NOTE

Bafilomycin K, a new antifungal macrolide from *Streptomyces flavotricini* Y12-26

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Because of the special living environment, marine-derived actinomycetes, especially the genus *Streptomyces*, possess distinct and complex metabolic capabilities, resulting in wide diversity of their secondary metabolites in chemical structures and biological activities.¹ Among them, many valuable lead compounds were obtained for discovery of new antibiotics.^{2–4} Bafilomycins are a family of polyene macrolides containing a 16-member lactone ring. Thirteen bafilomycin antibiotics have been isolated, including bafilomycins A₁, A₂, B₁, B₂, C₁ and C₂ from *Streptomyces griseus* sp. *sulphurus* (TU 1922),⁵ bafilomycins D and E from *S. griseus* TU 2599⁶ and bafilomycins F–J from *Streptomyces* spp.⁷ Among them, bafilomycins D and E contain the ring-opened side chain different from other bafilomycins. Bafilomycins have antibacterial, antifungal, antineoplastic and immunosuppressive activities.⁵ Some bafilomycins are specific inhibitors of vacuolar-type H⁺-ATPase (V-ATPase),^{8–11} in which bafilomycin A₁ is the most used. Being a specific inhibitor of V-ATPase, bafilomycin A₁ can prevent the re-acidification of synaptic vesicles once they have undergone exocytosis. In addition, bafilomycin A₁ has antimalarial activity.¹² Bafilomycins B₁ and C₁ have been mentioned as potential antiosteoporotic agents in treating bone lytic diseases.

In order to obtain new antibiotics from marine-derived actinomycetes, we screened the antifungal substances produced by *S. flavotricini* Y12-26 isolated from mangrove plant *Acanthus ilicifolius* grown in the intertidal zone of the Hainan province of China. We first found that the culture broth of *S. flavotricini* Y12-26 showed strong antifungal activity against *Pyricularia oryzae*, and a new antifungal macrolide named bafilomycin K (**1**, Figure 1) was then isolated from the fermentation broth of this strain. Its structure was established to be a new member of bafilomycins with a 16-member lactone ring by spectroscopic analysis. This paper describes the isolation, structure elucidation and antifungal bioassay of **1**.

The marine actinomycetes Y12-26, isolated from mangrove plant *A. ilicifolius* grown in the intertidal zone of the Hainan province of China, was identified as *Streptomyces flavotricini* according to its

morphological characters, biochemical characters and the partial sequence of its 16S rDNA. The strain was deposited in China General Microbiological Culture Collection Center, Beijing, China (CGMCC No. 3832).

The culture broth of *S. flavotricini* Y12-26 was conducted in shake flasks. The germination medium contained glucose 20.0 g, soybean grits 25.0 g, starch 10.0 g, yeast powder 4.0 g, NaCl 20.0 g, beef extract 1.0 g and K₂HPO₄ 50.0 mg in 1.0 l of tap water with pH 7.2. Each 250 ml flask containing 70 ml of this medium was incubated with 2 ml of the stock culture. The flasks were incubated at 28 °C on a rotary shaker at 220 r.p.m. for 12 days. Culture broth (30 l) of *S. flavotricini* Y12-26 was centrifuged at 4000 r.p.m. for 10 min to get the mycelia. The mycelia were then extracted three times with 80% acetone. The 80% acetone extracts were then concentrated *in vacuo* to afford residue, which was then extracted with *n*-BuOH three times. The *n*-BuOH extracts were further extracted with MeOH. The MeOH extract was then subjected to silica gel column eluted with CH₂Cl₂–MeOH system (60:1 v/v) to get the active component Y12-26-A, and the active fraction of Y12-26-A was further purified by pre-TLC eluted with CH₂Cl₂–MeOH (40:1 v/v) to get active component with R_f=0.35, and finally purified by HPLC (Nucleosil C18 (Macherey-Nagel, Düren, Germany), 5 µm, 30×250 mm, 16 ml min^{−1}, UV detection 247 nm) eluted with CH₃OH–MeCN–H₂O (40:38:22, v/v/v) to yield **1** (9.6 mg, retention time=63 min).

Compound **1** was obtained as colorless amorphous powder (UV (MeOH) λ_{max} 247.2 nm and 278.4 nm) by a multi-step chromatography procedure from the culture broth of the *S. flavotricini* Y12-26. It gave an [M+Na]⁺ peak in the high-resolution electrospray-ionization MS (HR-ESI-MS) at *m/z* 629.3929 (Calcd for C₃₅H₅₈O₈Na, 629.4024). The ¹³C NMR spectrum of **1** (Table 1) attributable to one ester carbonyl (δ_C 172.0) and eight olefinic carbons (δ_C 122.3, 146.5, 134.5, 144.6, 142.7, 125.3, 132.5, 127.5), accounted for seven of the degrees of unsaturation, suggesting that **1** is bicyclic. The ¹H NMR spectrum (Table 1) showed signals for 10 methyl groups at δ_H (2.05,

