文章编号:1001-6880(2016)4-0494-04

链霉菌 *Streptomyces* sp. neau-D50 中的一个新天然产物

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摘 要:对 borrelidin 产生菌 *Streptomyces* sp. neau-D50 发酵液中的化学成分进行研究并从中分离纯化得到一个新天然产物,*N*-acetylborrelidin B,其对人乳腺癌 MCF-7 细胞和小鼠黑色素瘤细胞株 B16 的 IC_{50} 值分别为 19.9 μM 和 36.3 μM。

关键词:borrelidin 类似物;链霉菌;次级代谢产物;细胞毒活性
中图分类号:R284.1 **文献标识码**:A DOI:10.16333/j.1001-6880.2016.4.005

A New Natural Product from *Streptomyces* sp. neau-D50

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Abstract: The chemical constituents of borrelidin-producing strain *Streptomyces* sp. neau-D50 was reinvestigated and a new natural product (*N*-acetylborrelidin B) was obtained. *N*-Acetylborrelidin B exhibited cytotoxic activity against human breast adenocarcinoma cell line MCF-7 and murine melanoma cell line B-16 with IC_{50} values of 19.9 μM and 36.3 μM, respectively.

Key words: borrelidin analogue; *Streptomyces*; secondary metabolite; cytotoxic activity

Introduction

Borrelidin (Fig. 1), an unusual nitrile-containing 18-membered macrolide, was firstly isolated from *Streptomyces rochei* in 1949 by Berger J[1]. It possesses interesting biological activity including antibacterial activity, antiviral activity, antiangiogenesis activity and inhibitory activity toward cyclin-dependent kinase Cdc28/Cln2 of *Saccharomyces cereisiae*[2]. Even though it has a wide spectrum effect, it is not a successful drug in case of humans due to the levels of toxicity. Nevertheless, research has to be initiated to produce novel derivatives of borrelidin to be more targets specific either by fermentation using suitable precursors or by synthetic methods in the laboratory because of the unusual chemical architecture and diverse biological profile of borrelidin[3]. In our previous investigation, a borrelidin-producing strain *Streptomyces* sp. neau-D50 was isolated from healthy soybean root by an *in vitro* screening technique and the antifungal activity of borrelidin against *Phytophthora sojae* was reported[4]. As part of our continuous effort to discover more secondary metabolites, we re-investigated the chemical constituents of the strain *Streptomyces* sp. neau-D50. As a result, a new natural borrelidin analogue (**1**) was obtained. This paper described the isolation, purification, characterization and bioactivity of this new natural product.

Materials and Methods

The strain *Streptomyces* sp. Neau-50 was maintained on the medium containing glucose 10 g, maltose 3 g, yeast extract 3 g, $K_2HPO_4 \cdot 3H_2O$ 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NaCl 0.5 g, KNO_3 1 g and agar 20 g in 1.0 L of tap water, pH 7.0. The seed medium consisted of glucose 4 g, maltodextrin 10 g, yeast extract 4 g, $CaCO_3$ 2 g in 1.0 L water and pH 7.2 - 7.4. All the media were sterilized at 121 ℃ for 20 min. Slant culture was incubated for 6 - 7 days at 28 ℃. Fermentation was carried out in 50 L of first seed fermentor (containing 30 L of seed medium), 500 L of second fermentor (containing 300 L of production medium) successively. The producing medium was composed of glucose 1%, soluble am-

Received: November 24, 2015 Accepted: January 5, 2016
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ylum 4%, yeast extract 0.5%, soybean powder 2.5%, peptone 0.5%, $CaCO_3$ 0.2%, $MgSO_4 \cdot 7H_2O$ 0.8%, $FeSO_4 \cdot 7H_2O$ 0.6%, $ZnSO_4 \cdot 7H_2O$ 0.2%, $MnSO_4 \cdot H_2O$ 0.2%, $CoCl_2 \cdot 6H_2O$ 0.05%, $Na_2MoO_4 \cdot 2H_2O$ 0.2%, and pH 7.0 before sterilization. The fermentation was conducted at 28 ℃ for 7 days stirred at 100 rpm with an aeration rate of 30 m^3 of air per hour.

