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NOTE

# Three new triterpenoids from *Panax ginseng* exhibit cytotoxicity against human A549 and Hep-3B cell lines

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**Abstract** Three new triterpenoid derivatives, 3-*O*-*β*-D-glucopyranosyl-20(*S*)-protopanaxtriol (**1**), 3-formyloxy-20-*O*-*β*-D-glucopyranosyl-20(*S*)-protopanaxtriol (**2**) and 26-hydroxyl-24(*E*)-20(*S*)-protopanaxtriol (**3**), along with six known ginsenosides, were isolated from leaves of *Panax ginseng*. Their structures were established on the basis of spectral analysis (IR, 1D and 2D NMR, HRESI-MS). Compounds **1**–**3** exhibited various degree of cytotoxicity towards human A549 pulmonary carcinoma cells and Hep3B hepatoma cells.

**Keywords** *Panax ginseng* · Dammarane · Cytotoxicity · Human A549 · Hep3B cells

## Introduction

*Panax ginseng* C.A.Mey. (Ginseng), an ancient and famous herbal drug in traditional Chinese medicines, has been widely used in China for more than 4000 years [1]. It is mainly distributed in the Changbai Mountain regions in northeast China. Ginseng is characterized by the presence of ginsenosides, which are triterpene saponins, considered to be the main bioactive principles of ginseng. Ginsenosides have been shown to interact with numerous membrane proteins such as ion channels, transporters and receptors, resulting in a broad range of physiological activities [2, 3]. In recent years, there is growing evidence in the literature that ginsenosides possess anti-tumor activity not only in studies in vitro but also in experiments in vivo [4–10]. Among these components, 20(*R*,*S*)-ginsenoside-Rg3, as one of the most effective cytostasis ginsenosides, can inhibit proliferation and induce cell apoptosis in mice or human tumor cell lines, and its pharmaceutical preparation "Shen Yi Capsule" in China [11, 12] is used for the treatment of lung, liver or other primary cancers in clinical treatments.

In order to search for promising anti-tumor agents from ginseng, in the studies, we aim to find some new structures from the cheap leaves of *Panax ginseng*. In this paper, three new protopanaxtriol (PPT) dammarane derivatives (**1**–**3**) were isolated and their cytotoxicity in vitro against human A549 and Hep3B cells was evaluated, and their much stronger inhibitory activity than that of 20(*R*)-ginsenoside-Rg3 was determined (Fig. 1).

## Results and discussion

### Structural determination of compounds

Nine compounds (**1**–**9**) were obtained from the 95 % EtOH (v/v) extract of leaves of *Panax ginseng*. During the process of chemical study of this plant, three new compounds and six known ones were isolated and identified as 3-*O*-*β*-D-glucopyranosyl-20(*S*)-protopanaxtriol (**1**), 3-formyloxy-20-*O*-*β*-D-glucopyranosyl-20(*S*)-protopanaxtriol (**2**), 26-hydroxyl-24(*E*)-20(*S*)-protopanaxtriol (**3**), 20(*S*)-ginsenoside $Rh_1$ (**4**) [13], 20(*R*)-ginsenoside $Rh_1$ (**5**) [13], ginsenoside $F_1$ (**6**) [14],

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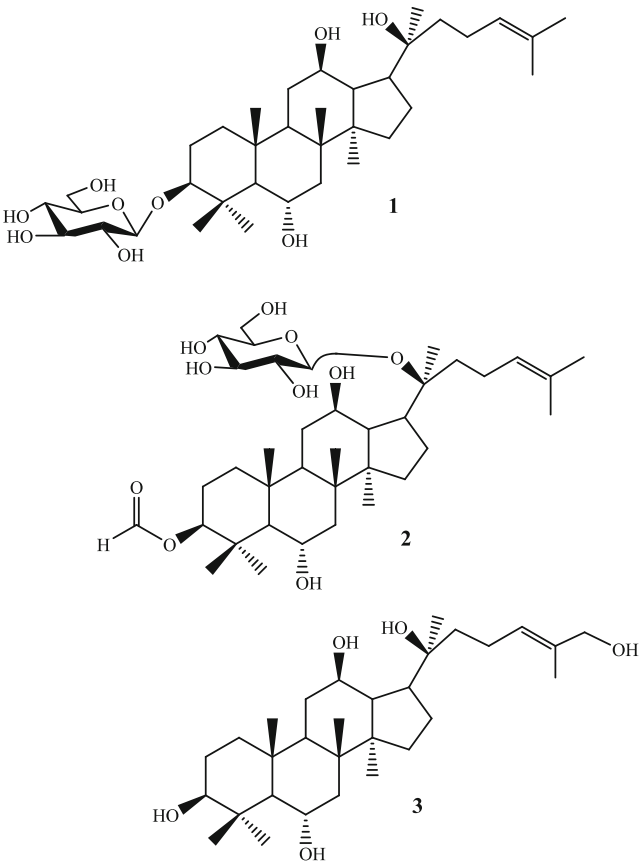


