Registry No. 1, 69-33-0; 2, 606-58-6; 3, 18417-89-5; 4, 6742-12-7; 5, 40627-14-3; 5·HCl, 40627-15-4; 6, 40725-90-4; 6·HCl, 40627-13-2; 7, 60129-59-1; 8, 15676-19-4; 9, 83379-28-6; 10, 64526-34-7; 11, 90813-71-1; 12, 90813-74-4; 13, 40725-89-1; 14, 105582-76-1; 15, 83379-31-1; 16, 64526-29-0; 17a, 42867-63-0; 17b, 40627-37-0; 17c, 40627-38-1; 18a, 40627-32-5; 18b, 105661-43-6; 18c, 40627-36-9; 19a, 105582-77-2; 19b, 105582-78-3; α-acetoxyisobutyryl bromide, 40635-67-4.

Multisubstrate Inhibitors of Dopamine β-Hydroxylase. 2.[1] Structure-Activity Relationships at the Phenethylamine Binding Site

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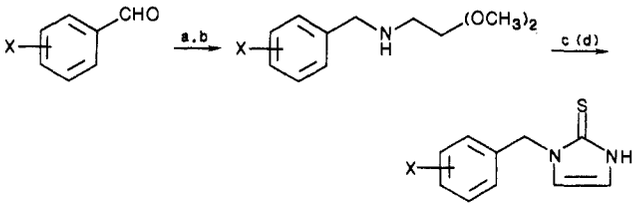
1-Aralkylimidazole-2-thiones have been shown to be potent multisubstrate inhibitors of dopamine β-hydroxylase (DBH; EC 1.14.17.1). In the present study, a series of 1-benzylimidazole-2-thiones was prepared to explore the effects of substitution in the benzyl ring on the inhibition of DBH. A detailed structure-activity relationship for in vitro activity was discovered and this was shown by a modified Hansch analysis to correlate (r = 0.91) with four key structural features of the benzyl ring: (1) the presence of a hydroxyl at the 4-position, (2) molar refractivity at the 3-, 4-, and 5-positions, (3) inductive effects of the substituents at the 3-, 4-, and 5-positions, and (4) π-electron density. The affinity (K_{is}) of eight substituted inhibitors for DBH was shown to correlate (r = 0.75) with the affinity (K_D) of comparably substituted tyramines for the ternary DBH-oxygen-tyramine complex. This correlate is used to support the hypothesis that binding of inhibitor to DBH occurs in a fashion that mimics the binding of tyramine substrates. The most potent inhibitors were selected for study in vivo in the spontaneously hypertensive rat model of hypertension. The changes in vascular dopamine and norepinephrine levels that resulted from oral administration of the inhibitors corresponded to the observed reduction in mean arterial blood pressure. A divergence between in vitro potency and in vivo efficacy upon oral dosing was noted and is suggested to result from an in vivo metabolic conjugation of the phenolic group of inhibitor.

Dopamine β-hydroxylase (DBH; EC 1.14.17.1), a copper-containing mixed-function oxidase that catalyzes the conversion of dopamine to norepinephrine, is an appealing target for the rational design of new agents of potential efficacy in the treatment of cardiovascular disorders.[2,3] We recently reported some 1-phenyl- and 1-phenyl-bridged imidazole-2-thiones to be potent inhibitors of DBH that appeared to act as multisubstrate mimics of the binding of oxygen and phenethylamine substrates to the reduced, catalytically active Cu^{1+} species of enzyme (Figure 1).[1] This study delineated a stringent set of structural requirements for maximal inhibitory activity, led to the optimization of chain length for the intersubstrate bridge between the portions of inhibitor that mimic oxygen and tyramine substrates, and identified 1-(4-hydroxybenzyl)- and 1-[(4-hydroxyphenyl)propyl]imidazole-2-thiones as optimal and equipotent DBH inhibitors.

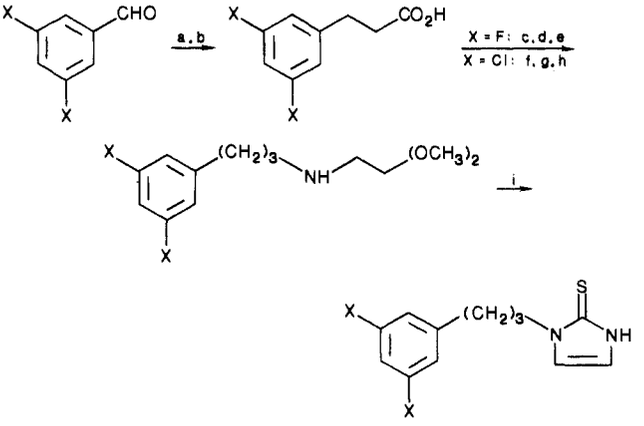
We report here the results of structure-activity relationship (SAR) studies on 1-benzylimidazole-2-thiones in which DBH inhibitory potency is compared to aryl substitution of the benzyl moiety, i.e., that portion of the inhibitor that binds the enzymatic phenethylamine site. The goals of this study were threefold. First, to generate SAR that would provide a basis for the design of multisubstrate inhibitors more potent than the parent compounds. Second, to identify a set of inhibitors of comparable in vitro potency but with differing lipophilicity and metabolic liability. It was anticipated that these would be useful in identifying the probable causes for the divergence between in vitro and in vivo potencies previously noted for several inhibitors.[3] Third, to compare the affinity of aryl-substituted inhibitors for DBH with the reported dissociation constants of identically substituted phen-

Scheme I[a]



[a] Reagents and conditions: (a) $NH_2CH_2CH(OCH_3)_2$; (b) $NaBH_4$, EtOH; (c) H_2O, HCl, KSCN, EtOH, reflux; (d) BBr_3, CH_2Cl_2.

Scheme II[a]



[a] Reagents and conditions: (a) $CH_2(CO_2H)_2$, piperidine, heat; (b) 10% Pd/carbon, THF, 50 psig hydrogen; (c) $SOCl_2$; DMF, 60 °C; (d) $NH_2CH_2CH(OCH_3)_2$, CH_2Cl_2, 0 °C; (e) $LiAlH_4$, Et_2O, 22 °C; (f) BH_3·THF, 0 °C; (g) $(COCl)_2$, Me_2SO, CH_2Cl_2, NEt_3, −78 °C; (h) $NH_2CH_2CH(OCH_3)_2$, hexane, EtOH, $NaBH_4$; (i) H_2O, HCl, KSCN, EtOH, reflux.

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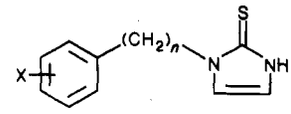
Table I. Structure, Physical Properties, and DBH-Inhibitory Activity of Some Substituted 1-Aralkylimidazole-2-thiones



