Metabolites From the Mangrove-Derived Fungus *Cladosporium* sp. HNWSW-1

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Two new benzoic acids, cladoslide A (**1**) and cladoslide B (**2**); one new β-carboline derivative, cladospomine (**3**); and one new pyridin-2(1*H*)-one, cladoslide C (**4**), were isolated from the fermentation cultures of the mangrove-derived fungus *Cladosporium* sp. HNWSW-1, along with the previously reported *N*-acetyl-β-oxotryptamine (**5**), (4*S*,5*S*,11*R*)-*iso*-cladospolide B (**6**), (4*S*,5*S*,11*S*)-*iso*-cladospolide B (**7**), and (4*R*,5*S*,11*R*)-*iso*-cladospolide B (**8**). Their structures were elucidated by spectroscopic analysis, $Rh_2(OCOCF_3)_4$-induced ECD experiments, and Marfey's method. Compound **1** showed cytotoxicity against the K562 cell line with IC_{50} values of $13.10 \pm 0.08 \mu M$. Moreover, compounds **1** and **5** exhibited inhibitory activity against α-glycosidase with IC_{50} values of 0.32 ± 0.01 mM and 0.17 ± 0.01 mM, respectively.

Keywords: mangrove-derived fungus, *Cladosporium* sp., metabolites, cytotoxicity, α-glycosidase inhibitor

INTRODUCTION

Mangrove-derived fungi are an important resource for structurally and biologically diverse substances for drug discovery, and in recent years, over 100 new molecules derived from mangrove-derived fungi have been discovered every year (Blunt et al., 2018; Carroll et al., 2019; Carroll et al., 2020). The genus *Cladosporium* (Cladosporiaceae) is one of the largest genera of dematiaceous hyphomycetes (Bensch et al., 2015). Many novel bioactive natural products were isolated from *Cladosporium* fungus, such as polyketides (Zhang et al., 2019; Zhu et al., 2018), macrolides (Huang et al., 2019), perylenequinones (Zhang et al., 2020), and indole alkaloids (Peng et al., 2013), which exhibited antimicrobial (Zhang et al., 2019), cytotoxic (Zhu et al., 2018), antiviral (Peng et al., 2013), and quorum-sensing inhibitory activities (Zhang et al., 2020).

As part of our previous research on novel biologically active natural products from mangrove-derived fungi, two novel succinimide-containing derivatives, cladosporitins A and B, have been isolated from the mangrove-derived *Cladosporium* sp. HNWSW-1 (Wang et al., 2019). Our further chemical investigations on this fungus led to the isolation of two new benzoic acids (**1** and **2**), one new β-carboline derivative (**3**), and one new pyridin-2(1*H*)-one (**4**), along with four known compounds (**5–8**) (Martínez-Luis et al., 2012; Reddy et al., 2012; Franck et al., 2001) from the EtOAc extract of its fermentation cultures. We describe the isolation, structure elucidation, and biological activities of these compounds in this article.

MATERIALS AND METHODS

General Experimental Procedures

Silica gel (60–80, 200–300 mesh, Qingdao Marine Chemical Co. Ltd.), ODS gel (20–45 m, Fuji Silysia Chemical Co. Ltd.), and Sephadex LH-20 (Merck, Kenilworth, NJ, United States) were used for column chromatography. Optical rotations were measured on a MCP 5100 modular compact polarimeter (Anton Paar, Austria). ECD spectra were recorded on a Bio-Logic Science MOS-500 spectrometer (Biologic, France). UV spectra were measured on a Beckman DU-640 spectrophotometer (Beckman Coulter, Inc., Brea, CA). IR absorptions were obtained on a Nicolet 380 FT-IR instrument (Thermo, Waltham, MA, United States) using KBr pellets. 1D and 2D NMR spectra were recorded on a Bruker AV III spectrometer (Bruker, United States; ^{1}H NMR at 500 MHz and ^{13}C NMR at 125 MHz for **1–3** and **5**; ^{1}H NMR at 600 MHz and ^{13}C NMR at 150 MHz for **4**, **6–8**; and HMBC spectrum for **3**) using TMS as the internal standard. ESIMS and HRESIMS were recorded with amaZon SL (Bruker, United States) or Compact QqTOF (Bruker, United States). Semipreparative HPLC was carried out using an ODS column and 5PFP column (Cosmosil-pack, 10 × 250 mm, 5 μm, 4 ml/min, Nacalai Tesque).

Fungal Material

The strain of *Cladosporium* sp. HNWSW-1 was isolated from the healthy tree root of *Ceriops tagal*, which was collected from the Dong Zhai Gang Mangrove Reserve in Hainan Province in July 2011 (Wang et al., 2019). The fungus was identified based on the DNA sequences (GenBank access No. MH 535968) of the 18Sr DNA gene (Wang et al., 2019).

Fermentation and Extraction

Cladosporium sp. HNWSW-1 was cultured in PDB (potato liquid media consisting of 200.0 g/L potato, 20.0 g/L glucose, and 1000 ml deionized water) at 28°C and 150 rpm for 72 h. Then, 5 ml seed broth was transferred to 1000 ml Erlenmeyer flasks (60 flasks) each containing rice medium (80.0 g rice, 120.0 ml water, and 120.0 mg tryptophan). The flasks were incubated at room temperature under static conditions for 60 days. The cultures were extracted three times by EtOAc, and the EtOAc solutions were combined and evaporated under reduced pressure to give a dark brown gum (40.0 g). Then, the extracts were dissolved in 90% CH_3OH, and the solution was extracted three times by petroleum ether. The methanol and petroleum ether solutions were evaporated under reduced pressure. The crude methanol extract (20.0 g) was obtained.