**Fig. 1** Structures of compounds **1**–**3**

ginsenoside $Rk_3$ (**7**) [15], ginsenoside $Rh_4$ (**8**) [16] and 20(*R*)-ginsenoside $Rg_3$ (**9**) [17], respectively.

Compound **1** was isolated as a white powder (MeOH). Its molecular formula was determined as $C_{36}H_{62}O_9$ by (HR) ESI-MS at *m/z* 661.4284 $[M+Na]^+$ (calcd. for $C_{36}H_{62}O_9Na$, 661.4286). The absorption bands at 3406, 1650 $cm^{-1}$ in the IR spectrum indicated the presence of hydroxyl and double-band functions in the structure. The $^1H$ NMR (600 MHz, $C_5D_5N$) spectrum (Table 1) exhibited signals due to eight methyls [$\delta_H$ 2.10, 1.67, 1.64, 1.08, 1.00, 0.95 (each 3H), 1.43 (3H × 2)], one olefinic proton [$\delta_H$ 5.34 (1H, t, $J = 6.0$ Hz)], an anomeric proton [$\delta_H$ 5.03 (1H, d, $J = 7.6$ Hz)] of a sugar unit and other alkyl groups. The $^{13}C$ NMR (150 MHz, $C_5D_5N$) spectrum exhibited 36 carbon signals including one glucose unit ($\delta_C$ 107.2, 78.7, 78.3, 75.9, 71.8, 63.0), two olefinic carbons ($\delta_C$ 130.7, 126.2), four O-bearing $sp^3$ carbons ($\delta_C$ 67.5, 70.9, 72.9, 89.4), eight methyls ($\delta_C$ 32.0, 26.5, 25.8, 17.6, 17.4, 17.3, 17.0, 16.9), and other alkyl groups consisting of eight methylene, four methine and four quaternary carbons. 30 carbon signals of aglycone, especially the signal at $\delta_C$ 61.8 (C-5) as a characteristic of a protopanaxtriol-type aglycone, suggested that compound **1** was a derivative of protopanaxtriol (PPT) [3]; moreover, signals at $\delta_C$ 67.5, 70.9 and

