

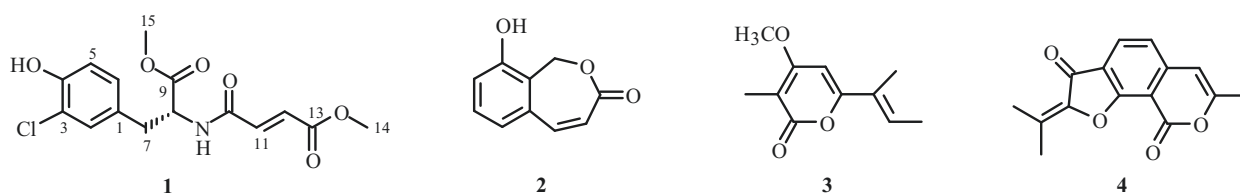
A NEW ANTIBACTERIAL CHLORINATED AMINO ACID DERIVATIVE FROM THE SPONGE-DERIVED FUNGUS *Aspergillus* sp. LS53

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A new chlorinated amino acid derivative with a rare 3-chloro-4-hydroxyphenyl unit named aspergamide A (**1**), along with three known compounds, xylarinol A (**2**), nectriapyrone (**3**), and asperisocoumarin A (**4**), were isolated from the sponge-derived fungus *Aspergillus* sp. LS53. Their structures were determined by combining detailed spectroscopic analysis and literature data. Compounds **1**, **3**, and **4** showed weak *Vibrio harveyi* inhibition with MIC values ranging from 16 to 64 $\mu\text{g/mL}$, whereas **2** was found to be inactive.

Keywords: *Aspergillus* sp., 3-chloro-4-hydroxyphenyl unit, sponge-derived fungi, antibacterial activity.

Marine fungi have been recognized as an abundant source of halogenated natural products with an impressive array of biological properties ranging from antimicrobial to enzyme-inhibitory [1]. However, chlorinated fungal metabolites containing the 3-chloro-4-hydroxyphenyl subunit are very rare. Only five halogenated compounds containing the 3-chloro-4-hydroxyphenyl moiety have so far been isolated from nature, including coniothyriomycin [2], 3-chloro-4-hydroxybenzeneethanol [3], 3-chloro-4-hydroxyphenylacetic acid [4, 5], 3-chloro-4-hydroxyphenylacetamide [5, 6], and (+)-xylariamide A [5, 7]. In the course of our ongoing investigation to find novel fungal bioactive compounds, a new aspergamide A (**1**) containing a 3-chloro-4-hydroxyl phenyl skeleton, together with three known compounds xylarinol A (**2**), nectriapyrone (**3**), and asperisocoumarin A (**4**), were isolated from a sponge-derived *Aspergillus* sp. LS53. The isolation, structure elucidation, and biological activities of these isolates are presented here.

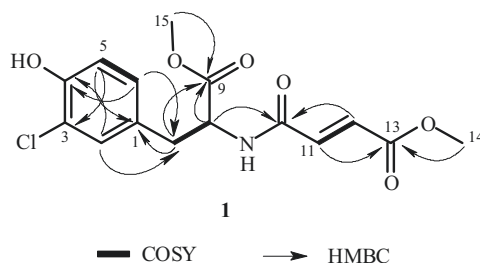


Compound **1** was obtained as a yellow powder. Its molecular formula was determined as $\text{C}_{15}\text{H}_{16}\text{NO}_6\text{Cl}$ by a combination of HR-ESI-MS and NMR data with seven degrees of unsaturation. The HR-ESI-MS peaks at m/z 342.0795 $[\text{M} + \text{H}]^+$ and 344.0688 $[\text{M} + \text{H}]^+$ in the ratio of 3:1 provided additional support for the presence of one chlorine atom in **1**. The ^1H NMR spectrum of **1** indicated the presence of three aromatic methine protons at δ 7.04 (d, $J = 1.7$ Hz, H-2), 6.93 (d, $J = 8.3$ Hz, H-5), and 6.88 (dd, $J = 8.3, 1.7$ Hz, H-6), implying a 1,3,4-trisubstituted benzene ring (Table 1). In combination with HR-ESI-MS data and the chemical shifts in the benzene ring, it can be deduced that compound **1** contains the 3-chloro-4-hydroxyphenyl subunit. Analysis of the ^{13}C NMR and HSQC spectra suggested the presence of one amide carboxyl carbonate δ 163.1 (C-10); two ester carbonyls at δ 171.5 (C-9) and 165.9 (C-13) (Table 1). The HMBC correlations of H_2 -7/C-1 and C-9, H-8/C-9, H-15/C-9, and H-2 and H-6/C-7, as well as the COSY correlations between H_2 -7 and H-8, established a 3-chloro-4-hydroxy-substituted phenylalanine methyl ester moiety. The additional HMBC correlations of H-11/C-10, C-12, and C-13 and H-12/C-10, C-11, and C-13 together with the large coupling constants (15.4 Hz) between H-11 and H-12 showed the presence of a fumaric acid moiety.

