2,3-Dihydro-1-benzofuran-5-ols as Analogues of α-Tocopherol That Inhibit *in Vitro* and *ex Vivo* Lipid Autoxidation and Protect Mice against Central Nervous System Trauma

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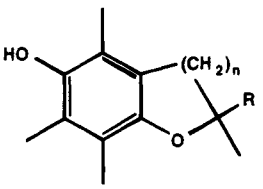
A series of α-tocopherol analogues was synthesized with potential therapeutic value for such pathological conditions as stroke and trauma. A set of criteria such as the inhibition of *in vitro* lipid peroxidation, superoxyl radical scavenging, and brain penetration, as measured by *ex vivo* inhibition of lipid peroxidation, was applied to select the most effective compound. 2,3-Dihydro-2,2,4,6,7-pentamethyl-3-[(4-methylpiperazino)methyl]-1-benzofuran-5-ol dihydrochloride (**22**) was selected because of its superior antioxidant properties and better brain penetration. This compound also protected mice against the effects of head injury. The criteria thus turned out to be useful for the characterization of a neuroprotective analogue of α-tocopherol.

The brain is particularly sensitive to oxidative damage because of its high concentration of polyunsaturated fatty acids and its high rate of oxygen consumption. The brain also has low concentrations of antioxidant protective agents such as α-tocopherol (vitamin E) and glutathione. Furthermore certain brain areas contain high amounts of iron, which promotes the formation of hydroxyl radicals from superoxyl radicals and hydrogen peroxide. Hence much attention is payed to the role of oxygen-derived free radicals in the aging process, in neurodegenerative conditions such as Alzheimer's and Parkinson's diseases, and in neurological injury occurring as the result of stroke and trauma. For reviews, see refs 1–3.

The structure of α-tocopherol (**1**) has often served as a starting point for analogues.[4] The 2,3-dihydro-1-benzofuran-5-ol analogue **2** has been shown to be more potent than **1** in the lipid phase and an *in vivo* assay.[5,6] A water-soluble analogue, Trolox (**3**), was synthesized by Scott et al.[7] We reported earlier on our successful



1, n = 2, R = -[$CH_2CH_2CH(CH_3)$]$_3$-CH_3

2, n = 1, R = -[$CH_2CH_2CH(CH_3)$]$_3$-CH_3

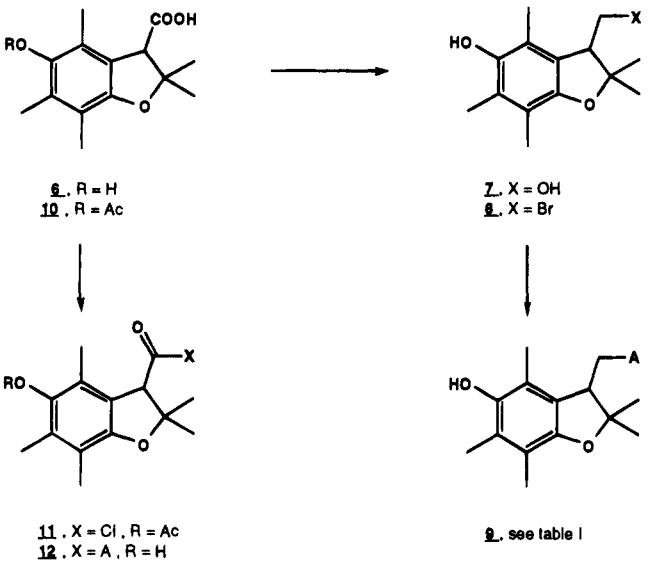
3, n = 2, R = -COOH

4, n = 2, R = -$CH_2CH_2N(CH_3)_3^+$ ^-OTs

5, n = 2, R = -$CH_2CH_2N(CH_3)_2 \cdot HCl$

targeting of an analogue (**4**), which is also hydrophilic, to accumulate in heart tissue,[8–10] reducing myocardial infarct size following reperfusion.[11,12] In looking for analogues that sufficiently penetrate the brain, we focused on compounds like **5**[8,13] containing a tertiary amino function, which is present in many central nervous system (CNS)-active compounds, such as tranquillizers, antidepressants, and the like. In this regard, it is important to note that a positively charged amino group has an advantage to interact with negatively charged brain lipids. Such an interaction is known to affect inhibition of lipid peroxidation.[14] We now report on the synthesis and evaluation of amino derivatives of 2,3-dihydro-1-benzofuran-5-ol shown in Table 1.

Scheme 1



Chemistry

The compounds listed in Table 1 were synthesized by the routes outlined in Scheme **1**. The acid **6** was reduced with borane to the alcohol **7** which was converted to the bromide **8** with bromotriphenylphosphonium bromide, both steps proceeding in good yield. Reaction of **8** with various primary and secondary amines gave compounds **9** (**18**–**28** in Table 1). This reaction requires relatively drastic conditions to overcome steric hindrance. The addition of phenol (stabilized with H_2PO_3) was found to improve the yield of **22**. The addition of sodium iodide was beneficial in the preparation of **27**. Only moderate yields in the range of 40–60% were obtained, however. The *O*-acetates **23** and **25** were obtained by acetylation of the corresponding phenols **22** and **24**. The acid chloride **11** was obtained by reaction of **10** with $Cl_3COCOCl$ (diphosgene) or $Cl_3COCOOCCl_3$ (triphosgene) in the presence of NEt_3 in CH_2Cl_2 at room temperature. Mixed anhydrides obtained by reaction of **6** or **10** with ethyl chloroformate or acetic or trifluoroacetic anhydride failed to give amides **12** on reaction with amines, presumably due to steric hindrance driving the reaction in the undesired

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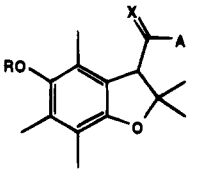
Table 1. Substituted 2,3-Dihydro-1-benzofuran-5-ols



