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Chemical constituents produced by the marine fungi *Penicillium* sp. M839 and their antimicrobial evaluation

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ABSTRACT

From the agar-based culture of the marine-derived *Penicillium* sp. M839 strain, nine known compounds were isolated and structurally determined, including fumiquinazoline D (1), fumiquinazoline B (2), cerevisterol (3), stoloniferol B (4), norhaman (5), 3,4-dihydroxy-6,7-dimethyl-quinolin-2-carboxylic (6), uracil (7), 3-methyl uracil (8), thymine (9). These compounds were characterized via 1D and 2D NMR spectroscopic and mass spectrometric analyses. Compounds 2 and 8 were first recognized from the genus *Penicillium*, while the remaining compounds were previously isolated from this genus. Compounds 1–6 were evaluated for their antimicrobial activity against a panel of test microorganisms (three Gram-positive bacteria: *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*; three Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella enterica* and one yeast strain *Candida albicans*). The results showed that compound 6 displayed strong antimicrobial activity against two Gram-negative bacteria *E. coli* and *S. enterica,* with the MIC values of 32 and 64 µg/mL, respectively. In addition, the remaining compounds 1–5 inhibited against all test microorganisms with MIC values ranging from 64 to 256 µg/mL.

Keywords: *Penicillium* sp. M839, marine fungi, alkaloid, sterol, antimicrobial.

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INTRODUCTION

Penicillium has gained significant recognition as one of the most well-known fungal genera due to the discovery of bioactive compounds [1, 2]. This genus is ubiquitous, found in diverse environments, and is especially prevalent in marine ecosystems, where it is one of the most widespread fungal groups [1, 2]. Notably, various *Penicillium* species have already led to the development of major clinical drugs [2]. It is impossible to discuss *Penicillium* without mentioning penicillin, a discovery that revolutionized medical treatment by introducing antibiotics and eradicating numerous human diseases caused by pathogenic bacteria [2]. Since terrestrial *Penicillium* species are known to produce many biologically active compounds, their abundance in marine environments (marine-derived fungi from the *Penicillium* genus) presents a promising source of novel agents with potential therapeutic applications [2, 3]. In Vietnam, there are limited publications on the chemical constituents of the marine fungi *Penicillium* [4]. During our screening program, the EtOAc extract of the *Penicillium* sp. M839 strain exhibited antimicrobial activity against two Gram-positive bacteria and one Gram-negative bacteria strain: *Staphylococus aureus* ATCC25923, *Enterococcus faecalis* ATCC29212, *Escherichia coli* ATCC25922 with MIC values of 32, 128 and 64 μg/mL, respectively. This paper reported the isolation and structural elucidation of nine compounds **1-9** from the marine-derived fungus *Penicillium* sp. M839 strain was isolated from sediment samples collected at the Ran Trao conservation area - Khanh Hoa sea area (Vietnam).

MATERIALS AND METHODS

Marine sediment sample

The marine sediment sample (Code: HP03/20-VP04-TT07) was collected in May 2021 at the Ran Trao conservation area, Khanh Hoa sea area, Vietnam. It was collected at 11 m depth with geographic coordination of 12.0^{o}37’34” - 109.0^{o}12’49” and water temperature of 28^{o}C. This sample was collected in a sterile 50 mL Falcon tube, preserved on ice, and processed within 24 hours.

Isolation and Identification of the fungus M839

The sediment sample was homogenized and subjected to a wet-heat treatment at 60^{o}C for 6 minutes. The suspension was diluted using a ten-fold dilution series down to 10^{-3}. Aliquots of 50 µL were then spread onto Petri dishes filled with PDA solid medium (30 g/L potato extract, 20 g/L dextrose, 30 g/L Instant Ocean, and 15 g/L agar). The plates were incubated at 28^{o}C for 7 days. A colony of the fungus M839 was subsequently transferred to a fresh Petri dish containing PDA medium for purification (Fig. 1).

The strain M839 was identified as belonging to the genus *Penicillium* through 18S rRNA gene sequence analysis, which was compared with fungal sequences in the GenBank database using the NCBI BLAST program.

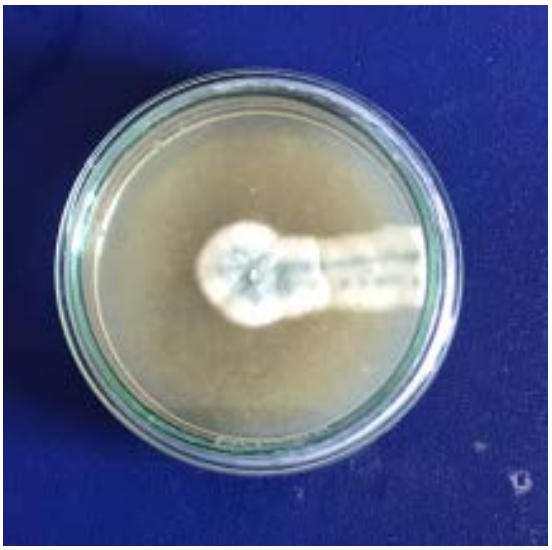

Figure 1. Morphological appearance of M839 strain’s colonies

Fermentation fungus M839

The strain M839 was activated and inoculated into 1 L of PDB broth medium pH 7.0 (comprising 30 g/L potato extract, 20 g/L

dextrose, 30 g/L instant ocean). After 7 days of incubation at 28°C with shaking at 100 rpm, the culture broth was spread on the medium surface of 50 flasks (each 3 L flask containing 1 L of PDA medium, pH 7.0). The flasks were incubated at 28°C and harvested on the twenty-one days.

