



## Discovery of certain benzyl/phenethyl thiazolidinone-indole hybrids as potential anti-proliferative agents: Synthesis, molecular modeling and tubulin polymerization inhibition study

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### ABSTRACT

A series of certain benzyl/phenethyl thiazolidinone-indole hybrids were synthesized for the study of anti-proliferative activity against A549, NCI-H460 (lung cancer), MDA-MB-231 (breast cancer), HCT-29 and HCT-15 (colon cancer) cell lines by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). We found that compound **G37** displayed highest cytotoxicity with IC<sub>50</sub> value of 0.92 ± 0.12 μM towards HCT-15 cancer cell line among all the synthesized compounds. Moreover, compound **G37** was also tested on normal human lung epithelial cells (L132) and was found to be safe in contrast to HCT-15 cells. The lead compound **G37** showed significant G2/M phase arrest in HCT-15 cells. Additionally, compound **G37** significantly inhibited tubulin polymerization with IC<sub>50</sub> value of 2.92 ± 0.23 μM. Mechanistic studies such as acridine orange/ethidium bromide (AO/EB) dual staining, DAPI nuclear staining, annexinV/propidium iodide dual staining, clonogenic growth inhibition assays inferred that compound **G37** induced apoptotic cell death in HCT-15 cells. Moreover, loss of mitochondrial membrane potential with elevated intracellular ROS levels was observed by compound **G37**. These compounds bind at the active pocket of the α/β-tubulin with higher number of stable hydrogen bonds, hydrophobic and arene-cation interactions confirmed by molecular modeling studies.

### 1. Introduction

Microtubule modulators have played an essential role in cancer chemotherapy over the past decades, where interference with microtubule assembly, either by down regulation of tubulin polymerization or by modulation of microtubule disassembly, that leads to an increase in the number of cells in metaphase arrest [1,2]. Tubulin protein is found to be α, β-heterodimer and forms heart of the microtubule network in cell. Moreover, cancer cells undergo mitosis rapidly as compared to normal cells, which means that they are more prone to tubulin-targeting agents [3]. Anti-mitotic compounds such as taxoids (paclitaxel and docetaxel) and vinca alkaloids (vinblastine and vincristine) have been widely using in the treatment of various human cancers since many years; however, these compounds have shown either one or multiple inabilities such as adverse side effects, drug resistance,

synthetic complexity and imperfect bioavailability [4,5]. To prevail the above limitations, medicinal chemists are encouraged to develop novel chemotherapeutic agents as modulators of microtubules/tubulin system with better safety profile.

4-Thiazolidinones are proven class of pharmacophores, which display a broad spectrum of biological activities. In view of tumor therapeutic potential, recent reports suggest that 4-thiazolidinones could express their antitumor property through various mechanisms featuring affinity towards tumor necrosis factor-α (TNF-α) [6], JNK stimulating phosphatase-1 (JSP-1) [7], BH3 domain and Bcl-X<sub>L</sub> [8], SHP-2 [9], integrin αvβ3 receptor [10], histone deacetylase [11] and microtubules/tubulin assembly [12], etc.

Molecular hybridization approach is an efficient and often used direction for investigations to find novel and highly active compounds in the current medicinal chemistry research [13–15]. Although several

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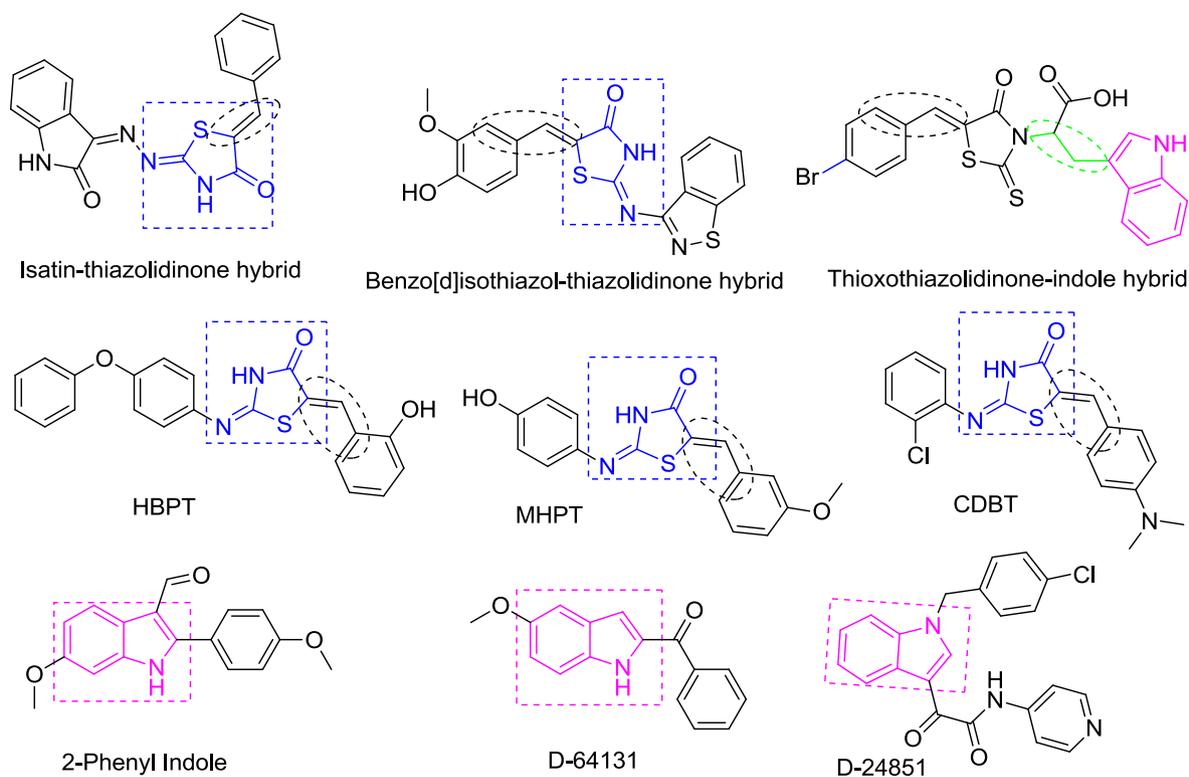


