Perylenequione Derivatives with Anticancer Activities Isolated from the Marine Sponge-Derived Fungus, Alternaria sp. SCSIO41014

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Original Data of Antibacterial Activity Assay

Compounds 1–28 were test for antibacterial activities against *Staphylococcus aureus* (ATCC 29213) using the agar filter paper diffusion [1]. *S. aureus* stored in glycerinum were activated in LB medium (10 g tryptone, 5 g yeast extract, 10 g NaCl, distilled water added up to 1,000 mL, pH 7.2–7.4) in a shaker-incubator at 37 °C and 180 r.p.m for 24 h. Then dilution-plate method was used to get effective concentration of 10^6–10^7 CFU ml^{-1}. The plate of LB medium was painted with 100 μL of *S. aureus* with effective concentration. The sterile filter paper (a diameter of 5 mm) was painted with each 10 μL of DMSO as negative control, ampicillin (0.5 mg/mL) as positive control and compounds 1–28 (5 mg/mL), respectively. Compounds 10 and 25 with 50 μg/disc displayed an inhibition zone with a diameter of about 21 and 15 mm, and ampicillin with 5 μg/disc showed an inhibition zone with a diameter of about 30 mm (Figure S1), respectively. Further, their minimum inhibitory concentrations (MIC) were evaluated in 96-well microtiter plates using a modification of the broth microdilution method [1]. Each well was added 100 μL of *S. aureus* with effective concentration, 90 μL of sterile LB medium and 10 μL of tested compounds, respectively. Compounds 10,25 and ampicillin with different concentration were added with final concentration as showed in Table S1 and replicated three times. The MIC value of compound 25 was 31.25 μg/mL, while compound 10 showed more than 500 μg/mL. Ampicillin was used as positive control with the MIC value of 6.25 μg/mL.

![Figure S1 Pictures of inhibition zones in the filter paper diffusion test.](image)

Table S1 The growth conditions of the *Staphylococcus aureus* after add different dilution concentration of compounds 10,25 and ampicillin.

<table>
<thead>
<tr>
<th>Concentration μg/mL</th>
<th>500</th>
<th>250</th>
<th>125</th>
<th>62.5</th>
<th>31.25</th>
<th>15.63</th>
<th>7.82</th>
<th>3.91</th>
<th>1.96</th>
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<table>
<thead>
<tr>
<th>Concentration μg/mL</th>
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<th>12.5</th>
<th>6.25</th>
<th>3.13</th>
<th>1.57</th>
<th>0.79</th>
<th>0.40</th>
<th>0.20</th>
<th>0.10</th>
<th>0.05</th>
<th>0.03</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

“+” indicated some *S. aureus* had grown. “−” indicated no *S. aureus* grew.

Detailed Process of Antitumor Activity Assay

Cytotoxicity was assayed with the CCK-8 (Dojindo, Kumamoto Prefecture, Japan) method [2]. Cell lines K562, SGC-7901 and BEL-7402 were purchased from Shanghai Cell Bank, Chinese Academy of Sciences. Cells were routinely grown and maintained in DMEM or RPMI media with 10% fetal bovine serum and with 1% streptomycin/penicillin. All cell lines were incubated in a Thermo/ Forma Scientific CO₂ water-jacketed incubator with 5% CO₂ in air at 37 °C. A cell viability assay was
determined with the CCK-8 assay. Cells were seeded at a density of 400–800 cells/well in 384-well plates and treated with various concentrations of compounds or solvent control. After 72 h incubation, CCK-8 reagent was added, and absorbance was measured at 450 nm using an Envision 2104 multilabel reader (PerkinElmer, Waltham, MA, USA). Dose–response curves were plotted to determine the IC₅₀ values using Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). Taxol was used as the positive control, with IC₅₀ values of 0.18 ± 0.20, 0.89 ± 0.15 and 0.54 ± 0.20 μg/mL, respectively.

**The 16S rRNA Gene Sequences Data of Alternaria sp. SCSIO41014**

CTGGATCTCTCGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGTTCCGGCACCACTAGGACAAACATAAACAAACACATAGATGGAATTGCCAGAATTCAGGATACGATTAAG
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**Theory and Calculation Details of Compound 1**

To determine the absolute configuration of 1, a computational modeling study was conducted using the Gaussian 03 program package [3,4]. The ECD of the lowest energy conformer was then calculated by the TDDFT method at the B3LYP/6-31G(d) level in methanol solution.

**References**

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Figure S36. HMBC spectrum of 6/7 in CD3OD.
Figure S37. NOESY spectrum of 6/7 in CD$_3$OD.

Figure S38. HRESIMS spectrum of 6/7 in CD$_3$OD.
Figure S39. Chiral HPLC analyses of compounds 6 and 7 (Phenomenex Lux Cellulose-2, 4.6 mm × 25 mm, eluent n-hexane–iso-propanol, 40:60 v/v, 1 mL/min).