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Cycloartane triterpenoids from the stems of *Schisandra glaucescens* and their bioactivity

Juan Zou [a,b], Jing Jiang [c], Yan-Yan Diao [c], Li-Bin Yang [a], Jin Huang [c], Hong-Lin Li [c], Xue Du [a], Wei-Lie Xiao [a,*], Jian-Xin Pu [a], Han-Dong Sun [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, People's Republic of China
[b] Graduate University of the Chinese Academy of Sciences, Beijing 100049, People's Republic of China
[c] Shanghai Key Laboratory of Chemical Biology, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, People's Republic of China

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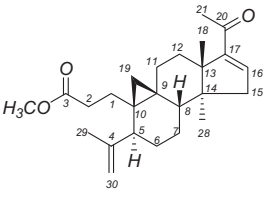
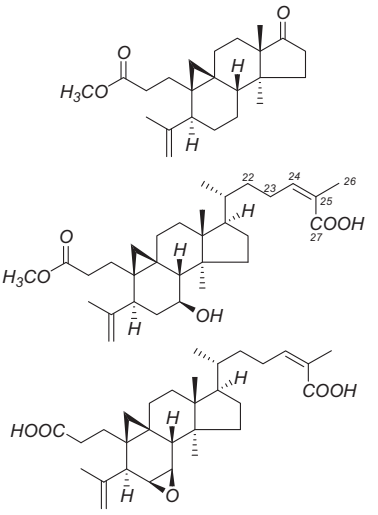
ABSTRACT

Five new cycloartane triterpenoids, schiglausins K–O (**1**–**5**), including one hexanortriterpenoid (**1**) and one octanortriterpenoid (**2**), as well as two known compounds (**6**–**7**), were isolated from the stems of *Schisandra glaucescens* Diels. Their structures were elucidated on the basis of spectroscopic methods, including extensive NMR spectra. Compounds **2**–**7** were tested for their FXR agonistic and antagonistic effects. Compound **7** exhibited significant antagonistic effect against FXR with IC_{50} of 1.50 μM.

1. Introduction

Schisandra glaucescens Diels (Schisandraceae) is a woodiness lianas, mainly distributed in Hubei and Sichuan provinces of China [1], which is used as folk medicine for the treatments of cough with dyspnea, spontaneous sweat, night sweat, chronic diarrhea, and neurasthenia [2]. Previous chemical investigations of *S. glaucescens* proved the presence of lanostane triterpenoids [3,4]. In this paper, we report the isolation, structure elucidation, and FXR agonistic and antagonistic effect evaluation of the new cycloartane triterpenoids schiglausins K–O (**1**–**5**). Schiglausins K (**1**) indicated as the first hexanortriterpenoid isolated from nature. The structures of **1**–**5** were established from their spectroscopic data.





* Corresponding authors. Tel.: +86 871 5223251; fax: +86 871 5216343.
E-mail addresses: xwl@mail.kib.ac.cn (W.-L. Xiao), hdsun@mail.kib.ac.cn (H.-D. Sun).

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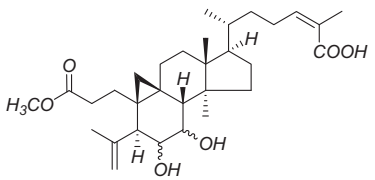

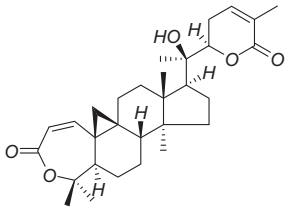

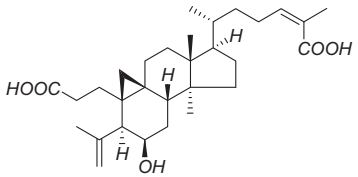

2. Experimental

2.1. General experimental procedures

Melting points were obtained on a XRC-1 micro melting point apparatus and are uncorrected. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. CD spectra were tested using Chirascan Circular Dichroism spectrometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. X-ray data was determined using a Bruker APEX DUO instrument. 1D and 2D NMR spectra were recorded on Bruker AM-400, DRX-500 and AVANCE III-600 MHz spectrometers. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Mass spectra were performed on a VG Autospec-3000 spectrometer at 70 eV. Column chromatography was performed using silica gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm × 25 cm column. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC-ODS (K) column.

