Secondary Metabolites of the Coral-Associated Fungus Cladosporium sp. SCSIO41206 from the Beibu Gulf

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Abstract

This study employs the OSMAC strategy to mine and investigate structurally novel compounds from the secondary metabolites of a coral-associated fungus, Cladosporium sp. SCSIO41206, isolated from the Beibu Gulf. Various chromatographic techniques were utilized for the isolation and purification of the secondary metabolites of this fungus, and NMR, MS, and comparison with relevant literature data were employed for structural elucidation of the compounds. The results indicate that this fungus produced a more abundant profile of metabolites when statically cultured at 26°C for 30 days using potato dextrose broth (PDB) liquid medium. Through isolation and identification, twelve known monomeric compounds were obtained, namely 3-isobutylhexahydropyrrolo(1,2-a)pyrazine-1,4-dione (1), 3-benzyl-7-hydroxyhexahydropyrrolo(1,2-a)pyrazine-1,4-dione (2), cyclo(L-alanine-L-4-hydroxy-proline)dipeptide (3), cyclo(L-proline-glycine)dipeptide (4), cyclo(D-Pro-D-Ile) (5), cyclo(L-phenylalanine-glycine)dipeptide (6), cyclo(4-S-hydroxy-R-proline-R-isoleucine) (7), N-phenethylacetamide (8), N-hydroxy-2-(hydroxyimino)-4-methylpentanamide (9), quinolactacin A1 (10), quinolactacin A2 (11), and dibutyl terephthalate (12). Compounds 1–12 were all isolated for the first time from the genus Cladosporium derived from Beibu Gulf corals. Compounds 2, 3, 4, 8, 10, 11, and 12 exhibited weak inhibitory activity against acetylcholinesterase.

Full Text

Preamble

Study on the Secondary Metabolites of the Coral-Associated Fungus *Cladosporium sp.* SCSIO41206 from the Beibu Gulf

Abstract

Based on the OSMAC (One Strain, Many Compounds) strategy, this study explores and investigates structurally novel compounds in the secondary metabolites of *Cladosporium sp.* SCSIO41206, a coral-associated fungus from the Beibu Gulf. Various chromatographic techniques were employed to isolate and purify the secondary metabolites, and compound structures were identified using NMR, MS, and comparison with relevant literature data. The results demonstrated that this fungus produced a richer array of metabolites when statically cultured in potato dextrose broth (PDB) liquid medium at 26°C for 30 days. Through isolation and identification, twelve known compounds were obtained: 3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione (1), 3-benzyl-7-hydroxyhexahydropyrrolo[1,2-a]pyrazine-1,4-dione (2), cyclo(L-alanine-L-4-hydroxyproline) dipeptide (3), cyclo(L-proline-glycine) dipeptide (4), cyclo(D)-Pro-(D)-Ile (5), cyclo(L-phenylalanine-glycine) dipeptide (6), cyclo-(4-S-hydroxy-R-proline-R-isoleucine) (7), N-phenethylacetamide (8), N-hydroxy-2-(hydroxyimino)-4-methylpentanamide (9), quinolactacin A1 (10), quinolactacin A2 (11), and dibutyl terephthalate (12). Compounds 1–12 were all isolated for the first time from *Cladosporium* species derived from Beibu Gulf corals. Compounds 2, 3, 4, 8, 10, 11, and 12 exhibited weak inhibitory activity against acetylcholinesterase.

Keywords: *Cladosporium sp.*; secondary metabolites; structural identification; acetylcholinesterase inhibitory activity

Marine habitats, with their unique environmental conditions and vast geographic extent, harbor richer biological resources than terrestrial environments. The enormous diversity of marine organisms provides a valuable resource for obtaining a large number of structurally diverse and biologically active natural products (Song, et al., 2021). In recent years, marine natural products have achieved new breakthroughs in drug development. To date, 20 drugs derived from marine sources are in clinical use (Haque, et al., 2022). Therefore, secondary metabolites produced by symbiotic fungi isolated from marine environments represent a highly promising research subject.

