

Article

Two New Spiro-Heterocyclic γ -Lactams from A Marine-Derived *Aspergillus fumigatus* Strain CUGBMF170049

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Abstract: Two new spiro-heterocyclic γ -lactam derivatives, cephalimysins M (1) and N (2), were isolated from the fermentation cultures of the marine-derived fungus *Aspergillus fumigatus* CUGBMF17018. Two known analogues, pseurotin A (3) and FD-838 (4), as well as four previously reported helvolic acid derivatives, 16-*O*-propionyl-16-*O*-deacetylhelvolic acid (5), 6-*O*-propionyl-6-*O*-deacetylhelvolic acid (6), helvolic acid (7), and 1,2-dihydrohelvolic acid (8) were also identified. One-dimensional (1D), two-dimensional (2D) NMR, HRMS, and circular dichroism spectral analysis characterized the structures of the isolated compounds.

Keywords: marine-derived *Aspergillus fumigatus*; spiro-heterocyclic γ -lactam; cephalimysins

1. Introduction

Marine-derived fungi are important resources of structurally and biologically diverse secondary metabolites in drug discovery [1–6]. A series of novel marine natural compounds have been isolated from marine-derived fungi of *Aspergillus fumigatus* strains, such as *E*- β -*trans*-5,8,11-trihydroxybergamot-9-ene and β -*trans*-2 β ,5,15-trihydroxybergamot-10-ene [7], diketopiperazines [8,9], indole alkaloids [10], fumigaclavine C [11], fumiquinazoline K [12], and gliotoxin analogues [13]. During our ongoing efforts to search for new bioactive metabolites from marine-derived fungi, an *Aspergillus fumigatus* strain CUGBMF170049 was isolated from a sediment sample that was collected from the Bohai Sea, China. Chemical investigations on an EtOAc-MeOH extracted fraction of its solid fermentation cultures resulted in the characterization of two new spiro-heterocyclic γ -lactam derivatives, cephalimysins M (1) and N (2), along with two known analogues, pseurotin A (3) [14], and FD-838 (4) [15], as well as four previously reported helvolic acid derivatives, 16-*O*-propionyl-16-*O*-deacetylhelvolic acid (5), 6-*O*-propionyl-6-*O*-deacetylhelvolic acid (6) [16], helvolic acid (7), and 1,2-dihydrohelvolic acid (8) [17] (Figure 1). Herein, we report the isolation and structural determination of the new compounds 1 and 2. The antibacterial activities of the compounds were also investigated against a panel of both Gram-positive and Gram-negative bacteria, *Mycobacterium bovis* bacillus Calmette Guérin (BCG) and *Candida albicans*. Compounds 5–7 showed significant antibacterial activities against both *Staphylococcus aureus* and methicillin resistant *S. aureus*.

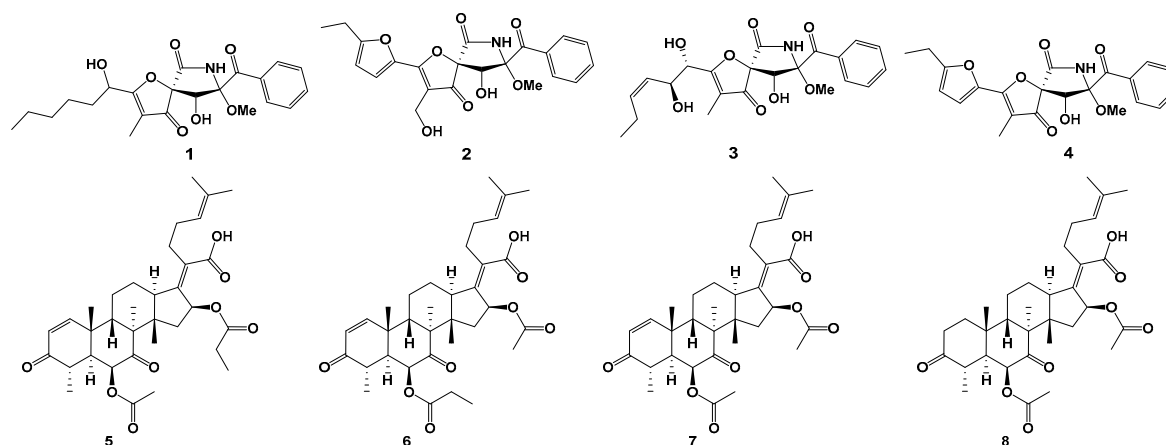


Figure 1. Chemical structures of 1–8.

