Triterpenoids from *Angiopteris palmiformis*

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Two new fernane triterpenoids, 7α-hydroxyfern-8-en-11-one (1) and 11β-hydroxyfern-8-en-7-one (2), and two new filicane triterpenoids, 3β-hydroxyfilic-4(23)-ene (3) and filicenol (5), together with one known filicane-type triterpenoid, 3α-hydroxyfilic-4(23)-ene (4), were isolated from the methyl alcohol extract of the leaves of *Angiopteris palmiformis*. Their structures were elucidated on the basis of extensive analyses of their spectroscopic data (NMR, MS, IR) and comparison with spectroscopic data in the literature.

Key words *Angiopteris palmiformis*; Marattiaceae; triterpenoid; fernane; filicane; cytotoxic

Angiopteris palmiformis (Cav.) C. Chr. belonging to the family Marattiaceae grows in the subtropical or tropic areas of Taiwan, Philippines, and Tahiti.[1)] It is a large-size fern with the pinnately compound fronds up to 2—3 m length. Extensive phytochemical investigation on *A. palmiformis* has not been performed yet, and only one fern glycoside, angiopteroside,[2)] and three flavonoids, violanthin, isoviolanthin, apigenin 6,8-di-*C*-α-L-arabinopyranoside,[3)] have been isolated from the *Angiopteris* genus. In continuation of our work on the discovery of secondary metabolites from Taiwanese plants, we have examined the methanolic extract of the leaves of *A. palmiformis* and isolated two new fernane-type triterpenoids, 7α-hydroxyfern-8-en-11-one (**1**) and 11β-hydroxyfern-8-en-7-one (**2**), and two new filicane-type triterpenoids, 3β-hydroxyfilic-4(23)-ene (**3**) and filicenol (**5**), together with one known filicane-type triterpenoid, 3α-hydroxyfilic-4(23)-ene (**4**) (Fig. 1).[4)] In this paper, we describe the extraction, purification, and structural elucidation of the four new constituents.

Compound **1** was obtained as a white needle crystal and its molecular formula was determined as $C_{30}H_{48}O_2$ by the HR-EI-MS spectrum, which revealed an $[M]^+$ ion peak at *m*/*z* 440.3625. A significant UV absorption maximum at 256 nm and the broad IR absorption band at 1640 cm^{-1} suggested the presence of an γ-hydroxy-α,β-conjugated enone moiety.[5)]

