($CDCl_3$; 300 MHz) 7.39 (d, 1 H, J = 7.3 Hz, Ar H), 7.21 (d, 1 H, J = 7.3 Hz, Ar H), 7.13 (t, 1 H, J = 7.2 Hz, H2), 3.32 (s, 1 H, H10), 3.29–3.10 (m, 2 H, H9 and H5), 2.74 (d, 1 H, J = 17.1 Hz, H9), 2.40–2.04 (m, 3 H, H8, exo H6, and exo H7), 1.84–1.73 (m, 1 H, endo H6 or endo H7), 1.54–1.42 (m, 1 H, endo H6 or endo H7), 1.23 (s, 2 H, exchangeable in D_2O, NH_2); IR (film) 3420, 3345, 2946, 1590, 1460, 1350, 1316, 1184, 1147, 1115, 1084, 829, 797, 766 cm^{-1}; ^{13}C NMR ($CDCl_3$; 75 MHz) 142.71, 135.49, 132.79, 126.43 (q, J_{CF} = 31.1 Hz, C4), 125.31, 124.46 (q, J_{CF} = 274.0 Hz, CF_3), 122.95 (q, J_{CF} = 6.1 Hz), 58.37, 44.18, 40.63, 39.82, 32.66, 27.42. **23**·HCl (recrystallized from EtOH): mp >300 °C; EIMS, m/z (relative intensity) 241 (M^+, 24.8), 224 (5.9), 209 (7.7), 196 (100), 177 (10.0), 129 (16.8), 128 (13.7), 115 (25.6). Anal. ($C_{13}H_{15}ClF_3N$) C, H, N.

***syn*-10-Amino-4-(trifluoromethyl)-5,6,7,8-tetrahydro-5,8-methano-9*H*-benzocycloheptene (27).** Formamide **73** (187 mg; 0.69 mmol) afforded, after bulb-to-bulb distillation (74 °C (0.35 mm)), 160 mg (0.66 mmol; 95%) of **27** as a clear oil: ^1H NMR ($CDCl_3$; 300 MHz) 7.44 (d, 1 H, J = 7.2 Hz, Ar H), 7.26 (d, 1 H, J = 7.9 Hz, Ar H), 7.16 (t, 1 H, J = 7.9 Hz, H2), 3.42–3.20 (m, 3 H, H10, H9, and H5), 2.61 (d, 1 H, J = 17.6 Hz, H9), 2.26–2.19 (m, 1 H, H8), 2.16–1.92 (m, 2 H, exo H6 and exo H7), 1.80–1.68 (m, 1 H, endo H6 or endo H7), 1.56–1.42 (m, 1 H, endo H6 or endo H7), 1.22 (s, 2 H, exchangeable in D_2O, NH_2); IR (film) 3425, 3320, 2940, 1589, 1460, 1446, 1352, 1316, 1186, 1113, 1073, 903, 827, 796, 721 cm^{-1}; ^{13}C NMR ($CDCl_3$; 75 MHz) 139.57, 136.04, 132.74, 128.32 (q, J_{CF} = 29.5 Hz, C4), 125.25, 124.45 (q, J_{CF} = 273.8 Hz, CF_3), 123.39 (q, J_{CF} = 6.2 Hz), 54.65, 42.09, 36.49, 33.99, 33.29, 28.59. **27**·HCl (recrystallized from EtOH/Et_2O): mp >300 °C; EIMS, m/z (relative intensity) 241 (M^+, 30.4), 224 (7.7), 209 (9.9), 196 (100), 177 (7.6), 129 (11.0), 115 (11.8). Anal. ($C_{13}H_{15}ClF_3N$) C, H, N.

Radiochemical Assay for PNMT Activity. The assay employed in this study has been described elsewhere.[28,51] Briefly, a typical assay mixture consisted of 50 μL of 0.5 M phosphate buffer (pH 8.0), 25 μL of a 10 μM solution of unlabeled AdoMet, 5 μL of [*methyl*-^{3}H]AdoMet, containing approximately 2×10^6 dpm (specific activity approximately 15 mCi/mmol), 25 μL of substrate solution, 25 μL of inhibitor solution (if applicable), 25 μL of the enzyme preparation, 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 the addition of 250 μL of 0.5 M borate buffer (pH 10) and was extracted with toluene/isoamyl alcohol (7:3). The organic layer was removed and transferred to a scintillation vial and diluted with cocktail for counting. The mode of inhibition was ascertained by inspection of the $1/V$ vs $1/S$ plot of the data.

Acknowledgment. We are grateful to Dr. Garland Marshall and to Tripos Associates (St. Louis, MO) for a grant of the SYBYL software system and for helpful discussions. Research support from the Kansas Advanced Technology Commission made possible some of the hardware purchases for the University of Kansas Molecular Graphics and Modeling Laboratory in the Department of Medicinal Chemistry. Research Grants GM 22988 and HL 34193 from the U.S. Public Health Service and financial support from the University of Kansas General Research Fund made this work possible.

Inhibition of Phenylethanolamine *N*-Methyltransferase (PNMT) by Aromatic Hydroxy-Substituted 1,2,3,4-Tetrahydroisoquinolines: Further Studies on the Hydrophilic Pocket of the Aromatic Ring Binding Region of the Active Site

Daniel J. Sall[1] and Gary L. Grunewald*

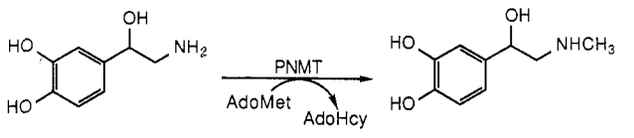
Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045. *Received March 6, 1987*

In a continuation of studies directed toward characterizing the hydrophilic pocket within the aromatic ring binding region of the active site of phenylethanolamine *N*-methyltransferase (PNMT), 5-, 6-, 7-, and 8-hydroxy-1,2,3,4-tetrahydroisoquinoline were prepared and evaluated as substrates and inhibitors of PNMT. In order to discern the necessity of an acidic hydrogen for interaction at this pocket the corresponding methyl ethers were also evaluated. The enhanced affinity of 7-hydroxy-1,2,3,4-tetrahydroisoquinoline (**16**) versus tetrahydroisoquinoline (**13**) itself indicates that a hydrophilic pocket exists off of carbon C7 in bound tetrahydroisoquinolines. The diminished affinity of the corresponding methyl ether is consistent with a requirement for the acidic hydrogen of **16** for interaction of the aromatic hydroxyl at this site. From the relative activities of the other regioisomeric aromatic hydroxyl-substituted tetrahydroisoquinolines, their corresponding methyl ethers, and 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, it appears that the hydrophilic pocket is spatially compact with respect to bound tetrahydroisoquinolines and is surrounded by larger areas of lipophilic character. To allow a comparison of the results of this study with previous data on bound β-phenylethylamines, the methyl ethers of 5-, 6-, 7-, and 8-hydroxy-*exo*-2-aminobenzonorbornene and of 5- and 6-hydroxy-*anti*-9-aminobenzonorbornene were also evaluated for their activity as substrates and inhibitors for PNMT. The results of this study are in agreement with previous findings for bound β-phenylethylamines and support the conclusion that the natural substrate for PNMT, norepinephrine, has a different active site binding orientation than most known substrates and competitive inhibitors of the enzyme.

In recent years, we have been engaged in the design of a potent yet selective inhibitor of phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28). This enzyme is known to catalyze the transfer of an active methyl group from *S*-adenosyl-L-methionine (AdoMet) to the primary amine of norepinephrine (NE, **1**) to produce the hormone/neurotransmitter epinephrine (Epi, **2**; Figure 1).[2] Since initial reports that PNMT exists within the mammalian central nervous system (CNS),[3-6] Epi has been implicated in a multitude of physiological functions including the regulation of blood pressure,[7-11] the release

(1) National Institutes of Health Predoctoral Trainee (Grant GM 07775) and 1986 recipient of the Robert Irsay–Norman Dahle Award in Medicinal Chemistry at the University of Kansas.
(2) Axelrod, J. *J. Biol. Chem.* **1962**, *237*, 1657.
(3) Diaz Borges, J. M.; Urbina, M.; Drujan, B. D. *Neurochem. Res.* **1978**, *3*, 15.
(4) Ciaranello, R. D.; Barchas, R. E.; Byers, G. S.; Stemmle, D. W.; Barchas, J. D. *Nature (London)* **1969**, *221*, 368.
(5) Hokfelt, T.; Fuxe, K.; Goldstein, M.; Johansson, O. *Brain Res.* **1974**, *66*, 235.
(6) Pohorecky, L. A.; Zigmond, M.; Karten, H.; Wurtman, R. J. *J. Pharmacol. Exp. Ther.* **1969**, *165*, 190.

0022-2623/87/1830-2208$01.50/0



Norepinephrine 1 Epinephrine 2

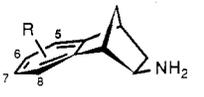
Figure 1. The last step in the biosynthetic pathway of epinephrine involving the transfer of an active methyl group from *S*-adenosyl-L-methionine (AdoMet) to the primary amine of norepinephrine. The enzyme that catalyzes this transformation is phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28).

(and inhibition of release) of pituitary hormones,[12-14] and the regulation of α_2-adrenoceptors.[15-17] The involvement of CNS Epi in such a variety of functions has made PNMT a popular target of inhibitor design, and to date, a number of potent inhibitors of this enzyme have been synthesized.[18-21] Unfortunately, all of these inhibitors interact at other biologically relevant sites (i.e., α_2-adrenoceptors[22-25]) and therefore suffer from a lack of selectivity. This drawback has severely limited their use as pharmacological tools in further elucidating and understanding the role of Epi within the CNS.

Our efforts toward the design of a potent yet highly selective inhibitor of PNMT have centered on determining the overall topography of the active site of the enzyme by defining those interactions that occur when substrates and competitive inhibitors bind. Enhanced selectivity may then result through the design of inhibitors that take advantage of those binding features that are found to be exclusively characteristic of the active site of PNMT.