General experiment procedures

Optical rotations were recorded on a JASCO P-2000 polarimeter using a sodium (589 nm, D line) lamp. Melting points were measured using a Thermo Mel-Temp 3.0 (Thermo Scientific). The NMR spectra were recorded on Bruker Avance 500 MHz and 600 MHz spectrometers, and tetramethylsilane (TMS) was used as an internal standard. Thin layer chromatography used pre-coated silica gel Merck 60 F_{254}. Column chromatography (CC) was performed on silica gel (Kiesel gel 60, 70–230 mesh, and 230–400 mesh), or Sephadex LH-20 resins (Sigma-Aldrich, USA), and reversed-phase C_{18} (RP-18, 30–50 µm). All solvents used in column chromatography were distilled before use.

Extraction and isolation

The culture of *Penicillium* sp. M839 (45 kg) were cut into small pieces and sonicated in ethyl acetate (30 L) at 45–50°C (5 times for 4 hours each). The filtrated solution was concentrated under reduced pressure to give the crude ethyl acetate extract (65.4 g). The EtOAc extract was subjected to a silica gel column chromatography (CC) and eluted with a solvent gradient mixture CH_2Cl_2/MeOH to yield six fractions F1-F6. Purification of fraction F2 (5.3 g) by a Sephadex LH-20 column using MeOH/CH_2Cl_2 (90/1, *v/v*), followed by a silica gel CC using CH_2Cl_2/acetone (5/1, 4/1, 3/1, *v/v*), afforded compound **4** (13 mg) and compound **5** (8.6 mg). Fraction F3 (534 mg) was subjected to CC on Sephadex LH-20 (CH_2Cl_2/MeOH (2/8, *v/v*)), followed by CC on a silica gel column (dichloromethane/ acetone (4/1, 3/1, *v/v*) to afford compound **8** (16 mg). Fraction F4 (2.1 mg) was separated into six subfractions (F4.1-F.4.6) by CC on silica gel using the solvent mixtures of CH_2Cl_2/MeOH gradient. Sub-fractions F4.3 (123 mg) and F4.4 (121 mg) were recrystallized in a solvent mixture of CH_2Cl_2/MeOH (9/1, *v/v*) to afford compound **9** (41 mg) and compound **7** (21 mg), respectively. Fraction F5 (4.7 g) was chromatographed on a reversed-phase C_{18} (RP- C_{18}) silica gel column using an H_2O/MeOH solvent system (2/1, 1/1, *v/v*) to obtain two subfractions (F5.1 and F5.2). Compounds **3** (7.8 mg) and **6** (29 mg) were purified from sub-fraction F5.1 (643 mg) by subjecting them to silica gel CC using a CH_2Cl_2/MeOH gradient. Compounds **1** (11 mg) and **2** (9 mg) were purified from sub-fraction F5.2 (1.3 g) after being subjected to a Sephadex LH-20 column with MeOH/CH_2Cl_2 (8/2, *v/v*), followed by a silica gel CC using CH_2Cl_2/acetone gradient.

Fumiquinazoline D (**1**): White solid, mp: 215–216°C, $[\alpha]_D^{25}$ = + 56.7 (*c* 1.73, $CHCl_3$). ^{1}H-NMR (acetone-d_6, 600 MHz): δ_H (ppm) 1.13 (3H, d, *J* = 6.6 Hz, CH_3-29), 2.18 (3H, s, CH_3-16), 2.32 (1H, dd, *J* = 15.0, 0.6 Hz, H_a-15), 3.21 (1H, dd, *J* = 15.0, 10.4 Hz, H_b-15), 4.25 (1H, td, *J* = 6.6, 1.2 Hz, H-20), 5.42 (1H, dd, *J* = 9.0, 0.2 Hz, H-14), 5.61 (OH-17), 5.76 (1H, d, *J* = 1.8 Hz, H-18), 7.16 (1H, td, *J* = 7.8, 1.2 Hz, H-26), 7.30 (1H, td, *J* = 7.8, 1.2 Hz, H-25), 7.43 (1H, d, *J* = 7.8 Hz, H-24), 7.52 (1H, d, *J* = 7.2 Hz, H-27), 7.54 (1H, td, *J* = 7.8, 1.2 Hz, H-9), 7.72 (1H, d, *J* = 7.8 Hz, H-7), 7.83 (1H, td, *J* = 7.8, 1.8 Hz, H-8), 8.18 (1H, dd, *J* = 7.8, 1.2 Hz, H-10).^{13}C-NMR (acetone-d_6, 150 MHz): δ_C (ppm) 17.9 (C-29), 19.3 (C-16), 44.1 (C-15), 54.0 (C-14), 59.5 (C-20), 71.9 (C-3), 84.4 (C-17), 86.5 (C-18), 115.8 (C-24), 121.7 (C-11), 125.1 (C-27), 125.9 (C-26), 127.4 (C-10), 128.0 (C-9), 128.5 (C-7), 130.1 (C-25), 135.3 (C-8), 139.1 (C-23), 139.7 (C-28), 147.6 (C-6), 154.4 (C-4), 161.2 (C-12), 169.6 (C-1), 172.9 (C-21).

