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Application of the Goldilocks Effect to the Design of Potent and Selective Inhibitors of Phenylethanolamine *N*-Methyltransferase: Balancing pK_a and Steric Effects in the Optimization of 3-Methyl-1,2,3,4-tetrahydroisoquinoline Inhibitors by β -Fluorination

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Abstract

3-Methyl-1,2,3,4-tetrahydroisoquinolines (3-methyl-THIQs) are potent inhibitors of phenylethanolamine *N*-methyltransferase (PNMT), but are not selective due to significant affinity for the α_2 -adrenoceptor. Fluorination of the methyl group lowers the pK_a of the THIQ amine from 9.53 (CH₃) to 7.88 (CH₂F), 6.42 (CHF₂), and 4.88 (CF₃). This decrease in pK_a results in a reduction in affinity for the α_2 -adrenoceptor. However, increased fluorination also results in a reduction in PNMT inhibitory potency, apparently due to steric and electrostatic factors. Biochemical evaluation of a series of 3-fluoromethyl-THIQs and 3-trifluoromethyl-THIQs showed that the former were highly potent inhibitors of PNMT, but were often non-selective due to significant affinity for the α_2 -adrenoceptor, while the latter were devoid of α_2 -adrenoceptor affinity, but also lost potency at PNMT. 3-Difluoromethyl-7-substituted-THIQs have the proper balance of both steric and pK_a properties and thus have enhanced selectivity versus the corresponding 3-fluoromethyl-7-substituted-THIQs and enhanced PNMT inhibitory potency versus the corresponding 3-trifluoromethyl-7-substituted-THIQs. Using the “Goldilocks Effect” analogy, the 3-fluoromethyl-THIQs are too potent (too hot) at the α_2 -adrenoceptor and the 3-trifluoromethyl-THIQs are not potent enough (too cold) at PNMT, but the 3-difluoromethyl-THIQs are just right. They are both potent inhibitors of PNMT and highly selective due to low affinity for the α_2 -adrenoceptor. This seems to be the first successful use of the β -fluorination of aliphatic amines to impart selectivity to a pharmacological agent while maintaining potency at the site of interest.

Introduction

Aliphatic amines are one of the most common functional groups in bioactive molecules² and are often crucial in the binding of these compounds by acting as a hydrogen bond donor or acceptor, or through electrostatic interactions when protonated. Fluorine can strongly influence the basicity of aliphatic amines. For example, the incorporation of a monofluoromethyl, difluoromethyl, or trifluoromethyl group at the 3-position of 1,2,3,4-tetrahydroisoquinoline (THIQ, **1**) lowers the pK_a of the amine by approximately 1.5 log units per fluorine (Table 1). The decrease in pK_a associated with the β -fluorination of aliphatic amines has been shown to have a profound effect on their biological properties. Some examples include increased substrate activity for lung *N*-methyltransferase,³ decreased inhibition of mitochondrial

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monoamine oxidase,⁴ increased *N*-dealkylation by CYP1A2⁵ and decreased aromatic hydroxylation by CYP2D6^{6,7} of fluorinated propranolol analogs, increased oral bioavailability,⁸ and changes in tissue distribution.^{4, 9}

Since the steric and pK_a changes caused by fluorination can simultaneously affect multiple biological properties, it is not uncommon to observe that the added benefits of fluorination are accompanied by negative effects. A decrease in pK_a that leads to enhanced oral absorption or improved metabolic properties may also result in a decrease in potency.¹⁰ While there are examples of varying the extent of fluorination to find an optimal pK_a at which pharmacokinetic properties are improved and potency is maintained,⁸ this approach has not been used to impart selectivity to a pharmacological agent while maintaining potency at the site of interest.^{7,11}

As one means of determining the function of epinephrine (**3**) in the CNS, our laboratory has targeted phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28). This enzyme catalyzes the terminal step in the biosynthesis of epinephrine (**3**), in which an activated methyl group is transferred from *S*-adenosyl-*L*-methionine (AdoMet; **4**) to the primary amine of norepinephrine (**2**) to form epinephrine (**3**) and the cofactor product *S*-adenosyl-*L*-homocysteine (AdoHcy; **5**).

The role of epinephrine (**3**) as a hormone in the periphery is well established. An acute stress response triggers the release of epinephrine (**3**) into the blood stream, which initiates the physiological changes associated with the “fight or flight syndrome”. In contrast to the vast understanding of the functions of epinephrine (**3**) in the periphery, its role in the CNS is much less clear. Significant levels of epinephrine (**3**) are present in the mammalian CNS,¹²⁻¹⁴ where it is postulated to act as a neurotransmitter. The most thoroughly studied physiological process to which epinephrine (**3**) in the CNS (central epinephrine) has been linked is the regulation of peripheral blood pressure. Administration of the PNMT inhibitor SK&F 64139¹⁵ (**6**; Table 2) to hypertensive rats resulted in a reduction in peripheral blood pressure.¹⁶ However, **6** also showed significant affinity for the α_2 -adrenoceptor.¹⁷ Thus, some of the decrease in peripheral blood pressure may be attributed to an α_2 -adrenergic effect rather than to decreases in central epinephrine concentrations.¹⁸⁻²⁰ Selective inhibitors of PNMT would be useful pharmacological tools for clearly defining the connection between central epinephrine concentrations and peripheral blood pressure.

THIQs having electron-withdrawing 7-substituents (either lipophilic or hydrophilic) are highly potent inhibitors of PNMT,²¹ and those with lipophilic electron-withdrawing 7-substituents, such as halides (**7** and **8**) are generally more potent than those having hydrophilic groups [**9** and SK&F 29661²² (**10**)]. Unfortunately, THIQs bearing lipophilic electron-withdrawing 7-substituents also have significant affinity for the α_2 -adrenoceptor. Addition of a methyl (**11d**) or a fluoromethyl (**11a**) group to the 3-position of **7** provides a slight increase in PNMT inhibitory potency while decreasing affinity for the α_2 -adrenoceptor. While compounds **11d** and **11a** still have significant affinity for the α_2 -adrenoceptor, the 3-trifluoromethyl analog (**11c**) shows a dramatic reduction in α_2 -adrenoceptor affinity. Previous studies suggest that this is due to the large drop in pK_a of the THIQ amine ($pK_a \approx 5$) making it mostly neutral at physiological pH. Unfortunately, **11c** is not particularly potent at PNMT, showing a greater than 100-fold loss of potency versus **11d** or **11a**. This has been attributed to the increased steric bulk of the trifluoromethyl group.^{23,24}

Analysis of the crystal structure of human PNMT (hPNMT) co-crystallized with inhibitors²⁵⁻²⁷ and substrates,²⁸ coupled with site directed mutagenesis studies,²⁷ has helped to identify the key amino acids at the hPNMT active site, some of which are shown in Figure 2. The aromatic rings of substrates or THIQ-type inhibitors are positioned between the side chains of Phe182 and Asn39. The β -hydroxyl of phenylethanolamine substrates or the amine

of THIQ inhibitors interacts with Glu219 and Asp267. The substrate β -hydroxyl group interacts through hydrogen bonds, but it is currently unknown whether the THIQ amine interacts through hydrogen bonds in its neutral form or through an electrostatic interaction in its protonated form. We have co-crystallized hPNMT with inhibitors containing either a lipophilic (**8**)²⁶ or hydrophilic (**10**)²⁵ 7-substituent. Lipophilic 7-substituents interact with the side chains of Val53 and Arg44. Hydrophilic 7-substituents interact with Lys57 through hydrogen bonds.²⁵ Examination of the hPNMT active site region surrounding the 3-position of co-crystallized THIQ inhibitors revealed the presence of a narrow lipophilic channel (Figure 2), which was consistent with THIQs having small 3-substituents (e.g., methyl, ethyl, or fluoromethyl) being more potent at PNMT than those having larger 3-substituents (e.g., isopropyl, butyl, pentyl, or trifluoromethyl). Docking studies with 3-trifluoromethyl-THIQs showed that the trifluoromethyl group did not fit into the lipophilic channel due to negative steric interactions.²⁴

We hypothesized that THIQs having a 3-substituent that (1) sufficiently lowers the pK_a of the THIQ amine to diminish affinity for the α_2 -adrenoceptor, and (2) is small enough to maintain potency at PNMT would be an ideal inhibitor. We reasoned that 3-difluoromethyl-THIQs could meet these criteria because (1) the pK_a of the THIQ amine is reduced below 6.5, and (2) docking studies indicated that the 3-difluoromethyl group would be able to fit into the lipophilic channel. Application of the “Goldilocks Effect”²⁹ suggested that while the 3-fluoromethyl-THIQs were too potent (too hot) at the α_2 -adrenoceptor and the 3-trifluoromethyl-THIQs were not potent enough (too cold) at PNMT, the 3-difluoromethyl-7-substituted-THIQs (**11b–25b**) would be just right—both highly potent at PNMT and highly selective due to low affinity for the α_2 -adrenoceptor.

Chemistry

The synthesis of 3-difluoromethyl-7-substituted-THIQs **11b–25b** and 7-(*N*-substituted aminosulfonyl)-3-trifluoromethyl-THIQs **14c**, **17c**, **23c**, and **25c** are shown in Schemes 1–4. While the synthesis of phenylpropylamine **30** has been reported previously (7 steps),³⁰ a more direct route is reported here. Difluoroacetamide (**28**) was reacted in a P_2O_5 melt to form difluoroacetonitrile (**29**; Scheme 1).³¹ This highly volatile liquid was immediately reacted with benzylmagnesium chloride to form the iminium salt intermediate, which was then reduced in situ with $NaBH_4$ to yield **30**. Phenylpropylamine **30** was treated with methyl chloroformate in CH_2Cl_2 and pyridine to afford carbamate **31**. Cyclization of **31** with polyphosphoric acid yielded lactam **32**, the key intermediate in the synthesis of all of the 3-difluoromethyl-THIQs listed in Table 3. Reduction of **32** with diborane formed 3-difluoromethyl-THIQ (**19b**). Nitration of lactam **32** with sulfuric acid and potassium nitrate afforded the desired regioisomer, **33**. Reduction of **33** with diborane yielded **15b**, which was reduced to the aniline and converted to **11b** via a Sandmeyer bromination reaction and to **18b** by Greis cyanation.³²

Lactam **33** was reduced to the aniline and then converted to lactam **34** via a Sandmeyer iodination reaction (Scheme 2).³³ Treatment of **34** with $FSO_2CF_2CO_2CH_3$ and CuI in DMF yielded **35**.³⁴ Compounds **34** and **35** were reduced with diborane to yield THIQs **13b** and **12b**, respectively.

The sulfonyl chloride group was introduced exclusively to the 7-position by reacting lactam **32** with chlorosulfonic acid (Scheme 3). Compound **36b** was then converted to the sulfonyl hydrazid, and reacted in situ with methyl iodide and sodium acetate in ethanol to form methyl sulfone **37**.³⁵ which was subsequently reduced with diborane to afford THIQ **20b**. Sulfonyl chloride **36b** was also treated with NH_4OH in acetonitrile to produce aminosulfonyl **38**, which was reduced with diborane to yield THIQ **16b**.

Sulfonyl chloride **36b** or **36c** (which was prepared by previously described methods)²³ was treated with the requisite amine in either a solution of CHCl_3 and pyridine for the synthesis of sulfonamides **39b**, **40b**, **45b**, **39c**, **40c**, and **45c** or a biphasic mixture of EtOAc and saturated sodium carbonate for the synthesis of sulfonamides **41b–44b** and **43c** (Scheme 4). These lactams were reduced with diborane to afford the corresponding 3-difluoromethyl-THIQs **14b**, **17b**, and **21b–25b** and 3-trifluoromethyl-THIQs **14c**, **17c**, **23c**, and **25c**.

Biochemistry

In the current study, human PNMT (hPNMT) with a C-terminal hexahistidine tag was expressed in *E. coli*.^{36, 37} The radiochemical assay conditions, previously reported for the bovine enzyme,³⁸ were modified to account for the high binding affinity of some inhibitors.^{36,39} Inhibition constants were determined using four concentrations of phenylethanolamine as the variable substrate, and three concentrations of inhibitor.

α_2 -Adrenergic receptor binding assays were performed using cortex obtained from male Sprague Dawley rats.⁴⁰ [³H]Clonidine was used as the radioligand to define the specific binding and phentolamine was used to define the nonspecific binding. Clonidine was used as the ligand to define α_2 -adrenergic binding affinity to simplify the comparison with previous results.

Results and Discussion

The biochemical data for the 3-difluoromethyl- (**11b–25b**) and the 3-trifluoromethyl-7-substituted-THIQs (**14c**, **17c**, **23c**, **25c**) are shown in Table 3, along with previously reported data for selected 3-mono- and 3-trifluoromethyl-THIQs. The pK_a of the 3-difluoromethyl-THIQs ($pK_a \approx 5.5–6.5$)⁴¹ is lowered to a level at which affinity for the α_2 -adrenoceptor is drastically reduced. 7-Bromo-3-difluoromethyl-THIQ (**11b**) has 1000-fold less affinity for the α_2 -adrenoceptor than 7-bromo-THIQ (**7**, Table 2) and 35-fold less affinity than 7-bromo-3-fluoromethyl-THIQ (**11a**). Although the α_2 -adrenoceptor affinity of 7-bromo-3-difluoromethyl-THIQ (**11b**) is quite low, it does have greater, α_2 -adrenoceptor affinity than 7-bromo-3-trifluoromethyl-THIQ (**11c**, $pK_a \approx 4.3$)⁴¹ since a higher percentage of the former compound is protonated at physiological pH. This trend is consistent for all of the compounds in Table 3. At the α_2 -adrenoceptor, the rank order of affinity is $\text{CH}_2\text{F} > \text{CHF}_2 > \text{CF}_3$.

At PNMT, previous studies have established that 3-fluoromethyl-7-substituted-THIQs are highly potent inhibitors,^{36, 42–44} while the corresponding 3-trifluoromethyl-7-substituted-THIQs are substantially, less potent.^{23, 24} The 3-difluoromethyl-7-substituted-THIQs were found to have PNMT inhibitory potencies that were quite similar to the corresponding 3-fluoromethyl-7-substituted-THIQs. This is especially evident for the most potent 3-fluoromethyl-7-substituted-THIQs (**11a–17a**), which have hPNMT K_i values of less than 300 nM. For these compounds, the corresponding 3-difluoromethyl-7-substituted-THIQs (**11b–17b**) have K_i values at PNMT ranging from statistically equipotent to 4-fold (average of 2.4-fold) less potent, whereas the corresponding 3-trifluoromethyl-7-substituted-THIQs (**11c–17c**) are between 15 and 140-fold (average of 58-fold) less potent. This data trend is consistent for all of the compounds in Table 3. At PNMT, the rank order of potency is $\text{CH}_2\text{F} \approx \text{CHF}_2 > \text{CF}_3$. The following sections analyze the data trends for the 3-mono-, di-, and trifluoromethyl-THIQs at the α_2 -adrenoceptor and PNMT.

