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Structure Elucidation of Sch 538415, a Novel Acyl Carrier Protein Synthase Inhibitor from a Microorganism

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Abstract—A novel acyl carrier protein synthase inhibitor, Sch 538415 (**1**), was isolated from an unidentified bacterial microbe. Structure elucidation of **1** was accomplished based on analysis of spectroscopic data including UV, MS and 2D-NMR spectra. Compound **1** exhibited inhibitory activity in the acyl carrier protein synthase (AcpS) assay with an IC_{50} value of 4.19 μM and showed antibacterial activity against *Staphylococcus aureus* in the agar diffusion assay.

Bacterial resistance to clinically approved antibiotics continues to pose a worldwide threat to public health.[1] Emergence of resistance to 'last line' therapies, such as vancomycin, has heightened awareness and concerns about bacterial pathogens that are potentially untreatable.[2] A renewed sense of urgency has been invoked for the discovery and development of new classes of antibacterial drugs. Mechanism-based drug discovery approaches are being explored to identify novel antimicrobial agents that may provide alternative treatments for bacterial infections. The bacterial acyl-carrier protein synthetase (AcpS) is a bacterial-specific protein that is broadly represented in many pathogens.[3,4] The AcpS enzyme is required for the covalent attachment of 4′-phosphopantetheine to a conserved serine residue on the apo form of the acyl-carrier protein (ACP) to generate functional holo-ACP.[5,6] ACP is required for de novo fatty acid biosynthesis and acyltransferase reactions; ACP and AcpS are both required for cellular viability and inhibition of AcpS may cause bactericidal responses.[7] A high-throughput assay has been devised for AcpS by measuring the incorporation of the radiolabeled 4′-phospho-pantetheine moiety of Coenzyme A into preparations of apo-ACP. During the process of search for novel AcpS inhibitors as potential leads for drug development, a large number of extracts from microbial sources have been tested in the high-throughput screening (HTS) program. As a result of the screening, Sch 538415 (**1**), has been isolated and identified from an unidentified bacterial microbe (culture # TG-10261). In this paper, we wish to report the isolation, structure elucidation and biological activity of **1** (Fig. 1).

Fermentation broth (200 mL) was extracted with ethyl acetate (2×400 mL) at harvest pH (~7.2). The EtOAc layer was concentrated in vacuo to obtain ~64 mg of crude extract. Purification of EtOAc extract was performed on normal phase HPLC (YMC PVA-Sil semi-preparative column 250×10 mm, S-5, 120 Å with a guard column 50×20 mm) using 2–10% MeOH in n-BuCl with a linear gradient for 30 min, 15 mL/min flow rate, UV detection at 220 nm. Two HPLC runs were conducted with 32 mg for each injection to afford ~6.6 mg of the enriched mixture. The mixture was purified by reversed-phase HPLC (YMC-ODS semi-preparative column, S-5, 120 Å with a guard column 50×20 mm) using 5–50% ACN in water with a linear gradient for 30

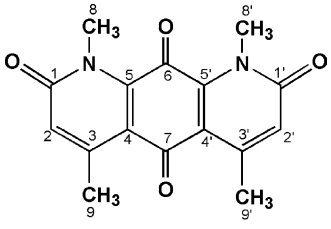

Figure 1. Structure of SCH 538415 (**1**).

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min, 15 mL/min flow rate, UV detection at 220 nm to obtain ~0.8 mg of pure **1** as a red-orange powder.

