Arch Pharm Res Vol 29, No 6, 469-475, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

# Synthesis and Evaluation of Biological Properties of Benzylideneacetophenone Derivatives

**Seikwan Oh[1,2], Soyong Jang[1], Donghyun Kim[2], Inn-Oc Han[3], and Jae-Chul Jung**

*Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, P.O. Box 1848, University, MS 38677-1848, U.S.A., [1]Department of Neuroscience, College of Medicine, Ewha Womans University, Seoul 158-710, Korea, [2]Medical Science Institute, Ewha Womans University, Seoul 158-710, Korea, and [3]Department of Physiology, College of Medicine, Inha University, Inchon, Korea*

*(Received February 20, 2006)*

A series of yakuchinone B **1f** and its analogs **1a-e** was synthesized and evaluated for free radical scavenging, suppression of LPS-induced NO generation, cytotoxicity and anti-excitotoxicity in vitro. Compound **1c** exhibited potent anti-excitotoxicity, while all compounds **1a-f** showed considerable effects of free radical scavenging, suppression of LPS–induced NO generation, and cytotoxicity in microglia.

**Key words:** Benzylideneacetophenones, Synthesis, Grignard reaction, NO generation, Free-radical scavenging, Cytotoxicity, Anti-excitotoxicity

## INTRODUCTION

Yakuchinone B **1f** and related enones **1a-e** (Fig. 1) showed several significant biological activities such as anti–inflammatory (Srimal and Dhawan, 1973), antitumor (Samaha *et al.*, 1997; Huang *et al.*, 1994; Ohtsu *et al.*, 2002), antibacterial (Hogale *et al.*, 1986), antiviral (Ninomiya *et al.*, 1990), and gastric protective activities (Murakami *et al.*, 1990). Recently, interest in these compounds has increased due to their ability to scavenge active oxygen (Sreejayan Rao, 1994), nematocidal activity (Kiuchi *et al.*, 1993), inhibition of lipid peroxidation (Arty *et al.*, 2000), and inhibition of acyl-CoA (Ohishi *et al.*, 2001). Furthermore, accumulating evidence suggests that endogenous excitatory amino acids, especially glutamate, play an important role in the neuronal degeneration associated with some neurological diseases, such as ischemia, Parkinson's disease, Alzheimer disease, and Huntington's disease.

In these neurodegenerative diseases, glutamate is excessively released and then activates the glutamate receptor. Overactivation of the glutamate receptors may induce elevation of intracellular $Ca^{2+}$ levels, resulting in activation of $Ca^{2+}$-dependent proteases and kinases. Also, high intracellular $Ca^{2+}$ may activate nitric oxide synthase, resulting in excessive production of nitric oxide (NO) and cytotoxicity.

Although many reagents, such as glutamate receptor antagonists, $Ca^{2+}$ channel antagonists, anti–inflammatory agents and nitric oxide synthase (NOS) inhibitors, have neuroprotective effects, their serious side effects limit their clinical application. Antioxidants also play an important role in biological defense mechanisms. According to the accumulated research data, activated oxygen is thought to be a major factor in cytotoxicity. Therefore, research was undertaken to search for novel compounds with better neuroprotective effects and less neurotoxicity. It has been shown that 4-hydroxy-3-methoxycinnamaldehyde (**2**) has antioxidant properties; thus we reasoned that its analogs, specifically 4-hydroxy-3-methoxycinnanamic acid (ferulic acid), could be applicable to neurodegenerative diseases.

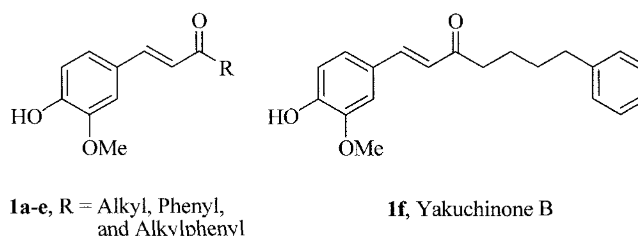



**Fig. 1.** Structures of yakuchinone B **1f** and related enones **1a-e**

Correspondence to: Jae-Chul Jung, Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, P.O. Box 1848, University, MS 38677-1848, U.S.A.
Tel: 1-662-915-1145, Fax: 1-662-915-5638
E-mail: jcjung@olemiss.edu

In this paper, we describe the synthesis of yakuchinone B **1f** and its derivatives **1a-e** and assess their biological activities for anti-excitotoxicity, free-radical scavenging, and suppression of LPS-induced NO generation *in vitro*.

