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CHEMICAL CONSTITUENTS OF MARINE-DERIVED ACTINOMYCETE *STREPTOMYCES FRADIAE* G650

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Abstract. In our continuing search for bioactive molecules, we collected a *Streptomyces fradiae* strain cultured from a prickly pen shell sample obtained at Van Phong, Khanh Hoa province, Vietnam. The ethyl acetate extract of *Streptomyces fradiae* G650 exhibited the strong inhibition against a panel of gram-positive bacteria including *Enterococcus faecalis* ATCC 29212, *Staphylococcus aurerus* ATCC 25923, *Bacillus cereus* ATCC 14579, and the yeast *Candida albicans* with the MIC values of 8, 32, 32, 8 µg/mL, respectively. As a result, from the marine-derived actinomycete *Streptomyces fradiae* G650, six compounds were isolated, including15*α*-hydroxyneoline (**1**), cyclo-(Leu-Tyr) (**2**), cyclo-(Pro-Gly) (**3**), 2'-deoxyuridine (**4**), uridine (**5**), and adenine (**6**). These compounds were structurally characterized via 1D and 2D NMR spectroscopic, mass spectrometric analyses and by comparison of the spectral data with those of previously reported data. To the best of our knowledge, 15*α*-hydroxyneoline (**1**) was reported to be isolated from microorganism for the first time.

Keywords: Streptomyces fradiae, aconitine-type diterpenoid alkaloid, cyclic dipeptide, pyrimidine, pyrimidine nucleoside.

Classification numbers: 1.1.1; 1.5.3.

1. INTRODUCTION

Marine microorganism has attracted a great deal of interest from the scientists in natural products field due to its potential as a valuable resource for a unique and large group of structurally diverse natural products [1, 2]. So far, there are more than ten thousand antibiotics produced by microorganism, more than 70 % of which are from actinomycetes [3]. Actinomycetes have produced natural products with novelly diverse structures and biological activities, including antibacterial, antifungal, antitumor, enzyme inhibition, immune regulation properties, etc [4]. In our continuing search for bioactive molecules, we collected a *Streptomyces fradiae* strain cultured from a sample obtained at Van Phong, Khanh Hoa province, Vietnam. The ethyl acetate extract of this strain exhibited the strong inhibition against a panel of gram-positive bacteria *E. faecalis* ATCC 29212, *S. aurerus* ATCC 25923, *B. cereus* ATCC 14579, and the yeast *C. albicans* with the MIC values of 8, 32, 32, 8 µg/mL, respectively. Herein, the fermentation, isolation, and structural elucidation of six known compounds (**1-6**) were reported. Their structures were shown in Figure 1.

2. MATERIALS AND METHODS

2.1. General experiment procedures

HR-ESI-MS were recorded on a FT-ICR 910-MS TQFTMS-7 T mass spectrometer. The ESI-MS were recorded on an Agilent 1100 LC-MSD Trap spectrometer. NMR spectra were recorded on a Bruker 500 and Bruker 600 MHz spectrometer operating at 125 and 150 MHz for ^{13}C-NMR, and at 500 and 600 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 solvent peak of at δ_C 77.1 ($CDCl_3$) and 49.0 (CD_3OD). For HMBC experiments the delay (1/2J) was 70 ms. TLC silica gel Merck 60 F_{254} was used as thin layer chromatography. Column chromatography (CC) was carried out using silica gel 40 - 63 µm, YMC RP-18 (30-50 µm) or Sephadex LH-20. Medium pressure liquid chromatography (MPLC) was performed on a Biotage-Isolera One system (Sweden).

2.2. Marine materials

The strain G650 was isolated from the prickly pen shell sample (*Pinna muricata* Linne, 1758) collected at the 8.5-meter depth on the coast of Van Phong, Khanh Hoa province (Vietnam) 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 G650

The prickly pen shell sample (0.5 g) was crushed by glass chopsticks 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 ^{o}C for 6 minutes). After that, 0.5 mL suspension was transferred to 4.5 ml sterile distilled water and vortexed for 1 minute afforded sample solution. Aliquots of 50 µL of sample solution was spread on ISP1 medium pH 7.0 (g/l): (2 g yeast extract, 5 g casitone, 30 g instant ocean 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 30 ^{o}C, the colony of the G650 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, 15 g agar) for purification (Figure 2). Further 16S rRNA gene sequence analysis identified G650 as *Streptomyces fradiae*.