One binding region within the active site of PNMT that has received considerable attention is the site at which the aromatic ring of substrates and competitive inhibitors interact. It has been shown that electron-withdrawing and lipophilic substituents on the aromatic ring greatly enhance the binding affinity of phenylethanolamines (substrates),[26-28] amphetamines (competitive inhibitors)[26,28,29] and benzylamines (also competitive inhibitors).[28,30] From these studies, it has been concluded that the aromatic ring binding region within the active site of PNMT contains a high degree of lipophilic character. In conflict with this conclusion, however, is the fact that the natural substrate for the enzyme, norepinephrine, displays high activity as a substrate (K_m = 4.7 ± 1.2 μM)[31] while possessing the hydrophilic catechol functionality. For this reason, we have been actively involved in defining those binding interactions that occur when ligands possessing aromatic hydroxyl groups bind at the active site of the enzyme. Ultimately, we hope to determine where, in the aromatic ring binding region of the active site of PNMT, the catechol portion of NE interacts.

As an initial approach to this problem, we have employed aryl hydroxy-substituted, conformationally defined amphetamine analogues modeled after parent systems **3** and **4**. Consistent with the amphetamine class, analogues **3** and **4** were shown to be competitive inhibitors of PNMT.[32] In addition, it was found that **3** and **4** displayed marginal activity as substrates.[26,31] It has been proposed that locking the ethylamine side chain of amphetamines into a fully extended conformation, as in **3** and **4**, serves the same purpose as the β-hydroxyl of phenylethanolamine substrates so as to anchor the amine in a region of the active site in which methylation can occur. With these results, catechol analogue **5** was prepared in an attempt to determine which orientation of the catechol hydroxyl groups, in substrates such as NE, leads to optimal interaction at the active site of PNMT.[31] It was expected that enhanced substrate activity, over parent analogue **3**, would result if the catechol nucleus interacted in a manner similar to NE. When it was found that catechol analogue **5** was rapidly oxidized under our assay conditions, we turned our attention to the synthesis and biological evaluation of the more stable phenol derivatives **6**–**11**.[33]



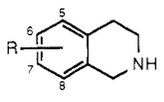
3, R = H
5, R = 6,7-$(OH)_2$
6, R = 5-OH
7, R = 6-OH
8, R = 7-OH
9, R = 8-OH

4, R = H
10, R = 5-OH
11, R = 6-OH

(7) Wijnen, H. J. L. M.; Versteeg, D. H. G. *Brain Res.* **1977**, *135*, 180.
(8) Saavedra, J. M.; Grobecker, H.; Axelrod, J. *Circ. Res.* **1978**, *42*, 529.
(9) Renaud, B.; Fourniere, S.; Denoroy, L.; Vincent, M.; Pujol, J.-F.; Sassard, J. *Brain Res.* **1978**, *159*, 149.
(10) Saavedra, J. M. *Brain Res.* **1979**, *166*, 283.
(11) Fuxe, K.; Ganten, D.; Bolme, P.; Agnati, L. F.; Hokfelt, T.; Anderson, K.; Goldstein, M.; Harfstrand, A.; Unger, T.; Rascher, W. *Central Adrenaline Neurons: Basic Aspects and Their Role in Cardiovascular Disease*; Permagon: New York, 1980; p 259.
(12) Crowley, W. R.; Terry, L. C. *Brain Res.* **1981**, *204*, 231.
(13) Kalra, S. P.; Crowley, W. R. *Endocrinology (Baltimore)* **1982**, *111*, 1403.
(14) Weiner, R. I.; Ganong, W. F. *Physiol. Rev.* **1978**, *58*, 905.
(15) Stolk, J. M.; Vantini, G.; Perry, B. D.; Guchhait, R. B.; U'Prichard, D. C. *J. Pharmacol. Exp. Ther.* **1984**, *230*, 577.
(16) Ruffolo, R. R.; Goldberg, M. R.; Morgan, E. L. *J. Pharmacol. Exp. Ther.* **1984**, *230*, 595.
(17) Perry, B. D.; Stolk, J. M.; Vantini, G.; Guchhait, R. B.; U'Prichard, D. C. *Science (Washington, D.C.)* **1983**, *221*, 1297.
(18) Bondinell, W. E.; Chapin, F. W.; Frazee, J. S.; Girard, G. R.; Holden, K. G.; Kaiser, C.; Maryanoff, C.; Perchonock, C. D.; Gessner, G. W.; Hieble, J. P.; Hillegass, L. M.; Pendleton, R. G.; Sawyer, J. L. *Drug Metab. Rev.* **1983**, *14*, 709.
(19) Grunewald, G. L.; Vincek, W. C.; Davis, D. P.; Borchardt, R. T. *Catecholamines: Basic Clin. Front. Proc. Int. Catecholamine Symp., 4th, 1978* **1979**, *1*, 189.
(20) Fuller, R. W.; Roush, B. W.; Molloy, B. B. *Adv. Enzyme Regul.* **1974**, *12*, 311.
(21) Kaiser, C.; Pendleton, R. G. *Intra-Sci. Chem. Rep.* **1974**, *8*, 43.
(22) Goldstein, M.; Saito, M.; Lew, J. Y.; Hieble, J. P.; Pendleton, R. G. *Eur. J. Pharmacol.* **1980**, *67*, 305.
(23) Pendleton, R. G.; Hieble, J. P. *Res. Commun. Chem. Pathol. Pharmacol.* **1981**, *34*, 399.
(24) Toomey, R. E.; Horng, J. S.; Hemrick-Luecke, S. K.; Fuller, R. W. *Life Sci.* **1981**, *29*, 2467.
(25) Biollaz, B.; Biollaz, J.; Kohlman, O., Jr.; Bresnahan, M.; Gavras, I.; Gavras, H. *Eur. J. Pharmacol.* **1984**, *102*, 515.
(26) Rafferty, M. F.; Grunewald, G. L. *Mol. Pharmacol.* **1982**, *22*, 127.
(27) Fuller, R. W.; Hemrick, S. K.; Molloy, B. B. *Res. Commun. Chem. Pathol. Pharmacol.* **1977**, *18*, 577.
(28) Rafferty, M. F.; Borchardt, R. T.; Grunewald, G. L. *J. Med. Chem.* **1982**, *25*, 1204.
(29) Fuller, R. W.; Mills, J.; Marsh, M. M. *J. Med. Chem.* **1971**, *14*, 322.
(30) Fuller, R. W.; Molloy, B. B.; Day, W. A.; Roush, B. W.; Marsh, M. M. *J. Med. Chem.* **1973**, *16*, 101.
(31) Grunewald, G. L.; Pleiss, M. A.; Rafferty, M. R. *Life Sci.* **1982**, *31*, 993.
(32) Grunewald, G. L.; Borchardt, R. T.; Rafferty, M. F.; Krass, P. *Mol. Pharmacol.* **1981**, *20*, 377.
(33) Grunewald, G. L.; Arrington, H. S.; Bartlett, W. J.; Reitz, T. J.; Sall, D. J. *J. Med. Chem.* **1986**, *29*, 1972.

While none of the analogues **6–11** displayed activity as substrates, varying degrees of competitive inhibition were observed.[33] The enhanced binding affinity of **7** and **11** (K_i = 304 and 108 μM, respectively) over the corresponding parent unsubstituted analogues **3** and **4** (K_i = 479 and 258 μM, respectively) suggested that the aromatic hydroxyl group interacts with a discrete hydrophilic site within the aromatic ring binding region of the active site of PNMT. The dramatic decrease in binding affinity (K_i = 1000–10 000 μM) when the aromatic hydroxyl group was substituted at any point on the aromatic ring other than carbon C6 (as in **6** and **8–10**) not only suggested that this hydrophilic pocket has a finite directionality with respect to the point of amine interaction in bound β-phenylethylamines, but that it is also spatially compact and surrounded by larger areas of lipophilic character. It was this apparent minimal hydrophilic character at the aromatic ring binding region of the active site of PNMT that led us to conclude that the natural substrate, norepinephrine, has a different active site binding orientation than most known substrates and competitive inhibitors of the enzyme.

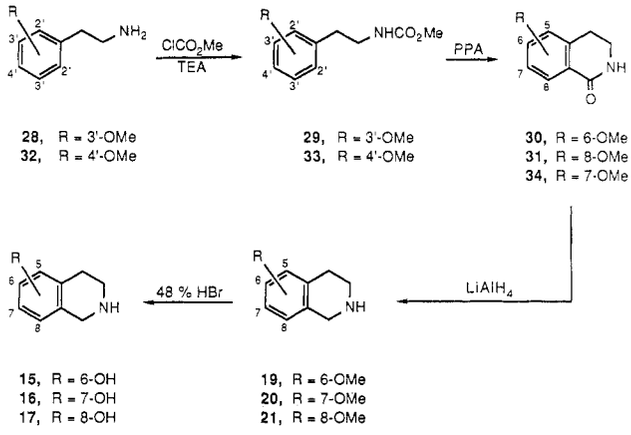
The fact that aromatic hydrophilic substituents also enhance the binding affinity of benzylamines (competitive inhibitors) was suggested by the pronounced enhancement in activity of SKF 29661[34] (**12**; K_i = 0.55 μM) over the parent 1,2,3,4-tetrahydroisoquinoline (**13**; K_i = 10.3 μM). That the acidic sulfonamido hydrogens are a necessary requirement for the high affinity of SKF 29661[35] may suggest that the sulfonamide acts as a hydrogen bond donor at the hydrophilic pocket. Unlike the case of bound β-phenylethylamines, however, little else is known about the existing hydrophilic pocket as it relates to the binding orientation of benzylamine inhibitors. Assuming that the C_{Ar}–S bond undergoes free rotation, the site of interaction of the sulfonamide NH_2 could exist in a region, both above and below the plane of the aromatic ring, extending from carbon C6 to C8. It is the purpose of the present study to define the spatial and directional limitations of the hydrophilic pocket in relationship to the point of amine interaction of bound tetrahydroisoquinolines and, presumably, of other bound benzylamines. Information of this type, coupled with the previous findings for bound conformationally defined β-phenylethylamines, may ultimately lead to an understanding of the active site binding orientation of the natural substrate norepinephrine.