2. Results

2.1. Structure Elucidation

Compound **1** was obtained as pale yellow amorphous powder. Its molecular formula was determined as $C_{22}H_{27}NO_7$ by HRESIMS m/z 440.1684 $[M + Na]^+$ (calcd. for $C_{22}H_{27}NO_7Na$ 440.1680, $\Delta m/mu + 0.4$) (Figure S1 in Supplementary Materials), which accounted for ten degrees of unsaturation. The 1H , ^{13}C , and HSQC NMR spectra (Figures S2–S4 in Supplementary Materials) of compound **1** showed signals of two ketone carbonyls at δ_C 196.7 (C-4) and 196.4 (C-17), one amide carbonyl at δ_C 166.4 (C-6), a mono-substituted benzene ring (δ_C 133.4, δ_C 130.3/ δ_H 8.25, δ_C 128.4/ δ_H 7.53, δ_C 133.9/ δ_H 7.64, δ_C 128.4/ δ_H 7.53, 130.3/ δ_H 8.25), two sp^2 quaternary carbons at δ_C 188.5 (C-2) and 109.6 (C-3), four sp^3 methylenes (δ_C 34.4/ δ_H 1.64, δ_C 31.1/ δ_H 1.24, δ_C 24.1/ δ_H 1.37, and δ_C 22.0/ δ_H 1.24), two sp^3 oxygenated methines (δ_C 74.9/ δ_H 4.38 and δ_C 67.0/ δ_H 4.50), two sp^3 oxygenated quaternary carbons at δ_C 91.2 (C-5) and δ_C 92.5 (C-8), and three methyls (δ_C 13.9/ δ_H 0.84, δ_C 5.4/ δ_H 1.64, and δ_C 51.7/ δ_H 3.24) (Table 1). A comparison of 1H and ^{13}C NMR data for compound **1** (Table 1) with those of previously reported pseurotin A (**3**) [14] revealed many similarities in their chemical structures, except for the oxygenated unsaturated side chain of **3** had been substituted with an oxygenated saturated fatty chain in **1**. Compound **1** had the same skeleton as that of pseurotin A, while the side chain of **1** is an oxygenated saturated fatty chain. The COSY correlations (Figure 2, and Figure S5 in Supplementary Materials) from H-19 (δ_H 8.25) to H-23 (δ_H 8.25), through H-20 (δ_H 7.53), H-21 (δ_H 7.64) and H-22 (δ_H 7.53) identified the mono-substituted benzene ring. Likewise, the fatty acid side chain was confirmed by COSY correlations from H-10 (δ_H 4.50) to H₃-15 (δ_H 0.84), through H-11 (δ_H 1.64), H-12 (δ_H 1.37), H-13 (δ_H 1.24), and H-14 (δ_H 1.24). The lactam ring was evidenced by the HMBC correlations (Figure 2, and Figure S6 in Supplementary Materials) from H-9-OH (δ_H 6.34) to C-5 (δ_C 91.2), C-8 (δ_C 92.5), and C-9 (δ_C 74.9), as well as from H-7-NH (δ_H 9.90) to C-5 (δ_C 91.2), C-6 (δ_C 166.4), C-8 (δ_C 92.5), and C-9 (δ_C 74.9). The phenylmethanone moiety was confirmed by the HMBC crossing peaks from H-19 (δ_H 8.25) and H-23 (δ_H 8.25) to C-17 (δ_C 196.4), and the connection from C-8 (δ_C 92.5) to C-17 (δ_C 196.4) was revealed by the HMBC correlation from H-9 (δ_H 4.38) to C-17 (δ_C 196.4). The HMBC correlation from H₃-24 (δ_H 3.24) to C-8 (δ_C 92.5) confirmed the methoxy at C-8. The connection from C-10 to C-4 through C-2 and C-3 was confirmed by the HMBC correlations from H-10-OH (δ_H 5.62) to C-10 (δ_C 67.0) and C-2 (δ_C 188.5), from H-10 (δ_H 4.50) to C-2 (δ_C 188.5) and C-3 (δ_C 109.6), as well as from H₃-16 (δ_H 1.64) to C-2 (δ_C 188.5), C-3 (δ_C 109.6), and C-4 (δ_C 196.7). In addition, the spirobicyclic moiety was suggested by the HMBC correlation from H-9 (δ_H 4.38) to C-4 (δ_C 196.7), the chemical shift of C-5 (δ_C 91.2) and the molecular formula. The *cis* configurations of 8-OCH₃ and 9-OH were supported by the chemical shift of H-9 and the coupling constant (δ_H 4.38, $J = 9.0$ Hz) between H-9 and 9-OH [15,18]. The circular dichroism (CD) spectrum (Figure S7 in Supplementary Materials) of **1** showed negative Cotton effects at around 230, 280, and 345 nm, and positive Cotton effects at around

