Benzylamine Antioxidants: Relationship between Structure, Peroxyl Radical Scavenging, Lipid Peroxidation Inhibition, and Cytoprotection

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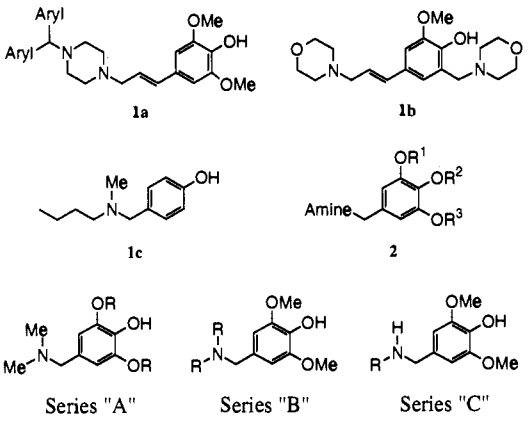
Three homologous series of 3,5-dialkoxy-4-hydroxybenzylamines were prepared and tested (1) as peroxyl radical scavengers in homogeneous aqueous solution, (2) as inhibitors of iron-dependent peroxidation of rabbit brain vesicular membrane lipids, and (3) as cytoprotective agents using primary cultures of rat hippocampal neurons exposed to hydrogen peroxide. The structural requirements for efficient radical trapping in homogeneous solution differed from those for effective lipid peroxidation inhibition: In homogeneous solution a kinetic preference existed for smaller, less sterically encumbered substituents flanking the reactive phenolic hydroxyl group. Lipid peroxidation inhibition, on the other hand, required longer more lipophilic substituents. Consequently, a lipophilic alkoxyl substituent at C3 and a small substituent at C5 appeared optimal for efficient radical scavenging activity in both lipid and homogeneous solution. Maximal cytoprotection of rat hippocampal neurons exposed to hydrogen peroxide was also associated with more lipophilic derivatives although substituent length and substituent bulk may represent independent parameters for relating structure and efficacy in this system.

Oxidants such as hydrogen peroxide and oxygen-derived free radicals may play an important role in the pathophysiology of tissue damage associated with acute ischemic or traumatic injury to the central nervous system.[1] While the mechanisms underlying postischemic cellular degeneration are likely to be multifactorial, alterations in lipid membrane structure and function as a consequence of destructive oxidative processes may be a significant factor in oxidant-mediated cell death and tissue necrosis.[2] Consequently, a number of structurally diverse antioxidants and lipid peroxidation inhibitors have been examined as potential therapeutic agents in models of cerebral[3] and myocardial ischemia[4-6] as well as models of posttraumatic head and spinal cord injury.[7]

Among the naturally occurring membrane antioxidants, α-tocopherol has been extensively investigated for its biological effects as a radical scavenger[8,9] where inhibition of lipid peroxidation in vitro appears to correlate with vitamin E activity in vivo.[10] Although water-soluble derivatives of α-tocopherol can block lipid peroxidation in vitro,[11] molecular lipophilicity as well as free radical scavenging efficiency represent two important determinants of activity. With respect to the former, the lipophilic phytyl "tail" of α-tocopherol enhances retention of the molecule in biological membranes and regulates mobility and distribution within the lipid bilayer.[12] Although the presence of branching methyl groups on the phytyl fragment has been proposed to be critical for membrane stabilization,[13-15] more recent evidence suggests that chain *length* rather than chain *branching* is the more important parameter for relating antioxidant structure with vitamin E activity and hence membrane protection.[16]

In connection with our interest in lipid peroxidation inhibitors, we wanted to examine possible relationships between antioxidant structure and cytoprotection that might aid in rationally designing simple aromatic membrane stabilizing agents for testing in models of oxidant injury. Since dialkoxyphenols and benzylamines such as compounds **1a** and **1b**, respectively, have been claimed in the patent literature as antioxidants with tissue-protecting activity,[17] and since benzylamines such as compound **1c** have been claimed as therapeutic agents for treatment of central nervous system disorders,[18] our initial goal was to determine how the topology of an alkyl substituent might alter the ability of a benzylamine (e.g. **2**) to act as a peroxyl radical scavenger, a lipid peroxidation inhibitor and a cytoprotective agent (Chart I). However, since aromatic amines (e.g. phenethylamines) can exert profound effects on the cardiovascular system and since significant alterations in systemic hemodynamics or impairment of cardiac function could offset a beneficial effect by lowering perfusion pressure to a postischemic organ, we evaluated representatives from this series for hypotensive liability in anesthetized normotensive rats.

Chart I



Chemistry

We began our synthetic efforts by targeting a dialkoxyphenol core structure that was readily amenable to systematic structural variation. This approach led us to initially assemble the three homologous series of benzyl-

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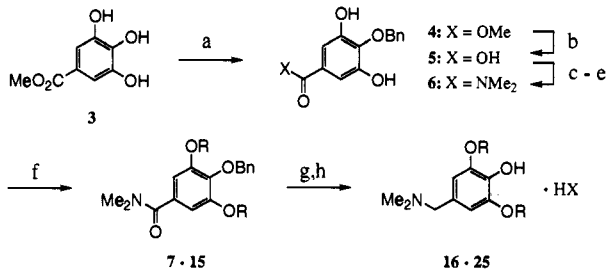
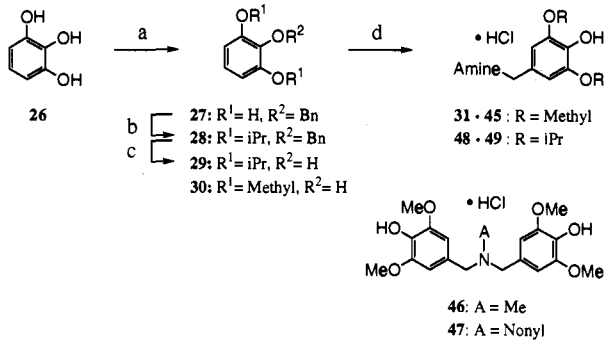
Scheme I[a]



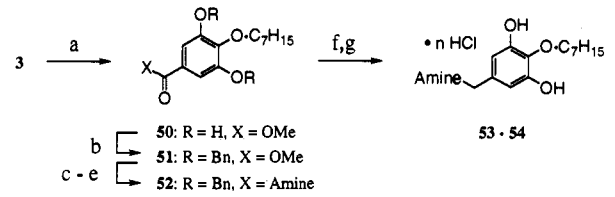
[a] (a) BnBr, NaH; (b) NaOH; (c) Ac_2O, pyridine; (d) $SOCl_2$; (e) Me_2NH; (f) RX, K_2CO_3; (g) LAH; (h) H_2, 10% Pd/C.

Scheme II[a]



[a] (a) BnBr, NaH; (b) 2-iodopropane, K_2CO_3; (c) H_2, 10% Pd/C; (d) amine, CH_2O.

Scheme III[a]



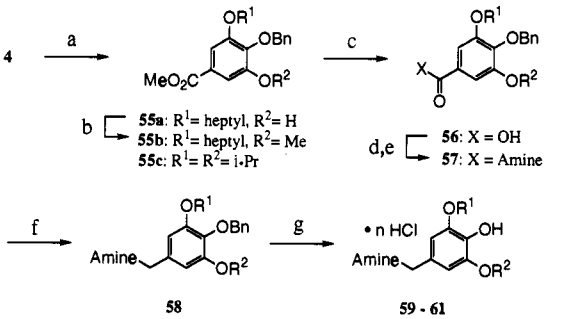
[a] (a) $C_7H_{15}I$, NaH; (b) BnBr, K_2CO_3; (c) NaOH; (d) $SOCl_2$; (e) amine; (f) LAH; (g) H_2, 10% Pd/C.

amines outlined in Chart I. The first (series A) was prepared by benzylating methyl gallate to yield compound **4** as the major product of the reaction (Scheme I). The desired phenol was readily separated from the accompanying less polar and more soluble dibenzylated material by recrystallization. Conversion of the methyl ester to a dimethylamide followed by alkylation, reduction, and deprotection furnished the desired benzylamine free bases. The corresponding methanesulfonate salts were initially prepared (e.g. **25**), but in our hands the hydrochloride salts were more easily handled. The second homologous series (series B) was prepared according to a literature procedure as outlined in Scheme II.[19] Although secondary amines furnished the desired products in good yield, primary amines often led to double addition products such as **46** and **47**. Using excess amine in the Mannich reaction, however, minimized formation of this side product and provided the targeted secondary benzylamines (series C) in a fashion analogous to series B albeit in lower yield.

Compounds **53** and **54** were prepared from methyl gallate as outlined in Scheme III by alkylating the C4 hydroxyl group with 1-iodoheptane. Functional group modification in a manner similar to series A provided the final products.

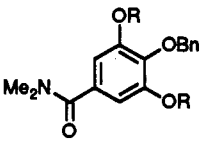
Synthesis of nonsymmetric benzylamine representatives was accomplished by successive O-alkylations as outlined in Scheme IV. Intermediate **55a** (R^1 = heptyl, R^2 = H) obtained as the minor alkylation product from compound

Scheme IV[a]



[a] (a) R^1X, K_2CO_3; (b) R^2X, K_2CO_3 if $R^1 \neq R^2$; (c) NaOH; (d) $SOCl_2$; (e) amine; (f) LAH; (g) H_2, 10% Pd/C.

Table I. Compound Numbering and Physical Data for Intermediate Amides **7–15**



no.	R	mp, °C	formula
7	ethyl	92–93.5	$C_{20}H_{25}NO_4$
8	*n*-propyl	53–55.5 dec	$C_{22}H_{29}NO_4$
9	*n*-butyl	37–39.5	$C_{24}H_{33}NO_4$
10	*n*-pentyl	oil	oil
11	*n*-hexyl	39–40	$C_{28}H_{41}NO_4$
12	*n*-heptyl	55–56	$C_{30}H_{45}NO_4$
13	isopropyl	oil	oil
14	cyclopentyl	94–96	$C_{26}H_{33}NO_4$
15	3-pentyl	oil	oil

4 using 1 equiv of 1-iodoheptane was methylated with iodomethane to provide **55b** (R^1 = heptyl, R^2 = Me). Subsequent ester hydrolysis, carboxylic acid activation, and coupling with 1-(2-hydroxyethyl)piperazine furnished derivative **61** after hydride reduction and hydrogenolysis.

