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ALKALOIDS AND CYCLOPEPTIDES FROM THE MARINE-DERIVED FUNGUS *ASPERGILLUS* SP. M512

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Abstract. In the course of our screening program, the EtOAc extract of an *Aspergillus* sp. (strain M512), from marine sediment collected in the sea of Co To-Thanh Lan (Quang Ninh, Viet Nam), exhibited antimicrobial activities against three gram positive bacteria (*Enterococcus faecalis* ATCC29212, *Stapphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC14579), and one yeast strain *Candida albicans* ATCC10231. This paper reported the isolation and structural elucidation of seven secondary metabolites including two alkaloids, 3,4-dihydroxy-6,7-dimethyl-quinoline-2-carboxylic (**1**), norharman (**2**) and five cyclodipeptides, cyclo-(Leu-*trans*-4-hydroxy-Pro) (**3**), cyclo-(Phe-*trans*-4-hydroxy-Pro) (**4**), Cyclo-(Phe-Pro) (**5**), cyclo (Pro-Tyr) (**6**), and cyclo-(Pro-Leu) (**7**) from culture broth of *Aspergillus* sp. M512. Their structures were determined by spectroscopic analysis including IR, MS, 1D-NMR and 2D-NMR, as well as comparison with literature data. All isolated compounds were evaluated for their antimicrobial activity against a panel of clinically significant microorganisms. Except compounds **1** and **2**, with MIC values ranged from 32 to 128 μg/mL, five other compounds do not show inhibition activities against tested microorganisms.

Keywords: Aspergillus, alkaloid, cyclodipeptide, antimicrobial activity.

Classification numbers: 1.1.1, 1.2.1, 1.5.3.

1. INTRODUCTION

Viet Nam, with a coastline of 3000 kilometers in length and total ocean surface areas of over one million square kilometers, has a unique ecosystem and is one of sixteen highest

biodiversity areas in the world. Studies in marine-derived fungus all over the world have been significantly developed and achieved many positive results [1 - 3]. However, research on secondary compounds from marine fungi in Viet Nam has just been started and there are not many publications [4 - 5].

As part of our study in the search for bioactive compounds from the Marine-Derived Fungus of Northern Viet Nam, the EtOAc extract of the *Aspergillus* sp. M512 strain, collected from marine sediment in the sea of Co To-Thanh Lan (Quang Ninh, Viet Nam), exhibited antimicrobial activities against three Gram positive bacteria (*Enterococcus faecalis* ATCC29212, *Stapphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC 14579), and one yeast strain *Candida albicans* ATCC10231 with a MIC value of 128, 64, 128, and 16 µg/ml, respectively. In this paper, we report the isolation, structural elucidation and antimicrobial activities of two alkaloids (**1-2**) and five cyclodipeptides (**3-7**) (Figure 1) isolated from the cultures broth of *Aspergillus* sp. M512 strain.

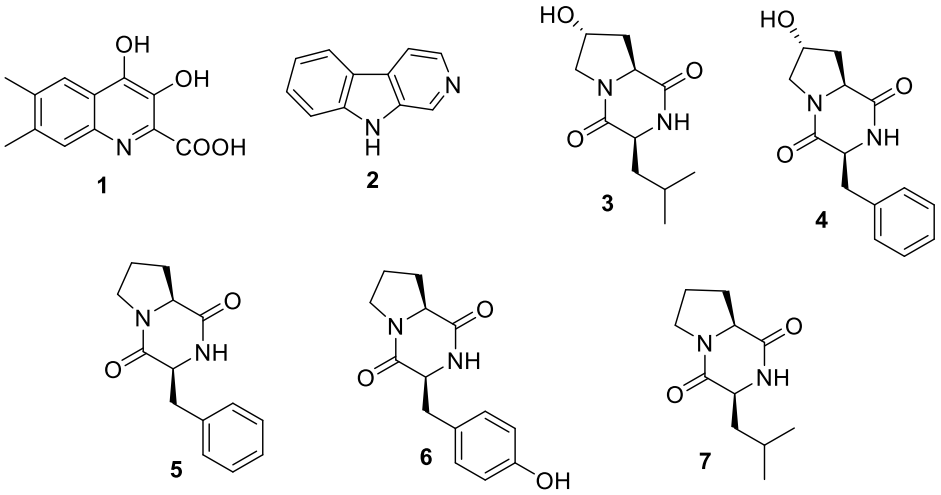

Figure 1. Compounds **1-7** from marine-derived fungus *Aspergillus* sp. M512.

