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Exploring the active site of phenylethanolamine *N*-methyltransferase with 3-hydroxyethyl- and 3-hydroxypropyl-7-substituted-1,2,3,4-tetrahydroisoquinolines

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Abstract—3-Hydroxyethyl- and 3-hydroxypropyl-7-substituted-tetrahydroisoquinolines (**9**, **10**, **16**, and **17**) were synthesized and evaluated for their phenylethanolamine *N*-methyltransferase (PNMT) inhibitory potency and affinity for the α_2-adrenoceptor. Although α_2-adrenoceptor affinity decreased for these compounds, selectivity was not gained over the parent 3-hydroxymethyl compounds (**1**, **2**) due to a loss in PNMT inhibitory potency.

1. Introduction

Epinephrine (Epi) in the central nervous system (CNS) constitutes approximately 5% of the catecholamine content in the mammalian brain,[1,2] yet its role therein remains unclear. It has been implicated in some of the neurodegeneration seen in Alzheimer's disease,[3,4] and in the regulation of blood pressure,[5] respiration,[6,7] body temperature,[6,7] as well as α_1-[8] and α_2-adrenoceptors.[9,10]

To elucidate the role of CNS Epi, we have targeted phenylethanolamine *N*-methyltransferase (PNMT: EC 2.1.1.28),[1] the terminal enzyme in the biosynthesis of Epi. Some problems associated with early PNMT inhibitors are 1) their lack of selectivity for PNMT versus the α_2-adrenoceptor, or 2) their inability to penetrate into the CNS. A compound possessing a calculated log *P*[11,12] value greater than 0.5 is likely required for 1,2,3,4-tetrahydroisoquinoline-type (THIQ-type) PNMT inhibitors to achieve significant CNS penetration.[13] A potent inhibitor of PNMT that is both selective and capable of crossing the blood–brain barrier (BBB) would be a useful pharmacological tool to help define the role of CNS Epi.

3-Hydroxymethyl-7-nitro-THIQ (**1**) and 7-bromo-3-hydroxymethyl-THIQ (**2**) are two highly potent PNMT inhibitors, but both lack significant selectivity for PNMT versus the α_2-adrenoceptor (Table 2).[14] Examination of the recently solved crystal structures of hPNMT co-crystallized with *S*-adenosyl-L-homocysteine (AdoHcy) and either 7-iodo-THIQ (**3**)[15] or SK&F 29661 (**4**; 7-aminosulfonyl-THIQ)[16] indicated that the active site should be able to accommodate longer 3-hydroxyalkyl groups. Docking of 3-hydroxyethyl- or 3-hydroxypropyl-7-substituted-THIQs into the PNMT active site indicated that the longer 3-hydroxyalkyl chains could bind favorably (Fig. 2 shows 3-hydroxyethyl-7-nitro-THIQ).

A previously reported α_2-adrenoceptor comparative molecular field analysis model on a set of THIQs showed that there was steric bulk intolerance around the 3-position of THIQ.[17] Thus, longer 3-hydroxyalkyl substituents (**9**, **10**, **16**, **17**) should be disfavored at the α_2-adrenoceptor and should increase selectivity for PNMT.

2. Chemistry

The synthesis of **9** and **10** is shown in Scheme 1. The synthesis of **5**[18] has been reported previously. Treatment of

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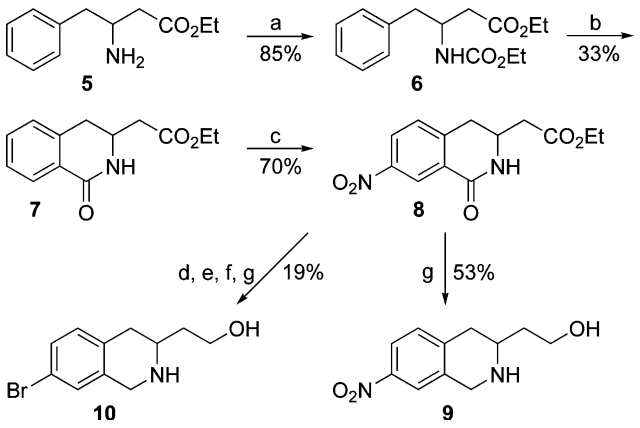
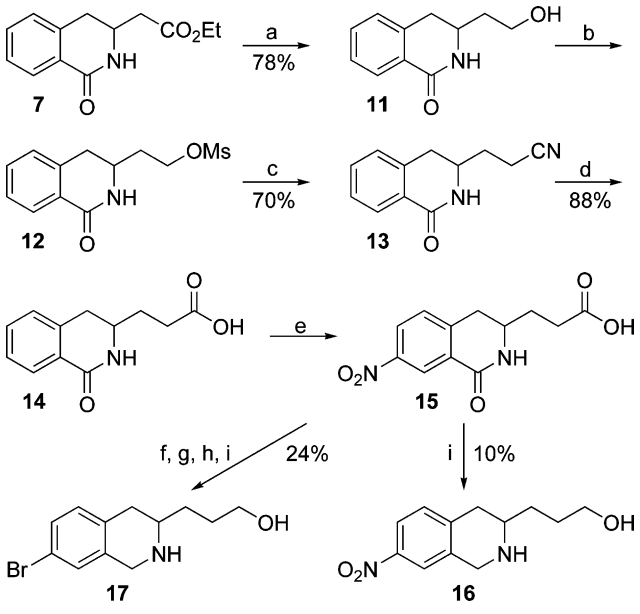


