Effect of Indole-3-acetic Acid Analogs on the Differentiation of HL-60 Cells

Jin-Cherng Lien* (連金城), Yu-Dong Jiang (蔣宇東), Chun-Jen Chen (陳俊仁),
Sheng-Chu Kuo (郭盛助) and Li-Jiau Huang* (黃麗嬌)
Graduate Institute of Pharmaceutical Chemistry, China Medical University,
91 Hsueh-Shin Road, Taichung, Taiwan, R.O.C.

In continuing search for novel cell differentiation agents, a series of derivatives of indole-3-acetic acid and indole-3-carboxylic acid were prepared and tested against HL-60 cells for their differentiation and antiproliferation activities. Among them, *N*-ethyl-1-benzylindole-3-carboxamide (**14**) was the most potent, whereas *N*-methyl 1-benzylindole-3-acetamide (**5**) and *N*-methyl 1-benzylindole-3-carboxamide (**13**) synergistically potentiated with all-*trans*-retinoic acid to induce cell differentiation as well as antiproliferation. Our results indicate that these compounds are effective cell differentiation and antiproliferation agents in combination with retinoic acid.

Keywords: Cell differentiation; Antiproliferation activity; Indole-3-acetic acids; Indole-3-carboxylic acids.

INTRODUCTION

Leukemia is a cancer of the bone marrow or blood and is characterized by an abnormal proliferation of blood cells, usually leukocytes. It is part of the broad group of diseases called hematological neoplasms. A trait of acute leukemia cells is their ability to differentiate into functional and mature cells. One possible tactic to the treatment of patients with acute leukemia is to induce differentiation of the leukemia cells. Recent studies have been focused on the human promyelocytic HL-60 leukemia cell line which provides a effective *in vitro* model system for studying the cellular and molecular consequences involved in the differentiation process.[1] An effective inducer for the differentiation of HL-60 cells is commonly recognized as having potential therapeutic importance. Reliable compounds known as efficient anticancer agents, for instance, all-*trans*-retinoic acid (ATRA), have been used to induce leukemia cell differentiation and have resulted in almost complete remission in a high proportion of patients with acute promyelocytic leukemia (APL).[2-4] However, the efficient dose of ATRA in clinical studies causes undesirable side effects, e.g. retinoic acid syndrome.[5] Besides, remission in APL patients resulting from ATRA treatment lasted for only a few months before the disease in variably recurred. The reason for recurrence is most likely because ATRA caused partial differentiation of the cancer cells.[6] Thus the search for novel differentiation agents and differentiation potentiators which are able of reducing both the dosage and side effects of ATRA and inducing complete differentiation is an important task for the improvement of cell differentiation therapy.

In our previous work,[7] we has been demonstrated that indole-3-acetic acid (IAA) can act as ATRA to induce terminal differentiation of HL-60. In the present work, a series of derivatives of indole-3-acetic acid and indole-3-carboxylic acid were prepared and tested against HL-60 cells for their differentiation and antiproliferation activities.