## MATERIALS AND METHODS

### Chemical synthesis

#### General methods

Reactions requiring anhydrous conditions were performed with the usual precautions for rigorous exclusion of air and moisture. Thin layer chromatography (TLC) was performed on precoated silica gel G and GP uniplates from Analtech and visualized with a 254 nm UV light. Flash chromatography was carried out on silica gel 60 [Scientific Adsorbents Incorporated (SAI), particle size 32-63 μm, pore size 60 Å]. $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Bruker DPX 400 at 400 MHz and 100 MHz, respectively. The chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane, and *J*-values were in Hz. Infrared (IR) spectra were obtained on an ATI Mattson FT/IR spectrometer. Mass spectra were recorded with a Waters Micromass ZQ LC-Mass system, and high resolution mass spectra (HRMS) were measured with a Bruker BioApex FTMS system by direct injection using an electrospray interface (ESI). When necessary, chemicals were purified according to the reported procedures (Perrin *et al.*, 1980).

### Chemistry

#### General procedure for the preparation of enones (1a-f)

Method A: To a stirred solution of **6a-f** (5.0 mmol) in dry THF (80 mL) was added dropwise TBAF (10.0 mmol, 1 M solution in THF) at room temperature under argon atmosphere, and the mixture was stirred at room temperature for 10 min. The reaction mixture was diluted with EtOAc (30 mL) and washed with brine (70 mL). The organic layer was separated, and the aqueous phase was extracted with EtOAc (2×30 mL). The combined organic phases were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give alcohols, which were purified by flash column chromatography (silica gel, 20-30% ethyl acetate/hexanes) to afford **1a-f**.

Method B: To a solution of enones **6a-f** (2.5 mmol) in dry dichloromethane (30 mL) was added dropwise TFA (5.6 g, 10% solution in dichloromethane) at 0°C under argon atmosphere, and the mixture was stirred at this temperature for 1 h. The reaction mixture was diluted with dichloromethane (10 mL) and treated with 50% aqueous NaCl solution (20 mL). The organic layer was separated, and the aqueous phase was extracted with dichloromethane (2×20 mL). The combined organic phases were washed with saturated aqueous $Na_2SO_4$ solution (20 mL) and again with brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give alcohols, which were purified by flash column chromatography (silica gel, 20-30% ethyl acetate/hexanes) to afford **1a-f**.

#### (3*E*)-4-(4-Hydroxy-3-methoxyphenyl)but-3-en-2-one (1a)

$R_f$ = 0.3 (30% ethyl acetate/hexanes); mp 126°C [(Bocchini *et al.*, 1992), 127-128°C]; IR (neat, NaCl) 3408, 2975, 1730, 1665, 1481, 1248, 1080 cm$^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J*=12.0 Hz, 1H), 7.07 (t, *J*=4.8 Hz, 2H), 6.93 (d, *J*=5.4 Hz, 1H), 6.59 (d, *J*=12.0 Hz, 1H), 5.71 (brs, 1H), 3.92 (s, 3H), 2.37 (s, 3H); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 198.9, 148.8, 147.4, 144.2, 127.2, 125.3, 123.9, 115.3, 109.9, 56.3, 27.6; HRMS calcd. for $C_{11}H_{13}O_3$: 193.0865 $[M+H]^+$, found: 193.0878.

#### (1*E*)-1-(4-Hydroxy-3-methoxyphenyl)hept-1-en-3-one (1b)

$R_f$ = 0.4 (20% ethyl acetate/hexanes); semisolid; IR (neat, NaCl) 3090, 2985, 2875, 1732, 1473, 1254, 1089 cm$^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J*=16.0 Hz, 1H), 7.12-7.04 (m, 2H), 6.94 (d, *J*=8.0 Hz, 1H), 6.62 (d, *J*=16.0 Hz, 1H), 5.43 (brs, 1H), 3.93 (s, 3H), 2.66 (t, *J*=7.5 Hz, 2H), 1.70–1.64 (m, 2H), 1.42–1.36 (m, 2H), 0.95 (t, *J*=7.5 Hz, 3H); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 200.4, 148.1, 146.8, 142.6, 126.9, 123.9, 123.3, 114.9, 109.5, 56.2, 40.7, 27.0, 22.6, 14.3; HRMS calcd. for $C_{14}H_{19}O_3$: 235.1334 $[M+H]^+$, found: 235.1351.