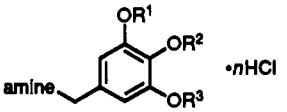
Compound numbering and physical data for intermediate amides **7–15** and all final benzylamines appear in Tables I and II, respectively.

Results

The oxidation potentials for compounds **31** and **16–20** were determined using cyclic voltammetry with a carbon paste disk electrode[20] against a Ag/AgCl reference electrode in pH 7.4 phosphate buffered saline at 37 °C (Table III). The half-wave potential (defined as the potential required for half-maximal oxidation under the experimental conditions) for these compounds were within 80 mV of one another, and all exhibited irreversible behavior. Thus, there do not appear to be dramatic electrochemical differences between short- and long-chain derivatives within this structural series. A representative voltammogram appears in Figure 1.

The three homologous series of benzylamines summarized in Chart I were evaluated as peroxyl radical scavengers in homogeneous aqueous solution using an assay described by Glazer for measuring the ability of compounds to block 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) induced decay of (*R*)-phycoerythrin (RPE) fluorescence emission under conditions where peroxyl radical formation is rate limiting (Table IV).[21] At a test agent concentration of 1 μM, compounds in series A scavenged peroxyl radicals in a structure dependent manner with lower homologues exhibiting greater activity (Figure 2).

Table II. Compound Numbering and Physical Data for Final Benzylamines[a]



no.	series	amine	R^1	R^2	R^3	n	mp, °C	yield,[b] %	recryst solvent[c]	formula[d]
16	A	$N(Me)_2$	ethyl	H	ethyl	1	192–195 dec	74		$C_{13}H_{22}NO_3Cl$
17	A	$N(Me)_2$	*n*-propyl	H	*n*-propyl	1	200–200.5 dec	72		$C_{15}H_{26}NO_3Cl$
18	A	$N(Me)_2$	*n*-butyl	H	*n*-butyl	1	163–164 dec	73		$C_{17}H_{30}NO_3Cl$
19	A	$N(Me)_2$	*n*-pentyl	H	*n*-pentyl	1	103–105	83		$C_{19}H_{34}NO_3Cl$
20	A	$N(Me)_2$	*n*-hexyl	H	*n*-hexyl	1	90–91	77		$C_{21}H_{38}NO_3Cl$
21	A	$N(Me)_2$	*n*-heptyl	H	*n*-heptyl	1	91–92	80		$C_{23}H_{42}NO_3Cl$
22	A	$N(Me)_2$	isopropyl	H	isopropyl	1	180–181 dec	67		$C_{15}H_{26}NO_3Cl$
23	A	$N(Me)_2$	cyclopentyl	H	cyclopentyl	1	205–207 dec	82		$C_{19}H_{30}NO_3Cl$
24	A	$N(Me)_2$	3-pentyl	H	3-pentyl	1	189–190 dec	79		$C_{19}H_{34}NO_3Cl$
25[a]		$N(Me)_2$	*n*-pentyl	H	*n*-pentyl	1	99–100			$C_{20}H_{37}NO_6S$
31	A,B	$N(Me)_2$	methyl	H	methyl	1	220–223	86		$C_{11}H_{18}NO_3Cl$
32	B	$N(Et)_2$	methyl	H	methyl	1	192–193	28		$C_{13}H_{22}NO_3Cl$
33	B	$N(n\text{-}Pr)_2$	methyl	H	methyl	1	131–132	56	I	$C_{15}H_{26}NO_3Cl$
34	B	$N(n\text{-}Bu)_2$	methyl	H	methyl	1	95–97	23	I	$C_{17}H_{30}NO_3Cl$
35	B	$N(n\text{-}Pent)_2$	methyl	H	methyl	1	87–91	27		$C_{19}H_{34}NO_3Cl$
36	B	$N(i\text{-}Bu)_2$	methyl	H	methyl	1	145–146	3	II	$C_{17}H_{30}NO_3Cl$
37	C	HN(Me)	methyl	H	methyl	1	191–192	37[e]	III	$C_{10}H_{16}NO_3Cl$
38	C	HN(Et)	methyl	H	methyl	1	210–211	47	IV	$C_{11}H_{18}NO_3Cl$
39	C	HN(*n*-Pr)	methyl	H	methyl	1	192–194	35	I	$C_{12}H_{20}NO_3Cl$
40	C	HN(*n*-Bu)	methyl	H	methyl	1	188–190 dec	29[f]	I	$C_{13}H_{22}NO_3Cl$
41	C	HN(*n*-Pent)	methyl	H	methyl	1	183–186	41[f]	IV	$C_{14}H_{24}NO_3Cl$
42	C	HN(*n*-Hex)	methyl	H	methyl	1	159–160	17	I	$C_{15}H_{26}NO_3Cl$
43	C	HN(*n*-Hept)	methyl	H	methyl	1	158–160	13	IV	$C_{16}H_{28}NO_3Cl$
44	C	HN(*n*-Oct)	methyl	H	methyl	1	162–163	17	V	$C_{17}H_{30}NO_3Cl$
45	C	HN(*n*-Non)	methyl	H	methyl	1	157–158	28[f]	V	$C_{18}H_{32}NO_3Cl$
48		HN(*n*-Pent)	isopropyl	H	isopropyl	1	120–122			$C_{18}H_{32}NO_3Cl$
49		HN(*n*-Hept)	isopropyl	H	isopropyl	1	133–134			$C_{20}H_{36}NO_3Cl$
53		NMe_2	H	*n*-heptyl	H	1	132–133			$C_{16}H_{28}NO_3Cl$
54		HEP[d]	H	*n*-heptyl	H	2	227–231			$C_{20}H_{36}N_2O_4Cl_2$
59		HEP[c]	isopropyl	H	isopropyl	2	192–193			$C_{19}H_{34}N_2O_4Cl_2$
60		NMe_2	*n*-heptyl	H	methyl	1	147–148			$C_{17}H_{30}NO_3Cl$
61		HEP[g]	*n*-heptyl	H	methyl	2	131–153			$C_{21}H_{38}N_2O_4Cl_2$

[a] All benzylamines were isolated and characterized as hydrochloride salts except for **25** (mesylate salt). [b] Yield for analogues **16–24** are for two steps starting with intermediates **7–15**, respectively. Yield for compounds **31–45** are based on 2,6-dimethoxyphenol. [c] Recrystallization solvents are as follows: (I) EtOAc/MeOH; (II) EtOAc/hexanes; (III) 2-PrOH/MeOH; (IV) EtOAc/EtOH; (V) EtOAc/2-PrOH. [d] Satisfactory C, H, N combustion analysis (±0.4%) were obtained for all final compounds. [e] Excess formaldehyde and amine (10 mol % and 5-fold, respectively) were used. See Scheme II and the Experimental Section. [f] Excess amine (4 to 5-fold) was used. See Scheme II and the Experimental Section. [g] 1-(2-Hydroxyethyl)piperazine.

Table III. Oxidation Potential of Compounds **31** and **16–21** (Series A) Determined by Cyclic Voltammetry[a]

compd	R	half-wave potential (mV)
31	methyl	280
16	ethyl	240
17	*n*-propyl	220
18	*n*-butyl	210
19	*n*-pentyl	200
20	*n*-hexyl	210
21	*n*-heptyl	–[b]

[a] Measurements were conducted using a carbon paste disk electrode against a Ag/AgCl reference electrode in pH 7.4 phosphate buffered saline at 37 °C. The initial potential was –0.1 V vs the Ag/AgCl reference with a scan rate of 10 mV/s in a positive direction. At approximately 500 mV, the scan was reversed. A new electrode surface was used for each compound. [b] Compound **21** was not sufficiently soluble in phosphate buffer solution for testing.

All of the compounds within series B and C, on the other hand, were approximately equipotent to one another. Since there were no apparent differences in oxidation potential measured electrochemically, the structure-dependent radical scavenging behavior for series A may be attributed to changes in steric environment around the reactive phenolic hydroxyl group with a kinetic preference for smaller alkoxyl substituents. Consistent with this hypothesis, the nonsymmetric benzylamines (e.g. **60**) exhibited efficient radical scavenging activity, suggesting that one but not two bulky flanking groups may be tolerated.

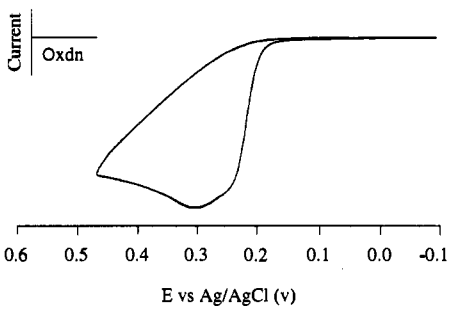


Figure 1. Cyclic voltammogram of compound **17**. Measurements were conducted using a carbon paste disk electrode against a Ag/AgCl reference electrode in pH 7.4 phosphate buffered saline at 37 °C. The initial potential was –0.1 V vs the Ag/AgCl reference with a scan rate of 10 mV/s in a positive direction. At approximately 500 mV, the scan was reversed. A new electrode surface was used for each compound.

The ability of these compounds to inhibit iron-dependent lipid peroxidation was determined using rabbit brain vesicular membrane lipids (Table IV). However, in contrast to the AAPH assay results, congeners with short alkoxyl groups were ineffective (MIC > 100 μM).[22] Since increasing chain length and clogP were both associated with greater inhibitory activity (eq 1), free-radical scavenging in this system may occur in the lipid phase or at the lipid–aqueous interface.

