0026-895X/89/010093-05$02.00/0

MOLECULAR PHARMACOLOGY, 35:93-97

Conformational and Steric Aspects of Phenylethanolamine and Phenylethylamine Analogues as Substrates or Inhibitors of Phenylethanolamine *N*-Methyltransferase

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Received May 31, 1988; Accepted October 18, 1988

SUMMARY

The conformational and steric aspects of binding to phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28) for phenylethanolamine substrates and phenylethylamine inhibitors were probed with three conformationally defined analogues (**11**, **12**, and **13**) of phenylethylamine (**1**) and phenylethanolamine (**6**) containing the benzobicyclo[3.2.1]octane skeleton. The 2-aminotetralin (2AT) moiety in conformationally defined analogues **11**, **12**, and **13** exists in a half-chair conformation with an equatorial amino group. Although conformationally restricted phenylethylamine analogue 2AT (**3**, K_i = 6.8 μM) and conformationally restricted phenylethanolamine analogues (*cis*)- and (*trans*)-2-amino-1-tetralol (**9**, K_m = 22 μM; V_{max} = 0.15; 100 × V_{max}/K_m = 0.68; **10**, K_i = 9.4 μM) are good ligands for PNMT, none of the analogues **11**, **12**, and **13** showed activity as a substrate of PNMT. The fact that **11** (K_i = 206 μM) is more potent than analogues **4** (K_i = 1296 μM) and **5** (K_i = 479 μM), with a half-boat 2AT moiety, suggests that PNMT preferentially binds the half-chair conformation of 2AT at the active site. This is consistent with previous findings that a fully extended conformation for the aminoethyl side chain of phenylethylamine inhibitors is optimal for PNMT binding. The reduced activity of **11**, **12** (K_i = 1246 μM), and **13** (K_i = 3000 μM), compared with 2AT and (*cis*)- and (*trans*)-2-amino-1-tetralol (**9** and **10**) is consistent with a negative steric interference from the extra ethano bridge in **11**, **12**, and **13**. The results from **11**, **12**, and **13**, combined with previous findings, suggest that PNMT interacts better with relatively planar ligands.

PNMT (EC 2.1.1.28) was first isolated and characterized from the adrenal medulla (1), in which it catalyzes the methyl transfer from AdoMet to norepinephrine, to generate epinephrine. Recent interest in this enzyme arose after PNMT and epinephrine were detected in the CNS and the existence of epinephrine neurons in the CNS was established (2). It has been suggested that epinephrine, as a CNS neurotransmitter, may be involved in several important biological processes, including blood pressure regulation, release of pituitary hormones, and the regulation of α_2-adrenoreceptors (3). Because PNMT catalyzes the last step in epinephrine biosynthesis, it is a promising target for studying the functional role played by epinephrine as a CNS neurotransmitter (3). An inhibitor of PNMT could function to regulate the level of epinephrine in the CNS without affecting levels of dopamine and norepinephrine.

In order to design selective inhibitors for PNMT, the knowledge of binding requirements at the active site for both substrates and inhibitors is required. Phenylethylamines, such as phenylethylamine (**1**; Fig. 1), amphetamine (**2**), and 2AT (**3**), represent one major class of PNMT ligands and are usually competitive inhibitors of the PNMT-catalyzed methyl transfer reaction. Phenylethylamine (**1**) and amphetamine (**2**) are molecules with a flexible aminoethyl side chain. Restriction of the side chain conformation by incorporation of the phenylethylamine moiety into 2AT greatly enhances the potency (4) (**1**, K_i = 854 ± 55 μM; **2**, K_i = 817 ± 21 μM; 2AT, K_i = 6.8 ± 0.2 μM). Apparently, the phenylethylamine moiety in one of the favored conformations of 2AT corresponds to the conformation by which phenylethylamine interacts with PNMT.

