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ANTIMICROBIAL DIKETOPIPERAZINE ALKALOIDS AND INDOLE DERIVATIVES FROM THE MARINE-DERIVED FUNGUS *PENICILLIUM* sp. M874

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Summary

From the solid-fermented product of the marine sediment-derived *Penicillium* sp. M874 collected in Ninh Thuan, Vietnam, eight secondary metabolites, including four diketopiperazine alkaloids, 6-methoxyspirotryprostatin B (**1**), tryprostatin B (**2**), 12,13-dihydroxy-fumitremorgin C (**3**), bis(dethio)bis(methylthio)gliotoxin (**4**), and four indoles, 1*H*-indole-3-ethanol (**5**), *N*-acetyltryptamine (**6**), 3-formylindole (**7**), and 3-hydroxyacetylindole (**8**) were isolated and structurally elucidated. The structures of these compounds were determined by MS and NMR spectra analyses and by comparison with literature data. Six compounds, **1-5** and **7**, displayed antimicrobial effects against at least one of the tested reference microorganisms, with MIC values ranging from 8 to 256 µg/mL.

Keywords: *Penicillium; Diketopiperazine alkaloids; Indoles; Secondary metabolites; Marine fungi.*

1. Introduction

Marine fungi have been recognized as an important source of biologically active natural products and are considered invaluable for the discovery of novel antibiotics, antitumor agents, and anti-inflammatory agents [1]. The genus *Penicillium* is among the most common and extensively studied groups of fungi by natural products chemists and is recognized as a significant source of bioactive compounds for drug discovery. Notable examples of fungal-derived drugs include the antibiotic penicillin and the antifungal agent griseofulvin [2],[3]. To date, *Penicillium* species continue to serve as prolific producers of secondary metabolites exhibiting a wide range of biological activities, as highlighted in numerous recent reviews [4],[5]. Vietnam is recognized as one of the countries with the richest marine biodiversity in the world. As part of our screening program, the crude extract of M874 fermentation exhibited antimicrobial activity against two Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923), one Gram-negative bacterium (*Salmonella enterica* ATCC 13076) and one yeast strain (*Candida albicans* ATCC 10231) with MIC values of 64, 32, 128, and 128 µg/mL, respectively. In this study, we report the isolation and structural elucidation of eight secondary metabolites (**1-8**) from the marine-derived fungus *Penicillium* sp. M874 strain, which was isolated from a sediment sample collected at a depth of 7 meters off the coast of Ninh Thuan province (Vietnam). The structures of these compounds are described in Fig. 1.

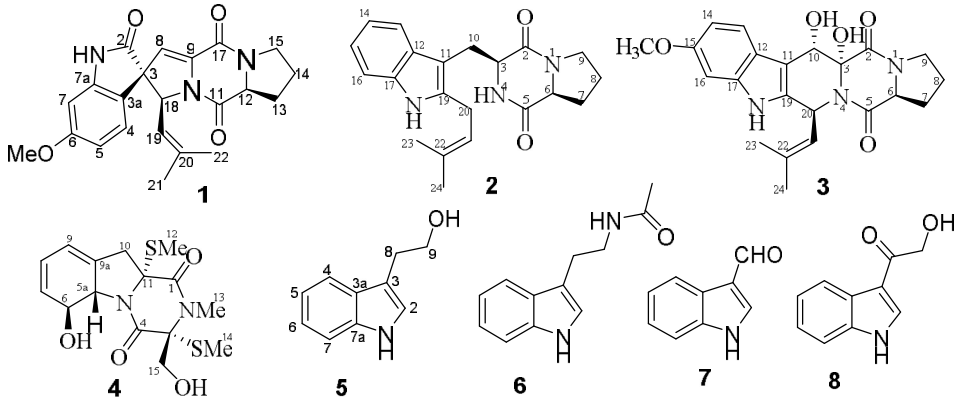

Fig. 1. Structures of compounds **1-8** isolated from *Penicillium* sp. M874

2. Materials and methods

2.1. General experimental procedures

Melting points were recorded using a Boetius apparatus. ESI-MS spectra were recorded on an Agilent 1100 LC-MSD Trap spectrometer. Optical rotations were measured on a JASCO DIP-1000 KUY Polarimeter. NMR spectra were recorded on a Bruker 500 MHz spectrometer using $CDCl_3$ as the solvent and TMS as the internal standard. Column chromatography (CC) was performed on silica-gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or Sephadex LH-20 (Sigma). Thin layer chromatography (TLC) was performed on pre-coated *silica gel* 60 F_{254} (0.25 mm, Merck) and RP-18 $F_{254}S$ (0.25 mm, Merck) plates. Spots were visualized under UV light (254 nm and 365 nm) and by heating *silica gel* plates sprayed with 10% sulfuric acid reagent.

