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New cytotoxic compounds from flowers of *Lawsonia inermis* L.

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ABSTRACT

Three new compounds, a bicoumarin A (**1**), a biflavonoid A (**2**), and a biquinone A (**3**), as well as 12 other known compounds, were isolated from the flower of *Lawsonia inermis* L. The structures were elucidated by spectral analysis and new compounds **2** and **3** then were further confirmed by ECD calculations and single-crystal X-ray diffraction crystallography respectively. The cytotoxicity of the compounds against four cancer cell lines, including MCF-7, Hela, HCT-116, and HT-29 were evaluated using MTT assay. The IC_{50} values of compounds **3** and **5** against MCF-7, Hela, HCT-116, and HT-29 were 2.24, 1.42, 24.29, and 7.02 μM and 6.1, 2.44, 5.58, and 10.21 μM respectively. The two compounds exhibited stronger inhibitory activities than the positive control 5-fluorouracil (IC_{50} = 7.34, 11.50, 36.17, 18.83 μM) against the four tested cell lines. These results demonstrated that compounds from the flowers of *L. inermis* L. showed cytotoxic activity on MCF-7, Hela, HCT-116, and HT-29 cell lines.

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1. Introduction

Lawsonia inermis L. (*L. inermis*), a single-species genus of the Lythraceae family, is distributed in dry tropical and subtropical regions, including North Africa, India, Sri Lanka, and the Middle East [1]. Modern pharmacological studies have demonstrated that the plant has anti-inflammatory [2], antioxidant [3], anti-osteoclastogenic [4], and protein glycation inhibition activities [5]. The plant has been used as a traditional medicine with a long history of use in the treatment of cancer. It has also been shown to be an effective anticancer agent [6]. Lawsone from *L. inermis* showed cytotoxic activities [7]. The crude dichloromethane extract of *L. inermis* leaves showed significant cytotoxic activity on MCF-7 and HepG2 [8]. The ethanolic extract of *L. inermis* increased the life span of Dalton's lymphoma ascites tumor bearing mice [9]. Because the crude extracts and fractions of *L. inermis* show cytotoxic activities, screening of plant extracts is necessary to isolate and characterize its active compounds.

In the present work, new bicoumarin, biflavonoid, and biquinone compounds, as well as twelve other known compounds **4** to **15** (Fig. 1), were isolated from the dichloromethane extract (DCM) of *L. inermis* flowers through repeated column chromatography (silica gel, sephadex LH-20, RP-18, and semi-preparative HPLC). All of the compounds were evaluated for cytotoxicity against the human cervical carcinoma (Hela), human breast cancer (MCF-7), and human colorectal cancer cell lines (HCT-116, HT-29) using MTT assay. 5-fluorouracil (5-FU) was used as positive control.

2. Results and discussion

2.1. Results

Compound **1** was obtained as a yellow powder (MeOH). The molecular formula was determined as $C_{21}H_{16}O_8$ by HR-ESI–MS at m/z 419.0734 $[M + Na]^+$ (calcd. for $C_{21}H_{16}O_8Na$ 419.0737).

