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Cytotoxic polycyclic polyprenylated acylphloroglucinols from *Hypericum attenuatum*

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ABSTRACT

Six new polycyclic polyprenylated acylphloroglucinols, attenuatumiones A–F (**1**–**6**), together with twelve known analogs (**7**–**18**) were isolated from the whole plant of *Hypericum attenuatum*. Their structures were elucidated by spectroscopic methods, and the absolute configuration of C-13 in attenuatumione C (**3**) was deduced via the circular dichroism datum of the in situ formed $[Rh_2(OCOCF_3)_4]$ complexes. All isolates were evaluated for the cytotoxic activities on three human cancer cell lines. Compound **3** showed moderate cytotoxic activities with IC_{50} values of 10.12 and 10.56 μM against SMMC7721 and U2OS, respectively.

1. Introduction

The polycyclic polyprenylated acylphloroglucinols (PPAPs), prominent secondary metabolites of the genus *Hypericum*, are well known for their fascinating chemical structures, which feature a phloroglucinol core densely decorated with prenyl, geranyl, or more highly substituted side chains. Apart from their structures, the exciting range of biological activities exhibited by these compounds is particularly striking [1]. Among their reported activities, such as anti-HIV, antidepressant, antibacterial, antimalarial, antioxidant, antiulcer, anti-inflammatory, and anti-neurodegenerative [2], their anticancer activities intrigued us indeed. In recent years, the anticancer activity of PPAPs has attracted much attention from both scientists and the wider public. Investigations of the constituents of *Hypericum sampsonii* and other plants from the genus *Hypericum* have revealed a number of PPAPs with modest to significant cytotoxicity on different cancer cell lines [3–8]. *Hypericum attenuatum* Choisy is widely distributed in China and its whole plant is a folk medicine for haemostasia, analgesia and stimulation of lactation [9,10]. To the best of our knowledge, no PPAPs have been reported from this plant. Therefore, a systematic chemical study focusing on the PPAPs of this species was conducted and eighteen PPAPs including six new ones (**1**–**6**) were isolated. Herein, we describe the isolation and structure elucidation of the new compounds as well as their cytotoxic activities.

2. Experimental

2.1. General

Optical rotations were obtained on a JASCO P-1020 polarimeter. CD spectra were recorded with a JASCO 810 spectropolarimeter. UV spectra were measured on a Shimadzu UV-2450 spectropolarimeter. A Bruker Tensor 27 spectrometer (KBr disks) was used to obtain the IR spectra. NMR spectra

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were acquired in $CDCl_3$ at 303 K on Bruker AV-500 (^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz) with TMS as the internal standard. ESIMS and HRESIMS data were carried out on an Agilent 1100 series LC/MSD Trap mass spectrometer (ESIMS) and an Agilent UPLC/Q-TOF (6520B) (HRESIMS), respectively. Silica gel (200–400 mesh, Qingdao Marine Chemical Co., Ltd.), Sephadex LH-20 (Pharmacia), and ODS (40–63 μm, Fuji) were used for open column chromatography. Preparative HPLC was carried out using Agilent 1100 Series equipped with Shim-park RP-C_{18} column (5 μm, 200 × 20 mm i.d., Shimadzu), Zorbax SIL column (5 μm, 250 × 4.6 mm i.d., Agilent) and 1100 Series Multiple Wavelength Detector.

2.2. Plant material

The whole plant of *H. attenuatum* Choisy was purchased from Bozhou, Anhui Province, China, on November 2011, and authenticated by Professor Mian Zhang, Department of Natural Medicinal Chemistry, China Pharmaceutical University. A voucher specimen (No. HA-201112) is deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