2.2. Marine materials

The strain M874 was isolated from a marine sediment sample collected at a depth of 7 meters, off the coast of Ninh Thuan province, Vietnam in May 2020. This strain was identified as *Penicillium* sp. by Dr. Le Thi Hong Minh, Institute of Chemistry, VAST.

2.3. Fermentation of the fungal strain Penicillium sp. *M874*

The strain M874 was activated and inoculated into 1 L of PDB (Potato Dextrose Broth) medium. After 7 days of incubation at 30 °C with shaking at 110 rpm, the culture broth was used to spread on the medium surface of 20 flasks (each 5L flask containing 1 L of PDA (Potato Dextrose Agar) solid medium). The flasks were incubated at 30°C and harvested on the fifteenth day.

2.4. Isolation of chemical constituents from the ethyl acetate extract of Penicillium sp. *M874*

The solid fermented product of *Penicillium* sp. M874 was cultured on the agar-based media for fifteen days and the agar was then cut into small pieces and extracted with methanol (4 times × 5 liters × 30 minutes each) at 40 °C. The solutions were concentrated *in vacuo* to give 101.2 g of methanolic residue. This MeOH residue was suspended in H_2O (500 mL) and then partitioned with EtOAc (5 times × 400 mL). The extracts were concentrated under pressure to give EtOAc (EM874, 16.2 g) and aqueous extracts (WM874), respectively.

The EtOAc extract (16,2 g) was subjected to *silica gel* column chromatography (CC) using a gradient of *n*-hexane - acetone (0-100% acetone, *v/v*) to yield 5 fractions F1-F5. Fraction F3 (1.22 g) was separated on *silica gel* CC with *n*-hexane - EtOAc (10-100% EtOAc, *v/v*) to yield 5 sub-fractions F3.1-F3.5. Sub-fraction F3.4 (0.1 g) was continuously separated by *silica gel* CC, eluted with *n*-hexane - acetone (3/1, *v/v*) to yield **1** (6 mg). Sub-fraction F3.5 (15 mg) was separated by preparative thin-layer chromatography (TLC) to give **2** (8 mg). Fraction F4 (4.14 g) was separated by *silica gel* CC, eluted with CH_2Cl_2 - MeOH gradient (0-100% MeOH, *v/v*) to give 5 sub-fractions F4.1-F4.5. Sub-fraction F4.4 (0.106 g) was continuously separated by *silica gel* CC with a mixture of CH_2Cl_2 - acetone gradient (5-100% acetone, *v/v*) as eluent to yield **3** (5 mg). Sub-fraction F4.5 (1.15 g) was purified by Sephadex LH-20 CC with MeOH as eluent to give 4 sub-fractions F4.5.1-F4.5.4. Sub-fraction F4.5.2 (0.16 g) was separated by *silica gel* CC with CH_2Cl_2 - MeOH gradient (5-100 % MeOH, *v/v*) as mobile phase to yield **4** (12 mg). Fraction F5 (5.42 g) was further purified by Sephadex LH-20 CC with MeOH to give 5 sub-fractions F5.1-F5.5. Sub-fraction F5.3 (1.28 g) was separated by *silica gel* CC with CH_2Cl_2 - MeOH gradient (5-100 % MeOH, *v/v*) to give four sub-fractions F5.3.1-F5.3.4. Sub-fraction F5.3.2 was separated by Sephadex LH-20 CC with MeOH to give **5** (5 mg). Sub-fraction F5.3.3 (0.36 g) was subjected to Sephadex LH-20 CC with MeOH as eluent to give **6** (7 mg). Sub-fraction F5.4 (0.84 g) was continuously separated by *silica gel* CC with a mixture of CH_2Cl_2 - MeOH gradient (5-100 % MeOH, *v/v*) as mobile phase to yield **7** (4 mg) and **8** (6 mg).

