Table VIII. Radioiodination of Glyceryl 1,3-Bis- and 1,2,3-Tris[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] in Pivalic Acid[a]

[^{125}I]compd	time, h	radiochemical yield,[b] %	R_f (solvent system)[c]
4a	2	83	0.36 (A)
4b	2	96	0.34 (A)
4c	3	94	0.39 (A)
4d	1.5	91	0.46 (A)
4e	2	90	0.43 (A)
5b	2	92	0.68 (B)
5c	6	70	0.41 (A)
5d	1.5	93	0.39 (C)
5e	2	80	0.44 (A)

[a] Temperature = 155–160 °C. [b] Based on TLC on reaction mixture. Isolated yields ranged from 5 to 20% less than that shown. [c] Solvent systems employed were as follows: A (hexane/EtOAc, 5:2), B (benzene/EtOAc, 1:1), C (chloroform).

capsules and counted (84% efficiency) for radioiodine content in a Searle 1185 well scintillation counter.

Plasma and Tissue Extraction. Radioactivity was extracted from plasma by a modified Folch procedure described previously.[20] Liver samples were homogenized, extracted, and analyzed by TLC with hexane/ethyl acetate (5:2) as eluent. Following development, the plates were air dried and then cut into 1-cm strips. Each strip was placed in a counting tube and assayed for radioactivity. In all cases, the unlabeled compound served as a reference standard. Results are expressed as a percentage of total radioactivity on each plate.

Plasma Electrophoresis. Polyacrylamide gel electrophoresis of plasma samples was performed according to the method previously described.[21] The amount of radioactivity associated with each lipoprotein class was determined by sectioning the gels and counting each section in a γ counter. The radioactivity associated with each lipoprotein band is expressed as a percentage of the total radioactivity applied to the gel.

Acknowledgment. This research was supported by USPHS Grant CA-08349. We thank Judi Wilson for preparation of the manuscript and Wendelyn Schmidt, Lisa Santay, Tammy Granroth, and Edie Quenby for their technical assistance.

Registry No. **1a**, 3119-17-3; **1b**, 1206-91-3; **1c**, 96-83-3; **1d**, 102831-71-0; **1e**, 102831-72-1; **2a**, 103959-66-6; **2b**, 103959-67-7; **2c**, 103959-68-8; **3a**, 103980-81-0; **3b**, 103959-69-9; **3c**, 103959-70-2; **4a**, 103959-71-3; **4b**, 103959-72-4; **4c**, 103959-73-5; **4d**, 103959-74-6; **4e**, 104013-73-2; **5b**, 103959-75-7; **5c**, 103959-76-8; **5d**, 103959-77-9; **5e**, 103980-82-1; $CO(CH_2OH)_2$, 96-26-4; $CH_3(CH_2)_{14}COOCH(CH_2OH)_2$, 23470-00-0; $CH_3(CH_2)_{14}CO_2H$, 57-10-3.

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Multisubstrate Inhibitors of Dopamine β-Hydroxylase. 1. Some 1-Phenyl and 1-Phenyl-Bridged Derivatives of Imidazole-2-thione

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Received March 17, 1986

The synthesis and characterization of some 1-(phenylalkyl)imidazole-2-thiones as a novel class of "multisubstrate" inhibitors of dopamine β-hydroxylase (DBH) are described. These inhibitors incorporate structural features that resemble both tyramine and oxygen substrates, and as evidenced by steady-state kinetics, they appear to bind both the phenethylamine binding site and the active site copper atom(s) in DBH. A series of structural congeners that incorporate different bridging chain lengths between the phenyl ring (dopamine mimic) and the imidazole-2-thione group (oxygen mimic) define the optimum distance for inhibitory potency and the likely intersite distance in the DBH active site. Additional bridging analogues were prepared to determine the active site bulk tolerance and the effects of heteroatom replacement.

The sympathetic nervous system and its neurotransmitter norepinephrine have long been implicated in the regulation of vascular tone and the pathophysiology of hypertension.[1-5] Indeed, some studies in hypertensive patients have correlated increased plasma levels of norepinephrine with elevated resting recumbent blood pressure.[2,6] Several investigations in animals[7-10] also correlate increases in circulating norepinephrine with high blood pressure. An increased level of circulating catecholamines has similarly been noted in patients with congestive heart failure.[11] Consequently, it is plausible that interference with the biosynthesis of norepinephrine might provide a means for treating cardiovascular disorders such as hypertension and congestive heart failure.

As an approach to such potential therapeutic agents, a search was undertaken[12] for inhibitors of the enzyme dopamine β-hydroxylase (EC 1.14.17.1, DBH), a copper-containing monooxygenase, localized in the adrenal me-

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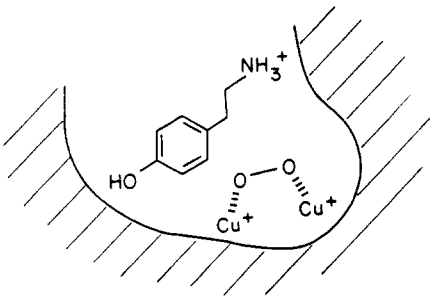


Figure 1. Hypothesized binding of phenethylamine (here tyramine) and oxygen substrates during DBH-catalyzed benzylic hydroxylation.

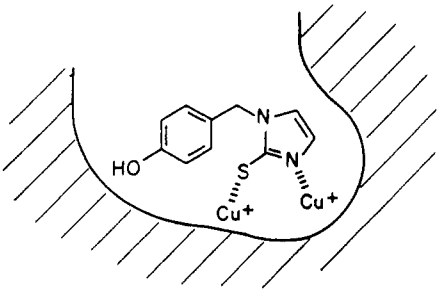


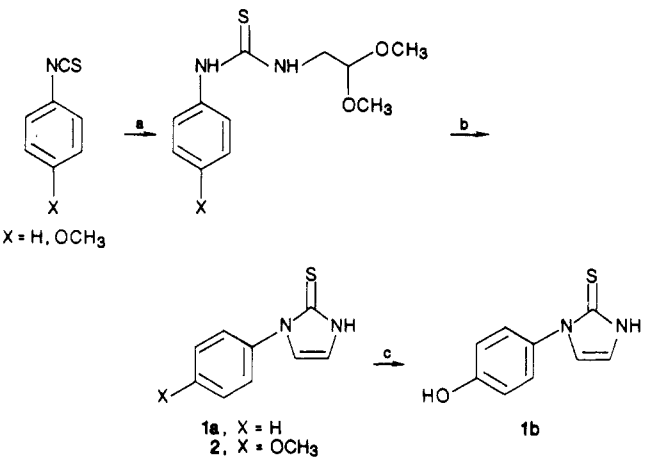
Figure 2. Postulated interaction of 1-substituted imidazole-2-thiones with hypothesized oxygen and aromatic binding sites of DBH.

