Daldinone Derivatives from the Mangrove-Derived Endophytic Fungus *Annulohypoxylon* sp.

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**Fig. S19** COSY spectrum of the new compound 2

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**Fig. S32** Structure and population of the low-energy B3LYP/6-31G(d) in vacuo conformers (>2%) of (1R,6bS,7R)-3 diastereomer

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**Fig. S36** Experimental ECD spectrum of 3 compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of (1$R$,6$S$,7$R$)-3 at various levels

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**Fig. S40** HPLC chromatograms of EtOAc extracts from co-culture experiments

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**Fig. S1.** Phylogenetic tree of *Annulohypoxylon* sp.

A. rDNA-derived phylogenetic tree

![rDNA-derived phylogenetic tree](image1)

rDNA-derived phylogenetic tree, based on alignment of rDNA sequences, which were restricted to bp 28-3357 of the rDNA sequence of the new fungus (acc.-no. KY190099)

B. beta-tubulin-derived phylogenetic tree

![beta-tubulin-derived phylogenetic tree](image2)

beta-tubulin-based phylogenetic tree, based on alignment of beta-tubulin sequences, which were restricted to bp 1-1415 of the beta-tubulin gene sequence of the new fungus (KY190100).
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Fig. S11 Structure and population of the low-energy B3LYP/6-31G(d) \textit{in vacuo} conformers (>2\%) of (1S,6bR)-1 diastereomer

![Image of conformers with percentages]

Fig. S12 Experimental ECD spectrum of 1 compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/6-31G(d) \textit{in vacuo} low-energy conformers of (1S,6bR)-1 at various levels

![Graph of ECD spectra]
**Fig. S13** Experimental ECD spectrum of 1 compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of (1R,6bR)-1 at various levels.

![Graph showing ECD spectra for different levels of theory.](image)

**Fig. S14** Experimental ECD spectrum of 1 compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of (1S,6bR)-1 at various levels.

![Graph showing ECD spectra for different levels of theory.](image)
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Fig. S16 Experimental ECD spectrum of 1 compared with the Boltzmann-weighted ECD spectra computed for the CAM-B3LYP/TZVP PCM/MeCN low-energy conformers of (1S,6bR)-1 at various levels.
**Table S1** Populations of conformers with equatorial and axial 1-H for (1R,6bR)-1 at various levels of theory

<table>
<thead>
<tr>
<th></th>
<th>B3LYP/6-31G(d) Boltzmann population / ax. or eq.</th>
<th>B3LYP/TZVP PCM/MeCN Boltzmann population / ax. or eq.</th>
<th>B97D/TZVP PCM/MeCN Boltzmann population / ax. or eq.</th>
<th>CAM-B3LYP/TZVP PCM/MeCN Boltzmann population / ax. or eq.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conf. A</td>
<td>46.1% / ax.</td>
<td>65.5% / eq.</td>
<td>68.9% / eq.</td>
<td>63.4% / eq.</td>
</tr>
<tr>
<td>Conf. B</td>
<td>26.6% / eq.</td>
<td>20.5% / eq.</td>
<td>15.1% / eq.</td>
<td>21.0% / eq.</td>
</tr>
<tr>
<td>Conf. C</td>
<td>20.3% / ax.</td>
<td>7.0% / ax.</td>
<td>8.0% / ax.</td>
<td>7.3% / ax.</td>
</tr>
<tr>
<td>Conf. D</td>
<td>4.4% / ax.</td>
<td>3.8% / ax.</td>
<td>5.8% / ax.</td>
<td>4.3% / ax.</td>
</tr>
<tr>
<td>Conf. E</td>
<td>2.6% / eq.</td>
<td>3.2% / ax.</td>
<td>2.2% / ax.</td>
<td>4.0% / ax.</td>
</tr>
<tr>
<td>ax./eq.</td>
<td>71/29</td>
<td>14 / 86</td>
<td>16/84</td>
<td>16 / 84</td>
</tr>
</tbody>
</table>

