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## Taraxastane-type triterpenoids from the medicinal and edible plant *Cirsium setosum*

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**[ABSTRACT]** Guided by TNF- $\alpha$  secretion inhibitory activity assay, four taraxastane-type triterpenoids, including two new ones, 22-oxo-20-taraxasten-3 $\beta$ , 30-diol (**1**) and 22 $\alpha$ -hydroxy-20-taraxasten-30 $\beta$ , 30-triol (**2**), have been obtained from an active fraction of the petroleum ether-soluble extract of the medicinal and edible plant *Cirsium setosum*. Their structures were elucidated by spectroscopic data and CD data analysis. In the TNF- $\alpha$  secretion inhibitory activity assay, compounds **1** and **2** were active with the IC<sub>50</sub> of 2.6 and 3.8  $\mu\text{mol}\cdot\text{L}^{-1}$ , respectively. In addition, compounds **1** and **2** showed moderately selective cytotoxicity against the human ovarian cancer (A2780) and colon cancer (HCT-8) cell lines.

**[KEY WORDS]** *Cirsium setosum*; Taraxastane-type triterpenoid; TNF- $\alpha$  secretion inhibitory activity; Cytotoxicity

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### Introduction

*Cirsium setosum* (Willd.) MB., a vegetable with thorns belonging to *Cirsium* plants of the Compositae family, is a wild perennial herb widely distributed in China, East Asia, Europe and other regions of the world<sup>[1]</sup>. *C. setosum* is not only an edible vegetable, but also a traditional herbal medicine in the Chinese pharmacopoeia<sup>[2]</sup>. At present, *C. setosum* has been used as a critical ingredient in many herbal teas, dry red wines, and black glutinous rice fermented beverages<sup>[3-6]</sup>. Modern pharmacological studies show that *C. setosum* extracts

have antihemorrhagic, anti-inflammatory, antimicrobial, anticancer, hepatoprotective, and sedative activities<sup>[7]</sup>. In our previous study on potential antitumor promoters from *C. setosum*, three  $\alpha$ -tocopheroid-type compounds<sup>[8]</sup> and seven triterpenoids<sup>[9-10]</sup> had been isolated from the petroleum ether (PE) fraction of the crude extract and showed potent cytotoxicity. Further bioassay-guided isolation of a remaining fraction with inhibitory activity against TNF- $\alpha$  secretion in mouse peritoneal macrophages led to the isolation of two new taraxastane-type triterpenoids, 22-oxo-20-taraxasten-3 $\beta$ , 30-diol (**1**) and 22 $\alpha$ -hydroxy-20-taraxasten-30 $\beta$ , 30-triol (**2**), and two known analogues (**3** and **4**) (Fig. 1). This paper deals with the isolation, structure elucidations and biological assays of these triterpenoids.

### Results and Discussion

#### Chemistry and structure elucidation

Guided by TNF- $\alpha$  secretion inhibitory activity assay, a fraction from the petroleum ether-soluble portion showed potent activity with an IC<sub>50</sub> of 22.5  $\mu\text{g}\cdot\text{mL}^{-1}$ . Then, column chromatography over silica gel, Sephadex LH-20, and reverse-phase HPLC were applied to isolate the constituents of the active fraction, resulting in the discovery of four tarax-

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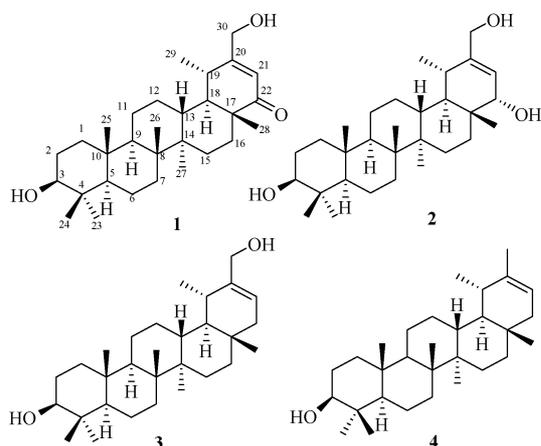
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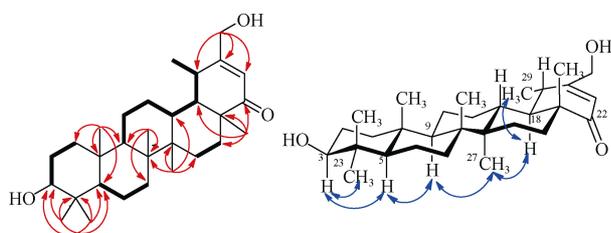
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astane-type triterpenoids **1–4** (Fig. 1).