RESULTS AND DISCUSSION

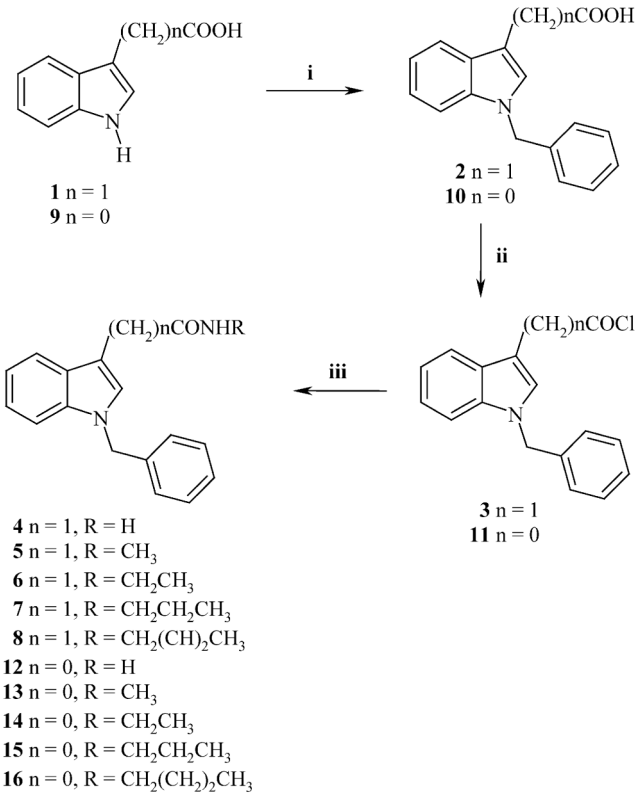
In this study, the target compounds were synthesized as shown in Scheme I. Briefly, indole-3-acetic acid (**1**) was treated with benzyl chloride in the presence of NaH, the corresponding compound **2** was obtained. Compound **3** obtained by chlorination of compound **2** with $SOCl_2$. Reaction of compound **3** with ammonia or variety of alkylamine afforded the target compounds **4-8**. Replacing **1** with indole-3-carboxylic acid (**9**), the target compounds **12-16** were prepared by the same methods as described above.

The Effect of Compounds 4-8 and 12-16 on the Differentiation of HL-60 Cells

The NBT cell differentiation assay was used to mea-

* Corresponding author. E-mail: jclien@mail.cmu.edu.tw

Scheme I



Reagents and conditions: (i) benzyl chloride, NaH, DMF, 0 °C; (ii) $SOCl_2$, diethyl ether, reflux; (iii) RNH_2, diethyl ether, 0 °C.

sure the potency of compounds **4-8** and **12-16** used alone or in combination with 5 nM of RA, in inducing the differentiation of HL-60 cells. As shown in Table 1, almost the tested compounds only showed slightly activity at a concentration range from 10 to 50 μM. In particular, the NBT reduction value of the most potent compound **14** at 50 μM was about 100 fold greater than the control. In the derivatives of *N*-substituted 1-benzylindole-3-acetamides (**4-8**), most compounds when used in combination with RA demonstrated potent activity in compared with control (RA). Among them, significant synergism was particularly noticeable with the N-methyl derivative (**5**) in which the NBT reduction value of RA was significant potentiated. To evaluation the activity of *N*-substituted 1-benzylindole-3-carboxamides (**12-16**), they displayed potent synergistic effects when combined with RA. Above all, the NBT reduction value of the most potent compound **13** at 50 μM was about 2 fold greater than the control. As a whole, based on the NBT assay, compounds **4-8** and **12-16** did not display significant efficacy when used alone. Nevertheless, these compounds used in combination with RA, all of them even though at a 10 μM concentration showed significant efficacy in potentiating RA-induced differentiation.

The Effect of Compounds 4-8 and 12-16 on the Proliferation of HL-60 Cells

The effect of compounds **4-8** and **12-16** alone on the proliferation of HL-60 cells is summarized in Table 1. In general, most of these compounds were nontoxic at low concentrations (10 μM). While at 50 μM concentration, compounds **13** and **15** suppressed proliferation about 50%. In particular, the MTT reduction value of the most potent compound **14** at 50 μM was about one-third less than control. There appeared to be correlation between their ability to affect cell differentiation and their ability to affect cell proliferation. When compounds **4-8** and **12-16** were used in combination with RA, most of them suppressed proliferation at 10-50 μM. Among them, compounds **5** and **13** displayed the best inhibitory effect at 50 μM. In general, these compounds combined with RA we found their ability to promote differentiation seemed to correlate with their ability to suppress proliferation.