2. MATERIAL AND METHODS

2.1. General experimental procedures

Optical rotations were recorded on a Polax-2L polarimeter in MeOH. ESI-MS spectra were recorded on an Agilent 1100 LC-MSD Trap spectrometer. 1D and 2D NMR spectra were recorded on a Bruker 500 MHz instrument (Avance 500). TLC silica gel Merck 60 F_{254} was used as thin-layer chromatography. Column chromatography (CC) was carried out using silica gel (Kieselgel 60, 40-63 µm) or Sephadex LH-20 (25 - 100 µm).

2.2. Marine materials

The marine sediment was collected from the sea of Co To-Thanh Lan (Quang Ninh, Viet Nam) in August 2019. A 0.5 g of sediment sample was suspended in 4.5 mL of sterile distilled water, homogenized by vortexing for 1 minute, and the suspension was heat-treated at 60 ^{o}C for 6 minutes. Next, 0.5 mL of the heat-treated suspension was used for serial dilution in sterile distilled water to 10^{-3} ratio. At the final dilution step, aliquots of 50 µL were spread on agar disk

pre-filled with PDA medium, including 30 g/L potato extract, 20 g/L dextrose 5 g/L soluble starch, 30 g/L instant ocean, 15 g/L agar). Plates were incubated at 28 °C for 7 - 15 days. Single colonies of fungi were transferred onto new petri dishes of PDA medium for further purification steps. The fermentation was carried out in a 5 L flask using a modification of the published method [5]. The identification of M512 by the 18S gene sequence of ribosomal RNA showed that strain M512 belongs to nenus *Aspergillus* with the 18S rRNA gene sequence identified (99 % similarity) of 18S rRNA gene sequences with nenus *Aspergillus* on GenBank database.

2.3. Extraction and isolation

The culture broth (50 L) of *Aspergillus* sp. M512 strain was filtered. Then this culture solution was subjected to amberlite-XAD 16 column (Ø 10) and eluted with 40 L of MeOH, combined with concentration under reduced pressure to obtain methanol residue (MM512, 160 g). The solution then was successively partitioned with EtOAc and CH_2Cl_2 to obtain the EtOAc (EM152, 30.0 g) and CH_2Cl_2 (CM512, 8.0 g) extracts after removal of the solvents *in vacuo.*

The extract MM512 was chromatographed by preparative reversed-phase MPLC, eluted with H_2O/MeOH gradient to give six fractions, MF1-MF6. The MF2 fraction was chromatographed on silica gel column eluting with CH_2Cl_2/MeOH gradient to give seven sub-fractions, MF2.1-MF2.7. Sub-fraction MF2.4 was purified by CC on silica gel (CH_2Cl_2/acetone gradient) to afford compound **1** (6 mg). Sub-Fraction F2.5 was separated by CC on Sephadex LH-20 with H_2O-MeOH (1:9) to furnish compound **2** (7 mg). The MF3 fraction was chromatographed on silica gel column eluting with EtOAc/MeOH gradient to give six sub-fractions, MF3.1-MF3.6. Sub-fraction MF3.3 was chromatographed on silica gel CC, eluting with a CH_2Cl_2/acetone (9/1) providing **3** (7 mg). Fraction MF4was subjected to Sephadex LH-20 CC (MeOH) to provide 6 sub-fractions, MF4.1-MF4.6. Compound **6** (5 mg) was purified from sub-fraction MF4.3 by CC on silica gel (CH_2Cl_2/acetone gradient). The MF5 fraction was chromatographed on silica gel column eluting with CH_2Cl_2/MeOH gradient to give six sub-fractions, MF5.1-MF5.6. Sub-fraction MF5.4 was purified by Sephadex LH-20 CC (MeOH) to give compound **5** (5 mg) and compound **7** (7 mg). Fraction MF6 was purified by CC on silica gel (CH_2Cl_2/MeOH gradient) to afford compound **4** (5 mg).

