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ORIGINAL PAPER

Eremophilane derivatives from *Senecio dianthus*

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Abstract From the aerial parts of *Senecio dianthus*, five eremophilane glucosides (**1**, **2**, **4**–**6**) and one new eremophilenolide (**7**) were isolated, together with sixteen known compounds (**3**, **8**–**22**). Their structures and relative configurations were elucidated on the basis of extensive spectroscopic analysis, including HR-ESI-MS, X-ray, CD, 1D- and 2D-NMR experiments.

Keywords Asteraceae · *Senecio dianthus* Franch. · Eremophilane glucosides · Eremophilane · Cytotoxicity

Crystallographic data for the structures **2** (CCDC 896956) and **4** (CCDC 896955) have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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Introduction

Senecio dianthus Franch., belonging to the Asteraceae family, is a herbaceous plant that is widely distributed in China, mostly distributed in Tibet and reputed for reducing fever and detoxification in Tibetan herbal medicine [1]. Previous investigations on the genus *Senecio* indicated that pyrrolizidine alkaloids and furo-eremophilanes are the typical compounds isolated from this genus [1]. Its chemical components were investigated by Li et al. [2], which led to the identification of seven eremophilenolides and an eremophilenolide alkaloid. In this paper, five new eremophilane glucosides (**1**, **2**, **4**–**6**) and one new eremophilenolide (**7**) (Fig. 1), together with sixteen known compounds (**3**, **8**–**22**) were isolated from this plant. Their structures and relative configurations were elucidated by use of various spectral methods (IR, HR-ESI-MS, 1D- and 2D-NMR) and by X-ray spectroscopy. Some of them were evaluated for their cytotoxic activity against K562, MCF-7, and Caco2 human cancer cell lines by the MTS method. Herein, we report the isolation and structural elucidation of these compounds, as well as their cytotoxicities (Fig. 2).

Results and discussion

Compound **1** was obtained as an amorphous solid. Its molecular formula was determined as $C_{21}H_{32}O_7$ by HR-ESI-MS at *m/z* 419.2053 $[M+Na]^+$ (calcd. for $C_{21}H_{32}O_7Na$, 419.2046). The IR spectrum displayed bands at 3391 and 1651 cm^{-1} and in the UV spectrum absorptions at 240 nm suggested the presence of a hydroxyl and conjugated carbonyl group. It exhibited very similar NMR spectroscopic data (Table 1) to those of known compound **3** [3]. The ^{13}C-NMR spectrum displayed 21 carbon signals,

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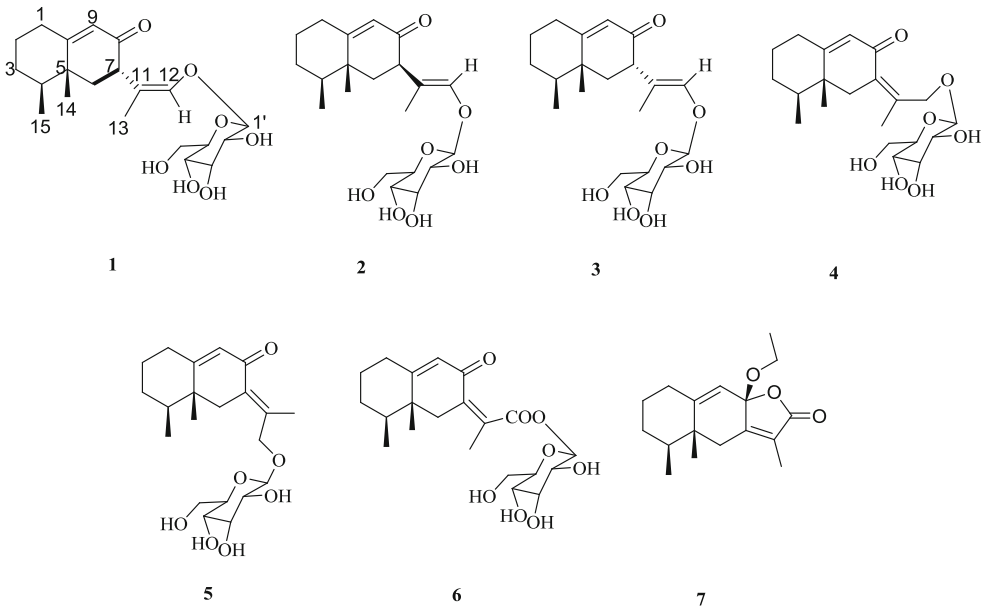

