Novel Nortriterpenes from *Phlomis umbrosa*

Pu LIU,[a,b] Zhi YAO,[c] Wen ZHANG,[a] Yoshihisa TAKAISHI,[d] and Hong-Quan DUAN*,[a]

[a] *School of Pharmaceutical Science, Basic Medical Research Center of Tianjin, Tianjin Medical University;* [c] *Department of Immunology, Tianjin Medical University; Tianjin 300070, China:* [b] *Chemical Engineering & Pharmaceutics College, Henan University of Science and Technology; Luoyang 471003, China: and* [d] *Faculty of Pharmaceutical Sciences, University of Tokushima; Tokushima 770–8505, Japan.*

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We isolated 8 novel 28-noroleanane-derived spirocyclic triterpenoids with unusual skeleton structures, phlomisone (1), phlomistetraol A (2), phlomistetraol B (3), phlomistetraol C (4), phlomispentaol (5), phlomishexaol A (6), phlomishexaol B (7), and phlomisin (8), from the ethanol extract of *Phlomis umbrosa* rhizomes. Their structures were elucidated on the basis of spectroscopic methods, including 1D, 2D nuclear magnetic resonance (NMR), high-resolution mass spectrometry (HR-MS), and X-ray analyses. Compounds 1—3, 5, and 7 exhibited positive cytotoxic activities against the carcinoma cell lines Hela and L929 *in vitro*, and these bioactive data suggested that the C-18 and C-21 positions had oxygenated functions that can improve the activity of the compound.

Key words *Phlomis umbrosa*; nortriterpenoid; cytotoxic activity; X-ray analysis

Phlomis umbrosa TURCZ, one of the plants of the genus *Phlomis* (Lamiaceae), is a perennial herbaceous plant growing in northern China. Its rhizome has been used in traditional Chinese medicine to reduce swelling and staunch bleeding, and it has anti-inflammatory and detoxification properties.[1,2] Some iridoids, phenylethanoids, and triterpenes have been previously isolated from the plant.[3—8] As reported previously,[9] we isolated 4 new nortriterpenoids from the plant. Further studies on the chemical constituents of the rhizomes of this plant enabled us to isolate 8 novel nortriterpenoids (**1**—**8**) with a rare skeleton.[10,11] In this paper, we describe the isolation, structure elucidation, and antitumor activities of compounds **1**—**8**, and for the first time, the skeleton of this type of compound has been confirmed by X-ray analysis.

Results and Discussion

The residue from the 95% ethanol extract of *P. umbrosa* was partitioned in H_2O and extracted with petroleum ether (P.E., 60—90 °C), EtOAc, and *n*-BuOH, successively. As the first screening of antitumor bioactivity, the P.E. extract revealed significant antitumor activity in Hela and L929 cell lines. After repeated column chromatography, including silica gel, Toyopearl HW-40C, and semi-preparative HPLC, compounds **1**—**8** were isolated from the P.E. extract of *P. umbrosa*.

Phlomisone (**1**), which was obtained as colorless needles, revealed an $[M+Na]^+$ ion at *m/z* 513.3183 $[M+Na]^+$ in the high-resolution positive Fourier transform mass spectrometer (FT-MS) consistent with a molecular formula of $C_{29}H_{46}O_6$ (calculated as 513.3186 for $C_{29}H_{46}O_6Na$). The IR spectrum of compound **1** revealed the presence of hydroxy (3393 cm^{-1}) and ketone (1719 cm^{-1}) groups. The ^{1}H-nuclear magnetic resonance (NMR) spectrum of compound **1** revealed 5 methyl groups [δ_H 1.17, 0.96, 1.08, 1.20, 1.37 (each 3H, s)], 3 oxygenated methine proton signals [δ_H 4.47 (1H, m), 4.88 (1H, br s), 4.23 (1H, br s)], 2 oxygenated methylene proton signals [δ_H 4.43, 4.63 (each 1H, d, J=11.0 Hz), 4.09, 4.25 (each 1H, d, J=11.1 Hz)], and an olefinic proton signal [δ_H 6.23 (1H, br s)]. Since the ^{13}C-NMR spectral data of this compound (Table 3) were similar to those of the known compounds from *Phlomis viscosa*,[11] compound **1** was assumed to be a pentacyclic nortriterpene.

