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Synthesis, biological evaluation, and molecular modeling of cinnamic acyl sulfonamide derivatives as novel antitubulin agents

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ABSTRACT

A series of novel cinnamic acyl sulfonamide derivatives (**9a**–**16e**) have been designed and synthesized and their biological activities were also evaluated as potential tubulin polymerization inhibitors. Among all the compounds, **10c** showed the most potent growth inhibitory activity against B16-F10 cancer cell line in vitro, with an IC_{50} value of 0.8 μg/mL. Docking simulation was performed to insert compound **10c** into the crystal structure of tubulin at colchicine binding site to determine the probable binding model. Based on the preliminary results, compound **10c** with potent inhibitory activity in tumor growth may be a potential anticancer agent.

1. Introduction

With the changes in the living environment, cancer has become the second major cause of death in developed countries.[1] Over 1 million cases of cancer occur in the United States annually and cancer-related deaths are estimated to reach 12 million worldwide, by the year 2015.[2] The inducement of cancer is complex involving many molecular mechanisms. Out of control in cell replication is one of the most important mechanisms that cause cancer. During the process of cell replication, microtubules, key cytoskeletal filaments and involved in numerous cellular functions, play an important role. During cell mitosis, the microtubules extend from the cell centrosome to form mitotic spindle and fasten to the kinetochore of chromosomes. Then, the kinetochores are gathered around the equatorial plate.[1] Microtubules are in dynamic equilibrium with tubulin dimers.[3] Disruption of the dynamic equilibrium will lead to cell cycle arrest or cell apoptosis. Given their significant roles in the growth and function of cells, microtubules or tubulins are among the most important molecular targets for cancer chemotherapeutic agents.

A number of small molecules were found to bind tubulins, interfering with microtubule polymerization or depolymerization, and then induce cell cycle arrest, leading to cell death.[4–7] Colchicine (Fig. 1) is the first drug that is well known to bind tubulin, and its binding site on tubulin has been characterized recently.[8] Several other new compounds have also been synthesized as antitubulin agents, such as E7010, CA-4, and HMN-214 (Fig. 1). They bound tubulins through the same site as colchicine.[9] Among these structures (MDL-27048, compound **8**, and compound **9**), chalcone is a promising skeleton that has shown the good antitubulin activity. Chalcones, precursors of flavonoids and isoflavones, are abundant in edible plants and display a variety of biological activities, such as anti-cancer,[10–12] anti-inflammatory,[13,14] anti-tuberculosis,[15] and anti-fungal activities.[16] Their broad biological properties are largely due to the α, β-unsaturated ketone moiety. The introduction of substituents on the two aromatic rings remains an area of pharmacological interest to screen active chalcones,[17] such as MDL-27048 (Fig. 1).[18]

As shown in Figure 2, chalcone and cinnamon amide own similar part of the active structure. Cinnamoyl moiety was found in a variety of biologically active substances.[19,20] Antitumor activities of various cinnamic acid derivatives were also explored by our research group.[21] In addition, many sulfonamides displayed potent biological activities and low toxicities in previous reports. Among them, some compounds showed their anticancer effects by inhibiting tubulin to form microtubulin, such as E7010 and HMN-241.[22,23] They are now undergoing human clinical trial against various tumor types.[24,25] The amide bond is not only active binding site in many active compounds but also the long-term research object in our laboratory.[26] Various cinnamic acid derivatives were synthesized and evaluated their antitumor activities in our previous work.[25] It encouraged us to continuously screen new cinnamic acyl sulfonamides as potential antitubulin agents. The two combined substructures, cinnamic acid and sulfonamide, might exhibit synergistic effect in anticancer activities. The objectives of present work are (1) to synthesize new

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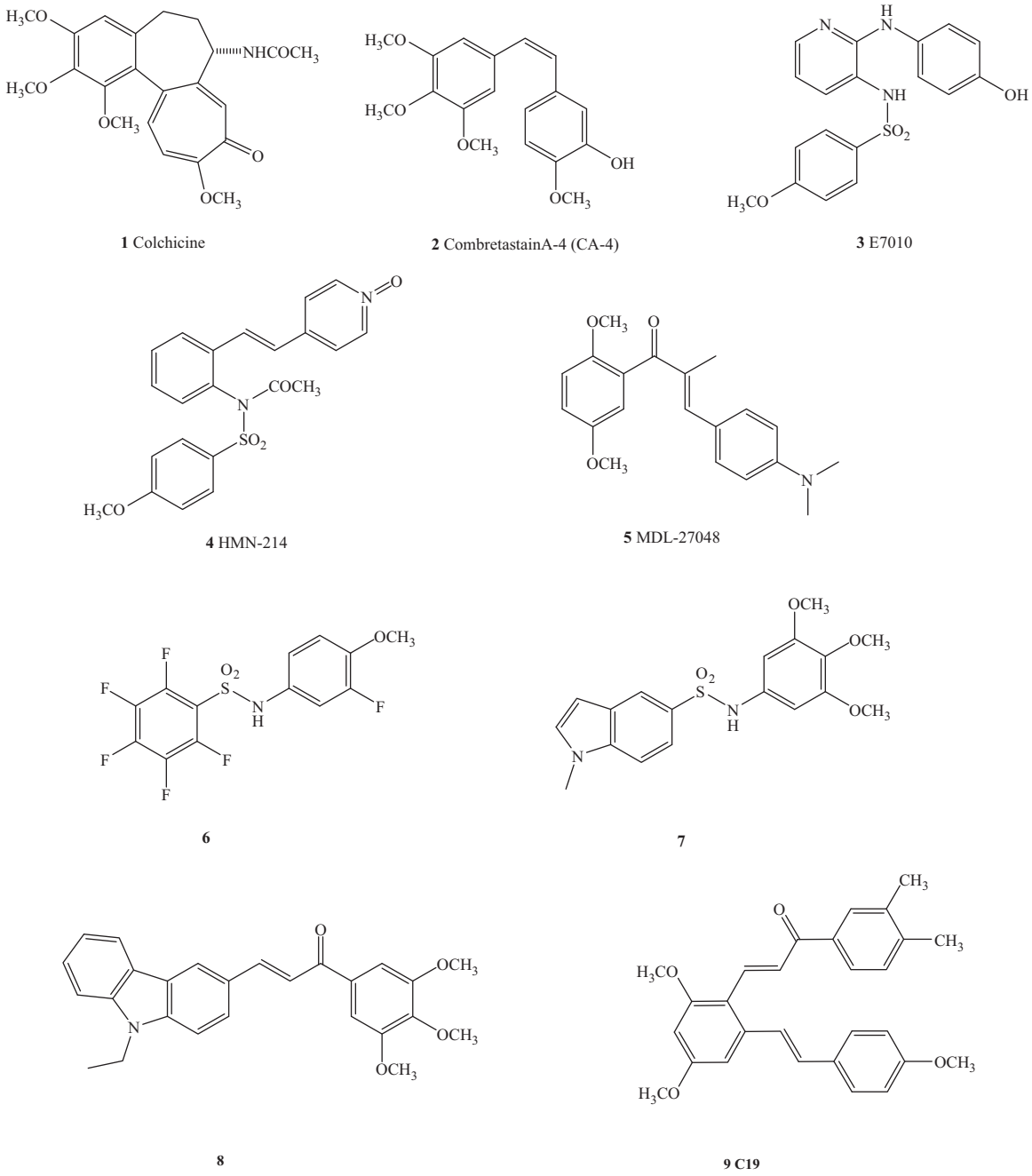


Figure 1. Chemical structures of antimitotic agents and lead tubulin inhibitors.

