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Synthesis of 1-substituted 3-pyridinylmethylidenylindolin-2-ones and 1-substituted 3-quinolinylmethylidenylindolin-2-ones as the enhancers of ATRA-induced differentiation in HL-60 cells

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Abstract—As part of our continuing search for potential differentiation agents, 1-benzyl-3-(4-pyridinylmethylidenyl)indolin-2-one (**14**) was selected as lead compound, and its new pyridinyl and quinolinyl analogs were synthesized and evaluated for differentiation-inducing activity toward HL-60 cells. Most of the tested compounds enhanced the ATRA-induced differentiation; among them, 1-(1-phenylethyl)-3-(3-quinolinylmethylidenyl)indolin-2-one (**25**) was the most promising one. The two isomers, **25*Z*** and **25*E***; consisting **25** were found to have similar differentiation activity. The combination of **25** with all *trans* retinoic acid (ATRA) was found to induce complete differentiation of HL-60 cells and arrest the cells in the G_0/G_1 phase of the cell cycle. Beside its excellent differentiation activity, **25** also exhibited relatively low cytotoxicity toward normal cells. Therefore, compound **25** is recommended as a candidate for further development of novel enhancer of ATRA-induced differentiation in HL-60 cells.

1. Introduction

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) characterized by the presence of a fusion gene which alters the gene of retinoic acid receptor (RAR)-α and the gene of promyelocytic leukemia (PML). Such fusion gene blocks the maturation of hematopoietic stem cells and results in APL. In 1988, the all *trans* retinoic acid (ATRA) was used to induce leukemic cell differentiation and resulted in almost complete remission in a high proportion of patients with APL.[1] But the effective dose of ATRA in clinical use also led to undesirable side effect.[2,3] Besides, the remission in APL patients who had received ATRA treatment lasted for only a few months before the disease invariably recurred.[4] The reason for recurrence is probably that ATRA induced incomplete differentiation of the leukemia cells.[5] The chemotherapeutic agents, cytarabine (Ara-C) and arsenic trioxide (As_2O_3), were also used in combination with ARTA to improve the APL treatment. But, Ara-C and As_2O_3 are highly toxic in nature. Thus, the search for novel differentiation enhancers that reduce both the dosage and side effects of ATRA and induce complete differentiation is an important task for improving the treatment of APL.

During a massive screening in our laboratory for new enhancers of ARTA-induced differentiation in HL-60 cells, 1-benzyl-3-(4-pyridinylmethylidenyl)indolin-2-one (**14**) was identified as a new and potent enhancer for ARTA-induced differentiation. The combined used of 2.5 μM of **14** with 5 nM of ATRA resulted in complete differentiation (>90% NBT-positive) of HL-60 cells. Moreover, the pyridine moiety of **14** makes it readily convertible to water soluble HCl salt which can be used readily in animal tests and clinical trials. Thus, **14** was selected as a lead compound and its new pyridinyl and quinolinyl analogs, which could be converted into HCl salts, were synthesized and studied for cell differentiation activity in the present work.

Keywords: Pyridinylidenylindolinone; Quinolinylidenylindolinone; Indolinone; ATRA; Differentiation; Leukemia; HL-60.
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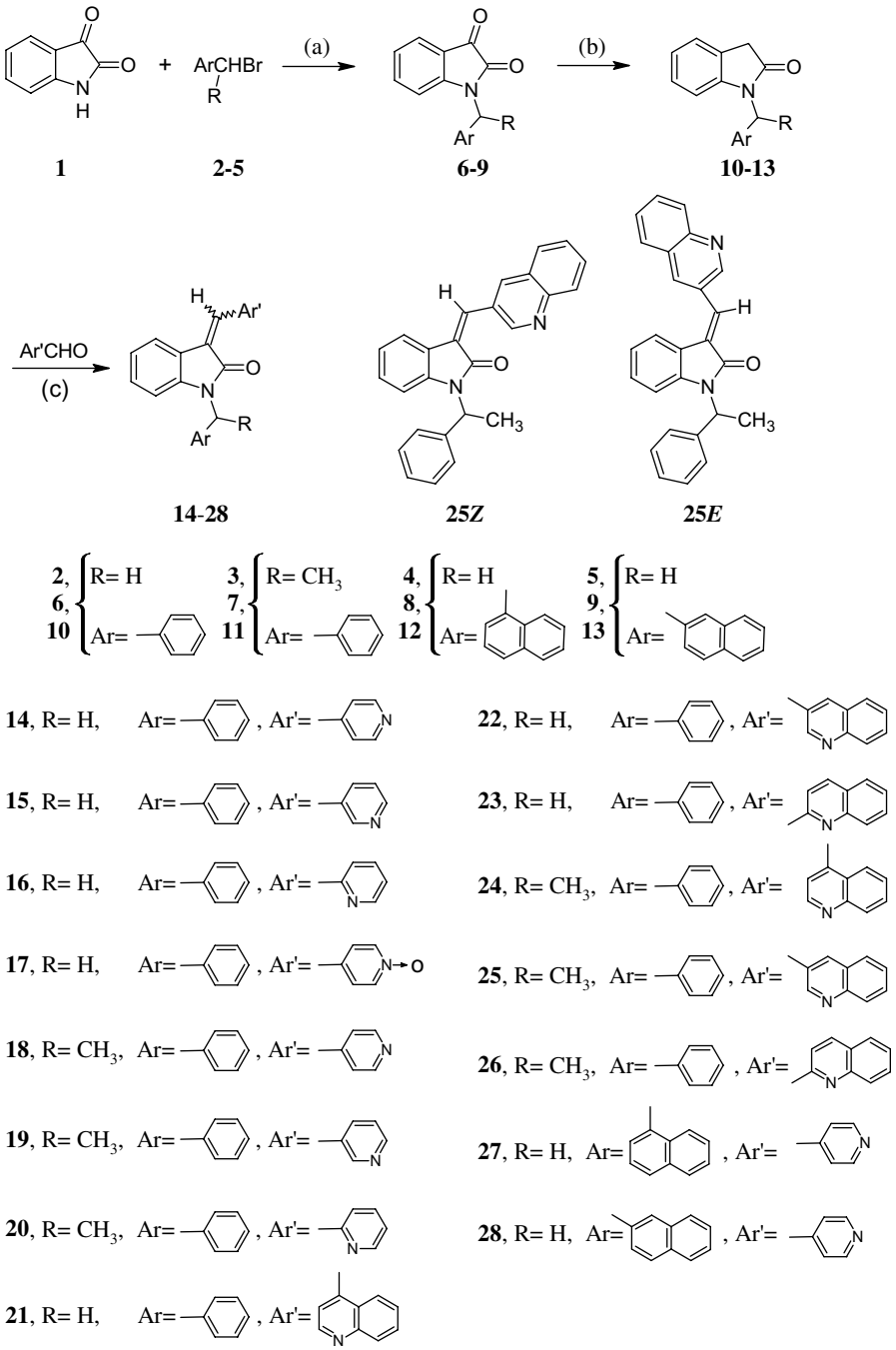
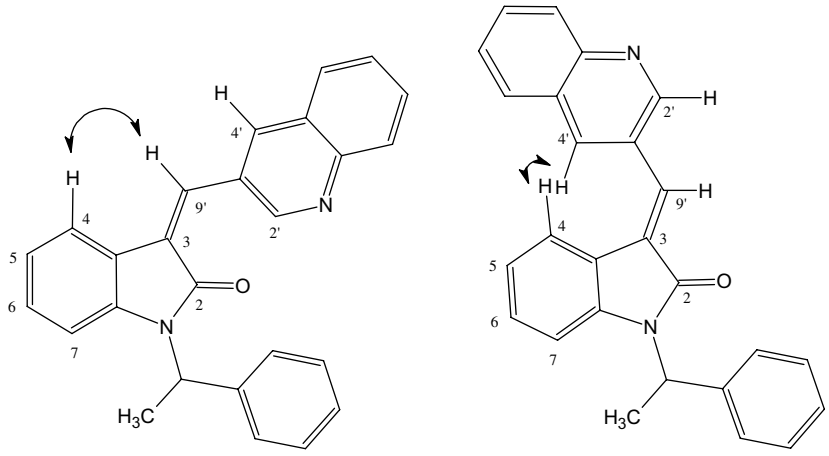
2. Results and discussion

