SUPPLEMENTARY MATERIAL

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

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Abstract

The gut actiobacteria of marine-inhabited fish is one of the most important reservoirs of novel natural products. Currently, the *Streptomyces* sp. MNU FJ-36 was isolated from the intestinal fabric of *Katsuwonus* sp. and determined by 16S rRNA analysis. From the cultures of the *S.* sp. MNU FJ-36, three new 2,5-diketopiperazines (2,5-DKPs) were discovered and identified as 3-(3-hydroxy-4-methoxybenzyl)-6-isobutyl-2,5-diketopiperazine (**1**), 3-(1,3-benzodioxol-5-ylmethyl)-6-isobutyl-2,5-diketopiperazine (**2**), and 3-(1,3-benzodioxol-5-ylmethyl)-6-isopropyl-2,5-diketopiperazine (**3**). Their structures were elucidated on the basis of spectroscopic data analysis. All compounds were also evaluated for their inhibitory activity against P388, A-549 and HCT-116 cell lines with the MTT assay.

Key words: Intestinal *Streptomyces*, diketopiperazine, cyclodipeptide, cytotoxicity

EXPERIMENTAL

General. TLC: Si gel G plates (Qingdao Marine Chemical Factory, Qingdao, P. R. China), visualization by spraying with 10% H₂SO₄ soln. followed by heating. Column chromatography (CC): silica gel (200-300 mesh; Qingdao Marine Chemical Factory); reversed phase C18 silica gel (40-63 μ m, Merk, Darmstadt, Germany); and Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd.). UV Spectra: $\lambda_{\max}$ (log ϵ) in nm. IR spectra: Bio-Rad FTS-135 spectrophotometer, in cm⁻¹. 1D- and 2D-NMR spectra: Bruker DRX-500 instrument at 500/125 MHz, resp.; δ in ppm, J in Hz. Mass spectra were recorded on VG Auto-Spec-3000 and Finnigan LCQ-Advantage mass spectrometers, resp.; in m/z (rel.%). Optical rotations: Jasco DIP-370 digital polarimeter.

Microbial Material. The fish was provided by Juhua Company, Fujian, P. R. China and stored at -20 °C. The dissection was performed in the sterilized environment, and the intestine was separated from the fish whose surface had been sterilized with 70% EtOH. Finally, the content of the intestine was transferred to a 50 mL Falcon tube. It was diluted with sterilized 0.8% saline in various concentrations and vortexed for 5 min. The suspension was applied in 2 media including ISP2 (yeast extract 0.4%, glucose 0.4%, malt extract 1.0%, agar 2.0%, sea water 1 L, pH 7.2) and ISP4 (DifcoTM) medium for bacterial isolation.

The strain was isolated from the intestinal fabric of *Katsuwonus* sp. using ISP4 medium, and identified as *Streptomyces* species by 16 S rRNA analysis (Li et al. 2015; Li et al. 2015). It was deposited at the Minnan Normal University, Fujian, P. R. China. *S.* sp. MNU-36 was cultivated on 8 L scale using solid ISP2 medium at 30°C for 7 days.

Compounds Extraction and Isolation. The 8 L solid culture of *S.* sp. was chopped up,

diced, and extracted 3 times with the solvent mixture EtOAc/MeOH/AcOH (80 : 15 :5) at room temperature. The crude extract (5.2 g) was filtered and subjected to MPLC on C18 reversed-phase silica gel (145 g), eluting with gradient H₂O/MeOH (100 : 0 → 0 : 100), to produce six fractions (Fraction A1-Fraction A6). Fraction A4 was applied to a Sephadex LH-20 using MeOH as elution followed by purification over a silica gel column (200-300 mesh, ø 1 × 40 cm) using CHCl₃-Me₂CO (100 : 6) as eluent to afford compound **1** (6 mg). Fraction A5 was further purified by a Sephadex LH-20 (Me₂CO), and then purified by column chromatography (silica gel, 200-300 mesh, ø 1.5 × 50 cm, petroleum ether-Me₂CO, 9 : 1) to yield compound **2** (15 mg) and **3** (5 mg).

