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BRIEF COMMUNICATION

A new macrolactam derivative from the marine actinomycete HF-11225

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Abstract

A new macrolactam derivative, designated as nivelactam B (**1**), was isolated from the fermentation broth of marine-derived actinomycete HF-11225. Its structure was determined on the basis of spectroscopic analysis, including 1D and 2D NMR techniques, as well as ESI-MS and comparison with data from the literature. Compound **1** showed weak cytotoxic and antifungal activities.

Marine environment, which represents approximately half of the global biodiversity, contains a rich source of structurally diverse and biologically active metabolites [1, 2]. Especially, the marine microorganisms are well-known to have the potential to produce a huge number of metabolites with interesting novel bioactivities [3, 4]. Recently, many secondary metabolites from marine microorganisms have become significant chemical entities in drug discovery [5–7]. During our effort to explore bioactive compounds from marine microorganisms, a new macrolactam derivative, nivelactam B (**1**) was isolated from the fermentation broth of marine-derived actinomycete HF-11225. Here we reported the purification, identification, and biological activities of **1**.

Strain HF-11225 was isolated from a deep ocean soil sample of the East Sea in China and was grown and maintained on the YMS medium containing malt extract (Becton, Dickinson and Company, Franklin Lake, NJ, USA) 10.0 g, yeast extract (Oxoid, Basingstoke, UK) 4.0 g, glucose (Sinopharm Chemical Reagent, Shanghai, China) 4.0 g, $CoCl_2{\cdot}6H_2O$ (Sinopharm Chemical Reagent) 0.005 g and agar (Becton, Dickinson and Company) 20.0 g in 1.0 l tap water at pH 7.0–7.2. The 16 S rRNA sequence of HF-11225 was analyzed by the method described previously [8]. The sequence was amplified using Taq polymerase (Promega, Madison, WI, USA) and the general primers (forward primer P1: 5′-AGAGTTTGA TCCTGGCTCAG-3′; reverse primer P2: 5′-AAGGAGGT GATCCAGCCGCA-3′). The amplified sequence (1492 bp) of HF-11225 was determined (accession no. MF772493). An NCBI BLAST search showed 16S rRNA of HF-11225 had identity with *Streptomyces varsoviensis* strain NRRL B-3589 (accession no. NR_043497.1). The seed medium consisted of glucose 4.0 g, malt extract 10.0 g and yeast extract 4.0 g in 1.0 l tap water, pH 7.0–7.2. All of the media were sterilized at 121 °C for 20 min. The producing medium was composed of mannitol (Shangdong Tianli Pharmaceutical, Weifang, China) 20.0 g and soybean powder (Ningbo Beilun Jiangnan Grease, Ningbo, China) 20.0 g in 1.0 l tap water, pH 6.8–7.0 before sterilization. Slant culture was incubated for 5–7 days at 28 °C. Then the slant culture was inoculated on 1 l Erlenmeyer flasks containing 250 ml of the seed medium and incubated at 28 °C for 24 h, shaken at 250 r.p.m. Fermentation was carried out in a 50 l fermentor containing 30 l of production medium at 28 °C for 7 days and stirred at 200 r.p.m. with an aeration rate of 1000 l of air per hour.

The final 30 l of fermentation broth was centrifuged to separate mycelia and supernatant. The mycelial cake was washed with water (3 l) and subsequently extracted with MeOH (10 l). The supernatant and the wash water were passed through a column of Diaion HP-20 resin (Mitsubushi

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Table 1 ^{1}H and ^{13}C NMR data of 1 (in CD_3OD)

Position	δ_H (J in Hz)	δ_C (p.p.m.)	Position	δ_H (J in Hz)	δ_C (p.p.m.)
1		177.5 s	16	3.91 m	68.7 d
2	3.66 br t (8.7)	55.7 d	17	3.92 m	76.2 d
3	5.46 dd (10.3, 8.7)	125.7 d	18	1.82 m	42.4 t
4	6.70 t (10.3)	133.6 d		2.51 m	
5	5.53 d (10.3)	131.0 d	19	3.94 m	53.5 d
6		133.0 s	20	2.46 m	38.1 t
7	2.09, m	53.8 d	21	5.60 dd (14.5, 7.5)	127.0 d
8	3.86 dd (11.8, 4.0)	71.0 d	22	6.13 dd (14.5, 10.4)	135.4 d
9	4.14 t (4.0)	67.9 d	23	6.08 dd (14.4, 10.4)	131.7 d
10	5.88 m	128.4 d	24	5.66 dd (14.4, 7.4)	134.4 d
11	5.91 m	133.9 d	25	2.07 m	35.7 t
12	2.84 t (10.7)	43.9 d	26	1.44 m	23.6 t
13	5.07 d (10.7)	133.3 d	27	0.94 t (7.4)	14.0 q
14		137.9 s	28	1.76 br s	12.7 q
15	3.24 t (8.7)	59.1 d	29	1.42 br s	17.0 q