dulla[13] and sympathetic nerve endings,[14] that catalyzes the conversion of dopamine to norepinephrine.[15,16] Although tyrosine hydroxylation is the putative rate-limiting step[17] in the four-stage sequence involved in the formation of norepinephrine from the essential amino acid phenylalanine, in principle this biotransformation might be reduced by adequate amounts of sufficiently potent inhibitors of any of the four intermediate reactions. A particular advantage resulting from specific inhibition of DBH is that levels of dopamine, which itself at appropriate concentrations has selective vasodilator actions, particularly in the renal[18] and mesenteric vasculature,[19] should be elevated as the production of norepinephrine is blunted. In addition, evidence derived primarily in cardiomyopathic hamsters indicates that a general manifestation of augmented sympathetic nerve traffic is a shift of the rate-limiting step from the hydroxylation of tyrosine to the DBH-catalyzed hydroxylation of dopamine.[20] A practical advantage of inhibiting the DBH-catalyzed reaction results from the "internal" position of this step in the catecholamine cascade, which suggests a lessened probability for enzymatic upregulation with subsequent tachyphylaxis upon chronic administration of the inhibitor.[21] Finally, it is noteworthy that fusaric acid, a prototypic copper-chelating DBH inhibitor, has potent antihypertensive and hypotensive actions in man[22] and other mammals,[23-25] although it is possible that it lowers blood pressure by mechanisms other than enzyme inhibition.[26]

Intense recent investigations[27,28] of the nature, properties, and mechanism of action of DBH serve as a further incentive to search for potent and selective inhibitors. Despite the considerable research directed toward DBH, however, relatively little is known about its catalytic site. We have hypothesized that the DBH-catalyzed oxidation involves simultaneous binding of oxygen to a Cu^{1+} site and of the phenethylamine to a proximate aromatic binding site as depicted in the model in Figure 1. A binuclear copper site, as drawn, remains speculative since the issue of a binuclear vs. a mononuclear copper site is still unresolved for DBH. Furthermore, the copper atoms in DBH appear magnetically noninteractive[28] to suggest a mononuclear site for the EPR active Cu^{2+} form of enzyme. Even so, the model draws support from the net two electron reaction stoichiometry and data for other, well-studied, binuclear copper-dependent monooxygenases such as tyrosinase.[29]

This model suggests a multisubstrate inhibitor as a rational means for deriving potent, competitive DBH inhibitors.[30] As the valency of oxygen prohibits its incorporation into such an inhibitor, the comparably soft, bidentate bridging ligand provided by the imidazole-2-thione system, several of whose 1-alkyl derivatives are weak inhibitors of DBH,[31-33] was selected as a replacement for the

Scheme I[a]



[a] Reagents and conditions: (a) $NH_2CH_2CH(OCH_3)_2$, $CHCl_3$; (b) 20% aqueous H_2SO_4, reflux; (c) BBr_3, CH_2Cl_2, 0 °C.

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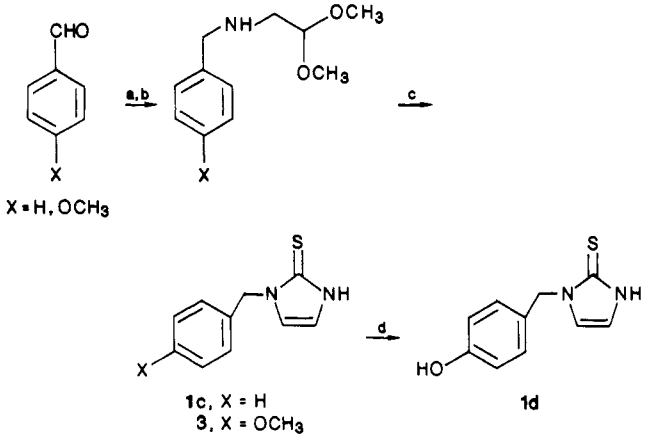
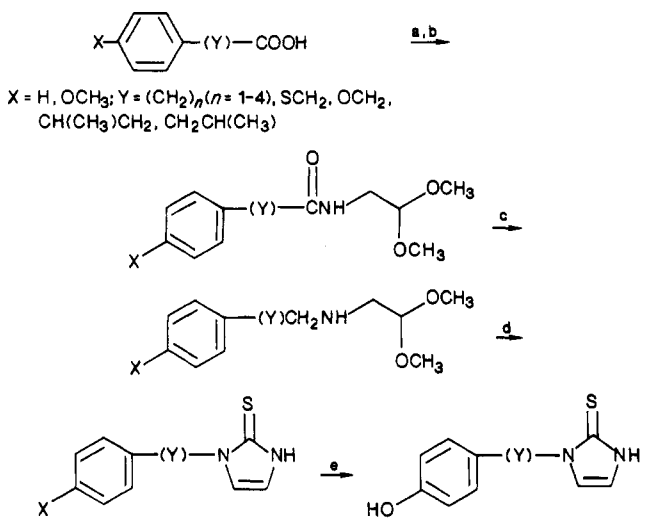
Scheme II[a]



[a] Reagents and conditions: (a) $NH_2CH_2CH(OCH_3)_2$, CH_3OH, reflux; (b) 10% Pd/carbon, ethanol, 50 psig of hydrogen; (c) aqueous HCl, KSCN, reflux; (d) BBr_3, CH_2Cl_2, 0 °C.

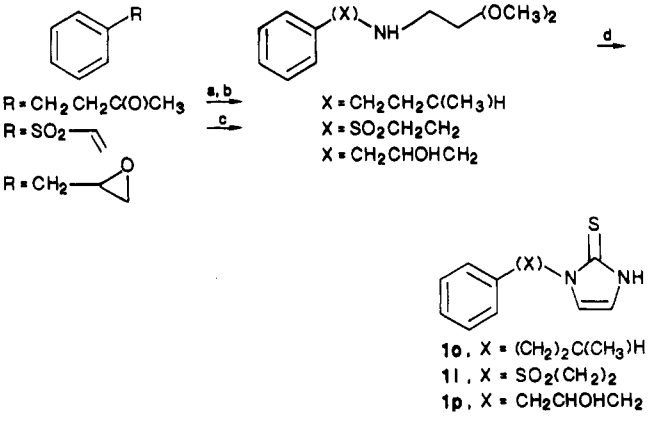
Scheme III[a]



[a] Reagents and conditions: (a) $(COCl)_2$, pyridine, CH_2Cl_2; (b) $NH_2CH_2CH(OCH_3)_2$, CH_2Cl_2; (c) $LiAlH_4$, Et_2O, THF, 22 °C; (d) aqueous HCl, KSCN, ethanol, reflux; (e) BBr_3, CH_2Cl_2.