**Table S2** Populations of conformers with equatorial and axial 1-H for (1S,6bR)-1 at various levels of theory

<table>
<thead>
<tr>
<th></th>
<th>B3LYP/6-31G(d) Boltzmann population / ax. or eq.</th>
<th>B3LYP/TZVP PCM/MeCN Boltzmann population / ax. or eq.</th>
<th>B97D/TZVP PCM/MeCN Boltzmann population / ax. or eq.</th>
<th>CAM-B3LYP/TZVP PCM/MeCN Boltzmann population / ax. or eq.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conf. A</td>
<td>57.8% / eq.</td>
<td>47.2% / eq.</td>
<td>47.4% / eq.</td>
<td>53.2% / eq.</td>
</tr>
<tr>
<td>Conf. B</td>
<td>22.6% / eq.</td>
<td>33.0% / eq.</td>
<td>30.6% / eq.</td>
<td>25.9% / eq.</td>
</tr>
<tr>
<td>Conf. C</td>
<td>13.1% / eq.</td>
<td>18.4% / eq.</td>
<td>21.2% / eq.</td>
<td>19.0% / eq.</td>
</tr>
<tr>
<td>Conf. D</td>
<td>5.4% / ax.</td>
<td>0.8% / ax.</td>
<td>0.6% / eq.</td>
<td>1.1% / eq.</td>
</tr>
<tr>
<td>Conf. E</td>
<td>1.1% / ax.</td>
<td>0.7% / ax.</td>
<td>0.1% / eq.</td>
<td>0.8% / eq.</td>
</tr>
<tr>
<td>ax./eq.</td>
<td>6/94</td>
<td>1 / 99</td>
<td>1/99</td>
<td>2 / 98</td>
</tr>
</tbody>
</table>
Fig. S17 $^1$H NMR (600 MHz, DMSO-$d_6$) spectrum of the new compound 2
Fig. S18 Expanded $^1$H NMR (DMSO-$d_6$, 600 MHz) spectrum of the new compound 2
Fig. S19 COSY spectrum of the new compound 2
Fig. S20 $^{13}$C NMR (DMSO-$d_6$, 150 MHz) spectrum of the new compound 2
Fig. S21 Expanded $^{13}\text{C}$ NMR (150 MHz, DMSO-$d_6$) spectrum of the new compound 2
Fig. S22 HSQC spectrum of the new compound 2
Fig. S23 HMBC spectrum of the new compound 2
Fig. S24 Expanded HMBC spectrum of the new compound 2
Fig. S25 HRESIMS spectrum of the new compound 2
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Fig. S30 HMBC spectrum of the new compound 3
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**Fig. S32** Structure and population of the low-energy B3LYP/6-31G(d) *in vacuo* conformers (>2%) of (1R,6bS,7R)-3 diastereomer

![Diagrams of conformers with populations](image)

**Fig. S33** Experimental ECD spectrum of 3 compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/6-31G(d) *in vacuo* low-energy conformers of (1R,6bS,7R)-3 at various levels

![ECD spectrum comparison](image)
**Fig. S34** Structure and population of the low-energy B3LYP/6-31G(d) conformers (>2%) of (1\text{S},6\text{b}\text{S},7\text{R})-3 diastereomer

![Structures and populations of low-energy B3LYP/6-31G(d) conformers](image)

- Conf. A 84.3%
- Conf. B 12.6%
- Conf. C 2.0%

**Fig. S35** Experimental ECD spectrum of 3 compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/6-31G(d) *in vacuo* low-energy conformers of (1\text{S},6\text{b}\text{S},7\text{R})-3 at various levels

![Experimental ECD spectrum with Boltzmann-weighted ECD spectra](image)

Δε (M^2 \cdot cm^{-1}) vs. wavelength (nm)
**Fig. S36** Experimental ECD spectrum of 3 compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of (1R,6bS,7R)-3 at various levels.

**Fig. S37** Experimental ECD spectrum of 3 compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of (1S,6bS,7R)-3 at various levels.
**Fig. S38** Experimental ECD spectrum of 3 compared with the Boltzmann-weighted ECD spectra computed for the CAM-B3LYP/TZVP PCM/MeCN low-energy conformers of (1R,6bS,7R)-3 at various levels.

![Graph](image1.png)

**Fig. S39** Experimental ECD spectrum of 3 compared with the Boltzmann-weighted ECD spectra computed for the CAM-B3LYP/TZVP PCM/MeCN low-energy conformers of (1S,6bS,7R)-3 at various levels.

![Graph](image2.png)
**Fig. S40** HPLC chromatograms of EtOAc extracts from co-culture experiments (detection at UV 235 nm)

A: (A1) *Annulohypoxylon* sp. control, (A2) co-cultivation of *Annulohypoxylon* sp. with viable *S. coelicolor*, (A3) co-cultivation of *Annulohypoxylon* sp. with viable *S. lividans*, (A4) *S. coelicolor* control, (A5) *S. lividans* control;

B: (B1) *Annulohypoxylon* sp. control, (B2) co-cultivation of *Annulohypoxylon* sp. with viable *B. cereus*, (B3) co-cultivation of *Annulohypoxylon* sp. with viable *B. subtilis*, (B4) *B. cereus* control, (B5) *B. subtilis* control
Fig. S41 Proposed biogenetic pathway of benzo[j]fluoranthene derivatives
Fig S42. Cytotoxic effect of compound 2 on human leukemia and lymphoma cell lines measured by MTT assay

(A) Jurkat J16 cells (acute T cell leukemia cells) and (B) Ramos cells (Burkitt's lymphoma B lymphocytes) were seeded at a density of $5 \times 10^5$ cells/mL and incubated with increasing concentrations of compound 2. Cells treated with DMSO (0.1% v/v) for 24 h were used as negative control. After incubation period of 24 h cell viability was monitored using the MTT Assay as described in methods. Relative viability in DMSO treated control cells was set to 100%. Data points shown are the mean of triplicates, error bars = SD. Viability and IC$_{50}$ values (IC$_{50}$ = half maximal inhibitory concentration) were calculated using Prism 6 (GraphPad Software). $R^2$ = coefficient of determination.
**Fig. S43** HPLC Chromatogram of compound 1 (compound 1 was isolated as a 1:1 mixture with compound 2)

UV absorption of compound 1 (peak 1, retention time: 22.230 min)

UV absorption of compound 2 (peak 2, retention time: 26.303 min)
Fig. S44 HPLC Chromatogram of compound 2

UV absorption of compound 2 (peak 1, retention time: 27.887 min)
**Fig. S45** HPLC Chromatogram of compound 3

UV absorption of compound 3 (peak 1, retention time: 24.303)