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Scytalidamides A and B, New Cytotoxic Cyclic Heptapeptides from a Marine Fungus of the Genus *Scytalidium*

Lik Tong Tan, Xing C. Cheng, Paul R. Jensen, and William Fenical*

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0204

wfenical@ucsd.edu

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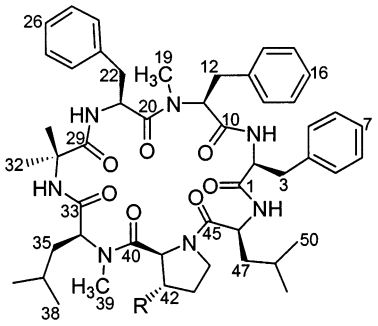
Two new cyclic heptapeptides have been isolated from the culture broth of a marine fungus, *Scytalidium* sp., collected from the Bahamas. The planar structures of scytalidamides A (**1**) and B (**2**) were assigned on the basis of 1D and 2D NMR spectroscopic techniques, while the absolute configuration of the amino acid residues in both molecules was determined by application of the advanced Marfey's method. The absolute stereochemistry of the uncommon 3-methylproline moiety in scytalidamide B (**2**) was confirmed by isolation and CD measurements, as well as application of the advanced Marfey's method. Scytalidamides A (**1**) and B (**2**) showed moderate in vitro cytotoxicity toward HCT-116 human colon adenocarcinoma with IC_{50} values of 2.7 and 11.0 μM, respectively.

Introduction

Marine fungi are proving to be a productive source of structurally unique and biologically active natural products.[1] Among the diverse classes of compounds being discovered from this group is a small but growing number of cyclic peptides and depsipeptides including the tetrapeptide JM47,[2] the pentadepsipeptides sansalvamide and *N*-methyl sansalvamide,[3] the hexadepsipeptides enniatin G and exumolides A, and B,[4] and the heptapeptides unguisins A−C.[5] Interestingly, with only one exception,[5] all of the fungi responsible for the production of these cyclic peptides and depsipeptides were isolated from marine plant material.

In this paper, we add to the list of biologically active cyclic peptides discovered from marine plant-derived fungi with the isolation and structure determination of two new heptapeptides, scytalidamides A (**1**) and B (**2**). These cytotoxic peptides were isolated from an alga-associated fungus (strain CNC-310) that has been identified as a member of the genus *Scytalidium*.[6] Although *Scytalidium* species are generally known to be associated with terrestrial plants, the scytalidamides join the exumolides[4b] as the second group of cyclic peptides reported from marine algae-associated *Scytalidium* species.



Scytalidamide A (**1**) R = H
Scytalidamide B (**2**) R = CH_3

The scytalidamides A and B (**1** and **2**) contain an α-aminoisobutyric acid (Aib) moiety in addition to other L-amino acid residues. The occurrence of Aib-containing peptides is common from microbial sources and many of these molecules, such as the cyclic peptide chlamydocin[7] and the linear peptides alamethicin, antiamoebin, emerimicin, and zervamicin,[8] possess antibiotic properties. Furthermore, it is well documented that Aib-containing molecules tend to adopt helical structures, either as α-helical or 3_{10}-helical structures, and that these structural elements are important for protein−protein and protein−nucleic acid interactions.[9] The advantage of

* To whom correspondence should be addressed. Tel: 858 534 2133, Fax: 858 558 3722.

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TABLE 1. NMR Spectral Data for Scytalidamide A (1) at 400 MHz (^{1}H) and 100 MHz (^{13}C) in Pyridine-d_5