The strain G650 was activated and inoculated into 1 L of A1 broth medium. After 7 days of incubation at 30 ^{o}C with shaking 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+ (soluble starch: 10 g/L, yeast extract: 4.0 g/L, peptone: 2.0 g/L, instant ocean: 30 g/L, 5 mL KBr (20 mg/mL), 5 mL $FeSO_4$ (8 mg/mL), $CaCO_3$: 1.0 g/L, agar: 15 g/L, and water: 1 L). The fermentation was incubated in an incubator at 30 ^{o}C and harvested on the twelfth day.

2.4. Extraction and isolation of the natural products from the extract of G650

The culture solid (50 L) of *Streptomyces* sp. G650 was extracted four times with ethyl acetate and methanol, respectively at room temperature to afford the corresponding extracts. These extracts were concentrated, *in vacuo*, to give 20.5 g of the EtOAC extract and 93.5 g of the MeOH extract. The EtOAc extract (20.5) was separated by chromatography on a silica gel column, using solvent mixtures of CH_2Cl_2/MeOH gradient (0 - 100 % MeOH) as the eluents to yield 8 fractions F1-F9. Fraction 3 (0.122 g) was separated on a silica gel column chromatography with CH_2Cl_2/MeOH gradient (0 - 100 % MeOH) as mobile phase to yield **2** (1.5 mg). Fraction 5 (1.16 g) was further purified by gel filtration over Sephadex LH-20 column chromatography with MeOH as eluent to give 4 fractions F5.1-F5.4. Fraction F5.1 (450 mg)

was subjected by silica gel column chromatography, eluted with CH_2Cl_2/MeOH gradient and then subfraction F5.1 was crystallized with MeOH/acetone (4/6) to give **1** (6.6 mg). Fraction 6 (2.8 g) was subjected to Sephadex LH-20 column chromatography, eluted with MeOH to give 7 fractions F6.1-F6.7. Fraction F6.3 (0.65 g) was purified using a Sephadex LH-20 column with MeOH as eluent to give **4** (7.0 mg). Fraction 6.5 (0.12 g) was separated on a Sephadex LH-20 column, eluting with MeOH, and followed by recrystallization in CH_2Cl_2/MeOH to give **6** (1.8 mg). The MeOH extract (93.5 g) was chromatographed by reversed phase column chromatography on medium pressure liquid chromatography system (MPLC), eluted with a solvent gradient MeOH/H_2O to yield 5 fractions F1-F5. Fraction 2 (0.88 g) was separated on Sephadex LH-20 column with a mixture of MeOH/H_2O (9/1) to yield 5 subfractions. Subfraction 2 (91 mg) was separated by silica gel column using a mixture of CH_2Cl_2/EtOH (9/1) to yield **3** (1.1 mg) and **5** (4.0 mg).

15α-Hydroxyneoline (**1**): White solid, HRESI-MS: *m/z* 454.2808 $[M+H]^+$ (calcd. for $C_{24}H_{40}NO_7$ *m/z* 454.2805), ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (150 MHz, CD_3OD): see Table 1.

Cyclo-(Leu-Tyr) (**2**): White solid, α_{25}^{D} 40.5° (c 0.40; MeOH), ESI-MS: *m/z* 277 $[M+H]^+$, ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H (ppm) 0.19 (1H, ddd, J = 13.8, 9.6, 4.8 Hz, H-5a), 0.64 (3H, d, J = 6.0 Hz, CH_3-7), 0.65 (3H, d, J = 6.6 Hz, CH_3-8), 0.78 (1H, ddd, J = 13.8, 9.6, 4.8 Hz, H-5b), 1.43 (1H, m, H-6), 2.70 (1H, dd, J = 4.8, 13.8 Hz, H-9a), 3.01 (1H, dd, J = 3.6, 13.8 Hz, H-9b), 3.45 (1H, m, H-1), 4.06 (1H, m, H-3), 6.64 (2H, d, J = 8.4 Hz, H-2', H-6'), 6.90 (2H, d, J = 8.4 Hz, H-3', H-5'), 8.00 (2H, br. s, 2 × NH), 9.20 (1H, br. s, OH), ^{13}C-NMR (150 MHz, DMSO-d_6): δ_C (ppm) 21.3 (CH_3-7), 22.8 (CH_3-8), 22.9 (C-6), 37.7 (C-9), 43.7 (C-5), 52.3 (C-1), 55.7 (C-3), 114.9 (C-3', C-5'), 125.9 (C-1'), 131.2 (C-2', C-6'), 156.4 (C-4'), 166.3 (C=O), 167.5 (C=O).

