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Synthesis of 1,3-diphenyl-2-propen-1-one derivatives and evaluation of their biological activities

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Abstract—A simple synthesis and biological properties of 1,3-diphenyl-2-propen-1-ones **18**–**22** and **25**–**26** are described. The key synthetic strategies involve Grignard reaction of aldehyde **2** and oxidation reaction of **8**–**12** in high yields. The prepared compounds **18**–**22** and **25**–**26** were evaluated for free-radical scavenging, suppression of LPS-induced NO generation, and anti-excitotoxicity in vitro. It was found that a couple of compounds, especially **21** and **26**, were potent suppressors of NO generation and demonstrated anti-excitotoxicity with the concentration range 10–20 μM in vitro.

1. Introduction

The naturally occurring Yakuchinones A and B or related 1,3-diphenyl-2-propen-1-ones exhibit a wide range of intriguing biological activities[1] such as anti-inflammatory,[2] antitumor,[3] antibacterial,[4] antiviral,[5] and gastric protective activities.[6] Recently, the Ryu group[1a] described that the optimal length of linker between two aryl groups played an important role for the biological activity. The Deck and Vander Jagt group[1d] reported the curcumin and related enones having significant anti-oxidant activities based on the formation of stable carbon centered-radicals. The Yakuchinones and their analogues are interesting due to their scavenging effect of active oxygen,[7] nematocidal activity,[8] inhibition of lipid peroxidation,[9] and inhibition of acyl-CoA[10] (Fig. 1). Furthermore, accumulating evidence implicates that endogenous excitatory amino acids, especially glutamate, play an important role in the neuronal degeneration associated with some neurological diseases such as ischemia, Parkinson's disease, Alzheimer's disease, and Huntington's disease. In these neurodegenerative diseases, glutamate is excessively released and then activates the glutamate receptor. Overactivation of the receptors may induce elevation of intracellular Ca^{2+} levels resulting in activation of Ca^{2+} dependent proteases and kinases. High intracellular Ca^{2+} may also activate nitric oxide synthase, resulting in excessive production of nitric oxide (NO) and cytotoxicity. Although many reagents, such as glutamate receptor antagonists, calcium channel antagonists, anti-inflammatory agents, and nitric oxide synthase (NOS) inhibitors, have neuroprotective effects, their serious side effects limit clinical application. Antioxidants also play an important role in biological defense mechanisms. According to the accumulated research data, activated oxygen is thought to be a major factor in cytotoxicity. Therefore, research was undertaken to search for novel compounds with better neuroprotective effects and less neurotoxicity. It is known that 4-hydroxy-3-methoxycinnamaldehyde has antioxidant properties and its analogues, 4-hydroxy-3-methoxycinnamic acid (ferulic acid), could be applicable in neurodegenerative diseases.[11]

In the context of our medicinal chemistry program dealing with the development of new Yakuchinone derivatives, we have introduced aromatic substrates having electron-withdrawing groups or electron-donating groups in order to generate antioxidant agents and anti-excitotoxicity compounds. Our previous work has examined the biological properties of

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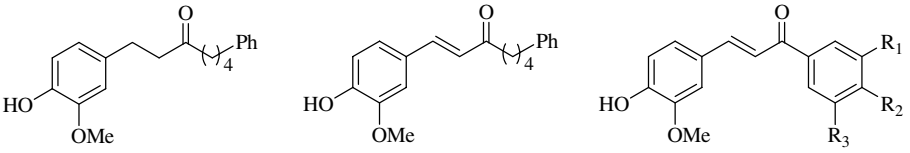


Yakuchinone A Yakuchinone B R_1, R_2, R_3 = H, Me, F, or OMe

Figure 1. Structures of Yakuchinone A, Yakuchinone B, and 1,3-diphenyl-2-propen-1-ones.

benzylideneacetophenone derivatives.[12] We wish to report herein the synthesis and biological activities of 1,3-diphenyl-2-propen-1-ones **18**–**22** and **25**–**26** for free-radical scavenging, suppression of LPS-induced NO generation in vitro, and anti-excitotoxicity starting from 4-hydroxy-3-methoxy cinnamaldehyde (**1**) via Grignard reaction of aldehyde **2** and oxidation reaction of **8**–**12** in high yields.