Fig. 1 Structure of compounds **1**–**7**

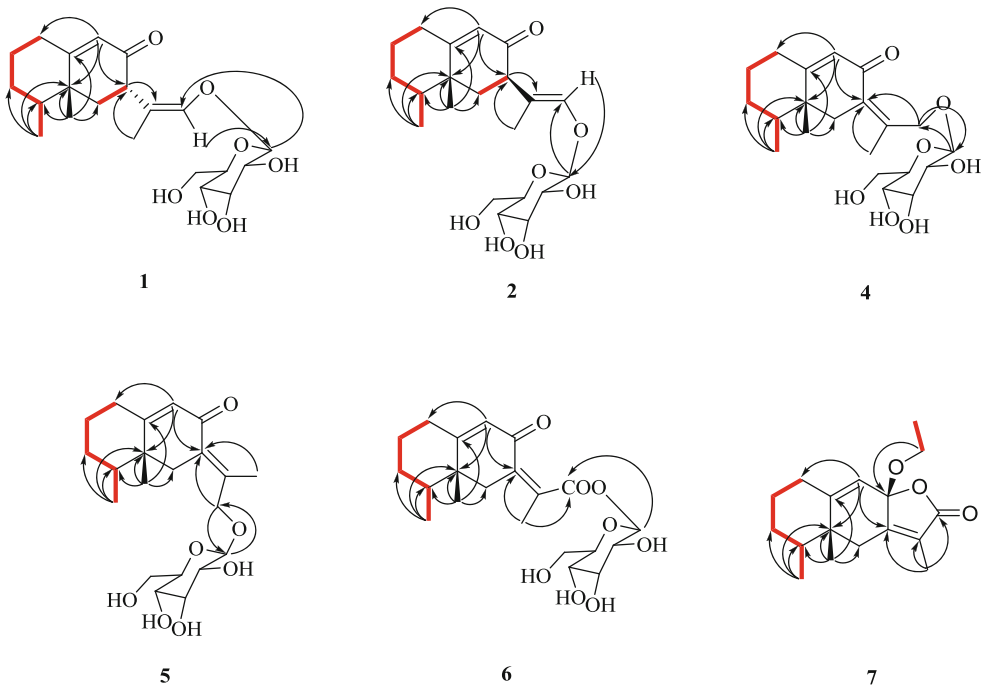

Fig. 2 Key HMBC and COSY interactions of compounds **1**, **2**, **4**, **5**, **6**, and **7**

including those of a sugar moiety: five CH (δ 104.3, 78.5, 78.1, 75.0, and 71.5) and one CH_2 (δ 62.8). The remaining 15 carbons were assigned, based on 1H- and 2D-NMR data, to an eremophilane-type sesquiterpene with a carbonyl group (δ 202.3) and two trisubstituted double bonds (δ 124.9 CH, 174.2 C and 115.1 C, 142.2 CH). The HMBC experiment placed the carbonyl group at C-8 and the double bonds at C-9 and C-11, respectively. Comparing the ^{13}C-NMR data of **1** with **3**, the main differences were that C-6 and C-7 were shifted upfield about $_{\Delta}\delta$ 1.7 and 5.0, and C-13 was shifted downfield about $_{\Delta}\delta$ 3.2. The sugar residue was identified as β-D-glucose by gas chromatography of the hydrolyzed product and by the coupling constant of its anomeric proton (δ 4.48, d, $J = 7.8$ Hz). The location of the sugar was determined by the cross-peak between the anomeric proton (δ 4.48) and C-12 (δ 142.2), observed in the HMBC experiment. Compared with compound **3**, the signal of C-13 in the ^{13}C-NMR spectrum appeared shifted downfield ($_{\Delta}\delta$ 3.2), and the correlation between H-7 and H-12 which disappeared in the ROESY implied the *Z* configuration of the C-11 double bond, and the correlation of H-7 with H_3-14 observed in the ROESY suggested

the β-orientation of H-7. Since the Cotton effects observed in the CD experiment were comparable to those reported for 4-ene-3-ketosteroids [3, 4], the structure of **1** was established to be 4*S*,5*R*,7*S*-12-*O*-β-D-glucopyranosyl-eremophil-9,11(12)(*Z*)-dien-8-one (Fig. 3).

Compound **2** had the same molecular formula ($C_{21}H_{32}O_7$) as compounds **1** and **3** [3]. In the ^{13}C-NMR spectrum (Table 1), the signals of C-4, C-6, and C-9 were displaced upfield ($_{\Delta}\delta$ 5.4, 4.6, 1.5 ppm, respectively) while those of C-2, C-5, C-10 and C-15 appeared shifted downfield ($_{\Delta}\delta$ 2.2, 0.9, 2.0, 5.6 ppm, respectively), in relation to those of **3**. Since the interactions observed in the ^{1}H–^{1}H COSY and HMBC experiments were the same for both compounds, it was evident that they should have different configurations. In the ROESY spectrum of compound **2**, the cross-peak between H-7 and H-12 indicated the *E* configuration of the C-11 double bond. Because the circular dichroism curve of **2** showed the same pattern as that reported for steroidal 4-en-3-ones [5], the structure of **2** was identified as 4*S*,5*R*,7*R*-12-*O*-β-D-glucopyranosyl-eremophil-9,11(12)(*E*)-dien-8-one. This structure was confirmed by the X-ray diffraction analysis of **2** (Fig. 4).

