Four new taraxastane-type triterpenoic acids from *Cirsium setosum*

Na Luana, Wen-Di Weia1, Ali Wanga, Xiu-Li Wub, Yan Qic, Jin-Jie Lia, Jian-Quan Zhenga and Xiao-Ya Shanga

aBeijing Key Laboratory of Bioactive Substances and Functional Foods, Beijing Union University, Beijing 100191, China; bCollege of Pharmacy, Ningxia Medical University, Yinchuan 750004, China; cBeijing Municipal Bureau of City Administration and Law Enforcement, Beijing 101500, China

**ABSTRACT**

Four new taraxastane-type triterpenoids acids 3β,22α-dihydroxy-20-taraxasten-30-oic acid (1), 3β-hydroxy-22-oxo-20-taraxasten-30-oic acid (2), 3-oxo-22α-hydroxy-20-taraxasten-30-oic acid (3), and 3β,19β-dihydroxy-20-taraxasten-30-oic acid (4) were isolated and characterized from *Cirsium setosum* (Willd.) MB. Their structures were determined by the combination of 1D and 2D NMR experiments (1H-1HCOSY, HSQC, HMBC and ROESY) and mass spectrometry. Compound 2 exhibited potent selective cytotoxicity against human ovarian cancer cell line A2780 with an IC$_{50}$ value of 3.9 μM.

**1. Introduction**

*Cirsium setosum* (Willd.) MB., a plant belonging to the Compositae family, is widely distributed in Anhui, Shandong, and Gansu provinces of China [1]. It has been used traditionally for treatments of various diseases, such as hemorrhage, hemostasis, hematuria, hemafecia, uterine bleeding, wounds healing, and swollen sores [1]. There were more than 300 patents reported on its medical uses. In 95% of the patents, *C. setosum* was used with other medicinal herbs in different ways and showed good efficacy. Its ethanol extract was examined for *in vitro* and *in vivo* pharmacological activities and demonstrated to have antioxidation, hemostasis, antibacterial, anti-inflammatory, antihypertension, anticancer, heart protection, and hepatoprotective activities [2–6]. Following *in vitro* MTT pharmacological screening, we found the petroleum ether (PE) fraction of the crude extract showed strong cytotoxicity [7]. By further fractionation guided by MTT pharmacological screening, we isolated and characterized three α-tocopheroid-type compounds from the PE fraction with potent cytotoxicity and the new chemical structure of the most potent one has not been reported before [8,9]. We have also isolated 5 steroids and 14 triterpenoids, among them three keto and alcohol derivatives of taraxerone of dandelion showed strongest cytotoxicity [10,11]. As a continuing effort, four new cytotoxic taraxastane-type triterpenoids acid 3β,22α-dihydroxytaraxast-20-en-30-oic...
acid (1), 3β-hydroxy-22-oxo-20-taraxasten-30-oic acid (2), 3-oxo-22α-hydroxy-20-taraxasten-30-oic acid (3), and 3β,19β-dihydroxy-20-taraxasten-30-oic acid (4) were isolated and characterized. Compound 2 showed potent selective cytotoxicity against human ovarian cancer cell line A2780 with an IC50 value of 3.9 μM.

2. Results and discussion

The PE-soluble portion of the ethanolic extract of C. setosum was subjected to column chromatography on macroporous adsorbent resin, normal phase and reversed phase silica gels and Sephadex LH-20, successively to afford three major components which were further purified by preparative reversed phase HPLC to yield compounds 1–4.

Compound 1 was obtained as white amorphous powder. Its molecular formula C30H48O4, with 7 degrees of unsaturation was indicated by HREIMS at m/z 472.3516 [M]+ combined with the NMR data (Table 1). The IR spectrum of 1 suggested that it contained hydroxyl groups (3358, 3599 and 3674 cm−1) and a conjugated carbonyl group (1695 and 1657 cm−1). The 1H NMR spectrum of 1 in CD5D5N displayed signals for six singlet methyl groups (δH 0.85, 0.93, 1.06, 1.09, 1.12 and 1.25), a doublet methyl group [δH 1.45 (3H, d, J = 6.5 Hz)], two carbinol methine protons [δH 3.48 (1H, dd, J = 10.3, 5.8 Hz) and 3.90 (1H, d, J = 6.5 Hz)], and an olefinic resonance at [δH 7.50 (1H, d, J = 6.5 Hz)]. The 13C NMR spectrum of 1 showed 30 carbon signals, and the DEPT experiment differentiated them to be seven CH3, eight CH2, eight CH, and seven C including two oxygen-bearing carbons at δC 78.1 and δC 72.5, two olefinic carbons at δC 135.5 and δC 141.4, and one carboxyl carbonyl carbon at δC 170.8. All above spectroscopic data suggested that 1 was an oxygenated triterpenoid bearing a carboxyl group.

