Dictyoquinazols A, B, and C, New Neuroprotective Compounds from the Mushroom *Dictyophora indusiata*

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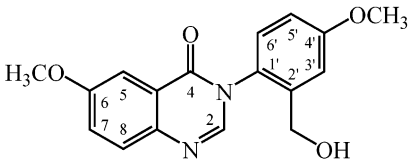
In an effort to identify neuroprotective compounds against excitatory neurotoxins from edible and medicinal mushrooms, dictyoquinazols A (**1**), B (**2**), and C (**3**) have been isolated from the methanolic extract of the mushroom *Dictyophora indusiata*. On the basis of NMR studies, their structures have been assigned as unique quinazoline compounds, which are very rare in nature. **2** and **3** existed as mixtures of rotamers (**2a**, **2b** and **3a**, **3b**) inseparable by HPLC because of fast interconversion. The *E*/*Z* isomers of these compounds were assigned by comparison of ^{1}H NMR, ^{13}C NMR, and NOESY spectra and by $^3J_{C,H}$ coupling constants. Dictyoquinazols protected primary cultured mouse cortical neurons from glutamate- and NMDA-induced excitotoxicities in a dose-dependent manner.

Hyperactivity of ionotropic glutamate receptors has been implicated in the development of neuronal cell death followed by many neurodegenerative processes including ischemic stroke, brain injury, and epilepsy.[1] *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors have been also proposed as one of the important contributors to neuronal death in brain.[2,3] Thus, intense efforts have been made on the discovery of compounds that block these receptors for a potential therapeutic remedy of these ailments.

In our research on novel neuroprotective compounds against excitatory neurotoxins including glutamate, NMDA, AMPA, and kainate using mouse primary cortical cell cultures, three new quinazoline compounds, named dictyoquinazols A (**1**), B (**2**), and C (**3**), were isolated from the mushroom *Dictyophora indusiata* (Vent.: Pers.) Fisch., which is an edible mushroom used in Chinese food and medicine. It is noteworthy that compounds **1**−**3** have a unique quinazoline moiety, rare in natural products, and we report their first isolation from the mushrooms. **2** was a mixture of unseparable rotamers **2a** and **2b**, and **3** was a mixture of rotamers **3a** and **3b**; thus **2** and **3** were analyzed and bioassayed as a mixture of rotamers. The regiochemistry of these compounds has been assigned by NMR experiments. In this paper, the isolation, structural determination, and biological evaluation of these compounds are described.



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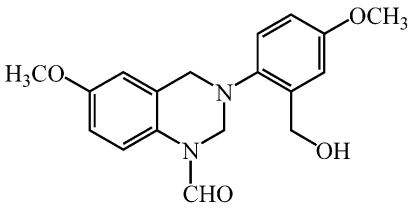

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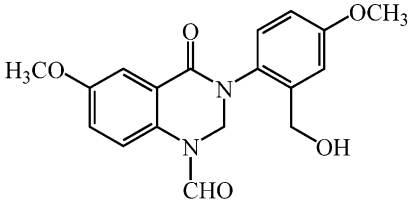

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Results and Discussion

A methanolic extract of the fresh mushroom *D. indusiata* was chromatographed on a column of Diaion HP-20 and partitioned between ethyl acetate and water. The organic fraction was further purified by Sephadex LH-20 column chromatography and preparative HPLC to give **1**−**3**.

Dictyoquinazol A (**1**) was obtained as a pale yellow oil, and its molecular formula was established as $C_{17}H_{17}O_4N_2$ by HRFABMS. IR absorptions at 3420 and 1655 cm^{-1} were attributed to the hydroxyl and amide carbonyl groups, respectively. The ^{1}H NMR spectrum in CD_3OD showed signals assignable to two aromatic methoxyl groups (δ 3.89 and 3.94), an asymmetric oxygenated methylene group (δ 4.45 and 4.40), and seven aromatic methine groups including two 1,2,4-trisubstituted benzenes from δ 7.0 to 7.8 (Table 1). The ^{13}C NMR spectrum suggested the presence of two methoxyl, an oxygenated methylene, an amide carbonyl, seven sp^2 methine, and six sp^2 quaternary carbons, as shown in Table 2. The characteristic chemical shifts of a methine proton (δ 8.09) and carbon (δ 147.1) of C-2 indicated close vicinity to nitrogen, and it was supported by a $^1J_{CH}$ coupling constant of C-2 of 212 Hz.[4] The structure of **1** was determined on the basis of HMBC correlations. The HMBC correlations of H-2 to C-4 and C-8a, H-5 to C-4 and C-8a, H-7 to C-8a, and H-8 to C-4a established a quinazolinone skeleton, and the long-range correlations of 2′-CH_2 to C-1′, C-2′, and C-3′, 4′-OCH_3 to C-4′, and H-2 to C-1′ revealed that a 2-hydroxymethyl-4-

