CYTOTOXIC CONSTITUENTS FROM *Viscum coloratum*

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The mistletoe is a perennial evergreen semiparasitic dwarf shrub, which usually parasitizes on more than 50 species of host plants from 29 families like Moraceae, Theaceae, Rutaceae, Rosaceae, etc. [1]. There are 11 species and one variety distributed in most provinces of China, but only *Viscum coloratum* (Kom.) Nakai is collected in China Pharmacopoeia [2]. As herbal drugs for complementary or alternative medicine, the mistletoe and its extracts were reported to have hypotensive, diuretic, and cardiac effects, as well as antitumor activities [3]. In order to find the bioactive constituents from mistletoe, we systematically investigated the extracts of Chinese mistletoe *Viscum coloratum* (Kom.) Nakai, and here we report the isolation of nine compounds along with their antitumor activities.

The air-dried stems and twigs of *Viscum coloratum* (Komar.) Nakai (10 kg), purchased from Chengde Medicinal Company of China National Group Corporation of Traditional & Herbal Medicine in July 2005, were extracted with 75% EtOH three times by refluxing. The EtOH extract was diluted in 1% HCl to obtain an acid solution and insoluble fraction. The acid solution alkalified with ammonia water was extracted with $CHCl_3$ to yield 45 g of residue. The insoluble fraction was suspended in a large amount of water, and then extracted with petroleum ether to yield 160 g of residue. The $CHCl_3$ extract (45 g) was subjected to silica gel column chromatography and eluted with EtOAc–MeOH–ammonia water in a gradient manner to give five fractions. Fraction 1 was further chromatographed on a silica gel column and eluted with PE–Me_2CO to afford compounds **2** (15 mg) and **4** (20 mg). Fraction 2 was further subjected to silica gel column chromatography and elution with PE–Me_2CO to give compounds **1** (10 mg) and **3** (16 mg). A portion of the petroleum ether extract (120 g) was subjected to silica gel column chromatography and eluted with PE–Me_2CO (100:0–0:100) to obtain 11 fractions. Fractions 2 and 3 were further chromatographed on a silica gel column and eluted with a gradient of PE–EtOAc to give compounds **5–6** and **7–9**, respectively.

The compounds were identified as loliolide (**1**) [4, 5], (+)-epipinoresinol (**2**) [6, 7], (+)-syringaresinol (**3**) [8, 9], 7,3′,4′-trimthylquercetin (**4**) [10], 3-*epi*-betulinic acid (**5**) [11, 12], betulonic acid (**6**) [13, 14], mixture of β-amyrin and lupeol (**7**) [15], pentacosanol (**8**) [16], and β-sitosterol (**9**) [17] by NMR and MS spectra. To the best of our knowledge, compounds **1–5** and **8** were obtained from genus *Viscum* for the first time, and compound **6** was obtained from *Viscum coloratum* (Kom.) Nakai for the first time.

Loliolide (1). Colorless needles, $C_{11}H_{16}O_3$, mp 156–157°C, positive ESI-MS *m/z* 219 $[M+Na]^+$; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.25 (3H, s, 9-CH_3), 1.45 (3H, s, 10-CH_3), 1.50 (1H, dd, J = 15.0, 3.6, H-2), 1.76 (3H, s, 11-CH_3), 1.78 (1H, dd, J = 13.2, 3.9, H-4), 1.94 (1H, dt, J = 14.7, 3.0, H-2), 2.43 (1H, dt, J = 14.1, 3.0, H-4), 4.31 (1H, quintet, J = 3.6, H-3), 5.67 (1H, s, H-7); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 35.9 (C-1), 47.3 (C-2), 66.8 (C-3), 45.6 (C-4), 86.7 (C-5), 182.4 (C-6), 112.9 (C-7), 171.9 (C-8), 30.6 (9-CH_3), 26.5 (10-CH_3), 27.0 (11-CH_3) [4, 5].

