Absolute structure and anti-oxidative activity of chaetochiversin C isolated from fungal strain Neocosmospora sp. FKI-7792 by physicochemical screening

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Summary

A new chaetochiversin analog, designated chaetochiversin C (1), was discovered from a cultured broth of fungal strain FKI-7792 by physicochemical screening. This strain was identified as a member of genus Neocosmospora based on morphology and DNA barcoding. The partially relative configuration of 1 was determined by $^{13}$C-NMR chemical shifts of the acetonide analog of 1. The absolute configuration was determined using an advanced Mosher’s method. Compound 1 was assessed for anti-tumor, anti-microbial, and anti-malarial activities, and its ability to scavenge or quench reactive oxygen species (ROS), such as superoxide anion radicals, hydroxy radicals and singlet oxygen ($^{1}$O$_2$). Compound 1 showed a quenching effect on $^{1}$O$_2$.

Keywords: absolute structure; antioxidant; chaetochiversin analog; Neocosmospora; physicochemical screening

Introduction
Many bioactive compounds, such as avermectin, cerulenin and pyripyropene, 

have been discovered as secondary metabolites in microorganisms by bioactivity-guided 

screening (Burg et al., 1979; Arison and Ōmura, 1974; Tomoda et al., 1996; Kim et al., 

1994). In some cases, biological assays can overlook valuable natural compounds that 
do not have a specific biological activity, or contain minor components at 

concentrations lower than those needed to exhibit activity. Conversely, many natural 
products have been isolated from cultured broths by physicochemical (PC) screening. 

For example, staurosporine was isolated from the cultured broth of Saccharothrix 
aerocolonigenes subsp. staurosporeus AM-2282T (Lentzea albida AM-2282) by 

Dragendorff’s reaction screening (Ōmura et al., 1977). After its initial discovery, 
staurosporine was found to have a protein kinase inhibitory activity and was released to 
the market as a protein kinase inhibitor (Tamaoki et al., 1986; Nakano et al., 1987; 
Nakano et al., 2009; Ōmura et al., 2018). Our research continues the search for new 
compounds via PC screening, which uses liquid chromatography-mass spectroscopy 
(LC/MS) analyses in combination with natural product databases, such as the 

Dictionary of Natural Products (http://dnp.chemnetbase.com/) and an in-house database,
to identify new compounds (Nakashima et al., 2017).

PC screening of four kinds of cultured broth of 560 fungal strains identified a new compound produced by the fungal strain FKI-7792. Strain FKI-7792 was isolated from the soil of Niijima Island (Tokyo, Japan). Based on its morphology and internal transcribed spacer (ITS) sequence, FKI-7792 was identified as a member of the genus *Neocosmospora*. A new compound, designated chaetochiversin C (1) (Fig. 1), was isolated using a guided LC/MS analysis of a cultured broth of *Neocosmospora* sp. FKI-7792. Compound 1 was evaluated for anti-tumor and anti-microbial activities, and for its ability to quench reactive oxygen species (ROS). Here, we report on the fermentation, isolation, structure elucidation and biological activity of 1.

**Materials and Methods**

**General experimental procedures**

All solvents were purchased from Kanto Chemical (Tokyo, Japan). Silica gel (Chromatorex FL100D) and ODS (Chromatorex ODS-DM1020MT) were purchased from Fuji Silysia Chemical (Aichi, Japan).
Nuclear magnetic resonance (NMR) spectra were measured using a JNM-ECA 1500 spectrometer (JEOL, Tokyo, Japan). $^1$H-NMR spectra were acquired at 500 MHz, and $^{13}$C NMR spectra at 125 MHz, in CD$_3$OD or CDCl$_3$. The chemical shifts are expressed in parts per million (ppm). The $^1$H-NMR spectra were referenced to residual CHD$_2$OD (3.31 ppm) and CHCl$_3$ (7.26 ppm), and the $^{13}$C NMR spectra were referenced to CD$_3$OD (49.0 ppm) and CDCl$_3$ (77.0 ppm). Electrospray ionization (ESI)-MS spectra were measured using a JMS-T100LP instrument (JEOL). Ultraviolet (UV) spectra were measured on a U-2810 spectrophotometer (Hitachi, Tokyo, Japan). Infrared (IR) absorption spectra were acquired on a FT-710 Fourier transform-IR spectrometer using potassium bromide as a sample matrix (Horiba, Kyoto, Japan). Melting point was measured with an OptiMelt instrument (Tokyo Instruments, Tokyo, Japan). Optical rotation was measured on a DIP-1000 polarimeter (Jasco, Tokyo, Japan).

