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Five new 5,6-β-epoxywithanolides from *Physalis minima*

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ABSTRACT

Five new 5,6-β-epoxywithanolides (**1–5**) were isolated from the whole plants of *Physalis minima* L. Their structural elucidations were achieved by the extensive spectroscopic analysis (IR, UV, HR-ESI-MS, 1D-NMR, and 2D-NMR). The isolates were evaluated for their anti-inflammatory activities on lipopolysaccharide-induced nitric oxide production in RAW 264.7 cells and cytotoxic activities against three cancer cell lines, *viz.* A549 lung adenocarcinoma cells, SMMC-7721 hepatic carcinoma cells and MCF-7 breast cancer cells by using the MTT-based assay. All of them possessed moderate inhibition to the production of nitric oxide with IC_{50} values from 42.18 to 73.26 μM, and the IC_{50} values of the cytotoxic activities were in the range of 31.25 to 80.14 μM.

1. Introduction

Physalis minima L. is a perennial herb belonging to the Solanaceae family [1]. In the traditional system of medicine, its fruit, named "Sunberry", is applied to cure the spleen disorder [2]. With anti-inflammatory, analgesic, diuretic, tonic and laxative activities [1,2], the whole plant is used for the treatment of gout and urinary diseases [3]. An activity-guided fractionation investigation showed that its anti-inflammatory activity is partly due to the presence of withanolides that mainly exist in genus *Physalis* [4]. Withanolides are structurally diverse steroids with an ergostane skeleton in which C-22 and C-26 or C-23 and C-26 are easily oxidized to form a δ or γ-lactone ring, which endow the compounds with anti-inflammatory [5,6]. The comprehensive investigations revealed that the anti-inflammatory activity of withanolides involved multiple pathways, including the inhibitions of the production of NO, prostaglandin E2, several pro-inflammatory cytokines, the suppression of nuclear translocations of NF-κB and p65, the phosphorylation of STAT3, and the upregulation of heme oxygenase-1expression [4].

In searching for herb-derived anti-inflammatory agents, we tested the 85% EtOH extract of the whole plant of *P. minima* in RAW 264.7 cells using the lipopolysaccharide (LPS)-induced nitric oxide (NO), and a moderate inhibitory activity was observed. Furthermore, the extract also exhibited remarkable cytotoxic activities through exerting apoptotic-programed cell death in the tumor cells [7,8]. With the bioassay-guided fractionation, five new withanolides were isolated by using column chromatography over D101 macroporous resins, silica gels, sephadex LH-20 and semi-preparative HPLC. Their structures were elucidated through extensive spectroscopic analyses. Furthermore, their inhibitory activities against NO production in RAW 264.7 cells and antiproliferative activities against three human tumor cell lines (A549, SMMC-7721, and MCF-7) were evaluated.

2. Experimental section

2.1. General experimental procedures

The optical rotations were measured on a Perkin-Elmer model 241 polarimeter (Waltham, Massachusetts, USA). IR spectra were determined with a Perkin-Elmer 983 G spectrometer (Waltham, Massachusetts, USA). The 1D-NMR and 2D-NMR spectra were obtained on a Bruker AVANCE III 600 spectrometer in CD_3OD using tetramethylsilane (TMS) as the internal standard. HR-ESI-MS spectra were taken on a Micromass Q-TOF2 spectrometer (AB SCIEX, Canada). HPLC analysis and purification were carried out on an SB-C18 semi-preparative HPLC column (250 × 9.4 mm i.d., 5 μm, Agilent Corp. Palo Alto, CA, USA) with a Shimadzu HPLC system composed of an LC-20AT pump and an SPD-20A detector (Shimadzu Corp., Kyoto, Japan). The wavelength for detection was 203 nm. Medium pressure liquid chromatography (MPLC) purification was performed on a Büchi Flash

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Chromatography system composed of a C-650 pump with a flash column (460 mm × 26 mm i.d., Büchi Corp., Flawil, Switzerland). Sephadex LH-20 for column chromatography was obtained from GE Corp (Pittsburgh, USA). The D101 macroporous resin was acquired from Xi'an Sunresin New Material Co.Ltd. Silica gel (200–300 mesh) for column chromatography and precoated silica gel TLC plates were purchased from Qingdao Marine Chemical Factory.

