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ANTIMICROBIAL COMPOUNDS PRODUCED BY THE MARINE ACTINOMYCETE *STREPTOMYCES* SP. G668

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Summary

Antimicrobial Compounds Produced by the Marine Actinomycete *Streptomyces* sp. G668

From the ethyl acetate extract of the *Streptomyces* sp. G668 strain, seven compounds were isolated: 9*H*-pyrido[3,4-b]indole (**1**), L-tryptophan (**2**), indole-3-carboxylic acid (**3**), 3-indolylacetic acid (**4**), cyclo-(Pro-Ala) (**5**), uracil (**6**), thymine (**7**). Their structures were elucidated by nuclear magnetic resonance spectroscopy (NMR), mass spectroscopy (MS) and comparison with the reference data. Compound **1** exhibited moderate antibacterial activity against *Escherichia coli* ATCC25922 and compound **5** strongly inhibited *Candida albicans* ATCC10231.

Keywords: *Marine actinomycetes, Streptomyces, Alkaloid, Indole, Cyclodipeptide.*

1. Introduction

Marine actinobacteria have been known as an important source of bioactive natural products which possess a wide range of bioactivities such as anticancer, antifungal, antiviral, etc. [1]. Among them, *Streptomyces* is the largest antibiotic-producing genus in the microbial world discovered so far [2]. In the framework of the project "Investigation of anti-tuberculosis and antimicrobial natural products from marine microorganisms in the South-Central region (Khanh Hoa - Binh Thuan Sea, Vietnam)", herein we report the isolation and structural elucidation of seven compounds from the ethyl acetate extract of the *Streptomyces* sp. G668 collected in Van Phong, Khanh Hoa province, Vietnam. Their structures are described in Fig. 1.

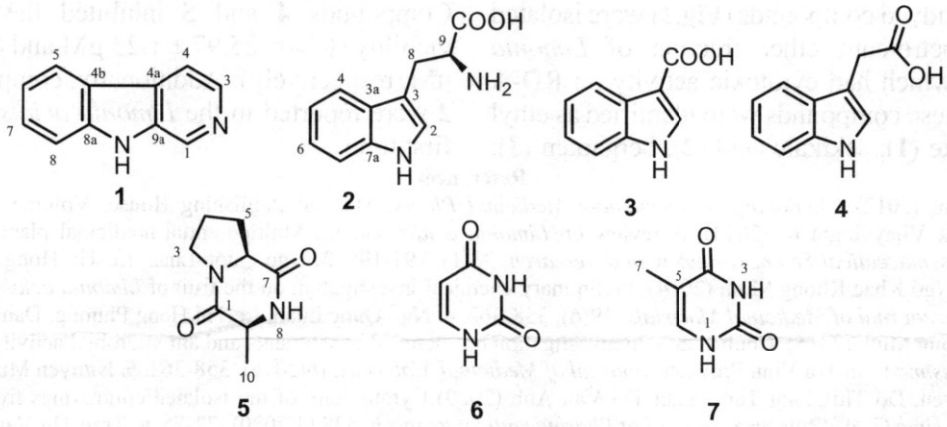

Fig. 1. Structures of compounds **1-7** isolated from *Streptomyces* sp. G668

2. Material and methods

2.1. General experimental procedures

The ESI-MS spectra were recorded on an Agilent 1100 LC-MSD Trap spectrometer. The NMR spectra were recorded on a Bruker 500.13 and 600.36 MHz spectrometer operating at 125.76 and 150.98 MHz for ^{13}C NMR, and at 500.13 and 600.36 MHz for ^{1}H-NMR. The ^{1}H chemical shifts were referenced to $CDCl_3$ and CD_3OD at δ_H 7.27 and 3.31 ppm, respectively, while the ^{13}C chemical shifts were referenced to the central peak at δ_C 77.1 ($CDCl_3$) and 49.0 (CD_3OD). TLC (thin-layer chromatography) *silica gel* Merck 60 F_{254} was used. Column chromatography (CC) was carried out using *silica gel* 40-63 μm, YMC RP-18 (30-50 μm) and Sephadex LH-20.

