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New phenylpropanoid-conjugated pentacyclic triterpenoids from the whole plants of *Leptopus lolonum* with their antiproliferative activities on cancer cells

Shi-Zhou Qi [a,b], Ting Liu [a,b], Miao Wang [a,b], Xin-Xin Zhang [a,b], Yi-Ren Yang [a], Wen-Hua Jing [a,b], Li-Ping Long [a,b], Kai-Ru Song [a,b], Yue Jin [a,b], Hui-Yuan Gao [a,b,*]

[a] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China
[b] Key Laboratory of Structure-Based Drug Design and Discovery of Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China

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ABSTRACT

Most of Euphorbiaceae plants are considered as folk medicinal plants because of their various pharmacological effects. However, there are eight *Leptopus* genus plants which belong to Euphorbiaceae have never be investigated. Thus, four *Leptopus* genus plants were collected to study their chemical constituents and pharmacological activities. In the present work, the cytotoxicities of the extracts of four *Leptopus* genus plants were evaluated before phytochemical experiments. And nine new phenylpropanoid-conjugated pentacyclic triterpenoids, along with twenty-two known compounds were isolated from the whole plants of *Leptopus lolonum*. The structures of these new compounds were unequivocally elucidated by HRESIMS and 1D/2D NMR data. All triterpenoids were screened for their cytotoxicities against four cancer cell lines including HepG2, MCF-7, A549 and HeLa. Among these isolates, the triterpenoid with a phenylpropanoid unit showed increasing cytotoxicity on cancer cells, which suggested the importance of the phenylpropanoid moiety.

1. Introduction

The family of Euphorbiaceae includes over 300 genus and 8000 species spread around the world, most of which are considered as medicinal plants for their multiple pharmacological effects. Among these genus, the *Leptopus* genus, also known as "Que-She-Mu" or "Hei-Gou-Ye" in China, is a small perennial shrub that grows as a tree 0.5–4 m tall which distributes in tropical and subtropical Asia [1]. There are almost 20 species plants belonging to *Leptopus* genus worldwide and 9 species distribute in the southern regions of China [2]. In Chinese traditional medicine, the leaves of *Leptopus pachyphyllus* are used to treat skin ulcers and hemostasis, and the roots of *Leptopus chinensis* could be used to treat diarrhea [1,3]. Few phytochemical and pharmacological investigations on *L. chinensis* were reported describing the presence of bioactive compounds such as triterpenoids and sterols, and some of these compounds displayed potential anti-cancer activity [4]. However, the main chemical constituents and biological activities of *L. chinensis* were not fully investigated. In addition, so far, there is no any study about the chemical constituents and pharmacological activities of other *Leptopus* genus plants. All of the above indicates that understanding the medicinal efficacy and material basis of *Leptopus* genus plants is of great value for the development of medicinal plant resources. Thus, four *Leptopus* genus plants including *Leptopus lolonum*, *Leptopus chinensis*, *Leptopus clarkei*, *Leptopus yunnanensis* were collected and their cytotoxic effects were also be evaluated. Additionally, the *L. lolonum* was selected to conduct further phytochemical experiments, and nine new phenylpropanoid-conjugated pentacyclic triterpenoids, along with twenty-two known compounds were isolated. Biological assays were conducted using human hepatocellular carcinoma cells (HepG2), human breast cancer cells (MCF-7), human lung cancer cells (A549), and human cervical cancer cells (HeLa). Furthermore, the synthetic route of the phenylpropanoid-conjugated triterpenoid was also be investigated. Therefore, in this article, we describe the isolation, structural determination, synthetic route of this kind of triterpenoid, and evaluation of cytotoxicities of these compounds and the extract of four *Leptopus* genus plants.

* Corresponding author.
E-mail address: sypugaohy@163.com (H.-Y. Gao).

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2. Materials and methods

2.1. General experimental procedures

Optical rotations were determined on a AUTOPOL IV automatic polarimeter (Rudolph ResearchAnalytical, USA). IR spectra were obtained with a Bruker IFS-55 Fourier transform infrared (FT-IR) spectrometer (Bruker, German). UV spectra were measured with Shimadzu UV-2401A spectrophotometer (Shimadzu, Japan). NMR spectra were obtained by Bruker AV-600 spectrometers (Bruker, German) using tetramethyl silane as an internal standard. HRESIMS spectra were obtained using a Bruker micro-TOF-Q mass spectrometer. Open-column chromatography was performed using silica gel (200–300 mesh, Qingdao Marine Chemical Co., Ltd.). TLC was performed with precoated silica gel GF_{254} glass plates (Qingdao Marine Chemical Co., Ltd). Preparative HPLC was conducted on an Agela P1050 pump with Agela UV1000D UV spectrophotometric detector at 210 and 254 nm using a YMC Pack ODS-A column column (5 μm, 20 × 250 mm).

2.2. Plant materials

Four *Leptopus* genus plants were purchased in august 2018 from Yunan Yuancai Biotechnology Co., Ltd. (Kunming, Yunnan, China), and identified by Associate Prof. Jiu-Zhi Yuan of Shenyang Pharmaceutical University. Four voucher specimens [LL-18-08-14 (*L. lolonum*); LY-18-08-14 (*L. yunnanensis*); LCH-18-08-27 (*L. chinensis*); LCL-18–08-27 (*L. clarkei*)] were deposited in the School of Traditional Chinese Medicine, Shenyang Pharmaceutical University, Shenyang, China.

2.3. Extraction and isolation

The dried whole plants of *L. lolonum* (18.0 kg) were repeatedly extracted with 75% EtOH to give 1.96 kg crude extract. Then the crude extract was suspended in H_2O and partitioned with petroleum ether (PE), $CHCl_3$, EtOAc and *n*-BuOH to yield PE (124.5 g), $CHCl_3$ (23.5 g), EtOAc (48.6 g) and *n*-BuOH (165.1 g) fractions. The $CHCl_3$ part was subjected to silica gel CC eluted with a gradient of PE-EtOAc (from 100:1 to 0:1) and CH_2Cl_2-MeOH (from 20:1 to 0:1) to obtain 18 fractions. Fraction 9 (1.8 g) was chromatographed over a silica gel column using a gradient of PE-EtOAc (from 1:0 to 0:1), and was separated into 13 fractions (Fr.9.1-Fr.9.13). Furthermore, Fr.9.5 (102 mg) was purified by HPLC, using a gradient solvent system 80%-95% MeOH in H_2O over 80 min yielded compounds **6** (7.3 mg, t_R = 33.2 min), **1** (15.2 mg, t_R = 46.2 min), **4** (6.3 mg, t_R = 59.4 min). Fr.9.7 (89.5 mg) was separated by HPLC, using an isocratic solvent system 90% MeOH in H_2O over 120 min yielded compounds **2** (7.2 mg, t_R = 35.5 min), **5** (15.9 mg, t_R = 44.7 min), **17** (5.7 mg, t_R = 56.4 min), **18** (8.2 mg, t_R = 85.1 min). Fr.9.8 (85.0 mg) was purified by Sephadex LH-20 column chromatography eluted with MeOH and then semipreparative HPLC using an isocratic solvent system 85% MeOH to yield **10** (4.3 mg, t_R = 45.1 min), **12** (3.9 mg, t_R = 55.1 min) and **31** (3.8 mg, t_R = 61.0 min). Fr.10 (1.7 g) was subjected to the RP-18 column and eluted with MeOH-H_2O (3:10 to 10:0) to afford 15 fractions (Fr.10.1-Fr.10.15). Fr.10.8 (115.3 mg) was separated by HPLC, using a gradient solvent system 80%-95% MeOH in H_2O yielded compounds **30** (10.8 mg, t_R = 38.7 min), **19** (7.8 mg, t_R = 44.6 min) and **20** (9.1 mg, t_R = 58.9 min). Fr.10.9 (88.7 mg) was purified via preparative HPLC using an isocratic solvent system of 80% MeCN in H_2O to yield compounds **9** (15.0 mg, t_R = 40.2 min), **11** (3.9 mg, t_R = 46.7 min) and **3** (5.2 mg, t_R = 49.5 min) and **15** (2.2 mg, t_R = 53.0 min). Fr.10.10 (106.9 mg) was purified via preparative HPLC using an isocratic solvent system of 90% MeOH in H_2O to yield compounds **7** (7.0 mg, t_R = 30.8 min), **8** (2.9 mg, t_R = 35.3 min), **23** (5.2 mg, t_R = 51.1 min) and **16** (3.2 mg, t_R = 55.7 min). Fr.10.11 (272.4 mg) was purified by HPLC, using an gradient solvent system 90%-95% MeOH in H_2O to yield compounds **22** (4.3 mg, t_R = 53.2 min), **25** (5.2 mg, t_R = 61.2 min), **26** (6.0 mg, t_R = 82.4 min) and **27** (2.8 mg, t_R = 88.0 min). Fraction 11 (2.2 g) was chromatographed over a silica gel column using a gradient of PE-Acetone (from 1:0 to 0:1), and was separated into 8 fractions (Fr.11.1-Fr.11.8). Fr.11.5 (455.3 mg) was separated by HPLC, using an gradient solvent system 85%-95% MeOH in H_2O yielded compounds **28** (4.8 mg, t_R = 41.3 min), **13** (17.0 mg, t_R = 45.5 min), **21** (10.2 mg, t_R = 64.2 min) **14** (9.0 mg, t_R = 78.3 min) and **24** (6.4 mg, t_R = 88.0 min). Fr.11.7 (305.3 mg) was separated by HPLC, using an isocratic solvent system 85% MeCN in H_2O yielded compound **29** (3.1 mg, t_R = 35.2 min).

