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ORIGINAL ARTICLE

New secondary metabolites from the marine-derived fungus *Penicillium* sp. MF20218001: Isolation and structural characterization

Yong Yang[1], Jianning Li[2], Ning Yan[1], Lei Liu[1], Xiaoyan Yang[4], Fuhang Song[3*] and Zhijun Song[2*]

[1]The Department of Orthopedics, General Hospital of Ningxia Medical University, Yinchuan, China
[2]School of Pharmacy, Key Laboratory of Protection, Development and Utilization of Medicinal Resources in Liupanshan Area, Ministry of Education, Ningxia Medical University, Yinchuan, China
[3]Key Laboratory of Geriatric Nutrition and Health, Ministry of Education of China; School of Light Indusy Science and Engineering, Beijing Technology and Business University, Beijing, China
[4]Guyuan Vocational and Technical College, Guyuan, China

Abstract: Marine-derived fungi have been regarded as rich and reliable sources of biologically active and chemically novel compounds, which may both directly and indirectly be used as potential lead compounds for drug research and development. In this study, one new discovered 2-pyridone derivative, penicinine C (**1**), a new phenylhydrazone derivative, penicinine D (**2**), together with four known compounds, (14β, 22*E*)-9,14-dihydroxyergosta-4,7,22-triene-3,6-dione (**3**), ergochrome F (**4**), nafuredin A (**5**) and altenusin (**6**) were isolated and purified from a marine-derived fungus *Penicillium* sp. MF20218001. Their chemical structures were elucidated through detailed 1D/2D NMR and HRESIMS spectroscopic analysis, computational calculation methods and comparison with previously reported data. Bioactivity assays showed that compounds **1** and **5** exhibited weak activity against *Candida albicans* with minimum inhibitory concentrations of 50 and 25 μg/mL, respectively.

Keywords: *Penicillium* sp, marine-derived fungus, 2-pyridone derivative, phenylhydrazone derivative, *Candida albicans*

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1 Introduction

As an important type of drug lead compounds, natural products play a significant role in drug discovery and innovation (Newman & Cragg, 2020). For example, paclitaxel, a well-known anticancer drugs, has been used to treat various types of cancer in clinical (Li et al., 2024), digitoxin is used to treat heart failure and certain types of arrhythmias diseases (Chen et al., 2024), mangiferin is used as a candidate drug for high blood pressure and high blood lipid diseases (Chen et al., 2018), etc. Therefore, the exploration of structural diversity and remarkable bioactivity of natural products has attracted increasing interest of researchers.

Marine-derived fungi as rich and reliable sources of biologically active and chemically novel compounds, which may both directly and indirectly be used as potential lead compounds for drug research and development (Banday et al., 2024). Among them, the genus *Penicillium* can produce a wide range of valuable secondary metabolites with multiple biological activities (Zhang et al., 2022), including polyketides (Zhang et al., 2023), alkaloids (Hu et al., 2023), terpenes (Huang et al., 2022), steroids (He et al., 2021), peptides (Youssef et al., 2022), and others, which show anti-inflammatory (Zeng et al., 2023), α-glucosidase inhibitory activity (Han et al., 2021), antimicrobial (Lai et al., 2023), and other biological activities. However, the current discovery is only the tip of the iceberg, a large number of natural products are unknown and need to be further explored. Our group has been working on the discovery of novel antibiotics from marine microbial natural product library, a series of bioactive natural products were identified from marine-derived fungus *Penicillium* sp., such as polyketide-derived penirubenones A and B (Xu et al., 2024), amino-bis-tetrahydrofuran derivatives, penirubenamides A and B (Xu et al., 2024), trienoic acid derivatives (Liu et al., 2023), etc.

As part of our continuous search for structurally new and bioactive secondary metabolites from marine microorganisms, we identified a marine-derived fungus *Penicillium* sp. MF20218001, collected from a sediment sample collected from South China Sea. A systematic chemical investigation on this strain led to the discovery of six compounds. Among them, one new discovered 2-pyridone derivative, penicinine C (**1**), a new phenylhydrazone derivative, penicinine D

*Corresponding Authors: Fuhang Song.
Email: songfuhang@btbu.edu.cn; Zhijun Song.
Email: songzhijun919@163.com

