Inhibitory Effect of Four Triterpenoids from *Trichilia connaroides* on Nitric Oxide Production in Lipopolysaccharide-Stimulated RAW264.7 Cells

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Two new highly rearranged trichiliton-type limonoids (trichilitons G–H, 1–2), and two new cycloartane-type triterpenoids, 21-(*E*)-coumaroyloxy-5 α -cycloart-24-ene-3,23-dione (3) and 21-(*E*)-feruloyloxy-5 α -cycloart-24-ene-3,23-dione (4), were isolated from the stem and bark of *Trichilia connaroides*. These isolates were elucidated on the basis of detailed spectroscopic analysis, and inhibitory effect of these compounds on lipopolysaccharide-induced nitric oxide production in mouse macrophage RAW264.7 cells was evaluated.

Key words *Trichilia connaroides*; Meliaceae; triterpenoid; nitric oxide production inhibition

Trichilia connaroides (WIGHT *et* ARNOTT) BENTV (Meliaceae), has been used as folk medicine in China for treatment of arthritis, pharyngitis, tonsillitis, and other ailments.¹⁾ Previous chemical investigations on the constituents of *T. connaroides* led to the discovery of many structurally diverse triterpenoids with complex ring systems,^{2–7)} which inspired a continuing interest of this plant. Searching for structurally unique and biologically interesting secondary metabolites, we investigated the dichloromethane extract of the stem and bark of this plant. As a result, two new highly rearranged trichiliton-type limonoids (trichilitons G–H, 1–2), and two new cycloartane-type triterpenoids, 21-(*E*)-coumaroyloxy-5 α -cycloart-24-ene-3,23-dione (3) and 21-(*E*)-feruloyloxy-5 α -cycloart-24-ene-3,23-dione (4) were isolated and identified. Herein we described the isolation, structure elucidation of these compounds, and their inhibitory effects on lipopolysaccharide-induced nitric oxide production in mouse macrophage

RAW264.7 cells.

Results and Discussion

The dichloromethane extract of the stem and bark of *T. connaroides* was chromatographed on MCI, silica gel, and octadecyl silica (ODS) and followed by semi-preparative HPLC to afford four new triterpenoids (1–4) (Fig. 1). The chemical structures of these compounds were determined on the basis of detailed spectroscopic analysis, including high resolution-electrospray ionization-mass spectrum (HR-ESI-MS), one dimensional (1D)- and 2D-NMR experiments.

Trichiliton G (1) was obtained as a white amorphous powder. Its molecular formula was determined to be C₃₂H₃₈O₁₀ by HR-ESI-MS, as indicated by a pseudo-molecular ion at *m/z* 581.2399 [M–H][–] (Calcd for C₃₂H₃₇O₁₀, 581.2392), suggesting 14° of unsaturation. The IR spectrum showed strong absorption bands corresponding to functionalities of

Fig. 1. Structures of Compounds 1–4

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Table 1. ¹H (500 MHz) and ¹³C (125 MHz) Data of Compounds 1–4 Measured in CDCl₃