2.3.1. 3β-O-(trans-p-coumaroyl)-lupane-28-al-20-ol (1)

White amorphous powder; [α] + 38.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 212 (2.19), 312 (2.70) nm; IR (KBr) ν_{max} 3425, 2920, 2840, 1721, 1632, 1589, 1510 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz)

Table 1
The ^1H NMR data of compounds **1**–**6** (600 MHz).

Position	1[a]	2[b]	3[a]	4[b]	5[b]	6[b]
1	1.69 m 0.84 m	1.67 m 0.95 m	1.67 m 0.95 m	1.75 m 1.04 m	1.74 m 1.00 m	1.81 m 0.91 m
2	1.68 *o* 1.51 m	1.65 *o* 1.56 m	1.65 m 1.49 m	1.64 m 1.07 m	1.62 *o* 1.57 m	1.67 m 1.59 m
3	4.60 dd (10.8, 4.8)	4.49 dd (11.4, 4.8)	4.60 dd (10.8, 4.2)	4.49 dd (11.4, 5.4)	4.62 dd (11.4, 5.4)	4.50 dd (12.0, 4.8)
5	0.81 m	0.84 m	0.85 m	0.84 m	0.85 m	0.76 m
6	1.45 *o* 1.35 m	1.58 m 1.39 m	1.42 m 1.28 m	1.56 *o* 1.30 m	1.62 *o* 1.39 *o*	1.60 m 1.39 *o*
7	1.37 *o*	1.36 *o*	1.37 m	1.48 *o*	1.39 *o*	1.39 *o*
9	1.26 m	1.36 *o*	1.28s	1.58 m	1.24 m	1.25 m
11	1.37 *o* 1.27 m	1.39 m 1.16 m	1.39 m	1.49 m 1.35 m	1.46 *o* 1.35 m	1.48 m 1.14 m
12	1.70 m 1.03 m	1.65 *o* 1.05 m	1.68 m 1.03 m	1.56 *o* 1.17 m	1.61 m	1.66 *o*
13	1.74 m	1.65 *o*	1.70 *o*	1.74 m	1.74 m	1.83 *o*
15	1.70 *o* 1.45 *o*	1.88 *o* 1.36 *o*	1.70 *o* 1.41 m	1.68 *o* 1.16 m	1.46 *o*	1.92 m 1.38 m
16	2.04 m 1.24 m	2.02 *o* 1.23 m	1.90 m 1.25 m	1.90 m 1.23 m	1.78 m 0.88 m	1.85 m 1.03 m
18	1.80 m	1.65 m	1.71 m	1.71 m	1.63 m	1.66 *o*
19	2.20 m	2.02 *o*	1.88 m	2.04 m	1.80 m	2.04 m
21	2.02 m 1.30 m	1.88 *o* 1.36 *o*	1.87 m 1.29 m	1.97 m	1.46 *o* 1.36 m	1.92 m 1.28 m
22	1.62 m	1.47 m	1.64 m	1.66 m	1.63 m 1.14 m	1.82 *o* 1.29 m
23	0.86 s	0.81 s	0.89 s	0.82 s	0.80 s	0.83 s
24	0.88 s	0.86 s	0.91 s	0.88 s	0.82 s	0.88 s
25	0.90 s	0.82 s	0.99 s	0.85 s	0.76 s	0.85 s
26	0.90 s	0.83 s	0.88 s	1.02 s	0.90 s	1.00 s
27	0.99 s	0.96 s	1.07, s	0.95 s	0.91 s	0.94 s
28	9.60 s	9.59 s	4.34 d (10.8) 3.83 d (10.8)	3.57 dd (10.2, 4.8) 3.04 dd (10.2, 4.8)		
29	1.16 s	1.03 s	1.14 s	0.98 s	1.05 s	1.01 s
30	1.27 s	1.10 s	1.24 s	1.09 s	1.07 s	1.23 s
32			2.07 s			
2′	7.42 d (8.4)	6.99 d (1.8)	7.43 d (9.0)	7.03 d (1.8)	7.62 d (8.4)	7.55 d (8.4)
3′	6.83 d (8.4)		6.83 d (8.4)		6.74 d (8.4)	6.78 d (8.4)
5′	6.83 d (8.4)	6.75 d (8.4)	6.83 d (8.4)	6.99 d (8.4)	6.74 d (8.4)	6.78 d (8.4)
6′	7.42 d (8.4)	6.98 dd (8.4,1.8)	7.43 d (9.0)	6.75 dd (8.4, 1.8)	7.62 d (8.4)	7.55 d (8.4)
7′	7.62 d (15.6)	7.45 d (15.6)	7.60 d (15.6)	6.87 d (12.6)	6.84 d (12.6)	7.53 d (15.6)
8′	6.29 d (15.6)	6.23 d (15.6)	6.29 d (15.6)	5.73 d (12.6)	5.77 d (12.6)	6.37 d (15.6)

"*o*" represents the overlapped peak.
[a] Measured in $CDCl_3$.
[b] Measured in DMSO-d_6.

spectral data in $CDCl_3$, see Table 1 and Table 2; HRESIMS: *m/z* 603.4037 $[M - H]^-$ (calcd for $C_{39}H_{56}O_5$, 604.4128).

*2.3.2. 3β-O-(trans-caffeoyl)-lupane-28-al-20-ol (**2**)*

White amorphous powder; [α] + 42.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 218 (3.08), 330 (2.91) nm; IR (KBr) ν_{max} 3468, 2904, 2861, 1752, 1634, 1602, 1521 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data in DMSO-d_6, see Table 1 and Table 2; HRESIMS: *m/z* 619.4032 $[M - H]^-$ (calcd for $C_{39}H_{56}O_6$, 620.4077).

*2.3.3. 3β-O-(trans-p-coumaroyl)-lupane-28-O-acetyl-20-ol (**3**)*

Light yellow amorphous powder; [α] + 45.9 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 215 (3.43), 317 (2.57) nm; IR (KBr) ν_{max} 3389, 2940, 2880, 1724, 1640, 1604, 1567, 1502 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data in $CDCl_3$, see Table 1 and Table 2; HRESIMS: *m/z* 647.4297 $[M - H]^-$ (calcd for $C_{41}H_{60}O_6$, 648.4390).

*2.3.4. 3β-O-(cis-caffeoyl)-20-ol-betulin (**4**)*

White amorphous powder; [α] + 57.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 213 (3.15), 313 (2.84) nm; IR (KBr) ν_{max} 3328, 2920, 2864, 1720, 1630, 1601, 1525 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data in DMSO-d_6, see Table 1 and Table 2; HRESIMS: *m/z* 621.4156 $[M - H]^-$ (calcd for $C_{39}H_{58}O_6$, 622.4233).

Table 2
The ^{13}C NMR data of compounds **1**–**6** (150 MHz).

