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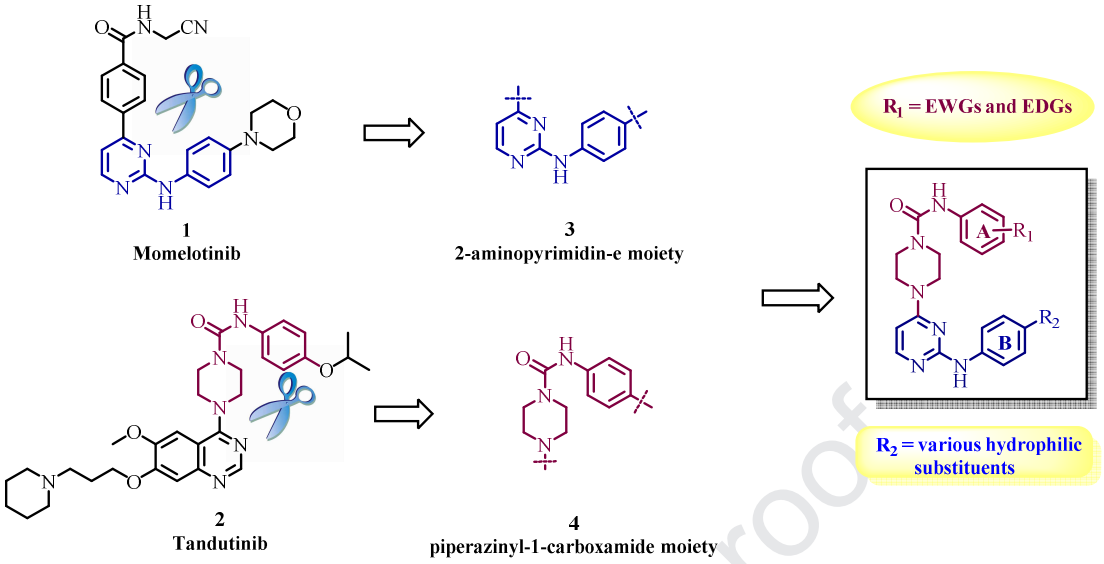
Graphical Abstract



A series of 4-piperazinyl-2-aminopyrimidine derivatives were designed, synthesized and evaluated for their biological activities.

Discovery of 4-piperazinyl-2-aminopyrimidine derivatives as dual inhibitors of JAK2 and FLT3

Yingxiu Li [a], Tianyu Ye [a], Le Xu [a], Yuhong Dong [a], Yong Luo [a], Chu Wang [a], Yufei Han [a], Ke Chen [a], Mingze Qin [a, b], Yajing Liu [a*], Yanfang Zhao [a†]

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Abstract: Hybridization strategy is an effective strategy to obtain multi-target inhibitors in drug design. In this study, we assembled the pharmacophores of momelotinib and tandutinib to get a series of 4-piperazinyl-2-aminopyrimidine derivatives. All compounds were tested for the inhibition of JAK2 and FLT3 enzymes, of which, compounds with potent enzyme activities were assayed for antiproliferative activities against three cancer cell lines (HEL, MV4-11, and HL60). The structure-activity relationship studies were conducted through variations in two regions, the "A" phenyl ring and "B" phenyl ring. Compound **14j** showed the most balanced *in vitro* inhibitory activity against JAK2 and FLT3 (JAK2 IC_{50}=27 nM, FLT3 IC_{50}=30 nM), and it also showed potent inhibition against the above tested cell lines. In the cellular context, **14j** strongly induced apoptosis by arresting cell cycle in the G_1/S phase, and was selected as a promising JAK2/FLT3 dual inhibitor.

Keywords: Hybridization strategy; 4-piperazinyl-2-aminopyrimidine derivatives; dual inhibitor.

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1. Introduction

Hematologic malignancies are a group of diseases caused by disorders of the hematopoietic system, which mainly include leukemia, lymphoma, myelodysplastic syndrome, and multiple myeloma diseases [1]. Current treatments of hematological malignancies, such as bone marrow transplantation, hormones, and chemotherapy, have known disadvantages such as serious side effects, low cure rate, and high recurrence rate. Researchers have conducted an in-depth study of the pathogenesis of hematologic malignancies and thus found that certain genetic mutations, including mutations in the JAK2 and FLT3 genes, lead to signal transduction disorders in different tissues and cells [2-3]. Therefore, based on the pathogenesis of such diseases, a variety of single-target and multi-target inhibitors have been developed [4-7] such as tofactinib, ruxolitinib, momelotinib, and pacritinib. Due to the unique mechanism of JAK2 and FLT3 kinases *in vivo* and their close relationship with hematologic malignancies, the development of JAK2/FLT3 dual inhibitors has brought new hopes for patients to cure such diseases safely and effectively.

The Janus kinases (JAK1, JAK2, JAK3, and TYK2) belong to the family of intracellular protein tyrosine kinases, which play an essential role in the signaling of a variety of cytokines. Activation of JAKs by different cytokines results in phosphorylation and dimerization of the STAT (signal transducers and activators of transcription) proteins, which further translocate to the nucleus and activate gene transcription [8]. JAK2-STATs pathway is one of the most important pathways of cell signal transduction, and plays an important role in regulating the normal physiological

and pathological responses of the human body. The abnormal activity of JAK2 can dysregulate the JAK2-STATs signaling pathway, which leads to various malignant diseases [3, 9-10].

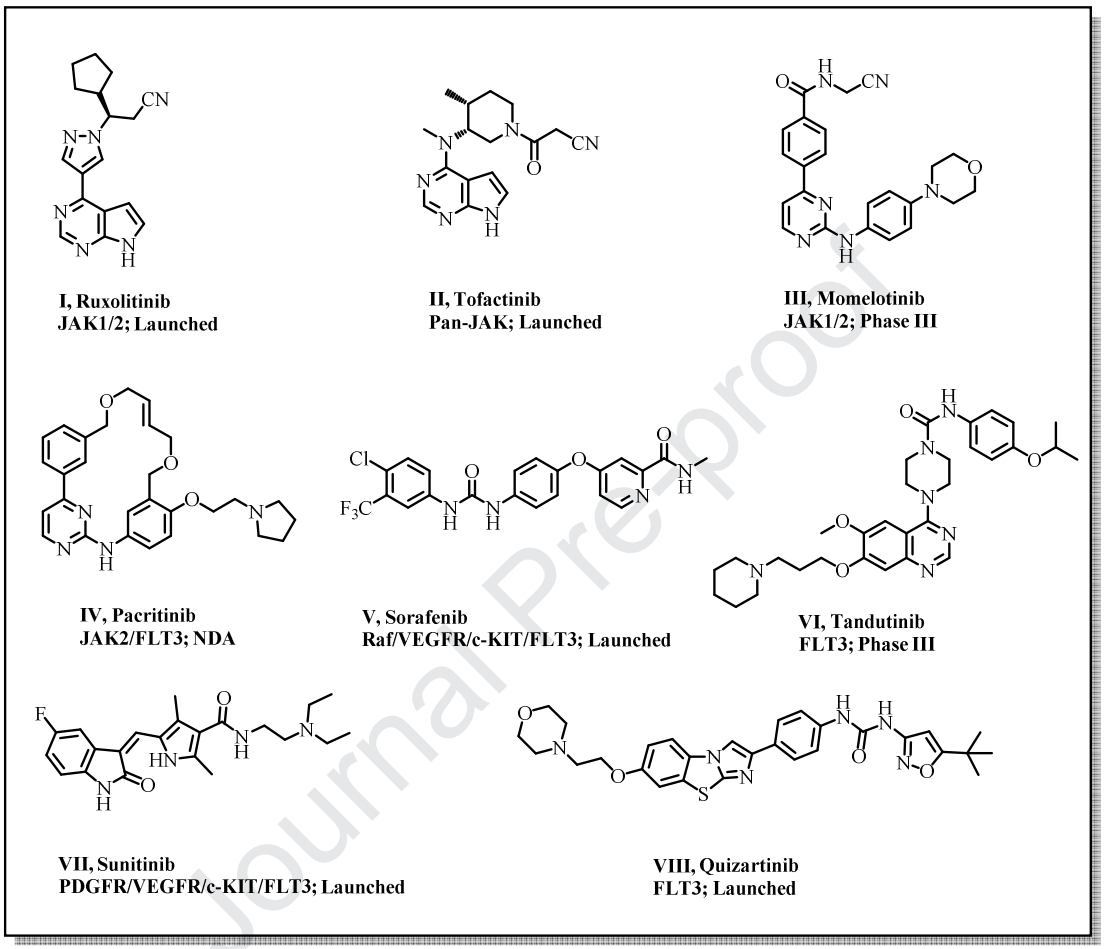


Fig. 1. Structures of representative JAK2 or FLT3 inhibitors

Currently, a number of pan-JAK and selective JAK inhibitors have been discovered (**Fig. 1**). JAK1/2 inhibitor ruxolitinib (**I**) and pan-JAK inhibitor tofacitinib (**II**) were successively approved by FDA for the treatment of myelofibrosis (MF), polycythemia vera (PV), and rheumatoid arthritis (RA), respectively. [11]. Another JAK1/2 inhibitor, momelotinib (**III**), is currently in phase III clinical tial [12]. Although JAK2 inhibitors provide a new option for the treatment of MF or other blood diseases, studies have shown that toxic side effects such as myelosuppression

and cytopenia are the main factors limiting the use of these drugs [13].

