Conformational Requirements of Substrates for Activity with Phenylethanolamine *N*-Methyltransferase

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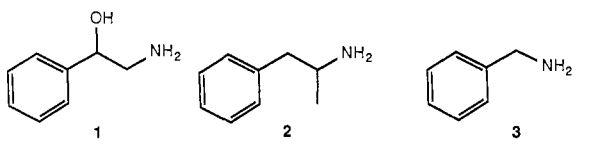
β-Phenylethanolamines have long been known to be substrates for the enzyme that converts norepinephrine to epinephrine (phenylethanolamine *N*-methyltransferase, PNMT, EC 2.1.1.28). In an effort to determine which, if any, particular conformation of the aminoethyl side chain of phenylethanolamines is required for PNMT active site binding and catalysis, we have prepared and evaluated conformationally restricted phenylethanolamine analogues **8–10**. The folded phenylethanolamine derivative 4-hydroxy-1,2,3,4-tetrahydroisoquinoline (**8**) is not a substrate and does not interact with the enzyme active site as an inhibitor as well as 1,2,3,4-tetrahydroisoquinoline (**6**). In the cyclic 2-aminotetralol systems, only *cis*-phenylethanolamine derivative **9** demonstrates activity as a PNMT substrate. The corresponding trans isomer **10** is not a substrate, in spite of enhanced active site interactions with respect to the parent analogue (2-aminotetralin, **4**). Comparison of the inhibition constants for the folded (**8**, K_i = 175 μM) and extended (**10**, K_i = 9 μM) phenylethanolamine analogues strongly suggests that simultaneous binding of both the amino and hydroxyl functionalities to the PNMT active site requires an extended aminoethyl side chain conformation.

Selective inhibition of the enzyme responsible for epinephrine biosynthesis, phenylethanolamine *N*-methyltransferase (PNMT, EC 2.1.1.28), is an objective that has not yet been attained. Our approach toward the design of a potent, yet selective, inhibitor of this enzyme involves the elucidation of the three-dimensional topography of the PNMT active site in terms of both bound substrates and competitive inhibitors.

Structure–activity relationship studies for aryl-substituted PNMT ligands indicate a similar mode of binding for the aromatic ring portion of phenylethanolamine (**1**) substrates[2] and amphetamine (**2**) inhibitors,[3] while the aromatic ring in benzylamine (**3**) inhibitors appears to bind in a slightly different way with respect to the others.[4] We



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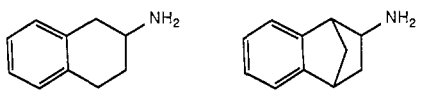
have explored the relationships that exist between ligand *conformation* and observed in vitro activity for PNMT inhibitors of both the amphetamine[5] and benzylamine[6] types. For amphetamine inhibitors, conformational restriction of the phenylethylamine pharmacophore through its incorporation into the 2-aminotetralin (2-AT, **4**) framework results in an enhancement in potency by a full order of magnitude over the fully flexible system.[7] Full conformational restriction of the 2-AT system as in **5** diminishes the in vitro activity as an inhibitor,[5] but also results in the remarkable ability for **5** to undergo PNMT catalysis.[8] This was the first example of a phenylethylamine not containing a heteroatom present in the benzylic position to exhibit this activity.[9,10] We have subsequently observed this phenomenon for other conformationally defined analogues of **2**.[11a,b]



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In the case of benzylamine inhibitors, conformational restriction of the aminomethyl side chain in **3** through its incorporation into the 1,2,3,4-tetrahydroisoquinoline (THIQ, **6**) framework results in a potency enhancement that closely parallels that observed in the case of amphetamine and 2-AT.[12] However, while full conformational

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restriction of the THIQ system resulted (as in the example of **4** and **5**) in a diminished ability for **7** to interact at the active site, this analogue (and four other conformationally defined benzylamines) did not display any activity as substrates for PNMT catalysis.[6]

The ability of **5** (and other conformationally defined analogues of amphetamine in which the aminoethyl side chain is held fixed in a fully extended conformation) to undergo PNMT catalysis suggested that a similar conformational requirement might be imposed on the more classical (phenylethanolamine) substrates for this enzyme. In this paper, we report the evaluation of conformationally restricted analogues of **1**, in which we have explored (1) the effect of side-chain conformation (folded in **8** vs a preferred fully extended one in **9** and **10**) and (2) the in-

