

PAPER

 View Article Online
View Journal | View Issue
Cite this: *RSC Adv.*, 2018, 8, 6425Isolation, identification and bioactivities of abietane diterpenoids from *Premna szemaoensis*†De-Bing Pu,[‡] Ting Wang,[‡] Xing-Jie Zhang,^a Jun-Bo Gao,^{cd} Rui-Han Zhang,^a Xiao-Nian Li,^c Yong-Mei Wang,^{cd} Xiao-Li Li,^{*a} He-Yao Wang^{*b} and Wei-Lie Xiao^{*ac}

Investigation of the leaves and stems of *Premna szemaoensis* resulted in the isolation of twelve new abietane diterpenoids, szemaoenoids A–L (1–12), together with four known abietane diterpenoids (13–16). The structures involved two rearranged-abietane skeletons: 17(15 → 16)-abeo-abietane (7, 10–12, 14 and 15) and 17(15 → 16),18(4 → 3)-diabeo-abietane (1–6, 13 and 16). The structures of the new compounds were established mainly by analyzing NMR and HRESIMS data. The absolute configurations of 1, 3 and 10 were confirmed by single crystal X-ray diffraction analysis. In bioactivity assays, compounds 11, 12, 14 and 15 were active against two human colon cancer cell lines (HCT-116 and HT-29) with IC₅₀ values ranging from 8.8 to 34.3 μM, and compounds 10, 13 and 14 exhibited effective free radical scavenging activity with IC₅₀ values ranging from 35.6 to 41.5 μM by DPPH experiment.

Received 13th December 2017
Accepted 23rd January 2018

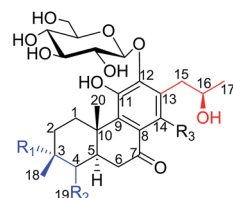
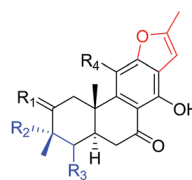
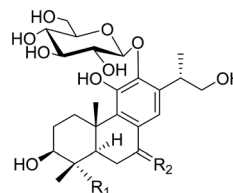
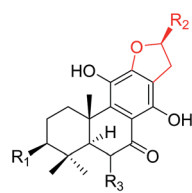
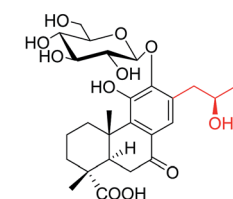
DOI: 10.1039/c7ra13309j

rsc.li/rsc-advances

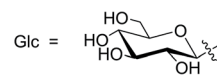
Introduction

The genus *Premna* (family: Verbenaceae) comprises approximately 200 species, which are mainly distributed in the tropical zone of Asia and Africa.¹ There are about 44 species and 5 varieties grown in the south of China, especially in Southwest China. The dried aerial parts of some *Premna* species have been used in traditional folk medicine for the treatment of pyogenic infections, trauma, fracture, dysentery, haemorrhoids, and rheumatic arthritis.² Previous phytochemical investigations of *Premna* have indicated the presence of diterpenoids,³ flavonoids,^{3–5} iridoid glycosides,^{4–7} xanthenes,⁸ phenylethanoid glycosides,⁹ triterpenoids,^{10,11} and lignins.¹² Their pharmacological effects, including neuroprotective,¹³ analgesic,^{14,15} anti-oxidative, cytotoxic,^{16,17} anti-inflammatory,¹⁸ and α-glucosidase

inhibition,¹⁹ have been reported for crude extracts and pure compounds from *Premna* plants.

1 R₁=OH R₂=CH₂ R₃=H Δ^{4,19}2 R₂=CH₂OH R₃=H Δ^{3,4}3 R₂=CH₂OH R₃=OH Δ^{3,4}16 R₂=COOH R₃=H Δ^{3,4}4 R₁=2H R₃=CH₂OH R₄=OGlc Δ^{3,4}5 R₁=O R₃=CH₂OH R₄=OGlc Δ^{3,4} Δ^{5,6}6 R₁=2H R₂=OH R₃=CH₂ R₄=OGlc Δ^{4,19}13 R₁=O R₃=CH₃ R₄=OH Δ^{3,4} Δ^{5,6}8 R₁=CH₃ R₂=O9 R₁=CH₂OH R₂=2H10 R₁=OH R₂=CH₃ R₃=H11 R₁=OH R₂=CH₃ R₃=H Δ^{15,16}12 R₁=H R₂=CH₂OH R₃=H Δ^{15,16}14 R₁=H R₂=CH₂OH R₃=H15 R₁=H R₂=CH₃ R₃=OCH₃ Δ^{5,6}

7



Glc =

^aKey Laboratory of Medicinal Chemistry for Natural Resource of Ministry of Education, School of Chemical Science and Technology, State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan University, Kunming 650091, People's Republic of China. E-mail: lixiaoli@ynu.edu.cn; xiaowei@ynu.edu.cn; Fax: +86 871-665033214; +86 871-67357014; Tel: +86 871-665033214; +86 871-67357014

^bState Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China. E-mail: hywang@sim.ac.cn; Fax: +86 021-50807088; Tel: +86 021-50805785

^cState Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China

^dUniversity of Chinese Academy of Sciences, Beijing 100049, People's Republic of China
† Electronic supplementary information (ESI) available: Detailed ¹D and ²D NMR, HRESIMS, IR, UV and X-ray crystallographic data. CCDC 1554050–1554052. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7ra13309j

‡ D.-B. Pu and T. Wang contributed equally to this work.

Premna szemaoensis Pei, locally called “simao dofu chai”, is mainly distributed in the south of Yunnan province of China.²⁰





Table 1 ^1H NMR data of compounds 1–12 measured at 600 MHz (δ in ppm, J in Hz)

Position	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^a	7 ^a	8 ^a	9 ^a	10 ^b	11 ^b	12 ^b
1 α	1.77 (overlap)	1.51 (td, 6.5)	1.53 (td, 12.6, 6.0)	1.68 (td, 12.6, 6.1)	2.54 (d, 16.9)	1.90 (td, 13.7, 4.1)	1.18 (overlap)	1.43 (overlap)	1.75 (m)	1.47 (br t, 14.2)	1.55 (br t, 14.0)	1.43 (overlap)
1 β	3.16 (br d, 13.1)	3.52 (overlap)	3.52 (overlap)	3.56 (overlap)	4.29 (d, 16.9)	3.22 (overlap)	3.38 (d, 12.9)	3.45 (overlap)	1.87 (overlap)	3.46 (br d, 13.7)	3.55 (br d, 13.6)	2.57 (br d, 17.0)
2 α	1.74 (overlap)	2.10 (dd, 6.1)	2.09 (dd, 18.5, 6.0)	2.08 (dd, 18.2, 5.5)		1.74 (overlap)	1.46 (overlap)	1.75 (overlap)	1.55 (qd, 12.4, 5.1)	1.74 (overlap)	1.73 (m)	1.55 (m)
2 β	1.74 (overlap)	2.27 (m)	2.28 (m)	2.32 (m)		1.74 (overlap)	2.12 (m)	1.81 (dd, 26.0, 12.8)	1.90 (overlap)	1.74 (overlap)	1.79 (overlap)	1.79 (overlap)
3 α							1.00 (td, 13.1, 3.4)	3.28 (overlap)	3.37	3.27	3.30 (dd, 11.5, 5.3)	1.33 (td, 13.5, 3.6)
3 β							2.25 (br d, 13.1)		(overlap)	(overlap)		1.50 (overlap)
5	3.32 (overlap)	2.94 (brd, 15.4)	2.90 (overlap)	2.88 (br d, 14.5)		3.28 (overlap)	1.81 (br d, 14.2)	1.73 (overlap)	1.29 (overlap)	1.71 (overlap)	1.80 (dd, 14.5, 1.7)	1.84 (dd, 14.7, 2.1)
6 α	2.37 (dd, 16.0, 2.4)	2.99 (dd, 2.9)	2.69 (br t, 12.0)	3.04 (dd, 17.3, 3.2)	6.80 (s)	2.41 (dd, 16.6, 2.4)	2.89 (br d, 16.9)	2.66 (br t, 15.5)	1.23 (overlap)	2.49 (d, 17.0)	2.57 (br d, 17.0)	2.57 (d, 17.0)
6 β	2.69 (overlap)	2.57 (t, 15.9)	3.01 (dd, 17.0, 2.7)	2.70 (dd, 17.3, 15.2)		2.85 (t, 15.7)	3.27 (overlap)	2.58 (d, 16.7)	3.40 (overlap)	2.70 (overlap)	2.78 (overlap)	2.76 (t, 15.9)
7 α									2.71 (overlap)			
7 β									2.76 (overlap)			
14	7.46 (s)	7.46 (s)					7.39 (s)	7.44 (s)		2.73	6.55 (s)	6.77 (s)
15 α	2.71 (overlap)	2.70 (dd, 6.6)	2.87 (overlap)	6.55 (s)	6.62 (d, 0.9)	6.55 (s)	2.66 (dd, 13.3, 6.9)	3.77 (m)	3.68 (overlap)	(overlap)		
15 β	3.20 (dd, 13.4, 6.6)	3.18 (dd, 6.6)	3.17 (dd, 13.0, 6.8)				3.20 (m)			3.26 (overlap)		
16 α	4.10 (ddd, 6.2, 6.4, 6.6)	4.11 (ddd, 6.6, 6.7)	4.15 (dd, 12.8, 6.4)				4.11 (ddd, 6.6, 6.4, 6.2)	3.50 (overlap)	3.42 (overlap)	5.09 (m)		
16 β								3.61 (dd, 10.5, 6.3)	3.58 (dd, 10.5, 6.3)			
17	1.12 (d, 6.2)	1.12 (d, 6.2)	1.12 (d, 6.3)	2.46 (s)	2.49 (s)	2.46 (s)	1.11 (d, 6.2)	1.15 (d, 6.9)	1.11 (d, 6.9)	1.43 (d, 6.2)	2.40 (s)	4.64 (d, 5.9)
18 α	1.41 (s)	1.26 (s)	1.24 (s)	1.68 (s)	2.06 (s)	1.40 (s)		1.03 (s)	3.45 (overlap)	1.05 (s)	1.07 (s)	0.99 (s)
18 β									4.18 (d, 11.2)			
19 α	4.74 (s)	4.07 (d, 11.7)	4.07 (d, 11.9)	4.09 (d, 12.0)	4.61 (overlap)	4.76 (s)	1.18 (s)	0.93 (s)	1.26 (s)	0.92 (s)	0.94 (s)	1.01 (s)
19 β	5.20 (s)	4.27 (d, 11.7)	4.27 (d, 11.9)	4.26 (d, 12.0)	4.71 (overlap)	5.20 (s)						
20	1.20 (s)	1.78 (s)	1.77 (s)	1.36 (s)	1.73 (s)	1.32 (s)	1.43 (s)	1.40 (s)	1.28 (s)	1.39 (s)	1.44 (s)	1.47 (s)
1'	4.57 (d, 7.9)	4.57 (d, 7.9)	4.65 (d, 8.0)	5.41 (d, 7.2)	5.90 (d, 7.3)	5.59 (d, 7.4)	4.55 (d, 7.9)	4.46 (d, 7.9)	4.37 (d, 7.9)			
2'	3.51 (t, 8.5)	3.51 (t, 8.5)	3.52 (m)	3.49 (overlap)	3.53 (overlap)	3.47 (overlap)	3.50 (t, 8.4)	3.50 (overlap)	3.45 (overlap)			
3'		3.29 (overlap)		3.20 (m)		3.22 (m)						