2.1. Chemistry

The synthetic procedure of the target compounds **14**–**28** was illustrated in Scheme 1. Isatin (**1**) was first alkylated with various arylalkyl halides (**2**–**5**) to afford the corresponding 1-substituted isatins (**6**–**9**) which were then reduced with hydrazine hydrate to yield the 1-substituted indolin-2-ones (**10**–**13**). Then the condensation of **10**–**13** with various pyridinyl aldehydes or quinolinyl aldehydes, in the presence of piperidine, afforded the target compounds **14**–**28**. Results of TLC and NMR spectrum analyses indicated that the reaction products **14**–**28** may be a mixture of *Z*- and *E*-form isomers. Column chromatography of product **25**, one of the most potent compounds, yielded two configurational isomers, **25*Z*** (mp 104.0–104.4 °C) and **25*E*** (mp 83.0–83.8 °C) (as shown in Fig. 1), with the same molecular formula $C_{26}H_{20}N_2O$. The two isomers could be recognized by NOESY experiment and by the analysis of chemical shifts. As shown in Figure 1, the *Z*-isomer (**25*Z***) has a NOESY correlation between H-4 (δ 7.66) and H-9′ (δ 7.75), whereas the *E*-isomer (**25*E***) exhibits a NOESY correlation as follows: H-4 (δ 7.60)/H-2′ (δ 9.25), H-4′ (δ 8.47). The chemical shifts of H-9′, H-2′ and H-4′ in



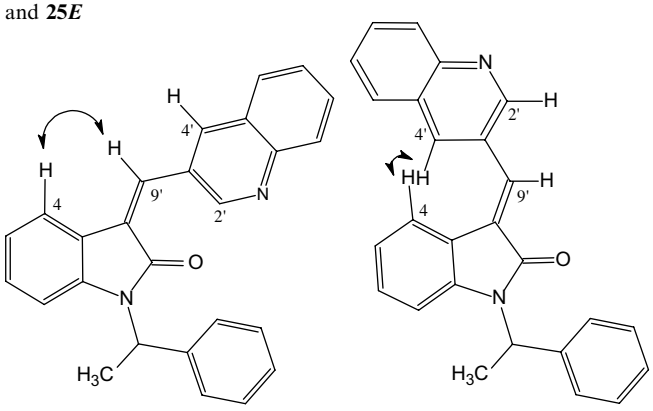
Scheme 1. Reagents and conditions: (a) *tert*-BuOK/THF; (b) $NH_2NH_2{\cdot}H_2O$; △ (c) piperidine/MeoH.



25Z **25E**

Figure 1. Key NOESY (↔) of **25*Z*** and **25*E***.

Table 1. Chemical shifts of H-2′, H-4′ and H-9′ in compounds **25*Z*** and **25*E***



25Z **25E**

Compound	Chemical shift (δ)		
	H-2′	H-4′	H-9′
25*Z*	9.87	9.33	7.75
25*E*	9.25	8.47	8.05

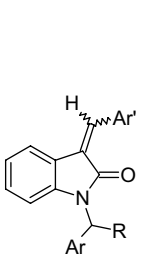
both **25*Z*** and **25*E*** were documented in Table 1. As shown, resonances of H-2′ and H-4′ of **25*Z*** displayed greater downfield shift than the corresponding protons of **25*E*** due to receiving the anisotropic effect from C-2 carbonyl group. Ascribing to the same reason, the signal of H-9′ of **25*E*** located on the lower field than the corresponding proton on the **25*Z***. Based on the above-mentioned result of NOESY experiment and chemical shifts of H-9′, H-2′ and H-4′ protons, **25*Z*** was assigned as *Z*-form of 1-(1-phenylethyl-3-(3-quinolinylmethylidenyl)indolin)-2-one, and **25*E*** existed as its geometrical isomer, *E*-form.

2.2. The effect of compounds 14–28 on the differentiation of HL-60 cells

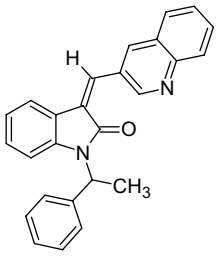
The nitroblue tetrazoliun reduction (NBT) cell differentiation assay was used to measure the potency of compounds **14**–**28**, either used alone or in combination with 5 nM of ATRA, in inducing the differentiation of HL-60 cells. As shown in Table 2, the lead compound (**14**) alone was inactive, but it significantly enhanced the ATRA- induced cell differentiation at 1.0 μM. Increasing the concentration of **14** to 2.5 μM resulted in 94.2% cell differentiation, which is about 5 folds the differentiation activity when 5 nM ATRA was used alone (19.0%). During a series of systematic structural modification, it was found that the replacement of pyridin-4-yl (**14**) with pyridin-3-yl (**15**) or pyridin-2-yl (**16**), or to replace the pyridin-4-yl group with a pyridinyl *N*-oxide (**17**), all resulted in slightly reduced activity. However, the replacement of benzyl group of **14** with 1-phenylethyl group (**18**) significantly enhanced the differentiation activity of ATRA at low concentration (0.5 μM). On the contrary, higher concentration of **18** (2.0 μM) caused death of cell completely. Another replacement of pyridin-4-yl group of **18** with pyridin-3-yl (**19**) or pyridin-2-yl (**20**) also weakened its activity. Based on the above structure–activity relationships, we demonstrated that the pyridin-4-yl derivatives (**14**, **18**) exhibited more differentiation activity than both pyridin-3-yl-(**15**, **19**) or pyridin-2-yl (**16**, **20**) derivatives.

Alternatively, the pyridin-4-yl group of **14** was replaced with quinolin-4-yl group (**21**) which resulted in lower activity, or replaced with quinolin-3-yl (**22**) or quinolin-2-yl (**23**) which not only reduced activity but also led to cell death at the concentration of 5 μM when combined with 5 nM ATRA. Replacing simultaneously the benzyl and pyridin-4-yl groups of **14** with 1-phenylethyl and quinolin-4-yl groups, respectively, gave **24** with considerable differentiation effect. However, at the concentration of 5 μM, **24** led to cell death. During subsequent modification, the quinolin-4-yl group of **24** was replaced with a quinolin-3-yl group to afford **25** which induced 33.9% cell differentiation at 2.5 μM, and induced 94.5% cell differentiation, when combined with 5 nM ATRA. We considered that **25** was the most promising one among all tested compounds. The two isomers (**25*Z*** and **25*E***), coexisting as **25**, were separated and evaluated, but no significant difference in their differentiation activity was found. Next, the substitution

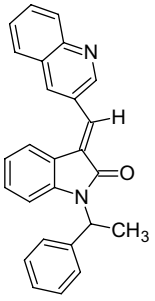
Table 2. Cell differentiation and antiproliferation effect of compounds **14**–**28** against human leukemia HL-60 cells



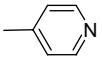
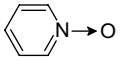
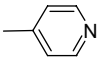
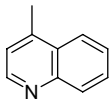
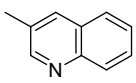
14-28



25*Z*

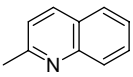
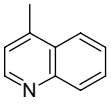
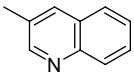
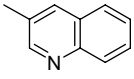
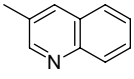
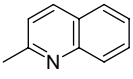
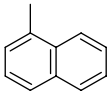
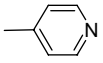
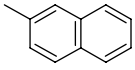
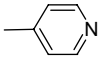


