Accepted Manuscript

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PII: S0223-5234(17)30266-0

DOI: 10.1016/j.ejmech.2017.04.011

Reference: EJMECH 9358

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 17 December 2016

Revised Date: 5 April 2017

Accepted Date: 6 April 2017

Please cite this article as: P.-C. Diao, Q. Li, M.-J. Hu, Y.-F. Ma, W.-W. You, K.H. Hong, P.-L. Zhao, Synthesis and biological evaluation of novel indole-pyrimidine hybrids bearing morpholine and thiomorpholine moieties, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.04.011.

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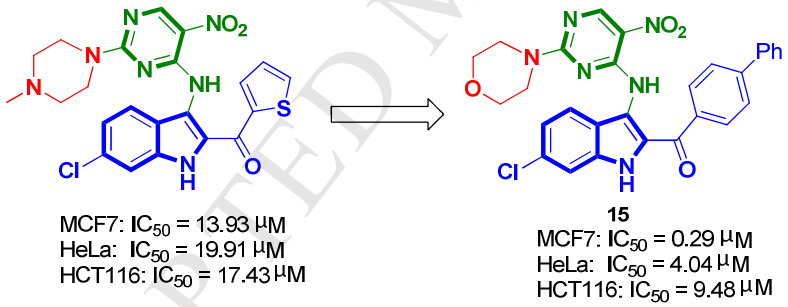

Synthesis and biological evaluation of novel indole-pyrimidine hybrids bearing morpholine and thiomorpholine moieties

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ABSTRACT: Based on our previous screening hit compound **1**, a series of novel indole-pyrimidine hybrids possessing morpholine or thiomorpholine moiety were synthesized *via* an efficient one-pot multistep synthetic method. The antiproliferative activities of the synthesized compounds were evaluated *in vitro* against four cancer cell lines including HeLa, MDA-MB-231, MCF-7, and HCT116. The results revealed that most compounds possessed moderate to excellent potency. The IC_{50} values of the most promising compound **15** are 0.29, 4.04, and 9.48 μM against MCF-7, HeLa, and HCT116 cell lines, respectively, which are 48.0, 4.9, and 1.8 folds more active than the lead compound **1**. Moreover, fluorescence-activated cell sorting analysis revealed that compound **14** showing the highest activity against HeLa (IC_{50} = 2.51 μM) displayed a significant effect on G_2/M cell-cycle arrest in a concentration-dependent manner in HeLa cell line. In addition, representative nine active hybrids were evaluated for tubulin polymerization inhibitory activities, and compound **15** exhibited the most potent anti-tubulin activity showing 42% inhibition at 10 μM. These preliminary results encourage a further investigation on indole-pyrimidine hybrids for the development of potent anticancer agents that inhibit tubulin polymerization.

Keywords: Indole-pyrimidine; Morpholine; Thiomorpholine; Antiproliferative activity; Tubulin polymerization

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1. Introduction

In recent decades, microtubules have been important molecular targets for the development of anticancer drugs due to their crucial roles in the regulating cancer cell survival and progression including cellular signaling, motility, cell shape maintenance, secretion, intercellular transport and spindle formation during mitosis [1-4]. Inhibiting tubulin polymerization or interfering with microtubule disassembly ultimately leads to cell cycle arrest or apoptosis of cancer cells [5-7]. Hence, there are commonly two major categories of anti-tubulin agents: inhibitors of the tubulin polymerization and stabilizers of the microtubule structure.

Recently, our research group discovered indole-pyrimidine hybrid **1**, having piperazine on the pyrimidine moiety (Fig. 1), as a potent inhibitor of the polymerization of tubulin with a low toxicity [8]. The agent shows low IC_{50} values ranged from 5.01 to 14.36 μM against four cancer cell lines and does not affect the normal human embryonic kidney cells, HEK-293. Interest towards morpholine- or thiomorpholine-containing indole or pyrimidine systems as anticancer agents has increased due to their important chemopreventive and chemotherapeutic effects on cancer [9-14]. As shown in Fig. **1**, pyrimidine derivative BKM-120 containing two morpholine groups manifests a great antiproliferative activity against PI3K-deregulated cell lines (GI_{50} values against A2780, U87MG, MCF7 and DU145 are 0.1-0.7 nM), and is currently in phase III clinical trials for the treatment of advanced breast cancer [15-16]. In addition, Kryštof and coworkers reported that thiomorpholine-containing pyrimidine compound **3** exhibited potential antitumor activities against a panel of cancer cell lines [17]. More interestingly, indole-pyrimidine hybrid **4** with a morpholine group was identified as a potent PI3K inhibitor with a low nanomolar IC_{50} value and used in the treatment of PI3K-mediated disorders such as inflammation and cancer [18].

*<Insert fig. **1** here>*

As the heterocyclic compounds consisting of multiple cores receive much attention in recent years [19-23], and in view of the above mentioned prominence, we have focused on the design and efficient synthesis of novel indole-pyrimidine systems bearing a morpholine or thiomorpholine moiety. In the present study, morpholine and thiomorpholine moieties replaced the piperazine group of the C-2 position of the pyrimidine moiety of compound **1** (Fig. 2) and a series of novel hybrids of

indole-pyrimidine containing a morpholine or thiomorpholine ring **11**–**32** were synthesized *via* an efficient one-pot multistep synthetic methodology. Furthermore, these compounds were evaluated for the antiproliferative and tubulin polymerization inhibitory activities.

*<Insert fig. **2** here>*

2. Chemistry

The synthesis of the target compounds **11**–**32** was illustrated in Scheme **1**. Substituted ethyl (2-cyanophenyl)carbamates **6** were obtained by condensation of the appropriately substituted 2-aminobenzonitriles **5** and ethyl chloroformate under reflux. The subsequent Thorpe–Ziegler cyclization with various α-bromoketones using K_2CO_3 as a base in dimethylformamide provided the common intermediates *N*-1-ethoxycarbonyl-2-substituted-3-aminoindoles **7**. These intermediates were subsequently *N*-deprotected by alkaline hydrolysis using NaOH in aqueous ethanol to generate the corresponding 3-amino-1H-indoles **8**, as illustrated in detail in our previously reported approach [24].

The expected indole-pyrimidine derivatives **11**–**32**, containing a morpholine or thiomorpholine ring, were generally obtained *via* a one-pot, multistep synthetic operation of equimolar amounts of substituted 3-amino-indoles **7** or **8**, 2,4-dichloro-5-nitropyrimidne **9** and morpholine or thiomorpholine in dry acetone (Scheme 1). The structures of target compounds **11**–**32** were characterized with spectroscopic techniques including ^{1}H NMR, ^{13}C NMR and HRMS, and the spectral data agree with the proposed structures.

<Insert scheme ***1** here>*

3. Results and Discussion

3.1 In vitro antiproliferative activity

The synthesized compounds **11**–**32** were evaluated for their *in vitro* antiproliferative activities against four human cancer cell lines including HeLa, MDA-MB-231, MCF-7, and HCT116 using

the MTT assay [25]. The assay results expressed as IC_{50} (μM) were summarized in Table **1** and compared with the inhibitory activities of two reference compounds, CA-4, a potent natural tubulin-binding anticancer agent, and our previously reported compound **1**. Here, the IC_{50} value represents the concentration of a compound resulting in 50% inhibition of cell growth after 48 h incubation, and is the average of three independent experiments.

<*Insert* table ***1*** *here*>

As shown in **Table 1**, it is clear that the first series of indole-pyrimidine hybrids (**11**–**29**) bearing a morpholine ring at the C-2 position of the pyrimidine, exhibit generally higher potency than the corresponding hybrids (**30**–**32**) with a thiomorpholine ring, indicating that the morpholine substitution on the pyrimidine ring might play a crucial role in modulating the antitumor activity.

