Steroids 92 (2014) 1–6

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Synthesis and anti-cancer cell activity of pseudo-ginsenoside Rh2

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ARTICLE INFO

Article history:
Received 23 February 2014
Received in revised form 9 August 2014
Accepted 17 August 2014
Available online 10 September 2014

Keywords:
Steroid derivatives
Human tumor cells
Ginsenoside
Synthesis

ABSTRACT

β-D-Glucopyranoside,(3β,12β,20E)-12,25-dihydroxydammar-20(22)-en-3-yl (pseudo-ginsenoside Rh2) and its 20Z-isomer were synthesized from ginsenoside Rh2 under a mild condition, via a simple three-step called acetylation, elimination–addition and saponification. In addition, their activities were evaluated by eight different human tumor cells, compared with ginsenoside Rh2 group. Results indicated that the reaction in the side chain might greatly enhance the anti-proliferative activity of ginsenosides.

1. Introduction

Ginseng, which is well-known as the king of herbs, has been an important medicinal resource all over the world [1]. A large number of experimental studies have shown this herb to have antidiabetic, anti-HIV protease, and antitumor activities [2,3]. Ginsenosides are considered as the major pharmacologically active ingredient of ginseng, which are defined as types of protopanaxadiol (PPD), protopanaxatriol (PPT), and oleanolic acid according to aglycone skeleton [4–6]. Among them, ginsenoside Rh2, which is synthesized via glycosylation of protopanaxadiol derivatives, has been proved to have a measurable effect on various cancer cells [7].

In the previous study, many dammarane-type derivatives have been prepared and investigated for their activities [8]. In current, we have described the synthesis of two dammarane-type derivatives, namely, β-D-Glucopyranoside,(3β,12β,20E)-12,25-dihydroxydammar-20(22)-en-3-yl (**3a**, Pseudo-G-Rh2) and β-D-Glucopyranoside,(3β,12β,20Z)-12,25-dihydroxydammar-20(22)-en-3-yl (**3b**), both of which bear a different side chain at C-17 by comparing with ginsenoside Rh2 (Scheme 1). Moreover, we have obtained the detailed structure elucidation of the new compounds by 1D, 2D and ROESY NMR, HRESIMS and their inhibitory effects on several tumor cells.

2. Experimental

2.1. General

Column chromatography (CC) was performed with silica gel (300–400 mesh, Qingdao Haiyang Chemical Co., Ltd., China) and spots were visualized by spraying the plates with 10% H_2SO_4 ethanol solution, followed by heating. Preparative HPLC was carried out on a LC-8A pump, a SPA-10A UV detector (Shimadzu) and a C18 column (20 mm × 200 mm), at an elution flow rate of 5.0 ml/min. The 1D, 2D and ROESY NMR spectroscopy were performed on a Bruker AVANCE-500 NMR spectrometer in Pyridine-d_5 and Methanol-d_4 using standard Bruker pulse programs. Optical rotations were obtained on a WZZ-15 polarimeter (Na 589 nm); ultraviolet (UV) spectra were determined on a Shimadzu UV-3600 spectrophotometer; infrared (IR) spectra were recorded with a PerkinElmer Spectrum One Fourier transform infrared (FTIR) spectrometer (KBr); and HRESI mass spectra were performed on an Agilent1290-micrOTOF Q II mass instrument. The absorbance was recorded on a Microplate Reader (SpectraMax® Plus384, Molecular Devices, USA) at a wavelength of 570 nm.

2.2. Plant material

The whole extraction of ginseng was purchased from Hongjiv Biotech Company, Jilin province, China, and identified by Prof. Yanping Chen. The material 20(S)-Rh2 was prepared and isolated from the crude product of ginseng in our laboratory [9].

