




## Secondary metabolites from Antarctic marine-derived fungus *Penicillium crustosum* HDN153086

Cong-Cong Liu, Zhen-Zhen Zhang, Yan-Yan Feng, Qian-Qun Gu, De-Hai Li & Tian-Jiao Zhu


To cite this article: Cong-Cong Liu, Zhen-Zhen Zhang, Yan-Yan Feng, Qian-Qun Gu, De-Hai Li & Tian-Jiao Zhu (2019) Secondary metabolites from Antarctic marine-derived fungus *Penicillium crustosum* HDN153086, Natural Product Research, 33:3, 414-419, DOI: 10.1080/14786419.2018.1455045



To link to this article: <https://doi.org/10.1080/14786419.2018.1455045>

 View supplementary material 

 Published online: 30 Mar 2018.

 Submit your article to this journal 

 Article views: 191

 View related articles 

 View Crossmark data 

 Citing articles: 2 View citing articles 



## Secondary metabolites from Antarctic marine-derived fungus *Penicillium crustosum* HDN153086

Cong-Cong Liu<sup>a</sup>, Zhen-Zhen Zhang<sup>a</sup>, Yan-Yan Feng<sup>a</sup>, Qian-Qun Gu<sup>a</sup>, De-Hai Li<sup>a,b</sup> and Tian-Jiao Zhu<sup>a,b</sup>

<sup>a</sup>Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao, P. R. China; <sup>b</sup>Laboratory for Marine Drugs and Bioproducts of Qingdao National Laboratory for Marine Science and Technology, Qingdao, P. R. China

### ABSTRACT

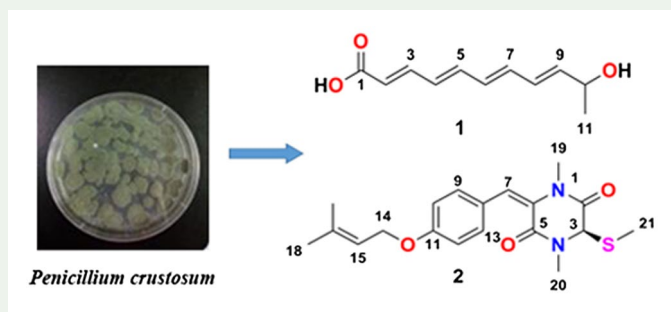
A new polyene compound (**1**) and a new diketopiperazine (**2**), as well as three known compounds (**3–5**), were isolated from the Antarctic marine-derived fungus *Penicillium crustosum* HDN153086. The structures of **1–5** were deduced based on MS, NMR and TD-DFT calculations of specific ECD spectra. These compounds were evaluated for their cytotoxic activities against K562 cell line and only compound **2** exhibited cytotoxicity against K562 cell, with IC<sub>50</sub> value of 12.7 μM.

### ARTICLE HISTORY

Received 19 January 2018  
Accepted 16 March 2018

### KEYWORDS


Secondary metabolites; Antarctic; marine-derived fungus; *Penicillium crustosum*; cytotoxicity



## 1. Introduction

Antarctic-derived microorganisms has become an important resource for investigation of structurally novel and biologically active compounds (Zoe and Margaret 2009; Ghiglione et al. 2012). In our on-going search for bioactive secondary metabolites from Antarctic-derived fungi (Li et al. 2012; Zhou et al. 2015, 2016), a *Penicillium crustosum* fungal strain HDN153086, isolated from a soil sample collected from Prydz Bay, was selected for its series of UV absorption peaks and cytotoxic activity (inhibitory rate was 27.69% against K562 cell

**CONTACT** Tian-Jiao Zhu  zhutj@ouc.edu.cn

 Supplemental data for this article can be accessed at <https://doi.org/10.1080/14786419.2018.1455045>.



