Synthesis and Evaluation of 4-Fluoro-8-substituted-2,3,4,5-tetrahydro-1*H*-2-benzazapines as Selective Inhibitors of Phenylethanolamine *N*-Methyltransferase versus the α_2-Adrenoceptor

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A small series of 4-fluoro-8-substituted-2,3,4,5-tetrahydro-1*H*-2-benzazapines (4-fluoro-THBAs; **12**−**15**) were synthesized and evaluated as inhibitors of phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28) and as inhibitors of the binding of clonidine at the α_2-adrenoceptor. 4-Fluoro-THBAs **13**−**15** displayed selectivity ratios (α_2 K_i/PNMT K_i) greater than 75 and 4-fluoro-8-nitro-THBA (**13**) was found to be one of the most selective inhibitors of PNMT known, with a selectivity ratio of greater than 900. These compounds are also quite lipophilic and according to previous results from this laboratory should be able to penetrate the blood−brain barrier. These 4-fluoro-THBAs represent important leads in the development of new, more selective, CNS-active inhibitors of PNMT.

Introduction

Phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28) catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine to the primary amine of norepinephrine (NE) as the final step in the biosynthesis of epinephrine (Epi).[2] Epi is known to comprise 5−10% of the total catecholamine content of the central nervous system (CNS).[3] Immunohistochemical and radiochemical studies have indicated that Epi and PNMT are colocalized within the CNS and are found in very specific regions of the brain (e.g., the hypothalamus, thalamus, and the C1 and C2 regions of the medulla oblongata[4]). However, very little is known about the pharmacology of Epi within the CNS. On the basis of the location of these Epi containing neurons and inhibition studies of PNMT, it has been postulated that Epi is involved in the regulation of a number of physiological processes: (1) blood pressure and respiration,[5] (2) food and water intake,[5] (3) regulation of body temperature,[5] (4) secretion of hormones from the pituitary gland,[6] (5) effects on ethanol intoxication,[7] (6) regulation of the α_2-adrenoceptor in the hypothalamus,[8] and (7) some of the neurodegeneration seen in Alzheimer's disease.[9] However, the inhibitors that have been used in the studies of PNMT (**1**: SK&F 64139,[10] **2**: LY 134046,[11] and **3**: CGS 19281A[12]) were found to display affinity for the α_2-adrenoceptor at concentrations required for the inhibition of PNMT (Table 1). Therefore, it is not known whether it is inhibition of PNMT or the interaction of these inhibitors with α_2-adrenoceptors that causes the effects observed in these studies. To define the role of Epi in these processes, the development of a highly selective inhibitor of PNMT [selectivity ratio (α_2 K_i/PNMT K_i) > 500] that is sufficiently lipophilic to penetrate the blood−brain barrier (BBB) is needed.

Table 1. In Vitro Activities of Selected Compounds for Inhibition of PNMT and the Binding of [^{3}H]Clonidine at the α_2-Adrenoceptor[a]

compd		$K_i \pm$ SEM (μM)		selectivity
		PNMT	α_2-adrenoceptor	α_2/PNMT
1[b]	SK&F 64139	0.22 ± 0.05	0.021 ± 0.005	0.095
2[b]	LY 134046	0.26 ± 0.03	4.5 ± 0.3	17
3[c]	CGS 19281A	2.7 ± 0.1	12 ± 1	4.4
4[d]	SK&F 29661	0.56 ± 0.04	100 ± 20	180
5[e]		0.34 ± 0.06	1400 ± 30	4100
6[f]		0.66 ± 0.10	680 ± 10	1000
7[f]		0.64 ± 0.1	6.40 ± 0.2	10
8[f]		1.1 ± 0.1	460 ± 10	420
9[f]		0.54 ± 0.06	76 ± 6	140
10[g]		5.3 ± 0.3	680 ± 10	130
11[e]		0.29 ± 0.04	19 ± 1	66

[a] PNMT and α_2-adrenoceptor K_i values for literature compounds were determined in our laboratory for consistent internal comparison. [b] Ref 23. [c] Ref 34. [d] Ref 35. [e] Ref 15. [f] Ref 18. [g] Ref 25.

On the basis of previous structure−activity relationship studies[13−15] and comparative molecular field analyses (CoMFA—a type of three-dimensional QSAR) of the PNMT active site and the α_2-adrenoceptor,[16,17] it has been determined that there are two areas on the 1,2,3,4-tetrahydroisoquinoline (THIQ) nucleus—surrounding the 3- and 7-positions of THIQ—that affect both potency and selectivity for PNMT. It was found that THIQs possessing an appropriate 3-substituent (e.g., 3-hydroxymethyl[15] or 3-fluoromethyl[18]) and a hydrophilic electron-withdrawing 7-substituent are highly selective inhibitors of PNMT. Compounds **5** and **6** are examples of this type of inhibitor and are two of the most selective inhibitors of PNMT known. Autoradiographic studies have shown that **4** does not penetrate the BBB, presumably due to the high polarity of the 7-aminosulfonyl substituent ($\pi = -1.84$).[19,20] The addition of a 3-hydroxymethyl group ($\pi = -0.69$)[20] to **4** would increase the polarity and make **5** even less likely to penetrate

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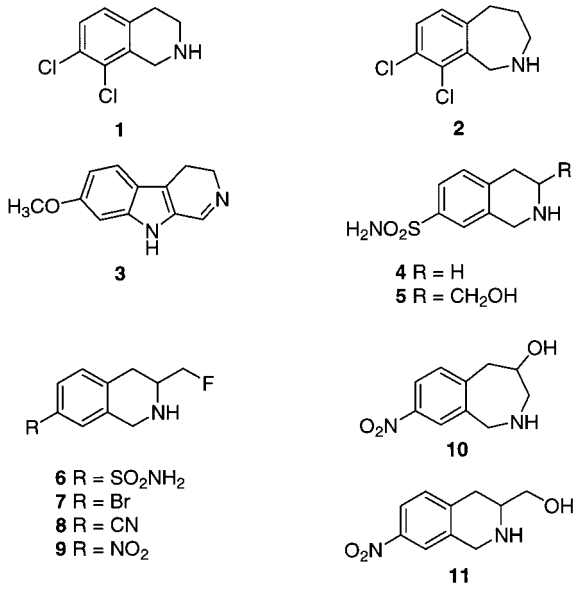