12, R = 7-SO_2NH_2
13, R = H
14, R = 5-OH
18, R = 5-OMe
15, R = 6-OH
19, R = 6-OMe
16, R = 7-OH
20, R = 7-OMe
17, R = 8-OH
21, R = 8-OMe

In this paper we report the synthesis and activity as inhibitors of PNMT of phenolic tetrahydroisoquinolines **14–17**. Analogues **14–17** possess the phenolic hydroxyl that is similar in acidity to the sulfonamide NH_2 (pK_a of 10.0 and 10.4, respectively) and is capable of acting as a hydrogen bond donor at the hydrophilic pocket. In addition, since the region of space where the acidic OH of **14–17** could interact is more limited than that of the acidic NH of the sulfonamide **12**, phenols **14–17** should provide a clear understanding of the spatial and directional limitations of the interaction of hydrophilic aromatic substituents of bound benzylamines. Further, in order to determine the necessity of the acidic hydrogens in the binding of **14–17**, the methyl ethers **18–21** were also evaluated. Finally, in an attempt to better understand the role of the phenolic hydroxyl in the binding of conformationally defined amphetamines **6–11**, the methyl ethers **22–27**, which were available as intermediates in the syntheses of **6–11**,[33] were tested for their activity as both substrates and inhibitors of PNMT.

Scheme I



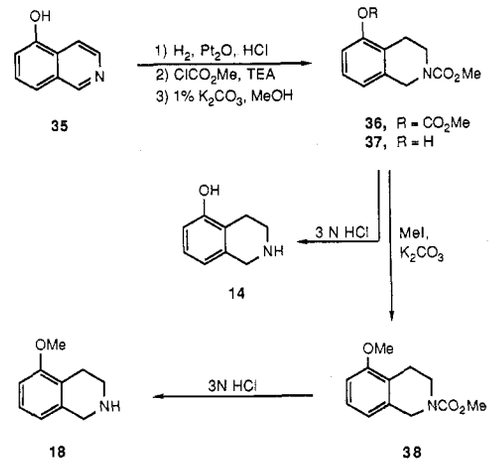
28, R = 3'-OMe
32, R = 4'-OMe
29, R = 3'-OMe
33, R = 4'-OMe
30, R = 6-OMe
31, R = 8-OMe
34, R = 7-OMe
15, R = 6-OH
16, R = 7-OH
17, R = 8-OH
19, R = 6-OMe
20, R = 7-OMe
21, R = 8-OMe

Chemistry. Tetrahydroisoquinolines **14–21** have been previously synthesized by a variety of methods.[36-42] However, we have employed an alternate approach toward the preparation of **15–17** and **19–21** based on the method of Lansbury et al.[43] for the closure of *N*-(methoxycarbonyl)-2-phenylethylamine to the 3,4-dihydroisoquinolin-1-one (Scheme I, R = H). This route was chosen on the basis of the availability of the starting methoxy-substituted 2-phenylethylamines as well as the ease with which they are converted to the requisite *N*-methoxycarbonyl derivatives. In addition, amines **15**, **17**, **19**, and **21** are derived from a divergent synthesis sharing the common intermediate *N*-(methoxycarbonyl)-2-(3′-methoxyphenyl)ethylamine and therefore are accessible in a limited number of steps.

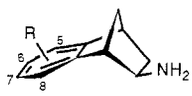
Synthesis of the 6-, 7-, and 8-substituted 1,2,3,4-tetrahydroisoquinolines, **15–17** and **19–21**, is shown in Scheme I. Preparation of amines **15**, **17**, **19**, and **21** begins with conversion of commercially available 2-(3′-methoxyphenyl)ethylamine (**28**) to methyl carbamate **29** by reaction with methyl chloroformate and triethylamine. Cyclization of **29** with polyphosphoric acid (PPA)[43] afforded a 75% yield of a mixture of 6-methoxy- and 8-methoxy-3,4-dihydroisoquinolin-1-one (**30** and **31**, respectively) in a ratio of 2:1, respectively. After separation by chromatography, lithium aluminum hydride reduction of **30** and **31** yielded

(34) Pendleton, R. G.; Gessner, G.; Weiner, G.; Jenkins, B.; Sawyer, J.; Bondinell, W.; Intoccia, A. *J. Pharmacol. Exp. Ther.* **1979**, *208*, 24.
(35) Blank, B.; Krog, A. J.; Weiner, G.; Pendleton, R. G. *J. Med. Chem.* **1980**, *23*, 837.
(36) Robinson, R. A. *J. Am. Chem. Soc.* **1947**, *69*, 1944.
(37) Schenker, F.; Schmidt, R. A.; Williams, T.; Brossi, A. *J. Heterocycl. Chem.* **1971**, *8*, 665.
(38) Schenker, F.; Schmidt, R. A.; Leimgruber, W.; Brossi, A. *J. Med. Chem.* **1966**, *9*, 46.
(39) Durand, S.; Lushinchi, X.; Moreau, R. C. *Bull. Soc. Chim. Fr.* **1961**, 270.
(40) Helfer, L. *Helv. Chem. Acta* **1924**, *7*, 945.
(41) Bobbit, J. M.; McNew Kiely, J.; Khanna, K. L.; Ebermann, R. *J. Org. Chem.* **1965**, *30*, 2247.
(42) Schlittler, E.; Muller, J. *Helv. Chem. Acta* **1948**, *31*, 914.
(43) Lansbury, P. T.; Colson, J. G.; Mancuso, N. R. *J. Am. Chem. Soc.* **1964**, *86*, 5225.

Scheme II



6-methoxy- and 8-methoxy-1,2,3,4-tetrahydroisoquinoline (**19** and **21**, respectively). Methyl ether cleavage with 48% HBr afforded the phenolic tetrahydroisoquinolines **15** and **17** as their hydrobromide salts.



22, R = 5-OMe
23, R = 6-OMe
24, R = 7-OMe
25, R = 8-OMe

26, R = 5-OMe
27, R = 6-OMe

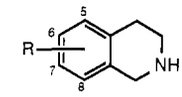
The isomeric purity of the 6- and 8-substituted series was determined at the methoxy amine stage (**19** and **21**) by gas chromatography. Each isomer was found to be at least 99.5% pure. Assignment of the regiochemistry (6- vs 8-substitution) was made at various stages in the synthetic pathway by analysis of the aromatic proton NMR resonances. In the case of lactams **30** and **31**, a deshielding effect by the carbonyl at carbon C1 shifts the adjacent aryl hydrogen H8 in **30** downfield relative to the other aryl hydrogens (8.05 ppm as opposed to 6.60–6.96 ppm). This effect is not seen for **31**, which lacks the adjacent H8 hydrogen. Finally, assignment of the aryl-substitution pattern in the hydrobromide salts of amines **15** and **17** follows from comparison of the chemical shifts and multiplicity of the aryl hydrogens with those published in the literature for the corresponding hydrochloride salts.[37]

Synthesis of 7-hydroxy- and 7-methoxy-1,2,3,4-tetrahydroisoquinoline (**16** and **20**, respectively) parallels the synthesis of the 6- and 8-substituted regioisomers and is also depicted in Scheme I. Conversion of commercially available 2-(4′-methoxyphenyl)ethylamine (**32**) to the corresponding *N*-methoxycarbonyl derivative **33**, followed by ring closure with PPA, afforded 7-methoxy-3,4-dihydroisoquinolin-1-one (**34**). For this particular regioisomer, cyclization proceeded in only a 60% yield. Neither extended nor shortened reaction times increased the yield significantly. The major byproduct in the reaction, 2-(4′-methoxyphenyl)ethylamine (**32**; 30% based on carbamate **33**), could result from hydrolysis either of the starting methyl carbamate or of an intermediary acylium-type ion. Reduction of **34** to the 1,2,3,4-tetrahydroisoquinoline **20**, followed by methyl ether cleavage, afforded 7-hydroxy-1,2,3,4-tetrahydroisoquinoline (**16**) as its hydrobromide salt. The chemical shifts and multiplicity of the aryl hydrogens in **16**·HBr were consistent with those published for the corresponding hydrochloride salt.[37]

The 5-hydroxy- and 5-methoxy-1,2,3,4-tetrahydroisoquinolines (**14** and **18**, respectively; Scheme II) were prepared from commercially available 5-hydroxyisoquinoline. Initial attempts to purify the starting 5-hydroxyisoquinoline (**35**; 90% technical grade) as well as the corresponding hydrochloride salt proved unsuccessful. The crude 5-hydroxyisoquinoline hydrochloride was reduced directly to 5-hydroxy-1,2,3,4-tetrahydroisoquinoline by catalytic hydrogenation. Attempted purification at this stage also proved futile. In order to facilitate the purification of a stable intermediate, the phenolic tetrahydroisoquinoline was converted to the *N*,*O*-bis(methoxycarbonyl) derivative **36**. Selective hydrolysis, with 1% K_2CO_3 in methanol,[44] afforded *N*-(methoxycarbonyl)-5-hydroxy-1,2,3,4-tetrahydroisoquinoline (**37**). This intermediate could easily be purified for spectral analysis but slowly decomposed with time.

Preparation of 5-hydroxy-1,2,3,4-tetrahydroisoquinoline (**14**) as its hydrochloride salt followed from acid hydrolysis of **37**. The methyl ether **18** was readily obtained by methylation of **37** with iodomethane[45] followed by acid hydrolysis to yield 5-methoxy-1,2,3,4-tetrahydroisoquinoline (**18**) as its hydrochloride salt. The proton NMR of **14**·HCl was in agreement with the literature.[37]

Table I. In Vitro Inhibition of PNMT by Aromatic-Substituted Tetrahydroisoquinolines **12–21**



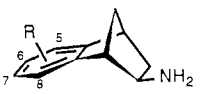
compound	$K_i \pm$ SEM, μM	compound	$K_i \pm$ SEM, μM
12, R = 7-SO_2NH_2	0.55 ± 0.04	**18**, R = 5-OMe	550 ± 40
13, R = H	10.3 ± 0.9	**19**, R = 6-OMe	169 ± 13
14, R = 5-OH	90 ± 4	**20**, R = 7-OMe	20 ± 1.6
15, R = 6-OH	7.8 ± 0.3	**21**, R = 8-OMe	3.8 ± 0.2
16, R = 7-OH	2.6 ± 0.1	**39**, R = 6,7-$(OH)_2$	5.7 ± 0.35[a]
17, R = 8-OH	1082 ± 60		

[a] Taken from ref 31.