A series of derivatives of indole-3-acetic acid (**4**-**8**) and indole-3-carboxylic acid (**12**-**16**) was synthesized and examined for inducing the differentiation of HL-60 cells. Among them, compound **14** was most potent. When used in combination with RA, compounds **5** and **13** demonstrated significant synergism in promoting cell differentiation. In terms of their proliferation activities, we found that, when used alone, compound **14** significantly suppressed proliferation. When used in combination with RA, most of these amide derivatives inhibited cell proliferation. The ability of these indole-3-carboxylic acid derivatives to affect proliferation does seem to correlate with their ability to affect cell differentiation. In general, the combinations that inhibited proliferation to a greater extent were also more potent inducers of cell differentiation. Our results indicate that these compounds are probably reducing both the dosage and side effects of ATRA for the improvement of cell differentiation therapy.

EXPERIMENTAL

Chemistry

IR spectra were recorded on a Nicolet Impact 400 FT-IR spectrophotometer as KBr pellets. NMR spectra were obtained on a Bruker Avance DPX-200 FT-NMR. MS were measured with HP 5995 GC-MS and VG PLATFORM II

Table 1. Effects of *N*-substituted 1-benzylindole-3-acetamides (**4-8**) and *N*-substituted 1-benzyl-indole-3-carboxamides (**12-16**) on differentiation and proliferation of HL-60 cells without (or with) all-*trans* retinoic acid

Compounds		**NBT** Assay (%)		**MTT** Assay (%)	
	Conc. (μM)	– RA	+ RA	– RA	+ RA
Control	--	0.3 ± 0.2	38.8 ± 2.6***	100.0 ± 0.1	66.6 ± 4.5***
4	10	0.3 ± 0.2	42.5 ± 1.9	99.8 ± 1.6	112.1 ± 8.8**
	20	0.4 ± 0.3	58.5 ± 4.7***	97.0 ± 6.8	55.2 ± 4.7*
	50	0.3 ± 0.2	75.7 ± 3.8***	78.9 ± 4.8***	53.0 ± 12.5***
5	10	0.3 ± 0.2	43.2 ± 1.4*	94.6 ± 5.0	53.6 ± 9.9*
	20	0.3 ± 0.2	65.6 ± 1.3***	88.6 ± 2.1***	23.6 ± 1.6***
	50	0.7 ± 0.3	79.4 ± 0.8***	75.1 ± 3.6***	15.5 ± 2.1***
6	10	0.3 ± 0.5	59.7 ± 3.4***	85.0 ± 6.0**	57.8 ± 5.2
	20	0.3 ± 0.2	65.4 ± 0.1***	95.5 ± 0.8***	49.5 ± 4.2*
	50	0.5 ± 0.4	77.6 ± 3.7***	94.3 ± 4.4*	37.3 ± 2.4***
7	10	0.3 ± 0.2	51.0 ± 1.4***	105.1 ± 4.4	74.7 ± 1.8*
	20	0.2 ± 0.3	58.7 ± 1.6***	117.5 ± 5.6**	68.7 ± 5.7
	50	0.3 ± 0.2	71.5 ± 2.2***	88.9 ± 2.0***	55.6 ± 0.1*
8	10	0.2 ± 0.3	39.6 ± 4.1	93.9 ± 4.6*	50.5 ± 3.6**
	20	0.3 ± 0.2	55.3 ± 5.2***	102.0 ± 8.0	49.5 ± 4.0**
	50	crystal out	crystal out	crystal out	crystal out
12	10	0.3 ± 0.2	40.5 ± 8.2	116.7 ± 2.1	88.6 ± 14.5
	20	0.3 ± 0.2	40.9 ± 6.1	108.6 ± 9.5	93.6 ± 9.6*
	50	0.4 ± 0.3	69.2 ± 4.6***	68.0 ± 1.3***	41.2 ± 3.2***
13	10	0	39.4 ± 1.9	89.7 ± 2.7***	92.4 ± 3.4***
	20	1.0 ± 0.8	45.6 ± 2.1**	84.8 ± 3.7***	70.4 ± 4.8
	50	1.2 ± 0.8	80.6 ± 0.3***	56.8 ± 4.1***	28.4 ± 5.6***
14	10	0.3 ± 0.2	45.8 ± 6.7*	103.7 ± 3.8	102.1 ± 3.7**
	20	0.7 ± 0.6	55.0 ± 1.1***	79.6 ± 6.3***	63.5 ± 4.9
	50	27.8 ± 1.7***	67.8 ± 2.8***	29.7 ± 4.2***	30.4 ± 3.8***
15	10	0	39.7 ± 4.5	110.1 ± 1.8***	65.7 ± 2.2
	20	0.2 ± 0.3	42.1 ± 1.4	84.5 ± 6.1**	57.2 ± 2.1*
	50	0.6 ± 0.3	62.1 ± 2.1***	51.9 ± 1.5***	34.0 ± 9.4***
16	10	0.6 ± 0.3	45.1 ± 2.1**	106.5 ± 6.8	69.6 ± 2.2
	20	0.3 ± 0.2	49.5 ± 2.3***	97.9 ± 3.7	59.7 ± 6.3
	50	crystal out	crystal out	crystal out	crystal out