into the CNS. The 3-fluoromethyl moiety of **6** is less polar ($\pi = 0.24$)[20] than the 3-hydroxymethyl group and can mimic some of the possible hydrogen-bond interactions[21] that may take place between PNMT and the 3-hydroxymethyl moiety of **5**. Unfortunately, using an in vitro BBB model developed by Borchardt and Audus at the University of Kansas,[22] **6** was determined to be unable to penetrate the BBB.[18] Further results of this study indicated that there was a good correlation between lipophilicity (calculated partition coefficient, ClogP) and BBB penetration for THIQs. It was concluded that a THIQ-type of inhibitor required a ClogP value of at least 0.13−0.57 to achieve some penetration into the CNS, while THIQs possessing ClogP values greater than 0.57 should be able to penetrate.[18] Compounds **7**−**9** are other 3-fluoromethyl-THIQs from this study and possess ClogP values greater than 0.57.

On the basis of our CoMFA studies of the PNMT active site and the α_2-adrenoceptor, we had proposed that THIQs may bind in one of two orientations at both PNMT and the α_2-adrenoceptor, and that this binding orientation was dependent on the lipophilicity of the 7-substituent (Figure 1).[16] Studies have shown that THIQs that contain hydrophilic electron-withdrawing 7-substituents are more selective than those containing lipophilic 7-substituents.[16,17] Compound **7**, which would bind in the proposed lipophilic orientation (ClogP = 2.63), is a nonselective inhibitor of PNMT (α_2 K_i/PNMT $K_i = 10$). On the other hand, compounds **8** and **9**, which possess hydrophilic 7-substituents (**8**: ClogP = 1.20; **9**: ClogP = 1.51), are selective PNMT inhibitors, with **8** being almost 3-fold more selective for PNMT than **9** (**8**: α_2 K_i/PNMT $K_i = 410$; **9**: α_2 K_i/PNMT $K_i = 140$). Also, compounds **8** and **9** should be able to penetrate the BBB according to our in vitro study.[18] Thus, these compounds represent important leads for the development of new, more CNS-active PNMT inhibitors.

2,3,4,5-Tetrahydro-1*H*-2-benzazepines (THBAs), such as LY 134046 (**2**), are potent inhibitors of PNMT (Table 1).[11] Examination of the selectivities of SK&F 64139 (**1**; α_2 K_i/PNMT $K_i = 0.095$) and LY 134046 (**2**; α_2 K_i/PNMT $K_i = 17$) shows that THBAs are often more selective than similarly substituted THIQs, apparently due to the additional methylene group of the THBA nucleus.[23]

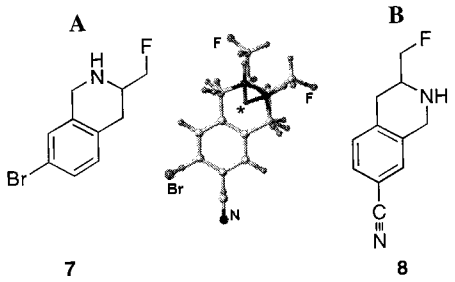


Figure 1. Proposed orientations of 7-substituted-THIQs at PNMT and the α_2-adrenoceptor. Orientation A is proposed for THIQs possessing lipophilic ($+\pi$) 7-substituents, while THIQs possessing hydrophilic ($-\pi$) 7-substituents are proposed to bind in orientation B. Between compounds **7** and **8** is a SYBYL-generated view of **7** in orientation A, superimposed on **8** in orientation B. The structures are energy minimized (Tripos force field with Gasteiger-Hückel charges) and were aligned using both ends of a 2 Å long normal through the centroids of the aromatic rings and the ends of the lone pairs (2.4 Å long). The asterisk marks the area in space where the lone pairs of **7** and **8** overlap.

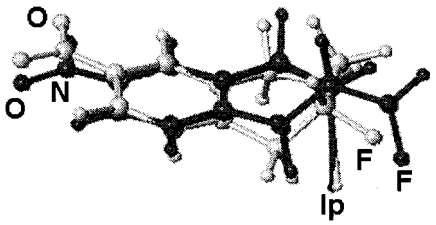
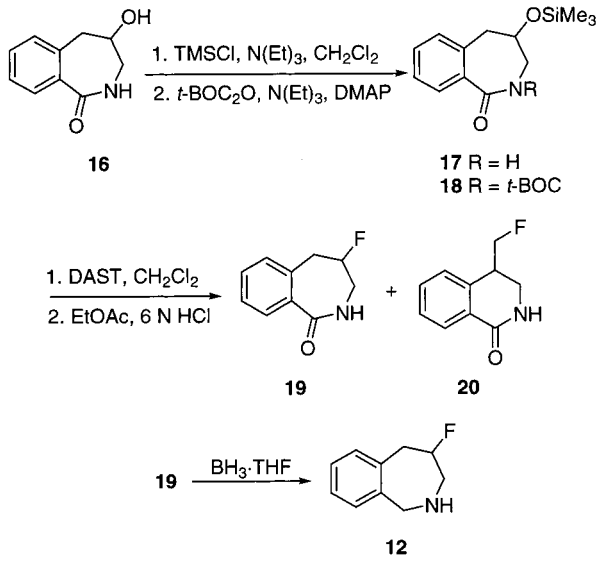


Figure 2. Molecular modeling overlay of the energy minimized structures (Tripos force field with Gasteiger-Hückel charges) of *R*-3-fluoromethyl-7-nitro-THIQ (*R*-**9**; dark) and *S*-4-fluoro-8-nitro-THBA (*S*-**13**; light). The structures were aligned using both ends of a 2 Å long normal through the centroids of the aromatic rings and the ends of the lone pairs (2.4 Å long). The "lp" indicates the point in space where the nitrogen lone pairs of *R*-**9** and *S*-**13** overlap. It should be noted that *R*-**9** and *S*-**13** have the same spatial orientation, but the opposite absolute configuration due to the Cahn−Ingold−Prelog rules for assigning priority.