Biochemistry. Amines **15–17** were evaluated as their hydrobromide salts for their activity as inhibitors of PNMT. Their respective methyl ethers, **19–21**, as well as amine **14** and its corresponding methyl ether, **18**, were evaluated as their hydrochloride salts. Methoxy-substituted, conformationally defined amphetamines **22–27** were tested as their hydrochloride salts for activity as both substrates and inhibitors of PNMT. Bovine adrenal PNMT,[46] which had been purified according to Connett and Kirshner through the isoelectric precipitation step,[47] was used. In vitro activity was assessed by use of a standard radiochemical assay that has been previously described for both substrates[48] and inhibitors.[32] For the determination of the kinetic constants for substrates, at least five concentrations of the variable substrate were assayed. Inhibition constants in this investigation were determined by using at least three different concentrations of the inhibitor with phenylethanolamine as the variable substrate.

(44) Meyers, A. I.; Tomioka, K.; Roland, D. M.; Comins, D. *Tetrahedron Lett.* **1978**, 1375.
(45) Vyas, G. N.; Shah, N. M. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, p 836.
(46) Adrenal PNMT has been found to be similar to the brain enzyme in terms of its susceptibility to inhibitors: Fuller, R. W. *Annu. Rev. Pharmacol. Toxicol.* **1982**, *22*, 31 and references therein.
(47) Connett, R. J.; Kirshner, N. *J. Biol. Chem.* **1970**, *245*, 329.
(48) Grunewald, G. L.; Grindel, J. M.; Vincek, W. C.; Borchardt, R. T. *Mol. Pharmacol.* **1975**, *11*, 694.

Table II. Aromatic-Substituted Conformationally Defined Phenylethylamines **3–11** and **22–27** as Substrates and Inhibitors of PNMT in Vitro



3, R = H
5, R = 6,7-$(OH)_2$
6, R = 5-OH
7, R = 6-OH
8, R = 7-OH
9, R = 8-OH
22, R = 5-OMe
23, R = 6-OMe
24, R = 7-OMe
25, R = 8-OMe

4, R = H
10, R = 5-OH
11, R = 6-OH
26, R = 5-OMe
27, R = 6-OMe

cmpd	$K_i \pm$ SEM, μM	$K_m \pm$ SEM, μM	V_{max}[a] $\pm$ SEM	$(V_{max}/K_m) \times 100 \pm$ SEM
3	479 ± 27[b]	392 ± 35[b]	0.04 ± 0.003[b]	0.01 ± 0.002[b]
4	258 ± 27[b]	151 ± 19[b]	0.17 ± 0.006[b]	0.12 ± 0.012[b]
5	487 ± 82[c]	686 ± 211[c]	0.44 ± 0.05[c]	0.06 ± 0.01[c]
6	>10000[d]			
7	304 ± 15[d]			
8	1114 ± 118[d]			
9	>3000[d]			
10	>4000[d]			
11	108 ± 5[d]			
22	>2000	970 ± 102	0.22 ± 0.01	0.02 ± 0.001
23	1259 ± 123	602 ± 160	0.06 ± 0.007	0.01 ± 0.001
24	>2000			
25	>2000			
26	1100 ± 50			
27	735 ± 93	294 ± 30	0.23 ± 0.006	0.08 ± 0.006

[a] Units of V_{max}: nanomoles of product formed per milligram of protein per minute. [b] Taken from ref 32. [c] Taken from ref 31. [d] Taken from ref 33.

Results and Discussion

Tetrahydroisoquinolines **14–21** were evaluated in vitro as inhibitors of PNMT. The results are shown in Table I. Because amphetamines in which the side chain is fixed in a fully extended conformation can display activity as substrates,[26,31] conformationally defined analogues **22–27** were evaluated in vitro as both substrates and inhibitors. These results are shown in Table II. The fact that both series of compounds in Tables I and II displayed competitive kinetics denotes binding to the same active site to which phenylethanolamine binds. At higher concentrations, mixed inhibition kinetics were observed for all compounds evaluated. Therefore, in order to ensure a competitive mode of binding, all of the analogues in this study were tested at concentrations below those producing a change to mixed inhibition kinetics.

The fact that phenolic tetrahydroisoquinolines **15** (6-OH) and **16** (7-OH) displayed enhanced activity as inhibitors (K_i = 7.8 and 2.6 μM, respectively) over parent **13** (K_i = 10.3 μM) suggests that an aromatic hydroxyl group at carbons C6 or C7 produces a positive contribution to binding. Given the sharp drop in activity when the hydrophilic hydroxyls are masked as the methyl ethers **19** and **20** (K_i = 169 and 20 μM, respectively), it appears that this enhancement in affinity is due to an interaction of the aromatic hydroxyl, as a hydrogen bond donor, with the hydrophilic pocket in the aromatic ring binding region of the active site of PNMT. These results are consistent with the findings of Blank et al.[35] in which it was shown that the acidic sulfonamido NH was necessary for the high affinity of SKF 29661 (**12**).

The data in Table I also reveal that **14** (5-OH) and **17** (8-OH) display diminished activity as inhibitors (K_i = 90 and 1082 μM, respectively) with respect to **13**. The hydroxyl group at carbons C5 and C8 thus lends a negative binding contribution. Bondinell et al.[49] have previously shown that aromatic chloro substituents at carbons C5 and C8 (as well as C6 and C7) enhance the binding affinity of 1,2,3,4-tetrahydroisoquinolines. In this light, the poor activity of **14** and **17** is easily understood. For these regioisomers, a C5 or C8 hydroxyl group would interact in a region containing a high degree of lipophilic character resulting in a negative binding contribution. Consistent with this finding, masking the hydrophilic hydroxyl group in **17** as the methyl ether **21**, greatly enhances the binding affinity for the 8-substituted isomer. The fact that the 5-substituted series does not follow a similar trend may be due, in part, to a negative steric interaction of the relatively bulky methoxy substituent. The drop in activity upon substitution of a hydroxyl at C5 and C8 in **13**, coupled with only a small enhancement of binding affinity upon substitution of the hydroxyl at carbon C6, suggests that, as in the case of β-phenylethylamines, the hydrophilic pocket is also spatially compact with respect to bound benzylamines. In addition, the fact that chloro substitution at any point on the aromatic ring enhances the binding affinity of 1,2,3,4-tetrahydroisoquinoline (**13**) itself, supports previous findings that this hydrophilic pocket is surrounded by or is part of a larger lipophilic region in the active site.

Additional evidence for the spatial compactness of the hydrophilic pocket arises from the relative activity of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**39**; see Table I).[31] Since **39** possesses hydroxyl groups at both carbons C6 and C7, it would be expected that this analogue would display a greater binding affinity than either **15** (6-OH) or **16** (7-OH). The fact that **39** exhibits a K_i value intermediate between those for **15** and **16** suggests that the hydrophilic pocket is only able to interact with one aromatic hydroxyl group.

The finding that **16** (7-OH) is a 3-fold better inhibitor than **15** (6-OH) suggests that **16** more nearly approaches the directional requirements necessary for simultaneous interaction of the aromatic hydroxyl and amino nitrogen for bound benzylamines at the active site of PNMT. This would suggest that the hydrophilic pocket in the aromatic ring binding region of the active site of the enzyme exists off of carbon C7 for bound tetrahydroisoquinolines (and, presumably, for other benzylamines). This would be consistent with **12** (7-SO_2NH_2) binding in a manner in which the favored rotamer places the acidic sulfonamide hydrogens nearest to C7. Further, the fact that **16** (7-OH) is not as active as **12** suggests that the hydroxyl group and amino nitrogen, while in the right orientation with respect to each other, are not far enough apart to approximate the distance between the hydrophilic pocket and the point of amine interaction for bound benzylamines. Molecular graphics analysis indicates that there is a 1.4-Å difference in the distances between the benzylamine nitrogen and the sulfonamido NH in **12** and the benzylamine nitrogen and the phenolic OH in **16**. Assuming that SKF 29661 (**12**) presently represents the best model that allows maximal simultaneous interaction of an aromatic hydrophilic substituent and the amino nitrogen of benzylamines, the distance between the hydrophilic pocket and the point of amine interaction is approximately 9 Å. These binding features are depicted with the aid of the SYBYL molecular graphics package[50] in Figure 2.

(49) Bondinell, W. E.; Chapin, R. W.; Girard, G. R.; Kaiser, C.; Krog, A. J.; Pavloff, A. M.; Schwartz, M. S.; Silvestri, J. S.; Vaidya, P. D.; Lam, B. L.; Wellman, G. R.; Pendleton, R. G. *J. Med. Chem.* **1980**, *23*, 506.

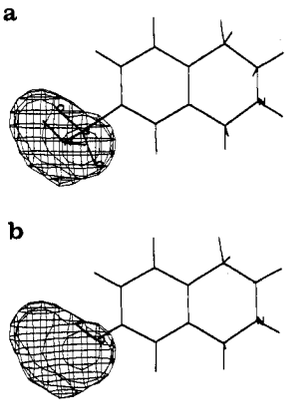


Figure 2. A representation of the hydrophilic pocket as it exists off of aromatic carbon C7 in bound tetrahydroisoquinolines at the active site of PNMT. The SYBYL molecular graphics system was used to generate these figures. By using a least-squares fit of the sulfonamido sulfur, the aromatic rings and the amino nitrogen (using the FIT command of SYBYL), SKF 29661 (**12**) was overlayed with a second molecule of **12** in which the sulfonamido NH_2 has been removed. Part a shows the volume resulting from substracting **12** (less NH_2) from the parent molecule of **12** and represents the hydrophilic pocket (webbed area) in respect to bound tetrahydroisoquinolines. The high activity of **12** (shown in a) results from a large degree of interaction of the sulfonamido NH_2 at this pocket. Interaction of 7-hydroxy-1,2,3,4-tetrahydroisoquinoline (**16**) at this site is shown in b. While the aromatic hydroxyl and the amino nitrogen are in the correct orientation with respect to each other, for simultaneous interaction they are not far enough apart to allow maximal interaction at both binding sites (the aromatic hydroxyl interacts only slightly with the hydrophilic pocket). This would account for the fact that **16** shows enhanced affinity over parent **13** but is less active than **12**.

