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Highly oxygenous trichilin-type limonoids from *Trichilia sinensis*

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[ABSTRACT] Six new trichilin-type limonoids (**1**−**6**) with C-19/29 lactol or acetal bridge and a new ring intact limonoid (**7**) were isolated from the desiccative ripe fruits of *Trichilia sinensis*. Their structures were determined by extensive spectroscopic methods including ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, ROESY experiments as well as HRESI-MS data. All isolated compounds were evaluated for toxicities against human pulmonary carcinoma A549 and Hela cell lines by sulforhodamine B (SRB) method. Compound **7** showed weak inhibitory activity in Hela cell line at 40 μmol·L^{-1}.

[KEY WORDS] Trichilin-type limonoids; *Trichilia sinensis*; Cytotoxic activity

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Introduction

Trichilia connaroides is used as a traditional Chinese plant medicine for local people against arthritis, pharyngitis, tonsillitis, and other ailments [1-2]. Its distribution is in the provinces of south of China: Yunnan, Guangxi, Hainan *et al*.. System chemical research showed a series of novel skeleton and/or bioactive limonoids were discovered and purified from *Trichilia* genus in past decade [1-6]. The desiccative ripe fruits of *Trichilia sinensis* were selected in our continue research for novel carbon skeleton limonoids [4, 7-9]. Eleven novel limonoids, trichisins A−K, with reverse multidrug resistance in MCF-7/DOX cells were isolated and reported in our previous research [4]. In this study, six characteristic trichilin-type limonoids with C-19/29 lactol bridge and a new ring intact limonoid (**7**) were explored and isolated from the residue extract (Fig. 1). Their structures were determined by extensive spectroscopic methods including ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, ROESY experiments as well as HRESI-MS data. All compounds were evaluated for toxicities against human pulmonary carcinoma A549 and Hela cell lines by SRB method [10]. Compound **7** showed weak cytotoxicity with 55% inhibitory rate in Hela cell line at concentration 40 μmol·L^{-1}. Herein, we report the isolation, structural elucidation, and cytotoxic activity evaluation of compounds **1**−**7**.

Results and Discussion

Trichisin L (**1**), a white amorphous powder, was obtained as a mixture of C-29 epimers, with the ratio of epimers being about 5 : 2. Its negative HRESI-MS gave a presudomolecular ion at *m/z* 613.2257, which suggested a molecular formula of $C_{30}H_{38}O_{12}$, indicating 12 degrees of unsaturation. The ^{1}H NMR (Table 1) data suggested presence of three methyl singlets at δ_H 0.75, 0.85 and 1.19, two acetyl methyl groups 1.91, and 2.03, a *β*-furyl ring hydrogen at δ_H 6.41 (1H, s, H-22), 7.34 (1H, s, H-23), and 7.50 (1H, s, H-21). Its ^{13}C NMR data (Table 2) showed 30 carbon resonances, which were further classified by analysis of its HSQC spectrum. Downfield carbon resonances in the ^{13}C NMR spectrum were assigned as three ketone carbonyls [δ_C 209.5 (C-11), 170.1 (2-AcO) and 169.4 (3-AcO)], a typical *β*-substituted furan δ_C 125.9 (C-20), 139.8 (C-21), 112.5 (C-22), and 142.1 (C-23)],

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These authors have no conflict of interest to declare.

