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Synthesis and Evaluation of 4-Hydroxyphenylacetic Acid Amides and 4-Hydroxycinnamamides as Antioxidants

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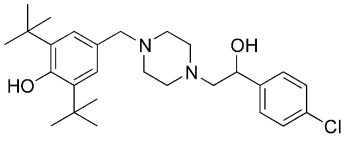
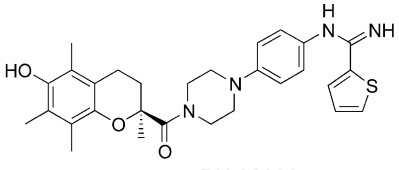
Abstract—4-Hydroxyphenylacetic acid amides and 4-hydroxycinnamamides were synthesized and their antioxidant and neuroprotective activities were evaluated. Among the prepared compounds, **6f**, **6g**, **8b**, and **9** exhibited potent inhibition of lipid peroxidation in rat brain homogenates, and marked DPPH radical scavenging activities. Furthermore, **6f**, **6g**, and **9** exhibited neuroprotective action against the oxidative damage induced by the exposure of primary cultured rat cortical cells to H_2O_2, xanthine/xanthine oxidase, or Fe^{2+}/ascorbic acid. Based on these results, we found that **6f** was the most potent antioxidant among the compounds tested.

Reactive oxygen species have been recognized to play an important role in the initiation and/or progression of various diseases such as ischemia-reperfusion injury, atherosclerosis, and inflammatory injury.[1] There is a growing interest in natural and unnatural antioxidants as a protective strategy against these diseases by block or removal of oxidative stresses.[2] α-Tocopherol is the most important and widely studied natural, lipid-soluble, and chain-breaking antioxidant. Recent studies provide evidences of protective effects of α-tocopherol against atherosclerosis[3] and reperfusion injury.[4] AM-36, a mixed antioxidant and sodium channel blocker, significantly reduced infarct volume and ischemia-induced increases in reactive oxygen species.[5] Intravenous administration of BN 80933, a dual inhibitor of neuronal nitric oxide synthase and lipid peroxidation, significantly reduced brain damage induced by head trauma in mice, global ischemia in gerbils, and transient focal ischemia in rats.[6]

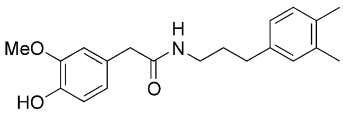
In the course of the development of new antioxidants as neuroprotective agents, we have been interested in novel 4-hydroxyphenylacetic acid amides and 4-hydroxycinnamamides based on our preliminary findings that a 4-hydroxyphenylacetic acid amide **1** has an inhibitory activity on lipid peroxidation. The structure of **1** is closely related with capsaicin, a vanilloid receptor agonist. Our previous studies suggested that **1** is a centrally acting agent with potent analgesic activity.[7] These findings prompted us to investigate the antioxidant and neuroprotective properties of a series of 4-hydroxyphenylacetic acid amides.



AM-36



BN 80933



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The present paper deals with the synthesis and the biological evaluation of a series of *N*-{3-(3,4-dimethylphenyl)-propyl}-4-hydroxyphenylacetic acid amides substituted at the C-3 position with various alkoxy groups and several *N*-{3-(3,4-dimethylphenyl)propyl}-4-hydroxy-cinnamamides.