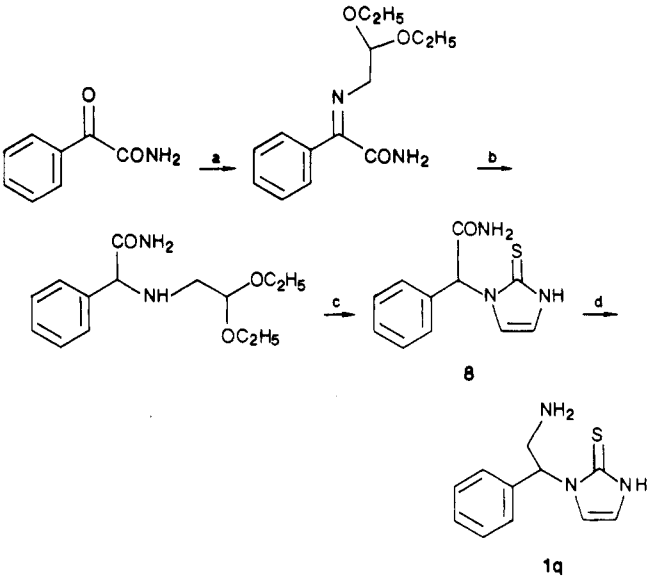
molecular oxygen substrate. A phenyl or 4-oxygenated phenyl group was selected as a mimic of dopamine's catecholic nucleus as a substrate for the aromatic binding site of the enzyme. These aryl systems were selected for synthetic simplicity, coupled with the observation that the related phenethylamines, i.e., *p*-tyramine and β-phenethylamine, are substrates for DBH.[34,35] Interaction of such 1-phenyl and phenyl-chain-linked imidazole-2-thiones with the oxygen and aromatic binding sites is envisioned

Scheme IV[a]



[a] Reagents and conditions: (a) $NH_2CH_2CH(OCH_3)_2$, hexane, 25 °C; (b) $NaBH_4$, hexane, EtOH; (c) $NH_2CH_2CH(OCH_3)_2$, Δ; (d) aqueous HCl, KSCN, EtOH reflux.

Scheme V[a]



[a] Reagents and conditions: (a) $NH_2CH_2CH(OC_2H_5)_2$, CH_2Cl_2, HOAc; (b) 5% Pd/carbon, ethyl acetate, 50 psig of hydrogen; (c) aqueous HOAc, KSCN; (d) $BH_3{\cdot}S(CH_3)_2$, $CHCl_3$, CH_2Cl_2.

as illustrated in Figure 2. Synthesis and testing for DBH inhibitory activity of these 1-substituted imidazole-2-thiones (**1a–q**, Table I) was undertaken in order to define the optimal spacing between the aryl system and the molecular oxygen mimic and to study the influence of steric and electronic alteration of the bridging chain. The results of this study are described in the present paper.

Chemistry

Synthesis of the compounds in Table I was carried out by the general methods outlined in Schemes I–V. 1-Phenylimidazole-2-thiones (**1a**) and 1-(4-methoxyphenyl)imidazole-2-thiones (**2**) were prepared as shown in Scheme I by the condensation of the appropriate phenyl isothiocyanate with aminoacetaldehyde dimethyl acetal followed by acid-catalyzed cyclization of the intermediate *N*-phenylthioureas.[36] The methoxyl group in **2** was cleanly deprotected with boron tribromide to yield **1b**.

The imidazole-2-thiones **1c–q** were prepared by cyclizing the appropriately substituted aminoacetaldehyde dimethyl

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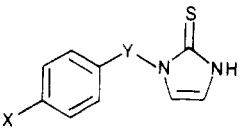
Table I. Structure, Physical Properties, and DBH-Inhibitory Activity of Some Substituted 1-Phenyl and Phenyl-Bridged Imidazole-2-thiones



no.	X	Y	synth method[a]	mp, °C	recrystn solvent	yield,[b] %	formula[c]	IC_{50},[d] μM
1a	H		A	180–182	EtOH	27	$C_9H_8N_2S$	110 (72–140)
2	CH_3O		A	215–217	EtOH	40	$C_{10}H_{10}N_2OS$	25%[e]
1b	HO		A, E	260–264	EtOH	57	$C_9H_8N_2OS$	370 (169–791)
1c	H	CH_2	B	144–145	EtOH	47	$C_{10}H_{10}N_2S$	32 (20–46)
3	CH_3O	CH_2	B	140	EtOH	68	$C_{11}H_{12}N_2OS$	202 (71–386)
1d	HO	CH_2	B, E	188	EtOH	42	$C_{10}H_{10}N_2OS$	2.6 (1.3–4.6)
4	CH_3O	$(CH_2)_2$	C	161–162	EtOH	26	$C_{12}H_{14}N_2OS$	68 (33–126)
1e	HO	$(CH_2)_2$	C, E	181–184	MeOH–H_2O	22	$C_{11}H_{12}N_2OS$	23 (15–33)
1f	H	$(CH_2)_3$	C	111–112	EtOH	26	$C_{12}H_{14}N_2S$	16 (10–24)
5	CH_3O	$(CH_2)_3$	C	108–109	EtOH	18	$C_{13}H_{16}N_2OS$	180 (130–190)
1g	HO	$(CH_2)_3$	C, E	185	EtOH	12	$C_{12}H_{14}N_2OS$	2.2 (1.8–2.8)
6	CH_3O	$(CH_2)_4$	C	96–98	MeCN–H_2O	69	$C_{14}H_{18}N_2OS$	152 (64–650)
1h	HO	$(CH_2)_4$	C, E	132–134	EtOH–H_2O	45	$C_{13}H_{16}N_2OS$	12%[e]
7	CH_3O	$(CH_2)_5$	C	67–69	PhMe–hexane	42	$C_{15}H_{20}N_2OS$	109 (61–220)
1i	HO	$(CH_2)_5$	C, E	157–161	MeOH–H_2O	19	$C_{14}H_{18}N_2OS$	30%[e]
1j	H	$O(CH_2)_2$	C	114–117	EtOH–H_2O	27	$C_{11}H_{12}N_2OS$	87 (77–97)
1k	H	$S(CH_2)_2$	C	140–142	EtOAc	34	$C_{11}H_{12}N_2S_2$	103 (88–121)
1l	H	$SO_2(CH_2)_2$	Ex[f]	112–114	EtOAc	25	$C_{11}H_{12}N_2O_2S_2$	5%[e]
1m	H	$CH(CH_3)(CH_2)_2$	C	100–103	MeOH–H_2O	50	$C_{13}H_{16}N_2S$	18 (11–25)
1n	H	$CH_2CH(CH_3)CH_2$	C	100–102	PhMe–hexane	51	$C_{13}H_{16}N_2S$	29 (24–34)
1o	H	$(CH_2)_2CH(CH_3)$	D	123–124	PhMe–hexane	20	$C_{13}H_{16}N_2S$	325 (269–393)
1p	H	$CH_2CH(OH)CH_2$	Ex	118–120	EtOAc–hexane	18	$C_{12}H_{14}N_2OS$	5%[e]
8	H	$CH(CONH_2)$	Ex	241–243	EtOAc	39	$C_{11}H_{11}N_3OS$	4%[e]
1q	H	$CH(CH_2NH_2)$	Ex	270–275	EtOH	15	$C_{11}H_{13}N_3S$·HCl	0%[e]

