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2-(2-Phenylethyl)chromones from Chinese eaglewood

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ABSTRACT

2-(2-Phenylethyl) chromones and a 2-(2-phenylethenyl) chromone, were isolated from the ethanolic extract of Chinese eaglewood. Their structures were determined on the basis of extensive analyses of spectroscopic data. Among those, one showed significant neuroprotective activities against both glutamate-induced and corticosterone-induced neurotoxicity in P12 pheochromocytoma and human U251 glioma cells at a concentration of 10 μM and increased cell viability by 82.2% and 86.9%, respectively.

1. Introduction

Chinese eaglewood (Chinese name: Chenxiang) is a resinous wood from the tree of *Aquilaria sinensis* (Lour.) Gilg (Thymelaeaceae), which is common in Southern China and is used as a type of agarwood. The resinous part of the agarwood is formed during the decomposition of trees from the *Aquilaria* species. Since ancient times, Chinese eaglewood has been widely used as a traditional sedative, analgesic, and digestive medicine in China (Xiao, 2002). In recent decades, researchers have isolated various sesquiterpenes and chromone derivatives from Chinese eaglewood (Yang et al., 1989, 1990, 1992; Yagura et al., 2003; Liu et al., 2008; Dai et al., 2010) and other agarwoods (Maheshiwali et al., 1963; Ishihara et al., 1993; Yoshii et al., 1978; Shimada et al., 1982; Hashimoto et al., 1985; Nakanish et al., 1986; Konishi et al., 1989, 1992, 2002; Yagura et al., 2005). These reports showed that the 2-(2-phenylethyl)chromone-type compound is one of the main chemical components in agarwood. A recent study reported that 2-(2-phenylethyl)chromone derivatives exhibit significant neuroprotective activities against glutamate-induced neurotoxicity in P12 pheochromocytoma cells (Yoon et al., 2006). In this context and in an effort to search for new 2-(2-henylethyl)chromomes with neuroprotective activity from Chinese eaglewood, a bioassay-guided fractionation was carried out in our laboratory. As a result, seven new 2-(2-phenylethyl)chromone derivatives (**1**–**7**) and one new 2-(2-phenylethenyl)chromone (**8**) (Fig. 1) were obtained from an EtOH extract of Chinese eaglewood that displayed neuroprotective activity. In this paper, the isolation and structural elucidation of these new compounds are described along with their neuroprotective activities against glutamate-induced neurotoxicity in P12 pheochromocytoma cells and corticosterone-induced neurotoxicity in human U251 glioma cells.

2. Results and discussion

Chinese eaglewood was first crushed into pieces and extracted with petroleum ether under reflux. The residual plant material was then extracted by refluxing in EtOH. The EtOH extract was first subjected to silica gel column chromatography to afford eight fractions. One of the fractions (Fr.4), with neuroprotective activities against glutamate- and corticosterone-induced neurotoxicity in P12 pheochromocytoma and human U251 glioma cells, which increased cell viability by 32.5% and 21.3% at 100 μg/ml, respectively, was repeatedly subjected to purification by silica gel, Sephadex LH-20, and semi-preparative HPLC. As a result, seven new 2-(2-phenylethyl)chromones (**1**–**7**) and one new 2-(2-phenylethenyl)chromone (**8**) were isolated.

Compound **1** was obtained as a white, amorphous, solid and was assigned the molecular formula $C_{19}H_{18}O_6$ (HRESIMS *m/z* 343.1168 $[M+H]^+$, calc. 343.1176, $C_{19}H_{19}O_6$) with eleven degrees

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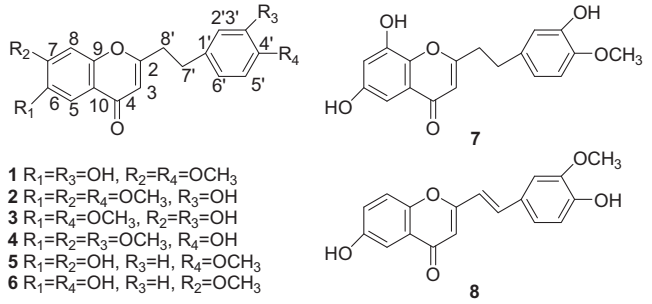

Fig. 1. Chemical structures of compounds **1–8**.