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Table 1. ^{1}H NMR Spectral Data for Dictyoquinazols A (**1**), B (**2**), and C (**3**) in CD_3OD

no.	1	2a	2b	3a	3b
1-CHO		8.66 (1H, s)	7.92 (1H, s)	8.69 (1H, s)	8.48 (1H, s)
2	8.09 (1H, s)[a]	4.84 (2H,s)	4.80 (2H, s)	5.41 (1H, d, J = 11.5)	5.35 (1H, d, J = 11.5)
				5.36 (1H, d, J = 11.5)	5.27 (1H, d, J = 11.5)
4		4.39 (2H, s)	4.47 (2H, s)		
5	7.69 (1H, d, J = 3.0)	6.81 (1H, d, J = 3.0)	6.81 (1H, d, J = 3.0)	7.59 (1H, d, J = 3.0)	7.57 (1H, d, J = 3.0)
6-OCH_3	3.94 (3H, s)	3.80 (3H, s)	3.81 (3H, s)	3.87 (3H, s)	3.90 (3H, s)
7	7.49 (1H, dd, J = 9.0, 3.0)	6.88 (1H, dd, J = 9.0, 3.0)	6.86 (1H, dd, J = 9.0, 3.0)	7.25 (1H, dd, J = 8.8, 3.0)	7.24 (1H, dd, J = 8.9, 3.0)
8	7.72 (1H, d, J = 9.0)	7.28 (1H, d, J = 9.0)	7.98 (1H, d, J = 9.0)	7.48 (1H, d, J = 8.8)	7.95 (1H, d, J = 8.9)
2′-CH_2	4.45 (1H, d, J = 13.1)	4.76 (2H, s)	4.74 (2H, s)	4.53 (1H, d, J = 13.7)	4.51 (1H, d, J = 13.7)
	4.40 (1H, d, J = 13.1)			4.49 (1H, d, J = 13.7)	4.47 (1H, d, J = 13.7)
3′	7.21 (1H, d, J = 3.0)	7.07 (1H, d, J = 3.0)	7.06 (1H, d, J = 3.0)	7.16 (1H, d, J = 2.8)	7.13 (1H, d, J = 2.9)
4′-OCH_3	3.89 (3H, s)	3.75 (3H, s)	3.76 (3H, s)	3.85 (3H, s)	3.85 (3H, s)
5′	7.03 (1H, dd, J = 8.6, 3.0)	6.67 (1H, dd, J = 8.7, 3.0)	6.69 (1H, dd, J = 8.6, 3.0)	6.94 (1H, dd, J = 8.7, 2.8)	6.97 (1H, dd, J = 8.6, 2.9)
6′	7.29 (1H, d, J = 8.6)	6.82 (1H, d, J = 8.7)	6.78 (1H, d, J = 8.6)	7.22 (1H, d, J = 8.7)	7.26 (1H, d, J = 8.6)

[a] Proton resonance integrals, multiplicities, and coupling constants (J = Hz) are in parentheses.

