Unusual Pyrrolyl 4-Quinolinone Alkaloids from the Marine-Derived Fungus *Penicillium* sp. ghq208

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One new pyrrolyl 4-quinolinone alkaloid, penicinoline E (1), together with three known derivatives, methyl-penicinoline (2), penicinoline (3), and quinolactacide (4), were isolated from the marine-derived fungus *Penicillium* sp. ghq208. The structures of these isolated compounds were elucidated by spectroscopic methods. Compounds (2, 3) exhibited moderate cytotoxicities against the HepG2 cell line with IC_{50} values of 11.3 and 13.2 μ M, respectively.

Key words *Penicillium*; marine-derived fungus; alkaloid; penicinoline

Alkaloids are a large group of secondary metabolites which produced by many organisms, which contain almost 40% metabolites of microorganisms.¹⁾ These N-containing metabolites usually biosynthesis by a convergence of multiple biosynthetic pathways from many kinds of amino acids,²⁾ and they have the novelty and diversity of structures. In particular, they have a variety of biological activities such as antitumor, antipredator, antiinflammatory, and antiviral *etc.* Therefore, investigation of structurally novel and biologically active alkaloids from marine-derived microorganisms has been an important research area in new drug discovery. As part of our programs to search for bioactive secondary metabolites from marine-derived microorganisms,^{3–5)} a fungal strain ghq208, identified as *Penicillium* sp., isolated from the sediment collected in Ji-aoshou Bay of China, was found to produce alkaloids by TLC visualizing with Dragendorff's reagent in a saline culture. The EtOAc extract showed significant cytotoxicity on P388 cell line. Chemical investigation on the extract led to the isolation of one new and unusual pyrrolyl 4-quinolinone alkaloid, named penicinoline E (1), along with three known derivatives, methyl-penicinoline (2),⁶⁾ penicinoline (3),⁷⁾ and quinolactacide (4)⁸⁾ (Fig. 1). In this paper, we describe the isolation, structural elucidation, and biological activities of these N-containing compounds.

Results and Discussion

Compound 1 was separated as a pale yellow powder, and its molecular formula was determined as $C_{13}H_{10}N_2O$ on the basis of its high resolution-electrospray ionization-mass spectra (HR-ESI-MS) peak at m/z 211.0863 $[M+H]^+$ (Calcd for $C_{13}H_{11}N_2O$: 211.0866), indicating 10 degrees of unsaturation.

1H - and ^{13}C -NMR data of 1 showed eight aromatic methines, one conjugated carbonyl group and four quaternary carbons (Table 1), which were very similar to those of penicinoline (3),⁷⁾ except for the difference of the absence of carboxyl group at C-3. In the 1H -NMR spectrum, the chemical shifts and the coupling constants of H-5 (δ_H 8.04, dd, $J=7.7, 1.1$ Hz), H-6 (δ_H 7.27, ddd, $J=7.7, 7.7, 1.1$ Hz), H-7 (δ_H 7.61, ddd, $J=7.7, 7.7, 1.1$ Hz), and H-8 (δ_H 7.72, dd, $J=7.7, 1.1$ Hz) indicated the presence of an *ortho*-disubstituted benzene system, which also supported by the 1H - 1H correlation spectroscopy (COSY) correlations (Fig. 2). The quinolinone moiety was deduced by heteronuclear multiple bond connectivity (HMBC) correlations from H-3 to C-2 and C-4, and from H-5 to C-4. The pyrrole ring was determined by comparison of the chemical shifts (δ_H 11.33, δ_C 125.3, $\delta_{C/H}$ 123.5/7.08, 111.0/7.05 and 110.4/6.27) with those of methyl-penicinoline (2)⁶⁾ and penicinoline (3),⁷⁾ and further confirmed by the 1H - 1H COSY correlations (H-2'/H-3'/H-4') and the HMBC correlations from H-2', H-3' and H-4' to C-5'. The pyrrol ring linked to the quinolinone nucleus was determined by connecting C-2 to C-5' by the HMBC correlations from H-3 to C-5', and from H-3' to C-2, and named as penicinoline E.

The cytotoxicities of all four compounds (1–4) were evaluated *in vitro* against the HepG2, P388 and HL-60 cell lines using the sulforhodanine B (SRB)⁹⁾ or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT)¹⁰⁾ methods. Only compounds 2 and 3 exhibited moderate cytotoxicities against the HepG2 cell line with the IC_{50} values of 11.3 and 13.2 μ M, respectively.

