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Discovery of 2,4-diarylaminopyrimidine derivatives bearing dithiocarbamate moiety as novel FAK inhibitors with antitumor and anti-angiogenesis activities

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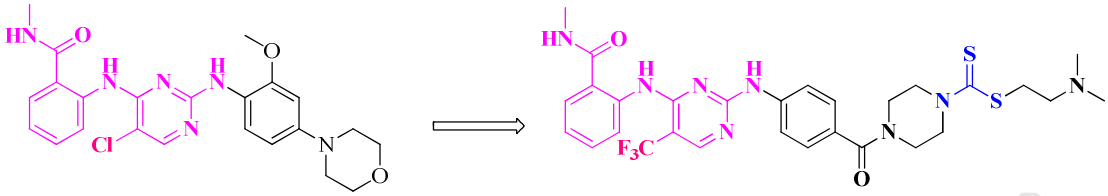
Graphical abstract



FAK activity: IC_{50} = 6.21 nM
4 Cell lines growth: IC_{50} > 450 nM

TAE226

FAK activity: IC_{50} = 0.07 nM
4 Cell lines growth: IC_{50} = 1~60 nM

14z

Discovery of 2,4-diarylaminopyrimidine derivatives bearing dithiocarbamate moiety as novel FAK inhibitors with antitumor and anti-angiogenesis activities

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Abstract

A series of 2,4-diarylaminopyrimidine derivatives containing dithiocarbamate moiety were designed by molecular hybridization strategy and synthesized for screening as inhibitors of focal adhesion kinase (FAK). Most of these compounds exhibit significant antiproliferative activities on human cancer cell lines expressing high levels of FAK at nanomolar concentrations. The compound **14z** was identified as the most potent FAK inhibitor among these candidates. **14z** has excellent anti-proliferative effect with IC_{50} values from 0.001 μM to 0.06 μM on HCT116, PC-3, U87-MG and MCF-7 cell lines and relatively less cytotoxicity to a nonmalignant cell line MCF-10A compared with MCF-7 cells (SI value > 10). **14z** also exhibits significant FAK inhibitory activity (IC_{50} = 0.07 nM). In addition, compound **14z** causes cell cycle arrest at G2/M and prompted apoptosis in both HCT116 and MCF-7 cells in a dose-dependent manner. Further studies show that compound **14z** inhibits migration of MCF-7 and has anti-angiogenesis effect on HUVEC cells.

Keywords:
FAK inhibitors; 2,4-Diarylaminopyrimidine; Dithiocarbamate; Anti-tumor activity; Anti-angiogenesis

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1. Introduction

Cancer is still one of the major causes of the death worldwide. However the traditional chemotherapy is facing the obstacles that lacking of selectivity towards tumor cells and causing drug resistance [1]. Thus a lot of efforts have been put into designing small molecular compounds with specific targets to enhance the selectivity and efficacy in recent years [2-4].

Focal adhesion kinase (FAK) is one of these targets. FAK is a non-receptor tyrosine kinase and plays a vital role in regulating cancer cell survival, proliferation, migration, and angiogenesis. It is overexpressed in a number of human cancers, including gliomas, breast, colon, prostate carcinomas and others [5-7]. Inhibition of FAK has exhibited efficacy in limiting tumor progression and metastasis. Therefore this kinase is emerging as a promising cancer therapeutic target.

In recent years, several FAK inhibitors have been developed and some of them have entered clinical trials (Fig.1) [8]. PF-573,228 is one of the first FAK inhibitors and was highly specific for FAK (IC_{50} = 4 nM) compared with other related enzymes. However it only inhibits cell migration rather than inhibits cell proliferation or induces apoptosis in tumor cells [9]. Another FAK inhibitor TAE226 (IC_{50} = 5.5 nM) exhibits antitumor activity *in vitro* and *in vivo* against several types of malignancies including glioma [10], breast cancer [11], ovarian cancer [12] and gastrointestinal stroma tumor [13]. But it has never entered clinical trials for its severe side effect in affecting glucose metabolism and glucose blood levels [14]. CEP-37440 and PF-562,271 have currently completed the phase □ study. CEP-37440 (IC_{50} = 2 nM)

displayed favorable *in vitro* ADME properties [15]. PF-562, 271 （IC_{50} = 1.5 nM）not only inhibits the proliferation of cancer cells but also exerts a novel mechanism in altering the tumor microenvironment [16]. In the phase I study of PF-562,271, although several patients have experienced prolonged disease stabilization of 6 months or longer, no objective response has been reported. Defactinib (VS-6063) is a second-generation inhibitor of FAK (IC_{50} = 0.6 nM). In the phase I dose-escalation study in patients with advanced solid malignancies, some adverse events have been reported, including nausea, diarrhea, hyperbilirubinemia and so on. Defactinib prevents tumor invasion and dissemination rather than reducing tumor volume [17]. Recently, the phase II clinical trials for Defactinib have been completed in patients with KRAS mutant non-small cell lung cancer. In summary of these currently available FAK inhibitors, their antitumor activity is not enough when used alone and needed to be combined with other therapies. Up till now, there is no FAK inhibitor coming into the market. So there is an urgent need to develop novel FAK inhibitors with higher antitumor activity to exert more potent antitumor effect in patients. Among the potent FAK inhibitors aforementioned, a panel of compounds possesses 2,4-diarylaminopyrimidine scaffold as a pivotal pharmacophore. Generally, the 2,4-diarylaminopyrimidine core may functionally interact with FAK at Cys502, Asp564 or other sites within the ATP binding pocket [8]. The researches of other FAK inhibitors with 1,2,4-triazine or imidazo[1,2‑a][1,3,5]triazinesas scaffold are underway [18, 19].

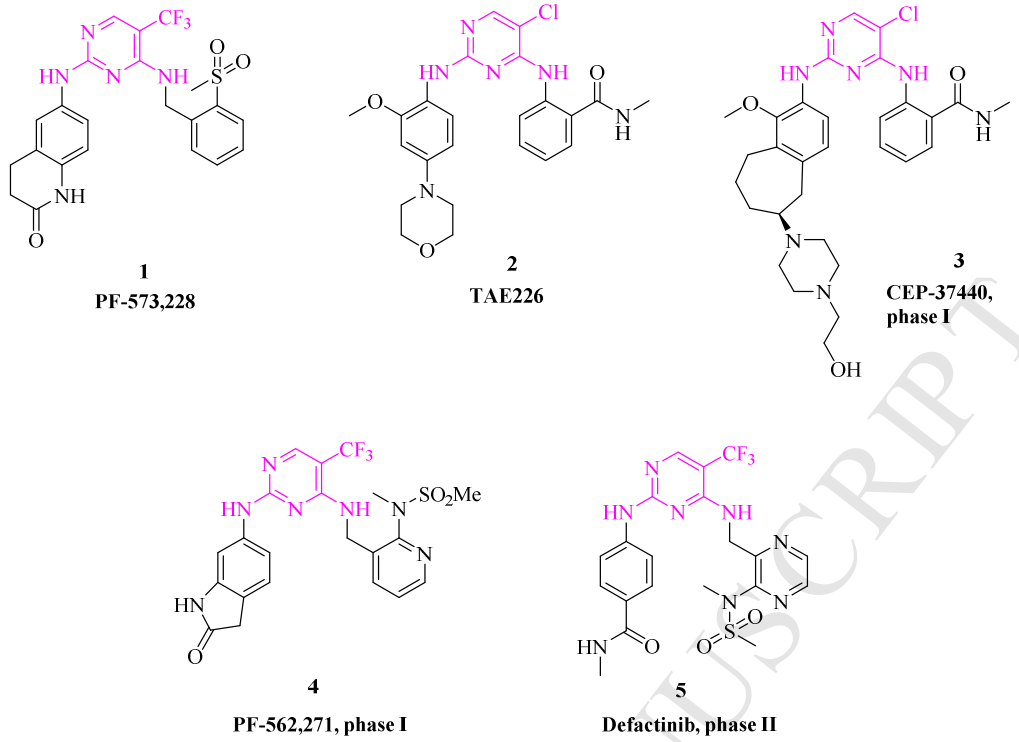


Fig.1. Structures of several known FAK inhibitors.

Molecular hybridization strategy is a common concept to design new drugs based on the combination of different bioactive moieties to produce a new hybrid with improved affinity and efficacy [20]. Recently, dithiocarbamates have received considerable attention due to their potent anticancer activities [21-26]. In this reasearch, molecular hybridization technique based on the incorporation of 2,4-diarylaminopyrimidine and dithiocarbamate into one molecular platform has the potential to improve the anti-FAK and antiproliferative efficacy.

Herein, a series of C-2 dithiocarbamate-substituted 2,4-diarylaminopyrimidine derivatives (Fig. 2) were synthesized to improve inhibitory potency toward FAK kinase and antiproliferative activity of cancer cells. To the best of our knowledge, there have been no reports regarding 2,4-diarylaminopyrimidine-dithiocarbamate

hybrids so far. These novel compounds showed significant inhibitory potency against FAK enzyme and antitumor activities in HCT116, PC-3, U87-MG, and MCF-7 cell lines. SAR of these compounds was discussed in this manuscript. The most effective compound **14z** was also evaluated for its effects on cell cycle arrest, apoptosis, cell migration and angiogenesis.

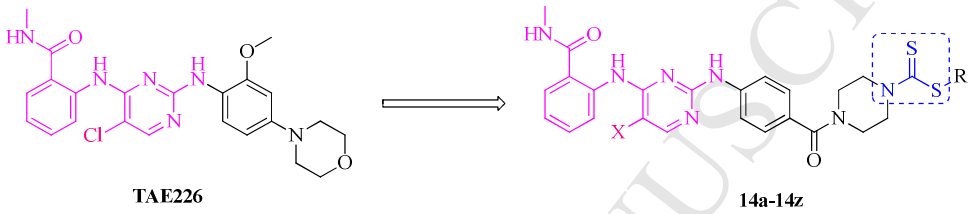
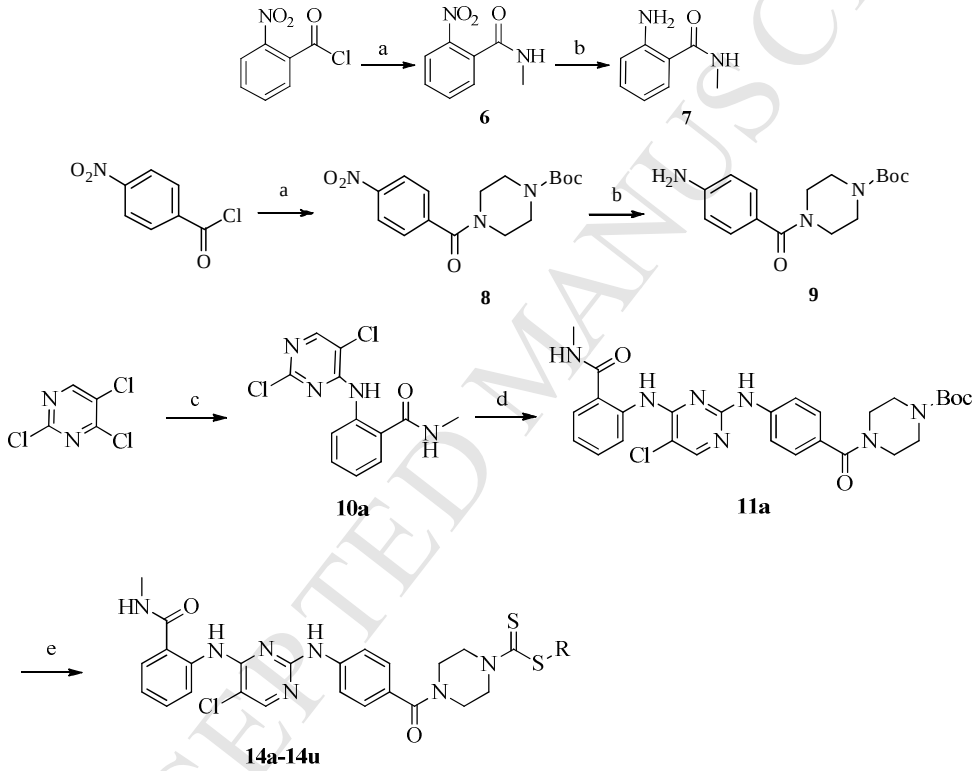


Fig.2. Designed strategy of the dithiocarbamate-substituted 2,4-diarylaminopyrimidine derivatives

2. Results and discussion

The synthesis of dithiocarbamate-substituted diphenylaminopyrimidine derivatives (**14a-14u**) was depicted in Scheme 1**.** 2-nitrobenzoyl chloride was reacted with methanamine in the presence of sodium bicarbonate to produce 2-nitro-(N-methyl)benzamide (**6**). 2-amino-N-methylbenzamide (**7**) was obtained via the reduction of compound **6** by using the Fe-NH_4Cl reduction reagent. Compound **8** was produced by the same way as compound **6** started from 4-nitrobenzoyl chloride. Compound **9** was produced by the same way as compound **7.** The C-4 chlorine atom of 2,4,5-trichloropyrimidine was first region selectively replaced by compound **7** to obtain **10a**. Then compound **9** was coupled with the C-2 of 2,5-dichloropyrimidine from compound **10a** and produced intermediate **11a**. The compound **11a** was stirred in the solution of trifluoroacetate and dichloromethane (1:1) to get rid of Boc group.