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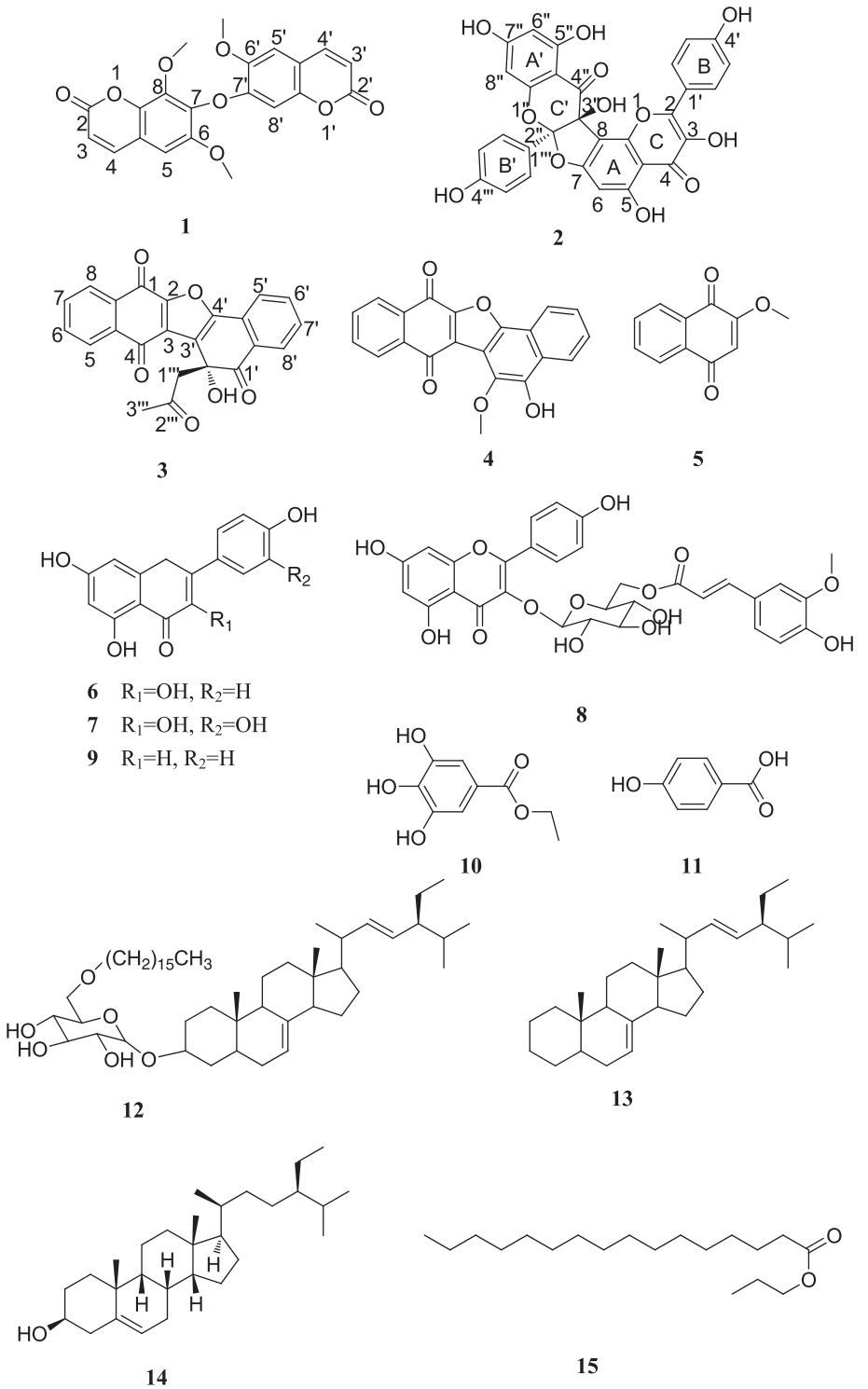

Fig. 1. Structures of compounds **1**–**15**.

The ^{1}H NMR spectrum (Table 1) showed a methoxyl group at δ_H 3.86 (3H, s), and two methoxyl groups at δ_H 3.81 (6H, s), while the appearance of δ_H 6.20 (1H, d, J = 9.3 Hz), 6.23 (1H, d, J = 9.3 Hz) and δ_H 7.98 (2H, d, J = 9.3 Hz) was the typical signals of coumarins. Apart from the carbon signals due to three methoxy groups (δ_C 56.0, 56.2 and 60.8), there remain sixteen aromatic carbons and two lactone carbons at δ_C 160.3 and 160.8 in the ^{13}C NMR spectrum (Table 1), suggesting that compound

Table 1
NMR data of compounds **1**–**3** in DMSO (^{1}H: 600 MHz, ^{13}C: 150 MHz).