Comparison of the 13C NMR data (Table 1) of 1 with those of the known (3β, 22α)-3,22-dihydroxytaraxast-20-en-30-al [12,13] suggested that 1 possesses the same skeletal structure with the exception that the C-20 aldehyde was replaced by a carboxyl group (Figure 1). The structure of 1 was finally confirmed by careful analysis of its 2D NMR spectroscopic data including 1H-1H COSY and HMBC (Figure 2), for spatial assignments of all the protons and carbons.

Five structural fragments as shown with bold lines in Figure 2 (C-1 to C-3; C-5 to C-7; C-9 through C-11 to C-13, C-13 through C-18 to C-29; C-15 to C-16; and C-21 to C-22) were first established by the correlations observed in the 1H-1H COSY spectrum. The connectivity study of the five structural fragments, quaternary carbons, and the other functional groups was mainly achieved by the analysis of the HMBC spectrum (Figure 2). Long-range HMBC correlations from H3-23 (δH 1.25) to C-3, C-4, C-5 and C-24, H3-24 (δH 0.93) to C-1, C-5, C-9 and C-10, H3-26 (δH 1.09) to C-7, C-8, C-9 and C-14, H3-27 (δH 1.12) to C-8, C-13, C-14 and C-15 not only confirmed the presence of A/B/C/D-rings system but also located the Me-25 at C-10, Me-26 at C-8, and Me-27 at C-14. In addition, H-21 (δH 7.50) to C-19, C-20 and C-30, H3-29 (δH 1.45) to C-18, C-19 and C-20 established the five-membered E-ring. The planar structure of 1 was, therefore, determined as 3,22-dihydroxytaraxast-20-en-30-oic acid (Figure 1).

The relative stereochemistry of 1 was elucidated by a careful analysis of the NOESY spectrum and compared with known structure of 3β,22α-dihydroxytaraxast-20-en-30-al [12] as shown in Figure 2. NOE correlations between H-3/H3-23 and H-5, H-5/H-9, H3-27/ H-18, H-18/ H3-29 revealed that these protons were cofacial and defined as having an α-orientation, whereas H3-24/H3-25, H3-25/H3-26, H3-26, H3-28 and H-19/H-13,
Table 1. $^{1}$H and $^{13}$C NMR data for compounds 1–4.

