

Enhancement of Chemically-Induced HL-60 Cell Differentiation by 3,3'-Diindolylmethane Derivatives

Tomomi NOGUCHI-YACHIDE,* Masashi TETSUHASHI, Hiroshi AOYAMA, and Yuichi HASHIMOTO

Institute of Molecular & Cellular Biosciences, The University of Tokyo; 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan.

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3,3'-Diindolylmethane (DIM, 1) and its derivatives have been prepared, and their enhancing effects on chemically-induced HL-60 cell differentiation were analyzed. Among the prepared compounds, IndDIM (12) showed the most potent enhancing effect on HL-60 cell differentiation induced by chemicals, including retinoids, 1,25-dihydroxyvitamin D₃, 12-*O*-tetradecanoyl phorbol-13-acetate and dimethyl sulfoxide.

Key words differentiation; diindolylmethane; tamibarotene

3,3'-Diindolylmethane (DIM, 1) is a major metabolite (spontaneously formed product in intragastric acid condition) of indole-3-carbinol (I3C, 2) which is a phytochemical expressed in brassica vegetables, including broccoli, brussels sprouts, kales and the cabbages, and has been thought to be associated with the anticancer/cancer chemopreventive activities of vegetable consumption.¹⁾ DIM (1) controls proliferation of various tumor cells, including cells of breast cancer, prostate cancer, ovary cancer, lung cancer and pancreatic cancer, by inducing G₁/S arrest of the cell cycle and apoptosis.²⁾ In addition, DIM (1) modulates metabolism of estrogen and testosterone,^{3,4)} which act as indigenous tumor promoters for several hormone-dependent cancers. DIM (1) is also reported to be a weak agonist for the arylhydrocarbon receptor (AhR) and blocks the effects of estrogens *via* inhibition of AhR-estrogen receptor cross talk.⁵⁾ In addition, DIM (1) possesses other various activities, including immunomodulatory effect.⁶⁾ All the experimental studies suggest that DIM (1) has incredible potential both for prevention and for treatment of cancer.

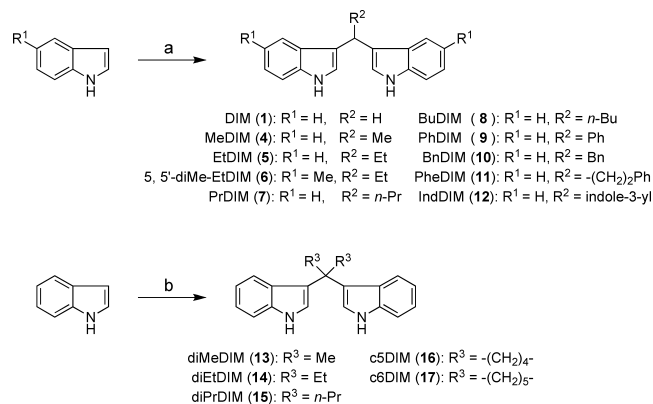
Although the molecular basis of cancer chemopreventive and cancer chemotherapeutic effects of DIM (1) is unclear, effects of DIM (1) on cell differentiation would be one of candidate mechanisms underlying the biological activities elicited by DIM (1). In fact, Kim *et al.* recently reported that indirubin, another AhR agonist with a structure related to that of DIM (1), and its derivatives show enhancing effect on 1,25-dihydroxyvitamin D₃ (1,25-VD₃)- and all-*trans* retinoic acid (ATRA)-induced cell differentiation of HL-60 leukemia cells.⁷⁾

Under such circumstances, we investigated the enhancing effect on chemically-induced HL-60 cell differentiation of DIM (1) and its derivatives in combination with various cell differentiation inducers, including ATRA, tamibarotene (Am80, 3), 1,25-VD₃, 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) and dimethyl sulfoxide (DMSO). All of these chemicals are known to induce terminal differentiation in leukemic cell lines, including HL-60 and U937 cells. ATRA and Am80 are therapeutic agents for acute promyelocytic leukemia

(APL) and induce differentiation of HL-60 cells to mature granulocytes.⁸⁾ On the other hand, 1,25-VD₃ and TPA have been established to induce differentiation of HL-60 cells to mature monocytes.⁹⁾ DMSO is another typical cell differentiation inducer.¹⁰⁾

Results and Discussion

Chemistry DIM (1), its derivatives (4–17), and isomers of DIM [2,2'-DIM (22) and 2,3'-DIM (25)] were synthesized using the methods reported by Nagarajan and Perumal¹¹⁾ and



Reagents and conditions: (a) R²CHO, KHSO₄, MeOH; (b) R³COR³, KHSO₄, MeOH.

Chart 1. Synthesis of DIM (1) and Its Derivatives (4–17)

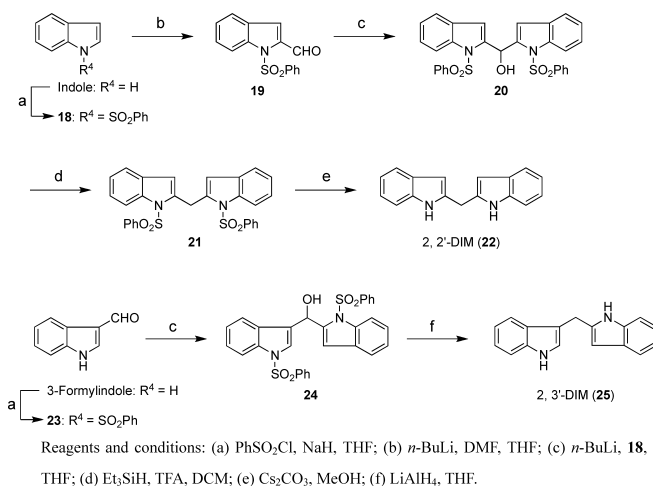


Chart 2. Synthesis of 2,2'-DIM (22) and 2,3'-DIM (25)

