Eremophilane sesquiterpenes from Hawaiian endophytic fungus *Chaetoconis* sp. FT087

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Bioassay-guided fractionation of an extract from a Hawaiian endophytic fungus *Chaetoconis* sp. led to the isolation of eight compounds (**1-8**), including six previously unknown sesquiterpenes, chaetopenoids A–F (**1-6**). Their chemical structures were determined by HRMS and NMR spectroscopy. All isolates were evaluated for their cytotoxicity against A2780 and cisplatin-resistant A2780 (A2780CisR) cancer cell lines. Dendryphiellin A1 (**7**) was active against A2780 and A2780CisR cell lines with IC₅₀ values of 6.6 and 9.1 µg/mL, respectively.

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ABSTRACT: ABSTRACT: Seven sesquiterpene derivatives, including chaetopenoids A–F and dendryphiellin A₁, and 6-methyl-(2*E*, 4*E*, 6*S*) octadienoic acid were isolated from the culture broth of *Chaetoconis* sp. FT087. Their structures were determined through the analysis of HRMS and NMR spectroscopic data. The absolute configurations of chaetopenoids A–D were elucidated by comparison of their CD and optical rotation data with those in the literature. Chaetopenoids A–C and E belong to the eremophilane type of sesquiterpenoids, while chaetopenoids D and F and dendryphiellin A₁ have a trinor-eremophilane skeleton. All compounds were tested against A2780 and cisplatin resistant A2780CisR cell lines, and dendryphiellin A₁ was moderately active with IC₅₀ values of 6.6 and 9.1 µg/mL, respectively.

Keywords: endophytic fungi, *Chaetoconis* sp. FT087, *Osmozylon novoguineensis*, Araliaceae, sesquiterpenes, Hawai'i, bioactivity

1. Introduction

Endophytic fungi are rich producers of secondary metabolites, (Li, et al., 2015a, 2015b), and many fungal secondary metabolites are biologically active (Cao, et al., 2010; Cao, et al., 2011; Cao, et al., 2012a, 2012b). As part of an ongoing search for new and bioactive compounds from fungi collected in Hawai'i, about 5000 semi-pure fungal samples were screened against cell-based STAT3 (Signal Transducer and Activator of Transcription 3), human ovarian cancer cell lines A2780, and cisplatin-resistant A2780 (A2780CisR). The results showed that some semi-pure fractions, including those from *Chaetoconis* sp. FT087, were active against A2780 and A2780CisR at 20 µg/mL. A bioassay-guided separation of the active fractions of *Chaetoconis* sp. FT087 led to isolation of eight compounds **1–8** (Figure 1), including the active sesquiterpene derivative (**7**).

2. Results and discussion

The fungus strain *Chaetoconis* sp. FT087 was isolated from a leaf of *Osmoxylon novoguineensis* (Scheff.) Becc. collected in the Waimea Valley on the Oahu island, Hawai'i. The strain was grown in a liquid culture for 30 days and the culture broth (3 L) was filtered through filter paper. The mycelia were then extracted with 80% acetone in water, followed by concentration under reduced pressure, to afford an aqueous solution. The filtered culture broth and the aqueous mycelial solution were combined and passed through a HP-20 column, eluted with MeOH-H₂O (10%, 30%, 50%, 70%, 90% MeOH in H₂O), to afford five fractions (Frs. A–E). All fractions were tested against A2780 and A2780CisR cancer cell lines. Fractions D and

E were active; therefore, both fractions were further fractionated by preparative HPLC and semi-preparative HPLC to afford compounds **1–8** (Figure 1).

