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Design, synthesis and biological evaluation of novel chalcone derivatives as antitubulin agents

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ABSTRACT

A series of novel chalcone derivatives have been designed and synthesized, and their biological activities were also evaluated as potential inhibitors of tubulin. These compounds were assayed for growth-inhibitory activity against MCF-7 and A549 cell lines in vitro. Compound **3d** showed the most potent antiproliferative activity against MCF-7 and A549 cell lines with IC_{50} values of 0.03 and 0.95 μg/mL and exhibited the most potent tubulin inhibitory activity with IC_{50} of 1.42 μg/mL. Docking simulation was performed to insert compound **3d** into the crystal structure of tubulin at colchicines binding site to determine the probable binding model. Based on the preliminary results, compound **3d** with potent inhibitory activity in tumor growth may be a potential anticancer agent.

1. Introduction

Dynamic mitotic-spindle microtubules are among the most successful targets for anticancer therapy.[1] Nowadays, anticancer agents that interfere with microtubulin function are in widespread use in man, such as colchicine and combretastatin A-4 (CA-4).[2] Colchicine is the first compound that binds to tubulin which is the dimeric subunit of microtubules and exerts its growth-inhibitory effects through the inhibition of microtubule dynamics, resulting in the growth arrest of tumor cells at the G2-M phase.[3] CA-4 possessed the most potent and interesting antitumor activity. And early work showed it inhibited tubulin polymerization and the proliferation of murine and human cancer cells.[4,5]

Chalcones, precursors of flavonoids and isoflavonoid, are an important pharmacophore of various natural products[6] and display a variety of biological activities, such as anti-cancer,[7–9] anti-inflammatory,[10,11] anti-tuberculosis,[12] and anti-fungal activities.[13] In the last few years, the development of chalcones as tubulin-binding agents has been drawn wide attention.[14] Recent studies have shown that some compounds containing the chalcone skeleton are believed to be a result of binding to tubulin and preventing it from polymerising into microtubules.[15,16] Structural modifications in chalcone scaffold lead to the improvement of their bioavailability and some synthetic chalcone derivatives show selective cytotoxicity against MCF-7 cells.[17] However, the single bond between C=O and C=C rotates with a relatively broad angle, which may increase binding energy of chalcone with the receptor. In order to attempt a better binding condition and improve biological activity, we introduce nitro group and trifluoromethyl group to the chalcone derivatives.

According to reports, the nitro group is a unique functional group with a diversity of chemical and biological actions. Its very strong electron attracting ability creates localized or regional electron deficient sites within molecules. When such compounds interact with living systems these electrophilic sites may then react with a variety of intra and extracellular biological nucleophiles, such as proteins, amino acids and enzymes. There are numerous drugs which exert their primary pharmacological action because of the presence of an aromatic nitro group. The structural types and functions are quite diverse and include antineoplastic, antibiotic and antiparasitic drugs.[18] In all cases, the nitro group attracting functionality is usually necessary for the specific biological effect desired.[19,20] On the other hand, trifluoromethyl group is confirmed to promote the penetration of ligands while moving through the cell membrane.[21,22] Moreover, it can provide high in vivo stability.[23]

The combined substructures (chalcone, dinitrobenzene and trifluoromethyl group) without wrecking their original effective characteristics, might exhibit synergistic effect to improve antitubulin and anticancer activities. Thus, we designed and synthesized chalcone compounds (**3a–3t**) (Table 1) bearing dinitrobenzotrifluoride, then evaluated their anticancer and antitubulin activities.

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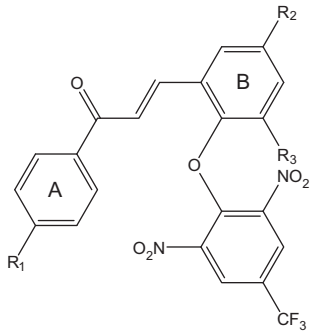
Table 1
Chemical structures of **3a–3t**



Compound	R_1	R_2	R_3
3a	CH_3	Br	Br
3b	CH_3	Cl	Cl
3c	CH_3	H	Br
3d	CH_3	H	Cl
3e	OCH_3	Br	Br
3f	OCH_3	Cl	Cl
3g	OCH_3	H	Br
3h	OCH_3	H	Cl
3i	F	Br	Br
3j	F	Cl	Cl
3k	F	H	Br
3l	F	H	Cl
3m	Cl	Br	Br
3n	Cl	Cl	Cl
3o	Cl	H	Br
3p	Cl	H	Cl
3q	Br	Br	Br
3r	Br	Cl	Cl
3s	Br	H	Br
3t	Br	H	Cl

2. Results and discussion

2.1. Chemistry

The synthesis of compounds (**3a–3t)** is followed the general pathway outlined in Scheme 1. They are prepared in two steps. Firstly, the chalcones (**1a–1t**) were obtained by the substituted salicylaldehydes and the substituted acetophenone, using 40% potassium hydroxide as catalyst in ethanol. Then, chalcones (**1a–1t**), 4-chloro-3,5-dinitro-α,α,α-trifluorotoluene (**2**) and potassium *tert*-butoxide were dissolved in DMF and reacted to obtain the desired compounds (**3a–3t)** at the room temperature. All the synthetic compounds were characterized by ^{1}H NMR, elemental analysis and mass spectrum, which were in full accordance with their depicted structures.

