Four New Neuroprotective Dihydropyranocoumarins from *Angelica gigas*

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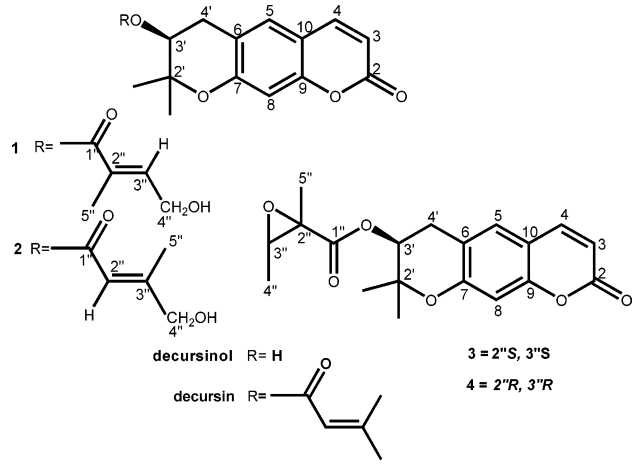
Four new dihydropyranocoumarins were isolated from *Angelica gigas* roots through neuroprotective activity-guided isolation and were characterized as decursinol derivatives 4″-hydroxytigloyldecursinol (**1**), 4″-hydroxydecursin (**2**), (2″*S*,3″*S*)-epoxyangeloyldecursinol (**3**), and (2″*R*,3″*R*)-epoxyangeloyldecursinol (**4**), respectively. All four new dihydropyranocoumarins and major coumarin derivatives of *A. gigas*, decursinol and decursin, exhibited signficant protective activity against glutamate-induced neurotoxicity when added to primary cultures of rat cortical cells at concentrations ranging from 0.1 to 10 μM.

L-Glutamate (Glu) is the major excitatory neurotransmitter in the CNS, involved in fast synaptic transmission, neuronal plasticity, outgrowth and survival, memory, learning, and behavior.[1] However, glutamate-mediated excitotoxicity appears to play a crucial role in neurodegenerative disorders, particularly in Parkinson's disease, Alzheimer's disease, epilepsy, spinal cord trauma, and ischemic stroke.[2] In the course of our search for compounds that protect against glutamate-induced injury on primary cultures of rat cortical cells from natural sources, both the methanolic extract of the roots of *Angelica gigas* Nakai (Umbelliferae) and its methylene chloride fraction exhibited significant neuroprotective activity. Previously, we found that both the methanolic extract of *A. gigas* roots and its coumarin derivatives inhibited acetylcholinesterase in vitro[3] and that both decursin and decursinol, major coumarin derivatives, excellently improved scopolamine-induced amnesia in vivo.[4] Regarding other biological activities of *A. gigas*, it has been reported that coumarins and/or extracts of *A. gigas* exhibited neuroprotective activity against kainic acid,[5] and antiamnesic,[6] antitumor,[7] antinociceptive,[8] antibacterial,[9] platelet antiaggregatory,[10] and protein kinase C (PKC) activating activities.[11,12] However, there is no report of protective activities against glutamate-induced neurotoxicity in primary cultures of rat cortical cells of *A. gigas* extract or its constituents. In the present study, we report the isolation and structural elucidation of four new dihydropyranocoumarins (**1**−**4**), as well as their neuroprotective activities.



Results and Discussion

The methanolic extract of *A. gigas* was suspended in water and partitioned with CH_2Cl_2. The resultant CH_2Cl_2 fraction was repeatedly subjected to silica gel and reverse-phase silica gel column chromatographies and reverse-phase HPLC and yielded four new dihydropyranocoumarins (**1**−**4**).

