Antifungal cyclic peptides from the marine sponge *Microscleroderma herdmani*

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Abstract: Screening natural product extracts from the National Cancer Institute Open Repository for antifungal discovery afforded hits for bioassay-guided fractionation. Using LC–MS analysis to generate chemical structure information on potentially active compounds, two new cyclic hexapeptides, microsclerodermins J (1) and K (2), were isolated from the deep-water sponge *Microscleroderma herdmani*, along with microsclerodermins A (3) and B (4), previously isolated from an unidentified *Microscleroderma* species. The structures of the new compounds were elucidated by spectroscopic analysis and chemical methods. In vitro antifungal testing showed that the four compounds possessed strong activities against the opportunistic fungal pathogens *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*.

Keywords: antifungal, microsclerodermins, *Microscleroderma herdmani*, opportunistic fungal pathogens

Introduction

Systemic mycoses caused by opportunistic fungal pathogens have increased over the past two decades.^{1,2} However, there are a limited number of antifungal drugs available in the current antifungal therapy, and resistance has been found in all antifungal drug classes.³ This highlights the need for the discovery of safer, broad-spectrum antifungal agents, preferably with novel modes of action.

To diversify the collection of antifungal scaffolds from natural sources is an important approach for antifungal drug discovery. As part of our antifungal discovery program, we have screened thousands of natural product extracts from marine organisms, microbes, and plants from the National Cancer Institute Open Repository. An organic extract from the deep-water sponge *Microscleroderma herdmani*, collected in Mauritius, showed potent in vitro antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*, three opportunistic fungal pathogens that may cause life-threatening systemic mycoses in immunosuppressed hosts, such as AIDS and cancer patients.⁴ Although microsclerodermins, as a class of cyclic hexapeptides, have been isolated from *Microscleroderma* sp. and *Theonella* sp. and have been shown to possess antifungal and antitumor activities,^{5–7} the presence of possible new microsclerodermin analogs in this sponge was indicated by preliminary LC–MS analysis. The new compounds might be responsible for the observed antifungal activity, prompting us to perform a bioassay-guided isolation. As a result, two new cyclic hexapeptides, microsclerodermins J (1) and K (2), were isolated, along with

the known microsclerodermins A (**3**) and B (**4**) (Figure 1). Herein, we report the isolation, structure elucidation, and in vitro antifungal activities of the four compounds.

Results and discussion

The organic extract of the sponge *M. herdmani*, obtained from the Natural Products Branch Repository Program at the National Cancer Institute (NCI), showed potent antifungal activities against *C. albicans*, *C. neoformans*, and *A. fumigatus*, with 50% growth inhibitory concentrations (IC₅₀s) of 4.0, 3.0, and 3.5 µg/mL, respectively. Fractionation of this extract by reversed-phase silica gel column chromatography afforded

active fractions that were analyzed by LC–MS. The known compounds microsclerodermins A (**3**) and B (**4**), previously isolated from *Microscleroderma* sp., were identified in one active fraction, and subsequent separation by reversed-phase HPLC afforded compounds **3** and **4**, whose structures were confirmed by NMR spectroscopy. In another active fraction, LC–MS indicated the presence of two major compounds with retention times of 16.12 and 16.51 min, corresponding to strong quasimolecular ion peaks at m/z 933.17 [M + H]⁺ and 917.29 [M + H]⁺, respectively, in their positive ion mode ESIMS spectra (Figure 2). In addition, two minor quasimolecular ion peaks at m/z 955.38 [M + Na]⁺ and 939.45 [M + Na]⁺ were observed for the two compounds. Thus, the molecular weights of the two compounds were deduced as 932 and 916, respectively, which do not match the molecular weights of previously reported microsclerodermins A–I, dehydromicrosclerodermin A, and dehydromicrosclerodermins C and D,^{5–7} potentially indicating new microsclerodermins. The UV spectra of these two compounds showed strong absorptions around 205 and 261.5 nm and a shoulder at 290 nm (Figure 2), similar to those of microsclerodermins A and B.⁵ This fraction was then subjected to reversed-phase HPLC separation to furnish compounds **1** and **2**, corresponding to the peaks with retention times of 16.51 and 16.12 minutes, respectively, in the LC–MS base peak chromatogram (Figure 2).

