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Design, synthesis and biological evaluation of novel indole-based oxalamide and aminoacetamide derivatives as tubulin polymerization inhibitors

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ABSTRACT: A series of novel indole-based oxalamide and aminoacetamide derivatives were designed, synthesized, and evaluated for antiproliferative activities. Preliminary results revealed that compound **8g** exhibited significant antiproliferative effect against PC-3, HeLa and HCT-116 cell lines. Flow cytometric analysis of the cell cycle demonstrated the compound **8g** induced the cell cycle arrest at G2/M phase in HeLa cell lines. Immunocytochemistry revealed loss of intact microtubule structure in cells treated with **8g** and inhibition of tubulin polymerization. Additionally, molecular docking analysis suggested that **8g** formed stable interactions in the colchicine-binding site of tubulin. These preliminary results demonstrated that a new class of novel indole-based oxalamide and aminoacetamide derivatives described in the investigation could be developed as potential scaffolds to new anticancer agents.

Keywords: Indoles; Synthesis; Tubulin polymerization; Antiproliferative activity.

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# These authors contributed equally to this work.

Tubulin, the basic protein component of microtubules, has been identified as a highly attractive molecular target of numerous anticancer agents. [1-3] Inhibition of tubulin polymerization or blocking microtubule disassembly disrupts essential cellular functions, such as maintenance of cell shape, cell signaling, cell division, and even transportation of vesicles and mitochondria. [4-8] In the former family of agents, colchicine, one of the earliest tubulin-targeting inhibitors, played a crucial role in clarifying the properties and functions of tubulin and microtubules, and its binding site on tubulin has attracted great attention. [9-12] However, the clinical development of colchicine as an anticancer agent did not succeed due to its serious side effects. [13] Therefore, it is an urgent need to develop new small moleculars as potent tubulin inhibitors with better therapeutic properties for clinical.

