3,5-Disubstituted-4-hydroxyphenyls Linked to 3-Hydroxy-2-methyl-4(1*H*)-pyridinone: Potent Inhibitors of Lipid Peroxidation and Cell Toxicity

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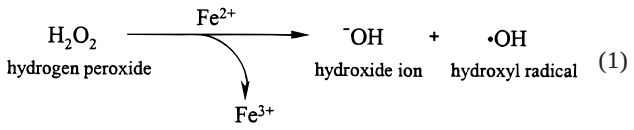
Introduction. For many years radical scavenging antioxidants have been successfully used to protect synthetic materials and food products from the degrading process of oxidation.[1] More recently a role as neuroprotective agents for the treatment of disorders known to involve oxidative stress (e.g. stroke, traumatic brain injury, Parkinson's disease, and Alzheimer's disease) has been proposed and supported by animal models.[2] The effectiveness of radical scavengers in reducing oxidative stress within a living biological environment is undermined, however, by the continual production of radicals via mechanisms such as that described by the Fenton reaction, a process that is catalyzed by the presence of Fe^{2+} (eq 1).[3] We postulated

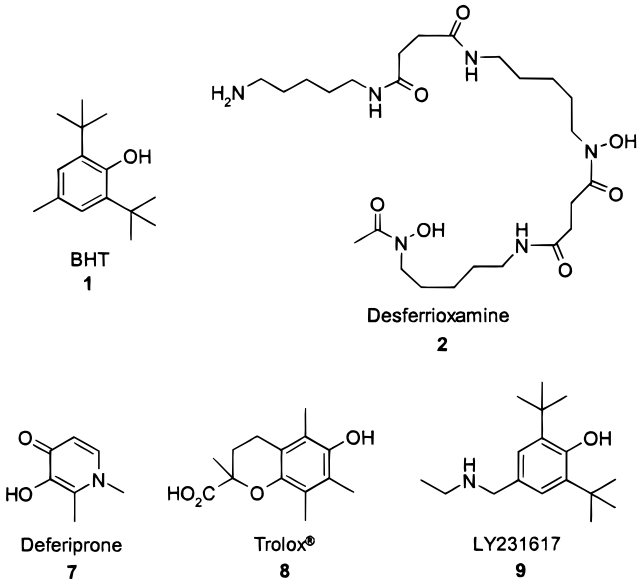


(1)

that treatment with both a radical scavenger and an Fe chelator might protect living tissue from oxidative stress to a greater degree than is achievable with either a radical scavenger or an Fe chelator alone (for examples of each, see Chart 1).[4]

A further rationale, that radical scavengers and Fe chelators may be able to interact synergistically,[5] is supported by reports of *tert*-butylphenolic antioxidants interacting with methoxyphenol,[6] phosphites,[7] ascorbate,[8] thiols,[8] and sulfites[9] to produce synergistic antioxidant effects. In addition it was hypothesized that a significant advantage might be gained over the combined administration of separate radical scavengers and Fe chelators, by combining the structural features for radical scavenging and Fe chelation within one "hybrid" molecule. First, a hybrid molecule could have the potential to behave synergistically via intramolecular interaction, a more favorable process than one which relies upon intermolecular interaction.[10] Second, by creating a hybrid molecule, it was likely that the antioxidant component of the molecule (typically lipophilic in nature, e.g. BHT (**1**)) would bestow more lipophilicity to the Fe chelator component (typically hydrophilic in nature, e.g. desferrioxamine (**2**)), giving the molecule a greater potential to penetrate and sequester Fe from areas susceptible to oxidative damage (i.e. lipids, proteins, and DNA).[11,12]

Chart 1. Structures of Typical Antioxidant (**1**, **8**, **9**) and Fe Chelator (**2**, **7**) Molecules



Chemistry. Favorable structural components for the hybrid molecules were selected by the evaluation of several classes of Fe chelator and radical scavenger in in vitro lipid peroxidation and cell toxicity assays.[13,14] This investigation, together with an assessment of amenability to synthetic manipulation, led to the identification of 3-hydroxy-4(1*H*)-pyridinone[15,16] and 2,6-disubstituted phenol[17] as preferred structural units for incorporation into hybrid molecules and to the design of target structures **3a**−**f**, where the two structural features are linked via simple alkyl or alkylthio chains. The key intermediate amines **4a**−**f** in the synthesis of **3a**−**f** were prepared in 15−63% overall yield from the commercially available phenols **5a**−**d**, using standard synthetic methods (Scheme 1). The amines **4a**−**f** were then reacted with benzyl maltol (**6**),[18] followed by deprotection, to give the target compounds **3a**−**f** in 17−56% yield from **4a**−**f** (Scheme 2).

