Chaetoglobins A and B, two unusual alkaloids from endophytic *Chaetomium globosum* culture†

Hui Ming Ge,‡[a] Wei Yun Zhang,‡[b] Gang Ding,[a] Patchareenart Saparpakorn,[c] Yong Chun Song,[a] Supa Hannongbua[c] and Ren Xiang Tan*[a]

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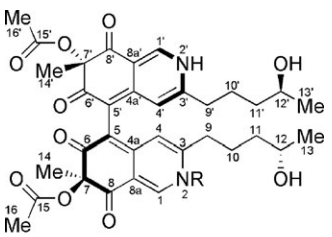
Chaetoglobins A (1) and B (2), two azaphilone alkaloid dimers with an unprecedented skeleton, were characterized from an endophytic fungus *Chaetomium globosum* with the former ascertained to be a significant cytotoxin valuable for anti-tumor drug discovery.

The field-growing plants harbor a wide range of symbiotic microorganisms named collectively "endophyte", which are actually fungi and bacteria living inter- and intra-cellularly in the apparently healthy plant tissues.[1] The existence of endophytes helps the hosts' overall adaptability to an array of stresses such as salinity,[2] drought, heat,[3] predators' attack[4] and pathogens' colonizations,[5] in return to the nutrition and protection the plants provide.[1] Endophyte has been assumed to be genetically unique owing to the high tendency that it might have acquired the host derived genes such as transposons. That is why endophytic microbes are being accepted as a fresh and rich source of functional biomolecules.[1,6] In our proceeding paper, we described the characterization of a series of bioactive macrocyclic indole alkaloids from the EtOAc extract of the solid substrate culture of *C. globosum* IFB-E019, an endophytic fungus residing inside the normal stem of *Imperata cylindrical*.[7] Moreover, our continuing test for the cytotoxic endophyte metabolites indicated that the *n*-BuOH extract derived from the regrown biomass of the endophyte showed substantial inhibitions to a set of cancer cell lines. The sequential chromatographies of the extract over silica gel and Sephadex LH-20 followed by final HPLC refinements gave two red alkaloids with an unprecedented carbon skeleton, named chaetoglobins A and B, respectively. We herewith wish to report the structure elucidation of the two metabolites accomplished by a combination of chemical (Mosher's reaction) and spectral evidences (IR, CD and HR-ESI-MS as well as 1D and 2D NMR techniques including ^{1}H and ^{13}C NMR, DEPT, COSY, NOESY, HMQC and HMBC), along with the cytotoxicity against cancer cell lines.

Chaetoglobin A (**1**)§ was isolated as orange–red gum. It gave the broadened IR absorption bands at 3261–3384 cm^{-1}, indicative of NH and/or OH, as well as those at 1732, 1692 and 1644 cm^{-1} characteristic of ester and α,β-unsaturated carbonyls. Compound **1** possessed a molecular formula of $C_{34}H_{40}N_2O_{10}$ (implying 16 unsaturation indices in the molecule) as evidenced from its positive-ion HRESIMS (*m*/*z* [M + H]$^+$ 637.2759, calc. for $C_{34}H_{41}N_2O_{10}$: 637.2756). However, the ^{13}C NMR spectrum of chaetoglobin A (aquired in [D$_4$]-MeOH) displayed only a total of 17 discrete carbon resonance lines arising from three methyl, three sp^3-hybridized methylene, one sp^3- and two sp^2-hybridized methine, one sp^3- and four sp^2-hybridized quaternary carbons, two conjugated ketones and an ester carbonyl group. And the ^{1}H NMR spectrum of metabolite **1** recorded in [D$_4$]-MeOH and [D$_6$]-DMSO afforded seemingly 18 and 20 hydrogens, respectively. The aforementioned NMR spectral "unusuality" could only be rationalized by assuming that chaetoglobin A consisted of two identical motifs ($C_{17}H_{20}N_1O_5\times2$), each having two deuterium exchangeable protons. Subtracting the twelve indices of unsaturation (corresponding to six carbonyl and twelve sp^2-hybridized carbons), the remaining double bond equivalents of **1** had to be expressed by four cycles in the molecule.



