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Bioorganic & Medicinal Chemistry Letters 15 (2005) 5319–5323
Bioorganic & Medicinal Chemistry Letters

Inhibitors of phenylethanolamine *N*-methyltransferase devoid of α_2-adrenoceptor affinity☆

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Received 5 July 2005; revised 5 August 2005; accepted 9 August 2005
Available online 19 September 2005

Abstract—A series of 3-trifluoromethyl-1,2,3,4-tetrahydroisoquinolines was synthesized and evaluated for their phenylethanolamine *N*-methyltransferase (PNMT) inhibitory potency and affinity for the α_2-adrenoceptor. Although their PNMT inhibitory potency decreased compared with corresponding 3-methyl-, 3-hydroxymethyl- or 3-unsubstituted-THIQs, some of them showed good selectivity due to their extremely low α_2-adrenoceptor affinity.

Phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28) catalyzes the last step of epinephrine biosynthesis.[2] Inhibitors of PNMT are potential pharmacological tools for the study of the function of epinephrine in the central nervous system (CNS).[3–6] Previous studies have found that 1,2,3,4-tetrahydroisoquinolines (THIQs) are potent inhibitors of PNMT (Table 1).[7–13] However, most of these inhibitors either display significant affinity for the α_2-adrenoceptor (e.g., **1**,**2**),[10,14] which complicates the interpretation of their biological effects, or those inhibitors that are selective for PNMT are too polar to pass the blood–brain barrier (BBB) (e.g., **3**, **4**).[8,12,15] In light of these limitations, there remains an ongoing interest in the development of a PNMT inhibitor that is both sufficiently lipophilic to penetrate the BBB and is devoid of affinity for the α_2-adrenoceptor.

Previously, several 3-trifluoromethyl-THIQs (i.e., **5a**, **5d**, **5f**, **5g**, and **5i** in Table 2) were studied.[16] Although these compounds displayed decreased inhibitory potency for PNMT compared to similarly substituted 3-methyl- or 3-hydroxymethyl-THIQs,[17] their potentially attractive features suggested a solution to the common problems of most PNMT inhibitors. First, this series of inhibitors showed extremely low affinity for the α_2-adrenoceptor (K_i, 400–3900 μM), possibly due to the low pK_a of the 3-trifluoromethyl-THIQ amine (e.g., for **5a**, pK_a = 4.86; it would be largely unprotonated at physiological pH, whereas the endogenous ligands at the α_2-adrenoceptor would be largely protonated) or the steric hindrance of the trifluoromethyl moiety.[16] Since THIQs with lipophilic 7-substituents usually show good PNMT inhibitory potency but also good α_2-adrenoceptor affinity,[10] compounds **5b**, **5c**, and **5e** were proposed to explore the possibility of increasing selectivity while maintaining the PNMT inhibitory potency of these THIQs. Second, the trifluoromethyl moiety would also increase the lipophilicity of these compounds thereby increasing the possibility of these THIQs to cross the BBB. Compounds **5h** and **5j** were proposed to take advantage of this feature as THIQs with 7-aminosulfonyl substituents generally possess good PNMT inhibitory potency and poor α_2-adrenoceptor affinity, but are usually too polar for BBB penetration.[8,12] To further enhance their PNMT inhibitory potency, the enantiomers of several of the most potent 3-trifluoromethyl-THIQs in this study were also prepared and evaluated.[18]

Compounds **5b** and **5c** were synthesized from amine **6** (Scheme 1). Lactam **8** had been synthesized previously,[16] but the yield of the cyclization of **7** was improved from 53% (with polyphosphoric acid at 140 °C) to 86% (with $POCl_3/SnCl_4$ at 110 °C). Hydrogenation of **9** gave aniline **10**, which was converted to iodo-lactam **11** via a Sandmeyer iodination.[15] Reduction of **11** with BH_3·THF gave **5b**. Treatment of **11** with $FSO_2CF_2CO_2CH_3$ and CuI in DMF gave the trifluoromethyl analogue (**12**), which was reduced to **5c**.