Table 2. ^{13}C NMR Spectral Data for Dictyoquinazols A (**1**) and B (**2**) in CD_3OD

no.	1	2a	2b
1-CHO		162.4	162.9
2	147.1	61.5	68.0
4	162.7	54.3	54.7
4a	124.2	129.0	129.8
5	107.4	112.6	111.7
6	160.8	158.9	158.5
6-OCH_3	56.4	56.0	55.9
7	125.7	114.6	113.5
8	130.0	119.2	124.9
8a	143.5	130.9	130.5
1′	129.5	141.2	141.2
2′	141.4	139.4	139.3
2′-CH_2	61.4	61.2	61.5
3′	115.2	114.8	115.8
4′	162.2	158.6	158.7
4′-OCH_3	56.1	55.8	56.0
5′	114.7	113.7	114.0
6′	130.4	123.6	124.8

methoxyphenyl group was attached to *N*-3 of the quinazolinone moiety. On the basis of these results, the structure of **1** was determined as 3-(2-hydroxymethyl-4-methoxyphenyl)-6-methoxyl-4-quinazolinone, which was a new quinazolinone compound. **1** was similar to methaqualone,[5] which was approved as a sedative-hypnotic and psychotropic substance.

Dictyoquinazol B (**2**) was obtained as a pale yellow oil and a single peak in the HPLC profile. However, a set of separated signals with different intensities in the ^{1}H NMR spectrum suggested that **2** was a mixture of two components, **2a** and **2b**, in the ratio of 1:0.8, and the mixture could not be separated by HPLC. The EIMS spectrum of the mixture revealed an ion peak at m/z 328 $[M]^+$, and the HREIMS established their molecular formulas to be $C_{18}H_{20}O_4N_2$, suggesting that **2a** and **2b** had the same molecular composition. IR absorptions at 3420 and 1676 cm^{-1} were attributed to the hydroxyl and amide carbonyl groups, respectively. The ^{1}H NMR spectrum of the major component **2a** revealed the presence of two aromatic methoxyl groups (δ 3.80 and 3.75), three methylenes (δ 4.84, 4.39, and 4.76), six aromatic methines for two 1,2,4-trisubstituted benzenes between δ 6.6 and 7.3, and a formyl group (Table 1). The ^{13}C NMR spectrum showed signals for two methoxyl groups, three methylenes, six sp^2 methines, six sp^2 quaternary carbons, and an aldehyde. An aldehyde peak at δ_H 8.66 and δ_C 162.4 implied that it was attached to nitrogen or oxygen. The linkage to nitrogen was based on its $^1J_{C,H}$ coupling constant of 198 Hz. The planar structure of **2a** was assigned by interpretation of a HMBC spectrum. H-2 was coupled with C-4 and C-8a, H-4 with C-4a, C-5, and C-8a, and H-7 with C-8a, respectively. These correlations established a 2,4-dihydroquinazoline moiety. Also the long-range correlations from H-2 and H-4 to C-1′ linked a 2-hydroxymethyl-4-methoxyphenyl group to *N*-3, and the correlation of the formyl proton at δ 8.66 with C-2 placed the formyl group on *N*-1. Thus, the structure of **2a** was assigned as 1-formyl-3-(2-hydroxymethyl-4-methoxyphenyl)-6-methoxyl-2,4-dihydroquinazoline. ^{1}H and ^{13}C NMR spectra of **2b** were very similar to those of **2a**. Namely, these spectra showed the presence of two aromatic methoxyl groups, three methylenes, two 1,2,4-trisubstituted benzenes, and a formyl group (Tables 1 and 2). The structure of **2b** was also assigned by the interpretation of the HMBC spectrum, which showed the long-range correlations from the formyl proton to C-8a, from H-2 to C-4, C-8a, and C-1′, and from H-4 to C-4a, C-5, C-8a, and C-1′. Therefore, the planar structure of **2b** was assigned as the same structure as **2a**, suggesting that **2b** was a close isomer of **2a**.

