SUPPLEMENTARY MATERIALS

Synthesis and Evaluation of Multi-functional Ferulic and Caffeic Acid Dimers for Alzheimer's Disease

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Abstract: In this study, a series of novel ferulic and caffeic acid dimers was designed and synthesized, and their multi-functional properties against Alzheimer's disease (AD) were evaluated. Results showed that our multi-functional strategy was great supported by enhancing the inhibition of A β ₁₋₄₂ self-induced aggregation. Moreover, **7b** also had potent protective effects against glutamate-induced cell death without significant cell toxicity in mouse hippocampal neuronal HT22 cells and **10c** effectively scavenged diphenylpicrylhydrazyl (DPPH) free radicals. Collectively, these data strongly encourage further optimization of **7b** as a new hit to develop multifunctional agents for the treatment of AD.

Keywords: Ferulic acid; Caffeic acid; Multi-functional neuroprotective agents; Amyloid protein; Aggregation; Free radicals.

1. Experimental

1.1. Chemistry

The ^1H NMR and ^{13}C NMR spectra were recorded with TMS as the internal standard on a Bruker Avance 400 spectrometer at 400 MHz and 100 MHz, respectively. Coupling constants were given in Hz. MS spectra were recorded on a ThermofisherLTQ Orbitrap Elite instrument with an ESI mass selective detector. All the solvents and reagents were purchased from vendors and were used after redistillation. Synthesis reactions were performed under non-aqueous condition, monitored by TLC on aluminium plate coated with silica gel. Column chromatography was performed by 200-300 mesh silica gel purchased from Qingdao Haiyang Chemical Co. Ltd.

1.1.1. General procedure for synthesis of compounds (3a-3d, 5a-5b, 7a-7b)

Hydroxycinnamic acids (2eq), diamine (1eq) and 0.13mL Et_3N were added into dried DMF 5mL. The mixture was stirred in ice bath under N_2 protection till the clean solution was formed. Then, the solution of BOP (2eq) in 2.5mL CH_2Cl_2 was added dropwise into the stirred mixture, and stirred reaction mixture in ice bath for 40min. After that, ice bath was removed and it was stirred at room temperature overnight. TLC played an important role to determine whether the reaction was complete or should be stopped. 1M HCl (80 mL) was added to the reaction mixture, and extracted with ethyl acetate (40 mL x 3). The combined organic layer was washed with 40mL water, 40mL saturated NaHCO_3 and 40mL water, successively, and dried over anhydrous Na_2SO_4 . After removal of solvent under reduced pressure yielded crude compound. Purification was performed by column chromatography (eluent: $\text{CH}_2\text{Cl}_2/\text{MeOH} = 9/1$, v/v) to afford desired compound as yellow solid.

Scheme 1. Reagents and conditions: (a) BOP, CH₂Cl₂, DMF, Et₃N, 0°C~r.t., overnight

1.1.1.1.(E)-3-(3,4-dihydroxyphenyl)-N-{4-[3-(3,4-dihydroxyphenyl)acryloylamino]phenyl}-acrylamide (3a)

CA (133.2mg, 0.74mmol) was reacted with *p*-phenylenediamine (50 mg, 0.37 mmol) in dried DMF to give the desired product as a yellow solid with a yield of 21.4%. ¹H-NMR (400 MHz, DMSO-*d*₆) δ10.05 (s, 2H, Ar-NH-CO), 8.32 (s, 1H, Ar-OH), 7.63 (s, 4H, Ar-H), 7.38 (d, *J*=15.5 Hz, 2H, Ar-CH=CH-), 7.01(d, *J*=1.0 Hz, 2H), 6.90 (dd, *J*=8.1,1.0 Hz, 2H), 6.78 (d, *J*=8.1 Hz, 2H), 6.54 (d, *J*=15.4 Hz, 2H, -CH=CH-CO-); ¹³C NMR (100 MHz, DMSO-*d*₆) δ163.89(-CONH-), 148.68(Ar-OH), 147.98(Ar-OH), 140.51(Ar-CH=), 134.99, 126.37, 122.03, 119.66, 119.00, 115.82, 110.97; HR-ESI-MS: *m/z* 431.12431 [M-H]⁻.