Fumiquinazoline B (2): Pale yellow solid, mp: 174–175°C, $[\alpha]_D^{25}$ = -176.8 (*c* 0.23, $CHCl_3$), ^{1}H-NMR (acetone-d_6, 600 MHz): δ_H (ppm) 1.23 (3H, d, *J* = 6.6 Hz, CH_3-29), 1.87 (3H, d, *J* = 7.2 Hz, CH_3-16), 2.47 (1H, dd, *J* = 13.8, 5.4 Hz, H_a-15), 2.64 (1H, dd, *J* = 13.8, 11.4 Hz, H_b-15), 4.17 (1H, td, *J* = 6.6, 1.2 Hz, H-20), 4.81 (1H, q, *J* = 14.4, 7.2 Hz, H-3), 5.36 (1H, d, *J* = 5.4 Hz, H-18), 5.70 (1H, td, *J* = 7.8, 1.2 Hz, H-26), 7.33 (1H, td, *J* = 7.8, 1.2 Hz, H-25), 7.48 (1H, d, *J* = 9.0 Hz, H-24), 7.51 (1H, td, *J* = 7.2, 1.2 Hz, H-9), 7.62 (1H,

d, *J* = 7.8 Hz, H-7), 7.63 (1H, d, *J* = 7.2 Hz, H-27), 7.81 (1H, td, *J* = 7.2, 1.8 Hz, H-8), 8.15 (1H, dd, *J* = 7.8, 1.2 Hz, H-10).^{13}C-NMR (acetone-d_6,150 MHz): δ_C (ppm) 18.4 (C-29), 24.9 (C-16), 39.8 (C-15), 52.9 (C-14), 53.4 (C-3), 59.5 (C-20), 80.9 (C-17), 87.4 (C-18), 115.2 (C-24), 121.2 (C-11), 125.5 (C-27), 126.2 (C-26), 127.3 (C-7), 127.5 (C-10), 127.8 (C-9), 129.9 (C-25), 135.4 (C-8), 138.2 (C-23), 140.5 (C-28), 148.4 (C-6), 153.0 (C-4), 161.0 (C-12), 171.0 (C-1), 171.7 (C-21).

Cerevisterol (3): White solid, mp: 225–226°C, $[\alpha]_D^{25}$ = -67 (0.07, MeOH), ^{1}H-NMR (DMSO-d_6, 600 MHz): δ_H (ppm) 0.54 (3H, s, CH_3-18), 0.80 (3H, d, *J* = 7.2 Hz, CH_3-27), 0.82 (3H, d, *J* = 7.8 Hz, CH_3-26), 0.88 (3H, d, *J* = 6.6 Hz, CH_3-28), 0.90 (3H, s, CH_3-19), 0.99 (3H, d, *J* = 6.6 Hz, CH_3-21), 3.36 (1H, d, *J* = 5.4 Hz, H-6), 3.58 (1H, s, OH-5), 3.77 (1H, m, H-3), 4.23 (1H, d, *J* = 5.4 Hz, OH-3), 4.48 (1H, d, *J* = 5.4 Hz, OH-6), 5.05 (1H, dd, *J* = 2.4, 5.4 Hz, H-7), 5.17 (1H, dd, *J* = 8.4, 15.6 Hz, H-22), 5.24 (1H, dd, *J* = 7.2, 15.6 Hz, H-23). ^{13}C-NMR (DMSO-d_6, 150 MHz): δ_C (ppm) 12.1 (C-18), 17.3 (C-28), 17.7 (C-19), 19.5 (C-26), 19.7 (C-27), 21.0 (C-21), 21.3 (C-11), 22.6 (C-15), 27.7 (C-16), 31.2 (C-2), 32.5 (C-1, C-25), 36.7 (C-10), 39.2 (C-12), 39.6 (C-4), 40.2 (C-20), 42.0 (C-24), 42.3 (C-9), 43.0 (C-13), 54.2 (C-14), 55.3 (C-17), 66.0 (C-3), 72.1 (C-6), 74.5 (C-5), 119.4 (C-7), 131.4 (C-23), 135.4 (C-22), 139.7 (C-8).

