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Synthesis, biological evaluation, and molecular docking studies of cinnamic acyl 1,3,4-thiadiazole amide derivatives as novel antitubulin agents

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ABSTRACT

A series of cinnamic acyl 1,3,4-thiadiazole amide derivatives (**6a**–**10e**) have been designed and synthesized, and their biological activities were also evaluated as potential antiproliferation and tubulin polymerization inhibitors. Among all the compounds, **10e** showed the most potent activity in vitro, which inhibited the growth of MCF-7 and A549 cell lines with IC_{50} values of 0.28 and 0.52 μg/mL, respectively. Compound **10e** also exhibited significant tubulin polymerization inhibitory activity (IC_{50} = 1.16 μg/mL). Docking simulation was performed to insert compound **10e** into the crystal structure of tubulin at colchicine binding site to determine the probable binding model. Based on the preliminary results, compound **10e** with potent inhibitory activity in tumor growth may be a potential anticancer agent.

1. Introduction

Cancer is one of the most serious threats against human health in the world, and the clinical prognosis remains relatively poor. Strategies to block cell division by affecting the mitotic spindle have historically been a successful area of research for the advancement of cancer drugs.[1,2] For example, Colchicine (Fig. 1) is the first drug that is well known to bind tubulin, and the colchicine binding site has been characterized recently.[3] Combretastatin A-4 (CA-4) (Fig. 1), which was isolated from the South African tree *Combretum caffrum*, was found to inhibit the polymerization of tubulin by binding to the colchicine site.[4,5] The development of ketones as tubulin binding agents such as chalcones was reviewed.[6]

Chalcones are an important pharmacophore of various natural products and synthetic precursors including flavonoids and isoflavonoids. A variety of pharmacological activities of chalcones have been reported, including anticancer,[7–9] anti-inflammatory,[10,11] anti-tuberculosis,[12] anti-fungal and antiproliferative activities.[13–19] Their broad biological properties are largely due to the α,β-unsaturated ketone moiety.[20] Cinnamon amide owns the similar part, α,β-unsaturated ketone moiety (Fig. 2), and cinnamoyl moiety was found in a variety of biologically active substances,[21,22] antitubulin activities of various cinnamoyl derivatives were also explored.[23,24]

On the other hand, thiadiazoles have gained prominence by exhibiting a wide variety of biological activities as well as producing useful intermediates in several organic preparations,[25–28] and are extensively investigated for their anticancer activity due to their therapeutic potential.[29–31] Furthermore, thiazoles and oxadiazoles, both being five-membered heterocycle, displayed potent biological activities and low toxicities in previous reports. Among them, some compounds showed their anticancer effects by inhibiting tubulin to form microtubule, such as compound A and A-105972 (Fig. 1).[32,33] As is known thiadiazoles are the bioisosters of thiazoles and oxadiazoles, it is supposed that thiadiazoles may also be a potential antitubulin agent.

These previous researches encouraged us to integrate cinnamon amide with thiadiazoles to screen new cinnamic acyl 1,3,4-thiadiazole amides as potential antitubulin agents. The two combined substructures, cinnamon amide and thiadiazoles, might exhibit synergistic effect in anticancer activities. The objectives of the present work are (1) to synthesize new cinnamic acyl 1,3,4-thiadiazole amides; (2) to evaluate their anticancer and antitubulin activities; (3) to explore the preliminary mechanism of their role in cell division cycle and (4) to investigate the potential inhibitor's interaction with tubulin by docking study.

2. Results and discussion

2.1. Chemistry

The synthetic route of the cinnamic acyl 1,3,4-thiadiazole amide derivatives **6a–10e** is outlined in Scheme 1. These compounds were synthesized from 2-amino- 1,3,4-thiadiazoles **6–10** and cinnamic acids **a–e**. Firstly, the different substituted benzoic acid **1–5** were treated with thiosemicarbazide in presence of phosphoryl chloride

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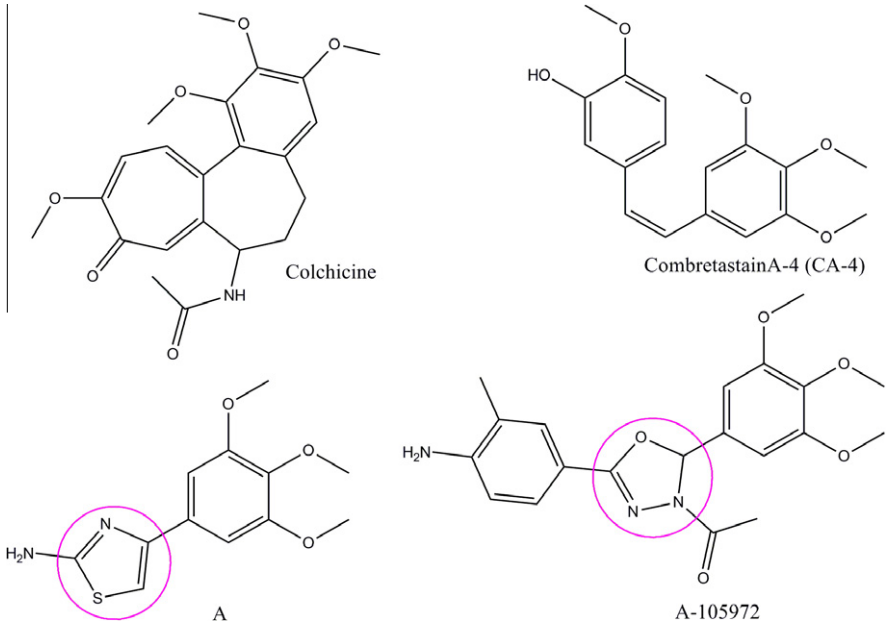


Figure 1. Chemical structures of antimitotic agents and lead tubulin inhibitors.

yielded 2-amino-1,3,4-thiadiazoles. Secondly, compounds **a–e** were prepared according to the procedure reported by Davis et al. with some modifications.[34] Aromatic aldehydes and malonic acid were dissolved in a mixture of pyridine and piperidine and refluxed for 12 h, and cinnamic acids were obtained with yields of 80–90%. Thirdly, the coupling reaction between the obtained different substituted 2-amino-1,3,4-thiadiazoles and cinnamic acids was performed by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCl) and *N*-hydroxybenzotriazole (HOBt) in anhydrous methylene dichloride, and refluxed to give the desired compounds **6a–10e** (Table 1). Among these compounds, **6c, 7b–7e, 8b–8e, 9b–9e** and **10b–10e** are reported for the first time. All of the synthetic compounds gave satisfactory elementary analytical and spectroscopic data. ^{1}H NMR and ESI-MS spectra were consistent with the assigned structures.