3,4-Dihydroxy-6,7-dimethyl-quinolin-2-carboxylic (1): Pale yellow powder, mp. 155 - 156 ^oC; ESI-MS: *m/z* 234 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6), δ_H (ppm): 7.89 (1H, s, H-5), 7.68 (1H, s, H-8), 2.50 (3H, s, CH_3-11), 2.47 (3H, s, CH_3-10); ^{13}C-NMR (125 MHz, DMSO-d_6), δ_C (ppm): 160.48 (C=O), 149.89 (C-3), 146.26 (C-2), 144.85 (C-7), 141.73 (C-4), 139.05 (C-6), 138.50 (C-8a), 129.78 (C-4a), 128.66 (C-5), 125.85 (C-8), 20.05 (C-11), 19.41 (C-10).

Norharman (2): White solid, mp.199 - 200 ^oC; ESI-MS: *m/z* 169 $[M+H]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6) δ_H (ppm): 8.90 (1H, s, H-1), 8.31 (1H, d, *J* = 5.5 Hz, H-3), 8.22 (1H, d, *J* = 8.0 Hz, H-5), 8.09 (1H, d, *J* = 5.0 Hz, H-4), 7.60 (1H, d, *J* = 8.0 Hz, H-8), 7.54 (1H, td, *J* = 0.5, 8.0 Hz, H-7), 7.23 (1H, td, *J* = 0.5, 8.0 Hz, H-6); ^{13}C-NMR (125 MHz, CD_3OD), δ_C (ppm): 140.66 (C-8a), 137,99 (C-3), 136.09 (C-8b), 134.10 (C-1), 128,06 (C-7), 127.43 (C-4a), 121.74 (C-5), 120.59 (C-4b), 119.18 (C-6), 114.62 (C-4), 112.04 (C-8).

Cyclo-(Leu-*trans*-4-hydroxy-Pro) (3): White powder; ESI-MS: *m/z* 227 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$), δ_H (ppm): 5.99 (1H, br. s, NH), 4.59 (1H, m, H-4), 4.50 (1H, dd, *J* = 11.0, 6.5 Hz, H-6), 4.06 (1H, dd, *J* = 3.5, 9.5 Hz, H-9), 3.73 (1H, dd, *J* = 4.5, 13.0 Hz, H-3b), 3.57 (1H, br d, *J* = 13.0 Hz, H-3a), 2.40 (1H, m, H-5b), 2.16 (1H, m, H-5a), 2.07 (1H, m, H-10b), 1.78 (1H, m, H-11), 1.53 (1H, m, H-10a), 1.00 (3H, d, *J* = 6.5 Hz, CH_3-13), 0.96 (3H, d, *J* = 6.5 Hz, CH_3-12); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 170.40 (C=O), 166.22 (C=O), 68.53 (C-

4), 57.34 (C-6), 54.37 (C-3), 53.43 (C-9), 38.60 (C-10), 37.49 (C-5), 24.73 (C-11), 23.26 (C-13), 21.24 (C-12).

