A new cytotoxic 12-membered macrolactone from the endophytic fungus Exserohilum rostratum LPC-001

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Conformational Analysis and ECD Calculations of Compound 1 and its Enantiomer

Conformational analysis and quantum computations of 1 were performed by Gaussian 16 program package. Conformational analysis of 1 showed 9 (C1–C9) lowest energy conformers with relative energy within 9.0 kcal/mol via the Discovery studio 2018 software package. The 9 conformers (C1-C9) were further optimized using TDDFT at B3LYP/6-31g(d) level in MeOH (Figure S1). And the frequency was calculated at the same level of theory. The ECD calculations of stable conformers without imaginary frequencies were calculated using the TDDFT method at the CAM-B3LYP/6-311+G (2d,p) level in MeOH. The final calculated ECD spectra of 1 were obtained according to the Boltzmann-calculated contribution of the 9 conformers (C1-C9). The ECD spectra of different conformers were simulated using SpecDis v1.70.1 with a half-bandwidth of 0.3 eV. Those of the enantiomer of 1 were depicted by inverting that of 1. In the 200-400nm region, the theoretically calculated ECD spectrum of 1 was in agreement with the experimental ECD spectra of 1 (Figure S2), supporting assignment of the 13R,14S,15R configuration for 1.

Figure S1  B3LYP/6-31G(d) optimized 9 lowest energy 3D conformers of (13R, 14S, 15R)-1 and their relative Gibbs free energies (ΔG < 9.0 kcal/mol)
Figure S2. The experimental ECD spectrum of 1 (black), and the calculated ECD spectra of (13R,14S,15R)-1 (red) and (13S,14R,15S)-1 (blue)

Figure S3. The CD Spectrum of Compound 1
**Figure S4. The HRESIMS Data of Compound 1**

| m/z     | z  | Abund | Formula      | Ion          |
|---------|----|-------|--------------|--------------|
| 141.063 | 1  | 10925 | C18 H22 Cl O6 | (M+H)+       |
| 369.1107| 1  | 131736| C18 H22 Cl O6 | (M+H)+       |
| 370.1136| 1  | 26483 | C18 H22 Cl O6 | (M+H)+       |
| 371.1083| 1  | 42931 | C18 H22 Cl O6 | (M+H)+       |
| 391.0923| 1  | 58666 | C18 H21 Cl Na O6 | (M+Na)+  |
| 392.0958| 1  | 112026| C18 H21 Cl Na O6 | (M+Na)+  |
| 393.0912| 1  | 20928 | C18 H21 Cl Na O6 | (M+Na)+  |
| 451.3434| 1  | 23780 | C18 H21 Cl Na O6 | (M+Na)+  |
| 475.3264| 1  | 119466| C18 H21 Cl Na O6 | (M+Na)+  |
| 476.3286| 1  | 122698| C18 H21 Cl Na O6 | (M+Na)+  |

**Formula Calculator Element Limits**

| Element | Min | Max |
|---------|-----|-----|
| C       | 0   | 100 |
| H       | 0   | 500 |
| O       | 0   | 90  |
| N       | 0   | 5   |
| S       | 0   | 5   |
| Cl      | 0   | 2   |
| Br      | 0   | 0   |
| F       | 0   | 0   |
| P       | 0   | 0   |

**Formula Calculator Results**

| Formula      | Best     | Mass     | Tgt Mass  | Diff (ppm) | Ion Species | Score |
|--------------|----------|----------|-----------|------------|-------------|-------|
| C19 H17 Cl N4 O2 | TRUE     | 368.1034 | 368.104    | 1.62       | C19 H18 Cl N4 O2 | 99.85 |
| C18 H21 Cl O6  | TRUE     | 368.1034 | 368.1027   | -1.99      | C18 H22 Cl O6  | 99.8  |
| C19 H25 Cl O5  | TRUE     | 368.1035 | 368.1035   | 0.31       | C19 H26 Cl O5  | 98.67 |
| C18 H21 Cl O6  | TRUE     | 368.1031 | 368.1027   | -1.22      | C18 H21 Cl Na O6 | 99.89 |
| C19 H17 Cl N4 O2 | 368.1031 | 368.104    | 2.39       | C19 H17 Cl N4 Na O2 | 99.67 |
| C19 H25 Cl O5  | 368.1031 | 368.1035   | 1.07       | C19 H25 Cl Na O5 | 99.14 |

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Figure S23. The NOESY Spectrum of (14S,15R)-deoxyoxacyclododecindione
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