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Prenylated phloroglucinol derivatives from *Hypericum sampsonii*

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ABSTRACT

Six new acylphloroglucinol derivatives, sampsonols A-F (**1**–**6**), were isolated from the petroleum ether extract of the aerial parts of *Hypericum sampsonii*. The structures and relative configurations of sampsonols A-F were elucidated by extensive spectroscopic analyses. All these compounds were tested for their *in vitro* cytotoxic and anti-inflammatory activities. Sampsonols A and B (**1** and **2**) showed significant cytotoxicity against four human tumor cell lines with IC_{50} values in the range of 13–28 μM, whereas sampsonols C and F (**3** and **6**) showed potent inhibitory activities against LPS-induced NO production in RAW 264.7 macrophages with IC_{50} values of 27.3 and 29.3 μM, respectively.

1. Introduction

Plants of the genus *Hypericum* have been used as traditional medicinal plants in various parts of the world, and some of them have antidepressant, antiviral, wound healing and antimicrobial bioactivities [1]. In China, *Hypericum sampsonii* is used in the treatment of numerous disorders such as backache, burns, diarrhoea, snakebites and swellings [2]. Due to its various bioactivities, *H. sampsonii* had been investigated and some xanthones and polyprenylated phloroglucinol derivatives had been isolated [3–12]. In the course of our investigations for bioactive constituents from *H. sampsonii*, we have previously isolated five new phenolic compounds, named sampsones A–C and hypericumxanthones A and B, which exhibited moderate antibacterial and cytotoxic activities in vitro [13,14]. Under great scientific interests to the complicated chemical structures and intriguing biological activities of polyprenylated phloroglucinol derivatives, we reinvestigated the petroleum ether extract of the aerial parts of *H. sampsonii*, which is rich in polyprenylated phloroglucinol derivatives. As a result, six new polyprenylated phloroglucinol derivatives (**1**–**6**) were identified from the petroleum ether extract. Briefly, they were all acylphloroglucinol derivatives possessing a spiro skeleton with one monoterpene moiety. By the way, C-3 and C-13 of **3**–**6** were linked by an oxygen ether bridge, which had never been reported before. Herein, we report the isolation, structure elucidation, and bioactivities of the isolated compounds.

2. Experimental

2.1. General

Optical rotations were measured on a KRÜSS P800-T polarimeter. UV spectra were tested on a Jasco UV–vis spectrometer. CD spectra were measured on a Jasco J-810 spectropolarimeter. IR spectra were recorded on a Nicolet™380 spectrometer from Thermo Electron using KBr pellets. HRESIMS spectra were performed using an APEXIII 7.0 TESLA FTMS mass

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spectrometer (Bruker Daltonics, Inc.) or IonSpec 4.7 Tesla FTMS. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker DRX-400 instrument with tetramethylsilane as an internal standard. Column chromatography (CC) was performed using silica gel (SiO_2, 200–300 mesh, Qingdao Haiyang Chemical & Special Silica Gel Co, Ltd., Qingdao, P. R. China), RP-18 silica gel (25–40 μm, Fuji Silysica Chemical Ltd.), Sephadex LH-20 (Pharmica Biotech AB, Uppsala, Sweden), and MCI GEL CHP20P (75–150 μm, Mitsubishi Chemical Industries) as packing materials, respectively. HSGF254 silica gel TLC (0.20–0.50 mm) plates (Yantai Chemical Industrial Institute, People's Republic of China) were used for analytical TLC and HSGF254 silica gel TLC (0.40–0.50 mm) plates (Yantai Chemical Industrial Institute, People's Republic of China) were used for preparative TLC.

2.2. Plant material

The plant material (stems and leaves) of *H. sampsonii* was collected from Jinhua, Zhejiang Province, PR China and was identified by Prof. Lu-Ping Qin, School of Pharmacy, Second Military Medical University, where the voucher specimen (No. 2008YBC2) was deposited.

2.3. Extraction and isolation

The whole air-dried and powdered plant material (2 kg) was extracted with petroleum ether (5 L×3) each for 24 h at room temperature. The extract was concentrated in vacuo to give a residue (18 g), which was stirred with 100 mL of acetone. After the insoluble solid was filtered, the filtration was concentrated to give a residue (12 g). The residue was chromatographed over MCI (400 g, 5×80 cm) with MeOH-H_2O (80:20, 6 L) to remove the chlorophyll part and then concentrated to get a residue (8 g). The residue was loaded on a silica gel (200–300 mesh, 300 g) column (8×46 cm) eluted with *n*-hexane-EtOAc (20:1, 10:1, 5:1, 3:1, 1:1, 0:1, each 4 L) to give six fractions (Fr.1-6) based on the TLC analysis. Fr.3 (800 mg) was rechromatographed on silica gel CC (300–400 mesh, 20 g, 1.5×50 cm), eluting with *n*-hexane-chloroform-acetone (75:20:5, 2 L) to give four fractions I-IV. Fraction II (45 mg) was subjected to chromatography over Sephadex LH-20 column (2×120 cm) with petroleum-chloroform-MeOH (45:45:10, 300 ml) to afford two new fractions Fr.IIa and Fr.IIb. Fr.IIa (22 mg) was purified by preparative TLC, developed with *n*-hexane-EtOAc-MeOH (30:78:2) to get **4** (5 mg; R_f 0.5) and **6** (8 mg; R_f 0.7). Fr.IIb (10 mg) was purified

Table 1
^{1}H and ^{13}C NMR data for **1** and **2** (in $CDCl_3$).

