Enantiomeric Isoflavones with neuroprotective activities from the Fruits of *Maclura tricuspidata*

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Seven pairs of enantiomeric isoflavones (1a/1b–7a/7b) were obtained from the ethyl acetate extract of the fruits of *Maclura tricuspidata* (syn. *Cudrania tricuspidata*), and successfully separated by chiral high-pressure liquid chromatography (HPLC). The structures and absolute configurations of the enantiomeric isoflavones were established on the basis of comprehensive spectroscopic analyses and quantum chemical calculation methods. Compounds 1, 1a, and 1b exhibited neuroprotective activities against oxygen-glucose deprivation/reoxygenation (ODG/R)-induced SH-SY5Y cells death with EC\(_{50}\) values of 5.5 \(\mu M\), 4.0 \(\mu M\), and 10.0 \(\mu M\), respectively. Furthermore, 1, 1a, and 1b inhibited OGD/R-induced reactive oxygen species generation in SH-SY5Y cells with IC\(_{50}\) values of 6.9 \(\mu M\), 4.5 \(\mu M\), and 9.5 \(\mu M\), respectively.

*Maclura tricuspidata* (Carr.) Bur. (syn. *Cudrania tricuspidata*) is a perennial plant, which is mainly distributed in the southern part of Korea. It has been used as folk remedies for gastritis, liver damage, and hypertension in Korean traditional medicine. Currently, its fruits are consumed fresh and in juices and jams. Further development as a dietary supplement and functional food ingredient has been actively accomplished in many fields.

According to previous reports, various types of flavonoids, including isoflavones, along with xanthones, are considered as the major bioactive constituents of *M. tricuspidata*, exhibiting antioxidant, antithrombotic, anti-inflammatory, cytotoxic, hepatoprotective, and neuroprotective activities.

Cerebral ischemia, also known as brain ischemia or ischemic stroke, is one of the most common causes of mortality and morbidity, conducing to major negative social and economic consequences. Accordingly, the prevention of this disease is clearly an important public health priority. It occurs as a result of the cerebral blood flow is disrupted, leading to the starvation of oxygen and glucose to the affected area, causing irreversible brain damage. Thus far, knowledge about the mechanisms of ischemic brain damage has increased considerably. In general, during ischemia a variety of pathophysiological mechanisms such as calcium influx, glutamate excitotoxicity, inflammation, mitochondrial dysfunction, and oxidative stress were activated, leading to neuronal cell death.

In present study, seven pairs of enantiomeric isoflavones (1a/1b–7a/7b) were obtained from the ethyl acetate extract of the fruits of *M. tricuspidata*. These enantiomeric isoflavones were further purified by using chiral high-pressure liquid chromatography (HPLC), their structures with absolute configurations were established based on interpretation of their 1D and 2D NMR, and HRESIMS data together with electronic circular dichroism (ECD) calculations. Furthermore, the neuroprotective potentials of the isolated compounds were evaluated.

**Results and Discussion**

Compound 1 was determined as C\(_{25}\)H\(_{26}\)O\(_{7}\) by the HRESIMS [M + H]\(^+\) ion at \(m/z\) 439.1742 (calcd. for C\(_{25}\)H\(_{25}\)O\(_{7}\), 439.1757). The \(^1\)H and \(^13\)C NMR spectra resembled those of cudraisoflavone D (Supplementary S.24, Table 1), except for the appearance of a 3-hydroxy-2,2-dimethylidydropyran group [\(\delta_\text{H} = 3.07\) (1 H, dd, \(J = 16.5, 5.5\) Hz, Ha-1\(''''\)), 2.73 (1 H, dd, \(J = 16.5, 7.5\) Hz, Hb-1\(''''\)), 3.89 (1 H, dd, \(J = 7.5, 5.0\) Hz, H-2\(''''\)), 1.35 (3 H, s, Me-4\(''''\))], and

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1.45 (3 H, s, Me-5′′′) at the C-7 and C-8 positions instead of the furan group, as deduced from the HMBC correlations H-1′′′-C-7 (δ_C 158.3), C-8 (δ_C 154.5), and C-9 (δ_C 154.5). Based on these, compound 1 was established as depicted (Fig. 1) and named cudraisoflavone U.