1 R = H **2** R = CH_2CH_2OH

The ^{1}H–^{1}H COSY spectrum of **1** indicated the presence of a side chain as shown in Fig. 1 marked with bold lines. Moreover, the presence of 2*H*-isoquinoline-6,8-dione moiety was evidenced from the HMBC correlations of 14-CH$_3$ (δ 1.64) with C-6 (δ 189.6), C-7 (δ 85.7) and C-8 (δ 197.8), of H-4 (δ 6.42) with C-3 (δ 151.2), C-5 (δ 103.7) and C-8a (δ 116.7), and of H-1 (δ 8.05) with C-3, C-4a (δ 153.2), C-8 and C-8a. In conjunction with the IR absorption band at 1732 cm^{-1}, the cross-peak between C-15 (δ 171.8) with 16-CH$_3$ (δ 2.19) disclosed the existence of an acetyloxy group whose anchorage at C-7 was based on the magnitude (δ 85.7) of the carbon signal.[8] The formulated linkage between 2*H*-isoquinoline-6,8-dione motif and the aliphatic chain was elucidated by the HMBC correlations of C-9 (δ 33.9) with H-4, of C-4 (δ 116.0) with H-9 (δ 2.49), and of C-3 with H-10 (δ 1.72). Another identical motif, displaying the same set of ^{1}H and ^{13}C NMR signals, had to be incorporated in the molecule through no bondage but that between C-5 and C-5′, both resonating at δ 103.6.

[a] *Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, P. R. China. E-mail: rxtan@nju.edu.cn; Fax: (+) 86-25-83302728*
[b] *Jiangsu Key Laboratory of Molecular Medicine, School of Medicine, 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*

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‡ These authors contributed equally to this work.

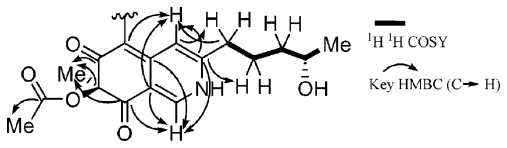


Fig. 1 Selected 2D NMR correlations for chaetoglobin A.

To understand the absolute stereochemistry, metabolite **1** was treated separately with (*R*)- and (*S*)-α-methoxy-α-trifluoromethyl phenylacetyl chloride (MTPA-Cl) by the in-NMR-tube Mosher's method.[9] The 12*S* configuration was subsequently unveiled by the $\Delta\delta_{S-R}$ values calculated from the ^{1}H NMR spectra of the resulted MTPA esters **1r** and **1s** to be negative for H-13, but positive for H-9, H-10 and H-11 (Fig. 2). Though Mosher's method became invalid, the 7*S*-configuration of **1** was eventually allocated on the basis of its CD absorption bands (MeOH, Fig. S9, ESI†) at 249 nm ($\Delta\varepsilon$ +5.9), 354 (+11.8) and 302 (−5.2), which were matches for those of (7*S*)-7-acetoxy-3,5,7-trimethyl-2-benzopyrane-6,8-dione and rubiginosin C.[10,11] Furthermore, the CD spectrum of **1** showed a negative exciton split [395 nm ($\Delta\varepsilon$ −3.5) and 354 (+11.8)] due to the exciton coupling of isoquinoline-6,8(2*H*,7*H*)-dione moiety. Thus, the axial chirality around C-5 and C-5′ was determined to be *M*-helicity.[12]

The optimized structure of chaetoglobin A at B3LYP/6-31G(d) level of theory is shown in Fig. 3.[13] The dihedral angle 4a–5–5′–4a′ is 244°. Two intra H-bonds (2.426 and 2.792 Å) were found for stabilizing the structure. This structure was used to be the starting geometry in conformational analysis. The relative potential energy of dihedral angle 4a–5–5′–4a′ of chaetoglobin A is shown in Fig. S1 (ESI†). The dihedral angle at 244° was found to be the energy-lowest conformation that has the same torsion angle obtained from full optimization. Structures with other dihedral angles showed the steric clash between four rings in the structure and the two intra H-bonds were lost in some dihedral angles. The results confirmed that the dihedral angle 4a–5–5′–4a′ of 244° is the most stable form of chaetoglobin A.