**Taxonomic study and fermentation of screening broths and producing strain of 1**

Fungal strain FKI-7792 was isolated from a soil sample collected at Niijima Island (Tokyo, Japan). The ITS sequence of FKI-7792 was compared with sequences in
the GenBank database by a BLASTN 2.8.1 analysis (Altschul et al., 1997). The
sequence of FKI-7792 was 97.1% similar to that of CBS 101018 (ex-type of
*Neocosmospora rubicola*, GenBank accession number KM231800). The producing
strain FKI-7792 was identified as a member of genus *Neocosmospora* based on its
morphology and DNA barcoding.

Each fungal strains for PC screening were cultured by four different media as
follows; soybean meal medium (3.0% soluble starch, 1.0% glycerol, 2.0% soybean meal,
0.3% dry yeast, 0.3% KCl, 0.2% CaCO₃, 0.05% MgSO₄·7H₂O, 0.03% quercetin), rice
medium containing 0.01% seaweed tea powder (ITO EN, Tokyo, Japan), malt extract
medium (3.0% sucrose, 3.0% soluble starch, 1.0% malt extract, 0.3% Ebios, 0.5%
KH₂PO₄, 0.05% MgSO₄·7H₂O) and brown rice powder medium (3.0% brown rice
powder, 1.0% sucrose, 1.0% yeast extract, 0.5% glycerol, 0.5% NaNO₃, 0.2%
ammonium acetate, 0.001% FeSO₄ and 0.001% ZnSO₄).

Strain FKI-7792 was grown on a slant of modified Miura’s medium [LcA: 0.1%
glycerol, 0.08% KH₂PO₄, 0.02% K₂HPO₄, 0.02% MgSO₄·7H₂O, 0.02% KCl, 0.2%
NaNO₃, 0.02% yeast extract and 1.5% agar (adjusted to pH 6.0 before sterilization)]. A
loop of spores from the strain was inoculated into a 500-mL Erlenmeyer flask containing 100 mL of seed medium (2% glucose, 0.2% yeast extract, 0.5% hipolypeptone, 0.1% KH₂PO₄, 0.05% MgSO₄·7H₂O and 0.1% agar), which was shaken at 210 rpm on a rotary shaker at 27°C for 3 days. A 25-mL aliquot of the seed culture was transferred into 500 g of rice medium containing 50 mL of seaweed tea (0.1 mg/mL). The fermentation (500 g × 4) was carried out for 13 days at 25°C.

**Isolation of chaetochiversin C (1)**

To the cultured rice medium (2 kg) was added 2 L of ethanol (EtOH), and the mixture was filtered to separate solids from the EtOH extract. The EtOH was evaporated from the extract and the residuals were suspended in water. The suspension (500 mL) was partitioned with ethyl acetate (EtOAc) (500 mL × 3). The dried EtOAc extract (7.23 g) was purified via silica gel column chromatography (φ50 × 180 mm) (n-hexane/EtOAc = 8:2, 6:4 and CHCl₃:CH₃OH = 50:1, 25:1, 10:1, 9:1, 8:2, 6:4, 0:1, stepwise separation with 900 mL). The CHCl₃:CH₃OH = 9:1 fraction (168.0 mg) including 1 was purified on an ODS column (10–100% gradient system with CH₃OH aq
+ 0.1% formic acid for 60 min, φ25 × 100 mm, flow rate 10 mL/min, detection UV 254 nm). The collective fraction at retention time (rt) 25-35 min (25.8 mg) including I was purified by preparative reversed-phase high-performance liquid chromatography (HPLC) (Inertsil ODS-4, φ14 × 250 mm; GL Sciences, Tokyo, Japan) in an isocratic system using an eluent of 60% CH$_3$OH aq (0.1% formic acid) to obtain I (rt 13.2 min, 5.2 mg).