no.	X	n	method of synth[a]	mp, °C	recryst solvent	yield,[b] %	formula[c]	IC_{50},[d] μM
1	4-CO_2H	1	Ex[e]	250 dec	EtOH	40	$C_{11}H_{10}N_2O_2S$	1%[f]
2	2,6-Me_2	1	A	232–235	EtOH	43	$C_{12}H_{14}N_2S$	5%[f]
3	4-CH_2OH	1	Ex[e]	137–140	MeCN	28	$C_{11}H_{12}N_2OS$	6%[f]
4	2,6-Cl_2	1	A	242–243	EtOH–Et_2O	71	$C_{10}H_8Cl_2N_2S$	7%[f]
5	3-SO_2NH_2, 4-OMe	1	Ex,[e] A	174–176	AcOH	53	$C_{11}H_{13}N_3O_3S_2$	7%[f]
6	2,6-$(OMe)_2$	1	A	194.5–196	MeCN	45	$C_{12}H_{14}N_2O_2S$	10%[f]
7	2-Cl	1	A	206–207	Me_2CO–EtOH	48	$C_{10}H_9ClN_2S$	14%[f]
8	2-Me	1	A	194–195	EtOH	39	$C_{11}H_{12}N_2S$	15%[f]
9	3,4-$(OMe)_2$	1	A	118	EtOH	72	$C_{12}H_{14}N_2O_2S$	15%[f]
10	4-CF_3	1	A	148–148.5	EtOAc–hexane	15	$C_{11}H_9F_3N_2S$	25%[f]
11	3-CF_3, 4-OMe	1	A	161–163	EtOH	48	$C_{12}H_{11}F_3N_2OS$	29%[f]
12	2,6-Cl_2, 4-OMe	1	Ex,[e] A	215–218	$EtCO_2H$	68	$C_{11}H_{10}Cl_2N_2OS$	32%[f]
13	4-CH_3	1	A	160–161.5	EtOAc–MeCN	43	$C_{11}H_{12}N_2S$	34%[f]
14	4-Br	1	A	191–192	EtOAc–MeCN	12	$C_{10}H_9BrN_2S$	43%[f]
15	3-Br, 4-OMe	1	A	188	EtOH	72	$C_{11}H_{11}BrN_2OS$	60%[f]
16	3-F, 4-OMe	1	A	151	EtOH	55	$C_{11}H_{11}FN_2OS$	67%[f]
17	2-OMe	1	A	159	EtOH	63	$C_{11}H_{12}N_2OS$	68%[f]
18	3-Me, 4-OMe	1	A	157	EtOH	54	$C_{12}H_{14}N_2OS$	72%[f]
19	2-OH	1	A, B	158	EtOH–H_2O	30	$C_{10}H_{10}N_2OS$	580 (410–948)
20	3-NO_2, 4-OMe	1	A	173–175	EtOAc–hexane	68	$C_{11}H_{11}N_3O_3S$	359 (230–606)
21	4-OMe	1	A	140	EtOH	54	$C_{11}H_{12}N_2OS$	202 (71–386)
22	3-OMe	1	A	118	EtOH	71	$C_{11}H_{12}N_2OS$	157 (118–215)
23	3-OH	1	A, B	167	EtOH–H_2O	63	$C_{10}H_{10}N_2OS$	148 (102–213)
24	3-CF_3, 4-OH	1	Ex[e]	220 dec	EtOAc–hexane	18	$C_{11}H_9F_3N_2OS$	121 (77–204)
25	2,4,6-Cl_3	1	A	240–244	EtOH	47	$C_{10}H_7Cl_3N_2S$	102 (80–135)
26	2,5-Cl_2	1	A	265 dec	$EtCO_2H$	34	$C_{10}H_8Cl_2N_2S$	97 (63–156)
27	4-Cl	1	A	187–189	MeCN	40	$C_{10}H_9ClN_2S$	96 (64–133)
28	2,6-Cl_2, 4-OH	1	Ex[e]	255 dec	$EtCO_2H$	25	$C_{10}H_8Cl_2N_2OS$	75 (36–102)
29	2,3,5,6-F_4, 4-OH	1	A, B	203–205	H_2O	42	$C_{10}H_6F_4N_2OS$	62 (56–72)
30	4-NO_2	1	A	188–190	EtOH	69	$C_{10}H_9N_3O_2S$	53 (45–62)
31	2,3-Cl_2	1	A	195–197	EtOH	23	$C_{10}H_8Cl_2N_2S$	52 (37–70)
32	3-Me, 4-OH	1	A, B	214	EtOH–H_2O	49	$C_{11}H_{12}N_2OS$	49 (25–81)
33	4-F	1	A	167–169	EtOH	79	$C_{10}H_9FN_2S$	47 (40–56)
34	3,5-Cl_2, 4-OMe	1	A	177–178	EtOAc–hexane	21	$C_{11}H_{10}Cl_2N_2OS$	47 (38–58)
35	3,5-F_2, 4-OMe	1	A	156–158	EtOAc–hexane	77	$C_{11}H_{10}F_2N_2OS$	36 (27–48)
36	H-	1	A	144–145	EtOH	47	$C_{10}H_{10}N_2S$	32 (20–46)
37	3-NO_2, 4-OH	1	Ex[e]	225–227	EtOH	42	$C_{10}H_9N_3O_3S$	31 (23–41)
38	3,4-Cl_2	1	A	178	EtOH	81	$C_{10}H_8Cl_2N_2S$	28 (21–37)
39	2,4-Cl_2	1	A	185–187	2-PrOH	60	$C_{10}H_8Cl_2N_2S$	17 (7–38)
40	3-Br, 4-OH	1	A, B	181	MeCN	42	$C_{10}H_9BrN_2OS$	12 (7–18)
41	3-Cl	1	A	129–131	MeCN	29	$C_{10}H_9ClN_2S$	12 (10–14)
42	3-F	1	A	112.5–114	2-PrOH–H_2O	27	$C_{10}H_9FN_2S$	5.6 (4.9–6.5)
43	3,5-F_2	3	Ex[e]	131–132	EtOH	26	$C_{12}H_{12}F_2N_2S$	4.7 (4.2–5.3)
44	4-OH	1	A, B	188	EtOH	42	$C_{10}H_{10}N_2OS$	2.6 (1.3–4.6)
45	3,5-Cl_2	1	A	206–209	EtOH–Me_2CO	24	$C_{10}H_8Cl_2N_2S$	2.4 (1.9–3.0)
46	3,4-$(OH)_2$	1	A, B	209–212	MeCN	28	$C_{10}H_{10}N_2O_2S$	2.2 (1.1–3.8)
47	3,5-Cl_2	3	Ex[e]	98–99	EtOH	11	$C_{12}H_{12}Cl_2N_2S$	2.1 (1.8–2.6)
48	3-Cl, 4-OH	1	A, B	186	MeCN	42	$C_{10}H_9ClN_2OS$	2.0 (1.7–2.4)
49	3-F, 4-OH	1	A, B	172	EtOH–H_2O	80	$C_{10}H_9FN_2OS$	1.5 (0.9–2.2)
50	3,5-F_2	1	A	141–143	EtOAc–hexane	78	$C_{10}H_8F_2N_2S$	1.2 (1.0–1.9)
51	3,5-Cl_2, 4-OH	1	A, B	220–222 dec	EtOAc–hexane	62	$C_{10}H_8Cl_2N_2OS$	0.68 (0.42–1.1)
52	3,5-F_2, 4-OH	1	A, B	213–215	EtOAc–hexane	74	$C_{10}H_8F_2N_2OS$	0.074 (0.060–0.087)

[a] See Experimental Section for description of general methods. [b] The overall yield from substituted benzaldehyde is given. [c] All new compounds had C, H, N microanalyses within ±0.4% of the calculated values. [d] Values are given as IC_{50} in μM with upper and lower 95% confidence limits (mean ± SEM) shown in parentheses. [e] Ex = specific experimental procedure described. [f] Activity expressed as percent inhibition at a compound concentration of 10^{-4} M.

ethylamine substrates. Similar SARs for inhibitors and substrates would strengthen arguments[4] that the binding of multisubstrate inhibitors to DBH occurs by a direct binding to the phenethylamine substrate site.

Chemistry. Synthesis of the compounds in Table I was carried out by the general methodology shown in Schemes I and II. Appropriately substituted aromatic aldehydes were condensed with aminoacetaldehyde dimethyl acetal and the resulting Schiff bases were reduced in situ with sodium borohydride in ethanol. Subsequent cyclization

(1) For part 1, see: Kruse, L. I.; Kaiser, C.; DeWolf, W. E., Jr.; Frazee, J. S.; Garvey, E.; Hilbert, E. L.; Faulkner, W. A.; Flaim, K. E.; Sawyer, J. L.; Berkowitz, B. A. *J. Med. Chem.* **1986**, *29*, 2465.

(2) Presented in part at the 190th National Meeting of the American Chemical Society, Chicago, IL, Sept 1985; American Chemical Society: Washington, DC, 1985; MEDI 65, MEDI 66.

(3) Kruse, L. I.; Kaiser, C.; DeWolf, W. E., Jr.; Frazee, J. S.; Erickson, R. W.; Ezekiel, M.; Ohlstein, E. H.; Ruffolo, R. R., Jr.; Berkowitz, B. A. *J. Med. Chem.* **1986**, *29*, 887.

(4) Kruse, L. I.; DeWolf, W. E., Jr.; Chambers, P. A.; Goodhart, P. J. *Biochemistry* **1986**, *25*, 7271.

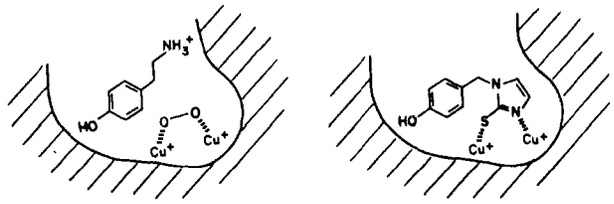


Figure 1. Hypothesized binding of substrates and multisubstrate inhibitor 44 to the DBH active site.

with acidic aqueous thiocyanate and, when appropriate, demethylation with boron tribromide, or other reagents as required, yielded the substituted 1-benzylimidazole-2-thiones in Table I.

