Fitoterapia 169 (2023) 105610

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Polycyclic polyprenylated acylphloroglucinols from *Hypericum sampsonii* Hance and their anti-inflammatory activity

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ARTICLE INFO

Keywords:
Hypericum sampsonii Hance
Polycyclic polyprenylated acylphloroglucinols
Anti-inflammatory activity

ABSTRACT

Phytochemical investigation of *Hypericum sampsonii* Hance resulted in the isolation of thirty-five polycyclic polyprenylated acylphloroglucinols including six new ones (**1**, **3**, **5**, and **15**–**17**). Their structures were elucidated by UV, IR, NMR, HRESIMS, and calculated ECD analysis. Some compounds were evaluated for their anti-inflammatory effects in LPS-induced RAW264.7 cells. Compounds **1** and **26** showed significant inhibitory effects on LPS-induced NO production, and markedly suppressed the protein expression of iNOS and COX-2 in LPS-activated RAW264.7 cells.

1. Introduction

Polycyclic polyprenylated acylphloroglucinols (PPAPs) are a type of natural products with fascinating structures and remarkable biological activities, including anti-inflammatory, anti-tumor, antiviral, *etc.* [1,2]. As a kind of hybrid natural products, PPAPs have been exclusively isolated from Guttiferous plants, especially from the genus *Hypericum* [1]. *Hypericum sampsonii* Hance, an herbaceous perennial plant belonging to *Hypericum* genus, has been commonly used as a folk medicine to treat enteritis, traumatic injury, and irregular menstruation, among others [3,4]. Previous phytochemical investigations on *H. sampsonii* led to the isolation of PPAPs, benzophenones, flavonoids, and so on [5,6]. Of these, PPAPs are the characteristic components possessing anti-inflammatory potential in *H. sampsonii*. Our previous studies have shown that the EtOAc extract of *H. sampsonii* possesses a preferable anti-colitis effect [7], while its detailed anti-inflammatory components remain unclear. Therefore, the potential anti-inflammatory PPAPs of this extract from *H. sampsonii* were investigated in this study.

In an effort to search for diverse and bioactive constituents from *H. sampsonii*, we conducted our investigation on the whole plant of *H. sampsonii*, which resulted in the isolation of 35 PPAPs, including six undescribed compounds (**1**, **3**, **5**, **15**–**17**). The structures of new isolates were determined by extensive analysis of UV, IR, NMR, HRESIMS, and calculated ECD analysis. Additionally, the screening for anti-inflammatory activity of isolated PPAPs was evaluated in LPS-activated RAW264.7 cells.

2. Experimental section

2.1. General experimental procedures

Optical rotations were obtained on an Insmark IP-digi 300 (InsMark, Shanghai, China). UV data was acquired using a Shimadzu UV-240 spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were carried out on a Shimadzu IR Affinity-1 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Circular dichroism (CD) spectra were obtained with a Jasco 820 spectropolarimeter (Jasco Corporation, Kyoto, Japan). NMR spectra were obtained by a Bruker Avance-400 spectrometer (Bruker Corporation, Fremont, CA, USA). HRESIMS was measured on an AB Sciex Triple-TOF 5600^{+} apparatus (Shimadzu Corporation, Tokyo, Japan). A Shimadzu LC-20AT equipped with an SPD-M20A PDA detector was used for HPLC analysis. A YMC-pack ODS-A column (250 × 10 mm, 5 μm, 12 nm, YMC Co., Ltd., Kyoto, Japan) and a H&E ODS-A column (250 × 20 mm, 5 μm, 12 nm, H&E Co., Ltd., Beijing, China) were used for semi-preparative and preparative HPLC separation. Silica gel (100–200 and 200–300 mesh, Marine Chemical Ltd., Qingdao,

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https://doi.org/10.1016/j.fitote.2023.105610
Received 27 May 2023; Received in revised form 10 July 2023; Accepted 12 July 2023
Available online 13 July 2023

Table 1
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data of compounds **1**, **3**, and **5**.

Position	1		3		5	
	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type
1	–	69.2, C	–	77.4, C	–	62.0, C
2	–	173.4, C	–	193.3, C	–	195.9, C
3	–	–	–	116.9, C	–	129.4, C
4	4.65, dd (10.5, 9.0)	93.7, CH	–	171.6, C	–	171.3, C
5	2.95, dd (15.0, 9.0), 2.80, dd (15.0, 10.5)	27.0, CH_2	–	–	–	–
6	–	118.7, C	5.03, dd (11.4, 6.3)	86.2, CH	–	87.0, C
7	–	190.8, C	2.18, dd (13.5, 5.6), 2.63, dd (11.4, 13.5)	33.7, CH_2	3.36, dt (13.0, 2.5)	71.6, CH
8	–	63.6, C	–	58.2, C	2.76, dd (13.0, 3.7), 1.79, t (13.0)	27.0, CH_2
9	2.17, m, 2.09, m	40.5, CH_2	2.19, m, 2.30 overlapped	35.9, CH_2	–	61.3, C
10	1.49, overlapped	48.9, CH	1.52, overlapped	47.7, CH	2.16, d (14.0), 2.06, dd (14.0, 7.0)	40.0, CH_2
11	–	48.7, C	–	49.5, C	1.57, m	46.3, CH
12	–	207.8, C	–	204.8, C	–	47.4, C
13	–	193.3, C	–	204.4, C	–	207.9, C
14	–	137.4, C	2.33, s	25.8, CH_3	2.54, dd (14.0, 7.9), 2.42, dd (14.0, 5.9)	31.0, CH_2
15	7.55, m	128.3, CH	3.04, dd (14.0, 7.3), 3.14, dd (14.0, 8.3)	22.3, CH_2	4.83, t (6.9)	124.9, CH
16	7.34, m	128.6, CH	5.08, t (7.3)	119.3, CH	–	133.3, C
17	7.48, m	131.5, CH	–	133.4, C	1.57, s	18.2, CH_3
18	7.34, m	128.6, CH	1.62, s	25.9, CH_3	1.64, s	26.3, CH_3
19	7.55, m	128.3, CH	1.54, s	17.9, CH_3	–	193.2, C
20	–	70.8, C	–	193.3, C	–	137.0, C
21	0.89, s	23.9, CH_3	–	132.2, C	7,67, m	129.1, CH
22	0.88, s	26.5, CH_3	7.24, m	128.1, CH	7.36, m	128.6, CH
23	2.59, dd (14.4, 7.0), 2.52, dd (14.4, 7.0)	29.8, CH_2	7.45, m	128.2, CH	7.50, m	133.5, CH
24	5.12, t (7.0)	119.2, CH	7.39, m	137.0, CH	7.36, m	128.6, CH
25	–	138.7, C	7.45, m	128.2, CH	7,67, m	129.1, CH
26	1.99, m	40.2, CH_2	7.24, m	128.1, CH	1.32, s	19.6, CH_3
27	1.71, s	16.7, CH_3	1.49, s	22.6, CH_3	0.78, s	28.1, CH_3
28	2.02, m	27.1, CH_2	1.42, s	27.1, CH_3	2.52, m, 2.42, m	30.1, CH_2
29	5.07, t (6.9)	124.4, CH	2.16, m	29.0, CH_2	5.08, t (7.2)	119.5, CH
30	–	132.9, C	4.87, t (7.1)	124.3, CH	–	135.0, C
31	1.67, s	25.8, CH_3	–	133.2, C	1.63, s	18.1, CH_3
32	1.57, s	17.8, CH_3	1.69, s	25.9, CH_3	1.69, s	26.0, CH_3
33	2.25, m, 2.01, m	29.8, CH_2	1.54, s	17.9, CH_3	0.97, s	27.1, CH_3
34	4.89, t (7.0)	124.8, CH	–	–	1.28, s	24.5, CH_3
35	–	132.9, C	–	–	–	–
36	1.55, s	18.1, CH_3	–	–	–	–
37	1.64, s	26.0, CH_3	–	–	–	–
38	1.47, s	23.7, CH_3	–	–	–	–
39	1.43, s	26.9, CH_3	–	–	–	–

China), reversed-phase C_{18} (RP-C_{18}) silica gel (12 nm, S-50 μm, YMC, Japan), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden), and MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.) were used for column chromatography (CC). All solvents were of analytical grade (Guangzhou Chemical Reagents Company, Guangzhou, China).