of unsaturation. The IR spectrum displayed absorption peaks indicating the presence of a hydroxy group (3313 cm^{-1}) and an unsaturated carbonyl group (1628 cm^{-1}). The ^1H NMR spectroscopic data (Table 1) of **1** showed the presence of two methoxy groups at δ_H 3.99 and 3.81 (each 3H, *s*) and two singlet aromatic protons at δ_H 7.37 and 7.05, which were assigned to H-5 and H-8. Additionally, a set of typical ABX coupling systems at δ_H 6.63 (1H, *dd*, *J* = 2.4, 8.4 Hz, H-6′), 6.79 (1H, *d*, *J* = 8.4 Hz, H-5′) and 6.69 (1H, *d*, *J* = 2.4 Hz, H-2′) and four methylene protons at δ_H 2.93 (*m*, H-7′ and H-8′) were observed. Analysis of the ^{13}C NMR spectroscopic data (Table 2) and DEPT experiments indicated that **1** had two methylene groups at δ_C 33.3 and 37.1, a tri-substituted double bond at δ_C 109.7 (CH) and 170.4 (*q*C), two methoxy groups at δ_C 56.9 and 56.4, and a carbonyl group at δ_C 179.9. Based on the above, **1** was deduced to be a 2-(2-phenylethyl)chromone derivative with two methoxy groups and two hydroxy groups substituted at C-6, C-7, C-3′ and C-4′, respectively. The HMBC correlations (Fig. 2) from the methoxy protons (δ_H 3.99) and phenyl protons (δ_H 7.37, 7.05) to the phenyl carbon (δ_C 155.3) in the chromone moiety indicated the location of a methoxy group at C-6 or C-7. According to the HMBC spectroscopic experiment and the typical ABX coupling system in the ^1H NMR spectrum, the other methoxy group was thought to be attached at C-3′ or C-4′. The exact loci of the two methoxy groups were determined by NOE spectral experiments (Fig. 2). Irradiation of the methoxy protons (δ_H 3.99, 3.81) enhanced the signal intensity of H-8 and H-5', respectively, indicating that the two methoxy groups are attached at C-7 and C-4′. Consequently, structure **1** was determined to be 6-hydroxy-7-methoxy-2-[2-(3′-hydroxy-4′-methoxyphenyl)ethyl]chromone.

The molecular formula of **2** was determined to be $C_{20}H_{20}O_6$ by combined analyses of HRESIMS (*m/z* 357.1333 $[M+H]^+$, calc. 357.1333, $C_{20}H_{21}O_6$), ^1H NMR and ^{13}C NMR spectroscopic data. The IR absorption at 3359 cm^{-1} indicated the presence of (a) hydroxy group(s). The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **2** were very similar to those of **1**, except for the presence of a signal from an additional methoxy group instead of a hydroxy group. Considering its molecular formula, it was inferred that **2** was a 2-(2-phenylethyl)chromone derivative with three methoxy groups and one hydroxy group in the same substituted pattern (C-6, C-7, C-3′ and C-4′) as that found in **1**. The HMBC and NOE correlations (Fig. 2) supported the conclusion that the substituted groups at C-6 and C-7 were methoxy groups. The other methoxy group was found to be attached at C-4′ by further NOE experiments (Fig. 2), in which the enhancement of H-5′ was observed when the methoxy protons at δ_H 3.86 was irradiated. Therefore, **2** was identified as 6,7-dimethoxy-2-[2-(3′-hydroxy-4′-methoxyphenyl)ethyl]chromone.

Compound **3** has the same molecular formula ($C_{19}H_{18}O_6$, HRESIMS *m/z* 343.1178 $[M+H]^+$, calc. for $C_{19}H_{19}O_6$, 343.1176) as **1**. Based on the ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2), **3** was also deduced to be a 2-(2-phenylethyl)chromone derivative, with two methoxy groups and two hydroxy groups in the same substituted pattern as that found in **1**. According to its HMBC and NOE spectra (Fig. 2), the two methoxy groups at the C-6 and C-4′ positions, respectively. Thus, structure **3** was determined to be 7-hydroxy-6-methoxy-2-[2-(3′-hydroxy-4′-methoxyphenyl)ethyl]chromone.

The HRESIMS, IR and NMR spectroscopic data indicated that compound **4** was also a 2-(2-phenylethyl)chromone derivative with three methoxy groups and one hydroxy group. From HMBC and NOE experiments (Fig. 2), the three methoxy groups were linked at the C-6, C-7 and C-3′ positions, respectively, and the hydroxy group was linked at C-4′ position. Accordingly, **4** was identified as 6,7-dimethoxy-2-[2-(4′-hydroxy-3′-methoxyphenyl) ethyl]chromone.

Compound **5** has a molecular formula of $C_{18}H_{16}O_5$ (HRESIMS *m/z* 313.1070 $[M+H]^+$, calc. for $C_{18}H_{17}O_5$, 313.1071), and its IR absorption at 3450 cm^{-1} showed the existence of hydroxy group(s). The ^1H NMR spectroscopic data (Table 1) of **5** displayed the presence of one methoxy group at δ_H 3.76 (3H, *s*) and two singlet protons at δ_H 7.37 and δ_H 6.89, which were assigned to H-5 and H-8, respectively. In addition, one 1, 4-substituted phenyl moiety with the typical A_2B_2 coupling systems [δ_H 7.10 and 6.81 (each 2H, *d*, *J* = 8.0 Hz)] designated to B ring was observed. On the basis of the above evidence, it was deduced that **5** was a 2-(2-phenylethyl)chromone derivative with one methoxy group and two