Compound **1** was obtained as an amorphous white powder, with the molecular formula C₄₆H₆₀N₈O₁₂, determined by a combination of high resolution ESIMS (m/z 917.4528 for [M + H]⁺) and ¹³C NMR spectra. Its ¹H NMR spectroscopic data in DMSO-*d*₆ (Table 1) displayed resonance signals of five amide NH at δ 7.12 (d, *J* = 9.0 Hz, NH3), 7.40 (m, NH24), 8.35 (d, *J* = 10.0 Hz, NH44), 8.38 (dd, *J* = 5.5, 6.5 Hz, NH26), and 8.70 (d, *J* = 5.0 Hz, NH28); an *N*-methyl group at δ 3.00 (s, C-40); an indole NH at δ 10.87 (s, N36); and an *N*-vinylamide NH at δ 10.42 (s, NH43), accounting for all eight nitrogen atoms. The ¹³C NMR spectroscopic data (Table 1) indicated the presence of seven carbonyl carbons at δ 168.1 (C-41), 168.9 (C-25), 170.9 (C-38), 171.5 (C-27), 172.4 (C-21), 174.1 (C-1), and 174.9 (C-46); four methines attached to hydroxy groups at δ 68.4 (C-4), 67.1 (C-23), 69.4 (C-2), and 72.8 (C-5); and one methoxy group at δ 55.1, making up all twelve oxygen atoms in the molecule. These NMR spectroscopic characteristics implied that compound **1** should be a member of the microsclerodermin family.^{5–7}

Comparison of its ¹H and ¹³C NMR data (Table 1) with those of reported microsclerodermins readily identified three amino acid residues, glycine (Gly), *N*-methyl

Figure 1 Structures of compounds **1**–**4**.

Figure 2 LC-MS analysis of antifungal column fraction D: **(A)** base peak chromatogram (BPC); **(B)** positive ESIMS spectrum for the peak with a retention time of 16.12 minutes, corresponding to compound **2**; **(C)** positive ESIMS spectrum for the peak with a retention time of 16.51 minutes, corresponding to compound **1**; **(D)** UV spectrum for the peak with a retention time of 16.12 minutes, corresponding to compound **2**; and **(E)** UV spectrum for the peak with a retention time of 16.51 minutes, corresponding to compound **1**.

glycine (NMeGly), and 4-amino-3-hydroxybutyric acid or gamma-amino-beta-hydroxybutyric acid (GABOB) moieties, which were invariable building blocks in all reported microsclerodermins.⁵⁻⁷ The presence of 3-amino-6-methyl-12-(*p*-methoxyphenyl)-2,4,5-trihydroxy-dodec-11-enoic acid (AMMTD), a unique amino acid residue present in microsclerodermins A and B,⁵ was evident by the resonance signals of an aromatic ring [$\delta_{\text{H/C}}$ 7.31 (d, $J = 8.5$ Hz)/126.9

and $\delta_{\text{H/C}}$ 6.86 (d, $J = 8.5$ Hz)/113.9], one *E*-double bond [$\delta_{\text{H/C}}$ 6.12 (m)/128.4, $\delta_{\text{H/C}}$ 6.32 (d, $J = 16.0$ Hz)/128.9], three oxygen-bearing methines [$\delta_{\text{H/C}}$ 4.43 (d, $J = 4.0$ Hz)/69.4, $\delta_{\text{H/C}}$ 3.48 (brs)/68.4, and $\delta_{\text{H/C}}$ 3.07 (m)/72.8], four methylenes (δ_{C} 25.9, 29.5, 32.5, and 32.8), and one methoxy group [$\delta_{\text{H/C}}$ 3.74 (s)/55.1]. DQF-COSY spectrum further confirmed the connections in the long aliphatic chain from C-2 to C-12. Eight aromatic carbons (δ_{C} 110.0, 111.4, 118.1, 118.4, 121.0,

Table 1 NMR spectroscopic data of compounds **1** and **2** in DMSO- d_6 (δ , ppm)^a