THIQs **5e**, **5h**, and **5j** were synthesized from aniline **10** (Scheme 2), which was converted to chloro-lactam **13**,

Keywords: Phenylethanolamine-*N*-methyltransferase; Inhibitor; α_2-Adrenoceptor; Selectivity; Blood–brain barrier.
☆ See Ref. 1.
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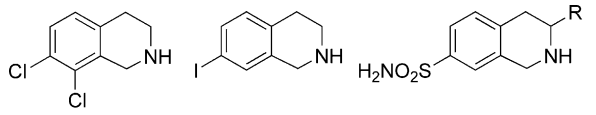
doi:10.1016/j.bmcl.2005.08.033

Table 1. In vitro human PNMT (hPNMT) and α_2-adrenoceptor affinities of some PNMT inhibitors

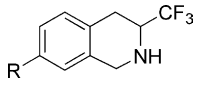


1 (SK&F 64139) **2** **3** R = H (SK&F 29661) **4** R = CH_2OH

Compound	K_i (μM ± SEM)		Selectivity α_2/hPNMT	C log P[b]
	hPNMT	α_2[a]		
1[c]	0.0031 ± 0.0006[d]	0.021 ± 0.005	7	2.90
2[e]	0.093 ± 0.007	0.22 ± 0.04	2.4	2.72
3[f]	0.28 ± 0.02[c]	100 ± 10	360	−0.29
4[g]	0.052 ± 0.004[h]	1400 ± 200	27,000	−0.93

[a] In vitro activities for the inhibition of [^{3}H]clonidine binding to the α_2-adrenoceptor.
[b] Calculated log P values (C log P function in SYBYL 6.9; Ref. 28).
[c] Ref. 7.
[d] Ref. 11.
[e] Ref. 10, previous data were for bovine PNMT.
[f] Ref. 8.
[g] Ref. 12.
[h] Ref. 13.

Table 2. In vitro activities of 3-trifluoromethyl-THIQs

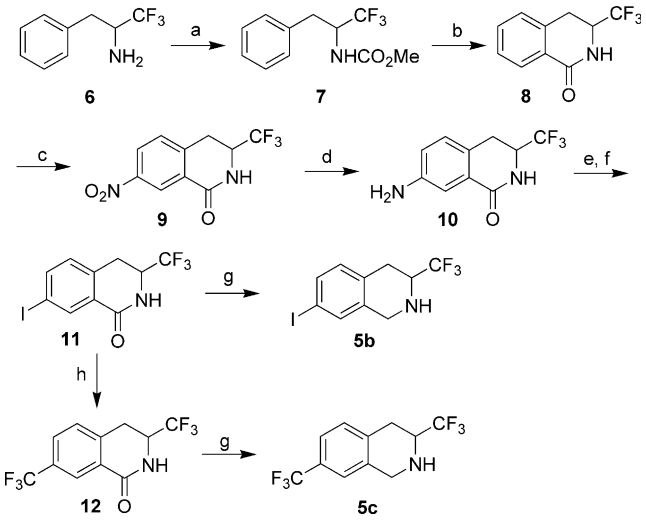


Compound	R	K_i (μM ± SEM)		Selectivity α_2/hPNMT	C log P[b]
		hPNMT	α_2[a]		
5a(±)[c]	H	23 ± 2	400 ± 40	17	2.87
5b(±)	I	1.9 ± 0.1	>1000	>530	4.00
R-(+)		0.94 ± 0.06	[d]		
S-(−)		4.2 ± 0.2	[d]		
5c(±)	CF_3	0.98 ± 0.11	>1000	>1000	3.76
R-(+)		0.50 ± 0.03	[d]		
S-(−)		1.9 ± 0.1	[d]		
5d(±)[c]	Br	3.2 ± 0.3	>1000	>310	3.74
5e(±)	Cl	0.99 ± 0.08	>1000	>1000	3.59
R-(+)		0.46 ± 0.02	[d]		
S-(−)		1.9 ± 0.2	[d]		
5f(±)[c]	NO_2	2.3 ± 0.1	1400 ± 200	610	2.62
R-(+)		1.2 ± 0.1	[d]		
S-(−)		4.4 ± 0.3	[d]		
5g(±)[c]	CN	21 ± 2	2900 ± 300	140	2.31
5h(±)	SO_2NHEt	60 ± 6	>1000	>17	2.19
5i(±)[c]	SO_2CH_3	41 ± 2	3900 ± 500	95	1.23
5j(±)	SO_2NH_2	8.0 ± 0.7	>1000	>125	1.04