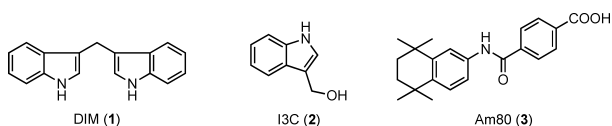


Fig. 1. Structures of DIM (1), I3C (2) and Am80 (3)

* To whom correspondence should be addressed. e-mail: noguchi@iam.u-tokyo.ac.jp

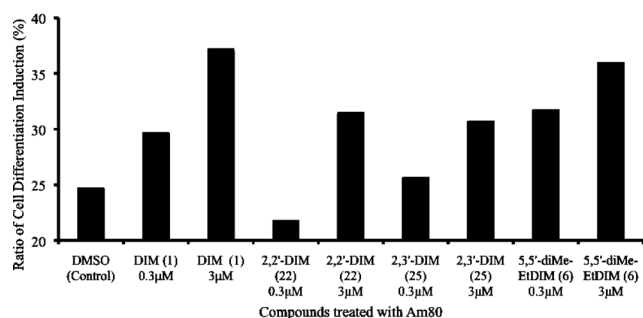


Fig. 2. Effects of DIM (1), Its Positional Isomers (22, 25) and Its Derivative (6) on 2 nM Am80 (3)-Induced HL-60 Cell Differentiation

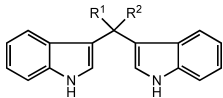
All the compounds were dissolved in DMSO, and the concentration of DMSO was adjusted to be 0.5% (v/v) in all cases.

usual organic synthetic methods as shown in Charts 1 and 2. Briefly, DIM (1) and its derivatives (4–17) were obtained by the reaction of indole or methylindole with various aldehydes or ketones (Chart 1). Bis(1-benzenesulfonyl-1*H*-indol-2-yl)methanol (20) and (1-benzenesulfonyl-1*H*-indol-2-yl)(1-benzenesulfonyl-1*H*-indol-3-yl)methanol (24) was obtained by the reaction of 2- (19) or 3-formyl-1-benzenesulfonyl-1*H*-indole (23) with 1-benzenesulfonyl-1*H*-indole (18), followed by reduction and desulfonylation to give 2,2'-DIM (22) or 2,3'-DIM (25) (Chart 2).

Effects on HL-60 Cell Differentiation At first, we investigated the enhancing effect on Am80-induced HL-60 cell differentiation of DIM (1), its derivative 6 and positional isomers of DIM (22, 25). HL-60 cell differentiation was assayed by the use of nitroblue tetrazolium (NBT) reduction assay, as described previously.¹²⁾ Of course, the population of NBT positive cells (%) deviates from experiments and experiments. However, the deviation of the value in one experiment (performed in duplicate or triplicate) is less than 5%, and the order of the activity of the test compounds was reproducible (repeated at least three times). The typical set of data obtained from one experiment performed in side-by-side was presented in Fig. 2. As expected, DIM (1) was revealed to show enhancing effect on 2 nM Am80 (3)-induced HL-60 cell differentiation (Fig. 2). Under our experimental conditions, the population of NBT-positive cells in untreated HL-60 cells is generally less than 5% (data not shown). Treatment of the cells with 2 nM Am80 (3) caused increase of the NBT-positive cells. Although the %-values of NBT-positive cell population of HL-60 cells treated with 2 nM Am80 (3) deviate among experiment to experiment, the %-values are generally between 15 to 30%. In Fig. 2, a typical set of data performed in one side-by-side experiment is shown. In this case, 2 nM Am80 (3)-treatment caused increase of the NBT-positive cells to approximately 25% (Fig. 2). But co-treatment of the cells with 0.3 μM and 3 μM DIM (1) caused drastic increase of the NBT-positive cells to approximately 30% and 37%, respectively. Its isomers, 2,2'-DIM (22) and 2,3'-DIM (25) also showed the enhancing effect, but were less potent than DIM (1). 5,5'-DiMe-EtDIM (6) showed the enhancing activity which is compatible to that of DIM (1).

Based on the results, we analyzed the effects of an alkyl/aryl substituent(s) introduced into the methylene carbon of DIM on the Am80 (3)-induced cell differentiation-enhancing effect. Also, the data obtained by one-side-by-side

Table 1. Effect of 3 μM DIM Derivatives (4–17) on 2 nM Am80 (3)-Induced HL-60 Cell Differentiation

Compound treated with Am80			Relative population of NBT-positive cells ^{a)}
	R ¹	R ²	
None	—	—	100
MeDIM (4)	Me	H	103
EtDIM (5)	Et	H	175
PrDIM (7)	<i>n</i> -Pr	H	164
BuDIM (8)	<i>n</i> -Bu	H	146
PhDIM (9)	Ph	H	136
BnDIM (10)	Bn	H	185
PheDIM (11)	-(CH ₂) ₂ Ph	H	176
IndDIM (12)	Indol-3-yl	H	251
diMeDIM (13)	Me	Me	168
diEtDIM (14)	Et	Et	134
diPrDIM (15)	<i>n</i> -Pr	Pr	128
c5DIM (16)	-(CH ₂) ₄ -		188
c6DIM (17)	-(CH ₂) ₅ -		140

a) The population of NBT-positive cells treated with 2 nM Am80 alone was defined as 100%. Test compounds were added at the concentration of 3 μM.

experiment was shown in Table 1. As shown in Table 1, the enhancing effect of DIM derivatives 4–8 [MeDIM (4), EtDIM (5), PrDIM (7), BuDIM (8)] seems to depend on the methylene-length of the introduced alkyl group. Although a methyl substitution [MeDIM (4)] caused almost no effect on the activity (103%), introduction of an alkyl group with a chain length longer than a methyl group, *i.e.*, EtDIM (5), PrDIM (7), and BuDIM (8), apparently caused increase of the activity (146–175%). Among the compounds 4–8, EtDIM (5) seemed to be the most potent in the enhancing effect (175%) on Am80 (3)-induced HL-60 cell differentiation.

