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

Xi Cao¹²†, Lei Guo²†, Caihong Cai¹, Fandong Kong³, Jingzhe Yuan¹, Cuijuan Gai¹, Haofu Dai¹, Pei Wang¹* and Wenli Mei¹*

¹Hainan Key Laboratory for Research and Development of Natural Products From Li Folk Medicine, Hainan Institute for Tropical Agricultural Resources, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, China, ²Jiangsu Key Laboratory of Marine Biosoressources and Environment, Co-Innovation Center of Jiangsu Marine Bio-Industry Technology, Jiangsu Ocean University, Liaoyang, China, ³Key Laboratory of Chemistry and Engineering of Forest Products, State Ethnic Affairs Commission, Guangxi Key Laboratory of Chemistry and Engineering of Forest Products, Guangxi Collaborative Innovation Center for Chemistry and Engineering of Forest Products, School of Chemistry and Chemical Engineering, Guangxi University for Nationalities, Nanning, China

Two new benzoic acids, cladoslide A (1) and cladoslide B (2); one new β-carboline derivative, cladospomine (3); and one new pyridin-2(1H)-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), (4S,5S,11R)-iso-cladospolide B (6), (4S,5S,11S)-iso-cladospolide B (7), and (4R,5S,11R)-iso-cladospolide B (8). Their structures were elucidated by spectroscopic analysis, Rh₂(OOCF₃)₄-induced ECD experiments, and Marfey’s method. Compound 1 showed cytotoxicity against the K562 cell line with IC₅₀ values of 13.10 ± 0.08 μM. Moreover, compounds 1 and 5 exhibited inhibitory activity against α-glycosidase with IC₅₀ 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 bioactively 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(1H)-one (4), along with four known compounds (5–8) (Martinez-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 µm, Fuji Silysa 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 instrument (Thermo, Waltham, MA, United States) using KBr or DMSO-d6 as the internal standard. ESIMS and HRESIMS were recorded using an ODS column and 5PFP column (Cosmosil-pack, 10 µm, 4.6 × 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% CH3OH, 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 CH3Cl2–petroleum ether (0–100%) and MeOH–CH2Cl2 (0–100%). Fr.6 (2.4 g) was subjected to an RP-C18 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-C18 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 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, tR 10.30 min) and 8 (4.7 mg, tR 13.20 min). Fr.6.6.4.1 was purified by a Sephadex LH-20 column and eluted with MeOH to yield 7 (4.0 mg). Fr.7 (853.1 mg) was submitted to an RP-C18 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 5 (2.0 mg, tR 16.9 min). Fr.7.5.1 was separated by a Sephadex LH-20 column and eluted with MeOH-water to give five fractions (Fr.7.5.2.1–Fr.7.5.2.5). Fr.7.5.2.1 was submitted to HPLC purification on a 5PFP column eluted with 50% MeOH (50% water added to 0.05% trifluoroacetic acid) to yield 6 (2.0 mg, tR 23.0 min). Fr.7.5.2.1 was separated by a Sephadex LH-20 column and eluted with MeOH to give fractions (Fr.7.5.2.2–Fr.7.5.2.5). Fr.7.5.2.2 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, tR 27.0 min). Fr.7.5.2.2 was separated by a Sephadex LH-20 column and eluted with MeOH-water to give eight fractions (Fr.7.5.2.3–Fr.7.5.2.8). Fr.7.5.2.3 was submitted to HPLC purification on a 5PFP column eluted with 40% MeOH (60% water added to 0.05% trifluoroacetic acid) to yield 3 (2.0 mg, tR 24.6 min).

