-50, $[\theta]_{650}$ -95, $[\theta]_{595-475}$ 0, $[\theta]_{290}$ 1650.

D-*threo*-(1*R*,2*R*)-1-*p*-**Bromophenyl-2-dichloroacetamido-1,3-propanediol (XIX)**: CD (*c* 1.042, Cupra A) $[\theta]_{700}$ 10, $[\theta]_{650}$ 90, $[\theta]_{595}$ 0, $[\theta]_{530}$ -100, $[\theta]_{470-450}$ 0, $[\theta]_{283}$ 1440, $[\theta]_{278}$ 1200*.

D-*threo*-(1*R*,2*R*)-1-*p*-**Iodophenyl-2-dichloroacetamido-1,3-propanediol (XX)**: CD (*c* 1.02, Cupra A) $[\theta]_{700}$ -150, $[\theta]_{640}$ -205, $[\theta]_{530-510}$ 0, $[\theta]_{480}$ 44, $[\theta]_{450-390}$ 0, $[\theta]_{297}$ 2425, $[\theta]_{288}$ 1625*; CD (*c* 0.102, Cupra A) $[\theta]_{350}$ 160, $[\theta]_{269}$ 7465, $[\theta]_{247}$ 560*.

D-*threo*-(1*R*,2*R*)-1-*p*-**Cyanophenyl-2-dichloroacetamido-1,3-propanediol (XXI)**: CD (*c* 1.09, Cupra A) $[\theta]_{700}$ -165, $[\theta]_{650}$ -200, $[\theta]_{515}$ 0, $[\theta]_{475}$ 25, $[\theta]_{430-390}$ 0, $[\theta]_{296}$ 2070, $[\theta]_{290}$ 1470*; CD (*c* 0.109, Cupra A) $[\theta]_{330}$ 240, $[\theta]_{277}$ 8065, $[\theta]_{272}$ 5700*.

D-*threo*-(1*R*,2*R*)-1-*p*-**Methylsulfonylphenyl-2-dichloroacetamido-1,3-propanediol (XXII)**: CD (*c* 1.10, Cupra A) $[\theta]_{700}$ -140, $[\theta]_{625}$ -200, $[\theta]_{515-370}$ 0, $[\theta]_{292}$ 2335, $[\theta]_{288}$ 2000*; CD (*c* 0.110, Cupra A) $[\theta]_{325}$ 500, $[\theta]_{266}$ 9550, $[\theta]_{248}$ 1290.

D-*threo*-(1*R*,2*R*)-1-*p*-**Ureidophenyl-2-dichloroacetamido-1,3-propanediol (XXIII)**: CD (*c* 1.07, Cupra A) $[\theta]_{700}$ -125, $[\theta]_{640}$ -140, $[\theta]_{500-470}$ 0, $[\theta]_{440}$ 20, $[\theta]_{420-410}$ 0, $[\theta]_{400}$ 13, $[\theta]_{305}$ 1620, $[\theta]_{298}$ 1370*; CD (*c* 0.107, Cupra A) $[\theta]_{350}$ 125, $[\theta]_{268}$ 6975, $[\theta]_{255}$ 2735.

D-*threo*-(1*R*,2*R*)-1-*p*-**Phenylureidophenyl-2-dichloroacetamido-1,3-propanediol (XXIV)**: CD (*c* 0.900, Cupra A) $[\theta]_{700}$ -215, $[\theta]_{625}$ -300, $[\theta]_{500}$ -125, $[\theta]_{400}$ -170, $[\theta]_{348}$ 0, $[\theta]_{320}$ 470, $[\theta]_{310}$ 210*; CD (*c* 0.090, Cupra A) $[\theta]_{350}$ 0, $[\theta]_{278}$ 7970, $[\theta]_{278}$ 3940.

D-*threo* (1*R*,2*R*)-1-*p*-**Carbomethoxyphenyl-2-dichloroacetamido-1,3-propanediol (XXV)**: CD (*c* 1.10, Cupra A) $[\theta]_{700}$ -120, $[\theta]_{625}$ -150, $[\theta]_{500-390}$ 0, $[\theta]_{295}$ 2245, $[\theta]_{292}$ 1925*; CD (*c* 0.11, Cupra A) $[\theta]_{350}$ 120, $[\theta]_{270}$ 7950, $[\theta]_{253}$ 2500*.

D-*threo*-(1*R*,2*R*)-1-*p*-**Methoxycarbonylaminophenyl-2-dichloroacetamido-1,3-propanediol (XXVI)**: CD (*c* 1.04, Cupra A) $[\theta]_{700}$ -120, $[\theta]_{650}$ -140, $[\theta]_{508}$ 0, $[\theta]_{485-475}$ 20, $[\theta]_{450-390}$ 0, $[\theta]_{300}$ 1925, $[\theta]_{295}$ 1550*; CD (*c* 0.104, Cupra A) $[\theta]_{300}$ 2600, $[\theta]_{269}$ 6280, $[\theta]_{254}$ 2465.

D-*threo*-(1*R*,2*R*)-1-*p*-**Nitrophenyl-2-amino-1,3-propanediol (XXVII)**: CD (*c* 1.13, Cupra A) $[\theta]_{700}$ +175, $[\theta]_{650}$ +190, $[\theta]_{530}$ 0, $[\theta]_{430}$ 0, $[\theta]_{360}$ -505*; CD (*c* 0.113, Cupra A) $[\theta]_{310}$ -3035*; CD (*c* 0.023, Cupra A) $[\theta]_{285}$ -4340, $[\theta]_{275}$ -2890.

D-*threo*-(1*R*,2*R*)-1-*p*-**Phenylthioureidophenyl-2-dichloroacetamido-1,3-propanediol (XXVIII)**. This material was incompletely soluble in Cupra A. It gave a broad negative band at about 650 nm and a strong positive peak at 280 nm: CD (*c* 0.479, methanol) $[\theta]_{350}$ 0, $[\theta]_{325}$ +170, $[\theta]_{322}$ +97*; CD (*c* 0.192, methanol) $[\theta]_{320}$ -304, $[\theta]_{317}$ -364, $[\theta]_{310}$ 0, $[\theta]_{305}$ +668*; CD (*c* 0.038, methanol) $[\theta]_{290}$ +607, $[\theta]_{285}$ -1820, $[\theta]_{255}$ 0, $[\theta]_{222}$ +12,135.