Then the product was concentrated and reacted with carbon disulfide in DMF with triethylamine as base as previously reported [27].Without further purification, a variety of halogen or alkene substituted side chains that were commercially available or prepared by literature [28] were reacted with the product mentioned above respectively, followed by chromatographic purification to generate the target compounds **14a-14u**.



Scheme 1. Synthesis of dithiocarbamate-substituted diphenylaminopyrimidine derivatives (**14a-14u**). Reagents and conditions:(a) Methanamine hydrochloride or tert-butyl piperazine-1-carboxylate, $NaHCO_3$, acetonitrile, reflux, 4 h; (b) Fe-NH_4Cl, EtOH-H_2O, reflux, 2 h; (c) Compound **7**; DIPEA, i-PrOH, reflux 6 h (d) Compound **9**, $Pd(OAc)_2$, Xantphos, K_3PO_4, DMF, Ar, reflux, 22 h; (e) i: TFA, DCM, rt., 2 h; ii: CS_2, Et_3N, DMF, rt., 1 h; iii: RBr/RCl/Methyl acrylate, DMF, 50 □, overnight.

In order to test our design rationale, we took **14a** which bears a propanenitrile moiety at R as our new lead to test the inhibitory activity against FAK. The result revealed that **14a** is a potent FAK inhibitor (inhibition rate = 50% at 1μM). Then

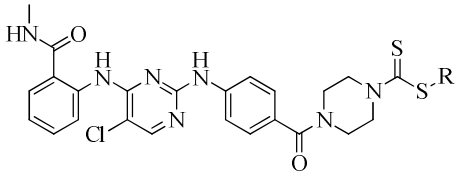
according to bioisosterism principles, the cyano group in **14a** was replaced with alkenyl, methoxy, ester, methanesulfonyl, hydroxyl, amines and five or six-membered aliphatic heterocycles, respectively (**14b-14p**). As a comparison, in order to investigate the influence of aromatic group on the inhibitory activity, we used benzyl group (**14q**), phenylethyl group (**14r**) and pyridinemethyl (**14s-14u**) to replace propanenitrile moiety. Compounds **14a-14u** were all evaluated as FAK inhibitors by ADP-Glo kinase assay [29] at the concentration of 1 μM. TAE226 and Defactinib were used as positive control. As shown in Table 1, most of the novel derivatives exhibited potent anti-FAK activity at 1 μM, especially compounds **14i** and **14j.** The replacement of cyano group with ester (**14d-14f**), hydroxyl (**14h, 14i**), amine (**14j, 14k, 14n**) or aliphatic heterocycle containing oxygen atom (**14o, 14p**) can increase the inhibitory activity against FAK. However, when the propanenitrile moiety of **14a** was replaced by phenylethyl group (**14r**) or pyridinemethyl (**14s-14u**), the inhibitory activity decreased.

To test the anticancer activity of synthesized compounds, we evaluated antiproliferative activities of compounds **14a-14u** against four FAK over-expressed human tumor cell lines derived from human colon cancer (HCT116), prostate cancer (PC-3), glioma (U87-MG), and breast cancer (MCF-7) by MTS assay. TAE226 and Defactinib were also used as positive control. As shown in Table 1, most compounds exhibited potent anti-cancer activities against selected cancer cell lines with IC_{50} less than 1μM. Among all of the compounds **14c**, **14h-14m** exhibited more significant efficacy against all of four cancer cell lines. Among these cancer cell lines, HCT116

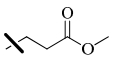
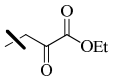
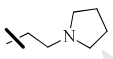
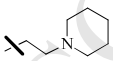
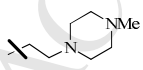
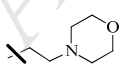
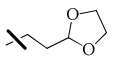
cell line was the most sensitive to these compounds with most IC_{50} values in a nanomolar range. Compared with TAE226 and Defactinib, **14a** significantly increased the activity against HCT116 cells (IC_{50} = 0.03 μM) and PC-3 cells (IC_{50} = 0.17 μM). Replacing the propanenitrile moiety of **14a** with allyl (**14b**), or ethyl acetate (**14e**) resulted in decrease in potency against HCT116, PC-3 and U87-MG cell lines. However, the replacements of propanenitrile by ethyl pyruvate (**14f**, IC_{50} = 0.52 μM), 2-(1,3-dioxolan-2-yl)ethyl (**14o**, IC_{50} = 0.21 μM) or (tetrahydro-2H-pyran-4-yl)methyl (**14p**, IC_{50} = 0.54 μM) significantly enhanced the inhibitory against MCF-7 cells. Replacements of cyano group of **14a** by methoxy (**14c**), methanesulfonyl (**14g**), hydroxyl (**14h, 14i**) or amine (**14j-14m**) resulted in a significant improvement of activity against U87-MG and MCF-7 cells and maintaining the inhibitory against HCT116 and PC3 cells except **14j** which increased the inhibitory against HCT116 and PC3 greatly. However, when the cyano group of **14a** was replaced by morpholine (**14n**), the potency against HCT116 cells decreased approximately 117-fold. Introducing the benzyl group (**14q**) or phenylethyl group (**14r**) at the R position led to significant or slight reduction of activities against all of the tested cancer cell lines. However, when pyridinemethyl (**14s-14u**) was introduced at the R position, the corresponding compounds (**14s-14u**) displayed 9~18-fold increases in inhibitory potency against MCF-7 cells compared with compound **14a**. The position of nitrogen atom in the pyridine ring has little effect on activity.

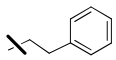
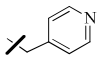
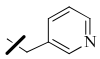
Table 1.

FAK inhibitory activities and antiproliferative activities of target compounds **14a-14u** against four human tumor cell lines.



14a-14u

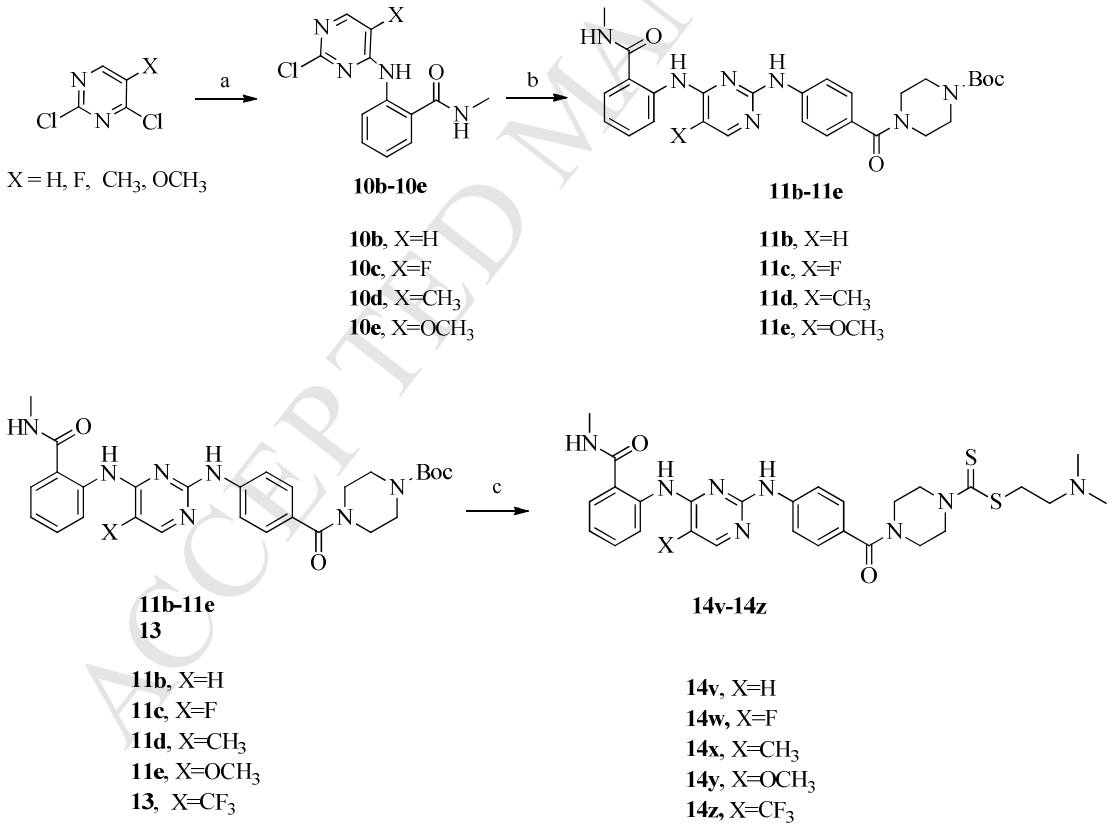
Compd.	R	FAK inhibition% (1 μM)	Antiproliferative activity (IC_{50} / μM)			
			HCT116	PC-3	U87-MG	MCF-7
14a		50	0.03±0.01	0.17±0.03	1.73±0.59	7.17±3.33
14b		45	0.11±0.01	1.02±0.47	>20	1.38±0.73
14c		69	0.04±0.01	0.46±0.02	0.61±0.09	0.30±0.08
14d		73	0.26±0.05	1.99±0.26	1.31±0.07	2.29±0.68
14e		55	4.69±0.81	3.53±0.91	7.54±0.22	9.93±0.10
14f		57	0.06±0.02	0.47±0.03	1.16±0.04	0.52±0.05
14g		20	0.03±0.01	0.52±0.14	0.92±0.43	0.40±0.07
14h		53	0.02±0.01	0.24±0.02	0.71±0.21	0.11±0.09
14i		71	0.03±0.01	0.20±0.04	0.87±0.41	0.11±0.10
14j		73	0.004±0.00	0.09±0.01	0.20±0.03	0.08±0.02
14k		55	0.01±0.00	0.22±0.06	0.20±0.02	0.16±0.01
14l		44	0.01±0.00	0.38±0.02	0.28±0.05	0.27±0.04
14m		50	0.01±0.00	0.26±0.01	0.62±0.28	0.22±0.02
14n		62	3.52±0.30	0.23±0.03	0.47±0.13	0.33±0.05
14o		63	0.05±0.00	0.69±0.37	2.74±1.13	0.21±0.01
14p		62	0.09±0.04	0.31±0.06	>20	0.54±0.17
14q		52	0.05±0.02	17.00±5.15	3.36±1.91	>20

14r		39	3.78±1.22	>20	>20	11.69±4.43
14s		0	0.06±0.03	0.36±0.15	1.70±0.42	0.77±0.22
14t		41	0.06±0.05	0.40±0.03	2.53±0.12	0.39±0.18
14u		23	0.03±0.02	0.45±0.05	1.53±0.60	0.39±0.18
TAE226	----	65	0.46±0.02	1.21±0.12	1.58±1.40	0.45±0.05
Defactinib	----	89	2.65±0.63	>20	10.27±1.04	8.97±1.47

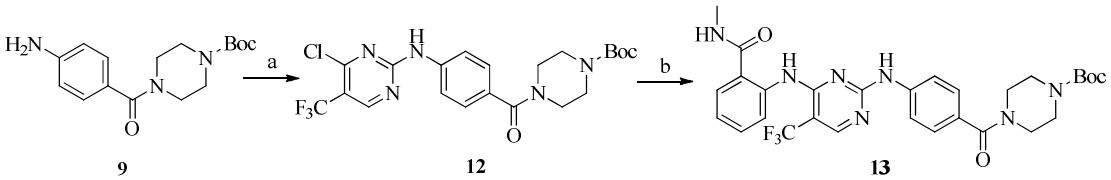
In view of the above results, we found that different structural units at R have great impact on enzymatic and cellular inhibitory activity. Compound **14j** exhibits the highest inhibitory activities against FAK and four tested tumor cell lines among all of target compounds modified at R. Therefore, compound **14j** was chosen as a new starting point for further optimization.