Cyclo-(Pro-Gly) (**3**): White solid, α_{25}^{D} -142.5° (c 0.40; MeOH), ESI-MS: *m/z* 155 $[M+H]^+$, ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 1.92 (1H, m, H-5a), 2.06 (2H, m, CH_2-4), 2.37 (1H, m, H-5b), 3.55 (1H, m, H-3a), 3.65 (1H, m, H-3b), 3.89 (1H, dd, J = 4.5, 16.5 Hz, H-6), 4.09 (1H, m, H-9a), 4.11 (1H, m, H-9b), 6.68 (1H, br. s, NH), ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 23.3 (C-4), 29.4 (C-5), 46.3 (C-3), 46.9 (C-9), 59.8 (C-6), 166.5 (C-1), 172.0 (C-7).

2'-Deoxyuridine (**4**): Yellow solid, ESI-MS: *m/z* 229 $[M+H]^+$, ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 2.20 (1H, m, H-2'a), 2.29 (1H, m, H-2'b), 3.70 (1H, dd, J = 3.5, 12.5 Hz, H-5'a), 3.77 (1H, dd, J = 3.0, 12.0 Hz, H-5'b), 3.92 (1H, dd, J = 3.0, 7.0 Hz, H-4'), 4.38 (1H, m, H-3'), 5.69 (1H, d, J = 8.0 Hz, H-5), 6.26 (1H, t, J = 6.5 Hz, H-1'), 7.97 (1H, d, J = 8.0 Hz, H-6), ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 41.4 (C-2'), 62.8 (C-5'), 72.2 (C-3'), 86.6 (C-4'), 89.0 (C-1'), 102.6 (C-5), 142.5 (C-6), 152.2 (C-2), 166.3 (C-4).

Uridine (**5**): White solid, ESI-MS: *m/z* 245 $[M+H]^+$, ^{1}H-NMR (600 MHz, CD_3OD): δ_H (ppm) 3.75 (1H, dd, J = 3.6, 12.6 Hz, H-5'a), 3.86 (1H, dd, J = 3.0, 12.6 Hz, H-5'b), 4.03 (1H, m, H-4'), 4.17 (1H, t, J = 4.8 Hz, H-3'), 4.20 (1H, t, J = 4.8 Hz, H-2'), 5.72 (1H, d, J = 8.4 Hz, H-5), 5.92 (1H, d, J = 4.8 Hz, H-1'), 8.01 (1H, d, J = 8.4 Hz, H-6).

Adenine (**6**): White solid, ESI-MS: *m/z* 136 $[M+H]^+$, ^{1}H-NMR (600 MHz, CD_3OD): δ_H (ppm) 8.13 (1H, s, H-8), 8.20 (1H, s, H-2).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as white solid. Its positive HR-ESI-MS showed the pseudomolecular ion peak at *m/z* 454.2808 $[M+H]^+$, which together with the ^{13}C-NMR data were consistent with the molecular formula of $C_{24}H_{39}NO_7$ (calcd. for $C_{24}H_{40}NO_7$ *m/z* 454.2805).