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http://dx.doi.org/10.1016/j.steroids.2014.08.021
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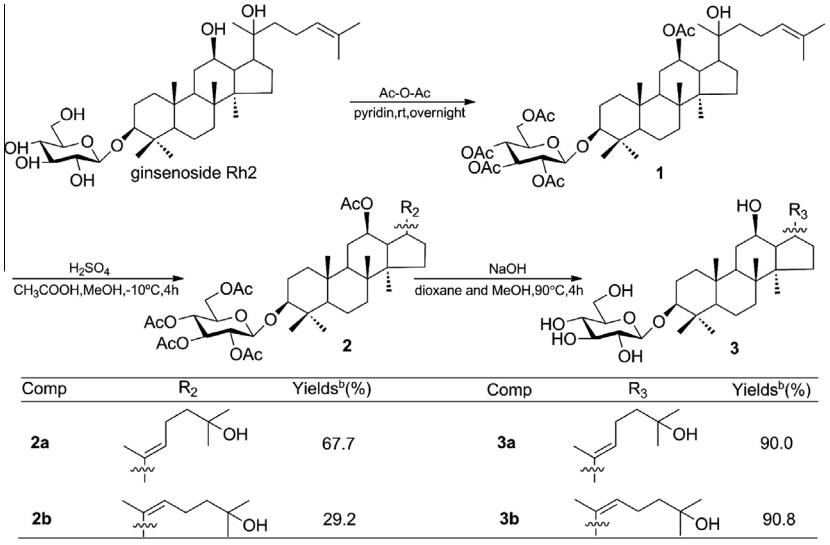

Comp	R_2	Yields[b](%)	Comp	R_3	Yields[b](%)
2a		67.7	**3a**		90.0
2b		29.2	**3b**		90.8

Scheme 1. Chemical transformation pathways of dammarane-type derivatives. [a]The yield of compound **1** is 93.4%; [b]Isolated yields.

2.3. Synthesis and isolation

Ginsenoside Rh2 (3.3 g) and acetic anhydride (24.3 ml) were dissolved and stirred in pyridine (25 ml) for 24 h at room temperature. Terminated by methanol (12.5 ml), the residue was taken up in ethyl acetate (25 ml) and water (200 ml). The organic phase was washed with brine (20 ml), water (20 ml), dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The dried (4.3 g) was rechromatographed on normal silica gel CC eluted with Et_2O/cyclohexane (4:1 → 3:1 → 2:1) to give compound **1** as a white solid (4.12 g).

To a solution of sulfuric acid/acetic acid solution (2%, 0.72 ml, 36 ml) in methanol (36 ml), compound **1** (4.0 g) was added in one portion which was stirred for 4 h at −10 °C. After being diluted with water (210 ml), the reaction mixture was extracted with an equal volume of ethyl acetate for three times. The combine organic layer was washed with a saturated aqueous solution of sodium carbonate and water, and then evaporated in vacuo to yield a white solid (3.89 g). The ethyl acetate extraction was submitted to silica gel CC eluted with Et_2O/cyclohexane (5:1 → 4:1) to yield a fraction. The fraction was further purified by preparative RP C18 HPLC [MeOH/H_2O (9:1), 5 ml/min, monitored at 203 nm] to obtain compounds **2a** (2.71 g) and **2b** (1.17 g), respectively.

Sodium hydroxide (2.7 g) was added to the solution of compound **2a** (2.7 g) in methanol (15 ml) and dioxane (15 ml). The resulting mixture was stirred for 4 h under reflux at 90 °C. Then the solution was diluted with water (90 ml) and extracted with an equal volume of *n*-Butanol for three times. The organic layer was washed with water until neutral and dried over anhydrous sodium sulfate. After evaporation of the solvent under reduced pressure and recrystallization from methanol, the desired white solid compound **3a** (1.82 g) was obtained. At the same time, compound **3b** (0.79 g) was prepared using the same procedure as described for the preparation of compound **3a** with the above reactants and solvents.

2.3.1. β-D-Glucopyranoside,(3β,12β,20S)-12-(acetyloxy)-20-hydroxydammar-24(25)-en-3-yl,2,3,4,6-tetraacetate (*1*)

Colorless amorphous solid; yield 93.4%; $[\alpha]_D^{25}$ −19.0 (c 0.05, MeOH); UV (MeOH) λ_{max} nm 202; IR (KBr) ν_{max} cm^{-1} 3340 (C–OH), 1753 (C=O), 1620 (C=C), 1037 (C–H); ESI-MS *m*/*z* 833.6 $[M+H]^+$, 855.7 $[M+Na]^+$, 871.6 $[M+K]^+$; for ^1H and ^{13}C NMR spectral data, see Table 1.