**Table 1** $^1H$-NMR and $^{13}C$-NMR spectral data for **1** and **2** ($\delta$: ppm, *J* in Hz, $C_5D_5N$)

| No. | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 1.56 (m), 0.90 (m) | 38.8 | 1.53 (m), 0.83 (td, 9.8, 3.4) | 38.5 |
| 2 | 1.60 (m), 1.43 (overlap.) | 27.0 | 1.58 (m), 1.43 (overlap.) | 30.9 |
| 3 | 3.50 (dd, 12.0, 4.6) | 89.4 | 4.76 (dd, 11.4, 4.8) | 81.4 |
| 4 | – | 39.0 | – | 38.8 |
| 5 | 1.19 (d, 10.2) | 61.7 | 1.14 (d, 10.8) | 61.3 |
| 6 | 4.37 (td, 10.2, 3.0) | 67.5 | 4.30 (overlap.) | 67.4 |
| 7 | 2.04 (overlap.) | 47.4 | 1.86 (dd, 10.8, 2.4) | 47.4 |
| | 1.98 (t, 10.8) | | 1.81 (overlap.) | |
| 8 | – | 40.5 | – | 41.2 |
| 9 | 1.55 (overlap.) | 50.0 | 1.47 (overlap.) | 49.7 |
| 10 | – | 41.0 | – | 39.1 |
| 11 | 2.07 (overlap.), 1.56 (overlap.) | 31.3 | 1.96 (overlap.), 1.45 (overlap.) | 30.8 |
| 12 | 3.96 (m) | 70.9 | 4.13 (m) | 70.2 |
| 13 | 2.04 (overlap.) | 48.2 | 1.92 (overlap.) | 49.2 |
| 14 | – | 51.6 | – | 51.7 |
| 15 | 1.64 (overlap.), 1.09 (overlap.) | 31.4 | 1.58 (overlap.), 0.97 (m) | 31.0 |
| 16 | 1.77 (m), 1.35 (m) | 26.8 | 1.78 (overlap.), 1.29 (m) | 26.7 |
| 17 | 2.36 (overlap.) | 54.7 | 2.50 (dd, 10.8, 8.0) | 51.4 |
| 18 | 1.08 (3H, s) | 17.4 | 1.60 (3H, s) | 17.9 |
| 19 | 0.95 (3H, s) | 17.3 | 0.93 (3H, s) | 17.4 |
| 20 | – | 72.9 | – | 83.4 |
| 21 | 1.43 (3H, s) | 26.5 | 1.60 (3H, s) | 22.4 |
| 22 | 2.05 (overlap.), 1.71 (m) | 35.8 | 2.35 (td, 13.8, 3.8), 1.76 (overlap.) | 36.2 |
| 23 | 2.63 (m), 2.30 (overlap.) | 23.0 | 2.48 (m), 2.22 (m) | 23.3 |
| 24 | 5.34 (t, 6.0) | 126.2 | 5.21 (t, 6.0) | 126.1 |
| 25 | – | 130.7 | – | 131.1 |
| 26 | 1.67 (3H, s) | 25.8 | 1.59 (3H, s) | 25.9 |
| 27 | 1.64 (3H, s) | 17.6 | 1.11 (3H, s) | 17.4 |
| 28 | 2.10 (3H, s) | 32.0 | 1.60 (3H, s) | 31.3 |
| 29 | 1.43 (3H, s) | 16.9 | 1.29 (3H, s) | 17.1 |
| 30 | 1.00 (3H, s) | 17.0 | 0.95 (3H, s) | 17.4 |
| HCO | – | – | 8.42 (1H, s) | 162.1 |
| Glc-1′ | 5.03 (d, 7.8) | 107.2 | 5.16 (d, 7.8) | 98.4 |
| 2′ | 4.11 (m) | 75.9 | 3.97 (t, 8.3) | 75.3 |
| 3′ | 4.28 (overlap.) | 78.7 | 4.21 (t, 8.3) | 79.5 |
| 4′ | 4.25 (overlap.) | 71.8 | 4.15 (t, 8.3) | 71.7 |
| 5′ | 4.03 (m) | 78.3 | 3.90 (ddd, 8.3 5.3, 2.5) | 78.5 |
| 6′ | 4.62 (dd, 11.5, 2.5), 4.44 (dd, 11.5, 5.4) | 63.0 | 4.30 (overlap.), 4.46 (dd, 5.7, 11.2) | 63.0 |

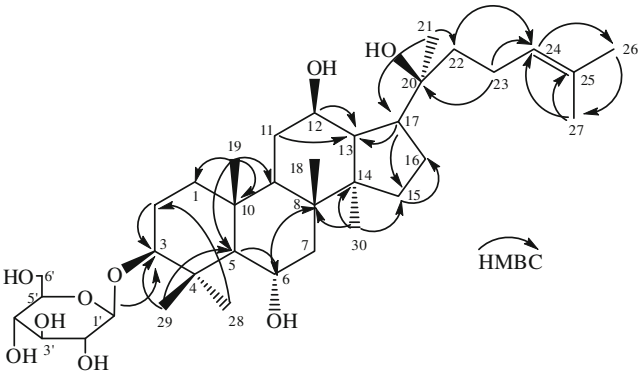


**Fig. 2** Key HMBC correlations of compound **1**

72.9 indicated that the hydroxyl groups were presented at C-6, C-12 and C-20 respectively. The D-glucose unit was identified by comparison with an authentic sample of D-glucose (No. 110833-200302) on TLC after enzymatic hydrolysis of **1** using $\beta$-D-glucosidase which could furnish the original unit [18]. According to the coupling constant of the anomeric proton [$\delta_H$ 5.03 (1H, d, $J = 7.8$ Hz)], the anomeric configuration was identified as the $\beta$ form. In the HMBC spectrum (Fig. 2), a crossing peak between the anomeric proton and carbon C-3 ($\delta_C$ 89.4) meant that glucose was linked with the C-3 position. On the basis of the coincidence of chemical shifts in C-17 ($\delta_C$ 54.7), C-21 ($\delta_C$ 26.5) and C-22 ($\delta_C$ 35.8) with those for 20(*S*)-ginsenoside $Rh_1$ (**4**), the absolute configuration of the C-20 was determined to be *S* orientation [13]. Finally, compound **1** was elucidated as 3-*O*-$\beta$-D-glucopyranosyl-20(*S*)-protopanaxtriol.

