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Polyprenylated acylphloroglucinols from the fruits of *Hypericum henryi*

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Hyphenrones R–X (**1**–**7**), seven new polyprenylated acylphloroglucinols derivatives, were isolated from the fruits of *Hypericum henryi*, together with eight know analogues. Compounds **1** and **2** were elucidated to possess complex caged skeleton, while compounds **3**–**6** shared a common 3,9-epoxy moiety deriving from the normal polyprenylated acylphloroglucinols with a bicyclo[3.3.1]nonane-2,4,9-trione core. The new structures were elucidated on the basis of the interpretation of nuclear magnetic resonance (NMR) data, circular dichroism (CD) comparison, and single-crystal X-ray diffraction. In the bioassay, several compounds exhibited inhibitory activities against human tumor cell lines *in vitro*.

polyprenylated acylphloroglucinols, *Hypericum henryi*, hyphenrones R–X, antitumor

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1 Introduction

Polycyclic polyprenylated acylphloroglucinols (PPAPs), reported only from the plants of family Guttiferae, are a group of structurally fascinating natural products possessing highly oxygenated acylphloroglucinol core and densely substituted with prenyl or geranyl side chains [1–3]. They exhibit a broad range of biological activities including anti-inflammatory, antibacterial, and antitumor effects, as well as central nervous system effects such as antidepressant and memory-enhancing properties [1,4]. Biogenetically, PPAPs are derived from a "mixed" mevalonate/methylerythritol phosphate and polyketide biosynthetic pathway [1,5,6]. Their acylphloroglucinol core structure is produced by a characteristic polyketide-type biosynthesis involving the condensation of one acyl-CoA and three malonyl-CoA units [1,5,6]. Prenylation of this core structure affords monocyclic polyprenylated acylphloroglucinols (MPAP), which may be further cyclized to PPAP-type metabolites with diverse carbon skeletons [1,7].

Hypericum henryi H. Lév & Vaniot (Guttiferae) is a traditional Chinese medicinal plant used for the treatment of hepatitis and "dampness-heat" disease [8]. Previous phytochemical investigations on the aerial parts (excluding the fruits) of this plant have led to the characterization of numbers of PPAPs with diverse carbon skeletons [7,9,10]. In order to explore the chemical constituents of its fruits and further compare the difference of PPAPs from the mentioned two botanic parts, the phytochemical research on the fruits was carried out. As a result, seven new PPAPs (hyphenrones R–X, **1**–**7**) were isolated (Scheme 1), together with eight know analogues: 3-hydroxyhyperforin-3,9-hemi-ketal (**8**) [11], furohyperforin [12], uralodin A [13], plukenetione B [14], hyphenrone M [7], hyphenrone N [7], tomoeone A [15], and

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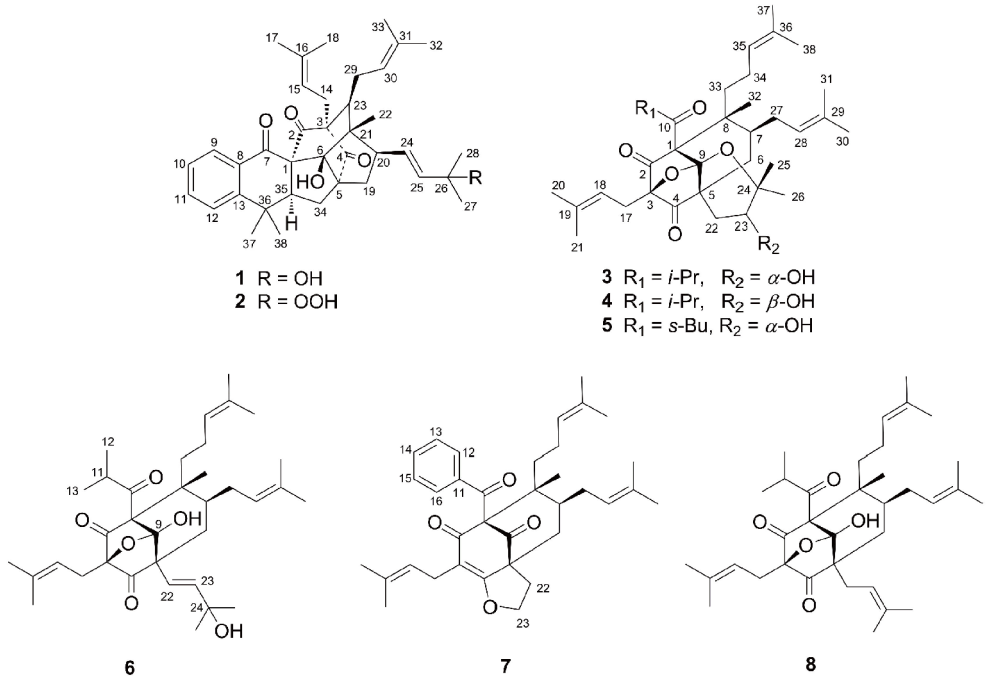

Scheme 1 Structures of compounds **1**–**8**.

tomoeone E [15]. Our finding suggested that the carbon skeletons of the obtained PPAPs from the fruits showed great similarity with those of PPAPs from aerial parts, which indicated that the fruits could be used as another source of PPAPs. Herein, we report the isolation, structural elucidation, and the bio-evaluation of these new metabolites.