250 and 310 nm, which were consistent with the reported CD data for pseurotin A [18]. Thus, the structure of compound **1** was established, as shown in Figure 1, and was named cephalimysin M, its absolute configurations for C-5, C-8, and C-9 being assigned the same as those of pseurotin A. The absolute configuration of C-10 was not defined.

Table 1. NMR data for **1** and **2** (DMSO-*d*₆).

Position	1		2	
	δ_C , Type	δ_H , mult (J in Hz)	δ_C , Type	δ_H , mult (J in Hz)
2	188.5, C		172.2, C	
3	109.6, C		111.6, C	
4	196.7, C		193.4, C	
5	91.2, C		92.1, C	
6	166.4, C		166.4, C	
8	92.5, C		92.6, C	
9	74.9, CH	4.38, d (9.0)	75.0, CH	4.50, s
10	67.0, CH	4.50, td (7.2, 5.4)	141.8, C	
11	34.4, CH ₂	1.64, m (overlap)	120.0, CH	7.40, d (3.6)
12	24.1, CH ₂	1.37, m	108.6, CH	6.52, d (3.6)
13	31.1, CH ₂	1.24, m	163.4, C	
14	22.0, CH ₂	1.24, m	21.0, CH ₂	2.76, q (7.8)
15	13.9, CH ₃	0.84, t (7.2)	11.8, CH ₃	1.22, t (7.8)
16	5.4, CH ₃	1.64, s	50.2, CH ₂	4.25, s
17	196.4, C		196.4, C	
18	133.4, C		133.4, C	
19	130.3, CH	8.25, dd (8.4, 1.2)	130.4, CH	8.27, d (7.2)
20	128.4, CH	7.53, ddd (8.4, 8.4, 1.2)	128.4, CH	7.53, dd (7.2, 7.2)
21	133.9, CH	7.64, dddd (8.4, 8.4, 1.2, 1.2)	133.9, CH	7.67, dd (7.2, 7.2)
22	128.4, CH	7.53, ddd (8.4, 8.4, 1.2)	128.4, CH	7.53, dd (7.2, 7.2)
23	130.3, CH	8.25, dd (8.4, 1.2)	130.4, CH	8.27, d (7.2)
24	51.7, CH ₃	3.24, s	51.7, CH ₃	3.26, s
7-NH		9.90, s		9.98, s
9-OH		6.34, d (9.0)		
10-OH		5.62, d (5.4)		

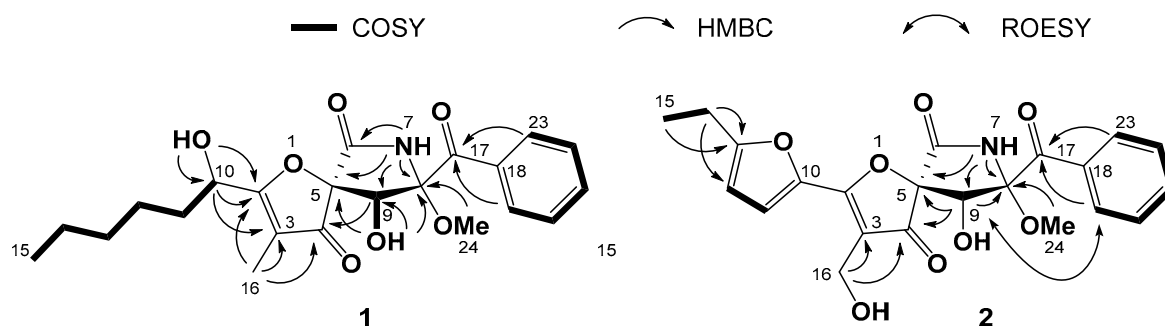


Figure 2. Key two-dimensional (2D) NMR correlations for **1** and **2**.

