



Alkaloid Constituents of *Ficus hispida* and Their Antiinflammatory Activity

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Abstract

Four new alkaloids, ficuhismines A–D (1–4), together with three known ones, were isolated from *Ficus hispida*. Their structures were elucidated by spectroscopic analysis and chemical method. The new compounds represent the first amine alkaloids with a rhamnosyl moiety (1–2) or with a *N*-oxide motif (2–4) from the genus *Ficus*. Compound 2 showed potent inhibitory effect in nuclear factor- κ B (NF- κ B) pathway luciferase assay with IC₅₀ value of 0.52 ± 0.11 μM.

Keywords *Ficus hispida* · Moraceae · Alkaloids · Antiinflammation · Nuclear factor- κ B

1 Introduction

The genus *Ficus* (Moraceae) comprises approximately 1000 species all over the world. They mainly distribute in tropical and subtropical areas and show diversity particularly in Southeast Asia [1]. In addition to the well-known fig (“Wu Hua Guo”) and banyan trees, many *Ficus* plants possess important medicinal values. The plant *Ficus hispida* L.f. is a herbal medicine that has been used in China and India as a remedy for bronchitis, dysentery, rheumatism, and skin disorders [2, 3]. The extracts from its different parts have been reported to show antiinflammatory, antidiabetic, antitumor, and hepatoprotective activities [4–7]. Primarily known for the rich flavonoids and triterpenoids [8], alkaloids

with antitumor and vasorelaxant activities have also been discovered from *F. hispida* [9, 10]. Some alkaloids and α -glucosidase inhibitory flavonoids from this plant have been reported previously by our group [11].

Nuclear factor- κ B (NF- κ B) is a key regulator of inflammation. Activation of NF- κ B initiates inflammation-associated metabolic disease, such as obesity, type 2 diabetes, and atherosclerosis [12]. In order to discover antiinflammatory natural products against metabolic diseases, alkaloid constituents of *F. hispida* were reinvestigated, and their inhibitory effects in NF- κ B pathway luciferase assay were evaluated. Seven alkaloids including four new ones (ficuhismines A–D, 1–4) were isolated from the twigs and leaves of *F. hispida* (Fig. 1). The new compounds represent the first amine alkaloids with a rhamnosyl moiety (1–2) or with a *N*-oxide motif (2–4) from the genus *Ficus*. Compound 2 showed potent NF- κ B inhibitory activity. In this paper, the structural identification and bioactivity evaluation of the alkaloids are discussed.

Dedicated to Professor Han-Dong Sun on the occasion of his 80th birthday.

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2 Results and Discussion

Compound 1 was assigned the molecular formula C₂₀H₃₁NO₆ by the (+)-HRESIMS ion at *m/z* 382.2232 [M+H]⁺ (calcd for C₂₀H₃₂NO₆, 382.2224). The IR spectrum of 1 showed absorption bands for OH (3385 cm⁻¹), carbonyl (1675 cm⁻¹), and aromatic (1599 cm⁻¹) functionalities. The 1D NMR (Table 1) and HSQC spectra displayed signals of a carbonyl group (δ_C 201.0, C-7), a *para*-substituted benzene ring (δ_H