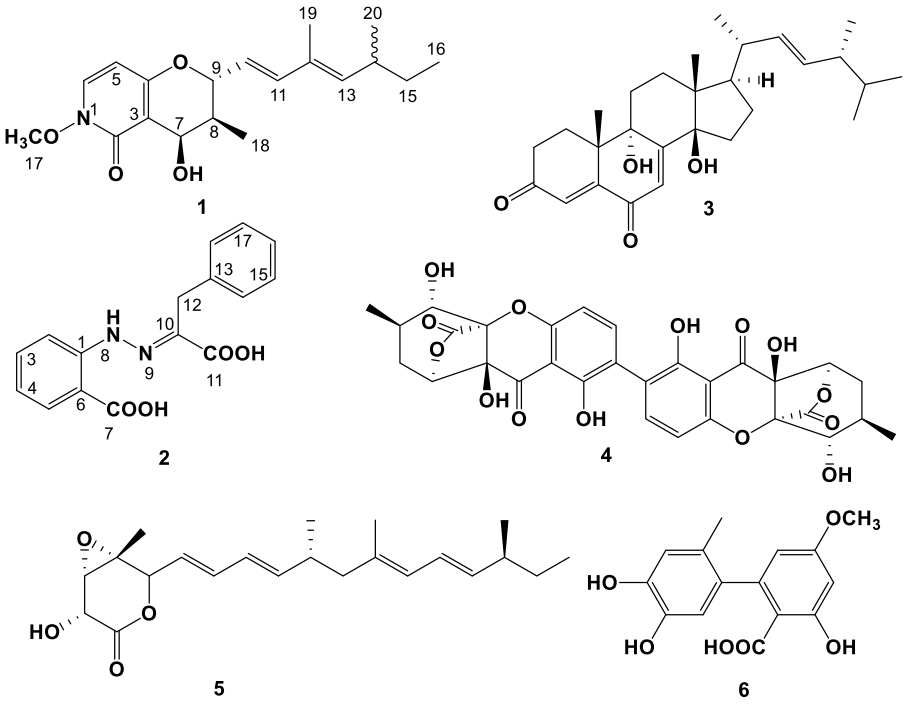

Figure 1. Structure of compounds **1**–**6** isolated from *Penicillium* sp.MF20218001

(**2**), together with four known compounds, (14*β*, 22*E*)-9,14-dihydroxyergosta-4,7,22-triene-3,6-dione (**3**), ergochrome F (**4**), nafuredin A (**5**) and altenusin (**6**) (Figures 1 and S1). In this report, the isolation, purification, structure determination, and evaluation of the antimicrobial activity of the compounds are presented.

2 Materials and Methods

2.1 General Experimental Procedures

Optical rotations $[\alpha]_D^{25}$ were recorded on an Anton Paar MCP 200 Modular Circular Polarimeter (Austria) in a 100 × 2 mm cell. NMR spectra were measured on a Bruker Avance 500 spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C) using DMSO-d_6 as solvent, with residual solvent peaks as references (δ_H 2.50, δ_C 39.5). High-resolution electrospray ionization mass spectroscopy (HR-ESI-MS) was performed on a Bruker microTOF mass spectrometer in positive ion mode (capillary voltage 4500 V, nebulizer pressure 0.4 bar, dry gas flow 4.0 L/min, dry temperature 180°C, mass range *m/z* 50–1500). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., Qingdao, China) and Sephadex LH-20 (Amersham Bioscience company) were used for column chromatography (CC). Semi-preparative reversed phase HPLC was performed using an Agilent 1200 Series system equipped with a diode array detector (DAD), an Agilent 1260 Series fraction collector and an Agilent ZORBAX SB-C_{18}/Eclipse XDB-C_{18} column (250 × 9.4 mm, 5 μM). Analytical TLC was carried out on pre-coated silica gel GF_{254} plates (Qingdao Marine Chemical Industry, Qingdao, China), and spots were visualized under UV light and by spraying with 10% H_2SO_4 in EtOH followed by heating at 120°C.

2.2 Fungal Material

The fungal strain MF20218001 was isolated from a sediment sample collected from South China Sea. The strain was preserved at the Key Laboratory of Geriatric Nutrition and Health, Ministry of Education of China; School of Light Industry Science and Engineering, Beijing Technology and Business University. The strain MF20218001 was identified as *Penicillium* sp. based on its ITS sequence, which shares 99.65% similarity with *Penicillium oxalicum* MN795755.1. The fungal strain used in this study (*Penicillium* sp. MF20218001) has been deposited at the China General Microbiological Culture Collection Center **(CGMCC, NO:** CGMCC 3.29844**)**, which is an internationally recognized culture collection.

