ORIGINAL ARTICLE

Artemlavanins A and B from *Artemisia lavandulaefolia* and Their Cytotoxicity Against Hepatic Stellate Cell Line LX2
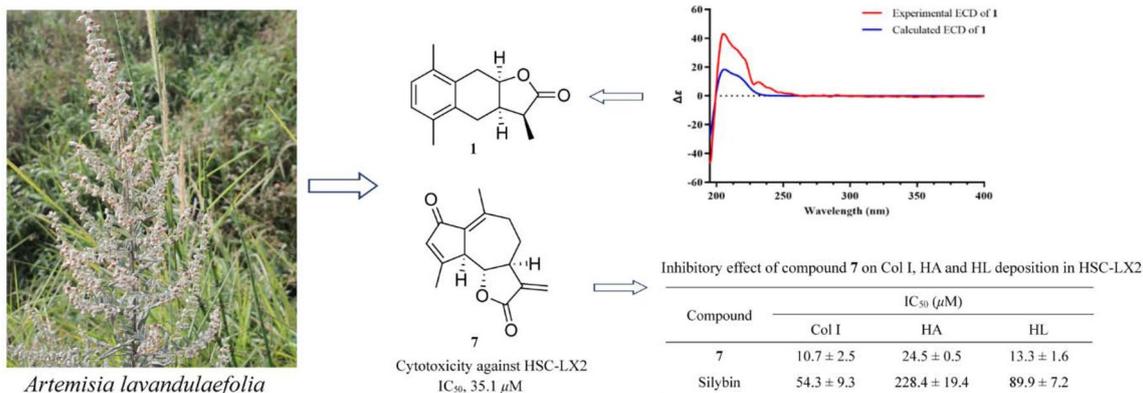
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Abstract

Two new sesquiterpenoids, artemlavanins A (**1**) and B (**3**), together with fifteen known compounds (**2** and **4−17**) were isolated from the EtOH extract of *Artemisia lavandulaefolia*. The structures of new compounds were elucidated by extensive spectroscopic analyses (HRESIMS, 1D and 2D NMR) and ECD calculations. Compound **1** was a sesquiterpenoid lactone possessing a rearranged eudesmane skeleton; compounds **2–5**, **6–8**, **9** and **10–12** belonged to the eudesmane, guaiane, oppositane and farnesane sesquiterpenoids, respectively; compounds **13–17** were the phenyl derivatives with a 4-hydroxy-acetophenone moiety. Twelve compounds (**1–3**, **5–7**, **10–12**, **14**, **15** and **17**) displayed cytotoxicity against hepatic stellate cell line LX2 (HSC-LX2) with IC_{50} values ranging from 35.1 to 370.3 μM. Compounds **2**, **7**, **10–12** and **17** exhibited the stronger cytotoxicity than silybin (IC_{50}, 169.6 μM) with IC_{50} values of 82.1, 35.1, 95.0, 83.8, 81.6 and 90.1 μM. Compound **7** as the most active one showed significant inhibition on the deposition of human collagen type I (Col I), human hyaluronic acid (HA) and human laminin (HL) with IC_{50} values of 10.7, 24.5 and 13.3 μM.

Graphic Abstract

Artemisia lavandulaefolia

Cytotoxicity against HSC-LX2
IC_{50}, 35.1 μM

Inhibitory effect of compound 7 on Col I, HA and HL deposition in HSC-LX2

Compound	IC_{50} (μM)		
	Col I	HA	HL
7	10.7 ± 2.5	24.5 ± 0.5	13.3 ± 1.6
Silybin	54.3 ± 9.3	228.4 ± 19.4	89.9 ± 7.2

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1 Introduction

Hepatic fibrosis is characterized by the abnormal accumulation of extracellular matrix (ECM), which results from the liver diseases such as viral hepatitis and alcoholic or nonalcoholic steatohepatitis. The global prevalence of alcohol-use disorders, diabetes and hepatitis B virus were approximately 75 million, 422 million and 257 million people [1], thus increasing the risks for liver cirrhosis or cancer and leading to a significant burden for liver diseases and medical costs. Although over 20 candidate molecules for hepatic fibrosis involving the inhibition of inflammatory response, ECM production and HSCs activation have achieved positive progress in recent years, most of which are being assessed in clinical trials and none of them have been commercialized as antifibrotic drugs [2–5]. Therefore, the discovery of antifibrotic agents with high efficacy and low side effect are urgently required. Recently, a review of 60 natural products with antihepatic fibrosis activity has been reported, indicating alkaloids, polysaccharides, flavonoids, polypeptides, terpenoids and polyphenols as the active ingredients [6].