Dedicated to Professor SUN Han-Dong on the Occasion of His 80th Birthday

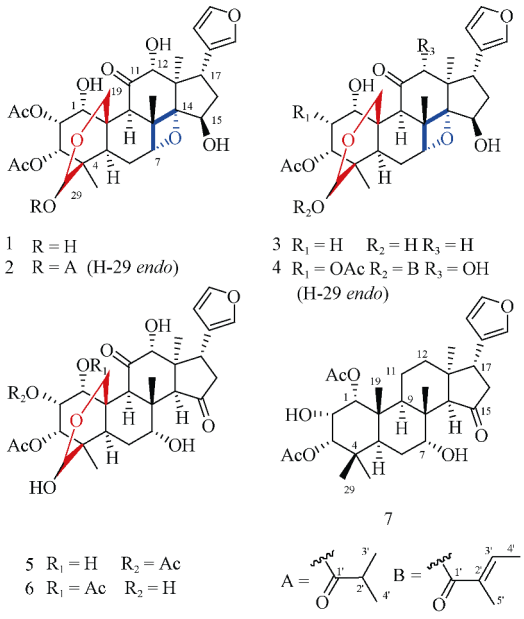

Fig. 1 Structures of the new isolated limonoids 1–7

accounting for six sites of unsaturation. The presence of ^{1}H NMR and ^{13}C NMR evidences for an additional hemiketal [δ_H 4.67 (1H, d, J = 4.5 H-29) and δ_C 94.7 (C-29)] implied that **1** possessed a hexatomic ring skeleton, which allowed the assignment of a trichilin-type limonoid skeleton. Detailed analysis of the typical oxygenated carbons [δ_C 94.7 (C-29)] and 2D NMR correlations indicated that the structure of **1** was similar to that of the known compounds 12a-hydroxymeliatoosenin I and trichisinlin E [6], and was consisted by the ring intact limonoid containing C-19/29 lactol bridge, which were derived from the HMBC correlations from H-29 to C-4 (δ_C 41.5), from H-19 to C-10 (δ_C 39.5), and from H-29 to C-19 (δ_C 57.5) (Fig. 2). A rare C-7/8/14 oxetane ring moiety appeared in the structure of **1**, which was accurately recognized by its special carbon resonance (δ_C 96.2, C-14) and assigned by the key HMBC correlations from H_3-30 to C-7, C-14 and from H-15 to C-14 [6, 11]. The key HMBC correlations from H-2 (δ_H 5.58) and H-3 (δ_H 4.85) to carbonyl carbons (δ_C 169.4 and 170.1) suggested that two acetoxyl groups were located at C-2 (δ_C 69.1) and C-3 (δ_C 74.7), respectively.

Table 1 ^{1}H NMR (500 MHz) data of compounds 1–7

Position	**1** [a]	**2** [c]	**3** [b]	**4** [b]	**5** [b]	**6** [c]	**7** [c]
	δ_H, milt	δ_H, milt	δ_H, milt	δ_H, milt	δ_H, milt	δ_H, milt	δ_H, milt
1	4.59 t (5.0)	4.95 brd s	4.51 m	5.01 brd s	4.73 d (5.0)	5.71 d (5.0)	4.72 t (3.0)
2	5.58 t (5.0)	5.90 t (5.0)	2.74 dt (16.0, 5.0) 1.91 m	5.97 t (5.0)	5.82 t (5.0)	4.77 t (5.0)	4.12 t (3.0)
3	4.85 d (5.0)	5.54 d (5.0)	5.22 d (5.0)	5.65 d (5.0)	5.48 d (5.0)	5.51 d (5.0)	4.93 t (3.0)
5	2.43 m	2.79 dd (13.0, 5.5)	2.67 m	2.85 dd (13.0, 5.5)	2.80 dd (14.0, 4.0)	2.85 dd (13.5, 3.5)	2.25 m
6	a 2.41 m b 1.63 m	a 1.97 m b 1.79 ddd (17.0, 13.0, 4.0)	1.83 m	a 2.04 m b 1.83 m	a 2.10 m b 1.70 dt (14.0, 4.0)	a 2.01 m b 1.71 dt (14.0, 4.0)	a 2.31 m b 1.67 m
7	4.81 brd s	4.99 brd s	5.02 brd s	50.5 brd s	4.09 brd s	3.98 t (3.0)	3.75 t (3.0)
9	4.44 s	4.59 s	4.49 m	4.64 s	3.75 s	3.49 s	2.00 m
11							a 1.98 m b 1.72 m
12	3.65 d (5.5)	3.92 s	a 2.46 d (19.0) b 2.30 d (19.0)	3.97 s	4.27 s	3.96 s	a 2.00 m b 1.11 dd (15.0 4.0)
14					3.42 s	3.38 s	2.89 s
15	4.66 d (3.5)	4.95 s	4.48 m	5.01 brd s			
16	a 1.83 dt (9.0, 4.5) b 1.75 td (11.5, 3.5)	1.99 m	1.92 m	a 2.04 m b 1.83 m	a 2.73 dd (17.0, 9.0) b 2.51 d (17.0, 6.0, 2.0)	a 2.66 dd (18.0, 8.5) b 2.56 dd (18.0, 8.0)	2.49 d (9.5)
17	3.30 m	3.57 d (10.0, 6.5)	3.15 dd (10.0, 6.0)	3.63 d (10.0, 6.5)	3.48 dd (7.5, 7.0)	3.37 m	4.25d (10.0)
18	0.85 s	0.96 s	1.06 s	1.02 s	0.91 s	0.89 s	0.76 s
19	a 4.15 d (11.5) b 3.49 d (11.5)	a 4.08 d (12.0) b 3.96 d (12.0)	a 4.06 d (11.5) b 3.90d (11.5)	a 4.15 d (12.0) b 4.02 d (12.0)	4.18 s	a 4.42 d (12.0) b 4.28 d (12.0)	1.37 s
21	7.34 s	7.22 s	7.30 s	7.28 s	7.34 s	7.38	7.28 s
22	6.41 s	6.45 s	6.30 s	6.51 s	6.35 s	6.39	6.31 s
23	7.50 s	7.33 s	7.44 s	7.39 s	7.42 s	7.46	7.39 s
28	0.75 s	0.79 s	0.84 s	0.85 s	0.84 s	0.87 s	0.95 s