Scheme 1. Reagents and conditions: (a) $ClCO_2Et$; (b) PPA, 145 °C; (c) H_2SO_4, KNO_3; (d) H_2, PtO_2/C; (e) HBr, $NaNO_2$; (f) CuBr, HBr; (g) BH_3·THF.

5 with ethylchloroformate and K_2CO_3 in THF produced ethylcarbamate **6** that was easily purified by Kugelrohr distillation and was cyclized with polyphosphoric acid heated to 145 °C to yield lactam **7**. Nitration of **7** with potassium nitrate afforded **8** that was recrystallized in EtOAc/hexanes. Reduction of lactam **8** with BH_3·THF yielded THIQ **9**. The nitro moiety of **8** was reduced to the aniline, Sandmeyer chemistry was used to transform the aniline to a bromide, and subsequent reduction of the lactam yielded THIQ **10**.

Compound **7** was used to synthesize **16** and **17** (Scheme 2). Compound **7** was reduced to alcohol **11**, which was transformed to nitrile **13** by conversion to mesylate **12** followed by displacement with cyanide (KCN). Hydrolysis of nitrile **13** with sulfuric acid afforded **14** in good yield. Compound **14** was nitrated and subsequent reduction of both the acid and lactam moieties yielded THIQ **16**. The nitro group of **15** was reduced to the aniline, converted to the bromide under Sandmeyer conditions, and reduction of the acid and lactam yielded THIQ **17**. Compounds **9**, **10**, **16**, and **17** were purified by flash chromatography before conversion to their HCl salts that were purified by recrystallization from EtOH/hexanes.[19,20]



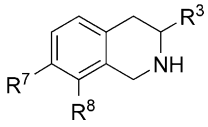
Scheme 2. Reagents and conditions: (a) $LiBH_4$; (b) CH_3SO_2Cl; (c) KCN, DMF; (d) H_2SO_4/H_2O; (e) KNO_3, H_2SO_4; (f) H_2, PtO_2/C; (g) HBr, $NaNO_2$; (h) CuBr, HBr; (i) BH_3·THF.

3. Biochemistry

In this study, human PNMT (hPNMT) with a C-terminal hexahistidine tag was expressed in *E. coli*.[21,22] The radiochemical assay conditions, previously reported for the bovine enzyme,[23] were modified to account for the high binding affinity of some inhibitors.[22,24] 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 either cortex obtained from male Sprague Dawley rats,[25] with [^{3}H]clonidine (2 nM) as the radioligand and phentolamine to define nonspecific binding, or membrane preparations from CHO cells stably transfected with human α_{2A}-adrenoceptors {radioligand: [^{3}H]RX821002 (0.2 nM)/nonspecific binding: (−)-norepinephrine}.[26,27] A comparison of the binding data for a related series of compounds (Table 1) shows a good correlation ($r^2 = 0.90$) between the inhibition of [^{3}H]clonidine binding to rat cortex α_2-adrenoceptors compared to the inhibition of [^{3}H]RX821002 binding to cloned human α_{2A}-adrenoceptors. This correlation

Table 1. α_2-Adrenoceptor affinity comparison



Compd	R^3	R^7	R^8	α_2-Adrenoceptor affinity (pK_i)	
				Rat cortex[a]	Human-α_{2A}[b]
18	H	Cl	Cl	7.68[c]	6.52
4	H	SO_2NH_2	H	4.00[d]	3.40
19	H	NO_2	H	5.37[e]	5.20
20	CH_3	NO_2	H	4.51[d]	4.84
1	CH_2OH	NO_2	H	4.12[d]	3.90
21	CH_2F	NO_2	H	3.00[f]	3.20
22[g]	CHF_2	NO_2	H	2.82	3.60
23	CF_3	NO_2	H	3.00[h]	3.40
24	CF_3	Br	H	4.72[h]	4.35

[a] Inhibition of [^{3}H]clonidine binding to rat cortex preparation.
[b] Inhibition of [^{3}H]RX821002 to cloned human α_{2A}-adrenoceptors.
[c] Ref. 28.
[d] Ref. 14.
[e] Ref. 29.
[f] Ref. 13.
[g] Unpublished results with M. R. Seim. The synthesis and hPNMT inhibitory data for **22** will be published separately.
[h] Ref. 30.

supports our continued use of [^{3}H]clonidine to define α_2-adrenoceptor affinity.