Position	1		2		3		4	
	δ_C	δ_H (<i>J</i> in Hz)	δ_C	δ_H (<i>J</i> in Hz)	δ_C	δ_H (<i>J</i> in Hz)	δ_C	δ_H (<i>J</i> in Hz)
1a	221.0		220.0		33.7	1.83, m	33.7	1.83, m
1b						1.52, m		1.51, m
2a	135.1/135.0	5.84/5.88, dd (12.5, 7.5)	135.2	5.87 ^{a)}	37.7	2.71, td (13.5, 6.0)	37.7	2.69, td (14.0, 6.5)
2b						2.30, dt (13.5, 3.5)		2.29, dt (14.0, 4.0)
3	76.9	4.82, d (7.5)	77.3	4.81, dd (5.5, 2.5)	216.8		216.6	
4	43.6		43.7		50.5		50.5	
5	40.1	3.78, m	41.2	3.71, brd (13.5)	48.7	1.70, dd (12.0, 4.0)	48.7	1.70, dd (12.0, 4.0)
6a	34.0	2.51, m	34.5	2.61/2.54, dd (15.5, 12.5)	21.7	1.56, m	21.7	1.54, m
6b		2.35, m		2.41 ^{a)}		0.97, m		0.96, m
7a	173.4		176.6		26.1	1.38, m	26.1	1.37, m
7b						1.15, m		1.15, m
8	133.3/133.4		132.5		48.0	1.61, m	48.1	1.61, m
9	44.8	2.70, brd (7.0)	44.4	2.72, d (5.5)	21.2		21.2	
10	54.4		54.6		26.3		26.3	
11a	19.5	(α) 1.65 ^{a)}	19.3	(α) 1.76, m	26.9	2.03, m	26.9	2.02, m
11b		(β) 1.90, m		(β) 2.00, m		1.19, m		1.19, m
12 α	28.4	1.13, m	28.4	1.31, m	32.0	1.63, m	32.0	1.63, m
12 β		1.65 ^{a)}		1.53, m				
13	38.2/38.3		38.8		45.6		45.6	
14	129.6/129.5		130.7		49.3		49.3	
15	33.1	3.13/3.11, brs	33.0	3.10/3.13, s	35.7	1.37 ^{a)}	35.7	1.36 ^{a)}
16a	169.1		168.1		28.1	1.84, m	28.1	1.84, m
16b						1.37 ^{a)}		1.36 ^{a)}
17	79.2/79.4	5.32/5.36, s	80.0	5.22, s	47.5	2.00, m	47.5	2.00, m
18	16.7/16.8	1.06/1.03, s	16.8	1.11, s	18.5	1.11, s	18.6	1.12, s
19a	22.9	1.00/0.99, s	23.3	1.02/1.00, s	29.7	0.80, d (4.0)	29.8	0.80, d (4.0)
19b						0.57, d (4.0)		0.58, d (4.0)
20	134.1/134.3		163.1		37.2	2.49, m	37.2	2.50 ^{a)}
21a	168.8		97.9	6.11, s	66.5	4.33, dd (11.5, 3.0)	66.6	4.34, dd (11.0, 2.5)
21b						4.02, dd (11.5, 6.5)		4.03, dd (11.0, 7.0)
22	150.0/150.2	7.35/7.36, s	122.2	6.28, s	46.7	2.50, m	46.7	2.50 ^{a)}
23	96.8/97.2	6.19/6.22, s	169.5		200.5		200.3	
24					124.5	6.06, brs	124.5	6.06, brs
25					155.5		155.4	
26					27.9	1.85, s	27.9	1.84, s
27					21.04	2.13, s	21.02	2.13, s
28	22.6	1.13, s	23.1	1.15/1.14, s	22.4	1.04, s	22.4	1.04, s
29a	47.0	2.05, d (17.5)	46.6	2.11/2.09, d (17.5)	21.0	1.10, s	21.0	1.10, s
29b		2.45, d (17.5)		2.41, d (17.5)				
30	129.9/129.8	5.85/5.87, d (12.5)	129.8	5.87 ^{a)}	19.6	0.93, s	19.7	0.93, s
OMe	52.1	3.68/3.69, s	52.9	3.76/3.72, s			56.3	3.93, s
1'	167.5		167.2		127.5		127.3	
2'	128.5		128.7		130.2	7.39, d (8.5)	109.7	7.01, d (2.0)
3'	139.7	7.34, q (7.0)	139.3	7.07, q (7.0)	116.2	6.84, d (8.5)	147.1	

Table 1. Continued

Position	1		2		3		4	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
4'	14.7	1.76, d (7.0)	14.6	1.76, d (7.0)	158.1		148.2	
5'	12.3	1.79, s	12.4	1.77, s	116.2	6.84, d (8.5)	115.0	6.91, d (8.0)
6'					130.2	7.39, d (8.5)	123.3	7.05, dd (8.0, 2.0)
7'					144.5	7.56, d (16.0)	145.0	7.55, d (16.0)
8'					115.8	6.22, d (16.0)	115.8	6.22, d (16.0)
9'					167.5		167.5	

a) Overlapped with other signals.