However, 2AT still can assume several conformations. Attempts have been made to further reduce the number of possible conformations by incorporating the carbon skeleton of 2AT into bicyclic systems like benzobicyclo[2.2.2]octane (i.e., compound **4**) and benzobicyclo[2.2.1]heptane (i.e., compound **5**)

The research work was supported by National Institutes of Health research grants HL 34193 and GM 22988. This paper is taken in large part from the dissertation submitted by Q.Y. to the Graduate School of the University of Kansas for the Ph.D. degree (April, 1988) and it is paper 14 in our series "Conformationally defined adrenergic agents"; for paper 13 see Ref. 23. D.J.S. (1986) and Q.Y. (1988) are recipients of the Robert Irsay-Norman Dahle Award in Medicinal Chemistry at the University of Kansas. During the research work, D.J.S. was a National Institutes of Health predoctoral trainee, Grant GM 07775. B. W. was the Sterling Winthrop Undergraduate Research Fellow, Department of Medicinal Chemistry, University of Kansas, summer 1986.

ABBREVIATIONS: PNMT, phenylethanolamine *N*-methyltransferase (EC 2.1.1.28); 2AT, 2-aminotetralin; AdoMet, *S*-adenosyl-L-methionine; CNS, central nervous system.

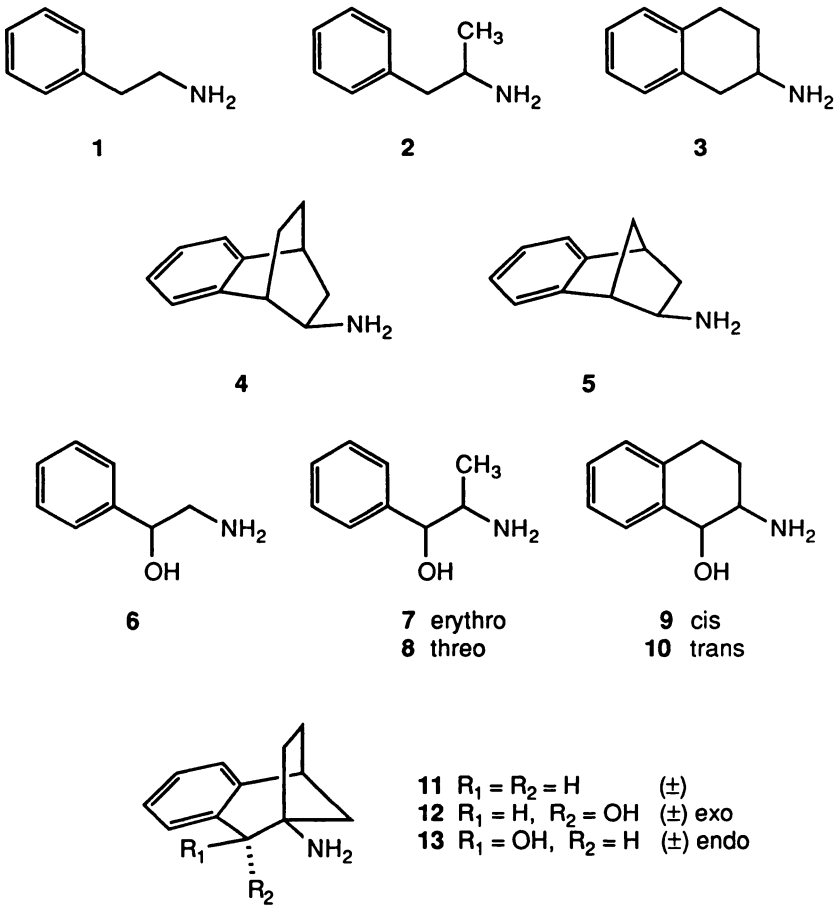


Fig. 1. Structures of the compounds mentioned in the text.