Comparison of NMR chemical shifts indicated that **2a** and **2b** were well matched except for 1-CHO and H-8 in the ^{1}H NMR spectrum, and C-2 and C-8 in the ^{13}C NMR spectrum. This implied that **2a** differed from **2b** in regiochemistry of the formyl−N-1 amide bond. The geometry of **2a** and **2b** was investigated by a NOESY experiment and interpretation of $^3J_{C,H}$ values. **2a** showed NOEs between H-2 and H-6′, H-4 and H-6′, and 1-CHO and H-8, and a weak NOE between 1-CHO and H-2, whereas **2b** exhibited NOEs between H-2 and H-6′, and H-4 and H-6′, and a significant NOE between 1-CHO and H-2. This suggested that in **2a** the formyl−N-1 amide bond was *Z*, while in **2b** it was *E*, as shown in Figure 1. In addition, a small $^3J_{1-CHO,C8a}$ value of <2 Hz in **2a** suggested a dihedral angle of 0°, and the very small $^3J_{1-CHO,C2}$ value of **2b** corresponded to a dihedral angle of 0°.[6] *E*/*Z* isomerism (interconversion of rotamers) of *N*-acyl compounds could be interpreted by physicochemical measurement. The slow interconversion rate of amide rotamers in solution usually is on the order of 10^{-1}−10^{-2}/s.[7,8] Thus, the two rotamers **2a** and **2b** can survive long enough to be detected by NMR experiments.

Dictyoquinazol C (**3**) was obtained in small amount as a mixture of two rotamers, **3a** and **3b**, in a ratio of 1:0.4 as well as **2**. Their molecular formulas were established as $C_{18}H_{18}O_5N_2$ by HREIMS. The IR absorptions near 3420 and 1654 cm^{-1} were attributed to hydroxyl and amide carbonyl groups, respectively. ^{1}H NMR and DQF-COSY spectra of the major component **3a** provided partial structures including a formyl group, two 1,2,4-trisubstituted benzenes, two methylenes, and two methoxyl groups. The general structure of **3a** was very similar to **2a** except for the disappearance of a methylene signal. The structure of **3a** was

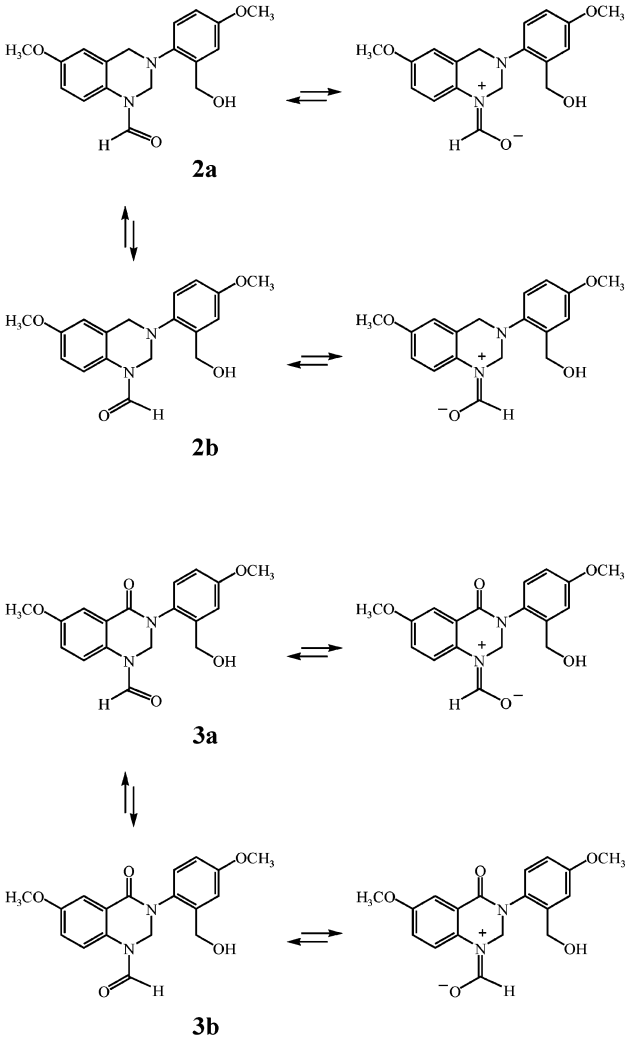

Figure 1. *E*/*Z* isomerism of dictyoquinazols B (**2**) and C (**3**) elucidated by the NOESY experiments and $^3J_{C,H}$ values.