Penicinolines is a kind of unusual pyrrolyl 4-quinolinone alkaloids biosynthesised from ornithine and tryptophan,⁷⁾

Fig. 1. Compounds 1–4 Isolated from *Penicillium* sp. ghq208

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Fig. 2. Selected ^1H - ^1H COSY and HMBC Correlations of Compound **1**

which is rare in nature. As far as our knowledge, totally three natural-occurring examples were isolated from several fungi strains such as *Auxarthron reticulatum*,⁶⁾ *Penicillium* sp.,⁷⁾ *Penicillium* sp.,⁸⁾ and one synthetical example.⁷⁾ There has been much recent interest arising from its biological activities, penicnoline (**3**) showed potent *in vitro* cytotoxicity toward 95-D and HepG2 cell lines with IC_{50} values of 0.57 and 6.5 $\mu\text{g}/\text{mL}$, respectively, and strong insecticidal activity against *A. gossypii*,⁷⁾ quinolactide (**4**) showed excellent insecticidal activity against *Myzus persicae*,⁸⁾ and the synthetical example penicnotam showed strong insecticidal activity against *A. gossypii* and *P. xylostella*.⁷⁾ These four penicnolines (**1**–**4**) were evaluated *in vitro* against three tumor cell lines, interestingly, we found that the substitution of carboxyl group at C-3 is an essential for the cytotoxicities of this kind unusual pyrrol-4-quinolinone alkaloids. This result indicated that the carboxyl group at C-3 in penicnolines showed be retained in further synthetical investigation.

Experimental

General Experimental Procedures IR spectra were taken on a NICOLET NEXUS 470 spectrophotometer (Beckman Coulter Inc., Brea, CA, U.S.A.) in KBr disks. The melting point were obtained on a Tianfen Melting Point RY-1 (Tianjin Tianfen Corporation, Tianjin, China). ^1H -, ^{13}C -NMR and distortionless enhancement by polarization transfer (DEPT) spectra and 2D-NMR were recorded on a JEOL JNM-ECP 600 spectrometer (JEOL Ltd., Tokyo, Japan) using tetramethylsilane (TMS) as internal standard and chemical shifts were recorded as δ values. ESI-MS was measured on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer (Waters

Corporation, Milford, MA, U.S.A.). Semi-preparative HPLC was performed using an ODS column (YMC-pak ODS-A (YMC Co., Ltd., Kyoto, Japan), 10 $\times$ 250 mm, 5 μm , 4 mL/min).

Fungal Material The fungal strain *Penicillium* sp. ghq208 was isolated from the sediments collected in Jiaozhou Bay of China. The voucher specimen is deposited in our laboratory at -20°C . Working stocks were prepared on potato dextrose agar slants stored at 4°C .

Fermentation, Extraction and Isolation Spores were directly inoculated into 1000 mL Erlenmeyer flasks containing 300 mL fermentation media (mannitol 20 g, maltose 20 g, glucose 10 g, monosodium glutamate 10 g, KH_2PO_4 0.5 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.3 g, yeast extract 3 g, and corn steep liquor 1 g, dissolved in 1 L sea water, pH 6.5). The fungus was grown under static conditions at 28°C . After 30 d of cultivation, 40 L of whole broth was filtered through cheesecloth to separate the broth supernatant, which was extracted with ethyl acetate, and concentrated under reduced pressure to give a crude extract (6.3 g).

The crude extract (6.3 g) was fractionated on a C-18 ODS column using a step gradient elution of MeOH - H_2O and was separated into 4 subfractions (Fr. 1–Fr. 4). Fraction 2 was further purified by semipreparative HPLC (50:50 MeOH - H_2O , 4 mL/min) to give compounds **1** (13 mg, t_R =9.8 min), and **2** (15 mg, t_R =12.0 min). Compounds **3** (19.3 mg, t_R =7.8 min), and **4** (23.5 mg, t_R =8.7 min) were obtained from Fr. 3, which was also purified by semi-preparative HPLC (60:40 MeOH - H_2O -TFA, 4 mL/min).

Penicnoline E (**1**): Pale yellow powder, mp $^\circ\text{C}$: 358–360, UV (HPLC, mobile phase) λ_{max} nm: 207, 271, 353, IR (KBr) cm^{-1} : 3412, 1659, 1564, 1465, 1297, 1144, 828, ^1H -NMR ($\text{DMSO}-d_6$, 600 MHz) and ^{13}C -NMR ($\text{DMSO}-d_6$, 150 MHz), see Table 1, HR-ESI-MS m/z : 211.0863 [$\text{M}+\text{H}$] $^+$ (Calcd for $\text{C}_{13}\text{H}_{11}\text{N}_2\text{O}$: 211.0866).