Position	1		2		3	
	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)
1					173.4	
2	160.8		147.5		153.1	
3	111.7	6.23 d (9.3)	136.2		129.3	
4	144.9	7.89 d (9.3)	176.3		180.2	
5	104.5	7.01 s	165.0		126.6	8.13 m
6	144.2		94.7	6.70 s	134.8	7.89 m
7	145.72		164.0		134.8	7.89 m
8	134.8		106.2		127.4	8.13 m
9	143.1		151.3		133.4	
10	110.3		105.3		132.3	
1′			121.5		196.9	
2′	160.3		130.3	8.15 d (8.9)	70.0	
3′	112.1	6.20 d (9.3)	115.4	6.94 d (8.9)	124.0	
4′	144.5	7.89 d (9.3)	159.7		152.8	
5′	109.7	7.19 s	115.4	6.94 d (8.9)	122.1	7.96 brd (7.15)
6′	145.4		130.3	8.15 d (8.9)	135.3	7.87 td(7.15,1.12)
7′	151.3				131.0	7.66 td (7.15,1.12)
8′	102.9	6.78 s			128.4	8.04 brd (7.15)
9′	149.6				128.9	
10′	110.6				128.3	
1″					54.0	4.38 d (18.30)
						3.73 d (18.30)
2″			117.9		207.6	
3″			80.0		29.9	1.94 s
4″			190.9			
5″			163.5			
6″			97.1	5.90 d (1.9)		
7″			168.0			
8″			95.3	5.93 d (1.9)		
9″			160.5			
10″			98.7			
1‴			123.4			
2‴			128.4	7.28 d (8.7)		
3‴			114.9	6.79 d (8.7)		
4‴			158.8			
5‴			114.9	6.79 d (8.7)		
6‴			128.4	7.28 d (8.7)		
2′-OH						6.29 s
6-OMe	56.0	3.80 s				
8-OMe	60.8	3.86 s				
6′-OMe	56.2	3.80 s				

1 possessed a bicoumarin skeleton. From the information provided, the NMR data of **1** resembled those of arteminorin B [10], except for some subtle differences. The lack of a hydroxyl group at C-3 in compound **1** was confirmed by its carbon signals with chemical shift of δ_C 111.7, together with signals of C-2, 4 downfielded from δ_C 156.7, 128.0 to δ_C 160.8, 144.9 respectively, while C-10 upfielded from 115.1 to δ_C 110.3. A NOESY experiment was a key step to finally confirm the structure of **1**. The results were presented in Fig. 2. The cross signals observed between H-3 and H-4 can reinforce the lack of a hydroxyl group at C-3, while cross signals observed between H-5 and the methoxy protons at δ_H 3.81, H-3′ and H-4′, H-4′ and H-5′ (δ_H 7.19), H-5′ and the methoxyl protons at δ_H 3.81, were very close to those of arteminorin B. Therefore, compound **1** was defined as 6,8-dimethoxy-7-(6′-methoxy-7′-coumarinyloxy) coumarin and named as *Lawsonia* bicoumarin A.

Compound **2** was isolated as a yellow powder (MeOH). Its molecular formula was determined as $C_{30}H_{18}O_{12}$ by HR-ESI–MS at m/z 571.0872 $[M + H]^+$ (calcd. for $C_{30}H_{19}O_{12}$ 571.0871). The ^{1}H NMR spectra exhibited, in the aromatic region, a 2,4,6-trioxyphenyl group at δ_H 5.91 (1H, d, $J = 1.0$ Hz) and 5.89 (1H, d, $J = 1.0$ Hz), two pairs of 4-oxyphenyl groups at δ_H 6.94 (2H, d, $J = 8.9$Hz), 8.15 (2H, d, $J = 8.9$Hz), 6.79 (2H, d, $J = 8.7$Hz), and 7.28 (2H, d, $J = 8.7$Hz) as well as four phenolic hydroxyl groups at δ_H 9.80, 10.20, 11.81, and 13.28 (each 1H, s) and one isolated proton at 6.70 (1H, s). The ^{13}C NMR spectrum (Table 1) showed 30 carbon signals, consisting of two carbonyl signals at δ_C 190.9 and 176.3. The HMBC experiment (Fig. 3) revealed long range coupling from δ5.89 to C-8″(δ_C 95.3), C-10″(δ_C 98.7), C-5″(δ_C 163.5), and C-7″(δ_C 168.0), and from δ5.90 to C-6″ (δ_C 97.1), C-10″ (δ_C 98.7), C-9″ (δ_C 160.5), and C-7″ (δ_C 168.0), reinforced the structure of A′-ring. Correlations from δ6.70 to C-10 (δ_C105.3), C-8 (δ_C106.2), C-7 (δ_C164.0), and C-5 (δ_C165.0) indicate the isolated proton attached to C-6. The comparison of the spectral data with a known compound daphnodorin H [11] revealed that compound **2** was a derivative of biflavonoid. Only some subtle changes of C-ring were observed (one more carbonyl signals at δ_C 176.3 and one olefinic carbon group at δ_C 147.5, 136.2), indicating the presence of flavonol moiety instead of flavan-3-ol moiety.