Initially, due to the positive of its specific rotation [α]_24^D +4.3 (c 0.01, MeOH)) together with the detection of Cotton effects (CE) in the ECD spectrum (Fig. 2), 1 was supposed to be an optically pure compound. Therefore, a modified Mosher’s experiment was carried out to establish the absolute configurations at the C-2′′′ and C-2″′′′ positions. Interestingly, when the (R) and (S)-MTPA esters of 1 were subjected to RP-C18 HPLC, two pairs of diastereomers including (+)-MTPA-1a/(−)-MTPA-1b and (−)-MTPA-1a/(+)-MTPA-1b were observed (Supplementary S.4), suggesting the racemic nature of 1. This suggestion was further confirmed by the detection of two peaks in the chiral HPLC analysis of 1. The enantiomeric separation of 1 by chiral HPLC let to the isolation depicted (Fig. 1) and named cudraisoflavone U.

Table 1. ^1H and ^13C NMR spectroscopic data of compounds 1–7.

| Compound | δ_C (in Hz) | δ_H (in Hz) | J (in Hz) |
|----------|-------------|-------------|-----------|
| 1 (Acetone-d_6) | 123.2 | 13.20 | 13.20, s |
| 2 (Acetone-d_6) | 154.0 | 8.24, s | 154.0, s |
| 3 (Acetone-d_6) | 124.2 | 12.4, s | 124.6, s |
| 4 (Acetone-d_6) | 158.5 | 158.6 | 158.8 |
| 5 (Acetone-d_6) | 110.2 | 110.2 | 109.5 |
| 6 (Acetone-d_6) | 158.3 | 158.3 | 166.2 |
| 7 (Acetone-d_6) | 99.2 | 99.3 | 100.5 |
| 8 (Acetone-d_6) | 154.5 | 154.4 | 156.7 |
| 9 (Acetone-d_6) | 106.2 | 106.2 | 106.9 |
| 10 (Acetone-d_6) | 123.2 | 123.2 | 123.1 |
| 1′′′ | 25.7 | 1.45, s | 25.9 |
| 2′′′′ | 149.2 | 149.2 | 71.6 |
| 3′′′′ | 110.3 | 4.73, brs | 148.9 |
| 4′′′′ | 17.7 | 1.3, s | 4.79, s |
| 5′′′′ | 25.7 | 1.45, s | 17.6 |

1H and 13C NMR spectroscopic data of compounds 1–7.
optimization in density functional methods. The ECD data of the selected conformers were calculated using the time-dependent DFT (TDDFT) method.

As shown in Fig. 2, the calculated ECD spectra for the (2''S,2'''R) and (2''R,2'''S)-isomers were well matched with the experimental spectra of 1a and 1b, respectively, and the simulated spectra for the (2''R,2'''R) and (2''S,2'''S)-isomers were highly consistent with the experimental spectra of 2a and 2b, respectively. Besides, in order to further confirm the results, the additional ECD calculations were carried out using the CAM-B3LYP and WB97XD functionals, which yielded consistent ECD results (Fig. 2). On this basis, the absolute configurations of 1a, 1b, 2a, and 2b were assigned as depicted, which were named as (2''S,2'''R)-cudraisoflavone U, (2''R,2'''S)-cudraisoflavone U, (2''R,2'''R)-cudraisoflavone U, and (2''S,2'''S)-cudraisoflavone U, respectively.

The HRESIMS of compound 3 was indicated the molecular formula of C_{25}H_{26}O_{7} ([M + H]^+).

