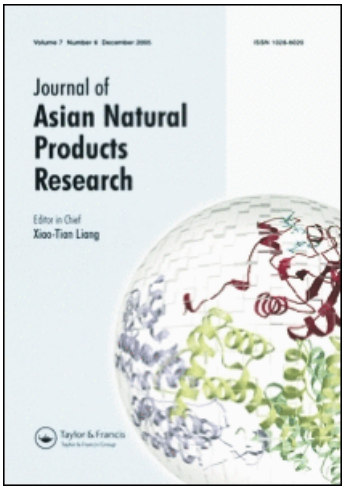

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Wen, Ping , Zhang, Xiao-Ming , Yang, Zhu , Wang, Nai-Li and Yao, Xin-Sheng(2008) 'Four new triterpenoid saponins from *Ardisia gigantifolia* Stapf. and their cytotoxic activity', Journal of Asian Natural Products Research, 10: 9, 873 — 880
To link to this Article: DOI: 10.1080/10286020802144719
URL: http://dx.doi.org/10.1080/10286020802144719

Journal of Asian Natural Products Research,
Vol. 10, No. 9, September 2008, 873–880

Four new triterpenoid saponins from *Ardisia gigantifolia* Stapf. and their cytotoxic activity

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(*Received 11 October 2007; final version received 8 April 2008*)

Four new oleanane-type triterpenoid saponins, cyclamiretin A 3β-*O*-α-L-rhamnopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside **(1)**, cyclamiretin A 3β-*O*-α-L-rhamnopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside **(2)**, cyclamiretin A 3β-*O*-α-L-rhamnopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-[β-D-6-*O*-acetylglucopyranosyl-(1 → 2)]-α-L-arabinopyranoside **(3)** and 3β-*O*-{α-L-rhamnopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside}-16,28-dihydroxy-30-acetoxyoleana-12-en **(4)**, along with one known triterpene saponin and ardisiacrispin A **(5)** were isolated from the rhizome of *Ardisia gigantifolia*. Their structures were established with the help of extensive spectroscopic techniques. Furthermore, the inhibitory effects of compounds on tumor cells (MTT based) *in vitro* were evaluated, and compounds **1**, **2**, and **5** showed potent anti-tumor activities.

Keywords: *Ardisia gigantifolia* Stapf; triterpenoid saponins; cytotoxic activity; MTT method

1. Introduction

Ardisia gigantifolia Stapf. is a widely occurring shrub in the southern parts of China. Its rhizomes have been used for the treatment of rheumatism, ostealgia, and traumatic injuries [1]. Previous chemical studies showed that triterpenoid saponins were the main components from this genus. From the rhizomes of *A. gigantifolia* Stapf., 32 triterpenoid saponins have been isolated over the last 20 years [2]. Some pharmacological studies of plants containing triterpenoid saponins showed that they had a significant cytotoxic activity [3]. The current study was conducted to extend our search for cytotoxic saponins from this plant. This paper describes the isolation and structure elucidation of four new triterpenoid saponins isolated from the ethanolic extract of the rhizome of the plant and their cytotoxicity against four human tumor cell lines.

2. Results and discussion

The dried and cut rhizome parts of *A. gigantifolia* were extracted and partitioned as described in Section 3. Successive chromatography of the *n*-butanol fraction on silica gel, macroporous resin HP-20, ODS column, and reversed phase HPLC afforded compounds **1**–**5**. Their structures were

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802144719
http://www.informaworld.com

established by analysis of their NMR and MS spectra.

