Synthesis and Evaluation of 3-Trifluoromethyl-7-substituted-1,2,3,4-tetrahydroisoquinolines as Selective Inhibitors of Phenylethanolamine *N*-Methyltransferase versus the α_2-Adrenoceptor[1]

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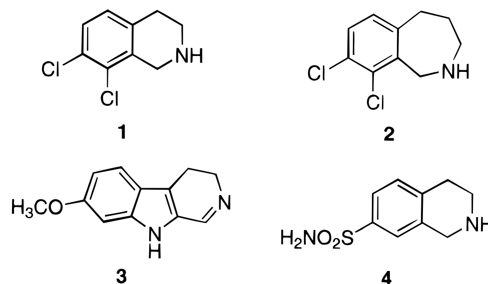
A series of 3-trifluoromethyl-1,2,3,4-tetrahydroisoquinolines was synthesized and evaluated as inhibitors of phenylethanolamine *N*-methyltransferase (PNMT) and as inhibitors of the binding of clonidine at the α_2-adrenoceptor. These compounds were found to be selective inhibitors of PNMT due to their decreased affinity for the α_2-adrenoceptor, which was attributed to steric bulk intolerance around the 3-position of 1,2,3,4-tetrahydroisoquinoline (THIQ) at the α_2-adrenoceptor and to the decreased pK_a of the THIQ amine due to the 3-trifluoromethyl moiety. Overall, these compounds displayed less affinity for PNMT compared to previously studied THIQ-type inhibitors, except for **16** which was found to have good affinity for PNMT (PNMT $K_i = 0.52\ \mu$M). Compounds **14** and **16** proved to be the most selective inhibitors in this small series of compounds and are some of the most selective inhibitors of PNMT known (**14**, selectivity α_2 K_i/PNMT $K_i = 700$; **16**, selectivity α_2 K_i/PNMT $K_i > 1900$). Compounds **14** and **16** are also quite lipophilic due to the 3-trifluoromethyl moiety and represent promising new leads for the development of new highly selective inhibitors of PNMT, which should be sufficiently lipophilic to penetrate the blood−brain barrier.

Introduction

Epinephrine (Epi) is known to comprise 5−10% of the catecholamines in the mammalian central nervous system (CNS).[2,3] The final step in the biosynthesis of Epi is the transfer of a methyl group from *S*-adenosyl-L-methionine (AdoMet) to the primary amine of norepinephrine (NE). This reaction is catalyzed by the enzyme phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28). PNMT and Epi are co-localized in the CNS, being sequestered principally in two specific regions (C1 and C2) of the medulla oblongata.[4,5] On the basis of this CNS localization, Epi neurons have been postulated to be involved in the regulation of a number of physiological processes.[4−13]

Undoubtedly the most thoroughly studied physiological process to which central Epi has been linked is the regulation of peripheral blood pressure. In vivo administration of centrally active PNMT inhibitors [SK&F 64139 (**1**),[14] LY 134046 (**2**),[15] and CGS 19281A (**3**)[16]] to hypertensive rats has demonstrated dramatic decreases in peripheral blood pressure. However, these results have been complicated by the observation that all of these inhibitors show low selectivity for the PNMT active site versus the α_2-adrenoceptor (Table 1). Thus, the decrease in peripheral blood pressure in hypertensive rat models by PNMT inhibitors may not be the result of their ability to decrease Epi concentrations within the medulla oblongata (i.e., to inhibit central PNMT) but rather their ability to interact with the α_2-adrenoceptor at concentrations required for PNMT inhibition.[17−19] To unambiguously define the functions of Epi within the CNS, the development of a potent yet



highly selective inhibitor of its biosynthesis is required (i.e., α_2 K_i/PNMT $K_i > 500$).[20]

Previous studies from our laboratory and others found that there were two areas on the tetrahydroisoquinoline (THIQ) nucleus where substitution can increase both potency for PNMT and selectivity versus the α_2-adrenoceptor. The first area is at the 7-position,[21] where it has been determined for monosubstituted chloro-THIQs that substitution at the 7-position was needed for optimal potency for PNMT.[22] Our laboratory has further examined how substitution at the 7-position of THIQ affects both potency and selectivity by developing comparative molecular field analysis[23] (CoMFA) models (a type of three-dimensional QSAR analysis) on a set of 30 7-substituted-THIQs[24] for both the PNMT active site and the α_2-adrenoceptor. These models indicate that, depending on the lipophilicity of the 7-substituent of THIQ, these compounds may bind in two different orientations at both the PNMT active site and the α_2-adrenoceptor (Figure 1). This study concluded that THIQs possessing hydrophilic electron-withdrawing 7-substituents would be both potent and selective for PNMT due to their

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

compd	K_i (μM) PNMT	α_2-adrenoceptor	selectivity α_2/PNMT
SK&F 64139[b] (**1**)	0.22 ± 0.05	0.021 ± 0.005	0.095
LY 134046[b] (**2**)	0.26 ± 0.03	4.5 ± 0.3	17
CGS 19281A (**3**)	2.7 ± 0.1	12 ± 1	4.4
SK&F 29661[c] (**4**)	0.56 ± 0.04	100 ± 20	180

[a] PNMT and α_2-adrenoceptor K_i values for literature compounds were determined in our laboratory for consistent internal comparison. [b] Reference 45. [c] Reference 46.

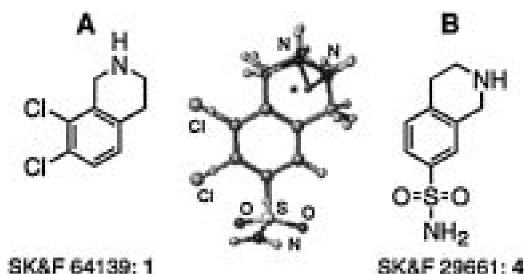


Figure 1. Two possible orientations of THIQ-type inhibitors at PNMT and the α_2-adrenoceptor.[24] Alignment A is proposed for lipophilic (+π) 7-substituents, while hydrophilic (−π) 7-substituents are proposed to bind in alignment B. Between these two structures is a SYBYL-generated view of SK&F 64139 (**1**) in alignment A superimposed on SK&F 29661 (**4**) in alignment B. The asterisk indicates the area in space where the lone pairs of **1** in alignment A and **4** in alignment B may overlap. (Adapted from ref 28, copyright 1999, with permission from Elsevier Science.)

