Tetrahedron Letters xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

# Novel antifungal janthinopolyenemycins A and B from a co-culture of marine-associated *Janthinobacterium* spp. ZZ145 and ZZ148

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ARTICLE INFO

*Article history:*
Received 31 May 2018
Revised 7 July 2018
Accepted 13 August 2018
Available online xxxx

*Keywords:*
Marine bacterium
*Janthinobacterium* spp.
Co-culture
Janthinopolyenemycins
Antifungal activity

ABSTRACT

Two rare polyketides, named as janthinopolyenemycins A (**1**) and B (**2**), were isolated from a co-culture of two marine-sourced bacteria *Janthinobacterium* spp. ZZ145 and ZZ148. Their structures were established by a combination of extensive NMR spectroscopic analyses, HRESIMS data, and ECD calculation. Both janthinopolyenemycins A and B showed activity in inhibiting the growth of *Candida albicans* with a MIC value of 15.6 μg/mL and a MBC value of 31. 25 μg/mL.



## Introduction

Marine microorganisms are important sources for the discovery of novel natural products with unique structures and diverse bioactivities [1–5]. For example, salinosporamide A from marine *Salinospora* strain CNB-392[1] was reported to be an irreversible inhibitor of the 20S proteasome and entered clinical trials against multiple myeloma only three years after its discovery [6], and abyssomicin C from marine *Verrucosispora* strain AB 18-032 is the first natural inhibitor of *p*-aminobenzoate (*p*-ABA) biosynthesis, a pathway used by microorganisms but not found in humans [2]. Recent advances in genome sequencing have revealed that microorganisms have the potential to produce even more structurally diverse secondary metabolites because of the presence of a large number of the putative biosynthetic gene clusters that encode for secondary metabolites that are not seen under classical cultivation conditions [7–9]. In the last ten years, several strategies have been proposed to activate these cryptic biosynthetic pathways to induce the production of new secondary metabolites [10]. Co-culture of two or more microorganisms is one of these strategies and has received increasing interest related to the potential discovery of new bioactive natural products [10,11]. Examples of the production of new natural products induced by co-culture of marine-derived microorganisms included a chlorinated benzophenone pestalone from a co-culture of marine-sourced *Pestalotia* strain CNL-365 with bacterium strain CNJ-328 [12], the diterpenoids libertellenones A–D from a mixed fermentation of the same strain CNL-365 with fungus *Libertella* strain CNL-52 [13], and the cyclic depsipeptides emericellamides A and B from a co-fermentation of marine fungus *Emericella* strain CNL-878 with marine bacterium *Salinispora arenicola* [14].

As a part of our ongoing project for the discovery of novel antimicrobial and anti-glioma agents from marine-sourced microorganisms [15–24], two marine bacteria strains ZZ145 and ZZ148 were isolated from a marine soil sample. Strain ZZ145 and strain ZZ 148 were initially cultured independently by using different media. Chemical analysis and antifungal active assay indicated that strain ZZ145 in EY medium produced some secondary metabolites with weak antifungal activity and strain ZZ148 in B medium produced less metabolites with good antifungal activity. Because the obtained results were not satisfied, therefore, co-cultures of the two strains in different media were attempted to induce the production of novel natural products. Interestingly, a co-culture of strains ZZ145 and ZZ148 in rice solid medium produced distinctive secondary metabolites with stronger bioactivity. Chemical investigation of a crude extract prepared from a large

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https://doi.org/10.1016/j.tetlet.2018.08.022
0040-4039/