Table 1
^{1}H NMR assignments of compounds **1–5**.

^{1}H	1 [a,d]	2 [b,d]	3 [c,e]	4 [b,d]	5 [a,e]
1	2.17 (overlap)	2.20 (m)	2.04 (m)	2.46 (m)	2.12 (m)
	1.01 (overlap)	1.50 (m)	1.42 (overlap)	1.66 (overlap)	1.31 (m)
2	2.66 (m)	2.65 (m)	2.48 (m)	2.89 (m)	2.33 (m)
	2.34 (m)	2.36 (m)	2.25 (m)	2.65 (m)	2.25 (m)
5	2.46 (m)	2.47 (m)	2.61 (m)	3.10 (overlap)	2.56 (overlap)
6α	1.44 (m)	1.44 (m)	1.55 (overlap)	3.09 (overlap)	3.95 (m)
6β	1.01 (overlap)	1.06 (m)	1.15 (m)	–	–
7α	1.01 (overlap)	0.98 (m)	3.66 (overlap)	2.92 (m)	3.60 (m)
7β	1.06 (m)	1.27 (m)	–	–	–
8	1.68 (overlap)	1.61 (m)	1.75 (m)	2.60 (m)	2.00 (m)
11α	2.17 (overlap)	1.98 (m)	1.43 (overlap)	1.31 (overlap)	2.18 (m)
11β	1.27 (overlap)	1.33 (m)	1.31 (overlap)	1.58 (overlap)	1.13 (overlap)
12α	1.94 (m)	1.90 (m)	1.55 (overlap)	1.43 (m)	1.52 (overlap)
12β	2.33 (m)	1.54 (overlap)	1.68 (overlap)	1.58 (overlap)	1.60 (m)
15α	1.95 (m)	2.20 (overlap)	1.63 (overlap)	1.58 (overlap)	1.68 (overlap)
15β	2.13 (m)	2.41 (m)	1.63 (overlap)	1.58(overlap)	1.68 (overlap)
16α	5.56 (m)	1.54 (overlap)	1.95 (m)	1.93 (m)	1.94 (m)
16β	–	1.64 (m)	1.33 (overlap)	1.33 (overlap)	1.52 (overlap)
17	–	–	1.54 (overlap)	1.54 (m)	1.54 (overlap)
18	1.29 (s)	1.10 (s)	0.97 (s)	0.81 (s)	1.02 (s)
19a	0.31 (d, 4.4)	0.26 (d, 4.4)	0.31 (overlap)	−0.11 (br. s)	0.63 (br. s)
19b	0.72 (d, 4.4)	0.70 (d, 4.4)	0.90 (br s)	1.88 (overlap)	1.13 (overlap)
20	–	–	1.42 (overlap)	1.43 (m)	1.41 (m)
21	2.25 (s)	–	0.91 (br. s)	0.94 (d, 6.2)	0.90 (d, 6.1)
22	–	–	1.15 (m)	1.31 (m)	1.13 (overlap)
			1.54 (m)	1.58 (m)	1.54 (overlap)
23	–	–	2.56 (m)	2.85 (m)	2.56 (overlap)
			2.46 (m)	2.78 (m)	2.46 (m)
24	–	–	6.09 (m)	6.03 (m)	6.08 (t)
26	–	–	1.92 (s)	2.15 (s)	1.91 (s)
28	0.97 (s)	0.82 (s)	0.97 (s)	0.98 (s)	1.06 (s)
29	1.68 (overlap)	1.68 (s)	1.68 (s)	1.97 (s)	1.83 (s)
30	4.82 (br. s)	4.82 (br. s)	4.75 (br. s)	4.95 (br. s)	5.12 (br. s)
	4.94 (br. s)	4.94 (br. s)	4.83 (br. s)	5.11 (br. s)	5.14 (br. s)
OMe	3.61 (s)	3.62 (s)	3.66 (s)	–	3.54 (s)

[a] Recorded at 400 MHz.
[b] Recorded at 500 MHz.
[c] Recorded at 600 MHz.
[d] Recorded in C_5D_5N.
[e] Recorded in $CDCl_3$.