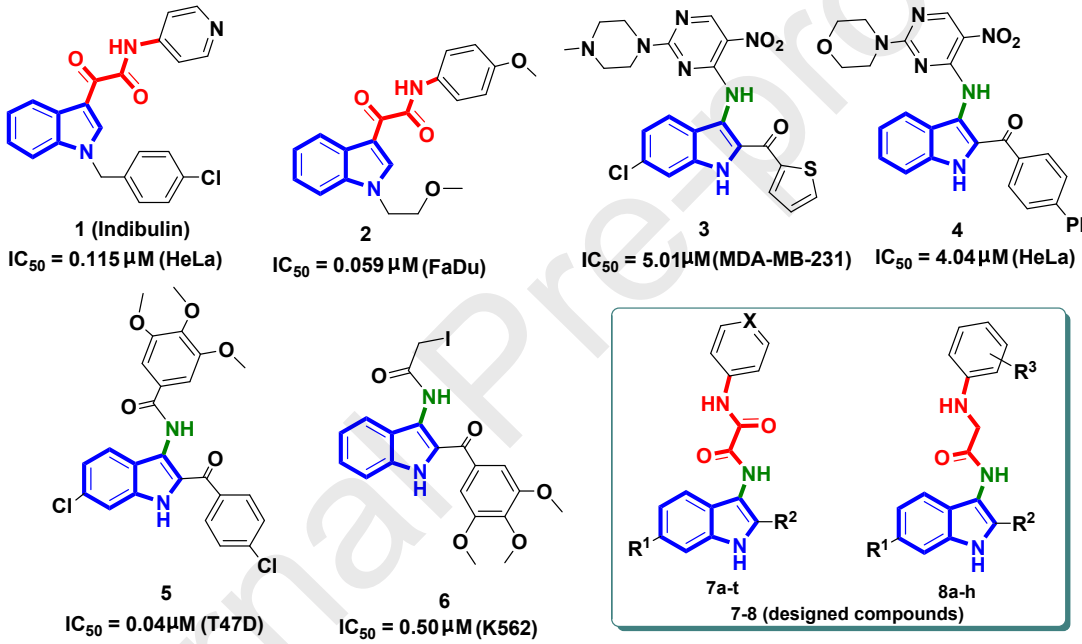
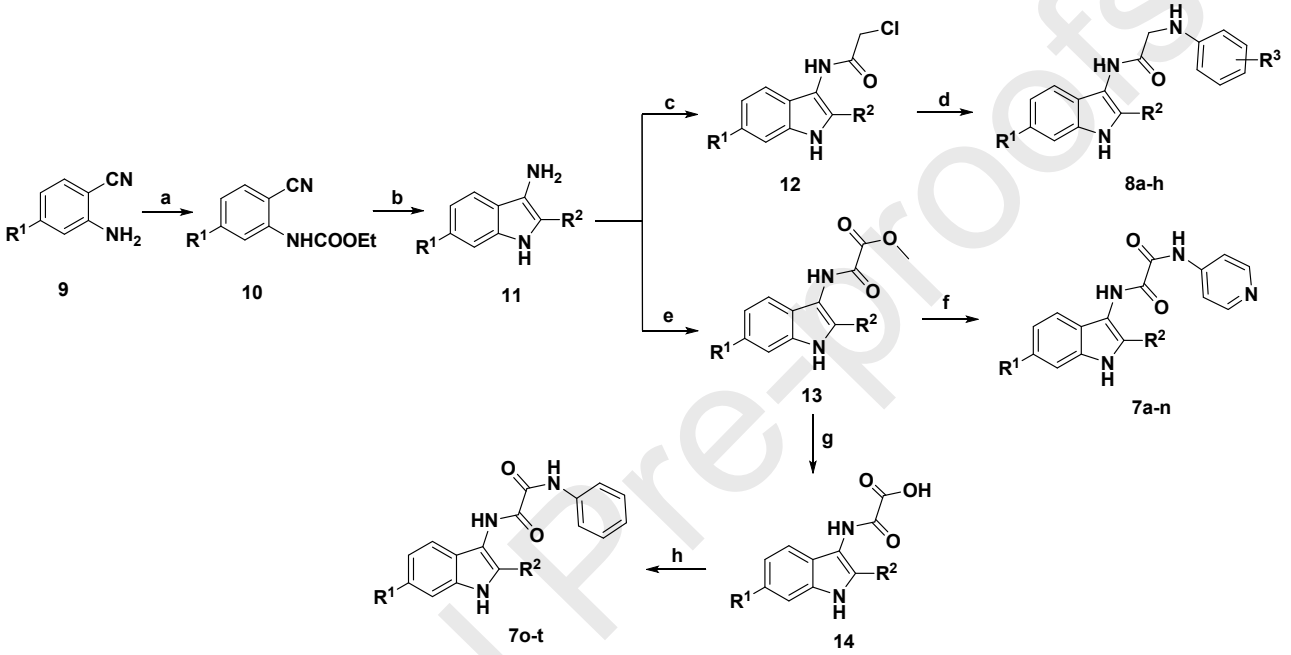


Fig. 1. Structures of representative 3-substituted indole derivatives **1**–**6** with antitumor activity, and newly designed compounds **7**–**8**.

Over the past few decades, a large number of structurally diverse anticancer agents have been identified as potent tubulin polymerization inhibitors. [14-16] Of which, indole core is the common structural feature of a great number of inhibitors, especially indolyl-3-glyoxamide derivatives exemplified by compound **1** (indibulin) possessing excellent antitumor activity with a minimal neurotoxicity in Phase I clinical trials for cancer chemotherapy, [17-18] and another recently reported analogue **2** (Fig. **1**) also displaying potent antiproliferative activities. [19] In our previous work, [20-22] a series of tubulin polymerization inhibitors were obtained by employing 3-aminoindole as the core structure, such as compounds **3**–**5** and another recently reported analogue **6** (Fig. **1**), [23] all of which

demonstrated remarkable antiproliferative activities against a panel of cancer cell lines. Encouraged by the therapeutic significance of indolyl-3-glyoxylamides and structural features of the 3-aminoindole scaffold, we are very interested in the indole-based oxalamide and aminoacetamide derivatives **7**–**8**, as shown in Fig. **1**, by incorporating glyoxylamide and acetamide moiety into the 3-aminoindole nucleus. Herein we report the detailed synthesis, antiproliferative evaluation and inhibition of tubulin polymerization of some promising analogues.