1.1.1.2.(E)-3-(4-hydroxy-3-methoxyphenyl)-N-{4-[3-(4-hydroxy-3-methoxyphenyl)acryloylamino]phenyl}-acrylamide (3b)

FA (143.6 mg, 0.74mmol) was reacted with *p*-phenylenediamine (50 mg, 0.37mmol) in dried DMF to give the desired product as a yellow solid with a yield of 30.2%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.06 (s, 2H, Ar-NH-CO), 9.51 (s, 2H, Ar-OH), 7.65 (s, 4H, Ar-H), 7.48 (d, *J*=15.5 Hz, 2H, Ar-CH=CH-), 7.18 (d, *J*=1.0 Hz, 2H), 7.06 (dd, *J*=8.1, 1.0 Hz, 2H), 6.83 (d, *J*=8.1 Hz, 2H), 6.64 (d, *J*=15.6 Hz, 2H, -CH=CH-CO-), 3.83 (s, 6H, -OCH₃); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 163.89(-CONH-), 148.68(Ar-OH), 147.98(Ar-OCH₃), 140.51(Ar-CH=), 134.99, 126.37, 122.03, 119.66, 119.00, 115.82, 110.97, 55.66(Ar-OCH₃); HR-ESI-MS: *m/z* 459.15640 [M-H]⁻.

1.1.1.3.(E)-3-(3,4-dihydroxyphenyl)-N-(4-{[3-(3,4-dihydroxyphenyl)acryloylamino]methyl}benzyl)-acrylamide (3c)

CA (133.2mg, 0.74mmol) was reacted with *p*-xylylenediamine (50 mg, 0.37 mmol) in dried DMF to give the desired product as a yellow solid with a yield of 25.6%. IR (KBr) ν 3306, 1649, 1591, 1529, 1448, 1371, 1262, 1204, 1176, 1113, 974, 848, 804, 688, 592, 569 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.45 (s, 2H, Ar-NH-CO), 7.27 (d, *J*=15.8 Hz, 2H, Ar-CH=CH-), 7.24 (s, 4H, Ar-H), 6.94 (d, *J*=0.9 Hz, 2H), 6.84 (dd, *J*=8.0, 0.9 Hz, 2H), 6.74 (d, *J*=8.0 Hz, 2H), 6.38 (d, *J*=15.6 Hz, 2H, -CH=CH-CO-), 4.35 (d, *J*=4.6 Hz, 4H, -NH-CH₂-Ar); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 165.50(-CONH-), 147.40(Ar-OH), 145.58(Ar-OH), 139.54(Ar-CH=), 138.17, 127.45, 126.41, 120.51, 118.35, 115.82, 113.92, 42.09(Ar-CH₂-NH-); HR-ESI-MS: *m/z* 459.15640 [M-H]⁻.

1.1.1.4.(E)-3-(4-hydroxy-3-methoxyphenyl)-N-(4-{[3-(4-hydroxy-3-methoxyphenyl)acryloylamino]methyl}benzyl)-acrylamide (3d)

FA (143.6mg, 0.74mmol) was reacted with *p*-xylylenediamine (50 mg, 0.37mmol) in dried DMF to give the desired product as a yellow solid with a yield of 28.6%. ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.51 (d, *J*=15.7 Hz, 2H, Ar-CH=CH-), 7.30 (s, 4H, Ar-H), 7.18 (d, *J*=1.0 Hz, 2H), 7.06 (d, *J*=8.2 Hz, 2H), 6.84 (dd, *J*=8.2, 1.0 Hz, 2H), 6.58 (d, *J*=15.6 Hz, 2H, -CH=CH-CO-), 4.49 (s, 4H, -NH-CH₂-Ar), 3.88 (s, 6H, -OCH₃); ¹³C NMR (100 MHz, DMSO-*d*₆)

δ 165.39(-CONH-), 148.33(Ar-OH), 147.83(Ar-OCH₃), 139.31(Ar-CH=), 138.12, 127.35, 126.40, 121.54, 118.83, 115.68, 110.88, 55.55(Ar-OCH₃), 42.06(Ar-CH₂-NH-); HR-ESI-MS: m/z 487.18691 [M-H]⁻.

1.1.1.5.