Preparation of acetonide analogs bis-isopropylidene acetal (2) and mono-isopropylidene acetal (3).

TsOH·H$_2$O (0.2 mg, 0.823 µmol) and 2-methoxypropene (7.7 µL, 82.3 µmol) were added to a solution of I (3.3 mg, 8.23 µmol) in dry acetone (0.82 mL) at room temperature. The reaction mixture was stirred for 45 min at room temperature and quenched with saturated aqueous NH$_4$Cl (3 mL). The mixture was then extracted with CHCl$_3$ (3 × 6 mL) and the combined extracts were dried over Na$_2$SO$_4$, filtered, and concentrated. The residue was purified by preparative thin-layer chromatography (TLC) (20 × 20 cm; CHCl$_3$/CH$_3$OH = 10/1). The collective fractions (Rf value 0.72 and 0.54)
were eluted with a mixture of CHCl₃ and CH₃OH (1:1) to yield bis-isopropylidene acetal (2) (2.6 mg, 66% yield) and mono-isopropylidene acetal (3) (0.7 mg, 19% yield) as colorless amorphous solids.

Bis-isopropylidene acetal (2): ¹H-NMR signals (CDCl₃, 500 MHz) δ: 11.13 (s, 1H, 8-OH), 6.63 (overlap, 2H, H-4 and H-2´), 6.62 (s, 1H, H-7), 6.36 (dd, J = 15.5, 1.4 Hz, 1H, H-1´), 4.58 (m, 1H, H-3´), 3.96 (m, 1H, H-8´), 3.85 (m, 1H, H-5´), 3.66 (dd, 14.3, 8.0 Hz, 1H, H-6´), 1.94 (ddd, J = 13.2, 2.3, 2.3 Hz, 1H, H-6´), 1.78 (ddd, J = 15.2, 9.2, 8.0 Hz, 1H, H-7´), 1.67 (ddd, J = 15.2, 9.7, 6.3 Hz, 1H, H-7´), 1.49 (s, 3H, acetonide CH₃), 1.46 (s, 3H, acetonide CH₃), 1.36 (s, 3H, acetonide CH₃), 1.34 (s, 3H, acetonide CH₃), 1.25 (m, 1H, H-4´), 1.21 (d, 3H, J = 6.3 Hz, 9´-Me), 13C-NMR signals (CDCl₃, 125 MHz) δ: 164.9 (C-1), 162.3 (C-8), 158.7 (C-6), 153.0 (C-3), 136.8 (C-2´), 135.9 (C-4a), 120.3 (C-1´), 105.8 (C-5), 102.8 (C-7), 102.3 (C-4), 100.9 (C-8a), 100.2 (acetal with C-6´ and C-8´), 99.0 (acetal with C-3´ and C-5´), 71.8 (C-5´), 69.5 (C-6´), 68.2 (C-3´), 62.8 (C-8´), 36.7 (C-7´), 34.1 (C-4´), 29.9 (acetonide CH₃ with C-3´ or C-5´), 25.0 (acetonide CH₃ with C-6´ or C-8´), 24.8 (acetonide CH₃ with C-6´ or C-8´), 21.6 (C-9´), 19.8 (acetonide CH₃ with C-3´ or C-5´).
Preparation of 6-methoxy analog (4)

Chaetochiversin C (1) (5.0 mg, 0.0125 mmol) dissolved in a CH\textsubscript{3}OH (1 mL) was treated with trimethylsilyl (TMS)-diazomethane (0.0624 mmol) (Tokyo Chemical Industry, Tokyo, Japan). The reaction was monitored by TLC while stirring at room temperature. After 1 hour, the reaction solution was evaporated and purified by preparative TLC (CHCl\textsubscript{3}:CH\textsubscript{3}OH = 10:1). The collective fraction (Rf value 0.32) was eluted with mixture of CHCl\textsubscript{3} and CH\textsubscript{3}OH (1:1) to yield the 6-methoxy analog (4) 4.9 mg, 95%) as a colorless solid.