**Fig. 1** Chemical structures of compounds **1–4**

Compound **1** was obtained as a white amorphous powder. Its molecular formula  $C_{30}H_{48}O_3$ , with 7 degrees of unsaturation, was indicated by HREIMS at  $m/z$  456.3638 ( $M^+$ ) (Calcd. 456.3603). The IR spectrum of **1** suggested that it contained hydroxyl groups ( $3438$  and  $3338\text{ cm}^{-1}$ ) and conjugated carbonyl ( $1655\text{ cm}^{-1}$ ) functionalities. The  $^1\text{H}$  NMR data (Table 1) of **1** in  $C_5D_5N$  displayed signals for six methyl singlet groups ( $\delta_H$  0.89, 0.92, 1.00, 1.04, 1.06 and 1.25), a methyl doublet group [ $\delta_H$  1.10 (3H, d,  $J = 6.5$  Hz)], one oxygenated methine [ $\delta_H$  3.48 (1H, dd,  $J = 6.5, 9.5$  Hz)], one oxygenated methylene [ $\delta_H$  4.62 (1H, d,  $J = 16.3$  Hz) and 4.46 (1H, d,  $J = 16.3$  Hz)] and a trisubstituted olefinic proton [ $\delta_H$  6.59 (1H, s)]. The  $^{13}\text{C}$  NMR spectrum displayed 30 carbon signals, which were classified as seven methyls, nine methylenes (one oxygenated), seven methines (one oxygenated and one olefinic carbon), and seven quaternary carbons (one carbonyl and one olefinic carbons) on the basis of DEPT and HMQC spectra. These data suggested that **1** was similar with one known taraxastane-type triterpenoid, 22-oxo-20-taraxasten-3 $\beta$ -ol<sup>[11]</sup>, except for an additional hydroxyl group located at C-30, which was confirmed by the comprehensive analysis of the 2D NMR spectra of **1**, especially  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC (Fig. 2).

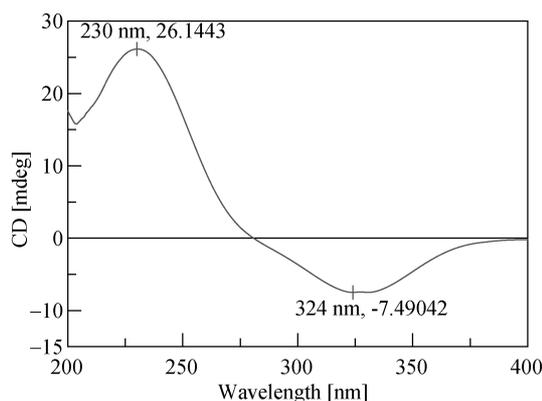


**Fig. 2** Main  $^1\text{H}$ - $^1\text{H}$  COSY (bold lines), HMBC (red arrows), and NOESY (blue arrows) correlations of compound **1**

Four structural fragments, as shown with bold lines in Fig. 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 and C-15 to C-16), were first established by the correlations observed in the  $^1\text{H}$ - $^1\text{H}$  COSY

spectrum. The connectivity study of the four structural fragments, quaternary carbons, and the other functional groups were mainly achieved by the analysis of the HMBC spectrum (Fig. 2). HMBC correlations from  $\text{H}_3$ -23 ( $\delta_H$  1.25) to C-3, C-4, C-5 and C-24 and from  $\text{H}_3$ -24 ( $\delta_H$  1.06) to C-3, C-4, C-5 and C-23 indicated that Me-23 and Me-24 were attached to C-4. The HMBC correlations of  $\text{H}_3$ -25 ( $\delta_H$  0.92) to C-1, C-5, C-9 and C-10,  $\text{H}_3$ -26 ( $\delta_H$  1.04) to C-7, C-8, C-9 and C-14,  $\text{H}_3$ -27 ( $\delta_H$  0.89) to C-8, C-13, C-14 and C-15,  $\text{H}_3$ -28 ( $\delta_H$  1.00) to C-16, C-17, C-18 and C-22, and  $\text{H}_2$ -30 ( $\delta_H$  4.46 and 4.62) to C-19, C-20 and C-21 not only confirmed the presence of A/B/C/D/E-ring systems but also located the Me-25 at C-10, Me-26 at C-8, Me-27 at C-14, Me-28 at C-17 and  $\text{CH}_2\text{OH}$ -30 at C-20, which completed the planar structure of **1**.