Purification and Identification

The crude methanol extract (20.0 g) was fractionated into 12 fractions (Fr.1–Fr.12) on a silica gel VLC column eluted with a gradient elution of CH_2Cl_2-petroleum ether (0–100%) and MeOH-CH_2Cl_2 (0–100%). Fr.6 (2.4 g) was subjected to an Rp-C_{18} silica gel column eluted with a gradient of water-MeOH (10–100%) to give 25 fractions (Fr.6.1–Fr.6.25). Fr.6.6 (535.0 mg) was further chromatographed on the Rp-C_{18} silica gel column using a step gradient with water-MeOH (10–100%) to obtain six fractions (Fr.6.6.1–Fr.6.6.6). Fr.6.6.4 (138.0 mg) was purified by a

TABLE 1 | ^{1}H and ^{13}C NMR data for **1** and **2** (500 and 125 MHz, δ in ppm) in CD_3OD.

No.	1		2	
	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)
1	159.4, C	-	159.3, C	-
2	122.2, C	-	122.2, C	-
3	132.9, CH	7.80, d, (2.1)	132.9, CH	7.78, s
4	123.2, C	-	123.3, C	-
5	130.4 CH	7.75, dd, (8.5, 2.1)	130.4, CH	7.73, d, (8.7)
6	118.2, CH	6.79, d, (8.5)	118.1, CH	6.75, d, (8.7)
7	22.7, CH_2	2.84, m	22.7, CH_2	2.84, m
8	31.7, CH_2	1.85, m	31.7, CH_2	1.84, m
9	77.7, C	-	77.6, C	-
10	36.0, CH_2	2.01, dt, (14.7, 7.4) 1.93, dt, (14.7, 7.9)	35.9, CH_2	2.03, dt, (14.3, 7.8) 1.94, dt, (14.3, 7.9)
11	29.4, CH_2	2.46, t, (7.9)	29.3, CH_2	2.50, t, (7.9)
12	177.4, C	-	175.8, C	-
13	24.0, CH_3	1.30, s	24.0, CH_3	1.29, s
14	170.1, C	-	170.1, C	-
15	-	-	52.2, OCH_3	3.65, s

Sephadex LH-20 column and eluted with MeOH to give three fractions (Fr.6.6.4.1–Fr.6.6.4.3). Fr.6.6.4.1 was submitted to HPLC purification on a 5PFP column eluted with 15% ACN (85% water added to 0.05% trifluoroacetic acid) to yield **7** (11.0 mg, t_R 10.30 min) and **8** (4.7 mg, t_R 13.20 min). Fr.6.10 (34.0 mg) was purified by a Sephadex LH-20 column and eluted with MeOH to yield **1** (4.0 mg). Fr.7 (853.1 mg) was submitted to an RP-C_{18} column and eluted with MeOH-water to give 30 fractions (Fr.7.1–Fr.7.30). Fr.7.5 (110.2 mg) was separated by a Sephadex LH-20 column and eluted with MeOH to give four fractions (Fr.7.5.1–Fr.7.5.4). Fr.7.5.1 (32.0 mg) was submitted to HPLC purification on a 5PFP column eluted with 30% MeOH (70% water added to 0.05% trifluoroacetic acid) to yield **6** (12.0 mg, t_R 23.0 min). Fr.7.12 (45.9 mg) was separated by a Sephadex LH-20 column and eluted with MeOH to give 5 fractions (Fr.7.12.1–Fr.7.12.5). Fr.7.12.5 was submitted to HPLC purification on a 5PFP column eluted with 50% MeOH (50% water added to 0.05% trifluoroacetic acid) to yield **5** (3.8 mg, t_R 27.0 min). Fr.7.13 (36.4 mg) was also separated by a Sephadex LH-20 column and eluted with MeOH to give eight fractions (Fr.7.13.1–Fr.7.13.8). Fr.7.13.6 was submitted to HPLC purification on a 5PFP column eluted with 40% MeOH (60% water added to 0.05% trifluoroacetic acid) to yield **4** (1.0 mg, t_R 27.0 min). Fr.7.18 (34.2 mg) was separated by a Sephadex LH-20 column and eluted with MeOH to give five fractions (Fr.7.18.1–Fr.7.18.5). Fr.7.18.3 was submitted to HPLC purification on a 5PFP column eluted with 50% MeOH (50% water added to 0.05% trifluoroacetic acid) to yield **2** (2.0 mg, t_R 16.9 min). Fr.7.20 was submitted to HPLC purification on a 5PFP column eluted with 30% MeCN (70% water added to 0.05% trifluoroacetic acid) to yield **3** (2.0 mg, t_R 24.6 min).

Characterization of Compounds 1–4

Cladoslide A (**1**): yellow, amorphous powder; $[\alpha]_D^{20}$ +26.5 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 259 (5.39) and 205 (5.42) nm;

TABLE 2 | ^1H and ^{13}C NMR data for **3** (500 and 125 MHz, δ in ppm) in DMSO and **4** (600 and 150 MHz, δ in ppm) in CD_3OD.

