The Journal of Antibiotics (2018) 71:609–612
https://doi.org/10.1038/s41429-018-0033-3

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

BRIEF COMMUNICATION

Fluvirucin B6, a new macrolactam isolated from a marine-derived actinomycete of the genus *Nocardiopsis*

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Received: 11 September 2017 / Revised: 22 December 2017 / Accepted: 14 January 2018 / Published online: 21 February 2018

Abstract

A new 14-membered macrolactam natural product, fluvirucin B6 (**1**), was isolated from a marine-derived actinomycete, *Nocardiopsis* sp. CNQ-115, via HPLC-UV guided isolation. The chemical structure of **1** was elucidated by 1D and 2D NMR spectroscopic data analysis. Compound **1** showed a weak activity against Gram-positive bacteria, whereas it was inactive against Gram-negative bacteria.

Note

Polyketides are one of the most commercially successful classes of natural products ranging from metabolites for the treatment of infectious disease to numerous cancers [1]. This useful class of secondary metabolites are synthesized through a specific series of proteins known as polyketide synthases (PKSs) [2]. The biogenetic engineering study on this class of the natural products was facilitated by PKSs, which consist of separate modules. The introduction of new building blocks is required as a substitution for PKSs modules, which can be employed easily.

The genus *Nocardiopsis* is a phylogenetically independent group within the order Actinomycetales, which can be found in a wide range of environment [3]. Various biologically active secondary metabolites, such as the cytotoxic apoptolidins [4], the antimicrobial nocardiamides [5], the antimicrobial nocarimidazoles [6], and the antiphotoaging-active compounds nocariones [7]. have been reported from this fertile genus.

During our investigations of new secondary metabolites from marine microorganisms, we encountered a *Nocardiopsis* sp., strain CNQ-115, which we now report produces a new 14-membered macrolactam, named fluvirucin B6 (**1**). This strain was also known to produce rare 4-aminoimidazole natural products [6]. Herein we report the detailed production, isolation, structure elucidation, and biological activity of fluvirucin B6 (**1**) (Fig. 1).

Fluvirocin B6 (**1**) was isolated as an optically active white oil ($[\alpha]^{25}_{D}$ −12.6, *c* 0.34, DMSO). The molecular weight of **1** was obtained from the HRESITOF mass spectrum, which showed pseudomolecular ions at *m/z* 485.3596 $[M + H]^+$ and at *m/z* 507.3533 as a sodium ion adduct $[M + Na]^+$. Interestingly, the sodium ion adduct was the major MS peak observed. (Figure S8) On this and upon NMR data, the molecular formula was defined as $C_{26}H_{48}N_2O_6$. The UV/vis spectrum of **1** showed only end absorption, while the IR (KBr) spectrum of **1** showed the presence of an amide (3436 and 1638 cm^{-1}) functionality.

The ^1H NMR spectrum of **1** included two overlapping methyl triplets at δ_H 0.89, methyl doublets at δ_H 0.92 and

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Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41429-018-0033-3) contains supplementary material, which is available to authorized users.

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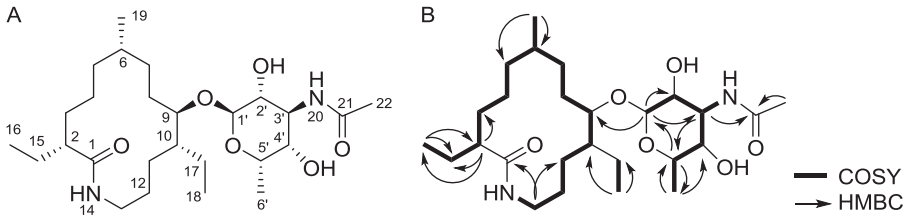

Fig. 1 **a** Chemical structure of fluvirucin B6 (**1**). **b** Key COSY and HMBC correlations of **1**