Amino acid	1		2	
	δ_c , mult	δ_H (J in Hz)	δ_c , mult	δ_H (J in Hz)
AMMTD				
1	174.1 s		174.0 s	
2	69.4 d	4.43 (d, 4.0)	69.4 d	4.48 (m)
3	53.7 d	4.11 (m)	53.7 d	4.10 (m)
4	68.4 d	3.48 (br s)	68.5 d	3.48 (br s)
5	72.8 d	3.07 (m)	72.8 d	3.07 (m)
6	34.0 d	1.59 (m)	34.5 d	1.59 (m)
7	32.8 t	2.16 (br s)	32.6 t	2.14 (br s)
8	25.9 t	1.24 (br s)	26.0 t	1.23 (br s)
		1.40 (m)		1.40 (m)
9	29.5 t	1.24 (br s)	29.5 t	1.23 (br s)
10	32.5 t	2.16 (br s)	32.4 t	2.14 (br s)
11	128.4 d	6.12 (m)	128.4 d	6.12 (m)
12	128.9 d	6.32 (d, 16.0)	128.9 d	6.31 (d, 15.5)
13	130.1 s		130.1 s	
14	126.9 d	7.31 (d, 8.5)	126.9 d	7.30 (d, 8.5)
15	113.9 d	6.86 (d, 8.5)	113.9 d	6.85 (d, 8.0)
16	158.3 s		158.3 s	
17	113.9 d	6.86 (d, 8.5)	113.9 d	6.85 (d, 8.0)
18	126.9 d	7.31 (d, 8.5)	126.9 d	7.30 (d, 8.5)
19	15.9 q	0.78 (d, 6.5)	15.4 q	0.77 (d, 6.5)
20	55.1 q	3.74 (s)	55.0 q	3.72 (s)
NH3		7.12 (d, 9.0)		7.20 (d, 9.5)
OH2		6.12		6.23
OH4		4.28		4.34
OH5		4.26		4.43
GABOB				
21	172.4 s		172.4 s	
22	41.4 t	2.06 (dd, 11.0, 13.5)	41.5 t	2.09 (m)
		2.34 (d, 13.5)		2.33 (d, 14.5)
23	67.1 d	3.72 (m)	66.6 d	3.79 (m)
24	45.7 t	2.77 (m)	45.3 t	2.73 (m)
		3.37 (m)		3.32 (m)
NH24		7.40 (m)		7.38 (m)
OH23		Not detected		
Gly				
25	168.9 s		169.0 s	
26	42.8 t	3.68 (dd, 5.5, 17.0)	42.6 t	3.66 (dd, 5.5, 16.5)
		3.55 (dd, 5.5, 17.0)		3.55 (dd, 5.5, 16.5)
NH26		8.38 (t, 5.5, 6.5)		8.38 (m)
Trp				
27	171.5 s		171.4 s	
28	55.7 d	4.09 (m)	55.6 d	4.10 (m)
29	25.7 t	3.09 (m)	25.7 t	3.06 (m)
		3.23 (m)		3.24 (d, 4.5)
30	123.9 d	7.24 (d, 0.5)	123.9 d	7.21 (s)
31	110.0 s		110.0 s	
32	118.1 d	7.54 (d, 8.0)	118.1 d	7.52 (d, 7.5)
33	118.4 d	7.01 (dd, 7.0, 7.5)	118.4 d	7.00 (dd, 7.5, 8.0)
34	121.0 d	7.08 (d, 7.5)	121.0 d	7.07 (dd, 7.5, 7.5)
35	111.4 d	7.36 (d, 8.0)	111.4 d	7.34 (d, 8.0)
36	136.2 s		136.1 s	
37	127.5 s		127.0 s	
NH28		8.70 (d, 5.0)		8.70 (d, 5.0)
NH36		10.87 (s)		10.85 (s)

(Continued)

Table 1 (Continued)