LC–MS analysis interfaced with atmospheric pressure chemical ionization (APCI+) indicated the presence of a protonated molecular ion $(M+H)^+$ at *m/z* 299 and a bi-molecular ion $(2M+H)^+$ at *m/z* 597. The molecular weight of **1** was further confirmed by negative mode ionization (APCI−) to show a molecular ion (M^-) at *m/z* 298. Elemental composition analysis revealed the molecular formula of **1** to be $C_{16}H_{14}N_2O_4$ based on HR-FABMS data (Calcd: *m/z* 299.1032 for $C_{16}H_{15}N_2O_4$. Found: *m/z* 299.1027). UV absorptions at 254, 268, 282, 302, 313, 348 and 435 nm suggested the presence of a highly conjugated chromophore related to anthraquinone class of compounds. The ^{1}H NMR spectrum of **1** (Table 1) was surprisingly simple with only three singlets at δ 2.57, 3.75 and 6.67 representing a double-bond attached CH_3, a nitrogen attached *N*-CH_3 and a vinyl CH, respectively. Since both singlets at δ 2.57 and 6.67 were slightly broad, further expansion of these signals revealed the presence of a very fine coupling constant with $J=1.1$ Hz. This observation suggested the allylic coupling of these two proton signals, therefore, the CH_3 group should be adjacent to the vinyl proton. In the ^{13}C NMR spectrum (Table 1), a total of nine carbon resonances were observed including three carbonyls, one vinyl methine, three vinyl/aromatic quaternary carbons, one nitrogen attached methyl and one double bond attached methyl carbon. Among the three carbonyls, two signals at δ 178.7 and 181.3 represented typical 1,4-quinone carbonyl carbons, while the other signal at δ 161.3 was considered as an amide carbonyl carbon. The resonance at δ 34.0 was assigned to a nitrogen attached methyl group.

The molecular weight of **1** had an even number indicating the presence of two nitrogen atoms in the molecule, however, only one *N*-CH_3 carbon was observed. This evidence suggested that the structure of **1** is symmetrical. There are three possible arrangements of the diazaquinone carbon skeleton that can be proposed as either C_2 (2-fold) or *i* (inversion) symmetries. The arrangement of *i* symmetry for **1** indicated that two 1,4-quinone carbonyl carbons should be identical in the ^{13}C NMR spectrum of **1** due to the presence of a center of inversion.[8] However, the ^{13}C NMR data of **1** revealed two 1,4-quinone carbonyl resonances at δ 178.7 and 181.3, therefore, the *i* symmetry is ruled out.

There are two configurations of C_2 symmetry for **1**. Besides the 2-fold symmetry C_2 along *y*-axis as shown in Figure 2, the other configuration of C_2 symmetry for **1** can be arranged along with *x*-axis, in which two nitrogen atoms are located in the same ring of the molecule,[9] This arrangement, again, did not agree with the ^{13}C NMR data of **1**, because the two 1,4-quinone carbonyl carbons should be identical. Therefore, the C_2 symmetry along with *x*-axis for **1** is also excluded. The 2-fold symmetry C_2 along *y*-axis was considered as the best candidate to be assigned the diazaquinone skeleton of **1** with both two nitrogen atoms located at the same side of the molecule in order to match the molecular formula established by HR-FABMS, as well as the ^{13}C NMR data.

Detailed assignments of each carbon and proton were accomplished by analysis of 2D-NMR data including NOESY and HMBC experiments, as shown in Figure 2. Since the only NOE correlation between H-2 and CH_3-9 was observed, the amide carbonyl functionality was assigned to position-1 to separate the *N*-CH_3 from H-2 and CH_3-9 due to the lack of NOE correlations between *N*-CH_3 and H-2, as well as *N*-CH_3 and CH_3-9.[10] The vinyl proton (H-2) and the methyl group (CH_3-9) should be assembled next to each other. In the HMBC spectrum, three-bond correlations of *N*-CH_3 to C-1 and C-5 confirmed the assignment of *N*-CH_3 at position-1. The correlation of H-2 to C-1 revealed that the vinylic proton was located at position-2. The other methyl group of (CH_3-9) was assigned to position 3 based on correlations of CH_3-9 to C-2, C-3 and C-4 without coupling to C-1.[11] Structure elucidation of **1** was completed on the basis of all above 2D-NMR data. Therefore, the structure of **1** was proposed as shown in Figure 1.

