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Triterpenoids from the fruit of *Schisandra glaucescens*

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ABSTRACT

Five new triterpenoids, named schiglausins P-T (**1**–**5**), together with twelve known analogues (**6**–**17**), were isolated from the fruit of *Schisandra glaucescens* Diels. Their structures were determined by various spectroscopic methods, including HRESIMS, 1D and 2D NMR spectra and CD experiment. Additionally, all these compounds were tested for their cytotoxicities against B16 mouse melanoma cell line. Compounds **8**, **11**, **14**, and **15** exhibited moderate anti-proliferative effects against B16 cells with IC_{50} values ranging from 3.64 to 27.00 μM.

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1. Introduction

Plants of the genus *Schisandra* are economically valuable and widely used in traditional Chinese medicine. *Schisandra glaucescens* Diels is a vine mainly found in western Hubei and southeastern Sichuan Provinces in China. In traditional Chinese medicine, the stems of this plant have been used for the treatment of various diseases, including contusions, rheumatism, and arthritis [1]. Recently, the stems of *S. glaucescens* have been studied extensively, and a number of triterpenoids and lignans have been isolated [2–8].

The ripe fruit of *S. glaucescens* is a sweet red berry that is consumed as a health food by villagers in mountains around Shennongjia, China. The berries are believed to be beneficial to the lungs and kidneys, relieve the symptoms of asthma, reduce sweating and night sweats, alleviate chronic diarrhea, and reduce neurasthenia. In our preliminary work, a number of lignans were isolated from *S. glaucescens* fruit, some of which exhibited significant antioxidant and/or neuroprotective effects [8]. As a continuation of our previous work, five new triterpenoids, along with twelve known ones, were isolated from *S. glaucescens* fruit. All isolates were tested for their *in vitro* cytotoxicities against B16 mouse melanoma cell line.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were measured on a Varian Cary 50 Scan UV/Vis spectrophotometer. CD spectra were recorded on a Jasco J-810 spectropolarimeter. IR spectra were recorded on a Bruker VERTEX 70 FT-IR microscopic spectroscopy. NMR spectra were recorded on a Bruker-AM-400 spectrometer. HRESIMS was performed on a Thermo Scientific LTQ-Orbitrap XL mass spectrometer. MPLC was performed using a Buchi pump module C-605. Column chromatography was performed with silica gel (200–300 or 300–400 mesh; Qingdao Marine Chemical Inc., Qingdao, China), Sephadex LH-20 gel (GE Healthcare, Uppsala, Sweden) and MCI gel (CHP20P, 75–150 μm; Mitsubishi Chemical Industries Ltd., Tokyo, Japan). HPLC was performed on an Agilent 1260 system. The reversed phase HPLC column (Outstand C_{18}, 250 × 4.6 mm i.d.; Intramax, Wuhan, China) was used for analytical purpose. YMC-Pack ODS-A HPLC column (C_{18}, 250 × 10 mm i.d.; YMC, Tokyo, Japan) was used for semi-preparative purpose. MTT assays were performed on a BioTek Synergy 2 multi-mode microplate reader. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-tetrazolium bromide (MTT) was purchased from Aladdin Industry Corporation, Shanghai, China. Doxorubicin hydrochloride for injection (the positive control drug, 10 mg, Shenzhen Main Luck Pharmaceuticals Inc.) was prescribed from Tongji Hospital, Wuhan, China.

2.2. Plant material

S. glaucescens fruit was collected in the Shennongjia mountain areas of Hubei Province, China in September 2011, and identified by Mr. Shi-

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Gui Shi (Shennongjia Institute for Drug Control). A voucher specimen (ID 20110905) was deposited in the Herbarium of Materia Medica, Faculty of Pharmacy, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China.

2.3. Extraction and isolation

The air-dried fruit of *S. glaucescens* (15 kg) was extracted with 95% ethanol at room temperature and concentrated *in vacuo* to give a crude extract (750 g). The extract was added with 1.5 L deionized water and sequentially partitioned with petroleum ether, EtOAc, and *n*-BuOH.