Analysis of the ^{13}C-NMR and DEPT spectra with the aid of HSQC experiment revealed the resonances of twenty-four carbons including three methoxy groups at δ_C 58.5 (6-OCH_3), 57.6 (16-OCH_3), 59.5 (18-OCH_3), one methyl group at δ_C 11.0 (20-CH_3)), six methylene groups at δ_C 79.9 (C-18), 58.9 (C-19), 50.5 (C-20), 29.0 (C-2), 28.5 (C-3) and 31.7 (C-12), eleven sp^3 methine groups at δ_C 72.2 (C-1), 76.1 (C-14), 79.3 (C-15), 83.6 (C-6), 92.7 (C-16), 64.4 (C-17), 43.8 (C-5), 48.8 (C-7), 49.0 (C-9), 45.3 (C-10), 41.8 (C-13), three sp^3 quaternary carbons at δ_C 39.4 (C-4), 51.0 (C-11) and one tertiary alcohol carbon at δ_C 79.2 (C-8). The chemical shifts of the C-1, C-6, C-8, C-15, C-16, C-17, C-18, C-19 and C-20 (Table 1) suggested their linkages to oxygen or nitrogen. The ^{1}H-NMR spectrum with the support of the HSQC spectrum of **1** revealed the signals which were consistent with the signals displayed on the ^{13}C and DEPT spectra of **1**, including three singlet methoxyls at δ_H 3.43 (3H, s, 6-OCH_3), 3.46 (3H, s, 16-OCH_3), 3.34 (3H, s, 18-OCH_3), eleven methines including five sp^3 methines bearing oxygen at δ_H 4.13 (1H, t, J = 4.2 Hz, H-14), 4.44 (1H, d, J = 6.6 Hz, H-15), 3.01 (1H, d, J = 6.6 Hz, H-16), 4.01 (1H, br. s, H-1), 4.32 (1H, d, J = 6.6 Hz, H-6) and other sp^3 methines at δ_H 2.35 (1H, d, J = 6.6 Hz, H-5), 2.51 (1H, br. d, J = 6.6 Hz, H-7), 2.19 (1H, overlap, H-9), 2.18 (1H, overlap, H-10), 3.32 (1H, overlap, H-17), 2.29 (1H, m, H-13), two methylenes bearing nitrogen at δ_H 2.99 (1H, d, J = 4.8 Hz, H-19a), 3.46 (1H, d, J = 5.4 Hz, H-19b), 3.03 (1H, m, H-20a), 3.33 (1H, overlap, H-20b), a methylene brearing oxygen at δ_H 3.56 (2H, s, H-18), three methylenes at δ_H 1.61(1H, m, H-2a), 1.64 (1H, m, H-2b), 1.84 (1H, td, J = 5.4, 14.4 Hz, H-3a), 2.04 (1H, dd, J = 5.4, 13.8 Hz, H-3b), 1.59 (1H, m, H-12a), 2.17 (1H, overlapped, H-12b). In the ^{1}H-^{1}H COSY spectrum of **1**, five spin-spin interaction systems of protons: H-1/H-2/H-3, H-5/H-6/H-7/H-17, H-15/H-16, H-9/H-10/H-12/H-13/H-14, CH_3-20/H-20 were recorded to afford five fragments shown in Figure 3. On the basis of these features, and the chemical shifts of the carbons and the protons of **1**, it was worthy to note that compound **1** possess an aconitine-type C_{19}-diterpenoid alkaloid skeleton [5 - 7].