Additional supportive evidence from the literature search for the structure of **1** was found by a comparison to diazaquinomycin A and its semi-synthetic *N*-methyl derivative.[12] Diazaquinomycin A possesses the same diazaquinone tricyclic carbon skeleton. The ^{13}C NMR spectral data of the *N*-methyl derivative of diazaquino-

Table 1. NMR spectral data of Sch 538415 (**1**)[a]

No.	^{13}C (δ)	^{1}H (δ)	HMBC
1, 1′	161.3 s[b]	—	—
2, 2′	126.4 d	6.67 br.s[c]	C1,C4,C9
3, 3′	148.9 s	—	—
4, 4′	116.8 s	—	—
5, 5′	142.8 s	—	—
6	178.7 s	—	—
7	181.3 s	—	—
8, 8′	34.0 q	3.75 s	C1,C5
9, 9′	22.5 q	2.57 br.s	C2,C3,C4

[a]Recorded at 500 and 125 MHz for ^{1}H and ^{13}C NMR in $CDCl_3$, respectively.
[b]Multiplicity was determined by APT data.
[c]Further examination of both broad singlets revealed the presence of a small coupling between H-2 and H-9 with $J_{H2,H9}=1.1$ Hz.

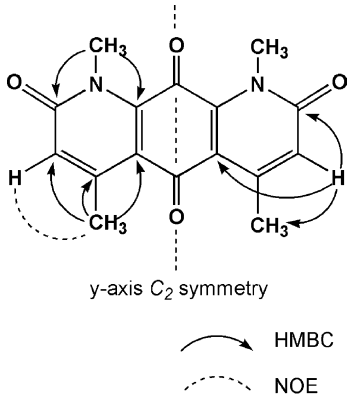

Figure 2. HMBC and NOE data of **1**.

mycin A were consistent with the data in Table 1 for **1**.[12b] To our best knowledge, only two related compounds reported previously in literature are diazaquinomycin A and nibomycin A.[13]

Compound **1** showed inhibitory activity against the bacterial acyl carrier protein synthase with an IC_{50} value of 4.19 μM in vitro. In a cell-based agar diffusion assay, **1** also demonstrated antibacterial activity against *Staphylococcus aureus* (FDA 209P strain) demonstrating a 12 mm inhibition zone with 5 μg on a paper disc (8-mm diameter).

Acknowledgements

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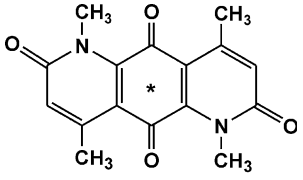
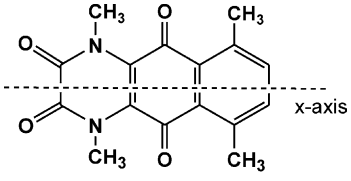
8. The arrangement of *i* symmetry for **1** with a center of inversion is shown below:

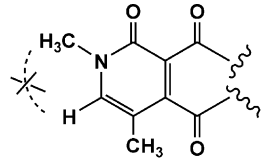


* Center of Inversion

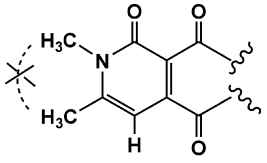
9. The C_2 symmetry along with *x*-axis for **1** is illustrated as follows:



10. The other two possible arrangements shown below were also excluded due to the lack of NOE correlations in **1**. In the case of **A**, the arrangement was incorrect because the NOE correlation of H-2 to *N*-CH_3 was not observed. In the case of **B**, the arrangement was also incorrect because the absence of the correlation of *N*-CH_3 to other methyl group (CH_3-9) in NOE experiments of **1**.



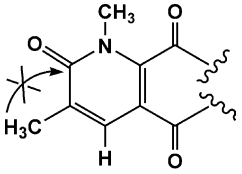
A



B

NOE correlation

11. The arrangement of the methyl group (CH_3-9) next to the amide carbonyl did not agree with the HMBC data, which should observe the correlation of CH_3-9 to C-1 as depicted as follows:



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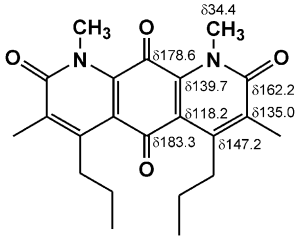

Structure and ^{13}C NMR data of *N*-methyl derivative of diazaquinomycin A

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