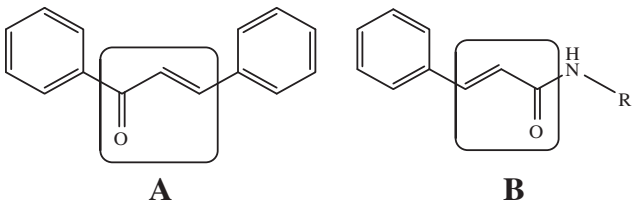


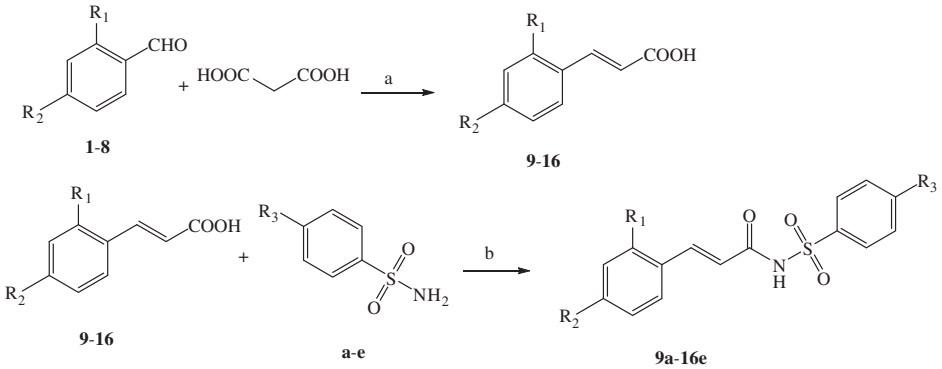
Figure 2. Similar part of chalcone and cinnamon amide.

cinnamic acyl sulfonamides; (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 inhibitor interaction with tubulin by docking study.

2. Results and discussion

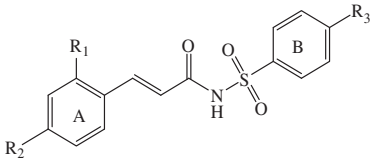
2.1. Chemistry

The synthetic route for the novel cinnamic acyl sulfonamide derivatives **9a**–**16e** is outlined in Scheme 1. These compounds were synthesized from cinnamic acids **9**–**16** and active sulfonamides **a**–**e**. Compounds **9**–**16** were prepared according to the procedure reported by Davis et al. with some modifications.[27] Aromatic aldehydes and malonic acid were dissolved in a mixture of pyridine and piperidine and refluxed for 24 h, and cinnamic acids were obtained with yields of 75–85%. Then, cinnamic acids **9**–**16**, active sulfonamides **a**–**e**, 4-dimethyaminopyridine (DMAP) and 1-[3-(dimethyamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDCI) were



Scheme 1. General synthesis of cinnamic acid sulfonamide derivatives (**9a**–**16e**). Reagents and conditions: (a) piperidine, pyridine, 80–90 °C, 24 h; (b) CH_2Cl_2, EDCI, DMAP, overnight.

Table 1
Structure of compounds **9a**–**16e**



Compounds	**9a**	**9b**	**9c**	**9d**	**9e**	**10a**	**10b**	**10c**	**10d**	**10e**
R_1	H	H	H	H	H	H	H	H	H	H
R_2	H	H	H	H	H	F	F	F	F	F
R_3	H	CH_3	F	Cl	Br	H	CH_3	F	Cl	Br
Compounds	**11a**	**11b**	**11c**	**11d**	**11e**	**12a**	**12b**	**12c**	**12d**	**12e**
R_1	H	H	H	H	H	H	H	H	H	H
R_2	Cl	Cl	Cl	Cl	Cl	Br	Br	Br	Br	Br
R_3	H	CH_3	F	Cl	Br	H	CH_3	F	Cl	Br
Compounds	**13a**	**13b**	**13c**	**13d**	**13e**	**14a**	**14b**	**14c**	**14d**	**14e**
R_1	H	H	H	H	H	H	H	H	H	H
R_2	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	$N(CH_3)_2$	$N(CH_3)_2$	$N(CH_3)_2$	$N(CH_3)_2$	$N(CH_3)_2$
R_3	H	CH_3	F	Cl	Br	H	CH_3	F	Cl	Br
Compounds	**15a**	**15b**	**15c**	**15d**	**15e**	**16a**	**16b**	**16c**	**16d**	**16e**
R_1	H	H	H	H	H	NO_2	NO_2	NO_2	NO_2	NO_2
R_2	NO_2	NO_2	NO_2	NO_2	NO_2	H	H	H	H	H
R_3	H	CH_3	F	Cl	Br	H	CH_3	F	Cl	Br

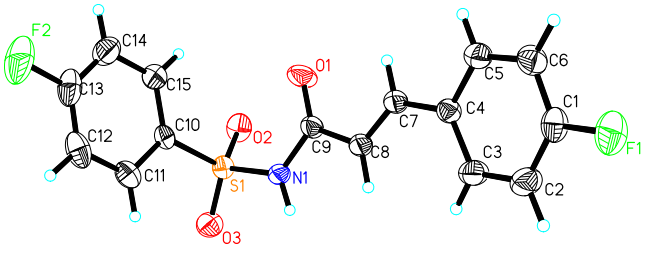


Figure 3. Crystal structure diagram of compound **10c**. H atoms are shown as small spheres of arbitrary radii.

dissolved in CH_2Cl_2 and refluxed to give the desired compounds **9a**–**16e** (Table 1).

Furthermore, the crystal structure of compound **10c** was determined by single crystal X-ray diffraction analysis in Figure 3.

2.2. Bioactivity

To test the anticancer activities of the synthesized compounds, we evaluated antiproliferative activities of compounds **9a**–**16e** against B16-F10 cancer cells. The results were summarized in Table 2. Most of the synthesized sulfonamide compounds bearing the cinnamoyl moiety showed remarkable antiproliferative effects. The compounds with *para* halogen substituted (**10a**, **11a**, **12a**) (IC_{50} = 2.1 μg/mL, 2.2 μg/mL, 3.9 μg/mL) exhibited significant antiproliferative activities in the order of F >Cl >Br. The results demonstrated that a hydrophobic and electron-withdrawing halogen group may have slightly improved antiproliferative activity and other electron donating substituted **13a**, **14a** (IC_{50} = 13.2 μg/mL, 2.3 μg/mL) had negative effects relative to those of **9a** (IC_{50} = 3.5 μg/mL). Compounds (**15a**, IC_{50} = 4.8 μg/mL) with *p*-substituted group showed slightly more potent activities than those of *o*-substituted (**16a**, IC_{50} = 6.0 μg/mL).