Results and Discussion. Lipid peroxidation in rat brain homogenates was used to measure the antioxidant capacity of the molecules in a biological environment (Table 1).[13] All the compounds (**3a**−**f**) were more potent inhibitors of lipid peroxidation than the Fe chelator deferiprone (**7**)[19] or the antioxidants BHT (**1**), Trolox (**8**), and LY231617 (**9**).[20] The two 2,6-di-*tert*-butyl-substituted compounds **3a**,**f** were the most potent inhibitors of lipid peroxidation within the series (**3a**−**f**). Replacement of the *tert*-butyl groups in compounds **3a**,**b** with methoxy groups led to compounds **3d**,**e** and a consequent reduction in potency. An increase in the linker chain length as seen in compounds **3a**−**c** also resulted in a decrease in potency. Substitution of the benzylic CH_2 group in **3b** with a sulfur atom gave the more potent compound **3f**.

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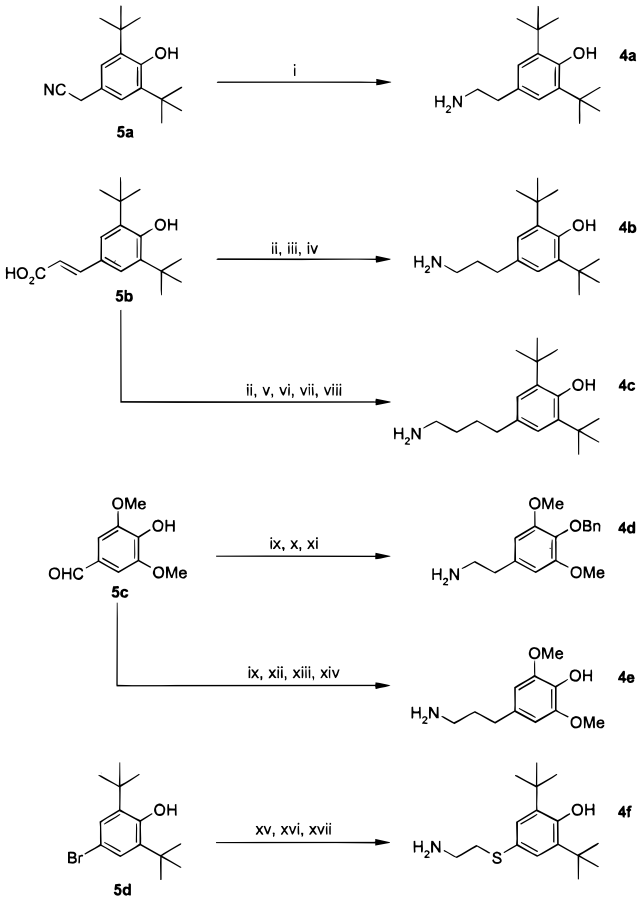
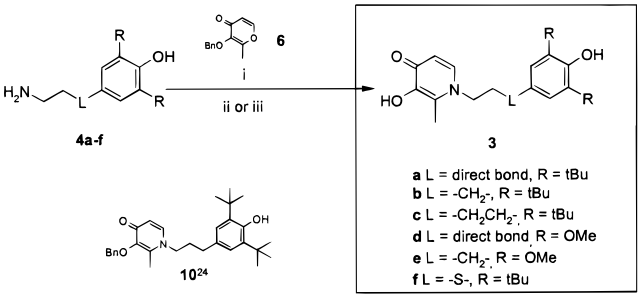
Scheme 1. Synthesis of Amines **4a**−**f**[a]



[a] Reagents and conditions: i. BH_3·SMe_2, THF, reflux, 63%; ii. H_2, Pd/C, EtOH, 50 psi, rt, 98%; iii. (a) $SOCl_2$, DMF (cat.), PhMe, CH_2Cl_2, rt, (b) NH_4OH, THF, 0 °C, 98%; iv. $LiAlH_4$, Et_2O, reflux, 65%; v. $LiAlH_4$, THF, reflux, 100%; vi. MsCl, Et_3N, CH_2Cl_2, rt, 74%; vii. NaCN, DMF, 100 °C, 63%; viii. BH_3·SMe_2, THF, reflux, 80%; ix. BnCl, K_2CO_3, DMF, 80 °C, 81%; x. $MeNO_2$, NH_4OAc, reflux, 76%; xi. $LiAlH_4$, THF, reflux, 86%; xii. MeCN, KOH, reflux, 46%; xiii. H_2, Pd/C, MeOH, rt, 94%; xiv. $LiAlH_4$, THF, reflux, 44%; xv. *n*-BuLi, TMSCl, THF, −78 °C to rt, 97%; xvi. (a) *t*-BuLi, S_8, THF, −78 to −30 °C, then 2-chloroacetamide, (b) Bu_4NF, MeOH, reflux, 57%; xvii. BH_3·SMe_2, THF, reflux, 100%.