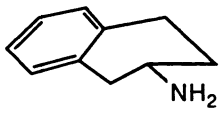
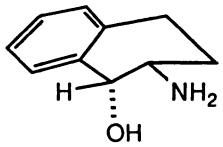
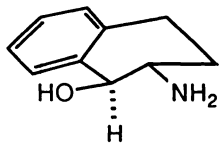
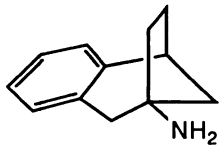
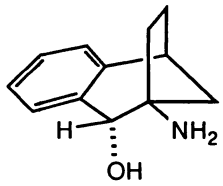
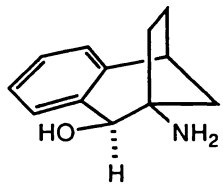
(5–8). However, analogues **4** and **5** showed reduced activities as PNMT ligands (**4**, K_i = 1296 μM; **5**, K_i = 479 μM) (9). Studies with 2AT derivatives using NMR experiments (10–13), X-ray crystallography (14, 15), and molecular orbital (16) and force field (12, 13, 16) calculations indicated that two low energy conformations for the aliphatic portion of 2AT are the half-chair conformations with the amino group in either an equatorial or an axial position. The energy difference between the two conformations is usually within 1 kcal/mol (12, 13, 16). In both the benzobicyclo[2.2.2]octane and the benzobicyclo[2.2.1] heptane ring systems, the carbon skeleton of 2AT exists in a half-boat conformation. Theoretical calculations (16) indicated that the half-boat conformation is unfavorable compared with the half-chair conformations by 3 to 5 kcal/mol. It is not clear whether the reduced activity is due to the steric interference in binding to PNMT from the extra bridging atoms in **4** and **5** or due to the unfavorable half-boat conformation for the 2AT moiety.

Phenylethanolamines are another class of PNMT ligands and they usually are substrates for the PNMT-catalyzed methyl transfer. Phenylethanolamine substrates differ from phenylethylamine inhibitors by the presence of a benzylic hydroxyl group. The conformational requirements for binding to PNMT for phenylethanolamine substrates are much less well studied than those for phenylethylamine inhibitors. Most phenylethanolamine substrates evaluated to date are molecules with a flexible aminoethyl side chain (17, 18). We recently studied several analogues of phenylethanolamine (**6**) for their activities as PNMT substrates or inhibitors. These analogues included (*cis*)- and (*trans*)-2-amino-1-tetralol (**9** and **10**) in both their racemic (19) and resolved (20) forms, as well as (20) optically active norephedrines (**7**) and norpseudoephedrines (**8**). The 2-amino-1-tetralols **9** and **10** (conformationally restricted phenylethanolamines) were more potent than were norephedrine and norpseudoephedrine (**7** and **8**; conformationally flexible phenylethanolamines), a result that is parallel with those from amphetamine and 2AT. This suggests that restriction of the side chain conformation of phenylethanolamines also enhances their ability to bind to the active site of PNMT. Only (*cis*)-2-amino-1-tetralol (**9**) showed activity as a PNMT substrate (K_m = 22 μM; V_{max} = 0.15; 100 × V_{max}/K_m = 0.68) whereas (*trans*)-2-amino-1-tetralol (**10**) was a potent inhibitor (K_i = 9.4 μM) (19), which suggests that not only the presence but also the orientation of the hydroxyl group is important for phenylethanolamine to act as a substrate for PNMT.

Although compound **5** showed reduced affinity (compared with 2AT) for PNMT (9), weak activity as a substrate was detected (18). Some analogues of **5**, with trifluoromethyl or methoxyl (but not hydroxyl) substitution at the aromatic ring, also showed varying degrees of activity as PNMT substrates (18, 21, 22). These results suggest that the benzylic hydroxyl group in phenylethanolamines might function as an "anchoring group" and help to achieve the side chain conformation required for the methyl transfer reaction to take place when the molecule binds to the enzyme. This "anchoring" effect may be needed for flexible molecules and also for the conformationally restricted 2-amino-1-tetralol but may no longer be needed in conformationally defined systems like the benzobicyclo[2.2.1] heptanes.