In order to evaluate the influence of different substituents on 5-position of the pyrimidine ring, we kept *N*,*N*-dimethylaminoethyl as R and modified the 5-position substitution of pyrimidine. Thus, analogs **14v-14z** with chlorine replaced by different substitutions at X were synthesized. The synthetic routes were depicted in Scheme 2 and Scheme 3. When the substitute at the 5-position of pyrimidine was H, F, CH_3, OCH_3, the C-4 chlorine atom of different 5-substituted-2,4-dichloropyrimidines was first region selectively replaced by compound **7** to obtain **10b-10e** respectively**.** Then compound **9** was coupled with the C-2 of different 5-substituted-2-chloropyrimidines from compounds **10b-10e** and produced intermediates **11b-11e** accordingly. As for the substitute at the 5-position of pyrimidine was trifluoromethyl (Scheme 3), compound **9** was first installed selectively to 2-position of

2,4-dichloro-5-trifluoromethylpyrimidine to generate intermediate **12** mediated by Lewis acid [30]. Then compound **12** was subjected to coupling with compound **7** to produce intermediate **13.** The compounds **11b-11e** and **13**were stirred in the solution of trifluoroacetate and dichloromethane (1:1) to get rid of Boc group as aforementioned. Then the products were concentrated and reacted with carbon disulfide in DMF with triethylamine as base. Without further purification, 2-bromo-N,N-dimethylethan-1-amine was reacted with the products mentioned above respectively followed by chromatographic purification to generate the target compounds **14v-14z**.



Scheme 2. Synthesis of compounds **14v-14z**. Reagents and conditions: (a) Compound **7**; X = H, F: DIPEA, i-PrOH, reflux 6 h; X = CH_3, OCH_3: NaH, DMF, 0 □→rt.,24 h; (b) Compound **9**, $Pd(OAc)_2$, Xantphos, K_3PO_4, DMF, Ar, reflux, 22 h; (c) i: TFA, DCM, rt., 2 h; ii: CS_2, Et_3N, DMF, rt., 1 h; iii: 2-bromo-N,N-dimethylethan-1-amine hydrobromide, DMF, 50□, overnight.



9 12 13

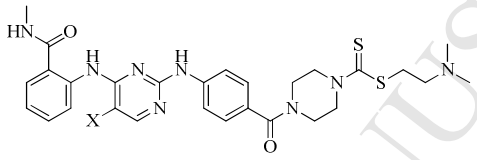
Scheme 3. Synthesis of intermediate **13.** Reagents and conditions:(a) i: 2,4-dichloro-5(trifluoromethyl)pyrimidine, $ZnCl_2$ (1.0 M solution in ether), DCE-tBuOH (1:1), 0 □, 1 h; ii: Et_3N, compound **9**, 0 □, 1.5 h; (b) Compound **7**, DIPEA, 1-butanol, reflux 24 h.

The newly synthesized compounds **14v-14z** were then evaluated against FAK and the same four human cancer cell lines. As shown in Table 2, anti-FAK activity of TAE226 was 6.21 nM and Defactinib was 0.77 nM, which were consistent with previously reported [14, 17]. Replacement of chlorine atom with fluorine atom (**14w**) caused slight decrease in FAK inhibition. When the chlorine group was removed (**14v**) or replaced with methyl group (**14x**) and methoxy group (**14y**), the kinase inhibitory activity decreased significantly. However, introducing a strong electron-withdrawing group such as trifluoromethyl group (**14z**) increased FAK inhibitory activity dramatically with an IC_{50} of 0.07nM. Compared with **14j**, the inhibitory effects of **14v** against the four tested cancer cell lines decreased dramatically. Compounds **14w**, **14x** and **14y** resulted in 4~40-fold decreases in potency. Nevertheless, **14z** increased the potency against HCT116, PC-3, U87-MG and MCF-7 cell lines by 3~4-fold. These results suggest that trifluoromethyl group may form an important interaction with the target protein. SAR analysis result of FAK inhibitory activity of these tested compounds was in agreement to that of their antitumor activities, indicating that the potent antitumor activities of these target compounds were likely related to their FAK inhibitory activities.

To evaluate the safety of the target compounds **14j** and **14v-14z**, they were also tested in human normal mammary epithelial cell MCF-10A (Table 2). The selectivity index (SI) values were calculated to determine the toxicities of these compounds on normal cells compared with breast cancer cell line MCF-7. Compounds **14x** and **14z** showed considerable safety (SI value >10).

Table 2.

FAK inhibitory activities and antiproliferative activities of target compounds **14j**, **14v-14z** against human tumor cell lines and normal cell line.



14j, 14v-14z

Compd.	X	FAK inhibition (IC_{50}/nM)	Antiproliferative activity (IC_{50}/μM)					SI value[a]
			HCT116	PC-3	U87-MG	MCF-7	MCF-10A	
14j	Cl	8.57	0.004±0.00	0.09±0.02	0.36±0.15	0.09±0.02	0.72±0.01	8.00
14v	H	316.30	0.44±0.05	3.01±0.43	2.80±1.05	2.01±0.14	6.19±0.42	3.08
14w	F	32.70	0.03±0.02	0.36±0.10	0.69±0.25	0.16±0.03	1.75±0.02	5.09
14x	Me	175.40	0.01±0.01	0.27±0.02	0.28±0.03	0.08±0.01	1.08±0.62	13.25
14y	OMe	506.70	0.16±0.05	0.92±0.11	1.61±0.36	0.36±0.02	2.43±0.04	6.75
14z	CF_3	0.07	0.001±0.00	0.03±0.00	0.06±0.01	0.02±0.01	0.28±0.05	14.00
TAE226	----	6.21	0.29±0.04	1.68±0.34	1.66±0.87	0.56±0.10	8.76±0.40	15.64
Defactinib	----	0.77	3.02±0.40	>20	10.21±1.02	9.76±1.53	18.42±1.21	1.89

[a]SI = IC_{50} for nonmalignant cell line / IC_{50} for MCF-7 cell line.

According to the results above, compound **14z** was chosen to further investigate the mechanism of the antiproliferative effect of these compounds. After exposure of MCF-7 to compound **14z** with different concentrations for 24 h, the cell cycle was analyzed using flow cytometry. As shown in Fig. 3, treatment of compound **14z** increased the fraction of cells arrested at G2/M phase. Compared with control (without **14z**), when MCF-7 cells were treated with increasing concentrations (0.005 μM, 0.01

μM and 0.05 μM) of compound **14z**, the percentage of cells in the G2/M phase increased from 11.48% to 86.08%, whereas cells in S and G0/G1 phase decreased concomitantly. Compound **14z** was also able to induce significant cell cycle arrest in HCT-116 in a dose-dependent manner (Fig. S1, Supporting Information).

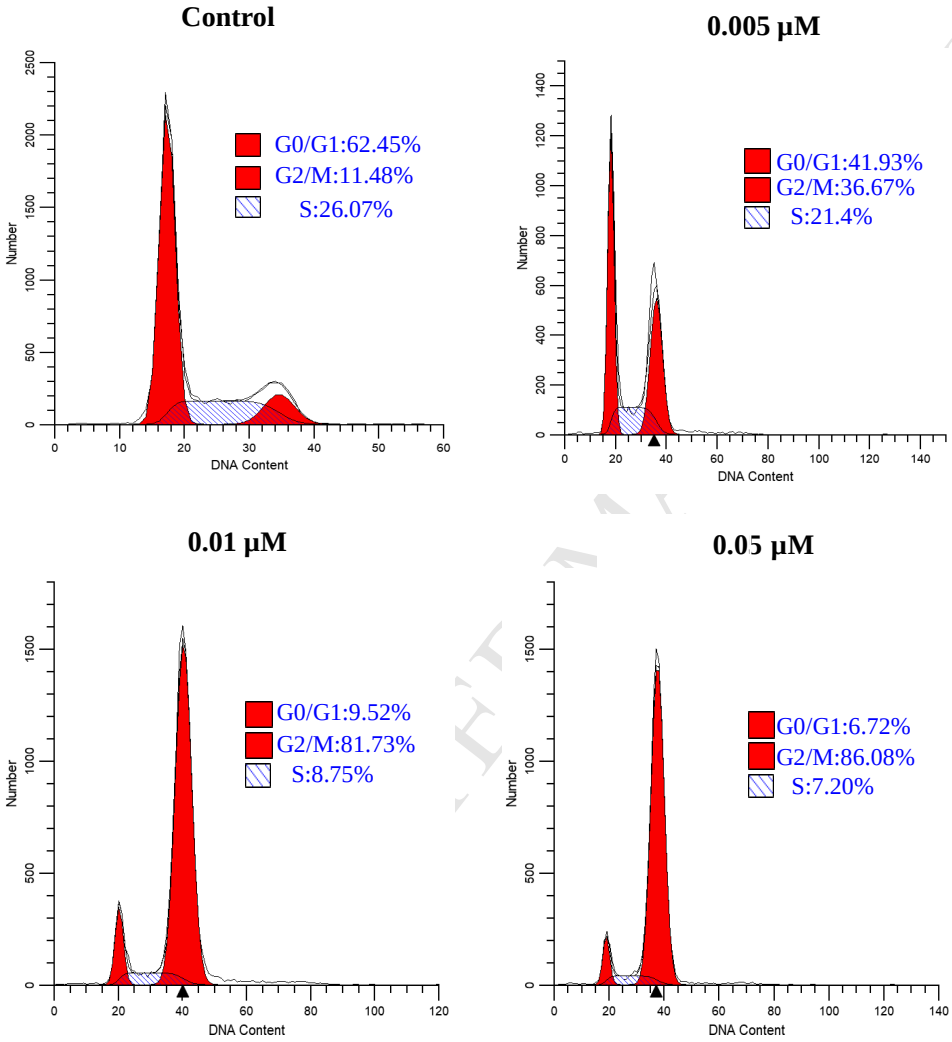


Fig.3. Compound **14z** induced cell cycle arrest in G2/M phase of MCF-7 cells in a dose-dependent manner. MCF-7 cells were treated with 0.005 μM, 0.01 μM and 0.05 μM compound **14z** for 24 h. Cells were fixed, stained with 7-AAD, and were assessed by flow cytometry. Cells untreated were used for control.

Next, in order to examine whether compound **14z** induces apoptosis in MCF-7 and HCT116 cell lines. Cells were treated with compound **14z** with increasing concentrations for 24 h and then examined for expression of both annexin V and

7-AAD by flow cytometry. As shown in Fig. 4, compound **14z** induced significant apoptosis in a dose-dependent manner compared with control. The result was consistent with pro-apoptosis effect of compound **14z** in HCT116 cell line (Fig. S2, Supporting Information**)**. These results suggested that compound **14z** may display antitumor activity by inducing both cell cycle arrest and cell apoptosis.

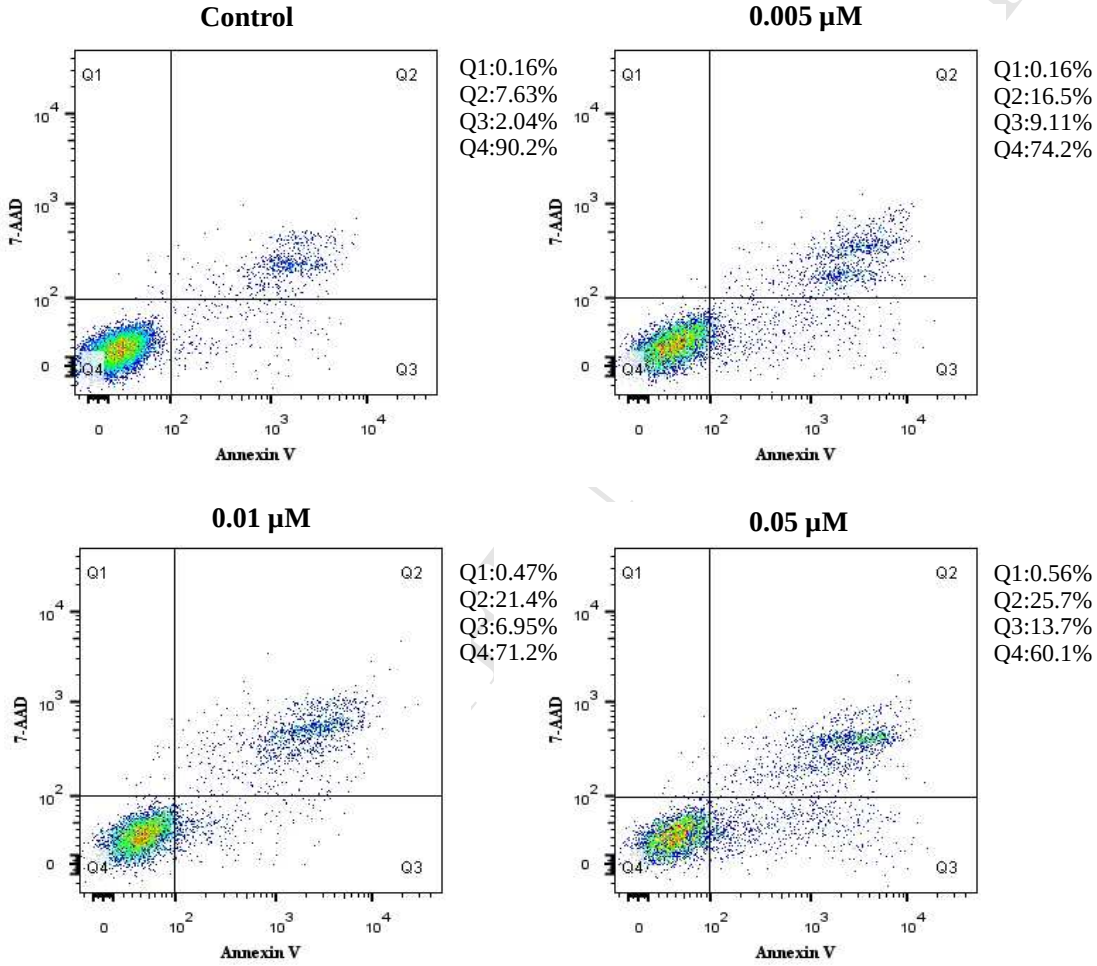


Fig.4. Compound **14z** induced apoptosis of MCF-7 cells in a dose-dependent manner. MCF-7 cells were treated with 0.005μM, 0.01μM and 0.05μM compound**14z** for 24 h. Cells were incubated with annexin V-FITC and 7-AAD and analyzed using flow cytometry.

