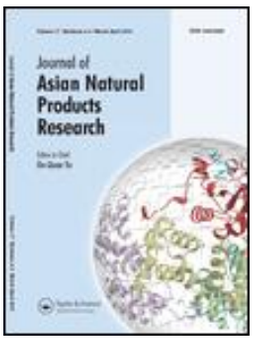

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Exopisiod B and farylhydrazone C, two new alkaloids from the Antarctic-derived fungus *Penicillium* sp. HDN14-431

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ABSTRACT

Two new compounds, exopisiod B (**1**) and farylhydrazone C (**2**), together with two known compounds (**3**–**4**), were isolated from the Antarctic-derived fungus *Penicillium* sp. HDN14-431. Their structures including absolute configurations were elucidated by spectroscopic methods and TDDFT ECD calculations. The cytotoxicity and antimicrobial activities of all compounds were tested.

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KEYWORDS
Exopisiod; farylhydrazone; Antarctic-derived fungus; absolute configuration; ECD calculations

1. Introduction

Antarctica has an area of approximately 4 million km^2 and is reported to be the most pristine environment on the planet [1]. The Antarctic-derived fungi survived in the extreme environments such as subzero temperatures, low water activity, low rates of nutrient, and low metabolite transfers [2–6]. Although very few chemical studies in the secondary metabolites of them were carried out, the Antarctic-derived fungi are attractive resource for exploring small molecules with structures and various bio-activities such as antiviral, antibacterial, antifungal, antitumoral, herbicidal, and antiprotozoal[6–11].

During our ongoing search for new secondary metabolites produced by Antarctic-derived fungi [9, 12], strain *Penicillium* sp. HDN14-431 was isolated from the soil of mesolittoral zone collected from the Antarctic. From the EtOAc extract, two new compounds named exopisiod B (**1**) and farylhydrazone C (**2**), together with two known compounds, namely *N*-acetyl-hydrazinobenzoic acid (**3**) and farylhydrazone B (**4**), were isolated (Figure 1). Herein, we report the details of their isolation, structure elucidation, and bioactivity assay.

2. Results and discussion

Exopisiod B **(1)** was isolated as a flaxen solid. Its molecular formula was determined to be $C_{14}H_{13}NO_4$ on the basis of HRESIMS ion peak at *m/z* 260.0920 $[M+H]^+$, indicating the presence of 9 degrees of unsaturation. The 1D NMR data of compound **1** displayed signals

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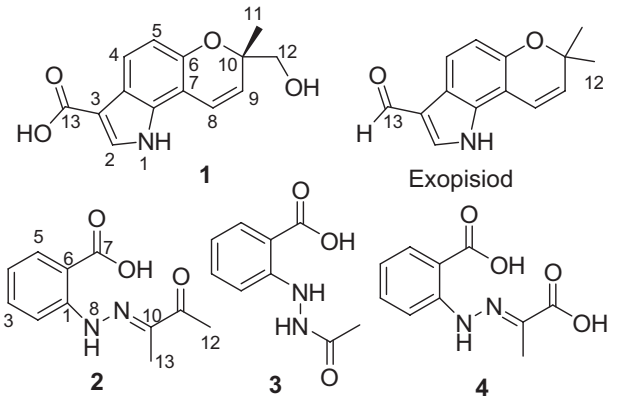

Figure 1. Structures of compounds **1**–**4** and the analog compound.

Table 1. ^{13}C and ^{1}H NMR spectral data for compounds **1** and **2** in DMSO-d_6.

	1		**2**	
No	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	11.80 s			146.3 C
2	7.86, d (2.7)	131.2 CH	7.76, d (8.0)	114.0 CH
3		109.6 C	7.60, t (8.0)	135.2 CH
3a		120.5 C		
4	7.67, d (8.5)	120.6 CH	6.98, t (8.0)	120.7 CH
5	6.61, d (8.5)	111.4 CH	7.91, dd (1.7, 8.0)	131.8 CH
6		148.2 C		112.4 C
7		105.4 C		170.3 C
7a		132.8 C		
8	6.92, d (9.9)	118.3 CH	11.45, s	
9	5.71, d (9.9)	127.1 CH		
10		78.7 C		143.4 C
11	1.28, s	22.5 CH_3		196.7 C
12	3.46, m	66.5 CH_2	2.39, s	24.5 CH_3
13		165.9 C	1.94, s	8.8 CH_3
12-OH	4.94, t (6.02)			