Artemisia is a large genus with over 300 species and belongs to the family *Asteraceae*, many of which were found to be effective for the liver diseases, such as *A. capillaris* [7, 8], *A. scoparia* [9], *A. sacrorum* [10], *A. absinthium* [11], *A. vulgaris* [12], *A. iwayomogi* [13] and *A. campestris* [14]. *A. lavandulaefolia* as a substitute of the traditional Chinese medicine *A. argyi* was used for the treatment of hemorrhage, menstruation-related symptoms, diarrhea, skin diseases, stomatitis, bronchitis and liver ailments in diverse cultures [15–17]. Phytochemical research of *A. lavandulaefolia* has led to reports on monoterpenoids [18, 19], sesquiterpenoids [19–21], triterpenoids [18, 22], steroids [18, 22], phenyl derivatives [18, 19], phenylpropanoids [18, 19, 23] and flavonoids [22, 24], as well as essential oils [17]. The present investigation on the EtOH extract of *A. lavandulaefolia* resulted artemlavanins A (**1**) and B (**3**) together with fifteen known compounds (**2** and **4–17**), which were evaluated on the hepatic stellate cell line LX2 (HSC-LX2). Herein, the isolation, structural elucidation and cytotoxicity of these compounds were reported (Fig. 1).

2 Results and Discussion

Artemlavanin A (**1**) was assigned a molecular formula of $C_{15}H_{18}O_2$ by the (+)-HRESIMS ion at *m/z* 231.1383 [M+H]$^+$ (calcd for $C_{15}H_{19}O_2$, 231.1380), implying seven degrees of unsaturation. Its IR spectrum indicated the

absorption bands for carbonyl (1752 cm^{-1}) and aromatic ring (1488 cm^{-1} and 1454 cm^{-1}) functionalities. The ^{1}H NMR data (Table 1) displayed the presence of five methines at δ_H 6.97 (H-2 and H-3), 4.92 (H-8), 2.93 (H-11) and 2.63 (H-7), two methylenes at δ_H 3.22 (H-9a), 2.87 (H-9b), 2.73 (H-6a) and 2.38 (H-6b), and three methyl protons at δ_H 2.30 (H-15), 2.29 (H-14) and 1.28 (H-13). The ^{13}C NMR (DEPT) data (Table 1) showed 15 signals for a carbonyl at δ_C 179.3, an aromatic ring at δ_C 134.8, 133.4, 132.1, 132.0 and 128.0 ($\times$2), three methines at δ_C 77.1, 38.3 and 38.0, two methylenes at δ_C 29.9 and 23.6, and three methyls at δ_C 19.4, 19.3 and 10.6. Two methyls were attached to C-1 and C-4 based on the HMBC correlations (Fig. 2) from H$_3$-14 (δ_H 2.29) to C-2 (δ_C 128.0) and C-10 (δ_C 132.1) and from H$_3$-15 (δ_H 2.30) to C-3 (δ_C 128.0) and C-5 (δ_C 134.8). The ^{1}H–^{1}H COSY cross-peaks of H-6/H-7/H-8/H-9 and H-7/H-11/H-13 together with the HMBC correlations from H$_2$-6 (δ_H 2.73 and 2.38) to C-4 (δ_C 132.0) and C-10 (δ_C 132.1) and from H$_2$-9 (δ_H 3.22 and 2.87) to C-1 (δ_H 133.4) and C-5 (δ_C 134.8) verified that the aromatic ring was fused with a hexatomic ring via C-10 and C-5 in compound **1**. In addition, the lactone ring was established by the HMBC correlations of H-7 (δ_H 2.63), H-11 (δ_H 2.93) and H-13 (δ_H 1.28) with C-12 (δ_C 179.3). The planar structure of compound **1** was therefore determined to be consistent with α-(1,4-dimethyl-7-hydroxy-5,6,7,8-tetrahydro-6-naphthyl)-propionic acid lactone as previously reported [25, 26], but the stereochemistry of this compound remained unclear. The coupling constant ($J = 6.4$ Hz) between H-7 and H-8 specified a *cis*-relationship [27, 28]. Furthermore, the ROESY correlation of H-8 with H-11 indicated that they were on the same side. Comparison of the experimental and calculated ECD spectra (Fig. 3) revealed that compound **1** was elucidated as $7\alpha,11\alpha$H-14(10$\rightarrow$1) abeo-eudesma-1,3,5(10)-trien-12,8β-olide.