Table 1 (Contd.)

Position	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^a	7 ^a	8 ^a	9 ^a	10 ^b	11 ^b	12 ^b
4'	3.29 (overlap) 3.44 (overlap)	3.45 (overlap) 3.42(overlap)	3.27 (overlap) 3.44 (t, 9.1) 3.42 (t, 9.1)	3.33 (overlap) 3.49 (overlap)	3.34 (overlap) 3.34 (overlap) 3.53 (overlap)	3.29 (overlap) 3.47 (overlap)	3.29 (overlap) 3.44 (overlap) 3.43 (overlap)	3.28 (overlap) 3.27 (overlap) 3.41 (m)	3.25 (overlap) 3.27 (overlap) 3.39 (overlap)			
6'α												
6'β	3.75 (dd, 12.0, 4.8) 3.84 (dd, 12.0, 1.8)	3.75 (dd, 12.0, 4.7) 3.85 (dd, 12.0, 1.9)	3.75 (dd, 12.0, 4.7) 3.84 (dd, 12.0, 1.6)	3.57 (overlap) 3.73 (dd, 11.8, 2.0)	3.70 (br, 11.9) 3.54 (overlap)	3.54 (dd, 12.0, 5.7) 3.70 (dd, 12.0, 2.0)	3.75 (dd, 10.3, 4.7) 3.83 (br d, 10.3)	3.66 (br d, 11.7) 3.89 (br d, 11.7)	3.65 (overlap) 3.89 (d, 12.0)	3.60 (br s) 7.27 (s) 13.39 (s)	3.63 (s) 8.16 (s) 13.8 (s)	8.28 (s) 13.8 (s) 4.50 (s)
OH-3												
OH-11												
OH-14												
OH-17												

^a In CD₃OD solution. ^b In acetone-d₆ solution.

This plant has drawn the attention of local farmers and has been cultivated to be an important commercial crop due to its various applications. Its fresh leaves can be rubbed and squeezed in water to yield a mucilaginous juice, which was used to prepare a food named “green tofu” by local people through addition of materials containing Ca²⁺. In addition, the local villagers also used the leaves of this plant to cure injuries and fracture.²⁰ However, the phytochemical investigation of this species was extremely rare, except a small number of flavonoids. In this investigation, we firstly afforded 12 new abietane diterpenoids (**1**–**12**) and four known abietane diterpenoids (**13**–**16**) from the aerial parts of *P. szemaoensis*. Most of the diterpenoids were with rearranged-abietane skeleton: 17(15 → 16)-*abeo*-abietane framework or 17(15 → 16),18(4 → 3)-*diabeo*-abietane framework, which were mainly isolated from the plants of genus *Clerodendron* and reported bioactivities including cytotoxic, angiotensin converting enzyme (ACE) inhibitory, antiviral actives.^{21–24} Herein, we describe the isolation and structural elucidation of these diterpenoids and the biological activities of selected compounds.

Results and discussion

The aerial parts of *P. szemaoensis* were extracted three times with 70% acetone aqueous. After recycling acetone, the rest of portion was partitioned by liquid–liquid extraction between *n*-butanol and H₂O. The *n*-butanol-soluble portion was repeatedly subjected to silica gel, Sephadex LH-20, and RP-C18 gel column chromatography (CC) and semi-preparative HPLC to afford 16 abietane diterpenoids, including 12 new compounds. The structures and stereochemistry of these isolates were elucidated mainly using spectroscopic analysis, X-ray diffraction analysis, and compared to data in the literature. Ultimately, the new compounds were named as szemaoenoids A–L (**1**–**12**), and known compounds were identified as teuvinenone F (**13**),²⁵ (16*R*)-12,16-epoxy-11,14,17-trihydroxy-17(15 → 16)-*abeo*-8,11,13-abieta-triene-7-one (villosin B) (**14**),^{26,27} 12,16-epoxy-11,14,17-trihydroxy-6-methoxy-17(15 → 16)-*abeo*-5,8,11,13-abietatetraene-7-one (**15**),²⁸ 11,16-dihydroxy-12-*O*-β-D-glucopyranosyl-17(15 → 16),18(4 → 3)-*diabeo*-4-carboxy-3,8,11,13-abietatetraene-7-one (**16**),²¹ respectively.

Compound **1** was isolated as an optically active, white monoclinic crystals (MeOH); [α]_D²⁵ +36.3 (*c* 0.10, MeOH). Its HRESIMS data showed a sodium adduct ion [M + Na]⁺ at *m/z* 531.2205 (calcd. for C₂₆H₃₆NaO₁₀, 531.2206), which together with ¹³C NMR (Table 2) and DEPT data were consistent with a molecular formula of C₂₆H₃₆O₁₀, representing nine indices of hydrogen deficiency. The ¹H NMR spectrum of **1** (Table 1) showed one doublet and two singlets for methyl groups at δ_{H} 1.12 (d, *J* = 6.2), 1.41 (s) and 1.20 (s), respectively; three olefinic proton signals at δ_{H} 7.46 (s), 5.20 (s), and 4.74 (s), and a double peak at δ_{H} 4.57 (d, *J* = 7.9). The ¹³C NMR and DEPT spectra of **1** (Table 2) showed signals of 20 carbons of an aglycon, attributable to a ketone group at δ_{C} 200.7, three methyls (two tertiary), five methylenes (one olefinic), three methines (one olefinic, one oxygenated), and eight quaternary carbons (six olefinic, one oxygenated), along with signals for a hexose unit. These data



Table 2 ^{13}C NMR data of compounds 1–12 measured at 150 MHz (δ in ppm)

