Five Novel Neuroprotective Triterpene Esters of *Ulmus davidiana* var. *japonica*

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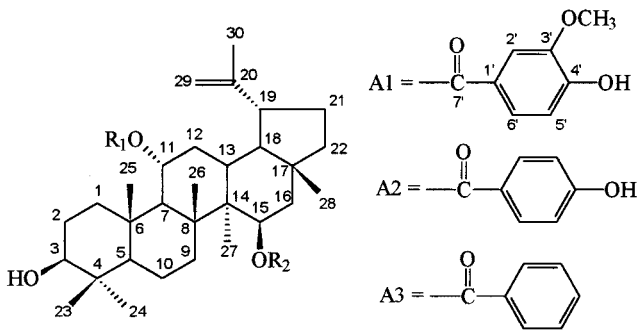
Investigation of the constituents of the stem and root barks of *Ulmus davidiana* var. *japonica* resulted in the isolation of five new triterpene esters named ulmicin A−E (**1**−**5**). Using several spectroscopic techniques, their structures were determined to be 3β,11α,15α-trihydroxylup-20(29)-ene-11-(3′-methoxy-4′-hydroxybenzoyl ester) (**1**), 3β,11α,15α-trihydroxylup-20(29)-ene-11-(4′-hydroxybenzoyl ester) (**2**), 3β,-11α,15α-trihydroxylup-20(29)-ene-11-(3′-methoxy-4′-hydroxybenzoyl)-15-(4′-hydroxybenzoyl ester) (**3**), 3β,11α,15α-trihydroxylup-20(29)-ene-11,15-di(3′-methoxy-4′-hydroxybenzoyl ester) (**4**), and 3β,11α,15α-trihydroxylup-20(29)-ene-11-(3′-methoxy-4′-hydroxybenzoyl)-15-(benzoyl ester) (**5**). All five compounds showed significant neuroprotective activities against glutamate-induced neurotoxicity in primary cultures of rat cortical cells.

We have searched for neuroprotective compounds from higher plants that can be developed as candidates for treatment of neurodegenerative diseases.[1−4] Glutamate-induced excitotoxicity is known to be a major contributor to pathological cell death within the nervous system and is a primary concern because of its involvement in stroke, trauma, neurodegeneration, and epilepsy.[5−7] In the course of screening, the methanolic extract of the stem and root barks of *Ulmus davidiana* Planch var. *japonica* Nakai (Ulmaceae) showed significant neuroprotective activity against glutamate-induced neurotoxicity. *U. davidiana* var. *japonica* is a widely distributed tree in Korea, and the stem and root barks of this species have been used as a traditional medicine for the treatment of edema, rheumatoid arthritis, and cancer.[8] Phytochemical studies of this species have resulted in the isolation of various sesquiterpenes, triterpenes, and flavonoids,[9−12] but none of these have been evaluated for neuroprotective activity. In the present study, we report the isolation and structural elucidation of five new triterpene esters, ulmicin A−E (**1**−**5**), as well as their neuroprotective activities.



	R_1	R_2
1	A1	H
2	A2	H
3	A1	A2
4	A1	A1
5	A1	A3

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Results and Discussion

This methanolic extract of *Ulmus davidiana* var. *japonica* was suspended in distilled water and partitioned with CH_2Cl_2. The CH_2Cl_2 layer was then suspended in 90% MeOH and partitioned with *n*-hexane. Repeated column chromatography of the 90% MeOH fraction over Si gel and Sephadex LH-20 and reverse-phase HPLC yielded five new triterpene esters, ulmicin A−E (**1**−**5**).