Separate chemical syntheses are reported for those benzaldehydes that were not readily accessible by known procedures. The two substituted phenylpropyl congeners **43** and **47** were prepared from the corresponding benzaldehydes by synthetic methodology similar to that employed earlier (Scheme II).[1]

Enzymology. The compounds in Table I were screened for DBH inhibitory activity with commercially available bovine enzyme. Enzymatic assays and data analyses were performed by the methods previously described[1] to yield the indicated IC_{50} values and confidence limits. Kinetic constants were determined for inhibitors of greatest interest using homogeneous DBH of specific activity 25–42 units/mg isolated by our new procedure.[5] The pH values used in the kinetic assays correspond to those of predominately ordered conditions (pH 4.5)[6] with phenethylamine binding preceding that of oxygen, random conditions (pH 6.6), or conditions (pH 6.0) comparable to those used by Klinman to calculate true dissociation constants for substituted tyramine substrates from the ternary DBH–oxygen–phenethylamine complex.[7] Kinetic data were analyzed by the standard COMP and NONCOMP programs of Cleland.[8]

Regression Analysis and Quantitation of in Vitro Structure–Activity Relationships. Computer-assisted multiple regression analyses were performed with the RS/1 program. The π, molar refractivity, F (inductive), and R (resonance) constants were those reported by Swain and Lupton.[9,10] In Table V and eq 1, the regression coefficients are reported with standard errors, n is the number of compounds, r is the correlation coefficient, F is a significance test, and S is the standard error of the estimate.

Pharmacology. Vascular catecholamine levels and blood pressure effects were both determined in adult male Okamoto-Aoki spontaneously hypertensive rats (SHR). Dopamine (DA) and norepinephrine (NE) levels were determined in the mesenteric artery by the method of DaPrada and Zürcher[11] as modified by Head and Berkowitz[12] after oral dosing with DBH inhibitors. Blood pressures were measured directly from indwelling cannulae in the femoral artery as previously described[3] and were related to control blood pressures of vehicle-dosed SHR run simultaneously with drug-treated animals. Drugs were initially administered intraperitoneally since this was found to produce the most reproducible changes in blood pressure. Subsequent oral dosing established efficacy by this route of administration.

Results and Discussion

A previous study of multisubstrate DBH inhibitors demonstrated optimal and identical inhibitory potency for 1-(4-hydroxybenzyl)- and 1-(4-hydroxyphenylpropyl)-imidazole-2-thiones where the bridging hydrocarbon chain between the phenethylamine and oxygen substrate mimics was (CH_2) and $(CH_2)_3$, respectively.[1] The present study employed benzyl-substituted inhibitors to reduce the degrees of rotational freedom available and thus minimize the potential for differently substituted inhibitors to interact with enzyme in different ways. An optimal substitution pattern, 3,5-dihalo, when extended to two homologous phenylpropyl inhibitors, compounds **43** and **47**, yielded the expected increase in potency relative to the unsubstituted parent phenylpropyl inhibitor.

The IC_{50} data in Table I demonstrate a 10^5-fold range for inhibitory potency that results from simple substituent changes at the benzyl moiety of 1-benzylimidazole-2-thiones, i.e., the portion of the inhibitor that mimics phenethylamine substrate. Thus the enzymatic site that recognizes and binds this portion of inhibitor is extremely sensitive toward substituent nature and placement. A hydroxyl group at the 4-position produces the optimal parent inhibitor **44**, whereas the isomeric 3-hydroxyl (**23**) and to a greater extent the 2-hydroxyl (**19**) substituted inhibitors are of diminished potency. The 3,4-dihydroxyl-substituted inhibitor **46** is of equal potency with the parent **44**. Interestingly, the 4-hydroxymethyl inhibitor **3** is of markedly reduced potency, despite the fact that this functional group has found considerable prior success as a mimic for catechol phenolic groups, for example, in β-adrenergic receptor agents.[13] The optimal activity found for the 4-hydroxyl-substituted inhibitor **44** is not adequately mimicked by compounds **14**, **27**, or **33** where the phenol is replaced by halogen, elements that have nonbonding electrons similar to those found on oxygen of the phenolic hydroxyl. These three halo-substituted inhibitors are considerably diminished in potency relative to the parent inhibitor **44**, and the decrease in potency appears to correspond to an increase in the van der Waals radii of these halogens. It is interesting to note that the 4-*O*-methyl ether counterparts to the phenolic inhibitors of Table I are 10^2–10^3-fold weaker than the related phenolic compounds. The optimal inhibitor in Table I, compound **52**, is a difluorophenol that is considerably more acidic (–OH pK_a = 8.0) than **44** (–OH pK_a = 10.65) or the monofluorophenol **49** (–OH pK_a = 9.42), which is of potency intermediate to that of **52** and **44**. While it is tempting to conclude that phenolic acidity directly influences inhibitor potency, this is not a totally satisfactory explanation since the potency of **36**, which *lacks* the phenolic group, is also increased by meta halogenation, i.e., 3-chloro (**41**) and 3-fluoro (**42**), and is increased still further by 3,5-dihalogenation, i.e., **45** and **50**. Furthermore, the acidic nitrophenol **37** is less potent than **44** although it is clear that other factors such as steric bulk of the nitro group or a strong intramolecular hydrogen bond[14] in this phenol may reduce potency.

(5) DeWolf, W. E., Jr.; Goodhart, P. J.; Kruse, L. I., unpublished results.
(6) Ahn, N.; Klinman, J. P. *Biochemistry* **1983**, *22*, 3096.
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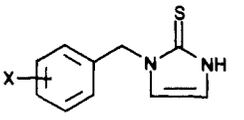
(14) (a) Pauling, L. *The Nature of the Chemical Bond*; Cornell University Press: Ithaca, NY, 1960; p 487. (b) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; p 327.

Table II. Kinetic Constants, Physical Properties, and Pharmacological Activity for Selected DBH Inhibitors



no.	X	IC_{50},[a] μM	K_{is},[b] μM pH 4.5	K_{is},[b] μM pH 6.0	K_{is},[b] μM pH 6.6	log *P*	% increase in DA/NE ratio[c]	mm decrease in BP[d] ip	mm decrease in BP[d] po
21	4-OMe	202 (71–386)		26.0 ± 1.0					
13	4-CH_3	34%[e]		9.77 ± 0.38					
10	4-CF_3	25%[e]		9.04 ± 0.29					
14	4-Br	43%[e]		3.42 ± 0.16					
27	4-Cl	96 (64–133)		2.74 ± 0.14					
33	4-F	47 (40–56)		0.99 ± 0.04					
36	H-	32 (20–46)		0.83 ± 0.03					
42	3-F	5.6 (4.9–6.5)	0.108 ± 0.003			1.70	364 ± 135		
44	4-OH	2.6 (1.3–4.6)	0.055 ± 0.002	0.088 ± 0.003	0.344 ± 0.016	1.02	92 ± 15	22 ± 4[f] (n = 3)	
45	3,5-Cl_2	2.4 (1.9–3.0)	0.024 ± 0.001			1.78	197 ± 32		
49	3-F, 4-OH	1.5 (0.9–2.2)	0.045 ± 0.002			1.12	78 ± 30	32 ± 13[g] (n = 4)	
50	3,5-F_2	1.2 (1.0–1.9)	0.041 ± 0.001			1.98	407 ± 30	50 ± 4[f] (n = 3)	22 ± 5[f] (n = 12) 28 ± 17[g] (n = 13)
51	3,5-Cl_2, 4-OH	0.68 (0.42–1.1)	0.018 ± 0.001				12 ± 16	28 ± 20[g] (n = 4)	
52	3,5-F_2, 4-OH	0.074 (0.060–0.087)	0.0057 ± 0.0006		0.039 ± 0.007	1.08	165 ± 45	36 ± 10[f] (n = 3)	no effect[g]

[a] Values are given as IC_{50} in μM with upper and lower 95% confidence limits (mean ± SEM) shown in parentheses. [b] K_{is} values (mean ± SEM) vs. tyramine substrate were calculated by using the computer programs of Cleland (*Methods in Enzymology*; Purich, D. L., Ed.; Academic: New York, 1979; Vol. 63, 103–138). [c] Change in DA/NE ratio after two 50 mg/kg po doses ca. 18 h apart. [d] Mean arterial BP at 4 h postdrug (mean ± SEM) for the indicated number of SHR relative to BP before dosing. [e] Percent inhibition at 10^{-4} M inhibitor concentration. [f] 50 mg/kg dose. [g] 100 mg/kg dose.