Fig. 1. Representative structures of bioactive thiazolidinones and indoles as anti-proliferative agents.

researchers explored for novel hybrid therapeutics, some of the research groups discovered that the thiazolidinone containing hybrids exhibit anti-cancer activity against different cancer cell lines [16,17]. These findings support that modifications of thiazolidinone ring at C-2 and C-5 positions have led to many new chemical entities with increased antitumor activity. On the other hand, indoles represent one of the most significant structural classes in drug discovery process. A number of indole derivatives used as tubulin polymerization inhibitors to induce apoptosis in cancer chemotherapy [18]. Some of the representatives are depicted in Fig. 1 such as 2-phenylindole, 2-arylindoles (D-64131) and indolyl-3-glyoxamide (D-24851) [19,20].

In search of target-specific therapeutics with the inspiration of molecular hybridization, we envision that the hybridization of 4-thiazolidinone moiety with indole structural motif into a single chemical entity, as depicted in Fig. 2, may produce synergistic anticancer activity. With our continued interest in the discovery of tubulin inhibitors [21], we synthesized the designed hybrid molecules and scrutinized for *in vitro* anti-proliferative activity against five human cancer cell lines: A549, NCI-H460 (lung cancer), MDA-MB-231 (breast cancer), HCT-29 and HCT-15 (colon cancer).

## 2. Material and methods

### 2.1. Chemistry

All the solvents and reagents were procured from commercial suppliers. Analytical thin layer chromatography (TLC) was performed on MERCK pre-coated silica gel 60-F-254 (0.5 mm) aluminium plates. Visualization of the spots on TLC plates was achieved using UV light.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on Bruker 500 MHz and 125 MHz spectrometers, respectively, using tetramethyl silane (TMS) as the internal standard. Chemical shifts for  $^1\text{H}$  and  $^{13}\text{C}$  are reported in parts per million (ppm) downfield from tetramethyl silane. Spin multiplicities are described as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet). Coupling constant ( $J$ ) values are reported in Hertz (Hz). Melting point was determined with the help of Stuart advanced melting point apparatus. HRMS were determined with Agilent QTOF mass spectrometer 6540 series instrument. Column chromatography was performed using silica gel 60–120.

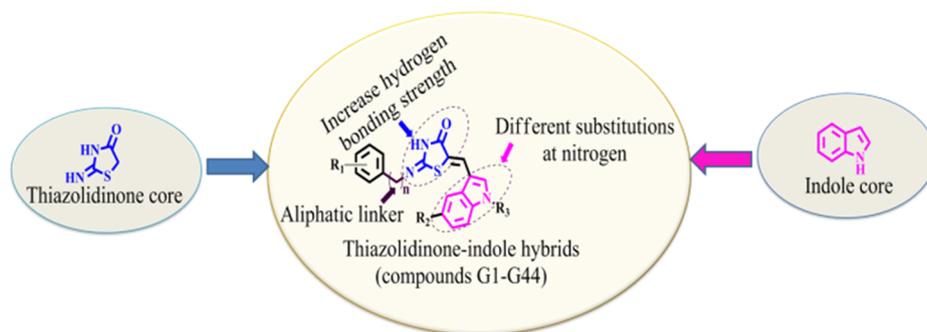


Fig. 2. Design of certain benzyl/phenethyl thiazolidinone-indole hybrids as anticancer agents.

## 2.2. Biology

### 2.2.1. Cell culture

Cell lines such as lung (A549 and NCI-H640), breast (MDA-MB-231), colon (HCT-15 and HCT-29) and normal lung epithelial cells (L132) were procured from National Centre for Cell Science (NCCS), Pune, India. All the cells were grown in appropriate DMEM and RPMI-1640 medium (Sigma-Aldrich, USA). Cells were supplemented with 10% fetal bovine serum stabilized with 1% antibiotic-anti mycotic solution (Sigma-Aldrich, USA) in a CO<sub>2</sub> incubator at 37 °C. When the cells reached up to 80–90% of confluency, they were sub-cultured using 0.25% trypsin/1 mM EDTA solution for further passage.

### 2.2.2. MTT assay

Cytotoxicity of synthesized compounds was evaluated by MTT assay. Briefly, cells were seeded in 96-well plates at a density of 4000 cells per well in 100 µL of complete medium and allowed to grow overnight for attachment onto the wells. Then the cells were treated with various concentrations of the compounds for a period of 72 h. After the treatment, 100 µL of MTT (0.5 mg/mL) was added and incubated at 37 °C for 4 h. Then MTT reagent was aspirated and the formazan crystals formed were dissolved by the addition of 200 µL of DMSO for 20 mins at 37 °C. The quantity of formazan product was measured by using a spectrophotometric microtiter plate reader (Spectra max, M4 molecular devices, USA) at 570 nm wavelength. Initially, cytotoxicity effects of the synthetic derivatives were screened by MTT assay at 30 µM concentration. Among these, the compound which showed IC<sub>50</sub> value < 30 µM was used for the dose dependent studies at various concentrations ranging from 0.78 µM to 30 µM in serial dilutions and the percentage of cytotoxicity was calculated.

### 2.3. Molecular modeling studies

Molecular modeling studies of the corresponding benzyl/phenethyl thiazolidinone-indole hybrid compounds were performed by using Maestro, version 10.4 of Schrödinger suite 2015-4. The 3D structures of the compounds were made by using Maestro Molecule Builder and optimized by means of LigPrep 3.6 version of Schrödinger suite 2015-4. Thus obtained 3D structures of the compounds were docked at the active site of tubulin protein (PDB ID: 1SA0).

