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CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF THE MARINE SEDIMENT-DERIVED FUNGUS *ASPERGILLUS* sp. M793

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Summary

In our ongoing search for bioactive compounds from marine-derived fungi, the ethyl acetate extract of *Aspergillus* sp. M793, isolated from marine sediment collected in Nha Trang - Khanh Hoa, Vietnam, exhibited strong antimicrobial activity against a panel of Gram-positive bacteria, including *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, and the yeast *Candida albicans* ATCC 10231, with the MIC values of 8, 32, 32, and 8 µg/mL, respectively. A chemical investigation of the ethyl acetate extract led to the isolation of seven known compounds, including fumigaclavine C (**1**), pyripyropene A (**2**), 20-hydroxycyclotryprostatin (**3**), spinasterol (**4**), cerevisterol (**5**), monomethylsulochrin (**6**), and phenol A (**7**). Their structures were elucidated by detailed spectroscopic analyses, including MS and NMR data, and comparison with literature data. Notably, compounds **4** and **5** are reported here for the first time from the genus *Aspergillus*. Among the isolates, compounds **5**-**7** exhibited moderate to strong antimicrobial activity, with compound **7** showing the most pronounced and broad-spectrum effects, particularly against *E. faecalis* and *C. albicans*. These findings enrich the chemical inventory of marine-derived *Aspergillus* species and support their potential as promising sources of antimicrobial natural products.

Keywords: *Marine-derived fungus; Aspergillus; Ergot alkaloid; Indole diketopiperazines; Meroterpenoid; Steroid; Polyketide.*

1. Introduction

Marine-derived fungi are a prolific source of chemically diverse secondary metabolites with a wide range of biological activities [1],[2],[3]. Vietnam's coastal ecosystems represent a rich yet underexplored reservoir of marine microbial biodiversity. Recent bioprospecting efforts in this region have revealed novel fungal strains capable of producing bioactive compounds with diverse mechanisms of action. Among these fungi, members of the genus *Aspergillus* have received considerable attention for their metabolic versatility and ability to biosynthesize structurally unique and pharmacologically relevant natural products, including alkaloids, meroterpenoids, polyketides, and sterols [4],[5],[6],[7]. As part of our ongoing research aimed at exploring the chemical diversity of marine-derived fungi from the coastal regions of Vietnam, the strain *Aspergillus* sp. M793 was isolated from marine sediment collected in Nha Trang, Khanh Hoa. The ethyl acetate extract of the fungal culture showed strong antimicrobial activity against a panel of Gram-positive bacteria, including *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, and the yeast *Candida albicans* ATCC 10231, with MIC values of 8, 32, 32, and 8 µg/mL, respectively, prompting further chemical investigation. Chromatographic separation of the EtOAc extract resulted in the isolation of seven known compounds: fumigaclavine C (**1**), pyripyropene A (**2**), 20-hydroxycyclotryprostatin (**3**), spinasterol (**4**), cerevisterol (**5**), monomethylsulochrin (**6**), and phenol A (**7**) (Fig. 1). The structures of these compounds were established based on spectroscopic analyses, including 1D and 2D NMR and ESI-MS, and by comparison with published data. To the best of our knowledge, this is the first report of spinasterol and cerevisterol from a fungus of the genus *Aspergillus*.

2. Materials and methods

2.1. General experimental procedures

The ESI-MS spectra were recorded on an Agilent 1100 LC/MSD Trap spectrometer. Optical rotations were recorded on a JASCO P-2000 polarimeter, using a sodium (589, D line) lamp. The NMR spectra were recorded on Bruker 500 and 600 MHz spectrometers, and tetramethylsilane (TMS) was used as an internal standard. Thin-layer chromatography was used with precoated *silica gel* Merck 60 F_{254}. Column chromatography (CC) was performed on *silica gel* (Kieselgel 60, 70–230 mesh and 230–400 mesh) or Sephadex LH-20 resins (Sigma-Aldrich, USA).

2.2. Marine material

The *Aspergillus* sp. M793 strain was isolated from marine sediment collected at an 8.5-meter

depth off the coast of Nha Trang, Khanh Hoa province, Vietnam, in May 2020, and has been preserved in 20% glycerol at -80°C for long-term storage.

2.3. Fermentation of Aspergillus sp. M793

The *Aspergillus* sp. M793 strain was activated and inoculated into 1 L of PDB medium (Potato Dextrose Broth; Himedia, Mumbai, India) to prepare the seed cultures. 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 solid medium (Potato Dextrose Agar; Himedia, Mumbai, India). The flasks were incubated at 30°C and harvested on the twelfth day.