6-methoxy analog (4): \textsuperscript{1}H-NMR signals (CD\textsubscript{3}OD, 500 MHz) \textdelta: 6.83 (s, 1H, H-4), 6.71 (s, 1H, H-7), 6.62 (dd, \textit{J} = 15.5, 6.1 Hz, 1H, H-2´), 6.44 (d, \textit{J} = 15.5 Hz , 1H, H-1´), 4.54 (ddd, \textit{J} = 6.7, 6.5, 6.1 Hz , 1H, H-3´), 4.00 (m, 1H, H-8´), 3.98 (s, 3H, 6-OMe), 3.70 (m, 1H, H-6´), 3.59 (m, 1H, H-5´), 1.88 (ddd, \textit{J} = 13.4, 6.7, 2.9 Hz , 1H, H-4´), 1.76 (ddd, \textit{J} = 13.8, 9.2, 6.9 Hz , 1H, H-4´), 1.64 (ddd, \textit{J} = 14.3, 9.7, 2.3 Hz , 1H, H-7´), 1.49 (ddd, \textit{J} = 14.3, 9.7, 2.9 Hz , 1H, H-7´), 1.21 (d, 3H, \textit{J} = 6.3 Hz, 9´-Me).
Preparation of (S)- and (R)-MTPA esters

A small amount of 4 (2.7 mg, 6.51 µmol) dissolved in 0.65 mL of tetrahydrofuran (THF) containing N,N'-dicyclohexylcarbodiimide (2.7 mg, 13.0 µmol) and 4-dimethylaminopyridine (0.08 mg, 0.651 µmol) was treated with (S)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid (MTPA-OH) (1.8 mg, 7.81 mmol) (Sigma-Aldrich, Tokyo, Japan). After stirring for 2 hours, the reaction was quenched with a solution of saturated NaHCO$_3$ and extracted with EtOAc. The organic layer was dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The MTPA esters were isolated by preparative TLC (CHCl$_3$:CH$_3$OH = 5:1) to afford a mixture of mono-MTPA esters. Further purification was carried out by preparative TLC (CHCl$_3$:CH$_3$OH = 10:1).

The two collective fractions (Rf values 0.80 and 0.84) were eluted with a mixture of CHCl$_3$ and CH$_3$OH (1:1) to yield a pure C-3´-(S)-MTPA ester (5a, 0.3 mg, 7.3%) and a mixture (0.7 mg, 17%) of C-8´-(S)-MTPA ester (6a), C-5´-(S)-MTPA ester, and C-6´-(S)-MTPA ester.

The (R)-MTPA ester was also prepared from 4 (3.0 mg, 7.23 mmol) using the same procedure used to prepare the (S)-MTPA esters described above. The reaction
mixture was separated by preparative TLC (CHCl₃:CH₃OH = 10:1). The two collective fractions (Rf value 0.45 and 0.52) were eluted with a mixture of CHCl₃ and CH₃OH (1:1) to yield a pure C-3´-(R)-MTPA ester (5b, 0.6 mg, 12%) and a mixture (0.5 mg, 10%) of C-8´-(R)-MTPA ester (6b) and an unidentified mono-MTPA ester.

C-3´-(S)-MTPA ester (5a): white amorphous solid; ¹H-NMR signals (CDCl₃, 500 MHz)

δ: 11.19 (s, 1H, 8-OH), 7.54-7.42 (m, MTPA-5H), 6.75 (s, 1H, H-4), 6.58 (s, 1H, H-7), 6.56 (dd, J = 15.5, 7.2 Hz, 1H, H-2´), 6.36 (d, J = 15.5 Hz, 1H, H-1´), 5.84 (q, 7.2 Hz, 1H, H-3´), 4.18 (m, 1H, H-8´), 3.98 (s, 3H, 6-OMe), 3.85 (m, 1H, H-6´), 3.62 (m, 1H, H-5´), 3.55 (s, 3H, MTPA-OMe), 2.21-2.13 (overlap, H-4´), 1.93 (m, 1H, H-4´), 1.78 (ddd, J = 15.2, 9.2, 3.4 Hz, 1H, H-7´), 1.46 (ddd, J = 15.2, 8.0, 3.4 Hz, 1H, H-7´), 1.27 (d, 3H, J = 6.3 Hz, 9´-Me)

C-3´-(R)-MTPA ester (5b): white amorphous solid; ¹H-NMR signals (CDCl₃, 500 MHz)