(E)-3-(3,4-dihydroxyphenyl)-N-{3-[3-(3,4-dihydroxyphenyl)acryloylamino]phenyl}-acrylamide (5a)

CA (166mg, 0.92mmol) was reacted with *m*-phenylenediamine (50 mg, 0.46mmol) in dried DMF to give the desired product as a yellow solid with a yield of 22.6%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.01 (s, 1H, Ar-OH), 7.48-7.40 (m, 3H), 7.37 (d, *J*=15.4 Hz, 2H, Ar-CH=CH-), 7.22 (t, *J*=8.1 Hz, 1H), 6.96 (d, *J*=1.9 Hz, 2H), 6.89-6.80 (dd, *J*=8.1, 1.9 Hz 2H), 6.66 (d, *J*=8.1 Hz, 2H), 6.51 (d, *J*=15.5 Hz, 2H, -CH=CH-CO-); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 164.69(-CONH-), 149.06(Ar-OH), 146.52(Ar-OH), 141.36(Ar-CH=), 140.28, 129.33, 126.30, 121.27, 118.69, 116.30, 114.73, 114.37; HR-ESI-MS: m/z 431.12431 [M-H]⁻.

1.1.1.6.

(E)-3-(3-hydroxy-4-methoxyphenyl)-N-{3-[3-(4-hydroxy-3-methoxyphenyl)acryloylamino]phenyl}-acrylamide (5b)

FA (178mg, 0.92mmol) was reacted with *m*-phenylenediamine (50mg, 0.46mmol) in dried DMF to give the desired product as a yellow solid with a yield of 28.7%. ¹H-NMR (400 MHz, CD₃OD) δ 8.06 (s, 1H, Ar-OH), 7.60 (d, *J*=15.6 Hz, 2H, Ar-CH=CH-), 7.43 (m, 2H), 7.34-7.26 (m, 2H), 7.19 (d, *J*=1.4 Hz, 2H), 7.08 (dd, *J* = 8.2, 1.4 Hz, 2H), 6.84 (dd, *J*=8.1, 2H), 6.65 (d, *J*=15.6 Hz, 2H, -CH=CH-CO-), 3.92 (s, 6H, -OCH₃); ¹³C-NMR (100 MHz, CD₃OD) δ 167.34(-CONH-), 150.12(Ar-OH), 149.33(Ar-OCH₃), 143.36(Ar-CH=), 140.60, 130.18, 128.27, 123.50, 119.11, 117.05, 116.58, 111.78, 56.44(Ar-OCH₃); HR-ESI-MS: m/z 459.15640 [M-H]⁻.

1.1.1.7.

(E)-3-(4-hydroxy-3-methoxyphenyl)-N-{4'-[3-(4-hydroxy-3-methoxyphenyl)acryloylamino]biphenyl-4-yl}-acrylamide (7a)

CA (97.2 mg, 0.54 mmol) was reacted with benzidine (50 mg, 0.27 mmol) in dried DMF to give the desired product as a yellow solid with a yield of 26.3%. ^1H NMR (400 MHz, DMSO- d_6) δ 10.22 (s, 2H, Ar-NH-CO), 7.77 (d, J =8.4 Hz, 4H), 7.61 (d, J =8.4 Hz, 4H), 7.50 (d, J =15.5 Hz, 2H, Ar-CH=CH-), 7.21 (s, 2H, Ar-OH), 7.15 (d, J =1.0 Hz, 2H), 7.08 (dd, J =8.1, 1.0 Hz, 2H), 6.83 (d, J =8.1 Hz, 2H), 6.69 (d, J =15.6 Hz, 2H, -CH=CH-CO-); ^{13}C NMR (100 MHz, DMSO- d_6) δ 164.14(-CONH-), 148.62(Ar-OH), 147.87(Ar-OH), 140.79(Ar-CH=), 127.21, 126.50, 126.21, 122.10, 120.38, 119.58, 118.76, 115.67, 110.86; HR-ESI-MS: m/z 507.15561 [M-H] $^-$.

1.1.1.8.

(E)-3-(3,4-dihydroxyphenyl)-N-{4'-[3-(3,4-dihydroxyphenyl)acryloylamino]biphenyl-4-yl}-acrylamide (7b)

