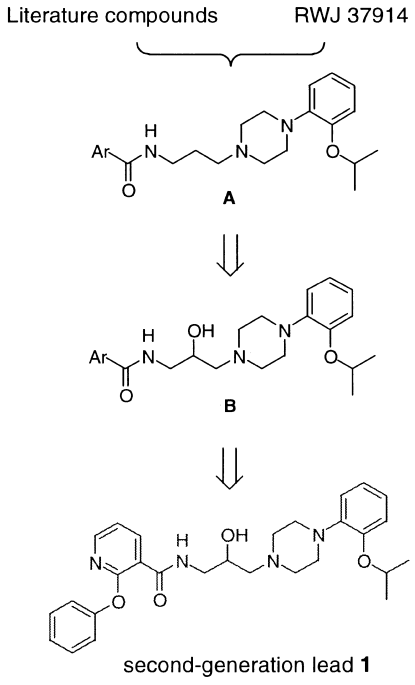


Scheme 1.

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 $\alpha_{1a} = 16.5$ nM, K_i of $\alpha_{1b} > 2000$ nM and K_i of $\alpha_{1d} = 2871$ nM) (Scheme 1). Therefore, RWJ 37914 had >121-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.

In an effort to improve the α_{1a}-AR binding affinity, we decided to modify the lead structure based upon other potent α_{1a}-AR antagonists in literature; 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 of the antagonists 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** ($\alpha_{1a} = 1.54$ nM, $\alpha_{1b} = 892$ nM and $\alpha_{1d} = 212$ nM) was identified.[20] Compound **1** had 579-fold higher affinity for the α_{1a}-AR over α_{1b}-AR and 137-fold higher affinity for the α_{1a}-AR over α_{1d}-AR. In comparison to the first-generation lead RWJ 37914, compound **1** exhibited improved binding affinity (11-fold) and maintained good selectivity at the α_{1a}-AR.



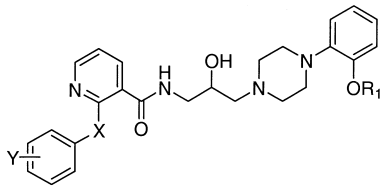
Scheme 2.

Chemistry

All of the racemic hydroxy compounds **1–7, 12–15** (Table 1) had been synthesized via the route shown in Scheme 3. Alkylation of either the commercially available alkoxyphenylpiperazines **26a–b** or the known **26c**[21] with the azido-tosylate **25**[22] gave azido-piperazines **27**. Hydrogenation of **27** gave the primary amines **28**. The primary amines **28** were coupled with either the arylcarbonyl chlorides (method A) or arylcarboxylic acids using EDCI, HOBT (method B) to give the desired products **1–7**, **12–15**. The ketone **24** was prepared by the Swern oxidation of the corresponding hydroxy compound **1** as shown in Scheme 3.

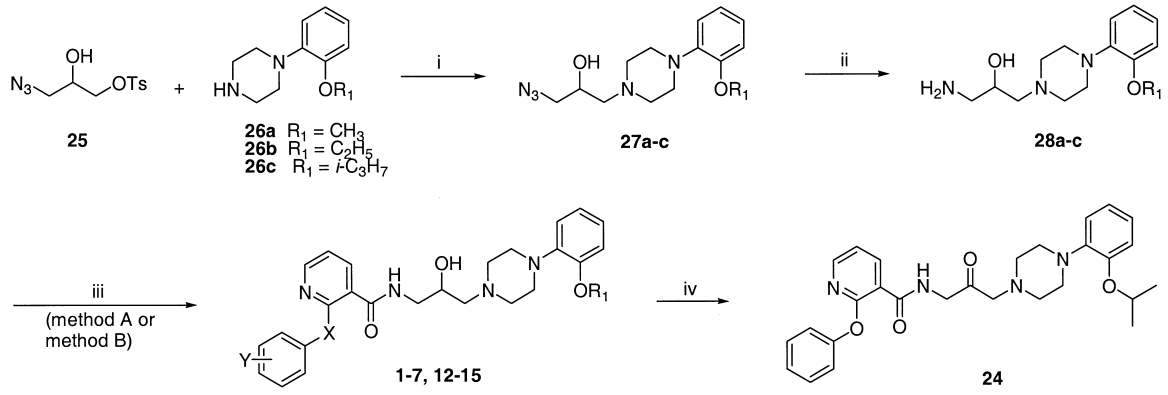
In the cases where neither the arylcarbonyl chlorides nor the arylcarboxylic acids were readily available, an

Table 1. Binding affinities at cloned human α_1-AR subtypes[a]



Compound	R_1	X	Y	$K_i \pm$ SEM[b] (nM)			K_i ratio	
				α_{1a}	α_{1b}	α_{1d}	α_{1b}/α_{1a}	α_{1d}/α_{1b}
1	*i*-C_3H_7	O	H	1.54±0.24	892±57	212±37	579	137
2	C_2H_5	O	H	8.87±0.65	136±102	207±22	15	23
3	CH_3	O	H	30.0±2.08	1570±346	147±33	52	5
4	*i*-C_3H_7	O	4-Cl	1.0±0.07	837±51	74±13	834	73
5	*i*-C_3H_7	O	3,4-Cl_2	2.07±0.19	>2000>2000	51±11	>1000	25
6	*i*-C_3H_7	O	4-CH_3	0.52±0.09	928±128	21±6	1797	40
7	*i*-C_3H_7	O	4-$C(CH_3)_3$	3.33±0.30	>2000>2000	72±8	>606	22
8	*i*-C_3H_7	O	3-$N(CH_3)_2$	1.93±0.55	956±160	59±11	495	31
9	*i*-C_3H_7	O	2-CH_3	1.07±0.33	730±79	1.9±0.4	684	2
10	*i*-C_3H_7	O	2-OCH_3	1.73±0.34	581±64	2.0±0.2	335	1
11	*i*-C_3H_7	O	4-OCH_3	2.38±0.12	1912±174	131±19	804	55
12	*i*-C_3H_7	S	H	2.0±0.15	1221±48	71±7	611	35
13	*i*-C_3H_7	S	4-Cl	2.60±0.15	807±44	301±16	310	116
14	*i*-C_3H_7	NH	H	25.3±1.8	735±32	25±7	29	1
15	*i*-C_3H_7	NH	3-CF_3	25.0±3.1	1020±13	79±7	41	3

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



Scheme 3. (i) NMP. Et_3N, 100°C; (ii) 10% Pd/C, H_2, CH_3OH; (iii) method A: ArCOCl, DMAP, CH_2Cl_2; method B: $ArCO_2H$, EDCI, HOBT, DMAP, CH_2Cl_2; (iv) DMSO, $(COCl)_2$, CH_2Cl_2, −78°C.

alternative synthetic route was pursued (Scheme 4). The primary amine **28c** was coupled with 2-chloronicotinic acid in the presence of EDCI, HOBT, DMAP to give the common intermediate **29**. Reaction of **29** with various substituted phenols at 110 °C in the presence of Cs_2CO_3 gave the products **8–11** (Table 1).

Optically active *S*-hydroxy compounds (**17, 19** and **20**) were prepared as shown in Scheme 5 starting from the optically active *S*-azido-tosylate *S*–**25**.[23] The *R*-hydroxy enantiomers (**16** and **18**) were prepared from the corresponding *R*-azido-tosylate.[23]

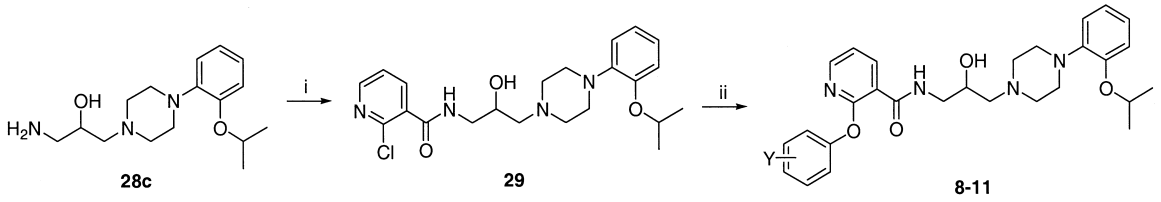
Preparation of the des-hydroxy compounds (**21** and **22**) were shown in Scheme 6. Alkylation of piperazine **26c** with *N*-Boc protected 3-bromopropylamine gave the piperazine derivative **31**. Removal of the Boc-protecting group with 25% TFA/CH_2Cl_2, followed by the coupling reaction with arylcarbonyl chlorides gave the des-hydroxy compounds.

The preparation of the *O*-methylated compound **23** was shown in Scheme 7. Methylation of the hydroxy-azido-piperazine **27c** with methyl iodide in the presence of sodium hydride gave **32**. Hydrogenation of **32** followed by amide bond formation with arylcarboxylic acid gave the *O*-methylated product **23**.