2.2. Plant material

The whole plant of *H. sampsonii* was gathered in Zisun Pharmaceutical Co., Ltd. (Guangzhou, China) and identified by Prof. Danyan Zhang (Guangzhou University of Chinese Medicine). A voucher specimen (GZYS-19117) was deposited at the School of Pharmaceutical Sciences at Guangzhou University of Chinese Medicine.

2.3. Extraction and isolation

The air-dried aerial parts of *H. sampsonii* (40 kg) were crushed and extracted with 95% ethanol (3 × 75 L) at room temperature three times to yield a crude extract (2.2 kg). The extract was further suspended in H_2O (20 L) and successively partitioned with petroleum ether (PE, 3 × 20 L) and EtOAc (3 × 20 L). After evaporation, the EtOAc (760 g) extract were chromatographed over silica gel eluting with PE/EtOAc (100:0 → 0:100) for 18 fractions (A-R), respectively.

Fr. F (168 g) was subjected to silica gel (200–300 mesh) column eluted with PE/CH_2Cl_2 (100:0 → 80:20) to give thirteen fractions (F1-F13). Fr. F6 (19.7 g) was separated by silica gel column eluted with PE/EtOAc (100:0 → 80:20), followed by Sephadex LH-20 eluted with CH_2Cl_2/MeOH (1:1, *v*/v) to give fifteen fractions (F6a-F6o). Fr. F6c (1.3 g) was fractionated by preparative HPLC (MeCN/H_2O, 93/7, 8 mL/min) to give compound **20** (3.8 mg). A mixture (2.1 g) obtained from subfraction F6f (5.3 g) by repeated silica gel chromatography was purified by preparative HPLC (MeCN/H_2O, 90/10, 8 mL/min) to give compound **6** (27.4 mg), **7** (27.4 mg), **25** (28.0 mg), and **27** (16.2 mg). Fr. F8 (22.8 g) was subjected to a silica gel column using a step gradient elution of PE/CH_2Cl_2 (100:0 → 70:30), and then subjected to a column of Sephadex LH-20 gel eluted with CH_2Cl_2/MeOH (1:1, v/v) to yield five fractions (F8a–F8e). Fr. F8a (3.7 g) was purified by preparative HPLC (MeCN/H_2O, 85:15, 8 mL/min) to give compound **15** (5.0 mg), **17** (10.0 mg), **18** (19.2 mg), **19** (9.6 mg), **21** (1.9 mg), **32** (4.5 mg), **34** (6.2 mg), and **35** (6.4 mg). Fr. F8b (0.4 g) was purified by preparative HPLC (MeCN/H_2O, 85:15, 8 mL/min) to give compound **3** (5.0 mg). Fr. F8c (2.3 g) was purified by preparative HPLC (MeCN/H_2O, 80:20, 8 mL/min) to give compound **8** (4.9 mg), **26** (20.3 mg), **29** (434.2 mg), and **30** (331.0 mg). Fr. F9 (16.1 g) was subjected to a silica gel column using a step gradient elution of PE/CH_2Cl_2 (100:0 → 60:40) to yield seven fractions (F9a–F9g). Fr. F9c (2.1 g) was first subjected to a column of Sephadex LH-20 gel eluted with CH_2Cl_2/MeOH (1:1, *v*/v), and then purified by preparative HPLC (MeCN/H_2O, 85:15, 8 mL/min) to furnish compound **1** (320 mg) and **2** (980 mg). Fr. F9e (4.5 g) was first subjected to a

Table 2
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data of compounds **15**–**17**.

Position	15		16		17	
	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type
1	–	77.4, C	–	81.4, C	–	82.6, C
2	–	200.6, C	–	207.3, C	–	198.8, C
3	–	79.3, C	–	75.0, C	–	78.3, C
4	–	200.9, C	2.09, d (15.1), 3.23, dd (5.1, 15.1)	38.0, CH_2	2.14, dd (14.0, 6.4), 2.42, dd (14.0, 9.6)	23.7, CH_2
5	–	69.2, C	4.01, d (5.1)	81.4, CH	3.06, m	52.2, CH
6	2.53, overlapped, 2.47, dd (2.7, 13.9)	41.0, CH_2	–	49.5, C	2.37, overlapped	56.2, CH
7	1.83, dd (2.7, 5.4)	47.7, CH	2.41, dd (6.2,14.4)	53.6, CH	3.09, m	59.1, CH
8	–	53.5, C	2.26, overlapped, 1.50, overlapped	28.8, CH_2	1.91, dd (2.4, 4.7)	44.4, CH
9	–	203.3, C	2.19, m	43.3, CH	2.36, m, 2.56, dt (13.9, 2.8)	39.3, CH_2
10	2.84, dd (6.5, 15.4), 2.74, dd (6.7, 15.4)	24.6, CH_2	2.55, m 1.83, d (13.8)	46.4, CH_2	–	68.4, C
11	6.22, t (6.5)	150.0, CH	–	68.4, C	–	201.8, C
12	–	141.2, C	–	204.9, C	–	57.9, C
13	9.29, s	194.9, CH	–	52.4, C	–	202.5, C
14	1.86, s	9.6, CH_3	–	204.4, C	–	193.3, C
15	–	192.8,C	–	192.7, C	–	135.1, C
16	–	135.5, C	–	135.1, C	7.20, m	129.1, CH
17	7.21, m	128.2, CH	7.27, m	128.0, CH	7.29, m	128.2, CH
18	7.31, m	129.1, CH	7.11, m	129.3, CH	7.42, m	132.6, CH
19	7.47, m	133.0, CH	7.41, m	132.3, CH	7.29, m	128.2, CH
20	7.31, m	129.1, CH	7.11, m	129.3, CH	7.20, m	129.1, CH
21	7.21, m	128.2, CH	7.28, m	128.0, CH	–	79.3, C
22	2.57, m	27.5, CH_2	0.89, s	19.2, CH_3	1.20, s	29.1, CH_3
23	5.20, t (7.2)	118.2, CH	1.00, s	22.8, CH_3	1.35, s	33.1, CH_3
24	–	135.6, C	2.55, m	30.2, CH_2	–	81.9, C
25	1.72, s	26.1, CH_3	4.92, t (7.0)	118.9, CH	1.29, s	32.0, CH_3
26	1.68, s	18.2, CH_3	–	135.4, C	1.22, s	26.3, CH_3
27	1.24, s	23.8, CH_3	1.65, s	26.2, CH_3	2.50, d (7.3)	27.9, CH_2
28	1.31, s	23.1, CH_3	1.66, s	18.3, CH_3	5.16, t (7.3)	118.3, CH
29	4.28, dt (8.0, 1.7)	51.2, CH	1.57, s	22.9, CH_3	–	135.5, C
30	5.03, dt (8.0, 2.9)	120.2, CH	1.46, s	26.5, CH_3	1.70, s	26.2, CH_3
31	–	134.7, C	–	–	1.67, s	18.2, CH_3
32	1.80, s	18.6, CH_3	–	–	1.50, s	23.5, CH_3
33	1.81, s	26.2, CH_3	–	–	1.54, s	23.5, CH_3

column of Sephadex LH-20 gel eluted with CH_2Cl_2/MeOH (1:1, v/v), and then purified by preparative HPLC (MeCN/H_2O, 88:12, 8 mL/min) to afford compound **13** (115.7 mg), **28** (22.6 mg), and **31** (37.2 mg).

