Research on bioactive natural products from the zoanthid-derived fungus *Exserohilum* sp.

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Abstract: Objective To isolate bioactive natural products from the zoanthid-derived fungus *Exserohilum* sp. Methods Silica gel column chromatography (CC), Sephadex LH-20 gel CC, and semi-preparative HPLC were used to isolate and purify the products. NMR and mass spectrum technology were used to confirm the structures of natural products. These natural products were tested for their antimicrobial activities against pathogenic fungi, bacteria, and marine fouling bacteria. Results Four natural compounds (1～4) were isolated from the fungus *Exserohilum* sp. Compound 3 showed moderate antimicrobial activity against the fungus *Candida albicans* (MIC=16, 3 μmol/L) and the marine fouling bacterium *Pseudomonas aeruginosa* (MIC=32, 6 μmol/L). Conclusion Bioactive natural products 1～4 were isolated from marine-derived fungus *Exserohilum* sp., with compound 3 displayed antimicrobial activity against pathogenic fungi and marine fouling bacteria.

Key words: natural product; zoanthid-derived fungus; *Exserohilum* sp.; antimicrobial activity


1 株六放珊瑚来源真菌*Exserohilum* sp. 中活性天然产物研究△*

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摘 要: 目的: 研究六放珊瑚来源的真菌*Exserohilum* sp. 中活性天然产物。方法: 综合使用硅胶柱层析、Sephadex LH-20凝胶柱层析和半制备型 HPLC 等分析和分离技术分离纯化天然产物, 利用核磁、质谱等现代波谱分析方法对化合物进行结构鉴定。以致病真菌、细菌和海洋微生物为活性模型对化合物进行抗微生物活性评价。结果: 从*Exserohilum* sp. 中分离得到了 4 个天然产物 (1～4), 化合物 3 对致病真菌 *Candida albicans* 和海洋污损细菌 *Pseudomonas aeruginosa* 显示中等抑制活性, MIC 分别为 16, 3 和 32, 6 μmol/L。结论: 从海洋来源的真菌中分离获得天然产物 1～4, 化合物 3 对致病真菌和海洋污损细菌显示中等抑制活性。

关键词: 天然产物; 六放珊瑚来源的真菌; *Exserohilum* sp.; 抗微生物活性

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Marine-derived fungi have been proven to be rich sources of structurally novel and biologically active natural products\cite{1}. As part of our ongoing investigation on bioactive natural products from marine invertebrates-derived fungi in the South China Sea\cite{27}, epicoccin A (1)\cite{31}, oxacyclodecindione (2)\cite{38}, methyl-6-acetyl-4-methoxy-5, 7, 8-trihydroxynaphthalene-2-carboxylate (3)\cite{30} and exserolide G (4)\cite{311} (Fig. 1), were isolated from the zoanthid-derived fungus Exserohilum sp. (CHNSCLM-0008). Herein, we report the extraction, isolation, structure elucidation, and biological activity of these compounds.

![Chemical structures of compounds 1-4](image)

**Fig. 1** Chemical structures of compounds 1-4

1 Experimental Section

1.1 Instruments

Agilent DD2 NMR spectrometer (500 MHz for $^1$H NMR and 125 MHz for $^{13}$C NMR, American Agilent Co.); ESI-MS spectra (Micromass Q-TOF spectrometer, American Waters Co.); Silica gel (200~300 mesh, Qingdao Haiyang Chemical Group Co.,); Sephadex LH-20 (Amersham Biosciences); TLC silica gel plates (GF-254, Yantai Zifu Chemical Group Co.,); Waters 1525 HPLC (American Waters Co.).

1.2 The Separation of 1-4

Fungal Material: The fungus Exserohilum sp. (CHNSCLM-0008) was isolated from the fresh inner tissue of the zoanthid Palystoa hadroni collected from the South China Sea, in April 2015.