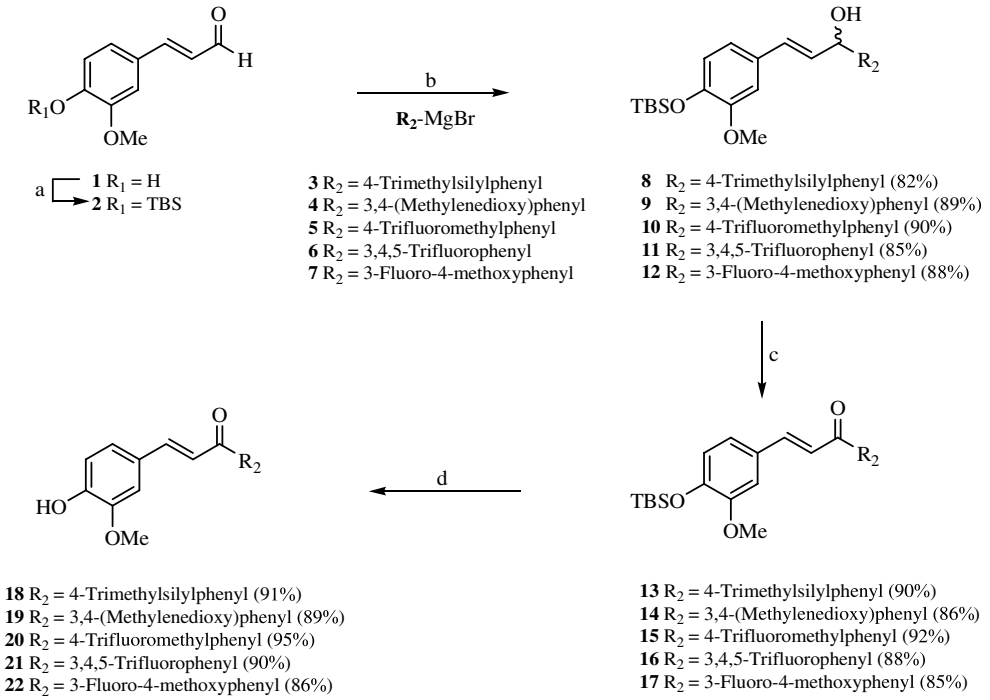
2. Results and discussion

2.1. Chemistry

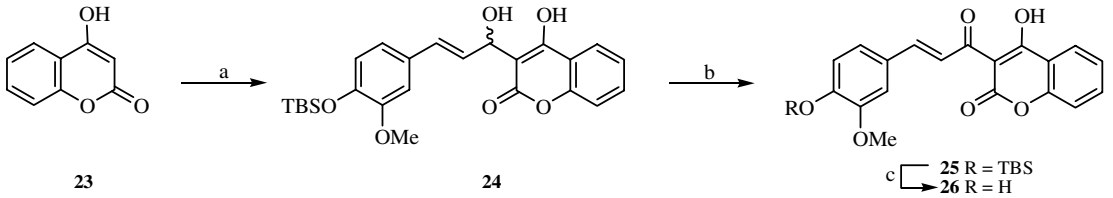
A series of benzylideneacetophenones **18**–**22** was prepared from commercially available 4-hydroxy-3-methoxy cinnamaldehyde (**1**) as a starting material, which was protected with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) in the presence of 2,6-lutidine to give aldehyde **2** in 95% yield. Grignard reaction of **2** with freshly prepared several phenyl magnesium halides **3**–**7** [phenylbromides were treated with activated magnesium (Mg) in 1,2-dichloroethane at 150 °C for 2 h, under argon atmosphere according to a literature procedure][10] gave racemic secondary alcohols **8**–**12** in high yields.

Oxidation of **8**–**12** was accomplished by manganese dioxide (MnO_2) condition to give **13**–**17** in good yields. In this stage, oxidation of **8**–**12** was also investigated using Dess–Martin periodinane (DMP),[13] tetrapropylammonium perruthenate (TPAP)/*N*-methylmorpholine (NMO),[14] and Swern [$(COCl)_2$, DMSO, TEA] conditions.[15] Although these latter conditions were more convenient for scale-up due to shorter reaction time and ease of handing, manganese dioxide oxidation afforded a superior yield. Subsequent deprotecting of **13**–**17** was accomplished with 2 equivalents of tetrabutyl ammonium fluoride (TBAF) in THF at room temperature to give 1,3-diphenyl-2-propen-1-ones **18**–**22** in good yields (Scheme 1).

On the other hand, 4-hydroxycoumarin (**23**) was treated with aldehyde **2** in ethanol to yield **24** which was subsequently oxidized using MnO_2 in *n*-pentane to give **25** in 75% yields in two steps. Removal of TBS group of **25** was achieved with 20% TFA in dichloromethane at



Scheme 1. Reagents and conditions: (a) TBSOTf (1.5 equiv), 2,6-lutidine (2.0 equiv), CH_2Cl_2, 0 °C, 30 min, 95%; (b) **3**–**7** (2.0 equiv), THF, −78 °C, 20 min, then −78 °C–rt, 30 min, (82–90%); (c) MnO_2 (10.0 equiv), *n*-pentane, reflux, 2 h, (85–92%); or Dess–Martin periodinane, CH_2Cl_2, rt, 30 min, (87% for **13**); or TPAP (5 mol %), NMO (1.5 equiv), molecular sieves (powder), CH_2Cl_2, 0 °C, 15 min, (88% for **13**); or DMSO (2.4 equiv), $(COCl)_2$ (1.2 equiv), TEA (4.8 equiv), CH_2Cl_2, −78 °C, 1 h, (85% for **13**); (d) TBAF (2.0 equiv), THF, rt, 10 min, (86–95%).



Scheme 2. Reagents and conditions: (a) **2**, EtOH, reflux, 30 min, (81%); (b) MnO_2 (10.0 equiv), *n*-pentane, reflux, 2 h, (84%); (c) 20% TFA, CH_2Cl_2, 0 °C, 1 h, (85%).

Table 1. Rate of scavenging DPPH radical of **18**–**22** and **25**–**26**

Compound	IC_{50} (DPPH) (μM)
18	52.8
19	46.0
20	43.8
21	53.2
22	52.5
25	45.5
26	52.5
1[a]	64.6

[a] Compound **1** was 4-hydroxy-3-methoxycinnamaldehyde as a compared material.

0 °C to generate 4-hydroxycoumarin moiety **26** in 85% yield (Scheme 2).