In the ROESY spectrum, the correlations of H-3/ $\text{H}_3$ -23, H-3/H-5, H-5/H-9, H-9/ $\text{H}_3$ -27,  $\text{H}_3$ -27/H-18, and H-18/ $\text{H}_3$ -29 revealed that these protons were cofacial and defined as  $\alpha$ -orientation (Fig. 2). The absolute configuration of **1** was proposed from the CD spectrum (Fig. 3). The CD spectrum of **1** showed a negative Cotton effect at 324 nm ( $\Delta\epsilon -7.49$ ) for  $n$ - $\pi^*$  and a positive Cotton effect at 230 nm ( $\Delta\epsilon +26.1$ ) for  $\pi$ - $\pi^*$ , suggested **1** possessed the absolute configuration as depicted in Fig. 4 based on the octant rules the cyclohexenones<sup>[12]</sup>. Thus, the structure of compound **1** was defined as 22-oxo-20-taraxasten-3 $\beta$ , 30-diol.



**Fig. 3** The CD spectrum of **1**

Compound **2** was obtained as a white amorphous powder. Its molecular formula  $C_{30}H_{50}O_3$ , with 6 degrees of unsaturation, was indicated by HREIMS at  $m/z$  458.3747 ( $M^+$ ) (Calcd. 458.3760). The IR spectrum of **2** suggested that it contained hydroxyl groups ( $3668$  and  $3324\text{ cm}^{-1}$ ) and a double bond ( $1641\text{ cm}^{-1}$ ). The  $^1\text{H}$  and  $^{13}\text{C}$  spectroscopic data of **2** (Table 1) resembled to those of **1**, except that the carbonyl signal ( $\delta_C$  205.4, C-22) in **1** was replaced by an oxymethine group ( $\delta_C$  73.2, C-22) in **2**. This was also supported by HMQC and HMBC experiments. The NOESY correlation between H-22 and  $\text{H}_3$ -28 indicated the OH-22 was  $\alpha$ -oriented. In addition, the ROESY spectra of **1** and **2** revealed that they had completely identical NOE correlations of H-3/ $\text{H}_3$ -23, H-3/H-5, H-5/H-9, H-9/ $\text{H}_3$ -27,  $\text{H}_3$ -27/H-18, and H-18/ $\text{H}_3$ -29.

On the basis of these data and biosynthetic considerations, the absolute configuration of **2** was proposed as depicted in Fig. 1

and its structure was elucidated as 22 $\alpha$ -hydroxy-20-taraxasten-30 $\beta$ , 30-triol.

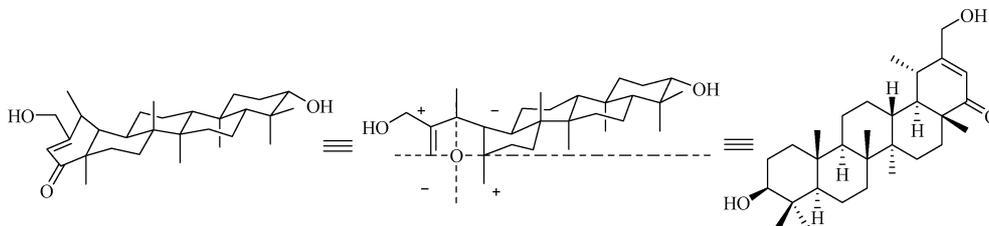


Fig. 4 The absolute configuration of **1** proposed on the basis of the octant rule for the cyclohexenone

Table 1  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR Data for compounds **1** and **2**<sup>a</sup>