2.4. Isolation and purification procedures from the EtOAc extract of Aspergillus sp. M793

The solid culture (20 L) of *Aspergillus* sp. M793 was cut into small pieces and extracted four times with ethyl acetate and methanol, respectively, under ultrasonic conditions at 45-50 °C to afford the corresponding extracts. These extracts were concentrated under reduced pressure to give 10.5 g of the ethyl acetate (EtOAc) extract and 43.5 g of the methanol (MeOH) extract. The EtOAc extract (10.5 g) was subjected to *silica gel* CC using a CH_2Cl_2/MeOH gradient (0-100% MeOH) to obtain seven fractions, F1-F7. Fraction F2 (0.86 g) was chromatographed on a *silica gel* CC using *n*-hexane - acetone gradient (0-100% acetone) to yield compound **4** (5 mg). Fraction F3 (1.02 g) was further purified by *silica gel* CC, using an *n*-hexane - acetone gradient (0-100% acetone), followed by Sephadex LH-20 CC (CH_2Cl_2 - MeOH (2/8, *v/v*)) to yield compounds **1** (3.8 mg) and **2** (3.5 mg). Fraction F4 (1.33 g) was separated into four subfractions (F4.1 - F4.4) by *silica gel* CC using CH_2Cl_2 - acetone (9/1, *v/v*) as the eluent. Subfraction F4.1 (115 mg) was subjected to Sephadex LH-20 CC (CH_2Cl_2/MeOH (1/9, *v/v*)), followed by *silica gel* CC (CH_2Cl_2 - acetone (85/15, *v/v*)) to afford compound **6** (4.3 mg). Subfraction F4.2 was purified by preparative TLC using CH_2Cl_2 - EtOAc (9/1, *v/v*) to afford compound **3** (4.7 mg). Fraction F5 (1.7 g) was subjected to Sephadex LH-20 CC (100% MeOH), followed by *silica gel* CC (CH_2Cl_2 - acetone gradient, 0-100% acetone) to afford compounds **5** (8 mg) and **7** (9 mg).

Fumigaclavine C (1): white solid; $[\alpha]_D^{25}$-50.2 (*c* 0.03, $CHCl_3$); ESI-MS $[M+H]^+$ *m/z* 367.1; ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 7.10 (1H, d, *J* = 8.0 Hz, H-14), 6.97 (1H, t, *J* = 8.0 Hz, H-13), 6.62 (1H, d, *J* = 8.0 Hz, H-12), 6.16 (1H, dd, *J* = 10.5, 17.0 Hz, H-22), 5.67 (1H, br. s, H-9), 5.09 (1H, d, *J* = 17.0 Hz, H_a-23), 5.06 (1H, dd, *J* = 10.5 Hz, H_b-23), 3.59 (1H, dd, *J* = 4.5, 14.0 Hz, H_a-4), 3.33 (1H, br. d, *J* = 7.5 Hz, H-10), 2.88 (1H, dd, *J* = 3.5, 12.0 Hz, H_a-7), 2.83 (1H, br. d, *J* = 12.0 Hz, H_b-7), 2.77 (1H, ddd, *J* = 4.5, 11.0, 15.5 Hz, H-5), 2.65 (1H, dd, *J* = 11.5, 14.0 Hz, H_b-4), 2.55 (3H, s, H-17), 2.15 (1H, m, H-8), 1.87 (3H, s, H-25), 1.55 (3H, s, H-20), 1.54 (3H, s, H-21), 1.37 (3H, d, *J* = 7.5 Hz, H-18). ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 172.1 (C-24), 147.3 (C-22), 139.3 (C-2), 134.4 (C-15), 129.0 (C-11), 128.5 (C-16), 122.7 (C-13), 113.2 (C-12), 111.8 (C-23), 109.7 (C-14), 105.1 (C-3), 72.1 (C-9), 63.6 (C-5), 58.2 (C-7), 43.2 (C-17), 40.2 (C-19), 39.4 (C-10), 33.7 (C-8), 28.1 (C-20), 28.0 (C-21), 28.2 (C-4), 20.8 (C-25), 16.0 (C-18).

Pyripyropene A (2): white powder; $[\alpha]_D^{25}$ + 60.2 (*c* 0.03, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 9.00 (1H, d, *J* = 2.0 Hz, H-2″), 8.68 (1H, dd, *J* = 2.0, 5.0 Hz, H-6″), 8.09 (1H, ddd, *J* = 2.0, 2.0, 8.0 Hz, H-4″), 7.40 (1H, dd, *J* = 5.0, 8.0 Hz, H-5″), 6.45 (1H, s, H-5′), 5.01 (2H, m, H-13, H-7), 4.80 (1H, dd, *J* = 4.5, 11.5 Hz, H-1), 3.78 (1H, d, *J* = 12.0 Hz, H_a-11), 3.71 (1H, d, *J* = 12.0 Hz, H_b-11), 2.16 (3H, s, 7-CH_3), 2.15 (1H, m, H_a-3), 2.09 (3H, s, 11-CH_3), 2.04 (3H, s, 1-CH_3), 1.92 (1H, m, H_a-2), 1.85 (1H, m, H_b-2), 1.81 (1H, m, H_a-8), 1.68 (3H, s, H-14), 1.61 (2H, m, H_b-8, H-9), 1.54 (1H, d, *J* = 4.0 Hz, H-5), 1.44 (3H, s, H-12), 1.37 (1H, m, H_b-3), 0.89 (3H, s, H-15). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 170.9 (11-C=O), 170.5 (1-C=O), 170.0 (7-C=O), 164.0 (C-2′), 162.2 (C-4′), 157.4 (C-6′), 151.6 (C-6″), 146.8 (C-2″), 132.9 (C-4″), 127.1 (C-3″), 123.6 (C-5″), 102.9 (C-3′), 99.4 (C-5′), 83.3 (C-6), 77.8 (C-7), 73.6 (C-1), 64.9 (C-11), 60.3 (C-13), 54.8 (C-5), 45.4 (C-9), 40.4 (C-10), 37.9 (C-4), 36.2 (C-3), 25.2 (C-8), 22.7 (C-2), 21.2 (7-CH_3CO), 21.0 (1-CH_3CO), 20.1 (11-CH_3CO), 17.4 (C-12), 16.3 (C-14), 13.2 (C-15).