Artemlavanin B (**3**) possessed a molecular formula of $C_{16}H_{26}O_3$ by the (+)-HRESIMS ion at *m/z* 289.1754 [M+Na]$^+$ (calcd for $C_{16}H_{26}O_3Na$, 289.1774), indicating four indices of hydrogen deficiency. The IR absorptions at 3455, 1713 and 1625 cm^{-1} suggested the existence of hydroxy, carbonyl and double bond groups. The ^{1}H NMR data (Table 1) exhibited characteristic signals for a methoxy at δ_H 3.70 (MeO-15) and three methyls at δ_H 1.10 (H$_3$-14) and 1.19 (H$_3$-12 and H$_3$-13). The ^{13}C NMR (DEPT) data (Table 1) showed 16 carbon resonances including a carbonyl at δ_C 171.0, a tetrasubstituted double bond at δ_C 150.5 and 124.3, two quaternary carbons at δ_C 72.7 and 35.6, a methine at δ_C 50.9, six methylenes at δ_C 41.9, 39.4, 28.9, 28.1, 23.0 and 18.6, three methyls at δ_C 27.1, 27.0 and 24.9, and a methoxy at δ_C 51.5. Its ^{1}H NMR and ^{13}C NMR spectra were similar with those of (+)-γ-eudesmol [29, 30], except that the methyl at C-4 in (+)-γ-eudesmol was replaced by a methoxycarbonyl
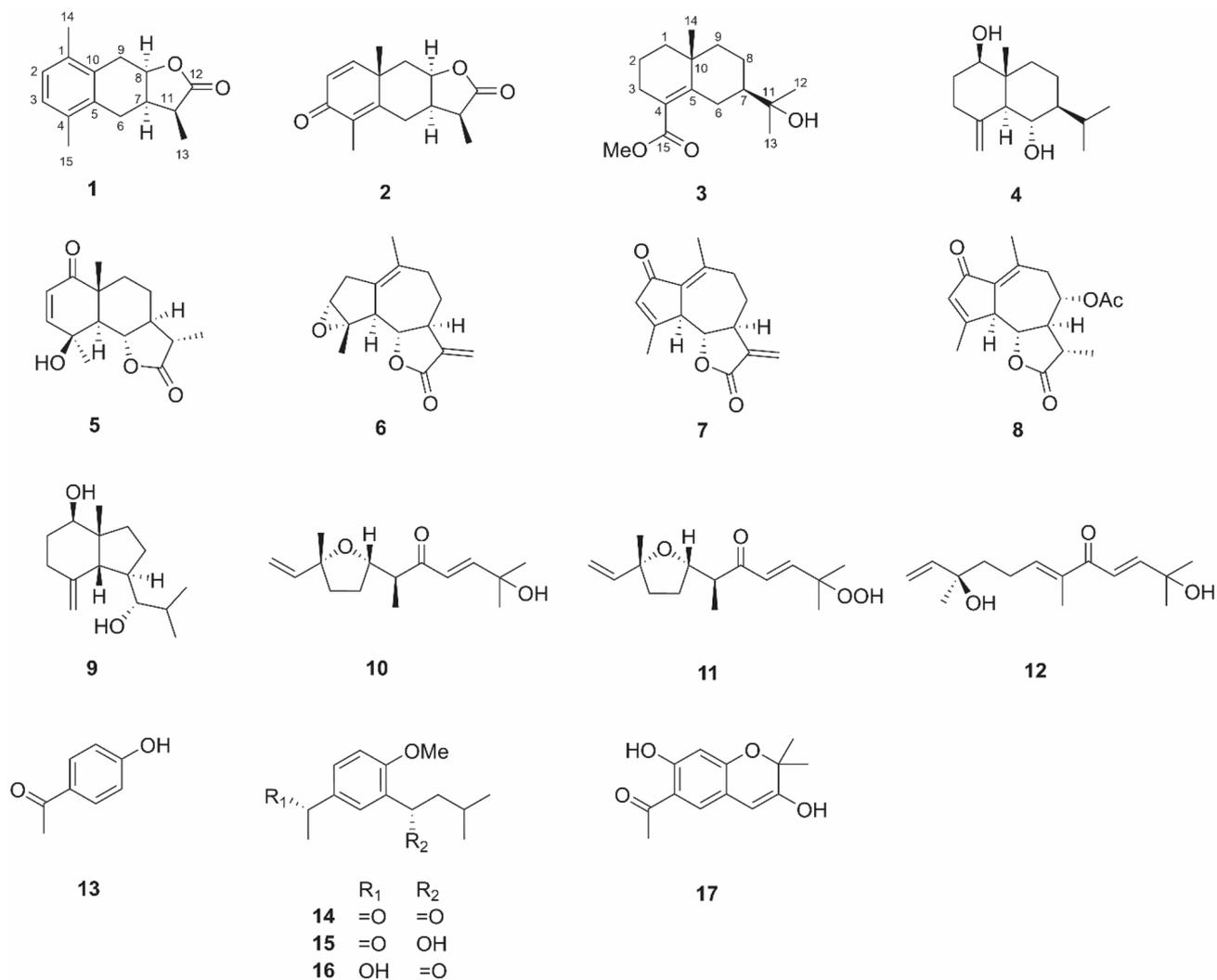
Fig. 1 Chemical structures of compounds **1–17**