[a] See Experimental Section for description of general methods. [b] The overall yield 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 shown in parentheses. [e] Activity expressed as percent inhibition at a compound concentration of 10^{-4} M. [f] Ex = specific experimental procedure described.

acetals with aqueous thiocyanate under acidic conditions.[37] The substituted aminoacetaldehyde acetal intermediates were themselves prepared by the general procedures outlined in Schemes II–IV. These intermediate amines were generally unstable oils and were therefore used immediately without purification. The imidazole-2-thione products resulting from the cyclization of the amine intermediates were highly crystalline and in most cases were easily purified from complex reaction mixtures simply by recrystallization. The condensation of benzaldehyde and 4-methoxybenzaldehyde with aminoacetaldehyde dimethyl acetal followed by a controlled catalytic hydrogenation of the Schiff base intermediates provided substituted (*N*-benzylamino)acetaldehyde dimethyl acetals (Scheme II). The homologous, or side-chain-functionalized, (*N*-aralkylamino)acetaldehyde dimethyl acetals were synthesized (Scheme III) by coupling the corresponding carboxylic acids with aminoacetaldehyde dimethyl acetal via the acid chloride. While borane reduction of the amide intermediates was completely unsatisfactory, a result attributed to complexation of this reagent with starting material and/or products, $LiAlH_4$ did produce reasonable amounts of the desired secondary amines. The three additional amine precursors to **1o**, **1l**, and **1p** were prepared by the methods described in Scheme IV. Boron tribromide was found to be the method of choice for deprotecting the penultimate *O*-methyl ether intermediates. Even though the use of this reagent produces only a slow cleavage of the methyl ethers, if the methanolic workup is conducted rapidly, the production of byproducts resulting from sulfur alkylation can be almost completely suppressed. Saturated aqueous hydrobromic acid was found to rapidly demethylate the methyl ethers, but under all conditions employed, the major reaction product was the corresponding S-methylated analogue of the desired phenolic product. Alkylation of the very nucleophilic sulfur thus appears to effectively compete with loss of the volatile bromomethane from these reactions. Presumably the formation of a stable boron complex with the imidazole-2-thione protects this group from methylation during the deprotection reaction with boron tribromide. The phenolic products are stable, crystalline solids that are only weakly acidic and are presumed to exist as an equilibrium mixture of thiol and thione tautomeric forms. The S-alkylated byproducts encountered in attempted hydrobromic acid demethylations readily form acid addition salts.

The small and deceptively simple aminomethyl congener **1q** proved, in fact, to be difficult to prepare. It was found that the dense placement of functional groups and the reactivity of the sulfur atom in intermediates to **1q** demanded a carefully executed synthetic sequence (Scheme V). The partial hydrolysis product of benzoyl cyanide, phenylglyoxylamide,[38] was condensed with aminoacetaldehyde diethyl acetal under acidic conditions which minimized both transamidation with this amine and hydrolysis of the product acetal and amide functional groups. A controlled hydrogenation of the intermediate imine and a subsequent mild acid-catalyzed cyclization with aqueous thiocyanate produced reasonable yields of amide **8**. The final step in the synthetic sequence, reduction of the amide to the primary amine **1q**, proceeded in only modest yield despite all attempts to improve the reaction. Side products in this reduction included the primary alcohol presumed to arise via intramolecular "transacylation" with loss of ammonia followed by reduction, and poorly characterized

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Table II. IC_{50} Data and Kinetic Constants for Selected DBH Inhibitors and Standards

no.	IC_{50}, μM[a]	K_i,[b] μM	
		pH 4.5	pH 6.6
1b	370 (169–791)		
1d	2.6 (1.3–4.6)	0.0549 ± 0.0016	0.344 ± 0.016
1e	23 (15–33)		
1g	2.2 (1.8–2.8)		
1h	12%[c]		
1i	30%[c]		
1-methylimidazole-2-thione	754 (532–1030)	45 ± 1.90	716 ± 126
5-butylpicolinic acid (fusaric acid)		0.149 ± 0.005	

[a] Values given are IC_{50} in μM with upper and lower 95% confidence limits shown in parentheses. [b] Inhibition constants are all K_{is} (mean ± SEM) values vs. tyramine substrate except that for fusaric acid, which is a K_{ii} (mean ± SEM) value. At pH 4.5 the K_m apparent for tyramine substrate is 3 mM. [c] Activity expressed as percent inhibition at a compound concentration of 10^{-4} M.

materials resulting from desulfurization.

Biochemistry

The compounds in Table I were initially screened for DBH inhibitory activity with use of commercially available bovine enzyme (Sigma). Compounds that inhibited enzyme activity by at least 30% at a concentration of 10^{-4} M were further tested to determine an IC_{50} value. Data points were determined in duplicate at a minimum number of drug concentrations in at least two separate experiments to obtain an IC_{50} value with 95% confidence limits. To measure enzyme activity, octopamine produced from tyramine substrate was subjected to periodate cleavage and treatment with ammonium hydroxide and the resulting 4-hydroxybenzaldehyde was measured by UV absorbance at 330 nm. Under the conditions of the standard assay, all substrates, oxygen, tyramine, and ascorbic acid, are present in near-saturating levels, yielding IC_{50} values that are substantially higher than the true K_i values.

Selected inhibitors were further kinetically characterized with use of homogeneous DBH isolated from whole bovine adrenal medulla. DBH is known to undergo pH-dependent changes in both kinetic mechanism and substrate binding constants,[29,40] and therefore one inhibitor from the present study, **1d**, was evaluated in detail at two different pH values. When ascorbate or tyramine was the varied substrate, enzyme activity was measured essentially as in the IC_{50} assay already described. When oxygen was the varied substrate, a Yellow Springs Instrument Model 53 oxygen electrode with a sample volume of 4 mL was used. Enzymatic rates were determined directly from the rate of oxygen uptake after correcting for nonenzymatic oxygen consumption by ascorbic acid autoxidation. Data were subjected to regression analysis with use of the standard COMP, NONCOMP, and UNCOMP computer programs of Cleland.[41]

Results and Discussion

The compounds synthesized in the present study were assayed initially against commercial DBH under conditions of substrate near-saturation and in the presence of the activator fumarate to determine potency and establish a quantitative measure of relative activity (Table I) under conditions of maximal enzyme activity. One inhibitor, **1d**, was chosen for a detailed comparison to both 1-methylimidazole-2-thione, the parent molecule, and fusaric acid, a DBH inhibitor that has undergone clinical trials (Table II). The data in Tables I and II establish the general class

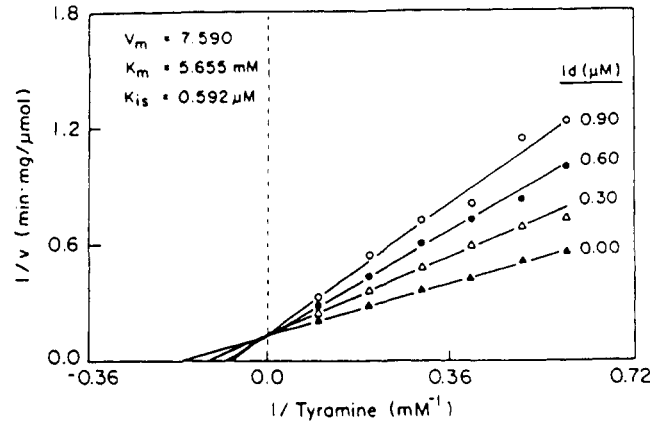

Figure 3. Compound **1d** vs. tyramine substrate at pH 6.6.