FLT3 (FMS-like tyrosine kinase-3), a class III RTK, is the most frequently mutated gene in acute myeloid leukemia (AML) and plays an important role in the maintenance, growth, and development of hematopoietic and non-hematopoietic cells [14]. The first wave of FLT3 inhibitors belongs to tyrosine kinase inhibitor (TKIs), which were initially developed for the treatment of solid tumors. These drugs were originally designed to inhibit other kinases, but occasionally found to be active against FLT3 (e.g., sorafenib, tandutinib, sunitinib) (**V-VII**, **Fig. 1**). Because these TKIs can inhibit a variety of kinases and FLT3, off-target effects and significant toxicity are inevitable [15]. In contrast to the numerous multi-kinase inhibitors that have been adopted as FLT3 inhibitors, quizartinib (**VIII**, **Fig. 1**) was designed specifically to target FLT3, which is in the New Drug Application (NDA) phase now. [16]

Studies have shown that JAK2/FLT3 dual inhibitors could regulate JAK2-STATs signaling pathway and inhibit phosphorylated FLT3 kinase. In the SET2 cell AML mouse models carrying JAK2-V617F and FLT3-ITD mutations, JAK2/FLT3 inhibitors could effectively inhibit the growth and transfer of tumor [18]. Pacritinib (SB1518, **IV, Fig. 1**), a JAK2/FLT3 inhibitor in the New Drug Application (NDA) phase, showed better therapeutic effects than ruxolitinib in patients with severe thrombocytopenia in its clinical trials, while its bone marrow toxicity and clinical response were comparable to ruxolitinib [19-20]. These results indicate that JAK2/FLT3 combined inhibitors have great potential on the treatment of MF and

AML.

In this paper, we utilized the hybridization strategy to design and synthesize a series of 4-piperazinyl-2-aminopyrimidine analogues as dual JAK2/FLT3 inhibitors. All compounds were assayed for the *in vitro* enzymatic inhibitory activities against JAK2 and FLT3. Based on the enzymatic inhibitory results, potent compounds were assayed for antiproliferative activities against three cancer cell lines, namely, HEL, MV4-11, and HL60. To further clarify the primary mechanism, cell apoptosis and cell cycles on HEL cell line were examined for **14j** by flow cytometry.

2. Results and discussion

2.1 JAK2/FLT3 dual inhibitors Designed by Hybridization strategy

Pharmacophore of JAK2 inhibitors is composed of two hydrophobic groups (two phenyl rings), a hinge-region binder (usually an amino-pyrimidine), and a solvent-exposed region [21]. FLT3 inhibitors (**Fig. 1**) such as quizartinib (**V**) and tandutinib (**VI**), usually consist of a urea linker, two hydrophobic groups (two phenyl rings), and a solvent-exposed tail. Given that the 2-aminopyrimidine derivatives have been widely reported as JAK2 inhibitors, the piperazinyl-1-carboxamide (**4, Fig. 2**), a bioisostere of diaryl urea based on tandutinib (**2**, **Fig. 2**) as a FLT3 inhibitor, was introduced to 4-position of pyrimidine moiety in order to obtain dual inhibitors against JAK2 and FLT3 (**Fig. 2**).

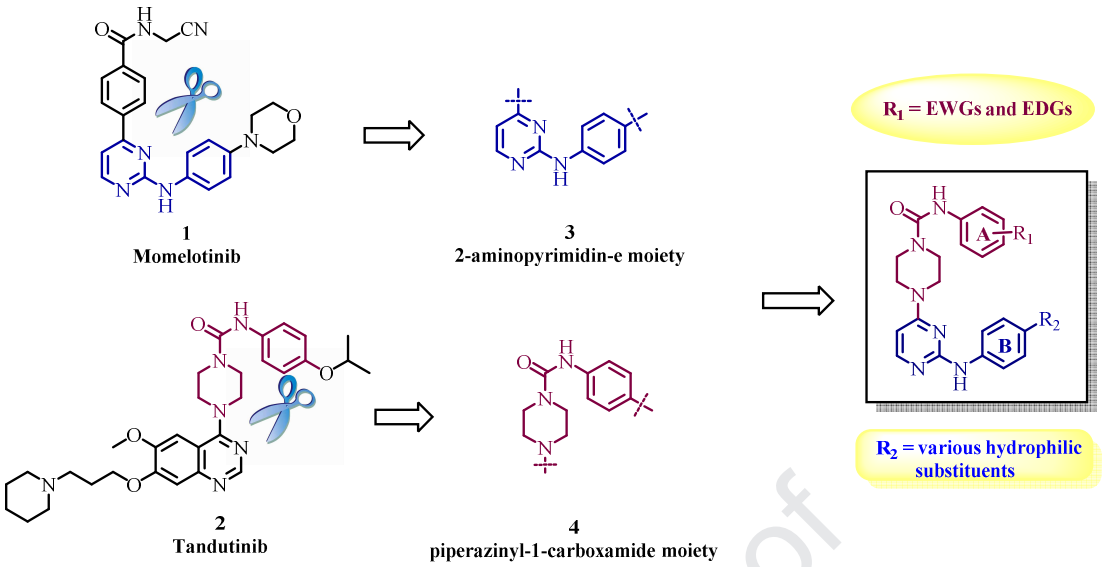


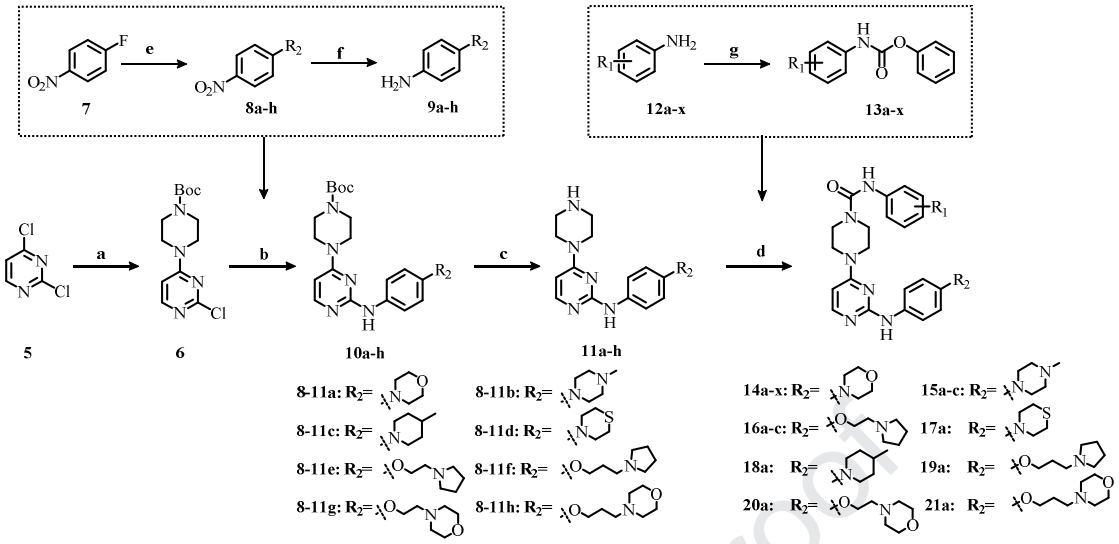
Fig. 2. Design strategy for the target compounds.

2.2 Chemistry

The synthetic route of target compounds is shown in **Scheme 1**. Commercially available starting material 2,4-dichloropyrimidine (**5**) reacted with *N*-Boc-piperazine to yield **6** [22], which was then reacted with various anilines **9a-h** in the catalysis of trifluoroacetic acid in *n*-butyl alcohol to yield intermediates **10a-h** [23]. After treating **10a-h** with trifluoroacetic acid in dichloromethane, intermediates **11a-h** were obtained. Subsequently, target compounds **14a-x**, **15a-c**, **16a-c**, **17-19a** were available *via* condensation of **11a-h** with different phenyl carbamates, respectively.

Anilines **9a-h** were prepared in two steps. Aromatic nucleophilic substitution of fluorine in 4-fluoromitrobenzene by reacting alcohols or secondary amines was processed to yield intermediates **8a-h** in DMF at 25 ^{o}C, which were subsequently reduced to corresponding anilines **9a-h** by using H_2-Pd/C in EtOH and generally used without further purification [24]. An assortment of phenylcarbamates was synthesized by treating different substituted anilines with phenyl chloroformate in the presence of

sodium carbonate at 25 °C [25].



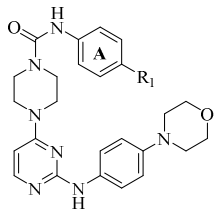
Scheme 1. General scheme for the synthesis of target compounds; Reagents and conditions: (a) *N*-Boc-piperazine, TEA, DMF, rt, 4 h; (b) TFA, *n*-BuOH, 120 °C, 1 h; (c) TFA, DCM, rt, 12 h; (d) DIPEA, DMF, 40 °C, 12 h; (e) TEA, acetonitrile, 80 °C, 3 h; (f) Pd-C, H_2, EtOH; (g) phenyl chloroformate, sodium carbonate, THF/EA/H_2O, rt, 12 h.

2.3 Bioactivity and discussion

2.3.1 Design Rationale and Structure−Activity Relationship (SAR) Exploration.

We first synthesized compound **14a**, which retains the *iso*-propoxyl group at "A" phenyl ring from **tandutinib**, and **14b-14h** with other different substituents. The results of the kinase-inhibition assays are listed in **Table 1** with pacritinib, momelotinib and tandutinib as the positive controls.

