Supplemental Material for

**Epicoccarines A, B and Epipyridone: Tetramic Acids and Pyridone Alkaloids from an *Epicoccum* sp. Associated with the Tree Fungus *Pholiota squarrosa***

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\[ R = H \]
\[ R = OH \]

**Figure S1**: Fragmentation pattern of epicoccarines A and B based on MS/MS

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**Experimental**

**General Experimental Procedures**

IR spectra (film) were recorded on a JASCO FT/IR-4100 spectrometer equipped with an ATR device. UV spectra were measured with a Spericord 200 Carl Zeiss spectrometer. Optical rotation was assessed with a JASCO P-1020 Polarimeter. High-resolution electron impact mass spectra (HR-EIMS) were recorded on an AMD 402 double-focussing mass spectrometer with BE geometry (AMD, Intesta, Harpstedt, Germany). NMR spectra were measured on a Bruker Avance 500 DRX spectrometer at 300.133 MHz for \(^1\)H and 75.475 MHz for \(^1\)C in CDCl\(_3\). Chemical shifts are given in ppm relative to TMS as internal standard. HSQC and NOESY (mixing time 0.7 s) data were obtained in the phase-sensitive mode TPPI. Column chromatography was performed using silica gel (60, Merck; 0.063-0.2 μm) and sephadex LH-20. Preparative HPLC was performed using a Gilson binary gradient HPLC system equipped with a UV detector (UV/VIS-151) monitoring at 300 nm; preparative column packed with nucleosil 100-7 C\(_{18}\). TLC was carried out with silica gel 60 F\(_{254}\) plates. Spots were visualized by spraying with vanilline/H\(_2\)SO\(_4\), followed by heating. All solvents used were spectral grade or distilled prior to use.

**Fungal Material**: The microorganism was isolated from the mushroom *Pholiota squarrosa*. Its microscopic features were similar to those described for *Epicoccum* sp. It was deposited in the fungal collection of the Leibniz-Institute for Natural Product Research and Infection Biology (HKI), Jena Germany.

The fungus was cultivated under the condition of surface fermentation at 25 °C in 500 ml Erlenmeyer flasks containing 100 ml medium composed of malt extract (30 g/l), glucose (10 g/l), yeast extract (1 g/l), and \((NH_4)_2SO_4\) (5 g/l), at pH 6.0. After cultivation for 28 days at 25 °C the mycelium cake from the culture medium (30 l) was extracted twice with ethyl acetate and methanol (each 10 l). The MeOH extract was treated with H\(_2\)O to eliminate H\(_2\)O-soluble substances and re-extracted with ethyl acetate (5 l). The ethyl acetate-soluble portion of the re-extraction residue (30 g) was submitted to open column chromatography on Silica gel (silica gel 60, Merk, 0.063 - 0.1
mm, column 4 x 60 cm), using stepwise CHCl3 and CHCl3-MeOH (9:1, 1:1, v/v) as eluents. Final purification was achieved by preparative HPLC using a Phenomenex Hydro-Rp 80 column, Synergi 10 μm, 250 x 21 mm, and acetonitrile-H2O (83:17, v/v) as eluent (flow rate 15 ml/min, UV-detection at 227 nm), yielding 1 (5 mg), 2 (6 mg), and 3 (16 mg).

**Epipyridone (1):** red oil; Rf 27 min, [α]D 25 123.3° (c 0.06, MeOH); UV (MeOH) λmax 213, 250 nm; IR (film): 3192, 2922, 1638, 1611, 1514, 1438, 1378, 1080, 833 cm⁻¹; 1H NMR (CDCl3, 500 MHz), see Table 1; 13C NMR (CDCl3, 125 MHz), see Table 1; ESI-MS (pos. ion mode) m/z 390.1 [M + Na]+, 368.2 [M + H]+; ESI-MS (neg. ion mode) m/z 384.2 [M - H]-; MS/MS (neg. ion mode) m/z 283.1, 268.0, 240.0, 214.0; HRESIMS m/z 390.1145 [M + Na]+ calcd m/z 390.1146 for C23H28O3N

**Epicoccarine A (2):** red oil; Rf 31 min, [α]D -45.8° (c 0.15, MeOH); UV (MeOH) λmax 228, 280 nm; IR (film): 3263, 2924, 1651, 1594, 1515, 1446, 1221 cm⁻¹; 1H NMR (CDCl3, 300 MHz), see Table 1; 13C NMR (CDCl3, 75 MHz), see Table 1; ESI-MS (neg. ion mode) m/z 384.3 [M - H]-; MS/MS (neg. ion mode) m/z 278.2, 260.1, 194.1, 152.9, 98.0; HRESIMS m/z 384.2149 [M - H]- calcd m/z 384.2147 for C23H30O4N

**Epicoccarine B (3):** red oil; Rf 28 min, [α]D -179.9° (c 0.1, MeOH); UV (MeOH) λmax 228, 280 nm; IR (film): 3271, 2923, 1651, 1594, 1516, 1454, 1213 cm⁻¹; 1H NMR (CDCl3, 300 MHz), see Table 1; 13C NMR (CDCl3, 75 MHz), see Table 1; ESI-MS (neg. ion mode) m/z 401.1 [M - H]-; MS/MS (neg. ion mode) m/z 278.1, 259.9, 194.2, 152.9, 98.0; HRESIMS m/z 400.2095 [M - H]- calcd m/z 400.2124 for C23H30O5N

**Biological Assays**

The in vitro antibacterial activity of the compound expresses in term of minimum inhibition concentration (MIC) was determined according to the NCCLS guidelines using the micro broth dilution method (Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard. NCCLS Document M7-A4, 4th ed, National Committee for Clinical Laboratory Standards, Villanova, PA, 1997.).

**Table S2:** Activity of epicoccarine A (2) against selected bacteria

<table>
<thead>
<tr>
<th>Organisms</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin-resistant Strep. aureus 15/8</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Multiresistant Strep. aureus 134/94</td>
<td>50</td>
</tr>
<tr>
<td>Mycobacterium vaccae IMET 10670</td>
<td>6.25</td>
</tr>
<tr>
<td>Mycobacterium smegmatis SG 987</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Mycobacterium aurum SB 66</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Mycobacterium fortuitum B.</td>
<td>50</td>
</tr>
</tbody>
</table>

Table S1. 1H and 13C NMR data of 1-3 (in CDCl3; chemical shifts in ppm, coupling constants (Hz) in parentheses, TMS as internal standard)