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Cytochalasin P_1, a new cytochalasin from the marine-derived fungus *Xylaria* sp. SOF11

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Abstract: A new cytochalasin, named cytochalasin P_1 (**1**), together with four known analogs (**2–5**) was isolated from marine-derived fungus *Xylaria* sp. SOF11 from the South China Sea. The structure of the new compound was elucidated on the basis of MS and NMR (^{1}H, ^{13}C, HSQC, HMBC, and NOESY) data analyses. Compounds **1–5** were tested for their cytotoxicities against four tumor cell lines (SF-268, MCF-7, NCI-H460, and HepG-2). Compounds **1–5** showed significant cytotoxicity against two tumor cell lines MCF-7 and SF-268, with the IC_{50} values varying between 0.33 and 4.17 μM.

Keywords: cytochalasin; cytotoxicity; marine-derived fungus; *Xylaria* sp.

1 Introduction

Marine-derived fungus has been recognized as a productive and important source of novel natural compounds that could potentially be used or modified as anticancer, antibacterial, and antiviral agents. Up to date, more than 1000 structurally interesting compounds have been isolated and characterized from marine fungi [1]. Among these compounds, a promising example is halimide, which demonstrated potent cytotoxic activities against human cancer cells [2]. A derivative of halimide, plinabulin (NPI-2358), is undergoing clinical trials as potential anticancer drug [3].

We focused our efforts on the investigation of bioactive secondary metabolites from the South China Sea-derived fungi with the intention of developing drug candidates. In the past few years, we have reported new cytochalasins from *Xylaria* sp. SCSIO 156, cytotoxic cycloheptapeptides from *Acremonium persicinum* SCSIO 115, immunosuppressive mycophenolic acid derivatives from *Penicillium* sp. SOF07 and halogenated anthraquinones from *Aspergillus* sp. SCSIO F063 [4–7]. Recently, a marine-derived fungus strain *Xylaria* sp. SOF11 attracted our attention because that the crude extract of *Xylaria* sp. SOF11 was lethal to brine shrimp (*Artemia salina*) and displayed cytotoxicity against three human tumor cell lines (A549, HCT15, and HEP3B). Chemical investigation of the fermentation products of *Xylaria* sp. SOF11 has resulted in the isolation of a new cytochalasin, named cytochalasin P_1 (**1**), as well as four known analogs, cytochalasin P (**2**) [8], 19, 20-epoxycytochalasin N (**3**), 19, 20-epoxycytochalasin D (**4**) [9], and zygosporin G (**5**) [10] (Figure 1). Herein, we report the isolation, structure elucidation, and cytotoxicity of the new compound.

2 Results and discussion

Compound **1** was obtained as a white powder. Its molecular formula was determined to be $C_{30}H_{39}NO_8$ based on the quasimolecular ion peak at *m/z* 540.2612 $[M-H]^-$ (calculated 540.2603) in HR-ESI-MS spectrum, indicating 12 degrees of unsaturation. The ^{1}H NMR spectrum of **1** displayed five methyl group proton signals at δ_H 0.91 (d, J = 6.5 Hz, H-11), 1.13 (d, J = 7.0 Hz, H-22), 1.17 (s, H-12), 1.49 (s, H-23), 2.09 (s, acetyl), 2 olefinic proton signals at δ_H 5.63 (dd, J = 15.8, 10.5 Hz, H-13), 5.60 (m, H-14), 2 pair of *ortho*-coupled aromatic proton signals at δ_H 7.13 (2H, d, J = 7.0 Hz, H-2′, 6′), 7.29 (2H, d, J = 7.0 Hz, H-3′, 5′), and several aliphatic proton signals between δ_H 1.89–3.55 (Table 1). Analysis of the ^{13}C NMR and HSQC data for **1** revealed 3