Position	**1**[a]	**2**[b]	**3**[a]	**4**[b]	**5**[b]	**6**[b]
1	38.6	38.1	38.6	38.3	38.4	38.4
2	24.0	23.5	24.0	24.0	23.8	24.0
3	81.0	79.8	81.0	80.3	80.4	80.3
4	38.2	37.6	38.3	37.0	37.9	37.1
5	55.5	54.5	55.0	55.0	55.3	55.1
6	18.4	17.8	18.4	18.3	18.2	18.3
7	34.8	34.0	34.6	34.4	34.0	34.6
8	41.5	40.8	41.7	41.5	41.2	41.3
9	50.5	49.6	50.3	50.1	50.7	50.3
10	37.2	36.6	36.8	36.4	36.5	35.3
11	21.5	20.9	21.5	21.4	21.6	21.6
12	28.4	27.3	27.4	27.4	27.2	27.5
13	38.4	37.9	37.2	38.1	37.1	38.1
14	38.4	43.1	43.6	43.4	42.3	42.8
15	29.4	28.8	29.2	28.2	28.2	28.2
16	29.7	29.0	30.5	28.4	31.1	29.2
17	61.8	60.7	48.1	49.0	78.7	90.6
18	47.2	46.3	49.0	48.7	49.7	49.3
19	49.6	48.9	49.8	49.0	54.0	48.3
20	73.8	71.2	73.7	71.9	70.8	71.8
21	29.3	28.3	28.5	30.1	23.4	29.4
22	33.2	32.9	34.2	33.5	40.5	32.9
23	28.2	27.7	28.2	27.9	28.1	28.0
24	16.9	16.5	16.9	17.1	16.9	17.1
25	16.5	15.8	16.4	16.3	16.8	16.7
26	16.4	16.0	16.4	16.4	15.8	16.4
27	14.7	14.4	15.2	15.5	14.5	14.6
28	207.2	207.7	63.1	58.9		
29	25.1	26.1	24.9	25.4	29.1	27.0
30	31.4	30.9	31.9	32.2	31.3	31.0
31			171.9			
32			21.3			
1′	127.5	125.5	127.7	126.0	126.0	125.5
2′	130.1	114.8	130.1	115.3	132.9	130.7
3′	116.1	145.6	116.0	146.0	115.3	116.2
4′	158.1	148.3	157.6	148.8	159.2	160.3
5′	116.1	121.3	116.0	121.8	115.3	116.2
6′	130.1	115.7	130.1	116.2	132.9	130.7
7′	144.3	144.8	144.0	145.3	143.4	144.9
8′	116.1	114.4	116.7	114.9	116.5	115.3
9′	167.5	166.3	167.4	166.8	166.3	166.9

[a] Measured in $CDCl_3$.
[b] Measured in DMSO-d_6.

*2.3.5. 3β-O-(cis-caffeoyl)-norlupane-17β,20-diol (**5**)*

White amorphous powder; [α] + 38.9 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 218 (2.89), 327 (2.58) nm; IR (KBr) ν_{max} 3455, 2937, 2875, 1737, 1630, 1605, 1485 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data in DMSO-d_6, see Table 1 and Table 2; HRESIMS: *m/z* 591.4053 $[M - H]^-$ (calcd for $C_{38}H_{56}O_5$, 592.4128).

*2.3.6. 3β-O-(trans-p-coumaroyl)-norlupane-17β-hydroperoxide-20-ol (**6**)*

White amorphous powder; [α] + 27.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 214 (3.26), 320 (2.86) nm; IR (KBr) ν_{max} 3547, 2964, 2866, 1725, 1633, 1608, 1464 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data in DMSO-d_6, see Table 1 and Table 2; HRESIMS: *m/z* 631.3970 $[M + Na]^+$ (calcd for $C_{38}H_{56}O_6$, 608.4077).

*2.3.7. Ethyl 3β-O-(trans-caffeoyl)-lupane-28-oate (**9**)*

White amorphous powder; [α] + 43.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 213 (2.27), 317 (2.95) nm; IR (KBr) ν_{max} 3397, 2940, 2883, 1733, 1629, 1593, 1514 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data in $CDCl_3$, see Table 2; HRESIMS: *m/z* 645.4128 $[M - H]^-$ (calcd for $C_{41}H_{58}O_6$, 646.4233).

*2.3.8. 24-O-(cis-p-coumaroyl)-3β-hydroxyl-olean-12-en-28-oic acid (**15**)*

White amorphous powder; [α] + 16.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 204 (3.19), 312 (2.90) nm; IR (KBr) ν_{max} 3360, 2913, 2887, 1723, 1634, 1601, 1501 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data in $CDCl_3$, see Table 2; HRESIMS: *m/z* 617.3846 $[M - H]^-$ (calcd for $C_{39}H_{54}O_6$, 618.3920).

*2.3.9. 24-O-(cis-p-feruloyl)-3β-hydroxyl-olean-12-en-28-oic acid (**16**)*

White amorphous powder; [α] + 14.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 211 (3.04), 263 (2.87) nm; IR (KBr) ν_{max} 3387, 2921, 2874, 1728, 1628, 1608, 1503 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data in $CDCl_3$, see Table 2; HRESIMS: *m/z* 647.3932 $[M - H]^-$ (calcd for $C_{40}H_{56}O_7$, 648.4026).

2.4. Cell viability assay

The MTT assay was used to evaluate the cytotoxicities of triterpenoids and extracts of four *Leptopus* genus plants according to the published method [5]. Four cancer cells of A549, HepG2, MCF-7 and HeLa were cultured in DMEM medium supplemented with 10% fetal bovine serum, 1 × penicillin–streptomycin in a humidified 37 ℃ incubator supplied with 5% CO_2, respectively. Briefly, cells were harvested with trypsin, seeded into 96-well plates at 5000 cells/well and then incubated for 12 h. The test samples which dissolved in DMSO were added to each well, and cells were incubated for another 48 h. Control wells were treated with 1% aqueous DMSO. And doxorubicin and ginsenoside Rg3 were used as two positive control. After 48 h incubation, 20 μL of MTT (5 mg/mL) was added to each well and incubated for 4 h at 37 ℃ with 5% CO_2. Next, the medium was aspirated and precipitated formazan crystals dissolved in DMSO (150 μL/well). The absorbance of each well was measured at 570 nm with a microplate reader (Thermo Scientific Multiskan MK3, Shanghai, China).

2.5. General procedure for synthesizing phenylpropanoid-conjugated triterpenoid

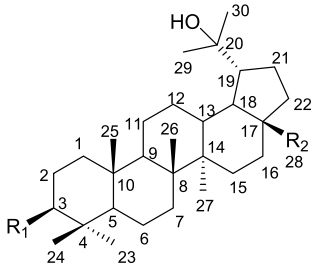
Tanachatchairatan provided a method to synthesize this kind of triterpenoid to study their antimycobacterial activities [6]. However, during the course of hydrolyzing the acetyl group with alkaline solution, the formed ester bond may also be hydrolyzed. In this study, the *tert*-butyldimethylsilyl chloride (TBDMSCl) was used to protect PhOH and tetrabutylammonium fluoride (TBAF) was used to remove this protective group. Briefly, 4-hydroxyl-cinnamic acid, caffeic acid or ferulic acid and 8 eq Et_3N were dissolved in anhydrous CH_2Cl_2 (15 mL). Then 4 eq TBDMSCl in anhydrous CH_2Cl_2 (15 mL) was slowly added to the

prepared cooled solution. The reaction mixture was stirred for 12 h at room temperature, washed with 1 M HCl (2 × 30 mL) and water (30 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. And formed brown oil was dissolved in MeOH (30 mL) and water (30 mL) before 1 eq K_2CO_3 was added. This reaction mixture was stirred for another 4 h and concentrated in vacuo to remove the MeOH. Then the EtOAc (2 × 50 mL) and concentrated HCl (4 mL, pH 0) were added and combined organic phase was washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was further purified by flash column chromatography (petroleum ether: EtOAc 5:1) to afford product. Next, linking with *tert*-butyldimethylsilyl acid chlorides were prepared by reacting the corresponding carboxylic acids with oxalyl chloride at reflux for 2 h, and the remaining oxalyl chloride was removed. The residue was dissolved in anhydrous CH_2Cl_2 (10 mL), added to a solution of betulinic acid, and heated to reflux for 2 h at 60 ℃. After the completion of the esterification and pure compound was obtained, 2 eq dry TBAF (1.0 M in THF) was added to reaction mixture and stirred for 4 h. Finally, solvent was removed and the crude product was dissolved in CH_2Cl_2 (3 × 20 mL), washed with water (3 × 20 mL), dried with anhydrous $MgSO_4$ and solvent was removed in vacuo to afford phenylpropanoid-conjugated triterpenoid, which was further purified by flash chromatography on silica gel (PE: EtOAc, 5:1 to 3:1).