Compound **1** was isolated as colorless needles from MeOH. The molecular formula of **1** was determined to be $C_{19}H_{20}O_6$ by positive HRFABMS. The positive FABMS of **1** showed $[M + Na]^+$, $[M + H]^+$, and $[M - C_5H_8O_3]^+$ at *m/z* 367, 345, and 229, respectively. The IR spectra of **1** showed absorption bands for hydroxyl groups (3440 cm^{-1}), a lactone carbonyl (1720 cm^{-1}), and an aromatic C=C (1400−1600 cm^{-1}). In the ^1H NMR spectra of **1**, characteristic signals were observed for a gem-dimethyl group (δ 1.34 and 1.38, 3H, s, each), a $-CH_2-CH$ system (δ 2.86 and 3.19 each 1H, H-4′ and δ 5.08, 1H H-3′), two aromatic *para* protons (δ 6.78 and 7.12, each 1H), and H-3 and H-4 of the coumarin nucleus (δ 6.21 and 7.55, each 1H, d), showing **1** to contain the decursinol moiety, a dihydropyranocoumarin.[13,14] In addition, ^1H NMR data of **1** showed that it has a hydroxylated tigloyl moiety at C-3′ by signals at δ 6.75 (1H, dq, J = 5.9 and 1.5 Hz) and 1.79 (3H, d, J = 1.1 Hz) and 4.32 (2H, br d, J = 5.9 Hz).[15,16] All carbon resonances of **1** were fully assigned by ^{13}C NMR, $^1H-^1H$ COSY, and $^{13}C-^1H$ COSY spectra. They confirmed the existence of a hydroxylated tigloyl moiety (δ 166.7, C-1″; δ 128.1, C-2″; δ 141.1, C-3″; δ 59.7, C-4″; δ 12.7, C-5″) in the structure of **1** (Table 1). Furthermore, the position of hydroxylation in the tigloyl moiety of **1** was determined as C-4″ by NOE interactions (Figure 1). The optical rotation value of **1** was $[\alpha]_D^{20}$ +56°, thus confirming the 3′*S* configuration.[12,13] Therefore, from the spectroscopic data above, the structure of **1** was defined as 4″-hydroxytigloyldecursinol.

Compound **2** was also obtained as colorless needles from MeOH. The molecular formula of **2** was determined as $C_{19}H_{20}O_6$ by positive HRFABMS. The spectroscopic data of **2** closely resembled those of **1** except for hydroxylated senecioyl protons at δ 5.98 (1H, m), 2.06 (3H, s), and 4.13 (2H, s). All carbon resonances of **2** were fully assigned by ^{13}C NMR, $^1H-^1H$ COSY, and $^{13}C-^1H$ COSY spectra. They confirmed the hydroxylated senecioyl moiety (δ 165.8, C-1″; δ 113.0, C-2″; δ 158.9, C-3″; δ 66.9, C-4″; δ 15.7, C-5″) in the structure of **2**[15] (Table 2). Similar to compound **1**, the position of hydroxylation in the senecioyl moiety of **2** was also determined as C-4″ by NOE interactions (Figure 2). The optical rotation value of **2** was $[\alpha]^{20}_D$ +59°, thus

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2** (in $CDCl_3$)

	1		**2**	
position	H (J = Hz)	C	H (J = Hz)	C
2		161.3		161.3
3	6.21 (1H, d, 9.5)	113.3	6.23 (1H, d, 9.5)	113.3
4	7.55 (1H, d, 9.5)	143.1	7.53 (1H, d, 9.5)	143.1
5	6.78 (1H, s)	128.6	6.79 (1H, s)	128.6
6		115.7		115.8
7		156.3		156.4
8	7.12 (1H, s)	104.7	7.15 (1H, s)	104.7
9		154.2		154.2
10		112.9		112.8
2′		76.5		77.2
3′	5.08 (1H, t, 4.9)	70.6	5.10 (1H, t, 4.9)	69.4
4′	2.86 (1H, dd, 17.0, 4.9)	27.7	2.87 (1H, dd, 17.0, 4.9)	27.9
	3.19 (1H, dd, 17.0, 4.9)		3.20 (1H, dd, 17.0, 4.9)	
gem$(CH_3)_2$	1.34 (3H, s)	23.1	1.36 (3H, s)	23.2
	1.38 (3H, s)	25.0	1.38 (3H, s)	25.0
1″		166.7		165.8
2″		128.1	5.98 m	113.0
3″	6.75 (1H, dq, 5.9, 1.5)	141.1		158.9
4″	4.32 (2H, br d, 5.9)	59.7	4.13 s	66.9
5″	1.79 (3H, d, 1.1)	12.7	2.06 s	15.7

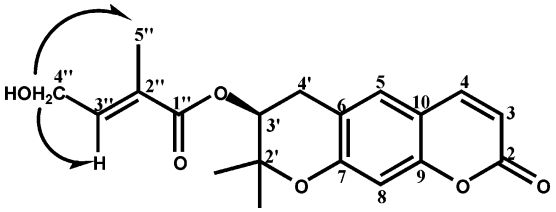

Figure 1. Key NOESY interactions of compound **1**.