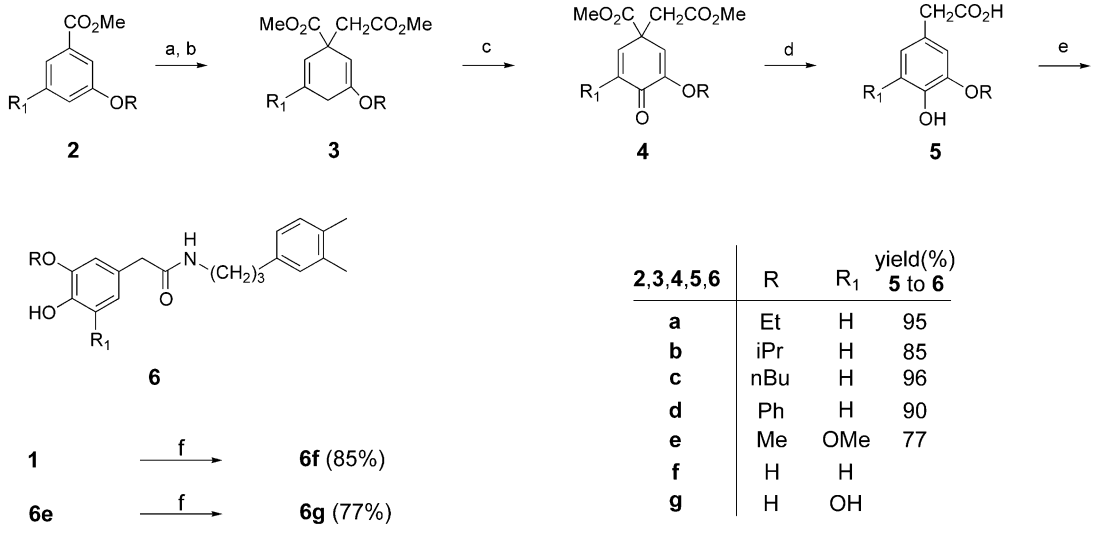
Chemistry

In the course of our research aimed at the development of new 4-hydroxyphenylacetic acid amides as antioxidants, we needed 4-hydroxyphenylacetic acids, which contain various alkoxy groups on the C-3 position of the ring. To the best of our knowledge, the synthesis of 3-alkoxy-4-hydroxyphenylacetic acids had not been reported, even though 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid) is commercially available. Thus, a general synthetic method of 3-alkoxy-4-hydroxyphenylacetic acids from methyl 3-alkoxybenzoates has been developed (Scheme 1).

Methyl 3-alkoxybenzoates **2** were easily obtained from the alkylations of methyl 3-hydroxybenzoate with the corresponding alkyl bromides or alkyl iodides for **2a**–**c** or the esterification of 3-phenoxybenzoic acid with methanol for **2d**. Reduction of methyl 3-alkoxybenzoates **2a**–**e** with potassium metal under Birch conditions, followed by alkylation with methyl bromoacetate resulted in the dienes **3a**–**e** in 64–100% yields. Allylic oxidation of the dienes **3a**–**e** with pyridinium dichromate (PDC) in benzene provided the dienones **4a**–**e** in moderate yields (43–66%). Treatment of **4a**–**e** with aqueous sodium hydroxide solution in MeOH at 0 °C followed by acidic aqueous workup provided the phenylacetic acids **5a**–**e** in high yields (64–86%). Detailed synthetic procedures from **2** to **5** were described in our previous report.[8] Coupling of the acids **5** with 3-(3,4-dimethylphenyl)propylamine using 1,3-dicyclohexycarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) in acetonitrile provided the phenylacetic acid amides **6** in 77–96% yield after flash column chromatography. Demethylation of the amide **1** and **6e** with BBr_3 in CH_2Cl_2 afforded benzenediol **6f** (85%) and benzenetriol **6g** (77%), respectively. 4-Hydroxycinnamamides (**8a**, **8b**) were also obtained from the corresponding acids (**7a**, **7b**) and 3-(3,4-dimethylphenyl)propylamine in 74 and 75% yields, and the demethylation of the amide **8a** with BBr_3 in CH_2Cl_2 afforded the amide **9** in 65% yield (Scheme 2).

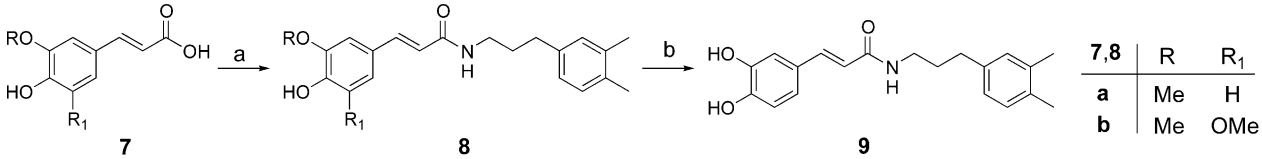
Results and Discussion

In order to evaluate antioxidant properties of the newly synthesized amides, we first examined the effects on lipid peroxidation in rat brain homogenate by thiobarbituric acid reactive substances (TBARS) assay[9] according to the method of Stocks.[10] The formation of lipid peroxides was significantly inhibited by the test compounds. The IC_{50} values are shown in Table 1. Regarding the alkoxy substituents at the C-3 position of amides (**1**, **6a**–**6c**), longer alkoxy groups enhanced the inhibition although the potency is still lower than that of 2,6-di-*tert*-butyl-4-methylphenol (BHT) or α-tocopherol (Table 1). However, phenoxy substituent (**6d**) retreated the activity. In the comparison of –OMe



2,3,4,5,6	R	R_1	yield(%) 5 to 6
a	Et	H	95
b	iPr	H	85
c	nBu	H	96
d	Ph	H	90
e	Me	OMe	77
f	H	H	
g	H	OH	

Scheme 1. (a) K, *t*-BuOH, NH_3, THF, −78 °C, 1 h; (b) $BrCH_2CO_2CH_3$, piperylene, rt, 30 min; (c) PDC, Celite, 90% *t*-BuOOH, C_6H_6, rt, 6 h; (d) NaOH, MeOH, 0 °C to rt, 4 h; (e) 3-(3,4-dimethylphenyl)propylamine, HOBT, DCC, acetonitrile, 0 °C to rt; (f) BBr_3, CH_2Cl_2, 0 °C.