^{1}H and ^{13}C NMR data were measured at 500 and 125 MHz, respectively.

for seven nonprotonated carbons, five olefinic methines, one methylene, and one methyl group (Table 1). The 1D and 2D NMR data of metabolite **1** were similar to those of exopisiod [13], suggested that they share the same skeleton 1,7-dihydropyrano[2,3-g]indole ring. The main differences between them were the replacements of methyl (CH_3-10) in exopisiod by the hydroxymethyl and the aldehyde group (C-13, δ_C 185.1) by carboxyl acid (C-13, δ_C 165.9). The structure of compound **1** was confirmed by 2D NMR correlation (Figure 2), especially the signals of COSY correlation between H_2-12 (δ_H 3.46) and OH-12 (δ_H 4.94), as well as the HMBC correlations from H-12 to C-9 (δ_C 127.1), C-10 (δ_C 78.7), C-11 (δ_C 22.5), together with the consideration of molecular formula.

The absolute configuration of **1** was deduced by comparison with the time-dependent density functional theory (TDDFT) computational electronic circular dichroism (ECD) with the experimental one. First, an MMFF (molecular mechanics force field) conformational search of the enantiomers was carried out. Then, the DFT reoptimization of the arbitrarily chosen (*S*) -**1** enantiomer was calculated at the B3LYP/6-31 + G (d) level, which afforded

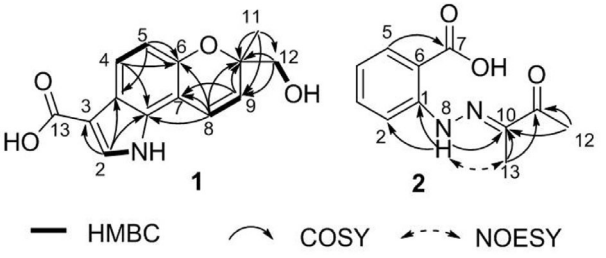

Figure 2. The key 2D NMR correlations of **1** and **2**.

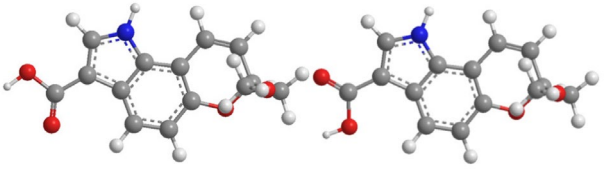

Figure 3. DFT-optimized structures for low-energy conformers of (S)-**1** at B3LYP/6–31 + G(d) level in vacuum methanol (PCM) (conformer populations were calculated using the Gibbs free energy and Boltzmann population at 298 K estimated thereof).

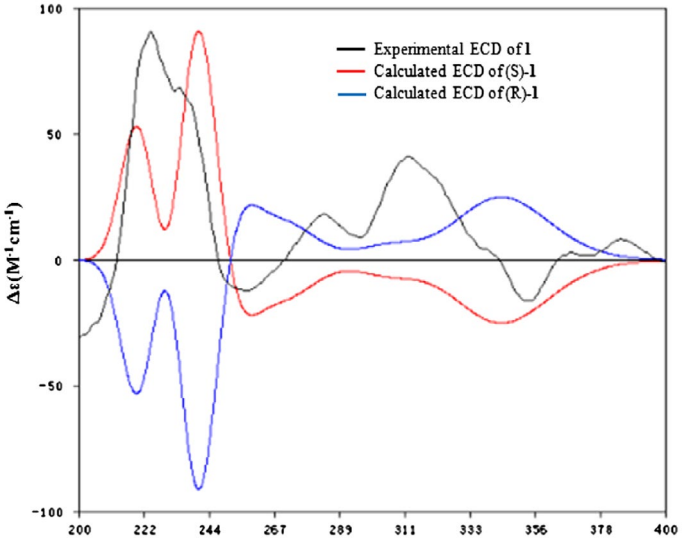

Figure 4. B3LYP/6–31 + G(d) calculated ECD spectra of (S)-**1** (red), calculated ECD spectra of (R)-**1** (blue), and the experimental ECD spectrum of **1** (black). (σ = 0.20 eV).

two major lowest-energy conformers with 65.5% (conf.A) and 34.5% (conf.B) population (Figure 3). The ECD spectra were calculated at the same basis set, which showed the best agreement with the experiment one (Figure 4) and allowed the determination of the absolute configuration of **1** as *S*.