α_2 -adrenoceptor

The 3-difluoromethyl-THIQs and 3-trifluoromethyl-THIQs show a significant reduction in affinity for the α_2 -adrenoceptor relative to the corresponding 3-fluoromethyl-THIQs, indicating that either an increase in the size of the 3-substituent, a decrease in the pK_a of the

THIQ amine, or a combination of these factors is responsible for the reduction in α_2 -adrenoceptor affinity. In order to delineate between steric and pK_a effects, the 7-bromo-THIQs (**11a–c**) and 7-nitro-THIQs (**15a–c**) were compared to 7-bromo- and 7-nitro-THIQs bearing increasingly larger alkyl groups on the 3-position (Table 4). Analysis of these data suggests that the steric bulk of the 3-substituent does not appear to significantly affect the affinity of THIQs for the α_2 -adrenoceptor. Despite considerable differences in the steric bulk of their 3-substituents [according to the Charton,⁴⁵ Taft (E_s),⁴⁶ or Dubois (E_s')⁴⁷ parameters], the 7-alkyl-3-bromo-THIQs (**11d–f**) and the 7-alkyl-3-nitro-THIQs (**15d–f**) have similar affinity for the α_2 -adrenoceptor.⁴⁸ This evidence alone suggests that changes in steric bulk at the 3-position of THIQ are not affecting α_2 -adrenoceptor affinity. This is also supported by comparing the 3-fluoroalkyl-THIQs with the 3-alkyl-THIQs and is best illustrated by comparing the 3-isopropyl-THIQs (**11f** and **15f**) and the 3-difluoromethyl-THIQs (**11b** and **15b**). Isopropyl and difluoromethyl groups are geometrically similar, but the isopropyl group is considered to have steric bulk that is either approximately equal to or greater than that of the difluoromethyl group. If an increase in the steric bulk of the 3-substituent is the determining factor in lowering α_2 -adrenoceptor affinity, the 3-isopropyl analogs would be expected to have α_2 -adrenoceptor affinity that is either approximately equal to or less than that of the 3-difluoromethyl analogs. This is not the case. The 3-isopropyl-THIQs **11f** and **15f** have, respectively, 59 and > 28-fold more affinity for the α_2 -adrenoceptor than the 3-difluoromethyl-THIQs **11b** and **15b**. These data demonstrate that the reduced α_2 -adrenoceptor affinity of the 3-difluoromethyl-THIQs, and by extension the 3-trifluoromethyl-THIQs, is likely a result of their reduced pK_a values. It appears that the most pronounced reduction of α_2 -adrenoceptor affinity occurs somewhere between a pK_a of approximately 8 and 6, the range between the 3-fluoromethyl-THIQs and the 3-difluoromethyl-THIQs.

The α_2 -adrenergic receptor data reported in this paper was obtained from the inhibition of [³H]clonidine binding to rat cortex α_2 -adrenoceptors. We recently evaluated a series of THIQs, which included 3-mono-, di- and trifluoromethyl-THIQs, for the inhibition of [³H]RX821002 binding to cloned human α_{2A} -adrenergic receptors.⁴⁹ The binding data for this series of THIQs showed good correlation ($r^2 = 0.90$) between the inhibition of [³H]clonidine binding to rat cortex α_2 -adrenoceptors and the inhibition of [³H]RX821002 binding to cloned human α_{2A} -adrenergic receptors. This correlation supports the use of structural models of the human α_{2A} -adrenergic receptor to explain the α_2 -adrenoceptor data trends reported in this paper.

α_{2A} -Adrenoceptors belong to the rhodopsin-like class of G-protein coupled receptors. Site directed mutagenesis studies and rhodopsin-based homology models have implicated an aspartate residue present in all G-protein coupled receptors as a key amino acid for ammonium ion binding.^{50,51} Numerous studies suggest that amine ligands for G-protein coupled receptors bind in their protonated form.⁵⁰⁻⁵² Recent studies with the human HT_{1D} receptor demonstrated that difluorination of amine ligands significantly lowers their binding affinity, apparently due to a reduction of protonated ligand at physiological pH.⁸ We expect that this same phenomenon is resulting in the low α_2 -adrenoceptor affinity of the 3-di- and 3-trifluoromethyl-THIQs, which exist primarily in the neutral form at physiological pH. Mutagenesis studies⁵² and rhodopsin-based homology models^{53,54} of the human α_{2A} -adrenoceptor predict that Asp113 provides an anchoring point for ligands containing a positively charged amine group. Structural models of the α_{2A} -adrenoceptor predict that the positively charged amine of phenylethylamines and the positively charged nitrogen in the imidazoline ring of clonidine analogs interact electrostatically with Asp113.⁵² The low affinity of the 3-di- and 3-trifluoromethyl-THIQs compared to 3-fluoromethyl- and 3-methyl-THIQs provides evidence that interaction between the protonated amine of THIQs and Asp113 is required for optimal α_{2A} -adrenergic receptor affinity.

The binding of aliphatic amines that are primarily neutral at physiological pH to cationic ligand binding G-protein coupled receptors has not been thoroughly investigated. The results in this paper coupled with the previous study of fluorinated HT_{1D} receptor ligands⁸ suggest that the fluorination of amine ligands for other G-protein coupled receptors may also result in reduced binding affinity. Since the key aspartate residue is highly conserved in all cationic ligand binding G-protein coupled receptors,⁵⁰ lowering the p*K*_a of amine ligands is likely to have a similar effect. Fluorination of amine ligands having undesired affinity for G-protein coupled receptors could be useful in the design of selective pharmacological agents.

PNMT

The rank order of PNMT inhibitory potency for the 3,7-disubstituted-THIQs in Table 3 is CH₂F ≈ CHF₂ > CF₃. The 3-mono- and difluoromethyl-7-substituted-THIQs have similarly high inhibitory potency while the corresponding 3-trifluoromethyl-7-substituted-THIQs have drastically reduced inhibitory potency, which poses an interesting question. Why does a simple hydrogen to fluorine substitution result in only a slight change in going from the fluoromethyl series to the difluoromethyl series, but has a profound effect when going from the difluoromethyl series to the trifluoromethyl series?

Structure-activity relationship studies on substituted benzylamines have found that electron-withdrawing aromatic substituents enhance PNMT inhibitory potency.⁵⁵ The inverse relationship observed between the biochemical activity and the p*K*_a of a series of benzylamines led to the hypothesis that the non-protonated form of benzylamine might be the active inhibitor species at PNMT.⁵⁵ However, there were two obvious problems with this hypothesis. First, the strong correlation between the σ value of the aromatic substituent and the p*K*_a of the benzylamines suggested that the electron-withdrawing substituents may facilitate the binding of benzylamines to PNMT not by lowering the p*K*_a, but by promoting an interaction between an electron deficient aromatic ring and the PNMT active site. Analysis of the X-ray crystal structure of hPNMT co-crystallized with THIQ inhibitors (which are conformationally constrained benzylamines) indicates that a π stacking interaction between the aromatic ring of THIQs (and by extension benzylamines) and Phe182 may be source of the enhanced potency of inhibitors having electron-withdrawing aromatic substituents. Secondly, even in the most thorough study of substituted benzylamines, the p*K*_a range was narrow and under the assay conditions (pH = 8.0) all of the compounds studied existed primarily as the protonated species.⁵⁵

This study of 3-mono-, di- and trifluoromethyl-THIQs compares compounds having a broad range of p*K*_a values significantly above and below the pH of the assay, and is thus superior for determining the ionization state in which these compounds bind. Compounds that are either protonated under the assay conditions, such as 3-methyl-THIQs **11d** and **15d** (Table 4), or are primarily neutral under the assay conditions, such as 3-difluoromethyl-THIQs **11b** and **15b** are highly potent inhibitors, indicating that THIQ inhibitors can bind to PNMT as either the protonated or neutral species. This is not inconsistent with the X-ray crystal structures of hPNMT that indicates that the THIQ amine could interact with Glu219 and Asp267 by either hydrogen bonding in its neutral form or through electrostatic interactions in its protonated form. The observed decrease in PNMT inhibitory potency of the 3-trifluoromethyl-THIQs does not appear to be due to the fact that they are primarily neutral at physiological pH, since we do not observe a significant drop in the inhibitory potency of the 3-difluoromethyl-THIQs.

It is well established that an increase in steric bulk at the 3-position of THIQ results in a decrease in PNMT inhibitory potency,⁴⁸ as illustrated by the biochemical results from a study of 7-bromo- and 7-nitro-THIQs having increasingly larger alkyl groups in the 3-position (Table 4, compounds **11d-f** and **15d-f**). However, when the 3-fluoroalkyl-7-substituted-THIQs (**11a-c** and **15a-c**) are compared to the corresponding 3-alkyl-THIQs (**11d-f** and **15d-f**), a

correlation between the steric bulk of the 3-substituent [according to the Charton,⁴⁵ Taft (E_s),⁴⁶ or Dubois (E_s')⁴⁷ parameters] and PNMT inhibitory potency is not observed (Table 4).

Docking studies suggest that this is because these steric parameters measure size only, without taking the geometry of the group or the electrostatic properties of the 3-substituent into account. Analysis of 7-bromo-3-mono-, di-, and trifluoromethyl-THIQ (**11a–c**) docked into the active site of hPNMT (Figure 3) suggests that the interaction of the 3-substituents of **11a–c** with Tyr222 dictates their relative potencies at hPNMT. The docking of 7-bromo-3-fluoromethyl-THIQ with hPNMT shows that the hydrogens on the fluoromethyl group are positioned in the face of Tyr222 while the fluorine is positioned away (Figure 3B). This result is supported by the co-crystallization of **14a** and **17a** with hPNMT, which show that the fluoromethyl groups of these compounds are positioned in the same manner as predicted by the docking study.⁵⁶ A similar result is observed when 7-bromo-3-difluoromethyl-THIQ (**11b**) is docked into the active site of hPNMT (Figure 3C). The hydrogen of the difluoromethyl group is positioned in the face of Tyr222 while the two fluorines are positioned away.

When 7-bromo-3-trifluoromethyl-THIQ (**11c**) is docked into the active site of hPNMT, a negative steric interaction is observed between one of the fluorines and Tyr222 (Figure 3D), resulting in a translational shift of **11c** in the binding pocket approximately 0.3 Å away from Tyr222,⁵⁷ relative to **11a** and **11b** (Figure 3A). It is also likely that electrostatic repulsion between the partially negatively charged fluorine atom and the π cloud of Tyr222 is contributing to this move away from Tyr222. All of the X-ray crystal structures reported to date indicate that it is not energetically favorable for Tyr222 to change its position in order to avoid this negative interaction as its position in the active site is stabilized by a hydrogen bond with Tyr27 and hydrophobic interactions with Leu229 and Ala224.^{25–28} Docking studies of 3-mono-, di-, and trifluoromethyl-THIQs having various hydrophilic and lipophilic 7-substituents produced similar results to those obtained from the docking studies of the 7-bromo analogs in Figure 3. The 3-trifluoromethyl-THIQs are shifted farther away from Tyr222 than the 3-mono- and difluoromethyl-THIQs. This is most likely preventing the THIQ amine of the 3-trifluoromethyl-THIQs from making optimal interactions with Glu219. It is also possible that this shift in the active site could be preventing optimal π -stacking interactions between the aromatic ring of the 3-trifluoromethyl-THIQs and Phe182. Clarification of this hypothesis awaits co-crystallization of 3-mono-, di-, and trifluoromethyl-THIQs having the same 7-substituent bound to hPNMT.

Conclusions

By varying the extent of fluorination on a series of highly potent, but nonselective 3-methyl-THIQ inhibitors of PNMT, we have dramatically increased their selectivity while maintaining their potency. This increase in selectivity (decrease in α_2 -adrenoceptor affinity) is apparently due to a decrease in the pK_a of the THIQ amine resulting from β -fluorination and seems to be the first successful application of this approach for the design of selective pharmacological agents. Affinity for the α_2 -adrenoceptor is dependent on the extent of fluorination, thus the 3-difluoromethyl- and 3-trifluoromethyl-THIQs have much less affinity for the α_2 -adrenoceptor than the 3-fluoromethyl-THIQs. However, increased fluorination also results in a reduction in PNMT inhibitory potency, apparently due to steric and electrostatic factors. 3-Difluoromethyl-7-substituted-THIQs have the proper balance of both steric and pK_a properties and thus have enhanced selectivity versus the corresponding 3-fluoromethyl-7-substituted-THIQs and enhanced PNMT inhibitory potency versus the corresponding 3-trifluoromethyl-7-substituted-THIQs. The observed superiority of the 3-difluoromethyl-THIQs can be summed up with a simple porridge analogy from the fairytale *Goldilocks and the Three Bears*.²⁹ While the 3-fluoromethyl-THIQs were too potent (too hot) at the α_2 -adrenoceptor and the 3-trifluoromethyl-THIQs were not potent enough (too cold) at PNMT, the 3-difluoromethyl-

THIQs are just right. They are both highly potent at PNMT due to minimal negative steric interaction with Tyr222 and highly selective due to low affinity for the α_2 -adrenoceptor.

Experimental

All reagents and solvents were of 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. Proton ($^1\text{H NMR}$) and carbon ($^{13}\text{C NMR}$) nuclear magnetic resonance spectra were taken on a Bruker DRX-400 or a Bruker AM-500 spectrophotometer. High resolution mass spectra (HRMS) were obtained on a Ribermag R 10-10 mass spectrophotometer. Flash chromatography was performed using silica gel 60 (230–400 mesh) supplied by Universal Adsorbents, Atlanta, Georgia.

Anhydrous methanol and ethanol were used unless stated otherwise, and were prepared by distillation over magnesium. Other solvents were routinely distilled prior to use. Anhydrous tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium-benzophenone ketyl. 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 or argon flow, and all glassware was either oven-dried or flame-dried before use. [*methyl*- ^3H]AdoMet and [^3H]clonidine were obtained from PerkinElmer (Boston, MA).

Radiochemical Assay of PNMT Inhibitors

A typical assay mixture consisted of 25 μL of 0.5 M phosphate buffer (pH 8.0), 25 μL of 50 μM unlabeled AdoMet, 5 μL of [*methyl*- ^3H]AdoMet, containing approximately 3×10^5 dpm (specific activity approximately 15 Ci/mmol), 25 μL of substrate solution (phenylethanolamine), 25 μL of inhibitor solution, 25 μL of enzyme preparation (containing 30 ng hPNMT and 25 μg of bovine serum albumin), and sufficient water to achieve a final volume of 250 μL . After incubation for 30 min at 37 °C, the reaction mixture was quenched by addition of 250 μL of 0.5 M borate buffer (pH 10.0) and was extracted with 2 mL of toluene/isoamyl alcohol (7:3). A 1 mL portion of the organic layer was removed, transferred to a scintillation vial and diluted with cocktail for counting. The mode of inhibition was ascertained to be competitive in all cases reported in Tables 2-4 by examination of the correlation coefficients (r^2) for the fit routines as calculated in the Enzyme Kinetics module (version 1.1) in SigmaPlot (version 7.0).³² While all K_i values reported were calculated using competitive kinetics, it should be noted that there was not always a great difference between the r^2 values for the competitive model versus the non-competitive model. All assays were run in duplicate with 3 inhibitor concentrations over a 5-fold range. K_i values were determined by a hyperbolic fit of the data using the Single Substrate – Single Inhibitor routine in the Enzyme Kinetics module (version 1.1) in SigmaPlot (version 7.0). For inhibitors with apparent IC_{50} values less than 0.1 μM (as determined by a preliminary screen of the compounds to be assayed), the Enzyme Kinetics Tight Binding Inhibition routine was used to calculate the K_i values.