Continued

Position	**1** [a]	**2** [c]	**3** [b]	**4** [b]	**5** [b]	**6** [c]	**7** [c]
	δ_H, milt	δ_H, milt	δ_H, milt	δ_H, milt	δ_H, milt	δ_H, milt	δ_H, milt
29	4.67 d (4.5)	5.71 s	4.86 s	5.84 s	4.81 s	4.81 s	0.91 s
30	1.19 s	1.33 s	1.26 s	1.39 s	1.29 s	1.10 s	1.59 s
OAc-1						2.11 s	2.01 s
OAc-2	1.91 s	2.07 s		2.14 s	1.99 s	2.07 s	
OAc-3	2.03 s	1.98 s	2.06 s	2.07 s	2.09 s		2.01 s
1-OH	4.56 d (5.0)						
12-OH	5.52 d (5.5)	2′ 2.65 p (7.0)		3′ 7.08 m			
15-OH	4.99 d (3.5)	3′ 1.21 d (6.5)		4′ 1.89 d (7.0)			
29-OH	6.42 d (4.5)	4′ 1.19 d (6.5)		5′ 1.92 s			

[a] Recorded at 500 MHz in DMSO-d_6; [b] Recorded at 500 MHz in CD_3OD; [c] Recorded at 500 MHz in $CDCl_3$