Position	1		2	
	δ_C	δ_H	δ_C	δ_H
1	176.7 (C)		174.4 (C)	
2	112.0 (C)		111.6 (C)	
3	195.9 (C)		198.2 (C)	
4	62.1 (C)		62.6 (C)	
5	208.2 (C)		207.4 (C)	
6	60.1 (C)		60.5 (C)	
7	24.9 (CH_2)	ax 1.52 (1 H, *m*) eq 2.13 (1 H, *m*)	26.1 (CH_2)	ax 1.75 (1 H, *m*) eq 1.86 (1 H, *m*)
8	48.0 (CH)	1.86 (1 H, *dd*, 12.2, 4.8)	47.7 (CH)	1.85 (1 H, *m*)
9	79.1 (C)		79.3 (C)	
10	40.2 (CH_2)	1.78 (2 H, *m*)	40.2 (CH_2)	1.76 (2 H, *m*)
11	21.8 (CH_2)	ax 1.51 (1 H, *m*) eq 1.82 (1 H, *m*)	21.7 (CH_2)	ax 1.48 (1 H, *m*) eq 1.83 (1 H, *m*)
12	51.3 (CH)	2.01 (1 H, *m*)	51.6 (CH)	1.84 (1 H, *m*)
13	73.6 (C)		73.2 (C)	
14	49.7 (CH_2)	ax 2.22 (1 H, *d*, 14.2) eq 1.52 (1 H, *m*)	50.2 (CH_2)	ax 2.30 (1 H, *d*, 14.0) eq 1.64 (1 H, *d*, 14.1)
15	21.8 (CH_3)	1.12 (3 H, *s*)	21.1 (CH_3)	0.95 (3 H, *s*)
16	26.6 (CH_3)	1.32 (3 H, *s*)	26.5 (CH_3)	1.31 (3 H, *s*)
17	32.0 (CH_2)	α 2.13 (1 H, *dd*, 15.3, 8.8) β 2.52 (1 H, *dd*, 15.3, 10.4)	31.6 (CH_2)	α 2.18 (1 H, *dd*, 15.5, 8.6) β 2.42 (1 H, *m*)
18	91.9 (CH)	4.51 (1 H, *dd*, 10.4, 8.9)	91.9 (CH)	4.44 (1 H, *dd*, 10.2, 8.6)
19	69.9 (C)		70.3 (C)	
20	26.9 (CH_3)	1.26 (3 H, *s*)	26.9 (CH_3)	1.17 (3 H, *s*)
21	24.7 (CH_3)	1.10 (3 H, *s*)	24.4 (CH_3)	1.10 (3 H, *s*)
22	39.1 (CH_2)	α 2.46 (1 H, *dd*, 13.5, 7.9) β 2.76 (1 H, *dd*, 13.5, 7.9)	38.4 (CH_2)	α 2.41 (1 H, *m*) β 2.66 (1 H, *dd*, 13.4, 7.6)
23	116.4 (CH)	4.97 (1 H, *t*, 7.9)	116.2 (CH)	5.03 (1 H, *t*, 7.6)
24	139.1 (C)		139.0 (C)	
25	26.2 (CH_3)	1.72 (3 H, *s*)	26.1 (CH_3)	1.75 (3 H, *s*)
26	18.1 (CH_3)	1.58 (3 H, *s*)	18.2 (CH_3)	1.60 (3 H, *s*)
27	192.3 (C)		191.4 (C)	
28	137.8 (C)		138.2 (C)	
29	129.9 (CH)	7.82 (1 H, *d*, 7.2)	129.8 (CH)	7.87 (1 H, *d*, 7.4)
30	128.2 (CH)	7.42 (1 H, *t*, 7.2)	128.3 (CH)	7.47 (1 H, *t*, 7.4)
31	133.5 (CH)	7.55 (1 H, *t*, 7.2)	133.4 (CH)	7.59 (1 H, *t*, 7.4)
32	128.2 (CH)	7.42 (1 H, *t*, 7.2)	128.3 (CH)	7.47 (1 H, *t*, 7.4)
33	129.9 (CH)	7.82 (1 H, *d*, 7.2)	129.8 (CH)	7.87 (1 H, *d*, 7.4)

by preparative TLC, developed with *n*-hexane-acetone (50:10) to get **5** (3 mg). Fr. III (200 mg) was purified with column chromatography over ODS column (3×50 cm, 40 g) eluted with acetone-water (70:30, 1 L) to give two further fractions (Fr.IIIa and Fr.IIIb). Fr.IIIa (40 mg) was purified by preparative TLC with *n*-hexane-chloroform-acetone (65:30:5) to get **3** (8 mg, R_f 0.4). Fr. IIIb (50 mg) was further purified by preparative TLC *n*-hexane-EtOAc-MeOH (46:46:8) to **1** (5 mg; R_f 0.4) and **2** (4 mg; R_f 0.6).

*2.3.1. Sampsonol A (**1**)*

White amorphous powder; $[\alpha]D^{20}$ −7.7 (*c* 0.5, CH_2Cl_2); UV (MeOH) λ_{max} nm (logε) 253 (4.4); CD (*c* = 0.5 g/L, MeOH) $\Delta\varepsilon$ (nm) 233.5 (−11.1), 251.6 (9.8), 276.8 (−2.4), 301.2 (3.9), 333.6 (−7.2); IR (KBr) ν_{max} 3442, 2926, 1718, 1664, 1647, 1449, 1376, 1286, 1208, 1177, 1117, 947 cm^{-1}; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data, see Table 1; HRESIMS *m/z* 573.2828 $[M+Na]^+$ (calcd for $C_{33}H_{42}NaO_7$, 573.2823).