#### (2*E*)-3-(4-Hydroxy-3-methoxyphenyl)phenylpro-2-en-1-one (1c)

$R_f$ = 0.4 (20% ethyl acetate/hexanes); mp 86-87°C [(Arty *et al.*, 2000), 85-90°C]; IR (neat, NaCl) 3321, 3001, 2960, 1739, 1656, 1420, 1285, 1038 cm$^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J*=5.4 Hz, 2H), 7.76 (d, *J*=11.7 Hz, 1H), 7.58 (t, *J*=5.1 Hz, 1H), 7.50 (t, *J*=5.4 Hz, 2H), 7.38 (d, *J*=11.7 Hz, 1H), 7.21 (d, *J*=6.0 Hz, 1H), 7.14 (s, 1H), 6.96 (d, *J*=6.0 Hz, 1H), 5.66 (brs, 1H), 3.95 (s, 3H); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 191.1, 149.0, 147.4, 145.7, 138.9, 132.9, 128.9, 128.8, 127.8, 123.8, 120.1, 115.4, 110.6, 56.4; HRMS calcd. for $C_{16}H_{15}O_3$: 255.1051 $[M+H]^+$, found: 255.1038.

#### (1*E*)-1-(4-Hydroxy-3-methoxyphenyl)-5-phenylpent-1-en-3-one (1d)

$R_f$ = 0.3 (20% ethyl acetate/hexanes); IR (neat, NaCl) 3365, 2957, 2868, 1733, 1665, 1471, 1254, 1092 cm$^{-1}$; $^1$H-NMR (Green *et al.*, 1982) (400 MHz, $CDCl_3$) δ 7.48 (d, *J*=16.1 Hz, 1H), 7.32-7.15 (m, 5H), 7.04 (t, *J*=5.7 Hz, 2H), 6.91 (d, *J*=8.1 Hz, 1H), 6.51 (d, *J*=16.1 Hz, 1H), 5.36 (brs,

1H), 3.89 (s, 3H), 2.99 (s, 4H); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 199.9, 149.0, 147.6, 143.6, 141.7, 128.9, 128.8, 127.2, 126.5, 124.2, 123.9, 115.5, 110.1, 56.3, 42.6, 30.7; HRMS calcd. for $C_{18}H_{19}O_3$: 283.2334 $[M+H]^+$, found: 283.2355.

### (1*E*)-1-(4-Hydroxy-3-methoxyphenyl)-6-phenylhex-1-en-3-one (1e)

$R_f$ = 0.3 (20% ethyl acetate/hexanes); IR (neat, NaCl) 3354, 3007, 2956, 1739, 1649, 1460, 1266, 1048 $cm^{-1}$; $^1$H-NMR (Green *et al.*, 1982) (400 MHz, $CDCl_3$) δ 7.44 (d, *J*=16.1 Hz, 1H), 7.28 (t, *J*=6.6 Hz, 2H), 7.19 (t, *J*=6.9 Hz, 3H), 7.04 (t, *J*=5.0 Hz, 2H), 6.91 (d, *J*=8.1 Hz, 1H), 6.58 (d, *J*=16.1 Hz, 1H), 5.39 (brs, 1H), 3.90 (s, 3H), 2.67 (q, *J*=7.6 Hz, 3H), 2.05-1.96 (m, 3H); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 200.7, 148.9, 147.4, 143.3, 142.1, 128.9, 128.8, 127.3, 126.3, 124.3, 123.8, 115.4, 110.0, 56.3, 40.1, 35.6, 26.3; HRMS calcd. for $C_{19}H_{21}O_3$: 297.1491 $[M+H]^+$, found: 297.1479.

### (1*E*)-1-(4-Hydroxy-3-methoxyphenyl)-7-phenylhept-1-en-3-one (1f)

$R_f$ = 0.3 (20% ethyl acetate/hexanes); mp 74-75°C [(Bicking *et al.*, 1976), 70-76°C); IR (neat, NaCl) 3043, 3002, 2975, 1731, 1667, 1458, 1292, 1086 $cm^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J*=16.1 Hz, 1H), 7.26 (t, *J*=6.8 Hz, 2H), 7.16 (t, *J*=7.2 Hz, 3H), 7.06 (t, *J*=6.1 Hz, 2H), 6.91 (d, *J*=8.1 Hz, 1H), 6.59 (d, *J*=16.1 Hz, 1H), 5.48 (brs, 1H), 3.90 (s, 3H), 2.65 (q, *J*=4.9 Hz, 3H), 1.77–1.55 (m, 3H), 1.27 (t, *J*=6.3 Hz, 2H); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 200.8, 148.9, 147.5, 143.2, 142.7, 128.8, 128.7, 127.3, 126.1, 124.3, 123.8, 115.4, 110.0, 56.3, 40.9, 36.2, 31.5, 24.6; HRMS calcd. for $C_{20}H_{23}O_3$: 311.1647 $[M+H]^+$, found: 311.1662.

## Biology

### DPPH (diphenylpicrylhydrazyl) radical scavenging effects

To test the free radical scavenging effects by using DPPH, compounds were adjusted with ethanol solution to final concentration of 0.1-100 μM. Acetic acid buffer (0.1 mM) was added, and the mixture was warmed in a water bath at 25°C. After 5 min, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical ethanol solution (1 mL, 0.2 mM) was added. After 30 min, absorbance was measured with a spectrophotometer (517 nm). The DPPH radical scavenging rate of each sample and the 50% scavenging concentration based on the DPPH radical scavenging rate were calculated using the following formula.

