Electronic Supplementary Information

Chaetoglobin A and B, Two Unusual Alkaloids from Endophytic Chaetomium globosum Culture

Hui Ming Ge,‡ Wei Yun Zhang,‡ Gang Ding, Patchareenart Saparpakorn, Yong Chun Song, Supa Hannongbua, Ren Xiang Tan

a Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, P. R. China, Fax: (+) 86-25-83302728, E-mail: rxtan@nju.edu.cn

b Jiangsu Key Laboratory of Molecular Medicine, Medical School, Nanjing University, Nanjing, 210093, P. R. China

c Department of Chemistry, Faculty of Science, Kasetsart University and Center of Nanotechnology, Kasetsart University, Cha-tu-chak, Bangkok 10900, Thailand

Experimental Section

Figure S1 Relative potential energy of dihedral angle 4a-5-5’-4a’ of chaetoglobin A at B3LYP/6-31G(d) level of theory, based on partial optimization.

Figure S2 1H-NMR spectrum of of 1 in [D₄]MeOH

Figure S3 13C-NMR spectrum of of 1 in [D₄]MeOH

Figure S4 DEPT135 spectrum of of 1 in [D₄]MeOH

Figure S5 HMQC spectrum of 1 in [D₄]MeOH

Figure S6 HMBC spectrum of 1 in [D₄]MeOH

Figure S7 1H-1H COSY spectrum of 1 in [D₄]MeOH

Figure S8 Positive-ion HR-ESIMS spectrum of 1

Figure S9 IR spectrum of 1

Figure S10 CD spectrum of 1 in MeOH

Figure S11 UV spectrum of 1 in MeOH

Figure S12 1H-NMR spectrum of of 2 in [D₄]MeOH

Figure S13 13C-NMR spectrum of of 2 in [D₄]MeOH

Figure S14 DEPT135 spectrum of of 2 in [D₄]MeOH

Figure S15 HMQC spectrum of 2 in [D₄]MeOH

Figure S16 HMBC spectrum of 2 in [D₄]MeOH

Figure S17 1H-1H COSY spectrum of 2 in [D₄]MeOH

Figure S18 Positive-ion HR-ESIMS spectrum of 2

Figure S19 IR spectrum of 2

Figure S20 CD spectrum of 2 in MeOH

Figure S21 UV spectrum of 2 in MeOH
Experimental Section

General Experimental Procedures: The UV spectrum was recorded on a Hitachi U-3000 spectrophotometer. CD spectra were obtained on a JASCO J-810 spectrometer, and the IR spectrum was measured on a Nexus 870 FT-IR spectrometer. ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer; NMR data were acquired in [D₄]MeOH and [D₆]DMSO on a Bruker DPX300, DRX500 NMR spectrometer with ¹H and ¹³C NMR with tetramethylsilane (TMS) and solvent signals as internal references. Silica gel (200-300 mesh) for column chromatography was produced by Qingdao Marine Chemical Factory, Qingdao, China. Sephadex LH-20 was purchased from Pharmacia Biotech, Sweden. Mosher’s acid chloride was purchased from Sigma-Aldrich. HPLC analyses were performed by using a column of Allsphere ODS-2.5 mm (250×4.6 mm), Hitachi pump L-7100, and a UV detector L-7400. All chemicals used in the study were of analytical grade.

Fungal Identification: Chaetomium globosum IFB-E019 was isolated from the fresh plant Imperata cylindrica collected in November 2001 from the seashore of Yancheng, Jiangsu, China. The specimen of Imperata cylindrica, authenticated by Prof. X. J. Tian, was preserved in the Herbarium of Nanjing University.