2.3. Extraction and isolation

The air-dried and powdered whole plant of *H. attenuatum* (8.5 kg) were extracted with 95% aqueous EtOH (3 × 20 L) under reflux. After the removal of the solvent under reduced pressure, the crude extract (633 g) was suspended in H_2O (2 L) and successively partitioned with petroleum ether (3 × 2 L) and CH_2Cl_2 (3 × 2 L). The petroleum ether extract (181.5 g) was subjected to a silica gel column, and eluted with a gradient of petroleum ether–acetone (1:0 to 0:1) to give four fractions (A–D). Fraction B (35 g) was chromatographed over a silica gel column using a gradient of petroleum ether–EtOAc (50:1 to 0:1) to yield six fractions (BI–BVI). Fraction BIV (12.4 g) was further separated by MPLC (MeOH–H_2O, 50:50 to 100:0) to get four fractions (BIV1–BIV4). Fraction BIV3 (5.5 g) was reseparated by MPLC (MeOH–H_2O, 85:15) to afford six fractions (BIV3a–BIV3f). Fraction BIV3b (1.4 g) was fractionated by a silica gel column eluted with petroleum ether–acetone (15:1) to give five fractions (BIV3bA–BIV3bE). BIV3bD (206 mg) was chromatographed on a LH-20 column ($CHCl_3$–MeOH, 1:1) and further purified by preparative HPLC (CH_3CN–H_2O 75:25) to afford **1** (3.9 mg) and **7** (3.7 mg). BIV3bB (356 mg) was isolated by passage over a LH-20 column ($CHCl_3$–MeOH, 1:1) and further purified by preparative HPLC (MeOH–H_2O 85:15) to yield **2** (9.4 mg) and **8** (8.5 mg). Fraction BIV3c (1.2 g) was subjected to a silica gel column, using petroleum ether–acetone (15:1) eluent and then preparative HPLC (MeOH–H_2O 85:15) to give **3** (4.9 mg), **9** (18.8 mg) and **10** (33.0 mg). Fraction BIV3d (1.2 g) was subjected to a LH-20 column ($CHCl_3$–MeOH 1:1) to yield seven fractions (BIV3dA–BIV3dG). BIV3dE (70 mg) was further isolated by preparative HPLC (MeOH–H_2O 85:15) to get **4** (4.1 mg), **12** (4.6 mg) and **13** (1.5 mg). BIV3dB (208 mg) was achieved by preparative HPLC with MeOH–H_2O (85:15), affording **14** (4.8 mg), BIV3dB-1 and BIV3dB-2. BIV3dB-1 (12 mg) was further purified by silica gel preparative HPLC with *n*-hexane–isopropanol (30:1) to give **5** (2.5 mg) and **6** (4.1 mg). BIV3dB-2 (15 mg) was further separated by silica gel preparative HPLC with *n*-hexane–EtOAc (10:1) to acquire **15** (5.4 mg) and **16** (4.3 mg). Fraction BIV3e (0.3 g) was run on a silica gel column (petroleum ether–acetone 15:1), yielding **11** (4.5 mg). Fraction BIV3a (0.4 g) was applied onto a LH-20 column ($CHCl_3$–MeOH 1:1) and further purified by preparative HPLC with MeOH–H_2O (80:20) to get **17** (4.2 mg). Fraction BV (9.8 g) was reseparated by MPLC (MeOH–H_2O, 85:15) to yield three fractions (BV1–BV3). BV3 (3.3 g) was eluted with petroleum ether–acetone (10:1) on a silica gel column to give four fractions (BV3a–BV3d). BV3c (305 mg) was further purified by preparative HPLC with MeOH–H_2O (85:15) to afford **18** (5.8 mg).

Attenuatumione A (**1**): colorless oil; $[\alpha]25$ D −19.3 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.09), 244 (3.75) nm; CD ($c = 3.0 \times 10^{-4}$, MeOH) λ_{max} nm ($\Delta\varepsilon$) 299 (−18.1), 248 (+52.0), 229 (−0.02), 218 (+15.6), 206 (−5.7); IR (KBr) ν_{max} cm^{-1} 3423, 2965, 2925, 1737, 1702, 1597, 1448, 1392, 1374, 1237, 1204, 1132, 1107, 1078, 1017, 971, 947, 796, 688, 623, 545; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; HRESIMS *m/z* 541.2928 $[M + Na]^+$ (calcd. for $C_{33}H_{42}NaO_5$, 541.2924).

Attenuatumione B (**2**): colorless oil; $[\alpha]25$ D +4.8 (*c* 0.19, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (3.22) nm; CD ($c = 3.0 \times$

Table 1
^{13}C NMR (125 MHz) spectral data of compounds **1–6** (in $CDCl_3$).

No.	1	2	3	4	5	6
1	71.1	171.9	58.9	81.5	74.2	74.8
2	30.6	198.1	31.1	203.4	171.7	171.7
3	59.6	48.5	89.3	71.1	120.8	120.4
4	46.7	40.1		30.5	190.8	191.0
5	56.4	36.9	172.1	59.6	64.1	63.9
6	22.9	56.9	115.7	46.7	39.7	39.9
7	42.5	206.6	194.0	56.5	42.5	44.4
8	48.1	95.3	77.5	22.9	47.8	47.3
9	81.5	63.2	49.5	42.5	205.8	205.9
10	203.3	206.6	48.0	35.4	26.9	26.5
11	203.8	42.5	36.9	67.8	94.1	93.4
12	67.8	18.9	205.6	204.8	71.3	71.1
13	35.6	19.8	73.1	48.0	27.0	26.1
14	204.8	15.7	21.3	203.8	25.5	25.0
15	72.9	40.1	39.6	193.0	208.8	209.1
16	30.5	29.0	22.5	135.2	41.0	41.0
17	31.2	122.6	123.8	128.6	21.2	20.7
18	17.1	135.0	132.8	128.5	21.2	20.9
19	29.8	26.0	25.8	132.4	29.4	29.4
20	22.7	18.3	17.9	128.5	119.7	119.7
21	25.5	143.3	22.5	128.6	134.3	134.3
22	193.0	120.8	120.1	72.9	26.0	26.0
23	135.2	70.8	133.0	31.2	18.2	18.0
24	128.6	30.0	25.9	30.5	28.3	28.2
25	128.5	30.0	17.9	30.6	122.5	122.5
26	132.3	26.0	193.7	17.0	133.6	133.6
27	128.5	113.7	137.2	29.1	26.1	25.8
28	128.6	139.0	128.3	119.2	17.9	18.0
29	29.3	25.9	128.0	139.1	14.6	12.9
30	119.0	18.2	132.1	40.2	38.3	39.4
31	135.4	27.6	128.0	16.5	24.5	25.3
32	26.2	117.7	128.3	26.8	124.6	124.5
33	18.2	136.4	22.5	124.4	131.8	132.3
34		25.9	27.2	131.6	25.8	27.0
35		18.1	29.2	25.9	18.1	18.2
36			124.7	17.8		
37			132.6	22.7		
38			26.0	25.5		
39			17.8			