20-Hydroxycyclotryprostatin B (3): light yellow solid; $[\alpha]_D^{25}$+ 35.7 (*c* 0.04, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 9.59 (1H, br. s, NH-1), 7.41 (1H, d, *J* = 8.5 Hz, H-4), 6.87 (1H, d, *J* = 2.0 Hz, H-7), 6.79 (1H, dd, *J* = 2.0, 8.5 Hz, H-5), 6.08 (1H, dd, *J* = 2.5, 8.0 Hz, H-18), 4.71 (1H, s, H-8), 4.37 (1H, dd, *J* = 6.0, 11.0 Hz, H-12), 3.84 (3H, s, OCH_3-6), 3.79 (1H, m, H_a-15), 3.69 (1H, m, H_b-15), 3.36 (3H, s, OCH_3-8), 2.49 (1H, m, H_a-13), 2.34 (1H, dd, *J* = 8.0, 15.0 Hz, H_a-19), 2.10 (1H, m, H_a-14), 2.05 (1H, dd, *J* = 2.0, 15.0 Hz, H_b-19), 1.99 (2H, m, H_b-14, H_b-13), 1.55 (3H, s, CH_3-22), 1.34 (3H, s, CH_3-21). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 166.8 (C-11), 166.1 (C-17), 156.4 (C-6), 136.4 (C-7a), 135.0 (C-

2), 122.3 (C-3a), 118.4 (C-4), 109.8 (C-5), 104.0 (C-3), 95.3 (C-7), 84.6 (C-9), 77.1 (C-8), 71.3 (C-20), 59.9 (C-12), 56.5 (OCH_3-8), 55.8 (OCH_3-6), 49.5 (C-19), 47.6 (C-18), 45.7 (C-15), 32.5 (C-21), 29.6 (C-13), 28.8 (C-22), 22.2 (C-14).

Spinasterol (4): white solid; $[\alpha]_D^{25}$ +25.7 (*c* 0.035, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H (ppm) 5.15 (1H, dd, *J* = 8.5, 15.0 Hz, H-22), 5.14 (1H, m, H-7), 5.02 (1H, dd, *J* = 8.5, 15.0 Hz, H-23), 3.61 (1H, m, H-3), 1.03 (3H, d, *J* = 6.5 Hz, H-21), 0.86 (3H, d, *J* = 6.0 Hz, H-26), 0.81 (3H, t, *J* = 6.5 Hz, H-29), 0.81 (3H, d, *J* = 6.0 Hz, H-27); 0.80 (3H, s, H-19), 0.54 (3H, s, H-18). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 139.6 (C-8), 138.2 (C-22), 129.5 (C-23), 117.5 (C-7), 71.1 (C-3), 55.9 (C-17), 55.1 (C-14), 51.3 (C-24), 49.4 (C-9), 43.2 (C-13), 40.8 (C-20), 40.2 (C-5), 39.5(C-12), 37.9 (C-4), 37.2 (C-1), 34.2 (C-11), 31.8 (C-25), 31.5 (C-2), 29.7 (C-6), 28.5 (C-16), 25.4 (C-28), 23.0 (C-15), 21.7 (C-21), 21.4 (C-27), 21.6 (C-10), 19.0 (C-26), 13.0 (C-19), 12.2 (C-29), 12.0 (C-18).

Cerevisterol (5): white solid, $[\alpha]_D^{25}$-10.0 (c 0.15, MeOH). ^{1}H-NMR (500 MHz. $CDCl_3$): δ_H (ppm) 5.35 (lH, m, H-7), 5.23 (1H, dd, J = 7.0, 15.0 Hz, H-23), 5.15 (1H, dd, J = 8.0, 15.0 Hz, H-22), 4.07 (lH, m, H-3), 3.63 (lH, m, H-6), 1.08 (3H, s, H-19), l.02 (3H, d, J = 6.5 Hz, H-21), 0.91 (3H, d, J = 7.0 Hz, H-28), 0.83 (3H, d, J = 7.0 Hz, H-27), 0.82 (3H, d, J = 7.0 Hz, H-26), 0.60 (3H, s, H-18). ^{13}C-NMR (125 MHz. $CDCl_3$): δ_C (ppm) 144.0 (C-8), 135.4 (C-22), 132.2 (C-23), 117.6 (C-7), 76.3 (C-5), 73.7 (C-6), 67.8 (C-3), 56.1 (C-17), 54.8 (C-14), 43.8 (C-13), 43.5 (C-9), 42.8 (C-24), 40.4 (C-20), 39.5 (C-4), 39.3 (C-12), 37.2 (C-10), 33.1 (C-25), 33.0 (C-1), 30.9 (C-2), 27.9 (C-16), 22.9 (C-15), 22.1 (C-11), 21.1 (C-21), 20.0 (C-26), 19.7 (C-27), 18.8 (C-19), 17.6 (C-28), 12.4 (C-18).

Monomethylsulochrin (6): Pale yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 12.94 (1H, s, OH-2′), 7.01 (1H, d, J = 2.0 Hz, H-6), 6.58 (1H, d, J = 2.0 Hz, H-5′), 6.46 (1H, d, J = 2.0 Hz, H-3′), 6.06 (1H, d, J = 2.0 Hz, H-4), 3.69 (3H, s, H-8), 3.67 (3H, s, H-9), 3.36 (3H, s, H-9′), 2.27 (3H, s, H-7′). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 199.7 (C-8′), 166.4 (C-7), 164.2 (C-2′), 161.0 (C-6′), 157.2 (C-5), 156.7 (C-3), 148.2 (C-4′), 128.4 (C-1), 127.6 (C-2), 111.0 (C-1′), 110.4 (C-3′), 108.1 (C-6), 103.4 (C-5′), 103.0 (C-4), 56.3 (C-9), 55.7 (C-9′), 52.3 (C-8), 22.4 (C-7′).