Cyclo-(Phe-*trans*-4-hydroxy-Pro) (4): White powder; ESI-MS: *m/z* 261 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$), δ_H (ppm): 7.27 (5H, m, H-aromatic), 4.52 (1H, m, H-9), 4.37 (1H, m, H-6), 4.30 (1H, m, H-4), 3.74 (1H, dd, *J* = 5.0, 13.5 Hz, H_a-3), 3.36 (1H, m, H_b-3), 3.33 (1H, m, H_a-10), 3.19 (1H, dd, *J* = 5.5, 15.0 Hz, H_b-10), 2.10 (1H, dd, *J* = 6.0, 13.5 Hz, H_a-5), 1.41 (1H, m, H_b-5); ^{13}C-NMR (125 MHz, $CDCl_3$), δ_C (ppm): 169.85 (C=O), 165.68 (C=O), 135.99 (C-1′), 129.59 (C-2′, 6′), 128.07 (C-3′, 5′), 126.66 (C-4′), 67.12 (C-4), 56.93 (C-6), 56.19 (C-9), 53.83 (C-3), 37.46 (C-5), 36.59 (C-10).

Cyclo-(Phe-Pro) (5): White powder; $[\alpha]^{29}_D$ = - 81.1 (*c* 0.4, MeOH); ESI-MS: *m/z* 245 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm): 7.20-7.33 (5H, m, H-aromatic), 4.22 (1H, m, H-9), 4.02 (1H, m, H-6), 3.58 (1H, m, H_a-10), 3.22 (1H, *J* = 5.0, 13.5 Hz, H_a-3), 3.01 (1H, *J* = 5.0, 13.5 Hz, H_b-3), 2.63 (1H, m, H_b-10), 2.07 (1H, m, H_a-5), 1.92 (1H, m, H_b-5), 1.69 (2H, m, H-4); ^{13}C-NMR (125 MHz,CD_3OD) δ_C (ppm): 169.91 (C=O), 166.01 (C=O), 135.30 (C-1′), 129.86 (C-2′, 6′), 128.24 (C-3′, 5′), 127.11 (C-4′), 58.36 (C-6), 57.70 (C-9), 44.73 (C-3), 39.56 (C-10), 28.42 (C-5), 21.06 (C-4).

Cyclo (Pro-Tyr) (6): White powder; $[\alpha]^{29}_D$ = - 90.4 (*c* 0.3, MeOH); ESI-MS: *m/z* 261 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD), δ_H (ppm): 7,00 (2H, d, *J* = 8.5 Hz, H-2′, 6′), 6.74 (2H, d, *J* = 8.5 Hz, H-3′, 5′), 4.38 (1H, m, H-9), 4.07 (1H, m, H-6), 3.57 (1H, m, H_a-10), 3.37 (1H, m, H_a-3), 3.08 (2H, m, H_b-10, H_b-3), 2.12 (1H, m, H_a-5); 1.82 (2H, m, H_b-5, H_a-4), 1.25 (1H, m, H_b-4); ^{13}C-NMR (125 MHz, CD_3OD), δ_C (ppm): 169.38 (C=O), 165.56 (C=O), 156.27 (C-4′), 130.70 (C-2′, 6′), 126.24 (C-1′), 114.79 (C-3′, 5′), 58.66 (C-6), 56.50 (C-9), 44.52 (C-3), 36.25 (C-10), 27.99 (C-5), 21.32 (C-4).

Cyclo-(Pro-Leu) (7): White solid, mp. 147-148 ^oC; $[\alpha]_D^{29}$ -77.7 (*c* 0.25, MeOH); ESI-MS: *m/z* 249 $[M+K]^+$, ^{1}H-NMR (500 MHz. $CDCl_3$), δ_H (ppm): 4.12 (1H, t,*J* = 8.0 Hz, H-6), 4.02 (1H, m, H-9), 3.60 (1H, m, H_b-3), 3.55 (1H, m, H_a-3), 2.34 (1H, m, H_a-5), 2.13 (1H, m, H_a-5), 2.05 (2H, m, H_b-4. H_b-10), 1.90 (1H, m, H_a-4), 1.53 (1H, m, H_a-10), 1.77 (1H, m, H-11), 0.99 (3H, d, *J* = 6.5 Hz, CH_3-12), 0.96 (3H, d, *J* = 6.5 Hz, CH_3-13); ^{13}C-NMR (125 MHz. CD_3OD), δ_C (ppm): 171.39 (C=O), 167.52 (C=O), 58.87 (C-6),53.23 (C-9), 45.03 (C-3), 38.00 (C-10), 27.66 (C-5), 24.36 (C-11), 22.24 (C-12), 21.88 (C-4), 20.79 (C-13).

