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

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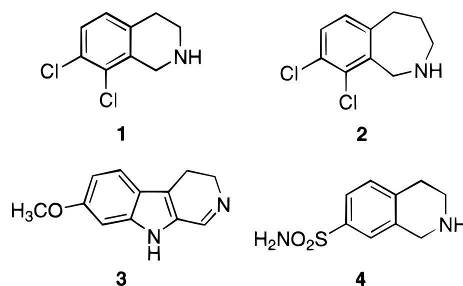
A series of 3-fluoromethyl-1,2,3,4-tetrahydroisoquinolines (3-fluoromethyl-THIQs) was proposed, and their phenylethanolamine *N*-methyltransferase (PNMT) and α_2-adrenoceptor affinities were predicted through the use of comparative molecular field analysis (CoMFA) models. These compounds were synthesized and evaluated for affinity at PNMT and the α_2-adrenoceptor. It was discovered that these compounds are some of the most selective inhibitors of PNMT versus the α_2-adrenoceptor known. To determine the ability of these compounds to penetrate the blood−brain barrier (BBB), a series of THIQs possessing a variety of calculated partition coefficients (Clog *P*) were assayed using an in vitro BBB model. This study found a good correlation between lipophilicity (Clog *P*) and BBB permeability, which indicated that THIQs possessing Clog *P* values of at least 0.13−0.57 should have some penetration into the brain. Two compounds [3-fluoromethyl-7-*N*-(4-chlorophenyl)aminosulfonyl-THIQ (**18**) and 3-fluoromethyl-7-cyano-THIQ (**20**)] possess calculated partition coefficients greater than 0.57 and display selectivities (α_2-adrenoceptor K_i/PNMT K_i) greater than 200 and thus represent promising leads in the development of highly selective inhibitors of PNMT with the ability to penetrate the BBB.

Introduction

To determine the function of epinephrine (Epi) in the brain, our laboratory has targeted the enzyme phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28).[2] This enzyme catalyzes the final step in the biosynthesis of Epi, the transfer of a methyl group from *S*-adenosyl-L-methionine to the primary amine of norepinephrine (NE). Epi constitutes 5−10% of the catecholamines found in the mammalian central nervous system (CNS)[3,4] and is co-localized with PNMT in very specific regions of the brain (most notably the C1 and C2 regions of the medulla oblongata).[5−7] Primarily on the basis of the localization of these Epi neurons and inhibition studies of PNMT, it has been speculated that Epi is involved in (1) the regulation of blood pressure and respiration,[8,9] (2) the secretion of hormones from the pituitary gland,[10,11] (3) the control of exercise tolerance,[12] (4) effects on ethanol intoxication,[13] (5) the regulation of the α_2-adrenoceptor[14,15] and (6) some of the neurodegeneration seen in Alzheimer's disease.[16−18]

The most thoroughly studied process that Epi has been associated with is the regulation of blood pressure. Studies from several laboratories including our own[19] have indicated that administration of centrally active PNMT inhibitors to hypertensive rats resulted in decreased brain Epi content[20,21] and concomitant reduction in peripheral blood pressure.[19,22] However, these results have been complicated by the fact that many of the most potent PNMT inhibitors studied (e.g., **1**, SK&F 64139;[23,24] **2**, LY 134046;[25] **3**, CGS 19281a[26]) were found to exhibit affinity for the α_2-adrenoceptor (Table 1). Therefore, to differentiate whether it is the inhibition of PNMT and subsequent reduction of central Epi or interaction with the α_2-adrenoceptor that is causing the reduction in blood pressure, an inhibitor with high potency and selectivity for PNMT is required.



1,2,3,4-Tetrahydroisoquinolines (THIQs) are potent inhibitors of PNMT (e.g., SK&F 64139, **1**). Studies have shown for monosubstituted THIQs that substitution at the 7-position of THIQ is required for optimum potency at PNMT.[27,28] Our laboratory performed a comparative molecular field analysis (CoMFA),[29] a type of three-dimensional QSAR that correlates steric and electrostatic interactions with biological activity, for a set of thirty 7-substituted-THIQs at PNMT and the α_2-adrenoceptor.[30] This study found that when all of the compounds in the data set were aligned in the same manner, a good predictive model could not be obtained. It was only when these THIQs were aligned in two different orientations—based on the lipophilicity of the

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Table 1. In Vitro PNMT and α_2-Adrenoceptor Affinities of Some PNMT Inhibitors[a]

compd	K_i (μM) PNMT	K_i (μM) α_2-adrenoceptor	selectivity 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[c]	2.1 ± 0.1	0.76 ± 0.08	0.36
6[c]	1.1 ± 0.1	6.6 ± 0.3	6.0
7[c]	24 ± 1	0.67 ± 0.11	0.028
8[d]	9.2 ± 0.4	2.8 ± 0.1	0.30
9[d]	0.64 ± 0.04	660 ± 10	1000
10[d]	0.34 ± 0.06	1400 ± 30	4100
11[c]	36 ± 3	3900 ± 100	110
12[c]	0.52 ± 0.05	>1000[e]	1900

[a] PNMT and α_2-adrenoceptor K_i values for literature compounds were determined in our laboratory for consistent internal comparison. [b] Reference 56. [c] Reference 35. [d] Reference 33. [e] **12** displayed solubility problems at higher concentrations.

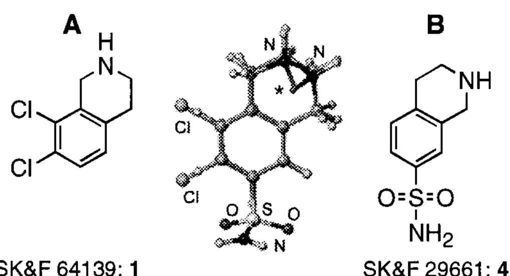
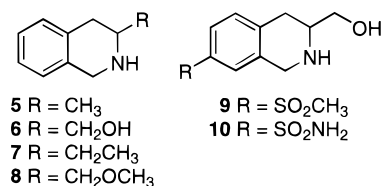


Figure 1. SK&F 64139 (**1**; A) and SK&F 29661 (**4**; B) showing the two proposed orientations of 7-substituted-THIQs at PNMT and the α_2-adrenoceptor. Orientation A is proposed for lipophilic ($+\pi$) 7-substituents, while B is proposed for hydrophilic ($-\pi$) 7-substituents. Between structures **1** and **4** is a SYBYL-generated view of **1** in orientation A, superimposed on **4** in orientation B. The asterisk marks the area in space where the lone pairs of the two molecules may overlap. This figure was adapted from ref 34, copyright 1999, with permission from Elsevier Science.

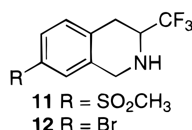
7-substituent—that a predictive model was found (Figure 1). The results of this study indicated that hydrophilic electron-withdrawing 7-substituents increased the potency of THIQs for PNMT while decreasing their affinity for the α_2-adrenoceptor. SK&F 29661 (**4**) is an example of this type of compound and is one of the more selective inhibitors of PNMT known (Table 1). However, autoradiographic studies[31a] using **4** have shown that it is unable to penetrate the blood−brain barrier (BBB), presumably due to its high polarity.

Later studies from our group examined substitution at others areas on the THIQ nucleus and found that substitution at the 3-position with either a 3-methyl (**5**) or a 3-hydroxymethyl (**6**) substituent increased both potency and selectivity of THIQs for PNMT (Table 1).[32] However, substitution of larger groups, such as a 3-ethyl (**7**) or a 3-methoxymethyl (**8**),[33] caused a dramatic decrease in PNMT activity (Table 1). It was later found that the combination of both 3- and 7-substituents resulted in synergistic increases in selectivity for PNMT.[33] 3-Hydroxymethyl-THIQs **9** and **10** are examples of this synergism, and **10** is the most selective inhibitor of PNMT (versus the α_2-adrenoceptor) known (Table 1). However, **9** and **10** are more polar than **4** (**4**, calculated partition coefficient Clog $P = -0.31$; **9**, Clog $P = -0.82$; **10**, Clog $P = -1.01$) due to the 3-hydroxymethyl moiety, making these compounds even less likely to penetrate the BBB. Nevertheless, these compounds did represent important leads for the development of new, more lipophilic PNMT inhibitors.



Our CoMFA models[30] were further refined with the addition of 3,7-disubstituted-THIQs, 8-substituted-2,3,4,5-tetrahydro-1*H*-2-benzazapines, and a variety of constrained benzylamine analogues to a total of 80 compounds for both PNMT and the α_2-adrenoceptor.[34] These models indicated that there were two areas that could be exploited to increase selectivity for PNMT versus the α_2-adrenoceptor. The first area surrounds the 3-position of THIQs in the proposed hydrophilic orientation (Figure 1) at the α_2-adrenoceptor, where our CoMFA model indicated an area of steric bulk intolerance. The second area of difference is found in the electrostatic preferences of PNMT and the α_2-adrenoceptor encompassing an area around the 7-position of THIQs in the proposed hydrophilic orientation (Figure 1). Our CoMFA models indicate that PNMT prefers electron density in this area, whereas the same area of the α_2-adrenoceptor disfavors electron density. From these updated models, a series of 3-trifluoromethyl-THIQs were designed, synthesized, and evaluated.[35] 3-Trifluoromethyl-THIQs containing a hydrophilic electron-withdrawing group (e.g., NO_2, CN, SO_2CH_3) were found to have decreased inhibitory potency for PNMT compared to similarly substituted 3-methyl- and 3-hydroxymethyl-THIQs. Sulfone **11** is an example of this type of compound and was found to be a fairly weak inhibitor of PNMT (K_i = 36 μM) compared to 3-hydroxymethyl-THIQ **9** (K_i = 0.64 μM). The decrease in PNMT potency of **11** was attributed to two possible factors. First, previous studies had indicated that the amount of steric bulk tolerance around the 3-position of THIQs was limited to a 3-methyl (**5**) or 3-hydroxymethyl (**6**) substituent (Table 1) and the 3-trifluoromethyl group may be too sterically demanding. Second, the 3-trifluoromethyl moiety is also very lipophilic (π = 0.88), and it may be placed in an area of space where a hydrophilic substituent that can participate in hydrogen bond donor interactions (e.g., the 3-hydroxymethyl of **9**) is preferred. However, **12** was found to display good inhibitory potency for PNMT (K_i = 0.52 μM). This difference in affinity between **11** and **12** was ascribed to the fact that these compounds may be bound differently at the PNMT active site due to the difference in lipophilicity of the 7-substituent as proposed previously (Figure 1).[30] This would place the 3-trifluoromethyl moiety of **12** in a different area of space where it may not come in contact with any of the negative interactions found for **11**. Fortunately, both **11** and **12** displayed dramatically decreased affinity for the α_2-adrenoceptor (**11**, K_i = 3900 μM; **12**, K_i > 1000 μM). The decreased affinity of **11** and **12** for the α_2-adrenoceptor was attributed to two factors. First, the pK_a of the THIQ amine of **11** and **12** is reduced (pK_a ca. 5)[36] due to the

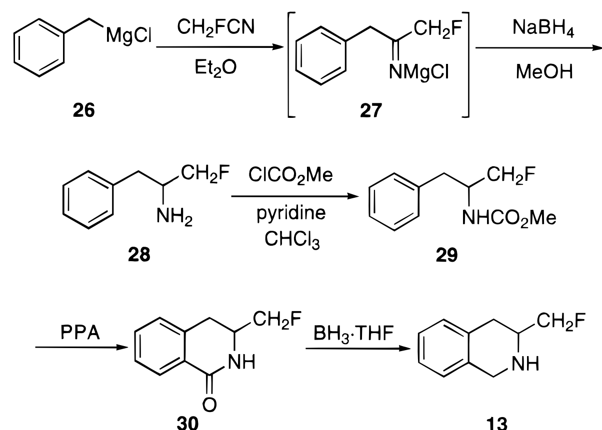
3-trifluoromethyl moiety, making them unprotonated at physiological pH, whereas the natural ligands of the α_2-adrenoceptor (NE and Epi) are protonated (pK_a ca. 10). Second, the 3-trifluoromethyl moiety of **11** may be interacting with the area of steric bulk intolerance surrounding the 3-position of THIQ at the α_2-adrenoceptor as indicated by our CoMFA study.[34] Due to their decreased affinity for the α_2-adrenoceptor, **11** and **12** are some of the more selective PNMT inhibitors known (**11**, α_2 K_i /PNMT K_i = 110; **12**, α_2 K_i /PNMT K_i > 1900). However, **12** displayed some solubility problems in aqueous media—presumably due to its high lipophilicity (Clog P = 3.67) and the reduced pK_a of the THIQ amine (unprotonated). Therefore, it was believed that the 3-substituent of THIQs **11** and **12** could be modified to test these factors and to further optimize potency and selectivity for PNMT.



