

Design, synthesis and structure-activity relationship studies of meridianin derivatives as novel JAK/STAT3 signaling inhibitors

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Abstract

Hyperactivation of Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) signaling is an attractive therapeutic target for tumor therapy. Herein, forty-eight novel meridianin derivatives were designed and synthesized, and their anti-tumor activity were evaluated *in vitro* both for activity optimization and structure-activity relationship (SAR) study. The results indicated that most derivatives exhibited significantly improved anti-tumor activity, especially for compound **6e**. The compound **6e** contains an isothiuronium linked by an alkyl chain consisting of 6 carbon atoms with IC₅₀ ranging from 1.11 to 2.80 μ M on various cancer cell lines. Consistently, **6e** dose dependently induced the apoptosis of A549 and DU145 cells, in which STAT3 are constitutively active. Western blotting assays indicated that the phosphorylation levels of JAK1, JAK2 and STAT3 were inhibited by **6e** at 5 μ M without significant change in total STAT3 level. Moreover, **6e** also suppressed the

expressions of STAT3 downstream genes, including c-Myc, Cyclin D1 and Bcl-XL at 10 μ M. An additional *in vivo* study revealed that **6e** at the dose of 10 mg/kg could potentially inhibit the DU145 xenograft tumor without obvious body-weight loss. These results clearly indicate that **6e** could be a potential anti-tumor agent by targeting JAK/STAT3 signaling pathway.

Key words: JAK/STAT3 signaling pathway; Meridianin derivatives; Isothiouronium; Anti-tumor activity

1. Introduction

The Janus kinase (JAK) and Signal transducer and activator of transcription (STAT) signaling pathway is essential in the regulation of various biological processes, including immune responses, cell division, hematopoiesis and tumor formation [1-4]. Various cytokines and growth factors transmit signals through the JAK/STAT signaling pathway, which consists of tyrosine kinase-associated receptors, JAKs and downstream transcription factor STATs [5,6]. After stimulated by cytokines, such as type I and type II interferons or IL-6, the JAKs phosphorylate each other at tyrosine residues and then phosphorylate and activate STAT proteins, which themselves dimerize and translocate to the nucleus where they regulate gene transcription. Aberrant activation of the JAK/STAT signaling pathway has been closely associated with many diseases. Four members of the JAK family have been identified in mammals, including JAK1, JAK2, JAK3, and TYK2. The mammalian STAT family has 7 members comprising of STAT1-4, STAT5(a/b) and STAT6. Among them, STAT3 is the most well studied, who is broadly hyperactivated in variety of cancers, and closely associated with tumor cell proliferation and metastasis. Therefore, the JAK/STAT signaling pathway, especially STAT3 signaling, has emerged as a promising drug target for cancer treatment strategy.

Meridianins A-G (Figureure. S1) are marine-derived indole alkaloids isolated from the South Atlantic tunicate *Aplidium meridianum*, which have demonstrated to exhibit a number of biological activities, such as antitumor activity, protein kinase

inhibitory activity in a low micromolar range, antimalarial activity, antituberculosis activity and anti-Alzheimer's disease activity [10-15]. In particular, they exhibit potent activity against the Clks and Dyrk kinase families, which are emerging as medically relevant targets involvement in cancer and Alzheimer's disease respectively [16]. Furthermore, Meijer and co-workers have found that the azaindole analogs of the meridianins, referred to as meriolins, are potent CDK9 inhibitors [17,18]. This potent activity, coupled with their antiproliferative properties, has established the meriolins as significant new leads for cancer therapeutics. Due to the promising biological activities and unique chemical structures, meridianins as lead compounds have attracted a great deal of interest in medicinal chemistry [11,16,19].

These promising results led us to expand our effort in the synthesis of new diversely substituted meridianin derivatives. Herein, forty-eight novel meridianin derivatives were obtained in this study, and JAK/STAT3 hyperactivated human cancer cell lines were evaluated. The results indicated that meridianins (A, C, D and G) displayed weak cancer cell growth inhibition in four tested cell lines. By comparison, **1a~g**, **2a~g**, **3a~g** and **4a~g** showed significantly increased inhibitory activities, but remained to be improved.

Isothiourea is a positively charged group with significant pharmacological activities, such as anesthesia, anti-bacterial and anti-tumor [19-25]. Our previous work showed that the isothiuronium-modified analogs had greatly enhanced anticancer activities in addition to their unique Golgi localization compared to the unmodified compounds [26].

With the aim of improving the anti-tumor activity, meridianins derivatives **5a~g** and **6a~g** were obtained by incorporating isothiourea groups at the N1-position with different length of carbon alkyl chains. The compound **6e** exhibited better anti-tumor activity than positive control Gefitinib. To further investigate the structure-activity relationship, the analogues of compound **6e** with different length of carbon alkyl chains or without isothiourea were obtained, and their anti-tumor activities were evaluated in the same cell lines. An additional *in vivo* study revealed that **6e** exhibited significant anti-tumor activity. Finally, the possible mechanism of **6e** was investigated

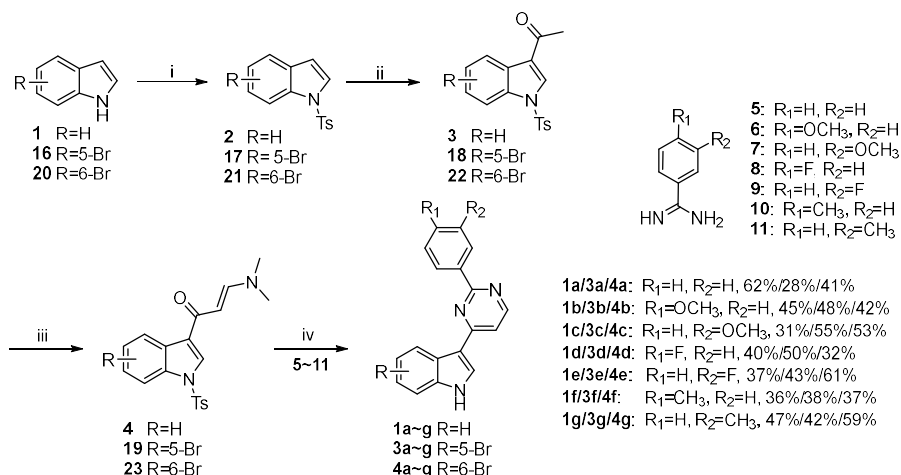
preliminarily by molecular docking, western blot, flow cytometry analysis assay and immunohistochemistry (IHC) analyses.

2. Results and Discussion

2.1. Chemistry

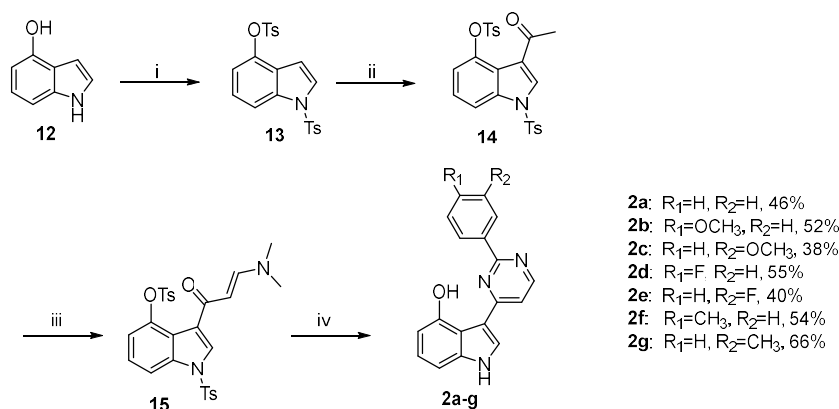
The reaction sequence employed to synthesize the target compounds is outlined in Scheme 1~4.

The marine natural products indole alkaloids (meridianin A, C, D and G) were synthesized in four steps starting from commercially available indoles [27]. Firstly, the indolic nitrogen was protected by reaction with tosyl chloride in the presence of NaOH and in Acetonitrile, leading to the formation of compounds **2**, **17** and **21** in 53% to 94% yields. Then, the C-3 position of indoles was acetylated using acetic anhydride and aluminum chloride in methylene chloride to give derivatives **3**, **18** and **22** in 73% to 79% yields. The enaminone intermediates proceeded with DMF/dimethylformamide-dimethylacetal (DMF-DMA) in 69% to 79% yield. Finally, compounds **1a~g**, **3a~g** and **4a~g** were obtained from enaminone intermediates using **5~11** in 2-methoxyethanol in the presence of potassium carbonate in considerable yield (Scheme 1). As depicted in Scheme 2, the indolic nitrogen and 4-hydroxyl of compound **12** were tosyl chloride protected to get compounds **13** in 85% yield. Then the preparation of the corresponding **2a~g** derivatives was undertaken using a similar synthetic pathway in 38% to 66% yields. As shown in Scheme 3 and Scheme 4, isothiuronium derivatives **5a~g**, **6a~g** and **6e-2~6** were synthesized in medium yield by using introducing 1,6-dibromohexane or dibromoalkyl chain with different carbon chains without any purification and then boiling isopropanol in the presence of thiourea. In addition, the compounds **6e-1** was prepared by using introducing 1-bromohexane in 63% yield.



Scheme 1. Synthesis of Final Target Compounds **1a~g**, **3a~g** and **4a~g**. Reagents and conditions:

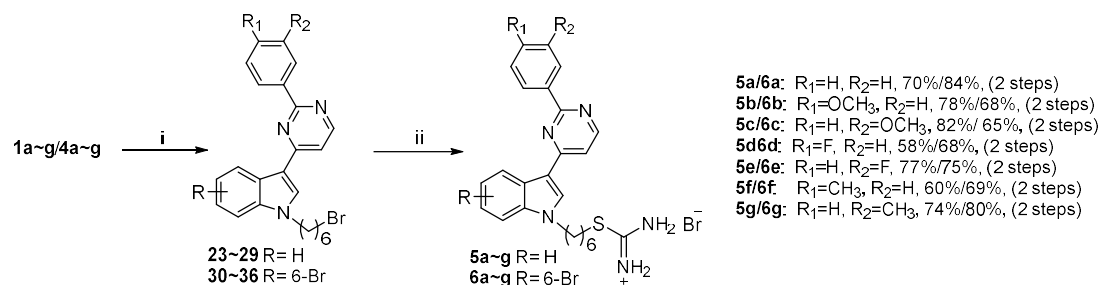
(i) TsCl, NaOH, CH₃CN, rt, 4 h; (ii) Ac₂O, AlCl₃, DCM, rt, 3 h; (iii) DMF-DMA, DMF, 110°C, 3 h; (iv) K₂CO₃, 2-methoxyethanol, 120°C, 12 h.



Scheme 2. Synthesis of Final Target Compounds **2a~g**. Reagents and conditions: (i) TsCl, NaH,

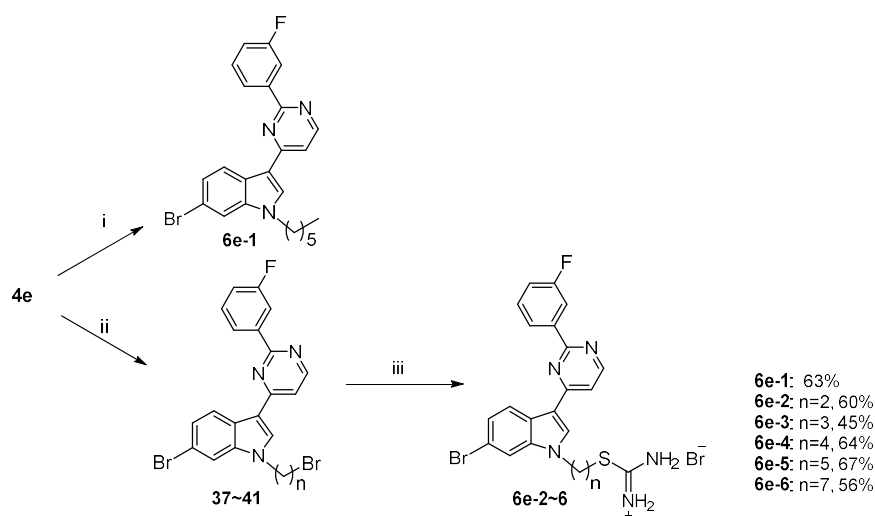
DMF, 0°C, 2 h; (ii) Ac₂O, AlCl₃, DCM, rt, 3 h; (iii) DMF-DMA, DMF, 110°C, 3 h; (iv) **5-11**,

K₂CO₃, 2-methoxyethanol, 120°C, 12 h.



Scheme 3. Synthesis of Final Target Compounds **5a~g** and **6a~g**. Reagents and conditions: (i)

1,6-dibromohexane, K₂CO₃, DMF, 50°C 12 h; (ii) Thiourea, isopropanol, 80°C, 14 h.



Scheme 4. Synthesis of Final Target Compounds **6e-1~6**. Reagents and conditions: (i) 1-bromohexane, K₂CO₃, DMF, 50°C 12 h; (ii) Dibromoalkyl chain, K₂CO₃, DMF, 50°C 12 h; (iii) Thiourea, isopropanol, 80°C, 14 h.

2.2. Biological activity assessments

2.2.1. Cell viability assay and SAR analysis

To evaluate the anti-tumor activities of meridianin A, C, D, G and their derivatives, four JAK/STAT3 over-activated human cancer cell lines HeLa, MDA-MB-231, A549 and DU145 were examined. Initially, meridianins (A, C, D and G) and their derivatives **1a~g**, **2a~g**, **3a~g** and **4a~g** were prepared and evaluated for cell growth inhibitory activities against cancer cell lines (Table 1). The results indicated that meridianin A, C, D and G displayed weak cancer cell growth inhibition in four tested cell lines. By comparison, meridianin derivatives **1a~g**, **2a~g**, **3a~g** and **4a~g** showed significantly increased inhibitory activities. With the aim of improving the antitumor activity, compounds **5a~g** and **6a~g** were obtained by incorporating isothiurea groups at the C1-position of meridianins D and G with different length of carbon alkyl chains. Notably, the anti-tumor effects of the compounds were significantly enhanced, almost all compounds with IC₅₀ less than 10 μM (Table 2).

