A New Griseofulvin Derivative from a Soft Coral-derived Fungus *Eupenicillium* sp. SCSIO41208

Yunqiu Li\(^a\), Yanhong Tan\(^a,\,b\), Juan Liu\(^b\), Xuefeng Zhou\(^b\), Siquan Zeng\(^b\), Junde Dong\(^b\), Yonghong Liu\(^b\), and Bin Yang\(^b\)*

\(^a\)School of Pharmacy, Guilin Medical University, Guilin 541004, P. R. China;

\(^b\)Key Laboratory of Marine Bio-resources Sustainable Utilization / Guangdong Key Laboratory of Marine Materia Medica / Research Center for Marine Microbes, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, P. R. China;

Yunqiu Li and Yanhong Tan contributed equally to this work

Abstract

A new griseofulvin derivative, eupenigriseofulvin (1), together with six known compounds, griseofulvin (2), dechlorogriseofulvin (3), dechloroisogriseofulvin (4), trichopyrone (5), 2-(4-hydroxyphenyl)-ethanol (6), and 1-phenylethane-1,2-diol (7), were isolated from the EtOAc extract of *Eupenicillium* sp. SCSIO41208. The structures of these compounds were elucidated by spectroscopic methods including NMR and mass spectrometry. The absolute configuration of 1 was determined on the basis of electronic circular dichroism (ECD) data analysis.

**Keywords:** eupenigriseofulvin; *Eupenicillium* sp.; griseofulvin;
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Experimental Section

1. Experimental

1.1. General Experimental Procedures

$^1$H-, $^{13}$C-NMR, DEPT and 2D-NMR spectra were recorded on a Bruker AC 500 NMR spectrometer with TMS as an internal standard. HR-ESI-MS data were measured on a Bruker microTOF-QII mass spectrometer. CD spectra were measured with a Chirascan circular dichroism spectrometer (Applied Photophysics). Optical rotation values were measured with a PerkineElmer 341 polarimeter. Column chromatography were performed on silica gel (200–300 mesh; Qingdao Marine Chemical Factory, China), YMC gel (ODS-A, 12 nm, S-50 µm) and Sephadex LH-20 (Amersham Biosciences, Sweden), respectively. The silica gel GF$_{254}$ used for TLC were supplied by the Qingdao Marine Chemical Factory, Qingdao, China. All solvents used were of analytical grade (Tianjin Fuyu Chemical and Industry Factory). HPLC was carried on Hitachi L-2400 with YMC ODS column. Spots were detected on TLC under UV light or by heating after spraying with 5% H$_2$SO$_4$ in EtOH ($v/v$).

1.2. Fungal Material

The culture of *Eupenicillium* sp. SCSIO41208 was isolated from a Soft Coral *Sinularia* sp., collected in Sansha, Hainan province of China, in May, 2015. The strain was identified as *Eupenicillium* sp. based on a molecular biological protocol calling for DNA amplification and ITS region sequence comparison with the GenBank database and shared a similarity of 100% with *Eupenicillium* sp. HKA22 (accession NO. DQ092508. The strain was deposited in the RNAM Center, South China Sea Institute of Oceanology, Chinese Academy of Sciences.

1.3. Fermentation and Extraction
Strain stored on PDA slants at 4 °C was cultured on MB agar (malt extract 15 g, sea salt 10 g, agar 15 g, distilled water 1 l, pH 7.4–7.8) plates. Seed medium (malt extract 15 g, sea salt 10 g, distilled water 1 l, pH 7.4–7.8) in 50-mL Erlenmeyer flasks was inoculated with strain SCSIO41208 and incubated at 25 °C for 48 h on a rotating shaker (180 rpm). Rice solid medium with 3% NaCl (200 g of rice, 200 g water, 6 g sea salt, 20_1 l flasks) was inoculated with liquid seed and incubated at 25 °C under daylight for 60 days.

The total rice solid culture was crushed and extracted with acetone three times. The acetone extract was evaporated under reduced pressure to afford an aqueous solution, and then the aqueous solution was extracted with EtOAc to yield 89 g of a crude gum.