*2.3.2. Sampsonol B (**2**)*

White amorphous powder; $[\alpha]D^{20}$ + 45.3 (*c* 0.5, CH_2Cl_2); UV (MeOH) λ_{max} nm (logε) 253 (4.4); CD (*c* = 0.5 g/L, MeOH) $\Delta\varepsilon$ (nm) 232.7 (10.3), 254 (−5.6), 266.9 (−2.2), 287.3 (−6.3), 336.8 (3.2); IR (KBr) ν_{max} 3413, 2967, 2927, 1719, 1666, 1649, 1449, 1377, 1282, 1208, 1177, 998 cm^{-1}; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data, see Table 1; HRESIMS *m/z* 573.2828 $[M+Na]^+$ (calcd for $C_{33}H_{42}NaO_7$, 573.2823).

*2.3.3. Sampsonol C (**3**)*

White amorphous powder; $[\alpha]D^{20}$ +16.5 (*c* 0.5, CH_2Cl_2); UV (MeOH) λ_{max} nm (logε) 256 (4.2); IR (KBr) ν_{max} 3510, 2937, 1711, 1664, 1635, 1452, 1378, 1350, 1318, 1246 cm^{-1}; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data, see Tables 2 and 3; HRESIMS *m/z* 485.2298 $[M+Na]^+$ (calcd for $C_{29}H_{34}NaO_5$, 485.2299).

*2.3.4. Sampsonol D (**4**)*

White amorphous powder; $[\alpha]D^{20}$ +35.2 (*c* 0.5, CH_2Cl_2); UV (MeOH) λ_{max} nm (logε) 258 (4.3); IR (KBr) ν_{max} 3445, 2925, 2853, 1721, 1676, 1625, 1449, 1352, 1317, 1243, 1186 cm^{-1}; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data, see Tables 2 and 3; HRESIMS *m/z* 501.2249 $[M+Na]^+$ (calcd for $C_{29}H_{34}NaO_6$, 501.2248).

*2.3.5. sampsonol E (**5**)*

Colorless oil; $[\alpha]D^{20}$ +25.8 (*c* 0.5, CH_2Cl_2); UV (MeOH) λ_{max} nm (logε) 286 (4.1), 234 (3.9); IR (KBr) ν_{max} 3479, 2963, 2931, 2872, 1720, 1696, 1620, 1454, 1381, 1350, 1315, 1008 cm^{-1}; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data, see Tables 2 and 3; HRESIMS *m/z* 443.2803 $[M+H]^+$ (calcd for $C_{27}H_{39}O_5$, 443.2792).

*2.3.6. Sampsonol F (**6**)*

Colorless oil; $[\alpha]D^{20}$ +12.4 (*c* 0.5, CH_2Cl_2); UV (MeOH) λ_{max} nm (logε) 283 (4.0), 233 (3.8); IR (KBr) ν_{max} 3479, 2970,

Table 2
^1H NMR data for **3**–**6** (δ, ppm, at 400 MHz) in $CDCl_3$.

Position	3	4	5	6
7	ax 1.63 (1 H, *m*) eq 2.10 (1 H, *m*)	ax 1.74 (1 H, *m*) eq 2.30 (1 H, *dd*, 12.7, 5.3)	ax 1.54 (1 H, *m*) eq 1.92 (1 H, *m*)	ax 1.55 (1 H, *m*) eq 1.80 (1 H, *m*)
8	1.35 (1 H, *m*)	1.38 (1 H, *dd*, 12.3, 4.9)	1.21 (1 H, *m*)	1.31 (1 H, *m*)
10	ax 1.71 (1 H, *m*) eq 1.86 (1 H, *m*)	ax 1.72 (1 H, *m*) eq 1.85 (1 H, *m*)	ax 1.72 (1 H, *m*) eq 1.89 (1 H, *m*)	ax 1.71 (1 H, *m*) eq 1.93 (1 H, *m*)
11	ax 1.74 (1 H, *m*) eq 1.26 (1 H, *m*)	ax 1.68 (1 H, *m*) eq 1.24 (1 H, *m*)	ax 1.78 (1 H, *m*) eq 1.40 (1 H, *m*)	ax 1.72 (1 H, *m*) eq 1.41 (1 H, *m*)
12	2.01 (1 H, *dd*, 11.8, 5.4)	2.05 (1 H, *ddd*, 12.0, 12.0, 5.6)	2.08 (1 H, *m*)	2.05 (1 H, *dd*, 11.8, 5.0)
14	ax 2.20 (1 H, *d*, 11.8) eq 2.05 (1 H, *m*)	2.18 (2 H, *m*)	ax 2.13 (1 H, *m*) eq 2.01 (1 H, *dd*, 12.0, 1.9)	ax 2.12 (1 H, *d*, 11.9) eq 1.90 (1 H, *m*)
15	1.45 (3 H, *s*)	1.45 (3 H, *s*)	1.54 (3 H, *s*)	1.53 (3 H, *s*)
16	1.33 (3 H, *s*)	1.29 (3 H, *s*)	1.26 (3 H, *s*)	1.29 (3 H, *s*)
17	1.43 (3 H, *s*)	1.48 (3 H, *s*)	1.32 (3 H, *s*)	1.38 (3 H, *s*)
18	2.65 (2 H, *d*, 7.9)	5.90 (1 H, *d*, 16.0)	2.62 (2 H, *d*, 7.8)	2.65 (1 H, *dd*, 13.4, 7.8) 2.42 (1 H, *dd*, 14.0, 7.8)
19	5.04 (1 H, *t*, 7.9)	5.71 (1 H, *d*, 16.0)	4.99 (1 H, *t*, 7.8)	4.84 (1 H, *t*, 7.6)
21	1.67 (3 H, *s*)	1.30 (3 H, *s*)	1.64 (3 H, *s*)	1.61 (3 H, *s*)
22	1.59 (3 H, *s*)	1.31 (3 H, *s*)	1.59 (3 H, *s*)	1.54 (3 H, *s*)
24			2.70 (1 H, *dd*, 15.6, 7.1) 2.52 (1 H, *dd*, 15.7, 6.8)	3.11 (1 H, *sept*, 6.8)
25	7.79 (1 H, *d*, 7.6)	7.79 (1 H, *d*, 7.2)	2.14 (1 H, *m*)	1.13 (3 H, *d*, 6.8)
26	7.42 (1 H, *t*, 7.6)	7.43 (1 H, *d*, 7.2)	0.91 (3 H, *d*, 7.6)	1.07 (3 H, *d*, 6.8)
27	7.55 (1 H, *t*, 7.6)	7.56 (1 H, *t*, 7.2)	0.95 (3 H, *d*, 7.6)	
28	7.42 (1 H, *t*, 7.6)	7.43 (1 H, *d*, 7.2)		
29	7.79 (1 H, *d*, 7.6)	7.79 (1 H, *d*, 7.2)		