7,8	R	R_1
a	Me	H
b	Me	OMe

Scheme 2. (a) 3-(3,4-Dimethylphenyl)propylamine, HOBT, DCC, acetonitrile, 0 °C to rt; (b) BBr_3, CH_2Cl_2, 0 °C.

versus $-(OMe)_2$ (**1** vs **6e**, **8a** vs **8b**), the compounds with two methoxy groups at C-3 and C-5 showed better inhibition than those with one methoxy group at the C-3 position. In general, 4-hydroxycinnamamides (**8**, **9**) exhibited higher potency of inhibition than phenylacetic acid amides **6**. Among the tested compounds, the amides with $-(OH)_2$ or $-(OH)_3$ (**6f**, **6g**, **9**) inhibited lipid peroxidation most potently.

The radical scavenging effects were also examined in the present study using radicals generated by 1,1-diphenyl-2-picryl hydrazyl (DPPH).[11] As shown in Table 1, the amides with $-(OH)_2$ or $-(OH)_3$ (**6f**, **6g**, **9**) were the most potent radical scavengers in this series.

Given the suggested roles of lipid peroxides and free radicals in neuronal death after ischemia or in neurodegenerative disorders,[12] we next examined whether the test compounds protect neurons from oxidative stress evoked by H_2O_2, xanthine/xanthine oxidase (X/XO), or Fe^{2+}/ascorbic acid (Fe^{2+}/AA)[13] in primary cultured rat cortical cells.[14] These oxidative insults generate reactive oxygen species including superoxide and hydroxyl radicals, which ultimately induces 70–90% neuronal damage measured by the leakage of cytoplasmic enzyme lactate dehydrogenase (LDH). The H_2O_2- and X/XO-induced neurotoxicity was dramatically inhibited by the simultaneous exposure to various concentrations of **6f**, **6g**, and **9** during the oxidative insults (Fig. 1A). The order of potency was **9** > **6f** > **6g**. As illustrated in Figure 1B, these three compounds also protected neurons from the toxicity induced by Fe^{2+}/AA. The neuroprotection was concentration-dependent at the range of 0.1 ~ 30 μM. However, **9** and **6g** were no more protective at 100 μM (Fig. 1B), implying their intrinsic toxicity at this concentration. In contrast, **6f** was highly potent and seemed to be nontoxic at this concentration because its neuroprotective effect was still maintained (Fig. 1B). The IC_{50} values were calculated from the data in Figure 1 by nonlinear regression using Prism (Table 2). The IC_{50} values of Trolox were included for comparisons. All the other compounds did not show any significant neuroprotective action, except that **6b**, **6c** and **8b** were shown to inhibit the Fe^{2+}/AA-induced toxicity with the IC_{50} values of 34.2, 4.2 and 5.4 μM, respectively.

Taken together, **6f**, **6g**, **8b**, and **9** were found to exhibit potent inhibition of lipid peroxidation. Among these compounds, **6f**, **6g**, and **9**, the amides with $-OH)_2$ or $-(OH)_3$, were the potent DPPH radical scavengers. Although all the three compounds exhibited protective action against oxidative stress-induced neurotoxicity in cortical cultures, **6f** was the most potent and appeared to be the most promising compound as a potential neuroprotective antioxidant.

Table 1. Inhibition of lipid peroxidation by the newly synthesized amides and their DPPH radical scavenging activities

Compd	R	R_1	Inhibition of lipid peroxidation (IC_{50}, μM)	DPPH radical scavenging activity (IC_{50}, μM)
1			25.90	178.6
6a	Et	H	23.96	182.1
6b	*i*Pr	H	19.38	>300
6c	*n*Bu	H	9.28	161.7
6d	Ph	H	32.01	>300
6e	Me	OMe	11.77	50.1
6f	H	H	2.60	31.0
6g	H	OH	1.81	21.3
8a	Me	H	9.94	82.2
8b	Me	OMe	3.41	89.1
9	H	H	1.11	34.3
α-Tocopherol			4.68	—
BHT			4.03	—

Table 2. IC_{50} values determined from inhibition of the H_2O_2-, X/XO-, or Fe^{2+}/AA-induced oxidative neuronal damage in primary cultured rat cortical cells

Compd	IC_{50}, μM (H_2O_2)	IC_{50}, μM (X/XO)	IC_{50}, μM (Fe^{2+}/AA)
6f	6.03	3.30	2.40
6g	34.99	34.82	26.80
9	5.91	2.54	23.70
Trolox[a]	329.61	201.84	430.53