Table 2 ^{13}C NMR (125 MHz) data of compounds 1−7

Position	**1** [a]	**2** [c]	**3** [b]	**4** [b]	**5** [b]	**6** [c]	**7** [c]
	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C
1	69.0	71.1	69.8	71.2	72.6	75.5	73.3
2	69.1	72.9	37.4	73.0	71.4	66.0	67.7
3	74.7	69.0	74.8	69.0	74.2	74.5	76.2
4	41.5	41.7	41.6	41.8	42.5	41.4	36.2
5	28.5	31.7	33.1	31.7	29.0	28.6	26.1
6	23.6	23.0	23.9	23.0	24.0	22.8	25.1
7	81.2	82.0	83.2	82.1	70.4	68.5	71.6
8	43.0	44.1	47.5	44.1	42.7	43.4	41.6
9	51.3	51.2	54.0	51.3	49.1	46.3	41.4
10	39.5	40.6	40.0	40.7	44.9	41.2	36.3
11	209.5	209.1	212.4	209.2	213.7	212.1	43.8
12	78.8	80.1	53.3	80.1	78.7	76.7	42.6
13	49.8	50.2	46.2	50.3	47.2	47.4	41.1
14	96.2	97.4	99.5	97.4	59.4	58.1	61.9
15	74.7	76.7	77.8	76.7	220.4	219.1	222.1
16	37.1	38.3	38.1	38.3	46.4	44.2	43.4
17	42.6	43.1	46.3	43.1	39.8	37.6	38.4
18	14.7	14.2	22.2	14.2	22.2	19.8	27.9
19	57.5	64.3	65.5	64.3	64.4	63.0	20.5
20	125.9	125.0	126.1	125.0	126.4	123.9	123.2
21	139.8	140.3	140.9	140.3	141.4	140.3	140.3
22	112.5	112.4	112.1	112.4	111.9	110.4	111.0
23	142.1	142.6	144.1	142.6	144.2	142.8	142.9
28	17.7	17.2	18.7	17.3	19.4	18.1	22.0
29	94.7	92.8	96.7	93.1	96.8	95.3	27.7
30	17.4	18.2	17.2	18.3	22.2	19.8	17.5
OAc-1				12.1 (C-5′)		171.7, 20.2	170.5, 21.5
OAc-2	169.4, 20.7	170.2, 21.0		170.2, 21.0	171.8, 20.9	171.5, 20.1	
OAc-3	170.1, 20.7	169.0, 20.9	172.8, 21.3	169.0, 20.9	172.8, 20.9		170.5, 21.5
1′		175.8		166.5			
2′		34.3		127.9			
3′		18.8		140.3			
4′		18.8		14.9			

[a] Recorded at 500 MHz in DMSO-d_6; [b] Recorded at 500 MHz in CD_3OD; [c] Recorded at 500 MHz in $CDCl_3$

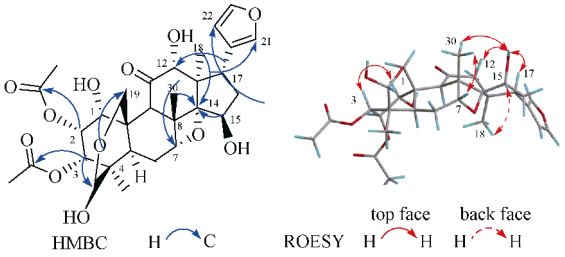

Fig. 2 Key HMBC and ROESY correlations of 1

The relative configuration of **1** was determined by characteristic vicinal coupling constant and ROESY data (Fig. 2). The obvious ROESY cross-peaks of H-17/H-30, H-30/H-7, H-30/H-12 indicated that all these protons were co-facial and located as *β*-orientation. The relative small vicinal coupling constant values (5.0 Hz) between H-1 and H-2, H-2 and H-3, revealed the *cis* relationships between these protons [6] and assigned their orientation as *β*, which were confirmed by the ROESY correlations of H1/H-3 and H-1/H-19. The ROESY cross-peaks of H-18/H-15, H-18/H-9 and H-28/H-5 indicated that these protons were arbitrarily determined in *α*-face. The cross-peaks of H-7/H-30 and H-30/15-OH determined the oxetane ring moiety to be *α*-oriented. Thus, the planar and relative structure of **1** was established as shown in Fig. 1.

Trichisin M (**2**), had the molecular formula $C_{34}H_{44}O_{13}$, as determined by HRESI-MS. The *β*-furyl ring resonances [δ_H 6.45 (1H, s, H-22), 7.33 (1H, s, H-23), and 7.22 (1H, s, H-21)], hemiketal group (δ_H 5.71, 1H, s, H-29; δ_C 92.8, C-29), and special carbon resonance (δ_C 97.4, C-14) in rare C-7/8/14 oxetane ring moiety in the ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **2** implied that it had the same trichilin-type limonoid skeleton as **1**. However, the tautomerism did not observed in compound **2** compared with **1** in methanol solution implying that hemiacetal group was replaced by hemiketal motif. The isobutyryl resonances were observed in 1D NMR in table 1, and designed its location as C-29 by the key correlations of HMBC from H-29 (δ_H 5.71 s) to its carbonyl (δ_C 175.8). The relative configuration of H-29 was determined as *endo* by the ROESY correlations of H-29/H-6 and H-29/H-19a (Fig. 3).