assigned by a HMBC spectrum. The HMBC spectrum showed critical correlations from H-2 to formyl carbonyl carbons at δ 162.0 and to C-4 at δ 164.3. Therefore, **3a** was assigned as a quinazolinone, 1-formyl-3-(2-hydroxymethyl-4-methoxyphenyl)-6-methoxyl-2-hydro-4-quinazolinone. Other HMBC cross-peaks were well matched to the structure of **3a**. The planar structure of **3b** was also determined to be the same as **3a** on the basis of the ^{1}H NMR, DQF-COSY, and HMBC spectra, revealing that **3b** was a rotamer of **3a**. The regiochemistry of **3a** and **3b** was deduced by comparison of ^{1}H NMR and ROESY spectral data. The ^{1}H NMR spectra of **3a** and **3b** were almost the same except for 1-CHO, H-2, and H-8, and this suggested that **3a** and **3b** differed in N-1 geometry. The ROESY spectrum of **3a** showed a significant NOE between 1-CHO and H-8 and a very weak NOE between 1-CHO and H-2, while **3b** exhibited a very strong NOE between 1-CHO and H-2 and no NOE between 1-CHO and H-8. These results implied that the formyl−N-1 amide bond was *Z* in **3a**, while in **3b** it was *E*. Also, small vicinal proton−carbon coupling constants of $^3J_{1-CHO,C8a}$ in **2a** and $^3J_{1-CHO,C2}$ in **2b** corresponded to a dihedral angle of 0°. The regiochemistry of **3** was in accordance with those of **2**.

The biological activity of compounds **1**−**3** to protect neuronal cells from excitotoxicity was estimated by observing primary cultured mouse cortical neurons upon treatment of excitatory neurotoxins including glutamate, AMPA, NMDA, and kainate. Although **1**−**3** were less active than

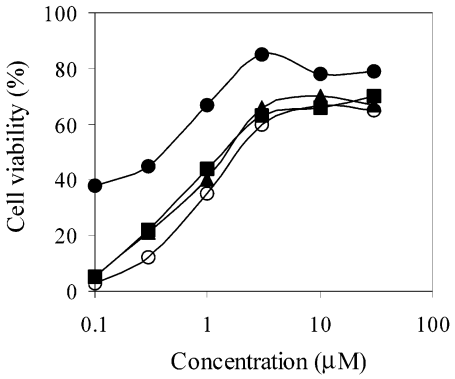

Figure 2. Neuroprotective activity of **1** (○), **2** (▲), **3** (■), and MK-801 (●) against excitotoxicity induced by glutamate.

the NMDA-receptor antagonist MK-801, they protected neurons from glutamate-induced neurotoxicity to a significant degree at concentrations ranging from 5 to 10 μM (Figure 2). Also these compounds protected neurons from toxicity induced by NMDA, in a dose-dependent manner, at concentrations ranging from 10 to 30 μM. However, **1**−**3** did not protect cortical neurons damaged by non-NMDA receptor agonists, AMPA and kainate. Recently, methaqualone derivatives with a C-2 enol side chain have been reported as a potent noncompetitive AMPA receptor antagonist, whereas its derivatives without a C-2 enol chain did not block AMPA receptor function.[9−11] Our results are in accordance with the results in that **1**−**3** without a C-2 enol side chain did not protect cortical cells against AMPA neurotoxicity. It has been known that glutamate neurotoxicity occurs through increase of intracellular Ca^{2+} influx by activation of glutamatergic receptors and, consequently, through the overproduction of free radicals via activation of Ca^{2+}-dependent enzymes such as phospholipase and/or nitric oxide synthase.[12−14] For the purpose of evaluating the antioxidative activity of **1**−**3**, superoxide and DPPH radical scavenging activities were investigated. In addition, inhibitory activity against glutamate toxicity in the neurohybridoma N18-RE-105 cell line was assessed. Glutamate in N18-RE-105 cells gives rise to inhibition of cystine uptake followed by the reduction of intracellular levels of glutathione, which acts as a reducing agent.[15] Consequently, intracellularly accumulated oxygen radicals cause neuronal cell death. **1**−**3** did not show radical scavenging activity and inhibitory effects against glutamate toxicity in N18-RE-105 cells (data not shown). These results suggested that **1**−**3** to protect neuronal cells against glutamate act as glutamate receptor antagonists, especially NMDA-receptor antagonists, but not antioxidants. On the other hand, it was known that neurodegeneration is also caused by H_2O_2 produced from accumulated catecholamines via monoamine oxidase (MAO) in the 5 min after 15 min global ischemia in rat.[16,17] Therefore, MAO inhibitors have been reported to block neuronal cell death induced by NMDA in cortical cultures.[18] **1**−**3** revealed moderate MAO inhibitory activity in the range 60−100 μM. Further biological activity and mode of action of these compounds for neuroprotection will be investigated.