2 Experimental

2.1 General experimental procedures

Melting points were obtained on an X-4 micro melting point apparatus. Optical rotations were measured on a Jasco P-1020 polarimeter (Japan). UV spectra were recorded on a Shmadzu UV-2401PC spectrometer (Japan). IR spectra were recorded on a Bruker FT-IR Tensor-27 infrared spectrophotometer (Germany) with KBr disks. 1D and 2D nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DRX-600 spectrometer (Germany) using tetramethylsilane (TMS) as an internal standard. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. Electrospray ionization mass spectrometry (ESIMS) and high resolution electrospray ionization mass spectrum (HREIMS) data were acquired on Waters Xevo TQS and Waters AutoSpec Premier P776 mass spectrometers (USA), respectively. X-ray data were generated using a Bruker Apex Duo instrument (Germany). Semi-preparative high performance liquid chromatography (HPLC) was performed on an Agilent 1100 HPLC with a Zorbarx SB-C_{18} (9.4 mm×250 mm) column (USA). Silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co., Ltd., China), and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Japan) were used for column chromatography. Fractions were monitored by thin-layer chromatography (TLC, GF 254, Qingdao Marine Chemical Co., Ltd., China), and spots were visualized by heating silica gel plates immersed in H_2SO_4 in ethanol.

2.2 Plant material

The fruits of *H. henryi* were collected at Hongtudi in the Dongchuan Prefecture, Yunnan province, China, in September 2013. The plant was identified by Dr. En-De Liu, and a voucher specimen 20130902 has been deposited at the Kunming Institute of Botany, China.

2.3 Extraction and isolation

The fruits of *H. henryi* (20.0 kg) were powdered and percolated with MeOH at room temperature and filtered. The filtrate was evaporated in vacuo to be concentrated. The crude extract (5.4 kg) was subjected to a silica gel column chromatography (CC) eluted with $CHCl_3$ to afford a fraction (840 g). This fraction was separated over a MCI-gel column (MeOH-H_2O, from 7:3 to 10:0) to produce seven fractions (Fr. A–G). Fr. A (120 g) was then chromatographed on a silica gel column, eluted with petroleum ether-acetone (from 200:1 to 0:1), to yield eight fractions (Fr. A1–A8). Fr. A3 (26.0 g) was separated over a RP-18 silica column (MeOH-H_2O, from 80:20 to 100:0) to obtained four fractions (Fr. A3a–A3d). Fr. A3b (5.6 g) was further isolated by Sephadex LH-20 CC ($CHCl_3$-MeOH, 1:1), preparative TLC, and semipreparative HPLC, to afford **1** (32 mg), **2** (14 mg),

7 (12 mg), and **8** (2.3 g). Fr. B (64 g) was chromatographed on a silica gel column, eluted with petroleum ether-acetone (from 50:1 to 0:1), to yield four fractions (Fr. B1–B4). Fr. B2 (16.2 g) was separated over a RP-18 silica column (MeOH-H_2O, from 70:30 to 100:0) to obtained four fractions (Fr. B2a–B2d). Compound **3** (18 mg), **4** (21 mg), **5** (9 mg), **6** (8 mg), furohyperforin (460 mg), uralodin A (320 mg), and plukenetione B (23 mg) were obtained from Fr. B2a (4.1 g), hyphenrone M (11 mg), hyphenrone N (18 mg), tomoeone A (33 mg), and tomoeone E (14 mg) were from Fr. B2b (2.2 g), by further preparative TLC and semipreparative HPLC.

Hyphenrone R (**1**): colorless gum; $[\alpha]^{17}_{D}$ –15 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (logε) 202 (3.87), 253 (3.44), 292 (2.62) nm; IR (KBr) ν_{max} 3440, 3432, 2968, 2925, 1738, 1709, 1672, 1631, 1452, 1382, 1113, 597 cm^{-1}; circular dichroism (CD) (0.0012 M, MeOH) λ_{max} ($\Delta\varepsilon$) 203 (+1.5), 217 (–1.6), 257 (–2.7), 291 (–1.1), 332 (+1.4) nm; ^1H and ^{13}C NMR data, see Table 1 and Table 2; negative ESIMS *m/z* 583 $[M-H]^-$; HRTOFMS *m/z* 607.3400 (calcd for $C_{38}H_{48}O_5Na$, 607.3399).