Compound **2** was obtained as a white powder (MeOH). Its (HR)ESI-MS at *m/z* 689.4238 $[M+Na]^+$ revealed a molecular formula of $C_{37}H_{62}O_{10}$ (calcd. for $C_{37}H_{62}O_{10}Na$, 689.4235). Its IR spectrum displayed absorption bands arising from hydroxyl (3406 $cm^{-1}$), carbonyl (1719 $cm^{-1}$) and double bond (1650 $cm^{-1}$). As well as these data from IR and HR-MS experiments, compound **2** could be deduced as a formyl ester derivative of ginsenoside $F_1$(**6**) not only because of the similar signals among their spectra but also because of the presence of a proton signal at $\delta_H$ 8.42 (1H, s) and a carbonyl group at $\delta_C$ 162.1 (600 MHz for $^1$H NMR, 150 MHz for $^{13}$C NMR, $C_5D_5N$) (Table 1). The HMBC spectrum showed a long-range correlation between $\delta_H$ 8.42 (1H, s) and a carbon at $\delta_C$ 81.4 (C-3), which indicted the formyloxy group linked with C-3. Compound **2** was therefore determined to be 3-formyloxy-ginsenoside $F_1$. The glucose unit was attached to C-20 ($\delta_C$ 83.4) on the basis of long-range correlation between C-20 and the anomeric proton [$\delta_H$ 5.16 (1H, d, $J = 7.8$ Hz)]. By means of the coincidence of chemical shifts in C-17 ($\delta_C$ 51.4), C-21 ($\delta_C$ 22.4) and C-22 ($\delta_C$ 36.2) with those for ginsenoside $F_1$, the absolute configuration of

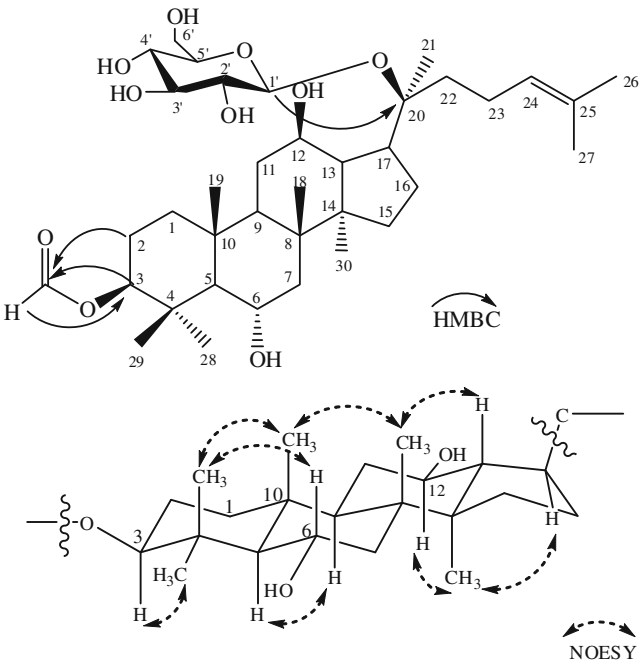


**Fig. 3** Key HMBC and NOE correlations of compound **2**

C-20 was determined to be *S* orientation. Moreover, the mutual correlations of $CH_3$-29/$CH_3$-19 and H-6 in the NOESY spectrum (Fig. 3) agreed with all $\beta$-orientations of $CH_3$-29, $CH_3$-19 and H-6 in the structure, and the correlation between $CH_3$-28 and H-3 confirmed that this formyloxy group should be placed with an $\beta$-orientation. Thus, compound **2** was identified as 3-formyloxy-20-*O*-$\beta$-D-glucopyranosyl-20(*S*)-protopanaxtriol.