2.2. Marine materials

The strain G668 was isolated from the seaweed (*Caulerpa serrulata* (Forsk.) J. Ag.) collected at the 6-meter depth on the coast of Van Phong -

Khanh Hoa in May 2020 and was identified by Prof. Do Cong Thung, Institute of Marine Environment and Resources -Vietnam Academy of Science and Technology (VAST). *2.3. Isolation, identification, and fermentation of the actinomycete strain G668*

The seaweed sample (0.5 g) was crushed by the glass rod in a falcon tube; 4.5 mL of sterile sea water was added. The mixture was then homogenized by vortexing for 1 minute, and the suspension was treated using a wet-heat technique (60°C for 6 minutes). After that, 0.5 mL of the suspension was transferred to 4.5 mL sterile distilled water and vortexed for 1 minute to afford the sample solution. Aliquots of 50 µL of the sample solution was spread on a petri dish of M1 medium pH 7.0 (g/L) (1 g soluble starch, 0.4 g yeast extract, 0.2 g peptone, 30 g sea salt, 15 g agar) supplemented with 50 µg/mL polymycin B and cycloheximide to inhibit Gram negative bacterial and fungal contaminations. After 7 days of aerobic incubation at 28°C, the colony of the G668 actinomycete strain was transferred onto a petri dish of the medium A1 (g/L) (5 g soluble starch, 2 g yeast extract, 1 g peptone, 30 g instant ocean sea salt, 15 g agar) for the purification purpose (Fig. 2) and G668 was identified belonging to the genus *Streptomyces* by using the 16S rRNA gene sequence analysis.

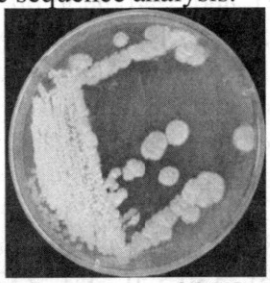

Fig. 2. Morphological appearance of G668 strain's colonies

The strain G668 was activated and inoculated into 1 L of A1 medium. After 7 days of incubation at 28°C with agitation of 150 rpm, the culture broth was used to spread on the medium surface of 50 flasks containing 1L of rich-nutrient solid medium A1+ (g/L) (soluble starch: 10 g, yeast extract: 4 g, peptone: 2 g, instant ocean sea salt 30 g, 5 mL KBr (20 mg/mL), 5 mL $FeSO_4$ (8 mg/mL), 1 g $CaCO_3$, 15 g agar). The fermentation was incubated in an incubator at 28°C and harvested on the ten days.

