New dammarane-type triterpenoids from the leaves of *Panax notoginseng* and their protein tyrosine phosphatase 1B inhibitory activity

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**A B S T R A C T**

Background: *Panax notoginseng* has been used as a general tonic agent to invigorate human body for millennia in China and continued to be used until present.

Methods: Some chromatographic methods were performed to isolate pure triterpenoids, and their structures were determined by nuclear magnetic resonance (NMR) experiments. Anti-diabetes activities of isolated compounds were evaluated through their inhibitory activity of protein tyrosine phosphatase 1B (PTP1B) enzyme.

Results and Conclusion: Three new dammarane-type triterpenoids, notoginsenoside-LX (1), notoginsenoside-LY (2), and notoginsenoside-FZ (3) together with eighteen known compounds were isolated from the *Panax notoginseng* leaves. The structure-activity relationship of the compounds with dammarane-type triterpenoids and their PTP1B inhibitory activity were also reported. Results showed that compounds 2, 15, 20, and 21 can significantly inhibit the enzyme activity of PTP1B in a dose-dependent manner, with inhibitory concentration 50 (IC50) values of 29.08 μM, 21.27 μM, 28.12 μM, and 26.59 μM, respectively. The results suggested that *Panax notoginseng* leaves might have potential as a new therapeutic agent for the treatment of diabetes.

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1. Introduction

*Panax notoginseng* (Chinese ginseng) or “sanqi” is a functional food in China [1]. Based on the United States (US) Dietary Supplement Health and Education Act (DSHEA) of 1994, notoginseng tea or capsules are being sold as over-the-counter dietary supplements in the US health food market [2]. *P. notoginseng* has been used for many years because of its beneficial anti-inflammatory and blood circulation properties [3,4]. *P. notoginseng* also possesses several interesting pharmacological activities, such as anti-aging, anti-tumor, immunostimulating, and radioresistance activities [5–8]. *P. notoginseng* belongs to the same genus as Korean ginseng (*Panax ginseng* Meyer) and American ginseng (*Panax quinquefolius* L.), and their main components are similar.

Dammarane triterpene saponins are the major bioactive ingredients of *P. notoginseng*. To date, more than 60 dammarane-type triterpenoids have been obtained from *P. notoginseng* [9]. The main constituents of these dammarane-type triterpenoids are ginsenosides that contain an aglycone with a dammarane skeleton. In continuing the search for the minor bioactive constituents from *P. notoginseng*, the leaves of this plant were chemically investigated.

Protein tyrosine phosphatase 1B (PTP1B) is a major non-transmembrane phosphotyrosine phosphatase in classical insulin-targeted tissues. PTP1B overexpression can inhibit the increased expression of insulin in insulin-resistant states [10]. A previous report suggested that PTP1B can be used to treat obesity and type-2 diabetes mellitus [11].

In the present study, 21 dammarane-type triterpenes (3 new and 18 known ones) were isolated from the leaves of *P. notoginseng*. Besides the isolation and structure elucidation of the new compounds, the inhibitory effects of all compounds on PTP1B activity were evaluated. The current data suggest that some compounds can be developed as antidiabetic agents in future translational studies.

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2. Materials and methods

2.1. General experimental procedure

Column chromatography (cc): silica gel (SiO₂; 300–400 mesh, Qingdao Marine Chemical Group Co., Qingdao, China); macroporous resin D 101 (Tianjin Chemical Co., Tianjin, China); RP C18 silica gel (300–400 mesh, Agela Technologies Co., Tianjin, China); Sephadex LH-20 (Pharmacia Co., Peapack, USA). Optical rotations were measured on a Perkin-Elmer 241MC polarimeter (Perkin-Elmer Co., Waltham, USA) using methanol (Concord Technology Co., Tianjin, China) as the solvent. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker ARX-600 (Bruker Co., Karlsruhe, Germany) (1H, 600 MHz; 13C, 150 MHz) spectrometer in CD3D2N with tetramethylsilane as internal standard. Infrared (IR) spectra on a Bruker Inter-Frame Space (IFS)-55 infrared spectrophotometer (Bruker Co., Karlsruhe, Germany) were recorded in Potassium bromide (KBr) disks. High-resolution electrospray ionization mass spectra (HRESIMS) were recorded on an Agilent 1100 LC-MSD (Mass Spectrometer Detector) TOF (time-of-flight) system (Agilent Technologies, Inc., Santa Clara, USA) [Ionization mode, positive; nebulizing gas (N2) pressure, 35 psi; drying gas (N2) flow, 12 L/min; temp, 325°C; capillary voltage, 3,000 V; fragmentor voltage, 225 V]. Gas chromatography (GC) was performed on the Agilent technologies 6890N apparatus (Agilent Technologies, Inc., Santa Clara, USA). Reversed-phase preparative HPLC was carried out on an octadecyl silica column [YMC-Pack ODS A (YMC Co., Kyoto, Japan) (250 mm × 10 mm, 5 μm)] at 25°C at a flow rate of 3.0 mL/min with the eluent MeOH/H2O 66:34 (HPLC system I), 70:30 (HPLC system II), 75:25 (HPLC system III), 80:20 (HPLC system IV), 82:18 (HPLC system V), or 9:1 (HPLC system VI). Ultraviolet (UV) spectrophotometric detection was carried out at 203 nm.

