SUPPLEMENTARY MATERIAL

Asymmetric synthesis and biological activities of natural product

(+)-balasubramide and its derivatives

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Abstract

The natural product (+)-balasubramide (**3j**) and its derivatives (**3a–3i**) were synthesized using a two-step asymmetric synthesis, and the biological activities of **3a–3j** were determined *in vitro*. Methyl (2S,3R)-(+)-3-phenyloxirane-2-carboxylate (**1h**), the asymmetric synthesis of which was described in a previous paper, was selected as the starting material. Compounds **3a–3j** were evaluated for their neuroprotective, antioxidative, and anti-neuroinflammatory effects. (+)-Balasubramide and its derivatives with different electronegative groups in the 6-phenyl ring produced little neuroprotection and antioxidation, but induced potent anti-neuroinflammatory effects in BV-2 microglial cells (with the exception of **3g**). Compound **3c**, with a trifluoromethyl group in its 6-phenyl ring, was a particularly potent anti-neuroinflammatory agent. These results demonstrated that the electronegativity of the 6-phenyl ring of (+)-balasubramide is an important determinant of its inhibitory effect on neuroinflammation. More electronegative substituents result in more potent anti-neuroinflammatory effects. Moreover, cytotoxicity assays indicated no significant effects of the tested compounds.

Key words: balasubramide; asymmetric catalysis; synthesis; biological screening; anti-neuroinflammatory effect

1. Experimental section

1.1. General chemistry

All the reagents for synthesis were commercially available and used without further purification or purified by standard methods prior to use. Melting points were determined using an X-5 apparatus and an uncorrected thermometer. Optical rotations were measured using a 1 mL cell with a 1 dm path length on a Perkin-Elmer 341 digital polarimeter (Perkin-Elmer, Norwalk, CT, USA). ^1H and ^{13}C NMR spectra were recorded on a Bruker Advance 500 MHz or Bruker Advance 400 MHz spectrometer (Bruker, Berlin, Germany). High-resolution mass spectra were obtained with Waters mass spectrometer (Waters, Milford, MA, USA). Enantiomeric excesses were determined by HPLC using a Daicel Chiralcel AD-H or OD-H column (4.6 mm $\times$ 25 cm) with n-hexane/i-PrOH eluent (Daicel, Osaka, Japan).

1.2. Biological assays

1.2.1. Protection of cerebellar granule neurons against glutamate- and nutrient deprivation-induced cell injury

Cerebellar granule neuronal cells (CGCs) were isolated from 8-day old Sprague Dawley rat pups as described previously (Pang et al. 2014). The neurons were plated in poly-L-lysine coated 96-well plates at a density of 1×10^6 cells/ml and cultured in the complete Neurobasal medium (Invitrogen) supplemented with 2% B27 (Invitrogen) and 0.5 mM GlutaMax (Invitrogen). Cultures were incubated in a humidified atmosphere of 5% CO_2 -95% air at 37°C. Cultures 6-8 days *in vitro* were used and cells were preincubated with compounds dissolved in DMSO and diluted with medium to the final concentrations for 24 h, followed by 100 μM glutamate incubation or nutrient deprivation (serum B27 deprivation, -B27). After 24 h, the cell viability was measured by MTT assay.

1.2.2. Protection of PC12 cells against H_2O_2 -induced cell injury

The PC12 cells were obtained from the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Fetal bovine serum (FBS), horse serum, penicillin, streptomycin, and the high-glucose DMEM medium were purchased from Invitrogen. H_2O_2 was purchased from Sigma. PC12 cells were cultured in high-glucose DMEM supplemented with 10% FBS, 5% horse serum, 100 U/ml penicillin and 100 U/ml streptomycin in a humidified atmosphere of 5% CO_2 at 37°C. PC12 cells were plated in 96-well plates at a density of 4×10^5 cells/ml. After cell attachment,

PC12 cells were preincubated with compounds dissolved in DMSO and diluted with medium to the final concentrations for 2 h, followed by 500 μ M H₂O₂ incubation. After 24 h, the cell viability was measured by MTT assay.

1.2.3. MTT assay

Cell viability was evaluated by incubating with 0.5 mg/ml MTT for 4 h under 5% CO₂/95% air at 37°C. Active mitochondrial dehydrogenases of living cells cause cleavage and reduction of soluble yellow MTT dye to the insoluble purple formazan, which was extracted in DMSO. Media were replaced with 100 μ l DMSO, and the optical density was measured at 570 nm in a BD plate-reader.

1.2.4. Inhibition of TNF α production of LPS-stimulated BV2 microglial cells

BV-2 microglial cells were obtained from the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. LPS was purchased from Sigma. BV2 microglial cells were maintained in DMEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 U/ml streptomycin in a humidified atmosphere of 5% CO₂ at 37°C. Cells were plated in 24-well plates at a density of 4×10^5 cells/ml. After cell attachment, the BV2 cells were preincubated with compounds dissolved in DMSO and diluted with medium to the final concentrations for 2 h, followed by 100 ng/ml LPS incubation. After incubation with LPS for 6 h, the TNF α production in the supernatant was determined by ELISA kit (R&D Systems, USA).

1.2.5. Quantitative PCR

Total RNA was extracted from BV2 cells using Trizol reagent (Invitrogen, Camarillo, CA). Isolated RNA was reverse transcribed into cDNA using cDNA synthesis kit (Vazyme, Jiangsu, China) following standard protocols. Quantitative PCR (qPCR) was performed using synthetic primers and SYBR Green (Thermo, Rockford, IL, USA) with a IQ5 Detection System. After incubation at 50°C for 2 minutes and 95°C for 10 minutes, samples were subjected to 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The final results were all normalized as fold change of the TNF α /GAPDH.

1.2.6. Statistical analysis

Statistical significance was determined using GraphPad Prism 5 Software (San Diego, CA). Data are represented as mean $\pm$ SD. Statistical analysis among multiple group was carried out using the One-way ANOVA followed by Bonferroni's post hoc test. The difference was considered

significant if the *P* value was below 0.05.

1.3. Procedure for the synthesis of compounds **1a-1i**, **2a-2i** and **3a-3j**

methyl (2S,3R)-3-(3-fluorophenyl)oxirane-2-carboxylate (**1a**)

To a solution of 3-fluorocinnamaldehyde (30 mmol) in CH₂Cl₂ (25 mL) was added catalyst (1.103 g, 3 mmol) and H₂O₂ (30 wt% in H₂O, 3.677 mL) at room temperature. After the reaction mixture was stirred for 2 h, anhydrous Na₂CO₃ (3.816 g, 36 mmol), CH₃OH (30 mL), and NBS (6.408 g, 36 mmol) were added sequentially. The mixture was stirred for another 3 h, concentrated under vacuum, and purified by flash column chromatography (ethyl acetate/petrol ether = 1/10). **1a** was obtained as a pale yellow oil with a 75% yield, $[\alpha]_D^{20} = +166.2$ (c=0.5, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.34 (td, *J* = 8.0, 5.7 Hz, 1H), 7.10 (d, *J* = 7.7 Hz, 1H), 7.04 (td, *J* = 8.4, 2.6 Hz, 1H), 7.01–6.95 (m, 1H), 4.10 (t, *J* = 3.3 Hz, 1H), 3.83 (s, 3H), 3.48 (d, *J* = 1.7 Hz, 1H); IR (KBr, cm⁻¹): 1752, 1634, 1442, 1292, 1233, 1210, 777, 686. The enantiomeric excess was determined by HPLC with a Chiralcel AD-H column (4.6 mm × 25 cm) (n-hexane/i-PrOH = 90/10, λ = 230 nm, 0.8 mL/min); *t*_{minor} = 8.6 min, *t*_{major} = 10.5 min, 97% ee.

methyl (2S,3R)-3-(4-fluorophenyl)oxirane-2-carboxylate (**1b**)