The relative activities of compounds in Table I underscore the degree of bulk intolerance at the phenethylamine binding site and furthermore the intolerance to substitution other than hydrogen at the positions ortho to the benzylic methylene. Potency is dramatically decreased upon substitution at the ortho position and is decreased by O-methylation at the 4-position or by substitution at the meta position by halogen larger than chlorine or by other substituents cited in Table I.

The optimal substitution patterns, found in **51** and **52**, combine the presence of an acidic 4-hydroxyl group with meta positions that are disubstituted with small halogens, either chlorine or fluorine. Other substitutions lead to less potent compounds than the parent inhibitor **44**. As indicated in Table I the substituent effects are additive both for compounds that are more potent than **44** and those that are much less potent. Once the optimum 3,5-difluoro or 3,5-dichloro substitution pattern is introduced into the inhibitor, the phenolic group, while optimizing in vitro potency, is not required for a high degree of activity. The 3,5-difluoro- and 3,5-dichloro-substituted phenylpropyl homologues **43** and **47** also show an increase in potency relative to that seen for the unsubstituted 1-(phenylpropyl)imidazole-2-thione (IC_{50} = 16 μM).[1]

A quantitative treatment of inhibition data by regression analysis was suggested by the apparent additivity of substituent effects in a series of relatively simple molecules that have only two degrees of rotational freedom and that probably interact with the enzyme active site in the same way. This analysis focused on 26 compounds from Table I substituted in the 3-, 4-, or 5-positions of the phenyl ring and for which IC_{50} values were available. As noted above, the presence of a hydroxyl at the 4-position appears to make a uniquely favorable contribution to the potency and therefore was included in the analysis as an indicator variable. Because of symmetry, there is no a priori basis for designating one of the meta positions as 3 and the other as 5. For this reason, and because the substituents lie close to each other in Cartesian space, the correlation examined regional effects expressed as the sum of the parameters for the 3-, 4-, and 5-positions. Four physicochemical descriptors, each summed over the 3-, 4-, and 5-positions, were examined: π, molar refractivity, and the F and R measures of inductive and resonance effects. Including the 4-OH indicator variable, a total of five variables were examined (Tables III, IV).

Preliminary analysis of the data showed compound **24** to be an outlier, with a residual typically more than twice as large as that of any other compound in the set. Anomolous behavior is not uncommon with fluorinated derivatives; hence this compound was excluded from further analysis. Stepwise correlation of the remaining 25 compounds as shown in Table V resulted in the following equation:

$$-\log IC_{50} = 1.28\ (\pm 0.22)I(\text{4-OH}) + 0.65\ (\pm 0.16)\pi_{345} - 0.14\ (\pm 0.02)MR_{345} + 1.42\ (\pm 0.33)F_{345} - 1.26 \quad (1)$$

$$n = 25,\ r = 0.91,\ F = 22.9,\ S = 0.44$$

The $-\log IC_{50}$ values calculated from eq 1 are plotted against the experimental values in Figure 2. Not surprisingly, the most important term in the equation is the presence of the *p*-OH group, which is seen from its coefficient to increase activity by over 1 order of magnitude. The region appears to be highly bulk sensitive, since even the relatively small substituents included in this study show a negative coefficient for molar refractivity in the regression. The equation also indicates a strong preference for lipophilicity in the 3,4,5 region.

σ-electron-withdrawing groups also impact favorably upon activity. It may be that the major role of electron-withdrawing groups is to decrease the pK_a of the hydroxyl. However, the considerable activity of the 3,5-dihalogenated compounds (**45** and **50**) that lack a 4-OH suggests that the principal effect is electron depletion from the ring itself.

Table III. Chemical Parameters of 3,4,5-Substituted DBH Inhibitors

no.	I_{4OH}	π_{345}	MR_{345}	F_{345}	R_{345}	-log IC_{50}[a]	-log IC_{50}[b] (calcd)
20	0	-0.30	16.23	0.93	-0.35	-2.55	-2.52
21	0	-0.02	9.87	0.26	-0.51	-2.30	-2.35
22	0	-0.02	9.87	0.26	-0.51	-2.19	-2.35
23	0	-0.67	4.85	0.29	-0.64	-2.17	-1.99
27	0	-0.71	8.03	0.41	-0.15	-1.98	-1.40
30	0	-0.28	9.36	0.67	0.16	-1.72	-1.86
32	1	-0.11	9.50	0.25	-0.77	-1.69	-1.09
33	0	0.14	2.92	0.43	-0.34	-1.67	-0.99
34	0	1.40	19.93	1.08	-0.81	-1.67	-1.75
35	0	0.26	9.71	1.12	-1.19	-1.55	-0.93
36	0	0.00	3.00	0.00	0.00	-1.50	-1.70
37	1	-0.95	11.21	0.96	-0.48	-1.49	-0.87
38	0	1.42	13.06	0.82	-0.30	-1.44	-1.10
40	1	0.19	12.73	0.73	-0.81	-1.07	-0.69
41	0	0.71	8.03	0.41	-0.15	-1.07	-1.40
42	0	0.14	2.92	0.43	-0.34	-0.74	-0.99
44	1	-0.67	4.85	0.29	-0.64	-0.41	-0.71
45	0	1.42	13.06	0.82	-0.30	-0.38	-1.10
46	1	-1.34	6.70	0.58	-1.28	-0.34	-1.00
48	1	0.04	9.88	0.70	-0.79	-0.30	-0.41
49	1	-0.53	4.77	0.72	-0.98	-0.17	0.00
50	0	0.28	2.84	0.86	-0.68	-0.07	-0.27
51	1	0.75	14.91	1.11	-0.94	-0.16	-0.10
52	1	-0.39	4.69	1.15	-1.32	1.13	0.71
9	0	-0.04	16.74	0.52	-1.02	-2.75	-3.01

[a] Experimental value from Table I. [b] Calculated from eq 1.

Table IV. Correlation Matrix

	-log IC_{50}	I_{4OH}	π_{345}	MR_{345}	F_{345}	R_{345}
-log IC_{50}	1.00					
I_{4OH}	0.58	1.00				
π_{345}	-0.03	-0.45	1.00			
MR_{345}	-0.35	-0.06	0.43	1.00		
F_{345}	0.40	0.21	0.23	0.45	1.00	
R_{345}	-0.40	-0.55	0.28	-0.10	-0.43	1.00

Table V. Development of Equation 1

equation	n	r^2	F	S
-log IC_{50}				
$= 1.14 \pm 0.32I^a - 1.61$	25	0.34	12.09	0.79
$= 1.10 \pm 0.31I - 0.06 \pm 0.03MR^b - 1.01$	25	0.44	8.79	0.74
$= 1.60 \pm 0.22I - 0.11 \pm 0.02MR + 0.79 \pm 0.22\pi^c - 0.82$	25	0.65	13.40	0.59
$= 1.28 \pm 0.22I - 0.14 \pm 0.02MR + 0.65 \pm 0.16\pi + 1.42 \pm 0.33F^d - 1.26$	25	0.82	22.96	0.44

[a] I is indicator variable for the presence of a 4-OH. [b] MR is the sum of the molar refractivity at the 3-, 4-, and 5-positions. [c] π is the sum of the lipophilicity at the 3-, 4-, and 5-positions. [d] F is the sum of the inductive effects at the 3-, 4-, and 5-positions.

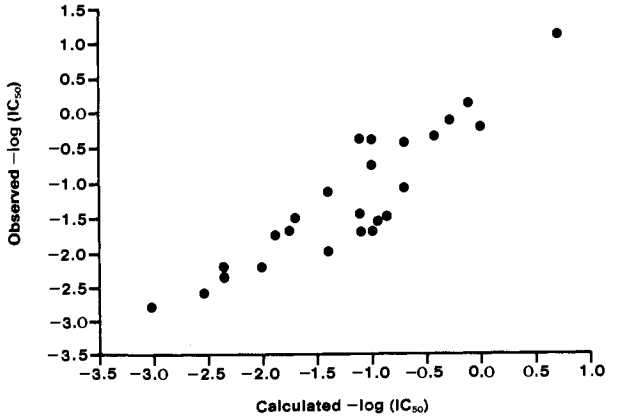


Figure 2. Plot of observed -log IC_{50} vs -log IC_{50} calculated by eq 1.