2.2. Plant material

P. notoginseng leaves were from the Yunnan province of the People's Republic of China and identified by Professor Jincai Lu of Shenyang Pharmaceutical University.

2.3. Extraction and isolation

Air-dried P. notoginseng leaves (35 kg) were extracted with 70% ethanol (2 × 350 L) and then evaporated under vacuum at 30°C. Ethanol extracts (1.6 kg) were applied on a macroporous resin column (10.5 kg) preconditioned with distilled water. Elution began with water to remove impurities and then with 70% ethanol (100 L) to isolate the saponin fraction, which was dried with a spray dryer to yield the total saponins (1 kg).

The total saponin (1 kg) was fractionated by silica gel column (300 mm × 1,600 mm, 30 kg) using a gradient of CH2Cl2/CH3OH (7:1 350 L–4:1 350 L–3:1 350 L) and CH3OH (300 L) to obtain 10 fractions, A–J. Fraction A (18 g) was subjected to chromatography on silica gel (70 mm × 800 mm, 400 g) and then eluted with ligarine and acetone in increasing polarity to yield 10 fractions, A1–A10, compounds 15 (20 mg), 16 (10 mg), and 17 (20 mg). Fractions B (20 g), C (25 g), D (23 g), and E (13 g) were subjected to chromatography on silica gel (70 mm × 800 mm, 500 g) and then eluted with methanol and dichloromethane in increasing polarity to yield seven fractions, B1–B7, eight fractions, C1–C8, 10 fractions, D1–D10, and five fractions, E1–E5. Fraction C4 (100 mg) was passed over a Sephadex LH-20 column (30 mm × 800 mm, 80 g) and then eluted with MeOH (500 mL) to obtain compound 20 (15 mg). Fraction E3 (1 g) was subjected to chromatography on ODS (30 mm × 150 mm, 50 g) and then eluted successively with solvents of decreasing polarity (MeOH/H2O, 4:6 600 mL–6:4 600 mL–7:3 600 mL–9:1 600 mL–1:0 600 mL) to yield seven fractions, E3–1–E3–7. Compound 21 (8 mg) was obtained as granulated crystal from E3–6. Fractions F (18 g), G (15 g), H (20 g), and I (20 g) were subjected to chromatography on ODS (50 mm × 250 mm, 250 g) and then eluted successively with solvents of decreasing polarity (MeOH/H2O, 3:7 3 L–5:5 3 L–7:3 3 L–9:1 3 L–1:0 3 L) to yield 11 fractions, F1–F11, eight fractions, G1–G8, seven fractions, H1–H7, and eight fractions, I1–I8. Compound 1 (10 mg) was obtained as granulated crystal from F–5. Isolation of the following 15 compounds was performed by preparative HPLC: compounds 2 (18 mg; tR 75.0 min), 3 (12 mg; tR 38.9 min), 13 (20 mg; tR 46.8 min), and 14 (60 mg; tR 53.5 min) were isolated from fraction D3 (500 mg) by HPLC system I; compound 18 (4 mg; tR 41.8 min) was isolated from fraction A2 (60 mg) by HPLC system II; compounds 3 (15 mg; tR 70.3 min), 4 (15 mg; tR 45.8 min), 5 (14 mg; tR 58.9 min), and 6 (14 mg; tR 65.9 min) were isolated from fraction I4 (200 mg) by HPLC system II; compounds 7 (40 mg; tR 33.5 min) and 8 (60 mg; tR 49.6 min) were isolated from fraction H5 (500 mg) by HPLC system III; compounds 9 (8 mg; tR 17.5 min), 10 (70 mg; tR 26.5 min), and 11 (65 mg; tR 33.7 min) were isolated from fraction G6 (1 g) by HPLC system IV; compound 19 (6 mg; tR 122.9 min) was isolated from fraction B2 (40 mg) by HPLC system V.