2.3.2. β-D-Glucopyranoside,(3β,12β,20E)-12-(acetyloxy)-25-hydroxydammar-20(22)-en-3-yl,2,3,4,6-tetraacetate (*2a*)

Colorless amorphous solid; yield 67.7%; $[\alpha]_D^{25}$ +15.2 (c 0.05, MeOH); UV (MeOH) λ_{max} nm 204; IR (KBr) ν_{max} cm^{-1} 3327 (C–OH), 1758 (C=O), 1633 (C=C), 1036 (C–H); HR ESI-MS *m*/*z* 833.5045 $[M+H]^+$, (calcd for 833.5046); for ^1H and ^{13}C NMR spectral data, see Table 1.

2.3.3. β-D-Glucopyranoside,(3β,12β,20Z)-12-(acetyloxy)-25-hydroxydammar-20(22)-en-3-yl,2,3,4,6-tetraacetate (*2b*)

Colorless amorphous solid; yield 29.2%; $[\alpha]_D^{25}$ −18.1 (c 0.05, MeOH); UV (MeOH) λ_{max} nm 203; IR (KBr) ν_{max} cm^{-1} 3349 (C–OH), 1760 (C=O), 1638 (C=C), 1037 (C–H); HR ESI-MS *m*/*z* 833.5029 $[M+H]^+$ (calcd for 833.5046); for ^1H and ^{13}C NMR spectral data, see Table 1.

2.3.4. β-D-Glucopyranoside,(3β,12β,20E)-12,25-dihydroxydammar-20(22)-en-3-yl (*3a*)

Colorless amorphous solid; yield 90.0%; $[\alpha]_D^{25}$ −23.9 (c 0.03, MeOH); UV (MeOH) λ_{max} nm 201; IR (KBr) ν_{max} cm^{-1} 3374 (C–OH), 1655 (C=C), 1051 (C–H); HR ESI-MS *m*/*z* 623.4511 $[M+H]^+$ (calcd for 623.4517); for ^1H, ^{13}C and HMBC NMR spectral data, see Table 2.

2.3.5. β-D-Glucopyranoside,(3β,12β,20Z)-12,25-dihydroxydammar-20(22)-en-3-yl (*3b*)

Colorless amorphous solid; yield 90.8%; $[\alpha]_D^{25}$ +23.9 (c 0.03, MeOH); UV (MeOH) λ_{max} nm 206; IR (KBr) ν_{max} cm^{-1} 3362 (C–OH), 1656 (C=C), 1044 (C–H); HR ESI-MS *m*/*z* 623.4515 $[M+H]^+$ (calcd for 623.4517); for ^1H, ^{13}C and HMBC NMR spectral data, see Table 2.

2.4. Cytotoxicity evaluation

Cytotoxicities of these derivatives were evaluated on eight different human tumor cell lines (SGC cell line, A549 cell line, HT1080 cell line, Hela cell line, A375 cell line, K562 cell line, HL-60 cell line and MCF-7 cell line) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay [10]. Briefly, after being seeded in a 96-well plate at a density of 1.2×10^4 cells/well and incubated for 24 h, these cells were treated with the tested compounds (0–150 μM) for 24 h. The cell viability was determined

Table 1
^{1}H and ^{13}C NMR spectroscopic data for compounds **1**, **2a** and **2b** in methanol-d_4.[a]