Compound **1** was isolated as a colorless solid. Its molecular formula was determined by HRESIMS (m/z 413.1960 $[M - H]^-$, calcd 413.1964) to be $C_{24}H_{30}O_6$, indicating 10 degrees of unsaturation. Comprehensive analysis of the 1H and ^{13}C NMR spectroscopic data (Table 1), as well as HSQC spectra indicated the presence of four methyls, three methylenes (one olefinic), ten methines (seven olefinic), and seven other carbons with no hydrogen attached (including one conjugated ketone (δ_C 199.8) and two carbonyl groups). Three partial structures, C-1–C-4–C-15, C-2'–C-8', and C-6'–C-9', were established by the 1H - 1H COSY spectrum as shown in Fig. 2. A literature search showed that the NMR data of compound **1** was very similar to those of KM-01, which was previously isolated from two fungi *Drechslera avenae* and *Pycnoporus coccineus* (Kim, et al., 1998). The only difference between **1** and KM-01 was the presence of a carboxyl functionality in **1** instead of an aldehyde in KM-01. The molecular formula of **1**, which has one more oxygen atom than that of KM-01, and the HMBC correlations from the protons of the terminal olefinic methylene (H_2 -12) to the carbonyl group (δ_C 170.3), suggested that the aldehyde in KM-01 was oxidized to a carboxyl moiety in **1**. Although no HMBC correlations between H_2 -12 and C-7, or between H_2 -6 and C-11, were observed, the functional group $CH_2=CRCOOH$ was most likely located at the 7-position because it was the only possible position. The key HMBC correlations shown in Fig. 2 further verified the proposed structure of compound **1**.

Compound **2** was isolated as a white powder. Its negative HRESIMS quasi-molecular ion peak at m/z 427.2117 $[M - H]^-$ (calcd 427.2121) corresponded to a molecular formula $C_{25}H_{32}O_6$, with one more carbon and two more hydrogen atoms than that of **1**. Comprehensive analysis of

the ^1H and ^{13}C NMR spectroscopic data (Table 1), as well as HSQC correlations, indicated the presence of five methyls, three methylenes (one olefinic), nine methines (six olefinic), and eight other carbons with no hydrogen attached (including one conjugated ketone (δ_{C} 199.8) and two carbonyl groups). Detailed comparison of the ^1H and ^{13}C NMR spectroscopic data of **2** with those of **1** showed that one more methyl group was present in **2** at the 4'-position. COSY and HMBC correlations (Fig. 2) suggested that the sesquiterpene moiety of **2** was the same as that of **1**. The observed HMBC correlations from the methyl at the 4'-position to the olefinic carbons C-3' (δ_{C} 151.8), C-4' (δ_{C} 133.2), and C-5' (δ_{C} 150.1) indicated that the C-10' methyl was located at C-4'.

Compound **3** was obtained as a white powder. Its molecular formula was determined to be $\text{C}_{24}\text{H}_{30}\text{O}_7$ by HRESIMS (m/z 429.1906 [$\text{M} - \text{H}$] $^-$, calcd 429.1913), with one more oxygen atom than that of **1**. Comprehensive analysis of the NMR spectra of **3** indicated the presence of three methyls, four methylenes (one olefinic, one oxygenated), ten methines (seven olefinic, and one oxygenated), as well as seven other carbons with no hydrogen attached. Comparison of the NMR data of **3** (Table 2) with those of **1** suggested that they were very similar except that one methyl in the latter was absent in the former, while one more oxygenated methylene (δ_{H} 3.35, δ_{C} 63.8) was observed in the molecule of **3**. The main difference between them was that the methyl (C-9') in **1** was replaced by an oxygenated methylene in **3**, which was proved by the HMBC correlations from $\text{H}_{2-7'}$ and $\text{H}-6'$ to the oxygenated methylene carbon C-9'.

Compound **4** was obtained as a white powder. The HRESIMS (m/z 385.1984 [$\text{M} + \text{Na}$] $^+$, calcd 385.1991) of **4** corresponded to a molecular formula $\text{C}_{21}\text{H}_{30}\text{O}_5$, three carbon and two oxygen atoms less than that of compound **3**. Comprehensive comparison of the NMR data for **4** (Table 2) and **3** suggested that both compounds had the same side-chain at 3-position. The main

