# Neuroprotective 2-(2-Phenylethyl)chromones of Imperata c ylindrica

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# Neuroprotective 2-(2-Phenylethyl)chromones of *Imperata cylindrica*

Jeong Seon Yoon, Mi Kyeong Lee, Sang Hyun Sung, and Young Choong Kim*

*College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, San 56-1, Sillim-Dong, Gwanak-Gu, Seoul 151-742, Korea*

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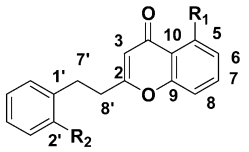
Bioactivity-guided fractionation of the methanolic extract of the rhizomes of *Imperata cylindrica* afforded a new compound, 5-hydroxy-2-(2-phenylethyl)chromone (**1**), together with three known compounds, 5-hydroxy-2-[2-(2-hydroxyphenyl)ethyl]chromone (**2**), flidersiachromone (**3**), and 5-hydroxy-2-styrylchromone (**4**). Among these four compounds, **1** and **2** showed significant neuroprotective activity against glutamate-induced neurotoxicity in primary cultures of rat cortical cells.

During our search for neuroprotective compounds from natural products, the MeOH extract of the rhizomes of *Imperata cylindrica* Beauv. (Gramineae) was found to significantly protect primary cultures of rat cortical cells from the toxicity induced by glutamate, an excitatory neurotransmitter. The rhizomes of *I. cylindrica* are widely distributed in Asia and have been described as a diuretic, anti-inflammatory, or antipyretic agent in Korean traditional herbal medicine.[1] Previous studies on the rhizomes of *I. cylindrica* have resulted in the isolation of various compounds such as arundoin,[2] cylindrin,[2] fernenol,[2] cylindol,[3] cylindrene,[4] graminones,[5] and imperarene.[6] To date, however, there has been no report related to neuroprotective constituents of this plant. Thus, we pursued the isolation of neuroprotective constituents from the MeOH extract of *I. cylindrica* rhizomes by bioactivity-guided fractionation. A new chromone, 5-hydroxy-2-(2-phenylethyl)chromone (**1**), and three known chromones (**2**−**4**) were obtained. Here, we report the isolation and characterization of these chromones and their neuroprotective activity against glutamate-induced neurotoxicity in primary cultures of rat cortical cells.




| | $R_1$ | $R_2$ | |
|---|---|---|---|
| 1 | OH | H | |
| 2 | OH | OH | |
| 3 | H | H | |
| 4 | OH | H | $\Delta^{7',8'}$ |

Compound **1** was obtained as a yellow powder. The positive HREIMS of **1** gave a molecular ion at *m*/*z* 266.0936, corresponding to the molecular formula $C_{17}H_{14}O_3$ (calcd *m*/*z* 266.0943). The UV absorption maxima at 344 and 252 nm and the IR absorption at 1655, 1621, and 1477 $cm^{-1}$ suggested the presence of a chromone ring.[7,8] The $^1H$ NMR data of **1** indicated the presence of 1,2,3-trisubstituted and monosubstituted aromatic rings from the signals at $\delta$ 7.44 (1H, t, $J$ = 8.4 Hz), 6.80 (1H, dd, $J$ = 8.4 and 0.9 Hz), and 6.71 (1H, dd, $J$ = 8.4 and 0.9 Hz) and signals at $\delta$ 7.12−7.27 (5H, m), respectively. The presence of two methylene groups was also deduced by the signals at $\delta$ 2.99 (2H, t, $J$ = 7.6 Hz) and 2.86 (2H, t, $J$ = 7.6 Hz). The other proton signals could be assigned as one hydroxyl group at $\delta$ 12.45 (1H, br s) and one olefinic proton at $\delta$ 6.00 (1H, s). The $^{13}C$ NMR and DEPT spectra of **1** revealed the presence of two methylenes, nine methines, and six quaternary carbons including one carbonyl carbon at $\delta$ 183.5 (s). In addition, HMBC correlations were observed between the H-7′ methylene protons at $\delta$ 2.99 and C-2′, C-6′, C-8′ and between the H-8′ methylene protons at $\delta$ 2.86 and C-3, C-7′. On the basis of these findings, compound **1** was suggested as a 2-(2-phenylethyl)-chromone with one hydroxyl group. The location of the hydroxyl group at $\delta$ 12.45 was defined as C-5. Taken together, compound **1** was assigned as the new 5-hydroxy-2-(2-phenylethyl)chromone.

* To whom correspondence should be addressed: Tel: 82-2-880-7842. Fax: 82-2-888-2933. E-mail: youngkim@snu.ac.kr.