| No. | 1                  | 2                  | 3                  | 4                  |
|-----|--------------------|--------------------|--------------------|--------------------|
| 1   | $^{1}$H (mult, J, Hz) | $^{13}$C (mult)    | $^{1}$H (mult, J, Hz) | $^{13}$C (mult)    |
|     | $^{1}$H (mult, J, Hz) | $^{13}$C (mult)    | $^{1}$H (mult, J, Hz) | $^{13}$C (mult)    |
| 1   | (a) 0.99, ddd (13.0, 13.0, 3.5) | 39.2 | (a) 0.99, m | 39.2 | (a) 1.38, m | 39.6 | (a) 0.95, m | 39.2 |
| 2   | 1.71, dt (13.0, 3.5) | (b) 1.70, dt (12.5, 3.0) | 1.89, m | 1.88, m | 28.3 | 1.88, m | 28.5 | (a) 1.43, m | 34.3 |
| 3   | 3.48, dd (10.3, 5.8) | 3.49, dd (10.5, 5.5) | 78.1 | 3.49, dd (10.5, 5.5) | 78.0 | 1.36, m | 54.8 | (a) 0.83, d (11.5) | 55.8 |
| 4   | 0.83, br d (10.0) | 0.79, dd (11.5, 2.0) | 1.40, m | 18.7 | 1.42, m | 19.8 | (a) 1.45, m | 18.8 |
| 5   | (a) 1.43, m$^a$ | (b) 1.59, m | 34.6 | 1.37, m | 34.5 | 1.42, m | 33.7 | (a) 1.35, m | 34.4 |
| 6   | 1.42, m | (a) 1.43, ma | 1.87, m | 18.7 | 1.40, m | 18.7 | (a) 1.42, m | 18.3 |
| 7   | 2.55, m | 1.43, m$^a$ | 34.6 | 1.37, m | 34.5 | 1.42, m | 33.7 | (a) 1.35, m | 34.4 |
| 8   | 3.49, dd (10.5, 5.5) | 78.0 | 1.36, m | 54.8 | (a) 0.83, d (11.5) | 55.8 |
| 9   | 0.83, br d (10.0) | 0.79, dd (11.5, 2.0) | 1.40, m | 18.7 | 1.42, m | 19.8 | (a) 1.45, m | 18.8 |
| 10  | (a) 1.23, m | (b) 1.54, m | 21.7 | 1.63, m | 28.1 | 1.24, m | 22.2 | (a) 1.36, m | 21.6 |
| 11  | (a) 1.19, m | (b) 1.82, m | 27.9 | 1.24, m | 21.7 | (a) 1.18, m$^a$ | 27.8 | (a) 1.87, m$^a$ | 28.3$^a$ |
| 12  | (a) 1.82, m | (b) 1.51, m | 1.81, m | 2.66, d (14.5) | 1.83, m | 1.97, m | 35.5 | 43.0 |
| 13  | 38.9 | 1.91, m | 38.5 | 1.82, m | 39.0 | 1.97, m | 35.5 | 43.0 |
| 14  | 42.5 | 42.0 | 42.5 | 42.5 | 42.5 | 42.5 | 42.5 | 42.5 |
| 15  | (a) 1.17, m$^a$ | (b) 1.84, m | 27.1 | 1.09, m | 26.5 | (a) 1.18, m$^a$ | 27.1 | (a) 1.03, m | 27.1 |
| 16  | 30.2 | (a) 1.75, m | 28.2 | (a) 1.35, m | 30.1 | (a) 1.18, m | 39.0 | 43.0 |
| 17  | (b) 2.53, td (14.0, 4.5) | (b) 1.89, m | 38.7 | 45.7 | 39.7 | 1.97, m | 35.5 | 43.0 |
| 18  | 2.08, dd (10.8, 7.8) | 40.9 | 1.51, m | 45.2 | 2.08, m | 40.9 | 1.97, m | 50.0 |
| 19  | 2.77, m | 32.5 | 2.87, m | 32.6 | 2.76, m | 32.5 | 1.97, m$^a$ | 50.0 |
| 20  | 141.8 | 141.4 | 154.8 | 141.8 | 141.8 | 141.8 | 141.8 | 141.8 |
| 21  | 7.50, d (6.5) | 135.5 | 6.98, d (1.5) | 127.3 | 7.48, d (4.8) | 134.5 | 7.18, s | 136.9 |
| 22  | 3.90, d (6.5) | 72.5 | 206.1 | 3.88, d (4.8) | 72.5 | (a) 1.89, br d (19.0) | 43.0 | (b) 2.04, br d (19.0) | 43.0 |
| 23  | 1.25, s | 28.6 | 1.22, s | 28.6 | 1.15, s | 26.7 | 1.25, s | 28.6 |
| 24  | 1.06, s | 16.3$^a$ | 1.03, s | 16.3 | 1.07, s | 21.2 | 1.07, s | 16.3 |