δ: 11.19 (s, 1H, 8-OH), 7.55-7.42 (m, MTPA-5H), 6.62 (s, 1H, H-4), 6.58 (s, 1H, H-7), 6.49 (dd, J = 15.5, 7.2 Hz, 1H, H-2´), 6.12 (d, J = 15.5 Hz, 1H, H-1´), 5.85 (q, 7.2 Hz, 1H, H-3´), 4.21 (m, 1H, H-8´), 3.98 (s, 3H, 6-OMe), 3.90 (ddd, 9.2, 5.2, 2.9, 1H, H-6´), 3.71 (ddd, 10.3, 10.3, 4.0 Hz, 1H, H-5´), 3.60 (s, 3H, MTPA-OMe), 2.07-1.93 (m, 2H, 9´-Me)
H-4'), 1.76 (ddd, J = 13.8, 9.2, 2.9 Hz, 1H, H-7'), 1.51 (overlap, H-7’), 1.29 (d, 3H, J = 6.3 Hz, 9’-Me)

C-8’-(S)-MTPA ester 6a: white amorphous solid; Key $^1$H-NMR signals (CDCl$_3$, 500 MHz) $\delta$: 5.41 (m, 1H, H-8’), 3.82 (m, 1H, H-5’), 3.37 (m, 1H, H-6’), 1.42 (d, 3H, J = 6.9 Hz, 9’-Me)

C-8’-(R)-MTPA ester 6b: white amorphous solid; Key $^1$H-NMR signals (CDCl$_3$, 500 MHz) $\delta$: 5.40 (m, 1H, H-8’), 3.90 (m, 1H, H-5’), 3.56 (m, 1H, H-6’), 1.35 (d, J = 6.3 Hz, 9’-Me)

Quenching effect on singlet oxygen ($^1$O$_2$)

Singlet oxygen was generated by laser irradiation as described previously (Nakamura et al., 2011; Ishiyama et al., 2012). The output power of the laser was 40 mW. A semi-microcuvette containing 200 µL of the reaction mixture was irradiated by the laser over an area approximately 5 × 5 mm, resulting in an energy dose of 160 mW/cm$^2$. The light path of the cuvette was 10 mm. A reaction mixture was prepared with 30 µL of phosphate buffer (PB, pH 7.0), 50 µL of 200 mM
2,2,5,5-tetramethyl-3-pyrroline-3-carboxamide (TPC; Sigma Aldrich, St. Louis, MO, USA), 20 µL of 100 µM rose bengal in PB, and 100 µL of the sample dissolved in acetone. As a negative control, 50 µL of acetone without any sample was also prepared.

To ascertain $^1\text{O}_2$ generation, 100 µL of 5 mM NaN$_3$ (Wako Pure Chemical, Osaka, Japan) in PB was added instead of the sample. Immediately after mixing, the cuvette was irradiated by laser light for 60 s. After irradiation, the sample was transferred to a quartz cell and its electron spin resonance (ESR) spectrum was recorded on an X-band JES-FA-100 ESR spectrometer (JEOL). Measurement conditions for ESR were: field sweep, 330.50–340.50 mT; field modulation frequency, 100 kHz; field modulation width, 0.05 mT; amplitude, 200; sweep time, 2 min; time constant, 0.03 s; microwave frequency, 9.420 GHz; and microwave power, 4 mW. To calculate the spin concentration of the nitroxide radical generated through TPC oxidation by $^1\text{O}_2$, 2 µM (1-oxyl-2,2,6,6-tetramethyl-4-hydroxypiperidine) TEMPOL was used as a standard and the ESR spectrum of manganese ion, which was equipped in the ESR cavity, was used as an internal standard. All tests were performed in triplicate.
Statistical analysis

Values are expressed as means ± standard deviation (SD). Statistical analyses of these data were carried out using Student's t tests. In all analyses, P values < 0.05 were taken to indicate statistical significance.