2.3 Specimen, Extraction and Isolation of Compounds (**1**–**6**)

The fungi *Penicillium* sp. MF20218001 was inoculated on a potato dextrose agar (PDA) medium and cultured at 28°C for 4 days. Then, a slit of agar with fungus was cut from the plate and inoculated into 250 mL conical flasks of potato dextrose broth (PDB) as seeds and cultured at 28°C (180 rpm) for 3 days. Then, 10 mL of seed culture was added in to twenty 1 L Erlenmeyer flasks, each containing a solid medium consisting of rice (200 g) and distilled water (200 mL), and the flasks were incubated at 28°C under static conditions for 30 days.

After fermentation was complete, the cultures were extracted three times with a mixture of EtOAc:MeOH (*v/v*,

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80:20), and the combined extracts were evaporated in vacuo to yield a residue. The residue was subsequently suspended in distilled water and successively extracted three times with EtOAc to obtain EtOAc crude extracts. Then, the EtOAc crude extracts (20.45 g) was fractionated by vacuum liquid chromatography on silica gel (50 × 80 mm column, Silica gel 60 H for thin-layer chromatography) for elution with a gradient of EtOAc in petroleum ether (EtOAc/petroleum ether, 0–100%), and then with MeOH in dichloromethane ranging from 10 to 100% to give 12 fractions (fractions 1–12). Fraction 4 (0.23 g) was fractionated using an ODS gel column with a step gradient elution of MeOH–H_2O (60–90%) to afford five fractions (Fr. 4–1 to Fr. 4–5). Fraction 4–2 (0.13 g) was subsequently purified by HPLC (Agilent ZORBAX SB-C_{18}, 9.4 × 250 mm, 5 μM column, 3.0 mL/min, elution with 30% to 100% acetonitrile/H_2O) to yield **1** (t_R = 8.38 min, 15.8 mg) and **2** (t_R = 9.13 min, 16.3 mg). Fraction 5 (2.52 g) was subjected to a Sephadex LH-20 column using an isocratic elution of CH_2Cl_2:MeOH (*v*/*v*, 2:1) to yield 12 subfractions (Fr. 5–1 to Fr. 5–12), and fraction 5–4 (0.14 g) was further purified by HPLC (Agilent Eclipse XDB-C_{18}, 9.4 × 250 mm, 5 μM column, 3.0 mL/min, elution with 20% to 40% acetonitrile/H_2O) to yield **3** (t_R = 7.97 min, 7.2 mg) and **4** (t_R = 9.34 min, 9.3 mg). Fraction 7 (0.45 g) was subjected to a Sephadex LH-20 column using an isocratic elution of CH_2Cl_2:MeOH (*v*/*v*, 2:1) to yield 9 subfractions (Fr. 7–1 to Fr. 7–9). Subfraction 7–2 was further fractionated by HPLC (Agilent Zorbax RX-C_{18} 250 × 9.4 mm, 5 μM column, 3.0 mL/min, isocratic 30% MeCN/H_2O over 25 min with isocratic 0.1% TFA modifier) to yield compounds **5** (t_R = 8.10 min, 3.5 mg) and **6** (t_R = 8.37 min, 1.9 mg).

2.4 Physicochemical Data of New Compounds

Penicinine C (**1**): White amorphous powder; $[\alpha]_D^{25}$ −30.00 (*c* 0.05, CH_3OH); ^1H and ^{13}C NMR data, Tables 1 and S1; HRESIMS *m/z* 334.2018 $[M + H]^+$ (calcd for $C_{19}H_{28}NO_4^+$, 334.2013).

Penicinine D (**2**): Light yellow gum; ^1H and ^{13}C NMR data, Tables 1 and S2; HRESIMS *m/z* 299.1034 $[M + H]^+$ (calcd for $C_{16}H_{15}N_2O_4^+$, 299.1027).

2.5 ECD Calculations

The conformers of compound **1** were generated using the MMFF94s force field (Chen et al., 2025). Geometry optimization was performed using the semiempirical PM6 method as implemented in Gaussian 16. Then, conformational searching was carried out in GMMX using the MMFF94 force field with an energy cutoff of 3.5 kcal/mol. Subsequently, geometry optimizations and frequency analyses were implemented at the B3LYP/6-311G(d,p) level in IEFPCM methanol using Gaussian 16. The time-dependent density functional theory (TDDFT) calculations of their low-energy conformations were performed to simulate their ECD spectra at the B3LYP/6-311G (d, p) level.