Figure 1. Structures of enantiomeric isoflavones 1a–7b.
The formula of compound 5 was established as C_{25}H_{26}O_{7} by the HRESIMS ion [M + H]^+ at m/z 439.1740 (calcd. for C_{25}H_{27}O_{7}, 439.1757). The 1D NMR spectra resembled those of cudraisoflavone I (Supplementary S.24). However, they differed in the presence of a 2-(1-hydroxy-1-methylethyl)dihydrofuran group [δ_H 3.20 (2 H, d, J = 8.5 Hz, H-1′′′), 4.76 (1 H, t, J = 8.5 Hz, H-2′′′), 1.18 (3 H, s, Me-4′′′), and 1.15 (3 H, s, Me-5′′′)] at the C-7 and C-8 positions instead of the furan group, confirmed by the HMBC cross-peaks H-1′′′/C-7 (δ_C 162.2), C-8 (δ_C 103.5) and C-9 (δ_C 152.3). Based on these, the structure of compound 5 was determined to be cudraisoflavone W.

The HRESIMS spectra of 6 resulted as the same molecular formula as that of 5. It was a stereoisomer of 5, as elucidated directly from the 1D and 2D NMR spectra. Additionally, no CE curves were detected in the ECD spectra of 5 and 6 (Fig. 4), indicating that these compounds were racemic mixtures, respectively. The racemic nature of 5 and 6 was also confirmed by chiral HPLC analysis. The further purification of 5 and 6 achieved of two pairs of enantiomers 5a (t_R 8.06 min, [α]_{22}D +15.7) and 5b (t_R 10.16 min, [α]_{22}D −11.2) as well as 6a (t_R 12.95 min, [α]_{22}D +12.0) and 6b (t_R 20.36 min, [α]_{22}D −10.7) (Supplementary S.23), respectively.

Similar to the case for 1–4, the experimental ECD spectra of 5a, 5b, 6a, and 6b were highly consistent with the calculated ECD spectra of the (2′′R,2′′′R), (2′′S,2′′′R), (2′′R,2′′′S), and (2′′R,2′′′R)-isomers, respectively (Fig. 4).
Consequently, the absolute configurations of $5a$, $5b$, $6a$, and $6b$ were determined as shown [(2′′$R$,2′′′$S$)-cudraisoflavone $W$, (2′′$S$,2′′′$R$)-cudraisoflavone $W$, (2′′$S$,2′′′$S$)-cudraisoflavone $W$, and (2′′$R$,2′′′$R$)-cudraisoflavone $W$, respectively].

The molecular formula of compound $7$ was $C_{25}H_{24}O_{6}$ (HRESIMS). The $1\text{H}$ and $13\text{C}$ NMR signals closely matched those of $6$. However, they differed in the replacement of a 2-(1-hydroxy-1-methylethyl)dihydrofuran group by a 2,2-dimethylpyran group [$\delta_{\text{H}}$ 6.68 (1H, d, $J = 10.0$ Hz, H-1′′′), 5.73 (1H, d, $J = 10.0$ Hz, H-2′′′), 1.43 (3H, s, Me-4′′′), and 1.45 (3H, s, Me-5′′′)] at the C-7 and C-8 positions, confirmed by the HMBC correlations H-1′′′/C-7 ($\delta_{\text{C}}$ 154.2), C-8 ($\delta_{\text{C}}$ 100.7), and C-9 ($\delta_{\text{C}}$ 151.6). Thus, compound $7$ was determined to be cudraisoflavone $X$.

Compound $7$ was also found to be a racemic mixture due to the presence of two peaks in the chiral HPLC analysis. Further HPLC separation led to the isolation of two enantiomers $7a$ ($t_{R}$ 11.40 min, $[\alpha]_{22}^{D}$ +18.0) and $7b$ ($t_{R}$ 18.58 min, $[\alpha]_{22}^{D}$ −13.2) (Supplementary S.23). $7a$ and $7b$ were assigned as (2′′$R$)-cudraisoflavone $X$ and (2′′$S$)-cudraisoflavone $X$, respectively, based on comparison of the experimental ECD spectral data with those of the (2′′$R$) and (2′′$S$)-isomers (Fig. 5).