DPPH radical-scavenging rate (%)

$$= \left\{1 - \frac{A-C}{B}\right\} \times 100$$

**DPPH radical–scavenging rate (%)**

Where A is the absorbance of the sample when a blank was substituted for ethanol, B is the absorbance of the sample when a color-contrast agent was substituted for ethanol in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-ethanol solution, and C is the absorbance of the color-contrast agent alone.

### Cell culture

Cerebral cortices were removed from the brains of 15-day-old fetal mice. The neocortices were triturated and plated on 24-well plates (with approximately $10^5$ cells/culture well) precoated with 100 μg/mL poly-D-lysine and 4 μg/mL laminine, in Eagle's minimal essential media (Earle's salts, supplied glutamine-free), and supplemented with horse serum (5%), fetal bovine serum (5%), 2 mM glutamine, and 21 mM glucose. Cultures were maintained at 37°C in a humidified atmosphere of 5% $CO_2$. After 7 days *in vitro* (DIV), the cultures were shifted to the plating media containing 10 μM cytosine arabinoside without fetal serum. Cultures were then fed twice per week. Mixed cortical cell cultures containing neurons and glia (DIV 16-22) were exposed to excitatory amino acid (glutamate) in Eagle's minimal essential media supplemented with 21 mM glucose and 26.5 mM bicarbonate. The morphology of the degenerating neurons was observed under a phase contrast microscope over the next 24 h.

The murine BV2 cell line (a generous gift from Tong Joh, Burke Institute, Cornell University, NY, U.S.A.), which becomes immortal after infection with a *v-raf/v-myc* recombinant retrovirus, exhibits phenotypic and functional properties of reactive microglial cells (Yoon *et al.*, 2001). BV2 cells were maintained at 37°C and 5% $CO_2$ in DMEM (dulbecco's modified eagle medium) supplemented with 10% heat-inactivated endotoxin-free FBS, 2 mM glutamine, 100 μg/mL streptomycin, and 100 U/mL penicillin. BV2 cells were grown in 24-well plates at a concentration of 5× $10^4$ cells/well followed by proper treatment. Morphological change was examined under phase-contrast microscopy.

### Nitrite assay

NO production from activated BV2 cells was determined by measuring the amount of nitrite, a relatively stable oxidation product of NO, as described previously (Connor and Flynn, 1990). Cells were incubated with or without LPS (lipopolysaccharide, 100 ng/mL) in the presence or absence of various concentrations of compounds for 24 h. The nitrite accumulation in the supernatant was assessed by Griess reaction. In brief, an aliquot of the conditioned medium was mixed with an equal volume of 1% sulfanilamide in water and 0.1% *N*-1-naphthylethylenediamine dihydrochloride in 5% phosphoric acid. The absorbance was determined at 570 nm in an automated microplate reader.

### Cell viability

To measure the BV2 cell viability, 50 μL of 5 mg/mL MTT [3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoilum bromide] in growth medium was added to each 24-well. After incubation for 90 min at 37°C with MTT, cell medium was removed. The precipitated formazan, a product of the MTT tetrazolium ring by the action of mitochondrial dehydrogenases, was solubilized with DMSO and quantified spectrophotometrically at 550 nm. Cortical neuronal cell number and viability were assessed using the reagent WST-1 (Roche, Indianapolis. IN). This colorimetric assay measures the metabolic activity of viable cells based on cleavage of the tetrazolium salt WST-1 substrate 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio-1,3-benzene disulphonate] into formazan by mitochondrial dehydrogenase in live cells. This was followed by incubation with WST-1 reagent at a dilution of 1:10 in the original conditioned media at 37°C for 2 h. After thorough shaking, the formazan produced by the metabolically active cells in each sample was measured at a wavelength of 450 nm and a reference wavelength 650 nm. Absorbance readings were normalized against control wells with medium alone. Neuronal death was analyzed 24 h later, and the percentage of neurons undergoing actual neuronal death was normalized to the mean value that is found after a 24 h exposure to 500 μM NMDA (N-methyl-D-aspartate, defined as 100) or a sham control (defined as 0).

### Scavenging activity

DPPH radicals are widely used for the preliminary screening of compounds capable of scavenging activated oxygen species, since they are much more stable and easier to handle than oxygen free radicals.

