Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2019, **17**(6): 0469–0474
Chinese Journal of Natural Medicines

Withaminimas A–F, six withanolides with potential anti-inflammatory activity from *Physalis minima*

WEI Shan-Shan[Δ], GAO Cai-Yun[Δ], LI Rui-Jun, KONG Ling-Yi[*], LUO Jun[*]

Jiangsu Key Laboratory of Bioactive Natural Product Research and State Key Laboratory of Natural Medicines, School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing 210009, China

Available online 20 June, 2019

[ABSTRACT] Withaminimas A–F (**1**–**6**), six new withaphysalin-type withanolides were isolated from the aerial parts of *Physalis minima* L.. The structures of these compounds were elucidated through a variety of spectroscopic techniques including HR-MS, NMR, and ECD. Compound **1** belongs to rare 18-norwithanolides, and **2**–**3** were 13/14-secowithanolides. According to the traditional usage of *P. minima*, inhibitory effects on nitric oxide (NO) production in lipopolysaccaride-activated RAW264.7 macrophages were evaluated, and compounds **1**–**4** exhibited significant inhibitory effects with IC_{50} values among 3.91–18.46 μmol·L^{-1}.

[KEY WORDS] *Physalis minima*; Withaphysalin-type withanolides; Anti-inflammatory

[CLC Number] R284.1 **[Document code]** A **[Article ID]** 2095-6975(2019)06-0469-06

Introduction

Withanolides are a group of modified C_{28} ergostane-type steroids with a C-22, C-26 *δ*-lactone side chain. These compounds are structurally diverse and distributed mainly in the genera *Withania*, *Physalis*, *Datura*, *Nicandra*, *Dunalia*, *Lycium*, *Tubocapsicum* and *Jaborosa* of the family Solanaceae [1]. Withanolides have been considered to be of great medicinal value since they display significant pharmacological activities such as anti-inflammatory [2], antitumor [3-4], immunosuppressive [5] and antioxidant properties [6]. *Physalis minima* L., known in China as 'xiaosuanjiang', is an annual herb and widely distributed in China. It has been used in Chinese folk medicine as tonic, diuretic, laxative, applied in inflammations, enlargement of the spleen, ascites, and as a helpful remedy in ulceration of the bladder [6-7]. In our previous study, withaphysalin A and 2, 3-dihydro-withaphysalin C, two major compounds with strong anti-inflammatory activities were isolated from this plant, which made a clarification of bioactive material basis of *P. minima* for inflammation treatment [8]. In order to find new withanolides with stronger biological activity, the remaining anti-inflammatory fractions of title plant were re-investigated. Consequently, six new withaphysalin-type withanolides (**1**–**6**) were discovered. Herein, the isolation, structure elucidation, and bioactivities evaluation of these compounds were reported.

Results and Discussion

Withaminima A (**1**) was isolated as amorphous solid with the molecular formula of $C_{27}H_{34}O_5$ by its HR-ESI-MS (*m/z* 439.247 6 $[M + H]^+$, calculated for 439.247 9) and 1D NMR data. The UV absorption maximum at 224 nm and strong IR absorption at 3435 and 1707 cm^{-1} indicated the presence of hydroxyl and *α*, *β*-unsaturated ketone groups [1]. The NMR spectroscopic data (Tables 1 and 2) of **1** showed four characteristic methyl singlets at δ_H 1.20 (s, 3H), 1.38 (s, 3H), 1.90 (s, 3H) and 1.95 (s, 3H), which were assigned to H3-19, H3-21, H3-27 and H3-28 [9], respectively. A double doublet at δ_H 4.83 (dd, J = 13.2, 3.0 Hz, H-22) and a set carbon signals (δc 166.0, 122.9 and 147.5) with two methyl singlets (H3-27, H3-28) above mentioned suggested the presence of a typical *α*, *β*-unsaturated-*δ*-lactone of withanolide skeleton in the side chain [9]. The vinylic proton signals at δ_H 5.86 (1H, dd, *J* = 10.0, 2.4 Hz) and δ_H 6.77 (1H, ddd, *J* = 10.0, 4.9, 2.4 Hz)

[Received on] 17- Apr.-2015

[Research funding] This work was supported in part by the National Natural Science Foundation of China (No. 81430092), the Outstanding Youth Fund of the Basic Research Program of Jiangsu Province (No. BK20160077), the Program for Changjiang Scholars and Innovative Research Team in University (No. IRT_15R63).