Position	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^a	7 ^a	8 ^a	9 ^a	10 ^b	11 ^b	12 ^b
1	31.9, CH ₂	32.6, CH ₂	32.7, CH ₂	33.7, CH ₂	47.9, CH ₂	33.4, CH ₂	37.4, CH ₂	35.8, CH ₂	29.2, CH ₂	35.6, CH ₂	36.1, CH ₂	37.6, CH ₂
2	37.8, CH ₂	31.1, CH ₂	31.2, CH ₂	31.2, CH ₂	201.1, C	38.1, CH ₂	21.2, CH ₂	28.4, CH ₂	20.5, CH ₂	28.7, CH ₂	18.8, CH ₂	19.7, CH ₂
3	71.5, C	133.8, C	134.0, C	133.9, C	138.4, C	71.3, C	39.9, CH ₂	78.7, CH	80.9, CH	77.5, CH	77.5, CH	41.8, CH ₂
4	153.7, C	129.6, C	129.3, C	129.4, C	149.6, C	153.6, C	45.7, C	40.2, C	44.3, C	39.8, C	39.9, C	34.0, C
5	44.0, CH	44.5, CH	44.0, CH	43.9, CH	161.9, C	43.7, CH	53.6, CH	51.4, CH	55.0, CH	50.4, CH	50.4, CH	50.9, CH
6	38.6, CH ₂	38.0, CH ₂	38.3, CH ₂	38.5, CH ₂	124.9, CH	38.7, CH ₂	39.2, CH ₂	36.2, CH ₂	34.4, CH ₂	35.6, CH ₂	36.0, CH ₂	36.2, CH ₂
7	200.7, C	201.1, C	207.1, C	207.5, C	191.8, C	207.0, C	203.2, C	201.3, C	35.6, CH ₂	205.2, C	207.0, C	207.0, C
8	130.0, C	130.2, C	114.2, C	112.1, C	110.1, C	111.9, C	130.1, C	130.2, C	135.6, C	110.7, C	111.2, C	111.5, C
9	139.5, C	139.7, C	137.3, C	139.9, C	133.7, C	139.1, C	141.1, C	140.3, C	134.1, C	140.6, C	135.1, C	135.6, C
10	42.0, C	39.1, C	39.2, C	39.9, C	44.2, C	42.7, C	42.3, C	41.4, C	40.1, C	41.2, C	41.2, C	41.6, C
11	149.9, C	149.7, C	141.7, C	133.7, C	133.1, C	134.0, C	150.4, C	149.3, C	149.1, C	132.7, C	133.5, C	133.4, C
12	150.5, C	150.5, C	153.3, C	153.4, C	151.3, C	153.0, C	149.7, C	150.0, C	143.2, C	156.3, C	148.3, C	152.1, C
13	132.8, C	132.7, C	121.0, C	119.8, C	120.6, C	119.9, C	132.2, C	138.2, C	136.6, C	111.7, C	117.9, C	117.3, C
14	121.7, CH	122.1, CH	157.4, C	156.8, C	154.1, C	156.7, C	121.3, CH	117.2, CH	118.2, CH	155.9, C	153.6, C	154.1, C
15	40.9, CH ₂	40.9, CH ₂	33.6, CH ₂	101.5, CH	101.2, CH	101.4, CH	41.0, CH ₂	35.1, CH	34.9, CH	34.5, CH ₂	101.6, CH	102.5, CH
16	68.3, CH	68.3, CH	68.3, CH	156.5, C	157.4, C	155.7, C	68.3, CH	68.8, CH ₂	69.1, CH ₂	83.3, CH	155.9, C	158.8, C
17	22.8, CH ₃	22.9, CH ₃	22.9, CH ₃	13.7, CH ₃	13.8, CH ₃	13.7, CH ₃	22.8, CH ₃	18.2, CH ₃	18.3, CH ₃	22.0, CH ₃	13.7, CH ₃	57.4, CH ₂
18	27.8, CH ₃	19.0, CH ₃	18.9, CH ₃	18.9, CH ₃	11.4, CH ₃	27.8, CH ₃	185.1, C	28.5, CH ₃	65.3, CH ₂	28.7, CH ₃	28.4, CH ₃	21.9, CH ₂
19	108.2, CH ₂	59.2, CH ₂	59.2, CH ₂	59.4, CH ₂	59.7, CH ₂	108.4, CH ₂	30.1, CH ₃	16.0, CH ₃	23.6, CH ₃	16.7, CH ₃	16.0, CH ₃	33.4, CH ₂
20	14.2, CH ₃	15.6, CH ₃	15.4, CH ₃	18.3, CH ₃	25.9, CH ₃	15.8, CH ₃	15.6, CH ₃	17.4, CH ₃	20.4, CH ₃	17.9, CH ₃	18.4, CH ₃	18.4, CH ₂
1'	107.6, CH	107.6, CH	107.1, CH	101.5, CH	101.9, CH	102.4, CH	107.6, CH	107.4, CH	107.8, CH			
2'	75.4, CH	75.4, CH	75.4, CH	75.9, CH	75.7, CH	75.9, CH	75.4, CH	75.5, CH	75.6, CH			
3'	78.6, CH	78.6, CH	78.7, CH	78.3, CH	78.6, CH	78.4, CH	78.6, CH	79.1, CH	79.0, CH			
4'	70.8, CH	70.8, CH	70.7, CH	71.8, CH	71.4, CH	71.7, CH	70.8, CH	71.4, CH	71.5, CH			
5'	77.9, CH	77.9, CH	78.0, CH	78.1, CH	78.4, CH	78.2, CH	77.9, CH	77.9, CH	77.9, CH			
6'	62.1, CH ₂	62.1, CH ₂	62.1, CH ₂	62.6, CH ₂	62.3, CH ₂	62.6, CH ₂	62.1, CH ₂	63.0, CH ₂	63.0, CH ₂			

^a In CD₃OD solution. ^b In acetone-*d*₆ solution.

suggested that **1** is a diterpene glycoside and in accordance with the characteristics of a 17(15 → 16),18(4 → 3)-*diabeo*-8,11,13-abietatriene.

The proton and protonated carbon NMR signals of **1** were assigned unambiguously by the HSQC experiment. Partial structures and the whole connection were deduced from correlations observed in the ¹H-¹H COSY and HMBC spectra (Fig. 1). The HMBC correlations of CH₃-18 (δ_H 1.41) to C-2 (δ_C 37.8), C-3 (δ_C 71.5, an oxygenated quaternary carbon), and C-4 (δ_C 153.7, an olefinic quaternary carbon) and of H₂-19 (δ_H 4.74, 5.20) to C-3/C-4/C-5 (δ_C 44.0) suggested the presence of a 18(4 → 3)-*abeo*-abietane structural unit, established the location of an OH group at C-3 and an exocyclic double bond at C-4 and C-19. The ketone group was placed at C-7 from correlations of H-5 (δ_H 3.32), H₂-6 (δ_H 2.37, 2.69), H-14 (δ_H 7.46, one aromatic proton) to a ketone-group carbon (δ_C 200.7). ¹H-¹H COSY correlations from H₂-15 (δ_H 2.71, 3.20) through H-16 (δ_H 4.10, an oxygenated proton) to CH₃-17 (δ_H 1.12), in combination with HMBC correlations from H₂-15 to C-12 (δ_C 150.5) and C-14 (δ_C 121.7), from CH₃-17 to C-16 (δ_C 68.3) and C-15 (δ_C 40.9), were suggestive of 17(15 → 16)-*abeo*-abietane moiety in this structure, and an hydroxyl group at C-16. The location of the sugar moieties was determined by the HMBC correlation of the anomeric proton H-1' (δ_H 4.57) to C-12. In addition, ¹H-¹H COSY correlations: H₂-1/H₂-2, H-5/H₂-6, and HMBC correlations of CH₃-20 to C-1, C-9 and C-10, of H-5 to C-4 and C-10 were also key interactions to support this gross structure.

The β-D-configurational glucose unit was confirmed through coupling constant of H-1' (d, *J* = 7.9), acid hydrolysis and comparison with reference standard. In the case of abietane diterpenoid derivatives, relative configuration of OH at C-3 could be assigned for the α- and β-epimers, by NOE effect between H-3/H-5 or H-3/CH₃-20 respectively. However, the interaction of CH₃-18/H-5 or CH₃-18/CH₃-20 was not observed in **1**. Its ROESY spectrum provided interactions of CH₃-18/H-2 and CH₃-20/H-2 (Fig. 1), but α- and β-H connected to C-2 displayed an overlapped signal at 1.74 in the ¹H NMR, which was not enough evidence to assign the C-3 configuration. Fortunately, appropriate crystals have been obtained, and the absolute configuration was assigned as 3*R*, 5*R*, 10*S*, 16*R* on the basis of the Flack parameter [0.17(14)] and Hooft parameter [0.10(6)] for 1225 Bijvoet pairs obtained by low-temperature [100(2) K] Cu Kα radiation X-ray crystallography (Fig. 2).^{29,30} Therefore, the

structure of **1** was elucidated as (3*R*,16*R*)-12-*O*-β-D-glucopyranosyl-3,11,16-trihydroxy-17(15 → 16),18(4 → 3)-*diabeo*-4(19),8(9),11(12),13(14)-abietatetraene-7-one, named *sze-maoenoid A*.

Compound **2** was isolated as a white amorphous powder with [α]_D²⁵ -8.8 (c 0.14, MeOH). The HRESIMS gave a quasi-molecular ion at *m/z* 531.2203 [M + Na]⁺ (calcd. for C₂₆H₃₆NaO₁₀, 531.2206). Thus, in conjunction with ¹³C NMR and DEPT data, the molecular formula was established as C₂₆H₃₆O₁₀, representing nine indices of hydrogen deficiency. The ¹H and ¹³C NMR data of **2** (Tables 1 and 2) were similar to those of compound **1**, with the differences being the presence of an oxygenated methylene [δ_H 4.07 (d, *J* = 11.7), 4.27 (d, *J* = 11.7), δ_C 59.2] and two olefinic quaternary carbons (δ_C 133.8, δ_C 129.6) in **2** vs. an oxygenated quaternary carbon and an exocyclic double bond group in **1**. Through HMBC experiment, observed correlations from H₂-19 (δ_H 4.07, 4.27) to C-3/C-4/C-5, from CH₃-18 (δ_H 1.26) to C-2/C-3/C-4 indicated that an OH group at C-19 and a double bond at C-3 and C-4. There was another hydroxyl group at C-16 (assigned the configuration as *R*) *via* comparing chemical shift and coupling constant of **2** with **1** [δ_H-16 4.11 (ddd, *J* = 6.2, 6.6, 6.7), δ_C-16 68.3 for **2**; δ_H-16 4.10 (ddd, *J* = 6.2, 6.4, 6.6), δ_C-16 68.3 for **1**], and the connecting correlations of ¹H-¹H COSY and HMBC data.

So far, the vast majority of natural abietane-type diterpenes from plants share the same carbon skeleton, with a *trans*-fused system of two six-membered rings A and B, a β-oriented methyl at C-10 and an α-oriented proton at C-5.³¹ From biogenetic considerations, **2** was inferred as possessing an identical absolute configuration to **1**. Thus, the structure of **2** was established as (16*R*)-12-*O*-β-D-glucopyranosyl-11,16,19-trihydroxy-17(15 → 16),18(4 → 3)-*diabeo*-3(4),8(9),11(12),13(14)-abietatetraene-7-one, named *sze-maoenoid B*.