2.2. Bioactivity and molecular modeling

To test the anticancer activities of the synthesized compounds, we evaluated antiproliferative activities of compounds **3a–3t** against MCF-7 and A549 cells. The results were summarized in Table 2. These chalcone compounds showed remarkable antiproliferative effects. Among them, compound **3d** displayed the most potent inhibitory activity (IC_{50} = 0.03 μg/mL for MCF-7 and IC_{50} = 0.95 μg/mL for A549), comparable to the positive control colchicine (IC_{50} = 0.51 μg/mL for MCF-7, IC_{50} = 0.74 μg/mL for A549, respectively).

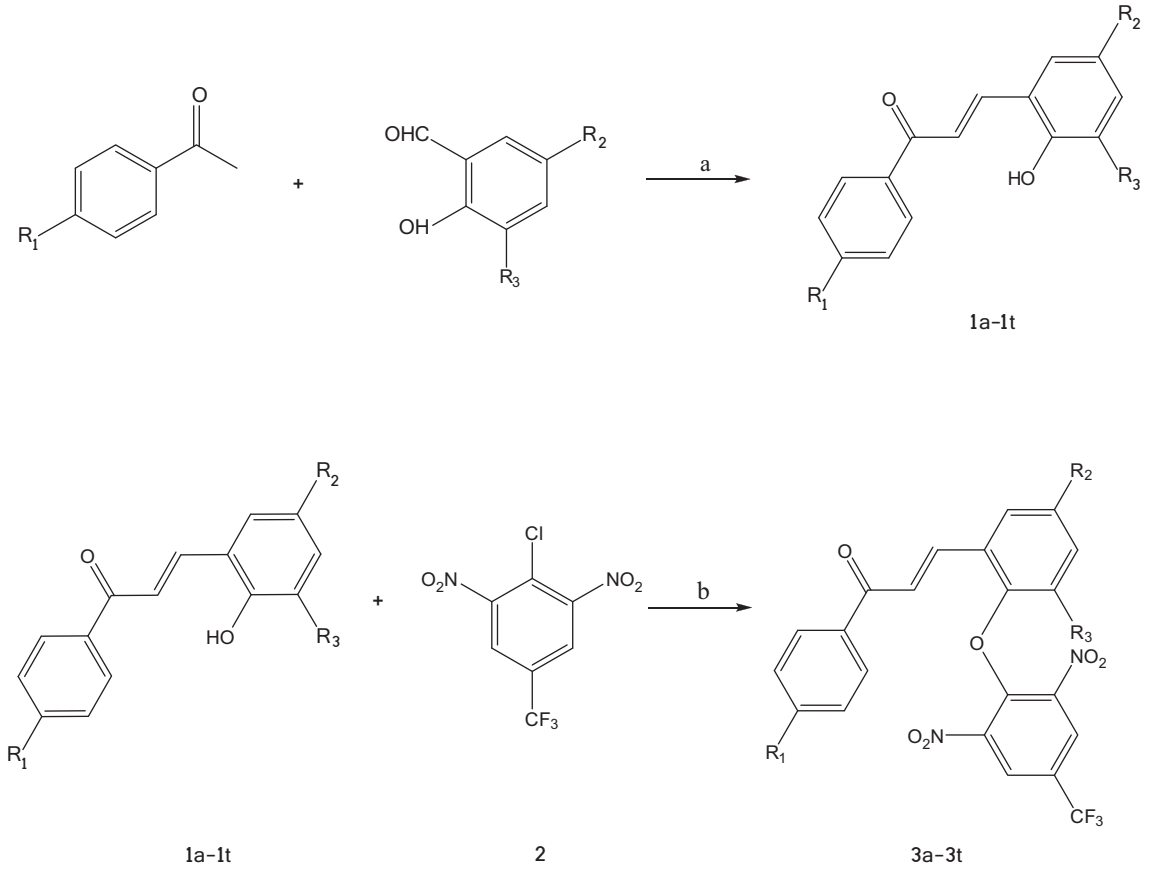
Structure-activity relationships in these chalcone derivatives demonstrated that compounds with *para* electron-donating substituents (**3a–3h**) showed more potent activities than those with electron-withdrawing substituents (**3i–3t**) in the A-ring. A comparison of the *para* substituents on the A-ring demonstrated that an electron-donating group (**3a–3h**) have slightly improved antiproliferative activity and the potency order is $CH_3 > OCH_3$, whereas, F (**3i–3l**), Cl (**3m–3p**) and Br (**3q–3t**) substituent had minimal effects compared with CH_3 (**3a–3d**) and OCH_3 (**3e–3h**). In the case of constant A-ring substituents, change of substituents on B-ring could also affect the activities of these compounds. We found that compounds with a halogen atom on the 5-position of salicylaldehyde displayed higher anticancer activity than compounds with two halogen atoms on the 3- and 5-position of salicylaldehyde. Among compounds with a halogen atom on the 5-position of salicylaldehyde, the strength order is Cl > Br. Thus, the compound **3d** with *para*-Me group in the A-ring and Cl in the B-ring showed the best activity. 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, compound **3d** showed the most potent anti-tubulin polymerization activity and 50% tubulin polymerization inhibition about 1.42 μg/mL, respectively (the positive control colchicine with an IC_{50} of 1.70 μg/mL for tubulin). This result indicated the anti-proliferative effect was produced by direct connection of tubulin and the compound.