Compound **1** was obtained as a white powder, $[\alpha]_D^{26}$ −25.2 (*c* 0.1, MeOH). The molecular formula of compound **1** was deduced as $C_{58}H_{94}O_{26}$ by HRESIMS at *m/z* 1205.5928 $[M - H]^-$. The ^1H NMR spectrum displayed signals for six methyl groups at δ 1.52 (3H, s), 1.27 (3H, s), 1.17 (3H, s), 1.02 (3H, s), 1.01 (3H, s), and 0.82 (3H, s), attributable to aglycone moiety, and five anomeric singals appearing at δ 5.93 (1H, br s, Rha-1), 5.39 (1H, d, $J = 7.6$ Hz, Glc-1), 5.01 (1H, d, $J = 6.8$ Hz, Xly-1), 4.87 (1H, d, $J = 7.7$ Hz, Glc-1), and 4.82 (1H, d, $J = 5.6$ Hz, Ara-1). In the ^{13}C NMR spectrum 58 carbon signals were detected, and based on the DEPT analysis 30 of them were assigned to aglycone moiety including six methyls, 11 methylenes (including one oxygenated methylenes), five methenyls (including two oxygenated methenyls), seven quaternary carbons (including one oxygenated quaternary), and one aldehyde carbon. Partital structures comprising the oleanane skeleton were identified with the help of HSQC, HMBC, and ^1H–^1H COSY correlations. With comparison of the corresponding carbon shifts of **5,** the triterpene aglycone of **1** was identified as cyclamiretin A [4]. The chemical shift assignments for the triterpene aglycone are shown in Table 1.

The monosaccharides obtained after acid hydrolysis of **1** were derivatized into aldononitrile peracetate derivative and analyzed by GC–MS using authentic samples as references. L-Rhamaose, L-arabinose, D-xylose, and D-glucose in the relative proportions of 1:1:1:2 were detected. The attachment points of the sugar chain and interglycosidic linkage were established by an HMBC experiment. Long-range correlations were observed between H-1 (δ 4.82) of arabinosyl and C-3 (δ 89.0) of the aglycone, H-1 (δ 5.39) of glucosyl and C-2 (δ 79.5) of arabinosyl, H-1 (δ 4.87) of glucosyl and C-4 (δ 78.2) of arabinosyl, H-1 (δ 5.01) of xylosyl and C-2 (δ 81.0) of glucosyl, and H-1 (δ 5.93) of rhamaosyl and C-3 (δ 85.8) of glucosyl. In the ^1H NMR spectrum of **1**, the relatively large $^3J_{H\text{-}1,\ H\text{-}2}$ coupling constant of the anomeric protons for the Glc, Xyl, and Ara (between 6.0 and 8.0 Hz) moieties indicated a β-configuration for Glc and Xyl and an α-configuration for Ara. The broad singlet of the anomeric proton of the Rha unit indicated an α-orientation [3]. On the basis of the above data, the structure of **1** was established as cyclamiretin A 3β-*O*-α-L-rhamnopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside.

Compound **2** was also obtained as a white powder, $[\alpha]_D^{26}$ −23.7 (*c* 0.1, MeOH). The positive-ion HRESIMS of compound **2** displayed a pseudo-molecular ion at *m/z* 1391.6425 $[M + Na]^+$, indicating the molecular formula as $C_{64}H_{104}O_{31}$. In the ^1H and ^{13}C NMR spectra of **2**, the chemical shifts of the aglycone moiety were similar to those of **1**. Therefore, the aglycone of **2** was identified as cyclamiretin A [4]. Acid hydrolysis of **2** with 2 M HCl was followed by GC–MS analysis of its aldononitrile peracetate derivatives using authentic samples as references. The result showed the L-rhamnose, L-arabinose, D-xylose, and D-glucose were presented in a ratio of 1:1:1:3. In the HMBC spectrum, the anomeric protons at δ 4.82 (H-1 of the arabinosyl), 5.34 (H-1 of the glucosyl), 4.85 (H-1 of the glucosyl), 5.01 (H-1 of the xylosyl), 5.89 (H-1 of the rhamaosyl), and 5.14 (H-1 of the glucosyl) showed long-range correlations with carbon signals at δ 89.0 (C-3 of the aglycone), 79.7 (C-2 of the arabinosyl), 78.4 (C-4 of the arabinosyl), 81.1 (C-2 of the glucosyl), 85.5 (C-3 of the glucosyl), and 86.8 (C-4 of the xylosyl), respectively. Consequently, the structure of **2** was determined as cyclamiretin A 3β-*O*-α-L-rhamnopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside.

Compound **3** was also obtained as a white powder, $[\alpha]_D^{26}$ −24.5 (*c* 0.1, MeOH). The positive-ion HRESIMS of compound **3**

Table 1. ^{13}C and ^{1}H NMR spectral data for the aglycone moiety of compounds **1**–**4**[a,b].