2.4. Isolation of the secondary metabolites from the ethyl acetate extract of G668

The *Streptomyces* sp. G668 was cultured on the agar-based media for ten days and the agar was cut into small pieces and extracted with ethyl acetate (3 times x 5 liters x 30 minute each) at 40°C. The combined ethyl acetate extracts were concentrated under reduced pressure to obtain 30 g of the crude extract. The ethyl acetate extract (30 g) was separated by *silica gel* column chromatography on a Medium-Performance Liquid Chromatography (MPLC) equipment with a gradient of CH_2Cl_2/MeOH (0-100% MeOH) to give 12 fractions F1-F12. Fraction F2 (2.5 g) was further purified by *silica gel* column chromatography with CH_2Cl_2/acetone gradient (0-100% acetone) to give 8 fractions F2.1-F2.8. Fraction F2.3 (42 mg) was separated by preparative TLC with *n*-hexane/acetone (1/1) to give compound **2** (2.0 mg). Fraction F2.5 (500 mg) was further purified by gel filtration over Sephadex LH-20 with MeOH to give 10 fractions F2.5.1- F2.5.10. Fraction F2.5.1 (0.94 g) was washed with *n*-hexane/acetone to furnish compound **1** (5 mg). Fraction F2.3 (42 mg) was separated by preparative TLC with *n*-hexane/acetone (1/1) to give compound **2** (2.0 mg). Fraction F2.5 (500 mg) was further purified by gel filtration over Sephadex LH-20 with MeOH to give 10 fractions F2.5.1- F2.5.10. Fraction F2.5.3 (100 mg) was separated on *silica gel* CC, eluting with CH_2Cl_2/MeOH (0-30% MeOH), to give compound **3** (3 mg) and compound **5** (7.1 mg). Fraction F4 (1.2 g) was further purified by gel filtration over Sephadex LH-20 (MeOH/CH_2Cl_2: 9/1) to give 7 fractions F4.1-F4.7. Fraction F4.3 (110 mg) was separated by *silica gel* column chromatography with CH_2Cl_2/MeOH (0-50% MeOH) to give compound **4** (2.4 mg). Fraction F3 (3.1 g) was subjected to CC on *silica gel* with CH_2Cl_2/MeOH (0-50% MeOH), to give 8 fractions F3.1-F3.8. Fraction F3.5 (125 mg) was separated by Sephadex LH-20 CC using MeOH to give compound **6** (3 mg) and **7** (5 mg).

9H-Pyrido[3,4-b]indole (1): White solid, mp. 198-200°C, ESI-MS: *m/z* 169 $[M+H]^+$. ^1H NMR (500 MHz, CD_3OD): δ_H (ppm) 7.28 (1H, dt, J = 1.0, 8.0 Hz, H-6), 7.57 (1H, dd, J = 1.0, 8.5 Hz, H-7), 7.58 (1H, d, J = 8.5 Hz, H-8), 8.12 (1H, d, J = 5.5 Hz, H-4), 8.22 (1H, d, J = 8.0 Hz, H-5), 8.31 (1H, d, J = 5.5 Hz, H-3), 8.81 (1H, s, H-1). ^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm): 112.8 (C-8), 116.1 (C-4), 120.9 (C-6), 122.2 (C-4b), 122.7 (C-5),

129.8 (C-7), 130.5 (C-4a), 134.1 (C-1), 137.8 (C-9a), 138.4 (C-3), 142.8 (C-8a).

L-Tryptophan (2): brown solid, mp. 59-60^{0}C, ESI-MS: *m/z* 205 $[M+H]^+$, ^{1}H-NMR (500 MHz, DMSO-*d6*): δ_H (ppm) 2.96 (1H, dd, *J* = 9.0, 15.0 Hz, H-8a), 3.31 (1H, dd, *J* = 9.0, 15.0 Hz, H-8b), 3.56 (1H, m, H-9), 6.97 (1H, dt, *J* = 1.0, 7.5 Hz, H-5), 7.06 (1H, dt, *J* = 1.5, 8.0 Hz, H-6), 7.17 (1H, br.s, H-2), 7.34 (1H, d, *J* = 8.0 Hz, H-7), 7.60 (1H, *J* = 7.5 Hz, H-4), 10.74 (1H, br.s, OH). ^{13}C-NMR (125 MHz, DMSO-*d6*): δ_C (ppm) 27.5 (C-8), 55.4 (C-9), 109.4 (C-3), 112.1 (C-7), 119.2 (C-4), 119.4 (C-5), 121.9 (C-6), 124.9 (C-2), 127.7 (C-3a), 137.0 (C-7a), 171.9 (C=O).