Metastasis is a major cause of mortality in patients with cancer [31]. Therefore, the potency of compound **14z** to inhibit tumor cell migration was also evaluated using Transwell migration assay as reported [32]. As shown in Fig.5, when MCF-7 cells

was exposed to compound **14z** with concentrations of 0.05 μM or 0.1 μM for 24 h, the number of the cells that penetrate through the filters was significantly decreased compared with control. This observation indicates that compound **14z** possesses the potency to inhibit the migration of tumor cells.

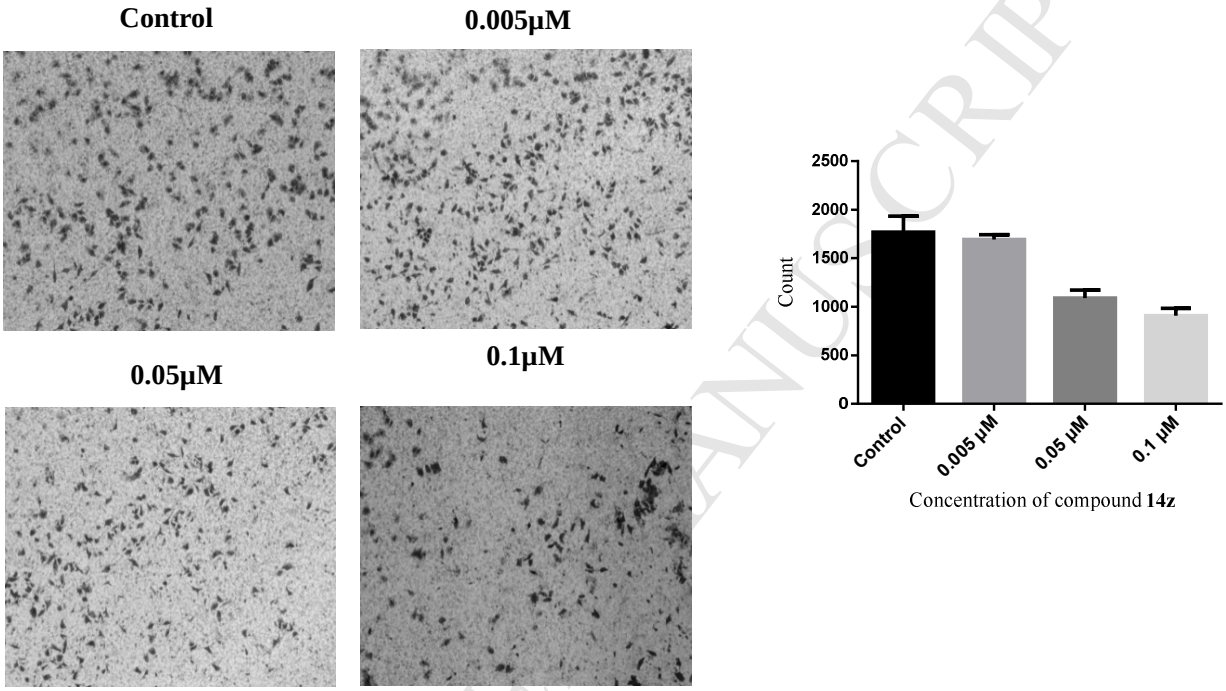


Fig.5. Effects of compound **14z** on the migrations of MCF-7. Cells seeded onto the upper chamber in serum-free medium were exposed to compound **14z** for 24 h.

Angiogenesis also plays an important role in development of tumor [33]. HUVEC (human umbilical vein endothelial cells) is a kind of cell line that has been widely used to imitate the neovascularization of tumor in vitro [34-36]. In order to investigate whether compound **14z** could interrupt the angiogenesis of tumor, HUVEC cells tube formation assay was carried out. After incubation for 6 h, the untreated endothelial cells formed tube-like networks (Fig.6). At the concentration of 0.005 μM, 0.05 μM and 0.1 μM, compound **14z** significantly inhibited the tube formation by reducing the

tube-like structure in a dose-dependent manner.

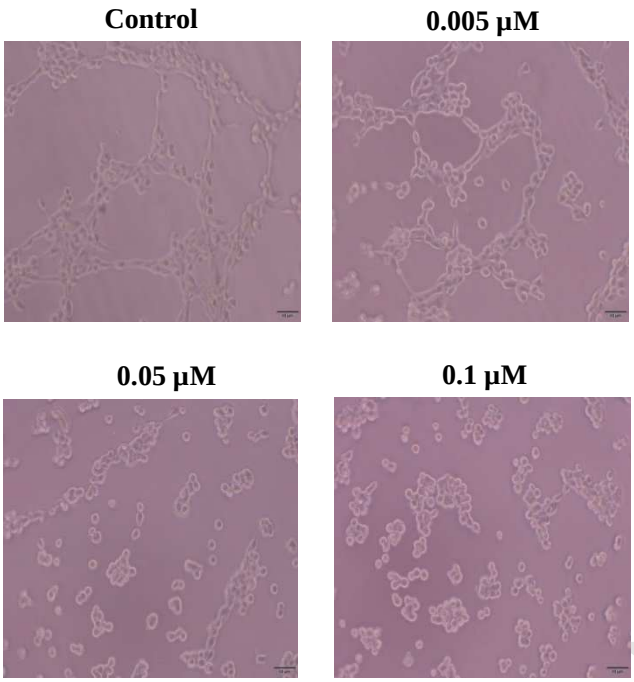


Fig.6. Anti-angiogenesis potency of compound **14z** by inhibiting the tube formation in HUVECs. The cells were incubated with or without compound **14z** in the Matrigel coated 96-wells plate for 6 h and then the images were taken.

3. Conclusion

A new series of 2,4-diarylaminopyrimidine derivatives containing dithiocarbamate moiety were synthesized and evaluated for their *in vitro* FAK enzyme and antitumor inhibitory activities. Most of the compounds showed obvious activity promotion compared with Defactinib while some of them exhibited better bioactivities than TAE226. Particularly, the most effective compound **14z** displayed superior antiproliferative effect with IC_{50} values from 0.001 to 0.06 μM against four FAK over expressed human tumor cell lines, which were more potent 10~100-fold than TAE226 and more potent 100~1000-fold than Defactinib.

Accordingly, compound **14z** inhibited FAK enzyme activity with an IC_{50} of 0.07

nM. In addition, compound **14z** showed good safety for the selectivity between human normal cells and MCF-7 tumor cells with SI value > 10. Compound **14z** was thus identified as the most potent antitumor agent and was used for studies of its antitumor mechanism in this paper. In summary, compound **14z** exerted antitumor effect by arresting cell cycle in G2/M phase and inducing apoptosis in both MCF-7 and HCT116 cell lines. In addition to antiproliferative activities, it also showed the potency of inhibiting the migration of tumor cells and disrupting the angiogenesis within nanomolar concentrations. Therefore, we propose that compound **14z** is a promising candidate for further preclinical studies.

4. Experimental Procedures

4.1. Chemistry

All reagents and solvents were purchased from commercial suppliers and were used without further purification. The positive control moleculars TAE226 and Defactinib were purchased from TargetMol. Melting points were determined on X4 microscope and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCEⅢ 400 MHz and 100 MHz spectrometer respectively. High resolution mass spectrum (HRMS) was recorded on a Thermo Scientific Orbitrap Elite MS. Thin Layer Chromatography (TLC) was performed on TLC Silica gel 60 F_{254} aluminium sheets (Merck, Darmstadt, Germany) to check the purity of the compounds.

*4.1.1. Procedure for the synthesis of N-methyl-2-nitrobenzamide (**6**)*

A mixture of 2-nitrobenzoyl chloride (3.9 g, 30 mmol), methylamine hydrochloride

(3 g, 45 mmol) and sodium bicarbonate (12.6 g, 150 mmol) in acetonitrile (30 mL) was heated under reflux for 4 h. The reaction was cooled to room temperature. The mixture was concentrated under vacuum and extracted with ethyl acetate (20 mL × 3). The organic phase was washed with 1N hydrochloric acid (15 mL × 3) and water (15 mL × 2), dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford compound **6** as white solid (4.55 g, yield: 84%). Mp: 86-87 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (d, J = 2.5 Hz, 1H), 8.03 – 8.01 (m, 1H), 7.77 (td, J = 7.6, 0.8Hz, 1H), 7.68 (td, J = 8.4, 1.6 Hz, 1H), 7.60 (dd, J = 7.6, 1.2 Hz, 1H), 2.76 (d, J = 4.4 Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ165.78, 147.16, 133.50, 132.52, 130.65, 128.98, 124.03, 26.10.

4.1.2. Procedure for the synthesis of 2-amino-N-methylbenzamide (7)

To a solution of compound **6** (4.80 g, 27 mmol) in H_2O (8 mL) and EtOH (24 mL) was added Fe (6.7 g, 120 mmol) and NH_4Cl (2.25 g, 42 mmol). The mixture was heated under reflux for 2 h. The solid was filtered off and the liquid was concentrated under vacuum to afford the crude product. The crude product was purified by column chromatography to provide compound **7** as white solid (3.73 g, yield: 92%). Mp: 66-67 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.27 (m, 1H), 7.20 – 7.15 (m, 1H), 6.66 (dd, J = 8.0, 0.8 Hz, 1H), 6.62 – 6.58 (m, 1H), 6.30 (s, 1H), 5.51 (s, 2H), 2.92 (d, J = 4.8 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ169.99, 148.41, 132.03, 127.10, 117.14, 116.49, 116.18, 26.37.

4.1.3. *Procedure for the synthesis of* tert-butyl 4-(4-nitrobenzoyl)piperazine-1-*carboxylate (**8**)*

A mixture of 4-nitrobenzoyl chloride (3.7 g, 20 mmol), tert-butyl piperazine-1-carboxylate (5.6 g, 30 mmol) and sodium bicarbonate (8.4 g, 100 mmol) in acetonitrile (30 mL) was heated under reflux for 4 h. The reaction was cooled to room temperature. The mixture was concentrated under vacuum and extracted with ethyl acetate (20 mL × 3). The organic phase was washed with 1N hydrochloric acid (15 mL × 3) and water (15 mL × 2), dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford compound **8** as white solid (6.0 g, yield: 90%). Mp: 133-134 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 8.4 Hz, 2H), 3.64 – 3.28 (m, 8H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ167.25, 153.76, 147.80, 142.02, 128.31, 123.71, 79.20, 43.11, 42.81, 27.95.

*4.1.4. The procedure for the synthesis of tert-butyl 4-(4-aminobenzoyl)piperazine-1-carboxylate (**9**)*

To a solution of compound **8** (6.0 g, 20 mmol) in H_2O (8 mL) and EtOH (24 mL) was added Fe (4.5 g, 80 mmol) and NH_4Cl (1.5 g, 28 mmol). The mixture was heated under reflux for 2 h. The solid was filtered off and the liquid was concentrated under vacuum to afford the crude product. The crude product was purified by column chromatography to provide compound **9** as yellow solid (5.7 g, yield: 93%). Mp: 155-156 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, J = 8.4 Hz, 2H), 6.65 (d, J = 8.4 Hz, 2H), 3.94 (s, 2H), 3.59 (s, 4H), 3.44 (s, 4H), 1.47 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ171.02, 154.54, 148.36, 129.26, 124.44, 114.10, 80.15, 44.01, 43.30, 28.29.