## 3. Results and discussion

### 3.1. Chemistry

We began our study with the synthesis of designed target molecules **G1-G44** using the multi-step reaction protocol as delineated in [Scheme 1](#). The designed target compounds **G1-G44** comprise of two core structural elements: (i) benzyl/phenethyl thiazolidinone, and (ii) a substituted indole moiety. Initially, substituted benzyl/phenethyl thiazolidinone (**C**) moiety was prepared from commercially available benzyl/phenethylamine (**A**) using two simple synthetic steps. Firstly, benzyl/phenethylamine (**A**) was treated with chloroacetyl chloride in DMF to afford the corresponding amide (**B**), which undergoes cyclization in the presence of ammonium thiocyanate at reflux temperature in ethanol to afford the desired fragment-thiazolidinone (**C**) in good to excellent yields [22,23].

On the other hand, the 5-substituted-1*H*-indole-3-carboxaldehyde (**E**) was prepared according to Vilsmeier-Haack reaction by the treatment of 5-substituted-1*H*-indole (**D**) with phosphorus oxychloride in DMF at 0–60 °C [24], and then *N*-alkylation/arylation was carried out using appropriate alkyl/aryl halide and sodium hydride in DMF to obtain the desired *N*-substituted indole-3-carboxaldehyde (**F**) [25–27]. Finally, the thiazolidinone (**C**) was coupled with the synthesized indole-3-carboxaldehyde derivative (**F**) using piperidine in ethanol at 60 °C to furnish the target compound (**G1-G44**) in moderate to very good yields

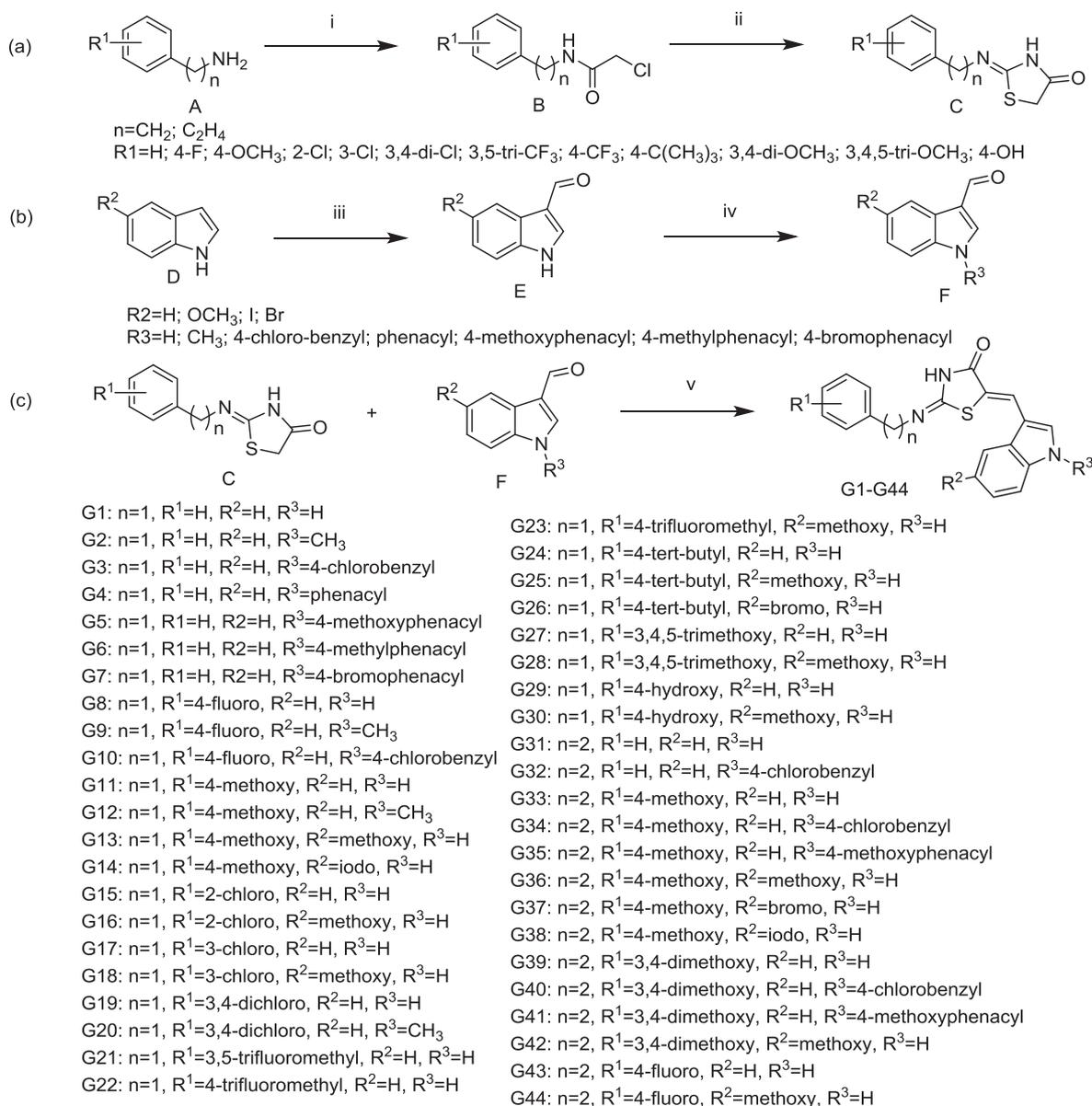
([Scheme 1](#)) as per our previous protocol [28]. All the synthesized compounds (**G1-G44**) were well characterized by using HRMS, <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy and all the data are produced in [supporting information](#). The theoretical existence of tautomeric forms of thiazolidinone (**C**) was represent in [Scheme 2](#). The <sup>1</sup>H NMR spectrum of **G1** showed a broad singlet of indole N–H proton at δ 12.02, which disappeared upon methylation, as seen in **G2**. A singlet was observed at δ 9.71, which is characteristic of amide N–H proton. Based on this observation as well as reported literature [29] supporting our prediction of the existence of thiazolidinone as phenylimino rather than phenylamino form.

