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Natural Hydroxamate-Containing Siderophore Acremonpeptides A−D and an Aluminum Complex of Acremonpeptide D from the Marine-Derived *Acremonium persicinum* SCSIO 115

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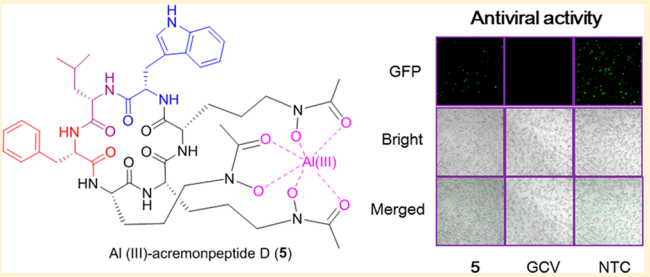
Supporting Information

ABSTRACT: Four new hydroxamate-containing natural product cyclopeptides designated acremonpeptides A−D (**1**−**4**), together with Al(III)−acremonpeptide D (**5**) were obtained from the marine fungus *Acremonium persicinum* SCSIO 115. The planar structures of **1**−**5** were established on the basis of HRMS as well as 1D and 2D NMR data sets. Moreover, the amino acid absolute configurations were determined using Marfey's method. Compounds **1**−**5** all feature three 2-amino-5-(*N*-hydroxyacetamido)pentanoic acid (N^5-hydroxy-N^5-acetyl-L-ornithine) metal ion chelating moieties. Beyond their discovery and structure elucidation, *in vitro* bioassays revealed acremonpeptides A (**1**), B (**2**), and Al(III)−acremonpeptide D (**5**) as moderate antiviral agents for herpes simplex virus 1 with EC_{50} values of 16, 8.7, and 14 μM, respectively.



Despite its very low abundance in earth's aerobic environments, iron (generally Fe^{2+} or Fe^{3+}) is an important nutritional element for almost all organisms on the planet. To facilitate iron acquisition and bioavailability, microorganisms have evolved elegant siderophore-based systems wherein organic agents enhance the availability and handling of iron in living systems.[1,2] Natural product siderophores are generally low-molecular-mass (500−1500 Da) iron chelators that enable the uptake of soluble iron(III) from both terrestrial and aquatic environments.[3] Structurally speaking, these agents are typically biosynthesized by non-ribosomal peptide synthetases (NRPSs) and are functionalized with metal-chelating groups such as phenols, catechols, hydroxamates, carboxylates, and mixed-type (heterocyclic) metal binders such as oxazolines and thiazolines.[2,4,5] The sideromycins are excellent examples of these NRPS-derived compounds.[6] Produced by *Actinomycetes* and displaying antibiotic activities, the sideromycins are readily taken up by cells via the exploitation of specific siderophore/Fe uptake systems.[6] As a result, the sideromycins are characterized by very low minimal inhibitory concentrations (MIC) values in antimicrobial assays. For instance, ferrimycins and albomycins, typical sideromycins isolated from *Streptomyces*, were originally identified on the basis of their potent abilities to target both Gram-positive and Gram-negative bacteria.[7,8] These findings have inspired significant drug delivery efforts to exploit siderophoric uptake systems in "Trojan horse" approaches wherein cell permeability limits of specific agents can be surpassed. Simply put, a target cell's need to import iron (and things associated with it) can be used to override permeability factors that might otherwise restrict a drug's cellular entry; drugs can be smuggled into a target cell by virtue of their association with cellularly valuable iron.[9] This logic has inspired dramatically increased reports of the discovery, synthesis, and utilization of siderophores over the last 10−20 years.[2]

Although seawater is abundant in some metal ions such as sodium and potassium, iron levels in seawater are extremely low. Indeed, there are numerous examples of marine microorganisms that biosynthesize and use siderophores just

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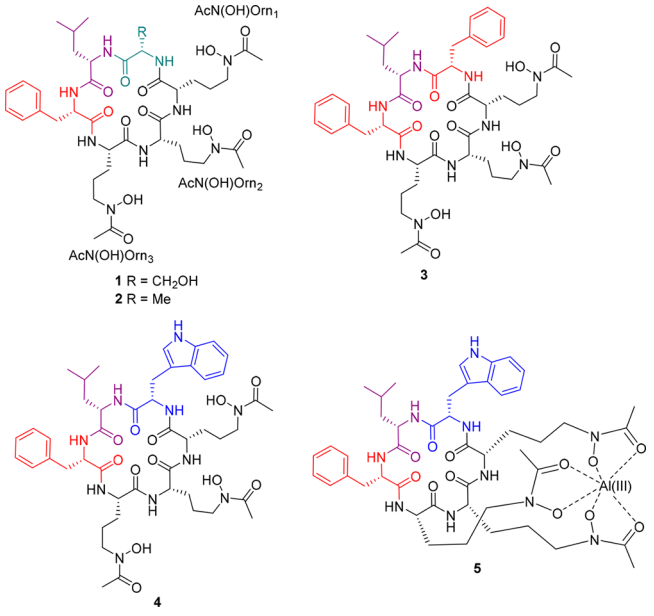
as their terrestrial counterparts do in order to acquire iron from their habitat.[10] In the course of our continuing investigations into bioactive secondary metabolites from marine-derived microorganisms, we have reported the cytotoxic heptapeptide cordyheptapeptides C−E from the marine fungus *Acremonium persicinum* SCSIO 115.[11] Recently, this strain was cultured on solid rice medium and HPLC-DAD analysis of the culture extracts revealed an array of compounds showing similar UV absorptions. Subsequent chemical investigations led to the purification of new cyclohexapeptide siderophores herein named acremonpeptides A−D (**1**−**4**) and Al(III)−acremonpeptide D (**5**). We report here the isolation, structure elucidation, and antiviral activities of these marine fungal metabolites.