Table 1
^1H NMR spectroscopic data for compounds **1–8** (*J* in Hz).[a]

Position	**1**[b]	**2**[c]	**3**[d]	**4**[e]	**5**[b]	**6**[d]	**7**[e]	**8**[d]
3	6.06, *s*	6.11, *s*	6.33, *s*	6.03, *s*	6.02, *s*	6.36, *s*	6.00, *s*	6.52, *s*
5	7.37, *s*	7.51, *s*	7.82, *s*	7.37, *s*	7.37, *s*	8.11, *s*	6.79, *d* (2.8)	8.10, *brs*
7							6.68, *d* (2.8)	7.51, *brd* (8.4)
8	7.05, *s*	6.87, *s*	7.24, *s*	7.03, *s*	6.89, *s*	7.12, *s*		7.58, *d* (8.4)
2′	6.69, *d* (2.4)	6.79, *d* (1.8)	7.17, *d* (1.8)	6.69, *d* (2.0)	7.10, *d* (8.0)	7.23, *d* (8.0)	6.64, *d* (2.0)	7.44, *brs*
3′					6.81, *d* (8.0)	7.15, *d* (8.0)		
5′	6.79, *d* (8.4)	6.75, *d* (8.4)	6.89, *d* (8.4)	6.62, *d* (8.0)	6.81, *d* (8.0)	7.15, *d* (8.0)	6.74, *d* (8.0)	7.25, *d* (7.8)
6′	6.63, *dd* (2.4, 8.4)	6.65, *dd* (1.8, 8.4)	6.76, *dd* (1.8, 8.4)	6.56, *dd* (2.0, 8.0)	7.10, *d* (8.0)	7.23, *d* (8.0)	6.57, *dd* (2.0, 8.0)	7.32, *brd* (7.8)
7′	2.93 (2H), *m*	2.96 (2H), *t* (7.8)	2.95 (2H), *t* (7.8)	2.93 (2H), *m*	2.99 (2H), *t* (7.2)	2.98 (2H), *t* (7.6)	2.91 (2H), *m*	7.77, *d* (15.6)
8′	2.92 (2H), *m*	2.87 (2H), *t* (7.8)	2.85 (2H), *t* (7.8)	2.89 (2H), *m*	2.91 (2H), *t* (7.2)	2.87 (2H), *t* (7.6)	2.91 (2H), *m*	7.02, *d* (15.6)
$-OCH_3$(*s*)	3.99 (C-7)	3.96 (C-6)	3.71 (C-6)	3.84 (C-6)	3.76 (C-4′)	3.86 (C-7)	3.74(C-4′)	3.81(C-3′)
	3.81 (C-4′)	3.99 (C-7)	3.68 (C-4′)	3.90 (C-7)				
		3.86 (C-4′)		3.68 (C-3′)				

[a] Recorded at 400 MHz for compounds **4–7** and 600 MHz for other compounds.
[b] Data recorded in $CD_3OD/CDCl_3$ (1/1, *v/v*).
[c] Data recorded in $CDCl_3$.
[d] Data recorded in pyridine-d_5.
[e] Data recorded in CD_3OD.

Table 2
^{13}C NMR spectroscopic data for compounds **1–8**.[a]

Position	**1**[b]	**2**[c]	**3**[d]	**4**[e]	**5**[b]	**6**[d]	**7**[f]	**8**[d]
2	170.4	167.7	167.8	170.9	170.0	167.9	170.3	162.6
3	109.7	109.6	109.7	110.0	109.4	109.7	109.7	109.2
4	179.9	177.5	177.2	179.8	180.0	177.3	179.9	177.9
5	108.4	104.4	105.3	104.8	108.3	109.1	99.2	109.2
6	146.4	147.4	147.6	149.3	145.6	146.6	156.2	156.2
7	155.3	154.3	154.4	156.6	153.8	154.2	109.4	123.7
8	100.6	99.5	104.2	101.0	103.5	100.5	148.9	119.8
9	153.4	152.5	153.4	154.4	153.5	151.9	141.9	150.0
10	117.7	116.9	116.8	117.3	116.8	118.3	126.0	125.9
1′	134.0	133.0	133.8	132.7	132.9	131.0	134.3	127.6
2′	116.3	114.4	116.9	113.1	130.1	129.9	116.8	111.2
3′	147.3	145.6	148.3	148.9	114.7	116.4	147.4	149.1
4′	147.4	145.2	147.3	146.1	159.3	157.5	147.7	150.7
5′	112.7	110.7	112.6	116.2	114.7	116.4	112.8	117.0
6′	120.4	119.6	119.3	121.8	130.1	129.9	120.4	123.1
7′	33.4	32.4	32.6	33.8	33.0	32.5	33.2	137.4
8′	37.1	36.1	36.2	37.3	37.0	36.4	36.9	117.8
–OCH_3	56.9, (C-7) 56.4, (C-4′)	56.3 (C-6) 56.4 (C-7) 56.0 (C-4′)	55.9 (C-6) 56.0 (C-4′)	56.6 (C-6) 57.0 (C-7) 56.3 (C-3′)	55.6 (C-4′)	56.2 (C-7)	56.4 (C-4′)	55.9 (C-3′)