Molecular modeling of the THBA nucleus indicates that there is a "pucker" in the ring, which corresponds to the 4-position of the benzazapine ring. This "pucker" overlays the same area of space as the 3-position of THIQ,[23] an area where substitution has been previously shown to increase selectivity for PNMT.[18] A comparison of (±)-4-hydroxy-8-nitro-THBA (**10**)[24,25] and (±)-3-hydroxymethyl-7-nitro-THIQ (**11**),[15] two compounds previously synthesized by this laboratory, indicates that THBA **10** is nearly twice as selective (α_2 K_i/PNMT $K_i = 130$) as THIQ **11** (α_2 K_i/PNMT $K_i = 67$). Even though **11** is more potent at PNMT, it is not as selective as **10** due to its increased α_2-adrenoceptor affinity (Table 1). As discussed previously, 3-fluoromethyl-THIQs (**6**, **8**, and **9**) are potent and selective inhibitors of PNMT (Table 1). Molecular modeling studies on 3-fluoromethyl-THIQ and 4-fluoro-THBA have shown that a fluorine at the 4-position of THBA can occupy the same area of space as a fluorine of the 3-fluoromethyl moiety of THIQ (Figure 2). Therefore, we proposed, synthesized, and evaluated a small series 4-fluoro-8-substituted-THBAs (compounds **12**−**15**) to compare their activities to similarly substituted 3-fluoromethyl-THIQs.

Chemistry. The syntheses of **12**−**15** are outlined in Schemes 1−3. The formation of the silyl-ether derivative (**17**) of secondary alcohol **16**[24] was readily effected with

Scheme 1



TMSCl and $N(Et)_3$, and the amide was protected with *t*-BOC_2O to form **18**. Treatment of **18** with diethylaminosulfur trifluoride (DAST), followed by deprotection of the amide in a biphasic mixture of 6 N HCl and EtOAc, yielded **19** and **20** as a (3:1) mixture, as determined by ^{1}H NMR (Scheme 1). It was found that this protection sequence was necessary because treatment of secondary alcohol **16** or TMS-protected alcohol **17** with DAST gave **19** and **20** in very low yields (<5%), with a variety of elimination and rearrangement byproducts. Attempts to isolate the *t*-BOC protected lactams of **19** and **20** by flash column chromatography and recrystallization were unsuccessful. Separation of **19** and **20** also proved to be very difficult with only small quantities of pure **19** being isolated after flash column chromatography and recrystallization (Scheme 1). However, **20** could not be isolated in pure form even after chromatography and multiple recrystallizations. The structures of **19** and **20** were confirmed by two-dimensional NMR (HMBC and HMQC). Reduction of **19** with BH_3·THF formed **12** (Scheme 1). We suggest that **20** is formed in the manner shown in Figure 3.

Because the separation of **19** and **20** proved to be difficult, the mixture was carried forward in the synthesis. Nitration of the mixture of **19** and **20** with H_2SO_4 and KNO_3 yielded lactams **24** and **25**, which were easily separated by flash column chromatography (Scheme 2). The structure of **25** was confirmed by X-ray crystallography (see Supporting Information for ORTEP diagram and crystal coordinates).

Compounds **24** and **25** were reduced individually with BH_3·THF to form **13** and **26**, respectively (Scheme 3). Hydrogenation of the nitro group of **13** with 10% Pd/C in MeOH formed the amine, which was immediately used in a Sandmeyer bromination reaction to form **14** (Scheme 3).[26] A similar reaction sequence using a modified Sandmeyer reaction was performed for the synthesis of nitrile **15** (Scheme 3).[27]

Biochemistry. All compounds were evaluated as their hydrochloride or hydrobromide salts for their activity as inhibitors of PNMT and of the binding of [^{3}H]clonidine to the α_2-adrenoceptor. Bovine adrenal PNMT was prepared using the method of Connett and

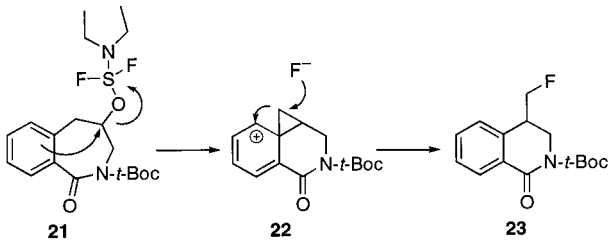
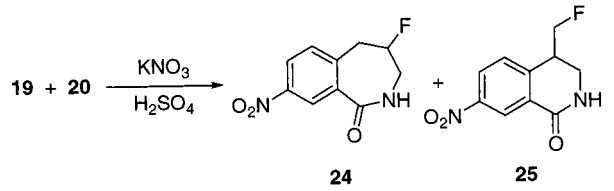
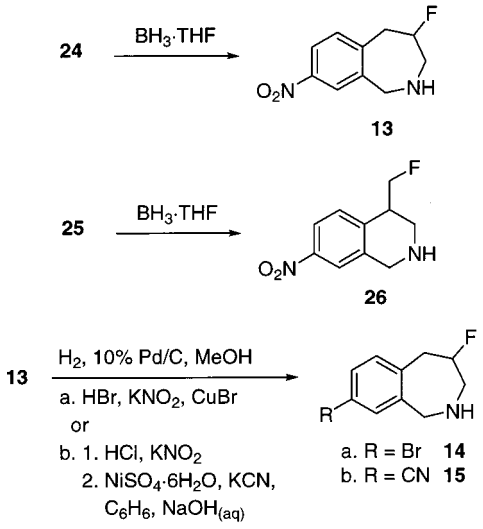


Figure 3. Proposed rearrangement for the formation of the 4-fluoromethyl-THIQ nucleus.

Scheme 2



Scheme 3



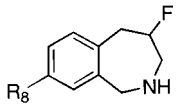
Kirshner through the isoelectric precipitation step.[28] The in vitro activity of these compounds was determined using a standard radiochemical assay that has been described previously.[29] Inhibition constants were determined by using three different concentrations of the inhibitor with phenylethanolamine as the substrate.

α_2-Adrenoceptor binding assays were performed using a standard radiochemical assay developed by U'Prichard et al.,[30] which uses [^{3}H]clonidine as the radioligand to define specific binding and phentolamine to determine the nonspecific binding affinity. This method was used to simplify the comparison with previous results.

