Full Paper

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

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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 ¹³C-NMR chemical shifts of the acetone 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 (¹O₂). Compound **1** showed a quenching effect on ¹O₂.

Key Words: 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 (Arison and Ōmura, 1974; Burg et al., 1979; Kim et al., 1994; Tomoda et al., 1996). 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-2282^T (*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 (Nakano et al., 1987; Nakano and Ōmura, 2009; Ōmura et al., 2018; Tamaoki et al., 1986). 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 Niiijima 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**.

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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 500 spectrometer (JEOL, Tokyo, Japan). ^1H -NMR spectra were acquired at 500 MHz, and ^{13}C NMR spectra at 125 MHz, in CD_3OD or CDCl_3 . The chemical shifts are expressed in parts per million (ppm). The ^1H -NMR spectra were referenced to residual CHD_2OD (3.31 ppm) and CHCl_3 (7.26 ppm), and the ^{13}C NMR spectra were referenced to CD_3OD (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% CaCO_3 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 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% KH_2PO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) and brown rice powder medium (3.0% brown rice powder, 1.0% sucrose, 1.0% yeast extract, 0.5% glycerol, 0.5% NaNO_3 , 0.2% ammonium acetate, 0.001% FeSO_4 and 0.001% ZnSO_4).

Strain FKI-7792 was grown on a slant of modified Miura's medium [LcA: 0.1% glycerol, 0.08% KH_2PO_4 , 0.02% K_2HPO_4 , 0.02% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02% KCl, 0.2% NaNO_3 , 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% KH_2PO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 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 $\times$ 4) was carried out for 13 days at 25°C.

Fig. 1. Absolute structure of **1**.

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 $\times$ 3). The dried EtOAc extract (7.23 g) was purified via silica gel column chromatography ($\phi 50 \times 180$ mm) (*n*-hexane/EtOAc = 8:2, 6:4 and CHCl_3 : CH_3OH = 50:1, 25:1, 10:1, 9:1, 8:2, 6:4, 0:1, stepwise separation with 900 mL). The CHCl_3 : CH_3OH = 9:1 fraction (168.0 mg) including **1** was purified on an ODS column (10–100% gradient system with CH_3OH aq + 0.1% formic acid for 60 min, $\phi 25 \times 100$ mm, flow rate 10 mL/min, detection UV 254 nm). The collective fraction at retention time (rt) 25–35 min (25.8 mg) including **1** was purified by preparative reversed-phase high-performance liquid chromatography (HPLC) (Inertsil ODS-4, $\phi 14 \times 250$ mm; GL Sciences, Tokyo, Japan) in an isocratic system using an eluent of 60% CH_3OH aq (0.1% formic acid) to obtain **1** (rt 13.2 min, 5.2 mg).

Preparation of acetonide analogs bis-isopropylidene acetal (2**) and mono-isopropylidene acetal (**3**).** $\text{TsOH} \cdot \text{H}_2\text{O}$ (0.2 mg, 0.823 μmol) and 2-methoxypropene (7.7 μL , 82.3 μmol) were added to a solution of **1** (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_4Cl (3 mL). The mixture was then extracted with CHCl_3 (3 $\times$ 6 mL) and the combined extracts were dried over Na_2SO_4 , filtered, and concentrated. The residue was purified by preparative thin-layer chromatography (TLC) (20 $\times$ 20 cm; CHCl_3 / CH_3OH = 10/1). The collective fractions (R_f value 0.72 and 0.54) were eluted with a mixture of CHCl_3 and CH_3OH (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 (CDCl_3 , 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-4'), 1.78 (ddd, J = 15.2, 9.2, 6.3 Hz, ^1H , H-7'), 1.67 (ddd, J = 15.2, 9.7, 6.3 Hz, ^1H , H-7'), 1.49 (s, 3H, acetonide CH_3), 1.46 (s, 3H, acetonide CH_3), 1.36 (s, 3H, acetonide CH_3), 1.34 (s, 3H, acetonide CH_3), 1.25 (m, ^1H , H-4'), 1.21 (d, 3H, J = 6.3 Hz, 9'-Me), ^{13}C -NMR signals (CDCl_3 , 125 MHz) δ : 164.9 (C-1), 162.3

Table 1. Physico-chemical properties of chaetochiversin C (**1**).

Chaetochiversin C (1)	
Appearance	Colorless powder
Molecular formula	C ₁₈ H ₂₁ ClO ₈
Molecular weight	400
ESI-MS (<i>m/z</i>)	
Calcd.	399.0842 (for C ₁₈ H ₂₀ ClO ₈)
Found	399.0847 [M-H] [−]
[α] _D ²³	+6.0 (<i>c</i> = 0.5, MeOH)
UV (MeOH) λ _{max} (log <i>e</i>)	367 (2.98), 351 (3.05), 318 (3.03), 306 (3.06), 262 (3.70), 204 (3.12)
IR (KBr) ν _{max} cm ^{−1}	3412, 1739, 1369, 1226
Melting point (°C)	94.5–103.8 decomp.
Soluble	Acetone, MeOH, DMSO
Insoluble	CHCl ₃ , EtOAc, H ₂ O

Table 2. ¹H and ¹³C-NMR chemical shifts of chaetochiversin C (**1**).