Stoloniferol B (4): Colorless needle, mp: 140–141°C, $[\alpha]_D^{25}$ = +93.3 (*c* 0.05, $CHCl_3$), ^{1}H-NMR ($CDCl_3$, 600 MHz): δ_H (ppm) 1.31 (3H, d, *J* = 6.6 Hz, CH_3-13), 1.33 (3H, d, *J* = 6.6 Hz, CH_3-12), 2.10 (3H, s, CH_3-11), 2.97 (1H, dt, *J* = 7.2, 6.0 Hz, H-4), 4.68 (1H, dt, *J* = 7.8, 6.6 Hz, H-3), 6.30 (1H, s, H-7), 11.35 (1H, s, OH-8). ^{13}C-NMR ($CDCl_3$,150 MHz): δ_C (ppm) 9.9 (C-11), 19.7 (C-12), 20.0 (C-13), 34.8 (C-4), 80.0 (C-3), 100.4 (C-9), 101.4 (C-7), 113.3 (C-5), 143.1 (C-10), 161.0 (C-6), 162.4 (C-8), 168.6 (C-1).

Norharman (5): White solid, mp: 198–200°C, ^{1}H NMR (500 MHz, CD_3OD): δ_H (ppm) 7.29 (1H, dd, *J* = 8.0, 2.5 Hz, H-6), 7.58 (1H, dd, *J* = 8.0, 0.5 Hz, H-7), 7.59 (1H, d, *J* = 8.0 Hz, H-8), 8.11 (1H, d, *J* = 5.0 Hz, H-4), 8.21 (1H, d, *J* = 8.0 Hz, H-5), 8.32 (1H, d, *J* = 5.5 Hz, H-3), 8.81 (1H, s, H-1). ^{13}C NMR (125 MHz, CD_3OD): δ_C (ppm) 112.9 (C-8), 116.3 (C-4), 120.7 (C-6), 122.4 (C-4b), 122.5 (C-5), 129.7 (C-7), 130.2 (C-4a), 134.2 (C-1), 137.7 (C-9a), 138.5 (C-3), 142.8 (C-8a).

3,4-Dihydroxy-6,7-dimethyl -quinolin-2-carboxylic (6): Pale yellow solid, mp: 154–155°C, ^{1}H-NMR ($CDCl_3$+CD_3OD, 600 MHz): δ_C (ppm) 2.48 (3H, s, C-10), 2.51 (3H, s, C-11), 7.69 (1H, s, H-8); 7.96 (1H, s, H-5).^{13}C-NMR ($CDCl_3$+ CD_3OD, 150 MHz): δ_C (ppm) 19.2 (C-10), 20.3 (C-11), 125.7 (C-8), 128.5 (C-5), 129.7 (C-4a), 138.6 (C-8a), 139.1 (C-7), 141.5 (C-4), 144.7 (C-6), 146.2 (C-3), 149.8 (C-2), 160.6 (C=O).

Uracil (7): White solid, mp: 155–156°C, ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H (ppm) 5.46 (1H, d, *J* = 7.6 Hz, H-5); 7.35 (1H, d, *J* = 7.5 Hz, H-6).

3-methyluracil (8): White solid, mp: 155–156°C, ^{1}H NMR (500 MHz, CD_3OD): δ_H 3.22 (3H, s, CH_3), 5.52 (1H, d, *J* = 8.0 Hz, CH-5), 7.63 (1H, d, *J* = 8.0 Hz, CH-6).

Thymine (9): White solid, mp: 320–323°C; ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 1.88 (3H, s, CH_3), 7.13 (1H, s, H-6).

Evaluating antimicrobial activity of compound 1-6

The antimicrobial activity of compounds **1-6** was evaluated using the serial dilution method described by Andrews (2001) at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology [4, 5]. The samples were diluted in DMSO in decreasing concentrations of 256, 128, 64, 32, 16, 8, 4, and 2 µg/mL. Subsequently, 50 µL of bacterial and yeast suspension at a concentration of 2 × 10^5 CFU/mL was added, and the mixture was incubated at 37°C for 24 hours. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the sample that completely inhibited microbial growth after 24 hours. Streptomycin and nystatin were positive controls for bacteria and yeast, respectively. Seven tested strains used in this study were provided by the American Type Culture Collection (ATCC), including three Gram-negative strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076; three Gram-positive strains: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579; and one yeast

strain: *Candida albicans* ATCC 10231. The independent experiments were performed in triplicate.

RESULTS AND DISCUSSION

From the agar-based culture of the marine-derived Penicillium sp. M839 strain, nine known compounds were isolated and structurally determined, including fumiquinazoline D (1), fumiquinazoline B (2), cerevisterol (3), stoloniferol B (4), norhaman (5), 3,4-dihydroxy-6,7-dimethyl-quinolin-2-carboxylic (6), uracil (7), 3-methyl uracil (8), thymine (9). These compounds were characterized via 1D and 2D NMR spectroscopic and mass spectrometric analyses (Fig. 2).