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 **9a**–**9e**, these compounds with the halogen atom substituent (**9c**, **9d**, **9e**) showed stronger anticancer activity and the strength order was similar with A ring: F >Cl >Br, followed that **9a** and **9b** had the lowest activities. The explanation theory of A ring could be also applied on B ring. Among all the compounds, **10c** (IC_{50} = 0.8 μg/mL) showed the best activity while the substitu-

Table 2
Inhibition of B16-F10 cells proliferation and tubulin polymerization by compounds **9a–16e**

Compound	IC_{50} ± SD (μg/mL)		Compound	IC_{50} ± SD (μg/mL)	
	B16-F10[a]	Tubulin[b]		B16-F10[a]	Tubulin[b]
9a	3.5 ± 0.2	37 ± 5	**13b**	12.2 ± 4	146 ± 5
9b	13.8 ± 2.2	152 ± 3	**13c**	5.1 ± 0.43	45 ± 7
9c	2.1 ± 0.3	4.5 ± 0.4	**13d**	5.3 ± 0.3	52 ± 3
9d	2.2 ± 0.15	14.8 ± 0.3	**13e**	5.8 ± 0.54	53 ± 8
9e	3.9 ± 0.3	63 ± 1	**14a**	2.3 ± 0.17	26 ± 2
10a	2.1 ± 0.1	4.5 ± 0.5	**14b**	4.3 ± 0.65	37 ± 4
10b	4.8 ± 0.14	40 ± 8	**14c**	2.3 ± 0.18	10 ± 5
10c	0.8 ± 0.14	2.4 ± 0.4	**14d**	2.8 ± 0.12	14 ± 3
10d	2.6 ± 0.12	4.5 ± 0.2	**14e**	4.2 ± 0.29	34 ± 5
10e	2.8 ± 0.26	14.7 ± 0.7	**15a**	4.8 ± 0.5	50 ± 1
11a	2.2 ± 0.12	15.3 ± 0.7	**15b**	13.5 ± 6	151 ± 12
11b	4.6 ± 0.4	30 ± 1	**15c**	2.2 ± 0.13	6.3 ± 0.3
11c	2.0 ± 0.17	8.9 ± 0.2	**15d**	13.7 ± 3	134 ± 14
11d	2.5 ± 0.13	9.3 ± 0.5	**15e**	13.9 ± 2	163 ± 12
11e	2.8 ± 0.25	10.8 ± 0.4	**16a**	6.0 ± 0.12	74 ± 9
12a	3.9 ± 0.12	74 ± 8	**16b**	10.3 ± 0.74	102 ± 13
12b	12.1 ± 3	137 ± 14	**16c**	6.0 ± 0.24	69 ± 5
12c	1.2 ± 0.1	3.8 ± 0.2	**16d**	6.6 ± 0.57	78 ± 3
12d	5.2 ± 0.27	55 ± 1	**16e**	12.6 ± 2	134 ± 16
12e	5.1 ± 0.35	53 ± 6	**Colchicine**	0.5 ± 0.04	1.3 ± 0.4
13a	13.2 ± 2.7	160 ± 9	**CA-4**	0.4 ± 0.15	0.7 ± 0.2

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

ents in the A ring and B ring were both F and F atom was *p*-substituted in the A ring. The results showed that electron-withdrawing groups could enhance the activity of compounds. It could be a promising lead for the further development of novel tubulin inhibition agents.

To examine whether the compounds interact with tubulin and inhibit tubulin polymerization in vitro, we performed the tubulin assembly assay. As shown in Table 2, compounds **9c**, **10c**, **12c**, showed strong inhibitory effect and their 50% tubulin polymerization inhibition about 4.5 μg/mL, 2.4 μg/mL, 3.8 μg/mL, respectively. Compound **10c** displayed the most potent anti-tubulin polymerization activity. This result indicated the anti-proliferative effect was produced by direct connection of tubulin and the compound.

Furthermore, compound **10c** was performed cell cycle analysis using flow cytometry (Fig. 4). The result showed that compound **10c** strongly induced G2/M arrest in B16-F10 cells, and the effect was observed in a dose-independent manner after treatment for 24 h with increasing dose of the compound. About 30.2% of the cells were arrested in the G2/M phase while 49.0% of the cells were found to be in the G2/M phase in the presence of 1 and 5 μg/mL **10c**, respectively. These findings indicated a continuing impairment of cell division and confirmed compound **10c** was a potent antitubulin agent.

To gain better understanding on the potency of compound **10c** and guide further SAR studies, we proceeded to examine the interaction of compound **10c** with tubulin (PDB code: 1SA0). The molecular docking was performed by simulation of compound **10c** into the colchicine binding site in tubulin. All docking runs were applied the Lamarckian genetic algorithm of Auto-Dock 4.0.[28] The binding model of compound **10c** and tubulin was depicted in Figure 5. The amino acid residue which had interaction with tubulin was labeled. In the binding mode, compound **10c** was nicely bound to the colchicine binding site of tubulin via hydrophobic interaction and binding was stabilized by a hydrogen bond. The calculated binding energies were used as the parameters for the selection of the cluster of docking posed to be evaluated, in which the binding mode of the lowest energy structure located in the top docking cluster. The selected pose of **10c** had an estimated binding free energy of −12.46 kcal/mol (binding free energy of control compounds colchicine and CA-4 are −8.86 kcal/mol and −7.62 kcal/mol, respectively). The oxygen atom of the sulfonamide system formed one hydrogen bond with the amino hydrogen of LYS352 (bond length: LYS352 N–H···O = 2.092 Å; bond angle: LYS352 N–H···O = 142.18°). This residue (LYS352) was also found to be involved in the binding of other tubulin inhibitors.[29] 3D model of the interaction between compound **10c** and the colchicine binding site was depicted in Figure 6. The model was similar with the models between colchicine, CA-4 and the colchicine binding site.[15]

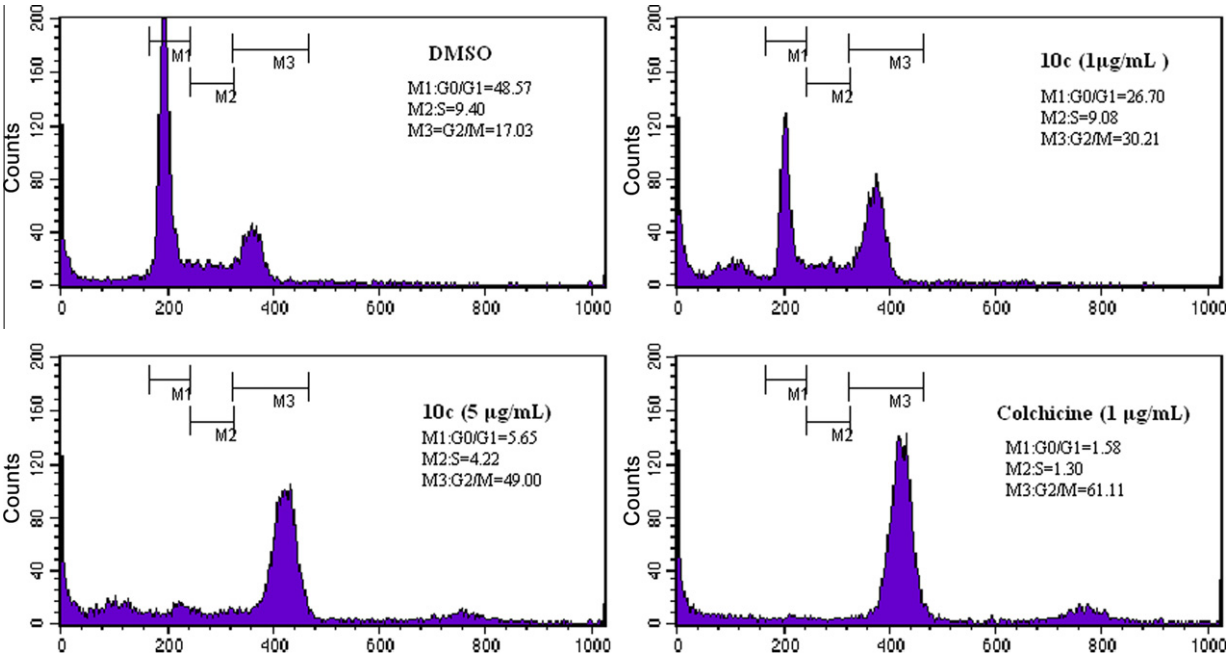


Figure 4. Effects of compound **10c** on cell cycle progression of B16-F10 cells were determined by flow cytometry analysis. B16-F10 cells were treated with different concentrations of **10c** for 24 h. The percentage of cells in each cycle phase was indicated.