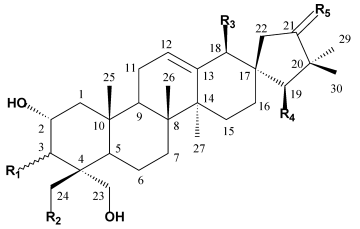
The partial structure of the pentacyclic E ring was obtained from the heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) spectra, and combined to the triterpene skeleton by a spiro-carbon atom of δ_C 50.9 (Fig. 2). According to the HMBC spectral correlations, the hydroxyl groups deduced from the molecular formula could be located at C-2, C-3, C-18, C-23, and C-24: H-3 (δ 4.88) to C-1, C-2, C-5, C-23, and C-24; H_2-23 to C-3, C-4, C-5, and C-24; and H-18 to C-13 and C-17. Furthermore, in the NOESY spectrum, the proton signal of H-2 correlated with that of H-24 and H-25; that of H-3 correlated with H-24; and that of H-18 correlated with H-27. Thus, the 3 hydroxy groups should be assigned as 2α-, 3α-, and 18β-oriented configurations. The structure of compound **1** was confirmed by X-ray analysis (Fig. 3), and designated as (17*R*)-19(18→17)-abeo-2α,3α,18β,23,24-pentahydroxy-28-norolean-12-ene-21-one (Fig. 1).

HR-FT-MS determined that phlomistetraol A (**2**) and B (**3**) had the same molecular formula $C_{29}H_{48}O_4$. The ^{1}H-NMR spectral data of compounds **2** and **3** (Table 1) revealed the presence of 6 tertiary methyls, 3 oxygenated methines, 1 oxygenated methylene, and 1 vinyl proton signal. The ^{13}C-NMR spectrum of these compounds revealed 29 carbons (Table 3) similar to those of compound **1**, except for the absence of a hydroxyl and a ketone group in compounds **2** and **3**. The hydroxy groups of compounds **2** and **3** could be located at C-2, C-3, C-18, and C-23 (or C-24) according to the HMBC correlation as follows: H-3 with C-1, C-2, C-5, C-23, and C-24; H_2-23 with C-3, C-4, C-5, and C-24; and H-18 with C-17 and C-13. In the NOESY spectrum, the correlation of H_3-24 with H_3-25 indicated that the hydroxyl group was located at C-23. On the other hand, H-18 correlated with H_3-27 in compounds **2** and **3**, H-3 (δ_H 3.61, br s) correlated with H-24 in compound **2** and H-3 (δ_H 3.36, d, J=9.6 Hz) correlated with H-5 and H-23 in compound **3**. Accordingly, the configuration of the C-3 and C-18 hydroxy groups had an 3α,18β-orientation in compound

* To whom correspondence should be addressed. e-mail: duanhq@tijmu.edu.cn

2 and an 3β,18β-orientation in compound **3**. Therefore, the structure of phlomistetraol A (**2**) was determined to be (2α,3α,17*R*,18β)-19(18→17)-abeo-28-norolean-12-ene-2,3,18,23-tetraol and that of phlomistetraol B (**3**) was assigned as (2α,3β,17*R*,18β)-19(18→17)-abeo-28-norolean-12-ene-2,3,18,23-tetraol.

Phlomistetraol C (**4**) had a molecular formula identical to that of **3**, *i.e.*, $C_{29}H_{48}O_4$ (HR-FT-MS). The NMR spectrum of compound **4** revealed 5 tertiary methyl groups, 2 oxygenated methines, 2 oxygenated methylenes, and a tri-substituted double bond. The ^{13}C-NMR spectral data of compound **4** was similar to those of compound **3**, except for C-18 and C-24 (Table 3). The HMBC correlation of H_2-24 with C-3, C-4, C-5, and C-23 confirmed that the hydroxy group was located at C-24. Phlomispentaol A (**5**) had the molecular formula $C_{29}H_{48}O_5$ and the NMR spectral data of this compound was similar to those of compound **4**, except for an additional hydroxyl group. Compound **5** was assumed to be 18-hydroxy phlomistetraol C, and the NOESY correlation of H-18 (δ_H 4.05) with H_3-27 (δ_H 1.12) proposed that the hydroxy group had 18β-orientation. Thus, the structures of compounds **4** and **5** were assigned as (2α,3β,17*R*)-19(18→17)-abeo-28-norolean-12-ene-2,3,23,24-tetraol and (2α,3β,17*R*,18β)-19(18→17)-abeo-28-norolean-12-ene-2,3,18,23,24-pentaol,



