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•Research article•

Ascyrones A−E, type B bicyclic ployprenylated acylphloroglucinol derivatives from *Hypericum ascyron*

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[ABSTRACT] Five new polycyclic polyprenylated acylphloroglucinols (**1**−**5**), ascyrones A−E, and four known compounds (**6**−**9**) were isolated from the aerial parts of *Hypericum ascyron*. All of the isolates containing a bicyclo[3.3.1]nonane-2,4,9-trione core and a benzoyl group, belonged to type B bicyclic polyprenylated acylphloroglucinols (BPAPs). Their structures and absolute configurations were established based on spectroscopic analyses and calculated electronic circular dichroism (ECD) data. The anti-inflammatory, neuroprotective and cytotoxicity activities of compounds **1**−**4** and **6**−**9** were evaluated. Compound **6** exhibited obvious anti-inflammatory activity in lipopolysaccharide (LPS)-induced RAW264.7 cells. Compounds **1** and **9** exhibited slight cytotoxicity against Hep3B cells. Meanwhile, compound **1** showed mild neuroprotective activity against corticosterone (CORT)-induced PC12 cell damage at 10 μmol·L^{-1}.

[KEY WORDS] *Hypericum ascyron*; Acylphloroglucinol; Anti-inflammatory activity; Neuroprotective activity
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Introduction

Polycyclic polyprenylated acylphloroglucinols (PPAPs), exclusively isolated from the plants of the *Hypericaceae* and *Clusiaceae* families, are attractive targets for chemical and pharmacological communities due to their fascinating structures and various biological activities [1-5]. *Hypericum ascyron* (family *Hypericaceae*), widely distributed in China, has been used as an herbal tea and a folk medicine to treat diarrhea, hematemesis and uterine bleeding [6]. In previous studies, a series of PPAPs have been reported from this plant, which possess extensive bioactivities, such as anti-inflammatory, antitumor, antidepressant, antibacterial, and neuroprotective activities [7-11]. As a part of our search for new and bioactive natural acylphloroglucinol derivatives [12, 13], the aerial parts of *H. ascyron* were phytochemically investigated. Five new PPAPs, ascyrones A−E (**1**−**5**), along with four known derivatives, longistyliones B–D (**8**, **6**, **7**) [14] and hyperascyrin N (**9**) [15], were obtained. All of the isolates contained a bicyclo[3.3.1]nonane-2,4,9-trione core and a benzoyl group at C-8, which are classified as type B bicyclic polyprenylated acylphloroglucinols (BPAPs) [1]. Moreover, BPAPs derived from this plant feature a methyl substituent at C-6 rather than a prenyl or geranyl group in most of other PPAPs, which suggests that a methylation may occur during the formation of acylphloroglucinol cores. Their structures were elucidated by analysis of the spectroscopic data and ECD data. Compounds **1**−**4** and **6**−**9** were evaluated for their cytotoxicity, neuroprotective and anti-inflammatory activities. Herein, the isolation, structural elucidation, and bio-evaluation of these compounds are reported.

Results and Discussion

Ascyrone A (**1**) was obtained as colorless oil, and its molecular formula, $C_{34}H_{44}O_6$, was determined by HRESIMS (*m/z* 549.3211 $[M + H]^+$), indicative of 13 sites of unsaturation. The UV spectrum showed the typical absorbances of a benzoyl group at 202 (log ε 3.64) and 252 (log ε 3.12) nm, corresponding to the ^{1}H NMR signals (δ_H 7.46, 7.58 and 7.86). Analysis of its ^{1}H NMR spectrum also revealed the presence of three olefinic protons (δ_H 5.11, 5.69 and 5.92) and eight methyl groups (Table 1). The ^{13}C NMR data showed three carbonyl resonances (δ_C 206.6, 193.5 and 194.1), four olefinic carbons in prenyl groups (δ_C 118.8, 121.9, 134.1 and 144.3), and four methylene groups (δ_C 42.3,

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These authors have no conflict of interest to declare.

Table 1 ^{1}H NMR and ^{13}C NMR spectra data for compounds 1−3 (*J* in Hz, δ in ppm)