Table 2
^{1}H NMR (500 MHz) spectral data of compounds **1**–**6** (in $CDCl_3$, *J* in Hz).

No.	1	2	3	4	5	6
2	2.52 m		2.73 dd (13.0, 10.5) 1.79 dd (13.0, 5.5)			
3	1.88 dd (12.5, 8.0)		4.66 dd (10.5, 5.5)			
4		2.20 d (6.5)		2.53 m		
5	1.92 d (5.5)	1.52 d (16.5)		1.85 dd (12.5, 7.5)		
6	1.97 t (13.0) 1.69 m				1.84 dd (14.0, 4.0) 1.38[a]	1.84 dd (13.5, 4.5) 1.56
7	2.10 dd (9.0, 7.0)			1.90 dd (13.5, 6.5)	1.68	1.55
8				1.97 t (13.0) 1.68 m		
9		4.38 s		2.06[a]		
10			1.50 m	2.49 dd (14.5, 6.5) 2.20 d (14.5)	2.99 d (10.5)	3.06 dd (15.0, 10.0) 2.90 dd (15.0, 10.0)
11		2.52 m	2.32 d (14.5) 2.22 m		4.77 t (10.5)	4.66 t (10.0)
12		1.02 d (6.5)				
13	2.46 dd (14.5, 6.5) 2.21 d (14.5)	1.08 d (6.5)			1.38[a]	1.32 s
14		1.12 s	1.18 s		1.24 s	1.23 s
15		1.49 m	2.16[a] 1.66 m			
16	1.37 s	2.08 m 1.72 m	2.16[a]		2.49 dd (13.0, 6.5)	2.41 d (6.5)
17	1.32 s	4.93 t (7.0)	5.15 t (7.0)	7.06 d (7.5)	1.15 s	1.07 d (6.5)
18	1.00 s			7.28 d (7.5)	1.15 s	1.16 d (6.5)
19	1.13 s	1.74 s	1.64 s	7.39 t (7.5)	2.44 dd (15.0, 7.0) 1.26	2.44 dd (13.0, 6.5) 1.26
20	1.39 s	1.59 s	1.66[a]	7.28 d (7.5)	5.00 dd (14.0, 7.0)	5.00 t (6.5)
21	1.42 s	5.68 d (15.5)	3.10 dd (14.0, 8.0) 2.98 dd (14.0, 8.0)	7.06 d (7.5)		
22		5.60 m	5.04 t (7.0)		1.67 s	1.66[a]
23				1.32 s	1.65[a]	1.66[a]
24	7.05 d (7.5)	1.39 s	1.71 s	1.37 s	2.07 dd (12.0, 6.0) 1.70 m	2.07 m 1.66[a]
25	7.28 d (7.5)	1.38 s	1.63 s	1.00 s	4.94 t (8.0)	4.92 t (7.0)
26	7.39 t (7.5)	2.85 dd (15.0, 7.0) 2.60 dd (15.0, 7.0)		1.12 s		
27	7.28 d (7.5)	4.93 t (7.0)		2.62 t (6.5)	1.66[a]	1.58 s
28	7.05 d (7.5)		7.46 d (7.5)	5.30 t7.5	1.55 s	1.53 s
29	2.62 dd (17.0, 8.0)	1.64 s	7.21 t (7.5)		1.13 s	1.09 s
30	5.27 t (7.5)	1.66[a]	7.38 t (7.5)	2.08[a]	1.66[a]	1.80 dd (13.0, 5.5) 1.41 d (13.0)
31		2.48 dd (15.0, 7.5) 2.12 m	7.21 t (7.5)	1.66[a]	2.01 dd (13.0, 6.5) 1.94 dd (12.5, 6.5)	2.18 m 2.00 m
32	1.75 s	4.70 t (7.0)	7.46 d (7.5)	2.08[a]	5.00 dd (14.0, 7.0)	5.00 t (6.5)
33	1.67 s		1.49 s	5.05 t (6.5)		
34		1.60 s	1.41 s		1.65[a]	1.66[a]
35		1.66[a]	2.18[a]	1.66[a]	1.57 s	1.66[a]
36			4.88 t (7.0)	1.59 s		
37				1.39 s		
38			1.69 s	1.42 s		
39			1.55 s			

[a] Overlapped signals.