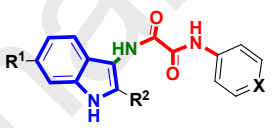
Scheme 1. Synthesis of the target compounds **7**–**8**. Reagents and conditions: (a) $ClCO_2Et$, reflux; (b) i) K_2CO_3/ DMF/EtOH, 80°C, 1 h; ii) 12% NaOH, 80°C, 15-30 min (c) Chloroacetyl chloride, K_2CO_3, THF, rt, 6 h; (d) R^3-NH_2, DIPEA, 2-methoxyethanol, 120°C, 20 h; (e) Methyl chlorooxoacetate, K_2CO_3, THF, rt, 6 h; (f) 4-aminopyridine, TBD (30 mol%), DMF, N_2, 85°C, overnight; (g) i) THF, 1% NaOH (aq), rt, 1 h ii) HCl, rt, 10 min; (h) i) CDI, THF, rt, 1h; ii) phenylamine, rt, overnight.

The synthetic sequence employed for the designed compounds **7**–**8** was illustrated in Scheme **1**. The required diverse intermediates 2,6-disubstituted-3-aminoindoles **11** were synthesized *via* a highly efficient one-pot method starting from ethyl 2-cyanophenylcarbamate **10** and α-bromoketones, which had been demonstrated in detail in our previously reported approach. [24] The 3-aminoindoles were then treated with methyl chlorooxoacetate in the presence of potassium carbonate (K_2CO_3) as base to give corresponding methyloxamates **13**, followed by aminolysis reaction with 4-aminopyridine to provide the target indole-based oxalamide derivatives **7a–n** in moderate to good isolated yields. Some phenyl-substituted oxalamide analogues **7o–t** not

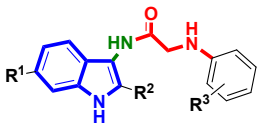
accessible through direct aminolysis were also generated using a straightforward two-step procedure including alkaline hydrolysis and nucleophilic substitution reaction. Similarly, aminoacetamides **8a–h** derived from 3-amine indoles were successfully obtained by a nucleophilic substitution reaction of suitable phenylamine with 2-chloroacetamide derivatives **12** which was prepared by acylation of substituted 3-amine indoles **11** with chloroacetyl chloride. The structures of the newly prepared indole-based oxalamide **7a–t** and aminoacetamide derivatives **8a–h** were characterized by ^{1}H NMR, ^{13}C NMR, IR and HRMS spectroscopic techniques, and the results are shown in the supplementary section.

All the synthesized target compounds were initially evaluated for *in vitro* antiproliferative activities against the human cervical cancer cells (HeLa) through MTT screening assay. Moreover, some potent analogues were further tested against HCT116 and PC-3 cells, which originated from different tissues of human. For comparison, a well-known tubulin-binding anticancer agent Colchicine was employed as a positive control, and the results expressed as IC_{50} (μM) were summarized in Table **1** and **2**. Here, the IC_{50} value represents the concentration of a compound resulting in 50% inhibition of cell growth after 48 h incubation with the compound, and is the average of three independent experiments.

Table 1 Antiproliferative activities of compounds 7–8 against HeLa cells.



7a-t



8a-h

Comp.	X	R^1	R^2	R^3	IC_{50} (μM) [a]
					HeLa
7a	N	H		\	85.26±5.88
7b	N	H		\	56.79±4.21
7c	N	H		\	>100
7d	N	H		\	62.41±5.09
7e	N	Cl		\	>100
7f	N	Cl		\	21.80±2.12