Dialkyl substitution at the methylene carbon of DIM [diMeDIM (13), diEtDIM (14), diPrDIM (15)] also increased the activity, with the dimethyl derivative, diMeDIM (13), seemed to be the most potent (168%). The cycloalkyl derivatives, c5DIM (16) and c6DIM (17), also showed rather potent enhancing activity, with the former (16) possessing the higher activity (188%) than the latter (17: 140%). These results suggest that appropriate size of hydrophobic group is critical for the enhancing effect on Am80 (3)-induced HL-60 cell differentiation.

The effect of a substituent with an aromatic group [PhDIM (9), BnDIM (10), PheDIM (11), IndDIM (12)] was also investigated. All of aromatic group-bearing DIM derivatives (9–12) showed potent enhancing effect (136–251%) on Am80 (3)-induced HL-60 cell differentiation induction. The order of the activity of the compounds bearing a phenyl group was: PhDIM (9) < PheDIM (11) < BnDIM (10). Among the compounds we prepared, IndDIM (12) showed the most potent enhancing effect on Am80 (3)-induced HL-60 cell differentiation (251% at 3 μM and 186% at 0.3 μM).

To examine whether the HL-60 cell differentiation-enhancing activity of DIM derivatives is specific to the cell differentiation induction by Am80 (3), the effects of typical DIM derivatives [EtDIM (5), BnDIM (10), PheDIM (11), IndDIM (12) and c5DIM (16), all of which showed rather po-

Table 2. Cell Differentiation-Enhancing Effect of DIM Derivatives

Compound	Ratio of cell differentiation induction (%) at 0.3 μ M			
	2 nM ATRA	4 nM 1,25-VD ₃	500 pM TPA	DMSO
None	31.3	28.7	12.7	7.4
EtDIM (5)	40.7	31.0	10.7	8.1
BnDIM (10)	40.2	35.7	13.7	15.4
PheDIM (11)	44.1	22.4	16.8	16.5
IndDIM (12)	43.7	46.0	14.3	20.5
c5DIM (16)	34.7	28.2	12.3	16.2

tent Am80 (3)-induced HL-60 cell differentiation-enhancing activity] were investigated in combination with other typical cell differentiation inducers, including ATRA, 1,25-VD₃, TPA and DMSO.

As shown in Table 2, all of the compounds examined showed enhancing effect on ATRA-induced HL-60 cell differentiation. Although all the test compounds did not possess HL-60 cell differentiation-inducing activity by themselves, coexistence of the physiological level concentration (2 nM) of ATRA caused cell differentiation, suggesting that these compounds are able to act as cell differentiation inducers in our body/under the physiological conditions where 1–3 nM ATRA exists. Especially, PheDIM (11) and IndDIM (12) showed potent enhancing effect on ATRA-induced HL-60 cell differentiation.

Concerning other cell differentiation inducers, there seems to be a tendency that all of the examined compounds enhance the cell differentiation, though the structure–activity relationships are different among the employed inducers. IndDIM (12) showed the most potent enhancing effect on 4 nM 1,25-VD₃-induced HL-60 cell differentiation, but it seems to be not so effective toward TPA-induced HL-60 cell differentiation. PheDIM (11) and c5DIM (16) seem to be negative toward 1,25-VD₃-induced HL-60 cell differentiation, though they are effective toward ATRA-induced cell differentiation. On the other hand, PheDIM (11) showed the most potent enhancing effect on 500 pM TPA-induced HL-60 cell differentiation. BnDIM (10) and IndDIM (12) showed weak activity, and EtDIM (5) and c5DIM (16) did not show the activity toward TPA-induced HL-60 cell differentiation. Concerning DMSO-induced HL-60 cell differentiation, IndDIM (12) showed the most potent enhancing activity. BnDIM (10), PheDIM (11) and c5DIM (16) showed moderate activity, and EtDIM (5) showed weak activity.

Although we could not extract clear structure–activity relationships in each chemically induced HL-60 differentiation system, our results suggest that the HL-60 cell differentiation-inducing systems employed seem to be classified into two groups. One group is cell differentiation system induced by ATRA or DMSO, both of which have been known as granulocytic differentiation inducers for HL-60 cells, in which almost all the examined compounds showed cell differentiation-enhancing activity. Another group is cell differentiation system induced by 1,25-VD₃ or TPA, both of which have been known as monocytic differentiation inducers, in which each examined compounds showed different effects from those observed in ATRA/DMSO-induced granulocytic differentiation system. This difference of structure–activity relationships between above-mentioned two groups might be

interpreted by the difference of cell differentiation direction induced by the chemicals, *i.e.*, granulocytic or monocytic. Interestingly, AhR has been reported to be expressed in the monocyte, whereas it is not expressed in the granulocyte.¹³⁾ Moreover, the expression of AhR in HL-60 cells was potentially-induced by TPA, moderately-induced by 1,25-VD₃, and not induced by ATRA. In addition, 1,25-VD₃-induced AhR expression was strongly enhanced by transforming growth factor (TGF)- β 1. Because our synthesized DIM derivatives possess AhR-agonistic feature (details will be published elsewhere), induction and/or function of AhR might underlie the above-mentioned difference, at least in part. Concerning the effect of AhR agonists on cell differentiation, it has been reported that AhR agonists inhibit differentiation of monocyte into macrophage.¹⁴⁾ Therefore, different effects of our synthesized DIM derivatives on HL-60 cell differentiation among those induced by Am80/ATRA/DMSO, 1,25-VD₃ and TPA might be attributed to the difference of AhR agonistic feature of our DIM derivatives and AhR induction manner of the employed chemical cell differentiation inducers.

