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Oryzamides A–E, Cyclodepsipeptides from the Sponge-Derived Fungus *Nigrospora oryzae* PF18

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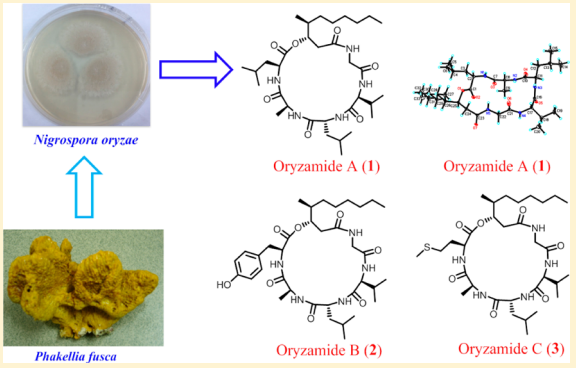
Supporting Information

ABSTRACT: Three new cyclohexadepsipeptides, oryzamides A–C (**1**–**3**), two isolation artifacts, oryzamides D (**4**) and E (**5**), and the known congener scopularide A (**6**), all possessing a rare 3-hydroxy-4-methyldecanoic acid (HMDA) substructure, were isolated from the mycelial extract of the sponge-derived fungus *Nigrospora oryzae* PF18. Their planar structures were elucidated by spectroscopic analysis and comparison with the literature data. The absolute configurations were determined using the advanced Marfey's method and single-crystal X-ray diffraction analysis. Among them, oryzamides D (**4**) and E (**5**) were a pair of diastereomers at the sulfur atom of the L-methionine sulfoxide residue, which showcased the possible separation of a pair of methionine sulfoxide diastereomers. The X-ray crystal structure of scopularide A (**6**) was obtained for the first time, thereby establishing its relative and absolute configuration at C-4 of the HMDA residue. Oryzamides A–C (**1**–**3**) did not display cytotoxic, antibacterial, antiparasitic, and NF-κB inhibitory activities.



Cyclodepsipeptides are a large group of cyclic peptides that are characterized by the presence of at least one ester bond as part of their backbone.[1,2] They have received enduring attention for a wide range of bioactivities, such as anticancer,[3] immunosuppressant,[4] anthelmintic,[5] antibiotic,[6,7] anti-inflammatory,[8] and antiviral[9] properties. Remarkably, several representative members have been approved for pharmaceutical use, such as emodepsin,[10] or are in clinical trials, as exemplified by kahalalide F[11] and aplidin.[12] Structurally, the diversity of hydroxy acid building blocks has been well characterized in cyclodepsipeptides.[13] As a rare member, the 3-hydroxy-4-methyldecanoic acid (HMDA) moiety was only encountered in beauverolides A, B, Ba, L, and La,[14−16] beauveriolide VIII,[17] and scopularide A[18] from fungi, as well as in arenamides A and C from an actinomycete strain.[19]

Our previous chemical investigation of the marine sponge *Phakellia fusca* collected from Yongxing Island led to the isolation of four new cyclopeptides, phakellistatins 15−18.[20] That result prompted us to examine and identify the presence of cyclopeptides from *P. fusca*-derived microbes, allowing for the fact that sponge-derived natural products are typically associated with microbial symbiotic consortia.[21,22] Consequently, six HMDA-containing cyclodepsipeptides, including new oryzamides A–E (**1**–**5**) and one known analogue, scopularide A (**6**), were isolated from the CH_2Cl_2 extract of *Nigrospora oryzae* PF18 obtained from the marine sponge *P. fusca*. Apart from an amino acid residue difference, cyclohexadepsipeptides **1**–**6** bear the same four amino acid and unusual HMDA residues. Scopularide A (**6**), whose complete stereostructure was not determined before, was previously isolated from a marine sponge-associated fungus.[18] It is noteworthy that the X-ray crystal structures of **1** and **6** enabled the rigorous assignments of the corresponding absolute configurations. Furthermore, a pair of methionine sulfoxide diastereomers, oryzamides D (**4**) and E (**5**), were readily separated from each other. Herein, we describe the detailed isolation process, structure elucidation, and biological activities of these cyclodepsipeptides.

RESULTS AND DISCUSSION

Oryzamide A (**1**) was isolated as a white crystal. The molecular formula $C_{33}H_{59}N_5O_7$ was deduced from the HRESIMS data (m/z 638.4504 $[M + H]^+$) in combination with the 1D and 2D

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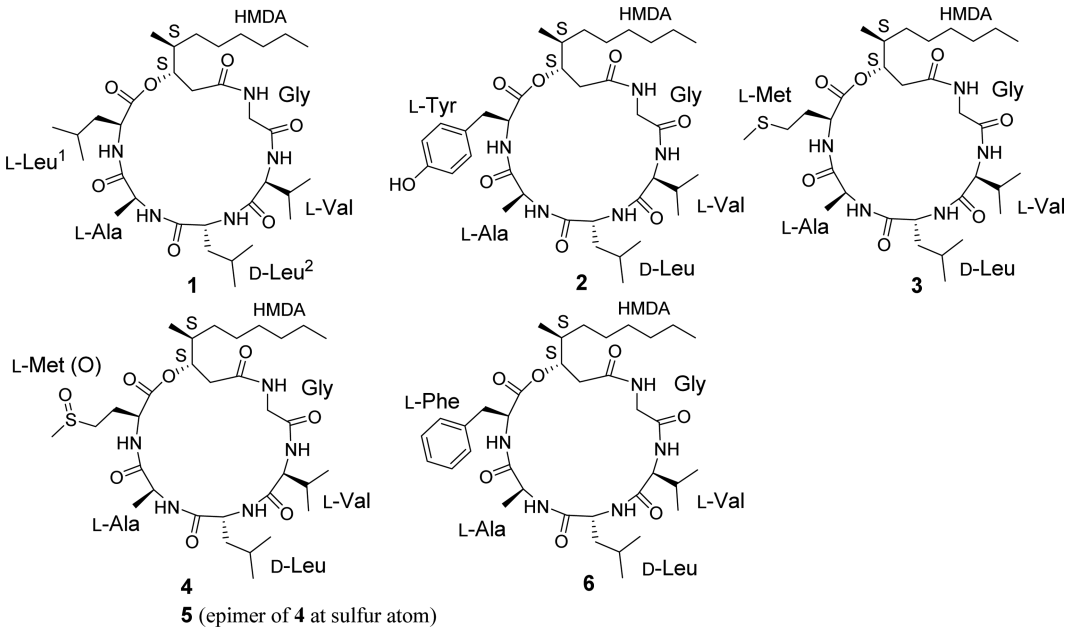
Chart 1