### Anti–neurotoxicity

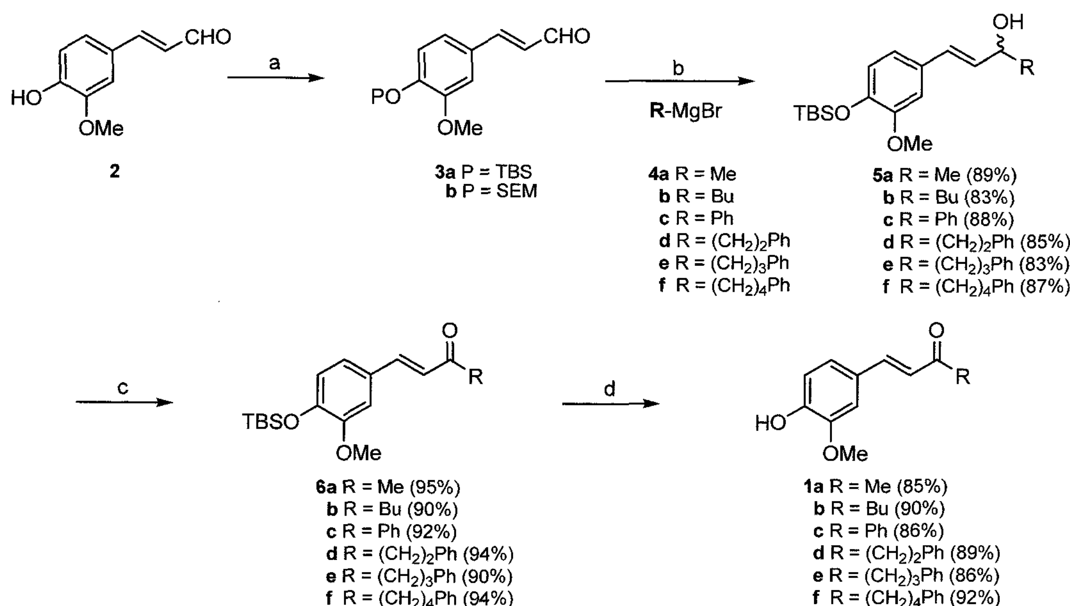
During tests for the inhibition ofneurotoxicity, exposure of cortical cell cultures to 500 μM NMDA (the prototypical glutamate receptor agonist) resulted in a rapid swelling of the neuronal cell body within 2 h and caused 90 to 100% neuronal death over the next day. Glutamate at 60 mM produced 60% neurotoxicity after 24 h exposure in cultured neurons.

## RESULTS AND DISCUSSION

### Synthesis

The synthesis of enones **1a**-**f** was initiated by protecting the 4-hydroxy-3-methoxy cinnamaldehyde (**2**) with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) in the presence of 2,6-lutidine or 2-(trimethylsilyl)ethoxymethyl chloride (SEM-Cl)/*N*,*N*-diisopropylethylamine (DIPEA) to form aldehydes **3a**-**b** in 95% and 97% yields, respectively.

Treatment of aldehyde **3a** with several Grignard reagents [(**4a**-**f**), commercially available; methylmagnesium bromide, butylmagnesium bromide, and phenylmagnesium bromide; **4a**-**c**, freshly prepared; phenethylmagnesium bromide, 3-phenylpropylmagnesium bromide, and 3-phenylbutylmagnesium bromide, **4d**-**f**; phenylalkyl bromides were treated




**Scheme 1.** (a) TBSOTf (1.5 equiv), 2,6–lutidine (2.0 equiv), $CH_2Cl_2$, 0°C, 30 min, 95%, or SEM–Cl (1.2 equiv), DIPEA (1.5 equiv), $CH_2Cl_2$, 0°C to rt, 1 h, 97%; (b) R-MgBr (2.0 equiv), THF, -78°C, 20 min, then -78°C to rt, 30 min, (83%-89%); (c) $MnO_2$ (10.0 equiv), pentane, reflux, 2 h, (90%-95%); or Dess-Martin periodinane, $CH_2Cl_2$, rt, 30 min, (88%); or TPAP (5 mol %), NMO (1.5 equiv), molecular sieves (powder), $CH_2Cl_2$, 0°C, 15 min, (90%); or DMSO (2.4 equiv), $(COCl)_2$ (1.2 equiv), TEA (4.8 equiv), $CH_2Cl_2$, -78°C, 1 h, (85%); (d) TBAF (2.0 equiv), THF, rt, 10 min, or 10% TFA, $CH_2Cl_2$, 0°C, 1 h, (85%-92%).