In conclusion, we synthesized DIM and its derivatives. Almost all of these compounds enhance chemically induced HL-60 cell differentiation induction. IndDIM (12) seemed to be the most potent enhancer in our derivatives and enhances HL-60 cell differentiation induced by Am80 (3), ATRA, 1,25-VD₃ and DMSO. c5DIM (16) might be a selective enhancer of granulocytic HL-60 cell differentiation, because it was potent in differentiation-inducing system using Am80 (3), ATRA and DMSO, while it did not enhance HL-60 cell differentiation induced by 1,25-VD₃ and TPA which cause monocytic differentiation.

Experimental

Chemicals. General ¹H-NMR spectra were recorded on a JEOL JNM-GX500 (500 MHz) spectrometer. Chemical shifts are expressed in δ (ppm) values with tetramethylsilane (TMS) as an internal reference. High-resolution mass spectra (HR-MS) and mass spectra (MS) were recorded on a JEOL JMS-DX303 spectrometer with *m*-nitrobenzyl alcohol. Flash column chromatography was performed on silica gel 60 (Kanto Kagaku, 40–100 μ m).

3,3'-Diindolylmethane (DIM: 1)^{11,15)}: General Procedure for the Synthesis of Compounds 1, 4–17 To a stirred solution of indole (351 mg, 3.00 mmol) in a mixture of MeOH (10 ml) and H₂O (0.5 ml) were added paraformaldehyde (90% purity, 45.4 mg, 1.36 mmol) and KHSO₄ (204 mg, 1.50 mmol) at the room temperature, and the reaction mixture was stirred for 40 h at the same temperature. The volatile solvents were removed under reduced pressure. The resulting residue was added to H₂O, and then extracted with CH₂Cl₂. The organic layer was dried over MgSO₄, and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (*n*-hexane/AcOEt=4/1) to give 1 (201 mg, 818 μ mol, 60%) as colorless crystal; mp 168–170 °C (lit.¹⁵⁾ 164 °C). ¹H-NMR (CDCl₃) δ : 4.23 (2H, s), 6.929 (1H, s), 6.931 (1H, s), 7.08 (2H, dd, *J*=6.7, 8.0 Hz), 7.17 (2H, dd, *J*=6.7, 8.0 Hz), 7.34 (2H, d, *J*=8.0 Hz), 7.61 (2H, d, *J*=8.0 Hz), 7.89 (2H, brs). FAB-MS *m/z*: 246.1143 (Calcd for C₁₇H₁₄N₂; 246.1157).

1,1-(3,3'-Diindolyl)ethane (4)^{11,15)} Yield 24%, white solid; mp 165–166 °C (lit.¹⁵⁾ 156 °C). ¹H-NMR (CDCl₃) δ : 1.81 (3H, d, *J*=7.3 Hz), 4.69 (1H, q, *J*=7.3 Hz), 6.94 (2H, d, *J*=1.8 Hz), 7.04 (2H, dd, *J*=7.3, 7.9 Hz), 7.16 (2H, dd, *J*=7.3, 7.9 Hz), 7.36 (2H, d, *J*=7.9 Hz), 7.58 (2H, d, *J*=7.9 Hz), 7.89 (2H, brs). FAB-MS *m/z*: 260.1343 (Calcd for C₁₈H₁₆N₂; 260.1313).

1,1-(3,3'-Diindolyl)propane (5)¹⁶⁾ Yield 16%, white solid; mp 128–130 °C. ¹H-NMR (CDCl₃) δ : 1.02 (3H, t, *J*=7.3 Hz), 2.26 (2H, qd, *J*=7.3, 7.3 Hz), 4.39 (1H, t, *J*=7.3 Hz), 7.05–7.01 (4H, m), 7.15 (2H, dd, *J*=7.9, 8.5 Hz), 7.34 (2H, d, *J*=7.9 Hz), 7.59 (2H, d, *J*=7.9 Hz), 7.90 (2H brs). EI-MS *m/z* 274.1472 (Calcd for C₁₉H₁₈N₂; 274.1470).

1-(5-methylindol-3-yl)-1-(5'-methylindol-3'-yl)propane (6) Yield

12%, colorless crystal; mp 132–133 °C. $^1\text{H-NMR}$ (CDCl_3) δ : 1.01 (3H, t, $J=7.3$ Hz), 2.23 (2H, qd, $J=7.3$, 7.3 Hz), 2.41 (6H, s), 4.33 (2H, t, $J=7.3$ Hz), 6.95 (2H, d, $J=1.8$ Hz), 6.98 (2H, dd, $J=7.9$, 1.8 Hz), 7.23 (2H, d, $J=7.9$ Hz), 7.40 (2H, s), 7.80 (2H, brs). FAB-MS m/z : 302.1757 (Calcd for $\text{C}_{21}\text{H}_{22}\text{N}_2$: 302.1783).

1,1-(3,3'-Diindolyl)butane (7)^{11,16} Yield 40%, pale yellow solid; mp 154–156 °C. $^1\text{H-NMR}$ (CDCl_3) δ : 0.96 (3H, t, $J=7.3$ Hz), 1.44 (2H, qt, $J=7.3$, 7.3 Hz), 2.21 (2H, td, $J=7.3$, 7.3 Hz), 4.50 (1H, t, $J=7.3$ Hz), 7.01 (2H, d, $J=1.2$ Hz), 7.04 (2H, dd, $J=7.9$, 7.9 Hz), 7.15 (2H, dd, $J=7.9$, 7.9 Hz), 7.33 (2H, d, $J=7.9$ Hz), 7.60 (2H, d, $J=7.9$ Hz), 7.89 (2H, brs). FAB-MS m/z : 288.1636 (Calcd for $\text{C}_{20}\text{H}_{20}\text{N}_2$: 288.1626).

