A New Lignan Glycoside and Phenylethanoid Glycosides from *Strobilanthes cusia* Bremek

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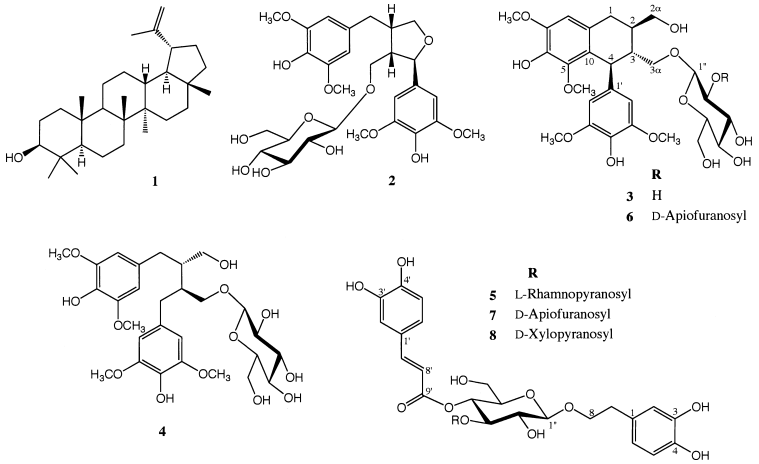
The root of *Strobilanthes cusia* Bremek. (Acanthaceae), popularly known as Da-Ching-Yeh, has been commonly used in traditional Chinese medicine. It is used to treat influenza, epidemic cerebrospinal meningitis, encephalitis B, viral pneumonia, mumps, and severe acute respiratory syndrome (SARS). In this study, we found a new lignan glycoside (6) and two new phenylethanoid glycosides (7, 8) together with five known compounds as chemical constituents of *Strobilanthes cusia* root. Some samples were examined for anti-herpes simplex virus type-1 (HSV-1) activity. Among the tested samples, lupeol showed anti-HSV-1 activity (EC_{50}: 11.7 μM) and showed 100% inhibition of virus plaque formation at 58.7 μM.

Key words *Strobilanthes cusia*; Acanthaceae; lignan; phenylethanoid; anti-herpes simplex virus type-1 activity

The root of *Strobilanthes cusia* Bremek. (Acanthaceae), popularly known as Da-Ching-Yeh, has been commonly used in traditional Chinese medicine.[1)] It is used to treat influenza, epidemic cerebrospinal meningitis, encephalitis B, viral pneumonia, and mumps. Recently, this crude drug has also been used in severe acute respiratory syndrome (SARS). It is also used to treat sore throat, aphthae, inflammatory diseases with redness of the skin, *etc*. However, the chemical constituents of *Strobilanthes cusia* are not clear,[2)] and we began a study to isolate the constitutes and elucidate the structure of this crude drug. Some compounds obtained from *S. cusia* were tested for anti-herpes simplex virus type-1 (HSV-1) activity.

The MeOH extract of *S. cusia* root was chromatographed on porous-polymer polystyrene resin (Diaion HP-20), and the MeOH eluate was subjected to column chromatography on silica gel and octadecylsilanized (ODS) silica gel and on Sepadex LH-20, giving compounds **1**—**8**. Compounds **1**—**5** were identified as lupeol (**1**),[3)] (+)-5,5′-dimethoxy-9-*O*-β-D-glucopyranosyl lariciresinol (**2**),[4—6)] (+)-9-*O*-β-D-glucopyranosyl lyoniresinol (**3**),[7,8)] (+)-5,5′-dimethoxy-9-*O*-β-D-glucopyranosyl secoisolariciresinol (**4**),[9)] and acteoside (**5**),[10)] respectively. The structural identities of these compounds were established by comparing the physical and spectroscopic data with values reported in the literature.