2.2. Bioactivity

To test the anticancer activities of the synthesized compounds, we evaluated antiproliferative activities of compounds **6a–10e** against MCF-7 and A549 cells. The results were summarized in Table 2. These 1,3,4-thiadiazole compounds bearing the cinnamoyl moiety showed remarkable antiproliferative effects. Among them, compound **10e** displayed the most potent inhibitory activity (IC_{50} = 0.28 μg/mL for MCF-7 and IC_{50} = 0.52 μg/mL for A549), comparable to the positive control colchicine (IC_{50} = 0.53 μg/mL for MCF-7, IC_{50} = 0.75 μg/mL for A549, respectively).

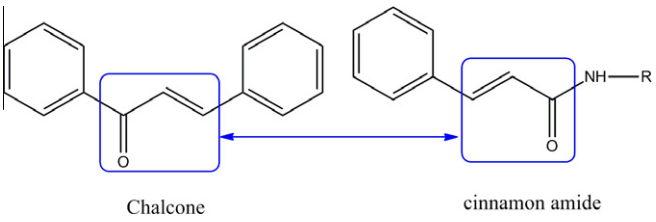
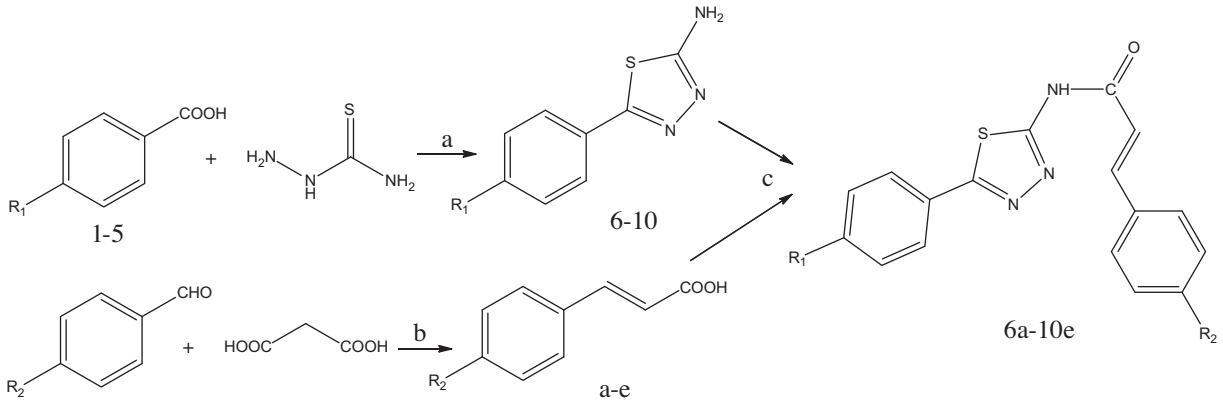


Figure 2. Similar part of cinnamon amide and colchicine.

Structure–activity relationships in these cinnamic acyl 1,3,4-thiadiazole amides derivatives demonstrated that compounds with *para* electron-donating substituents (**9a,10a**) showed more potent activities than those with electron-withdrawing substituents (**7a, 8a**) in the A-ring. A comparison of the *para*-substituents on the A-ring demonstrated that an electron-donating group (**9a,10a**) have slightly improved antiproliferative activity and the potency order is OMe > Me, whereas a Cl (**7a**) and Br (**8a**) group substituent had minimal effects compared with **6a**. In the case of constant A-ring substituents, change of substituents on B-ring could also affect the activities of these compounds. Among the compounds **10a–10e**, these compounds (**10d, 10e**) with *para* electron-donating substituted showed stronger anticancer activity and the strength order was similar with A-ring: OMe > Me, followed that **10b** and **10c** led to a noteworthy poor activity. Among all the compounds, **10e** with *para*- OMe group in the both A-ring and B-ring showed the best activity. To examine whether the compounds interact with tubulin and inhibit tubulin polymerization, we performed the tubulin in vitro assembly assay. As shown in Table 2, compounds **9e, 10d** and **10e** showed strong inhibitory effect and their 50% tubulin polymerization inhibition about 2.64 μg/mL, 2.56 μg/mL, 1.16 μg/mL, respectively (the positive control colchicine with an IC_{50} of 1.72 μg/mL for tubulin). Compound **10e** displayed the most potent anti-tubulin polymerization activity. This result indicated the anti-proliferative effect was possibly produced by direct connection of tubulin and the compound.

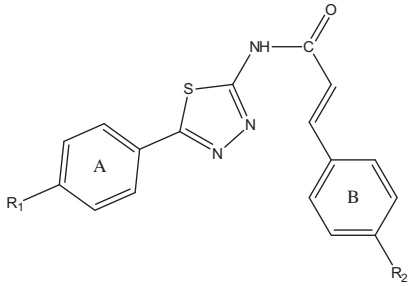
Furthermore, compound **10e** was further assayed for their effects on cell cycle using flow cytometry (Fig. 3). As shown in Figure 3, 68% of these cells were arrested at G2/M period after treatment with **10e** of 5 μg/mL for 24 h. These findings indicated a continuing impairment of cell division and confirmed compound **10e** was a potent antitubulin agent.