Cladosporium fungi represent a rich resource, with diverse structural types of chemical constituents in their secondary metabolites and prominent biological activities, including alkaloids, polyketides, macrolides, steroids, and terpenoids, most of which exhibit antimicrobial, antiviral, and cytotoxic activities (Dong Jinrun, et al., 2021), providing an important source of bioactive drug molecules. Johanna Silber et al. (Silber, et al., 2014) isolated four malettinin compounds (1–4) from a marine *Cladosporium* strain, which showed activity against the human pathogenic dermatophyte *Trichophyton rubrum* with IC50 values of 28.3–37.9 M. Zhu et al. (Zhu, et al., 2018) isolated a novel hybrid polyketide from a marine-derived *Cladosporium* fungus that exhibited cytotoxic activity against MCF-7, HeLa, HCT-116, and HL-60 human cancer cell lines with half-maximal inhibitory concentration values of 18.7, 19.1, 17.9, and 9.1 μM, respectively.

Gu et al. (Gu, et al., 2015) used high-speed counter-current chromatography to isolate three sulfur-containing diketopiperazine compounds from a marine *Cladosporium* fungus, all of which showed inhibitory effects on the proliferation of Hep G2 liver cancer cells. However, reports on marine-derived *Cladosporium* fungi both domestically and internationally remain limited.

1.1 Instruments and Reagents

The main instruments included a rotary evaporator (EYELAN-1100V-W, Tokyo Rikakikai Co., Ltd., Japan), AV500, AV600, and AV-700 NMR spectrometers (Bruker Corporation, Germany), a HITACHI L-2400 semi-preparative HPLC (Hitachi, Ltd., Japan), a ZYJ-S clean bench (Suzhou Purification Equipment Co., Ltd.), a medium-pressure preparative column chromatography system (Buchi C615/605), an HR-ESM-MS (Bruker Corporation, Germany), 96-well cell culture plates (Corning Incorporated, USA), Sephadex LH-20 dextran gel (Pharmacia, USA), and tacrine (Lot#07220AV, Aldrich, USA).

The main reagents included thin-layer chromatography silica gel (Qingdao Marine Chemical Factory), 10–40 mesh and 100–200 mesh normal-phase silica gel (Yantai Jiangyou Silica Gel Development Co., Ltd.), reversed-phase silica gel (Merck), and reagents from Guangzhou Chemical Reagent Factory and Tianjin Fuyu Fine Chemical Co., Ltd.

1.2 Strain Fermentation and Culture

The strain was collected from the Beibu Gulf in Guangxi and identified as *Cladosporium sp.* through DNA amplification and ITS region sequencing, designated as SCSIO41206. The specimen is currently preserved at the Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences. The activation of the strain and seed culture fermentation were performed as previously reported (She, et al., 2022). The prepared seed culture was inoculated into 200 sterilized flasks containing potato dextrose broth (PDB) liquid medium (24 g PDB powder in 1000 mL pure water) and statically cultured for 30 days.

1.3 Extraction and Isolation

An equal volume of ethyl acetate was added to the liquid fermentation medium, sonicated for 30 minutes, soaked overnight, and extracted five times. After concentration, 28.5 g of ethyl acetate extract was obtained. The crude extract was separated by medium-pressure normal-phase silica gel column chromatography, using 100–200 mesh silica gel for sample loading. The mobile phase consisted of petroleum ether:ethyl acetate (V:V = 100:0 to 1:1, flow rate 100 $mL \cdot min^{-1}$) for gradient elution to obtain the first fraction, followed by petroleum ether:ethyl acetate:methanol (V:V:V = 20:20:1 to 0:0:100, flow rate 100 $mL \cdot min^{-1}$) for gradient elution to obtain the second fraction. These two portions were combined and analyzed by thin-layer chromatography (TLC) to yield seven frac-