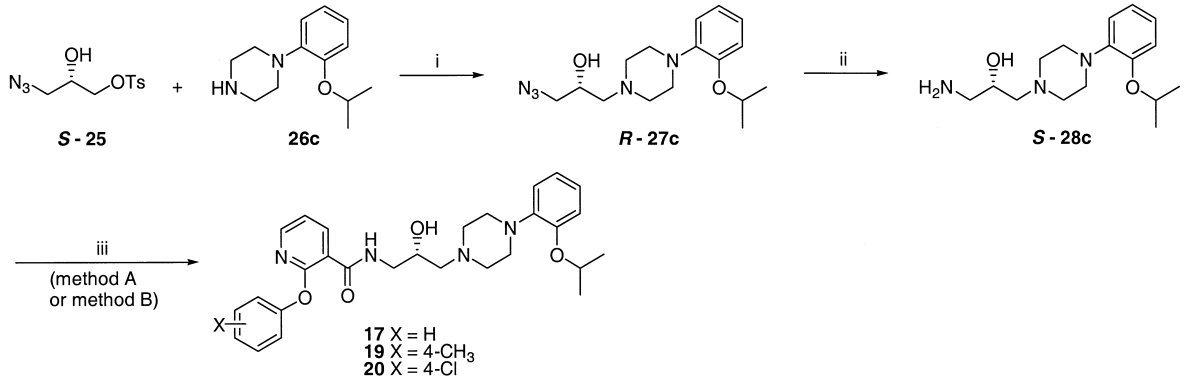
Results and Discussion

Lead optimization

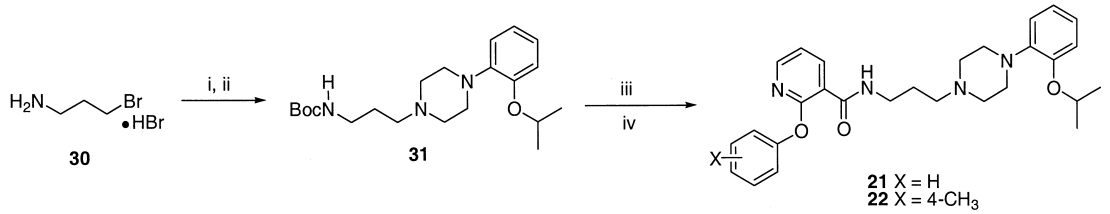
With the second-generation lead **1** in hand, we first re-examined the structure–activity relationships (SAR) of the phenylpiperazines at the R_1 site (Table 1). Indeed,



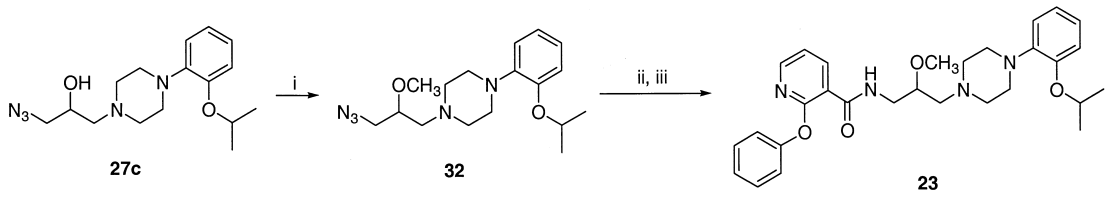
Scheme 4. (i) 2-chloronicotinic acid, EDCI, HOBT, DMAP, CH_2Cl_2; (ii) ArOH, Cs_2CO_3, NMP, 110°C.



Scheme 5. (i) NMP, Et_3N, 100°C; (ii) 10% Pd/C, H_2, CH_3OH; (iii) method A: ArCOCl, DMAP, CH_2Cl_2; method B: $ArCO_2H$, EDCI, HOBT, DMAP, CH_2Cl_2.



Scheme 6. (i) $(Boc)_2O$, CH_2Cl_2; (ii) **26c**, Cs_2CO_3, CH_3CN, reflux; (iii) 25% TFA/CH_2Cl_2; (iv) ArCOCl, DMAP, CH_2Cl_2.



Scheme 7. (i) NaH, CH_3I, THF; (ii) 10% Pd/C, H_2, CH_3OH; (iii) $ArCO_2H$, HATU, CH_2Cl_2.

compound **1** which has the bulkier *o*-isoproxy 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 ($K_i = 1.54$ nM versus 8.87 or 30 nM) and selectivity ($\alpha_{1b}/\alpha_{1a} = 579$-fold versus 15- or 52-fold; $\alpha_{1d}/\alpha_{1a} =$ 137-fold versus 23- or 5-fold respectively).

We then turned our focus optimizing at the Y site employing the Topliss approach[24,25] while maintaining the optimal isopropyl group at R_1 (Table 1). The Topliss approach took into account the electronic, lipophilic and steric factors for substitution on a phenyl ring. We first prepared 4-chlorophenyl analogue **4**. As compared to **1**, compound **4** showed slight improvement at α_{1a}-AR affinity ($K_i = 1.0$ nM versus 1.54 nM) and maintained the high selectivity for α_{1a}-AR over either α_{1b}-AR or α_{1d}-AR. Following the Topliss scheme, we prepared 3,4-dichlorophenyl **5** and 4-methylphenyl **6**. While compound **5** showed no improvement in binding affinity, compound **6** did increase potency by 3-fold of **1** ($K_i = 0.52$ nM versus 1.54 nM) at α_{1a}-AR. Meanwhile, compound **6** also showed higher selectivity for α_{1a}-AR over α_{1b}-AR ($\alpha_{1b}/\alpha_{1a} = 1797$-fold versus 579-fold) at the expense of lower selectivity for α_{1a}-AR over α_{1d}-AR ($\alpha_{1d}/\alpha_{1a} = 40$-fold versus 137-fold). To further increase the potency of **6**, we synthesized 4-*tert*-butylphenyl **7**. However, no improvement was observed for either potency ($K_i = 3.33$ nM) or selectivity at α_{1a}-AR ($\alpha_{1d}/\alpha_{1a} = 22$-fold). A bulky group at the *para*-position is unfavorable.

To ensure that we did not miss the optimal substituents at the Y site, we also prepared 3-dimethylaminophenyl **8**, 2-methylphenyl **9** and 2-methoxyphenyl **10** under the branch of **6** of the Topliss tree. Compounds **8**, **9** and **10** all possessed similar α_{1a}-AR potency ($K_i = 1.93$ nM, 1.07 nM and 1.73 nM, respectively) but much lower selectivity

over α_{1d}-AR (α_{1d}/α_{1a} = 32-, 2- and 1-fold, respectively) when compared to compound **1**. For the same reason, 4-methoxyphenyl **11** under the branch of **4** was prepared. Indeed, no improvement for the potency or selectivity at α_{1a}-AR was observed.

Preparation of the bioisosteric sulfur analogues of **1** and **4** gave the corresponding **12** and **13**. No advantage was obtained for the potency and selectivity in either sulfur analogues. Replacement of the oxygen atom with a more basic nitrogen atom (compound **14** and **15**) led to a significant decrease of α_{1a}-AR potency (K_i = 25 nM) and selectivity (α_{1b}/α_{1a} = 29- and 41-fold, α_{1d}/α_{1a} = 1- and 3-fold).

Based upon the potency and selectivity, compounds **1**, **4** and **6** were selected for further evaluation at the R_2 site (Table 2). Both enantiomers of **1** and **6** were synthesized. For compound **1**, the *S*-enantiomer **17** was about 14-fold more potent than the *R*-enantiomer **16** at α_{1a}-AR (K_i = 0.79 nM versus 11.17 nM), 10-fold more selective for α_{1a}-AR over α_{1b}-AR (800 versus 82) and 4-fold more selective for α_{1a}-AR over α_{1d}-AR (104 versus 28). It is interesting to note that the same trend was also observed for compound **6**. The *S*-enantiomer **19** was about 52-fold more potent than the *R*-enantiomer **18** at α_{1a}-AR (K_i = 0.48 nM versus 24.93 nM), 42-fold more selective for α_{1a}-AR over α_{1b}-AR (593 versus 14) and around 2.4-fold more selective for α_{1a}-AR over α_{1d}-AR (102 versus 42). 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. Among the three *S*-enantiomers (**17**, **19** and **20**) synthesized, they all possessed comparable potency at α_{1a}-AR but **17** showed slightly better selectivity over all.