Among the morpholine series (**11**–**29**), most compounds showed moderate to excellent antiproliferative activities against the four tested cancer cell lines. The IC_{50} values of the most promising compound **15** were 0.29, 4.04, and 9.48 μM against MCF-7, HeLa, and HCT116 cell lines, respectively, which indicated that this compound was 48.0, 4.9, and 1.8 folds more active than the lead compound **1**. More interestingly, compound **17** showed potent *in vitro* antiproliferative activities against all the tested cancer cell lines with the IC_{50} values ranged from 2.13 to 5.52 μM. Notably, compound **17** was two folds more active than positive control CA-4 in inhibiting HCT116 cell proliferation with IC_{50} value of 2.99 μM. It was worth noting that, the compound did not affect the normal human embryonic kidney cells, HEK-293.

Further analysis clearly revealed that different antiproliferative activities were observed when various substituents R^1, R^2, R^3 were introduced into the indole ring. Compounds **11**–**17**, without any substituent at 6-position (R^1) of the indole ring, displayed strong antiproliferative activity against the four tested cancer cell lines. The introduction of an electron-withdrawing or electron-donating group at the position led to an obvious decline in antitumour activities, which could be further confirmed by compounds **18**–**23**, and **24**–**29**. For the substituent R^2, compounds with a bulky 4-phenylphenyl substituent, exhibit much higher antiproliferative activities against HeLa and MCF-7 cell lines: **15**, **23**, **29** versus **11**–**13**, **18–21**, **24**–**28**, respectively. However, replacement of the phenyl ring (R^2) with a cyclopropyl group resulted in a decrease in the antiproliferative

activities (**16** versus **11**–**15**). It should be noted that introduction of ethoxycarbonyl group (R^3) enhanced the inhibitory activity against MDA-MB-231, MCF-7 and HCT116 (**17** versus **13**, **18**, **22**).

To study the effect of the synthesized compounds on the cell cycle progression, fluorescence-activated cell sorting analysis (FACS) was performed. Compound **14** was selected and tested against HeLa cell lines at given concentrations (1.25, 2.5, 5 μM). As shown in Fig. **3** and Table **2**, the G2/M peak significantly increased from 9.12% to 12.63% (1.25 μM), 58.34% (2.5 μM), and 88.58% (5 μM) after 12 h of incubation with the compound. These cell cycle analysis results revealed that the compound arrested the cell cycle at G2/M phase in a concentration-dependent manner when compared to untreated control cells.

*<Insert fig. **3** here>*

<Insert table ***2** here>*

3.2. Inhibition of in vitro tubulin polymerization

Based on the obtained IC_{50} values of target hybrids against the four cancer cell lines employed in this study, nine representative hybrids were selected for the evaluation of their inhibitory activities against *in vitro* tubulin polymerization at 10 μM and CA-4 was also used as a reference. The results were summarized in Table **3**. Notably, there is a clear correlation between anti-tubulin activity and antiproliferative activity of the tested compounds. For example, compounds **15** and **17** exhibited approximately 40% inhibition of the tubulin polymerization at 10 μM and antiproliferative activity in low micromolar concentrations against all four cell lines tested. Meanwhile, thiomorpholine-linked indole-pyrimidine hybrid **30** lost its inhibitory potential against tubulin polymerization as well as anticancer activities against all four tested cancer cell lines. Additionally, compound **15** displayed an anti-tubulin activity with an IC_{50} value of 19.3 μM, which was comparable with that of compound **1** (IC_{50} = 11.2 μM).

*<Insert table **3** here>*

3.3. Molecular docking study

Understanding of the binding mode of a lead compound in a molecular target provides an opportunity to rationally design inhibitors of the target more potent than the lead compound in a drug discovery project. We, therefore, performed docking simulations and the relative binding free energy calculation of the most active compound **15** and its relative binding free energy calculation in order to investigate its potential binding mode (PDB ID: 1SA0) [26]. Compound **15** was docked in the colchicine binding pocket in the beta chain of tubulin using Glide and the pose with the best emodel score(docking score -6.8, glide emodel -103.6 kcal/mol) was used for MM-GBSA relative binding free energy calculation using Prime (Glide, version 6.9, Schrodinger, LLC, New York, NY, 2015 and Prime, version 4.2, Schrodinger, LLC, New York, NY, 2015.). As shown in Fig. 4, docking studies revealed various hydrogen bonding and hydrophobic interactions that appear to play a role in the binding mode. 1-NH of the indole moiety of compound **15** forms a hydrogen bonding interaction with Thr353. Meanwhile, the indole moiety forms hydrophobic interaction with Leu248. The biaryl moiety of compound **15** occupies the hydrophobic pocket formed by Tyr202, Val238, Cys241, Leu242, Ala250, Leu252, Leu255, Ala316, Ala317 and Ile378. The morpholine moiety is located at close proximity to Asn101, Ser178, Thr179, and Tyr224 of the alpha chain of tubulin.

*<Insert fig. **4** here>*

4. Conclusion

In the present study, the piperazine ring of the lead compound **1** was replaced by a morpholine or thiomorpholine group to generate a series of novel indole-pyrimidine hybrids. The antiproliferative activities of the synthesized compounds were evaluated *in vitro* against HeLa, MDA-MB-231, MCF-7, and HCT116 cell lines. Most compounds demonstrated significant antiproliferative activities against four human cancer cell lines employed in this study. The IC_{50} values of the most promising compound **15** were 0.29, 4.04, and 9.48 μM against MCF-7, HeLa, and HCT116 cell lines, respectively, which was 48.0, 4.9, and 1.8 folds more active than the lead compound **1**. Moreover, the result from FACS analysis revealed that compound **14**, showing the highest activity against HeLa cell lines (IC_{50} = 2.51 μM) among the synthesized compounds, displayed a significant effect on G_2/M cell-cycle arrest in a concentration-dependent manner in

HeLa cells. Notably, the compound **15** also exhibited the most potent anti-tubulin activity showing 42% inhibition at 10 μM. These preliminary results encourage further investigation on indole-pyrimidine hybrids aiming to the development of new potential tubulin polymerization inhibitors and anticancer agents.

5. Experimental protocols

5.1 Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Mercury-Plus 400 spectrometer in $CDCl_3$ or DMSO-d_6 solution and chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. MS spectra were obtained using a Micromass ZQ 4000 mass spectrometer, and signals were given in m/z. Elemental analyses were performed on a Vario EL III elemental analysis instrument. Melting points (mp) were taken on a Buchi B-545 melting point apparatus and are uncorrected. Unless otherwise noted, reagents were purchased from commercial suppliers and used without further purification while all solvents were redistilled before use.

General procedure for the one-pot reactions of substituted 3-aminoindoles, 2,4-dichloro-5-nitro-pyrimidine, *and* morpholine or thiomorpholine.

2,4-Dichloro-5-nitro-pyrimidine **9** (1.0 mmol) was added to a stirred solution of substituted 3-aminoindoles **7** or **8** (1.0 mmol) in 5.0 mL of anhydrous acetone, and the mixture were heated at 40°C for 2–3h. Morpholine or thiomorpholine **10** (1.1 mmol) was then added and stirred for about 30 min. After the reaction was complete according to the TLC detection, solvent was removed under reduced pressure and the residue was purified by column chromatography using a mixture of petroleum ether and acetone as an eluent to give the target compounds in yields of 56.4–78.5%.