[a] Recorded at 100 MHz for compounds **4–7** and 150 MHz for others.
[b] Data recorded in $CD_3OD/CDCl_3$ (1/1, *v/v*).
[c] Data recorded in $CDCl_3$.
[d] Data recorded in pyridine-d_5.
[e] Data recorded in CD_3OD.
[f] Data recorded in CD_3OD/CD_3COCD_3 (1/1, *v/v*).

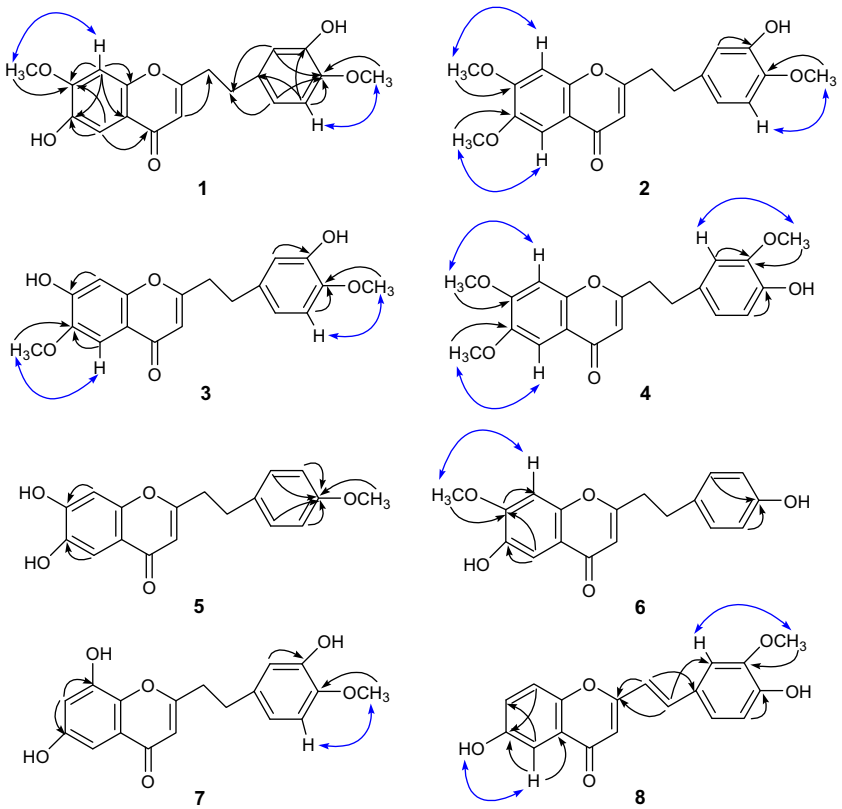

Fig. 2. Key HMBC (→) and NOE (↔) correlations of compounds **1–8**.

hydroxy groups. The position of an attached methoxy group at C-4′ was determined using the HMBC spectrum, in which the correlations of the methoxy protons (δ_H 3.76), H-2′/H-6′ and H-3′/H-5′ to the aromatic carbon (δ_C 159.3, C-4′) were observed (Fig. 2). Therefore, compound **5** was determined to be 6,7-dihydroxy-2-[2-(4′-methoxyphenyl)ethyl]chromone.

Compound **6** was thought to be another chromone derivative by the combined analyses of IR, HRESIMS, and NMR spectroscopic data, all of which were very similar to those of **5**. In the HMBC spectrum, correlations of the methoxy protons (δ_H 3.86), H-5 (δ_H 8.11) and H-8 (δ_H 7.12) with the aromatic carbon (δ_C 154.2) were observed (Fig. 2), indicating the presence of the methoxy group at C-6 or C-7. Compar-

ing the ^{13}C NMR spectroscopic data (δ_C 146.6, 154.2) of the chromone ring moiety with those of the reported compounds (Konishi et al., 2002) and compound **1**, it was concluded that the methoxy group was attached at C-7, which was further supported by the observation of H-8 enhancement when the methoxy protons were irradiated in the NOE experiment. Thus, **6** was identified as 6-hydroxy-7-methoxy-2-[2-(4′-hydroxyphenyl)ethyl]chromone.