Fermentation, Extraction and Isolation: The fungal strain Exserohilum sp. was cultivated in solid medium (each containing 80 g of rice, 120 mL of H$_2$O, and 3.6 g of natural sea salt from Yangkou Saltern, China) in 1 000 mL Erlenmeyer flasks (30 flasks) for 45 d. The fermented solid medium was extracted three times with EtOAc and three times with CH$_2$Cl$_2$-MeOH (v/v, 1:1). The combined organic extract were aerated to dryness under reduced pressure to give crude extract (5.4 g). The extract was subjected to silica gel column chromatography (CC) (petroleum ether-EtOAc, gradient elution) to afford five fractions (Fr. 1~Fr. 5). Fr. 4 was further subjected to silica gel CC (petroleum ether-EtOAc, gradient elution) to afford three sub-fractions (C1~C3). Fr. C3 was subjected to Sephadex LH-20 gel CC eluting with mixtures of CHCl$_3$-MeOH (v/v, 1:1) and purified repeatedly by semi-preparative HPLC to give 1 (3.5 mg) and 3 (4.2 mg). Fractions C2 and C3 were subjected to an ODS column eluting with 50% MeOH-H$_2$O, and finally purified by semi-preparative HPLC to afford 2 (6.3 mg) and 4 (4.0 mg).

2 Results and Discussion

2.1 Structural Determination

Compound 1 (epicoccin A): $^1$H NMR (500 MHz, DMSO-$d_6$, $\delta$, J/Hz); 6.19 (1H, br s, 8-
OH), 6.08 (1H, d, J = 3.3 Hz, 8-OH), 4.62 (2H, overlapped, H-8, H-9'), 4.48 (1H, br d, J = 8.5 Hz, H-9), 3.98 (1H, br s, H-8'), 3.74 (2H, overlapped, H-7, H-7'), 3.11 (2H, dd, J = 17.0, 11.0 Hz, Hb-6'), 3.10 (1H, br d, J = 8.5 Hz, H-4'), 3.09 (2H, dd, J = 18.0, 12.0 Hz, H-6), 3.05 (1H, br d, J = 6.8 Hz, H-4), 2.88 (4H, overlapped, Hb-3', Hb-6'), 2.85 (2H, d, J = 13.0 Hz, Hb-3), 2.76 (2H, d, J = 13.0 Hz, H-3), 2.57 (2H, d, J = 13.0 Hz, H-3), 2.46 (2H, d, J = 18.0 Hz, H-6); 13C NMR (125 MHz, DMSO-d6, δ, J/Hz); 207.8 (C, C-5), 207.4 (C, C-5'), 162.0 (C, C-1), 159.8 (C, C-1'), 74.4 (C, C-2), 70.5 (C, C-2'), 64.8 (CH, C-8), 64.8 (CH, C-8'), 62.3 (CH, C-9), 60.2 (CH, C-9'), 45.6 (CH2, C-3), 45.3 (CH3, C-7), 45.0 (CH, C-4'), 43.4 (CH, C-4), 43.3 (CH3, C-3'), 41.7 (CH, C-7'), 41.3 (CH2, C-6'), 38.0 (CH2, C-6); ESIMS m/z 454, 03 ([M + H]+).

Compound 2 (oxacyclocedindione), 1H NMR (500 MHz, acetone-d6, δ, J/Hz); 6.56 (m, 2H, H-6, H-6), 4.81 (q, J = 6.3 Hz, 1H, H-15), 3.54 (d, J = 16.7 Hz, 1H, H-2), 3.30 (d, J = 16.8 Hz, 1H, H-2), 2.46 (m, 1H, H-12), 2.35 (m, 1H, H-12), 2.08 (m, 1H, H-13), 1.86 (s, 3H, 10-CH3), 1.60 (br m, 1H, Hb-13), 1.13 (m, J = 3.7 Hz, 6H, 14-CH3, 15-CH3); 13C NMR (125 MHz, acetone-d6, δ, J/Hz); 199.2 (C, C-9), 170.0 (C, C-1), 152.5 (C, C-5, C-7), 152.4 (CH, C-11), 136.6 (C, C-10), 132.6 (C, C-3), 122.0 (C, C-8), 113.9 (C, C-4), 103.6 (CH, C-6), 77.2 (CH, C-15), 72.7 (C, C-14), 39.2 (CH2, C-2), 37.2 (CH2, C-13), 30.1 (14-CH3), 27.4 (CH2, C-12), 14.4 (15-CH3), 10.5 (10-CH3); ESIMS m/z 368, 10 ([M + H]+).

