Phytochemistry 111 (2015) 141–148

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Isoflavones with neuroprotective activities from fruits of *Cudrania tricuspidata*

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ARTICLE INFO

Article history:
Received 24 July 2014
Received in revised form 30 September 2014
Available online 6 December 2014

Keywords:
Cudrania tricuspidata
Moraceae
Isoflavones
Neuroprotection
Parkinson's disease
6-OHDA
Human neuroblastoma SH-SY5Y

ABSTRACT

Ten isoflavones, cudraisoflavones B–K (**1**–**10**), together with 27 known isoflavones, were isolated from the EtOAc soluble extract of fruits of *Cudrania tricuspidata*. The structures of compounds **1**–**10** were elucidated on the basis of MS and NMR spectroscopic data, including 2D NMR experiments. Compounds **7**–**9** and three known (**11**–**13**) compounds showed neuroprotective activity against 6-hydroxydopamine induced cell death in human neuroblastoma SH-SY5Y cells, with EC_{50} values of 0.5–9.2 μM.

1. Introduction

Cudrania tricuspidata (Carr.) Bur., a deciduous tree belonging to the family Moraceae, is distributed widely within East Asia, mainly in the southern part of Korea. The cortex and root bark of this plant have been used for the treatment of gonorrhea, hepatitis, inflammation, jaundice and neuritis (Jung and Shin, 1990). Prenylated flavonoids (Fujimoto et al., 1984; Fujimoto and Nomura, 1985; Hano et al., 1990) and xanthones (Lee et al., 2005; Zou et al., 2005, 2004) are the major classes of compounds isolated from *C. tricuspidata*. Previous biological studies have shown that the constituents of this plant are antioxidant (Zou et al., 2005), antiherosclerotic, anti-inflammatory (Park et al., 2006), cytotoxic (Zou et al., 2004), and hepatoprotective (An et al., 2006).

Parkinson's disease is a neurodegenerative disorder characterized by a selective loss of dopaminergic neurons in the nigrostriatal pathway, resulting in a clinical syndrome characterized by stiffness, tremor, slowness of movement, and postural instability. Although the precise mechanism of nigral cell death in Parkinson's disease remains unknown, oxidative stress has been strongly implicated (Wang et al., 2009).

As a part of an ongoing research program for the discovery of neuroprotective compounds from higher plants (Kwon et al., 2014), the EtOAc soluble extract of the fruits of *C. tricuspidata* was found to exhibit significant neuroprotective activity against SH-SY5Y cells death induced by 6-hydroxydopamine (6-OHDA) with an EC_{50} value of 7.7 μg/mL. Ten new isoflavones along with 27 known isoflavones were isolated, and their structures were determined on the basis of MS as well as 1D and 2D NMR spectroscopic data. Described herein are the isolation, structure determination, as well as neuroprotective activity, of these compounds.

Fresh fruits of *C. tricuspidata* were extracted with MeOH, and the dried extract was successively partitioned with *n*-hexane, EtOAc and H_2O. Repeated column chromatography and preparative HPLC of the EtOAc soluble extract resulted in the isolation of ten new isoflavones, cudraisoflavones B–K (**1**–**10**), as well as 27 known isoflavones, 5,7,3′,4′-tetrahydroxy-6,8-diprenylisoflavone (**11**), warangalone, auriculasin (Nkengfack et al., 1989), erythrinin B (**12**) (Jain and Sharma, 1974), gancaonin B (**13**), gancaonin A (Fukai et al., 1990), osajin, euchrenone b_8, euchrenone b_9 (Mizuno et al., 1990), alpinumisoflavone, 4′-*O*-methyl-alpinumisoflavone (Olivares et al., 1982), 5,7,4′-trihydroxy-6,8-diprenylisoflavone, erysenegalensein

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http://dx.doi.org/10.1016/j.phytochem.2014.10.021
0031-9422/

E (Sekine et al., 1999), anagyroidisoflavone A (Sato et al., 1995), euchrenone b_{10}, senegalensin (Tanaka et al., 2001), eryvarin B (Tanaka et al., 2000), 5,7-dihydroxy-6-(2″-hydroxy-3″-methylbut-3″-enyl)-4′-methoxylisoflavone (Han et al., 2009), 3′,5-dihydroxy-4′-methoxyl-2″,2″-dimethylpyrano[6″,5″-*h*]isoflavone, derrone (Tsukayama et al., 1992), 4′-*O*-methyl-2″-hydroxydihydroalpinumisoflavone (Abdel-Kader et al., 2006), isoerysenegalensein E (El-Masry et al., 2002), biochanin A (Klier et al., 2012), lupiwighteone (Tahara et al., 1991), 5,3′,4′-trihydroxy-6″,6″-dimethylpyrano-[2″,3″;7,6]isoflavone (Jain et al., 1978), erythrinin C (Tahara et al., 1985), and flemiphilippinin G (Fu et al., 2012), respectively (see Fig. 1).

2. Results and discussion

Compound **1** had a molecular ion peak at *m*/*z* 363.1215 $[M+H]^+$ (HRESIMS), corresponding to a molecular formula of $C_{22}H_{18}O_5$. Its ^{1}H NMR spectrum had a sharp singlet at δ_H 8.38 (1H, *s*, H-2) and a downfield signal at δ_H 13.73 (1H, *s*, OH-5) that were characteristic of a 5-hydoxyisoflavone skeleton (Tahara et al., 1989). Two sets of doublet resonances at δ_H 7.50 (2H, *d*, *J* = 8.5 Hz, H-2′, 6′) and 6.93 (2H, *d*, *J* = 8.5 Hz, H-3′, 5′) were assigned to a *p*-disubstituted benzene ring (Sekine et al., 1999). A group of signals at δ_H 3.72 (2H, *d*, *J* = 7.0 Hz, H-1‴), 5.35 (1H, *t*, *J* = 7.0 Hz, H-2‴), 1.67 (3H, *s*, Me-4‴), and 1.86 (3H, *s*, Me-5‴) suggested the presence of a 3,3-dimethylallyl (prenyl) group (Oh et al., 1999). Furthermore, there were two doublets at δ_H 7.05 (1H, *d*, *J* = 2.0 Hz, H-1″) and 7.90 (1H, *d*, *J* = 2.0 Hz, H-2″), which were assigned to the two protons of the furan group fused to the aromatic ring this being confirmed by the ^{13}C NMR spectrum that showed C-1″ and C-2″ resonances, respectively, at δ_C 105.0 and 146.5 (Table 1) (Wandji et al., 1995). In the HMBC spectrum, the correlations from H-2 to C-9 (δ_C 152.0), as well as those from H-1‴ to C-7 (δ_C 158.3), C-8 (δ_C 104.8), and C-9, allowed deduction that the position of the prenyl group was at C-8. Besides, the furano group was located at C-6 and C-7 positions on the basis of HMBC correlations between H-1″ and C-5 (δ_C 158.3), C-6 (δ_C 113.2) and C-7. All of the NMR assignments were obtained using ^{1}H–^{1}H COSY, HSQC, and HMBC NMR correlations. Accordingly, compound **1** was elucidated as shown and has been given trivial name cudraisoflavone B (Sun et al., 1988).