*5.1.1 (3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl)(phenyl)methanone (**11**).* Yield, 70.3%; mp 178.0–179.0°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.50 (d, *J* = 4.4 Hz, 2H, CH_2), 3.58 (d, *J* = 4.4 Hz, 2H, CH_2), 3.74 (d, *J* = 4.4 Hz, 2H, CH_2), 3.93 (d, *J* = 4.4 Hz, 2H, CH_2), 7.19 (t, *J* = 7.4 Hz, 1H, ArH), 7.39–7.45 (m, 3H, ArH), 7.47–7.54 (m, 2H, ArH), 7.66 (d, *J* = 8.0 Hz, 1H, ArH), 7.75 (t, *J* = 4.2 Hz, 2H, ArH), 8.92 (s, 1H, NH), 8.93 (s, 1H, pyrimidine-H), 10.28 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 187.9, 159.9, 157.9, 154.2, 138.2, 136.3, 132.6, 129.1, 128.6, 126.8, 126.2, 122.9, 122.7, 120.4, 120.2, 119.5, 113.4, 66.2, 44.7. HRMS (ESI) m/z: calcd for $C_{23}H_{20}N_6O_4$ $[M+H]^+$ 445.1546, found 445.1630.

*5.1.2 (4-bromophenyl)(3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl)methanone (**12**).* Yield, 57.5%; mp 235.1–236.0°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.50 (s, 2H, CH_2), 3.58 (s, 2H, CH_2), 3.73 (s, 2H, CH_2), 3.95 (s, 2H, CH_2), 7.19 (t, *J* = 7.4 Hz, 1H, ArH), 7.42–7.49 (m, 2H, ArH), 7.55 (d, *J* = 8.4 Hz, 2H, ArH), 7.66 (t, *J* = 8.6 Hz, 3H, ArH), 8.89 (s, 1H, NH), 8.99 (s, 1H, pyrimidine-H), 10.39 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 186.5, 159.5, 157.3, 154.1, 136.4, 135.7, 131.7, 130.0, 127.6, 127.1, 126.0, 123.1, 122.5, 120.8, 120.5, 119.7, 112.5, 66.7, 44.7. HRMS (ESI) m/z: calcd for $C_{23}H_{19}BrN_6O_4$ $[M+H]^+$ 525.0631, found 525.0718.

*5.1.3 (4-methoxyphenyl)(3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl)methanone (**13**).* Yield, 78.3%; mp 164.7–165.5°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.50 (d, *J* = 3.6 Hz, 2H, CH_2), 3.58 (s, 2H, CH_2), 3.73 (s, 2H, CH_2), 3.85 (s, 3H, CH_3), 3.92 (d, *J* = 7.6 Hz, 2H, CH_2), 6.88 (d, *J* = 8.8 Hz, 2H, ArH), 7.18 (t, *J* = 7.4 Hz, 1H, ArH), 7.41 (t, *J* = 7.6 Hz, 1H, ArH), 7.48 (d, *J* = 8.4 Hz, 1H, ArH), 7.66 (d, *J* = 8.4 Hz, 1H, ArH), 7.78 (d, *J* = 8.8 Hz, 2H, ArH), 8.95 (s, 1H, NH), 8.99 (s, 1H, pyrimidine-H), 10.37 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 186.6, 163.1, 159.9, 158.0, 154.1, 136.1, 131.6, 130.6, 126.8, 125.9, 122.8, 122.7, 120.5, 120.0, 119.1, 114.0, 113.4, 66.2, 56.0, 44.7. HRMS (ESI) m/z: calcd for $C_{24}H_{22}N_6O_5$ $[M+H]^+$ 475.1652, found 475.1740.

*5.1.4 (3-bromophenyl)(3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl)methanone (**14**).* Yield, 59.2%; mp 233.0–234.7°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.49 (s, 2H, CH_2), 3.63 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 7.11 (t, *J* = 7.6 Hz, 1H, ArH), 7.36 (dd, *J* = 12.0 and 7.6 Hz, 2H, ArH), 7.50 (d, *J* = 8.4 Hz, 1H, ArH), 7.59 (d, *J* = 8.0 Hz, 1H, ArH), 7.72 (dd, J_1 = 8.0 Hz, J_2 = 12.4 Hz, 2H, ArH), 7.79 (s, 1H, ArH), 8.90 (s, 1H, NH), 10.38 (s, 1H, pyrimidine-H), 11.90 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 186.3, 159.9, 158.0, 154.3, 140.3, 136.5, 135.0, 131.6, 130.7, 127.9, 126.8, 126.5, 123.2, 122.5, 121.6, 120.4, 120.3, 119.7, 113.4, 66.2, 44.7. HRMS (ESI) m/z: calcd for $C_{23}H_{19}BrN_6O_4$ $[M+H]^+$ 525.0631, found 525.0720.

*5.1.5 biphenyl-4-yl(3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl)methanone (**15**).* Yield, 61.7%; mp 116.3–117.9°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.39 (s, 2H, CH_2), 3.52 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 7.12 (t, *J* = 7.4 Hz, 1H, ArH), 7.36 (t, *J* = 7.6 Hz, 1H, ArH), 7.44 (t, *J* = 7.2 Hz, 1H, ArH), 7.52 ((t, *J* = 7.2 Hz, 3H, ArH), 7.64–7.70 (m, 5H, ArH), 7.85 (d, *J* = 8.0 Hz, 2H, ArH), 8.85 (s, 1H, NH), 10.64 (s, 1H, pyrimidine-H), 11.88 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 187.3, 160.0, 157.6, 154.4, 145.6, 139.7, 136.4, 135.6, 129.0, 128.9, 128.2, 127.3, 127.0, 126.8, 126.5, 123.3, 122.2, 120.7, 120.5, 119.3, 112.4, 66.7, 44.6. HRMS (ESI) m/z: calcd for $C_{29}H_{24}N_6O_4$ $[M+H]^+$ 521.1859, found 521.1960.

*5.1.6 cyclopropyl(3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl)methanone (**16**).* Yield, 74.6%; mp 234.8–236.9°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.03–1.07 (m, 2H, cyclopropane-H), 1.28–1.33 (m, 2H, cyclopropane-H), 2.58–2.62 (m, 1H, cyclopropane-H), 3.43 (d, *J* = 4.0 Hz, 2H, CH_2), 3.50 (d, *J* = 4.0 Hz, 2H, CH_2), 3.72 (s, 2H, CH_2), 3.96 (s, 2H, CH_2), 7.15 (t, *J*

= 7.4 Hz, 1H, ArH), 7.39 (t, *J* = 7.6 Hz, 1H, ArH), 7.44 (d, *J* = 8.4 Hz, 1H, ArH), 7.57 (d, *J* = 8.0 Hz, 1H, ArH), 9.08 (s, 1H, NH), 9.14 (s, 1H, pyrimidine-H), 10.52 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 193.4, 160.0, 158.3, 153.6, 135.9, 126.6, 126.2, 124.2, 121.9, 120.8, 119.7, 119.3, 113.3, 66.1, 44.8, 18.5, 11.5, 9.5. HRMS (ESI) m/z: calcd for $C_{20}H_{20}N_6O_4$ $[M+H]^+$ 409.1546, found 409.1636.