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Inhibition of Phenylethanolamine *N*-Methyltransferase by Benzylamines. 1. Structure–Activity Relationships

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In searching for inhibitors of phenylethanolamine *N*-methyltransferase (PNMT) as potentially useful pharmacologic agents for reducing epinephrine biosynthesis, we have found that the enzyme from rabbit adrenals is strongly inhibited by benzylamines. Among compounds with various ring substitutions, the 2,3-dichloro and 2-chloro-3-trifluoromethyl compounds were the most active inhibitors. Excellent correlation of inhibitor potency with Hansch π values and Hammett σ values associated with the aromatic substituent was obtained within single-substituent subseries. An α-methyl group in some cases reduced inhibitor activity but in other cases increased the inhibitor activity, perhaps through a steric influence. Other α-alkyl groups reduced inhibitor potency. Substitution on the nitrogen generally reduced inhibitor activity. Lengthening the alkyl chain connecting the phenyl group to the amine decreased inhibitor activity. PNMT from human or rat adrenal glands was inhibited by several of the benzylamines to a degree paralleling the inhibition of the rabbit enzyme.

Phenylethanolamine *N*-methyltransferase (PNMT) is chiefly localized in the adrenal medulla, where it has the physiological role of converting norepinephrine to epinephrine.[1] Tyrosine hydroxylase, the first and apparently rate-limiting enzyme in catecholamine biosynthesis, has often been considered an ideal target for inhibition of catecholamine production. However, inhibition of that enzyme (or of the decarboxylase or dopamine β-hydroxylase) would interfere with norepinephrine formation in the brain and in the peripheral sympathetic nervous system as well as in the chromaffin cells of the adrenal medulla. PNMT, on the other hand, represents a site for enzyme inhibition that would directly suppress formation only of the adrenal medullary hormone, epinephrine, without interfering with enzymic steps in norepinephrine formation in the adrenal gland or in the sympathetic nervous system. No information is available concerning the pharmacologic effects of inhibiting PNMT, and relatively few compounds are known to inhibit the enzyme even *in vitro*.

Previously studied inhibitors include phenylethylamines,[2-4] sulfhydryl-binding agents,[1,5,6] aminobenzimidazoles[7] and other substituted imidazoles,[8] and a few miscellaneous compounds.[3] We report here that benzylamines are potent competitive inhibitors of PNMT. Since these compounds can be viewed as structural analogs of PNMT substrates, it is not surprising that they should inhibit the enzyme competitively; however, the high degree of potency of the benzylamines as inhibitors was unexpected.

Inhibition by Benzylamines with Ring Substitutions. Table I shows the degree of PNMT inhibition by 28 compounds in the benzylamine series. Depending upon the substitution on the aromatic ring, the potency of the inhibitors covered a range of at least 10,000-fold. All but one of the compounds were better inhibitors than benzylamine itself. Seven of the compounds were more potent inhibitors than any of a group of amphetamines that we had previously reported to inhibit PNMT.[4]

We attempted to correlate structure and activity by using

Table I. Inhibition of PNMT by Benzylamines

Compd no.	Arom subst	% inhibition 0.1	0.3	1	3	10	32	100	317	1000	3170	Molar pI_{50}[a]
1	2-Cl, 3-CF_3	44	71	90								6.82
2	2,3-Cl		31	68	87	94						6.23
3	3-CF_3			32	57	90	94					5.64
4	2-I			10	41	77	93					5.37
5	3-I			6	37	69	91	99				5.30
6	4-CF_3			6	29	64	88	97				5.20
7	3-Br				23	62	85	94				5.17
8	3-Cl				21	54	84	94				5.07
9	3,4-Cl				19	48	77	90	98			4.97
10	2-Br				13	48	80	95	99			4.97
11	2,5-Cl				13	37	71	88	96			4.82
12	2,4-Cl					29	62	88	98			4.68
13	2-Cl					25	62	83	94			4.66
14	2-CF_3					27	59	88	97			4.64
15	4-I					14	44	84	98			4.42
16	4-Br					12	43	72	89			4.37
17	2,3-CH_3					11	32	67	87	95		4.24
18	2-CH						31	60	82	96		4.17
19	2,6-Cl						24	56	84	97		4.08
20	4-Cl						27	55	79	93		4.07
21	3-F						20	50	83			4.00
22	3,5-Cl						18	45	78			3.93
23	2-F						19	43	73			3.87
24	3-CH_3						11	39	71			3.82
25	4-F							12	27	60	85	3.17
26	4-CH_3							6	26	61	83	3.16
27	None							0	24	58	85	3.12
28	4-OCH_3								9	32	72	2.78

[a] Negative logarithm of the molar concentration of inhibitor required for 50% inhibition.

the Hammett σ, the Hansch π, and the Taft E_s values. The 2-CF_3 derivative was excluded because the substituent constants for it were not available. Statistically significant correlations for all of the remaining 27 compounds could be obtained, but the best equation only accounted for about $^2/_3$ of the variance. When the compounds were grouped in single-substituent subseries (ortho, meta, or para), excellent correlations were observed (Table II). In the ortho series ($N = 5$), eq 1 fits the data with an r^2 of 0.997 and with s (standard deviation) = 0.07. In the para series ($N = 7$), eq 2

$$pI_{50} = 1.691\ (\pm 0.099)\ \pi + 2.986\ (\pm 0.432)\ \sigma + 3.115\ (\pm 0.073) \quad (1)$$

$$pI_{50} = 0.670\ (\pm 0.262)\ \pi + 2.058\ (\pm 0.564)\ \sigma + 3.161\ (\pm 0.120) \quad (2)$$

had $s = 0.19$ and r^2 was 0.968. In the two preceding series, the addition of a π^2 term did not improve the fit, but in the meta series ($N = 7$) some improvement with a π^2 term was noted (eq 3). In this case, r^2 was 0.985 and $s = 0.16$. All the

$$pI_{50} = -0.597\ (\pm 0.478)\ \pi^2 + 2.131\ (\pm 0.582)\ \pi + 1.971\ (\pm 0.413)\ \sigma + 3.054\ (\pm 0.144) \quad (3)$$

equations were statistically significant by the F test at $P = 0.01$. The numbers in parentheses are the standard errors of the coefficients.