[*Corresponding authors] Tel/Fax: 86-25-8327-1405; cpu_lykong@126.com (KONG Ling-Yi); Tel/Fax: 86-25-8327-1402; luojun@cpu.edu.cn (LUO Jun)

[Δ]These authors contributed equally to this work.

These authors have no conflict of interest to declare.

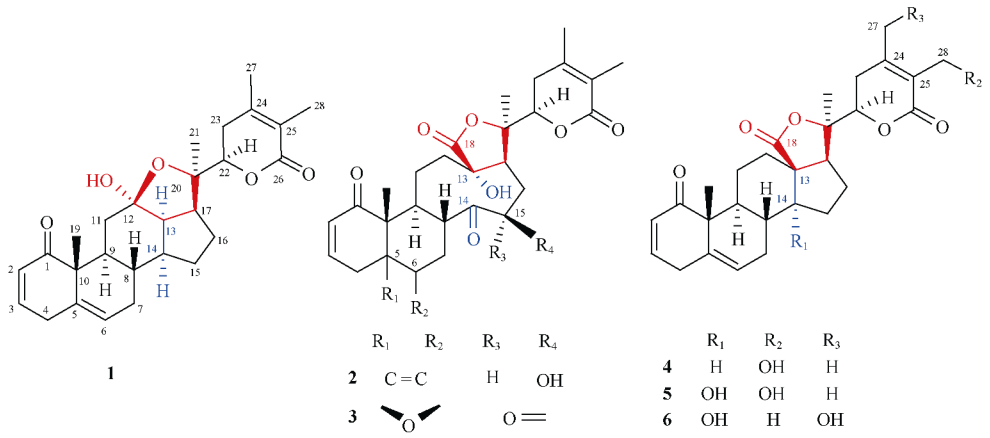

Fig. 1 Structures of isolated withanolides

Table 1 ^{1}H NMR (500 MHz) data for compounds 1–6 in $CDCl_3$

Position	1	2	3	4	5	6
2	5.86 (dd, 10.0, 2.4)	5.95 (dd, 10.0, 2.5)	6.12 (dd, 10.0, 2.6)	5.88 (dd, 9.9, 2.4)	5.89 (dd, 10.0, 2.4)	5.89 (dd, 10.0, 2.5)
3	6.77 (ddd, 10.0, 4.9, 2.4)	6.83 (ddd, 10.0, 4.6, 2.5)	6.91 (ddd, 10.0, 5.9, 2.6)	6.78 (ddd, 9.9, 4.6, 2.4)	6.81 (ddd, 10.0, 4.8, 2.4)	6.80 (ddd, 10.0, 4.0, 2.5)
4	3.27 (dd, 21.4, 2.4) 2.82 (dd, 21.4, 4.9)	3.34 (dt, 21.8, 2.5) 2.92 (dd, 21.8, 4.6)	3.07 m 1.93 m	3.52 (dt, 21.0, 2.4) 2.84 (dd, 21.0, 4.6)	3.30 (dd, 21.4, 2.4) 2.86 (dd, 21.4, 4.9)	3.30 (dd, 20.4, 2.5) 2.85 (dd, 20.4, 4.0)
6	5.60 (dt, 4.9, 2.5)	5.60 (d, 5.5)	3.15 (d, 5.5)	5.57 (d, 5.6)	5.61 (d, 5.9)	5.61 (d, 3.8)
7	2.16 m 1.64 m	2.06 m 1.64 m	2.19 m 2.08 m	1.78 m 1.19m	2.34 m 2.01 m	2.32 m 1.99 m
8	1.52 m	2.69 m	2.75 m	2.39 m	1.98 m	1.98 m
9	2.13 m	3.17 m	3.55 m	1.72 m	1.91 m	1.89 m
11	2.52 m 1.26 m	2.35 m 2.27 m	2.26 m 2.23 m	2.34 m 2.04 m	2.30 m 1.95 m	2.20 m 1.97 m
12		2.32 m 2.16 m	2.24 m 2.12 m	1.91 m 1.70 m	2.35 m 2.04 m	1.77 m 1.50 m
13	3.84 m					
14	2.01 m			1.36 m		
15	2.08 m 1.52 m	4.59 (t, 7.2)		2.04 m 1.57 m	1.83 m 1.77 m	2.18 m 1.65 m
16	2.03 m 1.86 m	2.60 m 1.88 m	3.56 m 2.41 (dd, 12.2, 4.3)	2.34 m 1.84 m	2.20 m 1.59 m	2.16 m 1.35 m
17	2.02 m	2.78 m	3.04 m	2.28 m	2.53 m	2.55 m
19	1.20 s	1.28 s	1.36 s	1.31 s	1.30 s	1.29 s
21	1.38 s	1.50 s	1.54 s	1.53 s	1.53 s	1.53 s
22	4.83 (dd, 13.2, 3.0)	4.24 (dd, 12.8, 3.6)	4.24 (dd, 12.8, 3.7)	4.59 (dd, 13.1, 3.1)	4.64 (dd, 13.0, 3.5)	4.55 (dd, 12.6, 3.1)
23	2.48 m 2.08 m	2.78 m 2.17 m	2.80 m 2.18 m	2.54 m 2. 25m	2.79 m 2.30 m	2.72 m 2.24 m
27	1.90 s	1.83 s	1.85 s	4.38 (2H, d, 12.6)	4.38 (2H, d, 12.6)	1.83 s
28	1.95 s	1.93 s	1.95 s	2.06 s	2.06 s	4.38 (2H, d, 15.2)