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Figure 1 Structure of **1**.Table 1 ^1H (400 MHz) and ^{13}C (100 MHz) NMR data for **1** in CDCl_3

Position	δ_{C}	δH (J in Hz)
1	172.0	
2	122.3	
3	146.5	7.24 (1H, s)
4	134.5	
5	144.6	5.85 (1H, s)
6	36.7	2.57 (1H, m)
7	81.3	3.32 (1H, s)
7-OH		1.59 (1H, d, $J=6.5$)
8	39.8	1.95 (1H, s)
9	41.2	2.17 (1H, m), 1.95(1H, s)
10	142.7	
11	125.3	5.88 (1H, s)
12	132.5	6.52 (1H, dd, $J=14.8, 10.8$)
13	127.5	5.21 (1H, dd, $J=14.8, 9.6$)
14	82.6	3.93 (1H, t, $J=8.8$)
14-OMe	55.6	3.27 (3H, s)
15	76.0	4.98 (1H, d, $J=8.1$)
16	37.6	2.10 (1H, m)
17	70.4	4.11 (1H, d, $J=10.5$)
17-OH		4.86 (1H, s)
18	41.8	1.76 (1H, d, $J=7.2$)
19	98.9	
19-OH		5.64 (1H, s)
20	43.4	2.30 (1H, dd, $J=12.0, 4.1$), 1.15 (1H, s)
21	71.0	3.70 (1H, m)
22	41.1	1.33 (1H, m)
23	75.7	3.50 (1H, d, $J=10.1$)
24	27.9	1.90 (1H, m)
25	14.4	0.78 (3H, d, $J=6.6$)
26	13.7	2.05 (3H, s)
27	15.2	2.00 (3H, s)
28	17.5	1.10 (3H, d, $J=6.8$)
29	21.5	0.96 (3H, d, $J=5.6$)
30	20.1	1.95 (3H, s)
31	9.8	0.83 (3H, d, $J=6.7$)
32	6.9	1.05 (3H, d, $J=7.0$)
33	12.1	0.96 (3H, d, $J=5.6$)
34	20.8	0.92 (3H, d, $J=6.7$)

2.00, 1.95, 1.10, 1.05, 0.96, 0.96, 0.92, 0.83, 0.78, 3H each) and one methoxy group at δ_{H} (3.27, 3H). The ^{13}C NMR spectrum also showed the presence of 10 methyl groups at δ_{C} (21.5, 20.8, 20.1, 17.5, 15.2, 14.4, 13.7, 12.1, 9.8, 6.9) and one methoxy group at δ_{C} 55.6. These data also pointed to a bafilomycin derivative skeleton. The structure of **1** (Figure 1) was further elucidated by 2D-NMR analyses including HMQC, ^1H - ^1H COSY and HMBC experiments (Figure 2). Finally, **1**

Figure 2 COSY (bold lines) and HMBC (arrows) correlations of **1**.Table 2 MIC value for phytopathogens of **1**

Plant pathogens	MIC ($\mu\text{g ml}^{-1}$)		
	1	Bafilomycin A ₁	Bafilomycin D
<i>Pyricularia oryzae</i>	31	16	125
<i>Botrytis cinerea</i>	250	125	> 500
<i>Rhizoctonia solani</i>	> 500	250	> 500
<i>Collectotrichum lagenarium</i>	62	125	250
<i>Helminthosporium maydis</i>	500	250	> 500
<i>Gibberella zeae</i>	> 500	NT	NT

Abbreviation: NT, not tested.

Bafilomycins A₁ (>95.0%, HPLC) and D (>99.0%, HPLC) were both isolated from *Streptomyces flavotricini* Y12-26.

was established to be a new tetraene macrolide containing a 16-member lactone ring. The structure of **1** was nearly identical to that of bafilomycin J.⁷ The only difference between bafilomycin J and **1** was that the methyl ether at C-21 in bafilomycin J (21-OCH₃) was replaced by a hydroxy group in **1** (21-OH), accounting for the different numbers of methyl in their formulas.

The three olefins at C-2,C-4 and C-12 were all determined to be *E*-configuration on the basis of their high-field ^{13}C chemical shifts of the methyl residues 2-CH₃ (δ_{C} 13.7) and 4-CH₃ (δ_{C} 15.2) and the coupling constants between 12-H/13-H ($J_{12\text{-H}-13\text{-H}}$, 14.8). Another olefin at C-10 also possessed *E*-configuration when ^{13}C chemical shifts at 10-CH₃ (δ_{C} 21.5) were compared with those of known analogs.^{7,10} The relative configuration of **1** may be deduced by comparison of the coupling constant with bafilomycin A₁,¹³ but further assignments will be supported by NOESY correlations and X-ray diffraction analysis.