RESULTS AND DISCUSSION

The fungus *A. persicinum* SCSIO 115 was cultured on autoclaved rice medium for 30 days. The mycelium and medium were extracted with MeOH, and the combined extracts were subsequently subjected to a combination of silica gel column chromatography (CC), Sephadex LH-20 CC, and semipreparative HPLC to yield compounds **1**−**5**. The structures of **1**−**5** were identified on the basis of HRESIMS in addition to 1D and 2D NMR data analyses, as well as Marfey's method for determinations of the absolute configurations.



Acremonpeptide A (**1**) was obtained as a white solid with a molecular formula of $C_{39}H_{61}N_9O_{13}$ as established by the HRESIMS signal at *m*/*z* 886.4288 ([M + Na]$^+$), indicating 14 degrees of unsaturation. The ^{1}H and ^{13}C NMR signals for **1** (Tables 1 and 2) unveiled the presence of three acetyl groups (δ_H 2.12, δ_C 20.3, δ_C 173.8; δ_H 2.09, δ_C 20.4, δ_C 173.7; δ_H 2.11, δ_C 20.3, and δ_C 173.8) with the aid of the HMBC correlations of δ_H 2.12/δ_C 173.8, δ_H 2.09/δ_C 173.7, and δ_H 2.11/δ_C 173.8. The remaining six carbonyl resonances at δ_C 172.1, 172.8, 173.3, 173.4, 174.7, and 175.0, together with six α-methine carbon resonances at δ_C 54.0, 55.5, 55.9, 56.2, 57.1, and 57.4 revealed **1** to be a hexapeptide. The ^{1}H NMR resonances at δ_H 7.24 (2H, d, *J* = 7.5 Hz), 7.30 (2H, t, *J* = 7.5 Hz), and 7.22 (1H, d, *J* = 7.5 Hz), as well as the ^{13}C NMR signals at δ_C 139.1, 127.9, 129.7 (2 × C), and 130.4 (2 × C) revealed the presence of a phenyl group in **1**. COSY and HSQC data sets revealed ^{1}H−^{1}H spin systems consistent with one −CH−CH$_2$−CH−(CH$_3$)$_2$, two −CH−CH$_2$−, and three −CH−(CH$_2$)$_2$−CH$_2$− moieties. HMBC correlation analyses confirmed the presence of one phenylalanine (Phe), one leucine (Leu), one serine (Ser), and three ornithine (Orn) residues within the structure of **1** (Figure 1). Further HMBC correlations from the protons at the terminal methylenes in the Orn units to the acetyl carbonyls (δ_H 3.57−3.75/δ_C 173.8 and δ_H 3.57−3.75/δ_C 173.7) confirmed the connectivity of the three acetyl groups (previously established) to the 5-*N* atom of each Orn via amide bonds. Furthermore, there were three hydroxy groups in **1** as dictated in large part by the molecular formula. The three hydroxy groups were linked to each Orn 5-*N* atom to form three hydroxylamine groups, which was determined by comparison of the ^{13}C NMR chemical shift value for C-5 of the Orn unit in **1** ($\delta_{C\text{-}5}$ 48.5) with those of the N^5-hydroxy-Orn unit ($\delta_{C\text{-}5}$ ∼ 46) and unsubstituted Orn unit ($\delta_{C\text{-}5}$ 38.6) in known compounds.[5,12−14] The elucidated benzene ring and nine carbonyl moieties accounted for 13 degrees of unsaturation. The remaining unsaturation and absence of other functionalities that might account for it suggested that **1** might be a cyclic scaffold, specifically a cyclohexapeptide. The HMBC spectrum gave corrections of δ_H 4.53/δ_C 175.0, δ_H 4.07/δ_C 172.1, δ_H 4.43/δ_C 173.4, δ_H 4.44/δ_C 173.3, δ_H 4.13/δ_C 174.7, and δ_H 4.03/δ_C 172.8, establishing the sequence of amino acid residues as cyclo-(Phe-Leu-Ser-AcN(OH)Orn$_1$-AcN(OH)Orn$_2$-AcN(OH)Orn$_3$) for **1**. Rigorous review of the literature revealed reports of an identical planar structure in a Japanese patent, but the amino acid configurations had not been disclosed.[15] Accordingly, we determined the amino acid absolute configurations for **1** using Marfey's method. HPLC analyses of the DAA derivatives of the HCl and HI hydrolysates of **1** confirmed that **1** is composed of L-Phe, L-Leu, L-Ser, and L-Orn (Figures S31 and S36). Consequently, cyclohexapeptide **1** was elucidated as shown and named acremonpeptide A.