The molecular formula of compound **7** was determined to be $C_{18}H_{16}O_6$ by HRESIMS (*m/z* 329.1013 $[M+H]^+$, calc. for $C_{18}H_{17}O_6$, 329.1020). Based on the combined analyses of the IR, ^1H NMR, and ^{13}C NMR spectroscopic data, **7** was another 2-(2-phenylethyl)chromone derivative with one methoxy group and three hydroxy groups. The ^1H NMR spectroscopic data (Table 1) of **7** showed the signals of two *meta*-coupled doublets at δ_H 6.79 (1H, *d*, *J* = 2.8 Hz) and δ_H 6.68 (1H, *d*, *J* = 2.8 Hz) in the chromone ring moiety, a methoxy group at δ_H 3.74 (3H, *s*), and a set of ABX coupling systems at δ_H 6.64 (1H, *d*, *J* = 2.0 Hz), 6.74 (1H, *d*, *J* = 8.0 Hz), and 6.57 (1H, *dd*, *J* = 2.0, 8.0 Hz) in the phenylethyl moiety. The linked position of the methoxy group at C-4′ was determined by analyses of the NOE spectrum, in which the integration value of H-5′ was enhanced when the methoxy protons at δ_H 3.74 was irradiated (Fig. 2). Thus, the position of the hydroxy group substituted in the phenylethyl moiety was designated as C-3′. The upfield of C-4 at δ_C 179.9 and downfield of C-10 at δ_C 126.0 confirmed that the two hydroxy groups were linked at C-6 and C-8 instead of C-5 and C-7. This deduction was supported by the comparison of its NMR spectroscopic data with those of reported compounds (Konishi et al., 2002; Nakanish et al., 1986; Shimada et al., 1982; Yagura et al., 2003; Yang et al., 1990; Yoon et al., 2006). Accordingly, **7** was identified as 6,8-dihydroxy-2-[2-(3′-hydroxy-4′-methoxyphenyl)ethyl]chromone.

Compound **8** was obtained as a yellow amorphous solid with the molecular formula of $C_{18}H_{14}O_5$, determined by combined analyses of HRESIMS, ^1H NMR, and ^{13}C NMR spectroscopic data. The IR spectrum exhibited the presence of hydroxy group(s) at 3373 cm^{-1}. Its ^1H NMR spectroscopic data (Table 1) showed two *trans*-olefinic protons at δ_H 7.77 (1H, *d*, *J* = 15.6 Hz, H-7′) and δ_H 7.02 (1H, *d*, *J* = 15.6 Hz, H-8′), two *ortho*-coupled aromatic doublets at δ_H 7.51 (1H, *brd*, *J* = 8.4 Hz) and δ_H 7.58 (1H, *d*, *J* = 8.4 Hz) and a broad singlet aromatic proton (δ_H 8.10, 1H, *brs*) in the chromone ring moiety. These data also indicated the presence of two *ortho*-coupled aromatic doublets at δ_H 7.32 (1H, *brd*, *J* = 7.8 Hz) and δ_H 7.25 (1H, *d*, *J* = 7.8 Hz), a broad singlet aromatic proton (δ_H 7.44, 1H, *brs*) in the phenylethenyl moiety, and methoxy protons (δ_H 3.81, 3H, *s*). Based on the analyses of the above data and the comparison of the ^1H and ^{13}C NMR spectroscopic data of **8** (Tables 1 and 2) with those of styrylchromones (Gerick et al., 1986; Gerick 1989), **8** was identified as a 2-(2-phenylethenyl)chromone derivative with one methoxy group and two hydroxy groups. The position of the attached methoxy group was determined to be at C-3′ using HMBC and NOE spectral experiments (Fig. 2). Therefore, one hydroxy group was attached at C-4′. The location of the other hydroxy group was determined to be at C-6 by HMBC and NOE experiments (Fig. 2). This result was supported by the comparison of the ^{13}C NMR spectroscopic data (Table 2) of the chromone ring moiety with those of a reported compound (Konishi et al., 2002; Shimada et al., 1982). Thus, the structure of **8** was identified as 6-hydroxy-2-[2-(4′-hydroxy-3′-methoxyphenyl)ethenyl]chromone.

Interestingly, it was concluded that these compounds may be biogenetically correlated with each other by comparing their chemical structures. For example, compounds **1**–**4** hypothetically originated from the same intermediate, 6,7-dihydroxy-2-[2-(3′,4′-dihydroxyphenyl)ethyl]chromone, being perhaps biosynthesized by *O*-methylation in different patterns by various *O*-methyltransferase in planta. Likewise, **5** and **6** maybe originate from the same intermediate, 6,7-dihydroxy-2-[2-(4′-hydroxyphenyl)ethyl]chromone. All the compounds are considered by biogenetically formed from the same precursor through different chemical reactions, such as reduction, hydroxylation and *O*-methylation, etc.