Chemical Co, Ltd., Tokyo, Japan) equilibrating with water and then eluting with 95% EtOH (5 l). The MeOH extract and the EtOH eluents were evaporated under reduced pressure at 50 °C to yield a mixture (36 g). The mixture was subjected to a silica gel (Qingdao Haiyang Chemical Group, Qingdao, Shandong, China; 100–200 mesh) column and eluted stepwise with $CHCl_3$/MeOH (100:0, 98:2, 95:5, 90:10, 85:15, and 80:20, v/v) to give five fractions (Fr.1 to Fr.5) based on the TLC profiles. TLC was performed on silica-gel plates (HSGF254, Yantai Chemical Industry Research Institute, Yantai, China) with solvent system of $CHCl_3$/MeOH (9:1, v/v). The developed TLC plates were observed under a UV lamp at 254 nm, or by heating after spraying with sulfuric acid/ethanol, 5:95 (v/v). After the Fr.2 eluted with $CHCl_3$/MeOH (95:5, v/v) was concentrated in vacuo, the material (1.2 g) was subjected to Sephadex LH-20 gel (GE Healthcare, Glies, UK) column eluted with $CHCl_3$/MeOH (1:1, v/v) and detected using TLC to afford three fractions (Fr.2-1 and Fr.2-3). The Fr.2-1 was further purified by preparative HPLC (Shimadzu LC-8A, Shimadzu-C18, 5 μm, 250 × 20 mm inner diameter; 20 ml min^{-1}; 220 nm/254 nm; Shimadzu, Kyoto, Japan) with a 30 min gradient program of 30–45% CH_3CN in H_2O to obtain compound **1** (t_R 31.8 min, 13.6 mg). ^{1}H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer (Rheinstetten, Germany). The ESIMS and HRESIMS spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Milford, MA, USA).

Compound **1** was isolated as white amorphous powder with $[\alpha]_D^{25}$ + 24.2 (c 0.06, EtOH) and UV (EtOH) λ_{max} nm (log ε): 232 (3.70). Its molecular formula $C_{29}H_{39}NO_4$ was deduced from the pseudo molecular ion at m/z 466.2950 $[M + H]^+$ (calcd as 466.2952 for $C_{29}H_{40}NO_4$) in its HRESIMS, which indicated 11 degrees of unsaturation. The IR spectrum showed absorption bands assignable to hydroxy group (3391 cm^{-1}) and carbonyl (1670 cm^{-1}). The ^{1}H NMR spectrum (Table 1) of **1** displayed ten olefinic proton signals from δ_H 5.07 to δ_H 6.70, one triplet aliphatic methyl at δ_H 0.94 (3 H, t, J = 7.4 Hz), two olefinic methyls at δ_H 1.42 (3 H, br. s) and 1.76 (3 H, br. s). The ^{13}C NMR spectrum (Table 1) showed signals from 29 carbon atoms. Combined with the DEPT experiment, the 29 carbon resonances can be categorized as one amide carbonyl (δ_C 177.5), ten sp^2 methines (δ_C 125.7, 127.0, 128.4, 131.0, 131.7, 133.3, 133.6, 133.9, 134.4, and 135.4), two sp^2 tetrasubstituted carbons (δ_C 133.0 and 137.9), four oxygenated sp^3 methines (δ_C 67.9, 68.7, 71.0 and 76.2), five sp^3 methines (δ_C 43.9, 53.5, 53.8, 55.7 and 59.1), four sp^3 methylenes (δ_C 23.6, 35.7, 38.1 and 42.4) and three methyls (δ_C 12.7, 14.0 and 17.0). The ^{1}H and ^{13}C NMR data assignment were supported by the ^{1}H–^{1}H COSY, HMQC, and HMBC experiments. Detailed comparison of the ^{1}H and ^{13}C NMR spectral data of **1** with those of nivelactam [9] showed that **1** was very similar to nivelactam except for an extra 1, 2-disbstituted double bond in **1**. The ^{1}H–^{1}H COSY correlations (Fig. 1) of H_2-20/H-21/H-22/H-23/H-24/H_2-25/H_2-26/H_3-27 indicated that the 2-hexenyl moiety in nivelactam was replaced by 2,4-octadienyl group in **1**. Consequently, the gross structure of **1** was established and named nivelactam B. Furthermore, the ^{1}H–^{1}H COSY correlations of H-2/H-3/H-4/H-5, H-2/H-15/H-16, H-7/H-8/H-9/H-10/H-11/H-12/H-13, H-17/H_2-18/H-19 and the observed HMBC (Fig. 1) correlations from H-2/H-3 to C-1 (δ_C 177.5), from H_3-28 to C-5, C-6, and C-7, from H_3-29 to C-13, C-14, and C-15 and from H_2-18 to C-16 (δ_C 68.7) confirmed the structure of **1**. In **1**, the large coupling constants of H-21 (δ_H 5.60, dd, J = 14.5, 7.5 Hz) and H-23 (δ_H 6.08, dd, J = 14.4,

