SYNTHESIS AND CYTOTOXICITY OF SOME ACETYLATED ALKALOIDS OF *HYMENOCALLIS LITTORALIS*

Dinh Thi Phuong Anh[1], Tran Bach Duong[2], Vu Dinh Hoang[1*]

[1]*School of Chemical Engineering, Hanoi University of Science and Technology*

[2]*Vietnam Institute of Industrial Chemistry*

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Abstract

11-*O*-acetyltazettine (**2**), 2-*O*-acetyllycorine (**4**), 1,2-*O,O'*-diacetyllycorine (**5**), and 11-*O*-acetylhaemanthamine (**7**) were synthesized from tazettine (**1**), lycorine (**3**) and haemanthamine (**6**) isolated from the whole plant of *Hymenocallis littoralis*. Structures of the compounds were elucidated by means of spectroscopic methods. All the alkaloids and their derivatives were tested for cytotoxicity activities to KB and Hep G2 cell lines.

Keywords: *Hymenocallis littoralis,* alkaloid, acetylated derivatives, cytotoxicity activity.

1. INTRODUCTION

In our phytochemical study of *Hymenocallis littoralis* (Amaryllidaceae), eight alkaloids including tazettine, 11,12β-epoxy-4a-dehydroxy-littoraline, lycorine, haemanthamine, trisphaeridine, 5,6-dihydrobicolorine, 3-epimacronine, and *O*-methylpretazettine were isolated [1, 2]. Among them, tazettine was isolated in the biggest amounts; lycorine and haemanthamine showed good cytotoxic activities of ephithelloma and hepatoma. In this paper, we report the synthesis of tazettine, lycorine and haemanthamine to afford some derivatives for searching their biological activities. The biological activities of the synthesized compounds were tested.

2. EXPERIMENTAL

2.1. Instruments and chemicals

All chemicals and reaction solvents were purchase from Merck and Aldrich. Melting points were determined in open capillaries on Electrothermal B 540 Büchi (Switzerland) apparatus and uncorrected. ESI-MS spectra were recorded on an Agilent 1200 series LC-MSD ion trap apparatus Infrared spectroscopy were recorded on FT-IR Nicolet 6700 using KBr discs. NMR experiments were performed on a Bruker AM500 FT-NMR Spectrometer using TMS as internal standard. Thin layer chromatography was carried out on precoated plates DC-Alufolien 60 F_{254} and RP18 F_{254} (Merck-Germany) and visualized by UV light (254 nm) and sprayed with Dragendorf freagent. Column chromatography was carried out with normal phase (silica gel 90-240 mesh, 240-430 mesh, Merck). CO_2 incubator (INNOVA CO-170), biological safety cabinets implant grade II, centrifuge (Universal 320R), contrast microscope (Zeizz), deep freezer, chamber cell count (Fisher, USA), spectrometer (Genios Tecan), average liquid nitrogen were used to test cytotoxicity.

2.2. Isolation

The air-dried and powdered materials (18.5 kg) were extracted with methanol by Soxhlet for 10h. The extract was concentrated under vacuum to yield crude residue (268.36 g) which was then acidified by 2% H_2SO_4 and ultrasoniced for getting homogenous mixture. After removing sediment complex, the mixture was extracted by chloroform, gradiented with 5 % Na_2CO_3 to pH = 8 and concentrated to give total alkaloid residue (8.76 g) (HLA). Tazettine (150 mg), lycorine (40mg) and haemanthamine (33 mg) were isolated from the alkaloid residue (HLA, 8.76 g) [1].

2.3. Preparation of the acetylated derivatives

2.3.1. Preparation of the acetylating reagent

A mixture of acetic anhydide (0.06 g, 0.06 mol), phosphoric acid 80 % (0.03 g) was stirred and then quieted for 2 hour at room temperature [3-5].

2.3.2. Preparation of O-acetyltazettine

A suspension of tazettine **1** (31.5 mg, 0.1 mmol), the reagent (60 µL), in 3.5 mL of acetic anhydride was stirred for 24 hours at room temperature. The product was neutralized with Na_2CO_3 5 % (200 mL), extracted with $CHCl_3$, dried by Na_2SO_4 and concentrated under vacuum pressure, providing the residue (41.4 mg). The resulting residue was purified by column chromatography on silica gel, using chloroform as eluent to yield the product **2** (17 mg, 0.048 mmol) [3-5].