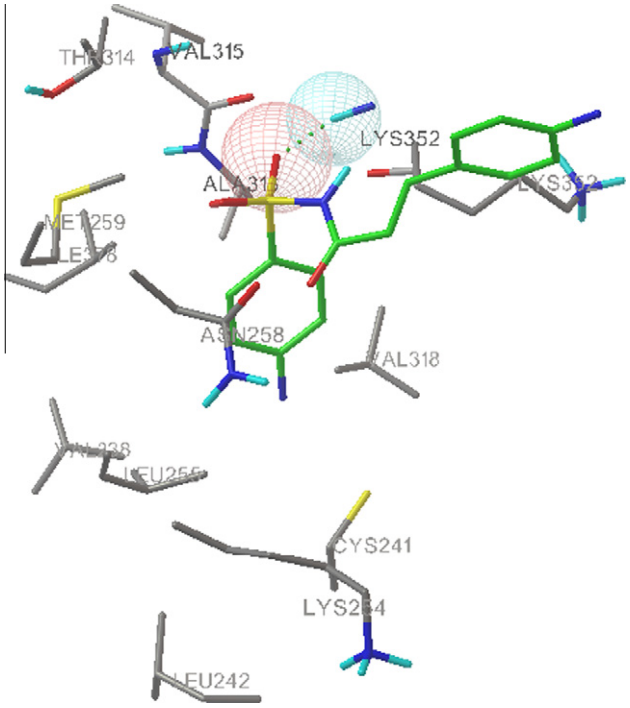


Figure 5. Binding mode of compound **10c** (colored by atom: carbons: green; oxygens: red) with tubulin (entry 1SA0 in the Protein Data Bank). The dotted lines show the hydrogen bonds.

Comparing these models, it was found that the hydrophobic pockets of colchicine binding site were occupied by compounds, and the difference was the arrangement on the enzyme surface. In the **10c** binding model, more details revealed that there were some key roles of the interaction of **10c** and tubulin (Fig. 6). The fluorophenyl moiety of B ring occupied a pocket bounded by ASN258, LEU255, and LYS352. It should be noted that the static interaction between F atom and VAL238 might played an important role. The fluorophenyl moiety of A ring was embedded in a hydrophobic pocket constructed by the side chains of residue of LYS352. Furthermore, the binding of compound **10c** to tubulin was stabilized by hydrogen bond with LYS352. Overall, these results suggested that compound **10c** could be well inserted into tubulin, similar with colchicine and CA-4.[30]

3. Conclusion

In this paper, a series of novel antitubulin polymerization inhibitors (**9a**–**16e**) containing cinnamoyl skeleton and sulfonamide moiety had been synthesized and evaluated their biological activities. These compounds exhibited potent tubulin polymerization inhibitory activities and antiproliferative activities against B16-F10 mouse melanoma cell line. Compound **10c** showed the most potent inhibition activity with an IC_{50} value of 0.8 μg/mL and antitubulin polymerization activity with an IC_{50} of 2.4 μg/mL. Molecular docking was further performed to study the inhibitor-tubulin protein interactions. After analysis of the binding model of compound **10c** with tubulin, it was found that several interactions 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 current study were of analytical grade. All the ^1H NMR spectra were recorded on a Bruker DPX 300 model Spectrometer in $CDCl_3$ 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 glass-backed silica gel sheets (Silica Gel 60 GF254) and visualized 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 cinnamic acids

A mixture of aromatic aldehydes (3.2 mmol), malonic acid (3.87 mmol), piperidine (0.387 mmol) was dissolved in pyridine and stirred on 80–90 °C for 24 h. The pyridine was removed at the vacuum. The reaction mixture was poured into water and washed with HCl. And the precipitate was filtered and washed with hexane for three times, and dried under vacuum to afford the cinnamic acids (Scheme 1).

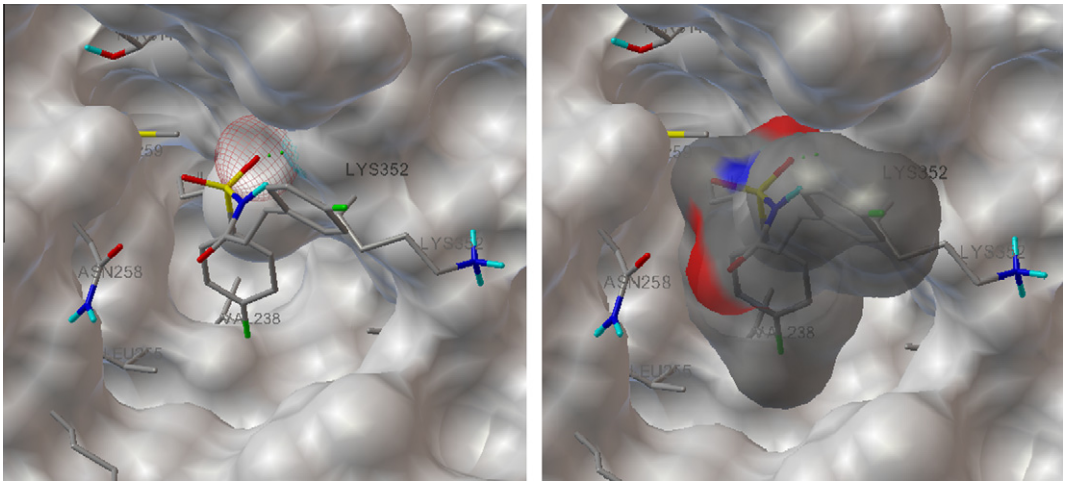


Figure 6. Comparison of compound **10c** binding model with tubulin complex. Left: 3D model of the interaction between compound **10c** and the colchicine binding site. The protein is represented by molecular surface. **10c** is depicted by sticks and balls. Right: 3D model of interaction between compound **10c** and the colchicine binding site. The protein is represented by molecular surface. **10c** is depicted by molecular surface, sticks and balls.

4.3. General procedure for synthesis of cinnamic acylsulfonamides

To a round-bottom flask (500 mL) that contained a solution of aryl sulfonamide (6 mmol), 4-dimethyaminopyridine (DMAP, 13 mmol), and 1-[3-(dimethyamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 13 mmol) in CH_2Cl_2 (150 mL) was added the synthesized cinnamic acid (6 mmol) at room temperature. The resulting mixture was stirred at room temperature for 12 h, then cooled to 5 °C, and acidified to pH 1 with addition of HCl aqueous solution (10%), which was followed by extraction with CH_2Cl_2/MeOH (9:1, 3 × 100 mL). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was subjected to silica gel chromatography or crystallization if necessary to afford the compounds (**9a**–**16e**) (Scheme 1).