	1		**2**		**3**		**4**	
Position	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)
1	39.2	0.85 (o)	39.1	0.85 (o)	39.2	0.81 (o)	38.8	0.86 (o)
		1.59 (o)		1.61 (o)		1.59 (o)		1.47 (o)
2	26.5	1.72 m 1.96 m	26.5	1.77 (o)	26.6	1.72 (o)	26.3	1.76 (o)
				1.94 (o)		1.96 (o)		1.93 (o)
3	89.0	3.12 m	89.0	3.11	89.2	3.12 (o)	88.9	3.12 (o)
4	39.7		39.6		39.7		39.5	
5	55.7	0.65 d (10.7)	55.6	0.65 d (10.7)	57.4	0.70 d (11.0)	55.8	0.71 d (11.8)
6	17.9	1.40 (o)	17.9	1.41 (o)	18.0	1.40 (o)	18.5	1.30 (o)
								1.44 (o)
7	34.3	1.19 (o)	34.3	1.20 (o)	34.4	1.19 (o)	33.2	1.33 (o)
		1.51 (o)		1.51 (o)		1.51 (o)		1.57 (o)
8	42.5		42.5		42.6		40.0	
9	50.4	1.26 (o)	50.4	1.27 (o)	50.5	1.26 (o)	47.0	1.73 (o)
10	36.9		36.8		36.9		36.8	
11	19.1	1.70 m	19.1	1.71 m	19.1	1.70 (o)	23.8	1.84 (o)
								2.23 (o)
12	32.7	1.43 (o)	32.6	1.42 (o)	32.7	1.43 (o)	122.9	5.35 br s
13	86.3		86.3		86.4		144.6	
14	44.6		44.6		44.6		41.9	
15	36.8	1.42 (o)	36.8	1.42 (o)	36.8	1.42 (o)	34.6	1.62 (o)
		2.18 m		2.17 m		2.18 (o)		2.16 (o)
16	76.9	4.21 (o)	76.9	4.21 (o)	76.9	4.21 (o)	73.8	4.31 (o)
17	44.0		44.0		44.0		40.9	
18	53.3	1.36 (o)	53.3	1.39 (o)	53.3	1.36 (o)	41.8	2.48 (o)
19	33.4	2.12 (o)	33.4	2.09 (o)	33.4	2.12 (o)	43.2	1.61 (o)
		2.84 t (13.5)		2.84 t (13.5)		2.84 t (13.5)		2.69 t (13.3)
20	48.3		48.3		48.3		34.7	
21	30.5	2.08 (o)	30.5	2.08 (o)	30.5	2.08 (o)	32.4	1.66 (o)
		2.58 m		2.51 m		2.58 m		2.31 m
22	32.3	1.56 (o)	32.3	1.56 (o)	32.3	1.56 (o)	29.2	2.28 (o)
		1.96 (o)		1.96 (o)		1.96 (o)		
23	28.1	1.17 s (3H)	28.1	1.17 s (3H)	28.0	1.23 s (3H)	28.1	1.17 s (3H)
24	16.6	1.02 s (3H)	16.6	1.02 s (3H)	16.5	1.07 s (3H)	16.8	1.03 s (3H)
25	16.4	0.82 s (3H)	16.3	0.82 s (3H)	16.4	0.85 s (3H)	15.7	0.84 s (3H)
26	18.5	1.27 s (3H)	18.5	1.28 s (3H)	18.6	1.29 s (3H)	17.0	0.92 s (3H)
27	19.7	1.52 s (3H)	19.7	1.52 s (3H)	19.8	1.52 s (3H)	27.3	1.80 s (3H)
28	77.6	3.15 (o)	77.6	3.15 (o)	77.7	3.15 (o)	69.5	3.62 (o)
		3.53 (o)		3.53 (o)		3.53 (o)		3.68 (o)
29	24.1	1.01 s (3H)	24.1	1.00 s (3H)	24.1	1.01 s (3H)	27.9	1.10 s (3H)
30	207.5	9.62 s	207.5	9.61 s	207.6	9.62 s	69.0	4.28 (o) 4.50 (o)
CH_3							20.8	2.07 s (3H)
CO							171.1	

[a]Assignments based on ^{1}H, ^{13}C, DEPT, HSQC, HMBC, ^{1}H–^{1}H COSY, TOCSY, and NOESY experiments.
[b]Overlapped signals are indicated by (o).

showed an accurate $[M + Na]^+$ ion at *m/z* 1271.6026, corresponding to the molecular formula $C_{60}H_{96}O_{27}$. In the ^{1}H NMR and ^{13}C NMR spectra of **3**, the chemical shifts of the aglycone moiety were similar to those of **1**. Therefore, the aglycone of **3** was identified as cyclamiretin A [4].