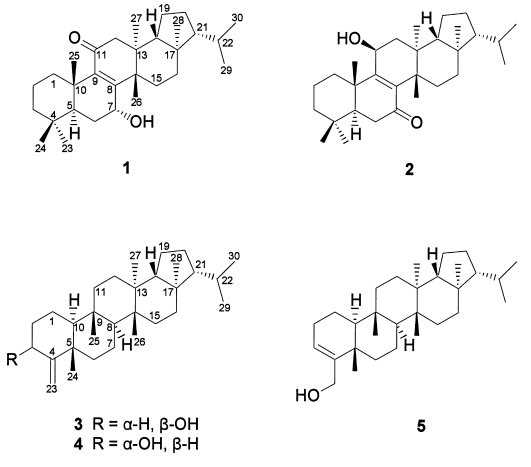

Fig. 1. Structures of Compounds **1**—**5** from *A. palmiformis*

The ^{1}H- and ^{13}C-NMR spectra of **1** (Tables 1, 2) exhibited signals characteristic for the presence of six methyl singlets [δ_H 0.77, 0.85, 0.91, 0.97, 1.08, 1.17 (3H each, s)], two methyl doublets of an isopropyl group [δ_H 0.82, 0.88 (3H each, d, J=6.4 Hz); showing ^{1}H–^{1}H correlation spectroscopy (COSY)], one set of α,β-unsaturated carbonyl system [δ_C 142.6 (s), 157.9 (s), 200.3 (s)], and one oxymethine [δ_H 4.33 (1H, br s)]. The 30 carbon signals found in the ^{13}C-NMR spectrum of **1** (Table 2) were differentiated as eight methyl, nine methylene, four methine, five quaternary, one oxymethine, two quaternary olefinic, and one carbonyl carbons by using distortionless enhancement by polarization transfer (DEPT) experiments. The ^{1}H- and ^{13}C-NMR data were generally similar to those of supinenolone A isolated from *Euphorbia supina*,[5)] except for the signals of the ring A (C-1—C-5, C-23—C-25). The NMR signal of oxymethine [δ_H 3.33 (dd, J=10.0, 6.7 Hz); δ_C 78.6 (d)] at C-3 in supinenolone A was replaced by those of a methylene [δ_H 1.21 (m), 1.44 (m); δ_C 41.4 (t)] in **1**. The structure of ring A was confirmed by the heteronuclear multiple bond correlation (HMBC) correlations between H-3 (δ_H 1.21)/C-2 (δ_C 19.0), C-23 (δ_C 33.3), and C-24 (δ_C 22.0) (Fig. 2). The HMBC correlations between H-7 (δ_H 4.33)/C-5 (δ_C 46.0) and C-9 (δ_C 142.6), together with the nuclear Overhauser effect (NOE) correlation between H-7 and Me-26 (δ_H 1.08) indicated that the hydroxy group was attached to C-7 in α orientation. The significant NOE correlations between H-5 (δ_H 1.34)/Me-23 (δ_H 0.91), H_α-15 (δ_H 2.42)/Me-27 (δ_H 0.97), H_α-15/Me-28 (δ_H 0.77), H-18 (δ_H 1.64)/Me-26, H-22 (δ_H 1.44)/Me-28, Me-24 (δ_H 0.85)/Me-25 (δ_H 1.17), and Me-27/Me-28 permitted definition of the relative configurations of sterogenic carbon atoms in the pentacyclic rings. Thus, compound **1** was assigned as 7α-hydroxyfern-8-en-11-one. ^{1}H- and ^{13}C-NMR chemical shifts were established by ^{1}H–^{1}H COSY, heteronuclear multiple quantum coherence (HMQC), HMBC, and nuclear Overhauser effect spectroscopy (NOESY) spectra.

The HR-EI-MS of **2** gave a molecular ion peak at *m*/*z* 440.3649 that corresponded to a molecular formula, $C_{30}H_{48}O_2$, indicating seven degrees of unsaturation. The UV and IR spectra showed absorption bands attributable to hydroxyl (3335 cm^{-1}), and γ-hydroxy-α,β-conjugated enone (254 nm; 1645 cm^{-1}) functionalities.[5)] The ^{1}H- and ^{13}C-NMR

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Table 1. ^{1}H-NMR Data for **1**—**3** and **5** (400 MHz in $CDCl_3$)

Position	1	2	3	5
1	0.81 m, 2.40 m	1.46 m, 2.22 m	1.50 m, 1.78 m	1.86 m
2	1.45 m, 1.64 m	1.57 m, 1.70 m	1.56 m, 1.98 m	2.12 m
3	1.21 m, 1.44 m	1.20 m, 1.48 m	4.29 br s	5.56 br t (3.2)
5	1.34 m	1.62 m		
6	1.62 m, 1.82 m	2.39 m	1.48 m, 1.78 m	1.19 m , 2.21 m
7	4.33 br s		1.39 m, 1.50 m	1.24, 1.42 m
8			1.28 m	1.29 m
10			1.01 m	1.24 m
11		4.71 t (8.1)	1.40 m	1.46 m
12	2.05 d (18.8), 2.17 d (18.8)	1.48 m, 2.02 dd (8.1, 8.8)	1.05 m, 1.22 m	1.02 m, 1.20 m
15	1.46 m, 2.42 m	1.48 m, 2.38 m	1.22 m, 1.41 m	1.21 m, 1.38 m
16	1.54 m, 1.80 m	1.36 m, 1.65 m	1.60 m, 1.70 m	1.58 m, 1.66 m
18	1.64 m	1.62 m	1.55 m	1.52 m
19	1.26 m, 1.40 m	1.28 m, 1.40 m	1.20 m, 1.35 m	1.19 m, 1.34 m
20	1.25 m, 1.83 m	1.24 m, 1.84 m	1.41 m, 1.83 m	1.39 m, 1.82 m
21	1.01 m	0.98 m	0.98 s	0.96 m
22	1.44 m	1.45 m	1.45 m	1.45 m
23	0.91 s	0.86 s	4.77 d (1.6), 4.82 d (1.6)	4.10 d (13.2), 4.22 d (13.2)
24	0.85 s	0.92 s	1.19 s	1.07 s
25	1.17 s	1.16 s	0.92 s	0.86 s
26	1.08 s	1.34 s	0.89 s	0.84 s
27	0.97 s	0.79 s	0.88 s	0.91 s
28	0.77 s	0.72 s	0.75 s	0.75 s
29	0.82 d (6.4)	0.80 d (6.4)	0.86 d (6.4)	0.85 d (6.4)
30	0.88 d (6.4)	0.87 d (6.4)	0.80 d (6.4)	0.79 d (6.4)

