Discovery of 5-(4-hydroxy-phenyl)-3-oxo-pentanoic acid [2-(5-methoxy-1H-indol-3-yl)-ethyl]-amide as a neuroprotectant for Alzheimer's disease by hybridization of curcumin and melatonin

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Chemistry. Reagents and solvents were obtained from commercial suppliers and used as received unless otherwise indicated. Reactions were monitored by thin-layer chromatography (TLC) (precoated silica gel 60F254 plates, EMD Chemicals) and visualized with UV light or by treatment with phosphomolybdic acid (PMA) or ninhydrin. Flash chromatography was performed on silica gel (200-300 mesh, Fisher Scientific) using solvents as indicated. ^{1}HNMR and ^{13}CNMR spectra were routinely recorded on a Bruker ARX 400 spectrometer. The NMR solvent used was $CDCl_3$ or CD_3OD as indicated. Tetramethylsilane (TMS) was used as the internal standard. HRMS were recorded on PerkinElmer AxION 2 TOF mass spectrometer. The purity of target compounds was determined by HPLC using a Varian 100-5 C18 250×4.6 mm column with UV detection (280 nm and 360 nm) (50% H_2O in acetonitrile and 0.1% TFA, and 30-50% H_2O in methanol and 0.1% TFA, two solvent systems) to be $\geq$ 95%.

Ethyl 4-(triphenylphosphoranylidene)acetoacetate (9). Triphenylphosphene (14.42 g, 55.25 mmol) was added to a solution of ethyl 4-chloroacetoacetate (8.39 g, 60.76 mmol) in benzene (35 mL) and stirred for 24 h at 55 °C. The solution was then cooled to

room temperature, and the precipitate was collected by filtration and washed with benzene. The solid precipitate was then dissolved in H_2O (10 mL). To this solution a 1 N $NaHCO_3$ solution (10 mL) was added, and the resulting precipitate was collected by filtration, washed with H_2O, and then dried under reduced pressure to afford **5** as a white solid (15.31 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 - 7.60 (m, 6H), 7.60 - 7.50 (m, 3H), 7.45 (m, 6H), 4.19 (q, J = 7.13 Hz, 2H), 3.81 (m, 1H), 3.35 (s, 2H), 1.28 (t, J = 7.13 Hz, 3H).

Preparation of 10. Compound **9** (5.04 g, 13.00 mmol) and 5-methoxytryptamine (2.60 g, 13.69 mmol) were added together in xylene (25 mL), and the solution was heated to reflux for 3 h. The solution was then cooled to room temperature and concentrated under reduced pressure. The crude residue was purified by flash chromatography (MeOH/CH_2Cl_2: 2/98) to give **7** (3.93 g, 57%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (br. s., 1H), 7.89 (br. s., 1H), 7.70 - 7.51 (m, 9H), 7.50 - 7.39 (m, 6H), 7.19 (d, J = 8.76 Hz, 1H), 7.04 (d, J = 2.42 Hz, 1H), 6.93 (d, J = 2.06 Hz, 1H), 6.82 (dd, J = 8.76, 2.42 Hz, 1H), 3.91 (m, 1H), 3.87 (s, 3H), 3.55 (m, 2H), 3.31 (s, 2H), 2.89 (t, J = 7.46 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.87, 169.47, 153.97, 133.07, 132.97, 132.37, 131.37, 129.06, 128.94, 127.89, 126.63, 125.73, 122.69, 113.28, 112.30, 111.77, 100.46, 100.00, 55.95, 39.45, 25.66.

Procedure A. Preparation of 3. Compound **10** (0.25 g, 0.47 mmol) was added to a solution of NaH (0.075 g, 1.87 mmol) in DMPU/THF (2 mL/2.2 mL) and cooled to 0 °C for 30 min. To this vanillin (0.085 g, 0.56 mmol) in THF (0.5 mL) was added dropwise. The solution was heated to 40°C for 3 h. The solution was then cooled to room temperature and stirred overnight. The reaction was then quenched using NH_4Cl (0.5