Experimental Section

General Experimental Procedures. Specific rotation was determined by using a JASCO DIP-370 polarimeter. EIMS and HREIMS were taken on a Micromass Autospec mass spectrometer operating at 70 eV, and FABMS and HRFABMS spectra were obtained on a JEOL JMS-HX 110A mass spectrometer in the FAB mode using a glycerol matrix with poly-

(ethylene glycol) as internal standard. UV and IR spectra were recorded on a Shimadzu UV-300 and a FT-IR Equinox 55 spectrometer, respectively. NMR spectra were obtained on a Bruker DMX 600 NMR spectrometer with ^{1}H NMR at 600 MHz and ^{13}C NMR at 150 MHz. Chemical shifts are given in ppm (δ) using TMS as internal standard. HPLC was run on a Waters 510 instrument equipped with a 991 photodiode array detector using YMC ODS-H80 (250 × 20 mm i.d) for preparative column, Cosmosil 5C 18-AR for analytical column, and CHIREX (R)-NGLY and DNB for chiral column.

Mushroom Material. The fresh mushroom *D. indusiata* (1.2 kg) was collected at Gochang-kun, Korea, in September 2000, and identified by staff at the Korea Research Institute of Bioscience and Biotechnology (KRIBB). The voucher specimen (No. 3725) is deposited in the herbarium of the Department of Biology, Woosuk University.

Extraction and Isolation. *D. indusiata* was extracted twice with methanol at room temperature for 2 days. After removal of methanol under reduced pressure, the resulting solution was subjected to a column of Diaion HP-20. The column was washed with H_2O and eluted with 30% aqueous methanol. The eluate was concentrated in vacuo to evaporate methanol, and the concentrate was partitioned between ethyl acetate and water. The ethyl acetate-soluble portion was chromatographed on a column of Sephadex LH-20 eluting with 70% aqueous methanol, followed by preparative HPLC using a YMC ODS-H80 column with 60% aqueous methanol as mobile phase to give **1** (4.0 mg), **2** (1.5 mg), and **3** (1.0 mg).

Dictyoquinazol A (1): pale yellow oil; UV λ_{max} (MeOH) (log ϵ) 224 (3.90), 274 (3.43), 319 (2.78) nm; IR ν_{max} 3420, 2927, 1655, 1460 cm^{-1}; for ^{1}H NMR and ^{13}C NMR in CD_3OD, see Tables 1 and 2, respectively; ^{1}H NMR (acetone-d_6) δ 7.99 (1H, s, H-2), 7.69 (1H, d, J = 8.9, H-8), 7.63 (1H, d, J = 3.0, H-5), 7.45 (1H, dd, J = 8.9, 3.0, H-7), 7.34 (1H, d, J = 8.6, H-6′), 7.25 (1H, d, J = 3.0, H-3′), 7.02 (1H, dd, J = 8.6, 3.0, H-5′), 4.48 (1H, d, J = 13.5, 2′-CH_2), 4.43 (1H, d, J = 13.5, 2′-CH_2), 3.94 (3H, s, 6-OCH_3), 3.89 (3H, s, 4′-OCH_3); FABMS m/z 313 [M + H]$^+$; HRFABMS m/z 313.1203 [M + H]$^+$ ($C_{17}H_{17}O_4N_2$ requires 313.1188).

