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BRIEF COMMUNICATION

Albumycin, a new isoindolequinone from *Streptomyces albus* J1074 harboring the fluostatin biosynthetic gene cluster

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Abstract

Heterologous expression of the fluostatin biosynthetic gene cluster from the marine-derived *Micromonospora rosaria* SCSIO N160 in *Streptomyces albus* J1074 led to the isolation of a novel isoindolequinone albumycin (**1**) and a known isoquinolinequinone mansouramycin A (**2**). The structure of **1** was elucidated on the basis of detailed 1D and 2D NMR spectroscopic analysis. Mansouramycin A (**2**) is active against methicillin-resistant *Staphylococcus aureus* ATCC 43300, with a MIC of 8 μg ml^{-1}, while albumycin (**1**) displayed negligible antibacterial activities. This study represents another example of activation of secondary metabolites that are non-relevant to the heterologously introduced biosynthetic gene cluster in a bacterial host.

Marine-derived actinomycetes are an important source of a variety of novel natural products with comprehensive biological activities. In recent years, the rediscovery rate for known compounds from marine-derived actinomycetes is increasing [1]. Thus, novel methods have been developed for the rapid dereplication of known natural products and targeted identification of novel compounds, such as the imaging mass spectrometry method [2], the strain prioritization by real-time polymerase chain reaction [3], the resistance gene-guided genome mining [4], the bioinformatics-guided search strategy [5], and the CRISPR/Cas9-based editing-directed activation of silent clusters [6]. Nevertheless, heterologous expression is still thought as a promising tool for natural products studies and has been widely applied to enable the discovery of new compounds from silent biosynthetic gene clusters, and to improve the yield of natural products [7].

During our previous studies, the intact fluostatin gene cluster (*fls*) from South China Sea-derived *Micromonospora rosaria* SCSIO N160 has been successfully expressed in different heterologous hosts, such as *Streptomyces coelicolor* YF11 [8], and *Streptomyces albus* J1074 [9], to afford a variety of C–C/C–N-coupled dimeric or trimeric fluostatins, and several aromatic polyketides derived from different chain lengths and diverse cyclization patterns [9, 10]. A further careful inspection of the metabolite extracts of the recombinant strain *S. albus* J1074 harboring the fluostatin biosynthetic gene cluster revealed the presence of two minor untapped peaks, the UV spectra of which were strikingly distinct from the identified aromatic polyketides. Subsequent-targeted isolation afforded to two products, a new isoindolequinone albumycin (**1**) and a known isoquinolinequinone mansouramycin A (**2**) [11–13]. Herein we reported the detailed isolation, structural elucidation, and antibacterial activities of **1** and **2**.

The 20 l of fermentation cultures of the recombinant strain *S. albus* J1074/pCSG5033 have been previously described, which led to the discovery of a number of dimeric or trimeric fluostatin analogues, and several aromatic polyketides differed in chain lengths and cyclization

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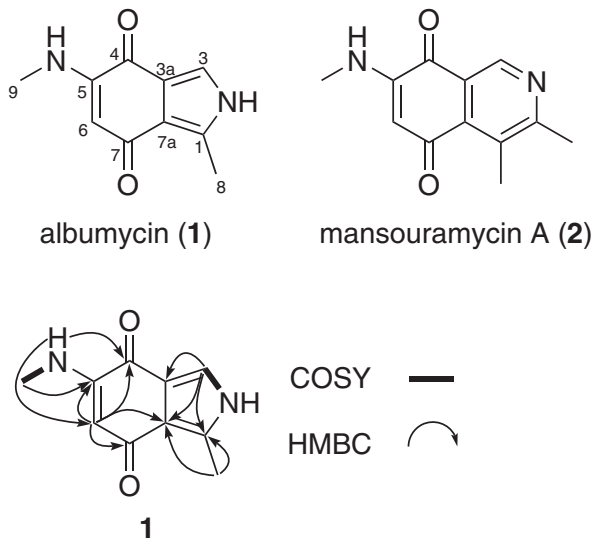

Fig. 1 Chemical structures of compounds **1** and **2**, and selected key COSY and HMBC correlations of **1**

patterns [9, 10]. Further careful and detailed investigation on the crude extracts enabled the isolation and characterization of two trace compounds, including a new isoindolequinone albumycin (**1**) and the known isoquinolinequinone mansouramycin A (**2**) (Fig. 1).