*4.1.5. Methods for the synthesis of compounds **10a-10e***

For the 5-subsititution was H, F or Cl, the procedure was as follows: A mixture of

compound **7** (0.79 g, 5.25 mmol), 5-substituted-2,4-dichloropyrimidine (5 mmol) and DIPEA (1.05 mL, 6 mmol) in i-PrOH was heated under reflux for 6 h. Water (100mL) was added and the solid was filtered off, washed with water anddried to obtain compounds **10a-10c**. For the 5-subsititution was methyl or methoxy, the procedure was as follows: To a mixture of compound **7** (1.26 g, 8.4 mmol) and5-substituted-2,4-dichloropyrimidine (8 mmol) was added NaH (60%, 0.38 g, 9.6 mmol) in DMF (6 mL) under ice bath. Then the mixture was stirred for 24 h at room temperature. Water was added dropwise to stop the reaction. The reaction mixture was extracted with dichloromethane (20 mL × 3), and the combined organic layer was washed with brine (15 mL × 2), dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford the crude product. The residue was purified by column chromatography to obtain compound **10d** and **10e**.

*4.1.5.3. 2-((3,6-dichloropyridin-2-yl)amino)benzamide (**10a**)*

Yield 80%. MP: 222-223 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27 (s, 1H), 8.88 (d, J = 4.4 Hz, 1H), 8.55 (d, J = 8.0 Hz, 1H), 8.46 (s, 1H), 7.82 (dd, J = 7.6, 0.8 Hz, 1H), 7.62 – 7.58 (m, 1H), 7.24 – 7.21 (m, 1H), 2.84 (d, J = 4.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.70, 156.63, 156.12, 155.25, 138.33, 131.84, 128.11, 123.06, 120.92, 120.77, 114.93, 26.36.

*4.1.5.1. 2-((6-chloropyridin-2-yl)amino)benzamide (**10b**)*

Yield 65%. Mp: 167-168°C.^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 8.65 (d, J = 4.4 Hz, 1H), 8.20 (d, J = 5.6 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.69 (dd, J = 8.0, 1.2 Hz, 1H), 7.56 – 7.52 (m, 1H), 7.22– 7.18 (m, 1H), 6.87 (d, J = 5.6 Hz, 1H), 2.79

(d, J = 4.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ168.43, 161.13, 159.28, 157.58, 137.70, 131.31, 128.27, 124.19, 123.23, 122.10, 106.52, 26.21.

*4.1.5.2. 2-((6-chloro-3-fluoropyridin-2-yl)amino)benzamide (**10c**)*

Yield 82%.Mp: 219-220 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27 (d, *J* = 2.0 Hz, 1H), 8.86 (d, *J* = 4.0 Hz, 1H), 8.53 (d, *J* = 8.0 Hz, 1H), 8.39 (d, *J* = 3.2 Hz, 1H), 7.82 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.62 – 7.58 (m, 1H), 7.23 – 7.19 (m, 1H), 2.82 (d, *J* = 4.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.79, 152.72 (d, *J* = 3.6 Hz), 150.37 (d, *J* = 10.8 Hz), 146.92, 144.36, 141.35 (d, *J* = 20.8 Hz), 138.41, 132.03, 128.13, 122.85, 120.49 (d, *J* = 15.8 Hz), 26.27.

*4.1.5.4. 2-((6-chloro-3-methylpyridin-2-yl)amino)benzamide (**10d**)*

Yield 40%. MP: 232-233 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.74 (s, 1H), 8.83 (d, *J* = 4.0 Hz, 1H), 8.64 (d, *J* = 8.0 Hz, 1H), 8.14 (s, 1H), 7.80 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.60 – 7.55 (m, 1H), 7.18 – 7.14 (m, 1H), 2.83 (d, *J* = 4.4 Hz, 3H), 2.18 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ169.07, 159.46, 156.63, 156.19, 139.50, 131.89, 128.05, 122.09, 120.45, 120.17, 115.40, 26.31, 12.98.

*4.1.5.5. 2-((6-chloro-3-methoxypyridin-2-yl)amino)benzamide (**10e**)*

Yield 41%. MP: 220-221 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 8.77 (d, *J* = 4.0 Hz, 1H), 8.67 (d, *J* = 8.4 Hz, 1H), 8.02 (s, 1H), 7.78 (d, *J* = 7.2 Hz, 1H), 7.58 (t, *J* = 7.2 Hz, 1H), 7.16 (t, *J* = 7.2 Hz, 1H), 3.99 (s, 3H), 2.83 (d, *J* = 4.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ168.71, 151.66, 149.16, 140.23, 138.84, 135.80, 131.78, 128.10, 122.11, 120.61, 120.14, 56.82, 26.29.

*4.1.6. The general procedure for the synthesis of compounds **11a-11e***

The mixture of compound **10a-10e** (3.24 mmol), compound **9** (1.04g, 3.4 mmol), $Pd(OAc)_2$ (0.11g, 0.5 mmol), Xantphos (0.28g, 0.5 mmol), and K_3PO_4（1.38g, 6.48 mmol）in DMF (8 mL) was heated 22 h at 125°C under argonfollowed by cooling to room temperature.The mixture was extracted with dichloromethane (20 mL × 3), the combined organic phase was washed with water (15 mL × 2), dried over anhydrous Na_2SO_4 and concentrated to afford the crude product. The crude product was purified by column chromatography to obtain compounds **11a-11e.**

*4.1.6.3. Tert-butyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin -2-yl)amino)benzoyl)piperazine-1-carboxylate (**11a**)*

Yield 62%. MP: 227-228 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 9.73 (s, 1H), 8.77 (dd, *J* = 17.2, 4.4 Hz, 2H), 8.28 (s, 1H), 7.78 (d, *J* = 8.8 Hz, 3H), 7.56 – 7.52 (m, 1H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.17 (t, *J* = 7.2 Hz, 1H), 3.50 – 3.36 (m, 8H), 2.83 (d, *J* = 4.4 Hz, 3H), 1.42 (s, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ169.32, 168.87, 157.39, 155.07, 154.53, 153.83, 141.78, 139.15, 131.45, 127.99, 122.13, 121.58, 121.01, 118.47, 105.80, 79.16, 43.56, 42.90, 28.02, 26.31.

*4.1.6.1. Tert-butyl4-(4-((4-((2-(methylcarbamoyl)phenyl)amino) pyrimidin-2-yl)amino) benzoyl)piperazine-1-carboxylate (**11b**)*

Yield 60%. MP: 206-207 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H), 9.55 (s, 1H), 8.68 (d, *J* = 4.8 Hz, 1H), 8.47 (d, *J* = 8.4 Hz, 1H), 8.13 (d, *J* = 5.6 Hz, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 7.2 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.12 (t, *J* = 7.2 Hz, 1H), 6.37 (d, *J* = 6.0 Hz, 1H), 3.51 (s, 3H), 3.41 (s,5H). 2.82 (d, *J* = 4.4 Hz, 3H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6)

*δ*169.47, 168.97, 159.89, 159.28, 156.68, 153.86, 142.34, 139.58, 131.33, 128.11, 128.03, 127.54, 121.90, 121.62, 118.15, 99.85, 79.17, 43.73, 42.98, 28.03, 26.24.

*4.1.6.2. Tert-butyl 4-(4-((5-fluoro-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin -2-yl)amino)benzoyl)piperazine-1-carboxylate (**11c**)*

Yield 62%. MP: 216-217 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (d, J = 2.0 Hz, 1H), 9.62 (s, 1H), 8.82 (t, J = 4.8 Hz, 2H), 8.23 (d, J = 3.2 Hz, 1H), 7.82 – 7.79 (m, 3H), 7.57 – 7.53 (m, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.18 – 7.14 (m, 1H), 3.50 – 3.38 (m, 8H), 2.83 (d, J = 4.4 Hz, 3H), 1.42 (s, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ 169.41, 169.06, 155.09 (d, J = 3.0 Hz),153.84, 149.07 (d, J = 10.0 Hz), 142.40 (d, J = 25.0 Hz), 140.51 – 140.06 (m), 139.47, 131.80, 128.06, 127.63, 121.82, 120.78, 120.78, 117.87, 79.17, 43.81, 42.82, 28.02, 26.30.

*4.1.6.4. Tert-butyl 4-(4-((5-methyl-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin -2-yl)amino)benzoyl)piperazine-1-carboxylate (**11d**)*

Yield 58%. MP: 232-233 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.26 (s, 1H), 9.41 (s, 1H), 8.87 (d, J = 8.4 Hz, 1H), 8.76 (d, J = 4.4 Hz, 1H), 8.02 (s, 1H), 7.82 (d, J = 8.4 Hz, 2H), 7.77 (d, J = 8.0 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.34 (d, J = 8.8 Hz, 2H), 7.10 (t, J = 7.2 Hz, 1H), 3.50 – 3.36 (m, 8H), 2.83 (d, J = 4.4 Hz, 3H), 2.09 (s, 3H), 1.42 (s, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ169.49, 169.32, 158.47, 157.85, 155.53, 153.84, 142.65, 140.50, 131.58, 128.07, 127.09, 121.10, 119.95, 117.75, 107.34, 79.16, 43.58, 42.93, 30.68, 28.03, 26.33.

*4.1.6.5. tert-butyl 4-(4-((5-methoxy-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin -2-yl)amino)benzoyl)piperazine-1-carboxylate (**11e**)*

Yield 59%. MP: 220-221 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.43 (s, 1H), 9.15z (s, 1H), 8.93 (d, *J* = 8.4 Hz, 1H), 8.70 (d, *J* = 4.4 Hz, 1H), 7.95 (s, 1H), 7.84 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 6.8 Hz, 1H), 7.55 – 7.51 (m, 1H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.11 (t, *J* = 7.2 Hz, 1H), 3.92 (s, 2H), 3.52 – 3.39 (m, 8H), 2.84 (d, *J* = 4.4 Hz, 3H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) *δ*169.56, 168.93, 153.84, 153.04, 150.95, 143.01, 139.74, 136.13, 135.50, 131.49, 128.13, 126.66, 121.19, 120.51, 117.12, 79.14, 56.89, 43.55, 42.94, 28.01, 26.29.

*4.1.7. The procedure for the synthesis of tert-butyl4-(4-((4-chloro-5-(trifluoromethyl) pyrimidin-2-yl)amino)benzoyl)piperazine-1-carboxylate (**12**)*

To a solution of 2,4-dichloro-5-(trifluoromethyl)pyrimidine (0.46 mL,3.4 mmol) in DCE (12 mL) and tBuOH (12 mL) was added 4 mL $ZnCl_2$ solution (1M in diethyl ether). The mixture was stirred under ice bath for 1 h. Then the mixture was added compound **21**(1.04 g, 3.4 mmol in 3mL DCE: tBuOH = 1:1) and stirred for another 1.5 h under ice bath. Then the mixture was added Et_3N (0.52 mL, 3.7 mmol) dropwise. The mixture was concentrated under vacuum and extracted with dichloromethane (20 mL × 3). The organic phase was washed with brine (15 mL × 3) and dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by column chromatography to get compound **12** as white solid (0.76 g, 46%). Mp: 182-183 °C. ^{1}H NMR (400 MHz, DMSO-d_6) *δ* 10.90 (s, 1H), 8.85 (s, 1H), 7.82 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 3.40 (s, 8H), 1.42 (s, 9H).^{13}C NMR (100 MHz, DMSO-d_6) *δ* 168.96, 160.37, 158.08 (d, *J* = 5.0 Hz),157.73, 153.81, 139.80, 130.26, 128.07, 119.55, 112.32 – 111.32 (m), 79.14, 43.49, 42.90, 27.99.

*4.1.8. Procedure for the synthesis oftert-butyl 4-(4-((4-((2-(methylcarbamoyl)phenyl) amino)pyrimidin-2-yl)amino)benzoyl)piperazine-1-carboxylate (**13**)*

The mixture of compound **7** (0.24 g, 1.58 mmol), compound **12** (0.73 g, 1.5 mmol) and DIPEA (0.31 mL, 1.8 mmol) in 1-Butanol was heated under reflux for 24 h. The mixture was concentrated under vacuum and extracted with dichloromethane (20 ml×3). The organic phase was washed with brine (15 mL × 3), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by column chromatography to get compound **13** as yellow solid (0.51 g, 55%). Mp: 203-204 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.34 (s, 1H), 10.07 (s, 1H), 8.76 (d, J = 3.6 Hz, 1H), 8.50 (s, 2H), 7.75 (d, J = 6.0 Hz, 3H), 7.53 (t, J = 7.6 Hz, 1H), 7.34 (d, J = 8.0 Hz, 2H), 7.21 (t, J = 7.2 Hz, 1H), 3.49 – 3.36 (m, 8H), 2.80 (d, J = 3.6 Hz, 3H), 1.42 (s, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ 169.16, 168.73, 160.59, 156.24, 155.94, 153.83, 140.89, 138.44, 131.12, 129.08, 127.85, 125.83, 123.23 (d, J = 17.4 Hz),122.82, 122.53 – 122.41 (m), 119.43, 79.16, 28.01, 26.21.