5 (epimer of **4** at sulfur atom)

NMR spectra, implying seven degrees of unsaturation. The IR spectrum of **1** showed strong absorption bands at 1724 and 1667 cm^{-1}, revealing the presence of ester and amide functionalities. The ^1H spectrum displayed resonances for five NH doublets (δ_H 7.72, 7.96, 8.65, 7.79, and 7.95) and six α-H multiplets (δ_H 3.38−4.18), indicating the peptidic nature of **1** (Table 1). In addition, the ^{13}C NMR spectrum exhibited signals for six ester/amide-type carbonyls (δ_C 169.1−171.9) and one oxygenated sp^3-hybridized carbon (δ_C 74.7), thus identifying **1** as a depsipeptide (Table 1). A detailed analysis of the COSY, HSQC, and HMBC data allowed the construction of an alanine (Ala), a glycine (Gly), a valine (Val), a 3-hydroxy-4-methyldecanoic acid (HMDA), and two leucine (Leu^1 and Leu^2) residues. The residue sequence of Leu^1−Ala−Leu^2−Val−Gly−HMDA in **1** was established by the HMBC correlations of Leu^1-NH (δ_H, 7.72)/Ala C-7 (δ_C, 171.9), Ala-NH (δ_H, 7.96)/Leu^2 C-10 (δ_C, 171.1), Leu^2-NH (δ_H, 8.65)/Val C-16 (δ_C, 171.7), Val-NH (δ_H, 7.79)/Gly C-21 (δ_C, 169.1), and Gly H_2-22 (δ_H, 4.10 and 3.38)/HMDA C-23 (δ_C, 169.9) (Figure 1). The assignments were further confirmed by the MS/MS fragment ion series at m/z 525, 454, and 341, corresponding to cleavages of the amide bonds between Leu^1/Ala, Ala/Leu^2, and Leu^2/Val, respectively (Figures 2 and S8). Given that six of the seven degrees of unsaturation were attributed to six carbonyl carbons, a monocyclic structure of **1** was inferred from the remaining degree of unsaturation, suggesting that the Leu^1 residue was attached to the HMDA moiety by an ester bond. Thus, **1** was identified as *cyclo*-(Leu^1−Ala−Leu^2−Val−Gly−HMDA). The absolute configuration of **1** was determined by a single-crystal X-ray diffraction experiment using Cu Kα radiation, which allowed the assignments of amino acid residues as L-Leu^1, L-Ala, D-Leu^2, and L-Val and gave a 3*S*,4*S* configuration for the HMDA moiety (Figure 3)

The molecular formula of oryzamide B (**2**) was determined to be $C_{36}H_{57}N_5O_8$ by the HRESIMS data, establishing seven degrees of unsaturation. The ^1H NMR spectrum of **2** was similar to that of **1**, with five NH protons (δ_H 7.78, 7.88, 7.90, 8.01, and 8.64) and six α-H protons (δ_H 3.42−4.24), confirming the cyclic hexadepsipeptide nature of **2** (Table 1). The major differences between the ^1H NMR spectra of **2** and **1** were the resonances associated with aromatic protons. Interpretation of the 2D NMR data further demonstrated that the Leu^1 residue in **1** was replaced by the Tyr residue in **2**, and the latter residue accounted for those aromatic signals. Therefore, oryzamide B (**2**) encompassed the Ala, Gly, HMDA, Leu, Val, and Tyr residues. The connection of the residues was established as *cyclo*-(Tyr−Ala−Leu−Val−Gly−HMDA) (Figures 2 and S18), according to the COSY and HMBC correlations in the same manner as **1** (Figure 1), which was further supported by the corresponding ESIMS/MS fragments. The absolute configurations of the amino acid residues were deciphered by the advanced Marfey's method.[23−25] The acid hydrolysates of **2** were derivatized with 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (L-FDLA) and L-/D-FDLA, and the amino acid derived products were detected by UPLC-MS. The absolute configurations of all of the amino acid residues were assigned to be L, except for D-Leu (Figure S21), based on the retention times of the L-FDLA derivatives with L-/D-FDLA derivatives. Regarding the HMDA unit of **2**, the absolute configuration was deduced as the same as that of **1**, owing to a shared biogenesis.