Compound **3** was obtained as white monoclinic crystals (MeOH). Its molecular formula assigned was determined to be C₂₆H₃₆O₁₁ based on the negative HRESIMS (*m/z* 523.2177 [M - H]⁻). The NMR data for this compound were highly close to those of **2** (Tables 1 and 2), except for presence of one olefinic quaternary carbon (δ_C 157.4) in **3**, correspondingly absence of an olefinic methine (δ_H 7.46, δ_C 122.1) vs. **2**. In the HMBC spectrum, the correlations of H₂-15 (δ_H 2.87, 3.17) to C-12 (δ_C 153.3), C-13 (δ_C 121.0), C-14 (δ_C 157.4) indicated that an OH group was at C-14, which made the chemical shifts of aromatic

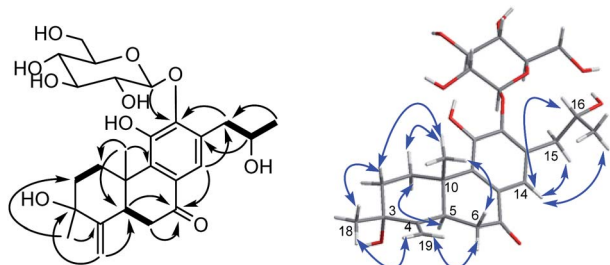


Fig. 1 ¹H-¹H COSY (bold), selected HMBC (arrow), and key ROESY (double arrow) correlations of **1**.

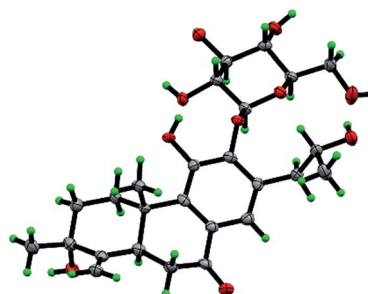


Fig. 2 ORTEP plot for the molecular structure of **1** drawn with 30% probability displacement ellipsoids.



fields and ketone group (δ_{C} 207.1) obviously change in ^{13}C NMR spectrum vs. **2**. Ultimately, the absolute configuration was confirmed by single crystal X-ray diffraction analysis, which assigned C-16 as *R* configuration (Fig. 3). Therefore, the structure of **3** was determined as (16*R*)-12-*O*- β -D-glucopyranosyl-11,14,16,19-tetrahydroxy-17(15 \rightarrow 16),18(4 \rightarrow 3)-*diabeo*-3(4),8(9),11(12),13(14)-abietatetraene-7-one, named szemaoenoid C.

Compound **4**, a yellowish amorphous powder, had a molecular formula of $\text{C}_{26}\text{H}_{32}\text{O}_{10}$ according to HRESIMS (m/z 527.1888 $[\text{M} + \text{Na}]^+$). The ^1H NMR and ^{13}C NMR data were closely related to those of **3** (Tables 1 and 2). The differences were emergence of an olefinic double bond: one methine at C-15 (δ_{H} 6.55, s; δ_{C} 101.5), one quaternary carbon at C-16 (δ_{C} 156.5). The HMBC correlations from CH_3 -17 (δ_{H} 2.46, s) to C-15/C-16 and from H-15 to C-12 (δ_{C} 153.4)/C-13 (δ_{C} 119.8)/C-14 (δ_{C} 156.8) powerfully confirmed the locations of the double bond between C-15 and C-16, and an OH group at C-16. In consideration of its molecular formula as $\text{C}_{26}\text{H}_{32}\text{O}_{10}$, and chemical stability, this structure must be absence of one H_2O , and linked from C-12 to C-16 through a vinyl ether bond, likewise the known compound **13** with a furan ring. The sugar moieties were determined to be located at C-11, by the HMBC correlation of the anomeric proton H-1' (δ_{H} 5.41, d, 7.2) to C-11 (δ_{C} 133.7). Thus, compound **4** was established as 11-*O*- β -D-glucopyranosyl-12,16-epoxy-14,19-dihydroxy-17(15 \rightarrow 16),18(4 \rightarrow 3)-*diabeo*-3(4),8(9),11(12),13(14),15(16)-abietapentaene-7-one, named szemaoenoid D.

Compound **5** was obtained as a brown amorphous powder. Its molecular formula was determined to be $\text{C}_{26}\text{H}_{28}\text{O}_{11}$ by HRESIMS data, indicating 12 degrees of unsaturation (two more than that of compound **4**). The ^1H and ^{13}C NMR resonances of **5** closely resembled those of **4** (Tables 1 and 2), except for appearance of a ketone group at C-2 and a double bond at C-5/C-6. The HMBC correlations from H_2 -1 (δ_{H} 2.54, d, J = 16.9 and δ_{H} 4.29, d, J = 16.9) and CH_3 -18 (δ_{H} 2.06, s) to C-2 (δ_{C} 201.1) and from H-6 (δ_{H} 6.80, s) to C-4 (δ_{C} 149.6)/C-8 (δ_{C} 110.1)/C-10 (δ_{C} 44.2) demonstrated above inference. Therefore, the structure of **5** was elucidated as 11-*O*- β -D-glucopyranosyl-12,16-epoxy-14,19-dihydroxy-17(15 \rightarrow 16),18(4 \rightarrow 3)-*diabeo*-3(4),5(6),8(9),11(12),13(14),15(16)-abietahexaene-2,7-dione, named szemaoenoid E.

Compound **6** was isolated as a yellowish amorphous powder. The HRESIMS gave its molecular formula as $\text{C}_{26}\text{H}_{32}\text{O}_{10}$ (being identical with **4**). The NMR data of **6** (Tables 1 and 2) were

similar to those of **4**, except for an oxygenated quaternary carbon (δ_{C} 71.3) and an exocyclic double bond [δ_{C} 153.6, δ_{C} 108.4; δ_{H} 4.76, 5.20] in **6** (in accord with compound **1**) vs. an oxygenated methylene and two olefinic quaternary carbons in **4**. In the HMBC spectrum, the cross-connection signals of CH_3 -18 (δ_{H} 1.40) to C-2 (δ_{C} 38.1), C-3 (δ_{C} 71.3) and C-4 (δ_{C} 153.6), and of H_2 -19 [δ_{H} 4.76 (s), 5.20 (s)] to C-3, C-4 and C-5 (δ_{C} 43.7), supported the presence of an OH group at C-3 and a double bond at C-4/C-19. In consideration of identical NMR data and biogenesis, absolute configuration of C-3 in **6** was identical with **1** (assigned as *R* configuration). Consequently, compound **6** was established as (3*R*)-11-*O*- β -D-glucopyranosyl-12,16-epoxy-3,14-dihydroxy-17(15 \rightarrow 16),18(4 \rightarrow 3)-*diabeo*-4(19),8(9),11(12),13(14),15(16)-abietapentaene-7-one, named szemaoenoid F.

Compound **7**, a white amorphous powder, had a molecular formula of $\text{C}_{26}\text{H}_{36}\text{O}_{11}$ on the basis of the HRESIMS. From the NMR data, it was similar to those of **2** (Tables 1 and 2), except for absence of two olefinic quaternary carbons (δ_{C} 133.8, 129.6) and an oxygenated methylene (δ_{C} 59.2) vs. **2**, correspondingly presence of an obvious carboxyl (δ_{C} 185.1), a quaternary carbon (δ_{C} 45.7) and a methylene (δ_{C} 39.9) in **7**. The HMBC correlations of CH_3 -19 (δ_{H} 1.18, s) to C-3 (δ_{C} 39.9)/C-4 (δ_{C} 45.7)/C-5 (δ_{C} 53.6) and the carboxyl C-18 (δ_{C} 185.1), of H-5 (δ_{H} 1.81, br d, J = 14.2) to C-4/C-18/ CH_3 -19 (δ_{C} 30.1), and the ^1H - ^1H COSY correlations of H_2 -1/ H_2 -2/ H_2 -3, all indicated that CH_3 -19 and the carboxyl C-18 were both linked to C-4. CH_3 -19 was assigned as β -orientation from the NOE effect of CH_3 -19/ CH_3 -20. In view of identical NMR data and biogenesis, absolute configuration of C-16 in **7** was also identical with **1** (assigned as *R* configuration). Therefore, the structure of **7** was determined as (16*R*)-12-*O*- β -D-glucopyranosyl-11,16-dihydroxy-17(15 \rightarrow 16)-*abeo*-8(9),11(12),13(14)-abietatriene-7-one-18-acid, named szemaoenoid G.

