SUPPLEMENTARY MATERIAL

Pholiotone A, a new polyketide derivative from Pholiota sp.

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Abstract: Pholiotone A (1), a new polyketide derivative, with tetrahydrobenzofuran-4(2H)-one skeleton, together with four known compounds, trichodermatides A (2) and B (3) and koninginins B (4) and E (5), were isolated from the crude extract of Pholiota sp. The structures of all the isolated compounds were determined mainly by NMR experiments, the modified Mosher method and electronic circular dichroism (ECD) calculations. The antifungal and cytotoxicity of all isolates were evaluated.

Keywords: structural elucidation; polyketide derivatives; Pholiota sp.
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1. Experimental Section

1.1 General Experimental Procedures

Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. CD spectra were recorded on a JASCO J-815 spectropolarimeter using MeOH as solvent. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. $^1$H and $^{13}$C NMR data were acquired with Varian Mercury -500 and -600 spectrometer using solvent signals (CDCl$_3$; $\delta_H$ 7.26/$\delta_C$ 77.7) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000$^{\text{plus}}$ spectrometer, and HRESIMS data were obtained using Bruker APEX III 7.0 T and APEX II FT-ICR spectrometers, respectively.

1.2 Fungal Material

The culture of Pholiota sp. was isolated from a soil sample on the surface of the fruiting body of C. sinensis (Berk.) Sacc. collected in Kangding, Sichuan, People’s Republic of China, in May, 2005. The isolate was identified by one of the authors (X.L.) based on morphology and sequence (Genbank Accession No. JQ411813) analysis of the ITS region of the rDNA and assigned the accession number SCK05-7-ZP19 in X.L.’s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. Agar plugs were cut into small pieces (about $0.5 \times 0.5 \times 0.5$ cm$^3$) under aseptic conditions, 15 pieces were used to inoculate three Erlenmeyer flasks (250 mL), each containing 50 mL of media (0.4% glucose,
1% malt extract, and 0.4% yeast extract); the final pH of the media was adjusted to 6.5 and sterilized by autoclave. Three flasks of the inoculated media were incubated at 25 °C on a rotary shaker at 170 rpm for five days to prepare the seed culture.

Fermentation was carried out in eight Fernbach flasks (500 mL), each containing 80 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H₂O to give a final spore/cell suspension of 1 × 10⁶/mL. Distilled H₂O (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

1.3 Extraction and Isolation

The fermented material was extracted repeatedly with EtOAc (4 × 1.0 L), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (3.1 g), which was fractionated by silica gel VLC using petroleum ether–EtOAc gradient elution. The fraction (128 mg) eluted with 65% EtOAc was also separated by RP HPLC (50% MeOH in H₂O for 5 min, followed by 80–90% for 35 min) to afford 1 (3.1 mg, tR 17.28 min). The fraction (86 mg) eluted with 55% EtOAc was also separated by RP HPLC (50% MeOH in H₂O for 5 min, followed by 80–90% for 30 min) to afford 2 (12.1 mg, tR 21.40 min), 3 (6.4 mg, tR 25.60 min), 4 (3.8 mg, tR 26.20 min), and 5 (2.8 mg, tR 20.20 min).

1.4 Spectroscopic Data (uv and IR, ms)

**Pholiotide A (1):** white powder; [a] D \( ^{25} \) +21.5 (c 0.4, MeOH); UV (MeOH) λmax (log ε) 273 (3.29) nm; CD (c 1.0 × 10⁻³ M, MeOH) λmax (Δε) 299 (–0.75), 268 (+1.82); IR
(neat) ν\text{max} 3284, 2925, 2850, 1588, 1369, 1232, 1063 cm\(^{-1}\); \(^1\)H, \(^{13}\)C NMR, and HMBC data see Table 1; HRESIMS \(m/z\) 283.1907 (calcd for \(\text{C}_{16}\text{H}_{27}\text{O}_4\), 283.1904).