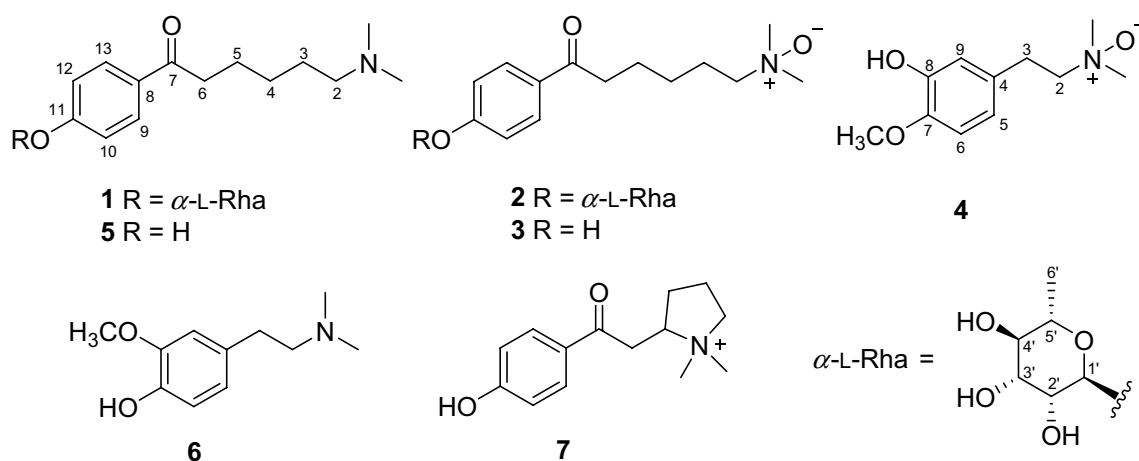


Fig. 1 Structures of compounds 1–7

Table 1 ^1H (400 MHz) and ^{13}C (150 MHz) NMR data for compounds 1–4 (in methanol- d_4)

Pos.	1		2		3		4	
	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)	δ_{C}
2H	3.10 (m)	58.9	3.63 (m)	70.5	3.61 (m)	70.5	3.43 (m)	73.0
3	1.72 (m)	25.5	1.92 (m)	23.9	1.90 (m)	23.9	3.04 (m)	30.4
4	1.43 (m)	27.0	1.47 (m)	26.7	1.45 (m)	26.8		129.1
5	1.74 (m)	24.8	1.78 (m)	24.8	1.76 (m)	25.0	6.67 (br d, 8.0)	122.5
6	3.01 (t, 6.8)	38.6	3.04 (t, 7.2)	38.6	2.99 (t, 7.2)	38.4	6.70 (d, 8.0)	116.7
7		201.0		200.9		201.0		149.5
8		132.3		132.3		130.0		147.3
9	7.96 (d, 8.8)	131.4	7.97 (d, 8.8)	131.4	7.87 (d, 8.8)	131.8	6.85 (br s)	113.6
10	7.13 (d, 8.8)	117.1	7.15 (d, 8.8)	117.1	6.82 (d, 8.8)	116.3		
11		161.8		161.8		163.9		
12	7.13 (d, 8.8)	117.1	7.15 (d, 8.8)	117.1	6.82 (d, 8.8)	116.3		
13	7.96 (d, 8.8)	131.4	7.97 (d, 8.8)	131.4	7.87 (d, 8.8)	131.8		
1'	5.52 (br s)	99.6	5.53 (br s)	99.6				
2'	3.98 (br s)	71.8	4.00 (br s)	71.8				
3'	3.81 (dd, 9.6, 3.2)	72.1	3.82 (dd, 9.3, 3.3)	72.2				
4'	3.44 (t, 9.6)	73.7	3.46 (overlap)	73.7				
5'	3.54 (m)	71.0	3.55 (m)	71.0				
6'	1.18 (d, 6.0)	18.0	1.20 (d, 6.0)	18.0				
Me ₂ N	2.85 (s)	43.4	3.46 (s)	56.4	3.45 (s)	56.4	3.17 (s)	58.6
MeO							3.82 (s)	56.4

7.96, H-9/H-13; 7.13, H-10/H-12; δ_{C} 132.3, C-8; 131.4, C-9/C-13; 117.1, C-10/C-12; 161.8, C-11), a rhamnopyranosyl moiety (δ_{H} 5.52, H-1'; 3.98, H-2'; 3.81, H-3'; 3.44, H-4'; 3.54, H-5'; 1.18, H₃-6'; δ_{C} 99.6, C-1'; 71.8, C-2'; 72.1, C-3'; 73.7, C-4'; 71.0, C-5'; 18.0, C-6'), a dimethylamino moiety (δ_{H} 2.85; δ_{C} 43.4), and five methylenes. The anomeric configuration of the L-rhamnopyranosyl moiety was determined as α by the chemical shifts of C-3' and C-5' [13]. The aforementioned information indicated **1** was an amine alkaloid

rhamnoside. In the HMBC spectrum (Fig. 2), the correlations of H₂-2/Me₂N, C-3; H₂-4/C-2, C-3, C-5, C-6; H₂-6/C-5, C-7; H-9/C-7, C-10, C-11, C-13; and H-10/C-8, C-11, C-12 constructed the aglycone motif as a 6-(dimethylamino)-1-(4-hydroxyphenyl)-1-hexanone. The H-1'/C-11 HMBC correlation indicated that the sugar was connected to C-11. Acid hydrolysis of **1** afforded the aglycone and sugar units. The sugar was verified as L-rhamnose by TLC analysis and comparing its specific rotation ($[\alpha]_{\text{D}}^{25} = +6.0$) with that of the