Compound **1** was obtained as an amorphous powder. (+) HRFABMS showed the $[M + H]^+$ ion at *m*/*z* 609.4141, corresponding to the molecular formula $C_{38}H_{56}O_6$. Its IR spectrum suggested the presence of hydroxyl (3440 cm^{-1}) and carbonyl (1680 cm^{-1}) groups. Compound **1** was deduced to be a triterpenoid through a positive Liebermann-Buchard test and proton and carbon signals typical for a triterpenoid observed in ^1H and ^{13}C NMR spectra. The singlet signals for six methyls [δ 0.67, 0.73, 0.94, 0.97, 1.07, and 1.15] and signals for an isopropylene group [δ 1.65 (3H, s), δ 4.65 (1H, br s), and δ 4.69 (1H, br s)] in the ^1H NMR spectrum and a characteristic fragment ion at *m*/*z* 189 in the FABMS indicated the presence of a lupene skeleton in **1**.[13,14] Additional signals for a 1,2,4-trisubstituted aromatic moiety [δ 7.59 (1H, dd, J = 2.0, 8.3 Hz, H-6′), δ 7.53 (1H, d, J = 2.0 Hz, H-2′), and δ 6.93 (1H, d, J = 8.3 Hz, H-5′)] and the methoxyl group [δ 3.93 (3H, s)] in the ^1H NMR spectrum together with the fragment ion at *m*/*z* 167 consistent with $C_8H_7O_4$ in the FABMS suggested the presence of a benzoyl ester substituted with a methoxyl and a hydroxyl group in **1**. The placement of substitutions on the benzoyl ester was determined to be a 3-methoxy-4-hydroxy benzoate since correlation of the methoxyl signal at δ_H 3.93 to C-3′ (δ_C 46.36) was observed in the HMBC spectrum. These results suggested that **1** possesses an ester linkage between a lupene-type triterpene and a 3-methoxy-4-hydroxybenzoic acid.

The ^{13}C NMR spectrum revealed three oxygenated methines [δ 73.16, 73.42, and 78.59], and among them the signal at δ_C 73.42 was assigned as ester linked due to the relatively downfield proton signal (δ_H 5.50).[15] The positions of two hydroxyl groups were confirmed at C-3 and C-15, respectively, since correlation of the oxymethine proton signal at δ_H 3.19 to C-2 (δ_C 27.87), C-4 (δ_C 39.67), and C-24 (δ_C 15.74) and correlation of the oxymethine proton signal at δ_H 3.80 to C-14 (δ_C 46.36) and C-27 (δ_C 12.34) were observed in the HMBC spectrum (Table 1).[16] The proton bearing the ester linkage (δ_H 5.50) showed interaction with C-9 (δ_C 53.31) and the carbonyl (δ_C 165.73) of 3-methoxy-

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Table 1. ^{13}C NMR Data and HMBC Correlations of Compound **1** (100 MHz, $CDCl_3$)

position	^{13}C (δ)	HMBC	position	^{13}C (δ)	HMBC
1	41.28	H-25	20	147.59	H-30
2	27.87	H-3	21	28.13	
3	78.59	H-23, H-24	22	32.67	
4	39.67	H-3, H-23, H-24	23	28.61	H-24
5	55.06	H-23, H-24, H-25	24	15.74	H-3, H-24
6	18.79		25	16.78	
7	37.75	H-26	26	19.10	
8	45.65	H-26, H-29	27	12.34	H-15
9	53.31	H-11, H-25, H-26	28	14.88	
10	39.23	H-25	29	110.34	H-30
11	73.42		30	20.05	H-29
12	31.35		1′	123.47	
13	47.58	H-28	2′	112.31	H-6′
14	46.36	H-15, H-26	3′	146.61	H-2′, H-5′, $-OCH_3$
15	73.16	H-27	4′	150.40	H-2′, H-5′, H-6′
16	39.71	H-28	5′	113.98	
17	44.34	H-28	6′	124.72	H-2′, H-5′
18	51.56	H-28	7′	165.73	H-2′
19	47.58	H-29, H-30	$-OCH_3$	56.51	

4-hydroxy benzoate, confirming the position of the ester linkage at C-11. The positions of the oxymethine carbons were also supported by the downfield shifts of neighboring carbon signals in comparison with literature data.[17]