The EtOAc-soluble fraction (150 g) was chromatographed on a silica gel column using PE-EtOAc (from 99:1 to 1:2) as elution solvent to give 14 fractions. Fraction 2 (12.5 g) was chromatographed on a Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1) column to give three subfractions (2-1 to 2-3). Fraction 2-1 (6.5 g) was chromatographed on a silica gel (PE-EtOAc, from 3.5:1 to 2.5:1) column to yield compounds **6** (250 mg) and **7** (180 mg). Fraction 3 (4.8 g) was chromatographed on a Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1) column to give two subfractions (2-1 to 3-2). Fraction 3-1 (2.5 g) was chromatographed on ODS (MeOH-H_2O, from 30:70 to 90:10) and silica gel (PE-EtOAc, 2:1) columns to yield compounds **8** (76 mg), **9** (43 mg), **11** (3 mg), and a mixture of two compounds. Then the mixture was separated using a semi-preparative HPLC (MeOH-H_2O, 80:20) column to yield compounds **1** (12 mg) and **10** (18 mg). Fraction 4 (15.3 g) was chromatographed on a Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1) column to give two subfractions (4-1 to 4-2). Fraction 4-1 (4.0 g) was chromatographed on ODS (MeOH-H_2O, from 30:70 to 90:10) and semi-preparative HPLC (MeOH-H_2O, 75:25) columns to yield compounds **12** (13 mg), **13** (5 mg), **14** (5 mg) and **15** (2 mg). Fraction 5 (5.3 g) was chromatographed on a Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1) column to give two subfractions (5-1 to 5-2). Fraction 5-1 (3.3 g) was chromatographed on a semi-preparative HPLC (MeOH-H_2O-CH_3COOH, 80:20:0.05) column to yield compounds **2** (15 mg), **3** (12 mg), **4** (18 mg), **5** (13 mg), **16** (9 mg), and **17** (22 mg).

*2.3.1. schiglausin P (**1**)*

Colorless oil; [α]20 D + 49.3 (*c* 0.49, $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 203 (4.21) nm; CD (*c* 1.49×10^{-3}, CH_3OH) λ_{max} (θ) 206 (75,851, pk), 237 (2550, tr), 255 (7063, pk) nm; IR (Film) ν_{max} 3503, 2930, 2869, 1718, 1559, 1439, 1373, 1238, 1123, 1032, 759 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 581.3412 $[M + Na]^+$ (calcd. for $C_{33}H_{50}O_7Na$, 581.3454).

*2.3.2. schiglausin Q (**2**)*

Colorless oil; [α]20 D + 65.9 (*c* 0.61, $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 203 (4.29) nm; CD (*c* 2.22×10^{-3}, CH_3OH) λ_{max} (θ) 210 (29,401, pk), 236 (2715, tr), 254 (7264, pk) nm; IR (Film) ν_{max} 2970, 2934, 2877, 1717, 1636, 1448, 1379, 1245, 1121, 1031, 982, 895, 858, 841, 811, 762, 683 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 491.3121 $[M + Na]^+$ (calcd. for $C_{30}H_{44}O_4Na$, 491.3137).

*2.3.3. schiglausin R (**3**)*

White powder; [α]20 D + 21.6 (*c* 0.39, $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 204 (4.37) nm; CD (*c* 1.58×10^{-3}, CH_3OH) λ_{max} (θ) 207 (22,023, pk), 223 (−4160, tr), 249 (−512, pk), 305 (−1432, tr) nm; IR (Film) ν_{max} 2974, 2944, 2924, 2870, 1733, 1707, 1375, 1241, 1027, 961, 898, 742, 712, 683, 658, 631 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 535.3383 $[M + Na]^+$ (calcd. for $C_{32}H_{48}O_5Na$, 535.3399).

*2.3.4. schiglausin S (**4**)*

Colorless oil; [α]20 D + 3.5 (*c* 0.64, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 202 (4.21) nm; CD (*c* 2.34×10^{-3}, CH_3OH) λ_{max} (θ) 203 (2450, pk), 213 (−19,736, tr), 249 (−103, pk), 294 (−2964, tr) nm; IR (Film) ν_{max} 2955, 2932, 2870, 1731, 1707, 1530, 1426, 1371, 1242, 1081, 1026, 972, 933, 894, 797, 758, 640, 569 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 511.3416 $[M - H]^-$ (calcd. for $C_{32}H_{47}O_5$, 511.3423).