Table 2. ^{13}C-NMR Data for **1**—**3** and **5** (100 MHz in $CDCl_3$)

Position	1	2	3	5
1	35.8	35.9	15.8	16.6
2	19.0	18.7	34.7	23.4
3	41.4	41.5	74.8	124.8
4	32.8	33.5	161.1	143.2
5	46.0	48.8	40.2	36.3
6	28.8	37.4	40.1	37.8
7	64.1	201.1	17.9	20.1
8	157.9	142.1	49.5	49.2
9	142.6	161.0	38.1	39.1
10	38.9	39.8	59.4	56.2
11	200.3	65.8	35.6	35.1
12	50.9	42.2	28.4	28.4
13	43.2	39.5	38.9	38.8
14	37.5	40.5	40.2	40.0
15	27.2	25.8	29.0	29.3
16	35.8	35.8	35.6	35.6
17	42.7	42.4	42.7	42.7
18	51.9	51.3	51.7	51.7
19	20.3	20.3	19.9	19.9
20	28.0	28.1	28.4	28.4
21	59.5	59.7	60.0	60.1
22	30.6	30.7	30.8	30.8
23	33.3	32.5	109.3	65.1
24	22.0	21.6	23.8	35.0
25	18.0	20.2	20.5	19.8
26	23.9	23.8	16.1	16.5
27	19.7	17.1	15.6	15.4
28	13.8	14.6	16.3	16.4
29	22.9	22.9	21.9	21.9
30	22.0	22.0	22.9	22.9

data (Tables 1, 2) of **2** showed close resemblance with those observed for **1**, except for the signals of rings B and C. The signals of γ-hydroxy-α,β-conjugated enone [δ_H 4.71 (1H, t, J=8.1 Hz); δ_C 65.8 (d), 142.1 (s), 161.0 (s), 201.1 (s)] were also observed in the ^{1}H- and ^{13}C-NMR spectra of **2**.[5] The

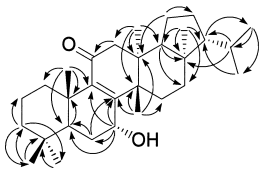

Fig. 2. Main HMBC Correlations of **1**

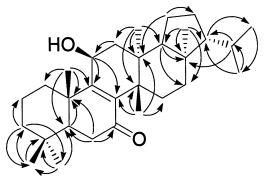

Fig. 3. Main HMBC Correlations of **2**

HMBC correlations between H-6 (δ_H 2.39)/C-5 (δ_C 48.8), C-7 (δ_C 201.1), and C-10 (δ_C 39.8) and H-11 (δ_H 4.71)/C-8 (δ_C 142.1) and C-9 (δ_C 161.0) (Fig. 3) confirmed that the α,β-conjugated enone and hydroxy groups were located at C-8, 9, and 11 and C-7, respectively. The β orientation of 11-OH was assured by the NOE effect between H-11 and Me-27 (δ_H 0.79) in the NOESY spectrum. The relative configurations of sterogenic carbon atoms in the pentacyclic rings were determined by significant NOE correlations between H-5 (δ_H 1.62)/Me-23 (δ_H 0.86), H-11 (δ_H 4.71)/H_α-15 (δ_H 1.48), H_α-15/Me-27, H_α-15/Me-28 (δ_H 0.72), H-18 (δ_H 1.62)/Me-26 (δ_H 1.34), H-18/H-21 (δ_H 0.98), Me-24 (δ_H 0.92)/Me-25 (δ_H 1.16), Me-27/Me-28 in the NOESY spectrum. On the basis of the above data, compound **2** was defined as 11β-hydroxy-fern-8-en-7-one.