To a solution of 4-fluorocinnamaldehyde (30 mmol) in CH₂Cl₂ (25 mL) was added catalyst (1.103 g, 3 mmol) and H₂O₂ (30 wt% in H₂O, 3.677 mL) at room temperature. After the reaction mixture was stirred for 2 h, anhydrous Na₂CO₃ (3.816 g, 36 mmol), CH₃OH (30 mL), and NBS (6.408 g, 36 mmol) were added sequentially. The mixture was stirred for another 3 h, concentrated under vacuum, and purified by flash column chromatography (ethyl acetate/petrol ether = 1/10). **1b** was obtained as a pale yellow oil with a 73% yield, $[\alpha]_D^{20} = +132.2$ (c=1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.22 – 7.16 (m, 2H), 7.02 – 6.95 (m, 2H), 4.02 (t, *J* = 3.7 Hz, 1H), 3.76 (d, *J* = 5.6 Hz, 3H), 3.42 (dd, *J* = 7.8, 2.2 Hz, 1H); IR (KBr, cm⁻¹): 1749, 1637, 1442, 1290, 1243, 1214, 775, 686. The enantiomeric excess was determined by HPLC with a Chiralcel AD-H column (4.6 mm × 25 cm) (n-hexane/i-PrOH = 90/10, λ = 230 nm, 0.8 mL/min); *t*_{major} = 8.38 min, *t*_{minor} = 10.73 min, 99% ee.

methyl (2S,3R)-3-(4-(trifluoromethyl)phenyl)oxirane-2-carboxylate (**1c**)

To a solution of 4-trifluoromethylcinnamaldehyde (30 mmol) in CH₂Cl₂ (25 mL) was added

catalyst (1.103 g, 3 mmol) and H₂O₂ (30 wt% in H₂O, 3.677 mL) at room temperature. After the reaction mixture was stirred for 2 h, anhydrous Na₂CO₃ (3.816 g, 36 mmol), CH₃OH (30 mL), and NBS (6.408 g, 36 mmol) were added sequentially. The mixture was stirred for another 3 h, concentrated under vacuum, and purified by flash column chromatography (ethyl acetate/petrol ether = 1/10). **1c** was obtained as white solid with a 79% yield, m.p. 52-54 °C, [α]_D²⁰ = +124.1 (c=0.9, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.64 (d, J= 8.1 Hz, 2H), 7.43 (d, J= 8.1 Hz, 2H), 4.17 (d, J= 1.6 Hz, 1H), 3.85 (s, 3H), 3.50 (d, J= 1.6 Hz, 1H); IR (KBr, cm⁻¹): 1755, 1636, 1405, 1328, 1125, 700, 662. The enantiomeric excess was determined by HPLC with a Chiralcel AD-H column (4.6 mm×25 cm) (n-hexane/i-PrOH = 90/10, λ = 230 nm, 0.8 mL/min); t_{minor} = 8.8 min, t_{major} = 9.2 min, 96% ee.

methyl (2S,3R)-3-(2-nitrophenyl)oxirane-2-carboxylate (**1d**)

To a solution of 2-nitrocinnamaldehyde (30 mmol) in CH₂Cl₂ (25 mL) was added catalyst (1.103 g, 3 mmol) and H₂O₂ (30 wt% in H₂O, 3.677 mL) at room temperature. After the reaction mixture was stirred for 2 h, anhydrous Na₂CO₃ (3.816 g, 36 mmol), CH₃OH (30 mL), and NBS (6.408 g, 36 mmol) were added sequentially. The mixture was stirred for another 3 h, concentrated under vacuum, and purified by flash column chromatography (ethyl acetate/petrol ether = 1/10). **1d** was obtained as white solid with a 80% yield, m.p. 60-62 °C, [α]_D²⁰ = +149.6 (c=1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.22 (d, J= 8.2 Hz, 1H), 7.72–7.52 (m, 3H), 4.70 (s, 1H), 3.88 (s, 3H), 3.39 (d, J= 1.7 Hz, 1H); IR (KBr, cm⁻¹): 1748, 1525, 1462, 1348, 1709, 725, 678. The enantiomeric excess was determined by HPLC with a Chiralcel AD-H column (4.6 mm×25 cm) (n-hexane/i-PrOH = 90/10, λ = 254 nm, 0.8 mL/min); t_{minor} = 13.2 min, t_{major} = 45.2 min, 97% ee.

methyl (2S,3R)-3-(4-chlorophenyl)oxirane-2-carboxylate (**1e**)

To a solution of 4-chlorocinnamaldehyde (30 mmol) in CH₂Cl₂ (25 mL) was added catalyst (1.103 g, 3 mmol) and H₂O₂ (30 wt% in H₂O, 3.677 mL) at room temperature. After the reaction mixture was stirred for 2 h, anhydrous Na₂CO₃ (3.816 g, 36 mmol), CH₃OH (30 mL), and NBS (6.408 g, 36 mmol) were added sequentially. The mixture was stirred for another 3 h, concentrated under vacuum, and purified by flash column chromatography (ethyl acetate/petrol ether = 1/10). **1e** was obtained as colorless oil with a 77% yield, [α]_D²⁰ = +155.1 (c=1.0, CHCl₃); ¹H NMR (500 MHz,

CDCl₃) δ 7.36 – 7.33 (m, 1H), 7.33 – 7.31 (m, 1H), 7.24 – 7.22 (m, 1H), 7.22 – 7.20 (m, 1H), 4.08 (d, J = 1.6 Hz, 1H), 3.82 (s, 3H), 3.47 (d, J = 1.7 Hz, 1H); IR (KBr, cm⁻¹): 2955, 1752, 1496, 1445, 1290, 1211, 1091, 835, 739. The enantiomeric excess was determined by HPLC with a Chiralcel OD-H column (4.6 mm $\times$ 25 cm) (n-hexane/i-PrOH = 90/10, λ = 227 nm, 0.8 mL/min); t_{minor} = 8.6 min, t_{major} = 9.7 min, 96% ee.

methyl (2S,3R)-3-(4-bromophenyl)oxirane-2-carboxylate (**1f**)

To a solution of 4-bromocinnamaldehyde (30 mmol) in CH₂Cl₂ (25 mL) was added catalyst (1.103 g, 3 mmol) and H₂O₂ (30 wt% in H₂O, 3.677 mL) at room temperature. After the reaction mixture was stirred for 2 h, anhydrous Na₂CO₃ (3.816 g, 36 mmol), CH₃OH (30 mL), and NBS (6.408 g, 36 mmol) were added sequentially. The mixture was stirred for another 3 h, concentrated under vacuum, and purified by flash column chromatography (ethyl acetate/petrol ether = 1/10). **1f** was obtained as white solid with a 84% yield, m.p. 69-70 °C, $[\alpha]_{\text{D}}^{20}$ = +127.6 (c=1.0, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.52 – 7.50 (m, 1H), 7.50 – 7.48 (m, 1H), 7.19 – 7.17 (m, 1H), 7.17 – 7.15 (m, 1H), 4.07 (d, J = 1.6 Hz, 1H), 3.83 (s, 3H), 3.47 (d, J = 1.7 Hz, 1H); IR (KBr, cm⁻¹): 1751, 1493, 1425, 1339, 1211, 1091, 835, 793. The enantiomeric excess was determined by HPLC with a Chiralcel AD-H column (4.6 mm $\times$ 25 cm) (n-hexane/i-PrOH = 90/10, λ = 230 nm, 0.8 mL/min); t_{minor} = 8.6 min, t_{major} = 10.5 min, 96% ee.

methyl (2S,3R)-3-(3-chlorophenyl)oxirane-2-carboxylate (**1g**)

To a solution of 3-chlorocinnamaldehyde (30 mmol) in CH₂Cl₂ (25 mL) was added catalyst (1.103 g, 3 mmol) and H₂O₂ (30 wt% in H₂O, 3.677 mL) at room temperature. After the reaction mixture was stirred for 2 h, anhydrous Na₂CO₃ (3.816 g, 36 mmol), CH₃OH (30 mL), and NBS (6.408 g, 36 mmol) were added sequentially. The mixture was stirred for another 3 h, concentrated under vacuum, and purified by flash column chromatography (ethyl acetate/petrol ether = 1/10). **1g** was obtained as pale yellow oil with a 83% yield, $[\alpha]_{\text{D}}^{20}$ = +147.7 (c=1.0, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.31 (dd, J = 6.5, 4.5 Hz, 2H), 7.27 (t, J = 3.7 Hz, 1H), 7.19 (dt, J = 7.0, 1.6 Hz, 1H), 4.08 (d, J = 1.6 Hz, 1H), 3.83 (s, 3H), 3.48 (d, J = 1.7 Hz, 1H); IR (KBr, cm⁻¹): 1747, 1638, 1436, 1401, 1209, 784, 686. The enantiomeric excess was determined by HPLC with a Chiralcel AD-H column (4.6 mm $\times$ 25 cm) (n-hexane/i-PrOH = 90/10, λ = 220 nm, 0.8 mL/min); t_{minor} = 8.5 min,