*5.1.7 ethyl 2-(4-methoxybenzoyl)-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indole-1-carboxylate (**17**).* Yield, 60.5%; mp 183.5–184.9°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.10 (t, *J* = 7.0 Hz, 3H, CH_3), 3.43 (s, 2H, CH_2), 3.54 (s, 2H, CH_2), 3.71 (s, 2H, CH_2), 3.88 (s, 3H, CH_3), 3.93 (s, 2H, CH_2), 4.16 (dd, *J* = 14.4 and 7.2 Hz, 2H, CH_2), 6.94 (d, *J* = 8.8 Hz, 2H, ArH), 7.32 (d, *J* = 7.6 Hz, 1H, ArH), 7.51 (t, *J* = 7.8 Hz, 1H, ArH), 7.57 (d, *J* = 8.0 Hz, 1H, ArH), 7.85 (d, *J* = 8.8 Hz, 2H, ArH), 8.29 (d, *J* = 8.4 Hz, 1H, ArH), 9.02 (s, 1H, pyrimidine-H), 10.37 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 185.4, 163.9, 159.8, 158.1, 154.7, 150.5, 135.0, 131.3, 130.4, 129.0, 127.7, 125.2, 123.6, 123.5, 122.7, 120.6, 115.1, 114.5, 66.3, 56.0, 49.0, 44.7, 13.8. HRMS (ESI) m/z: calcd for $C_{27}H_{26}N_6O_7$ $[M+H]^+$ 547.1863, found 547.1951.

*5.1.8 (6-chloro-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl)(phenyl)methanone (**18**).* Yield, 56.6%; mp 210.2–211.5°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.50 (d, *J* = 4.4 Hz, 2H, CH_2), 3.60 (d, *J* = 4.0 Hz, 2H, CH_2), 3.74 (d, *J* = 4.0 Hz, 2H, CH_2), 3.94 (d, *J* = 4.0 Hz, 2H, CH_2), 7.15 (d, *J* = 8.4 Hz, 1H, ArH), 7.41 (t, *J* = 7.8 Hz, 2H, ArH), 7.52 (dd, *J* = 16.2 and 9.0 Hz, 2H, ArH), 7.59 (d, *J* = 8.8 Hz, 1H, ArH), 7.74 (d, *J* = 7.6 Hz, 2H, ArH), 8.94 (s, 1H, NH), 9.08 (s, 1H, pyrimidine-H), 10.26 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 187.8, 159.8, 157.9, 154.2, 137.9, 136.4, 132.8, 130.7, 129.1, 128.6, 127.6, 124.4, 121.8, 120.8, 120.4, 119.6, 112.7, 66.4, 44.7. HRMS (ESI) m/z: calcd for $C_{23}H_{19}ClN_6O_4$ $[M+H]^+$ 479.1156, found 479.1245.

*5.1.9 (6-chloro-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl)(4-chlorophenyl) methanone (**19**).* Yield, 53.3%; mp 247.8–248.5°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.36 (s, 2H, CH_2), 3.51 (s, 2H, CH_2), 3.63 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 7.13 (dd, *J* = 8.8 and 1.6 Hz, 1H, ArH), 7.49 (dd, *J* = 9.8 and 5.0 Hz, 3H, ArH), 7.67 (d, *J* = 8.4 Hz, 1H, ArH), 7.76 (d, *J* = 8.4 Hz, 2H, ArH), 8.91 (s, 1H, NH), 10.54 (s, 1H, pyrimidine-H), 12.00 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 186.6, 159.8, 157.9, 154.2, 137.7, 136.6, 136.5, 130.9, 128.7, 127.4, 124.5, 121.8, 120.9, 120.4, 119.8, 112.7, 66.2, 46.1. HRMS (ESI) m/z: calcd for $C_{23}H_{18}Cl_2N_6O_4$$[M+H]^+$ 513.0767 found 513.0856.

*5.1.10 (6-chloro-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl)(4-methoxyphenyl) methanone (**20**).* Yield, 58.4%; mp 232.6–234.1°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.51 (d, *J* = 4.0 Hz, 2H, CH_2), 3.60 (s, 2H, CH_2), 3.73 (s, 2H, CH_2), 3.86 (s, 3H, CH_3), 3.94 (s, 2H, CH_2), 6.89 (d, *J* = 8.8 Hz, 2H, ArH), 7.15 (dd, *J* = 8.8 and 1.6 Hz, 1H, ArH), 7.48 (d, *J* = 1.2 Hz, 1H, ArH), 7.59 (d, *J* = 8.8 Hz, 1H, ArH), 7.77 (d, *J* = 8.8 Hz, 2H, ArH), 8.92 (s, 1H, NH), 8.96 (s, 1H, pyrimidine-H),

10.34 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 186.4, 163.3, 159.9, 158.0, 154.1, 136.2, 131.6, 130.4, 127.7, 124.4, 121.7, 120.6, 119.1, 114.0, 112.6, 66.2, 56.0, 44.7. HRMS (ESI) m/z: calcd for $C_{24}H_{21}ClN_6O_5$ $[M+H]^+$ 509.1262, found 509.1369.

*5.1.11 (6-chloro-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl)(thiophen-2-yl) methanone (**21**).* Yield, 52.8%; mp 268.4–269.7°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.40 (s, 2H, CH_2), 3.50 (s, 2H, CH_2), 3.62 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 7.14 (dd, *J* = 8.4 and 1.6 Hz, 1H, ArH), 7.25 (t, *J* = 4.2 Hz, 1H, thiophene-H), 7.53 (d, *J* = 1.6 Hz, 1H, ArH), 7.69 (d, *J* = 8.8 Hz, 1H, ArH), 7.98 (d, *J* = 3.6 Hz, 1H, thiophene-H), 8.07 (d, *J* = 4.8 Hz, 1H, thiophene-H), 8.98 (s, 1H, NH), 11.01 (s, 1H, pyrimidine-H), 12.02 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 178.9, 159.9, 158.2, 153.8, 142.9, 136.5, 135.7, 134.6, 130.7, 129.0, 126.7, 124.9, 121.3, 120.7, 120.6, 119.8, 112.8, 66.4, 44.8. HRMS (ESI) m/z: calcd for $C_{21}H_{17}ClN_6O_4S$ $[M+H]^+$ 485.0721, found 485.0815.

*5.1.12 ethyl 2-benzoyl-6-chloro-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indole-1-carboxylate (**22**).* Yield, 54.5%; mp 193.6–195.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.00 (t, *J* = 14.0 Hz, 3H, CH_3), 3.47 (s, 2H, CH_2), 3.60 (s, 2H, CH_2), 3.66 (s, 2H, CH_2), 3.82 (s, 2H, CH_2), 4.14 (dd, J_1 = 7.2 Hz, J_2 = 14.4 Hz, 2H, CH_2), 7.42 (dd, *J* = 8.6 and 1.6 Hz, 1H, ArH), 7.49 (t, *J* = 7.6 Hz, 2H, ArH), 7.62 (d, *J* = 7.6 Hz, 1H, ArH), 7.70 (d, *J* = 8.4 Hz, 1H, ArH), 7.77 (d, *J* = 8.0 Hz, 2H, ArH), 8.15 (d, *J* = 1.6 Hz, 1H, ArH), 8.93 (s, 1H, pyrimidine-H), 10.28 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 186.5, 159.8, 158.1, 154.6, 150.1, 137.5, 135.5, 134.0, 132.5, 129.2, 128.8, 124.5, 124.1, 124.0, 120.7, 115.0, 66.2, 64.8, 44.7, 13.7. HRMS (ESI) m/z: calcd for $C_{26}H_{23}ClN_6O_6$ $[M+H]^+$ 551.1368, found 551.1458.