Co-existent with metabolite **1** was its congener chaetoglobin B (**2**)¶ obtained also as orange red gum. The ^{1}H and ^{13}C NMR signals of **2** were well comparable to those of **1**. The HRESIMS spectrum of **2** gave a protonated molecular ion peak at *m*/*z* 681.3014 indicating that the molecular formula was $C_{36}H_{44}N_2O_{11}$, an extra elemental composition "C_2H_4O" in comparison to that of chaetoglobin A. Surprisingly, the molecule of **2** was made asymmetric by the two-carbon extra unit as evidenced from the partial superposition between the ^{1}H and ^{13}C NMR signals of the two motifs. This observation accommodated the presence of an *N*-(2-hydroxy)ethyl group

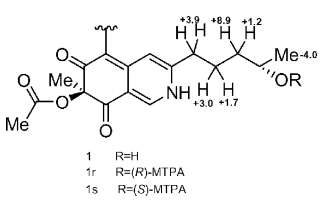


Fig. 2 $\Delta\delta$ (= $\delta_S - \delta_R$, expressed in Hz) values obtained for the MTPA esters of chaetoglobin A. MTPA = Methoxy (trifluoromethyl)-phenylacetic acid.

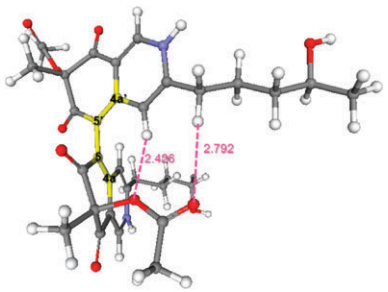
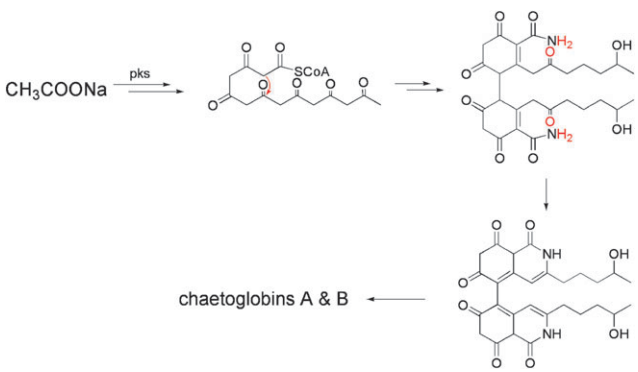


Fig. 3 Optimized structure of chaetoglobin A at B3LYP/6-31G(d) level of theory. A dihedral angle 4a–5–5′–4a′ of 244° was found as the most stable conformation.

in one of the two motifs if correlated with the two mutually coupled broadened triplets (J = 5.03 Hz) at δ_H 4.13 and 3.84 that showed HMQC correlations with two methylene carbon signals at δ_C 55.1 and 60.8, respectively. This assignment was reinforced by the HMBC correlation of H-a with C-1 and C-3. According to its CD spectrum, the absolute configuration of chaetoglobin B was just like that of **1**, which agreed with its presumable biosynthetic congeniality.

Chaetoglobins A and B both belong to the azaphilone-type compounds, which are a structurally diverse family of natural products containing a highly oxygenated bicyclic core and chiral quaternary center.[10,14] Previous biosynthetic studies with ^{13}C labelled acetate sodium tracer experiments on azaphilones (*e.g.* sclerotiorin, monascorubrin, monascoflavin, ochrephilone and chaetoviridin B) revealed the polyketide origin.[15] Thus, chaetoglobins A and B could be postulated to be acetate derived polyketides with a unique framework (Scheme 1).

