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

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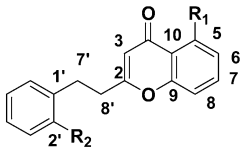
Bioactivity-guided fractionation of the methanolic extract of the rhizomes of *Imperata cylindrica* afforded a new compound, 5-hydroxy-2-(2-phenylethyl)chromone (**1**), together with three known compounds, 5-hydroxy-2-[2-(2-hydroxyphenyl)ethyl]chromone (**2**), flidersiachromone (**3**), and 5-hydroxy-2-styrylchromone (**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 δ 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 δ 7.12−7.27 (5H, m), respectively. The presence of two methylene groups was also deduced by the signals at δ 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 δ 12.45 (1H, br s) and one olefinic proton at δ 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 δ 183.5 (s). In addition, HMBC correlations were observed between the H-7′ methylene protons at δ 2.99 and C-2′, C-6′, C-8′ and between the H-8′ methylene protons at δ 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 δ 12.45 was defined as C-5. Taken together, compound **1** was assigned as the new 5-hydroxy-2-(2-phenylethyl)chromone.

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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 (μM)	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 (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

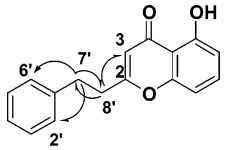

Figure 1. Key HMBC correlations for **1**.

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 μM 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 μm, Pharmacia Co. Ltd), and LiChroprep RP-18 (40−63 μm, 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) λ_{max} (log ϵ) 344 (3.83), 252 (4.41), 220 (4.43) nm; IR νKBr max cm^{-1} 2926, 1655, 1621, 1477, 1412, 1255, 848, 805; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 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) δ 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 μg/mL streptomycin at 37 °C in a humidified atmosphere of 95% air−5% CO_2. Cytosine-β-D-arbinofuranoside (1 μM) 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 μM 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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