Furthermore, compound **3d** was further assayed for its effect on cell cycle using flow cytometry (Fig. 1). As shown in Figure 1 and 67.93% of these cells were arrested at G2/M after treatment with a 5 μg/mL concentration of **3d** for 24 h. These findings indicated a continuing impairment of cell division and confirmed compound **3d** 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 **3d** with tubulin (PDB code: 1SA0). The molecular docking was performed by simulation of compound **3d** 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 **3d** and tubulin were depicted in Figure 2. All the amino acid residues which had interactions with tubulin were exhibited in Figure 2b. In the binding mode, compound **3d** is nicely bound to the colchicine binding site of tubulin via hydrophobic interactions and binding is stabilized by one hydrogen bond and one π-cation interactions. The oxygen atom on B-ring of **3d** formed one hydrogen bond with the amino hydrogen of Asn C:101 (bond length: Asn C:101 N–H···O = 1.604 Å; bond angle: Asn C:101 N–H···O = 145.3°). Overall, these results of the molecular docking study showed that the compound **3d** could act synergistically to interact with the colchicine binding site of tubulin, suggested that compound **3d** was a potential inhibitor of tubulin.

3. Conclusion

In this study, a series of novel antitubulin polymerization inhibitors (**3a–3t**) containing chalcone skeleton had been synthesized and evaluated their biological activities. These compounds exhibited potent tubulin polymerization inhibitory activities and antiproliferative activities against MCF-7 and A549 cells. Among all of the compounds, compound **3d** showed the most potent inhibition activity which inhibited the growth of MCF-7 and A549 cell lines with IC_{50} values of 0.03 and 0.95 μg/mL and inhibited the polymerization of tubulin with IC_{50} of 1.42 μg/mL. Molecular docking was further performed to study the inhibitor-tubulin protein interactions. After analysis of the binding model of compound **3d** with tubulin, it was found that one hydrogen bond and one π-cation interaction with the protein residues in the colchicine binding site might play a crucial role in its antitubulin polymerization and antiproliferative activities. In summary, compound **3d** shows



Scheme 1. The synthetic routes of compounds **3a**–**3t**. Reagents and conditions: (a) ethanol, NaOH, rt, 5 h; (b) DMF, potassium *tert*-butoxide, rt, 4 h.

Table 2
Inhibition (IC_{50}) of MCF-7 and A549 cells proliferation and inhibition of tubulin polymerization by compounds **3a**–**3t**

Compound	IC_{50} ± SD (μg/mL)		
	MCF-7[a]	A549[a]	Tubulin[b]
3a	1.44 ± 0.06	10.12 ± 0.51	5.68 ± 0.12
3b	0.70 ± 0.02	10.05 ± 0.23	4.87 ± 0.21
3c	0.55 ± 0.01	6.85 ± 0.42	6.41 ± 0.10
3d	0.03 ± 0.002	0.95 ± 0.04	1.42 ± 0.06
3e	3.53 ± 0.11	13.16 ± 0.48	6.02 ± 0.22
3f	2.69 ± 0.09	12.55 ± 0.61	6.56 ± 0.31
3g	2.32 ± 0.12	12.65 ± 0.34	4.08 ± 0.13
3h	1.83 ± 0.07	11.52 ± 0.51	4.83 ± 0.15
3i	8.06 ± 0.23	120.22 ± 9.1	15.31 ± 0.65
3j	5.02 ± 0.15	29.35 ± 1.6	9.62 ± 0.34
3k	6.01 ± 0.22	26.34 ± 1.3	12.31 ± 0.52
3l	5.17 ± 0.24	23.82 ± 1.1	11.66 ± 0.35
3m	6.76 ± 0.17	19.43 ± 0.9	12.6 ± 0.51
3n	4.54 ± 0.17	17.23 ± 1.0	6.34 ± 0.23
3o	4.74 ± 0.13	16.50 ± 0.71	7.14 ± 0.31
3p	3.21 ± 0.21	11.69 ± 0.52	4.68 ± 0.21
3q	2.87 ± 0.12	16.82 ± 0.62	10.01 ± 0.46
3r	2.60 ± 0.09	14.50 ± 0.67	9.46 ± 0.32
3s	2.17 ± 0.15	13.73 ± 0.54	6.25 ± 0.24
3t	1.41 ± 0.08	10.86 ± 0.41	4.62 ± 0.15
Colchicine	0.51 ± 0.06	0.74 ± 0.08	1.70 ± 0.12
CA-4	0.43 ± 0.04	0.53 ± 0.04	0.81 ± 0.08