hydroxyl (3450 cm^{-1}), carbonyl (1735 cm^{-1}) and double bond (1641 cm^{-1}). Apart from the easily distinguishable signals for a methoxyl [δ_H 3.68/3.69 (3H, s)], and a tigloyl group [δ_H 1.76 (3H, d, $J=7.0\text{ Hz}$), 1.79 (3H, s), and 7.34 (1H, q, $J=7.0\text{ Hz}$)], the ^1H -NMR spectrum of **1** (Table 1) also presented three singlet methyl protons at δ_H 1.06/1.03, 1.00/0.99 and 1.13. Its ^{13}C -NMR spectrum (Table 1) resolved 32 carbon resonances, after deduction of the carbons belonging to the methoxyl and the tigloyl group, 26 carbons remained in the skeleton, suggesting a tetranortriterpenoid nature of **1**. Nine degrees of unsaturation were accounted by four double bonds, four ester carbonyls, and one ketone carbonyl, the remaining five degrees of unsaturation requiring five rings for compound **1**. The signals for a typical lactone ring D was observed in the NMR spectrum, characterized by the following NMR data [δ_H 3.13/3.11 (2H, br s), 5.32/5.36 (1H, s); δ_C 38.2/38.3, 129.6/129.5, 33.1, 169.1, 79.2/79.4]. Its presence was also evidenced by the heteronuclear multiple bond connectivity (HMBC) correlations (Fig. 2) of H_2 -15/C-14, H_2 -15/C-16, H_2 -15/C-13 and H-17/C-13. A carbon signal at δ_C 47.0 corresponded in the heteronuclear single quantum coherence (HSQC) spectrum to a pair of geminal doublets at δ_H 2.05 and 2.45, which had the HMBC correlations with ketone carbonyl C-1, C-4, C-10, C-5 and C-28, was assigned to C-29. In the HMBC spectrum, correlations from a multiplet at δ_H 3.78 (H-5) to C-6, C-10, C-1 and C-4, and from a singlet methyl proton at δ_H 1.00/0.99 (Me-19) to C-10, C-5 and C-1, suggested the construction of a cyclopentanone ring A by C-1, C-29, C-4, C-5, and C-10, characteristic of a trichiliton-type limonoids. Detailed analysis of its NMR data revealed that it was structurally similar to chisomicine A,⁸ except for ring E: the β -furan ring in chisomicine A was replaced by a γ -hydroxybutenolide group,^{9,10} characterized by a pair of protons at δ_H 7.35/7.36 (H-22) and 6.19/6.22 (H-23), and carbon resonances in pair at δ_C 134.1/134.3 (C-20), 168.8 (C-21), 150.0/150.2 (C-22), and 96.8/97.2 (C-23). HMBC cross-peaks from H-17 to C-20, C-21 and C-22, located the γ -hydroxybutenolide group at C-17; the strong HMBC correlation between the proton at δ_H 4.82 (H-3) and the carbonyl carbon of the tigloyl group at δ_C 167.5 allowed its position at C-3. Thus, the planar structure of **1** was established. The relative configuration of **1** was determined by the rotating frame Overhauser effect correlation spectroscopy (ROESY) experiment. As shown in Fig. 2, the observed correlations of Me-28/H-5, H-5/H-17, H-17/H-12 β , and H-5/Me-19 revealed

Fig. 2. Key HMBC and ROESY Correlations of **1**

that H-5, H-17, Me-19, and Me-28 were cofacial and thus were arbitrarily assigned to be β -oriented. The assignment of Me-18 as α -orientation was deduced from the ROESY correlations of Me-18/H-22 and Me-18/H-11 α . The correlations of H-3/H-29 α , Me-19/H-29 β , and Me-19/H-9 indicated that H-3, H-9, and H_2 -29 were α -oriented. The relative configurations of all the chiral centers of **1** are in agreement with trichiliton A obtain from this plant.²⁾ The molecule adopt a cage-like conformation, and the lactone ring D is in a half-chair conformation. Thus, the structure of **1** was established as trichiliton G.