25*E*

Compounds	R_1	Ar	Ar′	Concn (μM)	NBT (%)		MTT (%)	
					−5 nM ATRA	+5 nM ATRA	−5 nM ATRA	+5 nM ATRA
Control					0.7 ± 0.3	19.0 ± 1.2	100.0 ± 1.0	69.4 ± 1.7
14	H			0.5	0.3 ± 0.3	38.2 ± 5.6*	101.0 ± 5.8	61.4 ± 3.2
				1.0	1.2 ± 0.3	51.3 ± 2.6*	85.6 ± 6.1#	48.1 ± 2.9*
				2.5	7.0 ± 0.3#	94.2 ± 4.1*	24.4 ± 5.1#	11.6 ± 0.5*
							IC_{50} = 1.9 μM	
15	H			1.0	0.7 ± 0.3	21.7 ± 3.3*	93.2 ± 2.5#	58.1 ± 3.5*
				2.5	0.8 ± 0.3	29.4 ± 1.7*	75.9 ± 3.3#	48.8 ± 3.8*
				5.0	2.7 ± 0.3	74.3 ± 3.4*	25.4 ± 1.9#	15.1 ± 5.8*
							IC_{50} = 3.8 μM	
16	H			1.0	0.7 ± 0.3	22.7 ± 5.5*	116.6 ± 4.9	63.4 ± 2.5*
				2.5	0.7 ± 0.3	32.3 ± 4.5*	98.5 ± 6.4	46.3 ± 1.5*
				5.0	3.4 ± 1.4	76.0 ± 3.1*	24.7 ± 4.9#	11.8 ± 1.6*
							IC_{50} = 3.6 μM	
17	H			1.0	1.6 ± 1.2	34.4 ± 0.9*	90.0 ± 3.8*	58.7 ± 14.0
				2.5	3.2 ± 0.6	67.2 ± 3.8*	43.4 ± 3.9#	30.9 ± 1.1*
				5.0	11.5 ± 1.9#	88.0 ± 3.5*	22.0 ± 0.9#	15.4 ± 0.8*
							IC_{50} = 2.9 μM	
18	CH_3			0.5	1.0 ± 0.5	55.3 ± 3.7*	66.7 ± 2.7#	47.0 ± 1.1*
				1.0	0.8 ± 0.6	72.5 ± 2.6*	49.2 ± 5.8#	42.1 ± 1.0*
				2.0	Death	Death	Death	Death
19	CH_3			1.0	0.8 ± 0.3	28.6 ± 2.7*	88.0 ± 0.4#	63.7 ± 1.0*
				2.5	2.5 ± 0.5	41.9 ± 2.0*	87.0 ± 2.4#	50.5 ± 0.6*
				5	19.2 ± 2.1#	58.0 ± 3.0#	19.6 ± 5.5#	9.0 ± 1.3*
							IC_{50} = 3.3 μM	
20	CH_3			1.0	0.7 ± 0.3	27.1 ± 2.7*	90.0 ± 1.9#	73.7 ± 2.2
				2.5	0.7 ± 0.3	32.3 ± 4.6*	87.1 ± 4.5#	58.9 ± 3.3*
				5.0	2.0 ± 0.9	80.6 ± 2.5*	55.7 ± 0.8#	27.0 ± 1.9*
							IC_{50} = 5.9 μM	
21	H			1.0	0.8 ± 0.3	21.5 ± 0.3	85.2 ± 7.7#	64.4 ± 4.8
				5.0	2.1 ± 0.3	37.6 ± 3.4*	73.8 ± 4.4#	56.7 ± 5.5*
				10.0	4.7 ± 0.3	87.5 ± 1.4*	51.2 ± 0.6#	35.7 ± 1.6*
							IC_{50} = 10.6 μM	
22	H			1.0	0.7 ± 0.3	12.2 ± 1.9	117.4 ± 2.6	70.2 ± 3.9
				2.5	0.8 ± 0.3	20.7 ± 3.8*	70.6 ± 5.0#	48.4 ± 1.5*
				5.0	23.6 ± 0.9#	Death	10.9 ± 2.8#	Death
							IC_{50} = 3.2 μM	

(*continued on next page*)

Table 2 (*continued*)

Compounds	R_1	Ar	Ar′	Concn (μM)	NBT (%)		MTT (%)	
					−5 nM ATRA	+5 nM ATRA	−5 nM ATRA	+5 nM ATRA
23	H			1.0 2.5 5.0	2.0 ± 0.5 2.0 ± 0.5 4.4 ± 1.9	32.4 ± 0.6* 53.7 ± 0.3* Death	82.7 ± 3.7# 55.7 ± 9.3# 12.2 ± 1.7# IC_{50} = 2.8 μM	63.7 ± 0.6 33.7 ± 3.3# Death
24	CH_3			1.0 2.5 5.0	1.2 ± 0.7 20.8 ± 2.4# Death	30.3 ± 2.0* 84.2 ± 3.5* Death	72.6 ± 3.0# 31.0 ± 2.6# Death	61.3 ± 2.7* 36.9 ± 1.4* Death
25	CH_3			0.5 1.0 2.0	0.8 ± 0.3 0.3 ± 0.3 33.9 ± 0.8#	54.8 ± 4.2* 79.3 ± 2.3* 94.5 ± 0.5*	89.5 ± 5.1# 70.9 ± 2.0# 24.7 ± 4.2# IC_{50} = 1.4 μM	45.1 ± 2.0* 41.4 ± 2.3* 20.4 ± 0.6*
25*Z*	CH_3			0.5 1.0 2.0	1.3 ± 0.3 3.8 ± 1.0 40.8 ± 1.8#	53.1 ± 4.2* 75.5 ± 4.9* 93.7 ± 3.2*	116.1 ± 4.0 82.3 ± 0.3# 20.3 ± 2.9# IC_{50} = 1.5 μM	72.8 ± 6.4* 60.2 ± 2.6* 32.2 ± 2.0*
25*E*	CH_3			0.5 1.0 2.0	0.8 ± 0.6 4.3 ± 0.3 37.7 ± 2.9#	52.1 ± 2.3* 72.1 ± 1.7* 90.2 ± 2.6*	109.9 ± 0.1 82.9 ± 2.6# 25.5 ± 0.8# IC_{50} = 1.4 μM	71.0 ± 2.5* 57.2 ± 3.8* 29.0 ± 1.3*
26				1.0 2.5 5.0	0.8 ± 0.3 1.0 ± 0.5 15.0 ± 2.2#	30.0 ± 1.5* 41.5 ± 3.5* 75.6 ± 0.4*	103.2 ± 1.3 81.3 ± 1.1# 18.5 ± 3.2# IC_{50} = 3.4 μM	62.2 ± 1.6* 50.8 ± 2.8* 15.7 ± 0.7#
27	H			0.5 1.0 2.5	0.8 ± 0.6 0.5 ± 0.5 Death	16.2 ± 2.8 24.0 ± 2.0 Death	103.6 ± 0.4 90.8 ± 4.2 Death	71.3 ± 4.9 59.4 ± 4.1 Death
28	H			0.1 0.5 1.0	0.8 ± 0.3 0.7 ± 0.3 Death	46.8 ± 4.4* 66.8 ± 1.2* Death	78.2 ± 8.0# 59.6 ± 1.4# Death	39.2 ± 3.8* 28.2 ± 3.5* 10.1 ± 0.9

HL-60 cells (4×10^4 cells/mL) were cultured with various concentrations of 14–28 for 72 h. After treatment, cells were harvested and examined by NBT- and MTT-assay. Data are represented as means ± SD from four independent experiments.

* $P < 0.001$ compared with 5 nM ATRA used alone.

\# $P < 0.001$ compared with vehicle control.

of the quinolin-3-yl group of **25** with quinolin-2-yl group (**26**) resulted in attenuated activity. Finally, the benzyl group of **14** was replaced with either α-naphthalinylmethyl group to yield **27** with lowered activity, or replaced with β-naphthalinylmethyl group to form **28**, which at concentration of 1 μM caused cell death.

