Fitoterapia 119 (2017) 40–44

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Scubatines A–F, new cytotoxic *neo*-clerodane diterpenoids from *Scutellaria barbata* D. Don

Qing-Qing Yuan [a,b], Wei-Bin Song [a], Wen-Qiong Wang [a], Li-Jiang Xuan [a,*]

[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 501 Haike Road, Shanghai 201203, PR China
[b] University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing 100049, PR China

ARTICLE INFO

Article history:
Received 8 November 2016
Received in revised form 23 March 2017
Accepted 24 March 2017
Available online 27 March 2017

Keywords:
Scutellaria barbata D. Don
Lamiaceae
neo-Clerodane diterpenoids
Cytotoxic activity

ABSTRACT

Phytochemical investigation of the 70% acetone extract of the whole plant of *Scutellaria barbata* D. Don afforded six new *neo*-clerodane diterpenoids, scubatines A–F (**1**–**6**), and four known analogues (**7**–**10**). Their structures were elucidated on the basis of extensive spectroscopic analyses. Cytotoxic activity against the HL-60 and A549 cell lines was assessed for all isolated compounds. Compound **9** exhibited moderate activity against HL-60 with an IC_{50} value of 5.6 μM. Compound **6** showed weak cytotoxic activity against A549 and HL-60 with IC_{50} values of 10.4 and 15.3 μM, respectively.

1. Introduction

Scutellaria barbata D. Don belonging to the Lamiaceae family, is widely distributed in mainland China, Korea, India and other Asian countries. This herb, known as 'Banzhilian' in traditional Chinese medicine, has been used therapeutically to treat cancer, hepatitis and other diseases [1]. Previous phytochemical investigations of *S. barbata* revealed the presence of a series of *neo*-clerodane diterpenoids, which possessed cytotoxic [2–11], anti-inflammatory [12], antiviral [13] and antioxidative activities [14]. As part of our efforts to discover cytotoxic diterpenoids from Chinese traditional medicinal plants, 70% acetone extract of the whole plant of *S. barbata* was investigated. Six new *neo*-clerodane diterpenoids (**1**–**6**) and four known analogues (**7**–**10**) were identified (Fig. 1). Herein, we report the isolation, structural elucidation of these compounds, and their cytotoxic activities against two human tumor cell lines.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Perkin-Elmer 577 spectrophotometer, respectively. NMR spectra were recorded on a Varian Mercury NMR spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. HRESIMS was measured on an Agilent G6224A TOF mass spectrometer. TLC was performed on pre-coated silica gel GF254 plates (Qingdao Marine Chemical Factory). Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Factory), Sephadex LH-20 (20–80 μm, Amersham Pharmacia Biotech AB), and RP-18 silica gel (20–45 μm; Fuji Silysia Chemical Ltd.). All solvents were spectroscopic grade (Adamas-beta Reagent Co. Ltd.) or distilled prior to use.

2.2. Plant material

The whole plants of *S. barbata* D. Don were collected in Jiangsu province, China, in May 2014, and authenticated by Prof. He-Ming Yang. A specimen (SIMMXLJ-608) was deposited at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, PR China.

2.3. Extraction and isolation

The air-dried and powdered whole plants of *S. barbata* (10 kg) were extracted with 70% acetone (3 × 25 L) at room temperature and concentrated under vacuum to yield a residue, which was dissolved in H_2O and extracted successively with EtOAc and *n*-BuOH. The EtOAc fraction (489 g) was subjected to silica gel column (200–300 mesh, 1.2 kg, 12 × 60 cm) and eluted with petroleum ether/ethyl acetate (15:1, 10:1, 5:1, 2:1, 1:1, 0:1, each 8 L) to yield fractions 1–5. Fraction 3 (108 g) was subjected to silica gel CC (200–300 mesh, 500 g, 6 × 35 cm) eluted with petroleum ether/actone (10:1, 5:1, 2:1, 1:1, each 1 L) to give fractions 3A–3E. Fraction 3D (32 g) was subjected to an RP-18 column (4.5 × 35 cm) eluted with an MeOH-H_2O gradient system (0:1, 1:4, 2:3, 3:2, 4:1, 1:0, each 800 mL) to give fractions 3D1–3D9. Fraction