The final 300 L of broth was filtered. The resulting cake was washed with water, and both filtrate and wash were discarded. The washed cake was extracted twice for about 24 h with 100 L of EtOH. The EtOH extract was diluted to about 30% EtOH and subjected to a Diaion HP-20 resin column eluting with 30%, 40%, 50%, 60%, 70%, 80% EtOH (each concentration eluted 2 bed volumes). The eluents eluting with 70% and 80% EtOH were pooled and concentrated *in vacuo* at 50 ℃ to give a mixture (100 g). Then one-fifth of the mixture (20 g) was subjected to a silica gel column (200-300 mesh, Qingdao Marine Chemical Factory, Qingdao, China) and successively eluted with a stepwise gradient of petroleum ether/acetone (100:0-50:50, v/v) to afford four fractions (I-IV) based on the TLC profiles. The fraction III was chromatographed on a silica gel column using petroleum ether/acetone (90:10-60:40, v/v). During this step, three fractions (A1-A3) were obtained and Fraction A2 was borrelidin (2.6 g). Fraction A3 was subjected to a Sephadex LH-20 (GE Healthcare, Glies, UK) eluting with EtOH and detected by TLC to give six fractions (B1-B6). Fraction B3 was separated by semi-preparative HPLC (Agilent 1100, ZorbaxSB-C18, 5 μm, 250 x 9.4 mm i. d.; 1.5 mL/min; 254 nm; Agilent, Palo Alto, CA, USA) eluting with CH_3CN/H_2O (65:35, v/v) to give compound **1** (t_R 11.2 min, 56 mg). UV spectra were obtained on a Varian CARY 300 BIO spectrophotometer (Varian, Palo Alto, CA, USA). IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrometer (Nicolet Magna, Madison, WI, USA). ^1H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer (Rheinstetten, Germany). ESI-MS and HR-ESI-MS spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Waters Co., Milford, MA, USA).

Results and Discussion

Compound **1** (Fig. 2) was obtained as colorless oil with UV (EtOH) λ_{max} nm (log ε): 240 (4.20). The IR spectrum of **1** indicated the presence of a hydroxy group (3336 cm^{-1}) and carbonyl group (1717 cm^{-1}). The molecular formula was determined to be $C_{30}H_{49}NO_7$ by positive HRESIMS (m/z: 558.3383 $[M + Na]^+$, calcd. for 558.3401) in conjunction with NMR data (Table 1). The ^{13}C NMR and DEPT spectra exhibited 30 carbon signals comprising three carbonyl groups at δ_C 180.1, 173.5, 170.7, three sp^2 methines at δ_C 132.4, 131.1, 128.1, a sp^2 quaternary carbon at δ_C 136.8, three oxygenated methines at δ_C 84.4, 75.6, 70.7 and five methyl resonances at δ_C 23.2, 20.2, 18.6, 16.9, 16.0 in addition to nine methylene carbons and six methine carbons. Analysis of the ^1H NMR data for **1** revealed the presence of four aliphatic methyl doublets at δ_H0.79 (3H, d, J = 6.2 Hz), 0.80 (3H, d, J = 7.2 Hz), 0.82 (3H, d, J = 7.3 Hz) and 1.00 (3H, d, J = 6.3 Hz), a methyl singlet at δ_H 1.99 (3H, s), three olefinic proton signals at δ_H 6.37 (1H, dd, J = 14.4, 11.1 Hz), 5.95 (1H, d, J = 11.1 Hz) and 5.59 (1H, m), a downfield proton signal at δ_H 6.73 (1H, dd, J = 6.6, 3.8 Hz). An acetyl group was present in **1** corroborated by the HMBC correlation from δ_H 1.99 (3H, s) to δ_C 170.7 and their chemical shifts. The hydrogen resonance at δ_H 6.73 was assigned to the NH proton because it lacked correlation in the HMQC spectrum. Further correlated signals from δ_H 4.23, 3.64 to δ_C 170.7 in the HMBC spectrum and the correlations of δ_H 6.73 and δ_H 4.23, 3.64 in the ^1H-^1H COSY experiment indicated the presence of an acetylaminomethylene group in **1**. Comparison of the ^1H and ^{13}C NMR data of **1** with those of borrelidin [4] suggested that compound **1** was an analogue of borrelidin. The only difference between **1** and borrelidin was that the nitrile group in borrelidin was replaced by an acetylaminomethylene group in **1**. This result was supported by the HMBC correlations from the H-19 methylene protons at δ_H 4.23, 3.64 to C-6, C-7 and C-8. Consequently, the gross structure of **1** was established. The geometry of $\Delta^{4,5}$ was assigned *trans* based on the large coupling

constant (14.4 Hz) of H-4 and H-5. The other relative stereochemistry of **1** was assigned by analogy with borrelidin. Hence, compound **1** was designed as *N*-Acetyl-borrelidin B. Though compound **1** had been prepared by synthetic method[5], it was firstly isolated from natural resources.