Albumycin (**1**) was obtained as a red powder. Its molecular formula was established as $C_{10}H_{10}N_2O_2$ (*m/z* 191.0817 $[M+H]^+$, calcd. for 191.0815) with seven degrees of unsaturation by high-resolution electrospray ionization mass spectroscopy (HRESIMS). The UV maximum absorption at 375 nm indicated a highly delocalized conjugated system. Analysis of the ^{1}H NMR spectrum of **1** (Table 1) revealed the presence of a singlet methyl at δ_H 2.40, a doublet methyl at δ_H 2.68 (d, $J = 5.1$ Hz), and two olefinic protons at δ_H 5.12 (s) and 7.43 (d, $J = 2.5$ Hz). In addition, the presence of two amino proton signals were implied by a broad quartet at δ_H 7.01 (q, $J = 5.1$ Hz) and a broad singlet at δ_H 11.90. The DEPT 135 spectrum classified the 10 carbons in **1** as two methyls, two olefinic methines, and six quaternary carbons (four olefinic ones, and two keto signals at δ_C 177.0 and 181.7). The presence of a member of isoindole-4,7-diones [14] in **1** was supported by HMBC correlations from H-3 (δ_H 7.43) to C-1/C-3a/C-7a and H-6 (δ_H 5.12) to C-4/C-5/C-7/C-7a, and the COSY correlation between NH-2 and H-3 (Fig. 1). Location of the singlet methyl (Me-8, δ_H 2.40, s) at C-1 was strongly supported by HMBC correlation from H_3-8 to C-1/C-7a (Fig. 1). Furthermore, the presence of the methylamino group (CH_3NH-) was confirmed by the COSY correlation between H_3-9 (δ_H 2.68, d, $J = 5.1$ Hz) and 5-NH (δ_H 7.01, q, $J = 5.1$ Hz) (Fig. 1). Location of the methylamino group at C-5 was based on HMBC correlations from H_3-9 to C-5 and from 5-NH to C-4/C-6 (Fig. 1). Taken together, the structure of **1** was unambiguously determined to be 1-methyl-5-methylamideisoindole-4,7-dione, designated albumycin (**1**).

Table 1 ^{1}H and ^{13}C NMR data for **1** and **2** (TMS, δ in ppm)

Position	1[a]		Position	2[b]	
	δ_C, type	δ_H, multi (*J* in Hz)		δ_C, type	δ_H, multi (*J* in Hz)
1	132.8, C		1	145.6, CH	8.93, s
3	121.6, CH	7.43, d, 2.5	3	167.9, C	
3a	119.1, C		4	133.5, C	
4	177.0, C		4a	137.1, C	
5	150.9, C		5	186.2, C	
6	100.0, CH	5.12, s	6	103.3, CH	5.71, s
7	181.7, C		7	149.8, C	
7a	116.4, C		8	182.4, C	
8	11.6, CH_3	2.40, s	8a	125.2, C	
9	28.8, CH_3	2.68, d, 5.1	3-CH_3	24.7, CH_3	2.69, s
NH-2		11.90, br s	4-CH_3	16.7, CH_3	2.76, s
5-NH		7.01, q, 5.1	NCH_3	29.5, CH_3	2.92, br s
			7-NH		7.59, br s

[a]^{1}H and ^{13}C NMR were recorded at 500 and 125 MHz in DMSO-d_6, respectively

[b]^{1}H and ^{13}C NMR were recorded at 700 and 176 MHz in CD_3OD, respectively

Detailed analysis of the ^{1}H and ^{13}C NMR data of compound **2** (Table 1) revealed that it was identical to isoquinolinequinone mansouramycin A (**2**), previously reported from a marine-derived *Streptomyces* sp. Mei37 [12]. ^{1}H NMR (700 MHz, CD_3OD) δ 8.93 (1H, s, H-1), 7.59 (1H, br s, 7-NH), 5.71 (1H, s, H-6), 2.92 (3H, br s, 7-NCH_3), 2.76 (3H, s, 4-CH_3), 2.69 (3H, s, 3-CH_3). ^{13}C NMR (176 MHz, CD_3OD) δ 186.2 (C-5), 182.4 (C-8), 167.9 (C-3), 149.8 (C-7), 145.6 (C-1), 137.1 (C-4a), 133.5 (C-4), 125.2 (C-8a), 103.3 (C-6), 29.5 (NCH_3), 24.7 (3-CH_3), 16.7 (4-CH_3).

