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Triterpene saponins with *a*-glucosidase and PTP1B inhibitory activities from the leaves of *Aralia elata*

Yan Zhang, Feng-Ying Han, Jie Wu, Shao-Jiang Song*

School of Traditional Chinese Materia Medica, Key Laboratory of Structure-Based Drug Design and Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang, 110016, People's Republic of China

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ABSTRACT

Two new triterpene saponins named congmuyenoside V (**1**) and congmuyenoside VI (**2**), together with eleven known ones (**3-13**) were isolated from the leaves of *Aralia elata*. Structures of compounds **1-13** were determined by comprehensive spectroscopic analyses, principally including 1D, 2D NMR and HRESIMS techniques. In addition, all compounds were evaluated for their inhibitory effects on *α*-glucosidase and protein tyrosine phosphatase 1B (PTP1B). Compounds **1, 7** and **9-13** exhibited stronger *α*-glucosidase inhibitory activities than acarbose (the positive control). Simultaneously compounds **1** and **13** also demonstrated significant inhibitory effects on PTP1B.

1. Introduction

Aralia elata, one species of the genus *Aralia* (*Araliaceae*), was widely distributed in northeastern China and rarely in Korea and Japan (Shikov et al., 2016). It has been used as a traditional Chinese herb to treat diabetes mellitus (Chung et al., 2005), rheumatoid arthritis, neurasthenia, and hepatitis (Lee and Jeong, 2009; Nhiem et al., 2011; Tomatsu et al., 2003). Previous phytochemical investigations of *A. elata* revealed the presence of triterpene saponins, flavones and polysaccharides (Clement and Clement, 2014). As reported, triterpene saponins with different structure types were regarded as the main bioactive constituents (Liu et al., 1995; Stavropoulou et al., 2017), most of which displayed significant biological activities. This made triterpene saponins from *A. elata* an attractive target.

Type 2 diabetes (T2DM) is a major health-threatening disease worldwide, which accounts for about 90 percent of diabetes (Bandorowicz-Pikula, 2017). It has been reported that *α*-glucosidase inhibitors which could inhibit carbohydrates hydrolysis could be used as anti-diabetic drugs to treat T2DM (Bischoff, 1995). Protein-tyrosine phosphatase 1B (PTP1B) is a negative regulator of the insulin signaling pathway, which is also considered as a promising potential therapeutic target for T2DM (Combs, 2010). Triterpene saponins especially oleanane-type saponins have been demonstrated to possess anti-diabetic effects (Wang et al., 2011). Recently, some triterpenoids derived from oleanolic acid exhibit potent PTP1B inhibitory effect in studies for searching new PTP1B inhibitors (Liu et al., 2013). Furthermore, triterpenoid saponins isolated from *Gypsophila paniculata* were found to have potential *α*-glucosidase inhibitiory activity (Yin et al., 2014). These findings might provide some evidences for the relationship of oleanane-type saponins and these two enzymes.

In our continuing research to seek new bioactive constituents from *A. elata*, two new oleanane-type triterpene saponins and eleven known ones were isolated from the leaves of *A. elata.* Their structures were demonstrated by spectroscopic methods, and the inhibitory activities of all compounds against *α*-glucosidase and PTP1B were tested. Given their inhibition of T2DM drug targets, triterpene saponins from *A. elata* might have great potential.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder from CH_3OH, and provided a positive result in the Liebermann-Burchard and Molish reactions. The molecular formula of **1** was determined as $C_{48}H_{78}O_{20}$ with ten degrees of unsaturation, which showed a pseudo molecular ion peak $[M + Na]^+$ at *m/z* 997.4769 (calcd. for 997.4769) by HRESIMS. The IR absorption bands were observed at 3424, 1077 and 1036 cm^{-1}, which were characteristic of glycosidic-type structures. The ^1H NMR spectrum showed characteristic signals of an olean-12-ene skeleton with six tertiary methyl groups at δ_H 0.94 (3H, s, H-24), 0.96 (3H, s, H-25), 1.15 (3H, s, H-26), 1.77 (3H, s, H-27), 0.96 (3H, s, H-29), 1.02 ppm (3H, s, H-30) and a sp^2 proton at δ_H 5.60 (1H, br s, H-12). Two olefinic carbons at δ_C 122.7 (C-12) and 144.4 (C-13) indicated a typical $\triangle^{12}$ pentacyclic triterpene derivative. The ^{13}C NMR spectrum showed signals of six angular methyls at δ_C 13.7, 16.3, 17.6, 24.6, 27.2,

* Corresponding author.
E-mail address: songsj99@163.com (S.-J. Song).

