



## Research Article

New dammarane-type triterpenoid saponins from *Panax notoginseng* saponins

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## ARTICLE INFO

## Article history:

Received 27 January 2018

Received in Revised form

4 December 2018

Accepted 6 December 2018

Available online 13 December 2018

## Keywords:

Cytotoxicity

Fingerprint

New dammarane-type triterpenoid saponins

*Panax Notoginseng* saponins

## ABSTRACT

**Background:** *Panax notoginseng* saponin (PNS) is the extraction from the roots and rhizomes of *Panax notoginseng* (Burk.) F. H. Chen. PNS is the main bioactive component of Xuesaitong, Xueshuantong, and other Chinese patent medicines, which are all bestselling prescriptions in China to treat cardiocerebrovascular diseases. Notoginsenoside R<sub>1</sub> and ginsenoside Rg<sub>1</sub>, Rd, Re, and Rb<sub>1</sub> are the principal effective constituents of PNS, but a systematic research on the rare saponin compositions has not been conducted. **Objective:** The objective of this study was to conduct a systematic chemical study on PNS and establish the HPLC fingerprint of PNS to provide scientific evidence in quality control. In addition, the cytotoxicity of the new compounds was tested.

**Methods:** Pure saponins from PNS were isolated by means of many chromatographic methods, and their structures were determined by extensive analyses of NMR and HR-ESI-MS studies. The fingerprint was established by HPLC-UV method. The cytotoxicity of the compounds was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

**Results and Conclusion:** Three new triterpenoid saponins (**1–3**) together with 25 known rare saponins (**4–28**) were isolated from PNS, except for the five main compounds (notoginsenoside R<sub>1</sub> and ginsenoside Rg<sub>1</sub>, Rd, Re, and Rb<sub>1</sub>). In addition, the HPLC fingerprint of PNS was established, and the peaks of the isolated compounds were marked. The study of chemical constituents and fingerprint was useful for the quality control of PNS. The study on antitumor activities showed that new Compound **2** exhibited significant inhibitory activity against the tested cell lines.

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## 1. Introduction

*Panax notoginseng* (Burk.) F.H. Chen (*P. notoginseng*), commonly called “Sanqi” or “Tianqi” in Chinese is a species of the genus *Panax*, family Araliaceae [1]. *P. notoginseng* is one of the most widely used Chinese herbal drugs for the treatment of cardiovascular diseases, such as occlusive vasculitis, coronary diseases, atherosclerosis, and cerebral infarction in China, Korea, and Russia for a long time [2]. There are about 200 chemical compositions that have been isolated from *P. notoginseng*, including saponins, flavonoids, and cyclopeptides [3]. Dammarane triterpenoidal saponins are the major bioactive ingredients of *P. notoginseng* [4].

*Panax notoginseng* saponin (PNS) are developed into the traditional Chinese medicine agents with the trademarks of Xuesaitong injection, Xueshuantong injection, and Xuesaitong tablet, which are all bestselling prescriptions used for treatment of cardiovascular and cerebrovascular diseases in China [5,6]. Notoginsenoside R<sub>1</sub> and ginsenoside Rg<sub>1</sub>, Rd, Re, and Rb<sub>1</sub> are regarded as the main active constituents [7,8]. But, there is a lack of references about the extraction and structure identification of the rare ginsenosides in PNS. As the study on microconstituents is important to control the quality and safety of clinical medication, we investigated the rare content in the PNS; three new and 25 known dammarane-type triterpenoids were isolated and identified (Fig. 1). We also

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**Table 1**  
<sup>1</sup>H [  $\delta$  in ppm, multiplicity (*J* in Hz)] and <sup>13</sup>C NMR ( $\delta$  in ppm) spectroscopic data of Compounds 1–3.<sup>1)</sup>