No.	3		4	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
1	142.3, C [a]	-	-	-
2	-	-	163.3, C [a]	-
3	131.0, C [a]	-	117.8, CH	6.40, s
4	119.8, CH	9.14, s	153.2, C	-
5	122.8, CH	8.47, d, (7.8)	109.8, CH	6.31, d, (6.4)
6	121.2, CH	7.35, t, (7.8)	137.4, CH	7.53, d, (6.4)
7	129.9, CH	7.64, t, (7.8)	48.4, CH_2	4.03, t, (6.8)
8	113.9, CH	7.85, d, (8.1)	24.2, CH_2	2.03, m
9	-	NH, 12.22, s	30.2, CH_2	2.36, t, (7.3)
10	136.5, C	-	174.9, C	-
11	132.2, C	-	19.8, CH_3	2.25, s
12	120.7, C	-	-	-
13	142.7, C	-	-	-
14	165.2, C	-	-	-
15	-	NH, 9.59, s	-	-
16	50.9, CH	4.69, t, (6.40)	-	-
17	174.6, C	-	-	-
18	40.6, CH_2	1.87, m, 1.77, m	-	-
19	25.0, CH	1.76, m	-	-
20	23.5, CH_3	0.98, d, (6.0)	-	-
21	21.8, CH_3	0.96, d, (5.9)	-	-
22	166.2, C	-	-	-

[a]*Assigned from HMBC and HSQC spectra.*

IR (KBr) ν_{max}: 3247, 2941, 1700, 1610, 1261, and 1161 cm^{-1}; HRESIMS m/z 305.1006 [M + Na]$^+$ (calcd. for $C_{14}H_{18}O_6Na$: 305.0996); ^1H and ^{13}C NMR data (see **Table 1**).

Cladoslide B (**2**): yellow oil; $[\alpha]_D^{20}$ +6 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 259 (5.46) and 205 (5.44) nm; IR (KBr) ν_{max}: 3394, 2937, 1722, 1613, 1260, and 1186 cm^{-1}; HRESIMS m/z 319.1139 [M + Na]$^+$ (calcd. for $C_{15}H_{20}O_6Na$: 319.1152); ^1H and ^{13}C NMR data (see **Table 1**).

Cladospomine (**3**): yellow, amorphous powder; $[\alpha]_D^{20}$ -4 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 362 (4.53), 279 (5.28), and 217 (5.18) nm; IR (KBr) ν_{max}: 3341, 2958, 1721, 1653, 1531, 1366, and 1235 cm^{-1}; HRESIMS m/z 392.1227 [M + Na]$^+$ (calcd. for $C_{19}H_{19}N_3O_5Na$: 392.1217); ^1H and ^{13}C NMR data (see **Table 2**).

Cladoslide C (**4**): yellow oil; UV (MeOH) λ_{max} (log ε) 297 (4.68), 229 (4.72), and 203 (5.08) nm; IR (KBr) ν_{max}: 3421, 2955, 1724, 1656, 1570, and 1196 cm^{-1}; HRESIMS m/z 218.0779 [M + Na]$^+$ (calcd. for $C_{10}H_{13}NO_3Na$: 218.0788); ^1H and ^{13}C NMR data (see **Table 2**).

$Rh_2(OCOCF_3)_4$-Induced ECD Experiments of **1** and **2**

The samples of compounds **1** and **2** (0.1 mg) were dissolved in a dry solution of the stock [$Rh_2(OCOCF_3)_4$] complex (1.5 mg) in CH_2Cl_2 (1 ml). The first induced ECD spectra of the compounds were recorded immediately after mixing, and their time evolution was monitored until stationary (about 10 min after mixing) (Frelek and Szczepek, 1999). The inherent ECD spectra were subtracted. The absolute configurations of the C-9 tertiary alcohol in **1** and **2** were identified by the observed sign of the E-band at ca. 350 nm in the induced ECD spectra (Gerards and Snatzke, 1990; Frelek and Szczepek, 1999).

Preparation of FDAA Derivatives of the Acid Hydrolysate of 3 and the Derivatives of Two Authentic Leucine Samples (L- and D-) and Marfey's Analysis

Compound **3** (1.0 mg, 2.71 μmol) was dissolved in 6 M HCl (1 ml) in a sealed tube, and the mixture was heated at 105°C for 11 h. Then, the solution was cooled and evaporated to dryness. The residue was dissolved in H_2O (250 μl). Meanwhile, L-Leu and D-Leu were also dissolved in H_2O (50 mM each), and 50 μl of each solution was treated with 200 μl of 1% FDAA in acetone followed by 1.0 M $NaHCO_3$ (40 μl). The reaction was maintained for 1 h at 45°C and then quenched by the addition of 2.0 M HCl (10 μl). The corresponding FDAA derivatives of the hydrolysate of **3**, L- Leu, and D-Leu were analyzed by HPLC on an ODS column maintained at 30 °C using the following programs: solvent A, H_2O + 0.1% TFA; solvent B, MeCN; linear gradient, 0 min 25% B (75% A), 40 min 60% B (40% A), and 45 min 100% B; UV detection at 340 nm. The retention times for the FDAA derivatives of the hydrolysate of **3**, L-Leu, and D-Leu were 24.28, 24.28, and 28.59 min, respectively (Marfey, 1984).

TABLE 3 | IC_{50} values of cytotoxicity and α-glycosidase inhibitory activity of compounds **1–3** and **5–8**.