FA (104.8 mg, 0.54mmol) was reacted with benzidine (50 mg, 0.27mmol) in dried DMF to give the desired product as a yellow solid with a yield of 22.3%. IR (KBr) ν 3276, 2923, 1649, 1592, 1500, 1448, 1248, 1123, 1031, 812 cm^{-1} . ^1H NMR (400 MHz, CD_3OD) δ 7.72 (d, J =8.6 Hz, 4H, Ar-H), 7.61 (d, J =15.6 Hz, 2H, Ar-CH=CH-), 7.55 (d, J =8.6 Hz, 4H, Ar-H), 7.43 (d, J =8.4 Hz, 2H), 7.23 (d, J =1.8 Hz, 2H), 7.14 (dd, J =8.2, 1.9 Hz, 2H), 6.87 (d, J =8.1 Hz, 2H), 6.80 (d, J =8.3 Hz, 2H), 6.67 (d, J =15.6 Hz, 2H, -CH=CH-CO-), 3.94 (s, 6H, Ar-OCH $_3$); ^{13}C NMR (100 MHz, DMSO- d_6) δ 164.14(-CONH-), 148.14(Ar-OH), 147.87(Ar-OCH $_3$), 140.79(Ar-CH=), 127.21, 126.50, 126.35, 122.01, 120.11, 119.58, 118.76, 115.29, 110.48, 55.87(Ar-OCH $_3$); HR-ESI-MS: m/z 535.18691 [M-H] $^-$.

1.1.2. General procedure for synthesis of compounds (10a-10d)

Hydroxycinnamic acid derivatives (1 eq), amine (1 eq) and 0.13mL Et $_3$ N were added into 5mL dried DMF. The mixture was stirred in ice bath under N $_2$ protection till a clean solution was formed. Then, the solution of BOP (1eq) in 2.5 mL CH $_2$ Cl $_2$ was added dropwise into the stirred mixture, and continued stirring in ice bath for 40min. After that, ice bath was removed and it was stirred at room temperature overnight. TLC was used to determine whether the reaction was complete or should be stopped. 1M HCl (80 mL) was added to the reaction mixture, and extracted with ethyl acetate 40 mL x 3. The combined organic layer was washed with 40 mL water, 40mL saturated NaHCO $_3$ and 40 mL water, successively, and dried over anhydrous

Na₂SO₄. After removal of the solvent under reduced pressure yielded crude compound. Purification was performed by column chromatography (eluent: CH₂Cl₂/MeOH =93/7, v/v) to afford desired compound as white solid.

Scheme 2. Reagents and conditions: (a) BOP, CH₂Cl₂, DMF, Et₃N, 0°C~r.t, overnight

1.1.2.1. (E)-3-(3,4-dihydroxyphenyl)-N-phenethyl-acrylamide (10a)

CA (50mg, 0.28mmol) was reacted with 2-phenylethylamine (33.7mg, 0.28mmol) in dried DMF to give the desired product as a white solid with a yield of 32.8%. ¹H NMR (400 MHz, Acetone-*d*₆) δ8.31 (s, 1H, Ar-OH), 7.45 (d, *J*=15.6 Hz, 1H, Ar-CH=CH-), 7.36 (s, 1H, -NH-CO), 7.32-7.23 (m, 4H, Ar-H), 7.22-7.16 (m, 1H, Ar-H), 7.09 (d, *J*=2.0 Hz, 1H), 6.94 (dd, *J*=8.2, 2.0 Hz, 1H), 6.85 (d, *J*=8.2 Hz, 1H), 6.45 (d, *J*=15.6 Hz, 1H, -CH=CH-CO), 3.60-3.50 (dt, *J* = 12.9, 6.9 Hz, 2H, -NHCH₂-), 2.91-2.81 (t, *J* = 6.9 Hz, 2H, Ar-CH₂-); ¹³C NMR (100 MHz, Acetone-*d*₆) δ166.86(-CONH-), 147.88(Ar-OH), 146.25(Ar-OH), 140.79(Ar-CH=), 140.51, 129.59, 129.21, 128.34, 126.97, 121.53, 119.51, 119.46, 116.31, 114.90, 41.71(-NHCH₂-), 36.54(Ar-CH₂-); HR-ESI-MS: *m/z* 282.11343 [M-H]⁻.

1.1.2.2. (E)-3-(4-hydroxy-3-methoxyphenyl)-N-phenethyl-acrylamide (10b)

FA (161mg, 0.83mmol) was reacted with 2-phenylethylamine (100mg, 0.83mmol) in dried DMF to give the desired product as a white solid with a yield of 35.2%. ¹H NMR (400 MHz, CDCl₃) δ7.54 (d, *J*=15.5 Hz, 1H, Ar-CH=CH-), 7.33-7.23 (m, 5H, Ar-H), 7.04 (dd, *J* =8.2, 1.8