Chaetoglobin A can significantly inhibit the proliferation ability of human breast cancer cell line MCF-7 and colon cancer cell line SW1116 with their IC_{50} values 26.8 and 35.4 μg ml^{-1}, respectively. It also inhibited expressions of tumor-related genes *bcl*-2, *c-myc* and *β-catenin* (Fig. 4). The proto-oncogene *bcl-2* is frequently expressed in cancer cells and expression of bcl-2 protein may inhibit apoptosis of cells.[16] Overexpression of the proto-oncogene *c-myc* is also common in human and animal cancers.[17] Elevated expression of β-catenin has been detected in cancers, which may result in constitutive activation of numerous β-catenin/Tcf target genes such as *c-myc* and *c-jun*, and may elevate bcl-2 protein expression as well.[18] Chaetoglobins A treated cancer cells expressed less proteins of bcl-2, c-myc and β-catenin than did untreated cells. Our



Scheme 1 Postulated biosynthesis of chaetoglobins A and B.

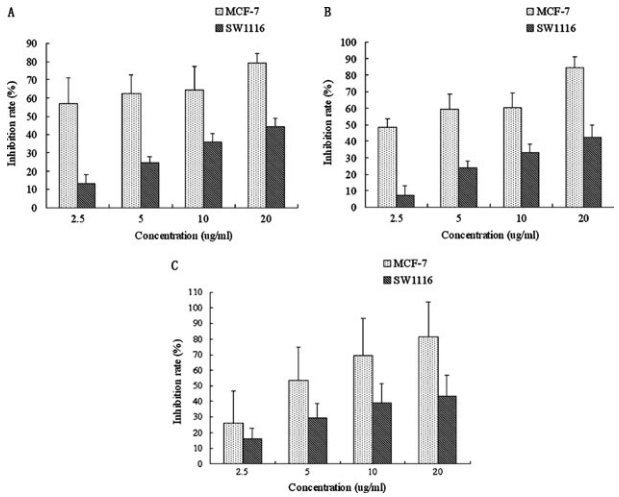


Fig. 4 Inhibition of chaetoglobin A on tumor-related gene expressions of MCF-7 and SW1116. (A) Effects of chaetoglobin A on *bcl-2* expression of MCF-7 and SW1116 cancer cells, $n = 10$. (B) Effects of chaetoglobin A on *c-myc* expression of MCF-7 and SW1116 cancer cells, $n = 10$. (C) Effects of chaetoglobin A on *β-catenin* expression of MCF-7 and SW1116 cancer cells, $n = 10$.

results suggested that chaetoglobin A could down-regulate all the three genes' expression in a dose dependent manner. As the decline in bcl-2, c-myc and β-catenin protein levels was thought to be related to the inhibition of tumor growth, it is therefore believed that chaetoglobin A can significantly inhibit the growth of those tumors.

In summary, chaetoglobins A (**1**) and B (**2**) isolated from an endophytic fungus *C. globosum* represent the first member of a novel class of azaphilone alkaloids unique in its dimerization through the bondage between C-5 and C-5′. The former has been demonstrated to be significantly cytotoxic against cancer cells used as targets in the study although the latter failed to be tested owing to its paucity of sample. In addition, chaetoglobin A is unique both in its azaphilone dimer based alkaloidal architecture, and in its cytotoxicity resulted from its inhibition of bcl-2, c-myc, β-catenin expressions in the test cancer cells, shedding light on its value as a new potential lead compound for the anti-tumor drug discovery.