Compound **3** gave the molecular formula $C_{30}H_{52}O_5$ by its pseudo molecular ion $[M+H]^+$ at *m/z* 493.3888 (calcd. for $C_{30}H_{53}O_5$, 493.3893) in the (HR)ESI-MS spectrum. The IR (KBr) spectrum showed hydroxyl absorption at 3406 $cm^{-1}$ and double bond at 1667 $cm^{-1}$. In its $^1$H NMR (600 MHz, $C_5D_5N$) (Table 2) spectra, seven methyls were observed at $\delta_H$ 0.97, 1.00, 1.10, 1.44, 1.47, 1.83, 2.00 (3H each, s), respectively. In addition, one olefinic proton signal at $\delta_H$ 5.86 (1H, t, $J = 6.0$ Hz) and two protons at $\delta_H$ 4.31 (2H, br s) for one oxomethylene were found. The $^{13}$C NMR (150 MHz, $C_5D_5N$) spectrum exhibited 30 carbon signals. Two signals at $\delta_C$ 125.7 (C-24), 136.2 (C-25) and five signals at $\delta_C$ 61.8 (C-5), 78.5 (C-3), 67.7 (C-6), 71.1 (C-12), 73.0 (C-20) indicated that compound **3** was also a derivative of protopanaxtriol (PPT) [19], which was completely confirmed by its HSQC and HMBC spectral analysis (Table 2). Moreover, the long-range correlations between $H_{a,b}$-26 ($\delta_H$ 4.31) with C-27 ($\delta_C$ 14.0), and H-24 ($\delta_H$ 5.86) with C-26 ($\delta_C$ 68.2) suggested the presence of a hydroxyl group at C-26. In addition, the NOE correlation between $H_{a,b}$-26 and H-24 in the ROESY spectrum (Fig. 4) indicated that the double bond at C-24 was a "*trans*-form".

**Table 2** $^{1}$H-NMR and $^{13}$C-NMR spectral data for compound **3** ($\delta$: ppm, $J$ in Hz, $C_5D_5N$) and carbon spectral data for 27-hydroxyl-20(*S*)-protopanaxatriol as reference

| No. | Compound 3 | | | 27-Hydroxyl-20(*S*)-protopanaxatriol [20][a] |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | HMBC (H → C) | $\delta_C$ |
| 1 | 1.68 (overlap.), 1.02 (overlap.) | 39.3 | C-10, 19 | 39.3 |
| 2 | 1.92 (overlap.), 1.88 (overlap.) | 28.1 | C-1, 3, 4, 28 | 28.1 |
| 3 | 3.52 (dd, 12.0, 6.0) | 78.5 | C-29 | 78.4 |
| 4 | – | 40.3 | | 40.3 |
| 5 | 1.24 (d, 10.5) | 61.8 | C-6, 7, 19, 29 | 61.8 |
| 6 | 4.42 (td, 15.0, 6.0) | 67.7 | C-4, 5, 7 | 67.7 |
| 7 | 1.99 (dd, 17.5, 4.0), 1.92 (dd, 17.5, 5.0) | 47.4 | C-8, 14 | 47.5 |
| 8 | – | 41.1 | | 41.1 |
| 9 | 1.60 (overlap.) | 50.1 | C-1, 10 | 50.1 |
| 10 | – | 39.3 | | 39.3 |
| 11 | 2.17 (dd, 6.6, 3.0), 1.59 (overlap.) | 32.0 | C-9, 13 | 32.0 |
| 12 | 3.94 (m) | 71.1 | C-13 | 71.0 |
| 13 | 2.04 (overlap.) | 48.2 | C-17 | 48.2 |
| 14 | – | 51.6 | | 51.6 |
| 15 | 1.58 (overlap.), 1.05 (overlap.) | 31.3 | C-17, 30 | 31.3 |
| 16 | 1.87 (overlap.), 1.38 (overlap.) | 26.8 | C-15 | 26.8 |
| 17 | 2.35 (dd, 9.0, 3.0) | 54.7 | C-20, 21 | 54.7 |
| 18 | 1.10 (3H, s) | 17.4 | C-7, 8, 14 | 17.6 |
| 19 | 1.00 (3H, s) | 17.5 | C-1, 5, 9, 10 | 17.5 |
| 20 | – | 73.0 | | 72.9 |
| 21 | 1.44 (3H, s) | 27.1 | C-17, 20, 22 | 27.0 |
| 22 | 2.10 (overlap.), 1.75 (overlap.) | 35.7 | C-21, 24 | 36.2 |
| 23 | 2.70 (m), 2.38 (m) | 22.6 | C-20 | 22.5 |
| 24 | 5.86 (t, 6.0) | **125.7** | C-21, 23, 26, 27 | **127.9** |
| 25 | – | 136.2 | C-26, 27 | 136.2 |
| 26 | 1.83 (3H, s) | **68.2** | C-27, | **21.9** |
| 27 | 4.31 (2H, br s) | **14.0** | C-26 | **60.8** |
| 28 | 2.00 (3H, s) | 32.0 | C-2 | 32.0 |
| 29 | 1.47 (3H, s) | 16.4 | C-3, 5 | 16.5 |
| 30 | 0.97 (3H, s) | 17.0 | C-8, 14, 15 | 17.0 |