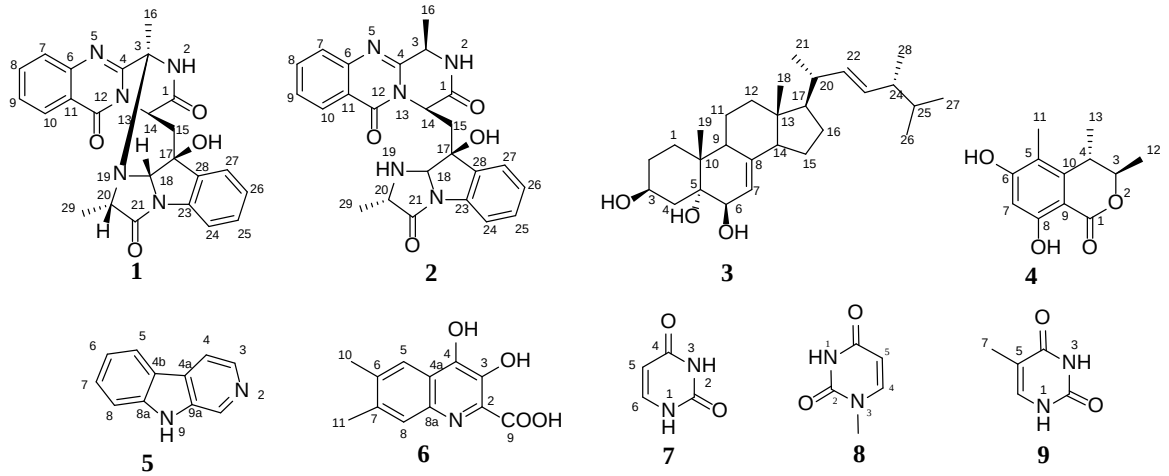

Figure 2. Secondary metabolites **1**-**9** from *Penicillium* sp. M839

Compound **1** was obtained as a white solid with a positive specific rotation $[\alpha]_D^{25}$ = +56.7 (*c* 1.73, $CHCl_3$). The ^{1}H NMR spectrum with the aid of the HSQC spectrum showed the signals of eight aromatic protons at δ_H 7.16–8.18, two methyl groups at δ_H 1.13 (d, *J* = 6.6 Hz, CH_3-29), 2.18 (s, CH_3-16); three sp^3 methine groups at δ_H 4.25 (td, *J* = 6.6, 1.2 Hz, H-20), 5.42 (dd, *J* = 9.0, 0.2 Hz, H-14), 5.76 (d, *J* = 1.8 Hz, H-18) and two methylene protons at δ_H 2.32 (dd, *J* = 15.0, 0.6 Hz, H_a-15) and 3.21 (dd, *J* = 15.0, 10.4 Hz, H_b-15). The chemical shifts of the three methine groups indicated their direct connection to nitrogen atoms. Analysis of the ^{13}C-NMR spectrum with the aid of the HSQC spectrum revealed the presence of 24 carbon atoms, including three carbonyl groups, two methyl groups, one sp^3 methylene carbon, three sp^3 methine carbons, eight sp^2 methine carbons, one quaternary carbon, one oxygen bearing tertiary carbon and five non-protonated carbons. The chemical shifts of the carbonyl groups at δ_C 161.2 (C-12), 169.6 (C-21), and 172.9 (C-1) are related to the carbonyls of the amide groups. Similarly, the chemical shifts of C-3 (δ_C 71.9) and C-17 (δ_C 84.4) suggested their direct linkage to nitrogen or oxygen atoms. In the COSY spectrum of **1,** two *ortho*-disubstituted benzene rings were indicated by the COSY correlations of the spin-spin coupling systems: H-7/H-8/H-9/H-10 and H-24/H-25/H-26/H-27 (Figure 3). The interpretation of the HMBC correlations of H-10 (δ_H 8.18) with C-12 (δ_C 161.2)/C-8 (δ_C 135.3)/C-6 (δ_C 147.6), CH_3-16 (δ_H 2.18) with C-3 (δ_C 71.9)/C-4 (δ_C 154.4), H-14 (δ_H 5.42) with C-12 (δ_C 161.2)/C-4 (δ_C 154.4)/C-1 (δ_C 169.6), along with the reasonable arrangement of two nitrogen atoms (N-13, N-5) confirmed the presence of the 6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione moiety (A moiety) in compound **1** (Fig. 3). Similarly, the imidazo[1,2-a]indole moiety (B moiety) was established through the second *ortho*-disubstituted benzene ring (from H-24 to H-27),

