Cytotoxic polyketides from the deep-sea-derived fungus 
Aspergillus fischeri FS452

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ABSTRACT
Six globoscin derivatives (1–6) including two new ones fischerins A (1) and B (2) were isolated from the deep-sea-derived fungus Aspergillus fischeri FS452. Their structures were elucidated by comprehensive spectroscopic analysis and the absolute configurations were determined by the quantum chemical ECD calculations. The in vitro cytotoxicity assays indicated that fischerin B (2) exhibited potential activities against the four tested human cancer cell lines (SF-268, MCF-7, HepG-2 and A549) with the IC50 values in the range of 7–10 μM.

1. Introduction
Globoscin derivatives are a small class of polyketides constructing a phenylbutyrolactone or phenylbutyric acid core with different chiral center at C-4. The first examples discovered are antafumicins A and B, which were isolated from Aspergillus niger in 1993 with antimicrobial activity (Fujimoto et al. 1993). Then, another two analogues, globoscin and globoscinic acid were discovered from fungi Xylaria globosa and X. obovate (Adeboya et al. 1995), and eight new analogues were reported from fungi Neosartorya spinosa and Aspergillus sp. (Rajachan et al. 2016; Wang et al. 2018). However, none of them exhibited significant bioactivities.
Marine fungus-derived natural products are considered as a great potential resource for new drug development in the 21st century, of which the marine-derived fungi are recognized to be the promising source for the discovery of novel biological active agents such as anticancer, anti-inflammatory and antitubercular (Carroll et al. 2021, 2020, 2019) during the past years. Moreover, the genera *Penicillium* and *Aspergillus* are considered to be the main contributors of bioactive metabolites (Wang et al. 2019; Amin et al. 2021; Salendra et al. 2021). In our previously searching for bioactive metabolites from deep-sea-derived fungi, the strain *Aspergillus fischeri* FS452 isolated from Indian Ocean sludge (81° 00.00’ N, 1° 59.987’ E; depth 3000 m) attracted our attention since its ability to produce the novel polypropionates with MptpB inhibitory activity when fermented in rice medium (Liu et al. 2019a). In order to dig out the chemical diversity of this strain, the fermentation in potato dextrose broth (PDB) medium was carried out in this study and two new polyketides belonging to globoscin family were obtained together with four known analogues. Herein, the details of the isolation, structure identification and cytotoxic activities of 1–6 are discussed.

### 2. Results and discussions

The methanol extract of the deep-sea derived-fungus *Aspergillus fischeri* FS452 was concentrated under reduced pressure and the obtained crude extract was further subjected to repeated column chromatography and semi-preparative HPLC to afford compounds 1–6 (Figure 1).

Fischerin A (1) was obtained as a colorless oil, of which the molecular formula was deduced to be C_{14}H_{18}O_{7} based on the deprotonated molecule ion peak at m/z 297.0979 [M−H]−. The 1H NMR spectrum collected in chloroform-d (Table S1) indicated the presence of a chelating proton at δH 13.13, three methyls including two methoxyl at δH 3.51/3.46 (Me-13/14, respectively), one AB coupling methylene group at δH 2.28 (ddd, J = 14.6, 10.4, 3.1) and 2.09 δH 2.28 (ddd, J = 14.6, 10.5, 3.1), four methine groups including two aromatic ones (δH 7.59/6.41, H-8/H-9) and two O-bearing ones (δH 4.11/5.14, H-2/H-4). The 13C NMR spectrum resolved 14 signals composing of two carbonyl carbons (δC 175.3 and 202.8, C-1 and C-11, respectively), six aromatic
carbons and six sp3 ones. By comparing the 1D NMR data to those of the known compounds spinoates suggested that compound 1 was a demethylation product of spinosate.