	1		**2**		**3**	
No.	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		206.6		206.2		206.4
2		65.3		65.0		64.2
3		44.6		44.4		44.7
4	2.52 (1H, m)	36.6	3.19 (1H, m)	39.5	3.12 (1H, m)	39.3
5	2.29 (1H, dd, 14.0, 5.0)	42.3	2.09 (1H, dd, 14.0, 5.0)	42.3	2.04 (1H, dd, 14.0, 5.0)	42.5
	1.54 (1H, t, 13.3)		1.82 (1H, t, 13.3)		1.74 (1H, t, 13.5)	
6		61.0		60.8		60.4
7		193.5		193.3		193.8
8		129.2		129.1		129.6
9		173.1		173.1		173.4
10	5.92 (1H, d, 16.5)	118.8	5.90 (1H, d, 16.5)	118.4	3.12 (1H, m)	24.7
					2.46 (1H, dd, 15.0, 7.4)	
11	5.69 (1H, d, 16.5)	144.3	5.67 (1H, d, 16.5)	144.6	4.92 (1H, t, 6.8)	118.7
12		71.4		70.9		134.9
13	1.39 (3H, s)	29.9	1.40 (3H, s)	30.3	1.72 (3H, s)	18.4
14	1.31 (3H, s)	29.5	1.31 (3H, s)	29.4	1.69 (3H, s)	26.2
15	0.92 (3H, s)	17.4	0.92 (3H, s)	17.5	0.83 (3H, s)	17.2
16	2.01 (1H, dd, 15.0, 6.0)	35.3	1.78 (1H, m)	36.4	1.83 (1H, m)	36.0
	1.67 (1H, m)		1.64 (1H, m)		1.71 (1H, overlap)	
17	1.73 (1H, overlap)	24.0	1.77 (1H, m)	23.8	1.84 (2H, m)	23.5
	1.62 (1H, m)		1.62 (1H, m)			
18	4.23 (1H, d, 9.5)	88.8	4.23 (1H, d, 8.0)	88.6	4.13 (1H, m)	90.0
19		72.4		72.5		72.7
20	0.89 (3H, s)	25.3	0.90 (3H, s)	25.5	0.95 (3H, s)	25.6
21	0.89 (3H, s)	25.3	0.89 (3H, s)	25.1	0.95 (3H, s)	25.5
22	2.17 (1H, m)	27.9	5.41 (1H, dd, 15.5, 8.5)	123.7	5.38 (1H, dd, 15.0, 8.5)	123.9
	1.72 (1H, overlap)					
23	5.11 (1H, t, 7.0)	121.9	5.77 (1H, d, 15.5)	143.0	5.76 (1H, d, 15.5)	142.9
24		134.1		71.2		70.9
25	1.61 (3H, s)	18.1	1.31 (3H, s)	30.2	1.31 (3H, s)	30.3
26	1.75 (3H, s)	26.1	1.30 (3H, s)	30.1	1.30 (3H, s)	30.1
27		194.1		194.2		195.7
28		137.4		137.3		137.1
29	7.86 (1H, d, 8.0)	129.0	7.86 (1H, d, 7.5)	129.0	7.82 (1H, d, 7.0)	129.2
30	7.46 (1H, t, 7.5)	129.0	7.47 (1H, t, 7.5)	129.0	7.43 (1H, t, 7.5)	128.9
31	7.58 (1H, t, 7.5)	134.0	7.59 (1H, t, 7.5)	134.0	7.55 (1H, t, 7.0)	134.0
32	7.46 (1H, t, 7.5)	129.0	7.47 (1H, t, 7.5)	129.0	7.43 (1H, t, 7.5)	128.9
33	7.86 (1H, d, 8.0)	129.0	7.86 (1H, d, 7.5)	129.0	7.82 (1H, d, 7.0)	129.2
34	1.26 (3H, s)	16.8	1.28 (3H, s)	16.7	1.29 (3H, s)	16.6

500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$.

35.3, 27.9, 24.0). The characteristic signals of two carbonyls at δ_C 206.6 (C-1) and 193.5 (C-7), two quaternary carbons at δ_C 61.0 (C-6) and 65.3 (C-2), two methyl groups at δ_C 18.1 (C-25) and 26.1 (C-26) indicated that **1** could be a PPAP derivative with a bicyclo[3.3.1]nonane core [1].

A comparison of its 1D NMR data with those of longistyliones C (**6**) [14], a known BPAP with bicyclo[3.3.1] nonane core, revealed that an isopentene group at C-2 in **6** was replaced by a 3-hydroxy-3-methyl-1-butene group in **1**. The presence of H-10 (δ_H 5.92), H-11 (δ_H 5.69) and C-12 (δ_C 71.4) was in accordance with this deduction. In the HMBC spectrum, the correlations (Fig. 2) between H_3-13 and C-12/C-11, between H-10 and C-2/C-3/C-9, and between H-11 and C-2 indicated the 3-hydroxy-3-methyl-1-butene group attached to C-2.

The relative configuration of **1** was established by its ROESY spectrum (Fig. 3). The cross-peaks from H_3-15 (δ_H 0.92) to H-22 (δ_H 2.17), H-5 (δ_H 1.54), and H-10 (δ_H 5.92) indicated that these protons were on the same side and arbitrarily designated as *α*-orientation. The correlations of H-18 (δ_H 4.23) with H-10 (δ_H 5.92) and H_3-25 (δ_H 1.61) suggested that H-18 was *α*-oriented. The absolute configuration of **1** was established by comparing experimental and calculated ECD data. The calculated ECD spectrum (Fig. 6) of **1** was in agreement with experimental data, indicating a (2*R*,3*S*,4*R*,6*R*,18*R*) absolute configuration for **1**. Thus, the structure of **1** was established as shown in Fig. 1.