Oryzamide C (**3**) has the molecular formula $C_{32}H_{57}N_5O_7S$, as indicated by the HRESIMS data. Signals observed at 169.0, 169.6, 170.7, 171.0, 171.5, and 171.7 ppm in its ^{13}C NMR spectrum (Table 1) accounted for the presence of six amide/ester-type carbonyls, revealing that the structure of **3** was similar to that of **1**. This was further supported by the strong IR absorption bands of ester and amide functionalities. The ^1H and COSY NMR spectra of **3** revealed the presence of a spin system containing a two-proton doublet of triplets at δ_H 1.97, a two-proton multiplet at δ_H 2.54, and a methyl singlet at δ_H 2.04, consistent with a $-CH_2CH_2SCH_3$ unit of methionine.[16] Considering that the resonances for the Leu^1 group were missing, oryzamide C (**3**) was composed of a methionine moiety instead of the Leu^1 counterpart in **1** (Figure 1). This planar structure was further confirmed by the analysis of the ESIMS/MS fragments of m/z 525, 454, and 341 (Figures 2 and S29), arising from the cleavage of the amide bonds between

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Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data for Oryzamides A–C (1–3) in DMSO-d_6

oryzamide A (1)				oryzamide B (2)				oryzamide C (3)			
unit	position	δ_C, type	δ_H (J in Hz)	unit	position	δ_C, type	δ_H (J in Hz)	unit	position	δ_C, type	δ_H (J in Hz)
L-Leu[1]	1	171.7, C		L-Tyr	1	170.9, C		L-Met	1	170.7, C	
	2	51.0, CH	4.14, m		2	54.9, CH	4.24, td (7.2, 6.8)		2	51.4, CH	4.30, dt (7.4, 7.1)
	3	39.5, CH_2	1.62, m		3	36.0, CH_2	2.85, m		3	30.2, CH_2	1.97, td (7.4, 7.1)
			1.47, m		4	127.1, C			4	29.4, CH_2	2.54, m
	4	24.1, CH	1.68, m		5/9	130.0, CH	7.03, d (8.4)		5	14.3, CH_3	2.04, s
	5	21.5, CH_3	0.83, d (6.6)		6/8	115.0, CH	6.64, d (8.4)		2-NH		7.54, d (7.2)
	6	22.7, CH_3	0.88, d (6.3)		7	156.0, C		L-Ala	6	171.7, C	
	2-NH		7.72, d (7.0)		2-NH		8.01, d (6.8)		7	47.6, CH	4.19, dq (8.2, 7.1)
L-Ala	7	171.9, C		L-Ala	10	171.8, C			8	16.8, CH_3	1.24, d (7.1)
	8	47.6, CH	4.18, m		11	47.6, CH	4.19, m		7-NH		8.03, d (8.2)
	9	17.4, CH_3	1.20, d (7.0)		12	17.8, CH_3	1.15, d (7.0)	D-Leu	9	171.0, C	
	8-NH		7.96 d (4.8)		11-NH		7.78, d (8.1)		10	52.2, CH	3.98, m
D-Leu[2]	10	171.1, C		D-Leu	13	171.0, C			11	38.7, CH_2	1.50, m
	11	52.2, CH	3.98, m		14	51.9, CH	4.05, m		12	24.0, CH	1.66, m
	12	38.7, CH_2	1.47, m		15	38.7, CH_2	1.46, m		13	22.6, CH_3	0.91, d (6.5)
	13	24.2, CH	1.64, m		16	24.1, CH	1.63, m		14	21.0, CH_3	0.84, d (7.0)
	14	23.0, CH_3	0.89, d (6.4)		17	23.0, CH_3	0.89, d (6.6)		10-NH		8.52, d (5.9)
	15	21.1, CH_3	0.81, d (6.6)		18	21.1, CH_3	0.82, d (6.6)	L-Val	15	171.5, C	
	11-NH		8.65, d (5.9)		14-NH		8.64, d (6.3)		16	58.2, CH	4.09, t (8.0)
L-Val	16	171.7, C		L-Val	19	171.7, C			17	29.8, CH	1.89, m
	17	58.3, CH	4.09, m		20	58.3, CH	4.10, t (8.6)		18	18.7, CH_3	0.90, d (6.6)
	18	30.0, CH	1.84, m		21	29.8, CH	1.87, m		19	18.5, CH_3	0.85, d (6.8)
	19	19.0, CH_3	0.88, d (6.7)		22	19.0, CH_3	0.88, d (7.4)		16-NH		7.69, d (7.6)
	20	18.7, CH_3	0.83, d (6.6)		23	18.8, CH_3	0.84, d (6.8)	Gly	20	169.0, C	
	17-NH		7.79, d (7.9)		20-NH		7.90, d (8.5)		21	42.2, CH_2	4.13, dd (16.4, 7.1)
Gly	21	169.1, C		Gly	24	168.9, C					3.38, dd (16.3, 4.1)
	22	42.4, CH_2	4.10, m		25	42.3, CH_2	4.05, m		21-NH		7.89, dd (7.0, 4.2)
			3.38, m				3.42, dd (16.7, 4.2)	HMDA	22	169.6, C	
	22-NH		7.95, d (2.9)		25-NH		7.88, d (6.3)		23	36.9, CH_2	2.50, m
HMDA	23	169.9, C		HMDA	26	169.8, C					2.27, dd (15.0, 2.2)
	24	36.9, CH_2	2.50, d (15.0)		27	37.2, CH_2	2.43, d (9.3)		24	74.6, CH	4.98, m
			2.24, dd (15.2, 1.9)				2.24, dd (14.9, 2.1)		25	36.1, CH	1.65, m
	25	74.7, CH	4.95, m		28	75.0, CH	4.90, m		26	31.6, CH_2	1.35, m
	26	36.3, CH	1.63, m		29	36.1, CH	1.51, m				1.01, m
	27	31.7, CH_2	1.33, m		30	31.5, CH_2	1.21, m		27	26.3, CH_2	1.30, m
			0.99, m				0.90, m				1.21, m
	28	26.6, CH_2	1.28, m		31	26.5, CH_2	1.21, m		28	28.7, CH_2	1.24, m
			1.18, m				1.11, m		29	30.9, CH_2	1.25, m
	29	29.0, CH_2	1.23, m		32	29.0, CH_2	1.23, m		30	21.8, CH_2	1.27, m
	30	31.2, CH_2	1.23, m		33	31.2, CH_2	1.24, m		31	13.6, CH_3	0.87, t (6.8)
	31	22.2, CH_2	1.25, m		34	22.1, CH_2	1.26, m		32	14.5, CH_3	0.83, d (7.7)
	32	14.0, CH_3	0.85, t (6.9)		35	14.0, CH_3	0.86, t (7.4)				
	33	14.8, CH_3	0.81, d (6.9)		36	14.6, CH_3	0.69, d (6.8)				

