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N-Arylsulfonylsubstituted-*1H* indole derivatives as small molecule dual inhibitors of signal transducer and activator of transcription 3 (STAT3) and tubulin

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ABSTRACT

Signal transducer and activator of transcription (STAT3) is a proposed therapeutic target for the development of anti-cancer agents. In this report, a series of *N*-arylsulfonylsubstituted-1*H* indole derivatives were designed and synthesized as STAT3 inhibitors, their anti-proliferative activities were evaluated against a number of tumor cells, some potent compounds exhibited IC_{50} values less than 10 μM. The most potent compound **4a** was further confirmed to inhibit STAT3 phosphorylation at Tyr705. It was further revealed that **4a** arrested the cell cycle at the G2/M phase and inhibited tubulin polymerization. This study describes a series of *N*-arylsulfonylsubstituted-*1H* indole derivatives as potent anti-cancer agents targeting both STAT3 and tubulin.

1. Introduction

The cytoplasmic signal transducer and activator of transcription (STAT) family proteins regulate the gene expression related to the cell proliferation, differentiation, apoptosis, immune-response, and angiogenesis.[1] There are seven different isoform members in this family: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6. Although structurally related,[2] they participate in different cellular processes. In particular, the STAT3 protein attracted a great attention for its crucial function in cancer development. As part of Janus kinase (Jak) signaling pathway, STAT3 is activated by both receptor and non receptor tyrosine kinase via the phosphorylation cascade.[3] The SH2 domain Tyr705 phosphorylation would transform inactive STAT3 into its active form, resulting in protein dimerization. STAT3 dimer then dissociated from the receptor and translocated from the cytosol into the nucleus to combine with the target genes, and stimulate the expression of the target proteins.[4] The instantaneous activation of STAT3 in normal cell differs from the continuous activation in the cancer cell. Due to its important role in malignant transformation and tumorgenesis, STAT3 was considered as a promising anticancer drug target.[5]

Most reported small molecule inhibitors of STAT3 were divided into two categories: DNA-binding domain inhibitors and/or SH2-binding domain inhibitors.[5] As the first reported nonpeptide small molecule STAT3 inhibitor, **STA-21**[6] (Fig. 1), was discovered through virtual screening. On the basis of **STA-21**, more synthetic compounds like **LLL-3**, **LLL-12**[7] (Fig. 1) were designed and revealed. **Stattic** was one of the most potent STAT3 inhibitors.[8] Derived from **Stattic**, compound **HJC0146**[9] (Fig. 1) displayed even higher potency and promoted cell apoptosis. In addition to these above mentioned compounds, other structurally distinct molecules were investigated, including: **S3I-201**[10], platinum (IV) complexes[11], **InS3-54**[12], **NSC-743380**[13] (Fig. 1). Despite the above mentioned research effort, none of these molecules was advanced to a successful clinical trial, probably due to the lack of ideal physical and chemical properties. Therefore, novel STAT3 inhibitors with distinctive scaffold need to be further explored.

Microtubule/tubulin is aggregated by the α- and β-tubulin heterodimer through so called "head to tail" manner to assemble into a tubular structure.[14] In the cell mitosis (M) phase, microtubules presented as a spindle to guide the genetic material distribute evenly into two daughter cells.[15] The proper dynamic balance between polymerization and depolymerization of microtubules is critical for the maintenance of cell morphology, cell signal transduction, and cell division.[16] Either promotion or inhibition of tubulin polymerization would disrupt mitosis and lead to cell death. The

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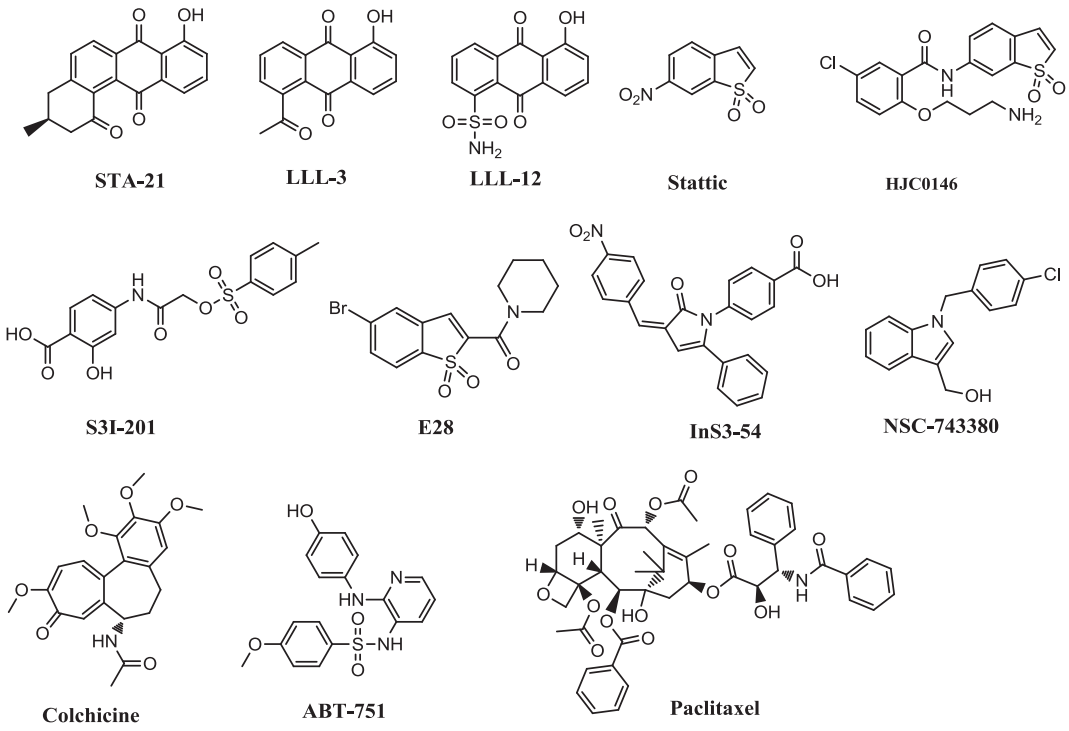


Fig. 1. Representative inhibitors of STAT3 and microtubule.

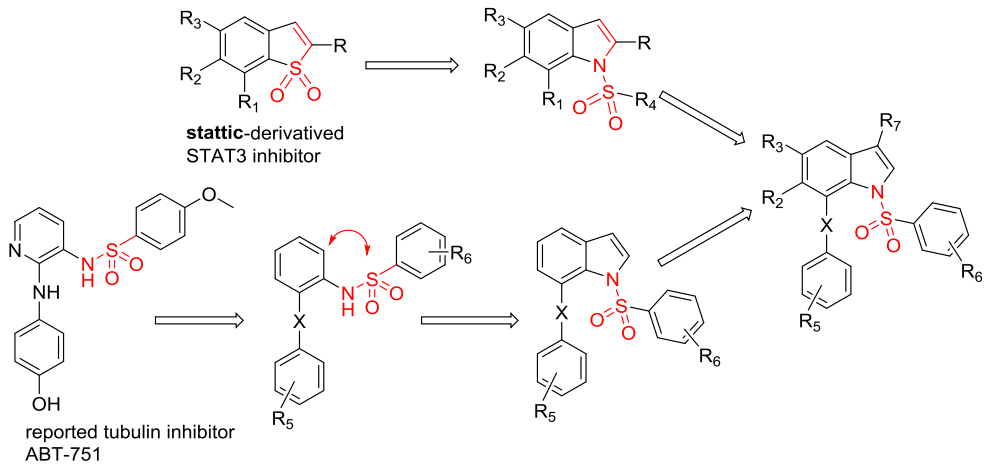


Fig. 2. Design rationale of prepared compounds.

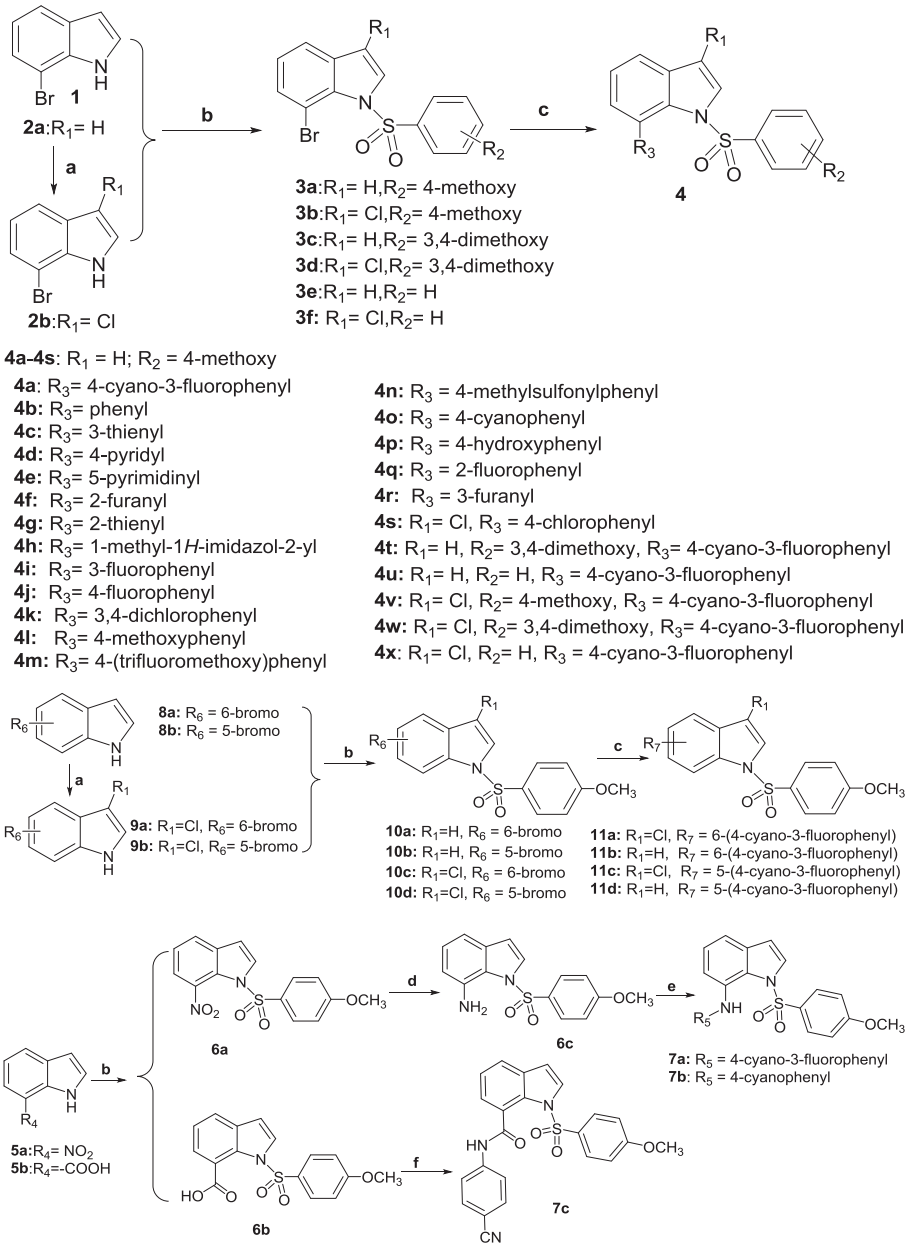
rapid growth of cancer cell makes this polymerization/depolymerization process more active than that in the normal cell. Consequently, microtubule has been considered a good target for anticancer drug development. A number of natural products, like paclitaxel, vincristine, and colchicine, have been demonstrated to bind to microtubule and exerted disruptive effect to tubulin dynamic process.[17] Specifically, paclitaxel functions as microtubule-stabilizer, while colchicine behaves as microtubule-destabilizer.