Dictyoquinazol B (2): pale yellow oil; $[\alpha]_D$ −80° (c 0.05, MeOH); UV λ_{max} (MeOH) (log ϵ) 206 (3.70), 239 (3.85), 290 (3.2) nm; IR ν_{max} 3420, 2922, 1676, 1501 cm^{-1}; for ^{1}H NMR and ^{13}C NMR in CD_3OD, see Tables 1 and 2, respectively; ^{1}H NMR (acetone-d_6) **2a** δ 8.71 (1H, s, 1-C*H*O), 7.35 (1H, d, J = 8.9, H-8), 7.12 (1H, d, J = 3.0, H-3′), 7.08 (1H, d, J = 3.0, H-5), 6.89 (1H, dd, J = 8.9, 3.0, H-7), 6.87 (1H, d, J = 8.7, H-6′), 6.68 (1H, dd, J = 8.7, 3.0, H-5′), 4.85 (2H, s, H-2), 4.75 (2H, s, 2′-CH_2), 4.39 (2H, s, H-4), 3.80 (3H, s, 6-OCH_3), 3.74 (3H, s, 4′-OCH_3), **2b** δ 8.14 (1H, d, J = 8.9, H-8), 8.05 (1H, s, 1-C*H*O), 7.09 (1H, d, J = 3.0, H-3′), 6.85 (1H, d, J = 3.0, H-5), 6.85 (1H, dd, J = 8.9, 3.0, H-7), 6.81 (1H, d, J = 8.7, H-6′), 6.69 (1H, dd, J = 8.7, 3.0, H-5′), 4.84 (2H, s, H-2), 4.75 (2H, s, 2′-CH_2), 4.45 (2H, s, H-4), 3.81 (3H, s, 6-OCH_3), 3.75 (3H, s, 4′-OCH_3); EIMS m/z 328 M$^+$; HREIMS m/z 328.1424 M$^+$ ($C_{18}H_{20}O_4N_2$ requires 328.1423).

Dictyoquinazol C (3): pale yellow oil; $[\alpha]_D$ +60° (c 0.033, MeOH); UV λ_{max} (MeOH) (log ϵ) 228 (3.9), 319 (2.9) nm; IR ν_{max} 3420, 2947, 2836, 1654 cm^{-1}; for ^{1}H NMR, see Table 1; EIMS m/z 342 M$^+$; HREIMS m/z 342.1218 M$^+$ ($C_{18}H_{18}O_5N_2$ requires 342.1216).

Cortical Cell Culture. Mouse cortical cell cultures were established from gestation day 15 ICR mouse embryos as described by Xie.[19] The cortices were incubated in 0.8 mg of trypsin/mL HBSS (Hank's balanced salts solution) for 7 min and then rinsed three times in 10 mL of HBSS. Cells were then centrifuged at 1500 rpm for 5 min, and after removal of media, the pellet was resuspended by trituration through the narrowed bore of a fired-polished Pasteur pipet in B27-supplemented neurobasal medium. The cortical cells were plated on 48-well plates (1×10^6 cells/dish) precoated with 100 μg/mL poly-D-lysine. The cortical cells were grown in neurobasal medium containing B27-supplement, L-glutamine (final concentration, 0.5 mM), 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. Cultures were allowed to mature for 12 days before they were used for experiments. Neurobasal medium, which is derived from Dulbecco's Modified Eagle's Medium, was optimized for cell survival and the almost complete absence of glial cells.[20] To assess neuroprotective activity, cortical cultures were replaced with neurobasal medium without supplement.

Assessment of Neurotoxicity. Test compounds were dissolved in methanol (final concentration, 0.1%). To assess the neuroprotective activity of **1**−**3** against excitotoxin-induced damage, 12-day-old cortical cell cultures were used. Cultures were pretreated with compounds for 1 h before being exposed to 20 μM glutamate, 80 μM AMPA, 40 μM NMDA, and 200 μM kainate, respectively. The cultures were then maintained for an additional 24 h in neurobasal medium. Neuronal viability was measured by the MTT assay, which reflects mitochondrial enzyme function. MTT tetrazolium salt (1 mg/mL) was added to neurons grown in 48-well microplates followed by incubation for 1 h at 37 °C in 5% CO_2 and in saturated humidity. The reaction medium was gently aspirated, and then DMSO was added to solubilize the blue formazan product. Neuronal viability was estimated by quantifying soluble formazan using a Molecular Devices tunable microplate reader at 540 nm. Cell viability was calculated as 100 × (OD of excitotoxin + compound-treated − OD of excitotoxin-treated)/(OD of control − OD of excitotoxin-treated).

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