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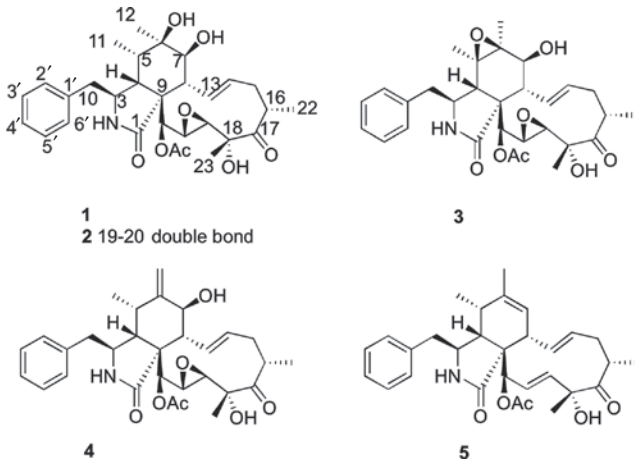

Figure 1: Structures of compounds **1–5**.

carbonyls (C-1, C-17, and acetyl), 4 quaternary carbons, including 2 oxygen-bearing quaternary carbons (C-6 and C-18), 16 methines, including 3 oxymethines (C-7, C-19, and C-20) and 7 aromatic methines (C-2′, C-3′, C-4′, C-5′, C-6′, C-13, and C-14), 2 methylenes (C-10 and C-15) and five methyls (C-11, C-12, C-22, C-23, and acetyl).

The ^{1}H and ^{13}C NMR data of **1** were similar to those of the known cytochalasin P (**2**), except that the signals of the double bond at δ_H 5.10 (1H, dd, J = 16.2, 2.0 Hz, H-19), 6.08 (1H, dd, J = 15.5, 3.0 Hz, H-20) and at δ_C 128.8 (C-19), 135.0 (C-20) in **2** were absent in **1**. Instead, two oxymethine signals were observed at δ_H 3.48 (1H, br s, H-19) and δ_C 54.3 (C-19), 3.09 (1H, d, J = 1.5 Hz, H-20), and δ_C 59.1 (C-20), suggesting the double bond at C-19 and C-20 in **2** was replaced by an epoxide bond in **1**. In the HMBC spectrum (Figure 2), the correlations of H-21/C-19, C-20, and H-23/C-19 substantiated the above conclusion.

The relative configuration of **1** was established by a combination of coupling constant and NOESY experiment. In the ^{1}H NMR spectrum, the large coupling constant (J = 12.0 Hz) for H-7 and H-8 suggested the *trans* orientation of the proton pair. The *E*-geometry of the Δ^{13}-double bond was deduced from the large coupling constant observed for H-13 and H-14 (J = 15.8 Hz). In the NOESY spectrum (Figure 2), the NOE correlation of H-3/H_3-11 and H_3-12, H_3-12/H-7, H-4/ H-5 and H-8 indicated that H-3, H_3-11, H-7, and H_3-12 were in the same orientation (α); while H-4, H-5 and H-8 were in the opposite direction (β). The NOE correlation of H-7/H-13, H-14/H-8 and H-15b, H-15a/H-22, H-16/H-23 indicated that H-13 and H-22 were α-oriented; H-16 and H-23 were β-oriented. The NOE correlation of H-19/H-13 and H-21, H-13/H-20 indicated that H-19, H-20, and H-21 were α-oriented. Thus, compound **1** was established to be a new cytochalasin, and named cytochalasin P_1.

Compounds **1–5** were biologically evaluated for in vitro cytotoxicity against four human tumor cell lines (SF-268, MCF-7, NCI-H460, and HepG-2) by using the SRB method with cisplatin as the positive control. Compounds **1–5** showed cytotoxic activities against SF-268 and MCF-7 cell lines, with the IC_{50} values varying between 0.33 and 4.17 μM, as listed in Table 2. However, Compounds **1–5** exhibited no cytotoxic activities against NCI-H460 and HepG-2 cell lines, those tentative results revealed that the tested compounds had excellent selective cytotoxic activities.