α_2 -adrenoceptor Radioligand Binding Assay

The radioligand receptor binding assay was performed according to the method of U'Prichard et al.²⁹ Male Sprague-Dawley rats were decapitated, and the cortexes were dissected out and homogenized in 20 volumes (w/v) of ice-cold 50 mM Tris/HCl buffer (pH 7.7 at 25 °C). Homogenates were centrifuged thrice for 10 min at $50,000 \times g$ 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 [^3H]clonidine (specific activity approximately 55 Ci/mmol, final concentration 2.0 nM), various concentrations of drugs and an aliquot of freshly resuspended tissue (800 μL) in 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 buffer (pH 7.7 at 25 °C). The filters were counted in vials containing premixed scintillation cocktail. Non-specific binding was defined as the concentration of bound ligand in the presence of 2 μM of phentolamine. All assays were run in quadruplicate with 5 inhibitor concentrations over a 16-fold range. IC₅₀ values were determined by a log-probit analysis of the data and K_i values were determined by the equation $K_i = IC_{50} / (1 + [Clonidine] / K_D)$, as all Hill coefficients were approximately equal to 1.

Molecular Modeling

Connolly surfaces were generated in SYBYL[®] on a Silicon Graphics Octane workstation.⁵⁸ Docking of the various inhibitors into the PNMT active site was performed using AutoDock 3.0.⁵⁹ The default settings for AutoDock 3.0 were used. The compound to be docked was initially overlaid with the cocrystallized ligand SK&F 64139 (**6**) and minimized with the Tripos force field. The docking of inhibitors into the hPNMT active site was performed on the *R*-enantiomer as a previous study on 3-substituted-THIQs indicated that the *R*-enantiomer is preferred over the *S*-enantiomer in the hPNMT active site.⁶⁰ Figure 3 was drawn with Molscrip⁶¹ and Raster3D.⁶²

(±)-Methyl *N*-(1-difluoro-3-phenyl-prop-2-yl) carbamate (**31**)

Difluoroacetonitrile (**29**) was prepared according to a procedure reported by Swarts.³¹ Difluoroacetamide (**28**, 2.30g, 24.2 mmol) and P₂O₅ (5.00 g, 17.6 mmol) were stirred to a homogeneous mixture in a 50 mL RB flask equipped with a short path distillation head. Et₂O (5 mL) was placed in the collection flask, which was cooled to -78 °C. The product (**29**) boils at 22 °C, so a tygon tube was fitted to the end of the distillation head and connected to a pipet to bubble the product directly into the Et₂O in the collection bulb. This solution of difluoroacetonitrile (**29**) in Et₂O was diluted with additional Et₂O (15 mL) and warmed to -40 °C. A solution of 1M benzylmagnesium chloride in Et₂O (27.0 mL, 27.0 mmol) was added dropwise over 30 min. The solution was stirred for 30 min at -40 °C and then allowed to warm to -20 °C and stirring was continued for 1.5 h. A grey precipitate (iminium salt) formed and the reaction mixture was transferred to a solution of NaBH₄ (1.51 g, 40.0 mmol) in MeOH (100 mL) and H₂O (1 mL) at -20 °C. The reaction mixture was stirred at -20 °C for 1 h and at 0 °C for an additional 1 h. 3N HCl (10 mL) was slowly added to the reaction mixture, which was then concentrated to approximately 20 mL. 3N HCl (30 mL) was added and the acidic aqueous mixture was washed with Et₂O (2 × 100 mL). The aqueous solution was made basic with 4N NaOH and extracted with Et₂O (4 × 75 mL). The combined organic extracts from the basic aqueous solution were washed with brine and dried over anhydrous K₂CO₃. The solvent was removed under reduced pressure to yield crude 1,1-difluoro-3-phenyl-2-aminopropane (**30**) as a dark oily residue (1.12 g, 6.54 mmol): ¹H NMR (400 MHz, CDCl₃) δ 7.4–7.28 (m, 5H), 5.71–5.41 (m, 1H), 3.35–3.15 (m, 1H), 3.00–2.59 (m, 2H). This crude product was dissolved in dry CH₂Cl₂ (25 mL) and pyridine (5 mL) and the solution was cooled to 0 °C. Methyl chloroformate (0.61 mL, 7.9 mmol) was added dropwise and the solution was stirred overnight at ambient temperature. Thin layer chromatography in hexanes/acetone (1:1), with visualization by ninhydrin staining, showed the presence of starting material so an additional aliquot of methyl chloroformate (0.40 mL, 5.2 mmol) was added and the reaction was stirred for 4 h at ambient temperature. Ice-cold water (25 mL) was added and the mixture was stirred for 30 min. The mixture was washed with 3N HCl (3 × 30 mL) and brine (30 mL). The organic phase was dried over anhydrous Na₂SO₄ and the solvent was removed under reduced pressure to yield a yellow oil. The oil was purified by flash chromatography eluting with hexanes/EtOAc (6:1) followed by recrystallization from EtOAc/hexanes to yield **31** as white crystals (1.26 g, 5.50 mmol, 23% from **28**): mp 83–85 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.36–7.23 (m, 5H), 5.83 (t, *J* = 55.7 Hz, 1H), 4.84 (b, 1H), 4.26 (b, 1H), 3.65 (s, 3H), 3.05–2.82 (m, 2H); ¹³C NMR

(400 MHz, CDCl₃) δ 156.9, 136.2, 129.5, 129.2, 127.5, 114.9 (t, *J* = 245 Hz), 54.2 (t, *J* = 45 Hz), 52.9, 34.4; HRMS (FAB⁺) *m/z* calcd for C₁₁H₁₄F₂NO₃ (MH⁺) 230.0993, obsd 230.0991.

(±)-3-Difluoromethyl-3,4-dihydroisoquinolin-1-(2*H*)-one (**32**)

Carbamate **31** (1.40 g, 6.11 mmol) was dissolved in polyphosphoric acid (PPA, 25 g) and heated to 120 °C. After stirring for 2 h, the reaction was poured onto ice water (100 mL) and stirred vigorously for 5 min. This aqueous mixture was extracted with EtOAc (4 × 50 mL). The combined organic extracts were washed with water (50 mL) and brine (50 mL) and dried over anhydrous Na₂SO₄. The solvent was removed to yield an off-white solid, which was purified by flash chromatography eluting with EtOAc/hexanes (1:1) followed by recrystallization from hexanes to yield **32** as white crystals (840 mg, 4.26 mmol, 70%): mp 98–100 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.10 (d, *J* = 7.6 Hz, 1H), 7.53–7.49 (m, 1H), 7.42–7.38 (m, 1H), 7.26 (d, *J* = 7.5 Hz, 1H), 6.91 (b, 1H), 5.88–5.59 (m, 1H), 3.96 (b, 1H), 3.31–3.06 (m, 2H); ¹³C NMR (400 MHz, CDCl₃) δ 166.2, 135.9, 133.3, 128.5, 128.4, 128.1, 128.0, 115.3 (t, *J* = 245 Hz), 53.1 (t, *J* = 25 Hz), 27.5 (t, *J* = 2.4 Hz); HRMS (FAB⁺) *m/z* calcd for C₁₀H₁₀F₂NO (MH⁺) 198.0730, obsd 198.0759.

General Procedure for Lactam Reduction. Synthesis of **11b–25b**, **14c**, **17c**, **23c**, and **25c** (Selected procedure for **19b**)

Lactam **32** (130 mg, 0.66 mmol) was dissolved in THF (10 mL) and 1M BH₃·THF (4.4 mL, 4.4 mmol) was added. The solution was heated to reflux for 4 h, cooled to ambient temperature, and MeOH (15 mL) was added dropwise. The solvent was removed under reduced pressure and to the remaining residue a solution of MeOH (15 mL) and 6N HCl (15 mL) was added. The mixture was heated to reflux for 3 h and the MeOH was removed under reduced pressure. Water (25 mL) was added to the mixture, which was then made basic (pH ≈ 10) with 10% NaOH. The basic solution was extracted with CH₂Cl₂ (4 × 30 mL) and the combined organic extracts were dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure to yield the free amine, which often required purification by flash chromatography eluting with EtOAc/hexanes. The free amine was dissolved in CH₂Cl₂ or Et₂O and dry HCl_(g) or HBr_(g) was bubbled through the solution to form the hydrochloride or hydrobromide salt, which was recrystallized from MeOH/CH₂Cl₂, EtOH/Et₂O or EtOH/hexanes.

(±)-3-Difluoromethyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (**19b-HBr**)

The hydrobromide salt was recrystallized from EtOH/hexanes to yield **19b-HBr** as white crystals (122 mg, 0.46 mmol, 70%). mp 242–244 °C; ¹H NMR (500 MHz, CD₃OD) δ 7.37–7.29 (m, 4H), 6.51–6.29 (m, 1H), 4.59–4.49 (m, 1H), 4.22–4.11 (m, 2H), 3.34–3.18 (m, 2H); ¹³C NMR (500 MHz, CD₃OD) δ 129.4, 128.8, 128.1, 127.2, 127.1, 126.2, 113.6 (t, *J* = 244 Hz), 54.4 (t, *J* = 22 Hz), 44.9, 24.5 (t, *J* = 4.0 Hz); HRMS (FAB⁺) *m/z* calcd for C₁₀H₁₂F₂N (MH⁺) 184.0938, obsd 184.0931. Anal. (C₁₀H₁₂BrF₂N) C, H, N.

(±)-3-Difluoromethyl-7-nitro-3,4-dihydroisoquinolin-1-(2*H*)-one (**33**)

Lactam **32** (560 mg, 2.84 mmol) was dissolved in concentrated H₂SO₄ (8 mL) and cooled to 0 °C. Potassium nitrate (314 mg, 3.12 mmol) was added in small portions to the solution, which was stirred overnight at ambient temperature. The mixture was poured slowly onto ice (50 g) and extracted with CH₂Cl₂ (4 × 40 mL). The combined organic extracts were washed with brine and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography eluting with hexanes/EtOAc (3:1). Recrystallization from CHCl₃/hexanes yielded **33** as light yellow crystals (520 mg, 2.15 mmol, 76%). mp 199–201 °C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.70 (s, 1H), 8.45 (b, 1H), 8.25–8.23 (m, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 5.92–5.69 (m, 1H), 3.96–3.88 (m, 1H), 3.34–3.15 (m, 2H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 162.8, 147.2, 143.2, 130.0, 129.1, 126.5, 122.5, 115.0

(t, $J = 246$ Hz), 51.5 (t, $J = 24$ Hz), 26.2; HRMS (FAB⁺) m/z calcd for C₁₀H₉F₂N₂O₃ (MH⁺) 243.0581, obsd 243.0573.

(±)-3-Difluoromethyl-7-nitro-1,2,3,4-tetrahydroisoquinoline hydrochloride (**15b-HCl**)

Compound **33** (490 mg, 2.02 mmol) was reduced to THIQ **15b** according to the general procedure for lactam reduction. The crude amine was purified by flash chromatography eluting with hexanes/EtOAc (1:1). The hydrochloride salt was recrystallized from EtOH/hexanes to yield **15b-HCl** as white crystals (382 mg, 1.44 mmol, 72%): mp 102–104 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.44 (b, 1H), 8.28 (s, 1H), 8.16–8.14 (m, 1H), 7.60 (d, $J = 8.4$ Hz, 1H), 6.70–6.43 (m, 1H), 4.57–4.47 (m, 2H), 4.24–4.18 (m, 1H), 3.36–3.13 (m, 2H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 146.6, 138.8, 131.1, 130.8, 122.6, 122.3, 114.1 (t, $J = 243$ Hz), 52.8 (t, $J = 24$ Hz), 44.4, 24.8; HRMS (FAB⁺) m/z calcd for C₁₀H₁₁F₂N₂O₂ (MH⁺) 229.0788, obsd 229.0781. Anal. (C₁₀H₁₁ClF₂N₂O₂) C, H, N.

(±)-3-Difluoromethyl-7-bromo-1,2,3,4-tetrahydroisoquinoline hydrochloride (**11b-HCl**)

THIQ **15b-HCl** (109 mg, 0.413 mmol) in dry EtOH (20 mL) was hydrogenated over 10% Pd/C (50 mg) for 2.5 h at 50 psi. The suspension was filtered through Celite and washed with EtOH. This solution was evaporated to dryness to yield the crude aniline, which was dissolved in a solution of 48% HBr (1.0 mL) and water (3.0 mL). A solution of sodium nitrite (32.0 mg, 0.464 mmol) and water (1 mL) was added dropwise to the HBr solution. After 30 min, excess HNO₂ was destroyed by the addition of urea (25 mg). The diazonium salt solution was added to a mixture of copper(I) bromide (180 mg, 1.25 mmol), 48% HBr (2.5 mL) and water (5.0 mL). The reaction was warmed to 75–80 °C and was stirred for 1.5 h. The reaction was stirred overnight at ambient temperature and then cautiously made basic with a 50% NaOH. The formation of blue copper salts was observed at this time. Ethyl acetate (50 mL) was added and the resulting solution was filtered through Celite and washed with EtOAc (3 × 20 mL). The organic phase was separated and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine and dried over anhydrous K₂CO₃. The solvent was removed under reduced pressure to yield a dark oil which was purified by flash chromatography eluting with hexanes/EtOAc (1:1). The free amine was dissolved in Et₂O and dry HCl (g) was bubbled through the solution to form the hydrochloride salt, which was recrystallized from MeOH/Et₂O to yield **11b-HCl** as white crystals (86 mg, 0.29 mmol, 69%): mp 240–242 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.35 (b, 1H), 7.56 (s, 1H), 7.50–7.48 (m, 1H), 7.27 (d, $J = 8.3$ Hz, 1H), 6.68–6.40 (m, 1H), 4.42–4.32 (m, 2H), 4.17–4.09 (m, 1H), 3.14–2.96 (m, 2H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 131.6, 131.4, 130.8, 130.1, 129.6, 120.0, 114.2 (t, $J = 243$ Hz), 53.0 (t, $J = 22$ Hz), 44.1, 24.1; HRMS (FAB⁺) m/z calcd for C₁₀H₁₁BrF₂N (MH⁺) 262.0043, obsd 262.0035. Anal. (C₁₀H₁₁BrClF₂N) C, H, N.