Position	1		2		3	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
1	39.9	1.70 (1H, m) 1.05 (1H, m)	39.7	1.65 (1H, m) 1.02 (1H, m)	40.0	1.71 (1H, m) 1.05 (1H, m)
2	28.4	1.94 (1H, m) 1.87 (1H, m)	28.2	1.91 (1H, m) 1.84 (1H, m)	28.4	1.95 (1H, m) 1.88 (1H, m)
3	79.0	3.55 (1H, m)	78.7	3.52 (1H, m)	79.0	3.55 (1H, m)
4	40.9		40.7		40.9	
5	61.8	1.46 (1H, m)	61.7	1.43 (1H, m)	61.9	1.46 (1H, m)
6	80.5	4.46 (1H, m)	80.2	4.45 (1H, m)	80.6	4.45 (1H, m)
7	45.8	2.59 (1H, m) 1.98 (1H, m)	45.6	2.57 (1H, m) 1.94 (1H, m)	45.8	2.55 (1H, m) 1.96 (1H, m)
8	41.7		41.5		41.7	
9	51.1	1.59 (1H, m)	50.9	1.59 (1H, m)	51.1	1.58 (1H, m)
10	40.1		40.0		40.2	
11	32.7	1.50 (2H, m)	32.8	1.88 (1H, m)	31.5	1.98 (1H, m)
12	80.2	4.29 (1H, m)	80.0	4.25 (1H, m)	80.2	4.28 (1H, m)
13	59.1	2.13 (1H, m)	55.4	2.29 (1H, m)	52.8	2.16 (1H, m)
14	52.2		51.7		51.6	
15	33.4	1.92 (1H, m) 1.26 (1H, m)	32.9	1.52 (1H, m) 1.22 (1H, m)	33.0	1.76 (1H, m) 1.16 (1H, m)
16	34.7	2.16 (1H, m) 1.60 (1H, m)	35.9	2.21 (1H, m) 1.43 (1H, m)	35.1	2.18 (1H, m) 2.05 (1H, m)
17	40.2	3.05 (1H, m)	38.4	2.91 (1H, m)	48.7	2.29 (1H, m)
18	17.8	1.25 (3H, s)	17.6	1.28 (3H, s)	17.8	1.24 (3H, s)
19	18.2	1.04 (3H, s)	18.1	1.03 (3H, s)	18.2	1.05 (3H, s)
20	160.2		156.3		156.5	
21	111.2	5.31 (2H, d, <i>J</i> = 12.5)	113.9	5.29 (2H, d, <i>J</i> = 12.5)	108.4	5.15 (1H, s) 4.97 (1H, s)
22	77.0	4.52 (1H, m)	91.3	4.71 (1H, t, <i>J</i> = 7.1)	33.1	2.77 (1H, m) 2.51 (1H, m)
23	36.3	2.73 (1H, m) 2.54 (1H, m)	31.3	2.60 (1H, m) 2.32 (1H, m)	33.2	2.06 (1H, m) 2.46 (1H, m)
24	122.4	5.42 (1H, t, <i>J</i> = 6.9)	121.1	5.34 (1H, t, <i>J</i> = 6.9)	75.5	4.46 (1H, m)
25	132.8		133.4		150.0	
26	26.4	1.71 (3H, s)	26.1	1.65 (3H, s)	110.5	5.29 (1H, s) 4.96 (1H, s)
27	18.6	1.65 (3H, s)	18.3	1.59 (3H, s)	18.7	1.91 (3H, s)
28	32.2	2.10 (3H, s)	32.0	2.08 (3H, s)	32.2	2.10 (3H, s)
29	16.8	1.63 (3H, s)	16.7	1.60 (3H, s)	16.8	1.63 (3H, s)
30	17.1	0.88 (3H, s)	16.9	0.82 (3H, s)	17.2	0.82 (3H, s)
6-O-sugar						
1	106.5	5.07 (1H, d, <i>J</i> = 8.0)	106.3	5.04 (1H, d, <i>J</i> = 7.8)	106.4	5.05 (1H, d, <i>J</i> = 7.8)
2	73.0	3.99 (1H, m)	73.2	3.93 (1H, m)	73.0	3.91 (1H, m)
3	78.7	3.99 (1H, m)	78.5	3.96 (1H, m)	78.6	3.98 (1H, m)
4	72.3	4.26 (1H, m)	72.1	4.22 (1H, m)	72.3	4.27 (1H, m)
5	75.9	4.13 (1H, m)	75.7	4.10 (1H, m)	75.9	4.13 (1H, m)
6	63.5	4.57 (1H, m) 4.41 (1H, m)	63.4	4.54 (1H, m) 4.36 (1H, m)	63.5	4.54 (1H, m) 4.44 (1H, m)

NMR, nuclear magnetic resonance; s, singlet; d, doublet; t, triplet; m, multiplet

<sup>1)</sup> Measured in pyridine-*d*<sub>5</sub>, 500 MHz for <sup>1</sup>H, 125 MHz for <sup>13</sup>C. The assignment was based on DEPT, correlation spectroscopy (COSY), HSQC, and HMBC experiments.

established the HPLC fingerprint and marked the compounds isolated. In addition, we tested the cytotoxic activity of the three new compounds against three human cancer cell lines.

## 2. Materials and methods

### 2.1. General experimental procedures

UV spectra: Shimadzu UV-2401A spectrophotometer (Shimadzu Instruments Co., Ltd, Tokyo, Japan). IR spectra: Nicolet FT-IR-360 spectrometer (Thermo Nicolet, Inc., Waltham, MA, USA). NMR spectra: Bruker ARX-400 spectrometers (Bruker Ltd, Karlsruhe, Germany). High resolution electrospray ionization mass spectrum (HR-ESI-MS) were taken using, Agilent G6230 instruments (Agilent Technologies, Santa Clara, CA, USA). Preparative HPLC was performed on an Agilent 1100 apparatus equipped with a Rheodyne injector and with UV detectors using a Thermo C<sub>18</sub> column (10 × 250 mm, 5 μm).

Column chromatography was performed using silica (200–300 mesh and 100–200 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China) and octadecylsilyl (ODS) (50 μm; YMC Co., Kyoto, Japan). The reagents were of HPLC grade or analytical grade (Sinopharm Chemical Reagent Co., Ltd, Beijing, China).

### 2.2. Plant material

The PNS was provided by Yunnan Baiyao Group Co., Ltd.

### 2.3. Extraction and isolation

The PNS (2 kg) was separated by silica gel column using a gradient of CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH (100:1 → 50:1 → 25:1 → 10:1 → 5:1 → 2:1 → 1:1, v/v) to obtain eight fractions (Fr.1–Fr.8). Fr.1 (10 g) was chromatographed subsequently over silica gel chromatography with CHCl<sub>3</sub>-MeOH (30:1 → 20:1 → 10:1, v/v) to get five major