Results and Discussion

Structure elucidation of chaetochiversin C (1)

The physico-chemical properties of 1 are summarized in Table 1. Compound 1 was obtained as a white powder. A combination of HRESIMS and $^{13}$C-NMR data revealed a chemical formula of C$_{18}$H$_{21}$ClO$_8$. The $^1$H-NMR data indicated the presence of two aromatic protons, trans-olefinic protons, and four oxymethine protons (Table 2). The gross structure of 1 was elucidated from detailed analyses of two-dimensional (2D)-NMR data, including $^1$H–$^1$H correlation spectroscopy (COSY), heteronuclear multiple-quantum correlation (HMQC) and heteronuclear multiple–bond correlation (HMBC) spectra in CD$_3$OD (Figure 2). The $^1$H–$^1$H COSY and HMQC spectra revealed the presence of partial structure a (C-1’ to C-9’). The presence of an isocoumarin
moiety and the position of the chlorine atom were revealed by comparing the $^1$H and $^{13}$C NMR data of chaetochiversins A and B (Wijeratne et al., 2006), and HMBC correlations between H-7 ($\delta_H$ 6.47) and C-5 ($\delta_C$ 108.2), C-6 ($\delta_C$ 163.0), C-8 ($\delta_C$ 163.2) and C-8a ($\delta_C$ 100.4), and between H-4 ($\delta_H$ 6.81) and C-3 ($\delta_C$ 153.9), C-5 and C-8a (Fig. 2). The connectivity between partial structure a and the isocoumarin moiety was revealed by HMBC correlations between H-4 and C-1´ ($\delta_C$ 122.5), H-1´ ($\delta_H$ 6.44) and C-4 ($\delta_C$ 103.6), and between H-2´ ($\delta_H$ 6.61) and C-3. Thus, a planar structure was determined as shown in Fig. 2.

Determination of absolute stereochemistry of chaetochiversin C (1)

Compound 1 has four chiral carbon centers at C-3’, C-5’, C-6’ and C-8’. The absolute configuration of 1 was determined by Mosher’s method at C-3’ after revealing the relative configuration of C-3’, C-5’, C-6’ and C-8’. The relative configuration of a 1,3-diol type compound can be assigned based on the $^{13}$C-NMR chemical shifts of acetal methyl groups and the acetal carbon of its acetonide analog (Rychnovsky et al., 1993). Acetonide analogs of 1, bis-isopropylidene acetal (2) and mono-isopropylidene
acetal (3) were prepared as shown in Fig. 3 and the gross structure of 2 was assigned based on one-dimensional (1D)- and 2D-NMR data (Figs. S6–S9). In general, the $^{13}$C NMR spectrum in CDCl$_3$ of syn-1,3-diol acetonide contains chemical shifts corresponding to the acetal dimethyl moiety at approximately 19 and 30 ppm and a peak at approximately 98.5 ppm, corresponding to the acetal carbon. In contrast, the NMR spectrum of anti-1,3-diol contains a peak at approximately 25 ppm, corresponding to both acetal dimethyl groups, and a peak corresponding to the acetal carbon at approximately 100.5 ppm. Acetonide analog 2 contains two acetal carbons and acetal dimethyl groups. One of the acetal carbons and acetal dimethyl groups, connected at C-3' and C-5', resulted in chemical shifts at 99.0 ppm, and at 19.8 and 29.9 ppm, respectively, while the remaining acetal carbon and acetal dimethyl group, connected at C-6' and C-8', yielded chemical shifts at 100.2 ppm, and at 24.8 and 25.0 ppm. These data indicate that the C-3',5'-diol exhibits a syn-stereochemistry, while the C-6',8'-diol exhibits an anti-stereochemistry. However, the relative configuration of compound 3 between C-5' and C-6' was unclear.

Two MTPA esters at C-3' and C-8' were prepared to determine the absolute
configuration at C-3’ and C-8’. To protect its additional hydroxyl group, 1 was
derivatized with TMS diazomethane to yield a 6-methoxy analog (4) (Fig. 4A).

Reaction of 4 with (S)-MTPA-OH afforded the pure C-3’-(S)-MTPA ester (5a) and a
mixture of the C-8’-(S)-MTPA ester (6a), C-5’-(S)-MTPA ester, and C-6’-(S)-MTPA
ester. The same reaction using (R)-MTPA-OH, yielded pure C-3’-(R)-MTPA ester (5b)
and a mixture of the C-8’-(R)-MTPA ester (6b), C-5’-(S)-MTPA ester, and
C-6’-(S)-MTPA ester. The chemical shifts in the NMR spectra of 5a and 5b, obtained as
pure mono-MTPA esters, confirmed an absolute S stereochemistry at C-3’ (Fig. 4B). A
detailed analysis of \(^1\)H–\(^1\)H COSY spectra of 6a and 6b allowed identification of key
proton signals (Figs. S16 and S17). Chemical shifts in the NMR spectra of 6a and 6b
confirmed an absolute R stereochemistry at C-8’ (Fig. 4C). The absolute configuration
of 1 was thus established to be 3’S, 5’R, 6’S and 8’R.