group in compound **3**. The location of methoxycarbonyl was verified at C-4 by the HMBC correlations (Fig. 2) from MeO-15 (δ_C 3.70) and H_2-3 (δ_H 2.31 and 2.16) to C-15 (δ_C 171.0). The relative configuration of compound **3** was identical with (+)-γ-eudesmol based on the ROESY correlation of H-8β with H-6β, H_3-14 and H_3-12(13). Combined with the positive optical rotation, compound **3** was established as methyl 11-hydroxyeudesm-4-en-7αH-15-oate.

The known compounds were identified as 11α,13-dihydroyomogin (**2**) [31], 1β,6α-dihydroxyeudesm-4(15)-ene (**4**) [32], 4-*epi*-vulgarin (**5**) [33], ludartin (**6**) [34], dehydroleucodin (**7**) [35], matricarin (**8**) [36], (7R*)-5-*epi*-opposit-4(15)-ene-1β,7-diol (**9**) [37], *cis*-hydroxydavanone (**10**) [38], (6S,7S,l0R)-2-hydroperoxy-2,6,10-trimethyl-7,10-epoxydodeca-3,11-dien-5-one (**11**) [39], 11-hydroxy-8-oxo-9,10-dehydro-10,11-dihydronerolidol (**12**) [40], 4-hydroxyacetophenone (**13**) [41], espeletone (**14**) [42],

(S)-3-(1-hydroxy-3-methylbutyl)-4-methoxyacetophenone (**15**) [43], (R)-1-[5-(1-hydroxyethyl)-2-methoxyphenyl]-3-methyl-1-butanone (**16**) [44], and 6-acetyl-3,7-dihydroxy-2,2-dimethyl-chromene (**17**) [45] by comparison of their spectroscopic data with those previously reported.