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

promising biological activity. Also detailed investigations are continuing to study the mechanisms of the inhibitory activity reported here.

4. Experiments

4.1. Materials and measurements

All chemicals and reagents used in current study were of analytical grade. All the ^{1}H NMR spectra were recorded on a Bruker DPX300 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 synthetic procedure of chalcones (1a–1t)

Equimolar portions of the appropriately substituted salicylaldehydes (2 mmol, 1 equiv) and substituted acetophenone (2 mmol, 1 equiv) were dissolved in approximately 20 mL of ethanol. The mixture was allowed to stir for several minutes at 0 °C to let dissolve. Then, a 0.5 mL aliquot of a 40% aqueous sodium hydroxide solution was slowly added dropwise to the reaction flask via a self-equalizing addition funnel. The reaction solution was allowed to stir at room temperature for approximately 4–6 h. The mixture was adjusted to pH 5.0 with dilute hydrochloric acid until the reaction was complete. The reaction was monitored by TLC. Most commonly, a precipitate formed and was then collected by suction filtration.

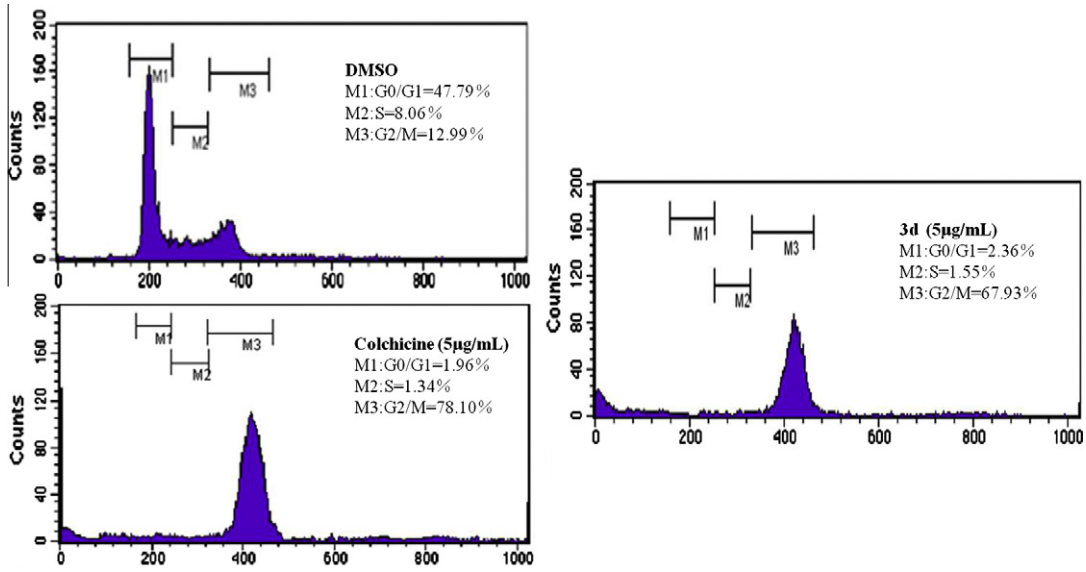


Figure 1. Effects of compound **3d** on cell cycle progression of MCF-7 cells were determined by flow cytometry analysis. MCF-7 cells were treated with **3d** for 24 h. The percentage of cells in each cycle phase was indicated.