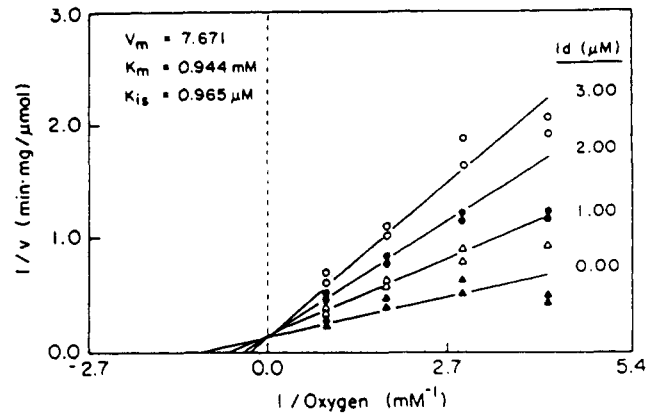

Figure 4. Compound **1d** vs. oxygen substrate at pH 6.6.

of compounds as potent DBH inhibitors. Compound **1d** binds to DBH approximately 10^5-fold more tightly than does tyramine substrate and appears significantly more potent than fusaric acid, the standard DBH inhibitor. As anticipated from the active-site models hypothesized in Figures 1 and 2, the nature of the substituent at position 1 of imidazole-2-thione has a profound impact upon DBH-inhibiting potency. Thus, while simple branched alkyl substituted imidazole-2-thiones have already been described to be reasonably potent inhibitors of DBH,[31-33] the optimum compound of the present study, **1d**, binds fully 10^3-fold more tightly to DBH than does the parent imidazole-2-thione as evidenced by the kinetic constants reported in Table II. The IC_{50} data for these compounds show a comparable but slightly smaller, 300-fold increase in potency for **1d** relative to 1-methylimidazole-2-thione.

As noted earlier in a detailed kinetic study,[29] the steady-state kinetics of the interaction of **1d** with DBH are strongly supportive of a specific interaction with the catalytic site. A multisubstrate inhibitor, by definition,

(39) Pisano, J. J.; Creveling, C. R.; Udenfriend, S. *Biochim. Biophys. Acta* **1960**, *43*, 566–568.
(40) Ahn, N.; Klinman, J. P. *Biochemistry* **1983**, *22*, 3096.
(41) Cleland, W. W. In *Methods in Enzymology*; Purich, D. L., Ed.; Academic: New York, 1979; Vol. 63, pp 103–138.

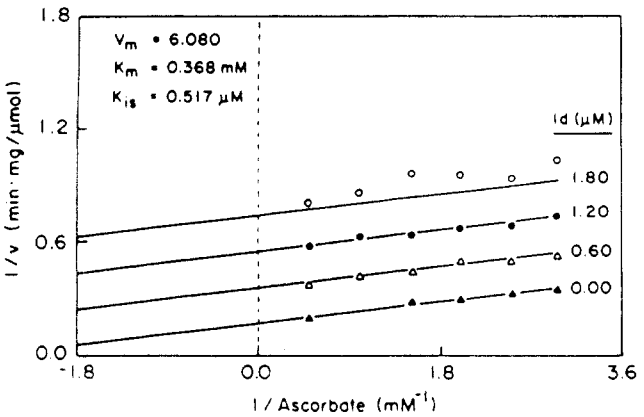

Figure 5. Compound **1d** vs. ascorbic acid substrate at pH 6.6.

must bind enzyme with complete mutual exclusion of substrates; i.e., it must bind competitively with the substrates mimicked.[30] Kinetic experiments were conducted at pH 6.6 (conditions of random tyramine and oxygen binding) to establish this for one prototypical inhibitor, **1d**. Compound **1d**, by nature of its competitive binding with respect to both tyramine and oxygen substrates (Figures 3 and 4), appears to fulfill this criterion and further demonstrates by its uncompetitive inhibition vs. ascorbate substrate (Figure 5) that it binds to the reduced (Cu^{1+}) form of DBH downstream from ascorbate, i.e., in a manner similar to that observed for the mimicked substrates.[42]

The active site in DBH that recognizes and binds (albeit weakly, millimolar K_m) the phenethylamine substrate appears to require a *p*-hydroxyl group for optimal binding of substrate.[43] A similar structural requirement for optimal binding of this series of inhibitors is evident by comparing **1c** to **1d** and **1f** to **1g**. In both cases the presence of the 4-hydroxyl group increases potency approximately 10-fold.

The series of compounds with varied bridging methylene chain length listed in Table II was synthesized in an attempt to define the optimum intersite distance. The relative potencies of these closely related congeners afford a further demonstration of the specificity with which these compounds interact with the enzyme active site. The 1-phenyl congener **1b** shows only a slight increase in potency over the 1-methyl parent, presumably a consequence of its inability to simultaneously bind to both enzymatic sites. This may also explain why for this series there is no advantage to having a hydroxyl group in position 4 of the phenyl ring (cf. **1b** and **1a**). Lengthening the chain as in the C_1 homologue **1d** yields a 300-fold increase in potency relative to the simple ligand, while a further lengthening to a two-carbon bridge, **1e**, lowers activity 10-fold relative to **1d**. The lowered activity of **1e** relative to **1d** and **1g** is interesting. It is conceivable that **1e** is unable to attain an optimum conformation to facilitate the binding of DBH. Only a very small change in conformational energy (~1.5 kcal/mol) would be required to produce the observed decrease in activity. With the addition of a further methylene group, the (4-hydroxyphenyl)propyl analogue **1g** once again yields maximum potency. Any further lengthening as in **1h** and **1i** virtually abolishes activity. This may derive from the increased bulk of these compounds, which are unable to bind a site extremely intolerant to bulk (vide infra), or more likely from the now-large number of degrees of rotational freedom, which negate the entropic advantages of combining two inhibitory molecules into a single multisubstrate inhibitor.

The intermediate O-methylated compounds **2–7** were also examined, and all were found to be only weakly active. While the activity of the "poorly fitting" pair of compounds **1b** and **2** or the higher homologues **6** and **7** is so weak as to make any significant conclusions difficult, the results with the C_1 and C_3 chain compounds are most revealing. In both cases, O-methylation so decreases inhibitory activity that the resulting compounds are less active than the unsubstituted phenyl congeners. Interestingly, true dissociation constants have been calculated for the correspondingly substituted phenethylamine substrates,[43] and these show the same relative binding affinity 4-HO > 4-H $\gg$ 4-CH_3O. Apparently the region of the phenethylamine binding site that accommodates the 4-position of this substrate is extremely bulk intolerant.