With respect to the choice of substituents, the correlation coefficients (Table IV) were reasonable, but halogens were heavily represented. To investigate the robustness of the conclusions, we examined three subsets of the data, viz., the nine compounds with a 4-OH, the 16 compounds without a 4-OH, and the 19 compounds with only 3,4-substitution. All of the subsets led to the same conclusions as the total set with respect to the important structural features (data not shown). Predicted activity values were also calculated (Table VI) from eq 1 for the less active 3,4,5-substituted compounds (activity expressed as percent inhibition at 100 μM in Table I). Although these compounds were not included in the correlation, eq 1 does distinguish the inactive compounds **1**, **3**, and **5** from the slightly active compounds **11**, **15**, **16**, and **18**.

Table VI. Calculated IC_{50} Values for Low-Potency DBH Inhibitors Not Included in the Regression Analysis

no.	substitution	% inhibn (100 μM)	predicted IC_{50}, μM
1	4-COOH	1	>300 000 (anion)
3	4-CH_2OH	6	1900
5	3-SO_2NH_2, 4-OMe	7	>900 000
11	3-CF_3, 4-OMe	29	69
15	3-Br, 4-OMe	60	208
16	3-F, 4-OMe	67	43
18	3-Me, 4-OMe	72	467

In earlier publications we have used kinetic data for inhibitors **44** and **52** together with data for the DBH substrates tyramine and 3,5-difluorotyramine to argue in support of a direct binding of multisubstrate inhibitor to

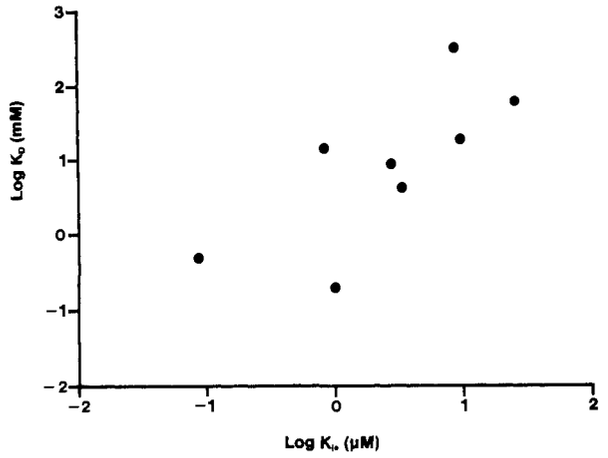


Figure 3. Plot of tyramine substrate dissociation constant log K_D vs. log K_{is} for substituted substrates and inhibitors.

the enzymatic site that binds phenethylamine substrate during normal catalysis.[1,2,4] Recently, true dissociation constants, K_D, have been calculated for a series of substituted tyramines.[7] These dissociation constants, which were determined at pH 6.2 under predominately random conditions, reflect the true affinity of variously substituted tyramines for the *ternary* DBH–oxygen–tyramine complex. The phenethylamines employed in this study had aromatic substitution patterns identical with those found in several of the inhibitors in Table I. Even though the dissociation constants calculated in this study reflect the relative affinities of substituted tyramines for enzyme with the oxygen site occupied, i.e., the ternary complex, it was of interest to compare these affinities (K_D) with affinities (K_{is}) of the identically substituted inhibitors for the reduced Cu^{1+} form of enzyme not bound to oxygen. Values of K_{is} were determined for inhibitors under random conditions, pH 6.0 (Table II), and a plot of log K_D vs log K_{is} (Figure 3) was found to show a correlation ($r = 0.75$) that improved ($r = 0.81$) when the outlying 4-fluoro-substituted inhibitor and substrate were not included. The affinity of inhibitor for the reduced Cu^{1+} form of DBH and the affinity of substrate for the reduced Cu^{1+} form of DBH appear to depend upon the same structural features. As noted previously,[4] under random conditions K_{is} values of inhibitor vs. tyramine substrate will be overestimates of the true K_i value since a fraction of enzyme will be present in the oxygen-bound form. However, so long as the concentration of nonvaried oxygen substrate remains constant, the relative magnitudes of K_{is} are an accurate reflection of the relative true K_i values for inhibitor. Despite the effects caused by oxygen binding at the DBH active site (presumably a tightening of the ternary complex), the same structural changes that increase substrate affinity for enzyme also increase inhibitory affinity, thereby strengthening the argument that both classes of compounds bind the same enzymatic site in a similar fashion.

Those inhibitors that showed the most promising activity against bovine DBH in vitro were evaluated for their in vivo blood pressure effects in SHR. Initial dosing was carried out by intraperitoneal (ip) injection since this route was found to give the most reproducible results. The more potent inhibitors listed in Table II effectively lowered blood pressure when administered by ip injection although the magnitude of decrease hinted at a divergence from relative in vitro DBH inhibitory potency. The divergence between in vitro and in vivo potency amplified upon oral dosing. The compound that showed the most effective oral antihypertensive activity, **50**, was clearly not the most effective in vitro. While the blood pressure effects of these inhibitors in SHR suggested efficacy against DBH from a second species, the lower in vivo activity of **51** and **52** in the SHR relative to their in vitro activity against bovine enzyme suggested either subtle interspecies differences in enzyme or a lessened oral absorption and/or bioavailability for the phenolic inhibitors. A more quantitative assessment of relative oral activity can be made by comparing the increases in the dopamine/norepinephrine (DA/NE) ratio produced by the inhibitors (Table II). It becomes apparent that the phenolic group, which confers optimal in vitro potency, is decidedly detrimental to oral activity of the inhibitors. Thus, phenol **49** is much less effective than **42**, phenol **51** is less effective than **45**, and **52** is considerably less effective than **50** in increasing the DA/NE ratio.

It became of interest to identify the reason(s) for the relatively poor in vivo activity shown upon oral dosing by the phenolic inhibitors in Table II. Differences in potency seemed unlikely to arise from differences in charged state (and resulting differences in oral absorption) since under gut conditions all of the molecules were expected to be essentially neutral. Of the phenolic compounds, only **52** was appreciably acidic and even this fairly acidic phenol ($pK_a = 8.0$) was expected to be un-ionized in the gut and appreciably un-ionized in the body. Differences in oral activity could not be directly related to changes in polarity, since all of the inhibitors were of roughly comparable lipophilicity, as evidenced by log P data (Table II). The most likely origin for the relative lack of oral activity for the phenolic inhibitors was thought to be conjugation of this functional group.[15] If the phenolic group were conjugated to a sulfate ester or glucuronide ether, these would be inactive, as clearly demonstrated by the SAR data in Table I, which underscore the bulk intolerance at the inhibitor 4-position. Preliminary experiments with ^3H-labeled **52** have suggested that the poor in vivo activity of this phenol derives from metabolic inactivation. Oral doses of **52** are readily absorbed in adult normotensive rats, but the bulk of the inhibitor rapidly appears in biliary excretions, suggestive of a conjugation to sulfate or glucuronide.[16]

Conclusion

The present study has defined a distinct structure–activity relationship for the portion of multisubstrate inhibitor that binds to the DBH phenethylamine binding site. The most potent inhibitors to result from this study bind enzyme approximately 10^6-fold more tightly than tyramine substrate. Quantitative regression analysis has identified and quantified the physical parameters that optimize inhibitor binding to enzyme, and selected inhibitor dissociation constants (K_{is} values) demonstrate a modest correlation to published substrate dissociation constants (K_D values).

Detailed pharmacological evaluation of the optimal inhibitors has suggested a correspondence between an elevated DA/NE ratio and a decrease in blood pressure in SHR. Furthermore, a lack of oral efficacy for some of the most potent inhibitors was discovered, and this was found to be associated with the phenolic group, a likely site for metabolic conjugation in vivo. Fortunately, as a result of the detailed SAR studies conducted, the 3,5-dihalo-substitution pattern was found to be a viable replacement for

(15) (a) Roth, J. A.; Rivett, A. J. *Biochem. Pharmacol.* **1982**, *31*, 3017. (b) Elchisak, M. A.; Carlson, J. H. *Life Sci.* **1982**, *30*, 2325.

(16) We thank Dr. T. Leonard, SK&F Department of Drug Metabolism, for these preliminary experiments.

the metabolically liable 4-hydroxyl group.

Experimental Section

Chemistry. When appropriate, all solvents used in reaction mixtures were dried and/or purified by standard procedures.[17] IR spectra were recorded on a Perkin-Elmer 727 spectrophotometer as neat oils or Nujol mulls calibrated with the 1601-cm^{-1} absorption of polystyrene film. NMR spectra were obtained as $CDCl_3$ solutions on a Hitachi Perkin-Elmer R-24 spectrometer and/or a Varian EM390 spectrometer. IR and NMR spectra were obtained for all new compounds and were judged to be consistent with the assigned structure. Solutions were dried over anhydrous magnesium sulfate and concentrated with a Büchi Rotovapor at ca. 10 torr before pumping at 0.5 torr.