Among them, the most potent compound **6e** inhibited the growth of HeLa, MDA-MB-231, A549 and DU145 cells with IC₅₀ values of 1.11, 1.22, 2.80 and 1.13 μ M, which exhibited better activity than positive control (Gefitinib), respectively. Based on the above results, the analogues of compound **6e** with different carbon chain lengths or without isothioureia were obtained, and the anti-tumor activity results in Table 3. The structure-activity relationship suggesting that reducing or increasing the number of carbon atoms will lead to the decrease of antiproliferative activity. On the other hand, compound **6e-1** was found to be completely inactive with IC₅₀ values greater than 100 μ M, indicating that the substitution of isothioureia group significantly contributed to the antitumor activity.

Table 1. Antiproliferative activity of meridianin A, C, D, G and their derivatives against human cancer cell lines.

Cpd.	IC ₅₀ \pm SD (μ M) ^a			
	HeLa	MDA-MB-231	A549	DU145
1a	4.50 \pm 0.41	4.39 \pm 0.23	10.86 \pm 0.52	39.16 \pm 2.21
1b	11.72 \pm 2.14	4.75 \pm 0.28	>100	46.92 \pm 1.31
1c	2.33 \pm 0.03	4.23 \pm 0.68	7.46 \pm 0.54	12.61 \pm 2.07
1d	4.35 \pm 0.20	5.25 \pm 0.27	11.45 \pm 0.95	16.71 \pm 2.28
1e	6.18 \pm 0.16	>100	7.10 \pm 0.01	>100
1f	>100	14.48 \pm 0.59	8.84 \pm 0.45	23.88 \pm 2.82
1g	1.56 \pm 0.09	8.57 \pm 0.65	7.11 \pm 0.31	11.23 \pm 0.65
2a	3.76 \pm 0.28	8.46 \pm 1.24	7.63 \pm 0.73	34.17 \pm 3.72
2b	4.16 \pm 0.11	8.59 \pm 0.64	9.40 \pm 0.64	29.15 \pm 3.45
2c	2.15 \pm 0.11	3.03 \pm 0.50	4.25 \pm 0.23	17.79 \pm 0.83
2d	9.05 \pm 0.61	13.11 \pm 1.51	28.74 \pm 8.43	>100
2e	2.86 \pm 0.27	5.21 \pm 0.24	5.12 \pm 0.23	13.70 \pm 4.37
2f	1.81 \pm 0.04	8.13 \pm 0.15	3.50 \pm 0.08	8.86 \pm 0.68
2g	2.70 \pm 0.18	35.22 \pm 2.40	17.15 \pm 1.41	22.61 \pm 2.47

3a	2.99±0.44	11.30±1.38	8.95±0.68	19.08±1.19
3b	4.86±0.16	6.25±0.34	7.73±0.38	10.46±0.45
3c	4.95±0.04	7.46±1.76	15.78±1.11	27.57±3.75
3d	26.63±1.05	25.48±1.70	>100	>100
3e	6.58±0.16	9.69±0.72	9.75±0.87	12.85±0.56
3f	7.37±1.22	>100	10.02±2.05	45.16±2.44
3g	25.04±0.82	>100	>100	>100
4a	25.07±3.57	20.23±2.09	25.07±3.57	>100
4b	8.05±0.85	8.60±0.64	21.68±1.70	>100
4c	2.55±0.33	4.76±0.37	9.49±0.10	24.65±0.50
4d	10.56±0.42	9.06±0.32	22.50±2.78	18.08±2.44
4e	17.53±1.05	27.46±1.26	>100	32.60±1.42
4f	5.03±0.70	8.56±0.87	11.84±1.29	26.01±2.83
4g	1.67±0.11	4.23±0.06	10.04±0.44	19.81±0.28
Meridianin A	25.39±0.35	>100	>100	>100
Meridianin C	24.07±0.16	14.03±2.63	23.54±3.20	>100
Meridianin D	13.17±2.71	>100	26.68±0.63	>100
Meridianin G	22.52±3.55	>100	>100	>100

^aThe inhibitory effects of the compounds on the proliferation of the four cell lines were determined by the MTT assay. Results are expressed as means ± SD. All experiments were independently performed at least three times.

Table 2. Antiproliferative activity of meridianin (D and G) derivatives against human cancer cell lines.

Cpd.	IC ₅₀ ± SD (μM) ^a			
	HeLa	MDA-MB-231	A549	DU145
5a	14.56±2.05	18.61±1.10	8.68±0.27	22.70±2.03
5b	4.21±0.15	4.67±0.17	2.68±0.30	6.04±0.10
5c	7.53±0.41	7.99±0.82	8.85±0.06	18.56±3.40

5d	6.92 ± 0.23	5.46 ± 0.10	5.76 ± 0.12	6.67 ± 0.21
5e	8.31 ± 1.00	5.87 ± 0.21	8.23 ± 0.64	10.20 ± 0.15
5f	4.70 ± 0.08	2.95 ± 0.11	5.84 ± 0.25	6.47 ± 0.28
5g	2.19 ± 0.09	1.92 ± 0.11	3.65 ± 0.07	3.86 ± 0.31
6a	3.28 ± 0.08	4.59 ± 0.13	4.81 ± 0.21	5.04 ± 0.06
6b	9.23 ± 0.99	6.80 ± 1.33	6.94 ± 0.07	8.91 ± 0.55
6c	3.49 ± 0.13	3.20 ± 0.02	5.00 ± 0.31	5.46 ± 0.09
6d	7.73 ± 0.26	5.61 ± 0.16	9.86 ± 0.46	7.01 ± 0.32
6e	1.11 ± 0.04	1.22 ± 0.34	2.80 ± 0.18	1.13 ± 0.17
6f	9.34 ± 0.72	4.89 ± 0.16	7.32 ± 0.35	4.74 ± 0.03
6g	2.42 ± 0.15	5.51 ± 0.03	8.93 ± 1.07	6.38 ± 0.31
Gefitinib	7.51 ± 0.45	32.65 ± 0.31	13.08 ± 0.28	9.18 ± 0.06

^a The inhibitory effects of the compounds on the proliferation of the four indicated cell lines were determined by the MTT assay. Results are expressed as means ± SD. All experiments were independently performed at least three times.

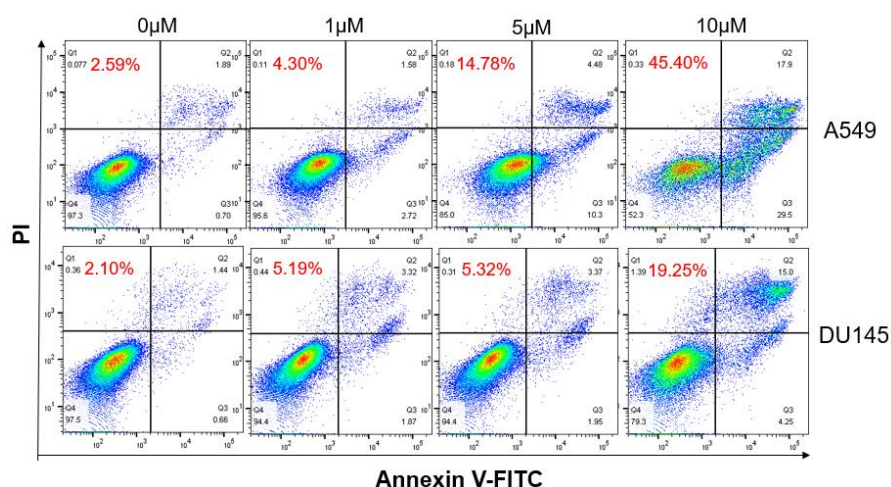
Table 3. Antiproliferative activity of compound **6e** analogs against human cancer cell lines.

Cpd.	IC ₅₀ ± SD (μM) ^a			
	HeLa	MDA-MB-231	A549	DU145
6e-1	>100	>100	>100	>100
6e-2	>100	24.31 ± 0.77	>100	29.18 ± 0.85
6e-3	11.28 ± 0.77	9.65 ± 0.30	9.49 ± 1.18	15.30 ± 3.10
6e-4	8.19 ± 0.08	8.38 ± 1.07	8.85 ± 0.30	10.73 ± 0.91
6e-5	4.92 ± 0.09	7.88 ± 0.11	7.80 ± 0.19	7.55 ± 0.73
6e-6	12.08 ± 1.26	14.21 ± 0.37	10.42 ± 0.27	10.32 ± 2.16

^aThe inhibitory effects of the compounds on the proliferation of the four cell lines were determined by the MTT assay. Results are expressed as means ± SD. All experiments were independently performed at least three times.

2.2.2. Compound **6e** induces cell apoptosis

The effect of compound **6e** on inducing tumor cell apoptosis was analyzed in Figureure. 1. A549 and DU145 cells were incubated with **6e** at different concentrations for 24 h. Annexin V-FITC/PI staining was carried out and the percentages of apoptotic cells were further determined using flow cytometry. The results showed that **6e** dose dependently induced the apoptosis of A549 and DU145 cells. As shown in Figure. 1, in A549, the induced apoptosis rates at 0, 1, 5, and 10 μ M were 2.59%, 4.30%, 14.78% and 45.40%, respectively. In DU145, the induced apoptosis rates at 0, 1, 5, and 10 μ M were 2.10%, 5.19%, 5.32% and 19.25%, respectively.

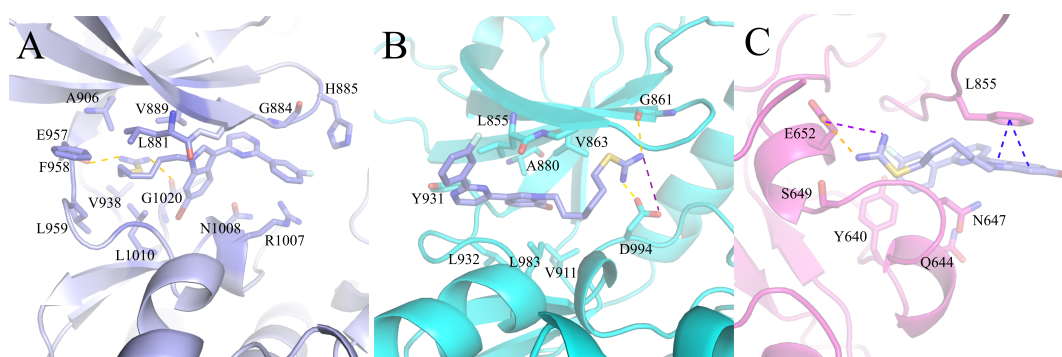


Figureure. 1. Compound **6e** induced A549 and DU145 cancer cells apoptosis *in Vitro*. A549 and DU145 cells were incubated with **6e** at different concentrations (0-10 μ M) for 24 h. Annexin V/PI staining was carried out and the percentages of apoptotic cells were further determined using flow cytometry.

2.2.3. Molecular docking

Molecular docking was performed for understanding the interaction mechanisms between compound **6e** with JAK1 (PDB ID:4I5C), JAK2 (PDB ID:5CF5) and STAT3 (PDB ID:1BG1) [28], respectively. The results showed that the hydrophobic fatty chain was accommodated at a hydrophobic pocket mainly defined by residues Leu881, Val889, Ala906, Val938, Phe958, Leu959 and Leu1010 (Figureure. 2 A). The

hydrogen in the imine of **6e** engages H-bonds formation with Glu957 and Gly1020. For JAK2, the docking poses suggested that the imine group of **6e** interacts with a carboxyl group of Asp994 and carbonyl group of Gly861 by forming two hydrogen bonds (Figureure. 2 B). In addition, nitrogen of imine on **6e** also forms salt bridge interaction with Asp994. The Phe710 forms π - π interactions with the indole ring of **6e**, while the Glu652 forms a hydrogen bond and a salt-bridge with the H atom and the N atom on imine, respectively (Figureure. 2 C) [29].



Figureure. 2. Binding modes of the compound **6e** with JAK1, JAK2 and STAT3. The compound **6e** was shown in blue, while the JAK1 (A), JAK2 (B) and STAT3 (C) were shown in purple, blue and pink, respectively. The H-bonds, π - π or salt-bridges were shown in dashed lines.

2.2.4 Compound **6e** inhibit expression of JAK/STAT3 target genes

The ability of compound **6e** to inhibit the phosphorylation of JAK/STAT3 was determined in A549 and DU145 cells. As shown in Figure. 3A and 3B, after 24 h treatment with 5 μ M **6e**, the decreased levels of JAK1, JAK2 and STAT3 were observed at both in A549 and DU145 cells, but no significant change was with total STAT3 protein expression. Moreover, compound **6e** significantly inhibited the expression of JAK/STAT3 downstream genes c-Myc, Cyclin D1 and Bcl-XL at 10 μ M after 24 h treatment (Figureure. 3C). Therefore, pretreatment with compound **6e** suppressed JAK/STAT3 signaling pathway and its downstream genes expression, which were consistent with the above results obtained *in vitro*.