**Preparation of (R)- (1a) and (S)-MTPA Esters (1b).** A sample of 1 (1.0 mg, 0.004 mmol), (S)-MTPA Cl (2.0 µL, 0.011 mmol), and pyridine-\(d_5\) (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 24 h. The mixture was evaporated to dryness and purified by RP HPLC (Agilent Zorbax SB-C\(_{18}\) column; 5 µm; 9.4 × 250 mm; 20% MeCN in \(\text{H}_2\text{O}\) for 5 min, followed by 20–100% for 38 min; 2 mL/min) to afford di-ester 1a (1.0 mg, \(t_R\) 36.0 min): colorless oil; \(^1\)H NMR (CD\(_3\)OD, 500 MHz) \(\delta\) 5.98 (1H, m, H-4), 5.26 (1H, m, H-8), 4.96 (1H, m, H-10), 2.98 (1H, m, H-7a), 2.53 (1H, m, H-7b), 2.41 (1H, m, H-3a), 2.41 (1H, m, H-3b), 2.41 (1H, m, H-2a), 2.23 (1H, m, H-9a), 2.09 (1H, m, H-2b), 2.05 (1H, m, H-9b), 1.65 (2H, m, H-11), 1.23 (2H, m, H-12), 1.16 (2H, m, H-13), 1.16 (2H, m, H-14), 1.16 (2H, m, H-15), 0.86 (3H, t, \(J = 7.1\), H-16).

Another sample of 1 (1.0 mg, 0.004 mmol), (R)-MTPA Cl (2.0 µL, 0.011 mmol), and pyridine-\(d_5\) (0.5 mL) were processed as described above for 1a to afford di-ester 1b (1.2 mg): colorless oil; \(^1\)H NMR (CD\(_3\)OD, 500 MHz) \(\delta\) 6.01 (1H, m, H-4), 5.21 (1H, m, H-8), 4.58 (1H, m, H-10), 2.78 (1H, m, H-7a), 2.46 (1H, m, H-7b), 2.46 (1H, m, H-3a), 2.46 (1H, m, H-3b), 2.46 (1H, m, H-2a), 2.22 (1H, m, H-9a), 2.12 (1H, m, H-2b), 1.96 (1H, m, H-9b), 1.73 (2H, m, H-11), 1.32 (2H, m, H-12), 1.30 (2H, m, H-13), 1.30 (2H, m, H-14), 1.30 (2H, m, H-15), 0.89 (3H, t, \(J = 7.1\), H-16).

1.5 **Computational Details.**
Systematic conformational analyses for 1’\textit{a} and 1’\textit{b} were performed via the Molecular Operating Environment (MOE) ver. 2009.10. (Chemical Computing Group, Canada) software package using the MMFF94 molecular mechanics force field calculation. The MMFF94 conformational analyses were further optimized using DFT at the B3LYP/6-31+G(d,p) basis set level. The stationary points have been checked as the true minima of the potential energy surface by verifying they do not exhibit vibrational imaginary frequencies. The 50 lowest electronic transitions were calculated at the B3LYP/6-31G(d) level, and the rotational strengths of each electronic excitation were given using both dipole length and dipole velocity representations. ECD spectra were stimulated using a Gaussian function with a half-bandwidth of 0.4 eV. Equilibrium populations of conformers at 298.15 K were calculated from their relative free energies (\(\Delta G\)) using Boltzmann statistics. The overall ECD spectra were then generated according to Boltzmann weighting of each conformer. The systematic errors in the prediction of the wavelength and excited-state energies are compensated for by employing UV correction (Berova et al. 2007).

1.6 Antifungal Assays.

Antifungal assays were conducted in triplicate following the National Center for Clinical Laboratory Standards (NCCLS) recommendations. (Bérdy 2005) The fungi, \textit{Aspergillus flavus} (CGMCC 3.0951), \textit{Piricularia oryzae} (CGMCC 3.3283), and \textit{Fusarium nivale} (CGMCC 3.4600) were obtained from China General Microbial Culture Collection (CGMCC) and were grown on PDA. Targeted fungi (3–4 colonies) were prepared from broth culture (\textit{A. flavus}: 28 °C for 36 h; the plant pathogens: 28
°C for 48 h) and the final suspensions contained 10⁴ hyphae/mL (in PDB medium).

Test samples (4 mg/mL as stock solution in DMSO and serial dilutions) were transferred to 96-well clear plate in triplicate, and the suspensions of the test organisms were added to each well, achieving a final volume of 200 μL. Alamar blue (10 μL of 10% solution) was added to each well as an indicator and amphotericin B and carbendazim were used as the positive controls. After incubation (A. flavus: 28 °C for 36 h; the plant pathogens: 28 °C for 48 h), the fluorescence intensity was measured at Ex/Em = 544/590 nm. The inhibition was calculated and plotted versus test concentrations to afford the IC₅₀.