Table 2
^{13}C NMR assignments of compounds **1–5**.

^{13}C	1 [a,d]	2 [a,d]	3 [b,e]	4 [a,d]	5 [c,e]
1	29.2 (t)	29.4 (t)	28.8 (t)	30.8 (t)	30.1 (t)
2	31.7 (t)	31.6 (t)	31.2 (t)	32.2 (t)	32.1 (t)
3	174.0 (s)	174.0 (s)	176.6 (s)	176.9 (s)	174.1 (s)
4	149.9 (s)	151.6 (s)	148.1 (s)	148.1 (s)	143.9 (s)
5	45.8 (d)	45.7 (d)	44.4 (d)	44.0 (d)	48.2 (d)
6	27.4 (t)	27.2 (t)	36.0 (t)	56.5 (d)	71.7 (d)
7	25.7 (t)	23.6 (t)	69.8 (d)	53.7 (d)	72.6 (d)
8	46.1 (d)	45.6 (d)	54.0 (d)	39.3 (d)	46.5 (d)
9	23.2 (s)	21.5 (s)	21.4 (s)	20.6 (s)	20.7 (s)
10	28.1 (s)	27.9 (s)	28.0 (s)	28.1 (s)	25.7 (s)
11	27.0 (t)	26.3 (t)	36.7 (t)	28.0 (t)	26.2 (t)
12	27.5 (t)	25.5 (t)	36.4 (t)	33.3 (t)	38.5 (t)
13	49.2 (s)	44.4 (s)	45.8 (s)	45.8 (s)	46.0 (s)
14	50.8 (s)	52.5 (s)	48.7 (s)	48.3 (s)	47.6 (s)
15	43.1 (t)	34.2 (t)	33.0 (t)	33.8 (t)	32.7 (t)
16	143.9 (d)	32.2 (t)	28.5 (t)	28.1 (t)	28.7 (t)
17	152.7 (s)	220.0 (s)	51.4 (d)	50.4 (d)	51.5 (d)
18	24.9 (q)	20.19 (q)	17.0 (q)	14.6 (q)	18.7 (q)
19	31.7 (t)	29.0 (t)	27.3 (t)	24.0 (t)	32.4 (t)
20	196.3 (s)	–	36.2 (d)	36.6 (d)	35.9 (d)
21	27.1 (q)	–	18.4 (q)	18.7 (q)	18.1 (q)
22	–	–	36.5 (t)	36.6 (t)	35.8 (t)
23	–	–	27.1 (t)	27.0 (t)	26.9 (t)
24	–	–	147.4 (d)	142.6 (d)	147.1 (d)
25	–	–	125.9 (s)	130.1 (s)	125.8 (s)
26	–	–	20.9 (q)	21.6 (q)	20.6 (q)
27	–	–	172.2 (s)	172.0 (s)	172.8 (s)
28	20.6 (q)	19.9 (q)	19.1 (q)	18.3 (q)	18.9 (q)
29	19.9 (q)	19.8 (q)	18.4 (q)	21.4 (q)	23.2 (q)
30	112.0 (t)	112.1 (t)	112.4 (t)	113.8 (t)	114.6 (t)
3-OMe	51.4 (q)	51.5 (q)	51.6 (q)	–	51.7 (q)

[a] Recorded at 125 MHz.
[b] Recorded at 150 MHz.
[c] Recorded at 100 MHz.
[d] Recorded in C_5D_5N.
[e] Recorded in $CDCl_3$.

Fractions were monitored by TLC and spots were visualized by heating the silica gel plates sprayed with 10% H_2SO_4 in CH_3CH_2OH.