binding in a hydrophilic orientation at both PNMT and the α_2-adrenoceptor. SK&F 29661 (**4**) is an example of this type of compound and is one of the most potent and selective PNMT inhibitors currently reported (Table 1). However, **4** has been shown in autoradiographic studies to be unable to penetrate the blood−brain barrier (BBB), presumably due to the high polarity of the 7-aminosulfonyl substituent.[25]

The other area where substitution on the THIQ nucleus was found to affect both the potency and selectivity for PNMT is the 3-position.[26] These studies indicated that substitution of a methyl or hydroxymethyl substituent at the 3-position of THIQ (**5**−**7**) increased the potency of THIQs for PNMT while slightly decreasing their potency at the α_2-adrenoceptor (Table 2). However, this area of steric bulk tolerance at PNMT was limited, with either a 3-methyl- or a 3-hydroxymethyl moiety (**6** and **7**) being ideal for potency, whereas 3-ethyl or 3-methoxymethyl[27] substitution (**8** and **9**) resulted in reduced potency for PNMT (Table 2).

It was later discovered that a synergistic effect was achieved by combining both 3- and 7-substituents.[27] Compounds **10** and **11** are examples of this synergism and are the two most selective inhibitors of PNMT yet known. However, these compounds are more polar than **4**, due to the 3-hydroxymethyl moiety, and thus even less likely to penetrate the BBB. Nonetheless, these compounds did represent promising leads for the development of new, more lipophilic inhibitors that have the potential to penetrate into the CNS.

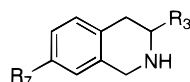
These compounds and others were added to our previous CoMFA models for 7-substituted-THIQs and were aligned according to the rules used in the original CoMFA study.[24] The final PNMT CoMFA model contained 80 compounds with 7 components being optimal, a cross-validated r^2 of 0.656 with a "press s" of 0.670 and a nonvalidated r^2 of 0.906 with a standard error of 0.326.[28] The α_2-adrenoceptor CoMFA model contained 80 compounds, with 4 components being optimal, a cross-validated r^2 of 0.633 with a "press s" of 0.592 and a nonvalidated r^2 of 0.845 with a standard error of 0.385.[28] The structures of the compounds used in this study were constructed using the SYBYL 6.4 software package, and the minimum energy conformations were calculated with electrostatics using the Tripos force field and charges calculated by the AM1 method in MOPAC (SYBYL 6.4 implementation). The conformations of compounds containing side chains were calculated by the "systematic search" option in SYBYL to locate the global minimum energy conformation. Side chains were aligned so that they occupied the same region of space, even though this sometimes resulted in the use of a local minimum energy conformation (within 2 kcal/mol of the corresponding global minimum).

Using these updated CoMFA models, compounds **12**−**16** were proposed (Table 3).[28] It should be noted that CoMFA does not possess the ability to predict the K_i values for racemic mixtures. Therefore, the K_i predictions are given for each enantiomer. We have previously investigated the enantioselectivity of other 3-substituted-THIQs (for examples, see ref 27). The 3-trifluoromethyl moiety found in these compounds was chosen[29] because (1) it is less bulky than the 3-ethyl group, which showed decreased PNMT activity (Table 2), but still large enough to take advantage of the area of steric bulk intolerance around the 3-position of THIQ at the α_2-adrenoceptor indicated by our CoMFA model, (2) it is more lipophilic (CF_3, $\pi = 0.88$) than the 3-hydroxymethyl moiety (CH_2OH, $\pi = -1.03$) of compounds **10** and **11**, thereby increasing the probability that these compounds may penetrate the BBB, (3) fluorine can act as a hydrogen bond acceptor, as shown by studies of fluorinated carbohydrates,[30] thereby mimicking the potential hydrogen-bonding interactions of the 3-hydroxymethyl substituent, and (4) studies from our group[31] and others[32] have indicated that benzylamines and THIQs appear to bind to PNMT in a neutral form. The 3-trifluoromethyl moiety would decrease the pK_a of the THIQ amine to ca. 5.[33] The 7-substituents that were chosen for this study of 3-trifluoromethyl-THIQs are 7-SO_2CH_3 (**13**), 7-NO_2 (**14**), and 7-CN (**15**). These substituents are all hydrophilic and electron-withdrawing, which was previously shown in our CoMFA study to increase the selectivity of THIQs for PNMT versus the α_2-adrenoceptor.[24] These compounds were predicted by our CoMFA models to have moderate to good selectivity for PNMT (Table 3) and are less polar than THIQs **4**, **10**, and **11**. The 7-bromo-THIQ **16** was also included in this study to further test the ability of our CoMFA models to predict activity of lipophilic 7-substituted-THIQs at both PNMT and the α_2-adrenoceptor. The CoMFA models predict that **16** should not be selective for PNMT (Table 3).

Chemistry

Compounds **12**−**16** were synthesized as shown in Schemes 1−3. Trifluoromethyl ketone **17** was formed

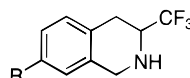
Table 2. In Vitro Inhibitory Potency of Various PNMT Inhibitors at PNMT and Binding of [^{3}H]Clonidine at the α_2-Adrenoceptor[a]



compd	R_3	R_7	K_i (μM) PNMT	K_i (μM) α_2-adrenoceptor	selectivity α_2/PNMT
5	H	H	10 ± 1	0.35 ± 0.1	0.035
6	CH_3	H	2.1 ± 0.1	0.76 ± 0.08	0.36
7	CH_2OH	H	1.1 ± 0.1	6.6 ± 0.3	6.0
8	CH_2CH_3	H	24 ± 1	0.67 ± 0.11	0.028
9	CH_2OCH_3	H	9.2 ± 0.4	2.8 ± 0.1	0.30
10	CH_2OH	SO_2CH_3	0.64 ± 0.04	660 ± 10	1000
11	CH_2OH	SO_2NH_2	0.34 ± 0.06	1400 ± 30	4100

[a] Reference 27.