Met/Ala, Ala/Leu, and Leu/Val, respectively. Thus, oryzamide C (**3**) was identified as *cyclo*-(Met–Ala–Leu–Val–Gly–HMDA), which possesses the same planar structure as arenamide C.[19] The absolute configurations of the amino acid residues in **3** were determined as L-Met, L-Ala, D-Leu, and L-Val (Figure S32) via the advanced Marfey's method. Accordingly, the structure of oryzamide C (**3**) was different from that of arenamide C owing to the opposite absolute configuration of the Leu residue. The stereogenic centers (C-3 and C-4) in the HMDA substructure probably possess the same configurations as those of **1**, allowing for its co-occurrence with **1**.

Oryzamides D (**4**) and E (**5**), two minor metabolites, were found to have the same molecular formula of $C_{32}H_{57}N_5O_8S$ via HRESIMS measurements, possessing an additional oxygen atom compared to oryzamide C (**3**). The ^{1}H and ^{13}C NMR spectra (Table 2) of **4** and **5** were almost identical to each other

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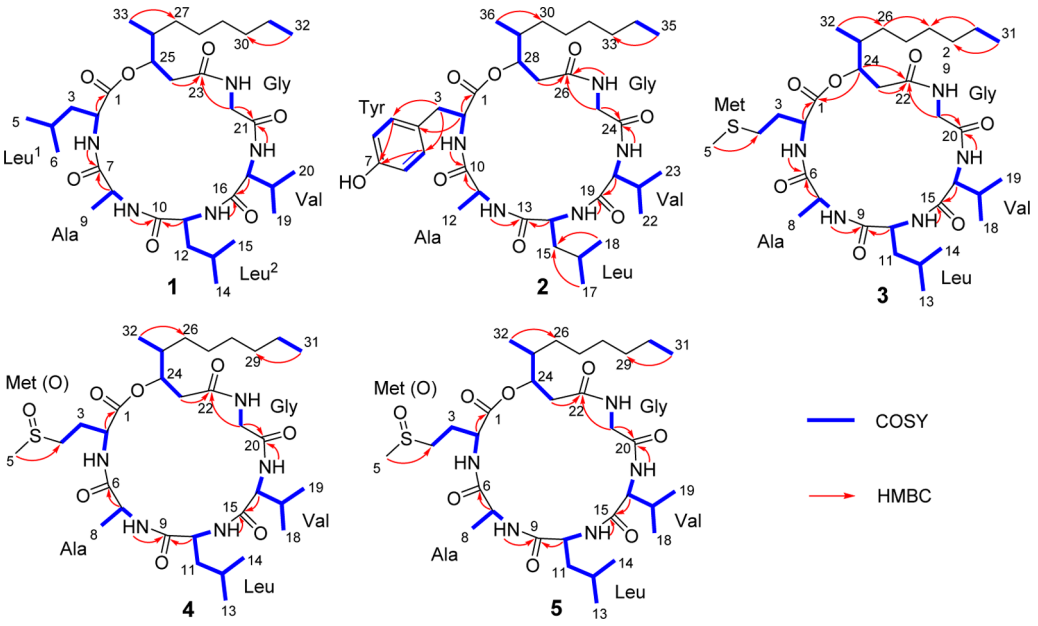

Figure 1. Key COSY and HMBC correlations of oryzamides A–E (**1**–**5**).