the reasonable arrangement of two nitrogen atoms (N-19, N-22), and the long-range correlations in the HMBC spectrum as follows: H-27 (δ_H 7.52) with C-17 (δ_C 84.4)/C-23 (δ_C 139.1)/C-25 (δ_C 130.1), H-24 (δ_H 7.43) with C-28 (δ_C 139.7), H_3-29 (δ_H 1.13) with C-20 (δ_C 59.5)/C-21 (δ_C 172.9), H-20 (δ_H 4.25) with C-21 (δ_C 172.9) (*Figure 3*). Furthermore, the HMBC spectrum showed the long-range correlations of H-18 (δ_H 5.76) with C-3 (δ_C 71.9), H-14 (δ_H 5.42) with C-15 (δ_C 44.1)/C-17 (δ_C 84.42), and H-15 (δ_H 2.32 and 3.21) with C-17 (δ_C 84.4)/C-1 (δ_C 172.9) were observed. These HMBC correlations confirmed that the A moiety was connected to the B moiety through a methylene bridge (CH_2-15) at C-14 and C-17 and the linkage between N-19 and C-3. Thus, the planar structure of compound **1** was determined, as shown in Figure 2. The relative configuration of **1** was elucidated based on the NOESY correlations from H-20 (δ_H 4.25) to H-16 (δ_H 2.18), from H-18 (δ_H 5.76) to OH-17 (δ_H 5.61). Thus, based on the analyses of the NMR spectra, optical rotation value, and comparison with reported data [6, 7], compound **1** was identified as fumiquinazoline D, which was previously isolated from the fungus *Aspergillus fumigatus* (1995) [6].

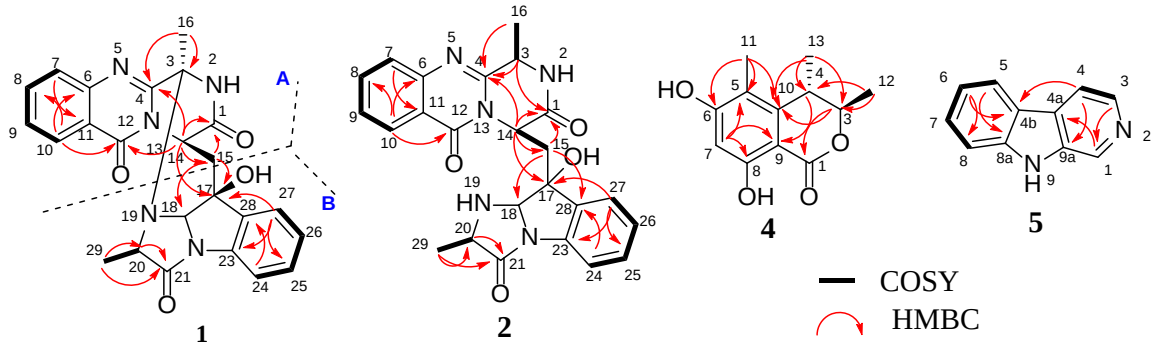

Figure 3. Key HMBC and COSY correlations of compounds **1**, **2**, **4** and **5**

Compound **2** was obtained as a white solid and optically active $[\alpha]_D^{25}$ = -176.8 (*c* 0.23, $CHCl_3$). The ^{13}C NMR and HSQC spectra of compound **2** displayed the signals of 24 carbons, including three carbonyl groups, two doublet methyl groups, one sp^3 methylene group, four sp^3 methine groups, eight sp^2 methine groups, one oxygen-bearing tertiary carbon and five non-protonated carbons. The ^1H NMR spectrum with the aid of the HSQC and COSY spectra showed the signals of two *ortho*-disubstituted benzene rings (from H-7 to H-10 and from H-24 to H-27), two methyl groups at δ_H 1.23 (d, *J* = 6.6 Hz, CH_3-29), 1.87 (d, *J* = 7.2 Hz, CH_3-16); two sp^3 methine groups at δ_H 4.17 (td, *J* = 6.6, 1.2 Hz, H-20), 4.81 (q, *J* = 14.4, 7.2 Hz, H-3); one sp^3 methylene at δ_H 2.47 (dd, *J* = 13.8, 5.4 Hz, H_a-15), 2.64 (dd, *J* = 13.8, 11.4 Hz, H_b-15). Comparison of the 1D and 2D-NMR spectra of compound **2** with those of **1** indicated that compound **2** had the same fumiquinazoline skeleton as compound **1,** except the presence of the nitrogen-bearing tertiary carbon in **1** was instead of one sp^3 methine group (δ_H 4.81, δ_C 53.4) in the structure of compound **2**. Thus, compound **2** was determined to be fumiquinazoline B, which was confirmed by the HMBC correlations (Fig. 3) and comparison with the literature [6].