* $p < 0.05$ compared with control without (with) RA5 nM.
** $p < 0.01$ compared with control without (with) RA5 nM.
*** $p < 0.001$ compared with control without (with) RA5 nM.
Data was presented as mean ± SD from three separate experiments

GC-MS instruments. Elemental analyses of C, H, and N were carried out on a Perkin-Elmer 2400 Series II CHNS/O Analyzer and were accurate within ± 0.4% of theoretical values.

1-Benzylindole-3-acetic acid (2)

A mixture of indole-3-acetic acid (1.0 g, 5.7 mmol) (**1**) and sodium hydride (0.2 g, 8.3 mmol) in N,N-dimethyl-formamide (DMF, 10 mL) was stirred at ice bath (0 °C) for about 30 min, and then benzyl chloride (1.4 g, 11.4 mmol) was added dropwise with stirring. The reaction mixture thus obtained was further agitated for 1 h at room temperature. The mixture was pour into ice water, and acidifies with conc. HCl. The resulting precipitate was filtered, dried and crystallized from ethanol. Yield 81%; mp: 144-145 °C; EIMS *m/z*: 265 (M^+); ^{1}H-NMR (DMSO-d_6): δ 7.50 (1H, *dd*, J = 0.8, 7.8 Hz, H-5), 7.42-7.00 (1H, *m*, H-6, 7, 8, H-2′, 3′, 4′, 5′, 6′), 6.96 (1H, *s*, H-2), 5.35 (2H, *s*, H-10), 3.65 (2H, *s*, H-11). ^{13}C-NMR (DMSO-d_6, 50 MHz): δ 173.34, 138.47, 136.15, 128.75 (2 × CH), 127.57 (C, 2 × CH), 127.30 (2 × CH), 121.59, 119.21, 119.03, 110.25, 107.90, 49.17, 31.10.

Anal. Calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 76.90; H, 5.72; N, 5.27.

1-Benzylindole-3-acetic chloride (3)

A mixture of compound **2** (1.0 g, 3.8 mmol) and thionyl chloride (20 mL) in anhydrous diethyl ether (100 mL) was reflux for 1 h. The solvent was evaporated to dryness under reduced pressure to afforded **3**.

1-Benzylindole-3-acetamide (4)

Ammonia gas was pass into the solution of the 1-benzylindole-3-acetic chloride (**3**) (1.0 g, 3.5 mmol) in anhydrous diethyl ether (20 mL) at 0 °C for 1.5 h. The reaction mixture was treated with ice water and extracted with ethyl acetate. The extract was dried with magnesium sulfate and evaporated to dryness. The residue was purified by column chromatography (silica gel/ethyl acetate) to afforded **4**. Yield 24%; mp: 144-145 °C; EIMS *m/z*: 264 (M^+); ^{1}H-NMR ($CDCl_3$): δ 7.61 (1H, *d*, *J* = 7.1 Hz, H-5), 7.29-7.12 (1H, *m*, H-6, 7, 8, H-2′, 3′, 4′, 5′, 6′), 7.08 (1H, *s*, H-2), 5.80 (1H, *s*, -NHa), 5.69 (1H, *s*, -NHb), 5.29 (2H, *s*, H-10) , 3.72 (2H, *s*, H-11). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 174.13, 136.90, 136.59, 128.63 (2 × CH), 127.59 (C, 2 × CH), 126.68 (2 × CH), 122.22, 119.67, 118.76, 109.78, 108.23, 49.84, 32.75. Anal. Calcd for $C_{17}H_{16}N_2O$: C, 77.25; H, 6.10; N, 10.60. Found: C, 77.23; H, 6.11; N, 10.62.