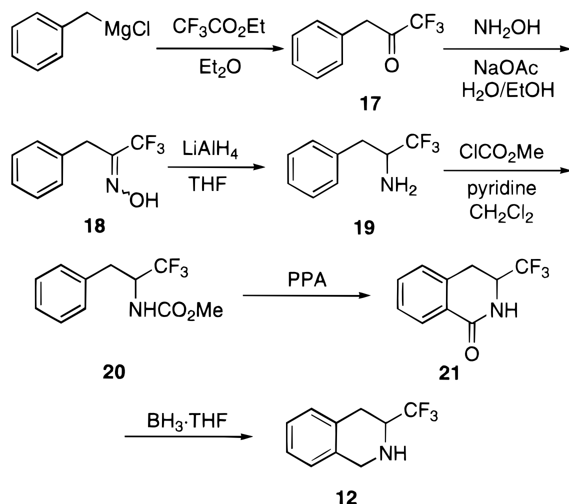
Table 3. Predicted and Actual in Vitro Activities of 3-Trifluoromethyl-7-substituted-THIQs as Inhibitors of PNMT and Binding of [^{3}H]Clonidine at the α_2-Adrenoceptor



		K_i SEM (μM)					
		PNMT		α_2-adrenoceptor		selectivity α_2/PNMT	
compd	R	pred	obsd	pred	obsd	pred	obsd
12 (±)	H		15 ± 1		400 ± 10		27
R		4.0		2.0		0.50	
S		44		2.1		0.050	
13 (±)	SO_2CH_3		36 ± 3		3900 ± 100		110
R		0.93		180		190	
S		8.1		180		22	
14 (±)	NO_2		2.0 ± 0.2		1400 ± 100		700
R		0.11		26		240	
S		1.3		32		25	
15 (±)	CN		13 ± 1		2900 ± 100		220
R		0.40		5.8		15	
S		4.8		6.8		1.4	
16 (±)	Br		0.52 ± 0.05		>1000[a]		>1900
R		0.46		0.38		0.83	
S		0.18		0.46		2.6	

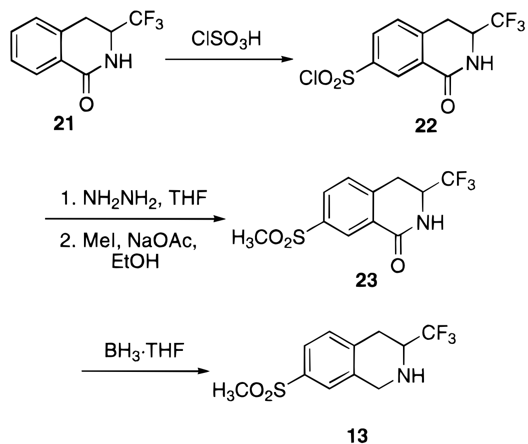
[a] Precipitate was noted at 1000 μM in the α_2-adrenoceptor assay.

Scheme 1



by the reaction of ethyl trifluoroacetate with benzyl Grignard.[34] Treatment of **17** with hydroxylamine and NaOAc in EtOH/water at reflux formed oxime **18**, which was reduced with $LiAlH_4$ in THF to form **19**.[35] Amine **19** was treated with methyl chloroformate to form **20**, which was cyclized with PPA to form lactam **21**.[36]

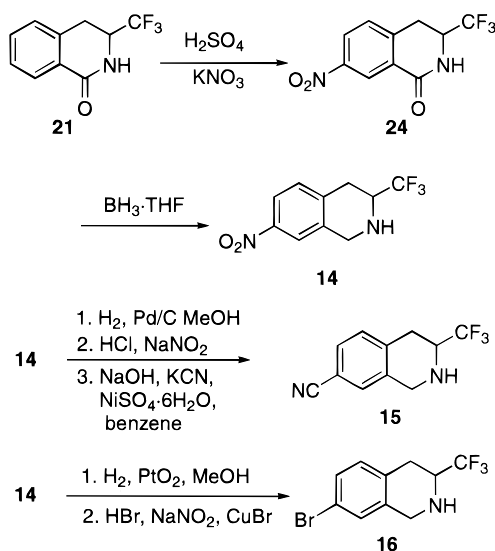
Scheme 2



Reduction of the lactam with BH_3·THF formed 3-trifluoromethyl-THIQ (**12**) (Scheme 1).

Chlorosulfonylation of lactam **21** with chlorosulfonic acid (neat) formed **22**. Chlorosulfone **22** was treated with hydrazine in THF to form the hydrazinosulfone, which was not isolated but was converted directly to methyl sulfone **23** with NaOAc and MeI in EtOH.[37]

Scheme 3



Reduction of lactam **23** with BH_3·THF formed **13** (Scheme 2).

Electrophilic nitration of **21** formed lactam **24**, which was reduced with BH_3·THF to form **14** (Scheme 3). Hydrogenation of the nitro moiety of **14** to the amine followed directly by diazotization and a modified Sandmeyer reaction formed 7-cyano-THIQ **15**.[38] Similar procedures utilizing a Sandmeyer bromination were used for the synthesis of **16** from **14** (Scheme 3).[39]

Biochemistry

All compounds were evaluated as their hydrochloride or hydrobromide salts for their activity as inhibitors of PNMT and the binding of [^{3}H]clonidine to the α_2-adrenoceptor. Bovine adrenal PNMT was prepared using the method of Connett and Kirshner through the isoelectric precipitation step.[40] The in vitro activity of these compounds was determined using a standard radiochemical assay which has been described previously.[41] Inhibition constants were determined by using three different concentrations of the inhibitor utilizing phenylethanolamine as the variable substrate.

α_2-Adrenoceptor binding assays were performed using a standard radiochemical assay developed by U'Prichard et al.[42] that uses [^{3}H]clonidine as the radioligand to define specific binding and phentolamine to determine the nonspecific binding affinity in order to simplify the comparison with previous results.

Results and Discussion

This series of 3-trifluoromethyl-THIQs (**12**−**16**) was found to contain some of the most selective inhibitors of PNMT known and represents promising new leads for the development of even more selective inhibitors. The high degree of selectivity for these compounds is due to their decreased affinity for the α_2-adrenoceptor and not to their potency for PNMT (Table 3). All of the compounds (**12**−**16**) in this study were found to display decreased affinity for the α_2-adrenoceptor (K_i > 400 μM). These results are generally consistent with our α_2-adrenoceptor CoMFA model, which had predicted an area of steric bulk intolerance around the 3-position of THIQs aligned in the hydrophilic orientation B (Figure 1). However, the affinities of **12**−**16** were dramatically underpredicted for the α_2-adrenoceptor (Table 3). Nevertheless, our CoMFA models were able to predict that significant decreases in affinity for the α_2-adrenoceptor could be obtained through the combination of a hydrophilic electron-withdrawing 7-substituent and the appropriate 3-substituent as found in **13**−**15**.