2.4. Notoginsenoside-LX (1)

(20S,23R)-3β-hydroxy-12β,23-epoxy-dammar-24-ene 3-O-β-D-glucopyranoside-20-O-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside (notoginsenoside-LX): white amorphous powder; [α]D 20 = −20.8 (c = 0.30, MeOH); IR νmax 3425, 2930, 1631, 1450, 1384, 1079, 620 cm⁻¹; Libermann-Burchard and Molish reactions were positive; 1H and 13C NMR: see Table 1; HRESIMS m/z 937.5097 [M+Na]+ (calculated for C47H78O17Na, 937.5137).

2.5. Notoginsenoside-LY (2)

(20S,23R)-3β-hydroxy-12β,23-epoxy-dammar-24-ene 20-O-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside (notoginsenoside-LY): white granulated crystal; [α]D 20 = −11.4 (c = 0.45, MeOH); IR νmax 3419, 2942, 1631, 1452, 1384, 1043, 621 cm⁻¹; Libermann-Burchard and Molish reactions were positive; 1H and 13C NMR: see Table 1; HRESIMS m/z 775.4577 (calculated for C43H62O12Na, 775.4608).

2.6. Notoginsenoside-FZ (3)

20(S)-protopanaxadiol 3-O-β-D-xylpyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (notoginsenoside-FZ): white granulated crystal; [α]D 20 = −12.2 (c = 0.44, MeOH); IR νmax 3426, 2924, 1650, 1458, 1384, 668 cm⁻¹; Libermann-Burchard and Molish reactions were positive; 1H and 13C NMR: see Table 1; HRESIMS m/z 1233.6235 (calculated for C58H86O32Na, 1233.6244).

2.7. Acid hydrolysis of 1, 2, and 3

Solutions of compounds 1, 2, and 3 (5 mg each) in 2M HCl/MeOH (4:1) (8 mL) were stirred at 90°C for 2 hours. After cooling, each
Table 1

| Position | 1       | 2       | 3       |
|----------|---------|---------|---------|
|          | δc      | δH      | δc      | δH      | δc      | δH      |
| 1        | 39.3    | 0.95 m  | 39.5    | 0.96 m  | 39.1    | 0.73 m  |
| 2        | 26.8    | 1.61 m  | 28.0    | 1.31 m  | 26.7    | 1.34 m  |
| 3        | 88.6    | 3.36 dd | 77.8    | 3.40 dd | 88.7    | 3.28 dd |
|          | (120, 4.8) |         | (108, 5.4) |         | (114, 4.2) |         |
| 4        | 39.6    |         | 39.4    |         |         |         |
| 6        | 56.2    | 0.68 m  | 56.2    | 0.78 m  | 56.2    | 0.66 m  |
| 12       | 75.4    | 4.03 m  | 82.9    | 4.10 m  | 76.4    | 4.15 m  |
| 14       | 32.4    | 1.13 m  | 32.4    | 1.13 m  | 30.7    | 0.97 m  |
| 16       | 25.5    | 2.08 m  | 25.4    | 2.12 m  | 26.5    | 1.80 m  |
| 17       | 46.4    | 3.16 ddd| 46.4    | 3.16 ddd| 51.5    | 2.57 m  |
| 18       | 15.4    | 0.80 s  | 15.4    | 0.87 s  | 15.9    | 0.78 s  |
| 19       | 16.4    | 0.92 s  | 16.4    | 0.95 s  | 16.2    | 0.93 s  |
| 20       | 81.9    |         | 81.9    |         | 83.4    |         |
| 21       | 24.8    | 1.48 s  | 24.5    | 1.48 s  | 22.2    | 1.63 s  |
| 22       | 51.9    | 2.24 dd | 51.9    | 2.24 dd | 36.1    | 1.82 m  |
|          | (160, 9.6) |         | (160, 9.6) |         |         |         |
| 23       | 72.6    | 4.82 br d| 72.6    | 4.83 br d| 72.3    | 2.38 m  |
|          | (174, 7.4) |         | (174, 7.4) |         |         |         |
| 24       | 129.1   | 5.52 d (7.8) | 129.1 | 5.52 d (7.8) | 125.8  | 5.31 m  |
| 25       | 131.2   |         | 131.1   |         | 131.0   |         |
| 26       | 25.6    | 1.82 s  | 25.6    | 1.81 s  | 25.7    | 1.61 s  |
| 27       | 18.9    | 1.65 s  | 18.8    | 1.65 s  | 17.8    | 1.64 s  |
| 28       | 28.0    | 1.29 s  | 28.5    | 1.20 s  | 28.0    | 1.26 s  |
| 29       | 16.7    | 0.99 s  | 16.2    | 1.02 s  | 16.6    | 1.09 s  |
| 30       | 16.9    | 1.15 s  | 16.9    | 1.10 s  | 17.3    | 0.94 s  |

