Supporting Information for

Chaetominine, a cytotoxic alkaloid produced by endophytic *Chaetomium* sp. IFB-E015

Rui H. Jiao[†], Shu Xu[†], Jun Y. Liu, Hui M. Ge, Hui Ding, Chen Xu, Hai L. Zhu, Ren X. Tan*

Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China,

rxtan@nju.edu.cn

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1 General

Melting point was measured on an XT-4 apparatus and was uncorrected. Optical rotation was determined in MeOH on a WXG-4 disc polarimeter, and IR spectrum in KBr disks on a Nexus 870 FT-IR spectrometer. The UV spectrum was recorded on a Hitachi U-3000 spectrophotometer. ESI-MS and HR-ESI mass spectra were taken on a Mariner Mass 5304 instrument. EI (70 eV) mass spectrum was obtained on a VG-ZAB-HS mass spectrometer. All NMR experiments were performed on a Bruker DRX-500 NMR spectrometer using TMS and as an internal standard. CD spectrum was recorded on a Jasco J-810 circular dichroism spectrometer. HPLC analyses were performed with a Phenomenex Luna C18 (250 × 4.6 mm, 5 μm) column on a Waters 600E HPLC instrument with the detector 996 PAD. Silica gel (200-300 mesh) for CC and silica GF₂₅₄ (10-20 μ) for TLC were produced by Qingdao Marine Chemical Company, China. Sephadex LH-20 was purchased from Pharmacia Biotech, Sweden, and authentic L- and D-alanine samples from Amresco and ACROS ORGANICS, respectively. All other chemicals used in the study were of analytical grade.

2 Isolation, identification and cultivation of the endophytes

According to the methods detailed elsewhere,^{5a} *Chaetomium* sp. IFB-E015 was isolated from the surface-sterilized leaves of apparently healthy *Adenophora axilliflora* collected in Oct. 2001 from the Zijin Mountain in the suburb of Nanjing, China. The endophytic fungus (strain IFB-E015), identified by Associate Prof. Dr. W. X. Zou via microscopic morphological character,⁶ was cultured by a solid-matrix steady method described in the literature.^{2b}

3 Extraction and isolation

A total of approximately 3 kg dehydrated fermentation batches were extracted with MeOH:CHCl₃ (1:1, 9 L×4) to give a brown residue (237 g), which was then suspended in H₂O (3.17 L). The suspension was partitioned successively with EtOAc (1.5 L × 4) and *n*-butanol (1.2 L × 4). The EtOAc soluble part was concentrated *in vacuo* to give a residue (181.5 g), which was subjected to column chromatography over a Si gel column (200-300 mesh, 1 kg) eluting with CHCl₃-MeOH mixture of a steadily growing polarity (100% CHCl₃→100% MeOH) to give nine fractions (cl1: 120.0 g; cl2: 8.9 g; cl3: 6.9 g; cl4: 13.1 g; cl5: 12.0 g; cl6: 13.0 g; cl7: 7.1 g; cl8: 2.0 g; cl9: 1.5 g). The bioactive fraction cl4 whose ¹H NMR spectrum gave a broadened singlet around δ 8.3 was chromatographed further over a Si gel column eluted with the CHCl₃-MeOH gradient (100:1→100:8) to give four parts (cl4-1: 0.1 g; cl4-2: 7.5 g; cl4-3: 1.5 g; cl4-4: 4.1 g). The bioactive one cl4-3, signifying around δ 8.3 in its ¹H NMR spectrum, was further refined by repeated gel filtrations over Sephadex LH-20 eluting with CHCl₃:MeOH (1:1), followed by recrystallization in MeOH at room temperature to yield chaetominine (**1**, 18.4 mg) as colorless crystals. Chaetominine was confirmed to be a fungal metabolite according to the method in literature.^{2b}