To gain better understanding on the potency of the studied compounds and guide further SAR studies, we proceeded to examine the interaction of compound **10e** with tubulin crystal structure (PDB code: 1SA0). The molecular docking was performed by inserting compound **10e** into the colchicine binding site of tubulin. All docking runs were applied LigandFit Dock protocol of Discovery Studio 3.1. The binding modes of compound **10e** and tubulin were depicted in Figure 4. All the amino acid residues which had inter-



Scheme 1. General synthesis of cinnamic acyl 1,3,4-thiadiazole amide derivatives (**6a**–**10e**). Reagents and conditions: (a) $POCl_3$, reflux, 8 h, 50% NaOH; (b) piperidine, pyridine, 80–90 °C, 12 h; (c) EDCl, HOBt, dichloromethane, reflux, 8 h.

Table 1
Structure of compounds **6a**–**10e**



Compounds	**6a**	**6b**	**6c**	**6d**	**6e**	**7a**	**7b**	**7c**	**7d**	**7e**
R_1	H	H	H	H	H	Cl	Cl	Cl	Cl	Cl
R_2	H	F	CF_3	CH_3	OCH_3	H	F	CF_3	CH_3	OCH_3
Compounds	**8a**	**8b**	**8c**	**8d**	**8e**	**9a**	**9b**	**9c**	**9d**	**9e**
R_1	Br	Br	Br	Br	Br	CH_3	CH_3	CH_3	CH_3	CH_3
R_2	H	F	CF_3	CH_3	OCH_3	H	F	CF_3	CH_3	OCH_3
Compounds	**10a**	**10b**	**10c**	**10d**	**10e**					
R_1	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3					
R_2	H	F	CF_3	CH_3	OCH_3					

actions with tubulin were exhibited in Figure 4c. In the binding mode, compound **10e** is nicely bound to the colchicine binding site of tubulin via hydrophobic interactions and binding is stabilized by two hydrogen bonds and two π-cation interactions. The nitrogen atom of the amide bond formed one hydrogen bond with the amino hydrogen of Thr C:179 (bond length: Thr179 N–H···N = 2.774 Å; bond angle: Thr179 N–H···N = 154.4°) and the oxygen atom of one of the methoxyl groups on B-ring of **10e** formed another one with the amino hydrogen of Lys D:254 (bond length: Lys D:254 N–H···O = 2.232 Å; bond angle: Lys D:254 N–H···O = 151.8°). The enzyme surface model was showed in Figure 4a, which revealed that the molecule was well embedded in the active pocket. Overall, these results of the molecular docking study showed that the 1,3,4-thiadiazole skeleton and cinnamon amide could act synergistically to interact with the colchicine binding site of tubulin, suggesting that compound **10e** is a potential inhibitor of tubulin.

3. Conclusion

In this study, a series of novel antitubulin polymerization inhibitors (**6a–10e**) bearing 1,3,4-thiadiazole skeleton and cinnamon amide had been synthesized and evaluated their biological activities. These compounds exhibited potent antiproliferative activities against MCF-7 and A549 cells and tubulin polymerization inhibitory activities. Among all of the compounds, **10e** showed the most potent inhibition activity which inhibited the growth of MCF-7 and A549 cell lines with IC_{50} values of 0.28 and 0.52 μg/mL and inhibited the polymerization of tubulin with IC_{50} of 1.16 μg/mL. Molecular docking was further performed to study the inhibitor-tubulin protein interactions. After analysis of the binding model of compound **10e** with tubulin, it was found that two hydrogen bond and two π-cation interaction with the protein residues in the colchicine binding site might play a crucial role in its antitubulin polymerization and antiproliferative activities. The information of this work might be helpful for the design and synthesis of tubulin polymerization inhibitors with stronger activities.

4. Experiments

4.1. Materials and measurements

All chemicals and reagents used in the current study were of analytical grade. All the ^1H NMR spectra were recorded on a Bruker DPX300 model Spectrometer in DMSO-d_6 and chemical shifts were reported in ppm (δ). ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument. TLC was performed on the glassbacked silica gel sheets (Silica Gel 60 GF254) and visualized

Table 2
Inhibition (IC_{50}) of MCF-7 and A549 cells proliferation and inhibition of tubulin polymerization by compounds **6a**–**10e**

Compound	IC_{50} ± SD (μg/mL)		
	MCF-7[a]	A549[a]	Tubulin[b]
6a	1.47 ± 0.17	1.75 ± 0.15	5.68 ± 0.74
6b	1.45 ± 0.19	1.82 ± 0.12	5.87 ± 0.58
6c	1.52 ± 0.12	1.78 ± 0.18	5.52 ± 0.54
6d	1.23 ± 0.19	1.58 ± 0.09	4.49 ± 0.35
6e	0.83 ± 0.08	0.76 ± 0.07	3.02 ± 0.42
7a	1.42 ± 0.14	2.02 ± 0.21	6.56 ± 0.86
7b	1.58 ± 0.15	2.06 ± 0.26	7.08 ± 0.97
7c	1.65 ± 0.09	3.09 ± 0.24	7.83 ± 1.05
7d	1.44 ± 0.14	1.25 ± 0.12	4.79 ± 0.55
7e	0.91 ± 0.09	1.38 ± 0.07	5.68 ± 0.60
8a	1.50 ± 0.07	2.18 ± 0.15	7.05 ± 0.85
8b	1.49 ± 0.08	2.83 ± 0.18	8.66 ± 1.03
8c	1.58 ± 0.13	4.68 ± 0.32	10.6 ± 1.66
8d	1. 32 ± 0.13	1.51 ± 0.13	5.02 ± 0.72
8e	1.13 ± 0.11	1.04 ± 0.08	4.11 ± 0.53
9a	1.19 ± 0.06	1.38 ± 0.09	4.83 ± 0.41
9b	1.27 ± 0.07	1.23 ± 0.14	5.08 ± 0.68
9c	1.10 ± 0.05	1.61 ± 0.16	5.16 ± 0.63
9d	0.61 ± 0.07	1.08 ± 0.10	4.15 ± 0.62
9e	0.35 ± 0.05	0.66 ± 0.05	2.64 ± 0.28
10a	0.78 ± 0.06	0.68 ± 0.08	3.08 ± 0.33
10b	0.85 ± 0.07	0.75 ± 0.11	3.05 ± 0.45
10c	0.79 ± 0.05	0.69 ± 0.10	3.17 ± 0.47
10d	0.31 ± 0.03	0.58 ± 0.05	2.56 ± 0.31
10e	0.28 ± 0.02	0.52 ± 0.06	1.16 ± 0.36
Colchicine	0.53 ± 0.07	0.75 ± 0.08	1.72 ± 0.18
CA-4	0.41 ± 0.04	0.55 ± 0.04	0.81 ± 0.09