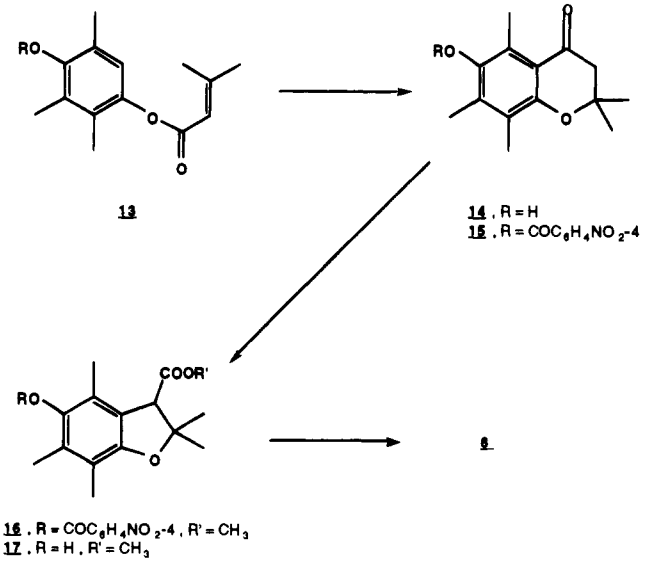
No.	A	R	X	formula	mp °C	*in vitro* lipid autoxidation in rat brain homogenate IC_{50} (μM)[a]	$O_2^{\cdot-}$ scavenging reaction rate constant (10^4 M^{-1} s^{-1})[b]
6	OH	H	O	$C_{14}H_{18}O_4$	152 dec	27 (1)	6.7 ± 0.5 (3)
7	OH	H	H_2	$C_{14}H_{20}O_3$	89–90	2.6 ± 0.6 (3)	14 ± 6 (3)
17	OCH_3	H	O	$C_{15}H_{20}O_4$	126	3.2 ± 0.8 (2)	not determined
18	$N(CH_3)_2$	H	H_2	$C_{16}H_{25}NO_2$·HCl	254 dec	0.7 ± 0.1 (2)	15 ± 5 (3)
19	$N^+(CH_3)_3{}^-OTs$	H	H_2	$C_{17}H_{28}NO_2{}^+C_7H_7O_3S^-$	244–5	3.6 ± 0.2 (2)	4.9 ± 0.6 (3)
20	$NHCH(CH_3)_2$	H	H_2	$C_{17}H_{27}NO_2$·HCl	274–6 dec	1.4 ± 0.5 (3)	33 ± 4 (3)
21	$N(CH_2)_5$	H	H_2	$C_{19}H_{29}NO_2$·HCl·0.5H_2O	273–5 dec	0.4 ± 0.2 (3)	9 ± 1 (3)
22[c]	$N(CH_2CH_2)_2NCH_3$	H	H_2	$C_{19}H_{30}N_2O_2$·2HCl·H_2O	268–9 dec	0.45 ± 0.05 (5)	11 ± 3 (3)
R-(+)-**22**[c]	$N(CH_2CH_2)_2NCH_3$	H	H_2	$C_{19}H_{30}N_2O_2$·2HCl·H_2O	265 dec	0.4 (1)	not determined
S-(−)-**22**[c]	$N(CH_2CH_2)_2NCH_3$	H	H_2	$C_{19}H_{30}N_2O_2$·2HCl·H_2O	267 dec	0.4 (1)	not determined
23	$N(CH_2CH_2)_2NCH_3$	Ac	H_2	$C_{21}H_{32}N_2O_3$·2$C_4H_4O_4$[d]	172 dec	no inhibition	no reaction
24	$N(CH_2CH_2)_2NCH_2CH_2OH$	H	H_2	$C_{20}H_{32}N_2O_3$·2$C_4H_4O_4$[d]	113–9 dec	0.5 ± 0.1 (5)	9 ± 2 (3)
25	$N(CH_2CH_2)_2NCH_2CH_2OAc$	Ac	H_2	$C_{24}H_{36}N_2O_5$·2$C_4H_4O_4$[d]	161 dec	no inhibition	no reaction
26	$N(CH_2CH_2)_2NCH_2CH_2OCH_2CH_2OH$	H	H_2	$C_{22}H_{36}N_2O_4$·$C_4H_4O_4$[d]	125 dec	1.2 ± 0.4 (2)	8.5 ± 0.9 (3)
27	$N(CH_2CH_2)_2NC_4H_3N_2$[e]	H	H_2	$C_{22}H_{30}N_4O_2$·$C_4H_4O_4$[d]·0.7-*i*-PrOH	161 dec	0.4 ± 0 (3)	not determined
28	$N(CH_2CH_2)_2NCH_2C_6H_5$	H	H_2	$C_{25}H_{34}N_2O_2$·2$C_4H_4O_4$[d]	134–7	0.3 ± 0.1 (2)	2.0 ± 0.2 (3)
29	$OCH_2CH_2N(CH_2CH_2)_2NCH_3$	H	O	$C_{21}H_{32}N_2O_4$·2$C_4H_4O_4$[d]	150–4	3.9 ± 0.9 (2)	not determined
30	$N(CH_2CH_2)_2NCH_3$	H	O	$C_{19}H_{28}N_2O_3$·$C_4H_4O_4$[d]	227 dec	12 (1)	8.3 ± 0.7 (3)
31	$N(CH_2CH_2)_2NCH_2CH_2OH$	H	O	$C_{20}H_{30}N_2O_4$·$C_4H_4O_4$[d]	184 dec	19 (1)	4.3 ± 0.3 (3)
4	see structure					2 ± 1 (9)	2.4 ± 0.2 (3)
5	see structure					0.7 ± 0.4 (4)	2.7 ± 0.2 (3)
	U-78517F					0.4 (1)	
	α-tocopherol					14 ± 4 (4)[f]	