mL). The solvent was removed under reduced pressure and the residual oil was purified by flash chromatography (Hexanes/Acetone: 50/50) to give **3** (0.06 g, 31%) as a light yellow solid. ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.56 (d, J = 16.04 Hz, 1H), 7.23 (d, J = 8.80 Hz, 1H), 7.09 (dd, J = 8.28 Hz, 1.84 Hz, 1H), 7.04-6.99 (m, 3H), 6.93 (d, J = 8.20 Hz, 1H), 6.85 (dd, J = 8.80 Hz, 2.4 Hz, 1H), 6.59 (d, J = 16.04 Hz, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.63 (q, J = 5.76 Hz, 2H), 3.58 (s, 2H), 2.96 (t, J = 6.88 Hz, 2H); ^{13}CNMR (100 MHz, $CDCl_3$) δ 195.31, 165.96, 154.09, 149.00, 147.00, 145.68, 131.56, 127.73, 126.48, 124.18, 123.30, 122.89, 115.00, 112.62, 112.43, 111.95, 109.83, 100.54, 56.03, 55.96, 47.30, 39.79, 25.24. HRMS (m/z) (M−H): calcd. for $C_{23}H_{23}N_2O_5$ 407.1613, found 407.1624.

Preparation of 4. 3-Methoxybenzaldehyde (0.076 g, 0.56 mmol) was reacted with **10** (0.47 mmol) following Procedure A to give **4** (0.06 g, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (br. s., 1H), 7.71 (d, J = 7.53 Hz, 1H), 7.60 (d, J = 12.80 Hz, 1H), 7.37 (t, J = 8.03 Hz, 1H), 7.32 (t, J = 8.00 Hz, 1H), 7.24 (d, J = 8.78 Hz, 1H), 7.14 (d, J = 8.28 Hz, 1H), 7.04 (s, 1H), 7.03 (br. s., 1H), 6.98 (d, J = 1.76 Hz, 1H), 6.85 (dd, J = 2.51, 8.78 Hz, 1H), 6.72 (d, J = 16.31 Hz, 1H), 3.86 (s, 6H), 3.60 - 3.67 (m, 4H), 2.97 (t, J = 7.03 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.5, 166.1, 159.9, 159.6, 154.0, 145.3, 135.3, 131.5, 130.0, 129.4, 127.7, 125.8, 122.9, 121.4, 120.1, 117.1, 113.4, 112.3, 111.9, 100.5, 55.9, 55.4, 55.3, 47.1, 39.8, 25.1. HRMS (m/z) (M−H): calcd. for $C_{23}H_{23}N_2O_4$ 391.1663, found 391.1675.

Preparation of 5. 4-Hydroxybenzaldehyde (0.035 g, 0.29 mmol) and **10** (0.25 g, 0.47 mmol) were added together in a DMSO/H_2O (5 mL/1 mL) solution, and then heated to 100 °C for 24 h. The reaction was cooled to room temperature, and the product was

extracted into EtOAc. The EtOAc layer was washed extensively with H_2O and then concentrated under reduced pressure. The residual was twice purified by flash chromatography (1. MeOH/CH_2Cl_2: 5/95; 2. Hexanes/Acetone: 50/50) to give **5** (0.045 g, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (br. s., 1H), 8.21 (br. s., 1H), 7.41 (d, J = 16.06 Hz, 1H), 7.24 (d, J = 8.53 Hz, 2H), 7.09 - 7.16 (m, 2H), 6.91 (dd, J = 2.26, 5.52 Hz, 2H), 6.74 (d, J = 8.78 Hz, 2H), 6.69 - 6.73 (m, 1H), 6.43 (d, J = 16.06 Hz, 1H), 3.73 (s, 3H), 3.49 (q, J = 6.78 Hz, 2H), 3.45 (s, 2H), 2.84 (t, J = 6.78 Hz, 2H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ 194.7, 173.2, 168.2, 154.8, 154.8, 144.5, 135.0, 132.9, 131.3, 129.8, 128.5, 124.1, 121.1, 116.6, 113.1, 112.5, 101.2, 55.9, 55.9, 49.3, 40.3, 26.3. HRMS (m/z) (M−H): calcd. for $C_{22}H_{21}N_2O_4$ 377.1507, found 377.1521.