10^{-4}, MeOH) λ_{max} nm ($\Delta\varepsilon$) 373 (+8.8), 333 (−0.9), 301 (+3.5), 252 (−3.0), 224 (+3.6); IR (KBr) ν_{max} cm^{-1} 3433, 2973, 2930, 1813, 1761, 1735, 1463, 1383, 1261, 1127, 1022, 980, 842, 800; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; HRESIMS *m/z* 591.3657 $[M + Na]^+$ (calcd. for $C_{35}H_{52}NaO_6$, 591.3656).

Attenuatumione C (**3**): colorless oil; [α]25 D +20.9 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.12), 247 (3.88), 274 (3.74) nm; CD ($c = 3.0 \times 10^{-4}$, MeOH) λ_{max} nm ($\Delta\varepsilon$) 395 (+9.8), 380 (+14.8), 303 (−20.0), 269 (+110.4); IR (KBr) ν_{max} cm^{-1} 3433, 2970, 2925, 2854, 1729, 1697, 1624, 1449, 1376, 1224, 1186, 1130, 1016, 845, 795, 690, 629, 537; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; HRESIMS *m/z* 587.3732 $[M + H]^+$ (calcd. for $C_{38}H_{51}O_5$, 587.3731).

Attenuatumione D (**4**): colorless oil; [α]25 D −10.8 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.38) nm; CD ($c = 3.0 \times 10^{-4}$, MeOH) λ_{max} nm ($\Delta\varepsilon$) 375 (+3.4), 297 (−11.7), 249 (+35.8), 228 (−0.7), 218 (+5.7); IR (KBr) ν_{max} cm^{-1} 3420, 2966, 2925, 1737, 1702, 1597, 1448, 1391, 1374, 1236, 1204, 1132, 1078, 1016, 971, 946, 794, 754, 688, 623, 539; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; HRESIMS *m/z* 609.3551 $[M + Na]^+$ (calcd. for $C_{38}H_{50}NaO_5$, 609.3550).

Attenuatumione E (**5**): colorless oil; [α]25 D +39.7 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.10), 278 (3.61) nm;

CD ($c = 3.0 \times 10^{-4}$, MeOH) λ_{max} nm ($\Delta\varepsilon$) 378 (+12.0), 307 (−56.4), 277 (+131.2), 207 (−44.5); IR (KBr) ν_{max} cm^{-1} 3432, 2965, 2925, 2854, 1726, 1640, 1617, 1463, 1392, 1383, 1239, 1157, 1079, 963, 909, 843, 800, 696, 584, 478; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; HRESIMS *m/z* 575.3706 $[M + Na]^+$ (calcd. for $C_{35}H_{52}NaO_5$, 575.3707).

Attenuatumione F (**6**): colorless oil; [α]25 D +19.6 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (3.94), 281 (3.78) nm; CD ($c = 3.0 \times 10^{-4}$, MeOH) λ_{max} nm ($\Delta\varepsilon$) 379 (+13.0), 307 (−66.8), 278 (+106.6), 208 (−30.3); IR (KBr) ν_{max} cm^{-1} 3440, 2969, 2926, 2855, 1725, 1640, 1616, 1446, 1392, 1379, 1354, 1248, 1177, 1155, 1131, 1084, 964, 950, 912, 885, 850, 767, 695, 681, 628, 596, 547, 447; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; HRESIMS *m/z* 575.3709 $[M + Na]^+$ (calcd. for $C_{35}H_{52}NaO_5$, 575.3707).

2.4. Absolute configuration of C-13 in 3

The in situ formed $[Rh_2(OCOCF_3)_4]$ complex method was used according to the published procedure [11,12]. Compound **3** (0.45 mg) was dissolved in dried solution of the dirhodium trifluoroacetate $[Rh_2(OCOCF_3)_4]$ complex (1.0 mg) in CH_2Cl_2 (400 μL). After mixing, the first CD spectrum was recorded immediately, and the time evolution was monitored until stationary (about 10 min). The inherent CD spectrum was subtracted. The sign of the E band at around 350 nm in the induced CD data was correlated to the absolute configuration of the tertiary alcohol [11,12].

2.5. Cytotoxicity assay

Human hepatoma cell lines SMMC7721, human osteosarcoma cell line U2OS, and human breast adenocarinoma cell line MCF-7 were obtained from the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). All the cells were cultured in RPMI-1640 medium (GIBCO Invitrogen Corp., Carlsbad, CA) supplemented with 10% fetal bovine serum (Sijiqing, Hangzhou, China), 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C with 5% CO_2. Cells were gathered and seeded in 96-well plates at a density of 5×10^3 cells per well in 200 μL medium for 24 h at 37 °C. The tested compounds were added at different concentrations (0–50 μM) for 48 h using *cis*-platinum (Sigma-Aldrich Company, USA) as a positive control. After the treatment, 20 μL of MTT solution (5 mg/mL) was added and cultured for 4 h. Then the supernatant was discarded and DMSO was added (150 μL/well). Absorbance was determined at 570 nm by a Universal Microplate Reader (SpectramaxPlus 384; Molecular Devices, Sunnyvale, CA).