In the course of searching potent STAT3 inhibitors, our laboratory conducted structural modification based on the reported compound **Stattic** (Fig. 1), and discovered one of our most potent STAT3 inhibitor **E28**.[18] **E28** inhibited STAT3 Tyr705 phosphorylation and induced tumor cell apoptosis. Unfortunately, **E28** structurally bears the α, β-unsaturated Michael acceptor like moiety[19], which could result in a broad spectrum toxicity. Aiming to remove this moiety in **E28**, a nitrogen atom was introduced to replace the sulfonyl group in the five member ring. Consequently, A number of *N*-arylsulfonylsubstituted-1*H* indoles (Fig. 2) were designed as a novel type of STAT3 inhibitors. Interestingly, this new skeleton is structurally similar to a reported microtubule inhibitors **ABT-751**[15,20] (Fig. 1). Therefore, we further evaluated the compounds biological activity as tubulin polymerization inhibitors.

2. Results and discussion

2.1. Chemistry

The synthetic routes of the designed compounds **4a–4x**, **7a–7c**, **11a–11d** were shown in Scheme 1. To prepare the reaction intermediates **2b**, **9a** and **9b**, the chlorine was introduced to the



Scheme 1. Synthetic route of *N*-arylsulfonylsubstituted-*1H* indoles. Reagents and conditions: (a) NCS, 1 h, room temperature, DMF; (b) substituted benzene sulfonyl chloride, NaOH, TEBAC, DCM; (c) corresponding boronic acid, $PdCl_2$(dppf), K_2CO_3. 86 °C, 4–12 h; (d) Fe, CH_3COOH/H_2O, 65 °C; (e) $Pd_2(dpa)_3$, BINAP, sodium *tert*-butoxide, 80 °C; (f) 4-cyanoaniline, EDC, pyridine, room temperature.

β-position of the indole ring by reaction of indole with *N*-chlorosuccinimide (NCS) in DMF. The sulfonamides **3a–3f**, **6a**, **6b** and **10a–10d** were obtained through reactions of benzenesulfonyl chloride with the corresponding substituted indoles. The final target compounds **4a–4x**, **11a–11d** were achieved through Suzuki coupling reaction of bromo-substituted arylsulfonyl 1*H*-indole with the corresponding boronic acids at moderate yields. Reduction of nitro group to amino in **6a** gave **6c**, which was reacted with 4-bromo-2-fluorobenzonitrile or 4-bromobenzonitrile to furnish the synthesis of **7a** or **7b**. The reaction of carboxylic acid **6b** with 4-cyanoaniline using coupling reagent EDC provided compound **7c**. All target compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS (ESI). High pressure liquid chromatography was performed to guarantee the compound purity is greater than 98% before further biological evaluation.

2.2. Biological study

2.2.1. MTT assay and SAR analysis

To study the compound inhibitory activity against cancer cell proliferation, MTT assay was performed and the result was shown in Table 1. Cell lines with continuous activation of STAT3 were selected, including A549, DU145, MCF-7, MDA-MB-231, KB and HepG2. One of our reported highly potent compound **E28** was used as positive control. The IC_{50} values for all prepared compounds were shown in Table 1. For those compounds displaying less than 50% inhibition at 20 μM, their IC_{50} values were not further determined. Otherwise, compounds were diluted and tested to calculate IC_{50} values. Overall, six compounds (**4a**, **4f**, **4n**, **4o**, **4v**, and **7c**) displayed potent inhibitory activity against cell proliferation for all six cancer cells, with IC_{50} values less than 10 μM. Compounds **4a** and

Table 1
Antiproliferative activity of the designed compounds.

Comp	MTT, IC_{50} ± SD(μM)[a]					
Cells	**A549**	**MCF-7**	**DU145**	**KB**	**HepG2**	**MDA-231**
4a	3.31 ± 1.64	3.20 ± 0.09	8.88 ± 0.51	6.17 ± 0.12	3.29 ± 0.86	2.23 ± 0.12
4b	10.25 ± 1.40	5.33 ± 0.29	10.66 ± 4.41	8.35 ± 2.03	5.51 ± 0.64	>10
4c	10.61 ± 1.12	>10	14.14 ± 1.36	>10	5.94 ± 0.96	>10
4d	5.30 ± 0.34	3.01 ± 0.14	7.06 ± 0.53	>10	4.68 ± 0.73	2.69 ± 0.25
4e	>10	>10	>10	>10	>10	>10
4f	5.30 ± 1.11	7.31 ± 0.176	4.44 ± 1.45	4.78 ± 0.13	9.07 ± 3.14	8.86 ± 0.36
4g	4.97 ± 0.96	>10	12.43 ± 1.26	>10	>10	>10
4h	4.61 ± 0.72	>10	6.01 ± 0.38	2.31 ± 0.36	5.10 ± 0.28	>10
4i	7.29 ± 1.51	>10	>10	>10	4.17 ± 0.14	>10
4j	11.42 ± 1.01	>10	>10	9.59 ± 0.15	6.21 ± 0.25	>10
4k	>20	>10	6.21 ± 0.58	>10	>10	>10
4l	11.67 ± 0.01	>10	>20	7.56 ± 0.35	5.38 ± 0.68	>10
4m	3.01 ± 1.28	>10	10.43 ± 0.69	5.86 ± 0.54	>10	9.23 ± 1.02
4n	2.89 ± 0.75	4.75 ± 0.97	7.25 ± 0.24	5.85 ± 1.65	3.65 ± 0.74	3.39 ± 0.89
4o	3.32 ± 1.03	1.27 ± 0.277	6.16 ± 1.98	2.63 ± 0.27	2.65 ± 0.12	2.54 ± 0.85
4p	8.44 ± 1.06	>10	10.66 ± 0.21	>10	>10	>10
4q	4.68 ± 0.24	>10	8.49 ± 0.23	>10	>10	>10
4r	>10	>10	>10	>10	>10	>10
4s	8.96 ± 1.26	6.63 ± 2.10	6.25 ± 1.50	>10	4.82 ± 0.83	8.36 ± 0.95
4t	>10	>10	>10	3.7 ± 1.35	>10	19.3 ± 0.67
4u	>10	6.95 ± 2.17	>10	7.31 ± 0.48	>10	>10
4v	4.83 ± 0.94	5.58 ± 0.39	8.77 ± 0.29	6.55 ± 0.09	2.77 ± 0.11	2.75 ± 0.15
4w	10.19 ± 3.27	8.36 ± 2.63	ND[b]	ND	7.15 ± 0.42	4.30 ± 0.43
4x	9.39 ± 1.72	2.46 ± 0.95	ND	ND	>20	>10
7a	4.06 ± 0.56	10.50 ± 2.47	8.75 ± 0.62	>10	>10	5.51 ± 0.23
7b	3.64 ± 0.89	7.59 ± 0.96	>10	7.22 ± 0.07	8.64 ± 1.19	2.28 ± 1.22
7c	3.47 ± 0.32	3.08 ± 0.77	8.19 ± 0.51	4.42 ± 0.22	2.91 ± 0.07	7.75 ± 0.58
11a	>20	>20	ND	ND	>10	>10
11b	>20	>10	>20	>10	>10	>10
11c	>10	>10	N	N	>10	>10
11d	>20	>10	>20	>10	>10	>10
E28	1.70 ± 0.29	0.91 ± 0.07	1.03 ± 0.29	ND	ND	0.70 ± 0.34

[a] Cell proliferation experiment determined by MTT method, The data are the mean ± SD from at least three independent experiments.
[b] Not determined.

4v displayed similar inhibitory activity for all cell lines, indicating chlorine at 3-position of indole had no favorable effect. Compared to **4u** (without methoxy group at the *para*- position of sulfonamide), compound **4a** (*para*-methoxy) displayed higher potency against all cancer cell lines, suggesting electron-donating methoxy group at sulfonamide *para*- position could increase the compound inhibitory ability. However, for compound **4s**, with two methoxy groups at *meta*- and *para*- position, low solubility in the assay solution was observed, which may lead to the reduced potency compared to **4a**. Apparently, the position of the aryl group on the phenyl ring of indole was critical for the compound potency, compound **4a**, with Ar at 7-position, demonstrated much higher inhibitory activity compared to **11b** (Ar at position 6) and **11d** (Ar at position 5) as indicated by their IC_{50} values. In addition, heterocycles at position 7 were tolerated. For example, compounds **4c** (3-thienyl), **4d** (4-pyridyl-) and **4f** (2-furanyl) displayed similar IC_{50} values with compound **4b** (phenyl), while compound **4g** (2-thienyl) exhibited the lowest activity. A variety of substitutes at R3 position were investigated, and it was observed that the electron withdrawing cyano group (**4o**) or methylsulfonyl (**4n**) was more favorable for compound inhibitory activity comparing to chloro- (**4s**) or fluoro- (**4j**). Both **4s** and **4j** were more potent than compound **4l**, with electron donation methoxy- substitute. Additionally, introduction of substituent at 3-(**4i**) or 4-position (**4j**) position of the phenyl ring decreased the compound inhibitory activity. Finally, insertion of amino linkage (**7a**, **7b**) or amide linkage (**7c**) would not add favorable effect to the compound potency.

2.2.2. Inhibition the phosphorylation of STAT3 (Tyr705)

To investigate the compound interaction with STAT3 protein, compounds **4a**, **4n** and **7a** were selected and western blot analysis was performed to detect the amount of p-STAT3 at Tyr 705 and the T-STAT3 using MDA-MB-231 breast cancer cell, **E28** was used as control compound. As shown in Fig. 3**A**, after 12 h treatment, all three compounds (**4a**, **4n** and **7a**) reduced the amount of p-STAT3 in a concentration depended manner, while the amount of T-STAT3 was not changed at the selected concentration range, indicating these compounds exerted inhibitory effect against STAT3 phosphorylation, and the increase of p-STAT3 was hardly related to the reduction of the T-STAT3. This inhibitory manner is similar to that of **E28**, though the activity is slightly weaker.

2.2.3. Compound *4a* inhibited IL-6 induced STAT3 phosphorylation

To further study the compound inhibitory effect toward STAT3 phosphorylation, MDA-MB-231 cell was starved by cultivating in serum-free medium overnight, then treated with **4a** for 2 h before being stimulated by IL-6 (25 ng/mL). The result is shown in Fig. 3**B**. IL-6 stimulation significantly elevated the level of phosphorylated STAT3, while **4a** treatment markedly reduced the overexpression of STAT3 phosphorylation. This result further demonstrated **4a** could inhibit STAT3 phosphorylation.