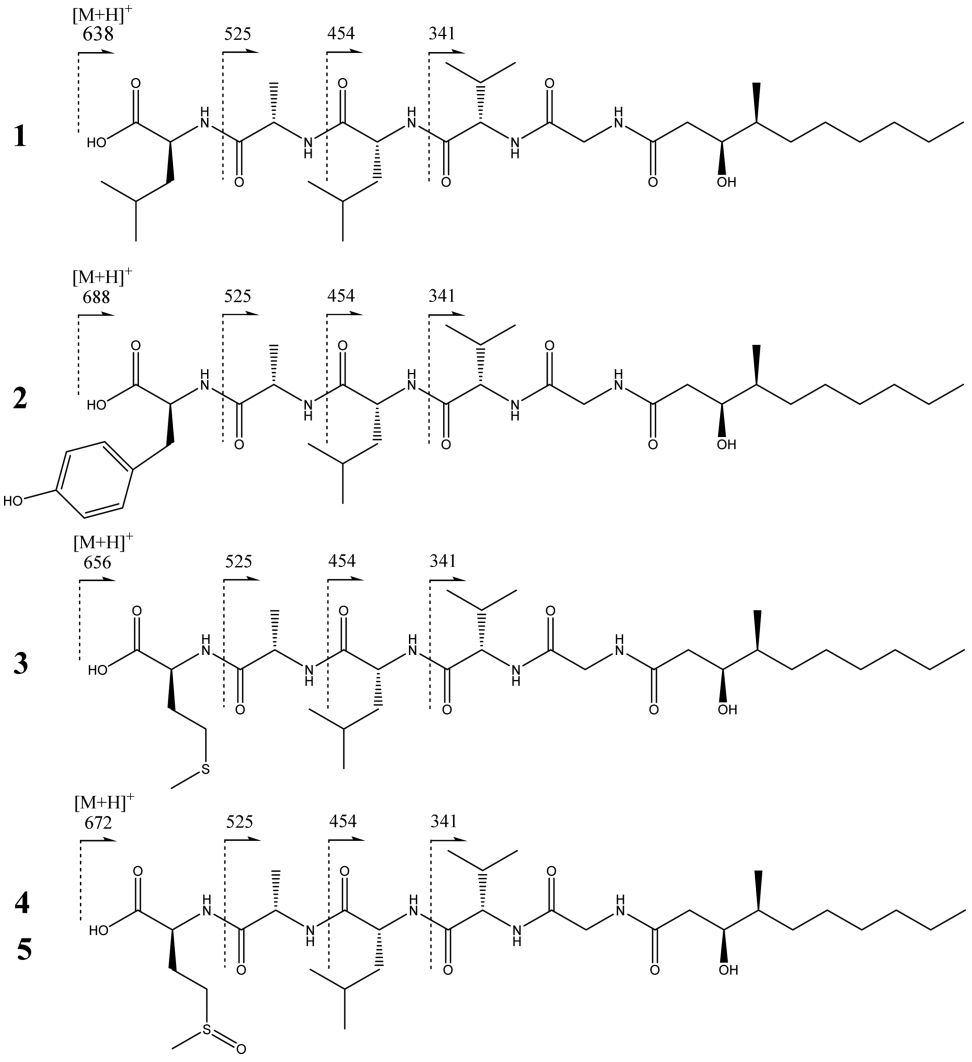

Figure 2. ESIMS/MS fragment ions (m/z) of oryzamides A–E (**1**–**5**).

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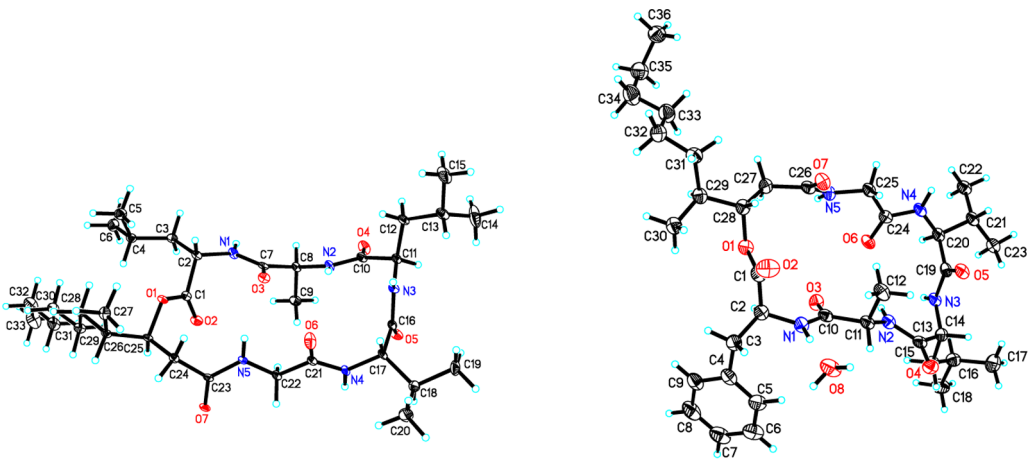

Figure 3. ORTEP drawings of **1** and **6** (Cu Kα).

and very similar to those of **3**, except for the deshielded resonances of C-4 at δ_C 48.5 or 49.1 and C-5 at δ_C 37.5 or 37.9, respectively. This deshielding effect indicated the presence of a methionine sulfoxide group,[26] which was also confirmed by the strong IR absorption bands at 1022 and 1025 cm^{-1},[24] respectively. Careful comparisons of the 1D and 2D NMR data between **4** and **5** showed the overall similarity but only slight differences in the chemical shifts of C-3, C-4, and C-5, implying that **4** and **5** are two diastereomers at a methionine sulfoxide residue, which was further supported by the same ESIMS/MS fragments (Figures 2, S40, and S51). The methione sulfoxide residue is generally recognized as an artifact formed via oxidation of methionine-containing natural products, resulting from the multistep purification process.[27,28] Compound **3** was treated with a mild oxidant (*m*-chloroperbenzoic acid), resulting in compounds **4** and **5**. Thus, compounds **4** and **5** were oxidative products of compound **3**. Interestingly, *R* and *S* sulfoxide diastereomers are typically isolated as a mixture,[28] but methionine sulfoxide diastereomers are not inherently resistant to separation.[29] Compounds **4** and **5** were easily obtained in pure form. The absolute configurations of compounds **4** and **5** were partially determined as L-Ala, L-Val, and D-Leu by the advanced Marfey's method, and the α-carbon C-2 of the methionine sulfoxide was assigned the same configuration as compound **3**, considering that compounds **4** and **5** originated from compound **3**.

Fortunately, scopularide A (**6**) yielded a crystal from 80% $MeOH/H_2O$, which was suitable for X-ray crystal structure determination. The stereocenters of scopularide A except at C-4 of the HMDA residue were determined previously by Marfey's and Mosher's methods. Those identified stereocenters are in agreement with the X-ray diffraction data. Notably, the crystallographic analysis of **6** allowed the unambiguous assignment of a 4*S* configuration of the HMDA unit for the first time (Figure 3).

Oryzamides A–E (**1**–**5**) and scopularide A (**6**) are structurally and biogenetically related cyclohexdepsipeptides with the L-Val, D-Leu, L-Ala, Gly, and HMDA residues, which constitute the same sequence (−Ala−Leu−Val−Gly−HMDA−). Prior to this study, only nine cyclodepsipeptides with an HMDA unit have been reported.[14−19] Oryzamides A–E (**1**–**5**) are probably hybrid peptide−polyketide natural products based on the recent identification of the scopularide A (**6**) biosynthetic gene cluster.[30] Therefore, the chemodiversity of these compounds is presumably due to the substrate promiscuity of the adenylation domain of the nonribosomal peptide synthetase.[31,32]