Compared with ^{13}C NMR spectral data of the saccharide region of **1**, the carbon signal due to C-6 of glucosyl was shifted

downfield by +1.9 ppm, while the carbon signal due to C-5 of glucosyl was shifted upfield by −2.9 ppm. In the HMBC spectrum, the long-range correlation between protons at δ 4.93 (H-6 of glucosyl), 4.80 (H-6 of glucosyl), 2.05 ($CO\mathit{CH_3}$), and the carbon signal at δ 171.1 indicated that the OH-6 of glucosyl was acetylated. Consequently, the sugar chain was established as S_3 (see Figure 1). Correlations observed in the HMBC spectrum between an anomeric signal at δ 4.79 (H-1 of arabinosyl) and C-3 confirmed the substitution at C-3 of the aglycone. Based on the above data, the structure of **3** was determined as cyclamiretin A 3β-*O*-α-L-rhamnopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 2)]-β-D-galactopyranosyl-(1 → 4)-[β-D-6-*O*-acetylglucopyranosyl-(1 → 2)]-α-L-arabinopyranoside.

Compound **4** was obtained as a white powder, $[\alpha]^{26}_{D}$ −10.9 (*c* 0.1, MeOH). The molecular formula of compound **4** was deduced as $C_{60}H_{98}O_{27}$ by HRESIMS at *m/z* 1273.6172 $[M + Na]^+$. The ^{1}H NMR spectrum of **4** showed signals for seven methyl groups as singlets, a trisubstituted olefinic proton at δ 5.35 (1H, br t, $J = 3.2$ Hz), which was typical of the oleanolic skeleton. Compared with ^{13}C NMR spectral data of pridentigenin E,[5] the carbon signal due to C-30 was shifted downfield by +1.9 ppm, while the carbon signal due to C-20 was shifted upfield by −1.5 ppm. In the HMBC spectrum, the long-range correlation between protons at δ 4.28 (H-30), 4.50 (H-30), 2.07 (CH_3), and the carbon signal at δ 171.1 indicated that the hydroxyl group of C-30 was acetylated. Consequently, the triterpene aglycone of **4** was established as 3β,16,28-trihydroxy-30-acetoxyoleana-12-en. The chemical shift assignments for the triterpene aglycone are shown in Table 1.