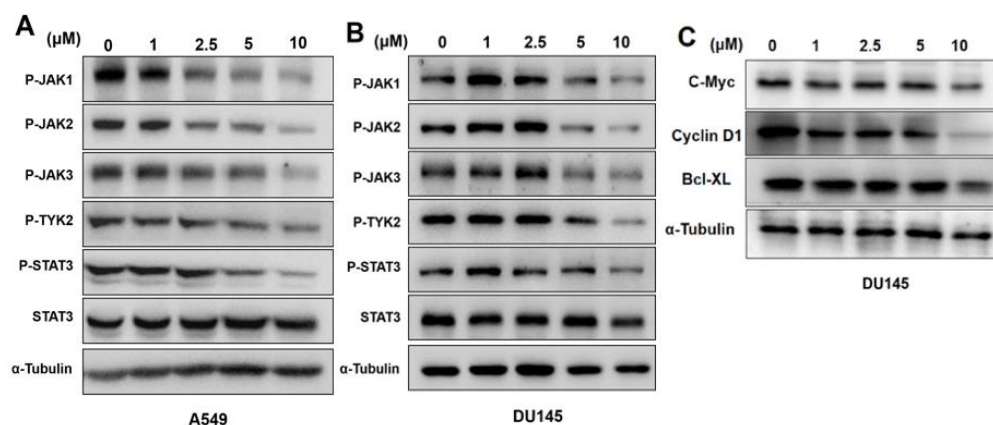


Figure. 3. The effect of **6e** on overexpression protein in JAK/STAT3 signaling pathway by western blot analysis

2.2.5. Compound **6e** inhibited tumor growth in a mouse breast cancer model

To further investigate the antitumor potential *in vivo*, we evaluated the effects of compound **6e** in nude mice tumor model (Figure. 4). After the solid tumor was established, twenty-four nude mice were randomly divided into four groups, which were vehicle control group, **6e** groups (5mg/kg and 10 mg/kg) and Gefitinib positive control group. All the compound were taken by intragastric gavage. Compound **6e** at the dose of 10 mg/kg could significantly inhibit tumor growth, and the tumor inhibition rate of **6e** was over 40%, which was comparable to that of the positive control (Figure. 4A, 4B and 4C). Subsequently, Ki67 and TUNEL staining were performed on tumor sections, which showed that tumor proliferation marker Ki67 was significantly inhibited, and the proportion of apoptotic cells that were marked by TUNEL positive staining also significantly increased with **6e** treatment at a dose of 10 mg/kg (Figure. 4D). Moreover, during the administration period, the weight of nude mice did not increase or decrease significantly (Figure. 4E), indicating that there is no obvious biological toxicity of **6e**.

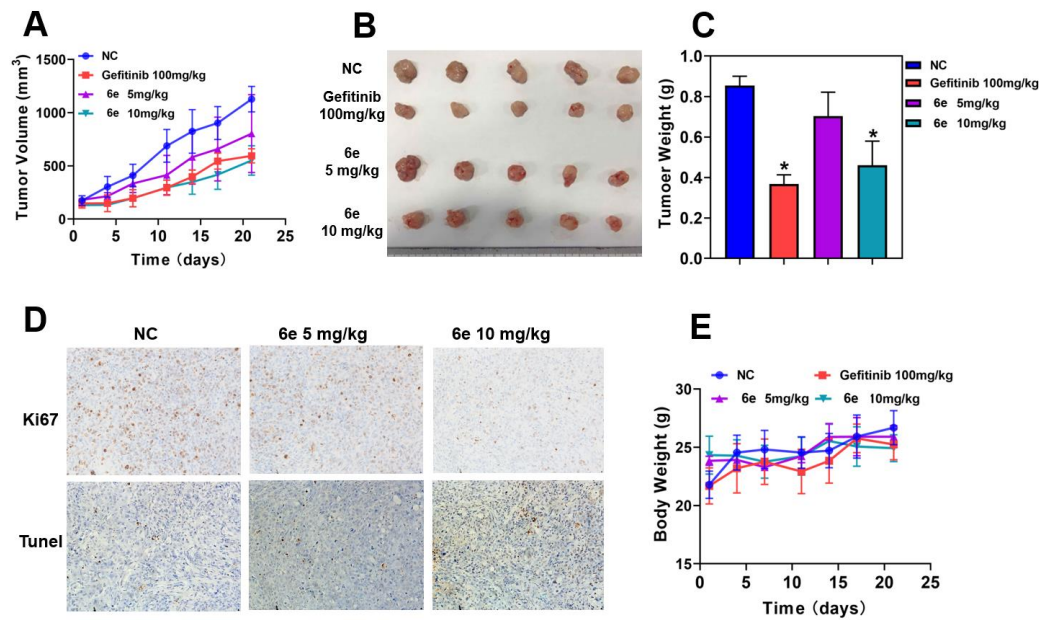


Figure 4. The results of **6e** on DU145 tumor bearing model *in vivo* therapeutic. (A) Growth curve of subcutaneous tumor bearing DU145 treated with **6e**. (B) Anatomical mice's tumor tissues treated with **6e**. (C) The weight of tumors in each group treated with **6e**. (D) Ki67 and TUNEL staining in DU145 tumors treated with different doses of **6e**. (E) Changes in body weight of mice in each group during administration.

2.2.6. Immunohistochemical (IHC) analysis

To further test the inhibitory effects of **6e** to JAK/STAT3 signaling, IHC analysis of nude mice inoculated with DU145 tumor cells were performed. As shown in Figure. 5, after **6e** treatment, the intratumoral staining of p-STAT3, Cyclin D1 and c-Myc in DU145 inoculated mice were significantly lower than those in the NC group, and their staining levels decreased while the dose of **6e** increasing. Therefore, compound **6e** may exert antitumor effects by inhibiting the JAK/STAT3 signaling pathway both *in vitro* and *in vivo*.

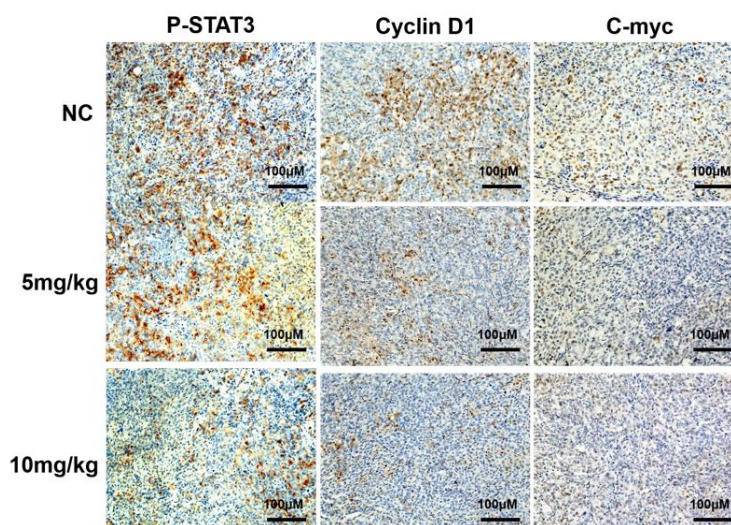


Figure. 5. Immunohistochemistry of protein expressions in DU145 xenograft model. Formalin-fixed paraffin-embedded sections from DU145 xenograft tumors were stained with H&E stain, p-STAT3, Cyclin D1 and c-Myc antibodies.

3. Conclusions

In summary, a novel series of meridianin derivatives were obtained and biologically evaluated. Initially, meridianins (A, C, D and G) and their four series derivatives of compounds **1a~g**, **2a~g**, **3a~g** and **4a~g** were prepared, the results indicated that meridianins (A, C, D and G) displayed weak inhibitory activity on four JAK/STAT3 over-activated human cancer cell lines HeLa, MDA-MB-231, A549 and DU145, whereas most of the meridianin derivatives exerted promising inhibitory activity on tested cell lines. To improve the anti-tumor activity, meridianins derivatives **5a~g** and **6a~g** were designed and synthesized by incorporating isothioureia groups at the N1-position with different length of carbon alkyl chains. Surprisingly, the anti-tumor effects of the isothiuronium-modified compounds were significantly enhanced, with IC_{50} less than 10 μ M. Among them, the most potent compound **6e** with alkyl chain of six carbon atoms had an IC_{50} ranged in 1.11-2.80 μ M in various cancer cell lines, which is superior to the positive control Gefitinib. Structure-activity relationship (SAR) study indicated that isothiuronium-modified by N-alkylation with 6C alkyl chains may contribute most to anti-tumor activity. Western

blotting assays suggested that treatment with compound **6e** could decrease the phosphorylation level of JAK1, JAK2 and STAT3 at 5 μ M, but did not affect the total STAT3 level. Moreover, **6e** also suppressed the expressions of STAT3 downstream gene, including c-Myc, Cyclin D1 and Bcl-XL. Consistently, **6e** dose dependently induced the apoptosis of A549 and DU145 cells. Molecular docking studies demonstrated that H-bond is the main type of interaction between compound **6e** and JAK1/JAK2 kinases, and **6e** could also tightly bind to STAT3 SH2 domain. An additional *in vivo* study revealed that application of **6e** at the dose of 10 mg/kg could significantly inhibit the DU145 xenograft tumor growth without obvious body-weight loss, which was comparable to that of the positive control. Taken together, these results clearly indicated that **6e** could be a highly potent anti-tumor agent by targeting JAK/STAT3 signaling pathway.

4. Materials and Methods

4.1. Chemistry

All commercially available starting materials and solvents were purchased from commercial vendors and used without further purification. Reactions were monitored using analytical thin layer chromatography (TLC) on precoated silica gel GF254 plates (Qingdao Haiyang Chemical Plant, Qingdao, China) plates and visualized under ultraviolet light (254 nm and 365 nm). Column chromatography was performed on silica gel (200-300 mesh). Melting points were determined on a Mitamura-Riken micro-hot stage and uncorrected. ^1H and ^{13}C NMR spectra were recorded on the Broker AVANCE NEO and Agilent DD2 500 with 400 or 500 MHz for proton (^1H NMR), and 100 or 125 MHz for carbon (^{13}C NMR), respectively. The chemical shifts (δ) were expressed in parts per million (ppm) downfield and coupling constants (J) values were described as hertz. High resolution (ESI) MS spectra were recorded using a QTOF-2 Micromass spectrometer. The purity of final compounds for biological evaluation was higher than 95% by analytical HPLC analysis with Primaide 1210 system.

Compounds **1-4**, **16-19** and **20-23** were prepared according to the procedure

published by Dong et al. and the spectroscopic data for intermediates were identical to those described in literature [30].

4.1.1. General procedure for synthesis of **1a~g**, **3a~g** and **4a~g** meridianin analogs.

To the solution of intermediates **4**, **19** or **23** (1.0 equiv.) in 2-methoxyethanol (5 mL) were added **5~11** (2.5 equiv.) and potassium carbonate (2.0 equiv.) respectively. The reaction mixture was stirred at 120°C for 20 h under a nitrogen atmosphere. Then, the mixture was poured into ice water and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine and dried over magnesium sulfate anhydrous. After filtration, the solvent was removed under vacuum and the residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate 10:1) to give get the final target compounds **1a~g**, **3a~g** and **4a~g**.

3-(2-phenylpyrimidin-4-yl)-1H-indole (1a). Yield: 62%; pale yellow solid; m.p. 137-139 °C; ¹H NMR (500 MHz, DMSO-*d*₆): δ 11.94 (s, 1H), 8.75–8.71 (m, 1H), 8.68 (d, *J* = 7.6 Hz, 1H), 8.54 (d, *J* = 7.0 Hz, 2H), 8.50 (d, *J* = 2.5 Hz, 1H), 7.83 (d, *J* = 5.3 Hz, 1H), 7.60 (t, *J* = 7.2 Hz, 2H), 7.57–7.55 (m, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.31–7.22 (m, 2H); ¹³C NMR (125 MHz, DMSO-*d*₆): δ 163.78, 162.85, 157.10, 138.72, 137.86, 131.09, 130.25, 129.25, 128.23, 125.80, 122.92, 122.25, 121.60, 114.68, 113.87, 112.81; HRMS: *m/z* [M + H]⁺ calcd for C₁₈H₁₃N₃, 272.1182; found, 272.1189.

3-(2-(4-methoxyphenyl)pyrimidin-4-yl)-1H-indole (1b). Yield: 45%; white solid; m.p. 194-196 °C; ¹H NMR (500 MHz, DMSO-*d*₆): δ 11.90 (d, *J* = 8.4 Hz, 1H), 8.66 (s, 1H), 8.51–8.44 (m, 1H), 7.74 (dd, *J* = 5.5, 3.0 Hz, 1H), 7.54–7.49 (m, 1H), 7.27 (d, *J* = 7.0 Hz, 1H), 7.13 (dd, *J* = 8.4, 3.4 Hz, 1H), 3.85 (d, *J* = 3.2 Hz, 1H); ¹³C NMR (125 MHz, DMSO-*d*₆): δ 163.49, 162.56, 161.76, 156.86, 137.71, 131.10, 129.95, 129.71, 125.69, 122.75, 122.14, 121.39, 114.47, 113.89, 113.81, 112.66, 55.75; HRMS: *m/z* [M + H]⁺ calcd for C₁₉H₁₅ON₃, 302.1288; found, 302.1296.

3-(2-(3-methoxyphenyl)pyrimidin-4-yl)-1H-indole (1c). Yield: 31%; white

solid; m.p. 188-190 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 11.91 (s, 1H), 8.71 (d, J = 5.4 Hz, 1H), 8.68–8.63 (m, 1H), 8.49 (d, J = 2.9 Hz, 1H), 8.12 (d, J = 7.7 Hz, 1H), 8.08 (s, 1H), 7.82 (d, J = 5.4 Hz, 1H), 7.50 (dd, J = 9.3, 6.7 Hz, 2H), 7.26–7.20 (m, 2H), 7.12 (dd, J = 8.1, 2.2 Hz, 1H), 3.89 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.38, 162.67, 160.00, 156.94, 140.06, 137.72, 130.23, 130.13, 125.68, 122.81, 122.08, 121.42, 120.46, 116.90, 114.64, 113.69, 112.98, 112.72, 55.56; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{15}\text{ON}_3$, 302.1288; found, 302.1295.