(±)-3-Fluoromethyl-7-cyano-1,2,3,4-tetrahydroisoquinoline hydrobromide (**18b-HBr**)

THIQ **15b-HCl** (150 mg, 0.548 mmol) in dry EtOH (20 mL) was hydrogenated over 10% Pd/C (50 mg) for 2.5 h at 50 psi. The suspension was filtered through Celite and washed with EtOH. This solution was evaporated to dryness to yield the crude aniline, which was dissolved in a solution of concentrated HCl (1.6 mL) and water (2 mL) and cooled in an ice bath. A solution of sodium nitrite (41.5 mg, 0.601 mmol) in water (2 mL) was added dropwise. After 30 min, excess HNO₂ was destroyed by the addition of urea (20 mg). In a separate flask, a solution of NaOH (66 mg) in water (1 mL) and KCN (179 mg) water (5 mL) was prepared. Benzene (5 mL) was added to the basic KCN solution and the suspension was cooled to 0 °C. A solution of Ni₂SO₄·6H₂O (143 g, 0.55 mmol) and water (1.5 mL) 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 this yellow-brown solution. Brisk evolution of N₂ was observed and the reaction mixture was allowed to warm to ambient temperature over a period of 2 h.

The mixture was warmed to 50 °C for 1 h, cooled to ambient temperature, made basic with 10% NaOH, and filtered through Celite. The Celite was washed with CH₂Cl₂ (2 × 100 mL). The organic phase was removed and the aqueous filtrate was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic extracts were washed with brine and dried over anhydrous K₂CO₃. Removal of the solvent yielded a dark oil which was purified by flash chromatography eluting with EtOAc/hexane (5:1) to yield **18b** as a pale brown solid. The free amine was dissolved in Et₂O and dry HBr_(g) was bubbled through the solution to form the hydrobromide salt, which was recrystallized from MeOH/Et₂O to yield **18b**·HBr as off-white crystals (31.1 mg, 0.107 mmol, 20%) mp 246–248 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.76 (b, 1H), 7.83 (s, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 6.63–6.36 (m, 1H), 4.50–4.41 (m, 2H), 4.24–4.17 (m, 1H), 3.30–3.03 (m, 2H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 136.6, 131.4, 131.0, 130.6, 130.6, 118.8, 114.3 (t, *J* = 243 Hz), 53.0 (t, *J* = 23 Hz), 44.5, 25.1; HRMS (FAB⁺) *m/z* calcd for C₁₁H₁₁F₂N₂ (MH⁺) 209.0890, obsd 209.0875. Anal. (C₁₁H₁₁BrF₂N₂) C, H, N.

(±)-3-Difluoromethyl-7-iodo-3,4-dihydroisoquinolin-1-(2*H*)-one (**34**)

Lactam **33** (900 mg, 4.56 mmol) in dry MeOH (75 mL) was hydrogenated over 10% Pd/C (120 mg) for 3.5 h at 50 psi. The suspension was filtered through Celite and evaporated to dryness to yield the aniline intermediate as an oily residue. CH₂Cl₂ (50 mL) was added to the oily residue and a white precipitate formed. A solution of concentrated HCl (1.6 mL) and water (2 mL) was added, which dissolved the precipitate. This biphasic mixture was cooled to 0 °C and a solution of sodium nitrite (282 mg, 4.09 mmol) in water (3 mL) was added. After stirring for 15 min, excess HNO₂ was destroyed by the addition of urea (20 mg). The diazonium salt solution was added in small portions to a vigorously stirred biphasic mixture of CH₂Cl₂ (25 mL), K₁ (1.30 g, 29.0 mmol), CuI (50 mg, 0.26 mmol) and water (8 mL). The reaction mixture was stirred overnight at ambient temperature. The resulting brown suspension was diluted with CH₂Cl₂ (75 mL). The aqueous phase was removed and the organic phase washed with 10% Na₂S₂O_{3(aq)} (3 × 40 mL). The organic phase was dried over anhydrous Na₂SO₄ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography eluting with hexanes/EtOAc (3:1) and recrystallized from CHCl₃/hexanes to yield lactam **34** as a white solid (950 mg, 2.94 mmol, 65%) mp 144–146 °C; ¹H NMR (500 MHz, CDCl₃) δ 8.44 (d, *J* = 8.4 Hz, 1H), 7.85–7.83 (m, 1H), 7.03 (d, *J* = 8.0 Hz, 1H), 5.84–5.61 (m, 1H), 4.02–3.91 (m, 1H), 3.25–3.02 (m, 2H); ¹³C NMR (500 MHz, CDCl₃) δ 163.7, 141.6, 137.0, 134.6, 129.5, 129.3, 114.7 (t, *J* = 246 Hz), 92.4, 52.6 (t, *J* = 25 Hz), 26.9 (t, *J* = 2.8 Hz); HRMS (FAB⁺) *m/z* calcd for C₁₀H₉F₂N₂O₃ (MH⁺) 323.9697, obsd 323.9669.

(±)-3-Difluoromethyl-7-iodo-1,2,3,4-tetrahydroisoquinoline hydrochloride (**13b**·HCl)

Compound **34** (490 mg, 2.02 mmol) was reduced to THIQ **13b** according to the general procedure for lactam reduction. The crude amine was purified by flash chromatography eluting with hexanes/EtOAc (3:1). The hydrochloride salt was recrystallized from MeOH/Et₂O to yield **13b**·HCl as white crystals (112 mg, 0.324 mmol, 89%): mp dec 282–284 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.32 (b, 1H), 7.70 (s, 1H), 7.64 (d, *J* = 8.1 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 1H), 6.67–6.39 (m, 1H), 4.39–4.29 (m, 2H), 4.16–4.07 (m, 1H), 3.11–2.95 (m, 2H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 136.5, 135.4, 131.6, 131.4, 130.4, 114.2 (t, *J* = 243 Hz), 92.7, 53.0 (t, *J* = 24 Hz), 43.8, 24.2; HRMS (FAB⁺) *m/z* calcd for C₁₀H₁₁F₂IN (MH⁺) 309.9904, obsd 309.9901. Anal. (C₁₀H₁₁ClF₂IN) C, H, N.

(±)-3-Difluoromethyl-7-trifluoromethyl-3,4-dihydroisoquinolin-1-(2*H*)-one (**35**)

To a solution of **34** (370 mg, 1.15 mmol) in DMF (2 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl) acetate (0.32 mL, 2.5 mmol) and CuI (252 mg, 1.32 mmol). The reaction mixture was stirred at 80 °C for 5 h. The reaction mixture was filtered through Celite, which

was washed with CH₂Cl₂ (2 × 25 mL). The filtrate was evaporated under reduced pressure and the residue was purified by flash chromatography eluting with CH₂Cl₂/Et₂O (3:1) to give a mixture of **34** and **35**. The mixture was separated by flash chromatography eluting with hexanes/EtOAc (3:1) to yield **35** as a white solid (177 mg, 0.67 mmol 58%); ¹H NMR (400 MHz, CDCl₃) δ 8.40 (s, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 6.45 (b, 1H), 5.90–5.61 (m, 1H), 4.08–3.96 (m, 1H), 3.39–3.15 (m, 2H); HRMS (FAB⁺) *m/z* calcd for C₁₁H₉F₅NO (MH⁺) 266.0604, obsd 266.0598.

(±)-3-Difluoromethyl-7-trifluoromethyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (12b·HCl)

Compound **35** (490 mg, 2.02 mmol) was reduced to THIQ **12b** according to the general procedure for lactam reduction. The crude amine was purified by flash chromatography eluting with hexanes/EtOAc (3:1). The hydrochloride salt was recrystallized from MeOH/Et₂O to yield **12b**·HCl as white crystals (125 mg, 0.435 mmol, 82%): mp 248–250 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.38 (b, 1H), 7.75 (s, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 6.69–6.42 (m, 1H), 4.46 (s, 2H), 4.26–4.13 (m, 1H), 3.28–3.10 (m, 2H); ¹³C NMR (400 MHz, DMSO-*d*₆) δ 137.2, 136.2, 130.3, 129.1 (q, *J* = 32.4 Hz), 124.5 (q, *J* = 272 Hz), 123.6 (q, *J* = 3.6 Hz), 123.4 (q, *J* = 3.8 Hz), 117.1 (t, *J* = 243 Hz), 55.3 (t, *J* = 23 Hz), 47.8, 27.7 (t, *J* = 3.7 Hz); HRMS (FAB⁺) *m/z* calcd for C₁₁H₁₁F₅N (MH⁺) 252.0812, obsd 252.0809. Anal. (C₁₁H₁₁ClF₅N) C, H, N.

(±)-7-Chlorosulfonyl-3-difluoromethyl-3,4-dihydroisoquinolin-1-(2H)-one (36b)

Lactam **32** (1.00 g, 5.07 mmol) was treated with chlorosulfonic acid (10 mL) and heated to 50 °C for 16 h. The solution was cooled and carefully added via pipet onto ice (100 mL). A white precipitate formed. The mixture was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic extracts were washed with brine and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure to yield a light brown residue, which was purified by flash chromatography eluting with EtOAc to yield a white solid. Recrystallization from EtOAc/hexanes yielded **36b** as white crystals (1.05 g, 3.55 mmol, 70%) mp 165–167 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.79 (d, *J* = 2.1 Hz, 1H), 8.18–8.16 (m, 1H), 7.54 (d, *J* = 8.1 Hz, 1H), 6.45 (b, 1H), 5.94–5.65 (m, 1H), 4.15–4.02 (m, 1H), 3.46–3.17 (m, 2H); ¹³C NMR (500 MHz, CDCl₃) δ 164.0, 144.5, 137.8, 129.6, 128.4, 127.7, 125.1, 115.2 (t, *J* = 245 Hz), 51.6 (t, *J* = 24 Hz), 26.2; HRMS (FAB⁺) *m/z* calcd for C₁₀H₉ClF₂NO₃S (MH⁺) 295.9960, obsd 295.9957.

(±)-3-Difluoromethyl-7-methylsulfonyl-3,4-dihydroisoquinolin-1-(2H)-one (37)

Sulfonyl chloride **36b** (228 mg, 0.77 mmol) was dissolved in THF (10 mL) and cooled to 0 °C. Hydrazine (0.072 mL, 2.32 mmol) was added dropwise to the solution, which was stirred overnight at ambient temperature. The solution was cooled to 0 °C and a white precipitate (hydrazidosulfone) was collected by filtration. The precipitate was dissolved in EtOH (5 mL), then NaOAc (0.316 g, 3.85 mmol) and iodomethane (0.240 mL, 3.85 mmol) were added. The mixture was heated at reflux overnight. Water (30 mL) was added and the solution was extracted with EtOAc (3 × 40 mL). The combined organic extracts were washed with brine and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure to yield a white solid, which was recrystallized from EtOAc/hexane to yield **37** as white needles (150 mg, 0.55 mmol, 71%) mp 215–217 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.68 (d, *J* = 2.0 Hz, 1H), 8.12–8.10 (m, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 6.27 (b, 1H), 5.88–5.65 (m, 1H), 4.06–4.01 (m, 1H), 3.42–3.19 (m, 2H), 3.12 (s, 3H); ¹³C NMR (500 MHz, CDCl₃) δ 163.0, 141.0, 140.6, 131.1, 129.2, 128.9, 127.6, 114.5 (t, *J* = 246 Hz), 52.5 (t, *J* = 24 Hz), 44.2, 27.3; HRMS (FAB⁺) *m/z* calcd for C₁₁H₁₁F₂NO₃S (MH⁺) 276.0506, obsd 276.0492.

(±)-3-Difluoromethyl-7-methylsulfonyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (20b·HCl)

Compound **37** (150 mg, 0.55 mmol) was reduced to THIQ **20b** according to the general procedure for lactam reduction. The crude amine was purified by flash chromatography eluting with hexanes/EtOAc (3:2). The hydrochloride salt was recrystallized from EtOH/hexanes to yield **20b·HCl** as white crystals (120 mg, 0.40 mmol, 74%): mp 243–245 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.32 (b, 1H), 7.92 (s, 1H), 7.84 (d, *J* = 8.1 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 1H), 6.69–6.42 (m, 1H), 4.53–4.45 (m, 2H), 4.24–4.16 (m, 1H), 3.30–3.11 (m, 2H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 139.8, 136.8, 130.5, 130.5, 126.2, 125.8, 114.2 (t, *J* = 243 Hz), 52.9 (t, *J* = 23 Hz), 44.5, 43.9, 24.8; HRMS (FAB⁺) *m/z* calcd for C₁₁H₁₄F₂N₂O₂S (MH⁺) 262.0713, obsd 262.0695. Anal. (C₁₁H₁₄ClF₂N₂O₂S) C, H, N.

(±)-7-Aminosulfonyl-3-difluoromethyl-3,4-dihydroisoquinolin-1-(2H)-one (38)

Sulfonyl chloride **36b** (200 mg, 0.68 mmol) was dissolved in acetonitrile (5 mL), concentrated ammonium hydroxide (5 mL) was added, and the reaction was stirred overnight at ambient temperature. The solvent was removed under reduced pressure and the residue was partitioned between water (50 mL) and EtOAc (50 mL). The organic layer was removed and the aqueous layer was extracted with EtOAc (2 × 30 mL). The organic layers were pooled, washed with brine, and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the resulting oil was recrystallized from EtOH/hexanes to yield **38** as white crystals (180 mg, 0.65 mmol, 96%): mp 205–207 °C; ¹H NMR (500 MHz, CD₃OD) δ 8.49 (d, *J* = 1.9 Hz, 1H), 8.04–8.02 (m, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 6.01–5.78 (m, 1H), 4.88 (s, 1H), 4.06–4.00 (m, 1H), 3.46–3.20 (m, 2H); ¹³C NMR (500 MHz, CD₃OD) δ 164.5, 143.0, 140.6, 129.5, 128.6, 128.3, 124.8, 115.3 (t, *J* = 244 Hz), 51.5 (t, *J* = 24 Hz), 25.8 (t, *J* = 3.8 Hz); HRMS (FAB⁺) *m/z* calcd for C₁₀H₁₁F₂N₂O₃S (MH⁺) 277.0458, obsd 277.0456.

(±)-7-Aminosulfonyl-3-difluoromethyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (16b·HCl)

Compound **38** (150 mg, 0.55 mmol) was reduced to THIQ **16b** according to the general procedure for lactam reduction. The crude amine was purified by flash chromatography eluting with hexanes/EtOAc (3:2). The hydrochloride salt was recrystallized from EtOH/hexanes to yield **16b·HCl** as white crystals (120 mg, 0.40 mmol, 74%): mp dec 239–242 °C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.30 (b, 1H), 7.76 (s, 1H), 7.73 (d, *J* = 8.1 Hz, 1H), 7.50 (d, *J* = 8.1 Hz, 1H), 7.43 (b, 2H), 6.67–6.45 (m, 1H), 4.47 (s, 2H), 4.24–4.14 (m, 1H), 3.32–3.09 (m, 2H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 143.1, 134.7, 130.0, 129.8, 125.1, 124.2, 114.2 (t, *J* = 243 Hz), 53.0 (t, *J* = 24 Hz), 44.5, 24.6; HRMS (FAB⁺) *m/z* calcd for C₁₀H₁₃F₂N₂O₂S (MH⁺) 263.0666, obsd 263.0668. Anal. (C₁₀H₁₃ClF₂N₂O₂S) C, H, N.