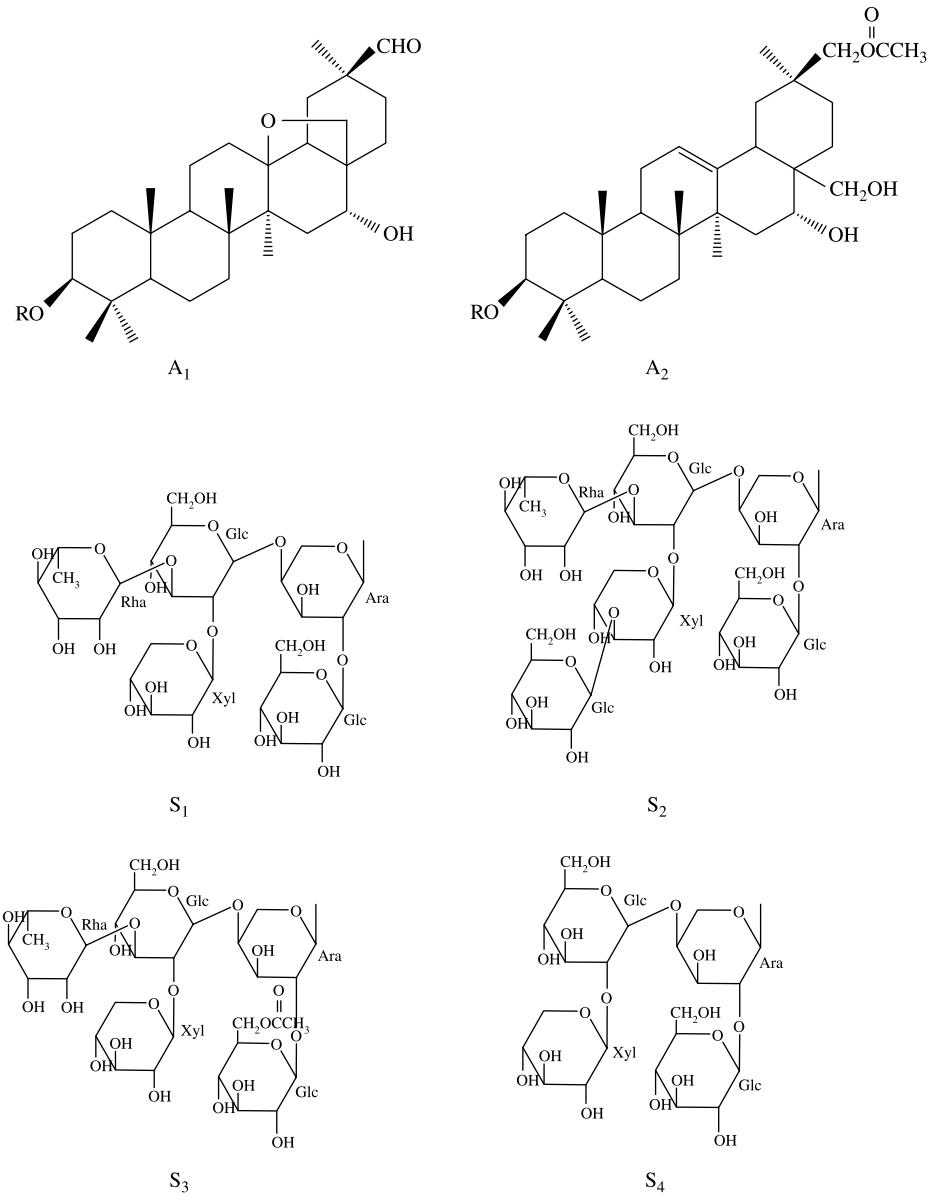
The ^{13}C NMR spectral data of saccharide moiety was similar to the ones of compound **1**. The sugar chain was established as S_1 (see Figure 1). Correlations observed in the HMBC spectrum between an anomeric signal at δ 4.80 (H-1 of arabinosyl) and C-3 confirmed the substitution at C-3 of the aglycone. On the basis of the above data, the structure of **4** was identificated as 3β-*O*-{α-L-rhamnopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside}-16,28-dihydroxy-30-acetoxyoleana-12-en.

Compounds **1**–**5** were examined for their inhibitory effects on NCI-H460, SF-268, MCF-7, and HepG2 tumor cells (MTT based) *in vitro*. Three triterpenoid saponins, compounds **1**, **2**, and **5**, showed potent antitumor activities, and the IC_{50} values for these compounds are shown in Table 3.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a P-1020 digital polarimeter (Jasco Corp.). ESIMS spectra were recorded on a Bruker Esquire 2000 mass spectrometer. HRESIMS spectra were recorded using a Micromass Q-TOF mass spectrometer. 1D and 2D NMR spectra were taken on a Bruker AV-400 (400 MHz for ^{1}H NMR) spectrometer in pyridine-d_5. Chemical shifts were expressed in δ using partially deuterated solvent chemical shifts at δ 149.8 and 8.71 as reference for ^{13}C and ^{1}H NMR signals, respectively. The GC–MS experiment was carried out on a Shimadzu GC–MS chromatograph using a RTX-5 capillary column (30 m × 0.25 mm). Purification of the extract was carried out using column chromatography with silica gel (Qingdao Haiyang Chemical Co., Ltd., China) and macroporous resin HP-20. Further purification of column fractions was then carried out using low-pressure column chromatography with ODS-A120-S150 (YMC Co., Ltd., Japan). Compounds were finally isolated with the help of a Shimadzu LC-8A preparative HPLC system equipped with a Shimadzu RID-10A detector using an ODS column (PRC-ODS, Shim-pack, 20 × 250 mm, 5 μm). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from



Compouds	**1**	**2**	**3**	**4**	**5**
aglycone moiety	A_1	A_1	A_1	A_2	A_1
saccharide moiety	S_1	S_2	S_3	S_1	S_4

Figure 1. Structures of compounds **1**–**5**.