3-(2-(4-fluorophenyl)pyrimidin-4-yl)-1H-indole (1d). Yield: 40%; white solid; m.p. 126-128 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 11.94 (s, 1H), 8.70 (d, J = 5.4 Hz, 1H), 8.62 (dd, J = 6.4, 2.2 Hz, 1H), 8.56 (dd, J = 8.8, 5.8 Hz, 2H), 8.49 (d, J = 2.3 Hz, 1H), 7.81 (d, J = 5.4 Hz, 1H), 7.53–7.49 (m, 1H), 7.41 (t, J = 8.8 Hz, 2H), 7.24 (pd, J = 7.0, 3.5 Hz, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 165.24, 163.27, 162.75, 156.99, 137.73, 135.06, 130.44, 130.37, 130.23, 125.62, 122.81, 122.06, 121.48, 116.13, 115.96, 114.48, 113.65, 112.70; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{12}\text{N}_3\text{F}$, 290.1088; found, 290.1096.

3-(2-(3-fluorophenyl)pyrimidin-4-yl)-1H-indole (1e). Yield: 37%; white solid; m.p. 128-130 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 11.95 (s, 1H), 8.73 (d, J = 5.4 Hz, 1H), 8.61 (d, J = 7.5 Hz, 1H), 8.51 (d, J = 2.9 Hz, 1H), 8.38 (d, J = 7.8 Hz, 1H), 8.21 (d, J = 9.8 Hz, 1H), 7.85 (d, J = 5.5 Hz, 1H), 7.64 (dd, J = 14.1, 7.9 Hz, 1H), 7.52 (d, J = 7.4 Hz, 1H), 7.39 (td, J = 8.4, 2.4 Hz, 1H), 7.29–7.23 (m, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.97, 162.82, 162.43 (d, J = 3.2 Hz), 162.04, 157.05, 141.18 (d, J = 7.6 Hz), 137.75, 131.23 (d, J = 8.1 Hz), 130.39, 125.60, 124.12 (d, J = 2.5 Hz), 122.85, 121.94, 121.54, 117.87, 117.70, 115.04, 114.53, 114.34, 113.55, 112.76; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{12}\text{N}_3\text{F}$, 290.1088; found, 290.1096.

3-(2-(p-tolyl)pyrimidin-4-yl)-1H-indole (1f). Yield: 36%; white solid; m.p. 193-195 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 11.90 (s, 1H), 8.68 (d, J = 5.1 Hz, 1H), 8.64 (d, J = 7.2 Hz, 1H), 8.45 (s, 1H), 8.40 (d, J = 7.8 Hz, 2H), 7.77 (d, J = 5.0 Hz, 1H), 7.51 (d, J = 7.6 Hz, 1H), 7.38 (d, J = 7.5 Hz, 2H), 7.28–7.21 (m, 2H), 2.39 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.71, 162.60, 156.93, 140.72, 137.70, 135.89, 129.96, 129.75, 128.08, 125.64, 122.80, 122.09, 121.46, 114.28, 113.78,

112.69, 21.48; HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{15}N_3$, 286.1339; found, 286.1346.

3-(2-(m-tolyl)pyrimidin-4-yl)-1H-indole (1g). Yield: 47%; white solid; m.p. 121-123 °C; 1H NMR (500 MHz, DMSO- d_6): δ 11.92 (s, 1H), 8.71 (d, $J = 5.4$ Hz, 1H), 8.66 (d, $J = 7.5$ Hz, 1H), 8.48 (d, $J = 2.5$ Hz, 1H), 8.34 (d, $J = 8.1$ Hz, 2H), 7.81 (d, $J = 5.4$ Hz, 1H), 7.52 (d, $J = 7.9$ Hz, 1H), 7.47 (t, $J = 7.5$ Hz, 1H), 7.36 (d, $J = 7.3$ Hz, 1H), 7.25 (dd, $J = 14.6, 7.3$ Hz, 2H), 2.45 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.73, 162.63, 156.94, 138.56, 138.14, 137.73, 131.58, 130.06, 129.02, 128.74, 125.67, 125.31, 122.77, 122.08, 121.44, 114.50, 113.79, 112.70, 21.71; HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{15}N_3$, 286.1339; found, 286.1347.

5-bromo-3-(2-phenylpyrimidin-4-yl)-1H-indole (3a). Yield: 28%; white solid; m.p. 201-203 °C; 1H NMR (500 MHz, DMSO- d_6): δ 12.11 (s, 1H), 8.86 (d, $J = 2.0$ Hz, 1H), 8.75 (d, $J = 5.4$ Hz, 1H), 8.55 (d, $J = 3.0$ Hz, 1H), 8.50 – 8.46 (m, 2H), 7.83 (d, $J = 5.4$ Hz, 1H), 7.58 (tdd, $J = 6.8, 3.8, 1.7$ Hz, 3H), 7.49 (d, $J = 8.6$ Hz, 1H), 7.37 (dd, $J = 8.6, 2.0$ Hz, 1H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.68, 162.17, 157.25, 138.52, 136.40, 131.39, 131.11, 129.14, 127.99, 127.43, 125.31, 124.51, 114.71, 114.61, 114.07, 113.30; HRMS: m/z $[M + H]^+$ calcd for $C_{18}H_{12}N_3Br$, 350.0287; found, 350.0287.

5-bromo-3-(2-(4-methoxyphenyl)pyrimidin-4-yl)-1H-indole (3b). Yield: 48%; pale yellow solid; m.p. 97-99 °C; 1H NMR (500 MHz, DMSO- d_6): δ 12.08 (s, 1H), 8.84 (s, 1H), 8.68 (d, $J = 5.4$ Hz, 1H), 8.51 (s, 1H), 8.43 (d, $J = 8.7$ Hz, 2H), 7.75 (d, $J = 5.4$ Hz, 1H), 7.49 (d, $J = 8.6$ Hz, 1H), 7.36 (dd, $J = 8.6, 1.6$ Hz, 1H), 7.12 (d, $J = 8.8$ Hz, 2H), 3.86 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.52, 162.01, 161.85, 157.13, 136.37, 131.23, 130.99, 129.59, 127.42, 125.26, 124.49, 114.68, 114.49, 114.00, 113.89, 113.41, 55.82; HRMS calcd for $C_{19}H_{14}N_3^{81}Br$ $[M + H]^+$ 382.0373, found: 382.0382.

5-bromo-3-(2-(3-methoxyphenyl)pyrimidin-4-yl)-1H-indole (3c). Yield: 55%; white solid; m.p. 192-194 °C; 1H NMR (500 MHz, DMSO- d_6): δ 12.11 (s, 1H), 8.91 (s, 1H), 8.73 (d, $J = 5.4$ Hz, 1H), 8.55 (d, $J = 2.9$ Hz, 1H), 8.08 (dd, $J = 10.7, 4.5$ Hz, 2H), 7.83 (d, $J = 5.4$ Hz, 1H), 7.52 – 7.45 (m, 2H), 7.37 (dd, $J = 8.6, 1.7$ Hz, 1H), 7.12

(dd, $J = 8.1, 2.4$ Hz, 1H), 3.95 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.42, 162.18, 160.04, 157.14, 139.95, 136.40, 131.44, 130.20, 127.45, 125.33, 124.58, 120.45, 117.82, 114.72, 114.64, 114.07, 113.23, 112.01, 55.67; HRMS calcd for $\text{C}_{19}\text{H}_{14}\text{N}_3^{81}\text{Br}$ $[\text{M} + \text{H}]^+$ 382.0373, found: 382.0382.

5-bromo-3-(2-(4-fluorophenyl)pyrimidin-4-yl)-1H-indole (3d). Yield: 50%; white solid; m.p. 88-90 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 12.13 (s, 1H), 8.79 (d, $J = 1.2$ Hz, 1H), 8.73 (d, $J = 5.4$ Hz, 1H), 8.55 (d, $J = 2.8$ Hz, 1H), 8.51 (dd, $J = 8.5, 5.9$ Hz, 2H), 7.82 (d, $J = 5.4$ Hz, 1H), 7.49 (d, $J = 8.6$ Hz, 1H), 7.44 – 7.34 (m, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 165.29, 163.32, 162.80, 162.18, 157.28, 136.41, 135.03, 131.48, 130.27, 127.35, 125.33, 124.34, 116.17, 116.00, 114.74, 114.56, 114.09, 113.23; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{11}\text{N}_3\text{BrF}$, 368.0193; found, 368.0189.

5-bromo-3-(2-(3-fluorophenyl)pyrimidin-4-yl)-1H-indole (3e). Yield: 43%; white solid; m.p. 205-206 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 12.13 (s, 1H), 8.81 (s, 1H), 8.74 (d, $J = 5.4$ Hz, 1H), 8.55 (d, $J = 2.8$ Hz, 1H), 8.31 (d, $J = 7.8$ Hz, 1H), 8.17 (d, $J = 10.3$ Hz, 1H), 7.85 (d, $J = 5.4$ Hz, 1H), 7.62 (dd, $J = 14.1, 7.8$ Hz, 1H), 7.49 (d, $J = 8.6$ Hz, 1H), 7.43 – 7.33 (m, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.98, 162.45, 162.25, 162.05, 157.27, 141.09 (d, $J = 7.6$ Hz), 136.41, 131.59, 131.18 (d, $J = 8.1$ Hz), 127.36, 125.34, 124.42, 123.97 (d, $J = 2.3$ Hz), 117.95, 117.78, 115.06, 114.73, 114.51, 114.32, 114.14, 113.13; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{11}\text{N}_3\text{BrF}$, 368.0193; found, 368.0198.

5-bromo-3-(2-(p-tolyl)pyrimidin-4-yl)-1H-indole (3f). Yield: 38%; pale yellow solid; m.p. 197-199 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 12.09 (s, 1H), 8.86 (d, $J = 1.7$ Hz, 1H), 8.71 (d, $J = 5.4$ Hz, 1H), 8.53 (s, 1H), 8.37 (d, $J = 8.1$ Hz, 2H), 7.79 (d, $J = 5.4$ Hz, 1H), 7.49 (d, $J = 8.6$ Hz, 1H), 7.41 – 7.35 (m, 3H), 2.41 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.75, 162.07, 157.19, 140.89, 136.38, 135.85, 131.27, 129.75, 127.97, 127.44, 125.27, 124.55, 114.69, 114.32, 114.03, 113.35, 21.44; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{14}\text{N}_3\text{Br}$, 364.0444; found, 368.0444.

5-bromo-3-(2-(m-tolyl)pyrimidin-4-yl)-1H-indole (3g). Yield: 42%; pale

yellow solid; m.p. 167-169 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 12.09 (s, 1H), 8.94 (s, 1H), 8.73 (d, J = 5.4 Hz, 1H), 8.54 (d, J = 2.8 Hz, 1H), 8.35 (s, 1H), 8.27 (d, J = 7.7 Hz, 1H), 7.81 (d, J = 5.4 Hz, 1H), 7.51 – 7.44 (m, 2H), 7.40 – 7.35 (m, 2H), 2.47 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.68, 162.08, 157.19, 138.44, 138.21, 136.37, 131.68, 131.32, 129.06, 128.73, 127.50, 125.25, 125.18, 124.75, 114.69, 114.45, 114.05, 113.30, 21.68; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{14}\text{N}_3\text{Br}$, 364.0444; found, 368.0446.

6-bromo-3-(2-phenylpyrimidin-4-yl)-1H-indole (4a). Yield: 41%; pale yellow solid; m.p. 175-177 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 12.03 (s, 1H), 8.74 (d, J = 5.4 Hz, 1H), 8.60 (d, J = 8.5 Hz, 1H), 8.51 (t, J = 4.4 Hz, 3H), 7.82 (d, J = 5.4 Hz, 1H), 7.70 (d, J = 1.5 Hz, 1H), 7.60 – 7.53 (m, 3H), 7.40 (dd, J = 8.5, 1.6 Hz, 1H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.68, 162.26, 157.16, 138.58, 138.40, 131.05, 130.98, 129.15, 128.13, 124.70, 124.37, 123.85, 115.46, 115.27, 114.66, 113.90; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{13}\text{N}_3\text{Br}$, 350.0287; found, 350.0288.

6-bromo-3-(2-(4-methoxyphenyl)pyrimidin-4-yl)-1H-indole (4b). Yield: 42%; white solid; m.p. >210 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 12.00 (s, 1H), 8.68 (d, J = 5.4 Hz, 1H), 8.58 (d, J = 8.5 Hz, 1H), 8.47 (dd, J = 13.9, 5.7 Hz, 3H), 7.74 (d, J = 5.4 Hz, 1H), 7.69 (s, 1H), 7.38 (d, J = 8.5 Hz, 1H), 7.11 (d, J = 8.7 Hz, 2H), 3.85 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.54, 162.08, 161.80, 157.07, 138.56, 130.93, 130.79, 129.75, 124.71, 124.27, 123.85, 115.41, 115.25, 114.49, 114.00, 113.92, 55.76; HRMS calcd for $\text{C}_{19}\text{H}_{14}\text{ON}_3^{81}\text{Br}$ $[\text{M} + \text{H}]^+$ 382.0373, found: 382.0384.

6-bromo-3-(2-(3-methoxyphenyl)pyrimidin-4-yl)-1H-indole (4c). Yield: 53%; yellow solid; m.p. 180-181 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 12.03 (s, 1H), 8.74 (d, J = 5.4 Hz, 1H), 8.60 (d, J = 8.5 Hz, 1H), 8.51 (d, J = 2.8 Hz, 1H), 8.09 (d, J = 7.7 Hz, 1H), 8.05 (s, 1H), 7.82 (d, J = 5.4 Hz, 1H), 7.70 (s, 1H), 7.49 (t, J = 7.9 Hz, 1H), 7.40 (d, J = 8.5 Hz, 1H), 7.12 (dd, J = 8.1, 1.6 Hz, 1H), 3.89 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.45, 162.20, 160.00, 157.16, 139.90, 138.58, 130.96, 130.26, 124.71, 124.30, 123.76, 120.49, 117.11, 115.47, 115.31, 114.75, 113.85, 112.83,

55.58; HRMS calcd for $C_{19}H_{14}ON_3^{81}Br$ $[M + H]^+$ 382.0373, found: 380.0377.