***N*-Methyl-1-benzylindole-3-acetamide (5)**

Compound **3** (1.0 g, 3.5 mmol) was reaction with methylamine as described for the preparation of **4** to afford **5**. Yield 49%; mp: 126-127 °C; EIMS *m/z*: 278 (M^+); ^{1}H-NMR ($CDCl_3$): δ 7.58 (1H, *d*, *J* = 7.2 Hz, H-5), 7.34-7.12 (1H, *m*, H-6, 7, 8, H-2′, 3′, 4′, 5′, 6′), 7.06 (1H, *s*, H-2), 5.85 (1H, *s*, -NH), 5.29 (2H, *s*, H-10), 3.75 (2H, *s*, H-11), 2.72 (3H, *d*, *J* = 4.8 Hz, H-1″). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 171.94, 136.90, 136.61, 128.63 (2 × CH), 127.55 (C, 2 × CH), 126.74 (2 × CH), 122.18, 119.65, 118.78, 109.79, 108.02, 49.86, 32.96, 26.16. Anal. Calcd for $C_{18}H_{18}N_2O$: C, 77.69; H, 6.52; N, 10.06. Found: C, 77.67; H, 6.49; N, 10.04.

***N*-Ethyl-1-benzylindole-3-acetamide (6)**

Compound **3** (1.0 g, 3.5 mmol) was reaction with ethylamine as described for the preparation of **4** to afford **6**. Yield 53%; mp: 108-109 °C; EIMS *m/z*: 292 (M^+); ^{1}H-NMR ($CDCl_3$): δ 7.60 (1H, *dd*, *J* = 1.5, 7.3 Hz, H-5), 7.34-7.10 (1H, *m*, H-6, 7, 8, H-2′, 3′, 4′, 5′, 6′), 7.07 (1H, *s*, H-2), 5.41 (1H, *s*, -NH), 5.30 (2H, *s*, H-10), 3.72 (2H, *s*, H-11), 3.29-3.16 (2H, *m*, H-1″), 1.01 (3H, *t*, *J* = 7.2 Hz, H-2″). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 171.06, 136.96, 136.61, 128.61 (2 × CH), 127.57 (C, 2 × CH), 126.68 (2 × CH), 122.15, 119.62, 118.80, 109.76, 108.12, 49.83, 34.14, 33.16, 14.54. Anal. Calcd for $C_{19}H_{20}N_2O$: C, 78.05; H, 6.89; N, 9.58. Found: C, 78.06; H, 6.85; N, 9.55.

***N*-n-Propyl-1-benzylindole-3-acetamide (7)**

Compound **3** (1.0 g, 3.5 mmol) was reaction with propylamine as described for the preparation of **4** to afford **7**. Yield 53%; mp: 93-94 °C; EIMS *m/z*: 306 (M^+); ^{1}H-NMR ($CDCl_3$): δ 7.60 (1H, *dd*, *J* = 0.9, 7.4 Hz, H-5), 7.34-7.11 (1H, *m*, H-6, 7, 8, H-2′,3′,4′,5′,6′), 7.06 (1H, *s*, H-2), 5.73 (1H, *s*, -NH), 5.31 (2H, *s*, H-10), 3.72 (2H, *s*, H-11), 3.14 (2H, *m*, H-1″), 1.47-1.21 (2H, *m*, H-2″), 1.01 (3H, *t*, *J* = 7.0, 14.6 Hz, H-3″). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 171.14, 136.93, 136.61, 128.61 (2 × CH), 127.58 (C, 2 × CH), 126.65 (2 × CH), 122.18, 119.64, 118.78, 109.74, 108.16, 49.83, 40.94 (C-1′), 33.15, 22.47, 15.05. Anal. Calcd for $C_{17}H_{16}N_2O$: C, 78.40; H, 7.23; N, 9.14. Found: C, 78.35; H, 7.26; N, 9.16.