1,1-(3,3'-Diindolyl)pentane (8)¹⁷ Yield 26%, white solid; mp 97–99 °C (lit.¹⁷ 80–82 °C). $^1\text{H-NMR}$ (CDCl_3) δ : 0.87 (3H, t, $J=6.7$ Hz), 1.42–1.36 (4H, m), 2.22 (2H, td, $J=7.3$, 7.3 Hz), 4.48 (1H, t, $J=7.3$ Hz), 7.05–7.01 (4H, m), 7.15 (2H, dd, $J=7.9$, 8.5 Hz), 7.34 (2H, d, $J=7.9$ Hz), 7.60 (2H, d, $J=7.9$ Hz), 7.90 (2H, brs). FAB-MS m/z : 302.1803 (Calcd for $\text{C}_{21}\text{H}_{22}\text{N}_2$: 302.1783).

3,3'-Diindolyl(phenyl)methane (9)¹¹ Yield 98%, pink foam; $^1\text{H-NMR}$ (CDCl_3) δ : 5.89 (1H, s), 6.67 (2H, d, $J=1.2$ Hz), 7.00 (2H, t, $J=7.3$ Hz), 7.17 (2H, t, $J=8.5$ Hz), 7.21 (1H, t, $J=7.3$ Hz), 7.28 (2H, t, $J=7.3$ Hz), 7.34–7.37 (4H, m), 7.39 (2H, d, $J=8.5$ Hz), 7.91 (2H, brs). FAB-MS m/z : 322.1455 (Calcd for $\text{C}_{23}\text{H}_{18}\text{N}_2$: 322.1470).

1,1-(3,3'-Diindolyl)-2-phenylethane (10) Yield 55%, white foam; $^1\text{H-NMR}$ (CDCl_3) δ : 3.55 (2H, t, $J=7.9$ Hz), 4.80 (1H, d, $J=7.9$ Hz), 6.95 (2H, d, $J=1.8$ Hz), 7.02 (2H, dd, $J=7.9$, 7.9 Hz), 7.09–7.17 (7H, m), 7.33 (2H, d, $J=7.9$ Hz), 7.57 (2H, d, $J=7.9$ Hz), 7.87 (2H, brs). FAB-MS m/z : 335.1563 (Calcd for $\text{C}_{24}\text{H}_{19}\text{N}_2$: 335.1548).

1,1-(3,3'-Diindolyl)-3-phenylpropane (11)¹⁸ Yield 27%, white solid; mp 158–159 °C. $^1\text{H-NMR}$ (CDCl_3) δ : 2.54–2.59 (2H, m), 2.73 (2H, t, $J=8.5$ Hz), 4.52 (1H, t, $J=7.3$ Hz), 7.01–7.05 (4H, m), 7.14–7.19 (5H, m), 7.27 (2H, dd, $J=7.9$, 7.9 Hz), 7.35 (2H, d, $J=8.5$ Hz), 7.55 (2H, d, $J=7.9$ Hz), 7.92 (2H, brs). FAB-MS m/z : 350.1783 (Calcd for $\text{C}_{25}\text{H}_{22}\text{N}_2$: 350.1783).

3,3',3''-Triindolylmethane (12)^{18,19} Yield 69%, beige solid; mp 251–253 °C (lit.¹⁹ 245–247 °C). $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 6.03 (1H, s), 6.83 (3H, dd, $J=7.9$, 7.9 Hz), 6.91 (3H, d, $J=2.4$ Hz), 6.99 (3H, dd, $J=7.9$, 7.9 Hz), 7.31 (3H, d, $J=7.9$ Hz), 7.37 (3H, d, $J=7.9$ Hz), 10.69 (3H, brs). FAB-MS m/z : 361.1546 (Calcd for $\text{C}_{25}\text{H}_{19}\text{N}_3$: 361.1579).

2,2-(3,3'-Diindolyl)propane (13)¹⁶ Yield 47%, pale yellow foam; $^1\text{H-NMR}$ (CDCl_3) δ : 1.90 (6H, s), 6.87 (2H, dd, $J=7.5$, 7.5 Hz), 7.05 (2H, d, $J=2.0$ Hz), 7.06 (2H, dd, $J=7.5$, 7.5 Hz), 7.30 (2H, d, $J=8.5$ Hz), 7.40 (2H, d, $J=8.5$ Hz), 7.89 (2H, brs). FAB-MS m/z : 274.1476 (Calcd for $\text{C}_{19}\text{H}_{18}\text{N}_2$: 274.1470).

3,3-(3,3'-Diindolyl)pentane (14)²⁰ Yield 91%, white foam; $^1\text{H-NMR}$ (CDCl_3) δ : 0.66 (6H, t, $J=7.5$ Hz), 2.29 (4H, q, $J=7.5$ Hz), 6.73 (2H, dd, $J=7.5$, 7.5 Hz), 6.99 (2H, dd, $J=7.5$, 7.5 Hz), 7.175 (2H, s), 7.180 (2H, d, $J=8.5$ Hz), 7.27 (2H, d, $J=8.5$ Hz), 7.95 (2H, brs). FAB-MS m/z : 302.1831 (Calcd for $\text{C}_{21}\text{H}_{22}\text{N}_2$: 302.1783).

4,4-(3,3'-Diindolyl)heptane (15) Yield 17%, white solid; mp 158–160 °C. $^1\text{H-NMR}$ (CDCl_3) δ : 0.82 (6H, t, $J=7.3$ Hz), 1.05–1.13 (4H, m), 2.24–2.27 (4H, m), 6.75 (2H, dd, $J=7.9$, 7.3 Hz), 7.01 (2H, dd, $J=7.9$, 7.3 Hz), 7.16 (2H, d, $J=2.4$ Hz), 7.22 (2H, d, $J=7.9$ Hz), 7.28 (2H, d, $J=7.9$ Hz), 7.94 (2H, brs). FAB-MS m/z : 330.2137 (Calcd for $\text{C}_{23}\text{H}_{26}\text{N}_2$: 330.2096).