1-(4-Carboxybenzyl)imidazole-2-thione (1). A mixture of methyl 4-formylbenzoate (5 g, 0.03 mol) and aminoacetaldehyde dimethyl acetal (3.15 g, 0.03 mol) was heated on a steam bath for 5 min and diluted with EtOH (25 mL). Sodium borohydride (1.5 g, 0.04 mol) was added in small portions and the solution was stirred at ambient temperature for 12 h. The mixture was diluted with H_2O (100 mL) and extracted with EtOAc. The organic extracts were dried and concentrated under reduced pressure to yield the crude intermediate amine. A solution of amine (2.8 g, 0.01 mol) and KSCN (1 g, 0.01 mol) in a mixture of H_2O (10 mL), EtOH (20 mL) and 3 N HCl (3.6 mL) was heated at reflux for 1 h. The solution was diluted with H_2O (25 mL) and cooled. The solid product was filtered and recrystallized from EtOH to yield 0.94 g (40%) of 1 (Table I).

Method A. 1-(2,6-Dimethylbenzyl)imidazole-2-thione (2). A mixture of 2,6-dimethylbenzaldehyde (13.4 g, 0.1 mol) and aminoacetaldehyde dimethyl acetal (10.5 g, 0.1 mol) was heated on a steam bath for 20 min, then cooled to ambient temperature, and diluted with EtOH (75 mL). Sodium borohydride (3.8 g, 0.1 mol) was added in small portions and the mixture was stirred at ambient temperature for 24 h. The mixture was concentrated under reduced pressure, the residue was partitioned between H_2O and EtOAc, and the organic layer was washed with H_2O, dried, and concentrated under reduced pressure. A solution of the crude amine and KSCN (10.7 g, 0.11 mol) in H_2O (100 mL), EtOH (150 mL), and 12 N HCl (10 mL) was heated at reflux for 2 h and then concentrated under reduced pressure to a volume of ca 100 mL. The residue was cooled and diluted with H_2O (200 mL) and the product was filtered. Recrystallization from EtOH yielded 9.37 g (43%) of 2 (Table I).

1-[4-(Hydroxymethyl)benzyl]imidazole-2-thione (3). A solution of 1 (2.34 g, 0.01 mol) in THF (50 mL) was treated dropwise with 1 M BH_3·THF solution (10 mL, 0.01 mol) and the resulting mixture was stirred at ambient temperature for 3 h. Methanol (100 mL) was added dropwise, the resulting solution was concentrated under reduced pressure, and the residue was recrystallized from MeCN to yield 1.54 g (70%) of 3 (Table I).

4-Methoxy-3-(aminosulfonyl)benzoic Acid (53). Chlorosulfuric acid (33 mL, 0.5 mol) was cooled to 0 °C and 4-methoxybenzoic acid (15.2 g, 0.1 mol) was added portionwise (10 min) with stirring. After the HCl evolution ceased, the viscous mixture was warmed to ambient temperature and then heated on a steam bath. The mixture was poured into ice (3 kg) and the product was filtered and washed with H_2O (250 mL). The crude product was dissolved in Me_2CO (100 mL), H_2O (30 mL) was added, and the mixture was warmed on a steam bath and then diluted with H_2O (450 mL). The product was filtered and dissolved in 14 N NH_4OH (50 mL). After standing at ambient temperature for 1 h, the solution was acidified with 12 N HCl and the product was filtered and recrystallized from EtOH to give 4.06 g (18%) of 53.

4-Methoxy-3-(aminosulfonyl)benzyl Alcohol (54). A mixture of 53 (2.31 g, 0.01 mol) in THF (10 mL) was stirred as a 0.97 M solution of BH_3·THF (25 mL, 0.024 mol) was added dropwise. The reaction mixture was stirred at ambient temperature for 3 h and quenched by the addition of MeOH (150 mL). The resulting solution was heated to reflux and then concentrated under reduced pressure. The residue was recrystallized from EtOH to give 1.66 g (76%) of 54.

4-Methoxy-3-(aminosulfonyl)benzaldehyde (55). A solution of 54 (4.9 g, 0.023 mol) in Me_2CO (340 mL) was stirred with activated MnO_2 (23 g) at ambient temperature for 18 h. The mixture was filtered, the filtrate was concentrated under reduced pressure, and the residue was recrystallized from Me_2CO–Et_2O to give 5.61 g (78%) of 55.

2,6-Dichloro-4-methoxybenzaldehyde (56). A solution of 3,5-dichloroanisole (17.7 g, 0.1 mol) in CH_2Cl_2 (240 mL) was treated at 0 °C with anhydrous $SnCl_4$ (17 mL, 0.15 mol) and dichloromethyl methyl ether (13.6 mL, 0.15 mol). The solution was stirred at 0 °C for 30 min and ambient temperature for 3 h. Ice (150 g) was added and the CH_2Cl_2 solution was washed sequentially with H_2O and saturated $NaHCO_3$. The solution was dried and concentrated under reduced pressure and the residue was purified by flash chromatography[18] on silica gel with 3:1 $CHCl_3$–hexane as eluant to yield 5.75 g (28%) of 56.

Method B. 1-(2-Hydroxybenzyl)imidazole-2-thione (19). A solution of 17 (2.2 g, 0.01 mol) in CH_2Cl_2 (60 mL) was treated with a solution of BBr_3 (7.0 g, 0.028 mol) in CH_2Cl_2 (10 mL). After 1.5 h, the reaction mixture was cooled (0 °C) and *cautiously* treated with MeOH (50 mL). The mixture was concentrated under reduced pressure and the residue was recrystallized from EtOH–H_2O to yield 0.97 g (47%) of 19 (Table I).

1-[4-Hydroxy-3-(trifluoromethyl)benzyl]imidazole-2-thione (24). A mixture of pyridine hydrochloride (15 g) and 11 (2.0 g, 0.007 mol) was heated in the melt at 210 °C for 30 min and then diluted with H_2O (75 mL) and extracted with EtOAc. The organic extracts were washed with H_2O, decolorized with carbon, and concentrated under reduced pressure. The residual oil was treated sequentially with THF (3 mL), Et_2O (6 mL), and hexane (35 mL) to yield crude crystalline product. The crude product was filtered and recrystallized from EtOAc–hexane to give 0.9 g (37%) of 24 (Table I).

1-(2,6-Dichloro-4-hydroxybenzyl)imidazole-2-thione (28). A mixture of 12 (1 g, 0.0035 mol) in constant boiling HBr (50 mL) was heated at reflux for 1.25 h and cooled. The crystalline product was filtered and recrystallized from $EtCO_2H$ to give 0.36 g (37%) of 28 (Table I).

1-(4-Hydroxy-3-nitrobenzyl)imidazole-2-thione (37). A solution of 20 (1.59 g, 0.006 mol) in 10% NaOH (200 mL) was heated at reflux for 2 h and then cooled, filtered, and acidified with 12 N HCl. The crystalline product was filtered and recrystallized from EtOH to give 0.933 g (62%) of 37 (Table I).

2-Bromo-4,6-difluoroaniline Hydrobromide (57).[19] A solution of 2,4-difluoroaniline (100 g, 0.775 mol) in AcOH (600 mL) was stirred in ice as a solution of Br_2 (40 mL, 0.78 mol) in AcOH (600 mL) was added (5 min). The mixture was stirred at ambient temperature for 15 min, Et_2O (1 L) was added, and the product was filtered. The product was washed sequentially with Et_2O and hexane and dried to yield 203 g (91%) of 57: mp 222–225 °C.

3,5-Difluorobromobenzene (58). A mixture of 57 (203 g, 0.703 mol), 12 N HCl (293 mL), and ice (350 g) was stirred and cooled in a 3-L flask during the addition (30 min) of a solution of $NaNO_2$ (51 g, 0.74 mol) in H_2O (125 mL). Ice was added to the reaction mixture from time to time to maintain the temperature below 5 °C. Ice and 50% H_3PO_2 (400 mL) were added simultaneously over 1 h while the temperature was maintained below 5 °C by efficient cooling. The resulting solution was stirred at 0 °C for 3 h and then extracted twice with CH_2Cl_2. The organic extracts were washed with saturated Na_2CO_3. They were then dried and concentrated under reduced pressure. The residual oil was distilled to yield 85.8 g (61%) of 58 as a clear oil: bp 130 °C (760 torr).