Compound **3** was deduced to be a triterpenoid due to a positive Liebermann–Burchard test. Its HR-EI-MS showed a molecular ion peak at m/z 426.3875, in agreement with the

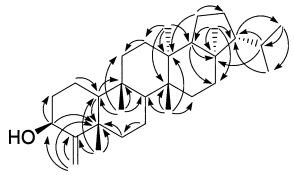

Fig. 4. Main HMBC Correlations of **3**

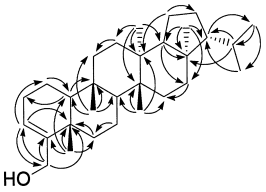

Fig. 5. Main HMBC Correlations of **5**

molecular formula $C_{30}H_{50}O$. The IR spectrum of **3** revealed the informative absorption bands at around 3374, 3087, 1638 and 906 cm^{-1} duo to hydroxy and double bond, respectively. Inspection of the ^{1}H- and ^{13}C-NMR spectra (Tables 1, 2) showed the signals for five tertiary methyls [δ_H 0.75, 0.88, 0.89, 0.92, 1.19 (3H each, s)], two secondary methyls [δ_H 0.80 (3H, d, J=6.4 Hz), 0.86 (3H, d, J=6.4 Hz)], an olefinic methylene of an exocyclic double bond [δ_H 4.77 (1H, d, J=1.6 Hz), 4.82 (1H, d, J=1.6 Hz); δ_C 109.3 (t)], and an oxymethine [δ_H 4.29 (1H, br s)]. Altogether, 30 carbon signals were found in the ^{13}C-NMR spectrum of **3**, and were resolved by DEPT experiments as seven methyl, ten methylene, one olefinic methylene, five methine, one oxymethine, five quaternary, and one quaternary olefinic carbons. The EI-MS spectrum of **3** showed the fragment ions at *m/z* 411, 408, 393, 356, 341, 323, 273, 229, 207, 201, and 191 corresponding to those of **4** and indicated **3** to be a filicene derivative.[4] Comparisons of the ^{1}H- and ^{13}C-NMR signals of **3** in rings C—D and isopropyl side chain were very similar to those of filic-3-ene.[6] As observed in the HMBC spectrum, the long-range correlations between H-3 (δ_H 4.29)/C-2 (δ_C 34.7), C-5 (δ_C 40.2) and H-23 (δ_H 4.77, 4.82)/C-3 (δ_C 74.8) (Fig. 4) confirmed that the hydroxy was attached to C-3. A broad singlet signal of H-3 at δ_H 4.29 (1H, br s) suggested that the oxymethine at C-3 had an equatorial α-orientation in **3** as opposed to an axial β-orientation in **4** [δ_H 4.28 (1H, dd, J= 5.4, 11.6 Hz)].[4] Compound **3** was thus determined as 3β-hydroxyfilic-4(23)-ene.

Compound **5** was obtained as a white needle crystal. Its HR-EI-MS showed a molecular $[M]^+$ ion at *m/z* 426.3855, indicative of a molecular formula of $C_{30}H_{50}O$. The IR spectrum displayed absorption bands for a hydroxy (3520 cm^{-1}) and a double bond (3045, 1653, 835 cm^{-1}). Characteristic resonances in the ^{1}H- and ^{13}C-NMR spectra of **5** (Tables 1, 2) exhibited the presence of five methyl singlets [δ_H 0.75, 0.84, 0.86, 0.91, 1.07 (3H each, s)], two secondary methyl doublets [δ_H 0.79, 0.85 (3H each, d, J=6.4 Hz)], an oxymethylene [δ_H 4.10, 4.22 (1H each, d, J=13.2 Hz; δ_C 65.1 (t)], and a trisubstituted double bond [δ_H 5.56 (1H, br t, J=3.2 Hz); δ_C 124.8 (d), 143.2 (s)]. Comparison of the ^{1}H- and ^{13}C-NMR data with those of **3**, indicated that both compounds exhibited identical structure in rings B—E. The HMBC correlations between H-3 (δ_H 5.56)/C-1 (δ_C 16.6), C-5 (δ_C 36.3), and C-23 (δ_C 65.1), and H-23 (δ_H 4.10, 4.22)/C-3 (δ_C 124.8), C-4 (δ_C 143.2), and C-5 (Fig. 5) confirmed that the trisubstituted double bond was located at C-3 and C-4 and the hydroxy group was attached on C-23. From the above evidence, compound **5** was characterized as filic-3-en-23-ol.