Table IV. Activity of Final Benzylamines

no.	AAPH (% inhib)[a]	lipid peroxidation[b]	cytoprotection (% cell viability)[c]	MAP effects (ED_{20}, mg/kg)[d]
16	71.6	100	14.7 ± 4.4	no effect
17	91.8	30	31.3 ± 3.8	1 mg/kg
18	73.3	10	35.8 ± 2.2	1 mg/kg
19	44.9	2.3	51.2 ± 4.7	NT[e]
20	17.6	1	73.1 ± 5.1	0.36 mg/kg
21	7.2	1	75.7 ± 5.1	not saline soluble
22	69.3	>100	35.8 ± 4.2	no effect
23	74.3	10	28.9 ± 3.7	study[f]
24	66.8	10	15.2 ± 5.2	0.42 mg/kg
25	NT[e]	NT[e]	NT[e]	study[g]
31	69.0	>100	5.2 ± 0.7	no effect
32	66.7	>100	3.7 ± 1.7	no effect
33	63.7	>100	5.4 ± 1.8	no effect
34	66.6	>100	26.4 ± 5.0	no effect
35	50.6	30	35.5 ± 4.2	>1 mg/kg
36	63.7	30	28.7 ± 1.3	no effect
37	75.8	>100	7.5 ± 3.6	no effect
38	71.8	>100	3.1 ± 3.3	no effect
39	80.1	>100	13.3 ± 1.7	no effect
40	62.4	>100	17.1 ± 5.1	no effect
41	61.6	>100	29.0 ± 2.8	no effect
42	65.2	100	42.6 ± 5.2	no effect
43	63.5	30	48.3 ± 2.3	no effect
44	60.9	10	59.8 ± 4.3	pressor[h]
45	52.9	5.3	47.1 ± 5.2	pressor[h]
48	NT[e]	30	42.8 ± 7.0	>1 mg/kg
49	NT[e]	10	43.5 ± 7.4	>1 mg/kg
53	43.8	30	47.0 ± 6.0	study[i]
54	60.3	30	50.3 ± 7.5	NT[e]
59	71.6	>100	45.3 ± 8.5	no effect
60	62.5	2.3	44.0 ± 5.3	>1 mg/kg
61	64.4	2.3	63.0 ± 7.2	>1 mg/kg
propyl gallate	40.0	3	NT[e]	NT[e]

[a] Percent inhibition of 2,2′-azobis(2-amidinopropane)dihydrochloride induced decay of (*R*)-phycoerythrin fluorescence emission in pH 7.0 phosphate buffer at 37 °C (15-min time point, $n = 4$). [b] Inhibition of iron-dependent peroxidation of rabbit vesicular membrane lipids expressed as an MIC (minimum tested concentration of test agent that gave ≥50% inhibition, μM, $n = 3$). [c] Percent viability ($n = 3$, mean ± SD) of rat hippocampal neurons exposed to H_2O_2 (50 μM) in the presence of test agent (5 μM). [d] Hypotensive effect in anesthetized normotensive rats. Compounds were evaluated at 1, 10, 100, and 1000 μg/kg iv using saline as vehicle. [e] Not tested. [f] Hemodynamic study conducted (see Figure 5). [g] Hemodynamic study conducted (see Figure 4). [h] No effect up to 0.1 mg/kg, slight pressor response (~16–17%) at 1 mg/kg. [i] Hemodynamic study conducted (see Figure 6).

$$-\log \text{MIC (series A, B, C)} = 0.43(\pm 0.06)\text{clogP} - 3.06(\pm 0.26) \quad (1)$$

$$n = 14 \quad r = 0.91 \quad F = 55.17 \quad p < 0.001$$

Cytoprotective activity (5 μM) was evaluated using primary cultures of rat hippocampal neurons exposed to hydrogen peroxide (50 μM) where cell survival was assessed by vital dye staining 18 h later. As summarized in Table IV, there is an apparent relationship between alkyl chain length and activity for compounds **16–24** (series A), **31–36** (series B), and **37–45** (series C) that favors derivatives with longer, more lipophilic appendages as indexed by clogP (eq 2). This parameter, however, is a global term

$$\%\ \text{cell viability (series A, B, C)} = 10.51(\pm 1.42)\text{clogP} - 4.71(\pm 5.31) \quad (2)$$

$$n = 24 \quad r = 0.85 \quad F = 55.04 \quad p < 0.001$$

that describes lipophilicity of the entire molecule. We therefore correlated properties of individual substituents with cytoprotection using values representing substituent bulk (MR), length (L1), and width (B4). Using these terms, regression analysis of series A (compounds **31**, **16–23**)[23] gave significant correlations as follows (eqs 3a–c):

$$\%\ \text{cell viability (series A)} = 2.59(0.44)\text{MR} - 16.28(9.15) \quad (3a)$$

$$n = 8 \quad r = 0.92 \quad F = 34.69 \quad p = 0.001$$

$$\%\ \text{cell viability (series A)} = 10.60(\pm 1.49)\text{L1} - 31.47(\pm 10.39) \quad (3b)$$

$$n = 9 \quad r = 0.94 \quad F = 50.22 \quad p < 0.001$$

$$\%\ \text{cell viability (series A)} = 15.99(\pm 1.80)\text{B4} - 35.62(\pm 8.74) \quad (3c)$$

$$n = 9 \quad r = 0.96 \quad F = 79.22 \quad p < 0.001$$

In a similar manner, series B (compounds **31–36**) and C (compounds **37–45**) were evaluated. However, since the number of published substituent parameters for the substructure $CH_2NR(R')$ was extremely limited, we used parameters for a C1–C9 alkyl chain assuming the contribution of the $-CH_2N-$ linker to remain constant. Thus, regression analysis of these two series provided significant correlations as follows (eqs 4a–c and 5a–c):[24]

$$\%\ \text{cell viability (series B)} = 1.88(\pm 0.45)\text{MR} - 12.02(\pm 7.68) \quad (4a)$$

$$n = 6 \quad r = 0.90 \quad F = 17.09 \quad p < 0.05$$

$$\%\ \text{cell viability (series B)} = 8.06(\pm 2.79)\text{L1} - 23.46(\pm 14.67) \quad (4b)$$

$$n = 6 \quad r = 0.82 \quad F = 8.33 \quad p < 0.05$$

$$\%\ \text{cell viability (series B)} = 11.95(\pm 3.05)\text{B4} - 26.47(\pm 11.61) \quad (4c)$$

$$n = 6 \quad r = 0.89 \quad F = 15.32 \quad p < 0.05$$

$$\%\ \text{cell viability (series C)} = 1.52(\pm 0.20)\text{MR} - 7.09(\pm 5.33) \quad (5a)$$

$$n = 9 \quad r = 0.95 \quad F = 59.36 \quad p < 0.001$$

$$\%\ \text{cell viability (series C)} = 7.96(\pm 0.79)\text{L1} - 25.28(\pm 5.56) \quad (5b)$$

$$n = 8 \quad r = 0.97 \quad F = 101.98 \quad p < 0.001$$

$$\%\ \text{cell viability (series C)} = 11.12(\pm 1.18)\text{B4} - 24.47(\pm 5.87) \quad (5c)$$

$$n = 8 \quad r = 0.97 \quad F = 88.87 \quad p < 0.001$$

Since membrane stabilization is more dependent upon substituent length rather than branching for antioxidants related to α-tocopherol, we plotted clogP against cytoprotective activity for the three homologous series and treated straight and branched/cyclic members separately

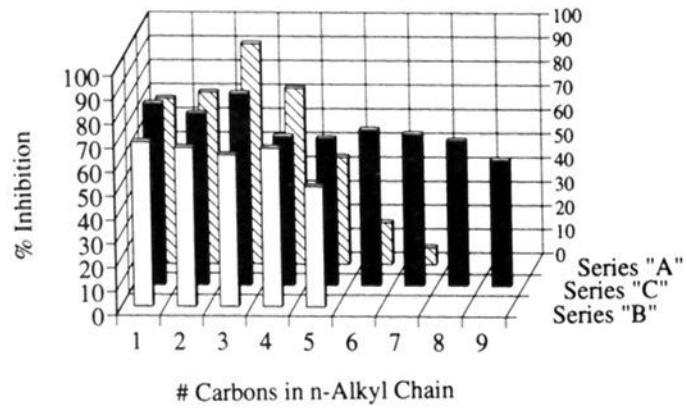


Figure 2. Effect of compounds **31, 16–21** (series A), **31–35** (series B), and **37–45** (series C) on (R)-phycoerythrin (R-PE) fluorescence emission (λ_{ex} = 544 nm, λ_{em} = 590 nm) decay induced by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) in phosphate buffer (pH 7.0) at 37 °C. Average relative fluorescence of each sample (normalized to fluorescence at 0 min) was calculated and percent inhibition at 15 min (n = 4) was determined.

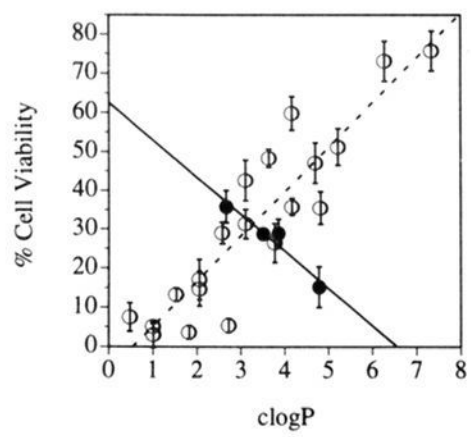


Figure 3. *Relationship between clogP and protection of rat* hippocampal neurons in vitro for compounds **16–24** (series A), **31–36** (series B), and **37–45** (series C). Linear regression lines for branched/cyclic (solid) and *n*-alkyl (dashed) derivatives were calculated separately. Each analogue was tested at n = 3 with primary cultures prepared on different days. Each point represents the mean ± SD.

in the regression analysis (Figure 3). Interestingly, two nearly orthogonal regression lines were obtained. While the number of branched examples is small, the data raises the possibility that lipophilic straight chain derivatives may be superior as cytoprotective agents relative to their branched counterparts.