4.3.1. N-(4-Fluorophenylsulfonyl)cinnamamide (9c)

Yield 85%; mp. 139 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.40 (d, J = 15.75 Hz, 1H), 7.20–7.26 (t, J = 9.42 Hz, 2H, ArH), 7.38 (d, J = 6.96 Hz, 3H), 7.47–7.49 (t, J = 3.93 Hz, 2H, ArH), 7.70 (d, J = 15.72 Hz, 1H, ArH), 8.14–8.19 (m, 2H, ArH), 8.44 (s, 1H, NH). ESI-MS: 306.05($C_{15}H_{13}FNO_3S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{12}FNO_3S$: C, 59.01; H, 3.96; N, 4.59. Found: C, 59.34; H, 4.00; N, 4.57.

4.3.2. N-(4-Bromophenylsulfonyl)cinnamamide (9e)

Yield 90%; mp. 166–167 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.39 (d, J = 15.57 Hz, 1H), 7.35-7.42 (m, 3H), 7.44–7.50 (m, 2H, ArH), 7.67–7.72 (dd, J_1 = 3.3 Hz, J_2 = 12.06 Hz, 3H, ArH), 8.00 (d, J = 8.79 Hz, 2H, ArH), 8.49 (s, 1H, NH). ESI-MS: 365.97 ($C_{15}H_{13}BrNO_3S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{12}BrNO_3S$: C, 49.19; H, 3.30; N, 3.82. Found: C, 49.35; H, 3.84; N, 3.82.

4.3.3. (E)-3-(4-Fluorophenyl)-N-(phenylsulfonyl)acrylamide (10a)

Yield 82%; mp. 165–166 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.34 (d, J = 15.54 Hz, 1H), 7.04–7.10 (m, 2H, ArH), 7.45–7.50 (m, 2H), 7.54–7.57 (m, 2H, ArH), 7.59–7.68 (m, 2H, ArH), 8.10–8.17 (m, 3H). ESI-MS: 306.05 ($C_{15}H_{13}FNO_3S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{12}FNO_3S$: C, 59.01; H, 3.96; N, 4.59. Found: C, 59.23; H, 4.03; N, 4.56.

4.3.4. (E)-3-(4-Fluorophenyl)-N-tosylacrylamide (10b)

Yield 86%; mp. 193–194 °C. ^{1}H NMR (300 MHz,$CDCl_3$): δ 2.45(s, 3H), 6.37(d, J = 15.54 Hz, 1H), 7.07 (t, J = 8.60 Hz, 2H, ArH), 7.37 (d, J = 8.04 Hz, 2H, ArH), 7.46–7.51 (m, 2H, ArH), 7.67 (d, J = 15.57 Hz, 1H), 8.01 (d, J = 8.43 Hz, 2H, ArH), 8.56 (s, 1H, NH). ESI-MS: 320.07 ($C_{16}H_{15}FNO_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{14}FNO_3S$: C, 60.18; H, 4.42; N, 4.39.C, 60.25; H, 4.58; N, 4.46. Found: C, 60.26; H, 4.45; N, 4.60.

4.3.5. (E)-3-(4-Fluorophenyl)-N-(4-fluorophenylsulfonyl) acrylamide (10c)

Yield 80%; mp. 185–186 °C. ^{1}H NMR (300 MHz,$CDCl_3$):δ 6.33 (d, J = 15.54 Hz, 1H), 7.09 (t, J = 8.60 Hz, 2H, ArH), 7.25 (t, J = 8.69 Hz, 2H, ArH), 7.47–7.52 (m, 2H, ArH), 7.68 (d, J = 15.72 Hz, 1H), 8.15–8.20 (m, 2H, ArH), 8.36 (s, 1H, NH). ESI-MS: 324.04 ($C_{15}H_{12}F_2NO_3S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}F_2NO_3S$: C, 55.72; H, 3.43; N, 4.33. Found: C, 55.65; H, 3.45; N, 4.31.

4.3.6. (E)-N-(4-Chlorophenylsulfonyl)-3-(4-fluorophenyl) acrylamide (10d)

Yield 84%; mp. 198–200 °C. 1H NMR (300 MHz, $CDCl_3$): δ 6.31 (d, J = 15.75 Hz, 1H), 7.09 (t, J = 8.51 Hz, 2H, ArH), 7.47–7.57 (m, 4H, ArH), 7.67 (d, J = 15.54 Hz, 1H), 8.08 (d, J = 8.43 Hz, 2H, ArH), 8.16 (s, 1H, NH). ESI-MS: 340.01 ($C_{15}H_{12}ClFNO_3S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}ClFNO_3S$: C, 53.02; H, 3.26; N, 4.12. Found: C, 53.17; H, 3.24; N, 4.09.

4.3.7. (E)-N-(4-Bromophenylsulfonyl)-3-(4-fluorophenyl) acrylamide (10e)

Yield 88%;mp. 194–195 °C. 1H NMR (300 MHz,$CDCl_3$): δ 6.30 (2d, J = 15.54 Hz, 1H), 7.07 (t, J = 8.51 Hz, 2H, ArH), 7.46–7.50 (m, 2H, ArH), 7.63 (s, 1H), 7.70 (d, J = 8.97 Hz, 2H, ArH), 7.98 (d, J = 8.76 Hz, 2H, ArH), 8.42 (s, 1H, NH). ESI-MS: 383.96 ($C_{15}H_{12}BrFNO_3S$, $[M+H]^+$).Anal. Calcd for $C_{15}H_{11}BrFNO_3S$: C, 46.89; H, 2.89; N, 3.65. Found: C, 46.63; H, 2.86; N, 3.62.

4.3.8. (E)-3-(4-Chlorophenyl)-N-(phenylsulfonyl)acrylamide (11a)

Yield 88%; mp. 206–210 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.38 (d, J = 15.54 Hz, 1H), 7.34–7.42 (m, 4H, ArH), 7.54–7.66 (m, 4H, ArH), 8.11(t, J = 4.29 Hz, 2H), 8.23 (s, 1H, NH). ESI-MS: 322.02 ($C_{15}H_{13}ClNO_3S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{12}ClNO_3S$: C, 55.99; H, 3.76; N, 4.35. Found: C, 56.25; H, 3.73; N, 4.32.

4.3.9. (E)-3-(4-Chlorophenyl)-N-(4-fluorophenylsulfonyl) acrylamide (11c)

Yield 82%; mp. 213–215 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.36 (d, J = 15.54 Hz, 1H), 7.23 (t, J = 9.42 Hz, 2H, ArH), 7.34–7.43 (m, 4H, ArH), 7.63 (d, J = 15.54 Hz, 1H), 8.12–8.17 (m, 2H, ArH), 8.32 (s, 1H, NH). ESI-MS: 340.01 ($C_{15}H_{12}ClFNO_3S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}ClFNO_3S$: C, 53.02; H, 3.26; N, 4.12. Found: C, 53.32; H, 3.24; N, 4.15.

4.3.10. (E)-N-(4-Bromophenylsulfonyl)-3-(4-chlorophenyl) acrylamide (11e)

Yield 88%; mp. 192–196 °C ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.35 (d, J = 15.75 Hz, 1H), 7.34–7.43 (m, 4H, ArH), 7.61–7.71 (m, 3H), 7.98 (dd, J_1 = 1.83 Hz, J_2 = 6.75 Hz, 2H, ArH), 8.29 (s, 1H, NH). ESI-MS: 399.93 ($C_{15}H_{12}BrClNO_3S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}BrClNO_3S$: C, 44.96; H, 2.77; N, 3.50. Found: C, 45.14; H, 2.80; N, 3.53.