(Continued)
| No. | $\delta_1$ (mult, $J$, Hz) | $\delta_C$ (mult) | $\delta_2$ (mult, $J$, Hz) | $\delta_C$ (mult) | $\delta_3$ (mult, $J$, Hz) | $\delta_C$ (mult) | $\delta_4$ (mult, $J$, Hz) | $\delta_C$ (mult) |
|-----|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 25  | 0.93, s         | 16.6            | 0.90, s         | 16.5            | 0.91, s         | 16.1            | 0.94, s         | 16.7            |
| 26  | 1.09, s         | 16.3$^a$        | 1.03, s         | 16.1            | 1.06, s         | 16.0            | 1.11, s         | 16.0            |
| 27  | 1.12, s         | 14.9            | 0.88, s         | 14.5            | 1.09, s         | 14.7            | 1.04, s         | 15.9            |
| 28  | 0.85, s         | 18.4            | 1.01, s         | 18.2            | 0.83, s         | 18.4            | 0.92, s         | 20.8            |
| 29  | 1.45, d (6.5)   | 24.1            | 1.27, d (6.5)   | 23.8            | 1.43, d (6.5)$^a$ | 24.1            | 1.76, s         | 22.8            |
| 30  | 170.8           |                 | 169.9           | 171.5           |                 |                 |                 |                 |

Notes: $^1$H and $^13$C data were measured in C$_6$D$_5$N for 1–4 at 500 and 125 MHz. The assignments were based on DEPT, $^1$H-$^1$H COSY, HSQC, and HMBC experiments. $^a$overlapped NMR data.
H$_3$-28/H-22 were in β-orientation, and the OH-22 possessed α-configuration, respectively. Based on the above spectral evidence, the structure of 1 was unambiguously established as 3β,22α-dihydroxytaraxast-20-en-30-oic acid.

Compound 2 was obtained as a white amorphous powder. The chemical structure of 2 was very similar to compound 1, except the C-22 oxygen-bearing carbons at δ$_C$ 72.5 were replaced by a carbonyl carbon at δ$_C$ 206.1, and the proton signal of H-22 at δ$_H$ 3.90 (1H, d, J = 6.5 Hz) disappeared in $^1$H NMR spectrum (Table 1). The HREIMS at m/z 470.3359 [M]$^+$, corresponded a formula of C$_{30}$H$_{46}$O$_4$, indicating eight degrees of unsaturation which was in agreement with the NMR spectrum. Therefore, the structure of 2 was unambiguously established as 3β-hydroxy-22-oxo-20-taraxasten-30-oic acid, and the full assignments of $^1$H and $^{13}$C NMR data (Table 1) were achieved.

Compound 3 was obtained as a white amorphous powder. The NMR spectral data of 3 were very similar to those of compound 1 with the exception of C-3 oxygen-bearing carbon at δ$_C$ 78.1 was replaced by a carbonyl carbon at δ$_C$ 216.5, and the proton signal of H-3 at δ$_H$
3.48 (1H, dd, J = 10.3, 5.8 Hz) disappeared in $^1$H NMR spectrum (Table 1). The molecular formula of C$_{30}$H$_{46}$O$_4$, with eight degrees of unsaturation was supported by HREIMS at m/z 470.3404 [M]$^+$. In combination with the NMR data (Table 1), the structure assignment of 3 was unambiguously established as 3-oxo-22α-hydroxy-20-taraxasten-30-oic acid which is in full agreements of $^1$H and $^{13}$C NMR data shown in Table 1.

Compound 4 was obtained as a white amorphous powder. The molecular formula of C$_{30}$H$_{48}$O$_4$, as determined by HREIMS at m/z 472.3542 [M]$^+$ with seven degrees of unsaturation was exactly the same with compound 1, and the spectroscopic data were very close to 1, indicating that compound 4 is an isomer of 1. Careful analysis of spectroscopic data of these two compounds revealed that the hydroxyl group is located at C-19 in compound 4 and at C-22 in compound 1. The key HMBC correlations from Me-28 to C-16, C-17, C-18 and C-22, H-18 to C-13, C-14, C-17, C-19, C-22, C-28 and C-29, H-21 to C-17, C-19, C-20, C-22 and C-30, Me-29 to C-18, C-19 and C-20, located the Me-29 and a hydroxyl group at C-19 (Figure 3). The planar structure of 4 was thus established as shown in Figure 1.

The relative stereochemistry of 4 was elucidated by the analysis of the NOESY spectrum as shown in Figure 3, and compared with 1, 2, and 3 based on the same NOE correlations (Figure 3). Accordingly, the structure of 4 was determined to be 3β,19β-dihydroxy-20-taraxasten-30-oic acid.