The racemic compounds 1–7 were evaluated for neuroprotective activity against oxygen-glucose deprivation/reoxygenation (ODG/R)-induced neuronal cell death in SH-SY5Y cells. Of these, 1 exhibited a significant protective effect with an EC_{50} value of 5.5 $\mu$M (carnosine was used as a positive control, EC_{50} 13.4 $\mu$M) (Table 2)21. The rest of the compounds were inactive (EC_{50}>20 $\mu$M). Accordingly, enantiomers $1a$ and $1b$ were further separately examined for their neuroprotective potential and both were found to attenuate ODG/R-induced neurotoxicity with EC_{50} values of 4.0 $\mu$M and 10.0 $\mu$M, respectively (Table 2).

Moreover, although the causes of neurodegenerative diseases have not been clearly elucidated, many experimental evidences suggested that oxidative stress resulting in the generation of reactive oxygen species (ROS) plays a pivotal role in neurodegenerative diseases16,17,22. Furthermore, recent biological studies indicate that several isoflavones are beneficial for reducing oxidative stress in neurons and protecting against neurodegenerative diseases22–25. Consequently, the inhibitory effect of 1, $1a$, and $1b$ on the ODG/R-induced intracellular oxidation of SH-SY5Y cells was further investigated. The EC_{50} values of 1, $1a$, and $1b$ were 5.5 $\mu$M, 4.0 $\mu$M, and 10.0 $\mu$M, respectively. These data suggest that these compounds have potential for the treatment of neurodegenerative diseases.
were obtained by preparative HPLC (MeOH–H₂O, 60–81%, MeOH in H₂O) of fraction TH3-19-3 (40.5 mg).

Consequently, the isolated compounds from *M. tricuspidata* could be promising candidates for the treatment of cerebral ischemia and more investigations are needed to understand their cellular mechanisms of action in the brain for fully exploring their neuroprotective potential.

### Methods

#### General experimental procedures

IR spectra were recorded on a Varian 640-IR spectrometer. Optical rotation was measured on a JASCO P-2000 polarimeter. UV spectra were recorded on an OPTIZEN POP spectrophotometer. ECD measurements were performed using a JASCO J-1100 spectrometer. 1D and 2D NMR spectra were recorded on a Varian VNMRs 500 MHz system. HRESIMS data were obtained on a Waters Q-TOF micromass spectrometer. Column chromatography (CC) was carried out using Kieselgel 60 silica gel (40–60 μm, 70–230 mesh, Merck) and reverse-phase (RP) C18 silica gel (12 μm, YMC, Kyoto, Japan). The HPLC system consisted of a Varian Prostar 210 system, a YMC/p/sphere ODS-H80 column (10 × 250 mm, 4 μm, YMC Co., Ltd., Kyoto, Japan), along with Chiralpak IA and IB columns (4.6 × 250 mm, 5 μm, Daicel, Osaka, Japan).

#### Plant materials

The collection of fruits of *Maclura tricuspidata* and deposition of voucher specimen (KH1-5-09004) were carried out as previously described.

#### Extraction and Isolation

Fresh fruits of *M. tricuspidata* (10.7 kg) were extracted in 100% MeOH (3 × 10 L) at room temperature over the course of ten days. The extracts were concentrated under vacuum to afford a residue (TH1-1-1, 630.9 g), which was further extracted with n-hexane (48.43 g) and EtOAc (27.8 g).