Phenol A (7): Pale yellow solid; $[\alpha]_D^{25}$-30.6 (*c* 0.2, MeOH); ESI-MS *m/z* 197 $[M+H]^+$; ^{1}H-NMR (600 MHz, CD_3OD): δ_H (ppm) 6.30 (1H, d, *J* = 2.4 Hz, H-4), 6.19 (1H, d, *J* = 2.4 Hz, H-6), 3.88 (1H, quint, *J* = 6.0 Hz, H-3′), 3.08 (1H, quint, *J* = 6.6 Hz, H-2′), 2.10 (3H, s, 2-CH_3), 1.16 (3H, d, *J* = 6.6 Hz, H-1′), 1.14 (3H, d, *J* = 6.0 Hz, H-4′). ^{13}C-NMR (150 MHz, CD_3OD): δ_C (ppm) 156.8 (C-3), 156.2 (C-5), 145.5 (C-1), 115.1 (C-2), 105.7 (C-6), 101.0 (C-4), 71.9 (C-3′), 42.9 (C-2′), 19.4 (C-4′), 16.2 (C-1′), 10.6 (2-CH_3).

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 25923), *Bacillus cereus* (ATCC 14579), and *Candida albicans* (ATCC 10231). Stock solutions of samples were prepared in DMSO at a decreasing concentration range: 256, 128, 64, 32, 16, and 8 µg/mL, and the antimicrobial assays were carried out in 96-well microtiter plates against the microbial strains (2×10^5 CFU/mL) using a modification of the published method [8]. 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. 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 white solid, with a specific optical rotation of $[\alpha]_D^{25}$-50.2 (*c* 0.03, $CHCl_3$). The ^{1}H-NMR spectrum of compound **1** revealed the presence of a 1,2,3-trisubstituted benzene ring at δ_H 7.10 (1H, d, *J* = 8.0 Hz, H-14), 6.97 (1H, t, *J* = 8.0 Hz, H-13), and 6.62 (1H, d, *J* = 8.0 Hz, H-12), one terminal double bond at δ_H 5.09 (1H, d, *J* = 17.0 Hz, H_a-23), 5.06 (1H, dd, *J* = 10.5 Hz, H_b-23) and 6.16 (dd, *J* = 10.5, 17.0 Hz, H-22), and one oxygenated methine at δ_H 5.67 (1H, br. s, H-9). Five methyl groups were observed at δ_H 2.55 (3H, s, H-17), 1.87 (3H, s, H-25), 1.55 (3H, s, H-20), 1.54 (3H, s, H-21), and 1.37 (3H, d, *J* = 7.5 Hz, H-18). The remaining protons resonated in the aliphatic region between δ_H 2.15 and 3.59. Analyses the ^{13}C-NMR spectrum of compound **1**, along with the support of the HSQC spectrum displayed the signals corresponding to twenty-three carbons, including: one carbonyl group at δ_C 172.1 (C-24), five sp^2 quaternary carbons at δ_C 139.3 (C-2), 134.4 (C-15), 128.5 (C-16), 129.0 (C-11), and 105.1 (C-3), one sp^3 quaternary carbon at δ_C 40.2 (C-19), four sp^2 methines at δ_C 147.3 (C-22), 122.7 (C-13), 113.2 (C-12), and 109.7 (C-14), four sp^3 methines at δ_C 72.1 (C-9),

63.6 (C-5), 39.4 (C-10), and 33.7 (C-8), one sp^2 methylene at δ_C 111.8 (C-23), two sp^3 methylenes at δ_C 58.2 (C-7) and 27.2 (C-4), and five methyls at δ_C 43.2 (C-17), 28.1 (C-20), 28.0 (C-21), 20.8 (C-25), and 16.0 (C-18). The COSY spectrum of compound **1** revealed four spin-spin coupling systems: a fragment consisting of H-5/H-10/H-9/H-8/H-7, an aromatic system involving H-12/H-13/H-14, a coupling between H-8 and H-18, and a double bond spin system between H-22 and H-23. The key HMBC correlations from H-7 to C-5, C-9, C-17, and C-18, from H-9 to C-7 and C-24, from H-25 to C-24, from H-9 to C-24, from H-18 to C-7, C-8, and C-9, and from H-17 to C-5 and C-7, along with the chemical shifts of C-25 (δ_H 1.87, δ_C 20.8) confirmed a six-membered ring composed of C-5/N-6/C-7/C-8/C-9/C-10 with an acetoxy group at C-9 (Fig. 2). Further HMBC correlations from H-5 to C-3 and C-4, H-4 to C-3, C-5 to C-10 and C-16, and from H-14 to C-16 supported a tricyclic structure for compound **1**. In addition, the HMBC correlations from H-23 to C-19 and C-22, from H-22 to C-2, C-19, C-20, C-21, and from H-20/H-21 to C-19, C-22, and C-2, as well as a COSY correlation between H-23 and H-22, confirmed the presence of an isopentenyl group at C-2. Based on these spectroscopic data and comparison with the literature values, the structure of compound **1** was definitively established as fumigaclavine C [9],[10].