Compounds **1–17** were evaluated for their cytotoxicity against HSC-LX2. Twelve compounds showed activities with inhibitory ratios higher than 50% at 400 μM (Table 2) and IC_{50} values ranging from 35.1 to 370.3 μM (Table 3). Compounds **2**, **7**, **10–12** and **17** displayed potent cytotoxicity with IC_{50} values of 82.1, 35.1, 95.0, 83.8, 81.6 and 90.1 μM, indicating more potent cytotoxicity than the positive control silybin (IC_{50}, 169.6 μM); compound **14** exhibited moderate cytotoxicity with the IC_{50} value of 151.7 μM; compounds **1**, **3**, **5**, **6** and **15** demonstrated less effective than silybin with the IC_{50} values in the range of 204.9–370.3 μM. In order to better comprehend the cytotoxic mechanism, the effects of
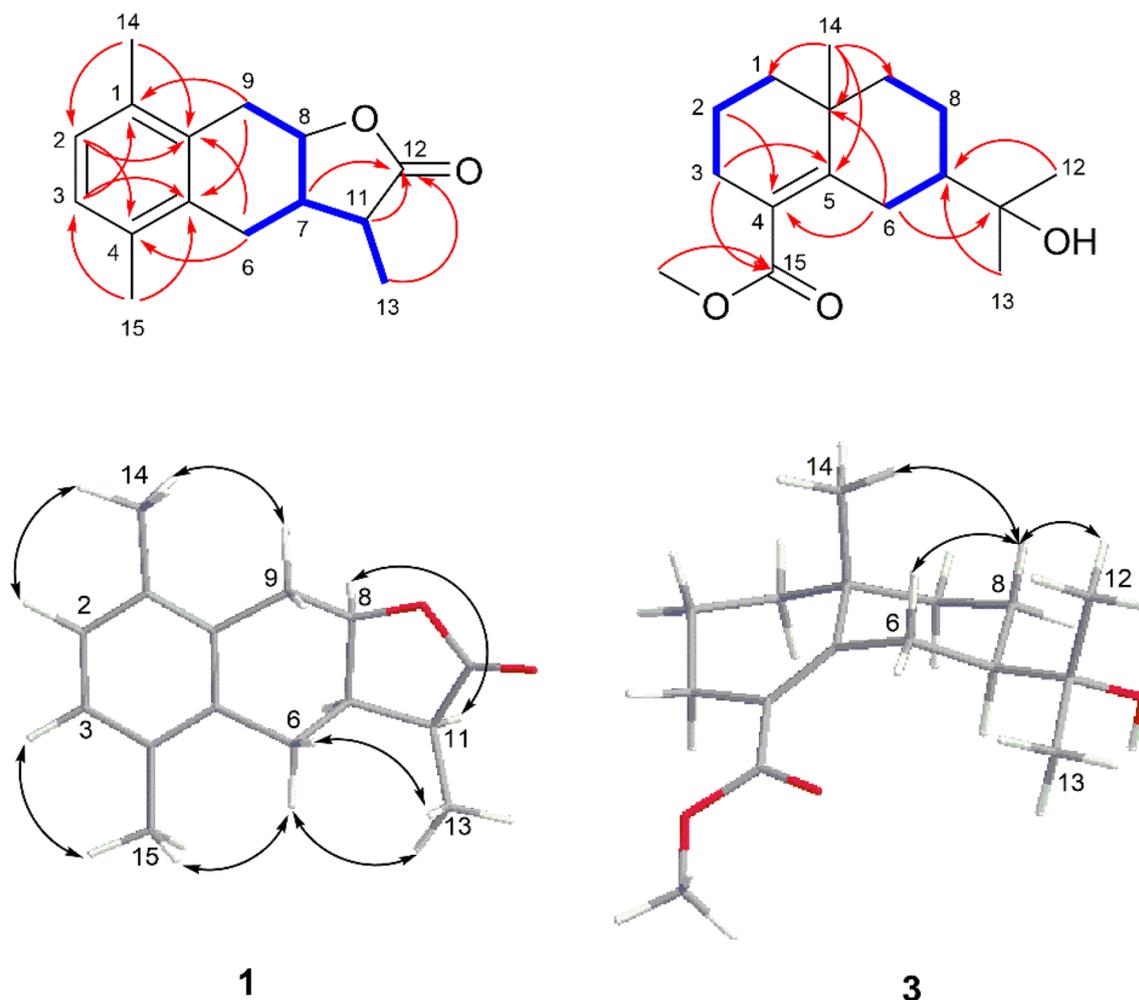
Fig. 2 Key HMBC (red), ^{1}H–^{1}H COSY (blue) and ROESY (black) correlations of compounds **1** and **3**
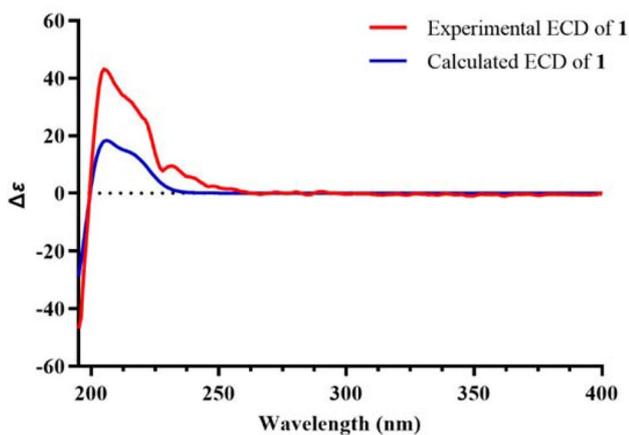

Fig. 3 Experimental and calculated ECD spectra for compound **1** in MeOH ($\sigma = 0.25$, shift $= -3$ nm)

active compounds on the deposition of Col I, HA and HL in HSC-LX2 were assayed by ELISA analysis. Notably, compound **7** as the most active one against HSC-LX2 showed obvious inhibitory effects on the secretion of Col I, HA and HL with IC_{50} values of 10.7, 24.5 and 13.3 μM, which were significantly superior to silybin with IC_{50} values of 54.3, 228.4 and 89.9 μM (Table 4).