6-Methoxyspirotryprostatin B (1): Pale yellow, amorphous powder, $[\alpha]_D^{25}$ -36.2 (*c* 0.05, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 8.16 (1H, s, NH), 6.94 (1H, d, *J* = 8.0 Hz, H-4), 6.52 (1H, dd, *J* = 2.0, 8.5 Hz, H-5), 6.44 (1H, d, *J* = 2.0 Hz, H-7), 5.75 (1H, s, H-8), 5.37 (1H, d, *J* = 9.0 Hz, H-18), 5.20 (1H, d, *J* = 9.0 Hz, H-19), 4.33 (1H, dd, *J* = 5.5, 10.5 Hz, H-12), 3.86 (1H, m, H_a-15), 3.79 (3H, s, OCH_3), 3.56 (1H, m, H_b-15), 2.48 (1H, m, H_a-13), 2.11 (1H, m, H_a-14), 1.99 (2H, m, H_b-14, H_b-13), 1.58 (3H, s, CH_3-21), 1.30 (3H, s, CH_3-22). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 178.9 (C-2), 162.5 (C-11), 160.6 (C-6), 155.3 (C-17), 141.6 (C-7a), 138.2 (C-20), 138.0 (C-9), 128.6 (C-4), 120.4 (C-19), 118.9 (C-3a), 116.9 (C-8), 107.2 (C-5), 97.2 (C-7), 64.1 (C-18), 61.6 (C-12), 61.4 (C-3), 55.6 (OCH_3), 44.8 (C-15), 29.2 (C-13), 25.4 (C-21), 22.2 (C-14), 18.3 (C-22).

Tryprostatin B (2): Pale yellow solid, m.p.

102-104 °C, $[\alpha]_D^{25}$ -56.3 (*c* 0.05, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 8.01 (1H, s, NH), 7.47 (1H, d, *J* = 7.5 Hz, H-13), 7.31 (1H, d, *J* = 7.5 Hz, H-16), 7.17 (1H, t, *J* = 7.5 Hz, H-15), 7.08 (1H, t, *J* = 7.5 Hz, H-14), 5.63 (1H, s, NH), 5.31 (1H, t, *J* = 8.0 Hz, H-21), 4.36 (1H, dd, *J* = 2.5, 11.0 Hz, H-3), 4.05 (1H, t, *J* = 7.5 Hz, H-6), 3.70 (1H, m, H_a-9), 3.67 (1H, m, H_a-10), 3.60 (1H, m, H_b-9), 3.48 (2H, m, H-20), 2.96 (1H, dd, *J* = 11.5, 15.0 Hz, H_b-10), 2.32 (1H, m, H_a-7), 2.05 (1H, m, H_b-7), 2.02 (1H, m, H_a-8), 1.90 (1H, m, H_b-8), 1.79 (3H, s, H-23), 1.74 (3H, s, H-24). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 169.4 (C-5), 165.7 (C-2), 136.4 (C-19), 135.5 (C-17), 135.4 (C-22), 128.1 (C-12), 121.8 (C-15), 119.9 (C-14), 119.8 (C-21), 117.7 (C-13), 110.8 (C-16), 104.6 (C-11), 59.4 (C-6), 54.7 (C-3), 45.4 (C-9), 28.4 (C-7), 25.7 (C-23), 25.5 (C-10), 25.1 (C-20), 22.6 (C-8), 18.1 (C-24).

12,13-Dihydroxy-fumitremorgin C (3): Pale yellow solid, $[\alpha]_D^{25}$ +21.8 (*c* 0.05, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 7.79 (1H, d, *J* = 8.5 Hz, H-13), 7.76 (1H, br. s, NH), 6.82 (1H, d, *J* = 1.5 Hz, H-16), 6.80 (1H, dd, *J* = 2.0, 8.5 Hz, H-14), 5.87 (1H, d, *J* = 9.5 Hz, H-20), 5.72 (1H, s, H-10), 4.79 (1H, d, *J* = 9.5 Hz, H-21), 4.43 (1H, dd, *J* = 2.0, 9.5 Hz, H-6), 3.81 (3H, s, OMe), 3.62 (2H, m, H_a-9, H_b-9), 2.46 (1H, m, H_a-7), 2.11 (1H, m, H_a-8), 2.05 (1H, m, H_b-7), 1.98 (3H, s, H-24), 1.93 (1H, m, H_b-8), 1.66 (3H, s, H-23). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 171.1 (C-5), 166.3 (C-2), 156.6 (C-15), 137.5 (C-17), 134.5 (C-22), 130.2 (C-19), 124.0 (C-21), 121.3 (C-13), 120.6 (C-12), 109.8 (C-14), 105.4 (C-11), 95.1 (C-16), 83.1 (C-3), 68.8 (C-10), 58.9 (C-6), 55.9 (15-OMe), 50.1 (C-20), 45.3 (C-9), 29.2 (C-7), 25.7 (C-23), 22.6 (C-8), 18.3 (C-24).