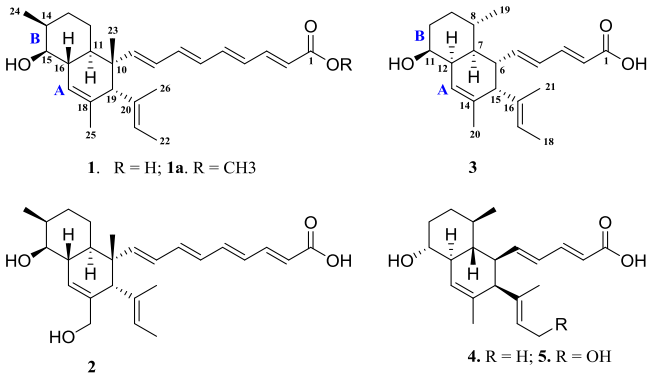


**Fig. 1.** Structures of janthinopolyenemycins A (**1**) and B (**2**).

scale co-culture of the two strains ZZ145 and ZZ148 in rice medium led to the isolation of two new polyketides, named as janthinopolyenemycins A (**1**) and B (**2**) (Fig. 1). In this article, we described the isolation and co-culture of strains ZZ145 and ZZ148, the structural elucidation of new janthinopolyenemycins A and B, and their bioactivities against the growth of methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Candia albicans*.

## Results and discussion

Strains ZZ145 and ZZ148 isolated from marine soil were assigned as *Janthinobacterium* sp. ZZ145 and *Janthinobacterium* sp. ZZ148 (Supplementary Data, Fig. $S_1$) based on the results (Figs. $S_3$ and $S_4$; Tables $S_1$ and $S_2$) of their 16S rDNA sequence analysis. A large scale co-culture (Fig. $S_2$) of the two strains ZZ145 and ZZ148 was conducted in rice solid medium and a crude extract prepared from the co-culture was separated by column chromatography, following by HPLC purification, to afford compounds **1** and **2**.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tetlet.2018.08.022.

Compound **1** has a molecular formula of $C_{26}H_{36}O_3$ deduced from its HRESIMS $[M-H]^-$ ion at *m/z* 395.2569 (calcd for $C_{26}H_{35}O_3$, 395.2564) and $^{13}C$ NMR data. Its UV spectrum showed a maximum absorption at 336 nm, suggesting the presence of the five conjugated double bonds. Interpretation of its $^{13}C$, $^1H$, and HSQC NMR spectra concluded that **1** is composed of one carbonyl ($\delta_C$ 167.8), six pairs of double bonds ($\delta_C$ 147.4, 144.4, 141.1, 138.0, 135.1, 133.3, 129.9, 129.2, 127.2, 122.7, 122.1, 120.9; $\delta_H$ 7.17, 6.67, 6.43, 6.36, 6.27, 6.08, 5.82, 5.74, 5.51, 5.17), one oxymethine ($\delta_C$ 74.3; $\delta_H$ 3.24), one non-protonated carbon ($\delta_C$ 41.6), four methines ($\delta_C$ 61.4, 40.1, 37.3, 34.5; $\delta_H$ 2.09, 1.96, 1.73, 1.22), two methylenes ($\delta_C$ 30.7, 20.4; $\delta_H$ 1.49, 1.42, 1.01), and five methyls ($\delta_C$ 22.8, 19.1, 18.1, 13.6, 11.9; $\delta_H$ 1.56, 1.53, 1.49, 0.96, 0.85) (Table 1). The six pairs of double bonds and one carbonyl accounted for seven out of the nine degrees of unsaturation required by the molecular formula and the remaining two came from rings A and B. Further analysis of its $^1H$-$^1H$ COSY and HMBC spectra demonstrated that the closest structural analogue of **1** is phomopsidin (**3**, Fig. 1) [25], but their structures have three differences. The first difference is the side chain of conjugated double bonds with a pentadienoic acid group for **3** and a nonatetraenoic acid group for **1**. The presence of the nonatetraenoic acid at C-10 was confirmed by $^1H$-$^1H$ COSY and HMBC correlations as depicted in Fig. 2. The second difference is the substitute group of ring A. In compound **3**, the hydrogen atom at C-6 was replaced by a methyl at C-10 in **1**. HMBC correlations of H-23 ($\delta_H$ 0.96, 3H, s) with C-9 ($\delta_C$ 147.4), C-10 ($\delta_C$ 41.6), C-11 ($\delta_C$ 40.1), and C-19 ($\delta_C$ 61.4) confirmed the methyl group at C-10 position. The third difference is the position of a methyl group of ring B. The methyl connected at C-14 in **1**, which was different from the methyl at C-8 in **3**, was established by HMBC correlations of H-24 ($\delta_H$ 0.85, 3H, d, 7.1 Hz) with C-13 ($\delta_C$ 30.7), C-14 ($\delta_C$ 34.5), C-15 ($\delta_C$ 74.3) and COSY correlations of H-14 ($\delta_H$ 1.96, 1H, m) with H-13 ($\delta_H$ 1.42, 1H, m), H-15 ($\delta_H$ 3.24, 1H, m), and H-24. So far, the planar structure of **1** was determined,