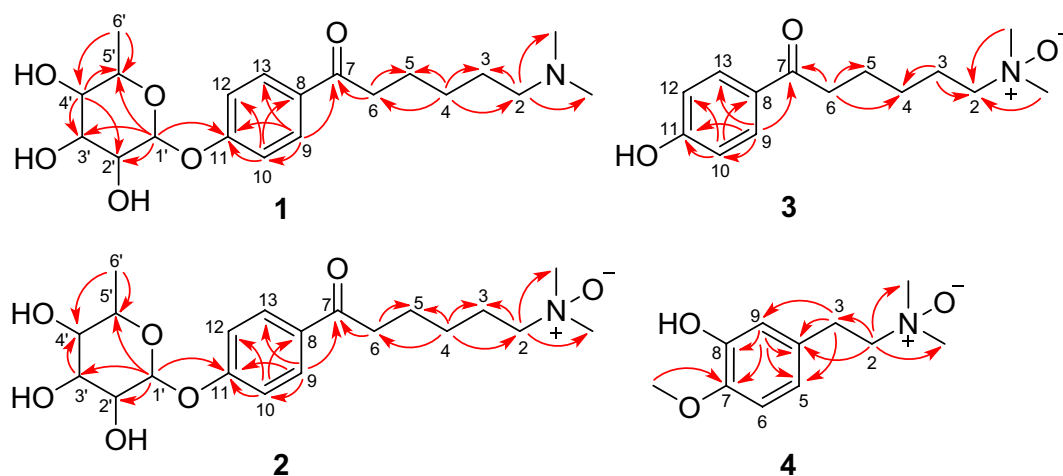


Fig. 2 Key HMBC (H→C) correlations of compounds **1–4**

authentic *L*-rhamnose ($[\alpha]_{\text{D}}^{25} = +8.0$). Thus, the structure of **1** was elucidated as depicted, and the compound was given the name ficuhismine A.

Compound **2** was assigned the molecular formula $\text{C}_{20}\text{H}_{31}\text{NO}_7$ by the (+)-HRESIMS (m/z 398.2165 $[\text{M}+\text{H}]^+$; calcd for $\text{C}_{20}\text{H}_{32}\text{NO}_7$, 398.2173), indicating six indices of hydrogen deficiency. Analysis of its NMR data (Table 1) implied that **2** possessed the same α -rhamnopyranosyl and *para*-substituted benzene moieties as **1**. The only difference was the 6-(dimethylamino)-1-hexanone side chain. In the ^{13}C NMR spectrum of **2**, the obviously deshielded carbon signals of the dimethylamino moiety ($\Delta\delta_{\text{C}} + 13.0$) and the methylene at C-2 ($\Delta\delta_{\text{C}} + 11.6$) were observed. In consideration the indices of hydrogen deficiency and an additional oxygen atom in the molecular formula of **2**, the presence of a *N*-oxide motif was inferred. The HMBC data shown in Fig. 2 confirmed the structure of **2**. The sugar was identified as *L*-rhamnose by the same protocol as that of **1**. Compound **2** was thus elucidated as an *N*-oxide of **2** and named ficuhismine B.

Compound **3** was assigned the molecular formula $\text{C}_{14}\text{H}_{21}\text{NO}_3$ by the (+)-HRESIMS ion at m/z 252.1595 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{14}\text{H}_{22}\text{NO}_3$, 252.1594). Its 1D (Table 1) and 2D NMR data (Fig. 2) indicated that **3** was an aglycone of **2**. The structure of **3** was thus elucidated, and the compound was named ficuhismine C.