Fusaperazine F (**2**) was obtained as a white powder. The molecular formula was determined as  $C_{19}H_{24}N_2O_3S$  on the basis of HRESIMS protonated peak at  $m/z$  361.1581  $[M + H]^+$  ion (Calcd for  $C_{19}H_{25}N_2O_3S$ , 361.1580), indicating nine degrees of unsaturation. The 1D NMR data (Table S2) of **2** suggested the presence of five methyls, one methylenes, seven methines, and six non-protonated carbons with two carbonyls ( $\delta_c$  163.1 and  $\delta_c$  164.6). The  $^1H$  and  $^{13}C$  NMR spectra of **2** are close to those of the known compound fusaperazine E (Guimarães et al. 2010). The major differences were the chemical shifts of C-5 ( $\delta_c$  163.1 vs.  $\delta_c$  159.2), C-19 ( $\delta_c$  35.1 vs.  $\delta_c$  32.5) and C-20 ( $\delta_c$  32.5 vs.  $\delta_c$  29.7), indicating the absolute configuration of C-3 or the geometry of double bond  $\Delta^{6,7}$  was different. The absolute configuration of C-3 was determined the same as fusaperazine E based on the similar optical rotation between **3** (+159.0) and fusaperazine E (+10.2) (Guimarães et al. 2010). In order to establish the geometry of double bond  $\Delta^{6,7}$ , NOEs were performed. The  $NOE_5$  correlation between H-7 and H-19 clearly suggests an *E* stereochemistry for  $\Delta^{6,7}$  which was different with fusaperazine E, resulting in the different chemical shifts of C-5, C-19 and C-20. Therefore, **2** was assigned as (*R*, *E*)-1, 4-dimethyl-3-(4-((3-methylbut-2-en-1-yl)oxy)benzylidene)-6-(methylthio)piperazine-2,5-dione (Figure S1).

Compound **3** was obtained as a white powder. The positive ESI-MS protonated peak at  $m/z$  183.08  $[M + H]^+$  and corresponding to the molecular composition of  $C_{10}H_{14}O_3$ . Further interpretation of 1D NMR demonstrated that the planar structure of **3** was same as the known compound xylariolide D (Hu et al. 2010). However, the absolute configuration of C-5 was not reported previously. To figure out the absolute configuration of the C-5 in **3**, the computational ECD calculations were attempted using a truncated model compound **3a**. A molecular mechanics conformational analysis of **3** was performed using Spartan software, followed by reoptimization using DFT at the B3LYP/6-31+G(d) level with Gaussian 09 software. According to the good agreement of the Boltzmann-weighted CD curve of the truncated model (5*S*)-**3a** with the experimental one (Figure S16), the absolute configuration of **3** was determined as 5*S*. Thus, the structure of **3** was established to be (5*S*)-5-(1-hydroxybutyl)-6-methyl-2H-pyran-2-one.

Compounds **1–5** were evaluated for their antitumor activities *in vitro* against the K562 cell line by the MTT method. Compound **2** showed cytotoxicity with the  $IC_{50}$  value of 12.7  $\mu M$ .

### 3. Experimental

#### 3.1. General experimental procedures

Optical rotations were obtained on a JASCO-1020 digital polarimeter. UV spectra were recorded on Waters 2487, while the ECD spectrum was measured on JASCO J-815 spectropolarimeter.  $^1H$  NMR,  $^{13}C$  NMR, DEPT and 2D NMR spectra were recorded on an Agilent 500 MHz DD2 spectrometer. HRESIMS and ESIMS data were obtained using a Thermo Scientific LTQ Orbitrap XL mass spectrometer. Column chromatography (CC) was performed with Sephadex LH-20 (Amersham Biosciences). MPLC was performed using a  $C_{18}$  column (Agela Technologies, YMC-Pack ODS-A, 3  $\times$  40 cm, 5  $\mu m$ , 20 mL/min). Preparative HPLC collection used a  $C_{18}$  column (Waters, YMC-Pack ODS-A, 10  $\times$  250 mm, 5  $\mu m$ , 3 mL/min). Chiral-phase HPLC used a Daicel Chiralpack IC column (4.6  $\times$  250 mm, 5  $\mu m$ , 1 mL/min).