[a] Concentration that inhibits TBARS formation by 50% in a rat brain homogenate incubated at 37 °C for 30 min; see methods; mean ± SD, number of experiments in parentheses. [b] Competition with nitro-blue tetrazolium of superoxyl radicals formed by xanthine oxidase in the presence of xanthine; see methods; mean ± SD, number of experiments in parentheses. [c] See ref 23. [d] Maleate. [e] 2-Pyrimidyl. [f] In the presence of 0.5 mM sodium dodecyl sulfate.

direction. The ester **29** was obtained by reaction of **11** with 4-methylpiperazine-1-ethanol[15] in refluxing toluene followed by hydrolysis of the *O*-acetate. Compounds **30** and **31** were obtained from **11** by reaction with 1-methylpiperazine or piperazine-1-ethanol, the hydroxyl group of the latter having been protected by a trimethylsilyl group, which was subsequently removed with $Bu_4N^+F^-$ in THF, followed by *O*-acetate hydrolysis.

Synthesis of the acid **6** is shown in Scheme 2. Fries rearrangement of the bis-dimethylacrylate ester **13** with 1 equiv of $AlCl_3$ at 135–145 °C gave **14** in somewhat better yield than previously reported.[16] Ring contraction of the *p*-nitrobenzoyl ester **15** to **16** was achieved in 85–90% yield with 1 equiv of thallium(III) nitrate in trimethyl orthoformate/methanol (1/1) at room temperature in 4–7 days. This reaction had been studied before on variously substituted 4-chromanones giving from 0 to 61% ring contraction, depending on substituents, besides α-methoxylation and dehydrogenation products.[17,18] We first studied the *O*-acetate of **14** and obtained up to 60% of ring contraction product, but the result was poorly reproducible. We then tried the *O*-benzyl derivative of **14** and obtained no (or less than 10% of) ring contraction product. We then used the more electron-withdrawing (*p*-nitrobenzoyl)oxy group (**15**) and obtained good results, as described. The parent reaction, i.e., the conversion of aromatic ketones into arylalkanoic acids by thallium(III) nitrate, originally reported by McKillop, Swann, and Taylor,[19] was studied by Higgins and Thomas.[20] They propose that formation of the dimethyl acetal of the α-dinitrothallyl ketone is

Scheme 2



the rate-determining step of this rearrangement reaction. Our reaction conditions for the formation of **16** from **15** are such that both starting material and product are nearly insoluble in the solvent mixture (trimethyl orthoformate/methanol = 1/1). Perhaps it is this property, rather than the electron-withdrawing property of the (*p*-nitrobenzoyl)oxy substituent, that favors the ring contraction reaction over competing side reactions. In any case, the 85–90% formation of **16** obviates the need for chromatographic separation of the

product with a minimum of handling of the highly toxic thallium reagent. Naturally, handling thallium(III) nitrate in large scale synthesis poses problems, and alternative reagents have been explored.[20] We are currently studying these alternatives for the synthesis of **16** as well as other routes to obtain **22**. Hydrolysis of **16** to provide **6** was carried out in two steps, possibly due to the sterically hindered ester group, which allows for easy separation of the *p*-nitrobenzoic acid formed in the first step.

Resolution of **10** with (*S*)-(−)- and (*R*)-(+)-α-methylbenzylamine gave, after several recrystallizations, the two diasteromeric salts, the latter of which was shown by X-ray crystallography to have the *S*-configuration. The enantiomers of **22** were obtained by the methods described for the racemate.

Biological Evaluation

The compounds listed in Table 1 were first evaluated *in vitro* for inhibition of lipid autoxidation of rat brain homogenate and for superoxyl radical ($O_2^{\cdot-}$) scavenging. The results are shown in Table 1. To assess their *in vivo* efficacy, *ex vivo* inhibition of lipid autoxidation was determined. The compounds were administered to mice at a subcutaneous dose of 20 μmol/kg of body weight, and the analysis was performed 1 h postadministration. For some compounds, drug concentration was also determined by HPLC in the same mouse brain homogenates. These results are shown in Table 2.

The mouse head injury model developed by Hall and co-workers[21] was used to determine pharmacologic activity. In this model a blow of measured severity is inflicted to the head of the mice and the effect is measured 1 h later by determining their ability to hold on to a horizontal string. Results are given in Table 3.

Results and Discussion

***In Vitro* Inhibition of Lipid Autoxidation.** Table 1 shows that all the 2,3-dihydro-1-benzofuran-5-ol analogues were potent inhibitors of spontaneous lipid peroxidation in rat brain homogenate. An enhanced potency of 5-ring over 6-ring analogues as previously observed,[5,6] e.g., if comparing **18** with **5**, was not apparent in this assay. The two enantiomers of **22** were equipotent.

***In Vitro* Superoxyl Radical Scavenging.** Table 1 also shows a strong superoxyl radical-scavenging effect of the test compounds. The benzopyran (6-ring) analogue **5** was significantly less active than the corresponding 5-ring analogues **18, 20**−**22, 24**, and **26**.

***Ex Vivo* Inhibition of Brain Lipid Autoxidation.** The *ex vivo* data given for the test compounds in Table 2 allow an estimate of their relative brain penetration as well as their potential antioxidant effect in a living organism. It was not possible to select a preferred compound among the analogues in Table 1 on the basis of the *in vitro* data alone. With two exceptions (**20** and **21**), the test compounds were administered to mice at a subcutaneous dose of 20 μmol/kg of body weight and the brains analyzed for relative inhibition of TBARS formation 1 h postadministration. This *ex vivo* evaluation in normal mouse brains revealed **22** and **24** to be more potent than the other analogues in spite of identical antioxidant potencies *in vitro*. The two enantiomers of **22** again were equipotent in this *ex vivo* test.