**Table 1.** Protective Activity of Compounds **1**−**4** Isolated from *I. cylindrica* against Glutamate-Induced Neurotoxicity in Primary Cultures of Rat Cortical Cells

| compound | concentration ($\mu$M) | protection (%)[a] |
|---|---|---|
| control | | 100.0 ± 2.1 |
| glutamate-treated[b] | | 0.0 ± 2.2 |
| **1** | 10.0 | 67.0 ± 5.6*** |
| **2** | 10.0 | 63.6 ± 5.6*** |
| **3** | 10.0 | 36.1 ± 5.4* |
| **4** | 10.0 | −11.3 ± 4.9 |
| MK-801[c] | 10.0 | 82.2 ± 6.9*** |

[a] Protection (%) was calculated as 100 × [optical density (OD) of test compound + glutamate-treated culture − OD of glutamate-treated culture]/[OD of control culture − OD of glutamate-treated culture]. The ODs of control and glutamate-injured cultures were 1.00 ± 0.01 and 0.72 ± 0.01, respectively. [b] Glutamate-treated value differs significantly from the untreated control at a level of $p < 0.001$. [c] MK-801: dizocipline maleate, a noncompetitive antagonist of NMDA receptor. The values are expressed as mean ± SD of triplicate experiments (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

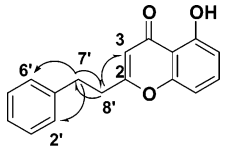



**Figure 1.** Key HMBC correlations for **1**.

Three known compounds were identified from their spectroscopic data by comparison with literature values as 5-hydroxy-2-[2-(2-hydroxyphenyl)ethyl]chromone (**2**),[10] flidersiachromone (**3**),[9] and 5-hydroxy-2-styrylchromone (**4**).[11] Although the synthesis of compound **4** has been described,[11] this is the first time that it has been isolated from natural resources. In addition, compounds **2** and **3** were isolated for the first time from this plant.

The neuroprotective activity of compounds **1**−**4** against glutamate-induced neurotoxicity in primary cultures of rat cortical cells was evaluated by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay as described in our previous reports.[12,13] As shown in Table 1, compounds **1** and **2** showed significant neuroprotective activity against glutamate-induced neurotoxicity at 10.0 $\mu$M concentration. Interestingly, however,

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compound **4**, which has a structure identical to compound **1** except a double bond at C-7′ and C-8′, showed little effect against glutamate-induced neurotoxicity. These results suggested that the absence of the double bond at C-7′ and C-8′ seems to be important for the neuroprotective activity of 2-(2-phenylethyl)chromone. In addition, the neuroprotective activity of compounds **1** and **2** was more potent than that of compound **3**, suggesting the importance of the 5-hydroxy group in the 2-(2-phenylethyl)chromone for neuroprotective activity. Although our present study demonstrated that the presence of the 5-hydroxy group and the absence of the double bond at C-7′ and C-8′ seem to be important for the neuroprotective activity of 2-(2-phenylethyl)chromone, further studies with more 2-(2-phenylethyl)chromone derivatives are required for the assessment of the relevant structure−activity relationships.

## Experimental Section

**General Experimental Procedures.** UV spectra were recorded on a Shimadzu UV-201 spectrometer using MeOH as solvent. FT-IR spectra were recorded on a Perkin-Elmer 1710 spectrometer. NMR spectra were obtained with Bruker AMX 300 and JEOL JNM-GSX 400 spectrometers. Solvent signals were used as internal references. $^1$H−$^1$H COSY, HMQC, and HMBC NMR experiments were performed on the same spectrometer. EI-mass spectra were obtained on a VG Trio 2 spectrometer with a 70 eV ionizing potential. TLC and column chromatography were carried out on precoated silica gel $F_{254}$ plates (Merck, art. 5715), RP-18 $F_{254}$ plates (Merck, art. 15423), silica gel 60 (230−400 mesh, Merck), Sephadex LH-20 (18−110 $\mu$m, Pharmacia Co. Ltd), and LiChroprep RP-18 (40−63 $\mu$m, Merck).

**Plant Material.** The rhizomes of *I. cylindrica* were purchased from a commercial supplier in Seoul, Korea, and identified by Dr. Jong Hee Park, a professor of the College of Pharmacy, Pusan National University. A voucher specimen (SNU-0185) has been deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