### 3.2. Biological material

The fungal strain was isolated from the Antarctic sediment from Prydz Bay (E 77.57°, S 68.34°, collected in February, 2015) and identified as *Penicillium crustosum* based on sequence of ITS region (GenBank No. MG728115) with 100% similarity. The strain was deposited at the Key Laboratory of Marine Drugs, the Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, People's Republic of China.

### 3.3. Fermentation, extraction and isolation

Erlenmeyer flasks (500 mL) containing 150 mL 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 syrup (1 g),  $\text{KH}_2\text{PO}_4$  (0.5 g), and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.3 g) dissolved in 1 L of naturally collected seawater (Huiquan Bay, Yellow Sea). The flasks were cultured with shaking at 180 rpm and 28 °C for 9 days.

The whole fermentation broth (120 L) was filtered through cheese cloth to separate the supernatant from the mycelia. The supernatant was extracted with EtOAc ( $3 \times 120$  L) and the extract was evaporated under reduced pressure to give a crude gum (40 g). The crude extract was subjected to a silica gel (300–400 mesh) CC and was separated into six fractions (Fr.1–Fr.6) using a step gradient elution of  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ . The fraction Fr.1 (2.6 g), eluted with  $\text{CH}_2\text{Cl}_2$ , was fractionated on a Sephadex LH-20 CC using  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  (7:3) as the eluting solvent to afford three fractions, Fr.1.1–Fr.1.3 (243.5 mg, 980.0 mg and 867.3 mg, respectively). Fr.1.2 was separated MPLC (C-18 ODS) using a stepped gradient elution of MeOH/ $\text{H}_2\text{O}$  (20:80 to 100:0) to yield seven subfractions (Fr.1.2.1 to Fr.1.2.7), Fr.1.2.6 (386.7 mg) was then separated by semi-preparative HPLC eluted with MeOH/ $\text{H}_2\text{O}$  (70:30) to obtain compounds **2** (3.0 mg,  $t_R = 33.5$  min), **4** (60.0 mg,  $t_R = 25.0$  min), and **5** (110.0 mg,  $t_R = 29.0$  min). Fr.1.3 was separated by MPLC (C-18 ODS) using a stepped gradient elution of MeOH/ $\text{H}_2\text{O}$  (5:95 to 100:0) to yield seven subfractions (Fr.1.3.1 to Fr.1.3.7), Fr.1.3.4 (681.5 mg) was separated by semi-preparative HPLC eluted with MeOH/ $\text{H}_2\text{O}$  (46:54) to yield three subfractions (Fr.1.3.4.1 to Fr.1.3.4.3) and Fr.1.3.4.2 (119.3 mg) was then separated by semi-preparative HPLC  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (27:73) to obtain compound **3** (10.0 mg,  $t_R = 22.5$  min). The fraction Fr.2 (1.5 g) was separated on Sephadex LH-20 CC using  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  (7:3) as the eluting solvent to afford five fractions (Fr.2.1–Fr.2.5), Fr.2.3 (132.8 mg) was applied on Sephadex LH-20 CC using  $\text{CH}_3\text{OH}$  to provide three subfractions (Fr.2.3.1–Fr.2.3.3). Fr.2.3.2 (18.7 mg) was further purified by semi-preparative HPLC  $\text{CH}_3\text{OH}/\text{H}_2\text{O}$  (57:43) to obtain compound **1** (3.0 mg,  $t_R = 16.0$  min).

#### 3.3.1. Compound 1

Pale yellow amorphous powder;  $[\alpha]_D^{25} -7.5$  ( $c$  0.2, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log $\epsilon$ ): 229 (1.26), 334 (4.19);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz) and  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 125 MHz): see Table S1; HRESIMS  $m/z$  193.0874  $[\text{M} - \text{H}]^-$  (Calcd for  $\text{C}_{11}\text{H}_{13}\text{O}_3$ , 193.0870).