$t_{\text{major}} = 10.5$ min, 93% ee.

methyl (2S,3R)-3-phenyloxirane-2-carboxylate (**1h**)

To a solution of trans-cinnamaldehyde (30 mmol) in CH_2Cl_2 (25 mL) was added catalyst (1.103 g, 3 mmol) and H_2O_2 (30 wt% in H_2O , 3.677 mL) at room temperature. After the reaction mixture was stirred for 2 h, anhydrous Na_2CO_3 (3.816 g, 36 mmol), CH_3OH (30 mL), and NBS (6.408 g, 36 mmol) were added sequentially. The mixture was stirred for another 3 h, concentrated under vacuum, and purified by flash column chromatography (ethyl acetate/petrol ether = 1/10). **1h** was obtained as pale yellow oil with a 87% yield, $[\alpha]_{\text{D}}^{20} = +156.3$ ($c=1.3$, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 7.40 – 7.34 (m, 3H), 7.32 – 7.28 (m, 2H), 4.11 (t, $J = 2.7$ Hz, 1H), 3.83 (s, 3H), 3.53 (d, $J = 1.8$ Hz, 1H); IR (KBr, cm^{-1}): 2955, 1750, 1634, 1440, 1413, 1201, 760, 696. The enantiomeric excess was determined by HPLC with a Chiralcel AD-H column (4.6 mm $\times$ 25 cm) (n-hexane/i-PrOH = 90/10, $\lambda = 220$ nm, 0.8 mL/min); $t_{\text{major}} = 18.4$ min, $t_{\text{minor}} = 19.7$ min, 95% ee.

methyl (2S,3R)-3-(4-nitrophenyl)oxirane-2-carboxylate (**1i**)

To a solution of 4-nitrocinnamaldehyde (30 mmol) in CH_2Cl_2 (25 mL) was added catalyst (1.103 g, 3 mmol) and H_2O_2 (30 wt% in H_2O , 3.677 mL) at room temperature. After the reaction mixture was stirred for 2 h, anhydrous Na_2CO_3 (3.816 g, 36 mmol), CH_3OH (30 mL), and NBS (6.408 g, 36 mmol) were added sequentially. The mixture was stirred for another 3 h, concentrated under vacuum, and purified by flash column chromatography (ethyl acetate/petrol ether = 1/10). **1i** was obtained as white solid with a 78% yield, m.p. 137-138 °C, $[\alpha]_{\text{D}}^{20} = +150.1$ ($c=1.0$, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 8.25 (s, 1H), 8.23 (s, 1H), 7.49 (s, 1H), 7.47 (s, 1H), 4.22 (d, $J = 1.5$ Hz, 1H), 3.85 (s, 3H), 3.50 (d, $J = 1.6$ Hz, 1H); IR (KBr, cm^{-1}): 1750, 1606, 1518, 1349, 1216, 863, 735, 690. The enantiomeric excess was determined by HPLC with a Chiralcel AD-H column (4.6 mm $\times$ 25 cm) (n-hexane/i-PrOH = 90/10, $\lambda = 254$ nm, 0.8 mL/min); $t_{\text{minor}} = 20.2$ min, $t_{\text{major}} = 22.6$ min, 99% ee.

(2S,3R)-N-(2-(1H-indol-3-yl)ethyl)-3-(3-fluorophenyl)oxirane-2-carboxamide (**2a**)

To a solution of tryptamine (1.6 g, 10 mmol) in CH_3OH (40 mL) was added MeONa (0.54 g, 10 mmol) and compound **1a** (2.36 g, 12 mmol) at -18 °C. The resulting mixture was stirred at -18 °C.

After the reaction is over, 5 mL distilled water was added. The pH was adjusted to 8-9 by the addition of 0.2N hydrochloric acid. The aqueous layer was washed 3 times with CH₂Cl₂(30 mL). The organic layer was collected and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (eluted using chloroform). **2a** was obtained as a white solid with an 81% yield, m.p. 138–140 °C, $[\alpha]_D^{20} = +45.1$ (c=0.5, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.18 (s, 1H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.45–7.37 (m, 1H), 7.30 (td, *J* = 8.0, 5.7 Hz, 1H), 7.24 (t, *J* = 7.5 Hz, 1H), 7.16 (dd, *J* = 7.8, 7.1 Hz, 1H), 7.06 (d, *J* = 2.0 Hz, 1H), 7.05–6.97 (m, 2H), 6.91–6.85 (m, 1H), 6.29 (s, 1H), 3.72–3.59 (m, 3H), 3.43 (d, *J* = 1.8 Hz, 1H), 3.11–2.95 (m, 2H); IR (KBr, cm⁻¹): 3313, 1638, 1557, 1458, 1248, 743, 610.

(2S,3R)-N-(2-(1*H*-indol-3-yl)ethyl)-3-(4-fluorophenyl)oxirane-2-carboxamide (**2b**)

To a solution of tryptamine (1.6 g, 10 mmol) in CH₃OH (40 mL) was added MeONa (0.54 g, 10 mmol) and compound **1b** (2.36 g, 12 mmol) at -18 °C. The resulting mixture was stirred at -18 °C. After the reaction is over, 5 mL distilled water was added. The pH was adjusted to 8-9 by the addition of 0.2N hydrochloric acid. The aqueous layer was washed 3 times with CH₂Cl₂(30 mL). The organic layer was collected and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (eluted using chloroform). **2b** was obtained as a white solid with an 89% yield, m.p. 127-130 °C, $[\alpha]_D^{20} = +36.2$ (c=0.5, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.12 (s, 1H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.45 – 7.39 (m, 2H), 7.37 (d, *J* = 7.7 Hz, 1H), 7.24 (d, *J* = 7.3 Hz, 1H), 7.17 (t, *J* = 7.4 Hz, 1H), 7.08 (d, *J* = 2.2 Hz, 1H), 6.28 (s, 1H), 3.74 – 3.59 (m, 3H), 3.45 (d, *J* = 1.9 Hz, 1H), 3.13 - 2.96 (m, 2H); IR (KBr, cm⁻¹): 3414, 1638, 1558, 1322, 1140, 746, 611.

(2S,3R)-N-(2-(1*H*-indol-3-yl)ethyl)-3-(4-(trifluoromethyl)phenyl)oxirane-2-carboxamide (**2c**)

To a solution of tryptamine (1.6 g, 10 mmol) in CH₃OH (40 mL) was added MeONa (0.54 g, 10 mmol) and compound **1c** (2.96 g, 12 mmol) at -18 °C. The resulting mixture was stirred at -18 °C. After the reaction is over, 5 mL distilled water was added. The pH was adjusted to 8-9 by the addition of 0.2N hydrochloric acid. The aqueous layer was wa

shed 3 times with CH₂Cl₂(30 mL). The organic layer was collected and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (eluted using chloroform). **2c** was obtained as a white solid with an 89% yield, m.p. 127-130 °C, $[\alpha]_D^{20} = +36.2$ (c=0.5, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.12 (s, 1H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.45 – 7.39 (m, 2H), 7.37 (d, *J* = 7.7 Hz, 1H), 7.24 (d, *J* = 7.3 Hz, 1H), 7.17 (t, *J* = 7.4 Hz, 1H), 7.08 (d, *J* = 2.2 Hz, 1H), 6.28 (s, 1H), 3.74 – 3.59 (m, 3H), 3.45 (d, *J* = 1.9 Hz, 1H), 3.13 – 2.96 (m, 2H); IR (KBr, cm⁻¹): 3414, 1638, 1558, 1322, 1140, 746, 611.