Compounds 1–4 were evaluated in vitro for their cytotoxicities against five human cancer cell lines: colon cancer (HCT8), hepatoma (Bel7402), stomach cancer (BGC823), lung adenocarcinoma (A549), and ovarian cancer (A2780) using the MTT method with topotecan as a positive control and human epithelial WISH cell line as a normal control. In this test, IC$_{50}$ values of greater than 20 μM were defined as inactive. The results showed that 2 were highly selective and potent cytotoxicity against human cancer cell line A2780 with an IC$_{50}$ value of 3.9 μM (Table 2).

![Figure 3. Main HMBC (arrows), and NOESY (double arrows) correlations of compound 4.](image-url)

| Compound | HCT-8 | Bel7402 | BGC-823 | A549 | A2780 |
|----------|-------|---------|---------|------|-------|
| 1        | >20   | >20     | >20     | >20  | 10.2  |
| 2        | >20   | >20     | >20     | >20  | 3.9   |
| 3        | >20   | >20     | >20     | >20  | 17.8  |
| 4        | >20   | >20     | >20     | >20  | 12.4  |
| Topotecan| 1.7   | 1.3     | 4.5     | 3.2  | 1.5   |

Table 2. Cytotoxic data for compounds 1–4.
3. Experimental

3.1. General experimental procedures

IR spectra were determined as KBr disks on a Nicolet Impact 400 FT-IR spectrophotometer (Nicolet Instrument. Inc., Madison, WI, U.S.A). 1D- and 2D-NMR Spectra were acquired in C$_5$D$_5$N with TMS (HWRK Chem Co., Beijing, China) as an internal standard on Varian 500 MHz spectrometers (Bruker Corporation, Billerica, MA, U.S.A) ($^1$H, 500.06MHZ, $^{13}$C, 125.75 MHZ). Mass spectra including high-resolution mass spectra were recorded on a JEOL JMS AX-500 spectrometer (JEOL Ltd. Company, Tokyo, Japan). Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc. China), cyanopropyl silica gel (43–60 μM), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). LPLC separation was performed with Combiflash (ISCO Companion, Lincoln, NE, U.S.A). HPLC separation was performed on an instrument consisting of a Waters 2545 controller, a Waters 2545 pump, and a Waters 2487 dual λ absorbance detector (Waters Corporation, Milford, Massachusetts, U.S.A) with an x-bridge (Waters, 250 × 10 mm i.d.) preparative column packed with C$_{18}$ (5 μm) (Alltech Associates, Inc., Bannockburn, IL, U.S.A). TLC was carried out with precoated silica gel GF$_{254}$ plates (Qingdao Marine Chemical Plant, Qingdao, China). Spots were visualized under UV light or by spraying with 8% H$_2$SO$_4$ in 95% EtOH followed by heating.

3.2. Plant material

The stems of Cirsium setosum were collected at Jiuhua Mountain, Anhui province, China, in September 2008. The plant was identified by Mr. Yun-wu Ke (Chizhou Huangjing Instituent of Jiuhua Moutain, Anhui, China). A voucher specimen (20081028) has been deposited at Beijing Union University, Beijing Key Laboratory of Bioactive Substances and Functional Foods, Beijing, China.

3.3. Extraction and isolation

The air-dried stems of C. setosum (20 kg) were ground into powder and extracted with 90, 80, and 60% aqueous EtOH sequentially, at room temperature for 120 min under sonication. The extract was evaporated under reduced pressure to yield a dark brown residue, which was suspended in H$_2$O and then partitioned with PE and EtOAc, respectively. The PE-soluble portion (468.5 g) showing cytotoxic activity (IC$_{50}$ < 50 μg/ml) was fractionated via silica gel column chromatography, eluting with a gradient of acetone (0–100%) in PE (60–90 °C), to give 11 fractions (Sh1–Sh11).