The EtOAc fraction (TH1-2-2, 27.8 g) was fractionated by silica gel CC using CHCl₃–MeOH (40–60 μm, 70–230 mesh, Merck) and reverse-phase (RP) C₁₈ silica gel (12 μm, YMC, Kyoto, Japan). The HPLC system consisted of a Varian Prostar 210 system, a YMC/p/sphere ODS-H80 column (10 × 250 mm, 4 μm, YMC Co., Ltd., Kyoto, Japan), along with Chiralpak IA and IB columns (4.6 × 250 mm, 5 μm, Daicel, Osaka, Japan).

| Compound | Protective effect against cell death (EC₅₀ μM) | Inhibitory effect against ROS generation (IC₅₀ μM) |
|----------|---------------------------------------------|-------------------------------------------------|
| 1        | 5.5 ± 1.4 †                              | 6.9 ± 1.2 †                                    |
| 1a       | 4.0 ± 1.0 †                              | 4.5 ± 2.5 †                                    |
| 1b       | 10.0 ± 2.1                                | 9.5 ± 3.2                                      |
| 2        | >20                                        | —                                              |
| 3        | >20                                        | —                                              |
| 4        | >20                                        | —                                              |
| 5        | >20                                        | —                                              |
| 6        | >20                                        | —                                              |
| 7        | >20                                        | —                                              |
| Carnosine| 13.4 ± 1.5                                | 14.2 ± 2.3                                     |

Table 2. Neuroprotective and inhibitory of ROS generation activities of isolated compounds. EC₅₀ and IC₅₀ values were determined in a semi-logarithmic graph with 4 different concentrations. *IC₅₀ value not determined. (†p < 0.05, ‡p < 0.01, and §§p < 0.001 versus carnosine, a control compound.)
3 (Chiralpak IA; n-hexane–ethanol, 80:20), and 4 (Chiralpak IB; n-hexane–ethanol, 90:10) by chiral preparative HPLC afforded 1a (1.3 mg, tR 11.14 min), 1b (1.4 mg, tR 14.49 min), 2a (1.6 mg, tR 21.48 min), 2b (1.9 mg, tR 23.52 min), 3a (1.1 mg, tR 14.70 min), 3b (1.4 mg, tR 27.68 min), 4a (1.5 mg, tR 15.13 min), and 4b (1.4 mg, tR 16.36 min), respectively. Purification of fractions TH3-9-2 (24.4 mg) and TH3-9-3 (9.1 mg) via preparative HPLC (MeOH–H2O, 60–85%, MeOH in H2O) yielded the racemic mixture 7 (17.4 mg). The enantiomers 7a (1.6 mg, tR 11.40 min) and 7b (1.6 mg, tR 18.58 min) were obtained by chiral HPLC (Chiralpak IA; n-hexane–ethanol, 85:15). Fraction TH1-74-14 (240.4 mg) was separated into four subfractions TH3-3–1 (TH3-3–4 with a RP-C18 silica gel CC using MeOH–H2O (1:1 to 8:2). Fraction TH3-3–2 (96.2 mg) was separated into the racemic mixtures 5 (14.7 mg) and 6 (7.4 mg) with preparative HPLC (MeOH–H2O, 55–75%). Further purification of mixtures 5 (Chiralpak IA; n-hexane–ethanol, 80:20) and 6 (Chiralpak IA; n-hexane–ethanol, 85:15) by chiral preparative HPLC afforded 5a (1.6 mg, tR 8.06 min), 5b (1.6 mg, tR 10.16 min), 6a (1.8 mg, tR 12.95 min), and 6b (1.1 mg, tR 20.36 min), respectively.

Cudratisflavone U (1): Yellow oil; [α]D +4.3 (c 0.01, MeOH); UV (MeOH) λmax nm (log ε): 213 (4.22), 271 (4.31); IR (ATR) νmax cm⁻¹: 3324 (>OH), 1649 (>C=O); 1H and 13C NMR data see Table 1; HRESIMS m/z 439.1742 [M + H]+ (calcd. for C23H25O7, 439.1757).