Position	**1**		**2a**		**2b**	
	δ_H (J in Hz)	δ_C, mult	δ_H (J in Hz)	δ_C, mult	δ_H (J in Hz)	δ_C, mult
1	1.61 (1H, m), 1.01 (1H, m)	39.95, CH_2	1.61 (1H, m), 1.07 (1H, m)	40.10, CH_2	1.67 (1H, m), 1.03 (1H, m)	40.10, CH_2
2	1.71 (1H, m), 1.32 (1H, m)	27.77, CH_2	1.77 (1H, m), 1.39 (1H, m)	29.58, CH_2	1.73 (1H, m), 1.37 (1H, m)	29.39, CH_2
3	3.18 (1H,dd, J = 11.7, 4.4 Hz)	91.21, CH	3.22 (1H,dd, J = 11.5, 4.5 Hz)	91.17, CH	3.22 (1H,dd, J = 11.5, 4.5 Hz)	91.17, CH
4		41.06, qC		41.35, qC		41.40, qC
5	0.82 (1H,d, J = 10.7 Hz m)	57.39, CH	0.86 (1H,d, J = 9.7 Hz m)	57.39, CH	0.86 (1H,d, J = 9.9 Hz m)	57.38, CH
6	1.57 (1H, m), 1.54 (1H, m)	19.37, CH_2	1.57 (1H, m), 1.54 (1H, m)	19.37, CH_2	1.56 (1H, m), 1.53 (1H, m)	19.37, CH_2
7	1.61 (1H, m), 1.32 (1H, m)	35.77, CH_2	1.61 (1H, m), 1.35 (1H, m)	36.08, CH_2	1.61 (1H, m), 1.34 (1H, m)	36.12, CH_2
8		38.14, qC		38.28, qC		38.29, qC
9	1.57 (1H, m)	51.41, CH	1.57 (1H, m)	51.83, CH	1.47 (1H, m)	51.88, CH
10		40.21, qC		40.22, qC		40.22, qC
11	1.71 (1H, m), 1.30 (1H, m)	29.69, CH_2	1.92 (1H, m), 1.45 (1H, m)	29.72, CH_2	1.76 (1H, m), 1.34 (1H, m)	29.41, CH_2
12	4.81 (1H, m)	76.92, CH	4.94 (1H, m)	76.03, CH	4.93 (1H, m)	76.14, CH
13	2.11 (1H, m)	46.77, CH	2.13 (1H, m)	48.3, CH	2.11 (1H, m)	48.43, CH
14		53.81, qC		52.34, qC		52.52, qC
15	1.65 (1H, m), 1.09 (1H, m)	32.43, CH_2	1.74 (1H, m),1.16 (1H, m)	33.19, CH_2	1.73 (1H, m), 1.18 (1H, m)	33.23, CH_2
16	1.92 (1H, m), 1.61 (1H, m)	26.06, CH_2	1.92 (1H, m), 1.71 (1H, m)	27.12, CH_2	1.89 (1H, m), 1.67 (1H, m)	27.12, CH_2
17	2.14[b]	53.40, CH	2.55 (1H,dd, J = 10.7, 6.5 Hz)	51.00, CH	3.06 (1H,dd, J = 17.3, 10.3 Hz)	41.32, CH
18	0.74 (3H, s)	16.84, CH_3	0.79 (3H, s)	16.91, CH_3	0.78 (3H, s)	16.90, CH_3
19	0.95 (3H, s)	16.76, CH_3	0.96 (3H, s)	16.91, CH_3	0.95 (3H, s)	16.90, CH_3
20		75.39, qC		138.79, qC		138.92, qC
21	1.13 (3H, s)	27.08, CH_3	1.65 (3H, s)	12.54, CH_3	1.70 (3H, s)	19.81, CH_3
22	1.98 (1H, m),1.57(1H, m)	38.99, CH_2	5.10 (1H,t, J = 6.6 Hz)	125.86, CH	5.11 (1H,t, J = 7.0 Hz)	126.55, CH
23	2.11 (1H, m), 1.92 (1H, m)	23.59, CH_2	2.14 (1H, m), 1.98 (1H, m)	24.23, CH_2	2.20 (1H, m), 2.07 (1H, m)	23.57, CH_2
24	5.14 (1H,t, J = 7.1 Hz)	126.35, CH	1.50 (2H, m)	44.95, CH_2	1.45 (2H, m)	45.49, CH_2
25		132.16, qC		71.38, qC		71.48, qC
26	1.69 (3H, s)	26.05, CH_3	1.22 (3H, s)	29.30, CH_3	1.21 (3H, s)	29.14, CH_3
27	1.63 (3H, s)	18.11, CH_3	1.22 (3H, s)	29.24, CH_3	1.21 (3H, s)	29.14, CH_3
28	1.03 (3H, s)	28.39, CH_3	0.99 (3H, s)	28.44, CH_3	0.99 (3H, s)	28.44, CH_3
29	0.98 (3H, s)	16.21, CH_3	1.11 (3H, s)	16.31, CH_3	1.10 (3H, s)	16.34, CH_3
30	0.90 (3H, s)	17.89, CH_3	1.02 (3H, s)	17.29, CH_3	1.06 (3H, s)	17.21, CH_3
3-glc-1'	4.72 (1H,d, J = 8.0 Hz)	103.83, CH	4.75 (1H,d, J = 8.0 Hz)	103.84, CH	4.76 (1H,d, J = 8.0 Hz)	103.83, CH
2'	5.25 (1H, m)	74.48, CH	5.29 (1H, m)	74.45, CH	5.28 (1H, m)	74.46, CH
3'	4.98 (1H, m)	70.20, CH	5.01 (1H, m)	70.21, CH	5.01 (1H, m)	70.20, CH
4'	4.90 (1H, m)	73.41, CH	4.97 (1H, m)	73.39, CH	4.97 (1H, m)	73.40, CH
5'	3.86 (1H, m)	72.76, CH	3.91 (1H, m)	72.74, CH	3.90 (1H, m)	72.75, CH
6'	4.29 (1H, m), 4.11 (1H, m)	63.38, CH_2	4.33 (1H, m), 4.14 (1H, m)	63.40, CH_2	4.32 (1H, m), 4.14 (1H, m)	63.39, CH_2