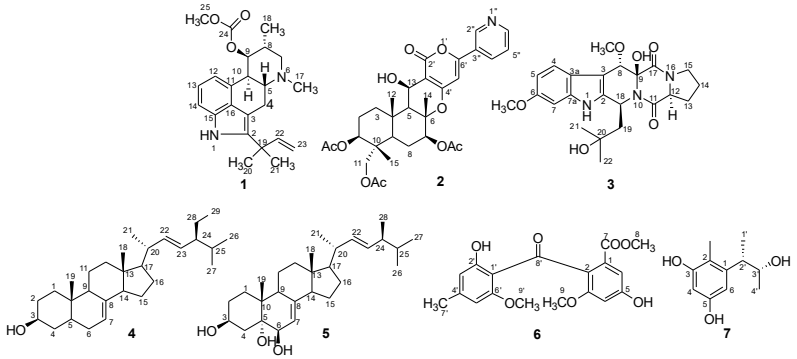

Fig. 1. Structures of the isolated compounds **1**–**7**

Compound **2** was isolated as a white powder, with a specific optical rotation of $[\alpha]_D^{25}$ +60.2 (*c* 0.03, $CHCl_3$). The ESI-MS spectrum of compound **2** displayed a *pseudo*-molecular ion peak at *m/z* 584 $[M+H]^+$. The ^1H NMR spectrum of 2 exhibited the presence of four aromatic proton signals at δ_H 9.00 (1H, d, J = 2.0 Hz, H-2″), 8.68 (1H, dd, J = 2.0, 5.0 Hz, H-6″), 8.09 (1H, ddd, J = 2.0, 2.0, 8.0 Hz, H-4″), and 7.40 (1H, dd, J = 5.0, 8.0 Hz, H-5″), indicating a 3-substituted pyridine ring. A singlet proton at δ_H 6.45 (1H, s, H-5′), three de-shielded methyl singlets at δ_H 2.16, 2.09, and 2.04, and three shielded singlets at δ_H 1.68, 1.44, and 0.89 were also observed. In addition, a series of oxygenated methine and methylene protons appeared in the range of δ_H 5.01-3.71 [5.01 (2H, m, H-13, H-7), 4.80 (1H, dd, J = 4.5, 11.5 Hz, H-1), 3.78 (1H, d, J = 12.0 Hz, H_a-11), and 3.71 (1H, d, J = 12.0 Hz, H_b-11)]. The ^{13}C-NMR spectrum of compound **2** revealed thirty-one carbon signals, including four carbonyl carbons at δ_C 170.9, 170.5, 170.0, and 164.0, four sp^2 quaternary carbons at δ_C 162.2 (C-4′), 157.4 (C-6′), 127.1 (C-3″), and 102.9 (C-3′), three sp^3 quaternary carbons at δ_C 83.3 (C-6), 40.4 (C-10), and 37.9 (C-4), five sp^2 methines at δ_C 151.6 (C-6″), 146.8 (C-2″), 132.9 (C-4″), 123.6 (C-5″), and 99.4 (C-5′), five sp^3 methines at δ_C 77.8 (C-7), 73.6 (C-1), 60.3 (C-13), 54.8 (C-5), and 45.4 (C-9), four sp^3 methylene at δ_C 64.9 (C-11), 36.2 (C-3), 25.2 (C-8), and 22.7 (C-2), and six methyls at δ_C 21.2 (7-CH_3CO), 21.0 (1-CH_3CO), 20.1 (11-CH_3CO), 17.4 (C-12), 16.3 (C-14), and 13.2 (C-15), as supported by the HSQC data. The characteristic carbon signals at δ_C 162.2 (C-4′), 157.4 (C-6′), 102.9 (C-3′), and 99.4 (C-5′), along with the singlet proton at δ_H 6.45 (H-5′), indicated the presence of a 3-oxo-α-pyrone moiety conjugated, oxygen-containing six-membered heterocycle bearing an α,β-unsaturated carbonyl group in the structure of compound **2**. The fusion of this rare heterocycle with a 3-substituted pyridine ring was further confirmed by detailed 2D NMR analysis. Analysis of the COSY spectrum of 2 established four spin-spin systems, including H-4″/H-5″/H-6″, supporting the substituted pyridine ring, and three fragments H-1/H-2/H-3, H-7/H-8/H-9, and H-5/H-13, indicative of a fused sesquiterpene scaffold. The key HMBC correlations from the proton H-5′ (δ_H 6.45) to C-3′, C-3″, C-4′, and C-6′, and from the proton H-4″ to C-6′ supported the presence of a 3,4-disubstituted 6-(3-pyridyl)-α-pyrone moiety. The additional HMBC interactions from H-12 to C-3, C-4, C-5, and C-9, from H-14 to C-5, C-6, and C-7; and from H-15 and H-11 to C-1, C-9, and C-10

established the positions of the methyl groups at C-4, C-6, and C-10, respectively. The connectivity between the sesquiterpene and pyrone units was confirmed by the HMBC correlations from H-13 to C-2′, C-3′, and C-4′ and from H-5 to C-3′. Furthermore, the HMBC correlations from H-11, H-7, and H-1 to the corresponding carbonyl carbons (δ_C 170.9 (11-C=O), 170.5 (1-C=O), and 170.0 (7-C=O)) verified the locations of three acetoxy groups at C-11, C-7, and C-1, respectively. The relative configuration of the sesquiterpene moiety was established by the NOESY experiments (Fig. 2). Strong NOESY effects were observed between Me-12 (δ_H 1.44) and Me-14 (δ_H 1.68), Me-15 (δ_H 0.89), as well as between Me-14 and Me-15, Ac-7 (δ_H 2.16), and between Me-15 and Ac-1 (δ_H 2.04), indicating that these groups are located on the same face of the molecule. On the basis of these 1D and 2D NMR spectroscopic data and comparison with reported spectral data, compound **2** was identified as pyripyropene A, a known fungal meroterpenoid. This compound was originally isolated from *Aspergillus fumigatus* and has been reported as a potent ACAT2 inhibitor with high isozyme selectivity [11],[12],[13].