Fr. H (152 g) was subjected to a silica gel column (PE/CH_2Cl_2, 100:0 → 1:1) to give nineteen fractions (Ha-Hs). Fr. Hc (5.4 g) was first subjected to a column of Sephadex LH-20 gel eluted with CH_2Cl_2/MeOH (1:1, v/v), and then separated by preparative HPLC (MeOH/H_2O, 93:7, 8 mL/min) to give compound **12** (24.0 mg) and **24** (103.0 mg). Fr. Hf (15.4 g) was subjected to a silica gel column (PE/EtOAc, 15:1 → 5:1) to give six fractions (Hf1-Hf6). Fr. Hf3 (1.8 g) was first subjected to a column of Sephadex LH-20 gel eluted with CH_2Cl_2/MeOH (1:1, v/v), and then purified on preparative HPLC (MeOH/H_2O, 90:10, 10 mL/min) to give compound **10** (14.8 mg), **14** (4.5 mg), and **22** (68.2 mg). Fr. Hf5 (3.9 g) was applied to a RP C_{18} silica gel column eluted with MeOH/H_2O (70:30 → 100:0, v/v) to return four fractions (Hf5a-Hf5d). Fr. Hf5a was separated by preparative HPLC (MeCN/H_2O, 83:17, 8 mL/min) to give compound **4** (610.0 mg) and **9** (22.9 mg), while Fr. Hf5b was purified by preparative HPLC (MeOH/H_2O, 93:7, 8 mL/min) to give compound **11** (15.2 mg), **23** (20.2 mg), and **33** (56.1 mg). Fr. Hf5d was separated by preparative HPLC (MeCN/H_2O, 80:20, 8 mL/min) to give compound **5** (10.3 mg) and **16** (5.0 mg).

Hypersampsonone H (**1**): yellow oil; $[\alpha]_D^{25}$ + 7.5 (*c* 1.0, CH_3OH); UV (MeOH) λ_{max} (log ε) 203 (4.57), 248 (4.23), 282 (4.11) nm; IR ν_{max} 3442, 2925, 1724, 1699, 1627, 1446, 1389, 1235 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; (+)-HRESIMS at *m/z* 609.3543 $[M + Na]^+$ (calcd for $C_{38}H_{50}O_5Na$ 609.3556).

Hypersampsonone I (**3**): yellow oil; $[\alpha]_D^{25}$ + 17.5 (*c* 1.0, CH_3OH); UV (MeOH) λ_{max} (log ε) 202 (4.37), 246 (4.08), 275 (3.91) nm; IR ν_{max} 2923, 1729, 1697, 1629, 1448, 1367, 1224, 1183, 1080 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; (+)-HRESIMS at *m/z* 503.2779 $[M + H]^+$ (calcd for $C_{32}H_{39}O_5$ 503.2797).

Hypersampsonone J (**5**): yellow oil; $[\alpha]_D^{25}$ - 96.3 (*c* 1.0, CH_3OH); UV (MeOH) λ_{max} (log ε) 202 (4.58), 249 (4.30) nm; IR ν_{max} 3464, 2925, 1728, 1681, 1652, 1598, 1448, 1327, 1087 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; (+)-HRESIMS at *m/z* 541.2912 $[M + Na]^+$ (calcd for $C_{33}H_{42}O_5Na$ 541.2930).

Hypersampsonone K (**15**): yellow oil; $[\alpha]_D^{25}$ - 7.5 (*c* 1.0, CH_3OH); UV (MeOH) λ_{max} (log ε) 202 (4.16), 235 (3.89) nm; IR ν_{max} 2926, 1700, 1447, 1377, 1249 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 2; (+)-HRESIMS at *m/z* 537.2590 $[M + Na]^+$ (calcd for $C_{33}H_{38}O_5Na$ 537.2617).

Hypersampsonone L (**16**): yellow oil; $[\alpha]_D^{25}$ + 10 (*c* 1.0, CH_3OH); UV (MeOH) λ_{max} (log ε) 203 (4.23), 246 (3.89) nm; IR ν_{max} 2926, 1733, 1695, 1447, 1374, 1222 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 2; (+)-HRESIMS at *m/z* 477.2627 $[M + Na]^+$ (calcd for $C_{30}H_{37}O_5$ 477.2641).

Hypersampsonone M (**17**): yellow oil; $[\alpha]_D^{25}$ + 10 (*c* 1.0, CH_3OH); UV (MeOH) λ_{max} (log ε) 203 (4.52), 246 (4.17) nm; IR ν_{max} 2928, 1743, 1700, 1447, 1377, 1257, 1142 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 2; (+)-HRESIMS at *m/z* 539.2765 $[M + Na]^+$ (calcd for $C_{33}H_{40}O_5Na$ 539.2774).

2.4. ECD calculations

The absolute configurations of new compounds were established by ECD calculations. The 3D structures were generated by Chem3D 18.1, while the conformational search was performed by the software Spartan's 14 under the mechanics models with Merck Molecular Force Field (MMFF). Stable conformers were subsequently submitted to ECD calculations by Time-dependent Density functional theory (TD-DFT) at the B3LYP/6–31 + g (2d, p) level. After correcting for the Boltzmann distribution of the stable conformation, the calculated CD spectrum was composed using SpecDis software with a UV shift to the ECD spectra.

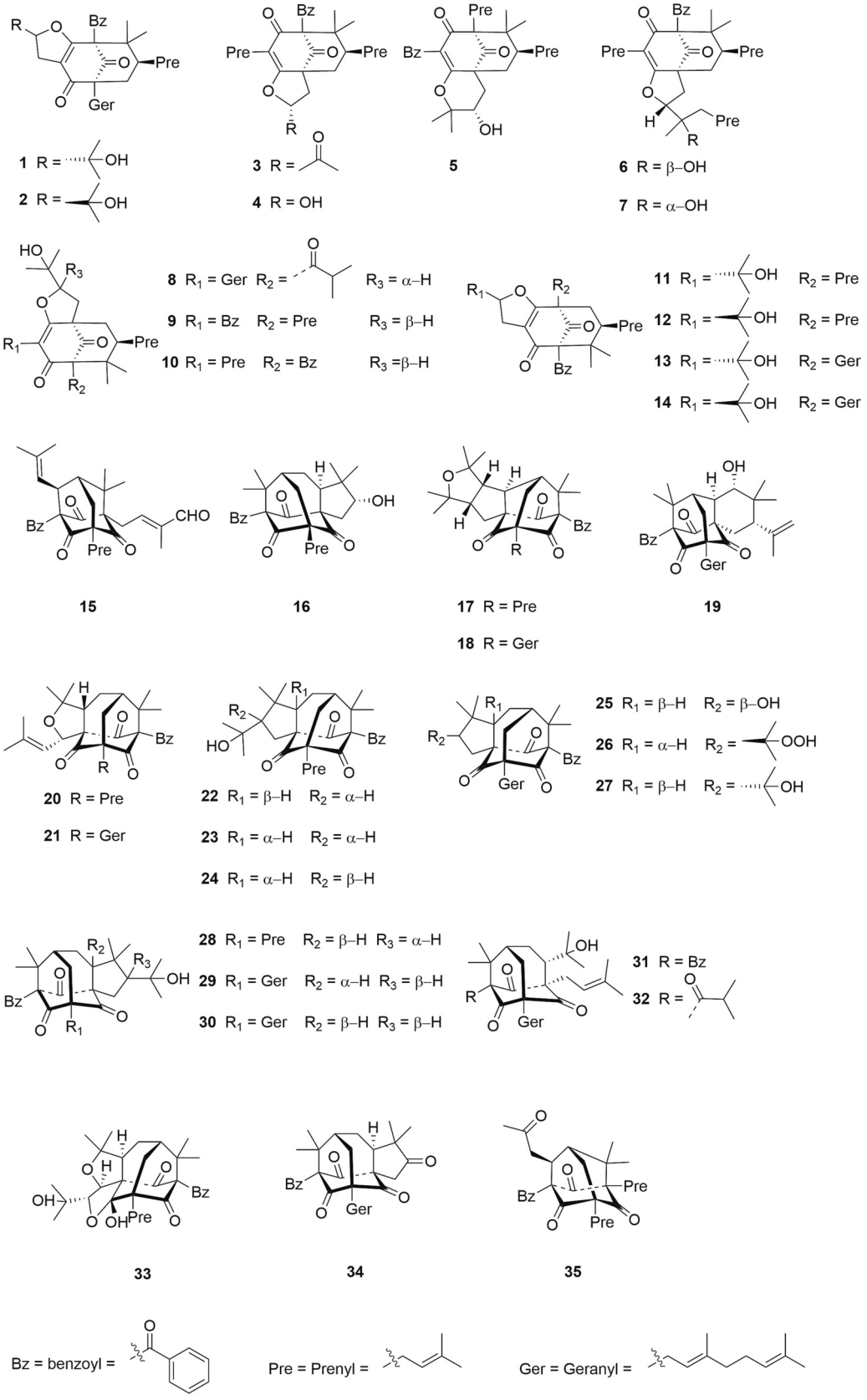

Fig. 1. Structures of isolated PPAPs (**1**–**35**).