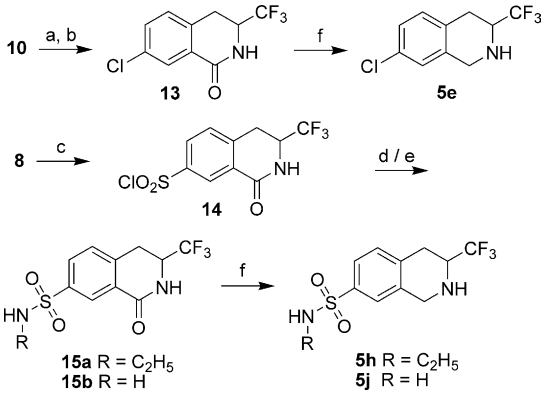
[a] In vitro activities reported for the inhibition of binding of [^{3}H]clonidine at the α_2-adrenoceptor.
[b] Calculated log P values (C log P function in SYBYL 6.9; Ref. 28).
[c] Ref. 16, previous data were for bovine PNMT.
[d] These values were not determined because of the extremely low α_2-adrenoceptor affinity or the solubility of the corresponding racemates.

followed by reduction with BH_3·THF to afford **5e**. Treatment of lactam **8** with chlorosulfonic acid (neat) gave **14**, which was reacted with ethylamine or ammonium hydroxide to give **15a** or **15b**, followed by reduction with BH_3·THF to give **5h** or **5j**.[15]

The enantiomers of **5b**, **5c**, **5e**, and **5f** were separated by chiral HPLC.[19] The absolute configurations of **5c** and **5e** were established by the X-ray structure analysis of (*R*)-**5c**·HCl and (*R*)-**5e**·HCl.[20] To establish the absolute configurations of **5b** and **5f**, (*R*)-**5b** and (*R*)-**5f** were prepared



Scheme 1. Reagents: (a) $ClCO_2Me$, pyridine, $CHCl_3$, 92%; (b) $POCl_3$, $SnCl_4$, 86%; (c) H_2SO_4, KNO_3, 90%; (d) H_2, PtO_2, MeOH, 99%; (e) HCl, $NaNO_2$; (f) CuI, KI, 61% from **10**; (g) BH_3·THF, THF, 78%; (h) $FSO_2CF_2CO_2Me$, CuI , DMF, 27%.



Scheme 2. Reagents: (a) HCl, $NaNO_2$; (b) CuCl, KCl, 76% from **10**; (c) HSO_3Cl, 77%; (d) $EtNH_2$·HCl, EtOAc/Na_2CO_3, 87% for **15a**; (e) NH_4OH, CH_3CN, 71% for **15b**; (f) BH_3·THF, THF, 85% for **5e**, 94% for **5h**, 63% for **5j**.

from amine (*R*)-**6** (97% ee), which was synthesized according to literature procedures.[21]

Radiochemical assays described previously were used to determine human PNMT (hPNMT) inhibition constants[11,22] and α_2-adrenoceptor binding affinities.[23,24]

In general, the 3-trifluoromethyl-THIQs (Table 2) showed decreased hPNMT inhibitory potency as compared to the similarly 7-substituted 3-methyl-,[25] 3-hydroxymethyl-[12,13] or 3-unsubstituted-THIQs.[11] For example, compared with THIQs **2** (K_i = 0.093 μM) and **3** (K_i = 0.28 μM), both 3-trifluoromethyl-THIQ analogues show decreased potency at hPNMT (**5b**, K_i = 1.9 μM; **5j**, K_i = 8.0 μM). For the hPNMT inhibitory potency of different 3-trifluoromethyl-THIQs, compounds bearing a lipophilic 7-substituent (e.g., **5b**, **5c**, and **5e**) showed higher potency than compounds bearing a hydrophilic 7-substituent (e.g., **5g**, **5i**, and **5j**).