Compound 3 (methyl 6-acetyl-4-methoxy-5, 7,8-trihydroxyanaphthalene-2-carboxylate), 1H NMR (500 MHz, DMSO-d6, δ, J/Hz); 10.80 (2H, br s, OH-5, OH-7), 9.51 (1H, br s, OH-8), 7.07 (1H, d, J = 2.0 Hz, H-1), 6.65 (1H, d, J = 2.0 Hz, H-3), 3.72 (3H, s, OCH3-2), 3.70 (3H, s, OCH3-4), 2.64 (3H, s, CH3-6); 13C NMR (125 MHz, DMSO-d6, δ, J/Hz); 200.3 (C, C-5), 166.2 (C, C-2), 158.9 (C, C-4), 157.2 (C, C-8), 155.1 (C, C-5), 142.1 (C, C-7), 134.2 (C, C-2), 128.7 (C, C-9), 125.6 (C, C-6), 113.9 (C, C-10), 108.6 (CH, C-3), 103.7 (CH, C-1), 56.1 (CH3, OCH3-4), 52.2 (CH2, OCH2-2), 19.0 (CH3, 5-CH3); ESIMS m/z 306, 07 ([M + H]+).

Compound 4 (exserolide G), 1H NMR (500 MHz, CDCl3, δ, J/Hz); 5.34 (1H, s, H-3), 3.72 (3H, s, O-CH3), 2.21 (2H, t, J = 6.2 Hz, H-7), 2.11 (3H, s, H-11), 1.26 (2H, m, H-8), 1.01 (3H, t, J = 6.8 Hz, H-10); 13C NMR (125 MHz, CDCl3, δ, J/Hz); 170.8 (C, C-4), 164.5 (C, C-2), 157.8 (C, C-6), 111.6 (C, C-5), 87.6 (C, C-3), 55.9 (O-CH3), 31.3 (CH2, C-8), 23.8 (CH2, C-7), 22.4 (CH2, C-9), 17.0 (CH2, C-11), 13.8 (CH3, C-10); ESIMS m/z 196.11 ([M + H]+).

2.2 Biological Evaluation

The antimicrobial activity against three antibiotic-resistant microorganisms, including the fungus Candida albicans, the bacteria Escherichia coli and Staphylococcus aureus, and six marine fouling bacteria, including Pseudomonas fulva, Photobacterium halotolerans, Enterobacter cloacae, Enterobacter hormaechei, Pseudomonas aeruginosa, and Aeromonas salmonicida, were evaluated by a serial dilution technique using 96-well microtiter plates[13]. Ciprofloxacin and Sareine 211 were used as positive controls. Compound 3 showed moderate antimicrobial activity against the fungus Candida albicans and marine fouling bacteria Pseudomonas aeruginosa with MIC values of 16.3 and 32.6 μmol/L, respectively (Table 1). No antimicrobial activity was observed for compounds 1, 2 and 4 in initial screenings at 50 μmol/L.
Table 1  Antimicrobial activities of compound 3 (MIC)  \( \mu \text{mol} \cdot \text{L}^{-1} \)

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<th>Compd.</th>
<th>fungus</th>
<th>bacterium</th>
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<tr>
<td></td>
<td>Candida albicans</td>
<td>Pseudomonas aeruginosa</td>
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<tr>
<td>3</td>
<td>16, 3</td>
<td>32, 6</td>
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<tr>
<td>Ciprofloxacin*</td>
<td>0, 2</td>
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<tr>
<td>Seanine 211 *</td>
<td>0, 2</td>
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Note: * Ciprofloxacin and Seanine 211 were used as positive controls.

References