*4.1.9. General procedure for the synthesis of compounds **14a-14z***

To the suspension of compound **11a-11e** or **13** (1.5 mmol) in DCM (3 mL) was added TFA (3 mL). The mixture was stirred at room temperature for 2 h and was concentrated under vacuum. The product was then dissolved in DMF (6 mL) and added Et_3N (1 mL) without further purification. The reaction mixture was stirred for 1 h and was added CS_2 (0.14 mL, 2.25 mmol) to continuously stir for 30 min. A series of different of halogen or alkene substituted compounds (1.5 mmol) were added respectively and the mixture was stirred at 50 °C overnight. The mixture was cooled

to room temperature and extracted with dichloromethane (20 mL × 3). The organic phase was washed with brine (15 mL × 3), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography to provide the target products.

*4.1.9.1. 2-cyanoethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino) pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate(**14a**)*

Yield 76%. Mp: 287-288 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 9.76 (s, 1H), 8.77 (dd, J = 18.4, 4.0 Hz, 2H), 8.28 (s, 1H), 7.79 (t, J = 7.6 Hz, 3H), 7.55 (t, J = 7.6 Hz, 1H), 7.42 (d, J = 8.4 Hz,2H), 7.18 (t, J = 7.2 Hz, 1H), 4.33 (s, 2H), 4.04 (s, 2H), 3.70 (s, 4H), 3.57 (t, J = 6.8 Hz, 2H), 2.97 (t, J = 6.8 Hz, 2H), 2.83 (d, J = 4.4Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6)δ193.92, 169.38, 168.88, 157.37, 155.08, 154.54, 142.01, 139.14, 131.48, 128.21, 128.04, 127.66, 122.17, 121.61, 121.04, 119.19, 118.40, 105.87, 50.84, 49.46, 31.41, 26.34, 17.28.HRMS m/z: calcd. for $C_{27}H_{28}ClN_8O_2S_2$ $[M+H]^+$: 595.1465; found: 595.1439.

*4.1.9.2.allyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin-2-yl) amino)benzoyl)piperazine-1-carbodithioate (**14b**)*

Yield 84%. Mp: 247-248 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H), 9.75 (s, 1H), 8.78 (t, J = 8.8 Hz, 2H), 8.28 (s, 1H), 7.82 (dd, J = 11.2, 8.8 Hz, 3H), 7.55 (t, J = 7.6 Hz, 1H), 7.43 (d, J = 8.4 Hz, 2H), 7.18 (t, J = 7.2 Hz, 1H), 5.96 – 5.85 (m, 1H), 5.34 (d, J = 16.8 Hz, 1H), 5.16 (d, J = 10.0 Hz, 1H), 4.34 – 3.70 (m, 6H), 3.70 (s, 4H), 2.85 (d, J = 4.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ195.05, 169.37, 168.86, 157.34, 155.05, 154.45, 142.00, 139.16, 131.40, 128.15, 127.98, 127.63, 122.06,

121.57, 120.99, 118.72, 118.39, 105.88, 54.87, 50.53, 49.24, 26.30. HRMS m/z: calcd. for $C_{27}H_{29}ClN_7O_2S_2$ $[M+H]^+$:582.1513;found: 582.1506.

*4.1.9.3.2-methoxyethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino) pyrimidin -2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14c**)*

Yield 82%. Mp: 215-216 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 9.76 (s, 1H), 8.80 – 8.73 (m, 2H), 8.28 (s, 1H), 7.82 – 7.77 (m, 3H), 7.55 (t, J = 7.2 Hz, 1H), 7.42 (d, J = 8.8 Hz, 2H), 7.18 (t, J = 7.2 Hz, 1H), 4.33 (s, 2H), 4.06 – 4.03 (m, 2H), 3.68 (s, 4H), 3.58 – 3.55 (m, 2H), 3.50 (t, J = 6.0 Hz, 2H), 3.27 (s, 3H), 2.83 (d, J = 4.4 Hz, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ195.54, 169.40, 168.89, 157.36, 155.07, 154.49, 142.04, 139.19, 131.45, 128.20, 128.02, 127.64, 122.10, 121.60, 120.99, 118.40, 105.92, 69.86, 57.89, 50.70, 49.18, 35.82, 26.33. HRMS m/z: calcd. for $C_{27}H_{31}ClN_7O_3S_2$ $[M+H]^+$: 600.1618 ; found: 600.1602.

*4.1.9.4. Methyl 3-((4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin -2-yl)amino)benzoyl)piperazine-1-carbonothioyl)thio)propanoate(**14d**)*

Yield 83%.Mp: 180-181 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.62 (s, 1H), 9.74 (s, 1H), 8.78 – 8.72 (m,2H), 8.28 (s, 1H), 7.80 – 7.76 (m, 3H), 7.57 – 7.52 (m, 1H), 7.40 (d, J = 8.4 Hz, 2H), 7.19 – 7.15 (m, 1H), 4.31 (s, 2H), 4.00 (s, 2H), 3.65 (d, J = 17.2 Hz, 7H), 3.48 (t, J = 7.2 Hz, 2H), 2.82 (d, J = 4.4 Hz, 3H), 2.78 (t, J = 6.8 Hz, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 195.86, 169.36, 168.88, 157.37, 155.08, 154.55, 142.00, 139.15, 131.48, 128.20, 128.04, 127.70, 122.15, 121.60, 121.01, 118.38, 105.87, 66.76, 50.48, 49.48, 34.19, 32.02, 26.33.HRMS m/z: calcd. for $C_{28}H_{31}ClN_7O_4S_2$ $[M+H]^+$: 628.1568; found:628.1561.

*4.1.9.5. Ethyl 2-((4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin-2-y l)amino)benzoyl)piperazine-1-carbonothioyl)thio)acetate (**14e**)*

Yield 83%. Mp: 120-121 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ11.66 (s, 1H), 9.76 (s, 1H), 8.80 – 8.74 (m, 2H), 8.28 (s, 1H), 7.80 (t, J = 9.6 Hz, 3H), 7.56 (t, J = 7.6 Hz, 1H), 7.43 (d, J = 8.4 Hz, 2H), 7.18 (t, J = 7.2 Hz, 1H), 4.31 (s, 2H), 4.22 (s, 2H), 4.16 – 4.01 (m, 4H), 3.71 (s, 4H), 2.84 (d, J = 4.4 Hz, 3H), 1.22 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ194.63, 169.40, 168.89, 168.00, 157.37, 155.08, 154.53, 142.02, 139.16, 131.47, 128.21, 128.03, 127.66, 122.15, 121.61, 121.02, 118.40, 105.88, 61.11, 51.02, 49.36, 38.27, 26.34, 14.06.HRMS m/z: calcd. for $C_{28}H_{31}ClN_7O_4S_2$ $[M+H]^+$: 628.1568; found:628.1564.

*4.1.9.6. Ethyl 3-((4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino) pyrimidin-2 -yl)amino)benzoyl)piperazine-1-carbonothioyl)thio)-2-oxopropanoat (**14f**)*

Yield 76%. Mp: 148-149 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.62 (s, 1H), 9.74 (s, 1H), 8.78 – 8.72 (m, 2H), 8.28 (s, 1H), 7.78 (t, J = 6.4 Hz, 3H), 7.55 (t, J = 8.0 Hz, 1H), 7.41 (d, J = 8.0 Hz, 2H), 7.18 (t, J = 7.6 Hz, 1H), 4.60 (s, 1H), 4.31 – 4.02 (m, 6H), 3.70 (s, 4H), 2.82 (d, J = 4.4 Hz, 3H), 1.32 – 1.16 (m, 4H).^{13}C NMR (100 MHz, DMSO-d_6) δ 194.37, 190.19, 186.73, 169.36, 168.88, 157.37, 155.08, 154.56, 142.01, 139.12, 131.50, 128.21, 128.04, 127.66, 122.19, 121.61, 121.05, 118.39, 105.85, 62.05, 61.47, 51.21, 50.04, 26.33, 13.86.HRMS m/z: calcd. for $C_{29}H_{31}ClN_7O_5S_2$ $[M+H]^+$: 656.1517; found: 656.1497.

*4.1.9.7.2-(methylsulfonyl)ethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl) amino)pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14g**)*

Yield 76%. Mp: 223-224 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 9.74 (s, 1H), 8.78 – 8.74 (dd, 2H), 8.28 (s, 1H), 7.80 (t, J = 8.0 Hz, 3H), 7.55 (t, J = 7.6 Hz, 1H), 7.42 (d, J = 8.4 Hz, 2H), 7.18 (t, J = 7.6 Hz, 1H), 4.34 (s, 2H), 4.03 (s, 2H), 3.70 – 3.65 (m, 6H), 3.53 – 3.38 (m, 2H), 3.09 (s, 3H), 2.84 (d, J = 4.0 Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 194.22, 169.38, 168.86, 157.35, 155.06, 154.50, 142.00, 139.13, 131.44, 128.18, 128.01, 127.63, 122.13, 121.58, 121.01, 118.40, 105.88, 52.78, 50.65, 49.33, 40.57, 28.59, 26.31.HRMS m/z: calcd. for $C_{27}H_{31}ClN_7O_4S_3$ $[M+H]^+$: 648.1288; found: 648.1633.

*4.1.9.8. 2-hydroxyethyl4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino) pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14h**)*

Yield 77%. Mp: 162-163 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 9.76 (s, 1H), 8.80 – 8.74 (m, 2H), 8.28 (s, 1H), 7.80 (t, J = 8.8 Hz, 3H), 7.55 (t, J = 7.6 Hz, 1H), 7.43 (d, J = 8.4 Hz, 2H), 7.18 (t, J = 7.6 Hz, 1H), 5.02 (t, J = 5.6 Hz, 1H), 4.34(s, 2H), 4.14 – 4.01 (m, 2H), 3.69 – 3.62 (m, 6H), 3.41 (t, J = 4.0 Hz, 2H), 2.84 (d, J = 4.4 Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ195.95, 169.45, 168.93, 157.40, 155.11, 154.54, 142.04, 139.18, 131.50, 128.24, 128.05, 127.71, 122.18, 121.65, 121.06, 118.46, 105.92, 59.31, 50.75, 49.23, 30.71, 26.37.HRMS m/z: calcd. for $C_{26}H_{29}ClN_7O_3S_2$ $[M+H]^+$: 586.1462; found: 586.1456.

*4.1.9.9. 2-hydroxypropyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino) pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14i**)*

Yield 78%. Mp: 258-259 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 9.76 (s, 1H), 8.80 – 8.73 (m, 2H), 8.28 (s, 1H), 7.79 (t, J = 8.0 Hz, 3H), 7.55 (t, J = 7.6 Hz,

1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.18 (t, *J* = 7.6 Hz, 1H), 4.33 (s, 2H), 4.03 (s, 2H), 3.68 (s, 5H), 3.38 (s, 2H), 2.83 (d, *J* = 4.4Hz, 3H), 2.60 (s, 3H), 2.51 (s, 1H).^{13}C NMR (100 MHz, DMSO-d_6) δ 196.89, 169.38, 168.88, 157.37, 155.08, 154.54, 141.99, 139.14, 131.49, 128.20, 128.04, 127.71, 122.16, 121.61, 121.03, 118.40, 105.86, 54.93, 50.56, 49.16, 30.70, 26.34, 19.49.HRMS m/z: calcd. for $C_{27}H_{31}ClN_7O_3S_2$ $[M+H]^+$: 600.1618; found: 600.1615.

*4.1.9.10. 2-(dimethylamino)ethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl) amino)pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14j**)*

Yield 78%. Mp: 178-179 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (s, 1H), 9.76 (s, 1H), 8.80 – 8.76 (m, 2H), 8.28 (s, 1H), 7.82 (t, *J* = 10.0 Hz, 3H), 7.55 (t, *J* = 7.2 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.18 (t, *J* = 7.2 Hz, 1H), 4.34 (s, 2H), 4.05 (dd, *J* = 14.0, 6.8 Hz, 2H), 3.70 (s, 4H), 3.40 (t, *J* = 7.2 Hz, 2H), 2.85 (d, *J* = 4.4 Hz, 3H), 2.55 – 2.52 (m, 2H), 2.19 (s, 6H).^{13}C NMR (100 MHz, DMSO-d_6) δ 196.08, 169.38, 168.88, 157.36, 155.07, 154.48, 142.02, 139.19, 131.44, 128.20, 128.02, 127.65, 122.09, 121.58, 120.97, 118.39, 105.90, 57.30, 50.46, 49.20, 44.77, 34.46, 26.33.HRMS m/z: calcd. for $C_{28}H_{15z}ClN_8O_2S_2$ $[M+H]^+$: 613.1935; found: 613.1920.