The molecular formula $C_{39}H_{61}N_9O_{12}$ for acremonpeptide B (**2**) was determined on the basis of HRESIMS. Signals at *m*/*z* 848.4519 ([M + H]$^+$) and *m*/*z* 870.4331 ([M + Na]$^+$), indicated a 16 mass unit deficiency relative to **1** suggesting one O atom difference between structures **1** and **2**. Comparisons of the ^{1}H and ^{13}C NMR spectroscopic data of **2** with those of **1** (Tables 1 and 2) revealed that the hydroxymethyl signals at δ_H 3.76, 3.99, and δ_C 63.0 in **1** were replaced by methyl signals at δ_H 1.37 and δ_C 18.4 in **2** suggesting that **2** harbors an Ala residue within the shared cyclohexapeptide scaffold where structure **1** houses a Ser residue. Detailed analyses of HMBC correlations of **2** confirmed the presence of the Ala residue and the sequence of all amino acid residues (Figure 1). Application of Marfey's method revealed that **2** is composed of L-Phe, L-Leu, L-Ala, and L-Orn (Figures S32 and S36). On the basis of these data, compound **2** was established to be cyclo-(-L-Phe-L-Leu-L-Ala-L-AcN(OH)Orn$_1$-L-AcN(OH)Orn$_2$-L- AcN(OH)-Orn$_3$-) and named acremonpeptide B.

The HRESIMS spectrum for acremonpeptide C (**3**) showed a protonated molecule peak at *m*/*z* 924.4831 ([M + H]$^+$) and a sodium adduct ion peak at *m*/*z* 946.4647 ([M + Na]$^+$), indicating a molecular formula of $C_{45}H_{65}N_9O_{12}$. The ^{1}H and ^{13}C NMR data of **3** were similar to those of **1**, except that signals attributed to the Ser residue in **1** were absent and appeared to be replaced with a set of signals consistent with a

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Table 1. ^{1}H NMR (600 MHz) Spectroscopic Data for Compounds 1−5 in CD_3OD

	δ_H, multi. (*J* in Hz)				
position	acremonpeptide A (**1**)	acremonpeptide B (**2**)	acremonpeptide C (**3**)	acremonpeptide D (**4**)	Al(III)−acremonpeptide D (**5**)
Phe					
2	4.53, dd (11.1, 4.3)	4.48, dd (9.0, 4.2)	4.49, t (8.2)	4.45, dd (8.3, 5.5)	4.06, dd (11.4, 4.2)
3	3.37, dd (13.8, 4.3); 3.05, dd (13.8, 11.1)	3.36, dd (13.5, 4.2); 3.15, dd (13.5, 9.0)	3.17, overlapped; 3.28, dd (13.5, 8.2)	3.30, dd (12.5, 5.5); 3.17, dd (12.5, 8.3)	3.57, d (13.8); 3.53, overlapped
5, 9	7.24, d (7.5)	7.23, d (7.2)	7.28−7.30, overlapped	7.26, overlapped	7.18, d (7.2)
6, 8	7.30, t (7.5)	7.27, t (7.2)	7.28−7.30, overlapped	7.28, overlapped	7.26, t (7.2)
7	7.22, t (7.5)	7.19, t (7.2)	7.22, t (6.6)	7.20, t (6.6)	7.20, overlapped
Leu					
2	4.07, dd (8.5, 6.3)	4.01, t (7.2)	3.90, dd (7.0, 5.7)	3.86, m	3.67, dd (8.4, 6.0)
3	1.27, m; 1.40, m	1.52, m; 1.37, m	1.59, m; 1.43, m	1.44, m; 1.30, m	0.99, m; 1.05, m
4	1.34, m	1.31, m	1.20, m	0.96, br s	0.64, m
5	0.78, d (6.0)	0.77,d (6.0)	0.75, d (6.4)	0.63, d (6.0)	0.53, d (6.0)
6	0.83, d (6.0)	0.81, d (6.0)	0.80, d (6.4)	0.67, d (6.0)	0.56, d (6.0)
	Ser	Ala	Phe	Trp	Trp
2	4.43, dd (9.3, 4.7)	4.27, q (7.2)	4.43, t (7.2)	4.55, t (6.8)	4.57, m
3	3.99, dd (11.4, 4.7); 3.76, dd (11.4, 9.3)	1.37, d (7.2)	3.17, overlapped; 3.10, dd (13.3, 7.2)	3.28, d (6.8)	2.87, br d (13.8); 3.37, dd (13.8, 4.0)
4				7.19, s	7.43, s
5			7.28−7.30, overlapped		
6			7.28−7.30, overlapped		
7			7.22, t (6.6)	7.65, d (7.8)	7.96, d (7.8)
8			7.28−7.30, overlapped	7.04, t (7.8)	7.05, t (7.8)
9			7.28−7.30, overlapped	7.11, t (7.8)	7.14, t (7.8)
10				7.35, d (7.8)	7.37, d (7.8)
AcN(OH) Orn_1					
2	4.44, dd (7.6, 3.8)	4.36, br s	4.21, t (5.0)	4.60, br s	4.55, m
3	1.90−2.09, m; 1.67−1.88, m	1.95−2.08, m; 1.66−1.90, m	1.85−1.95, m; 1.76−1.62, m	1.85−1.95, m; 1.76−1.62, m	1.90−2.10, m; 1.64−1.85, m
4	1.70−1.85, m; 1.56−1.68, m	1.70−1.90, m; 1.32−1.60, m	1.63−1.85, m; 1.40−1.55, m	1.50−1.73, m; 1.30−1.50, m	1.80−1.95, m; 1.60−1.75, m
5	3.53−3.75, m	3.57−3.66, m	3.56−3.66, m	3.40−3.60, m	3.50−3.80, m
Me	2.12, s	2.12, s	2.11, s	2.10, s	2.15, s
AcN(OH) Orn_2					
2	4.13, dd (10.4, 3.8)	4.08, t (6.6)	4.09, t (6.0)	4.15, m	4.39, dd (13.0, 2.4)
3	1.90−2.09, m; 1.67−1.88, m	1.95−2.08, m; 1.66−1.90, m	1.85−1.95, m; 1.76−1.62, m	1.75−1.90, m; 1.50−1.66, m	1.90−2.10, m; 1.64−1.85, m
4	1.70−1.85, m; 1.56−1.68, m	1.70−1.90, m; 1.32−1.60, m	1.63−1.85, m; 1.40−1.55, m	1.50−1.73, m; 1.30−1.50, m	1.80−1.95, m; 1.60−1.75, m
5	3.53−3.75, m	3.57−3.66, m	3.56−3.66, m	3.40−3.60, m	3.50−3.80, m
Me	2.09, s	2.09, s	2.08, s	2.09, s	2.17,s
AcN(OH) Orn_3					
2	4.03, t (7.0)	4.18,dd (8.9, 3.2)	4.15, t (6.0)	4.23, m	4.34, dd (11.0, 9.4)
3	1.90−2.09, m; 1.67−1.88, m	1.95−2.08, m; 1.66−1.90, m	1.85−1.95, m; 1.76−1.62, m	1.75−1.90, m; 1.50−1.66, m	1.90−2.10, m; 1.64−1.85, m
4	1.70−1.85, m; 1.56−1.68, m	1.70−1.90, m; 1.32−1.60, m	1.63−1.85, m; 1.40−1.55, m	1.50−1.73, m; 1.30−1.50, m	1.80−1.95, m; 1.60−1.75, m
5	3.53−3.75, m	3.57−3.66, m	3.56−3.66, m	3.40−3.60, m	3.50−3.80, m
Me	2.11, s	2.10, s	2.09, s	2.10, s	2.16, s