Compound (**1**): white amorphous powder. Molecular formula : C₁₆H₂₂N₂O₄. ESI-MS: 307.3 (100, [M + H]⁺). $[\alpha]_D^{20} = -65.2^\circ$ (*c* = 0.62, MeOH). UV nm⁻¹: $\lambda_{\max}^{\text{MeOH}}(\log \epsilon)$: 209 (4.16). IR (KBr) cm⁻¹: 3410 (OH), 3218 (NH), 1712 (C=O), 1602 (arom.). ¹H-, ¹³C-NMR: Table 1.

Compound (**2**): an amorphous powder. Molecular formula: C₁₆H₂₀N₂O₄. ESI-MS: 327.2 (100, [M + Na]⁺), 305.4 (50, [M + H]⁺). $[\alpha]_D^{20} = -38.1^\circ$ (*c* = 0.32, MeOH). UV nm⁻¹: $\lambda_{\max}^{\text{MeOH}}(\log \epsilon)$: 212 (2.36). IR (KBr) cm⁻¹: 3233 (NH), 3213 (NH), 1698 (C=O), 1597 (arom.). ¹H-, ¹³C-NMR: Table 1.

Compound **3**: an amorphous powder. Molecular formula: C₁₅H₁₈N₂O₄. ESI-MS 313.1 (100, [M + Na]⁺). $[\alpha]_D^{20} = -42.1^\circ$ (*c* = 0.46, MeOH). UV nm⁻¹: $\lambda_{\max}^{\text{MeOH}}(\log \epsilon)$: 218 (3.03). IR (KBr) cm⁻¹: 3220 (NH), 3208 (NH), 1700 (C=O), 1605 (arom.). ¹H-, ¹³C-NMR: Table 1.

Biological Assays. The cytotoxic activity for the P388, A-549 and HCT-116 cell lines was evaluated by the MTT assay (Mosmann 1983).

Table S1. 1D- and 2D-NMR spectroscopic data of compounds **1**. At 500 and 125 MHz, resp., CDCl₃, δ in ppm, *J* in Hz, Weak (w).

No.	¹ H	¹³ C	HMBC	COSY	NOESY
1	/	/	/	/	/
2	/	169.5 qC	/	/	/
3	4.91 (1H, dd, 7.1, 3.9)	56.4 CH	C-2, C-5, C-7, C-8	H-7	
4	/	/	/	/	/
5	/	170.9 qC	/	/	/
6	4.49-4.52 (1H, m)	51.9 CH	C-2, C-5, C-14, C-15 (w)	H-14a/b	H-3, H-16
7	2.83 (1H, dd, 10.9, 5.1)	40.6 CH ₂	C-2, C-3, C-8, C-9	H-3, H-7b	H-14a, H-9
	3.17-3.19 (1H, m)		C-2, C-3, C-8, C-9, C-13	H-3, H-7a	H-14b, H-16
8	/	133.1 qC	/	/	
9	7.08 (1H, d, 1.5)	121.5 CH	C-7, C-8, C-10, C-11, C-13	/	H-7a, H-12, H-13
10	/	148.9 qC	/	/	/
11	/	149.9 qC	/	/	/
12	7.15 (1H, d, 6.9)	119.5 CH	C-8, C-10, C-11, C-13	H-13	H-9, H-13
13	7.06 (1H, d, 7.1)	126.4 CH	C-8, C-9, C-11, C-12	H-12	H-12, H-9
14	1.49-1.52 (1H, m)	41.6 CH ₂	C-5, C-6, C-15, C-16, C-17	H-6, H-14b, H-15	H-7a, H-17
	1.91-1.94 (1H, m)		C-5, C-15, C-16, C-17	H-6, H-14a, H-15	H-7b, H-16
15	1.31-1.34 (1H, m)	25.2 CH	C-14, C-16, C-17	H-14a/b, H-16, H-17	H-16
16	0.79 (3H, d, 7.1)	22.2 CH ₃	C-14, C-15, C-17	H-15	H-6, H-3 (w)
17	0.83 (3H, d, 7.2)	21.5 CH ₃	C-14, C-15, C-16	H-15	H-14b, H-9
18-OCH ₃	3.91 (3H, s)	56.9 CH ₃	C-11	/	H-9, H-13

Table S2. 1D- and 2D-NMR spectroscopic data of compounds **2**. At 500 and 125 MHz, resp., CDCl₃, δ in ppm, *J* in Hz, Weak (w).