2.2. Plant material

The fresh whole plant materials of *P. minima* L. were collected from Huadu District (113.19 East longitude, 23.40 north latitude), Guangzhou, Guangdong Province of China, and dried under the sun. The plant materials were authenticated by Prof. Xiaoran Li, and a voucher specimen (No. 14–15–06-01) was deposited at the herbarium of College of Pharmaceutical Science, Soochow University.

2.3. Extraction and isolation

The dried plant materials (20 kg) were extracted with 85% EtOH (80 L) for three times at 80 °C under reflux. After removing the solvent under reduced pressure, the combined dark green extract (0.45 kg) was dissolved in distilled water, then passed through a D101 macroporous resin column (i.d. 30 cm × 200 cm, Xi'an Sunresin New Materials Co. Ltd.), eluting with step gradients of EtOH-H_2O (0%, 30%, 60%, 80%) to yield four fractions: Fr. A-D. Fr. C then suspended in distilled water and partitioned in turn with DCM, EtOAc, and n-BuOH. The DCM extract (65 g) was separated by silica gel (200–300 mesh) column (30 × 18 cm) and eluted using a step gradient of CH_2Cl_2-MeOH solvent system (25:1, 15:1, 10:1, 5:1, and 1:1) to obtained five fractions. Fr. C-5 was subjected to a silica gel (200–300 mesh) column (30 × 18 cm) and eluted with CH_2Cl_2-MeOH solvent system (25:1, 15:1, 5:1, 1:1) to yield four fractions. Fr. C-5-1 (80 mg) was subjected to a Sephadex-LH 20 column eluting with MeOH, and then separated by using semi-preparative HPLC over an ODS column eluting with MeOH-H_2O (70:30) to yield compound **1** (12 mg, t_R 58.7 min), **2** (14 mg, t_R 56.4 min) and **3** (12 mg, t_R 50.2 min). Similarly, Fr. C-5-2 (62 mg) was purified by semi-preparative HPLC eluting with MeOH-H_2O (68:32) to yield compound **4** (6 mg, t_R 70.7 min) and **5** (8 mg, t_R 75.0 min). All the semi-preparative HPLC separations were conducted at a flow rate of 2 mL/min and the detection wavelength was 203 nm. The purities of the isolated withanolides were above 95% by HPLC analysis with ELSD detection.

Physaliolide G (**1**): White amorphous powder; $[\alpha]_D^{22}$ + 14.4 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 203 (3.26), 224 (4.22) nm; IR ν_{max} (KBr): 3456, 2925, 1735, 1712, 1643, 1642, 1601, 1161 cm^{-1}; HR-ESI-MS $[M + FA\text{-}H]^-$ *m/z* 605.2626 (calcd. for $C_{31}H_{41}O_{12}$ 605.2598); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are given in Tables 1 and 2.

Physaliolide H (**2**): White amorphous powder; $[\alpha]_D^{22}$ + 17.2 (c 0.15, MeOH); UV (MeOH) λ_{max} (log ε): 203 (3.06), 224 (3.82) nm; IR ν_{max} (KBr): 3456, 2925, 1736, 1712, 1643, 1642, 1605, 1160 cm^{-1}; HR-ESI-MS $[M + FA\text{-}H]^-$ *m/z* 607.2795 (calcd. for $C_{31}H_{43}O_{12}$ 607.2755); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are given in Tables 1 and 2.

Physaliolide I (**3**): White amorphous powder; $[\alpha]_D^{22}$ + 11.8 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 203 (3.23), 224 (3.62) nm; IR ν_{max} (KBr): 3456, 2926, 1735, 1713, 1645, 1640, 1602, 1160 cm^{-1}; HR-ESI-MS $[M + FA\text{-}H]^-$ *m/z* 561.2743 (calcd. for $C_{30}H_{41}O_{10}$ 561.2700); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are given in Tables 1 and 2.