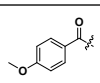
7g	N	Cl		\	80.65±4.02
7h	N	Cl		\	69.57±4.56
7i	N	CH_3		\	75.13±3.92
7j	N	CH_3		\	56.87±3.48
7k	N	CH_3		\	>100
7l	N	CH_3		\	>100
7m	N	H	H	\	>100
7n	CH	H		\	62.92±4.39
7o	CH	H		\	>100
7p	CH	Cl		\	29.16±3.39
7q	CH	Cl		\	>100
7r	CH	CH_3		\	55.20±2.25
7s	CH	CH_3		\	82.33±6.31
7t	CH	H	H	\	76.71±4.49
8a	\	H		2-CH_3	31.09±3.53
8b	\	H		4-CH_3O	16.20±0.63
8c	\	H		2-CH_3	27.88±4.13
8d	\	Cl		4-CH_3	18.30±1.30
8e	\	Cl		4-F	16.74±2.45
8f	\	Cl		3,4,5-$(CH_3O)_3$	＞100
8g	\	Cl		H	15.41±1.80
8h	\	Cl		3,4,5-$(CH_3O)_3$	＞100
		Colchicine			0.03±0.004

[a] IC_{50} values are presented as mean values of three independent experiments done in quadruplicates.

In general, antiproliferative activities obviously vary with respect to substitution on the position-3 of indole ring. Almost all of the indole derivatives bearing oxalamide group **7a–t** displayed inactive (IC_{50} >100 μM) or poor potency against the tested cell lines, while analogues **8b, 8d**, **8e**, and **8g**, which incorporate aminoacetamide moiety at the position-3 of indole ring, exhibited strong antiproliferative activities. The observed results obviously demonstrate that aminoacetamide derivatives **8a–h** manifest greater antitumor activities against tested cell lines than oxalamide analogues **7a–t**. Within the series of oxalamide derivatives **7a–t**, replacement of phenyl ring with pyridine nucleus leads to slight increase of antiproliferative activities (**7a** vs **7n**, **7e** vs **7p, 7i** vs **7r, 7k** vs **7s, 7m** vs **7t**). Meanwhile in the series of aminoacetamide analogues **8a–h**, antiproliferative activities were obviously dependent on the substitution pattern of the phenyl ring in aminoacetamide part. It is interesting that introduction of a 3,4,5-trimethoxy group in phenyl ring (**8f, 8h**), a well-defined pharmacophore for the inhibitors of tubulin found in colchicine and combretastatin A-4, dramatically decreased growth inhibitory properties.

To further explore the antiproliferative potential, the effect of highly active analogues **8b, 8d**, **8e**, and **8g** against a panel of two tumor cell lines derived from different tissues of human tumors was screened. All compounds showed good to excellent antiproliferative activities against HCT-116 and PC-3 cell lines (Table **2**). To confirm the growth inhibitory effect of the novel compounds was related to an interaction with the tubulin system, we investigated them direct inhibitory effects on tubulin polymerization *in vitro* at 10 μM concentration. The results obtained with the test agents are summarized in Table **2**. Among them, compound **8g,** exerted the most excellent antiproliferative activities, was aslo found to display the best inhibition activity (46%), while Colchicine exhibited a 77% inhibition effect at the tested concentration.

Table 2 Antiproliferative activities and tubulin polymerization inhibitory activities of representative selected compounds 8b, 8e, 8g, and 8h.

Comp.	R^1	R^2	R^3	IC_{50}(μM)		Tubulin polymerization
				HCT116	PC-3	% inhibition [a]
8b	H		4-CH_3O	46.89±2.14	43.71±2.88	7
8d	Cl		4-CH_3	20.42±3.75	15.82±1.93	29
8e	Cl		4-F	18.24±3.70	21.78±2.49	43

8g	Cl		H	11.99±1.62	14.43±2.12	46
		Colchicine		0.25±0.11	0.04±0.02	79

[a] Compounds were tested at a final concentration of 10μM.

In order to confirm the mode of action of these analogues on cancer cells, the influence of different concentrations of the highly active analogue **8g** on cell cycle progression was studied with HeLa cells which were treated with compound **8g** at the concentrations of 7.5, 15, and 30 μM for 24h. As illustrated in Fig. **2**, there was an accumulation of 14.20% (7.5 μM), 20.70% (15.0 μM), and 25.20% (30 μM) of cells in G2/M phase of the cell cycle observed in contrast to that of untreated control (11.76%). These observations indicated that compound **8g** induced a significant block in the G_2/M phase via a concentration-dependent manner.