Table 1. SAR exploration of different substituents in "A" phenyl ring



Cpd	R_1	JAK2 inhibition @ 100nM[a]	FLT3 inhibition @ 100nM[a]
14a		**11.1%**	**22.9%**

14b		**53.7%**	**27.8%**
14c		**54.8%**	**27.8%**
14d		**53.3%**	**27.4%**
14e		**48.7%**	**21.7%**
14f		**41.2%**	**0%**
14g		**79.3%**	**84.4%**
14h		**78.0%**	**70.8%**
14i		**35.2%**	**36.5%**
14j		**86.7%**	**80.7%**
14k		**64.4%**	**62.8%**
14l		**86.3%**	**81.1%**
14m		**68.1%**	**35.8%**
14n		**40.6%**	**33.1%**
14o		**20.0%**	**17.2%**
14p		**75.8%**	**53.9%**
14q		**86.0%**	**79.7%**
momelotinib	-	**94.7%**	**ND**[b]
tandutinib	-	**ND**[b]	**46.1%**
pacritinib	-	**92.0%**	**96.0%**

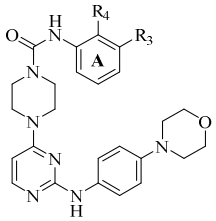
[a] All compounds were assayed at least twice, and the inhibitory values were averaged.
[b] Not determined.

As shown in **Table 1**, compounds with electron-withdrawing groups (EWGs), such as **14g** and **14h**, showed more potent inhibitory activities against JAK2 and

FLT3 than those with electron-donating groups (EDGs, see **14a-f**). Base on the results, a hypothesis was proposed that EWGs confers greater potency against both JAK2 and FLT3 than EDGs on the "A" phenyl ring. Therefore, some other EWGs were introduced to the "A" phenyl ring and compounds **14i-q** were obtained (**Table 1**).

When the carbonyl-like groups were introduced (**14j, 14l,** and **14q)**, both JAK2 and FLT3 inhibition rates increased up to 80%. The resulting increase in inhibition rates might be attributed to the H-bond formed between the carbonyl and ARG980 in JAK2 and ASP829 in FLT3 (**Fig. 3A**). However, when the "length" of amide in **14l** was enhanced, such as compounds **14m-o**, a decrease in their inhibitory activities against JAK2 and FLT3 was observed, especially against FLT3. The probable cause was the tail group of compounds located in a quite narrow channel which was formed by VAL675, PHE691, CYS828 and ASP829 in FLT3 (**Fig. 3C**). Therefore, the size of the tail group cannot be too large.

Table 2. SAR exploration of EWG substituents in "A" phenyl ring



Cpd	R_3	R_4	JAK2 inhibition @ 100nM[a]	FLT3 inhibition @ 100nM[a]
14r	CN	H	**8.1%**	**15.9%**
14s	CF_3	H	**35.2%**	**36.5%**
14t	H	CF_3	**21.8%**	**15.0%**
14u		H	**2.0%**	**3.3%**

14v			**18.9%**	**13.8%**
14w			**74.0%**	**39.8%**
14x			**9.5%**	**12.5%**

[a] All compounds were assayed at least twice, and the inhibitory values were averaged.

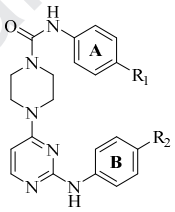
To investigate the effects of the substituents and substituent positions at "A" phenyl ring, compounds **14r-x** were synthesized (**Table 2**). After a comparison among **14j**, **14u,** and **14v**, it can be easily concluded that the compound with the *para*-substituent, **14j,** showed the greatest potency against JAK2 and FLT3, and a similar trend can be found among other analogues with their parent compounds. Therefore, *para*-substituted compounds **14j**, **14l,** and **14q** were chosen for further modifications. Although compound **14g** showed similar activities to **14j**, it was not included because the nitro group is a well-known structural alert in drug design.

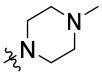
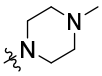
In the following modifications, we paid attention to the hydrophilic groups at 4-position of "B" phenyl ring, and the HEL cell line harboring the JAK2-V617F mutation was applied in further cellular profiling (**Table 3**). Replacement of the morpholino with *N*-methylpiperazinyl group (**15a-c**) retained the activities against JAK2 (inhibition rates at 100 nM range from 80.8% to 86.0%) and FLT3 (inhibition rates at 100 nM range from 85.0% to 87.5%), but replacement of morpholino group with 4-(2-pyrrolidinyl)ethoxy (**16a-c**) decreased activities against JAK2 (inhibition rates at 100 nM range from 45.0% to 59.0%) and FLT3 (inhibition rates at 100 nM range from 69.0% to 82.0%). It is noteworthy that compounds with amide moiety (**14l**, **15b**, and **16b**) or sulfonamide moiety (**14q**, **16c**, and **15c**) lose their antiproliferative activities against HEL cell line (>50 μM). This situation might be caused by an

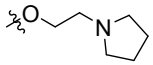
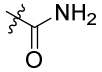
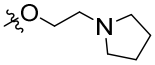
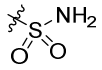
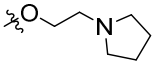
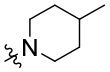
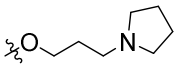
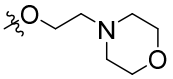
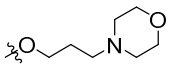
increase in the compounds' t-PSA, which influences their permeability (**Table 3**). Therefore, amide and sulfonamide moieties were not considered in the next modification.

Compounds **17-21a** were synthesized to investigate the effects of other hydrophilic groups, such as thiomorpholinyl, 4-methylpiperidinyl, and 4-(2-pyrrolidinyl)ethoxy. The results showed that they all have a decrease in inhibitory activities against JAK2 and FLT3 at 100 nM; similar trend was observed in cellular potency against HEL (GI_{50} values ranged from 3.3 μM to 26.4 μM). As a result, the morpholinyl group was found to be the best hydrophilic substituent in the designed compounds.

Table 3. SAR optimization of hydrophilic groups at "B" phenyl ring



Cpd	R_1	R_2	JAK2 inhibition @ 100nM[a]	FLT3 inhibition @ 100nM[a]	HEL GI_{50}[b]	t-PSA[c]
14j			**86.7%**	**80.7%**	**4.3μM**	**102.93**
14l			**86.3%**	**81.1%**	**>50μM**	**128.95**
14q			**86.0%**	**79.7%**	**>50μM**	**154.40**
15a			**80.8%**	**87.5%**	**14.1μM**	**96.94**
15b			**84.0%**	**85.0%**	**>50μM**	**122.96**
15c			**76.0%**	**86.0%**	**>50μM**	**148.41**

16a			**45.0%**	**69.0%**	**30.3μM**	**102.93**
16b			**53.0%**	**74.0%**	**>50μM**	**128.95**
16c			**59.0%**	**82.0%**	**>50μM**	**154.40**
17a			**73.9%**	**77.6%**	**4.7μM**	**93.70**
18a			**53.0%**	**54.0%**	**26.4μM**	**93.70**
19a			**60.7%**	**83.2%**	**3.3μM**	**102.93**
20a			**61.7%**	**70.6%**	**8.9μM**	**112.16**
21a			**60.9%**	**70.9%**	**10.7μM**	**114.96**

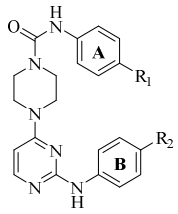
[a] All compounds were assayed at least twice, and the inhibitory values were averaged.

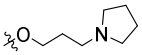
[b] All GI_{50} values are the average of three replicates.

[c] The t-PSA values were calculated using Marvin Sketch, version 6.1.0, with standard settings.

Among the series of compounds mentioned above, compounds having more than 80% inhibition rates against JAK2 and FLT3 were selected for further *in vitro* kinase and cellular profiling (**Table 4**). Compared to **14j**, compounds **14l** and **14q** both had higher t-PSA, and the aforementioned decrease in their permeability resulted in the loss of antiproliferation against HEL, MV4-11, and HL60 cell lines; therefore, amide and sulfonamide moieties at "B" phenyl ring were unfavorable. In summary, compound **14j** exhibited an overall balanced profile meeting all target criteria. It was shown to be active against JAK2 with IC_{50} value of 27 nM and FLT3 with IC_{50} value of 30 nM. Moreover, **14j** showed good inhibition against HEL with IC_{50} value of 4.3 μM and moderate inhibition against MV4-11 and HL60 (11.0 μM and 16.5 μM, respectively). Thus, **14j** was selected as the candidate for further study.

Table 4. In *vitro* profiling leading to 14j as a candidate



Cpd	R_1	R_2	JAK2 IC_{50}[a]	FLT3 IC_{50}[a]	HEL GI_{50}[b]	MV4-11 GI_{50}[b]	HL60 GI_{50}[b]
14j			**27nM**	**30nM**	**4.3μM**	**11.0μM**	**16.5μM**
14l			**11nM**	**21nM**	**>50μM**	**>50μM**	**>50μM**
14q			**9.3nM**	**30nM**	**>50μM**	**>50μM**	**>50μM**
15a			**13.8nM**	**12nM**	**14.1μM**	**32.0μM**	**46.4μM**
17a			**29.8nM**	**40nM**	**4.7μM**	**17.8μM**	**>50μM**
19a			**65.0nM**	**13nM**	**3.3μM**	**11.0μM**	**29.4μM**
Pacritinib	-	-	**23.0nM**[c]	**22nM**[c]	**1.1μM**	**1.6μM**	**1.4μM**

[a] All compounds were assayed at least twice, and the inhibitory values were averaged.

[b] All GI_{50} values are the average of three replicates.

[c] Data from *J. Med. Chem*, **2012**, *55*, 2623−2640.

*2.3.2 Effect of compound **14j** on cell cycle phases*

The effect of compound **14j** on various phases of cell cycle progression was tested in HEL cells. After treatment of HEL cells with compound **14j** for 72 h at varied concentrations (0.33μM, 1.0μM, 3.0μM, 9.0 μM), the cells were fixed and stained with propidium iodide (PI) and the DNA content was analyzed by flow cytometry. The obtained results were compared with those of non-treated HEL cells as control. As shown in **Fig. 3**, the percentage of cells in the G_1 populations increased from 43.12% (as control group) to 54.07%, 61.44%, 71.85%, and 78.05%, respectively. These results confirmed that compound **14j** arrested the cell-cycle

progression of the former cell line into the G_1/S phases in a dose-dependent manner.