unit	position	δH mult (J, Hz)	δC	HMBC
L-Phe-1	1		172.0	
	2	5.21 dt (9.2, 5.2)	57.5	C-3
	3a	3.92 m	37.5	C-2, C-4
	3b	3.68 m		C-2, C-4
	4		139.8	
	5/9	7.51 d (8.0)	129.8	C-3, C-7
	6/8	7.33 t (8.0)	129.4	C-4, C-5/9
	7	7.28 t (6.8)	127.4	C-5/9
	*N*H	8.21 d (8.4)		C-2, C-10
L-*N*Me Phe	10		171.4	
	11	3.70 d (5.0)	69.3	C-12
	12a	3.55 m	34.4	C-11, C-13, C-14/18
	12b	3.10 dd (12.0, 3.2)		C-11, C-13, C-14/18
	13		138.4	
	14/18	6.56 d (7.6)	129.9	C-12, C-15/17, C-16
	15/17	7.05 t (6.8)	129.0	C-13
	16	7.15 t (6.8)	127.1	C-14/18
	19 *N*Me	2.79 s	40.7	C-11, C-20
L-Phe-2	20		171.9	
	21	5.66 dt (10.4, 3.2)	51.4	C-20, C-29
	22a	3.72 m	37.9	C-21, C-23, C-24/28
	22b	3.04 dd (12.8, 3.2)		C-21, C-23, C-24/28
	23		139.6	
	24/28	7.46 d (8.0)	130.8	C-22, C-26
	25/27	7.35 t (8.0)	129.2	C-24/28
	26	7.25 t (6.4)	127.0	C-24/28
	*N*H	8.70 d (9.6)		C-21, C-29
Aib	29		174.3	
(2-aminoisobutyric acid)	30		57.6	
	31	1.63 s	28.4	C-29, C-30, C-32
	32	1.90 s	24.1	C-29, C-30, C-31
	*N*H	9.43 s		C-29, C-30, C-31, C-33
L-*N*Me Leu	33		170.3	
	34	4.76 brt (4.6)	56.9	C-33, C-35, C-39, C-40
	35a	1.80 m	39.8	
	35b	1.60 m		
	36	1.58 m	25.3	
	37	1.01 d (6.9)	22.7	C-35, C-36, C-38
	38	0.92 d (6.6)	22.3	C-35, C-36, C-37
	39 *N*Me	3.32 s	29.5	C-34, C-40
L-Pro	40		173.1	
	41	5.20 dd (8.0, 5.0)	56.4	C-40, C-43, C-44
	42a	2.06 m	26.1	
	42b	1.50 m		
	43ab	1.58 m	29.6	
	44a	3.95 m	47.9	
	44b	3.80 m		C-45
L-Leu	45		171.8	
	46	5.36 brt (9.2)	50.0	C-47
	47a	2.51 m	41.2	C-46, C-48
	47b	2.05 m		C-48
	48	2.50 m	24.1	
	49	1.02 d (6.0)	23.5	C-47, C-48, C-50
	50	1.09 d (5.4)	23.8	C-47, C-48, C-49
	*N*H	8.90 d (8.8)		C-1

having an Aib residue in a peptide is that it improves the molecular binding affinity by reducing the loss of conformational entropy upon binding.[10] In addition, since Aib is not part of the standard amino acid repertoire, peptides containing the Aib moiety may be more resistant to protease hydrolysis.[11] In view of these properties, Aib residues have been incorporated into a number of synthetic peptides resulting in enhanced bioactivity profiles.[9b,12] The presence of the Aib unit in the scytalidamides A and B may therefore account for the biological activities associated with these molecules.

Results and Discussion

Scytalidamide A (**1**) was purified as fine white crystals with a molecular composition of $C_{50}H_{67}N_7O_7$, as determined by HRFABMS (obsd $[M + Cs]^+$ at *m/z* 1010.4195). Several features of the ^{1}H NMR spectrum of **1**, specifically four amide-type protons at δ 9.43, 8.90, 8.70, and 8.21, as well as two *N*Me singlets at δ 3.32 and 2.79,

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TABLE 2. NMR Spectral Data for Scytalidamide B (2) at 400 MHz (^{1}H) and 100 MHz (^{13}C) in Pyridine-d_5