2.2. Biological activities

2.2.1. Radical scavenging activity. DPPH radicals are widely used for the preliminary screening of compounds capable of scavenging activated oxygen species since they are much more stable and easier to handle than oxygen free radicals. The test results of the scavenging ratio of DPPH radicals of each compound are shown in Table 1. By employing the DPPH radical test, favorable scavenging ratios were found in all of compounds **18**–**22** and **25**–**26** ranging from 43.8 to 53.2 μM in IC_{50}. We have found that the compounds **18**–**22** and **25**–**26** exhibited the similar DPPH scavenging activity.

2.2.2. Suppression of NO-generation and anti-neurotoxicity. NO production from activated microglial cells was determined by measuring the amount of nitrite after incubation with or without LPS (1 μg/mL) in the presence or absence of various concentrations of compounds for 24 h. Compounds **18**–**22** and **25**–**26** (1, 5, 10, 20 μM) showed considerable suppression of LPS-induced NO generation (Fig. 2). Exposure of cortical cell cultures to 300 μM NMDA (prototype of glutamate receptor agonist) resulted in a rapid swelling of the neuronal cell body within 2 h, and caused 90–100% neuronal death over the next day. The 60 μM of glutamate induced 50% of neurotoxicity after 24 h exposure in cultured neurons. These excitotoxic neuronal deaths were prevented by inclusion of 5 and 10 μM of compounds **19**, **21**, and **26** (Fig. 3).

3. Conclusion

In conclusion, an efficient preparation and biological evaluation of 1,3-diphenyl-2-propen-1-ones **18**–**22** and **25**–**26** have been described. Compounds **18**–**22** and **25**–**26** show the similar free radical scavenging activity. However, compounds **19**, **21**, and **26** showed better suppression effect on NO generation after LPS stimulation in microglial cells. In addition, compounds **21** and **26** showed inhibitory action on glutamate-induced neurotoxicity in cultured neurons.

4. Experimental

4.1. General

Reactions requiring anhydrous conditions were performed with the usual precautions for rigorous exclusion of air and moisture. Thin layer chromatography (TLC) was performed on precoated silica gel G and GP uniplates from Analtech and visualized with a 254-nm UV light. Flash chromatography was carried out on silica gel 60 [Scientific Adsorbents Incorporated (SAI), particle size 32–63 μm, pore size 60 Å]. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX 500 at 500 MHz and 125 MHz, respectively. The chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane, and *J*-values are in Hz. Infrared (IR) spectra were obtained on an ATI Mattson FT/IR spectrometer. Mass spectra were recorded with a Waters Micromass ZQ LC-Mass system and high resolution mass spectra (HRMS) were measured with a Bruker BioApex FTMS system by direct injection using an electrospray interface (ESI). When necessary, chemicals were purified according to the reported procedures.[16]

4.2. General procedure for the preparation of 8–12

To a stirred solution of **2** (2.9 g, 10.0 mmol) in dry THF (30 mL) were added dropwise freshly prepared Grignard reagents **3**–**7** (20.0 mmol) at −78 °C. The reaction mixture was stirred at same temperature for a further 20 min and then stirred to room temperature for 30 min. The reaction mixture was quenched by addition of sat'd aqueous NH_4Cl solution (20 mL) at 5 °C and diluted with ethyl acetate (30 mL). The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (20 mL). The combined organic phases were washed with brine (30 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure followed by purification of the crude products by flash column chromatography (silica gel, 10–15% ethyl acetate/hexanes) affording the desired compounds **8**–**12**.

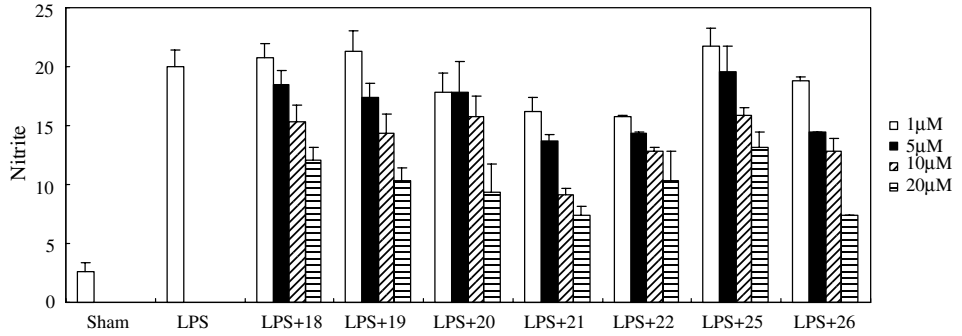


Figure 2. Suppression of NO production in LPS-treated microglia. The cells were treated with 1 μg/mL of LPS only or LPS plus different concentrations (1, 5, 10, 20 μM) of compounds **18**–**22** and **25**–**26** at 37 °C for 24 h. At the end of incubation, 50 μL of the medium was removed to measure nitrite production. All values represent mean ± SE of three-independent experiments performed in triplicate.

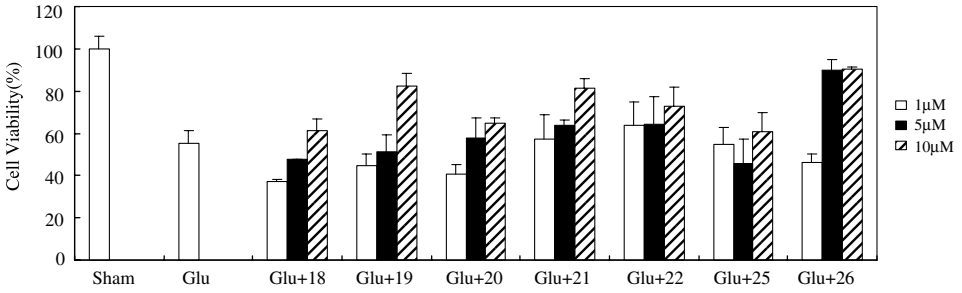


Figure 3. Inhibition of glutamate-induced neurotoxicity in cultured cortical neurons. Glutamate (60 μM), compounds **18**–**22** and **25**–**26** were applied for 24 h at 37 °C. After incubation of neurons with WST-1 for 2 h, its quantified spectrophotometrically. All values represent mean ± SE of three-independent experiments performed in triplicate.