Compound **2** exhibited the same molecular formula $C_{22}H_{18}O_5$ as **1**, established by HRESIMS at *m*/*z* 363.1221 $[M+H]^+$. The ^{1}H and ^{13}C NMR spectroscopic data of **2** were very similar with those of **1** (Table 1). In addition, the HMBC spectrum of **2** showed cross-peaks between H-2 at δ_H 8.36 (1H, *s*) and C-9 (δ_C 149.4), H-1‴ at δ_H 7.12 (1H, *d*, *J* = 2.0 Hz) of the furano group and C-7 (δ_C 158.8), C-8 (δ_C 108.6) and C-9, and also between H-1″ at δ_H 3.59 (2H, *d*, *J* = 7.0 Hz) of the prenyl group and C-5 (δ_C156.3), C-6 (δ_C109.1) and C-7; this enabled deduction that the position of the furan group was at the C-7 and C-8 positions, while the prenyl group was attached at the C-6 position. These data indicated clearly that the new compound **2** (cudraisoflavone C) is a regioisomer of cudraisoflavone B (**1**), and was represented by the structural formula **2**.

Compound **3** had a molecular ion $[M-H]^-$ at *m*/*z* 377.1032 (HRESIMS), consistent with the elemental formula of $C_{22}H_{18}O_6$. In its ^{1}H NMR spectrum (Table 1), characteristic proton signals for a 5-hydoxyisoflavone skeleton [δ_H 13.20 (1H, *s*, OH-5) and 8.58 (1H, *s*, H-2)], a 2-hydroxyl-3-methylbut-3-enyl group [δ_H 3.03 (1H, *dd*, *J* = 7.0, 13.0 Hz, Ha-1″), 2.98 (1H, *dd*, *J* = 7.5, 13.0 Hz, Hb-1″), 4.40 (1H, *dt*, *J* = 4.5, 7.0 Hz, H-2″), 4.94 (1H, *d*, *J* = 4.5 Hz, OH-2″), 4.64 (1H, *brs*, Ha-4″), 4.60 (1H, *brs*, Hb-4″), and 1.77 (3H, *s*, Me-5″)] (Oh et al., 1999), and a furan group [δ_H 7.21 (1H, *d*, *J* = 2.0 Hz, H-1‴), 8.06 (1H, *d*, *J* = 2.0 Hz, H-2‴)], were observed. These data suggested that **3** has a 5-hydoxyisoflavone skeleton with a 2-hydroxyl-3-methylbut-3-enyl group and a furan group. The HMBC correlations from OH-5 to C-5 (δ_C 155.2) and C-6 (δ_C 105.9), as well as from H-1″ to C-5, C-6, and C-7 (δ_C 158.0), indicated that the 2-hydroxyl-3-methylbut-3-enyl group was located at the C-6 position. Furthermore, the HMBC correlations from H-1‴ to C-7 and C-8 (δ_C 107.2) supported the concept that the furan group was linked at the C-7 and C-8 positions. Therefore, the structure of new compound **3** (cudraisoflavone D) was unambiguously formulated as shown.

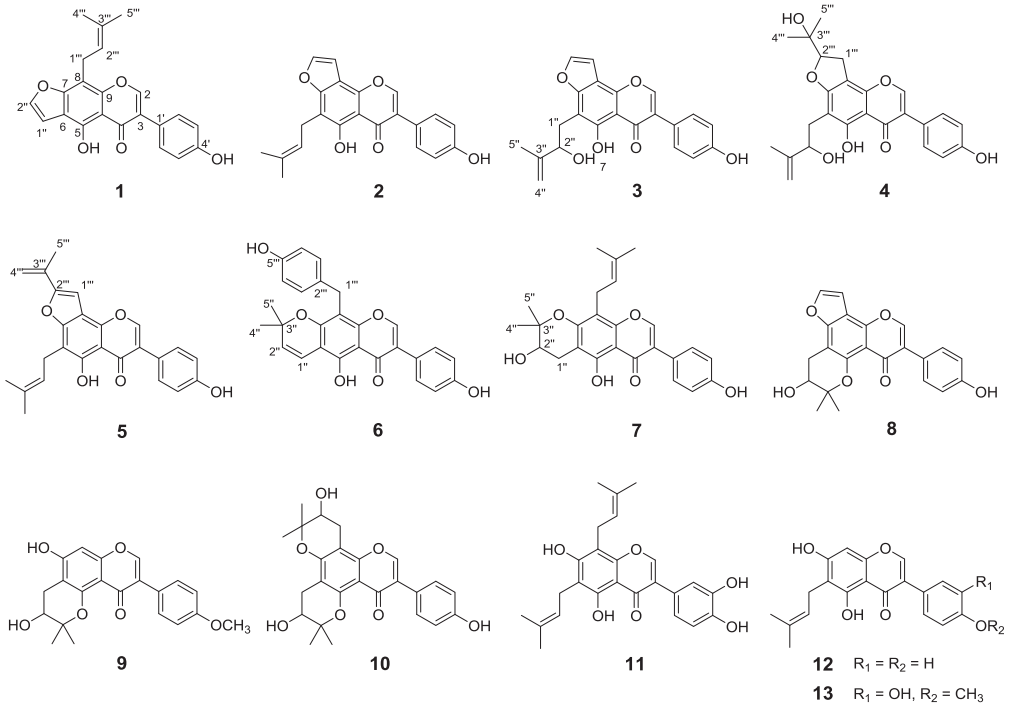

Fig. 1. Structures of compounds **1**–**13**.

Table 1
^{1}H NMR (500 Hz) and ^{13}C NMR (125 Hz) spectroscopic data of compounds **1**–**5**.[a]

	1[b]		**2**[b]		**3**[c]		**4**[c]		**5**[b]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	155.2	8.38, *s*	154.2	8.36, *s*	154.0	8.58, *s*	153.4	8.32, *s*	154.2	8.39, *s*
3	123.0		125.1		123.3		122.1		125.1	
4	183.8		182.7		181.3		180.3		182.6	
5	158.3		156.3		155.2		159.7		156.9	
6	113.2		109.1		105.9		104.3		108.8	
7	158.3		158.8		158.0		165.2		158.4	
8	104.8		108.6		107.2		102.6		110.2	
9	152.0		149.4		147.9		150.3		149.2	
10	107.4		108.8		107.2		104.4		108.8	
OH-5		13.73, *s*		13.20, *s*		13.20, *s*		13.43, *s*		13.31, *s*
1′	123.0		122.9		120.9		121.2		122.9	
2′,6′	131.2	7.50, *d*(8.5)	131.3	7.50, *d*(8.5)	130.3	7.42, *d*(8.5)	130.2	7.36, *d*(8.5)	131.3	7.50, *d*(8.5)
3′,5′	116.0	6.93, *d*(8.5)	116.0	6.93, *d*(8.5)	115.0	6.85, *d*(8.5)	114.9	6.82, *d*(8.5)	116.0	6.93, *d*(8.5)
4′	158.4		158.5		157.5		157.3		158.6	
1″	105.0	7.05, *d*(2.0)	22.5	3.59 (2H), *d*(7.0)	29.6	3.03, *dd* (7.0, 13.0) 2.98, *dd* (7.5, 13.0)	29.2	2.68, *dd* (6.5, 13.5) 2.80, *dd* (7.5, 13.5)	22.5	3.61 (2H), *d*(7.5)
2″	146.5	7.90, *d*(2.0)	122.2	5.36, *t*(7.0)	72.8	4.40, *dt* (4.5, 7.0)	73.0	4.28, *dt* (4.5, 7.0)	122.1	5.38, *t* (7.5)
3″			132.8		147.8		147.7		132.7	
4″			25.8	1.66, *s*	110.1	4.64, 4.60, *brs*	110.2	4.61,4.59, *s*	25.8	1.68, *s*
5″			17.9	1.83, *s*	17.0	1.77, *s*	16.7	1.73, *s*	17.9	1.87, *s*
OH-2″						4.94, *d*(4.5)		4.78, *d*(4.0)		
1‴	22.6	3.72 (2H), *d*(7.0)	104.6	7.12,*d*(2.0)	103.7	7.21, *d*(2.0)	26.3	3.20 (2H), *d*(9.0)	100.7	7.06, *s*
2‴	122.1	5.35, *t*(7.0)	145.8	7.89, *d*(2.0)	145.3	8.06, *d*(2.0)	90.8	4.73, *t* (8.5)	157.2	
3‴	133.2						70.1		133.5	
4‴	25.7	1.67, *s*					24.4	1.16, *s*	113.5	5.80, 5.26, *brs*
5‴	17.9	1.86, *s*					25.8	1.18, *s*	19.2	2.17, *s*