3-O-sugar

Glc

|            | G1      | G2      | G3      | G4      | G5      | G6      |
|------------|---------|---------|---------|---------|---------|---------|
|            | 106.0   | 75.7    | 78.7    | 72.2    | 78.3    | 63.0    |
|            | 4.94 d (7.8) | 4.03 m  | 4.22 m  | 4.03 m  | 4.03 m  | 4.62 dd |
|            | (120, 2.4) |         |         | (114, 5.4) |         |         |
| 4.41 dd    |         |         |         | 4.57 br d|         |         |
| 4.57 br d  |         |         |         | (120)    |         |         |

Glcα

|            | G1'     | G2'     | G3'     | G4'     | G5'     | G6'     |
|------------|---------|---------|---------|---------|---------|---------|
|            | 103.1   | 84.3    | 78.2    | 71.6    | 77.9    | 62.6    |
|            | 5.52 d (7.8) | 4.20 m  | 3.95 m  | 4.06 m  | 2.92 m  | 4.36 m  |
|            | (174, 7.8) |         |         |         |         |         |

Xyl

|            | X1      | X2      | X3      | X4      | X5      |
|------------|---------|---------|---------|---------|---------|
|            | 106.3   | 75.9    | 79.2    | 70.0    | 67.3    |
|            | 5.43 d (6.6) | 4.46 m  | 4.18 m  | 4.15 m  | 3.68 m  |
|            | (114, 5.4) |         |         |         |         |         |
| 4.47 m     |         |         |         |         |         |         |

2-O-sugars

Glcα

|            | G1'     | G2'     | G3'     | G4'     | G5'     | G6'     |
|------------|---------|---------|---------|---------|---------|---------|
|            | 99.2    | 75.9    | 79.2    | 70.0    | 67.3    | 4.30 m  |

Table 1 (continued)

| Position | 1       | 2       | 3       |
|----------|---------|---------|---------|
|          | δc      | δH      | δc      | δH      | δc      | δH      |
| 1        | 75.2    | 3.95 dd | 75.1    | 3.95 dd | 74.8    | 3.93 m  |
| 2        | 78.8    | 4.26 m  | 78.7    | 4.20 m  | 78.6    | 4.35 m  |
| 3        | 71.8    | 4.22 m  | 72.1    | 4.21 m  | 71.0    | 3.96 m  |
| 4        | 76.5    | 4.12 m  | 76.4    | 4.11 m  | 76.7    | 4.04 m  |
| 5        | 69.0    | 4.80 m  | 68.9    | 4.80 m  | 69.1    | 4.25 m  |
| 6        | 4.10 m  |         | 4.10 m  |         | 4.70 m  |         |

HMBC, heteronuclear multiple bond correlation; HSQC, heteronuclear single quantum correlation; NMR, nuclear magnetic resonance. m, multiplet; dd, double doublet; ddd, double double doublet; s, singlet; br d, broad doublet; br dd, broad double doublet. 1) The assignments were based on HSQC and HMBC experiments.

reaction mixture was diluted to 30 mL with water and then extracted with CH2Cl2 (30 mL × 3). The aqueous layer was neutralized with 1M KOH. After concentration, the residue was examined by thin layer chromatography (TLC, n-BuOH/H2O/HOAc 3:2:1) and compared with authentic samples [12]. The retention factor (Rf) values of glucose, arabinose, and xylose were 0.38, 0.43, and 0.51, respectively.

2.8. Determination of sugar components

Monosaccharide subunits were obtained as described above. The residue was dissolved in pyridine (0.5 mL) and then added to trimethylsilylrsosilane (0.2 mL) and hexamethyldisilazane (0.5 mL). The mixture was stirred at 20°C for 15 minutes. The mixture was then extracted with CH2Cl2 (2 mL) following the addition of H2O (2 mL). The CH2Cl2 layer was examined by GC [12].