Sigma (St. Louis, MO, USA). RPMI-1640 medium, fetal bovine serum (FBS), and trypsin–EDTA solution were obtained from Gibco-BRL (Grand Island, NY, USA).

3.2 Plant material

The dry rhizome of *A. gigantifolia* Stapf. was purchased in Guangzhou (China) in 2002 and was identified by Prof. Qishi Sun (Shenyang

Pharmaceutical University, China). A voucher specimen (No. 2002-Z-AG) has been deposited in Shenzhen Research Center of Traditional Chinese Medicines and Natural Products, Shenzhen, China.

3.3 Extraction and isolation

The rhizome of *A. gigantifolia* (5 kg) was extracted twice with 60% ethanol (40 L) under reflux for 2 h. The 60% ethanol extract (465 g) was suspended in water (5 L) and partitioned successively with EtOAc (5 L × 3), and *n*-BuOH (5 L × 3). The *n*-BuOH layer (100 g) was subjected to column chromatography on silica gel eluted with $CHCl_3$/MeOH (10:1–1:1, v/v) gradient to afford eight fractions. Fraction 5 (7.1 g) was separated with macroporous resin HP-20 eluted with EtOH/H_2O (30:70, 50:50, 70:30, and 95:5, v/v), medium-pressure liquid chromatography (MPLC) on ODS (φ 2.5 × 30.0 cm) eluted with MeOH/H_2O (50:50 and 70:30, v/v) and preparative HPLC [MeOH/H_2O (60:40, v/v), flow rate 10 ml/min, RID-10A detector] to give **3** (60 mg) and **5** (30 mg). Purification of fraction 6 (35.1 g) was carried out with the same method and gave rise to **1** (1.25 g), **2** (35 mg), and **4** (17.3 mg).

3.3.1 Compound *1*

White amorphous powder; $[\alpha]^{26}_{D}$ −25.2 (*c* 0.1, MeOH); ESIMS *m/z* 1205 $[M - H]^-$, 1073 $[M - H\text{-}132]^-$, 1059 $[M - H\text{-}146]^-$, 1043 $[M - H\text{-}162]^-$, 927 $[M - H\text{-}132\text{-}146]^-$, 911 $[M - H\text{-}132\text{-}162]^-$, 765 $[M - H\text{-}132\text{-}146\text{-}162]^-$, 603 $[M - H\text{-}132\text{-}146\text{-}162 \times 2]^-$; HRESIMS *m/z* 1205.5928 $[M - H]^-$ (calcd for $C_{58}H_{94}O_{26}Na$, 1205.5955). ^{13}C NMR (100 MHz, pyridine-d_5) and ^1H NMR (400 MHz) data, see Tables 1 and 2.

3.3.2 Compound *2*

White amorphous powder; $[\alpha]^{26}_{D}$ −23.7 (*c* 0.1, MeOH); ESIMS *m/z* 1391 $[M + Na]^+$, 1245 $[M + Na\text{-}146]^+$, 1229$[M + Na\text{-}162]^+$, 1097 $[M + Na\text{-}162\text{-}132]^+$, 951 $[M + Na\text{-}162\text{-}132\text{-}146]^+$, 789 $[M + Na\text{-}162 \times 2\text{-}132\text{-}146]^+$; HRESIMS *m/z* 1391.6425 $[M + Na]^+$ (calcd for $C_{64}H_{104}O_{31}Na$, 1391.6459). ^{13}C NMR (100 MHz, pyridine-d_5) and ^1H NMR (400 MHz) data, see Tables 1 and 2.

3.3.3 Compound *3*

White amorphous powder; $[\alpha]^{26}_{D}$ −24.5 (*c* 0.1, MeOH); ESIMS *m/z* 1271 $[M + Na]^+$, 1139 $[M + Na\text{-}132]^+$, 1125 $[M + Na\text{-}146]^+$, 993 $[M + Na\text{-}146\text{-}132]^+$, 935 $[M + Na\text{-}132\text{-}162\text{-}42]^+$, 831$[M + Na\text{-}162\text{-}132\text{-}146]^+$; HRESIMS *m/z* 1271.6026 $[M + Na]^+$ (calcd for $C_{60}H_{96}O_{27}Na$, 1271.6037). ^{13}C NMR (100 MHz, pyridine-d_5) and ^1H NMR (400 MHz) data, see Tables 1 and 2.