2.3.3. Preparation of the acetylated derivatives of lycorine

A solution of lycorine **3** (23 mg, 0.08 mmol), the reagent (96 mL), and acetic anhydride (7 mL) was stirred overnight at room temperature. Afterward, the reaction mixture was treated with aqueous Na_2CO_3 5% (350 mL). The resulting solution was extracted with $CHCl_3$. The organic layer was separated, dried over Na_2SO_4, and then concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$, 100) as eluent to afford **4** (10 mg, 0.027 mmol), and **5** (5 mg, 0.015 mmol) [3-5].

2.3.4. Preparation of O-acetylhaemanthamine

A mixture of haemanthamine **6** (15 mg, 0.05 mmol), the reagent (36 mL), and acetic anhydride (3 mL) was stirred for 24 h at room temperature. Afterward, the reaction mixture was treated with aqueous Na_2CO_3 5% (350 mL). The resulting solution was extracted with $CHCl_3$. The organic layer was separated, dried over Na_2SO_4, and then concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$, 100, v) as eluent to provide **7** (13 mg, 0.038 mmol) [3-5].

11-*O*-acetyltazettine (2): Yeild: 48 % (yellow solid). **IR** (KBr, υ (cm^{-1}): 2924.5, 1736.0, 1651.7, 1623.6-1436.9,1236.3, 1158.7, 1034.0. **^{1}H NMR** ($CDCl_3$), δ (ppm): 6.98 (1H, s, H-7); 6.49 (1H, s, H-10); 6.15 (1H, d, *J* = 10.5 Hz, H-1); 5.92 (2H, s, OCH_2O); 5.60 (1H, dt, *J* = 10.5; 1.5 Hz H-2); 4.74 (1H, s, Ha-6); 4.73 (1H, s, Hb-6); 4.14 (1H, m, H-3); 3.50 (1H, s, Ha-4); 3.47 (3H, s, 3-OCH_3); 3.34 (1H, d, *J* = 12.0 Hz Hb-12); 2.82 (1H, br s, H-4a) 2.44 (3H, s, NCH_3); 2.23 (1H, m, Ha-4); 1.61 (1H, m, Hb-4); 2.05 (3H, s, 11-OCH_3). **^{13}C NMR** (125 MHz, $CDCl_3$), δ (ppm): 171.1 (s, 11-O$\underline{C}$$OCH_3$); 146.73 (s, C-8); 146.70 (s, C-9); 131.5 (s, C-6a); 127.5 (d, C-1); 126.7 (d, C-2); 124.5 (s, C-10a); 109.5 (d, C-10); 104.9 (d, C-7); 103.8 (t, OCH_2O); 73.0 (d, C-3)); 68.1 (d, C-4a); 63.3 (t, C-12); 60.4 (t, C-6); 56.2 (q, 3-O$\underline{C}$$H_3$); 42.2 (q, N$\underline{C}$$H_3$); 26.0 (t, C-4); 21.0 (q, 11-OCO$\underline{C}$$H_3$)). **ESI-MS**, *m/z*: 374.05 $[M+H]^+$.

2-*O*-acetyllycorine (4): Yield: 15 % (yellow solid). **IR** (KBr, υ (cm^{-1}): 3434.8, 2922.1, 1714.8, 1643.0-1430.5, 1238.2, 1130.8, 1034.0. **^{1}H NMR** ($CDCl_3$), δ (ppm): 6.81 (1H, s, C-7); 6.60 (1H, s, C-10); 5.94 (1H, s, OCH_2O); 5.92 (1H, s, OCH_2O); 5.48 (1H, s, H-2); 5.32 (1H, s, H-3); 4.52 (1H, s, H-1); 4.14 (1H, d, *J* = 14.0 Hz, H-4a); 3.56 (1H, d, *J* = 14.0 Hz, H-10b); 3.36 (1H, t, *J* = 4.0 Hz, Hb-11); 2.86 (1H, d, *J* = 7.5 Hz, Ha-12); 2.72 (1H, d, *J* = 10.5 Hz, Hb-12); 2.66 (2H, br s, H-6); 2.42 (1H, m, Ha-11); 2.09 (3H, s, 2-OCO$\underline{C}$$H_3$). **^{13}C NMR** (125MHz, $CDCl_3$), δ (ppm): 170.6 (s, 2-O$\underline{C}$$OCH_3$); 146.7 (s, C-9); 146.4 (s, C-8); 146.1 (s, C-4); 129.5 (s, C-6a); 127.6 (s, C-10a); 113.9 (d, C-10); 107.7 (d, C-7); 104.7 (s, C-3); 101.1 (t, OCH_2O); 73.7 (d, C-2); 69.3 (d, C-1); 60.6 (d, C-4a); 56.8 (t, C-6); 53.7 (t, C-12); 41.6 (d, C10b); 28.8 (t, C-11); 21.2 (q, 2-OCO$\underline{C}$$H_3$). **ESI-MS**, *m/z*: 330.05 $[M+H]^+$.