### 3.2. In vitro anti-proliferative activity

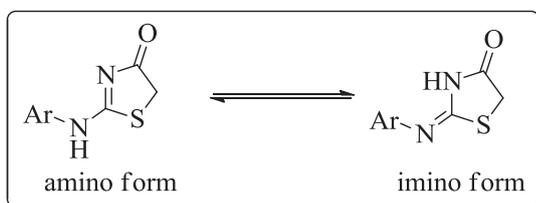
Anti-proliferative activity of all the synthesized compounds (**G1-G44**) were performed on various human cancer cell lines such as lung (A549 and NCI-H460), colon (HCT-29 and HCT-15), and breast cancer (MDA-MB-231) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Table S1 displays the IC<sub>50</sub> (µM) values (concentration required to inhibit 50% of cell growth) of tested compounds (**G1-G44**), as well as reference standard-podophyllotoxin. The screening of preliminary results suggests that few of the synthesized compounds displayed moderate to potent cytotoxicity against the tested cancer cells with IC<sub>50</sub> values in the range of 0.92 ± 0.12 to 12.28 ± 0.30 µM. Close examination of IC<sub>50</sub> values of the synthesized compounds showed that some of the compounds are sensitive towards colon (HCT-29 and HCT-15) and lung (A549 and NCI-H460) cancer cell lines and most of the compounds were inactive against MDA-MB-231 cancer cells. From these experimental results we observed that, compounds **G21**, **G26**, **G27**, **G33**, **G37**, and **G38** were the most active in the series and the remaining compounds were roughly moderate to inactive against the tested cancer cells. Interestingly, the compound **G37** was found to be most active in colon cancer (HCT-29 and HCT-15) cell lines and showed marked cytotoxicity with IC<sub>50</sub> of 0.92 ± 0.12 µM towards HCT-15 cells. To check the selectivity of our synthesized compounds towards cancerous cells, their cytotoxicity was evaluated against normal human lung epithelial cells (L132). To our delight, the compound **G37** showed IC<sub>50</sub> of 10.84 ± 0.94 µM on non-cancerous cells, thus, indicating 10 fold selectivity towards HCT-15 cells. Some of the common pharmacophoric features observed in active compounds **G33**, **G37**, and **G38** are the presence of the 4-methoxyphenyl group in the side chain connected to the thiazolidinone nucleus with an ethylene bridge and absence of the substitution of the nitrogen in indole. Furthermore, we noticed from the comparison of IC<sub>50</sub> values that the phenethylthiazolidinone-indolehybrids (**G31-G44**) have more potent anti-proliferative action than benzyl thiazolidinone-indole hybrids (**G1-G30**). In addition, it was also observed that *N*-alkyl/aryl substituted indole hybrids are relatively less active than unsubstituted indole hybrids. The electron donating group (methoxy) on the phenyl ring at the side chain of hybrid molecules has shown better anti-proliferative profile compared to those with halogen substitutions. Finally, due to the encouraging anti-proliferative activity, one of the most active compound **G37** was taken-up for further mechanistic investigation of cancer cell growth inhibition.

### 3.3. Structure-activity relationship (SAR)

The impact of substitution at benzyl/phenethyl, thiazolidinone and indole is highly significant. The structure–activity relationship (SAR) analysis of these molecules are: (1) The presence of electron donating (4-methoxy) group at the phenethyl group attached to the side chain of thiazolidinone (**G33**, **G37** and **G38**) showed significant cytotoxicity; (2) Compounds without any substitution at the benzyl/phenethyl group attached to the side chain of thiazolidinone (**G1-7** and **G31-32**) were nearly inactive at 30 µM; (3) Compounds with halogen (fluoro and chloro) substitutions at the benzyl/phenethyl group attached to the

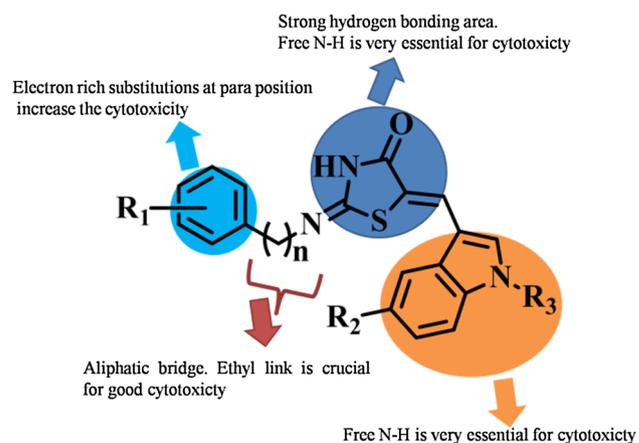


**Scheme 1.** Synthesis of benzyl/phenethyl thiazolidinone-indole hybrids (compound G1-G44).



**Scheme 2.** Tautomeric forms of 2-substituted thiazolidinone.

thiazolidinone (G8, G9, G15-20, G43 and G44) are less active; (4) From the SAR studies, we also observed that the presence of free N-H group in thiazolidinone and indole rings is essential for imparting cytotoxic activity (as depicted in Fig. 3.). Moreover, the compounds containing phenethyl side chain on thiazolidinone-indole core showed enhanced cytotoxicity than benzyl side chain.