Compound **2** had the same molecular formula of $\text{C}_{32}\text{H}_{38}\text{O}_{10}$ as that of **1**. The NMR spectroscopic data (Table 1) of **1** and **2** suggested a great structure resemblance of them except for ring E. The NMR spectroscopic data of **2** suggested the presence of a 21-hydroxybutenolide ring E characterized by protons at δ_H 6.28 (H-22) and 6.11 (H-21), and by carbon resonances at δ_C 163.1 (C-20), 97.9 (C-21), 122.2 (C-22), and 169.5 (C-23), which was confirmed by the HMBC correlation between H-17 and the hemiacetal carbon (C-21).^{10,11} The relative configuration of **2** was elucidated to be the same as that **1**. The structure of **2** was thus established as trichiliton H.

21-(*E*)-Coumaroyloxy-5 α -cycloart-24-ene-3,23-dione (**3**) was obtained as white amorphous powder and its molecular formula was deduced to be $\text{C}_{39}\text{H}_{52}\text{O}_5$ with 14° of unsaturation on the basis of its HR-ESI-MS positive-ion at m/z 623.3705 [$\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{39}\text{H}_{52}\text{O}_5\text{Na}$, 623.3707). Its IR spectrum showed strong absorption bands corresponding to functionalities of hydroxyl (3423 cm^{-1}), carbonyl (1704 cm^{-1}), and olefinic groups (1628 cm^{-1}). An A_2X_2 coupling system

Fig. 3. ^1H - ^1H COSY, Key HMBC and ROESY Correlations of **3**

existed through the signals at δ_{H} 7.39 and 6.84 (d, $J=8.5\text{ Hz}$) and an AX-type signals at δ_{H} 7.56 and 6.22 (d, $J=16.0\text{ Hz}$) in its ^1H -NMR spectrum (Table 1), indicated the presence of (*E*)-coumaroyl group.¹² Furthermore, the ^1H -NMR spectrum also showed the presence of a broad singlet of an olefinic methine at δ_{H} 6.06, four tertiary methyl singlets at δ_{H} 0.93, 1.10, 1.11, and 1.04 in the up-field region, two geminal vinyl-methyls at δ_{H} 1.85 and 2.13, as well as C-9/C-10 cyclopropyl methylene, featuring a pair of geminal protons at δ_{H} 0.80 and 0.57 (d, $J=4.0\text{ Hz}$) in the up-field region. Except for the nine carbons belonging to the (*E*)-coumaroyl moiety, the remaining 30 carbons in the ^{13}C -NMR spectrum, suggested **3** to be a normal cycloartane-type triterpene.¹³ Aided with the HSQC spectrum, these carbons were categorized into 6 tertiary methyls, 11 methylenes (one oxymethylene at δ_{C} 66.5), 5 methines (one olefinic carbon at δ_{C} 124.5), and 8 quaternary carbons (two ketone at δ_{C} 216.5 and 200.5, and one olefinic carbon at δ_{C} 155.5). The HMBC correlations from a ketone carbonyl carbon at δ_{C} 216.5 to H_2 -2 (δ_{H} 2.71 and 2.30), Me-28 (δ_{H} 1.04) and Me-29 (δ_{H} 1.10), located the ketone group at C-3. In the HMBC spectrum, correlations from an olefinic proton at δ_{H} 6.06 to two geminal vinylmethyls C-26 (δ_{C} 27.9) and C-27 (δ_{C} 21.04), and a ketone carbonyl carbon at δ_{C} 200.5, indicated that **3** possessed a $\Delta^{24,25}$ double bond, and the ketone carbonyl carbon was assigned to be C-23, which was also evidenced by the HMBC correlation from H_2 -22 (δ_{H} 2.50) to this ketone carbonyl carbon. HMBC cross-peaks from a pair of oxymethylene protons at δ_{H} 4.33 (dd, $J=11.5$, 3.0 Hz) and 4.02 (dd, $J=11.5$, 6.5 Hz) to the carbonyl carbon of the (*E*)-coumaroyl moiety C-9' (δ_{C} 167.5), C-20 (δ_{C} 37.2), and C-22 (δ_{C} 46.7), and ^1H - ^1H correlation spectroscopy (COSY) correlation between these two oxymethylene protons with H-20 (δ_{H} 2.49), attached the (*E*)-coumaroyloxy moiety to C-21. The full assignment and connectivity of protons and carbons in the structure were made by 2D-NMR experiments (Fig. 3). Therefore, the planar structure of **3** was established as shown.