2.2. Plant material

The stems of *S. glaucescens* were collected in Qinling Mountain, Shanxi Province, People's Republic of China, in August 2009. The specimen was identified by Prof. Xi-Wen Li and a voucher specimen (no. KIB 2009-08-08) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The plant material of *S. glaucescens* (6.5 kg) was powdered and exhaustively extracted with 70% aqueous Me_2CO at room temperature. The solvent was evaporated in vacuo, and the crude extract was partitioned between H_2O and EtOAc. The EtOAc extract (238.2 g) was chromatographed on a silica gel column eluted by $CHCl_3$–Me_2CO (1:0, 9:1, 8:2, 2:1, 1:1, and 0:1) to afford fractions I–VII. Fraction VI (30.1 g) was applied to RP-18, eluted with a MeOH–H_2O (45%–100%) gradient system, to afford five fractions. Fraction VI-3 was chromatographed over silica gel developed with petroleum ether-EtOAc (95:5–7:3) to afford five fractions, and VI-3-4 was subjected to preparative HPLC (MeOH–H_2O, 85:15) to afford two fractions. VI-3-4-1 and VI-3-4-2 were subjected to semi-preparative HPLC (CH_3CN:H_2O, 70:30) to yield compounds **4** (7.55 mg), **5** (4.3 mg) and **7** (18.07 mg). Fraction VII (30.1 g) was applied to RP-18, eluted with a MeOH–H_2O (45%–100%) gradient system, to afford eleven fractions. VII-11 was chromatographed over silica gel developed with (petroleum ether-EtOAc, 95:5–7:3) to afford five fractions. VII-11-2 was repeatedly chromatographed on silica gel (200–300 mesh) and Sephadex LH-20, and finally by semi-preparative HPLC (CH_3:H_2O, 80:20) to yield compounds **1** (1.2 mg), **2** (5.0 mg), **3** (3.9 mg) and **6** (15.2 mg).

Schiglausin K (**1**): colorless oil; $[\alpha]_D^{14.3} = +12.5$ (*c* 0.12, CH_3OH); UV (CH_3OH): λ_{max} (log ε) 241 (5.73) nm; IR (KBr): ν_{max} 3424, 2928, 1738, 1666, 1594, 1453, 1438, 1168, 1035, 890 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; positive ESIMS: *m/z* 407 $[M+Na]^+$; HRESIMS: *m/z* 407.2553 $[M+Na]^+$ (calcd. 407.2562 for $C_{25}H_{36}O_3Na$).

Schiglausin L (**2**): white powder; $[\alpha]_D^{25.2} = +52.7$ (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 203 (6.07) nm; IR (KBr) ν_{max} 3457, 2929, 1732, 1640, 1456, 1436, 1374, 1286, 1194, 1167, 1052, 1005, 888, 541 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; positive ESIMS *m/z* 381 $[M+Na]^+$; HRESIMS *m/z* 381.2403 $[M+Na]^+$ (calcd. 381.2405 for $C_{23}H_{34}O_3Na$).

Schiglausin M (**3**): white amorphous solid; $[\alpha]_D^{14.5} = +15.5$ (*c* 0.66, CH_3OH); UV (CH_3OH): λ_{max} (log ε) 207 (3.21) nm; IR (KBr): ν_{max} 3439, 2924, 1725, 1630, 1460, 1383, 1285, 1121, 558 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; positive ESIMS: *m/z* = 523 $[M+Na]^+$; HRESIMS: *m/z* 523.3390 $[M+Na]^+$ (calcd. 523.3399 for $C_{30}H_{46}O_5Na$).

Schiglausin N (**4**): white amorphous solid; $[\alpha]_D^{24.7} -16.0$ (*c* 0.97, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.17) nm; IR (KBr) ν_{max} 3432, 2950, 2934, 2873, 1723, 1638, 1452, 1376, 1280, 1122, 891, 749 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; positive ESIMS *m/z* 507 $[M+Na]^+$; HRESIMS *m/z* 507.3088 $[M+Na]^+$ (calcd. 507.3086 for $C_{30}H_{44}O_5Na$).