Table 3
The ^{13}C NMR data of compounds **3–6** (δ, ppm, at 100 MHz) in $CDCl_3$.

Position	3	4	5	6
1	197.5 (C)	195.7 (C)	197.9 (C)	198.2 (C)
2	111.1 (C)	111.8 (C)	112.4 (C)	111.1 (C)
3	177.2 (C)	177.6 (C)	177.8 (C)	177.5 (C)
4	58.9 (C)	60.4 (C)[a]	59.3 (C)	59.6 (C)
5	208.4 (C)	205.8 (C)	208.1 (C)	209.0 (C)
6	59.5 (C)	60.5 (C)[a]	59.6 (C)	58.4 (C)
7	32.7 (CH_2)	34.8 (CH_2)	32.4 (CH_2)	34.6 (CH_2)
8	48.9 (CH)	49.6 (CH)	48.9 (CH)	49.2 (CH)
9	77.9 (C)	77.8 (C)	77.3 (C)	77.3 (C)
10	40.1 (CH_2)	39.9 (CH_2)	40.1 (CH_2)	40.1 (CH_2)
11	22.4 (CH_2)	22.4 (CH_2)	22.5 (CH_2)	22.6 (CH_2)
12	47.8 (CH)	48.1 (CH)	47.8 (CH)	47.9 (CH)
13	91.9 (C)	92.7 (C)	91.9 (C)	91.6 (C)
14	45.0 (CH_2)	44.5 (CH_2)	44.9 (CH_2)	43.9 (CH_2)
15	21.6 (CH_3)	21.6 (CH_3)	21.7 (CH_3)	21.8 (CH_3)
16	26.7 (CH_3)	26.7 (CH_3)	26.7 (CH_3)	26.9 (CH_3)
17	25.9 (CH_3)	23.1 (CH_3)	26.2 (CH_3)	23.3 (CH_3)
18	35.4 (CH_2)	131.1 (CH)	34.7 (CH_2)	39.2 (CH_2)
19	119.9 (CH)	136.4 (CH)	120.1 (CH)	118.5 (CH)
20	135.3 (C)	81.9 (C)	135.1 (C)	135.6 (C)
21	25.9 (CH_3)	24.6 (CH_3)	25.9 (CH_3)	25.9 (CH_3)
22	17.9 (CH_3)	23.7 (CH_3)	17.9 (CH_3)	17.7 (CH_3)
23	192.2 (C)	192.2 (C)	199.8 (C)	203.7 (C)
24	137.7 (C)	137.3 (C)	52.3(CH_2)	39.9 (CH)
25	128.9 (CH)	129.0 (CH)	25.2 (CH)	17.7 (CH_3)
26	128.4 (CH)	128.5 (CH)	22.8 (CH_3)	18.2 (CH_3)
27	133.2 (CH)	133.5 (CH)	22.7 (CH_3)	
28	128.4 (CH)	128.5 (CH)		
29	128.9 (CH)	129.0 (CH)		

[a] Signals may be exchangeable.

2933, 1696, 1630, 1453, 1384, 1315 cm^{-1}; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data, see Tables 2 and 3; HRESIMS *m/z* 429.2634 $[M+H]^+$ (calcd for $C_{26}H_{37}O_5$, 429.2636).