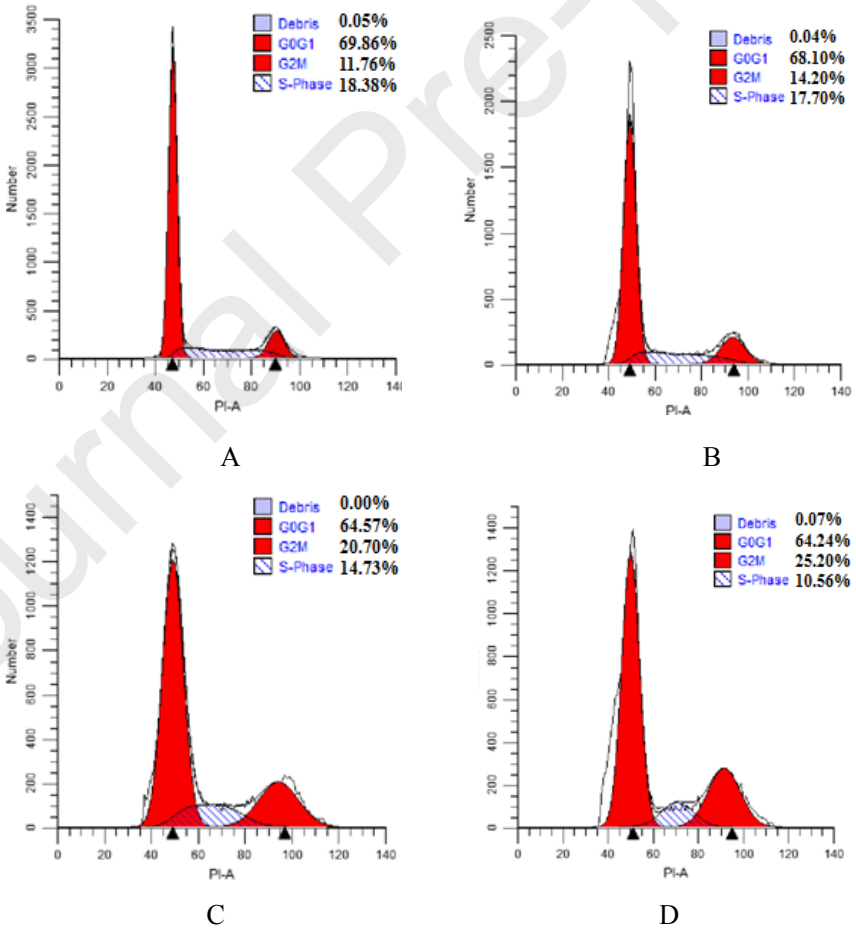


Fig. 2. Effect of compound **8g** on cell cycle in HeLa cells. Flow cytometry analysis of HeLa cells stained with propidium iodide and treated with **8g** for 24 h. (A) Control; (B) **8g**, 7.5 μM; (C) **8g**, 15 μM; (D) **8g**, 30 μM.

Since the tubulin−microtubule system plays a key role in maintenance of the cellular morphology, [5,6] to investigate whether the most active analogue **8g** could affect microtubule dynamics in living cells, we further explored the in situ effect of compound **8g** on tubulin organization in intact cells, and the microtubule structure of cells was visualized *via* immunocytochemistry. As shown in Fig. **3**, the control group demonstrated an intact network formed by *α*- and *β*-tubulin microtubules in the untreated HeLa cells, which were described with regularly assembled, and normal filiform microtubules wrapped around the uncondensed cell nucleus. Whereas cells treated with 15 and 30 μM of **8g** for 12 h caused obvious disruption of both tubulin subtypes, the microtubule spindles shrank significantly around the center of the cells, and formation of cell membrane rounding, thus exhibiting the inhibition of tubulin polymerization.