General Procedure for the Preparation of Compounds 39b, 40b, 45b, 39c, 40c, and 45c

Sulfonyl chloride **36b** or **36c** (between 0.25 and 1.0 mmol) was dissolved in CHCl₃ (20 mL) and cooled to 0 °C. The requisite amine (3 equivalents) and pyridine (3 equivalents) were dissolved in CHCl₃ (2 mL) and added dropwise to the sulfonyl chloride solution. The reaction was stirred for 12 h at ambient temperature and the solvent was removed under reduced pressure. The resulting residue was dissolved in EtOAc (50 mL) and this solution was washed with 3 N HCl (2 × 30 mL) and brine (30 mL), and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure to yield the crude product, which was purified by flash chromatography eluting with hexanes/EtOAc (if required), followed by recrystallization from EtOAc/hexanes or EtOH/hexanes.

(±)-3-Difluoromethyl-7-(*N*-2,2,2-trifluoroethylaminosulfonyl)-3,4-dihydroisoquinolin-1(2*H*)-one (39b)

According to the above procedure, sulfonyl chloride **36b** (170 mg, 0.58 mmol) was converted to **39b** (140 mg, 0.39 mmol, 68%): mp 208–210 °C; ¹H NMR (500 MHz, CD₃OD) δ 8.43 (d, *J* = 2.0 Hz, 1H), 8.01–7.99 (m, 1H), 7.56 (d, *J* = 8.0 Hz, 1H), 6.02–5.79 (m, 1H), 4.07–4.00 (m, 1H), 3.67 (q, *J* = 9.1 Hz, 2H), 3.46–3.22 (m, 2H); ¹³C NMR (500 MHz, CD₃OD) δ 164.3, 141.4, 140.2, 130.1, 128.9, 128.6, 125.4, 124.0 (q, *J* = 278 Hz), 115.2 (t, *J* = 244 Hz), 51.5 (t, *J* = 24 Hz), 43.5 (q, *J* = 35 Hz), 25.8 (t, *J* = 3.9 Hz); HRMS (FAB⁺) *m/z* calcd for C₁₂H₁₂F₅N₂O₃S (MH⁺) 359.0489, obsd 359.0485.

(±)-7-(*N*-2,2,2-Trifluoroethylaminosulfonyl)-3-trifluoromethyl-3,4-dihydroisoquinolin-1(2*H*)-one (39c)

According to the above procedure, sulfonyl chloride **36c** (100 mg, 0.32 mmol) was converted to **39c** (85 mg, 0.23 mmol, 71%): mp 203–205 °C; ¹H NMR (400 MHz, CD₃OD) δ 8.60 (s, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 4.36 (t, *J* = 8.0 Hz, 1H), 3.64 (q, *J* = 8.0, 2H), 3.57–3.28 (m, 2H); ¹³C NMR (400 MHz, CD₃OD) δ 165.6, 142.2, 142.0, 131.8, 130.0, 129.9, 127.0, 126.8 (q, *J* = 281 Hz), 125.6 (q, *J* = 281 Hz), 55.1 (q, *J* = 33 Hz), 45.1 (q, *J* = 35 Hz), 27.1.

(±)-7-(*N*-4-Chlorophenylaminosulfonyl)-3-difluoromethyl-3,4-dihydroisoquinolin-1(2*H*)-one (40b)

According to the above procedure, sulfonyl chloride **36b** (200 mg, 0.68 mmol) was converted to **40b** (245 mg, 0.63 mmol, 94%): mp 191–193 °C; ¹H NMR (500 MHz, CD₃OD) δ 8.36 (d, *J* = 1.8 Hz, 1H), 7.86–7.84 (m, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.24–7.22 (m, 2H), 7.11–7.09 (m, 2H), 5.98–5.75 (m, 1H), 4.04–3.96 (m, 1H), 3.40–3.16 (m, 2H); ¹³C NMR (500 MHz, CD₃OD) δ 164.2, 141.6, 138.7, 136.1, 130.4, 129.8, 128.8, 128.8, 128.4, 125.7, 122.2, 115.2 (t, *J* = 244 Hz), 51.4 (t, *J* = 24 Hz), 25.8 (t, *J* = 3.8 Hz).

(±)-7-(*N*-4-Chlorophenylaminosulfonyl)-3-trifluoromethyl-3,4-dihydroisoquinolin-1(2*H*)-one (40c)

According to the above procedure, sulfonyl chloride **36c** (300 mg, 0.960 mmol) was converted to **40c** (325 mg, 0.81 mmol, 84%): mp 230–232 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.68 (s, 1H), 8.34 (br s, 1H), 7.83–7.81 (m, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 6.9 Hz, 2H), 7.09 (d, *J* = 6.9 Hz, 2H), 7.03 (br s, 1H), 4.33–4.22 (m, 1H), 3.48–3.17 (m, 2H); ¹³C NMR (400 MHz, CDCl₃) δ 164.4, 140.3, 139.3, 135.4, 132.0, 131.5, 129.8, 128.7, 128.6, 127.6, 124.8 (q, *J* = 282 Hz), 123.7, 52.4 (q, *J* = 32 Hz), 26.8; HRMS (FAB⁺) *m/z* calcd for C₁₆H₁₃ClF₃N₂O₃S (MH⁺) 405.0288, obsd 405.0286.

(±)-3-Difluoromethyl-7-(*N*-4-nitrophenylaminosulfonyl)-3,4-dihydroisoquinolin-1(2*H*)-one (45b)

According to the above procedure, sulfonyl chloride **36b** (175 mg, 0.59 mmol) was converted to **45b** (135 mg, 0.34 mmol, 57%): mp dec 192–194 °C; ¹H NMR (500 MHz, CD₃OD) δ 8.45 (d, *J* = 2.0 Hz, 1H), 8.16–8.13 (m, 2H), 8.03–8.01 (m, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.35–7.32 (m, 2H), 5.98–5.75 (m, 1H), 4.05–3.95 (m, 1H), 3.41–3.16 (m, 2H); ¹³C NMR (500 MHz, CD₃OD) δ 164.1, 143.7, 143.4, 142.1, 138.7, 130.4, 129.0, 128.7, 125.7, 124.7, 118.2, 115.2 (t, *J* = 244 Hz), 51.4, 25.8; HRMS (FAB⁺) *m/z* calcd for C₁₆H₁₄F₂N₃O₅S (MH⁺) 398.0622, obsd 398.0624.

(±)-7-(*N*-4-Nitrophenylaminosulfonyl)-3-trifluoromethyl-3,4,-dihydroisoquinolin-1-(2*H*)-one (45c)

According to the above procedure, sulfonyl chloride **36c** (300 mg, 0.96 mmol) was converted to **45c** (370 mg, 0.89 mmol, 93%): mp 118–120 °C; ¹H NMR (400 MHz, CD₃OD) δ 8.44–7.65 (m, 4H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.32 (m, 2H), 4.36 (m, 1H), 3.60–3.25 (m, 2H); ¹³C NMR (400 MHz, CD₃OD) δ 164.2, 142.5, 141.9, 131.6, 131.0, 129.3, 129.0, 127.0, 126.8 (q, *J* = 281 Hz), 126.1, 125.2, 118.7, 51.0 (q, *J* = 31 Hz), 26.0; HRMS (FAB⁺) *m/z* calcd for C₁₆H₁₂F₃N₃O₅S (MH⁺) 416.0528, obsd 416.0536.

General Procedure for the Preparation of Compounds 41b–44b and 43c

Sulfonyl chloride **36b** or **36c** (between 0.5 and 1.0 mmol) was dissolved in a biphasic mixture of EtOAc (15 mL) and saturated Na₂CO₃ (10 mL). The requisite amine (3 equivalents) was added to the reaction, and the mixture was stirred for 4 h. The organic phase was removed, washed with 3 N HCl (3 × 50 mL) and brine (30 mL), and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure to yield the crude product, which was purified by flash chromatography (if required) eluting with hexanes/EtOAc, followed by recrystallization from EtOAc/hexanes or EtOH/hexanes.

(±)-3-Difluoromethyl-7-(*N*-ethylaminosulfonyl)-3,4-dihydroisoquinolin-1-(2*H*)-one (41b)

According to the above procedure, sulfonyl chloride **36b** (180 mg, 0.61 mmol) was converted to **41b** (160 mg, 0.53 mmol, 86%): mp 199–201 °C; ¹H NMR (500 MHz, CD₃OD) δ 8.42 (d, *J* = 1.9 Hz, 1H), 7.99–7.97 (m, 1H), 7.56 (d, *J* = 8.0 Hz, 1H), 6.02–5.79 (m, 1H), 4.08–3.98 (m, 1H), 3.46–3.21 (m, 2H), 2.93 (q, *J* = 7.3 Hz, 2H), 1.08 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (500 MHz, CD₃OD) δ 164.4, 141.0, 140.0, 130.3, 128.8, 128.5, 125.5, 115.2 (q, *J* = 244 Hz), 51.5 (t, *J* = 24 Hz), 37.5, 25.8 (t, *J* = 3.9 Hz), 13.8; HRMS (FAB⁺) *m/z* calcd for C₁₂H₁₅F₂N₂O₃S (MH⁺) 305.0771, obsd 305.0753.

(±)-3-Difluoromethyl-7-(*N*-propylaminosulfonyl)-3,4-dihydroisoquinolin-1-(2*H*)-one (42b)

According to the above procedure, sulfonyl chloride **36b** (180 mg, 0.61 mmol) was converted to **42b** (155 mg, 0.49 mmol, 80%): mp 197–199 °C; ¹H NMR (500 MHz, CD₃OD) δ 8.42 (d, *J* = 1.9 Hz, 1H), 7.99–7.97 (m, 1H), 7.56 (d, *J* = 8.0 Hz, 1H), 6.02–5.79 (m, 1H), 4.07–3.99 (m, 1H), 3.46–3.21 (m, 2H), 2.84 (m, *J* = 7.0 Hz, 2H), 1.52–1.45 (m, 2H), 0.88 (t, *J* = 7.4 Hz, 3H); ¹³C NMR (500 MHz, CD₃OD) δ 164.5, 140.9, 140.1, 130.2, 128.8, 128.4, 125.5, 115.3 (q, *J* = 244 Hz), 51.5 (t, *J* = 24 Hz), 44.4, 25.8 (t, *J* = 3.8 Hz), 22.5, 9.9; HRMS (FAB⁺) *m/z* calcd for C₁₃H₁₇F₂N₂O₃S (MH⁺) 319.0928, obsd 319.0931.

(±)-3-Difluoromethyl-7-(*N*-3-methoxypropylaminosulfonyl)-3,4-dihydroisoquinolin-1-(2*H*)-one (43b)

According to the above procedure, sulfonyl chloride **36b** (180 mg, 0.61 mmol) was converted to **43b** (185 mg, 0.53 mmol, 87%): mp 143–145 °C; ¹H NMR (500 MHz, CD₃OD) δ 8.42 (d, *J* = 1.8 Hz, 1H), 7.99–7.97 (m, 1H), 7.56 (d, *J* = 8.0 Hz, 1H), 6.02–5.79 (m, 1H), 4.08–4.00 (m, 1H), 3.46–3.21 (m, 2H), 3.39 (t, *J* = 6.1 Hz, 2H), 3.28, (s, 3H), 2.96 (t, *J* = 6.9 Hz, 2H), 1.73–1.68 (m, 2H); ¹³C NMR (500 MHz, CD₃OD) δ 164.4, 141.0, 139.9, 130.3, 128.8, 128.5, 125.5, 115.2 (q, *J* = 244 Hz), 69.2, 57.3, 51.5 (t, *J* = 24 Hz), 46.6, 29.2, 25.8 (t, *J* = 3.8 Hz); HRMS (FAB⁺) *m/z* calcd for C₁₄H₁₉F₂N₂O₄S (MH⁺) 349.1033, obsd 349.1047.

(±)-7-(*N*-3-Methoxypropylaminosulfonyl)-3-trifluoromethyl-3,4,-dihydroisoquinolin-1(2*H*)-one (43c)

According to the above procedure, sulfonyl chloride **36c** (320 mg, 1.02 mmol) was converted to **43c** (345 mg, 0.94 mmol, 92%): mp 146–147 °C; ¹H NMR (400 MHz, CD₃OD) δ 8.49 (s,

1H), 7.99 (d, $J = 8.0$ Hz, 1H), 7.57 (d, $J = 8.0$ Hz, 1H), 4.48–4.37 (m, 1H), 3.63–3.38 (m, 2H), 3.39–3.36 (m, 2H), 3.27 (s, 3H), 2.95 (t, $J = 6.8$ Hz, 2H), 1.73–1.67 (m, 2H); ^{13}C NMR (500 MHz, CD_3OD) δ 164.1, 140.4, 140.1, 130.6, 128.6, 128.4, 125.6, 124.7 (q, $J = 283$ Hz), 69.2, 57.4, 51.0 (q, $J = 31$ Hz), 39.8, 29.3, 25.6; HRMS (FAB⁺) m/z calcd for $\text{C}_{14}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_4\text{S}$ (MH⁺) 367.0939, obsd 367.0943.

(±)-3-Difluoromethyl-7-(*N*-butylaminosulfonyl)-3,4-dihydroisoquinolin-1-(2*H*)-one (44b)

According to the above procedure, sulfonyl chloride **36b** (180 mg, 0.61 mmol) was converted to **44b** (190 mg, 0.57 mmol, 94%): mp 194–196 °C; ^1H NMR (500 MHz, CD_3OD) δ 8.41 (d, $J = 1.9$ Hz, 1H), 7.99–7.97 (m, 1H), 7.56 (d, $J = 8.0$ Hz, 1H), 6.02–5.79 (m, 1H), 4.07–3.99 (m, 1H), 3.46–3.21 (m, 2H), 2.88 (t, $J = 7.0$ Hz, 2H), 1.45–1.41 (m, 2H), 1.34–1.30 (m, 2H), 0.88 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (500 MHz, CD_3OD) δ 164.5, 141.0, 140.0, 130.3, 128.8, 128.4, 125.5, 115.3 (q, $J = 244$ Hz), 51.5 (t, $J = 24$ Hz), 42.3, 31.3, 25.8 (t, $J = 3.8$ Hz), 19.2, 12.4; HRMS (FAB⁺) m/z calcd for $\text{C}_{14}\text{H}_{19}\text{F}_2\text{N}_2\text{O}_3\text{S}$ (MH⁺) 333.1084, obsd 333.1086.

(±)-3-Difluoromethyl-7-(*N*-2,2,2-trifluoroethylaminosulfonyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (14b-HCl)

Compound **39b** (150 mg, 0.55 mmol) was reduced to THIQ **14b** according to the general procedure for lactam reduction. The hydrochloride salt was recrystallized from EtOH/hexanes to yield **14b**·HCl as white crystals (120 mg, 0.40 mmol, 74%): mp 202–204 °C; ^1H NMR (500 MHz, CD_3OD) δ 7.84 (s, 2H), 7.84–7.83 (m, 1H), 7.56 (d, $J = 8.7$ Hz, 1H), 7.43 (b, 2H), 6.53–6.31 (m, 1H), 4.63 (s, 2H), 4.26–4.18 (m, 1H), 3.69 (q $J = 9.2$ Hz, 1H), 3.42–3.23 (m, 2H); ^{13}C NMR (500 MHz, CD_3OD) δ 140.3, 134.8, 129.9, 128.6, 126.0, 124.9, 124.0 (q, $J = 278$ Hz), 113.4 (t, $J = 244$ Hz), 53.9 (t, $J = 23$ Hz), 44.6, 43.5 (q $J = 35$ Hz), 24.6 (t, $J = 4.0$ Hz); HRMS (FAB⁺) m/z calcd for $\text{C}_{12}\text{H}_{14}\text{F}_5\text{N}_2\text{O}_2\text{S}$ (MH⁺) 345.0696, obsd 345.0687. Anal. ($\text{C}_{12}\text{H}_{14}\text{ClF}_5\text{N}_2\text{O}_2\text{S}$) C, H, N.