Compound **8**, a white amorphous powder, exhibited a molecular formula of $\text{C}_{26}\text{H}_{38}\text{O}_{10}$ according to HRESIMS. The ^1H and ^{13}C NMR spectra of **8** (Tables 1 and 2) were comparable with those of a known compound 12-*O*-D-glucopyranosyl-3,11,16-trihydroxy-8,11,13-abietatriene.²¹ The evident difference was appearance of carbonyl group (δ_{C} 201.3) in **8**, but the known compound was absent of this group. The HMBC correlations of H-5 (δ_{H} 1.73)/ H_2 -6 (δ_{H} 2.58, 2.66)/H-14 (δ_{H} 7.44) with C-7 (δ_{C} 201.3), of CH_3 -18 (δ_{H} 1.03)/ CH_3 -19 (δ_{H} 0.93) with C-3 (δ_{C} 78.7), and ^1H - ^1H COSY connections of H_2 -1/ H_2 -2/H-3, suggested the carbonyl group at C-7 and an OH group at C-3, respectively. ^1H - ^1H COSY correlations from CH_3 -17 (δ_{H} 1.15, d, J = 6.3) through H-15 (δ_{H} 3.77, m) to CH_2 -16 (δ_{H} 3.50, 3.61, an oxygenated methylene), and HMBC correlations from CH_3 -17 to C-13 (δ_{C} 138.2) and C-15 (δ_{C} 35.1), from H-15 to C-16 (δ_{C} 68.8), C-17 (δ_{C} 18.2), C-12 and C-14, were suggestive of an 1-hydroxy-isopropyl moiety linked to C-13 in this structure. Configurations of C-15 was established as *S*, by comparing the chemical shifts at C-13, C-15, C-16 with that of two known compounds (Table 3): (15*S*)-12-*O*-D-glucopyranosyl-3,11,16-trihydroxy-8,11,13-abietatriene²¹ and (15*R*)-cyrtophyllone B,³² whose structures were undoubtedly established by Mosher method and X-ray crystallography respectively. The β orientation of OH-3 was confirmed by the NOE effect of H-3/H-5. Therefore, this

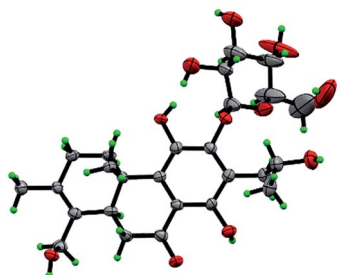


Fig. 3 ORTEP plot for the molecular structure of **3** drawn with 30% probability displacement ellipsoids.



Table 3 Comparison of partial NMR data of **8** and **9** with known compounds^a

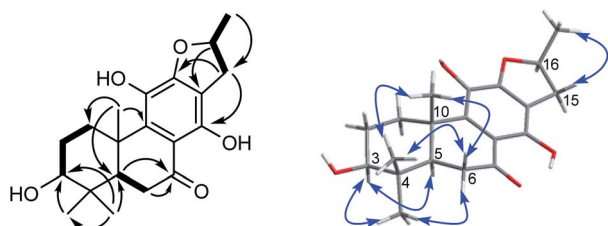
δ_{H}					δ_{C}				
Position	a	b	c	d	Position	a	b	c	d
H-15	3.69	3.15	3.77	3.68	C-13	135.7	130	138.2	136.6
H-16a	3.42	3.78	3.50	3.42	C-15	35.1	39.1	35.1	34.9
H-16b	3.58	3.94	3.61	3.58					

^a The **a** was (15*S*)-12-*O*- β -D-glucopyranosyl-3,11,16-trihydroxy-8,11,13-abietatriene;²¹ **b** was (15*R*)-cyrtophyllone B;³² **c** was szemaoenoid H (**8**); **d** was szemaoenoid I (**9**).

structure was established as (15*S*)-12-*O*- β -D-glucopyranosyl-3 β ,11,16-trihydroxy-8(9),11(12),13(14)-abietatriene-7-one, named szemaoenoid H.

The HREIMS and NMR data of compound **9** were consistent with the molecular formula of C₂₆H₄₀O₁₀. Its ¹H and ¹³C NMR spectra (Tables 1 and 2) were almost identical with those of the known compound: 12-*O*-D-glucopyranosyl-3,11,16-trihydroxy-8,11,13-abietatriene.²¹ In fact, the only difference was an oxygenated methylene (δ_{C} 65.3) in **9** instead of a methyl group (δ_{C} 17.0) in the known structure. Moreover, the oxygenated methylene signals at δ_{H} 3.45 and 4.18 showed the HMBC correlations with the signals at C-3 (δ_{C} 80.9), C-4 (δ_{C} 44.3) and C-5 (δ_{C} 55.0), and the NOE effect with H-5/H-3, which evidently inferred that the OH was located at C-18, in combination with NOE effect of CH₃-19/CH₃-20. The β orientation of OH-3 and *S* configuration of C-15 were confirmed like that of compound **8**. Consequently, compound **9** was assigned as (15*S*)-12-*O*- β -D-glucopyranosyl-3 β ,11,16,18-tetrahydroxy-8(9),11(12),13(14)-abietatriene, named szemaoenoid I.

Compound **10**, yellowish monoclinic crystals (MeOH), its chemical formula as C₂₀H₂₆O₅ was determined by HRESIMS, indicating 8 degrees of unsaturation in the structure. From the ¹H and ¹³C NMR spectrum (Tables 1 and 2), its data was extremely similar to known compound **14**, with a 17(15 \rightarrow 16)-*abeo*-abietane framework. Detailed HMBC and ¹H-¹H COSY NMR spectroscopic analyses (Fig. 4) suggested the appearance of an OH at C-3 [δ_{H} 3.27; δ_{C} 77.5] and the absence of a hydroxy group at C-17 in **10** by comparing with **14**. A correlation observed in the ROESY spectrum (Fig. 4) between H-3 and H-5 (δ_{H} 1.71) indicated the β -orientation of OH-3. The absolute configuration of this compound was established as 3*S*, 5*R*, 10*S*, 16*S* by single crystal X-ray diffraction analysis (Fig. 5).

**Fig. 4** ¹H-¹H COSY (bold), selected HMBC (arrow), and key ROESY (double arrow) correlations of **10**.

Accordingly, the structure of **10** was assigned as (3*S*,16*S*)-12,16-epoxy-3,11,14-trihydroxy-17(15 \rightarrow 16)-*abeo*-8(9),11(12),13(14)-abietatriene-7-one, named szemaoenoid J.

Compound **11** and **12** were assigned to an identical molecular formula of C₂₀H₂₄O₅ by HRESIMS data, with one more degree of unsaturation than **10**. As seen from ¹H NMR and ¹³C NMR spectrum (Tables 1 and 2), the structures of **11** and **12** were highly analogous to compound **10**, except for the appearance of a double bond between C-15 and C-16 in **11** and **12**, besides the presence of oxygenated methylene at C-17 [δ_{H} 4.64, d, *J* = 5.9; δ_{C} 57.4] and the absence of OH-3 in **12**. The positions of all functional groups in their structures were assigned by correlations of HMBC and ¹H-¹H COSY spectrum. The β -orientation of OH-3 in **11** was confirmed by ROESY correlation of H-3/H-5 and comparing with NMR data with those of **10**. Therefore, their structures were established as 12,16-epoxy-3 β ,11,14-trihydroxy-17(15 \rightarrow 16)-*abeo*-8(9),11(12),13(14),15(16)-abietatetraene-7-one (**11**), named szemaoenoid K; and 12,16-epoxy-11,14,17-trihydroxy-17(15 \rightarrow 16)-*abeo*-8(9),11(12),13(14),15(16)-abietatetraene-7-one (**12**), named szemaoenoid L.

In our cognition, many diterpenoids had been reported from plants of *Premna* genus,^{33–36} but this was also the first to report diterpenoids isolated from the *Premna* plants distributed in China, and the described rearranged-abietane skeletons were firstly isolated from *Premna* genus. Abietane diterpenoids represented a large group of secondary metabolites that have shown interesting biological activities.³¹ But the rearranged abietane diterpenoids with 17(15 \rightarrow 16)-*abeo*-abietane or 17(15 \rightarrow 16),18(4 \rightarrow 3)-*diabeo*-abietane were not common in nature. Structurally, the rearranged diterpenoids contain abundant hydroxyl groups and aromatic carbons, as well as *trans*-fused rings A and B according to biosynthetic pathway. However, the stereocenter of C-16 replaced with OH had never been established previously. Within this work, we firstly employed X-ray crystallography to assign absolute configuration of C-16 for a small series of 16-hydroxy-17(15 \rightarrow 16)-*abeo*-abietane diterpenoids, which might assist future unambiguous identification of structurally related compounds.

All the above compounds, except **3**, **4** and **10**, were evaluated for cytotoxicity *in vitro* against two human colon carcinoma cell lines (HCT-116 and HT-29). As a result (Table 4), compounds **11–15** showed antiproliferative activity against HCT-116 cell line, and compounds **11**, **12** and **15** also exhibited potent cytotoxicity on HT-29 colon carcinoma cell line. Impressively, the

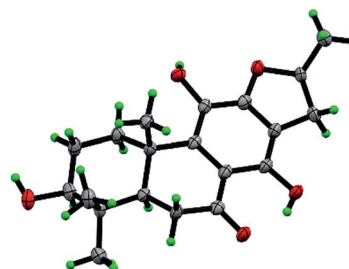
**Fig. 5** ORTEP plot for the molecular structure of **10** drawn with 30% probability displacement ellipsoids.

Table 4 IC₅₀ values (μM ± SD) obtained for the compounds against HCT-116 and HT-29 cell lines^a

Compound	IC ₅₀ (mean ± SD, μM)	
	HCT-116	HT-29
11	30.5 ± 4.7	21.3 ± 2.9
12	24.1 ± 4.5	34.6 ± 3.4
13	34.3 ± 2.8	NA
14	17.7 ± 4.6	8.7 ± 1.4
15	20.1 ± 3.0	14.2 ± 2.6
Sorafenib	8.5 ± 1.1	8.4 ± 1.3

^a NA means that compounds exhibited indistinctive activity against tumor cells, and IC₅₀ values were not evaluated.

antiproliferation activity of compound **14** was comparable (IC₅₀ 8.7 ± 1.4 μM) with positive control (sorafenib, IC₅₀ 8.4 ± 1.3 μM) (Table 4). Almost all the diterpene aglycones showed effective cytotoxicity, but none of the diterpene glucosides exhibited remarkable activity. The reason might be that the glucosides with strong chemical polarity failed to penetrate the liposoluble cell membrane.