Hyphenrone S (**2**): colorless gum; $[\alpha]^{18}_{D}$ –14 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (logε) 203 (4.42), 253 (3.96), 288 (3.16) nm; IR (KBr) ν_{max} 3441, 2970, 2928, 1738, 1709,

Table 1 ^{13}C NMR spectroscopic data for compounds **1**–**7** (δ in ppm, 150 MHz)

No.	**1** [a]	**2** [a]	**3** [a]	**4** [a]	**5** [a]	**6** [a]	**7** [b]
1	71.1, C	70.8, C	77.4, C	76.6, C	77.2, C	74.2, C	80.9, C
2	205.0, C	205.0, C	206.8, C	206.3, C	206.4, C	207.8, C	195.4, C
3	69.8, C	69.8, C	97.5, C	98.0, C	96.9, C	98.9, C	117.2, C
4	215.6, C	215.5, C	209.9, C	208.9, C	209.1, C	209.3, C	175.7, C
5	72.2, C	72.3, C	49.6, C	53.1, C	48.3, C	58.7, C	60.6, C
6	91.1, C	91.1, C	35.1, CH_2	33.0, CH_2	34.7, CH_2	33.6, CH_2	38.5, CH_2
7	199.8, C	199.7, C	46.8, CH	46.5, CH	46.2, CH	44.0, CH	44.4, CH
8	137.3, C	137.3, C	45.1, C	45.0, C	44.7, C	46.5, C	51.0, C
9	127.9, CH	127.9, CH	110.0, C	109.9, C	109.6, C	109.1, C	206.2, C
10	127.7 CH	127.7 CH	210.0, C	210.1, C	208.2, C	215.3, C	195.6, C
11	134.8, CH	134.8, CH	44.3, CH	44.3, CH	50.4, CH	42.3, CH	138.4, C
12	124.8, CH	124.8, CH	21.0, CH_3	21.1, CH_3	16.6, CH_3	19.7, CH_3	129.2, CH
13	152.3, C	152.3, C	19.6, CH_3	19.7, CH_3	28.0, CH_2	19.1, CH_3	128.9, CH
14	24.4, CH_2	24.4, CH_2			11.5, CH_3		133.2, CH
15	120.4, CH	120.4, CH					128.9, CH
16	134.9, C	134.9, C					129.2, CH
17	26.2, CH_3	26.2, CH_3	24.1, CH_2	24.1, CH_2	23.9, CH_2	24.5, CH_2	23.0, CH_2
18	17.9, CH_3	18.0, CH_3	117.1, CH	116.9, CH	116.8, CH	117.1, CH	121.9, CH
19	34.3, CH_2	34.2, CH_2	137.8, C	138.1, C	137.0, C	137.3, C	133.6, C
20	64.7, CH	64.9, CH	26.0, CH_3	26.1, CH_3	26.3, CH_3	25.9, CH_3	25.9, CH_3
21	48.4, C	48.5, C	18.0, CH_3	18.2, CH_3	18.1, CH_3	18.1, CH_3	17.9, CH_3
22	16.4, CH_3	16.4, CH_3	31.3, CH_2	31.9, CH_2	30.7, CH_2	122.3, CH	30.5, CH_2
23	54.3, CH	54.2, CH	69.0, CH	69.1, CH	68.6, CH	142.7, CH	74.0, CH_2
24	130.9, CH	134.3, CH	80.4, C	81.3, C	79.9, C	71.4, C	
25	139.3, CH	135.6, CH	27.9, CH_3	29.0, CH_3	27.6, CH_3	29.9, CH_3	
26	70.9, C	82.5, C	25.9, CH_3	21.0, CH_3	26.3, CH_3	29.6, CH_3	
27	30.3, CH_3	25.1, CH_3	28.8, CH_2	28.7, CH_2	28.5, CH_2	29.1, CH_2	28.2, CH_2
28	29.9, CH_3	24.8, CH_3	123.9, CH	123.6, CH	123.7, CH	123.4, CH	123.4, CH
29	27.7, CH_2	27.7, CH_2	133.9, C	134.3, C	133.2, C	134.6, C	134.7, C
30	126.7, CH	126.6, CH	26.1, CH_3	25.9, CH_3	25.9, CH_3	26.1, CH_3	26.0, CH_3
31	132.3, C	132.3, C	18.2, CH_3	17.8, CH_3	17.9, CH_3	18.1, CH_3	18.1, CH_3
32	25.9, CH_3	25.9, CH_3	15.3, CH_3	13.8, CH_3	13.4, CH_3	15.3, CH_3	14.7, CH_3
33	18.4, CH_3	18.4, CH_3	39.1, CH_2	39.2, CH_2	38.3, CH_2	38.6, CH_2	37.9, CH_2
34	29.6, CH_2	29.6, CH_2	26.6, CH_2	26.6, CH_2	26.1, CH_2	25.5, CH_2	26.0, CH_2
35	58.8, CH	58.8, CH	125.9, CH	125.8, CH	125.7, CH	125.4, CH	125.7, CH
36	38.4, C	38.4, C	132.0, C	132.1, C	131.3, C	132.5, C	132.1, C
37	30.3, CH_3	30.3, CH_3	26.1, CH_3	26.0, CH_3	25.8, CH_3	26.0, CH_3	26.0, CH_3
38	27.0, CH_3	27.0, CH_3	17.8, CH_3	18.0, CH_3	17.7, CH_3	17.9, CH_3	17.9, CH_3

a) Recorded in methanol-d_4; b) recorded in acetone-d_6.