2.3. The effect of compounds 14–28 on the proliferation of HL-60 cells

The effect of compounds **14**–**28** on the proliferation of HL-60 cells is summarized in Table 2. Most of the tested compounds showed significant antiproliferative activity with IC_{50} ranging between 0.5 and 5.0 μM. Their activity was significantly enhanced by the addition of ATRA. Comparing similar analogs, the pyridin-4-yl derivatives (**14**, **18**) demonstrated the best activity among pyridinyl derivatives (**14**–**20**), and the quinolin-3-yl derivative of **25** (**25*Z***, **25*E***) was found to be the most active among quinolinyl derivatives (**21**–**26**).

2.4. Morphological change of HL-60 cells responding to compound 25 alone or in combination with ATRA

Comparing the morphological change of HL-60 cells responding to **25** alone, or in combination with ATRA, we found that the morphology of HL-60 cells changed from round and smooth (Fig. 2A) before treatment into

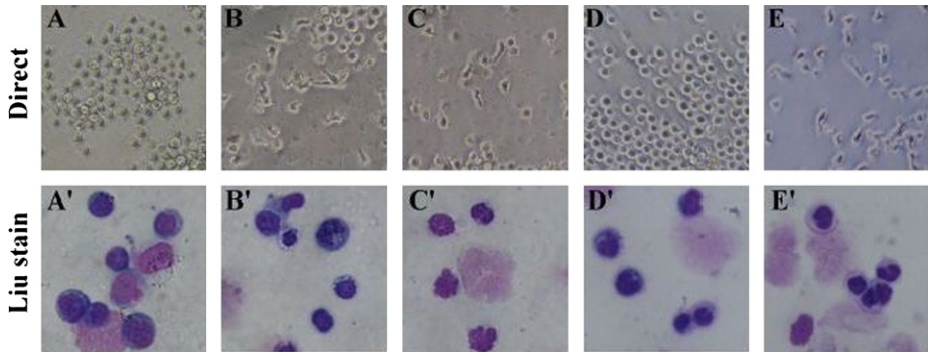


Figure 2. Morphological changes in response to **25**. HL-60 cells (4×10^4) were treated with vehicle control (A and A′), 2 μM **25** used alone (B and B′), co-treated 2 μM **25** and 5 nM ATRA together (C and C′), 5 nM ATRA (D and D′) or 1 μM ATRA (E and E′) for 72 h (magnification 200×).

spindle-shape after treatment with **25** (Fig. 2B). Such morphological change became more obvious (Fig. 2C) when **25** was used in combination with 5 nM ATRA. Similar morphological change was observed when higher concentration of 1 μM ATRA (Fig. 2E) was used alone.

To confirm the above observation, Liu's stain was also applied to the cells which were then examined under optical microscope. Again, the horseshoe morphology was not seen in the nuclei of untreated cells (Fig. 2A′), but was clearly seen in the nuclei of cells treated with **25** (Fig. 2B′). The population of horseshoe-shape nuclei increased significantly when **25** was used in combination with 5 nM ATRA (Fig. 2C′) or 1 μM ATRA used alone (Fig. 2E′). The morphological changes of cells, untreated or treated with the combination of **25** and 5 nM ATRA, determined after application of Liu's stain, agreed well with the previous observation without cell stain. These results indicated that the combined use of **25** with 5 nM ATRA or higher concentration (1 μM) of ATRA undoubtedly induced cell differentiation.

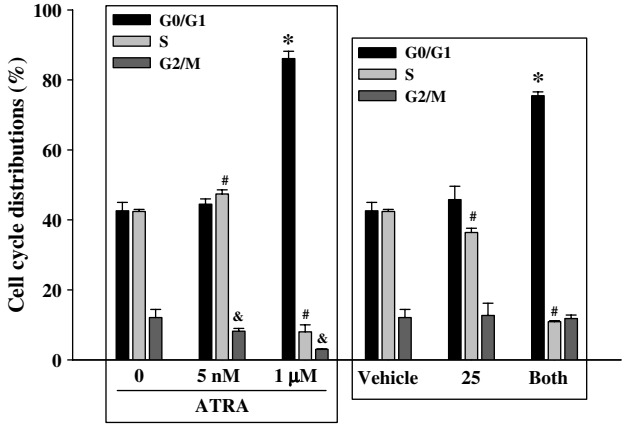


Figure 3. Cell cycle distributions of **25** used alone or combined with ATRA in HL-60 cells. HL-60 cells (4×10^4) were treated with vehicle control, ATRA (5 nM or 1 μM), 2 μM **25** used alone, or co-treated 2 μM **25** and 5 nM ATRA together (both) for 72 h. After treatment, cells were fixed and stained with PI and then the cell cycle distribution was examined by flow cytometry. Data are represented as means ± SD from four independent experiments. $^{*}P < 0.001$ compared with the G_0/G_1 phase of the vehicle control. $^{\#}P < 0.001$ compared with the S phase of the vehicle control. $^{\&}P < 0.001$ compared with the G_2/M phase of the vehicle control.

2.5. The effect of compound 25 alone or in combination with ATRA on the cell cycle distribution of HL-60 cells

The cellular DNA content was also measured by flow cytometry, and the result in Figure 3 indicated that, following 72 h treatment with **25**, the accumulation of cells in the G_0/G_1 phase of cell cycle increased to 49.8%. The combined use of **25** with 5 nM ATRA increased the G_0/G_1 phase percentage further to 76.8%. Similar cell cycle arrest in the G_0/G_1 phase was also observed when higher concentration (1 μM) of ATRA was used alone. These findings demonstrated that the combination of **25** with 5 nM ATRA arrested the HL-60 cells in the G_0/G_1 phase of cell cycle.

2.6. The effect of compound 25 alone or combined with ATRA on the growth of human normal cells

The excellent differentiation activity of compound **25** prompted us to examine the cytotoxicity of **25** used alone and combined with ATRA toward human normal leukocytes in order to assess selectivity to HL-60 leukemia cells. As shown in Figure 4, the IC_{50} value of **25** is

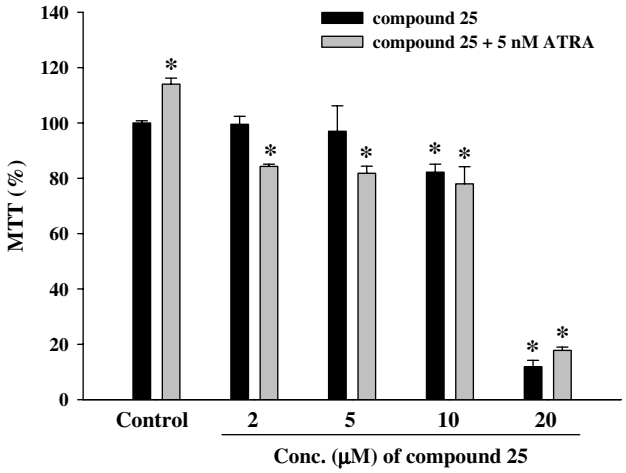


Figure 4. The cytotoxicity of **25** toward human normal leukocytes. Human normal leukocytes (2×10^6) were treated with vehicle control, **25** used alone, or co-treated **25** and 5 nM ATRA together for 48 h. After treatment, cells were harvested and examined using MTT assay. Data are represented as means ± SD from four independent experiments. $^{*}P < 0.001$ compared with control.

14.0 μM which is about 10-fold higher than its cytotoxicity against HL-60 cells (IC_{50} = 1.4 μM) (Table 2). We then tested the cytotoxicity of the use of **25** in combination with 5 nM ATRA toward human normal leukocytes and found that the concentration of compound **25** needed to accomplish 50% growth-inhibition of humane normal leukocytes to be 14.7 μM, which is about 30-fold higher than the concentration (0.5 μM) of **25** takes together with 5 nM ATRA, to accomplish 50% growth-inhibition of HL-60 cells (Table 2). Such finding indicated that the combined use of **25** with ATRA has relatively low toxicity toward normal cells.

3. Conclusion

A series of 1-substituted 3-pyridinylmethylidenylindolin-2-ones and 1-substituted 3-quinolinylmethylidenylindolin-2-ones were synthesized and evaluated for differentiation-inducing activity toward HL-60 cells. Among the target compounds, compound **25** was the most promising one. The combination of **25** with ATRA was found to induce complete differentiation of HL-60 cells. Also, **25** showed relatively low cytotoxicity against normal cells. Thus, compound **25** is recommended as a candidate for further development of novel enhancer of ATRA-induced differentiation in HL-60 cells.