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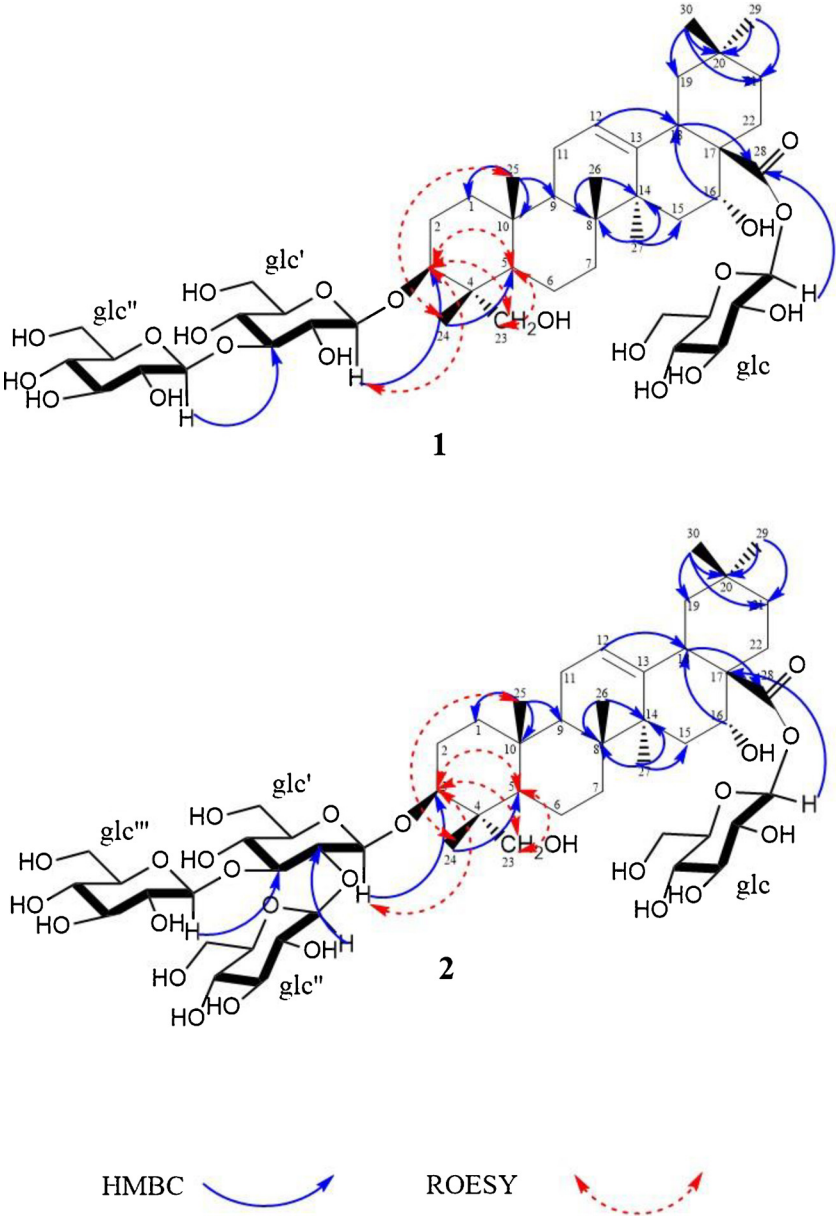

Fig. 1. Key HMBC and ROESY correlations of compounds **1**-**2**.