Amino acid	1		2	
	δ_c , mult	δ_H (J in Hz)	δ_c , mult	δ_H (J in Hz)
NMeGly				
38	170.9 s		170.7 s	
39	50.0 t	4.57 (d, 16.0) 3.45 (d, 16.0)	50.3 t	4.50 (d, 17.5) 3.55 (m)
40	36.9 q	3.00 (s)	37.0 q	3.00 (s)
pyrrolidone				
41	168.1 s		167.9 s	
42	87.9 d	5.26 (br s)	88.4 d	5.18 (s)
43	157.4 s		152.3 s	
44	45.9 d	5.24 (m)	55.2 d	4.82 (dd, 6.0, 7.0)
45	34.5 t	2.77 (m) 2.48 (m)	71.3 d	4.42 (m)
46	174.9 s		174.3 s	
NH43		10.42 (s)		10.42 (s)
NH44		8.35 (d, 10.0)		8.38 (m)
OH45				6.13

Notes: *Data recorded at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR. Assignments were based on DEPT and 2D NMR, including DQF-COSY, HMQC, HMBC, and ROESY. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses. For overlapped signals, only chemical shift values are given.

123.9, 127.5, and 136.2) and NH (δ_H 10.87) assembled an indole skeleton. HMBC correlations (Figure 3), from H-28 (δ_H 4.09) to C-30 (δ_C 123.9) and C-27 (δ_C 171.5), and from H-29 (δ_H 3.09 and 3.23) to C-28 (δ_C 55.7), C-30 (δ_C 123.9), C-31 (δ_C 110.0), and C-37 (δ_C 127.5), confirmed the presence of tryptophan (Trp), which was also an amino acid residue in microsclerodermins F and H.⁷ The last compositional amino acid residue was 3-hydroxy-4-amino-5-vinylpyrrolidone (pyrrolidone), which was indicated by the chemical shift of NH at δ 10.42 (NH43) and HMBC correlations from this NH to C-43 (δ_C 157.4), C-44 (δ_C 45.9), C-45 (δ_C 34.5), and C-46 (δ_C 174.9), and H-42 (δ_H 5.26, brs) to C-41 (δ_C 168.1) and C-44 (δ_C 45.9). These chemical shifts are consistent with those of the same pyrrolidone moiety in microsclerodermin E.⁶

The HMBC correlations between NH44 and C-1, NMe40 and C-41, NH28 and C-38, NH26 and C-27, NH24 and C-25,

and NH3 and C21 (Figure 3) determined the cyclic peptide chain, starting from AMMTD to pyrrolidone-NMeGly-Trp-Gly and closed by GABOB. As the ^1H and ^{13}C NMR chemical shifts of individual amino acid residues are identical to those of structurally close anhydromicrosclerodermin C, except for the achiral aromatic moiety of tryptophan,⁶ the absolute configurations of these amino acids should be the same as those of previously reported microsclerodermins that share similar taxonomic sources.⁵⁻⁷ The identical NMR data also suggest that these compounds should possess similar conformations. Compound **1** was named microsclerodermin J.

Compound **2**, named microsclerodermin K, has the molecular formula $\text{C}_{46}\text{H}_{60}\text{N}_8\text{O}_{13}$, which was determined by the high-resolution ESIMS (m/z 931.4124 [$\text{M} - \text{H}$] $^-$) and ^{13}C NMR spectra. Its NMR data (Table 2) are similar to those of compound **1**. However, the observed significant downfield shifting of C-45 at δ_C 71.3 (d) and H-45 at δ_C 4.42 (m) in **2**, compared to **1**, confirms that an α -hydroxy group is attached at C-45, giving the same pyrrolidone moiety as in dehydromicrosclerodermin A.⁵ Complete acid hydrolysis of **2** was performed, and the resultant common amino acid residue tryptophan was determined to possess a 2*R* absolute configuration, by Marfey's method.⁸ In conjunction with the NMR data, this further confirmed that the absolute configurations of the amino acid residues in **2**, as well as in **1**, should be the same as those of microsclerodermins A and B.⁵

Microsclerodermins J (**1**), K (**2**), A (**3**), and B (**4**) were tested for antifungal activity against *C. albicans*, *C. glabrata*, *C. krusei*, *A. fumigatus*, and *C. neoformans*, using the CLSI broth microdilution method.^{9,10} The minimum inhibitory

Figure 3 Key HMBC correlations for compounds **1** and **2**.