Compound **2** was obtained as pale yellow amorphous powder. Its molecular formula was determined as C₂₂H₂₁NO₈ by HRESIMS *m/z* 450.1159 [M + Na]⁺ (calcd. for C₂₂H₂₁NO₈Na 450.1159, Δ_{mmu} 0) (Figure S8 in Supplementary Materials), which accounted for thirteen degrees of unsaturation. The ¹H, ¹³C, and HSQC NMR spectra (Figures S9–S11 in Supplementary Materials) of compound **2** showed signals of two ketone carbonyls at δ_C 193.4 (C-4) and 196.4 (C-17), one amide carbonyl at δ_C 166.4 (C-6), a mono-substituted benzene ring (δ_C 133.4, δ_C 130.4/ δ_H 8.27, δ_C 128.4/ δ_H 7.53, δ_C 133.9/ δ_H 7.67, δ_C 128.4/ δ_H 7.53, δ_C 130.4/ δ_H 8.27), two sp² methines (δ_C 120.1/ δ_H 7.40, δ_C 108.6/ δ_H 6.52), four sp² quaternary carbons at δ_C 172.2 (C-2), 111.6 (C-3), 141.8 (C-10), and 163.4 (C-13), one sp³ methylene (δ_C 21.0/ δ_H 2.76), one sp³ oxygenated methylene (δ_C 50.2/ δ_H 4.25), one sp³ oxygenated methine

(δ_C 75.0/ δ_H 4.50), two sp^3 oxygenated quaternary carbons (δ_C 92.1 and δ_C 92.6), and two methyls (δ_C 11.8/ δ_H 1.22 and δ_C 51.7/ δ_H 3.26) (Table 1). A comparison of 1H and ^{13}C NMR data for compound 2 (Table 1) with that of the previously reported FD-838 (4) [15] revealed many similarities. Compound 2 had the same skeleton as that of FD-838, while methyl at C-3 of compound 4 was replaced by an oxygenated methylene in compound 2. The mono-substituted benzene ring was identified by the COSY correlations (Figure 2, and Figure S12 in Supplementary Materials) from H-19 (δ_H 8.27) to H-23 (δ_H 8.27), through H-20 (δ_H 7.53), H-21 (δ_H 7.67), and H-22 (δ_H 7.53), and the furan side chain was characterized by COSY correlations between H-11 (δ_H 7.40) and H-12 (δ_H 6.52), and between H₂-14 (δ_H 2.76) and H₃-15 (δ_H 1.22), along with the HMBC correlations (Figure 2, and Figure S13 in Supplementary Materials) from H₃-15 to C-13, and from H₂-14 to C-12 and C-13. The lactam ring was suggested by the HMBC correlations from H-9 (δ_H 4.50) to C-5 (δ_C 92.1) and C-8 (δ_C 92.6), as well as from H-7-NH (δ_H 9.98) to C-5 (δ_C 92.1), C-8 (δ_C 92.6) and C-9 (δ_C 75.0). HMBC crossing peaks from H-19 (δ_H 8.27) and H-23 (δ_H 8.27) to C-17 (δ_C 196.4) confirmed the phenylmethanone moiety, and the connection from C-8 (δ_C 92.6) to C-17 (δ_C 196.4) was revealed by the HMBC correlation from H-9 (δ_H 4.50) to C-17 (δ_C 196.4). The methoxy at C-8 was also confirmed by the HMBC correlation from H₃-24 (δ_H 3.26) to C-8 (δ_C 92.6). The spirobicyclic moiety was indicated by the HMBC correlation from H-9 (δ_H 4.50) to C-4 (δ_C 193.4) and the chemical shift of C-5 (δ_C 92.1). The ROESY correlations (Figure 2, and Figure S14 in Supplementary Materials) from H-9 (δ_H 4.50) to H-19 (δ_H 8.27) and H-23 (δ_H 8.27) indicated the relative configurations of C-8 and C-9. The chemical shifts of C-5, C-8, and C-9 for 2 were much closer to those of 1 and FD-838 [15], which defined the relative configurations of C-5, C-8, and C-9. The circular dichroism (CD) spectrum (Figure S15 in Supplementary Materials) of 2 showed a negative Cotton effect at around 318 nm and a positive Cotton effect at around 355 nm, which were consistent with the reported CD data for FD-838 [15]. Therefore, the structure of compound 2 was established, as shown in Figure 1, where absolute configurations were assigned the same as those of FD-838 and named cephalimysin N.

