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Neo-clerodane diterpenes and phytoecdysteroids from *Ajuga decumbens* Thunb. and evaluation of their effects on cytotoxic, superoxide anion generation and elastase release *in vitro*

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ABSTRACT

Five novel compounds, including four neoclerodane diterpenoids, named ajugacumbins K—N (**1**–**4**) along with a phytoecdysterone, named ajugacetalsterone E (**5**), were isolated from the whole herbs of *Ajuga decumbens* (Labiatae). Their structures were elucidated on the basis of detailed spectroscopic analysis including IR, HRESIMS, CD, 1D and 2D NMR spectroscopic experiments. Compounds **1**–**5** were evaluated for their cytotoxic activities and the effects on superoxide anion generation and elastase release in FMLP/CB-induced human neutrophils.

1. Introduction

Ajuga decumbens Thunb. (family Labiatae), a traditional Chinese medicine, distributed mainly in east, central south and southwest of China [1], is used to treat various diseases such as sore throat, phlegm and fever [2]. Previous investigation showed that *A. decumbens* possessed multiple biological activities and neoclerodane diterpenoids and phytoecdysteroids have been found to be responsible for such physiological activities [3, 4]. *Neo*-clerodane diterpenoid derivatives have been found to exhibit anti-inflammatory, antifeedants, antitumor, antifungal, antimicrobial and moluscicid activities [2, 5–8]. Phytoecdysteroids also have multiple pharmacology, such as regulating the organism behaviors, improving organism immunity, inducing hyperglycemia, potentiating the effects of insulin and preventing oxidation [4]. In addition, by bringing about positive shifts in metabolic processes, several phytoecdysterones (include ecdysterone and cyasterone) isolated from *A. decumbens* exert a considerable therapeutic on various experimental pathological states: in myocardial infarct, diabetes, hepatitis and atherosclerosis [9]. It must be mentioned that ecdysterone also possess the capacity for blocking processes of free-radical oxidation [9].

In the course of our search for neoclerodane diterpenoids and phytoecdysteroids with structural and biological diversity from the whole plant of *A. decumbens*, four new neoclerodane diterpenoids **(1–4)** and one new phytoecdysteroid (**5**) were isolated. The present paper reports the isolation and structural elucidation of compounds **1**–**5** and their cytotoxic activities and inhibitory activities on superoxide anion generation and elastase release in FMLP/CB-induced human neutrophils.

2. Results and discussion

The whole plant of *A. decumbens* was extracted with 85% EtOH under reflux. The crude extracts were partitioned sequentially with petroleum ether, CH_2Cl_2, and *n*-BuOH, successively. The CH_2Cl_2 extract was subjected repeatedly to ODS, MCI, silica gel, Sephadex LH-20, and preparative TLC to afford four neoclerodane diterpenoids (**1**–**4**) and one phytoecdysteroid derivative (**5**). The structures of compounds **1**–**5** (Fig. 1) were elucidated by using CD, IR, HRESIMS, 1D and 2D NMR, including ^{1}H—^{1}H COSY, HSQC, HMBC, and NOESY experiments.

2.1. Characterization of the novel compounds

Compound **1** was obtained as colorless crystals. The HRESIMS showed a positive ion peak at *m/z* 381.2260 $[M + H]^+$ (calcd for $[C_{21}H_{33}O_6]^+$, 381.2272), corresponding to a molecular formula of $C_{21}H_{32}O_6$. The ^{1}H NMR spectrum (Table 1) at δ_H 5.98 (1H, s, H-14) and 4.92 (2H, s, H-16), together with ^{13}C NMR spectrum (Table 1) at δ_C

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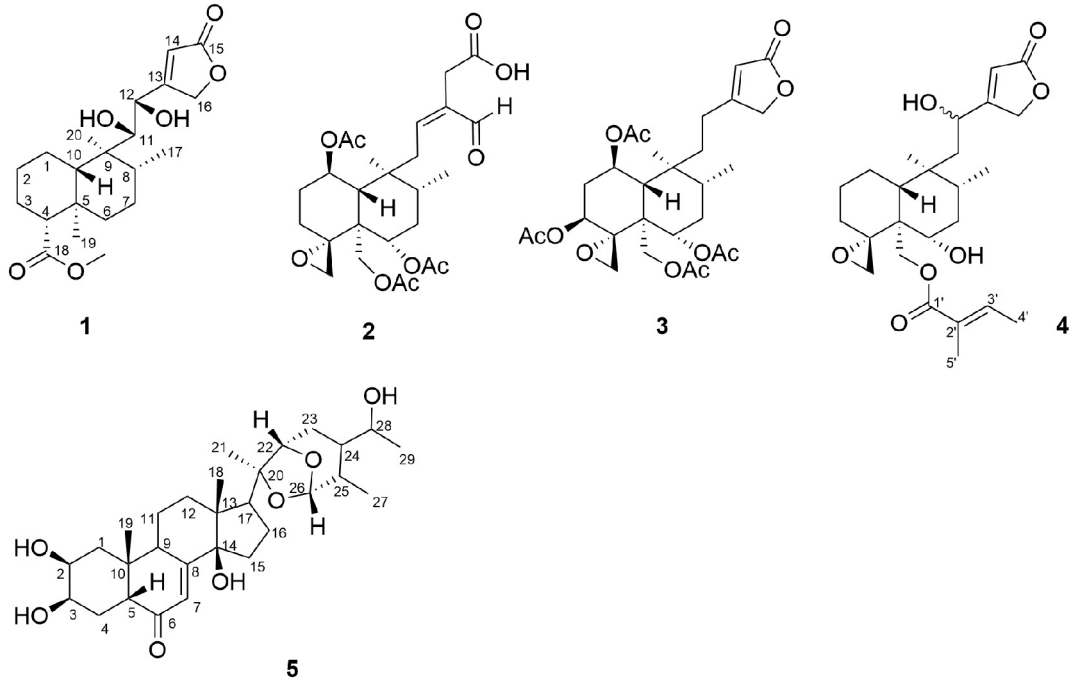