1 R_1 = α-OH R_2 = OH R_3 = OH R_4 = H R_5 = O

2 R_1 = α-OH R_2 = H R_3 = OH R_4 = H R_5 = H_2

3 R_1 = β-OH R_2 = H R_3 = OH R_4 = H R_5 = H_2

4 R_1 = β-OH R_2 = OH R_3 = H R_4 = H R_5 = H_2

5 R_1 = β-OH R_2 = OH R_3 = OH R_4 = H R_5 = H_2

6 R_1 = α-OH R_2 = OH R_3 = OH R_4 = OH R_5 = H_2

7 R_1 = α-OH R_2 = OH R_3 = OH R_4 = H R_5 = α-OH, β-H

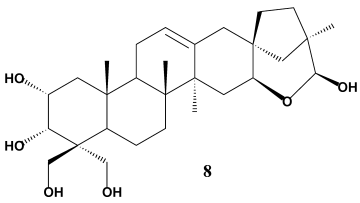


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Fig. 1. Structures of Compounds **1**—**8**

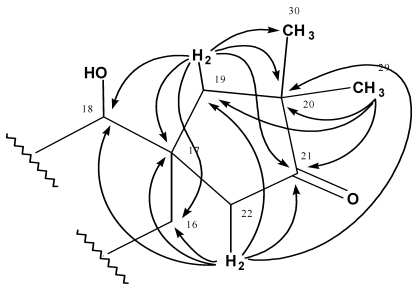

Fig. 2. HMBC Correlations of the E Ring in Compound **1**

Table 1. ^{1}H-NMR Data of Compounds **1**—**4**

No.	**1**[a)]	**2**[b)]	**3**[b)]	**4**[a)]
1	1.85, 2.06 m	1.31, 1.65 m	1.30, 0.94 m	1.42, 2.35 m
2	4.47 m	3.90 m	3.73 m	4.52 m
3	4.88 br s	3.61 br s	3.36 d, 9.6	4.40 d, 9.6
4				
5	2.16 m	1.55 m	2.01 m	2.03 m
6	1.63, 1.92 m	1.40 m	1.45 m	1.45, 1.75 m
7	1.55—1.75 m	1.40—1.50 m	1.40—1.50 m	1.70—1.80 m
8				
9	1.86 m	1.68 m	1.58 m	1.75 m
10				
11	2.08 m	2.02 m	2.02 m	2.05 m
12	6.23 br s	5.77 br s	5.77 br s	5.30 br s
13				
14				
15	1.12, 1.72 m	1.06, 1.70 m	1.10, 1.75 m	1.07, 1.71 m
16	1.55—1.75 m	1.65 m	1.63 m	1.63 m
17				
18	4.23 br s	3.90 br s	3.89 br s	2.60, 1.78 overlap
19	2.53, 1.70 d, 13.3	1.13, 1.97 d, 12.9	1.13, 1.97 d, 12.9	1.03, 2.56 d, 12.9
20				
21		1.36, 1.47 m	1.35, 1.46 m	1.85, 2.60 m
22	2.74, 2.27 d, 17.5	1.30 m	1.29 m	2.40 m
23	4.43, 4.63 d, 11.0	3.40, 3.54 d, 11.0	3.26, 3.50 d, 11.0	4.93, 4.33 d, 11.0
24	4.09, 4.25 d, 11.1	0.79 s	0.70 s	4.04, 4.67 d, 11.3
25	1.17 s	1.08 s	0.98 s	1.19 s
26	0.96 s	0.98 s	1.09 s	1.27 s
27	1.08 s	1.13 s	1.12 s	1.21 s
29	1.20 s	1.01 s	1.01 s	0.97 s
30	1.37 s	1.03 s	1.03 s	0.91 s

a) Measured in C_5D_5N, *b*) measured in CD_3OD.

respectively (Fig. 1).

Phlomishexaol A (**6**) and B (**7**) exhibited the same molecular formula of $C_{29}H_{48}O_6$ obtained from HR-FT-MS. Both compounds **6** and **7** revealed the presence of 5 methyl signals, 3 oxygenated methines, 2 oxygenated methylenes, and a vinyl proton in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectral data of compounds **6** and **7** were similar to those of compound **1**, except for the E ring (Table 3). Compounds **6** and **7** were assumed to be 19-hydroxy-21-deoxo-phlomisone or 21-hydroxy-21-deoxo-phlomisone. The HMBC correlations from δ_H 4.74 (1H, s, H-19 in compound **6**) to C-16, C-17, C-18, C-20, and C-29 were observed in compound **6**, while the HMBC correlations from δ_H 3.82 (1H, m, H-21 in compound **7**) to C-30 and C-19 and the ^{1}H–^{1}H COSY correlation between H-21 and H_2-22 were observed in compound **7**. In the NOESY spectrum, correlations of H_1-18 with H_3-27 in compounds **6** and **7** indicated the configurations of C-18 hydroxy groups had β-orientations in compounds **6** and **7**. In addition, the proton signal at δ_H 1.53 (H-16β) correlated with the methyl signal at δ_H 1.27 (H_3-30), while the signal at δ_H 4.74 (H-19) with δ_H 1.31 (H-29) in compound **6**, these observations suggested the presence of 19β-hydroxy group in compound **6**. On the other hand, the methyl proton signal at δ_H 1.06 (H_3-30) correlated with the signals at δ_H 1.44 (H-16β) and 3.82 (H-21) in compound **7**. Thus, the configuration of the C-21 hydroxy group was elucidated as an α-orientation in compound **7**. Therefore, structures of compounds **6** and **7** were assigned as (2α,3α,17*R*,18β,19β)-19(18→17)-abeo-28-norolean-12-ene-2,3,18,19,23,24-hexaol and (2α,3α,17*R*,18β,21α)-19(18→17)-abeo-28-norolean-12-ene-2,3,18,21,23,24-hexaol, respectively (Fig. 1).