2.4. Cytotoxicity bioassay

Compounds **1–6** were assayed for their cytotoxicity against human breast (MCF-7), hepatoma (HepG2), colon (HT-29) and lung (A549) tumor cell lines using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) method [15]. Doxorubicin was used as a positive control. Freshly trypsinized cell suspensions were seeded in the 96-well microtiter plates at densities of 4×10^4 cells per well. The cells were incubated in a 5% CO_2 atmosphere for 24 h at 37 °C. After incubation, 20 μL of DMSO solution containing the samples was added to give the final concentrations of 0.2–100 μg/mL; 20 μL of DMSO was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated by an MTT assay procedure. At the end of incubation, 10 μL of 5 mg/mL MTT (Sigma) in phosphate-buffered saline was added to each well, and the plate was further incubated in a 5% CO_2 atmosphere for 4 h at 37 °C. The plate was then centrifuged at 1500 g for 5 min to precipitate wells and formazan. An aliquot of 150 μL of the supernatant was removed from each well, and 200 μL of DMSO was added to dissolve the MTT formazan crystals. The plate was mixed on a microshaker for 10 min and then read on a microplate reader at 550 nm. The IC_{50} values were calculated by the concentration–inhibition curve. Values represent the mean of three independent experiments and are expressed as mean ± SD.

2.5. Anti-inflammatory activity assay

This assay was performed as previously described [16]. Briefly, RAW 264.7 macrophages were seeded in 96-well plates an initial density of 2×10^5 cells/well. The cells were co-incubated with the isolated compounds and LPS (1 μg/mL) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the cultured RAW 264.7 macrophage supernatants with Griess reagent. Aliquots of supernatants (100 μL) were incubated, in sequence, with 50 μL of 1% sulfanilamide and 50 μL of 0.1% naphthylethylenediamine in 2.5% phosphoric acid solution. The absorbance at 548 nm was read using a microplate reader (POLARstar). Cell viability was determined using the mitochondrial respiration-dependent MTT reduction method [16].

3. Results and discussion

The petroleum ether-soluble extract of *H. sampsonii* was successively subjected to silica gel and Sephadex LH20 column chromatography as well as preparative TLC to afford **1–6**, sampsonols A–F.

Compound **1** was obtained as a white amorphous powder. It was assigned a molecular formula of $C_{33}H_{42}O_7$ as inferred from its HRESIMS. The IR spectrum of **1** showed absorption bands of hydroxyl (3442 cm^{-1}) and carbonyl groups (1718 and 1664 cm^{-1}). The ^1H NMR, ^{13}C NMR and DEPT spectrum of **1** showed the presence of one mono-substituted benzene ring, one 3-methyl-2-butenyl group, a 2-methylbutane-2,3-diol moiety, an enol, three carbonyl groups, four sp^3 quaternary

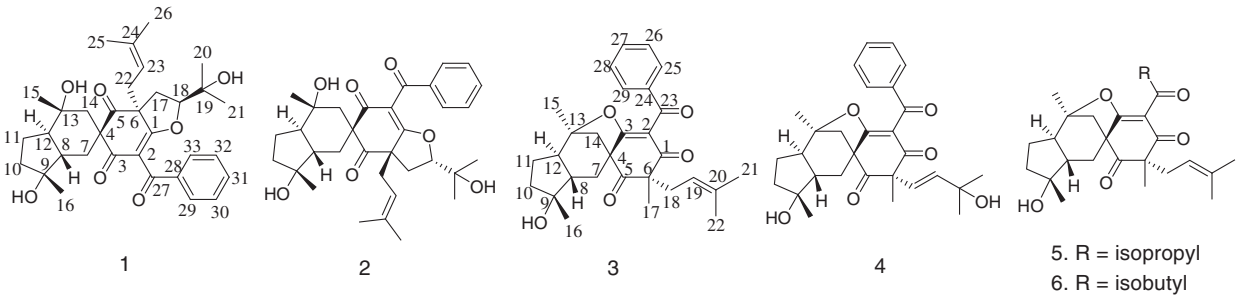

Fig. 1. Structures of sampsonols A–F (**1–6**).

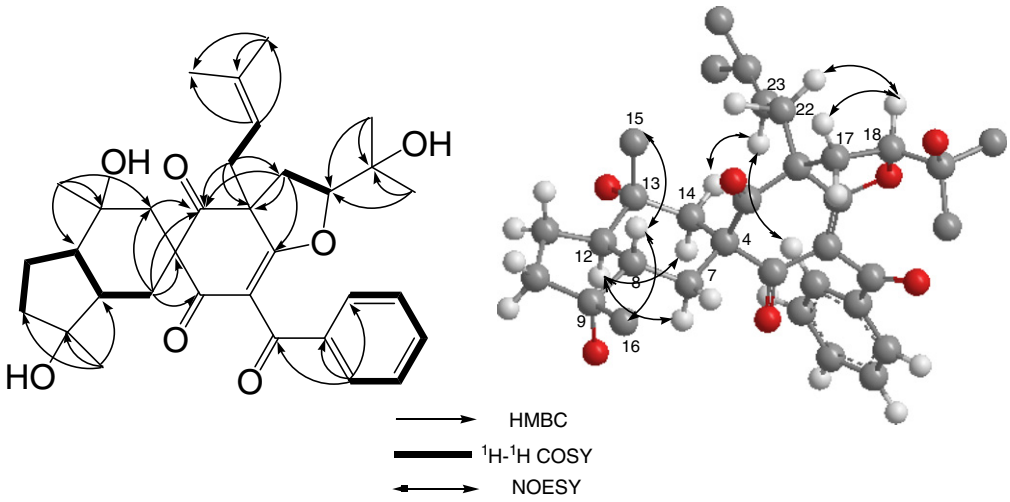

Fig. 2. The key HMBC, ^{1}H–^{1}H COSY and NOESY correlations of **1**.