4 The identification of chaetominine

Chaetominine (**1**), colorless crystals, Mp. 161-163 °C; [α]_D²⁰ -70 (c 0.48, MeOH); UV (MeOH): $\lambda_{\max}(\epsilon)$ 225 (2.87×10⁴) (sh), 205 (3.87×10⁴); IR $\nu_{\max}^{KBr}$ (cm⁻¹): 3506.8, 3411.4, 3239.6, 1729.9, 1664.7, 1609.2, 1480.2, 1461.7, 1376.1, 1279.9, 754.0 cm⁻¹; CD (c 0.0055 mg/mL in MeOH): λ 204.4 ($\Delta\epsilon$ +9.0), 214.9 ($\Delta\epsilon$ -6.9), 229.9 ($\Delta\epsilon$ +4.9), 241.9 ($\Delta\epsilon$ +2.6), 250.2 ($\Delta\epsilon$ +3.8), 281.9 ($\Delta\epsilon$ -2.9); EI-MS: *m/z* 402 (24.6 %), 384 (16.3 %), 238 (100 %), 210 (68.8 %), 199 (16.9 %), 146 (20.3 %), 66 (43.0 %), 44.1 (48.5 %); HR-ESI-MS: *m/z* 403.1404 [M+H]⁺, calcd. for C₂₂H₁₉N₄O₄, 403.1401; ¹H and ¹³C NMR data: see Table.

5 Determination of absolute configuration by Marfey's method

Chaetominine (**1**, 0.18 mg) was hydrolyzed with 1 mL of 6 M HCl in a sealed ampule at 110 °C for 20 h. After cooling down to room temperature, the hydrolysate was dried under a stream of N₂ with the residue re-suspended in H₂O (100 μL). An acetone solution (1% w/v, 100 μL each case) of the Marfey's reagent FDAA (1-fluoro-2,4-dinitrophenyl-5-L-alaninamide) was added separately to the resultant suspension, and to each of 50 mM solution (50 μL) of the standard amino acids. After addition of NaHCO₃ solution (1 M, 20 μL), the mixture was incubated for 1 h at 40 °C. The reaction was stopped by addition of HCl (1 M, 20 μL), and the subsequent evaporation of the solvent gave a dryness, which was re-dissolved in MeOH:H₂O (1:1, 1 mL). With UV detector set at 340 nm, an aliquot of this solution (2 μL) was analyzed by reversed phase HPLC eluted with mixtures of solvents A (triethylammonium phosphate, 50 mM, pH 5.3) and B (MeCN) at a flow rate of 1 mL/min with the solvent A/B proportions changed from 99/1 to 60/40 within 19 min, and then from 60/40 to 99/1 in the final minute. The analyses established the presence of L-Ala (12.51 min; D-Ala, 14.96 min) in the HCl hydrolysate of chaetominine (Figure S9).⁸

6 Cytotoxicity assay

The *in vitro* cytotoxic activity was performed by the MTT method as described in our previous paper.⁹

7 Single-crystal X-ray diffraction analysis

Crystal structure determination was carried out on a Nonius CAD4 diffractometer equipped with graphite-monochromated MoK α (λ = 0.71073) with Lorentz polarization and absorption corrections for a crystal of **chaetominine-MeOH** (0.35 mm × 0.35 mm × 0.38) sealed in a boron-capillary. The intensities were collected at 293 K using ω -scan mode with variable scan speed. A total of 4458 reflections were collected in the range of θ = 1.55-25.97°, of which 4095 were independent. 2391 observable reflections with $I \geq 2\sigma(I)$ were used in the structure solution and refinements. The structure was solved by direct methods and refined on F² by full-matrix least-squares methods using