Physaliolide J (**4**): White amorphous powder; $[\alpha]_D^{22}$ + 14.8 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 203 (3.03), 224 (3.22) nm; IR ν_{max} (KBr): 3460, 2930, 1738, 1710, 1642, 1640, 1601, 1162 cm^{-1}; HR-ESI-MS $[M + FA\text{-}H]^-$ *m/z* 547.2579 (calcd. for $C_{29}H_{39}O_{10}$ 547.2543);^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are given in Tables 1 and 2.

Table 1
^{13}C NMR (150 MHz) spectroscopic data for compounds **1–5** in CD_3OD.[a]

Position.	1	2	3	4	5
1	213	212.8	212.3	203.8	204.5
2	43.5	43.3	41.1	133.3	133.1
3	69.1	68.9	79.5	145.2	145.4
4	78.4	78.3	79.5	71.2	71.2
5	64.8	65.1	65.5	64.4	64.1
6	60.6	60.3	60.8	62.2	62.4
7	25.4	25.3	25.3	25.3	25.4
8	36.4	35.5	36.2	35.9	36.6
9	39.3	38.1	39.5	39.5	41.0
10	51.7	51.3	51.7	49.6	49.6
11	21.7	21.0	21.7	21.2	21.9
12	38.8	30.3	38.6	30.4	38.7
13	53.7	51.7	52.9	51.7	52.8
14	82.7	87.9	82.9	88.2	82.7
15	84.7	80.7	82.6	86.9	82.9
16	123.2	49.6	126.5	51.0	126.3
17	162.6	86.9	159.2	78.9	159.3
18	16.6	15.1	16.6	15.4	16.6
19	14.8	14.3	14.6	16.1	16.7
20	33.6	43.6	36.3	43.5	36.2
21	20.3	9.8	18.0	9.8	18.0
22	85.9	78.7	80.7	78.5	80.7
23	67.5	32.9	33.3	33.0	33.4
24	155.0	153.3	153.0	153.4	153.0
25	122.5	121.9	122.0	121.9	122.0
26	167.1	169.2	169.3	169.5	169.3
27	12.9	12.4	12.4	12.4	12.4
28	16.2	20.5	20.3	20.6	20.3
OMe			57.1		
CH_3CO-1′	171.8	171.4			
CH_3CO-2′	21.4	21.6			

[a] Chemical shifts are in ppm, and the assignments were based on HSQC, HMBC, and NOSEY spectra.

Physaliolide K (**5**): White amorphous powder; $[\alpha]_D^{22}$ + 14.3 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 203 (3.83), 224 (3.62) nm; IR ν_{max} (KBr): 3460, 2928, 1737, 1710, 1643, 1640, 1601, 1165 cm^{-1}; HR-ESI-MS $[M + FA\text{-}H]^-$ *m/z* 529.2743 (calcd. for $C_{29}H_{37}O_9$ 529.2438); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are given in Tables 1 and 2.

2.4. NO production bioassay

The inhibitory effects of five new withanolides on NO production were evaluated in LPS-activated murine macrophage RAW 264.7 cells based on the previous method [10]. The cells were seeded with 1 × 10^5 cells/well in 96-well plates and allowed to adhere at 37 °C for 24 h in a humidified atmosphere containing 5% CO_2. The cells were co-incubated with different concentrations of compounds ranging from 6.25 μM to 100 μM and L-NG-Monomethyl arginine methyl ester (L-NAME) was used as the positive control. The concentration of NO in the cultured medium was measured by Griess reagent [90 μL of 1% sulfanilamide and 90 μL of 0.1% N-(1-naphthyl)ethylenediamine in 2.5% H_3PO_4 in each well]. The absorbance was measured at 540 nm. All experiments were performed in three times. Cell viability was determined using MTT method to find whether the production was due to the cytotoxicity of all the tested compounds.