Employing the phenol analogues **6–11**, we have previously shown that the spatially compact hydrophilic pocket exists off of carbon C6 for bound β-phenylethylamines of type **3** and **4**.[33] Substitution of a hydroxyl at any point on the aromatic ring other than C6 led to a marked decrease in activity. While the hydrophilic pocket also displays spatial constraints for bound benzylamines of type **12–21**, the finding that a hydroxyl at both carbons C6 and C7 (i.e. **15** and **16**, respectively) enhances the binding affinity over **13** may suggest that the hydrophilic pocket within the aromatic ring binding region of the active site of PNMT is more readily accessible to bound benzylamines than to bound β-phenylethylamines.

In order to better define the role of the phenolic hydrogen in the binding of analogues **6–11** to the active site of PNMT, the corresponding methyl ethers **22–27** were evaluated (Table II). The finding that the 6-methoxy compounds **23** and **27** are weaker inhibitors than the corresponding phenol derivatives **7** and **11**, is consistent with a positive interaction of the 6-hydroxy substituent resulting from a hydrogen bonding effect of the acidic hydrogen. This is in agreement with the results found for the tetrahydroisoquinolines discussed above.

Given the positive interaction of the 6-hydroxy substituent and the fact that unsubstituted analogues **3** and **4** display activity as substrates, it is surprising that **7** and **11** do not also behave as substrates. The emergence of substrate activity in **23** and **27** suggests that the positive interaction of the C6 hydroxyl orients these molecules in a manner in which methylation cannot occur. It may be the case that interaction of the 6-hydroxy substituent displaces the aromatic ring into a slightly different binding orientation. Since this system is conformationally fixed, this displacement would necessarily translate to the bicyclic portion of the molecule and may then displace the amino nitrogen out of the region in which methylation takes place. Elimination of the interaction of the 6-hydroxy group could then result in **23** and **27** binding as substrates.

In the case of phenolic amines **6**, **9**, and **10**, an enhancement in binding affinity is seen for the corresponding methyl ethers **22**, **25**, and **26**. This would support our previous conclusion that an aromatic hydroxyl at any carbon other than C6 (as in analogues **6**, **9**, and **10**) lends a negative binding contribution due to interaction at sites of high lipophilic character. Elimination of this negative interaction results in enhanced binding affinity. Apparently, it is the masking of the hydrophilic hydroxyl group that allows methyl ether **27** to bind as a substrate. The fact that **25** and **26** do not also display activity as substrates indicates that the amino group of these molecules cannot gain access to the methylation zone.

In summary, we have synthesized and evaluated a series of aromatic hydroxy- and methoxy-substituted tetrahydroisoquinolines in order to probe the spatial and directional limitations of the hydrophilic pocket with respect to bound benzylamines. As in the case of bound β-phenylethylamines, the hydrophilic pocket with respect to bound benzylamines is spatially compact and surrounded by or part of a large area of lipophilic character. In the tetrahydroisoquinoline series, this pocket exists off of carbon C7. For both benzylamines and β-phenylethylamines, it appears that a substituent that can act as a hydrogen bond donor is required for interaction at this site. Finally, the results of this study confirm our original conclusion that there is relatively little hydrophilic character in the aromatic ring binding region of the active site of PNMT to which most known PNMT ligands bind and that the natural substrate, norepinephrine, binds in a manner different from most known substrates and competitive inhibitors of the enzyme. Studies are presently under way to incorporate this hydrophilic pocket into our current understanding of the overall topography of the active site of PNMT.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus calibrated with known compounds and are corrected accordingly. Proton nuclear magnetic resonance spectra (^1H NMR) were obtained on either a Varian FT-80A or XL-300 spectrometer with deuteriated chloroform ($CDCl_3$) as the solvent, and chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS; 0.0 ppm). Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded on a Varian XL-300 spectrometer with $CDCl_3$ as the solvent, and chemical shifts are reported in ppm relative to $CDCl_3$ (77.0 ppm). For the hydrobromide and hydrochloride salts of the phenolic amines, NMR spectra were taken in deuteriated dimethyl sulfoxide (Me_2SO-d_6) in which case chemical shifts are reported relative to Me_2SO (2.50 ppm for ^1H and 39.51 ppm for ^{13}C). Infrared spectra (IR) were recorded on either an IBM FT-IR 32 or a Perkin-Elmer IR-727 spectrometer. Electron-impact mass spectra (EIMS) were obtained on either a Varian Atlas CH-5 or Ribermag R 10-10 mass spectrometer. Combustion analyses were performed on a Hewlett-Packard Model 185B CHN Analyzer at the University of Kansas and were within 0.4% of the calculated values. Gas chromatography was performed on a Hewlett-Packard GC 5880A equipped with a 10% KOH on Apiezon column ($^1/_4$ in. × 72 in.) and an FID detector. Preparative centrifugal thin-layer

(50) SYBYL Molecular Modeling System Manual (Tripos Associates, Inc., St. Louis, MO), 1986. For a discussion of the use of unions of volumes in the development of the active analogue approach see: Marshall, G. R.; Barry, G. D.; Bossard, H. E.; Dammkoehler, R. A.; Dunn, D. A. *ACS Symp. Ser.* **1979**, *No. 112*, 205.

chromatography (PCTLC) was performed on a Harrison Model 7924 Chromatotron (Harrison Research, Palo Alto, CA) with Merck silica gel 60 PF254 containing $CaSO_4 \cdot 0.5H_2O$ binder on 1-, 2-, or 4-mm-thickness plates. Column chromatography was performed with Merck silica gel 60 (230–400 mesh). Analytical thin-layer chromatography (TLC) was performed with silica gel with fluorescent indicator coated on 1 × 3 in. glass plates in 0.2-mm thickness. Bulb-to-bulb distillations were carried out with a Kugelrohr distillation apparatus (Aldrich Chemical Co.).

5-Hydroxyisoquinoline, 2-(3′-methoxyphenyl)ethylamine, and 2-(4′-methoxyphenyl)ethylamine were purchased from Aldrich Chemical Co. (Milwaukee, WI). *S*-Adenosyl-L-methionine was obtained from Sigma Chemical Co. (St Louis, MO). [*methyl*-^{3}H]-*S*-Adenosyl-L-methionine, which was used in the radiochemical assays, was purchased from New England Nuclear Corp. (Boston, MA). Bovine adrenal glands, required for the purification of the enzyme used in this study, were obtained from Pel-Freez Biologicals (Rogers, AR). Solvents were routinely distilled prior to use; anhydrous tetrahydrofuran (THF) and ether (Et_2O) were distilled from sodium benzophenone ketyl; dry methanol (MeOH) and ethanol (EtOH) were obtained by distillation from magnesium. Unless otherwise stated, all MeOH and EtOH used was anhydrous. Where appropriate, amine hydrochloride salts were prepared by passing anhydrous HCl gas over a dry ethereal solution of the free base. All reactions requiring anhydrous conditions and/or an inert atmosphere were performed under a positive N_2 or Ar flow, and all glassware was oven dried and/or flame dried.

***N*-(Methoxycarbonyl)-2-(3′-methoxyphenyl)ethylamine (29).** To a solution of 10.0 g (66.1 mmol) of 2-(3′-methoxyphenyl)ethylamine (**28**) and 11.0 mL (78.9 mmol) of triethylamine in 300 mL of anhydrous THF at 0 °C under Ar was carefully added 32.0 g (339 mmol) of methyl chloroformate. The reaction mixture was stirred at room temperature under Ar for 24 h. H_2O (40 mL) was added, the aqueous and organic layers were separated, and the former was extracted with ether (3 × 30 mL). The combined organic fractions were washed with 1 N HCl (2 × 100 mL), H_2O (100 mL) and brine (100 mL), and dried over $MgSO_4$. Evaporation of the solvent in vacuo gave 14.1 g of orange oil, which was distilled bulb-to-bulb (106 °C; 0.05 mm) to give 13.4 g (64.0 mmol, 91%) of **29** as a clear oil: ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.29–7.08 (m, 1 H, Ar H), 6.83–6.63 (m, 3 H, Ar H), 5.10–4.75 (br, 1 H, NH), 3.75 (s, 3 H, CH_3), 3.63 (s, 3 H, CH_3), 3.39 (q, 2 H, J = 6.7 Hz, H1), 2.75 (t, 2 H, J = 7.0 Hz, H2); IR (film) 3395, 3045, 2995, 2875, 1720, 1610, 1590, 1535, 1490, 1472, 1460, 1265, 1160, 1045, 780, 700 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 159.56, 156.88, 140.23, 129.35, 120.88, 114.27, 111.57, 54.89, 51.78, 41.93, 35.95; EIMS, m/z (relative intensity) 209 (31.4, M$^+$), 194 (8.6), 178 (4.5), 150 (7.6), 134 (100), 121 (17.4), 91 (18.3), 88 (59.8). Anal. ($C_{11}H_{15}NO_3$) C, H, N.

***N*-(Methoxycarbonyl)-2-(4′-methoxyphenyl)ethylamine (33).** By using conditions analogous to those employed in the preparation of **29**, 10.0 g (66.1 mmol) of 2-(4′-methoxyphenyl)-ethylamine (**32**) afforded, after distillation (117 °C; 0.13 mm), 13.10 g (62.6 mmol, 95%) of **33** as a white solid: ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.08 (d, 2 H, J = 8.2 Hz, H2′), 6.80 (d, 2 H, J = 8.1 Hz, H3′), 5.15–4.75 (br, 1 H, NH), 3.74 (s, 3 H, CH_3), 3.62 (s, 3 H, CH_3), 3.36 (q, 2 H, J = 6.6 Hz, H1), 2.71 (t, 2 H, J = 7.0 Hz, H2); IR (KBr) 3357, 3004, 2946, 1699, 1612, 1535, 1516, 1308, 1283, 1271, 1250, 1198, 1007, 824 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 158.01, 156.85, 130.62, 129.46, 113.77, 54.96, 51.70, 42.23, 35.01; EIMS, m/z (relative intensity) 209 (10.5, M$^+$), 178 (2.6), 134 (90.6), 121 (100), 91 (8.1), 88 (11.8). Anal. ($C_{11}H_{15}NO_3$) C, H, N.

