Abstract

A compound was isolated from the fermented broth of *Pseudomonas aeruginosa*. The compound was determined as acridine-4-carboxylic acid based on nuclear magnetic resonance, mass spectrometry (MS), and single-crystal x-ray diffraction analysis. Acridine-4-carboxylic acid showed significant antibacterial activities against *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, and *Shinella* sp. Moreover, the compound showed certain antitumor activities against HCT116, ST486, HCC1806, and A549 with IC50 values of 38.38, 6.59, 20.69, and 82.81 μg/mL, respectively. In the test of nematicidal activity, acridine-4-carboxylic acid showed certain nematicidal activity against *Caenorhabditis elegans* and *Meloidogyne incognita*. In addition, the compound also exhibited 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity. Therefore, this compound may be used as a potential antibacterial, antitumor, nematicidal, and antioxidative agent.

Keywords

*Pseudomonas aeruginosa*, acridine-4-carboxylic acid, antibacterial, anti-tumor, nematicidal

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Results and Discussion

Strain Identification

Based on the 16S rRNA gene sequencing (GenBank MW463450), the strain was highly homologous to *P. aeruginosa* (99%) (http://blast.ncbi.nlm.nih.gov/Blast.cgi); therefore, strain B274 was considered to be *P. aeruginosa*. *Pseudomonas* belongs to the *Pseudomonadaceae* family, which contains 191 validly described species. *Pseudomonas aeruginosa* is a species of the genus *Pseudomonas* that is increasingly recognized as an emerging opportunistic pathogen of clinical relevance.

Compound Structure

The compound was isolated from the fermentation broth of *P. aeruginosa*. The compound was yellow solid with the following features: TOF-MS-222 [M-1]; 1H-NMR (500 MHz, CDCl3): 9.03 (1H, d, J = 7.0 Hz), 8.47 (1H, d, J = 8.7 Hz), 8.33 (1H, dd, J = 5.9 Hz, 1.9 Hz), 8.26 (1H, dd, J = 7.0 Hz, 1.9 Hz), 8.00 (1H, t, J = 8.4 Hz), and 7.94 (3H, m, overlap); and 13C-NMR (125 MHz, CDCl3): 128.5 (s), 129.1 (d), 129.4 (d), 130.2 (d), 131.5 (d), 131.9 (d), 131.9 (d), 134.1 (d), 136.1 (d), 140.8 (s), 141.6 (s), 142.6 (s), 142.9 (s), and 166.6 (s). Melting point: 202 to 204 °C (Solvent: acetone). According to the spectroscopic data and the available literature, the compound was identified as acridine-4-carboxylic acid (Figure 1). The structure was confirmed by a single-crystal x-ray diffraction analysis (Figure 1). This compound has been already obtained by chemical synthesis.12

Antibacterial Activity

The acridine-4-carboxylic acid was assayed in terms of antibacterial activity. Moreover, the acridine-4-carboxylic acid could effectively inhibit the growth of 3 tumor cells in vitro and exhibited the nematicidal activity against *C. elegans* and *M. incognita*. Therefore, this compound may be used as a potential antibacterial, antitumor, and nematicidal agent.

Antitumor Activity

The antitumor activity of acridine-4-carboxylic acid was investigated by a sulforhodamine B (SRB) assay. The inhibition activity against the tumor cells is presented in Table 2 and Figure 3. The results indicated that the acridine-4-carboxylic acid could effectively inhibit the growth of 3 tumor cells (HCT116, ST486, and HCC1806) in vitro with IC_{50} values of 38.38, 6.59, and 20.67 μg/mL, respectively. Whereas it displayed moderate antioxidant activity against DPPH radical with IC_{50} values of 440.64 μg/mL (Figure 4).

Nematicidal Activity

The nematicidal activity was detected for the different concentrations of acridine-4-carboxylic acid (Table 3 and Figure 5). The mortality on *C. elegans* of 50, 100, and 200 μg/mL of acridine-4-carboxylic acid was 5.13%, 14.97%, and 38.70%, respectively. Also, the mortality on *M. incognita* of 50, 100, and 200 μg/mL of acridine-4-carboxylic acid was 20.67%, 23.50%, and 26.90% after 24 h, respectively. The results demonstrate that the nematicidal activity of acridine-4-carboxylic acid will provide a possibility for biocontrol of *M. incognita*.

Discussion

In our study, the acridine-4-carboxylic acid compound was isolated from microorganisms for the first time. This compound showed stronger antibacterial activities to *S. aureus*, *B. subtilis*, *B. megaterium*, *Shinella* sp., *B. cereus*, and *E. coli*. The result showed that the compound had broad-spectrum and significant antibacterial activity. Moreover, the acridine-4-carboxylic acid could effectively inhibit the growth of 3 tumor cells in vitro and exhibited the nematicidal activity against *C. elegans* and *M. incognita*. Therefore, this compound may be used as a potential antibacterial, antitumor, and nematicidal agent.