Ascyrone B (**2**) was afforded as colorless oil, and its molecular formula was assigned as $C_{34}H_{44}O_7$ on the basis of its HRESIMS [*m/z* 565.3152 $[M + H]^+$ (Calcd. for 565.3160)]. The molecular formula showed an extra oxygen compared to **1** ($C_{34}H_{44}O_6$). The same benzoyl moiety and 3-hydroxy-3-methyl-1-butene group as those in compound **1** were also inferred from the characteristic proton and carbon signals of **2**

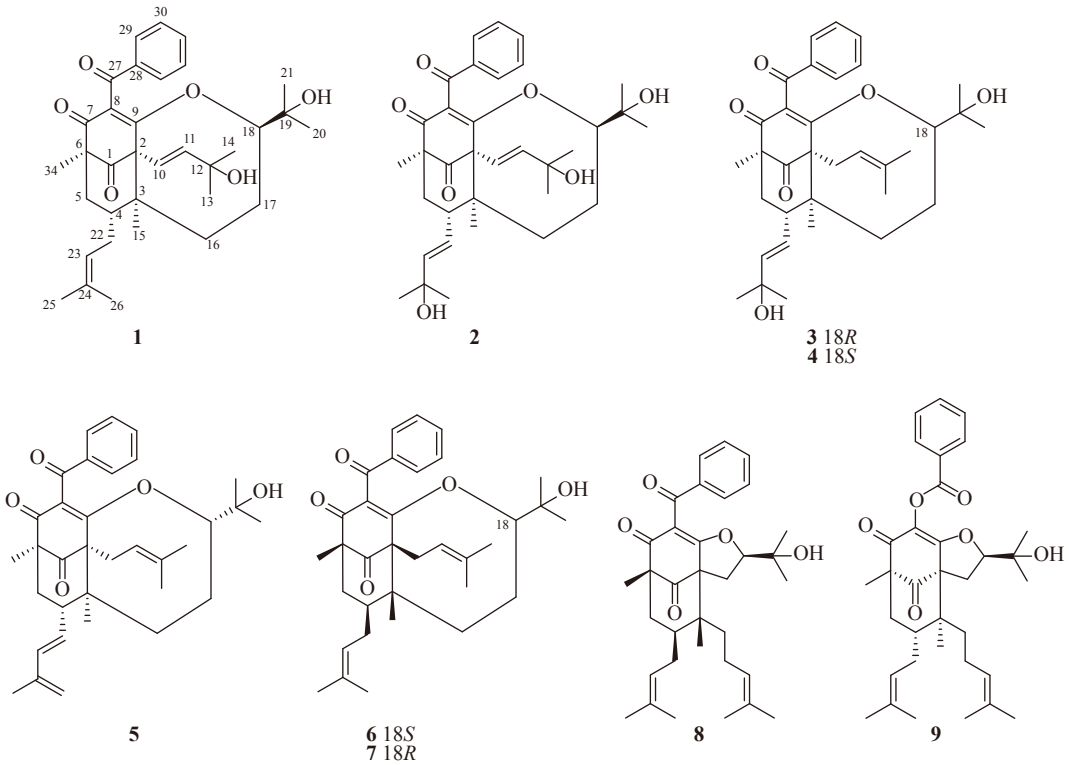

Fig. 1 Compounds 1−9 from the aerial parts of *H. ascyron*

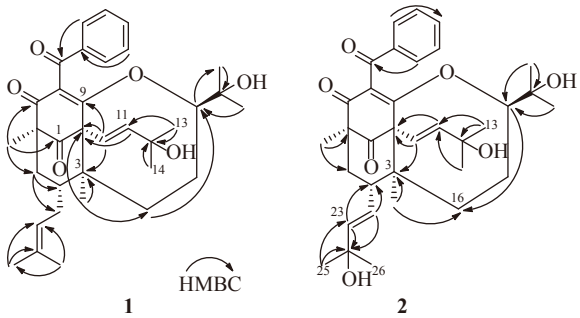

Fig. 2 Key HMBC correlations of 1 and 2

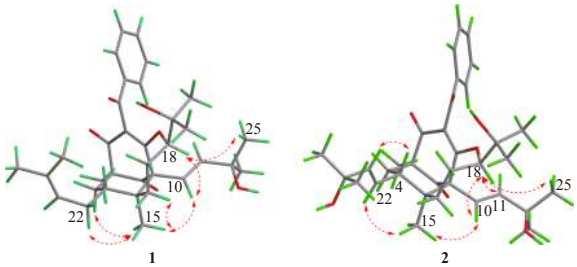

Fig. 3 Key ROESY correlations of 1 and 2

(Table 1). However, the signals for isopentene group at C-4 were absent in **2**. The presence of H-22 (δ_H 5.41), H-23 (δ_H 5.77) and C-24 (δ_C 71.2), combined with its molecular formula ($C_{34}H_{44}O_7$) indicated that the isopentene group at C-4 in **1** was replaced by a 3-hydroxy-3-methyl-1-butene group in **2**. This deduction was further confirmed by the cross-peak signals in the HSQC and HMBC spectra, especially HMBC correlations (Fig. 2) from H_3-25 (δ_H 1.31) to C-24 (δ_C 71.2), C-23 (δ_C 143.0), from H-23 (δ_H 5.77) to C-4 (δ_C 39.5), C-24 (δ_C 71.2), and from H-22 (δ_H 5.41) to C-24 (δ_C 71.2), C-4 (δ_C 39.5). Thus, the planar structure of **2** was demonstrated as shown in Fig. 1.