The relative configuration of **3** was determined on the basis of ROESY spectrum (Fig. 3). The significant cross-peaks from Me-29 to H-19a and H-19b, and from H-19a to H-8, suggested that H-8, H_2 -19, and Me-29 were co-facial and were arbitrarily assigned to be in β -orientation; correlations of H-5/Me-28, and H-17/Me-30 revealed an α -orientation for these protons. According to biosynthetic origin, Me-18 of cycloartane-type triterpenes was constantly in β -orientation.¹³⁻¹⁶ The NOE associations via a ROESY spectrum from ring to side chain protons could lead to erroneous configurational conclusions. Generally, H-17 and H-20 are biosynthetically in *trans* configuration for cycloartane-type triterpenes: H-17 was assigned to be α -orientated according to the ROESY correlation of H-17/

Me-30, H-20 was thus in β -orientation accordingly, consistent with those reported in the literature.¹³⁻¹⁶ Therefore, the structure of **3** is established as 21-(*E*)-coumaroyloxy-5 α -cycloart-24-ene-3,23-dione.

21-(*E*)-Feruloyloxy-5 α -cycloart-24-ene-3,23-dione (**4**) had a molecular formula of $\text{C}_{40}\text{H}_{54}\text{O}_6$, as determined by the observed pseudo-molecular ion at 631.3992 $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{40}\text{H}_{55}\text{O}_6$, 631.3993) in the HR-ESI-MS, 30 units more than that of **3**. Its NMR data exhibited a pair of doublets typical for cyclopropane methylene protons at δ_{H} 0.80 and 0.58, a broad singlet olefinic methine at δ_{H} 6.06, four tertiary methyl singlets at δ_{H} 0.93, 1.10, 1.12, and 1.04, and two geminal vinylmethyls at δ_{H} 1.85 and 2.13, suggesting **4** to be also a cycloartane-type triterpenoid like **3**. The great resemblance of their 1D-NMR data, especially those associated with rings A–D and the side chain combined with the 2D-NMR data revealed that they share the same carbon skeleton. The major difference was the appearance of an (*E*)-feruloyl moiety in **4** rather than a (*E*)-coumaroyl moiety at C-21 in **3**, as supported by an AMX-type signals at δ_{H} 7.01 (d, $J=2.0\text{ Hz}$), 7.05 (dd, $J=8.0$, 2.0 Hz), and 6.91 (d, $J=8.0\text{ Hz}$), ascribed to a 1,3,4-trisubstituted aromatic ring, an AX-type signals at δ_{H} 7.55 and 6.22 (d, $J=16.0\text{ Hz}$), and oxygenated methyl protons at δ_{H} 3.93.¹⁷ The similar ROESY correlations of **4** and **3** indicated the same relative configurations of them. Thus, the structure of **4** is established as 21-(*E*)-feruloyloxy-5 α -cycloart-24-ene-3,23-dione.