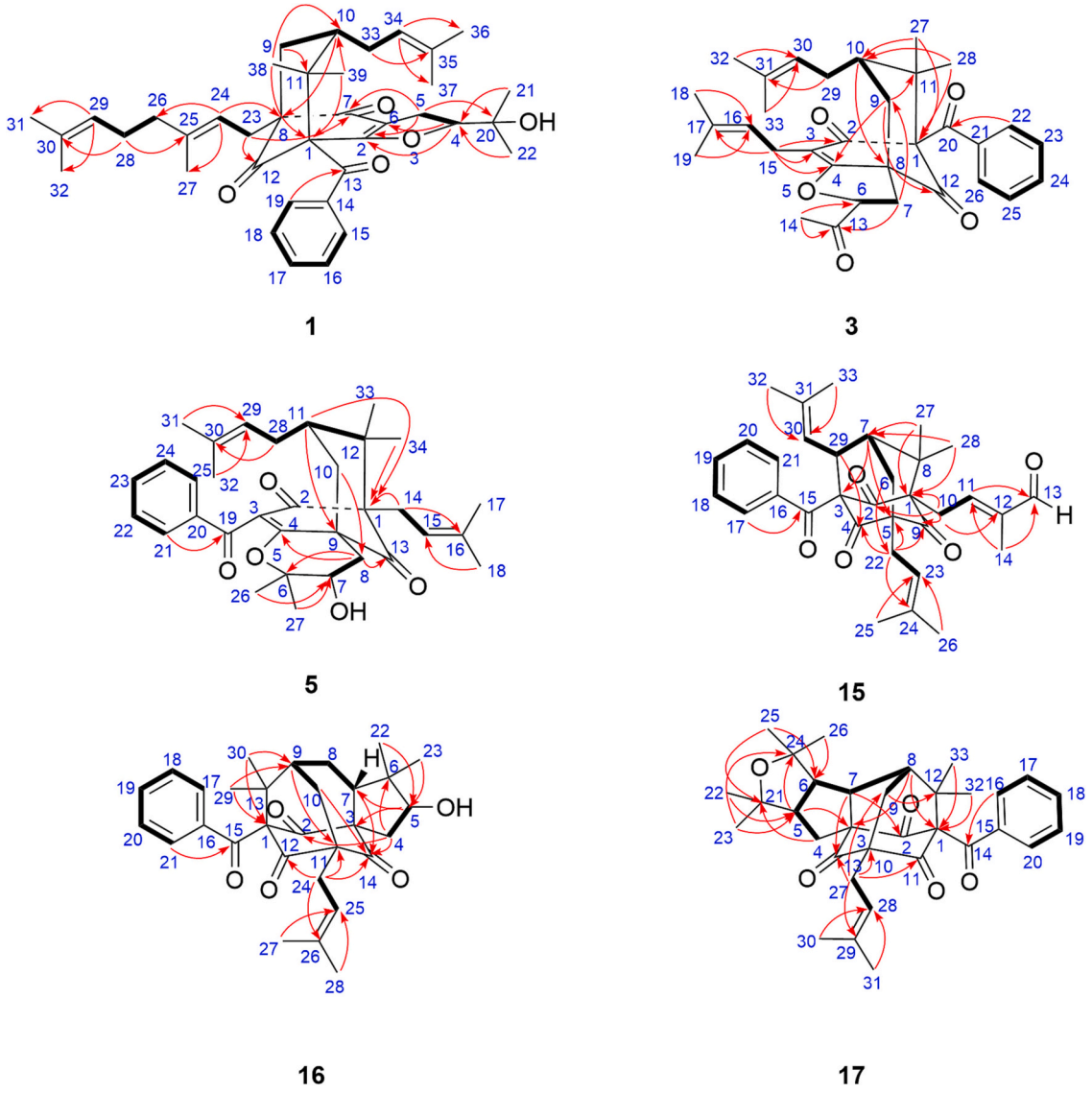

Fig. 2. Key $^{1}H—^{1}H$ COSY(—) and HMBC (→) correlations of compounds **1**, **3**, **5**, and **15**–**17**.

2.5. Cell culture and treatments

RAW264.7 cells were cultured in high-glucose DMEM supplemented with 10% FBS and 1% PSS at 37 °C with 5% CO_2. The isolated compounds were dissolved in DMSO before determination. To ensure that cell growth was not affected, the final DMSO concentration never exceeded 0.1%.

2.6. Cell viability assay

RAW264.7 cells (4×10^4/well) were seeded in 96-well plates and cultured overnight. Then, cells were pretreated with different compounds at the indicated concentrations for 24 h. After incubation, 10 μL of MTT (5 mg/mL) was added to each well and incubated for another 4 h. After discarding the supernatants, 120 μL of DMSO was added to dissolve formazan crystals, and the absorbance was determined at 490 nm.

2.7. Nitric oxide (NO) inhibitory assay

RAW264.7 cells (4×10^4/well) were seeded in 96-well plates and cultured overnight. Subsequently, cells were incubated with the tested compounds and continued to culture for 2 h, followed by the addition of LPS (1 μg/mL). After co-cultured for another 22 h, 50 μL of culture supernatants were measured by the Griess reaction method at the absorbance of 540 nm.

2.8. Western blot analysis

RAW264.7 cells (1×10^6/well) were seeded in 6-well plates and cultured overnight. Afterwards, cells were pretreated with compounds for 2 h and subsequently stimulated with LPS (1 μg/mL) to induce inflammation. After an incubation of 24 h, cells were harvested in cell lysis buffer. Protein concentrations were quantified using a Bestbio BCA Assay Kit before fractionation by SDS-PAGE on 8% precast gels and transferred to PVDF membranes (Millipore, Billerica, MA, USA). The PVDF membranes were blocked with 5% nonfat dry milk (CST, CA, USA) and incubated with primary and secondary antibodies according to the experimental protocols. The Image J software was used to analyze chemiluminescence signals.