Compound **3** was isolated as a light yellow solid, with a specific optical rotation of $[\alpha]_D^{25}$+35.7 (*c* 0.04, $CHCl_3$). The ^{1}H-NMR spectrum of compound **3** revealed the signals of an ABX aromatic ring system at δ_H 7.41 (1H, d, J = 8.5 Hz, H-4), 6.87 (1H, d, J = 2.0 Hz, H-7), and 6.79 (1H, dd, J = 2.5, 8.5 Hz, H-5), two methyl groups at δ_H 1.55 (3H, s, CH_3-22) and 1.34 (3H, s, CH_3-21), two methoxy groups at δ_H 3.84 (3H, s, OCH_3-6) and 3.36 (3H, s, OCH_3-8), and the remaining signals consistent with the methine and methylene groups at δ_H 1.99 – 4.71. The ^{13}C-NMR spectrum, in combination with the HSQC data of compound **3**, revealed twenty-three carbon signals, including two amide carbonyls at δ_C 166.1 (C-17) and 166.8 (C-11), five sp^2 quaternary carbons at δ_C 104.0 (C-3), 122.3 (C-3a), 135.0 (C-2), 136.4 (C-7a), and 156.4 (C-6), two sp^3 quaternary carbons at δ_C 71.3 (C-20) and 84.6 (C-9), three sp^2 methines at δ_C 95.3 (C-7), 109.8 (C-5), and 118.4 (C-4), three sp^3 methines at δ_C 77.1 (C-8), 59.9 (C-12), and 47.6 (C-18), four methylene groups at δ_C 22.2 (C-14), 29.6 (C-13), 45.7 (C-15), and 49.5 (C-19), two methyls at δ_C 28.8 (C-22) and 32.5 (C-21), and two methoxys at δ_C 55.8 (OCH_3-6) and 56.5 (OCH_3-8). The presence of an indole unit was supported by the ABX aromatic pattern, the chemical shift of C-7a (δ_C 136.4), and the HMBC correlations from H-4 to C-3, C-3a, and C-7a and from H-7 to C-3a, C-7a, and C-5. Further HMBC correlations from H-12 to C-13 and an amide carbonyl C-11, from H-13 to C-15, and from H-14 to C-12, together with the HMBC correlations from H-18 to C-2, C-3, and C-9, and from H-8 to C-2 and C-9, and the methoxy carbon (OCH_3-8) established a diketopiperazine ring linked to the indole moiety at C-2 and C-3. Moreover, the HMBC correlations from H-21 and H-22 to C-19 and C-20, from H-18 to C-19, and from H-19 to C-2 and C-18, confirmed the presence of a 2-methylbutan-2-ol side chain attached at C-18 of the diketopiperazine moiety. In addition, the methoxy group at δ_H 3.84 was assigned to C-6 of the indole ring, based on the HMBC correlation from its proton signal to C-6. Based on these data, the planar structure of compound **3** was determined as shown in Fig. 2. In addition, the diketopiperazine moiety also exhibited a *pseudo* C_2-symmetry axis (through C-11 and C-17), which, together with the NOESY correlations (H-12/H-18 and H-12/OCH_3-8), supported the assigned relative configuration. The absolute configuration of compound **3** was assumed to be identical to that reported for cyclotryprostatin derivatives, based on the close agreement of their NMR data and optical rotation values in the literature [13]. Taken together, analyses of the 1D and 2D NMR and comparison with literature values confirmed the structure of compound **3** as 20-hydroxycyclotryprostatin B [14],[15].