Results and Discussion

The results of the biochemical evaluation of these compounds are shown in Table 2. Overall, 4-fluoro-THBAs **12**−**14** were found to be more selective than similarly substituted 3-fluoromethyl-THIQs (Table 1). It was expected and found that 4-fluoro-THBAs containing hydrophilic electron-withdrawing 8-substituents (**13** and **15**) were selective PNMT inhibitors. 4-Fluoro-8-nitro-THBA (**13**) is the most selective inhibitor in this series with a selectivity ratio (α_2 K_i/PNMT K_i) greater than 900 and is one of the most selective PNMT inhibitors yet known. Compound **13** was found to

Table 2. In Vitro PNMT and α_2-Adrenoceptor Affinities of 4-Fluoro-THBAs **12-15** and 4-Fluoromethyl-7-nitro-THIQ (**26**)



		$K_i \pm$ SEM (μM)		selectivity	
compd	R_8	PNMT	α_2-adrenoceptor	α_2/PNMT	ClogP
12	H	2.0 ± 0.2	93 ± 2	47	1.93
13	NO_2	0.54 ± 0.05	510 ± 20	940	1.67
14	Br	0.39 ± 0.04	49 ± 2	130	2.79
15	CN	1.3 ± 0.1	100 ± 10	77	1.36
26		2.1 ± 0.1	35 ± 1	17	1.51

display a 7-fold decrease in α_2-adrenoceptor affinity as compared to its 3-fluoromethyl counterpart **9**, whereas nitrile **15** was 5-fold less selective as compared to **8** due to an increase in its α_2-adrenoceptor affinity (Table 2).

A comparison of 4-hydroxy-THBA **10** (Table 1) and 4-fluoro-THBA **13** (Table 2) revealed that substitution of a fluorine atom for a hydroxy-group at the 4-position produced a 10-fold increase in PNMT potency and a slight increase in α_2-adrenoceptor affinity.

An unexpected result was the finding that 8-bromo-THBA **14** is a selective PNMT inhibitor due to its high potency at PNMT and moderate affinity for the α_2-adrenoceptor (Table 2). However, **14** still had the highest affinity for the α_2-adrenoceptor in this series of compounds. A comparison of the PNMT and α_2-adrenoceptor affinities of 3-fluoromethyl-THIQ **7** (PNMT $K_i =$ 0.64) and 4-fluoro-THBA **14** (PNMT $K_i = 0.39$) shows that **14** displayed a 2-fold increase in PNMT potency and a 5-fold decrease in α_2-adrenoceptor affinity (**7**; α_2 $K_i = 6.4$ versus **14**; α_2 $K_i = 49$) corresponding to a 10-fold increase in selectivity for **14**. The decreased α_2-adrenoceptor affinity of **14** indicates that the 4-fluoro-THBA nucleus is interacting in a different manner with the α_2-adrenoceptor than are the 3-fluoromethyl-THIQs. This decrease in affinity is similar to that which was observed previously between SK&F 64139 (**1**) and LY 134046 (**2**) at the α_2-adrenoceptor (Table 1).

4-Fluoromethyl-7-nitro-THIQ (**26**) was found to have decreased PNMT potency ($K_i = 2.1$ μM) as compared to both 4-fluoro-8-nitro-THBA (**13**) ($K_i = 0.54$ μM) and 3-fluoromethyl-7-nitro-THIQ (**9**) ($K_i = 0.54$ μM). This decrease in potency was attributed to negative steric interactions surrounding the 4-fluoromethyl group, as substitution of a methyl group at the 4-position of THIQ has been shown to decrease the potency of THIQ at PNMT.[31] Compound **26** also displayed increased affinity for the α_2-adrenoceptor ($K_i = 35$ μM) as compared to **13** ($K_i = 510$ μM) and **9** ($K_i = 77$ μM), which makes **26** a relatively nonselective inhibitor of PNMT (α_2 K_i/PNMT $K_i = 17$).

Conclusions

Overall, these 4-fluoro-THBAs (**12**−**14**) were found to be more selective than similarly substituted 3-fluoromethyl-THIQs with the exception of nitrile **15**. 4-Fluoro-THBAs containing hydrophilic electron-withdrawing 8-substituents (**13** and **15**) were found to be selective inhibitors of PNMT as anticipated. However, an unexpected result was the finding that 8-bromo-THBA **14** was a selective PNMT inhibitor due to its excellent potency for PNMT and moderate affinity for the α_2-adrenoceptor (Table 2). 4-Fluoro-THBAs **13**−**15** displayed selectivities (α_2 K_i/PNMT K_i) greater than 75, and 4-fluoro-8-nitro-THBA (**13**) is one of the most selective inhibitors of PNMT yet known, with a selectivity ratio greater than 900. These compounds also possess calculated partition coefficients (ClogP) greater than 0.57 and, according to our in vitro BBB permeability studies,[18] should be able to penetrate into the CNS. These THBAs represent promising new leads in the development of new, more selective inhibitors of PNMT.

Experimental Section

All of the reagents and solvents used were reagent grade or were purified by standard methods before use.[32] Melting points were determined in open capillary tubes on a Thomas-Hoover melting point apparatus calibrated with known compounds, but are otherwise uncorrected. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on a Varian XL-300, a GE QE-300, a Bruker DRX-400, or a Bruker AM 500 spectrometer. Proton chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS, 0.00 ppm) and carbon chemical shifts are reported in ppm relative to $CDCl_3$ (77.0 ppm) unless otherwise noted. For the hydrochloride or hydrobromide salts, NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d_6) and the chemical shifts are reported relative to DMSO (2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C), or deuterated MeOH (CD_3OD) and the chemical shifts are reported relative to MeOH (3.31 ppm for ^{1}H and 49.15 ppm for ^{13}C). Multiplicity abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; ex, exchangeable. Infrared spectra were obtained on a Perkin-Elmer 1420 infrared spectrometer. Fast-atom bombardment mass spectra (FABMS), chemical-ionization mass spectra (CIMS), and high-resolution mass spectra (HRMS) were obtained on a ZAB HS or a Ribermag R 10-10 mass spectrometer. All CIMS used ammonia as the carrier gas. The intensity of each peak in the mass spectrum relative to the base peak is reported in parentheses. Microanalyses were performed on a Hewlett-Packard model 185B CHN analyzer at the University of Kansas. Bulb-to-bulb distillations were performed on a Kugelrohr distillation apparatus (Aldrich Chemical Co, Milwaukee, WI) and oven temperatures were recorded.