[a] TMS was used as the internal standard; chemical shifts are shown in the δ scale with *J* value (Hz) in parentheses.
[b] Acetone-d_6.
[c] DMSO-d_6.

Compound **4** (cudraisoflavone E) was obtained as a yellow powder, and the ^{1}H and ^{13}C NMR spectroscopic data of **4** were almost the same as those of **3** (Table 1), except for the absence of the furan group and the presence of a 2-(l-hydroxy-l-methylethyl)dihydrofuran group [δ_H 3.20 (2H, *d*, *J* = 9.0 Hz, H-1‴), 4.73 (1H, *t*, *J* = 8.5 Hz, H-2‴), 1.16 (3H, *s*, Me-4‴) and 1.18 (3H, *s*, Me-5‴)] (Sekine et al., 1999), consistent with the molecular formula ($C_{25}H_{26}O_7$; HRESIMS, *m*/*z* 437.1608 [M−H]$^-$) obtained. These observations suggested that **4** contains a 2-hydroxyl-3-methyl-but-3-enyl group at the C-6 position, this being confirmed by HMBC correlations from OH-5 at δ_H 13.43 (1H, *s*) to C-5 (δ_C 159.7) and C-6 (δ_C 104.3), as well as from H-1″ [δ_H 2.68 (1H, *dd*, *J* = 6.5, 13.5 Hz, Ha-1″) and 2.80 (1H, *dd*, *J* = 7.5, 13.5 Hz, Hb-1″)] to C-5, C-6 and C-7 (δ_c 165.2). In addition, the HMBC correlations of H-1‴ to C-7, C-8 (δ_C 102.6) and C-9 (δ_c 150.3) indicated the location of the 2-(l-hydroxy-l-methylethyl)dihydrofuran group was at the C-7 and C-8 positions. The structure of new compound **4** was thus established as shown.

The molecular formula of compound **5** was determined to be $C_{25}H_{22}O_5$ from HRESIMS (*m*/*z* 401.1370 [M−H]$^-$). Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **5** with those of **2** (Table 1) established that the furan group in the latter was replaced by a 2-(l-methylethenyl)furan group [δ_H 7.06 (1H, *s*, H-1‴), 5.80 (1H, *brs*, Ha-4‴), 5.26 (1H, *brs*, Hb-4‴), and 2.17 (3H, *s*, Me-5‴)] in **5** (Khalid and Waterman, 1983). Its HMBC showed cross-peaks between OH-5 at δ_H 13.31 (1H, *s*) and C-5 (δ_C 156.9), C-6 (δ_C 108.8), and between H-1″ at δ_H 3.61 (2H, *d*, *J* = 7.5 Hz) of the prenyl group and C-5, C-6, and C-7 (δ_C 158.4), supporting the attachment of the prenyl group at the C-6 position. Additionally, the HMBC correlations from H-1‴ to C-7, C-8 (δ_C 110.2) enabled deduction that the 2-(l-methylethenyl)furan was located at the C-7 and C-8 positions. The structure of new compound **5** (cudraisoflavone F) was finally elucidated as illustrated.

The HRESIMS spectrum of compound **6** (cudraisoflavone G) had a molecular ion peak at *m*/*z* 443.1488 [M+H]$^+$, corresponding to a molecular formula of $C_{27}H_{22}O_6$. In its ^{1}H NMR spectrum (Table 2), characteristic resonances were observed for a 5-hydroxylisoflavone skeleton [δ_H 13.40 (1H, *s*, OH-5) and 8.26 (1H, *s*, H-2)], a *p*-substituted benzyl group [δ_H 3.96 (2H, *s*, H-1‴), 7.15 (2H, *d*, *J* = 8.5 Hz, H-3‴, 7‴) and 6.72 (2H, *d*, *J* = 8.5 Hz, H-4‴, 6‴)] (Lee et al., 1996), and a 2,2-dimethylpyran group [δ_H 6.69 (1H, *d*, *J* = 10.0 Hz, H-1″), 5.75 (1H, *d*, *J* = 10.0 Hz, H-2″), and 1.42 (6H, *s*, Me-4″, 5″)] (Chen et al., 1991). The 2,2-dimethylpyran was located at the C-6 and C-7 positions, as confirmed by the HMBC correlations from OH-5 to C-5 (δ_C 156.0), C-6 (δ_C 105.9), as well as from H-1″ to C-5, C-6 and C-7 (δ_C 157.6). Besides, the position of a *p*-substituted benzyl group was determined as C-8 according to the HMBC correlations of the benzylic methylene protons at δ_H 3.96 (H-1‴) to C-7, C-8 (δ_C 108.5) and C-9 (δ_C 155.6). Accordingly, the structure of new compound **6** was determined as shown.

Analysis of the HRESIMS spectrum indicated that compound **7** had the molecular formula $C_{25}H_{26}O_6$ (*m*/*z* 423.1790, [M+H]$^+$). Its ^{1}H and ^{13}C NMR spectroscopic data were similar to those of **1** (Tables 1 and 2), except for the presence of the 3-hydroxy-2,2-dimethyldihydropyran group [δ_H 2.97 (1H, *dd*, *J* = 5.0, 17.0 Hz, Ha-1″), 2.63 (1H, *dd*, *J* = 7.5, 17.0 Hz, Hb-1″), 3.88 (1H, *dd*, *J* = 5.5, 7.5 Hz, H-2″), 1.43 (3H, *s*, Me-4″), and 1.33 (3H, *s*, Me-5″)] instead of the furan group as in **1** (Oh et al., 1999). The location of the 3-hydroxy-2,2-dimethyldihydropyran was determined to be at the C-6 and C-7 positions on the basis of HMBC correlations between OH-5 at δ_H 13.29 (1H, *s*) and C-5 (δ_C 158.5), C-6 (δ_C 104.9), and between H-1″ and C-6, C-7 (δ_C 157.7). Besides, the HMBC cross peaks from H-1‴ at δ_H 3.43 (2H, *d*, *J* = 7.5 Hz) of the prenyl group to C-7, C-8 (δ_C 107.6), and C-9 (δ_C 153.9), indicated that the prenyl group was attached to the C-8 position. Thus, the structure of new compound **7** (cudraisoflavone H) was established as shown.