Compounds **1** and **2** were evaluated for their antibacterial activities against five indicator strains: *Staphylococcus aureus* ATCC 29213, *Acinetobacter baumannii* ATCC 19606, *Bacillus subtilis* SCSIO BS01, *Micrococcus Luteus* SCSIO ML01, and methicillin-resistant *S. aureus* ATCC 43300 by measuring minimal inhibition concentrations (MIC). Compound **2** exhibited more potent antibacterial activities than compound **1** toward the tested strains (Table 2).

In conclusion, a novel compound albumycin (**1**) and the known mansouramycin A (**2**) were isolated and identified from *S. albus* J1074 harboring the heterologous fluostatin biosynthetic gene cluster (*fls*). A variety of mansouramycin analogues have been previously identified from several actinomycetal strains [11–13]. Mansouramycins (such as **2**) feature an isoquinolinequinone skeleton, in which a

Table 2 Antibacterial activities of **1** and **2**

	MIC (μg ml^{-1})				
	S. aureus ATCC 29213	*A. baumannii* ATCC 19606	*B. subtilis* SCSIO BS01	*M. Luteus* SCSIO ML01	Methicillin-resistant *S. aureus* ATCC 43300
1	>64	>64	>64	>64	>64
2	16	64	16	16	8
Trimethoprim	1	1	0.25	1	8