Schiglausin O (**5**): white amorphous solid; $[\alpha]_D^{15.3}$ +31.4 (*c* 0.13, MeOH), UV (MeOH) λ_{max} (log ε) 218 (5.81) nm; IR (KBr) ν_{max} 3448, 2954, 2933, 1737, 1456, 1439, 1377, 1255, 1201, 1168, 1050, 998, 891, 801 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; positive ESIMS *m/z* 539 $[M+Na]^+$; HRESIMS *m/z* 539.3338 $[M+Na]^+$ (calcd. 539.3348 for $C_{31}H_{48}O_6Na$).

2.4. Yeast two-hybrid system-based assay

The restriction and modification enzymes in this work were obtained from NEB. The *p*-nitrophenyl α-D-galactopyranoside, guggulsterone (GS), yeast nitrogen base without amino acids, agar, lithium acetate, dimethyl sulfoxide (DMSO) and glucose were all purchased from Sigma. The yeast expression plasmids pGADT7 and pGBKT7 were from Clontech (Palo Alto, CA). Chenodeoxycholic acid (CDCA) was from Merck. The dropout free from leucine and tryptophan (-Leu/-Trp DO) was bought from Takara. The yeast strain AH109 was purchased from Clontech (Palo Alto, CA). The agonistic or antagonistic activities of the compounds were tested by a yeast two-hybrid system for FXR constructed by yeast co-transformation with pGBKT7-FXR LBD and pGADT7-SRC1 according to the lithium acetate method [5]. Human FXR-LBD (200–473 aa) was sub-cloned into vector pGBKT-7 using NdeI and BamHI restrict enzyme sites. The

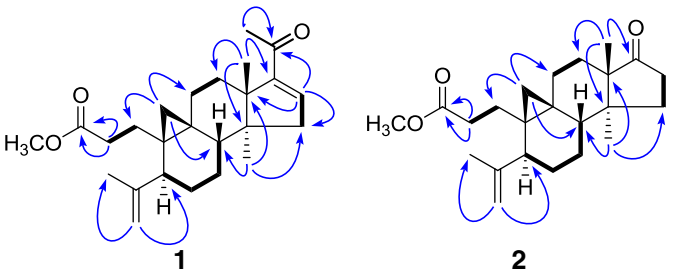

Fig. 1. Selected HMBC (⌒) and ^{1}H–^{1}H COSY (▬) correlations of **1** and **2**.

primers used for PCR amplification were listed as follows: FXR-LBD (sense) 5′-ATCATATGGAAATTCAGTGTAAATCTAAG-CG-3′, and (anti-sense) 5′-ATGGATCCTCACTGCACGTCCCA-3′. The combination plasmid pGADT7-SRC1 was prepared as described previously [6]. After co-transforming the two constructs into yeast strain AH109, we successfully evaluated FXR/SRC1 interactions by conducting a convenient α-galactosidase assay. Yeast transformations were incubated with either a control vehicle (DMSO) or the indicated compounds for 24 h in hFXR agonist testing, and in antagonist assays 10 μM CDCA was added. The α-galactosidase activity was then measured using *p*-nitrophenyl-D-galactopyranoside as the substrate [7]. The α-galactosidase activity was calculated according to the following formula:

$$\alpha - \text{galactosidase activity}[\text{milliunits}/(\text{mL}\times\text{cell})] = \frac{\text{OD410}\times Vf\times 1000}{(\varepsilon\times b)\times t\times Vi\times \text{OD600}}$$

where t is the elapsed time of incubation (min), V_f is the final volume of assay (200 μL), V_i is the volume of culture medium supernatant added (16 μL), OD_{600} is the optical density of overnight culture, and $\times b$ is the *p*-nitrophenol molar absorptivity at 410 nm × the light path (cm) = 10.5 mL/mol. Purities of guggulsterone (GS) and Chenodeoxycholic acid (CDCA) were greater than 96% and 97%, respectively.

3. Results and discussion

Powdered dried stems of *S. glaucescens* were extracted with 70% aqueous acetone. The filtrate was concentrated and partitioned between H_2O and EtOAc. The EtOAc fraction was dried under reduced pressure, and then subjected to successive chromatography on silica gel, MCI gel, Sephadex LH-20, and RP-18, and then purified by HPLC to afford five new 3, 4-*seco*-cycloartane triterpenoids, schiglausins K–O (**1**–**5**) and two known compounds (**6**–**7**). The ^{1}H and ^{13}C NMR spectroscopic data of **1**–**5** are listed (Tables 1, 2).