Quenching effects of chaetochiversin C on singlet oxygen

Compound 1 showed a concentration-dependent quenching effect on \(^1\)O₂. The
detected concentrations of \(^1\)O₂ were suppressed by up to 55% in the presence of 2.5 mM
Recently, PC screening has led to the discovery of a variety of new compounds, such as iminimycins (Nakashima et al., 2016a, b), mangromicins (Nakashima et al., 2014a; Nakashima et al., 2015; Nakashima et al., 2014b), trehangelins (Nakashima et al., 2013), sarcopodinols (Matsuo et al., 2018) and pochioniolides (Miyano et al., 2018), from actiniomycete and fungal strains. These compounds were shown to exhibit potentially useful biological activities. Iminimycin A is the first compound containing iminium ion to be isolated from microorganisms. The producing strain is *Streptomyces griseus*, which has been preserved in our laboratory for 40 years (Nakashima et al., 2016a). This discovery demonstrates that PC screening can be used to identify new compounds in natural resources that have been previously investigated.

In this study, we performed PC screening on cultured broths of fungal strains and discovered a new compound, chaetochiversin C (1), from a cultured broth of *Neocosmospora* sp. FKI-7792. Chaetochiversins A and B were isolated from *Chaetomium chiversii*. These compounds are thought to be biosynthesized from a common nonaketide precursor. Compound 1 can be considered a 3’,4´-olefin hydration
and 5′,6′-ring-opening analog of chaetochiversins A or B. The absolute configuration of

1 given herein is reasonable because 1 is likely biosynthesized by same pathway that

provides chaetochiversins A and B, which have the absolute configuration 5′R, 6′R and

8′R (Wijeratne et al., 2006). There are no reports of any activity of chaetochiversins A

and B. Although, the 1O2 quenching activity of 1 is weak, this study is the first report of

the activity of a chaetochiversin-type isocoumarin. The other useful activities of 1 are

also being investigated. PC screening is a helpful method for identifying new

compounds in natural resources.

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References

Altschul, S. F. et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of


Figure captions

Figure 1. Absolute structure of 1.

Figure 2. Selected two-dimensional nuclear magnetic resonance (2D-NMR) correlations of 1.

Figure 3. Structures of the bis-acetal analog (2) and mono-acetal analog (3) of 1.

Figure 4. The $\Delta \delta$ values [(Delta in parts per million) = $\delta_S - \delta_R$] obtained for (S)- and (R)-alpha-methoxy-alpha-(trifluoromethyl) (MTPA) esters.
Figure 5. Quenching effect of 1 on singlet oxygen ($^1$O$_2$). The $^1$O$_2$ spin concentration values were measured by electron spin resonance (ESR). NaN$_3$ was used as a positive control at 2.5 mM. Error bars indicate the standard deviations of measurements (n = 3) and asterisks indicate significant differences from the control, *$p < 0.05$ and **$p < 0.01$ (Student’s $t$-test).
<table>
<thead>
<tr>
<th>Property</th>
<th>Chaetochiversin C (I)</th>
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<tr>
<td>Appearance</td>
<td>Colorless powder</td>
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<tr>
<td>Molecular formula</td>
<td>C_{18}H_{21}ClO_{8}</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>400</td>
</tr>
<tr>
<td>ESI-MS (m/z) Calcd.</td>
<td>399.0842 (for C_{18}H_{20}ClO_{8})</td>
</tr>
<tr>
<td>ESI-MS (m/z) Found</td>
<td>399.0847 [M-H]</td>
</tr>
<tr>
<td>[α]_{D}^{23}</td>
<td>+6.0 (c = 0.5, MeOH)</td>
</tr>
<tr>
<td>UV (MeOH) λ_{max} (log e)</td>
<td>367 (2.98), 351 (3.05), 318 (3.03), 306 (3.06), 262 (3.70), 204 (3.12)</td>
</tr>
<tr>
<td>IR (KBr) ν_{max} cm^{-1}</td>
<td>3412, 1739, 1369, 1226</td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>94.5–103.8 decomp.</td>
</tr>
<tr>
<td>Soluble</td>
<td>Acetone, MeOH, DMSO</td>
</tr>
<tr>
<td>Insoluble</td>
<td>CHCl_{3}, EtOAc, H_{2}O</td>
</tr>
</tbody>
</table>
Table 2. $^1$H and $^{13}$C-NMR chemical shifts of chaetochiversin C (1)