[a] Inhibition of the growth of tumor cell lines.
[b] Inhibition of tubulin polymerization.

in UV light (254 nm). Column chromatography was performed using silica gel (200–300 mesh) eluting with ethyl acetate and petroleum ether.

4.2. General procedure for synthesis of 2-amino-1,3,4-thiadiazoles

A stirring mixture of substituted benzoic acid (10 mmol), thiosemicarbazide (10 mmol) and $POCl_3$ (10 mL) was heated at 75–80 °C for 6 h. After cooling down to room temperature, water was added. The reaction mixture was refluxed for 1 h. After cooling, the mixture was basified to pH 8–9 by the dropwise addition of 50% NaOH solution under ice bath. The precipitate was filtered and recrystallized from ethanol to gain compounds **6–10**.

4.3. General procedure for synthesis of cinnamic acids

A mixture of aromatic aldehydes (10 mmol), malonic acid (12 mmol), piperidine (12 mmol) was dissolved in pyridine and stirred on 80–90 °C for 12 h. The pyridine was removed at the vacuum. The reaction mixture was poured into ice water. And the precipitate was filtered and washed with 10% HCl, and dried under vacuum to afford the cinnamic acids **a–e**.

4.4. General procedure for synthesis of target compounds 6a–10e

Compounds **6a–10e** were synthesized by coupling substituted 2-amino-1,3,4-thiadiazoles with cinnamic acids, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCl) and N-hydroxybenzotriazole (HOBt) as condensing agent. The mixture was refluxed in anhydrous CH_2Cl_2 for 8–10 h. The products were extracted with ethyl acetate. The extract was washed successively with 10% HCl, saturated $NaHCO_3$ and water, respectively, then dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography using petroleum ether and ethyl acetate (3:1).

4.4.1. *N*-(5-Phenyl-1,3,4-thiadiazol-2-yl)cinnamamide (6a)

White powders, yield 80%, mp: 326–328 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 6.97 (d, J = 15.93 Hz, 1H); 7.47–7.51 (m, 3H); 7.54–7.57 (m, 3H); 7.66–7.69 (m, 2H); 7.82 (d, J = 15.72 Hz, 1H); 7.95–7.99 (m, 2H); 12.89 (s, 1H). ESI-MS: 308.37 ($C_{17}H_{14}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}N_3OS$: C, 66.43; H, 4.26; N, 13.67; Found: C, 66.31; H, 4.28; N, 13.71.

4.4.2. (*E*)-3-(4-Fluorophenyl)-*N*-(5-phenyl-1,3,4-thiadiazol-2-yl)acrylamide (6b)

White powders, yield 84%, mp: 348–350 °C, ^{1}H NMR(300 MHz, DMSO-d_6, δ ppm): 6.90 (d, J = 15.72 Hz, 1H); 7.33 (t, J = 8.87 Hz,

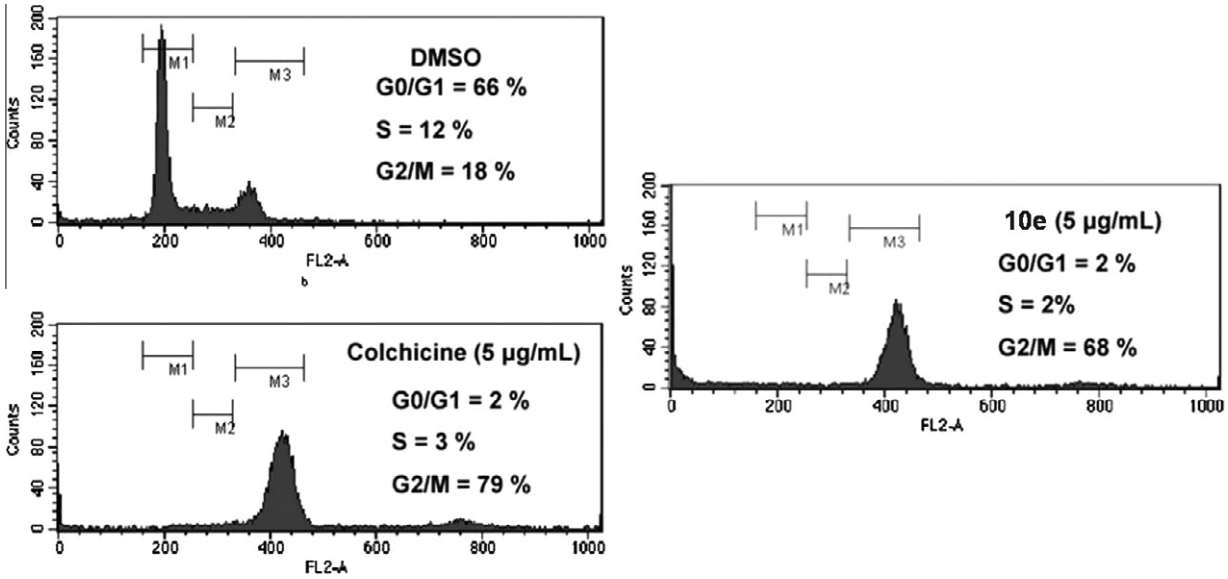


Figure 3. Effects of compound **10e** on cell cycle progression of MCF-7 cells were determined by flow cytometry analysis. MCF-7 cells were treated with **10e** for 24 h. The percentage of cells in each cycle phase was indicated.