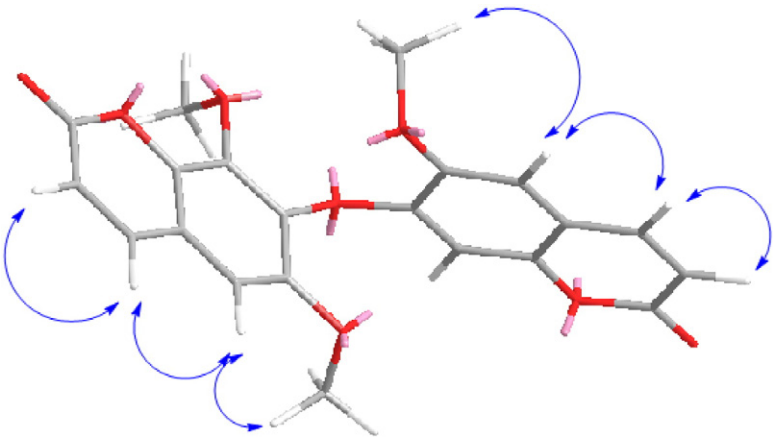

Fig. 2. NOESY correlations of compound **1**.

The determination of the absolute configuration of C-2″ and C-3″ in compound **2** was established circular dichroic (CD) spectra. The CD spectra of **2** showed a negative cotton effect similar to that of daphnodorin H [11]. ECD spectrum was calculated using the GAUSSIAN09 program to provide more evidence of the absolute configuration. In the 200–400 nm region, compared to the experimental negative cotton effects at 275, and 321 nm, the ECD calculated one showed the same pattern with two negative cotton effects at 280 nm (+5 nm), and 315 nm (−6 nm) respectively [16,17]. Therefore, the absolute configuration of C-2″and C-3″ was assigned as 2″S, 3″S. The above evidences allowed compound **2** to be determined as (2″S,3″S)-3,5,4′,3″,5″,7″,4‴-heptahydroxyl furano[2″,3′:7,8] diflavone and given the name of *Lawsonia* biflavone A.

Compound **3** was obtained as orange crystal (MeOH) and the molecular formula was determined as $C_{23}H_{14}O_6$ by HR-ESI–MS mass spectrum at m/z 409.0669 $[M + Na]^+$ (calcd. for $C_{23}H_{14}O_6Na$ 409.0683). ^1H NMR (Table 1) spectrum showed signals assignable to two pairs of 1,2-disubstituted benzene at δ_H 8.13 (2H, m), 7.09 (2H, m) and 8.04 (1H, brd, $J = 7.15$ Hz), 7.96 (1H, brd, $J = 7.15$ Hz), 7.87 (1H, td, $J = 7.15$ Hz, 1.12 Hz), 7.66 (1H, td, $J = 7.15$ Hz, 1.12 Hz), and a hydroxyl at δ_H 6.29 (2′-OH, s). The ^{13}C NMR (Table 1) and DEPT spectra of **3** exhibited twenty three carbon signals including four carbonyl carbon signals, eight methines, nine quaternary carbons, a methylene and a methyl. The existence of a $-CH_2COCH_3$ chain (a carbonyl signal at δ_C 207.6; a methylene at δ_C 54.0, δ_H 4.38 (1H, d, $J = 18.30$ Hz), 3.73 (1H, d, $J = 18.30$ Hz); a methyl at δ_C 29.9, δ_H 1.94 (3H, s)), a carbonyl signal at δ_C 196.9 and a hydroxyl at δ_H 6.29 (2′-OH, s) was also observed.