2.5. Cytotoxic bioassays

According to a method described previously [11], the cytotoxicities of the five new withanolides together with a positive control Norcantharidin were all tested *in vitro* by using the MTT assay against A549, SMCC-7721, and MCF-7 human tumor cell lines (Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences). Cells were plated in 96–well microplates with a concentration of

Table 2
^{1}H NMR(600 MHz) spectroscopic data for compounds **1–5** in CD_3OD.[a]

Position	1	2	3	4	5
2	*α*:2.54 dd (16.2, 3.6), *β*:2.86 dd (16.2, 7.2)	*α*: 2.56dd (16.2, 3.6), *β*: 2.87dd (16.2, 7.2)	*α*: 2.72 dd (16.2, 3.6), *β*: 2.80 dd (16.2, 7.2)	6.18 d (9.6)	6.15 d (9.6)
3	4.08 dt (7.2, 3.6)	4.10 dt (7.2, 3.6)	3.65 dt (7.2, 3.6)	7.08 dd (9.6, 6.0)	7.06 dd (9.6, 6.0)
4	3.34 d (3.6)	3.33 d (3.6)	3.45 d (3.6)	3.69 d (3.6)	3.69 d (3.6)
6	3.32 br. s	3.30 br. s	3.33 br. s	3.31 br. s	3.26 br. s
7	*α*: 1.80 m, *β*: 2.50 m	*α*: 1.66 m, *β*: 2.42 m	*α*: 1.82 m, *β*: 2.55 m	*α*: 1.83 m, *β*: 2.39 m	*α*: 1.76 m, *β*: 2.36 m
8	1.80 m	1.76 m	1.80 m	1.75 m	1.75 m
9	2.02 m	2.14 m	2.10 m	2.00 m	2.31 m
11	1.30 m,1.35 m	1.28 m, 1.35 m	1.26 m, 1.34 m	1.28 m, 1.34 m	1.28 m, 1.34 m
12	1.76 m, 1.47 m	1.56 m, 1.20 m	1.70 m, 1.53 m	1.72 m, 1.49 m	1.71 m, 1.49 m
15	5.25 d (2.4)	5.09 d (2.4)	5.34 d (2.4)	5.37 d (2.4)	5.28 d (2.4)
16	5.75 d (2.4)	1.89 m, 2.63 m	5.67 d (2.4)	5.67 d (2.4)	5.63 d (2.4)
18	1.03 s	1.10 s	1.02 s	1.20 s	1.02 s
19	1.23 s	1.24 s	1.25 s	1.38 s	1.37 s
20	2.72 dq (7.2, 3.6)	2.86 m	2.57 m	2.18 m	2.50 m
21	1.21 d (7.2)	1.06 d (7.2)	1.22 d (7.2)	1.07 d (7.2)	1.20 d (7.2)
22	4.18 dd (12.0, 3.6)	4.81 dd (12.0, 3.6)	4.37 dd (12.0, 3.6)	4.17 dd (12.0, 3.6)	4.35 dd (12.0, 3.6)
23	4.19 m	2.53 m, 1.36 m	2.54 m, 2.29 m	2.54 m, 2.28 m	2.51 m, 2.25 m
27	1.85 s	1.84 s	1.86 s	1.91 s	1.83 s
28	1.97 s	1.99 s	1.98 s	2.00 s	1.96 s
OMe			3.37 s		
CH_3CO	2.05 s	2.13 s			

[a] Chemical shifts are in ppm, and coupling constants (*J*) in Hz are given in parentheses. The assignments were based on HSQC, HMBC and NOSEY spectra.