1a: [α]D +12.7 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = -10.18 (222), +12.02 (276).
1b: [α]D +27.8 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = +9.06 (221), -9.34 (272).

Epi-cudratisflavone U (2): Yellow oil; [α]D +2.1 (c 0.01, MeOH); UV (MeOH) λmax nm (log ε): 214 (4.33), 271 (4.41); IR (ATR) νmax cm⁻¹: 3324 (>OH), 1648 (>C=O); 1H and 13C NMR data see Table 1; HRESIMS m/z 439.1741 [M + H]+ (calcd. for C23H25O7, 439.1757).

2a: [α]D +26.2 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = -0.82 (244), +0.68 (257), -5.97 (275), +4.75 (297).
2b: [α]D +12.0 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = +0.89 (233), +10.64 (272), -4.58 (298).

Cudratisflavone V (3): Yellow oil; [α]D +2.8 (c 0.01, MeOH); UV (MeOH) λmax nm (log ε): 216 (4.37), 270 (4.48); IR (ATR) νmax cm⁻¹: 3286 (>OH), 1660 (>C=O); 1H and 13C NMR data see Table 1; HRESIMS m/z 439.1753 [M + H]+ (calcd. for C23H25O7, 439.1757).

3a: [α]D +16.2 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = -0.41 (224), +15.28 (264), -1.01 (341).
3b: [α]D +6.2 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = +6.38 (220), -14.90 (268), +0.62 (338).

Epi-Cudratisflavone V (4): Yellow oil; [α]D +5.2 (c 0.01, MeOH); UV (MeOH) λmax nm (log ε): 216 (4.35), 270 (4.48); IR (ATR) νmax cm⁻¹: 3327 (>OH), 1660 (>C=O); 1H and 13C NMR data see Table 1; HRESIMS m/z 439.1754 [M + H]+ (calcd. for C23H25O7, 439.1757).

4a: [α]D +21.5 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = -1.13 (219), +0.70 (228), -0.10 (236), +5.47 (262), +4.01 (276), +4.54 (286), -0.53 (352).
4b: [α]D +22.5 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = +4.90 (215), -9.90 (260), -6.79 (268), -8.27 (277), +0.11 (399).

Cudratisflavone W (5): Yellow oil; [α]D +3.1 (c 0.01, MeOH); UV (MeOH) λmax nm (log ε): 213 (4.30), 263 (4.41); IR (ATR) νmax cm⁻¹: 3281 (>OH), 1639 (>C=O); 1H and 13C NMR data see Table 1; HRESIMS m/z 439.1740 [M + H]+ (calcd. for C23H25O7, 439.1757).

5a: [α]D +15.7 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = +5.81 (216), +1.02 (243), +3.80 (262), +0.14 (296), +0.43 (315), -0.58 (339).
5b: [α]D +11.2 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = -5.30 (218), -6.71 (241), -2.36 (255), -0.38 (297), -0.91 (314), +0.19 (337).

Epi-Cudratisflavone W (6): Yellow oil; [α]D +3.2 (c 0.01, MeOH); UV (MeOH) λmax nm (log ε): 214 (4.30), 263 (4.40); IR (ATR) νmax cm⁻¹: 3365 (>OH), 1640 (>C=O); 1H and 13C NMR data see Table 1; HRESIMS m/z 439.1744 [M + H]+ (calcd. for C23H25O7, 439.1757).

6a: [α]D +12.0 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = +5.23 (221), -0.60 (240), +3.91 (260), -2.95 (296), -1.29 (315), -2.26 (332), +0.48 (368).
6b: [α]D +10.7 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = -0.91 (223), +1.66 (247), -2.05 (265), +4.53 (292), +2.52 (313), +2.97 (328).

Cudratisflavone X (7): Yellow oil; [α]D -4.9 (c 0.01, MeOH); UV (MeOH) λmax nm (log ε): 210 (4.33), 268 (4.64), 344 (3.63); IR (ATR) νmax cm⁻¹: 3318 (>OH), 1630 (>C=O); 1H and 13C NMR data see Table 1; HRESIMS m/z 419.1476 [M – H]- (calcd. for C23H24O6, 419.1495).