The COSY correlations (Figure S1) from H-2 to H-4 indicated the only coupling unit of C-2/C-3/C-4 and the HMBC correlations from H-2/H3-2 to C-1 located the carboxyl group at C-2, which constructed a C4 aliphatic chain. The correlations from H-4 to C-5/C-6/C-10 suggested that the side chain was linked to C-5 of the benzene ring. Furthermore, the substitutions of the chelating hydroxyl and acetyl groups at C-6 and C-7 were elucidated through HMBC correlations (Figure S1) from H3-12 to C-7/C-11 and from 6-OH to C-5/C-6/C-7, respectively. Finally, based on the correlations from H-13 to C-2 and from H-14 to C-4, the methoxy groups Me-13 and Me-14 should be connected to C-2 and C-4, respectively. Hence, the planar structure was confirmed.

Fischerin B (2) was also obtained as a colorless oil and gave a molecular formula of C14H18O7 based on HRESIMS, The 1H/13C NMR data (Table S1) were nearly identical to those of 1 and one of the obvious differences detected was that the coupling constant between H2-3 and H-2/H-4 (JH-2/H-3l = 10.4 Hz, JH-4/H-3h = 10.5 Hz in 1 vs. JH-2/H-3h = 5.9 Hz, JH-4/H-3l = 9.9 Hz in 2), which suggested that they were isomers at C-2 or C-4. Analysis of the 2D NMR spectra (Figure S1) confirmed the planar structure of 2.

As to fischerins A (1) and B (2), the relative configuration between C-2 and C-4 was hard to be determined through the NOESY spectrum since they were in the flexible side chain. Thus, there are four possible absolute configurations: (2S,4R), (2S,4S), (2R,4R), (2R,4S). Since 1 and 2 were epimers but not enantiomers, they should present (2S,4R)/(2S,4S)-1/2 or (2R,4R)/(2R,4S)-1/2. According to the same biosynthetic pathway as those of compound 3–6, all of which constructed a 2S configuration, the absolute configuration of C-2 in 1 and 2 could be proposed to be S. In order to further confirm the stereochemistry, the theoretical ECD plots of the two possible conformers were calculated at b3lyp/6-311+g(d,p) level. As a result, the calculated plot of (2S,4R)-candidate exhibited a good fit to the experimental spectrum of 1 while the calculated plot of (2S,4S)-candidate fit well with the experimental spectrum of 2 (Figure S2). The above evidence suggested that the absolute configuration of 1 and 2 were 2S, 4R and 2S, 4S, respectively.

Compounds 3 and 4 were identified to be (2S,4R)- and (2S,4S)-spinosate, respectively (Rajachan et al. 2016). Compound 5 and 6 were identified to be antafumicins A and B, which were previously isolated from the fungus Aspergillus niger NH-401 in 1993. In this study, the single crystal of the mixture of 5 and 6 was obtained for the first time (Figure S3).

In the bioassays, compounds 1–6 were tested for their in vitro cytotoxicity against four human cancer cell lines (Table S2) and only fischerin B (2) showed potential activities against cell lines SF-268, MCF-7, HepG-2 and A549 with the IC50 values of 7.56, 8.45, 9.03 and 9.98 μM, respectively. Since no obvious cytotoxicity were detected for fischerin A (1) when compared to fischerin B (2), it could be concluded that the chiral center at C-4 might make a contribution to the cytotoxicity. Besides, a comparison of the cytotoxic activities results between compounds 2 and 4 (both of which presented a 4S configuration) indicated that the terminal carboxyl group at C-1 might also make a difference to the cytotoxic activities.
3. Experimental section

3.1. General experimental procedures

The optical rotation data were collected on an Anton Paar MCP-500 (Anton Paar, Graz, Austria). The circular dichroism (ECD) and the UV spectra were collected on Jakso 820 spectropolarimeter. IR spectra were recorded through Shimadzu IR Affinity-1 spectrophotometer. The 1D and 2D NMR spectra were recorded on a 600 MHz Bruker Avance-III HD spectrometer referenced to the signals of tetramethylsilane as an internal standard. HR-ESI-MS was measured on a Bruker maXis high-resolution mass spectrometer. A Shimadzu LC-20 AT with an SPD-M20A PDA detector was used for HPLC analysis and preparative separations. The ACE 5 AR-C_{18} column (250 × 10.0 mm, 5 μm, 12 nm) and the CHIRAL-MD (2)-RH column (250 × 10.0 mm, 5 μm) were used for semipreparative separation and chiral resolution, respectively (Guangzhou FLM Scientific Instrument Co., Ltd). Column chromatography material contained commercial silica gel (SiO_2; 200–300 mesh; Qingdao Marine Chemical Plant), Sephadex LH-20 gel (Amersham Biosciences) and different analytical grade solvents (Guangzhou Chemical Regents Company, Ltd.). The natural sea salt was purchased from Guangdong Yueyan saltern.