Unfortunately, while longer *n*-alkyl chains may be associated with enhanced cytoprotective activity in vitro, they are also associated with increased hypotensive liability when the compounds were administered intravenously to anesthetized normotensive rats (Table IV).[25] To gain direction in a structure–activity investigation for minimizing cardiovascular effects, hemodynamic studies with compounds **25, 23,** and **53** were conducted. As illustrated in Figure 4, compound **25** lowered mean arterial pressure (MAP) in a dose-dependent manner when administered by intravenous infusion over 10 min. The hypotensive action appears to be primarily related to a reduction in cardiac output (ABFI) since calculated systemic vascular resistance (SVR) remained unchanged. There was also a marked dose-related drop in heart rate (HR) that may account for the increased stroke volume (SVI) despite a decline in myocardial contractility as indexed by dP/dt_{max}.

The structurally isomeric analogue **23** also induced hypotension following intravenous administration (Figure 5). However, in contrast to **25**, this effect may be attributed to a reduction in both cardiac output and systemic vascular resistance, although only the latter was statistically significant. The higher dose of 10 mg/kg could not be evaluated due to toxicity. Thus, alterations in hemodynamic and cardiac function parameters appear to be highly structure dependent since closely related isomers **25** and **23** induce effects that differ qualitatively as well as quantitatively.

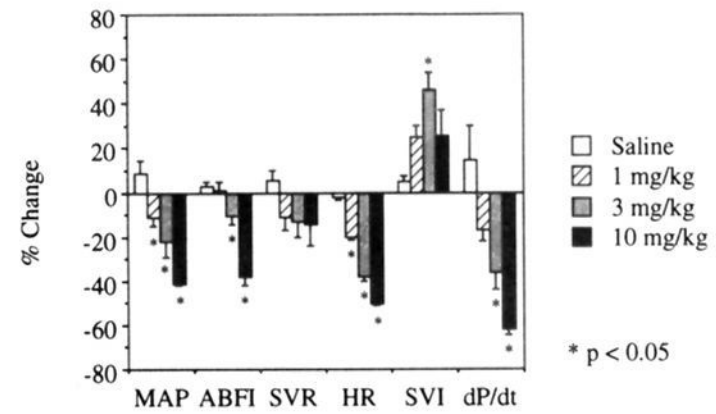


Figure 4. Peak hemodynamic alterations in anesthetized normotensive rats induced by compound **25.** Responses are expressed as percent change from the respective control value recorded just prior to infusion. Abbreviations are as follows: Aortic blood flow index (ABFI), systemic vascular resistance (SVR), heart rate (HR), stroke volume index (SVI). Each point represents the mean ± SE (n = 4–6).

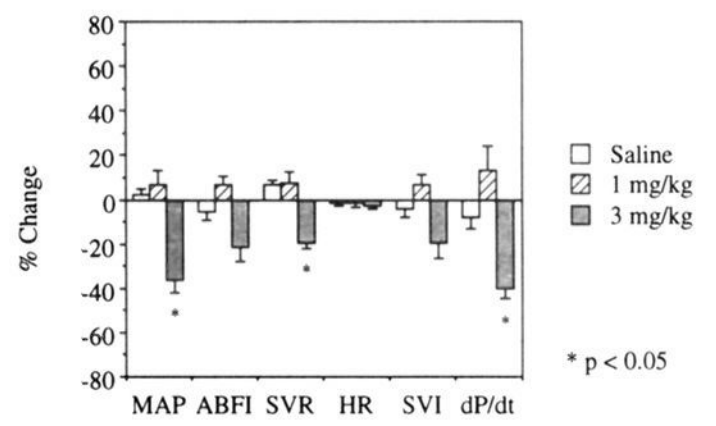


Figure 5. Peak hemodynamic alterations in anesthetized normotensive rats induced by compound **23.** Responses are expressed as percent change from the respective control value recorded just prior to infusion. Abbreviations are as follows: Aortic blood flow index (ABFI), systemic vascular resistance (SVR), heart rate (HR), stroke volume index (SVI). Each point represents the mean ± SE (n = 4–6).

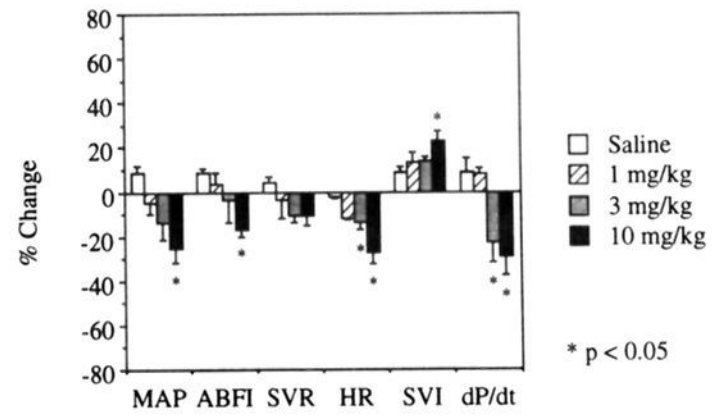


Figure 6. Peak hemodynamic alterations in anesthetized normotensive rats induced by compound **53.** Responses are expressed as percent change from the respective control value recorded just prior to infusion. Abbreviations are as follows: Aortic blood flow index (ABFI), systemic vascular resistance (SVR), heart rate (HR), stroke volume index (SVI). Each point represents the mean ± SE (n = 4–6).

The relationship between phenol structure and cardiovascular liability was further investigated using compound **53** (Figure 6). In this case, effects on measured and calculated hemodynamic parameters qualitatively mirrored those observed for compound **25.** However, statistical significance was achieved only at higher doses, suggesting that a 3,5-dihydroxyl congener may carry less hypotensive risk relative to a 3,5-dialkoxyl derivative.

Discussion

Structure–activity studies with α-tocopherol derivatives suggest that modifications in either the chroman nucleus or the C2 alkyl substituent influence vitamin E activity in the rat curative myopathy assay.[10,16] Since potency in this test system is related to in vitro lipid peroxidation inhibitory activity, structural perturbations may influence efficacy by altering oxidation potential and/or physicochemical properties (e.g. lipophilicity) important for lipid membrane interactions such as solubility, mobility, or distribution in a lipid bilayer. Under specific conditions membrane stabilization by lipophilic agents at relatively high concentrations can be achieved independent of antioxidant effects, and these results offer the possibility for a second cooperative mechanism by which lipophilic antioxidants can protect phospholipid membranes.[26]

A correlation between oxidation potential and molecular lipophilicity with iron-dependent lipid peroxidation in vitro has been reported for a series of dibenzo[1,4]-dichalcogenines (rat liver microsomes and hepatocytes)[27] and for antioxidants related to ascorbic acid (rat liver microsomes).[28,29] However, while lipophilicity represents an important parameter for relating structure with in vitro efficacy, hydrophilic antioxidants may also function as effective lipid peroxidation inhibitors provided they concentrate at the site of action. For example, a hydrophilic analogue of α-tocopherol (MDL-74270) reportedly prevents lipid peroxidation in vitro and decreases myocardial infarct size in anesthetized rats following coronary artery occlusion and reperfusion.[30,31] Thus, chemical structure and cytoprotective efficacy may be related by (1) antioxidant capacity/efficiency, (2) physicochemical properties that favor concentration in the lipid membrane or site of action, and (3) physical interactions that stabilize biomembranes independent of antioxidant mechanisms.

With respect to the described phenols exemplified by generic structure **2**, the structural requirements for efficient radical trapping in homogeneous solution differed from those for effective lipid peroxidation inhibition. In homogeneous solution a kinetic preference existed for smaller, less sterically encumbered substituents flanking the reactive phenolic hydroxyl group. Longer alkyl substituents on the amine group, on the other hand, had little if any effect on scavenging activity in this system. Since no dramatic differences in oxidation potential were observed electrochemically, the relationship between antioxidant structure and efficacy under these conditions is a reflection of steric rather than electronic factors. This observation is consistent with previous studies of phenols where bulky alkyl substituents ortho to the hydroxyl group diminished radical chain-breaking efficiency.[32,33] Consequently, a lipophilic alkoxyl substituent at C3 and a small substituent at C5 appeared optimal for efficient scavenging activity in both lipid and homogeneous solution. That only one sterically encumbered substituent ortho to the phenolic hydroxyl group is acceptable and not detrimental to membrane antiperoxidative activity has also been reported for chroman derivatives.[34]

Although these compounds could potentially block iron-dependent lipid peroxidation by scavenging the initiating radical species in aqueous solution, the statistically significant correlation between clogP and lipid peroxidation inhibitory activity supports their role as radical chain-breaking antioxidants in the lipid phase or lipid–aqueous interface. In contrast, chroman compounds

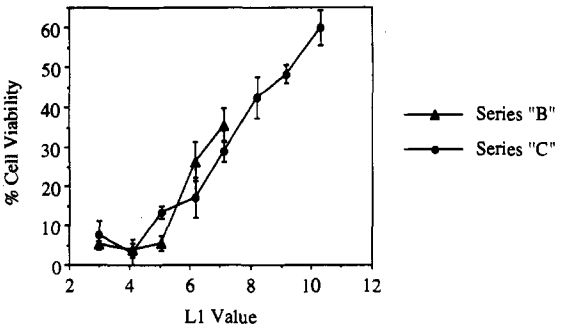


Figure 7. Relationship between substituent alkyl chain length (L1) and protection of rat hippocampal neurons exposed to hydrogen peroxide. Compounds **31–35** (series B) and **37–44** (series C) are shown. Each analogue was tested at $n = 3$ with primary cultures prepared on different days. Each point represents the mean ± SD.