Compound **3** was isolated as a white solid and optically active $[\alpha]_D^{25}$ = -72 (*c* 0.07, MeOH). The ^1H NMR spectrum of **3** indicated the presence of six methyl groups (two singlet signals and four doublet signals) at δ_H 0.54 (s, CH_3-18), 0.80 (d, *J* = 7.2 Hz, CH_3-27), 0.82 (d, *J* = 7.8 Hz, CH_3-26), 0.88 (d, *J* = 6.6 Hz, CH_3-28), 0.90 (s, CH_3-19), 0.99 (d, *J* = 6.6 Hz, CH_3-21), three olefinic protons at δ_H 5.05 (dd, *J* = 5.4, 2.4 Hz, H-7), 5.17 (dd, *J* = 15.6, 8.4 Hz, H-22), 5.24 (dd, *J* = 15.6, 7.2 Hz, H-23), two oxygenated methine groups at δ_H 3.36 (d, *J* = 5.4 Hz, H-6), 3.77 (1H, m, H-3), and of the overlapped

remaining protons in the aliphatic region at δ_H 1.43–2.03 (ppm). The ^{13}C NMR and HSQC spectra of compound **3** displayed the signals of 28 carbons, including six methyl groups, seven methylene groups, two oxymethine groups, three sp^2 methine carbons, six sp^3 methine carbons, two quaternary carbons, one oxygen bearing tertiary carbon and one non-protonated carbon. In addition, a coupling constant of 15.6 Hz between the olefinic protons H-22 and H-23 suggested an *E* configuration for the double bond. These NMR data indicated that compound **3** possessed a (22*E*)-ergosta-7,22-diene steroid skeleton, characterized by two hydroxyl groups bonded to the carbon at positions C-3 and C-6. Combining the 1D-NMR data, optical rotation value, and comparison with the reported literature confirmed the structure of compound **3** as cerevisterol [8].

Compound **4** was isolated as a colorless needle and optically active $[\alpha]_D^{25}$ = +93.8 (*c* 0.05, $CHCl_3$). The ^{1}H NMR spectrum of **4** indicated the presence of three methyl groups (one singlet and two doublets) at δ_H 1.31 (d, *J* = 6.6 Hz, CH_3-13), 1.33 (d, *J* = 6.6 Hz, CH_3-12), 2.10 (s, CH_3-11), two quintet sp^3 methine protons at δ_H 2.97 (dt, *J* = 7.2, 6.0 Hz, H-4), 4.68 (dt, *J* = 7.8, 6.6 Hz, H-3), and one singlet aromatic proton at δ_H 6.30 (s, H-7). The ^{13}C NMR spectrum of **4** indicated the presence of 12 carbons. The ^{1}H NMR and ^{13}C NMR spectra, with the aid of the HSQC spectrum, revealed the presence of a penta-substituted benzene ring, one carbon carbonyl at δ_C 168.6 (C-1), one sp^3 oxymethine carbon at δ_C 80.0 (C-3), one sp^3 methine carbon at δ_C 34.8 (C-4), and three methyl groups. The chemical shifts of the non-protonated aromatic carbons at δ_C 161.0 (C-6), and 162.4 (C-8) suggested that they were directly linked to the oxygen atoms. Detailed analyses of the 1D-NMR spectra of compound **4** indicated that compound **4** possessed an isochroman-1-one skeleton, which was supported by the HMBC correlations as follows: the HMBC correlations from H-7 (δ_H 6.30) to C-5 (δ_C 113.3)/C-6 (δ_C 161.0)/C-8 (δ_C 162.4)/C-9 (δ_C 100.4), from H-3 (δ_H 4.68) to C-4 (δ_C 34.8)/C-10 (δ_C 143.1)/C-1 (δ_C 168.6), from H-4 (δ_H 2.97) to C-5 (δ_C 113.3)/C-10 (δ_C 143.1)/C-9 (δ_C 100.4) (Fig. 3). Furthermore, the HMBC correlations of the protons H_3-11 (δ_H 2.10) with C-5 (δ_C 113.3)/C-6 (δ_C 161.0)/C-10 (δ_C 143.1), H_3-13 (δ_H 1.31) with C-4 (δ_C 34.8)/C-10 (δ_C 143.1)/C-3 (δ_C 80.0), and H_3-12 (δ_H 1.33) with C-3 (δ_C 80.0)/C-4 (δ_C 34.8) confirmed the positions of the methyl groups at C-5, C-4, C-3, respectively (Fig. 3). Therefore, based on the 1D and 2D-NMR data of compound **4** and comparison with the literature, compound **4** was determined to be stoloniferol B [9].

Compound **5** was isolated as a white solid. The ^{1}H NMR spectrum, with the aid of the COSY spectrum, showed the presence of seven aromatic protons, including one *ortho*-disubstituted benzene ring (from H-5 to H-8), two *ortho*-coupled aromatic protons at δ_H 8.32 (d, *J* = 5.5 Hz, H-3), 8.11 (d, *J* = 5.0 Hz, H-4). The ^{13}C NMR and HSQC spectra of compound **5** displayed the signals of 11 carbons, including seven aromatic methine carbons and four non-protonated aromatic carbons. The chemical shifts of the non-protonated aromatic carbons at δ_C 142.8 (C-8a), 137.7 (C-9a), and 138.5 (C-3) suggested their linkages to nitrogen atoms. The HMBC spectrum of compound **5** showed the cross-correlations of the protons H-4 (δ_H 8.11) with C-4b (δ_C 122.4)/C-9a (δ_C 137.7), H-3 (δ_H 8.32) with C-4a (δ_C 130.2)/C-1 (δ_C 134.2), H-1 (δ_H 8.81) with C-3 (δ_C 138.5)/C-9a (δ_C 137.7)/C-4a (δ_C 130.2), H-5 (δ_H 8.21) with C-8a (δ_C 142.8)/C-7 (δ_C 129.7), H-6 (δ_H 7.29) with C-4b (δ_C 122.4)/C-8 (δ_C 112.9), H-7 (δ_H 7.58) with C-8a (δ_C 142.8), which confirmed the positions of C-4, C-3, C-1, C-5, C-6, C-7, respectively. Based on the above evidence and compared with those reported in the reference [10], compound **5** was determined to be norharman.