4. Experimental

4.1. Chemistry

4.1.1. General. All starting materials were commercially available. Solvents and reagents were used without further purification. Reactions were monitored either by TLC on silica gel plastic sheets (Kieselgel 60 F_{254}, Merck) or by HPLC. Purification was performed by flash chromatography using silica gel (particle size 63–200 μm, Merck). NMR spectra were recorded on a Varian 500-MHz spectrometer. Chemical shifts are reported as ppm (δ) relative to TMS as internal standard. The ratio of *E*- to *Z*-isomer of compounds (**14**–**28**) was analyzed by ^{1}H NMR.[6,7] Mass spectra were recorded on a JEOL JMS-SX102A spectrometer (HREI). IR spectra were recorded on a Horiba FT-730 FT-IR spectrometer. Melting points were determined on a Buchi B-540 apparatus and are uncorrected.

4.2. General procedure of N-alkylation in base (6–9)

4.2.1. 1-Benzyl-isatin (6).[8] To a solution of isatin (**1**, 5.0 g, 34.0 mmol) in THF (100 mL) was added *tert*-BuOK (4.9 g, 43.8 mmol) at 0 °C. After stirring for 30 min, $PhCH_2Br$ (**2**, 7.0 g, 40.8 mmol) was added dropwise over 20 min at 0 °C. The reaction mixture was then warmed to room temperature and stirred for additional 16 h, and then concentrated under reduced pressure. The residue was dissolved in ethyl acetate, washed with water, dried over $MgSO_4$ and evaporated. The residue was then purified by flash column chromatography (EtOAc/*n*-hexane), and recrystallized from EtOAc/*n*-hexane to give 5.1 g (63%) of **6**: mp 126–127 °C; ^{1}H NMR ($CDCl_3$): δ 4.91 (s, 2H), 6.5–7.7 (m, 9H); HRMS: *m*/*z* 237.0805 (calculated for $C_{15}H_{11}NO_2$, 237.0790).

4.2.2. 1-(1-Phenylethyl)isatin (7).[9] Isatin, PhMeCHBr (**3**) and *tert*-BuOK in THF were allowed to react in the same manner as described in the preparation of compound **6** and the residue was purified by flash column chromatography (EtOAc/*n*-hexane), recrystallized from EtOAc/*n*-hexane to give **7** in 81% yield: mp 118–120 °C; ^{1}H NMR ($CDCl_3$): δ 1.85 (d, 3H, *J* = 7.0 Hz), 5.77 (q, 1H, *J* = 7.0 Hz), 6.53 (d, 1H, *J* = 8.5 Hz), 7.01 (t, 1H, *J* = 7.5 Hz), 7.27–7.39 (m, 6H), 7.58 (dd, 1H, *J* = 1.0, 7.5 Hz); HRMS: *m*/*z* 251.0926 (calculated for $C_{16}H_{13}NO_2$, 251.0946).

4.2.3. 1-(Naphthalen-1-ylmethyl)isatin (8). Isatin, 1-(chloromethyl)naphthalene (**4**) and *tert*-BuOK in THF were allowed to react in the same manner as described in the preparation of compound **6** and the residue was purified by flash column chromatography (EtOAc/*n*-hexane), recrystallized from EtOAc/*n*-hexane to give **8** in 83% yield: mp 192–194 °C; ^{1}H NMR ($CDCl_3$): δ 5.40 (s, 2H), 6.72 (d, 1H, *J* = 8.5 Hz), 7.06 (t, 1H, *J* = 8.0 Hz), 7.24–7.41 (m, 3H), 7.52–7.62 (m, 3H), 7.81 (dd, 1H, *J* = 2.0, 7.0 Hz), 7.88 (t, 1H, *J* = 8.0 Hz), 8.06 (d, 1H, *J* = 8.5 Hz); HRMS: *m*/*z* 287.0965 (calculated for $C_{19}H_{13}NO_2$, 287.0946).

4.2.4. 1-(Naphthalen-2-ylmethyl)isatin (9). Isatin, 2-(bromomethyl)naphthalene (**5**) and *tert*-BuOK in THF were allowed to react in the same manner as described in the preparation of compound **6** and the residue was purified by flash column chromatography (EtOAc/*n*-hexane), recrystallized from EtOAc/*n*-hexane to give **9** in 75% yield: mp 156 °C (dec); ^{1}H NMR ($CDCl_3$): δ 5.07 (s, 2H), 6.79 (d, 1H, *J* = 8.5 Hz), 7.06 (t, 1H, *J* = 7.5 Hz), 7.40–7.48 (m, 5H), 7.59 (d, 1H, *J* = 7.5 Hz), 7.77–7.82 (m, 2H), 8.14 (d, 1H, *J* = 8.5 Hz); HRMS: *m*/*z* 287.0935 (calculated for $C_{19}H_{13}NO_2$, 287.0946).

4.3. General procedure for reduction of *N*-aryl-oxindole with $NH_2NH_2{\cdot}H_2O$ (10–13)

4.3.1. 1-Benzyl-1,3-dihydroindol-2-one (10).[10] A mixture of *N*-benzylisatin (**6**, 0.5 g, 2.1 mmol) and $NH_2NH_2{\cdot}H_2O$ (12 mL) was refluxed for 30 min. The mixture was extracted with ethyl acetate, washed with water, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography (EtOAc/*n*-hexane), and recrystallized from EtOAc/*n*-hexane to give 0.39 g of **10** in 84% yield: mp 80.3–81.0 °C; ^{1}H NMR ($CDCl_3$): δ 3.60 (s, 2H), 4.93 (s, 2H), 6.76 (d, 1H, *J* = 7.5 Hz), 7.02 (t, 1H, *J* = 7.5 Hz), 7.18 (t, 1H, *J* = 7.5 Hz), 7.22–7.37 (m, 6H); HRMS: *m*/*z* 223.0985 (calculated for $C_{15}H_{13}NO$, 223.0997).

4.3.2. 1-(1-Phenylethyl)indolin-2-one (11). 1-(1-Phenylethyl)isatin (**7**) and $NH_2NH_2{\cdot}H_2O$ were allowed to react in the same manner as described in the preparation of compound **10** and the residue was purified by flash column chromatography (EtOAc/*n*-hexane), recrystallized from EtOAc/*n*-hexane to give **11** in 88% yield: mp 63.9–64.8 °C; ^{1}H NMR ($CDCl_3$): δ 1.784 (d, 3H), 3.61

(s, 2H), 5.88 (q, 1H), 6.49 (d, 1H), 6.92–7.03 (m, 2H), 7.21–7.36 (m, 6H); HRMS: *m*/*z* 227.1168 (calculated for $C_{16}H_{16}NO$, 237.1154).

4.3.3. 1-(Naphthalen-1-ylmethyl)indolin-2-one (12). 1-(Naphthalen-1-ylmethyl)-isatin (**8**) and $NH_2NH_2{\cdot}H_2O$ were allowed to react in the same manner as described in the preparation of compound **10** and the residue was purified by flash column chromatography (EtOAc/*n*-hexane), recrystallized from EtOAc/*n*-hexane to give **12** in 85% yield: mp 153–154 °C; ^{1}H NMR ($CDCl_3$): δ 3.68 (s, 2H), 5.39 (s, 2H), 6.66 (d, 1H, J = 8 Hz), 6.97 (t, 1H, J = 8 Hz), 7.09 (t, 1H, J = 7.5 Hz), 7.27–8.15 (m, 8H); HRMS: *m*/*z* 273.1133 (calculated for $C_{19}H_{15}NO$, 273.1154).