Disubstituted derivatives were evaluated with the above equations presumably valid for one of the substituent positions (Table III). These pI_{50} values fit well to one equation or the other except in those cases in which the substituents were on opposite sides of the ring (2,5-diCl, 2,6-diCl, and 3,5-diCl); in those cases, the fit was poor. The concept of a cleft in the enzyme surface in which the aromatic nucleus must rest could explain this observation. An enzyme-inhibitor assembly of this sort could also account for the apparent lesser or negligible contribution of a 3-OH group protruding from the cleft to stabilization through hydrogen bonding compared to the known stabilizing effect of a 4-OH group.[4]

Table II. Comparison of Calculated and Observed pI_{50} Values for Monosubstituted Benzylamines

Compd no.	Arom subst	π	σ	Calcd pI_{50}	Obsd pI_{50}
	a. Ortho-Substituted Benzylamines (Eq 1)				
27	None	0	0	3.11	3.12
4	I	0.92	0.210	5.30	5.37
10	Br	0.75	0.210	5.01	4.96
13	Cl	0.59	0.200	4.71	4.66
23	F	0.01	0.240	3.85	3.87
	b. Meta-Substituted Benzylamines (Eq 2)				
27	None	0	0	3.05	3.12
3	CF_3	1.07	0.415	5.46	5.64
5	I	1.15	0.352	5.40	5.30
7	Br	0.91	0.391	5.26	5.17
8	Cl	0.68	0.373	4.96	5.07
21	F	0.19	0.337	3.91	4.00
24	CH_3	0.15	−0.069	3.84	3.82
	c. Para-Substituted Benzylamines (Eq 3)				
27	None	0	0	3.16	3.12
6	CF_3	1.07	0.551	5.01	5.20
15	I	1.26	0.276	4.57	4.42
16	Br	1.02	0.232	4.32	4.37
20	Cl	0.70	0.227	4.10	4.07
25	F	0.14	0.062	3.38	3.17
28	CH_3O	−0.04	−0.268	2.58	2.78

Inhibition by Benzylamines with Side Chain Substitution. Table IV shows the effect of an α-methyl substituent on the inhibition by several benzylamines. In three of the four compounds examined with a substituent in the 2 position of the ring, the addition of an α-methyl group enhanced the degree of inhibition; thus, the 2,3-dichloro, the 2-chloro, and the 2-trifluoromethyl compounds with the α-methyl group were better inhibitors than the corresponding un-

Table III. Comparison of Calculated and Observed pI_{50} Values for Disubstituted Benzylamines

Compd no.	Arom subst	Calcd pI_{50} Eq 1	Eq 2	Eq 3	Obsd pI_{50}
1	2-Cl, 3-CF_3	7.76		6.75	6.82
2	2,3-diCl	6.97		6.11	6.23
9	3,4-diCl		5.32	6.32	4.97
11	2,5-diCl	6.97		6.11	4.82
12	2,4-diCl	6.57	4.91		4.68
17	2,3-diCH_3	4.45		4.41	4.24
19	2,6-diCl	6.31			4.08
22	3,5-diCl			6.57	3.93

Table IV. Inhibition by α-Methylbenzylamines

Compd no.	Arom subst	pI_{50}	Effect of α-methyl[a]
29	2-Cl, 3-CF_3	6.45	−0.37
30	2,3-Cl	6.42	+0.19
31	2-Cl	5.29	+0.63
32	2-CF_3	4.92	+0.28
33	3-Cl	4.72	−0.35
34	4-Cl	3.70	−0.37
35	None	3.04	−0.08
36	None (−)	3.17	
37	None (+)	2.21	

[a] Difference from pI_{50} for corresponding compounds lacking α-methyl (Table I).

Table V. Inhibition by α-Alkyl- and *N*-Alkylbenzylamines

Compd no.	Arom subst	pI_{50}	E_S	pK_a'
27	None	3.12	2.48[a]	9.1[b]
35	α-CH_3	3.04		
38	α-CH_2CH_3	2.25		
39	α-$CH(CH_3)_2$	<2		
40	α-Cyclopropyl	<2		
41	α-CF_3	<2		
42	*N*-Cyclopropyl	3.13	1.18 (estd)	7.5
43	*N*-Methyl	3.11	1.24	9.1
44	*N*-Propargyl	3.05	0.94 (estd)	6.5
45	*N*-Ethyl	2.86	1.17	9.1
46	*N*-Dimethyl	2.34	0.00	8.0
47	*N*-Isopropyl	2.31	0.77	9.1
48	*N*-*tert*-Butyl	2.19	−0.30	9.3
49	*N*-Benzyl	<2		

[a] E_S values from "Rates and Equilibria of Organic Reaction," J. E. Laffler and E. Grunwald, Ed., Wiley, New York, N. Y., 1963, p 228. [b] Apparent pK_a values by titration in 66% DMF.

branched benzylamines. A likely explanation is apparent by inspection of space-filling molecular models of these compounds. The presence of a bulky substituent in the 2 position of the ring of α-methylbenzylamines prevents free rotation of the side chain and may "lock" the molecule into a conformation favorable for combination with PNMT. The reason for the lack of enhanced activity as a result of the α-methyl group in the 2-chloro-3-trifluoromethyl compound is not obvious; as with compounds having no 2 substituent, the presence of the α-methyl group in that compound appreciably lowered the inhibitory potency. The (−) isomer of α-methylbenzylamine was nearly ten times as active as the (+) isomer.

3-Chlorophenylhydrazine and 3-bromophenylhydrazine, isosteres of the corresponding benzylamines, were weaker inhibitors with pI_{50} values of 3.76 and 3.98, respectively.