7a: [α]D +18.0 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = +2.14 (218), +5.59 (240), -0.70 (263), +1.83 (283), -2.24 (352).
7b: [α]D -13.2 (c 0.04, MeOH); CD (c 0.6 mM, ACN) Δε = +1.33 (209), -0.91 (229), +3.17 (264), -0.28 (294), +2.24 (350).
Computational details. The ECD calculations were performed as previously described with some modifications. The DFT/B3LYP/cc-pTVZ level was employed for optimizing and calculating the relative energies of the initial low-energy conformers. Calculation of the ECD spectra were carried out at the TDDFT/M062X/def2TZVP level. Additional ECD calculations were performed using the CAM-B3LYP and WB97XD functionals in order to further confirm the calculated results.

Measurement of cell viability and intracellular ROS and statistical analysis. The protective effects against ODG/R-induced cell death and intracellular ROS generation in SH-SY5Y cells of test compounds and statistical analysis were carried out as previously described. All experimental data are expressed as the mean value ± standard deviation from three replicates for each experiment. Statistical significance between multiple groups was determined by one-way ANOVA (PRISM Graph Pad, San Diego, CA, USA). When the ANOVA showed a significant difference, Bonferroni’s multiple comparison post hoc test was conducted. P values less than 0.05 were regarded as statistically significant.