unit	position	δH mult (*J*, Hz)	δC	HMBC
L-Phe-1	1		172.4	
	2	5.16 dt (9.6, 5.4)	57.4	C-3
	3a	3.85 m	37.6	C-2, C-4
	3b	3.65 m		C-2, C-4
	4		140.1	
	5/9	7.49 d (6.9)	130.1	C-3, C-7
	6/8	7.32 t (6.9)	129.7	C-4, C-5/9
	7	7.28 t (6.4)	127.6	C-5/9
	*N*H	8.13 d (8.4)		C-2, C-3, C-10
L-*N*Me Phe	10		171.5	
	11	3.68 m	69.6	C-12
	12a	3.60 m	34.7	C-11, C-13, C-14/18
	12b	3.20 dd (12.0, 3.2)		C-11, C-13, C-14/18
	13		138.7	
	14/18	6.52 d (6.9)	130.2	C-12, C-15/17, C-16
	15/17	7.04 t (7.5)	129.2	C-13
	16	7.15 t (7.5)	127.3	C-14/18
	19 *N*Me	2.78 s	40.9	C-11, C-20
L-Phe-2	20		172.5	
	21	5.67 dt (10.4, 3.2)	51.4	C-20, C-29
	22a	3.70 m	38.8	C-21, C-23, C-24/28
	22b	3.02 dd (12.6, 3.0)		C-21, C-23, C-24/28
	23		139.5	
	24/28	7.42 d (6.9)	131.0	C-22, C-26
	25/27	7.35 t (6.9)	129.4	C-24/28
	26	7.26 t (6.4)	127.3	C-24/28
	*N*H	8.70 d (9.9)		C-21, C-29
Aib	29		174.7	
(2-aminoisobutyric acid)	30		57.9	
	31	1.60 s	28.6	C-29, C-30, C-32
	32	1.90 s	24.4	C-29, C-30, C-31
	*N*H	9.40 s		C-29, C-30, C-31, C-33
L-*N*Me Leu	33		169.8	
	34	4.81 dd (9.0, 5.1)	58.1	C-33, C-35, C-39, C-40
	35a	2.05 m	41.1	
	35b	1.50 m		
	36	1.70 m	26.1	
	37	1.12 d (7.2)	23.3	C-35, C-36, C-38
	38	1.00 d (6.6)	23.9	C-35, C-36, C-37
	39 *N*Me	3.37 s	30.3	C-34, C-40
(2*S*,3*S*)-3-MePro	40		172.4	
	41	4.77 d (6.9)	62.7	C-40, C-42, C-45
	42	2.30 m	38.9	
	42-CH_3	0.76 d (6.9)	18.0	C-41, C-42, C-43
	43a	2.10 m	34.4	
	43b	1.05 m		
	44a	4.03 m	47.9	
	44b	3.75 m		C-46
L-Leu	45		172.3	
	46	5.35 brt (9.2)	50.2	C-48
	47a	2.50 m	40.9	
	47b	2.30 m		
	48	2.00 m	25.4	
	49	1.09 d (5.4)	22.4	C-48, C-49, C-51
	50	1.04 d (5.1)	24.3	C-48, C-49, C-50
	*N*H	8.90 d (9.0)		C-1

suggested the peptidic nature of this compound. In addition, seven low-field ^{13}C NMR signals, located between 170 and 175 ppm, were attributable to amide carbonyls. Analysis of 1D and 2D NMR spectra in pyridine-d_5 allowed construction of seven partial structures (Table 1). Six standard amino acid residues, two phenylalanine units (Phe-1 and Phe-2), one *N*-methylphenylalanine (*N*Me-Phe), one leucine (Leu), one *N*-methylleucine (*N*Me-Leu), and one proline (Pro) were resolved. An additional amino acid residue was identified as aminoisobutyric acid (Aib) from interpretation of HMBC data. Key HMBC correlations used to construct the Aib unit were from protons H_3-31 and H_3-32 to C-30 and the carbonyl carbon C-29 and an amide proton *N*H correlation (δ 9.43) to C-29, C-30, and C-31. A total of 20 degrees of unsaturation were accounted for with these seven residues. With only a single degree of unsaturation remaining, scytalidamide A was deduced to be a monocyclic peptide.

The sequential relationship of these seven residues was established mainly from HMBC data. For example, HMBC correlations were observed between *N*H-Leu (δ 8.90) to C-1 (carbonyl carbon of Phe-1); from *N*H-Phe-1 (δ 8.21) to C-10 (carbonyl carbon of *N*Me-Phe); from *N*CH_3-Phe (δ 2.79) to C-20 (carbonyl carbon of Phe-2); from *N*H-Phe-2 (δ 8.70) to C-29 (carbonyl carbon of Aib); from *N*H-Aib (δ 9.43) to C-33 (carbonyl carbon of *N*Me-Leu); and from *N*CH_3-Leu (δ 3.32) to C-40 (carbonyl