3. RESULTS AND DISCUSSION

Compound **1** was isolated as pale yellow powder. The ESI-MS of **1** showed the pseudo-molecular ion $[M+H]^+$ at *m/z* 234. The ^{1}H-NMR spectrum showed the signals of two singlet methyls at δ_H 2.47 (3H, s, CH_3-10) and 2.50 (3H, s, CH_3-11), two aromatic protons at δ_H 7.89 (1H, s, H-5), 7.68 (1H, s, H-8). The ^{13}C-NMR and DEPT spectra with the aid of the HSQC of **1** indicated the presence of 12 carbons, including one carboxylic carbon at δ_C160.5, two methyl groups at δ_C 20.05 (C-11), 19.41 (C-10), two sp^2 methine groups at δ_C 128.66 (C-5), 125.85 (C-8), and seven sp^2 quaternary nonprotonated carbons at δ_C 149.89 (C-3), 146.26 (C-2), 144.85 (C-7), 141.73 (C-4), 139.05 (C-6), 138.50 (C-8a), 129.78 (C-4a). The carbon chemical shifts of C-2, C-3, and C-4 suggested their linkages to oxygen or nitrogen atoms. In the HMBC spectrum of **1**, the correlation of the proton of methyl groups CH_3-10 (δ_H 5.35) with C-5,C-6 and C-7; CH_3-11 with C-6, C-7 and C-8, confirmed the position of methyl group CH_3-10 at C-6 and CH_3-11 at C-7. The HMBC correlation of H-8 with C-8a; H-5 with C-4, C-8a demonstrated the connection of C-4, C-5 and C-8a to C-4a (Figure 2). Detailed analysis of 2D-NMR spectra, especially HMBC

spectrum allowed structure determination of **1** as 3,4-dihydroxy-6,7-dimethyl-quinoline-2-carboxylic. This alkaloid was previously described from *Micromonospora* sp. G019 strain [6].

Compound **2** was isolated as white solid. The ESI-MS showed the pseudo-molecular ion $[M+H]^+$ at *m/z* 169. The ^{1}H-NMR spectrum showed the signals of seven aromatic protons at δ_H 8.90 (1H, s, H-1), 8.31 (1H, d, *J* = 5.5 Hz, H-3), 8.22 (1H, d, *J* = 8.0 Hz, H-5), 8.09 (1H, d, *J* = 5.0 Hz, H-4), 7.60 (1H, d, *J* = 8.0 Hz, H-8), 7.54 (1H, td, *J* = 0.5, 8.0 Hz, H-7), 7.23 (1H, td, *J* = 0.5, 8.0 Hz, H-6). The ^{13}C-NMR spectrum with the aid of the HSQC of **2** indicated the presence of 11 carbons, including seven sp^2 methine groups at δ_C 137,99 (C-3), 134.10 (C-1), 128,06 (C-7), 121.74 (C-5), 119.18 (C-6), 114.62 (C-4), 112.04 (C-8) and four sp^2quaternary nonprotonated carbons at δ_C 140.66 (C-8a), 136.09 (C-8b), 127.43 (C-4a), 120.59 (C-4b). Complete analyses of 2D NMR spectra established the structure of **2** as norharman which was previously reported [7].