The relative configuration of compound **2** was established through analysis of its ROESY spectrum (Fig. 3). The cross-peaks of H_3-15 with H-22/H-10 indicated that these protons were on the same side and arbitrarily designated as *α*–orientation. The correlations of H-18 with H-10, H_3-25, and H-11 suggested that H-18 was also *α*–oriented. The absolute configuration of **2** was determined by comparison of its experimental and calculated ECD data (Fig. 6), and shown to be 2*R*,3*S*,4*S*,6*R*,18*R*.

Ascyrone C (**3**) had the same molecular formula as **1**, according to the HRESIMS *m/z* 549.3208 $[M + H]^+$ (Calcd. for $C_{34}H_{45}O_6$, 549.3211). Comparison of the NMR data (Table 1) of **3** and **1** showed the presence of H-22 (δ_H 5.38) and H-11 (δ_H 4.92), the deshielded chemical shift of C-4 (Δδ +2.7), and the shielded chemical shift of C-2 (Δδ −1.1), which suggested that a 3-hydroxy-3-methyl-1-butene group was attached to C-4 and an isopentene group was positioned at C-2 in **3**. This assumption was supported by the HMBC correlations (Fig. 4) of H-5 to C-22, H-22 to C-4, H-25 to C-23, H-26 to C-24, H-10 to C-3/C-1, H-11 to C-2/C-10/C-13, and H_3-14 to C-12/C-11. Therefore, compound **3** was established as shown in Fig. 1.

In the ROESY spectrum of **3**, correlations between H-18 and H-10/H_3-13, and between H_3-15 and H-22/H-10 were observed, which were found to be in accordance with those of **1** and **2**. The 2*R*,3*S*,4*S*,6*R*,18*R* absolute configuration of **3** was defined by analysis of the experimental and calculated ECD data (Fig. 6).

Ascyrone D (**4**) was also obtained as colorless oil and its molecular formula was the same as **3**. A comparison of the ^{1}H and ^{13}C NMR data of **3** and **4** (Tables 1 and 4) suggested that the planar structure of **4** was identical to **3**. The chemical shift differences of H-11 (Δδ +0.12), H-18 (Δδ −0.23), H_3-14 (Δδ −0.09), and H_3-21 (Δδ −0.24) revealed that the orientation of H-18 was different in **4**. The *β*-orientation of H-18 in **4** was based on the ROESY correlations (Fig. 5) of H_3-13 (δ_H 1.75) to H_3-21 (δ_H 0.71) and H_3-20 (δ_H 0.98). As shown in Fig. 6, the predicted ECD spectrum for (2*R*,3*S*,4*S*,6*R*,18*S*)-**4** was in agreement with the experimental ECD spectrum of **4**. Thus, the structure of compound **4** was established as shown in Fig. 1.

Ascyrone E (**5**) has a molecular formula of $C_{34}H_{42}O_5$ deduced by HRESIMS. Compound **5** has one more site of unsaturation than compound **4**. The ^{1}H NMR data of **4** and **5** (Table 4) were similar except for the presence of H-23 (δ_H 6.35), H_2-25 (δ_H 4.98) and H_3-26 (δ_H 1.84) in compound **5**. The ^{13}C NMR data of **5** (Table 4) showed five carbon resonances: four olefinic carbons (δ_C 128.7, 137.2, 143.0, 116.9) and one methyl carbon (δ_C 18.6). The HMBC correlations (Fig. 4) from H_3-26 (δ_H 1.84) to C-24 (δ_C 143.0)/C-25 (δ_C 116.9), from H_2-25 (δ_H 4.98) to C-24 (δ_C 143.0)/C-23 (δ_C 137.2), and from H-22 (δ_H 5.48) to C-24 (δ_C 143.0)/C-25 (δ_C 116.9) were observed, which were typical of an isoprenyl moiety. The isoprenyl group is positioned at C-4, which was confirmed by the HMBC correlation (Fig. 4) between H-22 (δ_H 5.48) and C-5 (δ_C 44.8).

In the ROESY spectrum, the correlations (Fig. 5) of H_3-13 with H_3-21 and H_3-20 indicated that H-18 was *β*-oriented. Furthermore, the correlations of H_3-15 with H-22 and H-10 suggested that they were *α*-orientation. The absolute configuration of **5** was determined by comparison of its experimental and calculated ECD data (Fig. 6) and assigned as 2*R*,3*S*,4*S*,6*R*,18*S*.