Schiglausin K (**1**), obtained as colorless oil, showed the molecular ion peak $[M+Na]^+$ at *m/z* 407.2553 (calcd. 407.2562) in the HRESIMS, corresponding to the molecular formula $C_{25}H_{36}O_3$, requiring eight degrees of unsaturation. The IR spectrum exhibited the absorption bands at 1738 cm^{-1} (conjugated C═O stretching) and 1666 cm^{-1} (C═C stretching), which suggested that **1** had a conjugated ketone in the structure. The ^{13}C NMR data (Table 2) at δ_C 196.3 and 174.0 confirmed the presence of the conjugated carbonyl and the easter groups. A 9, 19-cyclo-3, 4-*seco*-cycloartane skeleton was deduced from the ^{1}H NMR data (Table 1), with typical geminal cyclo-propyl (δ_H 0.31 and 0.72, d, J = 4.4 Hz), three tertiary methyls (δ_H 0.97, 1.29 and 1.68, s), as well as an olefinic, terminal CH_2 moiety (δ_H 4.82 and 4.94, s) [8–10]. The ^{13}C NMR and DEPT spectra of **1** showed signals for 25 carbons, including eight quaternary carbons (one carbonyl, one easter, two olefinic), three methine group (one olefinic), nine methylene groups (one olefinic), four methyl groups, and one methoxyl group. Analysis of 1D NMR spectroscopic data indicated that **1** was structurally similar to the western hemisphere of nigranoic acid [8], and the main differences were the side chain in **7** cleavaged and the appearance of one methoxyl group with the signal at δ_C 51.4 in **1**. This was confirmed by detailed analysis of the 2D NMR data. Structure of **1** was elucidated by the following HMBC correlations, from one methyl singlet resonance at δ_H 1.29 (Me-18) to an olefinic carbon C-17 (δ_C 152.7), a methylene C-12 (δ_C 27.4), and two quaternary carbons C-13 (δ_C 50.8) and C-14 (δ_C 49.2); from another methyl proton signal at δ_H 0.97 (Me-30) to a methine C-8 (δ_C 45.8), a methylene C-15 (δ_C 43.1), C-13 and C-14; and from one olefinic hydrogen at δ_H 5.56 (H-16) to a quaternary carbon C-20 (δ_C 196.3), C-13, C-14 and C-15 (Fig. 1). Furthermore, the β-orientation of H-8 (δ_H 1.68) was deduced on the basis of

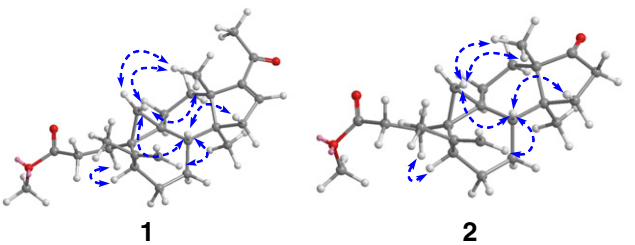

Fig. 2. Selected ROESY (↔) correlations of **1** and **2**.

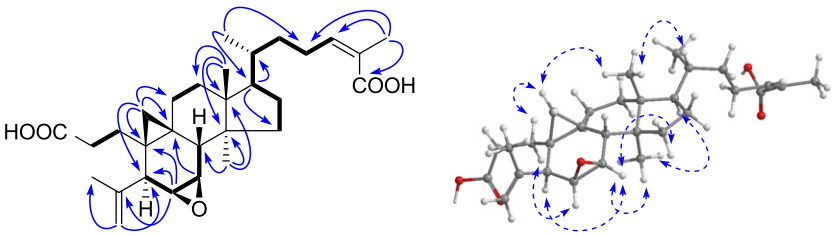

Fig. 3. Selected HMBC (⌒), ^{1}H–^{1}H COSY (▬) and ROESY(⌒) correlations of Selected correlations of **4**.