2.9. Inhibitory activity against PTP1B assay

The assay buffer (pH 7.4), consisting of 1 mM ethylene diamine tetra acetie acid (EDTA), 50 mM 3,3-dimethyl glutarate, 5 mM glutathione, and 0.5% fetal calf serum (FCS) (not heat inactivated) was adjusted to an ionic strength of 0.15M by the addition of NaCl [13]. Compounds (final concentration ranging from 0 µM to 200 µM) were added to the assay buffers containing PTP1B. The reaction mixtures were allowed to stand at 37°C for 5 minutes following the addition of the compounds. The reaction was started by the addition of p-nitrophenyl phosphate and incubated for another 30 min, and followed by the addition of 5 µL 0.5M NaOH solution to terminate the reaction. The absorbance at 405 nm was recorded using a microplate absorbance reader to test the enzyme activity.

3. Results and discussion

3.1. Structure elucidation of compounds 1–3

Compound 1 was obtained as white amorphous powder. The molecular formula of 1 was deduced to be C47H78O17 by positive
The proton NMR signals, eight methyl groups (δ 0.80 (3H, s), 0.92 (3H, s), 0.99 (3H, s), 1.15 (3H, s), 1.29 (3H, s), 1.48 (3H, s), 1.65 (3H, s), 1.82 (3H, s)), three oxygen substituted protons at H-3 (δ 5.36 1H, dd, J = 12, 4.8 Hz), H-12 (δ 3.66, 1H, m), H-23 (δ 4.82, 1H, br dd, J = 17.4, 7.8 Hz), and one olefin proton at H-24 (δ 5.52, 1H, d, J = 7.8 Hz), suggested that 1 was a dammarane-type triterpene. The 1H and 13C signals were fully assigned according to the NOESY spectrum (Table 1). The NMR data for the side-chain moiety of 1 were almost indistinguishable from those of ginsenosides Rb1 [14]. Otherwise, its NMR spectra were closely similar to those of notoginsenoside Rf [15], except the presence of the ether linkage between C-12 and C-23. In the heteronuclear multiple bond correlation (HMBC) spectrum (Fig. 1), the presence of long-range correlations between the proton signal at H-24 (δ 4.94, 1H, d, J = 7.8 Hz) and the carbon resonance signal at C-3 (δ 88.6), H-1Glc (δ 5.11, 1H, d, J = 7.8 Hz) and C-20 (δ 8.19), H-1Ara (δ 6.0 Hz), and C-6Glc (δ 6.90), which indicated that the C-1Glc-C-1Glc-C-20 of the aglycone, and C-6Glc-C-1Ara were linked to C-3, C-20 of the glycopyranose-20-α-D-L-arabinofuranosyl (1→6)-β-D-glucopyranosyl (otoginsenoside-LX).

Compound 2 was obtained as white granulated crystal and assigned the molecular formula C58H98O26 by GC-MS. The standard monosaccharides were subjected to the same reaction and GC analysis under the same conditions. Retention times were consistent. Three anomeric protons were observed at δ 5.49 (1H, d, J = 7.8 Hz), 5.11 (1H, d, J = 7.8 Hz), and 5.69 (1H, d, J = 1.8 Hz). On the basis of the NMR and HMBC data (Table 1) of 2 were closely similar to those of 1. Compared with 1, the shift to upfield of C-3 [−11 parts per million (ppm)] and shift to downfield of C-2 (-2 ppm) indicated that C-3 of 2 was linked to hydroxyl. The NMR data (Table 1) of 2 suggested that the only difference was the presence of one additional glucopyranosyl group in 1, and this was supported by the presence of more units of 162 in the molecular formula of 1 than that of 2. Besides, the NMR spectra of 2 were closely similar to those of ginsenoside M [16], except the presence of the ether linkage between C-12 and C-23. All the proton and carbon signals for compound 2 were assigned using 2D NMR spectra (Fig. 1). Therefore, compound 2 could be established to be (20S,23R)-3β-hydroxy-12β,23-epoxy-dammar-24-ene-3-O-β-D-glucopyranosyl-20-O-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl (notoginsenoside-LX).