***N*-n-Butyl-1-benzylindole-3-acetamide (8)**

Compound **3** (1.0 g, 3.5 mmol) was reaction with butylamine as described for the preparation of **4** to afford **8**. Yield 34%; mp: 76-77 °C; EIMS *m/z*: 320 (M^+); ^{1}H-NMR ($CDCl_3$): δ 7.60 (1H, *dd*, *J* = 1.6, 7.4 Hz, H-5); 7.35-7.11 (1H, *m*, H-6, 7, 8, H-2′,3′,4′,5′,6′), 7.06 (1H, *s*, H-2), 5.30 (2H, *s*, H-10), 5.80 (1H, *s*, -NH), 3.73 (2H, *s*, H-11), 3.23-3.13 (2H, *m*, H-1″), 1.40-1.18 (2H, *m*, H-2″, 3″), 0.84 (3H, *t*, *J* = 7.2 Hz, H-4″). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 171.15, 136.99, 136.61, 128.61 (2 × CH), 127.57 (C, 2 × CH), 126.66 (2 × CH), 122.17, 119.62, 118.79, 109.77, 108.18, 49.82, 39.03, 33.16, 31.31, 19.73, 13.49. Anal. Calcd for $C_{17}H_{16}N_2O$: C, 78.71; H, 7.55; N, 8.74. Found: C, 78.75; H, 7.57; N, 8.76.

1-Benzylindole-3-carboxylic acid (10)

Indole-3-carboxylic acid (1 g, 6.2 mmol) (**9**) was reaction with benzyl chloride (1.6 g, 12.4 mmol) as described for the preparation of **2** to afford **10**. Yield 84%; mp: 195-196 °C; EIMS *m/z*: 251 (M^+); ^{1}H-NMR (DMSO-d_6): δ 8.22 (1H, *s*, H-2), 8.02 (1H, *dd*, *J* = 3.1, 5.1 Hz, H-5), 7.51 (1H, *dd*, *J* = 2.1, 5.1 Hz, H-8), 7.34-7.23 (1H, *m*, H-3′, 4′, 5′, H-6, 7), 7.21-7.14 (1H, *m*, H-2′, 6′), 5.47 (2H, *s*, H-10). ^{13}C-NMR (DMSO-d_6, 50 MHz): δ 165.93, 137.36, 136.54, 135.76, 128.87 (2 × CH), 127.84, 127.48 (2 × CH), 126.92, 122.61, 121.67, 121.18, 111.31, 107.19, 49.77. Anal. Calcd for $C_{16}H_{13}NO_2$: C, 76.47; H, 5.21; N, 5.57. Found: C, 76.49; H, 5.23; N, 5.59.

1-Benzylindole-3-carboxylic chloride (11)

Compound **10** (1 g, 4.0 mmol) was reaction with thionyl chloride (20 mL) as described for the preparation of **3** to

afford **11**.

1-Benzylindole-3-carboxamide (12)

Compound **11** (1 g, 3.7 mmol) was reaction with ammonia gas as described for the preparation of **4** to afford **12**. Yield 63%; mp: 153-154 °C; EIMS *m/z*: 252 (M^+); ^{1}H-NMR (DMSO-d_6): δ 8.00 (1H, *dd*, *J* = 0.7, 7.1 Hz, H-5), 7.75 (1H, *s*, H-2), 7.37-7.24 (1H, *m*, H-3′, 4′, 5′, H-6, 7, 8), 7.11-7.13 (1H, *m*, H-2′, 6′), 5.74 (1H, *s*, -NH), 5.33 (2H, *s*, H-10). ^{13}C-NMR (DMSO-d_6, 50 MHz): δ 166.32, 137.68, 136.41, 131.99, 128.87 (2 × CH), 127.81, 127.35 (2 × CH), 126.97, 122.25, 121.58, 120.92, 110.78, 110.45, 49.64. Anal. Calcd for $C_{16}H_{14}N_2O$: C, 76.79; H, 5.64; N, 11.19. Found: C, 76.77; H, 5.62; N, 11.21.