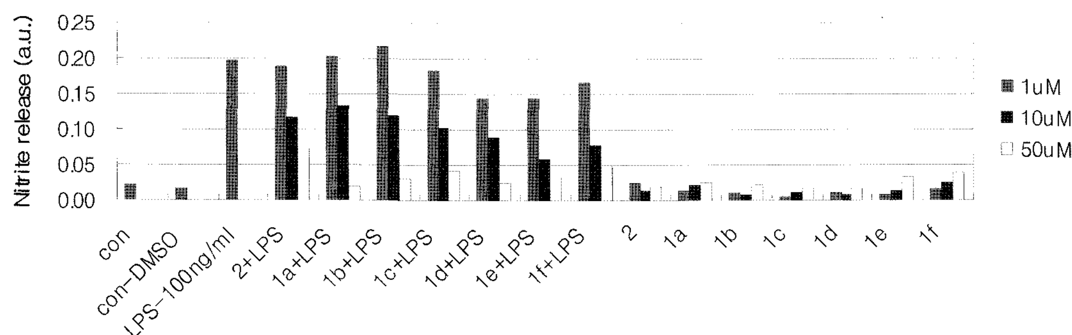



**Fig. 2**. Suppression of NO production in LPS–treated BV2 cells. The cells were treated with 100 ng/mL of LPS only or LPS plus different concentrations (1, 10, 50 μM) of compounds **1a-1f** and **2** at 37°C for 24 h. At the end of incubation, 50 μL of the medium was removed to measure nitrite production.

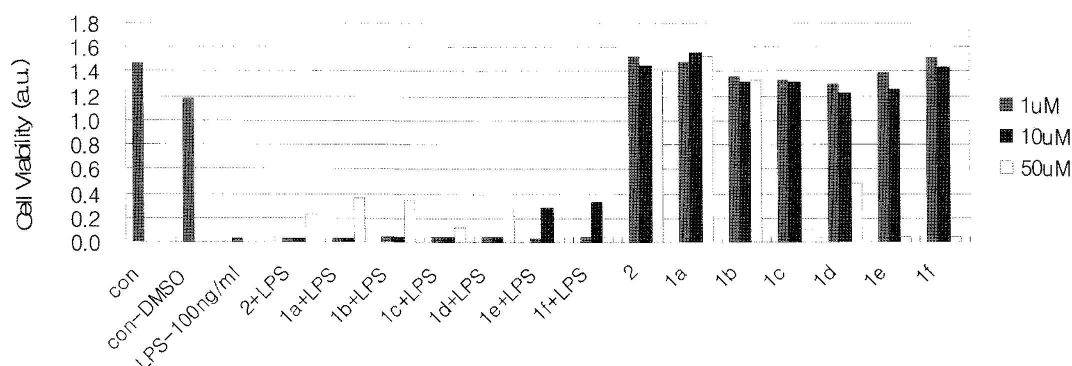



**Fig. 3**. Inhibition of cytotoxicity in LPS–treated BV2 cells. The cells were treated with 100 ng/mL of LPS only or LPS plus different concentrations (1, 10, 50 μM) of compounds **1e-f** and **1a-d** at 37°C for 24 h. After incubation of cell with 50 μL of 5 mg/mL MTT for 90 min, the precipitated formazan was solubilized with DMSO and quantified spectrophotometrically.

with activated magnesium (Mg) in 1,2-dichloroethane at 150°C for 2 h, under argon atmosphere] gave secondary alcohols **5a-f** in good yields. Alcohols **5a-f** were oxidized by manganese dioxide ($MnO_2$) to give ketones **6a-f** in excellent yields. In this stage, oxidation of **5a-f** was also accomplished using Dess-Martin periodinane (DMP) (Dess and Martin 1983), tetrapropylammonium perruthenate (TPAP)/*N*-methylmorpholine (NMO) (Ley *et al.*, 1994) and Swern [$(COCl)_2$, DMSO, TEA] conditions (Mancuso *et al.*, 1978; Jung *et al.*, 2004). Although these latter conditions were more convenient for scale-up due to shorter reaction time and ease of handling, manganese dioxide oxidation afforded a superior yield. Subsequent deprotection of ketones **6a-f** was accomplished with 2 equivalents of tetrabutylammonium fluoride (TBAF) in THF at ambient temperature or 10% trifluoroacetic acid (TFA) in dry $CH_2Cl_2$ at 0°C for 1 h, to afford 4-hydroxy-3-methoxyphenyl enones **1a-f** in high yields (Scheme 1).

## Biological properties

Compounds **1a-f** and **2** (10, 50 μM) showed considerable suppression of LPS-induced NO generation (Fig. 2).

Compounds **1e-f** (10 μM) and **1a-d** (50 μM) showed considerable suppression of LPS-induced cytotoxicity (Fig. 3).

The test results of the scavenging ratio of DPPH radicals of each compound are shown in Table I. By employing the DPPH radical test, favorable scavenging ratios were found in all of compounds **1a-f** and **2** ranging from 50-80% in 100 μM.