HR-FT-MS established the molecular formula of phlomisin (**8**) to be $C_{29}H_{46}O_6$ (*m/z* $[M+Na]^+$ 513.3221; that calculated for $C_{29}H_{46}O_6Na$, 513.3186). The ^{1}H-NMR spectrum for compound **8** showed 4 methyl groups [δ_H 1.18, 1.04, 1.02, 1.23 (each 3H, s)], 4 oxygenated methine proton signals δ_H [4.47 (1H, m), 4.89, 4.67, 5.17 (each 1H, br s)], 2 oxygenated methylene groups [δ_H 4.43, 4.63 (each 1H, d, J=11.0 Hz), 4.09, 4.25 (each 1H, d, J=11.1 Hz)], and an olefinic proton [δ_H 6.12 (1H, br s)]. Except for the D–E ring, the ^{1}H- and ^{13}C-NMR spectral data of compound **8** were similar to those of compound **7** (Tables 2, 3). The methyl proton signal at δ_H 1.02 (H_3-27) exhibited an HMBC correlation with C-8, C-13, C-14, and C-15, while the signal at δ_H 4.67 (1H, br s, H-16) coupled with H-15 (δ_H 2.05) in ^{1}H–^{1}H COSY spectrum. Thus, an oxygenated location was assigned at C-16. Furthermore, the acetal proton signal at δ_H 5.17 (H-30) revealed the HMBC correlation to C-16, and the unsaturated degrees of compound **8** obtained from HR-MS indi-

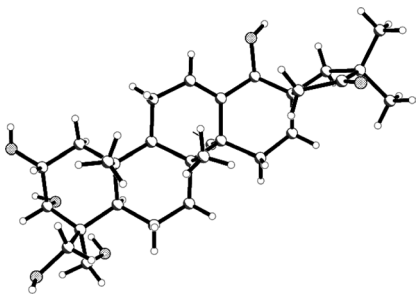

Fig. 3. The ORTEP Diagram of Compound **1**

Table 2. ^{1}H-NMR Data of Compounds **5**—**8**

No.	**5**[a)]	**6**[b)]	**7**[b)]	**8**[b)]
1	0.92, 1.41 m	1.87, 2.06 m	1.86, 2.05 m	1.84, 2.05 m
2	4.03 m	4.47 m	4.46 m	4.47 m
3	3.46 d, 9.6	4.92 br s	4.89 br s	4.89 br s
4				
5	2.02 m	2.15 m	2.18 m	2.15 m
6	1.42, 1.57 m	1.65, 1.88 m	1.69, 1.92 m	1.67, 1.93 m
7	1.38, 1.45 m	1.50, 1.73 m	1.53, 1.78 m	1.55, 1.75 m
8				
9	1.58 m	1.88 m	1.86 m	1.85 m
10				
11	1.98 m	2.13 m	2.05 m	2.07 m
12	5.77 br s	6.35 br s	6.25 br s	6.12 br s
13				
14				
15	1.02, 1.21 m	1.13, 1.87 m	1.10, 1.81 m	2.05 m
16	1.56, 1.63 m	1.53, 1.98 m	1.44, 1.72 m	4.67 br s
17				
18	4.05 br s	4.52 br s	4.16 br s	1.02, 1.81 m
19	1.97, 1.13 d, 12.7	4.74 s	2.45, 1.37 d, 13.1	2.27, 1.20 d, 12.5
20				
21	1.32, 1.46 m	1.55, 1.77 m	3.82 m	1.54, 2.25 m
22	1.31 m	1.73, 2.23 m	1.82 m	1.21, 1.62 m
23	3.52, 4.04 d, 11.4	4.48, 4.64 d, 11.0	4.45, 4.64 d, 11.0	4.43, 4.63 d, 11.0
24	3.63, 4.04 d, 11.4	4.14, 4.27 d, 11.0	4.11, 4.24 d, 11.1	4.09, 4.25 d, 11.1
25	1.08 s	1.19 s	1.20 s	1.18 s
26	0.96 s	1.11 s	1.03 s	1.04 s
27	1.12 s	1.08 s	1.09 s	1.02 s
29	1.03 s	1.31 s	1.31 s	1.23 s
30	1.01 s	1.27 s	1.06 s	5.17 s