Table 1: ^{1}H and ^{13}C NMR spectroscopic data for **1** in $CDCl_3$ at 500/125 MHz.

Position	δ_C, mult.	δ_H (J in Hz)	Position	δ_C, mult.	δ_H (J in Hz)
1	173.5 s		16	42.0 d	3.15 (m)
3	54.2 d	3.55 (m)	17	215.5 s	
4	50.3 d	2.17 t (10.0)	18	76.4 s	
5	38.5 d	1.89 t (6.5)	19	54.3 d	3.48 (br s)
6	71.9 s		20	59.1 d	3.09 d (1.5)
7	71.4 d	3.02 d (12.0)	21	74.5 d	5.26 (br s)
8	42.3 d	2.70 t (10.0)	22	18.9 q	1.13 d (7.0)
9	54.4 s		23	21.6 q	1.49 (s)
10	45.6 t	2.88 dd (13.5, 3.5)	1′	136.6 s	
		2.55 (m)	2′, 6′	128.9 d	7.13 d (7.0)
11	13.3 q	0.91 d (6.5)	3′, 5′	129.3 d	7.29 t (7.0)
12	24.8 q	1.17 (s)	4′	127.1 d	7.23 t (7.0)
13	129.9 d	5.63 dd (15.8, 10.5)	CH_3CO	20.5 q	2.09 (s)
14	133.7 d	5.60 (m)	CH_3CO	170.0 s	
15	37.6 t	2.52 (m)			
		2.08 (m)			

Chemical shifts (δ) are given in ppm and J in Hz.

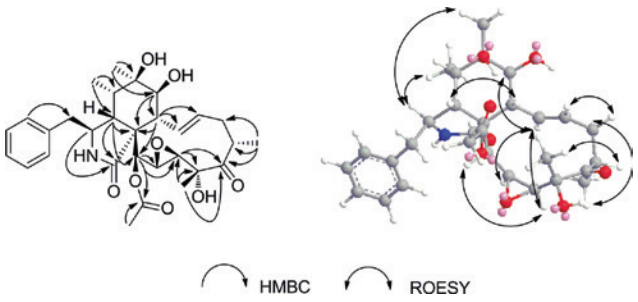

Figure 2: Key HMBC and NOESY correlations for compound **1**.

Table 2: Cytotoxicities of compounds **1–5** against four tumor cell lines (IC_{50}, μM).

Compounds	SF-268	MCF-7	NCI-H460	HepG-2
1	1.37 ± 0.06	0.71 ± 0.05	> 100	> 100
2	4.17 ± 0.08	3.28 ± 0.25	> 100	> 100
3	1.57 ± 0.10	2.67 ± 0.13	> 100	> 100
4	0.33 ± 0.04	0.53 ± 0.07	49.38 ± 2.47	> 100
5	1.38 ± 0.30	4.11 ± 0.29	> 100	> 100
Cisplatin[a]	1.93 ± 0.16	5.87 ± 0.45	1.32 ± 0.15	1.75 ± 0.17

[a]Positive control.

3 Experimental

3.1 General experimental procedures

Optical rotations were recorded on an Anton-Paar-MCP-300 polarimeter. ^1H, ^{13}C, and 2D NMR spectra were measured on a Bruker Avance-500 spectrometer; δ in ppm relative to Me_4Si as internal standard, J in Hz. ESI-MS was recorded on a Bruker Esquire 3000^{plus} spectrometer. HR-ESI-MS was recorded on a Waters-Q-TOF-micro-mass spectrometer. Column chromatography (CC) was carried out using silica gel (SiO_2, 100–200 mesh; Qingdao Haiyang Chemical Co., China), ODS (40–63 μm; YMC, Japan), and Sephadex LH-20 (GE Healthcare, Sweden). Thin layer chromatography was conducted using precoated SiO_2 GF_{254} plates (10–40 μm; Qingdao Haiyang Chemical Co., China). Semi-preparative HPLC was operated on a Varian ProStar 210 solvent delivery system equipped with a 335-PDA detector, using a YMC-Pack ODS-A column (250 × 10 mm, 5 μm), flow rate at 2.5 mL/min.