***N*-Methyl-1-benzylindole-3-carboxamide (13)**

Compound **11** (1 g, 3.7 mmol) was reaction with methylamine gas as described for the preparation of **4** to afford **13**. Yield 68%; mp: 135-136 °C; EIMS *m/z*: 264 (M^+); ^{1}H-NMR ($CDCl_3$): δ 8.04 (1H, *dd*, *J* = 2.0, 7.8 Hz, H-5), 7.69 (1H, *s*, H-2), 7.31-7.20 (1H, *m*, H-3′, 4′, 5′, H-6, 7, 8), 7.08-7.05 (1H, *m*, H-2′, 6′), 6.25 (1H, *s*, -NH), 5.19 (2H, *s*, H-10), 2.99 (3H, *d*, *J* = 4.6 Hz, H-1″), 0.99 (3H, *dt*, *J* = 7.4, 15.38, H-3″). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 165.74, 136.49, 135.97, 131.03, 128.66 (2 × CH), 127.75, 126.74 (2 × CH), 125.58, 122.43, 121.29, 120.30, 111.23, 110.26, 50.18, 26.09. Anal. Calcd for $C_{17}H_{16}N_2O$: C, 77.25; H, 6.10; N, 10.60. Found: C, 77.23; H, 6.11; N, 10.62.

***N*-Ethyl-1-benzylindole-3-carboxamide (14)**

Compound **11** (1 g, 3.7 mmol) was reaction with ethylamine gas as described for the preparation of **4** to afford **14**. Yield 58%; mp: 131-132 °C; EIMS *m/z*: 278 (M^+), ^{1}H-NMR ($CDCl_3$): δ 8.07 (1H, *dd*, *J* = 0.8, 5.9 Hz, H-5), 7.72 (1H, *s*, H-2), 7.36-7.19 (1H, *m*, H-3′, 4′, 5′, H-6, 7, 8), 7.09-7.04 (1H, *m*, H-2′, 6′), 6.32 (1H, *s*, -NH), 5.16 (2H, *s*, H-10), 3.57-3.43 (2H, *m*, H-1″), 1.24 (3H, *dt*, *J* = 7.2, 14.5 Hz, H-2″). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 165.02, 136.49, 136.03, 131.08, 128.64 (2 × CH), 127.73, 126.73 (2 × CH), 125.69, 122.43, 121.28, 120.37, 111.27, 110.27, 50.16, 34.14, 14.54. Anal. Calcd for $C_{18}H_{18}N_2O$: C, 77.67; H, 6.52; N, 10.06. Found: C, 77.69; H, 6.54; N, 10.66.

***N*-n-Propyl-1-benzylindole-3-carboxamide (15)**

Compound **11** (1 g, 3.7 mmol) was reaction with propylamine as described for the preparation of **4** to afford **15**. Yield 46%; mp: 113-114 °C; EIMS *m/z*: 292 (M^+); ^{1}H-NMR ($CDCl_3$): δ 8.04 (1H, *dd*, *J* = 0.8, 6.3, Hz, H-5), 7.72 (1H, *s*, H-2), 7.32-7.20 (1H, *m*, H-3′, 4′, 5′, H-6, 7, 8), 7.11-7.06 (1H, *m*, H-2′, 6′), 6.23 (1H, *s*, -NH), 5.21 (2H, *s*, H-10), 3.49-3.39 (2H, *m*, H-1″), 1.71-1.60 (2H, *m*, H-2″), 0.99 (3H, *dt*, *J* = 7.4, 15.4 Hz, H-3″). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 165.04, 136.52, 135.99, 131.21, 128.66 (2 × CH), 127.75, 126.74 (2 × CH), 125.52, 122.42, 121.29, 120.16, 111.37, 110.34, 50.21, 41.02, 22.96, 11.36. Anal. Calcd for $C_{19}H_{20}N_2O$: C, 78.05; H, 6.89; N, 9.58. Found: C, 78.08; H, 6.91; N, 9.57.