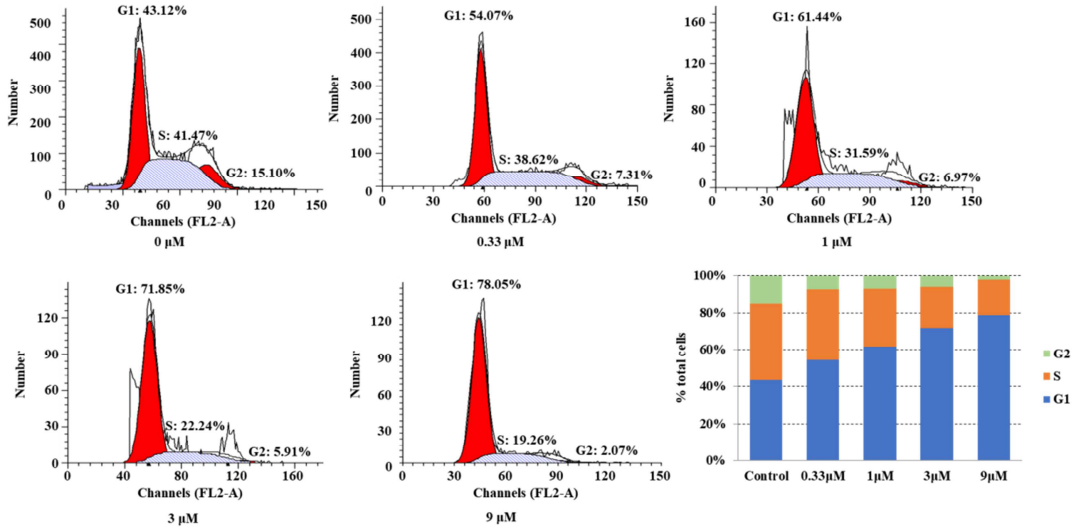


Fig. 3. Effect of compound **14j** on the cell cycle distribution of HEL cells. HEL cells treated with **14j** at 0.33 μM – 9 μM for 72 h were assayed by flow cytometer after staining with PI.

*2.3.3 Cell apoptosis study of compound **14j***

To characterize the mode of cell death induced by compound **14j**, biparametric-flow-cytometric analysis with Annexin-V and propidium iodide (PI) was performed. Flow cytometry was used to investigate whether the antiproliferative effect of **14j** was caused by the activation of cellular apoptosis in HEL cells. Quantitative analysis of early-apoptotic cells and advanced-apoptotic and necrotic cells was determined *via* an Annexin V-FITC and PI assay. HEL cells were stimulated with 0.33 μM−9 μM concentrations of compound **14j** for 72 h. DMSO was used as the negative control. As shown in **Fig. 4**, the apoptosis induced by compound **14j** was much greater than that by the control, and HEL cells underwent both early apoptosis (Annexin V+ and PI−, lower right) and advanced apoptosis and necrosis (Annexin V+ and PI+, upper right). The results showed that compound **14j** could effectively induce cell apoptosis starting from 1 μM with enhanced effects at higher concentrations. The apoptosis ratios of compound **14j** measured at different concentrations were 8.37%

(0.33 μM), 39.37% (1 μM), 65.47% (3 μM), and 65.93% (9 μM), and the apoptotic effect increased in a dose-dependent manner. From these results, we can conclude that **14j** caused effective apoptotic effects on the HEL cell line.

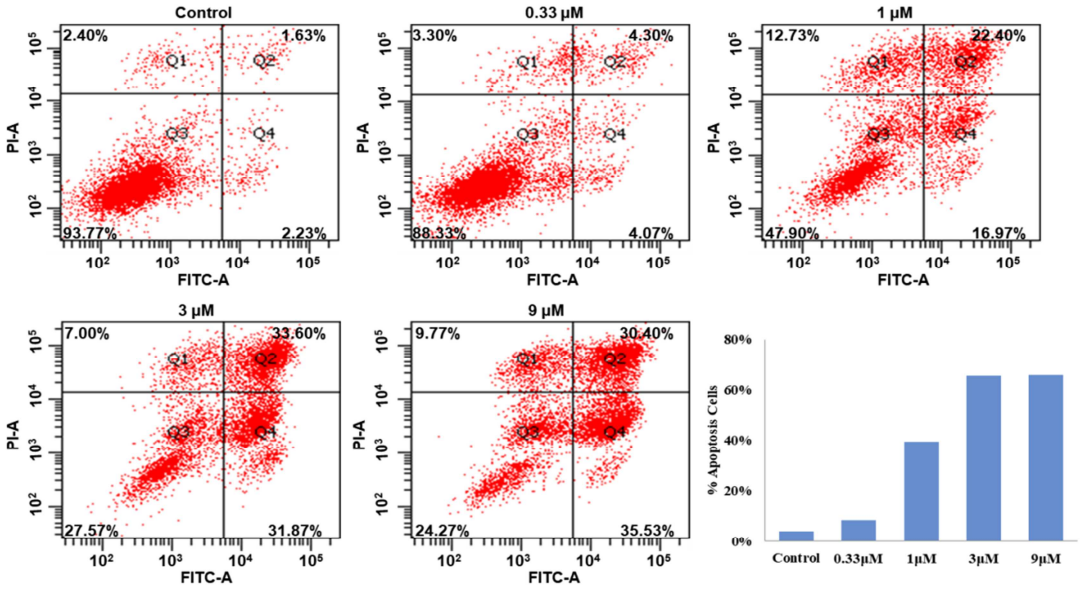


Fig. 4. Cell apoptosis induced by compound **14j**. HEL cells were treated with **14j** at 0.33 μM – 9 μM for 72 h. Apoptosis was examined by flow cytometer.

*2.3.4 Binding modes of compound **14j***

Compound **14j** shows optimal JAK2 and FLT3 inhibitory activities; hence, the binding mode of **14j** was elucidated using a docking model (**Fig. 5**). As shown in **Fig. 3A**, **14j** binds to the ATP-binding site of JAK2 in an orientation similar to that of pacritinib (**Fig. 5B**). The 2-aminopyrimidine moiety of **14j** forms two conserved hydrogen bonds with LEU932 in the hinge region of JAK2 which guaranteed the JAK2 potency; meanwhile the pyrimidinyl group together with adjacent ALA880, LEU855, VAL863, and LEU983 formed a netlike alkyl-π interaction. In addition, the morpholine moiety was exposed in the solvent area and formed a hydrogen bond with LYS943, and another hydrogen bond was formed between NH of

N-phenylpiperazine-1-carboxamide and LEU 855.

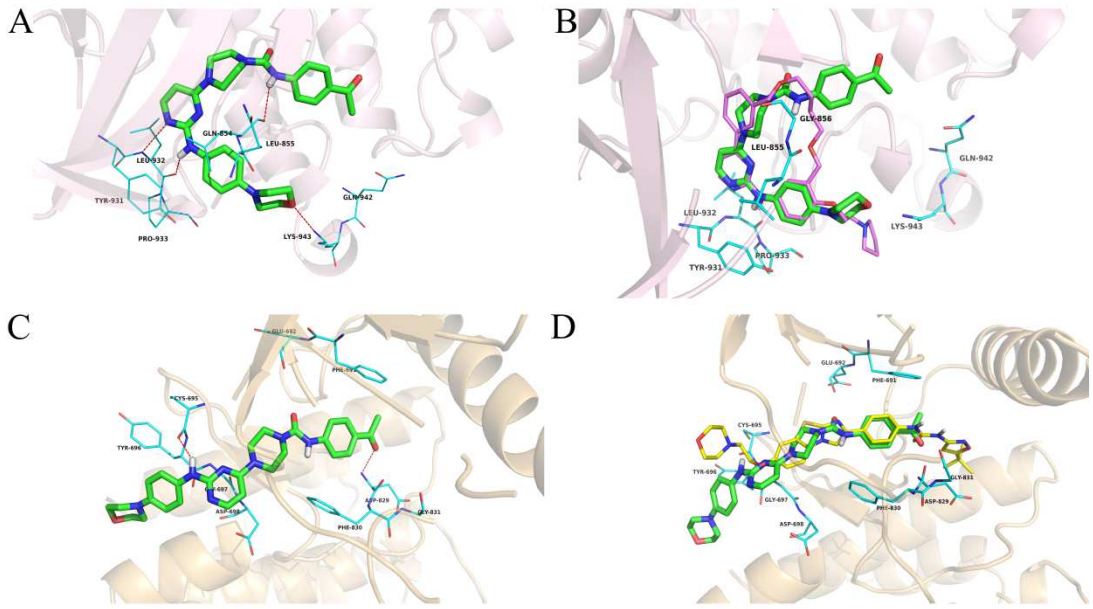


Fig. 5. Binding-mode analysis of compound **14j** to JAK2 kinase and FLT3 kinase, hydrogen bonds were shown as red dashes. (A) Compound **14j** docked into JAK2 kinase (PDB code 4AQC). (B) Overlapping images of compound **14j** (green) and pacritinib (violet) complexed with JAK2. (C) Compound **14j** docked into FLT3 kinase (PDB code 4XUF). (D) Overlapping images of compound **14j** (green) and quizartinib (yellow) complexed with FLT3.