(+)-Epipinoresinol (2). Colorless plates, $C_{20}H_{22}O_6$, mp 116–118°C, positive ESI-MS *m/z* 381 $[M+Na]^+$, negative ESI-MS *m/z* 357 $[M-H]^-$; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.30 (2H, m, H-1, 5), 3.74–4.12 (4H, m, H-4, 8), 3.88, 3.90 (each 3H, s, 3′,3″-OCH_3), 4.39 (1H, d, J = 7.8, H-6), 4.83 (1H, d, J = 5.1, H-2), 5.55 (1H × 2, br.s, 4′,4″-OH), 6.73–6.94 (6H, m, arom. H); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 54.5 (C-1), 87.7 (C-2), 69.7 (C-4), 50.1 (C-5), 82.1 (C-6), 70.9 (C-8), 133.0 (C-1′), 130.3 (C-1″), 108.5 (C-2′), 108.3 (C-2″), 146.7 (C-3′), 146.4 (C-3″), 145.3 (C-4′), 144.5 (C-4″), 114.2 (C-5′,5″), 119.2 (C-6′), 118.4 (C-6″) [6, 7].

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TABLE 1. Cytotoxicity of Compounds **1–6** against Five Human Tumor Cell Lines (ED_{50}, μM)[a]

Compound	Cell lines[b]				
	HO-8910	SMMC7721	T24	HepG2	SHG
1	100	100	100	100	100
2	61.27	64.52	52.08	44.72	100
3	100	–	100	76.69	100
4	100	–	100	54.00	100
5	50.00	24.20	100	42.70	100
6	30.20	–	54.76	38.34	49.31
5-Fluorouracil	62.74	100	47.40	100	50.60

[a]ED_{50} was defined as the concentration (μM) that caused 50% inhibition of cell growth *in vitro*.
[b]Cell lines: HO-8910, human ovary serous adenocarcinoma; SMMC7721, human hepatoma; T24, human bladder cancer; HepG2, human hepatoma; SHG, human glioma.

(+)-Syringaresinol (3). Colorless needles, $C_{22}H_{26}O_8$, mp 172–173°C, positive ESI-MS *m/z* 441 $[M+Na]^+$; ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.16 (1H × 2, t, J = 5.7, H-1,5), 3.54 (1H × 2, dd, J = 6.9, 9.3, H-4,8), 3.70 (1H × 2, dd, J = 1.8, 9.3, H-4,8), 3.89 (6H × 2, s, 3′,5′,3″,5″-OCH_3), 4.88 (1H × 2, d, J = 4.8, H-2,6), 6.59 (2H × 2, s, H-2′,6′,2″,6″); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 49.5 (C-1,5), 84.1 (C-2,6), 68.8 (C-4,8), 130.0 (C-1′,1″), 102.9 (C-2′,6′,2″,6″), 146.9 (C-3′,5′,3″,5″), 133.7 (C-4′,4″), 56.3 (3′,5′,3″,5″-OCH_3) [8, 9].

7,3′,4′-Trimethylquercetin (4). Yellow needles, $C_{18}H_{16}O_7$, mp 173–175°C, positive ESI-MS *m/z* 345 $[M+H]^+$, negative ESI-MS *m/z* 343 $[M-H]^-$; ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 12.6 (1H, br.s, 5-OH), 7.68 (1H, d, J = 1.8, H-2′), 7.65 (1H, dd, J = 1.8, 8.4, H-6′), 7.01 (1H, d, J = 8.4, H-5′), 6.42 (1H, d, J = 2.1, H-8), 6.34 (1H, d, J = 2.1, H-6), 5.98 (1H, br.s, 3-OH), 3.96 (3H, s, 3′-OCH_3), 3.86 (3H, s, 4′-OCH_3), 3.84 (3H, s, 7-OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 155.9 (C-2), 138.8 (C-3), 178.7 (C-4), 162.0 (C-5), 97.8 (C-6), 165.4 (C-7), 92.2 (C-8), 156.7 (C-9), 106.0 (C-10), 122.7 (C-1′), 122.4 (C-6′), 146.3 (C-3′), 148.3 (C-4′), 110.8 (C-2′), 114.5 (C-5′), 60.2 (7-OCH_3), 56.1 (4′-OCH_3), 55.8 (3′-OCH_3) [10].