Compounds	IC_{50} (μM)				IC_{50} (mM)
	Hela	BEL-7042	K562	SGC-7901	α-Glycosidase
1	>100	>100	13.10 ± 0.08	>100	0.32 ± 0.01
2	>100	>100	>100	>100	>1.0
3	>100	>100	>100	>100	>1.0
5	>100	>100	>100	>100	0.17 ± 0.01
6	>100	>100	>100	>100	>1.0
7	>100	>100	>100	>100	>1.0
8	>100	>100	>100	>100	>1.0
Adriamycin	0.28 ± 0.01	0.47 ± 0.01	0.10 ± 0.01	0.22 ± 0.01	ND[a]
Acarbose	ND[a]	ND[a]	ND[a]	ND[a]	0.72 ± 0.01

[a]*Not detected.*

Bioassay for Cytotoxicity

The cytotoxic activity of compounds **1–3** and **5–8** against human cervical cancer cell lines (Hela), human hepatic carcinoma cell lines (BEL-7402), leukemia cell lines (K562), and human gastric cell lines (SGC-7901) was assayed by the MTT method (Mosmann, 1983; Wang et al., 2013). These cell lines were purchased from Shang Hai Cell Bank of Chinese Academy of Sciences. Hela, BEL-7402, K562, and SGC-7901 cell lines were cultured in RPMI-1640 with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37°C, and 198 μl of the cell suspension was plated in 96-well microtiter plates. After being incubated for 24 h, 2 μl of the test solutions in DMSO was added to each well and further incubated for 36 h. The MTT solution (20 μl, 5 mg/ml in IPMI-1640 medium) was then added to each well and further incubated for 4 h. Finally, the medium containing MTT (150 μl) was gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a Multiskan FC photometric microplate reader (Thermo Fisher Scientific) at 570 nm. Adriamycin was used as the positive control drug.

Bioassay for α-Glycosidase Inhibitory Activity

α-Glucosidase inhibitory activity of compounds **1–3** and **5-8** was evaluated according to the literature experimental method (Ma et al., 2014). A mixture including 25 μl of different compounds (final concentrations of 0.0625, 0.125, 0.25, 0.5, and 1.0 mM), 25 μl of α-glucosidase (0.2 U/ml, from baker's yeast, Sigma), and 175 μl phosphate buffer (pH 6.8) was left to stand for 10 min at room temperature in a 96-well plate, and then 25 μl of 23.2 mM *p*-nitrophenyl *α*-D-glucopyranoside (Sigma-Aldrich) was added and further incubated at 37°C for 15 min. Finally, the absorbance was measured at 405 nm to determine the amount of *p*-nitrophenol cleaved by the enzyme using a Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Inc.). The control was prepared by adding phosphate buffer instead of the sample in the same way as the test. The blank was prepared by adding phosphate buffer instead of α-glucosidase using the same method. The inhibition rates (%) = [($OD_{control}$ − $OD_{control\ blank}$) − (OD_{sample} − $OD_{sample\ blank}$)]/($OD_{control}$ − $OD_{control\ blank}$) × 100%. Acarbose was used as the positive control with an IC_{50} value of 0.72 ± 0.01 mM.