The neuroprotective activities of compounds **1**–**4** and **6**–**9** were investigated against CORT-induced injury in PC12 cells. Compared with the negative group, compounds **1**, **2**, **8** and **9** exhibited neuroprotective effects, with cell viabilities of (75.6 ± 4.3)%, (73.0 ± 1.2)%, (69.6 ± 5.7)%, and (71.8 ± 4.0)%, respectively, under the concentration of 10 μmol·L^{-1} (Table 2). Compounds **1**–**4** and **6**–**9** were tested for anti-inflammatory activity by inhibiting NO production in LPS-activated RAW264.7 cells. As shown in Table 3, compounds **3, 6, 7, 8** and **9** showed anti-inflammatory effects, with IC_{50} value of (21.7 ± 1.8), (3.9 ± 3.3), (10.3 ± 2.4), (19.9 ± 2.1),

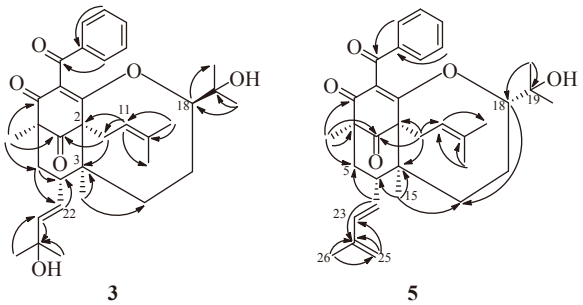

Fig. 4 Key HMBC correlations of 3 and 5

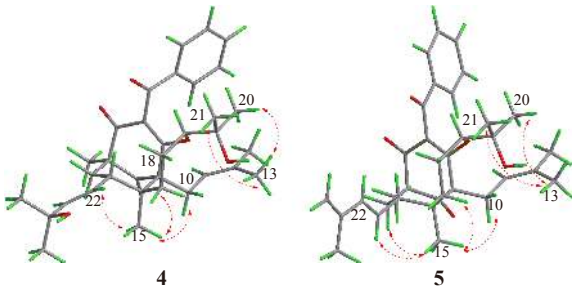

Fig. 5 Key ROESY correlations of 4 and 5

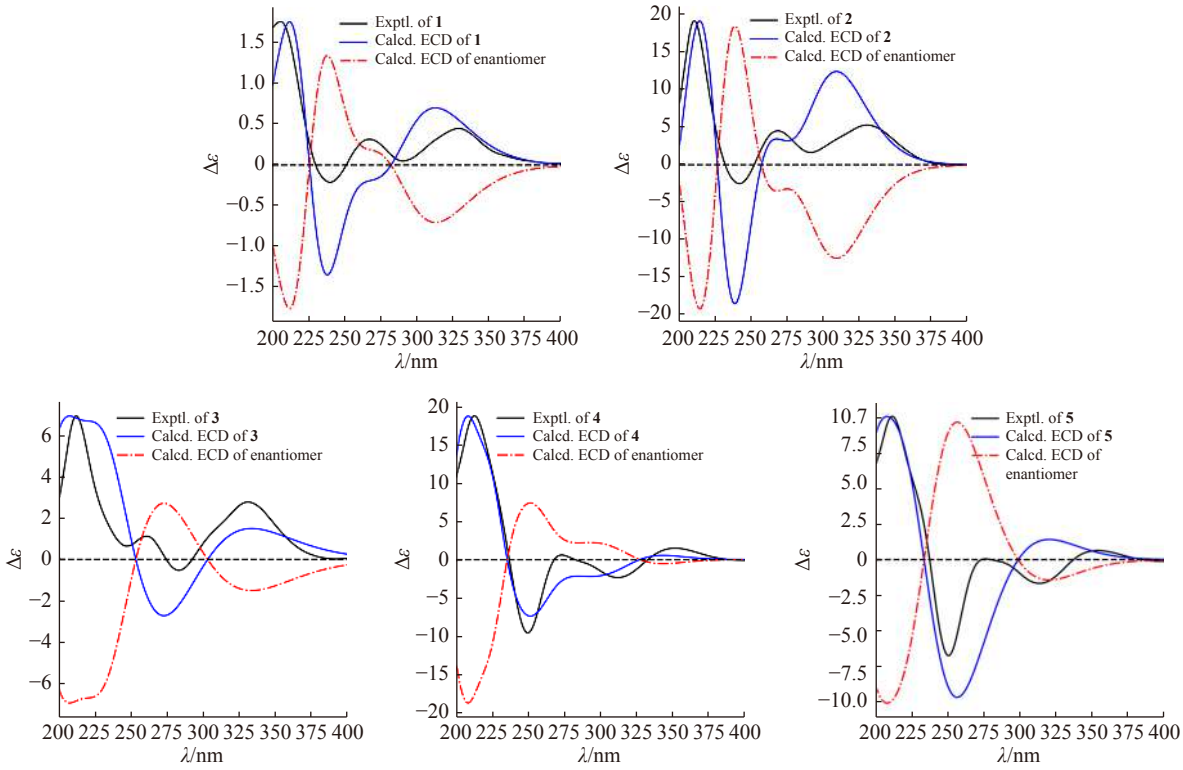

Fig. 6 Calculated and experimental ECD spectra of 1−5

Table 2 Neuroprotective effects of selected compounds (10 μmol·L^{-1}) against CORT-induced injury in PC12 cells[a]