Compound **4** showed a molecular formula $C_{21}H_{32}O_7$ by HR-ESI-MS. The sugar residue was identified as β-glucose by gas chromatography of the hydrolyzed product and by the coupling constant of its anomeric proton (δ 4.18, d, $J = 7.8$ Hz). In the ^{13}C-NMR spectrum (Table 2), the signal at δ 193.8 showed the presence of a carbonyl group, and signals (δ 126.2 CH, 173.3 C and 133.0 C, 144.1 C) were assigned to two double bonds. The UV spectrum of **4**

Table 1 ^{1}H- and ^{13}C-NMR data of compounds **1**, **2** and **7**

No.	**1**[a]		**2**[a]		**7**[b]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	2.41 m	34.2	2.54 m	34.2	2.14 m	32.4
1b	2.28 br.d (15.0)		2.19 br.d (11.3)		2.12 m	
2a	1.90 m	27.7	2.03 m	30.9	1.80 m	26.7
2b	1.48 m		1.44 m		1.45 m	
3a	1.55 m	31.6	1.59 m	31.5	1.52 m	30.8
3b	1.55 m		1.59 m		1.41 m	
4	1.47 m	45.2	2.02 m	39.9	1.61 m	43.8
5	–	41.0	–	42.0	–	46.0
6a	1.95 dd (13.4, 5.0)	41.6	2.02 dd (14.0, 5.0)	38.7	2.68 d (12.3)	36.7
6b	1.86 dd (13.4, 14.0)		1.90 dd (14.0, 13.4)		2.26 br.d (12.2)	
7	3.90 dd (14.0, 5.0)	43.5	3.02 dd (13.4, 5.0)	47.8	–	157.7
8	–	202.3	–	202.8	–	103.2
9	5.71 d (1.5)	124.9	5.57 s	123.3	5.67 s	118.0
10	–	174.2	–	176.1	–	152.1
11	–	115.1	–	114.6	–	123.9
12	6.32 d (1.3)	142.2	6.24 d (1.0)	143.0		171.9
13	1.49 d (1.3)	14.3	1.57 d (1.0)	11.2	1.87 d (1.2)	8.4
14	1.23 s	16.5	1.10 s	16.6	0.89 s	17.7
15	0.93 d (6.3)	15.6	0.96 d (6.4)	21.2	0.94 d (6.8)	15.5
1′	4.48 d (7.8)	104.3	4.50 d (7.6)	104.5	–	–
2′	3.25 m	75.0	3.30 m	75.0	–	–
3′	3.31 m	78.5	3.31 m	78.5	–	–
4′	3.30 m	71.5	3.30 m	71.4	–	–
5′	3.34 m	78.1	3.37 m	78.0	–	–
6′ a	3.87 dd (11.9, 1.6)	62.8	3.86 dd (11.9, 1.6)	62.7	–	–
6′ b	3.67 dd (11.9, 5.0)		3.67 dd (11.9, 5.0)		–	–
–O<u>CH$_2$</u>CH$_3$	–		–	–	a 3.50 m, b 3.34 m	59.0
–OCH$_2$<u>CH$_3$</u>	–		–	–	1.19 t (7.0)	15.6

Data are based on DEPT, HSQC, and HMBC experiments. ^{1}H-NMR (400 MHz, δ, J in Hz in parentheses), ^{13}C-NMR (100 MHz, δ)

[a] Data in CD_3OD

[b] Data in $CDCl_3$

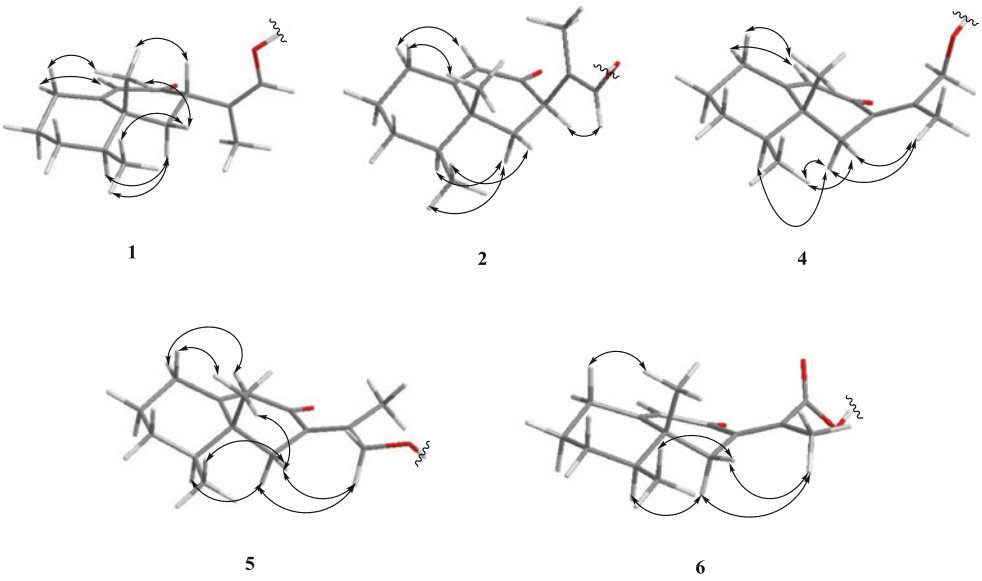

Fig. 3 Key ROESY correlations of compounds **1**, **2**, **4**, **5**, and **6**

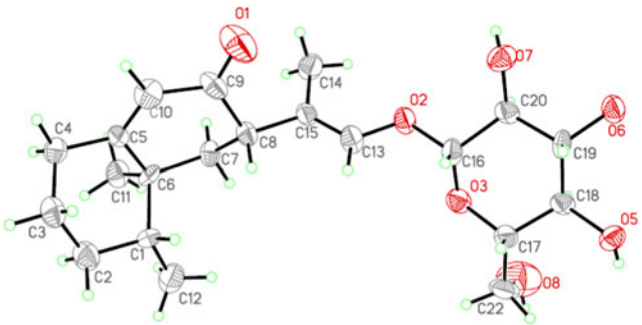

Fig. 4 ORTEP projection of compound **2** (crystallographic numbering)