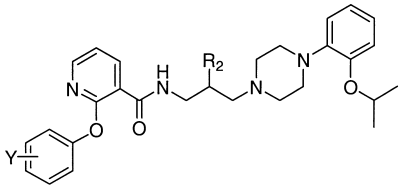
In order to examine the influence of the hydroxy group on the potency and selectivity at α_{1a}-AR, the des-hydroxy compounds **21** and **22** were prepared. In comparison to the des-hydroxy compounds **21** and **22**, the *S*-hydroxy enantiomers **17** and **19** had similar potency at α_{1a}-AR (K_i = 0.79 nM versus 0.64 and 0.48 nM versus 0.67 nM, respectively) but gained 3–4-fold selectivity for α_{1a}-AR over α_{1b}-AR, and an increase of 2-fold selectivity for α_{1a}-AR over α_{1d}-AR. Conversion of hydroxy compound **1** to the corresponding methoxy compound **23** led to a slight decrease of binding affinity at α_{1a}-AR (K_i = 1.54 nM versus 2.37 nM) and 14-fold lower selectivity over α_{1d}-AR (α_{1d}/α_{1a} = 137 versus 10). Conversion of hydroxy compound **1** to the conformationally more constrained carbonyl analogue **24** resulted in a significant loss of both potency and selectivity at α_{1a}-AR. The optimization at the R_1, R_2, X and Y position of **1** resulted in the identification of **19** and **17** that are 4–6-fold less potent than tamsulosin at α_{1a}-AR (K_i = 0.48 and 0.79 nM versus 0.13 nM) but 40–53-fold more selective over α_{1b}-AR (α_{1b}/α_{1a} = 593 and 800 versus 15) and ~74-fold more selective over α_{1d}-AR (α_{1d}/α_{1a} = 102 and 104 versus 1.4).

Lead selection

The ***S***-hydroxy compound **17**, Rec-15/2739 and tamsulosin were selected 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) were summarized in Table 3 and K_i values were measured. All three compounds exhibited significant binding affinities to selective dopamine and serotonin receptor subtypes. These results were not too surprising due to the fact that α-AR subtypes share about 45% identity with serotoninergic and dopaminergic receptors.[26]

All three compounds were further examined in the functional assay. Antagonist activity was assessed by

Table 2. Binding affinities at cloned human α_1-AR subtypes[a]



Compound	R_2	Y	$K_i \pm$ SEM[b] (nM)			K_i ratio	
			α_{1a}	α_{1b}	α_{1d}	α_{1b}/α_{1a}	α_{1d}/α_{1a}
16	*R*-OH	H	11.17±3.04	914±150	318±20	82	28
17	*S*-OH	H	0.79±0.18	630±29	82±7	800	104
18	*R*-OH	4-CH_3	24.93±1.91	350±62	1048±343	14	42
19	*S*-OH	4-CH_3	0.48±0.02	285±73	49±8	593	102
20	*S*-OH	4-Cl	0.87±0.07	804±670	57±2	928	65
21	H	H	0.64±0.08	151±5	41±4	235	63
22	H	4-CH_3	0.67±0.01	153±11	43±3	230	64
23	OCH_3	H	2.37±0.19	1377±338	25±11	582	10
24	=O	H	62.7±7.3	883±25	548±23	14	9
Rec-15/2739			1.06±0.24	35.7±2.4	6.43±0.97	34	6
Tamsulosin			0.13±0.02	1.92±0.17	0.18±0.02	15	1.4

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

Table 3. Receptor selectivity studies (Panlabs)

	17		Rec-15/2739		Tamsulosin	
Assay	K_i (nM)[a]	K_i ratio[b]	K_i (nM)	K_i ratio	K_i (nM)	K_i ratio
α_{1a}	0.76	1	1.1	1	0.13	1
α_{2A}	ND[c]	ND	61	55	63	485
α_{2B}	107	141	178	162	80	615
Dopamine D_{2L}	104	137	23	21	13	100
Dopamine D_{2S}	259	341	112	102	78	600
Dopamine D_3	108	142	18	16	0.28	2.2
Ca^{+2} channel, phenylalkylamine	ND	ND	1100	1000	ND	ND
Histamine H_1, central	39	51	900	818	ND	ND
Serotonin 5-HT_1	ND	ND	69	63	944	7261
Serotonin 5-HT_{1A}	133	175	0.6	(2)[d]	0.79	6.1
Serotonin 5-HT_7	3.1	4	8.8	8	84	646

[a]Values were the average of three determinations.
[b]The ratio of K_i (assay)/K_i (α_{1a}).
[c]K_i values not determined because low binding activity was seen in the initial 1 μM concentration screen.
[d]The ratio of K_i (α_{1a})/K_i (assay).

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 non-parallel shifts of dose–response curves with either tissue, affinities were reported as pK_B values in Table 4. Although antagonist **17** was less potent than tamsulosin in its ability to inhibit rat prostate contraction (pK_B = 7.75 versus 9.33), it displayed greater tissue selectivity for inhibition of rat prostate contraction over rat aorta contraction (ratio = 4.4 versus (26.9)). Tamsulosin actually exhibited 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). Antagonist **17** also showed very modest improvement over Rec-15/2739 in this tissue selectivity studies (4.4 versus 3.3). Currently, the apparent discrepancies between the binding affinity at α_{1a}-AR and functional potency in the rat prostate tissues of **17** versus tamsulosin 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]

We next examined the ability of **17** and tamsulosin to antagonize phenylephrine (PE)-induced increase in intraurethral pressure (IUP) and mean arterial pressure (MAP) in anesthetized beagle dogs (Fig. 1; Biosupport, Inc.). Tamsulosin was a very potent antagonist of both

Table 4. Rat prostate and aorta tissue contraction studies

	pK_B ± SEM[a]		
Compound	Rat aorta	Rat prostate	Ratio[b]
17	7.10 ± 0.07	7.75 ± 0.42	4.4
Rec-15/2739	7.58 ± 0.59	8.08 ± 0.14	3.3
Tamsulosin	10.76 ± 0.12	9.33 ± 0.43	(26.9)[c]

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

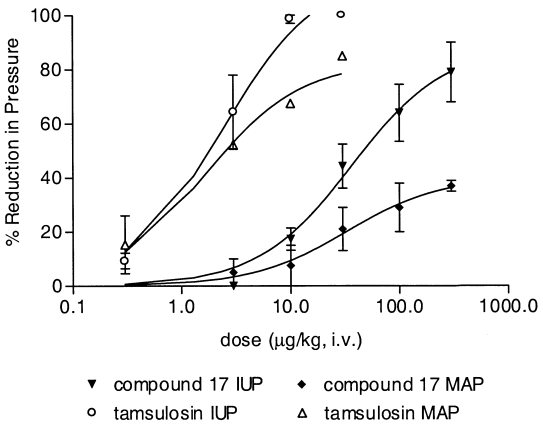


Figure 1. Compound **17** and tamsulosin effects upon IUP and MAP at 3 μg/kg PE in dogs.

the IUP and MAP response to the PE. The 6 ug/kg iv dose of tamsulosin caused 82% reduction in IUP and 61% reduction in MAP. Meanwhile, 300 ug/kg iv dose of **17** was required to cause 82% reduction in IUP but with 35% reduction in MAP observed. This in vivo evaluation indicated that compound **17** had lower potency than tamsulosin but with a modest improvement of uroselectivity in an anesthetized dog model.

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, the *S*-hydroxy enantiomers displayed comparable potency and better selectivity at α_{1a}-AR. In comparison to tamsulosin, the *S*-hydroxy enantiomer **17** was slightly less potent but much more selective at α_{1a}-AR. Compound **17** displayed higher selectivity in inhibiting rat prostate contraction than rat aorta contraction and also exhibited a modest improvement of uroselectivity although with lower potency in the anesthetized dog

model than tamsulosin. These data suggest that **17** may potentially be a useful agent for the treatment of BPH with minimal undesired side effects.

Experimental

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, New Jersey), 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 were 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 dimethylaminopyridine, '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 27a–c. α-(Azidomethyl)-4-[2-(1-methylethoxy)phenyl]-1-piperazineethanol (27c). The fumarate salt of 1-(2-isopropoxyphenyl)-piperazine **26c** (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 oil. A mixture of the oil and 1-azido-3-(p-toluenesulfonyloxy)propan-2-ol **25** (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 **27c** as a light-brown oily solid; ^{1}H NMR ($CDCl_3$) δ 6.91 (m, 4H), 4.59 (m, 1H), 3.93 (m, 1H), 3.67 (brs, 1H), 3.42 (dd, J = 12.6, 3.8 Hz, 1H), 3.23 (dd, J = 12.6, 5.4 Hz, 1H), 3.12 (m, 4H), 2.83 (m, 2H), 2.53 (m, 3H), 2.42 (dd, J = 12.2, 3.8 Hz, 1H), 1.34 (d, J = 6.0 Hz, 6H); 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 (27a). Brown oil (89%); ^{1}H NMR ($CDCl_3$) δ 6.85–7.04 (m, 4H), 3.94 (m, 1H), 3.87 (s, 3H), 3.42 (dd, J = 12.7, 3.8 Hz, 1H), 3.23 (dd, J = 12.7, 5.4 Hz, 1H), 3.09 (brs, 4H), 2.87 (m, 2H), 2.40–2.71 (m, 4H); 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 (27b). White oil (66%); ^{1}H NMR ($CDCl_3$) δ 6.92 (m, 4H), 4.06 (q, J = 7.0 Hz, 2H), 3.94 (m, 1H), 3.42 (dd, J = 12.8, 3.8 Hz, 1H), 3.24 (dd, J = 12.6, 5.3 Hz, 1H), 3.12 (m, 4H), 2.93 (m, 2H), 2.59 (m, 3H), 2.43 (dd, J = 12.2, 3.7 Hz, 1H), 1.45 (t, J = 6.9 Hz, 3H); FAB-HRMS ($M+H^+$). Calcd 306.1941, found 306.1941.