Fig. 1. Structures of compounds **1–5**.

Table 1
^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of compounds **1–4** in DMSO-d_6.

Position	**1**		**2**		**3**		**4**	
	δ_H[a] (*J* in Hz)	δ_C[b]	δ_H (*J* in Hz)	δ_C[b]	δ_H (*J* in Hz)	δ_C[b]	δ_H (*J* in Hz)	δ_C[b]
1	1.80–1.35 m, 1.35–1.20 m	21.3	5.47 ddd (5.6, 10.4, 16.0)	70.4	5.40 ddd (4.4, 10.0, 15.5)	70.3	1.35–1.50 m	20.7
2	1.80–1.35 m	25.6	2.33 m, 1.56 m	31.4	2.33 dd (8.4, 15.5)	40.8	1.35–1.50 m	24.3
3	1.80–1.35 m, 1.35–1.20 m	26.1	2.20 m	30.1	5.82 d (10.0)	67.0	1.35–1.50 m	31.9
4	2.01 dd (2.8, 12.8)	55.0	–	64.4	–	64.3	–	67.0
5	–	44.0	–	45.5	–	45.9	–	45.0
6	1.80–1.35 m	39.4	4.79 dd (4.4, 10.0)	71.4	4.65 dd (4.0, 12.0)	71.2	3.58 dd (4.5, 11.0)	72.3
7	1.80–1.35 m	35.0	1.62 m, 1.59 m	33.4	1.71 dd (4.0, 12.0)	30.2	1.65–1.73 m	34.0
8	1.80–1.35 m	36.9	1.38 dd (4.4, 10.0)	35.7	1.61 q (12.0)	34.6	1.80–1.87 m	34.2
9	–	43.6	–	40.1	–	39.0	–	39.0
10	1.40 t (8.0)	48.6	1.05 d (12.8)	49.2	1.07 d (13.2)	50.0	1.03 d (12.5)	46.7
11	4.25 d (6.4)	76.6	2.44 d (7.6)	38.5	1.30–1.50 m	31.7	1.90–2.05 m	42.5
12	5.33 d (6.4)	64.4	7.18 dd (4.8, 7.2)	154.8	2.16–2.25 m	33.0	5.38 d (6.0)	63.6
13	–	178.0	–	138.4	–	173.4	–	177.0
14	5.98 s	113.3	3.17 dd (16.4)	29.9	6.23 d (1.6)	115.0	5.96 s	112.8
15	–	173.9	–	171.6	–	171.1	–	173.5
16	4.92 s	71.5	9.50 s	194.9	5.00 dd (1.6, 18.4)	71.1	4.91 s	71.1
17	0.73 d (6.4)	16.3	0.73 d (6.0)	15.6	0.79 d (6.4)	15.8	0.76 d (7.0)	15.6
18	–	175.7	3.08 d (4.4), 2.35 d (4.4)	49.3	2.87 d (2.8), 2.33 d (2.8)	48.2	3.23 d (3.5), 2.46 d (3.5)	48.4
19	0.96 s	10.0	4.72 d (12.8), 4.37 d (12.8)	62.5	4.77 d (12.4), 4.37 d (12.4)	61.6	4.56 d (12.0), 4.45 d (12.0)	61.9
20	0.62 s	17.0	0.81 s	17.3	0.68 s	17.0	0.62 s	17.0
–COOH			12.30 s	–	–	–		
1-OAc			1.87 s	169.9, 21.4	2.13 s	170.3, 21.5		
3-OAc			–	–	2.05 s	170.3, 22.0		
6-OAc			1.90 s	169.4, 21.4	1.86 s	169.3, 21.4		
18-OAc			–	–	–	–		
19-OAc			2.06 s	170.3, 21.6	2.05 s	169.9, 21.5		
1′								167.2
2′								128.4
3′							6.91 d (6.4)	137.2
4′							1.76 d (6.4)	14.1
5′							1.79 s	11.8
6-OH							3.13 s	
11-OH	3.25 brs						–	
12-OH	4.56 brs						4.56 s	
$-OCH_3$	3.45 s	51.1					–	