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Regioselective one pot synthesis of 3,3′-diindolylethylene derivatives and study of their cytotoxic activity

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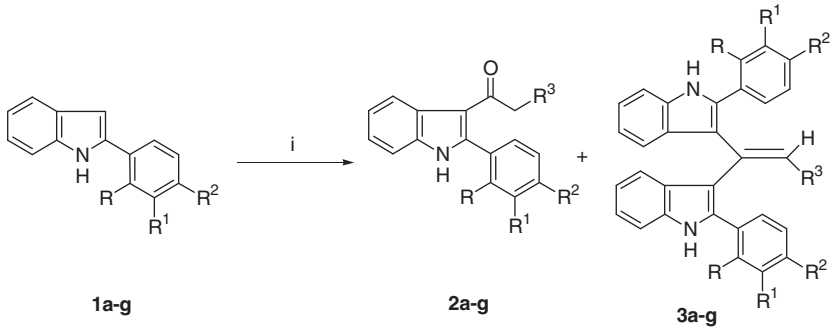
ABSTRACT

2,2′-Diphenyl-3,3′-diindolylethylene (DPDIE) derivatives **3a–g** were regioselectively prepared in one pot from indoles **1a–g** in the presence of Lewis acids and were subsequently evaluated for cytotoxic activity against human leukemic cell lines, U937 and K562. The most potent compound **3g** exhibited IC_{50} of 13.0–17.0 μM.

Indole and its derivatives possess a wide range of biological activities with interesting chemistry.[1] There has been a continuous efforts by synthetic organic chemists to develop simple and efficient synthetic methodologies for bis(indolyl)alkanes due to their excellent biological activities. Bis(indolyl)methanes[2,3] are the most active substances from cruciferous vegetables such as broccoli, kale, cabbage, etc. for promoting beneficial estrogen metabolism and inducing apoptosis in human cancer cells,[4] anti-leishmanial,[5] as well as plant growth promoters.[6] The 3,3′-diindolylethylene (DIE) and its derivatives are structurally similar to 3,3′-diindolylmethane (DIM). Although the 3-position of indole is the most reactive site for electrophilic attack,[7] low yields are always encountered due to the competitive formation of 1-acylated and/or 1,3-di-alkyl/acylated products because of the ambident character of the indole system.

Herein we report a Friedel–Crafts-type acylation of 2-phenylindoles to yield regioselectively 2,2′-diphenyl-3,3′-diindolylethylene



Scheme 1. Reagent and conditions: (i) CH_3COCl (1 equiv), $AlCl_3$ (1.2 equiv), $DCM/C_2H_5NO_2$ (1:0.5) 4–8 h.

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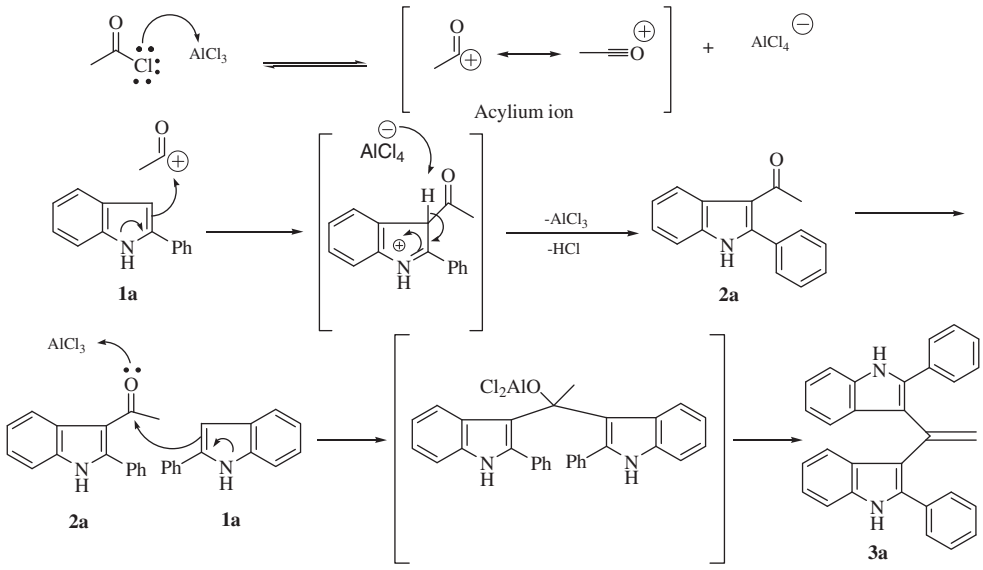
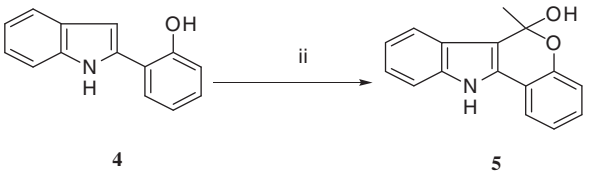