General procedure for the synthesis of 28a–c. α-(Aminomethyl)-4-[2-(1-methylethoxy)phenyl]-1-piperazineethanol (28c). 10% HCl (6 ml) was added to a mixture of **27c** (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 **28c** 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, 4H), 4.59 (m, 1H), 3.76 (m, 1H), 3.12 (m, 4H), 2.83 (dd, J = 12.7, 3.7 Hz, 2H), 2.82 (m, 1H), 2.25–2.68 (m, 8H), 1.34 (d, J = 6.1 Hz, 6H); MS (ES) m/z: 294 ($M+H^+$).

α-(Aminomethyl)-4-[2-(1-methoxy)phenyl]-1-piperazineethanol (28a). White solid (93%); ^{1}H NMR ($CDCl_3$) δ 6.95 (m, 4H), 3.87 (s, 3H), 3.66 (m, 1H), 3.10 (brs, 4H), 2.85 (m, 3H), 2.59 (m, 3H), 2.44 (m, 2H); MS (ES) m/z: 266 ($M+H^+$).

α-(Aminomethyl)-4-[2-(1-ethoxy)phenyl]-1-piperazineethanol (28b). Yellowish oil (87%); ^{1}H NMR ($CDCl_3$) δ 6.92 (m, 4H), 4.06 (q, J = 7.1 Hz, 2H), 3.73 (m, 1H), 3.12 (brs, 4H), 2.83 (m, 3H), 2.65 (m, 3H), 2.43 (m, 2H), 1.46 (t, J = 7.1 Hz, 3H); MS (ES) m/z: 280 ($M+H^+$).

General procedure for the synthesis of 1–7, 12–15 (Method A). 2-Phenoxy-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (1). A mixture of the amine **28c** (100 mg, 0.341 mmol), 2-phenoxypyridine-3-carbonyl chloride (81 mg, 0.341 mmol), DMAP (cat.) and *N,N*-diisopropylethylamine (0.23 ml) in methylene chloride (2 ml) was stirred at 20 °C for 16 h. The mixture was concentrated, diluted with water and extracted with EtOAc. The combined organic layer was dried (Na_2SO_4) and concentrated. The product was purified by column chromatography (silica gel) to give 116 mg (69%) of compound **1** as a foam; ^{1}H NMR ($CDCl_3$) δ 8.59 (d, J = 6.3 Hz, 1H), 8.32 (brs, 1H), 8.20 (d, J = 3.1 Hz, 1H), 7.44 (m, 2H), 7.21 (m, 4H), 6.87 (m, 4H), 4.57 (m, 1H), 3.98 (m, 1H), 3.75 (m, 1H), 3.50 (m, 1H), 3.06 (m, 4H), 2.79 (m, 2H), 2.48 (m, 4H), 1.33 (d, J = 5.9 Hz, 6H); MS (ES) m/z: 491 ($M+H^+$). Anal. ($C_{28}H_{34}N_4O_4 \cdot 0.1H_2O$) C, H, N.

2-Phenoxy-*N*-[2-hydroxy-3-[4-(2-ethoxyphenyl)-1-piperazinyl]propyl]-3-pyridinecarboxamide (2). Replacing **28c** with **28b** and following the same procedure as in the preparation of **1** gave **2** (60%) as a foam; ^{1}H NMR ($CDCl_3$) δ 8.60 (dd, J = 7.4, 1.7 Hz, 1H), 8.33 (m, 1H), 8.23 (dd, J = 5.0, 2.1 Hz, 1H), 7.45 (m, 2H), 7.29 (m, 1H), 7.18 (m, 3H), 6.90 (m, 4H), 4.06 (m, 3H), 3.74 (m, 1H), 3.51 (m, 1H), 3.09 (m, 4H), 2.88 (m, 2H), 2.58 (m, 4H), 1.45 (t, J = 6.8 Hz, 3H); MS (ES) m/z: 477 ($M+H^+$); FAB-HRMS ($M+H^+$). Calcd 477.2502, found 477. 2499.

2-Phenoxy-*N*-[2-hydroxy-3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-3-pyridinecarboxamide (3). Replacing **28c** with **28a** and following the same procedure as in the preparation of **1** gave **3** (69%) as a foam; ^{1}H NMR

($CDCl_3$) δ 8.60 (dd, J = 7.8, 2.0 Hz, 1H), 8.32 (m, 1H), 8.23 (dd, J = 4.7, 2.0 Hz, 1H), 7.46 (m, 2H), 7.29 (m, 1H), 7.17 (m, 3H), 7.02 (m, 1H), 6.90 (m, 3H), 4.01 (m, 1H), 3.86 (s, 3H), 3.75 (m, 1H), 3.49 (m, 1H), 3.06 (m, 4H), 2.87 (m, 2H), 2.56 (m, 4H); MS (ES) m/z: 463 ($M+H^+$). Anal ($C_{26}H_{30}N_4O_4 \cdot 1.1H_2O$) C, H, N.

2-(4-Chlorophenoxy)-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (4). (Method A, foam, 72%); ^1H NMR ($CDCl_3$) δ 8.59 (dd, J = 7.7, 1.9 Hz, 1H), 8.21 (m, 2H), 7.39 (d, J = 8.8 Hz, 2H), 7.16 (m, 3H), 6.87 (m, 4H), 4.58 (m, 1H), 3.99 (m, 1H), 3.77 (m, 1H), 3.47 (m, 1H), 3.07 (m, 4H), 2.79 (m, 2H), 2.51 (m, 4H), 1.33 (d, J = 6.1 Hz, 6H); MS (ES) m/z 525 ($M+H^+$). Anal ($C_{28}H_{33}ClN_4O_4$) C, H, N.

General procedure for the synthesis of 1–7, 12–15 (Method B). 2-(3,4-Dichlorophenoxy)-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (5). A mixture of the amine **28c** (100 mg, 0.341 mmol), 2-(3,4-dichlorophenoxy)-3-pyridinecarboxylic acid (97 mg, 0.341 mmol), EDCI (65 mg, 0.341 mmol), HOBT (46 mg, 0.341 mmol), DMAP (cat.) and triethylamine (0.23 ml) in methylene chloride (3 ml) was stirred at 20 °C for 18 h under nitrogen. The mixture was concentrated, diluted with water and extracted with methylene chloride. The combined organic layer was dried (Na_2SO_4) and concentrated. The product was purified by column chromatography (silica gel) to give 69 mg (36%) of compound **5** as a foam; ^1H NMR ($CDCl_3$) δ 8.60 (dd, J = 7.8, 1.9 Hz, 1H), 8.22 (m, 1H), 8.11 (m, 1H), 7.49 (d, J = 8.7 Hz, 1H), 7.36 (d, J = 2.6 Hz, 1H), 7.19 (m, 1H), 7.08 (dd, J = 8.8, 2.8 Hz, 1H), 6.89 (m, 4H), 4.58 (m, 1H), 3.98 (m, 1H), 3.79 (m, 1H), 3.43 (m, 1H), 3.08 (m, 4H), 2.82 (m, 2H), 2.50 (m, 4H), 1.33 (d, J = 6.0 Hz, 6H); MS (ES) m/z 559 ($M+H^+$). Anal ($C_{28}H_{32}Cl_2N_4O_4$) C, H, N.

2-(4-Methylphenoxy)-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (6). (Method A, foam, 83%); ^1H NMR ($CDCl_3$) δ 8.59 (dd, J = 7.8, 2.0 Hz, 1H), 8.33 (m, 1H), 8.22 (m, 1H), 7.25 (m, 2H), 7.1 (m, 3H), 6.87 (m, 4H), 4.58 (m, 1H), 4.0 (m, 1H), 3.73 (m, 1H), 3.50 (m, 1H), 3.06 (m, 4H), 2.79 (m, 2H), 2.49 (m, 4H), 2.38 (m, 3H), 1.33 (d, J = 6.3 Hz, 6H); MS (ES) m/z: 505 ($M+H^+$) Anal ($C_{29}H_{36}N_4O_4 \cdot 0.2H_2O$) C, H, N.