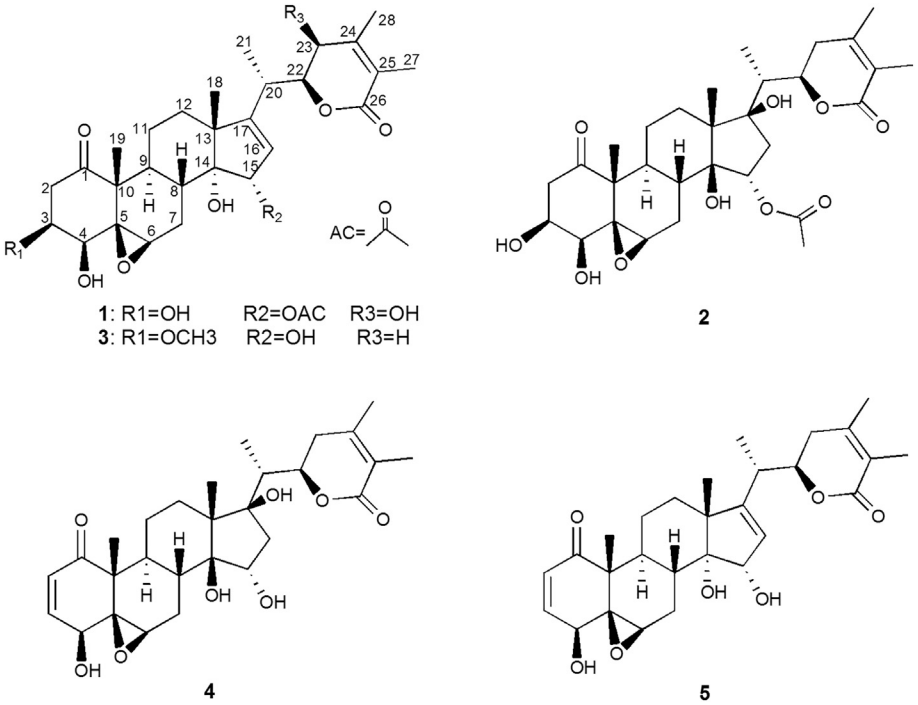

Fig. 1. The structures of new withanolides from the whole plants of *Physalis minima* L.

1×10^5cells per well in the fresh culture medium. After cultivation for 24 h, the test solutions of the new withanolides at 6.25, 12.5, 25, 50, 100 μM were applied to the cells, respectively. The cells were incubated with MTT solution (0.5 mg/mL) for four hours. And then, the medium was removed and resolved in DMSO (100 mL of each well). The assays were performed in triplicate, and the IC_{50} values of compounds **1–5** are given in Table 4.

3. Results and discussion

Compound **1** was isolated as amorphous powder, and its molecular formula was determined as $C_{30}H_{40}O_{10}$ based on its HR-ESI-MS spectrum ($[M + FA\text{-}H]^-$ at *m/z* 605.2626; calcd. 605.2598 for $C_{31}H_{41}O_{12}$), indicating eleven double-bond equivalents. The IR absorptions of **1** showed the presence of an *α*, *β*-unsaturated *δ*-lactone (1735 cm^{-1}), as well as an *α*, *β*-unsaturated ketone (1712 cm^{-1}) and a C═C bond group (1642 cm^{-1}). The analysis of its ^{13}C NMR and HSQC spectroscopic data revealed six methyls, four methylenes, ten methines and ten quaternary carbons in the structure. A comparison of the ^{1}H and ^{13}C NMR data of **1** with those of physaminimin F [5] suggested that **1** was 3*β*,4*β*-dihydroxy, 5*β*,6*β*-epoxy, 14*α*-hydroxy-15*α*-acetoxy-16-ene type of withanolide. The main difference between them was observed in the signals of ring E. The significant downfield shifts of C-23 (+33.9 ppm) at δ_C 67.5 indicated that a hydroxyl group linked to C-23 in **1**. The linkage was confirmed by the HMBC correlation between H-28 at δ_H 1.97 (3H, s) and C-23 at δ_C 67.5 (in Fig. 2). The assignment was further confirmed