33.1 on the basis of the HSQC spectrum. Characteristic chemical shifts at δ_C 47.3 (C-5), 74.4 (C-16) and 64.3 (C-23) further revealed that the nucleus of **1** was hederagenin with 16-OH. The chemical shifts of C-3 (δ_C 82.0) and C-16 (δ_C 74.4) indicated an *α*-orientation of H-3 and a *β*-orientation of H-16 (Liang et al., 2011). The orientation of H-3, H-16 and the hydroxymethyl at C-23 were also supported by the ROESY correlations (Fig. 1). The correlation of δ_H 3.67, 4.27 (d, 2H, H-23) with δ_H 1.68 (d, 1H, H-5) and δ_H 0.94 (s, 3H, H-24) with δ_H 0.96 (s, 3H, H-25), which indicated that a hydroxy group was connected to the C-23 carbon. And the correlation of δ_H 4.23 (d, 1H, H-3) with δ_H 1.68 (d, 1H, H-5), which indicated that H-3 was in an *α*-orientation. The multiplicity of H-16 (δ_H 5.27) as a broad singlet and the lack of ROESY correlation with protons of Me-27 confirmed that 16-OH group was *α*-oriented. Due to the glycosylation shift, the downfield shift of C-3 and upfield shifted of C-28 suggested that **1** was a bidesmoside saponin. The ^{1}H NMR spectrum of **1** showed three anomeric proton signals at δ_H 6.31 (d, J = 7.8 Hz, glc-H-1), 5.06 (d, J = 7.8 Hz, glc-H-1′), 5.21 (d, J = 7.8 Hz, glc-H-1″), which correlated in the HSQC spectrum to carbons at δ_C 95.8, 105.4 and 105.9 respectively, revealing the presence of three sugar units. In the HMBC spectrum, the anomeric proton at δ_H 6.31 (glc-H-1) showed correlation with the carbon signal at δ_C 175.9 (C-28), confirming a glucose was linked to C-28 through ether linkage. The glycosylation at C-3 of aglycone was confirmed as a glc (1→3)-glc moiety, through HMBC correlations of δ_H 5.06 (glc-H-1') to δ_C 82.0 (C-3), δ_H 5.21 (glc-H-1″) to δ_C 88.8 (glc-C-3′). According to the relatively large coupling constant (J = 7.8 Hz) and acid hydrolysis of **1**, the configuration of glucose was identified as D-glucose in *β* anomeric orientation of its pyranose form. Based on the above spectral data, the structure of **1** was identified as 3-O-[*β*-D-glucopyranosyl (1→3)-*β*-D-glucopyranosyl]-caulophyllogenin 28-O-*β*-D-glucopyranosyl ester.

Compound **2**, white amorphous powder from CH_3OH, the Molish and Liebermann-Burchard reactions were positive. After acid hydrolysis, compound **2** afforded sugar moieties that were identified as D-glucose based on gas chromatography (GC) analysis of chiral derivatives. And the coupling constants (7.2 or 7.8 Hz) for the anomeric protons in the ^{1}H NMR spectrum suggested *β*-configurations for the glucopyranosyl moieties. The ^{1}H and ^{13}C (Tables 1 and 2) NMR spectroscopic data were closely related to those of **1** except for the sugar

Table 1
C (150 MHz) and ^{1}H (600 MHz) NMR data for aglycone moieties of **1-2** in C_5D_5N [a,b].

Position	**1**		**2**	
	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)
1	38.8	0.98 (m), 1.47 (m)	38.7	0.98 (m), 1.46 (m)
2	26.0	1.91 (m), 2.22 (m)	25.9	1.90 (m), 2.20 (m)
3	82.0	4.23 (d, 9.6)	83.0	4.13 (d, 10.2)
4	43.5	–	43.5	–
5	47.6	1.68 (d, 11.4)	48.0	1.57 (d, 12.0)
6	18.2	1.32 (m), 1.67 (m)	18.2	1.37 (m), 1.68 (m)
7	33.1	1.38 (m), 1.75 (m)	33.1	1.33 (m), 1.69 (m)
8	40.1	–	40.0	–
9	47.3	1.86 (m)	47.2	1.84 (m)
10	36.9	–	36.9	–
11	23.8	1.93 (m), 1.99 (m)	23.8	1.98 (m)
12	122.7	5.60 (br s)	122.6	5.56 (br s)
13	144.4	–	144.4	–
14	42.0	–	42.0	–
15	36.2	1.71 (m), 2.52 (m)	36.1	1.71 (m), 2.52 (m)
16	74.4	5.27 (br s)	74.4	5.25 (br s)
17	49.1	–	49.0	–
18	41.3	3.51 (d, 7.8)	41.2	3.45 (dd, 3.6, 14.4)
19	47.1	1.32 (m), 2.73 (m)	47.1	1.33 (m), 2.75 (m)
20	30.8	–	30.8	–
21	35.9	1.20 (m), 2.38 (m)	35.9	1.23 (m), 2.40 (m)
22	32.2	2.08 (m), 2.35 (m)	32.2	2.12 (m), 2.38 (m)
23	64.3	3.67 (d, 10.2) 4.27 (d, 12.0)	64.7	3.66 (d, 11.4) 4.27 (d, 9.0)
24	13.7	0.94 (s)	13.3	1.06 (s)
25	16.3	0.96 (s)	16.2	0.89 (s)
26	17.6	1.15 (s)	17.5	1.10 (s)
27	27.2	1.77 (s)	27.2	1.73 (s)
28	175.9	–	175.9	–
29	33.1	0.96 (s)	33.1	0.93 (s)
30	24.6	1.02 (s)	24.5	0.98 (s)