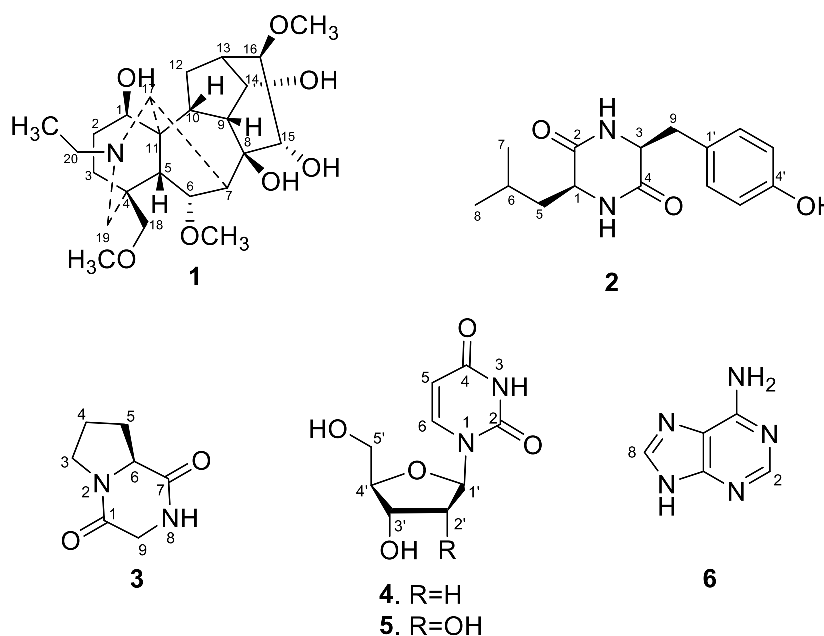

Figure 1. Structures of the isolated compounds **1-6.**

Figure 2. Morphological appearance of G650 strain's colonies.

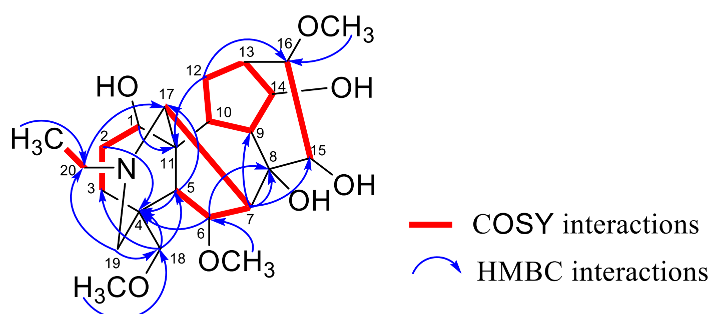

Figure 3. COSY and HMBC interactions of compound **1.**

Table 1. NMR data of compound **1** (CD_3OD, ^1H, 600 MHz; ^{13}C, 150 MHz).

Positions	δ_C mult.	δ_H mult. (J in Hz)	HMBC interaction (H-C)
1	72.2 CH	4.01 br.s	C-10, C-11, C-2, C-3
2	29.0 CH_2	1.61 m 1.64 m	C-4
3	28.5 CH_2	1.84 ddd (5.4, 13.8, 14.4) 2.04 br. dd (5.4, 13.8)	C-2
4	39.4 C	-	
5	43.8 CH	2.35 d (6.6)	C-4, C-17, C-18
6	83.6 CH	4.32 br. d (6.6)	6-OCH_3, C-4, C-7
7	48.8 CH	2.51 br. d (6.6)	C-8, C-9, C-15, C-17
8	79.2 C	-	
9	49.0 CH	2.19 overlapped	C-8
10	45.3 CH	2.18 overlapped	C-17, C-14, C-8
11	51.0 C	-	
12	31.7 CH_2	1.59 m 2.17 overlapped	C-11, C-16
13	41.8 CH	2.29 m	
14	76.1 CH	4.13 t (4.2)	C-15
15	79.3 CH	4.44 d (6.6)	C-8, C-9
16	92.7 CH	3.01 br d (6.6)	C-14, 16-OCH_3
17	64.4 CH	3.32 overlapped	C-19, C-5, C-8
18	79.9 CH_2	3.56 s	C-3, C-4, C-5, C-19, 18-OCH_3

19	58.9 CH_2	2.99 d (11.4) 3.46 d (11.4)	C-20, C-17, C-18
20	50.5 CH_2	3.03 m 3.33 overlapped	C-17, 20-CH_3
6-OCH_3	58.5 CH_3	3.43 s	C-6
16-OCH_3	57.6 CH_3	3.46 s	C-16
18-OCH_3	59.5 CH_3	3.34 s	C-18
20-CH_3	11.0 CH_3	1.45 t (7.2)	C-20