In Vitro Cytotoxicity Assays In the MTT assay, cell lines were grown in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) under a humidified atmosphere of 5% CO_2 and 95% air at 37°C . Cell suspensions, 200 μL , at a density of 5×10^4 cell/mL were plated in 96 well microtiter plates and incubated for 24 h. Then, 2 μL of the test solutions (in MeOH) were added to each well and further incubated for 72 h. Then,

Table 1. ^1H - and ^{13}C -NMR Data (δ in ppm, J in Hz) of **1** in $\text{DMSO}-d_6$

No.	$^{13}\text{C}^b$	$^1\text{H}^a$	COSY	HMBC
1		11.81 (s)		
2	143.3			
3	103.1	6.51 (s)		2, 4, 2'
4	177.4			
5	125.1	8.04 (dd, 7.7, 1.1)	6	4, 9
6	123.3	7.27 (ddd, 7.7, 7.7, 1.1)	5, 7	10
7	132.1	7.61 (ddd, 7.7, 7.7, 1.1)	6, 8	9
8	118.8	7.72 (dd, 7.7, 1.1)	7	10
9	141.2			
10	125.4			
1'		11.33 (brs)	5'	
2'	125.3			
3'	123.5	7.08 (m)	4'	2, 2'
4'	111.0	7.05 (m)	3', 5'	2'
5'	110.4	6.27 (m)	1', 4'	2'

a) Spectra measured at 400 MHz at 25°C . b) Spectra measured at 100 MHz at 25°C .

20 μ L of the MTT solution (5 mg/mL in IPMI-1640 medium) was added to each well and incubated for 4 h. Old medium containing MTT (150 μ L) was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a SPECTRA MAX PLUS plate reader (Molecular Devices, Sunnyvale, CA, U.S.A.) at 540 nm.

In the SRB assay, 200 μ L of the cell suspensions were plated in 96 cell plates at a density of 2×10^5 cell/mL. Then 2 μ L of the test solutions (in MeOH) was added to each well, and the culture was further incubated for 24 h. The cells were fixed with 12% trichloroacetic acid, and the cell layer was strained with 0.4% SRB. The absorbance of SRB solution was measured at 515 nm. Dose response curves were generated, and the IC_{50} values, the concentration of compound required to inhibit cell proliferation by 50%, were calculated from the linear portion of log dose response curves.

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References

- 1) Blunt J. W., Copp B. R., Keyzers R. A., Munro M. H. G., Prinsep M. R., *Nat. Prod. Rep.*, **29**, 144–222 (2012).
- 2) Khadem S., Marles R. J., *Molecules*, **17**, 191–206 (2012).
- 3) Du L., Ai J., Li D. H., Zhu T. J., Wang Y., Knauer M., Bruhn T., Liu H. B., Geng M. Y., Gu Q. Q., Bringmann G., *Chemistry*, **17**, 1319–1326 (2011).
- 4) Cai S. X., Sun S. W., Zhou H. N., Kong X. L., Zhu T., Li D. H., Gu Q. Q., *J. Nat. Prod.*, **74**, 1106–1110 (2011).
- 5) Du L., Liu H. C., Fu W., Li D. H., Pan Q. M., Zhu T. J., Geng M. Y., Gu Q. Q., *J. Med. Chem.*, **54**, 5796–5810 (2011).
- 6) Elsebai M. F., Rempel V., Schnakenburg G., Kehraus S., Muller C. E., Konig G. M., *ACS Med. Chem. Lett.*, **2**, 866–869 (2011).
- 7) Shao C. L., Wang C. Y., Gu Y. C., Wei M. Y., Pan J. H., Deng D. S., She Z. G., Lin Y. C., *Bioorg. Med. Chem. Lett.*, **20**, 3284–3286 (2010).
- 8) Abe M., Imai T., Ishii N., Usui M., Okuda T., Oki T., *Biosci. Biotechnol. Biochem.*, **69**, 1202–1205 (2005).
- 9) Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., Vistica D., Warren J. T., Bokesch H., Kenney S., Boyd M. R., *J. Natl. Cancer Inst.*, **82**, 1107–1112 (1990).
- 10) Mosmann T. J., *J. Immunol. Methods*, **65**, 55–63 (1983).