Compound **8** showed a molecular ion peak at *m*/*z* 379.1169 [M+H]$^+$ (HRESIMS), corresponding to a molecular formula of $C_{22}H_{18}O_6$. Its ^{1}H NMR spectroscopic data clearly indicated the presence of a furan group [δ_H 7.17 (1H, *d*, *J* = 2.0 Hz, H-1‴) and 8.02 (1H,

Table 2
^{1}H NMR (500 Hz) and ^{13}C NMR (125 Hz) spectroscopic data of compounds **6**–**10**.[a]

	6[b]		**7**[b]		**8**[c]		**9**[c]		**10**[b]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	154.4	8.26, *s*	154.3	8.25, *s*	150.0	8.24, *s*	150.2	8.03, *s*	150.5	7.98, *s*
3	123.8		123.5		125.6		124.7		126.4	
4	182.1		182.0		174.1		173.4		174.8	
5	156.0		158.5		150.4		154.1		153.2	
6	105.9		104.9		101.5		105.0		106.2	
7	157.6		157.7		155.5		159.8		155.6	
8	108.5		107.6		108.1		93.5	6.40,*s*	100.0	
9	155.6		153.9		149.8		157.1		156.1	
10	106.5		105.6		110.9		107.5		109.9	
OH-5		13.40, *s*		13.29, *s*						
1′	123.0		123.3		122.5		124.2		124.9	
2′,6	131.1	7.47, *d*(8.5)	131.1	7.47, *d*(8.5)	130.3	7.34, *d*(8.5)	130.2	7.40, *d*(8.5)	131.2	7.40, *d*(8.5)
3′,5′	116.0	6.91, *d*(8.5)	115.9	6.91, *d*(8.5)	114.7	6.80, *d*(8.5)	113.3	6.94, *d*(8.5)	115.5	6.86, *d*(8.5)
4′	158.4		158.3		157.0		158.6		157.9	
OCH_3-4′							55.0	3.77, *s*		
1″	116.1	6.69, *d*(10.0)	26.2	2.97, *dd* (5.0,17.0)	25.4	3.12, *dd*(5.5, 17.0)	26.0	2.76, *dd* (5.5, 17.0)	27.1	2.90, *dd* (5.5,17.0)
				2.63, *dd* (7.5, 17.0)		2.78, *dd* (6.5, 17.0)		2.40, *dd* (7.5, 17.0)		2.50, *dd* (8.0, 17.0)
2″	129.2	5.75, *d*(10.0)	68.8	3.88, *dd*(5.5, 7.5)	66.1	3.77, *q*(5.5)	66.8	3.63, *q*(5.5)	68.9	3.78, *dd*(6.0, 8.0)
3″	78.9		79.6		78.1		77.5		78.3	
4″	28.3	1.42, *s*	25.9	1.43, *s*	25.2	1.33, *s*	25.4	1.29, *s*	25.9	1.39, *s*
5″	28.3	1.42, *s*	21.0	1.33, *s*	20.8	1.26, *s*	20.4	1.18, *s*	20.3	1.25, *s*
OH-2″						5.27, *d*(4.5)		5.12, *d*(4.5)		
1‴	27.5	3.96 (2H), *s*	22.0	3.43 (2H), *d*(7.5)	104.1	7.17, *d*(2.0)			26.0	3.05, *dd* (5.5, 17.0)
										2.71, *dd*(7.0, 16.5)
2‴	132.3		123.1	5.22, *t*(7.0)	145.1	8.02, *d*(2.0)			68.9	3.89, *dd* (5.0,7.0)
3‴	130.3	7.15, *d*(8.5)	131.7						79.3	
4‴	115.8	6.72, *d*(8.5)	25.8	1.65, *s*					26.3	1.41, *s*
5‴	156.4		18.0	1.82, *s*					21.1	1.33, *s*
6‴	115.8	6.72, *d*(8.5)								
7‴	130.3	7.15, *d*(8.5)								

[a] TMS was used as the internal standard; chemical shifts are shown in the δ scale with *J* value (Hz) in parentheses.
[b] Acetone-d_6.
[c] DMSO-d_6.

d, *J* = 2.0 Hz, H-2‴)], and a 3-hydroxy-2,2-dimethyldihydropyran moiety [δ_H 3.12 (1H, *dd*, *J* = 5.5, 17.0 Hz, Ha-1″), 2.78 (1H, *dd*, *J* = 6.5, 17.0 Hz, Hb-1″), 3.77 (1H, *q*, *J* = 5.5 Hz, H-2″), 5.27 (1H, *d*, *J* = 4.5 Hz, OH-2″), 1.33 (3H, *s*, Me-4″), and 1.26 (3H, *s*, Me-5″)]. Additionally, the sharp proton signal at δ_H 8.24 (1H, s, H-2) indicated that **8** is based on a isoflavone skeleton with two substituents, a furan group and a 3-hydroxy-2,2-dimethyldihydropyran group (Fu et al., 2012). The HMBC cross-peaks from H-2 to C-9 (δ_C 149.8), and from H-1‴ to C-7 (δ_C 155.5), C-8 (δ_C 108.1) and C-9, suggested that the furan group was linked at the C-7 and C-8 positions. Besides, according to the HMBC correlations between H-1″ and C-5 (δ_C 150.4), C-6 (δ_C 101.5), and C-7, the location of the 3-hydroxy-2,2-dimethyldihydropyran was determined to be at the C-5 and C-6 positions. Therefore, the new compound **8** (cudraisoflavone I) was formulated as illustrated.

Compound **9** was formulated as $C_{21}H_{20}0_6$ from HRESIMS (*m*/*z* 369.1335 [M+H]$^+$). The ^{1}H and ^{13}C NMR spectroscopic data were almost the same as those of **8** (Table 2), except for the presence of a methoxyl group at δ_H 3.77 (3H, *s*, OMe-4′) and the absence of the furan group. In its ^{1}H NMR spectrum, the singlet signal at δ_H 6.40 (1H, *s*) was assigned to H-8, as confirmed by the HMBC correlations from H-2 at 8.03 (1H, *s*) to C-9 (δ_C 157.1) as well as from H-8 to C-6 (δ_C 105.0), C-7 (δ_C 159.8), C-9, and C-10 (δ_C 107.5). Besides, the HMBC correlations particularly from the methoxyl resonance to C-4′ (δ_C 158.6) supported the attachment of the methoxyl group at C-4′, and the correlations from H-1″ [2.76 (1H, *dd*, *J* = 5.5, 17.0 Hz, Ha-1″), 2.40 (1H, *dd*, *J* = 7.5, 17.0 Hz, Hb-1″)] to C-5 (δ_C 154.1), C-6 and C-7 indicated that the 3-hydroxy-2,2-dimethyldihydropyran group was located at the C-5 and C-6 positions. Accordingly, the new compound **9** (cudraisoflavone J) was finally established as despicted.