1,1-(3,3'-Diindolyl)cyclopentane (16)²¹ Yield 24%, white solid; mp 167–169 °C. $^1\text{H-NMR}$ (CDCl_3) δ : 1.82–1.85 (4H, m), 2.50–2.53 (4H, m), 6.90 (2H, dd, $J=7.3$, 7.9 Hz), 7.07 (2H, dd, $J=7.3$, 7.9 Hz), 7.10 (2H, d, $J=2.4$ Hz), 7.30 (2H, d, $J=7.9$ Hz), 7.51 (2H, d, $J=7.9$ Hz), 7.88 (2H, brs). FAB-MS m/z : 300.1621 (Calcd for $\text{C}_{21}\text{H}_{20}\text{N}_2$: 300.1626).

1,1-(3,3'-Diindolyl)cyclohexane (17)²¹ Yield 74%, white foam; $^1\text{H-NMR}$ (CDCl_3) δ : 1.57 (2H, quintet, $J=6.0$ Hz), 1.64 (4H, td, $J=5.5$, 6.0 Hz), 2.53 (4H, dd, $J=5.5$, 6.0 Hz), 6.87 (2H, dd, $J=7.5$, 8.0 Hz), 7.04 (2H, dd, $J=7.5$, 7.5 Hz), 7.09 (1H, s), 7.10 (1H, s), 7.28 (2H, d, $J=8.0$ Hz), 7.54 (2H, d, $J=7.5$ Hz), 7.90 (2H, brs). FAB-MS m/z : 314.1809 (Calcd for $\text{C}_{22}\text{H}_{22}\text{N}_2$: 314.1783).

1-Benzenesulfonyl-1H-indole (18)²² To a stirred solution of indole (3.50 g, 29.9 mmol) in dry THF (100 ml) was added NaH (55% in oil suspension, 1.44 g, 33.0 mmol) at 0 °C. After stirring at 0 °C for 30 min, benzenesulfonyl chloride (4.2 ml, 33 mmol) was added slowly and the reaction mixture was stirred for 18 h at room temperature. The reaction was quenched by adding water, and then extracted with AcOEt. The organic layer was washed with brine, dried over MgSO_4 , and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (*n*-hexane/AcOEt=9/1) to give **18** (6.80 g, 26.4 mmol, 88%) as a white solid:

$^1\text{H-NMR}$ (CDCl_3) δ : 6.67 (1H, d, $J=3.7$ Hz), 7.23 (1H, dd, $J=7.3$, 7.3 Hz), 7.31 (1H, dd, $J=7.3$, 7.3 Hz), 7.43 (2H, t, $J=7.6$ Hz), 7.51–7.54 (2H, m), 7.57 (1H, d, $J=3.7$ Hz), 7.87–7.89 (2H, m), 8.00 (1H, d, $J=8.5$ Hz). FAB-MS m/z : 257 ($\text{M}+\text{H}^+$).

2-Formyl-1-benzenesulfonyl-1H-indole (19)²³ To a stirred solution of **18** (1.50 g, 5.83 mmol) in dry THF (40 ml) was added *n*-BuLi (1.55 M in hexane, 4.52 ml, 7.00 mmol) at –78 °C. After stirring for 30 min at –78 °C and then for 1 h at room temperature, the solution was cooled again to –78 °C and DMF (1.00 ml, 12.9 mmol) was added dropwise, and the reaction mixture was stirred for 4 h at room temperature. The reaction was quenched by adding saturated aqueous NH_4Cl , and then extracted with AcOEt. The organic layer was washed with brine, dried over MgSO_4 , and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (*n*-hexane/AcOEt=10/1) to give **19** (1.07 g, 3.75 mmol, 64%) as a white solid: $^1\text{H-NMR}$ (CDCl_3) δ : 7.32 (1H, dd, $J=7.3$, 7.9 Hz), 7.41 (2H, dd, $J=7.3$, 7.3 Hz), 7.48 (1H, s), 7.52–7.56 (2H, m), 7.63 (1H, d, $J=7.9$ Hz), 7.78 (2H, dd, $J=8.5$, 1.2 Hz), 8.24 (1H, d, $J=8.5$ Hz), 10.53 (1H, s). FAB-MS m/z : 286 (M^+).

Bis(1-benzenesulfonyl-1H-indol-2-yl)methanol (20)²⁴ To a stirred solution of **18** (613 mg, 2.38 mmol) in dry THF (15 ml) was added *n*-BuLi (1.66 M in hexane, 1.58 ml, 2.62 mmol) at –78 °C. After stirring for 1 h at room temperature, the solution was cooled again to –78 °C and **19** (680 mg, 2.38 mmol) in dry THF (5 ml) was added dropwise, and then the reaction mixture was stirred for 18 h at room temperature. The reaction was quenched by adding 1 N HCl, and then extracted with AcOEt. The organic layer was washed with brine, dried over MgSO_4 , and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (*n*-hexane/AcOEt=4/1) to give **20** (965 mg, 1.78 mmol, 75%) as a yellow foam: $^1\text{H-NMR}$ (CDCl_3) δ : 3.97 (1H, d, $J=4.9$ Hz), 6.54 (2H, s), 7.21–7.24 (3H, m), 7.30–7.37 (6H, m), 7.40 (2H, d, $J=7.9$ Hz), 7.47 (2H, dd, $J=7.3$, 7.9 Hz), 7.84 (4H, d, $J=7.9$ Hz), 8.10 (2H, d, $J=8.5$ Hz). FAB-MS m/z : 542 (M^+).