Hz, 1H), 6.96 (d, $J=1.8$ Hz, 1H), 6.89 (d, $J=8.2$ Hz, 1H), 6.17 (d, $J=15.5$ Hz, 1H, -CH=CH-CO-), 5.91 (s, 1H, Ar-OH), 5.60 (s, 1H, Ar-OH), 3.90 (s, 3H, -OCH₃), 3.66 (dt, $J = 12.9, 6.9$ Hz, 2H, -NHCH₂-), 2.89 (t, $J = 6.9$ Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 166.17(-CONH-), 147.40(Ar-OH), 146.71(Ar-OCH₃), 141.08(Ar-CH=), 138.93, 128.82, 128.68, 127.34, 126.54, 122.12, 118.15, 114.72, 109.63, 55.92(Ar-OCH₃), 40.73(-NHCH₂-), 35.69(Ar-CH₂-); HR-ESI-MS: m/z 296.12867[M-H]⁻.

1.1.2.3. (E)-3-(3,4-dihydroxyphenyl)-N-[2-(3,4-dimethoxyphenyl)ethyl]-acrylamide (10c)

CA (59.5mg, 0.33mmol) was reacted with 2-(3,4-Dimethoxyphenyl)-ethylamine(60mg, 0.33mmol)in dried DMF to give the desired product as a white solid with a yield of 33.5%. ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.41 (d, $J=15.6$ Hz, 1H, Ar-CH=CH-), 7.06 (d, $J=2.0$ Hz, 1H), 6.93 (dd, $J=8.2, 2.0$ Hz, 1H), 6.89(d, $J=8.2$ Hz, 1H), 6.82 (d, $J=2.0$ Hz, 1H),6.76 (dd, $J=8.1, 1.9$ Hz, 1H), 6.43 (d, $J=15.6$ Hz, 1H, -CH=CH-CO-), 3.79 (s, 3H, -OCH₃), 3.78 (s, 3H, -OCH₃), 3.52 (dt, $J=12.7, 6.8$ Hz, 2H, -NHCH₂-), 2.82-2.71 (t, $J=6.8$ Hz, 2H, Ar-CH₂-); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 166.55(-CONH-), 150.34(Ar-OH),148.92(Ar-OH), 147.79(Ar-OCH₃), 146.24(Ar-OCH₃), 140.51(Ar-CH=), 133.17, 128.48, 121.57, 121.49, 119.78, 116.31, 114.84, 113.74, 113.04, 56.17(Ar-OCH₃), 56.04(Ar-OCH₃), 41.76(-NHCH₂-), 36.11(Ar-CH₂-); HR-ESI-MS: m/z 342.13415 [M-H]⁻.

1.1.2.4. N-[2-(3,4-dimethoxyphenyl)ethyl]-3-(4-hydroxy-3-methoxyphenyl)-acrylamide (10d)

FA (64mg, 0.33mmol) was reacted with 2-(3,4-Dimethoxyphenyl)-ethylamine(60mg, 0.33mmol)in dried DMF to give the desired product as a white solid with a yield of 36.2%. IR (KBr) ν 3562, 3333, 1646, 1581, 1542, 1515, 1468, 1466, 1259, 1140, 1020, 846, 812 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δ 7.89 (s, 2H, Ar-OH), 7.43 (d, $J=15.6$ Hz, 1H, Ar-CH=CH-), 7.05 (d, $J=1.7$ Hz, 1H), 6.98 (dd, $J=8.1, 1.7$ Hz, 1H), 6.89 (d, $J=8.1$ Hz, 1H), 6.86 (d, $J=1.9$ Hz, 1H), 6.78 (dd, $J=8.1, 1.9$ Hz, 1H), 6.71 (d, $J=8.1$ Hz, 1H), 6.32 (d, $J=15.6$ Hz, 1H, -CH=CH-CO-), 3.85 (s, 3H, Ar-OCH₃), 3.82 (s, 3H, Ar-OCH₃), 3.80 (s, 3H, Ar-OCH₃), 3.50 (dt, $J=12.7, 7.3$ Hz, 2H, -NHCH₂-), 2.80 (t, $J=7.3$ Hz, 2H, Ar-CH₂-); ¹³C NMR (100 MHz, CD₃OD) δ 169.87(-CONH-), 150.41(Ar-OH), 149.03(Ar-OCH₃), 143.06(Ar-OCH₃), 133.64, 124.32, 122.21, 118.01, 116.01, 113.87, 113.26, 111.06, 56.59(Ar-OCH₃), 56.46(Ar-OCH₃), 56.14(Ar-OCH₃), 42.33(-NHCH₂-), 36.25 (Ar-CH₂-CH₂-); HR-ESI-MS: m/z 356.14980 [M-H]⁻.