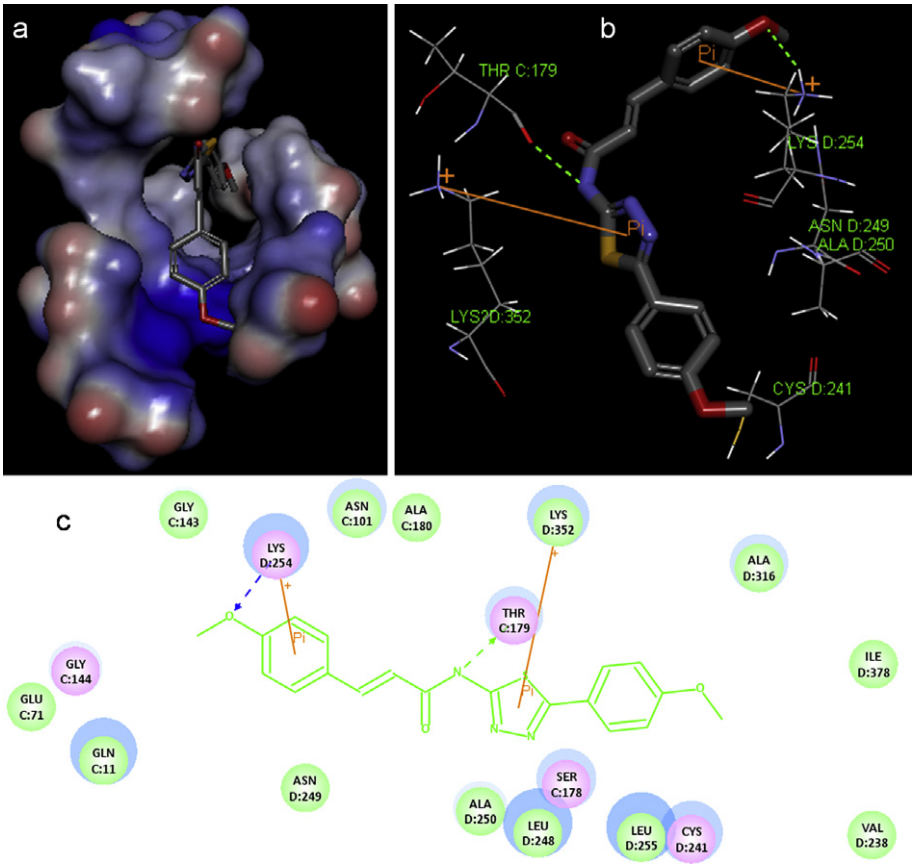


Figure 4. (a) The surface model structure of compound **10e** binding model with tubulin complex. (b) Compound **10e** (colored by atom: carbons: gray; nitrogen: blue; oxygen: red; sulfur: yellow) is bond into tubulin (entry 1SA0 in the Protein Data Bank). The dotted lines show the hydrogen bonds and the yellow line show the π-cation interactions. Thr C: 179 is the mean of Thr: 179 in C chain. (c) 2D Ligand interaction diagram of compound **10e** with tubulin using Discovery Studio program with the essential amino acid residues at the binding site are tagged in circles. The purple circles show the amino acids which participate in hydrogen bonding, electrostatic or polar interactions and the green circles show the amino acids which participate in the Van der Waals interactions.

2H); 7.54 (t, J = 2.84 Hz, 3H); 7.75 (t, J = 7.23 Hz, 2H); 7.82 (d, J = 15.72 Hz, 1H); 7.96–7.99 (m, 2H); 12.90 (s, 1H). ESI-MS: 326.36 ($C_{17}H_{13}FN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{12}FN_3OS$: C, 62.76; H, 3.72; N, 12.91; Found: C, 62.65; H, 3.75; N, 12.98.

4.4.3. (*E*)-*N*-(5-Phenyl-1,3,4-thiadiazol-2-yl)-3-(4-(trifluoromethyl)phenyl)acrylamide (6c)

White powders, yield 75%, mp: 315–316 °C, ^{1}H NMR(300 MHz, DMSO-d_6, δ ppm): 7.08 (d, J = 15.93 Hz, 1H); 7.54 (t, J = 2.93 Hz, 3H); 7.82–7.91 (m, 5H); 7.96 (t, J = 2.93 Hz, 2H); 12.96 (s, 1H). ESI-MS: 376.37 ($C_{18}H_{13}F_3N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{12}F_3N_3OS$: C, 57.59; H, 3.22; N, 11.19; Found: C, 57.75; H, 3.20; N, 11.14.

4.4.4. (*E*)-*N*-(5-Phenyl-1,3,4-thiadiazol-2-yl)-3-(*p*-tolyl)acrylamide (6d)

White powders, yield 87%, mp: 332–334 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.35 (s, 3H); 6.90 (d, J = 15.9 Hz, 1H); 7.29 (d, J = 8.07 Hz, 2H); 7.53–7.57 (m, 5H); 7.77 (d, J = 15.72 Hz, 1H); 7.95–7.98 (m, 2H); 12.85 (s, 1H). ESI-MS: 322.40 ($C_{18}H_{16}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{15}N_3OS$: C, 67.27; H, 4.70; N, 13.07; Found: C, 67.10; H, 4.72; N, 13.12.