In addition to compounds 1 and 2, known compounds were also identified in the fermentation products, such as pseurotin A (3) [14], FD-838 (4) [15], as well as four known helvolic acid derivatives, 16-O-propionyl-16-O-deacetylhelvolic acid (5), 6-O-propionyl-6-O-deacetylhelvolic acid (6) [16], helvolic acid (7), and 1,2-dihydrohelvolic acid (8) [17].

2.2. Biological Activity

Compounds 1–8 were tested against Gram positive bacteria *S. aureus* (ATCC 6538) and methicillin resistant *S. aureus* (MRSA) (ATCC 29213), Gram negative bacteria *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 15692), BCG, and *C. albicans*. Compounds 5–7 showed antibacterial activities against both *S. aureus* and MRSA. Comparing the antibacterial activities of compounds 5–7 with the inactive analogue 8 indicated that the α,β -unsaturated ketone appears to be a key functional group for antibacterial activity (Table 2). None of the isolated compounds exhibited antimicrobial activities against *E. coli*, *P. aeruginosa*, *C. albicans* (MIC > 100 $\mu\text{g/mL}$), nor BCG (MIC > 10 $\mu\text{g/mL}$).

Table 2. Antimicrobial activities of 1–8 ($\mu\text{g/mL}$).

Compounds	<i>S. aureus</i> ^a	MRSA ^a
1	>100	>100
2	>100	>100
3	>100	>100
4	>100	>100
5	12.5	25
6	6.25	12.5
7	0.78	0.78
8	>100	>100

^a Vancomycin was used as positive control with MIC value of 0.78 $\mu\text{g/mL}$.

3. Materials and Methods

3.1. General Experimental Procedures

The optical rotations ($[\alpha]_D$) were measured on Anton Paar MCP 200 Modular Circular Polarimeter (Austria) in a 100×2 mm cell at 22°C . CD spectra were recorded on an Applied Photophysics Chirascan spectropolarimeter (UK). NMR spectra were obtained on a Bruker Avance DRX600 spectrometer with residual solvent peaks serving as references (DMSO- d_6 : δ_{H} 2.50, δ_{C} 39.52). High-resolution ESIMS measurements were obtained on a Bruker micrOTOF mass spectrometer by direct infusion in MeCN at 3 mL/min using sodium formate clusters as an internal calibrate. HPLC was performed using an Agilent 1200 Series separation module that was equipped with Agilent 1200 Series diode array and Agilent 1260 Series fraction collector, and Agilent SB-C18 column (250×9.4 mm, $5 \mu\text{m}$).

3.2. Fungal Material

The *Aspergillus fumigatus* strain CUGBMF170049 was isolated from a sediment sample that was collected from the Bohai Sea, China and grown on a potato dextrose agar plate at 28°C . This strain was identified as *Aspergillus fumigatus* based on DNA sequence analysis of its internal transcribed spacer (ITS) region (Figure S16) (GenBank accession number MK453215) using a conventional primer pair of ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3').