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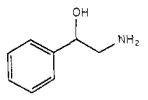
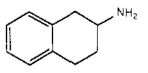
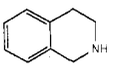
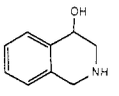
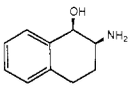
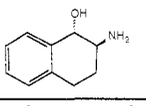
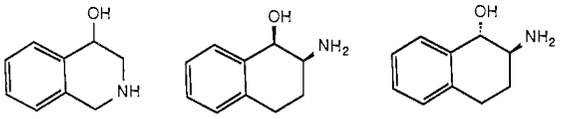
Table I. In Vitro Activity[a] of Phenylethanolamine and Some Conformationally Restricted Analogues of Phenylethanolamine and 2-Phenylethamine with PNMT

compd	no.	$K_i \pm$ SEM, μM	$K_m \pm$ SEM, μM	$V_{max} \pm$ SEM[b]	$100 \times V_{max}/K_m$
	1		70 ± 4.0	2.2 ± 0.07	3.1
	4	15 ± 2			
	6	10 ± 0.9			
	8	176 ± 26			
	9		22 ± 2.0	0.15 ± 0.007	0.68
	10	9.4 ± 0.5			

[a] Compounds 1, 4, and 8–10 were tested as racemates. [b] Units of V_{max} are nanomoles of product formed per milligram of protein per minute.

fluence of the relative stereochemistry (cis in **9** vs trans in **10**) on in vitro activity as substrates or inhibitors of PNMT.



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Chemistry. Compound 8 was prepared by the method of Ram,[13] and analogues **9** and **10** were prepared by the method of Thrift.[14] In each case, the final product was characterized by spectroscopic methods (infrared, proton nuclear magnetic resonance, and carbon nuclear magnetic resonance), as well as by electron impact mass spectrometry and melting point: **8**·HCl, mp 201–202 °C dec (lit.[15] mp 203 °C dec); **9**, mp 101–103 °C (lit.[14] mp 101–103 °C); **10**, mp 89–90 °C (lit.[14] mp 91–92 °C). Combustion analyses for the analytical samples (hydrochloride salts) were within 0.4% of the theoretical values.

Biochemistry. Conformationally restricted, racemic phenylethanolamines **8–10** were evaluated (as their hydrochloride salts) for activity as both substrates for PNMT and inhibitors of the PNMT-catalyzed methylation of **1**. For reference, racemic phenylethanolamine (**1**) and 2-AT (**4**) as well as THIQ (**6**) were also included. Bovine adrenal PNMT, which had been purified according to the method of Connett and Kirshner through the isoelectric precipitation step,[16] was used. In vitro activity was assessed by use of a standard radiochemical assay that has been previously described for both substrates[8] and inhibitors.[5] For the determination of the kinetic constants for substrates, at least five concentrations of the variable substrate were employed in the assay. Inhibition constants in this investigation were determined by using at least three different concentrations of the inhibitor with phenylethanolamine as the variable substrate.

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Results and Discussion

The observed in vitro activity as substrates or competitive inhibitors of PNMT for conformationally restricted phenylethanolamines **8–10**, as well as for compounds **1**, **4**, and **6**, is presented in Table I. With the exception of phenylethanolamine (**1**), the only compound examined in this study that displayed activity as a substrate (up to 2 mM) was analogue **9** The other compounds, although not undergoing PNMT catalysis, did inhibit the PNMT-catalyzed methylation of **1** by a competitive kinetic mechanism, denoting direct active-site interactions for each ligand.

From the data in Table I, several conclusions have been drawn. It is clear from the results of this study that, in the absence of strict conformational definition (as previously described for **5**),[8] the presence of a benzylic hydroxyl functionality is essential for activity of a 2-phenylethylamine as a substrate. The absence of the hydroxyl moiety in **4** results in a complete loss of this activity, in spite of the fact that active-site binding is apparently not affected (K_i = 15 μM). This result supports our contention that a hydroxyl group present in the benzylic position of 2-phenylethylamines serves to "anchor" the aminoethyl side chain in a particular conformation, which may then undergo PNMT catalysis. In the absence of this group, activity as a substrate is lost, unless (as in the case of **5**) this conformational bias is mimicked by locking the aminoethyl side chain in a fully extended arrangement, with the amino nitrogen residing close to the plane of the aromatic ring.[8]

The presence of a benzylic hydroxyl functionality in 2-phenylethylamines does not, however, in and of itself denote activity as a PNMT substrate. The aminoethyl side chain must be capable of assuming a fully extended arrangement with the aromatic ring. When this is not possible (as in the case of **8**), PNMT catalysis does not occur, and active-site binding is severely diminished (K_i = 176 μM) compared to that of the parent unsubstituted analogue **6** (K_i = 10 μM). This result strongly suggests that simultaneous binding of the aromatic ring, benzylic hydroxyl moiety, and the ring nitrogen to the PNMT active site is not allowed.

