Siderochelins with anti-mycobacterial activity from Amycolatopsis sp. LZ149

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[ABSTRACT] Three new compounds, namely siderochelins D (2), E (3), and F (4), together with one known siderochelin A (1), were isolated from Amycolatopsis sp. LZ149 and elucidated by spectroscopic analyses including 1D- and 2D-NMR and X-ray single crystal diffraction. Compounds 1−3 showed antibacterial activity against Mycobacterium smegmatis.

[KEY WORDS] Amycolatopsis sp. LZ149; Siderochelin; Bioassay-guided; Mycobacterium smegmatis; X-ray single crystal diffraction

[CLC Number] R284.1

Introduction

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis, which most commonly affects the lungs. It is transmitted from person to person via droplets from the throat and lungs of people with TB. In 2011, there were an estimated 8.7 million new cases of TB (13% co-infected with HIV) and 1.4 million people died from TB, including almost one million deaths among HIV-negative individuals and 430,000 among people who were HIV-positive[1]. Although TB is treatable, few alternative drugs are available to those with drug resistance. The second-line drugs currently used in the clinic, such as ethionamide, kanamycin, capreomycin, ofloxacin, and para-aminosalicylic acid, are either less effective or toxic. TB remains one of the most frequent and important infectious diseases causing morbidity and death worldwide. There is therefore an urgent need for discovery and development of new anti-TB drugs to overcome drug resistance and improve the treatment outcome of latent TB[2]. In the course of our screening program for anti-mycobacterial agents from micro-bial library, an isolated actinomycete strain, designated as LZ149, was selected for further investigation. The bioassay-guided fractionation and isolation process has led to the discovery of three new siderochelin analogs, siderochelins D (2), E (3), and F (4) (Fig. 1), together with a known compound, siderochelin A (1), from the culture extract of Amycolatopsis sp. LZ149. In this report, the fermentation, isolation, structure elucidation, and anti-mycobacterial activities of compounds 1−4 are described.

Results and Discussion

Compound 1 was obtained as yellowish crystal. Its molecular formula was determined to be C11H13N3O3 according to the HR Q-TOF MS data (m/z 236.1313 [M + H]+, 258.1138 [M + Na]+) and 1H- and 13C NMR data (Table 1). The coincidence of its 1H NMR spectroscopic properties with those reported in the literature revealed that 1 was siderochelin A[3−4]. The structure was confirmed by HMQC and HMBC spectral data and further corroborated with the X-ray single crystal structure data (Fig. 2), as described by Liu et al[3].

By comparison of the spectroscopic properties, the new compounds, siderochelins D (2), E (3), and F (4) (Fig. 2) were clearly homologues belonging to the siderochelin family.

The 1H and 13C NMR data of 2 were almost the same as those of 1 (Table 1). TLC analysis showed that it possessed acidity, and had a smaller Rf value than 1. Therefore, it was deduced that the NH2-14 in 1 was replaced by OH in 2, which
was corroborated with the HR Q-TOF MS data (m/z 237.1268 [M + H]+ and 259.1127 [M + Na]+) with the molecular formula of C11H12N2O4. The relative configuration of 2 was determined by the NOEs between H-15 and H-3β, and H-2 and H-3α, as those of 1. The chemical shift (δ 4.82) of CH(2) on the carboxylic-bearing carbon (X of ABX) was closer to that of the corresponding proton in siderochelin A (1, δ 5.00) than that of siderochelin B (δ 4.61), suggesting that the configuration of 2 was the same as 1 [4]. Therefore, the structure of 2 was elucidated as (2S,4R)-4-hydroxy-5-(3-hydroxypyridin-2-yl)-4-methyl-3,4-dihydro-2H-pyrrole-2-carboxamide.

Compound 3 was obtained as yellow crystal, and its molecular formula was determined as C12H12N2O4 by HR Q-TOF MS (m/z 251.1321 [M + H]+, 273.1158 [M + Na]+) and 13C NMR data. The 1H- and 13C NMR data of 3 (Table 1) were similar to that of 2, except for an additional signal at δ 3.82 (s) due to a methoxyl group, as confirmed by comparison of the 1H- and 13C NMR spectra of 2 and 3, and corroborated with an HMBC correlation between the proton at δ 3.82 and the carbon at δ 158.3.

The chemical shift (δ 4.69) of CH(2) (X of ABX) was closer to that of the corresponding proton in siderochelin B (δ 4.61) than that of siderochelin A (1, δ 5.00), and siderochelin A had a C-CH3 resonance at 1.70 and siderochelin B showed a C-CH3 resonance at 1.60 in the 1H NMR spectroscopy, suggesting that the configuration of 3 was the same as siderochelin B [3, 5]. Therefore, the structure of 3 was elucidated as (2R,4R)-4-hydroxy-5-(3-methoxy pyridin-2-yl)-4-methyl-3,4-dihydro-2H-pyrrole-2-carboxylic acid.