The HMBC correlations (Fig. 3) from 2′-OH (δ_H 6.29, s) to C-1‴ (δ_C 54.0), C-2′ (δ_C 70.0), C-3′ (δ_C 124.0), and δ_H 4.38 (1H, d, $J = 18.30$ Hz), 3.73 (1H, d, $J = 18.30$ Hz) to C-2′ (δ_C 70.0), C-3′ (δ_C 124.0), C-1′ (δ_C 196.9), C-2‴ (δ_C 207.6), were observed, indicating that the $-CH_2COCH_3$ chain and the hydroxyl were attached to C-2′.

The NMR data of compound **3** was similar to that of balsaminone C [12] which just described its relative configuration. In present study the absolute configurations of **3** were established by single crystal X-ray diffraction analysis (Fig. 4). Thus, the chiral center C-2′ of compound **3** was elucidated 2R, and named (2R) balsaminone C (Fig. 1).

Besides the three new compounds, the known compounds **4**–**15** (Fig. 1) were identified as balsaminone A (**4**) [13], 2-methoxyl-1,4-naphthoquinone (**5**) [14], kaempferol (**6**),

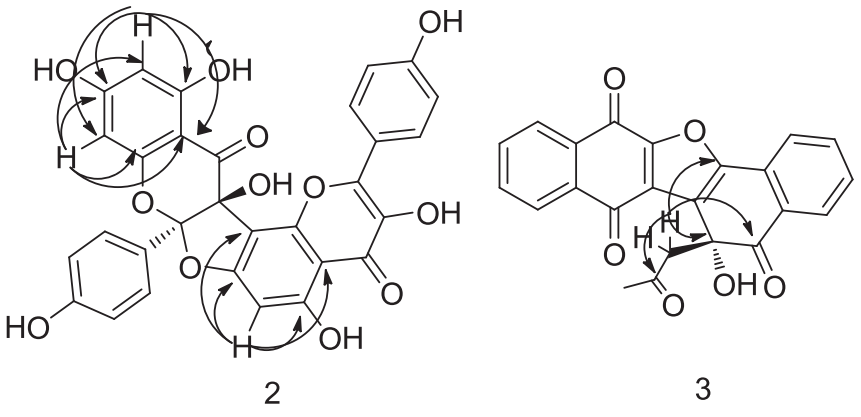

Fig. 3. Key HMBC corrections of compounds **2** and **3**.

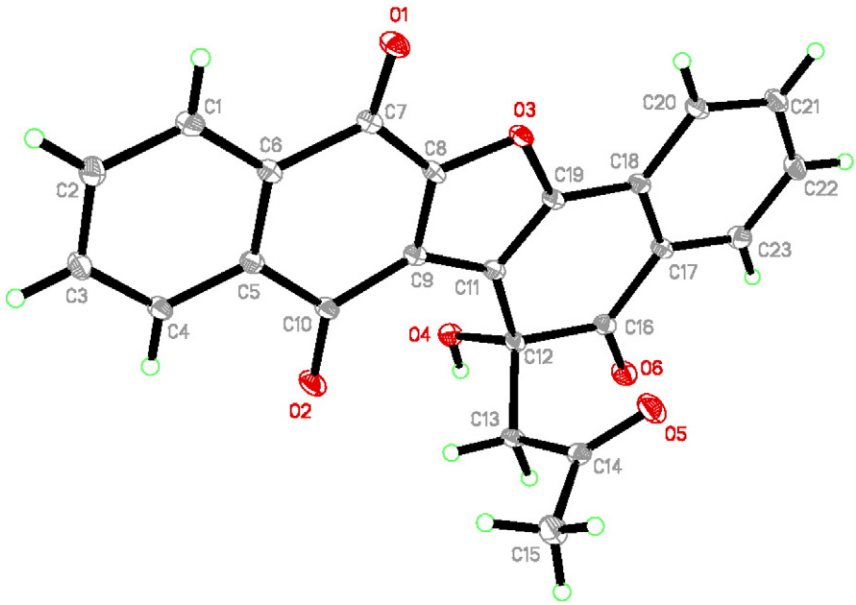

Fig. 4. X-ray crystal structure of compound **3**.

quercetin(**7**), β-sitosterol (**14**) [15], kaempferol-3-*O*-(6″-*O*-*E*-feruloyl)-β-*D*-glucopyranoside (**8**) [16–18], apigenin (**9**) [19] ethyl gallate (**10**) [20], p-hydroxybenzoicacid (**11**), 3-*O*-[6′-*O*-palmitoyl-β-*D*-glucosyl]-α-spinasterol (**12**), spinasterol (**13**) [21], and propyl heptadecanoate (**15**) [22] on the basis of the NMR data.