A small group of relatives in the C_3-chain-length series, **1j–p**, was prepared in order to study the effects of increased chain polarity, lipophilicity, and heteroatom substitution. With the exception of the simple methylated compound **1m**, all changes dramatically decreased activity, relative to the parent compound **1f**. With compound **1m**, the addition of a methyl group only marginally decreases activity. The portion of the DBH active site that accommodates the bridging chain between the substructures also appears sensitive to bulk. For example, the larger van der Waals radius and/or bond lengths for the sulfur atom relative to carbon in **1k** may make this compound more closely resemble the C_4 bridged analogue. In a final attempt to increase potency, an inhibitor, **1q**, that contained a basic side-chain nitrogen and that therefore closely resembles phenethylamine substrate was prepared. This compound was devoid of activity at 10^{-4} M. We have found the trivial phenethylamine analogue *p*-cresol to be an inhibitor of DBH that binds essentially as tightly as tyramine or dopamine substrate.[29] Apparently the basic side-chain nitrogen plays little, if any, role in the binding of phenethylamine substrates to the enzyme. In the case of **1q**, the presence of the aminomethyl group together with an "unnatural" oxygen mimic may lead to an extremely unfavorable "three-point" contact with a portion of the enzyme active site that is bulk or charge intolerant, to result in a substantial diminution of activity.[44]

Conclusions

The observed kinetic patterns for inhibitor **1d** vs. ascorbate, tyramine, and oxygen substrates, as well as the stringent structural requirements for optimal potency, are supportive of a rather specific interaction between this class of inhibitor and the enzyme active site. In this regard only inhibitors **1d** and **1g**, compounds of specific bridging chain length, are of maximal potency. Perhaps not surprisingly, in view of the small size of the oxygen, phenethylamine, and ascorbic acid substrates, which are normally bound during catalysis, the DBH active site appears to be extremely bulk intolerant. Inhibitors such as **3–7**, which have a methoxyl group at the 4-position of the aryl ring, show a substantial decrease in activity relative to the unsubstituted phenyl molecule. A similar effect has been reported for the calculated binding constants of comparably substituted phenethylamine substrates.[43] Bulk intolerance is shown not only in the region of the DBH active

(42) Although the precise kinetic mechanism of ascorbate substrate binding, i.e., ping pong vs. sequential, has not been unambiguously determined (see ref 29), this is immaterial for predicting an uncompetitive pattern of these multisubstrate inhibitors vs. this substrate.

(43) Miller, S. M.; Klinman, J. P. *Biochemistry* **1985**, *24*, 2114.

(44) Ogston, A. G. *Nature (London)* **1948**, *162*, 963.

site that binds the aromatic ring of substrate or inhibitor but also in the region of the bridging chain as evidenced in the 1-phenylpropyl and 1-benzyl series. Any substitution to these side chains reduces activity.

The present study has given rise to a novel class of multisubstrate inhibitors of a mixed-function oxidase, DBH. The concept of using a soft bidentate ligand as a mimic for molecular oxygen may find utility in the design of multisubstrate inhibitors of other mixed-function oxidases as well.

Experimental Section

Chemistry. When appropriate, all solvents used in reaction mixtures were dried and/or purified by standard procedures.[45] 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 an 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 structures. Solutions were dried over anhydrous magnesium sulfate and concentrated with a Büchi Rotovapor at ca. 10 torr before pumping at 0.5 torr.

Method A. 1-(4-Methoxyphenyl)imidazole-2-thione (2). A solution of (4-methoxyphenyl)isothiocyanate (10.0 g, 0.06 mol) in $CHCl_3$ (100 mL) was stirred at ambient temperature during the dropwise addition of aminoacetaldehyde dimethyl acetal (6.3 g, 0.06 mol). The resulting solution was concentrated, and the residue was recrystallized from EtOH to give 9.2 g (57%) of *N*-(4-methoxyphenyl)-*N'*-(2,2-dimethoxyethyl)thiourea. This thiourea was suspended in 25% aqueous H_2SO_4 (25 mL) and heated at reflux for 3 h. The resulting mixture was cooled, and the solid was filtered, washed with H_2O, and dried. Recrystallization from EtOH gave 4.9 g (40% overall) of **2** (Table I).

Method B. 1-(4-Methoxybenzyl)imidazole-2-thione (3). A mixture of 4-methoxybenzaldehyde (13.6 g, 0.1 mol), aminoacetaldehyde dimethyl acetal (10.5 g, 0.1 mol), and MeOH (1 mL) was heated at 95 °C for 10 min. The resulting mixture was dissolved in EtOH (150 mL) and hydrogenated over 10% Pd on carbon (1 g) until H_2 uptake slowed (about 2 h). The catalyst was filtered, and the filtrate was treated with 1.5 N HCl (80 mL) and KSCN (10.4 g, 0.11 mol). The resulting mixture was boiled until the volume was reduced to 100 mL, and then it was heated at reflux for an additional 1 h and cooled to 5 °C. The crystalline precipitate was filtered, washed with H_2O, and dried. Recrystallization from EtOH gave 15.0 g (68%) of **3** (Table I).

Method C. 1-[3-(4-Methoxyphenyl)propyl]imidazole-2-thione (5). A solution of 3-(4-methoxyphenyl)propionic acid (12.5 g, 0.07 mol) in CH_2Cl_2 (100 mL) and pyridine (0.1 mL) was treated with oxalyl chloride (9.8 g, 0.077 mol) and then stirred for 2.5 h and concentrated. A solution of the resulting crude acid chloride in CH_2Cl_2 (100 mL) was added to a cold (5 °C) solution of aminoacetaldehyde dimethyl acetal (14.7 g, 0.14 mol) in CH_2Cl_2 (300 mL) at such a rate that the temperature did not exceed 20 °C. After the addition was completed, the mixture was washed sequentially with H_2O, 5% aqueous Na_2CO_3, 0.5 N HCl, and H_2O, and then the organic phase was dried. The solution was concentrated under reduced pressure to give 10.3 g (55%) of *N*-(2,2-dimethoxyethyl)-3-(4-methoxyphenyl)propionamide. The unpurified amide was immediately reduced with $LiAlH_4$ (4.0 g) in a mixture of Et_2O (700 mL) and THF (300 mL) for 3.5 h at 22 °C. Following destruction of the excess $LiAlH_4$ by *cautious* sequential addition of H_2O (4 mL), 15% aqueous NaOH (4 mL), and H_2O (12 mL), the mixture was filtered. The filtrate was concentrated under reduced pressure to yield 4.6 g (52%) of [*N*-[3-(4-methoxyphenyl)propyl]amino]acetaldehyde dimethyl acetal as an unstable oil. A mixture of this amine (4.6 g, 0.018 mol) and KSCN (1.94 g, 0.02 mol) in EtOH (25 mL) and 3 N HCl (10 mL) was heated at reflux for 5 h. The mixture was cooled (5 °C) and diluted with H_2O (60 mL), and the resulting solid was collected by filtration, washed with H_2O, and dried. Recrystallization from EtOH gave 3.1 g (69% from amine, 18% overall) of **5** (Table I).