second Phe residue (Tables 1 and 2). All indications supported a Phe for Ser replacement in **3** relative to structure **1**. Indeed, HMBC corrections of δ_H 4.49/δ_C 173.6, 173.1, δ_H 3.90/δ_C 173.0, 173.6, δ_H 4.43/δ_C 173.8, 173.0, δ_H 4.21/δ_C 174.1, 173.8, δ_H 4.09/δ_C 174.1, and δ_H 4.15/δ_C 173.1, 174.1 confirmed the amino acid sequence of **3** to be cyclo-(-Phe_1-Leu-Phe_2-AcN(OH)Orn_1-AcN(OH)Orn_2-AcN(OH)Orn_3-). As with **1** and **2**, application of Marfey's method revealed **3** to be composed of L-Phe, L-Leu, and L-Orn (Figures S33 and S36).

Acremonpeptide D (**4**) was isolated as a white solid. The molecular formula of **4** was determined to be $C_{47}H_{66}N_{10}O_{12}$ based on the HRESIMS signals at *m/z* 963.4932 ($[M + H]^+$) and *m/z* 985.4735 ($[M + Na]^+$). Analyses of the ^{1}H and ^{13}C NMR signals of **4** made clear the presence of Phe, Leu, and three AcN(OH)Orn residues (Tables 1 and 2). Further comparisons of the 1D NMR data with those of **1**, revealed the absence of Ser in **4**, as had been noted for compounds **2** and **3**. Additionally, five aromatic methine carbon signals at δ_C 112.6, 119.6, 120.1, 122.7, and 124.9, three nonprotonated sp^2

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Table 2. ^{13}C NMR (150 MHz) Spectroscopic Data for Compounds 1−5 in CD_3OD