The stereochemistries of H-3, H-11, and H-15 were determined on the basis of the chemical shift, coupling constants, and NOE difference spectrum. The presence of β-OH substitution at C-3 was suggested by the value of the chemical shift of C-3 (δ_C 78.59), since the carbon signal is shifted upfield to δ_C 75.5 in α-OH substitution.[19] Irradiation of the signal at δ_H 5.50 (H-11) led to enhancement of the two methyl signals at δ_H 0.94 (CH_3-25) and δ_H 1.15 (CH_3-26), thus demonstrating the α-OH substitution at C-11.[20] The coupling constant of H-15 (δ_H 3.80, dd, $J = 10.2$ and 5.1 Hz) for diaxial and axial/equatorial interactions and a positive NOE with CH_3-26 (δ_H 1.15) were in accordance with an α-OH moiety at C-15.[16,19] Therefore, **1** was elucidated to be 3β,11α,15α-trihydroxylup-20(29)-ene-11-(3′-methoxy-4′-hydroxybenzoyl ester) and designated as ulmicin A.

Compound **2** was also obtained as an amorphous powder. The molecular formula was determined to be $C_{37}H_{55}O_5$ by HRFABMS. The ^1H NMR spectrum of **2** showed chemical shifts, multiplicities, and coupling constants similar to those of **1** except for replacement of the signals of the 1,3,4-trisubstituted benzoyl moiety of **1** with those of a 1,4-disubstituted benzoyl moiety and the absence of a methoxyl group. Therefore, **2** was determined to be 3β,11α,15α-trihydroxylup-20(29)-ene-11-(4′-hydroxybenzoyl ester) and designated as ulmicin B.

Compound **3** was also obtained as an amorphous powder. (+) HRFABMS showed the $[M + Na]^+$ ion at *m/z* 751.4193 corresponding to the molecular formula $C_{45}H_{60}O_8$. The ^1H NMR spectrum of **3** due to the triterpene portion was nearly identical to those of **1**and **2**, except for the downfield shift of the proton signal at δ_H 3.80 (H-15) to δ_H 5.11, suggesting that **3** is a lupene-skeleton triterpenoid bearing two ester linkages. Two carbonyl carbon signals at δ_C 165.77 and 165.93 in the ^{13}C NMR spectrum also supported two ester bonds in **3**. The presence of 1,2,4-trisubstituted and 1,4-disubstituted aromatic moieties was suggested by the additional proton signals in the ^1H NMR spectrum. The HMBC correlations of **3** (Figure 1) as well as the characteristic fragment ions at *m/z* 423 [consistent with loss of 3-methoxy-4-hydroxy benzoate and 4-hydroxy benzoate],

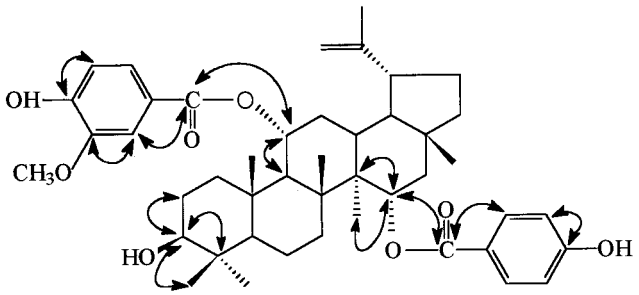

Figure 1. Key HMBC correlations of **3**.

167 [consistent with 3-methoxy-4-hydroxy benzoate], and 137 [consistent with 4-hydroxy benzoate] in the FABMS confirmed that 3-methoxy-4-hydroxy benzoate and 4-hydroxy benzoate were ester linked in **3**. The 3-methoxy-4-hydroxy benzoate and 4-hydroxy benzoate were determined to be attached to C-11 and C-15, respectively, by the HMBC correlations seen between δ_C 165.77 (C-7′) and δ_H 5.42 (H-11) and between δ_C 165.93 (C-7″) and δ_H 5.11 (H-15) in the HMBC spectrum. Therefore, **3** was determined to be 3β,11α,15α-trihydroxylup-20(29)-ene-11-(3′-methoxy-4′-hydroxybenzoyl)-15-(4′-hydroxybenzoyl ester) and designated as ulmicin C.