Table 2. *Ex Vivo* Inhibition of Mouse Brain Lipid Autoxidation and Brain Drug Concentration

	mouse brain, 1 h after 20 μmol/kg sc	
compd	% inhibition of lipid autoxidation[a,b]	brain concentration, nmol/g
18	no inhibition[c]	
20	37 ± 5[d]	
21	50 ± 11[d]	
22	63 ± 7	7.5 ± 1.0
23	57 ± 15	7.2 ± 1.6
24	57 ± 3	6.5 ± 0.8
25	35 ± 14	5.7 ± 0.8
26	no inhibition[c]	1.2 ± 0.3
27	29 ± 20[c]	0.8 ± 0.1
28	24 ± 17[c]	1.6 ± 0.3
29	10 ± 11[c]	
5	16 ± 10[c]	

[a] See the Experimental Section; mean values ± SD (five mice per group). [b] Compared to saline-treated control. [c] Significantly different from **22** ($p \leq 0.05$, ANOVA). [d] Tested at 0.1 mM.

Table 3. Percentage Number of Paraparetic Mice 1 h after Concussive Head Injury[a]

compd	% paraparetic[b]
22	30[c]
24	40
26	60
4	40
saline	50

[a] Prophylactic treatment 30 min before injury with 12 mg/kg sc of test compound. [b] The neurological status of the injured mice was evaluated blindly using a string test. The percentage number that was paraparetic reflects the number of mice that could not raise their hind limbs onto the string. [c] $p < 0.01$ when compared to saline-treated animals by means of a χ-square analysis; the number of mice in each group was between 20 and 25.

The brain levels of the test compounds after subcutaneous injection were determined by HPLC (Table 2), and these data indicate relative brain penetration which gives similar results to those estimated from measuring the *ex vivo* inhibition of lipid peroxidation. Compounds **22** and **24** were found to reach the highest brain levels after subcutaneous administration to mice (Table 2). Acetylation of the phenol did not affect the *ex vivo* potency of **22** and **24** (**23** and **25**, respectively) (Table 2), probably because of *in situ* hydrolysis. In adult Sprague-Dawley rats, compound **22** was similarly effective with respect to *ex vivo* inhibition of lipid peroxidation as in the mice (not shown).

The α-tocopherol analogue, lazaroid U-78517F from Upjohn, did not inhibit *ex vivo* lipid peroxidation under the present experimental conditions, although being as potent *in vitro* as **22** (Table 1). Its brain concentration has been previously determined by HPLC in head-injured mice to be around 2 μg/g of brain tissue after intravenous administration of 10 mg/kg.[22] The different route of administration as well as the injury may account for this discrepancy.

Reduction of Head Injury Trauma in Mice. The results of this study are shown in Table 3. Compound **22** significantly reduced the number of paraparetic mice following head trauma. The data indicate a relationship between the pharmacological effect and the potential of the compounds to inhibit lipid peroxidation. Compound **22** was selected for further evaluation. More detailed evaluation of this compound and its enantiomers will be published elsewhere at a later date.

Conclusion

2,3-Dihydro-2,2,4,6,7-pentamethyl-3-[(4-methylpiperazino)methyl]benzofuran-5-ol dihydrochloride (**22**)[23] was selected from a series of α-tocopherol analogues with strong antioxidant properties that penetrate the brain and reduce the effect of head injury in mice.

Experimental Section

Melting points are uncorrected. Elemental analyses for the elements indicated were within ±0.4% of calculated values. ^{1}H-NMR spectra were obtained at 360 MHZ with an AM-360 Bruker spectrometer, IR spectra were recorded with a Bruker IFS 48 FTIR spectrometer, and UV spectra were recorded with a Beckman DU-7 spectrometer. Enantiomeric excess (ee) was determined on an Ultron ES-OVM column (150 × 4 mm i.d., 5 μm particle size) supplied by Liquid Chromatography Columns, Rockland Technologies, IL. The mobile phase consisted of 0.025 M phosphate buffer at pH 6.5, containing 9% acetonitrile (v/v) at a flow rate of 1 mL/min. UV detection was used at 210 nm.

2,3,6-Trimethylhydroquinone Bis(3,3-dimethylacrylate) (13). To 100 g (1 mol) of 3,3-dimethylacrylic acid was added 73 mL (119 g, 1 mol) of thionyl chloride, and the mixture was stirred for 2 h at room temperature and for 1.5 h at 110 °C. The escaping HCl gas was trapped in 2 N NaOH. Distillation at 40 mmHg gave 107.7 g (90%) of 3,3-dimethylacryloyl chloride, bp 66–68 °C. It was added to a solution of 68.68 g (0.45 mol) of trimethylhydroquinone in 500 mL of toluene, and the mixture was slowly heated to reflux, while trapping the escaping HCl gas in 2 N NaOH. When gas evolution ceased (about 1 h of reflux), the solution was cooled, Et_2O was added, and the solution was washed with saturated $NaHCO_3$ solution, dried (Na_2SO_4), and evaporated. Crystallization of the resulting oil from 700 mL of *n*-hexane gave 133.16 g (93%) of 13.

3,4-Dihydro-2,2,5,7,8-pentamethyl-6-hydroxy-2*H*-1-benzopyran-4-one (14). The ester 13 (133.16 g, 0.42 mol) was pulverized and mixed with 61.74 g (0.46 mol) of powdered anhydrous $AlCl_3$ by means of a mechanical stirrer and heated to 135–145 °C for 1.5 h. On a larger scale, it was found advantageous to first melt a small portion of the mixture and then add the remainder of the mixture in small portions. The resulting melt was allowed to cool, dissolved in 200 mL of CH_2Cl_2, and stirred while 200 mL of 2 N HCl was added dropwise. The CH_2Cl_2 phase was separated, washed with $NaHCO_3$ and NaCl solutions, dried (Na_2SO_4), and evaporated. The residue was dissolved in 300 mL of CH_3OH and 300 mL of 2 N NaOH and refluxed for 1 h. The solution was cooled, acidified with 400 mL of 2 N HCl, and extracted twice with EtOAc. The extract was washed with H_2O and $NaHCO_3$ solution, dried (Na_2SO_4), filtered, and concentrated to about 300 mL. The product **14** crystallized and was recrystallized from EtOAc to give 53.84 g. A second crop of 13.29 g raised the yield to 68%.