exhibited maximum absorption at 252 nm and was consistent with the presence of a conjugated enone system. The HMBC experiment placed the carbonyl group at C-8 and the double bonds at C-9 and C-7, respectively. Linkage of the glucose moiety to C-12 was determined by the HMBC interaction observed between this carbon atom and the anomeric proton. The *Z* configuration of the 7(11) double bond was determined by the cross-peaks of H_3-13 with H-6β observed in the ROESY experiment. Because the circular dichroism curve of **4** showed the same pattern as that reported for steroidal 4-en-3-ones [3, 4], and further supported by the X-ray analysis (Fig. 5), the structure of **4** was established to be 4*S*,5*R*-12-*O*-β-D-glucopyranosyl-eremophil-7(11)(*Z*),9-dien-8-one.

Compound **5** also exhibited the same molecular formula and very similar spectroscopic data to those of compound **4**. Comparing the ^{13}C-NMR spectrum (Table 2) with compound **4**, the signal of C-11 was displaced upfield ($_{\Delta}\delta$ 2.1 ppm) and C-13 appeared shifted downfield ($_{\Delta}\delta$ 2.3). Since the COSY and HMBC experiments were the same for both compounds, **4** and **5** should have different configurations. In the ROESY spectrum of **5**, the cross-peaks between H-6 (δ 2.17, d, $J = 13.9$ Hz; 3.10, br.d, $J = 13.9$ Hz) and H-12 indicated the *E* configuration of the C-7(11) double bond. It was supported by the CD curve of **5**, which exhibited the same pattern as steroidal 4-en-3-ones [3, 4]. Thus, the structure of **5** was confirmed as 4*S*,5*R*-12-*O*-β-D-glucopyranosyl-eremophil-7(11)(*E*),9-dien-8-one.

The molecular formula of compound **6** was determined as $C_{21}H_{30}O_8$ by HR-ESI-MS at *m/z* 433.1830 $[M+Na]^+$ (calcd. for $C_{21}H_{30}O_8Na$, 433.1838). The UV spectrum displayed an absorption at 264 nm, indicating the presence of one or more conjugated enone systems. It was found to have a similar structure to compound **4** by comparison of their NMR data (Table 2), the main difference observed being the signal at δ 171.7 which showed the presence of a carbonyl group. The HMBC experiment placed the carbonyl group at C-12, and linkage of the glucose moiety to C-12. The *Z* configuration of the 7(11) double bond was determined by the cross-peaks of H_3-13 with H-6β observed in the ROESY experiment. The CD spectrum of compound **6** showed the similar profile as the one reported for **5**, which was comparable to those of 4-ene-3-ketosteroids [3, 4]. Thus, the structure of **6** was elucidated to be 4*S*,5*R*-12-*O*-β-D-glucopyranosyl-eremophil-7(11)(*Z*),9-dien-8-one-12-oic acid.

Compound **7** was obtained as a colorless oil. Its molecular formula was determined as $C_{17}H_{24}O_3$ by HR-ESI-MS at *m/z* 299.1626 $[M+Na]^+$ (calcd. for $C_{17}H_{24}O_3Na$, 299.1623). The ^{13}C-NMR spectrum of **7** showed one lactonic carbon at δ 171.9, a full-substituted double bond at δ 157.6 and δ 123.9, and a ketal quaternary carbon signal resonanced at δ 103.2

Table 2 ^{1}H- and ^{13}C-NMR data of compounds **4–6**

No.	**4**[a]		**5**[a]		**6**[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	2.36 m	33.8	2.36 m	33.7	2.47 m	34.2
1b	2.30 br.d (14.0)		2.28 br.d (13.6)		2.38 br.d (13.6)	
2a	1.90 m	27.8	1.90 m	27.7	1.92 m	27.7
2b	1.48 m		1.48 m		1.53 m	
3a	1.55 m	31.7	1.55 m	31.6	1.57 m	31.6
3b	1.55 m		1.55 m		1.57 m	
4	1.54 m	44.3	1.54 m	44.2	1.58 m	44.3
5	–	43.8	–	43.6	–	43.6
6a	3.00 d (13.8)	42.2	3.10 d (13.9)	41.9	2.96 d (14.3)	40.7
6b	2.19 br.d (13.8)		2.17 br.d (13.9)		2.32 br.d (14.3)	
7	–	133.0	–	133.0	–	135.0
8	–	193.8	–	194.7	–	190.6
9	5.73 d (1.4)	126.2	5.73 d (1.4)	126.2	5.83 d (1.5)	125.1
10	–	173.3	–	173.3	–	176.2
11	–	144.1	–	142.0	–	136.9
12	4.88 d (14.0)	70.0	4.40 s	70.0	–	171.7
	4.43 dd (2.0, 14.0)		4.40 s		–	
13	1.98 s	16.6	2.09 d (2.0)	18.9	2.03 d (1.8)	16.6
14	1.05 s	16.9	1.02 s	16.6	1.10 s	17.9
15	0.99 d (6.0)	15.8	0.99 d (6.4)	15.8	1.02 d (6.4)	15.9
1′	4.18 d (7.8)	103.2	4.25 d (7.8)	103.2	5.58 d (8.0)	96.4
2′	3.19 m	75.1	3.28 m	75.1	3.35 m	74.7
3′	3.31 m	78.3	3.33 m	78.1	3.40 m	79.2
4′	3.30 m	71.7	3.27 m	71.8	3.32 m	71.1
5′	3.19 m	78.1	3.27 m	78.1	3.49 m	77.9
6′ a	3.81 dd (12.0, 2.3)	62.8	3.87 dd (12.0, 1.6)	62.9	3.85 dd (12.2, 2.1)	62.5
6′ b	3.66 dd (12.0, 5.0)		3.67 dd (12.0, 5.0)		3.69 dd (12.2, 5.0)	