(2S,3R)-N-(2-(1*H*-indol-3-yl)ethyl)-3-(2-nitrophenyl)oxirane-2-carboxamide (**2d**)

To a solution of tryptamine (1.6 g, 10 mmol) in CH₃OH (40 mL) was added MeONa (0.54 g, 10 mmol) and compound **1d** (2.68 g, 12 mmol) at -18 °C. The resulting mixture was stirred at -18 °C. After the reaction is over, 5 mL distilled water was added. The pH was adjusted to 8-9 by the addition of 0.2N hydrochloric acid. The aqueous layer was washed 3 times with CH₂Cl₂(30 mL). The organic layer was collected and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (eluted using chloroform). **2d** was obtained as a pale yellow solid with an 81% yield, m.p. 127-129 °C, $[\alpha]_D^{20} = +65.2$ (c=0.5, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.25 (s, 1H), 8.17 (dt, *J* = 7.7, 1.8 Hz, 1H), 8.03 (d, *J* = 1.8 Hz, 1H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.54 – 7.46 (m, 2H), 7.41 (d, *J* = 8.1 Hz, 1H), 7.24 (t, *J* = 7.5 Hz, 1H), 7.16 (t, *J* = 7.5 Hz, 1H), 7.07 (d, *J* = 2.2 Hz, 1H), 6.30 (t, *J* = 5.6 Hz, 1H), 3.75 – 3.58 (m, 3H), 3.45 (d, *J* = 1.9 Hz, 1H), 3.14 – 2.94 (m, 2H); IR (KBr, cm⁻¹): 3320, 1641, 1532, 1352, 744, 647.

(2S,3R)-N-(2-(1*H*-indol-3-yl)ethyl)-3-(4-chlorophenyl)oxirane-2-carboxamide (**2e**)

To a solution of tryptamine (1.6 g, 10 mmol) in CH₃OH (40 mL) was added MeONa (0.54 g, 10 mmol) and compound **1e** (2.55 g, 12 mmol) at -18 °C. The resulting mixture was stirred at -18 °C. After the reaction is over, 5 mL distilled water was added. The pH was adjusted to 8-9 by the addition of 0.2N hydrochloric acid. The aqueous layer was washed 3 times with CH₂Cl₂(30 mL). The organic layer was collected and dried over anhydrous Na₂SO₄. The solvent was removed

under reduced pressure and purified by flash column chromatography (eluted using chloroform). **2e** was obtained as a white solid with an 83% yield, m.p. 160-163 °C, $[\alpha]_D^{20} = +40.1$ (c=0.5, CHCl₃); ¹H NMR (400 MHz, DMSO) δ 10.82 (s, 1H), 8.28 (t, *J* = 5.7 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.43 – 7.32 (m, 3H), 7.17 (d, *J* = 2.1 Hz, 1H), 7.08 (dd, *J* = 11.0, 4.0 Hz, 1H), 6.99 (dd, *J* = 11.0, 3.8 Hz, 1H), 4.03 (d, *J* = 1.7 Hz, 1H), 3.57 (d, *J* = 1.9 Hz, 1H), 3.41 (dd, *J* = 13.4, 7.2 Hz, 2H), 2.88 (t, *J* = 7.4 Hz, 2H); IR (KBr, cm⁻¹): 3313, 1639, 1551, 1431, 1092, 1012, 742, 614.

(2S,3R)-N-(2-(1*H*-indol-3-yl)ethyl)-3-(4-bromophenyl)oxirane-2-carboxamide (**2f**)

To a solution of tryptamine (1.6 g, 10 mmol) in CH₃OH (40 mL) was added MeONa (0.54 g, 10 mmol) and compound **1f** (3.08 g, 12 mmol) at -18 °C. The resulting mixture was stirred at -18 °C. After the reaction is over, 5 mL distilled water was added. The pH was adjusted to 8-9 by the addition of 0.2N hydrochloric acid. The aqueous layer was washed 3 times with CH₂Cl₂ (30 mL). The organic layer was collected and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (eluted using chloroform). **2f** was obtained as a white solid with an 89% yield, m.p. 177-179 °C, $[\alpha]_D^{20} = +55.3$ (c=0.5, CHCl₃); ¹H NMR (400 MHz, DMSO) δ 10.82 (s, 1H), 8.28 (t, *J* = 5.6 Hz, 1H), 7.57 (dd, *J* = 13.6, 8.1 Hz, 3H), 7.32 (dd, *J* = 18.6, 8.2 Hz, 3H), 7.17 (d, *J* = 1.7 Hz, 1H), 7.07 (t, *J* = 7.4 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 4.02 (d, *J* = 1.4 Hz, 1H), 3.56 (d, *J* = 1.7 Hz, 1H), 3.41 (dd, *J* = 13.4, 7.0 Hz, 2H), 2.87 (t, *J* = 7.3 Hz, 2H); IR (KBr, cm⁻¹): 3313, 1638, 1551, 1458, 1431, 1092, 742, 614.

(2S,3R)-N-(2-(1*H*-indol-3-yl)ethyl)-3-(3-chlorophenyl)oxirane-2-carboxamide (**2g**)

To a solution of tryptamine (1.6 g, 10 mmol) in CH₃OH (40 mL) was added MeONa (0.54 g, 10 mmol) and compound **1g** (2.55 g, 12 mmol) at -18 °C. The resulting mixture was stirred at -18 °C. After the reaction is over, 5 mL distilled water was added. The pH was adjusted to 8-9 by the addition of 0.2N hydrochloric acid. The aqueous layer was washed 3 times with CH₂Cl₂ (30 mL). The organic layer was collected and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (eluted using chloroform).

2g was obtained as a white solid with an 88% yield, m.p. 159-153 °C, $[\alpha]_D^{20} = +34.1$ (c=0.5, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.17 (s, 1H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.33 - 7.29 (m, 1H), 7.27 (d, *J* = 7.6 Hz, 1H), 7.24 (t, *J* = 6.6 Hz, 1H), 7.16 (q, *J* = 6.7 Hz, 2H), 7.10 - 7.04 (m, 2H), 6.28 (s, 1H), 3.70 - 3.61 (m, 2H), 3.60 (d, *J* = 1.9 Hz, 1H), 3.43 (d, *J* = 1.9 Hz, 1H), 3.11 - 2.95 (m, 2H); IR (KBr, cm⁻¹): 3313, 1638, 1556, 1458, 1430, 744, 617.

(2S,3R)-N-(2-(1*H*-indol-3-yl)ethyl)-3-phenyloxirane-2-carboxamide (**2h**)

To a solution of tryptamine (1.6 g, 10 mmol) in CH₃OH (40 mL) was added MeONa (0.54 g, 10 mmol) and compound **1h** (2.14 g, 12 mmol) at -18 °C. The resulting mixture was stirred at -18 °C. After the reaction is over, 5 mL distilled water was added. The pH was adjusted to 8-9 by the addition of 0.2N hydrochloric acid. The aqueous layer was washed 3 times with CH₂Cl₂(30 mL). The organic layer was collected and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (eluted using chloroform). **2h** was obtained as a white solid with an 88% yield, m.p. 131-133 °C, $[\alpha]_D^{20} = +33.1$ (0.5, CHCl₃), [lit^[1]: m.p. 125-127, $[\alpha]_D^{20} = +30$ (c=0.5, CHCl₃)]; ¹H NMR (500 MHz, CDCl₃) δ 8.11 (s, 1H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.37 - 7.30 (m, 3H), 7.23 (dd, *J* = 11.2, 4.0 Hz, 1H), 7.20 (dd, *J* = 6.5, 3.1 Hz, 2H), 7.16 (t, *J* = 7.4 Hz, 1H), 7.08 (d, *J* = 2.2 Hz, 1H), 6.31 (s, 1H), 3.66 (dt, *J* = 13.0, 4.2 Hz, 3H), 3.49 (d, *J* = 2.0 Hz, 1H), 3.10 - 2.96 (m, 2H); IR (KBr, cm⁻¹): 3310, 1638, 1556, 1458, 1432, 743, 616.