[a]These assignments are based on data from the ^{1}H-^{1}H COSY, HSQC, HSQC-TOCSY, HMBC and ROESY experiments.
[b]Overlapped ^{1}H-NMR signals are reported without designated multiplicity.

moieties at C-3. The signals of sugar moieties were assigned through HSQC, COSY, TOCSY and HSQC-TOCSY. Long-range couplings were observed between a proton signal at δ_H 4.98 (glc-H-1′) and the carbon signal at δ_C 83.0 (C-3), between a proton signal at δ_H 5.72 (glc-H-1″) and the carbon signal at δ_C 79.4 (glc-C-2′), and between a proton signal at δ_H 5.21 (glc-H-1‴) and the carbon signal at δ_C 87.6 (glc-C-3′) in the HMBC spectrum of **2**, which suggests glycosylation at C-3 with a glc (1→2)-[glc (1→3)]-glc moiety. Therefore, the structure of compound **2** was elucidated as 3-O-{[*β*-D-glucopyranosyl(1→2)]-[*β*-D-glucopyranosyl(1→3)]-*β*-D-glucopyranosyl}-caulophyllogenin 28-O-*β*-D-glucopyranosyl ester.

The other known compounds (**3-13**) were identified as glucocaulophyllogenin (Farias et al., 2010), acutoside A (Voutquenne et al., 2003), 3-O-{[*β*-D-glucopyranosyl (1→2)]-[*β*-D-glucopyranosyl (1→3)-*β*-D-glucopyranosyl (1→3)]-*β*-D-glucopyranosyl} oleanolic acid 28-O-*β*-D-glucopyranosyl ester (Zhang et al., 2012), aralia-saponin V (Song et al., 2001), lucynoside E (Magid et al., 2006), congmuyenoside B (Kuang et al., 1996), 3-O-[*β*-D-glucopyranosyl (1→3)-*β*-D-glucopyranosyl (1→3)]-[*β*-D-glucopyranosyl (1→2)]-*β*-D-glucopyranosyl hederagenin 28-O-*β*-D-glucopyranoside (Zhang et al., 2012), collinsonidin (Joshi et al., 1992), congmuyenoside III (Zhang et al., 2013), aralia-saponin IV (Song et al., 2000), 3-O-*α*-L-arabinopyranosyl echinocystic acid (Yakovishin et al., 1999), respectively, based on the NMR analyses and comparison with reported literatures.

All isolated compounds were evaluated for their inhibitory effects on *α*-glucosidase and PTP1B. As shown in Table 3, compounds **1, 7** and **9-13** displayed potent inhibitory effects on *α*-glucosidase than acarbose (the positive control). In addition, compounds **1** and **13** exhibited moderate inhibitory activities on PTP1B. Compound **13** displayed stronger inhibitory effect than others on both two enzymes.

Table 2
C (150 MHz) and ^{1}H (600 MHz) NMR data for sugar moieties of **1-2** in C_5D_5N [a,b].