As most of natural products possessing phenolic hydroxy exhibited antioxidant activity,^{37–39} some selective compounds of the diterpenosides were executed free radical scavenging activity assay in the DPPH experiment (Table 5). Among the tested compounds, diterpene aglycones **13**, **14** showed strong free radical scavenging activity with IC₅₀ values of 41.5 ± 17.0 and 39.9 ± 12.9 μM respectively, and compound **10** was especially the strongest activity with IC₅₀ 35.6 ± 9.8 μM (more potent than the positive control trolox and vitamin C). Compound **12** exhibited slightly weaken antioxidant activity with IC₅₀ values of 74.9 ± 6.9 μM. None of the tested diterpene glucosides (**1**, **2**, **3**, **5**, **9** and **16**) showed potent free radical scavenging activity. Concerning the structure–activity relationship, a reasonable conclusion was reasoned out that the more the structure possessed phenolic hydroxyl groups, the more its scavenging activity was strong.

Table 5 DPPH free radical scavenging activity for the compounds^a

Compound	IC ₅₀ (mean ± SD, μM)
1	>200
2	>200
3	>200
5	149.5 ± 51.1
9	>200
10	35.6 ± 9.8
12	74.9 ± 6.9
13	41.5 ± 17.0
14	39.9 ± 12.9
16	>200
Trolox	36.3 ± 5.6
Ascorbic acid (vitamin C)	39.1 ± 6.7

^a IC₅₀, 50% inhibitory concentration. Mean activity of IC₅₀ was exhibited by mean ± standard deviation, *n* ≥ 3.

Experimental section

General experimental procedures

X-ray data were collected using a Bruker APEX DUO instrument. Optical rotations were measured with Horiba SEPA-300 and JASCO P-1020 polarimeters. UV spectra were recorded on a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained on a Tenor 27 spectrophotometer with KBr pellets. One-dimensional (1D) and two-dimensional (2D) NMR spectra were recorded on Bruker DRX-600 spectrometers with TMS as the internal standard. Chemical shifts (δ) were expressed in parts per million with reference to the solvent signals. HRESIMS was performed on an Agilent G6230 TOF MS. Semi-preparative HPLC was performed on an Agilent 1260 liquid chromatograph with a Zorbax SB-C18 (9.4 mm × 25 cm) column. Column chromatography (CC) was performed on silica gel (100–200 mesh and 200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH-20 (Pharmacia). Fractions were monitored by TLC, and spots were visualized by UV light (254 nm) and sprayed with 8% H₂SO₄ in ethanol, followed by heating.

Plant materials

Aerial parts of *Premna szemaoensis* were collected in February 2012 from Puer city, Yunnan Province, People's Republic of China, and identified by Researcher Xi-Wen Li, Kunming Institute of Botany. A voucher specimen (XWL20140403) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The air-dried and powdered aerial parts of *P. szemaoensis* (10 kg) were extracted with 70% aqueous acetone (40 L) four times (two days each time) at room temperature and then filtered. The filtrate was evaporated under reduced pressure at 40 °C and then partitioned between *n*-butanol and H₂O. The *n*-butyl alcohol soluble portion (600 g) was subjected to silica gel CC (2.5 kg, 100–200 mesh), eluted with a CHCl₃–Me₂CO gradient system (1:0–0:1) that afforded fractions A–E. The fractions were then decolorized using MCI gel and eluted with 95% MeOH–H₂O.

Fraction B (33 g) was subjected to silica gel CC (200–300 mesh), eluted with a CHCl₃–MeOH gradient (150:1–1:1), to yield fractions B1–B5. Fraction B1 was purified by repeated silica gel CC (petroleum ether–Me₂CO gradient, 12:1–0:1) to yield compound **14** (10.2 mg). Fraction B3 was purified by Sephadex LH-20 (MeOH) to yield fractions B31–B34. Then compound **13** (75.0 mg) was crystallized from fraction B34, and compound **15** (8.4 mg) was isolated by HPLC (78% MeOH–H₂O, *R*_t = 15.2 min). B4 was purified by RP-18 CC (MeOH–H₂O gradient, 30–100%) to yield fractions B41–B45, then B43 was isolated by semi-preparative HPLC (72% MeOH–H₂O, *R*_t = 13.5 min) to obtain compound **10** (10.1 mg). B5 was subjected to Sephadex LH-20 CC (MeOH) to give B51–B55, then compounds **11** (8.7 mg)



and **12** (15.4 mg) were isolated from fraction B52 by semi-preparative HPLC (75% MeOH–H₂O, *R*_t = 13.1 and 14.8 min, respectively).

Fraction C (120 g) was separated by Sephadex LH-20 (MeOH) to give fractions C1–C5. Fraction C2 was subjected to repeated silica gel CC (200–300 mesh), eluted with CHCl₃–MeOH (gradient system: 120:1–1:1) to yield fractions C21–C27. C23 was isolated by semi-preparative HPLC (42% MeOH–H₂O, *R*_t = 15.1 and 13.2 min, respectively) to afford compounds **1** (8.5 mg) and **2** (11.7 mg). C25 was purified by HPLC (44% MeOH–H₂O, *R*_t = 10.3, 15.3 and 14.5 min, respectively) to yield compounds **4** (5.2 mg), **5** (4.6 mg), and **6** (2.3 mg).

Fraction D (100 g) was subjected to RP-18 CC (MeOH–H₂O, 10–100%) to give fractions D1–D6. Compound **16** (20.1 mg) was crystallized from fractions D2. Fraction D4 was separated to by Sephadex LH-20 eluted with MeOH to give D41–D47, then D43 was isolated by semi-preparative HPLC (41% MeOH–H₂O, *R*_t = 14.5 and 7.8 min, respectively) to yield compounds **3** (6.1 mg) and **7** (4.3 mg). Fraction D5 was separated to silica gel CC (200–300 mesh) eluted with CHCl₃–MeOH (50:1–1:2) to yield D51–D56, then D54 was followed by HPLC (44% MeOH–H₂O, *R*_t = 15.5 and 14.2 min, respectively) to afford compounds **8** (4.6 mg) and **9** (2.3 mg).

Szemaonoid A (1). White monoclinic crystals (MeOH); mp 257–259 °C (MeOH); [α]_D²² +36.3 (*c* 0.10, MeOH); UV (MeOH) λ_{\max} (log ϵ) 213 (4.23), 269 (3.91), 318 (3.49) nm; IR (KBr) ν_{\max} 3441, 1632, 1049 cm^{−1}; ¹H and ¹³C NMR data, see Tables 1 and 2; positive-ion ESIMS *m/z* 531 [M + Na]⁺; positive-ion HRESIMS [M + Na]⁺ *m/z* 531.2205 (calcd for 531.2206).

Szemaonoid B (2). White amorphous powder; [α]_D²² +28.8 (*c* 0.14, MeOH); UV (MeOH) λ_{\max} (log ϵ) 204 (4.17), 270 (3.96), 318 (3.32) nm; IR (KBr) ν_{\max} 3441, 1632, 1071 cm^{−1}; ¹H and ¹³C NMR data, see Tables 1 and 2; positive-ion ESIMS *m/z* 531 [M + Na]⁺; positive-ion HRESIMS [M + Na]⁺ *m/z* 531.2203 (calcd for 531.2206).

Szemaonoid C (3). White monoclinic crystals (MeOH); mp 275–278 °C (MeOH); [α]_D²² +17.8 (*c* 0.12, MeOH); UV (MeOH) λ_{\max} (log ϵ) 203 (4.55), 239 (1.55), 283 (1.51) nm; IR (KBr) ν_{\max} 3414, 1616, 1424, 1072 cm^{−1}; ¹H and ¹³C NMR data, see Tables 1 and 2; negative-ion ESIMS *m/z* 523 [M – H][−]; negative-ion HRESIMS [M – H][−] *m/z* 523.2177 (calcd for 523.2179).

Szemaonoid D (4). Yellowish amorphous powder; [α]_D²² −54.1 (*c* 0.04, MeOH); UV (MeOH) λ_{\max} (log ϵ) 204 (4.47), 239 (4.41), 254 (4.33), 355 (3.61) nm; IR (KBr) ν_{\max} 3425, 2924, 1631, 1384, 1069, 586 cm^{−1}; ¹H and ¹³C NMR data, see Tables 1 and 2; positive-ion ESIMS *m/z* 527 [M + Na]⁺; positive-ion HRESIMS [M + Na]⁺ *m/z* 527.1888 (calcd for 527.1893).

Szemaonoid E (5). Brown amorphous powder; [α]_D²² −53.0 (*c* 0.10, MeOH); UV (MeOH) λ_{\max} (log ϵ) 202 (4.13), 295 (4.06) nm; IR (KBr) ν_{\max} 3426, 1629, 1466, 1216, 580 cm^{−1}; ¹H and ¹³C NMR data, see Tables 1 and 2; positive-ion ESIMS *m/z* 539 [M + Na]⁺; positive-ion HRESIMS [M + Na]⁺ *m/z* 539.1519 (calcd for 539.1529).

Szemaonoid F (6). Yellowish amorphous powder; [α]_D²² +78.8 (*c* 0.06, MeOH); UV (MeOH) λ_{\max} (log ϵ) 202 (4.27), 240 (4.34), 254 (4.27), 356 (3.52) nm; IR (KBr) ν_{\max} 3422, 2924, 1635, 1357, 1201, 1077 cm^{−1}; ¹H and ¹³C NMR data, see Tables 1 and 2;

negative-ion ESIMS *m/z* 503 [M – H][−]; negative-ion HRESIMS [M – H][−] *m/z* 503.1912 (calcd for 503.1917).