2.6 Biological Activity

The antifungal and antibacterial screening were performed against a panel of microbial pathogens, including *C. albicans* ATCC 10231, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 by using a microdilution method according to Antimicrobial Susceptibility Testing Standards outlined by the Clinical and Laboratory Standards Institute document M07-A7 (2008) and previous report (Liu et al., 2023; Yang et al., 2024; Wang et al., 2024) Tested compounds (**1**–**6**) and the positive controls were dissolved in dimethyl sulfoxide (DMSO) by a two-fold serial dilution method with final concentrations ranged from 200 to 1.56 μg/mL. DMSO, rapamycin, methicillin and ciprofloxacin were run as negative and positive controls, respectively.

3 Results and Discussion

3.1 Compound Structure Identifcation

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{19}H_{27}NO_4$ based on the positive HR-ESI-MS ion peak at *m/z* 334.2018 $[M + H]^+$ (calc. for $C_{19}H_{28}NO_4^+$, 334.2013) (Figure S2) and ^1H and ^{13}C NMR spectroscopic data, suggesting 7 degrees of unsaturation. Analysis of 1D NMR date (Table 1, Figures S3–S5) of compound **1** indicated that the presence of one carbonyl carbon (δ_C 157.6), four olefinic bonds (δ_C/δ_H 140.3/5.37, δ_C/δ_H 139.5/6.37, δ_C/δ_H 135.5/7.81, δ_C/δ_H 123.9/5.56, δ_C/δ_H 98.2/5.85, δ_C 160.3, 131.3, 111.6), and combined with the degrees of unsaturation, meant that **1** contained two rings. Careful analysis of the 2D NMR (Table S1, Figures S6–S8) spectra of **1** indicated that the structure of compound **1** was very similar to those of YCM1008A (Koizumi et al., 2007), including a 3,4-substitued 2-pyridone ring A, a six-membered ether ring B and an alkyl side chain. Furthermore, the molecular weight of compound **1** was 26 amu less than that of YCM1008A, meaning that **1** lacks an olefinic bond at the alkyl side chain, which was confirmed by the COSY and the HMBC correlations (Table S3, Figure 2). In addition, the configurations of the two double bonds (Δ^{10} and Δ^{12}) in the alkyl chain in compound **1** were determined as *E* and *E*, respectively, according to the coupling constants of $J_{10,11}$ (J = 15.5 Hz) and $J_{12,13}$ (J = 10.0 Hz), respectively.

The relative configurations of C-7, C-8, and C-9 were deduced by analysis of coupling constants and by comparison with the known analogue YCM1008A, whose absolute configuration (2*R*, 3*R*, 4*S*, 8′*R*) was previously established by total synthesis (Koizumi et al., 2007; Tatsuta et al., 2007). Accordingly, the relative configurations of **1** are assigned as 7*R*, 8*R*, 9*S*. To further confirm the absolute configuration, ECD calculations were performed. The calculated ECD spectrum matched well with the experimental spectrum for the rigid core, supporting the assigned configurations at C-7, C-8, and C-9. However, due to the high conformational flexibility of the side chain, the absolute configuration of the remote chiral centers (e.g., on the alkyl chain) could not be reliably determined (Figure 3). Therefore, the structure of compound **1** is shown in Figure 1 and named penicinine C.

Compound **2** was isolated as a light yellow gum. Its molecular formula was determined as $C_{16}H_{14}N_2O_4$ based on the positive HRESIMS ion peak at *m/z* 299.1034 $[M + H]^+$ (calcd. for $C_{16}H_{15}N_2O_4^+$, 299.1027) (Figure S9) and ^1H and ^{13}C NMR

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) of **1** and **2** (J in Hz, δ in ppm)

Position	1		2	
	δ_H [a]	δ_C [b], type	δ_H [a]	δ_C [b], type
1				145.7, C
2		157.6, C	7.83, d (8.5)	114.0, CH
3		111.6, C	7.54, ddd (8.5, 7.0, 1.5)	111.6, CH
4		160.3, C	6.95, ddd (8.0, 7.0, 1.5)	120.1, CH
5	5.85, d (8.0)	98.2, CH	7.87, dd (8.0, 1.5)	131.2, CH
6	7.81, d (8.0)	135.5, CH		112.6, C
7	4.41, dd (5.5, 3.0)	60.4, CH		169.9, C
8	1.67, m	36.6, CH		
9	4.38, dd (11.0, 8.5)	78.0, CH		
10	5.56, dd (15.5, 8.5)	123.9, CH		137.3, C
11	6.37, d (15.5)	139.5, CH		166.2, C
12		131.3, C	3.92, s	30.7, CH_2
13	5.37, d (10.0)	140.3, CH		135.2, C
14	2.39, m	33.7, CH	7.27, m	128.2, CH
15	1.35, m1.24, m	29.7, CH_2	7.29, m	128.7, CH
16	0.81, t (7.5)	11.9, CH_3	7.20, m	126.6, CH
17	3.90, s	64.4, CH_3	7.29, m	128.7, CH
18	0.88, d (7.0)	13.0, CH_3	7.27, m	128.2, CH
19	1.74, d (1.0)	12.5, CH_3		
20	0.94, d (6.5)	20.5, CH_3		
7-OH	5.07, d (5.5)		11.80, s	