Compound **6** was isolated as a yellow solid. The ^{1}H NMR spectrum of compound **6** showed the presence of two singlet aromatic protons at δ_H 7.96 (s, H-5), 7.69 (s, H-8), two singlet methyl groups at δ_H 2.48 (s, CH_3-10) and 2.50 (s, CH_3-11). The ^{13}C NMR and DEPT of compound **6** displayed the signals of 12 carbons, including seven non-protonated aromatic carbons, two methyl groups at δ_C 19.2 (CH_3-10), 20.3 (CH_3-11), two sp^2 methine carbons at δ_C 125.7 (C-8), 128.5 (C-5), and one carbonyl carbon at δ_C

160.6 (COOH). The chemical shifts of four non-protonated aromatic carbons: δ_C 138.6 (C-8a), δ_C 149.8 (C-2), δ_C 146.2 (C-3) and δ_C 141.5 (C-4) suggested their linkages to oxygen or nitrogen atoms. Detailed analyses of 1D NMR data of compound **6** and comparison with the reported data [11] allowed for identifying compound **6** as 3,4-dihydroxy-quinolin-2-carboxylic.

Based on 1D NMR data, comparison with the literature, and co-TLC analysis of authentic samples, the remaining compounds were identified as uracil (**7**) [12], 3-methyl uracil (**8**) [13], thymine (**9**) [12, 13]. Compounds **2** and **8** were first recognized from the genus *Penicillium*, while the remaining compounds were previously isolated from this genus [4, 7, 14–16].

Antimicrobial assay

Compounds **1**-**6** were evaluated for their antimicrobial activity against a panel of test microorganisms (three Gram-positive bacteria: *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*; three Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella enterica* and one yeast strain *Candida albicans*). The results showed that compound **6** displayed strong antimicrobial activity against two Gram-negative bacteria *E. coli* and *S. enterica* with the MIC values of 32 and 64 µg/mL, respectively. Compound **1** exhibited selective inhibition against the Gram-positive bacteria *E. faecalis* with a MIC value of 64 µg/mL. In addition, the remaining compounds **2**-**5** had inhibitory activity against one to three Gram-positive and Gram-negative tested strains, with MIC values ranging from 128 to 256 µg/mL (Table 1).

Table 1. Antimicrobial activities of compounds **1**-**6** (MIC: µg/mL)

Compd.	Gram-positive			Gram-negative			Yeast
	E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C. albicans*
1	64	128	256	256	> 256	256	128
2	256	> 256	> 256	> 256	> 256	256	128
3	256	256	128	256	> 256	128	128
4	256	256	128	> 256	> 256	> 256	258
5	> 256	> 256	> 256	128	> 256	> 256	256
6	> 256	> 256	> 256	32	> 256	64	> 256
Streptomycin	256	256	128	32	256	128	-
Nystatin	-	-	-	-	-	-	8

Note: "-": not test.

CONCLUSION

From the agar-based culture of the marine-derived *Penicillium* sp. M839 strain, nine known compounds were isolated and structurally determined, including fumiquinazoline D (**1**), fumiquinazoline B (**2**), cerevisterol (**3**), stoloniferol B (**4**), norhaman (**5**), 3,4-dihydroxy-6,7-dimethyl-quinolin-2-carboxylic (**6**), uracil (**7**), 3-methyl uracil (**8**), thymine (**9**). These compounds were characterized via 1D and 2D NMR spectroscopic and mass spectrometric analyses. Compounds **2** and **8** were first recognized from the genus *Penicillium*, while the remaining compounds were previously isolated from this genus. Compounds **1**–**6** were evaluated for their antimicrobial activity against a panel of test microorganisms (three Gram-positive bacteria: *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*; three Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella enterica* and one yeast strain *Candida albicans*). The results showed that compound **6** displayed strong antimicrobial activity against two Gram-negative bacteria, *E. coli* and *S. enterica,* with the MIC values of 32 and 64 µg/mL, respectively. Compound **1** showed antimicrobial activity against all three Gram-positive and two Gram-negative strains (*S. enterica, C. albicans*) with MIC values from 64 to 256 µg/mL. In addition, the remaining compounds **2**-**5** had inhibitory

activity against one to three Gram-positive and Gram-negative tested strains with MIC values ranging from 128 to 256 µg/mL.

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