position	1	2	3	4	5
Phe					
CO	172.8, C	172.8, C	173.1, C	173.1, C	171.8, C
2	57.1, CH	57.0, CH	57.4, CH	57.3, CH	58.8, CH
3	38.2, CH_2	37.6, CH_2	37.9, CH_2	37.8, CH_2	35.4, CH_2
4	139.1, C	139.1, C	138.9, C	138.9, C	140.3, C
5, 9	130.4, CH	130.4, CH	130.6, CH	130.5, CH	130.3, CH
6, 8	129.7, CH	129.6, CH	129.7, CH	129.6, CH	129.6, CH
7	127.9, CH	127.8, CH	128.0, CH	127.7, CH	127.7, CH
Leu					
CO	175.0, C	174.9, C	173.6, C	173.9, C	174.9, C
2	55.5, CH	55.3, CH	55.2, CH	55.1, CH	55.5, CH
3	41.2, CH_2	40.8, CH_2	40.8, CH_2	40.7, CH_2	41.0, CH_2
4	25.7, CH	25.7, CH	25.6, CH	25.4, CH	25.3, CH
5	22.5, CH_3	22.5, CH_3	22.1, CH_3	22.0, CH_3	22.4, CH_3
6	23.0, CH_3	23.1, CH_3	23.5, CH_3	23.3, CH_3	22.9, CH_3
	Ser	Ala	Phe	Trp	Trp
CO	172.1, C	174.5, C	173.0, C	173.6, C	172.0, C
2	56.2, CH	50.9, CH	57.4, CH	56.5, CH	53.6, CH
3	63.0, CH_2	18.4, CH_3	38.3, CH_2	28.4, CH_2	30.5, CH_2
4			138.6, C	124.9, CH	125.9, CH
5			130.5, CH	110.9, C	111.5, C
6			129.7, CH	128.8, C	128.4, C
7			127.9, CH	119.6, CH	120.5, CH
8			129.7, CH	120.1, CH	120.6, CH
9			130.5, CH	122.7, CH	123.2, CH
10				112.6, CH	112.7, CH
11				138.1, C	138.4, C
$AcN(OH)Orn_1$					
CO	173.4, C	173.2, C	173.8, C	174.3, C	171.9, C
2	54.0, CH	54.7, CH	55.7, CH	56.5, CH	54.5, CH
3	30.8, CH_2	30.3, CH_2	29.2, CH_2	29.2, CH_2	26.6, CH_2
4	23.9, CH_2	24.7, CH_2	24.5, CH_2	24.4, CH_2	23.2, CH_2
5	48.5, CH_2	48.5, CH_2	48.6, CH_2	48.6, CH_2	50.3, CH_2
MeCO	173.8, C	173.8, C	173.7, C	173.8, C	163.7, C
MeCO	20.3, CH_3	20.6, CH_3	20.5, CH_3	20.5, CH_3	15.8, CH_3
$AcN(OH)Orn_2$					
CO	173.3, C	174.6, C	174.1, C	173.5, C	171.9, C
2	55.9, CH	57.0, CH	55.9, CH	56.4, CH	60.3, CH
3	28.1, CH_2	28.7, CH_2	29.1, CH_2	29.2, CH_2	26.3, CH_2
4	25.0, CH_2	24.1, CH_2	24.7, CH_2	24.7, CH_2	27.7, CH_2
5	48.5, CH_2	48.6, CH_2	48.5, CH_2	48.5, CH_2	50.2, CH_2
MeCO	173.7, C	173.9, C	173.7, C	173.9, C	163.9, C
MeCO	20.4, CH_3	20.5, CH_3	20.5, CH_3	20.5, CH_3	16.7, CH_3
$AcN(OH)Orn_3$					
CO	174.7, C	173.2, C	174.1, C	173.7, C	172.9, C
2	57.4, CH	55.4, CH	56.3, CH	55.5, CH	55.1, CH
3	28.9, CH_2	29.1, CH_2	28.9, CH_2	29.5, CH_2	28.7, CH_2
4	24.7, CH_2	24.7, CH_2	24.6, CH_2	24.5, CH_2	23.0, CH_2
5	48.5, CH_2	48.5, CH_2	48.6, CH_2	48.5, CH_2	49.2, CH_2
MeCO	173.8, C	173.7, C	173.7, C	173.8, C	163.9, C
MeCO	20.3, CH_3	20.5, CH_3	20.5, CH_3	20.5, CH_3	16.3, CH_3

hybridized carbon signals at δ_C 110.9, 128.8, and 138.1, one methylene signal at δ_C 28.4 and one α-methine signal at δ_C 56.5 were observed. Four coupled aromatic ^1H NMR signals at δ_H 7.04 (t, J = 7.8 Hz), 7.11 (t, J = 7.8 Hz), 7.35 (d, J = 7.8 Hz), and 7.65 (d, J = 7.8 Hz) indicated the presence of a 1,2-disubstituted benzene, which was supported by the COSY data (Figure 1). Also, a singlet signal at δ_H 7.19 defined the presence of an isolated aromatic proton. The HMBC correlations from the four coupled protons (δ_H 7.04, 7.11, 7.35, and 7.65) and the isolated aromatic proton at δ_H 7.19, as well as from the α-proton at δ_H 4.55 suggested the presence of a tryptophan (Trp) residue (Figure 1). The amino acid sequence of **4** was assigned as cyclo-(-Phe-Leu-Trp-AcN-(OH)Orn_1-AcN(OH)Orn_2-AcN(OH)Orn_3-) based on HMBC corrections of δ_H 4.45/δ_C 173.1 and 173.9, δ_H 3.86/δ_C 173.9 and 173.6, δ_H 4.55/δ_C 173.6 and 174.3, δ_H 4.60/δ_C

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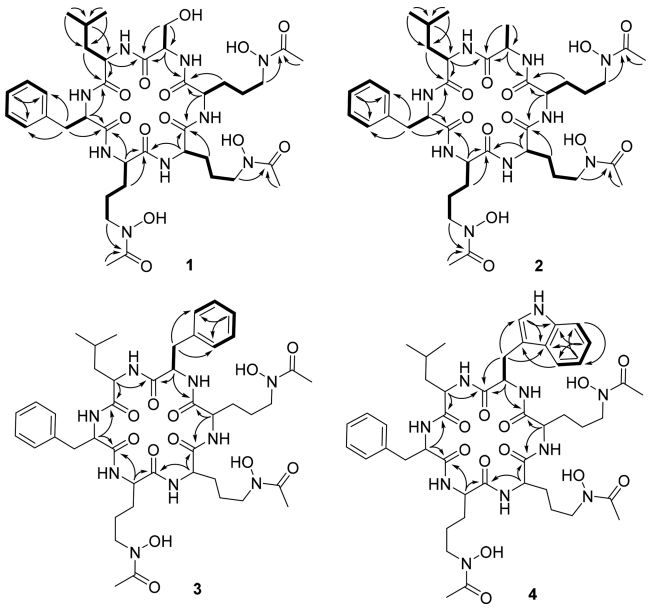

Figure 1. Key COSY and HMBC correlations of acremonpeptides A–D (**1**–**4**).