**Fig. 3.** SAR analysis of benzyl/phenethyl thiazolidinone-indole hybrids.

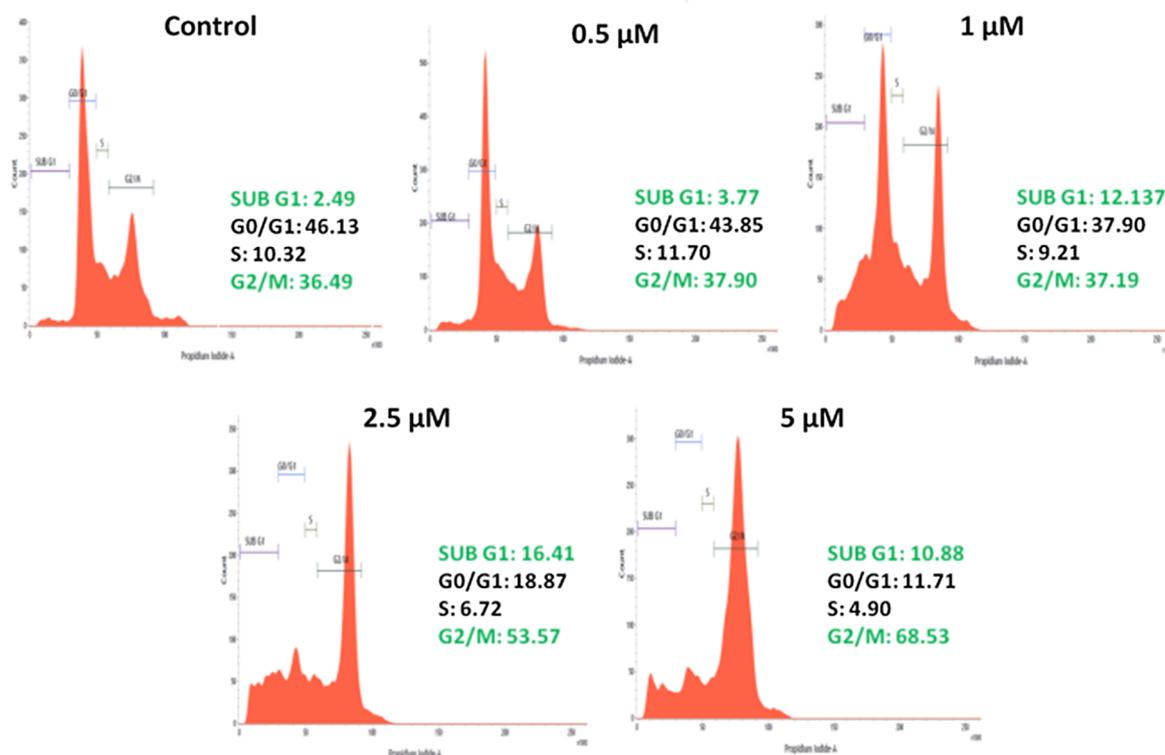


Fig. 4. Effect of **G37** compound on cell cycle progression of HCT-15. Cells were treated with compound **G37** (0.5, 1, 2.5 and 5  $\mu$ M) and cell cycle analysis was performed by flow cytometric analysis using PI staining after 24 h of incubation.

### 3.4. Cell cycle analysis

Most of the anticancer agents inhibit the cell proliferation of cancer cells by arresting the cell cycle at particular check point. In our study, we found that compound **G37** showed significant cytotoxicity against HCT-15 cells. To know the underlying mechanism of its cytotoxicity, we performed cell cycle analysis. Herein, cells were treated with compound **G37** (0.5, 1, 2.5 and 5  $\mu$ M) for 24 h and stained with propidium iodide (PI) and further analyzed by flow cytometer. Fig. 4 infers that the control cells exposed to DMSO showed 2.49% cells in sub G1 phase, whereas compound **G37** treatment resulted in increased sub G1 population to 10.88% with 5  $\mu$ M concentration. The percentage of cells in G2/M phases was 53.57 and 68.53% with 2.5 and 5  $\mu$ M of the compound, respectively, in comparison to the control cells where 36.49% was observed. Collectively, these results indicated that treatment of HCT-15 cells with compound **G37** arrested in sub G1 and G2/M phase.

### 3.5. Effect on tubulin polymerization

Microtubules are cytoskeletal filaments consisting of  $\alpha$  and  $\beta$  tubulin subunits and are involved in a wide range of cellular functions including cell division [30]. Further, tubulin is a well-established target for a variety of successful anticancer drugs. As evident from the literature, 4-thiazolidinones and indoles show anti-proliferative activity via inhibition of tubulin polymerization, thereby, disturbing the microtubule formation. In addition, as these hybrids significantly induced G2/M cell cycle arrest, their microtubule inhibitory functional aspects were also explored. The active compound **G37** was evaluated for tubulin polymerization inhibitory activity, where podophyllotoxin was taken as the positive control. Initially, compound **G37** was screened at 5  $\mu$ M concentration and the percentage of inhibition was found to be 81.46%. Fig. 5 infers that tubulin polymerization was significantly inhibited by compound **G37** in a concentration dependent manner with IC<sub>50</sub> value of  $2.92 \pm 0.23$   $\mu$ M. Collectively, these results showed that

tubulin might be the molecular target of 4-thiazolidinone-indole hybrids. Compound **G37** (0.31–5  $\mu$ M) concentration-dependently inhibited the tubulin polymerization compared to podophyllotoxin. Data represented as mean  $\pm$  SEM (n = 3).  $p < 0.001$  is significantly different from podophyllotoxin.

### 3.6. Apoptosis induction studies

As most of the anticancer agents show the anticancer activity by inducing apoptosis, we have investigated the apoptotic inducing activity of compound **G37** on HCT-15 cells. We performed various assays including acridine orange/ethidium bromide (AO/EB), 4',6-diamidino-2-phenylindole (DAPI), DCFDA, JC-1 staining and Annexin V binding assays and results are summarized. Initially, HCT-15 cells were treated with compound **G37** and were observed under phase contrast microscopy to examine the morphological changes of cells. Compound **G37** (0.5, 1 and 2.5  $\mu$ M) showed characteristic apoptotic morphological features such as cell shrinkage and decreased number of viable cells compared to the control group (Fig. S1 Supporting Information).