[a] ^{1}H and ^{13}C NMR data were measured at 500 and 126 MHZ, respectively.
[b] Overlapping signals.

by adding MTT (5 mg/ml) to each well followed by incubation at 37 °C in a humidified incubator containing 5% CO_2 gas for 4 h. After careful removal of the medium, all tested compounds were dissolved in 200 μl DMSO. The absorbance was recorded on a Microplate Reader at a wavelength of 570 nm. The inhibition efficiency of the compounds on cell proliferation was calculated as 100% × (1 − absorption of well treated with compounds/absorption of control treated with vehicle).

2.5. Statistical analysis

The count data expressed as a percentage and the measurement data expressed as ±SD. IC50 (median inhibitory concentration) were determined by multiple regression, with $p < 0.05$ considered to be statistically significant.

3. Results and discussion

In ^{1}H NMR spectrum, the proton signals at δ_H 4.72 (H-1′), 5.14 (H-24), 3.18 (H-3), 2.05–0.74 (13 singlet methyl signals), and in ^{13}C NMR spectrum, the carbon signals at δ_C 126.35 (C-24), 132.16 (C-25), 75.39 (C-20), 171.1–172.4 (five carbonyl carbon signals) (Table 1), their shape and the chemical shift completely determined **1** as ginsenoside Rh2 with the hydroxyl moiety of the sugar residue and C-12 being acetylated [11].

The structures of the synthesized compounds (**2a**, **2b** and **3a**, **3b**) were determined by NMR and HR ESI-MS measurements. Their characteristic signals of 20(22)-en-25-ol of the side chain at C-17 were observed at the ^{13}C NMR signals at C-20, C-22, C-24, C-25, C-26, C-27 (Tables 1 and 2) [12]. The stereochemistry of the alkene bond was determined by ROESY, in which the proton signal of H-22 was correlated with signal of H-17 or H-21, suggesting that **2a**, **3a** had the E-configuration and **2b**, **3b** were in the Z-configuration (Fig. 1), which was also supported by the chemical shift of the C-21 [13]. Analysis of the above NMR signals indicated **2a** was assigned to be **1** with a different side chain, and **2b** was elucidated as the 20(Z)-isomer of **2a** (Table 1), in agreement with the molecular formula $C_{46}H_{72}O_{13}$ established by HR ESI-MS. Same as above, the NMR data of **3a** and **3b** were closely resemble those of ginsenoside Rh2 except for the significant difference of signals appeared at the side chain, reflecting the similar features in the molecular structure (Table 2) [9,11]. From these data, the structure of **3a** was deduced to be β-D-Glucopyranoside,(3β,12β,20E)-12,25-dihydroxydammar-20(22)-en-3-yl, and **3b** was described as the 20(Z)-isomer of **3a**.