Excitotoxic neuronal death was prevented by inclusion of 10 μM and 100 μM of compounds **1a**, **1c**, and **2**. Among these compounds, the (2*E*)-3-(4-hydroxy-3-methoxyphenyl)

**Table I**. Rate of scavenging DPPH radical of derivatives **1a–f** and **2**

| Compds | DPPH radical scavenging activity (%) | | | |
|---|---|---|---|---|
| | 0.1 μM | 1 μM | 10 μM | 100 μM |
| **1a** | 0.7 | 4.9 | 6.80 | 53.8 |
| **1b** | 1.7 | 5.3 | 16.8 | 72.1 |
| **1c** | 2.6 | 6.6 | 17.3 | 73.0 |
| **1d** | 0.7 | 2.9 | 17.3 | 77.8 |
| **1e** | 3.5 | 3.0 | 16.8 | 77.8 |
| **1f** | 1.6 | 7.2 | 22.1 | 80.4 |
| **2**[a] | 1.4 | 2.8 | 18.5 | 64.6 |

[a]Compound **2** was 4-hydroxy-3-methoxycinnamaldehyde as a compared material.

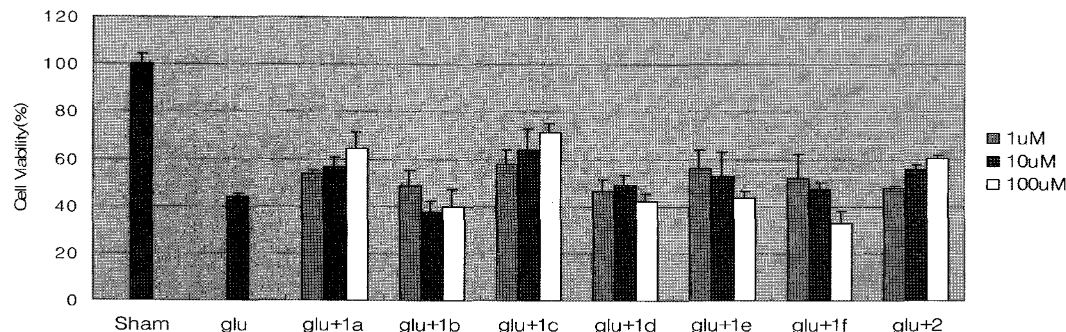



**Fig. 4.** Inhibition of glutamate-induced neurotoxicity in cultured cortical neurons. Glutamate (60 μM) or compounds **1a-f** and **2** were applied for 24 h at 37°C. After incubation of neurons with WST-1 for 2 h, the precipitated formazan was solubilized with DMSO and quantified spectrophotometrically. All values represent mean ± S. E. (or SD? Be precise.) of three independent experiments performed in triplicate.

phenylpro-2-en-1-one (**1c**) showed the most potent anti-neurotoxicity (Fig. 4).

In summary, an efficient preparation and biological evaluation of yakuchinone B **1f** and its derivatives **1a-e** have been described. Compounds **1a-f** and **2** show similar free radical scavenging activity and inhibition of nitrite generation. However, compounds **1a-f** and **2** show better anti-cytotoxicity after LPS stimulation in BV2 cells. Interestingly, compounds **1a**, **1c** and **2** exhibited the most potent preventive effect on glutamate–induced neurotoxicity in cultured neurons. These results suggest that benzylideneacetophenones **1a-f** show relatively good antioxidant effects, which might be due to the hydroxy group and methoxy group of benzene; however, the length of the R group in the benzylideneacetophenones **1a-f** would be important to show anti–excitotoxicity. We propose that compounds with a relatively short R group (i.e., hydrogen, methyl, phenyl) would show good anti–excitotoxicity in cultured neurons.

## ACKNOWLEDGEMENTS

This work has been supported by a KOSEF Brain Neurobiology Grant (2005).

## REFERENCES

Arty, I. S., Timmerman, H., Samhoedi, M., Sastrohamidjojo, Sugiyanto, and van der Goot, H., Synthesis of benzylideneacetophenones and their inhibition of lipid peroxidation. *Eur. J. Med. Chem.*, 35, 449-457 (2000).

Bicking, J. B., Robb, C. M., Watson, L. S., and Cragoe, E. J. Jr., (Vinylaryloxy)acetic acids. A new class of diuretic agents. 2. (4-(3-Oxo-1-alkenyl)phenoxy)acetic acids. *J. Med. Chem.*, 19, 544-547 (1976).

Bocchini, V., Mazzolla, R., Barluzzi, R., Blasi, E., Sick, P., and Kettenmann, H., An immortalized cell line expresses properties of activated microglial cells. *J. Neurosci. Res.*, 31, 616-621 (1992).