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4 difference between **3** and **4** was that the 2-substituted prop-2-enoic acid moiety in **3** was absent
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6 in **4**. Moreover, the oxygenated carbon (C-7) in the former was replaced by an oxygenated
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8 methine (δ_{H} 4.58, δ_{C} 70.3) in the latter. These observations suggested that the 2-substituted
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10 acrylic acid moiety in **3** was absent in **4**, forming a trinor-sesquiterpene moiety. The COSY and
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12 HMBC correlations shown in Fig. 3 further supported the above deduction.
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16 Compound **5** was isolated as a colorless oil. Its molecular formula was established by
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18 HRESIMS (m/z 287.1250 $[\text{M} + \text{Na}]^+$, calcd 287.1259) to be $\text{C}_{15}\text{H}_{20}\text{O}_4$, suggesting 6 degrees of
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20 unsaturation. The ^1H and ^{13}C NMR spectra of **5** showed two methyls, three methylenes (one
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22 oxygenated, and one olefinic), five methines (three olefinic, and one oxygenated), and five other
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24 carbons with no hydrogen attached in the molecules. Detailed comparison of the NMR data for **5**
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26 (Table 3) with those of dendryphiellin G (Guerriero, et al., 1989) suggested that both compounds
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28 had the same skeleton. The major difference between **5** and dendryphiellin G was that one
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30 oxygenated methine (δ_{H} 3.80, δ_{C} 67.9) at the 8-position was present in **5** instead of a ketone in
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32 dendryphiellin G (Guerriero, et al., 1989). The observed HMBC correlations from H_2 -6 and the
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34 olefinic proton H-9 to the oxygenated methine (Fig. 4) implied that position 8 was hydrogenated,
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36 which was further verified by the HMBC correlations from H-8 to C-6, C-7, C-9, and C-10.
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44 Compound **6**, isolated as a colorless oil, was determined to have a molecular formula of
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46 $\text{C}_{12}\text{H}_{16}\text{O}_3$ by HRESIMS (m/z 209.1183 $[\text{M} + \text{H}]^+$, calcd 209.1178). The ^1H , ^{13}C , and HSQC NMR
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48 spectra of **6** indicated the presence of two methyls, one methylene, six methine, and three other
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50 carbons with no hydrogen attached. Compared to **5**, the 2-substituted prop-2-en-1-ol group was
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52 absent in **6**. Meanwhile, two oxygenated methine (δ_{H} 3.57, δ_{C} 68.4; δ_{H} 3.92, δ_{C} 72.3) groups were
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54 observed in **6** (Table 3), instead of one oxygenated methine and one oxygenated carbon in **5**. All
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56 of the above observations suggested that the prop-2-en-1-ol group (C-11–C-13) was absent in **6**.
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The COSY correlations in the spin-system H-6–H-9, and the HMBC correlations from H₂-6 and H-9 to C-5 undoubtedly supported the supposition.

Compounds **7** and **8** were identified as dendryphiellin A₁ (**7**) (Guerriero, et al., 1990) and 6-methyl-(2E, 4E, 6S) octadienoic acid (**8**) (Kim, et al., 1998), respectively, based on comparison of their physical data with reported values.

The relative configuration of the new compounds **1–6** was determined by the coupling constants and by NOESY experiments (Fig. 5, key NOE correlations for compounds **2** and **3**). The *trans* configuration of the conjugated double bonds on the side-chain at 3-position in compounds **1**, **3**, and **4** were assigned based on the large coupling constants between H-2' and H-3', as well as H-4' and H-5' (Tables 1 and 2). Meanwhile, the large coupling constant between H-2' and H-3' in compound **2**, and the NOE correlations between H-5' and H-3', and between H₃-10' and H-2' indicated that both olefins on the side-chain were in a *trans* configuration. The NOE correlations between the two methyls (CH₃-14 and CH₃-15) in each of the six new compounds implied that they had the same orientation. The coupling constant between H-3 and H-4 ($J \approx 5.0$ Hz), and the NOE correlation between H-3 and H-4 suggested that they were on the same side of the ring system.

From biosynthetic considerations, the stereochemistry of C-7 in **1–3** and **5** should be the same as that of KM-01. The hydroxyl group at C-7 of compounds **1–3** and **5** was determined to be β -oriented based on the similarity of the NMR data of C-6/7/8 when compared with the reported compounds (Kim, et al., 1998). The observed NOE correlations of compound **5** from H-8 to H₂-12, from the two methyls to H_{eq}-6, and from H_{ax}-6 to H-8 suggested that both of the hydroxyl groups were co-facial and on the up-side of the ring system. For compounds **4** and **6**, the NOE correlations from H₃-14 to H_{eq}-6 and H-7, and from H_{ax}-6 to H-8 demonstrated that H-7

