Three new 2,5-diketopiperazines from the fish intestinal Streptomyces sp. MNU FJ-36

Yi-xin Oua,b, Jia-fu Huanga,b, Xiu-min Lia,b, Qian-jin Kangc,d and Yu-tian Pana,b

aEngineering Technological Center of Mushroom Industry, Minnan Normal University, Zhangzhou, P.R. China; bCollege of Life Sciences and Technology, Minnan Normal University, Zhangzhou, P.R. China; cState Key Laboratory of Microbial Metabolism, and School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, Shanghai, P.R. China; dJoint International Research Laboratory of Metabolic & Developmental Sciences, Shanghai Jiao Tong University, Shanghai, P.R. China

1. Introduction

Marine microorganisms are an important resource for the discovery of the various bioactive natural compounds (Haefner 2003; Zhang et al. 2005; Marris 2006; Gerwick & Moore 2012; Sun et al. 2014). Remarkably, the mutualistic symbiosis relationship between fish and its intestinal microorganisms (and plant and its endophytes) has been established for a long time.
evolutionary time (Yan et al. 2012; Zhou, Yang, Peng, et al. 2013; Zhou, Yang, Yang, et al. 2013; Zhang et al. 2014). The microorganisms of animal guts are known to play a significant role in regulating the physiology, nutrition and immune system of their hosts. The gut contains $10^7$–$10^{11}$ bacteria per gram in the content, including proteobacteria, firmicutes, actinobacteria and so on (Sanchez et al. 2012). It is noteworthy that the intestinal fabric from the ocean-originated fishes is important but remains an unexplored resource for discovery of actinomycetes producing new metabolites. Therefore, chemical investigations on the intestinal microbes of the ocean-originated fish might afford novel chemical structures and bioactive lead compounds for drug discovery.

2,5-Diketopiperazines (2,5-DKPs) results from the condensation of two α-amino acids forming a cyclodipeptide skeleton, on which various modifications afford their diversified chemicals and bioactivities (Nakao et al. 2014). They are widely distributed in bacteria, fungi, plants and mammals (Belin et al. 2012; Borthwick 2012; Giessen et al. 2013; Hayashi et al. 2013; Giessen & Marahiel 2015; Sano & Nakao 2015). The actinobacteria-derived natural products are well known for their pharmacological potentials and versatile molecular architectures (Ai et al. 2014; Pu et al. 2012; Shaaban et al. 2013; Yang et al. 2014). In order to obtain new natural compounds with interesting biological activities, our chemical investigation of *Streptomyces* sp. MNU FJ-36 isolated from *Katsuwonus* sp. intestine resulted in the discovery of three new 2,5-DKPs compounds (Figure 1).

**2. Results and discussion**

The cultures of *S*. sp. MNU FJ-36 were carried out on 8 L ISP2 solid medium at 30 °C for 7 days, and the cultures were extracted at room temperature. The crude extract was purified by successive column chromatography on silica gel and *Sephadex* LH-20 to afford compounds 1–3. Their structures were assigned by spectroscopic approaches.

Compound 1 was obtained as a white amorphous powder. Its molecular formula of C$_{16}$H$_{22}$N$_2$O$_4$ was established on the positive high-resolution electrospray ionization mass spectroscopy (HR-ESI-MS) ($m/z$: 329.1468, [M + Na]$^+$, Calcd for 329.1477). $^1$H and $^{13}$C NMR data displayed the presence of an ABX benzene ring system ($\delta_\text{H}$ 7.06 (H-13, d, $J = 7.1$ Hz, 1H), $\delta_\text{H}$ 7.15 (H-12, d, $J = 6.9$ Hz, 1H) and $\delta_\text{H}$ 7.08 (H-9, d, $J = 1.5$ Hz, 1H)). In the HMBC spectrum of 1, the correlations of H-3 ($\delta_\text{H}$ 4.91, dd, $J = 7.1$ and 3.9 Hz, 1H) with C-2 ($\delta_\text{C}$ 169.5), C-5 ($\delta_\text{C}$ 170.9), C-7 ($\delta_\text{C}$ 40.6) and C-8 ($\delta_\text{C}$ 133.1), H-9 with C-7, C-8, C-10 ($\delta_\text{C}$ 148.9), C-11 ($\delta_\text{C}$ 149.9) and C-13 ($\delta_\text{C}$ 126.4), suggested that an aromatic amino acid with 1,3,4-trisubstituted benzene
moiety was involved in the structure of 1. The extensive analysis of the HMBC spectra of 1 indicated that H-6 (δ_H 4.49, m, 1H) was correlated with C-2, C-5, C-14 (δ_C 41.6) and C-15 (δ_C 25.2), H-15 (δ_H 1.31, m, 1H) was in correlation to C-6 (δ_C 51.9), C-14, C-16 (δ_C 22.2) and C-17 (δ_C 21.5) (Table S1). These results showed that a leucine residue was also incorporated in the chemical structure of 1. The OCH_3-18 (δ_H 3.91, s, 3H) was substituted at C-11 by the corrections of the H-atom 3.91 with C-11 in the HMBC spectrum. In addition, the both of H-3 and H-6 were correlated with C-2 and C-5 in the HMBC spectrum indicated that the leucine and the aromatic acid was connected to a 2,5-piperazinedione skeleton (Figure 2) (Blaha & Fric 1970; Du et al. 1992). The relative configuration of 1 was established by the ROESY spectrum analysis. Crucial correlations of H-3 with H-6 and H-7a (δ_H 2.83, dd, J = 10.9 and 5.1 Hz, 1H) with Ha-14 (δ_H 1.49, m, 1H) displayed that the H-3 and H-6 were assigned as the α-orientation (Table S1, Figure 2). Finally, the structure of 1 was determined to be 3-(3-hydroxy-4-methoxybenzyl)-6-isobutyl-2,5-diketopiperazine.