3,5-Difluorobenzonitrile (59). A solution of 58 (85.8 g, 0.444 mol) and CuCN (80 g, 0.893 mol) in *N,N*-dimethylacetamide (350 mL) was heated at reflux for 4 h under argon. The hot solution was poured onto a mixture of ice (1 kg) and EtOAc (700 mL), the quenched reaction mixture was filtered through diatomaceous earth, and the organic layer of the filtrate was separated and washed three times with H_2O. The solution was dried and con-

(17) Perrin, D. D.; Armarego, W. F. L. *Purification of Laboratory Chemicals*; Pergamon: Oxford, 1966.

(18) Here and subsequently "flash chromatography" means the technique developed by Still et al.: Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(19) Roe, A.; Little, W. F. *J. Org. Chem.* **1955**, *20*, 1577.

centrated under reduced pressure, and the residue was recrystallized from hexane to yield 47.3 g (77%) of **59** as white crystals: mp 83–85 °C.

3,5-Difluorobenzaldehyde (60). A mixture of **59** (20 g, 0.144 mol) and Raney nickel alloy (20 g) in H_2O (20 mL) and 90% formic acid (200 mL) was stirred and heated at reflux for 2 h and filtered hot. The filter cake was washed with H_2O (250 mL) and hexane (500 mL), and the filtrates were extracted three times with hexane. The combined hexane washes and extracts were washed with water, dried, and concentrated under reduced pressure to yield 14.4 g (71%) of **60**.

3,5-Difluorocinnamic Acid (61). A mixture of **60** (5.5 g, 0.0387 mol), malonic acid (6.06 g, 0.0582 mol), pyridine (2.1 mL), and piperidine (0.105 mL) was heated at 100 °C for 2 h and then 155 °C for 1 h. The mixture was poured into cold 3 N HCl (50 mL) and the product was filtered and recrystallized from EtOH to yield 4.7 g (66%) of **61** as white needles: mp 199–201 °C.

3-(3,5-Difluorophenyl)propionic Acid (62). A solution of **61** (4.6 g, 0.025 mol) in THF (50 mL) was hydrogenated over 10% Pd on carbon (0.75 g) at 50 psi of H_2 pressure for 5 h. The mixture was filtered and concentrated under reduced pressure to yield 4.5 g (97%) of **62** as a low-melting solid: mp 56 °C.

3-(3,5-Difluorophenyl)propionyl Chloride (63). A solution of **62** (4.4 g, 0.0236 mol) in thionyl chloride (15 mL, 0.21 mol) was treated with DMF (0.1 mL) and heated at 60 °C for 3 h. The solution was concentrated under reduced pressure and the residue was distilled in vacuo to yield 4.1 g (85%) of **63** as a colorless oil: bp 122–130 °C (5–10 torr).

3-(3,5-Difluorophenyl)-*N*-(2,2-dimethoxyethyl)propionamide (64). A solution of **63** (4.0 g, 0.0196 mol) in CH_2Cl_2 (40 mL) was added dropwise to a stirred solution of aminoacetaldehyde dimethyl acetal (4.3 g, 0.0412 mol) in CH_2Cl_2 (100 mL) at 0 °C. After 1 h the reaction mixture was poured into H_2O (250 mL) and the organic layer was separated and washed sequentially with 5% Na_2CO_3, 0.5% HCl, and H_2O and then dried and concentrated under reduced pressure to yield 5.3 g (100%) of **64** as a colorless oil.

***N*-[3-(3,5-Difluorophenyl)propyl]aminoacetaldehyde Dimethyl Acetal (65).** A solution of **64** (5.3 g, 0.0194 mol) in Et_2O (100 mL) was slowly added to a slurry of $LiAlH_4$ (4.4 g, 0.116 mol) in Et_2O (200 mL), and the mixture was stirred for 16 h at ambient temperature. The reaction was quenched by the sequential addition of H_2O (4.5 mL), 10% NaOH (7 mL), and H_2O (11 mL) and then filtered. The filtrate was dried and concentrated under reduced pressure to yield 4.4 g (88%) of **65** as a colorless oil.

1-[3-(3,5-Difluorophenyl)propyl]imidazole-2-thione (43). A solution of **65** (4.3 g, 0.0166 mol) and KSCN (1.6 g, 0.0166 mol) in a mixture of H_2O (20 mL), EtOH (12 mL), and 12 N HCl (4 mL) was heated at reflux for 1 h and then cooled to ambient temperature and diluted with H_2O (75 mL). The crystalline product was filtered and recrystallized from ethanol to give 2.2 g (55%) of **43** (Table I).

3,5-Dichlorocinnamic Acid (66). The reaction of 3,5-dichlorobenzaldehyde (29.6 g, 0.154 mol), malonic acid (24.1 g, 0.232 mol), and piperidine (8 mL) as for **61** yielded 22.9 g (69%) of **66** after recrystallization from EtOH.

3-(3,5-Dichlorophenyl)propionic Acid (67). The hydrogenation of **66** (22.9 g, 0.106 mol) in THF (150 mL) over 3 g of 10% Pd on carbon as in the preparation of **62** yielded 22 g (95%) of **67** as a colorless oil.

3-(3,5-Dichlorophenyl)propan-1-ol (68). A solution of **67** (23 g, 0.106 mol) in dry THF (200 mL) was cooled at 0 °C during the slow (30 min) addition of 1 M BH_3·THF (0.233 L, 0.233 mol). The solution was stirred at ambient temperature for 2 h, MeOH (150 mL) was added, and the resulting solution was concentrated under reduced pressure to yield 21.2 g (98%) of **68** as a colorless oil.

3-(3,5-Dichlorophenyl)propan-1-al (69). A solution of oxalyl chloride (4.3 mL, 0.049 mol) in CH_2Cl_2 (15 mL) was cooled to −60 °C and stirred during the addition (20 min) of a solution of Me_2SO (6.1 mL, 0.0865 mol) in CH_2Cl_2 (15 mL). The reaction mixture was stirred for 2 min and then a solution of **68** (5 g, 0.0245 mol) in CH_2Cl_2 (20 mL) was added (10 min). After the mixture was stirred an additional 15 min, NEt_3 (22.5 mL, 0.160 mol) was added (15 min), and the mixture was stirred an additional 5 min and then warmed to ambient temperature and diluted with H_2O (250 mL). The CH_2Cl_2 layer was separated, washed with 3 N HCl, and then dried. The solvent was removed under reduced pressure to yield 4.8 g (97%) of **69** as a light yellow oil.

***N*-[3-(3,5-Dichlorophenyl)propyl]aminoacetaldehyde Dimethyl Acetal (70).** A solution of **69** (5.0 g, 0.025 mol) in hexane (10 mL) was stirred as aminoacetaldehyde dimethyl acetal (2.2 mL, 0.0197 mol) was added. After the mixture was stirred an additional 1 h, a solution of $NaBH_4$ (7.3 g, 0.193 mol) in EtOH (25 mL) was added, and the resulting mixture was cooled in ice and stirred for 18 h. The solution was diluted with H_2O (200 mL) and the product was extracted into EtOAc. The organic layer was washed with H_2O, then dried, and concentrated under reduced pressure to yield 6.8 g (93%) of **70** as a yellow oil.

1-[3-(3,5-Dichlorophenyl)propyl]imidazole-2-thione (47). A solution of **70** (6.5 g, 0.022 mol) and KSCN (2.2 g, 0.022 mol) in a mixture of H_2O (30 mL), EtOH (20 mL), and 12 N HCl (6.5 mL) was heated at reflux for 1 h. The solution was cooled and diluted with H_2O (75 mL) and the solid product was filtered. Purification of the crude product by chromatography on silica gel using a 0.5–1% MeOH gradient in CH_2Cl_2 followed by recrystallization from EtOH yielded 1.2 g (19%) of **47** (Table I).

Determination of Distribution Coefficients. The compounds were partitioned by shaking between 1-octanol and pH 7.4 phosphate buffer (ionic strength, μ, 0.01) prepared from Na_2HPO_4 (3.7 g) and KH_2PO_4 (1.6 g) in sufficient H_2O to make 9 L, adjusting the pH to 7.4 by addition of 0.02 M KH_2PO_4 or 0.01 M Na_2HPO_4 as required until equilibrium was attained. The phases were allowed to separate and were clarified by centrifugation. Aliquots from each phase were then assayed by UV detection. The compounds were partitioned at four different concentrations and the ratios [D = (mg of compound/mL of 1-octanol)/(mg of compound/mL of pH 7.4 phosphate buffer)] were plotted vs. concentration. The value of D (or log D) was determined at infinite dilution by linear regression.

pK_a determinations were obtained by titration of the compounds in 2:1 CH_3OH–H_2O (to overcome solubility constraints).