TABLE 1

In vitro activities of phenylethanolamine and phenylethylamine analogues as substrates or inhibitors of PNMT

	Compound[a]	K_i	K_m	V_{max}[b]
		μM		nmol/mg of protein/min
	**3**	6.8 ± 0.2		
	**9**, *cis*		22 ± 2[c]	0.15 ± 0.01[c]
	**10**, *trans*	9.4 ± 0.5[c]		
	**11**	206 ± 10		
	**12**, *exo*	1246 ± 81		
	**13**, *endo*	3000		

[a] All compounds tested were racemates.
[b] Units of V_{max} are nanomol of product/mg/min.
[c] Taken from Ref. 19.

To further study the binding requirements for phenylethanolamine substrates, as well as phenylethylamine inhibitors, we designed three conformationally defined analogues (**11**, **12**, and **13**) of phenylethylamine (**1**) and phenylethanolamine (**6**) with the benzobicyclo[3.2.1]octane skeleton. Two important features in these compounds are (i) the 2AT moiety exists in a half-chair conformation with an equatorial amino group, and (ii) the benzylic hydroxyl group has conformationally defined orientations. The evaluation of their properties as PNMT substrates or inhibitors and implications from these results are reported in this paper.

Materials and Methods

Chemicals. 2AT (**3**), (*cis*)- and (*trans*)-2-amino-1-tetralol (**9** and **10**), and the conformationally defined analogues **11**, **12**, and **13** were synthesized in this laboratory as racemates (19, 23). The structures of **12** and **13** were unambiguously assigned by NMR techniques (23) and X-ray crystallography (24). All samples were characterized by spectroscopic methods (IR, MS, ^{1}H NMR, and ^{13}C NMR). Combustion analyses for their HCl salts were within 0.4% of the theoretical values. AdoMet was obtained from Sigma Chemical Co. (St. Louis, MO) and [^{3}H]AdoMet (specific activity, 10–15 Ci/mmol) was purchased from New England Nuclear (Boston, MA).

***In Vitro* radiochemical assays.** Conformationally defined phenylethylamine analogue **11** and phenylethanolamine analogues **12** and **13** were evaluated for activity as both substrates and inhibitors of the PNMT-catalyzed methyl transfer. All compounds tested were racemates. Because PNMT in brain has similar properties to that in adrenal gland (3, 25), bovine adrenal PNMT was used in this study. It was purified according to the method of Connett and Kirshner through the isoelectric precipitation step (26). *In vitro* activity was assessed by use of a standard radiochemical assay that has been previously described for both substrates (27) and inhibitors (9). Briefly, a typical assay mixture consisted of 50 μl of 0.5 M phosphate buffer (pH 8.0), 25 μl of a 10 mM solution of unlabeled AdoMet, 5 μl of [^{3}H]AdoMet (approximately 300,000 dpm), 25 μl of substrate solution, 25 μl of inhibitor solution (if added), 25 μl of the enzyme preparation, and sufficient water to achieve a final volume of 250 μl. After incubation for 30 min at 37°, the reaction was terminated by the addition of 250 μl of 0.5 M borate buffer (pH 10) and extracted with 2 ml of toluene/isoamyl