tions (Fr1–Fr7). Fraction Fr3 (250 mg) was purified by semi-preparative HPLC (ChromCore 120 C18, 5 m, 10 × 250 mm; methanol:acidic water = 18:82, flow rate 3 mL · min^{-1}) to afford compound 12 (10.8 mg, tR = 10 min). Subfraction Fr4.2 (156.8 mg) was purified by semi-preparative HPLC (ChromCore 120 C18, 5 m, 10 × 250 mm; methanol:acidic water = 16:84, flow rate 3 mL · min^{-1}) to yield compound 8 (46.01 mg, tR = 9 min). Fraction Fr5 (1.2 g) was purified by semi-preparative HPLC (ChromCore 120 C18, 5 m, 10 × 250 mm; methanol:acidic water = 18:82, flow rate 3 mL · min^{-1}) to obtain compounds 1 (43.25 mg, tR = 15 min) and 2 (27 mg, tR = 18 min). Subfraction Fr5.3 (18.8 mg) was purified by semi-preparative HPLC (ChromCore 120 C18, 5 m, 10 × 250 mm; methanol:acidic water = 20:80, flow rate 3 mL · min^{-1}) to yield compounds 5 (2.01 mg, tR = 7.5 min), 6 (2.17 mg, tR = 9 min), and 7 (2.73 mg, tR = 12 min). Subfraction Fr5.2 (10 mg) was purified by semi-preparative HPLC (ChromCore 120 C18, 5 m, 10 × 250 mm; methanol:acidic water = 20:80, flow rate 3 mL · min^{-1}) to afford compound 9 (3.31 mg, tR = 8.6 min). Fraction Fr6 was subjected to Sepa flash column chromatography (Spherical C18, 20–45 m) with methanol and water gradient elution and combined into twelve subfractions (Fr.6-1–Fr.6-6). Fr.6-1 (200 mg) was purified by semi-preparative HPLC (ChromCore 120 C18, 5 m, 10 × 250 mm; methanol:acidic water = 60:40, flow rate 3 mL · min^{-1}) to yield Fr.6-1-2 and compound 4 (34.79 mg, tR = 12 min). Subfraction Fr.6-1-2 (72.1 mg) was further purified by semi-preparative HPLC (ChromCore 120 C18, 5 m, 10 × 250 mm; methanol:acidic water = 50:50, flow rate 3 mL · min^{-1}) to afford compound 3 (21.49 mg, tR = 15 min). Fraction Fr7 (500 mg) was purified by semi-preparative HPLC (ChromCore 120 C18, 5 m, 10 × 250 mm; acetonitrile:acidic water = 40:60, flow rate 3 mL · min^{-1}) to yield compounds 10 (2.78 mg, tR = 10 min) and 11 (2.45 mg, tR = 10.5 min). The structures of compounds 1–12 are shown in Figure 1 [Figure 1: see original paper].

1.4.1 Acetylcholinesterase Inhibition Activity Assay

The Ellman method was used to evaluate the acetylcholinesterase inhibitory activity of the compounds. All compounds were dissolved in methanol, with tacrine used as the positive control. Detailed procedures were performed as previously reported (Cai Jian, et al., 2021).

2.1 Structure Identification

Compound 1: White powder, ESI-MS m/z: 227.5 $[M+H]^+$. ^1H NMR (500 MHz, DMSO-d_6) δ 8.00 (1H, s, 2-NH), 5.13 (1H, s, 7-OH), 4.39 (1H, dd, J = 10.7, 6.6 Hz, H-7), 4.28 (1H, t, J = 4.4 Hz, H-3), 4.07–4.02 (1H, m, H-9), 3.48 (1H, dd, J = 12.4, 4.4 Hz, H-6a), 3.23 (1H, d, J = 12.4 Hz, H-6b), 2.04 (1H, ddt, J = 13.1, 6.7, 1.6 Hz, H-8a), 1.90 (2H, dddd, J = 25.5, 12.7, 9.5, 5.3 Hz, H-10), 1.34 (1H, ddd, J = 13.7, 7.5, 5.9 Hz, H-8b), 0.87 (3H, d, J = 3.5 Hz, H-12), 0.85 (3H, d, J = 3.6 Hz, H-13); ^{13}C NMR (125 MHz, DMSO) δ 170.8 (C-4), 166.7 (C-1), 67.1 (C-7), 57.1 (C-9), 53.8 (C-6), 52.6 (C-3), 37.8 (C-10),