TABLE 3. Retention Times (t_R, min) of L- and LD-FDLA-Derivatized Amino Acids from the Acid Hydrolyses of Scytalidamides A (1) and B (2) Detected by Positive-Mode ESI LC/MS

	scytalidamide A (**1**)			scytalidamide B (**2**)		destruxin analogue	
	m/z^a $[M + H]^+$	L-FDLA	LD-FDLA	L-FDLA	LD-FDLA	L-FDLA	LD-FDLA
Phe-1/-2	460.1	24.10	24.06 30.16	24.53	24.5 0 30.18		
*N*Me-Phe	474.1	25.70	25.64 26.98	25.67	25.6 2 26.96		
Leu	426.2	23.36	23.34 32.01	23.40	23.4 32.08		
*N*Me-Leu	440.1	27.00	26.92 30.56	26.90	26.83 30.31		
Pro	410.1	16.01	16.04 19.43				
3-MePro	424.1			19.33		19.34 (2*S*,3*S*)	19.35 (2*S*,3*S*) 23.69 (=2*R*,3*R*)[b]

[a] Detection in API-ES (positive) mode. [b] Retention time equivalent of (2*S*,3*S*)-3-MePro derivatized with D-FDLA.

carbon of Pro). These data revealed the following sequence: Leu/Phe-1/*N*Me-Phe/Phe-2/Aib/*N*Me-Leu/Pro. The final ring closure between Pro and Leu was indicated by an HMBC correlation of H_δ-44 of Pro (δ 3.80) to C-45 (carbonyl carbon of Leu). The trans conformation of the Pro amide bond in **1** was determined by a small $\Delta_{\beta\gamma}$ (differential value of ^{13}C chemical shifts of C_β and C_γ in Pro) value of 3.5 ppm.[13] The absolute stereochemistry of **1**, determined by application of the advanced Marfey method to the isolated amino acids using L/D-FDLA (1-fluoro-2,4-dinitrophenyl-5-L/D-leucinamide),[14] revealed that all possessed L-configurations (Table 3).

Scytalidamide B (**2**) was obtained as white crystals which analyzed for $C_{51}H_{69}N_7O_7$ by high-resolution FABMS. The peptide nature of **2** was also indicated by analysis of ^1H NMR data, which showed four exchangeable amide protons at δ 9.40, 8.90, 8.70, and 8.13 and two *N*-Me singlets at δ 3.37 and 2.78. In addition, the ^1H and ^{13}C NMR spectra of **2** were similar to those of scytalidamide A (**1**), indicating that **2** possessed similar amino acid residues.

Examination of the 2D NMR data derived from **2** revealed the presence of two Phe residues, one *N*Me-Phe, one Leu, one *N*Me-Leu, one Aib, and one 3-methylproline (3-MePro) residue (Table 2). The placement of the methyl functionality on C-42 of 3-MePro in **2** was facilitated mainly by interpretation of COSY and HMBC data. For instance, HMBC correlations were observed between CH_3-42 to C-41, C-42, and C-43. In addition, a cross-peak between CH_3-42 and H-42 was observed in the $^1H-^1H$ COSY spectrum of **2**.

HMBC correlations between *N*H protons to carbonyl carbons of adjacent amino acid residues established the connectivity of the seven amino acids. A key HMBC correlation between H_δ-44b of 3-MePro and the carbonyl carbon of Leu closed the ring structure of scytalidamide B. As in **1**, Marfey analysis of the isolated amino acids from **2** showed that all of the residues possessed L-configurations (Table 3).[14]

The trans configuration of the (2*S*,3*S*)-3-MePro residue in scytalidamide B (**2**) was first suggested by a ROESY correlation between the methyl protons at CH_3-42 and the α-proton at H-41. This was confirmed by the isolation of (2*S*,3*S*)-3-MePro from the hydrolysis mixture of **2**, which allowed the structure to be confirmed by interpretation of ^1H NMR data. The ^1H chemical shifts and coupling constants for (2*S*,3*S*)-3-MePro agreed well with those reported by Helms et al.[15a] and Mauger et al.[15b] for *trans*-3-MePro. Furthermore, the CD spectrum of pure (2*S*,3*S*)-3-MePro showed a single positive maximum at about 206 nm, consistent with that reported in the literature for a trans configuration.[16] The absolute stereochemistry of (2*S*,3*S*)-3-MePro in **2** was finally determined as 41*S* and 42*S* using the advanced Marfey's method[14] which showed a virtually identical retention time in comparison with an authentic sample of (2*S*,3*S*)-3-MePro obtained from the acid hydrolysis of a recently reported cyclic depsipeptide belonging to the destruxin class (Table 3).[17]

Solvent and Temperature Dependences of NH Protons. Variable-temperature NMR experiments were performed on scytalidamides A (**1**) and B (**2**) in order to probe the degree and nature of the amide protons participating in intramolecular hydrogen bonding within these molecules.[18] Proton NMR spectra of scytalidamides A and B were acquired over a range of temperatures in three different solvents, C_6D_6, $CDCl_3$, and DMSO-d_6, and the respective $\Delta\delta/\Delta T$ (in ppb/°C) values of the amide protons are summarized in Figure 1 and Table 4.

