Original Articles

SYNTHESIS AND CYTOTOXICITY OF 1,6,8,9-SUBSTITUTED-α-CARBOLINE DERIVATIVES

Running title: SYNTHESIS AND CYTOTOXICITY OF α-CARBOLINES

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原創文章

1,6,8,9-取代-α-咔吧啉衍生物之合成與細胞致毒活性

簡標： α-咔吧啉之合成與細胞毒性

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Abstract

α-Carboline (pyrido[2,3-*b*]indole) was selected as the basic scaffold for development of anti-leukemia agents by structure modification. From the structure-antileukemia activity study, it was found that sequential introduction of 6-acetyl and 9-substituted benzyl groups onto α-carboline scaffold resulted in 6-acetyl-9-(3,5-dimethoxybenzyl) -9*H*-pyrido[2,3-*b*]indole (**6**) and 6-acetyl-9-(3,4,5-trimethoxybenzyl)-9*H*-pyrido[2,3-*b*] indole (**7**) with potent cytotoxicity against HL-60 cell line. These two compounds will be used as new lead compounds for further investigation.

Key Words: α-carboline, HL-60, structure-antileukemia activity

摘要

α-咔吧啉(吡啶駢[2,3-*b*]吲哚)經由結構上的修飾被選擇當做開發抗血癌藥物之基本骨架。從其結構抗癌活性研究發現，將乙醯基及取代苄基導入α-咔吧啉基本骨架的第六位及第九位中，所得到的 6-乙醯基-9-(3,5-二甲氧基苄基)-9*H*-吡啶駢[2,3-*b*]吲哚(**6**)及 6-乙醯基-9-(3,4,5-三甲氧基苄基)-9*H*-吡啶駢[2,3-*b*]吲哚(**7**)對 HL-60 血癌細胞株具有優異的細胞致毒活性。這兩個化合物將作為新型先導藥物及進一步的研究。

關鍵詞: α-咔吧啉，HL-60，結構-抗血癌活性

Leukemia has long been recognized as one of the serious diseases. The number of new leukemia cases in 2007 worldwide was projected to be 330,963 with an estimated 245,871 death [1]. No doubt, there is an urgent need of novel therapeutical agents for effective treatment of leukemia. One of the effective approach in searching for new drugs is to synthesize new derivatives of naturally existed chemicals by modification of their structural skeleton.

Tricyclic β-carboline alkaloids are well known natural alkaloids, found in various plants [2], marine creatures [3], insects [4], mammalians as well as human tissues and body fluids [5, 6]. These alkaloids are of great interest due to their diverse biological activities [7]. Since Ishida and coworkers [8] reported in 1999 that harmine, a β-carboline alkaloid, and its analogs significantly inhibit the drug-resistant KB cells, a lot of research works on the anticancer activity of β-carboline derivatives have been reported [9 − 13]. However, the research works on the anticancer activity of tricyclic α-carboline (pyrido[2,3-*b*]indole) derivatives are relatively rare [14 − 16]. In the present work, we decided to study the anticancer activity of new tricyclic α-carbolines. We selected α-carbolineas the basic scaffold and synthesized a series of its 1,6,8,9-substituted derivatives for cytotoxicity evaluation against HL-60 leukemia cell line, and found that some of them demonstrated promising cytotoxicity. These findings are reported herein.

MATERIALS AND METHODS

Reagents and apparatus

All of the solvents and reagents were obtained commercially and used without further purification. Reactions were monitored by thin-layer chromatography, using Merck plates. Column chromatography was performed on silica gel.

Melting points were determined with a Yanaco MP-500D melting point apparatus and were uncorrected. NMR spectra were recorded on Bruker Advance DPX-200 FT-NMR spectrometer. Chemical shifts are expressed in ppm using tetramethylsilane (TMS) as an internal standard. The IR spectra were taken in potassium bromide (KBr) pellets using Shimadzu IR Prestige-21/FTIR-8400 spectrometer. The UV spectra were measured in methanol with a HITACHI U2800 spectrometer. The MS spectra were obtained with VG Platform II GS-MS or Finnigan/Thermo Quest MAT 95XL apparatus (-70 eV). Elemental microanalyses were performed by Elementar vario EL III Heraeus CHNOS Rapid F002 Analyzer (the elements were within ± 0.4 % of the theoretical values). X-ray diffractometer was performed by Bruker AXS SMART-1000. Column chromatography was carried out using Merck Reagents Silica Gel 60 (partical size 0.063-0.200 mm, 70-230 mesh ASTM). The purity and identity of the compounds were checked out by precoated plates (silica gel 60 F_{254}) purchased from Merck Inc.

RPMI-1640 medium, fetal bovine serum (FBS), *L*-glutamine and penicillin/streptomycin were obtained from GIBCO BRL (Grand Island, NY, USA). 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), RNase A and Triton X-100 were obtained from Sigma Chemical Co. (St. Louis, MO. USA).