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4 and H-8 were on opposite of the ring. The large coupling constant ($J = 7.9$ Hz) between H-7 and
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6 H-8 in compounds **4** and **6** implied that both of them should be in an axial orientation.
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9 The absolute configurations of the new compounds **1–3** were determined by comparison of
10 their CD spectra and optical rotations with that of KM-01 (Kim, et al., 1998). The CD spectra of
11 compounds **1–3** showed obvious positive Cotton effects between 280 and 300 nm, and **2** and **3**
12 exhibited negative Cotton effects between 240 and 255 nm, which were similar to that of KM-01
13 (Fig. 6). Thus, the absolute configuration of C-3 in compounds **1–3** were deduced to be *S*.
14 However, the CD spectra of compound **4** and KM-01 were different, perhaps due to the distinct
15 substituents at the 7- and 8-positions.
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25 The absolute configuration for the only chiral center on the side-chain in compound **1** was
26 determined by comparison of the optical rotation of the hydrolyzed product 6-methyl-(2*E*, 4*E*)
27 octadienoic acid of compound **1** with that reported in the literature (Kim, et al., 1998). The
28 hydrolyzed product had a positive optical rotation of +24.7, which is similar to that of the
29 synthesized analog (2*E*, 4*E*, 6*S*) octadienoate (+52.8). Thus, the absolute configuration of C-6'
30 was determined to be *S*, which is the same as that of KM-01. Meanwhile, the optical rotation of
31 compound **8** was measured and the value was +45.4, implying the absolute configuration of the
32 only chiral center in **8** should also be *S*. From a biosynthetic point of view, the other analogs (**2–4**)
33 should have the same stereochemistry at C-6' as those of compounds **1** and **8**.
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48 All the compounds were tested against A2780 and A2780CisR, but only compound **7**
49 showed antiproliferative activity with IC₅₀ values of 6.6 and 9.1 µg/mL, respectively. The
50 compounds were also tested against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*,
51 and *Pseudomonas aeruginosa*, but no activity was observed on all tested strains at the tested
52 concentration (100 µg/mL).
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3. Conclusions

The bioassay (A2780 and A2780CisR) guided separation of the secondary metabolites of the endophytic fungus *Chaetoconis* sp. FT087, obtained from a plant of *Osmoxylon novoguineensis* (Scheff.) Becc. in the Waimea Valley on the Oahu Island, Hawai'i, led to isolation of eight compounds including seven sesquiterpene derivatives. Among them, compounds **1–3**, and **5** belong to the eremophilane type of sesquiterpenoids, while compounds **4**, **6**, and **7** have a trinor-eremophilane skeleton. Their structures and absolute configurations were elucidated on the basis of spectroscopic data as well as CD analysis. Compound **5** might be the precursor of the C-15 sesquiterpenoids **1–3**, while **6**, which has a trinor-eremophilane skeleton, should be the precursor of compounds **4** and **7**. The branched unsaturated fatty acid **8** is likely to be the precursor for the substituent at 3-position of compounds **1–4**. Sesquiterpenoids, similar to compounds **1–7**, have been reported as brassinolide inhibitors (Kim, et al., 1998), antifouling (Watanabe, et al., 1995) and immunosuppressive (Fujimoto, et al., 2000) agents. Compounds **1–8** were evaluated for their activity against A2780S and cisplatin resistant A2780CisR cell lines, of which only **7** exhibited moderate antiproliferative activity. None of the compounds has antibacterial activity against *S. aureus*, *E. coli*, *B. subtilis*, and *P. aeruginosa*.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured with a DIP-370 digital polarimeter (JASCO). CD spectra were recorded on Olis DSM 20 CD spectrophotometer in methanol. UV spectra were acquired on a Beckman Coulter DU730 UV spectrophotometer, and IR spectra were measured on an IR Affinity-1 Fourier Transform Infrared FT/IR 620 spectrometer using ATR method. All NMR experiments were carried out on a Unity INOVA 500 NMR spectrometer. ESIMS spectra were obtained by LC/MS analysis that was performed on an Agilent 1100 Series HPLC/1100 Series mass spectrometer. High resolution mass spectra were acquired on a Waters Micromass Q-Tof Ultima ESI-TOF mass spectrometer. All of the compounds were purified on an Agilent 1100 series HPLC (Agilent Technologies) using a Phenomenex Luna C18 column (100 mm $\times$ 21.2 mm, 5 μ particle size), a Phenomenex Luna phenyl-hexyl column (250 $\times$ 10 mm, 10 μ m particle size) and a Phenomenex Luna C18 HPLC column (250 $\times$ 10 mm, 5 μ particle size), respectively.