Table 2 ^{1}H NMR spectroscopic data for compounds **1**–**7** (600 MHz, δ in ppm, J in Hz)

No.	**1** [a]	**2** [a]	3 [a]	4 [a]	**5** [a]	**6** [a]	7 [b]
6			2.30, t (13.8)	1.75, overlap	2.43, t (13.8)	1.89, dd (14.2, 3.5)	2.16, dd (13.8, 4.3)
			1.75, overlap	1.69, overlap	1.67, overlap	1.50, m	1.71, overlap
7			0.70, m	0.75, m	0.74, m	0.92, m	1.81, m
9	7.68, d (7.8)	7.68, d (7.8)					
10	7.36, t (7.8)	7.36, t (7.8)					
11	7.57, t (7.8)	7.57, t (7.8)	3.02, sept (6.5)	3.00, sept (6.5)	2.80, m	3.08, sept (6.5)	
12	7.44, d (7.8)	7.44, d (7.8)	0.90, d (6.5)	0.90, d (6.5)	1.12, d (6.6)	1.02, d (6.5)	7.43, d (7.8)
13			1.10, d (6.5)	1.10, d (6.5)	1.53, m	0.99, d (6.5)	7.26, t (7.8)
					1.18, m		
14	2.46, dd (15.7, 9.7)	2.46, overlap			0.81, t (7.4)		7.42, t (7.8)
	2.21, overlap	2.21, overlap					
15	5.00, m	4.99, m					7.26, t (7.8)
16							7.43, d (7.8)
17	1.67, s	1.67, s	2.64, dd (15.2, 7.5)	2.66, dd (15.2, 7.5)	2.63, dd (15.4, 7.5)	2.69, dd (15.4, 7.4)	3.06, dd (14.0, 7.5)
			2.54, dd (15.2, 7.5)	2.59, dd (15.2, 7.5)	2.56, dd (15.5, 7.5)	2.56, dd (15.4, 7.4)	2.98, dd (14.0, 7.5)
18	1.57, s	1.57, s	5.11, t (7.5)	5.11, t (7.5)	5.11, t (7.5)	5.05, overlap	5.11, t (7.5)
19	1.89, dd (11.4, 7.9)	1.91, dd (11.2, 7.6)					
	1.78, overlap	1.77, overlap					
20	2.43, m	1.57, s	1.68, s	1.69, s	1.67, s	1.65, s	1.64, s
21			1.66, s	1.67, s	1.66, s	1.65, s	1.63, s
22	1.30, s	1.29, s	2.05, overlap	1.84, t (12.8)	2.12, overlap	5.81, d (16.1)	2.67, m
			1.69, overlap	1.54, dd (12.8, 4.0)	1.71, overlap		2.01, dd (13.0, 5.6)
23	1.78, overlap	1.79, dd (8.6, 3.1)	3.73, m	3.71, dd (12.8, 4.0)	3.84, m	5.61, d (16.1)	4.71, t (9.4)
							4.56, m
24	5.64, dd (15.5, 9.4)	5.66, dd (15.7, 9.3)					
25	5.45, d (15.5)	5.46, d (15.6)	1.43, s	1.34, s	1.47, s	1.24, s	
26			1.30, s	1.37, s	1.34, s	1.24, s	
27	1.27, s	1.29, s	2.07, overlap	2.09, m	2.08, overlap	2.13, m	2.21, m
			1.68, overlap	1.76, overlap	1.74, m	1.75, m	1.89, m
28	1.27, s	1.29, s	4.90, t (7.3)	4.92, overlap	4.92, t (6.9)	4.90, overlap	5.06, overlap
29	2.14, m	2.15, m					
	1.60, overlap	1.63, overlap					
30	4.87, m	4.88, m	1.68, s	1.69, s	1.66, s	1.70, s	1.71, s
31			1.56, s	1.57, s	1.55, s	1.58, s	1.61, s
32	1.65, s	1.66, s	1.29, s	1.24, s	1.34, s	1.13, s	1.17, s
33	1.62, s	1.61, s	1.72, m	1.71, m	1.81, m	1.72, m	2.07, td (13.2, 4.3)
			1.28, m	1.28, m	1.34, overlap	1.47, m	1.49, m
34	2.21, overlap	2.21, overlap	2.06, m	2.03, m	2.11, overlap	2.16, m	2.20, overlap
	1.94, dd (11.6, 8.8)	1.94, dd (11.3, 7.8)	1.87, m	1.87, m	1.94, m	1.89, m	2.01, overlap
35	2.62, t (8.8)	2.62, dd (9.2, 8.3)	5.01, t (7.3)	5.01, t (7.3)	5.04, t (7.5)	5.02, overlap	5.05, overlap
37	1.14, s	1.14, s	1.65, s	1.65, s	1.62, s	1.67, s	1.66, s
38	1.35, s	1.35, s	1.57, s	1.67, s	1.57, s	1.59, s	1.63, s

a) Recorded in methanol-d_4; b) recorded in acetone-d_6.