No.	<b>1</b>		<b>2</b>	
	$\delta_{\text{H}}$ (mult, $J$ , Hz)	$\delta_{\text{C}}$ (mult)	$\delta_{\text{H}}$ (mult, $J$ , Hz)	$\delta_{\text{C}}$ (mult)
1	(a) 1.00 (1H, m) (b) 1.72 (1H, m)	39.2	(a) 0.98 (1H, dt, $J = 5.5, 12.5$ Hz) (b) 1.71 (1H, dt, $J = 3.5, 12.5$ Hz)	39.2
2	1.89 (2H, m)	28.4	1.89 (2H, m)	28.3
3	3.48 (1H, dd, $J = 6.5, 9.5$ Hz)	78.0	3.48 (1H, dd, $J = 6.5, 10.0$ Hz)	78.1
4		39.5		39.5
5	0.81 (1H, br d, $J = 10.5$ Hz)	55.7	0.81 (1H, br d, $J = 9.0$ Hz)	55.8
6	(a) 1.38 (1H, m) (b) 1.57 (1H, m)	18.7	(a) 1.41 (1H, m) (b) 1.57 (1H, m)	18.7
7	(a) 1.38 (2H, m)	34.6	1.41 (2H, m)	34.7
8		41.3		41.3
9	1.28 (1H, m)	50.5	1.36 (1H, m)	50.8
10		37.3		37.4
11	(a) 1.26 (1H, m) (b) 1.55 (1H, m)	21.8	(a) 1.21 (1H, m) (b) 1.53 (1H, m)	21.8
12	(a) 1.56 (1H, m) (b) 1.87 (1H, m)	28.2	(a) 1.29 (1H, m) (b) 1.70 (1H, m)	28.0
13	1.88 (1H, m)	38.6	1.79 (1H, m)	39.1
14		42.1		42.6
15	(a) 1.09 (1H, m) (b) 1.65 (1H, m)	26.6	(a) 1.14 (1H, m) (b) 1.84 (1H, m)	27.3
16	(a) 1.38 (1H, m) (b) 1.75 (1H, m)	28.6	(a) 1.17 (1H, m) (b) 2.53 (1H, td, $J = 13.5, 4.5$ Hz)	30.6
17		45.2		38.9
18	1.50 (1H, m)	45.8	2.00 (1H, dd, $J = 11.3, 7.8$ Hz)	41.3
19	2.29 (1H, m)	33.3	2.19 (1H, m)	32.7
20		166.7		148.3
21	6.59 (1H, s)	119.8	6.38 (1H, d, $J = 6.5$ Hz)	122.2
22		205.4	3.79 (1H, d, $J = 6.5$ Hz)	73.2
23	1.25 (3H, s)	28.6	1.24 (3H, s)	28.6
24	1.06 (3H, s)	16.3	1.06 (3H, s)	16.3
25	0.92 (3H, s)	16.6	0.91 (3H, s)	16.6
26	1.04 (3H, s)	16.2	1.05 (3H, s)	16.3
27	0.89 (3H, s)	14.6	1.10 (3H, s)	14.9
28	1.00 (3H, s)	18.8	0.87 (3H, s)	18.8
29	1.10 (3H, d, $J = 6.5$ Hz)	23.1	1.25 (3H, d, $J = 7.0$ Hz)	22.8
30	(a) 4.46 (1H, d, $J = 16.3$ Hz) (b) 4.62 (1H, d, $J = 16.3$ Hz)	62.9	(a) 4.46 (1H, d, $J = 16.3$ Hz) (b) 4.62 (1H, d, $J = 16.3$ Hz)	64.3

<sup>a</sup> Data were recorded on a Bruker Avance 500 spectrometer. Chemical shifts ( $\delta$ ) are given in parts per million with references to the center peak of  $\text{C}_5\text{D}_5\text{N}$  with  $\delta$  7.20 for  $^1\text{H}$  and  $\delta$  123.44 for  $^{13}\text{C}$ . \* Data can be interchanged.

By comparison of spectroscopic data (IR, EIMS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR) with those reported in the corresponding literature<sup>[11]</sup>, the two known compounds were identified as 20-taraxasten-3 $\beta$ , 30-diol (**3**) and 20-taraxasten-3 $\beta$ -ol (**4**).

Compounds **1–4** were tested for inhibitory activity against TNF- $\alpha$  secretion in mouse peritoneal macrophages. Compounds **1** and **2** were active with the  $\text{IC}_{50}$  of 2.6 and 3.8  $\mu\text{mol}\cdot\text{L}^{-1}$ , respectively. Dexamethasone was used as positive control with an  $\text{IC}_{50}$  of 0.83  $\mu\text{mol}\cdot\text{L}^{-1}$ . At concentrations up to 100  $\mu\text{mol}\cdot\text{L}^{-1}$ , compounds **1** and **2** were not cytotoxic to LPS-induced mouse peritoneal macrophages. Also, these isolates were subjected to cytotoxicity against five human cancer cell lines: colon cancer (HCT8), hepatoma (Bel7402), stomach cancer (BGC823), lung adenocarcinoma (A549) and ovarian cancer (A2780), by using the MTT method with topotecan as a positive control. Only compounds **1** and **2** showed moderately selective cytotoxicity against A2780 and HCT-8 cell lines (Table 2).