4.2. Fungal material

Fungal strain *Chaetoconis* sp. FT087 was isolated on PDA medium from a leaf of *Osmoxylon novoguineensis* (Scheff.) Becc. collected in the Waimea Valley on the Oahu Island, Hawai'i. The fungus (Code number: UHCCFT087) has been deposited at the strain bank of University of Hawai'i Cancer Center. The colony of the strain was flat, spreading and soft. The mycelia were white, and ten days later became dark green with appearance of velvety white mycelia on the surface of colony. The strain was identified by analysis of the large subunit RNA (LSU) sequence amplified by primers LR5 and LROR. Mycelia were retrieved by filtration and ground to a fine powder in liquid N₂. Genomic DNA was extracted using the SurePrep RNA/DNA/protein purification kit (Fisher Bioreagents), and large subunit rDNA was amplified by PCR using primers LR5 (5'-TCCTGAGGGAACTTCG-3') and LROR (5'-ACCCGCTGAACTTAAGC-3'). PCR

products were sequenced at Genewiz (<http://www.genewiz.com/>). The DNA sequence data obtained from fungal strain FT087 have been deposited at GenBank with accession number KT307969.

4.3. Fermentation

The fungus was grown under static conditions at room temperature for 30 days in a one 1L conical flask containing liquid medium (300 mL/flask) composed of mannitol (20 g), glucose (10 g), monosodium glutamate (5 g), KH_2PO_4 (0.5 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.3 g), yeast extract (3 g), corn steep liquor (2 mL) for 1L distilled H_2O ; pH 6.5 prior to sterilization.

4.4. Extraction and isolation

The fermentation broth (3 L) was filtered through filter paper to separate the supernatant from the mycelia. The latter was extracted using acetone/ H_2O (80:20, v/v) and the resulting extract was concentrated under reduced pressure to afford an aqueous solution. The filtered culture broth and the aqueous mycelial extract was passed through a HP-20 column (Diaion, Sigma), eluted with $\text{MeOH-H}_2\text{O}$ (10%, 30%, 50%, 70%, 90% methanol in H_2O) to afford five fractions (Frs. A–E). All fractions were tested against A2780 and A2780CisR cell lines, and fractions D and E were active against both A2780 and A2780CisR at 20 $\mu\text{g/mL}$ (>50% inhibition). Frs. D and E were combined (83.20 mg) due to their similar HPLC chromatograms and subjected to preparative HPLC (C18 column, 5 μ ; 100.0 $\times$ 21.2 mm; 15 mL/min) eluted with 30%-100% MeOH in H_2O in 30 min to afford 30 sub-fractions (DE1-30). All the sub-fractions

were prepared for bioassay (DE-i: DE1-5; DE-ii: DE6-15; DE-iii: 16-25; DE-iv: 26-30) and tested against the A2780 and A2780CisR cell lines, and DE-iii (DE16-25) were active at 20 $\mu\text{g/mL}$ (>50% inhibition). Subfraction DE17 (8.38 mg) was subjected to semi-preparative HPLC (Phenyl-hexyl column, 5 μ ; 250.0 $\times$ 10.0 mm; 3 mL/min; with 0.1% HCO_2H in $\text{MeCN}:\text{H}_2\text{O}$ (30/70, v/v) to obtain compound **6** (1.78 mg, t_R 45.8 min) and **7** (1.56 mg, t_R 51.6 min). Sub-fraction DE18 (6.75 mg) was separated by semi-preparative HPLC (Phenyl-hexyl column, 5 μ ; 250.0 $\times$ 10.0 mm; 3 mL/min) eluted by a linear gradient program of 10%-50% MeCN in H_2O with 0.1% HCO_2H in 50 min to afford compounds **3** (2.18 mg, t_R 33.1) and **2** (0.95 mg, t_R 38.9). DE19 (4.26 mg) was separated by semi-preparative HPLC (Phenyl-hexyl column, 5 μ ; 250.0 $\times$ 10.0 mm; 3 mL/min) eluted by a liner gradient program of 30%-50% MeCN in H_2O with 0.1% HCO_2H in 40 min to afford compound **8** (1.20 mg, t_R 27.6). DE24 (3.63 mg) was further purified by semi-preparative HPLC (Phenyl-hexyl column, 5 μ ; 250.0 $\times$ 10.0 mm; 3 mL/min; with 0.1% HCO_2H in 40% MeCN in H_2O) to give compound **1** (1.49 mg, t_R 44.2 min). DE25 (2.50 mg) was further purified by semi-preparative HPLC (Phenyl-hexyl column, 5 μ ; 250.0 $\times$ 10.0 mm; 3 mL/min, with 0.1% HCO_2H in 40% MeCN in H_2O) to afford compound **2** (0.67 mg, t_R 48.7 min). Fr. C (142.7 mg) was separated by preparative HPLC (C18 column, 5 μ ; 100.0 $\times$ 21.2 mm; 15 mL/min, 30-80% MeOH in H_2O in 25 min) to yield compounds **4** (3.10 mg, t_R 14.7) and **5** (3.38 mg, t_R 17.3).

