Supporting Information

New citrinin derivatives from the deep-sea-derived fungus
Cladosporium sp. SCSIO z015

Muhammad Amin\textsuperscript{a,b}, Xiao-Yong Zhang,\textsuperscript{a} Xin-Ya Xu,\textsuperscript{a} Shu-Hua Qi\textsuperscript{a,*}

\textsuperscript{a}CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou, 510301, Guangdong, China

\textsuperscript{b}University of Chinese Academy of Sciences, Beijing, 100049, China

\textbf{ABSTRACT}

During the course of our search for novel bioactive compounds from marine fungi, four new citrinin derivatives, cladosporins A-D (1-4) were isolated from a culture broth of the deep-sea-derived fungus \textit{Cladosporium} sp. SCSIO z015. Their complete structural assignments were elucidated by the extensive spectroscopic investigation. The absolute configurations of 1-3 were established by quantum chemical calculations of the electronic circular dichroism (ECD) spectra. Compounds 1-4 showed weak toxicity towards brine shrimp nauplii with \textit{LC}_{50} values of 72.0, 81.7, 49.9 and 81.4 \textmu M, respectively. And 4 also showed significant antioxidant activity against \textalpha,\textalpha-diphenyl-picrylhydrazyl (DPPH) radicals with an \textit{IC}_{50} value of 16.4 \textmu M.

\textbf{Keywords}: Deep-sea-derived fungus, \textit{Cladosporium} sp. SCSIO z015, citrinin derivative, toxicity, antioxidant

To whom correspondence should be addressed. Tel: (86) 020-89022112. Email: shuhuaqi@scsio.ac.cn.
List of Supporting Information

Figure S1 The 1H NMR spectrum of 1 in DMSO-d6 .................................................................
Figure S2 The 13C NMR spectrum of 1 in DMSO-d6 ............................................................... 
Figure S3 The DEPT135 spectrum of 1 in DMSO-d6 ............................................................... 
Figure S4 The HSQC spectrum of 1 in DMSO-d6 ................................................................. 
Figure S5 The HMBC spectrum of 1 in DMSO-d6 ............................................................... 
Figure S6 The 1H-1H NOESY spectrum of 1 in DMSO-d6 .................................................... 
Figure S7 The HRESIMS spectrum of 1 .................................................................................. 
Figure S8 The IR spectrum of 1 ............................................................................................. 
Figure S9 The UV spectrum of 1 ............................................................................................. 
Figure S10 The 1H NMR spectrum of 2 in DMSO-d6 ................................................................
Figure S11 The 13C NMR spectrum of 2 in DMSO-d6 ........................................................... 
Figure S12 The DEPT135 spectrum of 2 in DMSO-d6 ........................................................... 
Figure S13 The HSQC spectrum of 2 in DMSO-d6 ............................................................... 
Figure S14 The HMBC spectrum of 2 in M DMSO-d6 ............................................................
Figure S15 The 'H-'H NOESY spectrum of 2 in DMSO-d6 ....................................................
Figure S16 The HRESIMS spectrum of 2 ..............................................................................
Figure S17 The IR spectrum of 2..............................................................................................
Figure S18 The UV spectrum of 2 ..............................................................................................
Figure S19 The 1H NMR spectrum of 3 in DMSO-d6 ........................................................... 
Figure S20 The 13C NMR spectrum of 3 in DMSO-d6 ........................................................... 
Figure S21 The DEPT135 spectrum of 3 in DMSO-d6 ........................................................... 
Figure S22 The HSQC spectrum of 3 in DMSO-d6 ............................................................... 
Figure S23 The HMBC spectrum of 3 in DMSO-d6 ............................................................... 
Figure S24 The 'H-'H NOESY spectrum of 3 in DMSO-d6 ....................................................
Figure S25 The HRESIMS spectrum of 3 ..............................................................................
Figure S26 The IR spectrum of 3 .............................................................................................
Figure S27 The UV spectrum of 3 ..............................................................................................
Figure S28 The 'H NMR spectrum of 4 in DMSO-d6 ............................................................. 
Figure S29 The 13C NMR spectrum of 4 in DMSO-d6 ........................................................... 
Figure S30 The DEPT135 spectrum of 4 in DMSO-d6 ........................................................... 
Figure S31 The HSQC spectrum of 4 in DMSO-d6 ............................................................... 
Figure S32 The HMBC spectrum of 4 in DMSO-d6 ............................................................... 
Figure S33 The 'H-'H NOESY spectrum of 4 in DMSO-d6 ....................................................
Figure S34 The HRESIMS spectrum of 4 ..............................................................................
Figure S35 The IR spectrum of 4 .............................................................................................
Figure S36 The UV spectrum of 4 ..............................................................................................
Figure S37. The key HMBC correlations of 1, 3, and 4 ................................................................
Figure S38 The key NEOSY correlations of 1, 2 and 3 ..............................................................