Bis(dethio)bis(methylthio)gliotoxin (4): White solid, m.p. 204-206 °C, $[\alpha]_D^{25}$ -43.8 (*c* 0.05, $CHCl_3$). ESI-MS: *m/z* 357 $[M+H]^+$ ($C_{15}H_{21}N_2O_4S_2$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 5.93 (1H, m, H-9), 5.87 (1H, dd, *J* = 5.0, 9.0 Hz, H-7), 5.71 (1H, br. d, *J* = 9.5 Hz, H-8), 4.91 (2H, m, H-6, H-5a), 4.35 (1H, d, *J* = 12.0 Hz, H_a-15), 3.88 (1H, d, *J* = 12.0 Hz, H_b-15), 3.13 (3H, s, CH_3-13), 3.06 (1H, d, *J* = 15.5 Hz, H_a-10), 2.94 (1H, br. d, *J* = 16.0 Hz, H_b-10), 2.26 (3H, s, CH_3-14), 2.24 (3H, s, CH_3-12). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 166.8 (C-4), 166.0 (C-1), 131.7 (C-9a), 129.9 (C-8), 123.2 (C-7), 120.1 (C-9), 74.4 (C-6), 72.2 (C-3), 71.5 (C-11), 69.6 (C-5a), 63.6 (C-15), 38.9 (C-10), 28.5 (C-13), 14.8 (C-12), 13.6 (C-14).

1*H*-Indole-3-ethanol (5): White solid, m.p. 58-59 °C (ref. 56-59 °C [6]). ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 7.64 (1H, d, *J* = 8.0 Hz, H-4), 7.35 (1H, d, *J* = 8.0 Hz, H-7), 7.22 (1H, t, *J* = 8.0 Hz, H-6), 7.15 (1H, t, *J* = 8.0 Hz, H-5), 7.05 (1H, s, H-2), 3.93 (2H, t, *J* = 6.5 Hz, CH_2-9), 3.04 (2H, t, *J* = 6.5 Hz, CH_2-8). ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 136.6 (C-7a), 127.5 (C-3a), 122.5 (C-2), 122.2 (C-6), 119.3 (C-5), 118.8 (C-4), 112.2 (C-3), 111.4 (C-7), 62.6 (C-9), 28.6 (C-8).

***N*-Acetyltryptamine (6):** White solid, m.p. 79-80 °C (ref. 78-79 °C [7]). ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 7.13-7.61 (4H, m, H-aromatic), 7.03 (1H, d, *J* = 2.0 Hz, H-2), 3.60 (2H, q, *J* = 6.5 Hz, CH_2), 2.99 (2H, t, *J* = 6.5 Hz, CH_2), 1.92 (3H, s, CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 170.3 (C=O), 136.4 (C-7a), 127.3 (C-3a), 121.1 (C-6), 119.3 (C-5), 118.5 (C-4), 112.7 (C-3), 111.3 (C-7), 39.8 (CH_2N-), 25.4 (CH_2-1′), 23.4 (CH_3).

3-Formylindole (7): Pale yellow solid, ESI-MS: *m/z* 146.1 $[M+H]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 10.2 (-CHO), 8.33 (1H, m, H-4), 7.85 (1H, d, *J* = 3.0 Hz, H-2), 7.44 (1H, m, H-7), 7.33 (1H, m, H-6), 7.31 (1H, m, H-5). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 185.1 (CHO), 136.8 (C-3a), 135.1 (C-2), 124.4 (C-6), 124.0 (C-3), 123.1 (C-5), 122.1 (C-4), 120.1 (C-7a), 111.3 (C-7).

3-Hydroxyacetylindole (8): Pale yellow solid, ESI-MS: *m/z* 176 $[M+H]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 8.25 (1H, m, H-4), 8.20 (1H, d, *J* = 3.0 Hz, H-2), 7.48 (1H, m, H-7), 7.23-7.28 (2H, m, H-5, H-6), 4.74 (2H, s, CH_2). ^{13}C-NMR (125 MHz; $CDCl_3$): δ_C (ppm) 193.2 (C=O), 136.3 (C-7a), 130.5 (C-2), 125.2 (C-3a), 124.3 (C-6), 123.1 (C-5), 122.1 (C-4), 114. 3 (C-3), 111.6 (C-7), 65.5 (CH_2-9).

2.5. Antimicrobial assay

The antimicrobial assay was carried out using *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* (ATCC 13076), *Enterococcus faecalis* (ATCC 13124), *Staphylococcus aureus* (ATCC 299212), *Bacillus cereus* (ATCC 14579), and *Candida albicans* (ATCC 10231). The samples were initially diluted in DMSO at a decreasing concentration range: 256, 128, 64, 32, 16, and 8 μg/mL for the assays. The final concentration of microbial inoculum was adjusted to 2×10^5 CFU/mL in each well. All microplates were incubated at 37°C with shaking at 120 rpm. After 24 h, the minimum inhibitory concentration (MIC) was determined as the lowest concentration of the antimicrobial agent that

completely inhibited visible microbial growth [6]. Streptomycin (256, 128, 64, and 32 µg/mL) and nystatin (32 µg/mL) were used as reference compounds and subjected to the same dilution series as the test samples.