Scheme 2. Proposed mechanism for the formation of 2,2′-diphenyl-3,3′-diindolyl ethylene (**3a**) and 2-phenyl 3-acetyl indole (**2a**).



Scheme 3. Reagents and conditions: (ii) CH_3COCl (1 equiv), $AlCl_3$ (1.2 equiv), DCM/$C_2H_5NO_2$ (1:0.5), 6 h.

Table 1
$AlCl_3$ catalyzed synthesis of 3-acetyl indole derivatives **2a–g** and 3,3′-diindolyl ethylene derivatives **3a–g**

Entry	R	R^1	R^2	R^3	Time (h)	Yield[a] (%)	
						2	**3**
a	H	H	H	H	4	25[b]	62
b	H	Cl	H	H	6	20	60
c	Cl	H	Cl	H	4.5	23	63
d	OMe	H	H	H	4	26	62
e	H	OMe	OMe	H	3	21[c]	60
f	H	Br	H	H	6	26	67
g[d]	H	H	H	Cl	8	14[e]	69

[a] Isolated yield.
[b] See Ref. 10.
[c] See Ref. 11.
[d] Chloroacetyl chloride (1 equiv) was used for the formation of **3g** and **2g** instead of acetyl chloride.
[e] See Ref. 12.

Table 2
Effect of different Lewis acids in synthesis of 2-phenyl-3-acetyl indole (**2a**) and 2,2′-diphenyl-3,3′-diindolyl ethylene (**3a**)

Lewis acids	Time (h)	Yield[a] (%)	
		2a	**3a**
$AlCl_3$	4	25	62
$SnCl_2$	5	20	50
$FeCl_3$	4	30	60
$ZnCl_2$	6	25	45
$ZnBr_2$	10	20	40
$InCl_3$	15	22	30

[a] Isolated yield.

Table 3
Effect of different co-solvents in the formation of 2-phenyl-3-acetyl indole (**2a**) and 2,2′-diphenyl-3,3′-diindolyl ethylene (**3a**)

Co-solvents	Time (h)	Yield[a] (%)	
		2a	**3a**
Nitroethane	4	25	62
Nitromethane	4.5	29	67
Nitrobenzene	8	20	5

[a] Isolated yield.

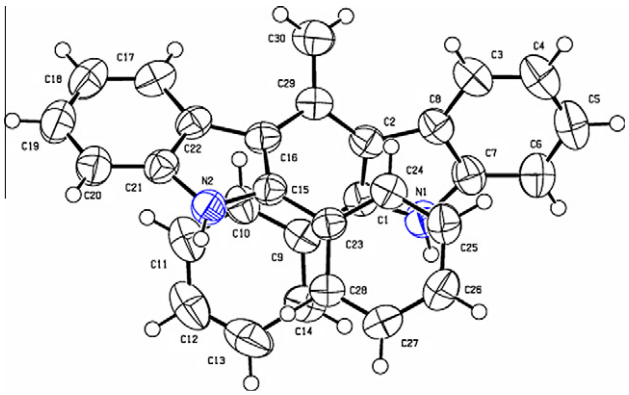


Figure 1. X-ray structure of **3a**.