fractions (Fr.1-1–Fr.1-5) based on thin-layer chromatography (TLC) analysis. Fr.1-2 was purified by ODS eluted with MeOH-H<sub>2</sub>O (40:60→55:45→70:30, v/v) to provide Compounds **2** (5.1 mg) and **4** (75.5 mg). Fr.1-3 was then separated into three major fractions (Fr.1-3a–Fr.1-3c) by silica gel chromatography with CHCl<sub>3</sub>-MeOH (15:1 and 13:1, v/v) as eluent. Fr.1-3a and Fr.1-3b were further separated by preparative HPLC (p-HPLC) eluting with MeCN-H<sub>2</sub>O 28:72 to yield Compounds **3** (10.2 mg) and **16** (25.3 mg), while **25** (3.0 mg) and **8** (4.5 mg) were prepared from Fr.1-3c with MeCN-H<sub>2</sub>O (29:71, v/v) as a solvent system. Fr.2 (18.8 g) was subjected to chromatography on ODS gel to afford 10 fractions (Fr.2-1–Fr.2-10). Fr.2-3 was subjected to chromatography on silica gel to yield Compound **6** (7.7 mg). Compounds **7** (5.3 mg) and **14** (3.6 mg) were isolated from Fr.2-6 by p-HPLC with MeCN-H<sub>2</sub>O (30:70, v/v), and Compounds **18** (9.8 mg) and **20** (5.6 mg) were isolated from Fr.2-7 by p-HPLC with MeCN-H<sub>2</sub>O (28:72, v/v). Fr.2-8 was chromatographed over silica gel to obtain 10 major fractions with CHCl<sub>3</sub>-MeOH (50:1 to 5:1, v/v) as eluent (Fr.2-8-1–Fr.2-8-10). Fr.2-8-5 was separated subsequently by ODS chromatography eluting with MeOH-H<sub>2</sub>O (40:60→55:45→70:30, v/v) to afford **19** (2.3 mg). Fr.2-4 was fractionated by ODS eluted with MeOH-H<sub>2</sub>O (40:60→60:40→80:20, v/v) to afford five major fractions (Fr.2-4-1–Fr.2-4-5). The analysis of a combined fraction of Fr.2-4-1–Fr.2-4-3 was performed by p-HPLC. Compound **27** (9.5 mg) was isolated from Fr.2-4-1 by HPLC system of MeCN-H<sub>2</sub>O (28:72, v/v). Compounds **10** (8.5 mg) and **15** (3.3 mg) were prepared from Fr.2-4-2 with MeCN-H<sub>2</sub>O (20:80, v/v) as a solvent system, whereas **9** (11.2 mg) and **12** (3.5 mg) were obtained from Fr.2-4-2 with MeCN-H<sub>2</sub>O (37:63, v/v) as a solvent system. Compounds **11** (3.8 mg) and **28** (2.2 mg) were got from Fr.2-4-3 by HPLC system of MeCN-H<sub>2</sub>O (43:57 and 35:65, v/v). Fr.5 (23.5 g) was subjected to chromatography on ODS gel to provide ten fractions (Fr.5-1–Fr.5-10). Compound **5** (1115.8 mg) was purified by recrystallizing from Fr.5-1. The analysis of other compounds isolated from Fr.5 was performed by p-HPLC: Compound **22** (mg) was purified from Fr.5-2, and Compounds **17** (51.1 mg) and **21** (12.7 mg) were isolated from Fr.5-3 by HPLC system of MeCN-H<sub>2</sub>O (30:70, v/v). Compound **13** (13.4 mg) were isolated from Fr.5-5 HPLC system of MeCN-H<sub>2</sub>O (34:66, v/v). Compounds **1** (4.3 mg) and **26** (8.5 mg) were prepared from Fr.5-7 with MeCN-H<sub>2</sub>O (27:73, v/v) as a solvent system, whereas **23** (36.7 mg) and **24** (8.9 mg) were prepared from Fr.5-8 with MeCN-H<sub>2</sub>O (37:63, v/v) as a solvent system.

#### 2.4. Notoginsenoside Ab<sub>1</sub> (**1**)

3 $\beta$ ,6 $\alpha$ ,12 $\beta$ ,22S-tetrahydroxy-dammar-20(21),24-diene-6-O- $\beta$ -D-glucopyranoside: white amorphous powder; [ $\alpha$ ]<sub>20D</sub> : +10.5, (c = 0.20, MeOH); IR  $\nu_{\max}$  3420, 2931, 1634, 1454, 1384, 1074, 1032 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR: see Table 1; HR-ESI-MS *m/z* 659.4130 [M+Na]<sup>+</sup> (calculated for C<sub>36</sub>H<sub>60</sub>O<sub>9</sub>Na, 659.4135).

#### 2.5. Notoginsenoside Ab<sub>2</sub> (**2**)

22S-hydroperoxy-3 $\beta$ ,6 $\alpha$ ,12 $\beta$ -trihydroxy-dammar-20(21),24-diene-6-O- $\beta$ -D-glucopyranoside: white amorphous powder; [ $\alpha$ ]<sub>20D</sub> : +11.6, (c = 0.18, MeOH); IR  $\nu_{\max}$  3422, 2933, 1637, 1452, 1384, 1075, 1031 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR: see Table 1; HR-ESI-MS 675.4077 [M + Na]<sup>+</sup> (calcd. for C<sub>36</sub>H<sub>60</sub>O<sub>10</sub>Na 675.4084).

#### 2.6. Notoginsenoside Ab<sub>3</sub> (**3**)

3 $\beta$ ,6 $\alpha$ ,12 $\beta$ ,24R-tetrahydroxy-dammar-20(21),25-diene-6-O- $\beta$ -D-glucopyranoside: white amorphous powder; [ $\alpha$ ]<sub>20D</sub> : +3.4, (c = 0.25, MeOH); IR  $\nu_{\max}$  3416, 2941, 1636, 1452, 1386, 1163, 1076,

1041 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR: see Table 1; HR-ESI-MS 659.4132 [M+Na]<sup>+</sup> (calcd. For. C<sub>36</sub>H<sub>60</sub>O<sub>9</sub>Na 659.4135).