Four isolates were evaluated for their inhibitory effects of nitric oxide (NO) production stimulated by lipopolysaccharide (LPS) in RAW264.7 cells using *N*-monomethyl-L-arginine as the reference compound with IC_{50} value at $37.7\text{ }\mu\text{M}$. To avoid any false-positive results caused by the cytotoxicity of the tested samples, the effects of the four triterpenoids on the growth of RAW264.7 cells were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay before evaluating their nitric oxide production inhibitory activity. None of them showed any effect on cell growth with a concentration up to $100\text{ }\mu\text{M}$. Among them, compounds **1** and **2** expressed moderate NO inhibition with IC_{50} values of 46.5 and $62.1\text{ }\mu\text{M}$, respectively, which revealed that the trichiliton framework seem to produce a positive effect on the activity. The data were in agreement with those of previously found for analogous compounds.⁸ The other two compounds, both with IC_{50} values over $100\text{ }\mu\text{M}$, were regarded as inactive.

Experimental

General Experimental Procedures Optical rotations were determined with a JASCO P-1020 polarimeter. UV spectra were measured on a Shimadzu UV-2450 spectropo-

larimeter. IR spectra were obtained on a Bruker Tensor 27 spectrometer (KBr discs). NMR spectra were recorded on Bruker ACF-500 instrument (^1H : 500 MHz, ^{13}C : 125 MHz), with tetramethylsilane (TMS) as internal standard. Chemical shifts were given in values of ppm and coupling constants in Hertz. High-resolution mass spectra were recorded on an Agilent UPLC-Q-TOF (6520B). Analytical HPLC was performed on Agilent 1100 series (Agilent, Santa Clara, CA, U.S.A.) with SB-C₁₈ column (4.6×250 mm, 5 μm). Semi-preparative HPLC was carried out using a Shimadzu SCL-10A Series instrument with a Capcell-park RP-C₁₈ column (10×250 mm, 5 μm) and a SPD 6A variable wavelength detector. All solvents used were of analytical grade (Jiangsu Hanbang Science and Technology Co., Ltd.). Silica gel (Qingdao Haiyang Chemical Co., Ltd.), Sephadex LH-20 (Pharmacia), MCI gel (75–150 μm , Mitsubishi) and RP-C₁₈ (40–63 μm , Fuji) were used for column chromatography (CC). The pre-coated silica gel GF₂₅₄ plates (Qingdao Marine Chemical Plant, Qingdao, China) were used for TLC. Spots were visualized by heating silica gel plates immersed in 10% H₂SO₄ in ethanol.

Plant Material The stem and bark of *T. connaroides* (W. et A.) BENTV were collected from Xishuangbanna, Yunnan Province, China, in November 2011 and authenticated by Prof. Shun-Cheng Zhang of Xishuangbanna Botanical Garden, Chinese Academy of Sciences. A voucher specimen (No. ZGH-201111) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation The air-dried stem and bark of *T. connaroides* (10 kg) were extracted with 95% EtOH for 2 h three times under reflux and concentrated to give the extracts. The extracts (1280 g) were suspended in H₂O and partitioned with petroleum ether (PE), dichloromethane (CH₂Cl₂), and ethyl acetate (EtOAc), respectively. The CH₂Cl₂ (64.0 g) extract was subjected to silica gel column and eluted with PE–EtOAc in a gradient (1:0 to 0:1, v/v) to give seven fractions (Frs. 1–7).

Fraction 4 (12.4 g) was chromatographed on MCI gel eluted successively with MeOH–H₂O (2:8 to 9:1, v/v), to give four subfractions (Frs. 4a–d). Fraction 4b (4.0 g) was subjected to columns of silica gel, eluted with CH₂Cl₂–MeOH (100:1 to 5:1, v/v), five subfractions 4b-1 to 4b-5 obtained. Subfractions 4b-3a to 4b-3d was given by separated the fraction 4b-3 (340.0 mg) with reversed-phase ODS (MeOH–H₂O; 5:5 to 9:1, v/v). Fraction 4b-3b (12.0 mg) was further purified by successive RP-18 semi-preparative HPLC by elution with 83% acetonitrile in water. (10 mL/min), to afford **3** (4.0 mg, t_{R} =74.5 min) and **4** (5.0 mg, t_{R} =84.0 min).