2.2.4. Compound *4a* could induce cell apoptosis

To determine whether compound **4a** possessed the ability to induce cell apoptosis, MDA-MB-231 cell was treated with different concentration of **4a**, and the level of full PARP and cleaved PARP were characterized through western blot analysis. Consistent with the expectation, **4a** treatment significantly elevated the level of cleaved PARP in time- and concentration-dependent manner (Fig. 3**C**). While PARP is the characteristic protein for cell apoptosis, this result demonstrated that the antiproliferative activity of **4a** was partially attributed to its ability to induce cell apoptosis.

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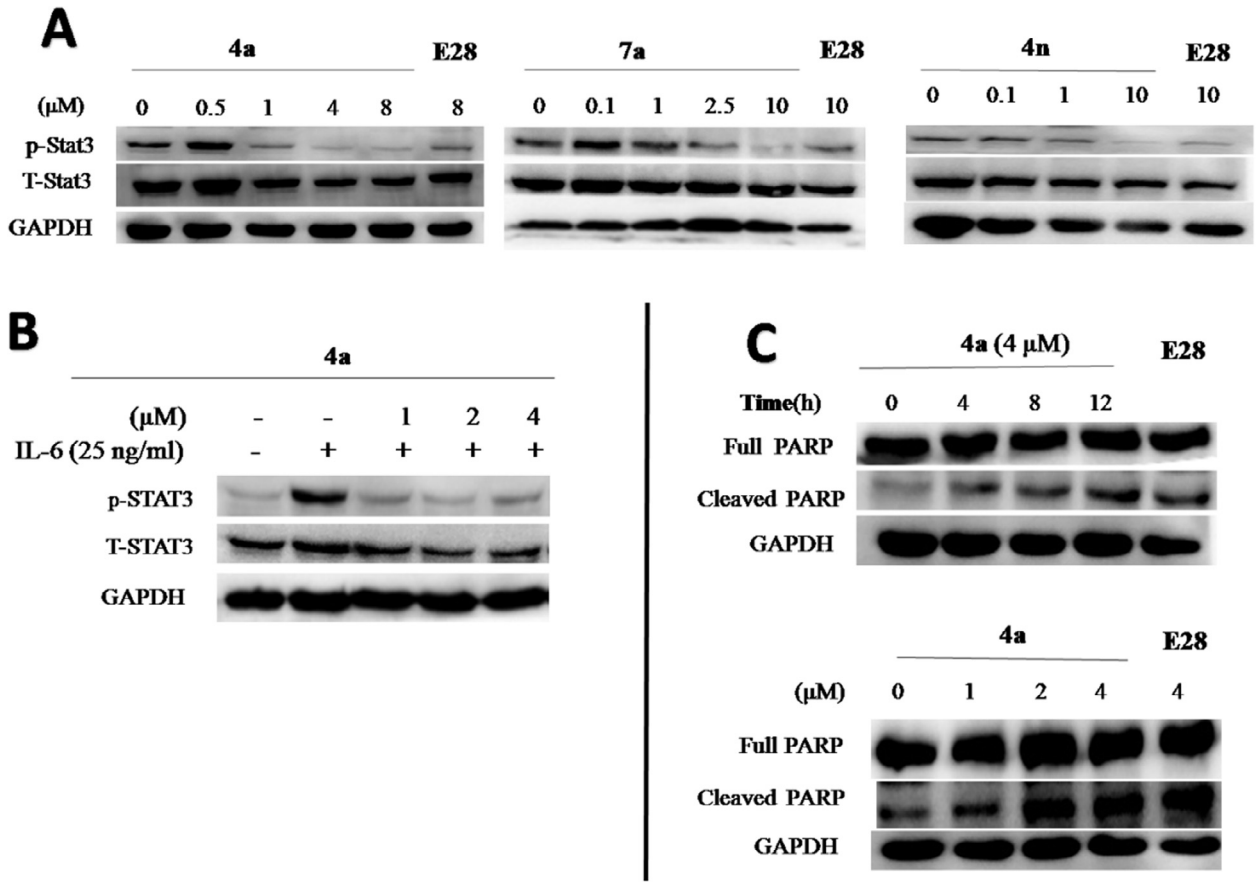

Fig. 3. Compounds affected STAT3-Y705 phosphorylation in MDA-MB-231 cell.

*2.2.5. Compound **4a** could block the cell cycle*

Based on the above observed results from cell apoptosis experiment, the effect of compound **4a** on cell cycle was examined by flow cytometry, and the result was shown in the Fig. 4. Treatment of A549 cell with **4a** for 12 h at different concentration resulted in a concentration-dependent accumulation of A549 cells in the G2/M phase, accompanied by losses of the G1 and S phases, especially the reduction of G1 phase. These results indicate cell cycle arrested in G2/M phase was induced by **4a**.

2.2.6. Fluorescence images of microtubules morphology

To investigate the compound effect on the cellular microtubule, the morphology of microtubules in A549 cells was examined by confocal microscope. Paclitaxel and colchicine were used as reference compounds. The results were shown in Fig. 5. As reported, paclitaxel promoted β-tubulin polymerization, while colchicine inhibited microtubule polymerization process. At 5 μM, compounds **4a** and **7c** exhibited similar behavior with colchicine by destroying the β-tubulin assembly. This result revealed that compound **4a** and **7c** could bind to β-tubulin and exert the inhibitory effect against microtubule polymerization. By comparison, compound E28 exerted no effect to tubulin polymerization/depolymerization.

2.2.7. Inhibition of tubulin polymerization in vitro

To further examine the compound inhibitory activity against tubulin polymerization, **4a** was evaluated for inhibition of tubulin polymerization using tubulin polymerization assay. Paclitaxel and colchicine were chosen as control compounds. The result was shown in Fig. 6. Contrary to the paclitaxel depolymerization effect, compound **4a** inhibited tubulin polymerization in a concentration-dependent manner, which is similar to that of colchicine, both **4a** and colchicine destroyed tubulin assembly process, consistent with the results from immunofluorescence staining experiment.

3. Conclusion

In summary, a class of *N*-arylsulfonyl substituted indole derivatives were synthesized and evaluated as dual inhibitors of STAT3 and tubulin. We have demonstrated that this series of compounds retained inhibitory activity to STAT3 phosphorylation, while empowered inhibitory ability to tubulin polymerization. Several compounds demonstrated strong antiproliferative activity in vitro. It was also confirmed the representative compound blocked the cell cycle in A549 with cell accumulation at G2/M phase. Additionally, immunofluorescence staining and inhibition of tubulin polymerization experiments confirmed the compound microtubule-destabilizing effect. Further study should be carried out to investigate these compounds in vivo antitumor activity, as well as the pharmacokinetic properties.

4. Materials and methods

4.1. Chemical synthesis

All chemicals were commercially available from J&K Scientific LTD, Sinopharm Chemical Reagent Co, Ltd and Energy Chemical. The chemical reaction process was monitored by thin layer chromatography (TLC) and observed under the UV light (254 nm). Reaction of post-processing contains silica gel chromatograph, recrystallization, salting out methods and so on. ^{1}H NMR and ^{13}C

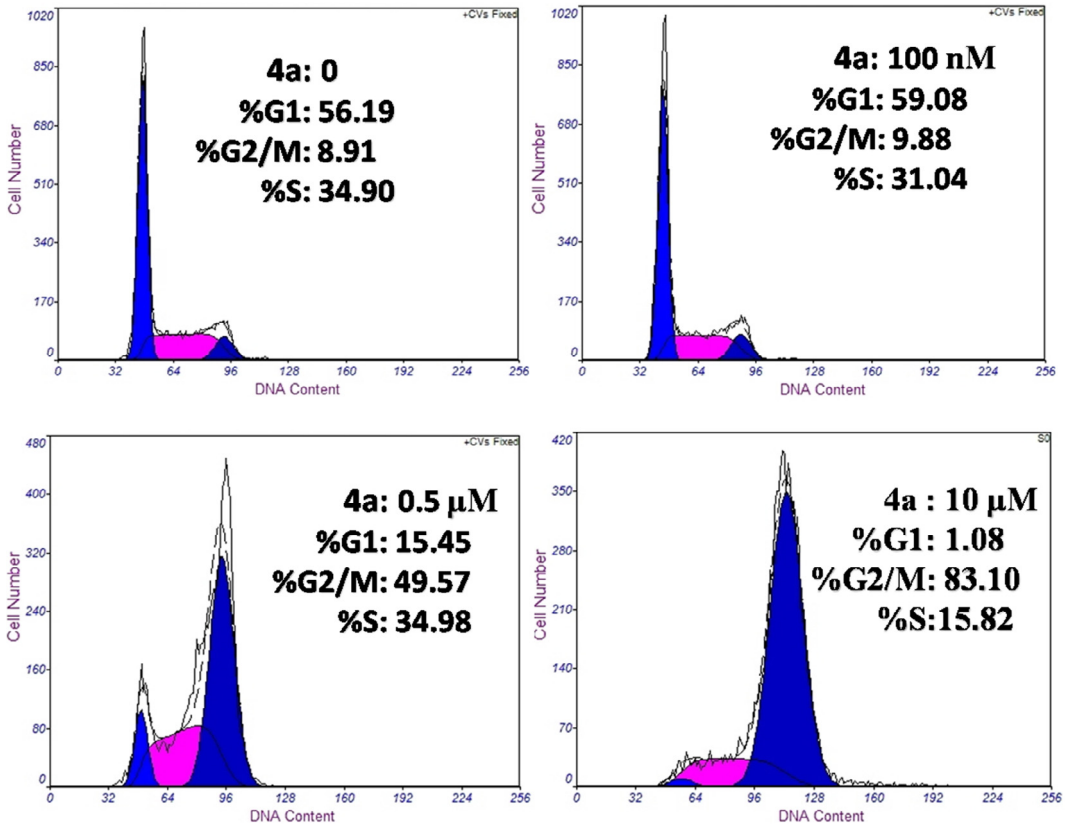

Fig. 4. Concentration effect of compound **4a** on cell cycle progress of A549 cells.

NMR spectra were measured on a Agilent 400 MHz or 600 MHz spectrometer and referenced to TMS. All final compounds were examined by HPLC analysis to assure the purity was higher than 98%. Mass spectra were obtained on an Agilent HRMS (ESI) spectrometer.

4.1.1. The synthesis of intermediates: ***2b***, ***9a***, *and* ***9b***

The solution of 5-, 6- or 7-bromo substituted indole (0.26 mmol) and NCS (0.31 mmol) in 5 mL DMF was stirred for 1 h, then water was added to terminate the reaction. The aqueous layer was extracted with DCM (3 × 15 mL). The combined organic layers were dried with Na_2SO_4, DCM was removed by rotary evaporator to afford intermediates **2b**, **9a**, and **9b**.

7-Bromo-3-chloro-1H-indole (**2b**). Yield 89%, liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H, NH), 7.59 (d, J = 8.0 Hz, 1H, CH=CH-N), 7.40 (d, J = 8.0 Hz, 1H, Ar-H), 7.25 (d, J = 8.0 Hz, 1H, Ar-H), 7.07 (m, 1H, Ar-H).

6-Bromo-3-chloro-1H-indole (**9a**). Yield 92%, liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H, NH), 7.63 (d, J = 7.8 Hz, 1H, CH=CH-N), 7.39 (d, J = 7.6 Hz, 1H, Ar-H), 7.04 (t, J = 7.7 Hz, 1H, Ar-H), 6.66 (s, 1H, Ar-H).