Data are based on DEPT, HSQC, and HMBC experiments. ^{1}H-NMR (400 MHz, δ, J in Hz in parentheses), ^{13}C-NMR (100 MHz, δ)

[a] Data in CD_3OD

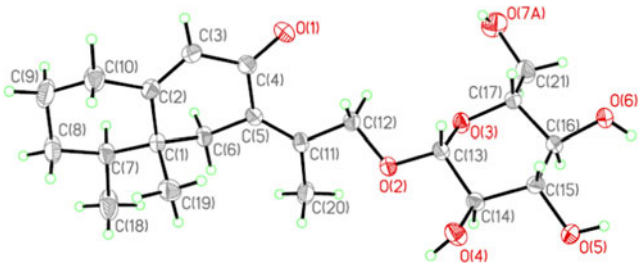

Fig. 5 ORTEP projection of compound **4** (crystallographic numbering)

(C-8) was visible. Its ^{1}H- and ^{13}C-NMR data (Table 1) were similar to those of tsoongianolide C [6], the difference being the signal of C-8 displaced downfield ($_{\Delta}\delta$ 2.8), and the presence of the signal of oxyethyl group at [δ 1.19 (3H, t, J = 7.0 Hz), 3.34 (1H, m), 3.49 (1H, m), δ 15.6 and 59.0]. Long-range correlations were observed between the oxyethyl group and C-8 from HMBC. The oxyethyl group was elucidated to be β-orientated by the splitting H-6α and H-6β appearing at δ 2.26 (br.d J = 12.2 Hz) and δ 2.68 (d J = 12.3 Hz) [6]. In addition, the optical rotation $[\alpha]_D^{20}$ −48.7 (c 0.150, $CHCl_3$) was in agreement with that of tsoongianolide C, $[\alpha]_D^{20}$ −26 (c 0.96, $CHCl_3$) [6]. Thus, compound **7** was determined as 4*S*, 5*R*-8β-ethoxy-eremophil-7(11),9-dien-8α,12-olide.

By comparing their physical and spectroscopic data with those reported in the literature, the known compounds were identified as 12-*O*-β-D-glucopyranosyl-eremophil-9,11(12)(*E*)-dien-8-one (**3**) [3], 10β-hydroxy-eremophil-7(11)-en-8α,12-olide (**8**) [7], phytol-stearate (**9**) [8],8β,10β-dihydroxyeremophil-7(11)-en-8α,12-olide (**10**) [2], 6β,8β-dihydroxyeremophil-7(11)-en-8α,12-olide (**11**) [10], 1β-hydroxy-12-*O*-β-D-glucopyranosyl-eremophil-9,11(12)(*E*)-dien-8-one (**12**) [3], farfugin A (**13**) [11, 12], farfugin B (**14**) [12],

phytol (**15**) [13], (4α*S*,5*S*)-5,6,7,8-tetrahydro-3-hydroxy-4α,5-dimethylnaphthalen-2 (4α*H*)-one (**16**) [14], β-sitosterol (**17**)[15], ligularenolide (**18**) [16], tsoongianolide B (**19**) [17], tsoongianolide D (**20**) [17], 6β-angeloyloxy-10β-hydroxy-eremophil-7(11)-en-8α,12-olide (**21**) [9], and 6β-hydroxy-eremophil-7(11)-en-8α,12-olide (**22**) [18]. In this paper, we also provided ^{13}C-NMR data of compounds **13** and **14** (Table 3) based on DEPT, HSQC, and HMBC experiments, which were not given in the literature.

Compounds **3**, **4**, **8**, **10**, **12**, **13**, **14**, **16**, **18**, **21** and **22** isolated from the aerial parts of *S. dianthus* were tested for their in-vitro cytotoxicities against K562, MCF-7, and Caco2 human cell lines by the MTS method, as described previously in the literature [19, 20]. However, none of the compounds showed significant inhibitory activity against the tumor cells used ($IC_{50} > 100$ μM, $n = 3$).