Here we proposed a low-temperature synthesis of **3a** and its 20Z-isomer (**3b**) as described in Scheme 1. Through the elimination and addition at different positions in a one-step reaction, the excellent yields of the title compounds were achieved conveniently. In addition, we found that if raise the temperature or alter the acid medium, the reaction was unsatisfactory and often resulted in a

Table 2
^{1}H, ^{13}C and HMBC NMR spectroscopic data for compounds **3a** and **3b** in pyridine-d_5.[a]

Position	**3a**			**3b**		
	δ_H (*J* in Hz)	δ_C, mult	HMBC	δ_H (*J* in Hz)	δ_C, mult	HMBC
1	0.77 (1H, m), 1.53 (1H, m)	39.66 CH_2	C-3,C-5,C-10,C-18	0.84, 1.54 (1H, m)	39.66 CH_2	C-3,C-5,C-10,C-18
2	1.80 (1H, m), 2.24 (1H, m)	27.13 CH_2	C-1,C-3	1.83 (1H, m), 2.25 (1H, m)	27.12 CH_2	C-1,C-3
3	3.40 (1H, dd, *J* = 11.8, 4.4 Hz)	89.14 CH	C-1',C-2,C-4	3.40 (1H, dd, J = 11.7, 4.2 Hz)	89.13 CH	C-1,C-1'
4		40.08 qC			40.07 qC	
5	0.76 (1H, d, *J* = 11.6 Hz)	56.78 CH	C-4,C-6,C-10,C-28	0.76 (1H, d, *J* = 11.8 Hz)	56.76 CH	C-3,C-6,C-10,C-28
6	1.41 (1H, m), 1.52 (1H, m)	18.84 CH_2	C-5,C-7	1.38 (1H, m), 1.52 (1H, m)	18.83 CH_2	C-5,C-7
7	1.24 (1H, m), 1.51 (1H, m)	35.73 CH_2	C-6,C-8,C-9,C-19	1.25 (1H, m), 1.49 (1H, m)	35.72 CH_2	C-6,C-8,C-9,C-19
8		40.65 qC			40.64 qC	
9	1.41 (1H, s)	51.16 CH	C-8,C-10,C-19	1.44 (1H, s)	51.21 CH	C-8,C-10
10		37.46 qC			37.46 qC	
11	1.09 (1H, m), 1.68 (1H, m)	33.03 CH_2	C-8,C-9	1.10 (1H, m), 1.69 (1H, m)	33.05 CH_2	C-8,C-9
12	3.93 (1H, td, *J* = 12.9, 5.0 Hz)	72.96 CH	C-13,C-17	3.92 (1H,td, *J* = 7.4 Hz)	72.79 CH	C-13,C-17
13	2.00 (1H, m)	50.93 CH	C-12,C-14,C-15,C-20	2.08 (1H, m)	50.91 CH	C-11,C-12,C-14,C-17
14		51.26 qC			51.57 qC	
15	1.44 (1H, m), 1.94 (1H, m)	32.62 CH_2	C-14,C-17,C-30	1.46 (1H, m), 1.91 (1H, m)	32.97 CH_2	C-14,C-17,C-30
16	1.03 (3H, s)	16.22 CH_3	C-15,C-17	1.51 (1H, m), 2.01 (1H, m)	28.8 CH_2	C-15,C-17,C-14,
17	2.82 (1H, dd, *J* = 10.6, 4.4 Hz)	51.36 CH	C-16,C-20,C-21,C-22	3.42[b]	41.28 CH	C-16
18	0.83 (3H, s)	16.85 CH_3	C-1,C-5,C-9,C-10	0.83 (3H, s)	16.82 CH_3	C-1,C-9,C-10
19	0.99 (3H, s)	17.44 CH_3	C-7,C-8,C-9	1.00 (3H, s)	17.42 CH_3	C-8,C-9,C-14
20		139.98 qC			139.53 qC	
21	1.84 (3H, s)	13.45 CH_3	C-17,C-20,C-22	1.95 (3H, s)	20.45 CH_3	C-17,C-20,C-22
22	5.62 (1H,t, *J* = 6.8 Hz)	125.96 CH_2	C-17,C-21,C-23,C-24	5.35 (1H,t, *J* = 7.0 Hz)	126.35 CH_2	C-17,C-21,C-23,C-24
23	2.38 (2H, m)	24.08 CH_2	C-20,C-22,C-24,C-25	2.57 (2H, m)	23.65 CH_2	C-20,C-22,C-24,C-25
24	1.75 (2H, m)	44.66 CH_2	C-22,C-23,C-25,C-27	1.82 (2H, m)	45.42 CH_2	C-22,C-23,C-25,C-27
25		69.89 qC			69.98 qC	
26	1.37 (3H, s)	30.39 CH_3	C-24,C-25,C-27	1.41 (3H, s)	30.41 CH_3	C-24,C-25,C-27
27	1.37 (3H, s)	30.15 CH_3	C-24,C-25,C-26	1.41 (3H, s)	30.27 CH_3	C-24,C-25,C-26
28	1.33 (3H, s)	28.53 CH_3	C-3,C-4,C-5	1.33 (3H, s)	28.51 CH_3	C-3,C-4,C-5
29	1.01 (3H, s)	17.17 CH_3	C-3,C-4,C-5,C-28	1.02 (3H, s)	17.18 CH_3	C-3,C-4,C-5,C-28
30	0.99 (3H, s)	17.44 CH_3	C-8,C-14,C-15	1.04 (3H, s)	16.22 CH_3	C-7,C-8,C-14
3-glc-1'	4.96 (1H,d, *J* = 7.5 Hz)	107.36 CH	C-3,C-3'	4.96 (1H,d, *J* = 7.8 Hz)	107.31 CH	C-2',C-3,C-5'
2'	4.07 (1H, m)	76.18 CH	C-1',C-3'	4.06 (1H, m)	76.17 CH	C-1',C-3'
3'	4.27 (1H, m)	79.15 CH	C-2',C-4'	4.27 (1H, m)	79.14 CH	C-2',C-4'
4'	4.23 (1H, m)	72.27 CH	C-5',C-6'	4.25 (1H, m)	72.25 CH	C-5',C-6'
5'	4.03 (1H, m)	78.77 CH	C-4',C-6'	4.03 (1H, m)	78.73 CH	C-4',C-6'
6'	4.42 (1H, m), 4.62 (1H, m)	63.48 CH_2	C-4',C-5'	4.42 (1H, m), 4.61 (1H, m)	63.45 CH_2	C-4',C-5'