RESULTS AND DISCUSSION

Identification of Compounds 1–4

Compound **1** was isolated as a yellow amorphous powder with the molecular formula of $C_{14}H_{18}O_6$ established by HRESIMS [*m/z* 305.1006 (M + Na)$^+$]. The analysis of its ^1H, ^{13}C, and HSQC NMR spectra (see **Supplementary Figures S1–S3** in the Supplementary Material) revealed the presence of six aromatic carbons (three of which were protonated), four methylene groups, one methyl group ($\delta_{C/H}$ 24.0/1.30), one oxygenated quaternary carbon (δ_C 77.7), and two carboxylic carbons (δ_C 177.4 and 170.1). These data combined with the molecular formula
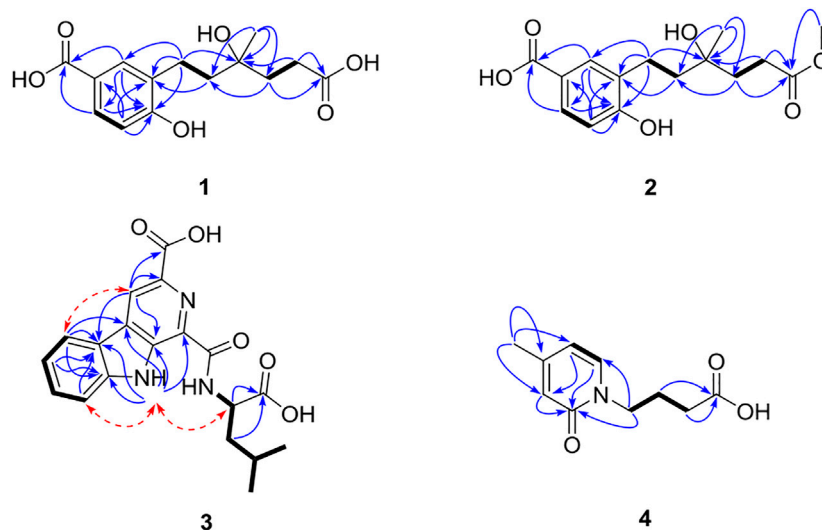
suggested four unobserved exchangeable protons, which plus the above data accounted for all the ^1H and ^{13}C NMR resonances for **1**. The hydroxyl groups were located at C-1, C-9, C-12, and C-14 by default supported by the chemical shift values for C-1 (δ_C 159.4), C-9 (δ_C 77.7), C-12 (δ_C 177.4), and C-14 (δ_C 170.1). The ^1H–^1H coupling patterns for the three aromatic protons, H-3 at δ_H 7.80 (1H, d, *J* = 2.1 Hz), H-5 at δ_H 7.75 (1H, dd, *J* = 8.5, 2.1 Hz), and H-6 at δ_H 6.79 (1H, d, *J* = 8.5 Hz), suggested the presence of a 1,2,4-trisubstituted benzene ring, which was confirmed by relevant ^1H–^1H COSY and HMBC correlations (**Figure 2**). The HMBC correlation from H-3 and H-5 to C-14 suggested a carboxylic carbon C-14 attached to C-4 (δ_C123.2) directly. The COSY correlations (in **Figure 2**) from H$_2$-7 (δ_H 2.84) to H$_2$-8 (δ_H 1.85) and from H$_2$-10 (δ_H 2.01, 1.93) to H$_2$-11 (δ_H 2.46), along with the key HMBC correlations (in **Figure 2**) from H$_2$-10 to C-8 (δ_C 31.7), from H$_2$-10 and H$_2$-11 to C-12, and from H$_3$-13 (δ_H 1.30) to C-8, C-9, and C-10 (δ_C 36.0), indicated a 4-hydroxy-4-methylhexanoic acid fragment. The above two fragments were connected by the key HMBC correlations from H$_2$-8 to C-2 (δ_C 122.2) and from H$_2$-7 to C-1 and C-3 (δ_C 132.9). Thus, the planar structure of **1** was determined as shown in **Figure 1**, and it was named as cladoslide A. An Rh$_2$(OCOCF$_3$)$_4$-induced electronic circular dichrosim (ECD) experiment (Frelek and Szczepek, 1999) was conducted in order to determine the absolute configuration of the C-9 chiral tertiary alcohol. The induced negative Cotton effect at approximately 350 nm (**Figure 3**) suggested the 9*S* configuration of **1** based on the bulkiness rule (Gerards and Snatzke, 1990; Frelek and Szczepek, 1999).

Compound **2** was isolated as a yellow oil, whose molecular formula was determined as $C_{15}H_{20}O_6$ by HRESIMS [*m/z* 319.1139 (M + Na)$^+$]. A detailed comparison of 1D NMR data of **2** (**Table 1**) with those of **1** indicated that **2** has a very similar chemical structure to that of **1**. The only difference between them was that the carboxyl group attached to C-11 in **1** was replaced by a carbomethoxy group in **2**, as evidenced by the presence of a methoxyl group ($\delta_{C/H}$ 52.2/3.65) in ^1H and ^{13}C NMR spectra of **2** and their difference of the molecular formula, along with the key HMBC correlations (in **Figure 2**) from H-10 (δ_H 2.03/1.94), H-11(δ_H 2.50), and H$_3$-15 (δ_H 3.65) to C-12 (δ_C 175.8). Therefore, the planar chemical structure of **2** was elucidated as shown in **Figure 1** and named as cladoslide B. The 9*S* configuration of **2** was also determined according to an induced negative Cotton effect at approximately 350 nm (**Figure 3**) by an Rh$_2$(OCOCF$_3$)$_4$-induced ECD experiment (Gerards and Snatzke, 1990; Frelek and Szczepek, 1999).