3. Results and discussion

Compound **1** was isolated as a pale yellow amorphous powder, with a specific optical rotation of $[\alpha]_D^{25}$ -36.2 (*c* 0.05, $CHCl_3$) (ref. -47.7 (*c* 0.26, $CHCl_3$)) [8]. The ^{1}H-NMR spectrum revealed the presence of ABX-type aromatic proton resonances at δ_H 6.94 (1H, d, *J* = 8.0 Hz, H-4), 6.52 (1H, dd, *J* = 2.0, 8.5 Hz, H-5), 6.44 (1H, d, *J* = 2.0 Hz, H-7), two olefinic protons at δ_H 5.75 (1H, s, H-8), and 5.20 (1H, d, *J* = 9.0 Hz, H-19), two singlet methyl groups at δ_H 1.58 (3H, s, CH_3-21), 1.30 (3H, s, CH_3-22), and one methoxy group at δ_H 3.79 (3H, s, OCH_3). The remaining protons in the aliphatic region at δ_H 1.99 - 4.33 were also noted. The ^{13}C-NMR and DEPT spectra, with the aid of the HSQC spectrum of **1**, showed the presence of twenty-two carbons, including three carbonyl groups (δ_C 178.9, 162.5, and 155.3), two methyl groups (δ_C 25.4 and 18.3), one methoxy group (δ_C 55.6), three methylene groups (δ_C 44.8, 29.2, and 22.2), five sp^2 methine groups (δ_C 128.6, 120.4, 116.9, 107.2, and 97.2), two sp^3 methine groups (δ_C 64.1 and 61.6), and six non-protonated carbons (δ_C 160.6, 141.6, 138.2, 138.0, 118.9, and 61.4). Analysis of the ^{1}H- and ^{13}C-NMR data of compound **1** revealed that it is a spirotryprostatin derivative, a type of compound previously isolated from marine-derived fungi. The COSY spectrum of **1** indicated the presence of three spin-spin coupling systems shown in bold link in Fig. 2, including H-18/ H-19, H-12/CH_2-13/CH_2-14/CH_2-15, and H-4/H-5. The HMBC correlations between H-12 with C-11/C-13, between the proton of CH_2-13 with C-11/C-14/C-15, between H-8 with C-17/C-18/C-3/C-9, and between H-18 with C-9/C-8/C-3/C-2 suggested that **1** was a diketopiperazine derivative. The presence of a 2-methylprop-1-enyl group at C-18 was confirmed by the HMBC correlations from H-19 (δ_H 5.20) to C-21 (δ_C 25.4)/C-22 (δ_C 18.3), from two methyl protons at δ_H 1.58 (CH_3-21) and 1.30 (CH_3-22) to C-20 (δ_C 138.2)/C-19 (δ_C 120.4) and from H-18 (δ_H 5.37) to C-19 (δ_C 120.4)/C-20 (δ_C 138.2) and the COSY correlations of H-18 (δ_H 5.37) and H-19 (δ_H 5.20). The methoxy group was confirmed at C-6 based on the correlation from the protons of this methoxy group (δ_H 3.80) to C-6 (δ_C 160.6) (Fig. 3). Careful analysis of the 2D NMR spectra of **1**, together with comparison with the reported data and the agreement of its optical rotation ($[\alpha]_D^{25}$ -36.2, *c* 0.05, $CHCl_3$) with the literature value ($[\alpha]_D^{25}$ -47.7, *c* 0.26, $CHCl_3$) [8], confirmed its structure as 6-methoxyspirotryprostatin B. Compound **1** has been reported to exhibit cytotoxicity against A-549 and HL-60 cell lines, with IC_{50} values of 8.29 and 9.71 µM, respectively [8].

