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Streptopyrazinones A–D, rare metabolites from marine-derived *Streptomyces* sp. ZZ446

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ABSTRACT

Secondary metabolites from marine-associated actinomycetes are important source for the discovery of novel bioactive compounds. In this study, an actinomycete *Streptomyces* sp. ZZ446 was isolated from coastal soils and different media were used to culture this isolated marine actinomycete. It has been found that this actinomycete in the liquid medium of 2216 E with sea salt produced five new compounds of streptopyrazinones A–D (**1**–**4**) and *N*-acetyl-L-isoleucine-L-leucinamide (**5**) as well as six known diketopiperazines (**6**–**11**) and one alkaloid (**12**). Structures of the new compounds were determined by extensive NMR analyses, HRESIMS data, electronic circular dichroism (ECD) calculation, chemical degradation, Marfey's method, and X-ray diffraction analysis. This type of streptopyrazinones A–D (**1**–**4**) is rarely found in the natural resources. New compounds **1**–**5** showed activity in inhibiting the growth of *Candida albicans* and methicillin-resistant *Staphylococcus aureus*.

1. Introduction

The marine environment has proven to be an important source of bioactive natural products, which have had nine approved drugs and 12 current clinical trial agents for the past over 50 years.[1–3] There has been a growing perception that many marine bioactive natural products found originally from collected biomass of macroorganisms, such as sponges, mollusks, and tunicates, are actually being produced by symbiotic or associated microorganisms.[1] Secondary metabolites from marine-associated microorganisms, especially marine actinomycetes, are abundant source for the discovery of novel bioactive compounds and drug leads.[4–7]

Over the course of our ongoing project for the discovery of novel bioactive compounds from marine microorganisms,[8–15] a strain ZZ446 was isolated from a sample of coastal soils. Ten different media were used to culture this marine actinomycete strain ZZ446 to induce different silent gene clusters to express and then produce different novel natural products.[15–17] A crude extract prepared from the culture of strain ZZ446 in the liquid medium of 2216 E with sea salt showed the most antimicrobial activity and contained more metabolites. Chemical investigation into this active crude extract resulted in the isolation and identification of 12 compounds including five new ones. In this article, we describe the isolation and culture of strain ZZ446, the isolation and structural elucidation of the new compounds, and their activities against the growth of methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Candida albicans* and the proliferation of different glioma cells.

2. Results and discussion

Strain ZZ446 (Supplementary Data Fig. S1) was assigned as *Streptomyces* sp. ZZ446 (Table S1) based on the result (Fig. S2) of its 16S rDNA sequence analysis. Ten different media a-j (Table S2) were used to culture this marine actinomycete and a crude extract prepared from the culture of strain ZZ446 in the liquid medium of 2216 E with sea salt (medium h) showed the most activity (Fig. S3) against the growth of methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Candida albicans* and had more metabolites as detected by HPLC (Fig. S4 and Fig. S5). A total of 60 L culture of strain ZZ446 was conducted in medium h and the crude extract made from this large culture was separated by column chromatography, followed by HPLC purification, to afford five new

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compounds **1**–**5** (Fig. 1) and seven known ones (Fig. S6). Based on the NMR and HRESIMS data and the results (Figs. S7–S13) from Marfey's analyses, the known compounds were identified as cyclo-L-proline-L-methionine (**6**),[18,19] cyclo-L-proline-L-valine (**7**),[19] cyclo-L-proline-L-leucine (**8**),[18] cyclo-L-proline-L-isoleucine (**9**),[20] cyclo-L-proline-L-phenylalanine (**10**),[21] cyclo-D-proline-L-phenylalanine (**11**),[21] and *N*-acetyl-β-oxotryptamine (**12**).[22] The ^{13}C NMR data of **6**–**12** were summarized in Table S3.