4.4.5. (*E*)-3-(4-Methoxyphenyl)-*N*-(5-phenyl-1,3,4-thiadiazol-2-yl)acrylamide (6e)

White powders, yield 83%, mp:308–311 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.82 (s, 3H); 6.81 (d, J = 15.75 Hz, 1H); 7.04 (d, J = 8.58 Hz, 2H); 7.54 (t, J = 2.84 Hz, 3H); 7.63 (d, J = 8.58 Hz, 2H); 7.76 (d, J = 15.75 Hz,1H); 7.94–7.98 (m, 2H); 12.79 (s, 1H). ESI-MS: 338.40 ($C_{18}H_{16}N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{15}N_3O_2S$: C, 64.08; H, 4.48; N, 12.45; Found: C, 64.21; H, 4.45; N, 12.51.

4.4.6. *N*-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)cinnamamide (7a)

White powders, yield 78%, mp: 333–335 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 6.96 (d, J = 15.72 Hz, 1H); 7.38 (t, J = 8.7 Hz, 2H); 7.46–7.51 (m, 3H); 7.65–7.69 (m, 2H); 7.81 (d, J = 16.26 Hz, 1H); 8.00–8.05 (m, 2H); 12.91 (s, 1H). ESI-MS: 342.81 ($C_{17}H_{13}ClN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{12}ClN_3OS$: C, 59.73; H, 3.54; N, 12.29; Found: C, 59.62; H, 3.56; N, 12.25.

4.4.7. (*E*)-*N*-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)-3-(4-fluorophenyl)acrylamide (7b)

White powders, yield 88%, mp: 354–356 °C, ^{1}H NMR(300 MHz, DMSO-d_6, δ ppm): 6.90 (d, J = 15.72 Hz, 1H); 7.33 (t, J = 9.0 Hz, 2H); 7.61 (d, J = 8.58 Hz, 2H); 7.73–7.77 (m, 2H); 7.82 (d, J = 15.9 Hz, 1H); 8.00 (d, J = 8.61 Hz, 2H); 12.94 (s, 1H). ESI-MS: 360.81 ($C_{17}H_{12}ClFN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{11}ClFN_3OS$: C, 56.75; H, 3.08; N, 11.68; Found: C, 56.86; H, 3.10; N, 11.64.

4.4.8. (*E*)-*N*-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)-3-(4-(trifluoromethyl)phenyl)acrylamide (7c)

White powders, yield 83%, mp: 328–330 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 7.06 (d, J = 15.90 Hz, 1H); 7.38 (t, J = 8.78 Hz,

2H); 7.82–7.90 (m, 5H); 8.00–8.05 (m, 2H); 13.00 (s, 1H). ESI-MS: 410.81 ($C_{18}H_{12}ClF_3N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{11}ClF_3N_3OS$: C, 52.75; H, 2.71; N, 10.25; Found: C, 52.81; H, 2.72; N, 10.20.

4.4.9. (*E*)-*N*-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)-3-(*p*-tolyl)acrylamide (7d)

White powders, yield 81%, mp: 333–334 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.35 (s, 3H); 6.90 (d, J = 15.93 Hz, 1H); 7.29 (d, J = 8.04 Hz, 2H);7.39 (t, J = 8.88 Hz, 2H); 7.56 (d, J = 8.22 Hz, 2H); 7.77 (d, J = 15.72 Hz, 1H); 8.00–8.05 (m, 2H); 12.85 (s, 1H). ESI-MS: 356.84 ($C_{18}H_{15}ClN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{14}ClN_3OS$: C, 60.76; H, 3.97; N, 11.81; Found: C, 60.81; H, 3.99; N, 11.86.

4.4.10. (*E*)-*N*-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)-3-(4-methoxyphenyl)acrylamide (7e)

White powders, yield 76%, mp: 330–332 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.82 (s, 3H); 6.81 (d, J = 15.72 Hz, 1H); 7.04 (d, J = 8.58 Hz, 2H); 7.38 (t, J = 7.78 Hz, 2H); 7.63 (d, J = 8.58 Hz, 2H); 7.76 (d, J = 15.90 Hz,1H); 8.00–8.04 (m, 2H); 12.79 (s, 1H). ESI-MS: 372.84 ($C_{18}H_{15}ClN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{14}ClN_3O_2S$: C, 58.14; H, 3.79; N, 11.30; Found: C, 58.08; H, 3.80; N, 11.34.

4.4.11. *N*-(5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl)cinnamamide (8a)

White powders, yield 87%, mp: 351–353 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 6.96 (d, J = 15.72 Hz, 1H); 7.47–7.49 (m, 3H); 7.66–7.69 (m,2H); 7.74 (d, J = 8.61 Hz, 2H); 7.82 (d, J = 15.72 Hz, 1H); 7.92 (t, J = 4.31 Hz, 2H); 12.92 (s, 1H). ESI-MS: 387.27 ($C_{17}H_{13}BrN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{12}BrN_3OS$: C, 52.86; H, 3.13; N, 10.88; Found: C, 52.95; H, 3.12; N, 10.93.

4.4.12. (*E*)-*N*-(5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl)-3-(4-fluorophenyl)acrylamide (8b)

White powders, yield 90%, mp: 357–359 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 6.90 (d, J = 16.11 Hz, 1H); 7.32 (t, J = 8.58 Hz, 2H); 7.72–7.76 (m,4H); 7.82 (d, J = 15.72 Hz, 1H); 7.92 (d, J = 8.58 Hz, 2H); 12.94 (s, 1H). ESI-MS: 405.26 ($C_{17}H_{12}BrFN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{11}BrFN_3OS$: C, 50.51; H, 2.74; N, 10.39; Found: C, 50.57; H, 2.75; N, 10.36.

