New chlorinated diphenyl ethers and xanthones from a deep-sea-derived fungus *Penicillium chrysogenum* SCSIO 41001

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ABSTRACT

Seven new compounds, including four new chlorinated diphenyl ethers, namely chrysines A–D (**1–4**), one new dichlorinated xanthone, chrysoxanthone (**5**), dichloroorcinol (**6**), and one new benzeneacetic acid derivative, 3-isopentyl-4-hydroxy phenylacetic acid methyl ester (**7**), along with fourteen known compounds (**8–21**), were isolated from a deep-sea-derived fungus *Penicillium chrysogenum* SCSIO 41001. Their structures were determined by extensive spectroscopic methods and X-ray single-crystal diffraction analysis. All of the isolated compounds (**1 − 21**) were evaluated for their α-glucosidase inhibitory activity using PNPG method. Among them, nine compounds (**2**, **3**, **5**, **6**, **8**, **9**, **13**, **17**, and **18**) exhibited inhibitory activity against α-glucosidase with IC_{50} values of 0.35, 0.20, 0.04, 0.16, 0.15, 0.09, 0.14, 0.14, and 0.12 mM, respectively (IC_{50} 0.28 mM for the positive control acarbose).

1. Introduction

Marine organisms are recognized as important sources of pharmacologically active metabolites. In particular, a growing number of marine-derived microorganisms have been reported to produce novel bioactive secondary metabolites [1–3]. Especially, marine-derived natural halogenated compounds possess a variety of bioactivities such as cytotoxic, antibacterial, antifungal, antiviral, antifouling, antifeedant, and insecticidal activities, which are widely used in the medicine and agricultural field [4,5]. To date, more than 5000 halogenated natural products have been discovered, these results have drawn the attention of both pharmaceutical and natural product chemists, and stimulated many biosynthesis and total synthesis programs [6,7].

Our previous investigation on the deep-sea-derived fungus *P. chrysogenum* SCSIO 41001 has resulted in the discovery of dimeric nitrophenyl *trans*-epoxyamides, chrysamides A–C, dimeric isocoumarin, and citrinin dimer in rice solid culture medium [8,9]. As part of our continuing efforts to explore the chemical diversity of microorganisms from the deep-sea for drug discovery, we reinvestigated the secondary metabolites of the fungus SCSIO 41001. When the culture conditions were changed from solid-static fermentation grown in rice culture medium to liquid-static fermentation grown in a nutrient-limited culture medium, its ethyl acetate extracts contained a variety of secondary metabolites with similar UV absorptions at 230 and 330 nm based upon high-performance liquid chromatography analysis. Further chemical investigations of the culture extracts afforded four new chlorinated diphenyl ethers, namely chrysines A–D (**1–4**), one new dichlorinated xanthone, chrysoxanthone (**5**), dichloroorcinol (**6**), and one new benzeneacetic acid derivative, 3-isopentyl-4-hydroxy phenylacetic acid methyl aster (**7**), along with nine known diphenyl ethers, methyl 3′-methoxy-3,5-dichloroasterric acid (**8**) [10], 2,4-dichloroasterric acid (**9**) [11], methyl dichloroasterrate (**10**) [12], geodin hydrate (**11**) [11], 5-chloroasterric acid (**12**) [13], methyl chloroasterrate (**13**) [11], iizukine A (**14**) [11], penicillither (**15**) [14], asterric acid (**16**) [11], mono-chlorosulochrin (**17**) [11], (+)-geodin (**18**) [15], and chrysamides A − C (**19–21**) [8].Their structures were determined by extensive spectroscopic methods and X-ray single-crystal diffraction analysis. To the best of our knowledge, dichloroorcinol (**6**) was firstly reported as the new natural product, which had been previously obtained as the important intermediate to synthesize pestalone [6,16]. All of the isolated compounds (**1–21**) were evaluated for their α-glucosidase inhibitory activities *in vitro*. Details of the isolation and structure

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elucidation of these compounds are reported herein.

2. Experimental

2.1. General experimental procedures

UV spectra were recorded on a UV-2600 spectrometer (Shimadzu). ^1H, ^{13}C NMR, DEPT, and 2D-NMR spectra were recorded on the Avance-700 spectrometer (Bruker). HRESIMS and ESIMS spectra data were recorded on a MaXis quadrupole-time-of-flight mass spectrometer and an amaZon SL ion trap mass spectrometer (Bruker), respectively. X-ray diffraction intensity data were collected on a CrysAlis PRO CCD area detector diffractometer with graphite-monochromated Cu Kα radiation (λ = 1.541 78 Å). Thin layer chromatography (TLC) and column chromatography (CC) were performed on plates precoated with silica gel GF$_{254}$ (10–40 μm) and over silica gel (200–300 mesh) (Qingdao Marine Chemical Factory), and Sephadex LH-20 (Amersham Biosciences, Sweden), respectively. Vacuum-liquid chromatography (VLC) used silica gel H (Qingdao Marine Chemical Factory). All solvents used were of analytical grade (Tianjin Fuyu Chemical and Industry Factory). Semipreparative HPLC was performed using an ODS column (YMC-pack ODS-A, 10 × 250 mm, 5 μm, 4 mL/min).