* Corresponding author at: State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, PR China.
E-mail address: ljxuan@simm.ac.cn (L.-J. Xuan).

http://dx.doi.org/10.1016/j.fitote.2017.03.012
0367-326X/

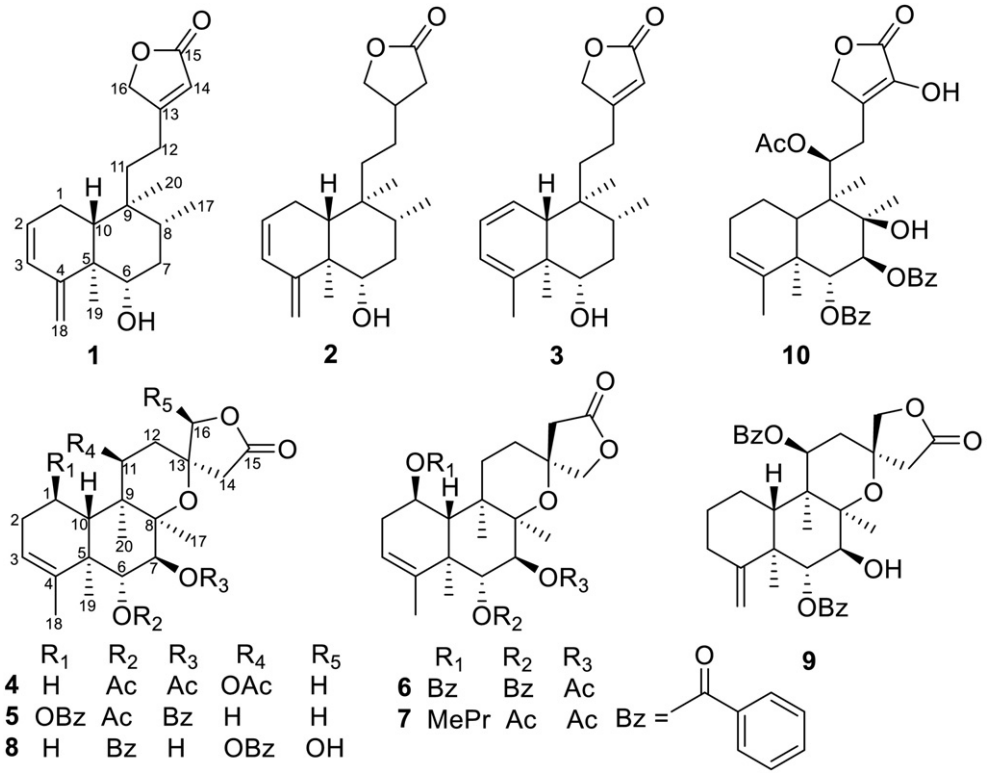

Fig. 1. Structures of compounds **1**–**10**.