Experimental

General

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. 1D and 2D NMR spectra were recorded on a Bruker AV 400 or a Varian Unity INOVA 400/54 NMR spectrometer instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C) with TMS as an internal standard. IR spectra were obtained with a ThermoFisher Nicolet 6700 spectrometer, KBr pellets in cm^{-1}. UV spectra were determined with a Shimadzu UV-2450 spectrophotometer. Circular dichroism was obtained on a Jasco J-720 spectropolarimeter. HR-ESI-MS were measured using a Q-TOF micro mass spectrometer (Waters, USA). Single-crystal X-ray diffraction analysis of compound **2** and **4** was carried out on a Bruker SMART APEX II CCD diffractometer. GC analyses were performed using a Hewlett Packard GC6890 instrument on a Agilent HP-5 column (0.25 mm, 30 m, i.d., 0.25 μm). Silica gel (Qingdao Haiyang Chemical Co., Ltd., China, 200–300 mesh), Sephadex LH-20 (Pharmacia Co.), RP-18 silica gel (Merck, 40–60 μm), and D-101 macroporous resin (Rohm & Haas) were used for column chromatography (CC). Semi-preparative HPLC was carried out on a Waters SymmetryPrep^{TM} C-18 column (7 μm, 300 × 19 mm) with a Waters 600 controller and Waters 2487 detector. TLC plates were precoated with silica gel GF_{254} (Qingdao Haiyang Chemical Co., Ltd., China) and visualized under a UV lamp at 254 nm or by spraying with 5 % vanillin-H_2SO_4 (w/v) or by iodine.

Table 3 ^{1}H- and ^{13}C-NMR data of compounds **13** and **14**

No.	**13**[a]		No.	**14**[a]	
	δ_H	δ_C		δ_H	δ_C
1	3.47 m	27.6	1	7.25 s	110.9
2a	1.98 m	29.7	2	–	130.1
2b	1.77 m		3	–	137.0
3a	1.93 m	18.8	4	7.29 s	120.0
3b	1.93 m		5	–	115.2
4a	2.80 dt (4.2, 3.6)	27.2	6	–	154.3
4b	2.63 m		7	7.33 d (1.2)	140.8
5	–	131.3	8	–	126.8
6	–	130.7	9	2.22 d (1.2)	8.0
7	7.20 s	117.1	10	2.41 s	19.6
8	–	115.1	11	2.74 m	33.8
9	–	152.8	12	2.30 m	33.4
10	–	126.0	13	5.50 m	130.7
11	–	125.8	14	5.50 m	125.4
12	7.37 s	140.1	15	1.68 d (4.8)	18.0
13	2.25 d (1.2)	7.9			
14	2.36 s	20.1			
15	1.45 d (6.4)	21.1			

Data are based on DEPT, HSQC, and HMBC experiments. ^{1}H-NMR (400 MHz, δ, *J* in Hz in parentheses), ^{13}C-NMR (100 MHz, δ)

[a] Data in $CDCl_3$

Plant material

S. dianthus specimens were collected in Lhasa, Tibet, China, in July 2010. The plant was identified by Prof. Gesangsuolang in the Tibet Autonomous Region Institute for Food and Drug Control, People's Republic of China. A voucher specimen (No. Z36151001) was deposited in the School of Life Science and Engineering, Southwest Jiaotong University, Sichuan, People's Republic of China.

Extraction and isolation

The aerial parts of *S. dianthus* (5.5 kg) were extracted with 95 % ethanol three times at room temperature, with each soaking process lasting a week. After removal of the solvent by evaporation, the ethanol extract (680 g) was recovered. The extract was then suspended in H_2O (2 L) and defatted successively with petroleum ether (60–90 °C) (1 L × 3) and EtOAc (1 L × 3) to afford petroleum ether extract (190 g), EtOAc extract (370 g) and the remaining water layer (1.8 L) respectively.

The water layer (1.8 L) was evaporated to remove the solvent, and then subjected to column chromatography (∅ 9.5 × 60 cm) using D101 resin and eluted with H_2O, 30, 50, 75 and 95 % EtOH sequentially, yielding five fractions (I, II, III, IV and V).

Fraction IV (10.0 g) was subjected to CC (200–300 mesh, ∅ 5.0 × 50 cm, 120 g) on silica gel column chromatography, eluted with CH_2Cl_2:MeOH in a gradient manner (9:1, 4:1, 0:1) to yield three fractions ($F_{IV.1}$–$F_{IV.3}$)

based on TLC analysis. $F_{IV.1}$ (1.5 g) was subjected to Sephadex LH-20 column chromatography (∅ 2.5 × 60 cm) eluted with MeOH to give three subfractions ($F_{IV.3.1}$–$F_{IV.3.3}$). $F_{IV.3.1}$ (876.0 mg) was subjected to further silica gel CC (200–300 mesh, ∅ 2.0 × 30 cm, 25 g) eluted with petroleum ether–EtOAc (10:1, 5:1), and $F_{IV.3.1.1}$ (170 mg) was purified by preparative semi-HPLC [Waters SymmetryPrep^{TM} C-18 column (7 μm, ∅ 19.0 × 300 mm), 8 mL/min, 55 % MeOH in H_2O] to afford compounds **8** (30.0 mg), and **10** (1.5 mg). $F_{IV.3.1.2}$ (108.0 mg) was further purified with the same HPLC system using 58 % MeOH in H_2O resulting in the isolation of compounds **10** (70.0 mg) and **11** (3.0 mg). $F_{IV.3.1.3}$ (38 mg) was subjected to the same HPLC procedures (50 % MeOH in H_2O) to afford compounds **6** (4.3 mg), **2** (5.0 mg) and **5** (4.3 mg). $F_{IV.3.1.4}$ (184.0 mg) was purified with HPLC to afford compounds **1** (11.0 mg), **4** (25.4 mg), and **3** (37.1 mg) by the above HPLC system (29 % CH_3CN in H_2O). $F_{IV.3.2}$ (205.0 mg) was separated by RP-18 silica gel column CC (∅ 1.5 × 30 cm, 8 g) using 35 % MeOH in H_2O, followed by HPLC (30 % CH_3CN in H_2O) to afford compound **12** (7.0 mg).