174.3 and 173.5, δ_H 4.15/δ_C 173.5 and 173.7, and δ_H 4.23/δ_C 173.7 and 173.1. The absolute configurations of the amino acid residues in **4** were determined to be L-Phe, L-Leu, L-Trp, and L-Orn on the basis of Marfey's method analyses (Figures S34 and S36).

Compound **5** displayed a molecular formula of $C_{47}H_{63}AlN_{10}O_{12}$ based on the HRESIMS peak at m/z 987.4514 ($[M + H]^+$). The ^1H and ^{13}C NMR spectroscopic data of **5** were very similar to those obtained for **4** (Tables 1 and 2). However, the ^{13}C NMR chemical shifts of the three acetyl group methyls were noted to shift from δ_C 20.5 in **4** to δ_C 15.8, 16.3, and 16.7 in **5**, respectively (Table 2), consistent with aluminum ion chelation by **4** to generate **5**; a similar phenomenon has been demonstrated with conversion of known compound AS2488059 into ASP2397.[14] As anticipated, the application of Marfey's method confirmed the presence of L-Phe, L-Leu, L-Trp, and L-Orn residues in **5** (Figures S35 and S36). Compound **5** was thus identified as shown and named Al(III)–acremonpeptide D, the aluminum complex of **4**.

Compounds **1**–**5** are natural hydroxamate-containing siderophores containing N^5-hydroxy-N^5-acetyl-L-ornithine units derived from L-ornithine. Thus far, all siderophores originating from fungi are hydroxamate-based; the only exception known is the polycarboxylate rhizoferrin.[2] The structures of **1**–**5** are closely related to the known agent AS2488059, which originates from the same species as the titled fungus,[14] and readily complexes with Al(III) (ASP2397), Fe(III) (AS2488053), and Ga(III) (AS2529132) ions. Notably, AS2488059 and its derivatives display *in vitro* antifungal activities against the clinical isolate *Aspergillus fumigatus* FP1305. The most potent antibiotic in the hydroxamate siderophore family is albomycin. Albomycin was initially isolated from *Streptomyces griseus* in 1947,[16] completely elucidated in 1982,[17] and generated by total synthesis by He and co-workers in 2018.[18] Importantly, albomycin is more potent than penicillin G and vancomycin against clinical isolates of *Streptococcus pneumoniae* and *Staphylococcus aureus*. Inspired by these realizations and shared structural properties, we assayed acremonpeptides A–D (**1**–**4**) and Al(III)–acremonpeptide D (**5**) for antimicrobial activities against a panel of bacteria and fungi. Somewhat surprisingly, **1**–**5** proved inactive in these assays at concentrations up to 100 μg/mL. We then evaluated **1**–**5** for antiviral activities against herpes simplex virus 1 (HSV-1) and vesicular stomatitis virus (VSV) by measuring the inhibition of the green fluorescent protein (GFP) expression in HSV-1 and VSV infected Vero cells (Figure 2A). We found that acremonpeptides A (**1**), B (**2**), and Al(III)–acremonpeptide D (**5**) showed antiviral activities against HSV-1 with EC_{50} values of 16, 8.7, and 14 μM, respectively (Table 3). Further mechanism of action (MOA) studies revealed that **1**, **2**, and **5** inhibit the transcription of the UL42 gene in HSV; UL42 codes for a polypeptide essential to viral DNA replication (Figure 2B).

Table 3. Antiviral Activities of Compounds 1–5 against Herpes Simplex Virus 1 (EC_{50}, μM)

compound	EC_{50} value
1	16 ± 6
2	8.7 ± 1.2
3	27 ± 2
4	24 ± 3
5	14 ± 2
Ganciclovir (GCV)	0.025

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained with an MCP 500 polarimeter (Anton Paar). UV spectra were recorded with a U-2600 spectrometer (Shimadzu). NMR spectra were acquired with a DD2 spectrometer (Agilent) at 600 MHz for ^1H nuclei and 150 MHz for ^{13}C nuclei. Chemical shifts (δ) are given in ppm with reference to TMS. HRESIMS spectra were obtained using a

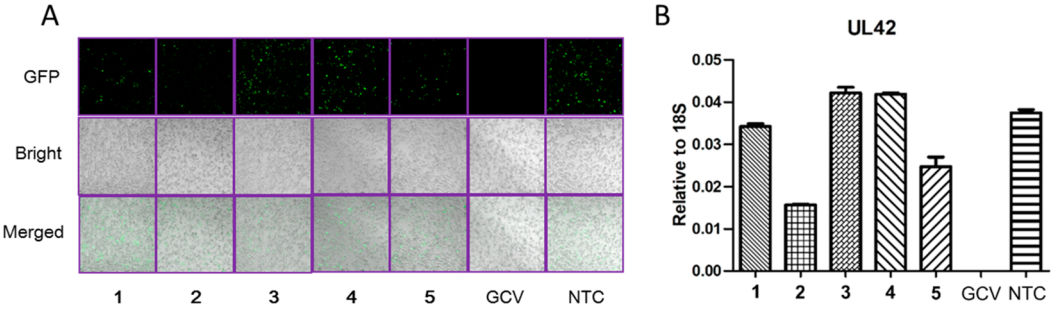

Figure 2. (A) Observed GFP expression in HSV-1 infected Vero cells incubated with compounds **1**–**5**; (B) compounds **1**–**5** inhibited the transcription of UL42 gene in HSV. Ganciclovir (GCV) was used as a positive control and NTC as a negative control.