**Table 1**
$^{13}C$ and $^1H$ NMR data of janthinopolyenemycins A (**1**) and B (**2**) (in DMSO $d_6$).

| No. | 1 | | 2 | |
|---|---|---|---|---|
| | C, type | H (*J* in Hz) | C, type | H (*J* in Hz) |
| 1 | 167.8, C | – | 168.3, C | – |
| 2 | 120.9, CH | 5.82, 1H, d (15.2) | 122.3, CH | 5.83, 1H, d (15.1) |
| 3 | 144.4, CH | 7.17, 1H, dd (15.2, 11.5) | 143.4, CH | 7.12, 1H, dd (15.1, 11.5) |
| 4 | 129.2, CH | 6.36, 1H, dd (14.9, 11.5) | 129.3, CH | 6.39, 1H, dd (14.9, 11.5) |
| 5 | 141.1, CH | 6.67, 1H, dd (14.9, 11.1) | 140.2, CH | 6.64, 1H, dd (14.9, 10.9) |
| 6 | 129.9, CH | 6.27, 1H, dd (14.9, 11.1) | 129.8, CH | 6.28, 1H, dd (14.9, 10.9) |
| 7 | 138.0, CH | 6.43, 1H, dd (14.9, 10.5) | 137.4, CH | 6.42, 1H, dd (14.9, 10.9) |
| 8 | 127.2, CH | 6.08, 1H, dd (15.6, 10.5) | 127.1, CH | 6.10, 1H, dd (15.5, 10.9) |
| 9 | 147.4, CH | 5.51, 1H, d (15.6) | 147.1, CH | 5.50, 1H, d (15.5) |
| 10 | 41.6, C | – | 41.1, C | – |
| 11 | 40.1, CH | 1.22, 1H, m | 39.6, CH | 1.29, 1H, m |
| 12 | 20.4, $CH_2$ | 1.01, 2H, m | 20.3, $CH_2$ | 1.01, 2H, m |
| 13 | 30.7, $CH_2$ | 1.42, 1H, m; 1.49, 1H, m | 30.6, $CH_2$ | 1.43, 1H, m; 1.48, 1H, m |
| 14 | 34.5, CH | 1.96, 1H, m | 34.4, CH | 1.97, 1H, m |
| 15 | 74.3, CH | 3.24, 1H, m | 74.4, CH | 3.23, 1H, m |
| 16 | 37.3, CH | 1.73, 1H, m | 37.3, CH | 1.77, 1H, m |
| 17 | 122.7, CH | 5.74, 1H, brs | 121.5, CH | 5.97, 1H, brs |
| 18 | 133.3, C | – | 138.4, C | – |
| 19 | 61.4, CH | 2.09, 1H, s | 56.4, CH | 2.30, 1H, s |
| 20 | 135.1, C | – | 135.2, C | – |
| 21 | 122.1, CH | 5.17, 1H, q (6.5) | 121.9, CH | 5.14, 1H, q (6.3) |
| 22 | 13.6, $CH_3$ | 1.56, 3H, d (6.5) | 13.5, $CH_3$ | 1.56, 3H, d (6.3) |
| 23 | 18.1, $CH_3$ | 0.96, 3H, s | 17.8, $CH_3$ | 0.96, 3H, s |
| 24 | 11.9, $CH_3$ | 0.85, 3H, d (7.1) | 11.8, $CH_3$ | 0.87, 3H, d (7.1) |
| 25 | 22.8, $CH_3$ | 1.49, 3H, s | 63.7, $CH_2$ | 3.66, 2H, s |
| 26 | 19.1, $CH_3$ | 1.53, 3H, s | 21.2, $CH_3$ | 1.53, 3H, s |
| OH-15 | – | 4.63, 1H, d (3.6) | – | – |