Compound **3** was isolated as a white powder. In its positive ESI mass spectrum, the pseudo-molecular ion was observed at *m/z* 227 $[M+H]^+$. Analyses of the ^{13}C-NMR and DEPT spectra with the aid of the HSQC of **3** indicated the presence of 11 carbons, including two carbonyl carbons at δ_C170.40 (C=O), 166.22 (C=O), three sp^3 methylene groups at δ_C 54.37 (C-3), 38.60 (C-10), 37.49 (C-5), four sp^3 methine groups at δ_C 68.53 (C-4), 57.34 (C-6), 53.43 (C-9), 23.26 (C-11), two methyl groups at δ_C 24.73 (C-13), 21.24 (C-12). The carbon chemical shifts of C-3, C-4, C-6 and C-9 suggested their linkages to oxygen or nitrogen atoms. The ^{1}H-NMR spectrum of **3** indicated the presence of two methyl group at δ_H1.00 (3H, d, *J* = 6.5 Hz, CH_3-13), 0.96 (3H, d, *J* = 6.5 Hz, CH_3-12), a oxymethine group at δ_H 4.59 (1H, m, H-4), two methine groups bearing nitrogen at δ_H4.50 (1H, dd, *J* = 11.0, 6.5 Hz, H-6), 4.06 (1H, dd, *J* = 3.5, 9.5 Hz, H-9), and one methylene group bearing nitrogen at δ_H 3.73 (1H, dd, *J* = 4.5, 13.0 Hz, H-3b), 3.57 (1H, br d, *J* = 13.0 Hz, H-3a), and 5 protons at δ_H 0.96-2.41. Analysis of ^{1}H-^{1}H COSY spectrum revealed the presence of two spin-spin coupling systems: CH_2-3/CH-4/CH_2-5/CH-6; and CH-9/CH_2-10/CH-11 (figure 2). Analyses of 2D NMR spectra, especially HMBC spectrum indicated the structure of **3** which was a cyclodipeptide, forming from a proline and a 4-hydroxy-phenyl unit. The NMR data of **3** were in agreement with those reported in the literature for cyclo-(Leu-*trans*-4-hydroxy-Pro) [8]. This compound was previously described from *Lactobacillus plantarum* MiLAB 393 strain, it showed antifungal activity against *A. fumigatus and P. roqueforti* [8].

Compound **4** was isolated as a white solid. The ESI-MS of **4** showed the pseudo-molecular ion $[M+H]^+$ at *m/z* 261. The ^{13}C-NMR and DEPT spectra of **4** indicated the presence of two carbonyl carbons at δ_C 169.85 (C=O), 165.68 (C=O), a phenyl ring at δ_C126.66-135.99, three methylene groups at δ_C 53.83 (C-3), 37.46 (C-5), 36.59 (C-10) and three methine groups at δ_C67.12 (C-4), 56.93 (C-6), 56.19 (C-9). The H-NMR spectrum of **4** indicated the presence of 5 aromatic protons at δ_H 7.27 (5H, m, H-aromatic) and nine protons in the aliphatic region at δ_H 1.41-4.52. This data strongly suggested that the leucine fragment in **3** was replaced by a phenyl unit in **4**. Analyses of ESI-MS and 1D-NMR, along with the comparison of spectral data in the literature established the structure of compound **4** as cyclo-(Phe-*trans*-4-hydroxy-Pro) [9].

Compound **5** was isolated as a white solid. The ESI-MS of **5** indicated the pseudo-molecular ion peak at *m/z* 245 $[M+H]^+$. The ^{1}H-NMR and ^{13}C-NMR spectra of compound **5** were similar to those of **4**, except for the presence of a methylene group instead of anoxymethine group of **4**. This observation suggested that the 4-hydroxy-proline fragment in **4** was replaced by a proline moiety in **5**. By comparing the obtained data of this compound with reported data, compound **5** was confirmed to be cyclo-(Phe-Pro) [10].

Compound **6** was isolated as a white solid. In its positive ESI mass spectrum, the pseudo-molecular ion was observed at *m/z* 261 $[M+H]^+$. The 1D-NMR spectra (^1H and ^{13}C) of compound **6** were close to those of **5**, except for the presence of an A_2B_2 system instead of a phenyl ring. The combination of the ESI-MS, 1D-NMR spectra analysis and comparison with data in the literature confirmed **6** as cyclo-(Pro-Tyr) [11].