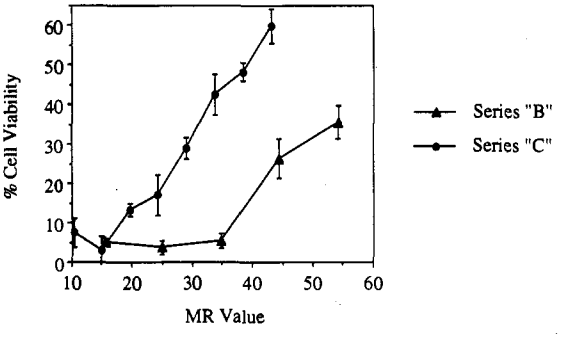
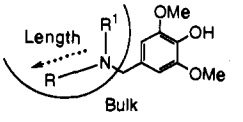


Figure 8. Relationship between amine substituent bulk (MR) and protection of rat hippocampal neurons exposed to hydrogen peroxide. Compounds **31–35** (series B) and **37–44** (series C) are shown. Each analogue was tested at $n = 3$ with primary cultures prepared on different days. Each point represents the mean ± SD.

related to α-tocopherol with hydrophobic side chains are reportedly less effective antiperoxidants in vitro than corresponding derivatives with shorter side chains.[34,35] These differences may be a function of the assay system and/or nature of the free-radical generator rather than a reflection of fundamentally different antiperoxidant mechanisms.

Maximal cytoprotection was also associated with more lipophilic derivatives. However, as illustrated in Figures 7 and 8, substituent length and substituent bulk (see **62**) as indexed by L1 and MR, respectively,[36] may represent independent parameters relating structure and efficacy in this system. Analogues in series B (e.g. **35**) and C (e.g.



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41) with an equal number of carbons in each individual R group (see Chart I) exhibited equivalent cytoprotective activity irrespective of the number of R groups attached to the nitrogen atom (Figure 7). Thus, members in series C were more potent than the corresponding derivatives in series B with approximately equivalent MR values (Figure 8), suggesting that substituent length may be more important than substituent bulk for cytoprotective activity. This qualitative trend is consistent with the regression analysis illustrated in Figure 3 for branched/cyclic derivatives. Thus, one interpretation is that bulky

(e.g. branched/cyclic) substituents may partially offset a protective effect by increasing membrane disordering relative to straight-chain derivatives. Conversely, the latter analogues may be more effective than the former in favorably altering the physical properties of phospholipid membranes to provide increased stability during oxidant exposure. Further studies may help address these possibilities since membrane perturbations by lipophilic agents are structure dependent and not necessarily related to octanol/water partition coefficients.[37]

Computer modeling and NMR studies suggest that *N*-alkylbenzylamines bind to phospholipid membranes in a structure dependent manner where a change in bound conformation occurs with analogues possessing an *N*-alkyl chain length greater than 5.[38] The benzylamines in the present study may have undergone a similar change in bound conformation with increasing alkyl chain length. However, as illustrated in Figure 7 cytoprotection and substituent length are linearly related beyond a threshold substituent L1 value. Thus, if a change in bound conformation did indeed occur with higher homologues, then it did not significantly impact cytoprotective efficacy as measured by cell viability.

From a mechanistic standpoint, acute exposure (15 min) of neuronal cultures to hydrogen peroxide (50 μM) induced few immediate morphological changes,[39] but in the ensuing 18-h period led to widespread degeneration that was partially blocked by these agents. Lipid peroxidation, however, represents only one possible outcome of oxidant-induced damage since hydrogen peroxide can penetrate cell membranes and potentially disrupt cellular metabolism by modifying and inactivating proteins.[40] Although these compounds enhanced cell survival, they did not completely block the effects of hydrogen peroxide exposure and therefore may not address events preceding membrane peroxidation. Thus, structurally modifying these compounds to allow blockade of earlier destructive processes during oxidative stress in addition to membrane protection and stabilization may ultimately furnish superior cytoprotective agents.

Experimental Section

Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded with a GE QE-300 and were consistent with the assigned structure. Mass spectra were recorded with a CEC 21-110 (EI) or with a Varian-MAT 731 (FD) spectrometer. SiO_2 (70–230 mesh) was used in all gravity column chromatographic separations. Medium-pressure liquid chromatography (MPLC) was performed with a Waters Prep 500 instrument using SiO_2 columns. Microanalytical data were provided by the Physical Chemistry Department of Lilly Research Laboratories. Where analyses are indicated only by symbols of the elements, results obtained were within ±0.4% of the theoretical values.

3,5-Dihydroxy-4-(phenylmethoxy)benzoic Acid Methyl Ester (4). NaH (50% oil dispersion, 8.00 g, 200 mmol) was washed with hexanes, suspended in THF (150 mL), and cooled to 0 °C under N_2. A solution of methyl gallate (36.46 g, 198 mmol) in THF (50 mL) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 10 min to give a clear green solution. Benzyl bromide (33.86 g, 198 mmol) in THF (50 mL) was rapidly added, and after being stirred for 16 h, the mixture was heated to reflux for 1 h, cooled, and concentrated under reduced pressure. The brown residue was suspended in EtOAc and washed three times with H_2O. The aqueous layers were combined and back-extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to leave a brown oily residue. This material was filtered through a pad of SiO_2 with 10% EtOAc/CH_2Cl_2 and concentrated under reduced pressure. The resulting yellow solid was dissolved in a minimum amount of hot EtOAc, and hexanes was added to the cloud point. After standing at room temperature, the desired product was collected by suction filtration to furnish 20.45 g of **4** as a white solid, mp 132–133.5 °C. Anal. ($C_{15}H_{14}O_5$) C, H, N. A second crop was obtained to give an additional 8.45 g, mp 132–133.5 °C.

3,5-Dihydroxy-4-(phenylmethoxy)benzoic Acid (5). A mixture of methyl 4-(benzyloxy)-3,5-dihydroxybenzoate (26.51 g, 96.7 mmol), 5 N NaOH (64 mL, 320 mmol), and MeOH (75 mL) was stirred at reflux under Ar for 2 h. The reaction mixture was cooled, concentrated under reduced pressure, and acidified with 5 N HCl (75 mL). After extracting with EtOAc, the organic layer was washed with brine, dried over Na_2SO_4, filtered through a pad of SiO_2, and concentrated in vacuo to furnish 24.72 g of **5** as a light tan solid. About 700 mg was triturated with Et_2O/hexanes and collected by suction filtration to give an analytically pure sample, mp 169.5–170.5 °C. Anal. ($C_{14}H_{12}O_5$) C, H, N.

3,5-Dihydroxy-*N*,*N*-dimethyl-4-(phenylmethoxy)benzamide (6). A mixture of **5** (23.99 g, 92.2 mmol), Ac_2O (150 mL), and pyridine (150 mL) was stirred at room temperature for 2.5 h. The volatiles were removed in vacuo, and the residue was concentrated two times from toluene under reduced pressure. $SOCl_2$ (100 mL) was added, and the mixture was allowed to stand at room temperature for 1.5 h. After removing excess $SOCl_2$ in vacuo, the intermediate acid chloride was dissolved in DMF (50 mL), added to a mixture of Me_2NH (40% aqueous solution, 52 g) and THF (200 mL) at 0 °C under N_2, and then stirred for 2 days at room temperature. Concentration under reduced pressure furnished the desired product. However, since at this scale not all of the phenolic acetate groups were removed by the excess Me_2NH, the product was fully deprotected by stirring in refluxing MeOH (100 mL) and K_2CO_3 under N_2 for 1 h. After solvent removal, the mixture was suspended in EtOAc, washed with 1 N HCl, saturated $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting solid was triturated with EtOAc/hexanes and collected by suction filtration to provide 19.14 g of **6** as an off-white solid, mp 169–170 °C. Anal. ($C_{16}H_{17}NO_4$) C, H, N.

3,5-Diethoxy-*N*,*N*-dimethyl-4-(phenylmethoxy)benzamide (7). The following is a representative experimental procedure for preparing compounds **7–15**: A mixture of **6** (2.00 g, 7 mmol), K_2CO_3 (4.81 g, 35 mmol), iodoethane (3.26 g, 21 mmol), and acetone (40 mL) was stirred at reflux under Ar for 22 h. The reaction mixture was cooled, filtered, and concentrated under reduced pressure. The resulting product was taken up in EtOAc, washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. Recrystallization from EtOAc/hexanes furnished 1.62 g of **7** as a white solid, mp 92–93.5 °C. Anal. ($C_{20}H_{25}NO_4$) C, H, N.

4-[(Dimethylamino)methyl]-2,6-diethoxyphenol Hydrochloride (16). The following is a representative experimental procedure for preparing compounds **16–25**: A mixture of intermediate amide **7** (2.20 g, 6.41 mmol) and THF (25 mL) was added dropwise to a suspension of LAH (486 mg, 12.8 mmol) and THF (25 mL) at 0 °C under N_2. The mixture was stirred at room temperature for 1 h and then at reflux for 1 h. After cooling to 0 °C, the excess hydride was destroyed by sequentially adding H_2O, 15% NaOH, and H_2O, and the salts were removed by filtration. Solvent removal in vacuo provided 2.78 g of the intermediate benzylamine as a light brown oil that was immediately dissolved in 95% EtOH (50 mL) and hydrogenated for 3 h over 10% Pd/C (210 mg) at atmospheric pressure. After the reaction mixture was filtered and concentrated under reduced pressure, the product was dissolved in Et_2O and washed with H_2O and brine. The ethereal solution was dried over Na_2SO_4, and HCl gas was passed over the surface of the solution. Suction filtration afforded 1.30 g (74% for two steps) of **16** as a white solid, mp 192–195 °C dec. Anal. ($C_{13}H_{22}NO_3Cl$) C, H, N.