4.3.11. (E)-3-(4-Bromophenyl)-N-(phenylsulfonyl)acrylamide (12a)

Yield 86%;mp. 222–224 °C. 1H NMR (300 MHz, $CDCl_3$): δ 6.39 (d, J = 15.72 Hz, 1H), 7.34 (d, J = 8.61 Hz, 2H, ArH), 7.51–7.69 (m, 6H), 7.96 (s, 1H, NH), 8.12 (d, J = 7.32 Hz, 2H, ArH). ESI-MS: 365.97 ($C_{15}H_{13}BrNO_3S$, $[M+H]^+$). Anal.Calcd for $C_{15}H_{12}BrNO_3S$: C, 49.19; H, 3.50; N, 3.94. Found: C, 49.35; H, 3.52; N, 3.97.

4.3.12. (E)-3-(4-Bromophenyl)-N-tosylacrylamide (12b)

Yield 90%; mp. 218–220 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.44 (s, 3H), 6.41 (d, J = 15.72 Hz, 1H), 7.32–7.37 (m, 4H, ArH), 7.51 (d, J = 8.4 Hz, 2H, ArH), 7.61 (d, J = 15.54 Hz, 1H), 8.00 (d, J = 8.4 Hz, 2H, ArH), 8.30 (s, 1H, NH). ESI-MS: 379.99 ($C_{16}H_{15}BrNO_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{14}BrNO_3S$: C, 50.54; H, 3.71; N, 3.68. Found: C, 50.68; H, 3.73; N, 3.64.

4.3.13. (E)-3-(4-Bromophenyl)-N-(4-fluorophenylsulfonyl) acrylamide (12c)

Yield 86%; mp. 212–213 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.36 (d, J = 15.72 Hz, 1H), 7.19–7.23 (m, 2H, ArH), 7.34 (d, J = 8.61 Hz, 2H, ArH), 7.52 (dd, J_1 = 1.65 Hz, J_2 = 6.6Hz, 2H, ArH), 7.62 (d, J = 15.72 Hz, 1H), 8.11–8.17 (m, 2H, ArH), 8.20 (s, 1H, NH). ESI-MS: 383.96 (C15$H_{12}BrFNO_3S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}BrFNO_3S$: C, 46.89; H, 2.89; N, 3.65. Found: C, 46.64; H, 2.86; N, 3.62.

4.3.14. (E)-3-(4-Bromophenyl)-N-(4-chlorophenylsulfonyl) acrylamide (12d)

Yield 92%; mp. 204–206 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.36 (d, J = 15.72 Hz, 1H), 7.33 (d, J = 8.43 Hz, 2H, ArH), 7.51–7.55 (m,

4H, ArH), 7.62 (d, *J* = 15.72 Hz, 1H), 8.00 (d, *J* = 8.79 Hz, 2H, ArH), 8.21 (s, 1H, NH). ESI-MS: 399.93 ($C_{15}H_{12}BrClNO_3S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}BrClNO_3S$: C, 44.96; H, 2.77; N, 3.50. Found: C, 44.72; H, 2.74; N, 3.47.

4.3.15. (E)-3-(4-Bromophenyl)-N-(4-bromophenylsulfonyl) acrylamide (12e)

Yield 93%; mp. 213–214 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.29 (d, *J* = 15.57 Hz, 1H), 7.54–7.69 (m, 5H), 8.10 (t, *J* = 10.61 Hz, 4H, ArH), 8.23 (s, 1H, NH). ESI-MS: 443.88 ($C_{15}H_{12}Br_2NO_3S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}Br_2NO_3S$: C, 40.47; H, 2.49; N, 3.15.C, 40.22; H, 2.37; N, 3.02. Found: C, 40.72; H, 2.46; N, 3.04.

4.3.16. (E)-3-(4-Methoxyphenyl)-N-(phenylsulfonyl)acrylamide (13a)

Yield 80%; mp. 173–175 °C. ^{1}H NMR (300 MHz,$CDCl_3$): δ 3.85 (s, 3H), 6.30 (d, *J* = 15.54 Hz, 1H), 6.90 (d, *J* = 8.76 Hz, 2H, ArH), 7.45 (d, *J* = 8.97 Hz, 2H, ArH), 7.57 (t, *J* = 7.50 Hz, 2H, ArH), 7.66 (t, *J* = 7.77 Hz, 2H), 8.13 (t, *J* = 2.93 Hz, 2H, ArH), 8.27 (s, 1H, NH). ESI-MS: 318.07 ($C_{16}H_{16}NO_4S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{15}NO_4S$:C, 60.55; H, 4.76; N, 4.41. Found: C, 60.61; H, 4.73; N, 4.43.

4.3.17. (E)-3-(4-Methoxyphenyl)-N-tosylacrylamide (13b)

Yield 86%; mp. 195–197 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H), 3.83 (s, 3H), 6.27 (d, *J* = 15.54 Hz, 1H), 6.88 (d, *J* = 8.79 Hz, 2H, ArH), 7.34 (d, *J* = 8.04 Hz, 2H, ArH), 7.43 (d, *J* = 8.79 Hz, 2H, ArH), 7.62 (d, *J* = 15.54 Hz, 1H), 7.99 (d, *J* = 8.22 Hz, 2H, ArH), 8.30 (s, 1H, NH). ESI-MS: 332.09 ($C_{17}H_{18}NO_4S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{17}NO_4S$: C, 61.61; H, 5.17; N, 4.23. Found: C, 61.83; H, 5.15; N, 4.25.

4.3.18. (E)-N-(4-Fluorophenylsulfonyl)-3-(4-methoxyphenyl) acrylamide (13c)

Yield 78%; mp. 175–177 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (s, 3H), 6.26 (d, *J* = 15.54 Hz, 1H), 6.87 (d, *J* = 8.76 Hz, 2H, ArH), 7.18–7.26 (m, 2H, ArH), 7.43 (d, *J* = 8.79 Hz, 2H, ArH), 7.65(d, *J* = 15.54 Hz, 1H), 8.13–8.18 (m, 2H, ArH), 8.50 (s, 1H, NH). ESI-MS: 336.06 ($C_{16}H_{15}FNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{14}FNO_4S$: C, 57.30; H, 4.21; N, 4.18. Found: C, 57.43; H, 4.24; N, 4.22.

4.3.19. (E)-N-(4-Chlorophenylsulfonyl)-3-(4-methoxyphenyl) acrylamide (13d)

Yield 84%; mp. 183–185 °C. ^{1}H NMR (300 MHz,$CDCl_3$): δ 3.83 (s, 3H), 6.24 (d, *J* = 15.54 Hz, 1H), 6.89 (d, *J* = 8.76 Hz, 2H, ArH), 7.43 (d, *J* = 8.61 Hz, 2H, ArH), 7.52 (d, *J* = 8.58 Hz, 2H, ArH), 7.64 (d, *J* = 15.54 Hz, 1H), 8.06 (d, *J* = 8.61 Hz, 2H, ArH), 8.43 (s, 1H, NH). ESI-MS: 352.03 ($C_{16}H_{15}ClNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{14}ClNO_4S$: C, 54.62; H, 4.01; N, 3.98. Found: C, 54.74; H, 4.03; N, 4.02.