a) Measured in CD_3OD, *b*) measured in C_5D_5N.

Table 3. ^{13}C-NMR Data of Compounds **1**—**8**

Carbon	**1**[a)]	**2**[b)]	**3**[b)]	**4**[a)]	**5**[b)]	**6**[a)]	**7**[a)]	**8**[a)]
1	43.8	42.6	48.3	48.2	48.2	43.8	43.8	43.8
2	66.8	67.3	69.8	69.4	70.0	66.9	66.8	66.8
3	74.2	78.8	78.2	80.2	79.5	74.2	74.2	74.2
4	48.0	42.7	44.2	48.2	48.0	48.0	48.0	48.0
5	45.3	44.4	48.5	48.9	48.9	45.3	45.3	45.3
6	19.4	19.0	19.1	19.7	19.7	19.5	19.5	19.4
7	35.1	34.7	34.6	34.0	35.0	35.2	35.1	35.0
8	40.2	40.8	40.7	40.5	40.7	40.3	40.3	40.2
9	48.4	48.5	48.7	48.6	48.5	48.5	48.5	48.4
10	38.7	39.2	39.0	38.7	38.8	38.7	38.7	38.7
11	23.9	24.1	24.1	24.8	24.3	24.1	23.9	23.9
12	119.8	119.2	119.2	122.9	119.1	119.1	119.6	119.5
13	142.6	143.6	143.6	146.1	143.6	144.1	143.3	139.4
14	44.5	45.3	45.3	42.5	45.2	44.4	44.5	44.6
15	28.2	28.2	28.1	26.1	28.2	27.2	28.1	28.1
16	36.0	37.0	37.0	31.6	37.0	28.3	38.0	75.1
17	43.6	51.2	51.1	49.6	51.2	51.8	49.6	47.1
18	75.4	76.4	76.4	28.4	76.3	73.6	74.6	29.3
19	50.9	53.1	53.1	49.3	53.1	82.6	49.7	45.0
20	45.6	39.8	39.8	37.5	39.8	43.0	44.8	44.7
21	222.5	43.1	43.0	31.6	43.1	38.7	81.2	36.5
22	41.7	30.8	30.7	26.8	29.6	29.2	39.6	31.8
23	69.6	71.4	66.3	63.3	62.8	69.5	69.6	69.6
24	64.6	17.7	14.1	64.8	64.6	64.6	64.6	64.6
25	17.8	17.9	18.1	17.7	18.0	17.9	17.8	17.8
26	18.1	18.2	18.2	18.2	18.0	18.3	18.1	18.1
27	23.4	23.6	23.6	26.1	23.6	23.6	23.4	23.3
29	29.4	30.5	30.4	24.5	30.5	29.7	29.3	23.0
30	26.0	30.6	30.4	33.3	30.4	22.4	24.8	100.3

a) Measured in C_5D_5N, *b*) measured in CD_3OD.