Bold values indicate an attention on the difference between compound 3 and the reference

[a] 125 MHz, in pyridine-$d_5$

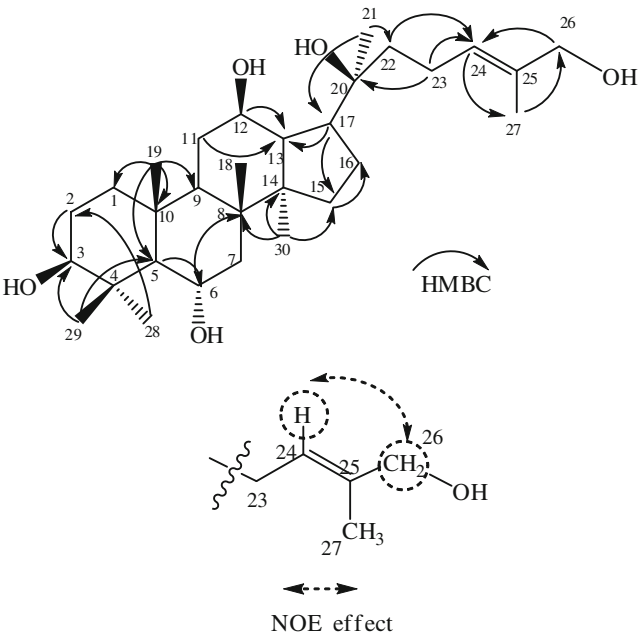


**Fig. 4** Key HMBC correlations of compound **3**, NOE correlation between $H_{a,b}$-26 and H-24 in the ROESY spectrum

Here, an interesting association between **3** and a known compound 27-hydroxyl-20(*S*)-protopanaxatriol [20] was found, and their almost identical signal patterns except for three carbons at C-24, C-26 and C-27 [$\delta_C$ 125.7, 68.2, 14.0 in compound **3** versus $\delta_C$ 127.9, 21.9, 60.8, respectively, in 27-hydroxyl-20(*S*)-protopanaxatriol (Table 2)] indicated that compound **3** was a isoformer of 27-hydroxyl-20(*S*)-protopanaxatriol of C-24 double bond. However, according to detailed information from refs. [21, 22], it was appropriate for the known structure to be changed to a *cis*-form at C-24 and named as 27-hydroxyl-24(*Z*)-20(*S*)-protopanaxtriol. This conclusion could be drawn by the coincident chemical shifts for C-26, 27, which were recorded as $\delta_C$ 21.8, 60.9 in the similar derivatives with a *cis*-form (*Z*-isomer) at the C-24 position, whereas in the *trans*-form (*E*-isomer) derivatives they were recorded as $\delta_C$ 68.2, 14.0, respectively. In addition, NOE correlations in the ROESY spectrum (Fig. 4) showed that compound **3** was a *trans*-isomer of 27-hydroxyl-24(*Z*)-20(*S*)-protopanaxtriol, and its structure was finally determined to be 26-hydroxyl-24(*E*)-20(*S*)-protopanaxtriol.

## Cell growth inhibition by compounds

Human A549 and Hep3B cells were cultured with 0.1–100 μM of compounds **1**–**3** and compound **9** [20(*R*)-ginsenoside $Rg_3$] for 72 h, and the effects on growth inhibition were measured. Compound **3** exhibited the most potent cytotoxicity towards the two cell lines, with $IC_{50}$ values of 27.9 μM and 31.4 μM, respectively. Compounds

**1** and **2** showed moderate inhibition or weak effect on cell growth at the same concentrations. It is worth mentioning that compounds **1**–**3** showed more effective inhibition than 20(*R*)-ginsenoside $Rg_3$. The concentrations of ginsenosides applied in some in-vitro experiments are as high as 100 μM level in order to produce any biological effects; moreover, a place for compound **9** in clinical treatments as an anti-tumor agent implies potential for compounds **1**–**3** with much greater inhibitory activity, but this conclusion must be proven by further experiments in vivo in the continuing work.