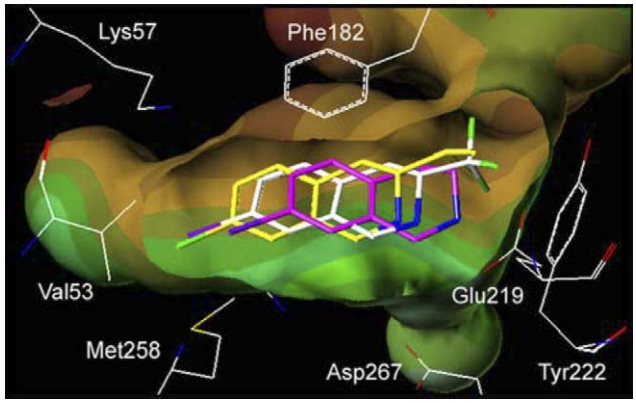
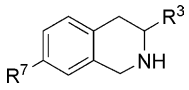


Figure 1. This figure shows the amino acids (carbon is white, nitrogen is blue, oxygen is red, sulfur is yellow, and bromine and fluorine are green) interacting with 7-iodo-THIQ (**2**; stick model, carbon is magenta) within the active site of hPNMT. Asn39 and Val269 are not shown. A Connolly (solvent accessible) surface shows lipophilic areas in brown, hydrophilic areas in blue, and neutral areas in green. The docking results of *R*-**5b** (stick model, carbon is white) and *R*-**18a** (stick model, carbon is yellow) are also shown. Hydrogens are not shown for clarity.

The crystal structure of hPNMT complexed with **2** and *S*-adenosyl-L-homocysteine (AdoHcy) has recently been published.[26] To help explain the reduced hPNMT inhibitory potency of the 3-trifluoromethyl-THIQs, molecular docking calculations were conducted using AutoDock 3.0[27] and Sybyl 6.9.[28] Compound **5b** was docked into the hPNMT active site based on the X-ray crystal structure of hPNMT-**2**-AdoHcy (Fig. 1). The docking study indicated that the binding orientation of **5b** is similar to that of **2** in the active site of PNMT, except that the THIQ ring of **5b** is shifted about 1 Å away from Glu219 and Tyr222 as compared with **2**.

Previous studies have shown that substitution of a methyl or hydroxymethyl group at the 3-position of THIQs increased the potency of THIQs for PNMT.[17] However, these studies also indicated that the active site of PNMT has a limited amount of steric bulk tolerance for 3-substituents of THIQs.[17] The comparisons of **5d** and **5f** with the corresponding 3-methyl, 3-ethyl, and 3-isopropyl substituted THIQs[25] are shown in Table 3. *A* values (conformational energies of cyclohexanes, $-\Delta G^0$) of these 3-substituents are also included as indicators of group sizes.[29] As the size of the 3-substituent increases from methyl to isopropyl (*A* value increases from 1.70 to 2.15 kcal/mole), the hPNMT inhibitory potency of these compounds decreases. Although a fluorine (van der Waals radius 1.47 Å) is a close isosteric substitution for a hydrogen (van der Waals radius 1.20 Å),[30] a trifluoromethyl group is considerably larger than a methyl group. Studies have shown that the trifluoromethyl moiety is close to the size of an isopropyl group.[31] Interestingly, when comparing their affinities for hPNMT, 3-trifluoromethyl-THIQs (i.e., **5d**, **5f**) displayed K_i values close to those of corresponding 3-isopropyl-THIQs (i.e., **18a**, **18b**). Furthermore, a docking study of **18a** (Fig. 1) also showed a similar shift of the molecule in the active site of PNMT as observed in the study of **5b**. In the crystal structure of the hPNMT–**2**–AdoHcy

Table 3. In vitro activities of (±)-3-alkyl-7-nitro and -7-bromo-THIQs



Compound	R^3	R^7	*A* value (R^3)[a]	K_i (μM ± SEM)	
				hPNMT	α_2 K_i (μM ± SEM)[b]
16a(±) [c]	CH_3	Br	1.70	0.017 ± 0.005	1.1 ± 0.1
17a(±)[c]	C_2H_5	Br	1.75	0.48 ± 0.02	1.2 ± 0.1
18a(±)[c]	$CH(CH_3)_2$	Br	2.15	4.4 ± 0.3	3.9 ± 0.3
5d(±)[d]	CF_3	Br	2.10	3.2 ± 0.3	>1000
16b(±)[c,e]	CH_3	NO_2	1.70	0.072 ± 0.005	31 ± 1
17b(±)[c]	C_2H_5	NO_2	1.75	0.49 ± 0.03	28 ± 0.3
18b(±)[c]	$CH(CH_3)_2$	NO_2	2.15	4.6 ± 0.3	36 ± 0.3
5f(±)[d]	CF_3	NO_2	2.10	2.3 ± 0.1	1400 ± 200