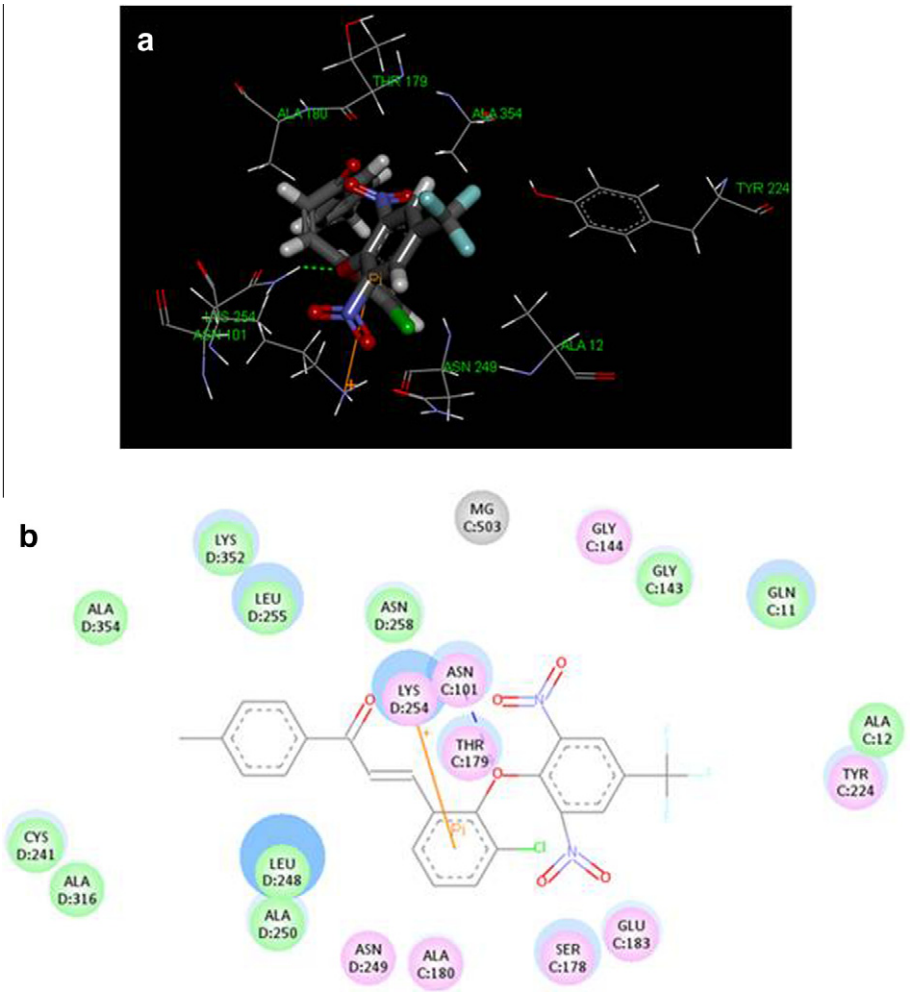


Figure 2. (a) Compound **3d** (colored by atom: carbons: gray; nitrogen: blue; oxygen: red; sulfur: yellow) is bond into tubulin (entry 1SA0 in the Protein Data Bank). The dotted lines show the hydrogen bond and the yellow line show the π-cation interactions. (b) 2D ligand interaction diagram of compound **3d** 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.

4.3. General procedure for synthesis of target compounds (3a–3t)

A mixture of chalcones (**1a–1t**) (1 mmol), 4-chloro-3,5-dinitro-α,α,α-trifluorotoluene (**2**) (1 mmol) and potassium *tert*-butoxide (0.75 mmol) was dissolved in DMF and stirred at the room temperature for 4 h. The products were extracted with ethyl acetate and water. The extract was dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography using petroleum ether and ethyl acetate (3:1).

4.3.1. (*E*)-3-(3,5-Dibromo-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-*p*-tolylprop-2-en-1-one (3a)

Yellow solid, yield 72.9%, mp: 145–147 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.42 (d, J = 11.0 Hz, 3H), 7.29 (t, J = 9.06 Hz, 2H), 7.55 (t, J = 16.38 Hz, 1H), 7.67 (d, J = 2.19 Hz, 1H), 7.78 (d, J = 2.19 Hz, 1H), 7.83 (s, 1H), 7.91 (t, J = 6.77 Hz, 2H), 8.27 (s, 2H). MS (ESI): 628.9 ($C_{23}H_{14}Br_2F_3N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{13}Br_2F_3N_2O_6$: C, 43.84; H, 2.08, N, 4.45. Found: C, 43.78; H, 2.11; N, 4.48.

4.3.2. (*E*)-3-(3,5-Dichloro-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-*p*-tolylprop-2-en-1-one (3b)

Yellow solid, yield 81.9%, mp: 170 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (s, 3H), 7.31 (d, J = 7.86 Hz, 2H), 7.36 (d, J = 2.4 Hz, 1H), 7.57 (d, J = 15.75 Hz, 1H), 7.64 (d, J = 2.4 Hz, 1H), 7.92 (t, J = 8.15 Hz, 3H), 8.30 (s, 2H). MS (ESI): 541.2 ($C_{23}H_{14}Cl_2F_3N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{13}Cl_2F_3N_2O_6$: C, 51.04; H, 2.42; N, 5.18. Found: C, 51.07; H, 2.39; N, 5.16.