Compound **4** was assigned the molecular formula $\text{C}_{11}\text{H}_{17}\text{NO}_3$ by the (+)-HRESIMS ion at m/z 212.1281 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{11}\text{H}_{18}\text{NO}_3$, 212.1281). The ^1H and ^{13}C NMR spectra of **4** (Table 1) revealed signals for a dimethylamino moiety, two methylenes, a methoxy group, and a 1, 2, 5-trisubstituted benzene motif. Similar to **2** and **3**, the downfield-shifted ^{13}C resonances of the dimethylamino moiety and the methylene at C-2 proved that **4** was also an amino alkaloid *N*-oxide. The HMBC correlations of H_2 -2/Me $_2\text{N}$,

C-3, C-4; H_2 -3/C-4, C-5, C-9; H-9/C-4, C-5, C-7, C-8; and MeO/C-7 (Fig. 2) confirmed the structure of **4** as depicted. The structure of **4** was thus elucidated, and the compound was named ficuhismine D.

The known compounds were identified as ficushispimine C (**5**) [11] magnosprengerine (**6**) [14], and ficushispimine A (**7**) [11] (Fig. 1) by comparison of their spectroscopic data with those reported.

All the isolated alkaloids were tested *in vitro* for their antiinflammatory effects. Ficuhismine B (**2**) exhibited significant inhibitory activity in NF- κB pathway luciferase assay with IC_{50} value of 0.52 ± 0.11 μM . Bortezomib (PS-341) was used as the positive control ($\text{IC}_{50} = 0.12 \pm 0.04$ μM) in this test. All the other compounds were inactive. It seemed that both *L*-rhamnosyl and *N*-oxide moieties are necessary for the NF- κB inhibition.

Alkaloids are one of the main bioactive ingredients of *F. hispida*. Amphetamine [9, 11], piperidine [9], pyrrolidine [10, 11], and isoquinoline [15] alkaloids have been reported from this plant previously. In this research, amine alkaloids with a rhamnosyl group (**1–2**) or with a *N*-oxide moiety (**2–4**) were discovered for the first time from the genus *Ficus*. Compound **2** with both rhamnosyl and *N*-oxide moieties showed potent NF- κB inhibitory activity, which may provide useful information for mining or designing drug leads against inflammation-related metabolic diseases.

3 Experimental Section

3.1 General Experimental Procedures

Optical rotations were recorded on a Rudolph Autopol IV-T polarimeter. UV spectra were recorded on a Hitachi U-2900 UV–Vis spectrophotometer. IR spectra were recorded on

a ThermoFisher Nicolet iS5 FT-IR spectrometer. NMR spectra were acquired on Varian Mercury Plus 400 instrument and Bruker Avance III HD 600 spectrometer using CD₃OD (δ_{H} 3.31 and δ_{C} 49.0). HRESIMS were obtained on an AB SCIEX 5600+ Q-TOF mass spectrometer. MCI gel CHP-20P (75–150 μm , Mitsubishi Chemical Corporation, Tokyo, Japan), ODS gel (50 μm , YMC Co., Ltd., Japan), and Sephadex LH-20 gel (GE Healthcare Bio-Sciences, USA) were used for column chromatography. Precoated silica gel GF254 plates (Qingdao Haiyang Chemical Co., Ltd., China) were used for TLC analysis. Semi-preparative HPLC was performed on a Shimadzu Essentia LC-16 with a UV detector (210 and 254 nm) and a Kromasil C₁₈ column (150 \times 10 mm, 5 μm , AkzoNobel, Co., Sweden).

3.2 Plant Material

The twigs and leaves of *Ficus hispida* were collected in Puer City, Yunnan Province, People's Republic of China, in September 2015. The plant material was identified by Dr. Yun Kang, School of Pharmacy, Fudan University. A voucher specimen (TCM 2015-09-02 Hou) has been deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Fudan University.