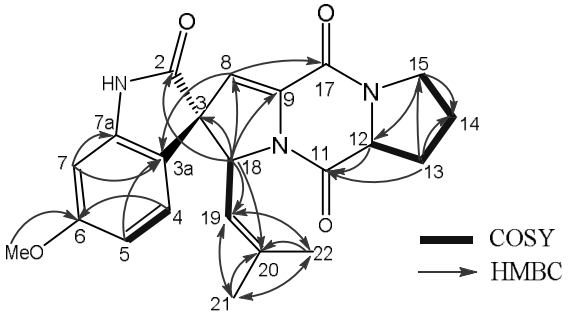

Fig. 2. Key COSY and HMBC correlations of compound **1**

Compound **2** was obtained as a pale yellow solid, with a specific optical rotation of $[\alpha]_D^{25}$ –56.3 (*c* 0.05, $CHCl_3$) (ref. -77 (*c* 0.6, $CHCl_3$) [8, 9]. The ^{1}H-NMR spectrum of **2** indicated thepresence of four aromatic protons of a 1,2-disubstituted benzene ring at δ_H 7.47 (1H, d, *J* = 7.5 Hz, H-13), 7.31 (1H, d, *J* = 7.5 Hz, H-16), 7.17 (1H, t, *J* = 7.5 Hz, H-15), and 7.08 (1H, t, *J* = 7.5 Hz, H-14), one olefinic proton at δ_H 5.31 (1H, t, *J* = 8.0 Hz, H-21), and two singlet methyl groups at δ_H 1.79 (3H, s, CH_3-23) and 1.74 (3H, s, CH_3-24). The remaining protons in the aliphatic region at δ_H 1.90-4.36 were also noted. Analyses of the ^{13}C-NMR, DEPT, and HSQC spectra of **2** showed the presence of twenty-one carbons, including two amide carbonyl groups at δ_C 169.4 (C-5) and 165.7 (C-2), two methyl groups at δ_C 25.7 (C-23) and 18.1 (C-24), five methylene groups at δ_C 45.4 (C-9), 28.4 (C-7), 25.5 (C-10), 25.1 (C-20), and 22.6 (C-8), five sp^2 methine groups at δ_C 121.8 (C-15), 119.9 (C-14), 119.8 (C-21), 117.7 (C-13), and 110.8 (C-16), two sp^3 methine groups at δ_C 54.7 (C-3) and 59.4 (C-6), and five non-protonated carbons. The 1D-NMR spectra of **2** suggested that it was a diketopiperazine derivative. The HMBC correlations from two methyl protons at δ_H 1.79 (CH_3-23), 1.74 (CH_3-24) to C-22/C-21, from H-21 to C-19/C-20/C-23/C-24, from methylene

protons of CH_2-20 to C-11/C-19/C-21/C-22 suggested the presence of an isoprenyl group at C-19 (Fig. 3). Complete analyses of the 2D NMR spectra of **2**, together with comparison with the reported data and the consistency of its optical rotation ($[\alpha]_D^{25}$ -56.3, *c* 0.05, $CHCl_3$) with the literature value ($[\alpha]_D^{25}$ -77, *c* 0.6, $CHCl_3$), confirmed its structure as tryprostatin B [8],[9]. Tryprostatin B was a diketopiperazine derivative described for the first time from *Aspergillus fumigatus* BM939 [10].

Compound **3** was isolated as a pale yellow solid, with a specific optical rotation of $[\alpha]_D^{25}$ +21.8 (*c* 0.05, $CHCl_3$) (ref. +9.0 (*c* 0.4, $CHCl_3$) [11]. Analyses of the ^{13}C-NMR and DEPT spectra, with the aid of the HSQC spectrum, revealed the presence of twenty two carbons, including two carbonyl groups at δ_C 171.1 (C-5) and 166.3 (C-2), four sp^2 methine groups at δ_C 121.3 (C-13), 109.8 (C-14), 95.1 (C-16), and 124.0 (C-21), three sp^3 methine groups at δ_C 68.8 (C-10), 58.9 (C-6), and 50.1 (C-20), three methylene groups at δ_C 45.3 (C-9), 29.2 (C-7), and 22.6 (C-8), two methyl groups at δ_C 25.7 (C-23) and 18.3 (C-24), one methoxy group at δ_C 55.9 (OMe), and seven non-protonated carbons. The ^{1}H-NMR spectrum of **3** showed signals of two methyl singlets at δ_H 1.66 (3H, s, CH_3-23) and 1.98 (3H, s, CH_3-24), one methoxy at δ_H 3.81 (3H, s, OMe), three aromatic protons of an ABX system at δ_H 7.79 (1H, d, *J* = 8.5 Hz, H-13), 6.82 (1H, d, *J* = 1.5 Hz, H-16), and 6.80 (1H, dd, *J* = 2.0, 8.5 Hz, H-14), one olefinic proton at δ_H 4.79 (1H, d, *J* = 9.5 Hz, H-21), one secondary amine group at δ_H 7.76 (1H, br. s, NH), and the remaining protons at δ_H 1.93 - 5.87. The position of the methoxy group at C-15 was confirmed by the HMBC correlations from the methoxy proton (δ_H 3.81) to C-15 (δ_C 156.6). The presence of a 2-methylprop-1-enyl group at C-20 and C-20 linked to C-19 and N-4 was confirmed by the HMBC correlations from the two methyl protons CH_3-23, CH_3-23 to C-22/C-21, from H-21 to C-19/C-20/C-23/C-24, and from H-20 to C-11/C-3/C-19/C-21 (Fig. 3). Detailed analysis of the 2D spectra of **3**, together with comparison with the literature data and the consistency of its optical rotation ($[\alpha]_D^{25}$ +21.8, *c* 0.05, $CHCl_3$) with the reported value ($[\alpha]_D^{25}$ +9.0, *c* 0.4, $CHCl_3$), confirmed its structure as 12,13-dihydroxy-fumitremorgin C [11]. This compound exhibited strong anti-tuberculosis activity towards *Mycobacterium tuberculosis* with an MIC_{50} value of 2.41 μM [12]. Compound **3** also exhibited cytotoxicity against human leukemia cancer cells (U-937), prostate cancer (PC-3), and human colon carcinoma (HCT-116) cell lines, with IC_{50} values of 1.8, 6.6, and 4.53 μM, respectively [13].