(±)-7-(*N*-2,2,2-Trifluoroethylaminosulfonyl)-3-trifluoromethyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (14c-HCl)

Compound **39c** (310 mg, 0.83 mmol) was reduced to THIQ **14c** according to the general procedure for lactam reduction. The hydrochloride salt was recrystallized from EtOH/Et₂O to yield **14c**·HCl as white crystals (200 mg, 0.51 mmol, 61%): mp dec 230–232 °C; ^1H NMR (400 MHz, CD_3OD) δ 7.80–7.77 (m, 2H), 7.52 (d, $J = 8.0$ Hz, 1H), 4.72–4.60 (m, 1H), 4.63 (s, 2H), 3.61 (q, $J = 9.0$ Hz, 2H), 3.50–3.32 (m, 2H); ^{13}C NMR (400 MHz, CD_3OD) δ 142.3, 135.6, 131.8, 130.1, 128.0, 126.7, 125.9 (q, $J = 280$ Hz), 125.2 (q, $J = 280$ Hz), 55.3 (q, $J = 33$ Hz), 46.8, 45.4 (q, $J = 35$ Hz), 26.3; HRMS (FAB⁺) m/z calcd for $\text{C}_{12}\text{H}_{13}\text{F}_6\text{N}_2\text{O}_2\text{S}$ (MH⁺) 363.0602, obsd 363.0602; Anal. ($\text{C}_{12}\text{H}_{13}\text{ClF}_6\text{N}_2\text{O}_2\text{S}$) C, H, N.

(±)-7-(*N*-4-Chlorophenylaminosulfonyl)-3-difluoromethyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (17b-HCl)

Compound **40b** (180 mg, 0.47 mmol) was reduced to THIQ **17b** according to the general procedure for lactam reduction. The hydrochloride salt was recrystallized from EtOH/hexanes to yield **17b**·HCl as white crystals (150 mg, 0.37 mmol, 79%): mp dec 250–252 °C; ^1H NMR (500 MHz, CD_3OD) δ 7.76–7.74 (m, 2H), 7.49 (d, $J = 8.6$ Hz, 1H), 7.23–7.21 (m, 2H), 7.12–7.10 (m, 2H), 6.48–6.26 (m, 1H), 4.54 (s, 2H), 4.19–4.09 (m, 1H), 3.36–3.16 (m, 2H); ^{13}C NMR (500 MHz, CD_3OD) δ 138.7, 136.1, 135.1, 129.9, 129.6, 128.8, 126.4, 125.4, 121.9, 113.4 (t, $J = 244$ Hz), 53.9 (t, $J = 22$ Hz), 44.6, 24.6; HRMS (FAB⁺) m/z calcd for $\text{C}_{16}\text{H}_{16}\text{ClF}_2\text{N}_2\text{O}_2\text{S}$ (MH⁺) 373.0589, obsd 373.0574. Anal. ($\text{C}_{16}\text{H}_{16}\text{ClF}_2\text{N}_2\text{O}_2\text{S}$) C, H, N.

(±)-7-(*N*-4-Chlorophenylaminosulfonyl)-3-trifluoromethyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (17c·HCl)

Compound **40c** (325 mg, 0.804 mmol) was reduced to THIQ **17c** according to the general procedure for lactam reduction. The hydrochloride salt was recrystallized from EtOH/Et₂O to yield **17c·HCl** as white crystals (288 mg, 0.67 mmol, 84%): mp 162–165 °C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.78 (s, 1H), 7.60 (d, *J* = 8.2 Hz, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.31 (d, *J* = 8.8 Hz, 2H), 7.15 (d, *J* = 8.8 Hz, 2H), 4.72–4.61 (m, 1H), 4.47–4.39 (m, 2H), 3.49 (b, 3H), 3.34–3.13 (m, 2H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 138.4, 137.0, 135.7, 130.8, 130.4, 129.6, 128.5, 125.8, 125.4, 124.1 (q, *J* = 282 Hz), 121.8, 52.6 (q, *J* = 32 Hz), 44.9, 25.2; HRMS (FAB⁺) *m/z* calcd for C₁₆H₁₅ClF₃N₂O₂S (MH⁺) 391.0495, obsd 391.0468; Anal. (C₁₆H₁₅Cl₂F₃N₂O₂S) C, H, N.

(±)-3-Difluoromethyl-7-(*N*-4-nitrophenylaminosulfonyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (25b·HCl)

Compound **45b** (115 mg, 0.29 mmol) was reduced to THIQ **25b** according to the general procedure for lactam reduction. The hydrochloride salt was recrystallized from EtOH/hexanes to yield **25b·HCl** as white crystals (83 mg, 0.20 mmol, 68%): mp dec 250–252 °C; ¹H NMR (500 MHz, CD₃OD) δ 8.14–8.11 (m, 2H), 7.91 (s, 1H), 7.88–7.86 (m, 1H), 7.53 (d, *J* = 8.2 Hz, 1H), 7.35–7.33 (m, 2H), 6.49–6.28 (m, 1H), 4.60 (s, 2H), 4.22–4.12 (m, 1H), 3.38–3.17 (m, 2H); ¹³C NMR (500 MHz, CD₃OD) δ 143.8, 143.3, 138.6, 135.6, 130.1, 128.9, 126.4, 125.5, 124.7, 118.1, 113.3 (t, *J* = 244 Hz), 53.9 (t, *J* = 23 Hz), 44.5, 24.5; HRMS (FAB⁺) *m/z* calcd for C₁₆H₁₆F₂N₃O₄S (MH⁺) 384.0830, obsd 384.0837. Anal. (C₁₆H₁₆ClF₂N₃O₄S) C, H, N.

(±)-7-(*N*-4-Nitrophenylaminosulfonyl)-3-trifluoromethyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (25c·HCl)

Compound **45c** (350 mg, 0.85 mmol) was reduced to THIQ **25c** according to the general procedure for lactam reduction. The hydrochloride salt was recrystallized from EtOH/Et₂O to yield **25c·HCl** as white crystals (210 mg, 0.48 mmol, 57%): mp 235–236 °C; ¹H NMR (400 MHz, CD₃OD) δ 8.14–8.12 (m, 2H), 7.93–7.88 (m, 2H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.35–7.33 (m, 2H), 4.67–4.61 (m, 3H), 3.50–3.28 (m, 2H); ¹³C NMR (500 MHz, CD₃OD) δ 143.8, 143.4, 138.8, 134.7, 130.2, 128.6, 126.6, 125.6, 124.7, 123.3 (q, *J* = 279 Hz), 118.2, 53.2 (q, *J* = 32 Hz), 45.0, 24.5; HRMS (FAB⁺) *m/z* calcd for C₁₆H₁₅F₃N₃O₄S (MH⁺) 402.0735, obsd 402.0723; Anal. (C₁₆H₁₅ClF₃N₃O₄S·EtOH) C, H, N.

(±)-3-Difluoromethyl-7-(ethylaminosulfonyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (21b·HCl)

Compound **41b** (140 mg, 0.46 mmol) was reduced to THIQ **21b** according to the general procedure for lactam reduction. The hydrochloride salt was recrystallized from EtOH/hexanes to yield **21b·HCl** as white crystals (146 mg, 0.45 mmol, 97%): mp 198–200 °C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.76 (s, 1H), 7.69 (d, *J* = 8.1 Hz, 1H), 7.65 (t, *J* = 5.7 Hz, 1H), 7.53 (d, *J* = 8.1 Hz, 1H), 6.66–6.44 (m, 1H), 4.52–4.45 (m, 2H), 4.25–4.14 (m, 1H), 3.26–3.10 (m, 2H), 2.81–2.76 (m, 2H), 1.00 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 139.6, 135.3, 130.3, 130.2, 125.8, 125.3, 114.2 (t, *J* = 243 Hz), 53.0 (t, *J* = 24 Hz), 44.5, 37.9, 24.7, 15.2; HRMS (FAB⁺) *m/z* calcd for C₁₂H₁₇F₂N₂O₂S (MH⁺) 291.0979, obsd 291.0956. Anal. (C₁₂H₁₇ClF₂N₂O₂S) C, H, N.

(±)-3-Difluoromethyl-7-(propylaminosulfonyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (22b·HCl)

Compound **42b** (140 mg, 0.44 mmol) was reduced to THIQ **22b** according to the general procedure for lactam reduction. The hydrochloride salt was recrystallized from EtOH/hexanes

to yield **22b**·HCl as white crystals (135 mg, 0.40 mmol, 90%): mp 186–188 °C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.75 (s, 1H), 7.70–7.68 (m, 1H), 7.66 (t, *J* = 5.9 Hz, 1H), 7.53 (d, *J* = 8.1 Hz, 1H), 6.65–6.42 (m, 1H), 4.52–4.45 (m, 2H), 4.25–4.13 (m, 1H), 3.27–3.08 (m, 2H), 2.72–2.68 (m, 2H), 1.43–1.36 (m, 2H), 0.81 (t, *J* = 7.4 Hz, 3H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 139.7, 135.2, 130.3, 130.2, 125.8, 125.3, 114.2 (t, *J* = 243 Hz), 53.0 (t, *J* = 24 Hz), 44.7, 44.6, 24.7, 22.8, 11.5; HRMS (FAB⁺) *m/z* calcd for C₁₃H₁₉F₂N₂O₂S (MH⁺) 305.1135, obsd 305.1137. Anal. (C₁₃H₁₉ClF₂N₂O₂S) C, H, N.

(±)-3-Difluoromethyl-7-(*N*-3-methoxypropylaminosulfonyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (**23b**·HCl)

Compound **43b** (160 mg, 0.46 mmol) was reduced to THIQ **23b** according to the general procedure for lactam reduction. The hydrochloride salt was recrystallized from EtOH/hexanes to yield **23b**·HCl as white crystals (150 mg, 0.40 mmol, 88%): mp 160–162 °C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.75 (s, 1H), 7.70–7.67 (m, 2H), 7.53 (d, *J* = 8.2 Hz, 1H), 6.65–6.43 (m, 1H), 4.52–4.45 (m, 2H), 4.25–4.14 (m, 1H), 3.29 (t, *J* = 6.1 Hz, 2H), 3.27–3.09 (m, 2H), 3.17 (s, 3H), 2.80–2.78 (m, 2H), 1.64–1.59 (m, 2H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 139.5, 135.3, 130.3, 130.2, 125.8, 125.3, 114.2 (t, *J* = 243 Hz), 69.3, 58.3, 53.0 (t, *J* = 24 Hz), 44.6, 29.6, 24.7; HRMS (FAB⁺) *m/z* calcd for C₁₄H₂₁F₂N₂O₃S (MH⁺) 335.1241, obsd 335.1245. Anal. (C₁₄H₂₁ClF₂N₂O₃S) C, H, N.

(±)-7-(*N*-3-Methoxypropylaminosulfonyl)-3-trifluoromethyl-1,2,3,4-tetrahydroisoquinoline (**23c**)

Compound **43c** (400 mg, 1.09 mmol) was reduced to THIQ **23c** according to the general procedure for lactam reduction. The free amine was recrystallized from EtOH/hexanes to yield **23c** as white crystals (297 mg, 0.84 mmol, 77%): mp 95–96 °C; ¹H NMR (400 MHz, CD₃OD) δ 7.65 (d, *J* = 8.0 Hz, 1H), 7.61 (s, 1H), 7.40 (d, *J* = 8.0, 1H), 4.15–4.06 (m, 2H), 3.73–3.64 (m, 1H), 3.39 (t, *J* = 6.0 Hz, 2H), 3.28 (s, 3H), 3.14–2.94 (m, 2H), 2.91 (t, *J* = 6.9 Hz, 2H), 1.72–1.66 (m, 2H); ¹³C NMR (500 MHz, CD₃OD) δ 138.4, 137.2, 136.1, 129.6, 125.9 (q, *J* = 279 Hz), 124.5, 124.2, 69.3, 57.3, 54.3 (q, *J* = 29), 46.3, 39.7, 29.3, 26.8; HRMS (FAB⁺) *m/z* calcd for C₁₄H₂₀F₃N₂O₃S (MH⁺) 353.1147, obsd 353.1136; Anal. (C₁₄H₁₉F₃N₂O₃S) C, H, N.

(±)-7-(*N*-Butylaminosulfonyl)-3-difluoromethyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (**24b**·HCl)

Compound **44b** (175 mg, 0.53 mmol) was reduced to THIQ **24b** according to the general procedure for lactam reduction. The hydrochloride salt was recrystallized from EtOH/hexanes to yield **24b**·HCl as white crystals (170 mg, 0.48 mmol, 91%): mp 206–208 °C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.75 (s, 1H), 7.70–7.68 (m, 1H), 7.65 (t, *J* = 5.8 Hz, 1H), 7.53 (d, *J* = 8.2 Hz, 1H), 6.64–6.42 (m, 1H), 4.52–4.45 (m, 2H), 4.25–4.14 (m, 1H), 3.26–3.10 (m, 2H), 2.75–2.71 (m, 2H), 1.40–1.34 (m, 2H), 1.29–1.21 (m, 2H), 0.82 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 139.6, 135.2, 130.3, 130.2, 125.7, 125.3, 114.2 (t, *J* = 243 Hz), 53.0 (t, *J* = 23 Hz), 44.6, 42.6, 31.5, 24.7, 19.6, 13.9; HRMS (FAB⁺) *m/z* calcd for C₁₄H₂₁F₂N₂O₂S (MH⁺) 319.1292, obsd 319.1297. Anal. (C₁₄H₂₁ClF₂N₂O₂S) C, H, N.

Supplementary Material

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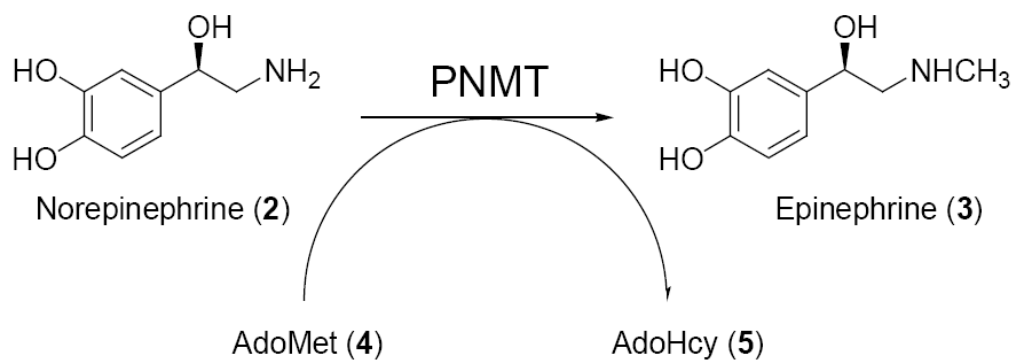


Figure 1.
The terminal step in epinephrine (3) biosynthesis is the transfer of a methyl group from AdoMet (4) to norepinephrine (2) to form epinephrine (3) and the cofactor product AdoHcy (5).