4.3.20. (E)-N-(4-Bromophenylsulfonyl)-3-(4-methoxyphenyl) acrylamide (13e)

Yield 88%; mp. 188–190 °C. ^{1}H NMR (300 MHz,$CDCl_3$): δ 3.83 (s, 3H), 6.23 (d, *J* = 15.54 Hz, 1H), 6.89 (d, *J* = 8.79 Hz, 2H, ArH), 7.43 (d, *J* = 8.79 Hz, 2H, ArH), 7.62 (s,1H), 7.69 (d, *J* = 8.79 Hz, 2H, ArH), 7.98 (d, *J* = 8.76 Hz, 2H, ArH), 8.22 (s, 1H, NH). ESI-MS: 395.98 ($C_{16}H_{15}BrNO_4S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{14}BrNO_4S$: C, 48.50; H, 3.56; N, 3.53. Found: C, 48.43; H, 3.60; N, 3.57.

4.3.21. (E)-3-(4-(Dimethylamino)phenyl)-N-(phenylsulfonyl) acrylamide (14a)

Yield 75%; mp. 202–204 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.05 (s, 6H, $N(CH_3)_2$), 6.20 (d, *J* = 15.54 Hz, 1H), 6.66 (d, *J* = 8.67 Hz, 2H, ArH), 7.32 (d, *J* = 8.43 Hz, 2H, ArH), 7.54 (d, *J* = 8.56 Hz, 2H, ArH), 7.62 (d, *J* = 15.54 Hz, 1H), 7.76 (d, *J* = 15.54 Hz, 1H, ArH), 8.01(d, *J* = 6.54 Hz, 2H, ArH), 8.12 (s, 1H, NH). ESI-MS: 331.10 ($C_{17}H_{19}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{18}N_2O_3S$: C, 61.80; H, 5.49; N, 8.48. Found: C, 62.02; H, 5.51; N, 8.45.

4.3.22. (E)-3-(4-(Dimethylamino)phenyl)-N-tosylacrylamide (14b)

Yield 85%; mp. 218–220 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43(s, 3H), 3.03 (s, 6H, $N(CH_3)_2$), 6.18 (d, *J* = 15.36 Hz, 1H), 6.68 (d, *J* = 8.79 Hz, 2H, ArH), 7.32–7.39 (m, 4H, ArH), 7.61 (d, *J* = 15.36 Hz, 1H), 7.93 (s, 1H, NH), 7.99 (d, *J* = 8.40 Hz, 2H, ArH). ESI-MS: 345.12 ($C_{18}H_{21}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{20}N_2O_3S$: C, 62.77; H, 5.85; N, 8.13. Found: C, 62.93; H, 5.89; N, 8.16.

4.3.23. (E)-3-(4-(Dimethylamino)phenyl)-N-(4-fluorophenyl-sulfonyl)acrylamide (14c)

Yield 77%; mp. 207–209 °C ^{1}H NMR (300 MHz,CDC_3): δ 3.04 (s, 6H, $N(CH_3)_2$), 6.15 (d, *J* = 15.47 Hz, 1H), 6.67 (d, *J* = 8.57 Hz, 2H, ArH), 7.32 (d, *J* = 7.78 Hz, 2H, ArH), 7.34-7.37 (m, 2H, ArH), 7.62 (d, *J* = 15.48 Hz, 1H), 7.73 (t, *J* = 4.56 Hz, 2H, ArH), 8.09 (s, 1H, NH). ESI-MS: 349.09 ($C_{17}H_{18}FN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{17}FN_2O_3S$: C, 58.61; H, 4.92; N, 8.04. Found: C, 58.73; H, 4.95; N, 8.01.

4.3.24. (E)-N-(4-Chlorophenylsulfonyl)-3-(4-(dimethylamino) phenyl)acrylamide (14d)

Yield 81%; mp. 220–222 °C. ^{1}H NMR (300 MHz,$CDCl_3$): δ 3.06 (s, 6H, $N(CH_3)_2$), 6.37 (d, *J* = 15.54 Hz, 1H), 6.67 (d, *J* = 8.85 Hz, 2H, ArH), 7.41 (d, *J* = 15.52 Hz, 1H), 7.64 (d, *J* = 8.94 Hz, 2H, ArH), 7.78 (d, *J* = 7.84 Hz, 2H, ArH), 7.84 (d, *J* = 7.73 Hz, 2H, ArH), 8.04(s, 1H, NH). ESI-MS: 365.06 ($C_{17}H_{18}ClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{17}ClN_2O_3S$: C, 55.96; H, 4.70; N, 7.48. Found: C, 55.86; H, 4.67; N, 7.51.

4.3.25. (E)-N-(4-Bromophenylsulfonyl)-3-(4-(dimethylamino) phenyl)acrylamide (14e)

Yield 84%; mp. 226–228 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.04 (s, 6H, $N(CH_3)_2$), 6.54 (d, *J* = 15.57 Hz, 1H), 6.67 (d, *J* = 8.86 Hz, 2H, ArH), 7.40 (d, *J* = 15.57 Hz, 1H), 7.70 (d, *J* = 6.45 Hz, 2H, ArH), 7.86–7.90 (m, 4H, ArH), 8.13 (s, 1H, NH). ESI-MS: 409.01 ($C_{17}H_{18}BrN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{17}BrN_2O_3S$: C, 49.89; H, 4.19; N, 6.84. Found: C, 49.76; H, 4.23; N, 6.81.

4.3.26. (E)-N-(4-Fluorophenylsulfonyl)-3-(2-nitrophenyl) acrylamide (15c)

Yield 75%; mp. 165–167 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.31 (d, *J* = 15.57 Hz, 1H), 7.21–7.27 (m, 2H, ArH), 7.54–7.57 (m, 2H, ArH), 7.65 (t, *J* = 6.86 Hz, 1H, ArH), 8.04 (t, *J* = 4.85, 2H, ArH), 8.10 (s, 1H, NH), 8.12–8.17 (m, 2H). ESI-MS: 351.04 ($C_{15}H_{12}FN_2O_5S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}FN_2O_5S$: C, 51.43; H, 3.16; N, 8.00. Found: C, 51.21; H, 3.13; N, 7.96.

4.3.27. (E)-N-(4-Chlorophenylsulfonyl)-3-(2-nitrophenyl)acrylamide (15d)

Yield 86%; mp. 178–180 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.36 (d, *J* = 15.57 Hz, 1H), 7.53–7.70 (m, 6H, ArH), 8.03–8.14 (m, 4H), 8.64 (s, 1H, NH). ESI-MS: 367.01 ($C_{15}H_{12}ClN_2O_5S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}ClN_2O_5S$: C, 49.12; H, 3.02; N, 7.64. Found: C, 50.16; H, 3.06; N, 7.67.

4.3.28. (E)-N-(4-Bromophenylsulfonyl)-3-(2-nitrophenyl) acrylamide (15e)

Yield 85%; mp. 175–176 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.29 (d, *J* = 15.54 Hz, 1H), 7.53–7.60 (m, 2H, ArH), 7.65 (d, *J* = 7.32 Hz, 1H, ArH), 7.72 (d, *J* = 8.79 Hz, 2H, ArH), 8.00 (d, *J* = 8.61 Hz, 2H, ArH), 8.05–8.12 (m, 2H), 8.49 (s, 1H, NH). ESI-MS: 410.96

($C_{15}H_{12}BrN_2O_5S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}BrN_2O_5S$: C, 43.81; H, 2.70; N, 6.81. Found: C, 43.56; H, 2.68; N, 6.84.