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Maxis quadrupole-time-of-flight mass spectrometer (Bruker). Column chromatography (CC) was performed with silica gel (200−300 mesh, Yantai Jiangyou Silica Gel Development Co., Ltd.). Medium-pressure liquid chromatography (MPLC) was performed with a CHEETAH 100 (Bonna-Agela) flash chromatography system. Preparative HPLC was carried out using a QuikSep-50 system (H&E Co., Ltd.) with an Innoval C18 column (250 × 21.2 mm, 5 μm, Bonna-Agela). Natural sea salt is a commercial product from Guangdong Province Salt Industry Group Co., Ltd., China.

Fungal Material, Fermentation, and Isolation. The fungal strain *A. persicinum* SCSIO 115 was isolated from a marine sediment sample collected in the South China Sea.[11] SCSIO 115 was cultured in potato dextrose broth medium supplemented with 3% sea salt at 28 °C on a rotary shaker at 200 rpm for 2 days as seed cultures, and then each 50 mL seed culture was transferred into a 2000 mL Erlenmeyer flask containing rice solid medium (300 g of rice, 0.2% yeast extract, 0.2% bacterial peptone, and 3% sea salt). The flasks were cultured statically at room temperature for 30 days. A total of 4.5 kg harvested medium and mycelium were extracted with 5 L MeOH (3 times) to afford a residue following solvent evaporation. The residue was divided into nine fractions (Fr.A1-Fr.A9) by silica gel CC using a gradient elution of petroleum ether/EtOAc/MeOH (10:0:0, 8:2:0, 6:4:0, 4:6:0, 2:8:0, 0:10:0, 0:9:1, 0:8:2, 0:7:3, v/v). Fr.A9 was purified by MPLC with an ODS column eluting with MeOH/H_2O (0−20 min, 10:90−50:50; 20−45 min, 50:50−100:0; 45−75 min, 100:0; v/v) at a flow rate of 15 mL min^{-1} to get Fr.B1−B11. Fr.B(4−6) were combined and subjected to MPLC using an ODS column eluting with MeOH/H_2O (0−30 min, 10:90−50:50; 30−70 min, 50:50−90:10; 70−100 min, 100:0; v/v) at a flow rate of 15 mL min^{-1} to obtain Fr.C1−C11. Fr.C (7−8) were combined and purified with preparative HPLC equipment with an Innoval C18 column (250 × 21.2 mm, 5 μm), eluting with a mixture of MeOH/H_2O (0−20 min, 40:60−60:40) at a flow rate of 9 mL min^{-1} to yield compounds **1** (20.3 mg) and **2** (18.5 mg) at t_R 8.3 min and t_R 8.6 min, respectively. Fr.C10 was prepared by preparative HPLC using an elution system of CH_3CN/H_2O (0−20 min, 45:55−85:15) at a flow rate of 9 mL min^{-1} to yield **4** (42.5 mg) and **3** (9.6 mg) at t_R 11.8 and 12.3 min, respectively. Fr.B(9−11) were combined and subjected to silica gel CC eluting with mixtures of EtOAc/MeOH (100:0, 98:2, 96:4, 94:6, 92:8, 90:10, 95:15, 80:20, v/v) to get Fr.D1-Fr.D10. Fr.D9 was further purified by preparative HPLC eluting with CH_3CN/H_2O (0−20 min, 50:50−80:20) at a flow rate of 9 mL min^{-1} to yield **5** (8.5 mg) at t_R 13.9 min.

*Acremonpeptide A (**1**).* White powder; $[\alpha]^{25}_D$ −27 (0.33, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.60) nm; ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data, Tables 1 and 2; HRESIMS *m/z* 864.4459 $[M + H]^+$ (calcd for $C_{39}H_{62}N_9O_{13}$, 864.4462), *m/z* 886.4288 $[M + Na]^+$ (calcd for $C_{39}H_{61}N_9NaO_{13}$, 886.4281).

*Acremonpeptide B (**2**).* White powder; $[\alpha]^{25}_D$ −24 (0.62, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.50) nm; ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data, Tables 1 and 2; HRESIMS *m/z* 848.4519 $[M + H]^+$ (calcd for $C_{39}H_{62}N_9O_{12}$, 848.4512), *m/z* 870.4331 $[M + Na]^+$ (calcd for $C_{39}H_{61}N_9NaO_{12}$, 870.4332).

*Acremonpeptide C (**3**).* White powder; $[\alpha]^{25}_D$ −48 (0.86, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.72) nm; ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data, Tables 1 and 2; HRESIMS *m/z* 924.4831 $[M + H]^+$ (calcd for $C_{45}H_{66}N_9O_{12}$, 924.4825), *m/z* 946.4647 $[M + Na]^+$ (calcd for $C_{45}H_{65}N_9NaO_{12}$, 946.4645).