In a nonpolar environment such as C_6D_6, the $\Delta\delta/\Delta T$ values of the amide protons in scytalidamides A (**1**) and B (**2**) were all greater than −4.0 indicating that the amide protons were exposed to solvent. However, when temperature-dependence experiments were conducted in

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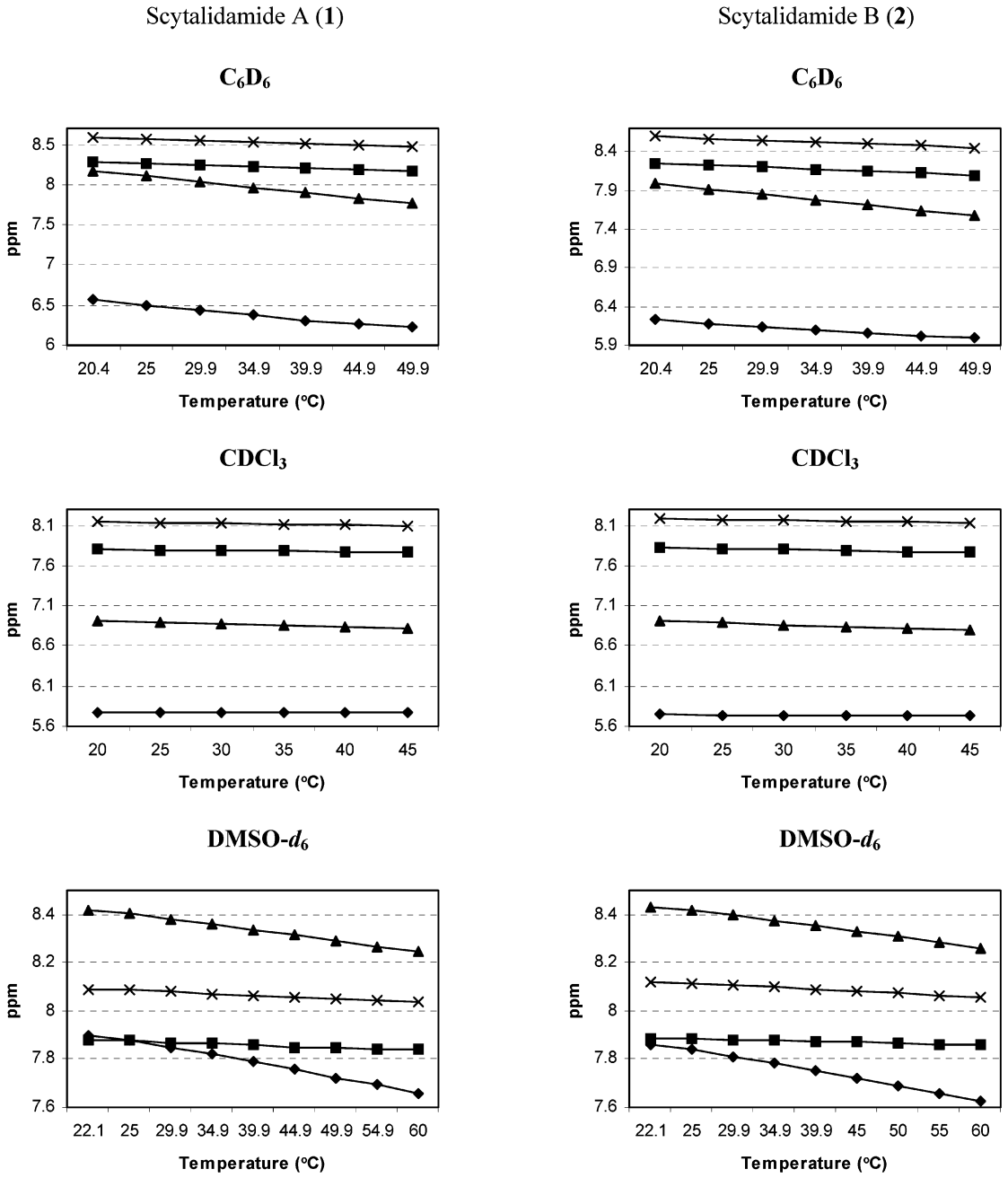


FIGURE 1. Graphical display of amide proton chemical shifts (ppm) in the scytalidamides (**1** and **2**) versus temperature (°C) taken in C_6D_6, $CDCl_3$, and DMSO-d_6 (Phe-1-*N*H, ◆; Phe-2-*N*H, ■; Aib-*N*H, ▲; Leu-*N*H, x).