2-(4-*tert*-Butylphenoxy)-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (7). (Method B, foam, 26%); ^1H NMR ($CDCl_3$) δ 8.60 (dd, J = 7.8, 2.0 Hz, 1H), 8.3 (m, 1H), 8.23 (dd, J = 4.8, 1.9 Hz, 1H), 7.46 (d, J = 8.7 Hz, 2H), 7.14 (m, 3H), 6.92 (m, 4H), 4.58 (m, 1H), 3.97 (m, 1H), 3.79 (m, 1H), 3.48 (m, 1H), 3.06 (m, 4H), 2.79 (m, 2H), 2.48 (m, 4H), 1.34 (s, 9H), 1.33 (d, J = 6.0 Hz, 6H); MS (ES) m/z 547 ($M+H^+$). Anal ($C_{32}H_{42}N_4O_4$) C, H, N.

2-(Phenylthio)-*N*-[3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]-2-hydroxypropyl]-3-pyridinecarboxamide (12). (Method B, foam, 71%); ^1H NMR ($CDCl_3$) δ 8.39 (dd, J = 4.9, 1.9 Hz, 1H), 7.83 (dd, J = 7.8, 1.9 Hz, 1H), 7.53 (m, 2H), 7.39 (m, 3H), 7.08 (dd, J = 7.8, 4.9 Hz, 1H), 6.89 (m, 5H), 4.59 (m, 1H), 4.00 (m, 1H), 3.73 (m, 1H), 3.46 (m, 1H), 3.10 (m, 4H), 2.86 (m, 2H), 2.53 (m, 4H), 1.34 (d, J = 6.0 Hz, 6H); MS (ES) m/z 507 ($M+H^+$). Anal ($C_{28}H_{34}N_4O_3S \cdot 0.2H_2O$) C, H, N.

2-[(4-Chlorophenyl)thio]-*N*-[3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]-2-hydroxypropyl]-3-pyridinecarboxamide (13). (Method B, foam, 70%); ^1H NMR ($CDCl_3$) δ 8.37 (dd, J = 4.5, 1.5 Hz, 1H), 7.81 (dd, J = 7.5, 1.5 Hz, 1H), 7.45 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 8.6 Hz, 2H), 7.08 (dd, J = 7.5, 4.7 Hz, 1H), 6.89 (m, 5H), 4.59 (m, 1H), 4.00 (m, 1H), 3.76 (m, 1H), 3.42 (m, 1H), 3.10 (m, 4H), 2.86 (m, 2H), 2.57 (m, 4H), 1.34 (d, J = 6.1 Hz, 6H); MS (ES) m/z 541 ($M+H^+$). Anal ($C_{28}H_{33}N_4O_3SCl \cdot 0.5H_2O$) C, H, N.

***N*-[3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]-2-hydroxypropyl]-2-(phenylamino)-3-pyridinecarboxamide (14).** (Method B, foam, 44%); ^1H NMR ($CDCl_3$) δ 10.42 (s, 1H), 8.28 (dd, J = 4.9, 1.5 Hz, 1H), 7.73 (dd, J = 7.7, 1.5 Hz, 1H), 7.68 (d, J = 7.6 Hz, 2H), 7.31 (t, J = 8.2 Hz, 2H), 7.20–6.84 (m, 6H), 6.65 (dd, J = 7.5, 4.7 Hz, 1H), 4.57 (m, 1H), 4.00 (m, 1H), 3.68 (m, 2H), 3.36 (m, 1H), 3.10 (m, 4H), 2.81 (m, 2H), 2.65–2.38 (m, 4H), 1.33 (d, J = 6.0 Hz, 6H); MS (ES) m/z 490 ($M+H^+$); FAB-HRMS ($M+H^+$). Calcd 490.2818, found 490.2825.

***N*-[3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]-2-hydroxypropyl]-2-[3-(trifluoromethyl)phenyl]amino]-3-pyridinecarboxamide (15).** (Method B, foam, 53%); ^1H NMR ($CDCl_3$) δ 10.76 (s, 1H), 8.33 (dd, J = 4.4, 1.2 Hz, 1H), 8.07 (s, 1H), 7.87 (d, J = 8.2 Hz, 1H), 7.78 (dd, J = 7.6, 1.5 Hz, 1H), 7.40 (t, J = 7.9 Hz, 1H), 7.23 (d, J = 7.7 Hz, 1H), 7.04 (brs, 1H), 6.90 (m, 4H), 6.74 (dd, J = 7.5, 4.7 Hz, 1H), 4.58 (m, 1H), 4.00 (m, 1H), 3.74 (m, 1H), 3.45 (m, 2H), 3.13 (m, 4H), 2.88 (m, 2H), 2.71–2.42 (m, 4H), 1.33 (d, J = 6.1 Hz, 6H); MS (ES) m/z 558 ($M+H^+$). Anal ($C_{29}H_{34}N_5O_3F_3 0.2H_2O$) C, H, N.

2-Chloro-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (29). A mixture of **28c** (900 mg, 3.07 mmol), 2-chloronicotinic acid (485 mg, 3.07 mmol), EDCI (589 mg, 3.07 mmol), HOBT (414 mg, 3.07 mmol), DMAP (cat.) and *N,N*-diisopropylethylamine (2 ml) in methylene chloride (20 ml) was stirred at 20 °C for 20 h. The mixture was concentrated, diluted with water and extracted with ether. The organic layer was dried (Na_2SO_4) and concentrated. The product was purified by column chromatography (silica gel) to give 580 mg (44%) of compound **29** as a white foam; ^1H NMR ($CDCl_3$) δ 8.47 (dd, J = 4.9, 2.0 Hz, 1H), 8.09 (dd, J = 7.6, 2.0 Hz, 1H), 7.35 (dd, J = 7.7, 2.8 Hz, 1H), 7.01 (m, 5H), 4.59 (m, 1H), 4.00 (m, 1H), 3.75 (m, 1H), 3.51 (m, 1H), 3.12 (m, 4H), 2.89 (m, 2H), 2.61 (m, 2H), 2.52 (d, J = 7.0 Hz, 2H), 1.33 (d, J = 6.0 Hz, 6H); MS (ES) m/z: 433 ($M+H^+$). Anal ($C_{22}H_{29}N_4O_3Cl \cdot 0.3H_2O$) C, H, N.

General procedure for the synthesis of 8–11. 2-(3-Dimethylaminophenoxy)-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (8). A mixture of compound **29** (95 mg, 0.22 mmol), 3-(dimethylamino)phenol (152 mg, 1.1 mmol) and cesium

carbonate (143 mg, 0.44 mmol) in NMP (2 mL) was stirred at 110 °C for 22 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 (silica gel) to give 91 mg (78%) of **8** as a foam; ^{1}H NMR ($CDCl_3$) δ 8.59 (dd, J = 7.4, 2.1 Hz, 1H), 8.35 (m, 1H), 8.25 (dd, J = 4.8, 1.9 Hz, 1H), 7.27 (t, J = 8.3 Hz, 1H), 7.13 (dd, J = 7.4, 4.6 Hz, 1H), 6.90 (m, 4H), 6.60 (brd, J = 7.5 Hz, 1H), 6.51 (m, 2H), 4.60 (m, 1H), 4.00 (m, 1H), 3.77 (m, 1H), 3.53 (m, 1H), 3.10 (m, 4H), 2.96 (s, 6H), 2.81 (m, 2H), 2.55 (m, 4H), 1.33 (d, J = 6.0 Hz, 6H); MS (ES) m/z: 534 ($M+H^+$); FAB-HRMS ($M+H^+$). Calcd 534.3080, found 534.3059.

2-(2-Methylphenoxy)-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (9). (Foam, 78%); ^{1}H NMR ($CDCl_3$) δ 8.61 (dd, J = 7.5, 1.6 Hz, 1H), 8.40 (brt, J = 5.3 Hz, 1H), 8.18 (dd, J = 4.6, 1.9 Hz, 1H), 7.25 (m, 3H), 7.11 (brt, J = 5.6 Hz, 2H), 6.87 (m, 4H), 4.57 (m, 1H), 4.00 (m, 1H), 3.80 (m, 1H), 3.50 (m, 1H), 3.06 (m, 4H), 2.80 (m, 2H), 2.52 (m, 4H), 2.19 (s, 3H), 1.33 (d, J = 6.0 Hz, 6H); MS (ES) m/z 505 ($M+H^+$). Anal ($C_{29}H_{36}N_4O_4 0.2H_2O$) C, H, N.

2-(2-Methoxyphenoxy)-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (10). (Foam, 80%); ^{1}H NMR ($CDCl_3$) δ 8.52 (dd, J = 7.5, 2.1 Hz, 1H), 8.38 (brt, J = 5.5 Hz, 1H), 8.16 (dd, J = 4.6, 1.9 Hz, 1H), 7.25 (m, 2H), 7.05 (m, 3H), 6.92 (m, 4H), 4.58 (m, 1H), 4.00 (m, 1H), 3.73 (m, 4H), 3.52 (m, 1H), 3.05 (m, 4H), 2.81 (m, 2H), 2.51 (m, 4H), 1.33 (d, J = 6.0 Hz, 6H); MS (ES) m/z 521 ($M+H^+$). Anal ($C_{29}H_{36}N_4O_5 0.6H_2O$) C, H, N.