Position	**1**		**2**	
	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)
3-O-glc				
1'	105.4	5.06 (d, 7.8)	103.8	4.98 (d, 7.2)
2'	75.5	4.02 (m)	79.4	4.01 (m)
3'	88.8	4.03 (m)	87.6	4.22 (m)
4'	69.6	4.09 (m)	69.8	4.01 (m)
5'	77.9	3.78 (m)	77.7	3.81 (m)
6'	62.4	4.26 (m), 4.50 (m)	63.1	4.30 (m), 4.42 (m)
glc				
1"	105.9	5.21 (d, 7.8)	103.7	5.72 (d, 7.8)
2'	74.4	4.10 (m)	76.3	4.08 (m)
3"	78.2	4.21 (m)	78.6	4.24 (m)
4"	71.5	4.14 (m)	72.1	4.17 (m)
5"	78.7	4.13 (m)	77.6	3.64 (m)
6"	62.3	4.06 (m), 4.12 (m)	62.1	4.25 (m), 4.42 (m)
glc				
1"'			105.2	5.08 (d, 7.8)
2"			75.4	4.02 (m)
3"'			78.5	4.95 (m)
4"			71.4	4.13 (m)
5"'			77.9	4.13 (m)
6"'			62.3	4.21 (m), 4.51 (m)
28-O-glc				
1	95.8	6.31 (d, 7.8)	95.8	6.29 (d, 7.8)
2	74.1	4.11 (m)	74.1	4.24 (m)
3	78.9	4.24 (m)	78.8	4.24 (m)
4	71.1	4.29 (m)	71.0	4.30 (m)
5	79.4	3.99 (m)	79.1	4.29 (m)
6	62.2	4.35 (m), 4.40 (m)	62.3	4.25 (m), 4.47 (m)

[a]These assignments are based on data from the ^{1}H-^{1}H COSY, HSQC, HSQC-TOCSY, HMBC and ROESY experiments.
[b]Overlapped ^{1}H-NMR signals are reported without designated multiplicity.

Table 3
α-glucosidase and PTP1B inhibitory assay results of compounds **1-13**.

Compound	*α*-glucosidase inhibitory activity (IC_{50}, *μ*M)[a]	PTP1B inhibitory activity (IC_{50}, *μ*M)[a]
1	2.96 ± 0.32	20.37 ± 0.46
2	6.82 ± 0.45	> 100
3	6.99 ± 0.22	> 100
4	12.82 ± 0.51	> 100
5	19.37 ± 0.23	> 100
6	6.97 ± 0.44	> 100
7	2.98 ± 0.56	> 100
8	12.54 ± 0.66	> 100
9	2.21 ± 0.43	> 100
10	0.87 ± 0.57	> 100
11	2.52 ± 0.48	> 100
12	3.32 ± 0.57	> 100
13	0.73 ± 0.39	11.22 ± 0.33
Acarbose	4.59 ± 0.41	ND[b]
Oleanolic acid	ND[b]	9.75 ± 0.66

[a] IC_{50} values represent the means ± SD of three parallel measurements.
[b] ND means not determined.

3. Experimental

3.1. General

Column chromatography was performed on a 200–300 mesh silica gel (Qingdao Marine Chemical Factory, P. R. China). Column chromatography was performed using YMC ODS-A gel (12 nm S-75 *μ*m, YMC Co., Ltd., Japan) and D101 Macro porous adsorption resin (Shanghai Hualing Resin Factory, P. R. China). TLC was performed with precoated silica gel GF254 plates (Qingdao Marine Chemical Factory, P. R. China). IR spectrum were acquired on Bruker EQUINOX55 spectrometer

(Bruker Co., Karlsruhe, Germany). NMR spectrum were performed on a Bruker ARX-300 or 600 spectrometer using TMS as the internal standard. HRESIMS were measured with a Bruker Daltonics Inc. micro-TOF-Q spectrometer. HPLC separations were performed on a Hitachi 655-15 series pumping system equipped with a Hitachi L-2490 refractive index detector using a YMC-Park ODS-A column (250 × 10 mm I.D, S-5 μm, 12 nm). GC separations were performed on an Agilent 7890 A Gas Chromatograph equipped with a SPH-300 A FID detector using an Agilent 19091J-413 capillary column (30 m × 320 μm × 0.25 μm).