Examination of the affinities of **12**−**16** for the α_2-adrenoceptor suggests that the receptor may not only be sensitive to substitution at the 3-position of THIQ but also to the pK_a of the THIQ amine. Therefore it may be possible to modulate the pK_a of THIQ in order to decrease its affinity for the α_2-adrenoceptor. For example, compare the α_2-adrenoceptor affinities of **12** (K_i = 400 μM) and **6** (K_i = 0.76 μM). These compounds display a 500-fold difference in affinity, which cannot be attributed only to unfavorable steric interactions around the 3-position of THIQ, as it has been shown previously that 3-ethyl-THIQ (**8**) has moderate affinity for the α_2-adrenoceptor (K_i = 0.67 μM). Therefore, the decreased affinity of **12** is most likely due to the decrease in pK_a for the THIQ amine (pK_a = 4.86 ± 0.02)[33] and not to deleterious steric interactions with the α_2-adrenoceptor. This is a logical assumption considering that the natural ligands for the α_2-adrenoceptor, NE and Epi, have a pK_a ca. 10 and would be protonated at physiological pH.

In general, this series of 3-trifluoromethyl-THIQs (**12**−**15**) displayed decreased affinity for PNMT compared to similarly substituted 3-methyl- and 3-hydroxymethyl-THIQs. A comparison of the PNMT affinity of 3-trifluoromethyl-THIQ (**12**, K_i = 15 μM) and 3-methyl-THIQ (**6**, K_i = 2.1 μM) shows a 7-fold decrease in affinity. Examination of the activities of **12** and 3-hydroxymethyl-THIQ (**7**, K_i = 1.1 μM) shows a 14-fold decrease in PNMT affinity. The decreased affinity of these 3-trifluoromethyl-THIQs for PNMT may be attributed to three factors. First, it has been shown that the amount of steric bulk tolerance at the 3-position of THIQ is limited (e.g., methyl or hydroxymethyl, but not ethyl) at the PNMT active site (Table 2). The trifluoromethyl moiety is larger (C−H bond = 1.09 Å; C−F bond = 1.34 Å) than a methyl group and may not be tolerated as well by the PNMT active site. Second, the 3-trifluoromethyl moiety is quite lipophilic (π = 0.88). It has been previously shown that 3-hydroxymethyl-THIQs **10** and **11** (Table 2)[27] are potent inhibitors of PNMT. In contrast, the 3-hydroxymethyl substituent is quite hydrophilic (π = −1.03). It is possible that by placing a trifluoromethyl substituent at the 3-position we have introduced a very lipophilic group into an area that prefers a hydrophilic moiety. Third, the 3-trifluoromethyl group can only act as a hydrogen bond acceptor, whereas the 3-hydroxymethyl moiety can act as both a hydrogen bond acceptor and a hydrogen bond donor. This argument is consistent with the decreased affinity of 3-trifluoromethyl-THIQs **12**−**15** for PNMT. Compound **16** is an exception to this trend and displayed good affinity for PNMT (K_i = 0.52 μM). Previously, it had been postulated that PNMT binds THIQs in two different orientations based on the lipophilicity of the 7-substituent (Figure 1).[24] Therefore, since the 7-bromo substituent has a positive π value (π = +0.86), **16** may be bound differently than **12**−**15**. If **16** is bound in the lipophilic alignment at the PNMT active site, this would

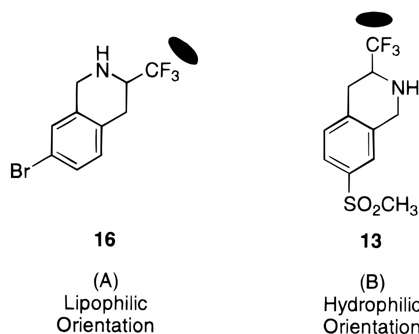


Figure 2. 3-Trifluoromethyl-THIQs **16** and **13** are shown in their proposed lipophilic and hydrophilic binding orientations, respectively. According to our hypothesis, the 3-trifluoromethyl moiety will occupy different regions in space (depicted by the black ellipses), depending upon the orientation of the molecule.

place the 3-trifluoromethyl moiety in a totally different area of space where it may not come in contact with any of the negative interactions found for **12**−**15** (Figure 2).

Although previous results have indicated that THIQs and benzylamines bind to PNMT in a neutral form,[31,32] it was still not clear if this hypothesis was valid. Further evidence in support of this hypothesis is that compounds **12**−**16** displayed moderate to good affinity for PNMT (Table 3). The estimated pK_a of these 3-trifluoromethyl-THIQs (pK_a ca. 5)[33] implies these compounds should be unprotonated at physiological pH when they interact with the PNMT active site.

Overall, our CoMFA models for PNMT and the α_2-adrenoceptor were able to predict that 3-trifluoromethyl-THIQs **12**−**15** would be selective inhibitors for PNMT. The rank order of potency for compounds **12**−**16** was predicted quite well by our PNMT CoMFA model, whereas the α_2-adrenoceptor model significantly under-predicted the inhibition constants. Compounds **15** and **16** had the poorest predictions by the α_2-adrenoceptor model. According to our CoMFA models, THIQs with lipophilic 7-substituents should be “nonselective” inhibitors of PNMT. Compound **16** was predicted to have very good potency at both PNMT and the α_2-adrenoceptor, but it was found to have almost no affinity for the α_2-adrenoceptor. This is an indication that some other factor, not measured by our CoMFA models (e.g., the decreased pK_a of these compounds or the lipophilicity of the 3-trifluoromethyl moiety), is affecting the potency of these compounds at both PNMT and the α_2-adrenoceptor.

Summary and Conclusion

Compounds **12**−**16** are some of the most selective PNMT inhibitors known due to their reduced affinity for the α_2-adrenoceptor. Of these compounds, **14** and **16** were found to display selectivities (α_2 K_i/PNMT K_i) greater than 500 for PNMT. Due to the 3-trifluoromethyl moiety, both **14** and **16** should be sufficiently lipophilic to penetrate the BBB, thus representing important new leads in the development of selective PNMT inhibitors.

Experimental Section

All of the reagents and solvents used were reagent grade or were purified by standard methods before use. 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 taken on a Varian XL-300, a GE QE-300, or a Bruker DRX-400 spectrophotometer. 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 salts of these THIQs, 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 in 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 spectrophotometer. Electron-impact mass spectra (EIMS), chemical-ionization mass spectra (CIMS), and high-resolution mass spectra (HRMS) were obtained on a Varian Atlas CH-5 or a Ribermag R 10-10 mass spectrophotometer. 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 chromatography was performed using silica gel 60 (230−400 mesh) supplied by Universal Adsorbents, Atlanta, GA.