**Table 2** Cytotoxic Data for compounds **1–2**

compounds	$\text{IC}_{50}$ ( $\mu\text{mol}\cdot\text{L}^{-1}$ )				
	HCT-8	Bel7402	BGC-823	A549	A2780
<b>1</b>	18.5	> 20	> 20	> 20	4.7
<b>2</b>	8.9	> 20	> 20	> 20	14.3
<b>3</b>	> 20	> 20	> 20	> 20	> 20
<b>4</b>	> 20	> 20	> 20	> 20	> 20
topotecan	1.5	1.4	4.7	3.5	1.4

## Experimental

### General experimental procedures

Mass spectra, including high-resolution mass spectra, were recorded on a JEOL JMS AX-500 spectrometer. 1D- and 2D-NMR spectra were acquired in  $\text{C}_5\text{D}_5\text{N}$  with TMS as internal standard on Bruker AV-III-500 MHz spectrometers. IR spectra were recorded on a Nicolet 5700 spectrometer by an FT-IR microscope transmission method. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc. China), cyanopropyl silica gel (43–60  $\mu\text{m}$ ) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala Sweden). LPLC separation was performed with Combiflash (ISCO Companion). HPLC separation was performed on an instrument consisting of a Waters 2545 controller, a Waters 2545 pump, and a Waters 2487 dual  $\lambda$  absorbance detector with an sunfire (waters, 250 mm  $\times$  10 mm i.d.) preparative column packed with  $\text{C}_{18}$  (5  $\mu\text{m}$ ). TLC was carried out with glass precoated silica gel GF<sub>254</sub> plates. Spots were visualized under UV light or by spraying with 8%  $\text{H}_2\text{SO}_4$  in 95% EtOH followed by heating.

### Plant materials

The stems of *Cirsium setosum* (Willd.) MB. were collected at Jiuhua Mountain, Anhui province, China, in September 2008. The plant was identified by Mr KE Yun-Wu (Chizhou Huangjing Institutent of Jiuhua Mountain, Anhui, China). A

voucher specimen (20081028) has been deposited at Beijing Union University, Beijing Key Laboratory of Bioactive Substances and Functional Foods, Beijing, China.

### Extraction and isolation

The air-dried stems of *Cirsium setosum* (20 kg) was 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  $\text{H}_2\text{O}$  and then partitioned with petroleum ether and EtOAc respectively. The petroleum ether-soluble portion (468.5 g) was fractionated via silica gel column chromatography, eluting with a gradient of acetone (0%–100%) in petroleum ether (60–90  $^\circ\text{C}$ ), to give eleven fractions (Sh1–Sh11). Fraction Sh7 (51.5 g) showing inhibitory activity to TNF- $\alpha$  ( $\text{IC}_{50}$  22.5  $\mu\text{g}\cdot\text{mL}^{-1}$ ).

Fraction Sh7 was chromatographed on normal phase LPLC using a gradient of acetone in petroleum ether (60–90  $^\circ\text{C}$ ) (2%–50%) to give nine (Sh7-1–Sh7-9) fractions. Subsequent separation of fraction Sh7-5 (7.6 g) over Sephadex LH-20 gel repeated, eluting with petroleum ether– $\text{CHCl}_3$ – $\text{CH}_3\text{OH}$  (5 : 5 : 1), afforded five subfractions (Sh7-5-1–Sh7-5-5). Subfraction Sh7-5-5 (1.3 g) was purified by LPLC over normal phase cyanopropyl silica, eluting with petroleum ether (60–90  $^\circ\text{C}$ )– $\text{Me}_2\text{CO}$  (10 : 1 to 0 : 100), to yield two fractions (Sh7-5-5-1, Sh7-5-5-2). Subfraction Sh7-5-5-1 was further purified by preparative reversed phase HPLC, eluting with  $\text{MeOH}$ – $\text{H}_2\text{O}$ – $\text{AcOH}$  (80 : 20 : 1, 18.0  $\text{mL}\cdot\text{min}^{-1}$ ) to afford **2** (105.0 mg,  $t_{\text{R}}$  21 min, monitor wavelength: 214 nm). Subfraction Sh7-5-5-2 was further purified by preparative reversed phase HPLC, eluting with  $\text{MeOH}$ – $\text{H}_2\text{O}$ – $\text{AcOH}$  (78 : 22 : 1, 18.0  $\text{mL}\cdot\text{min}^{-1}$ ) to afford **1** (185.0 mg,  $t_{\text{R}}$  19 min, monitor wavelength: 236 nm). Subfraction Sh7-5-1 (2.1 g) was purified by LPLC over normal phase cyanopropyl silica, eluting with petroleum ether (60–90 $^\circ\text{C}$ )– $\text{Me}_2\text{CO}$  (50 : 1 to 10 : 1), which was purified by preparative reversed phase HPLC, eluting with  $\text{MeOH}$ – $\text{H}_2\text{O}$ – $\text{AcOH}$  (90 : 10 : 1, 18.0  $\text{mL}\cdot\text{min}^{-1}$ ) to afford **3** (38.0 mg,  $t_{\text{R}}$  12 min, monitor wavelength: 206 nm) and **4** (763.0 mg,  $t_{\text{R}}$  35 min, monitor wavelength: 198 nm).