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_1$ (J in Hz)</th>
<th>$\delta_C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>166.4</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>153.9</td>
</tr>
<tr>
<td>4</td>
<td>6.81, s</td>
<td>103.6</td>
</tr>
<tr>
<td>4a</td>
<td></td>
<td>137.6</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>108.2</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>163.0$^a)$</td>
</tr>
<tr>
<td>7</td>
<td>6.47, s</td>
<td>103.5</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>163.2$^a)$</td>
</tr>
<tr>
<td>8a</td>
<td></td>
<td>100.4</td>
</tr>
<tr>
<td>1'</td>
<td>6.44, d (16.0)</td>
<td>122.5</td>
</tr>
<tr>
<td>2'</td>
<td>6.61, dd (16.0, 6.3)</td>
<td>139.6</td>
</tr>
<tr>
<td>3'</td>
<td>4.55, ddd (6.9, 6.3, 6.3)</td>
<td>71.0</td>
</tr>
<tr>
<td>4'</td>
<td>1.76, ddd (14.0, 9.7, 6.9)</td>
<td>40.6</td>
</tr>
<tr>
<td></td>
<td>1.88, ddd (14.0, 6.9, 2.9)</td>
<td></td>
</tr>
<tr>
<td>5'</td>
<td>3.59, m</td>
<td>74.2</td>
</tr>
<tr>
<td>6'</td>
<td>3.70, ddd (9.6, 5.6, 2.9)</td>
<td>72.8</td>
</tr>
<tr>
<td>7'</td>
<td>1.49, ddd (14.0, 9.6, 2.9)</td>
<td>42.5</td>
</tr>
<tr>
<td></td>
<td>1.64 ddd (14.0, 9.0, 2.3)</td>
<td></td>
</tr>
<tr>
<td>8'</td>
<td>4.00, m</td>
<td>65.5</td>
</tr>
<tr>
<td>----</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>9'</td>
<td>1.21, d (6.3)</td>
<td>24.5</td>
</tr>
</tbody>
</table>

a) assignment may be reversed
Fig. 1

![Chemical structure](image)

1
Fig. 3

$^{13}$C-NMR: $\Delta_2$ ppm

24.8 and 25.0

19.8 and 29.9

13

C-NMR: $\Delta_2$ ppm

99.0

24.8 and 25.0

3

$^{13}$C-NMR: $\Delta_2$ ppm

19.8 and 29.9
Fig. 4

A

\[
\begin{align*}
\text{MeO} & \quad \text{Cl} & \quad \text{OH} & \quad \text{O} \\
\text{Cl} & \quad \text{OH} & \quad \text{MeO} & \quad \text{O} \\
\text{OH} & \quad \text{OH} & \quad \text{OH} & \quad \text{OH} \\
\text{OH} & \quad \text{OH} & \quad \text{OR} & \quad \text{O}
\end{align*}
\]

5a \( R=\text{(S)-MTPA} \)
5b \( R=\text{(R)-MTPA} \)

B

\[
\begin{align*}
\text{MeO} & \quad \text{Cl} & \quad \text{OH} & \quad \text{O} \\
\text{Cl} & \quad \text{OH} & \quad \text{OR} & \quad \text{OH} \\
\text{OH} & \quad \text{OH} & \quad \text{OH} & \quad \text{OH} \\
\text{OH} & \quad \text{OH} & \quad \text{OR} & \quad \text{O}
\end{align*}
\]

6a \( R=\text{(S)-MTPA} \)
6b \( R=\text{(R)-MTPA} \)