Compound 3 was obtained as white amorphous powder. It was determined to have a molecular formula of C38H68O26 based on its [M+Na]+ ion at m/z 775.4577 (calculated for C38H68O26Na, 775.4608) in the HRESIMS. Its IR spectrum also exhibited strong absorption bands at 3419 cm−1, 1637 cm−1, and 1043 cm−1. The NMR data (Table 1) of 3 were closely similar to those of 1, except the presence of the ether linkage between C-12 and C-23. All the proton and carbon signals for compound 3 were assigned using 2D NMR spectra (Fig. 1). Therefore, compound 3 could be established to be (20S,23R)-3β-hydroxy-12β,23-epoxy-dammar-24-ene-3-O-α-L-arabinofuranosyl-1,6)-β-D-glucopyranosyl (notoginsenoside-LY).

Fig. 1. The important HMBC and NOESY correlations of compounds 1–3. HMBC, heteronuclear multiple bond correlation; NOESY, nuclear Overhauser effect spectroscopy.
of the aglycone and the glycosyl moieties linked to C-3 of aglycone were similar to those of notoginsenoside Fa [15], and glycosyl moieties linked to C-20 of aglycone were almost indistinguishable from those of ginsenoside Rb2[17].

The sugar moieties of 3 were determined to be D-glucose (Glc), D-xylose (Xyl), and L-arabinose (Ara) [tR (min): 26.60, 8.86, and 6.23] by GC. The standard monosaccharides were subjected to the same reaction and GC analysis under the same condition. Retention times were consistent. Five anomeric protons were observed at δ 4.93 (1H, d, J = 7.8 Hz), 5.52 (1H, d, J = 7.8 Hz), 5.43 (1H, d, J = 6.6 Hz), 5.14 (1H, d, J = 7.8 Hz), and 5.00 (1H, d, J = 6.0 Hz). On the basis of HSQC, HMBC, NOESY correlations and chemical reactions, three \( \beta \)-D-glucopyranose (δ 4.93, 5.52, and 5.14) (Glc, Glc\(_\text{II}\), and Glc\(_\text{III}\)), one \( \alpha \)-L-arabinopyranosyl (δ 5.00) (Ara), and \( \beta \)-D-xylopyranosy (δ 5.43) (Xyl) were identified. The above evidence suggested that 3 possesses the structure of 20(\( \beta \))-protopanaxadiol 3-O-\( \beta \)-D-xylopyranosyl-(1→2)-\( \beta \)-D-glucopyranosyl-(1→2)-\( \beta \)-D-glucopyranoside-20-O-\( \alpha \)-L-arabinopyranosyl-(1→6)-\( \beta \)-D-glucopyranoside (notoginsenoside-FZ).

### 3.2. Structure identification of known compounds

The known compounds were identified as notoginsenoside-Fa (4) [15], ginsenoside-Rh1 (5) [15], notoginsenoside-Fc (6) [15], vina-ginsenoside-R7 (7) [18], ginsenoside-Rc (8) [17], ginsenoside-Rd (9) [18], notoginsenoside-Fe (10) [15], gypenoside-IX (11) [15], 20(\( \beta \))-ginsenoside-Rh1 (12) [19], 20(\( \beta \))-ginsenoside-Rh1 (13) [19], ginsenoside-F1 (14) [20], 20(\( \beta \))-protopanaxadiol (15) [21], 20(\( \beta \))-protopanaxadiol (16) [21], protopanaxatriol (17) [22], panaxadiol (18) [21], 20(\( \beta \))-ginsenoside-Rh2 (19) [23], 20(\( \beta \))-ginsenoside-Rh2 (20) [23], and 20(\( \beta \))-ginsenoside-Mc (21) [16] by NMR and mass spectrometric analyses and by comparison of obtained values with literature values of the corresponding compounds.

### Table 2

| Compound | IC\(_{50}\) (pM) | IC\(_{50}\) (pM) |
|----------|----------------|----------------|
| 1        | 77.24          | 12             |
| 2        | 59.08          | 13             |
| 3        | >100           | >100           |
| 4        | >100           | 15             |
| 5        | >100           | 16             |
| 6        | >100           | 17             |
| 7        | >100           | >100           |
| 8        | >100           | >100           |
| 9        | >100           | 20             |
| 10       | >100           | 21             |
| 11       | >100           | Na\(_3\)VO\(_4\) 21.04 |

\( \text{IC}_{50} \): inhibitory concentration 50; PTP1B: protein tyrosine phosphatase 1B.