Scheme 2. Synthesis of Compounds **3a**−**f**[a]



[a] Reagents and conditions: i. 5 N NaOH, H_2O, EtOH, **6**,[18] reflux, 24−94%; ii. H_2, Pd/C, EtOH, rt, 18−100% for **3a**−**e**; iii. BCl_3·SMe_2, CH_2Cl_2, rt, 95% for **3f**.

The ability of the compounds to protect cerebellar granule cells (CGC) from iodoacetate (IAA)-induced toxicity was measured (Table 1).[14] The 2,6-di-*tert*-butyl-substituted compounds **3a**−**c**,**f** protected cells from IAA-induced toxicity at lower concentrations than deferiprone (**7**), BHT (**1**), Trolox (**8**), and LY231617 (**9**). The 2,6-dimethoxy-substituted compounds **3d**,**e** were less effective than BHT (**1**) and LY231617 (**9**) but more effective than deferiprone (**7**) and Trolox (**8**). The relative efficacy of compounds **3a**−**e** decreased with increasing chain length. Within the 2,6-di-*tert*-butyl-substituted series, **3a**,**b** provided the best protection against IAA-induced cellular toxicity, in terms of potency and relative efficacy. Using the oxidant-sensitive fluorescent dye 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), the neuronal toxicity induced by IAA was shown to be a result of oxidative stress, and **3b** inhibited the oxidation of DCFH to DCF in a similar concentration-dependent manner to its inhibition of IAA-induced cell death, confirming that **3b** protects neuronal cells from oxidative stress (Figure 1).[21]

In addition to the significant neuroprotection offered by the 2,6-di-*tert*-butyl compounds **3a**−**f**, compound **3b** showed marked enhancement in neuroprotection over the combination of the 3-hydroxy-2-methyl-4(1*H*)-pyridinone **7** and di-*tert*-butylphenol **9**. (Note: **9** was chosen as an appropriate comparative agent for **3b** because of their similar lipophilicity.) Thus, compound **3b** (mlog *P* = 3.9) showed significantly enhanced neuroprotection

Table 1. Biological Activities of Compounds

compd	inhibition of lipid peroxidation[a] IC_{50}, μM	protection of CGC from IAA-induced oxidative stress[b] EC_{50}, μM (rel efficacy)[c]
1	5.9	6.0 (0.9)
3a[d]	0.3	0.3 (0.9)
3b[e]	1.0	0.3 (0.8)
3c	2.9	0.6 (0.7)
3d	3.3	33.3 (0.9)
3e	2.0	26.8 (0.8)
3f[f]	0.4	0.4 (0.6)
7	3.9	46.7 (1.0)
8	28.7	77.8 (0.9)
9	14.8	5.0 (0.9)

[a] Compounds were tested in duplicate, and results are the average of at least two independent experiments. [b] Compounds were tested in duplicate, and results are the average of at least three independent experiments. [c] Rel efficacy: an indication of the percent of viable cells at the maximal efficacious concentration (i.e. 1.0 = 100% viability). [d] Tested as the mesylate salt. [e] Tested as the mesylate salt. Anal. ($C_{23}H_{33}NO_3$·CH_3SO_3H) C, H; N: calcd, 2.99; found, 2.46. [f] Tested as the hydrochloride salt. Anal. ($C_{22}H_{31}NO_3S$·HCl) C, N; H: calcd, 7.57; found, 7.07.

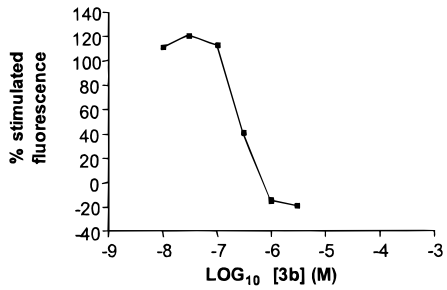


Figure 1. Inhibition of IAA-induced oxidative stress by compound **3b**. The intracellular oxidative stress induced during the IAA cell toxicity assay was measured using the oxidant-sensitive fluorescent dye 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). The nonfluorescent DCFH-DA readily crosses cell membranes whereupon it is trapped within the cytoplasm by deacetylation as the non-membrane-permeable form 2′,7′-dichlorodihydrofluorescein (DCFH). Upon oxidation, DCFH yields the highly fluorescent product 2′,7′-dichlorofluorescein (DCF). Compound **3b** was tested as its mesylate salt and inhibited IAA-induced oxidative stress with an EC_{50} = 0.28 μM.