Compound **6** was obtained as a white amorphous powder, with an $[\alpha]_D$ value of −2.5° (MeOH). The molecular formula of **6** was determined to be $C_{33}H_{46}O_{17}$ based on the NMR and high-resolution fast-atom bombardment mass spectrum (HR-FAB-MS) data ($[M+Na]^+$, *m*/*z*: 737.2632; Calcd for $C_{33}H_{46}O_{17}Na$ *m*/*z*: 737.2633). The ^{1}H-NMR spectrum of **6** in CD_3OD showed signals similar to those of **3**, except for an additional anomeric proton signal at δ 5.41 (d, J=2.0 Hz). The ^{13}C-NMR spectrum of **6** (Table 1) also showed carbon signals similar to those of **3**, except for an additional pentosyl moiety. Acid hydrolysis of **6** with 1 M HCl in dioxane–H_2O (1 : 1) afforded D-glucose and D-apiose in a ratio of 1 : 1 as carbohydrate components upon GLC analysis after conversion to the thiazolidine derivatives,[11)] while the aglycone (**6a**) was obtained by enzymatic hydrolysis and identified as (2*R*,3*R*)-(+)-lyoniresinol after comparison with an authentic sample.[7)] The NMR data could be assigned with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation spectroscopy (HMBC) experiments. The anomeric centers of the glucose moieties were determined to have the β-configuration from the large $^3J_{H1-H2}$ values. The $^4C_1$-conformation of glucose was shown by com-



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parison of the carbon resonance for monosaccharide. In the HMBC experiment, the anomeric proton signals at δ 4.33 (H-1″) and δ 5.41 (H-1‴) showed long-range correlation with the carbon signals at δ 70.5 (C-3α) and 77.6 (C-2″), respectively. From the evidence presented above, the structure of **6** was concluded to be (+)-lyoniresinol 3α-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside, as shown in the formula. This compound is a new lignan glycoside in our literature survey.

Compound **7** was obtained as a white amorphous powder, with an $[\alpha]_D$ value of −67.5° (MeOH). The molecular formula of **7** was determined to be $C_{28}H_{34}O_{15}$ based on the NMR and HR-FAB-MS data ($[M+H]^+$, *m/z*: 611.2041; Calcd for $C_{28}H_{35}O_{15}$ *m/z*: 611.1976). The ^{1}H- and ^{13}C-NMR spectra (see Table 2) are similar to those of acteoside (**5**), except for the signal due to a pentosyl group attached to 3″-*O* of the glucosyl moiety. Two hydroxymethyl groups were observed at δ 3.18 and 3.24 (each H, d, J=10.4 Hz) and 3.47 and 3.59 (each 1H, d, J=9.2 Hz) in **7** instead of the doublet methyl signals δ 0.96 (3H, d, J=6.1 Hz, rha-H$_3$-6) in **5**. Acid hydrolysis of **7** with 1 M HCl in dioxane–H_2O (1 : 1) afforded D-glucose and D-apiose in a ratio of 1 : 1 as carbohydrate components in GLC, as described above. The anomeric centers of the glucosyl moieties were determined to have the β-configuration from the large $^3J_{H1-H2}$ values (7.3 Hz). ^{1}H–^{1}H COSY, HMQC, and HMBC experiments were carried out for **7**. In the HMBC experiment, the anomeric proton signals at δ 4.35 (H-1″) and δ 5.25 (H-1‴) showed long-range correlations with the carbon signals at δ 70.1 (C-8) and 78.6 (C-3″), respectively. Additionally the H-4″ proton (δ 4.68) was correlated to C-9′ (δ 165.6) in the HMBC. From the evidence presented above, the structure of **7** was concluded to be [2-(3,4-dihydroxyphenylethyl)]-3-*O*-α-D-apiofuranosyl-(1→4)-(4-*O*-caffeoyl)-β-D-glucopyranoside, and designated cusianoside A.

Compound **8** was obtained as a white amorphous powder, with an $[\alpha]_D$ value of −41.9° (MeOH). The molecular formula of **8** was determined to be $C_{28}H_{34}O_{15}$, which was the same as that of **7**, based on the HR-FAB-MS data ($[M+H]^+$, *m/z*: 611.2041; Calcd for $C_{28}H_{35}O_{15}$ *m/z*: 611.1976). The ^{1}H- and ^{13}C-NMR spectra of **8** (see Table 2) are essentially the same as those of **7**, except for the signal due to a terminal pentosyl group attached to 3″-*O* of the glucosyl moiety. An anomeric proton was seen at δ 4.30 (1H, d, J=7.3 Hz) instead of δ 5.25 (1H, br s, apiose H-1) as in **7**. Acid hydrolysis of **8** with 1 M HCl in dioxane–H_2O (1 : 1) afforded D-glucose and D-xylose in a ratio of 1 : 1 as carbohydrate components in GLC, as described above. The anomeric centers of the glucosyl and xylosyl moieties were determined to have the β-configuration from the large $^3J_{H1-H2}$ values (7.9 and 7.3 Hz, respectively). In the HMBC experiment, the anomeric proton signals at δ 4.39 (H-1″) and δ 4.30 (H-1‴) showed long-