Compound	Cell viability (%)
Normal	100 ± 0.0
Negative control	65.3 ± 0.9[b]
Positive control	82.3 ± 1.4[c]
1	75.6 ± 4.3[c]
2	73.0 ± 1.2[c]
3	70.2 ± 4.9
4	67.2 ± 1.1
6	66.1 ± 5.8
7	69.2 ± 0.5
8	69.6 ± 5.7[c]
9	71.8 ± 4.0[c]

[a] Results are expressed as the means ± SD (n = 3; for normal and control, n = 6), desipramine was used as positive control (10 μmol·L^{-1}). [b]$P < 0.05$ *vs* normal. [c]$P < 0.05$ *vs* negative control

and (21.6 ± 1.5) μmol·L^{-1}, respectively. Meanwhile, Compounds **1**−**4** and **6**−**9** were also tested for their cytotoxic activities against human hepatoma Hep3B and SNU-387 cells. Most of the compounds tested did not show any discernible inhibitory effects against these two types of cells, with the exception of compounds **1** and **9**, which exhibited inhibitory effects on Hep3B cells with IC_{50} value of (23.3 ± 1.7) and (11.4 ± 0.9) μmol·L^{-1}, respectively. Compound **9** also showed cytotoxicity on SNU-387 cells with IC_{50} values of (35.9 ± 2.6) μmol·L^{-1}.

Table 3 IC_{50} vaules of selected compounds inhibiting NO production in LPS-induced RAW264.7 cells[a]

Compound	IC_{50} (μmol·L^{-1})
3	21.7 ± 1.8
6	3.9 ± 3.3
7	10.3 ± 2.4
8	19.9 ± 2.1
9	21.6 ± 1.5

[a] Other isolates with $IC_{50} > 40$ μmol·L^{-1} are not listed. Indomethacin was used as a positive control. Results are expressed as mean ± SD from triplicate experiments

The present phytochemical investigation of *H. ascyron* led to the isolation of five new and four known BPAPs. All these compounds were substituted with a methyl group at C-6 instead of a prenyl or geranyl group in many other PPAPs, which may be a key characteristic to identify the title plant. Their structures were elucidated on the basis of extensive NMR spectroscopic data analyses, and their absolute configurations were established by comparison of their experimental and calculated ECD spectra. Compound **1** displayed mild neuroprotective activity at 10 μmol·L^{-1}. Evaluation of their anti-inflammatory activity highlighted compound **6**, which showed potent anti-inflammatory effect on LPS-induced

Table 4 ^{1}H NMR and ^{13}C NMR spectra data for compounds 4 and 5 (*J* in Hz, δ in ppm)

	4		5	
No.	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		208.6		208.6
2		66.8		66.8
3		46.2		46.4
4	3.25 (1H, m)	39.1	3.30 (1H, overlap)	39.9
5	1.96 (1H, dd, 13.5, 5.0)	44.8	1.97 (1H, dd, 13.5, 5.0)	44.8
	1.80 (1H, t, 13.5)		1.85 (1H, t, 13.5)	
6		62.7		62.6
7		197.5		197.4
8		137.6		137.6
9		176.2		176.1
10	2.85 (1H, dd, 14.0, 6.5)	25.7	2.86 (1H, dd, 14.0, 6.5)	25.7
	2.61 (1H, dd, 14.0,7.0)		2.62 (1H, dd, 13.5, 6.5)	
11	5.04 (1H, t, 6.3)	121.6	5.06 (1H, t, 7.0)	121.6
12		135.4		135.4
13	1.75 (3H, s)	18.6	1.76 (3H, s)	18.6
14	1.78 (3H, s)	26.5	1.78 (3H, s)	26.5
15	0.85 (3H, s)	16.5	0.88 (3H, s)	16.7
16	2.10 (1H, m)	34.1	2.16 (1H, m)	34.4
	1.54 (1H, m)		1.48 (1H, m)	
17	2.07 (1H, m)	22.9	2.14 (1H, m)	23.0
	2.03 (1H, m)		2.04 (1H, m)	
18	3.90 (1H, m)	89.6	3.91 (1H, m)	89.6
19		73.3		73.4
20	0.98 (3H, s)	24.0	0.97 (3H, s)	24.0
21	0.71 (3H, s)	27.6	0.74 (3H, s)	27.6
22	5.48 (1H, dd, 15.5, 8.0)	125.3	5.48 (1H, dd, 15.5, 8.0)	128.7
23	5.80 (1H, d, 15.5)	143.0	6.35 (1H, d, 16.0)	137.2
24		71.3		143.0
25	1.28 (3H, s)	30.0	4.98 (2H, s)	116.9
26	1.28 (3H, s)	29.9	1.84 (3H, s)	18.7
27		195.4		195.4
28		133.3		133.4
29	7.90 (1H, d, 8.0)	130.7	7.90 (1H, d, 7.2)	130.7
30	7.50 (1H, t, 7.8)	129.9	7.50 (1H, t, 7.7)	129.9
31	7.67 (1H, t, 7.5)	135.6	7.67 (1H, t, 7.5)	135.6
32	7.50 (1H, t, 7.8)	129.9	7.50 (1H, t, 7.7)	129.9
33	7.90 (1H, d, 8.0)	130.7	7.90 (1H, d, 7.2)	130.7
34	1.24 (3H, s)	16.5	1.25 (3H, s)	16.5

500 MHz for ^{1}H and 125 MHz for ^{13}C in CD_3OD

RAW264.7 cells with an IC_{50} value of (3.9 ± 3.3) μmol·L^{-1}. Compounds **1** and **9** exhibited weak inhibitory activity on Hep3B cells, while compound **9** also exerted slight inhibitory activity against SNU-387 cells.