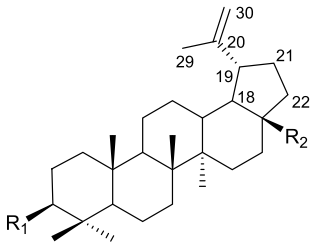
3. Results and discussion

3.1. Structure elucidation

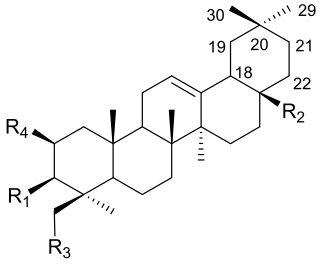
Compound **1** was obtained as a white amorphous powder. The



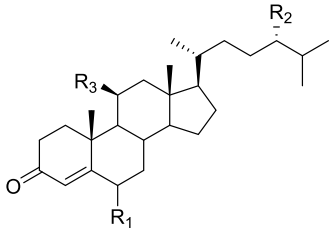
	R_1	R_2
1	A	CHO
2	C	CHO
3	A	CH_2OAc
4	D	CH_2OH
5	B	OH
6	A	OOH
7	OH	CH_3
8	C	CH_2OH



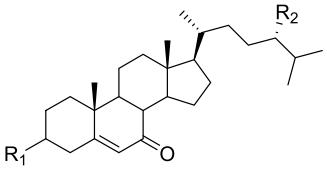
	R_1	R_2
9	C	$COOCH_2CH_3$
10	A	COOH
11	B	COOH
12	A	CH_2OH
13	C	CH_2OH
14	OH	COOH
H1	C	COOH
H2	E	COOH



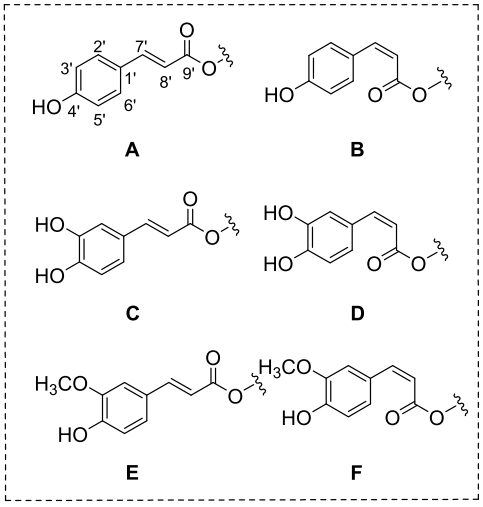
	R_1	R_2	R_3	R_4
15	OH	COOH	B	H
16	OH	COOH	F	H
17	A	COOH	H	H
18	C	COOH	H	H
19	OH	COOH	H	H
20	OH	$COOCH_3$	H	OH



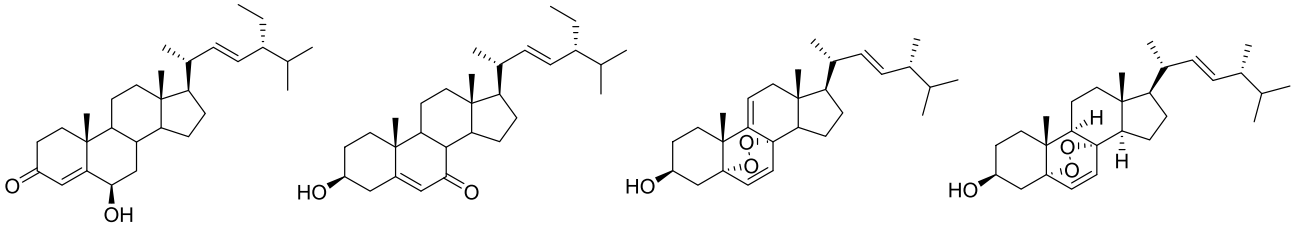
	R_1	R_2	R_3
21	β-OH	CH_3	H
22	α-OH	CH_3	H
23	α-OH	CH_2CH_3	H
24	H	CH_3	OH



	R_1	R_2
25	β-OH	CH_2CH_3
26	α-OH	CH_3
27	β-OH	CH_3



A **B** **C** **D** **E** **F**



28 **29** **30** **31**

Blue ink: Novel compounds

Red ink: Synthetic compounds

Fig. 1. The structures of compounds H1, H2 and **1**–**31**.

molecular formula was assigned as $C_{39}H_{56}O_5$ according to the HRESIMS (*m/z* 603.4037 $[M-H]^-$, calcd 604.4128) and ^{13}C NMR data. The IR spectrum showed typical absorption bands of hydroxy and carbonyl groups at 3425 and 1721 cm^{-1}, respectively. The ^1H NMR spectrum of **1** (Table 1) exhibited resonances for seven singlet methyl protons at δ_H 1.27, 1.16, 0.99, 0.90, 0.90, 0.88, 0.86, an oxygenated methine proton at δ_H 4.60 (1H, dd, $J = 10.8$, 4.8 Hz), two olefinic protons at δ_H 7.62 (1H, d, $J = 15.6$ Hz) and 6.29 (1H, d, $J = 15.6$ Hz), a set of AA'XX'-type aromatic protons at δ_H 7.42 (2H, d, $J = 8.4$ Hz) and 6.83 (2H, d, $J = 8.4$ Hz), and an aldehydic proton at δ_H 9.60. The ^{13}C NMR spectrum of **1** (Table 2) revealed 39 resonances including an aldehydic carbonyl carbon at δ_C 207.2, an ester group at δ_C 167.5, six aromatic and two olefinic carbons at δ_C 116.1–158.1, an oxygenated methine carbon at δ_C 81.0 and an oxygenated quaternary carbon at δ_C 73.8. The comparison of its ^1H and ^{13}C NMR data with those of lupane-3β,20,28-triol caffeate [7] indicated that **1** possesses the same skeleton, except for the presence of a *trans*-coumaroyl moiety and an aldehyde group which observed in **1**. And the position of ester bond between the *trans*-coumaroyl moiety and 3,20-dihydroxyl betulinic aldehyde was determined at C-3 by the key HMBC correlation of δ_H 4.60 (H-3) with δ_C 167.5 (C-9′) (Fig. 2). The 2D structure of **1** was established with the aid of HSQC and HMBC spectra. The β-orientation of C-3 was confirmed by the strong NOESY correlations of H-3α and H-23 (Fig. 3). Thus, the structure of **1** was assigned as 3β-*O*-(*trans*-*p*-coumaroyl)-lupane-28-al-20-ol.

Compound **2** was isolated as a white amorphous powder and assigned the molecular formula $C_{39}H_{56}O_6$ according to the HRESIMS (*m/z* 619.4032 $[M-H]^-$, calcd 620.4077) and ^{13}C NMR data. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **2** showed a close resemblance to those of **1**, suggesting that **2** was 3,20-dihydroxyl betulinic aldehyde with a *trans*-caffeoyl moiety. And it could be deduced by the NMR data at δ_H 7.45 (1H, d, $J = 15.6$ Hz), 6.99 (1H, d, $J = 1.8$ Hz), 6.98 (1H, dd, $J =$ 8.4, 1.8 Hz), 6.75 (1H, d, $J = 8.4$ Hz) and 6.23 (1H, d, $J = 15.6$ Hz), and δ_C 166.3, 148.3, 145.6, 144.8, 125.5, 121.3, 115.7, 114.8 and 114.4. The location of *trans*-caffeoyl moiety was confirmed at C-3 via the HMBC cross-peak of H-3/C-9′ (Fig. 2). The β-orientation of C-3 was also deduced by the NOESY correlations of H-3α and H-23 (Fig. 3). Collectively, the structure of compound **2** was confirmed and named 3β-*O*-(*trans*-caffeoyl)-lupane-28-al-20-ol.

Compound **3** was isolated as a light yellow amorphous powder, and the HRESIMS (*m/z* 647.4297 $[M-H]^-$, calcd 648.4390) and ^{13}C NMR data suggested that it possesses a molecular formula of $C_{41}H_{60}O_6$. The lupane nature of **3** was supported by a comparison of its NMR data with those of **1** and **2**, which showed signals of protons and carbons for the lupane triterpenoid and *trans*-coumaroyl moiety. However, the ^1H and ^{13}C NMR data of **3** (Tables 1 and 2) showed the presence of methyl acetate group [δ_H 4.34 (1H, d, $J = 10.8$ Hz), 3.83 (1H, d, $J = 10.8$ Hz), 2.07 (3H, s); δ_C 171.9, 63.1 and 21.3] replacing the aldehyde group (δ_C 207.2) of **1**. It could be further confirmed by the key HMBC correlations of δ_H 4.34/3.83 with δ_C 171.9 (C-31) and 48.1 (C-17), and of δ_H 2.07 with δ_C 171.9 (C-31) (Fig. 2). The structure of **3** was finally established as 3β-*O*-(*trans*-*p*-coumaroyl)-lupane-28-*O*-acetyl-20-ol.