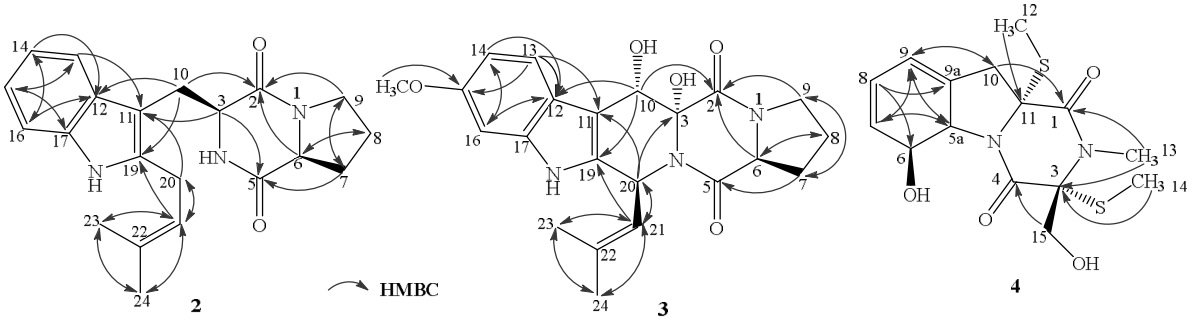

Fig. 3. Key HMBC correlations of compounds **2-4**

Compound **4** was isolated as a white solid, with a specific optical rotation of $[\alpha]_D^{25}$ -43.8 (*c* 0.05, $CHCl_3$) (ref. -51 (*c* 1.0, MeOH)) [14]. The ^{1}H NMR spectrum of **4** showed signals of three methyl groups at δ_H 2.24 (3H, s, CH_3-12), 3.13 (3H, s, CH_3-13), and 2.26 (3H, s, CH_3-14), 3 olefinic protons at δ_H 5.93 (1H, m, H-9), 5.87 (1H, dd, *J* = 5.0, 9.0 Hz, H-7), and 5.71 (1H, br. d, *J* = 9.5 Hz, H-8), one oxymethylene group at δ_H 3.88 (1H, d, *J* = 12.0 Hz, H_b-15) and 4.35 (1H, d, *J* = 12.0 Hz, H_a-15). In the ^{13}C-NMR and HSQC spectra of **4**, the presence of fifteen carbon signals were noted, including three methyl groups at δ_C 28.5 (C-13), 14.8 (C-12), and 13.6 (C-14), one sp^3 methylene group at δ_C 38.9 (C-10), one sp^3 oxymethylene group at δ_C 63.6 (C-15), three