3.2. Plant material

The leaves of *A. elata* were collected from Liaoning Province, China, in August 2009 and were identified by Prof. Lu Jincai of Shenyang Pharmaceutical University. A voucher specimen was deposited at the School of Traditional Chinese Materia Medica (No. 090811).

3.3. Extraction and isolation

The air-dried leaves (8 kg) of *A. elata* were extracted with 60% ethanol firstly for three times under reflux. The combined solution were concentrated under *vacuum* and subjected to macroporous resin D101 column chromatography, eluted with an EtOH-H_2O gradient. The solution eluted with 60% ethanol was evaporated to dryness under *vacuum* to give a residue (800 g). The residue was chromatographied on silica gel with a CH_2Cl_2-MeOH gradient to afford eight fractions (fr. A-H). Fr. D was subjected to column chromatography on silica gel and eluted using an increasing MeOH gradient in CH_2Cl_2 (20–100%), to provide twelve fractions (fr. D_1-D_{12}). Fr. D_5 was subjected to ODS column chromatography and eluted with a MeOH-H_2O gradient to yield 11 fractions (fr. $D_{5\text{-}1}$-$D_{5\text{-}11}$). Among them, fr. $D_{5\text{-}6}$ was purified by preparative HPLC using MeOH-H_2O (*v/v* = 8:2) to give **3** (10 mg), **4** (148 mg). Fr. D_9 was separated using ODS column chromatography with a solvent system MeOH-H_2O to afford sixteen fractions (fr. $D_{9\text{-}1}$-$D_{9\text{-}16}$). Fr. $D_{9\text{-}10}$ was further separated with silica gel column chromatography with a solvent system of CH_2Cl_2-MeOH-H_2O (*v/v/v* = 7:3:0.5) to obtain **7** (345 mg), **10** (10 mg) and **13** (20 mg). Fr. $D_{9\text{-}12}$ was purified using Sephadex column chromatography and eluted with MeOH-H_2O to give **8** (21 mg). Fr. E was fractionated using column chromatography on silica gel and eluted with an increasing MeOH gradient in CH_2Cl_2 (30–100%), led to nine fractions (fr. E_1-E_9). Fr. E_5 was applied to ODS column chromatograph using a solvent system MeOH-H_2O to afford eighteen fractions (fr. $E_{5\text{-}1}$-$E_{5\text{-}19}$). Of these fractions, fr. $E_{5\text{-}9}$ was separated using silica gel column chromatography with a solvent system of CH_2Cl_2-MeOH-H_2O (*v/v/v* = 8:2:0.25) to give **1** (24 mg), **2** (15 mg) and **12** (125 mg). Fr. E_6 was purified using ODS column chromatography with a solvent system MeOH-H_2O to obtain fifteen fractions (fr. $E_{6\text{-}1}$-$E_{6\text{-}15}$), the eighth fraction was subjected to silica gel column chromatography eluting with a solvent system of CH_2Cl_2-MeOH-H_2O (*v/v/v* = 6:1:0.1) to yield **5** (60 mg), **6** (32 mg), **9** (180 mg) and **11** (205 mg).

3.4. Characteristic data of the compounds *1* and *2*

Compound **1**: white amorphous powder, IR (KBr) ν_{max}: 3424, 1077 and 1036 cm^{-1}; HRESIMS at *m/z* 997.4769 $[M + Na]^+$ (calcd. for $C_{48}H_{78}O_{20}Na$, 997.4769); ^1H and ^{13}C NMR, see Tables 1 and 2.

Compound **2**: white amorphous powder, IR (KBr) ν_{max}: 3422, 1078 and 1032 cm^{-1}; HRESIMS at *m/z* 1137.5680 $[M+H]^+$ (calcd. for $C_{54}H_{89}O_{25}$, 1137.5687); ^1H and ^{13}C NMR, see Tables 1 and 2.