Indole-3-carboxylic acid (3): Brown powder; mp. 231-232°C, ESI-MS: *m/z* 162 $[M+H]^+$, ^{1}H-NMR (600 MHz, CD_3OD): δ_H (ppm) 8.08 (1H, dd, *J* = 7.2, 1.2 Hz, H-4), 7.96 (1H, s, H-2), 7.45 (1H, dd, *J* = 1.2, 7.2 Hz, H-7), 7.21 (1H, dt, *J* = 1.2, 7.2 Hz, H-6), 7.18 (1H, dt, *J* = 1.2, 7.2 Hz, H-5). ^{13}C-NMR (150 MHz, CD_3OD): δ_C (ppm) 168.8 (C=O), 138.2 (C-7a), 133.4 (C-2), 127.6 (C-3a), 123.6 (C-6), 122.4 (C-5), 122.0 (C-4), 112.9 (C-7), 108.9 (C-3).

3-Indolylacetic acid (4): brown solid, $[\alpha]_D^{25}$ -72° (*c* 0.42, $CHCl_3$), ESI-MS: *m/z* 176 $[M+H]^+$, 198 $[M+Na]^+$, ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 3.71 (2H, s, H-8), 6.99 (1H, t, *J* = 8.0 Hz, H-5), 7.09 (1H, t, *J* = 8.0 Hz, H-6), 7.15 (1H, s, H-2), 7.32 (1H, d, *J* = 8.0 Hz, H-7), 7.52 (1H, d, *J* = 8.0 Hz, H-4). ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 33.9 (C-8), 91.1 (C-3), 111.6 (C-7), 112.0 (C-4), 119.5 (C-5), 119.6 (C-6), 122.2 (C-2), 124.3 (C-7a), 128.9 (C-3a), 138.0 (C=O).

Cyclo-(Pro-Ala) (5): White solid, mp. 140-141°C, $[\alpha]_D^{25}$ -78.2° (*c* 0.32, MeOH), ESI-MS: *m/z* 207 $[M+K]^+$, ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H (ppm) 1.48 (3H, d, *J* = 7.2 Hz, CH_3-10), 1.89 (1H, m, H-4α), 2.02 (1H, m, H-4β), 2.13 (1H, m, H-5α), 2.36 (1H, m, H-5β), 3.55 (1H, m, H-3α), 3.61 (1H, m, H-3β), 4.13 (2H, m, H-6, H-9). ^{13}C-NMR (150 MHz, $CDCl_3$): δ_C (ppm) 16.0 (CH_3-10), 22.7 (C-4), 28.1 (C-5), 45.4 (C-3), 51.2 (C-9), 59.3 (C-6), 166.3 (C-1), 170.3 (C-7).

Uracil (6): White powder, mp. 320-321°C; ESI-MS: *m/z* 113 $[M+H]^+$, ^{1}H NMR (500 MHz, DMSO-*d6*): δ_H (ppm) 5.44 (1H, d, *J* = 7.5 Hz, H-5), 7.37 (1H, d, *J* = 7.5 Hz, H-6). ^{13}C NMR (125 MHz, DMSO-*d6*): δ_C (ppm) 100.2 (C-5), 142.2 (C-6), 151.6 (C-2), 164.6 (C-4).

Thymine (7): White powder, mp. 155-156°C, ESI-MS: *m/z* 127 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD & $CDCl_3$): δ_H (ppm) 1.87 (3H, s, CH_3), 7.15 (1H, s, H-6).

2.5. Antimicrobial assay

Antimicrobial assays were carried out using *Enterococcus faecalis* (ATCC299212), *Staphylococcus aureus* (ATCC25923), *Bacillus cereus* (ATCC14579), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), and *Candida albicans* (ATCC10231). Stock solutions of samples were prepared in DMSO, and the antimicrobial assays were carried out in 96-well microtiter plates against the microbial strains (5 x 10^5 CFU/mL) using a modification of the published method [3]. After incubation for 24 hours at 37°C, the absorbance at 650 nm was measured using a microplate reader. Streptomycin and cycloheximide were used as the positive reference compounds.