Furthermore, this hypothesis was confirmed in the HMBC spectrum of **1**. The positions of three methoxy groups were assigned to locate at C-6, C-16 and C-18 due to the correlations between the protons of three methoxy groups CH_3O-6, CH_3O-16, CH_3O-18 with C-6, C-16, and C-18, respectively. Next, the HMBC correlations between H-6 with C-8, H-7 with C-8/C-9/C-15, H-9 with C-8, H-15 with C-8/C-9 determined the position of C-8. Moreover, the HMBC correlations between H-6 with C-4/C-8, H-5 with C-4/C-17/C-18, H-2 with C-4, H-18 with C-3/C-4/C-5/C-19, H-19 with C-17/C-18/C-20, H-20 with C-17, H-1 with C-10/C-11, H-12 with C-11, C-16 located the positions of C-4, C-11, C-18, C-19 as shown in Figure 3. Thus, all of the carbons of **1** were assigned and the planar structure of **1** was established. The relative stereochemistry of **1** was elucidated by NOESY experiment. The correlations between H-9 (δ_H 2.19) and H-6 (δ_H 4.32), H-14 (δ_H 4.13) indicated that they were all oriented to the same molecular face and the proton H-9 was a β-oriented H atom, which is characteristic of most C_{19} diterpenoid alkaloids [8]. Similarly, the correlation between H-1 and H-9 suggested the β-orientation of H-1. On the other hand, the $J_{16\text{-}15}$ value observed in the ^{1}H-NMR of **1** suggesting the α-orientation of 15-OH [9]. In addition, the cross-peak between H-5 (δ_H 2.35) and H-9 in the NOESY spectrum indicated that H-5 was at β-position. Thus, H-1, H-5, H-6, H-9, H-10, H-16 were oriented at β-position. On the basis of the above mentioned 1D and 2D-NMR data and the literature data, the structure of compound **1** was identified as 15α-hydroxyneoline [10]. To the best of our knowledge, compound **1** was reported to be isolated from microorganism for the first time.

Compound **2** was obtained as white solid. Its positive ESI-MS showed the proton adduct $[M+H]^+$ at *m/z* 277 $[M+H]^+$. The ^{13}C-NMR and DEPT spectra of **2** exhibited the presence of fifteen carbons, including two carbonyl carbons at δ_C 166.3 and 167.5, seven methines including four sp^2 methines of the aromatic region at δ_C 114.9 (C-3', C-5'), 131.2 (C-2', C-6') and three sp^3 methines at δ_C 22.9 (C-6), 52.3 (C-1), 55.7 (C-3), two methylenes at δ_C 37.7 (C-9), 43.7 (C-5), two methyls at δ_C 21.3 (CH_3-7), 22.8 (CH_3-8), one quaternary carbon at δ_C 125.9 (C-1'), and one oxygenated aromatic carbon at δ_C 156.4 (C-4'). In the ^{1}H- NMR spectrum of **2**, the presences of two methyls at δ_H 0.64 (3H, d, J = 6.0 Hz, CH_3-7), 0.65 (3H, d, J = 6.6 Hz, CH_3-8), two methines at δ_H 3.45 (1H, m, H-1), 4.06 (1H, m, H-3), two methylenes at δ_H 0.19 (1H, m, H-5a), 0.78 (1H, m, H-5b), 2.70 (1H, dd, J = 4.8, 13.8 Hz, H-9a), 3.01 (1H, dd, J = 3.6, 13.8 Hz, H-9b), four aromatic protons of an A_2B_2- type disubstituted benzene ring at δ_H 6.64 (2H, d, J = 8.4 Hz, H-2'; H-6'), 6.90 (2H, d, J = 8.4 Hz, H-3'; H-5'), along with the signals of two secondary amine groups at δ_H 8.0 (2H, br. s, NH), one hydroxyl group at δ_H 9.20 (1H, br. s, OH) were observed. The chemical shifts of the ^{13}C and ^{1}H-NMR spectra of **2** revealed that compound **2** was a cyclic dipeptide. Detailed analysis of the 1D NMR spectra and MS data of **2** and comparison with the literature data indicated that compound **2** was cyclo (Leu-Tyr) [11, 12].