*5.1.13 biphenyl-4-yl(6-chloro-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl) methanone (**23**).* Yield, 75.0%; mp 276.5–277.7°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.39 (s, 2H, CH_2), 3.53 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 7.14 (dd, *J* = 8.6 and 1.6 Hz, 1H, ArH), 7.44 (t, *J* = 7.2 Hz, 1H, ArH), 7.52 (dd, *J* = 8.4 and 6.4 Hz, 3H, ArH), 7.65–7.70 (m, 5H, ArH), 7.84 (d, *J* = 8.0 Hz, 2H, ArH), 8.85 (s, 1H, NH), 10.63 (s, 1H, pyrimidine-H), 12.03 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 187.3, 159.9, 157.8, 154.3, 144.5, 139.5, 136.7, 136.5, 130.8, 129.8, 129.5, 128.7, 127.6, 127.4, 126.8, 124.5, 121.8, 120.8, 120.5, 119.7, 112.7, 66.2, 44.7. HRMS (ESI) m/z: calcd for $C_{29}H_{23}ClN_6O_4$ $[M+H]^+$ 555.1467, found 555.1558.

*5.1.14 (6-methyl-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl)(phenyl)methanone (**24**).* Yield, 63.7%; mp 210.1–210.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.44 (s, 3H, CH_3), 3.40 (s, 2H, CH_2), 3.52 (s, 2H, CH_2), 3.63 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 6.94 (d, *J* = 8.4 Hz, 1H, ArH), 7.28 (s, 1H, ArH), 7.42 (t, *J* = 7.6 Hz, 2H, ArH), 7.52 (dd, *J* = 7.8 and 5.0 Hz, 2H, ArH), 7.75 (d, *J* = 7.2 Hz, 2H, ArH), 8.87 (s, 1H, NH), 10.62 (s, 1H, pyrimidine-H), 11.69 (s, 1H, NH). ^{13}C NMR (100

MHz, DMSO-d_6) δ: 187.7, 159.9, 157.9, 154.1, 138.3, 136.9, 136.0, 132.5, 129.0, 128.5, 126.2, 122.6, 122.3, 120.9, 120.4, 119.9, 112.7, 66.4, 44.7, 22.1. HRMS (ESI) m/z: calcd for $C_{24}H_{22}N_6O_4$ $[M+H]^+$459.1703, found 459.1793.

*5.1.15 ((4-bromophenyl)(6-methyl-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl) methanone (**25**).* Yield, 65.2%; mp 258.6–259.9°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.44 (s, 3H, CH_3), 3.39 (s, 2H, CH_2), 3.51 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 6.95 (d, *J* = 8.4 Hz, 1H, ArH), 7.26 (s, 1H, ArH), 7.52 (d, *J* = 8.4 Hz, 1H, ArH), 7.62 (d, *J* = 8.4 Hz, 2H, ArH), 7.68 (d, *J* = 8.4 Hz, 2H, ArH), 8.91 (s, 1H, NH), 10.62 (s, 1H, pyrimidine-H), 11.70 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 186.6, 159.8, 157.9, 154.1, 137.4, 137.0, 136.3, 131.5, 131.0, 126.3, 125.9, 122.6, 122.5, 120.9, 120.4, 120.2, 112.7, 66.4, 44.7, 22.1. HRMS (ESI) m/z: calcd for $C_{24}H_{21}BrN_6O_4$ $[M+H]^+$ 538.0787, found 539.0872.

*5.1.16 (4-methoxyphenyl)(6-methyl-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl) methanone (**26**).* Yield, 62.6%; mp 231.5–232.5°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.44 (d, *J* = 2.4 Hz, 3H, CH_3), 3.42 (s, 2H, CH_2), 3.52 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.81 (s, 2H, CH_2), 3.82 (s, 3H, CH_3), 6.93–6.99 (m, 3H, ArH), 7.28 (s, 1H, ArH), 7.53 (t, *J* = 4.0 Hz, 1H, ArH), 7.79 (dd, *J* = 8.6 and 3.0 Hz, 2H, ArH), 8.91 (d, *J* = 3.2 Hz, 1H, NH), 10.75 (d, *J* = 2.4 Hz, 1H, pyrimidine-H), 11.64 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 186.4, 163.0, 159.9, 158.0, 154.0, 136.7, 135.6, 131.6, 130.8, 126.3, 122.6, 122.2, 120.8, 120.5, 119.5, 114.0, 112.7, 66.3, 56.0, 44.7, 22.0. HRMS (ESI) m/z: calcd for $C_{25}H_{24}N_6O_5$ $[M+H]^+$ 489.1808, found 489.1891.

*5.1.17 (4-chlorophenyl)(6-methyl-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl) methanone (**27**).* Yield, 71.4%; mp 248.9–249.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.44 (s, 3H, CH_3), 3.40 (s, 2H, CH_2), 3.51 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 6.95 (d, *J* = 8.4 Hz, 1H, ArH), 7.27 (s, 1H, ArH), 7.50 (dd, *J* = 17.2 and 8.4 Hz, 3H, ArH), 7.76 (d, *J* = 8.0 Hz, 2H, ArH), 8.91 (s, 1H, NH), 10.62 (s, 1H, pyrimidine-H), 11.71 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 186.5, 159.8, 157.9, 154.0, 137.4, 137.0, 136.3, 130.9, 128.6, 126.0, 122.7, 122.4, 120.9, 120.4, 120.2, 112.7, 66.4, 44.7, 22.1. HRMS (ESI) m/z: calcd for $C_{24}H_{21}ClN_6O_4$ $[M+H]^+$ 493.1313, found 493.1285.

*5.1.18 (6-methyl-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl)(thiophen-2-yl) methanone (**28**).* Yield, 72.3%; mp 281.2–282.6°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.46 (s, 3H, CH_3), 3.44 (s, 2H, CH_2), 3.52 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.87 (s, 2H, CH_2), 6.97 (d, *J* = 8.0 Hz, 1H, ArH), 7.29 (d, *J* = 16.0 Hz, 2H, ArH, thiophene-H), 7.57 (d, *J* = 8.4 Hz, 1H, ArH), 8.04 (t, *J* = 13.4 Hz, 2H, thiophene-H), 9.01 (s, 1H, NH), 11.12 (s, 1H, pyrimidine-H), 11.72 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 178.8, 159.9, 158.2, 153.6, 143.3, 137.1, 136.0, 135.2, 134.3, 128.9, 125.2, 123.2, 122.3, 120.6, 120.4, 120.3, 112.9, 66.3, 44.8, 31.1, 22.1. HRMS (ESI) m/z: calcd for $C_{22}H_{20}N_6O_4S$ $[M+H]^+$ 465.1267, found 465.1214.

*5.1.19 biphenyl-4-yl(6-methyl-3-(2-morpholino-5-nitropyrimidin-4-ylamino)-1H-indol-2-yl) methanone (**29**).* Yield, 58.7%; mp 256.7–258.2°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.44 (s, 3H, CH_3), 3.42 (s, 2H, CH_2), 3.53 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 6.95 (d, *J* = 8.4 Hz, 1H, ArH), 7.29 (s, 1H, ArH), 7.44 (t, *J* = 7.4 Hz, 1H, ArH), 7.50–7.55 (m, 3H, ArH), 7.65–7.70 (m, 4H, ArH), 7.84 (d, *J* = 8.0 Hz, 2H, ArH), 8.85 (s, 1H, NH), 10.68 (s, 1H, pyrimidine-H), 11.73 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 187.2, 159.9, 157.8, 154.2, 144.2, 139.6, 137.2, 137.0, 136.1, 129.8, 129.5, 128.6, 127.3, 126.8, 126.2, 122.6, 122.4, 120.9, 120.5, 120.0, 112.8, 66.2, 44.7, 22.1. HRMS (ESI) m/z: calcd for $C_{30}H_{26}N_6O_4$ $[M+H]^+$ 535.2016, found 535.2036.