2-(4-Methoxyphenoxy)-*N*-[2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (11). (Foam, 69%); ^{1}H NMR ($CDCl_3$) δ 8.58 (dd, J = 7.5, 1.7 Hz, 1H), 8.36 (brt, J = 5.5 Hz, 1H), 8.21 (dd, J = 5.0, 2.2 Hz, 1H), 7.13 (m, 3H), 6.91 (m, 6H), 4.58 (m, 1H), 4.00 (m, 1H), 3.82 (s, 3H), 3.79 (m, 1H), 3.50 (m, 1H), 3.07 (m, 4H), 2.81 (m, 2H), 2.52 (m, 4H), 1.33 (d, J = 6.0 Hz, 6H); MS (ES) m/z: 521 ($M+H^+$); FAB-HRMS ($M+H^+$). Calcd 521.2764, found 521.2759.

(*R*)-α-(Azidomethyl)-4-[2-(1-methylethoxy)phenyl]-1-piperazineethanol (*R*–27c). The fumarate salt of 1-(2-isopropoxyphenyl)-piperazine **26c** (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 oil. A mixture of the oil and (*2S*)-3-azido-2-hydroxypropyl *p*-toluenesulfonate ***S*-25** (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*-27c** as a off-white oily solid; $[\alpha]_D^{25} = -3.6°$ (c = 1, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 6.91 (m, 4H), 4.59 (m, 1H), 3.93 (m, 1H), 3.67 (brs, 1H), 3.42 (dd, J = 12.6, 3.8 Hz, 1H), 3.23 (dd, J = 12.6, 5.4 Hz, 1H), 3.12 (m, 4H), 2.83 (m, 2H), 2.53 (m, 3H), 2.42 (dd, J = 12.2, 3.8 Hz, 1H), 1.34 (d, J = 6.0 Hz, 6H); 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*-28c). Replacing **27c** with ***R*-27c** and following the same procedure as in the preparation of **28c** gave ***S*-28c** (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, 4H), 4.59 (m, 1H), 3.76 (m, 1H), 3.12 (m, 4H), 2.83 (dd, J = 12.7, 3.7 Hz, 2H), 2.82 (m, 1H), 2.69–2.24 (m, 8H), 1.34 (d, J = 6.1 Hz, 6H); MS (ES) m/z 294 ($M+H^+$).

2-Phenoxy-*N*-[(2*S*)-2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (17). Replacing **28c** with ***S*-28c** and following the same procedure as in the preparation of **1** (method A) gave **17** (62%) (~99% ee assay by reverse phase chiralcel OD column, 4.6×150 mm, mobile phase: 90:10:0.1 of hexane: isopropylalcohol:diethylamine, retention time = 22.900 min) as a white foam; $[\alpha]_D^{25} = +14.8°$ (c = 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.60 (dd, J = 7.5, 2.0 Hz, 1H), 8.31 (brs, 1H), 8.22 (dd, J = 4.7, 2.0 Hz, 1H), 7.43 (brt, J = 7.7 Hz, 2H), 7.30–7.14 (m, 4H), 6.87 (m, 4H), 4.58 (m, 1H), 3.97 (m, 1H), 3.75 (m, 1H), 3.51 (m, 1H), 3.06 (m, 4H), 2.80 (m, 2H), 2.49 (m, 4H), 1.33 (d, J = 6.0 Hz, 6H); MS (ES) m/z: 491 ($M+H^+$). Anal. ($C_{28}H_{34}N_4O_4$) C, H, N.

2-(4-Methylphenoxy)-*N*-[(2*S*)-2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (19). (Method A, foam, 57%); $[\alpha]_D^{25} = +13.2°$ (c = 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.59 (dd, J = 7.8, 2.0 Hz, 1H), 8.33 (m, 1H), 8.23 (m, 1H), 7.25 (m, 2H), 7.1 (m, 3H), 6.87 (m, 4H), 4.58 (m, 1H), 4.00 (m, 1H), 3.73 (m, 1H), 3.50 (m, 1H), 3.06 (m, 4H), 2.79 (m, 2H), 2.49 (m, 4H), 2.38 (m, 3H), 1.33 (d, J = 6.2 Hz, 6H); MS (ES) m/z 505 ($M+H^+$). Anal. ($C_{29}H_{36}N_4O_4$) C, H, N.

2-(4-Chlorophenoxy)-*N*-[(2*S*)-2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (20). (Method A, foam, 58%); $[\alpha]_D^{25} = +13.7°$ (c = 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.59 (dd, J = 7.7, 1.9 Hz, 1H), 8.21 (m, 2H), 7.39 (d, J = 8.8 Hz, 2H), 7.16 (m, 3H), 6.87 (m, 4H), 4.58 (m, 1H), 3.99 (m, 1H), 3.77 (m, 1H), 3.47 (m, 1H), 3.07 (m, 4H), 2.79 (m, 2H), 2.51 (m, 4H), 1.33 (d, J = 6.1 Hz, 6H); MS (ES) m/z 525 ($M+H^+$). Anal. ($C_{28}H_{33}N_4O_4Cl \cdot H_2O$) C, H, N.

(*S*)-α-(Azidomethyl)-4-[2-(1-methylethoxy)phenyl]-1-piperazineethanol (*S*-27c). Replacing (*2S*)-3-azido-2-hydroxypropyl *p*-toluenesulfonate ***S*-25** with (*2R*)-3-azido-2-hydroxypropyl *p*-toluenesulfonate ***R*-25** and following the same procedure as in the preparation of ***R*-27c** gave ***S*-27c** (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); ^{1}H NMR ($CDCl_3$) δ 6.92 (m, 4H), 4.59 (m, 1H), 3.92 (m,

1H), 3.67 (brs, 1H), 3.42 (dd, $J=12.6$, 3.8 Hz, 1H), 3.23 (dd, $J=12.6$, 5.4 Hz, 1H), 3.12 (m, 4H), 2.83 (m, 2H), 2.53 (m, 3H), 2.42 (dd, $J=12.2$, 3.8 Hz, 1H), 1.34 (d, $J=6.0$ Hz, 6H); MS (ES) m/z 320 ($M+H^+$).

(*R*)-α-(Aminomethyl)-4-[2-(1-methylethoxy)phenyl]-1-piperazineethanol (*R*-28c). Replacing **27c** with ***S*-27c** and following the same procedure as in the preparation of **28c** gave ***R*-28c** (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, 4H), 4.59 (m, 1H), 3.76 (m, 1H), 3.12 (m, 4H), 2.83 (dd, $J=12.7$, 3.7 Hz, 2H), 2.82 (m, 1H), 2.68–2.25 (m, 8H), 1.34 (d, $J=6.1$ Hz, 6H); MS (ES) m/z 294 ($M+H^+$).

2-Phenoxy-*N*-[(2*R*)-2-hydroxy-3-[4-[2-(1-methylethoxy)-phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (16). (Method B, foam 49%); $[\alpha]_D^{25}=-13.3°$ ($c=1$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.60 (dd, $J=7.4$, 1.9 Hz, 1H), 8.30 (brs, 1H), 8.21 (dd, $J=4.7$, 2.0 Hz, 1H), 7.42 (brt, $J=7.7$ Hz, 2H), 7.30–7.15 (m, 4H), 6.86 (m, 4H), 4.58 (m, 1H), 3.97 (m, 1H), 3.74 (m, 1H), 3.50 (m, 1H), 3.05 (m, 4H), 2.80 (m, 2H), 2.49 (m, 4H), 1.33 (d, $J=6.0$ Hz, 6H); MS (ES) m/z 491 ($M+H^+$).

2-(4-Methylphenoxy)-*N*-[(2*R*)-2-hydroxy-3-[4-[2-(1-methylethoxy)phenyl] - 1 - piperazinyl]propyl] - 3 - pyridinecarboxamide (18). (Method A, foam 60%); $[\alpha]_D^{25}=-12.8°$ (c = 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.59 (dd, $J=7.8$, 2.0 Hz, 1H), 8.33 (m, 1H), 8.23 (m, 1H), 7.25 (m, 2H), 7.10 (m, 3H), 6.87 (m, 4H), 4.58 (m, 1H), 4.00 (m, 1H), 3.73 (m, 1H), 3.50 (m, 1H), 3.06 (m, 4H), 2.79 (m, 2H), 2.49 (m, 4H), 2.38 (m, 3H), 1.33 (d, $J=6.1$ Hz, 6H); MS (ES) m/z 505 ($M+H^+$).