Table 2. ^{1}H and ^{13}C NMR Data of Compounds **3** and **4** (in $CDCl_3$)

	3		**4**	
position	H (J = Hz)	C	H (J = Hz)	C
2		161.1		161.1
3	6.22 (1H, d, 9.5)	113.5	6.22 (1H, d, 9.5)	113.6
4	7.55 (1H, d, 9.5)	143.0	7.56 (1H, d, 9.5)	143.0
5	7.12 (1H, s)	128.5	7.14 (1H, s)	128.5
6		115.2		115.2
7		156.2		156.2
8	6.79 (1H, s)	104.9	6.78 (1H, s)	104.8
9		154.3		154.3
10		113.0		113.0
2′		77.2		77.2
3′	5.10 (1H, t, 4.9)	71.5	5.12 (1H, t, 4.7)	71.5
4′	2.81 (1H, dd, 17.4, 4.9)	27.9	2.87 (1H, dd, 17.1, 4.7)	27.8
	3.20 (1H, dd, 17.4, 4.9)		3.20 (1H, dd, 17.1, 4.7)	
gem$(CH_3)_2$	1.36 (3H, s)	23.2	1.35 (3H, s)	23.0
	1.40 (3H, s)	25.0	1.36 (3H, s)	24.9
1″		169.3		169.4
2″		59.7		59.5
3″	2.99 (1H, q, 5.5)	59.8	3.02 (1H, q, 5.3)	60.0
4″	1.23 (1H, d, 5.5)	13.6	1.26 (1H, d, 5.3)	13.6
5″	1.53 (3H, s)	19.0	1.42 (3H, s)	19.1

confirming the 3′*S* configuration.[13,14] Therefore, **2** was characterized as 4″-hydroxydecursin.

Both compounds **3** and **4** were isolated as colorless needles from MeOH. The molecular formulas of both **3** and **4** were determined as $C_{19}H_{20}O_6$ by HREIMS. The EIMS of **3** and **4** showed identical peaks, $[M]^+$ and $[M - C_5H_8O_3]^+$ at *m/z* 344 and 228, respectively. The IR spectra of **3** and **4** showed similar absorption bands for lactone carbonyl

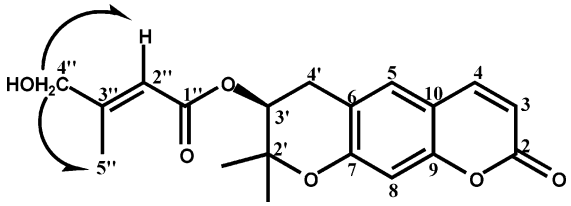

Figure 2. Key NOESY interactions of compound **2**.