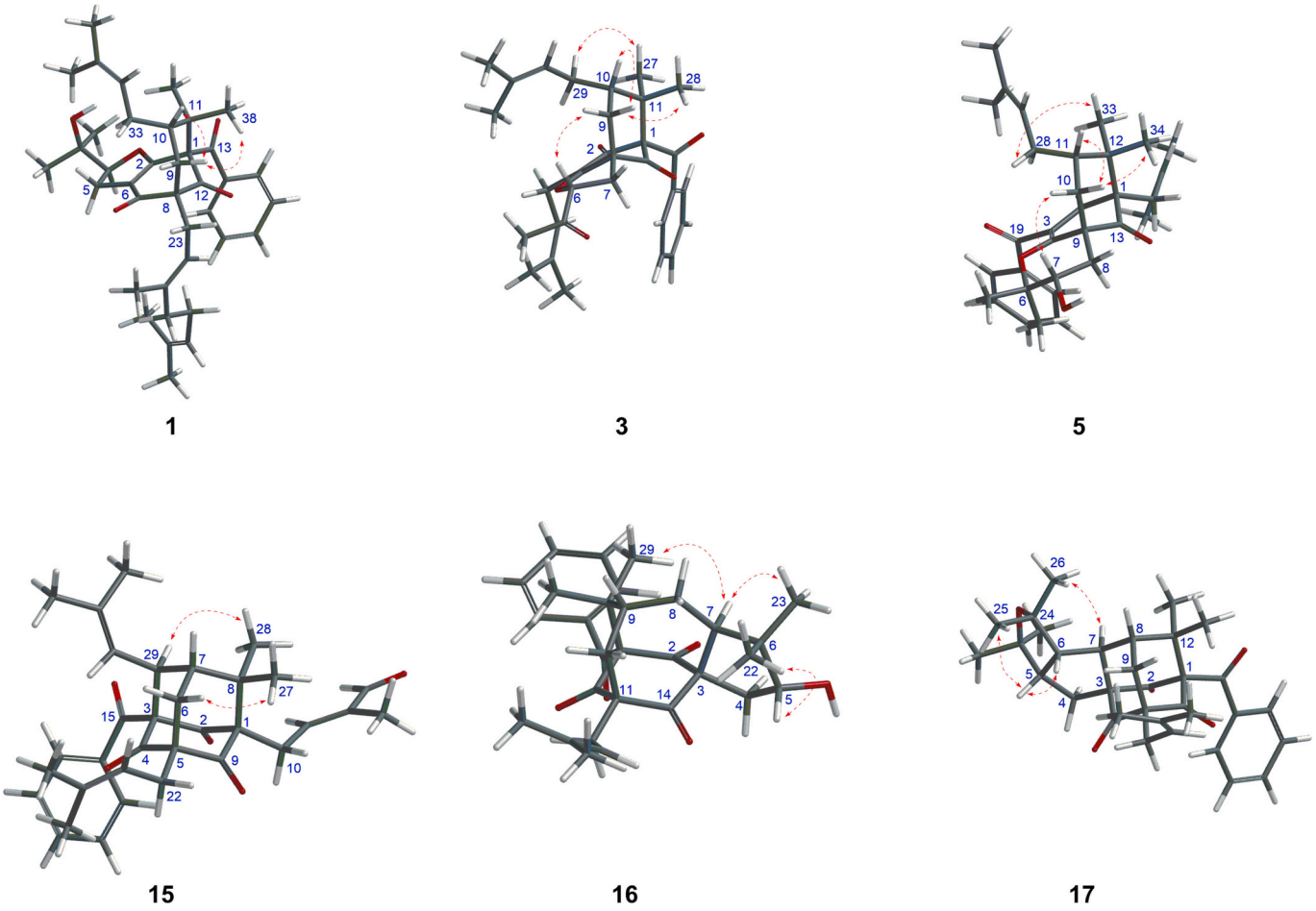

Fig. 3. Key NOESY (↔) correlations of compounds **1**, **3**, **5**, and **15**–**17**.

2.9. Statistical analysis

Statistical analysis was performed using the SPSS19.0 software (SPSS Inc., Chicago, USA). The data are expressed as mean ± standard errors of the mean (SEM). One-way analysis of variance (ANOVA) was used for multiple comparisons. Statistical significance was set at $p < 0.05$.

3. Results and discussion

Using various chromatographic methods, 35 compounds including 6 new ones were isolated from the EtOAc extract of *H. sampsonii* (Fig. 1).

Compound **1** was obtained as yellow oil with a molecular formula $C_{38}H_{50}O_5$ deduced by the high resolution electrospray ionization mass spectroscopy (HRESIMS). The result of (+)-HRESIMS at *m/z* 609.3543 $[M + Na]^+$ (calcd for $C_{38}H_{50}O_5Na$ 609.3556) indicated fourteen degrees of unsaturation. Analyses of the NMR data (Table 1) and HSQC spectrum disclosed a total of thirty-eight carbon signals, which were assigned to a benzoyl group [δ_H 7.55 (2H, m), 7.48 (1H, m), 7.34 (2H, m); δ_C 193.3, 137.4, 132.9, 128.6, 128.6, 128.3, 128.3], an isoprenyl group [δ_H 4.89 (1H, t, J = 7.0 Hz), 2.25 (1H, m), 2.01 (1H, m), 1.64 (3H, s), and 1.55 (3H, s); δ_C 25.8, 17.8, 132.9, 124.4, 27.1, 16.7, 40.2, 138.7, 119.2, and 29.8], a geranyl group [δ_H 5.12 (1H, t, J = 7.0 Hz), 5.07 (1H, t, J = 6.9 Hz), 1.71 (3H, s), 1.67 (3H, s), 1.57 (3H, s), 1.99 (2H, m), 2.02 (2H, m), 2.59 (1H, dd, J = 14.4, 7.0 Hz), and 2.52 (1H, dd, J = 14.4, 7.0 Hz); δ_C 25.8, 17.8, 132.9, 124.4, 27.1, 16.7, 40.2, 138.7, 119.2, and 29.8], two carbonyls (δ_C 207.8 and 190.8), an oxygenated quaternary carbons (δ_C 70.8), three sp^3 quaternary carbons (δ_C 69.2, 63.6, and 48.7), an oxymethine [δ_H 4.65 (1H, dd, J = 10.5, 9.0 Hz) and δ_C 93.7], two methylenes [δ_H 2.17 (1H, m), 2.09 (1H, m) and δ_C 40.5; δ_H 2.95 (1H, dd, J = 15.0, 9.0 Hz), 2.80 (1H, dd, J = 15.0, 9.0 Hz) and δ_C 27.0], a methine [δ_H 1.49 (1H, m) and δ_C 48.9], and four methyl groups [δ_H 1.47 (3H, s), 1.43 (3H, s), 0.89 (3H, s), and 0.88 (3H, s)]. The above observations indicated that **1** was a derivative of PPAPs which was similar to the structure of sampsonione M (**2**) according to their NMR data. The difference between compound **1** and **2** was the significant downfield shifted H-4 (δ_H 4.65 in **1**; δ_H 4.02 in **2**) and the obvious upfield shifted H_3–21/H_3–22 (δ_H 0.89 and 0.88 in **1**; δ_H 1.14 and 1.18 in **2**), which indicated that **1** was a C-4 epimer of **2**.

Analysis of 2D NMR spectra, including HSQC, HMBC, and COSY, revealed the planar structure of **1**. The $^1H—^1H$ COSY correlations of H-9/H-10/H-33/H-34 and HMBC correlations of H_2–23 (δ_H 2.59 and 2.52) to C-12 (δ_C 207.8), C-9 (δ_C 40.5), C-8 (δ_C 63.6) and C-7 (δ_C 190.8) revealed the isoprenyl group and geranyl group was attached to C-10 and C-8, respectively. HMBC correlations from H-4 to C-2 and C-6, from H-5 to C-2 and C-7, and from H_3–20 to C-4, C-5, C-6, and C-10 indicated that the fragment of C-5/C-4/C-20/C-21/C-22 was locked at C-6 (Fig. 2).

The relative configuration of **1** was investigated by NOESY experiment as shown in Fig. 3. The molecular model reveals the formation of the tricyclic system and establishes the relative configurations of C-1 and C-8 chiral carbons. NOESY crossing peaks from H_a-9 to H-10 and H-38 showed the chair conformation of the cyclohexanone ring and the axial orientation of the isopentenyl group on C-10. The β-orientation of H-4 was established by the furan ring fragments of **1** (C-5/C-4/C-20/C-21/C-22), which were highly similar to those of sampsonione N than