AO/EB staining is used to differentiate live, apoptotic and necrotic cells. Here, AO is a dye, which permeates the intact cell membrane and stains the nuclei with green colour, whereas EB stains the nucleus as red in colour. Fig. 6 demonstrated that control cells appeared green in colour without any morphological changes. However, cells treated with 2.5  $\mu$ M of compound **G37** clearly exhibited morphological changes such as cell shrinkage, nuclear condensation and apoptotic blebs formation. Collectively, these results indicate that compound **G37** induces apoptosis in HCT-15 cells in concentration dependent manner.

DAPI stain selectively binds to A-T rich sequences of DNA and helpful to visualize the nuclear changes in cells. It clearly differentiates live cells from apoptotic cells by bright condensed nuclei. Nuclear morphology of cancer cells after DAPI staining (Fig. S2 Supporting Information) demonstrated that the compound **G37** induced the formation of horse-shoe shaped or fragmented nuclei in cells in contrast to

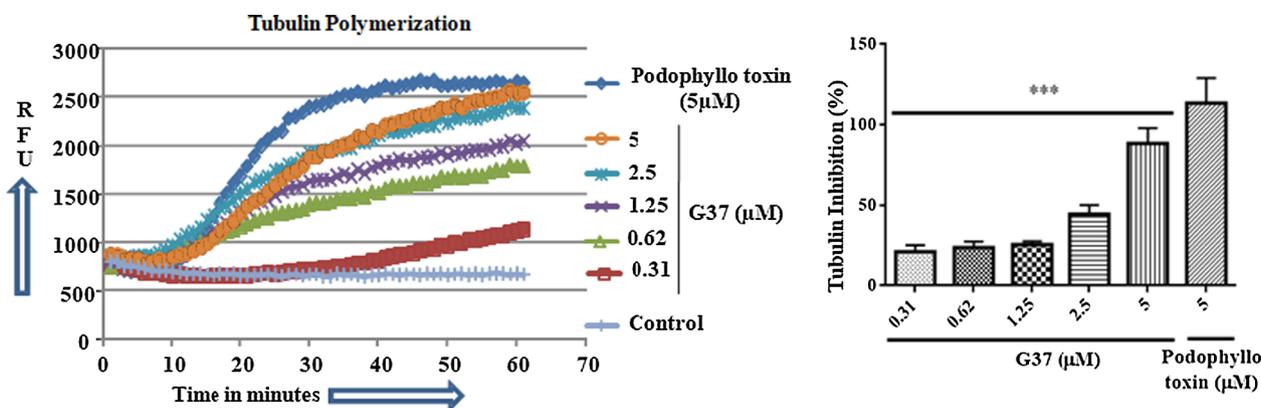


Fig. 5. Effect of compound G37 on tubulin polymerization.

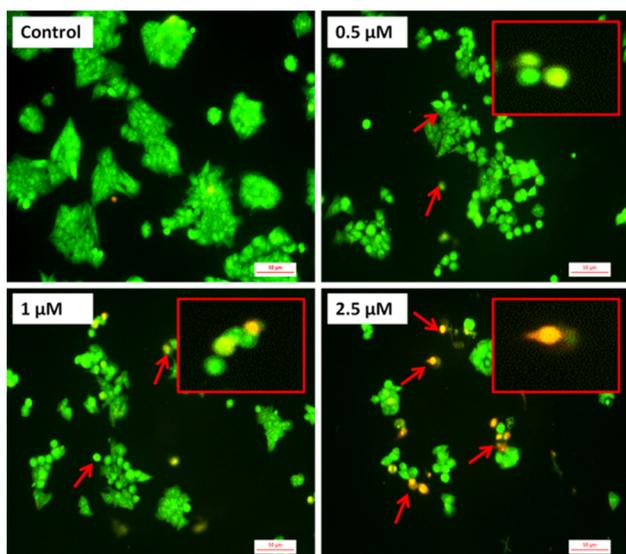


Fig. 6. AO/EB staining of compound G37. Cells were treated with G37 in the concentration of 0.5, 1 and 2.5  $\mu\text{M}$  and compared with control (DMSO treatment). Images were captured at 200X magnification. Arrows indicate the apoptotic changes such as nuclear condensation and membrane blebbing.

the control group. Loss of mitochondrial membrane potential (MMP) is an earliest stage of apoptotic signaling activation. We therefore used JC1 staining to assess the apoptotic activity of compound G37 on MMP. Treatment with compound G37 on HCT-15 cells for 48 h, resulted in increased loss of mitochondrial membrane potential in concentration dependent manner (Fig. S3 Supporting Information). Thus, mitochondrial membrane depolarization was significantly increased after treatment with compound G37 in colon cancer cells.

Quantitatively, the percentage of apoptosis induced by compound G37 was determined by Annexin V/PI dual staining. This staining facilitates for the quantification of live (LC; AV<sup>-</sup>/PI<sup>-</sup>), early apoptotic (EA; AV<sup>+</sup>/PI<sup>-</sup>), late apoptotic (LA; AV<sup>+</sup>/PI<sup>+</sup>) and necrotic (NC; AV<sup>-</sup>/PI<sup>+</sup>) cell population. The percentage of total apoptotic cells (both early and late apoptotic cells, respectively) was increased to 24.83% after treatment with 2.5  $\mu\text{M}$  concentration of compound G37 in comparison to the control (1.66%) cells (Fig. 7). Collectively, the percentage of early apoptotic cells was significantly increased by compound G37 in colon cancer cells.

We next determined the effect of compound G37 on cellular ROS levels in HCT-15 cancer cells using cell permeable fluorogenic dye-DCFDA. The compound G37 (0.5, 1 and 2.5  $\mu\text{M}$ ) increased the ROS levels in colon cells and increased the green fluorescence. The percentage of DCF intensity increased in concentration-dependent manner

(Fig. S4 Supporting Information). Anti-proliferative activity of the most potent compound G37 was further tested by colony formation assay which infers the ability of forming clone from single cells, which mimics the human tumor metastasis. The compound G37 significantly inhibited the clonogenic growth of HCT-15 cells at 0.5  $\mu\text{M}$ , which was the lowest tested concentration. However, at 2.5  $\mu\text{M}$  concentration of compound G37, the colony formation was completely inhibited as compared to the control group (Fig. S5A Supporting Information). The number of colonies was counted by molecular imaging system Vilber Fusion Fx software and values were represented as total number of colonies versus concentration (Fig. S5B Supporting Information).