sp^2 methine groups at δ_C 129.9 (C-8), 123.2 (C-7), and 120.1 (C-9), two sp^3 methine groups at δ_C 74.4 (C-6) and 69.6 (C-5a), two sp^3 non-protonated carbons at δ_C 72.2 (C-3) and 71.5 (C-11), one sp^2 non-protonated carbon at δ_C 131.7 (C-9a), and two carbonyl groups at δ_C 166.8 (C-4) and 166.0 (C-1). The ^{13}C chemical shifts of C-5a (δ_C 69.6), C-6 (δ_C 74.4), and C-15 (δ_C 63.6), together with the ^1H chemical shifts of the *N*-methyl group at δ_H 3.13 (CH_3-13), are characteristic of carbons bonded to heteroatoms, consistent with oxygen and nitrogen connections. In addition, the mass spectrum of **4** exhibited a molecular ion peak at *m/z* 357, along with the characteristic isotopic pattern indicative of two sulfur atoms, confirming the presence of methylthio groups in the structure of **4**. A detailed comparison of the NMR data with those reported for bis(dethio)bis(methylthio)gliotoxin revealed their similarity [15]. The HMBC correlations from the CH_3N and CH_3S protons at δ_H 2.24 (CH_3-12), 2.26 (CH_3-14), and 3.13 (CH_3-13) to C-11, C-3, and C-1/C-3, respectively, confirmed the attachment of these groups to their corresponding carbon atoms in the structure of **4**. Comparison with reference data confirmed the presence of two methyl groups attached to sulfur atoms. Thus, detailed analyses of the MS and 2D NMR spectra of **4**, together with comparison with the reported data and the agreement of its optical rotation $[\alpha]_D^{25}$ -43.8, *c* 0.05, $CHCl_3$) with the literature value ($[\alpha]_D^{25}$ -51, *c* 1.0, MeOH), confirmed its structure as bis(dethio)bis(methylthio)gliotoxin [15]. This compound showed inhibitory activity against PAF-induced platelet aggregation, with an IC_{50} value of 8.4 μM [16].

The known indole alkaloids were elucidated as 1*H*-indole-3-ethanol (**5**) [17], *N*-acetyltryptamine (**6**) [7], 3-formylindole (**7**) [18], and 3-hydroxyacetylindole (**8**) [19],[20] by comparison of their NMR spectral data with those reported in the literature.

All isolated compounds were evaluated for their antibacterial activity against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* (ATCC 13076), *Enterococcus faecalis* (ATCC 299212), *Staphylococcus aureus* (ATCC 25923), and *Bacillus cereus* (ATCC 14579), as well as for their antifungal activity against *Candida albicans* (ATCC 10231) (Table 1). Compounds **1** to **4** showed significant antimicrobial activity against the Gram-positive *E. faecalis* and the yeast *C. albicans* with MIC values ranging from 64 to 256 μg/mL (Table 1). In addition, compound **2** also displayed significant antibacterial activity against the Gram-negative *S. enterica* with an MIC value of 256 μg/mL. Compound **7** showed broad-spectrum activity, inhibiting all three Gram-positive strains, one Gram-negative strain (*S. enterica*), and the yeast *C. albicans* with MIC values ranging from 16 to 128 μg/mL. Meanwhile, compound **5** strongly inhibited *E. coli* with an MIC value of 8 μg/mL, but was inactive against the remaining test strains. These results suggest that compounds **5** and **7** may serve as potential antimicrobial agents.

Table 1. Antimicrobial activity of compounds **1**–**5** and **7**

Compounds	Gram (+)			Gram (-)			Yeast
	E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C. albicans*
	MIC (μg/mL)						
1	256	–	–	–	–	–	128
2	64	–	–	–	–	256	128
3	256	–	–	–	–	–	256
4	256	–	–	–	–	–	128
5	–	–	–	8	–	–	–
7	16	64	32	–	–	64	128
*Streptomycin**	256	256	128	32	256	128	–
*Nystatin**	–	–	–	–	–	–	32

*Positive control; (–) not active.

4. Conclusion

In summary, from the ethyl acetate extract of the marine-derived fungus *Penicillium* sp. M874, eight secondary metabolites (**1**-**8**) were isolated

and structurally determined by NMR spectroscopy analysis and by comparison of their NMR data with the literature. These compounds included four diketopiperazine alkaloids, 6-methoxyspirotryprostatin B (**1**), tryprostatin B (**2**), 12,13-dihydroxy-fumitremorgin C (**3**), and bis(dethio)bis(methylthio)gliotoxin (**4**), and four indoles, 1*H*-indole-3-ethanol (**5**), *N*-acetyltryptamine (**6**), 3-formylindole (**7**), and 3-hydroxyacetylindole (**8**). Compounds **1-5** and **7** exhibited antimicrobial activity against one to five tested strains, with MIC values ranging from 8 to 256 µg/mL. Notably, compound **5** exhibited selective activity against *E. coli*, with an MIC value of 8 µg/mL. Compound **1** is reported here for the first time from the *Penicillium* genus. These compounds are commonly found in fungi, particularly within the genera *Penicillium* and *Aspergillus*. This study highlights the potential of sediment-derived *Penicillium* sp. as a valuable source of antimicrobial secondary metabolites.

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