3.3. Fermentation and Extraction

A small spoonful of *Aspergillus fumigatus* (CUGBMF170049) spores growing on a potato dextrose agar slant was inoculated into four 250 mL conical flasks, each containing 40 mL of liquid medium consisting of potato infusion (20%), glucose (2.0%), artificial sea salt (3.5%), and distilled water. The flasks were incubated at 28°C for 3 d on a rotary shaker at 160 rpm. An aliquot (5 mL) of the resultant seed culture was inoculated into ten 1 L conical flasks, with each containing solid medium consisting of rice (120 g) and artificial seawater (3.5%; 80 mL), and the flasks were incubated stationary for 30 days at 28°C . The cultures were extracted three times by EtOAc:MeOH (80:20), and the combined extracts were reduced to dryness in vacuo and the residue was partitioned between EtOAc and H_2O . Subsequently, the EtOAc layer was dried in vacuo to yield a dark residue (11.3 g).

3.4. Isolation and Purification

The EtOAc fraction was fractionated by a reduced pressure silica gel chromatography (50×80 mm column, TLC H silica) using a stepwise gradient of 50–100% hexane/ CH_2Cl_2 and then 0–100% MeOH/ CH_2Cl_2 to afford 15 fractions. Fraction C was fractionated on a Sephadex LH-20 column (600×30 mm) while using an isocratic elution of hexane: CH_2Cl_2 :MeOH (5:5:1) to give five subfractions (F1–F5). Subfraction F3 (102.3 mg after drying in vacuo) was further fractionated by HPLC (Agilent Zorbax SB-C18 250×9.4 mm, $5 \mu\text{m}$ column, 2.0 mL/min, isocratic 65% MeOH/ H_2O) to yield FD-838 (**4**; t_{R} 10.4 min, 3.3 mg). Fraction J was fractionated on a Sephadex LH-20 column (600×30 mm) using an isocratic elution of CH_2Cl_2 :MeOH (2:1), to give four subfractions (F1–F4). Subfraction F1 was further fractionated by HPLC (Agilent Zorbax SB-C18 250×9.4 mm, $5 \mu\text{m}$ column, 2.0 mL/min, isocratic 65% MeOH/ H_2O) to yield helvolic acid (**7**, t_{R} 10.8 min, 1.3 mg), 16-*O*-propionyl-16-*O*-deacetylhelvolic acid (**5**, t_{R} 11.9 min, 1.2 mg), and 6-*O*-propionyl-6-*O*-deacetylhelvolic acid (**6**, t_{R} 12.4 min, 1.4 mg). Fraction K was fractionated on a Sephadex LH-20 column (600×30 mm) using an isocratic elution of CH_2Cl_2 :MeOH (2:1) to give five subfractions (F1–F5). Subfraction F3 was further fractionated by HPLC (Agilent Zorbax SB-C18 250×9.4 mm, $5 \mu\text{m}$ column, 2.0 mL/min, isocratic 65% MeOH/ H_2O) to yield 1,2-dihydrohelvolic acid (**8**, t_{R} 13.9 min, 1.6 mg). Fraction L was fractionated on a Sephadex LH-20 column (600×30 mm) using an isocratic elution of CH_2Cl_2 :MeOH (2:1) to give five subfractions (F1–F5). Subfraction F4 was further fractionated by an ODS column, which was eluted by a stepwise gradient (0–100% MeOH/ H_2O) to afford five subfractions (F1–F5). Subfraction F4 was further fractionated by HPLC (Agilent Zorbax SB-C18 250×9.4 mm, $5 \mu\text{m}$ column, 2.0 mL/min, isocratic 65% MeOH/ H_2O) to

yield pseurotin A (**3**, t_R 7.1 min, 3.2 mg), cephalimysins M (**1**, t_R 12.0 min, 1.5 mg), and N (**2**, t_R 8.9 min, 3.6 mg).

3.4.1. Cephalimysin M (**1**)

Pale yellow amorphous powder; $[\alpha]_D^{22}$ -21.3 (MeOH, 0.1); UV (MeOH) λ_{max} ($\log \epsilon$) 196 (4.43), 254 (4.14), 277(3.96) nm; (+)-ESIMS m/z 418.1 $[M + H]^+$; (+)-HRESIMS m/z 440.1684 $[M + Na]^+$ (calcd. for $C_{22}H_{27}NO_7Na$ 440.1680); 1H and ^{13}C NMR data: See Table 1.