Table 2 ^{13}C NMR (125 MHz) data for compounds 1–6 in $CDCl_3$

Position	1	2	3	4	5	6
1	204.5	204.5	200.8	204.7	204.2	204.2
2	128.1	127.8	129.3	128.1	127.9	127.8
3	145.6	146.1	145.5	145.5	145.7	145.8
4	33.6	33.3	32.9	33.6	33.3	33.4
5	134.3	135.8	62.5	136.3	135.5	135.2
6	125.4	122.7	60.2	124.3	124.5	124.6
7	23.1	27.7	19.9	27.6	27.2	27.0
8	43.6	51.2	42.6	31.3	40.2	39.8
9	38.7	35.0	33.8	42.4	38.2	38.2
10	50.6	51.6	52.2	50.9	51.6	51.6
11	28.6	27.5	22.0	23.5	25.3	24.9
12	105.1	28.2	29.7	26.1	26.8	26.9
13	59.6	77.4	77.4	56.1	60.8	60.7
14	46.4	217.1	206.7	55.6	83.6	83.9
15	21.6	69.7	202.6	31.3	34.9	35.0
16	23.2	33.9	33.8	35.6	35.7	35.7
17	42.4	57.7	57.2	52.9	57.4	57.6
18		178.6	178.6	177.7	177.4	177.4
19	18.9	18.6	18.9	19.1	18.9	18.9
20	84.1	83.6	84.1	83.5	83.2	83.6
21	20.5	23.3	22.8	23.4	23.1	23.2
22	78.4	76.4	76.5	79.5	78.8	79.2
23	32.9	30.7	30.7	31.7	32.0	26.2
24	147.5	148.4	148.2	151.2	151.9	151.3
25	122.9	121.9	122.0	126.5	126.1	121.7
26	166.0	163.9	163.6	165.3	165.0	164.9
27	12.6	12.4	12.4	57.5	57.5	12.0
28	20.8	20.6	20.6	20.1	20.1	61.5