### 3.7. Molecular modeling studies

#### 3.7.1. Docking simulation study

Molecular docking studies were performed for the representative compounds (G21, G26, G27, G33, G37, and G38) of synthesized benzyl/phenethyl thiazolidinone-indole hybrids and bound ligand against the active site of X-ray crystal structure of tubulin protein and the detailed interaction pattern of most active compound G37 with tubulin protein is shown in Fig. 8a and b. The docking model of the active compound G37 revealed that, compound G37 has shown four hydrogen bonding contacts with the key amino acid residues Thr145, Ala250, Asp251 and Lys254, and the results showing hydrogen bond, hydrophobic and  $\pi$ -cation interactions of synthesized compounds with tubulin protein are depicted in Table S2 (supporting information). The nitrogen atom of thiazolidinone moiety has shown H-bond interaction with side chain amino group of Lys254 with distance of 2.4 Å and oxygen atom of keto functional group of thiazolidinone ring has established two H-bond interactions with the back bone amino group of Ala250 ( $d = 1.9$  Å) and Asp251 ( $d = 3.3$  Å), respectively. Similarly, p-methoxy group of phenethyl side chain had shown hydrogen bond contact with Thr145 ( $d = 3.3$  Å). Furthermore, the benzene ring of this compound established one important  $\pi$ -cation interaction with Lys254. Additionally, compound G37 has shown several hydrophobic interactions with the key amino acid residues of  $\alpha/\beta$  tubulin. Moreover, docked compound G37 and cocrystallized ligand suggested that G37 also lodged at the active site in a comparable manner as that of cocrystallized ligand which demonstrates the superimposition of best docked pose and bound ligand at the active site of  $\alpha/\beta$  tubulin (Fig. S6 Supporting Information). This docking analysis gave us a discerning avocation to astounding tubulin polymerization inhibitory action of compound G37 and some significant information for future structural modifications.

#### 3.7.2. Prime MM/GBSA binding energy calculations

We observed that the synthesized compounds have shown comparable binding energies to that of bound ligand using MM/GBSA binding energy calculations (Table 1). The aim of MM-GBSA and

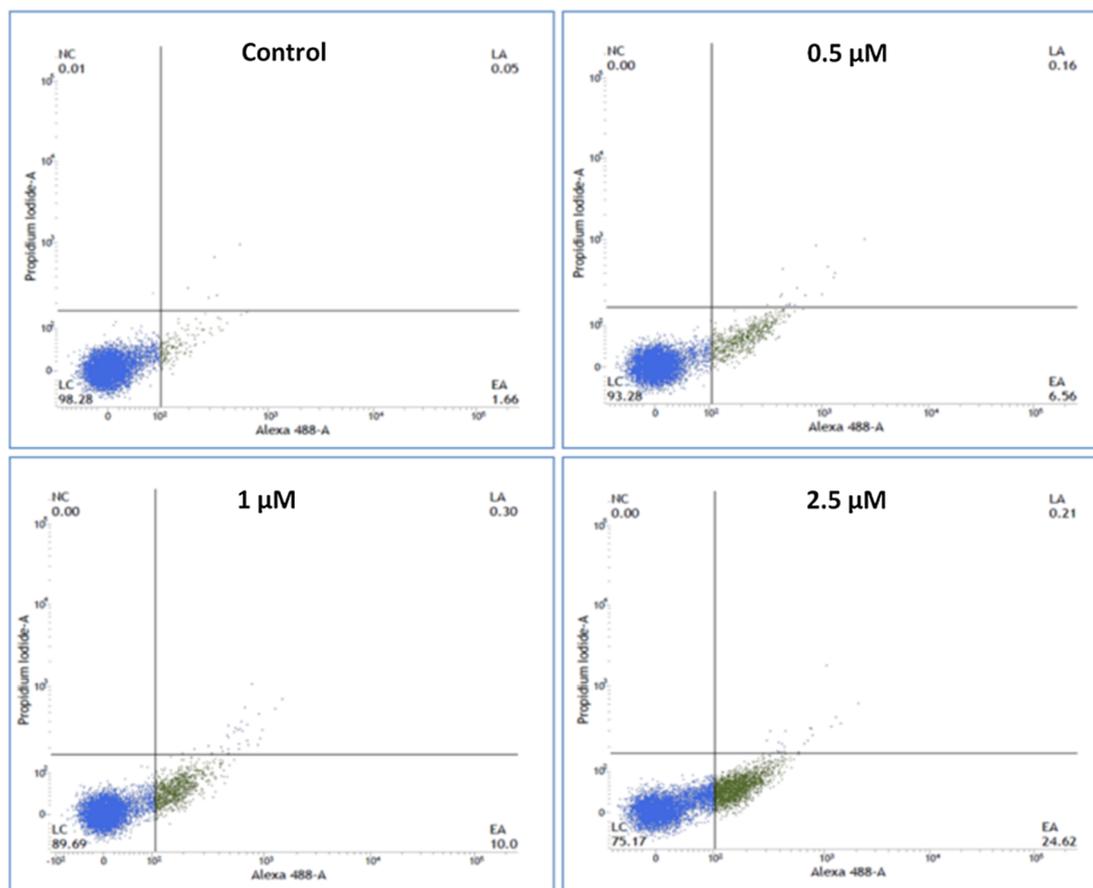


Fig. 7. Effect of compound G37 on apoptotic cell death in HCT-15 cells after 48 h. The compound G37 treated cells were stained with Annexin V/PI and analysed for apoptosis using flow cytometer. The 10,000 cells from each sample were analysed by flow cytometry.