Szemaonoid G (7). White amorphous powder; [α]_D²² +15.9 (*c* 0.13, MeOH); UV (MeOH) λ_{\max} (log ϵ) 215 (4.25), 269 (3.84), 316 (3.44) nm; IR (KBr) ν_{\max} 3441, 1632, 1068 cm^{−1}; ¹H and ¹³C NMR data, see Tables 1 and 2; negative-ion ESIMS *m/z* 523 [M – H][−]; negative-ion HRESIMS [M – H][−] *m/z* 523.2176 (calcd for 523.2179).

Szemaonoid H (8). White amorphous powder; [α]_D²² −8.57 (*c* 0.07, MeOH); UV (MeOH) λ_{\max} (log ϵ) 215 (4.11), 269 (3.76), 316 (3.33) nm; IR (KBr) ν_{\max} 3428, 1632, 1069 cm^{−1}; ¹H and ¹³C NMR data, see Tables 1 and 2; negative-ion ESIMS *m/z* 509 [M – H][−]; negative-ion HRESIMS [M – H][−] *m/z* 509.2388 (calcd for 509.2387).

Szemaonoid I (9). White amorphous powder; [α]_D²² +9.25 (*c* 0.09, MeOH); UV (MeOH) λ_{\max} (log ϵ) 204 (4.65), 275 (3.27) nm; IR (KBr) ν_{\max} 3424, 1632, 1422, 1063, 596 cm^{−1}; ¹H and ¹³C NMR data, see Tables 1 and 2; negative-ion ESIMS *m/z* 511 [M – H][−]; negative-ion HRESIMS [M – H][−] *m/z* 511.2540 (calcd for 511.2543).

Szemaonoid J (10). Yellowish monoclinic crystals (MeOH); mp 213–215 °C (MeOH); [α]_D²² +30.56 (*c* 0.12, MeOH); UV (MeOH) λ_{\max} (log ϵ) 203 (4.84), 212 (4.70), 298 (3.10), 353 (1.67) nm; IR (KBr) ν_{\max} 3427, 1632, 1462, 1351, 1015 cm^{−1}; ¹H and ¹³C NMR data, see Tables 1 and 2; negative-ion ESIMS *m/z* 345 [M – H][−]; negative-ion HRESIMS [M – H][−] *m/z* 345.1708 (calcd for 345.1702).

Szemaonoid K (11). Yellowish amorphous powder; [α]_D²² +61.3 (*c* 0.10, MeOH); UV (MeOH) λ_{\max} (log ϵ) 202 (4.23), 237 (4.25), 258 (4.18), 369 (3.52) nm; IR (KBr) ν_{\max} 3441, 1631, 1458, 1372, 1277, 1179, 1069, 602 cm^{−1}; ¹H and ¹³C NMR data, see Tables 1 and 2; negative-ion ESIMS *m/z* 343 [M – H][−]; negative-ion HRESIMS [M – H][−] *m/z* 343.1551 (calcd for 343.1546).

Szemaonoid L (12). Yellowish amorphous powder; [α]_D²² +43.3 (*c* 0.11, MeOH); UV (MeOH) λ_{\max} (log ϵ) 202 (3.99), 237 (4.06), 260 (4.02), 366 (3.36) nm; IR (KBr) ν_{\max} 3429, 2926, 1631, 1456, 1370 cm^{−1}; ¹H and ¹³C NMR data, see Tables 1 and 2; negative-ion ESIMS *m/z* 343 [M – H][−]; negative-ion HRESIMS [M – H][−] *m/z* 343.1548 (calcd for 343.1546).

X-ray crystal structure analysis

Crystals of **1**, **3** and **10** were obtained in MeOH, respectively. Intensity data were collected at 100 K on a Bruker APEX DUO diffractometer equipped with an APEX II CCD using Cu K α radiation. Cell refinement and data reduction were performed with Bruker SAINT. The structures were solved by direct methods using SHELXS-97.⁴⁰ Refinements were performed with SHELXL-97 and SHELXL-2014 using full-matrix least-squares, with anisotropic displacement parameters for all the non-hydrogen atoms. The H-atoms were placed in calculated positions and refined using a riding model. Molecular graphics were computed with PLATON.⁴¹ Crystallographic data (excluding structure factor tables) for the structures reported have been deposited with the Cambridge Crystallographic Data Center as supplementary publications no. CCDC 1554050 for **1**, CCDC 1554052 for **3**, and CCDC 1554051 for **10**.†



Crystal data for szemaoenoid A (1). $C_{26}H_{36}O_{10} \cdot H_2O$, $M = 526.56$, monoclinic, $a = 5.70480(10)$ Å, $b = 23.8602(5)$ Å, $c = 9.3419(2)$ Å, $\alpha = 90.00^\circ$, $\beta = 90.6040(10)^\circ$, $\gamma = 90.00^\circ$, $V = 1271.53(4)$ Å³, $T = 100(2)$ K, space group $P2_1$, $Z = 2$, $\mu(CuK\alpha) = 0.898$ mm⁻¹, 10 232 reflections measured, 3499 independent reflections ($R_{int} = 0.0328$). The final R_1 values were 0.0300 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0884 ($I > 2\sigma(I)$). The final R_1 values were 0.0300 (all data). The final $wR(F^2)$ values were 0.0885 (all data). The goodness of fit on F^2 was 1.113. Flack parameter = 0.17(14).²⁹ The Hooft parameter is 0.10(6) for 1225 Bijvoet pairs.³⁰

Crystal data for szemaoenoid C (3). $4(C_{26}H_{36}O_{11}) \cdot H_2O$, $M = 2116.20$, $a = 17.5477(6)$ Å, $b = 21.7199(7)$ Å, $c = 33.3592(12)$ Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$, $V = 12\ 714.3(8)$ Å³, $T = 100(2)$ K, space group $P2_12_12_1$, $Z = 4$, $\mu(CuK\alpha) = 0.728$ mm⁻¹, 112641 reflections measured, 23 429 independent reflections ($R_{int} = 0.0484$). The final R_1 values were 0.0744 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.2065 ($I > 2\sigma(I)$). The final R_1 values were 0.0759 (all data). The final $wR(F^2)$ values were 0.2083 (all data). The goodness of fit on F^2 was 1.044. Flack parameter = 0.11(3).⁴²

Crystal data for szemaoenoid J (10). $C_{20}H_{26}O_5$, $M = 346.41$, monoclinic, $a = 11.5843(7)$ Å, $b = 9.5501(6)$ Å, $c = 15.2093(10)$ Å, $\alpha = 90.00^\circ$, $\beta = 92.859(4)^\circ$, $\gamma = 90.00^\circ$, $V = 1680.53(18)$ Å³, $T = 100(2)$ K, space group $P2_1$, $Z = 4$, $\mu(CuK\alpha) = 0.794$ mm⁻¹, 10 384 reflections measured, 4801 independent reflections ($R_{int} = 0.0536$). The final R_1 values were 0.0627 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1682 ($I > 2\sigma(I)$). The final R_1 values were 0.0701 (all data). The final $wR(F^2)$ values were 0.1743 (all data). The goodness of fit on F^2 was 1.058. Flack parameter = 0.0(2).²⁹ The Hooft parameter is 0.01(14) for 1718 Bijvoet pairs.³⁰

Acid hydrolysis of szemaoenoid A

Compound **1** (4 mg) was hydrolyzed with 2 M HCl/dioxane (1 : 1, 4 mL) under reflux for 8 h, respectively. The reaction mixture was partitioned between H₂O and CHCl₃ (2 mL \times 3). The aqueous layer was neutralized with 2 M NaOH and then dried to give a monosaccharide. A solution of the sugar in pyridine (2 mL) was added to L-cysteine methyl ester hydrochloride (about 1.0 mg) and kept at 60 °C for 1 h. Then trimethylsilylimidazole (about 1.0 mL) was added to the reaction mixture and kept at 60 °C for 30 min. The mixture was subjected to GC analysis, run on a Shimadzu GC-14C gas chromatograph equipped with an H₂ flame ionization detector. The column was a 30 m \times 0.32 mm i.d. 30QC2/AC-5 quartz capillary column with the following conditions: column temperature, 180–280 °C; programmed increase, 3 °C min⁻¹; carrier gas, N₂ (1 mL min⁻¹); injector and detector temperature, 250 °C; injection volume, 4 µL; and split ratio, 1/50. The configuration of the sugar moiety was determined by comparing the retention time with the derivatives of the authentic samples. The retention times of D-/L-glucose were 21.115/21.565 min.⁴³ The configuration of the sugar moiety from compound **1** was D-glucose ($R_t = 21.117$ min).

Cytotoxicity assay

Human colon adenocarcinoma cell lines, HCT-116 and HT-29 were obtained from the American Type Culture Collection

(ATCC). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS in a 5% CO₂ atmosphere. HCT-116 (3×10^3 per well) and HT-29 (6×10^3 per well) were seeded onto 96-well plates and allowed to grow for 24 h prior to treatment. Different concentrations of compounds were then added and further incubated for 3 days. Sorafenib (purity > 99%; Medchem Express) was used as positive control. The culture medium was replaced by fresh DMEM containing 0.5 mg mL⁻¹ of MTT. After incubation for another 4 h, the medium was removed and the reduced formazan blue was solubilized by adding 100 µL DMSO to each well. The absorbance at 492 nm was measured using a microplate reader (Multiskan MK3, Thermo). The IC₅₀ values were calculated from concentration–response curves using Graphpad Prism software.