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TABLE 1. ^1H (600 MHz) and ^{13}C (150 MHz) NMR Data of **1** (CDCl_3 , δ , ppm, J/Hz)

C atom	δ_{H}	δ_{C}	C atom	δ_{H}	δ_{C}
1	–	129.7	8	4.91 (1H, m)	53.6
2	7.04 (1H, d, J = 1.7)	129.4	9	–	171.5
3	–	120.1	10	–	163.1
4	–	150.8	11	6.91 (1H, d, J = 15.4)	135.7
5	6.93 (1H, d, J = 8.3)	116.6	12	6.83 (1H, d, J = 15.4)	128.8
6	6.88 (1H, dd, J = 8.3, 1.7)	131.1	13	–	165.9
7	3.13 (1H, dd, J = 14.2, 5.8)	36.8	14	3.80 (3H, s)	52.4
	3.07 (1H, dd, J = 14.2, 5.8)		15	3.76 (3H, s)	52.8

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

Furthermore, the HMBC correlation of H_3 -14/ C -13 revealed that a methoxyl group was linked to the fumaric acid unit, constituting a fumaric acid methyl ester moiety. The linkage between the two units in **1** was examined based on the HMBC cross-peak of H-8/ C -10 (Fig. 1), which suggested that the fumaric acid methyl ester moiety was connected to the C-8 position of 3-chloro-4-hydroxy-substituted phenylalanine methyl ester moiety via an amide bond, leading to a planar structure assignment as shown for **1**. The absolute configuration at C-8 in **1** was assigned an *R* configuration based on comparison with literature [7]. Thus, the complete structure of **1** was established and named as aspergamide A.

The structure of three known compounds identified as xylarinol A (**2**) [8], nectriapyrone (**3**) [9], and asperisocoumarin A (**4**) [10] were deduced by comparison of their spectroscopic data with those reported in the literature. Their antimicrobial activity was tested against an aquatic pathogen, *Vibrio harveyi*, which commonly infects fish. Compounds **1**, **3**, and **4** displayed weak antibacterial activity with MIC values of 16, 64, and 32 $\mu\text{g}/\text{mL}$ against *V. harveyi*, respectively. However, compound **2** was appreciably inactive up to a concentration of 128 $\mu\text{g}/\text{mL}$ [11].

EXPERIMENTAL

General Experimental Procedures. NMR data were recorded on an Agilent 600 MHz NMR system, with TMS as internal standard. Optical rotation was recorded on a Perkin-Elmer model 341 polarimeter. The HR-ESI-MS spectrum was obtained on an Agilent 6224 TOF-MS mass spectrometer. Medium-pressure liquid chromatography (MPLC) was performed on an ODS column (15 μm). Vacuum liquid chromatography (VLC) was carried out with silica gel (200–300 mesh). Compound purification was carried out by semi-preparative HPLC separation on a Waters HPLC instrument (Alliance 2695, Milford, MA, USA) equipped with a Waters 2996 detector and a C_{18} column (250 \times 20 mm ID, 5 μm ; YMC Co. Ltd., Tokyo, Japan).

Fungal Material. The fungus *Aspergillus* sp. LS53 was isolated from the tissue of the sponge *Haliclona* sp. collected from Linshui, Sanya City, China. The fungus was identified by rDNA amplification and sequence analysis of the ITS region. The strain specimen in PDA medium was deposited at the College of Food and Pharmaceutical Sciences, Ningbo University, China.