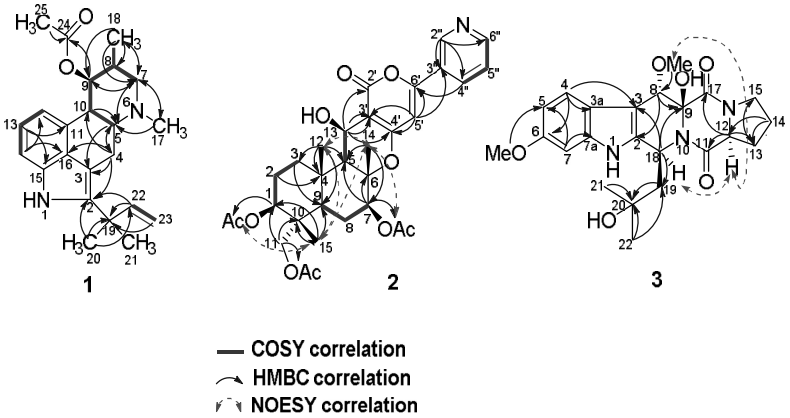

Fig. 2. COSY, NOESY, and HMBC correlations of compounds **1-3**

Compound **4** was isolated as a white solid, with a specific optical rotation of $[\alpha]_D^{25}$+25.7 (*c* 0.035, $CHCl_3$). The ^{1}H-NMR spectrum of compound **4** displayed the characteristic signals of a stigmastane skeleton. Three olefinic protons were observed at δ_H 5.15 (1H, dd, *J* = 8.5, 15.0 Hz, H-22), 5.14 (1H, m, H-7), and 5.02 (1H, dd, *J* = 8.5, 15.0 Hz, H-23), indicative of a trisubstituted double bond at C-7/C-8 and a disubstituted double bond at C-22/C-23. The large coupling constant (*J* = 15.0 Hz) between H-23 (δ_H 5.23 (1H, dd, *J* = 7.0, 15.0 Hz)) and H-22 (δ_H 5.15 (1H, dd, *J* = 8.0, 15.0 Hz)) was consistent with a *trans*-configuration of the C-22/C-23 double bond. A multiplet at δ_H 3.61 (1H, m) corresponded to the oxygenated methine proton at C-3. In the aliphatic region, six methyl resonances were observed, including three doublets at δ_H 1.03 (3H, d, *J* = 6.5 Hz, H-21), 0.86 (3H, d, *J* = 6.0 Hz, H-26), and 0.81 (3H, d, *J* = 6.0 Hz, H-27), one triplet at δ_H 0.81 (3H, t, *J* = 6.5 Hz, H-29), and two singlets at δ_H 0.80 (3H, s, H-19) and 0.54 (3H, s, H-18), all of which are characteristic of a sterol skeleton. The ^{13}C-NMR and DEPT spectra of compound **4** supported the proposed structure, showing the signals of twenty-nine carbons, including four olefinic carbons at δ_C 139.6 (C-8), 138.2 (C-22), 129.5 (C-23), and 117.5 (C-7), two sp^3 quaternary carbons at δ_C 43.2 (C-13) and 21.6 (C-10), eight sp^3 methine carbons at δ_C 71.1 (C-3), 55.9 (C-17), 55.1 (C-14), 51.3 (C-24), 49.4 (C-9), 40.8 (C-20), 40.2 (C-5), and 31.8 (C-25), nine sp^3methylene carbons at δ_C 39.5(C-12), 37.9 (C-4), 37.2 (C-1), 34.2 (C-11), 31.5 (C-2), 29.7 (C-6), 28.5 (C-16), 25.4 (C-28), and 23.0 (C-15), and six methyls at δ_C 21.7 (C-21), 21.4 (C-27), 19.0 (C-26), 13.0 (C-19), 12.2 (C-29), and 12.0 (C-18). The chemical shifts of C-3 (δ_H 3.61, δ_C 71.1) were consistent with literature values reported for steroids possessing a 3β-hydroxy configuration [16],[17]. Collectively, these spectroscopic data are in full agreement with literature values for spinasterol, thereby confirming the identity of compound **4** [18].

Compound **5** was isolated as a white solid, with a specific optical rotation of $[\alpha]_D^{25}$-10.0 (*c* 0.15, MeOH). The ^{1}H-NMR spectrum of compound **5** displayed six characteristic methyl resonances, indicative of an ergostane-type steroidal skeleton at δ_H 1.08 (3H, s, H-19), 1.02 (3H, d, *J* = 6.5 Hz, H-21), 0.91 (3H, d, *J* = 7.0 Hz, H-28), 0.83 (3H, d, *J* = 7.0 Hz, H-27), 0.82 (3H, d, *J* = 7.0 Hz, H-26), and 0.60 (3H, s, H-18). In addition, signals at δ_H 5.35 (1H, m, H-7), 5.23 (1H, dd, *J* = 7.0, 15.0 Hz, H-23), and 5.15 (1H, dd, *J* = 8.0, 15.0 Hz, H-22) suggested the presence of a trisubstituted double bond at C-7/C-8 and a disubstituted double bond at C-22/C-23. The large coupling constant (*J* = 15.0 Hz) between H-22 and H-23 indicated a *trans* configuration of the C-22/C-23 double bond. Two methine protons were observed at δ_H 4.07 (1H, m, H-3) and 3.63 (1H, m, H-6), along with the remaining aliphatic signals between δ_H 1.26-2.08. The ^{13}C-NMR spectrum of compound **5**, supported by DEPT spectrum analysis, confirmed a C_{28}-ergostane-type sterol skeleton, consisting of twenty-eight carbon signals: six methyls, seven methylenes, twelve methines, and three non-protonated carbons. The chemical shifts of C-3 (δ_C 67.8), C-5 (δ_C 76.3), and C-6 (δ_C 73.7) suggested their linkages to oxygen. Comparison of the 1D-NMR spectra of compound **5** with those of compound **4** revealed structural similarity in the sterol core. However, the key differences included the presence of an additional oxygenated methine and an oxygenated quaternary carbon in compound **5**, replacing the corresponding methine and methylene carbons in compound **4**. Furthermore, a triplet methyl group in compound **4** was replaced by a doublet methyl in compound **5**, suggesting a minor modification in the side chain. On the basis of the physical properties, spectroscopic data and comparison with reported literature values, compound **5** was identified as cerevisterol [19].

Compound **6** was isolated as a pale yellow solid. The ^{1}H-NMR spectrum of compound **6** revealed the presence of three methoxy groups at δ_H 3.69 (3H, s, H-8), 3.67 (3H, s, H-9), and 3.36 (3H, s, H-9′), one methyl group at δ_H 2.27 (3H, s, H-7′) and a downfield singlet at δ_H 12.94 (1H, s, OH-2′) which is characteristic of a hydrogen-bonded phenolic hydroxyl group, likely chelated to an adjacent carbonyl. In addition, four signals consistent with the *meta*-coupled protons: δ_H 7.01 (1H, d, *J* = 2.0 Hz, H-6) and 6.06 (1H, d, *J* = 2.0 Hz, H-4) on one aromatic ring, and δ_H 6.58 (1H, d, *J* = 2.0 Hz, H-5′) and 6.46 (1H, d, *J* = 2.0 Hz, H-3′) on a second ring were also observed in the ^{1}H-NMR spectrum of compound **6**. These splitting patterns and coupling constants are indicative of two 1,2,3,5-tetrasubstituted aromatic systems. Analyses of the ^{13}C-NMR spectrum along with the aid of the HSQC spectrum of compound **6** confirmed the presence of a conjugated ketone carbon at δ_C 199.7 (C-8′), an ester carbon at δ_C 166.4 (C-7), eight sp^2 quaternary carbons at δ_C 164.2 (C-2′), 161.0 (C-6′), 157.2 (C-5), 156.7 (C-3), 148.2 (C-4′), 128.4 (C-1), 127.6 (C-2), and 111.0 (C-1′), four sp^2