3,4-Dihydro-2,2,5,7,8-pentamethyl-6-hydroxy-2*H*-1-benzopyran-4-one 4-Nitrobenzoate (15). The *p*-nitrobenzoyl ester of **14** was prepared by portionwise addition of 38.98 g (0.21 mol) of *p*-nitrobenzoyl chloride to an ice-cooled solution of 46.86 g (0.2 mol) of **14** in 250 mL of pyridine and stirring at room temperature overnight. Water was added, and the precipitate was collected and washed with water and a little methanol. Recrystallization from $CHCl_3/CH_3OH$ gave 74.62 g (97%) of **15**.

Methyl 2,3-Dihydro-5-[(4-nitrobenzoyl)oxy]-2,2,4,6,7-pentamethyl-1-benzofuran-3-carboxylate (16). A heterogeneous mixture of 30.20 g (0.079 mol) of **15**, 36.86 g (0.083 mol) of thallium(III) nitrate trihydrate, 200 mL of trimethyl orthoformate, and 200 mL of methanol was stirred at room temperature for 4–7 days. The mixture remained heterogeneous. The solid was collected and washed with methanol. The remaining solid was slurried in 100 mL of $CHCl_3$ and filtered; this process was repeated three times. The combined filtrates were heated to boiling, and the $CHCl_3$ was gradually replaced by an equal volume of CH_3OH until crystallization occurred. On cooling, 27.44 g (84%) of **16** was obtained, mp 215–216 °C. Anal. C,H,N. This procedure was scaled up to 402 g, and 380.9 g (88%) of **16** was obtained.

Methyl 2,3-Dihydro-5-hydroxy-2,2,4,6,7-pentamethyl-1-benzofuran-3-carboxylate (17). To a boiling solution of 20.67 g (0.05 mol) of **16** in 200 mL of THF was added 50 mL of 2 N NaOH, and the mixture was stirred at reflux temperature for 50 min. The THF was evaporated (20 min), water was added, and the product was extracted with CH_2Cl_2 (three times). The extract was washed with H_2O and NaCl solution, dried (Na_2SO_4), and evaporated. The resulting solid was recrystallized from EtOAc/heptane to give 10.76 g (81%) of **17**, mp 145–146 °C. IR (KBr): 17.15 cm^{-1}. Anal. C,H.

2,3-Dihydro-5-hydroxy-2,2,4,6,7-pentamethyl-1-benzofuran-3-carboxylic Acid (6). A solution of 22.36 g (0.084 mol) of **17** in 100 mL of CH_3OH and 100 mL of 2 N NaOH was refluxed for 24 h. After addition of 120 mL of 2 N HCl, CH_3OH was removed by evaporation and the mixture was extracted twice with EtOAc. The extract was washed with water, and the acidic product was separated by washing with $NaHCO_3$ solution which upon acidification was reextracted into EtOAc. The extract was dried (Na_2SO_4) and evaporated, and the resulting solid was recrystallized from EtOAc/heptane to give 16.40 g (77%) of **6**, mp 161–164 °C. ^{1}H NMR (DMSO-d_6): δ 1.50 (3H, s, 2-C-CH_3), 1.52 (3H, s, 2-C-CH_3), 2.08 (3H, s, Ar-CH_3), 2.09 (3H, s, Ar-CH_3), 2.18 (3H, s, Ar-CH_3), 3.88 (1H, s, 3-H), 7.58 (1H, s, OH), 12.7 (1H, s, COOH). UV (CH_3CN): λ_{max} 298 nm (E = 4370), 200 (35 930). IR (KBr): 1722 cm^{-1}. Anal. C,H.

2,3-Dihydro-5-hydroxy-2,2,4,6,7-pentamethyl-1-benzofuran-3-methanol (7). To a stirred solution of 58.82 g (0.0235 mol) of **6** in 500 mL of THF was added dropwise over 30 min 50 mL of 10 M $BH_3{\cdot}S(CH_3)_2$, and the resulting mixture was stirred at reflux temperature for 7 h. After cooling, 120 mL of CH_3OH was added dropwise and the resulting solution was evaporated to dryness. The residue was taken up in EtOAc, washed with 2 N HCl, H_2O, $NaHCO_3$, and NaCl solution, dried (Na_2SO_4), and evaporated. Crystallization of the residue from EtOAc/heptane gave 37.85 g of **7**, mp 89–90 °C. ^{1}H NMR (DMSO-d_6): δ 1.32 (3H, s, 2-C-CH_3), 1.60 (3H, s, 2-C-CH_3), 2.05 (3H, s, Ar-CH_3), 2.12 (3H, s, Ar-CH_3), 2.20 (3H, s, Ar-CH_3), 3.02 (1H, dd, J = 3.77 Hz, J' = 8.28 Hz, 3-H), 3.52 (1H, dd, J = 8.41 Hz, J' = 11.21 Hz, CH_2OH), 3.58 (1H, dd, J = 3.80 Hz, J' = 11.28 Hz, CH_3OH), 4.72 (1H, dd, J = 4.30 Hz, J' = 5.73 Hz, OH), 7.48 (1H, s, ArOH). Anal. C,H. A second crop of 12.60 g was obtained raising the yield to 91%.