3.4.2. Cephalimysin N (**2**)

Pale yellow amorphous powder; $[\alpha]_D^{22}$ -21.5 (MeOH, 0.1); UV (MeOH) 197 (4.43), 252 (4.12), 329(3.56) nm; (+)-ESIMS m/z 428.0 $[M + H]^+$; (+)-HRESIMS m/z 450.1159 $[M + Na]^+$ (calcd. for $C_{22}H_{21}NO_8Na$ 450.1159); 1H and ^{13}C NMR data: See Table 1.

3.5. Antimicrobial Assays

The antimicrobial assays were performed according to the Antimicrobial Susceptibility Testing Standards that were outlined by the Clinical and Laboratory Standards Institute (CLSI) against *S. aureus* ATCC 6538, MRSA ATCC 29213, *E. coli* ATCC 11775, *P. aeruginosa* ATCC 15692, and *C. albicans* ATCC 10231 based on a 96 well microplate format in liquid growth. Briefly, the bacteria from glycerol stocks was inoculated on LB agar plate and cultured overnight at 37 °C. The glycerol stock of *C. albicans* was prepared on Sabouraud dextrose agar at 28 °C for 24 h. Afterwards, single colonies were picked and adjusted to approximately 10^4 CFU/mL with Mueller–Hinton Broth as bacterial suspension and with RPMI 1640 media as fungal suspension. 2 μ L of two-fold serial dilution of each compound (in DMSO) were added to each row on 96-well microplate, containing 78 μ L of bacterial or fungal suspension in each well. (Vancomycin and Ciprofloxacin were used as positive controls; Amphotericin B was used as positive for fungi; DMSO as negative control). The 96-well plate was aerobically incubated at 37 °C for 16 h. The 96-well plate of antifungal was aerobically incubated at 35 °C for 24 h. Here, MIC is defined as the minimum concentration of compound at which no bacterial growth is observed.

3.6. Anti-Bacillus Calmette Guérin (BCG) Assay

The anti-BCG assay was carried out by using a constitutive GFP expression strain (pUV3583c-GFP), according to previous published procedure (isoniazid was used as positive control with MIC value of 0.05 μ g/mL) [19]. The concentrations for the tested compounds were from 0.156 to 10 μ g/mL by using two-fold diluted solutions.

4. Conclusions

As part of our ongoing research program to discover novel secondary metabolites from the marine environment, eight compounds were isolated from the rice solid medium culture of the marine derived fungus CUGBMF170049 isolated from a sediment sample that was collected from the Bohai Sea, China. Two novel compounds (**1** and **2**) were isolated and characterized along with the previously reported analogues pseurotin A (**3**) and FD-838 (**4**), as well as four known helvolic acid derivatives, namely 16-*O*-propionyl-16-*O*-deacetylhelvolic acid (**5**), 6-*O*-propionyl-6-*O*-deacetylhelvolic acid (**6**), helvolic acid (**7**), and 1,2-dihydrohelvolic acid (**8**). All of the structures were confirmed by detailed analysis of the spectroscopic data. Compounds **5**–**7** showed antibacterial activity against both *S. aureus* and MRSA. Analogue **8** did not exhibit antibacterial activities that indicated that the α,β -unsaturated ketone of **5**–**7** is the key functional group for antibacterial activity. Structurally, cephalimysins M (**1**) and N (**2**) belong to a family of rare natural products with diverse biological activities, which contain an unusual spiro-heterocyclic γ -lactam core. To the best of our knowledge, 28 natural products of this family have been reported, including pseurotin A [14], 8-*O*-demethylpseurotin A [20] pseurotins A1 and A2 [18,21], pseurotins B – E [22], pseurotins F1 and F2 [23], 14-norpseurotin A [24] synerazol [25], azaspirene [26],

azaspirofurans A and B [27], and FD-838 and cephalimysins A–L [15,28,29]. **2** is the first cephalimysin analogue where the methyl of C-16 was oxidized to hydroxymethyl. The current research diversifies the structures of this class of natural products.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1660-3397/17/5/289/s1>, Figures S1–S15: The HRESIMS, UV, 1 D, 2D NMR, and CD spectra of compounds **1** and **2**, Figure S16 the phylogenetic tree of strain CUGBMF170049.

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Conflicts of Interest: The authors declare no conflict of interest.

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