Bis(1-benzenesulfonyl-1H-indol-2-yl)methane (21)²⁴ To a solution of **20** (303 mg, 0.558 mmol) in dry dichloromethane (DCM) (5.0 ml) was added Et_3SiH (2.00 ml, 12.5 mmol) followed by TFA (0.1 ml), and the reaction mixture was stirred for 18 h. The reaction was quenched by adding water, and then extracted with DCM. The organic layer was washed with brine, dried over MgSO_4 , and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (*n*-hexane/AcOEt=4/1) to give **21** (230 mg, 0.436 mmol, 78%) as a white foam: $^1\text{H-NMR}$ (CDCl_3) δ : 4.85 (2H, s), 6.22 (2H, s), 7.20 (2H, dd, $J=7.3$, 7.3 Hz), 7.29 (2H, dd, $J=7.9$, 8.5 Hz), 7.32–7.37 (6H, m), 7.47 (2H, dd, $J=7.3$, 7.3 Hz), 7.77 (4H, d, $J=8.5$ Hz), 8.16 (2H, d, $J=7.9$ Hz). FAB-MS m/z : 527 ($\text{M}+\text{H}^+$).

2,2'-Diindolylmethane (22)²⁴ To a stirred solution of **21** (60.0 mg, 0.114 mmol) in MeOH (3.0 ml) was added Cs_2CO_3 (190 mg, 0.570 mmol), and the reaction mixture was refluxed for 15 h. The reaction was quenched by adding water, and then extracted with AcOEt. The organic layer was washed with brine, dried over MgSO_4 , and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (*n*-hexane/AcOEt=6/1) and recrystallized from *n*-hexane/AcOEt to give **22** (6.7 mg, 27 mmol, 24%) as a colorless crystal: mp 168–169 °C (lit.²⁴ 168 °C). $^1\text{H-NMR}$ (CDCl_3) δ : 4.32 (2H, s), 6.45 (2H, s), 7.09 (2H, dd, $J=7.6$, 8.5 Hz), 7.14 (2H, dd, $J=7.6$, 7.9 Hz), 7.25 (2H, d, $J=8.5$ Hz), 7.57 (2H, d, $J=7.9$ Hz), 7.90 (2H, brs). FAB-MS m/z : 246.1203 (Calcd for $\text{C}_{17}\text{H}_{14}\text{N}_2$: 246.1157).

3-Formyl-1-benzenesulfonyl-1H-indole (23)²⁵ To a stirred solution of 3-formylindole (435 mg, 3.00 mmol) in dry THF (15 ml) was added NaH (55% in oil suspension, 160 mg, 3.67 mmol) at 0 °C. After stirring at 0 °C for 30 min, benzenesulfonyl chloride (0.430 ml, 3.36 mmol) was added slowly. The reaction mixture was stirred for 22 h at room temperature. The reaction was quenched by adding water, and then extracted with AcOEt. The organic layer was washed with brine, dried over MgSO_4 , and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (*n*-hexane/AcOEt=6/1 to 4/1) to give **23** (670 g, 2.35 mmol, 78%) as a pale yellow solid: $^1\text{H-NMR}$ (CDCl_3) δ : 7.35 (1H, dd, $J=7.3$, 7.9 Hz), 7.40 (2H, dd, $J=7.3$, 7.3 Hz), 7.50 (2H, t, $J=7.9$ Hz), 7.60 (1H, t, $J=7.3$ Hz), 7.93–7.96 (3H, m), 8.21 (1H, s), 8.24 (1H, d, $J=7.9$ Hz), 10.09 (1H, s). FAB-MS m/z : 286 (M^+).

(1-Benzenesulfonyl-1H-indol-2-yl)(1-benzenesulfonyl-1H-indol-3-yl)methanol (24)²⁶ To a stirred solution of **18** (386 mg, 1.50 mmol) in dry THF (10 ml) was added *n*-BuLi (1.55 M in hexane, 1.20 ml, 1.86 mmol) at –78 °C. After stirring for 1 h at room temperature, the solution was cooled again to –78 °C and **23** (427 mg, 1.50 mmol) in dry THF (5.0 ml) was added dropwise, and then the reaction mixture was stirred for 5 h at room tempera-

ture. The reaction was quenched by adding 1 N HCl, and then extracted with AcOEt. The organic layer was washed with brine, dried over MgSO_4 , and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (*n*-hexane/AcOEt=4/1) to give **24** (224 mg, 0.413 mmol, 28%) as a yellow foam: $^1\text{H-NMR}$ (CDCl_3) δ : 3.69 (1H, d, $J=4.8$ Hz), 6.20 (1H, s), 6.52 (1H, d, $J=4.8$ Hz), 6.92 (1H, d, $J=7.9$ Hz), 7.04 (1H, dd, $J=7.3$, 7.9 Hz), 7.21 (1H, dd, $J=6.7$, 7.9 Hz), 7.28–7.34 (3H, m), 7.41–7.48 (4H, m), 7.55–7.59 (2H, m), 7.72 (1H, s), 7.80 (2H, dd, $J=1.2$, 8.5 Hz), 7.92 (2H, dd, $J=1.2$, 8.5 Hz), 8.01 (1H, d, $J=8.5$ Hz), 8.15 (1H, d, $J=9.1$ Hz). FAB-MS m/z : 542 (M^+).

2,3'-Diindolylmethane (25)²⁷ To a stirred suspension of lithium aluminum hydride (LAH) (500 mg, 13.1 mmol) in dry THF (10 ml) was added **24** (225 mg, 0.415 mmol) slowly at -20°C . After the addition, the suspension was refluxed for 4 h and then cooled to 0°C and carefully quenched by adding saturated aqueous potassium sodium tartrate (50 ml). After stirring for 1 h, the reaction mixture was extracted with AcOEt. The organic layer was washed with brine, dried over MgSO_4 , and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (*n*-hexane/AcOEt=4/1) and recrystallized from *n*-hexane/AcOEt to give **25** (88.0 mg, 0.357 mmol, 86%) as a colorless needle: mp $139\text{--}140^\circ\text{C}$ (lit.²⁷ $141\text{--}143^\circ\text{C}$). $^1\text{H-NMR}$ (CDCl_3) δ : 4.15 (2H, s), 6.13 (1H, d, $J=1.2$ Hz), 6.96–6.86 (3H, m), 7.03 (1H, dd, $J=6.7$, 7.3 Hz), 7.20 (1H, d, $J=2.4$ Hz), 7.23 (1H, d, $J=8.5$ Hz), 7.33 (1H, d, $J=7.9$ Hz), 7.36 (1H, d, $J=7.3$ Hz), 7.46 (1H, d, $J=7.9$ Hz), 10.85 (2H, br s). FAB-MS m/z : 246.1193 (Calcd for $\text{C}_{17}\text{H}_{14}\text{N}_2$: 246.1157).