Farylhydrazone C (**2**) was isolated as a yellowish solid. Its molecular formula was deduced to be $C_{11}H_{12}N_2O_3$ evidenced by the HRESIMS protonated peak at *m/z* 221.0924 $[M+H]^+$, with 7 degrees of unsaturation. The NMR data of **2** were closely similar to the known farylhydrazone B (**4**) which had been isolated from cultures of the *Cordyceps*-colonizing fungus *Isaria farinosa* [14]. The only difference between them was that the hydroxy group at C-11 (δ_C 196.7) in **4** was replaced by a methyl group in **2**, which was confirmed by the HMBC correlations from the H-12 (δ_H 2.39, s) to C-10 (δ_C 143.4) and C-11 (Figure 2), as well as the downfield chemical shift of C-11 (δ_C 196.7). The *E*-geometry of the double bond was indicated by the NOESY correlation between NH-8 (δ_H 11.45) and H-13 (δ_H 1.94).

Compounds **3** and **4** were assigned as *N*-acetyl-hydrazinobenzoic acid [15] and farylhydrazone B [14] based on the comparison of spectroscopic data with those reported in the literature.

Compounds **1–4** showed no cytotoxicity against K562, A-549, HCT-116, and Hela cells ($IC_{50} > 10$ μM). Compound **2** showed inhibitory effect against the bacterium *Proteusbacillus vulgaris* with MIC 22.5 μM (chloramphenicol as positive control with MIC 3.13 μM).

In summary, a chemical study of an Antarctic-derived fungal strain led to the isolation of four compounds including the new indole alkaloid exopisiod B (**1**) and farylhydrazone C (**2**). It is notable that the naturally occurring phenylhydrazones are rare with only two structures (farylhydrazones A and B) discovered, and no activity is reported so far [14].

3. Experimental

3.1. General experimental procedures

Specific rotations were recorded on a JASCO P-1020 digital polarimeter (Jasco, Tokyo, Japan). UV spectra were recorded on Beckman DU640 spectrophotometer (Beckman, ALT, USA). IR spectra were recorded on a Nicolet NEXUS 470 spectrophotometer in KBr disks (Thermo Nicolet, USA). ECD spectra were recorded on a JASCO J-815 spectropolarimeter, using MeOH as solvent (Jasco, Tokyo, Japan). The melting point was determined on an RY-1 micromelting point apparatus and is uncorrected (Yu Tong Apparatus, Shanghai, China). All the 1D and 2D NMR spectra were recorded on an Agilent 500 MHz DD2 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ values (Agilent, Palo aito, USA). ESIMS was measured on a Micromass Q-TOF Ultima Global GAA076 LC mass spectrometer. HRESIMS was measured on a Micromass EI-4000 (Autospec-Ultima-TOF) (Thermo Fischer Scientific, Bermen, Germany). Analytical HPLC was performed with a C18 column [YMC-pak ODS-AM, 4.6 × 250 mm, 5 Yellowish solid; mp m, 1 ml/mim] (Waters, Texas, USA). Semiprepartive HPLC were carried on an ODS column [YMC-pak ODS-A, 10 × 250 mm, 5 μm, 3 ml/min] (Waters, Texas, USA). Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences), respectively.

3.2. Fungal material

The fungal strain was isolated from the soil of mesolittoral zone collected from the Antarctic and identified as *Penicillium* sp. based on sequencing of the ITS region (GenBank No.

KU204712). A voucher specimen is deposited in our laboratory at −20 °C. The producing strain was prepared on potato dextrose agar slants and stored at −4 °C.

3.3. Fermentation and extraction

Erlenmeyer flasks (500 ml) containing 150 ml of fermentation media were directly inoculated with spores. The media contained maltose (20 g), mannitol (20 g), glucose (10 g), sodium glutamate (10 g), yeast extract (3 g), corn steep liquor powder (1 g), KH_2PO_4 (0.5 g), and $MgSO_4 \cdot 7H_2O$ (0.3 g) dissolved in 1 L of naturally collected seawater (Jiaozhou Bay, Huanghai). The flasks were cultured with shaking at 180 rpm and 28 °C for 9 days.