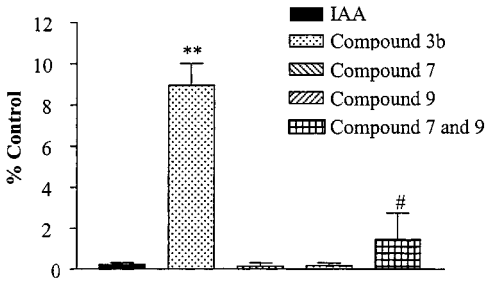


Figure 2. Inhibition of IAA-induced cell toxicity. CGC were exposed to 30 μM IAA for 30 min in a physiological salt solution. This was replaced with maintenance media containing 1 μM test compound, and the cells were tested for viability 24 h later. Compound **3b** was tested as its mesylate salt. The error bars represent SE bars. Statistical analysis was performed using two-tailed paired *t*-test. Statistical significance is defined as $p < 0.05$; **significantly different from **7** and **9** ($p < 0.01$); #significantly different from IAA alone ($p < 0.05$).

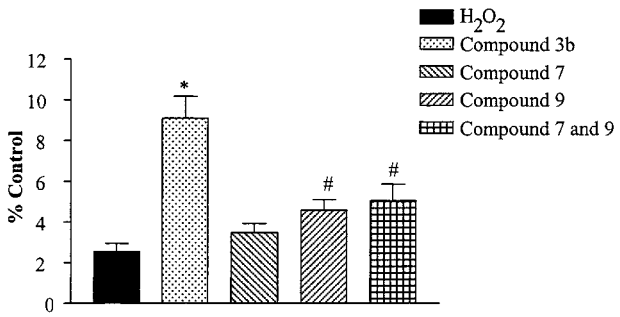


Figure 3. Inhibition of H_2O_2-induced cell toxicity. CGC were exposed to 100 μM H_2O_2 for 10 min prior to addition of test compounds at a concentration of 10 μM. The cells were tested for viability 24 h later. Compound **3b** was tested as its mesylate salt. The error bars represent SE bars. Statistical analysis was performed using two-tailed paired *t*-test. Statistical significance is defined as $p < 0.05$; *significantly different from **7** and **9** ($p < 0.05$); #significantly different from H_2O_2 alone ($p < 0.05$).

over the dual administration of **7** (mlog $P = -0.16$) and **9** (mlog $P = 3.8$) in two models of chemical-induced cell toxicity: the IAA cell toxicity assay[14] (Figure 2) and a H_2O_2 cell toxicity assay[22] (Figure 3).

Conclusion. A greater understanding of the complex multicomponent processes and mechanisms underlying neurodegenerative disorders such as stroke, traumatic brain injury, Parkinson's disease, and Alzheimer's disease has encouraged a growing trend to produce neuroprotective drugs with more than one mechanism of action.[23] This Communication describes the covalent linking of 3,5-disubstituted-4-hydroxyphenyls with 3-hydroxy-2-methyl-4(1*H*)-pyridinone, to produce molecules that are potent inhibitors of lipid peroxidation and cell toxicity. Compound **3b** (CEB-1370)[24] achieved its neuroprotective effect via inhibition of oxidative stress and displayed a superior neuroprotective action compared to the dual administration of the radical scavenger, di-*tert*-butylphenol **9**,[20] and the Fe chelator, 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridinone **7**.[19] Compounds of this series are currently under evaluation for the treatment of neurodegenerative disorders, and further data will be published in due course.[25]

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Supporting Information Available: Chemistry and biology experimental details are available free of charge via the Internet at http://pubs.acs.org.

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(24) The biological activity of the protected 3-hydroxy-4(1*H*)-pyridinone **10** (which theoretically has no potential to chelate Fe) indirectly provides an indication of the likely individual contributions afforded by the two structural units of **3b** to the inhibition of lipid peroxidation and cell toxicity (compd **10**: LP IC_{50} 4.5 μM; IAA cell toxicity EC_{50} 12.8 μM (relative efficacy 0.5)).

(25) This work is the subject of International Patent Number WO99/23075.

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