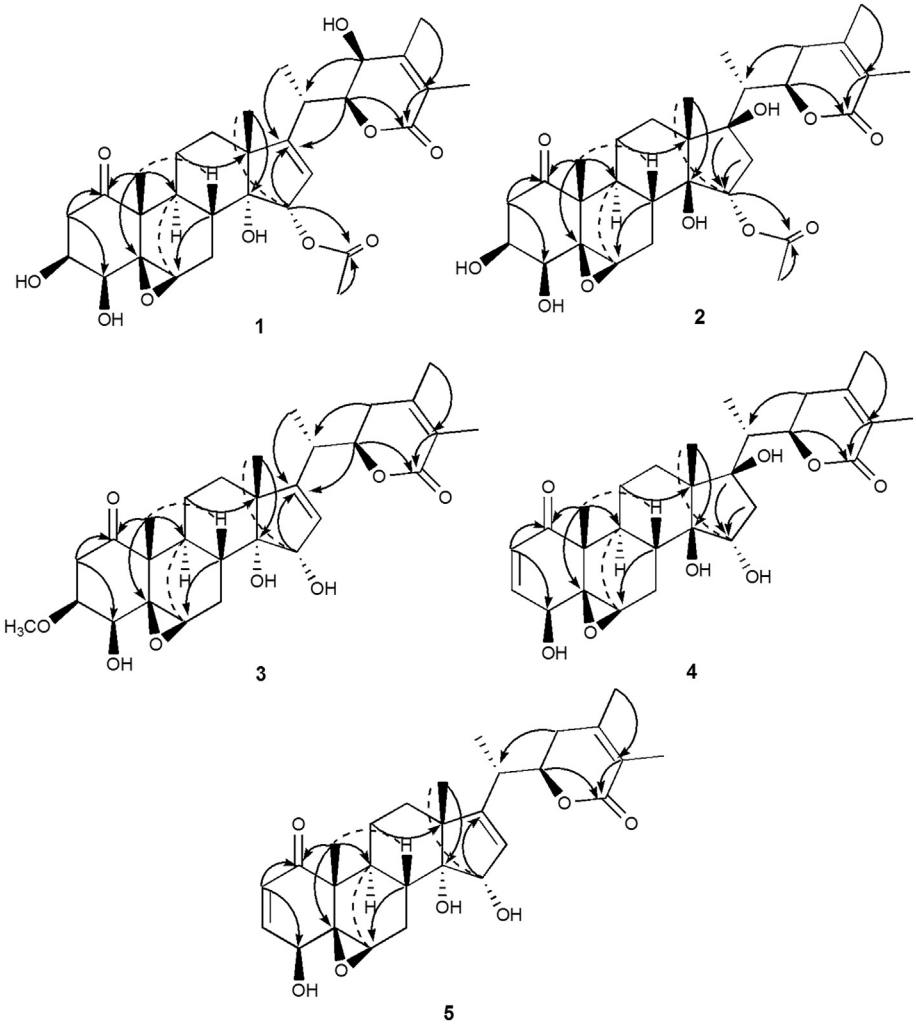

Fig. 2. Key HMBC and NOE correlations for compounds **1–5**.

Table 3
Inhibition of all tested compounds on the NO production in LPS-activated RAW264.7 cells. (n = 3).

Compounds	IC_{50} ± SD (μM)[a]
1	73.26 ± 2.53
2	58.37 ± 1.56
3	52.71 ± 2.41
4	43.26 ± 1.73
5	42.18 ± 1.59
L-NAME[b]	39.84 ± 1.58

[a] IC_{50} is means ± standard deviation of five new withanolides.
[b] L-NG-Monomethyl arginine methyl ester (L-NAME) was used as the positive control.

Table 4
In vitro cytotoxic activities of compounds **1–5** (IC_{50} values in μM).