3.3.4 Compound *4*

White amorphous powder; $[\alpha]^{26}_{D}$ −10.9 (*c* 0.1, MeOH); ESIMS *m/z* 1273 $[M + Na]^+$, 1141 $[M + Na\text{-}132]^+$, 1127 $[M + Na\text{-}146]^+$, 1111 $[M + Na\text{-}162]^+$, 995 $[M + Na\text{-}132\text{-}146]^+$, 833 $[M + Na\text{-}132\text{-}146\text{-}162]^+$, 775 $[M + Na\text{-}132\text{-}146\text{-}162\text{-}58]^+$; HRESIMS *m/z* 1273.6172 $[M + Na]^+$ (calcd for $C_{60}H_{98}O_{27}Na$, 1273.6193). ^{13}C NMR (100 MHz, pyridine-d_5) and ^1H NMR (400 MHz) data, see Tables 1 and 2.

3.3.5 Compound *5[5]*

White amorphous powder. ESIMS *m/z* 1083 $[M + Na]^+$, $C_{52}H_{84}O_{22}$.

3.4 Acid hydrolysis of 1 and 2

Each compound (10 mg) was heated in an ampule with 7 mL of 2 M HCl at 90°C for 2 h. The aglycone was extracted with EtOAC and H_2O. The H_2O layer was evaporated under reduced pressure. Pyridine (1.5 mL) and 3 mg of $NH_2OH{\cdot}HCl$ were added to the residue, and the mixtures was heated at 90°C for 1 h. After cooling, Ac_2O (2 mL) was added and the mixtures were heated at 90°C for 1 h. The reaction mixtures were evaporated under reduced pressure, and the resulting

Table 2. ^{13}C and ^{1}H NMR spectral data for the monosaccharide moiety of compounds **1**–**4**[a,b].

	1		**2**		**3**		**4**	
Position	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)
Ara- 1	104.4	4.82 d (5.6)	104.3	4.82 (o)	104.5	4.79 (o)	104.4	4.80 (o)
2	79.5	4.51 (o)	79.7	4.48 (o)	80.2	4.51 (o)	79.5	4.53 (o)
3	72.6	4.30 (o)	72.6	4.29 (o)	72.9	4.26 (o)	72.6	4.30 (o)
4	78.2	4.02 (o)	78.1	4.02 (o)	78.4	4.02 (o)	78.3	4.02 (o)
5	63.3	3.64 (o)	63.2	3.64 (o)	63.7	3.66 (o)	63.4	3.64 (o)
		4.53 (o)		4.53(o)		4.53 (o)		4.53 (o)
Glc-1	104.9	5.39 d (7.6)	104.9	5.34 d (7.6)	105.1	5.34 d (7.6)	104.9	5.41 d (7.6)
2	76.1	4.03 (o)	76.1	4.03 (o)	76.0	4.03 (o)	76.1	4.04 (o)
3	78.1	4.23 (o)	78.1	4.23 (o)	78.1	4.03 (o)	78.1	4.22 (o)
4	71.8	4.25 (o)	71.7	4.25 (o)	71.0	4.10 (o)	71.8	4.23 (o)
5	77.9	4.00 (o)	77.9	4.00 (o)	75.0	4.10 (o)	77.9	4.02 (o)
6	62.9	4.39 m	62.8	4.39 m	64.8	4.80 (o)	62.9	4.38 m
		4.52 (o)		4.49 (o)		4.92 (o)		4.53 (o)
$-CH_3$					21.0	2.05		
$-CO$					171.1			
Glc-1	103.8	4.87 d (7.7)	103.7	4.85 d (7.9)	103.9	4.89 d (7.4)	103.9	4.87 d (7.7)
2	81.0	3.98 (o)	81.1	3.96 (o)	81.0	4.02 (o)	81.1	3.99 (o)
3	85.8	4.09 (o)	85.5	4.09 (o)	85.9	4.09 (o)	85.7	4.09 (o)
4	69.6	4.27 (o)	69.6	4.27 (o)	69.6	4.27 (o)	69.6	4.27 (o)
5	78.0	3.67 (o)	78.0	3.67 (o)	78.0	3.67 (o)	78.0	3.67 (o)
6	62.1	4.28 (o)	62.0	4.28 (o)	62.1	4.28 (o)	62.0	4.28 (o)
		4.34 (o)		4.34 (o)		4.34 (o)		4.34 (o)
Xyl- 1	105.5	5.01 d (6.8)	105.1	5.01 d (6.5)	105.5	5.07 d (6.8)	105.5	5.02 d (6.8)
2	75.1	4.01 (o)	75.1	4.01(o)	75.1	4.01 (o)	75.1	4.01 (o)
3	78.0	4.23 (o)	86.8	4.03 (o)	77.9	4.02 (o)	78.0	4.23 (o)
4	70.7	4.26 (o)	69.1	4.06 (o)	70.7	4.26 (o)	70.7	4.26 (o)
5	66.9	3.56 (o)	66.2	3.51 (o)	66.9	3.56 (o)	66.9	3.56 (o)
		4.45 (o)		4.39 (o)		4.45 (o)		4.45 (o)
Rha-1	103.6	5.93 s	103.5	5.89 s	103.6	5.92 s	103.6	5.94 br s
2	72.2	4.97 (o)	72.1	4.94 (o)	72.2	4.97 (o)	72.2	4.97 (o)
3	72.6	4.49 (o)	72.6	4.49 (o)	72.6	4.49 (o)	72.6	4.49 (o)
4	73.8	4.30 (o)	73.8	4.30 (o)	73.8	4.30 (o)	73.8	4.30 (o)
5	70.5	4.79 (o)	70.4	4.82 (o)	70.5	4.79 (o)	70.5	4.79 (o)
6	18.5	1.63 d (6.2)	18.5	1.63 d (6.1)	18.5	1.63 d (6.1)	18.5	1.64 d (6.1)
Glc-1			104.8	5.14 d (7.8)				
2			73.9	4.03 (o)				
3			78.0	4.02 (o)				
4			71.5	4.28 (o)				
5			78.4	3.89 (o)				
6			62.3	4.04 (o)				
				4.42 (o)				