Preparation of compounds (1-8)

9*H*-pyrido[2,3-*b*]indole (α -carboline) (1) [17, 18]

The mixture of 1*H*-1,2,3-benzotriazole (3.57 g , 0.03 mol) and 2-chloropyridine (5.11 g , 0.045 mol) was heated under 150-160 ℃ for 1 h. The reaction mixture was cooled and quenched with 10 % Na_2CO_3 solution. The crude product was extracted with $CHCl_3$ and washed with H_2O, dried over $MgSO_4$, then evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc 4:1) and recrystallized from *n*-hexane/EtOH to afford 1-(pyridine-2-yl)-1*H*-benzo[*d*][1,2,3]triazole as white solid. Yield, 67 %; mp 98-100 ℃; Mass: 196 (*m*/*z*); found: C, 67.34; H, 4.11; N, 28.55. $C_{11}H_8N_4$ requires: C, 67.33; H, 4.16; N, 28.49; UV λ_{max} (log ε): 235 (3.79); IR (KBr): 1476 (C=N) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.47-7.55 (*m*, 2H), 7.64 (*dt*, 1H, *J* = 1.1, 7.1 Hz), 8.07-8.24 (*m*, 3H), 8.52 (*dd*, 1H, *J* = 0.7, 7.7 Hz), 8.65 (*d*, 1H, *J* = 4.9 Hz). ^{13}C NMR (50 MHz, DMSO-d_6): δ 114.7, 114.8, 120.0, 123.6, 125.8, 129.7, 131.3, 140.4, 146.5, 149.2, 151.2. Then 1-(pyridine-2-yl)-1*H*–benzo[*d*][1,2,3] triazole (5 g, 0.02 mol) and polyphosphoric acid (PPA) (16 g, 0.16 mol) were heated under 150-160 ℃ until N_2 gas evolution ceased and then heated to 180 ℃ for 15 min. After cooling, 5 % NaOH solution (400 mL) was poured into the reaction mixture, and the precipitate was collected, washed with water. The crude products were isolated and purified by silica gel column chromatography (*n*-hexane/EtOAc 1:1) and recrystallized from EtOH to give 9*H*-pyrido[2,3-*b*]indole (**1**) as white needles. Yield, 26 %; mp 177-180 ℃; Mass: 168 (*m*/*z*); found: C, 78.55; H, 4.79; N, 16.66. $C_{11}H_8N_2$ requires: C, 78.53; H, 4.42; N, 16.91; UV λ_{max} (log ε): 237 (3.56); IR (KBr): 1457 (C=N) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.11-7.17 (*m*, 2H), 7.40-7.46 (*m*, 2H), 8.08 (*d*, 1H, *J* = 7.8 Hz), 8.35 (*dd*, 1H, *J* = 1.6, 7.7 Hz), 8.41 (*dd*, 1H, *J* = 1.6, 4.8 Hz), 11.77 (*s*, 1H, NH).^{13}C NMR (50 MHz, DMSO-d_6): δ 111.7, 115.4, 115.6, 119.8, 120.8, 121.6, 127.0,

128.8, 139.2, 146.5, 152.3.

6-Acetyl-9*H*-pyrido[2,3-*b*]indole (**2**) [19] **and 8-Acetyl-9*H*-pyrido[2,3-*b*] indole** (**2a**)

To a stirred solution of compound **1** (0.2 g, 1.2 mmol) in dried CH_2Cl_2 (20 mL) were added $AlCl_3$ (0.72 g, 5.4 mmol) and acetyl chloride (0.2 g, 2.4 mmol) at 25 ± 2 ℃. The mixture was reflux for 4 h and then poured into iced water and extracted with EtOAc (300 mL). The organic layer was washed with water, dried over $MgSO_4$ and evaporated. The residue was chromatographed (silica gel/*n*-hexane/EtOAc) and recrystallized from EtOH/H_2O to give **2** and **2a**. Compound **2** was white needle. Yield, 62 %; mp 232-234 ℃; Mass: 210 (*m*/*z*); found: C, 74.27; H, 4.79; N, 13.33. $C_{13}H_{10}N_2O$ requires: C, 74.47; H, 4.69; N, 13.39; UV λ_{max} (log ε): 236 (3.85); IR (KBr): 1568 (C=N) cm^{-1}, 1602, 1667 (C=O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.63 (*s*, 3H), 7.21 (*dd*, 1H, *J* = 4.9, 7.7 Hz), 7.50 (*d*, 1H, *J* = 8.6 Hz), 8.01 (*dd*, 1H, *J* = 1.7, 8.6 Hz), 8.41 (*dd*, 1H, *J* = 1.6, 4.9 Hz), 8.59 (*dd*, 1H, *J* = 1.6, 7.7 Hz), 8.85 (*d*, 1H, *J* = 1.7 Hz), 12.24 (br *s*, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 27.1, 111.6, 116.0, 116.5, 120.6, 123.4, 127.5 129.5, 129.6, 142.2, 147.3, 152.9, 197.7. Compound **2a** was yellow-white needle. Yield, 3 %; mp 185-186 ℃; Mass: 210 (*m*/*z*); found: C, 74.27; H, 4.79; N, 13.33. $C_{13}H_{10}N_2O$ requires: C, 74.55; H, 4.47; N, 13.42; UV λ_{max} (log ε): 228 (3.71); IR (KBr): 1667 (C=O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.69 (*s*, 3H), 7.23-7.30 (*ddd*, 1H, *J* = 0.8, 4.8, 7.7 Hz), 7.30-7.38 (*dt*, 1H, *J* = 0.7, 7.7 Hz), 8.11-8.15 (*d*, 1H, *J* = 7.7 Hz), 8.43-8.50 (*m*, 2H,), 8.54-8.59 (*d*, 1H, *J* = 7.7 Hz), 11.53 (*s*, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 27.5, 114.7, 116.6, 119.7, 120.5, 122.6, 127.3, 129.4, 129.8, 137.3, 147.4, 152.7, 199.6.