All methanol (MeOH) and ethanol (EtOH) used were anhydrous unless stated otherwise and were prepared by distillation over magnesium. Solvents were routinely distilled prior to use. Anhydrous tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium−benzophenone ketyl. Methylene chloride (CH_2Cl_2) and chloroform ($CHCl_3$) were obtained by distillation from phosphorus pentoxide (P_2O_5). In some cases anhydrous solvents were used directly from Aldrich Sure Seal bottles. 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 used in the α_2-adrenoceptor assays was purchased from New England Nuclear Corp., Boston, MA. Bovine adrenal glands were obtained from Davis Meat Processing (Overbrook, KS).

1,1,1-Trifluoro-3-phenylpropan-2-one (17). Compound **17** has been prepared previously[34,43] but was synthesized using the following modified procedure: 2 M benzylmagnesium chloride in ether (24.5 mL, 49.0 mmol) was added dropwise to a solution of ethyl trifluoroacetate (5.83 mL, 49.0 mmol) in ether (100 mL) at −78 °C. After the addition, the reaction was warmed to room temperature and stirred for 30 min. A saturated solution of $NH_4Cl_{(aq)}$ (40 mL) was added, followed by the addition of 3 N HCl (25 mL). The mixture was stirred for 5 min. The phases were separated, and the aqueous phase was extracted with ether (2 × 25 mL). The organic extracts were combined, washed with brine (50 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel) eluting with hexanes/EtOAc (6:1) to yield a yellowish oil, which was distilled bulb-to-bulb (35 °C, 0.7 mmHg; lit.[35] bp 51−52 °C, 2 mmHg) to yield **17** as a colorless oil (7.9 g, 86%). All spectral data were consistent with literature values.[43]

1,1,1-Trifluoro-3-phenylpropan-2-one Oxime (18). Oxime **18** has been prepared previously[35] but was synthesized using the following modified procedure: ketone **17** (6.92 g, 36.8 mmol) was dissolved in a solution of hydroxylamine hydrochloride (19.0 g, 272 mmol), NaOAc (22.3 g, 272 mmol), water (100 mL), and EtOH (25 mL) and heated at reflux for 1 h. The

solution was cooled and extracted with $CHCl_3$ (3 × 100 mL). The combined organic extracts were washed with brine (100 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield a yellowish residue. The residue was purified by flash column chromatography (silica gel) eluting with hexanes/EtOAc (6:1) to yield an oily solid, which was distilled bulb-to-bulb (75−80 °C, 1 mmHg; lit.[35] bp 91−92 °C, 3 mmHg) to yield **18** as a colorless solid (6.80 g, 91.5%): mp 38−40 °C (lit.[35] mp 40−42 °C); IR (neat) 3300, 3100, 2920, 1700, 1600, 1500, 1450, 1190, 1130, 970, 750, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.5 (br ex s, 1H, NOH), 7.34−7.30 (m, 5H, ArH), 3.90 (s, 2H, $ArCH_2CN$); ^{13}C NMR ($CDCl_3$) δ 148.7 (q, J = 95 Hz, CO), 133.4, 128.5, 128.3, 126.9, 120.4 (q, J = 818 Hz, CF_3), 29.3; HRMS (FAB) m/z calcd for $C_9H_9NF_3O$ (M^+ + H) 204.0636, obsd 204.0610.

(±)-1,1,1-Trifluoro-3-phenyl-2-aminopropane Hydrochloride (19·HCl). Phenylpropylamine (**19**) has been prepared previously[35] but was synthesized using the following modified procedure: a solution of oxime **18** (1.09 g, 5.38 mmol) in THF (50 mL) was added dropwise to a mixture of $LiAlH_4$ (406 mg, 10.7 mmol) in THF (50 mL) at 0 °C. The reaction mixture was warmed to room temperature, after which it was heated at reflux for 14 h. The reaction mixture was cooled in an ice bath, and water was added dropwise until no H_2 evolution was observed. At this point, 10% NaOH (10 mL) was added and the solution was stirred for 30 min. The mixture was filtered through Celite, and the Celite pad was rinsed with Et_2O (2 × 100 mL). The rinses and filtrate were combined and dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The resulting residue was distilled bulb-to-bulb (35 °C, 0.05 mmHg; lit.[35] bp 65−66, 2 mmHg) to yield a colorless oil. The oil was dissolved in hexane, and anhydrous $HCl_{(g)}$ was bubbled through the solution to form the hydrochloride salt, which was collected by filtration to yield **19**·HCl as a white solid (0.91 g, 75%): mp dec 120 °C [lit.[35] mp 203−206 °C (sealed tube)]; IR (KBr) 3000, 2990, 2600, 1975, 1580, 1520, 1450, 1380, 1250, 1200, 1150, 1080, 1040, 740, 690, 660; ^{1}H NMR ($CDCl_3$) (free base) δ 7.42−7.20 (m, 5H, ArH), 3.48−3.39 (m, 1H, H-2), 3.16−3.09 (m, 1H, H-3), 2.65−2.59 (m, 1H, H-3), 1.28 (br ex s, 2H, NH_2); EIMS m/z (relative intensity) 190 (M^+ + 1, 12), 189 (M^+, 56), 118 (21), 109 (12), 98 (83), 91 (100), 78 (14), 65 (20). Anal. ($C_9H_{10}F_3N$·HCl) C, H, N.