In **Fig. 3C**, we found the configuration of **14j** resembles that of quizartinib (**Fig. 5D**). A hydrogen bond was observed between NH of 2-aminopyrimidine and CYS695, and the morpholine tail was fully stretched into the solvent-exposed area. Furthermore, a π-π stacked interaction was formed by "B" phenyl ring with TYR696. Another hydrogen bond was formed between the oxygen atom of acetophenone moiety with ASP829, and the "A" phenyl ring was strongly fixed at the narrow hydrophobic channel by two edge-to-face π-π stacked interactions with PHE691 and PHE830. In general, the docking results further confirm the rationality of our design strategy.

3. Conclusion

A series of novel 4-piperazinyl-2-aminopyrimidine derivatives were initially designed and synthesized as JAK2/FLT3 dual inhibitors. In particular, compound **14j**

showed the most balanced antiproliferative activity against JAK2 and FLT3 with IC_{50} value of 27 nM and 30 nM, respectively. It also showed good inhibition against HEL with GI_{50} value of 4.3 μM and moderate inhibition against MV4-11 and HL60 (GI_{50} values of 11.0 μM and 16.5 μM, respectively). Furthermore, cell cycle analysis of **14j** on HEL cells by flow cytometry showed that cell cycle was arrested in G_1/S phase, and thereby induced the apoptosis of HEL cells in a dose-dependent manner. All these reported results suggested that compound **14j** might be a "hit" compound for JAK2-FLT3 dual inhibitor.

4. Experimental procedures

4.1. Chemistry

All melting points were acquired on a Mettler Melting Point MP70 apparatus (Mettler, Toledo, Switzerland) without calibration. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC-MS (Agilent, palo Alto, CA, USA). High-resolution mass spectra (HRMS) were measured with an Agilent Accurate-Mass Q-TOF 6530 in ESI mode (Agilent, Santa Clara, CA, USA). The reverse phase HPLC was conducted on an Agilent 1260 Infinity chromatograph, which was equipped with ZORBAX SB-C18 column (250 mm × 4.6 mm). The mobile phase A was methanol, and mobile phase B was 30 mM NaH_2PO_4 in water (pH 2.5). The gradient of 5−95% A was run at a flow rate of 1.0 mL/min over 30 min. Reactions were monitored by thin layer chromatography (TLC) on silica plates (F-254) and visualized under UV light. ^{1}H NMR and ^{13}C NMR spectra were performed using Bruker spectrometers (Bruker Bioscience, respectively, Billerica, MA, USA) with TMS as an internal standard.

Column chromatography was run on silica gel (200-300 mesh) from Qingdao Ocean Chemicals (Qingdao, Shandong, China). Unless otherwise noted, all materials were obtained from commercially available sources and used without further purification.

4.1.1. General procedure for preparation of intermediate **6**

A solution of 2, 4-dichloropyrimidine (**5**) (10.0 g, 67.1 mmol) and Et_3N (11.1 mL, 80.5 mmol) in DMF (100 mL) was added dropwise to a cooled solution (-10 ^oC) of *N*-Boc-piperazine (13.1 g, 70.5 mmol) in DMF (100 mL). The mixture was then stirred for 4 h at room temperature. The resulting mixture was poured into stirring ice-water (500 mL), and then the white solid was filtered and dried under reduced pressure. The crude product was further purified by flash column chromatography using PE/EtOAc (1:1) as eluent to afford 15.3 g of **6** as a white solid; yield: 76.2%; ^1H NMR (600 MHz, DMSO-d_6) δ 8.10 (d, *J* = 6.2 Hz, 1H), 6.83 (d, *J* = 6.2 Hz, 1H), 3.62 (m, 4H), 3.47 – 3.37 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.76, 159.89, 157.86, 154.17, 102.85, 79.61, 43.57, 28.40; m.p.: 170.6 – 173.2 ^oC.

4.1.2 General procedure for preparation of intermediates **8a-h**

*4.1.2.1. 4-(4-nitrophenyl)morpholine (**8a**).* To a stirred solution of 1-fluoro-4-nitrobenzene **7** (2.0 g, 14.2 mmol) in 20 mL acetonitrile was added morpholine (1.9 g, 21.3 mmol) followed by triethylamine (4.3 g, 5.9 mL, 42.5 mmol). The mixture was stirred at reflux for 3 h. After the reaction was cooled to room temperature, it was poured into 80 mL water and extracted with ethyl acetate (2×80 mL). The combined organic layers were washed with brine (60 mL), dried over

sodium sulfate, concentrated in *vacuo,* then dried under vacuum to obtain 2.7 g of **8a** as yellow solid; yield: 92%; **8a** was used without further purification.

*4.1.2.2. 1-methyl-4-(4-nitrophenyl)piperazine (**8b**).* Synthesized using the procedure for **8a** using 2.0 g (14.2 mmol) of **7** and 2.1 g (21.3 mmol) of 1-methylpiperazine in 20 mL of acetonitrile and 5.9 mL of trimethylamine, and 2.8 g of **8b** was obtained as yellow solid; yield: 89%.

*4.1.2.3. 4-methyl-1-(4-nitrophenyl)piperidine (**8c**).* Synthesized using the procedure for **8a** using 2.0 g (14.2 mmol) of **7** and 2.1 g (21.3 mmol) of 4-methylpiperidine in 20 mL of acetonitrile and 5.9 mL of trimethylamine, and 2.8 g of **8c** was obtained as yellow solid; yield: 91%.

*4.1.2.4. 4-(4-nitrophenyl)thiomorpholine (**8d**).* Synthesized using the procedure for **8a** using 2.0 g (14.2 mmol) of **7** and 2.2 g (21.3 mmol) of thiomorpholine in 20 mL of acetonitrile and 5.9 mL of trimethylamine, and 2.7 g of **8d** was obtained as yellow solid; yield: 87%.

*4.1.2.5. 1-(2-(4-nitrophenoxy)ethyl)pyrrolidine (**8e**).* Synthesized using the procedure for **8a** using 2.0 g (14.2 mmol) of **7** and 2.5 g (21.3 mmol) of 2-(pyrrolidin-1-yl)ethan-1-ol in 20 mL of acetonitrile and 5.9 mL of trimethylamine, and 1.9 g of **8e** was obtained as yellow solid; yield: 58%.

*4.1.2.6. 1-(3-(4-nitrophenoxy)propyl)pyrrolidine (**8f**).* Synthesized using the procedure for **8a** using 2.0 g (14.2 mmol) of **7** and 2.8 g (21.3 mmol) of 3-(pyrrolidin-1-yl)propan-1-ol in 20 mL of acetonitrile and 5.9 mL of trimethylamine, and 2.2 g of **8f** was obtained as yellow oil; yield: 62%.

*4.1.2.7. 4-(2-(4-nitrophenoxy)ethyl)morpholine (**8g**).* Synthesized using the procedure for **8a** using 2.0 g (14.2 mmol) of **7** and 2.8 g (21.3 mmol) of 2-morpholinoethan-1-ol in 20 mL of acetonitrile and 5.9 mL of trimethylamine, and 2.6 g of **8g** was obtained as yellow oil; yield: 73%.

*4.1.2.8. 4-(3-(4-nitrophenoxy)propyl)morpholine (**8h**).* Synthesized using the procedure for **8a** using 2.0 g (14.2 mmol) of **7** and 3.1 g (21.3 mmol) of 3-morpholinopropan-1-ol in 20 mL of acetonitrile and 5.9 mL of trimethylamine, and 2.6 g of **8h** was obtained as yellow oil; yield: 69%.

4.1.3 General procedure for preparation of intermediates **9a-h**

*4.1.3.1. 4-morpholinoaniline (**9a**).* Pd-C (0.27g, 10% m/m) was added to a solution of **8a** (2.7g, 13.0 mmol) in ethanol (20ml) and hydrogenated for 12 h at room temperature. The resultant was filtered, washed with ethanol and concentrated. 2.2 g of **9a** as purple solid was obtained; yield: 94%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.68 (d, J = 8.5 Hz, 2H), 6.50 (d, J = 8.5 Hz, 2H), 4.62 (s, 2H), 3.69 (t, J = 4.8Hz ,4H),

2.87 (t, *J* = 4.8Hz ,4H). MS (ESI) m/z: 179.2 $[M+H]^+$.

*4.1.3.2. 4-(4-methylpiperazin-1-yl)aniline (**9b**).* Synthesized using the procedure for **9a** using 0.28 g (10% m/m) of Pd-C and 2.8 g (12.7 mmol) of **8b** in 20 mL of ethanol, and 2.1 g of **9b** was obtained as purple solid; yield: 88%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.67 (d, *J* = 8.8 Hz, 2H), 6.48 (d, *J* = 8.8 Hz, 2H), 4.57 (s, 2H), 2.89 (t, *J* = 4.8 Hz, 4H), 2.41 (t, *J* = 4.8 Hz, 4H), 2.19 (s, 3H). MS (ESI) m/z: 192.3 $[M+H]^+$.

*4.1.3.3. 4-(4-methylpiperidin-1-yl)aniline (**9c**).* Synthesized using the procedure for **9a** using 0.28 g (10% m/m) of Pd-C and 2.8 g (12.7 mmol) of **8c** in 20 mL of ethanol, and 2.3 g of **9c** was obtained as violet solid; yield: 96%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.67 (d, *J* = 7.2 Hz, 2H), 6.47 (d, *J* = 7.2 Hz, 2H), 4.57 (s, 2H), 3.29 (d, *J* = 10.2 Hz, 2H), 2.44 (t, *J* = 11.0 Hz, 2H), 1.65 (d, *J* = 11.7 Hz, 2H), 1.45 – 1.32 (m, 1H), 1.23 (q, *J* = 11.1 Hz, 2H), 0.92 (d, *J* = 6.5 Hz, 3H). MS (ESI) m/z: 191.3 $[M+H]^+$.