Table 3
Neuroprotective activity of compounds **1**–**8** against glutamate-induced neurotoxicity in P12 pheochromocytoma cells and corticosterone-induced neurotoxicity in human U251 glioma cells.[a]

Compound	Protective activity (%)[b]	
	Glutamate-induced neurotoxicity	Corticosterone-induced neurotoxicity
Control	100 ± 4.6	100 ± 2.4
Glutamate-treated	0 ± 3.5	–
Corticosterone-treated	–	0 ± 2.6
1	61.6 ± 2.3	32.9 ± 0.5
2	64.8 ± 1.5	−14.4 ± 2.3***
3	42.7 ± 2.5	−6.8 ± 2.2
4	34.6 ± 1.3	−10.5 ± 1.2***
5	65.8 ± 3.2	17.4 ± 1.2
6	25.2 ± 2.5	−2.8 ± 0.6
7	82.2 ± 2.0***	86.9 ± 2.6***
8	58.3 ± 2.8	−8.6 ± 1.7*
Fraction 4 (100 μg/ml)	32.5 ± 5.6	21.3 ± 3.5
Fluoxetine	92.5 ± 3.2***	93.7 ± 2.1***

[a] Concentrations of compound **1–8**, fluoxetine, glutamate and corticosterone were 10.0 μM.
[b] Protection (%) was calculated as 100 × [optical density (OD) of test compound + glutamate (corticosterone) − treated culture − OD of glutamate (corticosterone) − treated culture]/[OD of control culture − OD of glutamate(corticosterone) − treated culture]. The values are expressed as the mean ± SD of triplicate experiments.
* $P < 0.05$.
*** $P < 0.001$.

The neuroprotective activities of compounds **1**–**8** together with fluoxetine, a positive control, were evaluated against glutamate-induced neurotoxicity in P12 pheochromocytoma cells and corticosterone-induced neurotoxicity in human U251 glioma cells using the MTT assay, as described in the Section 3. As shown in Table 3, for the P12 cell model, all of the compounds exhibited positive activities, whereas for the U251 cell model, only **7** displayed remarkable activity. Compound **7** showed comparable neuroprotective activity with fluoxetine by increasing cell viability by 82.2% and 86.9% in the two cell models at 10 μM, respectively. The ED_{50} values of **7** and fluoxetine were 6.5/5.2 μM and 3.0/2.6 μM for the two cell types, respectively. Interestingly, when the structure of **7** was compared with those of the other seven compounds, it can be hypothesised that the more potent activity of **7** is attributed to its substituted pattern [6,8-disubstituted groups instead of 6,7-disubstituted groups (compounds **1**–**6**)] and/or a group [a 6-mono-substituted (compound **8**) group] in the chromone ring moiety. Furthermore, the hydroxy group at C-8 may be crucial for the neuroprotective activities of this type of compounds. Undoubtedly, the further extensive investigation is still necessary to validate this hypothesis.

3. Experimental

3.1. General experimental procedures

Melting point measurement were uncorrected UV spectra were recorded on a Cary 300 spectrometer, IR spectra were measured on a Nicolet 5700 FT-IR microscope spectrometer. 1D and 2D NMR spectra were acquired on a Varian MP-400 or VNS-600 spectrometer. EI mass spectra were obtained on an AutoSpec Ultima-TOF mass spectrometer (Micromass, UK), whereas HRESIMS spectra were measured on a Q-trap ESI mass spectrometer. Semi-preparative normal-phase HPLC was performed on a Shimadzu LC-6AD

instrument equipped with a Shimadzu RID-10A detector and a Grace Allsphere silica column (250 mm × 10 mm, i.d., 5 μm, flow rate 4 ml/min). Silica gel (200–300 mesh; Qingdao Mar. Chem. Inc., China) was used for column chromatography (cc). Sephadex LH-20 was purchased from Pharmacia (Amersham Biosciences). TLC was carried out on pre-coated silica gel GF-254 plates (Qingdao Mar. Chem. Inc., China) and visualised by illumination with an ultraviolet lamp and spraying with 5% H_2SO_4 in EtOH followed by heating at 105 °C.

3.2. Plant materials

Chinese eaglewood was purchased in January 2009 from an herbal medicine market in Wenchang County, Hainan Province, People's Republic of China, and was morphologically identified in these ways: Visually by one of the authors, Professor Shunxing Guo, similarity of an extract of the plant material using HPLC analyses and comparison with an authentic sample. A voucher specimen (No. 2009-01-AS) is deposited at the herbarium of the Institute of Medicinal Plant Development, Chinese Academy of Medicinal Sciences & Peking Union Medical College.