Compound **4** was also obtained as an amorphous powder. The molecular formula was determined to be $C_{46}H_{62}O_9$ by HRFABMS. The spectral data of **4** were very similar to those of **3**. The ^1H and ^{13}C NMR spectra of **4** due to the triterpene portion were almost identical to those of **3**, which suggested that **4** is also a lupene-type triterpene possessing two ester linkages. The ^1H and ^{13}C NMR spectra of **4** revealed the presence of two 3-methoxy-4-hydroxy benzoates, which is supported by the fragment ion at *m/z* 167 for 3-methoxy-4-hydroxy benzoate in the FABMS. Thus, **4** was determined to be 3β,11α,15α-trihydroxy-lup-20(29)-ene-11,15-di(3′-methoxy-4′-hydroxybenzoyl ester) and designated as ulmicin D.

Compound **5** was also obtained as an amorphous powder. The molecular formula was determined to be $C_{45}H_{60}O_7$ by HRFABMS. The spectral data of **5** closely resembled those of **3** and **4**, which suggested that **5** is also a lupene-type triterpene possessing two ester linkages. The ^1H and ^{13}C NMR spectra of **5** exhibited signals for 3-methoxy-4-hydroxy benzoate and benzoate.[20] The HMBC correlations seen between δ_C 165.75 (C-7′) and δ_H 5.45 (H-11) and between δ_C 165.87 (C-7″) and δ_H 5.17 (H-15) in the HMBC spectrum revealed that 3-methoxy-4-hydroxy benzoate and benzoate were attached to C-11 and C-15, respectively. Therefore, **5** was elucidated as 3β,11α,15α-trihydroxylup-20(29)-ene-11-(3′-methoxy-4′-hydroxybenzoyl)-15-(benzoyl ester) and designated as ulmicin E.

Compounds **1**−**5** were evaluated for their neuroprotective activities employing primary cultures of rat cortical cells injured with glutamate (Table 2). The neuroprotective activities were quantified by measuring the lactate dehydrogenase (LDH) leakage, a widely accepted measure of cell membrane integrity. Compounds **1**−**5** showed significant neuroprotective activities at concentrations ranging from 0.1 to 5 μM by blocking the LDH leakage from the glutamate-injured rat cortical cells into the medium. The neuroprotective activity of all five compounds was highest at the concentration of 5 μM, and no enhancement of neuroprotective activity was measured above the concentration of 5 μM (data not shown). From these results, the five triterpene esters, ulmicin A−E, isolated from *U. davidiana* var. *japonica* seem to be worthy candidates for protecting neurons from glutamate-induced neurotoxicity.

Table 2. ^{13}C NMR Data of Compounds **2**−**5**

position	**2**[a]	**3**[b]	**4**[a]	**5**[a]
1	41.56	41.17	41.02	41.02
2	27.51	26.71	26.78	26.78
3	78.72	77.40	77.35	77.35
4	39.68	38.92	38.52	38.87
5	55.48	54.50	54.44	54.35
6	18.37	18.06	18.04	18.00
7	37.76	35.99	36.19	35.99
8	45.78	45.04	45.01	45.02
9	53.70	52.76	52.76	52.72
10	39.33	38.54	38.52	38.51
11	73.26	72.33	72.25	72.26
12	31.54	27.67	27.59	27.57
13	47.45	46.31	46.28	46.28
14	46.83	46.11	46.01	46.11
15	73.15	75.33	75.54	75.85
16	39.68	38.78	38.75	38.74
17	44.37	43.53	43.50	43.49
18	51.85	50.55	50.53	50.50
19	47.74	47.25	47.14	47.13
20	147.81	147.40	147.45	147.38
21	27.94	27.15	27.12	27.12
22	32.38	30.47	30.44	30.41
23	28.14	27.29	27.26	27.23
24	15.41	14.61	14.54	14.56
25	16.25	15.45	15.38	15.36
26	18.15	17.76	17.70	17.72
27	11.76	12.31	12.26	12.24
28	14.28	13.61	13.55	13.54
29	110.48	109.40	109.33	109.36
30	18.96	18.24	18.19	18.19
1′	123.04	121.61	121.73	121.71
2′	132.25	112.32	112.28	112.31
3′	115.57	146.70	146.67	146.85
4′	162.84	151.21	151.82	151.53
5′	115.55	114.64	114.67	114.61
6′	132.25	123.90	123.90	123.85
7′	166.63	165.77	165.87	165.75
1″		121.74	121.73	131.66
2″		131.30	112.07	128.98
3″		114.88	146.67	128.25
4″		162.19	147.45	132.87
5″		114.88	114.67	128.25
6″		131.30	123.55	128.98
7″		165.93	165.75	165.87
−OCH_3		55.07	54.99	55.04
−OCH_3			55.01	