Table 1 1H (600 MHz) and 13C (150 MHz) NMR data of compounds 1–4 (in MeOD, J in Hz)

<table>
<thead>
<tr>
<th>Position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td></td>
<td>δ H</td>
<td>δ C</td>
<td>δ H</td>
<td>δ C</td>
</tr>
<tr>
<td>N-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.00 (dd, 3.5, 9.4)</td>
<td>70.6 (d)</td>
<td>4.82 (dd, 7.1, 9.2)</td>
<td>68.0 (d)</td>
</tr>
<tr>
<td>3</td>
<td>2.36 (dd, 3.5, 13.3)</td>
<td>40.8 (t)</td>
<td>2.53 (dd, 10.8, 12.5)</td>
<td>42.1 (t)</td>
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<tr>
<td>4</td>
<td></td>
<td>85.1 (s)</td>
<td></td>
<td>84.9 (s)</td>
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<tr>
<td>5</td>
<td></td>
<td>172.7 (s)</td>
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<td>173.3 (s)</td>
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<tr>
<td>6</td>
<td></td>
<td>180.2 (s)</td>
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<td>179.5 (s)</td>
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<tr>
<td>7</td>
<td></td>
<td>133.8 (s)</td>
<td></td>
<td>133.7 (s)</td>
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<tr>
<td>8</td>
<td></td>
<td>158.2 (s)</td>
<td></td>
<td>158.2 (s)</td>
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<tr>
<td>9</td>
<td>7.40 (dd, 1.8, 8.5)</td>
<td>125.7 (d)</td>
<td>7.43 (m)</td>
<td>125.3 (d)</td>
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<tr>
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<td>7.39 (dd, 4.0, 8.5)</td>
<td>127.6 (d)</td>
<td>7.42 (m)</td>
<td>127.3 (d)</td>
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<tr>
<td>11</td>
<td>8.20 (dd, 1.8, 4.0)</td>
<td>140.1 (d)</td>
<td>8.20 (m)</td>
<td>139.6 (d)</td>
</tr>
<tr>
<td>N-12</td>
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<tr>
<td>13=O</td>
<td></td>
<td></td>
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<tr>
<td>14-NH2 or OH</td>
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<tr>
<td>15</td>
<td>1.80 (s)</td>
<td>28.4 (q)</td>
<td>1.68 (s)</td>
<td>26.9 (q)</td>
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<tr>
<td>16-OH</td>
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<td></td>
<td></td>
<td>3.82 (s)</td>
</tr>
<tr>
<td>17-OH</td>
<td></td>
<td></td>
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</tbody>
</table>

Fig. 1 Chemical structure of compounds 1–4

Fig. 2 Crystal structure of compound 1

was corroborated with the HR Q-TOF MS data (m/z 251.1321 [M + H]+, 273.1158 [M + Na]+) and 13C NMR data. The 1H- and 13C NMR data of 3 (Table 1) were similar to that of 2, except for an additional signal at δ 3.82 (s) due to a methoxyl group, as confirmed by comparison of the 1H- and 13C NMR spectra of 2 and 3, and corroborated with an HMBC correlation between the proton at δ 3.82 and the carbon at δ 158.3.

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Compound 4 was obtained as yellow-green powder. The \(^{13}\)C NMR (DEPT) spectra of 4 (Table 1) displayed signals for six quaternary C-atoms, four CH groups, and one Me group. The presence of 8-OH substitute pyridine ring as in 1 and 2 was indicated by three doublet signals at J = 1.3, 8.2 Hz, 7.31 (dd, J = 1.3, 4.6 Hz) in the 1H NMR, and five aromatic carbons at 139.4 (C-11) in the 13C NMR spectrum. A pyrrole was deduced according to HMBC from the proton singlet at δ 6.75 (H-3) to Me(15), C-2, C-4 and C-5. The position of Me at C-4 was deduced by HMBCs from Me(15) to C-3, C-4 and C-5. The HR Q-TOF MS showed the quasi-molecular-ion peak [M + H]\(^+\) at m/z 218.103 4, establishing the molecular formula C\(_{11}\)H\(_{11}\)N\(_3\)O\(_2\). Therefore, compound 4 was determined as 5-(3-hydroxypyridin-2-yl)-4-methyl-4H-pyrrole-2-carboxamide.

In vitro anti-mycobacterial activity against Mycobacterium smegmatis MC\(_2\)155 was measured using the paper-disc diffusion assay \([7]\). The results suggested that the diameters of inhibition zone (mm) caused by 50 μg of compounds 1–4 were 23 (1), 10 (2), 15 (3), and 0 (4), respectively. Compound 1 also showed antimicrobial activities against Bacillus pumilus CMCC55051 (10 mm), Bacillus subtilis CMCC63501 (10 mm), Escherichia coli CMCC4103 (13 mm), and Staphylococcus aureus CMCC26003 (15 mm), but had no inhibitory activity against Candida albicans AS2.538 and Aspergillus niger ACCC30005 at 50 μg/disc.