3. Results and discussion

Attenuatumione A (**1**) was isolated as colorless oil. The molecular formula was assigned as $C_{33}H_{42}O_5$ on the basis of HRESIMS ion peak at *m/z* 541.2928 $[M + Na]^+$ (calcd. for $C_{33}H_{42}NaO_5$, 541.2924). The UV spectrum showed two absorption maxima at 204 and 244 nm. The IR spectrum showed absorption bands for hydroxy (3423 cm^{-1}) and carbonyl (1737 and 1702 cm^{-1}) groups. The ^{1}H NMR signals at δ 7.39 (1H, t, 7.5), 7.28 (2H, t, 7.5) and 7.05 (2H, d, 7.5) and the ^{13}C NMR resonances at δ 135.2, 132.3, 128.6 × 2, and 128.5 × 2 suggested the presence of an unsubstituted benzoyl moiety. Four ketone carbonyls (δ_C 204.8, 203.8, 203.3 and 193.0) signals in the ^{13}C NMR spectrum (Table 1) indicated the existence of the core tetracyclic system. The NMR signals of two gem-dimethyl groups [δ_H 1.39 (3H, s), 1.42 (3H, s), 1.13 (3H, s), 1.00 (3H, s); δ_C 29.8, 25.5, 22.7, 17.1], a 2-propanol group [δ_H 1.37 (3H, s), δ 1.32 (3H, s); δ_C 72.9, 31.2, 30.5], together with an isoprene unit [δ_H 5.27 (1H, t, 7.5), 1.75 (3H, s), 1.67 (3H, s); δ_C 135.4, 119.0, 29.3, 26.2, 18.2] suggested that **1** was a benzoylphloroglucinol derivative. The foregoing data was similar to that of plukenetione B [13]. In the HMBC spectrum, cross-peaks from H-29 (δ 2.62, dd, 17.0, 8.0) to C-12, -13, -14 and -30 and from H-30 (δ 5.27, t, 7.5) to C-33 indicated that the isoprene unit was attached to C-12. The HMBC correlations from H-2 (δ 2.52, m) to C-1, -3, -4 and -15, from Me-17 (δ 1.32, s) to C-3, -15 and -16, and from H-18 (δ 1.00, s) to C-3, -4, -5 and -19 confirmed that the cyclopentane-ring was fused at C-1 and C-5, the 2-propanol at C-3 and the gem-dimethyl group at C-4, respectively. The HMBC correlations observed between Me-21 (δ 1.42, s) and C-7, -8, -9 and -20 established the attachment of another gem-dimethyl group at C-8. The ROESY (Fig. 2) correlations of the proton signal at δ_H 1.97 (H-6a) with those at δ_H 1.88 (H-3), 1.39 (H-20) and 1.00 (H-18) indicated the 2-propanol group was α-orientated, while both Me-18 and Me-20 were at β-side. Thus, the structure of attenuatumione A (**1**) was assigned as shown in Fig. 1.

Attenuatumione B (**2**) was obtained as colorless oil, and its molecular formula was determined to be $C_{35}H_{52}O_6$ by the HRESIMS ion peak at *m/z* 591.3657 $[M + Na]^+$ (calcd. for $C_{35}H_{52}NaO_6$, 591.3656). The IR spectrum showed strong absorption bands for hydroxyl (3433 cm^{-1}) and carbonyl (1813, 1761 and 1735 cm^{-1}) groups. The ^{13}C NMR spectrum (Table 1) showed the characteristic signals for the core seven-membered ring system (δ_C 206.6, 95.3, 63.2, 56.9, 48.5, 40.1 and 36.9) that were only reported in perforatumone isolated from *H. perforatum* [14]. **2** exhibited prominent NMR signals showing the presence of three isoprenyl and one 3-hydroxyl-3-methylbutenyl groups, for example, five olefinic protons at δ_H 5.68 (d, 15.5), 5.60 (m), 4.93 (t, 7.0), 4.93 (t, 7.0) and 4.70 (t, 7.0) as well as four pairs of olefinic carbons between δ_C 113.7 and 143.3 (Table 1), and thus showed some similarities to perforatumone. Comparison of the ^{13}C NMR data of **2** with those of perforatumone revealed that they are structurally similar except for the side chain (C-21 to C-25). The HMBC spectrum showed correlations from δ_H 1.38 (H-25) to δ_C 120.8 (C-22) and 30.0 (C-24), and δ_H 5.60 (H-22) to δ_C 40.1 (C-4), 143.3 (C-21) and 70.8 (C-23). On the basis of these observations, **2** was determined to bear a 3-hydroxyl-3-methylbutenyl side chain (C-21 to C-25) at C-4 instead of the 3-methyl-2-butenyl side chain in perforatumone. Furthermore, except for the side chain, no significant differences in chemical shifts between them can be observed. Since according to plant biogenesis all derivatives should follow a common natural biosynthetic pathway, we are confident that their stereochemistry of C-6 and C-8 is identical and the lactone ring is fused to the seven-membered ring adopting a *cis* orientation [14]. The key ROESY correlations (Fig. 2) between δ_H 4.38 (1H, s, H-9) and 1.12 (3H, s, H-14) and 5.68 (1H, d, 15.5, H-21) indicated that C-14 and C-21 were on the same side of the seven-membered