2.2. Fungal material

The fungal strain, *P. chrysogenum* SCSIO 41001, was isolated from the deep sea sediment of Indian Ocean (Lat: 10.00371667°N, long: 88.72803333°E) at a depth of 3386 m in 2013. Fungal identification was carried out using ITS phylogenetic sequence analyses, which confirms that the fungal strain SCSIO 41001 was assigned as *Penicillium chrysogenum*. The ITS gene sequence data was deposited in NCBI Genbank, assigned with accession number KU556301. BLAST searching result showed that sequence has 99% sequence identity to that of *P. chrysogenum* strain QRF 370 (NCBI GenBank accession number KP278201). A reference culture is stored in our laboratory at − 80 °C. The producing strain was prepared on potato dextrose agar slants at 3.3% salt concentration and stored at 4 °C.

2.3. Fermentation and extraction

P. chrysogenum SCSIO 41001 was grown under static conditions at 26 °C for 35 days in 1000 mL × 142 conical flasks containing liquid medium (300 mL/flask) composed of soluble starch (10 g/L), polypeptone (1 g/L), and tap water after adjusting its pH to 7.5. The fermented whole broth (40 L) was filtered through cheesecloth to separate it into filtrate and mycelia. The filtrate was concentrated under vacuum to about a quarter of original volume and then extracted three times with EtOAc to give an EtOAc solution, while the mycelia were extracted three times with acetone. The acetone solution was evaporated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with EtOAc to give another EtOAc solution. Both EtOAc solutions were combined and concentrated under reduced pressure to give a dark brown gum (31.7 g).

2.4. Purification

The EtOAc extract (31.7 g) was subjected to VLC on a silica gel column using step gradient elution with MeOH–CH$_2$Cl$_2$ (0–100%) to separate into nine fractions based on TLC properties. Fraction 2 was further separated into five subfractions by Sephadex LH-20 eluting with MeOH-CH$_2$Cl$_2$ (1:1). Subfraction 2–3 was purified by semipreparative HPLC (72% MeOH-H$_2$O) to yield **8** (25.3 mg, t_R 18.9 min). Subfraction 2-3-1 was purified by semipreparative HPLC (47% CH$_3$CN-H$_2$O) to yield **3** (3.1 mg, t_R 34.2 min). Subfraction 2–4 was purified by semipreparative HPLC (65% MeOH-H$_2$O) to separate into six subfractions (Frs.2-4-1–2-4-6). Subfraction 2-4-3 was purified by semipreparative

HPLC (45% CH$_3$CN-H$_2$O + 0.1% CF$_3$CO$_2$H)) to yield **18** (6.8 mg, t_R 26.6 min) and **1** (31.9 mg, t_R 26.6 min). Subfraction 2-4-5 was purified by semipreparative HPLC (47% CH$_3$CN-H$_2$O + 0.1% CF$_3$CO$_2$H) to yield **7** (2.7 mg, t_R 26.3 min) and **5** (10.4 mg, t_R 39.6 min). Subfraction 2-5 was purified by semipreparative HPLC (60% MeOH-H$_2$O) to yield **6** (6.4 mg, t_R 19.8 min). Fraction 3 was further separated into four subfractions by Sephadex LH-20 eluting with MeOH-CH$_2$Cl$_2$ (1:1). Subfraction 3-2 was purified by semipreparative HPLC (68% MeOH-H$_2$O + 0.1% CF$_3$CO$_2$H) to separate into eight subfractions (Frs.3-2-1–3-2-8) and yield **20** (11.0 mg, t_R 19.2 min), **21** (18.8 mg, t_R 29.7 min), and **19** (20.5 mg, t_R 32.0 min). Subfraction 3-2-1 was purified by semipreparative HPLC (55% CH$_3$CN-H$_2$O + 0.1% CF$_3$CO$_2$H) to yield **4** (4.6 mg, t_R 21.4 min) and **10** (11.6 mg, t_R 42.7 min). Subfraction 3-3 was purified by semipreparative HPLC (79% MeOH-H$_2$O) to separate into five subfractions (Frs.3-3-1–3-3-6). Subfraction 3-3-1 was purified by semipreparative HPLC (45% CH$_3$CN-H$_2$O + 0.1% CF$_3$CO$_2$H) to yield **13** (8.4 mg, t_R 25.8 min) and **2** (9.3 mg, t_R 41.5 min). Fraction 4 was further separated into three subfractions by Sephadex LH-20 eluting with MeOH. Subfraction 4-3 was purified by semipreparative HPLC (42% CH$_3$CN-H$_2$O + 0.1% CF$_3$CO$_2$H) to separate into eight subfractions (Frs.4-3-1–4-3-8) and yield **17** (3.6 mg, t_R 25.6 min). Subfraction 4-3-1 was purified by semipreparative HPLC (32% CH$_3$CN-H$_2$O + 0.1% CF$_3$CO$_2$H) to yield **14** (5.5 mg, t_R 20.7 min). Subfraction 4-3-3 was purified by semipreparative HPLC (35% CH$_3$CN-H$_2$O + 0.1% CF$_3$CO$_2$H) to yield **12** (3.8 mg, t_R 31.2 min), **11** (46.0 mg, t_R 34.0 min), **15** (1.9 mg, t_R 39.0 min), and **16** (10.7 mg, t_R 42.7 min). Subfraction 4-3-6 was purified by semipreparative HPLC (40% CH$_3$CN-H$_2$O + 0.1% CF$_3$CO$_2$H) to yield **9** (53.1 mg, t_R 18.4 min).