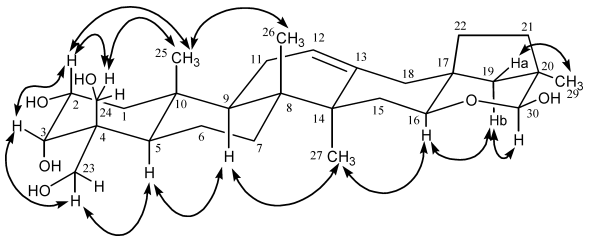

Fig. 4. Key NOESY Correlations of Compound **8**

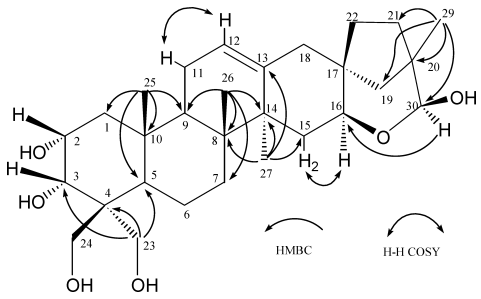

Fig. 5. Key HMBC and ^{1}H–^{1}H COSY Correlations of Compound **8**

cated that it contained another ring. These observations suggested that C-16 and C-30 should be combined by an ether bond. In the NOESY spectrum, the proton signal at δ_H 4.67 (H-16) correlated with δ_H 1.02 (H_3-27) and 2.27 (H-19b), while the signal at δ_H 2.27 (H-19b) with the signal at δ_H 5.17 (H-30). Thus, the configurations of C-16 and C-30 should be β-oriented (Fig. 4). Thus, the structure of compound **8** was assigned as (2α,3α,16β,17*R*,30β)-19(18→17)-abeo-16,30-epoxy-28-norolean-12-ene-2,3,23,24,30-pentaol (Fig. 1).

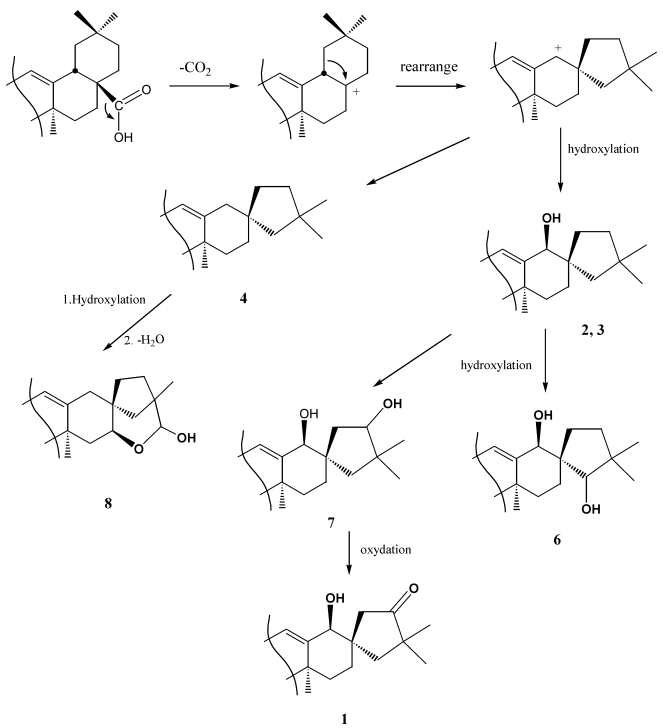

Chart 1. Plausible Biogenetic Pathway for Nortriterpenes

A plausible biogenetic pathway for above nortriterpenes was proposed on the basis of oleanolic acid isolated from the same plant, Chart 1.

The antitumor activity of compounds **1**—**8** was determined by using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-

Table 4. Cytotoxic Activities of Compounds **1**—**8**

Compd.	IC_{50} (μg/ml)	
	Hela	L929
1	25.14	21.99
2	43.04	9.03
3	75.29	61.81
4	>200	>200
5	30.01	18.18
6	150.13	91.73
7	31.21	3.14
8	>200	190.44

diphenyltetrazolium bromide) colorimetric assay with 2 tumor cell lines: Hela and L929. Compounds **1**—**3**, **5**, and **7** exhibited positive cytotoxic activities against the carcinoma cell lines Hela and L929 *in vitro* (Table 4), and these bioactive data suggested that the C-18 and C-21 positions had oxygenated functions that can improve the activity of the compound.

Experimental

General Experimental Procedures The melting point was uncorrected. NMR spectra were run on a Bruker AVANCE 300 instrument (^{1}H-NMR, 300 MHz; ^{13}C-NMR, 75 MHz) by using tetramethylsilane as an internal standard in both cases. MS data were obtained on an IonSpec 4.7 Tesla FTMS. Column chromatography was performed on silica gel (Qingdao Haiyang chemical Co., Ltd.) and Toyopearl HW-40 (TOSOH) columns. HPLC was performed with a JASCO Gulliver Series with PU-2089 (pump), RI-2031, and UV-2075 (detector). The preparative HPLC column of YMC-Pack ODS-A, SH-343-5 was used. IR spectra were recorded on a NICOLET 380 FT-IR spectrophotometer (Thermo Electron Corporation, U.S.A.). Optical rotation was measured with a MC 241 digital polarimeter (PERKIN-ELMER).

Plant Material *Phlomis umbrosa* TURCZ plants were collected from Jianshi, Hubei Province, China, in December 2005. The plant specimen was identified by Professor D. R. Wan (School of Life Sciences, South-Central University for Nationalities). A voucher specimen (D20050110) was deposited at the School of Pharmaceutical Sciences, Tianjin Medical University, China.