4.4.1. *Chaetopenoid A (I)*

White powder; $[\alpha]_D^{25}$ 259.7 (c = 1.49, MeOH); UV (MeOH) λ_{max} 278 nm; IR (KBr) ν_{max} 3415, 1587, 1386, 1352, 1304, 1247, 1187, 1139, 1009, 1032, 999 cm^{-1} ; For ^1H ($\text{DMSO}-d_6$, 500

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4 MHz) and ^{13}C NMR (DMSO- d_6 , 125 MHz) spectroscopic data, see Table 1; negative HR-ESIMS
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6 m/z 413.1960 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{24}\text{H}_{29}\text{O}_6$, 413.1964).
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10 11 4.4.2. Chaetopenoid B (2) 12 13

14 White powder; $[\alpha]_D^{25}$ 135.6 ($c = 0.67$, MeOH); UV (MeOH) λ_{max} 280 nm; IR (KBr) ν_{max}
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16 3417, 1593, 1354, 1293, 1243, 1163, 1127, 1110, 1039, 988 cm^{-1} ; For ^1H (DMSO- d_6 , 500 MHz)
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18 and ^{13}C NMR (DMSO- d_6 , 125 MHz) spectroscopic data, see Table 1; negative HR-ESIMS m/z
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20 427.2117 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{25}\text{H}_{31}\text{O}_6$, 427.2121).
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26 4.4.3. Chaetopenoid C (3) 27 28

29 White powder; $[\alpha]_D^{25}$ 144.7 ($c = 1.02$, MeOH); UV (MeOH) λ_{max} 280 nm; IR (KBr) ν_{max}
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31 3397, 1587, 1382, 1351, 1263, 1247, 1142, 1109, 1034, 999 cm^{-1} ; For ^1H (DMSO- d_6 , 500 MHz)
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33 and ^{13}C NMR (DMSO- d_6 , 125 MHz) spectroscopic data, see Table 2; negative HR-ESIMS m/z
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35 429.1906 $[\text{M} - \text{H}]^+$ (calcd for $\text{C}_{24}\text{H}_{29}\text{O}_7$, 429.1913).
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41 4.4.4. Chaetopenoid D (4) 42 43

44 White powder; $[\alpha]_D^{25}$ 161.0 ($c = 0.43$, MeOH); UV (MeOH) λ_{max} 240, 264 nm; IR (KBr) ν_{max}
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46 3406, 1594, 1352, 1261, 1177, 1139, 1085, 1054, 997 cm^{-1} ; For ^1H (DMSO- d_6 , 500 MHz) and
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48 ^{13}C NMR (DMSO- d_6 , 125 MHz) spectroscopic data, see Table 2; positive HR-ESIMS m/z
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50 385.1984 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{21}\text{H}_{30}\text{O}_5\text{Na}$, 385.1991).
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56 4.4.5. Chaetopenoid E (5) 57 58 59 60 61 62 63 64 65

Colorless oil; $[\alpha]_D^{25}$ 260.1 ($c = 0.50$, MeOH); UV (MeOH) $\lambda_{\max}$ 284 nm; IR (KBr) $\nu_{\max}$ 3392, 1659, 1652, 1588, 1398, 1386, 1352, 1320, 1216, 1165, 1122, 1102, 1080, 1024, 973 cm^{-1} ; For ^1H (DMSO- d_6 , 500 MHz) and ^{13}C NMR (DMSO- d_6 , 125 MHz) spectroscopic data, see Table 3; positive HR-ESIMS m/z 287.1250 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{15}\text{H}_{20}\text{O}_4\text{Na}$, 287.1259).