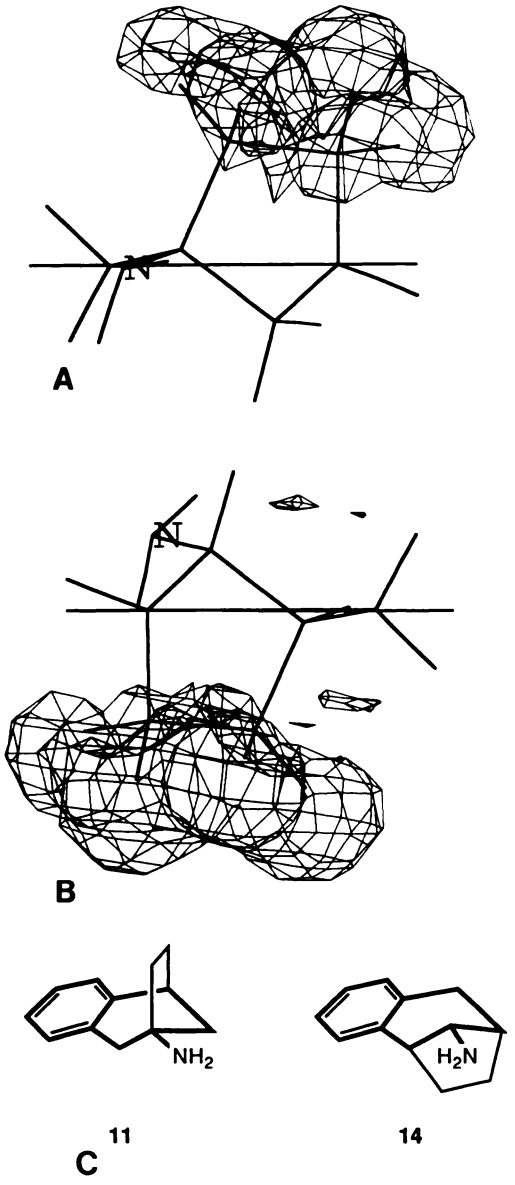


Fig. 2. Computer graphics-generated views of **11** (A) and **14** (B) along the plane of the aromatic ring to show the distorted half-chair conformation of the benzobicyclo[3.2.1]octane system. The figures A and B were generated with the SYBYL molecular graphics system (Tripos Associates, Inc., St. Louis, MO; version 3.4 was used). 2AT and compounds **11** and **14** were minimized in energy (MM2). Compounds **11** and **14** were fitted with 2AT with the FIT command and then two volume difference maps (subtracting 2AT from **11** and subtracting 2AT from **14**) were generated with the MVOLUME command to show the steric bulk compared with 2AT. Although both **11** and **14** have the half-chair conformation for the 2AT moiety, **11** has the steric bulk above the 2AT plane whereas **14** has the steric bulk below the 2AT plane. In both A and B, it is assumed that the 2AT moiety in **11** and **14** has the (2*S*)-configuration of the more potent enantiomer of 2AT, as shown in C with the *darkened lines*.

alcohol (7:3). The organic layer (1 ml) was removed, transferred to a scintillation vial, and diluted with cocktail for counting.

For the determination of kinetic constants for substrates, at least five concentrations of the variable substrate were employed in the assay. Inhibition constants were determined by using at least three different concentrations of the inhibitor with phenylethanolamine as the variable substrate.

Results and Discussion

The results for conformationally defined **11**, **12**, and **13** from *in vitro* testing on PNMT are summarized in Table 1. For reference, the more flexible 2AT (**3**) and (*cis*)- and (*trans*)-2-amino-1-tetralol (**9** and **10**) are also included. None of the three conformationally defined compounds, **11**, **12**, and **13**, showed activity as a PNMT substrate (up to 2 mM). Compound **11** (K_i = 206 μM) had more than 4 times the affinity for PNMT than did phenylethylamine (K_i = 854 μM). However, **12** and **13** exhibited only weak inhibitory activity, although the inhibition was competitive.

For the analogues examined in this study, two possible conformations of 2AT have been defined, a half-boat conformation in **4** and **5** and a half-chair conformation in **11**, **12**, and **13**. Compound **11** (K_i = 206 μM) was more active than either **4** (K_i = 1296 μM) or **5** (K_i = 479 μM), which suggests that the half-chair conformation of 2AT, as in **11**, is preferred by PNMT over the half-boat conformation present in **4** and **5**. It is reasonable to conclude that, inasmuch as 2AT is a potent inhibitor of PNMT (K_i = 6.8 μM), one of its low energy conformations corresponds to the active conformation. The half-chair conformation with an axial amino group would put the phenyl and amino groups of the phenylethylamine moiety in a *gauche* conformation. The *gauche* conformation for the phenylethylamine side chain has previously been found to show a considerably reduced affinity at binding to PNMT compared with the extended conformation (9). If, on the other hand, the active conformation of 2AT is a half-chair with an equatorial amino group, then one might expect **11**, in which this conformation is fixed, to be a more potent inhibitor than 2AT. Such was not observed. The extra ethano bridge in **11** introduces steric bulk above (or below) the planar 2AT moiety, which may interfere with binding of **11** to PNMT.