3-(Bromomethyl)-2,3-dihydro-2,2,4,6,7-pentamethyl-1-benzofuran-5-ol (8) and *O*-Acetate. To an ice-cooled solution of 41.89 g (0.16 mol) of triphenylphosphine in 120 mL of 4 Å molecular sieve-dried CH_2Cl_2 was added dropwise a solution of 24.33 g (0.15 mol) of bromine in 40 mL of CH_2Cl_2, and the resulting mixture was stirred at 0 °C for 1 h giving a white precipitate free of Br_2 coloration. To this mixture was added 34.26 g (0.145 mol) of **7**, and the resulting solution was allowed to warm to room temperature and stirred for 18 h. The solution was concentrated to a small volume and chromatographed on silica gel using CH_2Cl_2/hexane (1:2) as eluent. Fractions containing the product (as indicated by TLC) were combined and evaporated to give 43.28 g (99%) of an oil. A sample was crystallized from EtOAc/heptane to give **8**, mp 79–80 °C. The ^{1}H-NMR ($CDCl_3$) spectrum showed a splitting pattern similar to that of **7**. Anal. C,H. The *O*-acetate was obtained with Ac_2O in pyridine at room temperature (18 h) and recrystallized from EtOAc/heptane, mp 122–123 °C. Anal. C,H.

2,3-Dihydro-2,2,4,6,7-pentamethyl-3-[(dimethylamino)methyl]-1-benzofuran-5-ol Hydrochloride (18). Into 8 mL of dry DMF was bubbled dimethylamine gas until a volume of 10 mL was obtained. This solution was added to a solution of 4.98 g (16.6 mmol) of **8** in 20 mL of DMF, and the stoppered mixture was stirred at room temperature for 7 days. Water and $NaHCO_3$ solution were added, the mixture was extracted with Et_2O, and the extract was washed with H_2O. Basic product was separated by washing with 2 N HCl, basification with solid $NaHCO_3$, and reextraction into EtOAc. After drying (Na_2SO_4) and evaporation, 2.80 g (68%) of an oil was obtained, which was dissolved in *i*-PrOH to which isopropanolic HCl was added to pH < 3. The resulting crystals were recrystallized

from *i*-PrOH to give 1.90 g (41%) of **18**, mp 254 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.42 (3H, s, 2-C-CH_3), 1.80 (3H, s, 2-C-CH_3), 2.08 (3H, s, Ar-CH_3), 2.17 (3H, s, Ar-CH_3), 2.27 (3H, s, Ar-CH_3), 2.93 (3H, d, N-CH_3), 3.04 (3H, d, N-CH_3), 3.0–3.55 (2H, 2m, N-CH_2), 3.50 (1H, m, 3-H), 7.70 (1H, s, OH), 9.82 (1H, m, N-H). Anal. C,H,N.

Compound **20** was prepared by this procedure using 10 equiv of isopropylamine and compound **21** using 2 equiv of piperidine. See Table 1.

2,3-Dihydro-5-hydroxy-*N,N,N*,2,2,4,6,7-octamethyl-1-benzofuran-3-methanaminium (4-Methylbenzene)sulfonate (19). A solution of 4.28 g of **18** (free base; 16.3 mmol) and 3.3 g (10% excess) of methyl *p*-toluenesulfonate (17.7 mmol) in 60 mL of acetonitrile was refluxed for 18 h. The solvent was evaporated and the residue slurried in EtOAc. The semisolid was recrystallized twice from acetonitrile to give 3.7 g of **19**, mp 244–245 °C. Anal. C,H,N.

2,3-Dihydro-2,2,4,6,7-pentamethyl-3-[(4-methylpiperazino)methyl]-1-benzofuran-5-ol Dihydrochloride Hydrate (22). A solution of 81.0 g (0.27 mol) of **8**, 26.75 g (0.28 mol) of phenol (stabilized), and 28.47 g (0.28 mol) of 1-methylpiperazine in 300 mL of acetonitrile was stirred at reflux temperature for 60 h. The precipitate that formed was collected, washed with acetonitrile, and slurried in $NaHCO_3$ solution. The product was extracted twice with EtOAc, and the extract was washed with H_2O and NaCl solution, dried (Na_2SO_4), and evaporated. The resulting solid was dissolved in 150 mL of EtoH and 150 mL of 2 N HCl and evaporated to near dryness. The resulting solid was recrystallized from EtOH/EtOAc to give, after drying at 60 °C and 0.1 mmHg and equilibration in a moist atmosphere for 24 h, 48.60 g (44%) of **22**, mp 172–173 °C dec. ^{1}H NMR (D_2O): δ 1.39 (3H, s, 2-C-CH_3), 1.68 (3H, s, 2-C-CH_3), 2.10 (3H, s, Ar-CH_3), 2.16 (3H, s, Ar-CH_3), 2.22 (3H, s, Ar-CH_3), 3.00 (3H, s, N-CH_3), 3.01–3.35 (2H, dd, 3-C-CH_2), 3.35–3.7 (9H, m, 3H, piperazino-CH_2, 3-C-CH). UV (H_2O): λ_{max} 291 nm (E = 3605), 218 sh (9420), 202 (31 355). Anal. C,H,N. Weight loss from 50 to 165 °C (heating rate 40 °C/min): 4.49% = 1.02 mol H_2O. A second crop of 19.63 g (total yield 63%) could be obtained from the filtrate after purification of the base by chromatography on silica gel using CH_2Cl_2/CH_3OH (9:1) as eluent.

The *R*-(+)-enantiomer was obtained from the diasteromeric salt of **10** with (*R*)-(+)-α-methylbenzylamine (see below). *R*-(+)-**22**: mp 265 °C dec. $[\alpha]_D^{25}$ = +20.68° (*C* = 1.18 in H_2O, pH = 1.40). Anal. C, H, N. Weight loss on heating (40 °C/min, 40–175 °C): 4.22% = 1.02 mol H_2O.