4.3.29. (E)-3-(4-Nitrophenyl)-N-(phenylsulfonyl)acrylamide (16a)

Yield 78%; mp. 225–227 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.52 (d, J = 15.54 Hz, 1H), 7.35 (d, J = 8.73 Hz, 2H, ArH), 7.66 (d, J = 7.88 Hz, 2H, ArH), 7.73 (d, J = 8.54 Hz, 2H, ArH), 7.82 (d, J = 15.54 Hz, 1H), 8.01 (d, J = 4.52 Hz, 2H, ArH), 8.21 (s, 1H, NH). ESI-MS: 333.05($C_{15}H_{13}N_2O_5S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{12}N_2O_5S$: C, 54.21; H, 3.64; N, 8.43. Found: C, 54.33; H, 3.67; N, 8.45.

4.3.30. (E)-3-(4-Nitrophenyl)-N-tosylacrylamide (16b)

Yield 92%; mp. 189–190 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.45 (s,3H), 6.55 (d, J = 15.72 Hz, 1H), 7.37 (d, J = 8.22 Hz, 2H, ArH), 7.63 (d, J = 8.61 Hz,2H, ArH), 7.71 (d, J = 15.72 Hz, 1H), 8.00 (d, J = 8.40 Hz, 2H, ArH), 8.24 (d, J = 8.58, 3H). ESI-MS: 347.06 ($C_{16}H_{15}N_2O_5S$, $M+H]^+$). Anal. Calcd for $C_{16}H_{14}N_2O_5S$: C, 55.48; H, 4.07; N, 8.09. Found: C, 55.53; H, 4.05; N, 8.12.

4.3.31. (E)-N-(4-Fluorophenylsulfonyl)-3-(4-nitrophenyl) acrylamide (16c)

Yield 75%; mp. 139–141 °C. ^{1}H NMR (300 MHz, DMSO-D6): δ 6.75 (d, J = 15.90 Hz, 1H), 7.49 (t, J = 8.87 Hz, 2H, ArH), 7.68 (d, J = 16.08 Hz, 1H), 7.85 (d, J = 8.94 Hz, 2H, ArH), 8.03–8.08 (m, 2H, ArH), 8.27 (d, J = 8.76 Hz, 2H), 12.52 (s, 1H, NH). ESI-MS: 351.04 ($C_{15}H_{12}FN_2O_5S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}FN_2O_5S$: C, 51.43; H, 3.16; N, 8.00. Found: C, 51.72; H, 3.35; N, 8.28.

4.3.32. (E)-N-(4-Chlorophenylsulfonyl)-3-(4-nitrophenyl) acrylamide (16d)

Yield 81%; mp. 198–200 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.48 (d, J = 15.54 Hz, 1H), 7.55 (d, J = 8.76 Hz, 2H, ArH), 7.62 (t, J = 6.59 Hz, 2H, ArH), 7.73 (d, J = 15.54 Hz, 1H), 8.08 (d, J = 8.78 Hz, 2H, ArH), 8.11 (s, 1H, NH), 8.25 (d, J = 8.79 Hz, 2H, ArH).ESI-MS: 367.01 ($C_{15}H_{12}ClN_2O_5S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}ClN_2O_5S$: C, 49.12; H, 3.02; N, 7.64. Found: C, 49.25; H, 3.04; N, 7.60.

4.3.33. (E)-N-(4-Bromophenylsulfonyl)-3-(4-nitrophenyl) acrylamide (16e)

Yield 86%; mp. 209–212 °C. ^{1}H NMR (300 MHz,$CDCl_3$): δ 6.48(d, J = 15.75 Hz,1H), 7.63 (d, J = 8.79 Hz, 2H, ArH), 7.72 (d, J = 8.58 Hz, 2H, ArH), 7.75 (s, 1H), 7.98 (d, J = 8.79 Hz, 2H, ArH), 8.25 (d, J = 8.76, 2H, ArH), 8.28 (s, 1H, NH). ESI-MS: 410.96 ($C_{15}H_{12}BrN_2O_5S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}BrN_2O_5S$: C, 43.81; H, 2.70; N, 6.81. Found: C, 43.73; H, 2.67; N, 6.85.

4.4. Crystal structure determination

Crystal structure determination of compound **10c** were carried out on a Nonius CAD4 diffractometer equipped with graphite-monochromated Mo Kα (λ = 0.71073 Å) radiation. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares methods using SHELX-97.32. All the non-hydrogen atoms were refined anisotropically. All the hydrogen atoms were placed in calculated positions and were assigned fixed isotropic thermal parameters at 1.2 times the equivalent isotropic U of the atoms to which they are attached and allowed to ride on their respective parent atoms. The contributions of these hydrogen atoms were included in the structure-factors calculations. The crystal data, data collection and refinement parameters for the compound **10c** are listed in Table 3.

Table 3
Crystallographical and experimental data for compound **10c**

Compounds	**10c**
Empirical formula	$C_{15}H_{11}F_2NO_3S$
Formula weight	323.31
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	14.450(7)
b (Å)	10.908(5)
c (Å)	9.586(4)
a (°)	90
b (°)	92.800(4)
c (°)	90
V (Å)	1509.2(12)
Z	17
Dcalcd/g cm^{-3}	1.760
h range (°)	2.34–28.42
F(000)	799
Reflections collected/unique	8178/3563 [R(int) = 0.0239]
Data/restraints/parameters	3563/0/203
Absorption coefficient (mm^{-1})	0.730
R1; wR2 [I >2σ(I)]	0.0456/0.1033
R1; wR2 (all data)	0.0759/0.1203
GOF	1.024

4.5. Antiproliferation assay

The antiproliferative activities of the prepared compounds against B16-F10 mouse melanoma cell line were evaluated as described elsewhere with some modifications.[31] Target tumor cell lines were grown to log phase in RPMI 1640 medium supplemented with 10% fetal bovine serum. After diluting to 2×10^4 cells 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 colchicine and CA-4 coassayed 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.[32] To evaluate the effect of the compounds on tubulin assembly in vitro,[33] 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 turbidimetrically. 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

Molecular docking of compound **10c** into the 3D X-ray structure of tubulin (PDB code: 1SA0) was carried out using the Auto-Dock software (version 4.0) as implemented through the graphic user interface Auto-Dock Tool Kit (ADT 1. 4.6).[34]

The graphical user interface ADT was employed to set up the enzymes: all hydrogens were added, Gasteiger charges were calculated and nonpolar hydrogens were merged to carbon atoms. For macromolecules, generated pdbqt files were saved.

The 3D structures of ligand molecules were built, optimized (PM3) level, and saved in Mol2 format with the aid of the molecular modeling program SPARTAN (Wavefunction Inc.). These partial charges of Mol2 files were further modified by using the ADT package (version 1.4.6) so that the charges of the nonpolar hydrogens atoms assigned to the atom to which the hydrogen was attached. The resulting files were saved as pdbqt files.

Auto-Dock software (version 4.0) was employed for all docking calculations. The AUTODOCKTOOLS program was used to generate the docking input files. In all docking a grid box size of $42 \times 45 \times 43$ points in x, y, and z directions was built, the maps were center located (115.574, 89.495, 7.664) in the catalytic site of the protein. A grid spacing of 0.375 Å (approximately one forth of the length of carbon-carbon covalent bond) and a distances-dependent function of the dielectric constant were used for the calculation of the energetic map. Ten runs were generated by using Lamarckian genetic algorithm searches. Default settings were used with an initial population of 50 randomly placed individuals, a maximum number of 2.5×10^6 energy evaluations, and a maximum number of 2.7×10^4 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen. Results differing by less than 0.5 Å in positional root-mean-square deviation (RMSD) were clustered together and the results of the most favorable free energy of binding were selected as the resultant complex structures.

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