Compound 2 was isolated as an amorphous powder. Its molecular formula was C_{16}H_{20}N_{2}O_{4} with eight degree of unsaturation, which was validated by HR-eSI-MS (m/z: 327.1312 [M + Na]^+, Calcd for 327.1321) and in combination with NMR spectra analysis. The 1H and 13C NMR spectra of 2 were very similar to those of 1, except for a methylene group H-18 (δ_H 5.08, s, 2H, δ_C 56.9) carrying two oxygen atoms in 2 instead of the OCH_3-18 in 1 (Table S2). In the HMBC spectrum, the cross signals between the methylene protons at H-18 with C-10 (δ_C 150.1) and C-11 (δ_C 149.4) indicated that the methylene group was located between the C-10 and C-11 via O-atoms (Figure 2). The extensive 1-D and 2-D NMR analysis indicated that the leucine and aromatic acid residues in 2 were identical to 1. In the ROESY spectrum, correlations of H-3 (δ_H 4.93, dd, J = 7.2 and 3.8 Hz, 1H) with H-6 (δ_H 4.43, m, 1H), H-7a (δ_H 2.89, dd, J = 10.6 and 5.4 Hz, 1H) with Ha-14 (δ_H 1.46, m, 1H) revealed that 2 had the same α-orientation configuration to 1 (Table S2, Figure 2). Therefore, the structure of 2 was elucidated as 3-(1,3-benzodioxol-5-ylmethyl)-6-isobutyl-2,5-diketopiperazine.

Compound 3 was obtained as an amorphous powder. The HR-ESI-MS (m/z: 313.1157 [M + Na]^+, Calcd for 313.1164) of 3 indicated the molecular formula C_{15}H_{18}N_{2}O_{4}, which was further supported by its 1H, 13C and DEPT NMR spectra. Comparison of the NMR data of 3 with those of 2, suggested 3 had a very similar structure to 2. The HMBC correlations from H-14 (δ_H 2.35, m, 1H) to C-6 (δ_C 59.2), C-15 (δ_C 20.4) and C-16 (δ_C 19.6), together with the COSY correlations of H-14 with H-6 (δ_H 4.92, d, J = 7.8 Hz, 1H), H-7a (δ_H 2.89, dd, J = 10.6 and 5.4 Hz, 1H) with Ha-14 (δ_H 1.46, m, 1H) revealed that 2 had the same α-orientation configuration to 1 (Table S2, Figure 2). Therefore, the structure of 2 was elucidated as 3-(1,3-benzodioxol-5-ylmethyl)-6-isobutyl-2,5-diketopiperazine.

Figure 2. Key HMBC, 1H–1H COSY and ROESY correlations for 1, 2 and 3.
On the basis of the above evidence, compound 3 was defined as 3-(1,3-benzodioxol-5-ylmethyl)-6-isopropyl-2,5-diketopiperazine. All three new compounds were assayed for their cytotoxic effects on the P388, A-549 and HCT-116 cell lines by the MTT method with doxorubicin as the positive control. The three compounds displayed weak cytotoxicity against A-549 cell line, and compounds 2 and 3 also showed weak cytotoxicity against HCT-116 cell line (Table 1).

### Table 1. Cytotoxicity analysis of compounds 1–3 in three cancer cell lines.

| Cell lines | Cytotoxicity (IC50, μg/mL) | Doxorubicin | 1 | 2 | 3 |
|------------|-----------------------------|-------------|---|---|---|
| P388       | 0.011 ± 0.002               | >100        | >100 | >100 |
| A-549      | 0.255 ± 0.050               | 89.4 ± 5    | 35.4 ± 7 | 28.4 ± 5 |
| HCT-116    | 0.025 ± 0.005               | >100        | 75.4 ± 4 | 45.4 ± 6 |

ROESY spectrum (Table S3, Figure 2). On the basis of the above evidence, compound 3 was defined as 3-(1,3-benzodioxol-5-ylmethyl)-6-isopropyl-2,5-diketopiperazine.

### 3. Conclusions

The chemical investigation of cultures of the S. sp. MNU FJ-36 resulted in the isolation of three new 2,5-DKPs 1–3. All of the compounds displayed weak cytotoxicity against A-549 cell line, and compounds 2 and 3 also exhibited weak inhibitory activity against HCT-116 cell line.

### Supplementary material

Supplementary material relating to this article is available online, alongside the experimental part, Table S1–S3.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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