The *S*-(−)-enantiomer was obtained from the diastereomeric salt of **10** with (*S*)-(−)-α-methylbenzylamine (see below). S-(−)-**22**: mp 267 °C dec. $[\alpha]_D^{25}$ = −20.66 °C = 1.66 in H_2O, pH = 1). Anal. C,H,N. Weight loss on heating (40 °C/min, 40–175 °C): 3.95% = 0.9 mol H_2O.

The *O*-acetate of **22** was obtained by treatment of 1.90 g (6 mmol) of **22** (free base) with Ac_2O (10 mL) in pyridine (20 mL) overnight, addition of H_2O and $NaHCO_3$, extraction with EtOAc, evaporation of the dried (Na_2SO_4) extract, addition of 2 equiv of maleic acid in *i*-PrOH, and recrystallization from *i*-PrOH to give 2.73 g of **24**, mp 172–173 °C dec. Anal. C,H,N.

2,3-Dihydro-2,2,4,6,7-pentamethyl-3-[[4-(2-pyrimidyl)-piperazino]methyl]-1-benzofuran-5-ol Acid Maleate (27). To a solution of 2.61 g (0.011 mol) of 1-(2-pyrimidyl)piperazine dihydrochloride were added solid $NaHCO_3$ and NaCl, and the mixture was extracted five times with EtOAc. The extract was evaporated, 2.99 g (0.01 mol) of **8**, 1.50 g (0.01 mol) of NaI, 0.84 g (0.01 mol) of $NaHCO_3$, and 50 mL of CH_3CN were added, and the mixture was stirred at reflux temperature for 3 days. The solvent was evaporated, the residue taken up in EtOAc and washed with water, and the basic product separated by washing with 2 N HCl. Neutralization with $NaHCO_3$ and reextraction with EtOAc gave, after drying (Na_2SO_4) and evaporation of solvent, an oil to which 2.32 g (0.02 mol) of maleic acid was added. Crystallization and recrystallization from *i*-PrOH gave 3.50 g (65%) of **27**, mp 161–162 °C dec. Anal. C,H,N.

Compounds **24–26** and **28** were prepared by this procedure or that described for **18**, **22**, and **23**, or slight variations thereof. The yields obtained were in the range of 40–60%.

5-Acetoxy-2,3-dihydro-2,2,4,6,7-pentamethyl-1-benzofuran-3-carboxylic Acid (10). To a solution of 25.03 g (0.1 mol) of **6** in 200 mL of pyridine was added 100 mL of Ac_2O, and the mixture was stirred at room temperature for 24 h. Water and ice were added, and the mixture was stirred at about 30 °C for 30 min. The mixture was cooled in ice, and 450 mL of 6 N HCl was added. The resulting solid was collected, washed with H_2O, taken up in EtOAc, washed with 2 N HCl and H_2O, dried (Na_2SO_4), and evaporated. Recrystallization from EtOAc gave 23.6 g (81%) of **10**, mp 187–188 °C. Anal. C,H.

5-Acetoxy-2,3-dihydro-2,2,4,6,7-pentamethyl-1-benzofuran-3-carbonyl Chloride (11). To a solution of 9.59 g (0.047 mol) of trichloromethyl chloroformate in 80 mL of dry CH_2Cl_2 under N_2 was added dropwise over 3 h a solution of 13.75 g (0.047 mol) of **10** and 4.77 g (0.047 mol) of NEt_3 in 100 mL of CH_2Cl_2. The escaping gas was trapped over KOH. The mixture was stired at room temperature overnight and at reflux temperature for 1 h. Solvent was evaporated without heat, the residue was dissolved in toluene, and the NEt_3·HCl that precipitated was removed by filtration. The filtrate was evaporated and the residual oil crystallized from hexane, after filtration to remove additional NEt_3·HCl, to give 10.93 g (75%) of 11, mp 171–173 °C. Anal. C,H. To further exclude the possibility of this product being the trichloromethyl ester of **10**, a ^{13}C-NMR ($CDCl_3$) spectrum was obtained that showed no peak for CCl_3, all peaks being assignable to 11. The MS spectrum showed no large chlorine-containing fragments. The identical product was obtained when bis(trichloromethyl) carbonate (triphosgene) was used instead of diphosgene by the same procedure.

2,3-Dihydro-5-hydroxy-2,2,4,6,7-pentamethyl-1-benzofuran-3-carboxylic Acid Ester with 1-(2-Hydroxyethyl)-4-methylpiperazine Diacid Maleate (29). 1-(2-Hydroxyethyl)-1-methylpiperazine was prepared from 1-methylpiperazine and ethylene oxide in 49% yield by the procedure of Cannon.[15] One equivalent (1.62 g, 11.2 mmol) of this product was added to 3.48 g (11.2 mmol) of 11 in toluene, and the mixture was refluxed overnight. After cooling, the solution was washed with $NaHCO_3$ solution, dried (Na_2SO_4), and evaporated. The residue was refluxed in 50 mL of THF and 25 mL of 2 N NaOH for 45 min. After acidifying with 60 mL of 2 N HCl, the solution was washed with Et_2O, $NaHCO_3$ was added, and the product was extracted into EtOAc. To the residue, obtained after drying (Na_2SO_4) and evaporating the extract, was added 2.60 g (22.4 mmol) of maleic acid, and the resulting crystals were recrystallized from CH_3CN to give 2.00 g (29%) of **29**, mp 150–154 °C. Anal. C,H,N.