(1720 cm^{-1}) and aromatic C=C (1400−1600 cm^{-1}) groups. Their ^{1}H and ^{13}C NMR data showed similarity to those of compounds **1** and **2**, except for the moiety substituted at C-3′. In their ^{1}H NMR spectrum, characteristic signals representing a 2,3-epoxy-2-methylbutanoate (δ 2.99, 1H, q, J = 5.5 Hz, H-3″; δ 1.23, 3H, d, J = 5.5 Hz, H-4″; δ 1.53, 3H, s H-5″ in **3** and δ 3.02, 1H, q, J = 5.3 Hz, H-3″; δ 1.26, 3H, d, J = 5.3 Hz H-4″; δ 1.42, 3H, d, J = 5.3 Hz, H-5″ in **4**) were observed.[17,18] All carbon resonances were fully assigned by ^{13}C NMR, ^{1}H−^{1}H COSY, and ^{13}C−^{1}H COSY spectra. Furthermore, on the basis of reports of Torres-Valencia et al.,[17,18] both **3** and **4** turned out to possess a chiral epoxyangelate ((2*R*,3*R*) or (2*S*,3*S*)) moiety by comparison of signals at δ_H 2.99 (1H, q, J = 5.5 Hz, H-3″) and δ_C 19.0 (C-5″) of **3** and signals at δ_H 3.02 (1H, q, J = 5.3 Hz, H-3″) and δ_C 19.1 (C-5″) of **4** with literature values. It has also been reported that the configuration of the epoxyangelate residues can be assigned by comparison of the ^{1}H NMR data[17−19] (Torres-Valencia et al., 1998; Torres-Valencia, 1999; Zdero and Bohlmann, 1989). In the ^{1}H NMR data of **3** and **4**, a few differences of chemical shift were observed, thus permitting a clear distinction between the two enantiomers. The epoxyangelate methyl groups, Me-4″ and Me-5″, in **3** appeared at δ 1.23 and 1.53, respectively ($\Delta\delta_{5''-4''}$ = 0.3), while in **4** they appeared at δ 1.26 and 1.42 ($\Delta\delta_{5''-4''}$ = 0.16). This significant difference between chemical shifts of Me-4″ and Me-5″ is consistent with the report of Torres-Valrencia et al.,[17] in which in ^{1}H NMR spectra with $CDCl_3$ the gap between chemical shifts of epoxyangelate methyl groups in the (2*S*,3*S*)-epoxyangelate residue turned out to be wider than in the (2*R*,3*R*)-residue, and this trend was confirmed through comparison with data of the synthetic compounds. Concerning the signals of both H-3′ and H-4′ in the dihydropyran ring, they appeared at δ 5.10 and 2.81/3.20, respectively, in **3**, while in **4**, δ 5.12 and 2.87/3.20, respectively. Through comparison of such evidence with literature values,[16−18] it was concluded that **3** contained (2*S*,3*S*)-epoxyangelate and **4**, (2*R*,3*R*)-epoxyangelate (Table 2). On the other hand, since in each NOE difference spectrum of **3** and **4**, irradiation of each H-3′ resulted in the same interactions as those in decursinol, the absolute configuration of each C-3′ in both **3** and **4** was determined to be 3′*S*. From the spectroscopic data above, the structures of **3** and **4** were defined as (2″*S*,3″*S*)-epoxyangeloyldecursinol and (2″*R*,3″*R*)-epoxyangeloyldecursinol, respectively.

The protective activities against glutamate-induced neurotoxicity of the four new dihydropyranocoumarins, 4″-hydroxytigloyldecursinol (**1**), 4″-hydroxydecursin (**2**), (2″*S*,3″*S*)-epoxyangeloyldecursinol (**3**), and (2″*R*,3″*R*)-epoxyangeloyldecursinol (**4**), were measured by determining the lactate dehydrogenase activity in primary cultures of rat cortical cells as described previously.[20] In addition, to investigate differences among their neuroprotective activities depending on substitutions at C-3′ of decursinol, a major dihydropyranocoumarin of *A. gigas,* neuroprotective activities of both decursinol and decursin were also determined. As shown in Table 3, all six compounds, 4″-

Table 3. Neuroprotective Activities of Compounds **1**−**4**, Decursinol, and Decursin on Primary Cultures of Rat Cortical Cells Injured by Glutamate[a]

compound	cell viability (%)[b,d]		
	0.1 μM	1 μM	10 μM
control[c]		100.0	
glutamate-treated[c,e]		0.0	
4″-hydroxytigloyldecursinol (**1**)	33.2 ± 3.5*	19.9 ± 2.0	1.2 ± 4.0
4″-hydroxydecursin (**2**)	38.4 ± 4.0*	34.1 ± 3.5*	35.1 ± 4.5*
(2″*S*,3″*S*)-epoxyangeloyldecursinol (**3**)	70.0 ± 6.0***	52.5 ± 4.4**	49.0 ± 3.0**
(2″*R*,3″*R*)-epoxyangeloyldecursinol (**4**)	47.5 ± 4.0**	61.1 ± 5.0**	56.7 ± 2.8**
decursinol	33.4 ± 3.6*	69.6 ± 6.6***	40.7 ± 6.9*
decursin	39.0 ± 2.6*	50.0 ± 3.3**	65.1 ± 1.6***
MK-801[f]	54.0 ± 5.0**	65.0 ± 5.0***	70.0 ± 6.2***
APV[g]	10.0 ± 2.5	25.0 ± 3.0	39.0 ± 4.0*
CNQX[h]	29.0 ± 3.5*	40.5 ± 3.7*	50.5 ± 4.5**