The molecular formula of compound **10** (cudraisoflavone K) was established as $C_{25}H_{26}O_7$ by HRESIMS at *m*/*z* 439.1765 [M+H]$^+$. Its ^{1}H NMR spectrum showed a characteristic signal of an isoflavone skeleton at δ_H 7.98 (1H, *s*, H-2) and two sets of 3-hydroxy-2,2-dimethyldihydropyran groups at δ_H 3.05 (1H, *dd*, *J* = 5.5, 17.0 Hz, Ha-1‴), 2.71 (1H, *dd*, *J* = 7.0, 16.5 Hz, Hb-1‴), 3.89 (1H, *dd*, *J* = 5.0, 7.0 Hz, H-2‴), 1.41 (3H, *s*, Me-4‴), and 1.33 (3H, *s*, Me-5‴), as well as at δ_H 2.90 (1H, *dd*, *J* = 5.5, 17.0 Hz, Ha-1″), 2.50 (1H, *dd*, *J* = 8.0, 17.0 Hz, Hb-1″), 3.78 (1H, *dd*, *J* = 6.0, 8.0 Hz, H-3″), 1.39 (3H, *s*, Me-4″), 1.25 (3H, *s*, Me-5″). According to the HMBC correlations from H-2 to C-9 (δ_C 156.1), and from H-1‴ to C-7 (δ_C 155.6), C-8 (δ_C 100.0) and C-9, the first 3-hydroxy-2,2-dimethyldihydropyran group was located at C-7 and C-8 positions. Besides, the attachment of the second 3-hydroxy-2,2-dimethyldihydropyran group was determined to be at the C-5 and C-6 positions on the basis of the HMBC correlations between H-1″ and C-5 (δ_C 153.2), C-6 (δ_C 106.2), and C-7. Accordingly, the structure of new compound **10** was elucidated as shown.

In order to characterize the constituents responsible for the neuroprotective activity of the EtOAc extract of the fruits of *C. tricuspidata*, 37 compounds were assessed against 6-OHDA induced neuronal cell death in SH-SY5Y cells. Of these, six compounds **7**, **8**, **9**, **11**, **12**, and **13** exhibited neuroprotective activities, and their EC_{50} values are summarized in Table 3. The other compounds were inactive (EC_{50} > 20 μM). Cudraisoflavone J (**9**) (EC_{50} 0.5 μM) and gancaonin B (**13**) (EC_{50} 0.5 μM) were the most potent isoflavones obtained. Besides, cudraisoflavone H (**7**), cudraisoflavone I (**8**),

Table 3
Neuroprotective activity against 6-OHDA-induced cell death in SH-SY5Y cells.[a]

Compound	EC_{50} (μM)
7	4.5 ± 0.09
8	9.2 ± 0.07
9	0.5 ± 0.09
11	3.0 ± 0.10
12	3.1 ± 0.08
13	0.5 ± 0.11
Curcumin[b]	6.0 ± 0.14

[a] Data are presented as means ± standard deviation from three separate experiments.
[b] Curcumin was used as a positive control.

5,7,3′,4′-tetrahydroxy-6,8-diprenylisoflavone (**11**), and erythrinin B (**12**), possessed moderate neuroprotection potential with the EC_{50} values of 3.1–9.2 μM (Table 3).

3. Conclusion

As a part of our ongoing research program for the discovery of neuroprotective compounds from *C. tricuspidata*, 12 neuroprotective xanthones were discovered from the roots of this plant (Kwon et al., 2014). In the present study, six isoflavones isolated from the fruits of this plant also exhibited neuroprotective activities. Additionally, the neuroprotection was achieved at physiologically relevant concentrations of dietary isoflavones. Although no clear relationship was found between neuroprotective activities of isoflavones and the chemical structures of these compounds, the isoflavones, cudraisoflavone H (**7**), cudraisoflavone I (**8**), cudraisoflavone J (**9**), 5,7,3′,4′-tetrahydroxy-6,8-diprenylisoflavone (**11**), erythrinin B (**12**), and gancaonin B (**13**) show therapeutic potential in treating neurotoxicity, and could be considered as candidates for further research for therapeutic purposes in neural degenerative diseases such as Parkinson's disease.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a JASCO P-2000 polarimeter, using a 10-cm microcell. UV spectra were obtained using an OPTIZEN POP spectrophotometer. IR spectra were recorded using a Varian 640-IR spectrometer. NMR spectra were acquired using a Varian 500-MHz NMR spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed as δ values. ESI-MS was performed on a Waters Q-TOF micromass spectrometer. Column chromatography (CC) was performed using silica gel (Kieselgel 60, 70–230 and 230–400 mesh, Merck), and thin layer chromatography was performed using pre-coated silica gel 60 F254 plates (0.25 mm, Merck). HPLC was conducted using the Varian Prostar 210 system. For preparative HPLC, a YMC-Pack ODS-A (5 μm, 250 × 20 mm i.d., YMC Co., Ltd., Kyoto, Japan) column was used with a 8 mL/min flow rate. For semipreparative HPLC, a YMC J'sphere ODS-H80 (4 μm, 250 × 10 mm i.d., YMC Co., Ltd., Kyoto, Japan) column was used with a 4 mL/min flow rate. 6-OHDA and MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] were purchased from Sigma–Aldrich (St. Louis, MO, USA). Curcumin was obtained from Enzo Life Science (Farmingdale, NY, USA).

4.2. Plant material

The fruits of *C. tricuspidata* were collected from the Korea Forest Research Institute, Southern Forest Research Center, Jinju, Korea, since September 2008 (GPS coordinates: 35°12′46.7″N, 128′10′28.5″E). Voucher specimens (accession number: KH1-5-090904) of this plant are deposited at the Department of Biosystems and Biotechnology, Korea University.

4.3. Extraction and isolation

Fresh fruits of *C. tricuspidata* (10.7 kg) were sliced and extracted with MeOH (3 × 10 L) at room temperature for three to four days of each. The extracts were combined and evaporated *in vacuo* to afford a MeOH extract (TH1-1-1, 630.9 g), which was then suspended in H_2O (2 × 2.5 L). The aqueous solution was sequentially partitioned with *n*-hexane (4 × 2 L) and EtOAc (5 × 2 L) to afford dried *n*-hexane (TH1-2-1, 48.4 g) and EtOAc (TH1-2-2, 27.8 g) soluble extracts.