***N*-n-Butyl-1-benzylindole-3-carboxamide (16)**

Compound **11** (1 g, 3.7 mmol) was reaction with butylamine as described for the preparation of **4** to afford **16**. Yield 53%; mp: 110-111 °C; EIMS *m/z*: 306 (M^+); ^{1}H-NMR ($CDCl_3$): δ 7.97 (1H, *dd*, *J* = 0.6, 6.9 Hz, H-5); 7.71 (1H, *s*, H-2); 7.34-7.21 (1H, *m*, H-3′, 4′, 5′, H-6, 7, 8); 7.14-7.09 (1H, *m*, H-2′, 6′), 6.06 (1H, *s*, -NH), 5.27 (2H, *s*, H-10), 3.54-3.44 (2H, *m*, H-1″), 1.67-1.56 (2H, *m*, H-2″), 1.49-1.38 (2H, *m*, H-3″), 0.96 (3H, *dt*, *J* = 7.14, 14.36, H-3″). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 164.91, 136.54, 135.94, 131.25, 128.67 (2 × CH), 127.77, 126.75 (2 × CH), 125.36, 122.40, 121.27, 119.99, 111.46, 110.34, 50.25, 39.02, 31.81, 20.03, 13.62. Anal. Calcd for $C_{20}H_{22}N_2O$: C, 78.40; H, 7.24; N, 9.14. Found: C, 78.43; H, 7.26; N, 9.12.

Cells and Culture[8]

Human promyeloid leukemia HL-60 cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and the Culture Collection and Research Center (CCRC) (Taiwan, ROC). Human promyeloid leukemia HL-60 cells were cultured in suspension in RPMI-1640 medium (GIBCO, Grand Is land, USA), containing 10% fetal bovine serum (GIBCO, Grand Is land, USA), 100 unit/mL penicillin, 100 μL/mL streptomycin and 1% L-glutamine at 37 °C in a humidified atmosphere of 5% CO_2 in air. Cells were split every day to maintain the cell numbers between 2-5 × 10^5/mL. Cell numbers were assessed by the standard procedure of leukocyte counting using a hemocytometer and cell viability was checked by the ability of cells to exclude Trypan blue.

NBT Differentiation Assay[8]

Cellular differentiation was determined by the NBT reduction assay. Briefly, cells were incubated in a 96-well flat bottom plate (1 × 10^5 cells/well) for 4 days. The cells were then harvested by centrifugation and washed twice with HBSS (Hanks balanced salt solution). The cells were resuspended in 200 μL HBSS containing 1 mg/mL of NBT and 200 μg/mL of phorbol 12-myristate 13-acetate (PMA). After incubation at 37 °C for 30 min, the cells were pelleted and dissolved in 200 μL of DMSO, and their absorbance at

570 nm was determined.

MTT Proliferation Assay[8]

Cellular proliferation was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Briefly, 10 μL of MTT (5 μg/mL) was added to each well of 96-well plates containing 1×10^5 cells after treatment with different concentrations of samples for 4 days. The reaction was stopped after 2 h by adding 100 μL of 0.04 N HCl in isopropanol and the $OD_{570\ nm}$ was determined by a minicolorimetric reader. Each concentration treatment was performed in triplicate.

ACKNOWLEDGMENTS

This work was financially supported by the research grant CMU95-100, CMU96-049 and CMU96-230 from China Medicinal University and by grant 96-2815-C-039-049-B from National Science Council of Taiwan, R.O.C.

Received June 4, 2008.

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