Compounds **10** and **11** were added to our CoMFA models for both PNMT and the α_2-adrenoceptor in order to further refine the models:[37] PNMT [82 compounds, (cross-validated) r^2 = 0.638, "press s" = 0.691, 8 optimal components, (non-cross-validated) r^2 = 0.928, and s = 0.309] and α_2-adrenoceptor [82 compounds, (cross-validated) r^2 = 0.660, "press s" = 0.613, 4 optimal components, (non-cross-validated) r^2 = 0.856, and s = 0.398]. Structures of the compounds used in the data set were constructed using the SYBYL 6.4 software package,[38] 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). Compounds were aligned in either lipophilic orientation A or hydrophilic orientation B (Figure 1) depending on the lipophilicity (π) of the aromatic substituent.[30,34] The molecules were fit using three points: the two ends of a normal (2 Å long) passing through the centroid of the aromatic ring of the ligand and the end of the axial lone pair (2.4 Å long) on the benzylamine nitrogen. These updated models were used in the design of a series of 3-fluoromethyl-7-substituted-THIQs (**13**–**25**: Table 2).

The 3-fluoromethyl substituent was chosen for several reasons. First, it is smaller than the 3-trifluoromethyl moiety (C–H bond = 1.09 Å; C–F bond = 1.34 Å), due to the decreased number of fluorines, and it is less lipophilic (3-CH_2F π = 0.22; 3-CF_3 π = 0.88). Also, it has been shown in carbohydrate chemistry that a fluorine atom can mimic some of the hydrogen bond acceptor interactions of a hydroxyl moiety as found in sugars.[39] Therefore, the 3-fluoromethyl moiety may mimic some of the potential hydrogen-bonding interactions that can take place with the 3-hydroxymethyl substituent in compounds **9** and **10** while retaining the lack of affinity for the α_2-adrenoceptor demonstrated by **11**. However, decreasing the number of fluorines at the 3-position will also increase the pK_a of the THIQ amine (pK_a ca. 8),[36] which may increase the affinity of these compounds for the α_2-adrenoceptor. According to our CoMFA models for the PNMT active site and the α_2-adrenoceptor, 3-fluoromethyl-THIQs containing electron-withdrawing 7-substituents that are hydrophilic in nature (**14**–**16**, **19**, and **20**) are predicted to be selective for PNMT (Table 2). Due to the increased pK_a of these compounds (pK_a ca. 8),[36] 3-fluoromethyl-THIQs containing lipophilic electron-withdrawing groups [e.g., Br (**21**), I (**22**), CF_3 (**23**)] are predicted to be nonselective PNMT inhibitors (Table 2). Previously, our laboratory had found that 7-sulfonyl-THIQs displayed larger selectivities than those found for other types of 7-substituents.[40] Therefore, several other 3-fluoromethyl-7-sulfonyl-THIQs were proposed and predicted (**14**–**18**) in order to determine if this trend would be found in this series of compounds (Table 2). Compounds **24** and **25** were proposed for use as potential affinity labels and will also serve to further assess the predictive ability of our CoMFA models.

Scheme 1

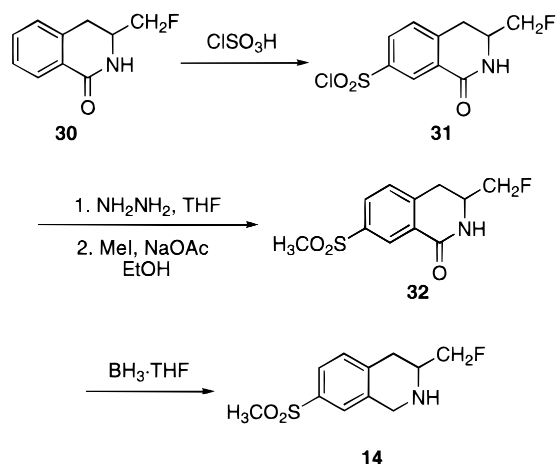


Chemistry

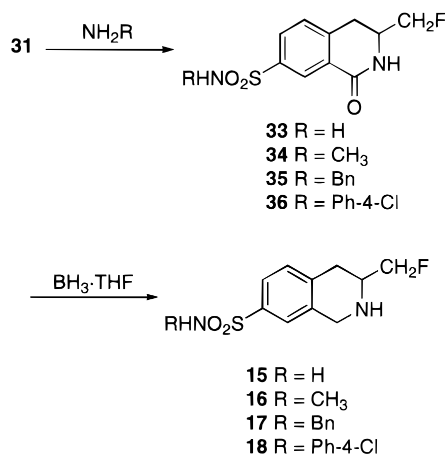
Compounds **13**–**25** were synthesized in the following manner. Benzyl Grignard **26** was reacted with commercially available fluoroacetonitrile to form imine salt **27** that was reduced in situ with $NaBH_4$ in MeOH to form **28**.[57] Phenylpropylamine **28** was treated with methyl chloroformate in $CHCl_3$ and pyridine to form **29**. Carbamate **29** was cyclized with polyphosphoric acid to yield lactam **30**.[32] Reduction of lactam **30** with BH_3·THF formed the unsubstituted 3-fluoromethyl-THIQ (**13**) (Scheme 1).

Treatment of lactam **30** with chlorosulfonic acid (neat) at 50 °C formed **31**. It should be noted that chlorosulfonylation was observed only at the 7-position and not at the 5-position. The regiochemistry was confirmed by examination of the coupling constants (J values) of the aromatic protons. Chlorosulfone **31** was converted to the methyl sulfone in a two-step process. First, treatment of **31** with hydrazine made the hydrazinosulfone, which was converted directly to **32** with MeI and sodium acetate in EtOH at reflux.[41] Reduction of lactam **32** with BH_3·THF yielded THIQ **14** (Scheme 2).

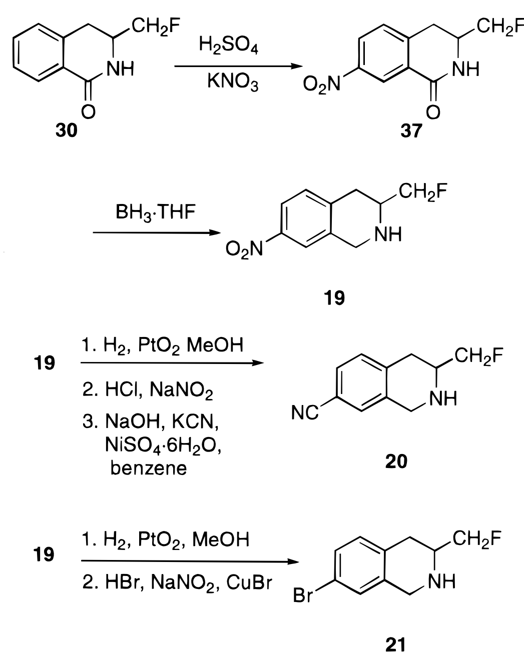
Scheme 2



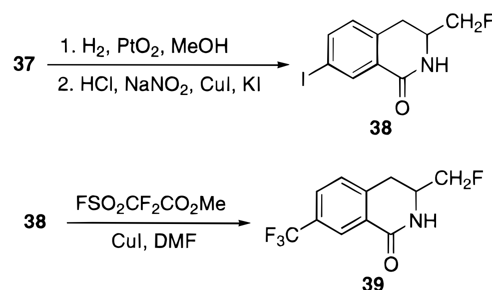
Scheme 3

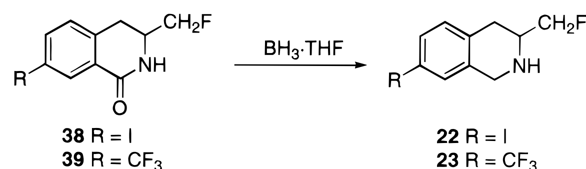


Scheme 4

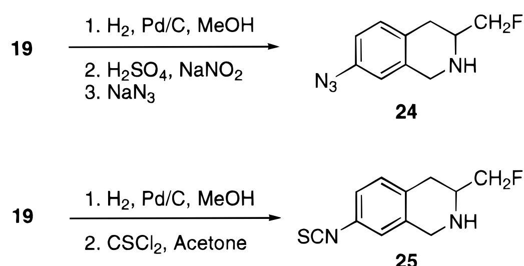


Scheme 5





Scheme 6



Chlorosulfone **31** was treated with NH_4OH in acetonitrile to produce **33,** which was reduced with BH_3·THF to form **15** (Scheme 3). Compound **16** was formed in a similar manner, reacting **31** with NH_2Me·HCl in a biphasic reaction mixture of EtOAc and 10% Na_2CO_3 to produce **34**, which was reduced with BH_3·THF to form **16** (Scheme 3). Compound **17** was synthesized using a similar protocol as **16**, except **31** was treated with benzylamine to yield **35,** which was reduced with BH_3·THF to form **17**. THIQ **18** was synthesized by treating chlorosulfone **31** with 4-Cl-aniline in pyridine to form **36**, followed by reduction with BH_3·THF to form **18** (Scheme 3).