Table 4
Inhibitory concentration of DIE derivatives **3a–g** against human leukemic cell lines

Entry	DIE derivatives	Cell growth inhibition in terms of IC_{50} (μM) ± SEM	
		U937	K562
1	**3a**	NA	NA
2	**3b**	46.93 ± 0.120	40.3 ± 0.0
3	**3c**	NA	NA
4	**3d**	71.6 ± 2.05	92.6 ± 6.56
5	**3e**	NA	NA
6	**3f**	NA	NA
7	**3g**	13.06 ± 1.29	18.21 ± 5.87

NA = not active (IC_{50} >100 μM)

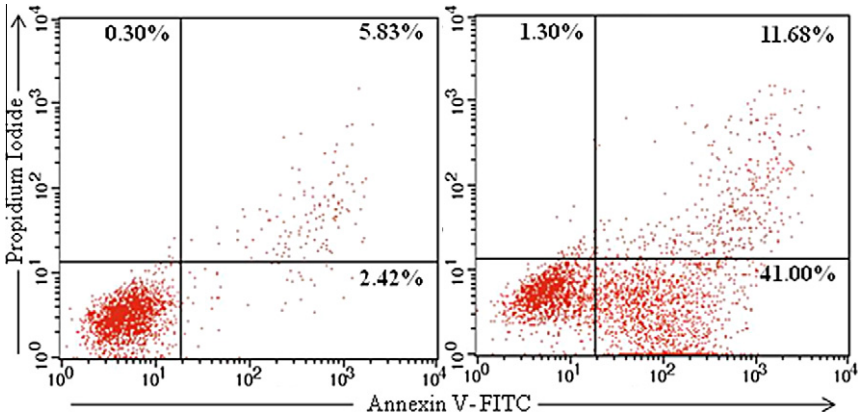


Figure 2. Externalization of phosphatidylserine by **3g**: (A) untreated U937 cells; (B) U937 cells were treated with an IC_{50} concentration of **3g** for 8 h, co-stained with PI and Annexin V-FITC and analyzed by flow cytometry.

(DPDIE)[8] in good yields. In the course of developing simple and efficient synthetic methodology for the preparation of bioactive indole derivatives required for one of our network projects of CSIR, Govt. of India involving multi-disciplinary R & D Institutions, it was observed that treatment of 2 equiv of 2-phenyl-indole (**1a**) with 1 equiv of acetyl chloride and 1.2 equiv of Lewis acid ($AlCl_3$) in dichloromethane/nitroethane (1:0.5) at 50 °C for 4 h resulted in 2,2′-diphenyl-3,3′-diindolylethylene (**3a**) in 62% yield along with expected 2-phenyl-3-acetyl indole (**2a**) in 25% yield (Scheme 1). It is pertinent to mention that the reaction becomes sluggish in the absence of co-solvents to produce the DPDIE (**3a–g**). The role of co-solvents nitroethane/nitromethane in the reaction may be explained to the fact that it increases the solubility of indole–Lewis acid complexes thereby accelerating the reactions.[9] The formation of **3a** by Friedel–Crafts acylation of 2-phenyl indole **1a** could be explained on the basis of the proposed mechanism as shown in Scheme 2. At first acylation of indole **1a** gives 3-acetyl indole **2a**, which further reacts with another moiety of indole **1a** to produce **3a** (DPDIE). The formation of DPDIE was confirmed when 3-acetyl-2-phenylindole (**2a**) was separately treated with 2-phenyl indole **(1a**) under similar reaction condition. When 2-(1H-indolyl-2-yl)-phenol (**4**) instead of 2-phenylindole (**1a**) was treated with acetyl chloride, resulted in the formation of 6-methyl-6,11-dihydro-5-oxa-11-aza benzo[*a*]fluoren-6-ol (**5**) in 60% yield (Scheme 3), which could be obviously formed due to intramolecular attack of phenolic –OH (nucleophile) of carbonyl group of acetyl moiety at 3-position of indole. In this case intramolecular attack of nucleophile on 3-acetyl-indole is more favored than intermolecular attack by another indole moiety of its 3-position. This efficient method was applied to prepare a number of DPDIEs **3a–g** in good yields (Table 1). The reaction has been studied using different Lewis acids in dichloromethane/nitroethane (1:0.5) solvent system at 50 °C (Table 2). $AlCl_3$, $FeCl_3$ and $SnCl_2$ provided better results in comparison to other Lewis acids investigated (entries 1–3). Among the other co-solvents used together with DCM at 50 °C in the presence of $AlCl_3$, both nitroethane and nitromethane seems to be better co-solvents (entries 1 and 2) to produce the regioselective formation of DPDIE (Table 3).