(2S,3R)-N-(2-(1*H*-indol-3-yl)ethyl)-3-(4-nitrophenyl)oxirane-2-carboxamide (**2i**)

To a solution of tryptamine (1.6 g, 10 mmol) in CH₃OH (40 mL) was added MeONa (0.54 g, 10 mmol) and compound **1i** (2.55 g, 12 mmol) at -18 °C. The resulting mixture was stirred at -18 °C. After the reaction is over, 5 mL distilled water was added. The pH was adjusted to 8-9 by the addition of 0.2N hydrochloric acid. The aqueous layer was washed 3 times with CH₂Cl₂(30 mL). The organic layer was collected and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (eluted using chloroform). **2i** was obtained as a pale yellow solid with an 81% yield, m.p. 167-169 °C, $[\alpha]_D^{20} = +73.2$ (c=0.5, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.23 - 8.21 (m, 1H), 8.21 - 8.19 (m, 1H), 8.11 (s, 1H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.41 (d, *J* = 8.1 Hz, 1H), 7.38 - 7.32 (m, 2H), 7.24 (dd, *J* = 8.1, 1.1 Hz,

1H), 7.20 – 7.14 (m, 1H), 7.09 (d, $J = 2.3$ Hz, 1H), 6.26 (s, 1H), 3.76 – 3.59 (m, 3H), 3.44 (d, $J = 1.9$ Hz, 1H), 3.14 – 2.95 (m, 2H); IR (KBr, cm^{-1}): 3310, 1639, 1522, 1348, 748, 612.

(5S,6R)-6-(3-fluorophenyl)-5-hydroxy-1,2,3,5,6,7-hexahydro-4*H*-azocino[5,4-*b*]indol-4-one (**3a**)

To a solution of **2a** (0.71 g, 2.19 mmol) in THF (30 mL) was added $\text{Yb}(\text{CF}_3\text{SO}_3)_3$ (271.56 mg, 0.438 mmol) at room temperature. The reaction was monitored by thin-layer chromatography (TLC). After the reaction is over, the solvent was removed under reduced pressure and the residue was added to CH_2Cl_2 (50 mL). The organic layer was washed 3 times using brine (10 mL) and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure and purified by flash column chromatography (ethyl acetate/petrol ether = 1/1). **3a** was obtained as a white powder with 76% yield, m.p. 221-223 °C; $[\alpha]_{\text{D}}^{16} = +6.2$ ($c=0.5$, MeOH); ^1H NMR (500 MHz, DMSO) δ 10.90 (s, 1H), 7.51 (d, $J = 7.9$ Hz, 1H), 7.35 – 7.28 (m, 2H), 7.21 – 7.14 (m, 3H), 7.05 – 6.94 (m, 3H), 5.14 (d, $J = 9.4$ Hz, 1H), 4.89 (t, $J = 9.8$ Hz, 1H), 4.27 – 4.17 (m, 1H), 3.66 (dd, $J = 16.8, 3.9$ Hz, 1H), 3.33 – 3.15 (m, 3H); IR (KBr, cm^{-1}): 3421, 1637, 1538, 1488, 1452, 1275, 1236, 1114, 789, 696; HRMS (ESI) calculated for $\text{C}_{19}\text{H}_{18}\text{O}_2\text{N}_2\text{F}$ $[\text{M}+\text{H}]^+ = 325.1352$, found = 325.1329. The enantiomeric excess was determined to be 97% by HPLC with a Daicel Chiralcel AD-H column (4.6 mm $\times$ 25 cm) (n-hexane/i-PrOH = 40/10, $\lambda = 254$ nm, 0.8 mL/min), $t_{\text{minor}} = 31.5$ min, $t_{\text{major}} = 35.1$ min.

(5S,6R)-6-(4-fluorophenyl)-5-hydroxy-1,2,3,5,6,7-hexahydro-4*H*-azocino[5,4-*b*]indol-4-one (**3b**)

To a solution of **2b** (0.71 g, 2.19 mmol) in THF (30 mL) was added $\text{Yb}(\text{CF}_3\text{SO}_3)_3$ (271.56 mg, 0.438 mmol) at room temperature. The reaction was monitored by thin-layer chromatography (TLC). After the reaction is over, the solvent was removed under reduced pressure and the residue was added to CH_2Cl_2 (50 mL). The organic layer was washed 3 times using brine (10 mL) and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure and purified by flash column chromatography (ethyl acetate/petrol ether = 1/1). **3b** was obtained as a white powder with 70% yield, m.p. 225-229 °C; $[\alpha]_{\text{D}}^{16} = +18.2$ ($c=0.5$, MeOH); ^1H NMR (500 MHz, DMSO) δ 10.86 (s, 1H), 7.51 (d, $J = 7.8$ Hz, 1H), 7.36 – 7.32 (m, 2H), 7.32 – 7.28 (m, 1H), 7.18 (d, $J = 7.9$ Hz, 1H), 7.10 (dd, $J = 12.4, 5.4$ Hz, 2H), 6.98 (dtd, $J = 14.8, 7.0, 1.1$ Hz, 2H), 5.10 (d, $J = 9.3$ Hz, 1H), 4.82 (t, $J = 9.8$ Hz, 1H), 4.19 (d, $J = 10.2$ Hz, 1H), 3.65 (t, $J = 10.2$ Hz, 1H), 3.32 –

3.15 (m, 3H); IR (KBr, cm^{-1}): 3492, 1634, 1506, 1116, 1088, 879, 745, 670; HRMS (ESI) calculated for $\text{C}_{19}\text{H}_{18}\text{O}_2\text{N}_2\text{F}$ $[\text{M}+\text{H}]^+ = 325.1352$, found = 325.1333; The enantiomeric excess was determined to be >99% by HPLC with a Daicel chiralcel AD-H column (4.6 mm $\times$ 25 cm)(n-hexane/i-PrOH = 40/10, $\lambda = 254\text{nm}$, 0.8 ml/min), $t_{\text{minor}} = 32.0\text{ min}$, $t_{\text{major}} = 37.3\text{ min}$.

(5S,6R)-5-hydroxy-6-(4-(trifluoromethyl)phenyl)-1,2,3,5,6,7-hexahydro-4*H*-azocino[5,4-*b*]indol-4-one (**3c**)

To a solution of **2c** (0.82 g, 2.19 mmol) in THF (30 mL) was added $\text{Yb}(\text{CF}_3\text{SO}_3)_3$ (271.56 mg, 0.438 mmol) at room temperature. The reaction was monitored by thin-layer chromatography (TLC). After the reaction is over, the solvent was removed under reduced pressure and the residue was added to CH_2Cl_2 (50 mL). The organic layer was washed 3 times using brine (10 mL) and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure and purified by flash column chromatography (ethyl acetate/petrol ether = 1/1). **3c** was obtained as a white powder with 65% yield, m.p. 229-332 $^\circ\text{C}$; $[\alpha]_{\text{D}}^{16} = +13.8$ ($c=0.5$, MeOH); ^1H NMR (500 MHz, DMSO) δ 10.92 (s, 1H), 7.69 – 7.63 (m, 2H), 7.54 (dd, $J = 13.1, 8.0\text{ Hz}$, 3H), 7.36 (d, $J = 6.6\text{ Hz}$, 1H), 7.19 (d, $J = 7.9\text{ Hz}$, 1H), 7.05 – 6.94 (m, 2H), 5.22 (d, $J = 9.3\text{ Hz}$, 1H), 4.90 (t, $J = 9.8\text{ Hz}$, 1H), 4.31 (d, $J = 10.2\text{ Hz}$, 1H), 3.72 – 3.63 (m, 1H), 3.33 – 3.15 (m, 3H); IR (KBr, cm^{-1}): 3436, 1647, 1342, 1122, 802, 746, 705; HRMS (ESI) calculated for $\text{C}_{20}\text{H}_{18}\text{O}_2\text{N}_2\text{F}_3$ $[\text{M}+\text{H}]^+ = 375.1320$, found = 375.1339; The enantiomeric excess was determined to be 96% by HPLC with a Daicel chiralcel AD-H column (4.6 mm $\times$ 25 cm)(n-hexane/i-PrOH = 40/10, $\lambda = 254\text{nm}$, 0.8 ml/min), $t_{\text{minor}} = 20.4\text{ min}$, $t_{\text{major}} = 21.4\text{ min}$.