[a] Rat cortical cell cultures were incubated with test compounds for 1 h. The cultures were then exposed to 100 μM glutamate for 24 h. After the incubation, the cultures were assessed for the extent of neuronal damage. [b] Cell viability was measured by LDH assay. [c] LDH released from control and glutamate-treated culures were 15.0 ± 2.3 and 50.5 ± 4.5 units/mL, respectively. [d] Cell viability was calculated as 100 × (LDH released from glutamate-treated − LDH released from glutamate+test compound-treated)/(LDH released from glutamate-treated − LDH released from control). The values shown are the mean ± STD of three experiments (5 or 6 cultures per experiment). Results differ significantly from the glutamate-treated: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. [e] Glutamate-treated value differed significantly from the untreated control at the level of $p < 0.001$. [f] MK-801: dizocilpine maleate, a noncompetitive antagonist of the NMDA receptor. [g] APV: DL-2-amino-5-phosphonovaleric acid, a competitive antagonist of the NMDA receptor. [h] CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione, non-NMDA receptor antagonist.

hydroxytigloyldecursinol (**1**), 4″-hydroxydecursin (**2**), (2″*S*,3″*S*)-epoxyangeloyldecursinol (**3**), (2″*R*,3″*R*)-epoxyangeloyldecursinol (**4**), decursinol, and decursin containing a decursinol moiety, were significantly effective in the protection against glutamate-induced neurotoxicity at the concentration range of 0.1−10 μM. Among the six compounds tested, (2″*S*,3″*S*)-epoxyangeloyldecursinol (**3**), (2″*R*,3″*R*)-epoxyangeloyldecursinol (**4**), decursinol, and decursin exhibited similar efficacies, showing a relative protection of 60−70% in neuroprotective activity, although significant differences in their potencies were observed. In particular, (2″*S*,3″*S*)-epoxyangeloyldecursinol (**3**) exhibited the highest neuroprotective effect, 70.0% at 0.1 μM, even showing significant protection of 60.2 ± 5.3% ($P < 0.01$) at 0.01 μM. From these results, (2″*S*,3″*S*)-epoxyangeloyldecursinol (**3**) was revealed to be very promising, as it was as effective as a noncompetitive antagonist of NMDA receptor, MK-801, and more potent than it. However, both 4″-hydroxytigloyldecursinol (**1**) and 4″-hydroxydecursin (**2**) showed modest neuroprotective effects, suggesting that the hydroxylated five-carbon units at C-3′ of decursinol reduced the neuroprotective activity of the decursinol moiety. From these results, the four dihydropyranocoumarins, (2″*S*,3″*S*)-epoxyangeloyldecursinol (**3**), (2″*R*,3″*R*)-epoxyangeloyldecursinol (**4**), decursinol, and decursin, from *A. gigas* roots seem to be worthy candidates for protecting neurons from glutamate-induced neurotoxicity.

On the other hand, generally, compounds/phytochemicals given orally or injected are often extensively metabolized. There is extensive first-pass metabolism in the intestinal enterocytes and then metabolism in the liver. As for coumarins, studies on the pharmacokinetics in humans have shown there is extensive first-pass hepatic conversion (by cytochrome P-450 enzymes) to 7-hydroxycoumarin followed by glucuronidation after oral administration, with only 1−5% absolute bioavailability of the parent drug.[21,22] Some evidence including these pharmacokinetic data has led to suggestions that coumarin is a prodrug, most likely active as the 7-hydroxy or, possibly, even as the 7-hydroxy-glucuronide form.[23,24] As for decursinol derivatives, there is no study about metabolism or/and biological activities of their metabolites in vivo. However, in the case where decursinol derivatives may extensively be metabolized in vivo, we suggest that they may exert their neuroprotective activities in vivo. Recent studies reported that orally administered decursinol exerted its neuroprotective and antinociceptive activities in vivo,[5,6,8] and both decursinol and decursin administered intraperitoneally possessed antiamnesic activity.[3] These data support our suggestion that although decursinol derivatives may undergo extensive first-pass metabolism, they may exert their activity in vivo. Moreover, 7-geranyloxycoumarin is a relatively metabolism-resistant substrate for cytochrome P-450 enzymes and, thus, stable in the liver compared with 7-ethoxycoumarin.[25] Since decursinol derivatives in the present study are 7-oxygenated coumarins, we cannot rule out the possibility that they could be more resistant to physiological metabolism than coumarin or 7-hydroxycoumarin.