1.4. Isolation and Purification

The EtOAc portion was subsequently separated by Si gel column chromatography using CHCl₃–MeOH gradient elution to give twenty-one fractions (Frs. 1-21). Fr.13 was subjected to ODS gel column chromatography, using a gradient of MeOH (10%→100%) in H₂O, to give 15 fractions (Frs. 13-1~13-15). Frs. 13-1 was purified by semipreparative RP HPLC (20% MeOH in H₂O) at a flow rate of 3 mL/min to afford 6 (17.5 mg, tᵣ = 15.8 min). Frs. 13-2 was purified by semipreparative RP HPLC (15% MeOH in H₂O) at a flow rate of 3 mL/min to afford 7 (5.4 mg, tᵣ = 24.9 min). Frs.14 was subjected to ODS gel column chromatography, using a gradient of MeOH (10%→100%) in H₂O, to give 17 fractions (Frs. 14-1~14-17). Frs. 14-6 was purified by semipreparative RP HPLC (50% MeOH in H₂O) at a flow rate of 3 mL/min to afford 1 (2.1 mg, tᵣ = 29.8 min), 2 (1059 mg, tᵣ = 41.7 min), 3 (152.1 mg, tᵣ = 24.6 min) and 4 (3.4 mg, tᵣ = 34.1 min). Frs.15 was subjected to ODS gel column chromatography, using a gradient of MeOH (10%→100%) in H₂O, to give 22 fractions (Frs.
Frs. 15-6 was purified by semipreparative RP HPLC (40% MeOH in H2O) at a flow rate of 3 mL/min to afford 5 (4.1 mg, tR = 41.7 min).

*Eupenigriseofulvin (1)*: Pale yellow amorphous solid; CD cm²mol⁻¹: Δε 316 +1.08, Δε 277 -0.12, Δε 234 -1.35, Δε 214 +1.40 (MeOH; c 0.86); ¹H and ¹³C NMR data: see Table 1; HRESIMS: m/z: 291.122617 [M + H]⁺ (calcd for C₁₆H₁₉O₅ 291.122700), m/z: 313.105227 [M + Na]⁺ (calcd for C₁₆H₁₈NaO₅ 313.104644), m/z: 603.220825 [2M + Na]⁺ (calcd for C₃₂H₃₆NaO₁₀ 603.220068).

**1.5 Biological Activity**

Antifungal Assay: A paper disk diffusion method was used with the organisms used consisting of *Colletotrichum asianum*, *Colletotrichum gloeosporioides*, *Colletotrichum acutatum*, *Fusarium oxysporum*, and *Pyriculariaoryae Cav*. Antifungal assay were prepared according to standard techniques. Paper discs (d = 5 mm) loaded with 50 μg samples were placed on the inoculated plate. Cycloheximide (2.5 μg/disc) were used as positive controls. The paper disc loaded with 2.5 μL of DMSO was used as the negative control. The inhibition zones (mm) were compared with controls after culturing at 30 °C for 2−5 days. Three replicates were maintained for each treatment.

**1.6. Computational Methods**

Molecular Merck force field (MMFF) and DFT/TDDFT calculations were performed with Spartan’14 software package (Wavefunction Inc., Irvine, CA, USA) and Gaussian09 program package (Frisch et al. 2010), respectively. MMFF conformational search generated low-energy conformers within a 10 kcal/mol energy window were subjected to geometry optimization and frequency calculations using the B3LYP/def2-SVP method and tight
convergence criteria. Energies of the low-energy conformers in MeOH were calculated at the B3LYP/def2-TZVP level. Solvent effects were taken into account by using polarizable continuum model (PCM). The TDDFT calculations were performed using the CAM-B3LYP (Yanai et al. 2004), M06 (Zhao and Truhlar 2008), and M06-2X (Zhao and Truhlar 2008), functionals, and Ahlrichs’ basis set TZVP (triple zeta valence plus polarization) (Schafer et al. 1994). The number of excited states per each molecule was 36. The ECD spectra were generated by the program SpecDis (Bruhn et al. 2013) using a Gaussian band shape from dipole-length dipolar and rotational strengths. Equilibrium population of each conformer at 298.15K was calculated from its relative free energies using Boltzmann statistics. The calculated spectra were generated from the low-energy conformers according to the Boltzmann weighting of each conformer in MeOH solution.

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| Position | $\delta_H$ (J in Hz) | $\delta_C$ |
|----------|----------------------|------------|
| 2        |                      | 94.5       |
| 3        |                      | 196.2      |
| 3a       |                      | 106.8      |
| 4        |                      | 158.9      |
| 5        | 6.12, d, 1.0         | 91.9       |
| 6        |                      | 170.3      |
| 7        | 6.29, d, 1.5         | 88.2       |
| 7a       |                      | 175.7      |
| 8        | 3.90, s              | 55.2       |
| 9        | 3.87, s              | 55.2       |
| 2'       | 4.62, brs            | 71.5       |
| 3'       | 5.55, m              | 126.8      |
| 4'       | 5.82, m              | 128.0      |
| 5'       | 2.47, m              | 30.8       |
|          | 2.28, m              |            |
| 6'       | 2.35, m              | 34.8       |
| 7'       | 0.84, d 4.5          | 13.3       |