4.2.1. (2*E*)-3-[(4-*tert*-Butyldimethylsilyloxy)-3-methoxyphenyl]-1-[4–(trimethylsilyl)phenyl]prop-2-en-1-ol (8). $R_f = 0.4$ (10% ethyl acetate/hexanes); IR (neat, NaCl) 3356, 3016, 2955, 2858, 1652, 1511, 1464, 1281, 1249, 1086 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.62–7.43 (m, 4H), 6.98 (d, $J = 5.5$ Hz, 1H), 6.92 (d, $J = 8.5$ Hz, 1H), 6.87 (d, $J = 8.0$ Hz, 1H), 6.68 (d, $J = 16.0$ Hz, 1H), 6.31 (dd, $J = 6.5$, 6.5 Hz, 1H), 5.41 (d, $J = 6.5$ Hz, 1H), 3.86 (s, 3H), 2.47 (br s, 1H), 1.09 (s, 9H), 0.36 (s, 9H), 0.25 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 150.9, 145.0, 143.5, 139.8, 133.6, 133.2, 130.6, 129.6, 128.7, 127.7, 125.6, 120.9, 120.0, 115.0, 75.5, 55.7, 26.2, 26.1, 18.9, −0.3, −0.6, −4.1; HRMS Calcd for $C_{25}H_{39}O_3Si_2$: 443.2438 $[M+H]^+$, found: 443.2421.

4.2.2. (2*E*)-3-[(4-*tert*-Butyldimethylsilyloxy)-3-methoxyphenyl]-1-[(3,4-methylenedioxy)phenyl]prop-2-en-1-ol (9). $R_f = 0.5$ (15% ethyl acetate/hexanes); IR (neat, NaCl) 3526, 3074, 2956, 2858, 1664, 1505, 1436, 1240, 1039 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.02–7.71 (m, 6H), 6.58 (d, $J = 7.0$ Hz, 1H), 6.45 (d, $J = 7.0$ Hz, 1H), 5.95 (s, 2H), 4.96 (d, $J = 8.5$ Hz, 1H), 3.81 (s, 3H), 2.37 (br s, 1H), 1.02 (s, 9H), 0.18 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 150.8, 147.3, 146.2, 130.3, 125.5, 124.9, 120.8, 119.7, 118.8, 107.6, 106.9, 101.0, 76.1, 55.7, 26.1 18.9, −4.1; HRMS Calcd for $C_{23}H_{31}O_5Si$: 415.1941 $[M+H]^+$, found: 415.1957.

4.2.3. (2*E*)-3-[(4-*tert*-Butyldimethylsilyloxy)-3-methoxyphenyl]-1-[4-(trifluoromethyl)phenyl]prop-2-en-1-ol (10). $R_f = 0.5$ (15% ethyl acetate/hexanes); IR (neat, NaCl) 3356, 3001, 2956, 2887, 1651, 1619, 1577, 1513, 1466, 1281, 1255, 1126, 1067 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65 (d, $J = 8.0$ Hz, 2H), 7.58 (d, $J = 8.0$ Hz, 2H), 6.92 (s, 1H), 6.86 (d, $J = 8.5$ Hz, 1H), 6.83 (d, $J = 8.5$ Hz, 1H), 6.63 (d, $J = 16.0$ Hz, 1H), 6.23 (dd, $J = 6.0$, 6.0 Hz, 1H), 5.43 (d, $J = 6.5$ Hz, 1H), 3.83 (s, 3H), 2.44 (br s, 1H), 1.03 (s, 9H), 0.19 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 150.9, 146.6, 145.3, 131.6, 129.9, 128.7, 127.0, 126.4, 125.4, 120.9, 120.0, 115.4, 109.9, 74.9, 55.7, 26.1, 18.9, −4.2; HRMS Calcd for $C_{23}H_{30}O_3F_3Si$: 439.1916 $[M+H]^+$, found: 439.1922.

4.2.4. (2*E*)-3-[(4-*tert*-Butyldimethylsilyloxy)-3-methoxyphenyl]-1-(3,4,5-trifluorophenyl)prop-2-en-1-ol (11). $R_f = 0.5$ (15% ethyl acetate/hexanes); mp 97–98 °C. IR (neat, NaCl) 3335, 3039, 2987, 2885, 1660, 1609, 1545, 1471, 1277, 1245, 1142, 1082 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.13–7.06 (m, 2H), 6.90 (s, 1H), 6.86 (d, $J = 8.0$ Hz, 1H), 6.82 (d, $J = 8.0$ Hz, 1H), 6.59 (d, $J = 16.0$ Hz, 1H), 6.10 (dd, $J = 6.0$, 6.0 Hz, 1H), 5.28 (d, $J = 6.5$ Hz, 1H), 3.83 (s, 3H), 2.34 (br s, 1H), 1.02 (s, 9H), 0.18 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 150.9, 145.4, 132.1, 129.6, 128.0, 121.0, 120.0, 110.2, 110.1, 109.9, 74.1, 55.7, 26.1, 18.9, −4.2; HRMS Calcd for $C_{22}H_{28}O_3F_3Si$: 425.1760 $[M+H]^+$, found: 425.1772.