*5.1.20 (3-(5-nitro-2-thiomorpholinopyrimidin-4-ylamino)-1H-indol-2-yl)(phenyl)methanone (**30**).* Yield, 56.7%; mp 172.6–173.8°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.49 (t, *J* = 4.6 Hz, 2H, CH_2), 2.68 (t, *J* = 4.6 Hz, 2H, CH_2), 3.76 (t, *J* = 4.6 Hz, 2H, CH_2), 4.21 (t, *J* = 4.6 Hz, 2H, CH_2), 7.19 (t, *J* = 7.6 Hz, 1H, ArH), 7.41 (dd, *J* = 16.0 and 8.0 Hz, 3H, ArH), 7.47–7.54 (m, 2H, ArH), 7.65 (d, *J* = 8.0 Hz, 1H, ArH), 7.75 (d, *J* = 7.6 Hz, 2H, ArH), 8.94 (s, 1H, NH), 9.07 (s, 1H, pyrimidine-H), 10.24 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 187.7, 159.9, 157.7, 154.4, 137.8, 135.6, 132.5, 128.4, 126.8, 126.4, 123.4, 122.5, 120.6, 120.4, 119.6, 112.4, 47.0, 46.9, 27.6. HRMS (ESI) m/z: calcd for $C_{23}H_{20}N_6O_3S$ $[M+H]^+$ 461.1318, found 461.1401.

*5.1.21 (4-bromophenyl)(3-(5-nitro-2-thiomorpholinopyrimidin-4-ylamino)-1H-indol-2-yl) methanone (**31**).* Yield, 59.0%; mp 265.3–266.6°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.51 (s, 2H, CH_2), 2.71 (s, 2H, CH_2), 3.77 (s, 2H, CH_2), 4.25 (s, 2H, CH_2), 7.21 (s, 1H, ArH), 7.47 (dd, *J* = 16.4 and 6.8 Hz, 2H, ArH), 7.57 (d, *J* = 7.6 Hz, 2H, ArH), 7.65 (d, *J* = 7.6 Hz, 3H, ArH), 8.96 (s, 1H, NH), 9.02 (s, 1H, pyrimidine-H), 10.45 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 186.4, 174.1, 159.3, 157.2, 154.2, 136.4, 135.7, 131.8, 130.0, 127.6, 127.1, 126.0, 123.2, 122.7, 120.9, 119.9, 112.5, 47.2, 27.7. HRMS (ESI) m/z: calcd for $C_{23}H_{19}BrN_6O_3S$ $[M+H]^+$ 540.0402, found 541.0483.

*5.1.22 (4-methoxyphenyl)(3-(5-nitro-2-thiomorpholinopyrimidin-4-ylamino)-1H-indol-2-yl) methanone (**32**).* Yield, 63.5%; mp 254.8–255.9°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.50 (s, 2H, CH_2), 2.70 (s, 2H, CH_2), 3.77 (s, 2H, CH_2), 3.85 (d, *J* = 3.6 Hz, 3H, CH_3), 4.24 (s, 2H, CH_2), 6.88 (d, *J* = 8.8 Hz, 2H, ArH), 7.19 (t, *J* = 7.4 Hz, 1H, ArH), 7.41 (t, *J* = 7.6 Hz, 1H, ArH), 7.50 (d, *J* = 8.4 Hz, 1H, ArH), 7.64 (d, *J* = 8.0 Hz, 1H, ArH), 7.79 (d, *J* = 8.4 Hz, 2H, ArH), 8.97 (s, 1H, NH), 9.14 (s, 1H, pyrimidine-H), 10.44 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 186.4, 163.4, 159.0, 156.7, 154.2, 135.4, 131.0, 130.1, 126.7, 126.5, 123.3, 122.3, 120.6, 118.7, 113.7, 112.5, 55.5, 47.2, 27.6. HRMS (ESI) m/z: calcd for $C_{24}H_{22}N_6O_4S$ $[M+H]^+$ 491.1423, found 491.1509.

5.2 Biological evaluation

5.2.1 Antitumor activity

The antitumor activities of compounds **11**–**32** were evaluated with HeLa, MDA-MB-231,

MCF-7, and HCT116 cell lines with the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay *in vitro*. The cancer cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were splitted at 70-80% confluence, about twice a week by trypsinization.

Exponentially growing cells were plated in 96-well plates (1×10^4 cells/well) and incubated at 37 °C for 24 h for attachment. Test compounds were prepared by dissolving in dimethyl sulfoxide (DMSO) at 10 mM and diluted with the medium into a series of concentrations. The culture medium was then changed, and cells grew in medium with the test compounds. DMSO (0.1%) was used as negative control. Cells were incubated at 37 °C for 48 h with or without compounds. Then the medium was replaced with MTT solution (5 mg/mL, 100 μL) followed by incubation for another 4 h. The medium was then aspirated and formazan crystals were dissolved in DMSO (100 μL) for about 10 min. The absorbance at 570 nm (Abs) of the suspension was measured with a spectrophotometer. The inhibition percentage was calculated using the following formula: % inhibition = $(Abs_{control} - Abs_{compound})/Abs_{control}\times100\%$. The IC_{50} values of the test compounds and CA-4 were measured by treating cells with various concentrations of compounds, and analyzed by use of the prism statistical package (GraphPad Software, San Diego, CA, U.S.A.).

5.2.2 In vitro tubulin polymerization assay

Pig brain microtubule protein was isolated by employing three cycles of temperature-dependent assembly/disassembly according to method described by Shelanski, et al [27]. Homogeneous tubulin was prepared from microtubule protein by phosphocellulose (P11) chromatography as has been described previously [28]. The purified proteins were stored in aliquots at -70 °C.

Microtubule polymerization of tubulin protein, in solutions containing different concentrations of compounds in PEM buffer (100 mM PIPES, 1 mM $MgCl_2$, and 1 mM EGTA), 1 mM GTP, and 5% glycerol, was monitored at 37 °C by using light scattering at 340 nm with a SPECTRA MAX 190 (MD) spectrophotometer. Plateau absorbance values were used for calculations. CA-4 was used as standard inhibitor of tubulin polymerisation, while DMSO was used as negative control. The percent inhibition values for selected compounds were compared to the value of CA-4 and measured the same day under the same conditions.

5.2.3 Molecular docking study

A tubulin pdb structure (PDB ID: 1SA0) was imported from the PDB data bank (www.rcsb.org). It was prepared for computational simulations using Protein Preparation Wizard that performed assignment of bond orders, reassignment of H atoms, generation of zero-order bonds to metals, removal of waters, generation of metal binding states, reassignment of H-bond networks and restrained minimization of H atoms [29]. A grid of the complex of tubulin alpha and beta was generated using Glide: van der Waals radius scaling factor 1.0, partial charge cutoff 0.25, ligand in the workspace as the grid centroid, and the grid box dimension of 10Å×10Å×10Å. LigPrep was used to prepare ligands for docking simulations (LigPrep, version 3.6, Schrodinger, LLC, New York, NY, 2015). Docking simulations were performed in the SP and XP modes using Glide MM-GBSA relative binding free energy calculation of the ligand was performed with Prime.