Preparation of 6. Benzaldehyde (0.060 g, 0.56 mmol) was reacted with **10** (0.25 g, 0.47 mmol) following Procedure A to give **6** (0.05 g, 29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (br. s., 1H), 7.59 (d, J = 16.06 Hz, 1H), 7.51 (dd, J = 1.80, 7.60 Hz, 2H), 7.29 - 7.45 (m, 3H), 7.21 (d, J = 8.78 Hz, 1H), 7.08 (br. s., 1H), 7.02 - 7.04 (m, 1H), 7.00 (d, J = 2.01 Hz, 1H), 6.84 (dd, J = 2.51, 8.78 Hz, 1H), 6.71 (d, J = 16.06 Hz, 1H), 3.84 (s, 3H), 3.61 (q, J = 6.80 Hz, 2H), 3.57 (s, 2H), 2.94 (t, J = 6.80 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.5, 165.6, 154.1, 145.3, 134.0, 131.5, 131.2, 129.1, 128.8, 128.7, 127.7, 127.4, 125.7, 122.9, 112.7, 112.5, 112.0, 100.5, 56.0, 47.4, 39.8, 25.2. HRMS (m/z) (M−H): calcd. for $C_{22}H_{21}N_2O_3$ 361.1558, found 361.1570.

Preparation of 7. Compound **5** (0.500 g, 1.32 mmol) was dissolved in MeOH (30 mL) under N_2. To this Pd/C (0.050 g) was added. The solution was then stirred under H_2 at normal pressure overnight. The solution was then filtered to remove Pd/C, and the filtrate was concentrated under reduced pressure. The residue was purified by flash

chromatography (MeOH/CH_2Cl_2: 2/98) to give **7** (0.360 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (br. s., 1H), 7.75 (br. s., 1H), 7.22 (d, J = 8.78 Hz, 1H), 7.01 (d, J = 2.26 Hz, 1H), 6.91 - 6.98 (m, 4H), 6.83 (dd, J = 2.26, 8.78 Hz, 1H), 6.74 (d, J = 8.28 Hz, 2H), 3.83 (s, 3H), 3.55 (q, J = 6.61 Hz, 2H), 3.25 (s, 2H), 2.90 (t, J = 6.78 Hz, 2H), 2.67 - 2.78 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.8, 165.8, 155.0, 153.9, 131.5, 131.3, 129.2, 127.6, 123.0, 115.5, 112.2, 112.0, 100.5, 55.9, 49.3, 45.2, 39.7, 28.5, 25.0. HRMS (m/z) (M−H): calcd. for $C_{22}H_{23}N_2O_4$ 379.1663, found 379.1665.

LC-MS/MS analysis. For brain samples, half a brain was weighed and diluted with 1.0 mL of acetonitrile and then mixed well. For plasma samples, 0.01 mL of plasma was diluted with 0.99 mL of acetonitrile and then mixed well. After mixing, samples were centrifuged at 15,000 rpm and the supernatant was transferred to a new tube and evaporated to dryness using spin vacuum. The samples were then reconstituted with an 80:20 solution of 1% acetic acid in acetonitrile: 1% acetic acid in water, and a volume of 0.025 mL was then injected into the LC-MS/MS. The LC/MS/MS method employed positive electrospray ionization (ESI) with a selected reaction monitoring (SRM) mode. Compound **9** was monitored using the following SRM transitions: 381→174, 130, and 159. Chromatographic separation was achieved under gradient conditions using a Waters Acquity® UPLC, with a reversed phase column (Gemini 5u C18 110Å, 100 mm x 2.0 mm; 5 um, Phenomenex Inc., Torrance, CA) with a mobile phase composition of 1% acetic acid in water (mobile phase A) and 1% acetic acid in acetonitrile (mobile phase B). The initial gradient consisted of 30% B for 1 min, 30% to 95% B from 1 to 3 min, hold for 1 min at 95% B, and then equilibrate at 30% B for 2.5 min. The total run time was 6.5 min. Results were processed using Analyst 1.5.2 software. Absolute recovery, precision

and accuracy, and matrix effects experiments produced an efficient method to continue sample analysis. Calibration curves were made with freshly prepared samples and calculated using peak area versus concentration with a linear or quadratic regression. Accuracy fell in the range of 85% to 115%.