[a] ^{1}H and ^{13}C NMR data were measured at 500 and 126 MHZ, respectively.
[b] Overlapping signals.

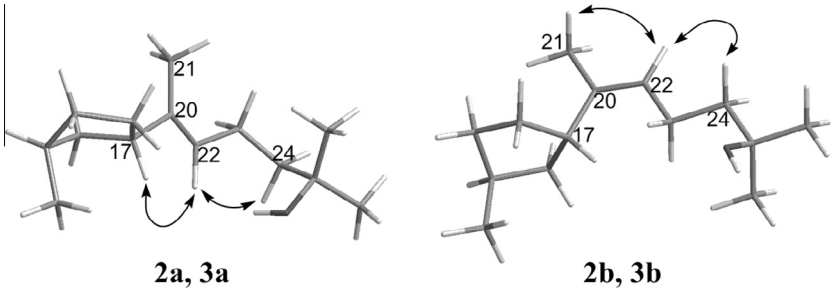

Fig. 1. The key ROESY correlations of compound **2a**, **3a** and **2b**, **3b**.

complicated mixture of products, which indirectly indicated the irreplaceability of the low-temperature and the acid catalysis.

Screening the inhibitory rate of the title compound on eight different human tumor cells (Fig. 2), the results showed that the proliferation of these human tumor cells was suppressed dose-dependently after treatment with **3a** for 24 h. Moreover the results of cytotoxic studies were shown in Fig. 3, which was expressed by the IC50 values. The testing indicated that **3b** showed a weaker cytotoxic activity in vitro, but **3a** had much stronger activity, compared with ginsenoside Rh2.

The conclusion we could get from the current data was that the side chain of ginseng sapogenin was crucial and a subtle change of ginsenoside Rh2 could result in a large variability of anti-tumor activity (Fig. 3). Previous studies on the structure–activity relationships of ginsenosides suggested that the anti-tumor activities were affected by the positions of the hydroxyl group and double band in the side chain, and the stereochemistry of the double bond also played a crucial role for their anti-tumor activities. For ginsenoside Rh2, with an appropriate change of the side chain at these atomic positions could increase the bioactivity, such as for **3a**. Currently, we are in the process of synthesizing and testing a much broader group of these types of molecules. Further analysis on these new artificial ginsenosides and their activities toward a broader spectrum of cancers will be published in a separate report in due course.