4. Results and discussion

Compounds **9**, **10**, **16**, and **17** (Table 2) were synthesized and evaluated for PNMT inhibitory potency and α_2-adrenoceptor affinity. Although the α_2-adrenoceptor affinity of these compounds decreased, their PNMT inhibitory potency also decreased such that no selectivity was gained.

Docking studies (AutoDock 3.0)[31] were performed to investigate the binding of these inhibitors in the PNMT active site, using the recently reported X-ray crystal structures of PNMT co-crystallized with AdoHcy and either SK&F 29661 (**4**)[16] or 7-iodo-THIQ (**3**).[15] The docking of inhibitors into the hPNMT active site was performed on the *R*-enantiomer as a previous study on 3-hydroxymethyl-7-substituted-THIQs indicated that the *R*-enantiomer is preferred over the *S*- in the hPNMT active site.[32] Residues Lys57 and Met258 move within the PNMT active site depending on the hydrophobicity of the 7-substitutent.[15] Inhibitors that contain a 7-nitro substituent were docked into the crystal structure of hPNMT co-crystallized with SK&F 29661 (**4**),[16] whereas inhibitors that possess a 7-bromo substituent were docked into the crystal structure of hPNMT co-crystallized with 7-iodo-THIQ (**3**).[15] The amino acid residues surrounding the 3-position of the co-crystallized ligands SK&F 29661 (**4**) and 7-iodo-THIQ (**3**) remain in similar positions in both crystal structures.

The docking of **1** (3-CH_2OH, 7-NO_2) or **2** (3-CH_2OH, 7-Br) into the hPNMT crystal structure shows that the hydroxy moiety appears to hydrogen bond to Glu219 (Fig. 1 shows **2**). The docking studies also show that the 7-nitro moiety of **1** (similar to Fig. 2) may hydrogen bond to Lys57 as does the sulfonamide substituent of SK&F 29661 (**4**) and the 7-bromo substituent of **2** (Fig. 1) may make hydrophobic contacts with Met258 and Val53 as does the iodo group of 7-iodo-THIQ (**3**). The docking studies indicated that a 3-hydroxyethyl (**9**) or a 3-hydroxypropyl (**16**) substituent could take advantage of hydrogen bonds to Tyr35 and/or the main chain

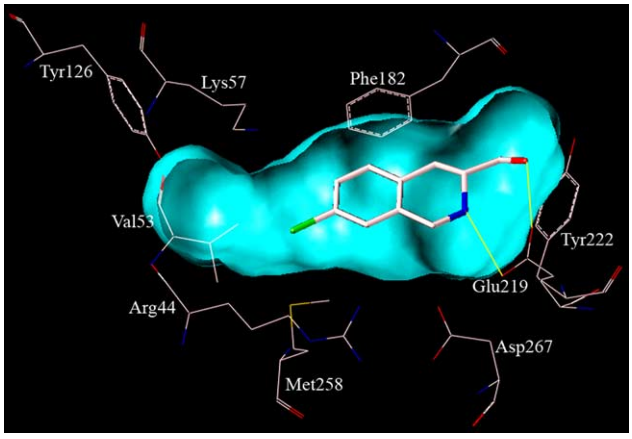


Figure 1. Compound **2** docked into the active site of hPNMT[15] and the amino acid residues that could interact with **2**, showing that the 3-hydroxymethyl moiety may hydrogen bond with Glu219. Yellow lines indicate possible hydrogen bonds. A Connolly (solvent accessible) surface of the active site is also shown. Carbon is white, nitrogen is blue, oxygen is red and bromine is green. Hydrogens are not shown for clarity.

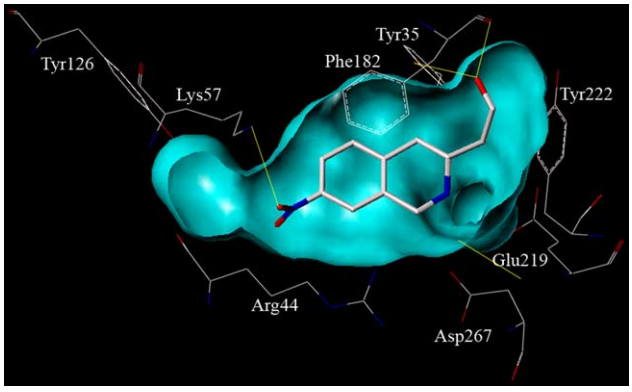
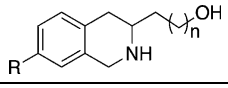


Figure 2. Compound **9** docked into the active site of hPNMT[16] and the amino acid residues that could interact with **9**, showing that the 3-hydroxyethyl moiety could hydrogen bond with Tyr35 and/or the main chain of Phe182. Yellow lines indicate possible hydrogen bonds. A Connolly (solvent accessible) surface of the active site is also shown. Carbon is white, nitrogen is blue, and oxygen is red. Hydrogens are not shown for clarity.