Fraction 6 (8.2 g) was subjected to column of MCI gel for removing chlorophyll eluted successively with MeOH–H₂O (2:8 to 9:1, v/v), to provide three major subfractions (Frs. 6a–c). Then fraction 6b (4.5 g) was fractionated by silica gel column chromatography, eluted with CH₂Cl₂–MeOH (100:1 to 5:1, v/v), to give five subfractions (Frs. 6b-1 to 6b-5), and fraction 6b-1 (1.9 g) was further separated by silica gel column chromatography (petroleum ether–acetone; 10:1 to 1:4, v/v), to yield five subfractions 6b-1a to 6b-1e. Fraction 6b-1b (760.0 mg) was chromatographed over successive reversed-phase ODS with MeOH–H₂O (3:7 to 8:2, v/v), to yield seven subfractions, and the fifth fraction (83.5 mg) eluted with 45% acetonitrile in water (10 mL/min) to give **1** (12.0 mg, t_{R} =33.4 min), and **2** (14.5 mg, t_{R} =39.3 min).

Trichiliton G (**1**): White, amorphous powder; $[\alpha]_{\text{D}}^{25}$ –2.3 (c =0.26, MeCN); UV (MeCN) λ_{max} (log ϵ) 196 (4.37), 280 (3.03) nm; IR (KBr) ν_{max} 3450, 2921, 2850, 1735, 1641, 1400, 1273, 1198, 1016 cm^{-1} ; ^1H - and ^{13}C -NMR data, see Table 1; HR-ESI-MS m/z 581.2399 $[\text{M}-\text{H}]^-$ (Calcd for C₃₂H₃₇O₁₀, 581.2392).

Trichiliton H (**2**): White, amorphous powder; $[\alpha]_{\text{D}}^{25}$ –11.9 (c =0.12, MeCN); UV (MeCN) λ_{max} (log ϵ) 198 (4.39), 281 (2.19) nm; IR (KBr) ν_{max} 3450, 2919, 2850, 1736, 1640, 1400, 1274, 1136, 1033 cm^{-1} ; ^1H - and ^{13}C -NMR data, see Table 1; HR-ESI-MS m/z 581.2386 $[\text{M}-\text{H}]^-$ (Calcd for C₃₂H₃₇O₁₀, 581.2392).

21-(*E*)-Coumaroyloxy-5 α -cycloart-24-ene-3,23-dione (**3**): White, amorphous powder; $[\alpha]_{\text{D}}^{25}$ +43.9 (c =0.13, MeCN); UV (MeCN) λ_{max} (log ϵ) 230 (4.09), 312 (3.99) nm; IR (KBr) ν_{max} 3423, 2925, 1704, 1628, 1608, 1513, 1459, 1399, 1275, 1252, 1159, 1046 cm^{-1} ; ^1H - and ^{13}C -NMR data, see Table 1; HR-ESI-MS m/z 623.3705 $[\text{M}+\text{Na}]^+$ (Calcd for C₃₉H₅₂O₅Na, 623.3707).

21-(*E*)-Feruloyloxy-5 α -cycloart-24-ene-3,23-dione (**4**): White, amorphous powder; $[\alpha]_{\text{D}}^{25}$ +38.1 (c =0.11, MeCN); UV (MeCN) λ_{max} (log ϵ) 203 (4.08), 235 (4.08), 323 (3.85) nm; IR (KBr) ν_{max} 3430, 2972, 2923, 1703, 1662, 1629, 1453, 1399, 1273, 1160, 1046 cm^{-1} ; ^1H - and ^{13}C -NMR data, see Table 1; HR-ESI-MS m/z 631.3992 $[\text{M}+\text{H}]^+$ (Calcd for C₄₀H₅₅O₆, 631.3993).

Cytotoxicity Assay The experimental process of cytotoxicity assay was afforded in our previously published paper.¹⁸⁾

Nitric Oxide (NO) Production Bioassays The details of the NO production bioassays were provided in a previously published paper.¹⁹⁾ *N*-Monomethyl-L-arginine was used as a positive control, and all experiments were performed in three independent replicates.

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