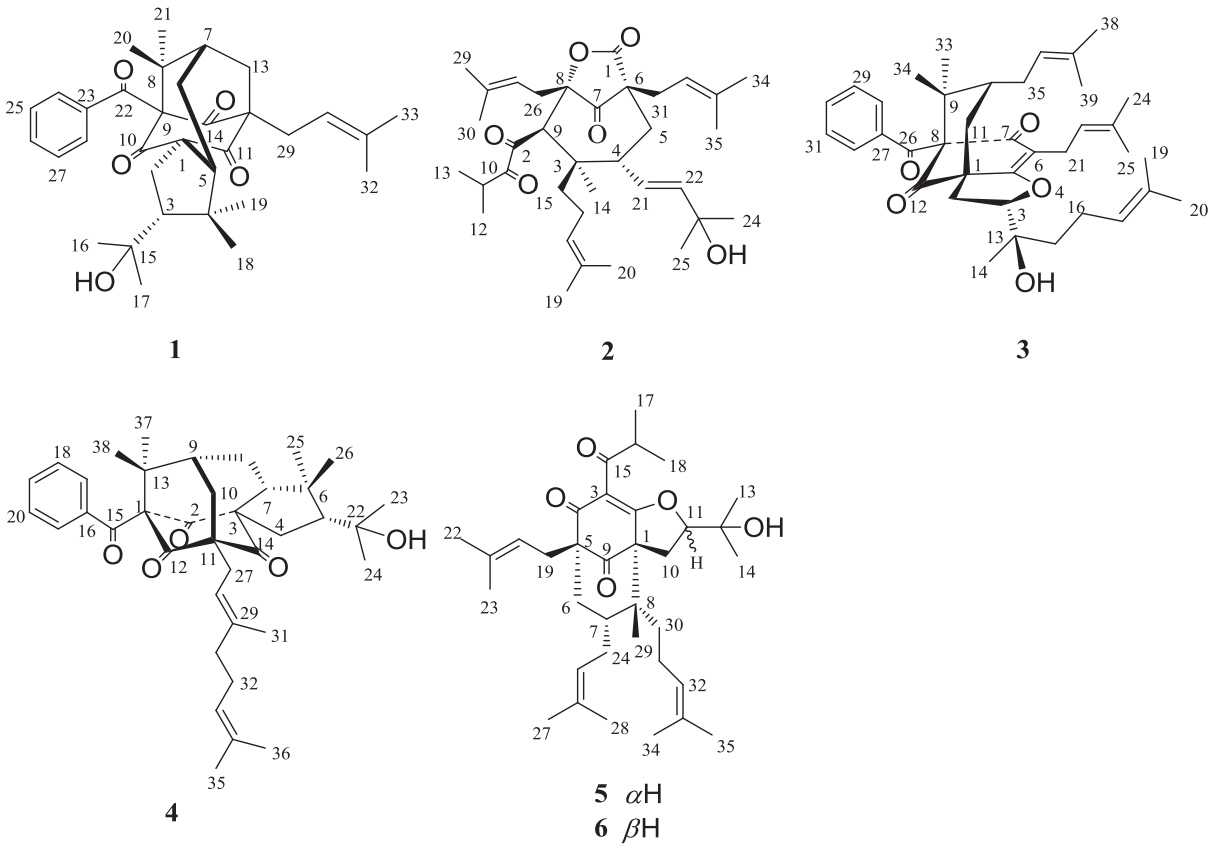

Fig. 1. Structures of compounds **1**–**6**.

ring. Therefore, the structure of **2** was assigned as shown in Fig. 1.