(±)-Methyl-*N*-(1,1,1-Trifluoro-3-phenylprop-2-yl)carbamate (20). Compound **19**·HCl (500 mg, 2.22 mmol) was dissolved in $CHCl_3$ (40 mL) and pyridine (0.80 mL, 9.0 mmol) and cooled to 0 °C in an ice bath. Methyl chloroformate (0.18 mL, 2.22 mmol) was added dropwise to the reaction mixture; the solution was warmed to room temperature and stirred for 14 h. Ice water (30 mL) was added slowly to the mixture, and the solution was stirred for 15 min. The organic phase was removed, and the aqueous phase was extracted with chloroform (2 × 30 mL). The organic phases and extracts were combined, washed with 3 N HCl (2 × 30 mL) and brine (30 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield a white solid which was recrystallized from hexane to yield **20** as white needles (535 mg, 97.0%): mp 94−95 °C; IR (KBr) 3300, 3060, 3010, 2950, 1690, 1600, 1540, 1490, 1440, 1360, 1300, 1250, 1200, 1160, 1120, 1050, 940, 870, 750, 700, 680, 640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.36−7.19 (m, 5H, ArH), 4.76 (br ex s, 1H, NH), 4.66−4.52 (m, 1H, H-2), 3.61 (s, 3H, OCH_3), 3.21−3.15 (m, 1H, H-3), 2.80−2.75 (m, 1H, H-3); ^{13}C NMR ($CDCl_3$) δ 156.8, 135.4, 129.7, 129.5, 129.1, 128.9, 127.6, 125.6 (q, J = 846 Hz, CF_3), 54.1 (q, J = 89 Hz, C-3), 53.0, 34.8; EIMS m/z (relative intensity) 248 (M^+ + 1, 10), 247 (M^+, 46), 172 (70), 91 (100), 65 (20), 59 (27). Anal. ($C_{10}H_{12}NF_3O_2$) C, H, N.

(±)-3-Trifluoromethyl-3,4-dihydroisoquinolin-1(2*H*)-one (21). Carbamate **20** (1.75 g, 7.08 mmol) was dissolved in cold polyphosphoric acid (45 g) and heated to 140 °C for 30 min. The mixture was poured onto ice water (100 mL). The aqueous phase was extracted with chloroform (3 × 75 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield a white solid, which was recrystallized from hexane to yield **21** as white needles (814 mg, 53.6%): mp 114−115 °C; IR (KBr) 3200, 3100, 2950, 1680, 1600, 1585, 1455, 1375, 1320, 1265, 1245, 1210, 1180, 1135, 1110, 1020, 900, 770, 740, 695 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.11−8.08 (m, 1H, ArH), 7.53−7.50 (m, 1H, ArH), 7.47−7.39 (m, 1H, ArH), 7.25−7.23 (m, 1H, ArH), 6.53 (br ex s, 1H, NH), 4.22−4.18 (m, 1H, H-2), 3.40−3.17 (m, 2H, H-3); EIMS m/z (relative intensity) 216 (M^+ + 1, 13), 215 (M^+, 48), 146 (100), 128 (70), 118 (25), 90 (45), 63 (20), 51 (12). Anal. ($C_{10}H_8NF_3O$) C, H, N.

(±)-3-Trifluoromethyl-1,2,3,4-tetrahydroisoquinoline Hydrochloride (12·HCl). Lactam **21** (60 mg, 0.30 mmol) in THF (5 mL) was added dropwise to a solution of BH_3·THF (1 M, 2.2 mL) in THF (10 mL). The reaction mixture was heated at reflux for 2 h and cooled to 0 °C. MeOH (10 mL) was added dropwise, and the solvent was removed under reduced pressure. The residue was dissolved in MeOH (10 mL) and 6 N HCl (10 mL) and heated at reflux for 3 h. The solution was made basic with the addition of 10% NaOH. The reaction mixture was extracted with CH_2Cl_2 (3 × 20 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) with $CHCl_3$ as the eluent. A white solid was obtained and dissolved in dry ether, and anhydrous $HCl_{(g)}$ bubbled through the solution to form the hydrochloride salt. The salt was recrystallized from EtOH/hexanes to yield **12**·HCl (35.8 mg, 50.3%): mp 195−196 °C; IR (KBr) 3500−3300 (broad), 2920−2400 (broad), 1575, 1400, 1270, 1260, 1205, 1130, 1095, 1000, 755; ^{1}H NMR (DMSO-d_6) δ 11.5−10.6 (br ex m, 2H, NH_2^+), 7.31 (m, 4H, ArH), 4.73−4.70 (m, 1H, H-3), 4.46−4.36 (m, 2H, H-1), 3.30−3.12 (m, 2H, H-4); EIMS m/z (relative intensity) 202 (M^+, 100), 185 (10), 133 (50), 105 (20), 91 (15), 77 (10). Anal. ($C_{10}H_{10}NF_3$·HCl) C, H, N.

(±)-7-Chlorosulfonyl-3-trifluoromethyl-3,4-dihydroisoquinolin-1(2*H*)-one (22). Lactam **21** (450 mg, 2.09 mmol) was dissolved in chlorosulfonic acid (3 mL) and heated at 80 °C for 16 h. The reaction mixture was cooled to room temperature, $CHCl_3$ (10 mL) was carefully added, and the mixture was slowly poured onto ice (25 g). The mixture was extracted with $CHCl_3$ (3 × 25 mL) and EtOAc (50 mL). The organic extracts were combined, washed with brine (100 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (silica gel) eluting with EtOAc/hexanes (3:1) to yield a white solid that was recrystallized from EtOAc/hexanes to yield **22** (455 mg, 69.6%) as white needles: mp 190−191 °C; IR (KBr) 3200, 3100, 2960, 1680, 1600, 1440, 1360, 1275, 1205, 1180, 1130, 1060, 990, 650; ^{1}H NMR δ 8.78−8.76 (m, 1H, ArH-8), 8.18−8.15 (m, 1H, ArH-6), 7.56−7.53 (m, 1H, ArH-5), 6.91 (br ex s, 1H, CONH), 4.38−4.25 (m, 1H, H-3), 3.58−3.34 (m, 2H, H-4); EIMS m/z (relative intensity) 313 (M^+, 20), 278 (83), 262 (15), 244 (100), 214 (45), 145 (60), 117 (20), 89 (50), 63 (40). Anal. ($C_{10}H_7NClF_3O_3S$) C, H, N.