6-bromo-3-(2-(4-fluorophenyl)pyrimidin-4-yl)-1H-indole (4d). Yield: 32%; white solid; m.p. 187-188 °C; 1H NMR (500 MHz, DMSO- d_6): δ 12.04 (s, 1H), 8.73 (d, $J = 5.4$ Hz, 1H), 8.59 – 8.52 (m, 3H), 8.52 (d, $J = 2.9$ Hz, 1H), 7.82 (d, $J = 5.4$ Hz, 1H), 7.70 (d, $J = 1.7$ Hz, 1H), 7.43 – 7.36 (m, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 165.27, 163.30, 162.81, 162.27, 157.22, 138.59, 130.46 (d, $J = 8.7$ Hz), 131.06, 130.50, 130.43, 124.65, 124.37, 123.79, 116.16, 115.99, 115.46, 115.29, 114.59, 113.83; HRMS: m/z $[M + H]^+$ calcd for $C_{18}H_{13}N_3Br$, 368.0193; found, 368.0193.

6-bromo-3-(2-(3-fluorophenyl)pyrimidin-4-yl)-1H-indole (4e). Yield: 61%; white solid; m.p. >210 °C; 1H NMR (500 MHz, DMSO- d_6): δ 12.06 (s, 1H), 8.76 (d, $J = 5.4$ Hz, 1H), 8.57 – 8.52 (m, 2H), 8.35 (d, $J = 7.8$ Hz, 1H), 8.17 (d, $J = 10.1$ Hz, 1H), 7.86 (d, $J = 5.4$ Hz, 1H), 7.70 (d, $J = 1.5$ Hz, 1H), 7.63 (dd, $J = 14.1, 7.9$ Hz, 1H), 7.41 (dd, $J = 8.5, 1.7$ Hz, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.96, 162.50 (d, $J = 3.2$ Hz), 162.35, 162.03, 157.28, 138.60, 131.26, 130.77, 127.17, 124.62, 124.44, 124.18, 123.66, 115.50, 115.33, 115.16, 114.55, 114.37, 113.72; HRMS: m/z $[M + H]^+$ calcd for $C_{18}H_{11}N_3BrF$, 368.0193; found, 368.0192.

6-bromo-3-(2-(p-tolyl)pyrimidin-4-yl)-1H-indole (4f). Yield: 37%; yellow solid; m.p. 192-194 °C; 1H NMR (500 MHz, DMSO- d_6): δ 12.02 (s, 1H), 8.71 (d, $J = 5.4$ Hz, 1H), 8.59 (d, $J = 8.5$ Hz, 1H), 8.49 (d, $J = 2.8$ Hz, 1H), 8.39 (d, $J = 8.0$ Hz, 2H), 7.78 (d, $J = 5.4$ Hz, 1H), 7.70 (d, $J = 1.1$ Hz, 1H), 7.39 (dd, $J = 13.5, 4.7$ Hz, 3H), 2.40 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.75, 162.16, 157.09, 140.78, 138.57, 135.75, 130.87, 129.76, 128.11, 124.71, 124.31, 123.84, 115.43, 115.26, 114.38, 113.95, 21.49; HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{14}N_3Br$, 364.0444; found, 364.0443.

6-bromo-3-(2-(m-tolyl)pyrimidin-4-yl)-1H-indole (4g). Yield: 59%; pale yellow solid; m.p. 203-204 °C; 1H NMR (500 MHz, DMSO- d_6): δ 12.02 (s, 1H), 8.73 (d, $J = 5.4$ Hz, 1H), 8.59 (d, $J = 8.5$ Hz, 1H), 8.50 (d, $J = 2.7$ Hz, 1H), 8.30 (d, $J = 9.2$ Hz, 2H), 7.81 (d, $J = 5.4$ Hz, 1H), 7.70 (d, $J = 1.1$ Hz, 1H), 7.46 (t, $J = 7.5$ Hz, 1H), 7.41 (dd, $J = 8.5, 1.3$ Hz, 1H), 7.36 (d, $J = 7.5$ Hz, 1H), 2.45 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.78, 162.15, 157.17, 138.57, 138.40, 138.21, 131.67, 130.89,

129.07, 128.73, 125.32, 124.70, 124.34, 123.80, 115.44, 115.27, 114.60, 113.93, 21.69; HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{14}N_3Br$, 364.0444; found, 364.0443.

4.1.2. General procedure for synthesis of **2a~g** meridianin analogs.

To a stirring solution of 4-hydroxyindole (**12**) in dry DMF (10 mL) was added sodium hydride (5.0 equiv.) at 0°C and the mixture was stirred for 30 min. Then, the *p*-toluenesulfonyl (3.0 equiv.) was added. After stirring for 4 h at room temperature. The reaction was quenched with saturated $NaHCO_3$ solution and extracted with ethyl acetate. (3×50 mL). The combined organic layers were washed with brine and dried over magnesium sulfate anhydrous. After filtration, the solvent was removed under vacuum and the residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate 10:1) to give compound **13**.

1-tosyl-1*H*-indol-4-yl 4-methylbenzenesulfonate (13). Yield: 85%; pale yellow solid; m.p. 100-102°C; 1H NMR (400 MHz, $DMSO-d_6$): δ 7.89 (d, $J = 8.5$ Hz, 1H), 7.88 (d, $J = 3.4$ Hz, 1H), 7.85 (s, 1H), 7.78 (t, $J = 4.1$ Hz, 1H), 7.71 (s, 1H), 7.69 (s, 1H), 7.38 (dd, $J = 15.4, 7.3$ Hz, 4H), 7.32 (d, $J = 8.2$ Hz, 1H), 6.93 (d, $J = 8.0$ Hz, 1H), 6.51 (d, $J = 3.7$ Hz, 1H), 2.38 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $DMSO-d_6$): δ 146.42, 146.38, 141.98, 135.99, 134.31, 131.72, 130.84, 130.62, 128.71, 128.33, 127.28, 125.88, 124.78, 117.02, 112.81, 105.63, 21.62, 21.52; HRMS: m/z $[M + H]^+$ calcd for $C_{22}H_{20}O_5NS_2$, 442.0777; found, 442.0789.

To a stirring solution of acetic anhydride (2.0 equiv.) in dry dichloromethane (8 mL) was added aluminium chloride (5.0 equiv.) at 0°C. Then, compound **13** in dry dichloromethane (8 mL) was added dropwise and the mixture was stirred for 2 h at room temperature. The reaction was quenched with saturated aqueous NH_4Cl and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with brine and dried over magnesium sulfate anhydrous. After filtration, the solvent was removed under vacuum and the residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate 8:1) to give compound **14**.

3-acetyl-1-tosyl-1*H*-indol-4-yl 4-methylbenzenesulfonate (14). Yield: 70%;

pale yellow solid; m.p. 163-165 °C; ^1H NMR (400 MHz, DMSO- d_6): δ 8.66 (s, 1H), 8.04 (s, 1H), 8.02 (s, 1H), 7.95 (d, J = 7.9 Hz, 1H), 7.52 (s, 1H), 7.50 (s, 1H), 7.47 (s, 1H), 7.45 (s, 1H), 7.39 (t, J = 8.2 Hz, 1H), 7.34 (s, 1H), 7.32 (s, 1H), 6.90–6.85 (m, 1H), 2.48 (s, 3H), 2.38 (s, 3H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 192.02, 147.08, 146.02, 142.26, 136.52, 134.25, 133.66, 131.87, 131.07, 130.27, 128.79, 128.67, 127.81, 126.84, 126.70, 122.26, 120.73, 119.30, 112.91, 105.45, 64.26, 29.61, 21.60; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{22}\text{O}_6\text{NS}_2$, 484.0883; found, 484.0891.

To a solution of compound **14** in DMF (5 mL) was added DMF-DMA (1.5 equiv.). The reaction mixture was stirred at 110°C for 5 h under a nitrogen atmosphere. Then, the mixture was poured into ice water and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with brine and dried over magnesium sulfate anhydrous. After filtration, the solvent was removed under vacuum and the residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate 2:1) to give intermediate **15**.

(*E*)-3-(3-(dimethylamino)acryloyl)-1-tosyl-1*H*-indol-4-yl

4-methylbenzenesulfonate (15). Yield: 64%; pale yellow solid; m.p. 129-131 °C; ^1H NMR (400 MHz, DMSO- d_6): δ 8.09 (s, 1H), 7.97 (s, 1H), 7.95 (d, J = 2.0 Hz, 2H), 7.93 (s, 1H), 7.60 (s, 1H), 7.58 (s, 1H), 7.43 (s, 2H), 7.41 (s, 1H), 7.36 (d, J = 8.2 Hz, 1H), 7.33 (s, 1H), 7.31 (s, 1H), 7.01 (d, J = 8.0 Hz, 1H), 5.40 (d, J = 12.5 Hz, 1H), 3.09 (d, J = 24.6 Hz, 3H), 2.81 (d, J = 16.5 Hz, 3H), 2.37 (s, 3H), 2.34 (d, J = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 170.81, 162.77, 154.45, 146.69, 145.76, 142.23, 136.55, 134.01, 132.10, 130.94, 130.23, 129.12, 128.76, 127.58, 126.13, 121.74, 118.13, 112.70, 60.22, 45.73, 37.16, 36.24, 36.24, 31.23, 21.58, 21.22, 14.54; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{27}\text{O}_6\text{N}_2\text{S}_2$, 539.1305; found, 539.1316.

To a solution of intermediate **15** in 2-methoxyethanol (5 mL) were added **5~11** (2.5 equiv.) and potassium carbonate (2.0 equiv.) respectively. The reaction mixture was stirred at 120°C for 20 h under a nitrogen atmosphere. Then, the mixture was poured into ice water and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with brine and dried over magnesium sulfate anhydrous.

After filtration, the solvent was removed under vacuum and the residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate 10:1) to give the final target compounds **2a-g**.

3-(2-phenylpyrimidin-4-yl)-1H-indol-4-ol (2a). Yield: 46%; pale yellow solid; m.p. 195-197 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 13.44 (s, 1H), 12.07 (s, 1H), 8.74 (d, J = 5.6 Hz, 1H), 8.53 (s, 1H), 8.23 (dd, J = 6.7, 1.7 Hz, 2H), 8.00 (d, J = 5.6 Hz, 1H), 7.61 (dd, J = 9.7, 4.9 Hz, 3H), 7.06 (t, J = 7.9 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 6.50 (d, J = 7.7 Hz, 1H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.56, 161.32, 157.80, 152.12, 139.93, 137.36, 131.38, 130.71, 129.39, 128.30, 125.23, 114.73, 114.59, 113.51, 106.42, 103.47; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{13}\text{ON}_3$, 288.1131; found, 288.1129.

3-(2-(4-methoxyphenyl)pyrimidin-4-yl)-1H-indol-4-ol (2b). Yield: 52%; pale yellow solid; m.p. >210 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 13.55 (s, 1H), 12.00 (s, 1H), 8.68 (d, J = 5.6 Hz, 1H), 8.50 (d, J = 2.8 Hz, 1H), 8.21 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 5.6 Hz, 1H), 7.15 (d, J = 8.8 Hz, 2H), 7.05 (t, J = 7.9 Hz, 1H), 6.89 (d, J = 7.9 Hz, 1H), 6.49 (d, J = 7.6 Hz, 1H), 3.86 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.24, 162.06, 161.15, 157.73, 152.16, 139.88, 130.49, 129.98, 129.64, 125.20, 114.79, 114.77, 113.83, 113.59, 106.32, 103.41, 55.86; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{15}\text{O}_2\text{N}_3$, 318.1238; found, 318.1237.

3-(2-(3-methoxyphenyl)pyrimidin-4-yl)-1H-indol-4-ol (2c). Yield: 38%; yellow solid; m.p. 207-209 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 13.39 (s, 1H), 12.04 (s, 1H), 8.73 (d, J = 5.6 Hz, 1H), 8.53 (d, J = 2.7 Hz, 1H), 8.00 (d, J = 5.6 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.78 (s, 1H), 7.51 (t, J = 7.9 Hz, 1H), 7.16 (d, J = 8.1 Hz, 1H), 7.05 (t, J = 7.8 Hz, 1H), 6.89 (d, J = 8.0 Hz, 1H), 6.49 (d, J = 7.7 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.34, 161.31, 160.10, 157.75, 152.13, 139.92, 138.74, 130.73, 130.52, 125.24, 120.57, 117.21, 114.70, 113.50, 113.47, 106.46, 103.47, 55.68; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{15}\text{O}_2\text{N}_3$, 318.1237; found, 318.1231.

3-(2-(4-fluorophenyl)pyrimidin-4-yl)-1H-indol-4-ol (2d). Yield: 55%; yellow

solid; m.p. 189-191 °C; ¹H NMR (500 MHz, DMSO-*d*₆): δ 13.39 (s, 1H), 12.04 (s, 1H), 8.72 (d, *J* = 5.6 Hz, 1H), 8.53 (d, *J* = 2.9 Hz, 1H), 8.27 (dd, *J* = 8.7, 5.6 Hz, 2H), 7.99 (d, *J* = 5.6 Hz, 1H), 7.44 (t, *J* = 8.8 Hz, 2H), 7.06 (t, *J* = 7.9 Hz, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.50 (d, *J* = 7.6 Hz, 1H); ¹³C NMR (125 MHz, DMSO-*d*₆): δ 165.37, 163.39, 162.59, 161.34, 157.79, 152.06, 139.92, 133.85, 130.75, 125.26, 116.50, 116.33, 114.72, 114.53, 113.46, 106.42, 103.52; HRMS: *m/z* [M + H]⁺ calcd for C₁₈H₁₂ON₃F, 306.1037; found, 306.1030.