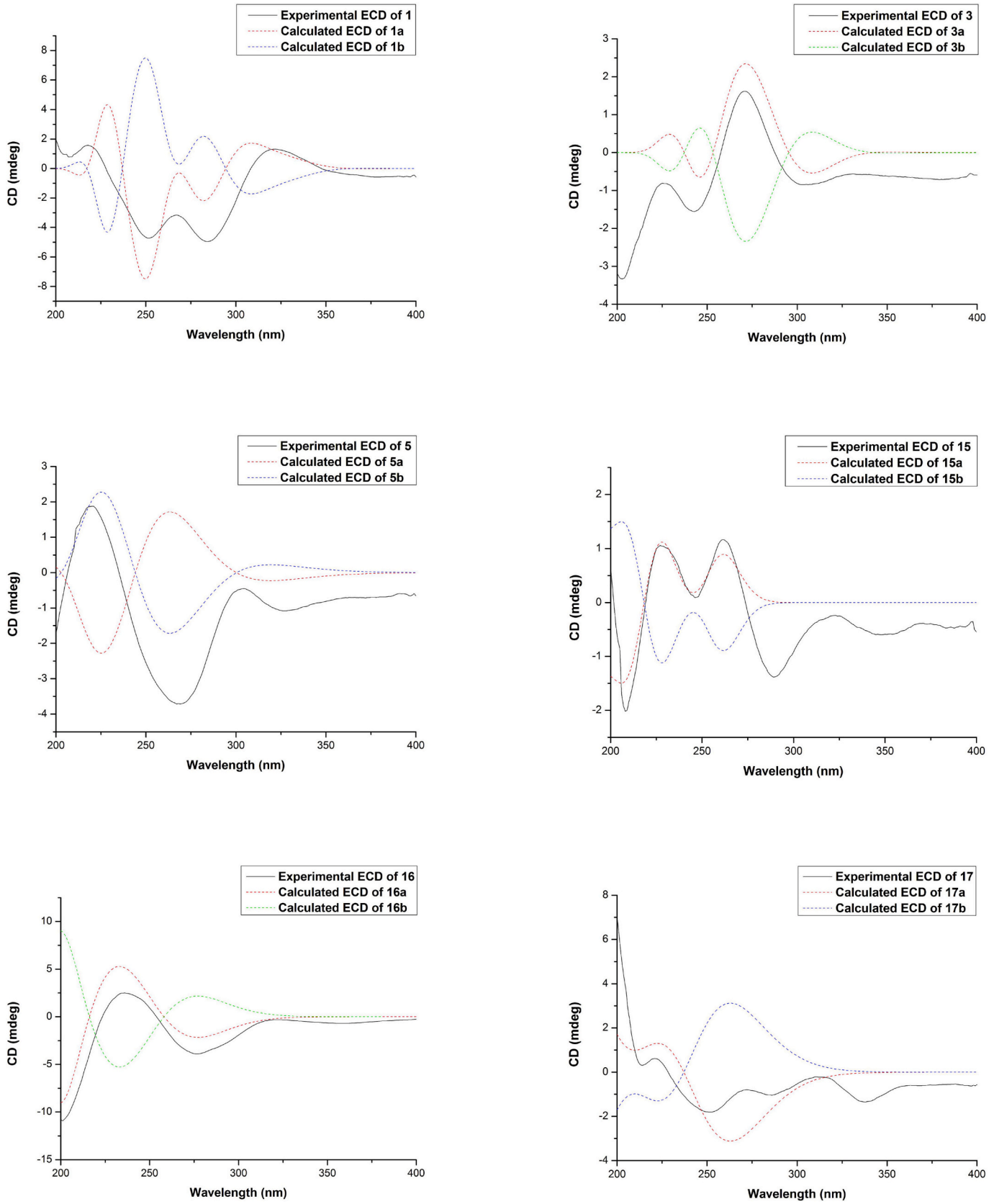

Fig. 4. Experimental and calculated ECD spectra of compounds **1**, **3**, **5**, and **15**–**17**.

Table 3
Cytotoxicity of all compounds in RAW264.7 cells at the concentration of 50 μM.

Compound (50 μM)	Cell viability (%)	Compound (50 μM)	Cell viability (%)
Control	100.00 ± 1.46	**18**	49.99 ± 0.41
1*	76.92 ± 5.46	**19**	74.12 ± 3.91
2	27.59 ± 4.04	**20**	63.37 ± 0.76
3*	51.63 ± 2.35	**21**	68.96 ± 2.98
4	42.77 ± 1.22	**22**	59.29 ± 1.75
5*	42.44 ± 1.31	**23**	49.86 ± 0.74
6	48.96 ± 2.35	**24**	49.92 ± 0.94
7	49.77 ± 1.52	**25**	49.99 ± 1.83
8	57.62 ± 1.78	**26**	60.41 ± 0.76
9	46.29 ± 1.16	**27**	66.52 ± 0.66
10	49.99 ± 4.80	**28**	45.89 ± 1.74
11	59.31 ± 2.96	**29**	49.99 ± 0.99
12	47.61 ± 1.88	**30**	49.50 ± 1.05
13	29.03 ± 0.90	**31**	56.51 ± 1.70
14	68.67 ± 2.62	**32**	74.52 ± 1.06
15*	61.86 ± 0.15	**33**	42.35 ± 0.97
16*	49.36 ± 1.81	**34**	88.67 ± 0.81
17*	72.07 ± 2.34	**35**	50.81 ± 1.96

Data representation: Mean ± SEM (n = 3); * New compound.

sampsonione M (**2**) [8]. The absolute configuration of **1** was deduced by ECD calculation. As shown in Fig. 4, the calculated ECD curves of **1a** and **1b** were 1*S*, 4*R*, 8*S*, 10*S* and 1*R*, 4*S*, 8*R*, 10*R* respectively. The experimental ECD curve was consistent with the curve of **1a**, implying the absolute configurations of 1*S*, 4*R*, 8*S*, 10*S*. Thus, this newly identified compound was named as hypersampsonone H.

Compound **3** was obtained as yellow oil and its molecular formula was established as $C_{32}H_{38}O_5$ according to (+)-HRESIMS at *m/z* 503.2779 $[M + H]^+$ (calcd for $C_{32}H_{39}O_5$ 503.2797), which possessed fourteen degrees of unsaturation. The 1D NMR data of **3** was very similar to those of hyperattenin A (**4**), except for the presence of additional carbonyl and methyl groups and the absence of hydroxyl group in **3** (Table 1). $^1H—^1H$ COSY correlations of H-30/H_2–29/H-10/H_2–9, H-16/H_2–15, and H-6/H_2–7, together with HMBC correlations from H_2–15 (δ_H 3.04 and 3.14) to C-2 (δ_C 193.3), C-3 (δ_C 116.9) and C-4 (δ_C 171.6), from H_3–14 (δ_H 2.33) to C-13 (δ_C 204.4) and C-6 (δ_C 86.2), and from H_3–28 (δ_H 1.42) to C-1 (δ_C 77.4) and C-10 (δ_C 47.7) were consistent with this structure (Fig. 2).

Molecular dynamics models confirmed the relative stereochemistry of chiral carbons (C-1 and C-8) in the tricyclic system. The relative configurations of the remaining chiral carbon C-6 and C-10 were determined by $^1H—^1H$ NOESY correlations (Fig. 3). The NOESY correlations of H_e-9 (δ_H 2.19) and H-6 (δ_H 5.03) inferred that H-6 was *β*-oriented. Based on the NOESY correlations of H_a-9 (δ_H 2.30)/H-10 (δ_H 1.52) and H_a-9/H_3–28 (δ_H 1.42), it is inferred that the isopentenyl group on the cyclohexanone ring was an axial configuration, which was further supported by comparing the 1D NMR data of garcinol and methylepigarcinol [9]. According to their 1D NMR data, the chemical shift of C-10 in **3** had an obvious downfield shift compared with methylepigarcinol (δ_C 43.9 in methylepigarcinol, δ_C 47.7 in **3**, and δ_C 48.0 in garcinol), which was more similar to garcinol. When the isoprenyl group at C-10 was equatorial, the axial methyl attached to C-11 was subjected to stronger shielding (δ_C 16.4 in methylepigarcinol; δ_C 49.5 in **3**) due to the γ-torsion interaction.

As shown in Fig. 4, the experimental ECD curve of compound **3** fitted in well with the calculated ECD spectra of **3a** (1*S*, 6*R*, 8*R*, 10*S*), suggesting that they had the same absolute configurations, which further confirmed by comparing the experimental ECD spectrum of **3** with that of hyperattenin A [10]. Thus, the structure of **3** was determined and named hypersampsonone I.

Compound **5** was purified as yellow oil, and its formula was established as $C_{33}H_{42}O_5$ according to (+)-HRESIMS at *m/z* 541.2912 $[M + Na]^+$ (calcd for $C_{33}H_{42}O_5Na$ 541.2930). The spectroscopic features revealed that **5** was similar to **3**, except for the presence of an additional methyl group (δ_H 0.78, H3–26) and the significant upfield shifted of oxymethine (δ_H 3.36, H-7) and methyl protons (δ_H 1.32, H_3–27) (Table 1). The information above was similar to that of oblongifolin S [11], indicating the presence of 2,2-dimethyl-3-hydroxypyran moiety

Table 4
Inhibitory effects of seventeen compounds on NO production in LPS-induced RAW264.7 cells at the maximum safe concentration.