Attenuatumione C (**3**) was obtained as colorless oil. It has the molecular formula of $C_{38}H_{50}O_5$ based on HRESIMS data *m/z* 587.3732 $[M + H]^+$ (calcd. for $C_{38}H_{51}O_5$, 587.3731). The UV spectrum showed maxima at 204, 247 and 274 nm. The IR spectrum exhibited the absorptions of hydroxyl (3433 cm^{-1}) and carbonyl (1729 and 1697 cm^{-1}) groups. The ^{13}C NMR spectrum (Table 1) showed the signals for an unconjugated carbonyl (δ 205.6), an enolized 1,3-dicarbonyl ether system (δ 194.0, 115.7 and 172.1) and a gem-dimethyl group (δ 22.5 and 27.2), which were characteristic chemical shifts and multiplicities for a benzoylphloroglucinol derivative with the same skeleton to sampsoniones K [15]. The ^1H NMR signals at δ 7.38 (1H, t, 7.5), 7.21 (2H, t, 7.5) and 7.46 (2H, d, 7.5) and the ^{13}C NMR resonances at δ 137.2, 132.1, 128.3 × 2, and 128.0 × 2 indicated the presence of an unsubstituted benzoyl moiety. The ^1H NMR signals at δ 5.15 (1H, t, 7.0), 5.04 (1H, t, 7.0), 4.88 (1H, t, 7.0) together with the ^{13}C NMR resonances (Table 1) revealed the existence of two 3-methyl-2-butenyl and a 4-methyl-3-pentenyl side chain. The ^{13}C NMR data of **3** were very similar to those of sampsoniones K except for the chemical shifts of C-14, -15, and -16, suggesting that **3** was a C-13 stereoisomer of sampsoniones K. The absolute

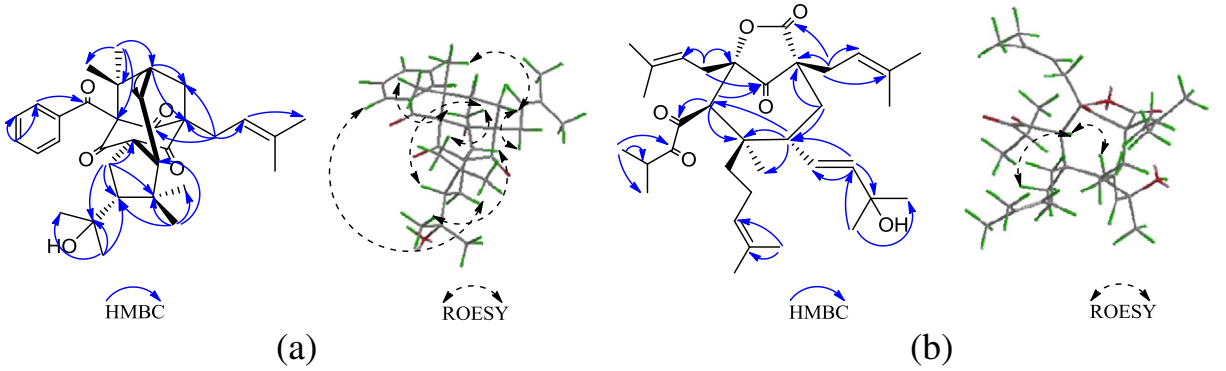

Fig. 2. Selected 2D NMR correlations of attenuatumione A (a) and attenuatumione B (b) (**1** and **2**).

configuration at C-13 was confirmed by the induced CD of the in situ formed $[Rh_2(OCOCF_3)_4]$ complex [11,12]. The Rh complex of **3** exhibited a negative E band correlating with the 13*R* configuration by applying the bulkiness rule [11,12,16]. Thus, the structure of **3** was deduced as shown in Fig. 1.

Attenuatumione D (**4**) was obtained as colorless oil. HRESIMS ion peak at *m/z* 609.3551 $[M + Na]^+$ (calcd. for $C_{38}H_{50}NaO_5$, 609.3550) indicated a molecular formula of $C_{38}H_{50}O_5$. The UV spectrum exhibited maximum at 204 nm. The IR spectrum revealed the presence of hydroxyl (3420 cm^{-1}) and carbonyl (1737 and 1702 cm^{-1}) groups. Extensive analyses of the ^1H NMR, ^{13}C NMR, HSQC and HMBC spectra of **4** led to the establishment of the structure of 4 as a diastereomer of sampsonione F [17]. The relative configuration of the C-7β methine proton and the C-5α 2-propanol group in **4** was established from the ROESY spectrum (Fig. 2), in which H-7 (δ 1.90) was correlated with Me-26 (δ 1.12) and H-10b (δ 2.20); H-10a (δ 2.49) was correlated with Me-38 (δ 1.42); Me-25 (δ 1.00) showed correlations with Me-23 (δ 1.32) and Me-24 (δ 1.37). Thus, the structure of **4** was deduced as shown in Fig. 1.