Compound **3** was obtained as a yellow amorphous powder, and it displayed a positive response toward *Dragendorff*'s reagent. Its molecular formula was assigned as $C_{19}H_{19}N_3O_5$ by HRESIMS [*m/z* 392.1227 (M + Na)$^+$]. The UV spectrum of **3** displayed the characteristic absorption maxima of β-carboline chromophore at 362, 278, and 217 nm (Chen et al., 2010; Cao et al., 2012). Interpretation of the ^1H NMR, ^{13}C NMR, and HSQC spectroscopic data of **3** (**Table 2**) displayed resonances for two exchangeable protons (δ_H 12.2 and 9.59), eleven aromatic carbons (five of which were protonated), two methyl groups ($\delta_{C/H}$ 23.5/0.98 and $\delta_{C/H}$ 21.8/0.96), one methylene group ($\delta_{C/H}$ 40.69/1.87, 1.77), two sp^3 methine groups (one of which was heteroatom-bonded at $\delta_{H/C}$ 4.69/50.9), and three carboxyl or amide carbonyls (δ_C 174.6, 166.2,
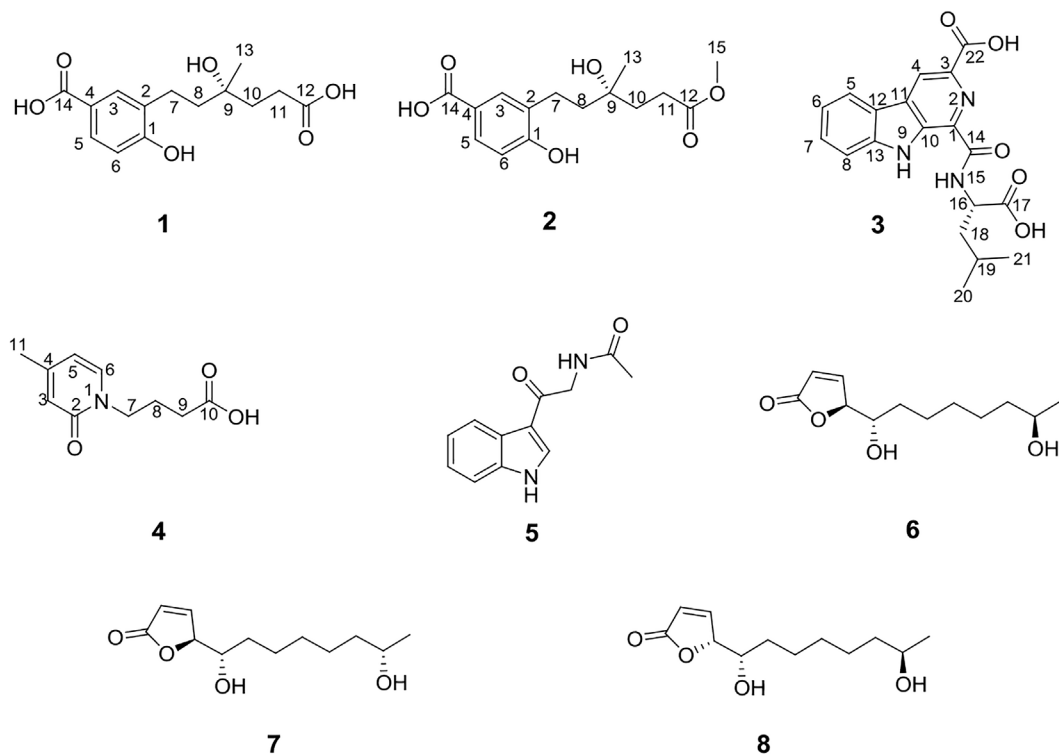
FIGURE 1 | Structures of compounds **1–8**.

FIGURE 2 | Key HMBC (→), ^{1}H–^{1}H COSY (−), and ROESY (← - →) correlations of compounds **1–4**.

and 165.2). These data and the two unobserved exchangeable protons accounted for all the ^{1}H and ^{13}C NMR resonances for **3**. The ^{1}H NMR spectra of **3** exhibited vicinally coupled aromatic proton signals at δ_H 8.47 (1H, d, J = 7.8 Hz, H-5), 7.85 (1H, d, J = 8.1 Hz, H-8), 7.64 (1H, t, J = 7.8 Hz, H-7), and 7.35 (1H, t, J = 7.8 Hz, H-6), which combined with the sequential COSY correlations of H-5/H-6/H-7/H-8 were indicative of a 1,2-disubstituted benzene ring of β-carboline alkaloid (Chen et al., 2010; Cao et al., 2012). A downfield aromatic proton singlet at δ_H 9.14 (s) was assigned as the characteristic H-4
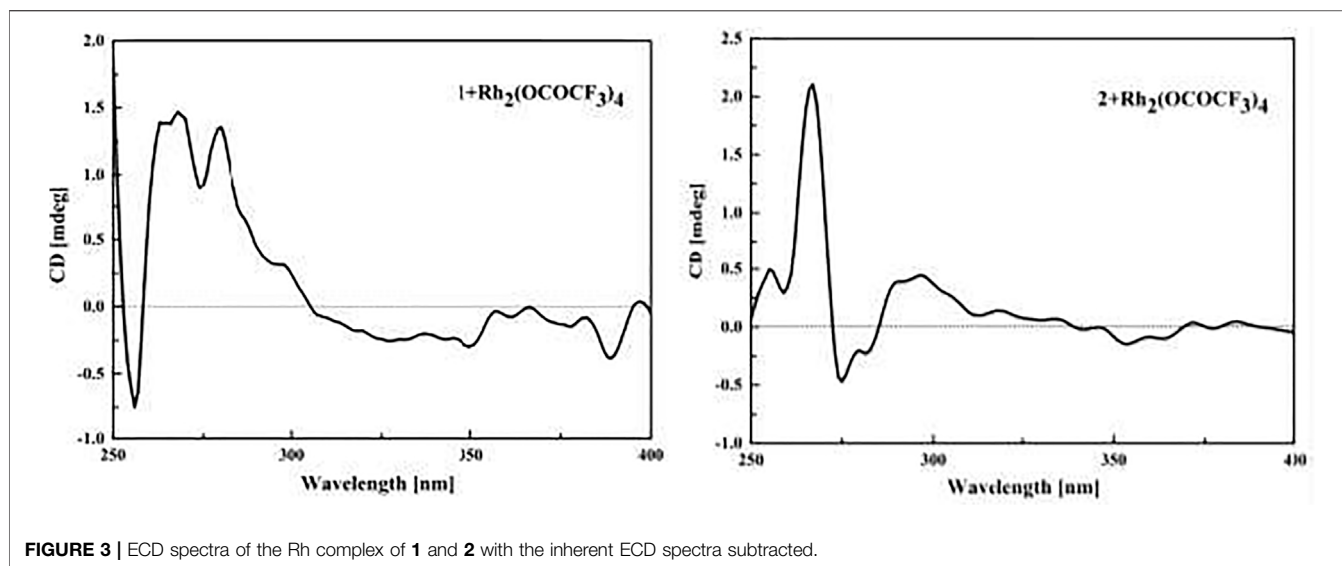
**FIGURE 3** | ECD spectra of the Rh complex of **1** and **2** with the inherent ECD spectra subtracted.