ROESY correlations of H-8 with both H-19 and H-18. In addition, H-5 (δ_H 2.46) showing ROESY correlation with H-2 and no ROESY cross peaks with H-8 and H-19 suggested that H-5 was α-oriented (Fig. 2). Thus, the structure of **1** was established.

Schiglausin L (**2**), obtained as colorless oil, gave the molecular formula $C_{23}H_{34}O_3$ from its HRESIMS data at *m/z* 381.2403 $[M+Na]^+$ (calcd. 381.2405), corresponding to seven degrees of unsaturation. Analysis of 1D NMR spectroscopic data showed that **2** was structurally similar to micranoic acid B [11]. The only difference was the appearance of one methoxyl group with the signal at δ_C 51.4 in **2**, which was established to be located at C-3 on the basis of the HMBC correlation of δ_H 3.62 (OMe) with C-3 (δ_C 174.0). Therefore, the structure of **2** was determined.

Structure determination of schiglausins M–O (**3**–**5**) was conducted by careful analyses of their 1D and 2D NMR spectral data and analogy to those of nigranoic acid [8].

Compound **3** (schiglausin M) was obtained as white amorphous solid. The molecular of **3** was determined as $C_{30}H_{46}O_5$ from its HRESIMS at *m/z* $[M+Na]^+$ 509.3237 (calcd. 509.3242), indicating eight degrees of unsaturation. The IR spectrum of **3** showed absorption bands for a hydroxyl group (3439 cm^{-1}) and two carbonyl bands (one of them indicative of a ester group (1725 cm^{-1}) and the other at 1630 cm^{-1} of an α, β-unsaturated carbonyl group), and these inferences were supported by the ^{13}C NMR data (δ_C 69.8, 174.4 and 172.2, respectively). The ^{1}H NMR data (Table 1) showed the presence of one isopropenyl group (methylene protons at δ_H 4.75 and 4.83, H-30a and H-30b), methyl group at δ_H 1.68 (H-29), an angelica acid moiety (δ_H 6.09, br. s, H-24; 1.92, H-26), two tertiary methyl groups (δ_H 1.29 and 0.97, H_3-18 and H_3-28) and one secondary methyl group (δ_H 0.91, br. s, H-21). Comparing the ^{13}C NMR data of **3** with the known compound nigranoic acid [8], indicated that they are analogous. The major differences were a hydroxyl group substituted at C-7 (δ_C 69.8 s) and one methoxyl group with signal at δ_C 51.9 in **3**. The HMBC correlation from H-8 to C-7, and ^{1}H–^{1}H COSY correlations of H-6/H-8/H-7, suggested a hydroxyl group was located at C-7. The ROESY correlations of H-7 with H-5 and H-28 established the H-7 was α-oriented. The obvious ROESY correlation of H-24 with H-26, determined the double bond between C-24 and C-25 to be *Z* configuration. The similarity of chemical shift and other observed ROESY correlations suggested other chiral centers possess the same relative stereochemistry as that of nigranoic acid. Consequently, the structure of **3** was established as shown.

Schiglausin N (**4**) was obtained as white amorphous solid, had a molecular formula of $C_{30}H_{44}O_5$ as determined by HRESIMS (*m/z* 507.3088$[M+Na]^+$), suggesting nine degrees of unsaturation. The ^{1}H NMR and ^{13}C NMR data were quite similar to those of **3**, and the main differences were C-6 and C-7 substituted by an epoxide ring group and the absence of one methoxyl group in **4**. The HMBC correlations from H-6 to C-4, C-5 and C-10, from H-7 to C-8 and C-9, together with ^{1}H–^{1}H COSY correlations of H-5/H-6/H-7/H-8 confirmed the above deduction (Fig. 3). ROESY correlations of H-7 with H-5 and H-28, and of H-6 with H-1 suggested the epoxide ring group was in β-orientation. The obvious ROESY correlation of H-24 with H-26, determined the double bond between C-24 and C-25 to be *Z* configuration (Fig. 3). Therefore, the structure of **4** was established as shown.