*4.1.3.4. 4-thiomorpholinoaniline (**9d**).* Synthesized using the procedure for **9a** using 0.27 g (10% m/m) of Pd-C and 2.7 g (12.0 mmol) of **8d** in 20 mL of ethanol, and 1.7 g of **9d** was obtained as light yellow solid; yield: 72%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.68 (d, *J* = 8.6 Hz, 2H), 6.48 (d, *J* = 8.6 Hz, 2H), 4.64 (s, 2H), 3.15 (t, *J* = 4.8 Hz, 4H), 2.68 (d, *J* = 4.8 Hz, 4H). MS (ESI) m/z: 195.2 $[M+H]^+$.

*4.1.3.5. 4-(2-(pyrrolidin-1-yl)ethoxy)aniline (**9e**).* Synthesized using the procedure for **9a** using 0.19 g (10% m/m) of Pd-C and 1.9 g (8.0 mmol) of **8e** in 20 mL of ethanol, and 1.2 g of **9e** was obtained as brown oil; yield: 73%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.64 (d, *J* = 8.7 Hz, 2H), 6.50 (d, *J* = 8.7 Hz, 2H), 4.59 (s, 2H), 3.90 (t, *J* = 6.0 Hz, 2H), 2.71 (t, *J* = 6.0 Hz, 2H), 2.48 (s, 4H), 1.67 (s, 4H). MS (ESI) m/z: 207.3 $[M+H]^+$.

*4.1.3.6. 4-(3-(pyrrolidin-1-yl)propoxy)aniline (**9f**).* Synthesized using the procedure for **9a** using 0.22 g (10% m/m) of Pd-C and 2.2 g (8.8 mmol) of **8f** in 20 mL of ethanol, and 1.8 g of **9f** was obtained as light yellow solid; yield: 92%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.62 (d, *J* = 8.8 Hz, 2H), 6.49 (d, *J* = 8.8 Hz, 2H), 4.59 (s, 2H), 3.84 (t, *J* = 6.4 Hz, 2H), 2.54 (t, *J* = 7.3 Hz, 2H), 2.47 (s, 4H), 1.82 (p, *J* = 6.6 Hz, 2H), 1.68 (p, *J* = 3.2 Hz, 4H). MS (ESI) m/z: 221.3 $[M+H]^+$.

*4.1.3.7. 4-(2-morpholinoethoxy)aniline (**9g**).* Synthesized using the procedure for **9a** using 0.26 g (10% m/m) of Pd-C and 2.6 g (10.3 mmol) of **8g** in 20 mL of ethanol, and 2.0 g of **9g** was obtained as purple solid; yield: 89%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.64 (d, *J* = 8.8 Hz, 2H), 6.49 (d, *J* = 8.8 Hz, 2H), 4.64 (s, 2H), 3.92 (t, *J* = 5.8 Hz, 2H), 3.57 (d, *J* = 4.8 Hz, 4H), 2.61 (t, *J* = 5.8 Hz, 2H), 2.44 (s, 4H). MS (ESI) m/z: 223.3 $[M+H]^+$.

*4.1.3.8. 4-(3-morpholinopropoxy)aniline (**9h**).* Synthesized using the procedure for **9a** using 0.26 g (10% m/m) of Pd-C and 2.6 g (9.8 mmol) of **8h** in 20 mL of ethanol, and

2.1 g of **9h** was obtained as brown solid; yield: 91%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.63 (d, *J* = 8.8 Hz, 2H), 6.49 (d, *J* = 8.8 Hz, 2H), 4.60 (s, 2H), 3.84 (t, *J* = 6.6 Hz, 2H), 3.56 (t, *J* = 4.5 Hz, 4H), 2.38 (t, *J* =6.6 Hz, 2H), 2.34 (s, 4H), 1.79 (p, *J* = 6.5 Hz, 2H). MS (ESI) m/z: 237.3 $[M+H]^+$.

4.1.4 General procedure for preparation of compounds **10a-h**

4.1.4.1.

tert-butyl-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxylate ***(10a)***. To a mixture of **6** (2.0 g, 6.7 mmol, 1 equiv.) and trifluoroacetate (2.3 g, 20.1 mmol, 3 equiv.) in 20 mL *n*-BuOH, **9a** (1.4 g, 8.0 mmol, 1.2 equiv.) was added and the resulted mixture was heated to 120 ^{o}C and stirred for 1 h. When the mixture was cooled to room temperature, it was poured into 40 mL water and the mixture was neutralized with 10% sodium hydroxide. Blue-gray precipitate was filtered off and washed with water; the crude product (**10a**) was dried in a vacuum desiccator and was then used directly without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.60 (s, 1H), 7.92 (d, *J* = 6.7 Hz, 1H), 7.44 (d, *J* = 9.0 Hz, 2H), 6.93 (d, *J* = 9.0 Hz, 2H), 6.39 (d, *J* = 6.7 Hz, 1H), 3.75 – 3.72 (m, 4H), 3.67 (t, *J* = 5.2 Hz, 4H), 3.43 (t, *J* = 5.2 Hz, 4H), 3.09 – 3.03 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.83, 155.70, 154.18, 131.04, 122.17, 115.94, 95.43, 79.66, 66.51, 49.31, 44.10, 28.42; m.p.: 197.3 – 200.6 ^{o}C. MS (ESI) m/z: 441.4 $[M+H]^+$.

4.1.4.2.

*tert-butyl-4-(2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)piperazine-1-carboxylate (**10b**).* Synthesized using the procedure for **10a** using 2.0 g (6.7 mmol) of **6** and 2.3 g (20.1 mmol) of trifluoroacetate and 1.5 g (8.0 mmol) of **9b** in 20 mL of *n*-BuOH, crude product of **10b** was obtained and directly used without purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.85 (s, 1H), 7.94 (d, *J* = 6.0 Hz, 1H), 7.54 (d, *J* = 9.0 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 6.19 (d, *J* = 6.0 Hz, 1H), 3.57 (s, 4H), 3.40 (s, 4H), 3.12 (s, 4H), 2.76 (s, 4H), 2.44 (s, 3H), 1.42 (s, 9H). m.p.: 175.2 – 177.5 ^{o}C. MS (ESI) m/z: 454.5 $[M+H]^+$.

4.1.4.3.

*tert-butyl-4-(2-((4-(4-methylpiperidin-1-yl)phenyl)amino)pyrimidin-4-yl)piperazine-1-carboxylate (**10c**).* Synthesized using the procedure for **10a** using 2.0 g (6.7 mmol) of **6** and 2.3 g (20.1 mmol) of trifluoroacetate and 1.5 g (8.0 mmol) of **9c** in 20 mL of *n*-BuOH, crude product of **10c** was obtained and directly used without purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.08 (s, 1H), 7.91 (d, *J* =4.2 Hz, 1H), 7.36 (d, *J* =1.2 Hz, 2H), 7.13(d, *J* =1.2 Hz, 2H), 6.52 (d, *J* = 7.2 Hz, 1H), 3.73 (s, 4H), 3.63 (d, *J* = 12.1 Hz, 2H), 3.45 (s, 4H), 2.72 (s, 2H), 1.71 (d, *J* = 11.0 Hz,2H), 1.53 (s, 1H), 1.42 (s, 9H), 1.26 (d, *J* = 11.0 Hz, 2H), 0.94 (d, *J* = 6.5 Hz, 3H). m.p.: 221.8 – 224.8 ^{o}C. MS (ESI) m/z: 453.5 $[M+H]^+$.

4.1.4.4.

tert-butyl-4-(2-((4-thiomorpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxyl

*ate (**10d**).* Synthesized using the procedure for **10a** using 2.0 g (6.7 mmol) of **6** and 2.3 g (20.1 mmol) of trifluoroacetate and 1.6 g (8.0 mmol) of **9d** in 20 mL of *n*-BuOH, crude product of **10d** was obtained and directly used without purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.23 (s, 1H), 7.92 (s, 1H), 7.34 (d, *J* = 7.8 Hz, 2H), 6.97 (d, *J* =7.8Hz, 2H), 6.56 (d, *J* =7.4 Hz, 1H), 3.76 (s, 4H), 3.51 (t, *J* = 4.8 Hz 4H), 3.45 (s, 4H), 2.67 (t, *J* = 4.8 Hz 4H), 1.42 (s, 9H). m.p.: 215.8 – 217.4 ^{o}C. MS (ESI) m/z: 457.4 $[M+H]^+$.

4.1.4.5.

*tert-butyl-4-(2-((4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)amino)pyrimidin-4-yl)piperazine-1-carboxylate (**10e**).* Synthesized using the procedure for **10a** using 2.0 g (6.7 mmol) of **6** and 2.3 g (20.1 mmol) of trifluoroacetate and 1.7 g (8.0 mmol) of **9e** in 20 mL of *n*-BuOH, crude product of **10e** was obtained and directly used without purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.89 (s, 1H), 7.94 (d, *J* = 6.0 Hz, 1H), 7.57 (d, *J* = 9.0 Hz, 2H), 6.84 (d, *J* = 9.0 Hz, 2H), 6.20 (d, *J* = 6.0 Hz, 1H), 4.02 (t, *J* = 5.9 Hz, 2H), 3.57 (s, 4H), 3.40 (s, 4H), 2.83 (t, *J* = 5.9 Hz, 2H), 2.59 (s, 4H), 1.70 (p, *J* = 3.2 Hz, 4H), 1.42 (s, 9H). m.p.: 141.0 – 143.1 ^{o}C. MS (ESI) m/z: 469.5 $[M+H]^+$.

4.1.4.6.