(5S,6R)-5-hydroxy-6-(2-nitrophenyl)-1,2,3,5,6,7-hexahydro-4*H*-azocino[5,4-*b*]indol-4-one (**3d**)

To a solution of **2d** (0.77 g, 2.19 mmol) in THF (30 mL) was added $\text{Yb}(\text{CF}_3\text{SO}_3)_3$ (271.56 mg, 0.438 mmol) at room temperature. The reaction was monitored by thin-layer chromatography (TLC). After the reaction is over, the solvent was removed under reduced pressure and the residue was added to CH_2Cl_2 (50 mL). The organic layer was washed 3 times using brine (10 mL) and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure and purified by flash column chromatography (ethyl acetate/petrol ether = 1/1). **3d** was obtained as a yellow

powder with 60% yield, m.p. 221-223 °C; $[\alpha]_D^{16} = +41.7$ (c=0.5, MeOH); ^1H NMR (500 MHz, DMSO) δ 10.93 (s, 1H), 8.20 (s, 1H), 8.09 (d, $J = 7.6$ Hz, 1H), 7.78 (d, $J = 7.5$ Hz, 1H), 7.60 (t, $J = 8.1$ Hz, 1H), 7.54 (d, $J = 7.5$ Hz, 1H), 7.33 (s, 1H), 7.20 (d, $J = 7.7$ Hz, 1H), 7.00 (dd, $J = 18.8, 7.1$ Hz, 2H), 5.24 (s, 1H), 4.92 (t, $J = 9.5$ Hz, 1H), 4.38 (d, $J = 10.4$ Hz, 1H), 3.69 (s, 1H), 3.40 – 3.16 (m, 3H); IR (KBr, cm^{-1}): 3293, 1650, 1528, 1436, 1346, 1113, 744, 696; HRMS (ESI) calculated for $\text{C}_{19}\text{H}_{18}\text{N}_3\text{O}_4[\text{M}+\text{H}]^+ = 352.1297$, found = 352.1313; The enantiomeric excess was determined to be >99% by HPLC with a Daicel chiralcel AD-H column (4.6 mm $\times$ 25 cm)(n-hexane/i-PrOH = 40/10, $\lambda = 254\text{nm}$, 0.8 ml/min), $t_{\text{minor}} = 30.6$ min, $t_{\text{major}} = 55.9$ min.

(5S,6R)-6-(4-chlorophenyl)-5-hydroxy-1,2,3,5,6,7-hexahydro-4*H*-azocino[5,4-*b*]indol-4-one (**3e**)

To a solution of **2e** (0.75 g, 2.19 mmol) in THF (30 mL) was added $\text{Yb}(\text{CF}_3\text{SO}_3)_3$ (271.56 mg, 0.438 mmol) at room temperature. The reaction was monitored by thin-layer chromatography (TLC). After the reaction is over, the solvent was removed under reduced pressure and the residue was added to CH_2Cl_2 (50 mL). The organic layer was washed 3 times using brine (10 mL) and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure and purified by flash column chromatography (ethyl acetate/petrol ether = 1/1). **3e** was obtained as a white powder with 75% yield, m.p. 112-116 °C; $[\alpha]_D^{16} = +29.0$ (c=0.5, MeOH); ^1H NMR (500 MHz, DMSO) δ 10.88 (s, 1H), 7.51 (d, $J = 7.8$ Hz, 1H), 7.34 (s, 4H), 7.33 – 7.30 (m, 1H), 7.18 (d, $J = 7.9$ Hz, 1H), 6.98 (dtd, $J = 14.8, 7.1, 1.1$ Hz, 2H), 5.14 (d, $J = 9.3$ Hz, 1H), 4.83 (t, $J = 9.8$ Hz, 1H), 4.21 (dd, $J = 15.3, 8.5$ Hz, 1H), 3.65 (t, $J = 10.0$ Hz, 1H), 3.31 – 3.16 (m, 3H); IR (KBr, cm^{-1}): 3366, 2956, 1638, 1538, 1491, 1458, 1413, 1091, 1036, 922, 814, 744; HRMS (ESI) calculated for $\text{C}_{19}\text{H}_{18}\text{O}_2\text{N}_2\text{Cl} [\text{M}+\text{H}]^+ = 341.1057$, found = 341.1046; The enantiomeric excess was determined to be >99% by HPLC with a Daicel chiralcel AD-H column (4.6 mm $\times$ 25 cm)(n-hexane/i-PrOH = 40/10, $\lambda = 254\text{nm}$, 0.8 ml/min), $t_{\text{minor}} = 31.9$ min, $t_{\text{major}} = 36.9$ min.

(5S,6R)-6-(4-bromophenyl)-5-hydroxy-1,2,3,5,6,7-hexahydro-4*H*-azocino[5,4-*b*]indol-4-one (**3f**)

To a solution of **2f** (0.84 g, 2.19 mmol) in THF (30 mL) was added $\text{Yb}(\text{CF}_3\text{SO}_3)_3$ (271.56 mg, 0.438 mmol) at room temperature. The reaction was monitored by thin-layer chromatography (TLC). After the reaction is over, the solvent was removed under reduced pressure

ure and the residue was added to CH₂Cl₂ (50 mL). The organic layer was washed 3 times using brine (10 mL) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (ethyl acetate/petrol ether = 1/1). **3f** was obtained as a white powder with 72% yield, m.p. 200-203 °C; [α]_D¹⁶ = +30.6 (c=0.5, MeOH); ¹H NMR (500 MHz, DMSO) δ 10.88 (s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.31 (s, 1H), 7.28 (d, *J* = 8.4 Hz, 2H), 7.18 (d, *J* = 7.9 Hz, 1H), 6.98 (dt, *J* = 14.6, 7.1 Hz, 2H), 5.14 (d, *J* = 9.3 Hz, 1H), 4.82 (t, *J* = 9.7 Hz, 1H), 4.18 (d, *J* = 10.1 Hz, 1H), 3.70 – 3.62 (m, 1H), 3.33 – 3.17 (m, 3H); IR (KBr, cm⁻¹): 3408, 1656, 1640, 1196, 1122, 804, 744, 667; HRMS (ESI) calculated for C₁₉H₁₈O₂N₂Br [M+H]⁺ = 385.0552, found = 385.0558; The enantiomeric excess was determined to be 98% by HPLC with a Daicel chiralcel AD-H column (4.6 mm × 25 cm)(n-hexane/i-PrOH = 40/10, λ = 254nm, 0.8 ml/min), *t*_{minor} = 34.9 min, *t*_{major} = 39.6 min.

(5*S*,6*R*)-6-(3-chlorophenyl)-5-hydroxy-1,2,3,5,6,7-hexahydro-4*H*-azocino[5,4-*b*]indol-4-one (**3g**)
To a solution of **2g** (0.75 g, 2.19 mmol) in THF (30 mL) was added Yb(CF₃SO₃)₃ (271.56 mg, 0.438 mmol) at room temperature. The reaction was monitored by thin-layer chromatography (TLC). After the reaction is over, the solvent was removed under reduced pressure and the residue was added to CH₂Cl₂ (50 mL). The organic layer was washed 3 times using brine (10 mL) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (ethyl acetate/petrol ether = 1/1). **3g** was obtained as a white powder with 72% yield, m.p. 202-206 °C; [α]_D¹⁶ = +90 (c=0.5, MeOH); ¹H NMR (500 MHz, DMSO) δ 10.90 (s, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.39 (d, *J* = 1.7 Hz, 1H), 7.33 – 7.26 (m, 4H), 7.19 (d, *J* = 7.9 Hz, 1H), 7.04 – 6.94 (m, 2H), 5.15 (d, *J* = 9.4 Hz, 1H), 4.89 (t, *J* = 9.9 Hz, 1H), 4.20 (d, *J* = 10.3 Hz, 1H), 3.72 – 3.62 (m, 1H), 3.32 – 3.14 (m, 3H); IR (KBr, cm⁻¹): 3425, 1634, 1536, 1430, 1114, 747, 706; HRMS (ESI) calculated for C₁₉H₁₈O₂N₂Cl [M+H]⁺ = 341.1057, found = 341.1057; The enantiomeric excess was determined to be 98% by HPLC with a Daicel chiralcel AD-H column (4.6 mm × 25 cm)(n-hexane/i-PrOH = 40/10, λ = 254nm, 0.8 ml/min), *t*_{minor} = 29.7 min, *t*_{major} = 34.0 min.