#### 2.7. Acid hydrolysis and HPLC analysis

The absolute configurations of the sugar moieties in Compounds **1–3** were determined by the method of literature reported [9]. Compounds **1–3** (2.0 mg/sample) were refluxed with 10 mL of 60% aqueous dioxane with 5% HCl for 2 h. The reaction mixture was evaporated under vacuum and then suspended in H<sub>2</sub>O and extracted with CHCl<sub>3</sub>. After drying in vacuum, the residue of aqueous layer was melted in 0.2 mL of C<sub>5</sub>H<sub>5</sub>N with 2 mg of L-cysteine methyl ester hydrochloride followed by warming at 60°C for 1 h. After that, 5 mL of *o*-tolylisothiocyanate is added and warmed up at 60°C for another hour. The reaction mixture was analyzed directly by reversed-phase HPLC on a Thermo C<sub>18</sub> column (250 × 4.6 mm, 5  $\mu$ m), with 20% CH<sub>3</sub>CN at a flow rate of 1.0 mL/min at 30°C, and the detection wavelength was 254 nm. The analysis of standard monosaccharide, D-glucose, followed the same procedure. The value of *t*<sub>R</sub> of the standard monosaccharide derivatives was 17.8 min, and the derivatives of **1–3** gave peaks at *t*<sub>R</sub> 17.7–17.9 min, respectively.

#### 2.8. Computational studies

Conformational searches were performed with Gaussian 09W program (Gaussian Inc., USA). The geometry of each conformer in the energy window of the conformational search was optimized with Gaussian 09W in vacuum, at the B3LYP-6-31g (d,p) level. Imaginary vibrational frequency of each conformer was checked, and no such frequency indicates true energy minima. Isotropic magnetic shielding was calculated with the GIAO (gauge-independent atomic orbital) method at the B3LYP/6-31G (d, P) level by using Gaussian 09W [10,11].

#### 2.9. Fingerprint analysis

Chromatographic conditions: Waters 1525 HPLC system (Waters Corp., Milford, Massachusetts, USA); Chromatographic column: VP ODS C<sub>18</sub> (250 mm × 4.6 mm, 5  $\mu$ m; Agilent Technologies, Santa Clara, CA, USA); volume flow: 1.0 mL/min; column temperature: 30°C; detection wavelength: 203 nm; injection volume: 10  $\mu$ L. The samples were eluted with the mixture of Solvent A (water) and Solvent B (acetonitrile). The elution rate using Solvent B was 20–45% for 0–60 min.

Preparation of samples: Accurately weighed 25 mg of powder sample was diluted with 10 mL of 70% methanol. Before injection, the samples were filtered through a 0.45- $\mu$ m membrane filter.

#### 2.10. Cell line

HepG-2 (human hepatic cancer cell line), NCI-H460 (human lung cancer cell), and MCF-7 (human breast cancer cell) were purchased from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). The HepG-2 and NCI-H460 cancer cells were maintained in Roswell Park Memorial Institute 1640 medium, and MCF-7 cancer cells were maintained in high-glucose Dulbecco's minimum essential medium, supplemented with 10% fetal bovine serum. The cells grew in a 5% CO<sub>2</sub> incubator at 37°C. The cells were routinely digested and passaged every 3 days.

#### 2.11. Cell viability assay

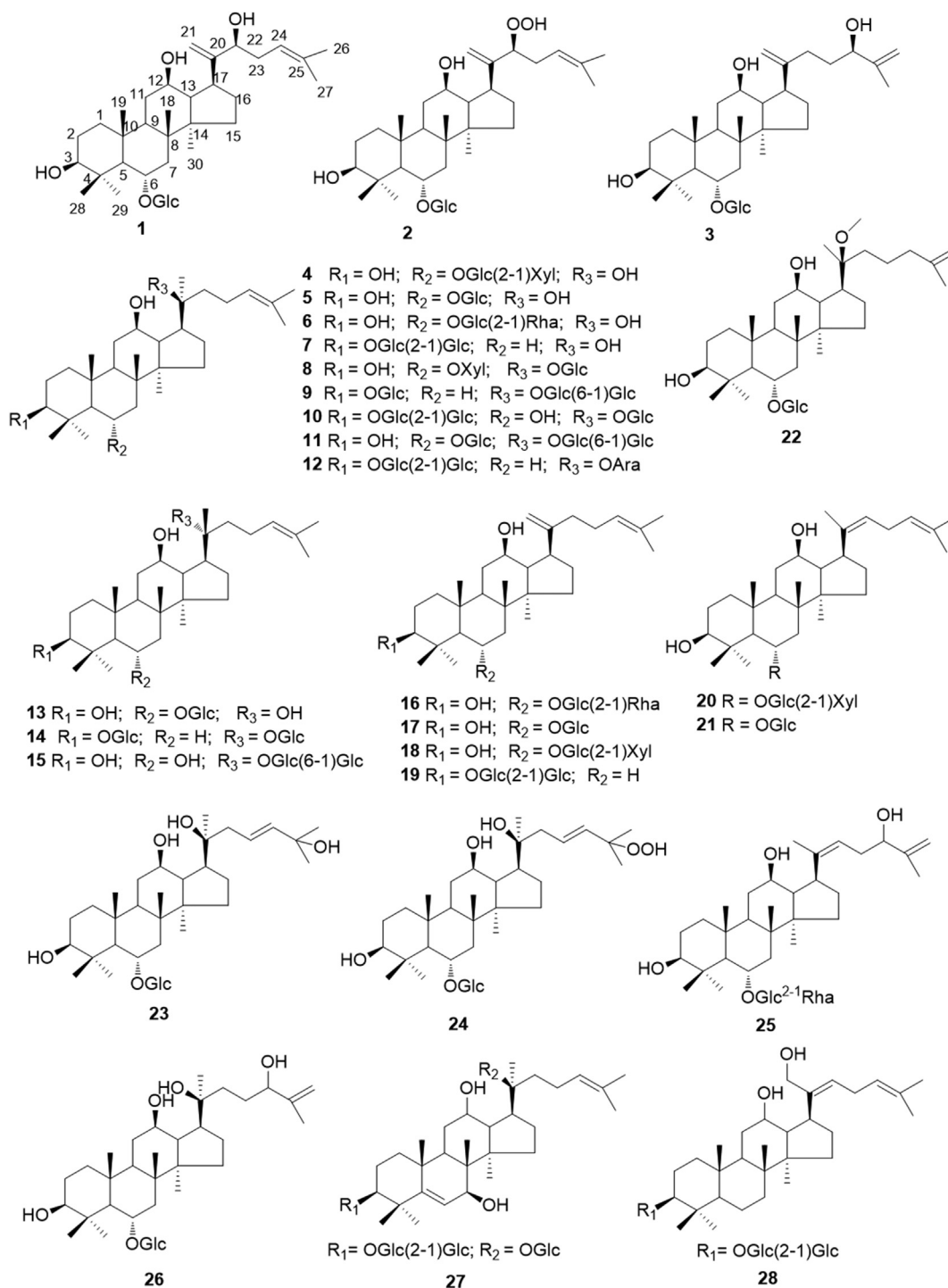
The cells were plated in 96-well plates (1 × 10<sup>4</sup> cells/well) overnight, then **1–3** at various concentrations of 0.01, 0.1, 1, 10, and