*Acremonpeptide D (**4**).* White powder; $[\alpha]^{25}_D$ −53 (0.33, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.75), 216 sh (4.67), 281 (3.85) nm; ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data, Tables 1 and 2; HRESIMS *m/z* 963.4932 $[M + H]^+$, (calcd for $C_{47}H_{67}N_{10}O_{12}$, 963.4934), *m/z* 985.4735 $[M + Na]^+$, (calcd for $C_{47}H_{66}N_{10}NaO_{12}$, 985.4754).

*Al(III)−Acremonpeptide D (**5**).* White powder; $[\alpha]^{20}_D$ +59 (0.58, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.64), 218 (4.56), 289 (3.74) nm; ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data, Tables 1 and 2; HRESIMS *m/z* 987.4514 $[M + H]^+$ (calcd for $C_{47}H_{64}AlN_{10}O_{12}$, 987.4515).

Marfey's Analysis. Compounds **1** (0.87 mg), **2** (0.61 mg), **3** (1.20 mg), **4** (0.58 mg), and **5** (0.60 mg) were separately dissolved into 1 mL of HCl (6 N) and heated at 110 °C for 18 h. The hydrolysates were dried and resuspended in 100 μL of H_2O. Then, 100 μL of 1% (w/v) 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA) in acetone and 50 μL of $NaHCO_3$ (1 N) were added. The mixtures were heated at 60 °C for 1 h, and then neutralized with 50 μL of HCl (1 N). MeOH was added to the quenched reaction to afford a total volume of 500 μL. Each sample of ∼ 0.5 mg of D- and L-amino acid standards were dissolved in 1 mL of $NaHCO_3$ (1 N); and 50 μL of amino acid solution was treated with FDAA in the same fashion. Derivatization products (20 μL) were then analyzed by HPLC with a Phenomenex column (Prodigy ODS (2), 150 × 4.6 mm, 5 μm) using an elution system consisting of solvent A (0.1% TFA in H_2O) and solvent B (0.1% TFA in CH_3CN). Samples were eluted with a linear gradient from 10% to 90% solvent B over the course of 30 min at a flow rate of 1 mL/min and UV detection of 340 nm. The retention times for DAA derivatives of the D-Phe, L-Phe, D-Leu, L-Leu, D-Ser, L-Ser, D-Ala, L-Ala D-Tyr, and L-Tyr were 23.30, 19.75, 24.25, 20.35, 7.82, 6.85, 13.45, 10.45, 21.85, and 19.15 min, respectively. The Phe and Leu residues in **1**−**5** were all assigned as L-Phe and L-Leu, respectively, the Ser residue in **1** was assigned as L-Ser, the Ala residue in **2** was assigned as L-Ala, and the Trp residue in **4** and **5** was assigned as L-Trp (Figures S31−S35).

For the determination of Orn residue, hydrolyses of compounds **1** (0.58 mg), **2** (0.61 mg), **3** (0.60 mg), **4** (0.59 mg), and **5** (0.60 mg) were conducted using 45% HI in place of HCl (6 N) to reduce N^5-acetyl-N^5-hydroxyornithine to ornithine for FDAA derivatization.[5] Similarly, D- and L-ornithine standards were treated with FDAA solution according to the aforementioned method. All the FDAA derivatization products were analyzed using the above-mentioned HPLC system with a linear gradient elution from 10% to 80% solvent B over the course of 40 min. The retention times for DAA derivatives (derivatization at the α-amine, the δ-amine, and both amines) of the D-Orn and L-Orn were 10.12, 12.20, and 21.13 min and 11.92, 12.23, and 22.32 min, respectively (Figure S36). The Orn residue present in **1**−**5** was determined to be L-Orn.

Antiviral Assays. Briefly, African green monkey kidney Vero cells were infected with recombinant HSV-1 in which a reporter gene green fluorecence protein (GFP) was inserted, at multiple of infection (MOI) of 1.0. Cells were treated with compounds at a final concentration of 10 μM as indicated in the figure or with equal amounts of DMSO, respectively. Ganciclovir (GCV), a clinically used anti-HSV medicine, was used as a positive control. Infected cells were monitored by a fluorescence microscope. Viral loads in infected cells were measured by $TCID_{50}$ assay, and viral gene transcriptions were measured by qRT-PCR assays. To estimate EC_{50} for each compound, infected cells were treated with compounds at different concentrations in triplicate and viral loads were monitored at 72 h postinfection.

Antimicrobial Assays. Compounds **1**−**5** were tested for their antimicrobial activities by the method previously reported.[19,20] Nine bacteria including *Bacillus thuringiensis* BT01, *B. thuringiensis* W102, *S. aureus* ATCC 29213, methicillin-resistant *S. aureus* shhs-A1 (a clinical isolate), *Enterococcus faecalis* ATCC 29212, *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, and drug resistant *E. coli* E11 (a clinical isolate), as well as two fungi, *Candida albicans* ATCC 96901 and *C. albicans* CMCC (F) 98001, were used.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.9b00545.

1D and 2D NMR spectra, HRESIMS spectra of compounds **1**−**5**, and HPLC chromatograms associated

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with Marfey's reaction analyses for absolute configuration determinations (PDF)

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Notes

The authors declare no competing financial interest.

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