3. Results and discussion

Compound **1** was isolated as white solid, mp. 198-200°C. The ESI-MS spectrum of **1** showed the protonated adduct $[M+H]^+$ at *m/z* 169. The ^{1}H-NMR spectrum showed the signals of an 1,2-disubstituted benzene ring at δ_H 7.28 (1H, dt, *J* = 1.0, 8.0 Hz, H-6), 7.57 (1H, dd, *J* = 1.0, 8.5 Hz, H-7), 7.58 (1H, d, *J* = 8.5 Hz, H-8), 8.22 (1H, d, *J* = 8.0 Hz, H-5), and 3 aromatic protons at δ_H 8.12 (1H, d, *J* = 5.5 Hz, H-4), 8.31 (1H, d, *J* = 5.5 Hz, H-3), 8.81 (1H, s, H-1). Analyses of the ^{13}C-NMR and DEPT spectra with the aid of the HSQC of **1** indicated the presence of 11 carbons, including 7 sp^2 methine carbons at δ_C 134.1 (C-1), 138.4 (C-3), 116.1 (C-4), 122.7 (C-5), 120.9 (C-6), 129.8 (C-7), 112.8 (C-8) and 4 aromatic carbons at δ_C 122.2 (C-4b), 130.5 (C-4a), 137.8 (C-9a) and 142.8 (C-8a). Analysis of the COSY spectrum revealed the presence of two spin–spin coupling systems: H-5/H-6/H-7/H-8 and H-3/H-4. In the HMBC spectrum, the interactions between H-1 with C-9a, C-4a, C-3; H-3 with C-1, C-4a; H-4 with C-4b, C-9a determined the positions of C-1, C-4a and C-9a (Fig. 3). The positions of C-4b and C-8b were confirmed through the interactions between H-6 with C-4b, H-5 with C-4b, C-8a, H-7 with C-8a, and H-8 with C-4b, C-8a. Thus, detailed analysis of the 1D, 2D-NMR spectra and MS data allowed determining the structure of **1** as 9H-pyrido[3,4-b]indole. Its NMR data were consistent with those reported in the literature [4].

Compound **2** was isolated as brown solid. The ESI-MS spectrum of **2** showed a pseudo-molecular ion peak at *m/z* 205 $[M+H]^+$. The ^{1}H-NMR spectrum of **2** indicated the presence of a 1,2-disubstituted benzene ring at δ_H 6.97 (1H, dt, J = 1.0, 7.5 Hz, H-5), 7.06 (1H, dt, J = 0.5, 8.0 Hz, H-6), 7.34 (1H, d, J = 8.0 Hz, H-7), 7.60 (1H, d, J = 7.5 Hz, H-4), an aromatic proton at 7.17 (1H, br.s, H-2), a methylene at δ_H 2.96 (1H, dd, J = 9.0, 15.0 Hz, H-8a), 3.31 (1H, dd, J = 9.0, 15.0 Hz, H-8b), and a methine linked to a nitrogen atom at δ_H 3.56 (1H, m, H-9). Analysis of the ^{13}C NMR and DEPT spectra of **2** revealed the presence of 11 carbons, including 5 methine groups at δ_C 112.1 (C-7), 119.2 (C-4), 119.4 (C-5), 121.9 (C-6), 124.9 (C-2), a methylene group at δ_C 27.5 (C-8), a methine group at δ_C 55.4 (C-9), 3 aromatic carbons at δ_C 109.4 (C-3), 127.7 (C-3a), 137.0 (C-7a), and a carbonyl group at δ_C 171.9 (C=O). Two spin-spin systems were observed in the COSY spectrum, including: H-4/H-5/H-6/H-7 and H-8/H-9 (Fig. 3). These information suggested that compound **2** was an indole derivative. In the HMBC spectrum of **2**, the correlation between H-4, H-5 with C-3a, H-6, H-7 with C-7a confirmed the positions of C-3a and C-7a, the interaction between H-8 and H-2 with C-3 located the position of C-3. Moreover, the correlation between H-9 with the carbonyl group (δ_C 171.9) and the chemical shift of C-9 determined the direct connection of C-9 and the carbonyl group. Complete analysis of MS, 1D, 2D-NMR data of **2** and comparison with reference data indicated compound **2** as L-tryptophan [5],[6].