*tert-butyl-4-(2-((4-(3-(pyrrolidin-1-yl)propoxy)phenyl)amino)pyrimidin-4-yl)piperazine-1-carboxylate (**10f**).* Synthesized using the procedure for **10a** using 2.0 g (6.7 mmol) of **6** and 2.3 g (20.1 mmol) of trifluoroacetate and 1.8 g (8.0 mmol) of **9f** in 20 mL of

n-BuOH, crude product of **10f** was obtained and directly used without purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.89 (s, 1H), 7.94 (d, *J* = 6.0 Hz, 1H), 7.57 (d, *J* = 9.0 Hz, 2H), 6.83 (d, *J* = 9.0 Hz, 2H), 6.20 (d, *J* = 6.0 Hz, 1H), 3.95 (t, *J* = 6.3 Hz, 2H), 3.58 (s, 4H), 3.41 (s, 4H), 2.62 (t, *J* = 7.0 Hz, 2H), 2.54 (s, 4H), 1.92 – 1.84 (m, 2H), 1.71 (s, 4H), 1.43 (s, 9H). m.p.: 150.9 – 153.1 ^{o}C. MS (ESI) m/z: 483.4 $[M+H]^+$.

4.1.4.7.

*tert-butyl-4-(2-((4-(2-morpholinoethoxy)phenyl)amino)pyrimidin-4-yl)piperazine-1-carboxylate (**10g**).* Synthesized using the procedure for **10a** using 2.0 g (6.7 mmol) of **6** and 2.3 g (20.1 mmol) of trifluoroacetate and 1.8 g (8.0 mmol) of **9g** in 20 mL of *n*-BuOH, crude product of **10g** was obtained and directly used without purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.88 (s, 1H), 7.94 (d, *J* = 6.0 Hz, 1H), 7.57 (d, *J* = 9.0 Hz, 2H), 6.84 (d, *J* = 9.0 Hz, 2H), 6.20 (d, *J* = 6.0 Hz, 1H), 4.02 (t, *J* = 5.4 Hz, 2H), 3.58 (t, *J* = 4.2Hz, 8H), 3.40 (s, 4H), 2.67 (t, *J* = 5.4Hz, 2H), 2.47 (s, 4H), 1.42 (s, 9H). m.p.: 184.5 – 186.4 ^{o}C. MS (ESI) m/z: 485.5 $[M+H]^+$.

4.1.4.8.

*tert-butyl-4-(2-((4-(3-morpholinopropoxy)phenyl)amino)pyrimidin-4-yl)piperazine-1-carboxylate (**10h**).* Synthesized using the procedure for **10a** using 2.0 g (6.7 mmol) of **6** and 2.3 g (20.1 mmol) of trifluoroacetate and 1.9 g (8.0 mmol) of **9h** in 20 mL of *n*-BuOH, crude product of **10h** was obtained and directly used without purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.88 (s, 1H), 7.94 (d, *J* = 6.0 Hz, 1H), 7.56 (d, *J* =

9.1 Hz, 2H), 6.83 (d, *J* = 9.1 Hz, 2H), 6.20 (d, *J* = 6.0 Hz, 1H), 3.94 (t, *J* = 6.4 Hz, 2H), 3.57 (s, 8H), 3.40 (s, 4H), 2.41 (s, 2H), 2.36 (s, 4H), 1.84 (p, *J* = 6.6 Hz, 2H), 1.42 (s, 9H). m.p.: 185.1 – 186.2 ^{o}C. MS (ESI) m/z: 499.5 $[M+H]^+$.

4.1.5 General procedure for preparation of compounds (**11a-h**)

*4.1.5.1. N-(4-morpholinophenyl)-4-(piperazin-1-yl)pyrimidin-2-amine (**11a**).* The crude product (**10a**) from the previous step was dissolved in 10 mL of dichloromethane; 10 mL of trifluoroacetate was then slowly added. After stirring overnight at room temperature, the mixture was evaporated under reduced pressure and the resultant was added to 20mL water, adjusting pH to 8 with saturated sodium hydrogen carbonate, the precipitate was filtered off and washed with water, then dried under vacuum to obtain 1.8 g of **11a** as blue-grey solid; yield: 77%; MS (ESI) m/z: 341.2 $[M+H]^+$.

*4.1.5.2. N-(4-(4-methylpiperazin-1-yl)phenyl)-4-(piperazin-1-yl)pyrimidin-2-amine (**11b**).* Synthesized using the procedure for **11a,** 10 mL of dichloromethane and 10 mL of trifluoroacetate were used, 2.0 g of **11b** were obtained; yield: 84%; MS (ESI) m/z: 354.2 $[M+H]^+$.

*4.1.5.3. N-(4-(4-methylpiperidin-1-yl)phenyl)-4-(piperazin-1-yl)pyrimidin-2-amine (**11c**).* Synthesized using the procedure for **11a,** 10 mL of dichloromethane and 10 mL of trifluoroacetate were used, 1.8 g of **11c** were obtained; yield: 78%; MS (ESI) m/z:

353.3 $[M+H]^+$.

*4.1.5.4. 4-(piperazin-1-yl)-N-(4-thiomorpholinophenyl)pyrimidin-2-amine (**11d**).* Synthesized using the procedure for **11a,** 10 mL of dichloromethane and 10 mL of trifluoroacetate were used, 1.9 g of **11d** were obtained; yield: 81%; MS (ESI) m/z: 357.4 $[M+H]^+$.

*4.1.5.5. 4-(piperazin-1-yl)-N-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)pyrimidin-2-amine (**11e**).* Synthesized using the procedure for **11a,** 10 mL of dichloromethane and 10 mL of trifluoroacetate were used, 1.8 g of **11e** were obtained; yield: 72%; MS (ESI) m/z: 369.3 $[M+H]^+$.

*4.1.5.6. 4-(piperazin-1-yl)-N-(4-(3-(pyrrolidin-1-yl)propoxy)phenyl)pyrimidin-2-amine (**11f**).* Synthesized using the procedure for **11a,** 10 mL of dichloromethane and 10 mL of trifluoroacetate were used, 1.7 g of **11f** were obtained; yield: 65%; MS (ESI) m/z: 383.3 $[M+H]^+$.

*4.1.5.7. N-(4-(2-morpholinoethoxy)phenyl)-4-(piperazin-1-yl)pyrimidin-2-amine (**11g**).* Synthesized using the procedure for **11a,** 10 mL of dichloromethane and 10 mL of trifluoroacetate were used, 1.7 g of **11g** were obtained; yield: 68%; MS (ESI) m/z: 385.2 $[M+H]^+$,

*4.1.5.8. N-(4-(3-morpholinopropoxy)phenyl)-4-(piperazin-1-yl)pyrimidin-2-amine (**11h**).* Synthesized using the procedure for **11a,** 10 mL of dichloromethane and 10 mL of trifluoroacetate were used, 1.9 g of **11h** were obtained; yield: 73%; MS (ESI) m/z: 399.2 $[M+H]^+$.

4.1.6 General procedure for preparation of compounds (**13a-x**)

*4.1.6.1. Phenyl-(4-isopropoxyphenyl)carbamate (**13a**).* Into a stirring mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL), water (4 mL), sodium carbonate (0.8 g, 7.9 mmol, 0.6 equiv.), and 4-isopropoxyaniline (2.0 g, 13.2 mmol, 1 equiv.), phenyl chloroformate (2.3 g, 14.5 mmol, 1.1 equiv.) was added dropwise at 0 °C. The reaction was stirred at room temperature overnight, then it was evaporated under reduced pressure and the resultant was added into 20 mL water, the precipitate was filtered and washed with water, then dried under vacuum to afford 3.2 g of **13a** as white solid; yield: 90%; MS (ESI) m/z: 286.1 $[M+H]^+$, 308.1 $[M+Na]^+$.

*4.1.6.2. Phenyl-phenylcarbamate (**13b**).* Synthesized using the procedure for **13a** using 2.0 g (21.5 mmol) of aniline and 1.4 g (12.9 mmol) of sodium carbonate and 3.7 g (23.7 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 4.3 g of **13b** (94.3% yield) was obtained; yield: 94%; MS (ESI) m/z: 214.1 $[M+H]^+$, 236.1 $[M+Na]^+$.

*4.1.6.3. Phenyl-(4-fluorophenyl)carbamate (**13c**).* Synthesized using the procedure for **13a** using 2.0 g (18.0 mmol) of 4-fluoroaniline and 1.1 g (10.8 mmol) of sodium carbonate and 3.1 g (19.8 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.8 g of **13c** was obtained; yield: 92%; MS (ESI) m/z: 232.1 $[M+H]^+$, 254.1 $[M+Na]^+$.

*4.1.6.4. Phenyl-(4-ethylphenyl)carbamate (**13d**).* Synthesized using the procedure for **13a** using 2.0 g (16.5 mmol) of 4-ethylaniline and 1.0 g (9.9 mmol) of sodium carbonate and 2.8 g (18.2 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 ml), tetrahydrofuran (4 mL) and water (4 mL), 3.4 g of **13d** was obtained; yield: 86%; MS (ESI) m/z: 242.1 $[M+H]^+$, 264.1 $[M+Na]^+$.

*4.1.6.5. Phenyl-(4-methoxyphenyl)carbamate (**13e**).* Synthesized using the procedure for **13a** using 2.0 g (16.2 mmol) of 4-methoxyaniline and 1.0 g (9.7 mmol) of sodium carbonate and 2.8 g (17.9 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.6 g of **13e** was obtained; yield: 92%; MS (ESI) m/z: 244.2 $[M+H]^+$, 266.2 $[M+Na]^+$.

*4.1.6.6. Phenyl-(4-(diethylamino)phenyl)carbamate (**13f**).* Synthesized using the procedure for **13a** using 2.0 g (12.2 mmol) of *N*,*N*-diethylbenzene-1,4-diamine and 0.7 g (7.3 mmol) of sodium carbonate and 2.1 g (13.4 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.0

g of **13f** was obtained; yield: 88%; MS (ESI) m/z: 285.2 $[M+H]^+$, 307.2 $[M+Na]^+$.