[a] CD_3OD at 75 MHz. [b] CD_3OD at 125 MHz.

Table 3. Neuroprotective Effects of Compounds **1**−**5** on Glutamate-Induced Toxicity[a]

	cell viability (%)
control	100.0 ± 4.2
glutamate-treated	0.0 ± 5.1
ulmicin A (**1**)	48.0 ± 5.6**
ulmicin B (**2**)	51.2 ± 5.1**
ulmicin C (**3**)	49.2 ± 4.2**
ulmicin D (**4**)	44.2 ± 4.7**
ulmicin E (**5**)	41.0 ± 3.9**

[a] Cortical cell cultures were pretreated with 5 μM compounds for 1 h before exposure to 100 μM glutamate and then maintained for 24 h. Cell viability was measured by LDH assay. LDH released from control and glutamate-treated cultures were 109.3 ± 7.2 and 189.2 ± 9.8 mU/mL, respectively. Cell viability was calculated as 100 × (LDH released from glutamate-treated − LDH released from glutamate + compound-treated)/(LDH released from glutamate-treated − LDH released from control). Results are significantly different from glutamate-treated (** $p < 0.01$).

Experimental Section

General Experimental Procedure. IR spectra were obtained on a Perkin-Elmer 1710 spectrometer. The NMR spectra were taken on either a JEOL GSX 400 (^1H, 400 MHz; ^{13}C, 100 MHz) or a JEOL LA 300 (^1H, 300 MHz; ^{13}C, 75 MHz) spectrometer. HRFABMS were taken on a JMS-SX 102A spectrometer (JEOL, Japan) and optical rotation on a Jasco DIP-1000 polarimeter. Column chromatography was performed over Si gel 60 (Merck, 230−400 mesh) and Sephadex LH-20 (Phamacia, Sweden).

Plant Material. The stem and root barks of *U. davidiana* var. *japonica* were purchased from Kyungdong Market, Seoul, Korea, and identified by Dr. Dae S. Han, an emeritus professor of the College of Pharmacy, Seoul National University. A voucher specimen has been deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

Extraction and Isolation. The dried stem and root barks of *U. davidiana* var. *japonica* (10 kg) were cut into pieces and extracted three times with 80% MeOH in an ultrasonic apparatus. Removal of the solvent in vacuo yielded a methanolic extract (936 g). This methanolic extract was then suspended in distilled water and partitioned with CH_2Cl_2. The CH_2Cl_2 layer was then suspended in 90% MeOH and partitioned with *n*-hexane. Column chromatography of the 90% MeOH fraction over Si gel using a $CHCl_3$−MeOH mixture with increasing polarity yielded 13 fractions (fractions 1−13). Column chromatography of fraction 4 over Sephadex LH-20 (MeOH) gave four fractions (fractions 4-1−4-4), and continuous column chromatography of fraction 4-2 over Sephadex LH-20 (*n*-hexane−CH_2Cl_2−MeOH, 5:5:1) gave nine fractions (fractions 4-2-1−4-2-9).

Compound **1** was purified from fraction 4-2-4 by semipreparative HPLC on RP_{18} eluted with AcCN−MeOH−H_2O (38:45:17, t_R 10.22). Compound **5** was isolated from fraction 4-2-1 by column chromatography over Sephadex LH-20 (*n*-hexane−CH_2Cl_2−MeOH, 10:10:1) and purified by semipreparative HPLC on RP_{18} (AcCN−MeOH−H_2O, 40:45:15, t_R 19.06).