Table 1. ^{13}C-NMR Data for **3**, **6**, and **6a** (**3**, **6** in CD_3OD, **6a** in $CDCl_3$)

C no.	**3**	**6**	**6a**	Sugar	**3**	**6**
1	33.8	33.9	33.5	glc-1	104.8	103.7
2	40.7	40.1	40.4	2	75.2	77.6
2a	66.4	65.7	64.1	3	78.0	78.0
3	46.7	46.6	49.4	4	71.7	71.5
3a	72.2	70.5	66.8	5	78.3	78.4
4	42.7	43.0	43.0	6	62.9	62.7
5	147.6	148.2	145.6			
6	139.0	138.5	137.1	api-1		111.1
7	148.7	147.2	146.3	2		79.6
8	108.0	107.5	106.0	3		80.3
9	130.2	129.9	128.7	4		75.0
10	126.4	126.2	125.3	5		65.9
1′	139.3	139.2	138.2			
2′	107.1	106.7	105.6			
3′	149.0	148.6	146.9			
4′	134.7	134.1	133.1			
5′	149.0	148.6	146.9			
6′	107.1	106.7	105.6			

Table 2. ^{13}C-NMR Data for **5**, **7**, and **8** (in DMSO-d_6)

C no.	**5**	**7**	**8**	Sugar	**5**	**7**	**8**
1	129.0	129.1	129.3	glc-1	102.2	102.4	102.2
2	116.2	116.2	116.3	2	74.4	74.0	73.2
3	144.9	144.9	144.9	3	79.0	78.6	84.2
4	143.4	143.4	143.5	4	69.0	69.2	69.4
5	115.7	115.7	115.8	5	74.4	74.5	74.5
6	119.4	119.4	119.5	6	60.6	60.7	60.8
7	34.9	35.0	35.0				
8	70.2	70.1	70.1	rha-1	101.1		
1′	125.4	125.5	125.7	2	70.4		
2′	114.6	114.7	114.6	3	70.3		
3′	145.5	145.5	144.8	4	71.6		
4′	148.4	148.4	143.5	5	68.6		
5′	115.4	115.4	115.5	6	18.0		
6′	121.3	121.2	121.2				
7′	145.5	145.2	145.6	api-1		109.5	
8′	113.5	113.9	114.2	2		76.1	
9′	165.6	165.6	165.6	3		78.9	
				4		73.6	
				5		63.8	
				xyl-1			105.7
				2			74.2
				3			76.9
				4			69.4
				5			65.9

Table 3. Anti-herpes Activities

Compound	Concentration (μg/ml)						EC_{50} (μM)	IC_{50} (μM)	Selective index (IC_{50}/EC_{50})
	100	50	25	12.5	6.25	3.13			
1	Toxic	Toxic	100	83	53	30	11.7	49.3	4.20
2	1	0	23	11	5	14	>172	N.T.[a)]	—
3	0	15	13	14	14	10	>172	N.T.[a)]	—
6	0	0	12	10	8	24	>141	N.T.[a)]	—
Acyclovir							1.72[b)]	15860[b)]	9220[b)]

a) Not tested. *b*) Ref. 13.

range correlations with the carbon signals at δ 70.1 (C-8) and 84.2 (C-3″), respectively. In addition, the H-4″ proton (δ 4.71) was correlated to C-9′ (δ 165.6) in the HMBC. From the evidence presented above, the structure of **8** was concluded to be [2-(3,4-dihydroxyphenylethyl)]-3-*O*-β-D-xylopyranosyl-(1→3)-(4-*O*-caffeoyl)-β-D-glucopyranoside, and designated cusianoside B.

It was noteworthy that the ^{13}C-NMR data of **6**, **7**, and **8** were different depending on the terminal saccharide.