5-Bromo-3-chloro-1H-indole (**9b**),[21] Yield 93%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H, NH), 7.78 (d, J = 1.8 Hz, 1H, CH=CH-N), 7.33 (dd, J = 8.7, 1.8 Hz, 1H, Ar-H), 7.24 (d, J = 8.7 Hz, 1H, Ar-H), 7.19 (d, J = 2.6 Hz, 1H, Ar-H).

4.1.2. The synthesis of intermediates: 3a-3f, 6a-6c and 10a-10d

A mixture of **2a** (0.765 mmol), or **2b**, **5a**, **5b**, **9a**, **9b**, TEBAC (0.383 mmol) and NaOH (3.06 mmol) in 10 mL THF was stirred for 30 min, then 4-methoxybenzenesulfonyl chloride (1.15 mmol) was slowly added under ice bath condition, and the reaction was stirred for another 30 min before adding water to terminate the reaction. The mixture was extracted with DCM (3 × 20 mL). The combined organic layers were dried with Na_2SO_4, the solvent was removed by rotary evaporators to afford reaction intermediates ***3a–3f***, ***6a***, ***6b***, *and* ***10a–10d***.

A mixture of compound **6a** (0.541 mmol) and Fe (3.63 mmol) was stirred in 5 mL of EtOH:H_2O = 3:1 for 10 min. To this mixture was added AcOH (7.31 mmol) slowly, and the resulting reaction mixture was stirred at 75 °C until the reaction was complete. The mixture was filtered through a pad of Celite, and the filtrate was extracted with EA (3 × 20 mL). The combined organic layers were washed with saline for three times, dried with Na_2SO_4, concentrated, and purified by silica gel chromatography to give **6c**.

7-Bromo-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**3a**). Yield 72%, white solid; ^{1}H NMR (400 MHz,$CDCl_3$) δ 7.89 (d, J = 4.0 Hz, 1H, CH=CH-N), 7.74 (d, J = 8.0 Hz, 2H, Ar-H), 7.51 (d, J = 8.0 Hz, 1H, Ar-H), 7.45 (d, J = 8.0 Hz, 1H, Ar-H), 7.06 (d, J = 4.0 Hz, 1H, Ar-H), 6.92 (d, J = 12.0 Hz, 2H, Ar-H), 6.70 (d, J = 4.0 Hz, 1H, CH=CH-N), 3.83 (s, 3H, CH_3)

7-Bromo-3-chloro-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**3b**). Yield 62%, white solid; ^{1}H NMR (400 MHz,$CDCl_3$) δ 9.13 (d, J = 3.8 Hz, 1H, CH=CH-N), 8.36 (d, J = 6.7 Hz, 1H, Ar-H), 7.70 (dd, J = 7.8, 4.7 Hz, 1H, Ar-H), 7.55 (t, J = 7.7 Hz, 2H, Ar-H), 7.45 (t, J = 7.4 Hz, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 6.42 (s, 1H, CH=CH-N), 3.59 (s, 3H, CH_3).

7-Bromo-1-((3,4-dimethoxyphenyl)sulfonyl)-1H-indole (**3c**). Yield 66%, white solid; ^{1}H NMR (400 MHz,$CDCl_3$) δ 8.14 (s, 1H, CH=CH-N), 7.46 (t, J = 6.0 Hz, 2H, Ar-H), 7.31 (t, J = 10.2 Hz, 2H, Ar-H), 7.22 (d, J = 12.0 Hz, 1H, Ar-H), 6.79 (d, J = 8.5 Hz, 1H, Ar-H), 6.55 (d, J = 3.3 Hz, 1H, CH=CH-N), 3.81 (s, 6H, CH_3).

7-Bromo-3-chloro-1-((3,4-dimethoxyphenyl)sulfonyl)-1H-indole (**3d**). Yield 68%, white solid; ^{1}H NMR (400 MHz,$CDCl_3$) δ 8.19 (s, 1H, CH=CH-N), 7.52 (d, J = 3.3 Hz, 1H, Ar-H), 7.38 (d, J = 8.3 Hz, 1H,

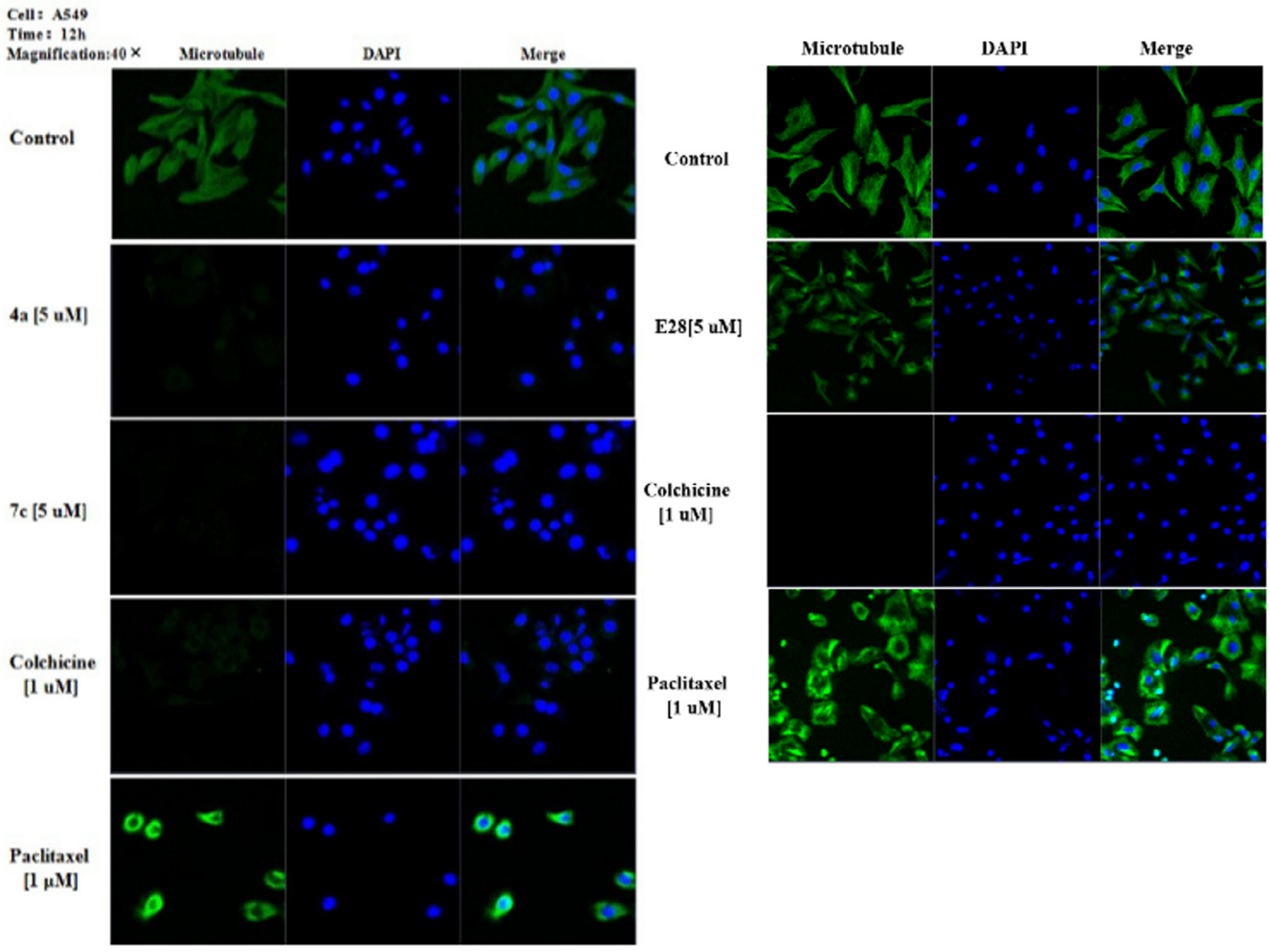

Fig. 5. Effect of **4a** and **7c** on the cellular microtubule network.

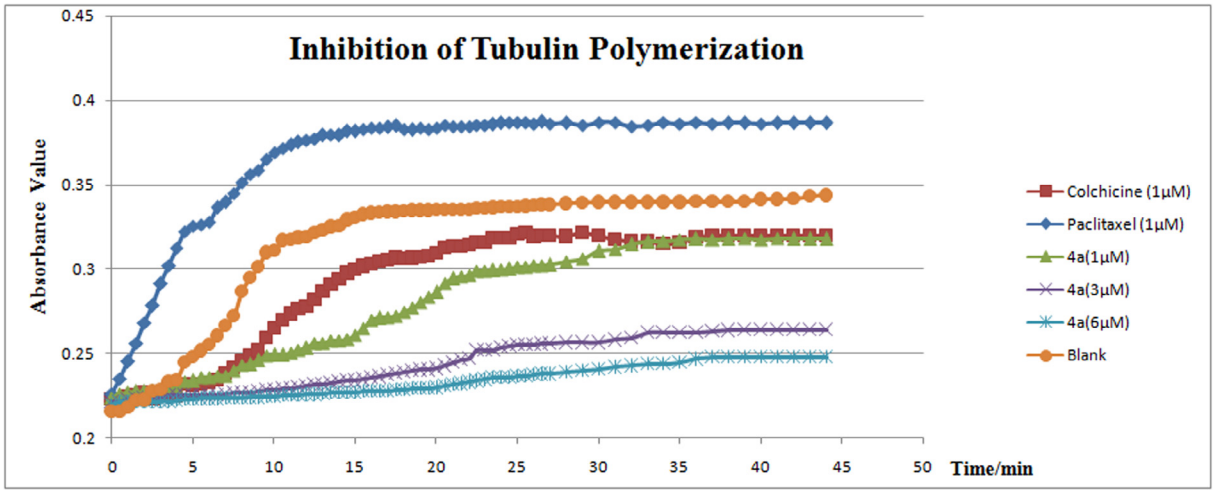

Fig. 6. Compound **4a** inhibits tubulin polymerization.

Ar-H), 7.35–7.28 (m, 2H, Ar-H), 6.85 (d, J = 8.5 Hz, 1H, Ar-H), 6.60 (d, J = 3.3 Hz, 1H, CH=CH-N), 3.86 (s, 6H, CH_3).

7-Bromo-1-(phenylsulfonyl)-1H-indole (**3e**). Yield 89%, white solid; ^{1}H NMR (400 MHz,$CDCl_3$) δ 7.92 (d, J = 3.5 Hz, 1H, CH=CH-N), 7.80 (d, J = 7.5 Hz, 2H, Ar-H), 7.59 (t, J = 7.4 Hz, 1H, Ar-H), 7.53 (d, J = 7.7 Hz, 1H, Ar-H), 7.47 (dd, J = 17.6, 8.0 Hz, 3H, Ar-H), 7.06 (t, J = 7.7 Hz, 1H, Ar-H), 6.73 (d, J = 3.5 Hz, 1H, CH=CH-N).

7-Bromo-3-chloro-1-(phenylsulfonyl)-1H-indole (**3f**). Yield 81%, white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 8.7 Hz, 2H, Ar-H, CH=CH-N), 7.86 (d, J = 1.2 Hz, 1H, Ar-H), 7.68 (d, J = 1.8 Hz,

1H, Ar-H), 7.58 (dd, *J* = 13.7, 6.3 Hz, 2H, Ar-H), 7.49–7.45 (m, 3H, Ar-H).