Compound **5** showed weak activity as a PNMT substrate (18) (K_m = 393 μM; V_{max} = 0.038; 100 × V_{max}/K_m = 0.0097) whereas **11** did not. The conformational definition of the half-chair of 2AT did not, in this case, convert an inhibitor into a substrate. This result is not surprising because conformationally defined **5** showed only weak activity as a substrate (18) and **4** showed no substrate activity at all (9). Thus, the presence of the half-chair conformation may enhance the binding to PNMT, but the steric hindrance from the extra ethano bridge might interfere with either the binding to PNMT or the methyl transfer catalyzed by PNMT, or both.

The conformations of (*cis*)- and (*trans*)-2-amino-1-tetralol derivatives have been studied by NMR (28, 29). The stable conformations for both the *cis*- and *trans*-isomers are the half-chair conformations for the cyclohexene portion with an equatorial amino group. Other studies on 1-tetralol derivatives by IR (30) and NMR (31) also indicated that the benzylic hydroxyl group prefers the pseudoaxial position. The chemical shift (31) for the benzylic carbon occurs at 65–67 ppm if the hydroxyl group is pseudoaxial or 69–70 ppm if the hydroxyl group is pseudoequatorial. We observed that the chemical shifts for the benzylic carbon were 65.19 and 69.27 ppm for (*cis*)- and (*trans*)-2-amino-1-tetralol (**9** and **10**), respectively, and 76.91 and 78.74 ppm for the conformationally defined *exo*-isomer **12** and *endo*-isomer **13**, respectively. These values are in agreement with the previous studies (31) and suggest that (*cis*)-2-amino-1-tetralol (**9**) has a half-chair conformation with an equatorial amino group and a pseudoaxial hydroxyl group, which has been defined in the *exo*-isomer **12**, and that (*trans*)-2-amino-1-tetralol (**10**) has a half-chair conformation with an equatorial amino group and a pseudoequatorial hydroxyl group, which has been defined in the *endo*-isomer **13**. Although both (*cis*)- and

(*trans*)-2-amino-1-tetralol (**9** and **10**) were potent PNMT ligands, both **12** and **13** had reduced affinity for the enzyme. The steric hindrance from the extra ethano bridge is a likely explanation. In comparing **12** and **13** with **11**, one sees that the presence of the benzylic hydroxyl group has an adverse effect on their binding to PNMT. It is possible that the enzyme can adapt conformationally to accommodate the ethano bridge in **11**, but this then places the benzylic hydroxyl group of **12** or **13** into a region of the enzyme where it cannot be accommodated.

We recently synthesized and evaluated another conformationally defined phenylethylamine analogue, **14** (32). Both analogues **11** and **14** contain a half-chair 2AT moiety and both are less active than 2AT (**11**, K_i = 206 μM; **14**, K_i = 106 μM; 2AT, K_i = 6.8 μM). We found (20) that the 2AT with the (2*S*)-configuration (K_i = 4.1 ± 0.1 μM) is more active than its (2*R*)-enantiomer (K_i = 10.0 ± 0.4 μM). If one draws the two conformationally defined analogues, **11** and **14**, from the same 2AT [(2*S*)- configuration], then both **11** and **14** will have different steric parameters because the extra ethano bridge resides on different faces of the 2AT moiety, as shown in Fig. 2. The results from **11**, **12**, and **13**, combined with that from **14**, suggest that the active site of PNMT might be a narrow groove or a slot, into which the relatively planar 2AT can fit easily but not our conformationally defined analogues with steric bulk above and below the 2AT plane.

Acknowledgments

We thank Dr. James A. Monn for helpful comments during the preparation of this manuscript.

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