1673, 1631, 1452, 1383, 1114, 574 cm^{-1}; CD (0.0005 M, MeOH) λ_{max} ($\Delta\varepsilon$) 204 (+3.0), 217 (–4.2), 257 (–8.9), 294 (–3.5), 331 (+4.4) nm; ^1H and ^{13}C NMR data, see Table 1 and Table 2; positive ESIMS *m/z* 623 $[M+Na]^+$; HRTOFMS *m/z* 623.3351 (calcd for $C_{38}H_{48}O_6Na$, 623.3349).

Hyphenrone T (**3**): colorless crystal; m.p. 146–148 °C; $[\alpha]^{19}_D$ –118 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (logε) 202 (4.38) nm; IR (KBr) ν_{max} 3453, 2968, 2929, 1774, 1742, 1710, 1635, 1448, 1383, 1171, 1058 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1 and Table 2; positive ESIMS *m/z* 591 $[M+Na]^+$; HRTOFMS *m/z* 591.3656 (calcd for $C_{35}H_{52}O_6Na$, 591.3662).

Hyphenrone U (**4**): colorless gum; $[\alpha]^{19}_D$ –46 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (logε) 202 (4.02) nm; IR (KBr) ν_{max} 3563, 3440, 2972, 2931, 1775, 1743, 1718, 1629, 1447, 1381, 1083, 1231, 1200, 1054 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1 and Table 2; positive ESIMS *m/z* 591 $[M+Na]^+$; HRTOFMS *m/z* 591.3653 (calcd for $C_{35}H_{52}O_6Na$, 591.3662).

Hyphenrone V (**5**): colorless gum; $[\alpha]^{21}_D$ –38 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (logε) 202 (4.18) nm; IR (KBr) ν_{max} 3456, 3441, 2971, 2930, 1774, 1741, 1631, 1452, 1383, 1114, 1052 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1 and Table 2; positive ESIMS *m/z* 583 $[M+H]^+$; HRTOFMS *m/z* 605.3817 (calcd for $C_{36}H_{54}O_6Na$, 605.3818).

Hyphenrone W (**6**): colorless gum; $[\alpha]^{17}_D$ +6 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (logε) 202 (3.97) nm; IR (KBr) ν_{max} 3443, 2972, 2929, 1730, 1709, 1631, 1449, 1384, 1153, 1115 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1 and Table 2; positive ESIMS *m/z* 591 $[M+Na]^+$; HRTOFMS *m/z* 591.3661 (calcd for $C_{35}H_{52}O_6Na$, 591.3662).

Hyphenrone X (**7**): colorless crystal; m.p. 124–126 °C; $[\alpha]^{21}_D$ –23 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (logε) 202 (4.42), 248 (4.16), 274 (4.05) nm; IR (KBr) ν_{max} 2971, 2930, 1739, 1710, 1672, 1632, 1453, 1383, 1115 cm^{-1}; CD (0.0002 M, MeOH) λ_{max} ($\Delta\varepsilon$) 201 (+15.9), 246 (+11.1), 270 (–21.8), 300 (+3.8) nm; ^1H and ^{13}C NMR data, see Table 1 and Table 2; positive ESIMS *m/z* 551 $[M+Na]^+$; HRTOFMS *m/z* 551.3129 (calcd for $C_{35}H_{44}O_4Na$, 551.3137).

Crystallographic data of **3**: $C_{35}H_{52}O_6$, *M*=568.77, orthorhombic, *a*=9.4718(2) Å, *b*=14.3354(4) Å, *c*=23.4620(6) Å, α=90.00°, β=90.00°, γ=90.00°, *V*=3185.72(14) $Å^3$, *T*=100(2) K, space group *P*212121, *Z*=4, μ(Cu-Kα)=0.628 mm^{-1}, 16002 reflections measured, 5583 independent reflections (R_{int}=0.0797). The final R_1 values were 0.0666 ($I>2\sigma(I)$). The final $wR(F^2)$ values were 0.1771 ($I>2\sigma(I)$). The final R_1 values were 0.0863 (all data). The final $wR(F^2)$ values were 0.2217 (all data). The goodness of fit on F^2 was 1.211. Flack parameter=0.2(3). The Hooft parameter is 0.19(16) for 2331 Bijvoet pairs. Crystallographic data for **3** have been deposited at the Cambridge Crystallographic Data Center (deposition number CCDC 1448224). Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk.