Figure S39. Comparison between calculated and experimental ECD spectra of compounds 1-3 in MeOH

Table S1. The NMR spectroscopic data for compounds 1-4 (DMSO-d6, δ in ppm, J in Hz)

Experimental section
Figure S1. $^1$H NMR spectrum of compound 1 in DMSO-d$_6$

![$^1$H NMR spectrum of compound 1 in DMSO-d$_6$]

Figure S2. $^{13}$C NMR spectrum of compound 1 in DMSO-d$_6$

![$^{13}$C NMR spectrum of compound 1 in DMSO-d$_6$]
Figure S3. DEPT-135 spectrum of compound 1 in DMSO-d$_6$

Figure S4. HSQC spectrum of compound 1 in DMSO-d$_6$
**Figure S5.** HMBC spectrum of compound 1 in DMSO-d6

**Figure S6.** $^1$H-$^1$H NOESY spectrum of compound 1 in DMSO-d6
Figure S7. HRESIMS spectrum of compound 1

Figure S8. IR spectrum of compound 1
Figure S9. UV spectrum of compound 1

|   | 283.00 | 0.328 |
|---|--------|-------|
| 2 | 207.40 | 2.269 |

Figure S10. $^1$H NMR spectrum of compound 2 in DMSO-d6
Figure S11. $^{13}$C NMR spectrum of compound 2 in DMSO-d6

Figure S12. DEPT-135 spectrum of compound 2 in DMSO-d6
Figure S13. HSQC spectrum of compound 2 in DMSO-d6

Figure S14. HMBC spectrum of compound 2 in DMSO-d6
Figure S15. $^1$H-$^1$H NOESY spectrum of compound 2 in DMSO-d6

Figure S16. HRESIMS spectrum of compound 2
**Figure S17.** IR spectrum of compound 2

![IR Spectrum Image]

**Figure S18.** UV spectrum of compound 2

|   | 285.80  | 0.768 |
|---|---------|-------|
| 1 |         |       |
| 2 | 214.20  | 3.089 |
Figure S19. $^1$H NMR spectrum of compound 3 in DMSO-d6

Figure S20. $^{13}$C NMR spectrum of compound 3 in DMSO-d6
Figure S21. DEPT-135 spectrum of compound 3 in DMSO-d6

Figure S22. HSQC spectrum of compound 3 in DMSO-d6
Figure S23. HMBC spectrum of compound 3 in DMSO-d6

Figure S24. $^1$H-$^1$H NOESY spectrum of compound 3 in DMSO-d6
Figure S25. HRESIMS spectrum of compound 3

Figure S26. IR spectrum of compound 3
**Figure S27.** UV spectrum of compound 3

|   | 321.00 | 0.182 |
|---|--------|-------|
| 2 | 286.60 | 1.370 |
| 3 | 223.80 | 3.626 |

**Figure S28.** $^1$H NMR spectrum of compound 4 in DMSO-d6
Figure S29. $^{13}$C NMR spectrum of compound 4 in DMSO-d6

Figure S30. DEPT-135 spectrum of compound 4 in DMSO-d6
Figure S31. HSQC spectrum of compound 4 in DMSO-d6

Figure S32. HMBC spectrum of compound 4 in DMSO-d6
Figure S33. $^1$H–$^1$H NOESY spectrum of compound 4 in DMSO-d$_6$.