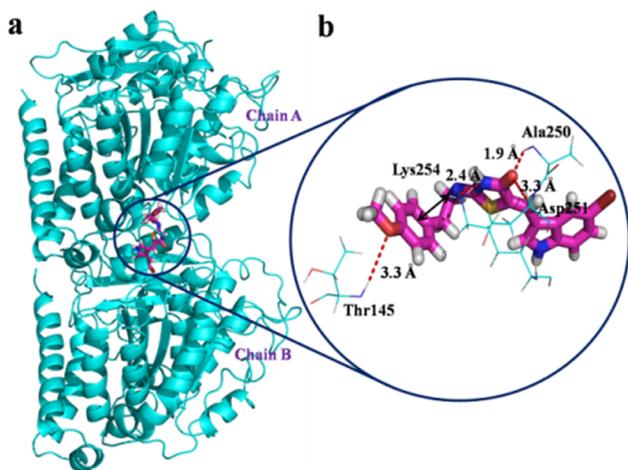


Fig. 8. (a) Predicted binding pose of G37 (ball and stick); (b) pattern of interactions in the active pocket of  $\alpha/\beta$  tubulin. The red colour lines represent hydrogen bond interactions and black line indicates cation-arene interaction with Lys254. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

docking studies in the present work is to examine the ligand-protein complexation energy and ligand affinity to  $\alpha/\beta$  tubulin, thereby giving more perceptive results of ligands' pose and pattern of interaction at active pocket of human  $\alpha/\beta$  tubulin. Some of the recognized ligands confirmed great binding energy similar to the bound ligand, signifying stable ligand-protein complex formation, leading to the stronger binding.

Table 1

Binding energies ( $\Delta G_{\text{bind}}$ ) obtained for some of the synthesized benzyl/phenethyl thiazolidinone-indole hybrids and co-crystallized ligand.

S. No.	Ligand ID	Binding Energy (Kcal/mol)
1	G21	-53.195
2	G26	-70.353
3	G27	-52.844
4	G33	-63.735
5	G37	-64.325
6	G38	-39.718
7	Cocrystallized ligand	-71.189

### 3.7.3. *In silico* ADME/T studies

In order to examine drug-likeness, physicochemically important descriptors and pharmacokinetically key properties of synthesized molecules were assessed through QikProp program of Schrödinger software. Some of the computed ADME/T parameters (Table 2) are within their recommended range and are mentioned in supporting information. From this *in silico* study, we can compare the ADME/T properties of the synthesized molecules with that of known drugs. ADME/T prediction studies depicts that the synthesized hybrids conform to the good PSA and that they have appropriate logP values and show no violation in the recommended ranges of physico-chemical descriptors.

## 4. Conclusion

In summary, we have synthesized a series of benzyl/phenethyl thiazolidinone-indole hybrid compounds (G1–G44) and characterized them by various analytical techniques such as HRMS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR.

**Table 2**

ADME/T Profile of some of the synthesized benzyl/phenethyl thiazolidinone-indole hybrids and other known tubulin inhibitors.

Ligand ID	Parameters									
	PSA	QPlogKhsa	Predicted octanol/water partition coefficient	Predicted polarizability	Predicted CNS Activity	Predicted apparent Caco-2 cell permeability in nm/s	Predicted brain/blood partition coefficient	Predicted skin permeability	Ionization Potential	Percent Human Oral Absorption
G21	68.428	0.944	5.706	43.656	0	649.268	-0.499	-2.280	8.573	100.000
G26	68.439	1.099	5.551	45.855	-1	656.605	-0.941	-2.190	8.600	96.911
G27	87.819	0.506	4.141	43.140	-2	649.812	-1.234	-2.012	9.005	100.000
G33	77.665	0.506	3.932	38.916	-2	536.906	-1.106	-2.167	8.467	100.000
G37	77.716	0.584	4.245	39.634	-1	531.574	-0.970	-2.324	8.627	100.000
G38	77.521	0.707	4.702	42.177	-2	549.938	-1.057	-2.183	8.563	90.565
C	92.246	-0.050	2.573	40.449	-1	542.309	-0.780	-2.798	8.571	90.950
P	97.519	-0.125	2.361	36.743	-1	1745.470	-0.314	-2.242	8.915	100.000
V	174.957	1.435	5.226	80.172	0	15.509	-0.918	-7.316	8.142	39.976

C # Colchicine; P # Podophyllotoxin; V # Vincristine.

Anti-proliferative activity of these compounds was evaluated in A549, NCI-H460 (lung cancer), MDA-MB-231 (breast cancer), HCT-29 and HCT-15 (colon cancer) cell lines using MTT assay. Amongst all the synthesized hybrids, compound **G37** showed excellent anti-proliferative activity on all the tested cell lines, especially in colon cancer cell lines HCT-15, HCT-29 ( $IC_{50}$  of  $0.92 \pm 0.12$  and  $2.802 \pm 0.02 \mu\text{M}$ , respectively). Additionally, compound **G37** significantly down-regulated the tubulin polymerization with  $IC_{50}$  value of  $2.92 \pm 0.23 \mu\text{M}$ . The cell cycle analysis disclosed that compound **G37** showed sub-G1 and significant G2/M phase arrest in HCT-15 cells. Further, compound **G37** impaired the morphological changes by inducing intracellular ROS and increased the loss of mitochondrial membrane potential, thus causing the apoptosis-mediated cell death in HCT-15 cells. The molecular modeling studies inferred that compound **G37** binds at the colchicine binding site of the tubulin with prominent binding affinity, stable ligand-protein complexation, good PSA as well as appreciative logP and physico-chemical descriptors. Taking everything into account, benzyl/phenethyl thiazolidinone-indole hybrids presented in this work signify the exciting development of new anti-proliferative agents through modifications in their chemical structure.

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### Declaration of Competing Interest

The authors have declared no conflict of interest.

### Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2019.103188>.

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