Compound **1** was obtained as colorless needle crystals (mp 177–178 °C) and has a molecular formula of $C_{13}H_{21}N_3O_2$ deduced from its HRESIMS ions at *m/z* $[M+H]^+$ 252.1711 (calcd. 252.1712) and $[M+Na]^+$ 274.1531 (calcd. 274.1531) as well as its ^{13}C NMR data. Its UV spectrum showed absorption at 228 and 325 nm, which are very close to the UV absorption of arglecin (**1a**)[23–25] and argvalin (**1b**),[26,27] two metabolites with a skeleton of 2(1*H*)-pyrazinone and a guanidine group that were previously isolated from *Streptomyces* sp. The carbon signals at δ_C 156.4 (C, C-3), 155.5 (C, C-2), 138.8 (C, C-4), 120.5 (CH, C-1) and proton signal at δ_H 7.08 (1H, s) observed in the ^{13}C and ^1H NMR spectra suggested the presence of a skeleton of 2(1*H*)-pyrazinone in **1**, which were confirmed by HMBC correlations (Fig. 2) and X-ray diffraction analysis (Fig. 3). The acetyl group was easily recognized by its NMR signals at δ_C 169.0 (C, C-12), 22.6 (CH_3, C-13) and δ_H 1.79 (3H, s, H_3-13) and HMBC correlation (Fig. 2) of H-13 with C-12; while the isobutyl (2-methylpropyl) group was indicated by its characteristic NMR signals at δ_C 41.0 (CH_2, C-5), δ_C 26.0 (CH, C-6), 22.5 (CH_3, C-7, C-8) and δ_H 2.45 (2H, d, 7.1 Hz, H-5), 2.05 (1H, m, H-6), 0.85 (6H, d, 6.7 Hz, H-7, H-8). HMBC correlations (Fig. 2) of H-5 with C-2 and C-3, NH-11 (δ_H 7.85, 1H, br t, 5.7 Hz) with C-11 (δ_C 37.8) and C-12, and H-11 (δ_H 2.99, 2H, m) with C-12 established that the acetyl group and the isobutyl group were connected to NH-11 and C-2, respectively. The ^{13}C NMR spectrum of **1** displayed 13 signals, of which four were assigned to the skeleton of 2(1*H*)-pyrazinone, four to the isobutyl group, two to the acetyl group, and the remaining three to a partial structure A of a trimethylene group ($-CH_2-CH_2-CH_2-$) located between C-4 and NH-11, which was confirmed by HMBC correlations as depicted in Fig. 2. The ^{13}C and ^1H NMR data (Tables 1 and 2) were assigned using HSQC and HMBC correlations. Based on the foregoing evidence, compound **1** was elucidated as a new compound, named as streptopyrazinone A, a pyrazinone derivative.

Compounds **2** and **1** have very similar UV spectra, suggesting that they are analogues. Compound **2** has a molecular formula of $C_{14}H_{23}N_3O_2$ as established by its HRESIMS ions at *m/z* $[M+H]^+$ 266.1864 and $[M+Na]^+$ 288.1679, 14 mass units higher than **1**, corresponding to a CH_2 group. Detailed comparison of the NMR data (Tables 1 and 2) of **2** with those of **1** indicated that both **2** and **1** share the common isobutyl and acetyl groups and the skeleton of 2(1*H*)-pyrazinone, but have a different partial structure A.

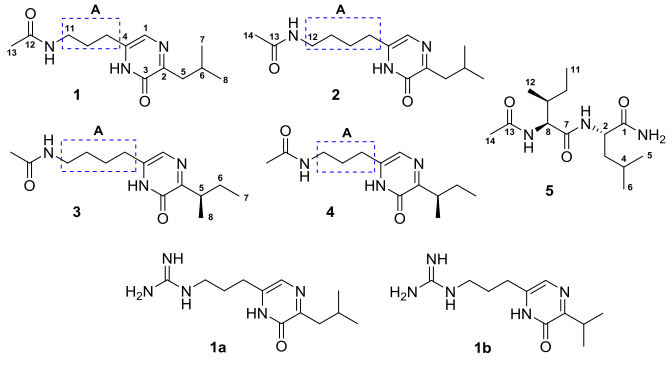

Fig. 1. Structures of compounds **1**–**5** isolated from the culture of *Streptomyces* sp. ZZ446.