carbons, two sp^3 methines, five sp^3 methylenes, and two tertiary methyls, among of which three sp^3 quaternary carbons {δ_C 79.1 (C-9), 73.6 (C-13), and 69.9 (C-19)} and a sp^3 methine {δ_C 91.9 (C-18)} were ascribed to those bearing an oxygen atom. Based on these data, **1** was considered as a benzoylphloroglucinol derivative with one 3-methyl-2-butenyl group, one 2-(2-hydroxypropyl)dihydrofuran ring, and one partial unit of a monoterpene moiety. The high similarity of the ^{1}H and ^{13}C NMR data (Table 1) of the C_{10} monoterpene unit of **1** with those of tomoeone A [17] established that **1** had the same C_{10} unit (a fused 5/6 ring system) as tomoeone A. The plane structure of the C_{10} unit was further confirmed by detailed interpretations of its 2D-NMR spectra (Fig. 2). Furthermore, the connectivities between the phloroglucinol skeleton (C-3 and C-5) and the monoterpene moiety (C-7 and C-14) via a spiro-carbon (C-4) were disclosed by HMBC cross-peaks of H_2-7 to C-14 and of H_2-7/H_2-14 to C-3, C-4, and C-5. A benzoyl group attached to C-2 was implied by the chemical shift of C-2 (δ_C 112.0) [17,18]. The presence of an ether linkage between C-1 and C-18 was deduced by the unsaturation degree of **1** and the chemical shifts of C-1 (δ_C 176.7) and C-18 (δ_C 91.9). The furan ring was further established to be fused to the C-1, 6 positions based on the HMBC correlations of H_2-17/C-1, C-5, C-6, and C-22. The 3-methyl-2-butenyl group was located at C-6 by the HMBC correlations of H_2-22 with C-1, C-5, C-6 and C-17 (Fig. 2). The relative configuration of **1** was elucidated by NOESY NMR analysis (Fig. 2). The NOESY interactions between H-8/H_3-15, H-12/H-7ax, and H-12/H-14ax suggested that Me-15 and H-8 were co-facial and H-12, H-7a and H-14ax were co-facial, all assigned in an ax-orientation. The above NOESY correlations confirmed the chair and envelope conformation of the six-membered and five-membered ring, respectively. The β-orientation for Me-16 was disclosed by the NOESY cross-peak of H-8/H_3-16. In addition, H-29 or H-33 due to the benzoyl group displayed NOESY correlations with H-23 and H_3-25, together with the NOESY correlation of H-23/H-14 eq, suggesting that the benzoyl group was at the lower side of C-4 spiro center and the 3-methyl-2-butenyl group was α-oriented, While the α-orientation of H-18 was confirmed by the strong NOESY correlations of H-18 with Hα-17 and Hα-22 (Fig. 2). Thus, the structure of **1** was established as illustrated in Fig. 1.

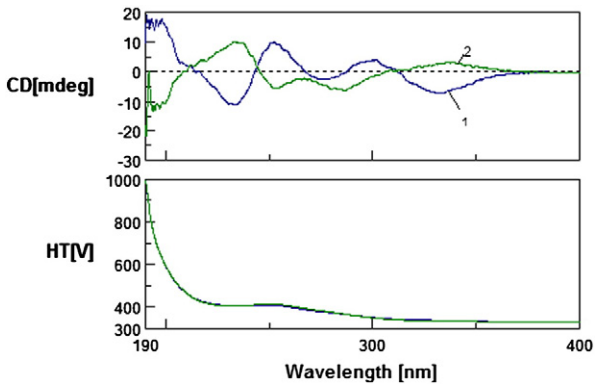

Fig. 3. The key HMBC, ^{1}H–^{1}H COSY and NOESY correlations of **2**.

Sampsonol B (**2**) had the same molecular formula as that of **1** from its HRESIMS. The ^{1}H and ^{13}C NMR data were similar to those of **1**, except for the signal of C-1 and C-5 (Table 1). Detailed analyses of the COSY and HMBC spectra indicated that **2** was a stereoisomer of **1**. Owing to the fact that the chemical shifts of the corresponding carbon signals of C-8, C-9, C-12 and C-13 in both compounds were very close (the differences were less than 0.4 ppm, Table 1), the conformations of the C_{10} unit of **2** were decided to be same as those of **1**. In addition, the NOESY correlations of the aromatic protons of H-29 or H-33 with H_3-15 and H-23 in the NOESY (Fig. 3) spectrum indicated the benzoyl group was at the upper side of the C-4 spiro center and the 3-methyl-2-butenyl group was β-oriented. The oxymethine proton of H-18 showed strong NOESY interactions with H-22 methylene protons implying its β-orientation. The different stereochemistry at the C-4 spiro center, C-6 and C-18 was further convinced by the totally opposite Cotton effects of **1** and **2**(Fig. 4). Thus, the structure of **2** was elucidated as shown in Fig. 1.