4.2.5. (2*E*)-3-[(4-*tert*-Butyldimethylsilyloxy)-3-methoxyphenyl]-1-(3-fluoro-4-methoxyphenyl)prop-2-en-1-ol (12). $R_f = 0.5$ (10% ethyl acetate/hexanes); mp 94–95 °C. IR (neat, NaCl) 3351, 3006, 2985, 2878, 1649, 1545, 1455, 1279, 1163, 1045 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.20 (d,

J = 12.0 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 7.01–6.89 (m, 2H), 6.83 (dd, J = 8.0, 8.0 Hz, 2H), 6.57 (d, J = 16.0 Hz, 1H), 6.20 (dd, J = 6.5, 6.5 Hz, 1H), 5.30 (d, J = 6.5 Hz, 1H), 3.90 (s, 3H), 3.82 (s, 3H), 2.25 (br s, 1H), 1.07 (s, 9H), 0.19 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 150.8, 146.9, 145.0, 136.0, 130.8, 130.2, 129.1, 127.6, 124.2, 121.9, 120.9, 119.9, 114.2, 113.3, 74.5, 56.4, 55.7, 26.1, 18.9, −4.1; HRMS Calcd for $C_{23}H_{32}O_4FSi$: 419.2054 $[M+H]^+$, found: 419.2062.

4.3. General procedure for the preparation of 13–17

To a stirred solution of **8**–**12** (5.9 mmol) in *n*-pentane (120 mL) was added portionwise manganese dioxide (59.0 mmol) and the mixture was refluxed for 2 h. The reaction mixture was cooled to room temperature and filtered through Celite and then washed successively with *n*-pentane (2times 10 mL). The filtrate was evaporated under reduced pressure to yield crude enones, which were purified by flash column chromatography (silica gel, 5% ethyl acetate/hexanes) to give the desired compounds **13**–**17**.

4.3.1. (2*E*)-3-[(4-*tert*-Butyldimethylsilyloxy)-3-methoxyphenyl]-1-(4-trimethylsilylphenyl)-2-propen-1-one (13). R_f = 0.5 (5% ethyl acetate/hexanes); IR (neat, NaCl) 3011, 2968, 2875, 1722, 1586, 1462, 1267, 1144, 1081 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.99 (t, J = 7.5 Hz, 1H), 7.78 (dd, J = 4.0, 4.0 Hz, 1H), 7.68 (t, J = 8.0 Hz, 2H), 7.46–7.38 (m, 3H), 7.17 (d, J = 8.5 Hz, 1H), 6.91 (dd, J = 4.0, 4.0 Hz, 1H), 3.91 (s, 3H), 1.04 (s, 9H), 0.34 (s, 9H), 0.28 (s, 3H), 0.22(s, 3H); ^{13}C NMR ($CDCl_3$) δ 190.5, 151.1, 147.7, 146.3, 145.0, 138.5, 133.4, 133.1, 128.7, 127.3, 125.0, 122.6, 121.2, 120.3, 111.5, 55.7, 26.0, 18.9, −0.64, −0.80, −4.1; HRMS Calcd for $C_{25}H_{37}O_3Si_2$: 441.2281 $[M+H]^+$, found: 441.2268.

4.3.2. (2*E*)-3-[(4-*tert*-Butyldimethylsilyloxy)-3-methoxyphenyl]-1-[(3,4-methylenedioxy)phenyl]-2-propen-1-one (14). R_f = 0.5 (5% ethyl acetate/hexanes); IR (neat, NaCl) 3008, 2981, 2883, 1715, 1544, 1465, 1268, 1185, 1042 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.09–6.75 (m, 6H), 6.58 (dd, J = 7.0, 7.0 Hz, 1H), 6.35 (dd, J = 6.5, 6.5 Hz, 1H), 6.02 (s, 2H), 3.86 (s, 3H), 1.03 (s, 9H), 0.24(s, 6H); ^{13}C NMR ($CDCl_3$) δ 189.3, 151.5, 146.8, 146.0, 145.2, 138.4, 133.3, 128.6, 127.7, 125.1, 121.9, 121.2, 120.3, 112.1, 101.8, 55.8, 26.0, 18.8, −4.1; HRMS Calcd for $C_{23}H_{29}O_5Si$: 413.1784 $[M+H]^+$, found: 413.1774.

4.3.3. (2*E*)-3-[(4-*tert*-Butyldimethylsilyloxy)-3-methoxyphenyl]-1-(4-trifluoromethylphenyl)-2-propen-1- one (15). R_f = 0.5 (5% ethyl acetate/hexanes); IR (neat, NaCl) 3006, 2979, 2856, 1720, 1551, 1476, 1285, 1164, 1062 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.11 (d, J = 7.5 Hz, 2H), 7.78 (d, J = 8.0. Hz, 3H), 7.35 (d, J = 15.5 Hz, 2H), 7.19 (d, J = 7.5 Hz, 1H), 7.16 (s, 1H), 3.91 (s, 3H), 1.04 (s, 9H), 0.22 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 187.5, 151.9, 147.8, 145.5, 131.0, 128.2, 126.1, 122.3, 120.4, 119.0, 115.8, 112.9, 111.3, 55.8, 26.0, 18.9, −4.2; HRMS Calcd for $C_{23}H_{28}O_3F_3Si$: 437.1760 $[M+H]^+$, found: 437.1785.