3.2 Fungal material

The strain of *Xylaria* sp. SOF11 was isolated from a marine sediment collected in the South China Sea (E114°14.709′, N20°44.239′). The fungus was identified by observing the morphological characteristics and analysis of the internal transcribed spacer (ITS) regions (GenBank accession number JF703668). A voucher strain of this fungus has been preserved at the RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences. The fungal strain was cultured on slants of potato dextrose agar at 25 °C for 10 days. Agar plugs were inoculated in 250-mL Erlenmeyer flask containing 50 mL of potato dextrose broth supplemented with 3% sea salt. Flask cultures were incubated at 28 °C on a rotary shaker at 200 rpm for two days as seed culture. Then 10 mL seed culture was inoculated to a 500-mL Erlenmeyer flask containing 100 g of rice, 100 mL of H_2O, and 3 g of sea salt. Totally, twenty 500 mL Erlenmeyer flasks were used. The Statistical incubation was carried out at 25 °C for 20 days.

3.3 Extraction and isolation

The fermented rice substrate was extracted repeatedly with ethanol (3 × 2 L), and the ethanol solvent was evaporated to dryness under vacuum to afford the crude extract. The crude extract was then suspended in water (1 L) and extracted repeatedly with butanone (3 × 1 L). The butanone-soluble fraction (24.0 g) was separated into six fractions (Fr.1 ~ Fr.6) on a silica gel CC using gradient elution of CH_2Cl_2–MeOH (from 100:0 to 0:100, V/V). Fr.2, eluted with CH_2Cl_2–MeOH (95:5), was separated into six subfractions (Fr.2A ~ Fr.2F) on a silica gel CC using gradient elution of petroleum ether – EtOAc (from 70:30 to 0:100, V/V). Frs. 2F was further separated into seven subfractions (Fr.2F1 ~ Fr.2F7) by silica gel CC eluting with $CHCl_3$–MeOH (100:0 to 95:5, V/V). Fr.2F5 was recrystallized with MeOH to yield compound **4** (63.0 mg). Fr. 2F6 was further purified by semipreparative HPLC to yield compounds **1** (5.6 mg), **2** (7.1 mg), **3** (8.3 mg), and **5** (7.5 mg).

Cytochalasin P_1 (**1**): a white powder. $[\alpha]_D^{25}$ – 10, (c = 0.2, MeOH). UV_{max} (MeOH): 210 nm. ^1H and ^{13}C NMR: see Table 1. ESI-MS (negative): 540.2 ($[M-H]^-$), ESI-MS (positive): 564.2 ($[M+Na]^+$). HR-ESI-MS (neg.): 540.2612 ($[M-H]^-$, calculated 540.2603, $C_{30}H_{39}NO_8$).

4 Cytotoxicity assay

The cell growth inhibitory activities of compounds **1–5** against the human tumor cell lines (SF-268, MCF-7, NCI-H460, and HepG-2) were determined using the SRB

method [11]. Briefly, cells (180 μL) with a density of 3×10^4 cells/mL of media were seeded onto 96-well plates and incubated for 24 h at 37 °C, 5% CO_2. Various concentrations of compounds (20 μL) were added to the plate wells, and plates were further incubated for 72 h. After incubation, cell monolayers were fixed with 50% (w/v) trichloroacetic acid (50 μL) and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by washing repeatedly with 1% acetic acid. The protein-bound dye was dissolved in 10 mM Tris base solution (200 μL) for OD determination at 570 nm using a microplate reader. Cisplatin was used as a positive control. All data were obtained in triplicate and are presented as means ± S.D. IC_{50} values were calculated with the Sigma Plot 10.0 software using a nonlinear curve-fitting method.

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