9-Benzyl-9*H*-pyrido[2,3-*b*]indole (**3**)

To a stirred solution of compound **1** (0.5 g, 2.97 mmol) in 11.9 mmol KOH/dehydrated THF at 50 ℃ for 10 min, then added 3.56 mmol benzyl bromide dropwisely. The reaction mixture was stirred refluxing for 6 h and then quenched with iced water. The solid precipitate was extracted with $CHCl_3$, dried over $MgSO_4$ and evaporated. The residue was chromatographed (silica gel/*n*-hexane/EtOAc) and recrystallized from EtOH to afford **3** as white needle. Yield, 53 %; mp 101-102 ℃; Mass: 258 (*m*/*z*); found: C, 83.69; H, 5.46; N, 10.84. $C_{18}H_{14}N_2$ requires: C, 83.88; H, 5.48; N, 10.82; UV λ_{max} (log ε): 238 (3.78); IR (KBr): 1740 (C=N) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 5.67 (*s*, 2H), 7.20 (*m*, 7H), 7.40 (*t*, 1H), 7.56 (*d*, 1H, *J* = 8.1 Hz), 8.16 (*d*, 1H, *J* = 7.8 Hz), 8.45 (*d*, 1H, *J* = 4.2 Hz), 8.52 (*d*, 1H, *J* = 7.6 Hz). ^{13}C NMR (50 MHz, DMSO-d_6): δ 44.5, 110.6, 116.0, 120.4, 121.3 127.3, 127.5, 127.7, 129.0, 129.2, 138.2, 139.7, 146.6.

6-Acetyl-9-benzyl-9*H*-pyrido[2,3-*b*]indole (**4**) **and 6-acetyl-1-benzyl-1*H*-pyrido [2,3-*b*]indole** (**4a**)

Compound **2** (0.5 g, 2.38 mmol), 9.52 mmol KOH/dehydrated THF and 2.86 mmol benzyl bromide were allowed to react as in the preparation of **3** to afford **4** and **4a**. Compound **4** was white needle. Yield, 46 %; mp 101-103 ℃; Mass: 300 (*m*/z); found: C, 79.98; H, 5.37; N, 9.33. $C_{20}H_{16}N_2O$ requires: C, 79.81; H, 5.39; N, 9.42; UV λ_{max} (log ε): 239 (4.01); IR (KBr): 1662 (C=O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.62 (*s*, 3H), 5.72 (*s*, 2H), 7.19 (*m*, 5H), 7.32 (*dd*, 1H, *J* = 4.9, 7.7 Hz), 7.66 (*d*, 1H, *J* = 8.7 Hz), 8.03 (*dd*, 1H, *J* = 1.7, 8.7 Hz), 8.51 (*dd*, 1H, *J* = 1.6, 4.9 Hz), 8.67 (*dd*, 1H, *J* = 1.6, 7.7 Hz), 8.90 (*d*, 1H, *J* = 1.4 Hz). ^{13}C NMR (50 MHz, DMSO-d_6): δ 27.2, 44.7, 110.4, 116.0, 116.9, 120.2, 123.4, 127.5, 127.8, 129.1, 129.9, 137.8, 142.2, 147.4, 152.1, 197.5. Compound **4a** was white solid. Yield, 6 %; mp 182-183 ℃; Mass: 300 (*m*/*z*);

found: C, 79.98; H, 5.37; N, 9.33. $C_{20}H_{16}N_2O$ requires: C, 79.90; H, 5.40; N, 9.37; UV λ_{max} (log ε): 237 (3.99); IR (KBr): 1653 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.60 (*s*, 3H), 5.88 (*s*, 2H), 7.10 (*t*, 1H), 7.27-7.43 (*m*, 5H), 7.59 (*d*, 1H, *J* = 8.6 Hz), 7.99 (*dd*, 1H, *J* = 1.8, 8.6 Hz), 8.40 (*dd*, 1H, *J* = 1.0, 5.5 Hz), 8.75 (*dd*, 1H, *J* = 1.0, 7.1 Hz), 8.85 (*d*, 1H, *J* = 1.2 Hz). ^{13}C NMR (50 MHz, DMSO-d_6): δ 27.0, 55.2, 107.4, 109.4, 117.6, 123.1, 123.6, 126.5, 127.8, 128.2, 128.4 (C×2), 128.5, 129.2 (C×2), 131.9, 136.0, 136.8, 157.0, 197.3.

6-Acetyl-9-(4-methoxybenzyl)-9*H*-pyrido[2,3-*b*]indole (**5**)

Compound **2** (0.5 g, 2.38 mmol), 9.52 mmol KOH/dehydrated THF and 2.86 mmol 4-methoxy benzyl chloride were allowed to react as in the preparation of **4** to afford **5** as white needle. Yield, 30 %; mp 119-120 ℃; Mass: 330 (*m*/*z*); found: C, 76.34; H, 5.49; N, 8.48. $C_{21}H_{18}N_2O_2$ requires: C, 76.38; H, 5.51; N, 8.44; UV λ_{max} (log ε): 240 (4.03); IR (KBr): 1678 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.62 (*s*, 3H), 3.62 (*s*, 3H), 5.64 (*s*, 2H), 6.76 (*d*, 2H, *J* = 8.0 Hz), 7.18 (*d*, 2H, *J* = 8.0 Hz), 7.29 (*dd*, 1H, *J* = 6.0, 8.0 Hz), 7.69 (*d*, 1H, *J* = 10.0 Hz), 8.04 (*dd*, 1H, *J* = 2.0, 10.0 Hz), 8.52 (*dd*, 1H, *J* = 2.0, 6.0 Hz), 8.66 (*dd*, 1H, *J* = 2.0 , 8.0 Hz), 8.89 (*d*, 1H, *J* = 2.0 Hz). ^{13}C NMR (50 MHz, DMSO-d_6): δ 27.2, 44.2, 55.5, 110.5, 114.4, 116.0, 116.9, 120.2, 123.4, 127.4, 129.0, 129.7, 129.9, 142.1, 147.3, 152.1, 159.0, 197.5.