### 22-oxo-20-taraxasten-3 $\beta$ , 30-diol (**1**)

Amorphous white powder;  $[\alpha]_{\text{D}}^{20} +59$  (c 0.1, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 230 (1.90); ECD (MeOH) 230 ( $\Delta\epsilon +26.1$ ), 324 ( $\Delta\epsilon -7.49$ ); IR (KBr)  $\nu_{\text{max}}$ : 3668, 3324, 2996, 2940, 2872, 1678, 1641, 1452, 1384, 1328, 1304, 1272, 1246, 1182, 1161, 1108, 1083, 1041, 1008  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{C}_5\text{D}_5\text{N}$ , 500 MHz) and  $^{13}\text{C}$  NMR ( $\text{C}_5\text{D}_5\text{N}$ , 125 MHz) spectroscopic data, see Table 1; EI-MS  $m/z$  440  $[\text{M} - \text{H}_2\text{O}]^+$ , 422, 407, 379, 189, 135, 107; HREI-MS  $m/z$  458.3747 (Calcd. for  $\text{C}_{30}\text{H}_{50}\text{O}_3$  458.3760).

### 22 $\alpha$ -hydroxy-20-taraxasten-3 $\beta$ , 30-triol (**2**)

Amorphous white powder;  $[\alpha]_{\text{D}}^{20} +80$  (c 0.1, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 206 (3.68); IR (KBr)  $\nu_{\text{max}}$ : 3438, 3338, 2927, 2874, 1655, 1447, 1384, 1303, 1191, 1146, 1083, 1044, 1034, 1012, 972  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{C}_5\text{D}_5\text{N}$ , 500 MHz) and  $^{13}\text{C}$  NMR ( $\text{C}_5\text{D}_5\text{N}$ , 125 MHz) spectroscopic data, see Table 1. EI-MS  $m/z$  456 ( $\text{M}^+$ ), 438, 393, 189, 135, 107; HREI-MS  $m/z$

456.3638 (Calcd. for C<sub>30</sub>H<sub>46</sub>O<sub>4</sub> 456.3603).

#### TNF- $\alpha$ secretion inhibition assay

Compounds 1–4 were evaluated for inhibitory activity against TNF- $\alpha$  secretion in mouse peritoneal macrophages, according to a previously described protocol [13].

#### Cytotoxicity assay

Human colon cancer (HCT-8), human hepatoma (Bel7402), human stomach cancer (BGC-823), human lung adenocarcinoma (A549), and human ovarian cancer (A2780) cell lines were obtained from ATCC. Cells were maintained in RRMI1640 supplemented with 10% fetal bovine serum (FBS), 100 U·mL<sup>-1</sup> penicillin, and 100  $\mu$ g·mL<sup>-1</sup> streptomycin. Cultures were incubated at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere. All the cells were seeded in 96-well microtiter plates at 1200 cells/well. After 24 h, the compounds were 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 Drogen) plate reader at 570 nm. All compounds were tested in five concentrations and were dissolved in 100% DMSO with a final DMSO concentration of 0.1% in each well. Each concentration of the compounds was tested in three parallel wells. IC<sub>50</sub> was calculated using Microsoft Excel software. Topotecan was used as a positive control.

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