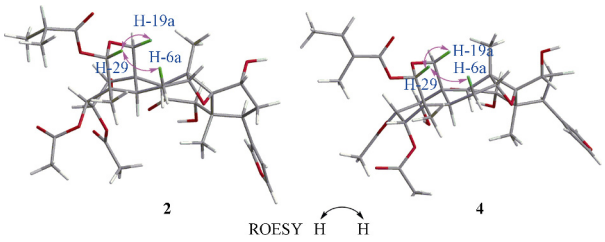

Fig. 3 Key ROESY correlations of 2 and 4

Trichisin N (**3**), a white amorphous powder, was obtained as a mixture of C-29 epimers, with the ratio of epimers being about 5 : 2, and trichisin O (**4**) had the same trichilin-type limonoid skeleton as **3**. The HRESI-MS data of **3** and **4** exhibited ions at *m/z* 517.2437 $[M + H]^+$ (Calcd. for $C_{28}H_{37}O_9$ 517.2443) and *m/z* 695.2675 $[M + Na]^+$ (Calcd. for $C_{35}H_{44}O_{13}Na$, 695.2674). NMR spectra of **3** were in general similar to those of **1**, especially in C-19/29 lactol bridge and C-17/8/14 oxetane ring moiety. Two more methenes [δ_H 2.74 dt *J* = 16.0, 5.0 Hz, 1.91 m (H-2); 2.46 d *J* = 19.0 Hz, 2.30 d *J* = 19.0 Hz (H-12)] appeared in ^{1}H NMR spectra and were classified as H-2 and H-12 based on HSQC and HMBC, respectively. The ROESY correlations of H-1/H_2-19, H-3/H-29 in **3** determined the orientations of H-1 and H-3 were *β*. Comparison of the NMR data of **4** with those of **3**, showed that they were structure congeners. The major differences between them were a tigloyl motif (tig) at C-29 and an OAc group at C-2 in **4** according to counterpart in **2**, which was supported by the HMBC correlation from H-29 to the carbonyl of tig motif and H-2 to the carbon of 2-OAc group. The small vicinal coupling constant values (5.0 Hz) of H-1/H-2 and H-2/H-3 in **4** revealed the *cis* relationships between these protons and assigned their orientation as *β*. The relative configuration of H-29 was determined as *endo* by the ROESY correlations of H-29/H-6 and H-29/H-19a in **4** (Fig. 3). The same correlation between H-7 and 30-Me in **3** and **4** determined the oxetane ring both in *β* face.

Trichisin P (**5**) and trichisin Q (**6**) were determined to be $C_{30}H_{38}O_{12}$ of the molecular formula by the HRESI-MS. The NMR spectroscopic data implided that the structure of **5** was closed related to the known compound meliatoxin B1 [6]. The differences were the absence of a 2-methylbutanoyloxy group at C-29 in **5** and a mixture of C-29 epimers with the ratio of epimers being about 5 : 2 in MeOH. Comparison of the ^{13}C NMR data of **1** with those of **5**, exhibited the absence of oxetane ring in **1** and the presence of typical C-15 carbonyl carbon (δ_C 220.4) in **5**. Thus, the structure of **5** was determined as shown in Fig. 1.The ^{1}H and ^{13}C NMR data of **5** exhibited most of the structure features in **6**, except for the different substitution patterns of functional groups. The structures of **5** and **6** were further established by detailed interpretation of its 2D NMR data. The location of two acetyl groups in **5** was assigned as C-2 (δ_C 71.4) and C-3 (δ_C 74.8) by the HMBC correlations from H-2 and H-3 to the carbons of 2-OAc and 3-OAc groups, respectively. The counterpart locations in **6** were determined as C-1 (δ_C 75.5) and C-3 (δ_C 74.5) by the HMBC correlations from H-1 and H-3 to the carbons of 1-OAc and 3-OAc groups, respectively. The relative configuration of **5** was identical to **6** demonstrated by the ROESY data and vicinal coupling constant value (5.0 Hz) between H-1/H-2 and H-2/H-3. Thus, the structures of **5** and **6** were conducted as shown in Fig. 1.