Column chromatography of fraction 5 over Sephdex LH 20 (MeOH) gave seven fractions (fractions 5-1−5-7). Compound **2** was separated from fraction 5-4 by column chromatography over Sephadex LH-20 (*n*-hexane−CH_2Cl_2−MeOH, 10:10:1) and purified by semipreparative HPLC on RP_{18} (AcCN−MeOH−H_2O, 20:40:40, t_R 16.68). Fraction 5-3 was rechromatographed over Sephadex LH-20 (*n*-hexane−CH_2Cl_2−MeOH, 10:10:1) to afford five fractions (fractions 5-3-1−5-3-5). Compound **3** was purified from fraction 5-3-2 by semipreparative HPLC on RP_{18} eluted with AcCN−MeOH−H_2O (40:40:20, t_R 17.22). Compound **4** was purified from fraction 5-3-3 by semipreparative HPLC on RP_{18} (AcCN−MeOH−H_2O, 40:40:20, t_R 18.18).

Compound 1: white amorphous powder (32 mg); $[\alpha]_D^{20}$ −0.87 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 263 (4.09), 291 (2.11) nm; IR (KBr) ν_{max} 3400, 1680 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.59 (1H, dd, J = 2.0, 8.3 Hz, H-6′), 7.53 (1H, d, J = 2.0 Hz, H-2′), 6.92 (1H, d, J = 8.3 Hz, H-5′), 5.50 (1H, m, H-11), 4.65, 4.69 (2H, each br s, H-29), 3.93 (3H, s, OCH_3), 3.80 (1H, dd, J = 10.2, 5.1 Hz, H-15), 3.19 (1H, br t, J = 8.0 Hz, H-3), 2.24 (1H, m, H-19), 1.65 (3H, s, H-30), 1.15 (3H, s, H-26), 1.07 (3H, s, H-27), 0.97 (3H, s, H-23), 0.94 (3H, s, H-25), 0.73 (3H, s, H-24), 0.67 (3H, s, H-28); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; FABMS *m*/*z* 609 $[M + H]^+$, 423, 189, 167; HRFABMS *m*/*z* 609.4141 (calcd for $C_{38}H_{57}O_6$ 609.4140).

Compound 2: white amorphous powder (18 mg); $[\alpha]_D^{20}$ −0.78 (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ϵ) 260 (4.15), 289 (2.13) nm; IR (KBr) ν_{max} 3440, 1680 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 7.86 (2H, d, J = 8.6 Hz, H-2′, 6′), 6.83 (1H, d, J = 8.6 Hz, H-3′, 5′), 5.53 (1H, m, H-11), 4.68, 4.70 (2H, each br s, H-29), 3.77 (1H, dd, J = 10.8, 5.1 Hz, H-15), 3.13 (1H, dd, J = 11.7, 4.8 Hz, H-3), 2.29 (1H, m, H-19), 1.67 (3H, s, H-30), 1.18 (3H, s, H-26), 1.11 (3H, s, H-27), 0.97 (6H, s, H-23, 25), 0.74 (3H, s, H-24), 0.72 (3H, s, H-28); ^{13}C NMR (CD_3OD, 75 MHz), see Table 2; FABMS *m*/*z* 601 $[M + Na]^+$, 423, 189, 137; HRFABMS *m*/*z* 601.3959 (calcd for $C_{37}H_{54}O_5Na$ 601.3925).