Compound **4** was purified as a white amorphous powder and the molecular formula was assigned as $C_{39}H_{58}O_6$ based on the HRESIMS ion signal at *m/z* 621.4156 $[M-H]^-$. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **4** were very similar to those of **2**, with the difference being the replacement of *trans*-olefinic protons with a large coupling constant [δ_H 7.45 (1H, d, $J = 15.6$ Hz), 7.45 (1H, d, $J = 15.6$ Hz)] by *cis*-olefinic protons with a smaller coupling constant [δ_H 6.87 (1H, d, $J = 12.6$ Hz), 5.73 (1H, d, $J = 12.6$ Hz)]. The position of *cis*-caffeoyl moiety was determined to be C-3 by the HMBC correlations of H-3/C-9′ (Fig. 2). Consequently, the structure of compound **4** was defined as shown and named 3β-*O*-(*cis*-caffeoyl)-lupane-20,28-diol.

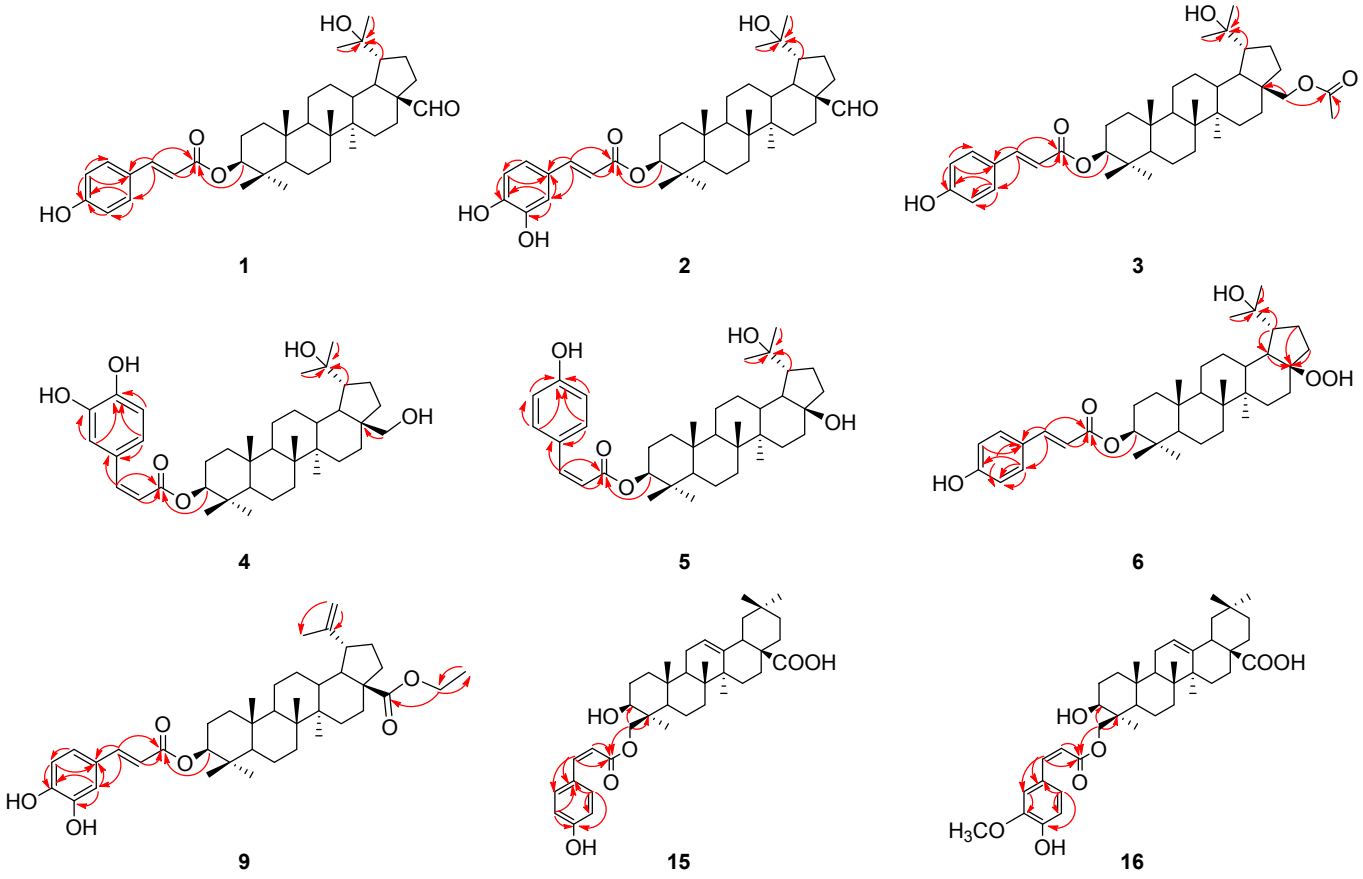


Fig. 2. Key HMBC correlations of compounds **1**–**6**, **9**, **15** and **16**.

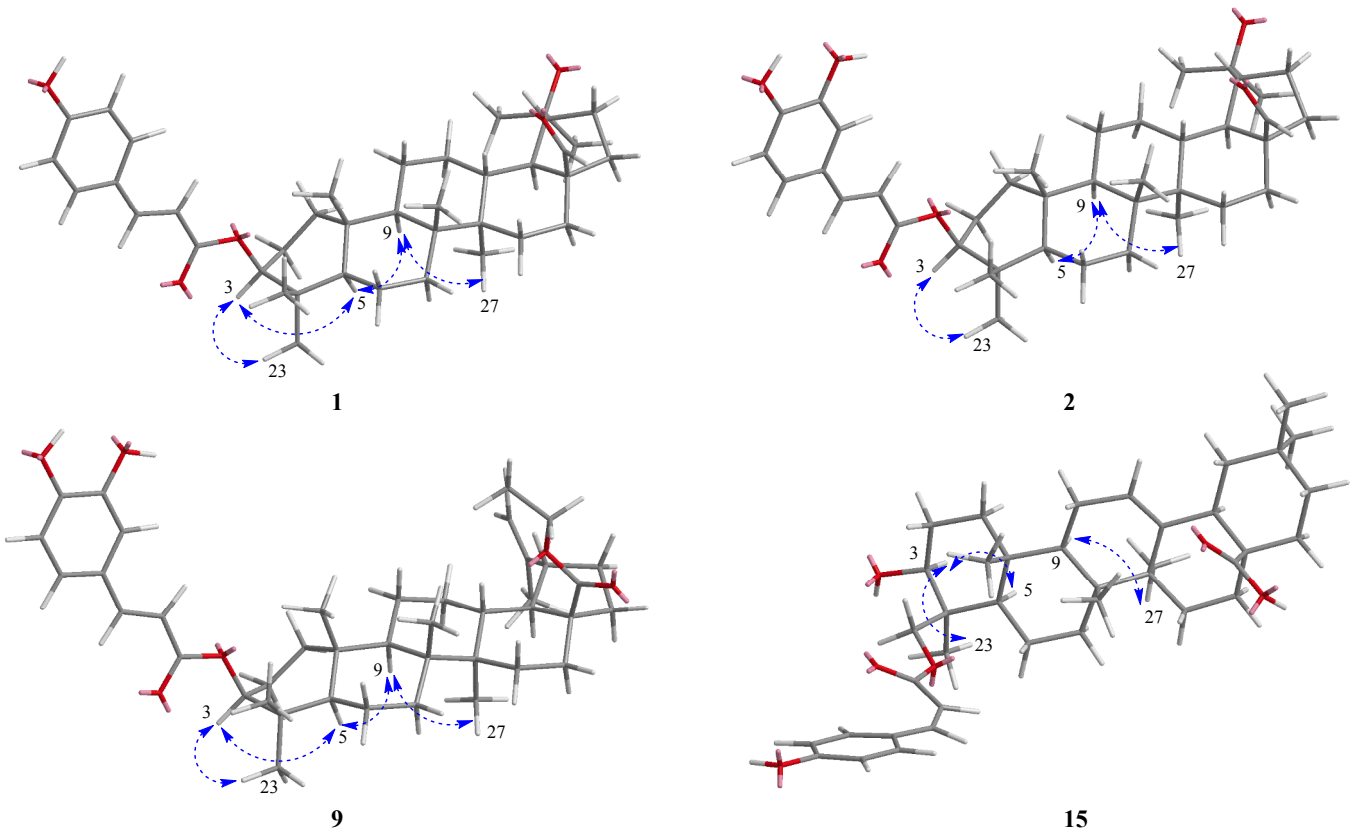


Fig. 3. Key NOESY correlations of compounds **1**, **2**, **9** and **15**.