4.3.4. 1-(Naphthalen-2-ylmethyl)indolin-2-one (13). 1-(Naphthalen-2-ylmethyl)-isatin (**9**) and $NH_2NH_2{\cdot}H_2O$ were allowed to react in the same manner as described in the preparation of compound **10** and the residue was purified by flash column chromatography (EtOAc/*n*-hexane), recrystallized from EtOAc/*n*-hexane to give **13** in 86% yield as oil; ^{1}H NMR ($CDCl_3$): δ 3.71 (s, 2H), 5.13 (s, 2H), 6.81 (d, 1H, J = 7.5 Hz), 7.04 (t, 1H, J = 7.5 Hz), 7.18 (t, J = 7.5 Hz, 1H), 7.30 (d, 1H, J = 7.5 Hz), 7.45–7.59 (m, 3H), 7.78–7.85 (m, 4H); HRMS: *m*/*z* 273.1142 (calculated for $C_{19}H_{15}NO$, 273.1154).

4.4. General procedure for condensation of indolin-2-one with heteroaryl-aldehydes (14–28)

4.4.1. 1-Benzyl-3-(4-pyridinylmethylidenyl)indolin-2-one (14). To a stirred solution of 1-benzyl-1,3-dihydroindol-2-one (**10**, 3.2 g, 13.5 mmol) in MeOH (30 mL) were added piperidine (50 mg) and 4-pyridinecarboxaldehyde (1.6 g, 14.8 mmol) at 0 °C. The mixture was heated under reflux for 4 h, then extracted with ethyl acetate, washed with water, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography (ethyl acetate/*n*-hexane), and recrystallized from EtOAc/*n*-hexane to give **14** in 53% yield: mp 60.0–61.1 °C; ^{1}H NMR ($CDCl_3$): δ 4.74, 4.76 (2s, 2H), 6.51 (d, 1H), 6.62 (t, 1H), 6.78–7.39, 7.81 (m+d, 9H), 7.55 (s, 1H), 8.48–8.53 (m, 2H); HRMS: *m*/*z* 312.1245 (calculated for $C_{21}H_{16}N_2O$, 312.1263).

4.4.2. 1-Benzyl-3-(3-pyridinylmethylidenyl)indolin-2-one (15). 1-Benzyl-1,3-dihydro-indol-2-one (**10**), 3-pyridinecarboxaldehyde and piperidine in MeOH were allowed to react in the same manner as described in the preparation of compound **14** and the residue was recrystallized from EtOAc/*i*-propyl ether to give **15** in 65% yield: mp 89.5–90.0 °C; IR (cm^{-1}) 3420, 1697; ^{1}H NMR ($CDCl_3$): δ 5.02, 5.03 (2s, 2H, $ArCH_2$-), 6.78 (d, 1H, J = 7.5 Hz), 6.89 (t, 1H, J = 7.5 Hz), 7.09 (t, 1H, J = 7.5 Hz), 7.07–7.61 (m, 8H), 7.87, 7.98 (2s, 1H), 8.65–9.23 (m, 2H); HRMS: *m*/*z* 312.1231 (calculated for $C_{21}H_{16}N_2O$, 312.1263).

4.4.3. 1-Benzyl-3-(2-pyridinylmethylidenyl) indolin-2-one (16). 1-Benzyl-1,3-dihydroindol-2-one (**10**), 2-pyridinecarboxaldehyde and piperidine in MeOH were allowed to react in the same manner as described in the preparation of compound **14** and the residue was recrystallized from EtOAc/*i*-propyl ether to give **16** in 81% yield: mp 46.0–46.4 °C; IR (cm^{-1}) 3379, 3076, 1697; ^{1}H NMR ($CDCl_3$): δ 5.06 (s, 2H), 6.76 (d, 1H, J = 7.5 Hz), 7.08 (t, 1H, J = 7.5 Hz), 7.24–7.38 (m, 7H), 7.67 (d, 1H, J = 7.5 Hz), 7.82–7.86 (m, 2H), 8.91 (d, 1H, J = 4.5 Hz), 9.06 (d, 1H, J = 7.5 Hz); HRMS: *m*/*z* 312.1281 (calculated for $C_{21}H_{16}N_2O$, 312.1263).

4.4.4. 1-Benzyl-3-(4-(N-oxy-pyridinylmethylidenyl))indolin-2-one (17). 1-Benzyl-1,3-dihydroindol-2-one (**10**), 4-pyridinecarboxaldehyde *N*-oxide and piperidine in MeOH were allowed to react in the same manner as described in the preparation of compound **14** and the residue was recrystallized from EtOAc/*i*-propyl ether to give **17** in 77% yield: mp 251 °C (dec); IR (cm^{-1}) 3419, 3082, 1682; ^{1}H NMR ($CDCl_3$): δ 5.02 (s, 2H), 6.79 (d, 1H, J = 7.5 Hz), 7.09 (d, 1H, J = 7.5 Hz), 7.26–7.41 (m, 7H), 7.56 (d, 1H, J = 7.5 Hz), 8.23 (s, 1H), 8.38 (d, 2H, J = 6.0 Hz); HRMS: *m*/*z* 328.1207 (calculated for $C_{21}H_{16}N_2O_2$, 328.1212).

4.4.5. 1-(1-Phenylethyl)-3-(4-pyridinylmethylidenyl)indolin-2-one (18). To a stirred solution of 1-(1-phenylethyl)indolin-2-one (**11**, 3.0 g, 13.5 mmol) in MeOH (30 mL) were added piperidine (50 mg) and 4-pyridinecarboxaldehyde (1.6 g, 14.8 mmol) at 0 °C. The mixture was refluxed for 4 h, concentrated, extracted with ethyl acetate, dried with $MgSO_4$, and concentrated again. The residue was purified by flash column chromatography (EtOAc/*n*-hexane), and recrystallized from EtOAc/*n*-hexane to give 3.0 g (73%) of **18**: mp 70.1–70.3 °C; IR (cm^{-1}) 3396, 3008, 2973, 1711; ^{1}H NMR ($CDCl_3$): δ 1.91 (d, 3H, J = 7.0 Hz), 5.96 (q, 1H, J = 7.5 Hz), 6.56 (d, 1H, J = 8 Hz), 6.81 (t, 1H, J = 7.5 Hz), 7.09 (t, 1H, J = 7.5 Hz), 7.32 (t, 1H, J = 7.5 Hz), 7.37–7.46 (m, 5H), 7.53 (d, 2H, J = 6.0 Hz), 7.80 (s, 1H), 8.78 (d, 2H, J = 6.0 Hz); HRMS: *m*/*z* 326.1402 (calculated for $C_{22}H_{18}N_2O$, 326.1419).

4.4.6. 1-(1-Phenylethyl)-3-(3-pyridinylmethylidenyl)indolin-2-one (19). 1-(1-Phenylethyl)indolin-2-one (**11**), 3-pyridinecarboxaldehyde and piperidine in MeOH were allowed to react in the same manner as described in the preparation of compound **14** and the residue was purified by flash column chromatography (EtOAc/*n*-hexane), recrystallized from EtOAc-*n*-hexane to give **19** in 67% yield as oil; IR (cm^{-1}) 3419, 1702; ^{1}H NMR ($CDCl_3$): δ 1.88–1.92 (dd, 3H), 5.98 (q, 1H), 6.53–6.57 (m, 1H), 6.83 (t, 1H), 7.02–7.13 (m, 2H), 7.29–7.60 (m, 7H), 7.87–7.98 (m, 1H), 8.65–9.22 (m, 2H); HRMS: *m*/*z* 326.1397 (calculated for $C_{22}H_{18}N_2O$, 326.1419).