3 Conclusion

In conclusion, the phytochemical investigations on *A. lavandulaefolia* revealed two new sesquiterpenoids and fifteen known compounds, of which artemlavanin A (**1**) was a rearranged eudesmanolide with an aromatic ring and artemlavanin B (**3**) was a methyl ester derivative of (+)-γ-eudesmol. In addition, the chemical constituents isolated from *A. lavandulaefolia* were composed of eudesmane (**2**, **4** and **5**), guaiane (**6–8**), oppositane (**9**) and farnesane (**10–12**)

Table 1 ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data for compounds **1** and **3** in CDCl$_3$ (δ in ppm, J in Hz)

No.	**1**		**3**	
	δ_C	δ_H	δ_C	δ_H
1	133.4, C	–	41.9, CH$_2$	1.65, m
				1.33, m
2	128.0, CH	6.97, s	18.6, CH$_2$	1.62, m
3	128.0, CH	6.97, s	28.1, CH$_2$	2.31, m
				2.16, m
4	132.0, C	–	124.3, C	–
5	134.8, C	–	150.5, C	–
6	23.6, CH$_2$	2.73, dd (14.9, 5.7)	28.9, CH$_2$	3.00, dt (13.4, 2.5)
		2.38, dd (14.9, 9.3)		1.79, tt (13.4, 2.7)
7	38.0, CH	2.63, m	50.9, CH	1.39, m
8	77.1, CH	4.92, td (6.4, 5.1)	23.0, CH$_2$	1.67, m
				1.46, m
9	29.9, CH$_2$	3.22, dd (16.0, 6.4)	39.4, CH$_2$	1.53, m
		2.87, dd (16.0, 5.1)		1.31, m
10	132.1, C	–	35.6, C	–
11	38.3, CH	2.93, dq (9.1, 7.4)	72.7, C	–
12	179.3, C	–	27.1, CH$_3$	1.19, s
13	10.6, CH$_3$	1.28, d (7.4)	27.0, CH$_3$	1.19, s
14	19.4, CH$_3$	2.29, s	24.9, CH$_3$	1.10, s
15	19.3, CH$_3$	2.30, s	171.0, C	–
–OMe	–	–	51.5, CH$_3$	3.70, s

Table 2 Inhibitory ratios of compounds **1–17** on HSC-LX2 at 400 μM

Compound	Inhibitory ratio (%)[a]	Compound	Inhibitory ratio (%)[a]
1	77.9 ± 1.0	**10**	101.3 ± 0.5
2	79.8 ± 1.1	**11**	101.4 ± 0.6
3	74.2 ± 3.6	**12**	102.9 ± 1.4
4	48.7 ± 10.1	**13**	30.0 ± 2.5
5	54.8 ± 3.5	**14**	76.0 ± 4.3
6	89.5 ± 0.6	**15**	64.2 ± 1.0
7	102.2 ± 0.3	**16**	47.3 ± 4.0
8	49.3 ± 3.9	**17**	77.4 ± 1.2
9	48.7 ± 1.9	Silybin[b]	76.7 ± 6.9

[a]Data were expressed as means ± SD (n = 3)

[b]Silybin was used as the positive control

sesquiterpenoids, together with the phenyl derivatives with a 4-hydroxyacetophenone moiety (**13–17**). Compounds **2**, **7**, **10–12** and **17** showed obvious cytotoxicity against HSC-LX2 with IC$_{50}$ values of 82.1, 35.1, 95.0, 83.8, 81.6 and

Table 3 IC$_{50}$ values of compounds against HSC-LX2

Compound	IC$_{50}$ (μM)[a]	Compound	IC$_{50}$ (μM)[a]
1	218.7 ± 4.0	**11**	83.8 ± 1.3
2	82.1 ± 7.2	**12**	81.6 ± 3.3
3	267.7 ± 8.2	**14**	151.7 ± 7.5
5	370.3 ± 23.6	**15**	219.9 ± 15.6
6	204.9 ± 4.0	**17**	90.1 ± 9.2
7	35.1 ± 0.3	Silybin[b]	169.6 ± 3.4
10	95.0 ± 6.9		

[a]Data were expressed as means ± SD (n = 3)

[b]Silybin was used as the positive control

Table 4 Inhibitory effect of compound **7** on Col I, HA and HL deposition in HSC-LX2

Compound	IC$_{50}$ (μM)[a]		
	Col I	HA	HL
7	10.7 ± 2.5	24.5 ± 0.5	13.3 ± 1.6
Silybin[b]	54.3 ± 9.3	228.4 ± 19.4	89.9 ± 7.2

[a]Data were expressed as means ± SD (n = 3)

[b]Silybin was used as the positive control

90.1 μM. Interestingly, compound **7** possessed inhibitory activity on not only HSC-LX2 but also the accumulation of Col I, HA and HL with IC$_{50}$ values of 10.7, 24.5 and 13.3 μM. These results provided the evidence for the anti-hepatic fibrosis potential of *A. lavandulaefolia*.