Schiglausin O (**5**) was obtained as white amorphous solid, the molecular of **5** was determined as $C_{31}H_{48}O_6$ from its HRESIMS at *m/z* $[M+Na]^+$ 539.3338 (calcd. 539.3348). Compared the ^{1}H NMR and ^{13}C NMR data of **5** with **3**, indicated that they are analogous. The major difference was the disappearance of a methylene group in **3** and the presence of a methine group in **5**. The HMBC correlations of H-30 and H-5 with C-6, H-8 with C-7, and the ^{1}H–^{1}H COSY correlations of H-6/H-5 and H-7/H-8 showed a hydroxyl group substituted at C-6 (δ_C 71.7 s). The obvious ROESY correlation of H-24 with H-26, determined the double bond between C-24 and C-25 to be *Z* configuration. However, there is not enough evidence in the ROESY spectrum for the determination of the configuration of C-6 and C-7. We also didn't get a suitable crystal for single crystal X-ray experiment. Therefore, the structure of **5** was currently established as shown.

Additionally, two known cycloartane-type triterpenoids kadsuphilactone B [12] (**6**) and 6β-hydroxy nigranoic acid [13] (**7**) were isolated.

Farnesoid X receptor (FXR), which is originally called retinoid X receptor-interacting protein 14 (RIP14), was renamed after the ability of its rat form to bind supra-physiological concentrations of farnesol. Since primary bile acids were identified as natural ligands, FXR was deorphanized in 1999. Highly expressed in the liver, intestine, kidney, adrenal glands, and

Table 3
Anti-FXR activities of compounds **2–7**.

Compounds	Activation (25 μM)	Inhibition rate % (25 μM)
DMSO	1	0
CDCA (10 μM)	2.70	ND
GS (25 μM)	ND	60.72 (IC_{50} = 6.47 μM)
2	0.95	16.35
3	1.15	12.96
4	1.13	21.86
5	1.09	19.49
6	1.08	12.84
7	0.91	92.90 (IC_{50} = 1.50 μM)

adipose tissue, FXR is a master regulator of the synthesis and pleiotropic actions of endogenous Bas [14] (e.g., chenodeoxycholic acid, CDCA, and cholic acid, CA). Several synthetic and semi-synthetic FXR agonists have been reported, such as GW4064 [15], 6 α-ethyl-chenodeoxycholic acid (6-ECDCA) [6], fexaramine [16] which have served as useful tools to investigate the role of FXR biology. While the above nonsteroidal analogs have suboptimal in vitro and in vivo characteristics compared to endogenous BAs, which limit their utility as small molecule drugs for treating FXR-mediated metabolic diseases. Most of the previously reported FXR antagonists have a steroid skeleton, such as the well-known steroidal FXR antagonist guggulsterones [17]. By regulating BAs, lipid and glucose homeostasis, FXR affects many aspects of our metabolism. This makes FXR an attractive pharmacological target for the management of diseases ranging from hyperlipidemia to diabetes, from cholestasis to enterohepatic tumors. Therefore, the discovery of novel FXR ligands is desirable. The fruits of *S. chinensis* have been reported to reduce the levels of CORT, Glu and protect the structure of the adrenal cortex, and can cure intrahepatic cholestasis of pregnancy with dexamethasone [15]. In order to find the related bioactive constituents from this plant, compounds **2**–**7** were tested for their FXR agonistic and antagonistic effects. Yeast two hybrid assay is usually used to screen for a gene encoding a novel protein that interacts with a known bait protein or to test two previously cloned proteins for interaction [18–19]. In this study, the α-galactosidase assay was performed by yeast co-transformation with pGBKT7-FXR LBD and pGADT7-SRC1 according to the lithium acetate method. As a result, compound **7** exhibited significant antagonistic effect against FXR with IC_{50} of 1.50 μM, and compound **4** showed weak inhibitory activity against FXR with inhibitory rate more than 20% at 25 μM, and other compounds didn't show bioactivity in current assay (Table 3). Compound **1** didn't test for its bioactivity for the quality limitation.

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