4.4.13. (*E*)-*N*-(5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl)-3-(4-(trifluoromethyl)phenyl)acrylamide (8c)

White powders, yield 88%, mp: 338–340 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 7.07 (d, J = 15.90 Hz, 1H); 7.75 (d, J = 8.61 Hz, 2H); 7.86 (t, J = 6.95 Hz, 4H); 7.93 (d, J = 8.61 Hz, 3H); 13.05 (s, 1H). ESI-MS: 455.26 ($C_{18}H_{12}BrF_3N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{11}BrF_3N_3OS$: C, 47.59; H, 2.44; N, 9.25; Found: C, 47.51; H, 2.43; N, 9.31.

4.4.14. (*E*)-*N*-(5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl)-3-(*p*-tolyl)acrylamide (8d)

White powders, yield 82%, mp: 341–343 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 3H); 6.89 (d, J = 15.72 Hz, 1H); 7.28 (d, J = 7.86 Hz, 2H); 7.55 (d, J = 8.04 Hz, 2H); 7.75 (t, J = 11.34 Hz, 3H); 7.91 (d, J = 8.40 Hz, 2H); 12.88 (s, 1H). ESI-MS: 401.29 ($C_{18}H_{15}BrN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{14}BrN_3OS$: C, 54.01; H, 3.53; N, 10.50; Found: C, 54.08; H, 3.51; N, 10.57.

4.4.15. (*E*)-*N*-(5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl)-3-(4-methoxyphenyl)acrylamide (8e)

White powders, yield 77%, mp: 336–338 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.82 (s, 3H); 6.80 (d, J = 15.75 Hz, 1H); 7.04 (d, J = 8.79 Hz, 2H); 7.63 (t, J = 8.94 Hz, 2H); 7.73–7.79 (m, 3H); 7.91 (d, J = 8.43 Hz, 2H); 12.83 (s, 1H). ESI-MS: 417.29 ($C_{18}H_{15}BrN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{14}BrN_3O_2S$: C, 51.93; H, 3.39; N, 10.09; Found: C, 51.80; H, 3.38; N, 10.04.

4.4.16. *N*-(5-(*P*-tolyl)-1,3,4-thiadiazol-2-yl)cinnamamide (9a)

White powders, yield 84%, mp: 322–324 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.38 (s, 3H); 6.96 (d, J = 15.93 Hz, 1H); 7.35 (d, J = 7.86 Hz, 2H); 7.48 (d, J = 2.48 Hz, 3H); 7.67 (d, J = 3.84 Hz, 2H); 7.83 (t, J = 12.81 Hz, 3H); 12.86 (s, 1H). ESI-MS: 322.40 ($C_{18}H_{16}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{15}N_3OS$: C, 67.27; H, 4.70; N, 13.07; Found: C, 67.38; H, 4.71; N, 13.13.

4.4.17. (*E*)-3-(4-Fluorophenyl)-*N*-(5-(p-tolyl)-1,3,4-thiadiazol-2-yl)acrylamide (9b)

White powders, yield 86%, mp: 340–342 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.38 (s, 3H); 6.89 (d, J = 15.75 Hz, 1H); 7.29–7.36 (m, 4H); 7.71–7.86 (m,5H); 12.84 (s, 1H). ESI-MS: 340.39 ($C_{18}H_{15}FN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{14}FN_3OS$: C, 63.70; H, 4.16; N, 12.38; Found: C, 63.62; H, 4.15; N, 12.44.

4.4.18. (*E*)-*N*-(5-(*P*-tolyl)-1,3,4-thiadiazol-2-yl)-3-(4-(trifluoromethyl)phenyl)acrylamide (9c)

White powders, yield 83%, mp: 345–347 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.38 (s, 3H); 7.07 (d, J = 15.90 Hz, 1H); 7.35 (d, J = 8.22 Hz, 2H); 7.84–7.90 (m, 7H); 12.97 (s, 1H). ESI-MS: 390.39 ($C_{19}H_{15}F_3N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{14}F_3N_3OS$: C, 58.60; H, 3.62; N, 10.79; Found: C, 58.69; H, 3.64; N, 10.75.

4.4.19. (*E*)-3-(*P*-tolyl)-*N*-(5-(*p*-tolyl)-1,3,4-thiadiazol-2-yl)acrylamide (9d)

White powders, yield 86%, mp: 330–332 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.36 (d, J = 6.96 Hz, 6H); 6.89 (d, J = 15.90 Hz, 1H); 7.29 (d, J = 8.04 Hz, 2H); 7.35 (d, J = 8.04 Hz, 2H); 7.56 (d, J = 8.22 Hz, 2H); 7.76 (d, J = 15.72 Hz, 1H); 7.85 (d, J = 8.25 Hz, 2H); 12.81 (s, 1H). ESI-MS: 336.42 ($C_{19}H_{18}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{17}N_3OS$: C, 68.03; H, 5.11; N, 12.53; Found: C, 68.16; H, 5.13; N, 12.48.

4.4.20. (*E*)-3-(4-Methoxyphenyl)-*N*-(5-(*p*-tolyl)-1,3,4-thiadiazol-2-yl)acrylamide (9e)

White powders, yield 92%, mp: 318–320 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.38 (s, 3H); 3.82 (s, 3H); 6.80 (d, J = 15.75 Hz, 1H); 7.04 (d, J = 8.79 Hz, 2H); 7.35 (d, J = 8.22 Hz, 2H); 7.62 (d, J = 8.97 Hz, 2H); 7.76 (d, J = 15.90 Hz,1H); 7.84 (d, J = 8.22 Hz,2H); 12.75 (s, 1H). ESI-MS: 352.42 ($C_{19}H_{18}N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{17}N_3O_2S$: C, 64.94; H, 4.88; N, 11.96; Found: C, 64.80; H, 4.86; N, 11.92.