Table 2 In vitro antifungal activity of compounds 1–4 (IC₅₀/MIC/MFC, µg/mL)^a

	<i>C. albicans</i> ATTC 90028	<i>C. glabrata</i> ATCC 90030	<i>C. krusei</i> ATCC 6258	<i>A. fumigatus</i> ATCC 90906	<i>C. neoformans</i> ATCC 90113
Microsclerodermin J (1)	9.0/20.0/20.0	11.0/20.0/20.0	10.6/20.0/20.0	5.3/10.0/- ^b	5.2/10.0/10.0
Microsclerodermin K (2)	5.3/10.0/10.0	5.7/10.0/10.0	5.7/10.0/10.0	4.5/10.0/- ^b	2.7/5.0/5.0
Microsclerodermin A (3)	1.8/5.0/5.0	2.8/5.0/5.0	2.9/5.0/5.0	0.8/1.3/- ^b	2.7/5.0/5.0
Microsclerodermin B (4)	1.4/5.0/5.0	3.0/5.0/5.0	3.3/5.0/5.0	0.4/0.6/- ^b	4.3/5.0/5.0
Amphotericin B	0.6/2.5/2.5	0.9/2.5/2.5	1.3/2.5/2.5	1.3/2.5/- ^c	2.3/5.0/5.0

Notes: The highest test concentration for compounds 1–4 were 20 µg/mL. The highest test concentration for amphotericin B was 5 µg/mL. ^aIC₅₀, 50% growth inhibition; MIC, minimum inhibitory concentration (lowest concentration that allows no detectable growth); MFC, minimum fungicidal concentration (the lowest concentration that kills the fungus); ^bnot fungicidal at 20 µg/mL; ^cnot fungicidal at 5 µg/mL.

concentration (MIC) and minimum fungicidal concentration (MFC) of the four compounds were determined in comparison with the positive control amphotericin B and are shown in Table 2. Compounds 1 and 2 are less potent than compounds 3 and 4. For example, compound 4 had MICs of 5.0, 5.0, 5.0, 0.6, and 5.0 µg/mL, comparable to amphotericin B, with MICs of 2.5, 2.5, 2.5, 2.5, and 5.0 µg/mL against *C. albicans*, *C. glabrata*, *C. krusei*, *A. fumigatus*, and *C. neoformans*, respectively. It is important to note that the four compounds are fungicidal against tested *Candida* species and *Cryptococcus neoformans*, which represent typical opportunistic fungal pathogens associated with immunosuppressed hosts.^{2,4}

Previous studies have described antifungal activity of microsclerodermins against *C. albicans* in a paper disk assay.^{5–7} Relative antifungal potencies of compounds were reported; eg, microsclerodermins A (3) and B (4) inhibited the growth of *C. albicans* at 2.5 µg/disk.⁵ Our antifungal testing method has shown that these two compounds indeed exhibited similar, strong antifungal potency when compared with the antifungal drug amphotericin B (Table 2), indicating that this class of compounds may warrant further study as potential antifungal leads.

Experimental section

General experimental procedure

Optical rotations were measured with an Autopol IV polarimeter. UV was obtained from an HP 8453 diode array spectrophotometer. IR spectra were recorded using a Thermo Nicolet IR 300 FT/IR spectrometer. The 1D and 2D NMR (DQF-COSY, HMQC, HMBC, and ROESY) spectra using standard pulse programs were recorded at room temperature on a Varian Oxford AS400 spectrometer, operating at 400 (¹H) and 100 (¹³C) MHz, or on a Bruker Avance DRX 500 FT spectrometer, operating at 500 (¹H) and 125 (¹³C) MHz. The chemical shift values are relative to the internal standard TMS. HRESIMS data were obtained on an Agilent Series 1100 SL mass spectrometer. Column chromatography was

performed using reversed-phase silica gel (RP-18, 40 µm; JT Baker, USA). Semi-preparative HPLC was conducted on a C18 column (Gemini, 250 × 10 mm id, 5 µm particle size; Phenomenex Inc, USA) with UV detection at 254 nm. TLC was carried out on silica gel sheets (Alugram® Sil G/UV₂₅₄; Macherey-Nagel, Germany) and reversed-phase plates (RP-18 F_{254S}; Merck, Germany) and visualized by spraying 10% H₂SO₄ followed by heating. *N*-α-(2,4-Dinitro-5-fluorophenyl)-L-alaninamide (L-FDAA), D- and L-tryptophan were purchased from Sigma (Milwaukee, WI).