#### 3.3.2. Compound 2

White powder;  $[\alpha]_D^{25} + 159.0$  ( $c$  0.2, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log $\epsilon$ ): 225 (4.25), 316 (4.47);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz): see Table S2; HRESIMS  $m/z$  361.1581  $[\text{M} + \text{H}]^+$  (Calcd for  $\text{C}_{19}\text{H}_{25}\text{N}_2\text{O}_3\text{S}$ , 361.1580).

### 3.4. Cytotoxic assay

Inhibition of K562 cell proliferation of compounds **1–5** were measured using the MTT assay (Mosmann 1983). Ninety microliters of those cell suspensions were plated in 96-cell plates at a density of 390,000 cell mL<sup>-1</sup>. Then 1  $\mu$ L of the test compound solutions (in DMSO) at different concentrations was added to each well and further incubated for 72 h. Following drug exposure, the cells were fixed with 20  $\mu$ L MTT (5 mg/mL), and then add 100  $\mu$ L triplex solution to the cell layer after 4 h. The absorbance of MTT solution was measured at 570 nm. Dose-response curves were generated, and the IC<sub>50</sub> values, the concentration of compound required to inhibit cell proliferation by 50%, were calculated from the linear portion of log-dose-response curve.

### 3.5. Computation section

Conformational searches were run by employing the 'systematic' procedure implemented in Spartan'14 using MMFF (Merck molecular force field). All MMFF minima were reoptimized with DFT calculations at the B3LYP/6-31+G(d) level using the Gaussian09 program (Frisch et al. 2010). 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 two lowest-energy conformations for (5S)-**3a** (>5% population) using 30 excited states, and using a polarizable continuum model (PCM) for MeOH. ECD spectra were generated using the program SpecDis (Bruhn et al. 2011) by applying a Gaussian band shape with 0.4 eV width for **3**, from dipolelength 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 100% of the weights. The calculated spectra were shifted by 5 nm to facilitate comparison to the experimental data.

## 4. Conclusion

Two new compounds (**1** and **2**), together with three known related compounds (**3–5**) were isolated from the Antarctic derived fungus *Penicillium crustosum* HDN153086, which including one polyene compound (**1**), three diketopiperazines (**2**, **4** and **5**), and xylariolide D (**3**). Compound **1** showed conjugated tetraene which was unusual in natural products (Komori and Itoh 1985; Fleury et al. 2009). Compound **2** showed cytotoxicity against K562 cells, while fusaperazine E (Guimarães et al. 2010), which was an isomer differed in the geometry of double bond  $\Delta^{6,7}$ , was inactive. The compounds **4** and **5** were discovered from various specific environment, such as terrestrial plant (Yang et al. 2017), inland soil (Rahbæk et al. 1998; Wang et al. 2015; Li et al. 2018) and marine organism (Capon et al. 2007; Wang et al. 2012) of Pacific coastal area. As far as we known, compounds **4** and **5** were isolated from Antarctic derived fungus for the first time.

### Supplementary material

Supplementary material relating to this article is available online, including HRESIMS and NMR spectra for compounds **1** and **2**, ECD spectra of compound **3**.

### Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

The work was financially supported by the National Natural Science Foundation of China [grant number 41376147] and NSFC-Shandong Joint Fund for Marine Science Research Centers [grant number U1606403], Fund of Key Laboratory of Marine bioactive substances and Modern Analytical Technology, State Oceanic Administration [grant number MBSMAT-2016-01], the Scientific and Technological Innovation Project Financially Supported by Qingdao National Laboratory for Marine Science and Technology [grant number 2015ASKJ02], Shandong province key research and development program [grant number 2016GSF201204].