1,2-*O,O'*-diacetyllycorine (5): Yield: 27 % (white solid); melting point: 222-224 ^oC. **IR** (KBr, υ (cm^{-1}): 2920.4, 1730.2, 1686.1, 1651.8-1430.4, 1238.4, 1133.5, 1036.8. **^{1}H NMR** ($CDCl_3$), δ (ppm): 6.75 (1H, s, C-10); 6.57 (1H, s, C-7); 5.92 (2H, s, OCH_2O) 5.73 (1H, s, H-1); 5.53 (1H, s, H-2); 5.25 (1H, t, *J* = 1.5 Hz, H-3); 4.16 (1H, d, *J* = 14.0 Hz, H-4a); 3.53 (1H, d, *J* = 14.0 Hz, H-10b); 3.37 (1H, m, Hb-11); 2.88 (1H, d, J = 10.5 Hz, H-12α); 2.78 (1H, d, J = 10.5 Hz, Hb-12); 2.66 (1H, d, *J* = 2.0 Hz, Ha-6); 2.65 (1H, d, *J* = 2.0 Hz, Hb-6); 2.42 (1H, t, *J* = 9.0 Hz, Ha-11); 2.08 (3H, s, 2-OCO$\underline{C}$$H_3$); 1.95 (3H, s, 1-OCO$\underline{C}$$H_3$). **^{13}C NMR** (125 MHz, $CDCl_3$), δ (ppm): 170.0 (s, 2-O$\underline{C}$$OCH_3$); 169.7 (s, 1-O$\underline{C}$$OCH_3$); 146.5 (s, C-9); 146.3 (s, C-8); 146.1 (s, C-4); 129.4 (s, C-6a); 126.6 (s, C-10a); 113.8 (d, C-10); 107.3 (d, C-7); 105.1 (s, C-3); 101.0 (t, OCH_2O); 70.9 (d, C-2); 69.3 (d, C-1); 61.2 (d, C-4a); 56.9 (t, C-6); 53.6 (t, C-12); 40.5 (d, C10b); 28.7 (t, C-11); 21.1 (q, 2-OCO$\underline{C}$$H_3$); 20.9 (q, 1-OCO$\underline{C}$$H_3$). **ESI-MS**, *m/z*: 372.05 $[M+H]^+$.

11-*O*-acetylhaemanthamine (6): Yield: 38 % (yellow solid). **IR** (KBr, υ (cm^{-1}): 2848.8, 1751.9, 1649.9, 1556.4-1458.2, 1208.4, 1118.9, 1035.4. **^{1}H NMR** ($CDCl_3$), δ (ppm): 6.90 (1H, s, H-10); 6.47 (1H, s, H-7); 6.35 (1H, d, *J* = 10.0 Hz, H-1); 6.16 (1H, dd, *J* = 10.0; 5.0 Hz, H-2); 5.90 (2H, s, OCH_2O); 4.97 (1H, dd, *J* = 7.0; 3.5 Hz, H-11); 4.35 (1H, d, *J* = 16.5 Hz, Ha-6); 3.85 (1H, t, *J* = 3.5 Hz, H-3); 3.72 (1H, d, *J* = 16.5 Hz, Hb-6); 3.40 (1H, dd, *J* = 14.5; 7.5 Hz, H-12_{ax}) 3.36 (3H, s, OCH_3); 3.34 (1H, t, *J* = 4.5 Hz, H-12_{eq}); 3.31 (1H, dd, *J* = 14.0; 3.5 Hz, H-4a); 2.04 (1H, dd, *J* = 13.5; 4.0 Hz, Ha-4); 1.98 (3H, s, 11-OCO$\underline{C}$$H_3$); 1.93 (1H, dd, *J* = 13.5;