4.3.3. (*E*)-3-(3-Bromo-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(*p*-tolyl)prop-2-en-1-one (3c)

Yellow solid, yield 77.2%, mp: 150 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.45 (s, 3H), 6.51 (d, J = 8.76 Hz, 1H), 7.24 (t, J = 6.86 Hz, 1H), 7.32 (d, J = 7.5 Hz, 2H), 7.61 (s, 1H), 7.77 (d, J = 2.25 Hz, 1H), 7.95 (d, J = 7.86 Hz, 2H), 8.07 (d, J = 15.18, 1H), 8.50 (s, 2H). MS (ESI): 551.0 ($C_{23}H_{15}BrF_3N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{14}BrF_3N_2O_6$: C, 50.11; H, 2.56; N, 5.08. Found: C, 50.07; H, 2.54; N, 5.11.

4.3.4. (*E*)-3-(3-Chloro-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(*p*-tolyl)prop-2-en-1-one (3d)

Yellow solid, yield 80.1%, mp: 145 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (s, 3H), 6.51 (d, J = 8.76 Hz, 1H), 7.24 (t, J = 6.86 Hz, 1H), 7.32 (d, J = 7.5 Hz, 2H), 7.61 (s, 1H), 7.77 (d, J = 2.25 Hz, 1H),7.95 (d, J = 7.86 Hz, 2H),8.07 (d, J = 15.18, 1H), 8.50 (s, 2H). MS (ESI): 507.0 ($C_{23}H_{15}ClF_3N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{14}ClF_3N_2O_6$: C, 54.51; H, 2.78; N, 5.53. Found: C, 54.43; H, 2.82; N, 5.48.

4.3.5. (*E*)-3-(3,5-Dibromo-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (3e)

Yellow solid, yield 76.1%, mp: 163 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.88 (t, J = 8.25 Hz, 3H), 6.96 (t, J = 13.64 Hz, 2H), 7.53 (d, J = 15.75 Hz,1H), 7.67 (d, J = 1.10 Hz,1H), 7.78 (d, J = 2.19 Hz,1H), 7.85 (d, J = 15.75 Hz, 1H), 8.02 (d, J = 8.79 Hz, 2H), 8.27 (s, 2H). MS (ESI):644.9 ($C_{23}H_{14}Br_2F_3N_2O_7$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{13}Br_2F_3N_2O_7$: C, 42.75; H, 2.03; N, 4.34. Found: C, 42. 66; H, 1.99; N, 4.30.

4.3.6. (*E*)-3-(3,5-Dichloro-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (3f)

Yellow solid, yield 80.2%, mp: 166 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.83 (t, J = 8.23 Hz, 3H), 7.16 (d, J = 2.30 Hz, 2H), 7.54 (d, J = 9.75 Hz, 2H), 8.11 (d, J = 6.75 Hz, 2H), 8.85 (m, 4H). MS (ESI): 557.0 ($C_{23}H_{14}Cl_2F_3N_2O_7$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{13}Cl_2F_3N_2O_7$: C, 49.57; H, 2.35; N, 5.03. Found: C, 49.65; H, 2.33; N, 5.10

4.3.7. (*E*)-3-(3-Bromo-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (3g)

Yellow solid, yield 72.5%, mp: 172 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.03 (s, 3H), 7.29 (d, J = 7.59 Hz, 1H), 7.56–7.59 (m, 2H), 7.84 (d, J = 2.58 Hz, 1H), 8.07 (d, J = 2.37 Hz, 2H), 8.46 (s, 1H), 8.54 (s, 4H). MS (ESI): 566.9 ($C_{23}H_{15}BrF_3N_2O_7$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{14}BrF_3N_2O_7$: C, 48.70; H, 2.49; N, 4.94. Found: C, 48.65; H, 2.48; N, 4.89.

4.3.8. (*E*)-3-(3-Chloro-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (3h)

Yellow solid, yield 67.2%, mp: 165 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.11 (s, 3H), 7.32 (d, J = 6.87 Hz, 1H), 7.60 (d, J = 7.21 Hz, 1H), 7.89 (d, J = 11.23 Hz, 2H), 8.14 (d, J = 4.62 Hz, 2H), 8.63 (s, 3H), 8.71 (d, J = 7.72 Hz, 2H). MS (ESI): 523.0 ($C_{23}H_{15}ClF_3N_2O_7$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{14}ClF_3N_2O_7$: C, 52.84; H, 2.70; N, 5.36. Found: C, 52.76; H, 2.74; N, 5.29.