proton signal of the β-carboline alkaloid, evidenced by its ROESY correlation with H-5. The above signals, together with HMBC correlations from H-4 to C-10 (δ_C 136.5) and C-12 (δ_C 120.7), from H-5 to C-11 (δ_C 132.2) and C-12, from H-6 and H-8 to C-12, from H-5 and H-7 to C-13 (δ_C 142.7), and from 9-NH (δ_H 12.22) to C-1 (δ_C 142.3), C-10, C-11, and C-12, suggested the presence of a β-carboline skeleton (Chen et al., 2010; Cao et al., 2012). A comparison of the ^1H and ^{13}C NMR data for **3** (**Table 2**) with those of the previously reported dichotomine H (Cao et al., 2012) suggested that **3** has a very similar chemical structure to that of dichotomine H (Cao et al., 2012). The main structural difference between them was that the glutamic acid unit in dichotomine H was replaced by leucine in **3**, as evidenced by the sequential COSY correlations of H-15 (NH, δ_H 9.59)/H-16 (δ_H 4.69)/H$_2$-18 (δ_H 1.87, 1.77)/H-19 (δ_H 1.76), H-20 (δ_H 0.98)/H-19, and H-21 (δ_H 0.96)/H-19, together with HMBC correlations from H$_2$-18 and H-16 to C-17 (δ_C 174.6). Moreover, the leucine unit was connected to C-14 (δ_C 165.2) rather than C-22 (δ_C 166.2) on the basis of the obvious ROESY correlations between 9-NH (δ_H 12.22) and H-16 (δ_H 4.69). A carboxyl group (C-22) was attached to C-3 (δ_C 131.0) in **3** according to the key HMBC correlation from H-4 to C-3 and C-22 combined with the molecular formula. The absolute configuration of the leucine was identified as L-leucine by Marfey's method (Marfey, 1984). The mixture obtained after hydrolyzing compound **3** and further derivatization with L-FDAA was analyzed by HPLC-DAD. The derivatives of two authentic leucine samples (L- and D-) were also prepared and analyzed by HPLC-DAD (see **Supplementary Figure S26** in Supplementary Material). The chromatogram of the derivative of **3** displayed the peak with the retention time (t_R 24.28 min), which was consistent with the retention time and the UV spectra obtained for the derivative of L-Leu (t_R 24.28 min) and different from the retention time obtained for the derivative of D-Leu (t_R 28.59 min). Finally, the leucine moiety in **3** was unambiguously identified as L-Leu, and the

structure of compound **3** was elucidated as shown in **Figure 1**, which was named cladospomine.

Compound **4** was obtained as a yellow oil and possessed a molecular formula $C_{10}H_{13}NO_3$ based on a prominent sodium adduct ion peak at *m/z* 218.0779 [M + Na]$^+$ in the HRESIMS spectrum. Its ^1H, DEPTQ, and HSQC NMR spectra (see **Supplementary Figures S20–S22** in Supplementary Material) showed signals for three sp^2 methine groups ($\delta_{C/H}$ 109.8/6.31, $\delta_{C/H}$ 137.4/7.53, and $\delta_{C/H}$ 117.8/6.40), three methylene groups ($\delta_{C/H}$ 48.4/4.03, $\delta_{C/H}$ 24.2/2.03, and $\delta_{C/H}$ 30.2/2.36), one methyl group ($\delta_{C/H}$ 19.8/2.25), one amide carbonyl signal (δ_C 163.3), one carboxyl carbonyl signal (δ_C 174.9), and one sp^2 quaternary carbon signal (δ_C 153.2). The sequential COSY correlations from H$_2$-7 (δ_H 4.03) through H$_2$-9 (δ_H 2.36) combined with the HMBC correlation from H$_2$-8 and H$_2$-9 to C-10 (δ_C 174.9) indicated a butyric acid fragment. The COSY correlations from H-5 (δ_H 6.31) to H-6 (δ_H 7.53), together with the HMBC correlations from H$_3$-11 (δ_H 2.25) to C-3 (δ_C 117.8), C-4 (δ_C 153.2), and C-5 (δ_C 109.8), from H-3 (δ_H 6.40) and H-6 to C-2 (δ_C 163.3), and from H-5 to C-3 displayed a 4-methylpyridin-2(1*H*)-one fragment. Finally, the key HMBC correlations from H$_2$-7 to C-2 and C-6 (δ_C 137.4) connected above two fragments. Thus, the chemical structure of compound **4** was identified as shown in **Figure 1** and named as cladoslide C.

The chemical structures of the previously reported *N*-acetyl-β-oxotryptamine (**5**) (Martínez-Luis et al., 2012), (4*S*,5*S*,11*R*)-*iso*-cladospolide B (**6**) (Reddy et al., 2012), (4*S*,5*S*,11*S*)-*iso*-cladospolide B (**7**) (Reddy et al., 2012), and (4*R*,5*S*,11*R*)-*iso*-cladospolide B (**8**) (Franck, et al., 2001) were identified by comparison of their spectroscopic data (see **Supplementary Tables S1, S2** in Supplementary Material) with those in the literature.

Compounds **1** and **2** were postulated to be produced biogenetically from the polyketide pathway. Condensation and redox reaction between one malony CoA unit and five acetyl-CoA units formed intermediate (**A**), which further underwent methylation to form compound **1**. Then, **1** underwent esterification to afford **2** (**Figure 4**).