3-(2-(3-fluorophenyl)pyrimidin-4-yl)-1*H*-indol-4-ol (2e). Yield: 40%; yellow solid; m.p. 159-161 °C; ¹H NMR (500 MHz, DMSO-*d*₆): δ 13.30 (s, 1H), 12.07 (s, 1H), 8.75 (d, *J* = 5.6 Hz, 1H), 8.55 (d, *J* = 3.1 Hz, 1H), 8.09 – 8.06 (m, 1H), 8.04 (d, *J* = 5.7 Hz, 1H), 7.67 (t, *J* = 8.0, 1H), 7.06 (t, *J* = 7.9, 2H), 6.89 (d, *J* = 8.1 Hz, 2H), 6.51 (d, *J* = 7.7 Hz, 1H); ¹³C NMR (125 MHz, DMSO-*d*₆): δ 163.91, 162.28, 161.98, 161.45, 157.79, 152.02, 139.94, 136.91, 131.57, 130.97, 125.29, 118.37, 118.20, 115.10, 113.38, 106.83, 106.51, 103.57; HRMS: *m/z* [M + H]⁺ calcd for C₁₈H₁₂ON₃F, 306.1037; found, 306.1033.

3-(2-(*p*-tolyl)pyrimidin-4-yl)-1*H*-indol-4-ol (2f). Yield: 54%; yellow solid; m.p. 175-177 °C; ¹H NMR (500 MHz, DMSO-*d*₆): δ 13.48 (s, 1H), 12.02 (s, 1H), 8.71 (d, *J* = 3.9 Hz, 1H), 8.51 (s, 1H), 8.12 (d, *J* = 6.9 Hz, 2H), 7.96 (d, *J* = 4.2 Hz, 1H), 7.40 (d, *J* = 7.3 Hz, 2H), 7.05 (t, *J* = 7.1 Hz, 1H), 6.89 (d, *J* = 7.2 Hz, 1H), 6.49 (d, *J* = 6.9 Hz, 1H), 2.40 (s, 3H); ¹³C NMR (125 MHz, DMSO-*d*₆): δ 163.57, 161.23, 157.77, 152.15, 141.32, 139.90, 134.63, 130.57, 129.99, 128.27, 125.21, 114.74, 114.30, 113.56, 106.39, 103.42, 21.44; HRMS: *m/z* [M + H]⁺ calcd for C₁₉H₁₅ON₃, 302.1288; found, 302.1281.

3-(2-(*m*-tolyl)pyrimidin-4-yl)-1*H*-indol-4-ol (2g). Yield: 66%; yellow solid; m.p. 144-146 °C; ¹H NMR (500 MHz, DMSO-*d*₆): δ 13.41 (s, 1H), 12.02 (s, 1H), 8.72 (d, *J* = 5.6 Hz, 1H), 8.52 (d, *J* = 3.0 Hz, 1H), 8.06 (s, 1H), 7.99 (t, *J* = 6.8 Hz, 2H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.40 (d, *J* = 7.5 Hz, 1H), 7.05 (dd, *J* = 9.5, 6.2 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.49 (d, *J* = 7.7 Hz, 1H), 2.43 (s, 3H); ¹³C NMR (125 MHz, DMSO-*d*₆): δ 163.67, 161.29, 157.75, 152.15, 139.92, 138.55, 137.34, 131.99, 130.64, 129.29,

129.03, 125.37, 125.22, 114.72, 114.52, 113.54, 106.44, 103.43, 21.61; HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{15}ON_3$, 302.1288; found, 302.1281.

4.1.3. General procedure for synthesis of **5a~g** meridianin analogs.

To the solution of **1a~g** in DMF (5 mL) was added 1,6-dibromohexane (5.0 equiv.) respectively, and the mixture was stirred at 50°C for 5 h. Then, the reaction mixture was removed under vacuum and the residue was poured into ice water and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with brine, dried over magnesium sulfate anhydrous and concentrated to give intermediates **23~29**, and used in the next step without further purification. To a stirring solution of compounds **23~29** in ethanol was added thiocarbamide (2.0 equiv.) and the mixture was stirred at 65°C for 3 h. Then, the solvent was removed under vacuum and the residue was purified by silica gel column chromatography (dichloromethane/methanol 10:1) to give get the final target compounds **5a-g**.

2-(6-(3-(2-phenylpyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiuronium (5a).

Yield: 70%; yellow oily substance; 1H NMR (500 MHz, $DMSO-d_6$): δ 8.97 (s, J = 28.0 Hz, 3H), 8.73 (d, J = 5.4 Hz, 1H), 8.68–8.64 (m, 1H), 8.56 (s, 1H), 8.53–8.49 (m, 2H), 7.77 (d, J = 5.4 Hz, 1H), 7.64–7.52 (m, 4H), 7.32–7.26 (m, 2H), 4.29 (t, J = 7.1 Hz, 2H), 3.11 (t, J = 7.3 Hz, 2H), 1.89–1.79 (m, 2H), 1.57 (dt, J = 14.9, 7.5 Hz, 2H), 1.46–1.36 (m, 2H), 1.31 (dt, J = 14.8, 7.3 Hz, 2H); ^{13}C NMR (125 MHz, $DMSO-d_6$): δ 170.30, 163.66, 162.26, 157.11, 138.51, 137.55, 133.02, 131.02, 129.13, 128.10, 126.13, 122.87, 122.35, 121.73, 114.51, 112.85, 111.20, 46.44, 30.49, 29.84, 28.71, 27.86, 26.06; HRMS calcd for $C_{25}H_{27}N_5S$ $[M + H]^+$ 430.2060, found: 430.2065.

2-(6-(3-(2-(4-methoxyphenyl)pyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiuronium (5b). Yield: 78%; pale yellow solid; m.p. 191-193 °C; 1H NMR (500 MHz, $DMSO-d_6$): δ 8.96 (s, 3H), 8.70 (d, J = 5.3 Hz, 1H), 8.64 (dd, J = 6.2, 2.7 Hz, 1H), 8.53 (s, 1H), 8.41 (d, J = 8.0 Hz, 2H), 7.73 (d, J = 5.4 Hz, 1H), 7.64–7.59 (m, 1H), 7.38 (d, J = 7.6 Hz, 2H), 7.33–7.25 (m, 2H), 4.29 (t, J = 6.6 Hz, 2H), 3.10 (t, J = 7.3 Hz, 2H), 2.40 (s, 3H), 1.88–1.80 (m, 2H), 1.56 (dd, J = 14.1, 6.9 Hz, 2H), 1.39 (dd, J

= 14.0, 6.8 Hz, 2H), 1.35–1.26 (m, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 170.27, 163.72, 162.17, 157.06, 140.73, 137.54, 135.86, 132.91, 129.74, 128.08, 126.13, 122.83, 122.34, 121.69, 114.23, 112.90, 111.18, 46.43, 30.48, 29.85, 28.71, 27.86, 26.06, 21.51; HRMS calcd for $\text{C}_{26}\text{H}_{29}\text{ON}_5\text{S}$ $[\text{M} + \text{H}]^+$ 460.2166, found: 460.2165.

2-(6-(3-(2-(3-methoxyphenyl)pyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiuronium (5c). Yield: 82%; pale yellow solid; m.p. 155–157 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 8.99 (s, 3H), 8.73 (d, J = 5.4 Hz, 1H), 8.66 (dd, J = 5.7, 3.2 Hz, 1H), 8.55 (s, 1H), 8.11 (d, J = 7.7 Hz, 1H), 8.07 (s, 1H), 7.77 (d, J = 5.4 Hz, 1H), 7.64–7.60 (m, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.29 (dd, J = 6.1, 3.1 Hz, 2H), 7.13 (dd, J = 8.1, 1.9 Hz, 1H), 4.29 (t, J = 6.9 Hz, 2H), 3.89 (s, 3H), 3.11 (t, J = 7.3 Hz, 2H), 1.90–1.80 (m, 2H), 1.61–1.51 (m, 2H), 1.40 (dt, J = 14.5, 7.3 Hz, 2H), 1.31 (dt, J = 14.5, 7.5 Hz, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 170.31, 163.40, 162.23, 160.00, 157.06, 139.99, 137.55, 133.02, 130.25, 126.14, 122.88, 122.31, 121.68, 120.46, 116.88, 114.59, 113.05, 112.80, 111.23, 55.58, 46.44, 30.48, 29.84, 28.71, 27.86, 26.06; HRMS calcd for $\text{C}_{26}\text{H}_{29}\text{ON}_5\text{S}$ $[\text{M} + \text{H}]^+$ 460.2166, found: 460.2166.

2-(6-(3-(2-(4-fluorophenyl)pyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiuronium (5d). Yield: 58%; yellow oily substance; ^1H NMR (500 MHz, DMSO- d_6): δ 8.99 (s, 3H), 8.71 (d, J = 5.4 Hz, 1H), 8.62 (dd, J = 6.0, 3.0 Hz, 1H), 8.58–8.53 (m, 3H), 7.77 (d, J = 5.5 Hz, 1H), 7.62 (dd, J = 6.0, 3.1 Hz, 1H), 7.40 (t, J = 8.8 Hz, 2H), 7.33–7.26 (m, 2H), 4.28 (t, J = 7.0 Hz, 2H), 3.11 (t, J = 7.3 Hz, 2H), 1.84 (dt, J = 14.5, 7.2 Hz, 2H), 1.57 (dt, J = 14.7, 7.5 Hz, 2H), 1.40 (dt, J = 14.5, 7.3 Hz, 2H), 1.31 (dt, J = 15.1, 7.6 Hz, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 170.32, 165.26, 163.29, 162.76, 162.30, 157.12, 137.55, 134.99, 133.13, 130.42 (d, J = 8.7 Hz), 126.08, 122.88, 122.28, 121.75, 116.14, 115.97, 114.43, 112.77, 111.22, 46.45, 30.48, 29.83, 28.72, 27.85, 26.05; HRMS calcd for $\text{C}_{25}\text{H}_{26}\text{N}_5\text{FS}$ $[\text{M} + \text{H}]^+$ 448.1966, found: 448.1968.

2-(6-(3-(2-(3-fluorophenyl)pyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiuronium (5e). Yield: 77%; yellow oily substance; ^1H NMR (500 MHz, DMSO- d_6): δ 9.00 (s, 3H), 8.75 (d, J = 5.5 Hz, 1H), 8.62–8.57 (m, 2H), 8.36 (d, J = 7.8 Hz, 1H), 8.19 (dd, J = 6.4, 5.4 Hz, 1H), 7.81 (d, J = 5.5 Hz, 1H), 7.67–7.61 (m, 2H), 7.40 (td, J = 8.4, 2.0 Hz, 1H), 7.33–7.26 (m, 2H), 4.29 (t, J = 7.0 Hz, 2H), 3.11 (t, J = 7.3 Hz, 2H),

1.88–1.80 (m, 2H), 1.57 (dt, $J = 14.8, 7.5$ Hz, 2H), 1.40 (dt, $J = 14.5, 7.3$ Hz, 2H), 1.31 (dt, $J = 14.9, 7.5$ Hz, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 170.33, 163.97, 162.40 (d, $J = 13.0$ Hz), 162.04, 157.21, 141.11 (d, $J = 7.6$ Hz), 137.58, 133.28, 131.26 (d, $J = 8.1$ Hz), 126.05, 124.14, 122.91, 122.15, 121.80, 117.92, 117.76, 114.99, 114.53, 114.35, 112.66, 111.27, 46.47, 30.47, 29.84, 28.72, 27.85, 26.06; HRMS calcd for $\text{C}_{25}\text{H}_{26}\text{N}_5\text{FS}$ $[\text{M} + \text{H}]^+$ 448.1966, found: 448.1974.

2-(6-(3-(2-(p-tolyl)pyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiuronium (5f).

Yield: 60%; white solid; m.p. 205–207 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 8.96 (s, 3H), 8.70 (d, $J = 5.3$ Hz, 1H), 8.64 (dd, $J = 6.2, 2.7$ Hz, 1H), 8.53 (s, 1H), 8.41 (d, $J = 8.0$ Hz, 2H), 7.73 (d, $J = 5.4$ Hz, 1H), 7.64–7.59 (m, 1H), 7.38 (d, $J = 7.6$ Hz, 2H), 7.33–7.25 (m, 2H), 4.29 (t, $J = 6.6$ Hz, 2H), 3.10 (t, $J = 7.3$ Hz, 2H), 2.40 (s, 3H), 1.88–1.80 (m, 2H), 1.56 (dd, $J = 14.1, 6.9$ Hz, 2H), 1.39 (dd, $J = 14.0, 6.8$ Hz, 2H), 1.35–1.26 (m, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 170.27, 163.72, 162.17, 157.06, 140.73, 137.54, 135.86, 132.91, 129.74, 128.08, 126.13, 122.83, 122.34, 121.69, 114.23, 112.90, 111.18, 46.43, 30.48, 29.85, 28.71, 27.86, 26.06, 21.51; HRMS calcd for $\text{C}_{26}\text{H}_{29}\text{N}_5\text{S}$ $[\text{M} + \text{H}]^+$ 444.2216, found: 444.2228.

2-(6-(3-(2-(m-tolyl)pyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiuronium (5g).