4.4.7. 1-(1-phenylethyl)-3-(2-pyridinylmethylidenyl)indolin-2-one (20). 1-(1-Phenylethyl)indolin-2-one (**11**), 2-pyridinecarboxaldehyde and piperidine in MeOH were allowed to react in the same manner as described in the preparation of compound **14** and the residue was purified by flash column chromatography (EtOAc/*n*-hexane), recrystallized from EtOAc-*n*-hexane to give **20** in 78% yield as oil; IR (cm^{-1}): 3425, 1700; ^{1}H NMR ($CDCl_3$): δ 1.92 (d, 3H, J = 7.5 Hz), 6.02

(q, 1H, J = 7.5 Hz), 6.55 (d, 1H, J = 7.5 Hz), 7.02 (t, 1H, J = 7.5 Hz), 7.13 (t, 1H, J = 7.5 Hz), 7.29–7.45 (m, 5H), 7.67 (d, 1H, J = 8.0 Hz), 7.83 (t, 1H, J = 7.5 Hz), 7.86 (s, 1H), 8.90 (d, 2H, J = 5.0 Hz), 9.04 (d, 1H, J = 7.5 Hz); HRMS: m/z 326.1431 (calculated for $C_{22}H_{18}N_2O$, 326.1419).

4.4.8. 1-Benzyl-3-(4-quinolinylmethylidenyl)indolin-2-one (21). 1-Benzyl-1,3-dihydroindol-2-one (**10**), 4-quinolinecarboxaldehyde and piperidine in MeOH were allowed to react in the same manner as described in the preparation of compound **14** and the residue was recrystallized from MeOH to give **21** in 87% yield: mp 94.8–95.2 °C; IR (cm^{-1}) 3415, 3060, 2941, 1707; ^1H NMR ($CDCl_3$): δ 5.07 (s, 2H), 6.73–6.79 (m, 3H), 6.97 (d, 1H, J = 7.5 Hz), 7.18 (t, 1H, J = 7.5 Hz), 7.30–7.43 (m, 4H), 7.63 (t, 1H, J = 7.5 Hz), 7.67 (d, 1H, J = 4.5 Hz), 7.84 (t, 1H, J = 7.5 Hz), 8.05 (d, 1H, J = 7.5 Hz), 8.26 (d, 1H, J = 8.5 Hz), 8.28 (s, 1H), 9.04 (d, 1H, J = 7.5 Hz); HRMS: m/z 362.1477 (calculated for $C_{25}H_{18}N_2O$, 362.1419).

4.4.9. 1-Benzyl-3-(3-quinolinylmethylidenyl)indolin-2-one (22). 1-Benzyl-1,3-dihydroindol-2-one (**10**), 3-quinolinecarboxaldehyde and piperidine in MeOH were allowed to react in the same manner as described in the preparation of compound **14** and the residue was recrystallized from EtOAc/*i*-propyl ether to give **22** in 85% yield: mp 228.1–228.4 °C; IR (cm^{-1}) 3429, 3060, 1685; ^1H NMR ($CDCl_3$): δ 5.07 (s, 2H), 6.80 (d, 1H, J = 7.5 Hz), 7.12 (t, 1H, J = 7.5 Hz), 7.25–7.40 (m, 7H), 7.62 (t, 1H, J = 7.5 Hz), 7.67 (d, 1H, J = 7.5 Hz), 7.80 (t, 1H, J = 7.5 Hz), 8.04 (d, 1H, J = 7.5 Hz), 8.16 (d, 1H, J = 8.0 Hz), 9.30 (d, 1H, J = 2.0 Hz), 9.83 (d, 1H, J = 2.0 Hz); HRMS: m/z 362.1432 (calculated for $C_{25}H_{18}N_2O$, 362.1419).

4.4.10. 1-Benzyl-3-(2-quinolinylmethylidenyl)indolin-2-one (23). 1-Benzyl-1,3-dihydroindol-2-one (**10**), 2-quinolinecarboxaldehyde and piperidine in MeOH were allowed to react in the same manner as described in the preparation of compound **14** and the residue was recrystallized from EtOAc/*n*-hexane to give **23** in 83% yield: mp 266.8–267.2 °C; IR (cm^{-1}) 3429, 3051, 1693; ^1H NMR ($CDCl_3$): δ 5.03 (s, 2H), 6.78 (d, 1H, J = 8.0 Hz), 7.11 (t, 1H, J = 8.0 Hz), 7.28–7.40 (m, 6H), 7.66 (t, 1H, J = 7.5 Hz), 7.75 (d, 1H, J = 8.0 Hz), 7.80–7.92 (m, 2H), 8.02 (s, 1H), 8.22 (d, 2H, J = 8.0 Hz), 9.26 (d, 1H, J = 8.0 Hz); HRMS: m/z 362.1477 (calculated for $C_{25}H_{18}N_2O$, 362.1422).

4.4.11. 1-(1-Phenylethyl)-3-(4-quinolinylmethylidenyl) indolin-2-one (24). 1-(1-Phenylethyl)indolin-2-one (**11**), 4-quinolinecarboxaldehyde and piperidine in MeOH were allowed to react in the same manner as described in the preparation of compound **14** and the residue was recrystallized from MeOH to give **24** in 90% yield: mp 149.5–150.1 °C; IR (cm^{-1}) 3396, 1710; ^1H NMR ($CDCl_3$): δ 1.95 (d, 3H, J = 7.0 Hz), 6.02 (q, 1H, J = 7.0 Hz), 6.56 (d, 1H, J = 8.0 Hz), 6.68 (t, 1H, J = 7.5 Hz), 6.96 (d, 1H, J = 7.5 Hz), 7.06 (t, 1H, J = 7.5 Hz), 7.33 (t, 1H, J = 7.5 Hz), 7.41 (t, 2H, J = 7.5 Hz), 7.47 (d, 2H, J = 7.0 Hz), 7.63 (t, 1H, J = 7.0 Hz), 7.68 (d, 1H, J = 4.5 Hz), 7.84 (t, 1H, J = 7.0 Hz), 8.06 (d, 1H, J = 8.0 Hz), 8.26–8.28 (m, 2H), 9.04 (d, 1H, J = 4.5 Hz); HRMS: m/z 376.1617 (calculated for $C_{26}H_{20}N_2O$, 376.1576).

4.4.12. 1-(1-Phenylethyl)-3-(3-quinolinylmethylidenyl) indolin-2-one (25). 1-(1-Phenylethyl)indolin-2-one (**11**), 3-quinolinecarboxaldehyde and piperidine in MeOH were allowed to react in the same manner as described in the preparation of compound **14** and the residue was recrystallized from MeOH to give **25**: (isomer ratio, *E*/*Z* = 3/1 by ^1H NMR analysis) in 88% yield. The *E*-/*Z*-form of **25** was separated by column chromatography [CH_2Cl_2/MeOH (95:5)], and recrystallized from EtOAc/*n*-hexane to give *Z*-isomer and *E*-isomer of **25**. *Z*-isomer (**25*Z***): mp 104.0–104.4 °C; IR (cm^{-1}) 3400, 3051, 1709; ^1H NMR ($CDCl_3$): δ 1.92 (d, 3H, J = 7.0 Hz), 6.06 (q, 1H, J = 7.0 Hz), 6.55 (d, 1H, J = 7.5 Hz), 7.06 (t, 1H, J = 7.5 Hz), 7.12 (t, 1H, J = 7.5 Hz), 7.32 (d, 1H, J = 7.5 Hz), 7.39 (t, 2H, J = 7.5 Hz), 7.44 (d, 2H, J = 7.5 Hz), 7.64 (t, 1H, J = 8.0 Hz), 7.66 (d, 1H, H_4, J = 8.8 Hz), 7.75 (s, 1H, H-9′), 7.82 (t, 1H, J = 8.0 Hz), 8.05 (d, 1H, J = 7.5 Hz), 8.18 (d, 1H, J = 7.5 Hz), 9.33 (s, 1H, $H_{4'}$), 9.87 (s, 1H, $H_{2'}$); ^{13}C NMR ($CDCl_3$): δ 16.23, 48.79, 110.89, 119.45, 121.80, 124.21, 126.72, 127.04, 127.16, 127.42, 127.63, 128.40, 128.69, 128.91, 129.20, 129.35, 131.06, 132.52, 139.16, 139.37, 140.63, 147.76, 153.10, 166.06; *E*-isomer (**25*E***): mp 83.0–83.8 °C; ^1H NMR ($CDCl_3$): δ 1.94 (d, 3H, J = 7.0 Hz), 6.01 (q, 1H, J = 7.0 Hz), 6.58 (d, 1H, J = 7.5 Hz), 6.83 (t, 1H, J = 7.5 Hz), 7.10 (t, 1H, J = 7.5 Hz), 7.31–7.39 (m, 2H), 7.41 (t, 2H, J = 7.5 Hz), 7.46 (d, 2H, J = 7.5 Hz), 7.60 (d, 1H, H_4, J = 8.0 Hz), 7.68 (t, 1H, J = 8.0 Hz), 7.85 (t, 1H, J = 8.0 Hz), 7.92 (d, 1H, J = 8.0 Hz), 8.05 (s, 1H, H-9′), 8.22 (d, 1H, J = 8.0 Hz), 8.47 (s, 1H, $H_{4'}$), 9.25 (s, 1H, $H_{2'}$); ^{13}C NMR ($CDCl_3$): δ 16.35, 49.35, 111.19, 121.32, 121.77, 122.66, 126.71, 127.47, 127.49, 127.52, 128.22, 128.46, 128.72, 129.15, 129.55, 130.08, 130.67, 132.92, 136.36, 139.33, 142.64, 148.06, 150.44, 167.97; HRMS: m/z 376.1552 (calculated for $C_{26}H_{20}N_2O$, 376.1576).