Finally, although the cellular and molecular mechanisms that underlie the neuroprotective action of decursinol derivatives should be studied further, considering the neuroprotective activity in the present study as well as anti-AChE and antiamnesic activities of both decursinol and decursin revealed in our previous studies,[3,4] these decursinol derivatives seem noteworthy as promising candidates for the treatment of neurodegenerative diseases with complicated pathology such as AD.[2]

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were run on a JEOL GSX 400 spectrometer at 400 and 100 MHz, respectively, with TMS as internal standard. FT-IR spectra were recorded on a Perkin-Elmer 1710 spectrophotometer. UV spectra were recorded on a Shimadzu UV-2100 spectrophotometer. EIMS spectra were obtained on a VG Trio II spectrometer, and FABMS spectra were obtained in a VG 70-VSEQ mass spectrometer with a direct inlet system using PEG 600/glycerol as a matrix. High-resolution mass spectral analyses were obtained on a JEOL JMS AX 505 WA spectrometer. Melting points were determined on an apparatus. Optical rotations were measured on a JASCO DIP 1000 digital polarimeter. Column chromatography was performed on Merck (9025) silica gel 60 (0.04−0.063 mm). Analytical TLC was performed on precoated Merck F_{254} silica gel plates and visualized by spraying with anisaldehyde-H_2SO_4. An HPLC system (Hitachi L-6200, Japan) equipped with a UV−visible detector and A-323 C_8 RP semipreparative column (YMC Co.) was used for purification.

Plant Material. The root of *A. gigas* was purchased in a local market for Oriental medicine in Chechon, Chung-Buk, Korea, and voucher specimens (SNUPH-0415) have been deposited in the Herbarium of the College of Pharmacy, Seoul National University.

Extraction and Isolation. The dried underground part (2 kg) of *A. gigas* was extracted with MeOH in an ultrasonic apparatus. Upon removal of solvent in vacuo, the MeOH extract yielded 150 g of residue. This was then suspended in H_2O and partitioned successively with CH_2Cl_2. Silica gel column chromatography of the CH_2Cl_2 fraction (90 g) with a mixture of *n*-hexane−$CHCl_3$−MeOH as eluent afforded seven fractions (F1−F7). F4 (4.5 g), the fraction with the highest neuroprotective activity, was subjected to silica gel column chromatography with a *n*-hexane−EtOAc−MeOH mixture and yielded 10 subfractions (F4.1−F4.9). F4.1 (900 mg) was eluted by C_{18} RP silica gel column chromatography with MeO−H_2O (20% MeOH in H_2O to 100% MeOH). The 50% MeOH eluate (90 mg) was eluted on Sepak with MeOH−H_2O, 60:40, and afforded pure compounds **3** (3 mg, t_R 56.2 min) and **4** (6 mg, t_R 58.4 min) and crude compounds **1** (10 mg, t_R 35.0 min) and **2** (13 mg, t_R 37.0 min) by HPLC on a A-323 C_8 5 mm column (250 × 10 mm) using AcCN−MeOH−H_2O (34:7:59, flow rate 2 mL/min). Then pure compounds **1** (5 mg) and **2** (7 mg) were obtained by consecutive HPLC on a A-323 C_8 5 μm column (250 × 10 mm) using AcCN−MeOH−H_2O (44:5:51, flow rate 2 mL/min, t_R 18.2 and 19.7 min, respectively).