Table 1 ^{1}**H NMR** (400 **MHz**) **and** ^{13}**C NMR** (100 **MHz**) **data for** 1 **and brrrelidin** (**in** $CDCl_3$)

No.	1		Borrelidin (ref. 4)	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2	5.18 (m)	75.6 (d)	4.98 (dt, 10.7, 3.2)	76.5 (d)
3	2.55 (m)	38.0 (t)	2.60 (m)	35.9 (t)
	2.32 (m)		2.57 (m)	
4	5.59 (m)	132.4 (d)	6.21 (m)	138.5 (d)
5	6.37 (dd, 14.4, 11.1)	128.1 (d)	6.39 (dd, 14.5, 11.2)	127.0 (d)
6	5.95 (d, 11.1)	131.1 (d)	6.83 (d, 11.2)	144.0 (d)
7	–	136.8 (s)	–	115.9 (s)
8	3.64 (d, 9.2)	84.4 (d)	4.12 (d, 9.6)	73.1 (d)
9	1.70 (m)	34.3 (d)	1.88 (m)	35.1 (d)
10	0.95 (m)	37.9 (t)	1.05 (m)	37.4 (t)
	0.62 (m)		0.73 (m)	
11	1.70 (m)	26.4 (d)	1.63 (m)	26.2 (d)
12	0.95 (m)	47.7 (t)	1.11 (m)	47.8 (t)
	1.16 (m)		0.98 (m)	
13	1.70 (m)	26.5 (d)	1.58 (m)	27.0 (d)
14	1.32 (m)	42.6 (t)	1.22 (m)	42.9 (t)
	0.93 (m)		0.94 (m)	
15	1.83 (m)	34.6 (d)	1.68 (m)	35.5 (d)
16	3.93 (m)	70.7 (d)	3.87 (br d, 9.7)	69.9 (d)
17	2.32 (m)	38.4 (t)	2.41 (dd, 15.8, 9.9)	39.2 (t)
			2.32 (d, 15.8)	
18	–	173.5 (s)		172.2 (s)
19	4.23 (dd, 14.4, 6.6)	35.4 (t)	–	118.3 (s)
	3.64 (dd, 14.4, 3.8)		–	–
20	–	170.7 (s)	–	–
21	1.99 (s)	23.2 (q)	–	–
1′	2.58 (m)	46.6 (d)	2.49 (m)	48.5 (d)
2′	2.55 (m)	48.1 (d)	2.71 (m)	45.8 (d)
3′	1.98 (m)	31.4 (t)	1.98 (m)	29.6 (t)
	1.86 (m)		1.38 (m)	
4′	1.73 (m)	25.6 (t)	1.82 (m)	25.2 (t)
5′	1.90 (m)	29.9 (t)	2.03 (m)	31.2 (t)
	1.35 (m)		1.92 (m)	
9-CH_3	1.00 (d, 6.3)	16.0 (q)	1.05 (d, 6.4)	14.9 (q)
11-CH_3	0.80 (d, 7.2)	20.2 (q)	0.84 (d, 6.4)	20.2 (q)
13-CH_3	0.79 (d, 6.2)	18.6 (q)	0.80 (d, 6.2)	18.2 (q)
15-CH_3	0.82 (d, 7.3)	16.9 (q)	0.83 (d, 6.7)	16.9 (q)
NH	6.73 (dd, 6.6, 3.8)	–	–	–
COOH	–	180.1 (s)	–	180.1 (s)

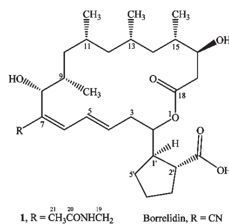

Fig. 1 Chemical structures of 1 and borrelidin

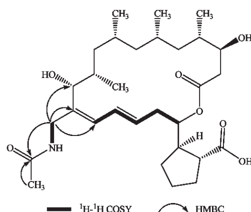

Fig. 2 Key ^{1}H-^{1}H COSY and HMBC correlations of 1

The cytotoxicity of **1** was assayed *in vitro* against the human breast adenocarcinoma cell line MCF-7 and the murine melanoma cell line B-16 by the CCK8 colorimetric method as described in our previous papers [6]. Compound **1** exhibited cytotoxic activity with IC_{50} values of 19.9 and 36.3 μM, respectively. The values of borrelidin were 0.31 and 0.05 μM, respectively.

Conclusion

In this study, *N*-Acetylborrelidin B (**1**) was isolated from a borrelidin-producing strain *Streptomyces* sp. neau-D50 as a new natural compound. The structure of **1** was successfully clarified by extensive NMR analysis. Bioassay results showed that borrelidin exhibited higher cytotoxic activity than compound **1**, hence nitrile group of borrelidin was important for its cytotoxicity.

Acknowledgement: This research work was financially supported by the Special Program for the Science and Technology Plan of Zhejiang Province of China (2012C03006-2).

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