Compound **7** was obtained as a white solid. The ESI mass spectrum (positive) of **7** showed a pseudo-molecular ion peak at *m/z* 249 $[M+K]^+$. The NMR data of compound **7** showed similar spectroscopic features as compound **3**. The difference between these two compounds was the presence of a methylene group instead of the oxymethine group. The NMR data of **7** were consistent with those previously reported of cyclo-(Pro-Leu) [12].

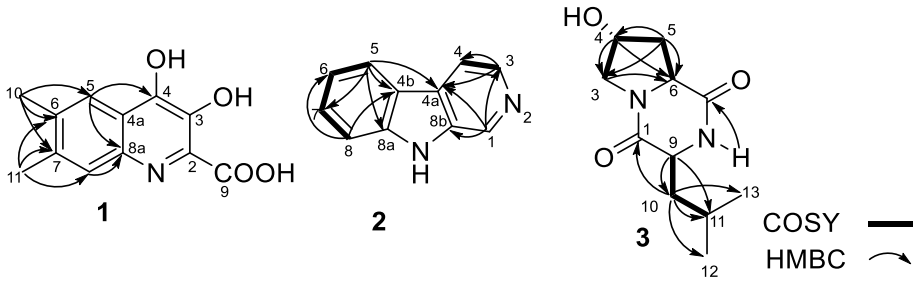

Figure 2. Selected HMBC and COSY correlations of compounds **1-3**.

Table 1: Antibacterial and antifungal activities of compounds **1-7** (MIC: µg/mL).

Compound	Gram-positive			Gram-negative			Yeast
	E. faecalis ATCC29212	*S. aureus* ATCC25923	*B. cereus* ATCC13245	*E. coli* ATCC25922	*P. aeruginosa* ATCC27853	*S. enterica* ATCC13076	*C. albicans* ATCC10231
1	**128**	> 256	> 256	**32**	➢ 256	**64**	➢ 256
2	> 256	> 256	> 256	**128**	> 256	> 256	> 256
3	> 256	> 256	> 256	> 256	> 256	> 256	> 256
4	> 256	> 256	> 256	> 256	> 256	> 256	> 256
5	> 256	> 256	> 256	> 256	> 256	> 256	> 256
6	> 256	> 256	> 256	> 256	> 256	> 256	> 256
7	> 256	> 256	> 256	> 256	> 256	> 256	> 256
Streptomycin	256	256	128	32	256	128	
Cycloheximide							32

All isolated compounds (**1-7**) were tested against 7 referenced microbial strains (Table 1). Streptomycin and cycloheximide were used as reference compounds. Compound **1** displayed inhibition against *E. faecalis, E. coli, andS.enterica* with MIC values of 128, 32, and 64 µg/mL, respectively. Compound **2** selectively inhibited *E. coli* with a MIC value of 128 µg/mL. Compounds **3-7** do not show activities against any tested microorganisms.

4. CONCLUSION

Analysis of an antimicrobial extract prepared from culture broth of the marine-derived fungus *Aspergillus* sp. (strain M512) led to the isolation of seven compounds identified as two alkaloids, 3,4-dihydroxy-6,7-dimethyl-quinoline-2-carboxylic (**1**) and norharman (**2**); five

cyclodipeptides, cyclo-(Leu-*trans*-4-hydroxy-Pro) (**3**), cyclo-(Phe-*trans*-4-hydroxy-Pro) (**4**), Cyclo-(Phe-Pro) (**5**), cyclo (Pro-Tyr) (**6**), and cyclo-(Pro-Leu) (**7**). Their structures were established by spectral data analysis, including MS, 1D, 2D-NMR and by comparison with literature data. All compounds were evaluated for their antimicrobial activity against a panel of clinically significant microorganisms. Compound **1** exhibited antimicrobial activity against several strains of both gram-positive and gram-negative bacteria (MIC values ranged from 32 to 128 μg/mL). Compound **2** selectively inhibited *E. coli* with a MIC value of 128 μg/mL.

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