Method D. 1-(1-Methyl-3-phenylpropyl)imidazole-2-thione (1o). A solution of 4-phenyl-2-butanone (7.4 g, 0.05 mol) in hexane (20 mL) was treated with aminoacetaldehyde dimethyl acetal (4.2 g, 0.04 mol) and stirred for 1 h. The resulting cloudy reaction mixture was diluted with EtOH (50 mL) and treated with $NaBH_4$ (1.51 g, 0.04 mol). After 18 h the reaction was diluted with H_2O, and volatile solvents were removed by concentration under reduced pressure. The residue was extracted with Et_2O, and the extracts were dried and concentrated under reduced pressure to yield 8.67 g (83%) of *N*-(1-methyl-3-phenylpropyl)aminoacetaldehyde dimethyl acetal as an oil. TLC and NMR analysis indicated that this was a mixture of desired product contaminated with 4-phenyl-2-butanol (ca. 20%). This crude mixture was dissolved in EtOH (40 mL) and H_2O (67 mL), treated with KSCN (2.91 g, 0.03 mol) and 12 N HCl (7.5 mL), and heated at reflux for 1 h. After addition of H_2O (500 mL), the mixture was cooled and extracted with Et_2O. The organic extracts were washed with H_2O, dried, and concentrated under reduced pressure. The resulting oily residue was dissolved in PhMe, and hexane was gradually added to induce crystallization. The mixture was chilled, and the white precipitate was filtered to give 1.82 g (20%) of **1o** (Table I).

2-Thioxo-α-phenyl-1-imidazole-1-acetamide (8). A solution of phenylglyoxylamide (15.7 g, 0.105 mol)[38] and aminoacetaldehyde diethyl acetal (13.9 g, 0.105 mol) in CH_2Cl_2 (100 mL) containing HOAc (0.66 g, 0.011 mol) was stirred at ambient temperature for 18 h. The mixture was concentrated under reduced pressure, and the residue was triturated with Et_2O to give 14.8 g (53%) of the Schiff base. The Schiff base was dissolved in EtOAc (100 mL) and hydrogenated over 2.7 g of 5% Pd on carbon for 2 h at 50 psi of H_2. The mixture was filtered, and the filtrate was concentrated under reduced pressure to yield 13.0 g (96%) of [*N*-[α-(aminocarbonyl)benzyl]amino]acetaldehyde diethyl acetal. A solution of this amine and KSCN (10.5 g, 0.11 mol) in AcOH (100 mL) was heated at reflux for 10 min and then poured into cold H_2O and extracted with EtOAc. The organic extracts were washed with 5% aqueous $NaHCO_3$, then dried, and then concentrated under reduced pressure. The residue was recrystallized from a small volume of EtOAc to give 4.5 g (39%) of **8** (Table I).

1-(2-Amino-1-phenylethyl)imidazole-2-thione (1q). A solution of **8** (9.0 g, 0.039 mol) in $CHCl_3$ (200 mL) was treated dropwise with a CH_2Cl_2 solution of BH_3·Me_2S complex (240 mL of a 1 M solution, 0.24 mol) and then heated at reflux for 60 h. The reaction mixture was cooled, MeOH (100 mL) was added, and the solution was concentrated under reduced pressure. The residue was dissolved in hot MeOH, and this solution was concentrated under reduced pressure. The residue was dissolved in EtOAc and extracted with 3 N HCl. The aqueous extracts were washed with Et_2O, basified to pH 9, and extracted with EtOAc. The organic extracts were dried and evaporated, and the residue was chromatographed over a silica gel column. Elution with 1:19 MeOH–CH_2Cl_2 yielded 1.2 g (15%) of 1-(2-amino-1-phenylethyl)imidazole-2-thione. A sample of this was converted to the hydrochloride salt by treatment with ethereal HCl. The salt was recrystallized from EtOH to yield **1q** (Table I).

1-[2-(Phenylsulfonyl)ethyl]imidazole-2-thione (1l). A solution of phenyl vinyl sulfone (4.71 g, 0.028 mol) in MeOH (100 mL) was stirred during the addition of a solution of aminoacetaldehyde dimethyl acetal (2.94 g, 0.028 mol) in MeOH (100 mL), and the resulting solution was stirred for 12 h at ambient temperature. The mixture was filtered and the filtrate was decolorized with activated carbon and concentrated under reduced pressure to yield the intermediate amine as a light yellow oil. The crude oil was dissolved in EtOH (36 mL), and a solution of KSCN (2.72 g, 0.028 mol) in H_2O (63 mL) and 12 N HCl (7 mL, 0.028 mol) was added. The resulting mixture was heated at reflux for 1 h, then diluted to a volume of 0.5 L with H_2O, and then cooled for 18 h. The resulting crystals were filtered and recrystallized from EtOAc to yield 1.88 g (25%) of **1l**.

1-(2-Hydroxy-3-phenylpropyl)imidazole-2-thione (1p). A solution of aminoacetaldehyde dimethyl acetal (6.3 g, 0.06 mol) and 1-phenylpropane 2,3-epoxide (6.71 g, 0.05 mol) in MeOH (200 mL) was heated at reflux for 18 h and then concentrated under reduced pressure to yield the crude amine as a light yellow oil.

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

A solution of the crude amine (8.41 g, 0.0352 mol) in EtOH (53 mL) was treated with a solution of KSCN (3.52 g, 0.0352 mol) in H_2O (88 mL) and 12 N HCl (8.81 mL), and the mixture was heated at reflux for 1 h. The solution was cooled, diluted with H_2O (100 mL), and extracted with Et_2O. The organic extracts were dried and concentrated under reduced pressure, and the residue was recrystallized from EtOAc–hexane to yield 1.48 g (18%) of **1p** as colorless crystals.

Method E. General Method for Cleavage of Aryl Methyl Ethers. 1-[3-(4-Hydroxyphenyl)propyl]imidazole-2-thione (1g). 1-[3-(4-Methoxyphenyl)propyl]imidazole-2-thione (**5**) (1.75 g, 0.007 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). The reaction mixture was stirred for 1.5 h at ambient temperature, cooled to 0 °C, and *cautiously* treated with MeOH (50 mL). The solvents were evaporated, and the residue was recrystallized from EtOH to yield 1.02 g (67%) of **1g**.