Attenuatumione E (**5**) and attenuatumione F (**6**) were acquired as colorless oils. Their molecular formulae were determined to be $C_{35}H_{52}O_5$, on the basis of the HRESIMS data. The IR spectrum exhibited absorptions for hydroxyl group (3432 cm^{-1}) in **5** and (3440 cm^{-1}) in **6** and carbonyl group (1726 cm^{-1}) in **5**, (1725 cm^{-1}) and in **6**. The ^1H- and ^{13}C NMR spectra of **5** and **6** resembled each other and indicated the presence of a 2-methylpropionyl group, three isoprenyl groups, two carbonyl groups, one enol moiety (Tables 1, 2). From these data, **5** and **6** were each assumed to be prenylated phloroglucinol derivatives having a 2-methylpropionyl group. Furthermore, the following characteristic spectral features are coincided with those of furohyperforins [18]: δ_H 4.77 (1H, t, 10.5), 2.99 (2H, d, 10.5), 1.38 and 1.24 (each 3H, s), δ_C 94.1, 71.3, 27.0, 26.9, and 25.5 in **5**; δ_H 4.66 (1H, t, 10.0), 3.06 (1H, dd, 15.0, 10.0), 2.90 (1H, dd, 15.0, 10.0), 1.32 and 1.23 (each 3H, s), δ_C 93.4, 71.1, 26.5, 26.1, and 25.0 in **6**. In the HMBC correlations in **5** from the proton signals at δ_H 2.99 (H-10a) to C-2 (δ_C 171.7), C-8 (δ_C 47.8) indicated that a dihydrofuran ring was formed between C-1 and C-2. Two isoprenyl groups were located at C-5 (δ_C 64.1) and C-7 (δ_C 42.5), suggested by the HMBC correlations from δ_H 2.44 (H-19) to δ_C 64.1 (C-5) and δ_C 190.8 (C-4), and δ_H 2.07 (H-24) to δ_C 42.5 (C-7), respectively. In addition, a methyl and a 4-methyl-3-pentenyl side chain were confirmed to be at C-8 by the correlations between δ_H 1.13 (Me-29) and 1.66 (H-30) and δ_C 47.8 (C-8). The analyses above indicated that the remaining 2-methylpropionyl group must be located to C-3 (δ 120.8). The relative stereochemistry of C-11 in **5** was deduced by the analysis of the ROESY spectrum. The correlations of the proton signals at δ_H 4.77 (H-11) with δ_H 1.66 (H-30) and 1.94 (H-31) suggested that the proton at C-11 in **5** was α-orientated. Correspondingly, **6** was regarded as a C-11 epimer of **5** due to the slight difference that appeared in their chemical shifts of C-11 to C-14 (Table 1), H-10 and H-11 (Table 2), and the ROESY correlation between δ_H 1.23 (Me-14) and 1.41 (H-30).

Twelve known compounds were identified as plukenetione B (**7**) [11], sampsonione N (**8**) [19], sampsonione K (**9**) [13], furohyperforin (**10**) [20], sampsonione M (**11**) [13], sampsonione C (**12**) [17], otogirinin D (**13**) [21], furoadhyperforin (**14**) [22], epifurohyperforin Isomer 1 (**15**) [23], furohyperforin Isomer 1 (**16**) [23], 2,4,6-Trihydroxy benzophenon-4-O-geranylether (**17**) [24], and sampsonione O (**18**) [19] by comparison of their spectroscopic data with those reported.

Table 3
Cytotoxicities of the isolates from *H. attenuatum* [a,b].

Compounds	SMMC7721	U2OS	MCF-7
1	15.10 ± 2.70	11.8 ± 2.45	>50
2	20.98 ± 1.30	>50	>50
3	10.12 ± 2.38	10.56 ± 0.78	29.70 ± 0.34
4	>50	>50	>50
5	>50	>50	>50
6	>50	>50	>50
7	11.12 ± 3.80	26.45 ± 3.44	30.45 ± 3.15
8	>50	>50	32.05 ± 1.91
9	32.68 ± 0.45	>50	>50
10	>50	12.93 ± 2.69	15.89 ± 2.12
11	>50	>50	>50
12	>50	>50	>50
13	31.89 ± 3.70	>50	>50
14	>50	>50	>50
15	19.73 ± 2.02	14.08 ± 3.62	22.32 ± 4.36
16	21.21 ± 0.56	15.15 ± 1.34	17.60 ± 0.19
17	>50	44.01 ± 1.37	27.20 ± 2.62
18	>50	>50	24.45 ± 0.92
cis-Platinum[c]	6.62 ± 1.57	6.19 ± 0.85	9.69 ± 0.64

[a] Results are expressed as IC_{50} values in μM.
[b] Compounds with IC_{50} >50 μM are not shown.
[c] Positive controls.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to measure the viability of three tumor cell lines after exposure to compounds **1–18**. As a result, twelve compounds **1–3**, **7–10**, **13**, and **15–18** exhibited IC_{50} values of 10.12–44.01 μM against one or more cancer cell lines (Table 3). Among them, **3**, **7**, **15** and **16** showed cytotoxic activities on all three cell lines. Compound **3** was most active with an IC_{50} value of 10.12 μM against SMMC7721 and 10.56 μM against U2OS but 29.70 μM against MCF-7 cell lines. Unfortunately, it is difficult to identify a common structural characteristic which might explain their activity as cytotoxic agents.

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Appendix A. Supplementary data

^1H and ^{13}C NMR spectra of compounds **1–6** and their selected 2D NMR and HRESIMS spectra are available as Supporting Information. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2014.02.011.

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