Table 1 NMR spectroscopic data for fluvirucin B6 (1) in MeOD

No.	$^{13}C^{a}$ mult.^{b}	$^{1}H^{a}$ mult., (*J* in Hz)	COSY	HMBC
1	179.2, C			
2	51.4, CH	2.10, m	3, 15	3, 15, 16
3	34.8, CH_2	1.41, m	2, 4	
4	26.3, CH_2	1.52, m	3, 5	
5	35.7, CH_2	1.10, m	4, 6	
6	32.6, CH	1.71, m	5, 7, 19	
7	26.6, CH_2	1.46, m	6, 8	
8	22.8, CH_2	1.61, m	7, 9	
9	78.8, CH	3.63, q, (5.5)	8, 10	
10	42.4, CH	1.60, m	9, 11	
11	26.6, CH_2	1.48, m	10, 12	
12a	28.9, CH_2	1.65, m	11, 13	
12b		1.41, m		
13a	40.0, CH_2	3.62, t (3.0)	12, 14	1, 11
13b		2.96, dq (3.0)		
14		8.16^{c}	13	
15	27.8, CH_2	1.60, 1.42, m	2, 16	
16	12.7, CH_3	0.89, t (7.0)	15	2, 15
17	22.5, CH_2	1.63, m	18	
18	9.7, CH_3	0.89, t (7.0)	17	10, 17
19	21.2, CH_3	0.92, d (6.5)	6	5, 6
20		8.12^{c}	3′	
21	173.3, C			
22	23.0, CH_3	2.05, s		21
1′	99.6, CH	4.87^{d}	2′	9, 2′, 3′, 5′
2′	71.5, CH	3.56, *t* (3.0)	1′, 3′	
3′	49.6, CH	4.17, *t* (3.0)	20, 2′, 4′	21, 5′
4′	72.5, CH	3.55, *t* (3.0)	3′, 5′	
5′	69.1, CH	4.05, *q* (5.5)	4′, 6′	1′, 3′, 6′
6′	17.3, CH_3	1.22, *d* (5.5)	5′	4′, 5′

[a] 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR

[b] Numbers of attached protons were determined by analysis of 2D spectroscopic data

[c] These resonances were assigned on the basis of their COSY correlations

[d] Signal overlapped with the solvent

1.22, and a methyl singlet at δ_H 2.05. Numerous signals between δ_H 3.4–4.2 p.p.m. as well as a doublet at δ_H 4.87 p.p.m., which indicated an overlapping with methanol solvent peak, revealed signals that were ascribable to a monosaccharide. The NMR DEPT spectrum indicated the presence of five methyl groups, ten methylene carbons, and nine methines. Two quaternary signals at δ_C 173.3 and 179.2, indicated the presence of two carbonyl groups. A proton at δ_H 8.16 that by HSQC analysis showed no correlation in the HSQC spectrum consists of an amide proton.

Analysis of COSY and HMBC spectroscopic data revealed two fragments. COSY NMR cross-peaks (H-6/H_2-5/H_2-4/H_2-3/H-2/H_2-15/H_3-16, H-14/H_2-13/H_2-12/H_2-11/H-10) and HMBC correlations (H-6/C-8, H_3-19/C-6, C-5, H_2-7/C-9, H_2-13/ C-1 (δ_C 179.2)) permitted the establishment of a branched 14-membered lactam moiety as one fragment. Further COSY cross-peaks (H-20/H-3′/H-2′/H-1′, H_3-6′/H-5′) and HMBC correlations (H-1′/C-2′ and C-3′, H-3′/C-5′, H-5′/C-1′, and C-3′) suggested the presence of amino sugar moiety for the second fragment.

The positions of a methyl and two ethyl groups were assigned by analysis of HMBC NMR correlations. The HMBC correlations from H_3-16 (δ_H 0.89) to C-2 (δ_C 51.4) and C-15 (δ_C 27.8), and form H-2 (δ_H 2.10) to C-16 (δ_C 12.7) and C-15 (δ_C 27.8) permitted establishing an ethyl group at C-2. The HMBC correlations from H_3-18 (δ_H 0.89) to C-10 (δ_C 42.4) and C-17 (δ_C 22.5), and from H-10 (δ_H 1.6) to C-18 (δ_C 9.7) and C-17 (δ_C 22.5) allowed another ethyl group to be positioned at C-10. The connectivity of C-19 methyl with C-6 was established from the observation of long-range HMBC correlations from H_3-19 (δ_H 0.92) to C-6 (δ_C 32.6) and C-5 (δ_C 35.7; Fig. 1b).