(±)-7-Methylsulfonyl-3-trifluoromethyl-3,4-dihydroisoquinolin-1(2*H*)-one (23). Chlorosulfone **22** (1.65 g, 5.27 mmol) was dissolved in THF (20 mL) and cooled to 0 °C. Hydrazine (0.47 mL, 14.7 mmol) was added dropwise to the solution, which was stirred for 16 h. The solvent was removed under reduced pressure and the residue dried under vacuum to yield a white solid. The white solid was dissolved in EtOH (25 mL). NaOAc (2.01 g, 24.5 mmol) and iodomethane (1.36 mL, 24.3 mmol) were added to the solution, and the resulting suspension was heated at reflux overnight. The mixture was cooled to room temperature, and water (50 mL) was added. The solution was extracted with EtOAc (3 × 50 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 recrystallized from EtOAc/hexane to yield **23** as white crystals (1.09 g, 70.6%): mp 226−227 °C; IR (KBr) 3200, 3100, 1680, 1600, 1440, 1375, 1275, 1180, 1130 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.67 (d, J = 2.0 Hz, 1H, ArH-8), 8.11 (dd, J = 2.0, 8.1 Hz, 1H, ArH-6), 7.49 (d, J = 8.1 Hz, 1H, ArH-5), 6.47 (br ex s, 1H, NH), 4.26 (m, 1H, H-3),

3.54−3.47 (m, 1H, H-4), 3.36−3.30 (m, 1H, H-4), 3.10 (s, 3H, CH_3); EIMS *m/z* (relative intensity) 294 ($M^+ + 1$, 50), 293 (M^+, 80), 224 (100), 145 (90). Anal. ($C_{11}H_{10}NF_3O_3S$) C, H, N.

(±)-7-Methylsulfonyl-3-trifluoromethyl-1,2,3,4-tetrahydroisoquinoline Hydrochloride (13·HCl). Lactam **23** (1.07 g, 3.66 mmol) was dissolved in THF (40 mL). BH_3·THF (1M) (9.15 mL) was added dropwise, and the solution was heated at reflux for 2 h. The solution was cooled to room temperature, and MeOH (15 mL) was added dropwise. The solvent was removed under reduced pressure, and MeOH (15 mL) and 6 N HCl (15 mL) were added. The solution was heated to reflux for 3 h. A solution of 10% NaOH was added to the mixture until the solution was basic (pH > 10). The solution was extracted with EtOAc (4 × 30 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 dissolved in $CHCl_3$. Dry $HCl_{(g)}$ was bubbled through the solution to form the HCl salt, which was recrystallized from EtOH/hexanes to yield **13**·HCl as white crystals (1.13 g, 97.9%): mp 253−254 °C; IR (KBr) 2900, 2700, 2600, 1425, 1300, 1180, 1130, 1010 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.5 (br ex s, 2H, NH_2^+), 7.90 (s, 1H, ArH-8), 7.84 (d, $J = 8.1$ Hz, 1H, ArH-6), 7.58 (d, $J = 8.1$ Hz, 1H, ArH-5), 4.60 (m, 1H, H-3), 4.45 (s, 2H, H-1) 3.35 (m, 1H, H-4), 3.22 (s, 3H, CH_3), 3.20 (m, 1H, H-4); EIMS *m/z* (relative intensity) 278 ($M^+ - 1$, 35), 210 (100), 130 (75). Anal. ($C_{11}H_{12}NF_3O_2S$·HCl) C, H, N.

(±)-3-Trifluoromethyl-7-nitro-3,4-dihydroisoquinolin-1(2*H*)-one (24). Lactam **21** (403 mg, 1.87 mmol) was dissolved in concentrated H_2SO_4 (5 mL) and cooled to 0 °C. KNO_3 (207 mg, 2.06 mmol) was added in small portions over the course of 30 min. The reaction mixture was warmed to room temperature and stirred overnight. The mixture was poured slowly onto ice (50 g). A white precipitate was formed, filtered off, washed with water, and dried under vacuum. The aqueous filtrate was collected and extracted with $CHCl_3$ (3 × 25 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 remaining yellow residue combined with the previously isolated precipitate. The crude reaction mixture was recrystallized from EtOAc/hexanes to yield **24** as light-yellow needles (410 mg, 84.4%): mp 219−221 °C; IR (KBr) 3190, 3090, 3010, 1675, 1600, 1510, 1430, 1370, 1340, 1250, 1200, 1170, 1120, 1050, 1000, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.91 (s, 1 H, H-8), 8.38 (d, $J = 8.1$ Hz, 1 H, H-6), 7.43 (d, $J = 8.1$ Hz, 1 H, H-5), 6.48 (s, 1 H, NH), 4.12 (m, 1 H, H-3), 3.52−3.22 (m, 2 H, H-4); CIMS *m/z* (relative intensity) 278 ($M + NH_4^+$, 4), 261 (MH^+, 20), 191 (100), 145 (30). Anal. ($C_{10}H_7F_3N_2O_3$) C, H, N.

(±)-3-Trifluoromethyl-7-nitro-1,2,3,4-tetrahydroisoquinoline Hydrochloride (14·HCl). This compound was prepared in a similar manner as **13** using the procedures outlined previously but using lactam **24** (1.08 g, 4.15 mmol) as the starting material. The hydrochloride salt was prepared in a similar manner as **13**·HCl and was recrystallized from MeOH/EtOAc to yield **14**·HCl as pale-yellow needles (1.02 g, 87.2%): mp dec 256−258 °C; IR (KBr) 3060, 2920, 2800−2400 (broad), 1570, 1510, 1420, 1345, 1275, 1255, 1170, 1125, 900 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.23 (s, 1H, ArH-8), 8.10 (d, $J = 8.6$ Hz, 1H, ArH-6), 7.53 (d, $J = 8.6$ Hz, 1H, ArH-5), 4.65 (m, 1H, H-3), 4.46 (s, 2H, H-1), 3.42−3.12 (m, 2H, H-4); CIMS *m/z* (relative intensity) 247 (MH^+, 100), 177 (30). Anal. ($C_{10}H_9N_2F_3O_2$·HCl) C, H, N.