Compounds	cell line IC_{50} (μM)[a]		
	A549	SMMC-7721	MCF-7
1	63.20 ± 1.77	78.56 ± 2.65	80.14 ± 1.66
2	58.71 ± 2.58	62.97 ± 1.98	70.15 ± 1.22
3	36.78 ± 4.25	42.01 ± 0.98	38.59 ± 2.01
4	31.25 ± 0.98	36.76 ± 1.59	35.17 ± 1.69
5	40.25 ± 1.87	45.36 ± 1.36	48.74 ± 0.98
Norcantharidin[b]	4.16 ± 0.28	7.93 ± 0.46	5.17 ± 0.38

[a] IC_{50} is the means ± standard deviation of five new withanolides.
[b] Norcantharidin was used as the positive control.

by the facts that the C-22 was de-shielded from 80.7 to 85.9 ppm, while C-20 was shielded from 36.6 to 33.6 ppm due to the β and γ effects of 23-hydroxyl group. The relative configuration of **1** was determined from the NOESY experiment. The NOE correlation from H-22 at δ_H 4.18 (1H, dd, J = 12.0, 3.6 Hz) to H-23 at δ_H 4.19 (1H, m) indicated the 23-hydroxyl group was β-oriented. Furthermore, the NOEs from H-3 at δ_H 4.08 (1H, dt, J = 7.2, 3.6 Hz) and H-4 at δ_H 3.34 (1H, d, J = 3.6 Hz) to H-2α at δ_H 2.54 [1H, dd, J = 16.2, 3.6 Hz)] implied that H-3, H-4 were co-facial and α-oriented. The NOE correlation of H-6 at 3.32 (1H, brs) with H-7α at 1.80 (1H, m) indicated the α-orientation of H-6, confirming that the epoxy was β-oriented. The NOE correlation between H-15 at δ_H 5.25 (1H, d, J = 2.4 Hz) and H-18 at δ_H 1.03 (3H, s) revealed that the 15-acetoxy was α-oriented. Based on the above analysis, the structure of **1** was established to be 15a-acetoxy-5,6-β-epoxy-3β,4β,14a,23β-tetrahydroxy-1-oxowitha-16,24-dienolide, named physaliolide G (Fig. 1).

Compound **2** has the molecular formula of $C_{30}H_{42}O_{10}$ according to the HR-ESI-MS ($[M + FA-H]^-$ ion peak at m/z 607.2795; calcd. 607.2755). The NMR spectroscopic data of **2** (Tables 1 and 2) were similar to those of **1**. The main differences were observed for C-17 (−75.7 ppm) at δ_C 86.9, C-16 (−73.6 ppm) at δ_C 49.6, and C-23 (−34.6 ppm) at δ_C 32.9, suggesting the hydroxylation at C-17 and dihydroxylation at C-23 in **2** The assignment was confirmed by HMBC

correlations from H-15 at δ_H 5.09 (1H, d, J = 2.4 Hz) to C-17 at δ_C 86.9 , and from H-18 at δ_H 1.10 (3H, s) to C-17 at δ_C 86.9 (in Fig. 2). Furthermore, by comparing the NMR data of compound **2** with those of Physagulin K [9], they had the identical D-E rings, suggesting that both OH-14 and OH-17 in **2** were co-facial and *β*-oriented. Besides, the NOESY experiment confirmed that the relative configuration of **2** was as same as **1**. Therefore, the structure of **2** was elucidated to be 15*α*-acetoxy-5,6-*β*-epoxy-3*β*,4*β*,14*β*,17*β*-tetrahydroxy-1-oxowitha-24-enolide, named physaliolide H (Fig. 1).

The HR-ESI-MS spectrum of **3** showed an $[M + FA\text{-}H]^-$ ion peak at 561.2743 (calcd. 561.2700), referring to a molecular formula of $C_{29}H_{40}O_8$. The ^1H and ^{13}C NMR data of **3** showed similarity to those of **1**, and the differences were observed for the replacements of the hydroxyl group by methoxy group at C-3 andthe OAC group by hydroxyl group at C-15, as well as the dehydroxylation at C-23. The assignment of a methoxy group to C-3 was confirmed by the HMBC correlation between H-OCH_3 at δ_H 3.37 (3H, s) and C-3 at δ_C 79.5, and the correlation between H-3 at δ_H 3.65 (1H, dt, J = 7.2, 3.6 Hz) and C-1 at δ_C 212.3 (in Fig. 2). The linkage of 15-OH was proved by the HMBC correlation between H-15 at 5.34 (1H, d, J = 2.4 Hz) and C-17 at δ_C 159.2. The E ring of **3** was identical to that of **2**. The structure of compound **3**, thus, was assigned to be 5,6-*β*-epoxy-4*β*,14*α*,15*α*-trihydroxy-3*β*-methoxy-1-oxowitha-16, 24-dienolide, named physaliolide I (Fig. 1).