[a] *A* value: conformational energy (kcal/mol) (Ref. 29).
[b] In vitro activities reported for the inhibition of binding of [^{3}H]clonidine at the α_2-adrenoceptor.
[c] Ref. 25.
[d] Ref. 16, previous data were for bovine PNMT.
[e] Ref. 12.

complex, the aliphatic amine of **2** forms a hydrogen bond to the carboxylate of Glu219.[26] In addition, the aromatic ring of **2** is sandwiched between Phe182 and Asn39,[26] suggesting the presence of an aromatic π–π stacking interaction between the aromatic ring of **2** and Phe182. The shift of the THIQ ring of **5b** as predicted by docking studies (Fig. 1), possibly induced by the unfavorable steric interaction of the 3-trifluoromethyl moiety, could disrupt both the H-bond interaction with Glu219 and the π–π stacking interaction with Phe182.

None of this series of 3-trifluoromethyl-THIQs displayed significant affinity toward the α_2-adrenoceptor, although the limited solubility of some compounds precluded determination of exact K_i values. Previous comparative molecular field analysis (CoMFA) studies on a series of THIQs indicated an area of steric bulk intolerance at the 3-position of THIQs for the α_2-adrenoceptor.[32] Interestingly, when comparing the α_2-adrenoceptor affinities of THIQs in Table 3, no significant changes are observed from the 3-methyl-THIQs (i.e., **16a**, **16b**) to the 3-isopropyl-THIQs (i.e., **18a**, **18b**). However, dramatic decreases in α_2-adrenoceptor affinities are noticed between 3-isopropyl-THIQs and 3-trifluoromethyl-THIQs (i.e., **5d**, **5f**) even though the trifluoromethyl moiety is close in size to the isopropyl group. This comparison reinforces the hypothesis that the significant reduction of the α_2-adrenoceptor affinities of the 3-trifluoromethyl-THIQs is due mainly to the decrease in pK_a of the THIQ amine rather than due to unfavorable steric interactions.[16]

Comparison of the hPNMT inhibitory activities of the enantiomers of the most potent racemates in Table 2 (i.e., **5b**, **5c**, **5e**, and **5f**) shows that the *R*-enantiomers are approximately 4-fold as potent as their corresponding *S*-enantiomers. This result is consistent with the observations of other 3-substituted THIQs.[18,33]

In conclusion, as a strong electron-withdrawing group at the 3-position of THIQ, the trifluoromethyl moiety can greatly decrease the affinity of THIQ for the α_2-adrenoceptor. On the other hand, the steric hindrance of the trifluoromethyl group is likely to be the major reason for the reduced PNMT inhibitory potency of these compounds. THIQs **5c** and **5e** are two highly selective sub-micromolar inhibitors of PNMT with high lipophilicity (C*log P* values greater than that of **1**) that should enable them to cross the BBB.

Acknowledgments

This research was supported by NIH Grant HL 34193. We thank David VanderVelde and Sarah Neuenswander of the University of Kansas Nuclear Magnetic Resonance Laboratory for their assistance. We thank Douglas Powell of the X-ray crystallography laboratory (National Science Foundation Grant CHE-0079282) for the crystal structures of (*R*)-**5c**·HCl and (*R*)-**5e**·HCl. We also thank Gerald Lushington of the University of Kansas Molecular Graphics and Modeling Laboratory and Todd Williams of the University of Kansas Mass Spectrometry Laboratory for their assistance.

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20. Crystallographic data of (*R*)-**5c**·HCl and (*R*)-**5e**·HCl have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 279450 and CCDC 279451. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 01223 336033 or e-mail: data_request@ccdc.cam.ac.uk].
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