Compounds **19**−**21** were synthesized from lactam **30** (Scheme 4). Nitration of lactam **30** yielded only 7-nitroisoquinolone **37**. The regiochemistry of **37** was confirmed by two-dimensional NMR (HMBC) and examination of the aromatic ^{1}H coupling constants (*J* values). Lactam **37** was reduced with BH_3·THF to form **19**. Hydrogenation of **19** with PtO_2 in MeOH formed the amine which was immediately diazotized with $HCl_{(aq)}$ and $NaNO_2$ and treated with $NiSO_4·6H_2O$, KCN, and $NaOH_{(aq)}$ to form **20**.[42] Compound **21** was synthesized in a similar manner using a Sandmeyer bromination reaction.[43]

7-Iodo-THIQ **22** could not be synthesized using a similar procedure as that for **21** due to decomposition during the Sandmeyer iodination reaction. Therefore, the synthesis of **22** was modified as outlined in Scheme 5. Hydrogenation of the 7-nitro substituent of **37** formed the amine, which was converted directly to lactam **38** via a Sandmeyer iodination reaction.[44] Reduction of **38** with BH_3·THF formed **22**. Treatment of **38** with $FSO_2CF_2CO_2CH_3$ and CuI in DMF formed **39** in low yields.[45] Reduction of **39** with BH_3·THF yielded **23** (Scheme 5).

Potential affinity labels **24** and **25** were synthesized as outlined in Scheme 6. Hydrogenation of **19** followed by diazotization with $NaNO_2$ and H_2SO_4 and treatment with NaN_3 formed **24**. Similarly, the nitro group of **19** was hydrogenated to the amine, which was immediately

treated with thiophosgene in acetone and water to form isothiocyanate **25**.[46]

Biochemistry

All compounds were evaluated as either their hydrochloride or hydrobromide salts for their activity as inhibitors of PNMT and as inhibitors of the binding of [^{3}H]clonidine at the α_2-adrenoceptor. Bovine adrenal PNMT was prepared using the method of Connett and Kirshner through the isoelectric precipitation step.[47] The in vitro activity of these compounds was determined using a standard radiochemical assay that has been described previously.[48] 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.[49] that uses [^{3}H]clonidine as the radioligand to define specific binding and phentolamine to determine the nonspecific binding affinity. [^{3}H]Clonidine was used in order to simplify the comparison with previous results.

In Vitro Blood Brain−Barrier Transport Studies. To estimate the permeability of some of these analogues through the BBB, we have used an in vitro BBB model developed at the University of Kansas.[50,51] This BBB model uses cultured bovine brain microvessel endothelial cells (BBMECs) (isolated from the cerebral gray matter of bovine brain) grown on porous polycarbonate membranes. After 10−12 days the cells reach confluency as determined by visual inspection under a microscope. Transport studies were performed in the following manner. The transwell device consists of two chambers (donor and acceptor) separated by the BBMEC/polycarbonate membrane. The acceptor chamber is filled with an appropriate buffer, while the donor chamber is filled with the buffer and the compound being analyzed. Aliquots were removed from the acceptor chamber at five time points. Each aliquot was analyzed by HPLC using a method developed for the quantitative determination of each particular compound using an internal standard. Paracellular transport or "leakiness" of the membrane was determined using [^{14}C]sucrose, which is unable to penetrate through the endothelial cells but which can penetrate through the "leaks". Using the known concentration of [^{14}C]sucrose that is allowed through the membrane, one can correct for this "leakiness." Six compounds were used in this study, in addition to SK&F 64139 (**1**) as the positive control and SK&F 29661 (**4**) as the negative control, as autoradiographic studies have shown that **1** is able and **4** is unable to penetrate the BBB in vivo.[31] Compounds **9**, **11**, **14**, **15**, **40**, and **41** were chosen because of their diversity of calculated partition coefficients (Clog P) as determined using the Clog P program.[38] These values are given below (Table 3) and range from −0.82 for **5** to 2.83 for SK&F 64139 (**1**). Equation 1 was used to calculate the permeability of these compounds through the membrane. The abbreviations used in eq 1 are P_{app} = permeability coefficient though the BBMEC monolayer (cm/min), k = flux rate (nmol/mL/min), V_D = volume of the donor chamber (1.5 mL), A = cross-sectional area of the cell surface (4.7 cm^2), and C_o = the initial concentration of the solute in the donor chamber (100 nmol/mL); flux rates (k) were determined by a linear fit of the permeability data:

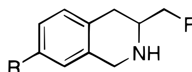
$$P_{app} = (k \times V_D)/(A \times C_o) \qquad (1)$$

Results and Discussion

Overall, the 3-fluoromethyl-THIQs compare favorably to similarly substituted 3-trifluoromethyl-THIQs and 3-hydroxymethyl-THIQs. A direct comparison of the PNMT inhibitory potencies of **11** (PNMT K_i = 36 μM) and **14** (PNMT K_i = 1.6 μM) or those of **12** (PNMT K_i = 0.52 μM) and **21** (PNMT K_i = 0.64 μM) indicates that decreasing the number of fluorines at the 3-methyl position increases the PNMT affinity 20-fold for **14**, while **12** and **21** displayed similar affinity. Previously we postulated that **11** displayed decreased inhibitory potency for PNMT (PNMT K_i = 36 μM) due to the increased lipophilicity and steric bulk of the 3-trifluoromethyl moiety. Decreasing the number of fluorines on the 3-methyl moiety decreases both the steric bulk and the lipophilicity at this position. 3-Fluoromethyl-THIQ **14** displayed increased affinity (PNMT K_i = 1.6 μM) for PNMT relative to **11**. However, both **14** (α_2 K_i = 230 μM) and **21** (α_2 K_i = 6.4 μM) displayed increased affinity for the α_2-adrenoceptor compared to their 3-trifluoromethyl counterparts **11** (α_2 K_i = 3900 μM) and **12** (α_2 K_i > 1000 μM). Previously, we had postulated that the α_2-adrenoceptor prefers a ligand that is protonated (i.e., NE or Epi). Decreasing the number of fluorines on the 3-methyl moiety increases the pK_a of **14** and **21** (pK_a ca. 8)[36] so that these compounds are mostly protonated at physiological pH. Compounds **14** and **21** both showed increased affinity for the α_2-adrenoceptor as compared to their 3-trifluoromethyl counterparts **11** and **12**. Our α_2-adrenoceptor CoMFA model was able to predict that **21** would display increased affinity, but the increased α_2-adrenoceptor affinity of **14** was not predicted. Overall, **14** was found to be more selective (α_2 K_i/PNMT K_i = 140) than **11** (α_2 K_i/PNMT K_i = 110) due to its increased PNMT affinity, while **21** (α_2 K_i/PNMT K_i = 10) was found to be around 200 times less selective than **12** (α_2 K_i/PNMT K_i > 1900) due to its increased affinity for the α_2-adrenoceptor. A comparison of the 7-aminosulfonyl-THIQs **15** (PNMT K_i = 0.66 μM; α_2 K_i = 680 μM; α_2 K_i/PNMT K_i = 1000) and **10** (PNMT K_i = 0.34 μM; α_2 K_i = 1400 μM; α_2 K_i/PNMT K_i = 4100) indicates that the 3-fluoromethyl moiety causes a slight decrease in potency for PNMT and a 2-fold increase in affinity for the α_2-adrenoceptor relative to **10**. This led to a 4-fold decrease in selectivity for PNMT for **15**. Nevertheless, **15** is still one of the most selective inhibitors of PNMT known.

By and large, our CoMFA model K_i predictions for this series of 3-fluoromethyl-THIQs at PNMT and the α_2-adrenoceptor were good (Table 2), being off by an average of 3−4 times the actual K_i values.[52] As predicted by our CoMFA models for PNMT and the α_2-adrenoceptor, compounds possessing hydrophilic electron-withdrawing 7-substituents (**14**−**16**, **19**, and **20**) were found to be the most selective inhibitors in this series of compounds (Table 2). Also, as predicted, THIQs containing lipophilic electron-withdrawing 7-substituents (**21** and **22**) were found to be less selective inhibitors of PNMT due to their increased affinity for the α_2-

Table 2. Predicted and Actual in Vitro Affinities of 3-Fluoromethyl-7-substituted-THIQs for PNMT and the α_2-Adrenoceptor



compd	R	PNMT $K_i \pm$ SEM (μM) pred[b]	PNMT $K_i \pm$ SEM (μM) exptl	α_2-receptor K_i + SEM (μM) pred[b]	α_2-receptor K_i + SEM (μM) exptl	selectivity α_2/PNMT pred[b]	selectivity α_2/PNMT exptl	clog P[a]
13 (±)	H		1.5 ± 0.1		3.8 ± 0.1		2.5	1.77
R		11		5.0		0.45		
S		44		4.4		0.10		
14 (±)	SO_2CH_3		1.6 ± 0.2		230 ± 10		140	0.13
R		1.5		650		430		
S		4.9		700		140		
15 (±)	SO_2NH_2		0.66 ± 0.10		680 ± 10		1000	−0.07
R		0.79		530		670		
S		2.7		520		190		
16 (±)	SO_2NHCH_3		2.4 ± 0.1		310 ± 10		130	0.55
R		0.53		880		1600		
S		2.0		730		370		
17 (±)	SO_2NHBn		6.5 ± 0.1		110 ± 10		17	2.31
R		10		39		3.9		
S		2.8		76		26		
18 (±)	SO_2NHPh-4-Cl		0.74 ± 0.07		160 ± 10		220	3.11
R		6.4		11		1.7		
S		2.2		11		6.8		
19 (±)	NO_2		0.54 ± 0.06		76 ± 6		140	1.50
R		0.30		70		230		
S		1.5		67		45		
20 (±)	CN		1.1 ± 0.1		460 ± 10		420	1.20
R		0.85		16		19		
S		4.6		14		3.0		
21 (±)	Br		0.64 ± 0.1		6.4 ± 0.2		10	2.63
R		0.58		0.49		0.84		
S		0.24		0.65		2.7		
22 (±)	I		0.21 ± 0.04		7.1 ± 0.5		34	2.89
R		0.69		0.43		0.62		
S		0.28		0.57		2.0		
23 (±)	CF_3		0.32 ± 0.03		41 ± 4		130	2.65
R		0.26		0.90		3.5		
S		0.11		1.2		11		
24 (±)	N_3		1.7 ± 0.1		47 ± 5		28	
R		0.89		5.7		6.4		
S		3.1		7.4		2.4		
25 (±)	NCS		0.57 ± 0.7[c]		22 ± 1[c]		39	2.18
R		1.2		8.2		6.8		
S		4.5		8.7		1.9		

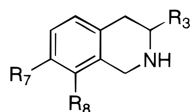
[a] Reference 38. [b] Although only racemates were synthesized and evaluated, the predicted values are given for each enantiomer as the alignment of each in the CoMFA was different. [c] **25** was assayed as described in the Experimental Section, and no attempt was made to determine if irreversible inhibition occurred.

adrenoceptor (Table 2). Compound **23** is an exception to this trend and has a selectivity ratio of 130. The high degree of selectivity of **23** compared to compounds **14**–**16**, **18**, and **20** is due more to its increased inhibitory potency for PNMT rather than to a reduction in its α_2-adrenoceptor affinity (Table 2).