Table V shows the result of substituting other alkyl groups besides methyl on the α-carbon of benzylamine. Although the α-methyl group only slightly reduced the inhibitor activity, larger α-alkyl groups markedly decreased the activity. The addition of an *N*-methyl substituent had no detectable effect on the potency of benzylamine as an inhibitor, but the activity of benzylamines with other N substituents was decreased. The N-substituted compounds permitted regression analysis of another feature of structural variation. The potency of the N-substituted benzylamines correlated fairly well with changes in the Taft steric constant (E_S) and in apparent pK_a. Excluding the benzyl derivative, we derived eq 4 which fits the data with a significant degree of correlation ($P < 0.05$). In this case, $n = 8$, $r^2 = 0.78$, and $s = 0.23$.

$$pI_{50} = -0.154pK_a\ (\pm 0.084) + 0.400E_S\ (\pm 0.101) + 3.696\ (\pm 0.715) \quad (4)$$

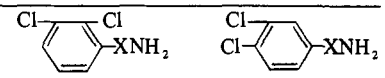
The effect of aromatic substitution on the pK_a of the amino group also points to the desirability of having more of the nonprotonated species available. Thus, the two most active compounds have apparent pK_a's at 6.95 (2-chloro-3-trifluoromethylbenzylamine) and 7.35 (2,3-dichlorobenzylamine), respectively. The series was not analyzable on this basis, however, because of the very narrow pK_a range of other compounds in the group (8.9–9.3).

Table VI. Inhibition by *N*-Methylbenzylamines

Compd no.	Arom subst	pI_{50}	Effect of *N*-methyl[a]
50	2,3-Cl	5.77	−0.46
51	2-Cl (α-methyl)	4.67	−0.62
52	3-Cl	4.59	−0.48
53	3-Cl (α-methyl)	4.41	−0.31
54	4-Cl	4.39	+0.22
55	2-Cl	4.23	−0.43
56	4-Cl (α-methyl)	3.50	−0.20
43	None	3.11	−0.01

[a] Difference from pI_{50} for corresponding compounds lacking *N*-methyl (Tables I and III).

Table VII. Influence of Side-Chain Length on the Inhibition of PNMT by 2,3-Dichlorophenyl- and 3,4-Dichlorophenylalkylamines

X = 

	pI_{50}[a]	
$-CH_2-$	6.23 (2)	4.97 (9)
$-CH_2CH_2-$	4.52 (57)	4.77 (60)
$-CH_2CH_2CH_2-$	4.18 (58)	3.79 (61)
$-CH_2CH_2CH_2CH_2-$		3.64 (62)
$-OCH_2CH_2-$	4.71 (59)	3.76 (63)

[a] Compound numbers are shown in parentheses.

The effect of the *N*-methyl group on several benzylamines with ring substitutions was examined (Table VI). With compounds of higher inhibitory potency than benzylamine, the *N*-methyl reduced inhibitor activity substantially in all cases but one (the 4-chloro compound). Thus, *N*-methyl compounds were dropped from further consideration as inhibitors.

Table VII shows the effect of the length of the connecting chain between the phenyl ring and the amine nitrogen. In the benzylamine series, the 2,3-dichloro compound was much more potent than the 3,4-dichloro derivative. In the phenethylamine series, the 3,4-dichloro member was more active. But in the phenylpropylamines or in the phenoxyethylamines, the 2,3-dichloro compound was again the more active inhibitor. Both with the 2,3-dichloro and the 3,4-dichloro substitution, lengthening the connecting carbon

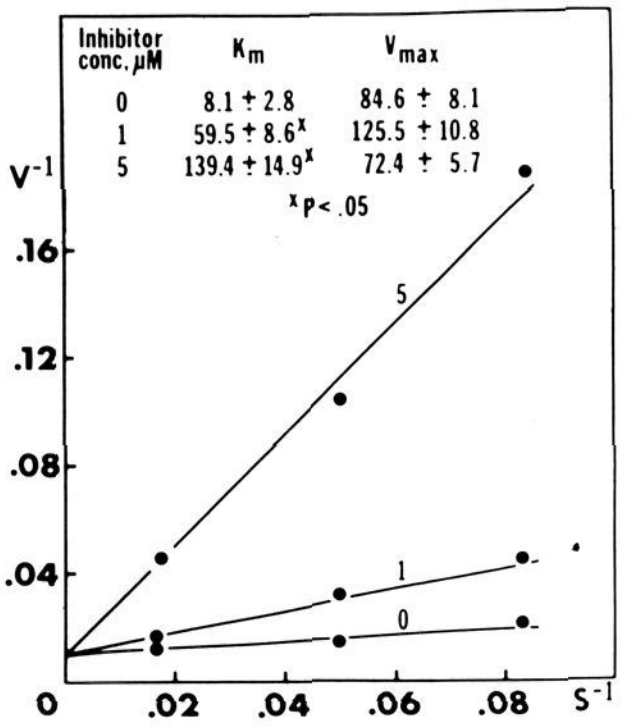


Figure 1. Competitive inhibition of PNMT by 3-trifluoromethylbenzylamine. The reciprocal of velocity (V) in picomoles of epinephrine formed per 30 min of incubation is plotted *vs.* the reciprocal of μM substrate concentration (S). K_m (μM) and V_{max} (picomoles per 30 min) values were calculated by the method of Wilkinson[12] with an Olivetti desk-top computer.