100 µg/mL and the positive control cisplatin at concentrations of 0.5, 1, 2, 4, and 8 µg/mL were treated in the plates for 72 h. Subsequently, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reagent (5 mg/mL) was added to each well for 4 h, and then 100 µL of triple liquid containing 10 mg SDS (sodium dodecyl sulfate), 1.2 µL of 36–37% concentrated hydrochloric acid, and 50 µL of isobutanol were added. After the coculture for 12 h, the reduction of cell viability was determined at 570 nm using a

microplate reader (Bio-Rad, USA). The cell proliferation inhibition rate was calculated according to the following formula: Inhibition rate (%) = (A<sub>control</sub> – A<sub>sample</sub>)/A<sub>control</sub> × 100 [12].

### 3. Result and discussion

Compound **1** was obtained as white amorphous powder. The molecular formula of **1** was deduced to be C<sub>36</sub>H<sub>60</sub>O<sub>9</sub> by positive



**Fig. 1.** The structure of the Compounds **1**–**28**. Glc, β-D-glucopyranosyl; Xyl, β-D-xylopyranosyl; Ara, α-L-arabinofuranosyl; Rha, α-L-rhamnopyranose.

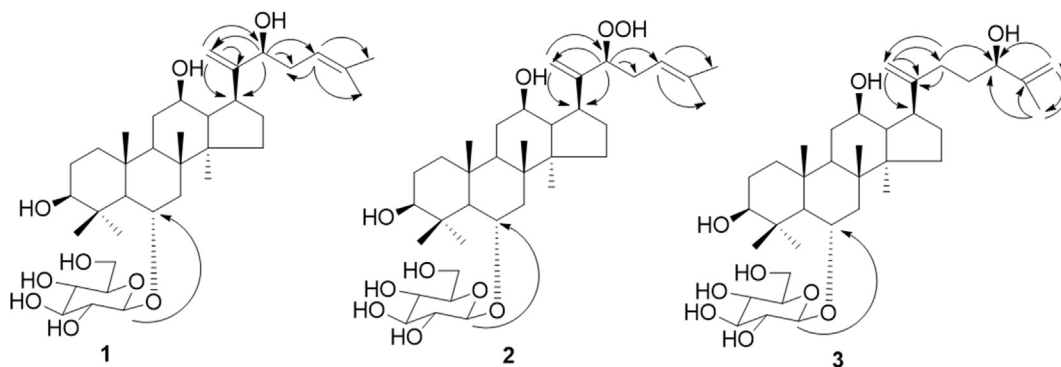


Fig. 2. The important HMBC correlations of Compounds 1–3. HMBC, heteronuclear multiplebond correlation.

mass spectrometry (HR-ESI-MS) data at  $m/z$  659.4130  $[M+Na]^+$  (calculated for  $C_{36}H_{60}O_9Na$ , 659.4135). The  $^{13}C$  NMR (Table 1) showed 36 carbon signals. The distortionless enhancement by polarization transfer (DEPT) spectrum exhibited 7 methyls, 9 methylenes, 14 methines, and 6 quaternary carbons signals. Four olefinic carbon signals at  $\delta_C$  160.2, 132.8, 122.4, and 111.2 ppm suggested two double bonds in the molecule. The  $^1H$  NMR showed signals of seven methyl groups at  $\delta_H$  0.88 (3H, s), 1.04 (3H, s), 1.25 (3H, s), 1.63 (3H, s), 1.65 (3H, s), 1.71 (3H, s), and 2.10 (3H, s); four oxygen substituted protons at  $\delta_H$  3.55 (1H, m), 4.29 (1H, m), 4.46 (1H, m), and 4.52 (1H, m); and one anomeric proton at  $\delta_H$  5.07 (1H, d,  $J = 8.0$ ). The  $^1H$  and  $^{13}C$  signals were fully assigned according to heteronuclear signal quantum correlation (HSQC) spectra (Table 1). Methylene carbon signal at  $\delta_C$  111.2 ppm showed correlation spots