Crystallographic data of **7**: $C_{35}H_{44}O_4$, *M*=528.70, orthorhombic, *a*=8.8788(2) Å, *b*=11.3127(3) Å, *c*=29.3926(7) Å, α=90.00°, β=90.00°, γ=90.00°, *V*=2952.29(12) $Å^3$, *T*=100(2) K, space group *P*212121, *Z*=4, μ(Cu-Kα)=0.595 mm^{-1}, 14377 reflections measured, 5052 independent reflections (R_{int}=0.0839). The final R_1 values were 0.0487 ($I>2\sigma(I)$). The final $wR(F^2)$ values were 0.1367 ($I>2\sigma(I)$). The final R_1 values were 0.0936 (all data). The final $wR(F^2)$ values were 0.1795 (all data). The goodness of fit on F^2 was 1.061. Flack parameter=0.0(2). The Hooft parameter is −0.07(10) for 2071 Bijvoet pairs. (CCDC 1448225).

2.4 Cytotoxicity bioassays

Colorimetric assays were performed to evaluate compound activity. The following human tumor cell lines were used: HL-60 human myeloid leukemia, SMMC-7721 human hepatocarcinoma, A-549 lung cancer, MCF-7 breast cancer, and SW-480 human pancreatic carcinoma. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Briefly, 100 μL adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before test compound addition, while suspended cells were seeded just before this step, both with initial density of 1×10^5 cells/mL in 100 μL of medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with *cis*-platin (Sigma, USA) as positive control. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were lysed with 100 μL of 20% sodium dodecyl sulfate (SDS)-50% DMF after removal of 100 μL of medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680, USA). The IC_{50} value of each compound was calculated by Reed and Muench's method.

3 Results and discussion

Hyphenrone R (**1**) was obtained as colorless gum and possessed a molecular formula $C_{38}H_{48}O_5$ as established by ^{13}C NMR and HRTOFMS data (*m/z* 607.3400, $[M+Na]^+$), indicating 15 degrees of unsaturation. The IR spectrum showed absorption bands due to hydroxyl (3440 and 3432 cm^{-1}), carbonyl groups (1738, 1709, and 1672 cm^{-1}), and an aromatic ring (1598 and 1446 cm^{-1}). The ^1H NMR spectrum (Table 1) exhibited four olefinic protons (δ_H 4.87, 5.00, 5.45, and 5.64), an *ortho*-disubstituted benzene moiety (δ_H 7.68, d; 7.36, t; 7.57, t; 7.44, d, *J*=7.8 Hz), and nine singlet methyls (δ_H 1.14–1.67). The ^{13}C and distortionless enhancement by polarization transfer (DEPT) spectra displayed 38 carbon

resonances (Table 2) attributable to twelve quaternary carbons (including three carbonyls), nine methines (including six olefinic ones), two methylenes, five methyls, and 10 other resonances assignable to two prenyl groups. Analysis of these data indicated the characteristic signals of three ketones (δ_C 205.0, C-2; 215.6, C-4; 199.8, C-7) and six quaternary carbons (δ_C 71.1, C-1; 69.8, C-3; 72.2, C-5; 91.1, C-6; 48.4, C-21; 38.4, C-36) for a caged PPAP, such as hyperuralone B [16]. Further comparison of the 1D NMR spectroscopic data of **1** with those of hyperuralone B revealed that they were structurally similar. The signals for two methylenes (δ_C 38.7, C-22; 42.0, C-23) in hyperuralone B were replaced by a methyl at δ_C 16.4 (C-22) and a methine at δ_C 54.3 (C-23) in **1**, which suggested that one isoprenyl unit was connected to C-23 in **1** rather than C-22 in hyperuralone B. This assumption was evidenced by the correlations from Me-22 (δ_H 1.30, s) to C-6, C-20 (δ_C 64.7), C-21, and C-23, from H_2-29 (δ_H 2.14 and 1.60) to C-3 and C-23 in the heteronuclear multiple bond correlation (HMBC) spectrum. Furthermore, an oxygenated isoprenyl group (δ_C 130.9, C-24; 139.3, C-25; 70.9, C-26; 30.0, C-27; 29.9, C-28) was connected to C-20 by the HMBC correlations from both Me-27 and Me-28 (δ_H 1.27, s) to C-25 and C-26, and from H-24 (δ_H 5.64) to C-19 (δ_C 34.3), C-20, and C-21, coupled with the proton spin system of H-25/H-24/H-20 in the ^{1}H-^{1}H COSY spectrum (Figure 1). The rest part of the structure of **1** was determined to be the same as that of hyperuralone B by analysis of the 2D NMR spectroscopic data. The correlations of Me-22/H_2-29, Me-22/H-24/H-19β/H-34β, and H-34α/H-35 in the ROESY spectrum, in combination with the rigid caged carbon skeleton, defined the relative configuration of **1**. Since the absolute configuration of hyperuralone B has been established by ECD calculation [16], the well matched experimental CD curves of **1** and hyperuralone B in Figure 2 suggested the absolute configuration of **1** to be 1*S*,3*S*,5*R*,6*S*,20*R*,21*S*,23*S*,35*S*.