4.4.6. Chaetopenoid F (**6**)

Colorless oil; $[\alpha]_D^{25}$ 25.0 ($c = 0.50$, MeOH); UV (MeOH) $\lambda_{\max}$ 287 nm; IR (KBr) $\nu_{\max}$ 3384, 1662, 1587, 1456, 1387, 1374, 1350, 1215, 1117, 1062, 1025, 965, 938, 913 cm^{-1} ; For ^1H (DMSO- d_6 , 500 MHz) and ^{13}C NMR (DMSO- d_6 , 125 MHz) spectroscopic data, see Table 3; positive HR-ESIMS m/z 209.1183 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{12}\text{H}_{17}\text{O}_3$, 209.1178).

4.5. Hydrolysis of compound **1**

Compound **1** (1.0 mg) was dissolved in MeOH (1 mL) and 0.1N-KOH (1 mL). The solution was stirred for 2 h at room temperature and the products were extracted with EtOAc (2 mL×3). Subsequently, 0.1N HCl was added to the aqueous solution and the pH was adjusted to 7 and the solution was again extracted with EtOAc (2 mL×3). The organic layers were collected, vacuum evaporated, and subjected to preparative HPLC to yield 6-methyl-(2*E*, 4*E*, 6*S*) octadienoic acid **8** (0.18 mg).

4.6. Antiproliferative Assays

Viability of normal mouse fibroblasts (NIH3T3) and two Stat3-non-activated/activated cancer cell lines, A2780/A2780CisR (ovarian cancer), was determined using the CyQuant assay according to the manufacturer's instructions (Life Technologies, CA, USA) (Delazar, A., et al., 2004; Sridhar, C., et al., 2004). Briefly, cells were cultured in 96-well plates at 6000 cells per well for 24 h and subsequently treated with compounds for 72 h and analyzed. Relative viability of the treated cells was normalized to the DMSO-treated control cells (Delazar, A., et al., 2004; Sridhar, C., et al., 2004). Cisplatin was used as a positive control. All experiments were performed in triplicate.

4.7. Antibacterial assay

Bacteria were grown on agar plates [YMG or Luria–Bertani (LB)] for 1–2 days at 30 or 37 °C and then added to a liquid medium (YMG for *S. aureus*, *B. subtilis*, and *P. aeruginosa* and LB for *E. coli*). After shaking at 200 rpm and 30 °C (37 °C for *E. coli*) for 5–20 h, the cultures were diluted with YMG or LB media to obtain an OD600 value of ~0.1. Bacterial cultures (100 µL) with the desired optical density value were added to the media (50 mL) and then mixed gently. The bacterium-containing media (98 µL) were then added to each well of 96-well plates, containing a DMSO solution (2 µL) of each compound. DMSO was used as a negative control, whereas ampicillin was used as a positive control. The 96-well plates were placed in an incubator for 17 to 48 h. An MTT solution (50 µL, 1 mg/mL) was then added to each well. After letting the mixtures stand for 30 min, a color change was observed. All experiments were performed in triplicate. The minimum inhibitory concentration values were determined. Ampicillin was used

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4 as a positive control, which was active against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli*
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6 with IC₅₀ values of 0.78, 0.78, 1.56, and 6.25 µg/mL, respectively.
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40 **Appendix A. Supplementary data**

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45 Supplementary data including 1D and 2D NMR spectra of compounds **1–6** associated
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47 with this article can be found in the online version at
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49 <http://dx.dio.org/10.1016/J.phytochem.2015.08.001>.
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Fig 1. Compounds identified from endophytic fungus *Chaetoconis* sp. FT087

Fig. 2. Key COSY (bolds) and HMBC (arrows) correlations of **1** and **2**

Fig. 3. Key COSY (bolds) and HMBC (arrows) correlations of **4**

Fig. 4. Key COSY (bolds) and HMBC (arrows) correlations of **5**

Fig. 5. Key NOE correlations of compounds **2** and **3**.

Fig. 6. CD spectra of compounds 1–4.

Table 1. ^1H and ^{13}C NMR spectroscopic data for compounds **1** and **2** in $\text{DMSO-}d_6$