with protons at  $\delta_H$  5.18 (H-21) and 4.52 (H-21) ppm in HSQC spectrum. These two proton signals showed connections with carbon signals at  $\delta_C$  160.2 (C-20), 77.0 (C-22), and 40.2 (C-17) ppm in heteronuclear multiple bond correlation (HMBC) spectrum, and  $\delta_H$  4.52 (H-22) showed connection with  $\delta_C$  111.2 (C-20), 122.4 (C-24), and 40.2 (C-17) (Fig. 2). Thus, the signals at  $\delta_C$  160.2, 111.2, and 77.0 ppm were assigned to be the signals of C-20, C-21, and C-22, respectively.  $\delta_H$  5.42 (H-24) showed connections with  $\delta_C$  132.8 (C-25), 26.4 (C-26), and 18.6 (C-27). Therefore, it was concluded that the two double bonds were at  $\Delta$  20(21) and  $\Delta$  24(25). The signals of Compound 1 were quiet similar to those of ginsenoside Rk<sub>3</sub>, except for the chemical shift of C-20, C-22, and C-23, which were at  $\delta_C$  160.2, 77.0, and 36.3 of Compound 1 but were at  $\delta_C$  155.4, 33.7, and 27.0 of ginsenoside Rk<sub>3</sub>, respectively [13]. The shift to