1-((4-Methoxyphenyl)sulfonyl)-7-nitro-1H-indole (**6a**). Yield 87%, white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.76 (d, *J* = 7.8 Hz, 1H, CH=CH-N), 7.72 (d, *J* = 3.7 Hz, 1H, Ar-H), 7.65 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.31 (t, *J* = 7.8 Hz, 1H, Ar-H), 6.98 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.80 (d, *J* = 3.7 Hz, 1H, CH=CH-N), 3.86 (s, 3H, CH_3).

1-((4-Methoxyphenyl)sulfonyl)-1H-indole-7-carboxylic acid (**6b**). Yield 65%, white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (m, 1H, COOH), 8.23–8.17 (m, 1H, CH=CH-N), 7.54 (dd, *J* = 7.8, 4.7 Hz, 1H, Ar-H), 7.40 (t, *J* = 7.7 Hz, 2H, Ar-H), 7.30 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.12 (d, *J* = 7.6 Hz, 2H, Ar-H), 6.26 (s, 1H, CH=CH-N), 3.44 (s, 3H, CH_3).

1-((4-Methoxyphenyl)sulfonyl)-7-amino-1H-indole (**6c**). Yield 59%, yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ7.78 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.55 (d, *J* = 3.7 Hz, 1H, CH=CH-N), 7.01 (t, *J* = 7.6 Hz, 1H, Ar-H), 6.88–6.83 (m, 3H, Ar-H, CH=CH-N), 6.57 (d, *J* = 7.7 Hz, 2H, Ar-H), 5.06 (s, 2H, NH_2), 3.77 (s, 3H, CH_3).

6-Bromo-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**10a**). Yield 86%, white solid; ^{1}H NMR (400 MHz,$CDCl_3$) δ 7.90 (d, *J* = 3.4 Hz, 1H, CH=CH-N), 7.74 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.51 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.45 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.04 (t, *J* = 7.7 Hz, 1H, Ar-H), 6.91 (d, *J* = 8.7 Hz, 2H, Ar-H), 6.70 (d, *J* = 3.5 Hz, 1H, CH=CH-N), 3.82 (s, 3H, CH_3).

5-Bromo-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**10b**). Yield 90%, white solid; ^{1}H NMR (400 MHz,$CDCl_3$) δ7.64 (d, *J* = 3.4 Hz, 1H, CH=CH-N), 7.49 (s, 1H, Ar-H), 7.47 (s, 1H, Ar-H), 7.26–7.16 (m, 2H, Ar-H), 6.78 (t, *J* = 7.7 Hz, 1H, Ar-H), 6.65 (d, *J* = 8.7 Hz, 2H, Ar-H), 6.44 (d, *J* = 3.5 Hz, 1H, CH=CH-N), 3.57 (s, 3H, CH_3).

6-Bromo-3-chloro-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**10c**). Yield 68%, white solid; ^{1}H NMR (400 MHz,$CDCl_3$) δ 7.85 (d, *J* = 8.8 Hz, 1H, CH=CH-N), 7.79 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.64 (s, 1H, Ar-H), 7.55 (d, *J* = 3.6 Hz, 1H, Ar-H), 7.38 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.88 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.57 (d, *J* = 3.6 Hz, 1H, CH=CH-N), 3.79 (s, 3H, CH_3).

5-Bromo-3-chloro-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**10d**). Yield 61%, white solid; ^{1}H NMR (400 MHz,$CDCl_3$) δ 7.84 (d, *J* = 8.8 Hz, 1H, CH=CH-N), 7.77 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.61 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.52 (s, 1H, Ar-H), 7.42 (dd, *J* = 8.8, 1.7 Hz, 1H, CH=CH-N), 6.85 (d, *J* = 9.0 Hz, 2H, Ar-H), 3.74 (s, 3H, CH_3).

4.1.3. Synthesis of the final compounds **4a–4x**, and **11a–11d**

General procedure. A solution of **3a–3f** (0.3 mmol), $PdCl_2$(dppf) (0.003 mmol), phenylboronic acid (0.446 mmol), and potassium carbonate (1.04 mmol) in 5 mL of DME:H_2O = 3.5:1 was heated at 86 °C for 2 h. Then, water was added to terminate the reaction, and the mixture was extracted with DCM (3 × 15 mL). Combined organic layers were washed with saline for three times, dried with Na_2SO_4, concentrated and purified by silica gel chromatography to afford the target compounds.

N-(4-Methoxyphenyl)sulfonyl-7-(3-fluoro-4-cyano)-phenyl-1H-indole (**4a**). Yield 77%, white solid; ^{1}H NMR (400 MHz,$CDCl_3$) δ 8.08 (d, *J* = 8.6 Hz, 1H, CH=CH-N), 7.85 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.73 (s, 1H, Ar-H), 7.65 (dd, *J* = 13.4, 5.6 Hz, 2H, Ar-H), 7.49 (dd, *J* = 13.5, 8.7 Hz, 2H, Ar-H), 7.41 (d, *J* = 10.3 Hz, 1H, Ar-H), 6.90 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.72 (d, *J* = 3.2 Hz, 1H, Ar-H), 3.79 (s, 3H, CH_3).^{13}C NMR (151 MHz, $CDCl_3$) δ 163.63, 163.33, 161.61, 148.62 (d, *J* = 8.5 Hz), 133.89, 133.06, 132.42, 131.14, 129.09, 128.55, 127.97, 127.72, 126.05 (d, *J* = 2.9 Hz), 124.21, 122.11, 117.21 (d, *J* = 20.0 Hz), 114.29, 114.08, 110.96, 99.77 (d, *J* = 15.4 Hz), 55.71. HRMS (ESI) *m/z* 429.0700 [M+Na$^+$] (calcd for 406.0787 $C_{22}H_{15}FN_2O_3S$).

1-((4-Methoxyphenyl)sulfonyl)-7-phenyl-1H-indole (**4b**). Yield 70%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 3.8 Hz, 1H, CH=CH-N), 7.48 (dd, *J* = 7.7, 1.1 Hz, 1H, Ar-H), 7.32 (dt, *J* = 17.0, 7.3 Hz, 5H, Ar-H), 7.21 (dd, *J* = 13.4, 8.3 Hz, 3H, Ar-H), 7.07 (d, *J* = 7.4 Hz, 1H, Ar-H), 6.74–6.72 (m, 2H, Ar-H), 6.71 (s, 1H, CH=CH-N), 3.79 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.17, 140.99, 133.61, 133.48, 131.05, 130.82, 129.64, 129.48, 128.81, 128.65, 127.45, 127.03, 123.75, 120.46, 113.81, 110.25, 55.57. HRMS (ESI) 386.0828 *m/z* [M+Na$^+$] (calcd for 363.0929, $C_{21}H_{17}NO_3S$).

1-((4-Methoxyphenyl)sulfonyl)-7-3-thienyl-1H-indole (**4c**). Yield 56%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, *J* = 3.6 Hz, 1H, CH=CH-N), 7.49 (d, *J* = 4.0 Hz, 1H, Ar-H), 7.40 (d, *J* = 1.2 Hz, 1H, Ar-H), 7.30 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.20 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.09–7.06 (m, 2H, Ar-H), 6.78 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.71 (d, *J* = 3.6 Hz, 1H, Ar-H), 6.48 (d, *J* = 0.8 Hz, 1H, CH=CH-N), 3.81 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.24, 141.10, 140.35, 134.19, 133.29, 130.45, 130.27, 128.83, 128.56, 124.39, 123.42, 121.13, 120.83, 113.88, 113.28, 109.22, 55.59. HRMS (ESI) 370.0475 *m/z* [M+H$^+$] (calcd for 369.0493, $C_{19}H_{15}NO_3S_2$).

1-((4-Methoxyphenyl)sulfonyl)-7-4-pyridinyl-1H-indole (**4d**). Yield 34%, white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.55 (s, 2H, Ar-H), 7.67 (d, *J* = 3.7 Hz, 1H, CH=CH-N), 7.57 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.30 (t, *J* = 5.9 Hz, 3H, Ar-H), 7.20 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.05 (d, *J* = 7.4 Hz, 1H, CH=CH-N), 6.78–6.75 (m, 3H, Ar-H), 3.81 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.50, 150.33, 147.53, 133.71, 132.86, 130.94, 129.31, 128.63, 127.82, 127.66, 124.70, 124.01, 121.96, 114.06, 110.51, 55.66. HRMS (ESI) 365.0905 *m/z* [M+H$^+$] (calcd for 364.0882, $C_{20}H_{16}N_2O_3S$).

1-((4-Methoxyphenyl)sulfonyl)-7-5-pyrimidinyl-1H-indole (**4e**), Yield 61%,. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H, N = CH-N), 8.64 (s, 2H, CH = N), 7.72 (d, *J* = 1.7 Hz, 1H, CH=CH-N), 7.59 (d, *J* = 7.3 Hz, 1H, Ar-H), 7.41–7.29 (m, 1H, Ar-H), 7.12 (d, *J* = 6.2 Hz, 2H, Ar-H), 7.00 (d, *J* = 6.1 Hz, 1H, CH=CH-N), 6.77 (s, 3H, Ar-H), 3.77 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.24, 141.10, 140.35, 134.19, 133.29, 130.45, 130.27, 128.83, 128.56, 124.39, 123.42, 121.13, 120.83, 113.88, 113.28, 109.22, 55.59. HRMS (ESI) 366.0817 *m/z* [M+H$^+$] (calcd for 365.0834, $C_{19}H_{15}N_3O_3S$).

7-2-Furanyl-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**4f**). Yield 68%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (d, *J* = 3.7 Hz, 1H, CH=CH-N), 7.50–7.44 (m, 2H, Ar-H), 7.41 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.29 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.23 (d, *J* = 7.5 Hz, 1H, Ar-H), 6.77 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.69 (d, *J* = 3.7 Hz, 1H, Ar-H), 6.52–6.48 (m, 1H, Ar-H), 6.43 (d, *J* = 3.2 Hz, 1H, CH=CH-N), 3.78 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.24, 141.10, 140.35, 134.19, 133.29, 130.45, 130.27, 128.83, 128.56, 124.39, 123.42, 121.13, 120.83, 113.88, 113.28, 109.22, 55.59. HRMS (ESI) 354.0798 *m/z* [M+H$^+$] (calcd for 353.0722, $C_{19}H_{15}NO_4S$).

1-((4-Methoxyphenyl)sulfonyl)-7-2-thienyl-1H-indole (**4g**). Yield 61%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 3.5 Hz, 1H, CH=CH-N), 7.49 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.40 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.30 (dd, *J* = 8.7, 3.0 Hz, 2H, Ar-H), 7.22–7.19 (m, 1H, Ar-H), 7.09–7.06 (m, 2H, Ar-H), 6.79 (dd, *J* = 8.7, 2.9 Hz, 2H, Ar-H), 6.72 (d, *J* = 3.7 Hz, 1H, Ar-H), 6.48 (s, 1H, CH=CH-N), 3.80 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.26, 141.12, 140.37, 134.21, 133.31, 130.47, 130.29, 128.85, 128.58, 124.42, 123.45, 121.15, 120.85, 113.90, 113.30, 109.24, 55.61. HRMS (ESI) 368.0422 *m/z* [M−H$^+$] (calcd for 369.0493, $C_{19}H_{15}NO_3S_2$).