indicated an *α*, *β*-unsaturated ketone in ring A as observed in many withanolides [10]. Another olefinic proton at δ_H 5.60 (1H, dt, *J* = 4.9, 2.5 Hz) along with two olefinic carbons at δc 134.3 and 125.4 in the ^{13}C NMR spectrum correspond to one double bond linked at C-5/6 [9-10]. In addition to these signals, the ^{1}H NMR spectrum displayed a proton signal at δ_H 3.84 (1H, m) linked at C-13 (δc 59.6), in conjunction with the ^{13}C NMR spectroscopic data showed 27 carbons, led to the conclusion that compound **1** was a C-18 norwithaphysalin- type withanolide [11]. The chemical shifts of C-12 (δc 105.1) and C-20 (δc 84.1) in the ^{13}C NMR spectrum and the hydroxyl group signal showed by characteristic UV spectrum indicated that **1** contained a 12, 20-*γ*-lactol moiety [11]. Above deductions were confirmed by the key HMBC correlations from H-13 (δ_H 3.84) to C-12 (δc 105.1), C-15 (δc 21.6) and C-17 (δc 42.4), and from H3-21 (δ_H 1.38) to C-17 and C-20 (δc 84.1), as shown in Fig. 2. Thus, the planar structure of **1**, C-18 norwithaphysalin-type withanolide featuring a rare 12, 20-*γ*-lactol moiety, was elucidated.

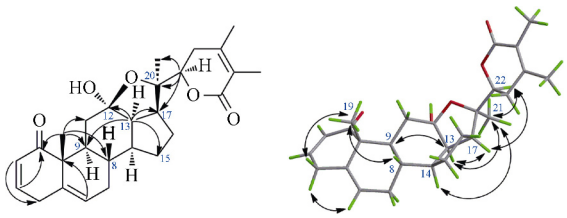

Fig. 2 The key HMBC and ROESY correlations of compound 1

The relative stereochemistry of **1** was established on the basis of relevant correlations observed in its ROESY spectrum (Fig. 2). The ROESY cross-peaks from H-8 to H3-19 and H-9 to H-13were observed which could deduce the configuration of C-8 and C-9. The correlations from H3-21 to H-13, H-14, and H-22, from H-17 to H-13 and H-22 revealed that these protons were adopted as *α* configuration and the C-12, C-20 oxygen ether bridge was *β*-orientation [11-12]. The configuration of C-22 was determined in the ECD spectrum by its positive Cotton effect at 250 nm based on the n→π* transition of an *α*, *β*-unsaturated *δ*-lactone having a 22*R* configuration [12-13]. Accordingly, the structure of **1** was established finally and named withaminima A.

The molecular formula of compound **2** was determined as $C_{28}H_{34}O_8$ by HR-ESI-MS (*m/z* 533.1951 [M + Cl^-, calculated for 533.1948). The ^{1}H and ^{13}C NMR data of **2** indicated that it was a withaphysalin-type withanolide contained an *α*, *β*-unsaturated ketone in ring A, a 5-en moiety and an *α*, *β*-unsaturated-*δ*-lactone side chain [10]. The obvious HMBC correlations (Fig. 3) from H3-21 (δ_H 1.50) to C-17 (δc 57.7), from H-12 (δ_H 2.32) to C-18 (δc 178.6) indicated that compound **2** contained the characteristic 18, 20-*γ*-lactone moiety [9]. Additionally, a carbonyl carbon signal at δc 217.1 (C-14), along with the HMBC correlations from H-16 (δ_H 2.60) to C-14, and from H-17 (δ_H 2.78) to C-12 (δc 28.2) and C-15 (δc 69.7) revealed that **2** was a C13/14 *seco*-with aphysalin and C-14 was existed as ketone and a hydroxyl was located at C-13 (δc 77.4) [9, 14]. In its ROESY spectrum, the correlations of H3-19 (*β*-orientation) with H-8, of H3-21 (*α*-orientation) with H-17, and of H-15 with H-17 and H-9 suggested the *trans* fusing of B/C rings and the *cis* junction of C (D) /18, 20-*γ*-lactone ring [3]. The *α*-orientation of 13-OH were deduced by the cis junctions of C (D) /18, 20-γ-lactone ring and the biogenetic arguments [3]. Correlations between H-17 and H-15, H3-21 suggested the *β*-orientation of 15-OH. The presence of the positive Cotton effect at 250 nm in its ECD

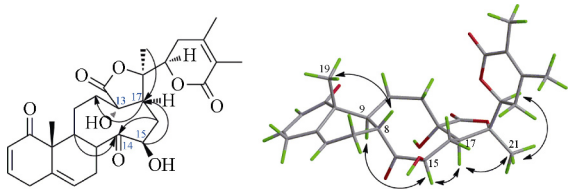

Fig. 3 The key HMBC and ROESY correlations of compound 2

spectrum indicated the *R* configuration at C-22 [12]. Thus, compound **2** was established as an (22R) C13/14 *seco*-with a-physalin and named withaminima B.