In the cases of the bioactivities, oryzamides A–C (**1**–**3**) were not cytotoxic (IC_{50} < 10 μM) against HeLa cells, with IC_{50} values of 25.7, 12.4, and 31.3 μM, respectively. None of them showed any NF-κB inhibitory, antibacterial (methicillin-sensitive *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, and *Escherichia coli*), or antiparasitic properties (*Ichthyophthirius multifiliis*). Oryzamides D (**4**) and E (**5**) were not evaluated for the aforementioned bioactivities because of the scarcity of material.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on an Electrothermal 9100 apparatus and are uncorrected. Optical rotations were measured with a PerkinElmer model 341 polarimeter with a 10 cm length cell at 589 nm. UV spectra were obtained on a Hitachi U-3010 spectrophotometer. IR spectra (film) were acquired on a Jasco FTIR-400 spectrometer. NMR spectroscopic data were recorded on an Agilent 600 MHz NMR instrument. HRESIMS and ESIMS/MS spectra were determined on a Waters Xevo G2-XS QTOF spectrometer. UPLC-MS spectra were obtained using a Waters Acquity UPLC system equipped with a Waters Xevo G2-XS QTOF spectrometer and a C18 Acquity UPLC BEH column (Waters, 2.1 × 50 mm, 1.7 μm). Preparative medium-pressure liquid chromatography (MPLC) was carried out on an Interchim Puriflash 450 Instrument. Semipreparative reversed-phased HPLC (RP-HPLC) was performed on a system composed of a Waters 1525 pump equipped with a 2998 photodiode array detector and a YMC-Pack Pro C18 column (YMC, 10 × 250 mm, 5 μm). 1-fluoro-2,4-dinitrophenyl-5-L-/D-leucinamide (L-/D-FDLA), and *m*-chloroperbenzoic acid were purchased from Sigma-Aldrich Chemical Corporation.

Fungal Material. The strain PF18 was isolated from the inner tissue of the sponge *Phakellia fusca* collected from Yongxing Island, China. The fungus was identified as *Nigrospora oryzae* by morphological characteristics and sequence analysis of the ITS region (GenBank accession no. FJ941865.1). A voucher strain was deposited at the School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, China.

Fermentation. After being maintained on PDA medium for 7 days, the strain PF18 was inoculated into 250 mL Erlenmeyer flasks each containing 100 mL of the seed medium (potato 200 g/L, dextrose 20 g/L, and artificial seawater salts[33] 33 g/L in tap water) on a rotatory shaker (180 rpm) at 28 °C for 48 h. The subsequent fermentation was performed in 48 × 2-L Erlenmeyer flasks. Each flask contained 12 g of mannitol, 12 g of maltose, 6 g of glucose, 6 g of

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Table 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data for Oryzamides D and E (4 and 5) in DMSO-d_6

oryzamide D (4)				oryzamide E (5)			
unit	position	δ_C, type	δ_H (J in Hz)	unit	position	δ_C, type	δ_H (J in Hz)
L-Met (O)	1	170.4, C		L-Met (O)	1	170.3, C	
	2	51.7, CH	4.27, m		2	52.1, CH	4.23, m
	3	23.1, CH_2	2.07, m		3	24.0, CH_2	2.10, m
			2.01, m				2.00, m
	4	48.5, CH_2	2.87, m		4	49.1, CH_2	2.81, m
			2.73, m				2.78, m
	5	37.5, CH_3	2.53, s		5	37.9, CH_3	2.54, s
	2-NH		7.40, d (6.5)		2-NH		7.48, d (4.2)
L-Ala	6	171.8, C		L-Ala	6	171.8, C	
	7	47.9, CH	4.16, dq (8.1, 7.0)		7	47.8, CH	4.19, dq (8.3, 7.0)
	8	16.5, CH_3	1.24, d (7.0)		8	16.6, CH_3	1.24, d (7.0)
	7-NH		8.23, d (8.1)		7-NH		8.26, d (8.3)
D-Leu	9	171.5, C		D-Leu	9	171.4, C	
	10	52.5, CH	3.94, m		10	52.6, CH	3.92, m
	11	38.8, CH_2	1.52, m		11	38.8, CH_2	1.52, m
			1.44, m				1.44, m
	12	24.1, CH	1.64, m		12	24.1, CH	1.64, m
	13	22.9, CH_3	0.90, d (6.6)		13	22.9, CH_3	0.90, d (6.4)
	14	21.1, CH_3	0.82, d (6.6)		14	21.1, CH_3	0.82, d (7.0)
	10-NH		8.70, d (5.8)		10-NH		8.69, d (5.5)
L-Val	15	172.2, C		L-Val	15	172.2, C	
	16	58.6, CH	4.03, m		16	58.5, CH	4.04, m
	17	29.8, CH	1.85, m		17	29.9, CH	1.84, m
	18	19.1, CH_3	0.90, d (6.6)		18	19.1, CH_3	0.90, d (6.6)
	19	18.8, CH_3	0.84, d (6.5)		19	18.7, CH_3	0.84, d (7.7)
	16-NH		8.03, d (7.1)		16-NH		7.93, d (7.4)
Gly	20	169.3, C		Gly	20	169.3, C	
	21	42.0, CH_2	4.24, m		21	42.1, CH_2	4.27, dd (16.0, 7.6)
			3.31, m				3.28, d (16.0)
	21-NH		8.01, d (3.5)		21-NH		8.04, d (7.2)
HMDA	22	169.6, C		HMDA	22	169.6, C	
	23	36.6, CH_2	2.55, dd (14.9, 4.4)		23	36.4, CH_2	2.55, m
			2.25, dd (14.8, 2.4)				2.25, dd (14.8, 2.1)
	24	75.0, CH	5.00, m		24	74.9, CH	4.99, m
	25	36.1, CH	1.64, m		25	36.0, CH	1.63, m
	26	31.6, CH_2	1.34, m		26	31.6, CH_2	1.34, m
			0.98, m				0.98, m
	27	26.6, CH_2	1.28, m		27	26.6, CH_2	1.28, m
			1.20, m				1.20, m
	28	29.0, CH_2	1.23, m		28	29.0, CH_2	1.23, m
	29	31.2, CH_2	1.24, m		29	31.2, CH_2	1.24, m
	30	22.1, CH_2	1.26, m		30	22.1, CH_2	1.26, m
	31	14.0, CH_3	0.86, t (6.8)		31	14.0, CH_3	0.86, t (7.0)
	32	14.8, CH_3	0.80, d (6.8)		32	14.8, CH_3	0.80, d (6.9)