Compounds **1**, **2**, **3**, and **6** were examined for anti-HSV-1 activity.[12)] Among the tested samples, lupeol (**1**) was exhibited anti-HSV-1 activity (EC_{50}: 11.7 μM) and showed 100% inhibition of virus plaque formation at 58.7 μM (Table 3). Although lupeol was less active than acyclovir and had a low selective index (IC_{50}/EC_{50}),[13)] lupeol was the major constituent (yield: 0.048%) and might be the active principle in this crude drug. We plan to synthesize lupeol derivatives and to test their anti-viral and/or antiinflammatory activity in the near future.

Experimental

The optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter. The NMR spectra were recorded at 500 MHz for ^{1}H- and 125 MHz for ^{13}C-NMR spectra on a JEOL α-500 spectrometer, and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as an internal standard. Standard pulse sequences were employed for the DEPT, HMQC, and HMBC experiments. NOESY spectra were measured with mixing times of 600 ms. The FAB-MS were measured with a JEOL DX-300 and/or SX102A spectrometer. The HR-FAB-MS were measured with a JEOL DX-303 HF spectrometer in a glycerol, triethylene glycol, and *m*-nitrobenzyl alcohol matrix. GLC was performed on an HP5890A gas chromatograph with a flame ionization detector. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck). Column chromatography was carried out on Kieselgel 60 (70—230 mesh and 230—400 mesh), Diaion HP-20 (Mitsubishi Chemical Industries), Sephadex LH-20 (Pharmacia), and Chromatorex ODS-DU 3050MT (Fuji Silysia). β-Glucosidase from almonds was purchased from Sigma Chemical. Fetal calf serum (FCS) was purchased from Gibco BRL. Sulfonated γ-globulin (Venilon) was supplied by the Chemo-Sero Therapeutic Institute.

Extraction and Isolation The root of *S. cusia* (2.0 kg) was extracted with MeOH, and the methanol extract (92.7 g) was subjected to Diaion HP-20 column chromatography (eluted with H_2O, MeOH, and acetone) to give three fractions. The acetone eluate (2.23 g) was purified by silica gel chromatography to give compound **1** (960 mg). The MeOH eluate (20.4 g) was further purified by Sephadex LH-20 (MeOH), chromatorex ODS (20% MeOH→50% MeOH), silica gel [$CHCl_3$–MeOH–H_2O=9 : 1 : 0.1→8 : 2 : 0.1] column chromatography to give compounds **2** (37 mg), **3** (559 mg), **4** (30 mg), **5** (42 mg), **6** (13 mg), **7** (66 mg), and **8** (27 mg), respectively.

Compound **6**: Amorphous powder, $[\alpha]_D^{25}$ −2.5° (*c*=0.12, MeOH), positive FAB-MS (*m/z*): 715 $[M+H]^+$, HR-FAB-MS (*m/z*): 737.2632 $[M+Na]^+$ (Calcd for $C_{33}H_{46}O_{17}Na$, 737.2633). ^{1}H-NMR (in CD_3OD) δ: 1.72 (1H, m, H-2), 2.04 (1H, m, H-3), 2.62 (1H, dd, *J*=11.7, 14.6 Hz, H*a*-1), 2.72 (1H, dd, *J*=4.4, 14.6 Hz, H*b*-1), 3.23 (1H, dd, *J*=7.9, 9.2 Hz, glc H-2), 3.30 (3H, s, 5-OCH_3), 3.31 (1H, overlapped, glc H-4), 3.38 (2H, overlapped, glc H-3, H*a*-3α), 3.47 (1H, d, *J*=9.8 Hz, api H*a*-4), 3.56 (1H, m, H*a*-2α), 3.59—3.62 (4H, overlapped, glc H-5, glc H*a*-6, api H*b*-4, H*b*-2α), 3.63 (1H, d, *J*=9.8 Hz, api H*a*-5), 3.75 (6H, s, 3′,5′-OCH_3), 3.79—3.81 (2H, overlapped, H*b*-3α, glc H*b*-6), 3.85 (3H, s, 7-OCH_3), 3.99 (1H, d, *J*=2.0 Hz, api H-2), 4.02 (1H, d, *J*=9.8 Hz, api H*b*-5), 4.33 (1H, d, *J*=7.9 Hz, glc H-1), 4.42 (1H, d, *J*=6.3 Hz, H-4), 5.41 (1H, d, *J*=2.0 Hz, api H-1), 6.42 (2H, s, H-2′,6′), 6.57 (1H, s, H-8). ^{13}C-NMR (in CD_3OD) δ: Table 1.