Compound **3** was obtained as amorphous solid with a molecular ion at *m/z* 547.1742 $[M + Cl]^-$ in the HR-ESI-MS, corresponding to an molecular formula of $C_{28}H_{32}O_9$. The characteristic signals at δc 77.4 (C-13), δc 206.7 (C-14) and δc 178.6 (C-18) indicated that **3** possessed the same 13, 14-*seco*-withaphysalin skeleton as **2** [14]. Comparison of its ^1H and ^{13}C NMR data (Table 1 and 2) with those of **2** revealed that the 5-en of **2** was replaced by 5, 6-epoxy unit in **3** (δ_H 3.15 (d, J = 5.5 Hz, H-6); δc 62.5, C-5; δc 60.2, C-6) [15]. And the C-15 carbonyl moiety (δc 202.6) in **3** was deduced by the upfield shifts of the C-14 from δc 217.1 in **2** to 206.7 and the key HMBC correlations from H-16 (δ_H 3.56) and H-17 (δ_H 3.04) to C-15 (δc 202.6). The relative configuration of the 5, 6-epoxy moiety in **3** was assigned as *β*-orientation by the ROESY correlations between H-4*α* (δ_H 1.93) with H-6. The absolute configuration of C-22 was established as *R* according to its positive Cotton effect at 255 nm in the ECD spectrum [12]. Thus, the structure of **3** was finally determined and named as withaminima C.

Withaminimas D–F (**4**–**6**) were also isolated as amorphous solid. The similar molecular formulas and 1D NMR features (Tables 1 and 2) suggested that they are typical C/D rings intact withaphysalin homologues with *α*, *β*-unsaturated ketone in ring A, 5-en moiety in ring B, and 18, 20-*γ*-lactone ring [1]. The main difference between these three was the number and location of hydroxyl moieties. For compound **4**, the presence of the oxygenated methylene proton signals at δ_H 4.38 (each 1H, d, J = 12.6 Hz) and its HMBC correlations with C-24 (δc 151.2), C-25 (δc 126.5), and δc C-26 (165.3) suggested that a hydroxymethyl group (CH_2OH-28) linked at C-25. Compared the NMR data of **4** and **5**, the downfield shifts of C-14 from δc 55.6 to δc 83.6 indicated the presence of a hydroxy moiety at C-14 in **5** [16-18]. The obvious HMBC correlations from two oxygenated methylene signals (δ_H 4.38) to C-24 (δc 151.9), C-25 (126.1), and C-26 (165.0) implied a hydroxymethyl group linked at C-25 in **5** as that of **4**. For compound **6**, these similar HMBC correlations were observed to C-24 (δc 151.3), C-25 (δc 121.7), and C-23 (δc 26.2), which implied the hydroxymethyl group (CH_2OH-27) linked at C-24 rather than at C-25 in **5**. The *α*-orientation of 14-OH in **5**/**6** were determined by the identical 1D NMR data with reported 14-hydroxywithaphysalins [2, 10]. The ECD spectra of **4**–**6** also showed a positive Cotton effect near 254 nm, indicating the 22*R* configuration of **4**–**6** [12]. Therefore, their structures were determined as shown in Fig. 1 and named as withaminimas D–F, respectively.

According to our and other previous researches, the crude methanol extract and chloroform fraction of the aerial parts of *P. minima* showed marked anti-inflammatory in mice [6, 8]. Thus, these six new withaphysalin-type withanolides were screened to demonstrate their anti-inflammatory activities by testing inhibitory effects of NO production on lipopolysaccharide-activated RAW264.7 macrophages [19]. MTT assay suggested that no significant cytotoxic effects (over 90% cell survival) at concentrations up to 100 μmol·L^{-1}. N^G-monomethyl L-arginine (L-NMMA) was used as a positive control and had an IC_{50} value of 39.20 μmol·L^{-1}. Based on the results summarized in table 3, compounds **1**–**4** effectively inhibited the NO production with IC_{50} values range from 4–20 μmol·L^{-1}, and **5**/**6** demonstrated equivalent inhibitory to positive drug. These results further proved that the withanolides in *P. minima* would be responsible for its anti-inflammatory effect and traditional usages [20].

