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Anti-MRSA actinomycins D_1-D_4 from the marine sponge-associated *Streptomyces* sp. LHW52447

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ABSTRACT

Actinomycins D_1–D_4 (**1**–**4**), four new D-type actinomycin analogues, were isolated from the fermentation broth of a strain of marine sponge-associated *Streptomyces* sp. LHW52447, together with actinomycin D (**5**). The structures of **1**–**4** were determined by a combination analysis of HRMS and NMR spectroscopic methods, and their absolute configurations of amino acids were determined by Marfey's analysis. Actinomycins D_1 (**1**) and D_2 (**2**) are the first two naturally occurring actinomycins with incorporation an oxazole unit into the central phenoxazinone chromophore. Both **1** and **2** showed more potent antibacterial activities against three strains of pathogenic methicillin-resistant *Staphylococcus aureus* (MRSA) with MIC values of 0.125–0.25 μg/mL than those of **3**–**5** with MIC values of 0.5–1.0 μg/mL, which suggested that the anti-MRSA activity might be enhanced by the incorporation of an additional oxazole unit. In addition, the cytotoxicity evaluation against WI-38 human diploid lung fibroblasts revealed that the incorporation of oxazole unit could decrease the cytotoxicity of actinomycins on human normal cells.

1. Introduction

Actinomycins are a well-known class of chromopeptide isolated from many species of *Streptomyces* [1]. These metabolites consist of two cyclic pentapeptide lactones (α- and β-rings) attached to a phenoxazinone chromophore through amide bonds. About 34 naturally occurring actinomycins have been reported so far [2], while more than 40 structural analogues have been obtained from precursor-directed biosynthesis and synthetic efforts [3]. This natural product class shows very potent antitumor and antibacterial activities and remains the subject of ongoing research [2,4,5]. These chromopeptides act as intercalators with the DNA double helix [6]. The phenoxazinone chromophore fits between guanine/cytosine base pairs, while the peptidolactone side chains lying inside the minor groove of the helix [7]. With some notable exceptions, the structure elucidation of several actinomycins relies on mass spectroscopic analyses, with absolute amino acid configurations often going unassigned [8,9]. Such incomplete assignments could make it difficult to determine the structure-activity relationship between structural analogues.

In an ongoing investigation of the chemical diversity of marine sponge-associated microbes [10], our laboratory has accumulated an extensive library of microbial isolates assembled from the marine sponges collected from the South China Sea. A strain of *Streptomyces* sp. LHW52447, isolated from the marine sponge *Phyllospongia foliascens*, was cultivated on a range of media, and metabolite production was profiled by HPLC-DAD-MS analysis. The analysis revealed an array of interesting metabolites ($m/z > 1200$) in an organic extract derived from ISP2 broth medium. Scaled up cultivation followed by solvent extraction and partitioning, and reversed phase chromatography, yielded a series of chromopeptides, actinomycins D_1–D_4 (**1**–**4**) and actinomycin D (**5**) (Fig. 1). In structural characterization of **1**–**4** we demonstrated a workflow

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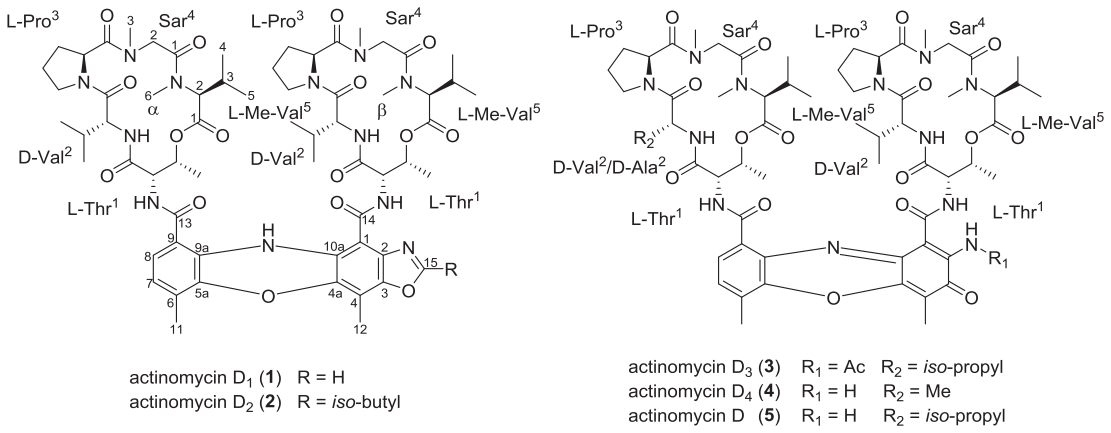

Fig. 1. Actinomycins D_1–D_4 (**1**–**4**) and actinomycin D (**5**) from the sponge-associated *Streptomyces* sp. LHW52447.

capable of complete structure assignments, including absolute configurations for all amino acid residues.