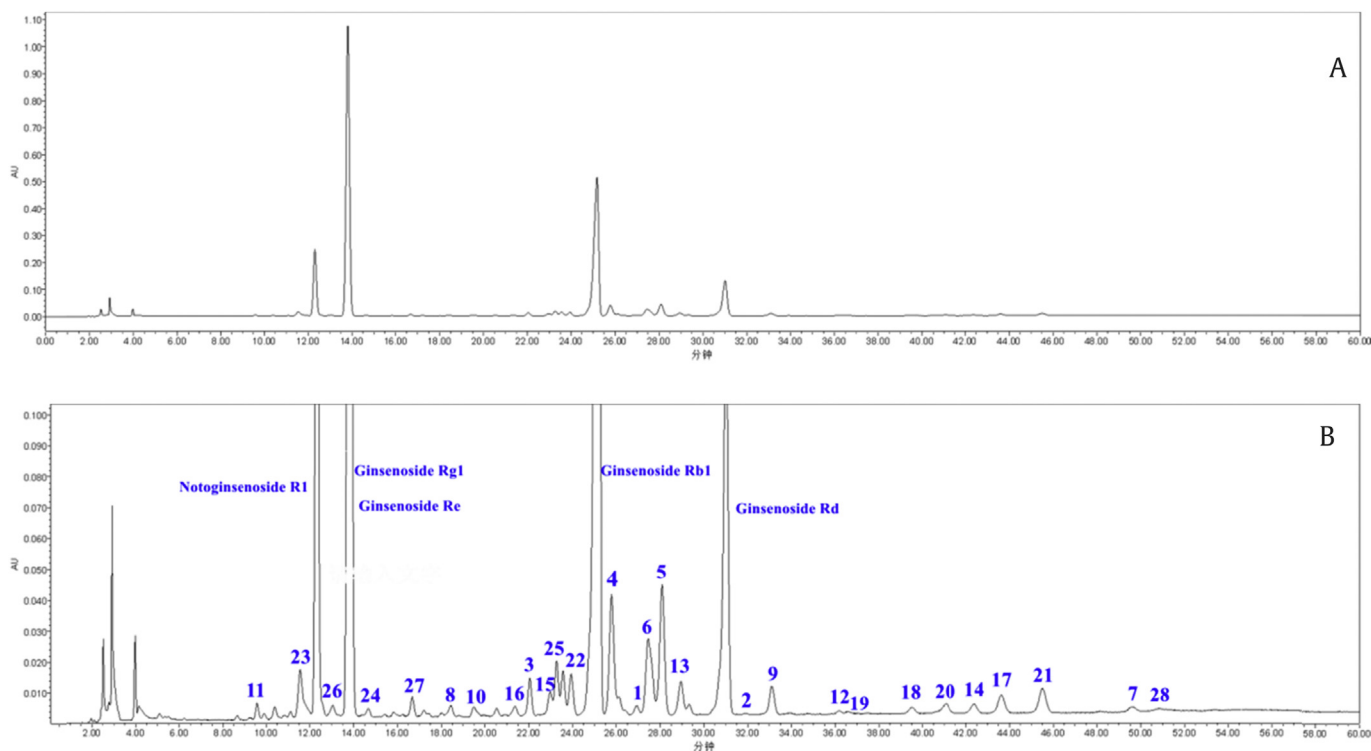


Fig. 3. (A) The HPLC fingerprint of PNS. (B) The magnified HPLC fingerprint of PNS with compounds isolated from PNS marked. (1, notoginsenoside Ab<sub>1</sub>; 2, notoginsenoside Ab<sub>2</sub>; 3, notoginsenoside Ab<sub>3</sub>; 4, notoginsenoside R<sub>2</sub>; 5, 20(S)-ginsenoside Rh<sub>1</sub>; 6, 20(S)-ginsenoside Rg<sub>2</sub>; 7, ginsenoside Rg<sub>3</sub>; 8, pseudoginsenoside RT<sub>3</sub>; 9, gypenoside-XVII; 10, vina-ginsenoside-R<sub>4</sub>; 11, notoginsenoside R<sub>3</sub>; 12, quinquenoside L<sub>14</sub>; 13, 20(R)-ginsenoside Rh<sub>1</sub>; 14, ginsenoside F<sub>2</sub>; 15, ginsenoside U; 16, ginsenoside Rg<sub>6</sub>; 17, ginsenoside Rk<sub>3</sub>; 18, notoginsenoside T<sub>5</sub>; 19, ginsenoside Rk<sub>1</sub>; 20, ginsenoside Rg<sub>4</sub>; 21, ginsenoside-Rh<sub>4</sub>; 22, ginsenoside-Rh<sub>5</sub>; 23, 20(S)-ginsenoside-ST<sub>2</sub>; 24, floralquinquenoside A; 25, ginsenoside-Rh<sub>14</sub>; 26, notopanaxoside A; 27, notopanaxoside G; 28, sanchirrhinoside D). All the Compounds 1–28 isolated from PNS and the sample of PNS were analysis by HPLC at the same condition. In addition, 1–28 were marked on the HPLC spectrum of PNS.

downfield of C-20 (+4.8 ppm), C-22 (+43.3 ppm), and C-23 (+9.3 ppm) indicated that C-22 of **1** was linked to hydroxyl. In addition, the  $\beta$  configuration was prompted by the large coupling constant observed for the anomeric proton  $\delta_H$  5.07 (1H, d,  $J = 8.0$ ). The absolute configurations of sugar was elucidated as D-glucose through acid hydrolysis and HPLC analysis. Moreover, the linkages between H-1" ( $\delta_H$  5.07) and C-6 ( $\delta_C$  80.5) were determined by HMBC correlations. The configuration of OH at C-6 was  $\alpha$  based on the correlations between H-6 with H-18 $\beta$ , 19 $\beta$  in rotating frame overhauser effect (ROESY) spectrum. In addition, H-17 was deduced as  $\alpha$ -forms by correlations between H-17 and Me-30 in the ROESY spectrum. The configuration of C-22 was identified by the comparison of the calculated and experimental chemical shifts of  $^{13}\text{C}$ . The calculated chemical shifts of C-22 about (22R)-**1** and (22S)-**1** were  $\delta_C$  72.0 and  $\delta_C$  77.8, whereas the experimental result was  $\delta_C$  77.0. Therefore, the configuration of C-22 was identified as S. On the basis of the aforementioned analyses, Compound **1** could be deduced to be 3 $\beta$ ,6 $\alpha$ ,12 $\beta$ ,22S-tetrahydroxy-dammar-20(21),24-diene-6-O- $\beta$ -D-glucopyranoside and named as notoginsenoside Ab<sub>1</sub>.

Compound **2** was isolated as white amorphous powder. The HR-ESI-MS spectrum showed  $[\text{M}+\text{Na}]^+$  at  $m/z$  675.4077 (calcd. for  $\text{C}_{36}\text{H}_{60}\text{O}_{10}\text{Na}$  675.4084). It was proposed to possess a hydroperoxyl group due to positive response to N,N-dimethyl-p-phenylenediammonium dichloride reagent [14]. The  $^{13}\text{C}$  NMR (Table 1) showed 36 carbon signals. The DEPT spectrum exhibited 7 methyls, 9 methylenes, 14 methines, and 6 quaternary carbons signals. The  $^1\text{H}$  NMR showed signals of seven methyl groups at  $\delta_H$  0.82 (3H, s), 1.03 (3H, s), 1.28 (3H, s), 1.59 (3H, s), 1.60 (3H, s), 1.65 (3H, s), and 2.08 (3H, s). The signals of Compound **2** were quiet similar to those of Compound **1**, except for the chemical shift of C-20 (-3.9 ppm), C-22 (+14.3 ppm), and C-23 (-5 ppm), indicating the hydroperoxyl substitution of C-22. C-22 ( $\delta_H$  4.71,  $\delta_C$  91.3) bearing hydroperoxyl group was also supported by chemical shift comparison with those of related hydroperoxylated triterpenes [15–17]. In addition, the long-range correlations between H-21 and C-17, 22, H-22 and C-17, 21, 23, 24, H-24 and C-26, 27, in the HMBC spectrum further confirmed the structure (Fig. 2). The monosaccharide was determined to be D-glucose by HPLC analysis of chiral derivatives of sugars in the acid hydrolyzate. The  $\beta$ -anomeric configuration for the glucosyl unit was established from the coupling constant for the anomeric proton  $\delta_H$  5.04 (d,  $J = 7.8$  Hz). Compared with the calculated chemical shifts of C-22 about (22R)-**2** ( $\delta_C$  82.8) and (22S)-**2** ( $\delta_C$  88.2), the configuration of C-22 in Compound **2** was identified as S. Compound **2** could be deduced to be 22S-hydroperoxyl-3 $\beta$ ,6 $\alpha$ ,12 $\beta$ -trihydroxy-dammar-20(21),24-diene-6-O- $\beta$ -D-glucopyranoside and named as notoginsenoside Ab<sub>2</sub>.