## Experimental section

### General experimental procedures

The infrared ray (IR) spectra were recorded on a Bruker IFS-55 Fourier transform infrared spectrometer. Optical rotations were recorded on a PerkinElmer 341 polarimeter at room temperature. NMR spectra ($^1$H, $^{13}$C, and 2D NMR) were obtained on Bruker Avance 300 and AV-600 instruments (Bruker, Rheinstetten, Germany) with TMS as internal standards. The high resolution electrospray ionization mass spectra (HRESI-MS) were recorded on a Bruker micro-TOF-Q mass spectrometer. HPLC were carried out on a Yonglin SP930D liquid chromatograph equipped with a UV730D UV/VIS detector using a Migtysil ODS-RP column [250 × 20 mm (5 μm); Kanto Chemical Inc.] and monitored at 210 nm. Column chromatography was performed on macroreticulated absorption resin of AB-8, Si gel G (200–300 mesh, Qingdao Ocean Chemical Inc., Qingdao, China), using Sephadex LH-20 (Pharmacia, Kalamazoo, MI, USA), and reversed-phase Si gel (Chromatorex C18, Fuji Silysia Chemical, Kasugai, Japan). A Microplate Reader (TECAN, Austria) was used for MTT assay. D-Glucose was purchased from the Natural Institute for the Control of Pharmaceutical and Biological Products (No. 110833-200302, China), and *β*-D-glucosidase was obtained from Sigma Company (Shanghai, China).

### Plant material

Leaves of *Panax ginseng* were collected in Jilin province in August 2008, and were provided by Fusong County Natural Biotechnology Co., Ltd. The specimen was identified by Professor Qishi Sun (Shenyang Pharmaceutical University, Shenyang, China) and a voucher specimen (No. 2008-PG-09) has been deposited in the same department.

### Extraction and isolation

Air-dried leaves of *P. ginseng* (2.5 kg) were extracted with 95 % ethanol three times at room temperature (15 L each time), and the solution was concentrated by vacuum to obtain an extract (752 g), which was subjected to a macroreticulated absorption resin in AB-8 column and thoroughly washed with water, then eluted with 30, 70 and 90 % EtOH (v/v) successively. The 70 % EtOH eluate (135 g) was chromatographed on a silica gel column with a gradient $CHCl_3$–MeOH solvent system (100:2, 100:5, 100:10, 100:20, 100:30, 100:50, 100:100, 0:100, each 3 L) to give seven fractions (Fr. 1–7). Fr. 3 ($CHCl_3$–MeOH 100:10, 13.7 g) was further subjected to reverse-phase ODS silica gel column chromatography using MeOH–$H_2O$ (45:55–70:30, v/v) to furnish three sub-fractions [SubFr. 3.1 (8.0 g), 3.2 (0.6 g), 3.3 (1.1 g)]. SubFr. 3.2 (0.6 g) was also applied to HPLC using an ODS column and 73 % MeOH to give compounds **1** (14 mg), **2** (30 mg), **7** (10 mg) and **8** (37 mg); 3 g of SubFr. 3.1 was repeatedly purified on HPLC (MeOH–$H_2O$, UV210 detection) using a ODS column and 78 % MeOH solution to furnish compound **3** (4 mg), and using 67 % MeOH solution to furnish compounds **4** (15 mg), **5** (15 mg) and **6** (110 mg); Fr. 4 ($CHCl_3$–MeOH 100:20, 27.6 g) was subjected to reverse-phase silica gel column chromatography using MeOH–$H_2O$ (40:60–70:30, v/v) to give four sub-fractions [SubFr. 4.1 (13.2 g), 4.2 (1.9 g), 4.3 (5.1 g), 4.4 (0.4 g)]. Compound **9** (17 mg) was obtained from SubFr. 4.3 by Si gel column chromatography with $CHCl_3$–MeOH (8:1) as an eluate and then recrystallized with mixed solvent ($CHCl_3$–MeOH 6:1).

