SUPPLEMENTARY MATERIAL

A novel cyclic dipeptide from deep marine-derived fungus

Aspergillus sp. SCSIOW2

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A novel cyclic dipeptide, 14-hydroxy-cyclopeptine (**1**) was purified from a deep sea derived fungal isolate identified as an *Aspergillus* sp. The structure was elucidated by detailed spectroscopic analyses using one and two dimensional NMR experiments and high resolution mass spectrometry. The absolute configuration of the amino acid was determined by Marfey's method. Two conformational isomers of **1** were established by ROE analyses. **1** inhibited nitric oxide production with IC₅₀ values at 40.3 µg/mL in a lipopolysaccharide (LPS) and recombinant mouse interferon-γ (IFN-γ) activated macrophage-like cell line, RAW 264.7 and showed no cytotoxic effect in the tested dose range up to 100 µg/mL.

Keywords: marine fungi; nitric oxide inhibitory activity; cyclodipeptide; *Aspergillus* sp.;

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Figure S1. ^1H -NMR spectrum of 14-hydroxy-cyclopeptide (**1**) in DMSO-d_6 at room temperature

Figure S2. ^1H -NMR spectrum of 14-hydroxy-cyclopeptine (**1**) in DMSO-d_6 at 50 $^\circ\text{C}$

Figure S3. ^1H -NMR spectrum of 14-hydroxy-cyclopeptine (**1**) in DMSO-d_6 at 85 $^\circ\text{C}$

Figure S4. HR-ESI-MS spectrum of 14-hydroxy-cyclopeptine (1)

Figure S5. Expansion of ^1H -NMR spectrum of 14-hydroxy-cyclopeptide (**1**) in DMSO-d_6

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Figure S8. HSQC spectra of 14-hydroxy-cyclopeptine (**1**) in DMSO-d₆

Figure S9. HMBC spectra of 14-hydroxy-cyclopeptide (**1**) in DMSO-d₆

Figure S10. Expansion of HMBC spectra of 14-hydroxy-cyclopeptide (**1**) in DM

Figure S11. ROESY spectrum of 14-hydroxy-cyclopeptine (1) in DMSO-d₆

Figure S12. HPLC Analysis of the Acid Hydrolysates of 14-hydroxy-cyclopeptine (**1**) using Marfey's Method. A: Acid Hydrolysates of 14-hydroxy-cyclopeptine (**1**); B: Mixture of Acid Hydrolysates of 14-hydroxy-cyclopeptine (**1**) and N-Me-L-Tyr standard; C: N-Me-D-Tyr standard; D: mixture of N-Me-L-Tyr and N-Me-D-Tyr standard; E: N-Me-L-Tyr standard

. HPLC Analysis of the Acid Hydrolysates of 1 Using Marfey's Method.

1 (0.7 mg) was dissolved in 6 N HCl (1 mL) and heated at 110 °C for 18 h. After cooling to room temperature (rt), the solvent was removed under reduced pressure. The remaining hydrolysate was resuspended in 50 µL of H₂O and treated with 128 µL of 1% (w/v) 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA) in acetone and 32 µL of 1 M NaHCO₃. The mixture was heated at 40 °C for 1.5 h. After cooling to rt, the contents were neutralized with 32 µL of 1 M HCl, and the resulting mixture was added to 300 µL of MeOH to afford a final hydrolysate volume of 500 µL. From this sample was then withdrawn a 50 µL aliquot, and its solvent removed followed by redissolution in 50 µL of MeOH. This sample was then analyzed by HPLC (Agilent, Poroshell 120 EC-C18 column; 4.6 × 50 mm, 2.7 µm) using a solvent of MeOH 35% in water over the

course of 30 min at 0.5 mL/min with detection at 254 and 210 nm. N-Me-D(L)-Tyr standards (4 μ M) were prepared by dissolving in 10 μ L of H₂O followed by addition of 20 μ L of FDAA and 5 μ L of 1 M NaHCO₃, reaction at 40 °C for 1.5 h, and neutralization with 5 μ L of 1 M HCl. Mixtures were then processed for HPLC in a fashion similar to that used for cyclopeptide analyses; the retention times for FDAA derivatives of N-Me-L-Tyr and N-Me-D-Tyr were 10.5 and 11.5 min. Accordingly, the amino acids were assigned in **1** as N-Me-L-Tyr (10.5 min).

Reference:

Marfey, P. (1984). Determination of D-amino acids. II. use of a bifunctional reagent, 1,5-difluoro-2,4-dinitrobenzene. Carlsberg Research Communication. 49, 591–596.