Note: [a]500 MHz in DMSO-d_6.[b]125 MHz in DMSO-d6.

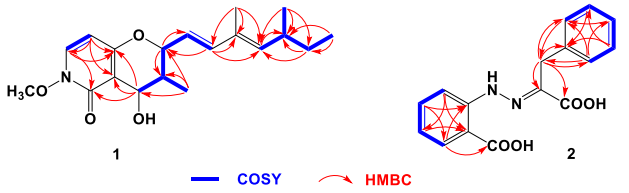

Figure 2. ^{1}H–^{1}H COSY and key HMBC correlations of compounds **1** and **2**

spectroscopic data (Figures S10–S12), suggesting 11 degrees of unsaturation. The 1D NMR data (Table 1) combined with HSQC spectra (Figure S13) of **2** indicated the presence of two carbonyl groups (δ_C 166.2, 169.9), one 1,6-substitued benzene ring A (δ_C/δ_H 111.6/7.54, δ_C/δ_H 114.0/7.83, δ_C/δ_H 120.1/6.95, δ_C/δ_H 131.2/7.87, δ_C 112.6, 145.7), one single substituted benzene ring B (δ_C/δ_H 126.6/7.20, δ_C/δ_H 128.2/7.27 (x2), δ_C/δ_H 128.7/7.29 (x2), δ_C 135.2), a quaternary carbon (δ_C 137.3) and one methylene (δ_C/δ_H 30.7/3.92). Further analysis of the 2D NMR (Table S2, Figures S13–S15) spectra of **2** indicated that the structure of compound **2** was very similar to those of farylhydrazones B (Ma et al., 2011, Table S4), including a 1,6-substitued benzoic acid unit and phenylpropionic acid unit, which was supported by the COSY and the HMBC correlations (Figure 2). Additionally, in combination with the molecular formula it is inferred that an azo unit was situated between C-1 and C-10. Thus, the whole connectivity of **2** was established, which was further confirmed by other COSY and HMBC correlations (Figure 2). No chiral centers are present in **2**, and the N=N double bond is assigned as *E*-configuration based on the chemical shift and aromatic ring deshielding effect, consistent with the known analogue farylhydrazone B (Ma et al., 2011). Thus, the structure of compound **2** was established as shown Figure 1, namely penicinine D.

In addition, four known compounds (Figures 1 and S1) were identified as (14β,22*E*)-9,14-dihydroxyergosta-4,7,22-triene-3,6-dione (**3**, Table S5, Figures S16–S19) (Wu et al., 2013), ergoflavin (**4**, Table S6, Figures S20-23-S19) (Anh et al., 2024), nafuredin A (**5**, Table S7, Figures S24–S27) (Ui et al., 2001) and altenusin (**6**, Table S8, Figures S28–S31) (Nakanishi et al., 1995; Elbermawi et al., 2022) by comparing their NMR data with those published previously (Figures 1 and S1).

3.2 Antimicrobial Activities Assay

In the bioassay, all compounds (**1–6**) were tested for their antimicrobial activities against *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, and only compounds **1** and **5** exhibited weak activity against *Candida albicans* with MICs of 50 and 25 μg/mL, respectively (Table 2).