Compound	Concentration (μM)	NO production (%)
Control	–	7.40 ± 0.62
LPS	–	100.00 ± 0.85##
DXMS	10	61.09 ± 0.54**
1	25	62.53 ± 0.85**
3	12.5	83.27 ± 0.81**
8	25	66.71 ± 1.81**
11	12.5	74.52 ± 0.50**
14	25	74.14 ± 1.32**
15	50	72.43 ± 1.31**
17	25	74.49 ± 2.48*
19	50	38.91 ± 1.33**
20	50	47.31 ± 1.04**
21	50	53.68 ± 0.50**
22	25	68.73 ± 2.03**
26	50	16.04 ± 1.26**
27	25	67.86 ± 4.38**
31	25	33.70 ± 1.00**
32	50	87.55 ± 6.58**
34	50	80.89 ± 2.09**
35	25	59.47 ± 0.77**

Data representation: Mean ± SEM (n = 3). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* Control; $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* LPS.

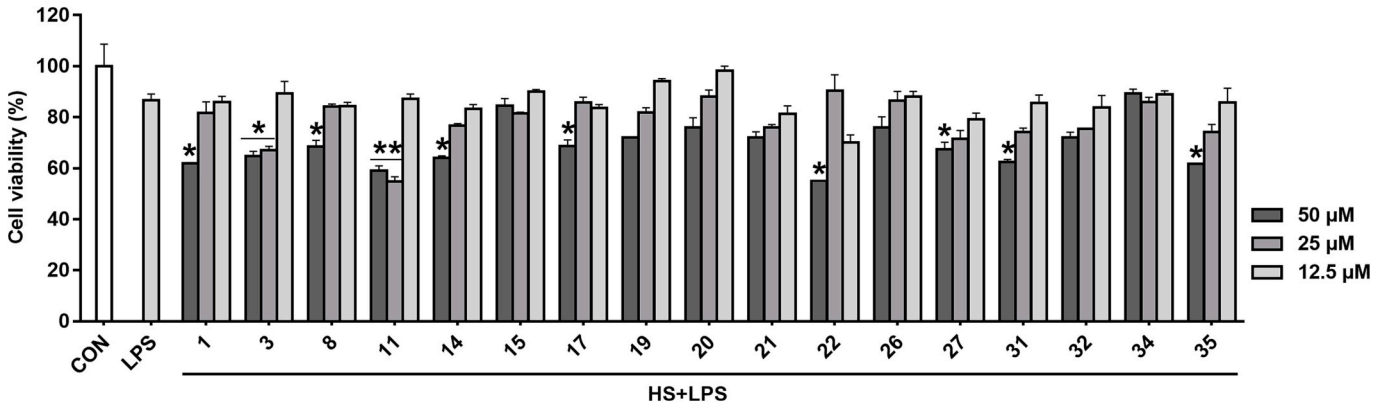

Fig. 5. Cell viability of seventeen compounds in LPS-induced RAW264.7 cells. Data representation: Mean ± SEM (*n* = 3). $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* LPS.

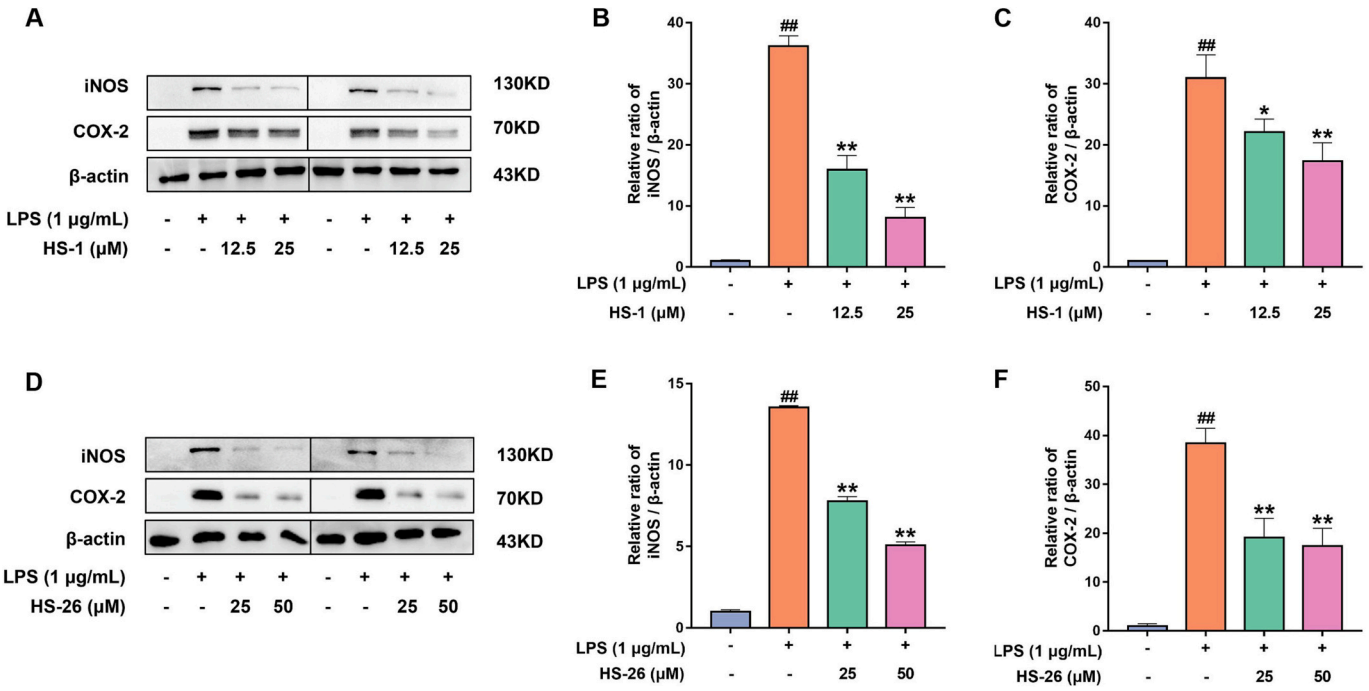

Fig. 6. Effects of compound **1** (HS-1) and **26** (HS-26) on the protein expression of iNOS and COX2 in LPS-activated RAW264.7 cells. (A-C) Effect of HS-1 on the protein expression of iNOS and COX2. (D—F) Effect of HS-26 on the protein expression of iNOS and COX2. Data representation: Mean ± SEM (n = 3). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* Control; $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* LPS.

fused with the phloroglucinol. Key $^{1}H—^{1}H$ COSY, HMBC and NOESY correlations revealed the consistent relative configuration of C-1/C-9/C-11 in **5** and **3**, while the NOESY correlations between H-7 (δ_H 3.36) and H_e-10 (δ_H 2.16) inferred the relative configuration of C-7 (Fig. 2 and Fig. 3). As seen in Fig. 4, the experimental ECD spectrum of **5** agreed well with the calculated ECD spectra of **5b**, namely 1*R*, 7*R*, 9*S*, 11*S*. Thus, the structure of **5** was determined and named hypersampsonone *J*.

Compound **15** was obtained as yellow oil with the molecular formula of $C_{33}H_{38}O_5$ established by HRESIMS (*m/z* 537.2612 $[M + Na]^+$, calcd for $C_{33}H_{38}O_5Na$ 537.2617), showing fifteen degrees of unsaturation. From the 1D NMR and HSQC spectrum, three carbonyl groups [δ_C 200.6 (C-2), 200.9 (C-4), and 203.3 (C-9)], a aldehyde group [δ_H 9.29 (H-13, 1H, s) and δ_C 194.9 (C-13)], a benzoyl group [δ_H 7.21 (H-17/21, m), δ_H 7.31 (H-18/20, m), δ_H 7.47 (H-19, m); δ_C 192.8 (C-15), 135.5 (C-16), 133.0 (C-19), 128.2 × 2 (C-17/21), and 129.1 × 2 (C-18/20)], an isoprenyl group [δ_H 2.57 (H-22, m), δ_H 5.20 (H-23, t, 7.2), δ_H 1.72 (H-25, s), δ_H 1.68 (H-26, s); δ_C 27.5 (C-22), 118.2 (C-23), 135.6 (C-24), 26.1 (C-25), and 18.2 (C-26)], five methyl groups [δ_H 1.86 (H-14, s), 1.72 (H-25, s), 1.68 (H-26, s), 1.24 (H-27, s), and 1.31 (H-28, s); δ_C 9.6 (C-14), 23.8 (C-27), 23.1 (C-28), 18.6 (C-32), and 26.2 (C-33)], two double bonds [δ_H 6.22 (H-11, t, 6.5) and 5.03 (H-30, dt, 8.0, 2.9); δ_C 150.0 (C-11), 141.2 (C-12), 120.2 (C-30), and 134.7 (C-31)], two sp^3 methylenes [δ_H 2.53 (H-6, m), 2.57 (H_a-22, m), and 5.03 (H_e-22, dd, 13.9, 2.7); δ_C 24.6 (C-6) and 27.5 (C-22)], four sp^3 quaternary carbons [δ_C 77.4 (C-1), 79.3 (C-3), 69.2 (C-5), 53.5 (C-8)], and two sp^3 methines (δ_C 47.7, 51.2) were observed (Table 2). The above data indicated that compound **15** was a type of caged PPAPs with adamantane skeleton, which possessed a benzoyl group, an isoprenyl group, and an isobutylene group.