All the new compounds **2a–g**, **3a–g**, and **5** have been characterized from their spectral data by NMR and mass spectra. The structure of **3a** was also confirmed by single crystal X-ray diffraction study (Fig. 1). Crystallographic data for the structure of **3a** have been deposited with the Cambridge Crystallographic Data Centre (CCDC No. 802362).

The cytotoxic activity of DPDIE derivatives **3a–g** against human leukaemic cell lines U937 and K562 was evaluated using MTS–PMS assay[13,14] and the results are shown in Table 4. The results are expressed as IC_{50} values, that is, concentration that inhibited 50% of cell growth, enumerated by graphic extrapolation using GRAPHPAD prism software (version 5). The most potent synthesized analogue **3g** inhibited the viability of U937 cells by 50% at concentration 13.06 μM. The lower IC_{50} values of **3g** encouraged us to delve into the mode of death incurred by it.

The binding of Annexin V, a Ca^{2+}-dependent phospholipid-binding protein known to have a strong affinity towards phosphatidylserine, is a proven measure of apoptosis. Propidium iodide (PI) can not enter within the cells with intact membranes. Annexin V and propidium iodide are used to differentiate between early apoptotic (Annexin V-positive, PI-negative), late apoptotic (Annexin V and PI double positive) and necrotic (Annexin V-negative, PI-positive).[15,16] In untreated U937 cells, the % binding of Annexin V was 8.25%. Following treatment with an IC_{50} of **3g** (13.06 μM) for 8 h, the percentage of Annexin V-FITC increased to 52.68% (Fig. 2). The percentage of PI-stained cells ranged from 0.30% to 1.30%, indicating that **3g** exerts its anti-proliferative activity primarily via apoptosis.

In conclusion, the present methodology developed by us offers an efficient regioselective one pot synthesis of biologically important DIE and its derivatives in very good yield. The cytotoxic activities of DIE derivatives against human leukemic cell lines, U937 and K562 were evaluated. The chemical modification and structure–activity relationship (SAR) of DIE compounds were investigated with different substituents at the 2-phenyl group and at the ethylene double bond based on biological evaluation against human leukemic cell lines U937 and K562. Substitution of ethylene double bond by chlorine atom (compound **3g**) contributes better anti-proliferative activity, the mode of cell death via apoptosis as evidenced by the six fold increase in Annexin V positivity. Therefore, the novel lead compound **3g** may be further studied to get potential anticancer agent.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.028.

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13. *Cell viability assay:* Novel synthetic compounds **3a**–**g** were first evaluated for its antiproliferative activity in U937 and K562 cells using MTS–PMS assay. Briefly, U937 and K562 cells (5×10^4/200 μl) were seeded in 96 well tissue culture plates and incubated with the compounds (0–100 μM) in triplicate for 48 h at 37 °C, 5% CO_2. To evaluate cell viability, MTS [3-(4,5 dimethylthiazol-2-yl) 5-(3-carboxymethoxy-phenyl)-2-(4-sulphonyl)-2*H*-tetrazolium, inner salt] (2.0 mg/ml) and PMS (Phenazine methosulfate) (0.92 mg/ml) were added in a ratio of 10:1 (20 μl per well) and plates incubated for 3 h at 37 °C. Resultant absorbance was measured at 490 nm in an ELISA reader. The specific absorbance that represented formazan production was calculated by substraction of background absorbance from total absorbance. The mean % viability was calculated as follows:

$$\frac{\text{Mean specific absorbance of treated cells}}{\text{Mean specific absorbance of untreated cells}} \times 100$$

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15. *Flow cytometric analysis of externalized phosphatidylserine:* U937 cells were incubated with an IC_{50} dose of **3g** (13.06 μM) for 8 h at 37 °C, 5% CO_2. Cells were centrifuged (4000 rpm for 4 min), washed twice in phosphate buffered saline (0.02 M, pH 7.2, PBS) and re-suspended in Annexin V binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4). Annexin V-FITC and propidium iodide were then added and incubated for 15 min in dark at 25 °C according to the manufacturer's instructions. Data acquisition was done on a FACS Calibur flow cytometer (BD Biosciences, USA) and analyzed with CELL QUEST PRO software.
16. For flow cytometric analysis of externalized phosphatidylserine, see: Saha, P.; Sen, R.; Hariharan, C.; Kumar, D.; Das, P.; Chaterjee, M. *Free Radical Res.* **2009**, *43*, 1101.