\( ^1 \) Na\(_3\)VO\(_4\) was used as a positive control. The detailed methods for inhibitory activity against PTP1B are described in Materials and methods. Values are means of triplicate experiments.
3.3. PTP1B inhibitory activity

The current data (Table 2) suggest that protopanaxadiol (PPD)-type aglycones are more effective than dammarane triterpenoids having more than three sugars and that the presence of sugar moieties reduces the PTP1B inhibitory activity of the compounds. Compound 15 [20(R)-PPD] was more effective than compound 16 [20(S)-PPD] with inhibitory concentration 50 (IC50) values of 21.27 μM and 57.14 μM, respectively, despite the fact that they differ from each other only by the absolute configuration of chiral carbon of C-20. Compound 20 [20(R)-ginsenoside-Rh2] was also more effective than compound 19 [20(S)-ginsenoside-Rh2]. These results suggest that 20(R)-PPD-type triterpenoids are more effective than 20(S)-PPD-type triterpenoids. The IC50 values of 12, 13, 14, and 17 showed that protopanaxatriol (PPT)-type triterpenoids exhibit no PTP1B inhibitory activity at all.

Ginsenosides possess antidiabetic activity, but their mechanisms are different. For example, ginsenoside Rb1 promotes adipogenesis through the regulation of peroxisome proliferator-activated receptor (PPAR)-γ and microRNA-27b, providing a good illustration to explain the antidiabetic effect of the ginsenoside [24]. The total saponins and ginsenoside Rb1 of ginseng stimulate the secretion of glucagon-like peptide-1 (GLP1) in vivo and in vitro, demonstrating an antidiabetic effect [25]. Ginsenoside Re reduces insulin resistance by activating the PPAR-γ pathway and inhibiting tumor necrosis factor (TNF-α) expression [26]. However, the current study shows that the antidiabetic effects of P. notoginseng may be a result of the inhibitory activity of some ginsenosides against PTP1B.

In the present study, we isolated three new dammarane-type triterpenoids, elucidated as notoginsenoside-LX (1), notoginsenoside-LY (2), and notoginsenoside-F2 (3), along with 18 known triterpenoids, elucidated as notoginsenoside-LX (1), notoginsenoside-RF, and notoginsenoside-FZ (3), along with 18 known triterpenoids, elucidated as notoginsenoside-LX (1), notoginsenoside-RF, and notoginsenoside-FZ (3), along with 18 known triterpenoids, elucidated as notoginsenoside-LX (1), notoginsenoside-RF, and notoginsenoside-FZ (3), along with 18 known triterpenoids, elucidated as notoginsenoside-LX (1), notoginsenoside-RF, and notoginsenoside-FZ (3). The IC50 values of 12, 13, 14, and 17 showed that protopanaxatriol (PPT)-type triterpenoids exhibit no PTP1B inhibitory activity at all.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