Compound **3** was isolated as white solid. The ESI-MS spectrum of **3** showed the pseudomolecular ion peak at m/z 155 $[M+H]^+$. Analysis of the ^{13}C- NMR, DEPT spectra of **3** showed the presence of seven carbons, including one sp^3 methine connected to a nitrogen atom at δ_C 59.8 (C-6), two carbonyl carbons at δ_C 166.5 (C-1), 172.0 (C-7), four methylenes at δ_C 23.3

(C-4), 29.4 (C-5), 46.3 (C-3), 46.9 (C-9). The chemical shifts of carbons at δ_C 46.3 (C-3), 46.9 (C-9) suggested their linkage to oxygen or nitrogen. The ^{1}H-NMR spectrum of **3** indicated the presence of one methine group at δ_H 3.89 (1H, dd, J = 4.5, 16.5 Hz, H-6), two methylenes at δ_H 1.92 (1H, m, H-5a), 2.06 (2H, m, CH_2-4,), 2.37 (1H, m, H-5b), two methylenes bearing nitrogen at δ_H 3.55 (1H, m, H-3a), 3.65 (1H, m, H-3b), 4.09 (1H, m, H-9a), 4.11 (1H, m, H-9b). Complete analysis of the NMR, mass spectra and comparison of the reference data indicated that compound **3** was cyclo-(Pro-Gly) [13].

Compound **4** was isolated as yellow solid. Its positive ESI mass spectrum showed the proton adduct ion $[M+H]^+$ at *m/z* 229 $[M+H]^+$. The ^{1}H-NMR spectrum of **4** presented the signals of two olefinic protons at δ_H 5.69 (1H, d, J = 8.0 Hz, H-5), 7.97 (1H, d, J = 8.0 Hz, H-6) and the protons of the aliphatic region at δ_H 2.20 (1H, m, H-2'a), 2.29 (1H, m, H-2'b), 3,70 (1H, dd, J = 3.5, 12.5 Hz, H-5'a), 3,77 (1H, dd, J = 3.0, 12.0 Hz, H-5'b), 3.92 (1H, dd, J = 3.0, 7.0 Hz, H-4'), 6.26 (1H, t, J = 6.5 Hz, H-1'). Analysis of the ^{13}C-NMR and DEPT spectra of **4** indicated the presence of nine carbons, including two methylenes at δ_C 41.4 (C-2'), 62.8 (C-5'), two sp^2 methines at δ_C 102.6 (C-5), 142.5 (C-6), two sp^3 methines at δ_C 86.6 (C-4'), 89.0 (C-1'), one sp^3 methine bearing oxygen at δ_C 72.2 (C-3') and two carbonyl carbons at δ_C 152.2 (C-2), 166.3 (C-4). The signals of the 1D-NMR spectra of **4** are characteristic of a pyrimidine nucleoside. Complete analysis of the MS, NMR spectra and comparison with reported NMR data indicated that compound **4** was 2'-deoxyuridine [14].

Compound **5** was obtained as white solid. The ESI-MS spectrum showed the pseudomolecular ion peak at *m/z* 245 $[M+H]^+$. In the ^{1}H-NMR spectrum of **5**, the proton signals were close to those of **4**, except for the presence of one methylene group in the aliphatic region of **4** replaced by an oxymethine group in **5.** Comparison of NMR data with the reference data and R_f value of commercially available uridine in the laboratory through using co-TLC method revealed the structure of **5** as uridine [15].

Compound **6** was obtained as white solid. The ESI-MS spectrum showed the pseudo molecular ion peak of **6** at *m/z* 245 $[M+H]^+$. In the ^{1}H-NMR spectrum of **6**, the singlet proton signals at δ_H 8.13 (1H, s, H-8), 8.20 (1H, s, H-2) were observed. Complete analysis of the ^{1}H-NMR, mass spectra and comparison of the ^{1}H-NMR data with the reported values indicated that this compound was adenine [9].

4. CONCLUSIONS

In summary, from the ethyl acetate and methanol extracts of the *Streptomyces fradiae* G650, six compounds were isolated and structurally elucidated, including: 15α-hydroxyneoline (**1**), cyclo-(Leu-Tyr) (**2**), cyclo-(Pro-Gly) (**3**), 2'-deoxyuridine (**4**), uridine (**5**), adenine (**6**). To the best of our knowledge, 15α-hydroxyneoline (**1**) was reported to be isolated from microorganism for the first time. Other remaining compounds were well-known to be isolated from *Streptomyces* genus.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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