[a]6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

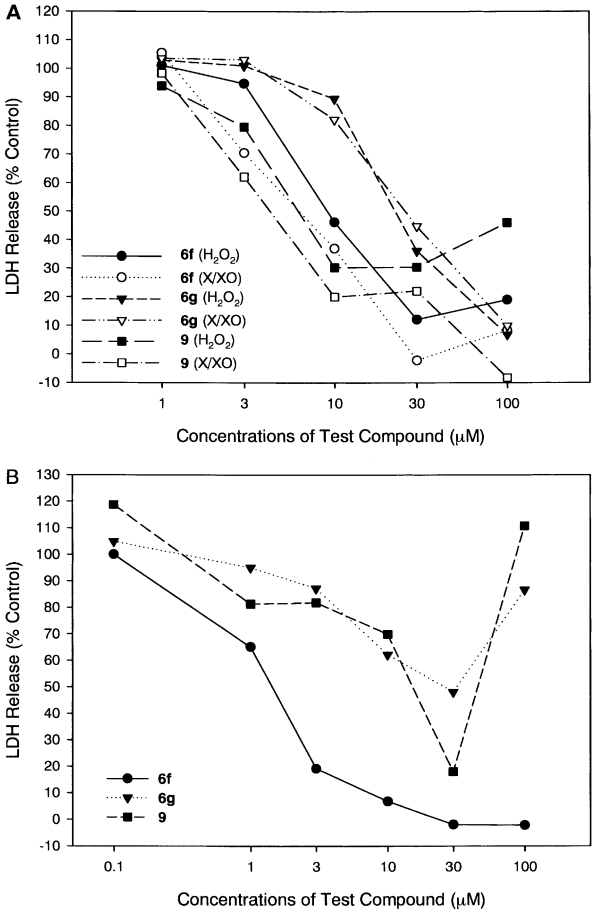


Figure 1. Inhibition of the oxidative neuronal damage induced by H_2O_2 or X/XO (A), and by Fe^{2+}/AA (B) in primary cultured rat cortical cells. Primary cultured rat cortical cells were exposed to H_2O_2, X/XO, or Fe^{2+}/AA in the absence or presence of various concentrations of test compounds, and the neuronal damage was determined as described.[13] Data were calculated as percent of control LDH activity released into the culture medium treated with H_2O_2, X/XO, or Fe^{2+}/AA, respectively. Each point represents the mean value from 4–6 measurements.

In conclusion, we have synthesized 4-hydroxyphenylacetic acid amides and 4-hydroxycinnamamides, and evaluated their antioxidant activities. Compounds exhibiting potent inhibition of lipid peroxidation as well as DPPH radical scavenging activity were selected, and their structure–activity relationships were discussed. Furthermore, we examined the ability to protect neurons from various oxidative stresses in primary cultured rat cortical cells. We found that **6f** is the most potent and promising antioxidant exerting prominent neuroprotective action. Based on these results, **6f** may provide a useful therapeutic potential for the treatment of neurodegenerative disorders caused by oxidative stress.

Acknowledgements

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9. **TBARS**: Brain tissue from male Sprague–Dawley rats (about 10-weeks old) was obtained after decapitation and homogenated in ice-cold 10 mM Tris–HCl buffer (pH. 7.4) using a Teflon-glass homogenizer. The homogenate was centrifuged for 10 min at 1000 × *g*, and the supernatant was used in the test. Lipid peroxidation was stimulated in assays containing 250 μL rat brain homogenate by additions of 0.02 mM $FeCl_2$ and 0.25 mM ascorbic acid, and the mixture was incubated for 30 min at 37 °C. The reaction was stopped by the addition of 0.05 mL of 35% perchloric acid. After centrifugation for 10 min at 1000 × *g*, 200 μL of the resulting supernatant was added to 100 μL of an aqueous solution containing 0.5% TBA and reacted at 80 °C for 1 h. Then, the reaction mixture was cooled to room temperature, and its absorbance at 532 nm was measured. Typical TBARS formation in brain homogenate was calculated from the absorbance at 532 nm using 1,1,3,3-tetraethoxypropane as an external standard.
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13. Cultures (12–14 days in vitro) were washed with HEPES-buffered salt solution (HBSS) three times and exposed to 100 M H_2O_2 for 5 min or X(0.5 mM)/XO(10 mU/mL) for 10 min, or Fe^{2+}(100 μM)/AA(25 μM) for 2 h. After the exposure, cultures were washed with HBSS and maintained in MEM supplemented with 21 mM glucose at 37 °C in a humidified atmosphere of 95% air/5% CO_2. After 20–24 h, neuronal damage was quantified by measuring LDH activity released into the culture media. For evaluation of neuroprotective action, cultures were exposed simultaneously to the various concentrations of test compounds during the oxidative insults.
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