The antifungal activities of **1**, bafilomycins A₁ and D against plant pathogens were determined by the serial two-fold agar dilution method.¹⁴ As shown in Table 2, the antifungal activities of **1** and bafilomycin A₁ with a bicyclic structure seem stronger than that of bafilomycin D containing the ring-opened side chain. Compound **1** showed medium activities against *P. oryzae* and *Collectotrichum lagenarium* with MICs of 31 and 62 $\mu\text{g ml}^{-1}$, respectively. It showed low activities against *Botrytis cinerea* and *Helminthosporium maydis* with MICs of not less than 250 $\mu\text{g ml}^{-1}$. However, it did not show antifungal activities against *Rhizoctonia solani* and *Gibberella zeae* at a concentration of 500 $\mu\text{g ml}^{-1}$.

Bafilomycin A₁, B₁ and C₁ possess a broad activity spectrum, including fungi, Gram-positive bacteria and yeasts at a concentration of 1 mg ml⁻¹.⁵ The antifungal activity of these three bafilomycins is more pronounced than the antibacterial effects by the disk-diffusion assay.⁵ Compound **1** and the three bafilomycins all exhibited antagonistic activity against *B. cinerea*,⁵ and bafilomycin A₁ also exhibited antagonistic activity against *B. cinerea* and *R. solani* in our research. However, compound **1** exhibited antifungal activities against plant

pathogens at different concentrations. Therefore, these bafilomycins may have a role in the biocontrol of different plant pathogens. *S. flavotricini* strain Y12-26 could possibly be used as a bafilomycin producer for regulating plant disease.

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- Fiedler, H. P. *et al.* Marine actinomycetes as a source of novel secondary metabolites. *Antonie van Leeuwenhoek*. **87**, 37–42 (2005).
- Maskey, R. P. *et al.* Anti-cancer and antibacterial trioxacarcins with high anti-malaria activity from a marine streptomycete and their absolute stereochemistry. *J. Antibiot.* **57**, 771–779 (2004).
- Gorajana, A. *et al.* 1-hydroxy-1-norresistomycin, a new cytotoxic compound from a marine actinomycete, *Streptomyces chibaensis* AUBN(1)/7. *J. Antibiot.* **58**, 526–531 (2005).
- Kock, I., Maskey, R. P., Biabani, M. A., Helmke, E. & Laatsch, H. 1-hydroxy-1-norresistomycin and resistoflavin methyl ether: New antibiotics from marine derived streptomycetes. *J. Antibiot.* **58**, 530–535 (2005).
- Werner, G., Hagenmaier, H., Drautz, H., Baumgartner, A. & Zähler, H. Metabolic products of microorganisms. 224. Bafilomycins, a new group of macrolide antibiotics. Production, isolation, chemical structure and biological activity. *J. Antibiot.* **37**, 110–117 (1984).
- Kretschmer, A., Dorgerloh, M., Deeg, M. & Hagenmaier, H. The structures of novel insecticidal macrolides bafilomycins D and E, and oxohydrogrolidin. *Agric. Biol. Chem.* **49**, 2509–2511 (1985).
- Carr, G. *et al.* Bafilomycins produced in culture by *Streptomyces* spp. isolated from marine habitats are potent inhibitors of autophagy. *J. Nat. Prod.* **73**, 422–427 (2010).
- Bowman, E. J., Siebers, A. & Altendorf, K. Bafilomycins: a class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells. *Proc. Natl Acad. Sci. USA*. **85**, 7972–7976 (1988).
- Drose, S. *et al.* Inhibitory effect of modified bafilomycins and concanamycins on P- and V-type adenosinetriphosphatases. *Biochem.* **32**, 3902–3906 (1993).
- Ueda, J. Y., Hashimoto, J., Yamamura, H., Hayakawa, M., Takagi, M. & Shin-ya, K. A new 16-membered tetraene macrolide JBIR-100 from a newly identified *Streptomyces* species. *J. Antibiot.* **63**, 627–629 (2010).
- Wang, Y. R., Inoue, T. & Forgac, M. Subunit a of the yeast V-ATPase participates in binding of bafilomycin[J]. *J. Biol. Chem.* **280**, 40481–40488 (2005).
- van Schalkwyk, D. A. *et al.* Inhibition of plasmodium falciparum pH regulation by small molecule indole derivatives results in rapid parasite death. *Biochem. Pharmacol.* **79**, 1291–1299 (2010).
- Baker, G. H., Brown, P. J., Dorgan, R. J. J. & Everett, J. R. The conformational analysis of bafilomycin A₁. *J. Chem. Soc., Perkin Trans.* **2**, 1073–1079 (1989).
- Zhang, D. J., Liu, R. F., Li, Y. G., Tao, L. M. & Tian, L. Two new antifungal cyclic lipopeptides from *Bacillus marinus* B-9987. *Chem. Pharm. Bull.* **58**, 1630–1634 (2010).