## References

- Bruhn T, Hemberger Y, Schaumlöffel A, Bringmann GS. 2011. version 153. Würzburg, Germany: University of Würzburg.
- Capon RJ, Stewart M, Ratnayake R, Lacey E, Gill JH. 2007. Citromycetins and bilains A–C: new aromatic polyketides and diketopiperazines from Australian marine-derived and terrestrial *Penicillium* spp. *J Nat Prod.* 70:1746–1752.
- Fleury E, Lannou MI, Bistri O, Sautel F, Massiot G, Pancrazi A, Ardisson J. 2009. Relative stereochemical determination and synthesis of the C1–C17 fragment of a new natural polyketide. *J Org Chem.* 74:7034–7045.
- Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, et al. 2010. Gaussian 09, Revision B01. Wallingford (CT): Gaussian, Inc.
- Ghiglione JF, Galand PE, Pommier T, Pedrós-Alió C, Maas EW, Bakker K, Bertilson S, Kirchman DL, Lovejoy C, Yager PL, Murray AE. 2012. Pole-to-pole biogeography of surface and deep marine bacterial communities. *Proc Natl Acad Sci.* 109:17633–17638.
- Guimarães DO, Borges WS, Vieira NJ, de Oliveira LF, da Silva CH, Lopes NP, Dias LG, Durán-Patrón R, Collado IG, Pupo MT. 2010. Diketopiperazines produced by endophytic fungi found in association with two Asteraceae species. *Phytochemistry.* 71:1423–1429.
- Hu ZY, Li YY, Lu CH, Lin T, Hu P, Shen YM. 2010. Seven novel linear polyketides from *Xylaria* sp. NCY2. *Helv Chim Acta.* 93:925–933.
- Komori T, Itoh Y. 1985. Chaetiacandin, a novel papulacandin. II Structure determination *J Antibiot.* 38:544–546.
- Li LY, Li DH, Luan YP, Gu QQ, Zhu TJ. 2012. Cytotoxic metabolites from the antarctic psychrophilic fungus *Oidiodendron truncatum*. *J Nat Prod.* 75:920–927.
- Li F, Guo WQ, Wu L, Zhu TJ, Gu QQ, Li DH, Che Q. 2018. Saroclazines A–C, thio-diketopiperazines from mangrove-derived fungi *Sarocladium kiliense* HDN11-84. *Arch Pharm Res.* 41:30–34.
- Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 65:55–63.
- Rahbæk L, Sperry S, Piper JE, Crews P. 1998. Deoxynortrichoharzin, a new polyketide from the saltwater culture of a sponge-derived paecilomyces fungus. *J Nat Prod.* 61:1571–1573.
- Wang JF, Liu PP, Wang Y, Wang H, Li J, Zhuang YB, Zhu WM. 2012. Antimicrobial aromatic polyketides from gorgonian-associated fungus, *Penicillium commune* 518. *Chin J Chem.* 30:1236–1242.
- Wang HF, Chen G, Liu YP, Hua HM, Bai J, Pei YH. 2015. 1H and 13C NMR assignments of two new piperazine-trione from the fungus *Penicillium crustosum* YN-HT-15. *Magn Reson Chem.* 53:620–623.
- Yang MH, Li TX, Wang Y, Liu RH, Luo J, Kong LY. 2017. Antimicrobial metabolites from the plant endophytic fungus *Penicillium* sp. *Fitoterapia.* 116:72–76.
- Zhou HB, Li LY, Wang W, Che Q, Li DH, Gu QQ, Zhu TJ. 2015. Chrodrimanins I and J from the antarctic moss-derived fungus *Penicillium funiculosum* GWT2-24. *J Nat Prod.* 78:1442–1445.
- Zhou HB, Li LY, Wu CM, Kurtán T, Mándi A, Liu YK, Gu QQ, Zhu TJ, Guo P, Li DH. 2016. Penipyridones A–F, pyridone alkaloids from *Penicillium funiculosum*. *J Nat Prod.* 79:1783–1790.
- Zoe EW, Margaret AB. 2009. Molecules derived from the extremes of life. *Nat Prod Rep.* 26:44–71.