3.5. Acid hydrolysis of *1* and *2*

Compounds **1** and **2** were heated with 2 M HCl (10 mL) at 95 °C for 4 h. The reaction mixture was extracted using $CHCl_3$ (3 × 5 mL). The residue was dissolved in pyridine (2 mL) after aqueous layer was evaporated to dryness. L-cysteine methyl ester hydrochloride (5 mg) was subsequently added to the solution to heat at 60 °C for 2 h. Then the chloro trimethyl silane (0.3 mL) was added to the mixture and stirred for another 2 h at 60 °C. After the reactions had been performed, the supernatant was diluted 20 times and analysed by GC under the following conditions: the column temperature was maintained at 80 °C for 5 min, then increased from 80 to 280 °C (25 °C/min) and maintained for 5 min; the carrier gas was N_2 (1.4 ml/min); the split ratio was 1/20; the injection temperature was 250 °C; and the injection volume was 1 μL. The absolute configurations of the monosaccharides of **1** and **2** were finally determined to be all D-glucose by comparing the retention times with hose of the standard samples D-glucose (14.489 min) and L-glucose (14.576 min).

3.6. α-glucosidase inhibition assay

The stock solutions of positive control and each test substances were prepared in DMSO and further diluted with PBS to obtain an experimental concentration. Enzyme solution was prepared using α-glucosidase (0.5 U/mL, Sigma, St. Louis, MO, USA) dissolved in 0.2 M potassium phosphate buffer (pH 6.8). The purified compound (80 μL) at different concentrations was mixed with enzyme solution (80 μL) then the mixture was incubated at 37 °C for 10 min. The *p*-nitrophenyl-α-D-glucopyranoside (40 μL, 1.5 mM) was added to the mixture as start of the reaction. After 30 min incubation at 37.0 °C, 0.1 M Na_2CO_3 (50 μL) was added to tube to terminate. The absorbance of yellow color produced due to the releasing of *p*-nitrophenol from the hydrolysis of *p*-nitrophenyl-α-D-glucopyranoside. The sample was evaluated through detecting the absorbance at 405 nm using a BIO-RAD Model 680 microplate reader. The non-enzymatic hydrolysis of *p*-nitrophenyl-α-D-glucopyranoside was corrected by detecting the addition in absorbance at 405 nm obtained in the absence of α-glucosidase. Acarbose (Sigma, St. Louis, MO, USA), a common α-glucosidase inhibitor, was used as a positive control. The inhibition activity was expressed as percentage inhibition of enzyme activity and was calculated using the following equation: $[1 - (A_{sample} - A_{blank}) / (A_{control} - A_{blank})] \times 100\%$. A_{sample} is the absorbance of the samples, $A_{control}$ is the absorbance of PBS that replaces the samples, A_{blank} is the absorbance of PBS that replaces the samples and the enzyme. The IC_{50} values were calculated plotting graphs with percentage inhibition on the y-axis and log concentrations on x-axis using graph pad prism v 6.0. The IC_{50} values are reported as best fit value after normalization of data (n = 3).

3.7. PTP1B inhibition assay

Buffer solution (pH 5.5) was composed of 50 mM citrate, 0.1 M NaCl, 1 mM EDTA and 1 mM dithiothreitol (DTT). Stock solution (in assay buffer containing 10% DMSO) of the test compounds (1 mM) and positive control (100 μM) were prepared. Taking suitable aliquots from the stock solution, five different concentrations were made by dilutions (50-3.125 μM). The sample (10 μL), 10 mM *p*-nitrophenyl phosphate (pNPP) (50 μL) and PTP1B (human recombinant) (40 μL, 0.1 U/mL, ProSpec-Tany TechnoGene Ltd., Ness Ziona, Israel) were added to each well of a 96-well plate (final volume: 100 μL). After incubation at 37 °C for 30 min, the reaction was terminated using 0.1 M NaOH (20 μL). *p*-Nitrophenyl, the product generated by dephosphorylation of pNPP, can be monitored at 405 nm using a BIO-RAD Model 680 microplate reader. The non-enzymatic hydrolysis of 10 mM pNPP was corrected by detecting the addition in absorbance at 405 nm obtained in the absence of PTP1B enzyme. The PTP1B inhibitor oleanolic acid was used as a positive control. The equation used was: $[(A_{blank} - A_{sample}) / A_{blank}] \times 100\%$. The IC_{50} values were calculated plotting graphs with percentage inhibition on the y-axis and log concentrations on x-axis using graph pad prism v 6.0. The IC_{50} values are reported as best fit value after normalization of data (n = 3).

Conflict of interest

The authors declared that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2018.06.002.

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