Table 3 Inhibitory effects of compounds 1−6 on NO production in LPS-activated RAW264.7 cells (Mean ± SD, *n* =3)

Compounds	IC_{50} (μmol·L^{-1})
1	18.46 ± 2.89
2	8.68 ± 2.95
3	14.54 ± 7.15
4	3.91 ± 0.49
5	51.48 ± 2.86
6	43.79 ± 5.19
L-NMMA [a]	39.20 ± 1.30

[a] N^G-Monomethyl-L-arginine (L-NMMA) was used as a positive control.

Experimental

General experimental procedures

UV spectra were recorded with a Shimadzu UV-2501 PC spectrophotometer. IR data were measured with a Bruker Tensor 27 spectrometer. Optical rotations were determined on a JASCO P-1020 polarimeter. 1D and 2D NMR spectra were performed on a Bruker Avance III NMR spectrometer at 500 MHz (^1H) and 125 MHz (^{13}C), using TMS as the internal standard. HRESI mass spectra were recorded on an Agilent 6520B UPLC-Q-TOF mass spectrometer. ECD spectra were recorded on a JASCO 810 spectropolarimeter. The HPLC analysis was run on an Agilent 1200 instrument equipped with multiple wavelength diode array detector (DAD). Preparative HPLC was proformed on a Agilent 1100 instrument equipped with a shim-pack RP-C_{18} column (20 mm × 250 mm, 10 μm), the flow rate was 10.0 mL·min^{-1}. Column chromatography (CC) was carried out using macroporous resin D-101 (pore size B 13–14 nm, 26–60 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), Silica gel (100–200 mesh and 200–300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China) and ODS RP-C_{18} (40−63 μm, FuJi, Japan).

Plant material

The plant of *Physalis minima* was obtained from Linyi City (Shandong Province, China) in August 2014, which was identified by Professor ZHANG Mian of the Research Department of Pharmacognosy, China Pharmaceutical University.

A voucher specimen (No. PM-201408-LY) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and isolation

The extraction and fraction using D-101 macroporous resin were as same as previous description [8]. Fr. C (21 g) was subjected to silica gel with increasing polarities of CH_2Cl_2–MeOH solvent system (40 : 1, 20 : 1, 10 : 1, 0 : 100 *V*/*V*) to obtain four subfractions (Frs. C1–C4). Fr. C1 was applied to ODS MPLC eluted with MeOH–H_2O (40 : 60 to 65 : 35, *V*/*V*) to afford four subfractions (Frs. C1A–D). Fr. C1A was applied to ODS MPLC eluted with isocratic MeOH–H_2O (40 : 60, *V*/*V*) to afford three subfractions (Frs. C1A1–3). Fr. C1A1 was separated by repeated preparative HPLC (MeOH–H_2O, 50 : 50, *V*/*V*) to afford **3** (17 mg). Fr. C1A2 was separated by repeated preparative HPLC (MeOH–H_2O, 50 : 50, *V*/*V*) to afford **2** (8.0 mg), **5** (2.3 mg), **6** (4.3 mg). Part of Fr. C1B was chromatography over ODS with MeOH–H_2O (45 : 55, *V*/*V*) to give two subfractions (Frs. C1B1–2). Fr. C1B1 was applied to preparative HPLC with MeOH–H_2O (50 : 50, *V*/*V*) to yield **4** (4.7 mg). Fr. C1D was purified by silica gel to afford **1** (14.1 mg).

withaminima A (**1**)

White amorphous solid; $[\alpha]_D^{22}$ +98.3 (*c* 0.12, MeOH); CD $\Delta\varepsilon_{250}$ +8.45, $\Delta\varepsilon_{337}$ −4.68 (MeOH; c 0.3); UV (MeOH) λ_{max} (log ε) 224 (4.78); IR (KBr) ν_{max} 3435, 2925, 1707, 1666, 1382, 1127 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; HR-ESI-MS *m/z* 439.2476 $[M + H]^+$ (Calcd. for $C_{27}H_{35}O_5$, 439.2479).