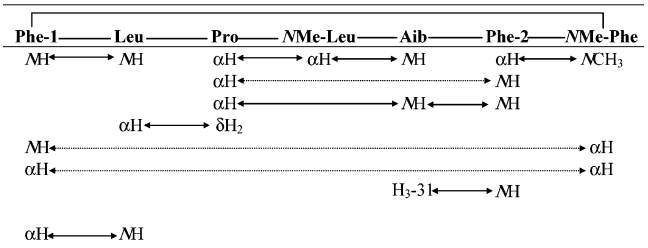
$CDCl_3$, only the Aib-*N*H in both molecules had $\Delta\delta/\Delta T$ values more than −4.0. The amide protons of both Phe-1 and -2 and Leu had small $\Delta\delta/\Delta T$ values suggesting that they were involved in intramolecular bonding. In a more polar environment such as in DMSO-d_6, only the amide protons of Phe-2 and Leu in both cyclic peptides were involved in intramolecular bonding. These series of experiments revealed the low rigidity of the peptide backbone in both scytalidamides A (**1**) and B (**2**).

The solution conformation of scytalidamide A (**1**) was examined in $CDCl_3$ based on several 2D NOESY as well as ROESY experiments using different mixing times. The observed NOE/ROE correlations for **1** are summarized in Table 5. The rigidity of scytalidamide A (**1**) was concluded on the basis of the vicinal coupling constants $^3J_{NH-CH\alpha}$ in Phe-1 (9.2 Hz), Phe-2 (9.6 Hz), and Leu (9.2 Hz). These data indicated a single dominant conformation as oppose to interconverting conformations. From temperature-dependent studies, three amide-protons (Phe-1-N^H, Phe-2-N^H, and Leu-N^H) were involved in intramolecular bonding, suggesting two turns within the backbone of molecule **1**. A transannular ROESY correlation between Pro-H^α and Phe-2-N^H indicated that one of the turns could be a type III β-turn (Figure 2). Evidence of such a tight β-turn was obtained from the following ROESY correlations: Phe-2-N^H (*i* position) to Aib-N^H (*i* + 1), Aib-N^H (*i* + 1) to *N*Me-Leu-H^α (*i* + 2), Aib-N^H (*i* + 1) to Pro-H^α (*i* + 3), and Pro-H^α (*i* + 3) to *N*Me-Leu-H^α (*i*

TABLE 4. Tabular Display of Amide Proton Temperature Coefficients ($\Delta\delta/\Delta T$) for Scytalidamides in C_6D_6, $CDCl_3$, and DMSO-d_6

	scytalidamide A (**1**)		scytalidamide B (**2**)	
	δ (ppm) at 25 °C (J in Hz)	$\Delta\delta/\Delta T$ (ppb/°C)	δ (ppm) at 25 °C (J in Hz)	$\Delta\delta/\Delta T$ (ppb/°C)
C_6D_6				
L-Phe-1-H^N	6.31 (8.8)	−11.6	6.17 (8.4)	−7.8
L-Phe-2-H^N	8.22 (9.6)	−5.1	8.20 (10.0)	−5.0
Aib-H^N	7.96	−14.0	7.91	−13.9
L-Leu-H^N	8.54 (9.2)	−4.6	8.56 (8.4)	−4.6
$CDCl_3$				
L-Phe-1-H^N	5.76 (9.2)	−0.1	5.73 (9.2)	−0.4
L-Phe-2-H^N	7.78 (9.6)	−1.7	7.79 (9.6)	−2.2
Aib-H^N	6.90	−4.2	6.88	−4.7
L-Leu-H^N	8.12 (9.2)	−1.8	8.16 (8.8)	−2.0
DMSO-d_6				
L-Phe-1-H^N	7.86 (8.8)	−6.2	7.82 (8.4)	−6.2
L-Phe-2-H^N	7.86 (10.4)	−1.0	7.85 (9.6)	−0.7
Aib-H^N	8.40	−4.5	8.41	−4.5
L-Leu-H^N	8.07 (9.2)	−1.5	8.10 (8.8)	−1.7

TABLE 5. Observed NOE/ROE Correlations for Scytalidamide A (1) in $CDCl_3$[a]



[a] Solid arrow: strong correlations. Dashed arrow: weak correlations.