Flash column chromatography was carried out using silica gel 60 (230−400 mesh) purchased from Universal Adsorbents, Atlanta, GA. Hexanes refers to the mixture of hexane isomers (bp 40−70 °C) and brine refers to a saturated solution of NaCl. All reactions that required anhydrous conditions were performed under a positive nitrogen (N_2) flow and all glassware was either oven-dried or flame-dried before use.

S-Adenosyl-L-methionine used in the radiochemical assays was obtained from Sigma Chemical Co. [^{3}H]-*S*-Adenosyl-L-methionine was purchased from American Radiolabeled Chemicals (St. Louis, MO). [^{3}H]Clonidine was purchased from DuPont New England Nuclear (Boston, MA) for use in the α_2-adrenoceptor assays. Bovine adrenal glands were obtained from Davis Meat Processing (Overbrook, KS).

Compound **16** has been synthesized previously by this laboratory.[24]

4-Trimethylsiloxy-2,3,4,5-tetrahydro-1*H*-2-benzazepin-1-one (17). Secondary alcohol **16**[24] (235 mg, 1.32 mmol) was dissolved in CH_2Cl_2 (25 mL) and NEt_3 (0.37 mL, 2.7 mmol) and $ClSiMe_3$ (0.33 mL, 2.7 mmol) were added dropwise to the solution. The reaction mixture was stirred overnight. The mixture was poured onto ice (5 g) and extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (silica gel) eluting with hexanes/

EtOAc (4:1) to yield **17** as a clear colorless oil that solidified on standing (329 mg, 99%): mp 92−94 °C; IR (KBr) 3200, 3060, 2950, 1650, 1340, 1300, 1240, 1180, 1090, 950, 860, 830, 750; ^{1}H NMR ($CDCl_3$) δ 7.75−7.72 (m, 1H, ArH-9), 7.40−7.33 (m, 2H, ArH-7,8), 7.20−7.18 (m, 1H, ArH-6), 6.25 (br ex, 1H, NH), 4.32 (m, 1H, H-4), 3.23−2.81 (m, 4H, 2H-3, 2H-5), 0.15 (s, 9H, $SiCH_3$); ^{13}C NMR ($CDCl_3$) 174.2, 136.2, 134.9, 131.3, 129.8, 129.1, 127.5, 73.6, 47.7, 40.8, 0.3; CIMS *m/z* (relative intensity) 250 (MH^+, 100), 234, (20), 205 (20), 178 (20), 159 (20), 119 (20), 73 (25). HRMS−FAB (*m/z*): $[M + H]^+$ calcd for $C_{13}H_{20}NO_2Si$, 250.1263; found, 250.1284.

2-*N*-(*t*-Butoxycarbonyl)-4-trimethylsiloxy-2,3,4,5-tetrahydro-1*H*-2-benzazepin-1-one (18). Silyl ether **17** (329 mg, 1.32 mmol) was dissolved in a solution of dry $CHCl_3$ (25 mL), 4-(dimethylamino)pyridine (161 mg, 1.32 mmol), and $N(Et)_3$ (0.18 mL, 1.32 mmol). The solution was cooled to 0 °C and *t*-BOC_2O (0.6 mL, 2.64 mmol) was added dropwise. The solution was allowed to warm to room temperature and was stirred for 16 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel eluting with hexanes/EtOAc (8:1) to yield **18** (438 mg, 95%) as a colorless oil that solidified on standing: mp 67−69 °C; IR (KBr) 2990, 2920, 1710, 1685, 1360, 1330, 1290, 1140, 1065, 975, 840, 790, 740; ^{1}H NMR ($CDCl_3$) 7.82−7.79 (m, 1H, ArH-9), 7.46−7.31 (m, 2H, ArH-7,8), 7.17−7.14 (m, 1H, ArH-6), 4.39−3.31 (m, 1H, H-4), 3.78 (dd, J = 14.6, 4.7 Hz, 1H, H-3), 3.39 (dd, J = 14.6, 6.2 Hz, 1H, H-3), 3.06 (dd, J = 13.8, 6.2 Hz, 1H, H-5), 2.82 (dd, J = 13.8, 4.7 Hz, 1H, H-5), 1.57 (s, 9H, $3CH_3$), 0.16 (s, 9H, $3CH_3$); FABMS *m/z* (relative intensity) 350 (MH^+, 100), 160 (43), 131 (27); HRMS−FAB (*m/z*): $[M + H]^+$ calcd for $C_{18}H_{28}NO_4Si$, 350.1787; found, 350.1790.

(±)-4-Fluoro-2,3,4,5-tetrahydro-1*H*-2-benzazepin-1-one (19) and (±)-4-Fluoromethyl-3,4-dihydroisoquiolin-1−2*H*-one (20). Silyl ether **18** (2.58 g, 7.38 mmol) was dissolved in dry CH_2Cl_2 (30 mL). Diethylaminosulfur trifluoride (2.89 mL, 14.8 mmol) was added dropwise to the mixture and the resulting solution was stirred at room temperature for 3 h. The reaction was quenched with ice water (10 mL). The solution was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with 3 N HCl (20 mL), 10% $NaHCO_{3(aq)}$ (20 mL) and brine (20 mL), and dried over anhydrous Na_2SO_4. The organic solvent was removed under reduced pressure and the yellowish residue was dissolved in a biphasic mixture of EtOAc (10 mL) and 6 N HCl (10 mL) and stirred for 6 h. The mixture was extracted with EtOAc (3 × 25 mL). The combined organic extracts were washed with water (25 mL), 5% $NaHCO_{3(aq)}$ (25 mL), and brine (25 mL) and dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel) eluting with hexanes/EtOAc (3:2) to yield **19** and **20** (897 mg, 68%) as a 3:1 mixture (estimated by ^{1}H NMR). A small amount of an enriched mixture of **19** could be obtained in the first few fractions off the column as a white solid. Two recrystallizations of this mixture from EtOAc/hexanes yielded pure **19**: mp 152−153 °C; IR (KBr); ^{1}H NMR ($CDCl_3$) δ 7.72−7.69 (m, 1H, ArH-9), 7.49−7.38 (m, 2H, ArH-7,8), 7.25−7.15 (m, 1H, ArH-6), 6.80 (br ex, 1H, NH), 5.07 (dm, J = 49.5 Hz, 1H, CHF), 3.44−3.17 (m, 4H, 2H-3 and 2H-5); ^{13}C NMR ($CDCl_3$) δ 173.0, 134.3, 134.1, 131.5, 129.6, 129.3, 128.0, 93.3 (d, J = 570 Hz, CH_2F), 45.1 (d, J = 82 Hz, C-3), 37.4 (d, J = 76 Hz, C-5); CIMS *m/z* (relative intensity) 180 (MH^+, 100), 159 (15), 150 (20), 122 (15). Anal. ($C_{10}H_{10}NFO$) C, H, N.