Compound **3**, was obtained as white amorphous powder. The molecular formula was assigned as  $\text{C}_{36}\text{H}_{60}\text{O}_9$  by the HR-ESI-MS spectrum at  $m/z$  659.4132  $[\text{M}+\text{Na}]^+$  (calcd. For.  $\text{C}_{36}\text{H}_{60}\text{O}_9\text{Na}$  659.4135). The  $^{13}\text{C}$  NMR (Table 1) showed 36 carbon signals, and the  $^1\text{H}$  NMR showed six methyl groups signals. The signals were similar to those of ginsenoside-Rh<sub>5</sub> [18], except for the position of the double bond. The corrections between H-21 ( $\delta_H$  4.97, 5.15) and C-20, 17, 22 in HMBC spectrum (Fig. 2) demonstrated there is a double bond in C-20, 21. What is more, the chemical shift of C-20, C-21, and C-22 were at  $\delta_C$  156.5, 108.4, and 33.1 in Compound **3** but that of ginsenoside-Rh<sub>5</sub> were at  $\delta_C$  142.5, 13.2, and 122.2 in ginsenoside-Rh<sub>5</sub>, further indicating that the double bond was in C-20, 21 instead of in C-20, 22. There was an additional  $\beta$ -D-glucose in Compound **3** based on the results of acid hydrolysis analysis and the coupling constant for the anomeric proton. The correlation of H-6/H-18 $\beta$ , H-6/H-19 $\beta$  in ROESY spectrum revealed the  $\alpha$ -orientation of OH at C-6. The correlation between H-17 and Me-30 in ROESY spectrum indicated the  $\alpha$ -forms of H-17. The configuration of C-24 was

**Table 2**IC<sub>50</sub> values of the new Compounds (**1–3**) against HepG2, NCI-H460, and MCF-7 cells.

Comp.	IC <sub>50</sub> (μg/mL) <sup>1)</sup>		
	HepG2	NCI-H460	MCF-7
<b>1</b>	>100	>100	>100
<b>2</b>	4.49	8.06	7.38
<b>3</b>	>100	>100	>100
Cisplatin <sup>2)</sup>	0.97	1.34	2.66

Cell inhibition activity was determined by the MTT assay. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

<sup>1)</sup> IC<sub>50</sub> is the concentration of the compound inhibiting 50% of cell proliferation.

<sup>2)</sup> Cisplatin was used as positive control.

identified as R by the comparison of the calculated chemical shifts of (24R)-**3** ( $\delta_C$  72.3) and (24S)-**3** ( $\delta_C$  67.9) with experimental chemical shift of C-24 ( $\delta_C$  75.5). Compound **3** could be deduced to be 3 $\beta$ ,6 $\alpha$ ,12 $\beta$ ,24R-tetrahydroxy-dammar-20(21),25-diene-6-O- $\beta$ -D-glucopyranoside and named as notoginsenoside Ab<sub>3</sub>.

The known compounds were identified as notoginsenoside R<sub>2</sub> (**4**) [19], 20(S)-ginsenoside Rh<sub>1</sub> (**5**) [20], 20(S)-ginsenoside Rg<sub>2</sub> (**6**) [21], ginsenoside Rg<sub>3</sub> (**7**) [22], pseudoginsenoside RT<sub>3</sub> (**8**) [23], gypenoside-XVII (**9**) [24], vina-ginsenoside-R<sub>4</sub> (**10**) [25], notoginsenoside R<sub>3</sub> (**11**) [26], quinquenoside L14 (**12**) [27], 20(R)-ginsenoside Rh<sub>1</sub> (**13**) [28], ginsenoside F<sub>2</sub> (**14**) [29], ginsenoside U (**15**) [30], ginsenoside Rg<sub>6</sub> (**16**) [31], ginsenoside Rk<sub>3</sub> (**17**) [13], notoginsenoside T<sub>5</sub> (**18**) [32], ginsenoside Rk<sub>1</sub> (**19**) [33], ginsenoside Rg<sub>4</sub> (**20**) [34], ginsenoside-Rh<sub>4</sub> (**21**) [35], ginsenoside-Rh<sub>5</sub> (**22**) [18], 20(S)-ginsenoside-ST<sub>2</sub> (**23**) [36], floralquinquenoside A (**24**) [37], ginsenoside-Rh<sub>14</sub> (**25**) [38], notopanaxoside A (**26**) [39], notopanaxoside G (**27**) [40], sanchirinoside D (**28**) [41] by comparison of their data with the literature (Fig. 1).

As typical multiple-constituent and multiple-action traditional Chinese medicine, the quality is difficult to control, so a systematic study about the complicated ingredients of PNS is much essential. This is the first study to explore the rare content of PNS comprehensively. In the study, we isolated 3 new and 25 known dammarane-type triterpenes from PNS. In addition, a method of HPLC-UV was successfully applied to the determination of rare ginsenosides in PNS. As the Fig. 3 showed, we also marked the compounds isolated in the fingerprint. The results of this study are significant for quality improvement and evaluation of clinical medicines made of PNS.

The new Compounds (**1–3**) were evaluated for their cytotoxic activity against HepG2, NCI-H460, and MCF-7 cancer cell lines by using the modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method, with cisplatin as the positive control (Table 2). New Compound **2** showed significant activity against HepG2, NCI-H460, and MCF-7 with IC<sub>50</sub> 4.49, 8.06, and 7.38 μg/mL, respectively.

## Conflicts of interest

All authors declare no conflicts of interest.

## Acknowledgments

Authors are glad to acknowledge the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences for the measurements of NMR, MS, HREIMS, and IR spectroscopic data. This work was supported by a grant from China national TCM standardization project (ZYBZH-Y-YN-44), Science and Technology Innovation Platform Construction Project (2015DC027), and Chemical Synthesis Platform Project (2016DC049) from Yunnan Provincial Science and Technology Department in China.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jgr.2018.12.001>.

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