no.	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	6.45, d, 9.8	132.5	6.46, d, 9.7	132.6
2	6.21, m	133.1	6.21, dd, 9.8, 5.3	133.2
3	5.40, t, 5.0	70.7	5.42, t, 5.0	70.7
4	2.18, m	42.5	2.02, m	42.5
5		37.4		37.4
6a	2.38, d, 13.8	47.0	2.41, d, 14.2	47.1
6b	1.98, d, 14.2		1.98, d, 14.2	
7		76.9		76.9
8		199.8		199.8
9	5.90, s	125.7	5.90, s	125.8
10		162.0		162.0
11		154.3		154.6
12	5.90, br. S	122.6	5.86 br. s	122.1
	6.22, br. s		6.20 br. s	
13		170.3		170.5
14	1.51, s	23.0	1.52, s	23.0
15	1.00, d, 7.1	10.6	1.01, d, 7.0	10.7
1'		168.4		168.4
2'	5.83, d, 15.3	119.8	5.80, d, 15.6	116.1
3'	7.25, dd, 15.3, 10.9	147.3	7.31, d, 15.6	151.8
4'	6.24, dd, 15.1, 10.9	128.1		133.2
5'	6.05, dd, 15.2, 8.1	152.0	5.69, d, 9.4	150.1
6'	2.18, m	40.2	2.50, m	36.2
7'	1.39, m	30.4	1.30, m	31.1
			1.43, m	
8'	0.88, t, 7.4	12.1	0.86, t, 7.5	12.3 q
9'	1.03, d, 6.8	19.9	0.99, d, 6.8	20.5 q
10'			1.80, s	12.6 q

Table 2. ^1H and ^{13}C NMR spectroscopic data for compounds **3** and **4** in $\text{DMSO-}d_6$

no.	3		4	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	6.44, d, 9.8	131.4	6.29, d, 10.9	124.4
2	6.12 (dd, 9.7, 5.1)	131.0	5.86, dd, 9.6, 5.0	125.2
3	5.34 (t, 4.9)	68.8	5.24, t, 5.0	68.3
4	1.85, m	40.8	1.83, m	40.4
5		35.4		35.3
6a	1.83, overlap	46.0	2.12, dd, 13.2, 7.0	47.7
6b	2.20 (d, 13.9)		1.02, overlap	
7		75.3	4.58 (m)	70.3
8		196.9	4.72, dd, 7.6, 3.5)	69.7
9	5.79, s	125.2	5.72, d, 3.9	123.9
10		157.9		nd
11		157.9 overlap		
12	5.47 br. s	119.0		
	5.94 br. s			
13		170.2		
14	1.40, s	21.7	1.04, s	19.3
15	0.89 d, 7.0	10.0	0.92, d, 7.1	10.6
1'		166.0		166.1
2'	5.90, d, 15.4	118.6	5.88, d, 15.7	118.7
3'	7.18, dd, 15.3, 11.0	145.5	7.15, d, 15.6, 10.9	144.9
4'	6.30, dd, 15.3, 11.0	128.4	6.26 (dd, 15.3, 10.2)	130.9
5'	6.08, dd, 15.4, 9.4	147.4	6.06, dd, 15.3, 8.6	146.7
6'	2.11, m	47.3	2.12, m	39.4
7'	1.50, m	23.2	1.49, m	23.5
	1.22, m		1.22, m	
8'	0.80, t, 7.5	11.6	0.79, t, 7.5	11.6
9'	3.35, m	63.8	3.30, m	63.8

Table 3. ^1H and ^{13}C NMR spectroscopic data for compounds **5** and **6** in $\text{DMSO-}d_6$

no.	5		6	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	7.10, d, 9.9	145.8	7.05, d, 9.9	144.4
2	5.85, d, 9.8	126.0	5.82, d, 9.8	125.7
3		200.7		200.0
4	2.39, q, 6.8	52.9	2.43, q, 6.8	51.4
5		39.3		40.2
6	1.89, d, 13.6	38.7	1.75, dd, 12.5, 3.8	42.5
	1.74, d, 13.5		1.50, t, 12.5	
7		73.2	3.57, ddd, 12.1, 7.9, 3.8	68.4
8	3.80, d, 5.0	67.9	3.92, dd, 7.9, 2.4	73.2
9	6.07, d, 5.1	132.5	5.82, d, 2.6	135.4
10		140.4		140.3
11		153.7		
12		60.8		
13		109.1		
14	1.06, s	20.2	0.93, s	19.5
15	0.98, d, 6.8	7.4	0.91, d, 6.9	7.0

Table 4 IC₅₀ values (µg/mL) of compounds **1–8** against A2780 and A2780CisR cell lines.

Cell lines	1	2	3	4	5	6	7	8	Positive control (Cisplatin)
A2780	>20	>20	>20	>20	>20	>20	6.6	>20	0.34
A2780CisR	>20	>20	>20	>20	>20	>20	9.1	>20	1.0