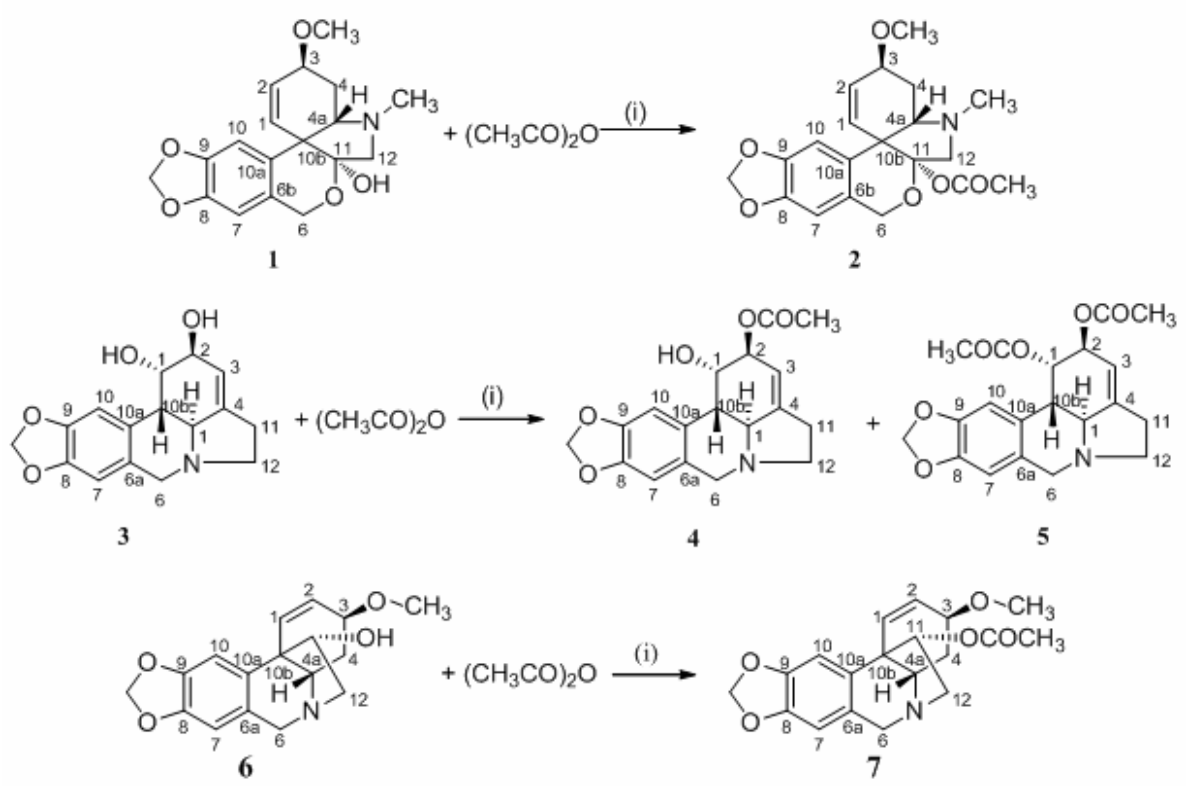
4.0Hz, Hb-4). **^{13}C NMR** (125 MHz, $CDCl_3$), δ (ppm): 170.0 (s, 11-O<u>C</u>OCH_3); 146.7 (s, C-9); 146.5 (s, C-8); 134.4 (s, C-10a); 129.5 (d, C-2); 127.7 (d, C-1); 126.6 (s, C-6a); 106.6 (d, C-7); 104.0 (d, C-10); 100.9 (t, OCH_2O); 80.4 (d, C-11); 72.6 (d, C-3); 62.8 (t, C-12); 61.3 (d, C-4a); 60.6 (d, C-6); 56.5 (q, 3-O<u>C</u>H_3); 49.2 (s, C10b); 28.4 (t, C-4); 21.1 (q, 11-OCO<u>C</u>H_3). **ESI-MS**, *m/z*: 344.06 $[M+H]^+$.