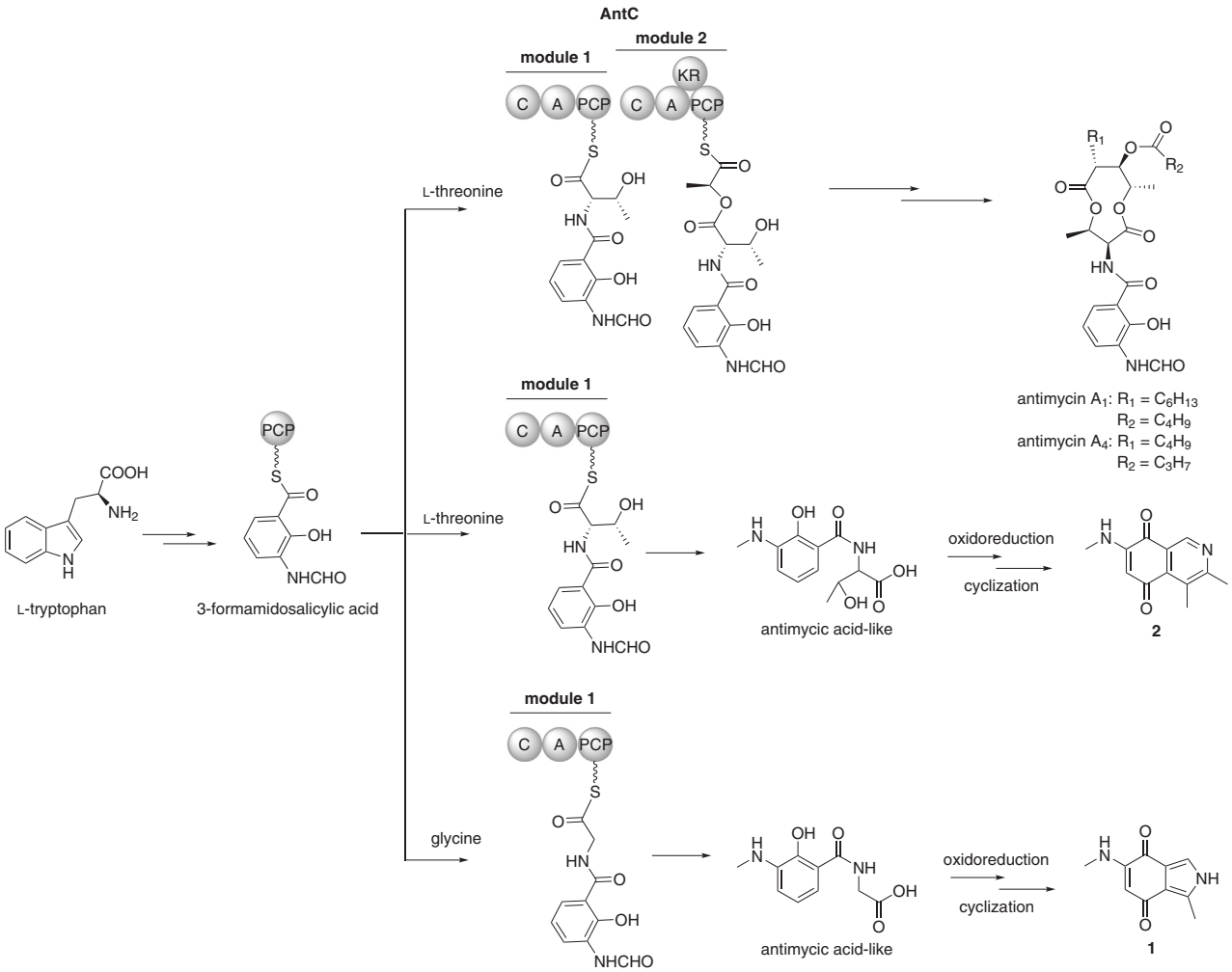

Fig. 2 Proposed antimycin-associated biosynthetic pathway of **1** and **2**

p-benzoquinone is fused to a six-membered pyridine ring. Albumycin (**1**) is different from mansouramycins by possessing an isoindolequinone scaffold, in which a five-membered pyrrole ring is fused to a *p*-benzoquinone. Obviously, no enzymes encoded in the *fls*-gene cluster could be found suitable to govern the biosynthesis of albumycin (**1**) and mansouramycin A (**2**), given the recent understanding of the fluostatin biosynthesis [8–10, 15, 16]. We proposed that the production of both **1** and **2** was probably associated with other biosynthetic gene clusters encoded in *S. albus* J1074, although **1** and **2** were not dectected in the native host *S. albus* J1074 under the same cultivation conditions. Consistent with this proposal, mansouramycin analogues have been reported from *S. albus* J1074 by activating silent gene clusters using chemical elicitors [13]. Also, there have been examples of producing secondary metabolites that are non-relevant to the heterologous biosynthetic gene clusters. For example, introduction of the thienamycin biosynthetic gene cluster from *Streptomyces cattleya* into *S. albus* J1074 led to the

production of paulomycins and paulomenols but not thienamycins [17]. Similarly, production of actinorhodins were strongly activated in *Streptomyces lividans* TK21 through the transformation of the same thienamycin biosynthetic gene cluster [17]. Heterologous expression of the positive regulatory gene *pimM* of the pimaricin cluster from *Streptomyces natalensis* activated the simultaneous production of candicidins and antimycins in *S. albus* J1074 [18]. The activation of a native biosynthetic pathway in the host through expressing a heterologous gene cluster reflects an extensive 'cross-talk' between pathway-specific regulators in different biosynthetic pathways that are previously reported in streptomycetes [19]. *S. albus* J1074 has been widely known as a heterologous host for expressing biosynthetic gene clusters from other actinomycetes [18]. Recent studies of genome mining and activation of 'silent' gene cluster have revealed that *S. albus* J1074 is also a producer for a variety diverse natural products [13, 18]. Herein, this study provides evidence that *S. albus* J1074 has the ability to produce isoindolequinone (albumycin, **1**). However, the biosynthetic mechanism of **1** and **2** has not been well understood. The production of **1** and **2** might be an interaction between the host genes (such as the antimycin biosynthetic genes) in *S. albus* J1074 and the heterologous fluostatin biosynthetic genes (probably the *fls* regulators). Plausibly, **1** and **2** were proposed to be derived from peptide carrier protein (PCP)-tethered 3-formamidosalicyclic acid, a well established precursor *en route* to the biosynthesis of antimycins (Fig. 2) [20, 21]. Alternatively, after condensation of 3-formamidosalicyclic acid with L-threonine (or glycine), the resulting product would be released from the nonribosomal peptide synthetase (NRPS) assembly of antimycins, to form antimycic acid-like precursors, which would undergo further oxidoreduction and cyclization to generate **1** and **2** (Fig. 2). Nonetheless, experimental data are required to understand the intriguing biosynthetic machinery for **1** and **2**. This study highlights again that heterologous expression plays an increasingly substantial role in the discovery of novel natural products.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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