Fractionation of the EtOAc soluble extract was initiated by silica gel CC as the stationary phase using a $CHCl_3$–MeOH gradient (1:0 to 1:1) as the mobile phase, to yield six fractions (TH1-4-1–TH1-4-6). Fraction TH1-4-3 (9.68 g) was also subjected to silica gel CC and eluted with a gradient of *n*-hexane–EtOAc (1:0 to 0:1), and seven subfractions (TH1-10-1–TH1-10-7) were obtained. Fraction TH1-10-4 (4.7 g) was eluted by silica gel CC with a gradient mixture of *n*-hexane-$CHCl_3$–MeOH (1:0:0 to 0:1:1), resulting in sixteen subfractions (TH1-74-1–TH1-74-16). Fraction TH1-74-4 (1354.8 mg) was separated using Sephadex LH-20 and eluted with $CHCl_3$–MeOH (1:1, v/v) to yield eight subfractions (TH1-84-1–TH1-84-8). Fraction TH1-84-3 (308.5 mg) was subjected to silica gel CC using $CHCl_3$–MeOH (1:0 to 1:1), to afford three subfractions (TH1-88-1–TH1-88-3). Fraction TH1-88-2 (192.5 mg) was applied to a silica gel column developed with a gradient of *n*-hexane–acetone (1:0 to 0:1) resulting in isolation of warangalone (22.4 mg) and osajin (28.2 mg). Fraction TH1-84-4 (552.0 mg) was separated using Sephadex LH-20 eluted with $CHCl_3$–MeOH (1:1, v/v), producing six subfractions (TH1-104-1–TH1-104-6). Fraction TH1-104-4 (191.7 mg) was purified by preparative HPLC (MeOH–H_2O, 50–90% MeOH in H_2O), to afford cudraisoflavone B (**1**, 1.4 mg), cudraisoflavone C (**2**, 3.2 mg), alpinumisoflavone (3.6 mg), and 5,7,4′-trihydroxy-6,8-diprenylisoflavone (4.0 mg), respectively. Fraction TH1-74-7 (301.9 mg) was subjected to silica gel CC with gradient mixtures of *n*-hexane–EtOAc (1:0 to 0:1), resulting in preparation of fractions TH1-194-1–TH1-194-12. Fraction TH1-194-7 (43.8 mg) was separated by preparative HPLC (MeOH–H_2O, 60–93%, MeOH in H_2O) to afford anagyroidisoflavone A (2.0 mg). Purification of TH1-194-8 (21.9 mg) was carried out by preparative HPLC (MeOH–H_2O, 60–85% MeOH in H_2O), resulting in isolation of cudraisoflavone D (**3**, 4.9 mg) and euchrenone b_8 (8.8 mg). Fraction TH1-194-11 (12.6 mg) was purified by semipreparative HPLC (MeOH–H_2O, 60–75% MeOH in H_2O) to yield cudraisoflavone E (**4**, 2.2 mg). TH1-74-9 (174.3 mg) and TH1-74-10 (109.1 mg) were subjected to passage over silica gel using *n*-hexane–EtOAc gradient (1:0 to 0:1), to afford eight subfractions TH3-5-1–TH3-5-8. Fraction TH3-5-5 (34.7 mg) was purified using preparative HPLC (MeOH–H_2O, 60–76% MeOH in H_2O), resulting in the isolation of cudraisoflavone H (**7**, 4.6 mg) and euchrenone b_{10} (3.0 mg). Further separation of the impure fraction (TH3-55-5, 20.6 mg) eluted by *n*-hexane–EtOAc gradient (1:0 to 0:1), yielded cudraisoflavone F (**5**, 1.9 mg) and cudraisoflavone G (**6**, 16.5 mg). Fraction TH1-74-12 (166.3 mg) was separated on a column of reversed-phase C_{18} silica gel, eluted with MeOH–H_2O (5:5 to 10:0, MeOH in H_2O), to give six subfractions (TH3-9-1–TH3-9-6). Fraction TH3-9-1 (71.1 mg) was then passed over a silica gel column and eluted with a *n*-hexane–EtOAc gradient (1:0 to 0:1), resulting in the preparation of fractions

TH3-19-1–TH3-19-5. Fraction TH3-19-3 (40.5 mg) and TH3-19-4 (12.3 mg) were purified by semipreparative HPLC (MeOH–H_2O, 60–81%, MeOH in H_2O), leading to the isolation of cudraisoflavone I (**8**, 4.2 mg) and cudraisoflavone J (**9**, 5.8 mg), respectively. Fraction TH1-74-14 (240.4 mg) was subjected to passage over reversed-phase C_{18} silica gel using MeOH–H_2O (5:5 to 8:2), producing subfractions (TH3-3-1–TH3-3-4). Fraction TH3-3-2 (96.2 mg) was purified by semipreparative HPLC (MeOH–H_2O, 55–75%), to give cudraisoflavone K (**10**, 1.8 mg) and eryvarin B (3.0 mg). Fraction TH1-10-5 (909.3 mg) was separated by Sephadex LH-20 (Sigma, St. Louis, MO) eluted with $CHCl_3$–MeOH (1:1, v/v) to yield seven subfractions (TH3-51-1–TH3-51-7). Fraction TH3-51-5 (311.3 mg) was eluted on silica gel using an *n*-hexane–EtOAc gradient (1:0 to 0:1) producing fractions TH3-69-1–TH3-69-8. Fraction TH3-69-3 (95.3 mg) was passed over a silica gel column and eluted with gradient of *n*-hexane–EtOAc (1:0 to 0:1) to yield six subfractions (TH3-79-1–TH3-79-6). Furthermore, the first fraction (29.3 mg) was purified by semipreparative HPLC (MeOH–H_2O, 60–95% MeOH in H_2O), leading to isolation of 5,7-dihydroxy-6-(2″-hydroxy-3″-methylbut-3″-enyl)-4′-methoxylisoflavone (2.6 mg), 3′,5-dihydroxy-4′-methoxyl-2″,2″-dimethylpyrano[6″,5″-*h*]isoflavone (3.9 mg) and gancaonin A (2.8 mg). Fraction TH3-79-4 (51.2 mg) was further separated by preparative HPLC (MeOH–H_2O, 60–95% MeOH in H_2O), to afford 4′-*O*-methyl-2″-hydroxydihydroalpinumisoflavone (2.8 mg) and isoerysenegalensein E (40.3 mg). Fraction TH3-51-6 (399.0 mg) was applied to a silica gel column eluted with gradient mixtures of *n*-hexane–EtOAc (1:0 to 0:1), resulting in the preparation of fractions TH3-81-1–TH3-81-7. Fraction TH3-81-3 (91.3 mg) was eluted on silica gel using an *n*-hexane–EtOAc gradient (1:0 to 0:1), producing fractions TH3-89-1–TH3-89-4. TH3-89-3 (25.2 mg) and TH3-89-4 (12.9 mg) were purified by semipreparative HPLC (MeOH–H_2O, 65–93% MeOH in H_2O) to afford biochanin A (1.6 mg). Fraction TH3-81-5 (49.6 mg) was further separated by preparative HPLC (MeOH–H_2O, 60–95% MeOH in H_2O), to yield lupiwighteone (2.6 mg), 5,7,3′,4′-tetrahydroxy-6,8-diprenylisoflavone (**11**, 2.4 mg), erysenegalensein E (20.4 mg), and auriculasin (3.0 mg). Purification of TH3-81-6 (38.2 mg) was carried out by preparative HPLC (MeOH–H_2O, 60–95% MeOH in H_2O), leading to the isolation of erythrinin B (**12**, 2.0 mg) and 5,3′,4′-trihydroxy-6″,6″-dimethylpyrano-[2″,3″;7,6]isoflavone (2.6 mg). Fraction TH1-10-6 (1672.0 mg) was passed over a column containing Sephadex LH20 (Sigma, St. Louis, MO) eluted with $CHCl_3$–MeOH (1:1), resulting in eleven subfractions (TH3-101-1–TH3-101-11). Fraction TH3-101-6 (307.0 mg) was subjected to silica gel CC with gradient mixtures of *n*-hexane–EtOAc (1:0 to 0:1), producing fractions TH3-113-1–TH3-113-9. Fraction TH3-113-5 (71.8 mg) was purified by semipreparative HPLC (MeOH–H_2O, 65–91% MeOH in H_2O), resulting in pure euchrenone b_9 (11.3 mg) and senegalensin (21.4 mg). Fraction TH3-101-7 (139.1 mg) was separated by silica gel CC using *n*-hexane–EtOAc (1:0 to 0:1), to give seven subfractions (TH3-123-1–TH3-123-7). Fraction TH3-123-5 (20.7 mg) was subjected to semipreparative HPLC (MeOH–H_2O, 65–91% MeOH in H_2O), to afford erythrinin C (2.0 mg) and flemiphilippinin G (9.0 mg). Fraction TH3-101-8 (243.8 mg) was eluted on silica gel using an *n*-hexane–EtOAc gradient (1:0 to 0:1), leading to preparation of fractions TH3-129-1–TH3-129-5. The purification of first fraction (16.7 mg) was carried out by semipreparative HPLC (MeOH–H_2O, 60–85% MeOH in H_2O), to give 4′-*O*-methyl-alpinumisoflavone (2.1 mg) and derrone (6.8 mg). Fraction TH3-129-2 (28.4 mg) was purified by semipreparative HPLC (MeOH–H_2O, 60–95% MeOH in H_2O), resulting in pure gancaonin B (**13**, 9.1 mg).