The $^{1}H—^{1}H$ COSY correlations of H-29/H-30/H-7/H_2–6 and HMBC correlations from H_3–32/H_3–33 to C-30 confirmed that the presence of the isobutylene group was locked at C-29. The location of the prenyl group at C-5 was elucidated in the HMBC correlations from H_2–22 to C-9/C-5/C-4 and from H_2–6 to C-22. Moreover, HMBC correlations from H_3–14 to C-11/C-13 and from H_2–10 to C-9/C-1/C-2/C-12, together with $^{1}H—^{1}H$ COSY correlations of H_2–10 and H-11 established the connectivity of C-10/C-11/C-12/C-13/C-14 and this fragment was located at C-1 (Fig. 2). The NOESY correlations of H-29/H_3–28, and H-6/H_3–27 implied that H-29 was α-orientation (Fig. 3). The absolute configuration of **15** was identified by ECD analysis (Fig. 4). The ECD curve of compound **15** was in good agreement with the calculated ECD curve of **15a**, namely 1*S*, 3*S*, 5*S*, 7*R*, 29*S*. Finally, the structure of **15** was confirmed and named hypersampsonone K.

Compound **16** was obtained as yellow oil. The formula of **16** was assigned as $C_{30}H_{36}O_5$ by HRESIMS (*m/z* 477.2636 $[M + H]^+$, calcd for $C_{30}H_{37}O_5$ 477.2641), indicating thirteen degrees of unsaturation. The 1D NMR data of **16** (Table 2) were similar to those of hyperattenin H (**25**), except that the geranyl group in **25** was replaced by isoprenyl group in **16** with an obvious downfield shift of C-10 (δ_C 35.4 in **25**; δ_C 46.4 in **16**). In addition, the relative configuration of H-7 in **16** was different from **25** [12]. The NOESY correlations of H-7/H-23, H-7/H-29, and H-5/H-22 implied the *α*-orientation of H-7 and *β*-orientation of H-5 in the molecule (Fig. 3). The absolute configuration of **16** was assigned as 1*S*, 3*S*, 5*S*, 7*R*, 9*R*, 11*S* by comparing the calculated ECD curve of **16a** (Fig. 4). Finally, the structure of **16** was named hypersampsonone L.

Compound **17** was obtained as yellow oil with the molecular formula of $C_{33}H_{40}O_5$ established by HRESIMS (*m/z* 539.2760 $[M + Na]^+$, calcd for $C_{33}H_{40}O_5Na$ 539.2774), possessing fourteen degrees of unsaturation. The 1D NMR characteristics of compound **17** were quite similar to hyperisampsin C (**18**), except for the absence of geranyl group and the appearance of isoprenyl group in **17** (Table 2). The planar structure of **17** was deduced by its $^{1}H—^{1}H$ COSY and HMBC correlations (Fig. 2). The NOESY correlations of H-5/H-6, H-5/H_3–25, and H-7/H_3–26 indicated that H-7 was α-oriented, while H-5 and H-6 were *β*-oriented (Fig. 3). To determine the absolute configuration of **17**, the ECD experiment was used. The experimental ECD spectrum of **17** was in good agreement with the calculated ECD curve of **17a**, which revealed that the absolute configuration of **17** was 1*R*, 3*R*, 5*S*, 6*R*, 7*R*, 8*R*, 10*S* (Fig. 4). Finally, the structure of **17** was confirmed and named hypersampsonone M.

Apart from six new compounds, the other known compounds were isolated and identified as sampsonione M (**2**) [9], hyperattenin A (**4**)

[10], attenuatumione C (**6**) [13], sampsonione K (**7**) [9], hyperacmosin A (**8**) [14], sampsonione P (**9**) [8], sampsonione L (**10**) [9], hyperibone A (**11**) [15], sampsonione O (**12**) [8], otogirinin D (**13**) [16], hyperattenin C (**14**) [10], hyperisampsin C (**18**) [17], sampsonione I (**19**) [18], hypersampsone O (**20**) [19], hyphenrone O (**21**) [20], attenuatumione A (**22**) [13], hypersampsone Q (**23**) [19], plukenetiones B (**24**) [21], hyperattenin H (**25**) [10], hypersampsonone F (**26**) [22], sampsonione F (**27**) [23], sampsonione G (**28**) [24], sampsonione C (**29**) [24], attenuatumione D (**30**) [13], otogirinin C (**31**) [16], hyperacmosin F (**32**) [25], dioxasampsone B (**33**) [26], sampsonione E (**34**) [24], and hyperibrin E (**35**) [27] by comparing their NMR data to those reported in literatures.

In the bioactivity assay, the isolated PPAPs were tested for their inhibitory effects against NO production in LPS-induced RAW264.7 macrophages. Before investigating their anti-inflammatory activity, the cell viability was examined to investigate the safe administrated concentration of all compounds. At first, all compounds were tested at a concentration of 50 μM. It is generally accepted that the cell viability of the test substances should be no <50% of the control viability *in vitro* [28].

As shown in Table 3, the cell viability of 35 compounds at 50 μM was first tested, of which 18 compounds showed cytotoxicity (<50%) and 17 compounds showed low cytotoxicity (>50%). Further, more concentrations of 17 compounds were evaluated to find the maximum safe dosage by decreasing the concentrations from 25 μM to 12.5 μM (Fig. 5). Finally, the compounds were tested for their inhibitory effects against NO production in LPS-induced RAW264.7 cells under safe administrated concentrations, and dexamethasone (DXMS) was used as the positive control (Table 4). Noteworthily, compounds **1** and **26** displayed lower rates of NO production ($p < 0.01$).

Since compounds **1** (HS-1) and **26** (HS-26) had promising inhibitory effects on LPS-stimulated NO production, they were further evaluated against pro-inflammatory enzymes (iNOS and COX-2) using western blot analysis under safe concentrations. As depicted in Fig. 6, the expression of iNOS and COX-2 was sharply decreased by HS-1 and HS-26 in LPS-treated RAW264.7 cells. Interestingly, HS-1 showed remarkable inhibition on the protein expression of iNOS and COX-2 at 12.5 μM ($p < 0.01$).

4. Conclusion

In summary, the phytochemical investigation of the whole plant of *Hypericum sampsonii* led to thirty-five PPAPs, including six new ones. Among the evaluated isolates, the new compound **1** and known compound **26** displayed strong inhibitory effects in NO production. Moreover, compound **1** showed significant inhibition of iNOS and COX-2 at a relatively low concentration. Therefore, further studies are necessary to explore its biological activities in the future.

Credit authorship contribution statement

Yanzhen Li: Bioactivity assay, reviewing and writing of the manuscript. Mingqiang Wang: Compounds purification and draft writing. Jianhui Su: Compounds isolation and structures elucidation. Yuanyuan Wang: Participating in revision. Zhongxiang Zhao: Funding acquisition and project administration. Zhanghua Sun: Structures elucidation, project administration, and funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the Science and Technology Program of Guangdong Province of China (2022B1212010014) and the Guangdong Province's Projects of High-Level University Construction Funds (No. A1-2601-21-414-001Z06).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2023.105610.

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