6-Acetyl-9-(3,5-dimethoxybenzyl)-9*H*-pyrido[2,3-*b*]indole (**6**)

Compound **2** (0.5 g, 2.38 mmol), 9.52 mmol KOH/dehydrated THF and 2.86 mmol 3,5-dimethoxybenzyl bromide were allowed to react as in the preparation of **4** to afford **6** as white needle. Yield, 31 %; mp 145-146 ℃; Mass: 360 (*m*/*z*); found: C, 73.32; H, 5.59; N, 7.77. $C_{22}H_{20}N_2O_3$ requires: C, 73.44; H, 5.61; N, 7.75; UV λ_{max} (log ε):

239 (4.08); IR (KBr): 1676 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.62 (*s*, 3H), 3.58 (*s*, 6H), 5.63 (*s*, 2H), 6.32 (*s*, 3H), 7.30 (*dd*, 1H, *J* = 4.8, 7.7 Hz), 7.66 (*d*, 1H, *J* = 8.7 Hz), 8.04 (*dd*, 1H, *J* = 1.7, 8.7 Hz), 8.50 (*dd*, 1H, *J* = 1.6, 4.8 Hz), 8.67 (*dd*, 1H, *J* = 1.6, 7.7 Hz), 8.90 (*d*, 1H, *J* = 1.5 Hz). ^{13}C NMR (50 MHz, DMSO-d_6): δ 27.2, 44.8, 55.5, 98.9, 105.7, 110.5, 115.9, 116.9, 120.2, 123.4, 127.5, 130.0, 140.1, 142.3, 147.4, 152.1, 161.1, 197.5.

6-Acetyl-9-(3,4,5-trimethoxybenzyl)-9*H*-pyrido[2,3-*b*]indole (**7**)

Compound **2** (0.5 g, 2.38 mmol), 9.52 mmol KOH/dehydrated THF and 2.86 mmol 3,4,5-trimethoxybenzyl chloride were allowed to react as in the preparation of **4** to afford **7** as white needle. Yield, 23 %; mp 149-151 ℃; Mass: 390 (*m*/z); found: C, 70.75; H, 5.68; N, 7.17. $C_{23}H_{22}N_2O_4$ requires: C, 70.77; H, 5.77; N, 7.20; UV λ_{max} (log ε): 239 (4.06); IR (KBr): 1667 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.63 (s, 3H), 3.52 (s, 3H), 3.57 (s, 6H), 5.62 (s, 2H), 6.64 (s, 2H), 7.32 (*dd*, 1H, *J* = 4.8, 7.7 Hz), 7.76 (*d*, 1H, *J* = 8.7 Hz), 8.05 (*dd*, 1H, *J* = 1.5, 8.7 Hz), 8.53 (*dd*, 1H, *J* = 1.4, 4.8 Hz), 8.67 (*dd*, 1H, *J* = 1.4, 7.7 Hz), 8.89 (*d*, 1H, *J* = 1.2 Hz). ^{13}C NMR (50 MHz, DMSO-d_6): δ 27.2, 45.1, 56.2, 60.4, 105.3, 110.5, 116.0, 116.9, 120.2, 123.4, 127.5, 129.9, 133.4, 137.3, 142.3, 147.3, 152.1, 153.4, 197.5.

9-Benzyl-6-(1-hydroxyethyl)-9*H*-pyrido[2,3-*b*]indole (**8**)

Compound **3** (0.5 g, 1.6 mmol) was added to a solution of 32 mmol $NaBH_4$ in dehydrated MeOH, stirred at 25 ℃ 24 h and then quenched with iced water. The solid precipitate was extracted with CH_2Cl_2, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane/EtOAc 1:1) and recrystallized from *n*-hexane/EtOAc to afford 9-benzyl-6-(1-hydroxyethyl)-9*H*-pyrido[2,3-*b*]indole (**8**) as

white solid. Yield, 29 %; mp 86-87 ℃; Mass: 302 (*m*/*z*); found: C, 79.44; H, 6.00; N, 9.26. $C_{20}H_{18}N_2O$ requires: C, 79.38; H, 6.09; N, 9.33; UV λ_{max} (log ε): 239 (3.76); IR (KBr): 3362 (OH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.34 (*d*, 1H, *J* = 6.4 Hz), 4.81 (*q*, 1H, *J* = 6.3 Hz), 5.14 (*d*, 1H, *J* = 3.6 Hz), 5.65(*s*, 2H), 7.14 (*m*, 6H), 7.39 (*dd*, 1H, *J* = 1.4, 8.5 Hz), 7.48 (*d*, 1H, *J* = 8.4 Hz), 8.14 (*s*, 1H), 8.42 (*dd*, 1H, *J* = 1.5, 4.8 Hz), 8.51 (*dd*, 1H, *J* = 1.5, 7.6 Hz). ^{13}C NMR (50 MHz, DMSO-d_6): δ 26.8, 44.5, 68.9, 110.1, 115.7, 115.9, 118.4, 121.1, 125.2, 127.5, 127.7, 128.9, 129.0, 138.3, 138.5, 139.9, 146.4.

Human leukemia HL-60 cell line and cell culture

Human leukemia HL-60 cells were obtained from ATCC. Cells were cultured in RPMI-1640 medium supplemented with 10 % FBS, penicillin (100 units/mL) /streptomycin (10 μg/mL), and 1 % *L*-glutamine at 37 ℃ in a humidified atmosphere containing 5 % CO_2. Cells were split every day to maintain the cell numbers between 2 and 5 × 10^5 cells/mL. The cell numbers were assessed by the standard procedure of leukocyte counting using a hemocytometer.

Anti-proliferative analysis

HL-60 cells were seeded at a density of 1 × 10^5 cells/mL in 24-well culture plates and treated with test compounds for 48 h. All of the test compounds were dissolved in DMSO, and the final concentration of DMSO in the culture medium was kept below 0.1 %.The anti-proliferative effect was assessed using the MTT assay. And the MTT assay was performed as described below. Inhibiting concentration (IC) was determined by plotting compound concentration versus cell viability. The IC_{50} value was then calculated.