FIGURE 4 | Hypothetical biogenetic pathway of compounds **1** and **2**.
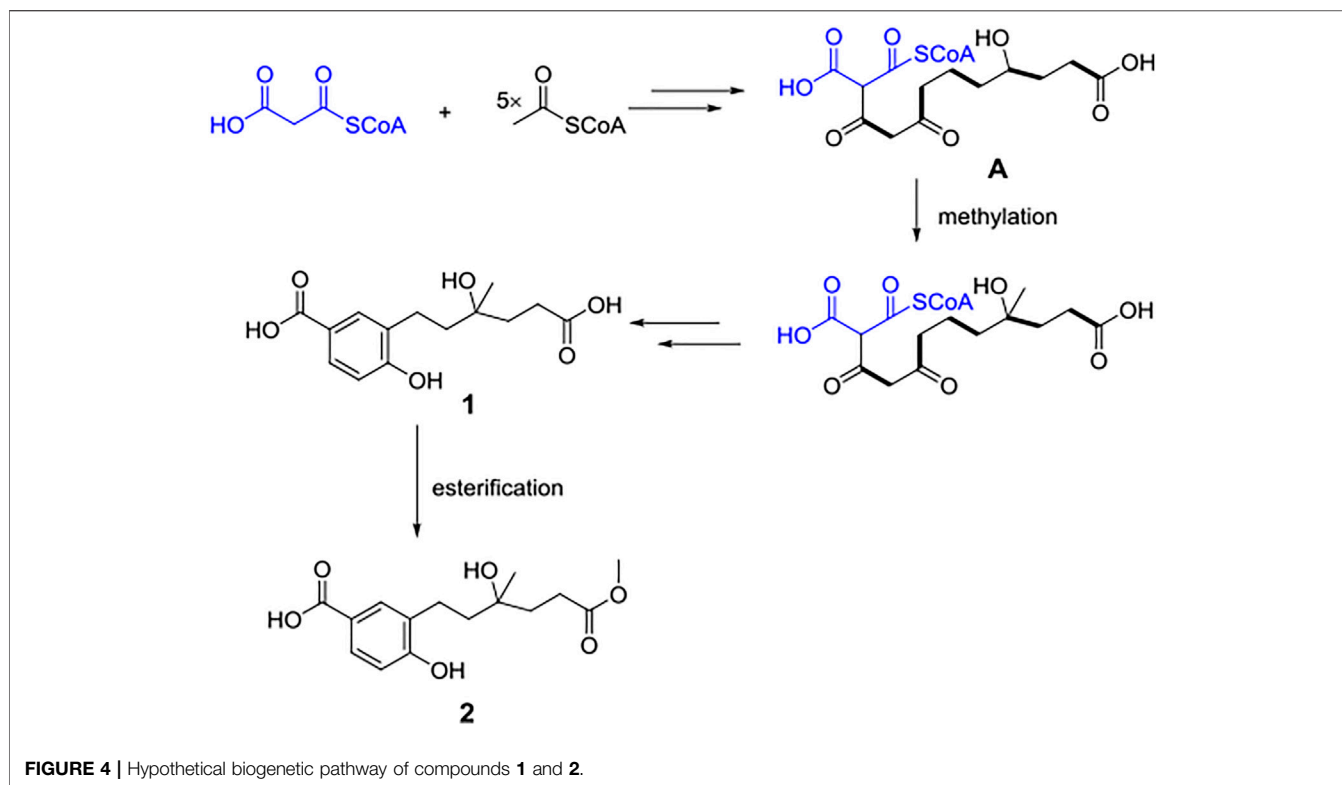
Biological Activity

Compounds **1–3** and **5–8** were tested for their cytotoxicity against Hela, BEL-7402, K562, and SGC-7901 cell lines and α-glycosidase inhibitory activity (**Table 3**). Compound **1** showed cytotoxicity against the K562 cell line with an IC_{50} value of $13.10 \pm 0.08\,\mu M$. Besides, compounds **1** and **5** exhibited inhibitory activity against α-glycosidase with IC_{50} values of $0.32 \pm 0.01\,mM$ and $0.17 \pm 0.01\,mM$, respectively.

CONCLUSION

In conclusion, four new compounds (**1–4**) were isolated from the rice medium culture of the mangrove-derived fungus *Cladosporium* sp. HNWSW-1, along with four previously reported *N*-acetyl-β-oxotryptamine (**5**), (4*S*,5*S*,11*R*)-*iso*-cladospolide B (**6**), (4*S*,5*S*,11*S*)-*iso*-cladospolide B (**7**), and (4*R*,5*S*,11*R*)-*iso*-cladospolide B (**8**). Compound **1** showed cytotoxicity against the K562 cell line with an IC_{50} value of $13.10 \pm 0.08\,\mu M$. Moreover, compounds **1** and **5** exhibited inhibitory activity against α-glycosidase with IC_{50} values of $0.32 \pm 0.01\,mM$ and $0.17 \pm 0.01\,mM$, respectively.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

PW, WM, and LG conceived and designed the experiments. XC, PW, CC, CG, and JY performed the experiments; PW and XC identified the structures of the compounds. PW and XC wrote the paper. WM, HD and FK reviewed the paper. All authors have approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2021.773703/full#supplementary-material

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