Position	δ _H (<i>J</i> in Hz)	δ _C
1		166.4
2		
3		153.9
4	6.81, s	103.6
4a		137.6
5		108.2
6		163.0*
7	6.47, s	103.5
8		163.2*
8a		100.4
1'	6.44, d (16.0)	122.5
2'	6.61, dd (16.0, 6.3)	139.6
3'	4.55, ddd (6.9, 6.3, 6.3)	71.0
4'	1.76, ddd (14.0, 9.7, 6.9)	40.6
	1.88, ddd (14.0, 6.9, 2.9)	
5'	3.59, m	74.2
6'	3.70, ddd (9.6, 5.6, 2.9)	72.8
7'	1.49, ddd (14.0, 9.6, 2.9)	42.5
	1.64 ddd (14.0, 9.0, 2.3)	
8'	4.00, m	65.5
9'	1.21, d (6.3)	24.5

*Assignment may be reversed.

(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 (acetone CH₃ with C-3' or C-5'), 25.0 (acetone CH₃ with C-6' or C-8'), 24.8 (acetone CH₃ with C-6' or C-8'), 21.6 (C-9'), 19.8 (acetone 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 CH₃OH (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 (CHCl₃:CH₃OH = 10:1). The collective fraction (R_f value 0.32) was eluted with mixture of CHCl₃ and CH₃OH (1:1) to yield the 6-methoxy analog (**4**) 4.9 mg, 95% as a colorless solid.

6-methoxy analog (**4**): ¹H-NMR signals (CD₃OD, 500

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

MHz) δ: 6.83 (s, ¹H, H-4), 6.71 (s, ¹H, H-7), 6.62 (dd, *J* = 15.5, 6.1 Hz, ¹H, H-2'), 6.44 (d, *J* = 15.5 Hz, ¹H, H-1'), 4.54 (ddd, *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, *J* = 13.4, 6.7, 2.9 Hz, ¹H, H-4'), 1.76 (ddd, *J* = 13.8, 9.2, 6.9 Hz, ¹H, H-4'), 1.64 (ddd, *J* = 14.3, 9.7, 2.3 Hz, ¹H, H-7'), 1.49 (ddd, *J* = 14.3, 9.7, 2.9 Hz, ¹H, H-7'), 1.21 (d, 3H, *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 NaHCO₃ and extracted with EtOAc. The organic layer was dried (Na₂SO₄) and concentrated under reduced pressure. The MTPA esters were isolated by preparative TLC (CHCl₃:CH₃OH = 5:1) to afford a mixture of mono-MTPA esters. Further purification was carried out by preparative TLC (CHCl₃:CH₃OH = 10:1). The two collective fractions (R_f values 0.80 and 0.84) were eluted with a mixture of CHCl₃ and CH₃OH (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

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

Fig. 4. The $\Delta\delta$ values [$(\Delta\delta \text{ in parts per million}) = \delta_S - \delta_R$] obtained for (S)- and (R)- α -methoxy- α -(trifluoromethyl) (MTPA) esters.

was separated by preparative TLC ($\text{CHCl}_3:\text{CH}_3\text{OH} = 10:1$). The two collective fractions (Rf value 0.45 and 0.52) were eluted with a mixture of CHCl_3 and CH_3OH (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 (CDCl_3 , 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 (CDCl_3 , 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, 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 ^1H -NMR signals (CDCl_3 , 500 MHz) δ : 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 ^1H -NMR signals (CDCl_3 , 500 MHz) δ : 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 ($^1\text{O}_2$). Singlet oxygen was generated by laser irradiation as described previously (Ishiyama et al., 2012; Nakamura et al., 2011). 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/cm². 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

Fig. 5. Quenching effect of **1** on singlet oxygen ($^1\text{O}_2$).

The $^1\text{O}_2$ 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).

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-oxy-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 $\pm$ 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 $\text{C}_{18}\text{H}_{21}\text{ClO}_8$. The ^1H -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 ^1H - ^1H correlation spectroscopy (COSY), heteronuclear multiple-quantum correlation (HMQC) and heteronuclear multiple-bond correlation (HMBC) spectra in CD_3OD (Fig. 2). The ^1H - ^1H 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 ^1H and ^{13}C NMR data of chaetochiversins A and B (Wijeratne et al., 2006), and HMBC correlations between H-7 (δ_{H} 6.47) and C-5 (δ_{C} 108.2), C-6 (δ_{C} 163.0), C-8 (δ_{C} 163.2) and C-8a (δ_{C}

100.4), and between H-4 (δ_{H} 6.81) and C-3 (δ_{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' (δ_{C} 122.5), H-1' (δ_{H} 6.44) and C-4 (δ_{C} 103.6), and between H-2' (δ_{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 indi-

cate 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 ¹H-¹H 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 ¹O₂. The detected concentrations of ¹O₂ were suppressed by up to 55% in the presence of 2.5 mM of compound **1**, assuming 100% generation in the control (Fig. 5).

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, 2014b, 2015), trehangelines (Nakashima et al., 2013), sarcopodinols (Matsuo et al., 2018) and pochoniolides (Miyano et al., 2018), from actinomycete 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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Supplementary Materials

Supplementary figures are available in our J-STAGE site (<http://www.jstage.jst.go.jp/browse/jgam>).

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