1-((4-Methoxyphenyl)sulfonyl)-7-(1-methyl-1H-imidazol-2-yl)-1H-indole (**4h**). Yield 57%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H, CH=CH-N), 7.99–7.89 (m, 1H, Ar-H), 7.76 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.72 (d, *J* = 9.1 Hz, 1H, Ar-H), 7.51 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.39 (m, 2H, Ar-H), 7.12 (s, 1H, Ar-H), 6.95 (s, 1H,

CH=CH-N), 6.90 (s, 2H, Ar-H), 3.82 (s, 6H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.10, 158.62, 133.81, 133.39, 133.18, 130.65 (d, J = 10.1 Hz), 130.42, 129.49, 128.62 (d, J = 12.5 Hz), 123.74, 120.16, 115.96, 114.56, 113.73, 112.73, 110.25, 55.52, 55.11. HRMS (ESI) 368.0975 m/z [M+H$^+$] (calcd for 367.0991, $C_{19}H_{17}N_3O_3S$).

7-(3-Fluorophenyl)-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**4i**). Yield 50%, white solid. ^1H NMR (600 MHz, $CDCl_3$) δ 7.73 (d, J = 3.8 Hz, 1H, CH=CH-N), 7.53 (dd, J = 7.8, 1.0 Hz, 1H, Ar-H), 7.28–7.26 (m, 1H, Ar-H), 7.24 (t, J = 1.7 Hz, 1H, Ar-H), 7.23 (d, J = 1.1 Hz, 2H, Ar-H), 7.22 (s, 1H, Ar-H), 7.18 (d, J = 8.4 Hz, 2H, Ar-H), 7.03 (dd, J = 7.3, 0.8 Hz, 1H, CH=CH-N), 6.77 (t, J = 6.9 Hz, 3H, Ar-H), 3.83 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.31, 162.81, 161.19, 142.97 (d, J = 8.0 Hz), 133.50, 133.29, 130.84, 129.56 (d, J = 4.0 Hz), 128.83 (d, J = 8.3 Hz), 128.67, 128.32, 125.53 (d, J = 2.4 Hz), 123.72, 120.96, 116.57, 116.43, 114.15–113.71, 110.07, 55.60. HRMS (ESI) 404.0736 m/z [M+Na$^+$] (calcd for 381.0835, $C_{21}H_{16}FNO_3S$).

7-(4-Fluorophenyl)-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**4j**). Yield 55%, white solid. ^1H NMR (600 MHz, $CDCl_3$): δ 7.71 (d, J = 3.7 Hz, 1H, CH=CH-N), 7.50 (d, J = 7.7 Hz, 1H, Ar-H), 7.25 (d, J = 7.5 Hz, 1H, Ar-H), 7.21 (dd, J = 11.5, 3.8 Hz, 4H, Ar-H), 7.03 (d, J = 7.4 Hz, 1H, Ar-H), 6.97 (t, J = 8.7 Hz, 2H, Ar-H), 6.75 (d, J = 6.0 Hz, 2H, Ar-H), 6.74 (d, J = 3.2 Hz, 1H, CH=CH-N), 3.79 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 163.33, 162.83, 161.21, 142.98 (d, J = 8.0 Hz), 133.51, 133.30, 130.85, 129.57 (d, J = 4.0 Hz), 128.85 (d, J = 8.3 Hz), 128.68, 128.33, 125.54 (d, J = 2.4 Hz), 123.74, 120.97, 116.59, 116.44, 114.16, 113.72, 110.09, 55.61. HRMS (ESI) 404.0739 m/z [M+Na$^+$] (calcd for 381.0835, $C_{21}H_{16}FNO_3S$).

7-(3,4-Dichlorophenyl)-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**4k**). Yield 60%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, J = 3.6 Hz, 1H, CH=CH-N), 7.55 (d, J = 7.8 Hz, 1H, Ar-H), 7.37 (d, J = 8.2 Hz, 1H, Ar-H), 7.23 (d, J = 7.7 Hz, 1H, Ar-H), 7.21–7.15 (m, 3H, Ar-H), 7.06 (s, 1H, Ar-H), 6.97 (d, J = 7.3 Hz, 1H, Ar-H), 6.79 (s, 1H, CH=CH-N), 6.76 (d, J = 5.2 Hz, 2H, Ar-H), 3.84 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.43, 140.54, 133.41, 133.01, 131.38, 131.18 (d, J = 12.9 Hz), 130.70, 129.75, 129.57, 129.21, 129.03, 128.37, 128.17, 127.91, 123.59, 121.35, 114.05, 109.46, 55.62. HRMS (ESI) 454.0044 m/z [M+Na$^+$] (calcd for 431.0150, $C_{21}H_{15}Cl_2NO_3S$).

7-(4-Methoxyphenyl)-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**4l**). Yield 64%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.70 (d, J = 3.7 Hz, 1H, CH=CH-N), 7.46 (d, J = 7.7 Hz, 1H, Ar-H), 7.20 (dd, J = 17.3, 8.3 Hz, 5H, Ar-H), 7.04 (d, J = 7.3 Hz, 1H, CH=CH-N), 6.82 (d, J = 8.5 Hz, 2H, Ar-H), 6.74–6.70 (m, 3H, Ar-H), 3.87 (s, 3H, CH_3), 3.79 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$):δ 163.10, 158.62, 151.06, 133.81, 133.39, 133.18, 130.65 (d, J = 10.1 Hz), 130.42, 129.49, 128.62 (d, J = 12.5 Hz), 123.74, 120.16, 115.96, 114.56, 113.73, 112.73, 110.25, 55.52, 55.11. HRMS (ESI) 416.0934 m/z [M+Na$^+$] (calcd for 393.1035, $C_{22}H_{19}NO_4S$).

1-((4-Methoxyphenyl)sulfonyl)-7-(4-(trifluoromethoxy)phenyl)-1H-indole (**4m**). Yield 57% white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, J = 3.7 Hz, 1H, CH=CH-N), 7.53 (d, J = 7.7 Hz, 1H, Ar-H), 7.24–7.19 (m, 3H, Ar-H), 7.16 (d, J = 8.9 Hz, 2H, Ar-H), 7.10 (d, J = 8.3 Hz, 2H, Ar-H), 7.01 (d, J = 7.4 Hz, 1H, Ar-H), 6.75 (d, J = 2.4 Hz, 2H, Ar-H), 6.73 (s, 1H, CH=CH-N), 3.80 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.67, 164.26, 162.55, 147.98 (d, J = 8.1 Hz), 137.58, 134.35 (d, J = 15.3 Hz), 134.13, 133.77, 132.29, 130.88, 129.56, 129.12, 128.81, 126.86, 125.02, 123.71–123.36, 117.90, 115.05, 114.91, 114.50, 114.00 (d, J = 12.0 Hz), 99.89 (d, J = 12.8 Hz), 65.54. HRMS (ESI) 470.0651 m/z [M+Na$^+$] (calcd for 447.0752, $C_{22}H_{16}F_3NO_4S$).

1-((4-Methoxyphenyl)sulfonyl)-7-(4-(methylsulfonyl)phenyl)-1H-indole (**4n**). Yield 50%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, J = 7.9 Hz, 2H, Ar-H), 7.68 (d, J = 3.7 Hz, 1H, CH=CH-N), 7.55 (d, J = 7.8 Hz, 1H, Ar-H), 7.44 (d, J = 8.0 Hz, 2H, Ar-H), 7.28 (d, J = 7.7 Hz, 1H, Ar-H), 7.17 (d, J = 8.8 Hz, 2H, Ar-H), 7.02 (d, J = 7.4 Hz, 1H, CH=CH-N), 6.75 (dd, J = 5.7, 3.0 Hz, 3H, Ar-H), 3.82 (s, 3H, CH_3), 3.12 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.09, 163.40, 146.60, 138.81, 133.65, 133.21, 130.89, 130.21, 129.45, 128.84, 128.41, 128.0, 126.48, 123.87, 121.53, 114.13, 110.28, 60.35, 55.67. HRMS (ESI) 464.0712 m/z [M+Na$^+$] (calcd for 441.0705, $C_{22}H_{19}NO_5S_2$).

N-(4-Methoxyphenyl)sulfonyl-7-(4-cyano)-phenyl-1H-indole (**4o**). Yield 76%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, J = 3.8 Hz, 1H, CH=CH-N), 7.59 (d, J = 8.2 Hz, 2H, Ar-H), 7.53 (d, J = 7.7 Hz, 1H, Ar-H), 7.41 (d, J = 8.1 Hz, 2H, Ar-H), 7.29 (d, J = 7.5 Hz, 1H, Ar-H), 7.19 (d, J = 8.9 Hz, 2H, Ar-H), 7.04 (d, J = 7.4 Hz, 1H, CH=CH-N), 6.77–6.73 (m, 3H, Ar-H), 3.81 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.45, 145.79, 133.76, 133.26, 131.33, 131.02, 129.99, 129.33, 129.15, 128.57, 128.03, 124.07, 121.54, 119.09, 113.93, 110.77 (d, J = 5.7 Hz), 55.65. HRMS (ESI) 411.0781 m/z [M+Na$^+$] (calcd for 388.0882, $C_{22}H_{16}N_2O_3S$).

N-4-Methoxyphenylsulfonyl-7-(4-hydroxyl)phenyl-1H-indole (**4p**). Yield 63%, white solid. ^1H NMR (600 MHz, $CDCl_3$): δ 8.41 (s, 1H, OH), 7.82 (d, J = 8.8 Hz, 2H, Ar-H), 7.66 (d, J = 7.7 Hz, 1H, CH=CH-N), 7.55 (d, J = 8.3 Hz, 2H, Ar-H), 7.20 (dd, J = 9.4, 5.2 Hz, 2H, Ar-H), 7.14 (dd, J = 13.8, 7.9 Hz, 3H, Ar-H), 7.00 (d, J = 8.7 Hz, 2H, Ar-H), 6.62 (s, 1H, CH=CH-N), 3.89 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.21, 148.78, 138.27, 133.45, 130.77, 129.35, 128.47, 126.64, 124.71, 124.12, 123.13, 121.94, 120.48, 120.27, 114.47, 103.11, 55.79. HRMS (ESI) 378.0805 m/z [M−H$^+$] (calcd for 379.0878, $C_{21}H_{17}NO_4S$).

7-(2-Fluorophenyl)-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**4q**). Yield 69%, white solid. ^1H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, J = 3.6 Hz, 1H, CH=CH-N), 7.49 (d, J = 7.7 Hz, 1H, Ar-H), 7.40 (d, J = 1.3 Hz, 1H, Ar-H), 7.30 (d, J = 8.8 Hz, 2H, Ar-H), 7.20 (t, J = 7.6 Hz, 1H, Ar-H), 7.08–7.07 (m, 2H, Ar-H), 6.78 (d, J = 8.9 Hz, 3H, Ar-H), 6.71 (d, J = 3.6 Hz, 1H, Ar-H), 6.48 (d, J = 0.7 Hz, 1H, CH=CH-N), 3.81 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.26, 141.11, 140.37, 134.21, 133.31, 130.47, 130.29, 128.85, 128.57, 124.41, 123.44, 121.15, 120.85, 113.90, 113.29, 109.23, 55.61. HRMS (ESI) 404.0739 m/z [M+Na$^+$] (calcd for 381.0835, $C_{21}H_{16}FNO_3S$).