MTT assay

We briefly added 10 μL MTT solution (5 mg/mL) with 50 μL cell suspension in HBSS into a 96-well plate and incubated at 37 ℃ in the dark for 4 h. Treatment of living cells with MTT produces a dark blue formazan product, whereas no such staining for us observed in dead cells. The formazan product was dissolved by adding 150 μL DMSO and then the absorbance was measured on an enzyme-linked immunosorbent assay (ELISA) reader at a best wavelength of 570 nm.

Flow cytometric analysis of DNA content for cell cycle

To estimate the proportion of HL-60 cells in different phases of cell cycle effect by various concentrations of compound **7**, cellular DNA contents were measured by flow cytometry. The 2×10^5 cells/well after with or without various concentrations of compound **7** cotreatment for 12 h. Cells were fixed by 70 % ethanol overnight at 4 ℃, washed twice and resuspended in PBS containing 20 μg/mL PI and 0.2 mg/mL RNase A and 0.1 % of Triton X-100 in dark room. After 30 min at 37 °C, cells were analyzed on a flow cytometry. Then the cell cycle was determined and analyzed and data were acquired with CellQuest software.

RESULTS AND DISCUSSION

Chemistry

As shown in Scheme 1, the starting α-carboline (**1**) was prepared according to published methods [17, 18], then, acetylated by the method of Cédric [19] to afford products **2** and **2a** that were found to have the same molecular formula $C_{13}H_{10}N_2O$ by mass spectroscopy and elemental analysis. The chemical structure of the major product **2** (62 %) was determined by ^{1}H NMR spectrum as 6-acetyl-9*H*-pyrido[2,3-*b*]indole which was the same product reported by Cédric. On the other hand, the analysis of various NMR spectra (^{1}H NMR, HMQC and HMBC) could not unambiguously determined whether the minor product **2a** (3 %) is 5- or 8-acetyl derivative. Finally, x-ray diffractometer assigned its structure as 8-acetyl-9*H*-pyrido[2,3-*b*]indole (Figure 1). Table 1 reports crystal data and refinement results for **2a**, while selected geometric parameters are listed in Table 2 and Table 3. Separately, compound **1** was benzylated by reacting with benzyl bromide in THF, in the presence of KOH, 9-benzyl-9*H*-pyrido[2,3-*b*]indole (**3**) was obtained. However, when compound **2** was subjected to alkylation by reacting with benzyl bromide in THF, in the presence of KOH, two products **4**, **4a** were isolated. The elemental analysis and mass spectra of both products were consistent with a molecular formula of $C_{20}H_{16}N_2O$, indicating that they are isomers of benzylation products. Based on the ^{1}H NMR analysis, the major product **4** was assigned as 6-acetyl-9-benzyl-9*H*- pyrido[2,3-*b*]indole. The structure of the minor product **4a** was determined to be 6-acetyl-1-benzyl-1*H*-pyrido[2,3-*b*]indole by the correlation between –CH_2-Ph (δ 55.17) and H-2 (δ 8.40) signal in its HMBC spectrum. Following the same synthetic procedure for compound **4**, three other substituted benzyl

derivatives **5-7** were prepared, although their accompanied minor products were not intentionally isolated. Then compound **4** was reduced with $NaBH_4$ to its corresponding alcohol **8**.

Cytotoxicity activity

All of the above synthesized α-carboline derivatives (**1-8**) were evaluated for cytotoxicity against HL-60 leukemia cell line. As shown by the results in Table 4, the non-substituted α-carboline (**1**) has almost none cytotoxicity (IC_{50} > 100 μM). But the introduction of an acetyl group into its 6-position resulted in compound **2** with dramatic increase of inhibitory activity against HL-60 (IC_{50} = 15.9 μM). Its positional isomer **2a**, however, exhibit poor cytotoxicity (IC_{50} > 50 μM). The introduction of benzyl group into the 9-position (**3**) of compound **1** resulted in significantly increased inhibitory activity, though not as potent as compound **2**. Next, our attempt to introduce 6-acetyl and 9-benzyl groups simultaneously onto α-carboline scaffold yielded compound **4** with IC_{50} = 2.3 μM. When the 9-benzyl was moved to 1-position (**4a**) or 6-acetyl group was reduced to alcohol **8** both resulted in weakened cytotoxicity.

We then focused on structural modification of benzyl group of compound **4**. Firstly, the addition of a methoxy group at the *para* position of the benzyl group **5** resulted in reduced cytotoxicity. On the contrary, introducing two methoxy groups onto both *meta* positions of the benzyl group **6** resulted in extraordinarily enhanced cytotoxicity (IC_{50} = 0.06 μM). The introduction of an additional methoxy group onto the *para* position on benzyl group **7** resulted in further enhanced cytotoxicity (IC_{50} = 0.03 μM). Both compounds **6** and **7** are potent α-carboline derivatives deserved further investigation.

Effects of various concentrations of compound 7 on G2/M arrest of HL-60 cells

To determine the stage at which compound **7**-induced growth inhibition occurs in the cell cycle progression of HL-60 cells, flow cytometric analysis was conducted. As shown in Fig 2, the compound **7**-treated cells showed a pattern of DNA content together with a sub-G1 phase (apoptosis cells). Compound **7** promoted G2/M increased % from 12.88 % to 62.20 % and it induced apoptosis from 1.48 % to 23.38 % and these effects are dose-dependent manners. Our data indicted that compound **7** increased the percentage of G2/M phase and induced cell apoptosis.