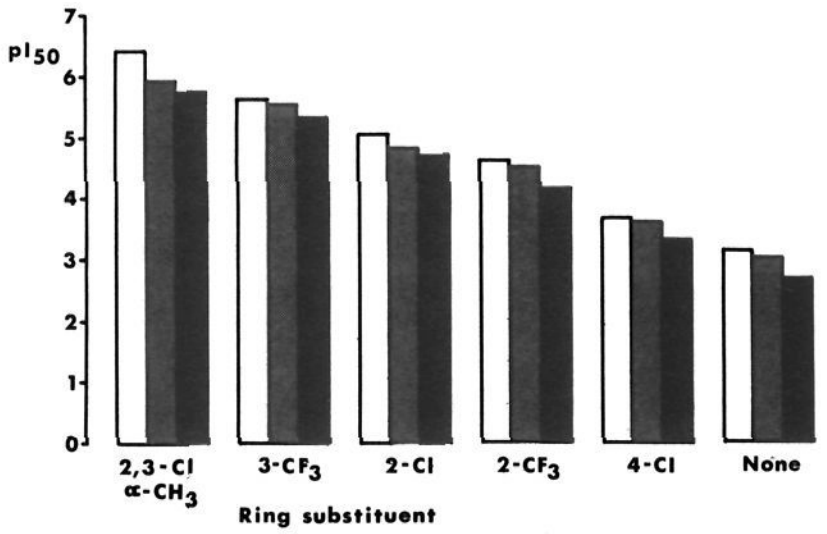


Figure 2. Parallel inhibition of PNMT from three species. Open bars represent inhibition of the enzyme from rabbit adrenals; shaded bars, the enzyme from rat adrenals; and solid bars, the enzyme from human adrenals.

chain diminished inhibitor potency (Table VII).

Kinetic Studies. Figure 1 shows that the inhibition by the most potent monosubstituted benzylamine in the series, 3-trifluoromethylbenzylamine, is competitive with respect to the methyl-accepting amine substrate, norepinephrine. There was a progressive increase in apparent K_m with increasing concentrations of inhibitor but no indication of a reduction in V_{max}. This finding suggests that the benzylamines occupy the same site on PNMT at which norepinephrine combines. The K_i value for 3-trifluoromethylbenzylamine calculated from the data in Figure 1 was $2\text{–}3 \times 10^{-7} M$, compared to the K_m for l-norepinephrine of $8 \times 10^{-6} M$, indicating that the inhibitor had about 40 times the affinity for PNMT as did its natural amine substrate.

Inhibition of PNMT from Three Different Species. Although most of our search for PNMT inhibitors is done with the rabbit adrenal enzyme, we anticipate testing any active compounds in other experimental animals, particularly in rats, and the ultimate aim is to obtain compounds that will be active in humans. For these reasons, we compared several benzylamines, chosen to cover a wide range of inhibitory potency, in terms of their effects on enzymes from the human and rat as well as from the rabbit (Figure 2). The order of inhibitory potency was exactly the same in all three species. All inhibitors were slightly less active against human PNMT than against rabbit PNMT, and all were least active against rat PNMT. The species differences were in all cases less than one pI_{50} unit.

Experimental Section

The following amines were commercially available: benzylamines **5, 8, 9, 12, 13, 18, 20, 21, 24,** and **26** (Aldrich), **2, 4, 10, 11, 15, 16,** and **22** (Sapon), **27** and **28** (Eastman), **25** (Pierce), **23** (Ames), **19** (Chemical Products); α-methylbenzylamines **34, 35, 36,** and **37** (Aldrich); *N*-alkylbenzylamines **44, 45, 47, 49, 54,** and **52** (Aldrich), **43** (Matheson), **46** (Eastman), **48** (K & K), **55** (Ames). 3-Chlorophenylhydrazine and 3-bromophenylhydrazine, as hydrochlorides, were purchased from Aldrich. All other amines were obtained from the appropriate starting materials by one of three procedures as shown in Table VIII.

General Procedure I. Amines from amides or nitriles were prepared by dropwise addition of a THF solution of the amide or nitrile (0.1 mol), at room temperature under N_2, to 300 ml of 1 *M* BH_3 in THF. The reaction mixture was refluxed for 16 hr. Excess borane was decomposed by the cautious dropwise addition of 2 *N* HCl at 0°. The THF was evaporated and the aqueous layer washed (Et_2O), basified (5 *N* NaOH), and extracted (Et_2O). The Et_2O layer was washed (H_2O) and extracted (2 *N* HCl). The aqueous extract was evaporated to yield the amine·HCl which was recrystallized.

General Procedure II. α-Methylbenzylamines were prepared by heating the appropriate acetophenone (0.1 mol), HCOOH (0.3 mol), and $HCONH_2$ (0.5 mol) at 160° for 16 hr. The mixture was diluted with Et_2O, washed (H_2O, 10% Na_2CO_3, saturated NaCl solution), dried (Na_2SO_4), and evaporated. The resulting oily *N*-formyl derivative was refluxed in 2 *N* HCl for 16 hr without further purification. The aqueous solution was washed ($CHCl_3$) until all color was removed. Evaporation of the aqueous solution yielded the α-methylbenzylamine HCl which was purified by recrystallization.

General Procedure III. *N*,α-Dimethylbenzylamines were prepared by reaction and isolation as in procedure II, with the exception that $HCONHCH_3$ was utilized in place of $HCONH_2$.

3-(α,α,α-Trifluoromethyl)anthranilic Acid. The anthranilic acid was prepared by the method of Baker, *et al.*,[9] from 7-trifluoromethylisatin (100 g, 0.47 mol) to yield 73.3 g (77%), mp 153–156°. *Anal.* ($C_8H_6F_3NO_2$).

2-Chloro-3-(α,α,α-trifluoromethyl)benzoic Acid. A solution of $NaNO_2$ was prepared by adding 23 g (0.33 mol) to 240 ml of concentrated H_2SO_4, warming to 70° to complete the solution, and cooling to 25°. A solution of 3-(α,α,α-trifluoromethyl)anthranilic acid (0.3 mol, 61.5 g) in 600 ml of warm AcOH was added dropwise to the previously prepared solution of $NaNO_2$ in concentrated H_2SO_4. The temperature of the mixture during addition was maintained below 40°. This solution was added dropwise to a solution of CuCl (0.66 mol, 66 g) in 40 ml of concentrated HCl at 0°. The mixture was warmed to 25° and 1500 ml of H_2O added to precipitate the product which was filtered to yield 52.7 g. Successive recrystallization from a total of 4 l. of H_2O yielded 43.2 g (64%), mp 130–132°. *Anal.* ($C_8H_4ClF_3O_2$).