1.7 MTS Assay.

The assay was run in triplicate. In a 96-well plate, each well was plated with 2–5 × 10³ cells (it depends on the cell multiplication rate). After cell attachment overnight, the medium was removed, and each well was treated with 100 μL of medium containing 0.1% DMSO, or appropriate concentrations of the test compounds and the positive control paclitaxel (Sigma) (100 mM as stock solution of a compound in DMSO and serial dilutions; the test compounds showed good solubility in DMSO and did not precipitate when added to the cells). The plate was incubated for 72 h at 37 °C in a humidified, 5% CO₂ atmosphere. Proliferation assessed by adding 20 μL of MTS (Promega) to each well in dark followed by a 90 min incubation at 37 °C. The assay plate was read at 490 nm using a microplate reader. (Jeong et al. 2009, Zhang et al. 2011)
Table S1. NMR data for compound 1

|   | $\delta_C^a$, mult. | $\delta_H^b$ (J in Hz) | HMBC$^b$ |
|---|-------------------|------------------------|----------|
| 1 | 194.8, qC         |                        |          |
| 2a| 34.7, CH$_2$      | 2.56, dt (13.9, 4.9)   | 1, 3, 4  |
| 2b|                   | 2.36, m                | 1, 3, 4  |
| 3a| 30.9, CH$_2$      | 2.34, m                | 1, 2, 4, 5|
| 3b|                   | 2.03, m                | 5        |
| 4a| 63.8, CH          | 4.59, m                | 5        |
| 4b|                   |                        |          |
| 5 | 174.8, qC         |                        |          |
| 6 | 113.2, qC         |                        |          |
| 7a| 32.3, CH$_2$      | 3.03, m                | 5, 6, 8, 9|
| 7b|                   | 2.53, m                | 5, 6, 8, 9|
| 8 | 85.8, CH          | 5.09, m                | 10       |
| 9a| 43.4, CH$_2$      | 2.01, m                | 7, 8, 10, 11|
| 9b|                   | 1.78, m                | 8, 10    |
| 10| 69.8, CH          | 3.82, m                |          |
| 11| 37.7, CH$_2$      | 1.52, m                | 9, 10, 12, 13|
| 12| 25.4, CH$_2$      | 1.42, m                | 11, 13   |
|   |                   | 1.32, m                | 14       |
| 13| 29.2, CH$_2$      | 1.28, m                |          |
| 14| 31.8, CH$_2$      | 1.28, m                | 15       |
| 15| 22.6, CH$_2$      | 1.28, m                | 14, 16   |
| 16| 14.0, CH$_3$      | 0.89, t (5.7)          | 14, 15   |

$^a$ Recorded at 150 MHz in acetone-$d_6$. $^b$ Recorded at 600 MHz in CDCl$_3$. 
Figure S1. Key $^1$H-$^1$H COSY, HMBC and NOESY correlations for 1.

Figure S2. $\Delta\delta$ Values Obtained for ($S$)- and ($R$)-MTPA Di-esters 1b and 1a.

Figure S3. CD spectrum of 1 in MeOH.
Figure S4. Experimental ECD spectra of 1 in MeOH and the calculated ECD spectra of 1’a–1’d.
Figure S5. $^1$H NMR Spectrum of Pholiotone A (1; 600 MHz, CDCl$_3$)
Figure S6. $^{13}$C NMR Spectrum of Pholiotone A (1; 150 MHz, CDCl₃)
**Figure S7.** $^1$H-$^1$H COSY Spectrum of Pholiotone A (1; 600 MHz, CDCl$_3$)
Figure S8. HMBC Spectrum of Pholiotone A (1; 600 MHz, CDCl₃)
Figure S9. NOESY Spectrum of Pholiotone A (1; 600 MHz, CDCl₃)
Figure S10. HSQC Spectrum of Pholiotone A (1; 600 MHz, CDCl₃)
Figure S11. $^1$H NMR (500 MHz, MEOD) spectrum of compound 4,10-di-(S)-MTPA-1.
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