The molecular formula of compound **5** was deduced as $C_{38}H_{56}O_5$ based on HRESIMS ($[M-H]^-$, *m/z* 591.4053), again corresponding to a lupane skeleton. However, several noticeable differences were observed in the NMR data (Tables 1 and 2) of **5** and **1**. The most conspicuous differences in their NMR signals were the replacement of *cis*-olefinic protons [δ_H 6.84 (1H, d, $J = 12.6$ Hz), 5.77 (1H, d, $J = 12.6$ Hz)] and the presence of an oxygenated quaternary at δ_C 78.7 in **5**. The structure of 28-norlupane skeleton was deduced by the HMBC (Fig. 2) and the comparison of its NMR data with those of 28-norlup-20(29)-ene-3β,17β-diol [8]. The β-orientation of C-17 can be deduced by the chemical shift (δ_C 37.1) of C-13 (17β: about 38 ppm; 17α: about 43 ppm) according the published study [8]. Thus, the structure of **5** was consequently established as 3β-*O*-(*cis*-*p*-coumaroyl)-norlupane-17β,20-diol.

Compound **6** has the molecular formula $C_{38}H_{56}O_6$ as determined by HRESIMS data ($[M+Na]^+$, *m/z* 631.3970). The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **6** suggested its close similarity to the known 28-norlup-20(29)-en-3-hydroxy-17-hydroperoxide [9], with an additional *trans*-*p*-coumaroyl moiety. The ^{13}C NMR spectrum of **6** showed a quaternary carbon signal at δ_C 90.6, assigned to C-17 based on HMBC data. Similarly with those of **5**, the relative configuration of C-17 in **7** could also be determined by ^{13}C NMR chemical shifts because of the presence of γ-substituent effect. The β-configuration of C-17 in **6** was deduced by the chemical shift value of its C-13 (δ_C 38.1) and C-19 (δ_C 48.3) [9]. The location of this additional *trans*-*p*-coumaroyl group was confirmed at C-3 by the HMBC correlations of δ_H 4.50 with 166.9 (C-9′) (Fig. 2). Therefore, the structure of **6** was determined as 3β-*O*-(*trans*-*p*-coumaroyl)-norlupane-17β-hydroperoxide-20-ol.

Compound **9** was obtained as a white amorphous powder with the identical molecular formula $C_{41}H_{58}O_6$ from its HRESIMS showing a deprotonated ion at *m/z* 645.4128 $[M-H]^-$ (calcd for $C_{41}H_{58}O_6$, 646.4233). The ^{1}H and ^{13}C NMR data (Table 3) of **9** were found to be closely comparable to those of **2**, but there were slight shifts in the presence of an oxygenated methine (δ_H 4.17; δ_C 59.9) and a methyl group [δ_H (1.27, t, 7.2 Hz); δ_C 14.4], and the absence of an oxygenated quaternary carbon at δ_C 71.2 in **9**. Combined with the HMBC spectrum (Fig. 2) could confirmed the exist of ethyl methanoate which linked with C-17. Thus, the structure of compound **9** was assigned as Ethyl 3β-*O*-(*trans*-caffeoyl)-lupane-28-oate.

Compound **15** was obtained as a white amorphous powder, and the molecular formula $C_{39}H_{54}O_6$ was calculated from the $[M-H]^-$ ion at *m/z* 617.3846 in its HRESIMS. Examination of the ^{1}H and ^{13}C NMR data (Table 3) of **15** suggested that this compound shares close structural similarities with (23*E*)-feruloylhederagenin, except that the *trans*-feruloyl moiety was replaced by a *cis*-*p*-coumaroyl moiety [10]. The long range HMBC correlations (Fig. 2) of **15** between H-24 (δ_H 4.63 and 4.25) with δ_C 167.4 (C-9′) and several key NOESY correlations (H-23/H-3; H-3/H-5) (Fig. 3) suggested that the *trans*-*p*-coumaroyl moiety attached to C-24 and relative stereochemistry of C-3 was in β-oriented. Hence, compound **15** was established as 24-*O*-(*cis*-*p*-coumaroyl)-3β-hydroxyl-olean-12-en-28-oic acid.

Compound **16** showed an $[M-H]^-$ ion at *m/z* 647.3932 (calcd for $C_{40}H_{56}O_7$, 648.4026) in the HRESIMS spectrum, corresponding to the molecular formula $C_{40}H_{56}O_7$. The NMR data (Table 3) of **16** were very similar with **15**, suggested that compound **16** possesses the same skeleton as that of **15**. And the major difference was the presence of a *trans*-feruloyl group replacing the *cis*-*p*-coumaroyl group. The location of the *trans*-feruloyl moiety was assigned to C-24 due to the key HMBC correlations of δ_H 4.37/4.18 with δ_C 166.4 (C-9′) (Fig. 2). The β-orientation of C-24 was deduced from the comparison of NMR data between **16** and **15** and the same biogenetic relationship. Thus, the structure of **16** was established as 24-*O*-(*cis*-feruloyl)-3β-hydroxyl-olean-12-en-28-oic acid.

Structure elucidation of known compounds was carried out by a combination of spectroscopic means and comparison with references. The compounds were determined to be monogynol A (**7**) [11], 3β-*O*-(*trans*-caffeoyl)-20-ol-betulin (**8**) [11], 3β-*O*-*trans*-coumaroylbetulinic acid (**10**) [12], 3β-*O*-(*cis*-coumaroyl)-betulinic acid (**11**) [13], 3β-*O*-

Table 3
The ^{1}H NMR and ^{13}C NMR data of compounds **9**, **15** and **16** in $CDCl_3$ (^{1}H: 600 MHz, ^{13}C: 150 MHz).

Position	9		15		16	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	1.70 *m* 1.01 *m*	38.3	1.58 *m* 0.98 *m*	38.7	1.66 *m* 0.99 *m*	38.5
2	1.68 *o*	23.8	1.62 *m* 1.17 *m*	22.9	1.77 *o* 1.08 *m*	23.0
3	4.60 dd (10.8, 6.0)	81.2	3.44 dd (12.0, 4.8)	80.6	3.28 dd (12.0, 4.2)	79.4
4		38.1		42.5		42.2
5	0.82 *m*	55.4	1.00 *m*	56.4	0.88 *m*	56.1
6	1.51 *m* 1.40 *o*	18.2	1.42 *m* 1.34 *m*	18.9	1.57 *o* 1.33 *m*	18.9
7	1.37 *m*	34.3	1.32 *m* 1.22 *m*	33.3	1.42 *m* 1.25 *m*	33.0
8		40.8		39.4		39.2
9	1.31 *m*	50.5	1.57 *m*	47.6	1.57 *o*	47.7
10		37.1		37.3		36.9
11	1.42 *m* 1.27 *m*	20.9	1.88 *m*	23.8	1.89 *m*	23.5
12	1.70 *m* 1.03 *m*	25.5	5.30 brs	123.0	5.28 brt (3.6)	122.5
13	1.69 *o*	38.4		143.7		143.5
14		42.4		41.2		41.1
15	1.40 *o* 1.15 *m*	29.6	1.70 *o* 1.09 *m*	27.2	1.76 *o* 1.08 *m*	27.1
16	2.22 *m* 1.39 *o*	32.2	1.70 *o*	27.9	2.00 *m* 1.63 *m*	27.6
17		56.4		46.7		46.5
18	1.60 *m*	49.4	2.86 dd (13.8, 4.2)	41.5	2.82 dd (13.8, 4.8)	41.6
19	3.03 *m*	47.0	1.59 *m* 1.21 *m*	45.9	1.57 *o* 1.23 *o*	45.9
20		150.6		30.9		30.7
21	1.88 *m*	30.6	1.37 *m* 1.26 *m*	33.9	1.33 *m* 1.23 *o*	33.8
22	1.89 *m* 1.40 *o*	37.1	1.80 *m* 1.64 *m*	32.7	1.80 *m* 1.57 *m*	32.4
23	0.88 s	28.0	1.24 s	22.7	1.13 s	22.4
24	0.90 s	16.2	4.63 d (11.4) 4.25 d (11.4)	66.3	4.37 d (11.4) 4.18 d (11.4)	65.4
25	0.87 s	16.7	1.06 s	15.9	0.93 s	15.6
26	0.93 s	15.9	0.72 s	17.6	0.74 s	16.8
27	0.97 s	14.7	1.14 s	26.2	1.12 s	25.8
28		176.4		184.4		181.6
29	4.74 s 4.60 s	109.6	0.92 s	32.8	0.91 s	32.9
30	1.69 s	19.4	0.95 s	23.8	0.92 s	23.5
31	4.17 *m*	59.9				
32	1.27 t (7.2)	14.4				
1′		127.6		126.2		127.1
2′	7.11 d (1.8)	114.4	7.01 d (7.8)	130.3	7.69 d (1.2)	112.8
3′		144.5	6.66 d (8.4)	116.1		146.0
4′		146.3		159.2		147.1
5′	6.88 d (7.8)	115.5	6.66 d (8.4)	116.1	6.88 d (8.4)	113.9
6′	7.01 dd (7.8, 1.8)	122.3	7.01 d (7.8)	130.3	7.12 dd (8.4, 1.2)	125.5
7′	7.56 d (15.6)	144.5	7.46 d (15.6)	145.2	6.80 d (13.2)	144.5
8′	6.27 d (15.6)	116.3	5.91 d (16.2)	114.5	5.78 d (13.2)	116.4
9′		167.6		167.4		166.4
OCH_3					3.92 s	56.0