Compounds **1**, **3**, **4** and **5** were evaluated for their cytotoxic activity against human hepatoma SK Hep-1 cells with fluorouracil (5-FU) as a positive control (IC_{50}=1.6 μM).[7] Forty-eight hrs after culture, compounds **1**, **3**, **4** and **5** showed 49.8, 46.1, 71.8, and 52.1% growth inhibitory activity at a concentration of 25 μg/ml, respectively.

Experimental

General Experimental Procedures Melting points were determined on a Fargo melting point apparatus (uncorrected) and optical rotations on a JASCO DIP-180 digital spectropolarimeter. UV spectra were measured in MeOH using a Shimadzu UV-1601PC spectrophotometer. IR spectra were recorded on a Nicolet 510P FT-IR spectrometer. NMR spectra were obtained in $CDCl_3$ at room temperature on a Varian Mercury plus 400 NMR spectrometer, and the solvent resonance was used as internal shift reference (tetramethylsilane as standard). The 2D NMR spectra were recorded by using standard pulse sequences. EI-MS and HR-EI-MS were recorded on Finnigan TSQ-700 and JEOL SX-102A mass spectrometers, respectively. TLC was performed by using Si gel 60 F_{254} plates (Merck). Column chromatography was performed on Si gel (230—400 mesh ASTM, Merck). HPLC was performed by using a Lichrosorb Si gel 60 (5 μm) column (250×10 mm).

Plant Material The leaves of *A. palmiformis* were collected in Pingtung County, Taiwan in June, 2003. The plant material was identified by Prof. Sheng-Zehn Yang, Curator of Herbarium, National Pingtung University of Science and Technology, where a voucher specimen (no. 19566) was deposited.

Extraction and Isolation The air-dried leaves (15 kg) of *A. palmiformis* were extracted with MeOH (3×80 l) at room temperature (7 d each). The combined MeOH extract was evaporated under reduced pressure to afford a green residue, which was suspended in H_2O (6 l), and then partitioned sequentially, using EtOAc and *n*-BuOH (3×4 l) as solvent. The EtOAc fraction (300 g) was chromatographed on Si gel column (120×10 cm), eluted with *n*-hexane and EtOAc of increasing polarity. Eleven fractions were collected as follows: 1 [10000 ml, *n*-hexane], 2 [8000 ml, *n*-hexane–EtOAc (49 : 1)], 3 [7000 ml, *n*-hexane–EtOAc (19 : 1)], 4 [8000 ml, *n*-hexane–EtOAc (9 : 1)], 5 [8000 ml, *n*-hexane–EtOAc (17 : 3)], 6 [9000 ml, *n*-hexane–EtOAc (8 : 2)], 7 [10000 ml, *n*-hexane–EtOAc (7 : 3)], 8 [9000 ml, *n*-hexane–EtOAc (5 : 5)], 9 [8000 ml, *n*-hexane–EtOAc (4 : 6)], 10 [7000 ml, *n*-hexane–EtOAc (2 : 8)], and 11 (10000 ml, EtOAc). Fraction 3 was further chromatographed on a Si gel column (5×45 cm), eluted with *n*-hexane–CH_2Cl_2–EtOAc (8 : 1 to 0 : 1) to obtain seven fractions (each about 600 ml), 7A—7G. Fr. 7D was subjected to column chromatography over Si gel eluted with *n*-hexane–CH_2Cl_2–EtOAc (3 : 3 : 1) and semipreparative HPLC eluted with *n*-hexane–EtOAc (7 : 3) to yield **3** (6 mg). Fraction 8 was further purified through a Si gel column (5×45 cm) eluted with CH_2Cl_2–EtOAc (7 : 1 to 0 : 1) to obtain six fractions (each about 500 ml), 8A—8F. Si gel column chromatography of fr. 8C eluted with a CH_2Cl_2–EtOAc gradient (100 : 1 to 0 : 1) yielded **1** (5 mg). Fr. 8E was subjected to column chromatography over Si gel eluted with *n*-hexane–CH_2Cl_2–EtOAc (3 : 3 : 1) and semipreparative HPLC eluted with CH_2Cl_2–EtOAc (3 : 2) to yield **2** (1 mg), **4** (9 mg) and **5** (15 mg).