Petroleum ether extract (150.0 g) was subjected to CC on silica gel (100–200 mesh, ∅ 8.0 × 60 cm, 600 g eluted with petroleum ether:EtOAc in a gradient manner (50:1, 20:1, 10:1, 5:1, 1:1, 0:1) to yield seven fractions ($F_{2.1}$–$F_{2.7}$) based on TLC analysis. $F_{2.1}$ (3.9 g) was also subjected to silica gel CC (200–300 mesh, ∅ 4.0 × 40 cm, 80 g) eluted with petroleum ether to obtain compounds **13** (38.0 mg) and **14** (15.0 mg). $F_{2.2}$ (5.0 g) was treated exactly like $F_{2.1}$, eluted with petroleum ether:EtOAc (80:1) to afford compounds **15** (29.0 mg), **7** (3.0 mg) and **22** (13.0 mg). $F_{2.3}$ (4.6 g) was separated by RP-18 silica gel column CC (∅ 3.5 × 60 cm, 40 g) using 90 % MeOH in H_2O, and $F_{2.3.2}$ was purified by silica gel column CC eluted with petroleum ether:EtOAc (30:1) to afford compounds **16** (4.0 mg) and **17** (119.0 mg), $F_{2.3.3}$ was purified by silica gel column CC eluted with petroleum ether:EtOAc (35:1) to afford compound **9** (19.0 mg). $F_{2.5}$ (6.6 g) was separated by silica gel column CC (∅ 4.5 × 60 cm, 70 g) eluted with petroleum ether:EtOAc (20:1). $F_{2.5.3}$ was purified by RP-18 silica gel column CC (∅ 1.5 × 60 cm, 8 g) using 42 % CH_3CN in H_2O to afford compounds **18** (81.0 mg), **19** (5 mg), and **20** (20 mg). $F_{2.5.3}$ was separated by Sephadex LH-20 (∅ 1.5 × 60 cm, MeOH) to get compound **21** (25.6 mg).

Compound **1**: Amorphous solid; $[\alpha]_D^{20}$ +31.3 (*c* 0.550, MeOH); UV (MeOH) λ_{max} (log ε), 240 (3.04) nm; CD (MeOH, *c* 5.50 × 10^{-4} M): 200 (+25.0), 239 (+8.7), 271 (-3.6) nm (Δε). IR (KBr) ν_{max} 3391, 2929, 1651, 1621, 1462, 1434, 1371, 1323, 1262, 1235, 1201, 1170, 1074, 1043, 892, 845, 654, 596 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 1. HR-ESI-MS at *m/z* 419.2053 $[M+Na]^+$ (calcd. for $C_{21}H_{32}O_7Na$, 419.2046).

Compound **2**: Colorless needles (MeOH); $[\alpha]_D^{20}$ −20.8 (*c* 0.125, MeOH); UV (MeOH) λ_{max} (log ε), 242 (2.96) nm; CD (MeOH, *c* 6.31 × 10^{-4} M): 205 (+11.5), 240 (−20.1) nm (Δε). IR (KBr) ν_{max} 3408, 2926, 2860, 1660, 1621, 1445, 1384, 1114, 1101, 1075, 1041, 641, 591 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 1. HR-ESI-MS at *m/z* 419.2049 $[M+Na]^+$ (calcd. for $C_{21}H_{32}O_7Na$, 419.2046).

Compound **4**: Colorless needles (MeOH); $[\alpha]_D^{20}$ +6.5 (*c* 0.750, MeOH); UV (MeOH) λ_{max} (log ε), 252 (2.96) nm; CD (MeOH, *c* 3.61 × 10^{-4} M): 202 (+21.5), 244 (+23.3), 280 (−12.3) nm (Δε). IR (KBr) ν_{max} 3395, 2915, 2872, 2855, 1657, 1617, 1461, 1430, 1372, 1306, 1229, 1162, 1085, 1048, 998, 885, 644, 580 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 2. HR-ESI-MS at *m/z* 419.2037 $[M+Na]^+$ (calcd. for $C_{21}H_{32}O_7Na$, 419.2046).

Compound **5**: Amorphous solid; $[\alpha]_D^{20}$ +75.8 (*c* 0.215, MeOH); UV (MeOH) λ_{max} (log ε), 251 (2.94) nm; CD (MeOH, *c* 4.32 × 10^{-4} M): 201 (+11.7), 245 (+21.6), 284 (−7.1) nm (Δε). IR (KBr) ν_{max} 3396, 2929, 2859, 1658, 1622, 1441, 1372, 1319, 1302, 1227, 1196, 1101, 1075,1037, 900,884, 634 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 2. HR-ESI-MS at *m/z* 419.2027 $[M+Na]^+$ (calcd. for $C_{21}H_{32}O_7Na$, 419.2046).