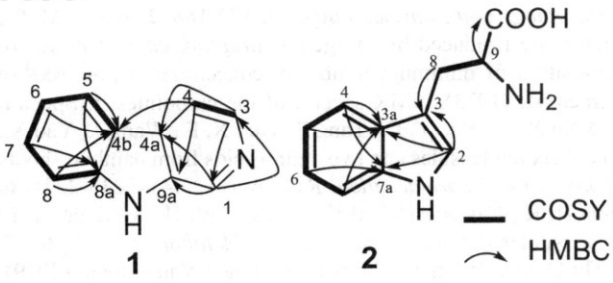

Fig. 3. Key COSY and HMBC interactions of compound **1** and **2**

Compound **3** was obtained as brown powder. The ESI-MS of **3** indicated a pseudo-molecular ion peak at *m/z* 162 $[M+H]^+$. The ^{13}C-NMR and DEPT spectra of **3** exhibited the signals of nine carbons including a carboxylic group (δ_C 168.8, C-1′), five methine groups at δ_C 112.9 (C-7), 122.0 (C-4), 122.4 (C-5), 123.6 (C-6), 133.4 (C-2), and three sp^2 aromatic carbons at δ_C 108.9 (C-3), 127.6 (C-3a), 138.2 (C-7a). Analysis of the ^{1}H-NMR spectrum of **3** showed the characteristic signals of an indole skeleton including a singlet proton at δ_H 7.96 (s, H-2) and a 1,2-disubstituted benzene ring at δ_H 7.18 (dt, J = 1.2, 7.2 Hz, H-5), 7.21 (dt, J = 1.2, 7.2 Hz, H-6), 7.45 (dd, J = 7.2, 1.2 Hz, H-7), 8.08 (dd, J = 7.2, 1.2 Hz, H-4). Thus, analysis of the MS and 1D-NMR spectra of **3** and comparison with reported data identified compound **3** as indole-3-carboxylic acid [7].

Compound **4** was isolated as brown solid, $[\alpha]_D^{25}$ -72° (c 0.42, $CDCl_3$). The ESI-MS spectrum of **4** showed a pseudo-molecular ion peak at *m/z* 176 $[M+H]^+$, 198 $[M+Na]^+$. The ^{1}H NMR spectra of **4** displayed the signals close to those of **3** in which five aromatic protons at δ_H 6.99 (1H, t, J = 8.0 Hz, H-5), 7.09 (1H, t, J = 8.0 Hz, H-6), 7.15 (1H, s, H-2), 7.32 (1H, d, J = 8.0 Hz, H-7), 7.52 (1H, d, J = 8.0 Hz, H-4), except the presence of a singlet methylene at δ_H 3.71 in the aliphatic region. These data suggested that compound **4** was an indole compound. Analysis of the ^{13}C-NMR and DEPT spectra of **4** displayed the signals of 10 carbons including five methine groups at δ_C 91.1, 112.0, 119.5, 119.6, 122.2, three sp^2 quaternary carbons at δ_C 111.6, 124.3, 128.9, a carboxylic group at δ_C 138.0 and a methylene at δ_C 33.9. Thus, detailed analysis of 1D-NMR, MS data of **4** and comparison with the reported values identified the structure of **4** as 3-indolylacetic acid [8].