Compound **20** could not be cleanly isolated: ^{1}H NMR ($CDCl_3$) δ 8.12−8.10 (m, 1H, ArH-8), 7.52−7.49 (m, 1H, ArH-6), 7.44−7.41 (m, 1H, ArH-7), 7.29−7.26 (m, 1H, ArH-5), 6.55 (br ex, 1H, NH), 4.54 (dm, J = 62 Hz, 2H, CH_2F), 3.76−3.66 (m, 2H, H-3) 3.33−3.31 (m, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 165.6, 136.4 (d, J = 27 Hz, C-4a), 132.6, 128.9, 128.4, 128.3, 127.7, 82.3 (d, J = 560 Hz, CH_2F), 40.3 (d, J = 12 Hz, C-3), 38.7 (d, J = 67 Hz, C-4).

4-Fluoro-2,3,4,5-tetrahydro-1*H*-2-benzazapine Hydrochloride (12·HCl). Lactam **19** (60.1 mg, 0.335 mmol) was dissolved in THF (10 mL), 1 M BH_3·THF (1.0 mL, 1.0 mmol) was added dropwise to the solution and the mixture was heated at reflux for 14 h. The solution was cooled to room temperature, MeOH (5 mL) was added, and the solvent was removed under reduced pressure. The residue was dissolved in MeOH (10 mL), 6 N HCl (10 mL) was added, and the solution was heated at reflux for 1 h. The MeOH was removed under reduced pressure and the remaining aqueous solution was made basic (pH > 10) with 10% NaOH. The basic solution was extracted with CH_2Cl_2 (3 × 30 mL), the organic extracts were combined and dried, and the solvent was removed under reduced pressure. The crude amine **12** was dissolved in $CHCl_3$ and the hydrochloride salt was formed using dry $HCl_{(g)}$, which was recrystallized from EtOH/hexanes to yield **12**·HCl (53.4 mg, 79%): mp 229−230 °C; ^{1}H NMR (DMSO-d_6) δ 10.10−9.53 (br ex m, 2H, NH_2^+), 7.44−7.28 (m, 4H, ArH-6,7,8,9), 5.20 (dm, J = 48.4 Hz, 1H, CHF), 4.45−4.24 (m, 2H, H-1), 3.59−3.33 (m, 4H, 2H-3 and 2H-5); ^{13}C NMR (DMSO-d_6) δ 136.9 (d, J = 23 Hz, C-5a), 133.8, 132.0, 131.5, 130.1, 128.0, 86.2 (d, J = 685 Hz, CHF), 51.6, 50.4, 38.5 (d, J = 90 Hz, C-5); CIMS *m/z* (relative intensity) 165 (MH^+, 100), 144 (50). Anal. ($C_{10}H_{12}FN$·HCl) C, H, N.

(±)-4-Fluoro-8-nitro-2,3,4,5-tetrahydro-1*H*-2-benzazepin-1-one (24) and (±)-4-Fluoromethyl-7-nitro-3,4-dihydroisoquiolin-1−2*H*-one (25). A mixture of lactams **19** and **20** (3:1) (606 mg, 3.38 mmol) was dissolved in H_2SO_4 (15 mL) and cooled to 0 °C. KNO_3 (376 mg, 3.72 mmol) was added in small portions over the course of 30 min. The mixture was allowed to warm to room temperature and stirred overnight. The solution was poured onto ice (20 g) where the formation of an off-white precipitate was noted. The precipitate was collected by filtration, washed with water (20 mL), and dried under vacuum. The aqueous filtrate was extracted with EtOAc (3 × 25 mL). The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure to yield a yellowish residue. This residue was combined with the previously isolated precipitate and purified by flash column chromatography (silica gel) eluting with EtOAc/hexanes (4:1) to yield **24** and **25** as off-white solids with **24** eluting first. Recrystallization of **24** from EtOAc/hexanes yielded **24** as white needles (386 mg, 1.72 mmol, 51%): mp 211−212 °C; IR (KBr) 3200, 3040, 2910, 1660, 1610, 1520, 1345, 1020, 1000, 820, 730; ^{1}H NMR ($CDCl_3$) δ 8.64 (d, J = 2.4 Hz, 1H, ArH-9), 8.31 (dd, J = 8.4, 2.4 Hz, 1H, ArH-7), 7.46 (d, J = 8.4, 1H, ArH-6), 6.31 (br ex s, 1H, NH), 5.15 (dm, J = 49.2 Hz, 1H, H-4), 3.42−3.28 (m, 4H, 2H-5, 2H-3); ^{13}C NMR (DMSO-d_6) 169.3, 147.0, 141.6, 136.9, 131.5, 125.2, 123.6, 93.0 (d, J = 700 Hz, C-4), 43.8 (d, J = 102 Hz, C-3), 36.5 (d, J = 97 Hz, C-5); CIMS *m/z* (relative intensity) 225 (MH^+, 100), 205 (40), 204 (10). Anal. ($C_{10}H_9N_2O_3F$) C, H, N.