2.4. Cytotoxicity assays

The bio-assays of acetylated derivatives were implemented at the Institute of Biotechnology, Vietnam Academy of Science and Technology. Two human cancer cell lines (human epidemic carcinoma line KB and hepatocellular carcinoma line Hep G2) were used to evaluate the toxicity of the alkaloid residue, pure alkaloids generated from *Hymenocallis littoralis* and the acetylated derivatives of alkaloids [6]. The cancer cell lines were grown in the appropriate culture medium supplemented with 10 % beef embryo serum (FBS) and required other components in standard conditions (5 % CO_2; 37 ^oC; humidity 98 %, absolute sterility). Depending on the characteristics of each different cell line, culture transfer time is also different. Cell development in the liquid phase was used to test toxicity. Samples were diluted in the concentration range of 128 (μg/ml), 0.5 (μg/ml), 2 (μg/ml), 8 (μg/ml), 32 (μg/ml). 200 μl Solution cells being diluted at concentrations of 3×10^4 (cells/ml) was added in each well (96-well disk) in RPMI 1640 environment. Solution was incubated at 37 ^oC/5 % CO_2. 50 μl MTT (1 mg/ml diluted in culture medium without serum) was added after three days, continuously incubated at 37 ^oC/4 hours, removed RPMI 1640 environment, added 100 μl DMSO and mixed and results were read at 540 nm wavelength on Genios TECAN spectrophotometer [6].

3. RESULTS AND DISCUSSION

The synthesis of acetylated derivatives of alkaloids of *Hymenocallis littoralis* (Amaryllidaceae) **2**, **4**, **5**, **7** was outlined in Scheme 1. Firstly, derivative of alkaloids was synthesized by acylation between alkaloids with acetic anhydride for 24 h in the presence of 80 % phosphoric acid as catalysts at room temperature. After a work-up solution of the reaction mixture by neutralization with 5 % Na_2CO_3 to pH 9, being dried over Na_2SO_4, extracted with $CHCl_3$, the resulting solids were purified by column chromatography using proper solvent system. The structure of compounds was confirmed by ^{1}H NMR, ^{13}C NMR, and IR spectra.



Scheme 1: Reagents and conditions: (i): 80% H_3PO_4, $(CH_3CO)_2O$, r.t., 24 h;
2. Products: **(2)**. 11-O-acetyltazettine; **(4)**. 2-O-acetyllycorine; **(5)**. 1,2-O,O'-diacetyllycorine; **(7)**. 11-O-acetylhaemanthamine

Infrared spectrum of compound **2** showed an absorption band at 1751.9 cm^{-1} characteristic of a carbonyl group. Besides, there were some differences in chemical shifts of ^1H NMR and ^{13}C NMR in comparison between compound **2** and tazettine [1]. In the ^1H NMR spectrum, it showed one singlet signal of methyl group at δ_H 2.05 (s, 11-$OCOCH_3$). The ^{13}C NMR, DEPT 90 and DEPT 135 spectra of **2** revealed twenty carbon signals including one carbonyl, three methyl, four methylene, six methine groups and six quaternary carbon atoms. In the ^{13}C NMR spectrum, it showed: (i) a signal at δ_C 170.0 assigned to the carbonyl group, (ii) chemical shift at δ_C 21.0 corresponding to the methyl group. This assignment confirms the proposed structure **2** as 11-*O*-acetyltazettine.

Compound **4** showed an absorption band at 3434.8 cm^{-1} and 1714.8 cm^{-1} characteristic of a hydroxyl and a carbonyl group. Besides, there were some differences in chemical shifts of ^1H NMR and ^{13}C NMR in comparison between compound **4** and lycorine [1]. In the ^1H NMR spectrum, it showed a singlet signal of a methyl group at δ_H 2.09 (s, 2-$OCOCH_3$). The ^{13}C NMR, DEPT 90 and DEPT 135 spectra of **4** revealed eighteen carbon signals including one carbonyl, one methyl, four methylene, seven methine groups and five quaternary carbon atoms. In the ^{13}C NMR spectrum, it showed: (i) a signal at δ_C 170.6 assigned to the carbonyl group, (ii) a signal with chemical shift at δ_H 21.2 corresponding to the methyl group. From this data and in comparison with the literature [7] compound **4** was confirmed to be 2-*O'*-acetyllycorine.