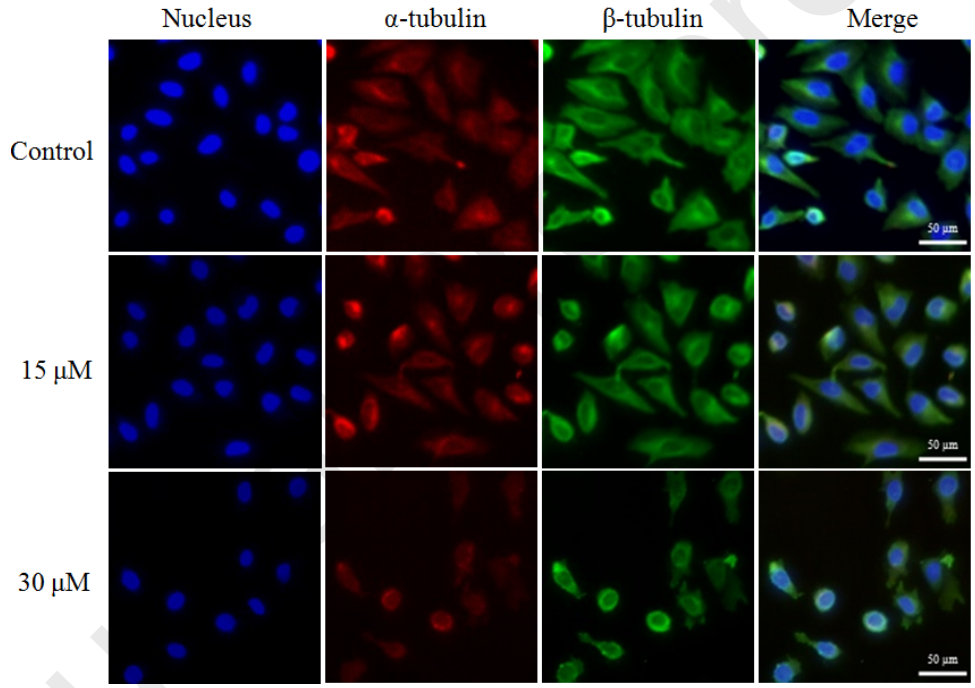


Fig.3. Effect of compound **8g** on tubulin expresion in HeLa cell. Cells were seeded on glass coverslips, incubated with compound **8g** (15 μM, 30 μM) for 12 h (Scale bar = 50 μm), then fixed and processed for confocal microscopy.

The possible binding mode for the most active derivative **8g** was carried out into the colchicine binding site of the tubulin crystal structure (PDB: 1SA0) using molecular docking studies. As depicted in Fig. **4**, indole ring and benzoyl of the analogue were located deeply into the β-subunit of tubulin, while phenylamine group extended toward the α/β-tubulin interface and analogue **8g** forms three important hydrogen bond interactions with amino acids of tubulin. An amide group of derivative **8g** established one hydrogen binding interaction in which carbonyl oxygen interacts with

βCys241 (2.2 Å). Furthermore, in the α/β-tubulin interface, the residues of αLys352 and βAla317 form two hydrogen bonds with the nitrogen of the phenylamine group, respectively. These molecular docking results demonstrated that compound **8g** could interact with the colchicine binding site betweenα and β subunits of tubulin.