36.7 (C-8), 24.1 (C-11), 22.9 (C-13), 21.9 (C-12). These data were consistent with literature values (Sun, et al., 2009; Fdhila, et al., 2003), confirming the structure as 3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione.

Compound 2: White powder, ESI-MS m/z: 261.6 $[M+H]^+$. ^1H NMR (500 MHz, DMSO-d_6) δ 7.99 (1H, s, 4-OH), 7.29–7.23 (4H, m, H-2 ,3 ,5 ,6), 7.20–7.16 (1H, m, H-4), 5.10 (1H, s, 8-NH), 4.40 (1H, t, J = 5.2 Hz, H-4), 4.31 (1H, ddd, J = 11.3, 6.3, 1.6 Hz, H-6), 4.19 (1H, t, J = 4.7 Hz, H-9), 3.52 (1H, dd, J = 12.5, 4.8 Hz, H-3), 3.16 (1H, d, J = 12.5 Hz, H-3), 3.11–2.97 (2H, m, H-10), 1.94 (1H, ddt, J = 13.0, 6.4, 1.5 Hz, H-5), 1.52 (1H, ddd, J = 12.8, 11.2, 4.5 Hz, H-5); ^{13}C NMR (125 MHz, DMSO) δ 169.6 (C-1), 165.3 (C-7), 137.3 (C-1), 129.8 (C-2 and C-6), 128.0 (C-3 and 5), 126.4 (C-4), 66.8 (C-4), 57.0 (C-6), 55.7 (C-9), 53.9 (C-3), 37.2 (C-5), 35.2 (C-10). These data were consistent with literature values (Fdhila, et al., 2003), confirming the structure as 3-benzyl-7-hydroxyhexahydropyrrolo[1,2-a]pyrazine-1,4-dione.

Compound 3: Colorless needle-like crystals, ESI-MS m/z: 185.3 $[M+H]^+$. ^1H NMR (500 MHz, DMSO-d_6) δ 8.14 (1H, s, 4-NH), 5.10 (1H, s, 8-OH), 4.37 (1H, ddd, J = 11.0, 6.6, 1.5 Hz, H-6), 4.28 (1H, t, J = 4.4 Hz, H-8), 4.17–4.08 (1H, m, H-3), 3.50 (1H, dd, J = 12.4, 4.5 Hz, H-9), 3.22 (1H, d, J = 12.4 Hz, H-9), 2.04 (1H, ddt, J = 13.0, 6.6, 1.5 Hz, H-7), 1.91 (1H, ddd, J = 12.9, 10.9, 4.3 Hz, H-7), 1.21 (3H, d, J = 6.9 Hz, H-10); ^{13}C NMR (125 MHz, DMSO) δ 170.4 (C-2), 166.7 (C-5), 67.1 (C-8), 57.3 (C-3), 53.8 (C-7), 50.1 (C-6), 36.9 (C-9), 15.3 (C-10). These data were consistent with literature values (Wang Yue, et al., 2022), confirming the structure as cyclo(L-alanine-L-4-hydroxyproline) dipeptide.