Compound **4** displayed an $[M + FA\text{-}H]^-$ ion peak at 547.2579 (calcd. 547.2543) in the HR-ESI-MS (negative mode) spectrum, revealing a molecular formula of $C_{28}H_{38}O_8$. Comparing the NMR spectroscopic data of **4** with **2**, they had similar structures except that **4** had a *α, β*-unsaturated ketone in the ring A [δ_C 133.3C-2, δ_H 6.18 (1H, d, J = 9.6 Hz); δ_C 145.2C-3, δ_H 7.08 (1H, dd, J = 9.6, 6.0 Hz)] and a hydroxyl group at C-15. The assignment was confirmed by the HMBC corrections from H-3 at δ_H 7.08 (1H, dd, J = 9.6, 6.0 Hz) to C-1 at δ_C 203.8, and from H-15 at δ_H 5.37 (1H, d, J = 2.4 Hz) to C-17 at δ_C 78.9 (in Fig. 2). Thus, the structure of compound **4** was assigned to be 5,6-*β*-epoxy-4*β*,14*β*,15*α*,17*β* -tetrahydroxy-1-oxowitha-2,24-dienolide, named physaliolide J (Fig. 1).

The molecular formula of compound **5** was established as $C_{28}H_{36}O_7$ according to its data HR-ESI-MS ($[M + FA\text{-}H]^-$ ion peak at 529.2473; calcd. 529.2438), indicating eleven double-bond equivalents in the structure. The NMR data of **5** showed similarity to those of 2, except the exist of one *α, β*-unsaturated ketone in the ring A [δ_C 133.1C-2, δ_H 6.15 (1H, d, J = 9.6 Hz); δ_C 145.4C-3, δ_H 7.06 (1H, dd, J = 9.6, 6.0 Hz)]. The assignment was confirmed by the HMBC correlation from H-3 at δ_H 7.06 (1H, dd, J = 9.6, 6.0 Hz) to C-1 at δ_C 204.5 (in Fig. 2). Therefore, the structure of compound **5** was assigned to be 5,6-*β*-epoxy-4*β*,14*α*,15*α*-trihydroxy −1-oxowitha-2,16,24-trienolide, named physaliolide K (Fig. 1).

All these five new 5,6-*β*-epoxywithanolides from the whole plant of *P. minima* were evaluated for their inhibitory effects on LPS-induced NO production in RAW 264.7 cells. Meanwhile, cell viability was conducted to explore whether the inhibition was due to the cytotoxicity of the tested compounds. The results (Table 3) showed all these withanolides possessed a moderate anti-inflammatory activity and exhibited no cytotoxicity against RAW 264.7 cells (data do not show here). Furthermore, their anti-tumor activity was evaluated *via* the cytotoxicity MTT-based assay, and the IC_{50} values (Table 4) indicated that all these new withanolides possessed moderate cytotoxic activity against A549 cells, SMMC-7721 cells, and MCF-7 cells.

4. Conclusion

In conclusion, five new 5,6*β*-epoxywithanolides were isolated and identified from *P. minima* L. At the same time, all the compounds were evaluated for their anti-inflammatory ability on NO production in LPS-stimulated murine macrophage RAW 264.7 cells and cytotoxic activities against A549, SMMC-7721 and MCF-7 cells. All of them possessed moderate inhibition to the production of nitric oxide with IC_{50} values from 42.18 to 73.26 μM, and the IC_{50} values of the cytotoxic activities were in the range of 31.25 to 80.14 μM.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.104413.

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