In conclusion, we have synthesized a series of α-carboline derivatives based on sequential introduction of acetyl and substituted benzyl groups onto its 1-,6-,8- and 9-position. These compounds were evaluated for their cytotoxicities against HL-60 cell line. Both compounds **6** and **7** demonstrated extraordinarily potent cytotoxicity that are identified as new lead compounds for further development of anticancer drugs. Besides, we chosen compound **7** to determine its mechanism against cytotoxicity of HL-60 cells, and we found compound **7** has an obvious effect on G2/M arrest in the cell cycle. The detail action mechanism of compound **7** will explored further.

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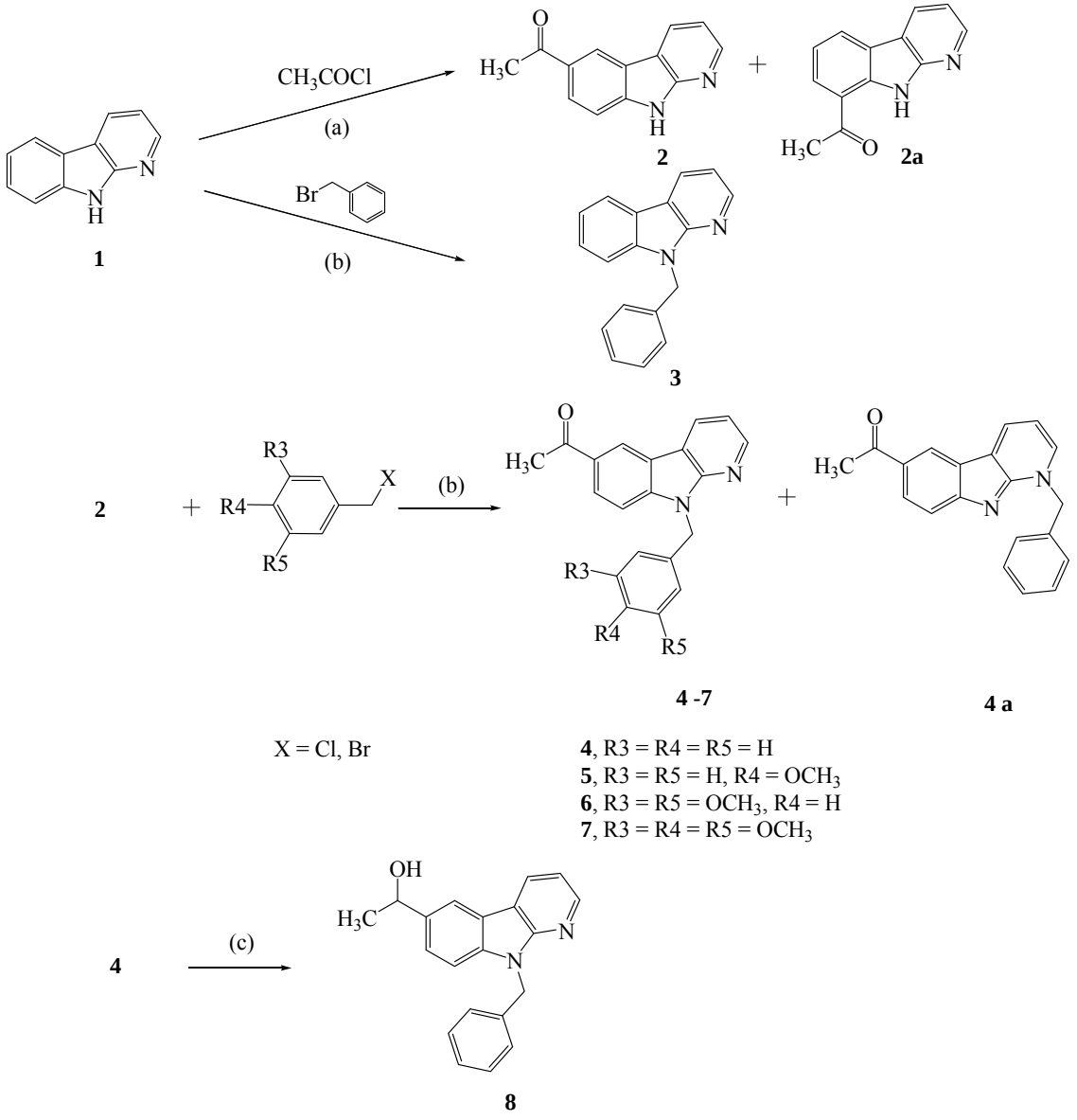


Scheme 1. Reagents and conditions: (a) $AlCl_3$ / CH_2Cl_2 (b) KOH / THF (c) $NaBH_4$ / MeOH

Table 1. Crystal data and refinement results for compound **2a**.

Identification code	**2a**
Empirical formula	$C_{13}H_{10}N_2O$
Formula weight (g mol^{-1})	210.23
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P21/n
Unit cell dimensions	
a (Å)	11.6087(8)
b (Å)	5.3734(3)
c (Å)	15.4052(9)
β °	92.075(6)
Volume ($Å^3$)	960.31(10)
Z	4
Density (calculated) (Mg/m^3)	1.454
Absorption coefficient, μ (mm^{-1})	0.095
F(000)	440
Crystal size	0.20 x 0.10 x 0.10 mm^3
θ range (°)	3.51 to 29.05
Index ranges	$-15 \leqq h \leqq 13$, $-7 \leqq k \leqq 6$, $-20 \leqq l \leqq 20$
Reflections collected/unique(R_{int})	4557 / 2210 (0.0555)
θ Completenes (°)	99.8 % (26)
Absorption correction	Semi-empirical from equivalents

Max. and min. transmission	0.9906 and 0.9813
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2210 / 3 / 145
Goodness-of-fit on F^2	0.684
Final R indices [I>2 σ (I)]	$R_1 = 0.0450$, $wR_2 = 0.0593$
R indices (all data)	$R_1 = 0.1386$, $wR_2 = 0.0684$
Largest diff. peak and hole (e.Å^{-3})	0.238 and -0.234