We had previously found that THIQs containing a 7-sulfonyl substituent were particularly selective for PNMT.[40] A similar trend was followed by the 3-fluoromethyl-THIQs. Compounds **14**–**15**, **16** and **18** were found to be some of the most selective inhibitors in this series (Table 2). Sulfonamide **15** is the most selective compound in this series of 3-fluoromethyl-THIQs (α_2 K_i/PNMT K_i = 1000). For the *N*-substituted sulfonamides, substitution of a methyl (**16**) or benzyl (**17**) group on the nitrogen of the aminosulfonyl decreased the potency of these compounds for PNMT, as compared to **15** while concomitantly increasing their affinity for the α_2-adrenoceptor (Table 2). However, substitution of a 4-chlorophenyl group on the sulfonamide (**18**) did not cause any significant decrease in PNMT affinity as compared to **15**. Sulfonamide **18** was found to display a 4-fold increase in affinity, relative to **15**, for the α_2-adrenoceptor, which is presumably due to the increased lipophilicity of the 7-substituent (Clog P = 3.11). It should be noted that the lipophilicity (π) of the 7-substituent of THIQ has been previously correlated with α_2-adrenoceptor affinity.[30] Nevertheless, **18** was still found to be one of the more selective compounds in this series (α_2 K_i/PNMT K_i = 220) and is much more lipophilic (Clog P = 3.11) than compound **15** (Clog P = −0.07).

An unexpected result was that nitrile **20** was found to be the second most selective compound in this series of inhibitors (α_2 K_i/PNMT K_i = 400). While nitrile **20** was predicted to be moderately selective for PNMT (Table 2), it was found to have a much lower affinity for the α_2-adrenoceptor (K_i = 460 μM) than predicted by our CoMFA model. This compound is also sufficiently lipophilic (Clog P = 1.20) to allow this compound to penetrate the BBB, and thus **20** is a promising lead in the development of highly selective PNMT

Table 3. In Vitro Blood−Brain Barrier Permeability of Some PNMT Inhibitors



compd	R_3	R_7	R_8	clog P[a]	$P_{app} \times 10^4$ (cm/min)
1 (SK&F 64139)	H	Cl	Cl	2.83	17.7 ± 1.9[b]
40[c]	CH_3	NO_2	H	1.79	13.9 ± 1.3
11[d]	CF_3	SO_2CH_3	H	1.16	9.89 ± 0.80
41[c]	CH_2OH	NO_2	H	0.57	12.5 ± 1.1
14	CH_2F	SO_2CH_3	H	0.13	9.00 ± 0.48
15	CH_2F	SO_2NH_2	H	−0.07	4.28 ± 0.38
4 (SK&F 29661)	H	SO_2NH_2	H	−0.29	3.51 ± 0.32
9[c]	CH_2OH	SO_2CH_3	H	−0.82	7.21 ± 0.70

[a] Reference 38. [b] C_0 for SK&F 64139 was 70 nmol/mL (see Experimental Section). [c] Reference 33. [d] Reference 35.

inhibitors that may have the ability to penetrate into the CNS.

The potential affinity labels **24** and **25**, which were included in this study to test the predictive ability of our CoMFA models, were also predicted fairly well at both PNMT and the α_2-adrenoceptor (Table 2).

In Vitro Blood−Brain Barrier Permeability. The results of this study are shown in Table 3 and indicate that there is a definite correlation between lipophilicity (Clog P) and potential BBB permeability (P_{app}) of these THIQ-type PNMT inhibitors (Clog P versus log P_{app}; correlation coefficient $r = 0.79$). A similar correlation between Clog P and BBB permeability has been previously reported for this type of system.[53] It has been well-established that most water-soluble materials (drugs) pass through the BBB by a passive diffusion mechanism. From previous in vivo studies, it has been established that the permeability of drug molecules across the BBB depends directly on their lipophilicity and inversely on their molecular size.[54] Positive control SK&F 64139 (**1**) was found to have the largest apparent permeability ($P_{app} = 17.7$ cm/min), as expected, due to its high lipophilicity (Clog $P = 2.83$), whereas negative control SK&F 29661 (**4**) was found to have the smallest P_{app} (3.51 cm/min). Methyl sulfone **14** (Clog $P = 0.13$; $P_{app} = 9.00$ cm/min) was found to display one-half the permeability of **1**. Examination of the permeabilities (P_{app}) of **41**, **14**, and **15** shows that there is a fairly significant decrease in P_{app} between **41** ($P_{app} = 12.5$ cm/min), **14** ($P_{app} = 9.00$ cm/min), and sulfonamide **15** ($P_{app} = 4.28$ cm/min). This decrease corresponds to a drop in the Clog P from 0.57 for **41** to 0.13 for **14** to −0.07 for **15**. Sulfonamide **15** and SK&F 29661 (**4**; negative control) were found to display relatively the same P_{app} and do not appear to display any penetration through the BBMEC monolayer. Therefore, the apparent minimum lipophilicity (Clog P) for partial permeability through the BBB is 0.13−0.57 for THIQs. It should be noted that removal of 3-hydroxymethyl-THIQs **41** and **9** from the linear regression analysis (Clog P versus log P_{app}) increases the correlation coefficient from $r = 0.79$ to $r = 0.91$. This may be an indication that another factor, which is not measured by this in vitro BBB model, is influencing the permeability of 3-hydroxymethyl-THIQs (**41** and **9**).

Summary and Conclusions

A series of 3-fluoromethyl-THIQs were synthesized and evaluated as inhibitors of PNMT and as inhibitors of the binding of clonidine at the α_2-adrenoceptor. Overall, our CoMFA models for PNMT and the α_2-adrenoceptor did a good job of predicting the activity of these compounds. As predicted by our CoMFA models, inhibitors containing 7-substituents that were both hydrophilic and electron-withdrawing were found to be selective for PNMT, whereas inhibitors containing lipophilic electron-withdrawing substituents were found to be nonselective PNMT inhibitors. Compounds **15**, **16**, **18**, and **20** are some of the most selective inhibitors of PNMT versus the α_2-adrenoceptor known. To determine the ability of these THIQs to penetrate into the brain, an in vitro BBB permeability study was performed. This study indicated that THIQs possessing Clog P values greater than 0.57 (**41**), possibly as low as 0.13 (**14**), should gain some penetration into the brain. Compounds **18** and **20** possess calculated partition coefficients greater than 0.57 and display selectivities (α_2-adrenoceptor K_i/PNMT K_i) greater than 200. These compounds represent promising leads in the development of a potent and selective PNMT inhibitor that can penetrate the BBB.

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 with $CDCl_3$ as the solvent unless otherwise noted in the text, and 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). For the hydrochloride salts of the 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 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: 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. 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. 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 out of 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. Basic brine refers to a (10:1) mixture of brine and 10% NaOH. All reactions that required anhydrous conditions were performed under a positive nitro-

gen (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-Fluoro-3-phenyl-2-aminopropane[55] Hemisulfate (28·1/2H_2SO_4). Fluoroacetonitrile (3.00 g, 50.8 mmol) in dry Et_2O (50 mL) was added dropwise over 30 min to a solution of 2 M benzyl Grignard in Et_2O (25.4 mL, 50.8 mmol) and dry Et_2O (75 mL) at −20 °C. After the addition, the solution was stirred for 30 min at −20 °C. A gray precipitate (iminium salt) formed and was transferred to a solution of $NaBH_4$ (1.92 g, 84.7 mmol) in MeOH (250 mL) and H_2O (2.00 mL) at −20 °C. Residual iminium salt was dissolved in anhydrous THF and added to the methanolic $NaBH_4$ solution. The reaction mixture was stirred 1 h at −20 °C and 1 h at 0 °C. 3 N HCl (20 mL) was slowly added to the reaction mixture and the reaction mixture was concentrated (ca. 20 mL). Additional 3N HCl (30 mL) was added and the acidic aqueous mixture was washed with Et_2O (2 × 200 mL). The aqueous solution was made basic with 4 N NaOH and extracted with Et_2O (4 × 75 mL). The combined organic extracts from the basic aqueous solution were washed with basic brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue distilled bulb-to-bulb (100 °C, 0.25 mmHg) to yield **28** as a colorless oil, which was dissolved in CH_2Cl_2. The sulfate salt was formed by the addition of methanolic H_2SO_4 (10%); the precipitate was collected by filtration and recrystallized from EtOH/hexanes to yield **28**·1/2H_2SO_4 (3.71 g, 36%): mp 210 °C dec; IR (KBr) 3400, 2900, 1600, 1580, 1510, 1450, 1100, 750, 690 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.39−7.28 (m, 5H, ArH), 4.72−4.39 (m, 2H, CH_2F), 3.85−3.68 (m, 1H, H-2), 3.17−2.86 (m, 2H, H-3); ^{13}C NMR (CD_3OD) δ 135.7, 129.5, 129.0, 127.4, 81.7 (d, *J* = 678 Hz, CH_2F), 52.9 (d, *J* = 74 Hz, C-2), 34.5 (d, *J* = 21 Hz, C-3); CIMS *m*/*z* (relative intensity) 154 (MH$^+$, 100), 62 (40); HRMS (FAB) *m*/*z* [M + H]$^+$ calcd for $C_9H_{12}FN$ 154.1032, found 154.1059.

(±)-Methyl *N*-(1-Fluoro-3-phenylprop-2-yl)carbamate (29). Compound **28**·1/2H_2SO_4 (1.60 g, 7.90 mmol) was dissolved in dry $CHCl_3$ (75 mL) and pyridine (2.63 mL, 31.6 mmol) and the solution cooled to 0 °C. Methyl chloroformate (0.61 mL, 7.90 mmol) was added dropwise and the solution was stirred at room temperature overnight. Ice water (25 mL) was added and the mixture was stirred for 30 min. The mixture was washed with 3 N HCl (3 × 50 mL) and brine (50 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to yield a yellowish oil, which was distilled bulb-to-bulb (111 °C, 0.25 mmHg) to yield **29** as a colorless oil (1.58 g, 95%): IR (neat) 3300, 2950, 1700, 1530, 1450, 1250, 1075, 1025, 750, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.34−7.21 (m, 5H, ArH), 4.95 (br ex s, 1H, NH), 4.40 (dm, *J* = 46.6 Hz, 2H, H-1), 3.98−4.17 (m, 1H, H-2), 3.66 (s, 3H, OCH_3), 2.91−2.88 (m, 2H, H-3); ^{13}C NMR ($CDCl_3$) δ 157.0 (CO), 137.6, 129.7, 128.8, 127.0, 83.8 (d, *J* = 680 Hz, CH_2F), 52.8 (d, *J* = 76 Hz, C-2), 52.0 (OCH_3), 37.1 (C-1); EIMS *m*/*z* (relative intensity) 212 (MH$^+$, 80), 120 (82), 91 (100), 76 (75). Anal. ($C_{11}H_{14}FNO_2$) C, H, N.

(±)-3-Fluoromethyl-3,4-dihydroisoquinolin-1(2*H*)-one (30). Carbamate **29** (1.44 g, 6.82 mmol) was dissolved in polyphosphoric acid (PPA; 15 g), heated to 130 °C, and stirred for 2 h. The mixture was cooled in an ice bath and ice-cold distilled water (100 mL) was added. This mixture was stirred until all of the PPA had dissolved. The aqueous mixture was extracted with EtOAc (4 × 50 mL). The organic extracts were combined, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed to yield an off-white solid, which was purified by chromatography on silica gel eluting with EtOAc/hexanes (1:1) to yield a white solid. Recrystallization from $CHCl_3$/hexanes yielded **30** as white crystals (665 mg, 55%): mp 117−119 °C; IR (KBr) 3400, 3200, 1710, 1680, 1460, 1400, 1075, 1025, 800, 740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.07 (d, *J* = 9.1 Hz, 1H, ArH-8), 7.48 (m, 1H, ArH-6), 7.37 (m, 1H, ArH-7), 7.22 (d, *J* = 7.7 Hz, 1H, ArH-5), 6.07 (br ex s, 1H, NH), 4.46 (dm, *J* = 45.9 Hz, 2H, CH_2F), 4.06 (m, 1H, H-3), 3.02 (m, 1H, H-4), 2.92 (m, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 166.2, 136.8, 133.0, 128.6, 128.5, 128.1, 127.8, 84.4 (d, *J* = 692 Hz, CH_2F), 51.0 (d, *J* = 82 Hz, C-3), 29.3 (d, *J* = 24 Hz, C-4); EIMS *m*/*z* (relative intensity) 179 (M$^+$, 50), 146 (100), 128 (55), 90 (25). Anal. ($C_{10}H_{10}FNO$) C, H, N.