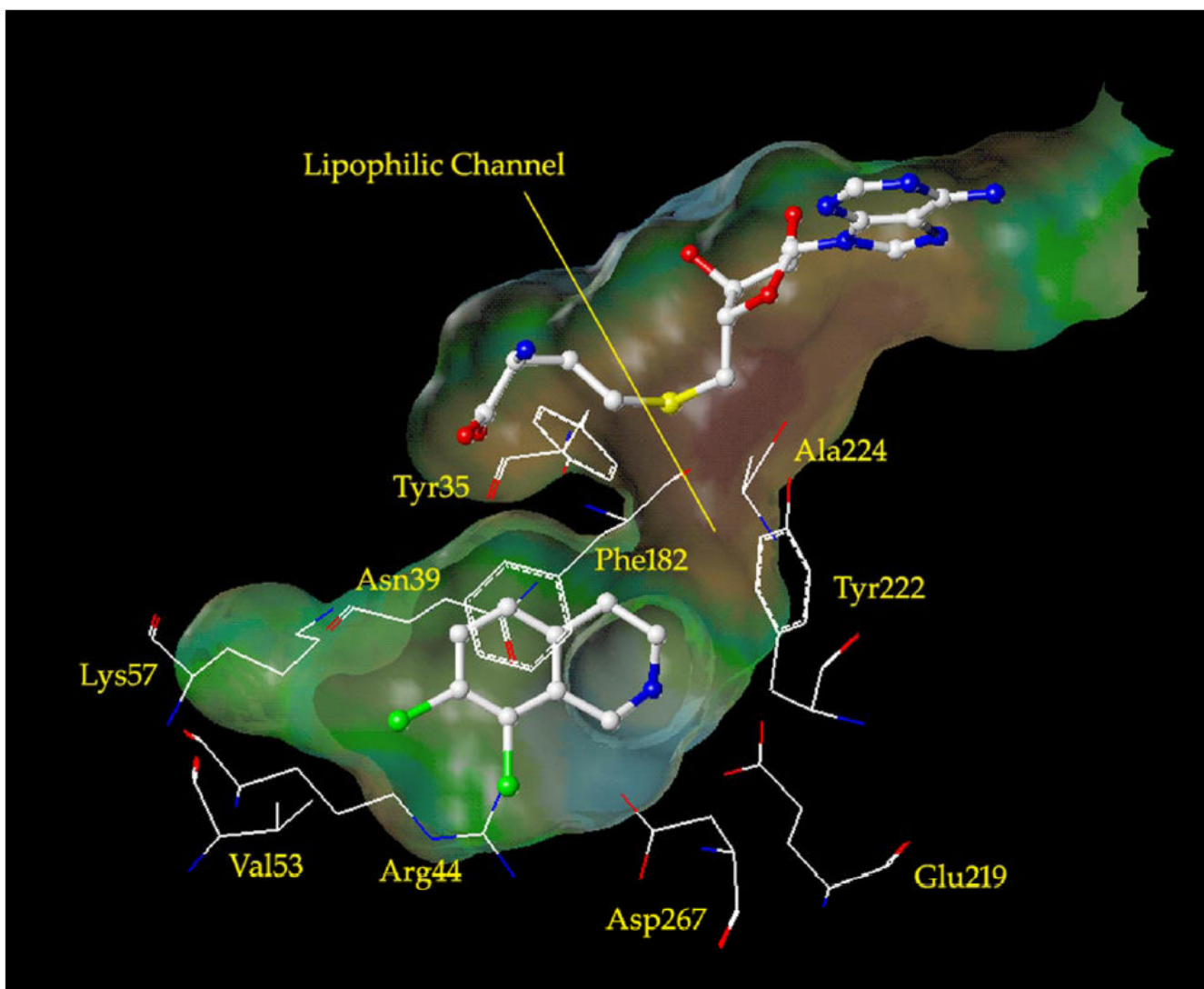
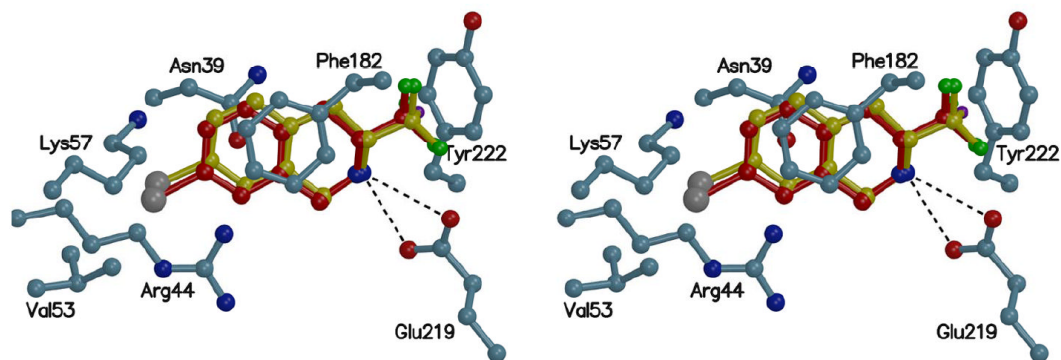
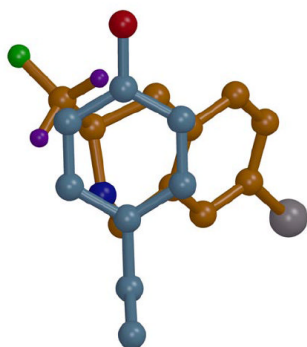


Figure 2. The active site of hPNMT co-crystallized with SK&F 64139 (**6**) and AdoHcy (**5**).²⁷ A Connolly (solvent accessible) surface exposing **6** and **5** is also shown and indicates the presence of a lipophilic channel adjacent to the 3-position of **6**. A lipophilic potential is mapped on the Connolly surface whereby the areas shown in green are neutral, blue are hydrophilic, and brown are lipophilic. Carbon is white, nitrogen is blue, oxygen is red, chlorine is green, and sulfur is yellow. Hydrogens are not shown for clarity.

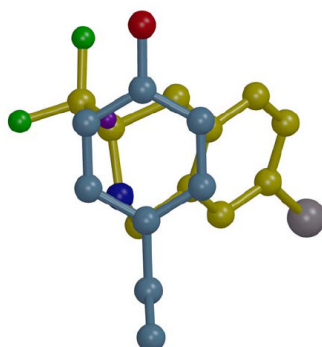
A



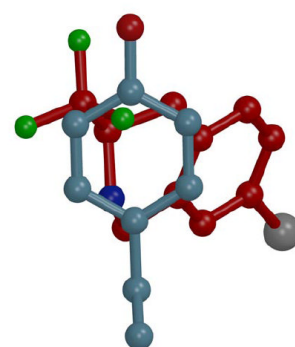
B



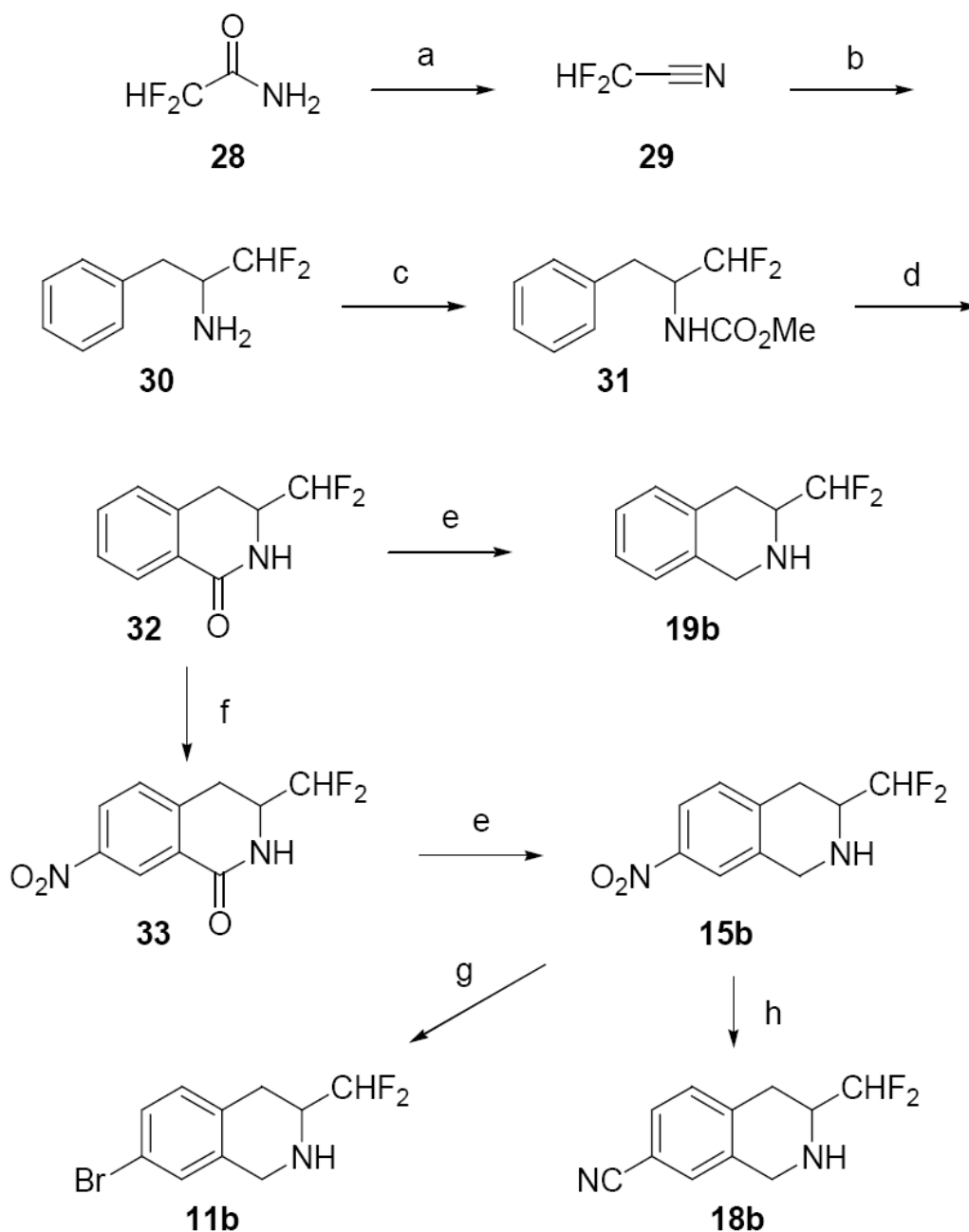
C



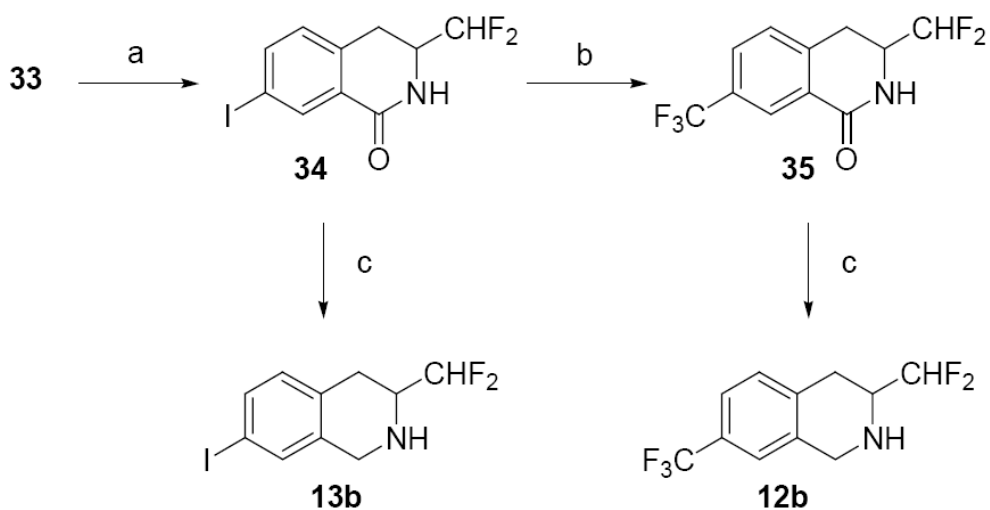
D

**Figure 3.**

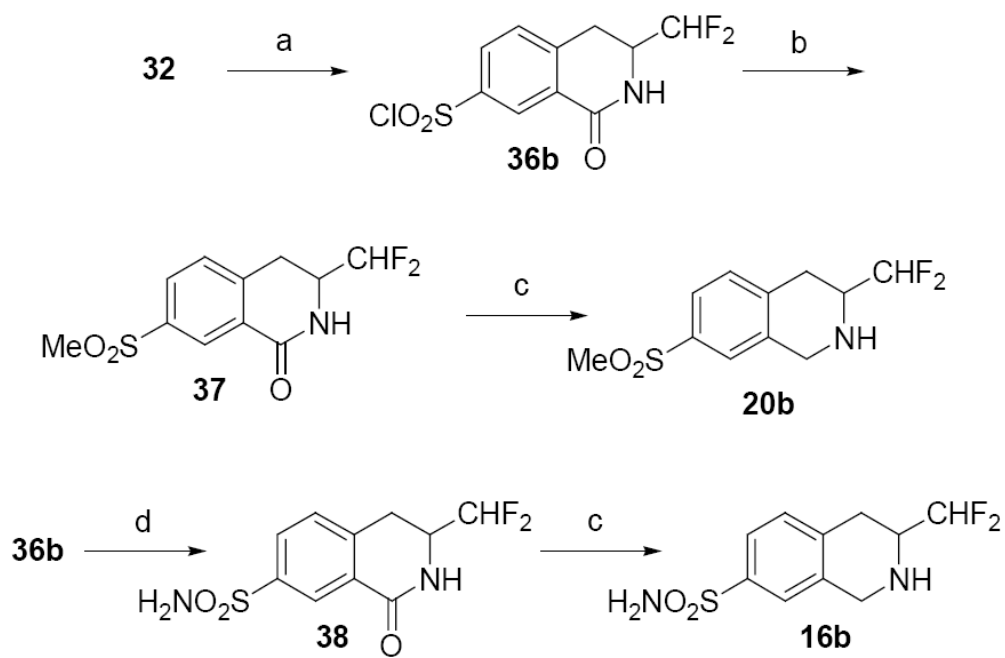
This figure shows 7-bromo-3-fluoromethyl-, difluoromethyl-, and trifluoromethyl-THIQ (**11a–c**) docked into the active site of hPNMT [from the hPNMT X-ray structure co-crystallized with AdoHcy (**5**) and SK&F 64139 (**6**)] and the amino acid residues that could interact with **11a–c**. Nitrogen is blue, oxygen is red, bromine is gray, fluorine is green, carbons of amino acid side chains are cyan, carbons of **11a** are orange, carbons of **11b** are yellow, carbons of **11c** are red, and hydrogens on the 3-substituents of **11a** and **11b** are purple. Other hydrogens are not shown for clarity. (A) This figure shows an overlay of **11b** and **11c**. **11a** is not shown for clarity but the THIQ portion of **11a** overlays almost identically with that of **11b**. **11c** is shifted approximately 0.3 Å away from Tyr222 relative to **11a** and **11b**.⁵⁷ (B) This figure shows the interaction of **11a** with Tyr222, the aromatic ring of which is shown in the plane of the paper. The hydrogens of the fluoromethyl group are positioned in the face of Tyr222 while the fluorine is moved away from Tyr222. (C) This figure shows the interaction of **11b** with Tyr222, the aromatic ring of which is shown in the plane of the paper. The hydrogen of the difluoromethyl group is positioned in the face of Tyr222 while the two fluorine atoms are moved away from Tyr222. (D) This figure shows the interaction of **11c** with Tyr 222, the aromatic ring of which is shown in the plane of the paper. One of the fluorine atoms is in the face of Tyr222.