4.3.1. Cudraisoflavone B (*1*)

Yellow oil; UV (MeOH) λ_{max} nm (log ε): 265 (4.17); IR (ATR) ν_{max} cm^{-1}: 3323 (>OH), 1681 (>C=O); For ^{13}C and ^{1}H NMR (Acetone-d_6, 500 MHz) spectroscopic data, see Table 1; HMBC correlations H-2/C-3, C-4, C-9, C-1′; H-2′/C-2, C-4′, C-6′; H-3′/C-1′, C-4′, C-5′; H-1″/C-5, C-6, C-7, C-2″; H-2″/C-6, C-7; H-1‴/C-7, C-8, C-9, C-2‴, C-3‴; Me-4‴/C-2‴, C-3‴, C-5‴; Me-5‴/C-2‴, C-3‴, C-4‴; ESIMS (positive) *m*/*z* 363 $[M+H]^+$; ESIMS (negative) *m*/*z* 361 $[M-H]^-$; HRESIMS *m*/*z* 363.1215 $[M+H]^+$ (calcd for $C_{22}H_{19}O_5$, 363.1232).

4.3.2. Cudraisoflavone C (*2*)

Yellow oil; UV (MeOH) λ_{max} nm (log ε): 220 (4.41), 259 (4.50); IR (ATR) ν_{max} cm^{-1}: 3345 (>OH), 1650 (>C=O); For ^{13}C and ^{1}H NMR (Acetone-d_6, 500 MHz) spectroscopic data, see Table 1; HMBC correlations H-2/C-3, C-4, C-9, C-1′; H-2′/C-2, C-4′, C-6′; H-3′/C-1′, C-4′, C-5′; OH-5/C-5, C-6, C-10; H-1″/C-5, C-6, C-7, C-2″, C-3″; Me-4″/C-2″, C-3″, C-5″; Me-5″/C-2″, C-3″, C-4″; H-1‴/C-7, C-8, C-9, C-2‴; H-2‴/C-7, C-8; ESIMS (positive) *m*/*z* 363 $[M+H]^+$; ESIMS (negative) *m*/*z* 361 $[M-H]^-$; HRESIMS *m*/*z* 363.1221 $[M+H]^+$ (calcd for $C_{22}H_{19}O_5$, 363.1232).

4.3.3. Cudraisoflavone D (*3*)

Yellow powder; $[\alpha]_D^{24}$ + 8.56 (*c* 0.03, MeOH); UV (MeOH) λ_{max} nm (log ε): 220 (4.22), 260 (4.28); IR (ATR) ν_{max} cm^{-1}: 3394 (>OH), 1649 (>C=O); For ^{13}C and ^{1}H NMR (DMSO-d_6, 500 MHz) spectroscopic data, see Table 1; HMBC correlations H-2/C-3, C-4, C-9, C-1′; H-2′/C-2, C-4′, C-6′; H-3′/C-1′, C-4′, C-5′; OH-5/C-5, C-6, C-10; H-1″/C-5, C-6, C-7, C-2″, C-3″; H-2″/C-6, C-1″, C-3″, C-4″, C-5″; OH-2″/C-1″, C-2″, C-3″; H-4″/C-2″, C-3″, C-5″; Me-5″/C-2″, C-3″, C-4″; H-1‴/C-7, C-8, C-9, C-2‴; H-2‴/C-7, C-8, C-1‴; ESIMS (positive) *m*/*z* 379 $[M+H]^+$; ESIMS (negative) *m*/*z* 377 $[M-H]^-$; HRESIMS *m*/*z* 377.1032 $[M-H]^-$ (calcd for $C_{22}H_{17}O_6$, 377.1025).

4.3.4. Cudraisoflavone E (*4*)

Yellow powder; $[\alpha]_D^{24}$ + 13.39 (*c* 0.02, MeOH); UV (MeOH) λ_{max} nm (log ε): 216 (3.88), 267 (3.91); IR (ATR) ν_{max} cm^{-1}: 3363 (>OH), 1651 (>C=O); For ^{13}C and ^{1}H NMR *(DMSO-d_6*, 500 MHz) spectroscopic data, see Table 1; HMBC correlations H-2/C-3, C-4, C-9, C-1′; H-2′/C-2, C-4′, C-6′; H-3′/C-1′, C-4′, C-5′; OH-5/C-5, C-6, C-10; H-1″/C-5, C-6, C-7, C-2″, C-3″; H-2″/C-6, C-1″, C-3″, C-4″, C-5″; OH-2″/C-1″, C-2″, C-3″; H-4″/C-2″, C-3″, C-5″; Me-5″/C-2″, C-3″, C-4″; H-1‴/C-7, C-8, C-9, C-2‴, C-3‴; H-2‴/C-7, C-8, C-1‴, C-4‴, C-5‴; Me-4‴/C-2‴, C-3‴, C-5‴; Me-5‴,C-2‴, C-3‴, C-4‴; ESIMS (positive) *m*/*z* 439 $[M+H]^+$; ESIMS (negative) *m*/*z* 437 $[M-H]^-$; HRESIMS *m*/*z* 437.1608 $[M-H]^-$ (calcd for $C_{25}H_{25}O_7$, 437.1600).

4.3.5. Cudraisoflavone F (*5*)

Yellow oil; UV (MeOH) λ_{max} nm (log ε): 210 (4.06), 275 (4.11); IR (ATR) ν_{max} cm^{-1}: 3339 (>OH), 1653 (>C=O); For ^{13}C and ^{1}H NMR (Acetone-d_6, 500 MHz) spectroscopic data, see Table 1; HMBC correlations H-2/C-3, C-4, C-9, C-1′; H-2′/C-2, C-4′, C-6′; H-3′/C-1′, C-4′, C-5′; OH-5/C-5, C-6, C-10; H-1″/C-5, C-6, C-7, C-2″, C-3″; Me-4″/C-2″, C-3″, C-5″; Me-5″/C-2″, C-3″, C-4″; H-1‴/C-7, C-8, C-2‴; H-4‴/C-2‴, C-3‴, C-5‴; Me-5‴/C-2‴, C-3‴, C-4‴; ESIMS (positive) *m*/*z* 403 $[M+H]^+$; ESIMS (negative) *m*/*z* 401 $[M-H]^-$; HRESIMS *m*/*z* 401.1370 $[M-H]^-$ (calcd for $C_{25}H_{21}O_5$, 401.1389).