Compound **25** was recrystallized from EtOAc/hexanes to yield **25** as pale yellow needles (128 mg, 0.571 mmol, 17%): mp 187−188 °C; IR (KBr) 3180, 3080, 2900, 1670, 1600, 1520, 1480, 1440, 1340, 1090, 1050, 1040, 930, 740; ^{1}H NMR (DMSO-d_6) δ 8.52 (d, J = 2.4 Hz, 1H, ArH-8), 8.33 (dd, J = 2.4, 8.4 Hz, 1H, ArH-6), 8.22 (br ex s, 1H, NH), 7.70 (d, J = 8.4 Hz, 1H, ArH-5), 4.72−4.44 (m, 2H, CH_2F), 3.61−3.37 (m, 3H, 2H-3, 1H-4); ^{13}C NMR (DMSO-d_6) 162.8, 148.1, 145.5, 131.6, 130.9, 127.1, 122.7, 83.8 (d, J = 683 Hz, CH_2F), 40.7 (d, J = 12 Hz, C-3), 38.3 (d, J = 79 Hz, C-4); CIMS *m/z* (relative intensity) 242 ($M+NH_4^+$, 20), 225 (MH^+, 100), 205 (75), 195 (25); HRMS−FAB (*m/z*): $[M + H]^+$ calcd for $C_{10}H_{10}N_2O_3F$, 225.0675; found, 225.0691. Anal. ($C_{10}H_9N_2O_3F$) C, H, N.

4-Fluoro-8-nitro-2,3,4,5-tetrahydro-1*H*-2-benzazapine Hydrochloride (13·HCl). Lactam **24** (293 mg, 1.31 mmol) was dissolved in THF (20 mL) and cooled to 0 °C. 1 M BH_3·THF (3.3 mL, 3.3 mmol) was added and the reaction mixture was heated at reflux overnight. The mixture was cooled in an ice bath, MeOH (2 mL) was added dropwise, and the solvent was removed under reduced pressure. The residue was dissolved in MeOH (10 mL), 6 M HCl (10 mL) was added, and the solution was heated at reflux for 3 h. The MeOH was removed under reduced pressure and the remaining aqueous phase was made basic (pH > 10) with 10% $NaOH_{(aq)}$. The basic

solution was extracted with CH_2Cl_2 (3 × 25 mL). The organic extracts were combined and dried over anhydrous K_2CO_3. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel) eluting with 100% EtOAc. The solvent was removed under reduced pressure to yield **13** as a white solid. The solid was dissolved in EtOH, and dry $HCl_{(g)}$ was used to form the hydrochloride salt, which was recrystallized from EtOH/hexanes to yield **13**·HCl as white needles (232 mg, 82%): mp 250 °C (dec); ^{1}H NMR (DMSO-d_6) δ 10.53 (br ex s, 2H, NH_2^+), 8.35 (d, J = 2.4 Hz, 1H, ArH-9), 8.15 (dd, J = 8.4, 2.4 Hz, 1H, ArH-7), 7.58 (d, J = 8.4 Hz, 1H, ArH-6), 5.30 (dm, J = 45.3 Hz, 1H, H-4), 4.55−4.44 (m, 2H, H-1), 3.62−3.58 (m, 2H, H-3), 3.53−3.46 (m, 2H, H-5); CIMS *m*/*z* (relative intensity) 211 (MH^+, 100), 195 (20). Anal. ($C_{10}H_{12}N_2O_2F$·HCl) C, H, N.

(±)-4-Fluoromethyl-7-nitro-1,2,3,4-tetrahydroisoquinoline Hydrochloride (26·HCl). Lactam **25** (90.7 mg, 0.405 mmol) was reduced using the same procedure outlined previously for the synthesis of **13**·HCl. Crude **26** was purified by flash chromatography (silica gel) eluting with 100% EtOAc to yield **26** as a white solid, which was dissolved in EtOH, and the hydrochloride salt was formed using dry $HCl_{(g)}$. The white solid was recrystallized from EtOH/hexanes to yield **26**·HCl as white needles (58.3 mg, 58%): mp 260 °C (dec); ^{1}H NMR (CD_3OD) δ 8.21−8.19 (m, 2H, ArH-6,8), 7.73 (d, J = 9.3 Hz, 1H, ArH-5), 4.82 (dm, J = 9.3 Hz, 2H, CH_2F), 4.46 (s, 2H, H-1), 3.71−3.53 (m, 3H, 2H-3, 1H-4); CIMS *m*/*z* (relative intensity) 211 (MH^+, 100), 181 (25). Anal. ($C_{10}H_{11}N_2O_2F$·HCl) C, H, N.

4-Fluoro-8-bromo-2,3,4,5-tetrahydro-1*H*-2-benzazapine Hydrobromide (14·HBr). Nitrobenzazapine **13** (116 mg, 0.520 mmol) was dissolved in MeOH, Pd/C (10 mg) was added, and the mixture was hydrogenated at 50 psi for 6 h. The solution was filtered through Celite and the Celite bed was washed with MeOH (2 × 20 mL). The solvent was removed under reduced pressure to yield a white solid, which was dissolved in 48% HBr (1 mL) and H_2O (2 mL). The solution was cooled to 0 °C, and $NaNO_2$ (43.1 mg, 0.624 mmol) was added in small portions over 15 min, followed by stirring for 30 min. Urea (20 mg, 0.33 mmol) was added to destroy excess HNO_2. This solution was added dropwise to a solution of CuBr (300 mg, 2.09 mmol) in 48% HBr (2 mL) and H_2O (1 mL). After the addition, the reaction mixture was heated to 75 °C and stirred for 2 h. The mixture was cooled to room temperature and stirred overnight. The mixture was made basic (pH > 10) with 20% NaOH, and the resulting blue copper salts were removed by filtration through Celite. The Celite bed was washed thoroughly with CH_2Cl_2 (3 × 50 mL), the organic phase was collected and the aqueous filtrate was extracted with CH_2Cl_2 (3 × 50 mL). All of the organic washes and extractions were combined and dried over K_2CO_3, and the solvent removed under reduced pressure to yield a yellow oil. The oil was purified by flash chromatography on silica gel eluting with hexanes/EtOAc (3:1) to yield **14** as a colorless oil. The oil was dissolved in EtOH and the hydrobromide salt was formed using dry $HBr_{(g)}$. The off-white solid was recrystallized from EtOH/hexanes to yield **14**·HBr as off-white plates (39.7 mg, 16%): mp 265 °C (dec); ^{1}H NMR ($CDCl_3$, free base) δ 7.32−7.29 (m, 1H, ArH-9), 7.26−7.25 (m, 1H, ArH-7), 7.09 (d, J = 7.8 Hz, 1H, ArH-6), 4.79 (dm, J = 47.1 Hz, 1H, H-4), 3.87 (s, 2H, H-1), 3.64−3.12 (m, 5H, NH, 2H-3 and 2H-5); CIMS *m*/*z* (relative intensity) 247 (MH^++3, 10), 246 (MH^++2, 90), 245, (MH^++1, 12), 244, (MH^+, 100), 225 (25), 224 (50), 223 (25), 222 (30), 115 (25). Anal. ($C_{10}H_{11}NBrF$·HBr) C, H, N.