Compound 4: Colorless needle-like crystals, ESI-MS m/z: 155.2 $[M+H]^+$. ^1H NMR (500 MHz, DMSO-d_6) δ 8.06 (1H, s, 7-NH), 4.15–4.08 (1H, m, H-3), 3.99 (1H, dd, J = 16.4, 1.8 Hz, H-3), 3.50 (1H, dd, J = 16.4, 4.5 Hz, H-7), 3.41 (1H, dt, J = 11.3, 7.7 Hz, H-5), 3.33 (1H, ddd, J = 11.6, 8.2, 3.3 Hz, H-5), 2.16–2.10 (1H, m, H-3), 1.89–1.75 (2H, m, H-4); ^{13}C NMR (125 MHz, DMSO) δ 169.3 (C-1), 163.9 (C-6), 58.0 (C-2), 45.9 (C-5), 44.7 (C-7), 27.9 (C-3), 22.1 (C-4). These data were consistent with literature values (Tang Jinshan, et al., 2008), confirming the structure as cyclo(L-proline-glycine) dipeptide.

Compound 5: White solid, 2.01 mg, ESI-MS m/z: 211.1 $[M+H]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ 7.96 (1H, s, 8-NH), 4.11 (1H, td, J = 7.1, 6.6, 3.6 Hz, H-6), 3.92 (1H, s, H-9), 2.34 (1H, pd, J = 7.1, 2.5 Hz, H-10), 2.16–2.11 (1H, m, H-5), 1.90–1.74 (2H, m, H-4), 1.01 (3H, d, J = 7.2 Hz, H-12), 0.85 (3H, d, J = 6.9 Hz, H-13); ^{13}C NMR (150 MHz, DMSO) δ 170.3 (C-1), 165.3 (C-7), 59.5 (C-6), 58.3 (C-9), 44.6 (C-3), 27.9 (C-10), 27.7 (C-5), 22.1 (C-4), 18.3 (C-13), 16.4 (C-12). These data were consistent with literature values (Fdhila, et al., 2003), confirming the structure as cyclo(D)-Pro-(D)-Ile.

Compound 6: White solid, 2.17 mg, ESI-MS m/z: 204.1 $[M+H]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ 8.15 (1H, d, J = 2.7 Hz, 5-NH), 7.89 (1H, s, 2-NH), 7.30–7.23 (3H, m, H-2 ,4 ,6), 7.18–7.15 (2H, m, H-3 ,5), 4.06 (1H, q, J = 4.2, 3.1 Hz, H-6), 3.09 (1H, dd, J = 13.5, 4.5 Hz, H-7), 2.88 (1H, dd, J = 13.5, 5.0

Hz, H-7), 2.76 (1H, d, J = 17.3 Hz, H-3); ^{13}C NMR (150 MHz, DMSO) δ 167.6 (C-1), 166.1 (C-4), 136.4 (C-1), 130.5 (C-2), 128.6 (C-3), 127.2 (C-4), 56.0 (C-5), 44.1 (C-3), 40.5 (C-7). These data were consistent with literature values (Qu Chenglei, et al., 2015), confirming the structure as cyclo(L-phenylalanine-glycine) dipeptide.

Compound 7: White solid, 2.73 mg, ESI-MS m/z: 226.13 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.96 (1H, s, 8-NH), 4.32 (1H, ddd, J = 11.2, 6.3, 1.7 Hz, H-6), 4.28 (1H, t, J = 4.4 Hz, H-4), 4.00 (1H, s, H-9), 3.52 (1H, dd, J = 12.5, 4.6 Hz, H-3), 3.21 (1H, d, J = 12.5 Hz, H-3), 2.03 (2H, dddd, J = 18.7, 9.5, 5.4, 1.9 Hz, H-5 and H-10), 1.88 (1H, ddd, J = 12.9, 11.2, 4.4 Hz, H-5), 1.34 (1H, dtd, J = 15.1, 7.5, 4.5 Hz, H-11), 1.29–1.22 (1H, m, H-11), 0.98 (3H, d, J = 7.1 Hz, H-13), 0.82 (3H, t, J = 7.4 Hz, H-12); ^{13}C NMR (150 MHz, DMSO) δ 170.5 (C-7), 165.4 (C-1), 66.9 (C-4), 59.1 (C-9), 56.7 (C-6), 53.8 (C-3), 37.2 (C-5), 34.8 (C-10), 23.9 (C-11), 15.0 (C-13), 12.3 (C-12). These data were consistent with literature values (Ovenden, et al., 2011), confirming the structure as cyclo-(4-S-hydroxy-R-proline-R-isoleucine).