Experimental

General experimental procedures

Optical rotation values were determined with an Autopol IV automatic polarimeter (Rudolph Research Co.). NMR spectra were recorded at an 500 MHz NMR instrument (Bruker 500, Germany) using TMS as the internal standard. HRESIMS data were carried out using an LTQ-FT Ultra ESI-FTICR-MS spectrometer (Thermo Fisher Scientific, CA, USA). UV spectra were performed on a JASCO V-650 spectrophotometer. ECD spectra were recorded on a JASCO 815 spectropolarimeter in MeOH. IR spectra were recorded on a Nicolet 5700 IR spectrometer (Thermo Nicolet, Waltham, MA, USA). Column chromatography (CC) was performed on silica gel (Qingdao marine Chemical Co., Ltd., China), ODS (40–63 μm, FuJi, Japan), MCI gel (35–75 μm, Mitsubishi Chemical Co., Japan), or Sephadex LH-20 (Pharmacia, Sweden). Preparative HPLC was carried out using a Shimadzu LC-20AR instrument with a shim-pack RP-C_{18} column (20 mm × 150 mm). Analytical LC and semipreparative HPLC were performed on a Thermo Scientific Ultimate 3000 instrument with a DAD detector using a shim-pack VP–ODS column (4.6 mm × 250 mm) and a SP ODS-A column (10 mm × 250 mm), respectively.

Plant material

The air-dried aerial parts of *H. ascyron* were collected from Anqing, Anhui Province, China, in July 2017. A voucher specimen (No. DX201707) was deposited in School of Pharmacy, Anhui Medical University.

Extraction and isolation

The air-dried aerial parts of *H. ascyron* (600 g) were powdered and extracted with CH_2Cl_2. After removal of the solvent, the CH_2Cl_2 extract (28 g) was subjected to an ODS column (i.d. 50 cm × 5 cm) using a gradient of MeOH−H_2O (60 : 40 to 100 : 0) to give five fractions (Fr. A–E) by TLC analysis. Fr. A (5.4 g) was loaded onto a silica gel column and eluted with PE−EtOAc (8 : 1 to 0 : 1) to yield four fractions (Fr. A.1−4). Fr. A.3 (1.8 g) was separated over an MCI-gel column to obtain six subfractions (Fr. A.3.1−3.6). Fr. A.3.4 (540.0 mg) was chromatographed over a Sephadex LH-20 column (i.d. 200 cm × 1.5 cm), eluting with MeOH to give four fractions (Fr. A.3.4.1−3.4.4). Fr. A.3.4.3 (40.0 mg) was further separately subjected to preparative HPLC using MeOH−H_2O (60 : 40, 10 mL·min^{-1}) to give compound **2** (5 mg). Fr. B (5.7 g) was then applied onto a silica gel column with PE−EtOAc (20 : 1 to 2 : 1) to give eight fractions (Fr. B.1−8). Fr. B.4 (1.2 g) and Fr. B.5 (1.3 g) were separately fractioned using an ODS column (i.d. 40 cm × 4 cm) followed by Sephadex LH-20 (i.d. 200 cm × 1.5 cm). Purification of Fr. B.4.3.2 (60 mg) was then performed on preparative HPLC with MeOH–H_2O (75 : 25, 10 mL·min^{-1}) to obtain **1** (2.9 mg), **3** (10 mg) and **6** (7 mg). Fr. B.5.4.2 (45 mg) was purified by semipreparative HPLC using MeOH–H_2O (80 : 20, 3 mL·min^{-1}) to afford **4** (4 mg), **5** (1.7 mg). Similarly, Fr. C (1.5 g) were chromatographed over silica gel column, ODS column, and Sephadex LH-20 column and finally purified by preparative HPLC with MeOH−H_2O (80 : 20, 10 mL·min^{-1}) to afford compounds **7** (6 mg), **8** (2.5 mg), and **9** (4 mg).

Ascyrone A (**1**): colorless oil; $[\alpha]_D^{20}$ +6 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.64), 252 (3.12), 273^{sh} (3.04) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 206 (1.75), 240 (−0.21), 267 (0.32), 328 (0.45); IR (KBr) ν_{max} 3415, 2960, 2920, 2850, 1732, 1678, 1649, 1584, 1461, 1450, 1376 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 549.3211 $[M + H]^+$ (Calcd. for $C_{34}H_{45}O_6$, 549.3211).