The inhibitory effects of the obtained compounds on MCF-7, Hela, HCT-116, and HT-29 cell lines were determined by MTT assay [23].

Table 2 shows that compounds **3**, **4**, and **5**, which could be structurally classified as 1,4-naphthoquinones, showed significant activities. In particular, compounds **3** and **5** displayed much stronger inhibitory activities than 5-FU. While the IC_{50} values of compound **3** were 2.24 μM for MCF-7 cells, 1.42 μM for Hela cells, 24.29 μM for HCT-116 cells, and 7.02 μM for HT-29 cells, those of compound **5** were 6.1 μM for MCF-7 cells, 2.44 μM for Hela cells, 5.58 μM for HCT-116 cells and 10.21 μM for HT-29 cells. All of these values are much lower than those of 5-FU (16.28, 7.34, 36.17, and 18.83 μM for MCF-7, Hela, HCT-116, and HT-29 cells, respectively).

Table 2
Inhibition effects of compounds **1**–**15** on the growth of tumor cells in vitro (IC_{50}, μM).

Compound	IC_{50} (μM)			
	MCF-7	Hela	HCT-116	HT-29
1	>50	49.19	>50	63.18
2	32.81	>50	>50	44.87
3	2.24	1.42	24.29	7.02
4	36.96	7.63	20.71	21.23
5	6.1	2.44	5.58	10.21
6	>50	20.63	>50	>50
7	>50	>50	>50	>50
8	>50	>50	>50	>50
9	>50	>50	>50	>50
10	>50	>50	>50	>50
11	>50	>50	>50	NA[a]
12	47.95	>50	>50	>50
13	>50	>50	>50	>50
14	>50	NA[a]	>50	>50
15	NA[a]	NA[a]	>50	>50
5-FU[b]	16.28	7.34	36.17	18.83

[a] Not active 5-FU[b] was used as positive control.

2.2. Conclusion

The present phytochemical study of the DCM extract of *L. inermis* flowers yielded three new compounds **1** to **3** and 12 other known compounds using chromatographic methods. The structures of the compounds were elucidated by spectroscopic analysis and their cytotoxicities against human cancer Hela, MCF-7, HCT-116, and HT-29 cell lines were evaluated by MTT assay. Study showed that 1,4-naphthoquinone and its derivatives could be potent inhibitors of human cancer cell growth [24]. Wang YC investigated the cytotoxicity of compound **5** against gastric adenocarcinoma (MKN45 cell line) and proposed the relevant mechanisms [25]. Our present study demonstrates that new compound **3** and compounds **4**, and **5** which could be structurally classified as 1,4-naphthoquinone, showed significant cytotoxicities against human cancer MCF-7, HCT-116 and HT-29 cell lines for the first time. Compound **3** displayed stronger cytotoxicities toward cell lines tested than compounds **4**, and **5**. The new compound **3** was considered to have the potential to be an antitumor agent, which could significantly inhibit the cancer cell growth in a dose-dependent manner.

3. Experimental

3.1. Plant material

The flowers of *L. inermis* were purchased from Nanjing Zelang Phar. CO. Ltd. (Nanjing, China) in November 2010. The

voucher specimen of this herb (No. 201010) was identified by Prof. Qishi Sun of Shenyang Pharmaceutical University.

3.2. General

Column chromatography (CC): silica gel (SiO_2: 300–400 mesh, Qingdao Marine Chemical Group, Co.); RP C18 silica gel (300–400 mesh, Agela Technologies Co.); Macroporous resin D 101 (Tianjin Chemical Co.) and Sephadex LH-20 (Pharmacia, Co.). Optical rotations were measured on a P-E 241 MC polarimeter using methanol as the solvent. IR spectra on a Bruker IFS-55 infrared spectrophotometer were recorded in MeOH and KBr disks, respectively. NMR spectra were recorded with a Bruker ARX-600 (^1H, 600 MHz; ^{13}C, 150 MHz) spectrometer in C_5D_5N with tetramethylsilane as internal standard. High-resolution electrospray ionization mass spectra (HRESIMS) were recorded on an Agilent 1100 LC–MSD TOF (time-of-flight) system [ionization mode, positive; nebulizing gas (N_2) pressure, 35 psi; drying gas (N_2) flow, 12 L/min, temp, 325 °C; capillary voltage, 3000 V; fragmentor voltage, 225 V].

MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Sino-American Biotechnology (Beijing, China). The analytical grade solvents were used for extraction and the mobile phase includes ethanol, petroleum ether, dichloromethane, ethyl acetate and n-butanol (Tianjing DaMao Chemical Reagents Co. Tianjing, China). HPLC grade methanol was from Concord Chemical Reagents Co. (Tianjin, China).

3.3. Extraction and isolation

The flowers of *L. inermis* (10.0 kg) were extracted with 75% ethanol (3 × 120 L, 2 h each) under refluxing at 78 °C. The extract was concentrated in vacuum and suspended in water (8 L), then partitioned with petroleum ether (3 × 5 L), dichloromethane (3 × 5 L), ethyl acetate (3 × 5 L) and n-butanol (3 × 5 L). After concentration of the solvents under vacuum, petroleum ether (110 g), dichloromethane (300 g), ethyl acetate (130 g), and n-butanol (300 g) soluble fractions were obtained.

The dichloromethane fraction (180.0 g) was subjected to a silica gel column chromatography (CC) (200–300 mesh, 1.4 kg), using a gradient of petroleum ether–acetone (PE–Acetone) as eluent to provide nine fractions (Fr. 1–Fr. 9) based on TLC analysis.

Fraction 6 (7 g) was repeatedly separated by silica gel column chromatography (CC) using a petroleum ether (PE)–acetone gradient. The final purification was done through polyamide CC with MeOH–H_2O as the eluent to obtain compound **1** (20 mg). Fraction 8 (8 g) was repeatedly chromatographed on a silica gel (200 to 300 mesh) column using PE–acetone and subjected to Sephadex LH-20 CC with MeOH as the eluent to obtain compounds **6** (10 mg), **7** (160 mg), and **2** (7 mg). Fraction 7 (8.5 g) was subjected to silica gel CC using PE–acetone elution to obtain three fractions G_1 to G_3. Fractions G_1 and G_2 were subjected to Sephadex LH-20 CC with $CHCl_3$–MeOH (1:1) as the eluting solvent to yield compounds **9** (11 mg) and **3** (10 mg), respectively. Fraction G_3 was purified using polyamide CC with MeOH–H_2O as the eluent to obtain compound **10** (15 mg). Fraction 2 (4.0 g) produced compounds **13** (125 mg) and **14** (9 mg) by repeated silica gel (200 to 300 mesh) CC with a PE–acetone gradient. Fraction 3 (1.7 g) was subjected to silica gel (200 to 300 mesh) CC with a PE–acetone gradient and then recrystallized to obtain compound **15** (28 mg). Fraction 4 (4 g) was subjected to repeated silica gel CC using PE ether–ethyl acetate (PE–EtOAC) elution. The final purification was conducted by Sephadex LH-20 CC with $CHCl_3$–MeOH (1:1) as the eluting solvent to obtain compound **4** (15 mg). Fraction 5 (6.7 g) was chromatographed on a silica gel (200 to 300 mesh) column with a PE–acetone gradient. Recrystallization yielded compound **5** (1.5 g) and a mixture with compound **11**. Preparative HPLC (YMC, ODS S-5, 10% MeOH) of this mixture yielded the pure compound **11** (12 mg). Fraction 9 (8 g) was subjected to repeated silica gel CC (PE–EtOAC) to obtain two fractions I_1 and I_2. Recrystallization of Fraction I_1 produced compound **12** (20 mg). Sephadex LH-20 ($CHCl_3$–MeOH 1:1) CC and preparative TLC were used for the separation of fraction I_2 to obtain compound **8** (6 mg).