Trichisin R (**7**) was obtained as white powder, and its molecular formula was determined to be $C_{30}H_{42}O_8$ by the positive HRESI-MS data, suggesting ten indices of hydrogen deficiency. The absence of characteristic hemiketal proton and the presence of an additional singlet methyl group indicated that compound **7** was a trichilin-type limonoid precursor with

ring intact skeleton. The location of acetyls was determined as C-1 and C-3 based on the HMBC correlations from H-1 and H-3 to the responding carbonyls. The relative configuration of **7** was identical to **5** at C-1, C-2 and C-3 according to their coupling constant values. The 7-OH was determined as *α*-orientation based on the ROESY correlation between H-7 and H-30. Thus, the structure of **7** was determined as shown in Fig. 1.

All compounds were evaluated for toxicities against human pulmonary carcinoma A549 and Hela cell lines by SRB method [10]. Trichisin R (**7**) showed weak cytotoxic activity with 55% inhibitory rate in Hela cell line at concentration 40 μmol·L^{-1}. Unfortunately, the other compounds showed no cytotoxicity at concentration 40 μmol·L^{-1}.

Experimental

General experimental procedures

All solvents used were of analytical grade (Jiangsu Hanbang Science and Technology. Co., Ltd., Nanjing, China). Silica gel (200−300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), MCI (Mitsubishi, Japan) and RP-C_{18} silica (40−63 μm, FuJi, Japan) were used for column chromatography. Optical rotations were measured on a JASCO P-1020 polarimeter (Japan) at 25 °C. ^{1}H NMR, ^{13}C NMR, HSQC, HMBC and ROESY spectra were measured on a Bruker AVIII-500 NMR instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz, Germany). HRESI-MS data were obtained on an Agilent 6529B Q-TOF mass instrument (USA) using electrospray ionization. HPLC (Agilent 1260, USA) equipped with a Shim-pack RP-C_{18} column (5 μm, 5 mm × 200 mm, i.d.) and Preparative HPLC was carried out using a Shimadzu LC-8A equipped with a Shim-pack RP-C_{18} column (10 μm, 20 mm × 200 mm, i.d.) with a flow rate of 10.0 mL·min^{-1}, detected by a binary channel UV detector. Fractions obtained from CC were monitored by TLC with precoated silica gel GF_{254} (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China) plates under UV detector at 254 and 365 nm.

Plant materials

Air-dried ripe fruits of *Trichilia connaroides* (5.0 Kg) were collected in 07/2014 from Xishuangbanna Dai Autonomous Prefecture, China, and were authenticated by Professor ZHANG Shun-Cheng, Xishuangbanna Tropical Botanical Garden. A voucher specimen (No. AA201408) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and isolation

The air-dried fruits (5.0 kg) were refluxed with 95% industrial ethanol (3 × 5 L). After removal of the solvent under reduced pressure, the crude extract (500.0 g) was suspended in H_2O (1.5 L) and partitioned with petroleum ether (3 × 1 L), ethyl acetate (3 × 1 L), successively. The ethyl acetate extract (100.0 g) was subjected to silica column, eluted with CH_2Cl_2/MeOH (100 : 1, 50 : 1, 25 : 1, 10 : 1, 5 : 1) to give five fractions (A1−A5). Fraction A5 (32.0 g) was chromatographed over a MCI column, eluted with a gradient of MeOH/H_2O (50 : 50, 70 : 30) to give two fractions (A1A−A1B). The A1B was chromatographed over a silica gel column (200−300 mesh) with CH_2Cl_2/MeOH system, and then purified by semi-prepare-HPLC with with 65% methanol aqueous solution to get **1** (5 mg), **2** (11 mg), **3** (7 mg), **4** (7 mg), **5** (13 mg), **6** (4 mg) and **7** (20mg).

*Trichisin L (**1**)*

Colorless powder: $[\alpha]_D^{25}$ +43.0 (*c*, 0.1, MeOH); UV (MeOH) λ_{max} 200 nm; ^{1}H and ^{13}C NMR (DMSO-d_6), see Table 1 and Table 2; negative ESI-MS *m/z* 625.6 [M + Cl]$^-$; positive ESI-MS *m/z* 608.4 [M + NH_4]$^+$; HRESI-MS *m/z* 613.2257 [M + Na]$^+$ (Calcd. for $C_{30}H_{38}O_{12}Na$, 613.2255).