Fraction Sh11 (36.9 g) was chromatographed on normal phase LPLC using a gradient of methanol in chloroform (1%-50%) to give seven (Sh11-1–Sh11-7) fractions. Subsequent separations of fraction Sh11-1 (1.3 g) over Sephadex LH-20 column, eluting with CHCl$_3$-CH$_3$OH (2.5:1), afforded three subfractions (Sh11-1-1, Sh11-1-2, Sh11-1-3). Subfraction Sh11-1-1 (0.4 g) was further purified by LPLC over normal phase cyanopropyl silica gel, eluting with PE (60–90 °C), to yield two fractions (Sh11-1-1-1, Sh11-1-1-2). Fraction Sh11-1-1-1 was heated and dissolved in chloroform. Upon cooling, the white precipitate was filtered to afford 1 (40 mg). Subsequent separations of fraction Sh11-2 (2.8 g) by preparative reversed
phase LPLC, eluting with a gradient of MeOH-H$_2$O (70:1–0:100), gave five subfractions (Sh11-2-1–Sh11-2-5). Further separation of fraction Sh11-2-4 (0.8 g) by Sephadex LH-20 column, eluting with CHCl$_3$-MeOH (2.5:1) and LPLC over normal phase cyanopropyl silica gel, eluting with PE (60–90 °C)–Me$_2$CO (50:1 to 0:100), which was then purified by preparative reversed phase HPLC, eluting with MeOH-H$_2$O-MeCOOH (850:150:1, 18.0 ml/min) to afford 2 (24.0 mg, $t_R$ 25 min) and 3 (8.0 mg, $t_R$ 14 min). Subfraction Sh11-2-4 (0.2 g) was purified by Sephadex LH-20 column, eluting with CHCl$_3$-MeOH (2.5:1) and further purified by preparative reversed-phase (C-18) HPLC using MeOH-H$_2$O-MeCOOH (900:100:1, 18.0 ml/min) as mobile phase to afford 4 (24.0 mg, $t_R$ 18 min).

3.3.1. 3β,22α-Dihydroxytaraxast-20-en-30-oic acid (1)
Amorphous white powder; IR $\nu_{\text{max}}$ KBr: 3674, 3599, 3358, 2938, 2658, 1695, 1657, 1444, 1386, 1296, 1276, 1265, 1196, 1179, 1141, 1075, 1038, 1030 cm$^{-1}$. $^1$H NMR spectral data (C$_5$D$_5$N, 500 MHz) and $^{13}$C NMR spectral data (C$_5$D$_5$N, 125 MHz): see Table 1. EI-MS $m/z$ 472 [M]+, 454, 436, 393,189, 56; HREI-MS: $m/z$ 472.3516 [M]+ (calcd for C$_{30}$H$_{48}$O$_4$, 472.3553).

3.3.2. 3β-Hydroxy-22-oxo-20-taraxasten-30-oic acid (2)
Amorphous white powder; IR $\nu_{\text{max}}$ KBr: 3459, 2942, 2874, 2824, 2629, 1720, 1693, 1633, 1455, 1416, 1385, 1261, 1244, 1187, 1158, 1123, 1089, 1027 cm$^{-1}$. $^1$H NMR spectral data (C$_5$D$_5$N, 500 MHz) and $^{13}$C NMR spectral data (C$_5$D$_5$N, 125 MHz): see Table 1. EI-MS $m/z$ 470 [M]+, 452, 437, 409, 207, 189, 135, 107; HREI-MS: $m/z$ 470.3359 [M]+ (calcd for C$_{30}$H$_{46}$O$_4$, 470.3396).

3.3.3. 3-Oxo-22α-hydroxy-20-taraxasten-30-oic acid (3)
Amorphous white powder; IR $\nu_{\text{max}}$ KBr: 3513, 3256, 2951, 2873, 2407, 1693, 1651, 1460, 1385, 1349, 1281, 1247, 1209, 1147, 1107, 1070, 1038, 1010 cm$^{-1}$. $^1$H NMR spectral data (C$_5$D$_5$N, 500 MHz) and $^{13}$C NMR spectral data (C$_5$D$_5$N, 125 MHz): see Table 1. EI-MS: $m/z$ 470 (M$^+$), 452, 434, 419, 404, 344, 308, 205, 163, 145; HREI-MS: $m/z$ 470.3403 [M]+ (calcd for C$_{30}$H$_{46}$O$_4$, 470.3396).