6-Methoxy-3,4-dihydroisoquinolin-1-one (30) and 8-Methoxy-3,4-dihydroisoquinolin-1-one (31). To 6.0 g of polyphosphoric acid at 145 °C was added 550 mg (2.63 mmol) of carbamate **29**. The reaction was stirred at 145 °C for 10 min and then poured onto ice. The mixture was extracted with CH_2Cl_2 (6 × 50 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated in vacuo to give 420 mg of a mixture of **30** and **31**, which were separated by PCTLC (4 mm) with 5% MeOH in ethyl acetate as the eluent (TLC R_f values in the same solvent: **30**, 0.43 and **31**, 0.19).

6-Methoxy-3,4-dihydroisoquinolin-1-one (30). Compound **30** (235 mg, 1.33 mmol, 51%) was obtained as a white solid: mp 141.8–142.2 °C (recrystallized from ethyl acetate); ^{1}H NMR ($CDCl_3$, 80 MHz) δ 8.01 (d, 1 H, J = 8.5 Hz, H8), 6.96–6.60 (m, 3 H, H5, H7, and NH), 3.85 (s, 3 H, OCH_3), 3.66–3.36 (m, 2 H, H3), 2.94 (t, 2 H, J = 6.6 Hz, H4); IR (KBr) 3210, 3069, 2963, 1655, 1607, 1480, 1404, 1317, 1273, 1253, 1157, 1113, 845, 808 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 166.62, 162.47, 140.96, 129.94, 121.77, 112.39, 112.23, 55.30, 40.12, 28.65; EIMS, m/z (relative intensity) 177 (71.4, M$^+$), 148 (76.7), 134 (11.6), 118 (100), 105 (59.2), 90 (88.9). Anal. ($C_{10}H_{11}NO_2$) C, H, N.

8-Methoxy-3,4-dihydroisoquinolin-1-one (31). Compound **31** (111 mg, 0.63 mmol, 24%) was obtained as a white solid: mp 147.7–148.8 °C (recrystallized from ethyl acetate); ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.41–7.22 (m, 1 H, Ar H), 6.88–6.69 (m, 2 H, Ar H), 3.89 (s, 3 H, OCH_3), 3.50–3.30 (m, 2 H, H3), 2.87 (t, 2 H, J = 6.0 Hz, H4), 1.84 (br s, 1 H); IR (KBr) 3216, 3005, 2937, 1646, 1597, 1475, 1452, 1340, 1298, 1271, 1092, 974, 806, 675 cm^{-1}; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.24, 159.97, 141.97, 132.62, 119.44, 117.60, 110.71, 56.07, 39.68, 30.09; EIMS, m/z (relative intensity) 177 (60.7, M$^+$), 148 (100), 134 (3.7), 120 (22.1), 91 (12.8). Anal. ($C_{10}H_{11}NO_2$) C, H, N.

7-Methoxy-3,4-dihydroisoquinolin-1-one (34). Employing conditions analogous to those used in the preparation of **30** and **31**, 1.1 g (5.26 mmol) of **33** gave rise to 550 mg (3.10 mmol, 59%) of **34** as a white solid: mp 112.8–114.3 °C (recrystallized from ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.59 (d, 1 H, J = 2.4 Hz, H8), 7.17–6.93 (m, 3 H, H5, H6, and NH), 3.84 (s, 3 H, OCH_3), 3.50 (dt, 2 H, J = 6.8 and 2.9 Hz, H3), 2.90 (t, 2 H, J = 6.7 Hz, H4); IR (KBr) 3206, 3067, 2990, 1667, 1611, 1498, 1485, 1431, 1395, 1333, 1261, 1039, 818 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 166.58, 158.61, 131.02, 129.81, 128.30, 119.50, 110.97, 55.45, 40.24, 27.33; EIMS, m/z (relative intensity) 177 (75.3, M$^+$), 148 (82.7), 134 (2.4), 120 (100), 105 (9.3), 91 (18.8). Anal. ($C_{10}H_{11}NO_2$) C, H, N.

The original PPA/water mixture was basified with solid KOH and extracted with Et_2O (4 × 20 mL). The combined organic fractions were dried over K_2CO_3, and the solvent was evaporated in vacuo to give 235 mg (1.55 mmol, 30%) of 2-(4′-methoxyphenyl)ethylamine (**32**).

6-Methoxy-1,2,3,4-tetrahydroisoquinoline (19). A solution of 2.0 g (11.3 mmol) of 6-methoxy-3,4-dihydroisoquinolin-1-one (**30**) in 50 mL of anhydrous THF was slowly dripped into 15 mL (15 mmol) of a $LiAlH_4$ solution (1 M in THF) at 0 °C under Ar. The mixture was heated at reflux for 2 h and was quenched by the sequential addition of 20 mL of H_2O and 20 mL of 3 N NaOH. The reaction mixture was filtered, and the residue was washed well with Me_2CO (2 × 50 mL) and Et_2O (2 × 50 mL). The combined organic/aqueous mixture was concentrated to $^1/_3$ volume and made acidic with 3 N HCl. The mixture was washed with Et_2O (2 × 30 mL) and made basic with solid KOH. The basic aqueous fraction was extracted with Et_2O (5 × 50 mL). The Et_2O extracts were dried over K_2CO_3 and evaporated in vacuo to give 2.69 g of a yellow oil, which was distilled bulb-to-bulb (82 °C, 0.05 mm) to give 1.80 g (11.0 mmol, 98%) of **19** as a clear oil: ^{1}H NMR ($CDCl_3$, 80 MHz) δ 6.86 (d, 1 H, J = 7.7 Hz, H8), 6.68 (d, 1 H, J = 2.7 Hz, H7), 6.57 (br s, 1 H, H5), 3.88 (br s, 2 H, H1), 3.73 (s, 3 H, CH_3), 3.05 (t, 2 H, J = 5.0 Hz, H3), 2.75 (t, 2 H, J = 6.2 Hz, H4), 1.64 (br s, 1 H, exchangeable in D_2O, NH); IR (film) 3300, 3040, 2960, 2870, 1612, 1504, 1460, 1300, 1272, 1265, 1157, 1125, 1040, 805 cm^{-1}; **19**·HCl (recrystallized from EtOH/Et_2O): mp 242.6–244.1 °C (lit.[37] mp 238–239 °C); EIMS, m/z (relative intensity) 163 (29.4, M$^+$), 162 (100), 147 (9.7), 134 (46.7), 118 (7.6), 104 (6.7), 91 (17.7). Anal. ($C_{10}H_{13}NO$·HCl) C, H, N.

7-Methoxy-1,2,3,4-tetrahydroisoquinoline (20). Employing conditions analogous to those used in the preparation of **19**, 1.20 g (6.77 mmol) of **34** afforded, after bulb-to-bulb distillation (115 °C, 0.1 mm), 1.02 g (6.25 mmol, 92%) of **20** as a clear oil: ^{1}H NMR ($CDCl_3$, 80 MHz) δ 6.94 (d, 1 H, J = 7.8 Hz, H5), 6.75–6.40 (m, 2 H, H6 and H8), 3.92 (s, 2 H, H1), 3.74 (s, 3 H, OCH_3), 3.07 (t, 2 H, J = 5.8 Hz, H3), 2.68 (t, 2 H, J = 5.8 Hz, H4), 2.40 (s, 1 H, exchangeable in D_2O, NH); IR (film) 3350, 3095, 2974, 2875, 1617, 1507, 1468, 1438, 1325, 1280, 1255, 1222, 1163, 1118, 1042, 805, 720, cm^{-1}; **20**·HCl (recrystallized from EtOH/Et_2O): mp 232.0–233.0 °C (lit.[37] mp 231–232 °C); EIMS, m/z (relative intensity) 163 (47.0, M$^+$), 147 (4.9), 134 (100), 118 (6.8), 104 (9.0), 91 (26.6). Anal. ($C_{10}H_{13}NO$·HCl) C, H, N.

8-Methoxy-1,2,3,4-tetrahydroisoquinoline (21). Employing conditions analogous to those used in the preparation of **19** and **20**, 1.10 g (6.21 mmol) of **31** afforded, after bulb-to-bulb distillation

(90 °C, 0.08 mm), 990 mg (6.10 mmol, 98%) of **21** as a clear oil: ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.07 (t, 1 H, J = 7.8 Hz, H6), 6.67 (d, 1 H, J = 7.2 Hz, H5), 6.61 (d, 1 H, J = 8.1 Hz, H7), 3.90 (s, 2 H, H1), 3.75 (s, 3 H, OCH_3), 3.03 (t, 2 H, J = 5.5 Hz, H3), 2.71 (t, 2 H, J = 5.5 Hz, H4), 1.53 (s, 1 H, exchangeable in D_2O, NH); IR (film) 3268, 3038, 2934, 1603, 1588, 1470, 1439, 1340, 1300, 1256, 1102, 1074, 964, 768 cm^{-1}; **21**·HCl (recrystallized from EtOH/Et_2O): mp 259.7–261.2 °C (lit.[37] mp 261–262 °C); EIMS, m/z (relative intensity) 163 (34.1, M^+), 162 (100), 147 (20.7), 134 (42.7), 118 (4.6), 104 (28.7), 91 (16.4). Anal. ($C_{10}H_{13}NO$·HCl) C, H, N.

General Procedure for the Preparation of the Hydrobromide Salts of Phenolic Amines 15–17. The free base of the respective aryl methoxy-substituted tetrahydroisoquinoline (**19–21**) was dissolved in 48% HBr (4 mL/mmol) and heated at 120 °C for 3 h. The reaction mixture was cooled and concentrated in vacuo to give a residue, which was further dried under vacuum. The hydrobromide salts of the phenolic tetrahydroisoquinolines (**15–17**) were isolated as tan solids.