(±)-3-Fluoromethyl-1,2,3,4-tetrahydroisoquinoline Hydrochloride (13·HCl). Lactam **30** (43.3 mg, 0.241 mmol) was dissolved in THF (5 mL), 1 M BH_3·THF (0.5 mL, 0.5 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 to 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 combined organic extracts were 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 EtOAc. Amine **13** was dissolved in $CHCl_3$ and dry $HCl_{(g)}$ was used to form the hydrochloride salt, which was recrystallized from EtOH/hexanes to yield **13**·HCl (32.0 mg, 66%): mp 214−215 °C; ^{1}H NMR (DMSO-d_6) δ 10.26−9.93 (br ex m, 2H, NH_2^+), 7.31−7.24 (m, 4H, ArH), 4.99−4.67 (m, 2H, CH_2F), 4.34−4.27 (m, 2H, H-1), 3.85−3.79 (m, 1H, H-3), 3.03 (d, *J* = 7.9 Hz, 2H, H-4); ^{13}C NMR (DMSO-d_6) δ 131.9, 129.7, 129.3, 128.4, 127.5, 127.4, 83.1 (d, *J* = 672 Hz, CH_2F), 53.0 (d, *J* = 74 Hz, C-3), 44.7, 26.9; EIMS *m*/*z* (relative intensity) 164 (M$^+$ − 1, 10), 132 (100), 130 (30), 104 (30), 77 (25), 65 (25), 51 (10). Anal. ($C_{10}H_{12}$-FN·HCl) C, H, N.

(±)-7-Chlorosulfonyl-3-fluoromethyl-3,4-dihydroisoquinolin-1(2*H*)-one (31). Lactam **30** (338 mg, 1.89 mmol) was dissolved in chlorosulfonic acid (10 mL) and heated to 50 °C for 16 h. The solution was cooled and poured carefully onto ice. A white precipitate formed, which was collected by filtration, washed with water (2 × 25 mL), and dried under vacuum. The aqueous filtrate was extracted with EtOAc (4 × 50 mL). The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield a light brown residue, which was combined with the precipitate isolated previously. The combined material was purified by chromatography on silica gel eluting with EtOAc to yield a white solid. Recrystallization from EtOAc/hexanes yielded **31** as white crystals (342 mg, 65%): mp 188−189 °C; IR (KBr) 3450, 2900, 1690, 1300, 1133, 1000 cm $^{-1}$; ^{1}H NMR ($CDCl_3$) δ 8.77 (s, 1H, ArH-8), 8.14 (d, *J* = 8.1 Hz, 1H, ArH-6), 7.52 (d, *J* = 8.1 Hz, 1H, ArH-5), 6.13 (br ex s, 1H, NH), 4.60−4.40 (m, 2H, CH_2F), 4.17−4.09 (m, 1H, H-3), 3.19−3.08 (m, 2H, H-4); ^{13}C NMR (DMSO-d_6) δ 164.8, 147.8, 138.4, 129.8, 128.7, 128.2, 125.2, 84.8 (d, *J* = 681 Hz, CH_2F), 50.4 (d, *J* = 81 Hz, C-3), 29.2; EIMS *m*/*z* 278 (relative intensity) (MH$^+$, 50), 244 (90), 145 (100), 89 (65). Anal. ($C_{10}H_9$-$NClFO_3S$) C, H, N.

(±)-3-Fluoromethyl-7-methanesulfonyl-3,4-dihydroisoquinolin-1(2*H*)-one (32). Chlorosulfone **31** (324 mg, 1.17 mmol) was dissolved in THF (10 mL) and cooled to 0 °C. Hydrazine (0.130 mL, 3.10 mmol) was added dropwise to the solution, which was stirred overnight at room temperature. The solution was cooled and the white precipitate (hydrazinosulfone) was collected by filtration. The precipitate was dissolved in EtOH (5 mL) and NaOAc (0.520 g, 6.34 mmol) and iodomethane (0.290 mL, 5.20 mmol) were added. The mixture was heated at reflux overnight. Water (25 mL) was added and the solution was extracted with EtOAc (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 to yield a white solid, which was recrystallized from EtOAc/hexanes to yield **32** as white needles (166 mg, 55%): mp 218−220 °C; IR (KBr) 3450, 2900, 1690, 1300,

1133, 1000 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.66 (d, J = 2.0 Hz, 1H, ArH-8), 8.07 (dd, J = 7.9, 2.0 Hz, 1H, ArH-6), 7.47 (d, J = 7.9 Hz, 1H, ArH-5), 6.33 (br ex s, 1H, NH), 4.58−4.37 (m, 2H, CH_2F), 4.15−4.04 (m, 1H, H-3), 3.15−3.00 (m, 2H, H-4), 3.10 (s, 3H, CH_3); EIMS m/z (relative intensity) 258 (MH^+, 100), 224 (95), 145 (60). Anal. ($C_{11}H_{12}FNO_3S{\cdot}0.5H_2O$) C, H, N.

(±)-3-Fluoromethyl-7-methanesulfonyl-1,2,3,4-tetrahydroisoquinoline Hydrochloride (14·HCl). Lactam **32** (125 mg, 0.485 mmol) was dissolved in THF (10 mL). 1 M BH_3·THF (3 mL, 3.00 mmol) was added dropwise to the solution and the reaction mixture was heated at reflux for 3 h. The solution was cooled, MeOH (10 mL) was added dropwise, and the solvent was removed under reduced pressure. A solution of 6 N HCl (10 mL) and MeOH (10 mL) was added slowly to the residue. The mixture was heated to reflux for 30 min. The solution was concentrated under reduced pressure, made basic with 4 N NaOH, and extracted with EtOAc (4 × 25 mL). The combined organic extracts were washed with basic brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield **14** as a white solid, which was dissolved in $CHCl_3$. Dry $HCl_{(g)}$ was used to form the HCl salt, which was collected by filtration and recrystallized from EtOH/hexanes to yield **14**·HCl as white crystals (78.3 mg, 58%): mp 222−223 °C; IR (KBr) 3450, 2900, 2750, 1425, 1300, 1125, 1010, 770 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.0 (br ex s, 2H, NH_2^+), 7.90 (s, 1H, ArH-8), 7.83 (d, J = 8.1 Hz, 1H, ArH-6), 7.55 (d, J = 8.22 Hz, 1H, ArH-5), 4.98−4.80 (m, 2H, CH_2F), 4.48 (s, 2H, H-1), 4.05−3.90 (m, 1H, H-3), 3.21−3.08 (m, 2H, H-4), 3.22 (s, 3H, CH_3); EIMS m/z (relative intensity) 243 (M^+, 5), 224 (100), 145 (60). Anal. ($C_{11}H_{14}NFO_2S{\cdot}HCl$) C, H, N.

(±)-7-Aminosulfonyl-3-fluoromethyl-3,4-dihydroisoquinolin-1(2*H*)-one (33). Chlorosulfone **31** (313 mg, 1.13 mmol) was dissolved in acetonitrile (5 mL), concentrated ammonium hydroxide (5 mL) was added, and the solution was stirred overnight. The solvent was removed under reduced pressure and the residue was recrystallized from EtOH to yield **33** as white crystals (252 mg, 87%): mp 240 °C dec; IR (KBr) 3250, 3190, 2910, 1680, 1325, 1150 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.38 (s, 1H, ArH-8), 7.93 (d, J = 7.9 Hz, 1H, ArH-6), 7.44 (d, J = 7.9 Hz, 1H, ArH-5), 4.47 (m, 1H, CHF), 4.30 (m, 1H, CHF), 3.90 (m, 1H, H-3), 3.13 (m, 1H, H-4), 2.98 (m, 1H, H-4); CIMS m/z (relative intensities) 276 (M + NH_4^+, 25), 259 (MH^+, 100), 225 (27). Anal. ($C_{10}H_{11}N_2FO_3S$) C, H, N.

(±)-3-Fluoromethyl-7-aminosulfonyl-1,2,3,4-tetrahydroisoquinoline Hydrochloride (15·HCl). Lactam **33** (252 mg, 0.997 mmol) was dissolved in THF (10 mL). 1 M BH_3·THF (6 mL, 6 mmol) was added dropwise to the solution and the reaction mixture was heated at reflux for 4 h. The solution was cooled in an ice bath and 3 N HCl (15 mL) was added slowly. The solution was concentrated under vacuum (ca. 15 mL) and washed with EtOAc (25 mL). The aqueous phase was treated with 6 N NaOH until the solution pH was ca. 9 (note pK_a of sulfonamide) and sodium chloride was added until the solution was saturated. The aqueous solution was extracted with EtOAc (4 × 50 mL). The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed and the residue was dissolved in dry MeOH. Dry $HCl_{(g)}$ was used to form the hydrochloride salt. The solvent was removed and the remaining white solid was recrystallized from EtOH/hexanes to yield **15**·HCl as off-white crystals (88.0 mg, 32%): mp 216−217 °C; IR (KBr) 3270, 3185, 2910, 1580, 1325, 1150 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.08 (br ex s, 2H, NH_2^+), 7.78−7.64 (m, 2H, ArH-6,8), 7.52 (d, J = 8.0 Hz, 1H, ArH-5), 7.46 (ex s, 2H, SO_2NH_2), 4.98−4.68 (m, 2H, CH_2F), 4.44 (s, 2H, H-1), 3.95−3.89 (m, 1H, H-3), 3.17−3.05 (m, 2H, H-4); CIMS m/z 245 (MH^+, 100), 225 (70), 211 (50). Anal. ($C_{10}H_{13}N_2FO_2S{\cdot}HCl$) C, H, N.

(±)-3-Fluoromethyl-7-(*N*-methylaminosulfonyl)-3,4-dihydroisoquinolin-1(2*H*)-one (34). Chlorosulfone 31 (99.7 mg, 0.360 mmol) was dissolved in a biphasic mixture of EtOAc (10 mL) and saturated Na_2CO_3 (10 mL). Methylamine hydrochloride (200 mg, 2.96 mmol) was added to the reaction and the mixture was stirred for 3 h. The organic phase was removed, washed with 3 N HCl (2 × 10 mL) and brine (10 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue recrystallized from EtOH/hexanes to yield 34 as white crystals (90.6 mg, 93%): mp 178−179 °C; IR (KBr) 3600, 3400, 3280−3190, 1680, 1160, 1000, 840, 640 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 8.38 (d, J = 2.0 Hz, 1H, ArH-8), 7.93 (dd, J = 8.0, 2.0 Hz, 1H, ArH-6), 7.58 (d, J = 8.0 Hz, 1H, ArH-5), 7.40 (br ex s, 1H, NH), 6.48 (br ex s, 1H, NH), 4.65−4.46 (m, 2H, CH_2F), 4.15−4.05 (m, 1H, H-3), 3.30−3.15 (m, 2H, H-4), 2.59 (d, J = 2.5 Hz, 3H, CH_3); CIMS m/z (relative intensity) 273 (MH^+, 15), 239 (100), 145 (25), 144 (20), 89 (20), 63 (20). Anal. ($C_{11}H_{13}N_2O_3S$) C, H, N.