1.2. Pharmacological evaluation

1.2.1 Materials

Dulbecco's modified Eagle's medium (DMEM), and fetal bovineserum (FBS) were purchased from Gibico-BRL (Grand Island, NY, USA). Thiazolyl blue tetrazolium bromide (MTT), DPPH and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and stored at -20°C.

1.2.2. Cell culture and treatment

HT22 cells were maintained in DMEM supplemented with 10% (v/v) FBS and incubated at 37 °C under 5% CO₂. To study the protective effect of test compounds on glutamate-induced neuronal death and their cell toxicity, HT22 cells were seeded into 96-well plates, and 6 wells were used for each treatment group. Cells in the control group were treated with vehicle alone. Cell morphology could be observed by an inverse phase contrast microscopy (Olympus, Japan).

1.2.3. Thioflavin T (Th-T) based fluorometric assay

After incubated at 37°C for 24 h, the solutions containing 10 µM A β ₁₋₄₂ oligomers plus 10 µM compounds or 0.215 M PBS were added to 50 mM glycine-NaOH buffer (pH 8.5) containing 1.67 µM *Th-T* in a final volume of 200 µL. Fluorescence was monitored with excitation at 446 nm and emission at 490 nm. A time scan of fluorescence was performed and the intensity values reached at the plateau (around 300s) were averaged after subtracting the background fluorescence from 1.5 µM *Th-T* and PBS. The percent inhibition of A β ₁₋₄₂ aggregation due to the presence of test compounds was calculated by the following expression: 100 - (IF_i/IF₀*100) where IF_i and IF₀ are the fluorescence intensities obtained for A β ₁₋₄₂ or A β ₁₋₄₂ plus test compound in the presence, respectively.

1.2.4. Assessment of cell viability by MTT assay

HT22 cells (10,000 cells / well) were seeded into 96-well culture plates. After 24 h incubation, cells were further treated with different concentrations of test compounds or

pretreated for 30 min and then added glutamate solution (final concentration, 2 mM). Cell growth was measured after 24h, MTT (5 mg/mL) 10 μ L was added to each well and the mixture was incubated for 2h at 37 °C. MTT reagent was then replaced with DMSO (100 μ L per well) to dissolve formazan crystals. After the mixture was shaken at room temperature for 15 min, absorbance was determined at 570 nm using a microplate reader (Bio-Tek, USA). Results were expressed as the percentage of MTT reduction and the absorbance of control cells was set as 100%.

1.2.5. DPPH assay

The compounds were added into a 1 mM ethanol solution of DPPH (final concentration, 100 μ M) to make final concentration of 10 μ M. The reaction tubes were wrapped in aluminum foil and kept at 25-26 °C for 60 min in dark. All measurements were done under dim light. Spectrophotometric measurements were operated at 517 nm by ultraviolet spectrophotometer. The capacity to scavenge free radicals was measured as follow: capacity = $(A_1 - A_2) / A_1 * 100\%$, where A_1 and A_2 are the absorbance of the control treatment and the twelve compounds or caffeic acid in 517 nm, respectively.

1.2.6. Lactate dehydrogenase (LDH) release assay

HT22 cells were plated in 96-well plates at a density of 1×10^4 . On the following day, cultures were exposed to various concentrations. After 24 h, the medium was collected and assayed for LDH activity using a cytotoxicity detection kit (Nanjing Jiancheng, China). Briefly, the release of LDH is measured with a coupled enzymatic reaction that results in the conversion of a tetrazolium salt into red-colored formazan. The amount of formazan synthesized correlated with LDH activity. The formazan product was measured with a microplate reader (Bio-Tek, USA) at 450 nm. Results were expressed as the percentage of LDH release and the absorbance of control cells was set as 100%.

1.2.7. Measurement of intracellular ROS accumulation

Intracellular ROS was detected by using fluorescent dyes, 2', 7'-dichlorodihydrofluorescein diacetate (H_2 -DCF-DA). After treatment, cells were washed with phosphate buffer saline (PBS, pH 7.4), and then stained with 10 μ M H_2 -DCF-DA in serum-free medium for 30 min at 37 °

Cin the dark. The cells were washed with PBS and then photographed using a fluorescence microscope (Olympus, Japan).