Acid Hydrolysis of 6, 7, and 8 Each sample (1 mg) was hydrolyzed with 2 mol/l of HCl in H_2O for 4 h at 80 °C. The reaction mixture was neutralized with 2 mol/l of NaOH in H_2O and extracted with $CHCl_3$. The aqueous layer was concentrated to dryness *in vacuo* to give a residue that was dissolved in dry pyridine, to which was added L-cysteine methyl ester hydrochloride.[11)] The reaction mixture was heated for 2 h at 60 °C and concentrated to dryness by blowing with N_2 gas. To the residue was added trimethylsilylimidazole, followed by heating for 1 h at 60 °C. The residue was extracted with hexane and H_2O, and the organic layer was analyzed using GLC: column, OV-17 (0.32 mm×30 m); detector, FID; column temperature, 230 °C; detector temp., 270 °C; injector temp., 270 °C; carrier gas, He (2.0 kg/cm^2). Peaks were observed at t_R (min) for **6** at 12.4 min (D-apiose) and 15.5 min (D-glucose), for **7** at 12.5 min (D-apiose) and 15.4 min (D-glucose), and for **8** at 7.3 min (D-xylose) and 15.6 min (D-glucose). The standard monosaccharides were subjected to the same reaction and GLC analysis under the same conditions.

Enzymatic Hydrolysis of Compound 6 A mixture of **6** (10 mg) and β-glucosidase (10 mg; Sigma Chemical, EC 3.2.1.21 from almonds) in acetate buffer (1.0 ml, 100 mM, pH 4.0) was incubated at 37 °C for 3 d. The mixture was concentrated *in vacuo* to dryness, and the residue was chromatographed over Chromatorex ODS (30% MeOH) to afford a **6a** (3.2 mg).

Compound **6a** [(2*R*,3*R*)-(+)-lyoniresinol]: Amorphous powder, $[\alpha]_D^{25}$ +67.2° (*c*=0.05, MeOH), EI-MS (*m/z*): 420 $[M]^+$, ^{1}H-NMR (in $CDCl_3$) δ: 1.76 (1H, m, H-2), 1.92 (1H, m, H-3), 2.60 (1H, dd, *J*=4.9, 11.0 Hz, H*a*-1), 2.66 (1H, dd, *J*=11.0, 15.3 Hz, H*b*-1), 3.29 (3H, s, 5-OCH_3), 3.57 (1H, dd, *J*=6.1, 11.0 Hz, H*a*-2α), 3.63 (1H, dd, *J*=6.7, 11.0 Hz, H*a*-3α), 3.75 (1H, dd, *J*=4.3, 11.0 Hz, H*b*-3α), 3.79 (6H, s, 3′,5′-OCH_3), 3.81 (1H, dd, *J*=4.9, 11.0 Hz, H*b*-2α), 3.88 (3H, s, 7-OCH_3), 4.01 (1H, d, *J*=7.3 Hz, H-4), 5.40 (2H, br s, 2×OH), 6.34 (2H, s, H-2′,6′), 6.44 (1H, s, H-8). ^{13}C-NMR (in $CDCl_3$) δ: Table 1.