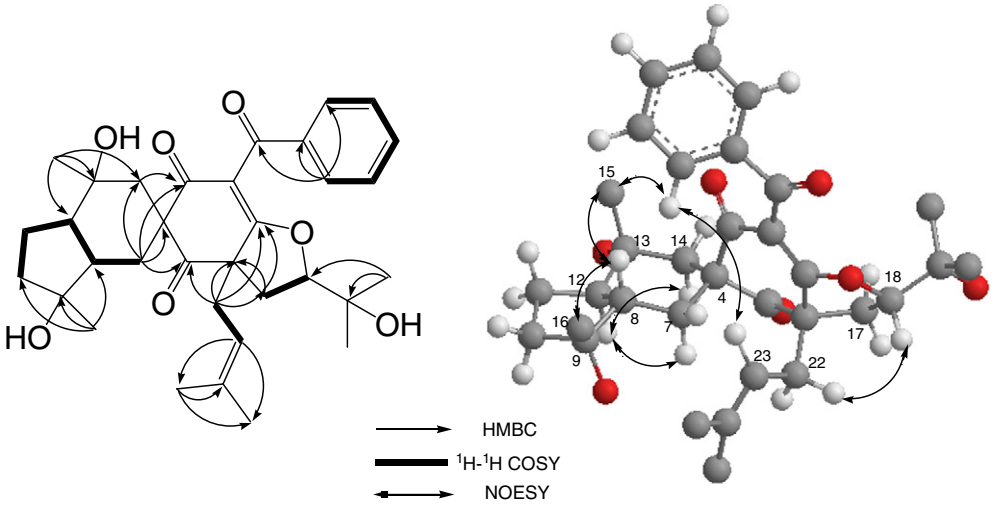

Fig. 4. CD and UV spectra of **1** and **2** (in MeOH).

Sampsonol C (**3**) was obtained as a white amorphous powder and had the molecular formula of $C_{29}H_{34}O_5$ with 13 degrees of unsaturation as determined by HRESIMS at *m/z* 485.2298 $[M+Na]^+$ (calcd for 485.2299). Analysis of the ^1H and ^{13}C NMR data of **3** (Table 2) indicated it to be a prenylated acylphloroglucinol derivative having a monoterpene moiety similar to **1** and **2**, as well as a prenyl group and a benzoyl group. The chemical shifts of C-1–C-6 were consistent with the phloroglucinol moiety of **3** being characterized by a ketone {δ_C 208.4 (C-5)}, a conjugated ketone {δ_C 197.5 (C-1)}, an enol {δ_C 177.2 (C-3) and 111.1 (C-2)}, and two sp^3 quaternary carbons {δ_C 58.9 (C-4) and 59.5 (C-6)}. A benzoyl group is connected to C-2 supported by the chemical shift of C-2 (δ_C 111.1) [17,18]. HMBC correlations for H_3-17 to C-1, C-5, C-6, and C-18 implied that C-6 is connected to C-1, C-5, the methyl group (C-17), and the prenyl group (C-18) (Fig. 5). The presence of an oxygen ether bridge between C-3 and C-13 was deduced by the unsaturation requirement of **1**, taking the chemical shifts of C-4, C-7, C-12, C-13, and C-14 into consideration, shifted by −2.2, +7.8, −3.5, +18.3, and −4.7 ppm, respectively, as compared with those of **1** (Table 3). Additionally, the HMBC correlations of H_2-7 and H_2-14 with C-3, C-4 and C-5, respectively, indicated that the C_{10} unit was located at C-4. Since the chemical shift of C-13 was significantly shifted to downfield (ca. 18 ppm) as compared with that of **1** and **2**, together with the NOESY correlations of H_3-15/H-12, the configuration of C-13 was deduced to be different from that of **1** and **2**. The chair conformation of the cyclohexane ring of the C_{10} unit and the eq-orientation of CH_3-16 were shown to be the same as those of **1** and **2** by the NOESY correlations of

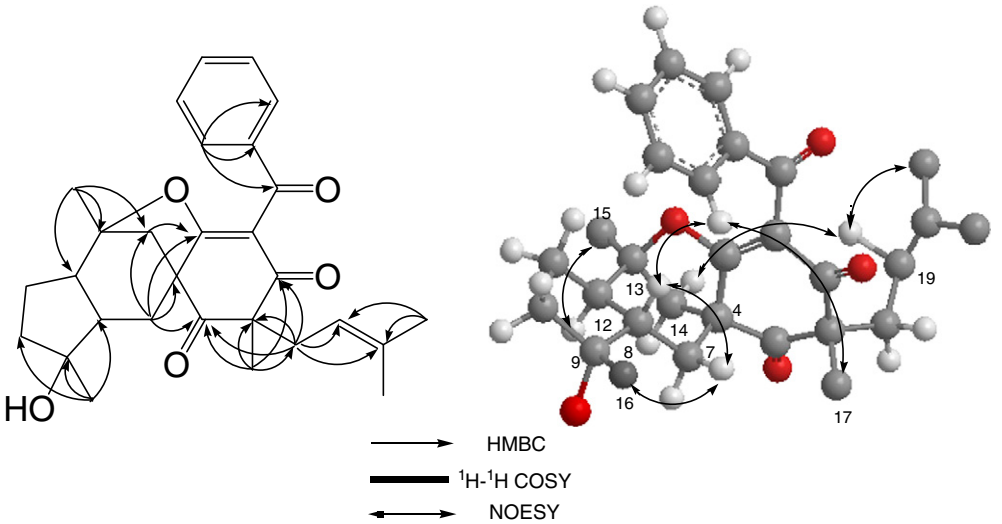

Fig. 5. The key HMBC, ^{1}H–^{1}H COSY and NOESY correlations of **3**.

Table 4
Cytotoxicity data (IC_{50}, μM) for **1** and **2** against human tumor cells[a].

Compound	Cell lines			
	MCF-7	HepG2	HT-29	A549
Sampsonol A (**1**)	16.5 ± 2.5	25.5 ± 0.7	15.9 ± 2.2	21.8 ± 1.4
Sampsonol B (**2**)	14.7 ± 0.8	17.3 ± 3.4	28.2 ± 1.3	13.5 ± 0.3
Doxorubicin	6.8 ± 0.1×10^{-2}	4.4 ± 0.2×10^{-2}	7.4 ± 0.1×10^{-2}	5.3 ± 0.4×10^{-2}

[a] Values are mean ± SD.