4.3.9. (*E*)-3-(3,5-Dibromo-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(4-fluorophenyl)prop-2-en-1-one (3i)

Yellow solid, yield 51.7%, mp: 157 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.18 (t, J = 8.51 Hz, 2H), 7.52 (d, J = 15.72 Hz, 2H), 7.68 (d, J = 2.04 Hz, 1H), 7.79 (d, J = 2.01 Hz, 2H), 7.88 (d, J = 15.72 Hz, 1H), 8.28 (s, 2H). MS (ESI): 632.9 ($C_{22}H_{11}Br_2F_4N_2O_6$, $[M+H]^+$). Anal. Calcd for: $C_{22}H_{10}Br_2F_4N_2O_6$: C, 41.67; H, 1.59; N, 4.42. Found: C, 41.55; H, 1.63; N, 4.38.

4.3.10. (*E*)-3-(3,5-Dichloro-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(4-fluorophenyl)prop-2-en-1-one (3j)

Yellow solid, yield 70.3%, mp: 163 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.18 (t, J = 8.52 Hz, 3H), 7.25 (s, 1H), 7.38 (d, J = 2.15 Hz, 1H), 7.49 (s, 1H), 7.67 (d, J = 15.7 Hz, 1H), 7.93 (d, J = 15.9 Hz, 1H), 8.06 (m, 2H). MS (ESI): 544.9 ($C_{22}H_{11}Cl_2F_4N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{10}Cl_2F_4N_2O_6$: C, 48.46; H, 1.85; N, 5.14. Found: C, 48.38; H, 1.81; N, 5.11.

4.3.11. (*E*)-3-(3-Bromo-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(4-fluorophenyl)prop-2-en-1-one (3k)

Yellow solid, yield 65.5%, mp: 157 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.44 (d, J = 8.79 Hz, 1H), 7.18 (t, J = 8.15 Hz, 2H), 7.37 (d, J = 8.76 Hz, 1H), 7.58 (d, J = 15.72 Hz, 1H), 7.91 (s, 1H), 8.03–8.10 (m, 3H), 8.50 (s, 2H). MS (ESI): 554.9 ($C_{22}H_{12}BrF_4N_2O_6$, $[M+H]^+$). Anal. Calcd for: $C_{22}H_{11}BrF_4N_2O_6$: C, 47.59; H, 2.00; N, 5.05. Found: C, 47.48; H, 1.99; N, 5.10.

4.3.12. (*E*)-3-(3-Chloro-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(4-fluorophenyl)prop-2-en-1-one (3l)

Yellow solid, yield 67.9%, mp: 190 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.38 (d, J = 2.37 Hz, 1H), 7.52 (d, J = 15.9 Hz, 1H), 7.64 (t, J = 10.34 Hz, 4H), 7.92 (t, J = 12.62 Hz, 3H), 8.30 (s, 2H). MS (ESI): 511.0 ($C_{22}H_{12}ClF_4N_2O_6$, $[M+H]^+$). Anal. Calcd for: $C_{22}H_{11}ClF_4N_2O_6$: C, 51.73; H, 2.17; N, 5.48. Found: C, 51.67; H, 2.21; N, 5.41.

4.3.13. (*E*)-1-(4-Chlorophenyl)-3-(3,5-dibromo-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)prop-2-en-1-one (3m)

Yellow solid, yield 72.7%, mp: 163 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.47–7.53 (m, 3H), 7.68 (d, J = 2.4 Hz, 1H), 7.80 (d, J = 2.37 Hz, 1H), 7.90 (d, J = 15.72 Hz, 1H), 7.93–7.98 (m, 2H), 8.28 (d, J = 0.57 Hz, 2H). MS (ESI): 648.8 ($C_{22}H_{11}Br_2ClF_3N_2O_6$, $[M+H]^+$). Anal. Calcd for: $C_{22}H_{10}Br_2ClF_3N_2O_6$: C, 40.62; H, 1.55; N, 4.31. Found: C, 40.67; H, 1.59; N, 4.37.

4.3.14. (*E*)-1-(4-Chlorophenyl)-3-(3,5-dichloro-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)prop-2-en-1-one (3n)

Yellow solid, yield 76.2%, mp: 165 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.50 (t, J = 7.97 Hz, 3H), 7.68 (d, J = 2.22 Hz, 1H), 7.80 (d, J = 2.22 Hz, 1H), 7.90 (d, J = 15.72 Hz, 1H), 7.96 (d, J = 8.61 Hz, 2H), 8.28 (s, 2H). MS (ESI): 560.9 ($C_{22}H_{11}Cl_3F_3N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{10}Cl_3F_3N_2O_6$: C, 47.04; H, 1.79; N, 4.99. Found: C, 47.11; H, 1.85; N, 4.98.