Extraction and Isolation The powered and dried rhizomes of *Phlomis umbrosa* (3400 g) was extracted with 95% ethanol (20 l×3, 6 h each time) under reflux. The extract was concentrated *in vacuo* to produce a residue (500 g) suspended in water, which was then partitioned with P.E. (60—90 °C), EtOAc, and *n*-BuOH, successively. The P.E.-partitioned extract (18 g) was chromatographed on a silica gel column, eluted with a solvent gradient system (petroleum ether–EtOAc [7 : 1, 5 : 1, 3 : 1, 1 : 1, 1 : 3], EtOAc, EtOAc–MeOH [19 : 1, 10 : 1, 5 : 1]) to produce 20 fractions (fractions 1—20). Fraction 14 (6.4 g) was chromatographed on a silica gel column with $CHCl_3$–MeOH (9 : 1, 85 : 15, 8 : 2) to produce 6 fractions (fractions 14.1—14.6). Fraction 14.4 (3.1 g) was chromatographed on a Toyopearl HW-40C column ($CHCl_3$–MeOH, 2 : 1) to produce 6 fractions (fractions 14.4.1—14.4.6). Fraction 14.4.5 (1.1 g) was separated by semi-preparative HPLC (ODS, MeOH–H_2O, 85 : 15, and then 8 : 2) to yield compounds **1** (10.1 mg), **2** (6.4 mg), **3** (9.8 mg), **4** (35.1 mg), and **5** (45.6 mg). Fraction 14.4.6 (0.6 g) was separated by semi-preparative HPLC (ODS, MeOH–H_2O, 85 : 15) to yield compounds **6** (71.5 mg), **7** (35.1 mg), and **8** (80.1 mg).

Phlomisone (**1**): Colorless needles, melting point (mp) 289—290 °C. $[\alpha]_D^{25}=+11.0°$ (c=1.07, C_5H_5N). IR (KBr): ν_{max}=3418, 2933, 1719, 1632, 1454, 1382, 1123, and 1039. For ^{1}H- and ^{13}C-NMR data, refer to Tables 1 and 3. Positive HR-FT-MS: *m/z*: 513.3183 $[M+Na]^+$ (Calcd for $C_{29}H_{46}O_6Na$: 513.3186).

Phlomistetraol A (**2**): Amorphous white powder, $[\alpha]_D^{25}=+10.0°$ (c=0.72, C_5H_5N). IR (KBr): ν_{max}=3393, 2927, 1457, 1383, 1138, and 1041. For ^{1}H- and ^{13}C-NMR data, refer to Tables 1 and 3. Positive HR-FT-MS: *m/z*: 483.3441 $[M+Na]^+$ (Calcd for $C_{29}H_{48}O_4Na$: 483.3450).

Phlomistetraol B (**3**): Amorphous white powder, $[\alpha]_D^{25}=+15.4°$ (c=0.27, CH_3OH). IR (KBr): ν_{max}=3403, 2934, 1457, 1382, 1138, and 1041. For ^{1}H- and ^{13}C-NMR data, refer to Tables 1 and 3. Positive HR-FT-MS: *m/z*: 483.3450 $[M+Na]^+$ (Calcd for $C_{29}H_{48}O_4Na$: 483.3450).

Phlomistetraol C (**4**): Amorphous white powder, $[\alpha]_D^{25}=+13.0°$ (c=0.24, CH_3OH). IR (KBr): ν_{max}=3423, 2926, 1458, 1384, 1124, and 1046. For ^{1}H- and ^{13}C-NMR data, refer to Tables 1 and 3. Positive HR-FT-MS: *m/z*: 483.3445 $[M+Na]^+$ (Calcd for $C_{29}H_{48}O_4Na$: 483.3450).

Phlomispentaol (**5**): Amorphous white powder, $[\alpha]_D^{25}=+9.7°$ (c=0.64, CH_3OH). IR (KBr): ν_{max}=3401, 2929, 1456, 1385, 1129, and 1049. For ^{1}H- and ^{13}C-NMR data, refer to Tables 2 and 3. Positive HR-FT-MS: *m/z*: 499.3387 $[M+Na]^+$ (Calcd for $C_{29}H_{48}O_5Na$: 499.3394).

Phlomishexaol A (**6**): Amorphous white powder, $[\alpha]_D^{25}=+11.4°$ (c=0.43, C_5H_5N). IR (KBr): ν_{max}=3411, 2934, 1632, 1452, 1376, 1262, and 1042. For ^{1}H- and ^{13}C-NMR data, refer to Tables 2 and 3. Positive HR-FT-MS: *m/z*: 515.3296 $[M+Na]^+$ (Calcd for $C_{29}H_{48}O_6Na$: 515.3343).