Ascyrone B (**2**): colorless oil; $[\alpha]_D^{20}$ +34 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.78), 253 (3.53), 274^{sh} (3.40) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 211 (19.09), 242 (−2.48), 268 (4.51), 330 (5.27); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 565.3152 $[M + H]^+$ (Calcd. for $C_{34}H_{45}O_7$, 565.3160).

Ascyrone C (**3**): colorless oil; $[\alpha]_D^{20}$ +78 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.26), 252 (4.10), 275^{sh} (3.94) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 212 (6.94), 261 (1.12), 283 (−0.52), 331 (2.78); IR (KBr) ν_{max} 3443, 2973, 2934, 2873, 1729, 1678, 1648, 1596, 1450, 1377, 1364, 1303 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 549.3208 $[M + H]^+$ (Calcd. for $C_{34}H_{45}O_6$, 549.3211).

Ascyrone D (**4**): colorless oil; $[\alpha]_D^{20}$ +11 (*c* 0.11, MeOH); UV (CH_2Cl_2) λ_{max} (log ε) 229 (3.83), 254 (3.81) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 213 (18.70), 250 (−9.56), 273 (0.59), 312 (−2.34), 351 (1.48); ^{1}H and ^{13}C NMR data, see Table 4; HRESIMS *m/z* 549.3207 $[M + H]^+$ (Calcd. for $C_{34}H_{45}O_6$, 549.3211).

Ascyrone E (**5**): colorless oil; $[\alpha]_D^{20}$ +3 (*c* 0.09, MeOH); UV (CH_2Cl_2) λ_{max} (log ε) 229 (3.81), 254 (3.46) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 211 (10.07), 250 (−6.73), 314 (−1.66), 354 (0.64); ^{1}H and ^{13}C NMR data, see Table 4; HRESIMS *m/z* 531.3102 $[M + H]^+$ (Calcd. for $C_{34}H_{43}O_5$, 531.3105).

Computational method

According to the conformation of each compound deduced from the ROESY spectrum, systematic conformational searches were first performed using Confab [16]. The initial conformations were optimized and re-optimized with the molclus program (version 1.9.9.5) [17] by invoking xtb program (version 6.4) [18, 19] and ORCA-4.2.1 [20, 21] at the B97-3c. The ECD spectra were calculated by ORCA-4.2.1 at the PBE0/def2-SV(P) level with a CPCM solvent model in methanol. The overall ECD curves of all the compounds were weighted by Boltzmann distribution.

Neuroprotective assay

The neuroprotective effects of compounds **1**−**4** and **6**−**9** were determined by the cell counting kit-8 (CCK-8) colorimetric assay in PC12 cells [22]. All the test compounds were dissolved in dimethyl sulfoxide (DMSO) as a 100 mmol·L^{-1} stock solution. According to the experimental purpose, selected 400 μmol·L^{-1} CORT as a damage model group. PC12 cells were randomly divided into the following groups: No-treatment (normal group); CORT (400 μmol·L^{-1}) (negative control group), CORT (400 μmol·L^{-1}) plus desipramine (10

μmol·L^{-1}) (positive control group), and CORT (400 μmol·L^{-1}) plus test compounds (10 μmol·L^{-1}). Each cell suspension (100 μL) was added into 96-well microplates at a density of 1 × 10^4 cells/well. After 24 h of preculturing at 5 % CO_2 and 37 °C, the compounds were added to the wells as previously described, and the cells were continuously cultured for 24 h. Then 100 μL of 10 % CCK-8 medium was added to each well after withdrawal of the culture medium and incubated for an additional 2 h. The absorbance was measured at 450 nm using a BioTek Synergy HTX muti-mode reader.

Anti-inflammatory assay

LPS-stimulated RAW264.7 cells were used for the anti-inflammatory assay. A nonsteroid anti-inflammatory agent, indomethacin, was used as a positive control. The cells were seeded into 96-well plate at 1 × 10^4 cells/well and precultured for 24 h at 37 °C humidified atmosphere containing 5% CO_2. RAW264.7 cells were pretreated with various concentrations of different compounds for 1 h, and then stimulated with LPS (0.5 μg·mL^{-1}) for 24 h. Cell viability was determined by MTS assay and the inhibition of NO production was tested by Griess Reagent System as previously reported[23].

Cytotoxicity assay

The cytotoxicity of compounds **1**−**4** and **6**−**9** gainst Hep3B and SNU-387 cells was investigated, using the MTT method previously described[7]. Hep3B and SNU-387 cells were precultured in 96-well plates at 1 × 10^4 cells/well for 24 h at 37 °C under 5 % CO_2 air environment, and the test compounds were added at different concentrations. After incubation for another 24 h, 0.5 mg·mL^{-1} MTT solution was added to each well and then incubated for additional 4 h. 150 μL DMSO was used to dissolve formazan in each well after discarding the culture medium. Then the absorbance was measured at 490 nm using a BioTek Synergy HTX muti-mode reader.

Supporting Materials

NMR, MS and CD spectra for all new compounds are available as Supporting Information, and can be requested by sending E-mail to the corresponding author.

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