3-Trifluoromethyl-7-cyano-1,2,3,4-tetrahydroisoquinoline Hydrochloride (15·HCl). THIQ **14**·HCl (1.23 g, 4.99 mmol) was dissolved in MeOH (50 mL) and hydrogenated with 10% Pd/C (25 mg) at 50 psi. The solution was filtered through Celite, the Celite bed was washed with MeOH (50 mL), and the filtrate was evaporated to dryness. The residue was dissolved in concentrated HCl (1.0 mL) and water (1 mL) and stirred in an ice bath. $NaNO_2$ (52.0 mg, 0.756 mmol), dissolved in water (1 mL), was added dropwise. After stirring for 15 min, a positive starch-iodide test was obtained and urea (20 mg, 0.34 mmol) was added to destroy any excess HNO_2. In a second flask, a solution of NaOH (0.20 g in 1.5 mL water) and KCN (325 mg, 4.99 mmol) in water (5 mL) was prepared. Benzene (5 mL) was added to the basic mixture and the suspension chilled in an ice bath. A solution of $Ni_2SO_4·6H_2O$ (192 mg, 0.110 mmol) in water (1 mL) was added to this suspension, and the color of the biphasic solution changed to yellow-brown. The diazonium salt solution was added dropwise to the basic KCN solution. Brisk evolution of N_2 was observed, and the reaction mixture was warmed to room temperature over a period of 2 h. The mixture was heated to 50 °C in an oil bath for 1 h, cooled to room temperature, made basic (pH > 10) with 1 N NaOH, and filtered through Celite. The Celite bed was rinsed with CH_2Cl_2 (3 × 20 mL). The filtrate was extracted with CH_2Cl_2 (3 × 30 mL). The organic rinses and extracts were combined, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by flash chromatography (silica gel) with hexanes/EtOAc (3:1) to give a brown oil, which was purified by flash column chromatography (silica gel) with CH_2Cl_2/hexanes (3:1) to yield a pale-yellow solid. The yellow solid was recrystallized from CH_2Cl_2/hexane to yield **15** as pale-yellow needles (116 mg, 30.0%): mp 116−118 °C. The hydrochloride salt was formed with $HCl_{(g)}$ in MeOH and was recrystallized from MeOH/EtOAc/hexanes to yield **15**·HCl as white crystals: IR (KBr) (salt) 2920, 2700, 2600, 2460, 2200, 1590, 1570, 1450, 1390, 1280, 1260, 1200, 1170, 1140, 1000, 900 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.85 (s, 1H, H-8), 7.76 (d, $J = 8.0$ Hz, 1 H, H-6), 7.28 (d, $J = 8.0$ Hz, 1H, H-5), 4.80−4.73 (m, 1H, H-3), 4.49−4.39 (m, 2H, H-1), 3.42−3.22 (m, 2H, H-4); ^{13}C NMR (DMSO-d_6) δ 136.5, 131.8, 131.4, 131.2, 130.9, 124.7 (q, $J = 838$ Hz, CF_3), 119.3, 110.6 (CN), 52.9 (q, $J = 95$ Hz, C-3), 45.0, 25.7; CIMS *m/z* (relative intensity) 227 (MH^+, 30), 226 (M^+, 10), 225 ($M^+ - 1$, 100), 155 (25), 129 (15). Anal. ($C_{11}H_9N_2F_3$·HCl) C, H, N.

(±)-3-Trifluoromethyl-7-bromo-1,2,3,4-tetrahydroisoquinoline Hydrochloride (16·HCl). Tetrahydroisoquinoline **14**·HCl (390 mg, 1.38 mmol) was placed in a Parr shaker bottle and dissolved in MeOH (50 mL), and concentrated HCl (3 mL) and water (8 mL) were added. After the addition of PtO_2 (90 mg), the mixture was hydrogenated at 50 psi (4 h). The solution was filtered and concentrated. The concentrated solution was made basic with 10% NaOH solution. The basic solution was extracted with CH_2Cl_2 (3 × 50 mL); the organic extracts were combined and dried over anhydrous Na_2SO_4. The solvent was removed, and the residue was dissolved in ice-cold 48% $HBr_{(aq)}$ (1.0 mL) in water (3.0 mL). Sodium nitrite (103 mg, 1.50 mmol) in water (2 mL) was added dropwise. After 30 min, excess HNO_2 was destroyed by the addition of urea (a negative starch-iodide test was obtained at this time). The diazonium salt solution was added to a mixture of CuBr (600 mg, 4.18 mmol), 48% $HBr_{(aq)}$ (2.5 mL), and water (5 mL) at 35 °C. After the addition of the diazonium salt solution, the reaction mixture was warmed to 75−80 °C and stirred for 1.5 h. The reaction mixture was allowed to stand overnight at room temperature and was cautiously made basic with a 50% NaOH solution. Ethyl acetate (50 mL) was added, and the blue copper salts were removed by filtration. The filtrate was extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated to give a dark oil. The crude product was purified by flash chromatography (silica gel) with hexanes/EtOAc (6:1) to yield a white solid, which was recrystallized from hexane to yield **16** as white crystals (292 mg, 75.8%): mp 77−79 °C. The hydrochloride salt was formed in the same manner as **12**·HCl and recrystallized from MeOH/EtOAc/hexane to give **16**·HCl as white crystals: IR (KBr) 2920, 2850−2400 (broad), 1590, 1570, 1450, 1400, 1390, 1270, 1250, 1205, 1180, 1140, 1300, 1000, 900 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.58 (s, 1H, ArH-8), 7.50−7.48 (m, 1H, ArH-6), 7.28−7.26 (m, 1H, ArH-5), 4.72−4.70 (m, 1 H, H-3), 4.44−4.34 (m, 2H, H-1), 4.37 (br ex s, 1 H, NH), 3.27−3.3.08 (m, 2 H, H-4); ^{13}C NMR (DMSO-d_6) δ 132.0, 131.8, 131.3, 130.0, 129.9, 124.7 (q, $J = 837$ Hz, CF_3), 120.7, 53.2 (q, $J = 96$ Hz, C-3), 45.0, 25.1; CIMS *m/z* (relative

intensity) 283, (MH^+ + 2, 10), 282 (MH^+ + 1, 65), 281 (MH^+, 15), 280 (M^+, 100), 212 (10), 210 (10). Anal. ($C_{10}H_9NBrF_3 \cdot HCl$) C, H, N.

Radiochemical Assay for PNMT Activity. The assay used for this study has been described previously.[41] A normal assay tube 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 approximately 15 mCi/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 and quenched by the addition of 250 μL of 0.5 M borate buffer (pH 10), and the mixture is extracted with 2 mL of toluene/isoamyl alcohol (7:3). A 1-mL aliquot of the organic layer is extracted, 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 $1/V$ vs $1/S$ 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.[42] 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 three 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 examined by a log–probit analysis of the data to determine the IC_{50} values, and K_i values were determined by the equation: $K_i = IC_{50}/(1 + [\text{clonidine}]/K_D)$, as all the Hill coefficients were approximately equal to 1.

Molecular Modeling. All molecular modeling and CoMFA studies were carried out on a Silicon Graphics Indigo^2 workstation running SYBYL 6.4.[44] All compounds in this updated CoMFA study were aligned according to the rules used in the previous study of 7-substituted-THIQs for PNMT and the α_2-adrenoceptor,[24] and the details of these studies may be found in ref 28.

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