4.4.13. 1-(1-Phenylethyl)-3-(2-quinolinylmethylidenyl) indolin-2-one (26). 1-(1-Phenylethyl)indolin-2-one (**11**), 2-quinolinecarboxaldehyde and piperidine in MeOH were allowed to react in the same manner as described in the preparation of compound **14** and the residue was purified by flash column chromatography (EtOAc/*n*-hexane), recrystallized from EtOAc/*n*-hexane to give **26** in 78% yield as oil; IR (cm^{-1}) 3416, 1703; ^1H NMR ($CDCl_3$): δ 1.92 (d, 3H, J = 7.5 Hz), 6.04 (q, 1H, J = 7.5 Hz), 7.05 (t, 1H, J = 7.5 Hz), 7.16 (t, 1H, J = 7.5 Hz), 7.31 (t, 2H, J = 7.5 Hz), 7.39 (t, 2H, J = 7.5 Hz), 7.46 (d, 2H, J = 8.0 Hz), 7.64 (t, 1H, J = 8.0 Hz), 7.74 (d, 1H, J = 8.0 Hz), 7.83 (t, 1H, J = 8.0 Hz), 7.89 (d, 1H, J = 8.0 Hz), 8.03 (s, 1H), 8.27–8.29 (m, 2H), 9.24 (d, 1H, J = 7.5 Hz); HRMS: m/z 376.1552 (calculated for $C_{26}H_{20}N_2O$, 376.1576).

4.4.14. 1-(1-Naphthylmethyl)-3-(4-pyridinylmethylidenyl) indolin-2-one (27). 1-1-(Naphthalenylmethyl)indolin-2-one (**12**), 4-pyridinecarboxaldehyde and piperidine in MeOH were allowed to react in the same manner as

described in the preparation of compound **14** and the residue was purified by flash column chromatography (EtOAc/*n*-hexane), recrystallized from EtOAc/*n*-hexane to give **27** in 89% yield: mp 102.3–102.7 °C; IR (cm^{-1}) 3417, 1711; ^1H NMR ($CDCl_3$): δ 5.52 (s, 2H), 6.74 (d, 1H, $J = 8.0$ Hz), 6.89 (t, 1H, $J = 7.5$ Hz), 7.17 (t, 1H, $J = 7.5$ Hz), 7.35 (d, 1H, $J = 7.0$ Hz), 7.43 (t, 1H, $J = 7.5$ Hz), 7.50 (d, 1H, $J = 8.0$ Hz), 7.56–7.60 (m, 3H), 7.65 (t, 1H, $J = 7.5$ Hz), 7.84 (d, 1H, $J = 8.0$ Hz), 7.87 (s, 1H), 7.94 (d, 1H, $J = 8.0$ Hz), 8.22 (d, 1H, $J = 8.0$ Hz), 8.84 (d, 2H, $J = 8.0$ Hz); HRMS: *m/z* 362.1422 (calculated for $C_{25}H_{18}N_2O$, 362.1419).

4.4.15. 1-(2-Naphthylmethyl)-3-(4-pyridinylmethylidenyl) indolin-2-one (28). 1-2-(Naphthalenylmethyl)indolin-2-one (**13**), 4-pyridinecarboxaldehyde and piperidine in MeOH were allowed to react in the same manner as described in the preparation of compound **14** and the residue was purified by flash column chromatography (EtOAc/*n*-hexane), recrystallized from EtOAc/*n*-hexane to give **28** in 80% yield: mp 85.1–85.4 °C; IR (cm^{-1}) 3417, 3059, 3020, 1707; ^1H NMR ($CDCl_3$): δ 5.20 (s, 2H), 6.82 (d, 1H, $J = 8.0$ Hz), 6.88 (t, 1H, $J = 8.0$ Hz), 7.20 (t, 1H, $J = 8.0$ Hz), 7.48–7.56 (m, 6H), 7.82–7.87 (m, 5H), 8.80 (d, 1H); HRMS: *m/z* 362.1417 (calculated for $C_{25}H_{18}N_2O$, 362.1419).

5. Bioassay

5.1. Materials and methods

5.1.1. Cell culture and treatment. The human leukemia cells HL-60 and normal leukocytes were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (GIBCO/BRL), penicillin (100 U/mL)/streptomycin (100 μg/mL) (GIBCO/BRL) and 1% L-glutamine (GIBCO/BRL) at 37 °C in a humidified atmosphere containing 5% CO_2. Logarithmically growing tumor cells were used for all experiments.

5.2. Cell differentiation assay

Cell differentiation effect was assessed using nitroblue tetrazoliun reduction (NBT) assay.[11] HL-60 cells (4×10^4 cells/mL) were cultured with various concentrations of **14**–**28** for 72 h. After treatment, the cells were collected, washed with cold PBS, and then incubated in 40 μL NBT solution containing 1 mg/mL NBT and 1 mg/mL phorbol phosphate 13-acetate (PMA) and incubated at 37 °C for 30 min. Then, cells were pelleted and a hematocytometer was used to determine the population of NBT-positive cells among a total number of at least 200 cells counted.

5.3. Cytotoxicity assay

The cytotoxicity was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide (MTT) assay.[12] HL-60 cells (4×10^4 cells/mL) were treated with **14**–**28**, and human normal leukocytes (2.5×10^5 cells/mL) were treated with **25** for 72 h. After treatment, the cells were collected, washed with cold phosphate buffered saline (PBS), and then 10 μL of MTT solution (5 mg/mL) with 50 μL of cells suspension in HBSS was added into each well of a 96-well plate and incubated at 37 °C in the dark for 2 h. Treatment of living cells with MTT produces a dark blue formazan product, whereas no such staining is observed in dead cells. The formazan product was dissolved by adding 140 μL DMSO and then the absorbance of the mixture was measured on an ELISA reader at a best wavelength of 570 nm.

5.4. Cell cycle distribution analysis

Cell cycle analysis by flow cytometry was performed as described in the previous paper.[13] HL-60 cells (4×10^4 cells/mL) were cultured with 2.5 μM compound **25** alone or in combination with 5 nM ATRA for 72 h. After treatment, the cells were collected, washed with cold PBS, and fixed with 70% ice-cold ethanol at −20 °C overnight. Then the cells were centrifuged and resuspended in staining solution, containing 1% Triton X-100 (Sigma), 0.1 mg/mL RNase (Sigma) and 4 μg/mL phopidium iodide (Sigma), for 30 min at 37 °C in the dark and then analyzed on a fluorescence-activated cell sorter flow cytometry (FACS-caliber, Becton Dickinson, San Jose, CA, USA) and all the resulted histograms were analyzed by ModFit Software.

5.5. Statistic evaluation

Values were expressed as means ± SD of three independent experiments. Student's *t* tests were used to assess the statistical significance of the differences, with '*P*' values of less than 0.05 being considered statistically significant.

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