The isolated endophytic fungus IFB-E019 was identified according the following morphological characters. Colonies of IFB-E019 on PDA grew slowly, attaining 50-53 mm in diameter in 9 days at 22 °C. The surface of the colony, first white turned off dust-color gradually, and the back of lawn looked black brown, with many granules in the middle of the colony in PDA plates. The surface of the colony had concentric rings. The shell of vesicles was ovate and had a taupe coloration, adhering to the culture plate, with interascicular pseudoparenchyma. Vesicles were claviform and transparent, with eight spores. These morphological characteristics suggested an identification of the endophytic fungus as Chaetomium globosum₁, which was reinforced by the sequence of its 18S rRNA that gave a 99% sequence similarity to those accessible by BLAST analyses of available C. globosum sequences.

**Fermentation and Isolation:** After growing on PDA medium at 28 °C for 5 days, the title endophyte *C. globosum* IFB-E019 was inoculated into Erlenmeyer flasks (1000 mL) containing 300 mL PDA medium. After incubation for 4 days at 28 ± 1 °C on a rotary shaker at 150 rpm, 20 mL of culture liquid was transferred as the seed into 250 mL flasks, each preloaded with the evenly mingled medium composed of 7.5 g grain, 7.5 g bran, 0.5 g yeast extract, 0.1 g sodium tartrate, 0.01 g FeSO₄·7H₂O, 0.1 g sodium glutamate, 0.1 mL pure corn oil, and 30 mL H₂O, and grown for 40-day at 28 ± 1 °C with the relative humidity in the range of 60-70 %. The harvested solid culture was dehydrated to a residue (3.5 kg, not completely dried) which was extracted at room temperature with MeOH/CHCl₃ (1:1, v/v) mixture (5 L × 5). Evaporation of the solvent under reduced pressure gave a brown oil (300 g), to which 1000 mL H₂O was added and thoroughly mixed to yield a suspension. This was extracted successively with petroleum ether (1000 mL × 3), EtOAc (1000 mL × 3) and *n*-butanol (1000 mL × 3). The *n*-butanol fraction, shown to be strong cytotoxic, was concentrated *in vacuo* to give a residue (30.0 g), which was chromatographed on a silica gel column (60 × 10 cm) eluted with a CHCl₃/MeOH gradient (CHCl₃:MeOH = 1:0; 100:1; 100:2; 100:4; 100:8; 100:16; 0:1, each 1.5 L) to afford 7 fractions. The bioactive fraction Fr-5 (CHCl₃:MeOH = 100:8) was chromatographed on a Sephadex LH-20 column (40 × 2.8 cm) with MeOH (80 mL) to give dark red gum, which was, then, separated by HPLC (H₂O: MeOH=70:30) to afford chaetoglobin B (5.2 mg, *t_R*=8.21 min) and at chaetoglobin A (42.3 mg, *t_R*=10.34 min).

**Cytotoxicity assay of chaetoglobin A against breast cancer cells and colon cancer cells in vitro**

The cancer cell lines were maintained as monolayer culture in RPMI-1640 medium containing 10 % fetal bovine serum, 100 units/mL penicillin and 100 µg/mL streptomycin. The cells were cultured at 37 °C in a 5 % CO₂, and water-saturated atmosphere. Exponentially growing cells were collected and diluted to 5×10⁴ /mL with medium. They were plated into a 96-well microplate in 100 µl volume aliquots and incubated overnight for attachment. Chaetoglobin A with different concentrations was added to each well and ultimate concentrations were 1.25, 2.5, 5, 10, 20, 40 µg/mL, respectively. Five wells were used for each concentration. Medium was used as control. The plate was placed at 37°C in a humidified 5 % CO₂, 95 % air atmosphere for 48 h. Cell numbers in each well were quantified by the MTT (3’-[4,5-Dimethylthiazol-2-yl]-2, 5-diphenyl
tetrazolium bromide, Sigma) method. The plate was incubated for additional 4 h after addition of
10 μl MTT per well, and then the medium was removed and formazan was dissolved in 150 μl
DMSO. Finally, the microplates were read on a Bio-Rad microplate reader (Model 550) using test
wavelength of 570 nm.