Table 1
^1H NMR (400 MHz) and ^{13}C NMR (101 MHz) data for compounds **1** and **2** in $CDCl_3$.

	1			2	
Position	δ_C, type	δ_H (*J* in Hz)	Position	δ_C, type	δ_H (*J* in Hz)
1a	39.0, CH_2	3.21, dd (16.2, 4.3)	1a	32.1, CH_2	1.98, overlap
1b		2.29, dd (16.2, 5.5)	1b		1.80, overlap
2	70.9, CH	5.26, dd (5.5, 4.3)	2	28.9, CH_2	2.45, m
3	173.2, C		3	174.4, C	
4	75.7, C		4	147.4, C	
5	49.9, CH	1.63, overlap	5	49.2, CH	2.05, overlap
6	26.6, CH_2	1.52, overlap	6a	27.8, CH_2	1.77, overlap
			6b		1.53, overlap
7a	26.8, CH_2	1.71, overlap	7a	26.8, CH_2	1.78, overlap
7b		1.37, overlap	7b		1.59, overlap
8	43.9, CH	2.05, overlap	8	42.5, CH	2.14, overlap
9	144.0, C		9	142.6, C	
10	45.3, C		10	42.5, C	
11	119.1, CH	5.52, d (5.5)	11	118.2, CH	5.34, d (4.7)
12a	37.8, CH_2	2.03, overlap	12a	37.5, CH_2	2.13, overlap
12b		1.94, overlap	12b		1.95, overlap
13	44.0, C		13	44.4, C	
14	46.9, C		14	46.8, C	
15	33.7, CH_2	1.38, overlap	15	33.7, CH_2	1.38, overlap
16	27.4, CH_2	1.63, overlap	16a	26.7, CH_2	1.42, overlap
			16b		1.23, overlap
17	46.7, CH	1.58, overlap	17	46.8, CH	1.61, overlap
18	14.5, CH_3	0.67, s	18	14.4, CH_3	0.70, s
19	27.3, CH_3	1.22, s	19	26.9, CH_3	1.07, s
20	39.1, CH	2.00, overlap	20	39.1, CH	2.02, overlap
21	13.1, CH_3	0.95, d (6.6)	21	13.2, CH_3	0.99, d (6.5)
22	80.5, CH	4.43, dt (13.0, 3.4)	22	80.6, CH	4.47, dt (13.1, 3.3)
23a	23.5, CH_2	2.35, m	23a	23.5, CH_2	2.37, overlap
23b		2.05, m	23b		2.09, overlap
24	139.4, CH	6.58, brd (6.3)	24	139.5, CH	6.62, dt (6.3, 1.5)
25	128.3, C		25	128.3, C	
26	166.5, C		26	166.6, C	
27	17.0, CH_3	1.89, s	27	17.0, CH_3	1.92, s
28	18.7, CH_3	0.67, s	28	18.4, CH_3	0.75, s
29	26.9, CH_3	1.22, s	29a	113.9 CH_2	4.88, s
30	34.3, CH_3	1.32, s	29b		4.72, s
3-OMe	52.6, CH_3	3.72, s	30	23.2, CH_3	1.77, s
1′	170.2, C				
2′	20.7, CH_3	2.05, s			

*2.3.5. schiglausin T (**5**)*

Colorless oil; [α]20 D + 41.8 (*c* 0.47, $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 202 (4.21) nm; IR (Film) ν_{max} 3458, 2962, 2932, 2878, 1694, 1642, 1446, 1377, 1261,1095, 1060, 1021, 802, 753, 634 cm^{-1}; ^1H and ^{13}C NMR data see Table 3; HRESIMS *m/z* 525.3517 $[M + Na]^+$ (calcd. for $C_{31}H_{50}O_5Na$, 525.3556).

2.4. Cytotoxicity assay

The viability of cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. B16 mouse melanoma cells were treated with compounds **1**–**17** at various concentrations (0, 1, 4, 8, 20, and 50 μM) for 24 h. Analysis was performed according to a previously published procedure [9]. The half-maximal inhibitory concentration values (IC_{50}) were obtained from the MTT viability curves using GraphPad Prism 4.0.

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