7α-Hydroxyfern-8-en-11-one (**1**): White needles, mp 278—280 °C (CH_2Cl_2); $[\alpha]_D^{25}$ +64.9 (c=0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) nm: 256 (3.1); IR (KBr) cm^{-1}: 3349, 2950, 2867, 1640, 1455, 1392, 1246, 1060, 959, 671; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; EI-MS *m/z*: 440 $[M]^+$ (46), 422 (100), 407 (39), 351 (8), 339 (20), 311 (10), 261 (18), 235 (18), 121 (16), 95 (20), 55 (34); HR-EI-MS *m/z*: 440.3625 (Calcd for $C_{30}H_{48}O_2$ 440.3656).

11β-Hydroxyfern-8-en-7-one (**2**): White needles, mp 275—277 °C (CH_2Cl_2); $[\alpha]_D^{25}$ +71.4 (c=0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ε) nm: 254 (3.0); IR (KBr) cm^{-1}: 3335, 2926, 2872, 1645, 1470, 1367, 1309, 959, 750; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; EI-MS *m/z*: 440 $[M]^+$ (68), 426 (38), 407 (100), 397 (17), 302 (23), 271 (29), 245 (15), 147 (16), 123 (21), 81 (17), 69 (29); HR-EI-MS *m/z*: 440.3649 (Calcd for $C_{30}H_{48}O_2$ 440.3656).

3β-Hydroxyfilic-4(23)-ene (**3**): White needles, mp 228—230 °C (CH_2Cl_2); $[\alpha]_D^{25}$ +65.7 (c=0.15, $CHCl_3$); IR (KBr) cm^{-1}: 3374, 3087, 2926,

2865, 1638, 1458, 1379, 1325, 1028, 906, 752; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; EI-MS *m*/*z*: 426 $[M]^+$ (4), 411 (6), 408 (3), 393 (2), 356 (1), 341 (12), 323 (3), 273 (6), 231 (7), 229 (6), 207 (8), 205 (16), 201 (15), 191 (70), 179 (30), 173 (14), 161 (35), 149 (42), 135 (50), 121 (80), 109 (88), 95 (96), 81 (92), 69 (100); HR-EI-MS *m*/*z*: 426.3875 (Calcd for $C_{30}H_{50}O$ 426.3864).

Filic-3-en-23-ol (**5**): White needles, mp 207—209 °C (CH_2Cl_2); $[\alpha]_D^{25}$ +4.5 (c=0.22, $CHCl_3$); IR (KBr) cm^{-1}: 3520, 3045, 2945, 2867, 1653, 1455, 1377, 1012, 987, 835, 764; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; EI-MS *m*/*z*: 426 $[M]^+$ (5), 411 (5), 408 (12), 393 (7), 356 (1), 341 (3), 323 (3), 273 (19), 231 (3), 229 (2), 207 (1), 205 (16), 201 (5), 191 (14), 179 (30), 173 (8), 161 (15), 149 (42), 135 (50), 121 (57), 107 (71), 95 (90), 81 (100), 69 (78); HR-EI-MS *m*/*z*: 426.3855 (Calcd for $C_{30}H_{50}O$ 426.3864).

Cytotoxicity Assay SK Hep-1 cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated for 24 h. Test samples were dissolved in dimethyl sulfoxide (DMSO) and added to the medium. Following a 48 h incubation, the wells were incubated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (100 μl/well concentrated at 5 mg/ml) at 37 °C for 4 h. The supernatant was aspired, and the 200 μl of DMSO was added to redissolve the formazan crystals. The optical density was measured by an enzyme-linked immunosorbent assay plate reader at 550 nm. The results were compared with that of the control and expressed as a percentage of cell viability. The experiments were repeated three times. The ratio of cell viability (%) was calculated by using the following formula: [(experimental absorbance−background absorbance)/(control absorbance−background absorbance)]×100.

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