Compound 8: Brown oil, ESI-MS m/z: 164.2 $[M+H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.93 (1H, s, NH), 7.32–7.25 (2H, m, H-3,5), 7.20 (3H, d, J = 7.4 Hz, H-2,4,6), 3.28–3.23 (2H, m, H-8), 2.70 (1H, t, J = 7.5 Hz, H-7), 1.79 (3H, s, H-10); ^{13}C NMR (125 MHz, DMSO) δ 169.2 (C-9), 139.6 (C-1), 128.6 (C-3,5), 128.3 (C-2,6), 126.1 (C-4), 40.3 (C-8), 35.2 (C-7), 22.6 (C-10). These data were consistent with literature values (Cao Yang, et al., 2020), confirming the structure as N-phenethylacetamide.

Compound 9: White solid, 3.31 mg, ESI-MS m/z: 114.09 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.53 (1H, s, 2-N-OH), 7.15 (2H, d, J = 75.5 Hz, 1-NH), 2.35 (2H, d, J = 7.4 Hz, H-3), 1.91 (1H, hept, J = 6.9 Hz, H-4), 0.83 (6H, d, J = 6.7 Hz, H-5 and 6); ^{13}C NMR (150 MHz, DMSO) δ 165.9 (C-1), 153.4 (C-2), 31.6 (C-3), 25.9 (C-4), 22.7 (C-5 and 6). These data were consistent with literature values (Su, et al., 2022), confirming the structure as N-hydroxy-2-(hydroxyimino)-4-methylpentanamide.

Compound 10: Yellow oil, ESI-MS m/z: 271.1 $[M+H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.26 (1H, d, J = 7.7 Hz, H-8), 7.83 (2H, d, J = 1.7 Hz, H-5,6), 7.49 (1H, t, J = 8.0 Hz, H-7), 4.91 (1H, s, H-3), 3.85 (3H, s, N-CH_3), 2.18 (1H, tt, J = 7.1, 3.6 Hz, H-1), 1.28–1.20 (2H, m, H-2), 1.01 (3H, t, J = 7.4 Hz, H-2 -CH_3), 0.44 (3H, d, J = 6.9 Hz, H-1 -CH_3); ^{13}C NMR (125 MHz, DMSO) δ 171.6 (C-9), 168.5 (C-1), 164.2 (C-3a), 141.3 (C-4a), 132.6 (C-6), 128.0 (C-8a), 125.9 (C-8), 124.4 (C-7), 117.2 (C-5), 110.3 (C-9a), 56.9 (C-3), 36.1 (N-CH_3), 35.7 (C-1), 27.3 (C-2), 12.0 (2 -CH_3), 11.5 (1 -CH_3). These data were consistent with literature values (Kozlovsky, et al., 2003; Kim, et al., 2010), confirming the structure as quinolactacin A1.

Compound 11: Yellow oil, ESI-MS m/z: 271.1 $[M+H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.26 (1H, d, J = 7.7 Hz, H-8), 7.83 (2H, d, J = 1.7 Hz, H-5,6), 7.49 (1H, t, J = 8.0 Hz, H-7), 4.83 (1H, s, H-3), 3.82 (3H, s, N-CH_3), 2.18 (1H,

tt, J = 7.1, 3.6 Hz, H-1), 1.13 (3H, d, J = 6.9 Hz, H-1 -CH_3), 0.86 (2H, td, J = 7.3, 2.8 Hz, H-2), 0.66 (3H, t, J = 7.4 Hz, H-2 -CH_3); ^{13}C NMR (125 MHz, DMSO) δ 171.6 (C-9), 168.7 (C-1), 164.6 (C-3a), 141.3 (C-4a), 132.6 (C-6), 128.0 (C-8a), 125.9 (C-8), 124.4 (C-7), 117.2 (C-5), 110.3 (C-9a), 59.0 (C-3), 36.1 (N-CH_3), 35.8 (C-1), 20.9 (C-2), 17.6 (1 -CH_3), 11.6 (2 -CH_3). These data were consistent with literature values (Kozlovsky, et al., 2003; Kim, et al., 2010), confirming the structure as quinolactacin A2.