Characterization of Compounds 1–4
Cladoslide A (1): yellow, amorphous powder; [α]20D +26.5 (c 0.2, MeOH); UV (MeOH) λmax (log ε) 259 (5.39) and 205 (5.42) nm;

| No. | δC | δH, mult. (J in Hz) | δC | δH, 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, CH2 | 2.84, m | 22.7, CH2 | 2.84, m |
| 8   | 31.7, CH2 | 1.85, m | 31.7, CH2 | 1.84, m |
| 9   | 77.7, C | - | 77.6, C | - |
| 10  | 36.0, CH2 | 2.01, dt, (14.7, 7.4) | 35.9, CH2 | 2.03, dt, (14.3, 7.8) |
|     |       | 1.93, dt, (14.7, 7.9) |       | 1.94, dt, (14.3, 7.9) |
| 11  | 29.4, CH2 | 2.46, t, (7.9) | 29.3, CH2 | 2.50, t, (7.9) |
| 12  | 177.4, C | - | 175.8, C | - |
| 13  | 24.0, CH2 | 1.30, s | 24.0, CH2 | 1.29, s |
| 14  | 170.1, C | - | 170.1, C | - |
| 15  | - | - | 52.2, OCH3 | 3.65, s |
Cladoslide C (4): yellow oil; UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)) 297 (4.68), 229 (4.72), and 203 (5.08) nm; IR (KBr) \( \nu_{\text{max}} \) 3421, 2955, 1724, 1656, 1570, and 1196 cm\(^{-1}\); HRESIMS \( m/z \) 218.0779 [M + Na]\(^+\) (calcld. for C\(_{10}\)H\(_{13}\)NO\(_3\)Na: 218.0788); \(^1\)H 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\(_2\)Cl\(_2\) (1 ml). The first induced ECD spectra of the compounds were recorded immediately after mixing, and the time evolution was monitored until stationary (about 10 min after mixing) (Frelek and Szczepk, 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 Szczepk, 1999).

### Preparation of FDAA Derivatives of the Acid Hydrolysate of 3 and the Derivatives of Two Authentic Leucine Samples (\(\alpha\)- and \(\delta\)-) and Marfrey’s Analysis

Compound 3 (1.0 mg, 2.71 \(\mu\)mol) 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\(_2\)O (250 \(\mu\)l). Meanwhile, L-Leu and D-Leu were also dissolved in H\(_2\)O (50 mM each), and 50 \(\mu\)l of each solution was treated with 200 \(\mu\)l of 1% FDAA in acetone followed by 2.0 M HCl (10 \(\mu\)l). The reaction was maintained for 1 h at 45°C and then quenched by the addition of 2.0 M HCl (10 \(\mu\)l). 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\(_2\)O + 0.1% TFA; solvent B, MeCN; linear gradient, 0 min 20% B, 40 min 50% B, and 45 min 100% B; UV detection at 205 (5.44) nm; IR (KBr) \(\nu_{\text{max}} \) 3341, 2958, 1721, 1653, 1531, 1366, and 1235 cm\(^{-1}\); HRESIMS \( m/z \) 392.1227 [M + Na]\(^+\) (calcld. for C\(_{15}\)H\(_{19}\)N\(_3\)O\(_5\)Na: 392.1217); \(^1\)H and \(^{13}\)C NMR data (see Table 2).

### Table 3 | IC\(_{50}\) values of cytotoxicity and \(\alpha\)-glycosidase inhibitory activity of compounds 1-3 and 5-8.