Table 2. In vitro activities of (±)-3-hydroxyethyl- and (±)-3-hydroxypropyl-7-substituted-THIQs



Compd	R	*n*	hPNMT K_i (μM ± SEM)	α_2[a] K_i (μM ± SEM)	Selectivity α_2/PNMT	C log *P*[b]
1[c]	NO_2	0	0.047 ± 0.006	19 ± 1	400	0.65
2[c]	Br	0	0.012 ± 0.001	1.0 ± 0.1	83	1.77
9	NO_2	1	0.51 ± 0.03	46 ± 4	90	0.91
10	Br	1	0.35 ± 0.01	1.3 ± 0.2	3.7	2.03
16	NO_2	2	1.4 ± 0.1	59 ± 6	42	0.93
17	Br	2	1.7 ± 0.2	4.8 ± 0.4	2.8	2.05

[a] Inhibition of [^{3}H]clonidine binding to rat cortex preparation.
[b] Calculated log *P*.
[c] The syntheses and PNMT (bovine adrenal enzyme) data for these compounds has been previously reported.[14]

of Phe182 (Fig. 2 shows **9**), rather than hydrogen bond to Glu219 as proposed for the 3-hydroxmethyl group (Fig. 1), and thus PNMT inhibitory potency should be retained. However, the biochemical data for these compounds indicates that no alternative interaction is gained in the hPNMT active site. Extension of the 3-hydroxyalkyl chain by just one carbon (**9**, **10**) resulted in a significant loss in PNMT inhibitory potency. The 3-hydroxymethyl substituent and THIQ nitrogen of **1** (3-CH_2OH, 7-NO_2) and **2** (3-CH_2OH, 7-Br) are in optimal positions to interact with Glu219 (Fig. 1 shows **2**). The loss of the hydrogen bond between Glu219 and the hydroxyl group of **9**, **10**, **16**, and **17** without the formation of a new hydrogen bond may be the reason why a significant loss in PNMT inhibitory potency is observed. Structure-based design is an iterative process and an X-ray crystal structure of PNMT co-crystallized with one of these inhibitors will be required to use these results for future structure-based design.

In conclusion, although the rationale for decreasing α_2-adrenoceptor affinity proved correct by extending the 3-hydroxyalkyl chain of **1** or **2** (**9**, **10**, **16**, **17**), these compounds showed no increase in selectivity. Molecular modeling suggested that longer 3-hydroxyalkyl chains should bind favorably in the PNMT active site, but the significant decrease in PNMT inhibitory potency indicates that an alternative hydrogen bond did not form. The 3-hydroxymethyl substituent of **1** or **2** appears to be an optimal group to interact with the PNMT active site.

Acknowledgements

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19. Compound **9**·HCl: mp 236–237 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.67 (s, 2H), 8.23 (s, 1H), 8.12 (d, J = 8.5 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 5.94 (s, 1H), 4.50–4.41 (m, 2H), 3.63 (m, 3H), 3.30–3.26 (m, 1H), 3.05–2.99 (m, 1H), 2.04 (m, 1H), 1.79 (m, 1H). Compound **10**·HCl: mp 235–236 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 2H), 7.51–7.46 (m, 2H), 7.19 (d, J = 8.2 Hz, 1H), 5.01 (s, 1H), 4.38–4.28 (m, 2H), 3.75–3.57 (m, 3H), 3.12–3.06 (m, 1H), 2.84–2.77 (m, 1H), 1.99–1.90 (m, 1H), 1.78–1.70 (m, 1H). Compound **16**·HCl: mp 237–243 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 2H), 8.22 (s, 1H), 8.12 (d, J = 8.7 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 4.50–4.37 (m, 2H), 3.46 (m, 3H), 3.29–3.24 (m, 2H), 2.93–2.86 (m, 1H), 1.79 (m, 1H), 1.67–1.59 (m, 3H). Compound **17**·HCl: mp 203–204 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 2H), 7.51 (s, 1H), 7.46 (d, J = 8.7 Hz, 1H), 7.19 (d, J = 8.5 Hz, 1H), 4.29 (m, 2H), 3.45 (m, 3H), 3.22 (m, 1H), 3.10–3.05 (m, 1H), 2.79–2.72 (m, 1H), 1.87–1.76 (m, 1H), 1.68–1.50 (m, 3H).
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