Compound 12: Yellow oil, ESI-MS m/z: 279.7 $[M+H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.73–7.69 (2H, m, H-2,6), 7.66 (2H, dt, J = 5.4, 3.7 Hz, H-3,5), 4.22 (4H, t, J = 6.6 Hz, H-9 and 9), 1.67–1.60 (4H, m, H-10 and 10), 1.41–1.33 (4H, m, H-11 and 11), 0.90 (6H, t, J = 7.4 Hz, H-12 and 12); ^{13}C NMR (125 MHz, DMSO) δ 167.0 (C-7 and 7), 131.7 (C-1 and 4), 131.5 (C-2 and 6), 128.7 (C-3 and 5), 65.0 (C-9 and 9), 30.0 (C-10 and 10), 18.7 (C-11 and 11), 13.5 (C-12 and 12). These data were consistent with literature values (Niu Feng, et al., 2006), confirming the structure as dibutyl terephthalate.

2.2 Activity Results

The twelve isolated compounds were tested for acetylcholinesterase inhibitory activity. Compounds 2, 3, 4, 8, 10, 11, and 12 exhibited weak inhibitory activity against acetylcholinesterase, with inhibition rates of 17.45%, 18.93%, 13.97%, 14.13%, 32.3%, and 26.2%, respectively, at a final concentration of 0.05 $g \cdot L^{-1}$ (the positive control tacrine showed 72.2% inhibition at a final concentration of 0.333 M).

The marine environment remains an underexplored treasure trove, with many marine-associated *Cladosporium* species demonstrating significant enzyme production capabilities (Mohamed and Ibrahim, 2021). This study investigated the secondary metabolites of *Cladosporium sp.* SCSIO41206, a coral-derived fungus from the Beibu Gulf, leading to the isolation of twelve compounds. The main types of metabolites were cyclic dipeptides and quinoline alkaloids; whether this fungus can metabolize other types of compounds remains to be explored through further modifications of fermentation conditions.

Compounds 1–12 were all isolated for the first time from *Cladosporium* species derived from the Beibu Gulf. According to literature reports, compound 10 exhibits antimalarial potential against chloroquine-sensitive *Plasmodium falciparum* 3D7, with an EC50 of 24.8 µM compared to artesunate (EC50 0.074 µM) in SYBR Green I assays (Mohamed and Ibrahim, 2021). Compounds 1, 2, 3, 4, 5, and 7 show strong inhibitory activity against *Vibrio anguillarum* with MIC values between 0.03–0.07 $\mu g \cdot mL^{-1}$, which is tenfold lower than some antibiotics currently used in aquaculture such as oxytetracycline (MIC 0.5 $\mu g \cdot mL^{-1}$) (Fdhila, et al., 2003). Moreover, compounds 1–7 are diketopiperazine compounds containing the important 2,5-diketopiperazine six-membered ring pharmacophore, a class of compounds with broad biological activities including antitumor, antiviral, anti-inflammatory, and antimicrobial properties (Xing

Nannan, et al.), indicating their potential as drug candidates or leads. This study tested the acetylcholinesterase inhibitory activity of the twelve isolated compounds and found that compounds 2, 3, 4, 8, 10, 11, and 12 showed weak activity, while other activities remain to be explored. These findings further enrich the structural diversity of natural products from coral-derived *Cladosporium* species in the Beibu Gulf region and provide a foundation for future studies on secondary metabolites from Beibu Gulf-derived *Cladosporium* species through biological activity testing of the isolated compounds.

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