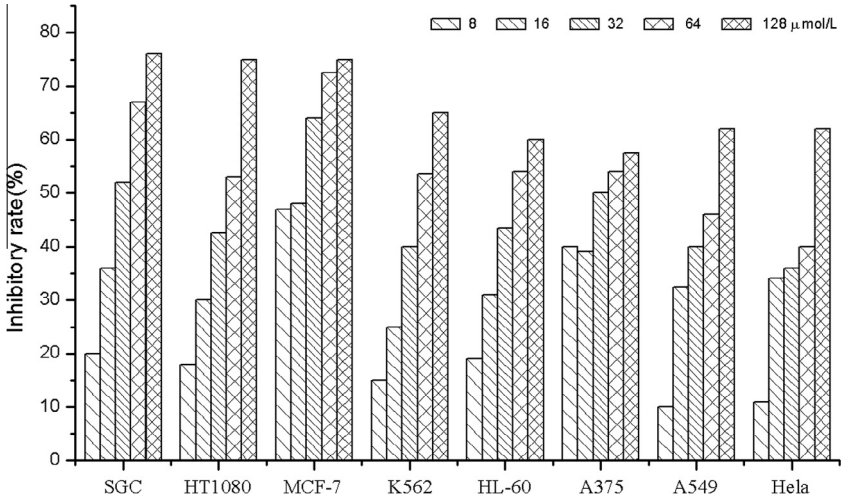

Fig. 2. The inhibitory rate of compound **3a** on different tumor cells. The cells were plated in 96-well plate, and treated with different doses of compound **3a** for 24 h, and then measured by MTT assay. It showed that compound **3a** inhibited SGC cell growth in a dose-dependent manner, and the inhibitory effect on SGC cells was more obvious than on the others. The experiment was repeated for at least three times. Each column represents the mean ± SD.

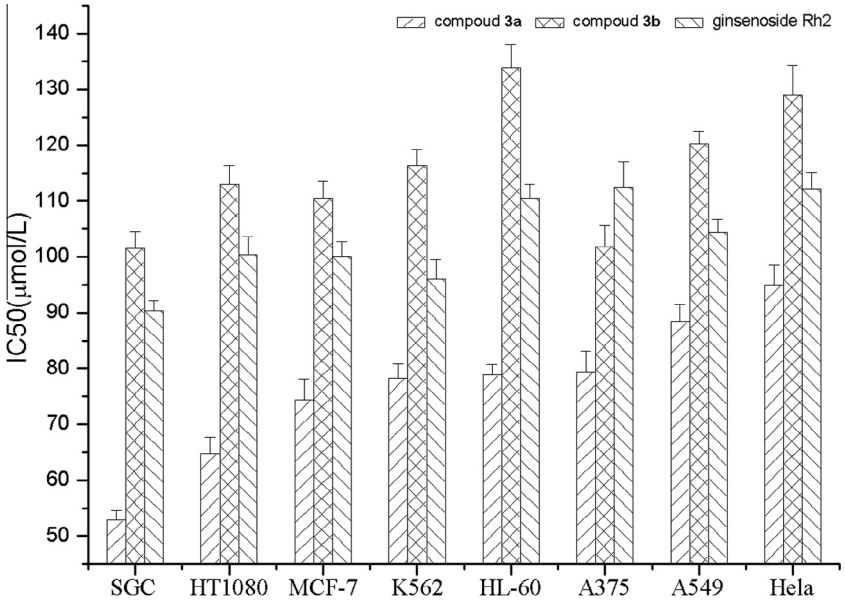

Fig. 3. The IC50 values of compound **3a**, compound **3b** and ginsenoside Rh2 on different tumor cells. The cells were plated in 96-well plate, and treated with different doses of compound **3a**, compound **3b** or ginsenoside Rh2 for 24 h, and then measured by MTT assay. The experiment was repeated for at least three times. Each column represents the mean ± SD.

Acknowledgements

The authors are grateful to the State Key Laboratory of Inorganic Synthesis and Preparative Chemistry, University of Jilin at Changchun for access to the Bruker AVANCE-500 NMR instrument for this study. The other chemical analysis was provided by the Alan. G. Macdiarmid Laboratory of Jilin University.

Appendix A. Supplementary data

Spectral data including 1D, 2D and ROESY NMR, HRESIMS data of compounds **1**, **2a**, **2b**, **3a** and **3b**.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2014.08.021.

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