4-Fluoro-8-cyano-2,3,4,5-tetrahydro-1*H*-2-benzazapine Hydrobromide (15·HBr). Nitrobenzazapine **13** (118 mg, 0.479 mmol) was dissolved in MeOH. Pd/C (10 mg) was added and the mixture was hydrogenated at 50 psi for 6 h. The solution was filtered through Celite and the solvent was removed to yield a white solid, which was dissolved in concentrated HCl (2 mL) and H_2O (1 mL). The solution was cooled to 0 °C and $NaNO_2$ (33.1 mg, 0.479 mmol) was added in small portions over the course of 15 min. Following the addition, the solution was stirred for 30 min. Urea (30 mg, 0.5 mmol) was added to destroy excess HNO_2.

In a separate flask, NaOH (250 mg, 6.25 mmol) was dissolved in H_2O (3 mL) and KCN (163 mg, 2.50 mmol) was added. Benzene (5 mL) was added to the basic KCN solution, and the solution was cooled in an ice bath. $NiSO_4$·$6H_2O$ (130 mg, 0.495 mmol) was added and the basic KCN mixture was changed from colorless to a yellow-brown. The diazonium salt solution was added dropwise to the basic KCN mixture. Nitrogen evolution was observed and the reaction mixture was allowed to warm to room temperature. After 2 h, the mixture was warmed to 50 °C for 1 h and subsequently cooled to room temperature. The solution was made basic (pH > 10) with 10% $NaOH_{(aq)}$ and filtered through Celite. The Celite was washed thoroughly with CH_2Cl_2 (2 × 50 mL) and the organic phase was collected. The remaining aqueous phase was extracted with CH_2Cl_2 (3 × 25 mL). The organic phases were combined and dried over anhydrous K_2CO_3. The solvent was removed to yield a brown oil which was purified by flash chromatography (silica gel) eluting with hexanes/EtOAc (1:1) to yield **15** as a colorless oil. The oil was dissolved in EtOH and 48% HBr was added dropwise to form the hydrobromide salt. The crude HBr salt was collected and recrystallized from EtOH/hexanes to yield **15**·HBr as yellowish needles (45.6 mg, 35%): mp 262 °C (dec); IR (KBr) 3400, 2910, 2800, 2700, 2220, 1550, 1425, 1100, 1060, 990; ^{1}H NMR ($CDCl_3$, free base) δ 7.49 (dd, J = 7.5, 1.5 Hz, 1H, ArH-9), 7.40 (d, J = 1.5 Hz, 1H, ArH-7), 7.33 (d, J = 7.5 Hz, 1H, ArH-6), 4.65 (dm, J = 51.0 Hz, 1H, H-4), 3.95 (s, 2H, H-1), 3.45−3.25 (m, 5H, NH, 2H-3 and 2H-5); CIMS *m*/*z* (relative intensity) 191 (MH^+, 100), 171 (30), 170 (25), 169 (45). Anal. ($C_{11}H_{11}N_2F$·HBr) C, H, N.

Radiochemical Assay for PNMT Activity. The assay used for this study has been described previously.[29] A normal assay mixture consists of 50 μL of 0.5 M phosphate buffer (pH 8.0), 25 μL of 10 mM AdoMet, 5 μL of [^{3}H]AdoMet that contains 3 × 10^5 dpm (specific activity ca. 15 Ci/mmol), 25 μL of substrate solution (phenylethanolamine), 25 μL of inhibitor solution, 25 μL of the enzyme preparation, and water to a achieve a total volume of 250 μL. The mixture is incubated for 30 min at 37 °C, quenched with the addition of 250 μL of 0.5 M borate buffer (pH 10) and the mixture extracted with 2 mL of toluene/isoamyl alcohol (7:3). A 1-mL aliquot of the organic layer is removed, transferred to a scintillation vial, and diluted with cocktail for counting. The mode of inhibition for all of the inhibitors assayed was determined to be competitive by inspection of the 1/*V* versus 1/*S* plots of the data. All assays were run in duplicate with three inhibitor concentrations over a 5-fold range. K_i values were determined by a hyperbolic fit of the data.

α_2-Adrenoceptor Radioligand Binding Assay. The radioligand binding assay was performed using the methods developed by U'Prichard et al.[30] Male Sprague Dawley rats were decapitated and the cortexes removed and homogenized with 20 volumes (w/v) of ice-cold 50 mM Tris/HCl buffer (pH 7.7 at 25 °C). Homogenates were centrifuged 3 times for 10 min at 50000*g* with resuspension of the pellet in fresh buffer between spins. The final pellet was homogenized in 200 volumes (w/v) of ice-cold 50 mM Tris/HCl buffer (pH 7.7 at 25 °C). Incubation tubes containing [^{3}H]clonidine (specific activity ca. 19.2 mCi/mmol, final concentration 2.0 nM), various concentrations of the inhibitors and an aliquot of freshly suspended tissue (800 μL) to a final volume of 1 mL were used. Tubes were incubated at 25 °C for 30 min and the incubation was terminated by rapid filtration under vacuum through GF/B glass fiber filters. The filters were rinsed with three 5-mL washes of 50 mM Tris/HCl buffer (pH 7.7 at 25 °C). The filters were counted in vials containing premixed scintillation cocktail. Nonspecific binding was determined as the concentration of ligand bound in the presence of 2 μM phentolamine. All assays were determined by a log-probit analysis of the data and K_i values were determined by the equation $K_i = IC_{50}/(1+[\text{Clonidine}]/K_D)$, as all of the Hill coefficients were approximately equal to one.

Molecular Modeling. All ClogP values were calculated and all molecular modeling studies were carried out on a Silicon Graphics O2 workstation running SYBYL 6.6.[33]

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Supporting Information Available: Details of the two-dimensional NMR experiments for **19** and **20**, which are contained in two tables and the details of the X-ray crystallographic study and tables of relevant crystal data for compound **25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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