Hyphenrone S (**2**) shared the same carbon skeleton and absolute configuration of **1** by analysis of its 1D (Table 1 and Table 2) and 2D NMR spectroscopic data, as well as its CD data (Figure 2). The increased 16 mass units of the molecular weight (*m/z* 623.3351, $[M+Na]^+$) observed in HRTOFMS and 11.6 ppm downshift of C-26 (δ_C 82.5) in the ^{13}C NMR spectra of **2** indicated that the hydroxyl in **1** was replaced by a hydroperoxyl in **2** [17].

Hyphenrone T (**3**) was isolated as a colorless gum. Its molecular formula was established from the ^{13}C NMR and HRTOFMS data (*m/z* 591.3656, $[M+Na]^+$) as $C_{35}H_{52}O_6$, indicating ten degrees of unsaturation. The ^{1}H NMR spectrum showed signals assignable to a isopropyl (δ_H 1.10, 3H, d; 0.90, 3H, d; 3.02, 1H, sept, *J*=6.5 Hz), three olefinic protons (δ_H 5.11, 5.01, and 4.90), and other nine singlet methyl groups (δ_H 1.29–1.68). The ^{13}C and DEPT NMR spectra exhibited 35 carbon resonances (Table 2) attributable to eight quaternary carbons (including three carbonyls), two methines, three methylenes, three methyls, and 19 other resonances assignable to one isobutyryl and three prenyl groups. The ^{1}H and ^{13}C NMR spectroscopic data of **3** were resemble to those of 3-hydroxyhyperforin-3,9-hemi-ketal (**8**) [11], a bicyclic polyprenylated acylphloroglucinols (BPAP) with a bicyclo[3.3.1]nonane-2,4,9-trione core, indicating that they are structural similar. However, the double bond of the isoprenyl group at C-5 in the known compound was oxidized (δ_C 69.0, C-23; 80.4, C-24) in **3**, as evidenced by the HMBC correlations of Me-25 (δ_H 1.43) and Me-26 (δ_H 1.30) with C-23 and C-24, and of H_2-22 (δ_H 2.05 and 1.69) with C-4 (δ_C 209.9), C-5 (δ_C 49.6), and C-9 (δ_C 110.0). An oxygen bridge between C-9 and C-13 was expected by the downfield chemical shifts of C-9 (δ_C 110.0) and C-24 (δ_C 80.4) and the degree of unsaturation, which put C-9 in a state of ketal. Other parts of **3** were identical to those of **8** by analysis of the 2D NMR spectroscopic data. The final refinement on the Cu-Kα data (CCDC 1448224) of the crystal of **3** (the Flack parameter is 0.2(3); the Hooft parameter is 0.19(16) for 2331 Bijvoet pairs) allowed an unambiguous assignment of the absolute configuration as 1*R*,3*R*,5*S*,7*S*,8*R*,9*S*,23*R* (Figure 3).

Hyphenrone U (**4**) shared the same planar scaffold as **3** by detailed analysis of its HRTOFMS, 1D and 2D NMR spectroscopic data. The ^{13}C NMR data of **4** (Table 2) were nearly the same to those of **3** except for the carbon resonances of C-5, C-6, C-25 and C-26, which supposed **4** as 23-epimer of

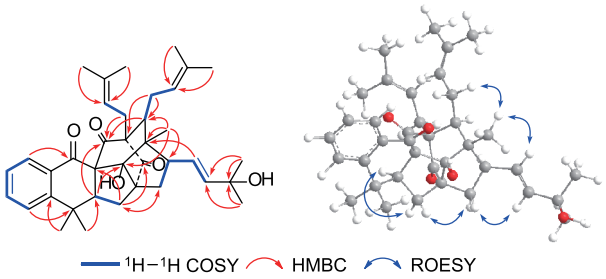

Figure 1 Key HMBC, ^{1}H-^{1}H COSY, and ROESY correlations of **1** (color online).

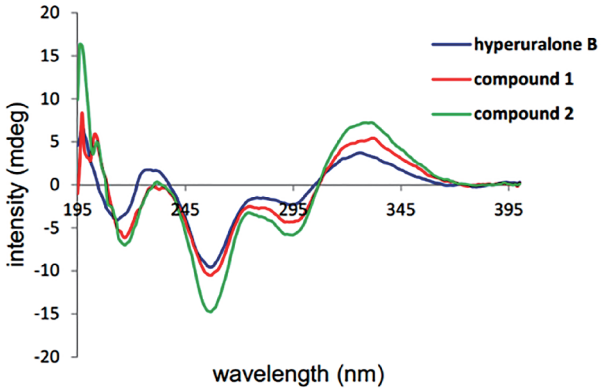

Figure 2 The experimental CD spectra of **1**, **2**, and hyperuralone B.

3. The correlation of H-23 (3.71) with H-6 (δ_H 1.75) in the ROESY spectrum (Figure 4) confirmed that a β-OH was substituted at C-23.