True partition coefficient, P, was calculated from the distribution coefficient D as follows: $P = D/(1 - \alpha)$, where $\alpha = 1/[1 + \text{antilog}(\text{pH} - \text{p}K_a)]$.

Enzymology. In vitro IC_{50} determinations were made as previously reported.[1] The IC_{50} is defined as the concentration of compound that produces a 50% inhibition of product formation when compared to uninhibited control.

Kinetic assays with purified DBH were made as previously reported[1] with tyramine as variable substrate.

Pharmacological Testing. Vascular catecholamine levels were determined in a procedure similar to that already reported.[12] Briefly, SHR were orally administered vehicle (5% PEG 400, 1% methocel) or vehicle suspension of compound in two doses at an 18-h interval. Two hours after the second dose, animals were sacrificed and mesenteric arteries were removed and homogenized into aqueous $HClO_4$ (0.4 M) containing $Na_2S_2O_5$ (5.3 mM) and EDTA (1.4 mM) at 0 °C. The homogenate was centrifuged (4250*g*, 10 min) and the supernatant was assayed for catecholamine content. Aliquots were incubated for 60 min at 37 °C with rat liver catechol-*O*-methyltransferase (COMT) and excess [methyl-^{3}H]-*S*-adenosylmethionine in 1 M pH 9.6 Tris·HCl buffer containing $MgCl_2$ (0.5 mM). The incubations were terminated by cooling and the addition of 1.5% sodium tetraphenylborate in 1 M sodium borate buffer. Carrier solutions of unlabeled *O*-methylcatecholamines were added, and the resulting solution was extracted with 7:3 toluene–isoamyl alcohol. The organic phase was extracted with 1 N AcOH and the resulting aqueous phase was washed with 0.5 M AcOH saturated butyl acetate and concentrated in vacuo. The residue was resolved by silica gel TLC with 16:3:2 $CHCl_3$–MeOH–70% Et_2NH/H_2O as eluant, and the catecholamine-containing bands were assayed for ^{3}H content by scintillation counting with correction for recovery based on internal standards. Values for DA and NE content were determined relative to starting tissue weight.

Blood Pressure Measurements. Male Okamoto-Aoki SHR (Taconic Farms, Germantown, NY), 16–18 weeks of age and weighing 275–310 g, were anesthetized with Brevital sodium (10 mg/kg, iv). The femoral artery was cannulated with Micro Bore PVC tubing (0.38 mm i.d., 0.76 mm o.d.), the cannula was ex-

ternalized at the nape of the neck, and the incision site was sutured closed with surgical staples. The cannula was flushed with heparinized saline (20 units/mL) and attached to a Gould Statham pressure transducer (Model P23ID), and blood pressures and heart rates were recorded on a Beckman R-611 polygraph recorder. Rats were placed unrestrained except for the catheter tether into individual cages and allowed 2 h to recover from surgery. Drugs were administered ip as solutions in 20% DMF or po as suspensions in 5% PEG 400/1% methocel. Blood pressures and heart rates were monitored for 5–6 h and recorded at 0.5-h intervals.

Registry No. 1, 105763-92-6; 2, 105763-93-7; 3, 105763-94-8; 4, 95333-69-0; 5, 105763-95-9; 6, 105763-96-0; 7, 95333-70-3; 8, 105763-97-1; 9, 105763-98-2; 10, 105763-99-3; 11, 95333-91-8; 12, 95333-90-7; 13, 105764-00-9; 14, 105764-01-0; 15, 95333-88-3; 16, 105764-02-1; 17, 95333-53-2; 18, 105764-03-2; 19, 95333-51-0; 20, 101913-05-7; 21, 95460-09-6; 22, 95333-54-3; 23, 95333-50-9; 24, 95333-61-2; 25, 95333-79-2; 26, 95333-71-4; 27, 95333-72-5; 28, 95333-59-8; 29, 95333-62-3; 30, 105764-04-3; 31, 95333-73-6; 32, 95333-52-1; 33, 95333-74-7; 34, 105764-05-4; 35, 104197-16-2; 36, 23269-10-5; 37, 95333-58-7; 38, 95333-75-8; 39, 95333-76-9; 40, 95333-48-5; 41, 95333-77-0; 42, 95333-80-5; 43, 95333-83-8; 44, 95333-64-5; 45, 95333-78-1; 46, 95333-66-7; 47, 95333-82-7; 48, 95333-68-9; 49, 95333-49-6; 50, 95333-81-6; 51, 95333-57-6; 52, 95333-60-1; 53, 20532-06-3; 54, 105764-06-5; 55, 105764-07-6; 56, 82772-93-8; 57, 101471-20-9; 58, 461-96-1; 59, 64248-63-1; 60, 32085-88-4; 61, 84315-23-1; 62, 84315-24-2; 63, 95333-92-9; 64, 95333-93-0; 65, 95333-94-1; 66, 90418-21-6; 67, 95333-95-2; 68, 95333-96-3; 69, 95333-97-4; 70, 101913-07-9; DBH, 9013-38-1; p-$OHCC_6H_4CO_2Me$, 1571-08-0; $NH_2CH_2CH(OMe)_2$, 22483-09-6; HO_3SCl, 7790-94-5; p-$MeOC_6H_4CO_2H$, 100-09-4; $(HO_2C)_2CH_2$, 141-82-2; $Ph(CH_2)_2NH_2$, 64-04-0; 2,6-dimethylbenzaldehyde, 1123-56-4; 3,5-dichloroanisole, 33719-74-3; 2,4-difluoroaniline, 367-25-9; 3,5-dichlorobenzaldehyde, 10203-08-4; dopamine, 51-61-6; norepinephrine, 51-41-2.

Dopamine Autoreceptor Agonists: Resolution and Pharmacological Activity of 2,6-Diaminotetrahydrobenzothiazole and an Aminothiazole Analogue of Apomorphine

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The enantiomers of the aminothiazole analogues of the known dopaminergic agonists apomorphine (1) and 2-aminohydroxytetralin (2) have been prepared. The absolute configurations of the enantiomers of 2,6-diaminotetrahydrobenzothiazole have been established by X-ray crystallographic analysis. Dopamine (DA) autoreceptor agonist activities of the compounds were evaluated. Testing revealed (-)-5, the *S* enantiomer, to be the most active compound tested (inhibition of GBL accelerated dopamine synthesis and inhibition of α-methyltyrosine-induced decline of DA). In addition (-)-5 does not exhibit stereotyped behavior, suggesting a pronounced selectivity for DA autoreceptors.

Dopamine (DA) receptor agonists that stimulate the DA autoreceptor in the brain represent a novel therapeutic approach in the treatment of schizophrenia by reducing the release of DA presynaptically.[1-3] Those selective DA agonists should be devoid of the untoward dyskinetic side effects of the classical neuroleptics. Apomorphine exerts presynaptic DA receptor activity.

Doses of (*R*)-(-)-apomorphine (1), at least 1 order of magnitude below that required for producing stereotyped behavior in rats, inhibit DA synthesis and also cause a reduction in DA release.[4-6] The *S*-(+) enantiomer of apomorphine has an approximately 10-fold lower affinity to presynaptic DA receptors.[7]

Investigation of hydroxy derivatives of 2-aminotetralins 2 that may be regarded as subunits of apomorphine revealed that dopaminergic activity appears to be dependent upon the configuration of carbon C-2 and upon the position of the phenolic hydroxy group.[8,9]

Recently, it has been shown by Andén that the aminothiazoloazepine derivative B-HT 920 (3) inhibits the synthesis and the α-methyltyrosine-induced decline of DA in the brain of rodents. From these findings Andén concluded that B-HT 920 is a relatively selective DA receptor agonist that acts presynaptically.[10,11]

In order to study the influence of the exchange of the catechol subunit of DA agonists for the aminothiazole moiety on presynaptic DA receptor activity, we prepared the enantiomers of aminothiazolo analogues 4 and 5[12] of apomorphine and aminotetralin, respectively.

Chemistry. The aminothiazole analogue 4 of apomorphine was synthesized by using a previously published procedure.[13] Optical resolution was accomplished with L- and D-tartaric acid, respectively. The enantiomeric tartrates thus obtained were converted into the corresponding dihydrochlorides.

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