2. Results and discussion

Compound **1** was isolated as orange amorphous powders. The HRESI(+)MS analysis of **1** showed a sodium adduct ion consistent with a molecular formula of $C_{63}H_{86}N_{12}O_{16}$ requiring 27 double-bond equivalents (DBE). The ^1H and ^{13}C NMR data for **1** (Tables 1 and 2) displayed typical features of an actinomycin compound. Detailed analysis of the ^1H, ^{13}C, COSY, HSQC, and HMBC NMR spectra allowed for the assignment of the amino acids within the α- and β-rings as Thr, Val, Pro, Sar, and MeVal (Fig. 2). The amino acid composition of both rings is identical with actinomycin D (**5**) [11]. However, the 12 amu difference between **1** and actinomycin D indicated adduct of a carbon atom in **1**, which was supported by an additional aromatic methine (δ_C 151.7/δ_H 8.19, CH-15). This methine proton showed HMBC correlations with C-2 and C-3, suggesting the phenoxazinone chromophore in actinomycin D was replaced by oxazolophenoxazine in **1**. This assignment was supported by HMBC correlations of 10-NH/C-1, C-4a, C-5a, and C-9 as well as H_3-12/C-3, C-4, and C-4a. Furthermore, the amino acid sequences of α-ring and β-ring in **1** were determined by the NOESY correlations depicted in Fig. 3, which was confirmed by the TOF-MS/MS fragmentation (Fig. S23). The absolute configurations of amino acid residues in **1** was analyzed by 6 M HCl, followed by Marfey's analysis as described previously [10]. The presence of L-Thr, D-Val, L-Pro, Sar, and L-MeVal was unambiguously determined by comparison with authentic standards (Fig. S24). Thus, the structure of **1** was determined as a new member of the actinomycin family and subsequently named actinomycin D_1 (see Fig. 1).

The molecular formula of **2** was determined to be $C_{67}H_{94}N_{12}O_{16}$ on the basis of HRESI(−)MS data. The NMR spectroscopic data for **2** (Tables 1 and 2) revealed characteristic features of actinomycins and were quite similar to those of actinomycin D_1 (**1**), except for the resonances for an *iso*-butyl group (δ_C/δ_H 36.5/2.81, 27.1/2.23, 22.2/1.03, and 22.1/1.01) in the high field of ^1H and ^{13}C NMR spectra, supported by the COSY correlations of H_2-16/H-17, H-17/H_3-18, and H-17/H_3-19. Moreover, the placement of the *iso*-butyl group at C-15 (δ_C 165.8) was determined by HMBC correlations of H_2-16/C-15, C-18, and C-19 as well as H-17/C-15, C-16, and C-18. The sequence and absolute configurations of amino acids in **2** were established by TOF-MS/MS fragmentation (Fig. S32) and Marfey's analysis (Fig. S33). The overall structure of **2** was assigned as a new analogue of the actinomycin series and designated as actinomycin D_2.

Compound **3** was assigned the molecular formula $C_{64}H_{88}N_{12}O_{17}$ by the HRESI(−)MS data. The NMR data showed the presence of the phenoxazinone chromophore and two identical pentapeptidolactones that contained Thr, Val, Pro, Sar, and MeVal moieties (Tables 1 and 2). Compound **3** differed with actinomycin D by an additional acetylation of 2-NH (δ_C 169.9, C-15, and δ_C 24.1/δ_H 2.16, CH_3-16), which was supported by the 2D NMR analysis. In combination with the TOF-MS/MS fragmentation (Fig. S41) and Marfey's analysis (Fig. S42), the data were consistent with structure of **3** as depicted and named actinomycin D_3.

The molecular formula of compound **4** was determined as $C_{60}H_{82}N_{12}O_{16}$ by the HRESI(−)MS data with less of 28 amu (C_2H_4) than actinomycin D. The NMR data recorded for **4** was nearly identical with those of actinomycin D, except for the replacement of Val by Ala in α-ring of **2** (Tables 1 and 2). Further COSY and HMBC correlations were full agreement with Ala in α-ring. Marfey's analysis revealed the D-configuration of Ala (Fig. S51). Thus the whole structure of **4** was assigned as shown and named actinomycin D_4.

The antibacterial activity of the isolated actinomycins was evaluated against three strains of pathogenic methicillin-resistant *Staphylococcus aureus* (MRSA) [12], using chloramphenicol and daptomycin as positive control (Table 3), the anti-MRSA activities of **1** and **2** are nearly 2–4 times potent than those of **3**–**5**, which suggested the anti-MRSA activity might be enhanced by incorporation of an additional oxazole unit into the phenoxazinone chromophore. In addition, the cytotoxic activity of the five isolates was evaluated against WI-38 human diploid lung fibroblasts (Table 3). The results showed that **1** and **2** showed less cytotoxicity against human normal WI38 cells than **3**–**5**, which suggested that the incorporation of oxazole unit into the chromophore could more dramatically decrease the cytotoxicity of actinomycins against human normal cells.

3. Conclusion

In summary, four new D-type actinomycin analogues, actinomycins D_1–D_4 (**1**–**4**), were isolated from the fermentation broth of a strain of marine sponge-associated *Streptomyces* sp. LHW52447. Antibacterial and cytotoxicity assays showed that the incorporation of oxazole unit into the phenoxazinone chromophore would enhance the antibacterial activity of actinomycins while decrease their cytotoxicity against human normal cells. This represents the first actinomycin compounds from a marine source and highlights the importance of continued efforts toward screening for chemical diversity within sponge-associated microbes.