(±)-3-Fluoromethyl-7-(*N*-methylaminosulfonyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (16·HCl). Lactam **34** (1.062 g, 3.87 mmol) was reduced to THIQ **16** using the same procedures described previously for the synthesis of THIQ **14** from lactam **32**. The hydrochloride salt of **16** was prepared in dry EtOH using dry $HCl_{(g)}$. The solvent was removed and the residue was recrystallized from EtOH/hexanes to yield **16**·HCl as white crystals (989 mg, 99%): mp 204−206 °C; IR (KBr) 3160, 2900, 2750, 2500, 1420, 1320, 1170, 1050, 1000, 720 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.5 (br ex s, 2H, NH_2^+), 7.38−7.65 (m, 2H, ArH-8, SO_2NH), 7.56−7.48 (m, 2H, ArH-5,6), 5.01−4.70 (m, 2H, CH_2F), 4.44 (s, 2H, H-1), 3.92−3.87 (m, 1H, H-3), 3.18−3.05 (m, 2H, H-4), 2.43 (s, 3H, NCH_3); ^{13}C NMR (DMSO-d_6) δ 138.7, 136.9, 130.7, 130.8, 126.3, 126.0, 83.0 (d, J = 683 Hz, CH_2F), 52.6 (d, J = 76 Hz, C-3), 44.5, 29.5, 27.0 (d, J = 22 Hz, C-4); CIMS m/z (relative intensity) 275 (M + NH_4^+, 6), 259 (MH^+, 100), 239 (30), 225 (40). Anal. ($C_{11}H_{15}N_2FO_2S{\cdot}HCl$) C, H, N.

(±)-3-Fluoromethyl-7-(*N*-benzylaminosulfonyl)-3,4-dihydroisoquinolin-1(2*H*)-one (35). Chlorosulfone **31** (100 mg, 0.361 mmol) was dissolved in a biphasic mixture of EtOAc (10 mL) and saturated Na_2CO_3 (10 mL). Benzylamine (0.3 mL, 3 mmol) was added and the reaction mixture was stirred for 3 h. The organic phase was removed, washed with 3 N HCl (2 × 10 mL) and brine (10 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue recrystallized from EtOH/hexanes to yield **35** as white crystals (104 mg, 83%): mp 225−226 °C; IR (KBr) 3450, 3250, 3100−2900, 1680, 1360, 1325, 1160, 1000, 900, 750, 640 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 8.44 (d, J = 2.0 Hz, 1H, ArH-8), 7.98 (dd, J = 2.0 Hz, 7.8 Hz, 1H, ArH-6), 7.57 (d, J = 7.8 Hz, 1H, ArH-5), 7.41 (br ex s, 1H, SO_2NH), 7.32 (m, 5H, Ph H-2,3,4,5,6), 7.12, (br ex s, 1H, CONH), 4.69−4.51 (m, 2H, CH_2F), 4.20 (s, 2H, $PhCH_2N$), 4.15−4.10 (m, 1H, H-3), 3.33−3.12 (m, 2H, H-4); CIMS m/z (relative intensity) 350 (MH^+ + 1, 15), 349 (MH^+, 100), 106 (75), 91 (12); HRMS (FAB) m/z [M + H]$^+$ calcd for $C_{17}H_{18}FN_2O_3S$ 349.1022, found 349.1044.

(±)-3-Fluoromethyl-7-(*N*-benzylaminosulfonyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (17·HCl). Lactam **35** (92 mg, 0.29 mmol) was reduced to THIQ **17** using the same procedures described previously for the synthesis of THIQ **14** from lactam **32**. The hydrochloride salt of **17** was prepared in anhydrous EtOH using dry $HCl_{(g)}$. The solvent was removed under reduced pressure and the crude hydrochloride salt was recrystallized from EtOH/hexanes to yield **17**·HCl as white crystals (73 mg, 75%): mp 220−221 °C; IR (KBr) 3450, 3280, 2900−2500, 1320, 1150, 1050, 1010, 700 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.74 (d, J = 8.2 Hz, 1H, ArH-6), 7.70 (s, 1H, ArH-8), 7.45 (d, J = 8.2 Hz, 1H, ArH-5), 7.23 (m, 5H, PhH-2,3,4,5,6), 4.95−4.70 (m, 2H, CH_2F), 4.51 (s, 2H, H-1), 4.08 (s, 2H, $PhCH_2N$), 3.97−3.88 (m, 1H, H-3), 3.24−3.17 (m, 2H, H-4); CIMS m/z (relative intensity) 335 (MH^+, 100), 301 (95), 130 (45), 115 (30), 106 (75), 91 (95), 77 (50). Anal. ($C_{17}H_{19}N_2FO_2S{\cdot}HCl{\cdot}0.25H_2O$) C, H, N.

(±)-3-Fluoromethyl-7-[*N*-(4-chlorophenyl)aminosulfonyl]-3,4-dihydroisoquinolin-1(2*H*)-one (36). Compound **31** (500 mg, 1.80 mmol) was added to a solution of 4-chloroaniline (260 mg, 2.05 mmol) in pyridine (15 mL). The solution was stirred for 6 h. The pyridine was removed under reduced pressure and the residue was dissolved in EtOAc (30 mL) and 3 N HCl (30 mL). The organic phase was separated, washed with 3 N HCl (30 mL), 10% $NaHCO_3$ (30 mL), and brine (30 mL), and dried over anhydrous Na_2SO_4. The solvent was

removed under reduced pressure to yield an orange residue, which was recrystallized from EtOAc/hexanes to yield **36** as light orange crystals (639 mg, 96%): mp 141−143 °C; IR (KBr) 3300, 3100, 3050, 2900, 1720, 1650, 1490, 1340, 1180, 1160, 1040, 640 cm^{-1}; ^1H NMR (acetone-d_6) δ 8.38 (d, J = 2.0 Hz, 1H, ArH-8), 7.85 (dd, J = 8.0, 2.0 Hz, 1H, ArH-6), 7.57 (d, J = 6.6 Hz, 2H, Ph H-3, H-5), 7.51 (d, J = 8.0 Hz, 1H, ArH-5), 7.37−7.24 (m, 4H, Ph H-2, H-6, CONH, SO_2NH), 4.61−4.42 (m, 2H, CH_2F), 4.08 (m, 1H, H-3), 3.23−3.11 (m, 2H, H-4); ^{13}C NMR (DMSO-d_6) δ 163.7, 143.6, 138.8, 137.4, 130.5, 130.3, 130.1, 129.2, 126.1, 122.5, 105.0, 85.0 (d, J = 680 Hz, CH_2F), 50.2 (d, J = 80 Hz, C-3), 29.3 (d, J = 19 Hz, C-3); CIMS m/z (relative intensity) 371 (MH^+, 60), 335 (30), 128 (100). Anal. ($C_{16}H_{14}N_2ClFO_3S$) C, H, N.

(±)-3-Fluoromethyl-7-[*N*-(4-chlorophenyl)aminosulfonyl]-1,2,3,4-tetrahydroisoquinoline Hydrochloride (18·HCl). Lactam **36** (639 mg, 1.74 mmol) was dissolved in anhydrous THF (20 mL) and BH_3·THF (1 M, 8.7 mL) was added dropwise to the solution. The mixture was heated at reflux for 15 h. It was cooled in an ice bath and MeOH (5 mL) was added dropwise. The solvent was removed under reduced pressure to yield a white residue, which was dissolved in MeOH (10 mL) and concentrated HCl (5 mL) and heated to reflux for 3 h. The MeOH was removed under reduced pressure and 15% KOH was added dropwise until the solution pH was ca. 8. Sodium chloride was added to the basic solution until it became saturated. The aqueous phase was extracted with EtOAc (4 × 50 mL). The combined organic extracts were dried over anhydrous K_2CO_3 and the solvent removed under reduced pressure to yield **18** as a white solid, which was dissolved in dry EtOH, and dry $HCl_{(g)}$ was used to form the HCl salt. The solvent was removed under reduced pressure to yield a white residue, which was recrystallized from EtOH/hexanes to yield **18**·HCl as white crystals (248 mg, 36%): mp 236−238 °C dec; IR (KBr) 3320, 3200−2900, 1700, 1650, 1600, 1340, 1240, 1150, 920, 840, 750, 640 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.58 (ex s, 1H, SO_2NH), 9.8−9.6 (br ex m, 2H, NH_2^+), 7.74 (s, 1H, ArH-8), 7.67 (d, J = 9.0 Hz, 1H, ArH-6), 7.46 (d, J = 9.0, 1H, ArH-5), 7.32 (d, J = 8.8 Hz, 2H, Ph H-3, H-5), 7.13 (d, J = 8.8 Hz, 2H, Ph H-2, H-6), 4.85−4.60 (m, 2H, CH_2F), 4.44 (s, 2H, H-1), 3.95 (m, 1H, H-3), 3.12 (m, 2H, H-4); CIMS m/z (relative intensity) 357 (MH^+ + 2, 60), 355 (MH^+, 100), 335 (75), 321 (25), 128 (30), 115 (25), 99 (25), 79 (75). Anal. ($C_{16}H_{16}N_2ClFO_2S$·HCl) C, H, N.

(±)-3-Fluoromethyl-7-nitro-3,4-dihydroisoquinolin-1-(2*H*)-one (37). Lactam **30** (3.14 g, 17.5 mmol) was dissolved in concentrated H_2SO_4 (20 mL) and cooled to 0 °C. KNO_3 (2.14 g, 21.2 mmol) was added in small portions over 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The mixture was poured slowly onto ice (100 g). A white precipitate formed, which was collected by filtration, washed with water (20 mL), and dried under vacuum. The aqueous filtrate was extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The resulting yellow residue was combined with the dried filtrate and the combined material recrystallized from $CHCl_3$/hexanes to yield **37** as light yellow needles (3.48 g, 89%): mp 202−203 °C; IR (KBr) 3450, 3200, 1680, 1510, 1350 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.94 (d, J = 2.4 Hz, 1H, ArH-8), 8.33 (dd, J = 8.3, 2.4, 1H, ArH-6), 7.44 (d, J = 8.3 Hz, 1H, ArH-5), 6.51 (br ex s, 1H, NH), 4.57 (m, 1H, CH_2F), 4.42 (m, 1H, CH_2F), 4.13 (m, 1H, H-3), 3.17 (m, 1H, H-4), 3.11 (m, 1H, H-4); ^{13}C NMR (DMSO-d_6) δ 163.3, 147.5, 146.0, 130.7, 130.6, 127.2, 122.4, 85.1 (d, J = 682 Hz, CH_2F), 50.2 (d, J = 79 Hz, C-3), 29.5 (d, J = 20 Hz, C-4); CIMS m/z (relative intensity) 242 (M + NH_4^+, 25), 225 (MH^+, 100), 191 (25). Anal. ($C_{10}H_9FN_2O_2$) C, H, N.