The whole fermentation broth (45 L) was filtered through cheese cloth to separate the supernatant from the mycelia. The supernatant was extracted with ethyl acetate (3 × 45 L), and the mycelia was macerated and extracted with acetone (3 × 15 L). All extracts were evaporated under reduced pressure to give a crude extract (15.0 g).

3.4. Purification

The extract (15 g) was separated by VLC on silica gel using a stepped gradient elution with petroleum ether-CH_2Cl_2-MeOH to give ten fractions (Fr.1 to Fr.10). The Fr.5 (30:1 CH_2Cl_2-MeOH 2.3 g) was further fractionated on a C-18 ODS column using a step gradient elution of MeOH-H_2O, resulting in 6 subfractions (Fr.5.1-Fr.5.6). The Fr.5.1 (139 mg) was applied on Sephadex LH-20 using CH_2Cl_2-CH_3OH (1:1) and further purified by semi-preparative HPLC (70:30 CH_3OH-H_2O, 3 ml/min) to yield compound **2** (10.0 mg, t_R = 21 min) and compound **4** (2 mg, t_R = 26 min). The Fr.6 (1.06 g) was separated by MPLC and then Fr.6.4 (206 mg) on a Sephadex LH-20 column eluting with CH_2Cl_2-MeOH (1:1) to provide six fractions (Fr.6.4.1-Fr.6.4.6). Fr.6.4.6 (45 mg) was purified by semi-preparative HPLC (20% CH_3CN in H_2O for 5 min, followed by 20–80% in 30 min) to afford compound **1** (2 mg, t_R = 22 min). Fr.6.4.5 (89 mg) was purified by semi-preparative HPLC (40:60 CH_3OH-H_2O, 3 ml/min) to afford compound **3** (12 mg, t_R = 16 min).

3.4.1. Exopisiod B (1)

Flaxen solid; $[\alpha]_D^{20}$ − 3.3 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε): 209 (2.58), 279 (2.81), 307 (2.86) nm; ^1H and ^{13}C NMR spectral data see Table 1; HRESIMS: *m/z* 260.0920 $[M+H]^+$ (calcd for $C_{14}H_{14}NO_4$, 260.0917).

3.4.2. Farylhydrazone C (2)

Yellowish solid; mp 226–228 °C; UV (MeOH) λ_{max} (log ε): 203 (3.08), 232 (2.81), 326 (2.86) nm; IR (KBr) ν_{max} 3304, 3008(br), 1655, 1582, 1504, 1448, 1406, 1361, 1329, 1265, 1224, 1153, 1113, 1039, 997, 910, 754, 628, 528 cm^{-1}; ^1H and ^{13} C NMR spectral data see Table 1; HRESIMS: *m/z* 221.0924 $[M+H]^+$ (calcd for $C_{11}H_{13}N_2O_3$, 221.0921).

3.5. Computation section

Conformational searches were run employing the "systematic" procedure implemented in Spartan'10 [16], using MMFF (Merck molecular force field). All MMFF minima were reoptimized with DFT calculations at the B3LYP/6-31+G(d) level using the Gaussian 09

program [17]. 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 2 lowest-energy conformations for **1** (>5% population) for each configuration using 20 excited states, and using a polarizable continuum model (PCM) for MeOH (Figure 3). ECD spectra were generated using the program SpecDis [18] by applying a Gaussian band shape with 0.20 eV width for **1**, from dipole-length rotational strengths. The dipole velocity forms yielded negligible differences. The spectra of the conformers were combined using Boltzmann weighting, with the lowest-energy conformations accounting for about 99% of the weights. The calculated spectrum was red-shifted by 10 nm for **1** to facilitate comparison with the experimental data.