methines at δ_C 110.4 (C-3′), 108.1 (C-6), 103.4 (C-5′), and 103.0 (C-4), three methoxy carbons at δ_C 56.3 (C-9), 55.7 (C-9′), and 52.3 (C-8), and one methyl group at δ_C 22.4 (C-7). The downfield shift of OH-2′ (δ_H 12.94) is thus attributed to intramolecular hydrogen bonding with the adjacent carbonyl at C-8′. The chemical shifts of C-2′, C-6′, C-3, and C-5 suggested their linkages to the oxygen atoms. Taken together, the NMR data indicated that compound **6** possesses a benzophenone-type skeleton with hydroxyl, methoxy, and methyl substitutions. Therefore, through comprehensive analysis of its 1D-NMR spectroscopic data and comparison with literature data, the structure of compound **6** was assigned as monomethylsulochrin, a known natural benzophenone derivative [20].

Compound **7** was isolated as a pale-yellow solid, with a specific optical rotation of $[\alpha]_D^{25}$ -30.6 (*c* 0.2, MeOH). The ESI-MS spectrum of compound **7** exhibited a *pseudo*-molecular ion peak at *m/z* 197 $[M+H]^+$. The ^{1}H-NMR spectrum of compound **7** displayed the signals of two *meta*-coupled aromatic proton at δ_H 6.30 (1H, d, *J* = 2.4 Hz, H-4) and 6.19 (1H, d, *J* = 2.4 Hz, H-6), two methines at δ_H 3.88 (1H, quint, *J* = 6.0 Hz, H-3′) and 3.08 (1H, quint, *J* = 6.6 Hz, H-2′), and three methyls at δ_H 2.10 (3H, s, 2-CH_3), 1.16 (3H, d, *J* = 6.6 Hz, H-1′), and 1.14 (3H, d, *J* = 6.0 Hz, H-4′). Analysis of the ^{13}C-NMR spectrum of compound **7** indicated the presence of eleven carbons, including four sp^2 quaternary carbons at δ_C 156.8 (C-3), 156.2 (C-5), 145.5 (C-1), and 115.1 (C-2), two sp^2 methines at δ_C 105.7 (C-6) and 101.0 (C-4), two sp^3 methines at δ_C 71.9 (C-3′) and 42.9 (C-2′), and three methyls at δ_C 19.4 (C-4′), 16.2 (C-1′), and 10.6 (2-CH_3). The chemical shifts of C-3, C-5, and C-3′ suggested their linkages to the oxygen atoms. On the basis of these spectroscopic data and comparison with literature data, compound **7** was identified as phenol A [21],[22].

The antimicrobial activities of compounds **1**-**7** were evaluated against a panel of Gram-positive and Gram-negative bacteria as well as the yeast *Candida albicans* using the microdilution broth method. Among the tested compounds, compound **7** exhibited the most potent and broad-spectrum antimicrobial activity, with minimum inhibitory concentrations (MICs) of 16 μg/mL against *Enterococcus faecalis* and *C. albicans*, and moderate activity against *Staphylococcus aureus* and *Bacillus cereus* (MICs of 128 and 256 μg/mL, respectively). Compound **6** also showed significant inhibition of *E. faecalis* with an MIC of 32 μg/mL. Notably, compound **5** demonstrated activity across both bacterial groups, including *E. faecalis*, *S. aureus*, *B. cereus*, *Escherichia coli*, and *Salmonella enterica*, with MIC values ranging from 128 to 256 μg/mL. In contrast, compounds **1**–**4** displayed weak or no antimicrobial activity at the tested concentrations (MIC ≥ 128 μg/mL). These results suggest that compounds **5**–**7**, particularly compound **7**, possess promising antimicrobial potential, warranting further pharmacological evaluation.

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

Compounds	Gram (+)			Gram (-)			Yeast
	E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C. albicans*
	MIC (μg/mL)						
1	128	–	–	–	–	–	–
2	64	256	–	–	–	256	256
3	256	–	–	–	–	–	–
4	128	–	–	–	–	–	256
5	256	256	128	256	–	128	128
6	32	–	–	–	–	256	–
7	16	128	256	–	–	–	16
*Streptomycin**	256	256	128	32	256	128	–
*Nystatin**	–	–	–	–	–	–	32

*Positive control; (–) MIC > 256 μg/mL. Streptomycin and nystatin are positive controls.

4. Conclusion

In summary, seven known compounds were isolated from the ethyl acetate extract of the marine sediment-derived fungus *Aspergillus* sp. M793, including fumigaclavine C (**1**), pyripyropene A (**2**), 20-hydroxycyclotryprostatin (**3**), spinasterol (**4**), cerevisterol (**5**), monomethylsulochrin (**6**), and phenol A (**7**).

Structural elucidation was accomplished through comprehensive spectroscopic analyses, including NMR and MS data. Notably, compounds **4** and **5** are reported here for the first time from the genus *Aspergillus*, whereas the remaining compounds have been previously reported from this genus. Antimicrobial evaluation revealed that compounds **5**-**7** exhibited notable activity, with compound **7** displaying the most potent and broad-spectrum effects, particularly against *Enterococcus faecalis* and *Candida albicans*. These results not only expand the chemical diversity reported from marine-derived *Aspergillus* species but also highlight their potential as a rich source of structurally diverse and biologically active natural products for future drug discovery efforts.

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