(±)-3-Fluoromethyl-7-nitro-1,2,3,4-tetrahydroisoquinoline Hydrochloride (19·HCl). Lactam **37** (2.07 g, 12.4 mmol) was reduced to THIQ **19** using the same procedures described previously in the synthesis of THIQ **14** from lactam **32**. The hydrochloride salt of **19** was prepared in anhydrous EtOH using dry $HCl_{(g)}$. The solvent was removed under reduced pressure and the crude hydrochloride salt was recrystallized from EtOH/hexanes to yield **19**·HCl as off-white crystals (2.03 g, 86%): mp 255 °C dec; IR (KBr) 2925, 2750, 1590, 1515, 1450, 1425, 1250, 1000 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.20−10.11 (br ex m, 2H, NH_2^+), 8.26 (d, J = 2.0 Hz, 1H, ArH-8), 8.19 (dd, J = 8.5, 2.0 Hz, 1H, ArH-6), 7.55 (d, J = 8.5 Hz, 1H, ArH-5), 5.02−4.47 (m, 2H, CH_2F), 4.47 (m, 2H, H-1), 3.95−3.80 (m, 1H, H-3), 3.24−3.12 (m, 2H, H-4); ^{13}C NMR (DMSO-d_6) δ 146.9, 140.3, 131.6, 131.2, 123.0, 122.8, 82.9 (d, J = 674 Hz, CH_2F), 52.4 (d, J = 76 Hz, C-3), 44.4, 27.2 (d, J = 24 Hz, C-4); CIMS m/z (relative intensity) 211 (MH^+, 100), 177 (25). Anal. ($C_{10}H_{11}FN_2O_2$·HCl) C, H, N.

(±)-3-Fluoromethyl-7-cyano-1,2,3,4-tetrahydroisoquinoline Hydrochloride (20·HCl). Compound **19**·HCl (1.23 g, 4.99 mmol) in MeOH (80 mL) was hydrogenated over PtO_2 (50 mg) for 2.5 h at 50 psi. The suspension was filtered and evaporated to dryness to yield the amine as a white residue. The residue was dissolved in a solution of concentrated HCl (1.6 mL) and water (2 mL) and the resulting acidic solution cooled in an ice bath. $NaNO_2$ (0.35 g, 5.1 mmol) dissolved in water (2 mL) was added dropwise to the acidic solution to form the diazonium salt. After stirring for 15 min, excess HNO_2 was destroyed by the addition of urea (20 mg). A negative starch−iodide test was obtained at this time. In a separate flask, a solution of NaOH (0.60 g, 15 mmol in 1.5 mL water) and KCN (1.63 g, 25 mmol in 5 mL water) was prepared. Benzene (5 mL) was added to the basic KCN solution and the suspension chilled in an ice bath. A solution of Ni_2SO_4·$6H_2O$ (1.30 g, 5.0 mmol in 2.5 mL water) was added to the basic KCN solution and the color of the 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 allowed to warm to room temperature over a period of 2 h. The mixture was warmed to 50 °C for 1 h, cooled to room temperature, made basic with 1 N NaOH, and filtered through Celite. The Celite bed was rinsed with CH_2Cl_2 (2 × 25 mL). The organic phase was separated and the aqueous filtrate was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic rinses and extracts were washed with basic brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield a dark oil which was purified by flash chromatography (silica gel) with EtOAc/hexanes (5:1) to yield **20** as a pale brown solid (256 mg, 27%): mp 121−122 °C. Dry $HCl_{(g)}$ was used to form the HCl salt in MeOH. The solvent was removed and the crude HCl salt was recrystallized from MeOH/EtOAc to yield **20**·HCl as off-white crystals: mp 251−253 °C; IR (KBr, HCl salt) 2940, 2800−2600, 2490, 2220, 1600, 1580, 1450, 1000 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.21 (br ex s, 2H, NH_2^+), 7.81 (s, 1H, ArH-8), 7.73 (d, J = 7.6 Hz, 1H, ArH-6), 7.47 (d, J = 7.6 Hz, 1H, ArH-5), 5.00−4.69 (m, 2H, CH_2F), 4.37 (s, 2H, H-1), 3.91−3.86 (m, 1H, H-3), 3.16−3.12 (m, 2H, H-4); ^{13}C NMR (DMSO-d_6) δ 138.2, 131.6, 131.5, 131.3, 130.9, 119.4, 110.2, 82.9 (d, J = 674 Hz, CH_2F), 52.5 (d, J = 75 Hz, C-3), 44.2, 27.2 (d, J = 23 Hz, C-4); CIMS m/z (relative intensity) 191 (MH^+, 100), 157 (80), 129 (15). Anal. ($C_{11}H_{11}FN_2$·HCl) C, H, N.

(±)-3-Fluoromethyl-7-bromo-1,2,3,4-tetrahydroisoquinoline Hydrochloride (21·HCl). THIQ **19**·HCl (804 mg, 3.26 mmol) was hydrogenated to the amine using the same procedures used in the synthesis of nitrile **20**. The diazonium salt was prepared by taking the amine residue and dissolving it in ice-cold 48% HBr (1.6 mL) in water (5.4 mL). Sodium nitrite (0.245 g, 3.55 mmol) in water (3 mL) was added dropwise. After 30 min, excess HNO_2 was destroyed by the addition of urea (20 mg). A negative starch−iodide test was obtained at this time. The diazonium salt solution was added to a mixture of CuBr (1.40 g), 48% HBr (3.3 mL), and water (8.1 mL) at 35 °C. The reaction mixture was warmed to 75−80 °C and stirred for 1.5 h. The reaction mixture was allowed to stand overnight and was made basic cautiously with 50% NaOH. The formation of blue copper salts was observed at this time. Ethyl acetate (50 mL) was added and the copper salts were removed by filtration through Celite. The Celite bed was rinsed with EtOAc (3 × 10 mL). The aqueous filtrate was extracted with

EtOAc (3 × 50 mL). The combined organic rinses and extracts were dried over anhydrous Na_2SO_4 and evaporated to yield a dark oil. This crude product was purified by flash chromatography (silica gel) with EtOAc/hexanes (2:1) to yield **21** as a white solid (527 mg, 66%): mp 91–92 °C. The hydrochloride salt of **21** was formed in MeOH using dry $HCl_{(g)}$. The solvent was removed and the crude HCl salt recrystallized from MeOH and water to yield **21**·HCl as white crystals: mp 265–267 °C; IR (KBr, HCl salt) 2940, 2730, 2500, 1590, 1570, 1370, 1060, 910, 825 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.32 (br ex s, 2H, NH_2^+), 7.76 (m, 1H, ArH-8), 7.53 (d, J = 7.8 Hz, 1H, ArH-6), 7.21 (d, J = 7.8, 1H, ArH-5), 4.98–4.68 (m, 2H, CH_2F), 4.31 (s, 2H, H-1), 3.86–3.38 (m, 1H, H-3), 3.03–2.92 (m, 2H, H-4); ^{13}C NMR (DMSO-d_6) δ 132.1, 131.8, 131.6, 131.1, 130.1, 120.2, 83.0 (d, J = 673 Hz, CH_2F), 52.7 (d, J = 76 Hz, C-3), 44.2, 26.5 (d, J = 23 Hz, C-4); CIMS m/z (relative intensity) 246 (MH^+ + 2, 100), 244 (MH^+, 100), 226 (70), 224 (70), 212 (35), 210 (35). Anal. ($C_{10}H_{11}BrFN$·HCl) C, H, N.

(±)-3-Fluoromethyl-7-iodo-3,4-dihydroisoquinolin-1-(2*H*)-one (38). Lactam **37** (934 mg, 4.17 mmol) was hydrogenated and the diazonium salt formed using the same procedures described previously for the hydrogenation and diazotization of THIQ **19** in the synthesis of nitrile **20**. The diazonium salt solution was added in small portions to a vigorously stirred biphasic mixture of CH_2Cl_2 (25 mL), KI (1.40 g, 31 mmol), CuI (57 mg, 0.30 mmol), and water (8 mL). The reaction mixture was stirred overnight at room temperature. The brown suspension was diluted with CH_2Cl_2 (50 mL), the aqueous phase was removed, and the organic phase was washed with 10% $Na_2S_2O_{3(aq)}$ (3 × 40 mL) to yield a yellow CH_2Cl_2 extract. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography with CH_2Cl_2/EtOAc (10:1) to yield **38** as a white solid (1.12 g, 67%): mp 160–161 °C; IR (KBr) 3180–2940, 1665, 1320, 1065, 890, 810, 775 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.42 (d, J = 1.8 Hz, 1H, ArH-8), 7.78 (dd, J = 7.9, 1.8 Hz, 1H, ArH-6), 6.92 (d, J = 7.9 Hz, 1H, ArH-5), 6.78 (s, 1H, NH), 4.56–4.39 (m, 2H, CH_2F), 4.08 (m, 1H, H-3), 3.05–2.87 (m, 2H, H-4); ^{13}C NMR ($CDCl_3$) δ 164.7, 141.7, 137.4, 136.2, 130.3, 129.9, 92.6, 84.3 (d, J = 694 Hz, CH_2F), 50.8 (d, J = 81 Hz, C-3), 29.0 (d, J = 24 Hz, C-4); EIMS m/z (relative intensity) 306 (MH^+, 25), 305 (M^+, 50), 272 (100), 145 (100), 89 (100), 63 (60). Anal. ($C_{10}H_9FINO$) C, H, N.

(±)-3-Fluoromethyl-7-iodo-1,2,3,4-tetrahydroisoquinoline Hydrochloride (22·HCl). THIQ **22**·HCl was synthesized from lactam **38** (130 mg, 0.426 mmol) using the same procedures for the reduction of lactam **32** to form THIQ **14**. The hydrochloride salt was formed in the same manner as **14**·HCl and recrystallized from MeOH to yield **22**·HCl as a white solid (108 mg, 78%): mp 272–273 °C dec; IR (KBr) 3420, 2910, 2800–2600, 2500, 1575, 1450, 1400, 1060, 910 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.36–10.00 (br ex m, 2H, NH_2^+), 7.73 (s, 1H, ArH-8), 7.40 (d, J = 8.1 Hz, 1H, H-6), 6.88 (d, J = 8.1 Hz, 1H, ArH-5), 4.97–4.66 (m, 2H, CH_2F), 4.31–4.29 (m, 2H, H-1), 3.95–3.80 (m, 1H, H-3), 2.97–2.94 (m, 2H, H-4); ^{13}C NMR (DMSO-d_6) δ 136.9, 135.9, 132.1, 131.9, 131.8, 92.9, 83.0 (d, J = 673 Hz, CH_2F), 52.7 (d, J = 76 Hz, C-3), 43.9, 26.5 (d, J = 24 Hz, C-4); CIMS m/z (relative intensity) 292 (MH^+, 100). Anal. ($C_{10}H_{11}FIN$·HCl) C, H, N.