Table 2. Selected bond lengths for **2a**

Atoms	Bond lengths (Å)
O-C(7)	1.222(3)
N(1)-C(2)	1.374(3)
N(1)-C(10)	1.398(2)
N(1)-H(1A)	0.8800
N(2)-C(10)	1.332(3)
N(2)-C(13)	1.358(2)
C(3)-C(4)	1.388(3)
C(3)-C(2)	1.416(3)
C(3)-C(9)	1.446(3)
C(1)-C(6)	1.389(3)
C(1)-C(2)	1.409(3)
C(1)-C(7)	1.473(3)
C(4)-C(5)	1.384(3)
C(4)-H(4A)	0.9500
C(5)-C(6)	1.403(3)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(7)-C(8)	1.509(3)
C(8)-H(8A)	0.9800
C(8)-H(8B)	0.9800
C(8)-H(8C)	0.9800
C(9)-C(11)	1.370(3)

C(9)-C(10)	1.412(3)
C(11)-C(12)	1.397(3)
C(11)-H(11A)	0.9500
C(12)-C(13)	1.378(3)
C(12)-H(12A)	0.9500
C(13)-H(13B)	0.9500

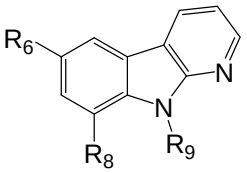
Table 3. Selected bond angles for **2a**

Atoms	Angles (°)
C(2)-N(1)-C(10)	108.71(19)
C(2)-N(1)-H(1A)	125.6
C(10)-N(1)-H(1A)	125.6
C(10)-N(2)-C(13)	112.65(18)
C(4)-C(3)-C(2)	120.4(2)
C(4)-C(3)-C(9)	132.6(2)
C(2)-C(3)-C(9)	107.0(2)
C(6)-C(1)-C(2)	117.0(2)
C(6)-C(1)-C(7)	121.5(2)
C(2)-C(1)-C(7)	121.4(2)
N(1)-C(2)-C(1)	130.2(2)
N(1)-C(2)-C(3)	109.0(2)
C(1)-C(2)-C(3)	120.8(2)
C(5)-C(4)-C(3)	119.4(2)
C(5)-C(4)-H(4A)	120.3
C(3)-C(4)-H(4A)	120.3
C(4)-C(5)-C(6)	119.9(2)
C(4)-C(5)-H(5A)	120.0
C(6)-C(5)-H(5A)	120.0
C(1)-C(6)-C(5)	122.4(2)
C(1)-C(6)-H(6A)	118.8
C(5)-C(6)-H(6A)	118.8

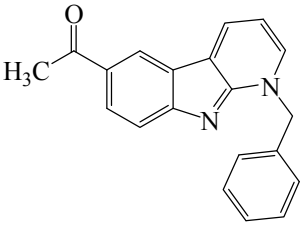
O-C(7)-C(1)	121.0(2)
O-C(7)-C(8)	119.8(2)
C(1)-C(7)-C(8)	119.2(2)
C(7)-C(8)-H(8A)	109.5
C(7)-C(8)-H(8B)	109.5
H(8A)-C(8)-H(8B)	109.5
C(7)-C(8)-H(8C)	109.5
H(8A)-C(8)-H(8C)	109.5
H(8B)-C(8)-H(8C)	109.5
C(11)-C(9)-C(10)	118.32(19)
C(11)-C(9)-C(3)	135.5(2)
C(10)-C(9)-C(3)	106.13(18)
N(2)-C(10)-N(1)	124.2(2)
N(2)-C(10)-C(9)	126.69(19)
N(1)-C(10)-C(9)	109.10(18)
C(9)-C(11)-C(12)	117.1(2)
C(9)-C(11)-H(11A)	121.4
C(12)-C(11)-H(11A)	121.4
C(13)-C(12)-C(11)	119.5(2)
C(13)-C(12)-H(12A)	120.2
C(11)-C(12)-H(12A)	120.2
N(2)-C(13)-C(12)	125.67(19)
N(2)-C(13)-H(13B)	117.2
C(12)-C(13)-H(13B)	117.2

The symmetry transformations used to generate equivalent atoms are bracketed.

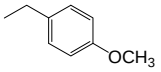
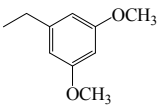
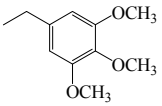
Table 4. Cytotoxicity of 1,6,8,9-substituted- α -carbolines (**1-8**)