4.3.4. (2*E*)-3-[(4-*tert*-Butyldimethylsilyloxy)-3-methoxyphenyl]-1-[3,4,5-trifluorophenyl]-2-propen-1-one (16). R_f = 0.5 (5% ethyl acetate/hexanes); mp 105 °C. IR (neat, NaCl) 3008, 2981, 2857, 1718, 1549, 1453, 1287, 1163, 1061 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.81 (d, J = 15.5 Hz, 1H), 7.68 (t, J = 7.5 Hz, 2H), 7.24 (d, J = 15.5 Hz, 1H), 7.19 (d, J = 8.0 Hz, 1H), 7.14 (s, 1H), 6.91 (d, J = 8.5 Hz, 1H), 3.91 (s, 3H), 1.03 (s, 9H), 0.22 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 186.2, 151.2, 148.4, 146.7, 128.1, 123.0, 121.2, 118.0, 112.9, 112.7, 111.5, 55.8, 26.0, 18.9, −4.1; HRMS Calcd for $C_{22}H_{25}O_3F_3SiNa$: 445.1423 $[M+Na]^+$, found: 445.1438.

4.3.5. (2*E*)-3-[(4-*tert*-Butyldimethylsilyloxy)-3-methoxyphenyl]-1-(3-fluoro-4-methoxyphenyl)-2-propen-1-one (17). R_f = 0.5 (5% ethyl acetate/hexanes); mp 145–146 °C. IR (neat, NaCl) 3010, 2959, 2868, 1722, 1538, 1465, 1272, 1154, 1050 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.86–7.74 (m, 3H), 7.35 (d, J = 15.5 Hz, 1H), 7.17 (d, J = 8.5 Hz, 1H), 7.14 (s, 1H), 7.05 (t, J = 8.5 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 3.98 (s, 3H), 3.90 (s, 3H), 1.03 (s, 9H), 0.21 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 187.4, 151.3, 147.8, 144.8, 131.5, 128.6, 125.5, 122.6, 121.4, 119.1, 116.2, 112.4, 111.5, 56.5, 55.8, 26.0, 18.9, −4.1; HRMS Calcd for $C_{23}H_{30}O_4FSi$: 417.1897 $[M+H]^+$, found: 417.1881.

4.4. General procedure for the preparation of 1,3-diphenyl-2-propen-1-ones (18–22)

To a stirred solution of **13**–**17** (5.0 mmol) in dry THF (80 mL) was added dropwise TBAF (10.0 mmol, 1 M solution in THF) at room temperature under argon atmosphere and the mixture was stirred at room temperature for 10 min. The reaction mixture was diluted with EtOAc (30 mL) and washed with brine (70 mL). The organic phase was separated, and the aqueous phase was extracted with EtOAc (2× 30 mL). The combined organic phases were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to yield alcohols, which were purified by flash column chromatography (silica gel, 20–30% ethyl acetate/hexanes) to give **18**–**22**.

4.4.1. (2*E*)-3-[4-Hydroxy-3-(methoxyphenyl)]-1-(4-trimethylsilylphenyl)-2-propen-1-one (18). R_f = 0.3 (30% ethyl acetate/hexanes); IR (neat, NaCl) 3374, 3070, 3018, 2956, 2857, 1654, 1579, 1512, 1430, 1272, 1185, 1032 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.00 (d, J = 8.5 Hz, 2H), 7.81 (d, J = 15.5 Hz, 1H), 7.69 (t, J = 7.5 Hz, 2H), 7.38 (d, J = 8.5 Hz, 1H), 7.25 (d, J = 8.5 Hz, 1H), 7.17 (s, 1H), 6.98 (dd, J = 4.5, 4.5 Hz, 1H), 3.98 (s, 3H), 0.36 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 190.7, 148.4, 146.8, 146.4, 145.2, 138.5, 133.4, 127.3, 123.4, 119.8, 115.0, 110.1, 56.2, −0.79; HRMS Calcd for $C_{19}H_{22}O_3SiNa$: 349.1236 $[M+Na]^+$, found: 349.1248.

4.4.2. (2*E*)-3-[4-Hydroxy-3-(methoxyphenyl)]-1-[(3,4-methylenedioxy)phenyl]-2-propen-1-one (19). R_f = 0.3 (30% ethyl acetate/hexanes); IR (neat, NaCl) 3349, 3007, 2955, 2876, 1645, 1554, 1477, 1243, 1186, 1045 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.11–6.72 (m, 7H), 6.59 (dd, J = 7.5, 7.0 Hz, 1H), 6.38 (dd, J = 7.0, 7.0 Hz, 1H), 6.05 (s, 2H), 3.88

(s, 3H); ^{13}C NMR ($CDCl_3$) δ 188.9, 151.1, 147.0, 146.3, 145.7, 138.3, 133.5, 127.9, 126.5, 125.4, 122.1, 121.4, 120.5, 112.1, 102.5, 55.9; HRMS Calcd for $C_{17}H_{15}O_5$: 299.0919 $[M+H]^+$, found: 299.0928.