Antioxidant activity assay

Trolox (purity > 98%; Sigma) and vitamin C (Ascorbic acid, purity > 98%; Sigma) were used as positive control. A 0.1 mM solution of DPPH radical in ethanol was prepared, and 100 µL of this solution was mixed with 100 µL of sample solution. The mixture was incubated for 5 min in a dark room at room temperature. Scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517 nm. DPPH scavenging activity (%) = $[1 - (S - B)/(C - B)] \times 100\%$, where S , B and C are the absorbencies of the sample, the blank and the control, respectively.⁴⁴

Conclusions

In summary, we have firstly reported twelve new abietane diterpenoids (**1–12**) isolated from *P. szemaoensis*, together with four known compounds (**13–16**). Structurally, these compounds involved two rearranged-abietane skeletons: 17(15 \rightarrow 16)-abeo-abietane and 17(15 \rightarrow 16),18(4 \rightarrow 3)-diabeo-abietane. Their structures with absolute configurations were characterized by a series of spectroscopic methods and X-ray diffraction. In bioactivity assays, compounds **11**, **12**, **14** and **15** were active against two human colon cancer cell lines (HCT-116 and HT-29) with IC₅₀ values ranging from 8.8 to 34.3 µM, and compounds **10**, **13** and **14** exhibited effective free radical scavenging activity with IC₅₀ values ranging from 35.6 to 41.5 µM by DPPH experiment. In short, the current study adds to understanding of the chemical composition and biological effects of this plant prepared for green food and ethnodrugs.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This project was supported financially by the NSFC (81422046, 21762048 and U1702286), the State Key Laboratory of Drug Research (SIMM1705KF-05), and Program for Changjiang Scholars and Innovative Research Team in University (IRT_17R94).



Notes and references

- 1 J. W. Kadereit, *The Families and Genera of Vascular Plants*, Springer, Berlin, 2004.
- 2 J. Pei and S. L. Chen, *Zhongguo Zhiwu Zhi*, Science Press, Beijing, 1982.
- 3 S. Habtemariam, *BMC Pharmacol.*, 2003, **3**, 6.
- 4 H. Sudo, T. Ide, H. Otsuka, E. Hirata, A. Takushi and Y. Takeda, *Phytochemistry*, 1997, **46**, 1231–1236.
- 5 H. Sudo, A. Takushi, E. Hirata, T. Ide, H. Otsuka and Y. Takeda, *Phytochemistry*, 1999, **52**, 1495–1499.
- 6 N. T. B. Hang, P. T. Ky, C. Van Minh, N. X. Cuong, N. P. Thao and P. Van Kiem, *Nat. Prod. Commun.*, 2008, **3**, 1449–1452.
- 7 V. Shukla, S. C. Phulara, D. Yadav, S. Tiwari, S. Kaur, M. M. Gupta, A. Nazir and R. Pandey, *CNS Neurol. Disord.: Drug Targets*, 2012, **11**, 984–992.
- 8 D. Y. Wang and S. Y. Xu, *Nat. Prod. Res.*, 2003, **17**, 75–77.
- 9 H. Sudo, A. Takushi, T. Ide, H. Otsuka, E. Hirata and Y. Takeda, *Phytochemistry*, 1997, **46**, 1147–1150.
- 10 S. B. Lirio, A. P. G. Macabeo, E. M. Paragas, M. Knorn, P. Kohls, S. G. Franzblau, Y. H. Wang and M. A. M. Aguinaldo, *J. Ethnopharmacol.*, 2014, **154**, 471–474.
- 11 K. Y. Niu, L. Y. Wang, S. Z. Liu and W. M. Zhao, *J. Asian Nat. Prod. Res.*, 2013, **15**, 1–8.
- 12 D. Yadav, N. Masood, S. Luqman, P. Brindha and M. M. Gupta, *Ind. Crops Prod.*, 2013, **41**, 397–402.
- 13 J. Lin, B. Yang and J. H. Chen, *J. Guangxi Med. Univ.*, 2001, **2**, 207–208.
- 14 N. Narayanan, P. Thirugnanasambantham, S. Viswanathan, M. Kannappa Reddy, V. Vijayasekaran and E. Sukumar, *Fitoterapia*, 2000, **71**, 147–153.
- 15 H. P. Pan, Y. Chen, S. H. Jing, M. Lu and G. H. Zhang, *Chin. J. Hosp. Pharm.*, 2007, **07**, 861–864.
- 16 G. Suresh, K. S. Babu, V. R. S. Rao, M. S. A. Rao, V. L. Nayak and S. Ramakrishna, *Tetrahedron Lett.*, 2011, **52**, 1273–1276.
- 17 M. Biradi and K. Hullatti, *J. Pharmacol.*, 2015, **10**, 205–208.
- 18 A. W. Salae, A. Rodjun, C. Karalai, C. Ponglimanont, S. Chantrapromma, A. Kanjana-Opas, S. Tewtrakul and H.-K. Fun, *Tetrahedron*, 2012, **68**, 819–829.
- 19 S. R. Ayinampudi, R. Domala, R. Merugu, S. Bathula and M. R. Janaswamy, *Fitoterapia*, 2012, **83**, 88–92.
- 20 Z. Y. Wu, *Yunnan Zhiwu Zhi*, Science Press, Yunnan province, 1977.
- 21 S. Liu, H. Zhu, S. Zhang, X. Zhang, Q. Yu and L. Xuan, *J. Nat. Prod.*, 2008, **71**, 755–759.
- 22 M. F. Xu, L. Q. Shen, K. W. Wang, Q. Z. Du and N. Wang, *J. Asian Nat. Prod. Res.*, 2011, **13**, 260–264.
- 23 W. X. Wang, J. J. Zhu, Y. K. Zou, Z. L. Hong, S. T. Liu, M. Li, Y. Huang, J. Xiong, Y. Zhao, G. X. Yang, G. Xia and J. F. Hu, *Tetrahedron Lett.*, 2013, **54**, 2549–2552.
- 24 Q. Liu, H. J. Hu, P. F. Li, Y. B. Yang, L. H. Wu, G. X. Chou and Z. T. Wang, *Phytochemistry*, 2014, **103**, 196–202.
- 25 X. Tian, Z. Min, N. Xie, Y. Lei, Z. Tian, Q. Zheng, R. Xu, T. Tanaka, M. Iinuma and M. Mizuno, *Chem. Pharm. Bull.*, 1993, **41**, 1415–1417.
- 26 M. F. Xu, L. Q. Shen, K. W. Wang, Q. Z. Du and N. Wang, *Nat. Prod. Commun.*, 2011, **6**, 3–5.
- 27 A. Ulubelen, G. Topcu and S. Olcal, *Phytochemistry*, 1994, **37**, 1371–1375.
- 28 W. X. Wang, J. Xiong, Y. Tang, J. J. Zhu, M. Li, Y. Zhao, G. X. Yang, G. Xia and J. F. Hu, *Phytochemistry*, 2013, **89**, 89–95.
- 29 H. D. Flack, *Acta Crystallogr.*, 1983, **39**, 876–881.
- 30 R. W. W. Hooft, L. H. Straver and A. L. Spek, *J. Appl. Crystallogr.*, 2008, **41**, 96–103.
- 31 M. A. Gonzalez, *Nat. Prod. Rep.*, 2015, **32**, 684–704.
- 32 T. Murata, Y. Ishikawa, E. Saruul, E. Selenge, K. Sasaki, K. Umehara, F. Yoshizaki and J. Batkhuu, *Phytochemistry*, 2016, **130**, 152–158.
- 33 Y. W. Chin, W. P. Jones, Q. Mi, I. Rachman, S. Riswan, L. B. Kardono, H. B. Chai, N. R. Farnsworth, G. A. Cordell, S. M. Swanson, J. M. Cassidy and A. D. Kinghorn, *Phytochemistry*, 2006, **67**, 1243–1248.
- 34 A. Hymavathi, K. Suresh Babu, V. G. Naidu, S. Rama Krishna, P. V. Diwan and J. Madhusudana Rao, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 5727–5731.
- 35 G. Suresh, K. S. Babu, M. S. A. Rao, V. R. S. Rao, P. A. Yadav, V. L. Nayak and S. Ramakrishna, *Tetrahedron Lett.*, 2011, **52**, 5016–5019.
- 36 A. W. Salae and N. Boonnak, *Tetrahedron Lett.*, 2013, **54**, 1356–1359.
- 37 T. Kallio, J. Kallio, M. Jaakkola, M. Maki, P. Kilpelainen and V. Virtanen, *J. Agric. Food Chem.*, 2013, **61**, 10720–10729.
- 38 H. M. Ge, W. H. Yang, J. Zhang and R. X. Tan, *J. Agric. Food Chem.*, 2009, **57**, 5756–5761.
- 39 Y. Yao, W. Sang, M. J. Zhou and G. X. Ren, *J. Agric. Food Chem.*, 2010, **58**, 770–774.
- 40 G. M. Sheldrick and T. R. Schneider, *Methods Enzymol.*, 1997, **277**, 319–343.
- 41 A. L. Spek, *Acta Crystallogr., Sect. C: Struct. Chem.*, 2015, **71**, 9–18.
- 42 H. D. Flack and G. Bernardinelli, *Chirality*, 2008, **20**, 681.
- 43 P. Y. Liao, D. Wang, Y. J. Zhang and C. R. Yang, Dammara-ne-type glycosides from steamed notoginseng, *J. Agric. Food Chem.*, 2008, **56**, 1751–1756.
- 44 S. B. Wu, J. Wu, Z. Yin, J. Zhang, C. Long, E. J. Kennelly and S. Zheng, *J. Agric. Food Chem.*, 2013, **61**, 4035–4043.