Connor, D. T. and Flynn, D. L., Preparation of diarylalkenones as lipoxygenase inhibitors. (Warner-Lambert Co., Ltd., U.S.A.), *USP* 4959503, 1-20 (1990).

Dess, D. B. and Martin, J. C., Readily accessible 12-I-5 oxidant for the conversion of primary and secondary alcohols to aldehydes and ketones. *J. Org. Chem.*, 48, 4155-4156 (1983).

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., and Tannenbaum, S. R., Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal. Biochem.*, 126, 131-138 (1982).

Hogale, M. B., Dhore, N. P., Shelar, A. R., and Pawar, P. K., Synthesis and biological activity of some urethane derivatives of chalcones. *Oriental. J. Chem.*, 2, 55-57 (1986).

Huang, M. T., Lou, Y. R., Ma. W., Newmark, H. L., Reuhl, K. R., and Conney, A. H., Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. *Cancer Res.*, 54, 5841-5847 (1994).

Jung, J. C., Kache, R., Vines, K. K., Zheng, Y. S., Bijoy, P., Valluri, M., and Avery, M. A., Total Syntheses of Epothilones B and D. *J. Org. Chem.*, 69, 9269-9284 (2004).

Kiuchi, F., Goto, Y., Sugimoto, N., Akao, N., Kondo, K., and Tsuda, Y., Studies on crude drugs effective on visceral larva migrans. XVI. Nematocidal activity of turmeric: synergistic action of curcuminoids. *Chem. Pharm. Bull.*, 41, 1640-1643 (1993).

Ley, S. V., Norman, J., Griffith, W. P., and Marsden, S. P., Tetrapropylammonium perruthenate, Pr4N+RuO4-, TPAP: a catalytic oxidant for organic synthesis. *Synthesis*, 639-666 (1994).

Mancuso, A. J., Huang, S. -L., and Swern, D., Oxidation of long-chain and related alcohols to carbonyls by dimethyl sulfoxide "activated" by oxalyl chloride. *J. Org. Chem.*, 43, 2480-2482 (1978).

Murakami, S., Kijima, H., Isobe, Y., Muramatsu, M., Aihara, H., Otomo, S., Baba, K., and Kozawa, M., Inhibition of gastric hydrogen ion-potassium ATPase by chalcone derivatives, xanthoangelol and 4-hydroxyderricin, from Angelica keiskei Koidzumi. *J. Pharm. Pharmacol.*, 42, 723-726 (1990).

Ninomiya, Y., Shimma, N., and Ishitsuka, H., Comparative

studies on the antirhinovirus activity and the mode of action of the rhinovirus capsid-binding agents, chalcone amides. *Antiviral Res.*, 13, 61-74 (1990).

Ohishi, K., Aiyama, R., Hatano, H., Yoshida, Y., Wada, Y., Yokoi, W., Sawada, H., Watanabe, and Yokokura, T., Structure-activity relationships of N-(3,5-dimethoxy-4-n-octyloxycinnamoyl)-N'-(3,4-dimethylphenyl)piperazine and analogues as inhibitors of acyl-CoA: cholesterol O-acyltransferase. *Chem. Pharm. Bull.*, 49, 830-839 (2001).

Ohtsu, H., Xiao, Z., Ishida, J., Nagai, M., Wang, H. -K., Itokawa, H., Su, C. -Y., Shih, C., Chiang, T., Chang, E., Lee, Y., Tsai, M. -Y., Chang, C., and Lee, K. -H., Antitumor Agents. 217. Curcumin Analogues as Novel Androgen Receptor Antagonists with Potential as Anti-Prostate Cancer Agents. *J. Med. Chem.*, 45, 5037-5042 (2002).

Perrin, D. D., Armarego, L. F., and Perrin, D. R., *Purification of Laboratory Chemicals*. 2$^{nd}$ ed. Pergamon Press: New York, (1980).

Samaha, H. S., Kelloff, G. J., Steele, V., Rao, C. V., and Reddy, B. S., Modulation of apoptosis by sulindac, curcumin, phenylethyl-3-methylcaffeate, and 6-phenylhexyl isothiocyanate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. *Cancer Res.*, 57, 1301-1305 (1997).

Sreejayan, Rao, M. N., Curcuminoids as potent inhibitors of lipid peroxidation. *J. Pharm. Pharmacol.*, 46, 1013-1016 (1994).

Srimal, R. C. and Dhawan, B. N., Pharmacology of diferuloyl methane (curcumin), a nonsteroidal antiinflammatory agent. *J. Pharm. Pharmacol.*, 25, 447-452 (1973).

Yoon, J. W., Ahn, H., Ryu, J. -H., and Kim, H. -D., Synthesis of yakuchinone derivatives and their inhibitory activities on nitric oxide synthesis. *Yakhak Hoechi*, 45, 16-22 (2001).