3.3. Extraction and isolation

Chinese eaglewood (7.5 kg, dry weight) was first crushed and extracted (3 × 8 h) with petroleum ether (60–90 °C) under conducted where reflux occurred. The residual plant materials were then further extracted by EtOH (3 × 8 L) under conductors of reflux to yield a brown powder (935 g) after removal of the solvent under reduced pressure. The EtOH extract (783 g) was subjected to silica gel (200–300 mesh) cc and successively eluted with petroleum ether (60–90 °C), petroleum (60–90 °C) ether–EtOAc (4:1, 1:1, 1:2, 1:5, *v/v*), EtOAc, acetone and MeOH. This resulted in eight fractions (Fr. 1–8). Silica gel cc of Fr. 4 (43.8 g) with petroleum ether–EtOAc (1:2) resulted in six sub-fractions (Fr. 4–1–Fr. 4–6). Fr. 4–5 (19.0 g) was subjected to silica gel cc and further purified by semi-preparative normal-phase HPLC with n-hexane–EtOAc (1:2, *v/v*) to yield **1** (86 mg, *R*t 9.9 min), **2** (58 mg, *R*t 11.7 min), **3** (16 mg, *R*t 15.1 min), and **4** (6 mg, *R*t 20.9 min). Subsequently, **5** (30 mg) was obtained from a Sephadex LH-20 column eluted with MeOH after normal-phase semi-preparative HPLC (*R*t 12.4 min). Fr. 4-2 (5.9 g) was subjected to silica gel cc by eluting with (60–90 °C) petroleum ether–EtOAc (2:1–1:3, *v/v*) to give six sub-fractions (Fr. 4-2-1–Fr. 4-2-6). Compound **7** (129 mg) was separated out as an amorphous solid from the solution of Fr. 4-2-3 in EtOAc–MeOH (1:1, *v/v*), and the 'mother liquor' solution was further purified by normal-phase semi-preparative HPLC with n-hexane–EtOAc (1:1, *v/v*) to give **6** (15 mg, *R*t 21.7 min). Fr. 4-2-2 was first separated using a Sephadex LH-20 column with MeOH as the eluent and then purified by normal-phase semi-preparative HPLC with n-hexane–EtOAc (3:2, *v/v*) to give **8** (4 mg, *R*t 37.5 min).

3.3.1. 6-Hydroxy-7-methoxy-2-[2-(3′-hydroxy-4′-methoxyphenyl) ethyl]chromone (**1**)

White, amorphous solid; UV (MeOH) λ_{max} (log ε) 231 (4.48), 281 (4.09), 321 (4.00) nm; IR ν_{max} cm^{-1}: 3313 (OH), 2465, 1628, 1585 (γ-pyrone), 1514, 1474, 1395, 1271, 1235, 1212, 1080, 977, 949, 842, 826; For ^1H NMR [$CDCl_3$/CD_3OD (1/1, *v/v*), 600 MHz] and ^{13}C NMR [$CDCl_3$/CD_3OD (1/1, *v/v*), 150 MHz] spectroscopic data, see Tables 1 and 2; EIMS, 70 eV, *m/z* (rel. int.): 342 $[M]^+$ (60), 324 (7), 253 (6), 206 (72), 149 (34), 137 (100), 83 (27), 72 (25), 58 (38); positive HRESIMS, *m/z*: 343.1168 $[M + H]^+$ (calc. for $C_{19}H_{19}O_6$, 343.1176).

3.3.2. 6,7-Dimethoxy-2-[2-(3′-hydroxy-4′-methoxyphenyl) ethyl]chromone (**2**)

Yellow, amorphous solid; UV (MeOH) λ_{max} (log ε) 232 (4.43), 280 (4.04), 316 (3.96) nm; IR ν_{max} cm^{-1}: 3359 (OH), 1639, 1595, 1505, 1473, 1429, 1386, 1337, 1270, 1237, 1196, 1080, 1037, 968, 841, 817; For ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) spectroscopic data, see Tables 1 and 2, respectively; EIMS, 70 eV, *m/z* (rel. int.): 356 $[M]^+$ (75), 338 (7), 220 (100), 191 (9), 149 (16), 137 (81), 83 (15), 72 (17), 58 (24); positive HRESIMS, *m/z*: 357.1333 $[M + H]^+$ (calc. for $C_{20}H_{21}O_6$, 357.1333).

3.3.3. 7-Hydroxy-6-methoxy-2-[2-(3′-hydroxy-4′-methoxy-phenyl) ethyl]chromone (**3**)

Colourless needles (MeOH); mp 199–201 °C; UV (MeOH) λ_{max} (log ε) 282 (4.23), 326 (4.15) nm; IR ν_{max} cm^{-1}: 3432 (OH), 1630, 1586, 1544, 1509, 1482, 1424, 1394, 1281, 1254, 1218, 974, 849, 815; For ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) spectroscopic data, see Tables 1 and 2, respectively; positive HRESIMS, *m/z*: 343.1178 $[M + H]^+$ (calc. for $C_{19}H_{19}O_6$, 343.1176).