3.6. Cytotoxic assay and antimicrobial assay

The cytotoxic assay was performed according to the SRB [19,20] and MTT [21] methods in 96-well microplates. Four human cancer cell lines such as leukemia cell line k562, lung cancer A-549, Hela cells, and colorectal cancer cell HCT-116 were used in the cytotoxicity assay. The antimicrobial assay was performed according to the dilution method [22] in 96-well microplates. Nine types of bacterial strains such as MRSA, MRCNS, *Staphylococcus aureus, Proteusbacillus Vulgaris, Mycobacterium tuberculosis, Escherichia coli, Bacillus subtilis, Mycobacterium tuberculosis, Pseudomonas aeruginosa,* and one fungus: *Candida albicans* were used in the antimicrobial assay.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] O.R. Azmi and R.D. Seppelt, *Polar Biochem*. **18**, 128 (1998).
[2] J.W. Fell, G. Scorzetti, L. Connell, and S. Craig, *Soil Biol. Biochem*. **38**, 3107 (2006).
[3] D. Skropeta, *Nat. Prod. Rep*. **25**, 1131 (2008).
[4] A.A. Stierle, D.B. Stierle, and K.J. Kelly, *Org. Chem*. **71**, 5357 (2006).
[5] L. Du, T.J. Zhu, Y. Fang, Q.Q. Gu, and W.J. Zhu, *J. Nat. Prod*. **71**, 1343 (2008).
[6] M.D. Lebar, J.L. Heimbegner, and B. Baker, *J. Nat. Prod. Rep*. **24**, 774 (2007).
[7] G.W. Wu, H.Y. Ma, T.J. Zhu, J. Li, Q.Q. Gu, and D.H. Li, *Tetrahedron*. **68**, 9745 (2012).
[8] G.W. Wu, A.Q. Lin, Q.Q. Gu, T.J. Zhu, and D.H. Li, *Mar. Drugs*. **11**, 1399 (2013).
[9] L.Y. Li, D.H. Li, Y.P. Luan, Q.Q. Gu, and T.J. Zhu, *J. Nat. Prod*. **75**, 920 (2012).
[10] F. Luis, J. Carlos, R.G. Jaime, A. Carlos, C. Renato, H.Q. Marlene, D.C. Mercedes, D. Caridad, S. Yuri, and V. Inmaculada, *J. Nat. Prod*. **78**, 919 (2015).

[11] J.F. Wang, W.J. He, X.C. Qin, X.Y. Wei, X.P. Tian, L. Liao, S.R. Liao, B. Yang, Z.C. Tu, B. Chen, F.Z. Wang, X.J. Zhou, and Y.H. Liu, *RSC Adv*. **5**, 68736 (2015).
[12] H.B. Zhou, L.Y. Li, W. Wang, Q. Che, D.H. Li, Q.Q. Gu, and T.J. Zhu, *J. Nat. Prod*. **78**, 1442 (2015).
[13] C.C. Wang, M. Liu, J.T. Wei, H. Wang, and X.K. Liu, *Chin. New Drugs J*. **21**, 1660 (2012).
[14] C. Ma, Y. Li, S.B. Niu, H. Zhang, X.Z. Liu, and Y.S. Che, *J. Nat. Prod*. **74**, 32 (2011).
[15] L. Spáčilová, J. Hlaváč, P. Hradil, I. Fryšová, M. Soural, and P. Krejčí, *J. Heterocycl. Chem*. **43**, 1065 (2006).
[16] Spartan 10, (Wavefunction Inc., Irvine, CA, 2010)
[17] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr, J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, and D.J. Fox, *Gaussian 09, Revision A.1*, (Gaussian Inc., Wallingford, CT, 2009).
[18] Bruhn, T., Hemberger, Y., Schaumlöffel, A., Bringmann, G., *SpecDis, Version 1.53*, (University of Wurzburg, Germany, 2011).
[19] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, and M.R. Boyd, *J. Natl. Cancer Inst*. **82**, 1107 (1990).
[20] L. Du, T. Feng, B.Y. Zhao, D.H. Li, X.S. Cai, T.J. Zhu, F.P. Wang, X. Xiao, and Q.Q. Gu, *J. Antibiot*. **63**, 165 (2010).
[21] S.X. Cai, D.H. Li, T.J. Zhu, F.P. Wang, X. Xiao, and Q.Q. Gu, *Helv. Chim. Acta*. **93**, 791 (2010).
[22] S.N. Aslam, P.C. Stevenson, T. Kokubun, and D.R. Hall, *Microbiol. Res*. **164**, 191 (2009).