Infrared spectrum of compound **5** showed an absorption band at 1730.2 cm^{-1} characteristic of carbonyl groups. Besides, there were some differences in chemical shifts of ^1H NMR and ^{13}C NMR in comparison between compound **5** and lycorine [1]. In the ^1H NMR spectrum, it showed singlet signals of two methyl groups at δ_H 2.08 (s, 1-$OCOCH_3$), 1.95 (s, 2-$OCOCH_3$). The ^{13}C NMR, DEPT 90 and DEPT 135 spectra of **5** revealed twenty carbon signals including two carbonyl, two methyl, four methylene, seven methine groups and five quaternary carbon atoms. In the ^{13}C NMR spectrum, it showed: (i) two signals at δ_C 170.0 and 169.7 assigned to two carbonyl groups, (ii) chemical shifts at δ_H 21.1 and 20.9 corresponding to two methyl groups. From this data in comparison with the literature [8] compound **5** was confirmed to be 1,2-*O,O'*-diacetyllycorine.

The molar product ratio of **4** and **5** is approximately 2:1 suggesting that in lycorine **3** 1-OH group is more reactive than 2-OH group in the acetylation. This can be explained by the higher reactivity of allylic OH group in esterification.

Compound **7** showed an absorption band at 1736.0 cm^{-1} characteristic of a carbonyl group. Besides, there were some differences in chemical shifts of ^1H NMR and ^{13}C NMR in comparison between compound **7** and haemanthamine [1]. In the ^1H NMR spectrum, it showed singlet signals of one methyl group at δ_H 1.98 (s, 11-$OCOCH_3$). The ^{13}C NMR, DEPT 90 and DEPT 135 spectra of **7** revealed nineteen carbon signals including one carbonyl, two methyl, four methylene, seven methine groups and six quaternary carbon atoms. In the ^{13}C NMR spectrum, it showed: (i) signal at δ_C 170.2 assigned to the carbonyl group, (ii) chemical shifts at δ_H 21.1 corresponding to the methyl group From this data in comparison with the literature [9] compound **7** was confirmed to be 11-*O*-acetylhaemanthamine.

Table 1: Cytotoxicity of alkaloid residue, pure alkaloids isolated from *Hymenocallis littoralis* **1**, **3**, **7**, and their acetylated derivatives **2**, **4, 5** and **7**

Compounds	Cytotoxicity to **KB** (IC_{50} μg/ml)	Cytotoxicity to **HepG2** (IC_{50} μg/ml)
HLA	1.15	1.29
1	> 100	> 100
2	74.44	67.48
3	1.14	0.98
4	10.54	10.28
5	10.66	10.58
6	1.27	1.10
7	25.04	21.67

During the initial screen, the total alkaloid residue of *Hymenocallis littoralis* showed *cytotoxicity* to cells. Consequently, the alkaloid residue, tazettine, lycorine, haemanthamine, and acetylated derivatives **2**, **4**, **5** and **7** were tested for in *vitro* inhibitory effects against *cytotoxicity* in KB and Hep G2. The data are listed in table 1. These results showed that: (i) tazettine and 11-*O*-acetyltazettine provided *cytotoxicity* with IC_{50} values of > 100 (μg/ml), 74.44 (μg/ml) in KB cell and > 100 (μg/ml), 67.48 (μg/ml) in Hep G2; (ii) lycorine, 2-*O*-acetyllycorine and 1,2-*O,O'*-diacetyllycorine demonstrated *cytotoxicity* with IC_{50} values of 1.44 (μg/ml), 10.54 (μg/ml), 10.66 (μg/ml) in KB cell and 0.98 (μg/ml), 10.28 (μg/ml), 10.58 (μg/ml) in Hep G2; (iii) haemanthamine and 11-*O*-acetylhaemanthamine afforded *cytotoxicity* with IC_{50}

values of 1.27 (µg/ml), 25.04 (µg/ml) in KB and 1.10 (µg/ml), 21.67 (µg/ml) in Hep G2 cell. These results suggest that the secondary hydroxyl groups may be important for the cytotoxicity of these alkaloids. So the acetylated derivatives have no significant activities.

4. CONCLUSIONS

Acetylated derivatives of alkaloids of the *Hymenocallis littoralis* were synthesized through a simple procedure and screened for cytotoxic activities. The result showed that the acetylation of the alkaloids decreased the cytotoxic activities to KB and Hep G2 cell lines.

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Corresponding author: **Vu Dinh Hoang**
School of Chemical Engineering
University of Science and Technology,
1 Dai Co Viet St., Hai Ba Trung Dist., Hanoi, Vietnam;
Email: vudinhhoanghn@gmail.com; Tel: 04 38684963