withaminima B (**2**)

White amorphous solid; $[\alpha]_D^{22}$ +29.3 (*c* 0.09, MeOH); CD $\Delta\varepsilon_{250}$ +4.19, $\Delta\varepsilon_{337}$ −2.09 (MeOH; c 0.3); UV (MeOH) λ_{max} (log ε) 225 (4.86); IR (KBr) ν_{max} 3436, 2959, 2922, 1771, 1710, 1459, 1379, 1136 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; HR-ESI-MS *m/z* 533.1951 $[M + Cl]^-$ (Calcd. for $C_{28}H_{34}ClO_8$, 533.1948).

withaminima C (**3**)

White amorphous solid; $[\alpha]_D^{22}$ +53.0 (*c* 0.14, MeOH); CD $\Delta\varepsilon_{255}$ +3.48, $\Delta\varepsilon_{338}$ −0.65 (MeOH; c 0.3); UV (MeOH) λ_{max} (log ε) 225 (4.90); IR (KBr) ν_{max} 3444, 2924, 1773, 1711, 1458, 1383, 1137 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; HR-ESI-MS *m/z* 547.1742 $[M + Cl]^-$ (Calcd. for $C_{28}H_{32}ClO_9$, 547.174).

withaminima D (**4**)

White amorphous solid; $[\alpha]_D^{22}$ +50.5 (*c* 0.11, MeOH); CD $\Delta\varepsilon_{253}$ +4.24, $\Delta\varepsilon_{311}$ −1.29 (MeOH; c 0.3); UV (MeOH) λ_{max} (log ε) 221 (4.73); IR (KBr) ν_{max} 3439, 2923, 2868, 1755, 1709, 1664, 1453, 1384, 1110 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; HR-ESI-MS *m/z* 501.2044 $[M + Cl]^-$ (Calcd. for $C_{28}H_{34}ClO_6$, 501.2049).

withaminima E (**5**)

White amorphous solid; $[\alpha]_D^{22}$ +38.0 (*c* 0.10, MeOH); CD $\Delta\varepsilon_{252}$ +5.74, $\Delta\varepsilon_{337}$ −3.52 (MeOH; c 0.3); UV (MeOH) λ_{max} (log ε) 220 (4.73); IR (KBr) ν_{max} 3430, 2923, 1752, 1701, 1385, 1127cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; HR-ESI-MS *m/z* 517.2001 $[M + Cl]^-$ (Calcd. for $C_{28}H_{34}ClO_7$, 517.1999).

withaminima F (**6**)

White amorphous solid; $[\alpha]_D^{22}$ +28.7 (*c* 0.14, MeOH); CD $\Delta\varepsilon_{254}$ +2.22, $\Delta\varepsilon_{327}$ −0.78 (MeOH; c 0.3); UV (MeOH) λ_{max} (log ε) 222 (4.31); IR (KBr) ν_{max} 3439, 2925, 1752, 1714, 1453, 1382, 1122 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; HR-ESI-MS *m/z* 517.1998 $[M + Cl]^-$ (Calcd. for $C_{28}H_{34}ClO_7$, 517.1999).

NO production bioassay

The protocol for NO production bioassays was provided *in vitro* as described previously, and N^G-Monomethyl-L-arginine (L-NMMA) was used as the positive control [19]. All experiments were carried out in triplicate.

References

[1] Chen LX, He H, Qiu F. Natural withanolides: an overview [J]. *Nat Prod Rep*, 2011, **28**(4): 705-740.

[2] Sun CP, Qiu CY, Yuan T, *et al*. Antiproliferative and anti-inflammatory withanolides from *Physalis angulata* [J]. *J Nat Prod*, 2016; **79**(6): 1586-1597.

[3] Ma L, Gan X, He QP, *et al*. Cytotoxic Withaphysalins from *Physalis minima* [J]. *Helv Chim Acta*, 2007, **90**(7): 1406- 1419.