[a]Assignments based on ^{1}H, ^{13}C, DEPT, HSQC, HMBC, $^{1}H-^{1}H$COSY, TOCSY, and NOESY experiments.
[b]Overlapped signals are indicated by (o).

aldononitrile peracetates were analyzed by GC–MS using standard aldononitrile peracetates as reference samples.

The GC–MS experiment was carried out on a Shimadzu GC–MS chromatograph using a RTX-5 capillary column (30 m × 0.25mm). Nitrogen (2.0 mL/min) was used as carrier gas. The initial column oven temperature was 100°C for 1 min; then the temperature was increased by 20°C/min to a value of 200°C for

Table 3. Results of inhibitory effects of compounds **1**–**5** on tumor cell bioassay (MTT based) *in vitro*.

	IC_{50} (μmol/L)			
Samples	NCI-H46	SF-268	MCF-7	HepG2
1	3.73	3.13	4.58	5.06
2	3.35	3.43	3.68	6.30
3	*	*	*	*
4	*	*	*	*
5	6.01	6.84	5.33	*

*The compounds showed no effects.

1 min; then the temperature was increased by 8°C/min to a final value of 280°C.

3.5 Cytotoxicity assay

We evaluated the cytotoxic activity of compounds **1**–**5** on NCI-H46, SF-268, MCF-7, and HepG2 cell lines (see Table 3). The cells were maintained in PRMI 1640 (Gibco-BRL) containing 10% FBS (Gibco), 2 mg/mL $NaHCO_3$, 100 μg/mL penicillin sodium salt, and 100 μg/mL streptomycin sulfate. Cells were grown to 70% for experimental use. In all experiments, cells were grown in RPMI-1640 medium with 10% FBS for 24 h prior to treatment. And then the cells were incubated for 48 h followed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at 570 nm [6].

Acknowledgements

The authors are grateful to Prof. QiShi Sun of Shenyang Pharmaceutical University (Liaoning province, China) for identifying the plant materials and Jinghui Huang of Shenzhen Research Center of Traditional Chinese Medicine and Natural Products (Shenzhen, China) for measuring all NMR measurements.

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