4-[(Dimethylamino)methyl]-2,6-dimethoxyphenol Hydrochloride (31). The following is a representative experimental procedure for preparing compounds **31–45**: A solution of 2,6-dimethoxyphenol (19.8 g, 130 mmol), Me_2NH (40% aqueous solution, 13.7 g), CH_2O (38% aqueous solution, 10.1 g), and MeOH (110 mL) was stirred at reflux under N_2 for 2 h. After cooling

and solvent removal, the residue was concentrated two times from toluene. The resulting dark oily residue was dissolved in EtOAc, and HCl gas was passed over the surface of the solution. The solid was collected by suction filtration and suspended in boiling EtOAc. After cooling, the product was again collected by suction filtration to furnish 27.3 g (86%) of **31** as a white powder, mp 220–223 °C dec. Anal. ($C_{11}H_{18}NO_3Cl$). Variations from this general procedure for individual analogues are summarized in Table II.

4,4'-[(Nonylimino)bis(methylene)]bis[2,6-dimethoxyphenol] Hydrochloride (47). *n*-Nonylamine (19.0 g, 133 mmol) under the general conditions described above (except using a 4-fold excess of amine) furnished 6.57 g and 2.36 g of compounds **45** (more polar component) and **47** (less polar component) free base, respectively, following column chromatography (20% EtOAc/hexanes, 30% EtOAc/hexanes, and finally 50% EtOAc/hexanes). Undesired hydrochloride salt **47** was isolated as a white solid, mp 151–152 °C. Anal. ($C_{27}H_{42}NO_6Cl$) C, H, N.

2-(Phenylmethoxy)-1,3-benzenediol (27). NaH (60% oil dispersion, 15.9 g, 400 mmol) was washed with hexanes, suspended in DMF (200 mL), and cooled in an ice bath. A solution of pyrogallol (50.0 g, 400 mmol) in DMF (150 mL) was added dropwise. When gas evolution ceased, benzyl bromide (68.0 g, 400 mmol) was added, and the reaction mixture was allowed to warm to room temperature. After 16 h, the solvent was removed in vacuo, and the residue was dissolved in EtOAc and H_2O. The aqueous layer was separated and extracted with EtOAc. The organic layers were combined, washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to leave a dark oil. Chromatography (MPLC, 5% EtOAc/hexanes followed by 10% EtOAc/hexanes) gave 29.06 g (33%) of **27** that was sufficiently pure for subsequent reactions. If desired, the product could be purified by short-path distillation, bp 175–179 °C (0.2 mmHg).

1,3-Bis(1-methylethoxy)-2-(phenylmethoxy)benzene (28). A mixture of compound **27** (15.5 g, 72 mmol), K_2CO_3 (19.8 g, 144 mmol), 2-iodopropane (24.4 g, 144 mmol), and acetone (300 mL) was stirred at reflux for 2 days. Additional K_2CO_3 (19 g) and 2-iodopropane (24.4 g) were added, and the reaction was allowed to proceed for 3 more days. After cooling, the mixture was concentrated under reduced pressure, and the resulting residue was partitioned between EtOAc and H_2O. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Chromatography (MPLC, hexanes followed by 2.5% EtOAc/hexanes) gave 17.94 g of **28** as a light yellow oil.

2,6-Bis(1-methylethoxy)phenol (29). A solution of compound **28** (16.8 g, 56 mmol) in MeOH (30 mL) was hydrogenated over 10% Pd/C (500 mg) at atmospheric pressure for 2 days. After removing the catalyst by filtration, the filtrate was concentrated in vacuo to furnish the crude phenol as an oil. Chromatography (MPLC, gradient elution: hexanes to 30% EtOAc/hexanes) gave 11.04 g of **29** as the more mobile product.

2,6-Bis(1-methylethoxy)-4-[(pentylamino)methyl]-phenol Hydrochloride (48). A mixture of compound **29** (5.0 g, 24 mmol), CH_2O (35% aqueous solution, 6.3 g), *n*-pentylamine (10.4 g, 120 mmol), and MeOH (50 mL) was stirred at reflux for 48 h. After solvent removal in vacuo, the product was chromatographed (MPLC, 1% EtOH/CH_2Cl_2 containing trace NH_4OH followed by 5% EtOH/CH_2Cl_2 containing NH_4OH) to give 2.8 g of an oil. The hydrochloride salt was formed by passing HCl gas over an EtOAc solution of the free base to furnish an oil that solidified upon trituration with Et_2O, mp 120–122 °C. Anal. ($C_{18}H_{32}NO_3Cl$) C, H, N.

2,6-Bis(1-methylethoxy)-4-[(heptylamino)methyl]-phenol Hydrochloride (49). In a fashion analogous to compound **48**, compound **29** (5.0 g, 24 mmol) and *n*-heptylamine (13.8 g, 120 mmol) furnished 3.83 g of free base after chromatography (MPLC, gradient elution: 1% EtOH/CH_2Cl_2 with trace NH_4OH to 5% EtOH/CH_2Cl_2 containing NH_4OH). Hydrochloride salt formation afforded 2.76 g of **49** as white crystals, mp 133–134 °C. Anal. ($C_{20}H_{36}NO_3Cl$) C, H, N.

4-(Heptyloxy)-3,5-dihydroxybenzoic Acid Methyl Ester (50). In a fashion analogous to compound **4**, methyl gallate (25.0 g, 140 mmol) was converted to 10.52 g of **50** as the less mobile major product after chromatography (MPLC, gradient elution: hexanes to 15% EtOAc/hexanes).

2-(Heptyloxy)-5-[(dimethylamino)methyl]-3-hydroxyphenol Hydrochloride (53). A mixture of compound **50** (10.5 g, 37.3 mmol), K_2CO_3 (25.8 g, 187 mmol), benzyl bromide (13.4 g, 78.3 mmol), and acetone (500 mL) was stirred at reflux for 16 h. After cooling, the solvent was removed in vacuo and the residue was dissolved in EtOAc and H_2O. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated under reduced pressure to leave 20.77 g of **51** that was saponified with 5 N NaOH (20 mL) in refluxing EtOH (150 mL). After 20 min, the solvent was removed and the residue was acidified with 5 N HCl (21 mL). Extraction with EtOAc and drying over Na_2SO_4 gave 15.13 g of the desired carboxylic acid.

The material so obtained (10 g, 22 mmol) was treated with $SOCl_2$ (25 mL), CH_2Cl_2 (25 mL), and DMF (0.5 mL). After stirring at room temperature for 3 h, the volatiles were removed in vacuo, and the residue was concentrated from toluene to furnish the corresponding acid chloride.

Half of this intermediate was dissolved in THF (50 mL), treated with methylamine (40% aqueous solution, 20 mL), and stirred for 30 min. After concentration under reduced pressure, the resulting amide was dissolved in EtOAc, washed with H_2O and brine, and dried over Na_2SO_4. Solvent removal afforded 5.6 g of **52**.

This amide (5.5 g, 11.6 mmol) was stirred with LAH (0.88 g, 23 mmol) in refluxing THF (30 mL) for 30 min. Excess hydride was destroyed by sequentially adding H_2O, 15% NaOH, and H_2O, and the salts were removed by filtration to give 5.07 g of the protected benzylamine.

The above product (5.0 g, 11 mmol) in EtOH (50 mL) was hydrogenated at atmospheric pressure over 10% Pd/C (500 mg) for 2 h. After the reaction mixture was filtered and concentrated under reduced pressure, the residue was dissolved in EtOAc, washed with H_2O and brine, and dried over Na_2SO_4. The hydrochloride salt was formed and crystallized from EtOAc/CH_2Cl_2. The solid so obtained was triturated with EtOAc and Et_2O to provide 2.74 g of **53** as a white solid, mp 132–133 °C. Anal. ($C_{16}H_{28}NO_3Cl$) C, H, N.

2-(Heptyloxy)-5-[[4-(2-hydroxyethyl)-1-piperazinyl]-methyl]-1,3-benzenediol Dihydrochloride (54). In a fashion analogous to **53**, the remaining half of the acid chloride prepared above was treated with 1-(2-hydroxyethyl)piperazine to finally yield 2.93 g of crude dihydrochloride salt. This material was triturated with hot THF and then hot acetone to furnish 2.67 g of **54** as a white solid, mp 227–231 °C. Anal. ($C_{20}H_{36}N_2O_4Cl_2$) C, H, N.

4-[[4-Hydroxy-3,5-bis(1-methylethoxy)phenyl]methyl]-1-piperzineethanol Dihydrochloride (59). In a fashion analogous to **7**, compound **4** (10.3 g, 37.4 mmol) and 2-iodopropane (25 g, 150 mmol) was converted to 15.6 g of **55c**. The ester was saponified with 1 N NaOH (70 mL) in refluxing EtOH (200 mL), and after 20 min, the solvent was removed. Acidification with 1 N HCl (70 mL), extraction with EtOAc, and drying over Na_2SO_4 gave 15.5 g of the desired carboxylic acid. The acid chloride was formed, coupled with 1-(2-hydroxyethyl)piperazine, and reduced with LAH in a fashion analogous to **53** to yield 16.73 g of protected benzylamine **58** ($R^1 = R^2$ = isopropyl). This material (8.1 g, 18.3 mmol) was hydrogenated as described for **53**, and the hydrochloride salt was formed and crystallized from hot EtOAc/MeOH. Recrystallization from EtOAc/MeOH afforded 2.89 g of **59** as a white solid, mp 192–193 °C. Anal. ($C_{19}H_{34}N_2O_4Cl_2$) C, H, N.