3.3.4. 3β,19β-Dihydroxy-20-taraxasten-30-oic acid (4)
Amorphous white powder; IR $\nu_{\text{max}}$ KBr: 3394, 2938, 2874, 2641, 1674, 1646, 1450, 1385, 1298, 1262, 1178, 1139, 1079, 1045, 1011 cm$^{-1}$. $^1$H NMR spectral data (C$_5$D$_5$N, 500 MHz) and $^{13}$C NMR spectral data (C$_5$D$_5$N, 125 MHz): see Table 1. EI-MS: $m/z$ 454 [M-H$_2$O]$^+$, 436, 393, 217, 189, 161, 133; HREI-MS: $m/z$ 472.3542 [M]+ (calcd for C$_{30}$H$_{48}$O$_4$, 472.3553).

3.4. In vitro cytotoxicity bioassays
Human colon cancer (HCT-8), hepatoma (Bel7402), stomach cancer (BGC-823), lung adenocarcinoma (A549), and ovarian cancer (A2780) cell lines were obtained from ATCC. Cells were maintained in RRM1640 supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin. Cultures were incubated at 37 °C in a humidified 5% CO$_2$ atmosphere. All the cells were seeded in 96-well microtiter plates at 1200 cells/well. After 24 h, the test compound was added to the cells. After 96 h of drug treatment, cell viability was determined by measuring the metabolic conversion of MTT.
(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) into purple formazan crystals by active cells. MTT assay results were read using a MK 3 Wellscan (Labsystem Drogon) plate reader at 570 nm. All compounds were tested in five concentrations and were dissolved in DMSO with a final DMSO concentration of 0.1% in each well. Each concentration of the compounds was tested in three parallel wells. IC$_{50}$ values were calculated using Microsoft Excel software.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was financially supported by the Beijing Natural Sciences Foundation [grant number 7142028]; National Natural Sciences Foundation of China (NNSFC) [grant number 31171669]; Beijing Key Laboratory of Bioactive Substances and Functional Foods, Beijing Union University [Zk60201601].

**References**

[1] L.L. Sun, Y. Chen, L. Zhang, and A.W. Ding, *Lishizhen Med. Mater. Med. Res.* 16, 1304 (2005).
[2] Y. Chen, A.W. Ding, X.H. Yang, and L. Zhang, *Chin. Arch. Tradit. Chin. Med.* 23, 614 (2005).
[3] Y. Li, Z.F. Wang, and R.Z. Jia, *Chin. Arch. Tradit. Chin. Med.* 26, 274 (2008).
[4] J. Liang, Z.N. Zhang, and L. Ye, *Shanxi J. TCM.* 27, 50 (2011).
[5] Q.H. Zeng, J.B. Zhao, J.J. Wang, X.W. Zhang, and J.G. Jiang, *LWT – Food Sci. and Tech.* 68, 595 (2016).
[6] Q. Ma, Y.M. Guo, B.M. Luo, W.M. Liu, C.X. Yang, C.H. Ding, X.F. Xu, M.H. He, and R.R. Wei, *Nat. Prod. Res.* 29, 1 (2015).
[7] X.Y. Shang, J.J. Li, and L.L. Li, CN 101564410 A 20091028 (2009).
[8] Z.Z. Yuan, H.M. Duan, Y.Y. Xu, A.L. Wang, L.S. Gan, J.J. Li, M.T. Liu, and X.Y. Shang, *Phytochem. Lett.* 8, 116 (2014).
[9] Z.Z. Yuan, L.L. Li, Z. Sun, J.J. Li, A.L. Wang, and X.Y. Shang, *J. Chin. Inst. Food Sci. Technol.* 14, 196 (2014).
[10] Z. Sun, L.L. Li, Z.Z. Yuan, A.L. Wang, J.J. Li, and X.Y. Shang, *Food Sci. Chin.* 33, 124 (2012).
[11] L.L. Li, Z. Sun, X.Y. Shang, J.J. Li, and R. Wang, *China J. Chin. Mater. Med.* 37, 30 (2012).
[12] X.F. He, X.N. Wang, C.Q. Fan, L.S. Gan, S. Yin, and J.M. Yue, *Helv. Chim. Acta.* 90, 783 (2007).
[13] S.G. Li, M.M. Li, B.X. Zhao, Y. Wang, and W.C. Ye, *J. Asian Nat. Prod. Res.* 17, 1153 (2015).