(5S,6R)-5-hydroxy-6-phenyl-1,2,3,5,6,7-hexahydro-4*H*-azocino[5,4-*b*]indol-4-one (**3h**)

To a solution of **2h** (0.67 g, 2.19 mmol) in THF (30 mL) was added Yb(CF₃SO₃)₃ (271.56 mg, 0.438 mmol) at room temperature. The reaction was monitored by thin-layer chromatography (TLC). After the reaction is over, the solvent was removed under reduced pressure and the residue was added to CH₂Cl₂ (50 mL). The organic layer was washed 3 times using brine (10 mL) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (ethyl acetate/petrol ether = 1/1). **3h** was obtained as a white powder with 75% yield, m.p. 237-238°C; [α]_D¹⁶ = +3.0 (c=0.5, MeOH); ¹H NMR (400 MHz, DMSO) δ 10.84 (s, 1H), 7.51 (d, *J* = 7.7 Hz, 1H), 7.36 (d, *J* = 7.5 Hz, 2H), 7.31 – 7.17 (m, 5H), 7.04 – 6.92 (m, 2H), 5.07 – 4.97 (m, 1H), 4.88 (d, *J* = 9.9 Hz, 1H), 4.20 (d, *J* = 9.9 Hz, 1H), 3.73 – 3.63 (m, 1H), 3.31 – 3.16 (m, 3H); ¹³C NMR (101 MHz, DMSO) δ 175.9, 141.6, 135.8, 135.3, 128.6, 128.4, 128.0, 126.4, 120.7, 118.2, 117.4, 110.5, 105.6, 70.4, 51.4, 38.9, 23.1; IR (KBr, cm⁻¹): 3402, 1641, 1536, 1457, 1449, 1118, 745, 703; HRMS (ESI) calculated for C₁₉H₁₉O₂N₂ [M+H]⁺ = 307.1447, found = 307.1436; The enantiomeric excess was determined to be >99% by HPLC with a Daicel chiralcel AD-H column (4.6 mm $\times$ 25 cm) (n-hexane/i-PrOH = 40/10, λ = 254nm, 0.8 ml/min), t_{minor} = 40.8 min, t_{major} = 25.9 min.

(5S,6R)-5-hydroxy-6-(4-nitrophenyl)-1,2,3,5,6,7-hexahydro-4*H*-azocino[5,4-*b*]indol-4-one (**3i**)

To a solution of **2i** (0.77 g, 2.19 mmol) in THF (30 mL) was added Yb(CF₃SO₃)₃ (271.56 mg, 0.438 mmol) at room temperature. The reaction was monitored by thin-layer chromatography (TLC). After the reaction is over, the solvent was removed under reduced pressure and the residue was added to CH₂Cl₂ (50 mL). The organic layer was washed 3 times using brine (10 mL) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (ethyl acetate/petrol ether = 1/1). **3i** was obtained as a yellow powder with 62% yield, m.p. 245-247 °C; [α]_D¹⁶ = +46.0 (c=0.5, MeOH); ¹H NMR (400 MHz, DMSO) δ 10.91 (s, 1H), 7.66 (d, *J* = 8.2 Hz, 2H), 7.55 (dd, *J* = 11.3, 8.1 Hz, 3H), 7.35 (s, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.00 (dq, *J* = 20.2, 6.8 Hz, 2H), 5.20 (d, *J* = 9.1 Hz, 1H), 4.91 (t, *J* = 9.7 Hz, 1H), 4.32 (d, *J* = 10.1 Hz, 1H), 3.74 – 3.62 (m, 1H), 3.32 – 3.18 (m, 3H); IR (KBr, cm⁻¹): 3309, 1642, 1522, 1352, 1113, 70; HRMS (ESI) calculated for C₁₉H₁₈N₃O₄ [M+H]⁺ = 352.1297, found = 352.1273; The enantiomeric excess was determined to be 97% by HPLC with a Daicel chiralcel

AD-H column (4.6 mm × 25 cm)(n-hexane/i-PrOH = 40/10, λ = 254nm, 0.8 ml/min), t_{minor} = 33.0 min, t_{major} = 40.1 min.

(5S,6R)-5-hydroxy-3-methyl-6-phenyl-1,2,3,5,6,7-hexahydro-4*H*-azocino[5,4-*b*]indol-4-one (**3j**)

To a solution of **2j** (0.70 g, 2.19 mmol) in THF (30 mL) was added Yb(CF₃SO₃)₃ (271.5 mg, 0.438 mmol) at room temperature. The reaction was monitored by thin-layer chromatography (TLC). After the reaction is over, the solvent was removed under reduced pressure and the residue was added to CH₂Cl₂ (50 mL). The organic layer was washed 3 times using brine (10 mL) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and purified by flash column chromatography (ethyl acetate/petrol ether = 1/1). **3j** was obtained as a white powder with 50% yield over two steps, m.p. 64–66 °C; $[\alpha]_{\text{D}}^{16}$ = +4 (c=0.5, MeOH); ¹H NMR (500 MHz, DMSO) δ 10.77 (s, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.34 – 7.27 (m, 4H), 7.23 – 7.16 (m, 2H), 7.03 – 6.93 (m, 2H), 5.22 (d, J = 8.3 Hz, 1H), 4.98 (t, J = 8.5 Hz, 1H), 4.27 (d, J = 8.6 Hz, 1H), 4.12 (dt, J = 15.1, 7.7 Hz, 1H), 3.57 – 3.48 (m, 1H), 3.43 (dt, J = 16.0, 8.0 Hz, 1H), 3.04 (ddd, J = 16.0, 7.3, 4.6 Hz, 1H), 2.59 (s, 3H); ¹³C NMR (126 MHz, DMSO) δ 174.3, 142.7, 135.5, 134.8, 129.6, 128.7, 128.6, 127.0, 121.3, 118.8, 117.9, 111.0, 105.6, 79.7, 52.6, 46.4, 34.5, 22.5; IR (KBr, cm⁻¹): 3455, 3245, 2361, 1651, 1624, 1496, 1459, 1072, 738, 699, 504; HRM S (ESI) calculated for C₂₀H₂₁O₂N₂ [M+H]⁺ = 321.1603; found: 321.1600; The enantiomeric excess was determined to be >99% by HPLC with a Daicel chiralcel AD-H column (4.6 mm × 25 cm)(n-hexane/i-PrOH = 40/10, λ = 254nm, 0.8 ml/min), t_{major} = 18.5 min, t_{minor} = 33.2 min.

Table S1. The neuroprotective effects of target compounds on nutrient deprivation (-B27)-induced neurotoxicity in primary rat cerebellar granule neuronal cells (CGCs)^a

Compound	Percent of Control (0.1 μ M)	Percent of Control (1 μ M)	Percent of Control (10 μ M)
3a	40.0 $\pm$ 1.7	46.2 $\pm$ 1.6	42.3 $\pm$ 1.2
3b	37.3 $\pm$ 0.8	45.6 $\pm$ 4.2	45.7 $\pm$ 1.1
3c	38.2 $\pm$ 3.4	41.8 $\pm$ 0.6	43.3 $\pm$ 3.9
3d	38.4 $\pm$ 1.3	48.3 $\pm$ 4.3	47.0 $\pm$ 2.6
3e	37.4 $\pm$ 1.5	49.2 $\pm$ 3.1	40.9 $\pm$ 2.5
3f	37.8 $\pm$ 1.1	48.8 $\pm$ 3.5	44.3 $\pm$ 0.9
3g	38.3 $\pm$ 0.8	48.6 $\pm$ 3.3	39.6 $\pm$ 2.2
3h	38.3 $\pm$ 1.3	51.7 $\pm$ 1.9	42.4 $\pm$ 3.4
3i	35.9 $\pm$ 3.9	49.0 $\pm$ 3.8	42.5 $\pm$ 2.5
3j	39.4 $\pm$ 3.9	40.9 $\pm$ 2.6	39.7 $\pm$ 1.5

^a The cell viability of the control (DMSO-treated) group was considered as 100%, and the average value of cell viability under nutrient deprivation (-B27) was 38.8 $\pm$ 1.4%. Cell viability was evaluated by the MTT assay.