In the present study, siderochelins 1–3 isolated from A. sp. LZ149 were found to be inhibitors of mycobacterial growth, and but 4 showed no activity against M. smegmatis at 50 μg/disc.

It is reported that the activity of siderochelin A is antagonized in the presence of Fe\(^{2+}\), Fe\(^{3+}\), Co\(^{2+}\) and Zn\(^{2+}\) ions, suggesting that the mode of action of siderochelin is related to its ability to chelate metal ions \([5]\). The structure–activity analysis has suggested that the hydroxyl at C-4 is likely to be a key site of iron chelation of siderochelin A and the substituent amide or carboxyl at C-2 has little effect on the antibacterial activity. Further, the aromatization of dihydropyrrrol ring to form pyrrol ring in siderochelin E is also important in loss of its activity.

In conclusion, these derivatives may serve as templates for further modifications to attain more effective antimycobacterial compounds.

Experimental

General experimental procedures

Silica gel (RP-18) was purchased from Merck (Darmstadt, Germany). Sephadex LH-20 gel was purchased from Amer sham Biosciences (Piscataway, New Jersey). The NMR spectra were measured using a DRX-600 spectrometer (Bruker Daltonics Inc., Billerica, Massachusetts), with \(^1\)H NMR at 600 MHz and \(^{13}\)C NMR at 150 MHz (J in Hz). HR Q-TOF MS were measured on a BioTOFTM-Q mass spectrometer (Bruker); in m/z (rel. %).

Isolation and identification of Strain LZ149

Strain LZ149 was isolated from the rhizosphere of Cynodon dactylon collected at Baicheng beach of Xiamen, Fujian, China. The BLAST analysis of 16S rRNA gene segment of strain LZ149 was highly homologous to Amycolatopsis orientalis (accession No. HM368600). Therefore, strain LZ149 was identified as an Amycolatopsis species, and designated as Amycolatopsis sp. LZ149 \([6]\).

Fermentation and isolation

The solid-state fermentation was performed with ISP2 medium (Yeast extract 4.0 g, Malt extract 10.0 g, glucose 4.0 g, agar 15.0 g, H\(_2\)O 1.0 L, pH 7.2; 10 L) at 28 °C for 11 d. The cultured agar was chopped, diced, and extracted with EtOAc–MeOH–AcOH (80 : 15 : 5; 3.5 liters) at room temperature overnight. The organic solution was collected through filtration, and the remaining agar residue was extracted several times as described above until the filtrate became colorless. The combined filtrates were concentrated under vacuum to remove organic solvents to afford crude extract (4.5 g). The crude extract was then partitioned with petroleum ether (0.5 l) and MeOH (0.5 l). The solvent was evaporated under reduced pressure to afford 3.8 g of a MeOH extract (brown solid). The crude extract was subjected to MPLC on RP-18 SiO\(_2\) (170 g), eluted with H\(_2\)O, MeOH extract (brown solid). The crude extract was further purified by crystallization and silica gel column chromatography to yield compounds 1 (20 mg), 2 (2 mg), and 4 (1.5 mg).

In order to obtain trace constituents from Amycolatopsis sp. LZ149, the second batch fermentation (8 L) was carried out with half-seawater ISP2 medium at 28 °C for 11 d. The new active siderochelin derivative, named siderochelin E (3), along with compounds 1, 2 and 4, was obtained.

Structural Identification

Siderochelin D \([\{(2R,4R)-4-hydroxy-5-(3-hydroxypyridin-2-yl)-4-methyl-3,4-dihydro-2H-pyrrole-2-carboxamide; 2]\)

Yellow solid. The \(^1\)H and \(^{13}\)C NMR are shown in Table 1. HR Q-TOF MS m/z 237.1268 ([M + H]\(^+\); C\(_{11}\)H\(_{12}\)N\(_2\)O\(_4\); Calcd. 237.231 4).

Siderochelin E \([\{(2R,4R)-4-hydroxy-5-(3-methoxy-pyridin-2-yl)-4-methyl-3,4-dihydro-2H-pyrrole-2-carboxylic acid; 3]\)

Yellow crystal. The \(^1\)H and \(^{13}\)C NMR are shown in Table 1.
HR Q-TOF MS $m/z$ 251.132 1 ([M + H]$^+$, C$_{12}$H$_{12}$N$_2$O$_4$$^+$, Calcd. 251.103 2).

**Siderochelin F** [5-(3-hydroxypyridin-2-yl)-4-methyl-4H-pyrrole-2-carboxamide; 4]. Yellow-green powder. The $^1$H- and $^{13}$C NMR are shown in Table 1. HR Q-TOF-MS $m/z$ 218.103 4 ([M + H]$^+$, C$_{11}$H$_{11}$N$_3$O$_2$$^+$, Calcd. 218.231 3).

**References**