Yield: 74%; yellow solid; m.p. 125–127 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 8.96 (s, 3H), 8.72 (d, $J = 5.4$ Hz, 1H), 8.68–8.63 (m, 1H), 8.54 (s, 1H), 8.32 (d, $J = 7.2$ Hz, 2H), 7.76 (d, $J = 5.4$ Hz, 1H), 7.65–7.59 (m, 1H), 7.47 (t, $J = 7.7$ Hz, 1H), 7.36 (d, $J = 7.4$ Hz, 1H), 7.32–7.26 (m, 2H), 4.29 (t, $J = 6.9$ Hz, 2H), 3.11 (t, $J = 7.3$ Hz, 2H), 2.45 (s, 3H), 1.89–1.80 (m, 2H), 1.59–1.53 (m, 2H), 1.40 (dt, $J = 14.7, 7.3$ Hz, 2H), 1.35–1.28 (m, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 170.30, 163.74, 162.19, 157.07, 138.48, 138.16, 137.54, 132.95, 131.64, 129.04, 128.72, 126.13, 125.31, 122.84, 122.31, 121.70, 114.45, 112.88, 111.20, 46.44, 30.49, 29.85, 28.71, 27.86, 26.06, 21.71; HRMS calcd for $\text{C}_{26}\text{H}_{29}\text{N}_5\text{S}$ $[\text{M} + \text{H}]^+$ 444.2216, found: 444.2219.

4.1.4. General procedure for synthesis of 6a~g meridianin analogs.

To the solution of 4a~g in DMF (5 mL) was added 1,6-dibromohexane (5.0 equiv.) respectively, and the mixture was stirred at 50°C for 5 h. Then, the reaction mixture

was removed under vacuum and the residue was poured into ice water and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with brine, dried over magnesium sulfate anhydrous and concentrated to give intermediates **30~36**, and used in the next step without further purification. To a stirring solution of compounds **30~36** in ethanol was added thiocarbamide (2.0 equiv.), and the mixture was stirred at 65°C for 3 h. Then, the solvent was removed under vacuum and the residue was purified by silica gel column chromatography (dichloromethane/methanol 10:1) to give the final target compounds **6a-g**.

2-(6-(6-bromo-3-(2-phenylpyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiuronium m (6a). Yield: 84%; white solid; m.p. $131\text{--}133^{\circ}\text{C}$; ^1H NMR (500 MHz, $\text{DMSO-}d_6$): δ 9.20 (s, 3H), 8.75 (d, $J = 5.4$ Hz, 1H), 8.62 – 8.55 (m, 2H), 8.52 – 8.47 (m, 2H), 7.92 (d, $J = 1.0$ Hz, 1H), 7.78 (d, $J = 5.4$ Hz, 1H), 7.56 (p, $J = 6.0$ Hz, 3H), 7.43 (dd, $J = 8.5, 1.4$ Hz, 1H), 4.27 (t, $J = 7.0$ Hz, 2H), 3.06 (t, $J = 7.2$ Hz, 2H), 1.82 (dt, $J = 14.5, 7.2$ Hz, 2H), 1.57 (dt, $J = 14.7, 7.3$ Hz, 2H), 1.45 – 1.36 (m, 2H), 1.35 – 1.27 (m, 2H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$): δ 169.33, 163.72, 161.83, 157.31, 138.45, 138.37, 133.72, 131.08, 129.16, 128.13, 125.07, 124.63, 124.02, 115.83, 114.62, 114.05, 113.10, 46.55, 30.35, 29.86, 28.85, 27.88, 26.02; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{26}\text{N}_5\text{BrS}$, 510.1145; found, 510.1130.

2-(6-(6-bromo-3-(2-(4-methoxyphenyl)pyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiuronium (6b). Yield: 68%; pale yellow solid; m.p. $54\text{--}56^{\circ}\text{C}$; ^1H NMR (500 MHz, $\text{DMSO-}d_6$): δ 9.32 (s, 3H), 8.74 (d, $J = 4.6$ Hz, 1H), 8.57 (dd, $J = 26.4, 14.5$ Hz, 2H), 8.13 – 7.99 (m, 2H), 7.92 (s, 1H), 7.78 (d, $J = 4.3$ Hz, 1H), 7.53 – 7.38 (m, 2H), 7.12 (d, $J = 7.4$ Hz, 1H), 4.27 (t, 2H), 3.88 (s, 3H), 3.03 (t, 2H), 1.81 (dt, 2H), 1.56 (dt, 2H), 1.40 (m, 2H), 1.30 (m, 2H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$): δ 168.64, 163.46, 161.80, 160.00, 157.26, 139.86, 138.44, 133.73, 130.26, 125.09, 124.56, 123.94, 120.49, 117.08, 115.84, 114.73, 114.08, 113.05, 112.89, 55.58, 46.54, 30.25, 29.86, 28.94, 27.87, 26.02; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{28}\text{N}_5^{81}\text{BrS}$, 540.1250; found, 540.1236.

2-(6-(6-bromo-3-(2-(3-methoxyphenyl)pyrimidin-4-yl)-1H-indol-1-yl)hexyl)is

othiuronium (6c). Yield: 65%; yellow solid; m.p. 120-122 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 9.32 (s, 3H), 8.68 (d, J = 5.3 Hz, 1H), 8.58 (d, J = 8.6 Hz, 1H), 8.54 (s, 1H), 8.44 (d, J = 8.8 Hz, 2H), 7.91 (s, 1H), 7.69 (d, J = 5.3 Hz, 1H), 7.41 (dd, J = 8.5, 1.4 Hz, 1H), 7.11 (d, J = 8.9 Hz, 2H), 4.26 (t, J = 6.9 Hz, 2H), 3.85 (s, 3H), 3.03 (t, J = 7.1 Hz, 2H), 1.80 (dd, J = 14.4, 7.2 Hz, 2H), 1.61 – 1.53 (m, 2H), 1.39 (dd, J = 14.3, 7.2 Hz, 2H), 1.31 (d, J = 6.6 Hz, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 168.51, 163.55, 161.81, 161.68, 157.18, 138.42, 133.58, 130.89, 129.76, 125.08, 124.52, 124.03, 115.78, 114.49, 114.01, 113.89, 113.20, 55.77, 46.52, 30.24, 29.87, 28.95, 27.88, 26.03; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{28}\text{N}_5^{81}\text{BrS}$, 540.1250; found, 540.1234.

2-(6-(6-bromo-3-(2-(4-fluorophenyl)pyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiuronium (6d). Yield: 68%; yellow oily substance; ^1H NMR (500 MHz, DMSO- d_6): δ 9.33 (s, 3H), 8.73 (d, J = 5.4 Hz, 1H), 8.60 – 8.48 (m, 4H), 7.92 (d, J = 1.2 Hz, 1H), 7.77 (d, J = 5.4 Hz, 1H), 7.44 – 7.34 (m, 3H), 4.26 (t, J = 7.0 Hz, 2H), 3.03 (t, J = 7.1 Hz, 2H), 1.80 (dd, J = 14.3, 7.2 Hz, 2H), 1.56 (dd, J = 14.1, 7.2 Hz, 2H), 1.44 – 1.37 (m, 2H), 1.34 – 1.26 (m, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 168.61, 165.28, 163.30, 162.80, 161.86, 157.31, 138.44, 134.87, 133.83, 130.50, 130.43, 125.02, 124.62, 123.97, 116.15, 115.90 (d, J = 19.0 Hz), 114.56, 114.05, 113.02, 46.55, 30.24, 29.85, 28.95, 27.86, 26.02; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{25}\text{N}_5^{81}\text{BrFS}$, 528.1050; found, 528.1035.

2-(6-(6-bromo-3-(2-(3-fluorophenyl)pyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiuronium (6e). Yield: 75%; brown solid; m.p. 124-126 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 9.31 (s, 3H), 8.76 (d, J = 5.4 Hz, 1H), 8.59 (s, 1H), 8.53 (d, J = 8.5 Hz, 1H), 8.34 (d, J = 7.8 Hz, 1H), 8.17 (d, J = 10.4 Hz, 1H), 7.92 (d, J = 1.3 Hz, 1H), 7.81 (d, J = 5.4 Hz, 1H), 7.63 (dd, J = 14.0, 7.9 Hz, 1H), 7.45 – 7.35 (m, 2H), 4.27 (t, J = 7.1 Hz, 2H), 3.05 (t, J = 7.2 Hz, 2H), 1.81 (dd, J = 14.5, 7.3 Hz, 2H), 1.57 (dt, J = 14.5, 7.4 Hz, 2H), 1.40 (dt, J = 14.5, 7.3 Hz, 2H), 1.35 – 1.27 (m, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 168.99, 163.96, 162.50 (d, J = 3.0 Hz), 161.97 (d, J = 13.8 Hz), 157.38, 140.97 (d, J = 7.7 Hz), 138.46, 133.96, 131.26 (d, J = 8.1 Hz), 124.99, 124.69, 124.16, 123.83, 117.98, 117.81, 115.86, 115.12, 114.56, 114.38, 114.10, 112.92, 46.56,

30.29, 29.85, 28.90, 27.87, 26.02; HRMS calcd for $C_{25}H_{25}N_5BrFS$ $[M + H]^+$ 528.1050, found: 528.1056.

2-(6-(6-bromo-3-(2-(p-tolyl)pyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiouronium (6f). Yield: 69%; yellow solid; m.p. 130-131 °C; 1H NMR (500 MHz, DMSO- d_6): δ 9.33 (s, 3H), 8.71 (d, J = 5.3 Hz, 1H), 8.61 – 8.53 (m, 2H), 8.38 (d, J = 8.1 Hz, 2H), 7.91 (s, 1H), 7.74 (d, J = 5.3 Hz, 1H), 7.39 (dd, J = 25.4, 8.2 Hz, 3H), 4.26 (t, J = 6.8 Hz, 2H), 3.04 (t, J = 7.1 Hz, 2H), 2.40 (s, 3H), 1.86 – 1.75 (m, 2H), 1.55 (dd, J = 14.1, 7.1 Hz, 2H), 1.39 (dd, J = 14.2, 7.2 Hz, 2H), 1.31 (d, J = 6.7 Hz, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 168.93, 163.77, 161.74, 157.24, 140.80, 138.42, 135.73, 133.63, 129.76, 128.11, 125.08, 124.56, 124.02, 115.80, 114.36, 114.02, 113.15, 46.53, 30.26, 29.86, 28.91, 27.87, 26.02, 21.50; HRMS calcd for $C_{26}H_{29}N_5^{81}BrS$ $[M + H]^+$ 524.1301, found: 524.1306.

2-(6-(6-bromo-3-(2-(m-tolyl)pyrimidin-4-yl)-1H-indol-1-yl)hexyl)isothiouronium (6g). Yield: 80%; brown solid; m.p. 125-127 °C; 1H NMR (500 MHz, DMSO- d_6): δ 9.19 (s, 3H), 8.73 (d, J = 5.4 Hz, 1H), 8.61 – 8.53 (m, 2H), 8.29 (d, J = 7.6 Hz, 2H), 7.92 (d, J = 1.3 Hz, 1H), 7.76 (d, J = 5.4 Hz, 1H), 7.49 – 7.41 (m, 2H), 7.36 (d, J = 7.6 Hz, 1H), 4.27 (t, J = 7.1 Hz, 2H), 3.08 (t, J = 7.3 Hz, 2H), 2.44 (s, 3H), 1.82 (dt, J = 14.6, 7.3 Hz, 2H), 1.57 (dt, J = 14.7, 7.4 Hz, 2H), 1.40 (dt, J = 14.6, 7.3 Hz, 2H), 1.30 (dt, J = 14.8, 7.4 Hz, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 169.66, 163.80, 161.76, 157.26, 138.43, 138.35, 138.21, 133.65, 131.70, 129.07, 128.73, 125.34, 125.07, 124.60, 123.99, 115.82, 114.56, 114.04, 113.13, 46.54, 30.38, 29.86, 28.82, 27.87, 26.02, 21.69; HRMS calcd for $C_{26}H_{29}N_5^{81}BrS$ $[M + H]^+$ 524.1301, found: 524.1310.

4.1.5. General procedure for synthesis of **6e-1**.

To a solution of **4e** in DMF (5 mL) was added 1-bromohexane (5.0 equiv.), and the mixture was stirred at 50°C for 5 h. Then, the reaction mixture was removed under vacuum and the residue was poured into ice water and extracted with ethyl acetate (3 \times 50 mL). The combined organic layers were washed with brine, dried over magnesium sulfate anhydrous. After filtration, the solvent was removed under vacuum

and the residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate 5:1) to give the final target compound **6e-1**.

2-(6-(6-bromo-3-(2-(3-fluorophenyl)pyrimidin-4-yl)-1*H*-indol-1-yl)hexyl)isothiuronium bromide (6e-1). Yield: 63%; pale yellow solid; m.p. 85-87°C; ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.78 (d, *J* = 5.4 Hz, 1H), 8.60 (s, 1H), 8.54 (d, *J* = 8.6 Hz, 1H), 8.36 (d, *J* = 7.9 Hz, 1H), 8.19 (ddd, *J* = 10.6, 2.5, 1.5 Hz, 1H), 7.94 (d, *J* = 1.6 Hz, 1H), 7.82 (d, *J* = 5.5 Hz, 1H), 7.68–7.60 (m, 1H), 7.42 (ddd, *J* = 10.6, 8.3, 1.9 Hz, 2H), 4.29 (t, *J* = 7.1 Hz, 2H), 1.26 (dd, *J* = 16.6, 10.1 Hz, 8H), 0.84 (d, *J* = 7.0 Hz, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 161.96, 157.41, 141.01, 138.52, 133.99, 131.30, 124.99, 124.70, 124.19, 123.83, 118.04, 117.82, 115.87, 115.11, 114.50, 114.16, 112.91, 46.65, 31.23, 30.03, 26.24, 22.47, 14.33; HRMS: *m/z* [M + H]⁺ calcd for C₂₄H₂₄N₃BrF, 452.1132; found, 452.1145.