Table S2. The neuroprotective effects of target compounds on glutamate-induced neurotoxicity in primary rat cerebellar granule neuronal cells (CGCs) ^a

Compound	Percent of Control (1 μ M)	Percent of Control (10 μ M)
3a	44.3 $\pm$ 1.6	45.8 $\pm$ 0.9
3b	45.2 $\pm$ 2.3	49.8 $\pm$ 0.2
3c	45.2 $\pm$ 1.1	42.6 $\pm$ 0.5
3d	43.5 $\pm$ 2.5	47.4 $\pm$ 2.5
3e	42.8 $\pm$ 2.1	41.9 $\pm$ 2.0
3f	41.1 $\pm$ 1.3	44.9 $\pm$ 2.3
3g	47.0 $\pm$ 1.2	41.5 $\pm$ 1.4
3h	47.2 $\pm$ 2.9	45.4 $\pm$ 3.6
3i	46.8 $\pm$ 2.8	47.2 $\pm$ 4.2
3j	52.2 $\pm$ 2.8	54.5 $\pm$ 2.3

^aThe cell viability of the control group was considered as 100%, and the average cell viability under glutamate (Glu) exposure was 48.1%. Cell viability was evaluated by the MTT assay.

Table S3. The neuroprotective effects of target compounds on hydrogen dioxide (H₂O₂)-induced neurotoxicity in PC12 neuronal cells ^a

Compound	Percent of Control (0.1 μ M)	Percent of Control (1 μ M)	Percent of Control (10 μ M)
3a	71.0 $\pm$ 6.8	61.5 $\pm$ 1.3	65.2 $\pm$ 0.7
3b	68.6 $\pm$ 2.5	60.2 $\pm$ 2.3	61.0 $\pm$ 1.7
3c	66.9 $\pm$ 2.1	61.3 $\pm$ 2.6	65.8 $\pm$ 0.8
3d	68.9 $\pm$ 2.8	61.8 $\pm$ 2.9	63.8 $\pm$ 2.0
3e	67.0 $\pm$ 2.1	65.4 $\pm$ 3.6	64.9 $\pm$ 4.2
3f	68.6 $\pm$ 1.5	61.0 $\pm$ 3.2	60.8 $\pm$ 3.8
3g	68.0 $\pm$ 2.3	63.4 $\pm$ 3.9	66.6 $\pm$ 3.2
3h	71.7 $\pm$ 4.2	66.8 $\pm$ 1.6	67.0 $\pm$ 1.2
3i	66.5 $\pm$ 2.3	62.4 $\pm$ 4.3	61.5 $\pm$ 0.9
3j	63.9 $\pm$ 3.0	60.7 $\pm$ 4.7	61.6 $\pm$ 1.8

^aThe cell viability of the control (DMSO-treated) group was considered as 100%, and the average cell viability under H₂O₂ exposure was 61.9 $\pm$ 3.8%. Cell viability was evaluated by the MTT assay.

Table S4. Effects of target compounds on LPS-induced TNF α release in BV-2 microglial cells ^a

Compound	Percent of LPS (1 μ M)	Percent of LPS (10 μ M)
3a	102.0 $\pm$ 7.7	51.4 $\pm$ 5.3
3b	96.3 $\pm$ 7.5	45.9 $\pm$ 3.4
3c	72.8 $\pm$ 5.4	26.9 $\pm$ 3.6
3d	108.0 $\pm$ 6.9	36.5 $\pm$ 3.7
3e	105.9 $\pm$ 7.6	58.0 $\pm$ 5.1
3f	94.5 $\pm$ 7.7	65.0 $\pm$ 6.3
3g	112.4 $\pm$ 11.8	97.1 $\pm$ 8.6
3h	104.5 $\pm$ 6.1	55.5 $\pm$ 5.6
3i	90.9 $\pm$ 5.3	63.6 $\pm$ 6.5
3j	105.9 $\pm$ 6.3	52.3 $\pm$ 5.2

^a BV-2 cells were incubated with 100 ng/mL LPS alone or after 2 h pre-incubation with different doses of the test compounds. The cumulative release of pro-inflammatory cytokine TNF α 6 h after LPS addition was determined by ELISA. The LPS-induced response was considered as 100%. Results are presented as mean $\pm$ SD and are expressed as a percentage of the LPS-induced response.

Figure S1 Compound **3c** dose-dependently inhibits LPS-induced TNF α mRNA expression in BV-2 microglial cells. *** P < 0.001 compared to Vehicle group; # P < 0.05, ## P < 0.01, ### P < 0.001, compared to LPS group.

Figure S2 Cytotoxicity of the test compounds in BV-2 microglial cells. Cells were treated with different concentrations of the test compounds for 24 h and cell viability was determined by the MTT assay. Data are expressed as mean $\pm$ SD.

Figure S3 ^1H NMR Spectra of compound **1a**

¹H NMR spectrum (CDCl₃) of compound 10. The spectrum shows a multiplet at 7.4-7.6 ppm (integration 1.00), a multiplet at 4.1-4.3 ppm (integration 1.00), a multiplet at 3.4-3.6 ppm (integration 1.00), a singlet at 1.9 ppm (integration 3.00), and a multiplet at 1.1-1.3 ppm (integration 3.00). A solvent peak for CDCl₃ is visible at 7.26 ppm.

[illegible][illegible]

¹H NMR spectrum (CDCl₃) of compound 10. The x-axis represents the chemical shift (δ) in ppm, ranging from 0.0 to 8.5. The spectrum shows several peaks: aromatic protons between 7.0 and 7.5 ppm, a broad peak around 4.0 ppm, a sharp singlet at approximately 3.7 ppm, and a cluster of peaks between 0.5 and 2.0 ppm. Integration values are shown below the baseline.

¹H NMR spectrum (CDCl₃) of 1,4-dichlorobenzene. The spectrum shows a doublet at 7.26 ppm (4H), a singlet at 4.26 ppm (2H), and a singlet at 3.26 ppm (2H). Integration values are 1.00, 1.00, and 0.99 respectively. The x-axis is labeled f1 (ppm) from 10.0 to 0.0.

Figure S12 H NMR Spectra of compound **2a**

Figure S13 H NMR Spectra of compound **2b**

Figure S14 H NMR Spectra of compound **2c**

Figure S15 H NMR Spectra of compound **2d**

[illegible]

¹H NMR spectrum of compound 1 in CDCl₃. The spectrum shows peaks at 11.02 (s, 1H), 8.28 (s, 1H), 7.33 (d, 2H), 7.29 (d, 2H), 7.17 (d, 2H), 3.94 (s, 2H), 3.42 (s, 2H), 3.33 (s, 2H), 3.19 (s, 2H), 2.67 (s, 2H), 2.56 (s, 2H), 2.33 (s, 2H), 1.73 (s, 2H), 1.48 (s, 2H), and 0.00 (s, 3H). Integration values are shown below the peaks.

Figure S18 H NMR Spectra of compound **2g**

Figure S19 H NMR Spectra of compound **2h**

Figure S20 H NMR Spectra of compound **2i**

Figure S21 H NMR Spectra of compound **3a**

Figure S22 H NMR Spectra of compound **3b**

Figure S23 H NMR Spectra of compound **3c**

Figure S24 H NMR Spectra of compound **3c**

Figure S25 H NMR Spectra of compound **3d**

Figure S26 H NMR Spectra of compound **3e**

Figure S27 H NMR Spectra of compound **3f**

Figure S28 H NMR Spectra of compound **3g**

Figure S29 H NMR Spectra of compound **3h**

Figure S30 ¹H NMR Spectra of compound **3i**

Figure S31 ¹H NMR Spectra of compound **3j**

Figure S32 ¹³C NMR Spectra of compound **3h**

Figure S33 ¹³C NMR Spectra of compound **3j**

Figure S34 HRMS of compound **3a**

Figure S35 HRMS of compound **3b**

Figure S36 HRMS of compound **3c**

Figure S37 HRMS of compound **3d**

Figure S38 HRMS of compound **3e**

Figure S39 HRMS of compound **3f**

Figure S40 HRMS of compound **3g**

Figure S41 HRMS of compound **3h**

Figure S42 HRMS of compound **3i**

Figure S43 HRMS of compound **3j**

Reference

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