SHELX-97.⁷ All the non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed in calculated positions and were assigned fixed isotropic thermal parameters at 1.2 times the equivalent isotropic U of the atoms to which they are attached and allowed to ride on their respective parent atoms. The contributions of these hydrogen atoms were included in the structure-factors calculations. The refinement gave the final $R_1 = 0.1026$ with $w = [\sigma^2(F_o)^2 + (0.1(\max(0, F_o^2) + 2F_c^2)/3)^2]^{-1}$. Some crystallographic and experimental data were listed in Table S1 and selected bond lengths and bond angles were listed in Table S2.⁷

8 Crystal structure description

The strong hydrogen bond (bond length O1-H...O5 = 2.728 Å and bond angle O1-H1-O5 = 162.8°) between the hydroxyl group in chaetominine (**1**) and the oxygen atom in the methanol molecule linked one molecule of **1** and one lattice methanol molecule to form **chaetominine·MeOH** single crystals. The structure of **chaetominine·MeOH** was shown in Figure 3.

Chaetominine·MeOH consisted of two subunits, which were jointed by a single C-N bond.

There was a dihydrogen indole in subunit B, interestingly, all the constituent atoms from the hydrogen indole were in a well-defined plane, indicating a strong cycle effect. The hydroxyl oxygen atom almost vertically stood above the indole plane at the separation of 1.176 Å. A tetrahydrogen imidazole ring was next to the indole plane by sharing a common side C2-N1 with it. The five atoms in the imidazole ring were un-coplanar, as expected. Four atoms (C2N1C10C11) of the five membered-imidazole ring constituted a fine plane with the deviation of 0.011 Å, and the fifth atom N16 was located 0.488 Å below the quadrilateral plane (C2N1C10C11). The carbonyl oxygen atom and the carbon atom attached to the tetrahydrogen imidazole ring were at the same side of the quadrangle at the distances of 0.094 and 0.841 Å, respectively, below the plane. The dihedral angle between the dihydrogen indole and the quadrangle plane was about 35°. A six-membered cyclo-amide ring formed by sharing three atoms (N16C2C3) with the previous rings, which finished the skeleton of subunit B in **1**. The cycloamide was in a severely distorted ship-conformation, with C13 and C16 being the poles and rest four atoms constituting the bottom of the ship. In order to minimize the steric effects from the neighboring cycles, the planarity of the ship-bottom was quite poor with the average deviation of 0.142 Å. Two carbonyl oxygen atoms in subunit B made two amides of N1 and N6.

Table S1. Crystallographic and experimental data for chaetominine·MeOH in diethyl ether solution

	chaetominine·MeOH
Formula	C ₂₂ H ₁₈ N ₄ O ₄ ·CH ₃ OH
FW	435.1246
Crystal shape/color	prism / colorless
Crystal size/ mm	0.35×0.35×0.38
Crystal system	Orthorhombic
Space group	P2 ₁ 2 ₁ 2 ₁
a/Å	8.4630(17)
b/Å	9.3650(19)
c/Å	26.305(5)
U/Å ³	2847.1(4)
Z	4
T/K	293(2)
μ/mm ⁻¹ (Mo-Kα)	0.099
Dx/g cm ⁻³	1.499
Reflections/parameters	4095/293
Independent reflections (R _{int})	2391
F(000)	980
T _{max}	0.863
T _{min}	0.755
Goodness of fit on F ²	1.154
R ₁ , wR [I ≥ 2σ(I)]	0.1026; 0.1707

Table S2. Selected bond lengths and bond angles

O1 C3	1.409(8)
O2 C10	1.233(9)
O3 C15	1.189(9)
O4 C17	1.191(9)
O5 C26	1.400(13)
N1 C10	1.380(9)