Figure S34. HRESIMS spectrum of compound 4.
Figure S35. IR spectrum of compound 4.

Figure S36. UV spectrum of compound 4.

1  362.80  0.385
2  284.40  2.042
3  210.20  2.519
**Figure S37.** The key HMBC correlations of 1, 3, and 4

![HMBC correlations](image)

**Figure S38.** The key NEOSY correlations of 1, 2, and 3

![NEOSY correlations](image)

**Figure S39.** Comparison between calculated and experimental ECD spectra of compounds 1-3 in MeOH.

![ECD spectra comparison](image)

- For 1: \( \sigma = 0.3 \text{ eV} \)  \shift = -1nm
- For 2: \( \sigma = 0.25 \text{ eV} \)  \shift = -5nm
Table S1. The NMR spectroscopic data for compounds 1-4 (DMSO-$d_6$, $\delta$ in ppm, $J$ in Hz)

| No | Compounds | Chemical Shifts |
|----|-----------|----------------|
| 1  | 1         | 5.58, s 57.5, CH 5.61, s 60.1, CH 4.79, dd (4.1, 8.8) 67.3, CH |
| 2  |           | 113.5, C |
| 3  | 4.14, q (6.7) 74.0, CH 3.80, q (6.2) 74.7, CH 3.54, dq (4.4, 4.5) 77.9, CH |
| 4  | 2.67, q (6.8) 33.3, CH 2.69, q (6.6) 35.1, CH 2.72, dq (4.8, 4.5) 36.6, CH |
| 5  | 112.3, C 112.6, C 112.4, C |
| 6  | 156.0, C 156.4, C 155.9, C 6.41, s 104.6, CH |
| 7  | 6.33, s 100.0, CH 6.32, s 99.8, CH 6.41, s 100.1, CH 2.95, dq (6.1, 6.2) 42.1, CH |
| 8  | 151.4, C 151.5, C 146.6, C 3.72, dq (6.9, 6.7) 69.3, CH |
| 9  | 103.6, C 106.0, C 118.3, C 1.02, d (6.2) 20.4, CH$_3$ |
| 10 | 136.7, C 137.6, C 138.0, C 1.07, d (6.9) 16.2, CH$_3$ |
| 11 | 1.30, d (6.8) 18.9, CH$_3$ 1.21, d (6.2) 19.9, CH$_3$ 1.29, d (4.4) 20.6, CH$_3$ 2.01, s 9.5, CH$_3$ |
| 12 | 1.07, d (6.9) 20.3, CH$_3$ 1.15, d (6.8) 19.2, CH$_3$ 1.12, d (4.8) 18.6, CH$_3$ |
| 13 | 1.95, s 9.5, CH$_3$ 1.95 (s, 3H) 10.8, CH$_3$ 2.04, s 11.7, CH$_3$ |
| 14 | 120.0, C 118.5, C 2.70, dd (10.8, 8.8) 2.94, dd (10.8, 4.1) 36.1, CH$_2$ |
|    | COO-      | 166.8, C  |
|    | OH-3      | 12.01, s  |
|    | OH-5      | 10.46, s  |
|    | OH-6      | 9.33, s 9.35, s 9.65, s |
|    | OH-8      | 9.76, s 9.75, s 4.50, br s |
|    | H-12      | 10.10, s  |
Experimental section

1. General experimental procedures

UV spectra were obtained using a Shimadzu UV-2600 spectrophotometer. IR spectra were recorded on a Shimadzu IR Affinity-1 Fourier transform infrared spectrophotometer. Optical rotations were measured using an Anton Paar MCP 500 polimeter. NMR spectra were recorded on a Bruker AV-500MHz or AVANCE III HD 700 MHz NMR spectrometer with TMS as an internal standard. HRESIMS spectra were obtained on a Bruker MaXis quadrupole-time-of-flight spectrometer. Preparative HPLC was operated using HPLC Shimadzu SPD-M20A equipped with gradient pump LC-20AT at room temperature on a Shimadzu LC-20AT pump with a Shimadzu SPD-M20A Photodiode Array Detector using a YMC-Pack ODS-A column (250*20 mm, 5 μm). Column chromatography (CC) was performed on Silica gel (200-300 mesh, Qingdao Marine Chemical), the unstable fractions were isolated using Sephadex LH-20 (GE Healthcare) and ODS (40-63 μm, YMC, Japan).