A. 2-Chloro-3-(α,α,α-trifluoromethyl)benzamide. A stirred solution of 2-chloro-3-(α,α,α-trifluoromethyl)benzoic acid (0.02 mol, 4.48 g) in 50 ml of C_6H_6 at room temperature was treated with oxalyl chloride (0.1 mol, 9 ml). The mixture was refluxed overnight, cooled, and evaporated. The resulting acid chloride was dissolved in 50 ml of Et_2O, cooled to 0°, and saturated with a dry NH_3 gas. The mixture was stirred at room temperature 4 hr, washed (2 *N* HCl, 10% Na_2CO_3, saturated NaCl), dried (Na_2SO_4), and evaporated. The resulting amide was recrystallized (C_6H_6–cyclohexane) to yield 1.7 g (38%), mp 112–113°. *Anal.* ($C_8H_5ClF_3NO$).

B. 2-Chloro-3-(α,α,α-trifluoromethyl)acetophenone. A solution of 2-chloro-3-α,α,α-trifluoromethylbenzoic acid (0.06 mol, 13.5 g) in 75 ml of THF was added dropwise to a solution of CH_3Li (0.132 mol, 0.97 g) in Et_2O under N_2 at −78°. The mixture was warmed to 0° for 1 hr and the excess CH_3Li decomposed with saturated NH_4Cl solution. The mixture was diluted with Et_2O, washed (saturated NH_4Cl solution, H_2O, saturated NaCl solution), dried ($MgSO_4$), and evaporated to yield 17.3 g (90%) of light yellow liquid which was used without further purification (pure by nmr).

Table VIII. Synthetic Methods and Physical Properties of New Compounds

Compd	Method[a]	Mp, °C[b]	Crystn solvent	% yield	Formula[c]
1	IA	237–238	*i*-PrOH + EtOAc	68	$C_8H_7ClF_3N \cdot HCl$
3	I[d]	216–218	*i*-PrOH + EtOAc	55	$C_8H_8F_3N \cdot HCl$
6	I[d]	>250	*i*-PrOH + EtOAc	60	$C_8H_8F_3N \cdot HCl$
7	I[d]	208–211	*i*-PrOH	22	$C_7H_8BrN \cdot HCl$
14	I[d]	>250	*i*-PrOH	38	$C_8H_8F_3N \cdot HCl$
17	I[d]	235–236	*i*-PrOH + EtOAc	63	$C_9H_{13}N \cdot HCl$
29	IIB	237–239	*i*-PrOH + EtOAc	41	$C_9H_9ClF_3N \cdot HCl$
30	IIC	210–215	EtOAc + MeOH	54	$C_8H_9Cl_2N \cdot HCl$
31	II[e]	86–91[g]		57	$C_8H_{10}ClN$
32	II[e]	217–220	EtOAc + MeOH	23	$C_9H_{10}F_3N \cdot HCl$
33	II[e]	170–172	*i*-PrOH + Me_2CO	58	$C_8H_{10}ClN \cdot HCl$
38	II[e]	189–191	EtOAc + MeOH	73	$C_9H_{13}N \cdot HCl$
39	II[e]	>250	EtOAc + MeOH	50	$C_{10}H_{15}N \cdot HCl$
40	II[f]	234–237	*i*-PrOH + EtOAc	68	$C_{10}H_{13}N \cdot HCl$
41	II[e]	235 (sub)	EtOAc + MeOH	9[h]	$C_8H_8F_3N \cdot HCl$
42	ID	158–160	*i*-PrOH + EtOAc	68	$C_{10}H_{13}N \cdot HCl$
50	IE	173–175	*i*-PrOH + EtOAc	68	$C_8H_9Cl_2N \cdot HCl$
51	III[e]	148–149	EtOAc + MeOH	35	$C_9H_{12}ClN \cdot HCl$
53	III[e]	190–192	EtOAc + MeOH	39	$C_9H_{12}ClN \cdot HCl$
56	III[e]	202–205	EtOAc + MeOH	51	$C_9H_{12}ClN \cdot HCl$
57	IF	195–196	*i*-PrOH + EtOAc	52	$C_8H_9Cl_2N \cdot HCl$
58	IG	207–209	*i*-PrOH + EtOAc	47	$C_9H_{11}Cl_2N \cdot HCl$
59	IH	190–192	*i*-PrOH + EtOAc	67	$C_8H_9Cl_2NO \cdot HCl$
60	I[d]	176–178	*i*-PrOH + EtOAc	61	$C_8H_9Cl_2N \cdot HCl$
61	II	141–143	EtOAc + MeOH	82	$C_9H_{11}Cl_2N \cdot HCl$
62	IJ	109–111	EtOAc	64	$C_{10}H_{13}Cl_2N \cdot HCl$
63	IH	238–240	*i*-PrOH + MeOH	52	$C_8H_9Cl_2NO \cdot HCl$

[a]Methods refer to the Experimental Section, the general procedure used, and preparation of starting material(s). [b]Melting points were detected in an open capillary in an oil bath and are uncorrected. [c]All compounds were analyzed for C, H, N, and halogen and are correct within ±0.3% of their calculated values. [d]Nitrile is commercially available. [e]Acetophenone is commercially available. [f]Cyclopropyl phenyl ketone is commercially available. [g]Boiling point (0.1 mm). [h]Recovered 88% α-trifluoromethylbenzyl alcohol.

C. 2,3-Dichloroacetophenone. A solution of 2,3-dichlorobenzonitrile (0.25 mol, 43 g) in 500 ml of Et_2O was added dropwise to a stirred solution of methyl Grignard (0.5 mol) in 500 ml of Et_2O and refluxed 2 hr. The mixture was carefully added to 250 ml of concentrated HCl in ice and allowed to stand overnight. The aqueous layer was extracted (Et_2O) and the extract washed (H_2O, 10% Na_2CO_3, saturated NaCl soln) and dried (Na_2SO_4). Evaporation yielded 45 g (96%) of a dark liquid which was used without purification (nmr showed no starting nitrile): ir C=O (1700 cm^{-1}), no C=N absorption.