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References

[1] Yang Y. Scientific substantiation of functional food health claims in China. J Nutr 2008;138:1199S–2055S.
[2] Sun S, Wang CZ, Tong R, Li X, Fishbein A, Wang Q, He TC, Du W, Yuan CS. Effects of steaming the root of Panax notoginseng on chemical composition and anticancer activities. Food Chem Toxicol 2010;118:307–14.
[3] Kwan CY, Kwan TK. Effects of Panax notoginseng saponins on vascular endothelial cells in vitro. Acta Pharmocol Sin 2000;21:1101–5.
[4] Li SH, Chu Y. Anti-inflammation effects of total saponins of Panax notoginseng. Acta Pharmocol Sin 1999;20:551–4.
[5] Lee JH, Han Y, Ginsenoside Rg1 helps mice resist to disseminated candidiasis by Th1 type differentiation of CD4+ T cell. Int Immunopharmacol 2006;6:1424–30.
[6] Lei J, Li X, Gong XJ, Zheng YN. Integration, synthesis and structures of cytotoxic ginsenoside derivatives. Molecules 2007;12:2140–50.
[7] Tamura T, Gui X, Sakaguchi N, Akashi M. Ginsenoside Rd prevents and reduces rat intestinal epithelial cells from irradiation-induced apoptosis. Food Chem Toxicol 2008;46:3080–9.
[8] Tung NH, Song GV, Minh CV, Kiem PV, Jin LG, Boo HJ, Kang HK, Kim YH. Steamed ginseng-leaf components enhance cytotoxic effects on human leukemia HL-60 cells. Chem Pharm Bull 2010;58:1111–5.
[9] Kim DH. Chemical diversity of Panax ginseng, Panax quinquifolium, and Panax notoginseng. J Ginseng Res 2012;36:1–15.
[10] Zhang J, Shen Q, Du J, Li Y, Liu WY, Yang JJ, Li J, Xiao K. Phenolic compounds from the leaves of Cyclocarya paliurus (Batal.) Iljinskaja and their inhibitory activity against PTP1B. Food Chem 2010;119:1496–501.
[11] Zhang XS, Bi XL, Xiao Y, Cao JQ, Xia XC, Xiao YP, Zhao YQ. Protein tyrosine phosphatase 1B inhibitory effect by dammarane-type triterpenes from hydrolyzate of total Gynostemma pentaphyllum saponins. Bioorg Med Chem Lett 2013;23:297–300.
[12] Li W, Cao J, Tang Y, Zhang L, Xie Q, Shen H, Zhao Y. Cyclic bisdesmosides from Actinostemma lobatum MAXIM (Cucurbitaceae) and their in vitro cytotoxicity. Fitoterapia 2012;83:147–52.
[13] Jain V, Viswanatha GL, Malnar D, Shivaraprasad NH. Isolation of antidiabetic principle from fruit rinds of Panax ginseng. Evid Based Complement Alternat Med 2012;2012:147202.
[14] Li KK, Yang XB, Yang XW, Liu JK, Gong XJ. New triterpenoids from the stems and leaves of Panax ginseng. Fitoterapia 2012;83:1030–5.
[15] Yang TR, Kasai R, Zhuo J, Tanaka O. Dammarane saponins of leaves and seeds of Panax notoginseng. Phytochemistry 1983;22:1473–8.
[16] Han Y, Sun H, Hu X, Zhang H, Jiang B, Spranger MI, Zhao Y. Transformation of bioactive compounds by Pusarium sacchari fungus isolated from the soil-cultivated ginseng. J Agric Food Chem 2007;55:9373–9.
[17] Besso H, Kasai R, Saruwatari Y, F Wu H, Tanaka O. Ginsenoside-Ra1 and ginsenoside-Ra2, new dammarane-saponins of ginseng roots. Chem Pharm Bull 1982;30:2830–5.
[18] Nguyen MD, Kasai R, Ohnita K, Ito A, Nguyen TN, Yamatsuki K, Tanaka O. Saponins from Vietnamese ginseng, Panax vietnensis Ha et Grushv. collected in central Vietnam. II. Chem Pharm Bull 1994;42:115–22.
[19] Teng RW, Li HZ, Chen JC, Wang DZ, He YN, Yang CG. Complete assignment of 1H and 13C NMR data for nine protopanaxatriol glycosides. Magn Reson Chem 2002;40:483–8.
[20] Yahara S, Kasai R, Tanaka O. New dammarane-type saponins of leaves of Panax japonicus C.A. Meyer. J. Chilussetusaponins-LS, -LSa and -L10. Chem Pharm Bull 1977;25:2041–7.
[21] Duc NM, Kasai R, Ohnita K, Ito A, Nham NT, Yamatsuki K, Tanaka O. Saponins from Vietnamese ginseng, Panax vietnensis Ha et Grushv. collected in central Vietnam. I. Chem Pharm Bull 1994;42:634–40.
[22] Cheng YJ, Su SX, Ma QF, Pei YP, Xie H, Yao X. Studies on new minor saponins isolated from leaves of Panax ginseng C. A. Meyer. Acta Pharm Sin 1987;22:685–9.
[23] Wang YJ, Li XG, Zheng YN, Yang XW. Iso-ginsenoside-Rh3, a new triterpenoid saponin from the fruits of Panax ginseng C. A. Meyer. J. Asian Nat Prod Res 2004;6:289–93.
[24] Chan LS, Yue PY, Kok TW, Keung MH, Mak NK, Wong RN. Ginsenoside-Rh1 promotes adipogenesis through regulation of PPARy and microRNA-27b. Horm Metab Res 2012;44:819–24.
[25] Liu C, Zhang M, Hu MY, Guo HF, Li J, Yu YL, Jin S, Wang XT, Liu L, Liu XD. Increased glucagon-like peptide-1 secretion may be involved in antidiabetic effects of ginsenosides. J Endocrinol 2013;217:185–96.
[26] Gao Y, Yang MF, Su YP, Jiang HM, You XJ, Yang YJ, Zhang HL. Ginsenoside Re reduces insulin resistance through activation of PPAR-γ pathway and inhibition of TNF-α production. J Ethnopharmacol 2013;147:509–16.