2.4.1. Chrysine A (1)

White crystal; UV (CH$_2$Cl$_2$) λ_{max} (log ε): 228 (4.18), 330 (3.72) nm; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS m/z 431.0278 [M + H]$^+$ (calcd for C$_{18}$H$_{17}$Cl$_2$O$_8$, 431.0295), 453.0105 [M + Na]$^+$ (calcd for C$_{18}$H$_{16}$Cl$_2$NaO$_8$, 453.0114), 883.0320 [2 M + Na]$^+$ (calcd for C$_{36}$H$_{32}$Cl$_4$NaO$_{16}$, 883.0337).

2.4.2. Chrysine B (2)

Colorless oil; UV (CH$_2$Cl$_2$) λ_{max} (log ε): 227 (4.11), 329 (3.59) nm; ^1H NMR data, see Table 1; HRESIMS m/z 445.0441 [M + H]$^+$ (calcd for C$_{19}$H$_{19}$Cl$_2$O$_8$, 445.0451), 467.0270 [M + Na]$^+$ (calcd for C$_{19}$H$_{18}$Cl$_2$NaO$_8$, 467.0271).

2.4.3. Chrysine C (3)

Colorless oil; UV (CH$_2$Cl$_2$) λ_{max} (log ε): 229 (4.24), 323 (3.70) nm; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS m/z 411.0838 [M + H]$^+$ (calcd for C$_{19}$H$_{20}$ClO$_8$, 411.0841), 433.0672 [M + Na]$^+$ (calcd for C$_{19}$H$_{19}$ClNaO$_8$, 433.0661).

2.4.4. Chrysine D (4)

Colorless oil; UV (CH$_2$Cl$_2$) λ_{max} (log ε): 229 (4.21), 326 (3.73) nm; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS m/z 397.0691 [M + H]$^+$ (calcd for C$_{18}$H$_{18}$ClO$_8$, 397.0685), 419.0504 [M + Na]$^+$ (calcd for C$_{18}$H$_{17}$ClNaO$_8$, 419.0504).

2.4.5. Chrysoxanthone (5)

Yellow gum; UV (MeOH) λ_{max} (log ε): 202 (4.07), 233 (3.93), 265 (3.70) nm; ^1H NMR and ^{13}C NMR data, see Table S1; HRESIMS m/z 384.9879 [M + H]$^+$ (calcd for C$_{16}$H$_{11}$Cl$_2$O$_7$, 384.9876), 406.9700 [M + Na]$^+$ (calcd for C$_{16}$H$_{10}$Cl$_2$NaO$_7$, 406.9696).

2.4.6. Dichloroorcinol (6)

Colorless oil; UV (CH$_2$Cl$_2$) λ_{max} (log ε): 228 (3.73), 287 (3.282) nm; ^1H NMR and ^{13}C NMR data, see Table S2; HRESIMS m/z 190.9673 [M – H]$^-$ (calcd for C$_7$H$_5$Cl$_2$O$_2$, 190.9672), 226.9438 [M + Cl]$^-$ (calcd for C$_7$H$_6$Cl$_3$O$_2$, 226.9439).