4-[(Dimethylamino)methyl]-2-(heptyloxy)-6-methoxyphenol Hydrochloride (60). In a fashion analogous to **7**, compound **4** (22.7 g, 82.7 mmol) and 1-iodoheptane (18.7 g, 82.7 mmol) were converted to 9.78 g of desired **55a** and 12.33 g of the undesired dialkylated product ($R^1 = R^2$ = heptyl; more mobile by TLC) following chromatography (MPLC, gradient elution: hexanes to 30% EtOAc/hexanes). The desired material (9.78 g, 32.6 mmol) was resubjected to the alkylation conditions in the presence of MeI (9.45 g, 65.2 mmol) to give 12.4 g of **55b** as an oil. Saponification with 5 N NaOH as described for **59** gave 8.51 g of the corresponding carboxylic acid **56** (R^1 = heptyl, R^2 = Me). An analytical sample was obtained by trituration with EtOAc/hexanes, mp 90–91 °C. Anal. ($C_{22}H_{28}O_5$) C, H, N. In a fashion analogous to **53**, the carboxylic acid was then converted to the protected benzylamine **58** (R^1 = heptyl, R^2 = Me). Hydrogenation and hydrochloride salt formation as described above furnished

2.13 g of **60** as a fluffy white solid, mp 147–148 °C. Anal. ($C_{17}H_{30}NO_3Cl$) C, H, N.

4-[[3-(Heptyloxy)-4-hydroxy-5-methoxyphenyl]methyl]-1-piperazineethanol Dihydrochloride (61). In a fashion analogous to **53**, carboxylic acid **56** (4.3 g, 11.3 mmol) where R^1 = heptyl, R^2 = Me provided 2.32 g of **61** after recrystallization from MeOH, mp 131–153 °C. Anal. ($C_{21}H_{38}N_2O_4Cl_2$) C, H, N.

Correlation Analysis. The dataset was composed of compounds **16–24** (series A), **31–36** (series B), and **37–45** (series C). The CLOGP program (Pomona College, Medchem Software, v. 3, 1984) was used to calculate octanol/water partition coefficients. Published aromatic substituent constants[41] for s, π, F, R, MR, and steric descriptors as defined by Verloop[42] (sterimol terms) were used in the regression analysis. Electronic, lipophilic, and sterimol values for only the alkyl portion of amine chains were used in calculations involving series B and C. The biological activity data and parameters used for QSAR as well as the correlation coefficient matrix of pertinent parameters are provided in the supplementary material. Regression analysis was performed on an Apple Macintosh IIcx using the program JMP, a statistical visualization program (SAS Institute, Inc.). In the equations, the numbers in parentheses are the 95% confidence intervals, n is the number of observations, r is the correlation coefficient, and F is the Fisher test for significance of the equation.

Cyclic Voltammetry. Cyclic voltammetry was carried out using a carbon paste disk electrode[20] against a Ag/AgCl reference electrode as previously described.[43]

Peroxyl Radical Scavenging. Inhibition of 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) induced decay of (*R*)-phycoerythrin (R-PE) fluorescence emission (λ_{ex} = 544 nm, λ_{em} = 590 nm) was measured by a modification of the method described by Glazer.[21] A 100-μL sample of standard R-PE solution (4.5×10^{-8} M solution of R-PE in 75 mM pH 7.0 phosphate buffer) and 100 μL of the test agent (3 μM in buffer) or buffer (AAPH control) were added to each well of a 96-well microtiter plate and equilibrated in a Fluoroskan II unit for 5 min at 37 °C. A 100-μL sample of an AAPH solution (12 mM in buffer) prepared immediately prior to use or buffer (control) was added, and the fluorescence in each well was measured immediately after addition of the AAPH (0 min) and then every 5 min thereafter for 25 min. The values were normalized to the fluorescence at 0 min, and the average relative fluorescence of each sample (n = 4) was calculated. The percent inhibition at 15 min was determined as follows: % inhibition = (SA − AAPH)/(CONTROL − AAPH) × 100 where SA = average relative fluorescence of the sample; AAPH = average relative fluorescence of the control in the presence of AAPH; and CONTROL = average relative fluorescence of the control in the absence of AAPH.

Lipid Peroxidation. Iron-dependent peroxidation of rabbit brain vesicular membrane lipids was assayed as previously described.[44]

Cytoprotection. H_2O_2 toxicity studies using primary cultures of rat hippocampal neurons prepared by modifications of the methods described by Banker and Cowan[45] and Novelli et al.[46] were performed as previously described.[43]

Arterial Blood Pressure and Hemodynamic Studies. Arterial blood pressure studies were conducted using adult male Sprague–Dawley rats (weighing approximately 300–380 g) as previously described.[47] Drug solutions were prepared fresh daily in saline in a dose volume of 1 mL/kg and injected intravenously. Group (n = 4–6) mean values ± SE were then calculated.

Hemodynamic studies were conducted using adult male Sprague–Dawley rats as previously described.[48] Drug solutions were prepared fresh daily in saline in a dose volume of 1 mL/kg and infused intravenously over 10 min. Hemodynamic alterations were recorded continuously during drug or saline infusion and for an additional 20 min. Responses were expressed as percent change from the respective control value recorded just prior to infusion. Group (n = 4–6) mean values ± SE were then calculated for saline and drug infused rats.

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Supplementary Material Available: Biological activity and parameters used in the QSAR analysis and correlation coefficient matrix of pertinent parameters (2 pages). Ordering information is given on any current masthead page.

References

(1) For recent reviews, see: (a) Halliwell, B.; Gutteridge, J. M. C.; Cross, C. E. Free Radicals, Antioxidants, and Human Disease: Where are We Now? *J. Lab. Clin. Med.* **1992**, *119*, 598–620. (b) Traystman, R. J.; Kirsch, J. R.; Koehler, R. C. Oxygen Radical Mechanisms of Brain Injury Following Ischemia and Reperfusion. *J. Appl. Physiol.* **1991**, *71*, 1185–1195. (c) Jesberger, J. A.; Richardson, J. S. Oxygen Free-Radicals and Brain-Dysfunction. *Int. J. Neuroscience* **1991**, *57*, 1–17. (d) Ikeda, Y.; Long, D. M. The Molecular Basis of Brain Injury and Brain Edema: The Role of Oxygen Free Radicals. *Neurosurgery* **1990**, *27*, 1–11. (e) Kontos, H. A. Oxygen Radicals in CNS Damage. *Chem. Biol. Interact.* **1989**, *72*, 229–255.

(2) See, for example: (a) Gutteridge, M. M. C.; Halliwell, B. The Measurement and Mechanism of Lipid Peroxidation in Biological Systems. *Trends Biochem. Sci.* **1990**, *15*, 129–135. (b) Braughler, J. M.; Hall, E. D. Central Nervous System Trauma and Stroke. I. Biochemical Considerations for Oxygen Radical Formation and Lipid Peroxidation. *Free Rad. Biol. Med.* **1989**, *6*, 289–301. (c) Hall, E. D.; Braughler, J. M. Central Nervous System Trauma and Stroke. II. Physiological and Pharmacological Evidence for Involvement of Oxygen Radicals and Lipid Peroxidation. *Free Rad. Biol. Med.* **1989**, *6*, 303–313.

(3) Hall, E. D.; Braughler, M. M.; Yonkers, P. A.; Smith, S. L.; Linseman, K. L.; Means, E. D.; Scherch, H. M.; Von Voigtlander, P. F.; Lahti, R. A.; Jacobsen, E. J. U-78517F: A Potent Inhibitor of Lipid Peroxidation with Activity in Experimental Brain Injury and Ischemia. *J. Pharmacol. Exp. Therap.* **1991**, *258*, 688–694.

(4) For an overview of structures, see: Yu, M. J.; Simpson, P. J. Treatment of Acute Myocardial Ischemia. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: New York, 1990; Vol. 25, pp 31–40.

(5) Campo, G. M.; Ioculano, M.; Avenoso, A.; Zingarelli, B.; Calandra, S.; Scuri, R.; Saitta, A.; Caputi, A. P. IRFI-016, A New Radical Scavenger, Limits Ischemic Damage Following Coronary Artery Occlusion in Rats. *Res. Commun. Chem. Pathol. Pharmacol.* **1992**, *76*, 287–303.

(6) Axford, Gatley, R. A.; Wilson, G. J. Reduction of Experimental Myocardial Infarct Size by Oral Administration of α-tocopherol. *Cardiovasc. Res.* **1991**, *25*, 89–92.

(7) For an overview of structures, see: Jacobsen, E. J.; McCall, J. M.; Panetta, J. A. Acute Ischemic and Traumatic Injury to the CNS. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: New York, 1990; Vol. 25, pp 31–40.

(8) Burton, G. W.; Ingold, K. U. Autooxidation of Biological Molecules. 1. The Antioxidant Activity of Vitamin E and Related Chain-Breaking Phenolic Antioxidants in Vitro. *J. Am. Chem. Soc.* **1981**, *103*, 6472–6477.

(9) Burton, G. W.; Hughes, L.; Ingold, K. U. Antioxidant Activity of Phenols Related to Vitamin E. Are there Chain-Breaking Antioxidants Better than α-Tocopherol? *J. Am. Chem. Soc.* **1983**, *105*, 5950–5951.

(10) Ingold, K. U.; Burton, G. W.; Hughes, L.; Foster, D. O.; Robillard, B. Vitamin E Activity of 1-Thio-α-Tocopherol as Measured by the Rat Curative Myopathy Bioassay. *Free Rad. Res. Commun.* **1990**, *11*, 207–211.

(11) See, for example: Bindoli, A.; Rigobello, M. P.; Musacchio, E.; Scuri, R.; Rizzoli, V.; Galzigna, L. Protective Action of a New Benzofuran Derivative on Lipid Peroxidation and Sulphydryl Groups Oxidation. *Pharmacol. Res.* **1991**, *24*, 369–375.

(12) See, for example: Niki, E.; Kawakami, A.; Saito, M.; Yamamoto, Y.; Tsuchiya, J.; Kamiya, Y. Effect of Phytyl Side Chain of Vitamin E on its Antioxidant Activity. *J. Biol. Chem.* **1985**, *260*, 2191–2196.