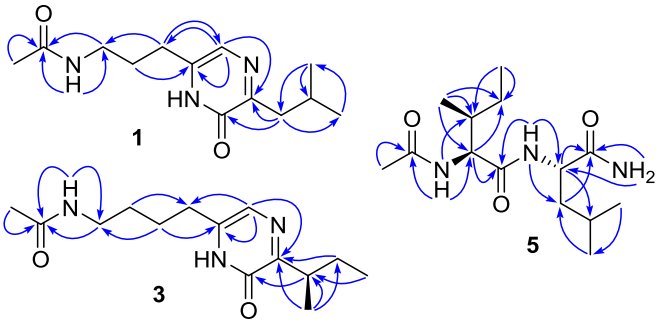

Fig. 2. HMBC correlations of compounds **1**, **3**, and **5**.

Consideration of the HRESIMS data, compound **2** must have an additional methylene (-CH_2-) with the partial structure A of a quadmethylene group ($-CH_2-CH_2-CH_2-CH_2-$). Accordingly, compound **2** was identified as streptopyrazinone B, a new pyrazinone derivative.

Compounds **3** and **2** have the same molecular formula of $C_{14}H_{23}N_3O_2$ and similar UV absorption. Comparison of the NMR data (Tables 1 and 2) of **3** and **2** proved that they are structurally different only in the substitute at C-2. In compound **3**, the substitute at C-2 was assigned as 1-methylpropyl as indicated by its NMR signals at δ_C 35.6 (CH, C-5), 27.0 (CH_2, C-6), 11.8 (CH_3, C-7), 17.8 (CH_3, C-8) and δ_H 3.05 (1H, m, H-5), 1.37 (1H, m, H-6), 1.67 (1H, m, H-6), 0.77 (3H, t, 7.4 Hz, H-7), 1.06 (3H, d, 6.9 Hz, H-8). HMBC correlations as shown in Fig. 2 also demonstrated the presence of this 1-methylpropyl group at C-2. The absolute configuration at C-5 of **3** was determined by electronic circular dichroism (ECD) calculation. The conformational analyses were carried out via random searching in the Sybyl-X 2.0 using the MMFF94S force field with an energy cutoff of 2.5 kcal/mol.[28] The results showed six lowest energy conformers for compound **3**. Subsequently, the two lowest conformers C_1 and C_2 (Table S4 and Fig. S63) weighing for more than 99% were further re-optimized using DFT at the b3lyp/6-31+g(d) level in gas phase by the GAUSSIAN 09 program.[29] The energies, oscillator strengths, and rotational strengths (velocity) of the first 60 electronic excitations were calculated using the TDDFT methodology at the b3lyp/6-311++g(d,p) level in vacuum. The ECD spectra were simulated by the overlapping Gaussian function (half the bandwidth at 1/e peak height, $\sigma = 0.2$).[30] To get the final spectra, the simulated spectra of the conformers were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy (ΔG). Theoretical ECD spectrum of the corresponding enantiomer was obtained through the direct inverse of the ECD spectrum of the calculated model molecule. By comparing the experiment spectrum with the calculated ECD spectra (Fig. 4), the absolute configuration at C-5 of **3** was determined to be *R*. Based on the above evidence, compound **3** was determined as streptopyrazinone C, a new pyrazinone derivative.

Compounds **4** and **1** share the same molecular formula of $C_{13}H_{21}N_3O_2$ and similar UV absorption, suggesting that **4** is also an analogue of streptopyrazinones A–B (**1**–**3**). Further analysis of the NMR data (Tables 1 and 2) of **4** and **1** demonstrated that the structural difference between **4** and **1** is only the substitute at C-2. This means that the isobutyl group at C-2 in **1** is replaced by a 1-methylpropyl group in **4**. The similar ^{13}C chemical shifts for C-1 to C-8 between **4** and **3** also supported that **4** has the 1-methylpropyl group at C-2. Just like **1** and **2**, the structural difference between **4** and **3** is due to the partial structure A with a trimethylene group for **4** and a quadmethylene group for **3**. The absolute configuration at C-5 of **4** was assigned as *R* also based on the result of ECD calculation (Fig. 5). Therefore, compound **4** was