6-Hydroxy-1,2,3,4-tetrahydroisoquinoline Hydrobromide (15·HBr). Methoxy amine **19** (1.05 g, 5.26 mmol) afforded 1.09 g (4.74 mmol, 90%) of **15**·HBr (recrystallized from EtOH/Et_2O): mp 196.7–197.8 °C; ^{1}H NMR (Me_2SO-d_6; 300 MHz) δ 9.44 (br s, 1 H, exchangeable in D_2O, OH), 9.11 (br s, 2 H, exchangeable in D_2O, NH_2^+), 7.02 (d, 1 H, J = 8.3 Hz, H8), 6.69 (d, 1 H, J = 1.95 Hz, H7), 6.61 (s, 1 H, H5), 4.15 (s, 2 H, H1), 3.33 (t, 2 H, J = 5.8 Hz, H3), 2.92 (t, 2 H, J = 6.5 Hz, H4); IR (KBr) 3290, 3120, 2945, 2845, 2735, 2670, 1580, 1498, 1372, 1270, 1233, 1148, 835 cm^{-1}; ^{13}C NMR (Me_2SO-d_6) δ 156.56, 132.93, 127.78, 118.83, 114.70, 114.15, 43.38, 40.59, 24.70; EIMS, m/z (relative intensity) 149 (31.3, M^+), 148 (100), 133 (10.3), 120 (64.9), 91 (23.5). Anal. ($C_9H_{11}NO$·HBr) C, H, N.

7-Hydroxy-1,2,3,4-tetrahydroisoquinoline Hydrobromide (16·HBr). Methoxy amine **20** (900 mg, 5.51 mmol) afforded 1.05 g (4.56 mmol, 83%) of **16**·HBr (recrystallized from EtOH): mp 210.3–211.4 °C; ^{1}H NMR (Me_2SO-d_6) δ 9.42–9.18 (br s, 3 H, exchangeable in D_2O, NH_2^+ and OH), 7.00 (d, 1 H, J = 8.31 Hz, H5), 6.70 (d, 1 H, J = 8.31 Hz, H6), 6.61 (s, 1 H, H8), 4.18 (s, 2 H, H1), 3.33 (t, 2 H, J = 5.37 Hz, H3), 2.88 (t, 2 H, J = 5.48 Hz, H4); IR (KBr) 3348, 2998, 2860, 2705, 2680, 2500, 1618, 1517, 1453, 1438, 1299, 1212, 1166, 852 cm^{-1}; ^{13}C NMR (Me_2SO-d_6) δ 155.81, 129.78, 129.54, 121.86, 115.12, 112.76, 43.67, 41.04, 23.84; EIMS, m/z (relative intensity) 149 (46.6), 148 (38.0), 133 (6.7), 120 (100), 91 (31.4). Anal. ($C_9H_{11}NO$·HBr) C, H, N.

8-Hydroxy-1,2,3,4-tetrahydroisoquinoline Hydrobromide (17·HBr). Methoxy amine **21** (600 mg, 3.67 mmol) afforded 685 mg (2.97 mmol, 81%) of **17**·HBr (recrystallized from EtOH/Et_2O): mp 257.5–258.7 °C; ^{1}H NMR (Me_2SO-d_6, 300 MHz) δ 9.87 (br s, 1 H, exchangeable in D_2O, OH), 9.13 (br s, 2 H, exchangeable in D_2O, NH_2^+), 7.08 (t, 1 H, J = 7.8 Hz, H6), 6.75 (d, 1 H, J = 8.4 Hz, H5), 6.66 (d, 1 H, J = 7.9 Hz, H7), 4.07 (s, 2 H, H1), 3.34 (t, 2 H, J = 5.6 Hz, H3), 2.94 (t, 2 H, J = 6.3 Hz, H4); IR (KBr) 3225, 3119, 3036, 2926, 2824, 2654, 1597, 1473, 1398, 1336, 1279, 657 cm^{-1}; ^{13}C NMR (Me_2SO-d_6) 153.87, 133.09, 127.85, 119.08, 115.66, 112.36, 40.30, 39.96, 24.56; EIMS, m/z (relative intensity) 149 (41.2, M^+), 148 (100), 132 (7.5), 120 (51.2), 91 (17.5). Anal. ($C_9H_{11}NO$·HBr) C, H, N.

***N,O*-Bis(methoxycarbonyl)-5-hydroxy-1,2,3,4-tetrahydroisoquinoline (36).** 5-Hydroxyisoquinoline (1.0 g, 5.50 mmol) was dissolved in 100 mL of MeOH saturated with HCl_g in a 500-mL Parr shaker bottle. Platinum oxide catalyst (250 mg), which had been prewetted with the same solvent, was added, and the mixture was hydrogenated at an initial pressure of 45 psi. After 3 h, the solvent was evaporated in vacuo to give 920 mg of crude 5-hydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride. The products from four of these runs were combined (3.5 g) and slurried in 200 mL of anhydrous THF. Dry triethylamine (17.5 mL, 125.5 mmol) was added, and the mixture was cooled to 5 °C. Methyl chloroformate (8.0 mL, 103.5 mmol) was slowly added, and the mixture was stirred at room temperature for 10 h. The reaction mixture was transferred to a separatory funnel with the aid of 50 mL of H_2O and 50 mL of Et_2O. The organic and aqueous layers were separated, and the latter was extracted with Et_2O (2 × 30 mL). The combined organic fractions were washed with H_2O (2 × 50 mL) and brine (50 mL) and dried over K_2CO_3. Evaporation of the solvent in vacuo gave 4.87 g of a light brown oil, which was purified by column chromatography with hexanes/ethyl acetate (3:1) as the eluent to afford 3.60 g (13.6 mmol, 72% based on crude 5-hydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride) of **36** as a clear oil: ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.27–6.87 (m, 3 H, Ar H), 4.63 (s, 2 H, H1), 3.88 (s, 3 H, CH_3), 3.73 (s, 3 H, CH_3), 3.68 (2 H, t, J = 6.1 Hz, H3), 2.73 (t, 2 H, J = 5.9 Hz, H4); IR (neat) 3005, 2957, 1765, 1705, 1469, 1446, 1412, 1372, 1271, 1238, 1208, 1089, 991, 939, 781 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 155.53, 153.54, 148.71, 134.99, 126.59, 123.83, 119.08, 55.15, 52.33, 45.13, 40.33, 22.51; EIMS, m/z (relative intensity) 265 (26.6, M^+), 250 (100), 206 (28.6), 190 (11.7), 160 (8.3), 146 (19.2), 104 (25.8), 91 (41.6). While **36** was readily purified for spectral analysis, it slowly decomposed with time and therefore was used immediately in the next reaction.

***N*-(Methoxycarbonyl)-5-hydroxy-1,2,3,4-tetrahydroisoquinoline (37).** A mixture of 4.90 g (18.5 mmol) of carbonate/carbamate **36** in 340 mL of MeOH containing 1% K_2CO_3 and 1 mL of H_2O was stirred at room temperature under Ar for 12 h. The reaction mixture was concentrated in vacuo to $^1/_5$ volume, dissolved in 100 mL of H_2O, and adjusted to pH 7 with 0.1 N aqueous HCl. The aqueous pool was extracted with Et_2O (4 × 50 mL). The combined organic fractions were washed with H_2O (2 × 50 mL) and brine (50 mL) and dried over K_2CO_3. Evaporation of the solvent gave 3.95 g of a viscous oil, which was triturated with petroleum ether to afford 3.65 g (17.6 mmol, 95%) of **37** as a white solid: mp 105.6–106.7 °C (recrystallized from ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.18–6.73 (m, 4 H, 1 H exchangeable in D_2O, Ar H and OH), 4.75 (s, 2 H, H1), 3.91 (s, 3 H, OCH_3), 3.85 (t, 2 H, J = 6.0 Hz, H3), 2.94 (t, 2 H, J = 5.9 Hz, H4); IR (film) 3326, 3025, 2957, 1674, 1592, 1470, 1453, 1414, 1373, 1283, 1246, 1119, 773, 722 cm^{-1}; EIMS, m/z (relative intensity) 207 (35.8, M^+), 192 (100), 148 (54.7), 133 (16.2), 120 (60.0), 91 (81.6). Anal. ($C_{11}H_{13}NO_3$) C, H, N.

***N*-(Methoxycarbonyl)-5-methoxy-1,2,3,4-tetrahydroisoquinoline (38).** Carbamate **37** (1.50 g, 7.24 mmol) and 10.0 g (72.3 mmol) K_2CO_3 were mixed in 45 mL of acetone, and the slurry was cooled to 5 °C. Iodomethane (4.50 mL, 72.3 mmol) was slowly added, and the reaction mixture was heated at reflux for 12 h. The reaction mixture was evaporated to an oily solid, and the residue was dissolved in 30 mL of H_2O and 30 mL of Et_2O. The two layers were separated, and the aqueous phase was extracted with Et_2O (2 × 20 mL). The organic pool was washed with H_2O (2 × 20 mL) and brine (20 mL) and dried over $MgSO_4$. Evaporation of the solvent in vacuo gave 1.78 g of a light yellow oil, which was distilled bulb-to-bulb (115 °C; 0.05 mm) to afford 1.49 g (6.73 mmol, 93%) of **38** as a clear oil: ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.12–6.93 (m, 1 H, Ar H), 6.65–6.43 (m, 2 H, Ar H), 4.46 (s, 2 H, H1), 3.70 (s, 3 H, CH_3), 3.63 (s, 3 H, CH_3), 3.57 (t, 2 H, J = 6.1 Hz, H3), 2.66 (t, 2 H, J = 5.9 Hz, H4); IR (film) 3036, 2984, 1701, 1589, 1472, 1445, 1407, 1343, 1381, 1260, 1238, 1110, 1080, 765 cm^{-1}; EIMS, m/z (relative intensity) 221 (37.8, M^+), 206 (100), 190 (10.1), 162 (38.8), 146 (20.2), 134 (32.7), 104 (37.6), 91 (34.7). Anal. ($C_{12}H_{15}NO_3$) C, H, N.