Methods

Cell Lines and Reagents

The cell lines HCT116, ST486, HCC1806, and A549 were obtained from Kunming institute of Zoology, CAS. Also, the RPMI-1640 medium was purchased from Gibco BRL (Life Technologies), and fetal bovine serum was purchased from HyClone Laboratories. Column chromatography (CC) was performed on silica gel G (200-300 mesh, Qingdao Marine Pharmacia), and GF254 silica gel (10-40 μm; Qingdao Marine Chemical Inc.). All solvents were distilled before use.

Isolation and Identification of Strain

The strain B274 was isolated from a soil sample of Kunming, China. The strain was deposited in the Southwest Natural Germplasm Center for Microbial Resources (China). The bacterium was identified based on its 16S rRNA sequence using universal bacterial primers, including 8F (5’-GAGTTTGATCCTGGCTCAG-3’) and 1492R (5’-GTTCACCTGGTTAGGACTT-3’).17 To identify the isolates, PCR amplicons were sequenced by BGI (China), and the resulting sequences were analyzed with the BLAST algorithm of the Ribosomal Database Project II.
The 16S rRNA gene sequences have been deposited in the NCBI nucleotide sequence database.

Extraction and Isolation of Compound

The strain B274 was grown in LB medium inside flasks (200 mL per 500 mL of Erlenmeyer flask) on a shaker incubator. After fermentation for 3 days at 37 °C at 180 rpm, the fermentation broth of 3 L B274 was centrifuged. The filtrate was then condensed to 1 L by rotary evaporation and extracted with an equal volume of butanol to obtain a crude extract (3.4 g). The crude extract was purified on a Sephadex LH-20 column (600 g), eluted with methanol to yield fractions A1-A9. The fraction A5 (543 mg) was subjected to silica gel G (15 g) CC eluting with EtOAc/methanol by increasing polarity from 100:1 to 20:1 to yield A5-1-A5-4 fractions. Fraction A5-2 (274 mg) was purified on the Sephadex LH-20 column (60 g), eluted in methanol to yield fractions A5-2-1-A5-2-3. The fraction A5-2-2 (78 mg) was further purified on the Sephadex LH-20 column (20 g) and eluted with acetone to obtain compound 1 (14 mg).

Determination of Antibacterial Activity

The antibacterial activity was determined using the disc diffusion method, according to Motta and Brandelli’s method with minor modifications. The strains *S. aureus*, *B. cereus*, *B. subtilis*, *E. coli*, and *Shinella sp.* were used in our study. They were grown in tubes containing 5 mL of LB medium, and then incubated for 12 h at 30 °C at 180 rpm. Then, 100 μL of bacterial broth was used to coat the LB medium plates. The compound acridine-4-carboxylic acid (200, 100, and 50 μg/disc) was added on a filter paper (diameter, 0.6 cm), and then, each filter paper was attached to the plate. The same amount of chloroform was used as the control. The size of the inhibition zone was measured after culturing for 24 h at 30 °C.

![Figure 1](image.png)

**Figure 1.** The structure of acridine-4-carboxylic acid. (a) The structure of acridine-4-carboxylic acid; (b) the single crystal structure of acridine-4-carboxylic acid.

**Table 1.** The Antibacterial Activity of Acridine-4-Carboxylic Acid.

| Compound dosage | Zone of inhibition (cm) |  |
|-----------------|-------------------------|---|
|                 | *S. aureus* | *B. cereus* | *B. subtilis* | *E. coli* | *B. megaterium* | *Shinella sp.* |
| 50 μg/disc       | 1.61 ± 0.12 | 1.81 ± 0.11 | 2.79 ± 0.12 | 1.34 ± 0.14 | 2.79 ± 0.14 | 2.44 ± 0.14 |
| 100 μg/disc      | 2.12 ± 0.11 | 1.93 ± 0.12 | 3.01 ± 0.14 | 1.44 ± 0.11 | 2.91 ± 0.12 | 3.30 ± 0.21 |
| 200 μg/disc      | 2.52 ± 0.14 | 2.14 ± 0.12 | 3.44 ± 0.13 | 2.13 ± 0.14 | 3.30 ± 0.14 | 4.00 ± 0.11 |

Antitumor Activity Assay

The antitumor activity was determined using the SRB method. Logarithmic growth phase cells were inoculated in 96-well plates (5 × 10³ cell/well) and incubated until the cells covered about 80% of the plate. Next, compound acridine-4-carboxylic acid (0, 6.25, 12.5, 25, 50, 100, and 200 μg/mL) was added into the wells. Each dose was treated in 4 wells and incubated for 48 h at 37 °C in 5% CO₂. Following that, the culture supernatant was discarded, and 100 μL/well of 10% precooled trichloroacetic acid was added. The cell was fixed at 4 °C for 1 h and naturally air-dried. Next, it was washed with distilled water 5 times, and 4 mg/mL of SRB solution (100 μL/well) was added for 15 min at room temperature.