3.2. Fungal material

The strain *Aspergillus fischeri* FS452 was isolated from the deep-sea sludge in the Indian Ocean (81° 00' N, 1° 59.987' E; depth 3000 m) in March 2016, which was identified according to morphological traits and ITS rDNA sequence analysis. The sequence data have been submitted to GenBank (accession number KF294264). The strain was deposited at the Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Institute of Microbiology, Guangdong Academy of Science. Working stocks were prepared on PDA slants at 4°C.

3.3. Fermentation, extraction, and isolation

A grown plate culture of *A. fischeri* FS452 was used for the preparation of the seed cultures. Mycelia were inoculated in PDB culture in a rotary shaker at 28°C for 4 days, which was next transferred into large-scale fermentation PDB medium (seven Erlenmeyer flasks each containing 1 L PDB and incubated in a rotary shaker at 28°C for 15 days. Then, the fermented liquid and mycelia were extracted with EtOAc and methanol for three times, respectively. A total of 84.5 g dark brown oily residue was obtained. After being subjected to silica gel column chromatography eluting with petroleum ether/EtOAc in a linear gradient (10:1 to 1:2), eight fractions (Fr.1–Fr.8) were obtained. Fr.7 was subjected to silica gel column eluting with MeOH/CH_2Cl_2 (5:95, v/v) to give the mixtures of 1 and 2, which was further purified by HPLC with CHIRAL-MD (2)-RH column (MeCN/H_2O, 65:35, 2 mL/min) to give 1 (7.3 mg, *t*_R = 9.9 min), 2 (7.1 mg, *t*_R = 12.7 min). The Fr. 4 was subjected to silica gel column eluting with MeOH/CH_2Cl_2 (2:98, v/v) to give the mixture of 3 and 4, which was purified by HPLC with same chiral column (MeCN/H_2O, 75:25, 2 mL/min; 3: 19.1 mg, *t*_R = 8.7 min; 4: 12.2 mg, *t*_R = 11.5 min). The mixture of compounds 5 and 6 were recrystallized from the purified...
fragment of Fr. 5 by Sephadex LH-20 column, which were further separated by HPLC with CHIRAL-MD (2)-RH column (MeCN/H2O, 60:40, 2 mL/min) to give the pure 5 (27.1 mg, \( t_R = 10.9 \) min) and 6 (16.5 mg, \( t_R = 13.1 \) min).

3.3.1. Fischerin A (1)

Colorless oil; \([\alpha]_D^{25} = -22 \) (c 0.01, MeOH); UV (MeOH) \( \lambda_{\max} \) (log \( e \)): 217 (4.29), 234 (4.01), 278 (4.13), 316 (3.80) nm; IR (KBr): 3233, 2926, 2811, 1749, 1653, 1494, 1373, 1250 cm\(^{-1}\); \(^1\)H and \(^{13}\)C NMR data, Table S1. HRESIMS \( m/z \) 297.0979 \([M - H]^-\) (calcd for \([C_{14}H_{17}O_7]^-\), 297.0980).

3.3.2. Fischerin B (2)

Colorless oil; \([\alpha]_D^{25} = -22 \) (c 0.01, MeOH); UV (MeOH) \( \lambda_{\max} \) (log \( e \)): 217 (4.28), 233 (4.01), 278 (4.11), 316 (3.75) nm; IR (KBr): 3235, 2933, 2817, 1750, 1663, 1456, 1375, 1223 cm\(^{-1}\); \(^1\)H and \(^{13}\)C NMR data, Table S1. HRESIMS \( m/z \) 297.0978 \([M - H]^-\) (calcd for \([C_{14}H_{17}O_7]^-\), 297.0980).