(±)-3-Fluoromethyl-7-trifluoromethyl-3,4-dihydroisoquinolin-1(2*H*)-one (39). To a solution of **38** (436 mg, 1.43 mmol) in DMF (2 mL) were added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.36 mL, 3.1 mmol) and CuI (310 mg, 1.62 mmol). The reaction mixture was stirred under N_2 at 80 °C for 5 h. The reaction mixture was filtered through Celite. The Celite bed was washed thoroughly with CH_2Cl_2 (2 × 25 mL). The filtrate was evaporated under reduced pressure and the residue was partially purified by flash chromatography (silica gel) with CH_2Cl_2/hexanes (5:1) to yield an off-white solid. The compound was further purified by flash column chromatography with EtOAc/hexanes (1:2) to yield **39** as a white solid (103 mg, 29%): mp 169–170 °C; IR (KBr) 3150–2800, 1670, 1600, 1360, 1310, 1250, 1150, 1100, 1050, 920 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.39 (s, 1H, ArH-8), 7.78 (d, J = 7.9 Hz, 1H, ArH-6), 7.42 (d, J = 7.9 Hz, 1H, ArH-5), 6.84 (br ex s, 1H, NH), 4.60–4.10 (dm, J = 51.5 Hz, 2H, CH_2F), 4.14–4.11 (m, 1H, H-3), 3.18–3.01 (m, 2H, H-4); CIMS m/z (relative intensity) 265 (M + NH_4^+, 10), 248 (MH^+, 100), 214 (25); HRMS (FAB) m/z [M + H]$^+$ calcd for $C_{11}H_{10}F_4NO_4$ 248.0698, found 248.0681.

(±)-3-Fluoromethyl-7-trifluoromethyl-1,2,3,4-tetrahydroisoquinoline Hydrobromide (23·HBr). THIQ **23**·HCl was synthesized from lactam **39** (60 mg, 0.243 mmol) using the same procedures described previously for the reduction of lactam **32** to form THIQ **14**. The crude product was purified by flash chromatography (silica gel) with EtOAc as the eluent to yield **23** (23 mg, 41%) as a white solid. The solid was dissolved in MeOH and concentrated HBr was added dropwise. The solvent was removed under vacuum and the hydrobromide salt was recrystallized with MeOH/EtOAc/hexanes to yield **23**·HBr as white crystals: mp 205–206 °C; IR (KBr) 3450, 2900–2600, 2500, 1575, 1560, 1425, 1325, 1300, 1170, 1145, 1095, 1060, 900 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.76 (s, 1H, ArH-8), 7.72 (d, J = 7.6 Hz, 1H, H-6), 6.88 (d, J = 7.6 Hz, 1H, ArH-5), 5.00–4.68 (m, 2H, CH_2F), 4.59 (s, 2H, H-1), 4.09–3.95 (m, 1H, H-3), 3.34–3.22 (m, 2H, H-4); CIMS m/z (relative intensity) 234 (MH^+, 100). Anal. ($C_{11}H_{11}F_4N$·HBr) C, H, N.

(±)-3-Fluoromethyl-7-azido-1,2,3,4-tetrahydroisoquinoline Hydrochloride (24·HCl). THIQ **19**·HCl (357 mg, 1.45 mmol) was dissolved in MeOH (50 mL) and 3 N HCl (3 mL), and PtO_2 (50 mg) was added to the solution. The suspension was hydrogenated at 50 psi for 4 h. The suspension was filtered and the solvent removed under reduced pressure. A solution of concentrated H_2SO_4 (1 mL) and water (14 mL) was added to the resulting residue. The solution was cooled in an ice bath and a solution of $NaNO_2$ (165 mg, 2.39 mmol) in water (5 mL) was added dropwise. After the addition was complete, the reaction was stirred for 30 min, followed by the addition of sodium azide (253 mg, 3.87 mmol) in water (5 mL). After stirring 1 h at room temperature, the reaction mixture was cautiously made basic with 10% KOH. The basic solution was extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were combined, washed with basic brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield a yellow oil, which was purified by flash chromatography (silica gel) eluting with CH_2Cl_2/EtOAc (2:1) to produce a yellow oil. The oil was dissolved in dry MeOH and dry $HCl_{(g)}$ was used to form the hydrochloride salt. The solvent was removed under reduced pressure and the salt was recrystallized from MeOH/EtOAc/hexanes to yield **24**·HCl as white crystals (350 mg, 67%): mp 205 °C; IR (KBr) 3320, 2940, 2720, 2400, 2100, 1610, 1500, 1300, 1000, 800 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.33 (d, J = 8.3 Hz, 1H, ArH-5), 7.06 (d, J = 8.3 Hz, 1H, ArH-6), 7.02 (s, 1H, ArH-8), 4.97–4.64 (m, 2H, CH_2F), 4.48 (d, J = 4.2 Hz, 2H, H-1), 3.95–3.89 (m, 1H, H-3), 3.14–3.10 (m, 2H, H-4); ^{13}C NMR (CD_3OD) δ 139.7, 130.9, 129.6, 127.6, 119.2, 117.0, 82.3 (d, J = 682 Hz, CH_2F), 53.9 (d, J = 75 Hz, C-3), 44.8, 25.9 (d, J = 24 Hz, C-4); CIMS m/z (relative intensity) 207 (MH^+, 100), 181 (40). Anal. ($C_{10}H_{11}FN_4$·HCl) C, H, N.

(±)-3-Fluoromethyl-7-isothiocyanato-1,2,3,4-tetrahydroisoquinoline Hydrochloride (25·HCl). THIQ **19**·HCl (509 mg, 2.07 mmol) was hydrogenated to the amine in a similar manner as described previously in the preparation of nitrile **20**. The crude amine was dissolved in water (10 mL) and added dropwise over 10 min to an ice-cold solution of thiophosgene (0.25 mL, 2.48 mmol) in acetone (30 mL). The solution was stirred overnight at room temperature. The solvent was evaporated under reduced pressure to yield a yellow solid, which was recystallized from MeOH and water to yield pale yellow needles. A second recrystallization was performed from MeOH/Et_2O/EtOAc to yield **25**·HCl as off-white crystals (365 mg, 69%): mp 271–274 °C dec; IR (KBr) 3310, 3000–2500 (broad), 2150, 1600, 1580, 1420, 1000, 876, 821 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.3 (s, 1H, NH_2^+), 9.99 (s, 1H, NH_2^+), 7.45–7.18 (m, 3H, ArH-5,6,8), 4.96–4.65 (m, 2H, CH_2F), 4.30 (s, 2H, H-1), 3.88–3.79 (m, 1H, H-3), 3.03–2.94 (m, 2H, H-4); EIMS m/z (relative intensity) 222 (M^+, 10), 202

(10), 189 (100), 161 (45), 130 (30), 77 (25). Anal. ($C_{11}H_{11}FN_2S \cdot HCl$) C, H, N.

Radiochemical Assay for PNMT Activity. The assay used for this study has been described previously.[48] A normal assay tube 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 mCi/mmol), 25 μL of substrate solution (phenylethanolamine), 25 μL of inhibitor solution, 25 μL of the enzyme preparation, and water to achieve a total volume of 250 μL. The mixture is incubated for 30 min at 37 °C and quenched with 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 $1/V$ 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.[49] Male Sprague–Dawley rats were decapitated, and the cortexes were 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 ice-cold 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, 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 1.

In Vitro Blood–Brain Barrier Permeability Assay. This assay was performed in the transwell device and has been described previously.[51] Bovine brain microvessel endothelial cells were isolated from the gray matter of cerebral cortices according to the protocol described by Borchardt and Audus.[51] Isolated bovine brain microvessel endothelial cells (BBMEC) were grown to confluent monolayers on polycarbonate membranes in the transwell device. The culture medium consisted of minimum essential medium/Eagle's modified, F12 nutrient mix, 10 mM HEPES, 13 mM sodium bicarbonate, pH 7.4, 10% plasma-derived equine serum, 100 μg/mL penicillin G, 100 μg/mL streptomycin, 2.5 μg/mL amphotericin B, and 100 μg/mL heparin. Cells were refed every 3 days until the development of confluent monolayers, which generally occurred 8–10 days after seeding. The BBMEC monolayers were washed three times with Hank's balanced salt solution (HBSS) buffer containing 10 mM HEPES, pH 7.4. The acceptor chamber contained 2.5 mL of HBSS buffer, and the donor chamber was filled with 1.5 mL HBSS buffer containing 100 μM of the compound being assayed. (Note: the concentration of SK&F 64139 used was 70 μM because at higher concentrations the viability of the BBMECs was decreased.) The acceptor chamber was sampled (200 μL) at 10, 20, 30, 40, and 60 min time intervals. After the chamber was sampled, it was replaced with an equal volume of fresh HBSS buffer. Each experiment was performed in triplicate. Each sample was analyzed by HPLC using a method previously developed, and sample concentrations were determined by comparison to an internal standard. SK&F 64139 (**1**) and SK&F 29661 (**4**) were used as positive and negative controls, as it has been shown in autoradiographic studies that SK&F 64139 (**1**) can penetrate the BBB while SK&F 29661 (**4**) cannot.[31] The formula that was used to calculate the permeability through the membrane of these compounds is shown in eq 1. Flux rates (k) were determined by a linear fit of the permeability data and were corrected for paracellular transport or "leakiness" by measuring the flux of [^{14}C]sucrose which can only cross the BBMEC monolayer paracellularly.

Molecular Modeling. All molecular modeling and CoMFA studies were carried out on a Silicon Graphics Indigo[2] workstation running SYBYL 6.4.[38] All compounds in this updated study were aligned according to the rules used in the previous CoMFA studies,[30,34] for PNMT and the α_2-adrenoceptor.

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Supporting Information Available: Listings of all predicted and observed activities with residuals for both PNMT and the α_2-adrenoceptor for the compounds used in the CoMFA studies and the statistical analyses of both models. This material is available free of charge via the Internet at http://pubs.acs.org.

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(37) In our previous CoMFA models for PNMT and the α_2-adrenoceptor developed with 80 compounds,[34] we noted that THIQs containing 7-sulfonyl substituents were poorly predicted at the α_2-adrenoceptor (e.g., **10** and **11** were predicted to have K_i values ca. 200–250-fold lower than found). Similarly, the observed PNMT activity of **11** was found to be underpredicted (36-fold) by the PNMT model.[34] Therefore, the data for **10** and **11** were added to both models in hopes of improving the predictive abilities of the models for compounds containing other 7-sulfonyl and 3-fluoroalkyl substituents. Since **10** and **11** were only available as racemates, the K_i value for the racemate was assigned to the *R*-enantiomer and only that enantiomer was added to the data set. Previous evaluation of the enantiomers of 3-substituted-THIQs[33] has indicated that the K_i value for the *R*-enantiomer is closer to that of the racemate than is the *S*-enantiomer.
(38) (a) *SYBYL,* version 6.4; Tripos Associates, Inc.: St. Louis, MO, 1997. (b) Clog *P* values were calculated using the *SYBYL* 6.4 implementation of the CLOGP algorithm. For further information, see: Chou, J. T.; Jurs, P. C. Computer-Assisted Computation of Partition Coefficients from Molecular Structures Using Fragment Constants. *J. Chem. Inf. Sci.* **1979**, *19*, 172–178.
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