2,3-Dihydro-2,2,4,6,7-pentamethyl-3-(4-methylpiperazino)-1-carboxy-1-benzofuran-5-ol Acid Maleate (30). A solution of 11, prepared from 4.38 g (16 mmol) of **10** with triphosgene, and 1.60 g (16 mmol) of 1-methylpiperazine in 100 mL of toluene was refluxed for 4 h. The product was washed into 2 N HCl and extracted into EtOAc after neutralization with $NaHCO_3$. The extract was dried (Na_2SO_4) and evaporated and the residue crystallized from EtOAc/heptane to give 4.35 g (77%) of the *O*-acetate of **30**, mp 171–173.5 °C. This material was refluxed in 25 mL of CH_3OH and 25 mL of 2 N NaOH for 45 min, CH_3OH was evaporated, and the residue was acidified and extracted with EtOAc. After drying (Na_2SO_4) and evaporation, 1 equiv of maleic acid in *i*-PrOH was added and crystallized product was recrystallized from *i*-PrOH/H_2O, mp 227 °C dec. Anal. C,H,N.

Compound 31 was prepared analogously. To 2-(hydroxyethyl)piperazine in toluene was added 1 equiv of $ClSiMe_3$ to protect the hydroxy group. After 3 h of standing at room temperature, 1 equiv of 11 was added and the mixture was refluxed for 5 h. The residue after workup as described above was treated with 1 M $Bu_4N^+F^-$ in THF at room temperature overnight to remove the trimethylsilyl group, evaporated, taken up in EtOAc, washed, dried, and evaporated. Hydrolysis of the *O*-acetate group as described above gave 31 in 30% overall yield from 11.

Resolution of 10. A solution of 15.27 g (0.0523 mol) of **10** and 6.65 g (0.0549 mol) of (*S*)-(−)-α-methylbenzylamine in 100 mL of *i*-PrOH, 2 mL of H_2O, and 300 mL of EtOAc was azeotroped to a volume of about 100 mL. The crystalline

material obtained was recrystallized twice from the same solvent system to give 6.02 g (56%) of diastereometric salt, $[\alpha]_D^{25} = -13.81°$ ($C = 0.99$ in CH_3OH), ee = 99.9%. Anal. C,H,N. The combined filtrates were suspended in H_2O, 50 mL of 2 N HCl was added, and acidic product was extracted twice with EtOAc. The extract was washed with 2 N HCl and NaCl solution, dried (Na_2SO_4), and evaporated to give 11.34 g of an oil. To this was added 4.70 g (0.0388 mol) of (*R*)-(+)-α-methylbenzylamine, and crystallization was obtained using the same solvent system. Two recrystallizations gave 7.90 g (73%) of the other diastereomeric salt, $[\alpha]_D^{25} = -14.21°$ ($C = 0.99$ in CH_3OH), ee = 99.9%. Anal. C,H,N. The two diastereomeric salts each were converted to free acid, reduced to **7** with BH_3·S-$(CH_3)_2$, converted to bromide **8** with triphenylphosphine/Br_2, and reacted with 1-methylpiperazine to give the enantiomers of **22**.

X-ray crystallography showed the salt with *R*-(+)-amine to have the *S*-configuration. Note that due to the nomenclature conventions, the enantiomers of **22**, derived from the *S*-acid, has the *R*-configuration.

Biological Methods. Lipid Autoxidation. The method has been described previously.[8,9]

Superoxyl Radical Scavenging. The method applied has been described previously.[9]

***Ex Vivo* Lipid Autoxidation.** Groups of five male CD1 mice (body weight = 30 g) were injected sc with either 20 μmol/kg test compound or with saline solution (control) and the animals killed exactly 1 h later. In the different experiments, one group treated with **22** was always included for comparison. The brains were excised quickly, frozen in liquid nitrogen, and then stored at −80 °C. The frozen brains were homogenized (1/19, w/v) in ice-cold water.

Incubations were done in a shaking water bath with 200 μL of homogenate (10 mg of tissue) plus 500 μL of KH_2PO_4 (40 mM, pH 7.3; 20 mM final) and 300 μL of 467 mM KCl (0.14 M final) at 37 °C for 30 min. Of each treatment group, part of the sample was left unincubated to determine the basal TBARS content.

A malondialdehyde (MDA) standard was prepared by dissolving MDA bis(dimethyl acetal) in water to which 0.7% $HClO_4$ was added. From this solution, a series of standard solutions ranging from 0.5 to 10 nmol/sample was prepared.

Following incubation, the reaction was stopped on ice and 200 μL of 35% $HClO_4$ was added. The samples were centrifuged at 3000*g* for 5 min, and 800 μL of the supernatant was mixed with 200 μL of 1% thiobarbituric acid in water. Samples were then placed in a boiling water bath for 15 min and subsequently cooled to room temperature. The TBA adduct was extracted into 1 mL of *n*-butanol. The fluorescence of the supernatant was read at 515 nm (excitation) and 553 nm (emission). The standard curve was fitted by second-order polynomial regression, and the corresponding contents of malondialdehyde equivalents (minus basal contents) were calculated for all the samples. Results are expressed as percent inhibition of *ex vivo* lipid peroxidation observed in the saline-treated control group. The data in Table 2 were recalculated, on the basis of the mean inhibition obtained after administration of **22**.

HPLC Analysis of the Brain Extracts. Of the brain homogenates, 450 μL was diluted with 1 mL of methanol containing 2% (w/v) ascorbic acid and centrifuged at 1000 rpm. Separation of 50 μL of the supernatant was achieved on an Ultrabase C_8 column (250 × 4.6 mm i.d., 5 μm particle size) supplied by Shandon-SFCC (Eragny, France) and isocratic elution with 0.1 M phosphate buffer containing 50−75% methanol. Electrochemical detection with a working potential of 0.6 V was used.

Head Injury Trauma in Mice. The experiments were performed as described by Hall and co-workers.[21]

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(23) Compound **22** as racemate has the code number MDL 74,180DA; the *R*-(+)-enantiomer is MDL 74,722DA, and the *S*-(−)-enantiomer is MDL 75,204DA.

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