LC–MS analysis

ESI–MS spectra were acquired using a FinniganMat LCQ™ (Thermo Scientific, Waltham, MA). The mass detector was an ion trap analyzer equipped with an ESI interface. The MS conditions were optimized with the sheath gas at 20 arbitrary units (AU), auxiliary gas at 5 AU, capillary temperature at 250°C, electrospray voltage of the ion source at 4.5 kV, capillary voltage at 30 V, and tube lens offset voltage at –20 V. ESI was operated in the positive ion mode. Full-scan spectra were acquired over a 300–2000 *m/z* range. Chromatographic analysis was conducted at ambient temperature, and the data was analyzed using Xcalibur software. The HPLC system consisted of a quaternary pump (model TSP P4000), an autosampler (model TSP AS3000), and a diode-array detector (model TSP UV6000). Separation was achieved on a Gemini C18 column (150 × 4.6 mm id, 5 µm particle size; Phenomenex Inc) at room temperature. The column was equipped with a 2 cm Phenomenex LC-18 guard column. The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in CH₃CN at a flow rate of 1.0 mL/min; the gradient elution was as follows: 0–25 min, 20% A:80% B to 65% A:35% B; 25–27 min, 65% A:35% B to 100% A; 27–35 min, 100% A. Each run was followed by an 8-minute wash with 100% CH₃CN and a re-equilibration with 20% A:80% B for 15 minutes, respectively. The injection volume was 10 µL at a concentration of 50 µg/mL.

Biological material

A specimen of the lithistid sponge *Microscleroderma herdmani* was collected in Mauritius (latitude 20°17.60' S, longitude 057°21.06' E) at a depth of 45 m under sea on November 13, 1999. A voucher specimen was deposited in the Smithsonian Institute, Washington DC (voucher #0CDN6936). The NCI received the material on December 2, 1999, from which a CHCl_3 –MeOH (1:1) extract (coded as C019611) was prepared by an extraction protocol described previously.¹¹

Isolation of antifungal compounds

The organic extract (2.770 g) was chromatographed on a reversed-phase C18 column (RP-18 silica gel, 70 g), using stepwise gradient elution of MeOH–H₂O (0%, 25%, 50%, 75%, and 100%) to afford five fractions (Fr. A–E). Fr. C (50% MeOH eluate, 0.203 g) showed IC_{50} s of 1.6, 9.3, and 1.9 $\mu\text{g/mL}$ against *C. albicans*, *C. neoformans*, and *A. fumigatus*, respectively, while Fr. D (75% MeOH eluate, 0.184 g) gave IC_{50} s of 2.9, 17.8, and 3.1 $\mu\text{g/mL}$ against the aforementioned three fungal pathogens. Upon semi-preparative, reversed-phase HPLC separation using 43% aqueous CH_3CN in 0.1% trifluoroacetic acid, Fr. C afforded microsclerodermins A (**3**, 54.6 mg) and B (**4**, 71.6 mg), while Fr. D gave microsclerodermins J (**1**, 10.4 mg) and K (**2**, 8.0 mg).

Microsclerodermin J (**1**): amorphous white powder, $[\alpha]_D^{25} -57.3$ (*c* 0.12, 1:5 0.1 N aqueous $\text{NH}_4\text{HCO}_3/\text{MeOH}$); UV (MeOH) λ_{max} (log ϵ) 205 (4.78), 264 (4.23); IR ν_{max} 3285, 2917, 2850, 1649, 1510, 1462, 1249, 1174, 1069, 867, 839, 789, 718 cm^{-1} ; NMR data (DMSO-*d*₆): Table 1; HRESIMS *m/z* 917.4528 (calcd for $[\text{C}_{46}\text{H}_{60}\text{N}_8\text{O}_{12} + \text{H}]^+$, 917.4409).