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Notes and references

§ Chaetoglobin A: orange red gum; UV/Vis (MeOH): λ_{max}/nm (log ε) = 246.0 (4.56), 372.0 (4.50); CD (c 4.2 × 10^{-5} g ml^{-1}, MeOH) λ_{max}/nm ($\Delta\varepsilon$): 249 (+5.9), 302 (−5.2), 354 (+11.8), 395 (−3.5); IR (KBr): ν = 3384, 3261, 3117, 3073, 2962, 2931, 2875, 1732, 1692, 1644, 1549, 1471, 1372, 1247, 1204, 1128, 1080 cm^{-1}; ^{1}H NMR (300 MHz, [D_4]MeOH): δ 1.16 (d, 6.2, H-13, 13′), 1.45 (m, H-11, 11′), 1.64 (s, H-14, 14′), 1.72 (m, H-10, H-10′) 2.19 (s, H-16, 16′), 2.49 (t, 7.4, H-9, 9′), 3.73 (m, H-12, 12′), 6.42 (s, H-4, 4′), 8.05 (s, H-1, 1′); ^{13}C NMR (75 MHz, [D_4]MeOH): δ 20.4 (C-16, 16′), 23.3 (C-13, 13′), 23.4 (C-14, 14′), 25.3 (C-10, 10′), 33.9 (C-9, 9′), 39.0 (C-11, 11′), 67.8 (C-12, 12′), 85.7 (C-7, 7′), 103.7 (C-5, 5′), 116.0 (C-4, 4′), 116.7 (C-8a, 8a′), 139.6 (C-1, 1′), 151.2 (C-3, 3′), 153.2 (C-4a, 4a′), 171.8 (C-15, 15′), 189.6 (C-6, 6′), 197.8 (C-8, 8′); (+) ESI m/z: $[M + H]^+$ = 637, $[M + Na]^+$ = 659; (−) ESI m/z: $[M - H]^-$ = 635; HR ESI m/z: $[M + H]^+$ = 637.2759, calc.: 637.2756 for $[C_{34}H_{41}N_2O_{10}]$.

¶ Chaetoglobin B: orange red gum; UV/Vis (MeOH): λ_{max}/nm (log ε) = 246 (0.65), 374 (0.58); CD (c 1.1 × 10^{-4} g ml^{-1}, MeOH) λ_{max}/nm ($\Delta\varepsilon$): 249 (+5.5), 304 (−4.7), 356 (+11.6), 392 (−4.7); IR (KBr): ν = 3412, 3260, 2961, 2926, 2877, 1733, 1690, 1640, 1562, 1481, 1373, 1240, 1131, 1079 cm^{-1}, ^{1}H NMR (300 MHz, [D_4]MeOH): δ 1.16 (d, J = 6.2 Hz, H-13, 13′), 1.50 (m, H-11, H-11′), 1.64 (s, H-14, 14′), 1.68 (m, H-10, 10′), 2.19 (s, H-16), 2.20 (s, H-16′), 2.48 (t, J = 7.54 H-9′), 2.63 (q, J = 6.17, H-9), 3.75 (m, H-12, 12′), 3.84 (br t, J = 5.03 Hz, H-a), 4.13 (br t, J = 5.03, H-b), 6.41 (s, H-4′), 6.47 (s, H-4), 8.06 (s, H-1′), 8.16 (s, H-1); ^{13}C NMR (75 MHz, [D_4]MeOH): δ 19.6 (C-16, 16′), 22.46 (C-13), 22.54 (C-13′), 22.6 (C-14), 22.7 (C-14′), 24.5 (C-10), 24.7 (C-10′), 31.8 (C-9), 33.1 (C-9′), 38.2 (C-11), 38.3 (C-11′), 55.1 (C-a), 60.8 (C-b), 67.1 (C-12, 12′), 85.0 (C-7, 7′), 102.7 (C-5, 5′), 115.2 (C-4′), 115.8 (C-8a), 116.1 (C-8a′), 117.1 (C-4), 138.7 (C-1′), 143.6 (C-1), 150.3 (C-4a′), 151.1 (C-4a), 151.6 (C-3′), 152.4 (C-3), 171.0 (C-15′), 171.1 (C-15), 188.9 (C-6′), 189.3 (C-6), 196.7 (C-8), 196.9 (C-8′); (+) ESIMS m/z: $[M + H]^+$ = 681, $[M + Na]^+$ = 703; HRESIMS m/z: $[M + H]^+$ = 681.3014, calc.: 681.3018 for $[C_{36}H_{45}N_2O_{11}]$.

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