3D4 (0.9 g) was subjected to silica gel column (200–300 mesh, 200 g, 2.5 × 30 cm) and eluted with petroleum ether/CH_2Cl_2 (1:3, 1:5, 1:10, each 400 mL) to yield compound **2** (8 mg). Fraction 3D6 (1.5 g) was subjected to silica gel column (200–300 mesh, 300 g, 3 × 30 cm) and eluted with CH_2Cl_2/acetone (200:1, 100:1, 50:1, 20:1, 10:1, each 500 mL) to afford compound **5** (7 mg) and **6** (8 mg). Fraction 3E (25 g) was subjected to silica gel CC (200–300 mesh, 600 g, 4.5 × 45 cm) eluted with petroleum ether/acetone (5:1, 3:1, 1:1, each 800 mL) to obtain a major fraction, which was further purified on a column of Sephadex LH-20 gel (2.5 × 120 cm, MeOH) to give compound **10** (80 mg). Fraction 4 (129 g) was subjected to silica gel CC (200–300 mesh, 500 g, 6 × 35 cm) eluted with petroleum ether/acetone (10:1, 8:1, 6:1, 4:1, 2:1, 1:1, each 1 L) to give fractions 4A–4E. Fraction 4B (45 g) was subjected to an RP-18 column (4.5 × 35 cm) eluted with an MeOH-H_2O gradient system (0:1, 1:4, 2:3, 3:2, 4:1, 1:0, each 800 mL) to give fractions 4B1–4B9. Fraction 4B6 (0.8 g) was subjected to silica gel column (200–300 mesh, 300 g, 3 × 30 cm) and eluted with CH_2Cl_2/acetone (100:1, 50:1, 20:1, each 500 mL) followed by a Sephadex LH-20 gel (2.0 × 80 cm, $CHCl_3$/MeOH 1:1) to give compound **1** (20 mg). Fraction 4B7 (1.2 g) was subjected to silica gel CC (200–300 mesh, 300 g, 3 × 30 cm) eluted with CH_2Cl_2/acetone (150:1, 100:1, 50:1, 20:1, 10:1, 5:1, each 500 mL) to give fractions 4B71–4B72. Fraction 4B71 (2.3 g) was subjected to an RP-18 column (2.5 × 35 cm) eluted with an MeOH-H_2O gradient system (3:2, 7:3, 4:1, each 400 mL) to give compound **3** (23 mg), **4** (10 mg), and **7** (25 mg). Fraction 4B8 (1.5 g) was subjected to silica gel column (200–300 mesh, 400 g, 3.5 × 30 cm) and eluted with CH_2Cl_2/MeOH (200:1, 100:1, 50:1, 20:1, each 600 mL) followed by a Sephadex LH-20 gel (2.0 × 80 cm, $CHCl_3$/MeOH 1:1) to give compound **8** (42 mg) and **9** (15 mg).

2.3.1. Scubatine A (*1*)

White amorphous powder; $[\alpha]_D^{20}$ − 7.3 (*c* 0.48, $CHCl_3$); IR (KBr) ν_{max} 3451, 1781, 1744, 1640, 1008 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 339.1934 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_3Na$, 339.1931).

2.3.2. Scubatine B (*2*)

White amorphous powder; $[\alpha]_D^{20}$ + 8.3 (*c* 0.36, $CHCl_3$); IR (KBr) ν_{max} 3454, 1779, 1640, 1173, 1013 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 341.2089 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_3Na$, 341.2087).

Table 1
^1H (500 MHz) and ^{13}C NMR (125 MHz) data of compounds **1**–**3** in $CDCl_3$ (δ in ppm, *J* in Hz).

No.	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.16 overlap 1.94 m	23.7	2.20 m 1.97 dt (18.6,5.1)	23.9	5.97 ddd (9.1,4.9,3.1)	126.4
2	5.66 m	127.0	5.72 t (7.4)	127.4	5.60 m	123.8
3	5.95 d (9.6)	129.6	6.01 d (9.7)	129.7	5.58 m	120.5
4		154.1		154.3		148.1
5		43.5		43.7		44.4
6	3.83 m	74.8	3.87 overlap	75.2	3.75 dd (11.2,4.3)	74.3
7	1.58 overlap	36.7	1.60 overlap	36.9	1.53 m 1.65 m	37.6
8	1.55 m	34.4	1.58 overlap	34.5	1.65 m	34.5
9		38.7		38.8		38.1
10	1.40 dd (11.7,4.5)	43.2	1.43 dd (11.3,4.8)	43.3	2.15 m	47.1
11	1.53 m	34.7	1.25 overlap	35.6	1.60 m 1.74 m	34.5
12	2.14 m	21.8	1.25 overlap	26.3	2.22 m	22.2
13		170.8	2.41 m	36.4		170.8
14	5.76 br s	114.9	2.14 m 2.62 dd (17.2,8.4)	34.8	5.83 br s	115.3
15		174.0		177.1		174.1
16	4.67 d (1.8)	73.1	3.87 overlap 4.42 t (8.2)	73.5	4.70 d (1.7)	73.2
17	0.80 d (7.3)	15.4	0.82 d (5.8)	15.5	0.85 d (6.5)	15.5
18	4.72 s 5.35 s	110.0	4.78 s 5.36 s	109.8	1.95 s	21.2
19	0.98 s	14.5	1.02 s	14.6	0.87 s	9.4
20	0.81 s	17.5	0.81 s	17.8	0.89 s	19.0