7-(Furan-3-yl)-1-((4-methoxyphenyl)sulfonyl)-1H-indole (**4r**). Yield 81%, white solid. ^1H NMR (600 MHz, $CDCl_3$) δ 7.75 (s, 1H, CH=CH-N), 7.49 (d, J = 7.7 Hz, 1H, Ar-H), 7.40 (d, J = 2.8 Hz, 1H, Ar-H), 7.30 (dd, J = 8.7, 3.0 Hz, 2H, Ar-H), 7.21–7.18 (m, 1H, Ar-H), 7.09–7.05 (m, 2H, Ar-H), 6.78 (dd, J = 8.7, 2.9 Hz, 2H, Ar-H), 6.72 (d, J = 3.7 Hz, 1H, Ar-H), 6.47 (s, 1H, CH=CH-N), 3.80 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.00, 140.86, 140.11, 133.96, 133.06, 130.21, 130.03, 128.60, 128.32, 124.16, 123.19, 120.89, 120.60, 113.65, 113.04, 108.98, 55.36. HRMS (ESI) 354.0784 m/z [M+H$^+$] (calcd for 353.0722, $C_{19}H_{15}NO_4S$).

3-Chloro-N-4-methoxylbenzenesulfonyl-7-(4-chlorophenyl)-1H-indole (**4s**). Yield 74%, white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 3.7 Hz, 1H, CH=CH-N), 7.51 (d, J = 7.7 Hz, 1H, Ar-H), 7.24–7.18 (m, 5H, Ar-H), 7.15 (d, J = 8.4 Hz, 2H, Ar-H), 7.01 (d, J = 7.3 Hz, 1H, CH=CH-N), 6.77–6.73 (m, 3H, Ar-H), 3.81 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.27, 139.18, 133.49 (d, J = 3.0 Hz), 132.99, 130.79 (d, J = 3.0 Hz), 129.84, 129.56, 128.55, 128.37, 127.51, 123.74, 120.84, 113.86, 109.97, 55.63. HRMS (ESI) 455.0027 m/z [M+Na$^+$] (calcd for 455.0042, $C_{21}H_{16}ClNO_3S$).

N-3,4-Dimethoxylbenzenesulfonyl-7-(4-cyano-3-fluorophenyl)-1H-indole (**4t**). Yield 79%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.64 (d, J = 3.2 Hz, 1H, CH=CH-N), 7.58 (dd, J = 13.1, 7.2 Hz, 2H, Ar-H), 7.33–7.27 (m, 2H, Ar-H), 7.08 (dd, J = 13.3, 8.8 Hz, 2H, Ar-H), 6.88 (d, J = 8.3 Hz, 1H,), 6.74 (dd, J = 12.3, 6.0 Hz, 2H, CH_3), 6.69 (s,

1H, CH=CH-N), 3.87 (s, 3H, CH_3), 3.74 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.34, 161.63, 153.37, 148.78, 148.54 (d, *J* = 8.4 Hz), 133.90, 133.22, 132.43, 131.26, 129.08, 128.05, 127.68, 125.94 (d, *J* = 2.9 Hz), 124.28, 122.13, 120.50, 117.12 (d, *J* = 20.0 Hz), 114.17, 111.08, 110.18, 108.47, 99.77 (d, *J* = 15.3 Hz), 56.21, 56.05. HRMS (ESI) 459.0795 *m/z* [M+Na$^+$] (calcd for 436.0893, $C_{23}H_{17}FN_2O_4S$).

N-Benzenesulfonyl-7-(4-cyano-3-fluorophenyl)-1H-indole (**4u**) Yield 86%, white solid; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.67 (d, *J* = 3.7 Hz, 1H, CH=CH-N), 7.57 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.55–7.51 (m, 2H, Ar-H), 7.35 (t, *J* = 7.9 Hz, 2H, Ar-H), 7.30 (dd, *J* = 11.2, 3.9 Hz, 3H, Ar-H), 7.22 (dd, *J* = 7.9, 1.1 Hz, 1H, Ar-H), 7.04 (t, *J* = 9.5 Hz, 2H, Ar-H), 6.78 (d, *J* = 3.8 Hz, 1H, CH=CH-N). ^{13}C NMR (151 MHz, $CDCl_3$): δ 163.26, 161.55, 148.39 (d, *J* = 8.4 Hz), 137.62, 133.76 (d, *J* = 8.7 Hz), 133.00, 132.44, 130.98, 128.94, 127.83 (d, *J* = 13.6 Hz), 126.15, 126.01 (d, *J* = 3.0 Hz), 124.28, 122.14,117.25 (d, *J* = 20.0 Hz), 114.20, 111.06, 99.81 (d, *J* = 15.4 Hz), HRMS (ESI) *m/z* 375.0626 [M−H$^+$] (calcd for 376.0682 $C_{21}H_{13}FN_2O_2S$).

3-Chloro-N-4-methoxylbenzenesulfonyl-7-(4-cyano-3-fluorophenyl)-1H-indole (**4v**). Yield 76%, white solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 (s, 1H, CH-N), 7.62–7.57 (m, 2H, Ar-H), 7.38 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.29 (dd, *J* = 8.0, 1.1 Hz, 1H, Ar-H), 7.24 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.14 (d, *J* = 7.4 Hz, 1H, Ar-H), 7.08 (d, *J* = 9.6 Hz, 1H, CH=CH-N), 6.80 (d, *J* = 8.9 Hz, 2H, Ar-H), 3.82 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.85, 163.34, 161.63, 147.75 (d, *J* = 8.4 Hz), 132.55 (d, *J* = 5.1 Hz), 131.54, 128.89, 128.67, 128.35 (d, *J* = 15.6 Hz), 126.96, 125.97 (d, *J* = 3.0 Hz), 124.73, 119.76, 117.23, 117.10, 115.68, 114.14 (d, *J* = 16.9 Hz), 100.14 (d, *J* = 15.3 Hz), 55.72. HRMS (ESI) *m/z* 458.0740 [M+NH$_4^+$] (calcd for 440.0398, $C_{22}H_{14}ClFN_2O_3S$)

3-Chloro-N-3,4-dimethoxylbenzenesulfonyl-7-(4-cyano-3-fluorophenyl)-1H-indole (**4w**) Yield 78%, white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.63–7.57 (m, 3H, Ar-H), 7.39 (t, *J* = 7.7 Hz, 1H, CH=CH-N), 7.29 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.15 (dd, *J* = 12.4, 8.6 Hz, 2H, Ar-H), 6.91 (dd, *J* = 8.5, 1.2 Hz, 1H, CH=CH-N), 6.73 (dd, *J* = 17.2, 4.8 Hz, 2H, Ar-H), 3.88 (s, 3H, CH_3), 3.76 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.41, 161.70, 153.66, 148.87, 147.74 (d, *J* = 8.4 Hz), 132.80, 132.63, 131.65, 128.88, 128.48, 128.23, 127.17, 125.84 (d, *J* = 3.1 Hz), 124.93, 120.77, 119.84, 117.13, 117.00, 116.06, 114.02, 110.25, 108.48, 100.14 (d, *J* = 15.4 Hz), 56.18, 56.12. HRMS (ESI) 471.0471 *m/z* [M+H$^+$] (calcd for 470.0503, $C_{22}H_{14}ClFN_2O_3S$).

3-*Chloro-N-benzenesulfonyl-7-(4-cyano-3-fluorophenyl)-1H-indole* (**4x**). Yield 73%, white solid: ^{1}H NMR(400 MHz, $CDCl_3$): δ 7.67 (s, 1H, CH-N), 7.59 (dd, *J* = 11.3, 4.3 Hz, 1H, Ar-H), 7.55 (dd, *J* = 11.5, 4.6 Hz, 2H, Ar-H), 7.38 (dd, *J* = 16.0, 8.2 Hz, 3H, Ar-H), 7.33–7.30 (m, 2H, Ar-H), 7.23 (dd, *J* = 7.9, 1.1 Hz, 1H, Ar-H), 7.14 (d, *J* = 7.4 Hz, 1H, Ar-H), 7.05 (d, *J* = 9.5 Hz, 1H, Ar-H). ^{13}C NMR (151 MHz, $CDCl_3$):δ 163.30, 161.58, 147.55 (d, *J* = 8.4 Hz), 137.04, 134.05, 132.54 (d, *J* = 19.6 Hz), 131.48, 129.04 (d, *J* = 11.6 Hz), 128.23, 126.80, 126.25, 125.95 (d, *J* = 3.1 Hz), 124.85, 119.83, 117.32, 117.24 (d, *J* = 20.1 Hz), 115.89, 114.03, 100.18 (d, *J* = 15.4 Hz), HRMS (ESI) *m/z* 428.0643 [M+NH$_4^+$] (calcd for 410.0292, $C_{21}H_{12}ClFN_2O_2S$).

3-*Chloro-N-(4-methoxybenzenesulfonyl)-6-(4-cyano-3-fluorophenyl)-1H-indole* (**11a**). Yield 62%, white solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.10 (d, *J* = 8.7 Hz, 1H, CH=CH-N), 7.85 (d, *J* = 8.8 Hz, 2H,Ar-H), 7.73 (s, 1H,Ar-H), 7.68 (t, *J* = 7.3 Hz, 1H,Ar-H), 7.61 (s, 1H,Ar-H), 7.58 (d, *J* = 8.7 Hz, 1H,Ar-H), 7.51 (d, *J* = 8.0 Hz, 1H,Ar-H), 7.45 (d, *J* = 10.1 Hz, 1H, CH=CH-N), 6.92 (d, *J* = 8.8 Hz, 2H, Ar-H), 3.81 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 164.24 (d, *J* = 5.5 Hz), 162.54, 148.07 (d, *J* = 8.1 Hz), 134.25, 133.91, 133.76, 129.24, 129.07, 128.90, 124.86, 123.73, 117.82, 115.01, 114.88, 114.73, 114.48, 113.99, 113.63, 99.80 (d, *J* = 15.6 Hz), 55.73. HRMS (ESI) *m/z* 458.0732 [M+NH$_4^+$] (calcd for 440.0398, $C_{22}H_{14}ClFN_2O_3S$).

N-(4-Methoxybenzenesulfonyl)-6-(4-cyano-3-fluorophenyl)-1H-indole (**11b**). Yield 76%, white solid. ^{1}H NMR(400 MHz, $CDCl_3$): δ 8.20 (s, 1H, CH=CH-N), 7.84 (d, *J* = 6.9 Hz, 2H, Ar-H), 7.70 (s, 1H, Ar-H), 7.64 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.53 (d, *J* = 6.5 Hz, 1H, Ar-H), 7.46 (d, *J* = 7.9 Hz, 2H, Ar-H), 6.91 (d, *J* = 6.8 Hz, 2H, Ar-H), 6.70 (s, 1H, CH=CH-N), 3.80 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 163.82, 163.52, 161.80, 148.81 (d, *J* = 8.5 Hz), 134.08, 133.25, 132.61, 131.33, 129.28, 128.74, 128.16, 127.91, 126.24 (d, *J* = 2.9 Hz), 124.40, 122.30, 117.40 (d, *J* = 20.0 Hz), 114.48, 114.27, 111.15, 99.96 (d, *J* = 15.4 Hz), 55.90. HRMS (ESI) *m/z* 424.1129 [M+NH$_4^+$] (calcd for 406.0787, $C_{22}H_{15}FN_2O_3S$).