1.2.8. Statistical analysis

All quantitative data and experiments described in this study were repeated at least three times. The data were presented as the means $\pm$ standard error of multiple independent experiments. Statistical analyses between two groups were performed by unpaired Student's *t*-test. Differences among groups were tested by one-way analysis of variance (ANOVA). Following ANOVA analyses, the Tukey's test was used and $P < 0.05$ was accepted to be statistically significant.

Table S1. The calculated logP (ClogP) of the compounds

Chemicals	ClogP ¹	Chemicals	ClogP ¹
CA	0.16	FA	0.60
3a	2.66	7a	4.55
3b	3.55	7b	5.44
3c	2.48	10a	2.64
3d	2.84	10b	3.08
5a	2.66	10c	2.30
5b	3.55	10d	2.74

¹ The values were calculated by the trial version of ChemBioOffice Ultra 12.0.

Figure S1. Cell toxicity and neuroprotective effects of test compounds in HT22 cells. (a) The chemical structures of test compounds. (b) Cells were treated with 30 μM test compounds for 24h. Cell viability was determined by using MTT assay (n = 6). (c) After pretreated with 10μM test compounds for 30 min, cell were incubated with 2 mM glutamate for 24 h. Cell viability was determined by using MTT assay (n = 6). **P* < 0.05 versus control; ***P* < 0.01 versus control; ****P* < 0.001 versus control; ##*P* < 0.01 versus glutamate-treated group, ###*P* < 0.001 versus glutamate-treated group.

Figure S2. 7b protects HT22 cells against glutamate-induced cytotoxicity. (a) HT22 cells were photographed using a photomicroscope ($\times 200$) after cells were pretreated with different concentrations for 30 min and then incubated with/without 2 mM glutamate for 24 h. (b) Cell viability was determined using the MTT assay ($n = 6$) and (c) LDH release was also determined ($n = 6$). * $P < 0.05$ versus control; ** $P < 0.01$ versus control; *** $P < 0.001$ versus control; #### $P < 0.001$ versus glutamate -treated group. (d) Cells were pretreated with 10 μ M **7a** or **7b** for 30 min and then exposed to 2 mM glutamate for 12 h followed by incubation with 10 μ M H₂-DCF-DA for 30 min. Cells were photographed using a fluorescence microscope ($\times 200$).

3. Characterization of Compounds

Figure S3 ^1H NMR of compound **3a** in DMSO

Figure S4 MS spectra of compound **3a**

Figure S5 ^1H NMR of compound **3b** in DMSO

Figure S6

Figure S8 ^1H NMR of compound **3c** in DMSO

Figure S11 IR spectra of compound **3c**

Figure S12 ¹H NMR of compound **3d** in DMSO

Figure S13 ¹³C NMR of compound **3d** in DMSO

Figure S14 MS spectra of compound **3d**

Figure S15 ^1H NMR of compound **5a** in DMSO

Figure S16 ^{13}C NMR of compound **5a** in DMSO

Figure S17 MS spectra of compound **5a**

Figure S18 ¹H NMR of compound **5b** in DMSO

Figure S19 ¹³C NMR of compound **5b** in DMSO

Figure S20 MS spectra of compound **5b**

Figure S21 ^1H NMR of compound **7a** in DMSO

Figure S22 MS spectra of compound **7a**

Figure S25 MS spectra of compound **7b**

Figure S26 IR spectra of compound **7b**

Figure S27 ^1H NMR of compound **10a** in DMSO

Figure S28 ^{13}C NMR of compound **10a** in DMSO

Figure S29 MS spectra of compound **10a**

Figure S30 ¹H NMR of compound **10b** in DMSO

Figure S31 MS spectra of compound **10b**

Figure S32 ¹H NMR of compound **10c** in DMSO

^{13}C NMR (101 MHz, DMSO) δ 165.50, 147.40, 145.58, 139.54, 138.17, 127.45, 126.41, 120.51, 118.35, 115.82, 113.92, 42.09.

Figure S34 IR of compound **10c**

Figure S35 MS spectra of compound **10c**

Figure S36 ¹H NMR of compound **10d** in DMSO

Figure S37 ^{13}C NMR of compound **10d** in DMSO

Figure S38 MS spectra of compound **10d**

1505A0565-5#5 RT: 0.07 AV: 1 NL: 4.60E5
T: FTMS - c ESI Full ms [100.00-1000.00]