3.3.3. The crystal data of compound 5 and 6

Data were collected on an Agilent Xcalibur Nova single-crystal diffractometer using Cu K\( \alpha \) radiation. The crystal structure was refined by full-matrix least-squares calculation with the SHELXL-97. Crystallographic data for the structure of 5 and 6 have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 2102015). Crystal data: \( C_{13}H_{14}O_6 \) (\( M = 266.24 \)); block crystal (0.08 \( \times \) 0.1 \( \times \) 0.2); space group P2\( \bar{1} \); unit cell dimensions \( a = 5.1977(2) \) \( \AA \), \( b = 24.0738(7) \) \( \AA \), \( c = 9.9527(3) \) \( \AA \), \( \alpha = 90^\circ \), \( \beta = 102.534(3)^\circ \), \( \gamma = 90^\circ \), \( V = 1215.68(7) \) \( \AA^3 \), \( Z = 4 \); \( T = 150(2) \) K; \( \rho_{\text{cald}} = 1.455 \text{ mg/m}^3 \); absorption coefficient 0.988 mm\(^{-1}\); \( F(000) = 560 \), a total of 6179 reflections were collected in the range 3.672 < \( \theta < 73.898^\circ \), independent reflections 3716 [R(int) = 0.0445]; the number of data/parameters/restraints were 3716/1/351; goodness-of fit on \( F^2 = 1.049 \); final R indices \( [I > 2\sigma(I)] R_1 = 0.0679, \omega R_2 = 0.1875; R \) indices (all data) \( R_1 = 0.0762, \omega R_2 = 0.1973 \).

3.4. Details of quantum chemical calculations

The pre-optimization through Merck molecular force field (MMFF) and DFT/TD-DFT calculations at b3lyp/6-311 + g(d,p) was proceeded by the Spartan’14 software (Wavefunction Inc.) and the Gaussian 09 program, respectively (Frisch et al. 2013). Pre-optimized conformers with an energy window lower than 5 kcal mol\(^{-1}\) were generated and optimized using at the b3lyp/6-31 + g(d,p) level. The frequency calculations were performed at the same level to confirm that each optimized conformer was a true minimum and to estimate their relative thermal free energy (\( \Delta G \)) at 298.15 K. Then, conformers with the Boltzmann distribution over 10% were chosen for energy calculations at the b3lyp/6-311 + g(d,p) level. The rotatory strengths for a total of 50 excited states were collected according to their Boltzmann distribution. The solvent effects were considered based on the self-consistent reaction field (SCRF) method with the polarizable continuum model (PCM). Finally, the spectra were
generated by the SpecDis program (Bruhn et al. 2013) with a Gaussian band shape with 0.35 eV exponential half-width from dipole-length dipolar and rotational strengths.

3.5. Cytotoxicity assay

The in vitro cytotoxicity assays were carried out according to our previously reported method (Liu et al. 2019b). Human cancer cells (SF-268, MCF-7, HepG-2, A549) purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) were selected to be the targets. The tested cell lines were injected into 96-well plates and incubated at 37°C under 5% CO₂ protection. After 24 h, different concentrations of the inhibitors were added and further co-incubated for another 72 h. Then, cell monolayers were fixed with 50 μL trichloroacetic acid (wt/v: 50%) and stained with 0.4% SRB (dissolved in 1% CH₃COOH) for 30 min, which were washed by 1% CH₃COOH three times to remove the unbound dye. Cisplatin was used as a positive control possessing potent cytotoxic activity. All data were obtained in triplicate experiment.

4. Conclusion

In conclusion, two new polyketides fischerins A (1) and B (2) together with four known analogues were isolated from the deep-sea-derived fungus Aspergillus fischeri FS452. In the bioassays, compounds 2 exhibited potential in vitro cytotoxicity against four human cancer cell lines (SF-268, MCF-7, HepG-2 and A549) with the IC₅₀ values in the range of 7–10 μM. We hope this study will pave the way for the discovery of anti-cancer polyketides from deep-sea-derived fungi.

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Disclosure statement

The authors declare no competing financial interest.

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