D. *N*-Cyclopropylbenzamide. The amide was prepared according to method of Aeberli, *et al.*,[10] in 62% yield, mp 96–98° (lit.[10] mp 95–97°). *Anal.* ($C_{10}H_{11}NO$).

E. *N*-Methyl-2,3-dichlorobenzamide. Oxalyl chloride (0.25 mol, 22 ml) was added slowly to a stirred solution of 2,3-dichlorobenzoic acid (0.05 mol, 9.55 g) in 100 ml of C_6H_6. The solution was dissolved in 150 ml of Et_2O at 0°, dry CH_3NH_2 introduced, and the resultant mixture stirred at room temperature 4 hr. The mixture was washed (2 *N* HCl, 10% Na_2CO_3, saturated NaCl solution), dried (Na_2SO_4), and evaporated. The resulting amide was recrystallized (C_6H_6–cyclohexane) to give 5.5 g (54%), mp 118–121°. *Anal.* ($C_8H_7Cl_2NO$).

2,3-Dichloro-α-bromotoluene. A solution of 2,3-dichlorotoluene (0.31 mol, 50 g), *N*-bromosuccinimide (0.34 mol, 60.5 g), and benzoyl peroxide (150 mg) in 1 l. of CCl_4 was refluxed 16 hr. The mixture was cooled to room temperature and the resulting succinimide filtered. The filtrate was washed (H_2O), dried (Na_2SO_4), and evaporated to yield 74 g (99%) of yellow liquid which was used without further purification (nmr showed no starting material).

F. 2,3-Dichlorophenylacetonitrile. A stirred solution of 2,3-dichloro-α-bromotoluene (0.15 mol, 36 g) in 150 ml of DMF–100 ml of H_2O at 0° was treated with KCN (0.225 mol, 14.6 g), which was added in portions. The mixture was allowed to warm to room temperature, stirred for 16 hr, and then diluted with 250 ml of H_2O and extracted (Et_2O). The Et_2O layer was washed (H_2O, saturated NaCl solution), dried (Na_2SO_4), and evaporated. The residue was distilled at 92–95° (0.5 mm) to yield 12.6 g (45%) of colorless liquid. A recrystallized sample (hexane) had mp 72–74°. *Anal.* ($C_8H_5Cl_2N$).

Ethyl 2,3-Dichlorobenzylcyanoacetate. A solution of ethyl cyanoacetate (0.32 mol, 36 g) in 50 ml of C_6H_6 was added dropwise to a suspension of NaH (0.24 mol, 5.8 g) in 150 ml of DMF–50 ml of C_6H_6. The mixture was allowed to stir at room temperature until gas evolution ceased. A solution of 2,3-dichloro-α-bromotoluene (39 g, 0.162 mol) in 50 ml of C_6H_6 was then added dropwise and the mixture stirred 48 hr. The excess NaH was destroyed with the addition of a few milliliters of EtOH. The mixture was then diluted with 500 ml of H_2O and extracted (C_6H_6) and the extracts were washed (H_2O, saturated NaCl solution), dried (Na_2SO_4), and evaporated. The resulting residue was distilled at 144–150° (0.1 mm) to yield 16.9 g (37%) of product. *Anal.* ($C_{12}H_{11}Cl_2NO_2$).

2,3-Dichlorobenzylcyanoacetic Acid. A solution of ethyl 2,3-dichlorobenzylcyanoacetate (0.06 mol, 16.9 g) in 250 ml of MeOH–260 ml of 10% Na_2CO_3 (0.24 mol) was heated at 65° for 16 hr. The MeOH was evaporated and the aqueous mixture diluted with 200 ml of H_2O. The aqueous layer was washed (Et_2O), acidified (concentrated HCl), and extracted (Et_2O). The extracts were washed (H_2O, saturated NaCl solution), dried (Na_2SO_4), and evaporated. The resulting solid was recrystallized (C_6H_6) to yield 9.2 g (62%), mp 159–162°. *Anal.* ($C_{10}H_7Cl_2NO_2$).

G. 3-(2,3-Dichlorophenyl)propionitrile. 2,3-Dichlorobenzylcyanoacetic acid (0.04 mol, 9.2 g) was heated at 180° for 2 hr until evolution of gas ceased. The resulting residue was distilled at 103–106° (0.1 mm) to yield 4.1 g (53%) of colorless liquid. *Anal.* ($C_9H_7Cl_2N$).

H. 2,3- and 3,4-Dichlorophenoxyacetonitriles. The respective phenol (0.1 mol, 16.3 g), $ClCH_2CN$ (7.5 g, 0.1 mol), and K_2CO_3 (0.1 mol, 13.8 g) were refluxed in 100 ml of acetone for 16 hr. The cooled mixture was diluted with H_2O, basified (NaOH), and extracted (EtOAc). The extract was washed (5 *N* NaOH, H_2O, saturated NaCl solution), dried ($MgSO_4$), and evaporated. The resulting solid was recrystallized (C_6H_6–cyclohexane) to yield 2,3-dichloro- [18 g (89%), mp 86–89°. *Anal.* ($C_8H_5Cl_2NO$)] and 3,4-dichlorophenoxyacetonitrile [17.8 g (88%), mp 59–61°. *Anal.* ($C_8H_5Cl_2NO$)].

β-(3,4-Dichlorophenyl)propionic Acid. Hydrogenation of 3,4-dichlorocinnamic acid (0.46 mol, 100 g) in dioxane over Pd/C catalyst yielded 80 g (80%) of the recrystallized (hexane) propionic acid, mp 86–89°. *Anal.* ($C_9H_8Cl_2O_2$).

I. β-(3,4-Dichlorophenyl)propionamide. A solution of β-(3,4-dichlorophenyl)propionic acid (0.1 mol, 21.9 g) in 200 ml of C_6H_6 was treated at room temperature with oxalyl chloride (0.5 mol, 64 g). The mixture was refluxed 3 hr, cooled, and evaporated *in vacuo*. The residue was dissolved in 250 ml of Et_2O and the ethereal solution saturated with dry NH_3 at 0°. The mixture was stirred at room temperature 4 hr, washed (H_2O, 2 *N* HCl, 10% Na_2CO_3, saturated

NaCl), and dried (Na_2SO_4). Evaporation (Et_2O) and recrystallization (C_6H_6–cyclohexane) yielded 17.5 g (81%) of the amide, mp 76–78°. *Anal.* ($C_9H_9Cl_2NO$).