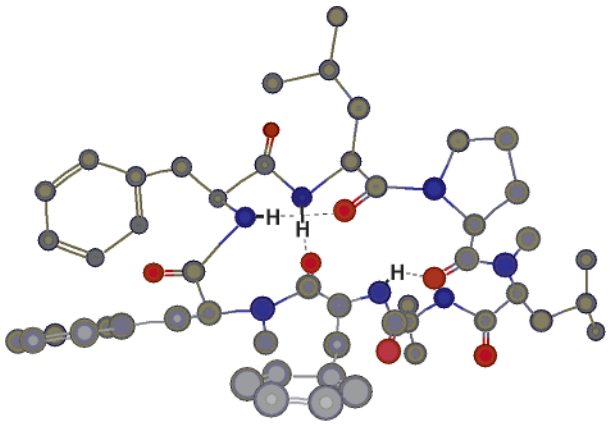


FIGURE 2. Proposed stereostructure of scytalidamide A (**1**) in $CDCl_3$ showing intramolecular amide hydrogen bondings.

+ 2). A second turn in scytalidamide A was indicated from NOE correlations between Leu-N^H and Phe-1-N^H, Leu-N^H and Phe-1-H^α, Phe-1-N^H and *N*Me-Phe-H^α, and Phe-1-H^α and *N*Me-Phe-H^α. These correlations suggest that the amide protons of Phe-1, Phe-2, and Leu are hydrogen-bonded to the carbonyl oxygen atoms of Leu, Pro, and Phe-1, respectively (Figure 2), resulting in a rigid solution conformation.

Scytalidamides A (**1**) and B (**2**) displayed in vitro cytotoxicity against the human colon carcinoma tumor cell line HCT-116 with IC_{50} values of 2.7 and 11.0 μM, respectively. Both compounds displayed moderate cytotoxicity in the NCI 60 cell-line panel with mean GI-50 values of 7.9 μM and 4.1 μM for **1** and **2**, respectively. The most sensitive cell lines were MOLT-4 leukemia (3.0 μM) for scytalidamide A (**1**) and Uacc-257 melanoma (1.2 μM) for scytalidamide B (**2**).

Experimental Section

Collection, Extraction, and Isolation. The fungal strain (CNC-310) was cultured from the surface of a sample of the green alga *Halimeda* sp. collected from a patch reef at a depth of 15 m from the northern end of Long Island, the Bahamas. The fungus was cultured without shaking in multiple 2.8 L Fernbach flasks each containing 1 L of the fermentation medium YGP (5 g yeast extract, 10 g glucose, 5 g peptone, 1 L seawater) for a total volume of 30 L. After 26 days, the mycelial mass was separated from the broth by filtration and extracted with 10 L of MeOH. The methanol extract was re-extracted with 5 L of EtOAc to give 2.25 g of brown solid. The broth was also extracted with 20 L of EtOAc to yield 1.06 g of oily material. Both mycelium and broth extracts were combined and subjected to a silica gel column (2 × 24 cm) eluting with 50% isooctane in EtOAc (200 mL) and finally in 100% EtOAc (200 mL) to obtain a mixture of compounds **1** and **2**. Final purification by repeated C-18 reversed-phase HPLC with 70% CH_3CN in H_2O provided 35.0 mg of scytalidamide A (**1**) and 62.0 mg of scytalidamide B (**2**).

Scytalidamide A (1): fine white crystals; mp 147−150 °C; $[\alpha]^{25}_D$ −151.2 (c = 0.6, MeOH); UV (MeOH) λ_{max} 202 nm (ϵ = 0.82); IR (neat) 3309, 2956, 1631, 1537, 1454 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) and ^{13}C NMR (100 MHz, pyridine-d_5) see Table 1; HR FABMS m/z $[M + Cs]^+$ 1010.4195 (calcd for $C_{50}H_{67}N_7O_7$ 1010.4156).