H_3-15/H-12, H_3-16/H-7 eq and H-8/H-7 eq (Fig. 5). Furthermore, the configurations of C-4 and C-6 in **3** were elucidated from the following NOESY correlations: H-25 or H-29/H-8 and Me-17; and H-14 eq/H-19, indicating the benzoyl group was at the upper side of the cyclohexane ring and α-orientation of the isoprenyl group (Fig. 5). Therefore, the structure of sampsonol C was elucidated as **3**.

Sampsonol D (**4**) had a molecular formula of $C_{29}H_{34}O_6$ based on its HRESIMS data (*m/z* 501.2249 $[M+Na]^+$). The ^1H and ^{13}C NMR data of **4** were similar to those of **3**, respectively, except for the presence of a 3-hydroxyl-3-methylbutenyl group in **4** instead of the 3-methyl-2-butenyl group in **3** deduced from the following ^1H and ^{13}C resonances: [δ_H 5.90 (1 H, *d*, *J* = 16.0 Hz), 5.71 (1 H, *d*, *J* = 16.0 Hz), 1.31 (3 H, *s*), 1.30 (3 H, *s*); δ_C 136.4, 131.1, 81.9, 24.6, 23.7] (Tables 2 and 3). These NMR observations suggested that the 3-methyl-2-butenyl group at C-6 in **3** was replaced by a 3-hydroxyl-3-methylbutenyl group in **4**, which was confirmed by the HMBC correlations of the proton signal at δ_H 5.90 (H-18) with the carbon signals at δ_C 195.7 (C-1), 205.8 (C-5), 60.5 (C-6) and 23.1 (C-17). The relative stereochemistry of **4** was decided to be same as **3** by the following NOE correlations: H-7 eq/H-8 and H-16; H-12/H_3-15; H-25 or H-29/H-8 and H_3-17. Thus, the structure of sampsonol D (**4**) was assigned as shown in Fig. 1.

Sampsonol E (**5**) was obtained as viscous colorless oil with the molecular formula of $C_{27}H_{38}O_5$ calculated from its HRESIMS. The ^1H and ^{13}C NMR data of **5** were quite similar to **3** except for the signals of one isobutyl group [δ_H 2.14 (1 H, *m*), 2.70 (1 H, *dd*, *J* = 15.6, 7.1 Hz), 2.52 (1 H, *dd*, *J* = 15.7, 6.8 Hz), 0.95 (3 H, *d*, *J* = 7.6 Hz), 0.91 (3 H, *d*, *J* = 7.6 Hz); δ_C 22.7, 22.8, 25.2, 52.3] in **5** instead of the mono-substituted benzene group in **3**. The chair conformation of the cyclohexane ring consisted of C-4, C-7, C-8, C-12, C-13 and C-14, as well as the ax-orientation of Me-15 and eq-orientation of Me-16 was elucidated from the following NOE correlations: H-7 eq with H_3-16 and H-8; H-12 with H-7ax and H_3-15; and H-14ax with H_3-15. Thus, **5** was elucidated as shown in Fig. 1.

Sampsonol F (**6**) was also obtained as viscous colorless oil. Its NMR data were quite resembled with those of **5** and a 2-methylpropanoyl group was deduced from the following NMR resonances: [δ_H 1.13 (3 H, *d*, *J* = 6.8 Hz), 1.07 (3 H, *d*, *J* = 6.8 Hz), 3.11 (1 H, *sept*, *J* = 6.8 Hz); δ_C 18.2, 17.7, 39.9, 203.7]. These NMR observations suggested that the 3-methylbutanoyl group at C-2 in **5** was substituted by a 2-methylpropanoyl group in **6**. The relative stereochemistry of **6** was deduced to be the same as that of **5** by NOESY experiment, and by comparing its NMR data with those of **5**. Therefore, **6** was elucidated as shown in Fig. 1.

Compounds **1**–**6** were screened for their *in vitro* cytotoxicity, antiinflammtory and antimicrobial activity. Sampsonols A and B (**1** and **2**) showed significant cytotoxicity against human breast (MCF-7), hepatoma (HepG2), colon (HT-29) and lung (A549) tumor cell lines with IC_{50} values in the range of 13–28 μM (see Table 4). And doxorubicin was used as a positive control. In a previous report, tomoeone F with the main skeleton of **1**–**6** demonstrated a significant cytotoxicity against KB cells with an IC_{50} value of 6.3 μM [17]. Combined with previous reports, we speculated that a 9, 13-dihydroxyl moiety and an eq-orientation of Me-15 might be the necessary elements for their cytotoxicity. Sampsonols C and F (**3** and **6**) showed potent inhibitory activities against LPS-induced NO production in RAW 264.7 macrophages with IC_{50} values of 27.3 and 29.3 μM, respectively. Indomethacin, a non-steroidal anti-inflammatory drug (IC_{50} = 23.6 μM) was used as a positive control. Since the cytotoxic effect was not observed in RAW 264.7 cells after sampsonols C and F treatment (data not shown), this result implied that sampsonols C and F inhibited nitrite release without causing cell death, which merits further studies regarding the precise site and the mechanism of their action. Taken together, our findings indicated that these phloroglucinol derivatives isolated from *H. sampsonii* might serve as lead compounds with promising therapeutic potential as antitumor and anti-inflammatory agents.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2012.08.022.

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