Microsclerodermin K (**2**): amorphous white powder, $[\alpha]_D^{25} -78.2$ (*c* 0.12, 1:5 0.1 N aqueous $\text{NH}_4\text{HCO}_3/\text{MeOH}$); UV (MeOH) λ_{max} (log ϵ) 204 (4.72), 262 (4.53); IR (KBr) ν_{max} 3298, 2920, 2851, 1650, 1510, 1461, 1336, 1248, 1174, 1087, 1023, 801, 745, 719 cm^{-1} ; NMR data (DMSO-*d*₆): Table 1; HRESIMS *m/z* 931.4124 (calcd for $[\text{C}_{46}\text{H}_{60}\text{N}_8\text{O}_{13} - \text{H}]^-$, 931.4202).

Determination of absolute configuration of tryptophan in microsclerodermin K (**2**)

A solution of compound **2** (0.9 mg) in 6 N HCl (1 mL) was heated in a sealed tube at 110°C for 24 hours. After cooling, the solvent was removed by a stream of dry nitrogen gas. The residue was further dried in vacuo and dissolved in 50 μL water. To this solution, 20 μL of 1 M sodium bicarbonate and 100 μL of 1% L-FDAA in acetone were added. The solution was incubated at 37°C for 1 hour. The reaction was

quenched by adding 20 μL 1 M HCl. The reaction mixture was diluted with 810 μL MeOH, and 5 μL of the resultant solution was subjected to HPLC analysis. The standard amino acids L- and D-tryptophan (0.3 mg each) were treated in the same manner. The HPLC analysis was carried out on a Discovery C18 column (150 $\times$ 4.6 mm, 5 μm ; Supelco, PA) at ambient temperature, using a mobile phase of CH_3CN and 0.1% trifluoroacetic acid in water at a flow rate of 1 mL/min under a linear gradient elution, starting from 25% to 65% CH_3CN , for 45 minutes. A diode-array detector at 340 nm was used for detection. The retention times for L-tryptophan-FDAA and D-tryptophan-FDAA were 22.9 and 25.8 minutes, respectively. Identification of L-tryptophan in compound **2** was made by spiking the standard L-tryptophan-FDAA and D-tryptophan-FDAA in the derivatized hydrolysis products.

Antifungal assay

The organisms were obtained from the American Type Culture Collection (Manassas, VA), and included *Candida albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 204305. All organisms were tested using modified versions of the CLSI (formerly NCCLS) methods.^{9,10} For all organisms, excluding *A. fumigatus*, optical density was used to monitor growth. Media supplemented with 5% Alamar Blue™ (BioSource International, Camarillo, CA) was used for growth detection of *A. fumigatus*. Samples (dissolved in DMSO) were serially-diluted in 20% DMSO/saline and transferred (10 μL) in duplicate to 96-well, flat-bottom microplates. Inocula were prepared by correcting the OD_{630} of microbe suspensions in incubation broth. Amphotericin B (ICN Biomedicals, OH) was included as a positive control in each assay. The growth of all organisms was read at either 530 nm, using the Biotek Powerwave XS plate reader (Bio-Tek Instruments, VT), or 544ex/590em, (*A. fumigatus*), using the Polarstar Galaxy Plate Reader (BMG LabTechnologies, Germany) prior to and after incubation at 35°C. IC_{50} s (concentrations that afford 50% inhibition relative to controls) were calculated using XLfit 4.2 software (IDBS, Alameda, CA), using fit model 201. The MIC is defined as the lowest test concentration that allows no detectable growth (for *A. fumigatus*, no color change from blue to pink). MFCs were determined by removing 5 μL from each clear (or blue) well, transferring to fresh media, and incubating at 35°C for 2–3 days. The MFC is defined as the lowest test concentration that kills the organism (allows no growth).

Acknowledgment

The authors would like to thank the Natural Products Branch Repository Program at the National Cancer Institute for providing the marine extract from the NCI Open Repository, Ms Marsha Wright for antifungal testing, Dr Bharathi Avula for recording HRESI-MS spectra, Mr Frank T Wiggers for obtaining some NMR spectra, and Dr Wenhui Xu and Mr Xiang Fu for technical assistance of HPLC analysis. This work was supported by the NIH, NIAID, Division of AIDS, Grant No AI 27094, and the USDA Agricultural Research Service Specific Cooperative Agreement No 58-6408-2-0009.

Disclosure

The authors report no conflicts of interest in this work.

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