3.3 Discussion

Despite the successful isolation and structural characterization of two new compounds, but several aspects remain unresolved. For instance, antimicrobial testing was limited to three reference strains—broader screening against drug-resistant isolates, biofilm-forming strains, or fungi with

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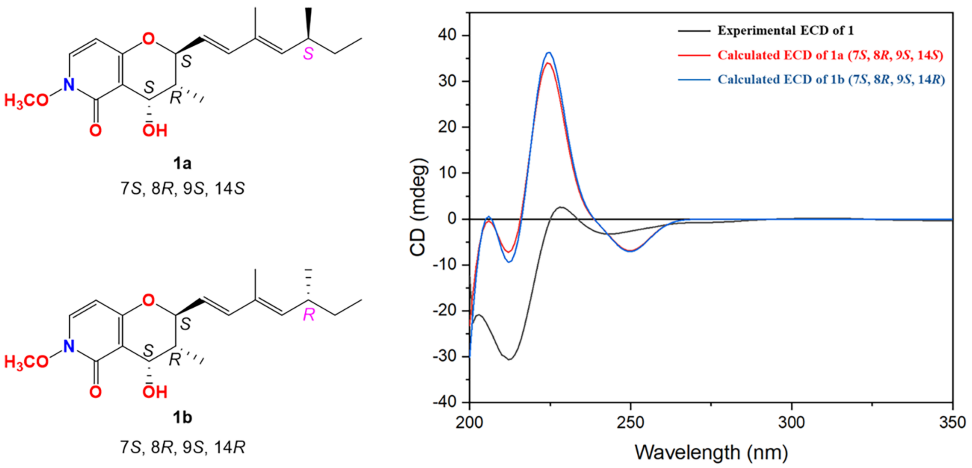

Figure 3. Experimental and calculated ECD spectra of **1**

Table 2. Antimicrobial activity of compounds **1**–**6**

Compounds	Pathogenic bacteria, Minimum inhibitory concentration (μg/mL)		
	Candida albicans[a] (SC5314)	*Staphylococcus aureus*[b] (ATCC 6538)	*Escherichia coli*[c] (ATCC25922)
1	50	>200	200
2	200	>200	200
3	>200	>200	>200
4	>200	>200	>200
5	25	>200	>200
6	>200	>200	>200

Note: **Control:** [a]1.0 μg/mL rapamycin, [b]1.0 μg/mL vancomycin, [c]0.5 μg/mL ciprofloxacin.

different susceptibility profiles would clearly offer a more complete picture. In the case of compounds **1** and **5**, both showed only weak antifungal activity, and the mechanisms involved are still unclear; further work, including target identification or synergy assays with existing antifungal agents, is needed to clarify their potential.

The presence of the unusual phenylhydrazone motif in **2** also invites further investigation into its biosynthesis. Whether this structure arises from an enzyme-catalyzed process or a non-enzymatic condensation event remains an open question, and could be addressed by combining genome mining with feeding experiments in follow-up studies.

Taken together, the isolation of penicinine C (**1**) and penicinine D (**2**) adds new structural diversity to the known metabolite profile of marine-derived *Penicillium* species. Although the antifungal activities observed here are modest, these compounds still represent useful additions to natural product collections and offer distinctive scaffolds that may be of interest for future drug discovery efforts.

4 Conclusions

In summary, we report two new compounds penicinines C and D (**1** and **2**), together with four known compounds, (14*β*,22*E*)-9,14-dihydroxyergosta-4,7,22-triene-3,6-dione (**3**), ergoflavin (**4**), nafuredin A (**5**) and altenusin (**6**), isolated from the marine-derived fungus *Penicillium* sp. MF20218001. To the best of our knowledge, penicinine C represents a rare 2-pyridone derivative with a fused ether ring isolated from marine-derived *Penicillium* species. Furthermore, the antimicrobial activities of the compounds (**1**–**6**) were evaluated and compounds **1** and **5** exhibited weak activity against *Candida albicans* with MICs of 50 and 25 μg/mL, respectively. The discovery of these compounds further enriches the structural diversity of the genus *Penicillium*.

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Author Contributions

Yong Yang: Data curation, Visualization, Writing–original draft. Jianning Li and Ning Yan: Validation, Methodology. Lei Liu and Xiaoyan Yang: Funding acquisition, Investigation, Fuhang Song: Conceptualization, Supervision, Formal analysis, Project administration, Writing–review & editing. Zhijun Song: Funding acquisition, Formal analysis, Writing–review & editing.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

Supplementary information for this article is available online at http://www.acgpubs.org/journals/records-of-natural-products.

ORCID

Yong Yang: 0000-0001-7642-1299
Jianning Li: 0009-0008-2147-1211
Ning Yan: 0000-0002-8190-1921
Lei Liu: 0009-0005-9726-3142
Xiaoyan Yang: 0009-0001-0683-4431
Fuhang Song: 0000-0002-9162-3355
Zhijun Song: 0009-0000-4972-2344

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