The amino sugar moiety of compound **1** was determined as an *epi*-mycosamine based on the small coupling constant values for H-2′ (*t*, $J = 3.0$ Hz), H-3′ (*t*, $J = 3.0$ Hz), H-4′ (*t*, $J = 3.0$ Hz), suggesting the equatorial orientation of these protons [8]. Furthermore, an equatorial orientation of H-1′ and H_3-6′ were deduced by analysis of NOESY NMR correlations with H-9 and H-4′. The acetyl group was located at C-3′ based on the long-range HMBC correlations of H-3′ (δ_H 4.17) and H_3-22 (δ_H 2.05) to a carbonyl carbon C-21 (δ_C 173.3). Finally, connectivity of the two fragments was achieved on the basis of a long-range HMBC correlation from H-1′ (δ_H 4.87) to C-9 (δ_C 78.8), thus completing the structure assignment of *N*-acetyl-2,5-*epi*-mycosamine (**1**) (Table 1 for NMR data).

To determine the absolute configuration of **1**, numerous attempts to crystalize the compound were employed. Unfortunately, a crystal of suitable size could not be

obtained for X-ray analysis. Therefore, we carefully compared the NMR spectroscopic data of **1** to those of the previous reported natural product, Sch 38516/fluvirucin B1. Most of the ^{1}H, and ^{13}C NMR signals of **1** and Sch 38516/fluvirucin B1 were very similar, except for the substitution of one amino proton with an acetyl group in the sugar moiety. The average carbon chemical shift differences in conserved areas are 0–0.7 p.p.m., whereas the differences around the attached acetyl group are 1.1–2.8 p.p.m. (Table S1) Moreover, the negative optical rotation values of compound **1** ($[\alpha]^{25}_{D}$ −12.6, *c* 0.34, DMSO), which compared favorably with the reported value for Sch 38516 ($[\alpha]^{26}_{D}$ −6.7, *c* 0.50, DMSO) [9] suggested these compounds could have the same configurations.

Compound **1** belongs to the fluvirucins class of polyketides, which possess a 14-membered macrolactam attached to a sugar. The fluvirucin A series have a sugar at the C-3 position whereas the B series have a sugar at the C-9 position [10–12]. Compound **1** is the acetylated derivative of fluvirucin B1, thus we have named this compound fluvirucin B6.

Fluvirucins are known to have various bioactivities such as antiviral, antibacterial, anthelmintic, and antifungal [10, 13–15]. Fluvirucin B6 (**1**) was tested against six pathogenic bacteria including three Gram-positive bacteria (*Bacillus subtillis* ATCC 6644, *Kocuria rhizophila* ATCC 9341, and *Staphylococcus aureus* ATCC 6538 P) and three Gram-negative bacteria (*Escherichia coli* ATCC 11775, *Salmonella typhimurium* ATCC 14028, and *Klebsiela pneumoniae* ATCC 4352). Compound **1** was found to exhibit weak antibacterial activities against *B. subtillis*, *K. rhizophila*, and *S. aureus* with MIC values of 64, 32, and 32 µg/L, respectively. Fluvirucin B6 (**1**) only showed an antibacterial activity against Gram-positive bacteria, any significant activity against Gram-negative bacteria was not observed up to 128 µg/L. The weak antibacterial activity of compound **1** could be due to the impact of the modified sugar moiety within 14-membered macrolactone/lactam class PKS products [16].

Lactam substitution for a lactone moiety has been employed to improve the bioactivity or pharmacological profiles of known-macrolactone structure [17], which was demonstrated with the azalides [18]. However, macrolactam structures are mainly acquired from low-yield synthetic methods which limit a more facile approach for these PKS products. For example, most improved enantioselective synthetic efforts for fluvirucin B1 aglycon–fluvirucinin B1 resulted in 12 linear synthetic steps with a 11% overall yield [19].

In combination with the recently revealed fluvirucin B1 biosynthetic pathway [20], *Nocardiopsis* sp. strain CNQ-115 may provide new macrolactam biosynthesis building block for this class of PKS product. This possibility can be employed to increase the range of novel 14-membered macrolactam analogs.

Acknowledgements This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2017R1D1A1B03028172 to S.N.) and by the U.S. National Cancer Institute (NIH) under grant R37CA044848 and instrument grant 1 S10 OD0106400-01A1 (HR-LC-MS, both to W.F.)

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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