sodium glutamate, 1.8 g of yeast extract, 0.6 g of corn syrup, 0.3 g of KH_2PO_4, 0.18 g of $MgSO_4{\cdot}7H_2O$, 19.8 g/L artificial seawater salts, and 600 mL of tap water, where 60 mL of the seed culture was transferred and incubated at 28 °C with shaking at 180 rpm for 12 days.

Extraction and Isolation. The fungal mycelium was separated from the fermentation broth by filtration and exhaustively extracted with CH_2Cl_2/MeOH (1:1, v/v). The resultant extract was suspended in 1 L of H_2O and partitioned with CH_2Cl_2 (4 × 1 L) to yield the CH_2Cl_2 extract (5.5 g), which was subjected to column chromatography on Sephadex LH-20 with CH_2Cl_2/MeOH (1:1, v/v) as eluent, affording four fractions (Fr.1−4). Fr.3 (2 g) was subjected to VLC over silica gel using gradients of CH_2Cl_2/MeOH (20:1, 10:1, 8:1, 5:1, 1:1, 0:1, v/v) to obtain six subfractions (Fr.3.A−F). Fr.3.C (100 mg) was further separated by reversed-phase MPLC (10−100% MeOH/H_2O, 180 min, flow rate 20 mL/min, UV detection at 210 nm) to afford six subfractions (Fr.3.C.1−6). Subsequently, the subfraction Fr.3.C.3 was purified by semipreparative RP-HPLC (YMC-Pack Pro C18, 50% MeCN/H_2O, 2.0 mL/min, UV detection at 210 nm) to yield oryzamide D (**4**, 2.8 mg, t_R 16.9 min) and oryzamide E (**5**, 2.5 mg, t_R 17.6 min). Subfraction Fr.3.C.4 was further separated by semipreparative RP-HPLC (YMC-Pack Pro C18, 60% MeCN/H_2O, 2.0 mL/min, UV detection at 210 nm) to obtain oryzamide B (**2**, 5.8 mg, t_R 31.8 min) and oryzamide C (**3**, 10.3 mg, t_R 33.9 min). Subfraction Fr.3.C.5 was purified by semipreparative RP-HPLC (YMC-Pack Pro C18, 55% MeCN/H_2O, 2.0 mL/min, UV detection at 210 nm) to afford oryzamide A (**1**, 18.2 mg, t_R 94.9 min) and scopularide A (**6**, 20.1 mg, t_R 95.7 min).

Oryzamide A (1): white crystals; mp 230 °C; $[\alpha]_D^{25}$ −30 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.69) nm; IR (film) ν_{max} 3314, 3259, 3060, 2957, 2927, 2866, 1724, 1667, 1637, 1540, 1464, 1444, 1377, 1276, 1235, 1186, 1169 cm^{-1}; ^{1}H and ^{13}C NMR data,

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Table 1; ESIMS/MS data, Figure 2; HRESIMS m/z 638.4504 $[M + H]^+$ (calcd for $C_{33}H_{60}N_5O_7$, 638.4493).

Oryzamide B (2): white powder; $[\alpha]_D^{25}$ −33 (c 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.97), 229 (3.93), 277 (3.75) nm; IR (film) ν_{max} 3304, 3061, 2958, 2928, 2857, 1738, 1649, 1538, 1518, 1460, 1380, 1231, 1176 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; ESIMS/MS data, Figure 2; HRESIMS m/z 688.4285 $[M + H]^+$ (calcd for $C_{36}H_{58}N_5O_8$, 688.4285).

Oryzamide C (3): white powder; $[\alpha]_D^{25}$ −33 (c 0.65, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.65) nm; IR (film) ν_{max} 3403, 3285, 3059, 2958, 2924, 2854, 1737, 1669, 1641, 1531, 1501, 1464, 1448, 1426, 1381, 1312, 1279, 1233, 1195, 1170, 1102, 1065, 1018 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; ESIMS/MS data, Figure 2; HRESIMS m/z 656.4068 $[M + H]^+$ (calcd for $C_{32}H_{58}N_5O_7S$, 656.4057).

Oryzamide D (4): white powder; $[\alpha]_D^{25}$ −28 (c 0.40, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.41) nm; IR (film) ν_{max} 3302, 3050, 2959, 2928, 2861, 1737, 1650, 1540, 1462, 1383, 1277, 1233, 1209, 1173, 1022 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; ESIMS/MS data, Figure 2; HRESIMS m/z 672.4016 $[M + H]^+$ (calcd for $C_{32}H_{58}N_5O_8S$, 672.4006).

Oryzamide E (5): white powder; $[\alpha]_D^{25}$ +4 (c 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.35) nm; IR (film) ν_{max} 3299, 3049, 2959, 2929, 2868, 1737, 1651, 1538, 1463, 1383, 1277, 1231, 1174, 1025 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; ESIMS/MS data, Figure 2; HRESIMS m/z 672.4015 $[M + H]^+$ (calcd for $C_{32}H_{58}N_5O_8S$, 672.4006).

Scopularide A (6): white crystals; mp 230 °C; $[\alpha]_D^{25}$ −33 (c 0.30, MeOH).