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After discarding the supernatant, it was washed with 1% glacial acetic acid 5 times and air-dried. Next, 150 μL/well of Tris solution (10 mM) was used for dissolution for 5 min. Absorbance was measured at 570 nm by a microplate reader. The rate of inhibited proliferation was measured as follows:

\[
\text{Proliferation inhibition rate (\%)} = (1 - \frac{\text{experimental A570}}{\text{control group A570}}) \times 100\%
\]

**Antioxidant Activity Assay**

The antioxidant activities of acridine-4-carboxylic acid were measured in the DPPH radical scavenging assay with a slight modification of the reported method. Briefly, 100 μL 0.2 mM DPPH prepared in MeOH was added to 96-well dishes with compound at different concentrations. The samples were incubated for 30 min in the dark at room temperatures, and the decrease of absorbance at 515 nm was measured against MeOH using a Microplate reader. The DPPH solution was freshly prepared and used within 12 h. All determinations were performed in more than triplicate. The radical scavenging activity of the tested samples was calculated as “inhibition percentage” according to the equation:

\[
\text{Inhibition percentage (\%)} = \left[\frac{A_r - A_b}{A_r - A_{ref}}\right] \times 100\%
\]

Note: \(A_r\) represents the OD value of the test sample and the DPPH mixture solution, \(A_b\) represents the OD value of the

**Figure 2.** The antibacterial activity of acridine-4-carboxylic acid. (a) 200 μg/disc; (b) 100 μg/disc; (c) 50 μg/disc; (d) control. The diameter of disc is 0.60 cm.

**Table 2.** The Antitumor Activity of Acridine-4-Carboxylic Acid Against Tumor Cells (n = 5).

| Compound                | Tumor cell lines (IC_{50}, μg/mL) |
|-------------------------|-----------------------------------|
|                         | HCT116   | ST486   | HCC1806  | A549     |
| Acridine-4-carboxylic   | 38.38 ± 2.70 | 6.59 ± 0.42 | 20.69 ± 1.94 | 82.81 ± 2.81 |
| acid                    |                      |          |           |          |

After discarding the supernatant, it was washed with 1% glacial acetic acid 5 times and air-dried. Next, 150 μL/well of Tris solution (10 mM) was used for dissolution for 5 min. Absorbance was measured at 570 nm by a microplate reader. The rate of inhibited proliferation was measured as follows:

After discarding the supernatant, it was washed with 1% glacial acetic acid 5 times and air-dried. Next, 150 μL/well of Tris solution (10 mM) was used for dissolution for 5 min. Absorbance was measured at 570 nm by a microplate reader. The rate of inhibited proliferation was measured as follows:

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\]

Note: \(A_r\) represents the OD value of the test sample and the DPPH mixture solution, \(A_b\) represents the OD value of the

**Figure 3.** The antitumor activity of acridine-4-carboxylic acid. The experiments were repeated 3 times.
DPPH and anhydrous ethanol mixture solution, \textit{Aref} represents the anhydrous ethanol OD value.

**Nematicidal Activity**

The nematicidal activity assay of the target compound against \textit{Meloidogyne incognita} and \textit{Caenorhabditis elegans} refers to the method in the literature.\textsuperscript{22,23} \textit{Caenorhabditis elegans} was synchronized and cultured at 20 °C. \textit{Meloidogyne incognita} used diluted sodium hypochlorite (NaOCl) solution to extract the eggs of \textit{Meloidogyne incognita} from the rhizomes of infected tomatoes (\textit{Solanum lycopersicum}). The strainer was placed in a petri dish containing water and incubated at 25 °C. The second instar larva of \textit{Meloidogyne incognita} (J2) and the fourth instar larva of \textit{Caenorhabditis elegans} (L4) were collected every day and used directly for biological activity testing. The target compound was dissolved in a mixture of sodium hydroxide: absolute ethanol: ddH2O = 2:9:9 (pH value was neutral), and diluted with water and M9 solution to prepare the test solution. The test solution was added to a 24-well tissue culture plate. The concentration of second-instar nematodes per well was about 100 second-instar nematodes per milliliter of water. Then covered the 24-well tissue culture plate cover and proceed at (25 ± 1) °C cultivation, each treatment was repeated 3 times, and the mortality of nematodes was observed with a stereo microscope after 24 h. If the nematode body was still (for example, the body was straight), even if the nematode remains still after being transferred to clean water for 12 h, it was considered that the nematode is dead.

**Declaration of Conflicting Interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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