Compound **5** was obtained as white solid, and **5** was optically active, $[\alpha]_D^{25}$ -78.2° (c 0.32, MeOH)) (ref 10. $[\alpha]_D$ -146.38 (c 9.66; EtOH), mp. 140-141°C. The ESI-MS spectrum of **5** showed a pseudo-molecular ion peak at *m/z* 207 $[M+K]^+$. The ^{1}H-NMR spectrum of **5** displayed signals of a methyl group at δ_H 1.48 (3H, d, J = 7.2 Hz, CH_3-10), two methine groups which were linked to a nitrogen atom at δ_H 4.13 (2H, m, H-6, H-9), a methylene group linked to a nitrogen atom at δ_H 3.55 (1H, m, H-3α), 3.61 (1H, m, H-3β), and two methylene groups at δ_H 1.89 (1H, m, H-4α), 2.02 (1H, m, H-4β), 2.13 (1H, m, H-5α), 2.36 (1H, m, H-5β). Analysis of the ^{13}C NMR and DEPT spectra of **5** revealed the presence of 8 carbons, including two sp^3 methine groups at δ_C 51.2 (C-9), 59.3 (C-6), three methylene groups at δ_C 45.4 (C-3), 22.7 (C-4), 28.1 (C-5), a methyl group at δ_C 16.0 (C-10), and two carbonyls at δ_C 166.3 (C-1), 170.3 (C-7). The chemical shifts of CH_2-3, CH-6 and CH-9 suggested their linkage to nitrogen atoms, which are consistent with the ^{1}H-NMR data of **5**. These

data suggested the presence of two amino acid units, L-proline, and L-alanine in the structure of **5**. Detailed analysis of the 1D-NMR spectra, MS data and comparison with reported values in the literature, the structure of **5** was determined to be Cyclo-(Pro-Ala) [9-10].

Compound **6** was obtained as white solid, mp. 320-321°C. Its ESI-MS showed a protonated molecular ion at *m/z* 113 $[M+H]^+$. The ^{1}H-NMR spectrum of **6** showed signals of two olefinic protons at δ_H 5.44 (1H, d, J = 7.5 Hz, H-5) and 7.37 (1H, d, J = 7.5 Hz, H-6). The ^{13}C-NMR and DEPT spectra of **6** exhibited signals of 4 carbons, including two carbonyl groups at δ_C 151.6 (C-2), 164.6 (C-4), two methine groups at δ_C 100.2 (C-5), 142.2 (C-6). Complete analysis of the 1D NMR spectra and MS data of **6** and comparison of the NMR data of **6** with the reference data indicated that compound **6** was uracil [11].

Compound **7** was isolated as white solid. Its ESI-MS data showed a protonated adduct ion $[M+H]^+$ at *m/z* 127. The ^{1}H-NMR spectrum of **7** exhibited the signals of an olefinic methine at δ_H 7.15 (1H, s, H-6) and a methyl group at δ_H 1.87 (3H, s, CH_3). The combination of ^{1}H-NMR, MS data and comparison with the reference data allowed toconclude that compound **7** was thymine [12].

Among the isolated compounds, compounds **1** and **5** were tested against a panel of gram-positive bacteria (*Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC14579), and gram-negative bacteria (*Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853) and fungi (*Candida albicans* ATCC10231). Compound **1** exhibited the selectively moderate antibacterial activity against *Escherichia coli* ATCC25922 with a MIC value of 128 µg/mL. Compound **5** strongly inhibited the fungus *Candida albicans* ATCC10231 with a MIC value of 2 µg/mL, but weakly inhibited *Enterococcus faecalis* ATCC299212 with a MIC value of 256 µg/mL. Unfortunately, both compounds did not show the inhibition against *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC14579, *Pseudomonas aeruginosa* ATCC27853.

4. Conclusion

From the ethyl acetate extract of *Streptomyces* sp. G668 strain, seven compounds were isolated. Their structures were identified by using the MS and NMR spectroscopy and comparing with the reference data, including: 9H-pyrido[3,4-b]indole (**1**), L-tryptophan (**2**), indole-3-carboxylic acid (**3**), 3-indolylacetic acid (**4**), cyclo-(Pro-Ala) (**5**), uracil (**6**), thymine (**7**). Compound **1** exhibited moderate antibacterial activity against *Escherichia coli* ATCC25922 and compound **5** strongly inhibited *Candida albicans* ATCC10231.

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