*4.1.9.11. 2-(pyrrolidin-1-yl)ethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl) phenyl)amino) pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14k**)*

Yield 74%. Mp: 145-146 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H), 9.75 (s, 1H), 8.79 –8.75 (m, 2H), 8.28 (s, 1H), 7.80 (t, *J* = 8.4 Hz, 3H), 7.55 (t, *J* = 7.6Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.18 (t, *J* = 7.2 Hz, 1H), 4.33 (s, 2H), 4.03 (s, 2H), 3.68 (s, 4H), 3.41 (t, *J* = 7.2 Hz, 2H), 2.84 (d, *J* = 4.4 Hz, 3H), 2.68 (t, *J* = 6.8 Hz, 2H),

2.50 (d, *J* = 19.6 Hz, 4H), 1.67 (s, 4H).^{13}C NMR (100 MHz, DMSO-d_6) δ 196.12, 169.37, 168.87, 157.36, 155.07, 154.50, 142.01, 139.17, 131.45, 128.19, 128.01, 127.67, 122.11, 121.58, 120.99, 118.39, 105.88, 54.06, 53.31, 50.52, 49.19, 35.61, 26.32, 23.13.HRMS m/z: calcd. for $C_{30}H_{36}ClN_8O_2S_2$ $[M+H]^+$: 639.2091; found: 639.2079.

*4.1.9.12. 2-(piperidin-1-yl)ethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl) phenyl) amino)pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14l**)*

Yield 84%. Mp: 153-154 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 9.74 (s, 1H), 8.78 – 8.73 (m, 2H), 8.28 (s, 1H), 7.79 (t, *J* = 6.0 Hz, 3H), 7.55 (t, *J* = 7.6 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 2H), 7.17 (t, *J* = 7.6 Hz, 1H), 4.31 (s, 2H), 4.04 (dd, *J* = 14.0, 6.8 Hz, 2H), 3.67 (s, 4H), 3.41 – 3.36 (m, 3H), 2.83 (d, *J* = 4.4 Hz, 3H), 2.55 – 2.38 (m, 3H), 2.38 (s, 4H), 1.49 – 1.46 (m, 4H).^{13}C NMR (100 MHz, DMSO-d_6) δ 196.08, 169.32, 168.83, 157.35, 155.05, 154.49, 141.95, 139.12, 131.42, 128.13, 127.98, 127.69, 122.10, 121.56, 121.00, 118.37, 105.83, 57.03, 53.72, 50.41, 49.33, 33.78, 26.28, 25.47, 23.96.HRMS m/z: calcd. for $C_{31}H_{38}ClN_8O_2S_2$ $[M+H]^+$: 653.2248; found: 653.2239.

*4.1.9.13. 2-(4-methylpiperazin-1-yl)ethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl) phenyl)amino)pyrimidin-2-yl)amino)benzoyl)piperidine-1-carbodithioate (**14m**)*

Yield 80%. Mp: 222-223 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 9.74 (s, 1H), 8.79 – 8.73 (m, 2H), 8.27 (d, *J* = 1.2 Hz, 1H), 7.81 – 7.77 (m, 3H), 7.56 –7.52 (m, 1H), 7.42 (d, *J* = 1.6 Hz, 2H), 7.19 – 7.15 (m, 1H), 4.32 (s, 2H), 4.06– 4.01 (m, 2H), 3.67 (s, 4H), 3.41(t, *J* = 5.4 Hz 2H), 2.83 (d, *J* = 4.8 Hz, 3H), 2.57 (t, *J* = 7.2 Hz,

2H), 2.52– 2.36 (m, 8H), 2.18 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ195.98, 169.33, 168.84, 157.34, 155.05, 154.49, 141.96, 139.11, 131.42, 128.14, 127.99, 127.69, 122.11, 121.56, 121.01, 118.38, 105.83, 56.22, 54.44, 52.14, 50.55, 49.19, 45.43, 33.69, 26.28.HRMS m/z: calcd. for $C_{31}H_{39}ClN_9O_2S_2$ $[M+H]^+$: 668.2358; found: 668.2134.

*4.1.9.14. 2-morpholinoethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino) pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14n**)*

Yield 80%. Mp: 145-146 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 9.74 (s, 1H), 8.78 – 8.73 (m, 2H), 8.28 (s, 1H), 7.79 (t, J = 6.8 Hz, 3H), 7.55 (t, J = 7.2 Hz, 1H), 7.41 (d, J = 8.8 Hz, 2H), 7.18 (t, J = 7.2 Hz, 1H), 4.32 (s, 2H), 4.03 (d, J = 7.2 Hz, 2H), 3.65 (d, J = 18.8 Hz, 5H), 3.58 – 3.55 (m, 3H), 3.50 – 3.36 (m, 3H), 2.83 (d, J = 4.4 Hz,3H), 2.58 (t, J = 7.2 Hz, 2H), 2.42 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 195.91, 169.33, 168.85, 157.35, 155.06, 154.50, 141.97, 139.13, 131.44, 128.15, 127.99, 127.69, 122.11, 121.57, 121.01, 118.38, 105.85, 66.10, 56.75, 53.01, 51.57, 50.66, 49.14, 26.29.HRMS m/z: calcd. for $C_{30}H_{36}ClN_8O_3S_2$ $[M+H]^+$: 655.2040; found: 655.2031.

*4.1.9.15. 2-(1,3-dioxolan-2-yl)ethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl) amino)pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate(**14o**)*

Yield 81%. Mp: 230-231 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.62 (s, 1H), 9.74 (s, 1H), 8.78 – 8.72 (m, 2H), 8.28 (s, 1H), 7.80 – 7.77 (m, 3H), 7.54 (t, J = 7.2 Hz, 1H), 7.40 (d, J = 8.4 Hz, 2H), 7.17 (t, J = 7.2 Hz, 1H), 4.89 (t, J = 4.8 Hz, 1H), 4.31 (s, 2H), 4.01 (s, 2H), 3.93 – 3.77 (m, 4H), 3.67 (s, 4H), 3.32 – 3.28 (m, 2H), 2.82 (d, J =

4.8 Hz, 3H), 1.99 – 1.94 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 195.57, 169.34, 168.84, 157.35, 155.06, 154.49, 141.96, 139.12, 131.42, 128.14, 127.98, 127.69, 122.10, 121.57, 121.02, 118.39, 105.84, 102.32, 64.33, 50.30, 49.15, 32.45, 30.97, 26.28.HRMS m/z: calcd. for $C_{29}H_{33}ClN_7O_4S_2$ $[M+H]^+$: 642.1724; found: 642.1717.

*4.1.9.16. (tetrahydro-2H-pyran-4-yl)methyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl) phenyl)amino)pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14p**)*

Yield 82%. Mp: 217-218 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 9.75 (s, 1H), 8.79 – 8.72(m, 2H), 8.28 (s, 1H), 7.78 (t, J = 5.6 Hz, 3H), 7.55 (t, J = 7.6 Hz, 1H), 7.41 (d, J = 8.4 Hz, 2H), 7.17 (t, J = 7.6 Hz, 1H), 4.32 (s, 2H), 4.03 (d, J = 6.8Hz, 2H), 3.85 – 3.82 (m, 2H), 3.67 (s, 4H), 3.27 – 3.22 (m, 4H), 2.82 (d, J = 4.4 Hz, 3H), 1.84 – 1.82(m, 1H), 1.66 (d, J = 12.8 Hz, 2H), 1.32 – 1.22 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 195.87, 169.37, 168.87, 157.36, 155.07, 154.50, 142.02, 139.18, 131.46, 128.20, 128.02, 127.66, 122.11, 121.58, 120.97, 118.37, 105.90, 66.76, 50.59, 49.21, 42.51, 34.18, 32.02, 26.33.HRMS m/z: calcd. for $C_{30}H_{35}ClN_7O_3S_2$ $[M+H]^+$: 640.1931; found: 640.1921.

*4.1.9.17. Benzyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin -2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14q**)*

Yield79%. Mp: 157-158 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 9.76 (s, 1H), 8.80 – 8.73 (m, 2H), 8.28 (s, 1H), 7.79 (t, J = 7.2 Hz, 3H), 7.55 (t, J = 7.6 Hz, 1H), 7.41 (d, J = 8.4 Hz, 4H), 7.34 – 7.25 (m, 3H), 7.17 (t, J = 7.6 Hz, 1H), 4.56 (s, 2H), 4.34 (s, 2H), 4.04 (dd, J = 14.4, 7.2 Hz, 2H), 3.68 (s, 4H), 2.83 (d, J = 4.8 Hz, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 195.19, 169.37, 168.88, 157.37, 155.07,

154.53, 142.00, 139.15, 136.13, 131.48, 129.25, 128.48, 128.19, 128.03, 127.68, 127.40, 122.15, 121.60, 121.02, 118.39, 105.87, 50.59, 49.19, 40.78, 26.33.HRMS m/z: calcd. for $C_{31}H_{31}ClN_7O_2S_2$ $[M+H]^+$: 632.1669; found: 632.1672.

*4.1.9.18. Phenethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin -2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14r**)*

Yield 79%. Mp: 232-233 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H), 9.77 (s, 1H), 8.80 – 8.75 (m, 2H), 8.28 (s, 1H), 7.81 (t, J = 9.6 Hz, 3H), 7.55 (t, J = 7.6 Hz, 1H), 7.43 (d, J = 8.4 Hz, 2H), 7.33 – 7.28 (m, 4H), 7.24 – 7.16 (m, 2H), 4.35 (s, 2H), 4.01 (s, 2H), 3.68 (s, 4H), 3.53 – 3.50 (m, 2H), 2.97 – 2.93 (m, 2H), 2.84 (d, J = 4.4 Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 195.60, 169.36, 168.87, 157.36, 155.07, 154.51, 142.01, 139.98, 139.17, 131.46, 128.46, 128.40, 128.19, 128.02, 127.67, 126.35, 122.11, 121.59, 120.99, 118.39, 105.89, 50.58, 49.29, 37.33, 34.46, 26.32.HRMS m/z: calcd. for $C_{32}H_{33}ClN_7O_2S_2$ $[M+H]^+$: 646.1826; found: 646.1816.

*4.1.9.19. Pyridin-4-ylmethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino) pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14s**)*

Yield 82%. Mp: 171-172 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.63 (s, 1H), 9.75 (s, 1H), 8.79 – 8.71 (m, 2H), 8.51 (d, J = 4.8 Hz, 2H), 8.28 (s, 1H), 7.79 (d, J = 8.0 Hz, 2H), 7.54 (t, J = 7.6 Hz, 2H), 7.42 (t, J = 10.0 Hz, 4H), 7.17 (t, J = 7.6 Hz, 1H), 4.64 (s, 2H), 4.32 (s, 2H), 4.04 (s, 2H), 3.68 (s, 4H), 2.82 (d, J = 4.0 Hz, 3H).^{13}C NMR (100 MHz,DMSO-d_6) δ 194.49, 169.41, 168.90, 157.36, 155.08, 154.50, 149.57, 146.01, 142.05, 139.18, 131.47, 128.21, 128.03, 127.64, 124.11, 122.13, 121.59, 120.99, 118.40, 105.92, 51.07, 49.35, 38.84, 35.79, 26.34. HRMS m/z: calcd. for

$C_{30}H_{30}ClN_8O_2S_2$ $[M+H]^+$: 633.1622;found: 633.1497.

*4.1.9.20. Pyridin-2-ylmethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino) pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14t**)*

Yield 78%. Mp: 161-162 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H),9.76 (s, 1H), 8.80 – 8.73 (m, 2H), 8.52 (d, J = 4.4 Hz, 1H), 8.28 (s, 1H), 7.82 – 7.73 (m, 4H), 7.57 – 7.49 (m, 2H), 7.42 (d, J = 8.4 Hz, 2H), 7.29 (dd, J = 6.8, 5.2 Hz, 1H), 7.18 (t, J = 7.6 Hz, 1H), 4.69 (s, 2H), 4.34 (s, 2H), 4.06 – 4.03 (m, 2H), 3.69 (s, 4H), 2.83 (d, J = 4.4 Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 195.08, 169.42, 168.90, 157.35, 156.03, 155.07, 149.20, 142.05, 139.19, 136.71, 128.20, 128.01, 127.63, 123.47, 122.43, 122.09, 121.59, 120.99, 118.41, 105.92, 50.78, 49.29, 42.69, 26.34.HRMS m/z: calcd. for $C_{30}H_{30}ClN_8O_2S_2$ $[M+H]^+$: 633.1622; found: 633.1666.