Phlomishexaol B (**7**): Amorphous white powder, $[\alpha]_D^{25}=+12.56°$ (c=0.97, CH_3OH). IR (KBr): ν_{max}=3381, 2930, 1455, 1378, 1131, and 1099. ^{1}H- and ^{13}C-NMR data, see Tables 2 and 3. Positive HR-FT-MS: *m/z*: 515.3354 $[M+Na]^+$ (Calcd for $C_{29}H_{48}O_6Na$: 515.3343).

Phlomisin (**8**): Amorphous white powder, $[\alpha]_D^{25}=+14.4°$ (c=0.45, CH_3OH). IR (KBr): ν_{max}=3415, 2928, 1453, 1379, 1263, and 1046. For ^{1}H- and ^{13}C-NMR data, refer to Tables 2 and 3. Positive HR-FT-MS: *m/z*: 513.3221 $[M+Na]^+$ (Calcd for $C_{29}H_{46}O_6Na$: 513.3186).

X-Ray Crystallographic Analysis Data of Compound 1 A monoclinic crystal was obtained from a CH_3OH solvent system. Crystal data: $C_{29}H_{46}O_6$, Mr=490.66, monoclinic. Crystal size=0.22×0.20×0.08 mm^3. Cell parameters: a=7.0637(10) Å, b=11.6334(17) Å, c=31.080(4) Å, V=2554.0(6) Å^3, space group $P2_12_12_1$. Data collection was performed on a SMART system (Bruker, 1997), the structure was resolved by direct methods (SHELXS-97), and the final R and R_w values were 0.45 and 0.119, respectively, for 3012 observed reflections. Copies of these data can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K.; fax: +44 1223 336 033; or deposit@ccdc.cam.ac.uk).

Procedure for Bioassay We seeded cancer cell lines (Hela and L929) at the concentration of 5×10^4 cells·ml^{-1} 180 μl in 96-well microplates, and incubated them for 24 h in order to allow cell attachment. The tested compound solutions were added to the cell cultures at proper concentrations, and the cells were incubated (37 °C, 5% CO_2) for 72 h. Supernatants were then removed using a micropipette, and 100 μl of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (0.5 mg·ml^{-1}) was then added to each well of 96-well plates and incubated for 4 h. The cell culture medium was removed with a pipette, and the purple formazan crystals were dissolved by adding 100 μl of DMSO to each well. The optical density (OD) was read at a wavelength of 490 nm with a microplate reader. The inhibitory ratio was calculated as follows: IR%=1−(OD with drug/OD without drug)×100%.[12)]

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References

1) Jiang Su New Medical College, "Dictionary of Chinese Materia Medica," Shanghai Scientific and Technical Publishing House, Shanghai, 1977.
2) Guo S. J., Gao L. M., Cheng D. L., *Pharmazie*, **2**, 178—180 (2001).
3) Jung K. Y., Do J. C., Son K. H., *Saengyak Hakhoechi*, **2**, 87—90 (1996).
4) Chung B. S., Kim J. W., Kim J. C., Kim Y. H., *Saengyak Hakhoechi*, **1**, 5—8 (1983).
5) Chung B. S., Kim J. W., Lee H. K., *Saengyak Hakhoechi*, **2**, 82—87 (1981).
6) Yang Y. L., Guo S. J., Sun K., Zhang J., Cheng D. L.. *Lanzhou Daxue Xuebao*, *Ziran Kexueban*, **2**, 67—71 (2004).
7) Liu S. W., Fu H. Z., Lin W. H., *Chin. Trad. Herb. Drugs*, **3**, 161—164 (1999).
8) Zhao J., Yang X. W., Fu H. Z., Li R. Z., Lou Z. C., *Chin. Trad. Herb. Drugs*, **2**, 90—93 (1999).
9) Liu P., Yao Z., Li H. Q., Duan H. Q., *Helv. Chim. Acta*, **2**, 601—606 (2007).
10) Calis I., Hasan K., Deniz T., Pete R., *Helv. Chim. Acta*, **3**, 611—619 (2004).
11) Luo Y. G., Chun F., Tian Y. J., Li B. G., Zhang G. L., *Tetrahedron*, **59**, 8227—8232 (2003).
12) Monks A., Carlos M. C., Ramon G., Concepcion A., Juan D. H., Pedro J. N., *J. Natl. Cancer Inst.*, **83**, 757—766 (1991).