### 3-*O*-*β*-D-glucopyranosyl-20(*S*)-protopanaxtriol (**1**)

White powder (MeOH), m.p. 220 °C, $\alpha_D^{21}$ +35.0 (*c* 0.1, MeOH), IR(KBr) $\nu_{max}$ 3406(OH), 2949, 1650 (C=C), 1453, 1385, 1030, 1019 cm$^{-1}$, HRESI-MS: *m/z* 661.4284 $[M+Na]^+$ (calcd. for $C_{36}H_{63}O_9Na$, 661.4286); $^1$H NMR (600 Mz, $C_5D_5N$) and $^{13}$C NMR (150 Mz, $C_5D_5N$) data see Table 1.

### Enzymatic hydrolysis of **1**

A solution of compound **1** (5.0 mg) in 0.2 M acetate buffer (pH 4.5, 3.0 mL) was treated with *β*-D-glucosidase (15 mg), and then stirred at 37 °C for 72 h. After treatment of the reaction mixture with EtOH, the mixture was evaporated to dryness under reduced pressure and the residue was dissolved in MeOH and analyzed by TLC with developing agent $CHCl_3$:MeOH:$H_2O$ (30:10:1); spots were visualized by spraying the plates with 10 % $H_2SO_4$ solution.

### 3-Formyloxy-20-*O*-*β*-D-glucopyranosyl-20(*S*)-protopanaxtriol (**2**)

White powder (MeOH), m.p. 202–204 °C, $\alpha_D^{21}$ +45.5 (*c* 0.1, MeOH). IR (KBr) $\nu_{max}$ 3406 (OH), 2956, 1720 (C=O),

**Table 3** Growth inhibition of compounds **1–3** and **9** against human A549 and Hep3B cells

| Cells | $IC_{50}^{a}$ (μM) | | | |
|---|---|---|---|---|
| | **1** | **2** | **3** | **9** |
| A549 | 63.9 | 48.7 | 27.9 | >100 |
| Hep3B | >100 | 66.5 | 31.4 | >100 |

[a] $IC_{50}$ values were calculated from dose-dependent inhibition curves

1650 (C=C), 1456, 1390, 1187, 1076, 1044, 1013 $cm^{-1}$, HRESI-MS: *m/z* 689.4238 $[M+Na]^+$ (calcd. for $C_{37}H_{62}O_{10}Na$, 689.4235); $^1H$ NMR (600 MHz, $C_5D_5N$) and $^{13}C$ NMR (150 Mz, $C_5D_5N$) data see Table 1.

26-Hydroxyl-24(*E*)-20(*S*)-protopanaxtriol (**3**)

White powder (MeOH), m.p. 153–155 °C, $\alpha_D^{21}$ +28.8 (*c* 0.1, MeOH), IR (KBr) $\nu_{max}$ 3270 (OH), 2926, 1673 (C=C), 1455, 1384, 1030 $cm^{-1}$, HRESI-MS: 493.3888 *m/z* $[M+H]^+$ (calcd. for $C_{30}H_{53}O_5$, 493.3893); $^1H$ NMR (600 Mz, $C_5D_5N$) and $^{13}C$ NMR (150 Mz, $C_5D_5N$) see Table 2.

Cell culture

Human A549 and Hep3B cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in RPMI-1640 medium (Gibco, NY, USA) supplemented with 10 % fetal calf serum (Shengma Yuanheng, Beijing, China), 100 mg/L streptomycin, 100 IU/mL penicillin, and 0.03 % L-glutamine, and maintained at 37 °C with 5 % $CO_2$ in a humidified atmosphere (Table 3).

Cytotoxicity assays

Compounds **1–3** and **9** were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions. The DMSO concentration was kept below 0.05 % throughout the cell culture period and did not exert any detectable effect on cell growth or cell death. Human A549 and Hep3B cells were incubated at $3 \times 10^4$ cells per well in 96-well plates (Nunc, Roskilde, Denmark). The cells were then treated with compounds **1–3** and **9** at various doses (0.1–100 μM) for 72 h. Cell growth was measured with a plate reader (Tecan Spectra, Wetzlar, Germany) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay [23, 24] at different time points. The percentage of cell growth inhibition was calculated as follows: Cell death (%) = $[A_{492}$ (control) − $A_{492}$ (compound)]/$A_{492}$ (control) × 100.

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