[3-[4-[2-(1-Methylethoxy)phenyl]-1-piperazinyl]propyl-carbamic acid, 1,1-dimethylethyl ester (31). 3-Bromopropylamine hydrobromide **30** (5 g, 22.8 mmol) was dissolved in 10% NaOH (50 ml), 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%), $NaHCO_{3(aq)}$, $NaCl_{(aq)}$, 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 **26c** (5.1g, 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 **31** 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 21–22. *N*-[3-[4-[2-(1-Methylethoxy)phenyl]-1-piperazinyl]-2-phenoxy-3-pyridinecarboxamide (21). The Boc-protected amine **31** (0.185 g, 0.53 mmol) was dissolved in 25% TFA/methylene chloride (5 ml) and stirred for 1.5 h. The solvent was removed and the TFA salt was washed with toluene (3 x) and then basified with 20% $NaOH_{(aq)}$ followed by extracted with methylene chloride, dried (Na_2SO_4) and concentrated to give a oil. This oil was dissolved in methylene chloride (4 ml). *N,N*-Diisopropylethylamine (0.34 g, 2.64 mmol), DMAP $_{(cat.)}$ and 2-phenoxypyridine-3-carbonyl chloride (0.12 g, 0.53 mmol) was added. The reaction was stirred at 20 °C under N_2 for 2 h and concentrated. The product was purified by column chromatography (silica gel) to give 0.2 g (80%) of **21** as a foam; ^1H NMR ($CDCl_3$) δ 8.61 (dd, $J=7.5$, 2.0 Hz, 1H), 8.20 (dd, $J=4.9$, 2.0 Hz, 1H), 8.05 (m, 1H), 7.46 (m, 2H), 7.29 (d, $J=7.4$ Hz, 1H), 7.15 (m, 3H), 6.88 (m, 4H), 4.56 (m, 1H), 3.59 (q, $J=6.3$ Hz, 2H), 3.03 (m, 4H), 2.56 (m, 4H), 2.49 (t, $J=7.0$ Hz, 2H), 1.87 (m, 2H), 1.32 (d, $J=6.1$ Hz, 6H); MS (ES) m/z 475 ($M+H^+$); FAB-HRMS ($M+H^+$). Calcd 475.2709, found 475.2721.

***N*-[3-[4-[2-(1-Methylethoxy)phenyl]-1-piperazinyl]-2-(4-methylphenoxy)-3-pyridinecarboxamide (22).** Replacing 2-phenoxypyridine-3-carbonyl chloride with 2-(4-methyl-phenoxypyridine)-3-carbonyl chloride and following the same procedure as in the preparation of **21** gave **22** (76%) as a foam; ^1H NMR ($CDCl_3$) δ 8.60 (dd, $J=7.4$, 2.1 Hz, 1H), 8.20 (dd, $J=5.0$, 2.1 Hz, 1H), 8.08 (m, 1H), 7.24 (d, $J=8.7$ Hz, 2H), 7.12 (dd, $J=7.4$, 4.6 Hz, 1H), 7.05 (d, $J=8.5$ Hz, 2H), 6.88 (m, 4H), 4.58 (m, 1H), 3.58 (q, $J=6.6$ Hz, 2H), 3.04 (m, 4H), 2.57 (m, 4H), 2.50 (t, $J=7.0$ Hz, 2H), 2.38 (s, 3H), 1.86 (m, 2H), 1.33 (d, $J=6.1$ Hz, 6H); MS (ES) m/z: 489 ($M+H^+$); FAB-HRMS ($M+H^+$) calcd 489.2866, found 489.2874.

1-(3-Azido-2-methoxypropyl)-4-[2-(1-methylethoxy)phenyl]piperazine (32). Compound **27c** (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 and 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 **32**; ^1H NMR ($CDCl_3$) δ 6.90 (m, 4H), 4.60 (m, 1H), 3.50 (m, 5H), 3.35 (m, 1H), 3.18 (m, 4H), 2.55 (m, 6H), 1.32 (d, $J=6.1$ Hz, 6H); MS (ES) m/z 334 ($M+H^+$).

2-Phenoxy-*N*-[2-methoxy-3-[4-[2-(1-methylethoxy)phenyl]-1-piperazinyl]propyl]-3-pyridinecarboxamide (23). 10% HCl (0.3 ml) was added to a mixture of **32** (0.64 g, 1.9 mmol) and 10% Pd/C (0.13 g) in methanol (5 ml) and the mixture was hydrogenated under H_2 (50 psi) in a Parr shaker overnight. The mixture was filtered through Celite and the filtrate was concentrated. The residue was basified with 20% $NaOH_{(aq)}$ and extracted with methylene chloride. The combined organic extract were dried (Na_2SO_4) and concentrated to give a yellow oil at quantitative yield. The amine was used directly without further purification; MS (ES) m/z 308 ($M+H^+$).

The amine was dissolved in methylene chloride (15 ml) and *N,N*-diisopropylethylamine (0.97 g, 7.6 mmol) was added. To this solution was added a mixture of HATU (0.72 g, 1.9 mmol) and 2-phenoxynicotinic acid (0.43 g, 1.9 mmol). The reaction was stirred under N_2 overnight at room temperature, the solvent was evaporated and the residue was dissolved in methylene chloride. This solution was washed with 3% K_2CO_3, the organic layer was dried (Na_2SO_4) and concentrated. The product was purified by column chromatography (silica gel) to give 0.62 g (64%) of **23** as an oil; ^{1}H NMR ($CDCl_3$) δ 8.61 (dd, $J=7.4$, 2.1 Hz, 1H), 8.27 (m, 1H), 8.23 (m, 1H), 7.44 (m, 2H), 7.26 (m, 1H), 7.17 (m, 3H), 6.87 (m, 4H), 4.58 (m, 1H), 3.92 (m, 1H), 3.55 (m, 2H), 3.42 (s, 3H), 3.03 (brs, 4H), 2.54 (m, 6H), 1.33 (d, $J=6.0$ Hz, 6H); MS (ES) *m/z*: 505 ($M+H^+$); FAB-HRMS ($M+H^+$). Calcd 505.2815, found 505.2832.

***N*-[3-[4-[2-(1-Methylethoxy)phenyl]-1-piperazinyl]-2-oxopropyl]-2-phenoxy-3-pyridinecarboxamide (24).** Oxalyl chloride (0.03 g, 0.22 mmol) was dissolved in 0.3 ml of methylene chloride. A mixture of DMSO (0.035 ml, 0.49 mmol) in methylene chloride (3 ml) was added dropwise to this solution at −78 °C. The mixture was stirred at −78 °C for 1 h. A solution of **1** (0.1 g, 0.2 mmol) in methylene chloride (0.4 ml) was added slowly. The reaction mixture was stirred for 30 min and triethylamine (0.14 ml, 1.02 mmol) was added slowly. The mixture was allowed to warm up to room temperature, water was added and the resulting mixture was extracted with methylene chloride. The combined organic layers were dried (Na_2SO_4) and concentrated to give 13 mg (13%) of **24** as a foam; ^{1}H NMR ($CDCl_3$) δ 8.69 (brs, 1H), 8.61 (dd, $J=7.6$, 2.1 Hz, 1H), 8.23 (dd, $J=4.6$, 1.8 Hz, 1H), 7.46 (m, 2H), 7.28 (m, 3H), 7.15 (m, 1H), 6.89 (m, 4H), 4.57 (m, 3H), 3.35 (s, 2H), 3.15 (brs, 4H), 2.70 (brs, 4H), 1.33 (d, $J=6.0$ Hz, 6H); MS (ES) *m/z* 489 ($M+H^+$).

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.[28,29] Each tissue culture dish was inoculated with 3.5×10^6 cells and transfected with 10 μg of DNA. 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 $1000\times g$ for 10 min at 4 °C. Supernatants were centrifuged at $34{,}500\times g$ 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. 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] 10 μM WB4101 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] 10 μM phentolamine 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] 10 μM haloperidol 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] 10 μM metergoline 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] 1 μM nifedipine 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] 1 μM pyrilamine 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 3–4 times prior to determining the level of radio-ligand binding by liquid scintillation counting. Competition curves were fitted, and IC_{50} values were estimated, by non-linear 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, $MgSO_4$:$7H_2O$ 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.