3.3 Extraction and Isolation

The plant material (10.0 kg) was ground and percolated with 95% EtOH (30 L) at room temperature. The filtrate was evaporated under reduced pressure to produce a crude extract (1.5 kg). It was dissolved in tartaric acid solution (pH 2.0), basified using saturated sodium carbonate solution to pH 13, and then partitioned with CHCl₃ to give a total alkaloid extract (1.2 g). This extract was subjected to MCI column chromatography (CC) (MeOH/H₂O, from 1:9 to 10:0) to provide fractions A–E. Fractions A–D were separated over CC (ODS gel, MeOH/H₂O, 10:90, 20:80, 30:70, 100:0) to give fractions A1–A4, B1–B4, C1–C4, and D1–D4, respectively. Fr. A1 was loaded on a Sephadex LH-20 column (CH₂Cl₂/MeOH, 1:1) and then purified by semi-preparative HPLC (MeOH/H₂O, 9:91, flow rate 2 mL/min) to afford **3** (2.3 mg, t_{R} 16.5 min). Fractions A2 and B1 were purified by semi-preparative HPLC to give **5** (MeCN/H₂O, 16:84, flow rate 2 mL/min, 2.3 mg, t_{R} 23 min) and **7** (MeCN/H₂O, 6:94, flow rate 2 mL/min, 2.5 mg, t_{R} 15 min), respectively. Compounds **4** (MeOH/H₂O, 3:97, flow rate 4 mL/min, 5.0 mg, t_{R} 31 min) and **6** (MeCN/H₂O, 10:90, flow rate 2 mL/min, 1.5 mg, t_{R} 26 min) were isolated by semi-preparative HPLC from fractions C1 and C2, respectively. Compounds **2** (MeOH/H₂O, 3:97, flow rate 4 mL/min, 2.0 mg, t_{R} 31 min) and **1** (MeCN/H₂O, 11:89, flow

rate 4 mL/min, 2.0 mg, t_{R} 16.0 min) were purified by semi-preparative HPLC from fractions D1 and D3, respectively.

3.4 Spectroscopic Data of Compounds

3.4.1 Ficuhismine A (1)

White solid; $[\alpha]_{\text{D}}^{25} = -61.0$ (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.28), 262 (4.32) nm; IR (KBr) ν_{max} 3385, 2915, 1675, 1599, 1383, 1181, 1141, 1075, 632 cm⁻¹; ¹H NMR and ¹³C NMR data: Table 1; HRESIMS m/z 382.2232 [M+H]⁺ (calcd for C₂₀H₃₂NO₆, 382.2224).

3.4.2 Ficuhismine B (2)

White solid; $[\alpha]_{\text{D}}^{25} = -70.0$ (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.32), 264 (4.41) nm; IR (KBr) ν_{max} 3372, 2935, 1675, 1600, 1421, 1196, 1179, 1140, 1073, 1014, 971, 835 cm⁻¹; ¹H NMR and ¹³C NMR data: Table 1; HRESIMS m/z 398.2165 [M+H]⁺ (calcd for C₂₀H₃₂NO₇, 398.2173).

3.4.3 Ficuhismine C (3)

White solid; UV (MeOH) λ_{max} (log ϵ) 204 (3.87), 216 (3.90), 274 (4.03) nm; IR (KBr) ν_{max} 3412, 2914, 1674, 1604, 1193, 1178, 1142, 1073, 637 cm⁻¹; ¹H NMR and ¹³C NMR data: Table 1; HRESIMS m/z 252.1595 [M+H]⁺ (calcd for C₁₄H₂₂NO₃, 252.1594).

3.4.4 Ficuhismine D (4)

White solid; UV (MeOH) λ_{max} (log ϵ) 204 (3.81), 216 (3.80), 274 (3.97) nm; IR (KBr) ν_{max} 3409, 2950, 2937, 2855, 2581, 1599, 1524, 1454, 1387, 1280, 1033 cm⁻¹; ¹H NMR and ¹³C NMR data: Table 1; HRESIMS m/z 212.1281 [M+H]⁺ (calcd for C₁₁H₁₈NO₃, 212.1281).

3.5 Acid Hydrolysis of Compounds 1 and 2

Compound **1** (1 mg) was added to a solution of H₂O (3 mL), dioxane (3 mL), and HCl (0.5 mL), and then kept at 60 °C for 4 h. The reaction mixture was quenched with NaHCO₃ and extracted with EtOAc. The aqueous phase was concentrated under reduced pressure. The residue was dissolved in H₂O for TLC analysis with the authentic L-rhamnose (EtOAc/MeOH/AcOH/H₂O, 10:3:3:2, v/v, R_f: 0.6) and for optical rotation measurement {the aqueous phase: $[\alpha]_{\text{D}}^{25} = +6.0$ (c 0.1 H₂O); L-rhamnose: $[\alpha]_{\text{D}}^{25} = +8.0$ (c 0.1 H₂O)}. Compound **2** was operated in the same way as **1**.

3.6 Luciferase Assay

HEK293/NF- κ B cells were prepared as reported [16]. The luciferase assay procedure was performed according to the reported methods [17]. The IC₅₀ values were calculated using Graphpad prism 8.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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