"o" represents the overlapped peak.

coumaroylbetulin (**12**) [14], 3*β*-*O*-caffeoylbetulin (**13**) [14], betulinic acid (**14**) [14], 3*β*-*O*-(*trans*-caffeoyl)-betulinic acid (**H1**) [15], 3*β*-*O*-*trans*-feruloylbetulinic acid (**H2**) [14], 3*β*-(*p*-hydroxy-*trans*-cinnamoyloxy)olean-12-en-28-oic acid (**17**) [16], 3-*O*-caffeoyloleanolic acid (**18**) [13], oleanolic acid (**19**) [13], Methyl olean-12-en-2*β*,3*β*-diol-28-oate (**20**) [17], 24-methylcholest-4-ene-6*β*-ol-3-one (**21**) [18], 24-methylcholest-4-ene-6*α*-ol-3-one (**22**) [18], 24-ethylcholest-4-ene-6*β*-ol-3-one (**23**) [18], 11*β*-hydroxy-ergost-4-en-3-one (**24**) [19], 7-oxo-*β*-sitosterol (**25**) [20], 7-oxocampesterol (**26**) [21], (3*α*, 24*R*)-3-hydroxyergost-5-en-7-one (**27**) [21], Stigmasta-4,22-dien-6*β*-ol-3-one (**28**) [22], 7-Ketostigmasterol (**29**) [23], ergosterol peroxide (**30**) [24], 9,11-dehydroergosterol peroxide (**31**) [24] (Fig. 1).

3.2. The synthetic route of the phenylpropanoid-conjugated triterpenoid.

Three common phenylpropionic acid were found to be randomly attach to triterpenoid skeleton. In order to systematically study the structure–activity relationships, further structural modification, and pharmaceutical effect of this kind of compounds, an efficient synthetic route has been researched as shown in Fig. 7, and compounds **H1**, **H2** were obtained.

3.3. The cytotoxicities of four Leptopus genus plants and the characteristic triterpenoids of L. lolonum

Cytotoxicities of four *Leptopus* genus plants toward four cancer cell lines A549, HepG2, MCF-7 and HeLa were examined. Results (Fig. 4, Table S1) showed that PE, $CHCl_3$ fractions displayed significant cytotoxicities on four cancer cells, and $CHCl_3$ fractions showed different level of cytotoxicities with IC_{50} values of 30.19 to 77.92 μg/mL. The very weak cytotoxicities of *n*-BuOH fractions (Table S1) of four plants were observed. And this phenomenon might be related with the attachment of phenylpropanoid at C-3 and C-24 of triterpenoid skeleton leading to the less triterpenoid saponins. Considering the timeliness of chemical isolation and the plant's Euphorbiaceae background, the constituents of $CHCl_3$ fraction of *L. lolonum* were first investigated. As a result, a series of compounds including triterpenoids and sterols were isolated and most triterpenoids were found to possess *trans* or *cis p*-coumaroyl, caffeoyl and feruloyl moieties at C-3 or C-24 of oleanene or lupane-type triterpenoid skeleton. Moreover, the major feature of the isolated lupane-type triterpenoids is that the double bond (C-20/29) are oxidized and a hydroxyl group is attached to C-20. This is the first time to report the main bioactive constituents of *L. lolonum* and the abundant presence of 20-hydroxyl-lupane triterpenoids.

3.4. The cytotoxicities of compounds *H1*, *H2* and *1–20*.

Compounds **H1**, **H2** and **1–20** were also evaluated their cytotoxicities on four cancer cells of HepG2, MCF-7, A549 and HeLa (Fig. 5, Table S2). And doxorubicin and ginsenoside Rg3 were used as the positive control. All the compounds except compounds **7**, **19** and **20** showed moderate cytotoxicities on cancer cells. A preliminary structure–activity relationship (Fig. 6) suggested that triterpenoids with a phenylpropanoid moiety exhibited more potent effects than those without such a unit, which was deduced from the results that compounds **10**, **11**, **H1**, **H2**, **17** and **18** showed relatively high cytotoxicities, but not compounds **14** and **19**. It is evident that the binding site of the phenylpropanoid moiety may be not play an important role in efficacy by comparing the effects of compounds **11** (at C-3), **H2** (at C-3), **15** (at C-24) and **16** (at C-24) with similar potency. And the comparison of IC_{50} values of compounds **8** and **13** indicated that the hydroxyl group at C-20 might play an negative effect on the cytotoxicity of triterpenoids. In addition, compound **10**, with a *trans*-coumaroyl moiety (A), showed more potent cytotoxicity than **11**, with *cis*-coumaroyl moiety (B). This showed that *trans*-coumaroyl moiety might be responsible for the increasing bioactivity of compound **10**. Taken together, phenylpropanoids in triterpenoids are important for their cytotoxicities on cancer cells.

3.5. The short discussion on phenylpropanoid-conjugated triterpenoids

To our knowledge, about 200 phenylpropanoid-conjugated pentacyclic triterpenoids have been reported until now. And they displayed a

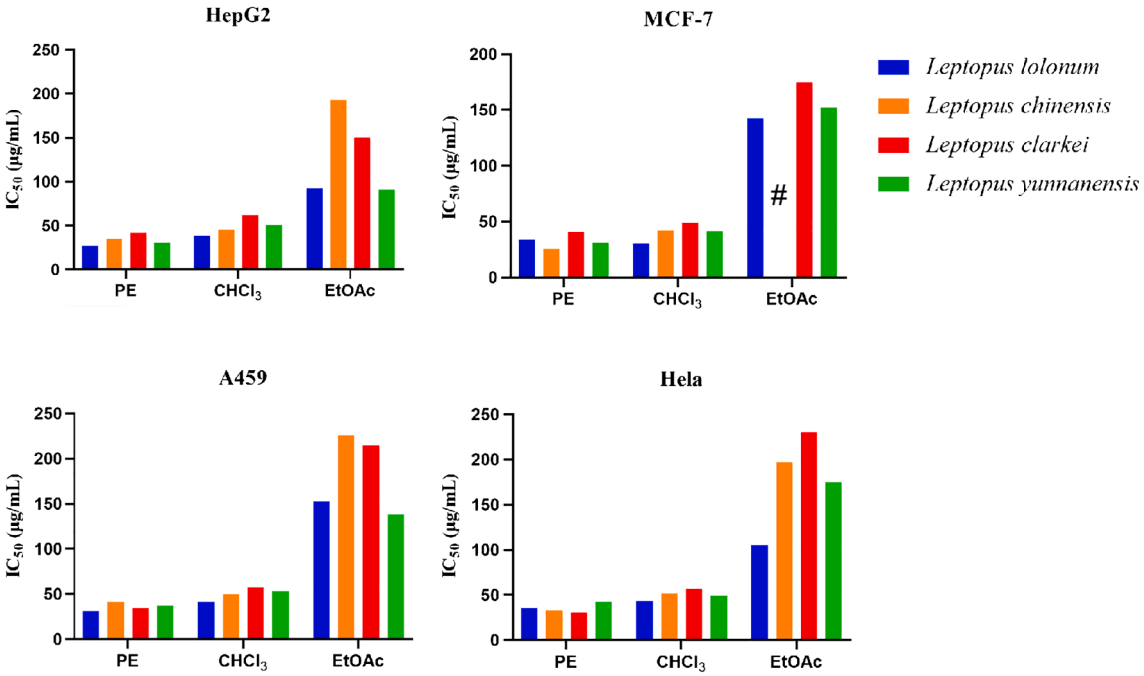


Fig. 4. The cytotoxicities of four *Leptopus* genus plants on four cancer cells. #: IC_{50} > 300 μg/mL.