Scytalidamide B (2): fine white crystals; mp 141−143 °C; $[\alpha]^{25}_D$ −156.9 (c = 0.6, MeOH); UV (MeOH) λ_{max} 202 nm (ϵ = 0.63); IR (neat) 3439, 2957, 1631, 1539, 1455 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) and ^{13}C NMR (100 MHz, pyridine-d_5) see Table 2; HR FABMS m/z $[M + Cs]^+$ 1024.4356 (calcd for $C_{51}H_{69}N_7O_7$ 1024.4313).

Isolation of 3-Methylproline. Scytalidamide B (**2**, 200 mg), obtained from several sequential fermentations, was dissolved in 27.0 mL of 6 N HCl and 5.5 mL of acetic acid, and the mixture was heated at 110 °C for 16 h. The hydrolysate was cooled to room temperature and extracted three times with EtOAc. The aqueous layer was dried in vacuo to yield a crude amino acid mixture that was fractionated by chromatography on a 20 × 1.5 cm column of Dowex 50W × 2-400 resin (hydrogen form) eluting with a linear gradient of water (200 mL) and 1.5 N HCl (150 mL) to give 3-methylproline and other amino acids. Further purification was carried out by reversed-phase C-18 HPLC eluting with H_2O (0.01% TFA) to yield 4.3 mg of 3-methylproline as a white amorphous solid that showed: $[\alpha]^{25}_D$ −8.84 (c = 0.28, H_2O); ^1H NMR (300 MHz, D_2O) δ 3.85 d (J = 8.4 Hz), 3.45 m, 2.52 m, 2.25 m, 1.75 m, 1.30 d (J = 7.8 Hz); ^{13}C NMR (100 MHz, D_2O) δ 173.5, 66.7, 46.3, 39.0, 33.1, 18.2; HR FABMS m/z $[M + H]^+$ 130.0866 (calcd for $C_6H_{11}NO$ 130.0868).

Advanced Marfey's Analyses of the Standard Amino Acid Residues in the Scytalidamides Using LC/MS. Approximately 0.5 mg of scytalidamides A (**1**) and B (**2**) were separately hydrolyzed with 6 N HCl (0.8 mL) in an Ace high-pressure tube for 16 h. The hydrolysates were evaporated to dryness and resuspended in H_2O (100 μL). To each a half portion (50 μL) were added 1 N $NaHCO_3$ (20 μL) and 1% 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (L-FDLA or D-FDLA solution in acetone, 100 μL), and the mixtures were heated at 40 °C for 40 min. The solutions were cooled to room temperature, neutralized with 2 N HCl (20 μL), and evaporated to dryness. The residues were resuspended in 500 μL of MeCN,

and about 8 μL of each solution of FDLA derivatives was analyzed by LC/MS.

The analysis of the L- and LD-FDLA (mixture of D- and L-FDLA) derivatives was performed on an Agilent Hypersil ODS column (5 μm, 4.6 × 100 mm). Aqueous MeCN containing 0.01 M TFA was used as a mobile phase under a linear gradient elution mode (MeCN 25−65% for 45 min) at a flow rate of 0.7 mL/min. A Hewlett-Packard Series 1100 MSD mass spectrometer was used for detection in API-ES (positive) mode. The fragmentor and capillary voltage were kept at 70 and 4000 V, respectively, and the ion source at 350 °C. Nitrogen gas was used as a sheath gas at 13 L/min. A mass range of *m*/*z* 100−1000 was scanned in 0.1 min. The retention times (t_R, min) of the Marfey-derivatized amino acids are summarized in Table 3.

Absolute Configuration of 3-Methylproline. A 0.5 mg portion of a destruxin class peptide, identified by X-ray analysis, containing (2*S*,3*S*)-3-methylproline[17] was subjected to acid hydrolysis and its acid hydrolysate derivatized with L- or L,D-FDLA as described above for the scytalidamides. The t_R of (2*S*,3*S*)-3-MePro derivatized with L-FDLA, detected in positive-mode API-ES on LC/MS (as described above), was compared to that of 3-MePro obtained from scytalidamide B (**2**) and are summarized in Table 3.

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Supporting Information Available: 1D and 2D NMR spectra of **1** and **2**; ^{1}H NMR and CD spectra of (2*S*,3*S*)-3MePro. This material is available free of charge via the Internet at http://pubs.acs.org.

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