Assay of Cell Differentiation-Inducing Activity Human leukemia cells, HL-60, were cultured in RPMI1640 medium containing 5% fetal bovine serum at 37°C in a humidified atmosphere of 5% CO_2 in the air. Cell differentiation was performed by the nitro blue tetrazolium (NBT) reduction method. For this assay, HL-60 cells in 1×10^5 cells/ml were incubated in RPMI1640 medium in the presence or absence of test compounds (test compounds were added as a DMSO solution with the final concentration of DMSO in the incubation mixture being adjusted to be 0.5% (v/v)) for 3 d. Treated HL-60 cells were harvested *via* centrifugation and incubated with an equal volume of 0.2% (w/v) NBT dissolved in a phosphate-buffered saline (PBS), containing 20 nM 12-*O*-tetradecanoylphorbol 13-acetate (TPA) at 37°C for 30 min. NBT positivity was measured by counting at least 200 cells and the results were expressed as the percentage of NBT-positive cells.

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References

- Aggarwal B. B., Ichikawa H., *Cell Cycle*, **4**, 1201–1215 (2005).
- Rahman K. W., Li Y., Wang Z., Sarkar S. H., Sarkar F. H., *Cancer Res.*, **66**, 4952–4960 (2006).
- Le H. T., Schaldach C. M., Firestone G. L., Bjeldanes L. F., *J. Biol. Chem.*, **278**, 21136–21145 (2003).
- Jellinck P. H., Forkert P. G., Riddick D. S., Okey A. B., Michnovicz J. J., Bradlow H. L., *Biochem. Pharmacol.*, **45**, 1129–1136 (1993).
- Chen I., McDougal A., Wang F., Safe S., *Carcinogenesis*, **19**, 1631–1639 (1998).
- Xue L., Pestka J. J., Li M., Firestone G. L., Bjeldanes L. F., *J. Nutr. Biochem.*, **19**, 336–344 (2008).
- Kim S. H., Kim S. W., Choi S. J., Kim Y. C., Kim T. S., *Bioorg. Med. Chem.*, **14**, 6752–6758 (2006).
- Kagechika H., *Curr. Med. Chem.*, **9**, 591–608 (2002).
- White S. L., Belov L., Barber N., Hodgkin P. D., Christopherson R. I., *Leuk. Res.*, **29**, 1141–1151 (2005).
- Collins S. J., Ruscetti F. W., Gallagher R. E., Gallo R. C., *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 2458–2462 (1978).
- Nagarajan R., Perumal P. T., *Chem. Lett.*, **33**, 288–289 (2004).
- Noguchi T., Shinji C., Kobayashi H., Makishima M., Miyachi H., Hashimoto Y., *Biol. Pharm. Bull.*, **28**, 563–564 (2005).
- Hayashi S., Okabe-Kado J., Honma Y., Kawajiri K., *Carcinogenesis*, **16**, 1403–1409 (1995).
- Grevenynghe J., Rion S., Ferrec E. L., Vee M. L., Amiot L., Fauchet R., Fardel O., *J. Immunol.*, **170**, 2374–2381 (2003).
- Kamal A., Qureshi A. A., *Tetrahedron*, **19**, 513–520 (1963).
- Bandgar B. P., Bettigeri S. V., Joshi N. S., *Monatsh. f. Chemie*, **135**, 1265–1273 (2004).
- Hasaninejad A., Zare A., Sharghi H., Shekouhy M., Khalifeh R., Beni A. S., Zare A. R. M., *Can. J. Chem.*, **85**, 416–420 (2007).
- Ramesh C., Banerjee J., Pal R., Das B., *Adv. Synth. Catal.*, **345**, 557–559 (2003).
- Nair V., Abhilash K. G., Vidya N., *J. Chem. Res.*, **2003**, S, 72–74 (2003).
- Shokoufeh M., Najmoddin A., Mohammad R. S., *Lett. Org. Chem.*, **3**, 161–164 (2006).
- Yadav J. S., Reddy B. V. S., Murthy Ch. V. S. R., Kumar G. M., Madan Ch., *Synthesis*, **2001**, 783–787 (2001).
- Naka H., Akagi Y., Yamada K., Imahori T., Kasahara T., Kondo Y., *Eur. J. Org. Chem.*, **2007**, 4635–4637 (2007).
- Mahboobi S., Uecker A., Sellmer A., Cnac C., Hcher H., Pongratz H., Eichhorn E., Hufsky H., Trmpler A., Sicker M., Heide F., Fischer T., Stocking C., Elz S., Bhmer F. D., Dove S., *J. Med. Chem.*, **49**, 3101–3115 (2006).
- Mahboobi S., Burgemeister T., Dove S., Kuhr S., Popp A., *J. Org. Chem.*, **64**, 8130–8137 (1999).
- Diana P., Carbone A., Barraja P., Montalbano A., Martorana A., Datelo G., Gia O., Via L. D., Cirrincione G., *Bioorg. Med. Chem. Lett.*, **17**, 2342–2346 (2007).
- Mahboobi S., Teller S., Pongratz H., Hufsky H., Sellmer A., Botzki A., Uecker A., Beckers T., Baasner S., Schchtele C., Berall F., Kassack M. U., Dove S., Bhmer F. D., *J. Med. Chem.*, **45**, 1002–1018 (2002).
- Wahlström N., Stensland B., Bergman J., *Synthesis*, **2004**, 1187–1194 (2004).