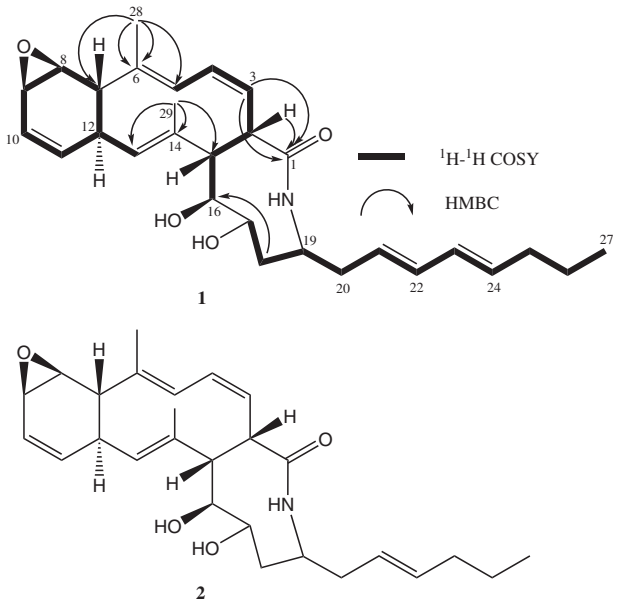

Fig. 1 Structures of **1** and nivelactam (**2**) and the key ^{1}H–^{1}H COSY and HMBC correlations of **1**

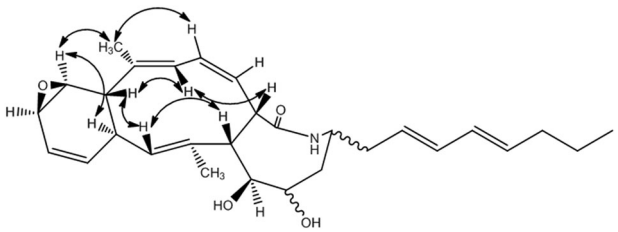

Fig. 2 The main NOESY correlations observed in **1**

10.4 Hz) and the NOESY crossing peaks (Fig. 2) between H_3-28 and H-4, and H-13 and H-15 indicated that the four double bonds at C-21, C-23, C-5, and C-13 were all *E*. The coupling constant of H-3 (δ_H 5.46 dd, $J = 10.3$, 8.7 Hz) revealed the double bond geometry at C-3 to be *Z*. The correlations of H-8/H-12, H-8/H_3-28 in the NOESY spectrum indicated these protons were cofacial. The observed NOESY correlations from H-5 to H-2 and H-7, from H-7 to H-13, and from H-13 to H-15 suggested that these protons having the same orientations as in nivelactam. The triplet of H-15 with a large coupling constant (8.7 Hz) revealed the relative configuration of H-15 and H-16 was anti relation. Due to the heavily signal overlapping, the configurations of C-17 and C-19 remained unassigned.

The cytotoxicity of **1** was assayed for growth-inhibition activity in vitro against human lung tumor cells A549 and human prostate cancer cells PC-3 using the sulforhodamine B (SRB) method [10]. As a result, **1** showed weak cytotoxicity against the A549 (IC_{50}: 37.21 μM) and PC-3 (IC_{50}: 49.55 μM) cells, whereas the IC_{50} values of the positive control (doxorubicin) were 1.32 μM and 1.97 μM, respectively. The antifungal activity of **1** was measured by disk diffusion method [11]. Compound **1** exhibited inhibition activity against *Sclerotinia sclerotiorum* with inhibition zone of 9 mm at 100 μg per 7 mm paper disks.

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Compliance with ethical standards

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

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