3.3.1. Compound *1*

Compound **1**: yellowish power, [α]25 D-15.2 (c = 1.00, $(CH_3)_2C{=}O$); UV(CH_3OH) λ_{max} log(ε) 206(2.57); IR, v_{max} (cm^{-1}) 3325, 2924, 1707, 1607, 1526, and 1511; ^1H NMR and ^{13}C NMR data, see Table 1; HRESI–MS 419.0734 $[M+Na]^+$ m/z (calcd. for $C_{21}H_{16}O_8Na$, 419.0737).

3.3.2. Compound *2*

Compound **2**: yellowish power, [α]25 D-46.2 (c = 1.53, CH_3OH); UV(CH_3OH) λ_{max} log(ε) 207(2.92), IR, v_{max} (cm^{-1}) 2957, 2924, 2853, 1730, 1637 and 1460. ^1H NMR and ^{13}C NMR data, see Table 1; HRESI–MS 571.0872 $[M+H]^+$ m/z (calcd. for $C_{30}H_{19}O_{12}H$, 571.0871).

3.3.3. Compound *3*

Compound **3**: orange yellow power, [α]25 D-25.0 (c 1.20, CH_3OH); UV(CH_3OH) λ_{max} log(ε) 257(2.40) IR, v_{max} (cm^{-1}) 3420, 2957, 2924, 1673, 1613, 1587, 1534 and 1440; ^1H NMR and ^{13}C NMR data, see Table 1; HRESI–MS 409.0669 $[M+Na]^+$ m/z (calcd. For $C_{23}H_{24}O_6Na$, 409.0683).

3.4. X-ray crystallographic analysis

The molecular structure and absolute configuration of **3** were established unambiguously by X-ray crystallographic analysis. Crystal data for compound **3**: formula $C_{23}H_{14}O_6$; Mr = 386.34; Crystal size: 0.32 × 0.24 × 0.18 mm. Cell parameters: the crystal of **3** belongs to a monoclinic system, space group: P-1, a = 7.5849 (6) Å, b = 8.2886 (7) Å, c = 14.5427 (12) Å, α = 86.8050 (10), β = 88.7970 (10), γ = 69.1510 (10) V = 853.08 (12) $Å^3$, Z = 2, Dc = 1.504 g/cm^3, T = 120(2) K, λ (Mo Kα) = 0.71073 Å, the final R1 = 0.0392, wR2 = 0.1005 (w = 1/σF2), S = 1.039 observed reflections with $I > 2\sigma(I)$. The structures were solved by direct methods and refined by full-matrix least-squares on F2 using SHELXTL-97 software package.

3.5. ECD experiment and calculations

Simulated ECD spectra were calculated using the Time-Dependent Density Functional Theory (TD-DFT) method at the B3LYP/6-31G (d) level applying the Polarizable Continuum

Solvation Model (CPCM) in MeOH for the solution phase. The calculated excitation energy and predicted rotatory strength of each conformer were then normalized, Boltzmann averaged, and applied to the Gaussian function to produce the calculated spectra for compound **2**.

3.6. Cytotoxic assay

MCF-7, Hela, BGC-823, HCT-116, and HT-29 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). MCF-7, Hela, and BGC-823 cells were maintained in RPMI 1640 medium (Hyclone, Beijing, China) and HCT-116, and HT-29 cell lines were maintained in high glucose DMEM (GIBCO, NY, USA), supplemented with 10% FBS (Biochrom AG, Berlin, Germany), 100 mg/L streptomycin, 100 IU/mL penicillin, and 0.03% L-glutamine, and grown in a humidified incubator at 37 °C under 5% CO_2. Cells were seeded in a 96-well microtiter plate (1×10^4 cells/well) overnight and then incubated with varying concentrations of the test compounds, for 48 h. The effects of the test compounds were determined through MTT assay. Briefly, MTT solution (5 mg/mL) was added into each well, and the plate was incubated for another 4 h at 37 °C. The supernatant was then removed and the formazan crystals that had formed were dissolved with 100 μL of DMSO. The absorbance at 490 nm was recorded using an OPTI max microplate reader. 5-fluorouracil (99% purity) was used as a positive control. The percentage of cell-growth inhibition was calculated using the following formula: cell death (%) = [(control) −(compound)] / (control) × 100.

Conflict of interest

There is no conflict of interest.

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