*4.1.9.21. Pyridin-3-ylmethyl 4-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino) pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14u**)*

Yield 80%. Mp: 150-151 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 9.75 (s, 1H), 8.79 – 8.74 (m, 2H), 8.64 (s, 1H), 8.47 (d, J = 3.6 Hz, 1H), 8.27 (s, 1H), 7.80 (t, J = 8.8 Hz, 4H), 7.55 (t, J = 7.6 Hz, 1H), 7.42 (d, J = 8.4Hz, 2H), 7.35 (dd, J = 7.6, 4.8 Hz, 1H), 7.17 (t, J = 7.2 Hz, 1H), 4.62 (s, 2H), 4.34 (s, 2H), 4.03 (s, 2H), 3.70 (s, 4H), 2.84 (d, J = 4.4 Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6) 194.65, 169.38, 168.87, 157.35, 155.06, 154.47, 150.08, 148.35, 142.01, 139.15, 136.68, 132.67, 131.43, 128.17, 128.00, 127.64, 123.42, 122.10, 121.58, 121.01, 118.39, 105.88, 50.76, 49.36, 38.89, 37.46, 26.31. HRMS m/z: calcd. for $C_{30}H_{30}ClN_8O_2S_2$ $[M+H]^+$: 633.1622; found: 633.1689.

*4.1.9.22. 2-(dimethylamino)ethyl 4-(4-((4-((2-(methylcarbamoyl)phenyl) amino) pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14v**)*

Yield 79%. Mp: 160-161 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H), 9.56 (s, 1H), 8.68 (s, 1H), 8.47 (d, *J* = 7.2 Hz, 1H), 8.13 (d, *J* = 3.6 Hz, 1H), 7.87 (d, *J* = 6.8 Hz, 2H), 7.71 (d, *J* = 6.8 Hz, 1H), 7.51 (s, 1H), 7.41 (d, *J* = 7.0 Hz, 1H), 7.12 (s, 1H), 6.37 (s, 1H), 4.33 (s, 2H), 4.04 (s, 2H), 3.69 (s, 4H), 3.40 (s, 2H), 2.81 (s, 3H), 2.53 (s, 2H), 2.18 (s, 6H).^{13}C NMR (100 MHz, DMSO-d_6) δ 196.10, 169.49, 168.96, 159.86, 159.28, 156.73, 142.55, 139.59, 131.34, 128.22, 128.11, 127.12, 121.84, 121.61, 118.05, 99.88, 57.33, 50.57, 49.17, 44.80, 34.49, 26.25.HRMS m/z: calcd. for $C_{28}H_{35}N_8O_2S_2$ $[M+H]^+$: 579.2324; found: 579.2319.

*4.1.9.23. 2-(dimethylamino)ethyl 4-(4-((5-fluoro-4-((2-(methylcarbamoyl)phenyl) amino)pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14w**)*

Yield 76 %. Mp: 241-242 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (s, 1H), 9.64 (s, 1H), 8.83 (d, *J* = 8.0 Hz, 2H), 8.23 (d, *J* = 2.4 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 3H), 7.56 (t, *J* = 7.6 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.16 (t, *J* = 7.6 Hz, 1H), 4.32 (s, 2H), 4.03 (d, *J* = 6.4 Hz, 2H), 3.68 (s, 4H), 3.40 (s, 2H), 2.83 (d, *J* = 3.9 Hz,3H), 2.53 (t, *J* = 5.6 Hz, 2H), 2.18 (s, 6H).^{13}C NMR (100 MHz, DMSO-d_6) δ 196.08, 169.43, 169.05, 155.07 (d, *J* = 3.2 Hz), 149.07 (d, *J* = 10.0 Hz), 142.50 (d, *J* = 6.9 Hz), 140.42 (d, *J* = 19.7 Hz), 140.07, 139.45, 131.82, 128.27, 128.07, 127.25, 121.85, 120.79, 120.09, 117.78, 57.33, 50.53, 49.34, 44.82, 34.50, 26.31.HRMS m/z: calcd. for $C_{28}H_{15z}FN_8O_2S_2$ $[M+H]^+$: 597.2230; found: 597.2222.

4.1.9.24. 2-(dimethylamino)ethyl 4-(4-((5-methyl-4-((2-(methylcarbamoyl)phenyl)

*amino)pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14x**)*

Yield 77%. Mp: 192-193 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.27 (s, 1H), 9.45 (s, 1H), 8.89 (d, J = 8.4 Hz, 1H), 8.77 (d, J = 4.8 Hz, 1H), 8.03 (s, 1H), 7.85 (d, J = 8.4Hz, 2H), 7.78 (d, J = 7.6 Hz, 1H), 7.53 (t, J = 7.6 Hz, 1H), 7.40 (d, J = 8.4 Hz, 2H), 7.11 (t, J = 7.6 Hz, 1H), 4.33 (s, 2H), 4.03 (d, J = 6.8 Hz, 2H), 3.69 (s, 4H), 3.39 (d, J = 6.8 Hz,2H), 2.84 (d, J = 4.4 Hz, 3H), 2.52 (t, J = 6.4 Hz, 2H), 2.17 (d, J = 9.6 Hz, 9H).^{13}C NMR (101 MHz, DMSO-d_6) δ196.11, 169.54, 169.32, 158.47, 157.83, 155.51, 142.87, 140.50, 131.58, 128.26, 127.97, 126.71, 121.19, 121.10, 119.96, 117.70, 107.40, 57.34, 50.50, 49.16, 44.82, 34.51, 26.34, 13.15.HRMS m/z: calcd. for $C_{29}H_{37}N_8O_2S_2$ $[M+H]^+$: 593.2481; found: 593.2476.

*4.1.9.25. 2-(dimethylamino)ethyl 4-(4-((5-methoxy-4-((2-(methylcarbamoyl) phenyl) amino)pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14y**)*

Yield 77%. Mp: 164-165 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (s, 1H), 9.36 (s, 1H), 8.93 (d, J = 8.4 Hz, 1H), 8.70 (d, J = 4.8 Hz, 1H), 7.95 (s, 1H), 7.85 (d, J = 8.8 Hz, 2H), 7.75 (d, J = 6.8 Hz, 1H), 7.53 (t, J = 7.2 Hz, 1H), 7.41 (d, J = 8.4 Hz, 2H), 7.11 (t, J = 7.6 Hz, 1H), 4.33 (s, 2H), 4.06 – 4.01 (m, 2H), 3.92 (s, 3H), 3.69 (s, 4H), 3.40 (t, J = 6.8 Hz, 2H), 2.83 (d, J = 4.4 Hz, 3H), 2.55 – 2.51 (m, 2H), 2.18 (s, 6H).^{13}C NMR (100 MHz, DMSO-d_6) δ196.07, 169.56, 168.91, 152.99, 150.93, 143.19, 139.72, 136.10, 135.51, 131.50, 128.32, 128.02, 126.26, 121.20, 120.70, 120.49, 117.03, 57.32, 56.88, 50.56, 49.22, 44.78, 34.48, 26.29.HRMS m/z: calcd. for $C_{29}H_{37}N_8O_3S_2$ $[M+H]^+$: 609.2423; found: 609.2423.

4.1.9.26.2-(dimethylamino)ethyl 4-(4-((4-((2-(methylcarbamoyl)phenyl)amino)-5-

*(trifluoromethyl)pyrimidin-2-yl)amino)benzoyl)piperazine-1-carbodithioate (**14z**)*

Yield 78%. Mp: 191-192 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (s, 1H), 10.11 (s, 1H), 8.79 (d, J = 4.4 Hz, 1H), 8.51 (s, 2H), 7.79 (dd, J = 14.4, 8.0 Hz, 3H), 7.55 (t, J = 7.6 Hz, 1H), 7.40 (d, J = 8.4 Hz, 2H), 7.22 (t, J = 7.6 Hz, 1H), 4.34 (s, 2H), 4.05 (dd, J = 14.0, 6.8 Hz, 2H), 3.68 (s, 4H), 3.41 (t, J = 6.8 Hz, 2H), 2.82 (d, J = 4.0 Hz, 3H), 2.53 (t, J = 6.4 Hz, 2H), 2.19 (s, 6H).^{13}C NMR (100 MHz, DMSO-d_6) δ 196.13, 170.31, 169.23, 168.76, 160.59, 156.25, 155.87, 141.14, 138.49, 131.15, 128.69, 128.00 (d, J = 14.9 Hz), 125.84, 123.26 (d, J = 19.0 Hz), 122.83, 122.45, 119.33, 59.8, 57.3, 50.5, 49.2, 44.8, 15z.5, 26.2. HRMS m/z: calcd. for $C_{29}H_{15z}F_3N_8O_2S_2$ $[M+H]^+$: 647.2198; found: 647.2188.

4.2 Biochemistry

4.2.1. In vitro FAK activity assay

The ADP-glo kinase assay (Promega, USA) was used to screen FAK inhibitors. The 5 μL reaction solution included 2.6 ng FAK, 0.4 μg/μL peptide substrate poly (4:1 Glu, Tyr), 25 μM ATP and test compounds at indicated final concentrations or DMSO as negative control. The test was performed in a 384-well plate according to the instructions of the manufacturer. Briefly, the reaction solution was incubated at room temperature for 1 h. Then 5 μL of ADP-Glo Reagent was added and incubated at room temperature for 40 min to stop the kinase reaction and deplete the unconsumed ATP. Finally, 10 μL of kinase detection reagent was added into the well and incubated for 30 min to convert ADP to ATP and produced a luminescence signal. The signal was measured by a microplate reader (Flexstation 3). The IC_{50} values were calculated

using Prism Graphpad software.

4.2.2. Cell culture and MTS assay

Cell lines (HCT116, PC-3, U87-MG, MCF-7) were cultured in Dulbecco's Modified Eagle Media (DMEM) supplement with 9% fetal bovine serum (FBS) as well as 1% (v/v) penicillin and streptomycin at 37 °C in 5% CO_2. Cell viability was detected with the MTS assay (Promega, USA) according to the manufacturer's instructions as we previously reported. Briefly, 2000 cells suspended in DMEM medium were plated into per well of 96-well plates and incubated at 37 °C in 5% CO_2 overnight. The cells were treated with the tested compounds at indicated final concentrations or DMSO (negative control) for 72 h. TAE226 and Defactinib were used as positive control. Then 20 μL MTS solution was added to each well and incubated at 37 °C for 3 h. Absorbance of each well was determined by a microplate reader (Flexstation 3) at a 490 nm wavelength. The IC_{50} values were calculated using Prism Graphpad software of the triplicate experiments.

4.2.3. Cell cycle analysis

MCF-7 cells (1×10^6 cells) were incubated in 6 cm dishes and incubated for 24 h. Then the cells were treated with DMSO or different concentrations of compound **14z**. After incubation of 24 h, the cells were harvested and washed twice with cold PBS. Then the cells were fixed in ethanol (70%) at 4 °C overnight. The cells were centrifuged to remove the fixing solution and washed twice with cold PBS. Finally the cells were stained with 5μL 7-AAD (BD) at room temperature in dark for 10 min. The cells were analyzed by flow cytometry (FACSVerse™, BD).

4.2.4. Cell apoptosis analysis

MCF-7 cells (1×10^6 cells) were incubated in 6 cm dishes and incubated for 24 h. Then the cells were treated with DMSO or different concentrations of compound **14z**. After incubation of 24 h, the cells were harvested and washed twice with cold PBS. Annexin V and 7-AAD staining protocols were applied according to the manufacturer's instructions. The cells were analyzed by flow cytometry (FACSVerse™, BD).

4.2.5. Cell migration analysis

MCF-7 cells (5×10^4 cells) with or without compound **14z (**0.005, 0.05, 0.1 μM) were suspended in serum-free medium and incubated for 30 min. Then the cells were seeded into the upper chamber (Corning Life Science), while the medium containing 15% fetal bovine serum was added in the lower compartment. After incubation for 24 h, the non-migrated cells on the upper side of the membrane were removed with a cotton swab. The migrated cells on the other side of the membrane were stained with 0.1% crystal violet. Attached cells were imaged under an inverted florescence microscope. The cells that were randomly chosen from three fields were analyzed using ImageJ and Prism Graphpad software.

4.2.6. Tube formation assay

HUVEC (3×10^4 cells) with or without compound **14z (**0.005, 0.05, 0.1 μM) were incubated for 30 min. Then the cells were plated into the 96-well plate coated with 50 μL Matrigel basement. After the incubation of 6 h, endothelial cell tubular structure was observed under an inverted florescence microscope.

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Highlights

- A series of 2,4-diarylaminopyrimidine derivatives containing dithiocarbamate moiety were synthesized as FAK inhibitors.
- Compound **14z** shows excellent antiproliferative effects with IC_{50} values from 0.001 μM to 0.06 μM on HCT116, PC-3, U87-MG and MCF-7 cell lines.
- Compound **14z** exhibits potent FAK inhibitory activity.
- Compound **14z** arrests cell cycle at G2/M phase and induces cell apoptosis.
- Compound **14z** inhibits cell migration and angiogenesis.