**Immunohistochemical analysis of bcl-2, c-myc and c-fos expression**

Exponentially growing cells were collected and 2×10⁴ cells in medium were pippeted onto
coverslips in 6-well microplates. After over night incubation, different concentrations of
chaetoglobin A (ultimate concentrations were 2.5, 5, 10, 20 μg/mL, respectively) were added and
then were incubated again at 37 °C in a 5% CO₂ atmosphere for 48h. The coverslips with cell
growing were taken out and were washed twice with PBS (pH7.4). The cells were fixed with 80%
pre-cold acetone for 15 min. Endogenous peroxidase was blocked with 3% (v/v) H₂O₂ for 10 min
at 25 °C. The coverslips were washed three times for 5 min with 0.1 M PBS (pH7.4) and
incubated for 30 min at 25 °C in a protein-blocking solution containing 5% bovine serum albumin.
After discarding extra blocking solution, the primary antibody, rabbit anti-human bcl-2, c-myc or
β-catenin (1:200), was applied to the coverslips for 1 h at 37 °C in a moist chamber. Then the
residual antibody was washed off with 0.1 M PBS (pH 7.4; three times, 5 min each time) and
allowed to react with the biotinylated secondary antibody, namely biotin–goat antirabbit IgG, for
30 min. After three 5 min washes with PBS, they were treated with streptavidin–peroxidase for 20
min and then were incubated with 3,3-diaminobenzidine solution for 3-5 min after washing.
Finally, the reaction was stopped by wash washing with distilled water. They were fixed on glass
slide with arabic gum. The experiment was repeated at least two times. Slides were examined
using a light microscope at ×400 magnifications. The percent of bcl-2, c-myc or β-catenin protein
positive cells was respectively determined by checking 10 visual fields for each concentration. All
values were expressed as mean ± S.D. Statistical analysis was performed by t-test. P < 0.05 was
accepted as statistically significant.

**Computational methods:** Starting geometry of chaetoglobin A was constructed by using
Sybyl7.2 program and then fully optimized at B3LYP/6-31G(d) level of theory by using Gaussian03 program. Conformational analysis of dihedral angle 4a-5-5’-4a’ was studied by the minimization of the geometries at fixed dihedral angle with a step size of 30 degree at B3LYP/6-31G(d) level of theory.

\[^2\] SYBYL 7.2; TRIPOS, Assoc., Inc.: St. Louis, MO

Figure S1. Relative potential energy of dihedral angle 4a-5-5’-4a’ of chaetoglobin A at B3LYP/6-31G(d) level of theory.
Figure S2  
$^1$H-NMR spectrum of of 1 in [D$_4$]MeOH
Figure S3  $^{13}$C-NMR spectrum of 1 in [D$_4$]MeOH
Figure S4  DEPT135 spectrum of of 1 in [D₄]MeOH
Figure S5  HMQC spectrum of 1 in [D₄]MeOH
Figure S6  HMBC spectrum of 1 in [D₄]MeOH
Figure S7  $^1$H-$^1$HCOSY spectrum of 1 in [D$_4$]MeOH
Figure S8  Positive-ion HR-ESIMS spectrum of 1
Figure S9  IR spectrum of 1
Figure S10  CD spectrum of 1 in MeOH

Figure S11  UV spectrum of 1 in MeOH
Figure S12  $^1$H-NMR spectrum of 2 in [D$_4$]MeOH
Figure S13  $^{13}$C-NMR spectrum of 2 in [D$_4$]MeOH
Figure S14  DEPT135 spectrum of of 2 in [D₄]MeOH
Figure S15  HMQC spectrum of 2 in [D₄]MeOH
Figure S16  HMBC spectrum of 2 in [D₄]MeOH
Figure S17 \textsuperscript{1}H-\textsuperscript{1}HCOSY spectrum of 2 in [D\textsubscript{4}]MeOH
Figure S18  Positive-ion HR-ESIMS spectrum of 2
Figure S19  IR spectrum of 2
Figure S20  CD spectrum of 2 in MeOH

Figure S21  UV spectrum of 2 in MeOH