Compound 1: colorless needles from MeOH; mp 104−106 °C; $[\alpha]^{20}_D$ +56° (*c* 0.5, $CHCl_3$); UV λ_{max} nm (log ϵ) 217 (4.51), 256 (3.64), 327 (4.34); IR ν_{max}cm^{-1} 3440 (OH), 1720 (C=O), 1400−1600 (aromatic −C=C−); ^{1}H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; FABMS *m/z* 367 $[M + Na]^+$, 345 $[M + H]^+$; HRFABMS *m/z* 345.1339 (calcd for $C_{19}H_{20}O_6$, 345.1338).

Compound 2: colorless needles from MeOH; mp 101−103 °C; $[\alpha]_D$ +59° (*c* 0.5, $CHCl_3$); UV λ_{max} nm (log ϵ) 256 (3.83), 290 (4.08), 325 (4.16); IR ν_{max}cm^{-1} 3440 (OH), 1720 (C=O), 1400−1600 (aromatic −C=C−); ^{1}H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; FABMS 367 $[M + Na]^+$, 345 $[M + H]^+$; HRFABMS *m/z* 345.1333 (calcd for $C_{19}H_{20}O_6$, 345.1338).

Compound 3: colorless needles from MeOH; mp 140−142 °C; $[\alpha]_D$ +91° (*c* 0.5, $CHCl_3$); UV λ_{max} nm (log ϵ) 255 (3.69), 328 (4.20); IR ν_{max}cm^{-1} 1730 (C=O), 1400−1600 (aromatic −C=C−); ^{1}H NMR (400 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; EIMS *m/z* (rel int) 344 $[M]^+$ (48), 228 $[M - C_5H_8O_3]^+$ (100), 213 $[M - C_5H_8O_3 - CH_3]^+$ (100) HREIMS *m/z* 344.1252 (calcd for $C_{19}H_{20}O_6$, 344.1260).

Compound 4: colorless needles from MeOH; mp141−143 °C; $[\alpha]_D$ +24° (*c* 0.5, $CHCl_3$); UV λ_{max} nm (log ϵ) 255 (3.23), 326 (3.87); IR ν_{max}cm^{-1} 1730 (C=O), 1400−1600 (aromatic −C=C-); ^{1}H NMR (400 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; EIMS *m/z* (rel int) 344 $[M]^+$ (12), 228 $[M - C_5H_8O_3]^+$ (28), 213 $[M - C_5H_8O_3 - CH_3]^+$ (100); HRFABMS *m/z* 344.1261 (calcd for $C_{19}H_{20}O_6$, 344.1260).

Cortical Cell Culture. Primary cultures of mixed cortical cells containing both neurons and glia were prepared from 17−19-day-old fetal rats (Sprague−Dawley) as described previously.[11] In brief, the trypsin-dissociated cortical cells were plated on 15 mm dishes (Falcon Primaria, Becton Dickinson, NJ) coated with collagen at a density of 5×10^5 cells/dish. The cortical cells were grown in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 100 IU/mL penicillin, and 10 μg/mL streptomycin at 37 °C in a humidified atmosphere of 95% air−5% CO_2. After 3 days in culture, cell division of non-neuronal cells was halted by adding 5-fluoro-2′-deoxyuridine (50 μM). Cultures were allowed to mature for at least 2 weeks before being used for experiments. Our mixed cortical cultures consisted of approximately 70−75% cells immunopositive for neuron-specific enolase and 25−30% cells immunopositive for glial fibrillary acidic protein as determined by immunocytochemical staining methods.[19]

Assessment of Cell Viability. Test compounds were dissolved in DMSO (final concentration in culture, 0.1%). Cortical cell cultures (17 days in vitro) were washed with DMEM and incubated with test compounds for 1 h. The cultures were then exposed to 100 μM glutamate and maintained for 24 h. After the incubation, the cultures were assessed for the extent of neuronal damage. The cultures were assessed for viability by measuring the efflux of LDH (lactic dehydrogenase), which reflects the integrity of cellular membranes.[19,26] Data are expressed as the percentage protection relative to vehicle-treated control cultures: 100 × [optical density (OD) of glutamate+sample-treated cultures − OD of glutamate-treated cultures]/[OD of control cultures − OD of glutamate-treated cultures].

Statistical Analysis. The evaluation of statistical significance was determined by the one-way ANOVA test with a value of $p < 0.05$ considered to be statistically significant.

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Supporting Information Available: Spectra of compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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