2. Antioxidant assay

**DPPH % radical and scavenging activity**

The DPPH free radical is reduced to corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in color. It is a discoloration assay, which is evaluated by the addition of the antioxidant to DPPH solution and a decrease in absorbance was measured at 515 nm.

*Stock solution*

2, 2-diphenyl 1-picrylhydrazyl (0.1 mM) 2.7 mg of DPPH was dissolved in 50 mL methanol. Test compounds and L-ascorbic acid (positive control) were dissolved in DMSO (1mg/mL). The negative control contained only DMSO and the blank contained methanol instead of the DPPH solution.

*Procedure*

The assay was carried out in a 96 well microtitre plate. To 190 μL of DPPH solution, 10 μL of each test sample or the standard solution was added to wells of a microtitre plate. The final concentration of the test samples and standard (positive control) were 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 μg/mL respectively. The plates were incubated at 37 °C for 30 min in the dark.
The absorbance was measured at 515 nm on a Tecan Genios microplate reader. The experiment was conducted in four replicates. Percent of radical scavenging activity was calculated using the following formula:\textsuperscript{17-18}

$$\text{% Radical scavenging activity} = \left( 1 - \frac{\text{absorbance of sample} - \text{blank}}{\text{control}} \right) \times 100$$

3. Brine shrimp bioassay

Brine shrimp (Artemia salina) eggs (Aquatic Lifeline, Inc., Utah, USA) were sprinkled into a shallow dish filled with seawater and incubated at 28-30 °C. The larvae (nauplii) hatched within 48 hrs. The assay was carried out in 96-well plates. A 10 µL of each tested compounds 1-5 and positive control toosendanin dissolved in DMSO were added to 190 µL seawater containing 20-30 nauplii. The final concentrations were 100, 50, 25, 12.5 and 6.25 µg/mL, respectively. Three replicates in each were done. After 24 hours of incubation at 28-30°C, the dead and total numbers of nauplii in each vial were counted under the microscope.\textsuperscript{19} The mortality rate was calculated by the following formula:

$$\text{Mortality rate} (\%) = \left( \frac{(T - C)}{(1 - C)} \right) \times 100$$

Where T is the mortality rate of the treatment and C is the mortality rate of the negative control. The data were analyzed by Data Processing System (DPS) software to find LC\textsubscript{50}.

4. Data Analysis

All the experiments were done in triplicates. Replicates were expressed as mean ± Standard Deviations and statistical analysis was subjected to one-factor analysis of variance performed with the Data Processing System (DPS) software. Values were expressed as α 95% confidence interval.

5. Computational methods

Conformational searches were performed by employing a systematic procedure implemented in Spartan’14 software package using Molecular Merck force field (MMFF) (Wavefunction Inc., Irvine, CA, 2013). DFT/TDDFT calculations were conducted with the Guassian09 program (Gaussian, Inc., Pittsburgh PA, 2011). The MMFF conformations were reoptimized to afford low-energy conformers within a 10 kcal/mol energy window, using density functional theory (DFT) calculation at the B3LYP/6-31G (d) level using the Guassians09.
Vibrational frequency calculations were run at the same level to estimate their relative thermal free energies (ΔG) at 298.15K. A series of single-point energy calculations for the conformers above were performed at the M06-2X/def2-TZVP level, supposing methanol as the solvent with the polarizable continuum model (PCM). The DFT optimized conformers with the Boltzmann distribution over 1% was then subjected to TDDFT calculations using the functional PBE1PBE and basis set 6-311G (d). ECD spectra were generated using the program SpecDis by applying a Gaussian band shape with 0.2-0.35 eV exponential half-width from dipole-length dipolar and rotational strength. The spectra of the conformers were combined using Boltzmann weighing, with the lowest-energy conformation. The calculated spectra were shifted by -1 nm for 1, -5 nm for 2, and -6 nm for 3 to facilitate comparison to experimental data.