4.4.3. (2*E*)-3-[4-Hydroxy-3-(methoxyphenyl)]-1-(4-trifluoromethylphenyl)-2-propen-1-one (20). $R_f = 0.3$ (30% ethyl acetate/hexanes); IR (neat, NaCl) 3384, 3011, 2964, 2876, 1658, 1585, 1512, 1452, 1323, 1272, 1125, 1033 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.09 (d, $J = 8.0$ Hz, 2H), 7.76 (dd, $J = 6.5$, 6.5 Hz, 3H), 7.34 (d, $J = 16.0$, Hz, 1H), 7.23 (dd, $J = 7.5$, 7.5 Hz, 1H), 7.15 (d, $J = 7.5$ Hz, 1H), 6.97 (d, $J = 8.0$ Hz, 1H), 5.66 (br s, 1H), 3.96 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 189.6, 149.4, 146.1, 145.9, 145.0, 138.5, 133.2, 128.3, 126.8, 124.1, 120.0, 115.6, 111.4, 56.1; HRMS Calcd for $C_{17}H_{13}O_3F_3Na$: 345.0714 $[M+Na]^+$, found: 345.0725.

4.4.4. (2*E*)-3-[4-Hydroxy-3-(methoxyphenyl)]-1-[3,4,5-trifluorophenyl]-2-propen-1-one (21). $R_f = 0.3$ (30% ethyl acetate/hexanes); IR (neat, NaCl) 3350, 3005, 2985, 2858, 1657, 1550, 1448, 1262, 1153, 1081 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.83 (d, $J = 16.0$ Hz, 1H), 7.66 (t, $J = 8.0$ Hz, 2H), 7.22 (d, $J = 16.0$ Hz, 1H), 7.20 (d, $J = 8.5$ Hz, 1H), 7.15 (s, 1H), 6.93 (d, $J = 8.0$ Hz, 1H), 5.58 (br s, 1H), 3.90 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 187.3, 151.0, 148.2, 146.5, 132.2, 128.3, 122.9, 121.7, 119.3, 113.1, 112.2, 111.9, 55.8. HRMS Calcd for $C_{16}H_{12}O_3F_3$: 309.0739 $[M+H]^+$, found: 309.0745.

4.4.5. (2*E*)-3-[4-Hydroxy-3-(methoxyphenyl)]-1-(3-fluoro-4-methoxyphenyl)-2-propen-1-one (22). $R_f = 0.3$ (40% ethyl acetate/hexanes); IR (neat, NaCl) 3359, 2987, 2875, 1662, 1578, 1455, 1278, 1164, 1086 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.85–7.72 (m, 3H), 7.33 (d, $J = 16.0$ Hz, 1H), 7.15 (d, $J = 8.5$ Hz, 1H), 7.13 (s, 1H), 7.04 (t, $J = 8.5$ Hz, 1H), 6.91 (d, $J = 8.0$ Hz, 1H), 5.45 (br s, 1H), 3.96 (s, 3H), 3.91 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 189.4, 150.3, 148.7, 145.2, 132.4, 129.6, 124.9, 123.1, 121.8, 119.5, 115.3, 113.2, 111.8, 56.8, 55.9; HRMS Calcd for $C_{17}H_{16}O_4F$: 303.1033 $[M+H]^+$, found: 303.1020.

4.5. (2*E*)-4-Hydroxy-3-[3-(4-*tert*-butyldimethylsilyloxy)-3-(methoxyphenyl)prop-2-enoyl]-2*H*-chromen-2-one (25)

To a stirred solution of 4-hydroxycoumarin (**23**, 0.62 g, 3.8 mmol) in ethanol (25 mL) was added portionwise **2** (1.1 g, 3.8 mmol) and the mixture was refluxed for 30 min. The reaction mixture was cooled to room temperature and evaporated under reduced pressure to yield **24**, which was treated without purification with manganese dioxide (3.9 g, 38.0 mmol) in *n*-pentane (50 mL) and the mixture was refluxed for 2 h. The reaction mixture was cooled to room temperature and filtered through Celite and then washed successively with *n*-pentane (2× 10 mL). The filtrate was evaporated under reduced pressure to yield crude enones, which were purified by flash column chromatography (silica gel, 10% ethyl acetate/hexanes) to give **25** as a brown solid (0.5 g, 75%). $R_f = 0.4$ (10% ethyl acetate/hexanes); mp 129–130 °C; IR (neat, NaCl) 3465, 3012, 2985, 2856, 1705, 1640, 1457, 1286, 1125, 1064 cm^{-1}cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.39 (dd, $J = 11.0$, 12.0 Hz, 1H), 8.09 (d, $J = 8.0$, 8.0 Hz, 1H), 7.64 (t, $J = 7.0$ Hz, 1H), 7.52 (dd, $J = 7.0$, 7.0 Hz, 1H), 7.25 (dd, $J = 6.0$, 7.0 Hz, 4H), 6.91 (d, $J = 8.0$ Hz, 2H), 3.91 (s, 3H), 1.01 (s, 9H), 0.22 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 186.7, 178.5, 160.2, 151.1, 149.8, 144.2, 138.9, 128.8, 127.2, 125.4, 123.9, 122.2, 120.9, 112.3, 101.2, 56.3, 26.1, 18.9, −4.1; HRMS Calcd for $C_{25}H_{29}O_6Si$: 453.1733 $[M+H]^+$, found: 453.1741.