No.	¹ H	¹³ C	HMBC	COSY	NOESY
1	/	/	/	/	/
2	/	169.2 qC	/	/	/
3	4.93 (1H, dd, 3.8, 7.2)	56.1 CH	C-2, C-5, C-7, C-8 (w)	H-7	
4	/	/	/	/	/
5	/	171.2 qC	/	/	/
6	4.42-4.45 (1H, m)	52.3 CH	C-2 (w), C-5, C-14, C-15	H-14a/b	H-3, H-16
7	2.89 (1H, dd, 5.4, 10.6)	40.9 CH ₂	C-2, C-3, C-8, C-9, C-13	H-3, H-7b	H-14a
	3.25(1H,dd, 5.6, 10.9)		C-2, C-3, C-8, C-9, C-13	H-3, H-7a	/
8	/	133.5 qC	/	/	/
9	7.10 (1H, d, 1.6)	121.8 CH	C-7,C-8,C-10, C-11, C-13	/	H-7a, H-12
10	/	150.1 qC	/	/	/
11	/	149.4 qC	/	/	/
12	7.13 (1H, dd, 1.6, 6.8)	119.3 CH	C-8, C-10 (w), C-11, C-13	H-13	H-9, H-13
13	7.09 (1H, d, 6.9)	126.7 CH	C-8, C-9, C-11, C-12	H-12	H-12, H-9
14	1.46 -1.48 (1H, m)	41.0 CH ₂	C-5, C-6, C-15, C-16, C-17	H-6, H-14b, H-15	H-7a, H-17
	1.99-2.01 (1H, m)		C-5, C-15, C-16, C-17	H-6, H-14a, H-15	H-7b, H-16
15	1.33-1.36 (1H, m)	25.0 CH	C-14, C-16, C-17, C-6 (w)	H-14a/b, H-16, H-17	H-16
16	0.78 (3H, d, 6.8)	21.9 CH ₃	C-14, C-15, C-17	H-15	H-6, H-3
17	0.82 (3H, d, 7.0)	20.8 CH ₃	C-14, C-15, C-16	H-15	H-14b, H-9
18-OCH ₂ -	5.08 (2H, s)	106.2 CH ₂	C-10, C-11	/	H-12

Table S3. 1D- and 2D-NMR spectroscopic data of compounds **3**. At 500 and 125 MHz, resp., CDCl₃, δ in ppm, J in Hz.

No.	¹ H	¹³ C	HMBC	COSY	NOESY
1	/	/	/	/	/
2	/	170.0 qC	/	/	/
3	4.95 (1H, d, 7.2)	55.3 CH	C-2, C-5, C-7, C-8	H-7	
4	/	/	/	/	/
5	/	172.1 qC	/	/	/
6	4.92 (1H, d, 7.8)	59.2 CH	C-2, C-5, C-14, C-15	H-14a/b	H-3
7	2.82 (1H, d, 5.6)	41.2 CH ₂	C-2, C-3, C-8, C-9, C-13	H-3, H-7b	H-14a, H-9
	3.04-3.07 (1H, d, 6.2)		C-2, C-3, C-8, C-9, C-13	H-3, H-7a	/
8	/	132.8 qC	/	/	/
9	7.09 (1H, d, 1.5)	121.4 CH	C-7, C-8, C-10, C-13	/	H-7a, H-12, H-13
10	/	149.7 qC	/	/	/
11	/	149.1 qC	/	/	/
12	7.12 (1H, d, 7.0)	118.9 CH	C-8, C-10, C-11, C-13	H-13	H-9, H-13
13	7.10 (1H, d, 6.9)	125.9 CH	C-8, C-9, C-11, C-12	H-12	H-12, H-9
14	2.35-2.37 (1H, m)	30.9 CH	C-5, C-6, C-14, C-16	H-6, H-15, H-16	H-7
15	1.07 (3H, d, 6.8)	20.4 CH ₃	C-6, C-14, C-16	H-14	/
16	1.12 (3H, d, 7.0)	19.6 CH ₃	C-6, C-14, C-15	H-14	H-13
17-OCH ₂ -	5.14 (2H, s)	105.6 CH ₂	C-10, C-11	/	/

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