Compound **6**: Amorphous solid; $[\alpha]_D^{20}$ +52.1 (*c* 0.215, MeOH); UV (MeOH) λ_{max} (log ε), 264 (2.68) nm; CD (MeOH, *c* 1.03 × 10^{-3} M): 216 (+12.4), 255 (+16.4), 282 (−7.2) nm (Δε). IR (KBr) ν_{max} 3386, 2928, 2859, 1761, 1743, 1662, 1619, 1384, 1306, 1260, 1241, 1199, 1101, 1074, 1033, 641, 564 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 2. HR-ESI-MS at *m/z* 433.1830 $[M+Na]^+$ (calcd. for $C_{21}H_{30}O_8Na$, 433.1838).

Compound **7**: Colorless oil; $[\alpha]_D^{20}$ −48.7 (*c* 0.150, $CHCl_3$); UV (MeOH) λ_{max} (log ε), 233 (3.01) nm; IR (KBr) ν_{max} 2972, 2927, 2858, 1761, 1701, 1649, 1441, 1386, 1307, 1284, 1204, 1112, 1100, 1045, 1022, 976, 945, 915, 749 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 1. HR-ESI-MS at *m/z* 299.1626 $[M+Na]^+$ (calcd. for $C_{17}H_{24}O_3Na$, 299.1623).

Crystal data of **2** (ref 22): $C_{21}H_{32}O_7$, M_r 396.47, orthorhombic, space group $P2_12_12_1$, a = 7.1264(5) Å, α = 90°, b = 7.6320(9) Å, β = 90°, c = 37.822(3) Å, γ = 90°, V = 2057.1(3) Å^3, Z = 4, D_c = 1.280 Mg/m^3, $F(000)$ = 856; crystal dimensions/shape/color 0.35 × 0.30 × 0.01 mm/block/colorless. Reflections collected 4694, independent reflections 3277. Number of parameters refined 265; final R indices $[I > 2\sigma(I)]$ R_1 = 0.0409, wR^2 = 0.0929; R indices (all data) R = 0.0484, wR^2 = 0.0982.

Crystal data of **4** (ref 22): $C_{21}H_{32}O_7$, M_r 224.75, monoclinic, space group $P2_1$, a = 7.412(2) Å, α = 90°, b = 7.943(3) Å, β = 92.45(3)°, c = 20.031(7) Å; γ = 90°, V = 1178.2(7) Å^3, Z = 4, D_c = 1.267 Mg/m^3, $F(000)$ = 486, crystal dimensions/shape/color 0.03 × 0.03 × 0.02 mm/block/colorless. Reflections collected 7289, independent reflections 3989.

Number of parameters refined 287; final R indices [$I > 2\sigma(I)$] $R_1 = 0.1295$, $wR_2 = 0.3090$; R indices (all data) $R = 0.1953$, $wR_2 = 0.3706$.

Acid hydrolysis

Compound **1** (2 mg) was heated in 1 M HCl–dioxane (1:1, 1.5 ml) at 80 °C for 4 h. After cooling, the solution was diluted with H_2O (3 mL), neutralized with 1 M NaOH, and then extracted with $CHCl_3$ (3 × 3 mL). The H_2O layer was evaporated under a stream of N_2. The residue was dissolved in anhydrous pyridine (0.1 ml), 0.1 M L-cysteine methyl ester hydrochloride (0.2 mL) was added, and the solution was stirred at 60 °C for 1 h. The trimethylsilylation reagent HMDS–TMCS–pyridine (hexamethyldisilazane–trimethylchlorosilane–pyridine, 2:1:10) (Acros Organics, Geel, Belgium) was added and warmed at 60 °C for 30 min. The thiazolidine derivatives were analyzed by GC for sugar identification. The retention time of D-glucose (t_R 11.84 min) was confirmed by comparison with the retention time of the authentic standard. The process was repeated for compounds **2**, **4**, **5** and **6** [21].

Cell culture and cytotoxicity assay

K562 (human leukaemia cancer), MCF-7 (human breast cancer) and Caco2 (human colon cancer) cell lines were obtained from ATCC. The cells were maintained in a growth medium containing RPMI 1640 or Dulbecco's MEM with glutamate supplemented with 10 % fetal bovine serum (FBS) and antibiotics. All cells were cultured at 37 °C, 5 % CO_2 (v/v). All media supplements for cell culture were from Invitrogen (Shanghai, China). Treatments with xenobiotics were carried out in growth medium minus serum. DMSO (0.1 % v/v) were used as negative controls. Adriamycin was used as the positive control. Cells were seeded in a 96-well plate at 1×10^4 cells per well. After 24 h, cells were treated with compounds **3**, **4**, **8**, **10**, **12**, **13**, **14**, **16**, **18**, **21** and **22** at different concentrations, respectively, in a medium minus FBS for 24 h and then compared to the untreated cells. Cell viability was determined with an MTS Cell Proliferating Assay Kit (Promega, China) [19, 20].

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