4.6. (2*E*)-4-Hydroxy-3-[3-(4-hydroxy)-3-(methoxyphenyl)prop-2-enoyl]-2*H*-chromen-2-one (26)

To a solution of **25** (0.3 g, 2.5 mmol) in dry dichloromethane (10 mL) was added dropwise TFA (2.6 g, 20% solution in dichloromethane) at 0 °C under argon atmosphere and the mixture was stirred at this temperature for 1 h. The reaction mixture was diluted with dichloromethane (10 mL) and treated with sat'd aqueous NaCl solution (20 mL). The organic layer was separated, and the aqueous phase was extracted with dichloromethane (2× 20 mL). The combined organic phases were washed with saturated aqueous Na_2SO_4 solution (20 mL), brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give alcohols, which were purified by flash column chromatography (silica gel, 20–30% ethyl acetate/hexanes) to afford **26**. $R_f = 0.4$ (30% ethyl acetate/hexanes); IR (neat, NaCl) 3521, 3345, 2976, 2845, 1710, 1645, 1464, 1265, 1133, 1072 cm^{-1}cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 13.2 (br s, 1H), 8.36 (dd, $J = 11.0$, 11.0 Hz, 1H), 8.11 (d, $J = 8.5$, 8.5 Hz, 1H), 7.61 (t, $J = 7.5$ Hz, 1H), 7.50 (d, $J = 6.5$, 6.5 Hz, 1H), 7.23 (d, $J = 7.0$ Hz, 4H), 6.92 (d, $J = 8.0$ Hz, 2H), 3.90 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 183.6, 176.1, 159.8, 150.4, 149.5, 145.3, 138.2, 133.1, 128.3, 126.9, 124.5, 123.0, 121.1, 115.4, 108.4, 56.3; HRMS Calcd for $C_{19}H_{15}O_6$: 339.0869 $[M+H]^+$, found: 339.0876.

4.7. DPPH radical-scavenging effects

To test the free radical-scavenging effects by using DPPH, compounds were adjusted with ethanol solution to final concentration of 0.1–100 μM. Acetic acid buffer (0.1 mM) was added, and the mixture was warmed in water bath at 25 °C. After 5 min, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical ethanol solution (1 mL, 0.2 mM) was added. After 30 min, absorbance was measured with a spectrophotometer (517 nm). The DPPH radical-scavenging rate of each sample was calculated and the 50% scavenging concentration based on the DPPH radical-scavenging rate was also calculated based on the following formula.

$$\text{DPPH radical-scavenging rate (\%)} = \left\{1 - \frac{A - C}{B}\right\} \times 100$$

where A is the absorbance of the sample when a blank was substituted for ethanol, B is the absorbance of the sample when a color-contrast agent was substituted for

ethanol in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-ethanol solution, and C is the absorbance of the color-contrast agent alone which is an absorbance change in order to detect the DPPH radical-scavenging rate.

4.8. Cell culture

Cerebral cortices were removed from the brains of 15-day-old fetal mice. The neocortices were triturated and plated on 24-well plates (with approximately 10^6 cells/mL) precoated with 100 μg/mL poly-D-lysine and 4 μg/mL laminine, in Eagle's minimal essential media (Earle's salts, supplied glutamine-free), and supplemented with horse serum (5%), fetal bovine serum (5%), 2 mM glutamine, and 20 mM glucose. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2. After 6 days in vitro (DIV), the cultures were shifted to the plating media containing 10 μM cytosine arabinoside without fetal serum. Cultures were then fed twice per week. Mixed cortical cell cultures containing neurons and glia (DIV 16–14) were exposed to excitatory amino acid, glutamate, in Eagle's minimal essential media without serum. The morphology of the degenerating neurons was observed under a phase contrast microscope over the next 24 h. Primary microglia were prepared from postnatal 1 day Sprague–Dawley rat as described previously[17] with some modifications. In brief, the rat cortices were triturated into single cells in minimal essential medium (MEM) containing 10% FBS and then plated into 75 cm^2 T-flask for 10–14 days. To prepare microglia, cells were removed from the T-flasks by mild shaking. Detached microglia was plated on 24-well plates (with approximately 5×10^5 cells/mL). Morphology was examined under a phase-contrast microscopy.

4.9. Nitrite assay

NO production from activated microglial cells was determined by measuring the amount of nitrite, a relatively stable oxidation product of NO, as described previously.[17] Cells were incubated with or without LPS (1 μg/mL) in the presence or absence of various concentrations of compounds for 24 h. The nitrite accumulation in the supernatant was assessed by Griess reaction. In brief, an aliquot of the conditioned medium was mixed with an equal volume of 1% sulfanilamide in water and 0.1% *N*-1-naphthylethylenediamine dihydrochloride in 5% phosphoric acid. The absorbance was determined at 540 nm in an automated microplate reader.

4.10. Cell viability

Cortical neuronal cell number and viability were assessed by using the reagent WST-1 (Roche, Indianapolis, IN). This colorimetric assay measures the metabolic activity of viable cells based on cleavage of the tetrazolium salt WST-1 substrate 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio-1,3-benzene disulfonate into formazan by mitochondrial dehydrogenase in live cells. This was followed by incubation with WST-1 reagent at a dilution of 1:10 in the original conditioned media at 37 °C for 2 h. After thorough shaking, the formazan produced by the metabolically active cells in each sample was measured at a wavelength of 450 nm and a reference wavelength of 650 nm. Absorbance readings were normalized against control wells with untreated cells. Neuronal death was analyzed 24 h later, and the percentage of neurons undergoing actual neuronal death was normalized to the mean value that is found after a 24-h exposure to 300 μM NMDA (defined as 0) or a sham control (defined as 100) (Fig. 3).

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