4.3.15. (*E*)-3-(3-Bromo-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(4-chlorophenyl)prop-2-en-1-one (3o)

Yellow solid, yield 80.1%, mp: 168 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.45 (d, J = 8.79 Hz, 1H), 7.31–7.40 (m, 1H), 7.47–7.50 (m, 2H), 7.55 (d, J = 15.93 Hz, 1H), 7.90 (d, J = 2.19 Hz, 1H), 7.97–7.80 (m, 2H), 8.05 (d, J = 15.75 Hz, 1H), 8.50 (s, 2H). MS (ESI): 570.9 ($C_{22}H_{12}BrClF_3N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{11}BrClF_3N_2O_6$: C, 46.22; H, 1.94; N, 4.90. Found: C, 46.31; H, 1.91; N, 4.86.

4.3.16. (*E*)-3-(3-Chloro-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(4-chlorophenyl)prop-2-en-1-one (3p)

Yellow solid, yield 77.2%, mp: 172 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.51 (d, J = 8.79 Hz, 1H), 7.23–7.27 (m, 1H), 7.48 (t, J = 4.31 Hz, 2H), 7.56 (t, J = 7.97 Hz, 1H), 7.76 (d, J = 2.55 Hz,1H), 7.98 (t, J = 2.66 Hz, 2H), 7.99–8.09 (m, 1H), 8.50(s, 2H). MS (ESI): 526.9 ($C_{22}H_{12}Cl_2F_3N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{11}Cl_2F_3N_2O_6$: C, 50.12; H, 2.10; N, 5.31. Found: C, 50.19; H, 2.06; N, 5.28.

4.3.17. (*E*)-1-(4-Bromophenyl)-3-(3,5-dibromo-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)prop-2-en-1-one (3q)

Yellow solid, yield 75.4%, mp: 185 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.49 (d, J = 15.72 Hz, 1H), 7.65 (d, J = 8.61 Hz, 3H), 7.80 (s, 1H), 7.90 (t, J = 7.86 Hz, 3H), 8.28 (s, 2H). MS (ESI): 692.8 ($C_{22}H_{11}Br_3F_3N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{10}Br_3F_3N_2O_6$: C, 38.02; H, 1.45, N, 4.03. Found: C, 38.11; H, 1.47; N, 4.01.

4.3.18. (*E*)-1-(4-Bromophenyl)-3-(3,5-dichloro-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)prop-2-en-1-one (3r)

Yellow solid, yield 69.6%, mp: 165 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.37 (d, J = 2.37 Hz, 1H), 7.52 (d, J = 15.72 Hz, 1H), 7.61–7.67 (m, 3H), 7.92 (t, J = 12.53 Hz, 3H), 8.31 (s, 2H). MS (ESI): 604.9 ($C_{22}H_{11}BrCl_2F_3N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{10}BrCl_2F_3N_2O_6$: C, 43.59; H, 1.66; N, 4.62. Found: C, 43.48; H, 1.65; N, 4.58.

4.3.19. (*E*)-3-(3-Bromo-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)-1-(4-bromophenyl)prop-2-en-1-one (3s)

Yellow solid, yield 71.9%, mp: 158 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.45 (d, J = 8.79 Hz, 1H), 7.38–7.41 (m, 1H), 7.54 (d, J = 15.93 Hz, 1H), 7.64 (t, J = 9.96 Hz, 2H), 7.89–7.92 (m, 3H), 8.06 (d, J = 15.72 Hz, 1H), 8.50 (s, 2H). MS (ESI): 614.9 ($C_{22}H_{12}Br_2F_3N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{11}Br_2F_3N_2O_6$: C, 42.89; H, 1.80; N, 4.55. Found: C, 42.98; H, 1.83; N, 4.49.

4.3.20. (*E*)-1-(4-Bromophenyl)-3-(3-chloro-2-(2,6-dinitro-4-(trifluoromethyl)phenoxy)phenyl)prop-2-en-1-one (3t)

Yellow solid, yield 76.2%, mp: 165 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.51 (d, J = 8.79 Hz, 1H), 7.16–7.22 (m, 3H), 7.58 (d, J = 15.72 Hz, 1H), 7.76 (d, J = 2.22 Hz, 1H), 8.03–8.10 (m, 3H), 8.50 (s, 2H). MS (ESI): 570.9 ($C_{22}H_{12}BrClF_3N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{11}BrClF_3N_2O_6$: C, 46.22; H, 1.94; N, 4.90. Found: C, 46.31; H, 1.91; N, 4.88.

4.4. Antiproliferation assay

The antiproliferative activities of the prepared compounds against MCF-7 and A549 cells were evaluated as described elsewhere with some modifications.[24] 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 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.

4.5. Effects on tubulin polymerization

Bovine brain tubulin was purified as described previously.[25] To evaluate the effect of the compounds on tubulin assembly in vitro,[26] 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.

4.6. Docking simulations

Molecular docking of compound **3d** into the three-dimensional X-ray structure of tubulin (PDB code: 1SA0) was carried out using LigandFit Dock protocol of 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 **3d** 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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