(13) Lucy, J. A. Functional and Structural Aspects of Biological Membranes: A Suggested Structural Role for Vitamin E in the Control of Membrane Permeability and Stability. *Ann. NY Acad. Sci.* **1972**, *203*, 4–11.

(14) Maggio, B.; Diplock, A. T.; Lucy, J. A. Interactions of Tocopherols and Ubiquinones with Monolayers of Phospholipids. *Biochem. J.* **1977**, *161*, 111–121.

(15) Gonzalez Flecha, B. S.; Repetto, M.; Evelson, P.; Boveris, A. Inhibition of Microsomal Lipid Peroxidation by α-Tocopherol and α-Tocopherol Acetate. *Xenobiotica* **1991**, *21*, 1013–1022.

(16) Ingold, K. U.; Burton, G. W.; Foster, D. O.; Hughes, L. Is Methyl-Branching in α-Tocopherol's "Tail" Important for its in Vivo Activity? Rat Curative Myopathy Bioassay Measurements of the Vitamin E Activity of Three 2*RS*-*n*-Alkyl-2,5,7,8-Tetramethyl-6-Hydroxychromans. *Free Radical Biol. Med.* **1990**, *9*, 205–210.

(17) Menta, E.; Spinelli, S.; Gandolfi, C. A.; Frigerio, M.; Tofanetti, O.; Tognella, S. New 1-Phenyl-3-amino-2-propene Derivs.—Having Antioxidation, Mucolytic, Antithrombotic and Tissue Protecting Activity. EP 266549, 1988.

(18) White, J. F.; Minchin, M. C. W.; Ennis, C. Preparation of Aromatic and Heteroaromatic Amines for Treating Depressions, Cerebral Deficiency Disorders or Dementia. EP 407032, 1991.
(19) Short, J. H.; Dunnigan, D. A.; Ours, C. W. Synthesis of Phenethylamines from Phenylacetonitriles Obtained by Alkylation of Cyanide Ion with Mannich Bases from Phenols and Other Benzylamines. *Tetrahedron* **1973**, *29*, 1931–1939.
(20) Adams, R. N. Carbon Paste Electrodes. *Anal. Chem.* **1958**, *30*, 1576.
(21) Glazer, A. N. Fluorescence-based Assay for Reactive Oxygen Species: A Protective Role for Creatine. *FASEB J.* **1988**, *2*, 2487–2491.
(22) The concentration–response curve for lipid peroxidation inhibition is extremely steep. Consequently, we report the inhibitory activity as an MIC (minimum tested concentration of inhibitor that gave ≥50% inhibition).
(23) Compound **24** was omitted from these equations since parameters for MR, L1, and B4 were not available for a 3-pentyloxyl substituent. Compound **21** was also omitted from eq 3a since MR values were not available for an heptyloxyl substituent.
(24) Compound **45** was excluded from eqs 5b–c since L1 and B4 parameters were not available for a nonyl substituent.
(25) Intravenous administration of compounds **23** and **53** did not produce a vasoconstrictor response in pithed rats over the concentration range of 0.1–10 mg/kg. Compound **23** produced arrhythmias at the higher doses examined.
(26) See, for example: (a) Urano, S.; Inomori, Y.; Sugawara, T.; Kato, Y.; Kitahara, M.; Hasegawa, Y.; Matsuo, M.; Mukai, K. Vitamin E: Inhibition of Retinol-induced Hemolysis and Membrane-stabilizing Behavior. *J. Biol. Chem.* **1992**, *267*, 18365–18370. (b) Janero, D. R.; Gurghardt, B. Prevention of Oxidative Injury to Cardiac Phospholipid by Membrane-Active "Stabilizing Agents". *Res. Commun. Chem. Pathol. Pharmacol.* **1989**, *63*, 163–173.
(27) Cotgreave, I. A.; Moldéus, P.; Engman, L.; Hallberg, A. The Correlation of the Oxidation Potentials of Structurally Related Dibenzo[1,4]dichalcogenines to their Antioxidance Capacity in Biological Systems Undergoing Free Radical-Induced Lipid Peroxidation. *Biochem. Pharmacol.* **1991**, *42*, 1481–1485.
(28) Kato, K.; Terao, S.; Shimamoto, N.; Hirata, M. Studies on Scavengers of Active Oxygen Species. 1. Synthesis and Biological Activity of 2-*O*-Alkylascorbic Acids. *J. Med. Chem.* **1988**, *31*, 793–798.
(29) Nihro, Y.; Miyataka, H.; Sudo, T.; Matsumoto, H.; Satoh, T. 3-*O*-Alkylascorbic Acids as Free-Radical Quenchers: Synthesis and Inhibitory Effect on Lipid Peroxidation. *J. Med. Chem.* **1991**, *34*, 2152–2157.
(30) Petty, M. A.; Dow, J.; Grisar, J. M.; De Jong, W. Effect of a Cardioselective α-tocopherol Analogue on Reperfusion Injury in Rats Induced by Myocardial Ischemia. *Eur. J. Pharmacol.* **1991**, *192*, 383–388.
(31) Grisar, J. M.; Petty, M. A.; Bolkenius, F. N.; Dow, J.; Wagner, J.; Wagner, E. R.; Haegele, K. D.; De Jong, W. A. Cardioselective, Hydrophilic *N,N,N*-Trimethylethanaminium α-Tocopherol Analogue that Reduces Myocardial Infarct Size. *J. Med. Chem.* **1991**, *34*, 257–260.
(32) Howard, J. A.; Ingold, K. U. The Inhibited Autoxidation of Styrene. Part III. The Relative Inhibiting Efficiencies of Ortho-Alkyl Phenols. *Can. J. Chem.* **1963**, *41*, 2800–2806.
(33) Mahoney, L. R. Antioxidants. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 547–555.
(34) Janero, D. A.; Cohen, N.; Burghardt, B.; Schaer, B. H. Novel 6-Hydroxychroman-2-Carbonitrile Inhibitors of Membrane Peroxidative Injury. *Biochem. Pharmacol.* **1990**, *40*, 551–558.
(35) Kagan, V. E.; Serbinova, E. A.; Bakalova, R. A.; Stoytchev, Ts. S.; Erin, A. N.; Prilipko, L. L.; Evstigneeva, R. P. Mechanisms of Stabilization of Biomembranes by Alpha-Tocopherol. The Role of the Hydrocarbon Chain in the Inhibition of Lipid Peroxidation. *Biochem. Pharmacol.* **1990**, *40*, 2403–2413.
(36) MR values for the substituent fragment NR(R′) were used in this analysis. Since L1 parameters were not available, we used values for only the alkyl portion.
(37) See, for example: Makriyannis, A.; DiMeglio, C. M.; Fesik, S. W. Anesthetic Steroid Mobility in Model Membrane Preparations as Examined by High Resolution ^1H and ^2H NMR Spectroscopy. *J. Med. Chem.* **1991**, *34*, 1700–1703 and references cited therein.
(38) Coats, E. A.; Wiese, M.; Chi, H.-L.; Cordes, H. P.; Seydel, J. K. Computer Modeling and NMR Studies of Interactions Between Phospholipids and Benzylamines. *Quant. Struct.-Act. Relat.* **1992**, *11*, 364–369.
(39) The time course for neuronal cell death and the morphological changes associated with H_2O_2 exposure under these assay conditions were presented at the American Society for Neurochemistry Meeting in Charleston, SC, March 10–15, 1991. Saunders, R. D.; Keith, P. T.; Luttman, C. A. Role of Oxidants in the Delayed Cell Death of Neurons In Vitro.
(40) Halliwell, B. Oxidants and the Central Nervous System: Some Fundamental Questions. Is Oxidant Damage Relevant to Parkinson's Disease, Alzheimer's Disease, Traumatic Injury or Stroke? *Acta Neurol. Scand.* **1989**, *126*, 23–33.
(41) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979.
(42) Verloop, A.; Hoogenstraaten, W.; Tipker, J. Development and Application of New Steric Substituent Parameters in Drug Design. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1976; Vol. VII, pp 165–207.
(43) Yu, M. J.; McCowan, J. R.; Thrasher, K. J.; Keith, P. T.; Luttman, C. A.; Ho, P. P. K.; Towner, R. D.; Bertsch, B.; Horng, J. S.; Um, S. L.; Phebus, L. A.; Saunders, R. A. Phenothiazines as Lipid Peroxidation Inhibitors and Cytoprotective Agents. *J. Med. Chem.* **1992**, *35*, 716–724.
(44) Yu, M. J.; McCowan, J. R.; Bertsch, B.; Ho, P. P. K. Enzymic and Nonenzymic Lipid Peroxidation: Inhibition by Substituted Phenoxazines. *Biomed. Chem. Lett.* **1991**, *1*, 107–111.
(45) Banker, G. A.; Cowan, W. M. Rat Hippocampal Neurons in Dispersed Cell Culture. *Brain Res.* **1977**, *126*, 397–425.
(46) Novelli, A.; Reilly, J. A.; Lysko, P. G.; Henneberry, R. C. Glutamate Becomes Neurotoxic via the *N*-Methyl-D-Aspartate Receptor when Intracellular Energy Levels are Reduced. *Brain Res.* **1988**, *451*, 205–212.
(47) Hahn, R. A.; MacDonald, B. R.; Martin, M. A. Antihypertensive Activity of LY141865, a Selective Presynaptic Dopamine Receptor Agonist. *J. Pharmacol. Exp. Ther.* **1983**, *224*, 206–214.
(48) Hahn, R. A.; MacDonald, B. R.; Morgan, E.; Potts, B. D.; Parli, C. J.; Rinkema, L. E.; Whitesitt, C. A.; Marshall, W. S. Evaluation of LY203647 on Cardiovascular Leukotriene D4 Receptors and Myocardial Reperfusion Injury. *J. Pharmacol. Exp. Ther.* **1992**, *260*, 979–989.