Acknowledgments

We should thank Dr. Hong-Bing Zhao (Shanghai TANG Bioscience Co., Ltd.) for the test of tubulin polymerisation activity. The present work was supported by National Natural Science Foundation of China (21372113 and 21102069), and the Special Funds for the Cultivation of Guangdong College Students' Scientific and Technological Innovation. ("Climbing Program" Special Funds.) (No. pdjh2016b0108)

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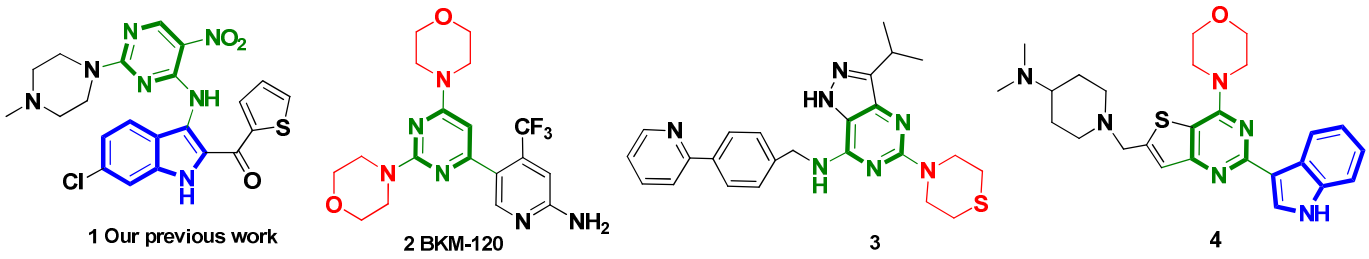
Fig. captions

Fig. 1. Our previously reported the indole-pyrimidine hybrid **1** and selected morpholine or thiomorpholine-linked pyrimidine and indole derivatives with antitumor activity.

Fig. 2. Design strategy of the title compounds.

Fig. 3. Effect of compound **14** on cell cycle and apoptosis in HeLa cells. Flow cytometry analysis of HeLa cells treated with **14** for 48 h. (A) Control; (B) **14**, 1.25 μM; (C) **14**, 2.5 μM; (D) **14**, 5 μM.

Fig. 4. Docking pose of compound **15** in tubulin with (a) and without (b) the surface of the pocket (PDP ID: 1SA0). Amino acid residues of tubulin beta chain are labeled in yellow and those in tubulin alpha in orange. MM-GBSA ΔG is -83.1 kcal/mol.



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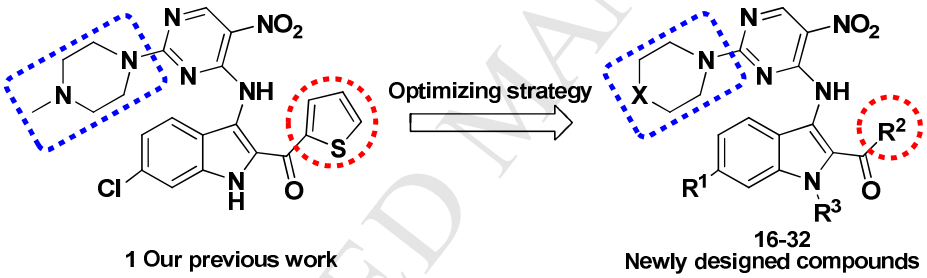

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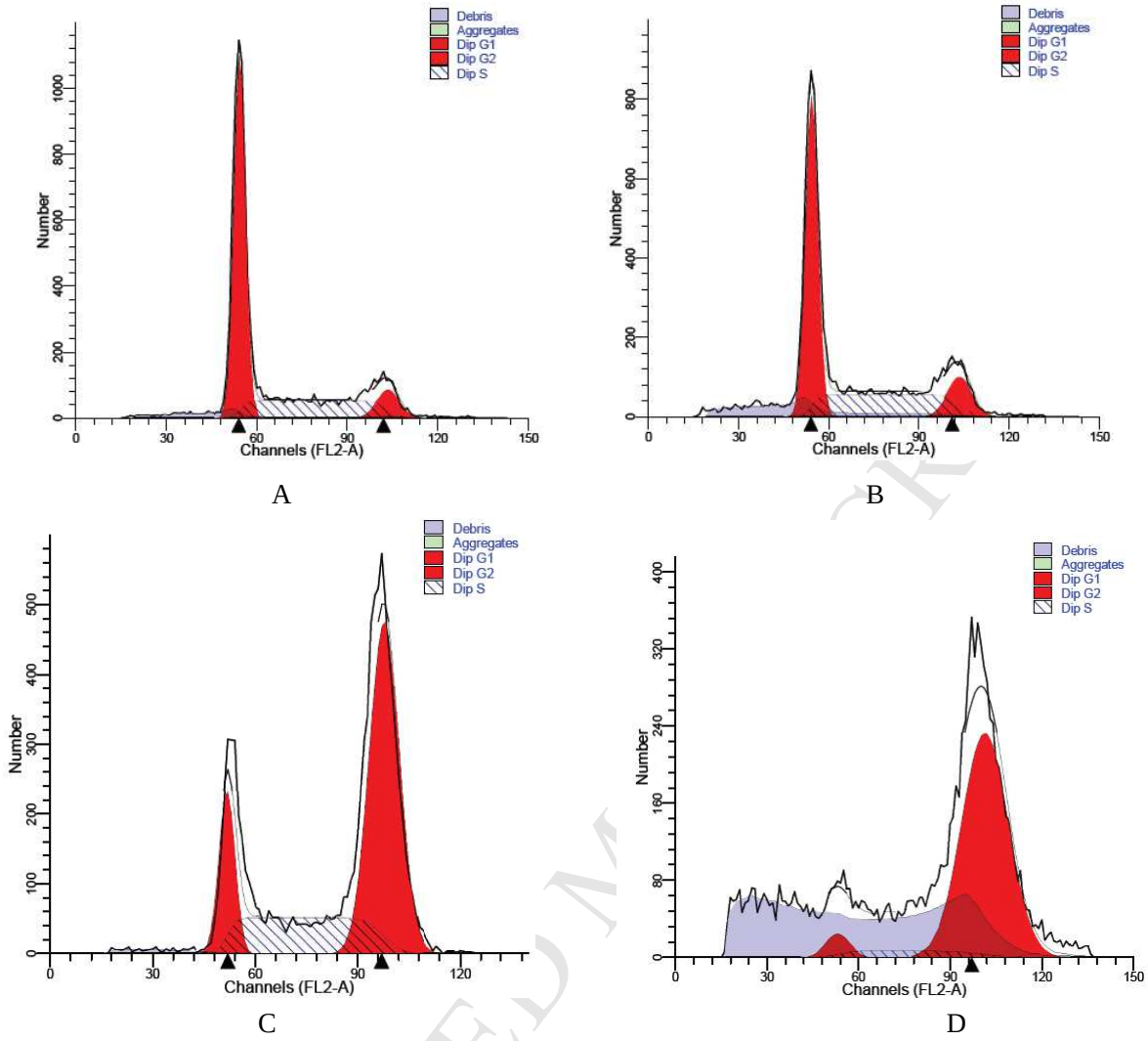

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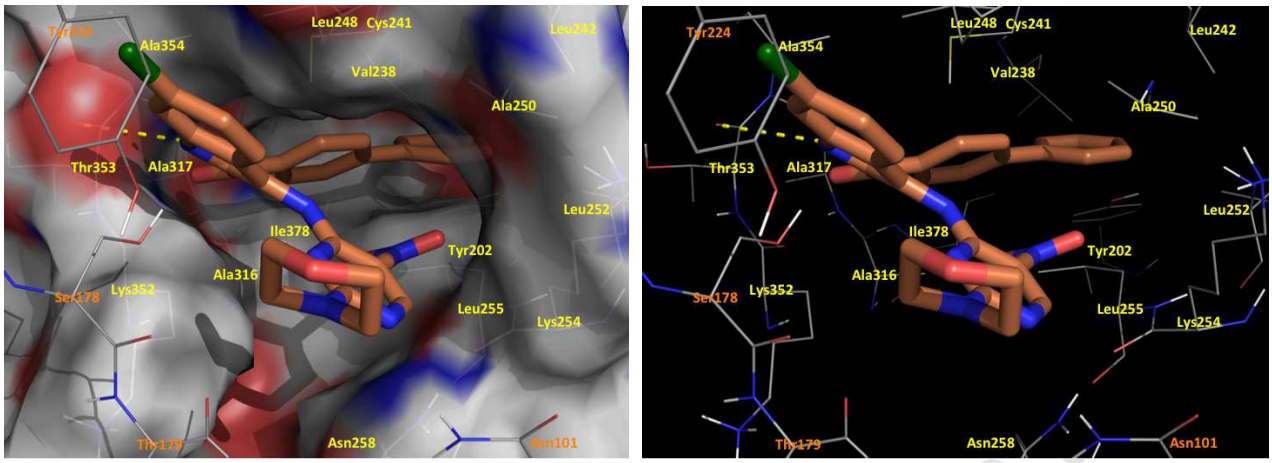