Considering 2-aminotetralols **9** and **10**, the conformation of the saturated ring portion of these molecules as well as

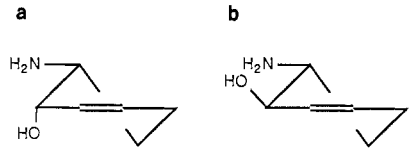

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Figure 1. Preferred conformations for *cis*- and *trans*-2-aminotetralol (**9** and **10**, respectively). (a) The low-energy form of **9** is represented by a half-chair conformation of the saturated ring with the primary amine occupying an equatorial position and the benzylic hydroxyl a pseudoaxial one. Interaction of **9** with PNMT results in methylation of the primary amine. (b) The low-energy form of **10** is represented by a half-chair conformation of the saturated ring, with the primary amine occupying an equatorial position and the benzylic hydroxyl functionality occupying a pseudoequatorial position. Interaction of **10** with PNMT does not result in catalysis (N-methylation), but does cause inhibition of the PNMT-catalyzed methylation of phenylethanolamine at lower concentrations than that observed for 2-aminotetralin (**4**), suggesting simultaneous binding of the aromatic ring, primary amine, and benzylic hydroxyl moieties to the PNMT active site.

the orientation of the hydroxyl and amino groups has been described by others.[17] Thus, in both cis and trans isomers, a half-chair conformation of the saturated ring is the preferred one, and the amino functionality resides in a equatorial (fully extended) position. This arrangement is also preferred in 2-AT (**4**),[18] its hydrochloride salt,[19] and its N-substituted derivatives.[20,21] In the case of the cis isomer **9**, the hydroxyl moiety is therefore oriented in a pseudoaxial position (Figure 1a), which has been previously shown by others to be the preferred orientation for 1-tetralol.[22,23] On the other hand, the benzylic hydroxyl group in trans isomer **10** is oriented in a pseudoequatorial position (Figure 1b). Thus, the principal difference between *cis*- and *trans*-2-aminotetralol is the orientation of the benzylic hydroxyl group (pseudoaxial in **9** and pseudoequatorial in **10**).

This result, combined with the observed activity as a PNMT substrate for **9** and lack of this activity for **10**, points to a specific side-chain orientation (Figure 1a) for phenylethanolamines which may have to be assumed for catalysis to occur. Thus, even though trans isomer **10** (K_i = 9 μM) retains all of the ability of 2-AT (K_i = 15 μM) to bind to the active site, it does not undergo the methyl transfer reaction. However, unlike the previous example where we observed a diminished ability for **8** to interact with the active site compared to **6**, simultaneous binding of the aromatic ring, benzylic hydroxyl moiety, and the primary amino group in **10** is apparently allowed.

Finally, a comparison of the conformationally restricted phenylethanolamine substrate **9** and phenylethanolamine **1** reveals the effect of conformational restriction on in vitro activity with PNMT. From the data in Table I, it is clear that **9** is converted by PNMT to N-methylated product 15 times less efficiently than is **1** (V_{max} for **9** = 0.15, V_{max} for **1** = 2.2); hence, the overall indicator of substrate activity ($100 \times V_{max}/K_m$) is correspondingly lower for **9** (0.68) than for **1** (3.1). However, cyclic ethanolamine **9** dissociates much more slowly from the enzyme–substrate complex, as reflected in the Michaelis constant (K_m = 22 μM) compared to that of the fully flexible **1** (K_m = 70 μM), denoting a stronger interaction between the PNMT active site and **9** compared to that for **1**.

In summary, we have explored the effect of phenylethanolamine conformation on in vitro activity as substrates or inhibitors of PNMT and have found that (1) in the absence of strict conformational definition of the side chain in phenylethylamines, a benzylic hydroxyl group is essential for activity as a PNMT substrate; (2) the presence of a benzylic hydroxyl moiety in cyclic 2-phenylethylamines does not, in and of itself, denote activity as a substrate; two other requirements must also be fulfilled, (a) the aminoethyl side chain must exist in a fully extended conformation, and (b) once in this form, the primary amine and the benzylic hydroxyl group must exist in a cis arrangement with respect to one another; (3) simultaneous binding of both of the heteroatoms in phenylethanolamine to the complementary active site surface requires a fully extended ethylamine side chain; and (4) restricting the fully flexible **1** through its incorporation into a cyclic framework (**9**) results in a substrate that binds more efficiently to the PNMT active site and is only slowly converted to N-methylated product with respect to **1**.

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Registry No. (±)-**8**, 75240-38-9; (±)-**8**·HCl, 105121-97-9; (±)-**9**, 64282-62-8; (±)-**9**·HCl, 110874-80-1; (±)-**10**, 64282-63-9; (±)-**10**·HCl, 110874-81-2; PNMT, 9037-68-7.

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