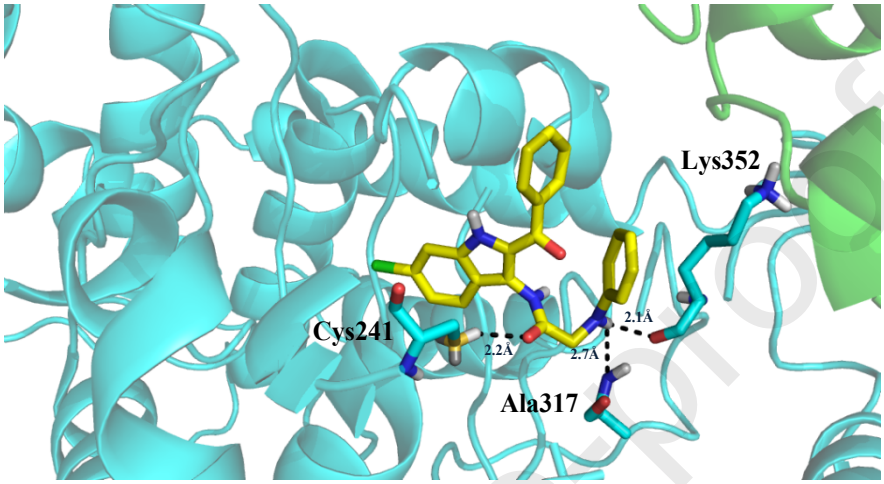


Fig.4. Proposed binding mode of compound **8g** in colchicine binding site of tubulin. The main interacting residues are shown and labeled. The black dashed lines are the potential H-bond between Cys241 (2.2 Å), Ala317 (2.7 Å), Lys352 (2.1 Å).

In conclusion, a new class of novel indole-based oxalamide and aminoacetamide derivatives were synthesized and evaluated for their antiproliferative activities. Among them, analogue **8g** displayed significant antiproliferative activities against PC-3, HeLa and HCT-116 cell lines. The flow cytometric analysis revealed that the compound caused cell cycle arrest at G2/M phase. Notably, the compound **8g** exhibited potent antitubulin activity and effectively inhibit microtubule assembly and disrupt the microtubule organization in the HeLa cells. Furthermore, docking studies revealed that **8g**, as a typical potent tubulin polymerization inhibitor, formed stable interactions in the colchicine binding site in the α/β-tubulin interface. These preliminary results encourage further investigation on these analogues as potential scaffolds to develop new anticancer agents.

Acknowledgments

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2019.xx.xxx.

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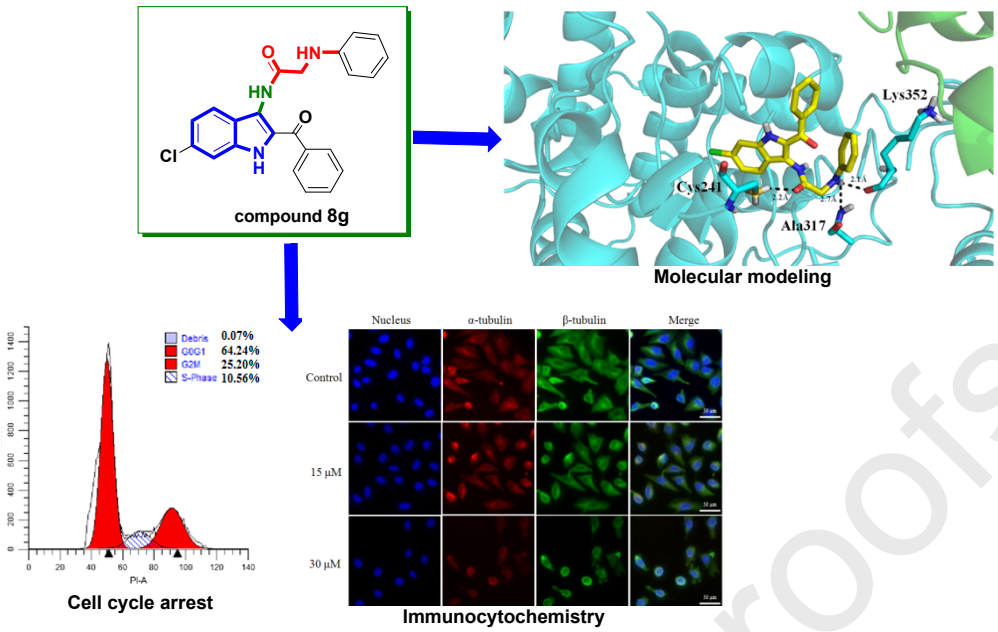

25.

Highlights

► A series of novel indole-based oxalamide and aminoacetamide derivatives were synthesized. ► **8g** exhibited significant antiproliferative effect against PC-3, HeLa and HCT-116 cell lines. ► Immunocytochemistry revealed loss of intact microtubule structure in cells treated with **8g**.

26.

Declaration of interests

☒ The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

27.