A B

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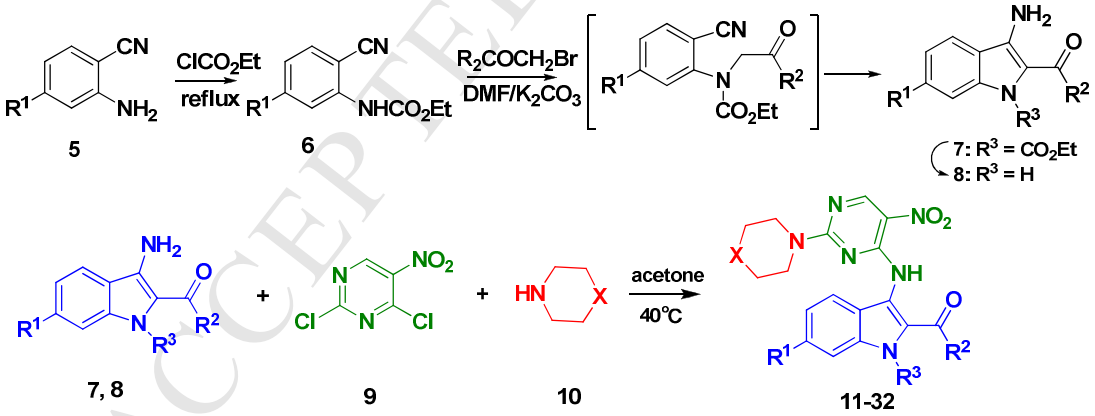

Scheme 1 One-pot synthesis of compounds **11–32**

660 **Table 1** Antiproliferative **activities of compounds 11–32 against a panel of human cancer and normal cell lines**

Comp.	X	R^1	R^2	R^3	*In vitro antiproliferative* IC_{50}(μM) [a]				
					HeLa	**MDA-MB-231**	**MCF-7**	**HCT116**	**HEK293** [b]
11	O	H	C_6H_5	H	82.55	92.63 ±0.96	89.2±1.26	55.77±2.13	NT [c]
12	O	H	4-BrC_6H_4	H	54.30±3.69	34.12±0.78	85.39±5.26	33.28±2.22	NT
13	O	H	4-$MeOC_6H_4$	H	4.32±0.29	8.69±0.33	57.12±2.36	6.40±0.14	14.77±0.15
14	O	H	3-BrC_6H_4	H	2.51±0.18	9.42±0.25	1.42±0.09	11.42±0.23	10.06±0.53
15	O	H	4-PhC_6H_4	H	4.04±0.37	10.35±0.23	0.29±0.02	9.48±0.11	78.63±1.25
16	O	H		H	49.65±2.56	89.36±2.34	89.36±2.39	73.49±2.13	NT
17	O	H	4-$MeOC_6H_4$	CO_2Et	5.52±0.26	3.01±0.18	2.13±0.14	2.99±0.13	>100
18	O	Cl	C_6H_5	H	>100	>100	>100	>100	NT
19	O	Cl	4-ClC_6H_4	H	>100	>100	83.65±3.25	>100	NT
20	O	Cl	4-$MeOC_6H_4$	H	47.05±3.65	>100	35.03±1.53	24.35±2.36	NT
21	O	Cl		H	94.65±0.99	>100	69.36±1.03	>100	NT
22	O	Cl	C_6H_5	CO_2Et	>100±0.	35.79±0.15	78.39±5.39	80.56±5.36	NT
23	O	Cl	4-PhC_6H_4	H	16.57±2.61	58.69±1.29	42.69±1.23	>100	NT
24	O	CH_3	C_6H_5	H	47.09±3.56	76.32±3.25	56.81±1.03	80.63±0.26	NT
25	O	CH_3	4-BrC_6H_4	H	>100	>100	>100	>100	NT
26	O	CH_3	4-$MeOC_6H_4$	H	90.53±2.15	89.36±0.15	>100	43.95±4.42	NT
27	O	CH_3	4-ClC_6H_4	H	>100	>100	98.68±4.26	>100	NT
28	O	CH_3		H	>100	93.65±0.32	>100	39.11±3.26	NT
29	O	CH_3	4-PhC_6H_4	H	7.46±0.21	>100	60.36±3.68	>100	NT
30	S	H	C_6H_5	H	>100	>100	>100	>100	NT
31	S	H	4-BrC_6H_4	H	>100	>100	>100	>100	NT
32	S	H	4-$MeOC_6H_4$	H	>100	>100	>100	>100	NT
			1		19.91±1.23	5.01±0.15	13.93±0.29	17.43±1.02	>100
			CA-4		0.23±0.018	270.0±1.65	0.041±0.013	6.10±0.14	NT

[a] 50% inhibitory concentration and mean ± SD of three independent experiments performed in duplicate. [b] Normal human embryonic kidney (HEK-293) cell lines. [c] NT: not tested.

665 **Table 2. Effect of compound 14 on cell cycle distribution in HeLa cells**

Concentration	**G_0/G_1(%)** [a]	**S(%)** [b]	**G_2/M(%)** [c]
0μM	61.74	29.14	9.12
1.25μM	53.32	34.05	12.63
2.5μM	15.31	26.35	58.34
5μM	5.02	6.40	88.58

[a] G0/G1: to prepare the cell for DNA synthesis. [b] S: DNA is manufactured during the phase.

[c] G2/M: is the phase in which DNA replication completed, start to mitosis.

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Table 3 Tubulin polymerization inhibitory activities of selected representative compounds

Comp.	X	R^1	R^2	R^3	Tubulin polymerization	
					% inhibition [a]	IC_{50}(μM)
11	O	H	C_6H_5	H	23	-[b]
13	O	H	4-$MeOC_6H_4$	H	14	-
14	O	H	3-BrC_6H_4	H	36	-
15	O	H	4-PhC_6H_4	H	42	19.3±1.18
17	O	H	4-$MeOC_6H_4$	CO_2Et	19	-
18	O	Cl	C_6H_5	H	9	-
20	O	Cl	4-$MeOC_6H_4$	H	14	-
21	O	Cl		H	19	-
30	S	H	C_6H_5	H	2	-
			1		47	11.2±1.13
			CA-4		80	4.22±0.22

[a] Compounds were tested at a final concentration of 10μM. [b] -: not tested.

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Highlights

• 22 novel indole-pyrimidine hybrids were designed and synthesized. • Antiproliferative activities of these compounds were evaluated. • Compound **14** arrested HeLa cells in G2/M phase of cell cycle. • Hybrid **15** exhibited powerful tubulin inhibitory activity. • Molecular modeling suggested that **15** binds well in the colchicine binding site of α,β-tubulin.