4.1.6. General procedure for synthesis of **6e-2~6**.

To a solution of **4e** in DMF (5 mL) was added 1,2-dibromoethane, 1,3-dibromopropane, 1,4-dibromobutane, 1,5-dibromopentane or 1,7-dibromoheptane (5.0 equiv.) respectively, and the mixture was stirred at 50°C for 5 h. Then, the reaction mixture was removed under vacuum and the residue was poured into ice water and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried over magnesium sulfate anhydrous and concentrated to give intermediates **37~41**, and used in the next step without further purification. To a stirring solution of compounds **37~41** in ethanol was added thiocarbamide (2.0 equiv.), and the mixture was stirred at 65°C for 3 h. Then, the solvent was removed under vacuum and the residue was purified by silica gel column chromatography (dichloromethane/methanol 10:1) to give the final target compounds **6e-2~6**.

2-(2-(6-bromo-3-(2-(3-fluorophenyl)pyrimidin-4-yl)-1*H*-indol-1-yl)ethyl)isothiuronium bromide (6e-2). Yield: 60%; pale yellow solid; m.p. 194-194 °C; ¹H NMR (400 MHz, DMSO-*d*₆): δ 9.15 (s, 2H), 9.01 (d, *J* = 25.1 Hz, 2H), 8.83 (d, *J* = 5.4 Hz, 1H), 8.62 (s, 1H), 8.54 (d, *J* = 8.6 Hz, 1H), 8.36 (d, *J* = 7.9 Hz, 1H), 8.22–8.17 (m, 1H), 8.02 (d, *J* = 1.6 Hz, 1H), 7.81 (d, *J* = 5.4 Hz, 1H), 7.65 (td, *J* = 8.0,

6.1 Hz, 1H), 7.49 (dd, $J = 8.6, 1.7$ Hz, 1H), 7.43 (td, $J = 8.3, 2.2$ Hz, 1H), 4.64 (t, $J = 6.4$ Hz, 2H), 3.79–3.67 (m, 2H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 169.30, 162.59, 161.71, 157.67, 140.89, 138.43, 134.21, 131.42, 131.33, 125.04, 124.22, 123.77, 118.15, 117.94, 116.20, 115.24, 114.62, 114.37, 113.46, 45.41, 30.84; HRMS: m/z [$\text{M} + \text{H}$] $^{+}$ calcd for $\text{C}_{21}\text{H}_{18}\text{N}_5^{81}\text{BrFS}$, 472.0424; found, 472.0427.

2-(3-(6-bromo-3-(2-(3-fluorophenyl)pyrimidin-4-yl)-1H-indol-1-yl)propyl)isothiuronium bromide (6e-3). Yield: 45%; pale yellow solid; m.p. 175–177 °C; ^1H NMR (400 MHz, DMSO- d_6): δ 9.12 (s, 2H), 8.98 (s, 2H), 8.80 (d, $J = 5.4$ Hz, 1H), 8.61 (s, 1H), 8.56 (d, $J = 8.6$ Hz, 1H), 8.36 (d, $J = 7.9$ Hz, 1H), 8.22–8.16 (m, 1H), 7.97 (d, $J = 1.6$ Hz, 1H), 7.83 (d, $J = 5.5$ Hz, 1H), 7.65 (td, $J = 8.0, 6.1$ Hz, 1H), 7.47 (dd, $J = 8.6, 1.7$ Hz, 1H), 7.42 (td, $J = 8.3, 2.1$ Hz, 1H), 4.41 (t, $J = 6.9$ Hz, 2H), 3.24 – 3.16 (m, 2H), 2.27–2.14 (m, 2H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 170.02, 164.18, 162.52, 161.86, 157.51, 140.98, 140.83, 138.49, 133.91, 131.39, 124.99, 124.22, 123.91, 117.90, 116.06, 115.24, 114.06, 113.32, 45.32, 29.73, 27.85; HRMS: m/z [$\text{M} + \text{H}$] $^{+}$ calcd for $\text{C}_{22}\text{H}_{20}\text{N}_5^{81}\text{BrFS}$, 486.0581; found, 486.0583.

2-(4-(6-bromo-3-(2-(3-fluorophenyl)pyrimidin-4-yl)-1H-indol-1-yl)butyl)isothiuronium (6e-4). Yield: 64%; pale yellow solid; m.p. 171–173 °C; ^1H NMR (400 MHz, DMSO- d_6): δ 9.05 (s, 2H), 8.95 (s, 2H), 8.79 (d, $J = 5.5$ Hz, 1H), 8.64 (s, 1H), 8.55 (d, $J = 8.6$ Hz, 1H), 8.36 (d, $J = 7.9$ Hz, 1H), 8.19 (ddd, $J = 10.5, 2.5, 1.4$ Hz, 1H), 7.99 (d, $J = 1.6$ Hz, 1H), 7.83 (d, $J = 5.5$ Hz, 1H), 7.65 (td, $J = 8.0, 6.1$ Hz, 1H), 7.49–7.44 (m, 1H), 7.41 (dd, $J = 8.5, 2.2$ Hz, 1H), 4.40–4.30 (m, 2H), 3.23 (dd, $J = 15.2, 8.0$ Hz, 2H), 1.99–1.89 (m, 2H), 1.64 (dt, $J = 14.9, 7.6$ Hz, 2H); ^{13}C NMR (100 MHz, DMSO): δ 170.17, 162.52, 161.92, 161.80, 157.42, 140.93, 138.48, 134.04, 131.38, 124.93, 124.23, 123.88, 118.06, 117.89, 116.00, 115.14, 114.59, 114.19, 113.05, 46.05, 30.06, 28.88, 26.37; HRMS: m/z [$\text{M} + \text{H}$] $^{+}$ calcd for $\text{C}_{23}\text{H}_{22}\text{N}_5^{81}\text{BrFS}$, 500.0737; found, 500.0740.

2-(5-(6-bromo-3-(2-(3-fluorophenyl)pyrimidin-4-yl)-1H-indol-1-yl)pentyl)isothiuronium (6e-5). Yield: 67%; pale yellow solid; m.p. 155–157 °C; ^1H NMR (400 MHz, DMSO- d_6): δ 9.02 (s, 2H), 8.92 (s, 2H), 8.78 (t, $J = 7.4$ Hz, 1H), 8.64 (s, 1H), 8.55 (d, $J = 8.6$ Hz, 1H), 8.36 (d, $J = 7.9$ Hz, 1H), 8.19 (ddd, $J = 10.5, 2.5, 1.5$ Hz,

1H), 7.96 (d, $J = 1.6$ Hz, 1H), 7.83 (d, $J = 5.5$ Hz, 1H), 7.65 (td, $J = 8.0, 6.1$ Hz, 1H), 7.45 (dd, $J = 8.6, 1.7$ Hz, 1H), 7.41 (dd, $J = 8.3, 2.2$ Hz, 1H), 4.37–4.28 (m, 2H), 3.15–3.11 (m, 3H), 1.87 (dd, $J = 14.8, 7.3$ Hz, 2H), 1.65 (dd, $J = 14.5, 7.4$ Hz, 3H), 1.43–1.37 (m, 2H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 170.30, 162.53, 161.96, 157.41, 140.96, 138.52, 134.06, 131.37, 125.01, 124.77, 124.22, 123.85, 117.87, 115.93, 115.13, 114.41, 114.40, 114.13, 112.96, 46.48, 30.32, 29.47, 28.52, 25.56; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{24}\text{N}_5^{81}\text{BrFS}$, 514.0894; found, 514.0898.

2-(7-(6-bromo-3-(2-(3-fluorophenyl)pyrimidin-4-yl)-1H-indol-1-yl)heptyl)isothiuronium (6e-6). Yield: 56%; pale yellow solid; m.p. 121–123 °C; ^1H NMR (400 MHz, DMSO- d_6): δ 9.00 (s, 2H), 8.90 (s, 2H), 8.79 (d, $J = 5.4$ Hz, 1H), 8.62 (s, 1H), 8.55 (d, $J = 8.6$ Hz, 1H), 8.36 (d, $J = 7.9$ Hz, 1H), 8.22–8.16 (m, 1H), 7.95 (d, $J = 1.6$ Hz, 1H), 7.83 (d, $J = 5.5$ Hz, 1H), 7.65 (td, $J = 8.0, 6.1$ Hz, 1H), 7.46 (dd, $J = 8.6, 1.7$ Hz, 1H), 7.41 (dd, $J = 8.3, 2.2$ Hz, 1H), 4.31 (t, $J = 7.0$ Hz, 2H), 3.11 (t, $J = 7.3$ Hz, 2H), 1.88–1.79 (m, 2H), 1.57 (d, $J = 6.7$ Hz, 2H), 1.32 (d, $J = 11.1$ Hz, 6H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 170.27, 161.96, 157.45, 141.50, 140.90, 138.52, 134.05, 131.41, 125.24, 124.87, 124.22, 123.86, 118.15, 117.84, 115.90, 115.12, 114.63, 114.19, 112.91, 46.61, 30.47, 30.00, 28.70, 28.38, 28.16, 26.42; HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{28}\text{N}_5^{81}\text{BrFS}$, 542.1207; found, 542.1206.

4.2. Biological evaluation

Antibodies against p-Tyr1022/1023-JAK1, p-Tyr1007/1008-JAK2, p-Tyr980/981-JAK3, p-Tyr1054/1055-TYK2, pTyr705-STAT3, STAT3, c-Myc, CyclinD1 and Bcl-XL were obtained from Cell Signaling Technology (Beverly, USA), and antibodies against α -tubulin and GAPDH were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). Transfection Reagent, protease inhibitor and phosphatase inhibitor were purchased from Millipore (Billerica, USA). Polyvinylidene difluoride (PVDF) membranes and chemiluminescent horseradish peroxidase (HRP) substrate were purchased from Millipore (Billerica, USA). Gefitinib was acquired from Selleckchem (Huston, USA).

In vitro inhibitory activity. The resazurin indicator was used to evaluate cell

viability. HeLa, MDA-MB-231, A549 and DU145 cells were seeded in 96-well plates in 50 μ L at plating densities ranging from 4000 to 8000 cells/well depending on the doubling time of individual cell lines. After incubation for 24 h, different concentrations of compounds were added, and then the cells were further cultured for 72 h, with 0.5% DMSO as the solvent control group and 10 μ L of resazurin solution (1 mg/mL) was directly added to each well as a redox indicator. Plates were incubated for 3 h to measure the absorbance of a SpectraMax@i3 (Molecular Devices, Madison, USA) of each well at a 595 nm emission wavelength (549 nm excitation wavelength). Each treatment was performed in triplicate to reduce the experimental error. Results were analyzed with GraphPad Prism 6 and data were shown as Mean \pm SD.

Molecular Docking. All calculations were performed using Molecular Docking Program of MOE (version MOE 2020.09). The crystal structures of the proteins involved in this article were retrieved from the Protein Data Bank (PDB). Firstly, all compounds were treated through energy minimization. The parameters and charges were assigned with the MMFF94x force field. Secondly, after removing water molecules, each selected protein structures, was treated by adding hydrogen-atoms. Finally, the small molecules were docked into the pockets of the proteins defined by the originally bound ligands in the crystal structures, respectively. The poses are ranked by the scores from the GBVI/WSA binding free energy calculation, and the results were analyzed using Pymol (<https://pymol.org/2/>).

Flow cytometry analysis of apoptotic cells. An Annexin V-FITC/PI apoptosis kit (Invitrogen) was used to detect cell apoptosis. A549 and DU145 cells were cells at a density of 5×10^5 per well were cultured in regular growth medium in 6-well plates for 24 h and disposed induplicate with various concentrations of compound **6e** for 24 h. After 48 h later, the A549 and DU145 were trypsinized, centrifuged and washed with precooled PBS twice with an Annexin V-FITC/PI apoptosis kit (Invitrogen) following the manufacturer's instructions.

Western blot analysis. A549 and DU145 cells were plated in 6-well plates and cultured overnight respectively, and different concentrations of compound **6e** were added for 2 h. The corresponding cells were collected, washed with PBS and lysed

with cell lysis buffer to extract the total proteins. The extracted protein was loaded, subjected to SDS-PAGE electrophoresis, and then the protein was transferred to a PDVF membrane and incubated in the corresponding Primary antibody overnight. The next day, the Primary antibody was recovered and labeled and the corresponding Secondary antibody was incubated. The immune complexes were detected using chemiluminescence HRP substrate (Millipore) and visualized by the Tanon 5200 Chemiluminescence Imaging System (Tanon Science and Technology Co., Ltd.).

In vivo studies. Six-week-old male nude mice (SPF degree, 17-20 g weight, nu/nu) were obtained from Beijing Vital River Laboratory Animal Technology Co. Ltd (Beijing, China). Nude mice were injected into the back with DU145 tumor cells (about 15×10^6). After 2 weeks, the mice were randomly divided into four groups: blank control group (NC, DMSO), positive control Gefitinib group (PC, 100 mg/kg), compound **6e** group (5 mg/kg) and compound **6e** group (10 mg/kg), with 6 mice per group. The compound **6e** groups and the PC group were intraperitoneally injected or intragastric administered every two days until the mice were sacrificed. The body weight of nude mice was recorded every three days, and the tumor weight was recorded on the day of death of nude mice. All of the procedures were approved by the Committee of Experimental Animals of the Ocean University of China and conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No 85-23, revised 1996).

Immunohistochemistry (IHC) analyses. The mouse tumor tissues were collected, fixed in 4% PFA for 72 h at 4 °C, embedded in paraffin, and cut into sections. The sections were deparaffinized in xylene, rehydrated in graded ethanol, boiled in antigen retrieval solution [31]. Then incubated with fresh 3% H₂O₂ to inactivate endogenous peroxidase. After PBS washing, the slides were blocked with fatty free milk and incubated with primary antibody at 4 °C overnight, followed by incubation with the HRP-conjugated secondary antibody at room temperature (Boster, Wuhan, China) according to the manufacturer's instructions. Finally, DAB colour developing solution was added dropwise. A brown colour in the cell membrane indicated positive staining. Images were captured using an upright fluorescence microscope (Olympus BX53,

Japan).

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Conflicts of interest

The authors declare no other conflicts of interest.

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