| Compounds | Hela IC\(_{50}\) (\(\mu\)M) | BEL-7042 IC\(_{50}\) (\(\mu\)M) | K562 IC\(_{50}\) (\(\mu\)M) | SGC-7901 IC\(_{50}\) (\(\mu\)M) | \(\alpha\)-Glycosidase IC\(_{50}\) (mM) |
|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1         | >100            | >100            | >100            | >100            | 0.32 ± 0.01     |
| 2         | >100            | >100            | >100            | >100            | >1.0            |
| 3         | >100            | >100            | >100            | >100            | >1.0 ± 0.01     |
| 5         | >100            | >100            | >100            | >100            | >10.0           |
| 6         | >100            | >100            | >100            | >100            | >1.0            |
| 7         | >100            | >100            | >100            | >100            | >1.0 ± 0.01     |
| 8         | >100            | >100            | >100            | >100            | >1.0 ± 0.01     |
| 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 Shanghai 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% CO₂ 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 enzyme. The inhibitor was prepared by adding phosphate buffer instead of α-glucosidase using the same method. The inhibition rates (% = [(ODcontrol - ODcontrol blank) - (ODsample - ODsample blank)]/(ODcontrol - ODcontrol blank) × 100%. Acarbose was used as the positive control with an IC₅₀ 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 C₁₉H₁₉N₅O₅ established 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 carboxylic group attached to C-11 in 1 was replaced by a carbomethoxy group in 2, as evidenced by the presence of a methoxyl group (δ₁H 3.65) and a 4-hydroxy-4-methylhexanoic acid fragment. The above two fragments were connected by the key HMBC correlations from H₂-8 to C-7 (δC 86.3), and from H₂-7 to C-1 and C-3 (δC 132.9). Thus, the planar structure of 1 was determined as shown in Figure 1, and it was named as cladoslide A. An Rh₂(OCOF₃)₄-induced electronic circular dichrosim (ECD) experiment (Frelke and Szczep, 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 9S configuration of 1 based on the bulkiness rule (Gerards and Snatzke, 1990; Frelke and Szczep, 1999).

Compound 2 was isolated as a yellow oil, whose molecular formula was determined as C₁₉H₁₈O₅ 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 (δ₁H 3.65) and a 4-hydroxy-4-methylhexanoic acid fragment. The above two fragments were connected by the key HMBC correlations from H₂-8 to C-7 (δC 86.3), and from H₂-7 to C-1 and C-3 (δC 132.9). Thus, the planar structure of 2 was elucidated as shown in Figure 1 and named as cladoslide B. The 9S configuration of 2 was also determined according to an induced negative Cotton effect at approximately 350 nm (Figure 3) by an Rh₂(OCOF₃)₄-induced ECD experiment (Gerards and Snatzke, 1990; Frelke and Szczep, 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 C₁₉H₁₉N₅O₅ 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, ¹³C NMR, and HMQC spectroscopic data of 3 (Table 2) displayed resonances for two exchangeable protons (δ₁H 12.2 and 9.59), eleven aromatic carbons (five of which were protonated), two methyl groups (δ₁C 23.5/0.98 and δ₁C 21.8/0.96), one methylene group (δ₁C 40.69/1.87, 1.77), two sp³ methine groups (one of which was heteroatom-bonded at δ₁C 4.69/50.9), and three carboxyl or amide carbonyls (δ₁C 174.6, 166.2,
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 $\delta_{\text{H}}$ 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 $\delta_{\text{H}}$ 9.14 (s) was assigned as the characteristic H-4
The 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 13C 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)/H2-18 (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 H2-18 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 deprotection of compound 3 and further derivatization with 1-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 (δ_C/H 109.8/6.31, 137.4/7.53, and 132.2/6.40), three methylene groups (δ_C/H 48.4/4.03, 24.2/2.03, and 30.2/2.36), one methyl group (δ_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 H2-7 (δ_H 4.03) through H2-9 (δ_H 2.36) combined with the HMBC correlation from H2-8 and H2-9 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 H2-11 (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(1H)-one fragment. Finally, the key HMBC correlations from H2-7 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), (4S,5S,11R)-iso-cladospolide B (6) (Reddy et al., 2012), (4S,5S,11S)-iso-cladospolide B (7) (Reddy et al., 2012), and (4R,5S,11R)-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 malonyl 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).
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\textsubscript{50} value of 13.10 ± 0.08 μM. Besides, compounds 1 and 5 exhibited inhibitory activity against α-glycosidase with IC\textsubscript{50} values of 0.32 ± 0.01 mM and 0.17 ± 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), (4S,5S,11R)-iso-cladospolide B (6), (4S,5S,11S)-iso-cladospolide B (7), and (4R,5S,11R)-iso-cladospolide B (8). Compound 1 showed cytotoxicity against the K562 cell line with an IC\textsubscript{50} value of 13.10 ± 0.08 μM. Moreover, compounds 1 and 5 exhibited inhibitory activity against α-glycosidase with IC\textsubscript{50} values of 0.32 ± 0.01 mM and 0.17 ± 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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