Compound **7**: Amorphous powder, $[\alpha]_D^{25}$ −67.5° (*c*=0.11, MeOH), positive FAB-MS (*m/z*): 611 $[M+H]^+$. HR-FAB-MS (*m/z*): 611.2041 $[M+H]^+$ (Calcd for $C_{28}H_{35}O_{15}$, 611.1976). ^{1}H-NMR (in DMSO-d_6) δ: 2.71 (2H, m, H_2-7), 3.18 (1H, d, *J*=10.4 Hz, api H*a*-4), 3.22 (1H, dd, *J*=7.3, 9.8 Hz, glc H-2), 3.24 (1H, d, *J*=10.4 Hz, api H*b*-4), 3.43—3.47 (3H, overlapped, glc H*a*-6, glc H*b*-6, glc H-5), 3.47 (1H, d, *J*=9.2 Hz, api H*a*-5), 3.59 (1H, d, *J*=9.2 Hz, api H*b*-5), 3.63 (1H, m, H*a*-8), 3.67 (1H, dd, *J*=9.2, 9.8 Hz, glc H-3), 3.71 (1H, br s, api H-2), 3.88 (1H, m, H*b*-8), 4.35 (1H, d, *J*=7.3 Hz, glc H-1), 4.68 (1H, dd, *J*=9.8, 9.8 Hz, glc H-4), 5.25 (1H, br s, api H-1), 6.21 (1H, d, *J*=15.9 Hz, H-7′), 6.50 (1H, d, *J*=7.9 Hz, H-6), 6.62 (1H, br s, H-2), 6.63 (1H, d, *J*=7.9 Hz, H-5′), 6.77 (1H, d, *J*=7.9 Hz, H-5), 6.99 (1H, d, *J*=7.9 Hz, H-6′), 7.05 (1H, br s, H-2′), 7.47 (1H, d, *J*=15.9 Hz, H-8′). ^{13}C-NMR (in DMSO-d_6) δ: Table 2.

Compound **8**: Amorphous powder, $[\alpha]_D^{25}$ −41.9° (*c*=0.09, MeOH), positive FAB-MS (*m/z*): 633 $[M+Na]^+$. HR-FAB-MS (*m/z*): 611.2041 $[M+H]^+$ (Calcd for $C_{28}H_{35}O_{15}$, 611.1976). ^{1}H-NMR (in DMSO-d_6) δ: 2.70 (2H, m, H_2-7), 2.92—2.96 (2H, overlapped, xyl H-2, xyl H*a*-5), 3.08 (1H, dd, *J*=8.6, 9.2 Hz, xyl H-3), 3.16 (1H, m, xyl H-4), 3.34 (1H, dd, *J*=7.9, 9.2 Hz, glc H-2), 3.42—3.50 (4H, overlapped, xyl H*b*-5, glc H*a*-6, glc H*b*-6, glc H-5), 3.62 (1H, m, H*a*-8), 3.72 (1H, dd, *J*=9.2, 9.2 Hz, glc H-3), 3.89 (1H, m, H*b*-8), 4.30 (1H, d, *J*=7.3 Hz, xyl H-1), 4.40 (1H, d, *J*=7.9 Hz, glc H-1), 4.71 (1H, dd, *J*=9.2, 9.8 Hz glc H-4), 6.22 (1H, d, *J*=15.9 Hz, H-7′), 6.50 (1H, d, *J*=7.9 Hz, H-6), 6.63 (1H, br s, H-2), 6.64 (1H, d, *J*=7.9 Hz, H-5′), 6.77

(1H, d, J=7.9 Hz, H-5), 6.99 (1H, d, J=7.9 Hz, H-6′), 7.05 (1H, br s, H-2′), 7.45 (1H, d, J=15.9 Hz, H-8′). ^{13}C-NMR (in DMSO-d_6) δ: Table 2.

Cell and Virus HSV-1 strain KOS and Vero cells were provided by the Chemo-Sero Therapeutic Institute.

Antiviral Assays The antiviral activity of test samples against HSV-1 (KOS) was determined using the plaque reduction assay.[12] Confluent monolayers of Vero cells in 6-well plates were infected with HSV-1 at 100 plaque-forming units per cell. After a 1 h adsorption period, the cultures were overlaid with Dulbecco's modified Eagle minimum essential medium (DMEM) containing 2% heat-inactivated FCS and 2% sulfonated γ-globulin including various concentrations of the test samples. The plates were incubated in the CO_2 incubator for 3 d, then fixed with formalin, and stained with crystal violet in methanol. Infectious HSV-1 production was quantified by observing the virus-induced cytopathic effect.

Cytotoxic Assays The anticellular activity was examined as described below. Confluent monolayers of Vero cells were seeded in 96-well plates at 5×10^4 cells per well. After 1 d, the cells were refed with DMEM containing 5% FCS and various concentrations of the test samples. After 3 d of incubation, cells were fixed with formalin and stained with crystal violet in methanol. Excess dye was washed off, and the dye incorporated by the viable cells was eluted with dimethyl sulfoxide. The optical densities were read at 560 nm.

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