1-8



4a

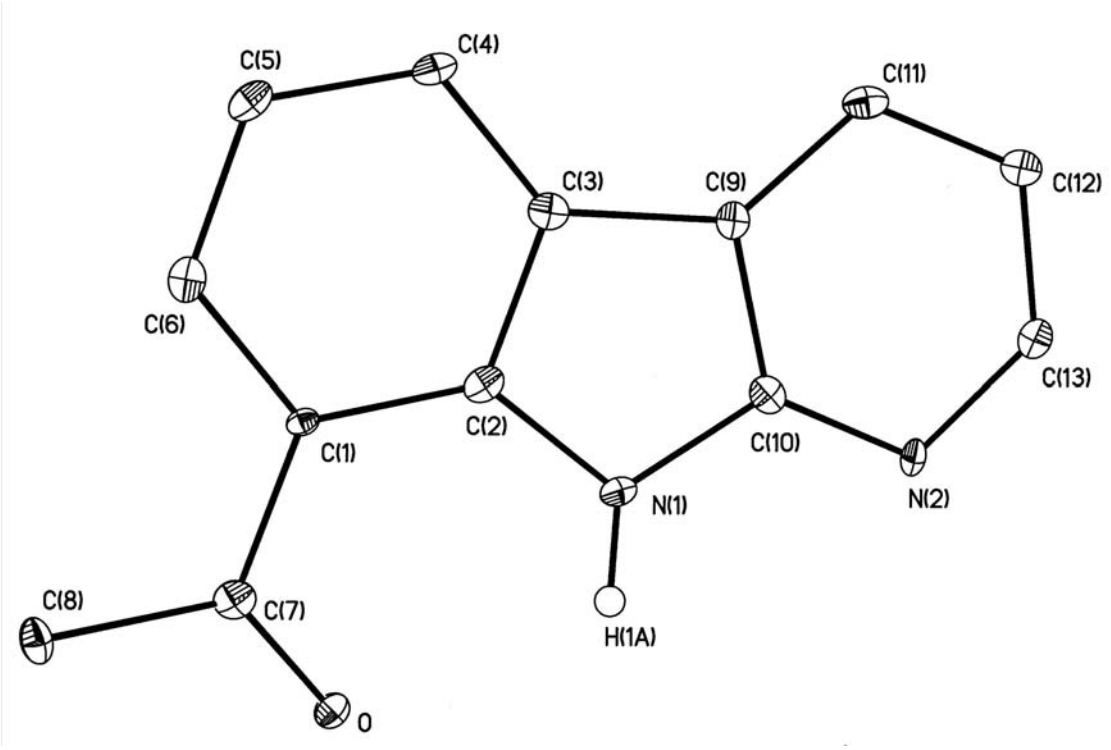
No.	R_6	R_8	R_9	IC_{50} (μ M)
1	H	H	H	>100
2	$COCH_3$	H	H	15.9
2a	H	$COCH_3$	H	>50
3	H	H		26.3
4	$COCH_3$	H		2.3
4a	-	-	-	34.9
8	$C(CH_3)HOH$	H		33.9
5	$COCH_3$	H		8.8
6	$COCH_3$	H		0.06
7	$COCH_3$	H		0.03

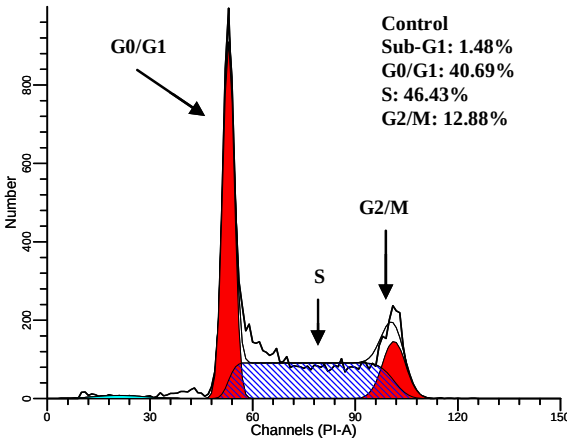
*HL-60 cells (1×10^5/mL) were treated with tested samples for 48 h.

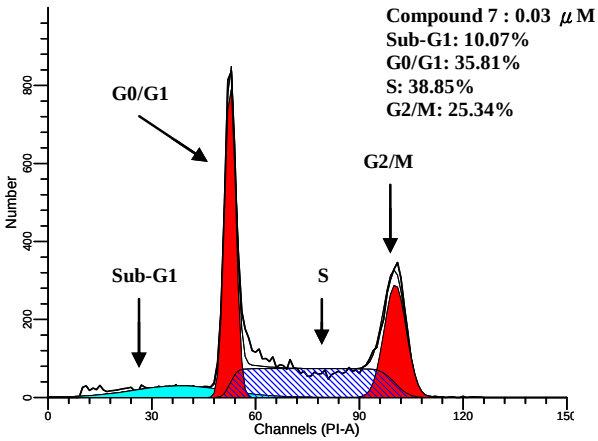
Legend of Figure:

Figure 1. The atom arrangements and atom numbering for compound **2a**

Figure 2. Effects of compound **7** on cell cycle of HL-60 cells. The HL-60 cells were incubated with various concentrations of compound **7** for 12 h, and they were harvested and were analysed by flow cytometry.


C(5)
C(4)
C(11)
C(3)
C(9)
C(12)
C(6)
C(13)
C(2)
C(1)
C(10)
N(1)
N(2)
C(8)
C(7)
H(1A)
O


Control
Sub-G1: 1.48%
G0/G1: 40.69%
S: 46.43%
G2/M: 12.88%
G0/G1
S
G2/M
Number
Channels (PI-A)


Compound 7 : 0.03 μM
Sub-G1: 10.07%
G0/G1: 35.81%
S: 38.85%
G2/M: 25.34%
G0/G1
G2/M
Sub-G1
S
Number
Channels (PI-A)

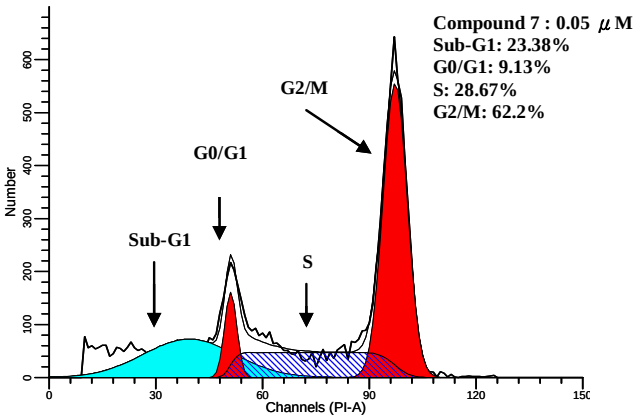

Compound 7 : 0.05 μM
Sub-G1: 23.38%
G0/G1: 9.13%
S: 28.67%
G2/M: 62.2%
G2/M
G0/G1
Sub-G1
S
Number
Channels (PI-A)

Figure 2