References
1. Kang, D. G. et al. Effects of Cudrania tricuspidata water extract on blood pressure and renal functions in NO-dependent hypertensive. Life Sci. 70, 2359–2369 (2002).
2. Geong, J. Y. et al. Optimization of pancreatic lipase inhibition by Cudrania tricuspidata fruits using response surface methodology. Bioorg. Med. Chem. Lett. 24, 2329–2333 (2014).
3. Fujimoto, T., Hano, Y., Nomura, T. & Uzawa, J. Components of root bark of Cudrania tricuspidata 2. Structures of two new isoprenylated flavones, cudraflavones A and B. Planta Med. 50, 161–163 (1984).
4. Fujimoto, T. & Nomura, T. Components of root bark of Cudrania tricuspidata, 3-12 Isolation and structure studies on the flavonoids. Planta Med. 51, 190–193 (1985).
5. Hano, Y. et al. Cudraflavone C and cudraflavone C, two new prenylflavones from the root bark of Cudrania tricuspidata (Carr) bert. Heterocycles. 31, 1339–1344 (1990).
6. Heip, N. T. et al. Isoflavones with neuroprotective activities from fruits of Cudrania tricuspidata. Phytochemistry. 111, 141–148 (2015).
7. Heip, N. T. et al. Neuroprotective constituents from the fruits of Maclura tricuspidata. Tetrahedron. 73, 2747–2759 (2017).
8. Zou, Y. S., Hou, A. J. & Zhu, G. F. Isoprenylated xanthones and flavonoids from Cudrania tricuspidata. Chem. Biodivers. 2, 131–138 (2005).
9. Lee, B. W. et al. Antioxidant and cytotoxic activities of xanthones from Cudrania tricuspidata. Bioorg. Med. Chem. Lett. 15, 5548–5552 (2005).
10. Zou, Y. S. et al. Cytotoxic isoprenylated xanthones from Cudrania tricuspidata. Bioorg. Med. Chem. 12, 1947–1953 (2004).
11. Kwon, J. et al. Chemical constituents isolated from the root bark of Cudrania tricuspidata and their potential neuroprotective effects. J. Nat. Prod. 79, 1938–1951 (2016).
12. Kwon, J. et al. Neuroprotective xanthones from the root bark of Cudrania tricuspidata. J. Nat. Prod. 77, 1893–1901 (2014).
13. Park, K. H. et al. Anti-atherosclerotic and anti-inflammatory activities of catecholic xanthones and flavonoids isolated from Cudrania tricuspidata. Bioorg. Med. Chem. Lett. 16, 5580–5583 (2006).
14. An, R. B., Sohn, D. H. & Kim, Y. G. Hepatoprotective compounds of the roots of Cudrania tricuspidata on tcarin-induced cytotoxicity in Hep G2 cells. Biol. Pharm. Bull. 29, 838–840 (2006).
15. Hori, M. et al. Unraveling the ischemic brain transcriptome in a permanent middle cerebral artery occlusion mouse model by DNA microarray analysis. Dis. Model. Mech. 5, 270–283 (2012).
16. Allen, C. L. & Bayraktutan, U. Oxidative stress and its role in the pathogenesis of ischaemic stroke. Int. J. Stroke. 4, 461–470 (2009).
17. Chen, H. et al. Oxidative stress in ischemic brain damage: Mechanisms of cell death and potential molecular targets for neuroprotection. Antioxid. Redox. Signal. 14, 1505–1517 (2011).
18. Moskowitz, M. A., Lo, E. H. & Iadecola, C. The science of stroke: Mechanisms in search of treatments. Neuron. 67, 181–198 (2010).
19. Suresh, L. M. & Raghu, V. Mechanisms of Stroke Induced Neuronal Death: Multiple Therapeutic Opportunities. Adv. Anim. Vet. Sci. 2, 438–446 (2014).
20. Ohtani, I., Kusumi, T., Kashman, Y. & Kakisawa, H. High-field FT NMR application of Mosher's method. The absolute configurations of marine terpenoids. J. Am. Chem. Soc. 113, 4092–4096 (1991).
21. Bae, O. N. & Majid, A. Role of histidine/histamine in carnosine-induced neuroprotection during ischemic brain damage. Brain Res. 1527, 246–254 (2013).
22. Gutierrez-Merino, C. et al. Neuroprotective actions of flavonoids. Curr. Med. Chem. 18, 1195–1212 (2011).
23. Vauzour, D. et al. The neuroprotective potential of flavonoids: A multiplicity of effects. Genes Nutr. 3, 115–126 (2008).
24. Inanami, O. et al. Oral administration of (−)-catechin protects against ischemia-reperfusion-induced neuronal death in the gerbil. Free Radic. Res. 39, 359–365 (1998).
25. Liang, H. W. et al. Genistein attenuates oxidative stress and neuronal damage following transient global cerebral ischemia in rats. Neurosci. Lett. 438, 116–120 (2008).
26. Hong, S. et al. The isoflavones and extracts from Maclura tricuspidata fruit protect against neuronal cell death in ischemic injury via induction of Nox4-targeting miRNA-25, miRNA-92a, and miRNA-146a. J. Funct. Foods. 40, 785–797 (2018).
27. Pilskova, L., Rieckyans, I. & Jagla, F. The physiological actions of isoflavone phytoestrogens. Physiol. Res. 59, 651–664 (2010).
28. Rendeiro, C. et al. Optimization of pancreatic lipase inhibition by flavonoid metabolites transported across a human BBB model. Food Chem. 149, 190–196 (2014).
29. Hong, S. et al. Mulberrofuran G protects ischemic injury induced cell death via inhibition of Nox4-mediated ROS generation and ER stress. Phytother. Res. 31, 321–327 (2017).
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Author Contributions
Dongho Lee and Woongchon Mar initiated the project. Nguyen Tuan Hiep, Jaeyoung Kwon, Nahyun Kim, and Sungun Hwang performed the extraction, isolation, structural identification, and biological assays of the compounds. Yuanqiang Guo and Bang Yeon Hwang supported data analysis. Nguyen Tuan Hiep, Dongho Lee, and Woongchon Mar wrote the manuscript. All authors reviewed and confirmed the manuscript.

Additional Information
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