3-(3,4-Dichlorophenyl)propanol-1. A solution of β-(3,4-dichlorophenyl)propionic acid (0.2 mol, 43.8 g) in 250 ml of THF was added dropwise to a solution of BH_3 (0.6 mol) in 600 ml of THF at 0° under N_2. The mixture was refluxed 3 hr and cooled to 0°, and the excess BH_3 was destroyed with 2 *N* HCl. The THF was removed *in vacuo;* the aqueous layer was neutralized (2 *N* NaOH) and extracted (Et_2O). The extracts were washed (saturated $NaHCO_3$, saturated NaCl solution), dried (Na_2SO_4), and evaporated. The resulting residue was distilled at 121–122° (0.5 mm) to yield 37.6 g (91%). *Anal.* ($C_9H_{10}Cl_2O$).

J. 4-(3,4-Dichlorophenyl)butyronitrile. The propanol (0.1 mol, 20.5 g) was dissolved in 75 ml of dry C_5H_5N and CH_3SO_2Cl (0.11 mol, 8.4 ml) was added dropwise, while maintaining the temperature below 20°. The mixture was stirred at room temperature for 3 hr and then added to 75 ml of concentrated HCl in ice. The aqueous layer was extracted (Et_2O) and the extract washed (10% Na_2CO_3, saturated NaCl solution), dried (Na_2SO_4), and evaporated. The resulting 28 g (99%) of light-yellow liquid sulfonate ester was immediately dissolved in 100 ml of DMF–25 ml of H_2O and cooled to 0°. Solid KCN (0.15 mol, 9.75 g) was added and the mixture stirred at room temperature 72 hr. The mixture was diluted with 100 ml of H_2O and extracted (Et_2O). The Et_2O extracts were washed (H_2O, saturated NaCl) and dried (Na_2SO_4). Evaporation of solvent yielded a red liquid which was distilled at 115–118° (0.1 mm) to yield 18.1 g (84%) of colorless liquid. *Anal.* ($C_{10}H_9Cl_2N$).

Enzyme Assay. The enzyme preparation and the method of enzyme assay have been previously described in detail.[11] An $(NH_4)_2SO_4$ fraction of the high-speed centrifugal supernatant fraction from a homogenate of whole adrenal glands from rabbits was used as the enzyme.[11] L-Norepinephrine (40 μ*M*) was the substrate. Enzyme activity was calculated as picomoles of epinephrine formed per 30 min of incubation. Several concentrations of each inhibitor were tested for enzyme inhibition; the concentrations were spaced on a log basis, *e.g.*, 1, 3, 10, 32, 100, 317 μ*M*, and were selected to achieve a range of inhibition on both sides of 50%. By interpolation, the molar concentration required for 50% inhibition was determined. The negative log of this concentration is defined as the pI_{50} value.

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Inhibition of Phenylethanolamine *N*-Methyltransferase by Benzylamines. 2. *In Vitro* and *In Vivo* Studies with 2,3-Dichloro-α-methylbenzylamine

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2,3-Dichloro-α-methylbenzylamine (DCMB) was a potent inhibitor of phenylethanolamine *N*-methyltransferase *in vitro*. The (+) isomer was more active than the (−) isomer, and the inhibition was reversible and competitive with respect to norepinephrine. The calculated K_i for DCMB was 0.09 μ*M*, compared to the K_m of 12 μ*M* for l-norepinephrine as the substrate. The α-methyl was required for biological stability of benzylamines. DCMB localized in tissues in the following order of concentration when injected intraperitoneally into rats: lung > kidney > liver > brain > adrenal > spleen > heart > blood. The half-life of the compound in the adrenal gland after injection of a dose of 0.2 mmol/kg was 3.4 hr. The concentration of the drug in the adrenal glands bore a nearly linear relation to dose over the dose range of 0.1–0.4 mmol/kg. Treatment of rats with DCMB prior to forced exercise caused a significant reduction of epinephrine, indicative of phenylethanolamine *N*-methyltransferase inhibition *in vivo*.

A specific inhibitor of phenylethanolamine *N*-methyltransferase (PNMT) in the adrenal medulla would suppress the formation of the adrenal medullary hormone, epinephrine, without directly interfering with norepinephrine biosynthesis. Such an inhibitor may find medicinal application, especially if some pathological states are associated with overproduction of epinephrine. Recently we have found that a group of benzylamines are unexpectedly potent inhibitors of PNMT *in vitro*.[1] This paper describes further *in vitro* studies with one of the most active members of that group, 2,3-dichloro-α-methylbenzylamine, and also reports the results of the initial *in vivo* studies with this compound.

In Vitro **Studies.** Figure 1 shows a comparison of the inhibition caused by the stereoisomers of 2,3-dichloro-α-methylbenzylamine. The (+) isomer was more active than the racemic mixture and hence much more active than the (−) isomer. The difference between the isomers was more than tenfold, the pI_{50} value for the (+) isomer being 6.81 and that for the (−) isomer being 5.72.

To determine whether the inhibition by 2,3-dichloro-α-methylbenzylamine was reversible, we measured the ability of dialysis in Visking tubing to remove the inhibitor and thereby restore enzyme activity after the inhibitor and enzyme were mixed *in vitro*. The results in Table I show that dialysis for 24 hr nearly completely restored enzyme activity, indicating that the amount of inhibitor which was sufficient to cause 62–65% inhibition had been removed by dialysis. Thus, the inhibitor appears to form a reversible complex with the enzyme.

Figure 2 shows a Lineweaver-Burk plot. The inhibition by 2,3-dichloro-α-methylbenzylamine was competitive with respect to the methyl-accepting substrate, norepinephrine. From the data in Figure 2, kinetic constants were calculated by the method of Wilkinson.[2] The V_{max} was not altered by the inhibitor, being 57 ± 6 for the control and 58 ± 2 and