5-Methoxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (18·HCl). A mixture of 1.1 g (4.97 mmol) of carbamate **38** in 15 mL of 3 N aqueous HCl was heated at reflux for 60 h. The reaction mixture was washed with Et_2O (25 mL) and concentrated in vacuo to afford 950 mg (4.76 mmol, 96%) of **18**·HCl as a white solid, a sample of which was converted to the free base for spectral analysis: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.08 (t, 1 H, J = 7.71 Hz, H7), 6.65 (d, 1 H, J = 8.3 Hz, Ar H), 6.61 (d, 1 H, J = 7.92 Hz, Ar H), 3.95 (s, 2 H, H1), 3.79 (s, 3 H, CH_3), 3.10 (t, 2 H, J = 5.86 Hz, H3), 2.63 (d, 2 H, J = 5.86 Hz, H4), 2.07 (s, 1 H, exchangeable in D_2O, NH); IR (film) 3240, 2973, 2881, 1594, 1472, 1440, 1346, 1263, 1234, 1134, 1078, 981, 847, 770 cm^{-1}; **18**·HCl: mp 234.9–236.0 °C (recrystallized from EtOH/Et_2O; lit.[36] mp 233–234 °C); EIMS, m/z (relative intensity) 163 (75.0, M^+), 162 (78.1), 146 (30.1), 134 (100), 132 (38.2), 104 (65.1), 91 (33.9). Anal. ($C_{10}H_{13}NO$·HCl) C, H, N.

5-Hydroxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (14·HCl). A mixture of 1.0 g (4.83 mmol) of carbamate **37** in 20 mL of 3 N aqueous HCl was heated at reflux for 20 h. The reaction mixture was washed with Et_2O (25 mL) and was evaporated in vacuo to afford 890 mg (4.79 mmol, 99%) of **14**·HCl as a white solid which was recrystallized from EtOH/Et_2O: mp 257.6–259.7 °C (lit.[36] mp 275–277 °C); the ^{1}H NMR (Me_2SO-d_6) for **14**·HCl was consistent with the literature;[37] IR (KBr) 3326,

2980, 2853, 1601, 1468, 1345, 1284, 1210, 1077, 982, 921, 778 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 154.84, 129.87, 126.96, 118.99, 116.95, 113.21, 43.49, 40.43, 19.59; EIMS, *m/z* (relative intensity) 149 (75.8, M^+), 148 (84.7), 132 (25.8), 120 (100), 91 (40.0). Anal. ($C_9H_{11}NO$·HCl) C, H, N.

Radiochemical Assay for PNMT Activity. The assay employed in this investigation has been described elsewhere.[32,48] Briefly, a typical assay mixture consisted of 50 μL of 0.5 M phosphate buffer (pH 8.0), 25 μL of a 10 μM solution of unlabeled AdoMet, 5 μL of [*methyl*-^{3}H]AdoMet, containing approximately 2×10^6 dpm (specific activity approximately 15 mCi/mmol), 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 °C, 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 alcohol (7:3). The organic layer was removed and transferred to a scintillation vial and diluted with cocktail for counting. The mode of inhibition was ascertained by inspection of the $1/V$ vs $1/S$ plot of the data.

Acknowledgment. We are grateful to Dr. Garland Marshall and to Tripos Associates (St. Louis, MO) for a grant of the SYBYL software system. Research support from the Kansas Advanced Technology Commission made possible some of the hardware purchases for the University of Kansas Molecular Graphics and Modeling Laboratory in the Department of Medicinal Chemistry. In addition, we thank Dr. J. Paul Hieble (Smith, Kline and French Laboratories, Philadelphia, PA) for his generous gift of SKF 29661. Research Grants HL 34193 and GM 22988 from the U.S. Public Health Service and financial support from the University of Kansas General Research Fund made this work possible.

Registry No. **12**, 31404-61-2; **13**, 91-21-4; **14**, 102877-50-9; **14**·HCl, 102879-34-5; **15**, 14446-24-3; **15**·HBr, 59839-23-5; **16**, 30798-64-2; **16**·HBr, 110192-19-3; **17**, 32999-37-4; **17**·HBr, 110192-20-6; **18**, 103030-70-2; **18**·HCl, 103030-69-9; **19**, 42923-77-3; **19**·HCl, 57196-62-0; **20**, 43207-78-9; **21**, 34146-68-4; **22**, 103904-80-9; **23**, 88081-58-7; **24**, 103904-88-7; **25**, 103904-81-0; **26**, 103904-85-4; **27**, 103904-89-8; **28**, 2039-67-0; **29**, 110192-21-7; **30**, 22246-62-4; **31**, 74904-29-3; **32**, 55-81-2; **33**, 91247-71-1; **34**, 22246-04-4; **35**, 2439-04-5; **36**, 110192-22-8; **37**, 110192-23-9; **38**, 110192-24-0; PNMT, 9037-68-7.

Novel 1*H*-Benzimidazol-4-ols with Potent 5-Lipoxygenase Inhibitory Activity

Derek R. Buckle,*† Keith A. Foster,† John F. Taylor,† John M. Tedder,† Veronica E. Thody,† Richard A. B. Webster,† Jose Bermudez,‡ Roger E. Markwell,‡ and Stephen A. Smith‡

Beecham Pharmaceuticals—Research Division, Biosciences Research Centre, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey, KT18 5XQ England, and Beecham Pharmaceuticals—Research Division, Medicinal Research Centre, Coldharbour Road, The Pinnacles, Harlow, Essex, CM19 5AD England. *Received May 19, 1987*

The synthesis and structure–activity profile of 2-substituted benzimidazol-4-ols as inhibitors of cell-free RBL-1 5-lipoxygenase are discussed, and their potency is compared with that of the standard inhibitors phenidone, AA 861, BW 755C, and nordihydroguaiaretic acid. In contrast to the standard compounds, most did not inhibit the release of slow-reacting substance of anaphylaxis (SRS-A) in vivo when administered at 200 μM ip to rats subjected to peritoneal anaphylaxis, although five compounds containing a methoxylated benzyl group (compounds **36**, **39**, **42**, and **43**) or hydroxylated benzyl group (**41**) showed similar activity to that of phenidone, nordihydroguaiaretic acid, and AA 861. Of the many compounds tested, two, 5-*tert*-butyl-7-methyl-2-(trifluoromethyl)-1*H*-benzimidazol-4-ol (**57**) and 2-(4-methoxybenzyl)-7-methyl-1*H*-benzimidazol-4-ol (**36**), like dexamethasone, inhibited monocyte accumulation in a pleural exudate model of inflammation. Standard lipoxygenase inhibitors such as phenidone, BW 755C, and AA 861 were inactive in this system.

Oxidative metabolites of arachidonic acid and their subsequent derivatives have been implicated in the pathology of a variety of inflammatory and allergic diseases,[1] and modulation of their formation has attracted considerable attention.[2] While the early work of Vane and others[3] showed that the nonsteroidal antiinflammatory drugs owed their activity to the inhibition of cyclooxygenase and the consequent reduction in the formation of thromboxanes and prostaglandins, little interest was shown in other oxidative pathways until the late 1970s. Indeed, it was the characterization of the slow-reacting substance of anaphylaxis (SRS-A), a potent bronchoconstrictor, as a mixture of the leukotrienes LTC_4, LTD_4, and LTE_4[1] and the identification of LTB_4, a potent chemotaxin for mononuclear cells[4] and polymorphonuclear cells,[5] that focused attention on the 5-lipoxygenase pathway of arachidonic acid metabolism and the range of products available through it. It was this increased awareness of the breadth of the arachidonic acid cascade and the enzymes involved that led to the development of inhibitors of leukotriene synthesis. Early inhibitors such as phenidone[6] and BW 755C[7] were shown to exhibit activity on both the major oxidative pathways, although subsequent work has identified an ever-increasing number of more selective inhibitors of 5-lipoxygenase.[8] Few of these, however, have shown useful in vivo activity in potentially relevant animal models of allergic or inflammatory disease.[9]

† Biosciences Research Centre.
‡ Medicinal Research Centre.

(1) Samuelson, B. *Science (Washington, D.C.)* **1983**, *220*, 568.
(2) See, for example: Musser, J. H.; Kreft, A. F.; Lewis, A. J. *Annu. Rep. Med. Chem.* **1985**, *20*, 71.
(3) Vane, J. R. *Nature (London) New Biol.* **1971**, *231*, 232.
(4) Higgs, G. A.; Mugridge, K. G. *Adv. Prostaglandin, Thromboxane, Leukotriene Res.* **1983**, *12*, 19.
(5) Ford-Hutchinson, A. W.; Bray, M. A.; Doig, M. V.; Shipley, M. E.; Smith, M. J. M. *Nature (London)* **1980**, *286*, 2646.
(6) (a) Blackwell, G. J.; Flower, R. J. *Prostaglandins* **1978**, *16*, 417. (b) Blackwell, G. J.; Flower, R. J. *Br. J. Pharmacol.* **1978**, *63*, 360P.
(7) Higgs, G. A.; Flower, R. J.; Vane, J. R. *Biochem. Pharmacol.* **1979**, *28*, 1959.
(8) (a) Nicolaou, K. C.; Petasis, N. A.; Seitz, S. P. *J. Chem. Soc., Chem. Commun.* **1981**, 1195. (b) Corey, E. J.; Kang, J. *Tetrahedron Lett.* **1982**, *23*, 1651. (c) Yoshimoto, T.; Furukawa, M.; Yamamoto, S.; Horie, T.; Watanabe-Kohno, S. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 612. (d) Sun, F. F.; McGuire, J. C.; *Prostaglandins* **1983**, *26*, 211. (e) Corey, E. J.; Cashman, J. R.; Kantner, S. S.; Wright, S. W. *J. Am. Chem. Soc.* **1984**, *106*, 1503. (f) Ashida, Y.; Saijo, T.; Kuriki, H.; Makino, H.; Terao, S.; Maki, Y. *Prostaglandins* **1983**, *26*, 955.

0022-2623/87/1830-2216$01.50/0