3-*Chloro-N-(4-methoxybenzenesulfonyl)-5-(4-cyano-3-fluorophenyl)-1H-indole* (**11c**). Yield 76%, white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09 (d, *J* = 8.6 Hz, 1H, CH=CH-N), 7.85 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.72 (s, 1H, Ar-H), 7.71–7.66 (m, 1H, Ar-H), 7.61 (s, 1H, Ar-H), 7.58 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.53–7.49 (m, 1H, Ar-H), 7.45 (d, *J* = 10.1 Hz, 1H, CH=CH-N), 6.92 (d, *J* = 8.9 Hz, 2H, Ar-H), 3.81 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.24 (d, *J* = 6.7 Hz), 162.55, 148.08 (d, *J* = 8.0 Hz), 134.25, 133.93, 133.76, 129.24, 129.08, 128.91, 124.85, 123.69–123.38, 117.82, 115.02, 114.89, 114.72, 114.48, 113.98, 113.63, 99.82 (d, *J* = 15.6 Hz), 55.72. HRMS (ESI) *m/z* 458.0736 [M+NH$_4^+$] (calcd for 440.0398, $C_{22}H_{14}ClFN_2O_3S$).

N-(4-Methoxybenzenesulfonyl)-5-(4-cyano-3-fluorophenyl)-1H-indole (**11d**). Yield 86%, white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 (d, *J* = 3.7 Hz, 1H, CH=CH-N), 7.59–7.54 (m, 2H, Ar-H), 7.29 (t, *J* = 7.7 Hz, 2H, Ar-H), 7.22 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.06 (dd, *J* = 15.3, 8.5 Hz, 2H, Ar-H), 6.76 (dd, *J* = 12.2, 6.3 Hz, 2H, Ar-H; 1H, CH=CH-N), 3.80 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.60, 163.30, 161.59, 148.59 (d, *J* = 8.5 Hz), 133.87, 133.04, 132.40, 131.11, 129.07, 128.52, 127.95, 127.70, 126.03 (d, *J* = 2.9 Hz), 124.18, 122.08, 117.18 (d, *J* = 20.0 Hz), 114.26, 114.06, 110.94, 99.74 (d, *J* = 15.4 Hz), 55.69. HRMS (ESI) *m/z* 424.1138 [M+NH$_4^+$] (calcd for 406.0787, $C_{22}H_{15}FN_2O_3S$).

4.1.4. Synthesis of *7a* and *7b*

A mixture of **6c** (0.25 mmol), $Pd_2(dba)_3$ (0.005 mmol), 4-bromo-2-fluorobenzonitrile or 4-bromobenzonitrile (0.25 mmol), *t*-BuONa (0.35 mmol), BINAP (0.015 mmol) in toluene (10 mL) was stirred under N_2 at 80 °C until the reaction was complete. The mixture was extracted with DCM (3 × 15 mL), and the combined organic layers were washed with saline for three times, dried with Na_2SO_4, concentrated and purified by silica gel chromatography to afford compound **7a** or **7b**.

1-((4-Methoxyphenyl)sulfonyl)-7-(N-(4-cyano-3-fluoro)phenyl) amino-1H-indole (**7a**). Yield 79%, yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 (d, *J* = 3.6 Hz, 1H, NH), 7.47–7.42 (m, 2H, Ar-H), 7.30–7.27 (m, 1H, CH=CH-N), 7.24 (t, *J* = 8.6 Hz, 3H, Ar-H), 7.17 (d, *J* = 7.7 Hz, 1H, Ar-H), 6.76 (d, *J* = 3.7 Hz, 1H, Ar-H), 6.57 (d, *J* = 8.9 Hz, 2H, Ar-H), 6.49 (dd, *J* = 8.6, 1.7 Hz, 1H, Ar-H), 6.30 (dd, *J* = 11.7, 1.6 Hz, 1H, CH=CH-N), 3.72 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 134.43, 133.91 (d, *J* = 1.9 Hz), 129.66, 128.45, 127.75, 125.87, 124.34, 120.43, 118.83, 115.06, 114.18, 110.89, 108.39, 100.41, 100.25, 89.52 (d, *J* = 16.1 Hz), 55.64. HRMS (ESI) 422.0971 *m/z* [M+H$^+$] (calcd for 421.0896, $C_{22}H_{16}FN_3O_3S$).

1-((4-Methoxyphenyl)sulfonyl)-7-(N-(4-cyano)phenyl)amino-1H-indole (**7b**) Yield 82%, yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (d, *J* = 3.6 Hz, 1H, CH=CH-N), 7.45 (s, 1H, NH), 7.36 (dd, *J* = 11.7, 5.7 Hz, 3H, Ar-H), 7.28 (s, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 7.22–7.19 (m, 2H, Ar-H), 6.74 (d, *J* = 3.6 Hz, 1H, CH=CH-N), 6.71 (d, *J* = 8.6 Hz, 2H, Ar-H), 6.53 (d, *J* = 8.9 Hz, 2H, Ar-H), 3.69 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.37, 148.32, 134.38, 133.46, 129.56 (d, *J* = 18.8 Hz), 128.15, 127.94, 126.87, 124.24, 119.87, 119.47, 117.84, 114.42, 114.15, 108.46, 100.93, 55.60. HRMS (ESI) 404.1063 *m/z* [M+H$^+$] (calcd for 403.0991, $C_{22}H_{17}N_3O_3S$).

4.1.5. Synthesis of **7c**

A mixture of **6b** (0.165 mmol), 4-cyanoaniline (0.165 mmol), EDC (0.248 mmol) in pyridine (5 mL) was stirred at room temperature overnight. Water was added to quench the reaction, and the mixture was adjusted to pH 3.0 by slow addition of diluted HCl. The resulting mixture was extracted with DCM (3 × 10 mL), and the combined organic layers were washed with saline for three times, dried with Na_2SO_4, concentrated, and purified by silica gel chromatography to afford compound **7c**.

N-(4-Cyanophenyl)-1-((4-methoxyphenyl)sulfonyl)-1H-indole-7-carboxamide (**7c**) Yield 74%, yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.47 (s, 1H, CONH), 8.26 (d, J = 7.2 Hz, 1H, CH=CH-N), 8.14 (d, J = 7.8 Hz, 2H, Ar-H), 7.83 (d, J = 8.0 Hz, 2H, Ar-H), 7.60 (d, J = 3.3 Hz, 1H, Ar-H), 7.36–7.28 (m, 4H, Ar-H), 6.73–6.70 (m, 2H, Ar-H), 6.68 (s, 1H, CH=CH-N), 3.73 (s, 3H, CH_3).^{13}C NMR (151 MHz, $CDCl_3$) δ 163.87, 163.68, 138.55, 134.33, 132.52, 129.56, 128.54, 128.39, 128.19, 126.01, 125.83, 124.95, 119.55, 118.50, 118.03, 115.44, 114.54, 111.11, 55.67. HRMS (ESI) 432.1012 m/z [M+H$^+$] (calcd for 431.0940, $C_{23}H_{17}N_3O_4S$).

4.2. MTT assay

All cell lines were purchased from Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China), including: Human non-small cell lung carcinoma cell (A549), human breast carcinoma cell (MCF-7) and (MDA-MB-231), Human liver cancer cells (HePG2), Oral epidermoid cancer cells (KB), Human prostate cancer cells (DU145). All cells were maintained in RPMI-1640 medium, supplemented with 10% fetal bovine serum containing 50 mg/mL penicillin and 50 mg/mL streptomycin. Cells were grown to 80% confluency in a tissue culture flask at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were then seeded in 96-well plates at a density of 3000–5000 cells per well and incubated at 37 °C for 16 h in a humidified 5% CO_2 incubator. Different concentrations of tested compounds (The total volume of 10 μL) were added in triplicate to the plates in 190 μL medium, the plates were incubated at 37 °C for 48 h. The percentage of DMSO in the medium not exceeded 0.1%. 3-(4, 5-Dimethylthiazolyl)-2, 5-diphenyltetrazoliumbromide (MTT) was added to evaluate cell viability. The absorbance was read by an ELISA reader (SpectraMax Plus384, Molecular Devices, Sunnyvale, CA) at a test wavelength of 570 nm and a reference wavelength of 570 nm.

4.3. Western blot

MDA-MB-231 cells were incubated with various concentrations of tested compounds for 12 h and harvested after trypsinisation. Cells were gathered in epoxide (EP) tubes under low-speed centrifuge, and then resuspended in PBS for three times, treated with RIPA (Radio-Immunoprecipitation Assay) lysis buffer (50 mM TrisHCl, pH 7.4, 150 mM NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM EDTA and protease inhibitors) to extract the total proteins. An aliquot of proteins from the total cell lysates was separated by sodium dodecyl sulfate (10%) polyacryl amide gel electrophoresis. The separated proteins were then transferred to PVDF membrane and blocked with 5% skim milk for 1 h. PVDF membrane was further washed with TBST (3 × 20 mL), and incubated with primary antibodies specific for objective stripe at 4 °C overnight. The objective stripe was observed by enhanced chemiluminescence (ECL) immunoblotting technique after the membrane was blotted with HRP-labeled second antibody for 1 h at room temperature.

4.4. IL-6 induction of STAT3 phosphorylation

MDA-MB-231 cells were seeded in 6-well plates and allowed to adhere overnight. The cells were serum-starved for 12 h, then left untreated or treated with **4a**. After 12 h, the untreated and **4a** treated cells were stimulated by IL-6 (25 ng/mL). The cells were harvested after 30 min and analyzed by western blot assay.

4.5. Immunofluorescence confocal microscopy

A549 cells were seeded sparsely in eight-well chamber slides and treated with or without identified compounds **4a** and **7c** for 24 h. Following treatment, cells were fixed with cold MeOH at −20 °C for 15 min, washed three times with PBS, and blotted with 1% PBS plus 0.1% Triton X-100 for 30 min at 37 °C. Microtubules were detected by incubation with a monoclonal anti-β-tubulin at 37 °C for 1 h. Then, the cells were washed with PBS and incubated with a FITC-conjugated antimouse IgG antibody. Nucleus were stained with DAPI, and microtubule distribution images were acquired with the Confocal Spectral Microscope.

4.6. In vitro tubulin polymerization assay

The effect of compound **4a** on tubulin polymerization was determined kinetically using a kit (BK006P, Cytoskeleton Inc, Denver, CO). Cold porcine tubulin protein (>99% purity) was added to G-PEM buffer (80 mM PIPES, 2 mM $MgCl_2$, 0.5 mM EGTA, and 1 mM GTP, pH 6.9) containing 15% glycerol with or without **4a**. The sample mixture was dotted onto a pre-warmed 96-well plate, which was immediately transferred to a 37 °C plate reader. The absorbance was read every minute for 30 min at 340 nm.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmc.2017.11.023.

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