Anesthetized dog model of intraurethral pressure and mean arterial pressure (Biosupport Inc)

Male beagle dogs between 10 and 18 months of age were pre-anesthetized with thiopental sodium, and an endotracheal tube was inserted transorally. They were attached to a closed volume cycle respirator and maintained at a surgical plane of anesthesia with isoflurane inhalation. Intraurethral pressure (IUP) was monitored with a 7–8 F Fogarty venous thrombectomy balloon catheter positioned in the prostatic urethra with the balloon port of the catheter connected to a pressure transducer. Mean arterial pressure (MAP) was monitored via a 20–18 gauge catheter placed in the femoral artery and connected to a pressure transducer. Heart rate and ECG patterns were monitored through lead II of the ECG monitor. Compounds were administered iv through the cephalic vein. To generate a control dose-response curve, test compound vehicle was administered intravenously, followed 15 min later by 3 ug/kg iv doses of PE. An bolus dose of antagonist was given, followed 15 min later by the PE challenge. The doses of antagonist that were tested were 3, 10, 30, 100 and 300 μg/kg for **17**; and 0.3, 3, 10 and 30 ug/kg for tamsulosin. Antagonist doses were given in ascending concentrations at no less than 45 min intervals. The IUP and MAP are presented as the percent of the maximal response following each PE challenge, with the maximal response as the IUP and MAP at the PE dose followings administration of vehicle only. Data from three experiments were used to calculate an average IUP or MAP response. Dose–response curves were generated using GRAPHPAD PRISM software.

Acknowledgements

We thank Linda Mulcahy and Deborah Loughney for helpful discussions.

References and Notes

1. Bylund, D. B.; Eikenberg, D. C.; Hieble, J. P.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Molinoff, P. B.; Ruffolo Jr., R. R.; Trendelenburg, U. *Pharmacol. Rev.* **1994**, *46*, 121.

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. *Pharmacol. Rev.* **1995**, *47*, 267.
3. Muramatsu, I.; Ohmura, T.; Kigoshi, S.; Hashimoto, S.; Oshita, M. *Br. J. Pharmacol.* **1990**, *99*, 197.
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. *Mol. Pharmacol.* **1994**, *45*, 703.
5. Marshall, I.; Burt, R. P.; Chapple, C. R. *Br. J. Pharmacol.* **1995**, *115*, 781.
6. Burt, R. P.; Chapple, C. R.; Marshall, I. *Br. J. Pharmacol.* **1995**, *116*, 2327.
7. Hatano, A.; Takahashi, H.; Tamaki, M.; Komeyama, T.; Koizumi, T.; Takeda, M. *Br. J. Pharmacol.* **1994**, *113*, 723.
8. (a) Kenny, B. A.; Chalmers, D. H.; Philpott, P. C.; Naylor, A. M. *Br. J. Pharmacol.* **1995**, *115*, 981. (b) Aboud, R.; Shafii, M.; Docherty, J. R. *Br. J. Pharmacol.* **1993,** *109*, 80.
9. Lepor, H.; Henry, D.; Laddu, A. R. *Prostate* **1991**, *18*, 345.
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. *Scand. J. Urol. Nephrol.* **1994**, *28*, 77.
11. Lepor, H. *J. Androl.* **1991**, *12*, 389.
12. (a) Kawabe, K.; Ueno, A.; Takimoto, Y.; Aso, Y.; Kato, H. *J. Urol*, **1990**, *144*, 908. (b) Rabasseda, X.; Fitzpatrick, J. M. *Drugs of Today*, **1996**, *32* (Suppl. C), 1.
13. (a) Djavan, B.; Marberger, *M. Eur. Urol*, **1999**, *36*, 1. (b) Lowe, F. *Prostate Cancer and Prostatic Diseases*, **1999**, *2*, 110. (c) Schulman, C. C.; Cortvriend, J.; Jonas, U.; Lock, T. M. T. W.; Vaage, S.; Speakman, M. J. *Eur. Urol*, **1996**, *29*, 145. (d) Jardin, A.; Andersson, K. E.; Caine, M. Alpha-blockers in the treatment of BPH. In The Fourth International Consultation on Benign Prostatic Hyperplasia (BPH). Denis, L.; Griffiths, K.; Khoury, S.; Cockett, A. T. K.; McConnell, J.; Chatelain, C.; Murphy, G.; Yoshida, O. Health Publication Ltd: Plymouth: UK, 1998; p599.
14. [^{125}I] HEAT: ((±)-β-(^{125}I Iodo-4-hydroxyphenyl)-1-ethyl-aminoethyl-tetralone) was obtained from NEN^{TM} 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. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 1237.
16. Shibata, K.; Foglar, R.; Horie, K.; Obika, K.; Sakamoto, A.; Ogawa, S.; Tsujimoto, G. *Mol. Pharmacol.* **1995**, *48*, 250.
17. Wetzel, J. M.; Miao, S. W.; Forray, C.; Borden, L. A.; Branchek, T. A.; Gluchowski, C. *J. Med. Chem.* **1995**, *38*, 1579.
18. Cui, W.; Nakanishi, H.; Wetzel, J. M.; Gluchowski, C. 210th ACS National Meeting, Chicago, IL, 20–24 August 1995, MEDI-045.
19. Hamaguchi, N.; True, T. A.; Saussy, D. L.; Jeffs Jr., P. W. *Biochemistry* **1996**, *35*, 14312.
20. Two second-generation leads were identified. The SAR and biological evaluation of the other second-generation lead, phthalimide-phenylpiperazine, is reported in Kuo, G.-H.; Prouty, C.; Murray, W. V.; Pulito, V.; Jolliff, L.; Cheung, P.; Varga, S.; Evangelisto, M.; Wang, J. *J. Med. Chem.* **2000**, *43*, 2183.
21. Martin, G. E.; Elgin Jr., R. J.; Mathiasen, J. R.; Davis, C. B.; Kesslick, J. M.; Baldy, W. J.; Shank, R. P.; DiStefano, D. L.; Fedde, C. L.; Scott, M. K. *J. Med. Chem.* **1989**, *32*, 1052.
22. Holy, A. *Collect. Czech. Chem. Commun.* **1989**, *54*, 446.
23. Juricova, K.; Smrckova, S.; Holy, A. *Collect. Czech. Chem. Commun.* **1995**, *60*, 237.
24. Topliss, J. G. *J. Med. Chem.* **1972**, *15*, 1006.
25. Topliss, J. G. *J. Med. Chem.* **1977**, *20*, 463.
26. Trumpp-Kallmeyer, S.; Hoflack, J.; Bruinvels, A.; Hibert, M. *J. Med. Chem.* **1992**, *35*, 3448.
27. (a) Leff, P. *Trends Pharmacol. Sci.* **1995**, *16*, 89. (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. *Mol. Pharmacol.* **1996**, *49*, 209.
28. McCutchan, J. H.; Pagano, J. S. *J. Natl. Cancer. Inst.* **1968**, *41*, 351.
29. Luthman, H.; Magnusson, G. *Nucleic Acids Res.* **1983**, *11*, 1295.
30. Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
31. Uhlen, S.; Porter, A. C.; Neubig, R. R. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1558.
32. Connaughton, S.; Docherty, R. *Br. J. Pharmacol.* **1990**, *101*, 285.
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. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 9762.
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. *Nature* **1988**, *336*, 783.
35. Hayes, G.; Biden, T. J.; Selbie, L. A.; Shine, J. *Molec. Endocrin* **1992**, *6*, 920.
36. Sokoloff, P.; Giros, B.; Martres, M. P.; Bouthenet, M. L.; Schwartz, J. C. *Nature* **1990**, *347*, 146.
37. Sibley, D. R.; Monsma, F. J. *Trends in Pharmacol. Sci.* **1992**, *13*, 60.
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. *Nature* **1992**, *358*, 149.
39. Van Tol, H. H. M.; Bunzow, J. R.; Guan, H. C.; Sunahara, R. K.; Seeman, P.; Niznik, H. B.; Civelli, O. *Nature* **1991**, *350*, 610.
40. Martin, G. R.; Humphrey, P. P. A. *Neuropharm* **1994**, *33*, 261.
41. Shen, Y.; Monsma, F. J. Jr.; Metcalf, M. A.; Jose, P. A.; Hamblin, M. W.; Sibley, D. R. *J. Biol. Chem.* **1993**, *268*, 18200.
42. Roth, B. L.; Craigo, S. C.; Choudhary, M. S.; Uluer, S.; Monsma, F. J. Jr.; Shen, Y.; Meltzer, H. Y.; Sibley, D. R. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 1403.
43. Middlemiss, D. N. *Eur. J. Pharmacol.* **1984**, *101*, 289.
44. Gould, R. J.; Murphy, K. M. M.; Snyder, S. H. *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 3650.
45. Ehlert, F. J.; Roeske, W. R.; Itoga, E.; Yamamura, H. I. *Life Sci.* **1982**, *30*, 2191.
46. Brown, C. M.; Mackinnon, A. C.; McGrath, J. C.; Spedding, M.; Kilpatrick, A. T. *Br. J. Pharmacol.* **1990**, *99*, 803.
47. Michel, M. C.; Ernsberger, P. *Trends. Pharmacol. Sci.* **1992**, *13*, 369.
48. Hill, S. J.; Emson, P. C.; Young, J. M. *J. Neurochem* **1978**, *31*, 997.
49. Dini, S.; Caselli, G. F.; Ferrari, M. P.; Giani, R.; Clavenna, G. *Agents and Actions* **1991**, *33*, 181.