Crystal Structure Determination. Single crystals of oryzamide A (**1**) and scopularide A (**6**) were obtained by slow evaporation from 80% MeOH/H_2O and were measured on a Bruker APEX-II CCD diffractometer using graphite-monochromated Cu Kα radiation (λ = 1.541 78 Å). The thermal ellipsoid diagram was generated by ORTEP.[34] The structure was solved by direct methods (Shelxs97) and refined by the full-matrix least-squares method on F^2.[35] Hydrogen atoms were located by geometric calculation and difference Fourier methods. Crystallographic data for compounds **1** and **6** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 1474001 and 1474000, respectively. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033, or e-mail: deposit@ccdc.cam.ac.uk).

Crystal Data for Compound 1. The molecular structure comprises six molecules of **1**, three in the asymmetric unit, and four molecules of water, one at half-occupancy. $C_{99}H_{184}N_{15}O_{24.5}$, M = 1976.6, monoclinic, space group = $P2(1)$, No. 4, a = 17.2997(3) Å, b = 16.9120(3) Å, c = 19.4202(3) Å, β = 90.6820(10)°, V = 5681.42(17) $Å^3$, T = 130 K; Z = 2, D_x = 1.155 mg/m^3, $F(000)$ = 2158, μ(Cu Kα) = 0.671 mm^{-1}, crystal dimensions 0.25 × 0.1 × 0.03 mm^3, 41 717 unique reflections collected (θ_{max} = 69.542°), 18 172 independent reflections (R_{int} = 0.0529). The final R_1 = 0.0620, wR_2 = 0.1642 ($w = 1/\sigma|F|^2$), and S = 1.008. The Flack parameter was −0.07(15).

Crystal Data for Compound 6. The molecular structure comprises one molecule of **6** and one molecule of water. $C_{36}H_{59}N_5O_8$, M = 689.88, monoclinic, space group = $C2$, No. 5, a = 26.172(2) Å, b = 9.2265(6) Å, c = 16.7839(13) Å, β = 107.326(5)°, V = 3869.0(5) $Å^3$, T = 130 K; Z = 4, D_x = 1.184 mg/m^3, $F(000)$ = 1496, μ(Cu Kα) = 0.679 mm^{-1}, crystal dimensions 0.25 × 0.12 × 0.02 mm^3, 13 510 unique reflections collected (θ_{max} = 69.263°), 5879 independent reflections (R_{int} = 0.0570). The final R_1 = 0.0595, wR_2 = 0.1513 ($w = 1/\sigma|F|^2$), and S = 1.022. The Flack parameter was −0.1(15).

Acid Hydrolysis of Oryzamides B–E (2–5). Approximately 0.5 mg of each of **2–5** was hydrolyzed with 6 N HCl (1 mL) for 16 h at 110 °C. After cooling to room temperature, the hydrolysate mixtures were evaporated to dryness, and traces of HCl were removed by repeated drying *in vacuo*.

Absolute Configurations of Amino Acids by the Advanced Marfey's Analysis. Each acid hydrolysate was resuspended in 100 μL of H_2O and separated into two equal portions. Each portion (50 μL) was treated with 1 N $NaHCO_3$ (20 μL) and 100 μL of L- or D-/L-FDLA (10 mg/mL in acetone). Each mixture was heated to 50 °C for 1 h. The reaction was quenched with 20 μL of 1 N HCl and dried under vacuum. The residue was redissolved in 300 μL of MeOH. The retention times of the L- and L-/D-FDLA-derivatized amino acids were measured by UPLC-MS using an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm, 0.5 mL/min) with a linear gradient from 10% to 100% MeCN/H_2O containing 0.1% formic acid over 10 min. Through comparison of the retention times of the L- and D-/L-FDLA derivatives of corresponding amino acids, the absolute configurations were established. The retention times of the L-FDLA amino acid derivatives were 3.53 min (L-Ala, m/z 384 $[M + H]^+$), 3.94 min (L-Val, m/z 412 $[M + H]^+$), 5.51 min (L-Tyr, m/z 770 $[M + H]^+$), 3.92 min (L-Met, m/z 444 $[M + H]^+$), and 5.23 min (D-Leu, m/z 426 $[M + H]^+$). The retention times of the D-/L-FDLA amino acid derivatives were 3.53 and 4.02 min (L-Ala, m/z 384 $[M + H]^+$), 3.94 and 4.78 min (L-Val, m/z 412 $[M + H]^+$), 5.51 and 5.92 min (L-Tyr, m/z 770 $[M + H]^+$), 3.92 and 4.63 min (L-Met, m/z 444 $[M + H]^+$), and 4.30 and 5.23 min (D-Leu, m/z 426 $[M + H]^+$) (Figures S21, S32, S43, and S54).

Cytotoxicity Assay. The cytotoxic activities of compounds **1–3** against HeLa cells were evaluated by the MTT method as described previously.[36] Cisplatin (IC_{50} = 4.2 μM) was used as the positive control.

NF-κB Luciferase Assay. For compounds **1–3**, the NF-κB inhibitory activity was assessed with the methods described by Jiao et al.[37]

Antibacterial Activity Assay. Compounds **1–3** were tested for antibacterial activities against methicillin-sensitive *Staphylococcus aureus* ATCC25923, methicillin-resistant *Staphylococcus aureus* ATCC43300, and *Escherichia coli* ATCC25922 in 96-well microplates according to a previously published protocol.[38,39] Chloramphenicol was used as the positive control and exhibited MICs of 2, 4, and 2 μg/mL, respectively.

Antiparasitic Activity Assay. Compounds **1–3** were evaluated for antiparasitic activity against *Ichthyophthirius multifiliis* according to the established protocol of Liang et al.[40]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00349.

1D and 2D NMR, HRESIMS, HRESIMS/MS, UV, IR spectra, and the advanced Marfey's analysis for compounds **1–5** (PDF)

X-ray crystallographic data of **1** and **6** (ZIP)

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Notes

The authors declare no competing financial interest.

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