Tetralone Derivatives From a Deep-Sea-Derived Fungus *Cladosporium* sp. HDN17-58

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Abstract

One new tetralone derivative, named aladothalen (1), and one known biogenetically related compound, (3S,4S,8)-3,4,8-trihydroxy-3,4-dihydronaphthalene-1(2H)-one (2), were isolated from a deep-sea-derived-fungal *Cladosporium* sp. HDN17-58. Their structures, including absolute configurations, were elucidated by extensive NMR, MS, and ECD analyses. Compound 1 exhibited potent bacteriostatic activity against *Bacillus cereus*, *Mycobacterium phlei* and methicillin-resistant coagulase-negative *Staphylococcus*, with a MIC value of 25 µM.

Keywords

tetralone derivative, marine-derived fungus, *Cladosporium* sp., bioactivity, deep sea

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Antimicrobial agents are the main basis for the treatment of microbial infections. However, the overuse of such agents has become a major problem due to the emergence and spread of several multidrug resistant microorganisms. For example, Methicillin-resistant *Staphylococcus aureus* (MRSA) has become one of the most common strains of drug resistant microorganisms in the world. In the United States alone, more than 11 000 people die from *Staphylococcus aureus* every year. As drug resistant bacteria continue to upgrade, modifications of existing scaffolds have become more and more challenging during the warfare between human beings and bacteria, and it is urgent to discover new antibiotic scaffolds. Within our ongoing work of searching for antibacterial compounds from marine derived fungi, we have isolated a series of compounds with antibacterial activity, such as amphiepicoccins E−F, prenylterphenyllin H, and penicilocycles A−E. A fungal strain, *Cladosporium* sp. HDN17-58, has recently been isolated from a sediment sample collected from the Western Pacific Ocean at a depth of 5,874 m. This was selected for study because of the UV absorption observed in its HPLC–UV profile (Supplemental Figure S12). Further chemical studies of this strain led to the isolation of one new antibacterial compound, namely aladothalen (1), together with a biogenetically related known compound, (3S,4S,8)-3,4,8-trihydroxy-3,4-dihydronaphthalene-1(2H)-one (2). Herein, we report the isolation, structure elucidation, and bioactivity of the new compound (Figure 1).

Results and Discussion

*Cladosporium* sp. HDN17-58 was incubated for 30 days in a rice medium in Erlenmeyer flasks under static conditions. The whole (about 60 L) culture was then extracted 3 times with MeOH, and the organic extract (112.0 g) was fractionated by repeated column chromatography using silica gel and high-performance liquid chromatography with octadecyl silane columns to afford compounds 1 (12.6 mg) and 2 (1.4 mg).

Aladothalen (1) was isolated as a brown powder. Its molecular formula was established as 

\[ \text{C}_{16}\text{H}_{16}\text{O}_{4} \]

on the basis of the deprotonated molecular HRESIMS peak at \( m/z \) 193.0502 [M−H]⁻, requiring 6 degrees of unsaturation. The UV absorption maxima (EtOH) at 213, 260, and 333 nm indicated the existence of an aromatic system. The IR bands (KBr) at 3600, 2920, 1540 and 1580 cm⁻¹ indicated the presence in the molecule of hydroxyl groups and a carbonyl group conjugated with an aromatic ring. The 

\[ \frac{\text{H}}{\text{C}} \]

NMR data (Table 1) of compound 1 were highly similar to those of scytalone, indicating that they shared the same skeleton. The only difference was the position of a hydroxyl group, which were located...
The antibacterial activity was tested of compound 1 against Bacillus cereus, Proteus sp., Mycobacterium phlei, Edwardsiella tarda, Bacillus subtilis, methicillin resistant coagulase-negative Staphylococci (MRCONS), MRSA, and Vibrio parahaemolyticus (Table 2). Compound 1 showed antimicrobial potencies against Mycobacterium phlei and MRCONS with a MIC value of 25 µM. The effect of 1 against MRCONS is comparable to that of the positive control ciprofloxacin.

Experimental

General Experimental Procedures

Optical rotations were obtained on a JASCOP-1020 digital polarimeter (JASCO Corporation, Tokyo, Japan). UV spectra were recorded on an Alltech 1500 (Beckman Coulter Inc., Brea, CA, USA), IR spectra on a Bruker tensor-27 spectrophotometer using KBr discs (Bruker Corporation, Billerica, MA, USA), 1H NMR, 13C NMR, DEPT, and 2D NMR spectra on an Agilent 500 MHz DD2 spectrometer (Agilent Technologies Inc, Santa Clara, CA, USA), HRESIMS and ESIMS using a Thermo Scientific LTQ Orbitrap XL mass spectrometer, and CD spectra on a JASCO J-715 spectropolarimeter (JASCO Corporation, Tokyo, Japan). Column chromatography (CC) was performed with Sephadex LH-20 (Amersham Biosciences). For preparative HPLC, a C18 column was used (Waters, YMC-Pack ODS-A, 250 × 10 mm, 5 µm, 12 nm, 3 mL min⁻¹).