4.4.21. *N*-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)cinnamamide (10a)

White powders, yield 81%, mp: 318–320 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.84 (s, 3H); 6.96 (d, J = 15.72 Hz, 1H); 7.10 (d, J = 8.97 Hz, 2H); 7.48 (t, J = 2.57 Hz, 3H); 7.65–7.69 (m, 2H); 7.80 (d, J = 15.72 Hz, 1H); 7.91 (d, J = 8.79 Hz, 2H); 12.82 (s, 1H). ESI-MS: 338.40 ($C_{18}H_{16}N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{15}N_3O_2S$: C, 64.08; H, 4.48; N, 12.45; Found: C, 64.01; H, 4.51; N, 12.41.

4.4.22. (*E*)-3-(4-Fluorophenyl)-*N*-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)acrylamide (10b)

White powders, yield 84%, mp: 321–323 °C, ^{1}H NMR(300 MHz, DMSO-d_6, δ ppm): 3.84 (s, 3H); 6.90 (d, J = 15.90 Hz, 1H); 7.10 (d, J = 8.61 Hz, 2H); 7.33 (t, J = 8.60 Hz, 2H); 7.74 (t, J = 7.23 Hz, 2H); 7.81 (d, J = 15.90 Hz, 1H); 7.91 (d, J = 8.58 Hz, 2H); 12.82 (s, 1H). ESI-MS: 356.39 ($C_{18}H_{15}FN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{14}FN_3O_2S$: C, 60.83; H, 3.97; N, 11.82; Found: C, 60.90; H, 3.96; N, 11.88.

4.4.23. (*E*)-*N*-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)-3-(4-(trifluoromethyl)phenyl)acrylamide (10c)

White powders, yield 76%, mp: 336–338 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.84 (s, 3H); 7.07 (d, J = 15.90 Hz, 1H); 7.10 (d,

J = 8.61 Hz, 2H); 7.84–7.89 (m, 4H); 7.95 (d, J = 8.58 Hz, 3H); 12.92 (s, 1H) ESI-MS: 406.39 ($C_{19}H_{15}F_3N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{14}F_3N_3O_2S$: C, 56.29; H, 3.48; N, 10.37; Found: C, 56.21; H, 3.49; N, 10.43.

4.4.24. (*E*)-*N*-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)-3-(*p*-tolyl)acrylamide(10d)

White powders, yield 80%, mp: 319–321 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.35 (s, 3H); 3.84 (s, 3H); 6.89 (d, J = 15.90 Hz, 1H); 7.09 (d, J = 8.79 Hz, 2H); 7.29 (d, J = 8.04 Hz, 2H); 7.56 (d, J = 8.04 Hz, 2H); 7.76 (d, J = 15.54 Hz, 1H); 7.90 (d, J = 8.79 Hz, 2H); 12.77 (s, 1H). ESI-MS: 352.42 ($C_{19}H_{18}N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{17}N_3O_2S$: C, 64.94; H, 4.88; N, 11.96; Found: C, 64.80; H, 4.89; N, 11.92.

4.4.25. (*E*)-3-(4-Methoxyphenyl)-*N*-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)acrylamide (10e)

White powders, yield 74%, mp: 308–310 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.84 (s, 6H); 6.80 (d, J = 15.75 Hz, 1H); 7.02–7.10 (m, 4H); 7.62 (d, J = 8.43 Hz, 2H); 7.74 (d, J = 15.93 Hz, 1H); 7.89 (d, J = 8.79 Hz, 2H); 12.70 (s, 1H). ESI-MS: 368.42 ($C_{19}H_{18}N_3O_3S$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{17}N_3O_3S$: C, 62.11; H, 4.66; N, 11.44; Found: C, 62.25; H, 4.68; N, 11.40.

4.5. Antiproliferation assay

The antiproliferative activities of the prepared compounds against MCF-7 and A549 cells were evaluated as described elsewhere with some modifications.[35] Target tumor cell lines were grown to log phase in RPMI 1640 medium supplemented with 10% fetal bovine serum. After diluting to 2×10^4cells mL^{-1}with the complete medium, 100 μL of the obtained cell suspension was added to each well of 96-well culture plates. The subsequent incubation was permitted at 37 °C, 5% CO_2 atmosphere for 24 h before the cytotoxicity assessments. Tested samples at pre-set concentrations were added to six wells with colchicines and CA-4 co-assayed as positive references. After 48 h exposure period, 40 μL of PBS containing 2.5 mg mL^{-1} of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)) was added to each well. Four hours later, 100 μL extraction solution (10% SDS-5% isobutyl alcohol-0.01 M HCl) was added. After an overnight incubation at 37 °C, the optical density was measured at a wavelength of 570 nm on an ELISA microplate reader. In all experiments three replicate wells were used for each drug concentration. Each assay was carried out for at least three times. The results were summarized in Table 2.

4.6. Effects on tubulin polymerization

Bovine brain tubulin was purified as described previously.[36] To evaluate the effect of the compounds on tubulin assembly in vitro,[37] varying concentrations were preincubated with 10 μM tubulin in glutamate buffer at 30 °C and then cooled to 0 °C. After addition of GTP, the mixtures were transferred to 0 °C cuvettes in a recording spectrophotometer and warmed up to 30 °C and the assembly of tubulin was observed turbid metrically. The IC_{50} was defined as the compound concentration that inhibited the extent of assembly by 50% after 20 min incubation.

4.7. Docking simulations

The three-dimensional X-ray structure of tubulin (PDB code: 1SA0) was chosen as the template for the modeling study of compound **10e** bound to tubulin. The crystal structure was obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do).

The molecular docking procedure was performed by using LigandFit protocol within Discovery Studio 3.1. For ligand preparation, the 3D structures of **10e** was generated and minimized using Discovery Studio 3.1. For protein preparation, the hydrogen atoms were added, and the water and impurities were removed. The whole tubulin was defined as a receptor and the site sphere was selected based on the ligand binding location of colchicine, then the colchicine molecule was removed and **10e** was placed during the molecular docking procedure. Types of interactions of the docked protein with ligand were analyzed after the end of molecular docking.

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