3.3.4. 6,7-Dimethoxy-2-[2-(4′-hydroxy-3′-methoxyphenyl)ethyl] chromone (**4**)

Yellow, amorphous solid; UV (MeOH) λ_{max} (log ε) 232 (4.44), 280 (4.03), 315 (3.93) nm; IR ν_{max} cm^{-1}: 3288 (OH), 2923, 1652, 1541, 1464, 1404, 1242, 1172, 1083, 972, 721; For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Tables 1 and 2, respectively; positive HRESIMS, *m/z*: 357.1314 $[M + H]^+$ (calc. for $C_{20}H_{21}O_6$, 357.1333).

3.3.5. 6,7-Dihydroxy-2-[2-(4′-methoxyphenyl)ethyl]chromone (**5**)

Blueviolet, amorphous solid; UV (MeOH) λ_{max} (log ε) 225 (4.70), 281 (4.12), 327 (4.16) nm; IR ν_{max} cm^{-1}: 3450 (OH), 1632, 1558, 1512, 1457, 1393, 1271, 1241, 1179, 1034, 973, 837, 562; For ^1H NMR [$CDCl_3$/CD_3OD (1/1, *v/v*), 400 MHz] and ^{13}C NMR [$CDCl_3$/CD_3OD (1/1, *v/v*), 100 MHz] spectroscopic data, see Tables 1 and 2, respectively; EIMS, 70 eV, *m/z* (rel. int.): 312 $[M]^+$ (12), 191 (8), 163 (6), 149 (16), 121 (100), 83 (23), 72 (37), 58 (53); positive HRESIMS, *m/z*: 313.1070 $[M + H]^+$ (calc. for $C_{18}H_{17}O_5$, 313.1071).

3.3.6. 6-Hydroxy-7-methoxy-2-[2-(4′-hydroxyphenyl)ethyl]chromone (**6**)

White, amorphous solid; UV (MeOH) λ_{max} (log ε) 229 (4.38), 279 (3.95), 323 (3.89) nm; IR ν_{max} cm^{-1}: 3178 (OH), 1635, 1621, 1566, 1509, 1487, 1383, 1276, 1215, 1081, 968, 847; For ^1H (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectroscopic data, see Tables 1 and 2, respectively; positive HREIMS, *m/z*: 313.1066 $[M + H]^+$ (calc. for $C_{18}H_{17}O_5$, 313.1071).

3.3.7. 6,8-Dihydroxy-2-[2-(3′-hydroxy-4′-methoxyphenyl) ethyl]chromone (**7**)

Sandybrown, amorphous solid; UV (MeOH) λ_{max} (log ε) 242 (4.52), 338 (3.74) nm; IR IR ν_{max} cm^{-1}: 3349 (OH), 2538, 1627, 1585, 1552, 1514, 1399, 1302, 1278, 1140, 1031, 987, 861, 845; For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR [CD_3OD/CD_3COCD_3 (1/1, *v/v*), 100 MHz] spectroscopic data, see Tables 1 and 2, respectively; positive HRESIMS, *m/z*: 329.1013 $[M + H]^+$ (calc. for $C_{18}H_{17}O_6$, 329.1020).

3.3.8. 6-Hydroxy-2-[2-(4′-hydroxy-3′-methoxyphenyl) ethenyl]chromone (**8**)

Golden red, amorphous solid; UV (MeOH) λ_{max} (log ε) 258 (3.75), 365 (3.92) nm; IR ν_{max} cm^{-1}: 3373 (OH), 3276, 1665, 1632, 1611, 1564, 1475, 1400, 1283, 1257, 1234, 1160, 1124, 1032, 983, 965, 849; For ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C

NMR (pyridine-d_5, 150 MHz) spectroscopic data, see Tables 1 and 2, respectively; positive HRESIMS, *m/z*: 311.0914 $[M + H]^+$ (calc. for $C_{18}H_{15}O_5$, 311.0914).

3.4. Assessment of neuroprotective activity

Glutamate, corticosterone and fluoxetine hydrochloride were purchased from Sigma Chemical Co. (St Louis MO, USA). Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, Sigma) reduction assay. Briefly, the PC12 pheochromocytoma or U251 glioma cells were plated on a 96-well plate and cultured with glutamate or corticosterone with or without each compound at 10^{-5} M for 2 days. After incubation with MTT for 4 h in the dark at 37 °C, the cells were lysed with DMSO, and the reduced MTT was measured in the 96-well plate at 570 nm using a spectrophotometer (Molecular Devices, Spectra Max 190). All readings were compared with the control, which represented 100% viability. The compound was considered neuroprotective when the cell viability of the compound group was significantly enhanced ($^*p < 0.05$) when compared with that of the glutamate/corticosterone group. The ED_{50} values of the agents, defined as the concentration that results in a 50% increase in the number of cells compared with glutamate/corticosterone-treated cells after 2 days of incubation were determined directly from the semi-logarithmic dose–response curves. All data are presented as the mean ± standard error of the mean. The data were subjected to Student's *t*-test to determine whether the means differed significantly from the control.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2011.11.017.

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