Fungal Material

The fungal strain was isolated from a deep-sea sediment sample collected from the western Pacific Ocean (depth 5874 m, E 127°51′19.31″, N 10°42′37.87″, collected in December, 2016) and identified as Cladosporium sp. based on sequencing of the ITS region (Supplemental Figure S11) (GenBank no. MW250204). The strain was deposited at the Key Laboratory of Marine Drugs, the Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, People’s Republic of China.

Fermentation

Cladosporium sp. HDN17-58 was cultured on a PDA plate at 28 °C for 5 days. The fresh mycelia and spores were inoculated into 1000 ml Erlenmeyer flasks (×200) each containing 80 g of rice and 120 mL of water to perform large-scale fermentation. The cultures were incubated statically at 28 °C for 30 days.
**Extraction and Purification**

The fermented broth was extracted first with MeOH, 3 times, and then with EtOAc, 3 times. The organic solvent was evaporated under reduced pressure to afford an organic extract (114.0 g).

The extract (114.0 g) was applied to a VLC column and fractionated using stepped gradient elution with MeOH–CH₂Cl₂, to yield 7 sub-fractions (Fr.1−Fr.7). Fr.2 was subjected to CC on ODS using H₂O MeOH (20%→100%) to obtain 7 fractions (Fr.2-1-Fr.2-7). Fr.2-2 was further fractionated on a LH-20 column to furnish 3 sub-fractions (Fr.2-2-1–Fr.2-2-3), and Fr.2-2-3 was further purified by semipreparative HPLC (25:75 MeOH–H₂O, 3 mL/min) to afford compound 1 (12.6 mg). Fr.2-1 was separated on a LH-20 column to furnish 4 sub-fractions (Fr.2-1-1–Fr.2-1-4), and Fr.2-1-4 was further purified by semipreparative HPLC (23:77 MeOH–H₂O, 3 mL/min) to afford compound 2 (1.4 mg).

**Spectroscopic Data**

Aladothalen (1): pale brown powder; [α] −36.5 (c 1.0, CH₃OH); ECD (MeOH) λ [nm] (Δε) 218 (0.5), 237 (-5.3), 273 (6.4), 296 (-3.0); IR (KBr) ν max 3196, 2933, 2362, 1630, 1580, 1540, 1257, 1164, 1110, 1036, 1006, 911 cm⁻¹; UV (MeOH) λ max (log ε) =213 (3.26), 260 (1.85), 333 (0.76) nm; ¹H and ¹³C NMR data, see Table 1; HRESIMS m/z 193.0502 [M−H]⁻ (calcd for C₁₀H₁₀O₄, 193.0506).

**Antimicrobial Activity Assay**

The antimicrobial activities of compound 1 against *Bacillus cereus*, *Proteus sp.*, *Mycobacterium phlei*, *Bacillus subtilis*, *Vibrio parahemolyticus*, *Edwardsiella tarda*, MRCNS, and MRSA were evaluated as previously reported by using the agar dilution method. All experiments were performed in triplicate and the values representing the mean of the three replicate experiments. The results are expressed as the minimum inhibitory concentrations (MICs) in μg/mL.

| Comp  | B. cereus | P. sp. | M. phlei | E. tarda | B. subtilis | MRCNS | MRSA | V. parahemolyticus |
|-------|-----------|--------|----------|----------|-------------|-------|------|-------------------|
| 1     | 50        | >50    | 25       | >50      | >50         | 25    | >50  | >50               |
| CPFX  | <0.4      | 0.8    | <0.4     | <0.4     | <0.4        | 25    | >50  | <0.39             |

*Figure 3.* Experimental electronic circular dichroism (ECD) spectrum of 1 (black curve), and calculated ECD spectrum of truncated model 1a (red curve).
performed in triplicate, and ciprofloxacin (CPFX) was used as a positive control. All strains were donated by the Qingdao municipal hospital.

**Computation Section**

Conformational searches were run by employing the “systematic” procedure implemented in Spartan’14 using the MMFF. All MMFF minima were reoptimized with DFT calculations at the B3LYP/6-31 + G(d) level using the Gaussian 09 program. The geometry was optimized starting from various initial conformations, with vibrational frequency calculations confirming the presence of minima. Time-dependent DFT calculations were performed on the 2 lowest-energy conformations for (2R)−1a (>5% population) using 30 excited states and using a polarizable continuum model for MeOH. Electronic circular dichroism spectra were generated using the program SpecDis by applying a Gaussian band shape with 0.16 eV width for 1a from dipole length rotational strengths. The dipole velocity forms yielded negligible differences. The spectra of 1a were combined using Boltzmann weighting, with rotational frequency calculations confirming the presence of minima. The calculated spectra were shifted by 2 nm for 1a to facilitate comparison with the experimental data.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**Supplemental Material**

Supplemental material for this article is available online.

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