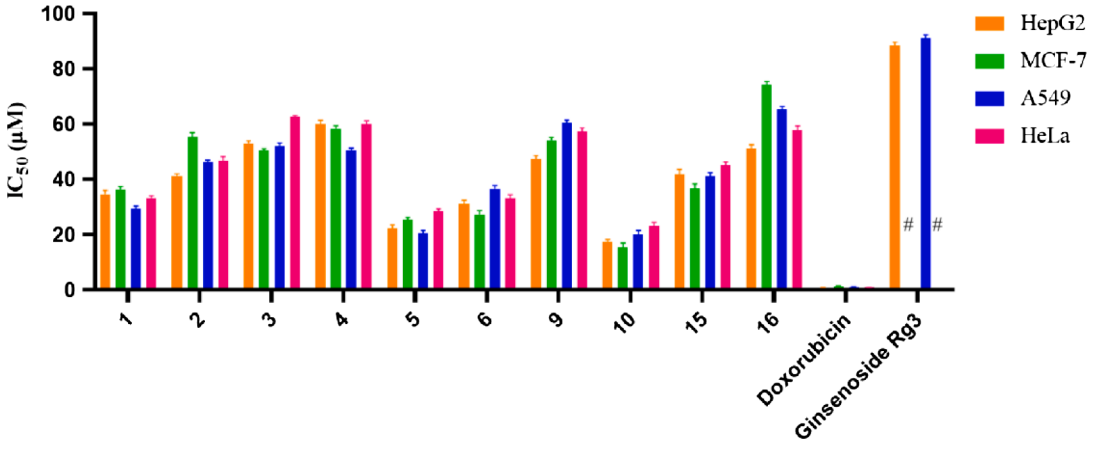


Fig. 5. The cytotoxicities of compounds **1**–**6**, **9**, **10**, **15** and **16**. #: IC_{50} > 100 μM.

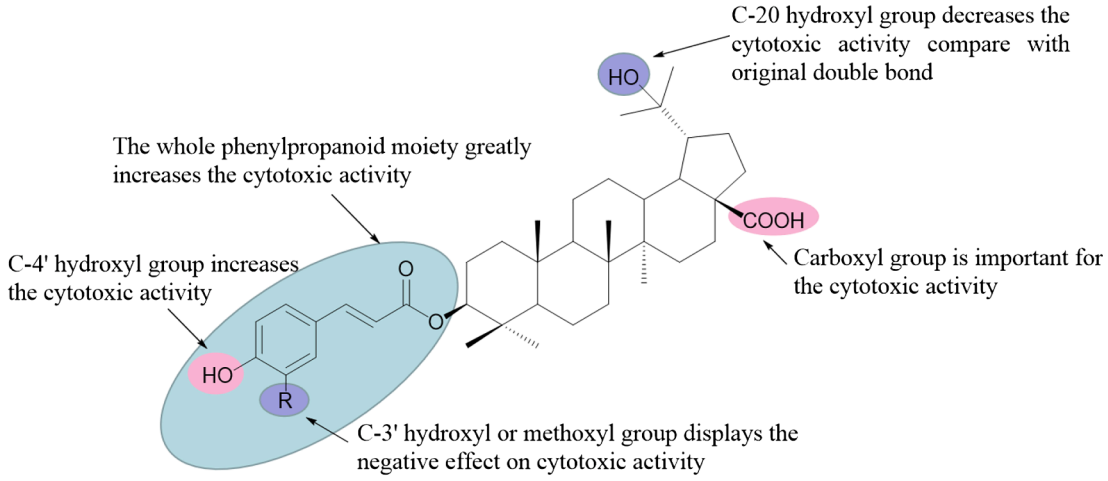


Fig. 6. The structure–activity relationships of phenylpropanoid-conjugated triterpenoid.

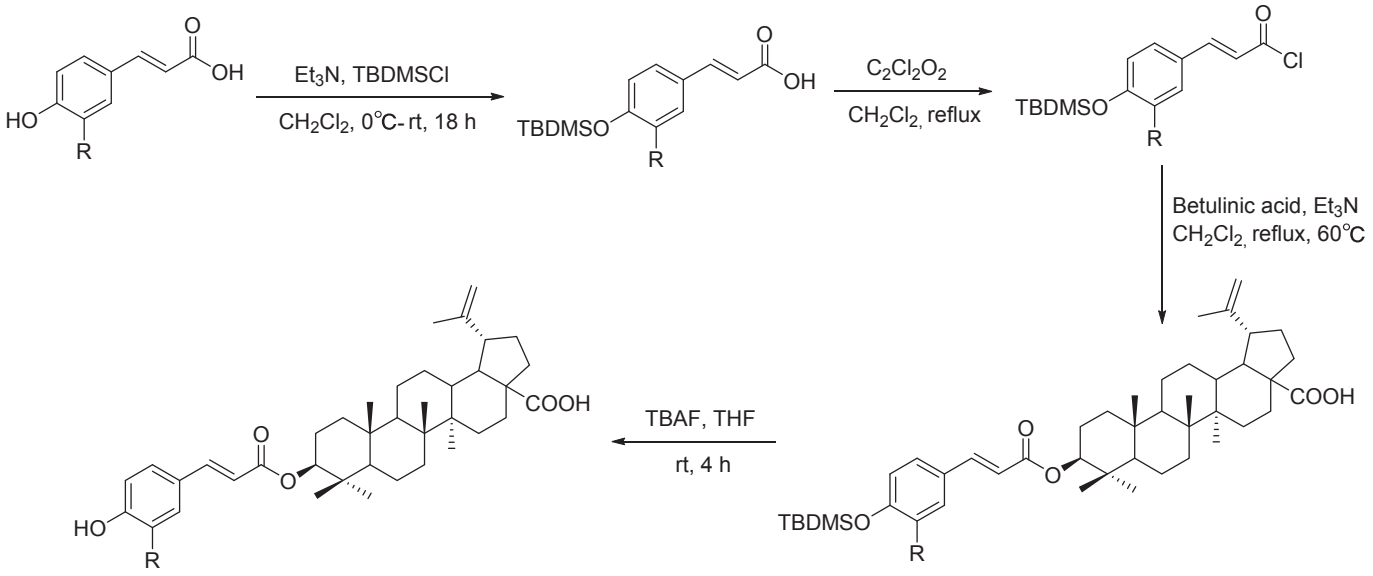


Fig. 7. The synthetic route of the phenylpropanoid-conjugated triterpenoid.

variety of biological activities including anti-inflammatory, antioxidant, anticancer, anti-hyperglycaemic, anti-ischemic stroke, antiosteoclastogenesis, anti-HIV as well as neuroprotective activity [25-28]. In most published articles, the significant increasing pharmacological activities are observed when phenylpropanoid moiety attached to triterpenoid skeleton. In addition, it is worth noting that this triterpenoid exists in some edible fruits such as apple, pear, actinidia arguta and jujube, and play a key role in respective reported bioactivity [13,29-31]. These findings showed that phenylpropanoid-conjugated triterpenoid might has potential possibility of being lead compound because of the less drug toxicity. Moreover, in the study of finding quality-marker ingredients of *Panax quinquefolius* [32], metabolomics analysis revealed the presence of phenylpropanoid-conjugated triterpenoids in the blood of mice. It indicated that this kind of triterpenoid could enter the blood to exert their effects and they have certain medicinal properties. Nevertheless, there is not much research to report the detailed mechanisms of anticancer *in vitro* and *in vivo*. Thus, more pharmacological investigations about phenylpropanoid-conjugated triterpenoids should be carried out.

4. Conclusion

In summary, the cytotoxicities of four *Leptopus* genus plants were first studied, and thirty-one compounds including nine new phenylpropanoid-conjugated triterpenoids were isolated from the whole plants of *L. lolonum*. All triterpenoids were tested their cytotoxicities on HepG2, MCF-7, A549 and HeLa cancer cells, and triterpenoid with phenylpropanoid moiety showed increasing cytotoxicity compared with betulinic acid and oleanolic acid. The structure–activity relationships of compounds **10**, **11**, **14**, **H1** and **H2** indicated that *trans-p*-coumaroyl group exhibited more potent effect compared with caffeoyl or feruloyl moiety. The present investigation has disclosed that *L. lolonum* possesses abundant triterpenoids with a phenylpropanoid moiety and this may provide potential support for the application of traditional Chinese medicine. Moreover, an efficient synthetic route of phenylpropanoid-conjugated triterpenoids has been studied. Thus, *in vivo* experiments will be needed in our further study to comprehensive evaluation of anticancer effect of phenylpropanoid-conjugated triterpenoids.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2021.104628.

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