[4] Rocha DD, Balgi A, Maia AI, *et al*. Cell cycle arrest through inhibition of tubulin polymerization by withaphysalin F, a bioactive compound isolated from *Acnistus arborescens* [J]. *Invest New Drug*, 2012, **30**(3): 959-966.

[5] Sun L, Liu J, Liu P, *et al*. Immunosuppression effect of withangulatin A from *Physalis angulata via* heme oxygenase 1-dependent pathways [J]. *Process Biochem*, 2011, **46**(2): 482- 488.

[6] Khan MA, Khan H, Khan S, *et al*. Anti-inflammatory, analgesic and antipyretic activities of *Physalis minima* Linn. [J]. *J Enzym Inhib Med Ch*, 2009, **24**(3): 632-637.

[7] Xu XM, Guan YZ, Shan SM, *et al*. Withaphysalin-type withanolides from *Physalis minima* [J]. *Phytochem Lett*, 2016, **15**: 1-6.

[8] Li RJ, Gao CY, Guo C, *et al*. The anti-inflammatory activities of two major withanolides from *Physalis minima via* acting on NF-κB, STAT3, and HO-1 in LPS-stimulated RAW264.7 cells [J]. *Inflammation*, 2017, **40**(2): 401-413.

[9] Cao CM, Wu X, Kindscher K, *et al*. Withanolides and sucrose esters from *Physalis neomexicana* [J]. *J Nat Prod*, 2015, **78**(10): 2488-2493.

[10] Zhang WN, Tong WY. Chemical constituents and biological activities of plants from the genus *Physalis* [J]. *Chem Biodivers*, 2016, **13**(1): 48-65.

[11] Su BN, Nikolic D, Santarsiero BD, *et al*. Activity-guided isolation of novel norwithanolides from *Deprea subtriflora* with potential cancer chemopreventive activity [J]. *J Org Chem*, 2003, **68**(6): 2350-2361.

[12] Yang BY, Guo R, Li T, *et al*. New anti-inflammatory withanolides from the leaves of *Datura metel* L. [J]. *Steroids*, 2014, **87**: 26-34.

[13] Kuroyanagi M, Shibata K, Umehara K. Cell differentiation including steroids from *Withania somnifera* L. [J]. *Chem*

Pharm Bull, 1999, **47**(11): 1646-1649.

[14] Kirson I, Zaretskii Z, Glotter E. Withaphysalin C, a naturally occurring 13, 14-*Seco*-steroid [J]. *J Chem Soc Perk T*, 1976, **7**(11): 1244-1247.

[15] Fang ST, Liu JK, Li B. Ten new withanolides from *Physalis peruviana* [J]. *Steroids*, 2012, **77**(1-2): 36-44.

[16] Guan YZ, Shan SM, Zhang W, *et al*. Withanolides from *Physalis minima* and their inhibitory effects on nitric oxide production [J]. *Steroids*, 2014, **82**: 38-43.

[17] Zhang H, Timmermann BN. Withanolide structural revisions by ^{13}C NMR spectroscopic analysis inclusive of the gamma-gauche effect [J]. *J Nat Prod*, 2016, **79**(4): 732-742.

[18] Sahai M, Kirson I. Withaphysalin D, a new withaphysalin from *Physalis minima* Linn. var. *indica* [J]. *J Nat Prod*, 1984, **47**(3): 527-529.

[19] Qiu L, Zhao F, Jiang ZH, *et al*. Steroids and flavonoids from *Physalis alkekengi* var. *franchetii* and their inhibitory effects on nitric oxide production [J]. *J Nat Prod*, 2008, **71**(4): 642-646.

[20] Yang MH, Wang JS, Luo JG, *et al*. Chisopanins A–K, 11 new protolimonoids from *Chisocheton paniculatus* and their anti-inflammatory activities [J]. *Bioorgan Med Chem*, 2011, **19**(4): 1409-1417.

Cite this article as: WEI Shan-Shan, GAO Cai-Yun, LI Rui-Jun, KONG Ling-Yi, LUO Jun. Withaminimas A–F, six withanolides with potential anti-inflammatory activity from *Physalis minima* [J]. *Chin J Nat Med*, 2019, **17**(6): 469-474.