The molecular formula of hyphenrone V (**5**) was determined as $C_{36}H_{54}O_6$ by analysis of its ^{13}C NMR (Table 2) and HRTOFMS data (*m/z* 605.3817, $[M+Na]^+$), 14 mass units more than that of **3**. Comparison of the ^1H and ^{13}C NMR spectroscopic data of **5** with those of **3** indicated that the isopropyl group in **3** is replaced by a *sec*-butyl group (C-11, δ_C 50.4; C-12, δ_C 16.6; C-13, δ_C 28.0; and C-14, δ_C 11.5) in **5**. Other parts of **5** were identical to those of **3** by analysis of the 2D NMR spectroscopic data.

Hyphenrone W (**6**) was assigned the molecular formula $C_{36}H_{54}O_6$ from its ^{13}C NMR and HREIMS data. The ^1H and ^{13}C NMR spectroscopic data of **6** (Table 1 and Table 2) resembled those of **8**. Instead of the olefinic quaternary carbon at δ_C 133.5 (C-24) and a methylene (δ_C 31.7, C-22) in **8**, an oxygenated quaternary carbon at δ_C 71.4 and an olefinic methine (δ_C 122.3) appeared in **6**, assuming hydroxylation of C-24 and formation of a $\Delta^{22,23}$ double bond. This assumption was supported by the correlations of H-22 (δ_H 5.81, *J*=16.1 Hz) with C-4 (δ_C 209.3), C-5, (δ_C 58.7), and C-9 (δ_C 109.1), and of both Me-25 and Me-26 (δ_H 1.24) with C-23 (δ_C 142.7) and C-24 in the HMBC spectrum. The 2D NMR data showed that other structural units of **6** are the same as those of **8**.

On the basis of analysis of its MS, 1D, and 2D NMR data (Table 1 and Table 2), hyphenrone X (**7**) was shown to possess the same backbone and relative configuration as deoxyfurohyperforin A [18], a PPAP isolated from *H. perforatum* in 2003. The structural novelty of **7** involves the presence of a phenyl attached to C-10 rather than an isopropyl group. The final refinement on the Cu-Kα data (CCDC 1448225) of the crystal of **7** (the Flack parameter is 0.0(2); the Hooft parameter is −0.07(10) for 2071 Bijvoet pairs) allowed an unambiguous assignment of the absolute configuration as 1*R*,5*S*,7*S*,8*R* (Figure 3).

Considering the fact that several cytotoxic PPAPs have been isolated previously [7,9,19,20], the inhibitory activities of the new compounds were examined against five human tumor cell lines HL-60, A-549, SMMC-7721, MCF-7, and SW-480. As shown in Table 3, compounds **1**, **4**, **5**, and **7** exhibited moderate cytotoxic activities (IC_{50} 3.3–23.3 μM) against the cancer cell lines using the MTT method [21].

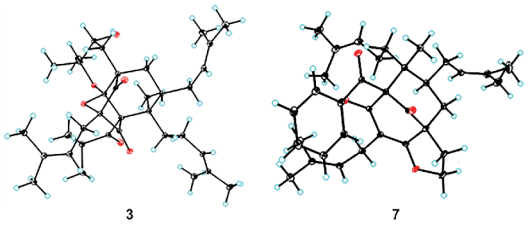

Figure 3 Single-crystal X-ray structures of **3** and **7** (color online).

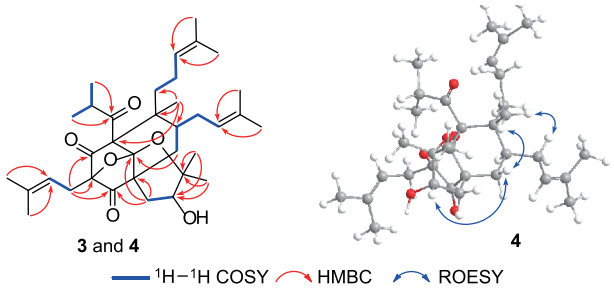

Figure 4 Key HMBC, ^{1}H-^{1}H COSY, and ROESY correlations of **3** and **4** (color online).

Table 3 Cytotoxicities of selected PPAPs against five cancer cell lines (IC_{50} in μM)

Compound [a]	HL-60	SMMC-7721	A-549	MCF-7	SW-480
1	3.3	22.1	17.6	16.2	22.5
4	6.1	13.7	12.6	5.5	16.2
5	13.8	19.8	17.0	13.1	>40
7	4.3	22.8	11.5	2.1	23.3
cis-Platin [b]	2.3	10.0	8.3	14.7	15.1
Taxol [b]	<0.008	<0.008	<0.008	<0.008	<0.008

a) Other isolates with IC_{50}>40 μM for all cell lines are not listed; b) *cis*-platin and taxol were used as positive controls.

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Conflict of interest The authors declare that they have no conflict of interest.

Supporting information The original MS, ^{1}H and ^{13}C NMR, HSQC, ^{1}H-^{1}H COSY, HMBC, and ROESY NMR spectra of compounds **1**–**7**, and crystallographic files for **3** and **7** in CIF format. The supporting information is available online at http://chem.scichina.com and http://link.springer.com/journal/11426. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

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