4.3.6. Cudraisoflavone G (*6*)

Yellow oil; UV (MeOH) λ_{max} nm (log ε): 227 (4.41), 283 (4.49); IR (ATR) ν_{max} cm^{-1}: 3374 (>OH), 1651 (>C=O); For ^{13}C and ^{1}H NMR (Acetone-d_6, 500 MHz) spectroscopic data, see Table 2; HMBC correlations H-2/C-3, C-4, C-9, C-1′; H-2′/C-2, C-4′, C-6′; H-3′/C-1′, C-4′, C-5′; OH-5/C-5, C-6, C-10; H-1″/C-5, C-6, C-7, C-2″, C-3″; Me-4″/C-2″, C-3″, C-5″; Me-5″/C-2″, C-3″, C-4″; H-1‴/C-7, C-8, C-9, C-2‴, C-3‴, C-7‴; H-3‴/C-1‴, C-5‴, C-7‴; H-4‴/C-2‴, C-5‴, C-6‴; ESIMS (positive) *m*/*z* 443 $[M+H]^+$; ESIMS (negative) *m*/*z* 441 $[M-H]^-$, 883 $[2M-H]^-$; HRESIMS *m*/*z* 443.1488 $[M+H]^+$ (calcd for $C_{27}H_{23}O_6$, 443.1495).

*4.3.7. Cudraisoflavone H (**7**)*

Yellow oil; $[\alpha]_D^{22}$ − 0.7 (*c* 0.02, MeOH); UV (MeOH) λ_{max} nm (log ε): 270 (4.49); IR (ATR) ν_{max} cm^{-1}: 3324 (>OH), 1646 (>C=O); For ^{13}C and ^{1}H NMR (Acetone-d_6, 500 MHz) spectroscopic data, see Table 2; HMBC correlations H-2/C-3, C-4, C-9, C-1′; H-2′/C-2, C-4′, C-6′; H-3′/C-1′, C-4′, C-5′; OH-5/C-5, C-6, C-10; H-1″/C-6, C-7, C-2″, C-3″; Me-4″/C-2″, C-3″, C-5″; Me-5″/C-2″, C-3″, C-4″; H-1‴/C-7, C-8, C-9, C-2‴, C-3‴; H-2‴/C-8, C-3‴; Me-4‴/C-2‴, C-3‴, C-5‴; Me-5‴/C-2‴, C-3‴, C-4‴; ESIMS (positive) *m*/*z* 423 $[M+H]^+$, 845 $[2M+H]^+$; ESIMS (negative) *m*/*z* 421 $[M-H]^-$, 843 $[2M-H]^-$; HRESIMS *m*/*z* 423.1790 $[M+H]^+$ (calcd for $C_{25}H_{27}O_6$, 423.1808).

*4.3.8. Cudraisoflavone I (**8**)*

Yellow powder; $[\alpha]_D^{22}$ − 4.52 (*c* 0.02, MeOH); UV (MeOH) λ_{max} nm (log ε): 256 (4.29); IR (ATR) ν_{max} cm^{-1}: 3316 (>OH), 1638 (>C=O); For ^{13}C and ^{1}H NMR (DMSO-d_6, 500 MHz) spectroscopic data, see Table 2; HMBC correlations H-2/C-3, C-4, C-9, C-1′; H-2′/C-2, C-4′, C-6′; H-3′/C-1′, C-4′, C-5′; H-1″/C-5, C-6, C-7, C-2″, C-3″; Me-4″/C-2″, C-3″, C-5″; Me-5″/C-2″, C-3″, C-4″; H-1‴/C-7, C-8, C-9, C-2‴; H-2‴/C-7, C-8; ESIMS (positive) *m*/*z* 379 $[M+H]^+$; ESIMS (negative) m/z 377 $[M-H]^-$, 801 $[2M+HCOO]^-$; HRESIMS *m*/*z* 379.1169 $[M+H]^+$ (calcd for $C_{22}H_{19}O_6$, 379.1182).

*4.3.9. Cudraisoflavone J (**9**)*

Yellow powder; $[\alpha]_D^{22}$ − 4.90 (*c* 0.01, MeOH); UV (MeOH) λ_{max} nm (log ε): 215 (4.62), 260 (4.73); IR (ATR) ν_{max} cm^{-1}: 3076 (>OH), 1637 (>C=O); For ^{13}C and ^{1}H NMR (DMSO-d_6, 500 MHz) spectroscopic data, see Table 2; HMBC correlations H-2/C-3, C-4, C-9, C-1′; H-2′/C-2, C-4′, C-6′; H-3′/C-1′, C-4′, C-5′; H-1″/C-5, C-6, C-7, C-2″, C-3″; Me-4″/C-2″, C-3″, C-5″; Me-5″/C-2″, C-3″, C-4″; H-8/C-6, C-7, C-9, C-10; OMe-4′/C-4′; ESIMS (positive) *m*/*z* 369 $[M+H]^+$; ESIMS (negative) *m*/*z* 367 $[M-H]^-$, 735 $[2M-H]^-$; HRESIMS *m*/*z* 369.1335 $[M+H]^+$ (calcd for $C_{21}H_{21}O_6$, 369.1338).

*4.3.10. Cudraisoflavone K (**10**)*

Yellow oil; $[\alpha]_D^{24}$ + 12.64 (*c* 0.02, MeOH); UV (MeOH) λ_{max} nm (log ε): 211 (4.25), 265 (4.34); IR (ATR) ν_{max} cm^{-1}: 3338 (>OH), 1635 (>C=O); For ^{13}C and ^{1}H NMR (Acetone-d_6, 500 MHz) spectroscopic data, see Table 2; HMBC correlations H-2/C-3, C-4, C-9, C-1′; H-2′/C-2, C-4′, C-6′; H-3′/C-1′, C-4′, C-5′; H-1″/C-5, C-6, C-7, C-2″, C-3″; Me-4″/C-2″, C-3″, C-5″; Me-5″/C-2″, C-3″, C-4″; H-1‴/C-7, C-8, C-9, C-2‴, C-3‴; Me-4‴/C-2‴, C-3‴, C-5‴; Me-5‴/C-2‴, C-3‴, C-4‴; ESIMS (positive) *m*/*z* 439 $[M+H]^+$; ESIMS (negative) *m*/*z* 437 $[M-H]^-$, 483 $[M+HCOO]^-$, 921 $[2M+HCOO]^-$; HRESIMS *m*/*z* 439.1765 $[M+H]^+$ (calcd for $C_{25}H_{27}O_7$, 439.1757).

4.4. Neuroprotective assay

The neuroprotective activity of extracts and test compounds against 6-OHDA-induced cell death in SH-SY5Y cells was evaluated in accordance with a protocol, described in our previous report (Kwon et al., 2014).

Acknowledgments

This work was supported by a grant from the Korea University, the Korea Forest Research Institute, and the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (NRF-2013R1A1A2008111).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2014.10.021.

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