Culture, Extraction and Isolation. The strain was cultivated in 250 mL Erlenmeyer flasks, each containing 100 mL PDB medium (potato 200 g/L, glucose 20 g/L, sea salt 100 g/L, H_2O 1L). After 2 days of incubation at 25°C with 180 rpm agitation, the 10% concentration of the cultures were used to inoculate Erlenmeyer flasks (250 mL), each containing 80 g rice solid medium (rice 80 g, sea salt 35 g, H_2O 120 mL) [12]. Fermentation under static cultivation was carried out at room temperature for 40 days. The fermentation products were extracted three times with ethyl acetate. The extracting solutions were merged by boiling away solvent under reduced pressure to afford 23 g of oily crude extract.

Then the crude extract was subjected to VLC on a silica gel column (6 × 15 cm, 200–300 mesh) using petroleum ether–ethyl acetate as gradient eluent (from 15:1 to 0:1), affording five fractions (Fr. 1–5). Fraction 3 (3.75 g) was applied to MPLC using MeOH–H₂O gradients (20–80% MeOH, UV detection at 254 nm) to give three subfractions Subfrs.3.A–3.C. Subfraction 3.A (116 mg) was further separated by semipreparative HPLC (40% MeOH–H₂O) to yield **1** (3.0 mg), **2** (5.7 mg), and **3** (8.9 mg). Subfraction 3.B (97 mg) was further purified by semipreparative HPLC (55% MeOH–H₂O) to yield **4** (12.5 mg).

Aspergamide A (1). C₁₅H₁₇NO₆Cl, yellow amorphous powder, $[\alpha]_D^{25} -12^\circ$ (c 0.10, MeOH). HR-ESI-MS, *m/z* 342.0795/344.0688 [M + H]⁺ (calcd for C₁₅H₁₈NO₆Cl, 342.0744/344.0715 in 3:1-intensity). For ¹H and ¹³C NMR, see Table 1.

Xylarinol A (2). C₁₀H₈O₃, white solid. ¹H NMR (600 MHz, CD₃OD, δ, ppm, J/Hz): 7.30 (1H, d, J = 12.1), 7.28 (1H, t, J = 7.9), 6.99 (1H, d, J = 7.9), 6.97 (1H, d, J = 7.9), 6.31 (1H, d, J = 12.1), 5.22 (2H, s). ¹³C NMR (150 MHz, CD₃OD, δ, ppm): 62.5 (C-1), 170.8 (C-3), 122.9 (C-4), 142.9 (C-5), 138.5 (C-5a), 121.9 (C-6), 131.3 (C-7), 118.3 (C-8), 156.0 (C-9), 122.9 (C-9a).

Nectriapyrone (3). C₁₁H₁₄O₃, colorless oil. ¹H NMR (600 MHz, DMSO-d₆, δ, ppm, J/Hz): 6.69 (1H, d, J = 7.2), 6.09 (1H, s), 3.90 (3H, s), 1.93 (3H, s), 1.84 (3H, s), 1.83 (3H, d, J = 7.2). ¹³C NMR (150 MHz, DMSO-d₆, δ, ppm): 165.2 (C-2), 103.0 (C-3), 166.1 (C-4), 91.6 (C-5), 160.2 (C-6), 127.0 (C-7), 129.9 (C-8), 14.4 (C-9), 8.7 (C-10), 56.2 (C-11), 12.3 (C-12).

Asperisocoumarin A (4). C₁₅H₁₂O₄, yellow solid. ¹H NMR (600 MHz, CDCl₃, δ, ppm, J/Hz): 7.91 (1H, d, J = 8.1), 7.23 (1H, d, J = 8.1), 6.59 (1H, s), 2.36 (1H, s), 2.30 (1H, s), 2.19 (1H, s). ¹³C NMR (125 MHz, CDCl₃, δ, ppm): 146.9 (C-2), 181.7 (C-3), 123.4 (C-3a), 130.5 (C-4), 120.4 (C-5), 146.0 (C-5a), 104.5 (C-6), 160.1 (C-7), 157.9 (C-9), 106.3 (C-9a), 165.7 (C-9b), 20.2 (C-10), 134.3 (C-11), 19.9 (C-12), 17.1 (C-13).

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