**Extraction and Isolation.** The dried rhizomes of *I. cylindrica* (38 kg) were extracted three times with MeOH in an ultrasonic apparatus. Upon removal of the solvent under vacuum, the MeOH extract yielded 8.7 kg of material (22.9% by dry weight). The extract was suspended in $H_2O$ and partitioned repeatedly with *n*-hexane. The *n*-hexane fraction (347.5 g), which showed significant neuroprotective activity, was subjected to column chromatography (CC) over silica gel (12 × 80 cm) eluted with an *n*-hexane−EtOAc−MeOH mixture (*n*-hexane; *n*-hexane−EtOAc, 50:1; 30:1; 10:1; 5:1; 3:1; 1:1; EtOAc; EtOAc−MeOH, 50:1; 30:1; 10:1; 5:1; 3:1; 1:1; MeOH; 2 L of each solvent) to afford 12 fractions (F1−F12). F3 (3.3 g) was subjected to reversed-phase (RP) CC with a MeOH−$H_2O$ step gradient (50% MeOH → 100% MeOH) to yield 10 fractions (F3-1−F3-10). Among these fractions, F3-6 (227.9 mg) was subjected to CC over Sephadex LH-20 using MeOH to yield five fractions (F3-6-1−F3-6-5). Compounds **1** (46.8 mg) and **4** (24 mg) were isolated from F3-6-2 (165.7 mg) by repeated semipreparative HPLC (YMC-Pack Pro $C_{18}$, 10 × 250 mm, $H_2O$−acetonitrile = 40:60, 2 mL/min, $t_R$ = 28.87 and 31.84, respectively) with UV detection at 254 nm. F8 (9.0 g) was subjected to RP CC with a MeOH−$H_2O$ step gradient (60% MeOH → 100% MeOH) to yield five fractions (F8-1−F8-5). Among these fractions, F8-3 (282.7 mg) was subjected to CC over Sephadex LH-20 using MeOH to yield five fractions (F8-3-1−F8-3-5). Compounds **2** (3.1 mg) and **3** (8.7 mg) were isolated from F8-3-2 (33.6 mg) by repeated semipreparative HPLC (Luna, 10 × 250 mm, $H_2O$−acetonitrile = 40:60, 2 mL/min, $t_R$ = 19.54 and 28.98, respectively) with UV detection at 254 nm.

**5-Hydroxy-2-(2-phenylethyl)chromone (1):** yellow powder; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 344 (3.83), 252 (4.41), 220 (4.43) nm; IR $\nu$KBr max cm$^{-1}$ 2926, 1655, 1621, 1477, 1412, 1255, 848, 805; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 12.45 (1H, br s, 5-O*H*), 7.44 (1H, t, $J$ = 8.4 Hz, H-7), 7.12−7.27 (5H, m, H-2′, 3′, 4′, 5′, 6′), 6.80 (1H, dd, $J$ = 8.4 and 0.9 Hz, H-8), 6.71 (1H, dd, $J$ = 8.4 and 0.9 Hz, H-6), 6.00 (1H, s, H-3), 2.99 (2H, t, $J$ = 7.6 Hz, H-7′), 2.86 (2H, t, $J$ = 7.6 Hz, H-8′); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 29.7 (C-7′), 32.8 (C-8′), 106.8 (C-8), 108.8 (C-3), 110.6 (C-10), 111.2 (C-6), 126.7 (C-4′), 128.2 (C-2′, 6′), 128.7 (C-3′, 5′), 135.2 (C-7), 139.4 (C-1′), 156.7 (C-9), 160.8 (C-5), 169.8 (C-2), 183.5 (C-4); HREIMS (positive) *m*/*z* 266.0936 [M]$^+$ (calcd for $C_{17}H_{14}O_3$, 266.0943).

**Cell Culture.** Primary cultures of rat cortical cells containing both neurons and non-neuronal cells were prepared from 17- to 19-day-old fetal rats (Sprague−Dawley) as previously reported.[12] Cortical cells were seeded onto a collagen-coated 48-well plate at a density of 1 × $10^6$ cells/mL. The cultures were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum, 100 IU/mL penicillin, and 100 $\mu$g/mL streptomycin at 37 °C in a humidified atmosphere of 95% air−5% $CO_2$. Cytosine-$\beta$-D-arbinofuranoside (1 $\mu$M) was added to the culture medium 3 days after plating to inhibit the proliferation of non-neuronal cells. Cultures were allowed to mature for 15 days before being used for experiments.

**Assessment of Neuroprotective Activity.** All tested compounds were dissolved in DMSO (final culture concentration, 0.1%). Cortical cell cultures were pretreated with test compounds for 1 h and then exposed to 100 $\mu$M glutamate. After incubation for an additional 24 h, cell viability of the cultures was assessed by the MTT assay, which reflects the mitochondrial enzyme function of cells. Protection (%) was calculated as 100 × [optical density (OD) of test compound + glutamate-treated culture − OD of glutamate-treated culture]/[OD of control culture − OD of glutamate-treated culture)].

**Statistical Analysis.** Data were evaluated for statistical significance using an analysis of variance (ANOVA) with a computerized statistical package. The data were considered to be statistically significant if the probability value was $<0.05$.

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**Supporting Information Available:** NMR spectra for compound **1**. This information is available free of charge via the Internet at http://pubs.acs.org.

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