**Scheme 1.**

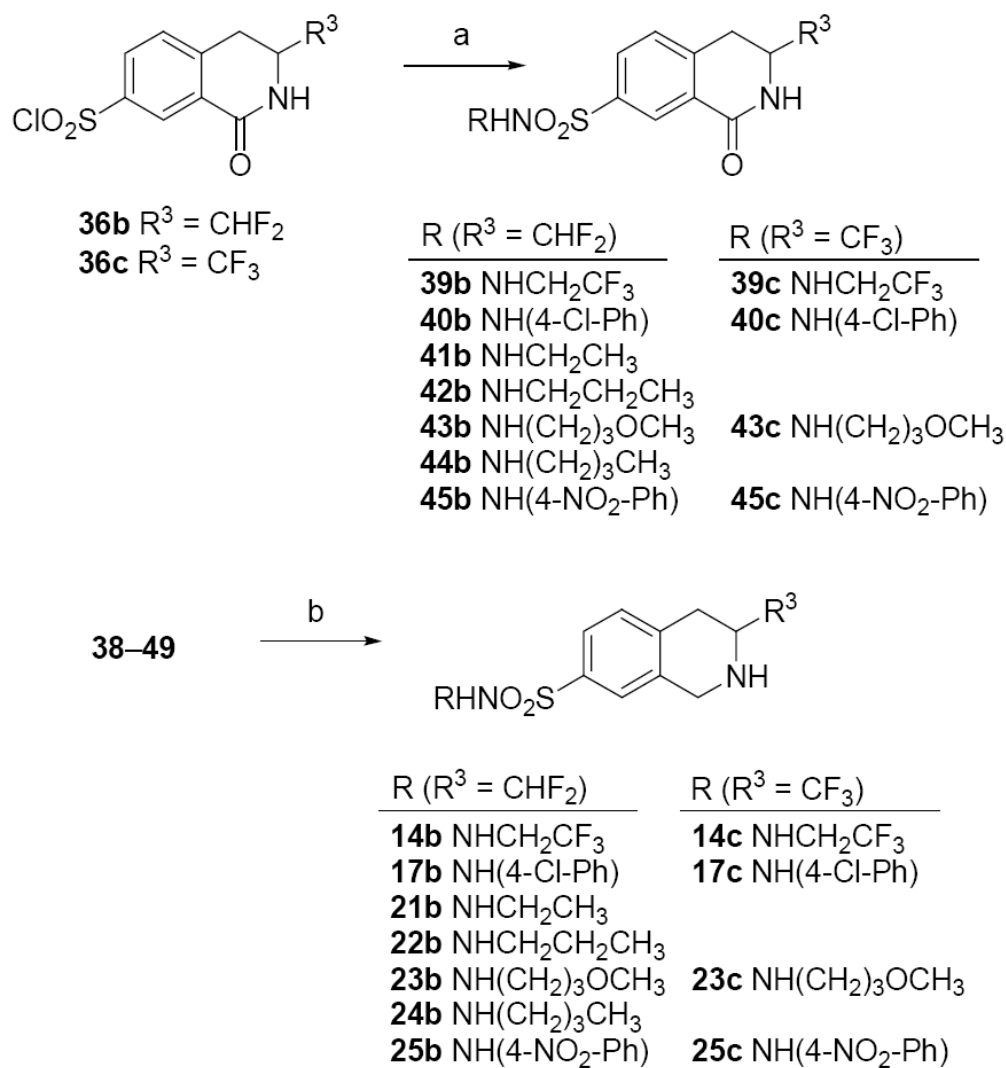
Reagents and conditions: (a) P_2O_5 ; (b) PhCH_2MgCl , Et_2O ; then NaBH_4 , MeOH ; (c) ClCO_2Me , pyridine, CH_2Cl_2 ; (d) polyphosphoric acid; (e) $\text{BH}_3\cdot\text{THF}$; (f) H_2SO_4 , KNO_3 ; (g) H_2 , Pd/C , MeOH ; then HBr , NaNO_2 , CuBr ; (h) H_2 , Pd/C , MeOH ; then HCl , NaNO_2 ; then NaOH , KCN , $\text{NiSO}_4\cdot 6\text{H}_2\text{O}$, benzene.

**Scheme 2.**

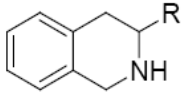
Reagents and conditions: (a) H_2 , Pd/C, MeOH; then HCl, $NaNO_2$, CuI, KI; (b) $FSO_2CF_2CO_2Me$, CuI, DMF; (c) $BH_3 \cdot THF$.

**Scheme 3.**

Reagents and conditions: (a) ClSO_3H ; (b) NH_2NH_2 , THF; then MeI, NaOAc, EtOH; (c) $\text{BH}_3\cdot\text{THF}$; (d) NH_4OH , CH_3CN .

**Scheme 4.**

Reagents and conditions: (a) amines, pyridine/ CHCl_3 or $\text{EtOAc}/\text{Na}_2\text{CO}_3$; (b) $\text{BH}_3\cdot\text{THF}$.

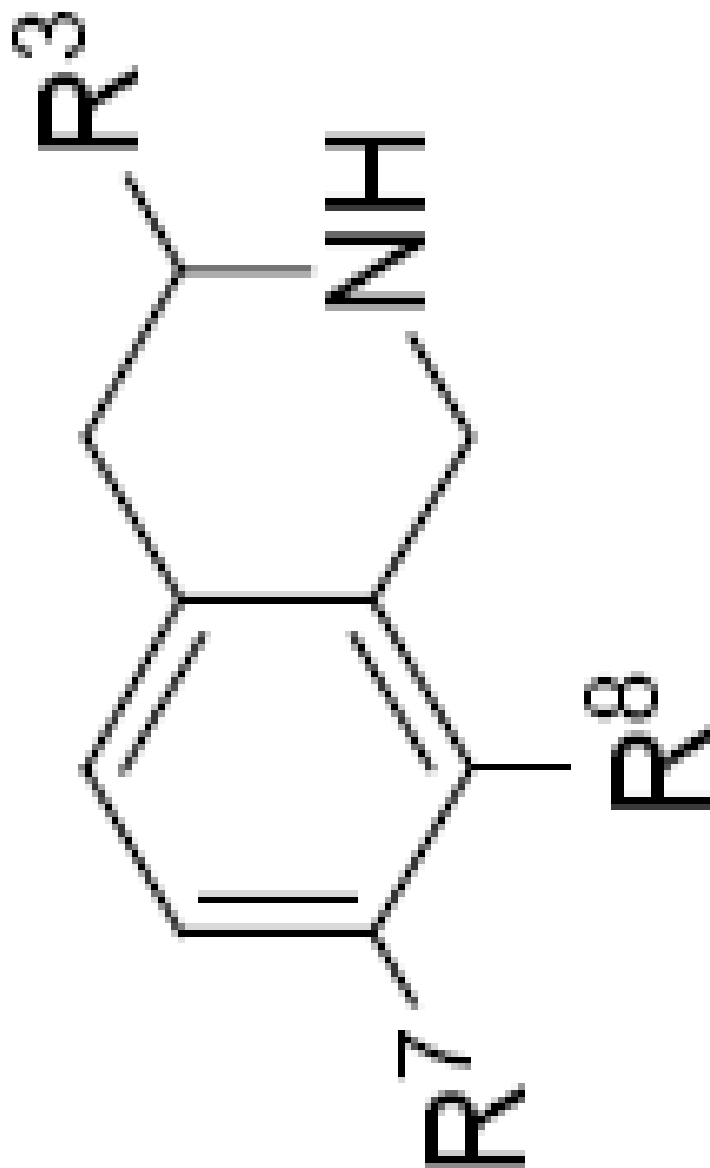
Table 1The Effect of Fluorine on the pK_a of THIQ.

Compd	R	pK_a^a
1	H	9.53
19d	CH ₃	9.42 ^b
19a	CH ₂ F	7.88 ^b
19b	CHF ₂	6.42
19c	CF ₃	4.88 ^b

^a pK_a values (± 0.02) were determined by potentiometric titration by pION, Inc., Cambridge, MA.^b Ref. 44.

Table 2

In Vitro Human PNMT (hPNMT) and α_2 -adrenoceptor Affinity of Some PNMT Inhibitors.



Compd	R ⁷	R ⁸	R ³	hPNMT	K _i (nM) ± SEM ^a	α_2 ^b	Selectivity α_2 /hPNMT
6 ^c	Cl	Cl	H	0.0031 ± 0.0006 ^d		0.021 ± 0.005	6.8
7 ^e	Br	H	H	0.056 ± 0.003 ^f		0.23 ± 0.02	4.1
8 ^e	I	H	H	0.093 ± 0.007 ^g		0.22 ± 0.04	2.4
9 ^e	NO ₂	H	H	0.12 ± 0.01 ^f		4.3 ± 0.3	36
10 ^g	SO ₂ NH ₂	H	H	0.28 ± 0.02 ^d		100 ± 10	360
11a ^h	Br	H	CH ₂ F	0.023 ± 0.003 ^f		6.4 ± 0.2	280
11c ⁱ	Br	H	CF ₃	3.2 ± 0.3 ^j		>1000	>310
11d ^f	Br	H	CH ₃	0.017 ± 0.005		1.1 ± 0.1	65

^a Standard error of the mean.

^b In vitro activities for the inhibition of [³H]clonidine binding to the α_2 -adrenoceptor.

^c Ref. 15.

^d Ref. 39.

^e Ref. 21.

^f Ref. 48.

^g Ref. 22.

^h Ref. 44.

ⁱ Ref. 23.

^j Ref. 24.

Table 3

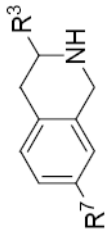
In Vitro Activities of 3-Mono-, Di-, and Trifluoromethyl-7-substituted-THIQs.

Compd	R ⁷	R ³ = CH ₂ F (a) ^d			R ³ = CHF ₂ (b) ^d			R ³ = CF ₃ (c) ^b		
		PNMT ^c	α ₂ c,d	Set ^e	PNMT ^c	α ₂ c,d	Set ^e	PNMT ^c	α ₂ c,d	Set ^e
11a-c	Br	0.023	6.4	280	0.094	230	2400	3.2	>1000	>310
12a-c	CF ₃	0.030	41	1400	0.067	190	2800	0.98	>1000	>1000
13a-c	I	0.054	7.1	130	0.20	>1000	>5000	1.9	>1000	>530
14a-c	SO ₂ NHCH ₂ CF ₃	0.13	1200	9200	0.25	>1000	>4000	9.4	>1000	>110
15a-c	NO ₂	0.15	76	510	0.17	>1000	>5800	2.3	1400	610
16a-c	SO ₂ NH ₂	0.15	680	4500	0.68	>1000	>1500	8.0	>1000	>125
17a-c	SO ₂ NH(<i>p</i> -C ₆ H ₄ Cl)	0.27	140	520	0.90	>1000	>1100	15	>1000	>67
18a-c	CN	0.80	460	570	3.1	>1000	>320	21	2900	140
19a-c	H	0.82	3.8	4.6	3.4	150	44	23	400	17
20a-c	SO ₂ CH ₃	1.1	230	210	6.0	2500	420	41	3900	95
21a-c	SO ₂ NHEt	1.4	550	390	3.2	>1000	>310	60	>1000	>17
22a-c	SO ₂ NHPr	1.7	610	360	2.1	>1000	>480	—	—	—
23a-c	SO ₂ NH(CH ₂) ₃ OCH ₃	2.6	750	290	5.3	>1000	>190	13	>1000	>77
24a-c	SO ₂ NHBu	3.4	260	76	5.6	>1000	>180	—	—	—
25a-c	SO ₂ NH(<i>p</i> -C ₆ H ₄ NO ₂)	7.7	>1000	>130	37	>1000	>27	110	>1000	>9.1

^aData for compounds **11a–25a** found in refs. 48 and 44.^bData for compounds **11c–13c**, **15c**, **16c**, and **18c–21c** found in refs. 23 and 24.^cStandard error of the mean was not greater than 10%.^dIn vitro activities for the inhibition of [³H]clonidine binding to the α₂-adrenoceptor.^eSelectivity α₂ K_i /hPNMT K_i.

Table 4

In Vitro Activities of (±)-7-Nitro- and -7-Bromo-3-substituted-THIQs.



Compd	R ⁷	R ₃	Charton ^d	E _s Taft ^b	E _s Dubois ^c	pK _a ^d	K _i (μM) ± SEM ^e PNMT	α ₂ ^f	Selectivity α ₂ /hPNMT
11a ^g	Br	CHF ₂	0.62	-1.48	1.32	7.77	0.023 ^h	6.4	280
11b	Br	CHF ₂	0.68	-1.91	1.44	6.12	0.094	230	2400
11c ⁱ	Br	CF ₃	0.91	-2.40	1.90	4.33	3.2 ^j	>1000	>310
11d ^h	Br	CH ₃	0.52	-1.24	1.12	9.29	0.017	1.1	65
11e ^h	Br	CH ₂ CH ₃	0.56	-1.31	1.20	9.44	0.48	1.2	2.5
11f ^h	Br	CH(CH ₃) ₂	0.76	-1.71	1.43	9.59	4.4	3.9	0.89
15a ^g	NO ₂	CHF ₂	0.62	-1.48	1.32	7.45	0.15 ⁱ	76	510
15b	NO ₂	CHF ₂	0.68	-1.91	1.44	5.83	0.17	>1000	>5900
15c ^j	NO ₂	CF ₃	0.91	-2.40	1.90	4.06	2.3 ^j	1400	610
15d ^h	NO ₂	CH ₃	0.52	-1.24	1.12	8.95	0.072	31	430
15e ^h	NO ₂	CH ₂ CH ₃	0.56	-1.31	1.20	9.11	0.49	28	57
15f ^h	NO ₂	CH(CH ₃) ₂	0.76	-1.71	1.43	9.27	4.6	36	7.9

^aRef. 45.^bRef. 46.^cScaled to H=0, Ref. 47.^dpK_a values were calculated using pKaPlugin from the JChem (version 3.1) software package (see ref. 41).^eStandard error of the mean.^fIn vitro activities for the inhibition of [³H]clonidine binding to the α₂-adrenoceptor^gRef. 44.^hRef. 48.ⁱRef. 23.^jRef. 24.