Compound 3: white amorphous powder (28 mg); $[\alpha]_D^{20}$ −7.0 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 260 (4.29) nm; IR (KBr) ν_{max} 3400, 1680 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 7.75 (2H, d, J = 8.7 Hz, H-2″, 6″), 7.47 (1H, dd, J = 8.7, 1.8 Hz, H-6′), 7.45 (1H, d, J = 1.8 Hz, H-2′), 6.76 (1H, d, J = 8.7 Hz, H-5′), 6.74 (2H, d, J = 8.7 Hz, H-3″, 5″), 5.42 (1H, m, H-11),

5.11 (1H, dd, J = 9.9, 5.2 Hz, H-15), 4.60 (2H, br s, H-29), 3.80 (3H, s, OCH_3), 3.03 (1H, dd, J = 13.9, 4.5 Hz, H-3), 2.23 (1H, m, H-19), 1.65 (3H, s, H-30), 1.30 (3H, s, H-27), 1.13 (3H, s, H-26), 0.79 (3H, s, H-25), 0.74 (3H, s, H-23), 0.68 (3H, s, H-28), 0.59 (3H, s, H-24); ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; FABMS m/z 751 $[M + Na]^+$, 423, 189, 167, 137; HRFABMS m/z 751.4193 (calcd for $C_{45}H_{60}O_8Na$ 751.4169).

Compound 4: white amorphous powder (39 mg); $[\alpha]_D^{20}$ −8.4 (c 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 260 (4.23) nm; IR (KBr) ν_{max} 3400, 1680 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.45 (2H, dd, J = 8.9, 1.9 Hz, H-6′, 6″), 7.40 (2H, d, J = 1.9 Hz, H-2′, 2″), 6.75 (2H, d, J = 8.9 Hz, H-5′, 5″), 5.43 (1H, m, H-11), 5.09 (1H, dd, J = 10.3, 5.3 Hz, H-15), 4.59 (2H, br s, H-29), 3.89 (6H, s, 2 × OCH_3), 3.02 (1H, dd, J = 11.2, 4.6 Hz, H-3), 2.21 (1H, m, H-19), 1.60 (3H, s, H-30), 1.29 (3H, s, H-27), 1.13 (3H, s, H-26), 0.85 (3H, s, H-25), 0.78 (3H, s, H-23), 0.70 (3H, s, H-28), 0.58 (3H, s, H-24); ^{13}C NMR (CD_3OD, 75 MHz), see Table 2; FABMS m/z 781 $[M + Na]^+$, 423, 189, 167; HRFABMS m/z 781.4263 (calcd for $C_{46}H_{62}O_9Na$ 781.4174).

Compound 5: white amorphous powder (17 mg); $[\alpha]_D^{20}$ −8.9 (c 0.7, MeOH); UV (MeOH) λ_{max} (log ϵ) 263 (4.19) nm; IR (KBr) ν_{max} 3420, 1690 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.90 (2H, m, H-2″, 6″), 7.49 (2H, m, H-5′, 6′), 7.41 (3H, m, H-3″, 4″, 5″), 6.76 (1H, d, J = 8.8 Hz, H-2′), 5.45 (1H, m, H-11), 5.17 (1H, dd, J = 10.1, 5.3 Hz, H-15), 4.60, 4.62 (2H, each br s, H-29), 3.81 (3H, s, OCH_3), 3.02 (1H, dd, J = 11.0, 4.7 Hz, H-3), 2.24 (1H, m, H-19), 1.61 (3H, s, H-30), 1.32 (3H, s, H-27), 1.15 (3H, s, H-26), 0.77 (3H, s, H-25), 0.72 (3H, s, H-23), 0.68 (3H, s, H-28), 0.59 (3H, s, H-24); ^{13}C NMR (CD_3OD, 75 MHz), see Table 2; FABMS m/z 735 $[M + Na]^+$, 423, 189, 167, 105; HRFABMS m/z 735.4238 (calcd for $C_{45}H_{60}O_7Na$ 735.4220).

Evaluation of Neuroprotective Activity. The neuroprotective activities of compounds were evaluated employing the primary cultures of rat cortical cells injured with glutamate. Primary cultures of rat cortical cells were prepared from 17- to 19-day-old fetal rats (Sprague−Dawley) as described previously.[1] To assess the neuroprotective activities against glutamate, cultures were allowed to mature for 2 weeks. Cultures were pretreated with compounds for 1 h and exposed to 100 μM glutamate. Cultures were then maintained for an additional 24 h for evaluation of neuroprotective activities. Neuronal integrity was assessed by measuring the release of LDH from primary cortical cells into the medium by the method described in our previous report.[2]

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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