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New *neo*-clerodane diterpenoids from *Scutellaria barbata* with cytotoxic activities

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ABSTRACT

Two new *neo*-clerodane diterpenoids have been isolated from the whole plant of *Scutellaria barbata* D. Don, and their structures were established by detailed spectral analyses as scutehenanine H (**1**) and 6-(2,3-epoxy-2-isopropyl-n-propoxyl)barbatin C (**2**). *In vitro*, the isolated two new compounds showed significant cytotoxic activities against three human cancer lines, and gave IC_{50} values in the range OF 2.0-4.2 μM.

1. Introduction

Scutellaria L. (Labiatae) is a large subcosmopolitan genus with 360 currently recognized species [1]. Recently, plants belonging to this genus have attracted attention owing to interesting biological activities observed for some *neo*-clerodane diterpenoids isolated from them. *Scutellaria barbata* D. Don is a perennial herb which is natively distributed throughout Korea and southern China. This herb is known in traditional Chinese Medicine as Ban-Zhi-Lian and traditional Korean medicine as Banjiryun, respectively, and has been used as an anti-inflammatory and antitumor agent [2–4]. In previous phytochemical studies on *S. barbata* collected from Linyi district (Shandong Province, China), we reported the isolation of a series of *neo*-clerodane diterpenoids [5–7]. From a chemotaxonomic point of view, it is of interest to note that these *neo*-clerodane diterpenoids lack an oxygenated substituent at C-19 found in almost all of the *neo*-clerodane diterpenoids from European *Scutellaria* species [8]. The oxidation pattern of the neo-clerodane diterpenoids indicates a phytogeographical and an evolutionary relation between some Asiatic and European Scutellaria species. This relation has been proposed previously, based on both botanical considerations and the global distribution of the genus in the reported literature [9].

As part of our ongoing search for more novel *neo*-clerodane diterpenoid, we have investigated the aerial parts of *S. barbata* collected from the Zhumadian district, Henan Province, China. The EtOH extract of this species was successively partitioned with $CHCl_3$ and EtOAc. The $CHCl_3$ fraction was subjected to extraction with 3% HCl, and the aqueous layer was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ fraction was concentrated in vacuo and sequentially subjected to column chromatography over silica gel, silica gel RP-18, and Sephadex LH-20 to give two new *neo*-clerodane diterpenoids. By means of extensive spectroscopic analyses, the structures of two new compounds **1**–**2** were elucidated as scutehenanine H (**1**), and 6-(2,3-epoxy-2-isopropyl-n-propoxyl)barbatin C (**2**). In addition, the isolated

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two new compounds were screened for cytotoxity against three tumor cell lines (HONE-1 nasopharyngeal, KB oral epidermoid carcinoma, and HT29 colorectal carcinoma cells), with IC_{50} values being in the range of 2.0–4.2 μM. Herein we report on the isolation, structure elucidation, as well as the evaluation of cytotoxic effects of these two new *neo*-clerodane diterpenoids.

2. Experimental

2.1. General experimental procedures

Melting points were measured on an XT-4 micro-melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Shimadzu UV-160 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 683 infrared spectrometer with KBr disks. FABMS and HR-FABMS were recorded on an Autospec-Ultima ETOF MS spectrometer. NMR spectra were recorded on a Varian Unity BRUKER 400 at 400 MHz (^{1}H) and 100 MHz (^{13}C), with TMS as the internal standard. Silica gel (200–300 mesh) for column chromatography was obtained from Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. Precoated plates of silica gel GF254 and silica gel RP-18 F254s (Merck) were used for TLC, and detected under UV light.

2.2. Plant material

S. barbata D. Don was collected in Zhumadian district, Henan Province, People's Republic of China, in September 2008, and identified by Professor Yan-yan Zhao, School of Pharmaceutical Science, Yantai University. The whole plants of *S. barbata* was harvested and air-dried at room temperature in the dark. A voucher specimen (YP08077) has been deposited at the Herbarium of School of Pharmaceutical Science, Yantai University.

2.3. Extraction and isolation

The air-dried whole plant (49.6 kg) of *S. barbata* was finely cut and extracted three times with refluxing EtOH. Evaporation of the solvent under reduced pressure provided the ethanolic extract. The extract was dissolved and suspended in H_2O, and

Table 1
NMR data of compounds **1–2** (in $CDCl_3$)[a,b].

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	1.76 (m, H_a-1) 2.03 (m, H_b-1)	18.3 CH_2	1.31 (m, H_a-1) 1.65 (m, H_b-1)	19.4 CH_2
2	2.68 (m, 2H)	26.2 CH_2	2.02 (m, 2H)	26.2 CH_2
3	5.31 (br s)	123.1 CH	5.21 (br s)	123.0 CH
4		141.5 C		141.1 C
5		42.8 C		43.0 C
6	5.33 (d, 10.0)	77.7 CH	5.34 (d, 9.8)	81.4 CH
7	3.72 (d, 10.0)	74.7 CH	3.85 (d, 9.8)	73.9 CH
8		84.7 C		77.5 C
9		43.3 C		47.6 C
10	2.46 (dd, 2.4, 12.2)	40.3 CH	2.24 (dd, 2.3, 12.0)	42.5 CH
11	6.05 (dd, 3.9, 12.0)	75.6 CH	6.39 (d, 16.8)	147.8 CH
12	2.09 (m, H_a-12) 2.35 (m, H_b-12)	30.7 CH_2	6.36 (d, 16.8)	121.5 CH
13		79.9 C		162.3 C
14	4.49 (s)	76.2 CH	5.91 (br s)	114.6 CH
15		175.3 C		173.9 C
16	4.26 (d, 8.7, H_a-16) 4.30 (d, 8.7, H_b-16)	75.8 CH_2	4.99 (dd, 1.3, 8.7, H_a-16) 5.02 (dd, 1.3, 8.7, H_b-16)	70.7 CH_2
17	1.72 (s, 3H)	18.0 CH_3	1.16 (s, 3H)	22.5 CH_3
18	1.61 (s, 3H)	20.9 CH_3	1.61 (s, 3H)	20.8 CH_3
19	1.38 (s, 3H)	17.2 CH_3	1.23 (s, 3H)	17.5 CH_3
20	1.14 (s, 3H)	17.0 CH_3	1.12 (s, 3H)	15.4 CH_3
1′		164.8 C	4.17 (br s, 2H)	61.6 CH_2
2′		125.9 C		78.4 C
3′	9.22 (br s)	150.7 CH	2.71 (d, 5.9, H_a-3′) 2.98 (d, 5.9, H_b-3′)	38.3 CH_2
4′			2.31 (sept, 6.8)	34.9 CH
5′	8.85 (br d, 4.5)	153.6 CH	0.97 (3H, d, 6.8)	17.1 CH_3
6′	7.44 (dd, 4.5, 7.6)	123.5 CH	1.07 (3H, d, 6.8)	16.9 CH_3
7′	8.29 (br d, 7.6)	137.5 CH		
1″		167.5 C		
2″		130.7 C		
3″ and 7″	8.07 (m, 2H)	129.7 CH		
4″ and 6″	7.48 (m, 2H)	128.4 CH		
5″	7.57 (br t, 7.7)	133.1 CH		

[a] Coupling constants (in Hz) were presented in parentheses.
[b] The assignments were based on DEPT, HMQC, HMBC, and ^{1}H–^{1}H COSY experiments.