4 Experimental Section

4.1 General Experimental Procedures

Optical rotations were measured on an Autopol VI automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). UV spectra were recorded on a Shimadzu UV2401PC spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were determined on a NICOLET iS10 (Thermo scientific, Madison, USA) spectrometers using ATR ITX-DIAMOND mode. HRESIMS data were acquired on an LCMS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). 1D and 2D NMR data were obtained on Avance III-600 spectrometers (Bruker, Bremerhaven, Germany). ECD spectra were collected on an Applied Photophysics Chirascan spectrometer (Applied Photophysics, Surrey, UK). Silica gel (200–300 mesh, Qingdao Makall group Co., Ltd., Qingdao, China) and Sephadex LH-20 (GE Healthcare Amersham Biosciences, Uppsala, Sweden) were used for column chromatography. MPLC separations was conducted on a Dr-Flash II apparatus (Lisure Science Co., Ltd., Suzhou,

China) using MCI gel CHP 20P column (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Semipreparative HPLC separations were performed on a Shimadzu LC-CBM-20 system (Shimadzu, Kyoto, Japan) with an Agilent Eclipse XDB-C$_{18}$ column (5 μm, 9.4×250 mm, Agilent Technologies, Santa Clara, USA) unless otherwise specified. TLC analyses were carried out using silica gel GF$_{254}$ plates (Jiangyou Silica Gel Development Co., Ltd., Yantai, China), and compounds were detected by heating after spraying with 10% H_2SO_4 in EtOH. Hepatic stellate cell line LX2 (Jining Industrial Co., Ltd., Shanghai, China) were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Gibco BRL, NY, USA). The cytotoxicity was assessed by MTT (BioFROXX, Guangzhou, China) method. The ELISA kits (Cusabio Biotech Co., Ltd., Wuhan, China) was used for the detection of the human collagen type I (Col I) and human hyaluronic acid (HA), and the ELISA kit (Boster Biological Technology Co., Ltd., California, USA) was applied to determine the human laminin (HL). The absorbance was measured on a microplate reader (Bio-Rad, Hercules, CA, USA).

4.2 Plant Materials

The aerial parts of *Artemisia lavandulaefolia* DC. were collected in October, 2018, from Dali, Yunnan Province, China, and authenticated by Prof. Dr. Li-Gong Lei (Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen (No. 20181104) was deposited at the Laboratory of Antivirus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

4.3 Extraction and Isolation

The air-dried aerial parts of *Artemisia lavandulaefolia* (20 kg) were powdered and extracted with 95% EtOH for two times (2×100 L×4 day) at room temperature. The combined extract was concentrated under reduced pressure to yield the residue, which was suspended in H_2O and subsequently partitioned with EtOAc. The EtOAc fraction (814 g) was subjected to silica gel column chromatography (CC) using EtOAc–petroleum ether (PE) gradient (0:100, 5:95, 10:90, 20:80, 40:60 and 100:0, v/v) to give fractions A–F (122, 75, 87, 34, 91 and 363 g).

Fraction C (87 g) was separated by silica gel CC with Me$_2$CO–PE gradient (5:95, 10:90 and 20:80) as the eluent to give fractions C1–C3 (36, 35 and 10 g). Fraction C1 was submitted on MPLC eluted with H_2O–MeOH gradient (10:90 and 0:100) to give two fractions (Frs. C1.1 and C1.2). Compounds **1** (12 mg, t_R = 31 min) and **14** (37 mg, t_R = 26 min) was obtained from fraction C1.1 by repeated silica gel CC (EtOAc–PE, 10:90) and semipreparative