*Trichisin M (**2**)*

Colorless powder: $[\alpha]_D^{25}$ +30.0 (*c*, 0.1, MeOH); UV (MeOH) λ_{max} 200 nm; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1 and Table 2; negative ESI-MS *m/z* 695.4 [M + Cl]$^-$; positive ESI-MS *m/z* 678.2 [M + NH_4]$^+$; HRESI-MS *m/z* 683.2670 [M + Na]$^+$ (Calcd. for $C_{34}H_{44}O_{13}Na$, 683.2674).

*Trichisin N (**3**)*

Colorless powder: $[\alpha]_D^{25}$ +40.0 (*c*, 0.1, MeOH); UV (MeOH) λ_{max} 200 nm; ^{1}H and ^{13}C NMR (CD_3OD), see Table 1 and Table 2; negative ESI-MS *m/z* 551.6 [M + Cl]$^-$; positive ESI-MS *m/z* 534.1 [M + NH_4]$^+$; HRESI-MS *m/z* 517.2437 [M + H]$^+$ (Calcd. for $C_{28}H_{37}O_9$ 517.2443).

*Trichisin O (**4**)*

Colorless powder: $[\alpha]_D^{25}$ +27.0 (*c*, 0.1, MeOH); UV (MeOH) λ_{max} 200 nm; ^{1}H and ^{13}C NMR (CD_3OD), see Table 1 and Table 2; negative ESI-MS *m/z* 707.1 [M + Cl]$^-$; positive ESI-MS *m/z* 690.3 [M + NH_4]$^+$; HRESI-MS *m/z* 695.2675 [M + Na]$^+$ (Calcd. for $C_{35}H_{44}O_{13}Na$, 695.2674).

*Trichisin P (**5**)*

Colorless powder: $[\alpha]_D^{25}$ +47.0 (*c*, 0.1, MeOH); UV (MeOH) λ_{max} 200 nm; ^{1}H and ^{13}C NMR (CD_3OD), see Table 1 and Table 2; HRESI-MS *m/z* 613.2257 [M + Na]$^+$ (Calcd. for $C_{30}H_{38}O_{12}Na$, 613.2255).

*Trichisin Q (**6**)*

Colorless powder: $[\alpha]_D^{25}$ +57.0 (*c*, 0.1, MeOH); UV (MeOH) λ_{max} 200, 288 nm; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1 and Table 2; negative ESI-MS *m/z* 625.4 [M + Cl]$^-$; HRESI-MS *m/z* 613.2257 [M + Na]$^+$ (Calcd. for $C_{30}H_{38}O_{12}Na$, 613.2255).

*Trichisin R (**7**)*

Colorless powder: $[\alpha]_D^{25}$ +26.0 (*c*, 0.1, MeOH); UV (MeOH) λ_{max} 200 nm; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1 and Table 2; negative ESI-MS *m/z* 565.4 [M + Cl]$^-$; positive ESI-MS *m/z* 548.4 [M + NH_4]$^+$; HRESI-MS *m/z* 553.2776 [M + Na]$^+$ (Calcd. for $C_{30}H_{42}O_8Na$, 553.2772).

In vitro cytotoxicity

Cytotoxic activities against A549 and Hela cell lines were tested based on the SRP method [11]. The cells were plated in a 96-well plate at a density of 5 × 10^3 cells per well in 190 μL medium for 24 h. Then the selected compounds and positive control were added to a final concentration of 40

μmol·L^{-1}. After 48 h exposure, cells were fixed by the addition of 50 % ice-cold trichloroacetic acid and incubated at 4 °C for 1 h. The plate was stained for 15 min with 0.4% SRB in 1% glacial acetic acid after washed and air-dried. Excessive dye was removed by washing in 1% glacial acetic acid, SRB was resuspended in 10 nmol·L^{-1} Tris solution and the absorbance was recorded at 560 nm using a microplate reader. All experiments were carried out in triplicate and repeated twice. Taxol was used as positive control.

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