Biology. In Vitro IC_{50} Determination. Each of the compounds in Table I was initially tested for DBH inhibitory activity at 1×10^{-4} M in duplicate assays. Where indicated, the IC_{50} value for a compound was determined in at least two separate experiments, each testing 4–6 concentrations of compound in duplicate. Each incubation vessel contained DBH (0.0145 unit/mL; Sigma D1893); test compound at the appropriate concentration; 0.2 M sodium acetate buffer, pH 5.0; 10 mM freshly prepared ascorbic acid; 10 mM freshly prepared sodium fumarate; crystalline catalase (65 000 units/mL); 1 mM pargyline; 30 mM freshly prepared *N*-ethylmaleimide; and 10 mM tyramine in a 1.0-mL volume. Samples were preincubated with compound for 2 h without tyramine in a shaking H_2O bath at 37 °C. Tyramine was added, and incubation was continued for 1 h. Tubes were removed and placed in ice, and then 0.2 mL of 3.0 M trichloroacetic acid was added and samples were centrifuged at 2000 rpm in a tabletop centrifuge for 10 min. The resulting supernatant was passed through Dowex 50 (H^+ form, 200–400 mesh, 0.3-mL packed volume) in a disposable column (1 × 11 cm). The sample tube and pellet were washed with 1.0 mL of H_2O, which was also poured onto the column. The column was washed two times with 2.0 mL of H_2O, and the washes were repassed through the column. The column was eluted with 1.5 mL of 4.0 M NH_4OH. The octopamine in the eluate was converted to 4-hydroxybenzaldehyde by the addition of 0.1 mL of 2% aqueous $NaIO_4$. After 5 min, 0.1 mL of 10% aqueous $Na_2S_2O_5$ was added to reduce excess $NaIO_4$. UV absorption was monitored against a reagent blank at 330 nm. An octopamine standard curve (50, 100, and 150 μM octopamine) and an H_2O blank were processed in each assay. Additionally, fusaric acid, a potent inhibitor of DBH, was processed with each assay as a control. Compounds whose H_2O solubilities were insufficient were dissolved in Me_2SO prior to dilution into the assay. Suitably treated controls were also assayed. A computer analysis of the data was used to establish the IC_{50} values and the 95% confidence limits. The IC_{50} value is defined as the concentration of the compound that produces a 50% inhibition of product formation when compared to the tyramine control.

Kinetic Assays with Purified DBH. The homogeneous bovine DBH (sp act. 25–42 μmol min^{-1} mg^{-1} at pH 5.0 in the tyramine assay) used for kinetic assays was isolated by our modified isolation procedure.[46] All experiments were carried out at 37 °C with incubation mixtures that contained 50 mM buffer (either NaOAc, pH 4.5, or NaH_2PO_4, pH 6.6), 200 μg/mL crystalline catalase, 10 μM (pH 4.5) or 5 μM (pH 6.6) $CuCl_2$, and enough NaCl to maintain a constant ionic strength of 0.2. The concentration of ascorbate was maintained at 10 mM when either tyramine or oxygen was the varied substrate. Tyramine was fixed at 1.0 mM when ascorbate or oxygen was varied. When tyramine or ascorbate was varied, oxygen was maintained at 0.24 mM by an equilibration in a reciprocating shaker-bath. When either tyramine or ascorbate substrates were varied, the production of octopamine was determined by using a procedure similar to the one described above. When oxygen was the varied substrate, a Yellow Springs Instrument Model 53 biological oxygen electrode was employed with 4-mL incubation mixtures. In a typical assay, buffer (with or without inhibitor) was stirred and equilibrated at 37 °C for 4 min while being saturated with oxygen or oxygen–nitrogen gas mixtures, the Lucite plunger holding the oxygen electrode was lowered into contact with the buffer, and crystalline catalase suspension (40 μL of 20 mg/mL) and 0.2 M aqueous ascorbic acid stock solution (20 μL) were added sequentially. After the rate of oxygen uptake due to nonenzymatic ascorbate autoxidation was monitored, DBH solution was added and oxygen uptake was followed for 1–4 min.

Registry No. **1a**, 17452-09-4; **1b**, 95333-67-8; **1c**, 23269-10-5; **1d**, 95333-64-5; **1e**, 95333-63-4; **1f**, 104489-48-7; **1g**, 95333-65-6; **1h**, 95333-55-4; **1i**, 104489-49-8; **1j**, 104489-50-1; **1k**, 104489-51-2; **1l**, 104489-52-3; **1m**, 104489-53-4; **1n**, 104489-54-5; **1o**, 104489-55-6; **1p**, 104489-56-7; **1q**, 104489-57-8; **1q**·HCl, 104489-61-4; **2**, 17452-14-1; **3**, 95460-09-6; **4**, 100134-69-8; **5**, 95333-89-4; **6**, 104489-45-4; **7**, 104489-46-5; **8**, 104489-47-6; 4-$MeOC_6H_4NCS$, 2284-20-0; 4-$MeOC_6H_4NHC{=}S(NHCH_2CH(OMe)_2)$, 95333-84-9; $H_2NCH_2CH(OMe)_2$, 22483-09-6; PhNCS, 103-72-0; 4-$MeOC_6H_4CHO$, 123-11-5; PhCHO, 100-52-7; 4-$MeOC_6H_4(CH_2)_2CONHCH_2CH(OMe)_2$, 95333-86-1; 4-$MeOC_6H_4(CH_2)_3NHCH_2CH(OMe)_2$, 95333-87-2; 4-$MeOC_6H_4(CH_2)_2COCl$, 15893-42-2; 4-$MeOC_6H_4(CH_2)_2CO_2H$, 1929-29-9; $CH_3CO(CH_2)_2Ph$, 2550-26-7; $(MeO)_2CHCH_2NHCH(CH_3)CH_2CH_2Ph$, 104489-58-9; $PhCOCONH_2$, 7505-92-2; $(EtO)_2CHCH_2N{=}C(Ph)CONH_2$, 104489-59-0; $PhCH(CONH_2)_2NHCH_2CH(OEt)_2$, 104489-60-3; $PhCH_2CH(OH)CH_2NHCH_2CH(OMe)_2$, 104489-62-5; $PhSO_2(CH_2)_2NHCH_2CH(OMe)_2$, 104489-63-6; phenyl vinyl sulfone, 5535-48-8; 1-phenylpropane 2,3-epoxide, 4436-24-2; dopamine β-hydroxylase, 9013-38-1.

(46) DeWolf, W. E., Jr.; Kruse, L. I., unpublished results.

Studies of the Antitumor Activity of (2-Alkoxyalkyl)- and (2-Alkoxyalkenyl)phosphocholines

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Analogues of the synthetic antitumor phospholipid ALP (1-octadecyl-2-methyl-*sn*-glycero-3-phosphocholine; alkyl lysophospholipid) in which the 1-ether oxygen atom has been removed have been prepared and evaluated for in vitro and in vivo anticancer activity. Compounds are presented in which the saturated long chain varies in length from 8 to 25 carbon atoms. Sites of unsaturation are also incorporated into the framework in some examples. In particular, *rac*-(2-ethoxyeicosyl)phosphocholine (**10**) displays the best in vivo activity of the chemical series against a variety of transplanted tumors and activates murine peritoneal macrophages to express tumor cytotoxicity in vitro. However, **10** does not offer the wide spectrum of antitumor activity we feel necessary to warrant further study.

The recent discovery of platelet activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, PAF) as a potent cell-derived mediator of allergy, inflammation, and cardiovascular function has provoked scientists of many

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