HPLC (H_2O–MeCN, 56:44). Fraction C2 was purified by MPLC using H_2O–MeOH gradient (50:50, 30:70, 10:90 and 0:100) to give four fractions (Frs. C2.1–C2.4). Fraction C2.2 was loaded on a silica gel CC and eluted with EtOAc–PE gradient (5:95, 10:90 and 20:80) to yield three fractions (Frs. C2.2.1–C2.2.3). Fraction C2.2.1 was separated by silica gel CC (EtOAc–CHCl$_3$, 2:98) to afford compounds **10** (296 mg) and **11** (753 mg), and subsequently purified by semipreparative HPLC (H_2O–MeCN, 67:33) to give compounds **4** (85 mg, t_R = 26 min) and **12** (10 mg, t_R = 29 min). Fraction C2.3 was chromatographed on a silica gel CC and eluted with Me$_2$CO–PE (5:95 and 10:90) to provide three fractions (Frs. C2.3.1–C2.3.3). Fraction C2.3.1 was carried out on silica gel CC and eluted with PE–CHCl$_3$ gradient (1:99 and 2:98) to produce two fractions (Frs. C2.3.1.1 and C2.3.1.2). Compounds **15** (8 mg, t_R = 50 min) and **16** (9 mg, t_R = 54 min) were obtained from fraction C2.3.1.1 by semipreparative HPLC (H_2O–MeCN, 73:27) using Opti-Chiral® C1-5 (RP) column (5 μm, 10×250 mm, ColumnTek, LLC, 200 Innovation Blvd., Suite 258A, State College, PA 16803 USA). Fraction C2.3.1.2 was chromatographed on a Sephadex LH-20 CC (MeOH–CHCl$_3$, 50:50) to provide compound **13** (10 mg) and subsequently purified by semipreparative HPLC (H_2O–MeCN, 53:47) to yield compound **17** (189 mg, t_R = 14 min). Compounds **3** (25 mg), **6** (57 mg) and **7** (16 mg) were obtained from fraction C2.3.2 by repeated silica gel CC (EtOAc–PE, 3:97 and 10:90) and Sephadex LH-20 CC (MeOH–CHCl$_3$, 50:50). Fraction C3 was separated by MPLC using H_2O–MeOH gradient (30:70, 10:90 and 0:100) to generate three fractions (Frs. C3.1–C3.3). Compound **9** (10 mg) was isolated from fraction C3.1 by repeated silica gel CC (EtOAc–PE, 10:90) and Sephadex LH-20 CC (MeOH–CHCl$_3$, 50:50).

Fraction D (34 g) was subjected to MPLC and eluted with H_2O–MeOH gradient (40:60, 60:40, 20:80, and 0:100) to yield fractions D1–D4 (0.9, 6.8, 9.0 and 11 g). Fraction D3 was separated by silica gel CC eluted with EtOAc–PE (20:80) and semipreparative HPLC (H_2O–MeCN, 70:30) to give compounds **5** (57 mg, t_R = 18 min) and **8** (31 mg, t_R = 34 min). Fraction D4 was chromatographed on a silica gel CC using EtOAc–CHCl$_3$ as eluent (5:95) to afford two fractions (Frs. D4.1 and D4.2). Compound **2** (46 mg, t_R = 18 min) was obtained from fraction D4.1 by semipreparative HPLC (H_2O–MeCN, 70:30).

Artemlavanin A (**1**): colorless oil; $[\alpha]_D^{22}$ + 15.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.91) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 205 (+ 43.2) nm; IR ν_{max} 1752, 1488, 1454, 1189 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Table 1; (+)-HRESIMS *m/z* 231.1383 [M+H]$^+$ (calcd for $C_{15}H_{19}O_2$, 231.1380).

Artemlavanin B (**3**): colorless oil; $[\alpha]_D^{20}$ + 68.9 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 225 (3.77) nm; IR ν_{max}

3455, 1713, 1625, 1241, 1218 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Table 1; (+)-HRESIMS *m/z* 289.1754 [M+Na]$^+$ (calcd for $C_{16}H_{26}O_3Na$, 289.1774).

4.4 Cytotoxicity Assay

The cytotoxicity of compounds was determined using the MTT method. Briefly, HSC-LX2 were cultured in 96-well plates at a density of 1×10^4 cells/well in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37 ℃ with 5% CO_2. After overnight incubation, cells were treated with compounds at different concentrations for 48 h. Subsequently, the culture medium was exchanged by 100 μL of MTT reagent (1 mg/mL). After co-incubation for 4 h at 37 °C, the solution was removed and 100 μL of DMSO was added to dissolve the formazan crystals. The absorbance was recorded on a microplate reader at 490 nm. All the experiments were carried out in triplicate. The inhibitory ratios were calculated as $[A_{(control)} - A_{(sample)}]/A_{(control)} \times 100\%$, and IC_{50} values were calculated using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA).

4.5 Col I, HA and HL Secretion Assay

HSC-LX2 were seeded at 8×10^4 cells/well in 24-well plates overnight and then treated with the compounds at different concentrations. After 72 h incubation, Col I, HA and HL levels of culture media were collected and determined using the commercial ELISA kits according to the manufacturer's instructions.

Compliance with Ethical Standards

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