N1 C9	1.417(10)
N1 C2	1.451(9)
C2 N16	1.456(9)
C2 C3	1.572(10)
C3 C4	1.488(10)
C3 C13	1.516(9)
C4 C5	1.384(10)
C4 C9	1.386(10)
C5 C6	1.340(12)
C6 C7	1.376(13)
C7 C8	1.376(12)
C8 C9	1.361(11)
C10 C11	1.519(12)
C11 N16	1.456(10)
C11 C12	1.532(12)
C13 C14	1.518(10)
C14 N26	1.468(9)
C14 C15	1.556(10)
C15 N16	1.359(9)
C17 N26	1.390(9)
C17 C18	1.466(11)
C18 C23	1.370(12)
C18 C19	1.406(11)
C19 C20	1.401(13)
C20 C21	1.395(15)
C21 C22	1.375(13)
C22 C23	1.407(11)
C23 N24	1.395(11)
N24 C25	1.297(10)
C25 N26	1.364(10)
C10 N1 C9	127.0(7)
C10 N1 C2	109.6(6)
C9 N1 C2	110.4(6)
N1 C2 N16	102.7(6)
N1 C2 C3	106.3(6)
N16 C2 C3	118.1(5)
O1 C3 C4	108.8(6)
O1 C3 C13	111.7(6)
C4 C3 C13	115.1(6)
O1 C3 C2	109.4(6)
C4 C3 C2	101.5(5)
C13 C3 C2	109.7(5)
C5 C4 C9	118.6(7)
C5 C4 C3	128.5(7)
C9 C4 C3	112.9(6)
C6 C5 C4	119.5(8)
C5 C6 C7	121.3(8)
C8 C7 C6	120.6(8)
C9 C8 C7	117.5(8)
C8 C9 C4	122.3(7)
C8 C9 N1	128.9(7)
C4 C9 N1	108.8(7)
O2 C10 N1	124.1(8)
O2 C10 C11	127.4(7)

N1 C10 C11	108.5(7)
N16 C11 C10	99.9(6)
N16 C11 C12	116.6(7)
C10 C11 C12	109.9(7)
C3 C13 C14	110.9(6)
N26 C14 C13	113.3(6)
N26 C14 C15	110.2(6)
C13 C14 C15	112.6(6)
O3 C15 N16	126.7(7)
O3 C15 C14	122.8(6)
N16 C15 C14	110.5(6)
C15 N16 C11	127.1(6)
C15 N16 C2	125.3(6)
C11 N16 C2	107.6(5)
O4 C17 N26	122.3(7)
O4 C17 C18	125.5(7)
N26 C17 C18	112.2(7)
C23 C18 C19	119.4(8)
C23 C18 C17	121.7(7)
C19 C18 C17	118.8(7)
C20 C19 C18	121.2(9)
C21 C20 C19	117.2(9)
C22 C21 C20	122.7(8)
C21 C22 C23	118.7(9)
C18 C23 N24	121.8(7)
C18 C23 C22	120.7(8)
N24 C23 C22	117.5(8)
C25 N24 C23	115.8(7)
N24 C25 N26	126.2(7)
C25 N26 C17	122.3(6)
C25 N26 C14	118.7(6)
C17 N26 C14	119.0(6)
O1...O5	2.728(10)
O1-H1...O5	162.8(8)

Figure S1. The EI-MS spectrum of chaetominine.

Figure S2. The CD spectrum of chaetominine in MeOH (c 0.0055 M).

Figure S3. The ^1H NMR spectrum of chaetominine in $\text{DMSO}-d_6$.

Figure S4. The ^{13}C NMR spectrum of chaetominine in $\text{DMSO}-d_6$.

Figure S5. The HMQC spectrum of chaetominine in DMSO- d_6 .

Figure S6. The HMBC spectrum of chaetominine in DMSO- d_6 .

Figure S7. The ^1H - ^1H COSY spectrum of chaetominine in $\text{DMSO}-d_6$.

Figure S8. The NOESY spectrum of chaetominine in DMSO-*d*₆.

(A) L-alanine FDAA derivative

(B) D-alanine FDAA derivative

(C) FDAA derivative of alanine released from chaetominine (**1**) (D) Overlaid chromatograms of (A), (B) and (C)

Figure S9. HPLC chromatograms of Marfey's derivatives.