3-*epi*-Betulinic Acid (5). White amorphous powder, $C_{30}H_{48}O_3$, mp 280–282°C, negative ESI-MS *m/z* 455 $[M-H]^-$; ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 0.80 (3H, s, H-23), 0.81 (3H, s, H-25), 0.91 (3H, s, H-24, 26), 0.97 (3H, s, H-27), 1.67 (3H, s, H-30), 2.99 (1H, m, H-19), 3.37 (1H, br.s, H-3), 4.59, 4.72 (each 1H, s, H-29); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 33.2 (C-1), 25.4 (C-2), 76.3 (C-3), 37.0 (C-4), 49.0 (C-5), 18.2 (C-6), 34.1 (C-7), 40.8 (C-8), 50.2 (C-9), 37.3 (C-10), 20.7 (C-11), 25.4 (C-12), 38.3 (C-13), 42.5 (C-14), 30.5 (C-15), 32.1 (C-16), 56.4 (C-17), 49.2 (C-18), 46.9 (C-19), 150.4 (C-20), 29.6 (C-21), 37.5 (C-22), 28.3 (C-23), 22.1 (C-24), 16.0 (C-25), 15.9 (C-26), 14.8 (C-27), 181.3 (C-28), 109.7 (C-29), 19.3 (C-30) [11, 12].

Betulonic Acid (6). White powder, $C_{30}H_{46}O_3$, mp 244–246°C, positive ESI-MS *m/z* 455 $[M+H]^+$; ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 0.86 (3H, s, H-23), 0.90 (3H, s, H-24), 0.92 (3H, s, H-25), 0.95 (3H, s, H-26), 1.00 (3H, s, H-27), 1.63(3H, s, H-30), 2.95 (1H, m, H-19), 4.55, 4.67 (each 1H, br.s, H-29); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 39.6 (C-1), 34.1 (C-2), 218.3 (C-3), 47.3 (C-4), 54.9 (C-5), 19.6 (C-6), 33.6 (C-7), 40.1 (C-8), 49.8 (C-9), 36.9 (C-10), 21.3 (C-11), 25.4 (C-12), 38.5 (C-13), 42.5 (C-14), 30.5 (C-15), 32.1 (C-16), 56.3 (C-17), 49.1 (C-18), 46.9 (C-19), 150.3 (C-20), 29.7 (C-21), 37.0 (C-22), 26.6 (C-23), 21.0 (C-24), 15.9 (C-25), 15.8 (C-26), 14.6 (C-27), 181.8 (C-28), 109.8 (C-29), 19.3 (C-30) [13, 14].

The cytotoxicities of compounds **1–6** were evaluated by the MTT method *in vitro* against five human tumor cell lines (Table 1). Compounds **2**, **5**, and **6** exhibited significant inhibitory activities against HO-8910 and selective cytotoxicities against four other human cancer cell lines. Compounds **1**, **3**, and **4** showed few activities against the five human cancer cell lines based on their higher or undetected ED_{50} values.

Previous literature data reported that betulonic acid showed high cytotoxic activity against several cancer cell lines [18, 19], and the cytotoxicity might be due to its inhibitory effect on DNA topoisomerases II [20]. In order to evaluate the quantification of potential antitumor activity of compound **6**, the cell apoptotic effect was assessed using human ovary serous adenocarcinoma HO-8910 cell line by Annexin V-EGFP/PI staining, and the results indicated that compound **6** induced moderate apoptosis in HO-8910 cells.

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