*4.1.6.7. Phenyl-(4-nitrophenyl)carbamate (**13g**).* Synthesized using the procedure for **13a** using 2.0 g (14.5 mmol) of 4-nitroaniline and 0.9 g (8.7 mmol) of sodium carbonate and 2.5 g (15.9 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.4 g of **13g** was obtained; yield: 91%; MS (ESI) m/z: 258.1 $[M+H]^+$, 280.1 $[M+Na]^+$.

*4.1.6.8. Phenyl-(4-cyanophenyl)carbamate (**13h**).* Synthesized using the procedure for **13a** using 2.0 g (16.9 mmol) of 4-aminobenzonitrile and 1.1 g (10.2 mmol) of sodium carbonate and 2.9 g (18.6 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.6 g of **13h** was obtained; yield: 89%; MS (ESI) m/z: 239.1 $[M+H]^+$, 261.1 $[M+Na]^+$.

*4.1.6.9. Phenyl-(4-(trifluoromethyl)phenyl)carbamate (**13i**).* Synthesized using the procedure for **13a** using 2.0 g (12.4 mmol) of 4-(trifluoromethyl)aniline and 0.8 g (7.4 mmol) of sodium carbonate and 2.1 g (13.7 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **13i** was obtained; yield: 94%; MS (ESI) m/z: 282.1 $[M+H]^+$, 304.1 $[M+Na]^+$.

*4.1.6.10. Phenyl-(4-acetylphenyl)carbamate (**13j**).* Synthesized using the procedure for **13a** using 2.0 g (14.8 mmol) of 1-(4-aminophenyl)ethan-1-one and 0.9 g (8.9

mmol) of sodium carbonate and 2.6 g (16.3 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **13j** was obtained; yield: 88%; MS (ESI) m/z: 256.1 $[M+H]^+$,278.1 $[M+Na]^+$.

*4.1.6.11. Methyl-4-((phenoxycarbonyl)amino)benzoate (**13k**).* Synthesized using the procedure for **13a** using 2.0 g (13.2 mmol) of methyl-4-aminobenzoate and 0.8 g (7.9 mmol) of sodium carbonate and 2.3 g (14.6 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **13k** was obtained; yield: 93%; MS (ESI) m/z: 272.2 $[M+H]^+$, 294.2 $[M+Na]^+$.

*4.1.6.12. Phenyl-(4-carbamoylphenyl)carbamate (**13l**).* Synthesized using the procedure for **13a** using 2.0 g (14.7 mmol) of 4-aminobenzamide and 0.9 g (8.8 mmol) of sodium carbonate and 2.5 g (16.2 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **13l** was obtained; yield: 88%; MS (ESI) m/z: 257.1 $[M+H]^+$, 279.1 $[M+Na]^+$.

*4.1.6.13. Phenyl-(4-(methylcarbamoyl)phenyl)carbamate (**13m**).* Synthesized using the procedure for **13a** using 2.0 g (13.3 mmol) of 4-amino-*N*-methylbenzamide and 0.8 g (8.0 mmol) of sodium carbonate and 2.3 g (14.6 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.1 g of **13m** was obtained; yield: 87%; MS (ESI) m/z: 271.1 $[M+H]^+$, 293.1 $[M+Na]^+$.

*4.1.6.14. Methyl-(4-((phenoxycarbonyl)amino)benzoyl)glycinate (**13n**).* Synthesized using the procedure for **13a** using 2.0 g (9.6 mmol) of methyl-(4-aminobenzoyl)glycinate and 0.6 g (5.8 mmol) of sodium carbonate and 1.7 g (10.6 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 2.9 g of **13n** was obtained; yield: 93%; MS (ESI) m/z: 329.1 $[M+H]^+$.

*4.1.6.15. Phenyl-(4-(morpholine-4-carbonyl)phenyl)carbamate (**13o**).* Synthesized using the procedure for **13a** using 2.0 g (9.7 mmol) of (4-aminophenyl)-morpholino-methanone and 0.6 g (5.8 mmol) of sodium carbonate and 1.7 g (10.7 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 2.8 g of **13o** was obtained; yield: 87%; MS (ESI) m/z: 327.2.

*4.1.6.16. Phenyl-(4-(methylsulfonyl)phenyl)carbamate (**13p**).* Synthesized using the procedure for **13a** using 2.0 g (11.7 mmol) of 4-(methylsulfonyl)aniline and 0.7 g (7.0 mmol) of sodium carbonate and 2.0 g (12.8 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.1 g of **13p** was obtained; yield: 90%; MS (ESI) m/z: 292.1 $[M+H]^+$, 314.1 $[M+Na]^+$.

*4.1.6.17. Phenyl-(4-sulfamoylphenyl)carbamate (**13q**).* Synthesized using the procedure for **13a** using 2.0 g (11.6 mmol) of 4-aminobenzenesulfonamide and 0.7 g

(7.0 mmol) of sodium carbonate and 2.0 g (12.8 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.0 g of **13q** was obtained; yield: 88%; MS (ESI) m/z: 293.1 $[M+H]^+$, 315.1 $[M+Na]^+$.

*4.1.6.18. Phenyl-(3-cyanophenyl)carbamate (**13r**).* Synthesized using the procedure for **13a** using 2.0 g (16.9 mmol) of 3-aminobenzonitrile and 1.1 g (10.2 mmol) of sodium carbonate and 2.9 g (18.6 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.4 g of **13r** was obtained; yield: 85%; MS (ESI) m/z: 239.1 $[M+H]^+$, 261.1 $[M+Na]^+$.

*4.1.6.19. Phenyl-(3-(trifluoromethyl)phenyl)carbamate (**13s**).* Synthesized using the procedure for **13a** using 2.0 g (12.4 mmol) of 3-(trifluoromethyl)aniline and 0.8 g (7.4 mmol) of sodium carbonate and 2.1 g (13.7 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.1 g of **13s** was obtained; yield: 89%; MS (ESI) m/z: 282.1 $[M+H]^+$, 304.1 $[M+Na]^+$.

*4.1.6.20. Phenyl-(2-(trifluoromethyl)phenyl)carbamate (**13t**).* Synthesized using the procedure for **13a** using 2.0 g (12.4 mmol) of 2-(trifluoromethyl)aniline and 0.8 g (7.4 mmol) of sodium carbonate and 2.1 g (13.7 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.2 g of **13t** was obtained; yield: 92%; MS (ESI) m/z: 282.1 $[M+H]^+$, 304.1 $[M+Na]^+$.

*4.1.6.21. Phenyl-(3-acetylphenyl)carbamate (**13u**).* Synthesized using the procedure for **13a** using 2.0 g (14.8 mmol) of 1-(3-aminophenyl)ethan-1-one and 0.9 g (8.9 mmol) of sodium carbonate and 2.6 g (16.3 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **13u** was obtained; yield: 88%; MS (ESI) m/z: 256.1 $[M+H]^+$, 278.1 $[M+Na]^+$.

*4.1.6.22. Phenyl-(2-acetylphenyl)carbamate (**13v**).* Synthesized using the procedure for **13a** using 2.0 g (14.8 mmol) of 1-(2-aminophenyl)ethan-1-one and 0.9 g (8.9 mmol) of sodium carbonate and 2.6 g (16.3 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.4 g of **13v** was obtained; yield: 91%; MS (ESI) m/z: 256.1 $[M+H]^+$, 278.1 $[M+Na]^+$.

*4.1.6.23. Phenyl-(3-carbamoylphenyl)carbamate (**13w**).* Synthesized using the procedure for **13a** using 2.0 g (14.7 mmol) of 3-aminobenzamide and 0.9 g (8.8 mmol) of sodium carbonate and 2.5 g (16.2 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.4 g of **13w** was obtained; yield: 90%; MS (ESI) m/z: 257.1 $[M+H]^+$, 279.1 $[M+Na]^+$.

*4.1.6.24. Phenyl-(2-carbamoylphenyl)carbamate (**13x**).* Synthesized using the procedure for **13a** using 2.0 g (14.7 mmol) of 2-aminobenzamide and 0.9 g (8.8 mmol) of sodium carbonate and 2.5 g (16.2 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **13x** was

obtained; yield: 88%; MS (ESI) m/z: 257.1 $[M+H]^+$, 279.1 $[M+Na]^+$.

4.1.7 General procedure for preparation of compounds (**14a-x, 15a-c, 16a-c, 17-21a**)

4.1.7.1.

*N-(4-isopropoxyphenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14a**).* To a mixture of **11a** (0.2g, 0.59 mmol, 1 equiv.) and **13a** (0.19 g, 0.71 mmol, 1.2 equiv.) in 5 mL DMF, DIPEA (0.23 g, 1.76 mmol, 3 equiv.) was slowly added, after stirring at 40 ^oC for 12 h, the mixture was poured into 10 mL water, the solution was neutralized with 1N hydrochloric acid, then the precipitate was filtered and washed with water, the crude product was dried in vacuum desiccator and further purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14a** as a pale yellow solid. ^1H NMR (400 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.43 (s, 1H), 7.95 (d, *J* = 6.0 Hz, 1H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.33 (d, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.81 (d, *J* = 8.7 Hz, 2H), 6.25 (d, *J* = 6.0 Hz, 1H), 4.50 (dt, *J* = 12.1, 6.0 Hz, 1H), 3.78 – 3.68 (m, 4H), 3.64 (s, 4H), 3.53 (s, 4H), 3.07 – 2.97 (m, 4H), 1.23 (d, *J* = 5.7 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.43, 155.72, 153.11, 146.27, 145.56, 133.73, 130.05, 122.11, 116.13, 116.10, 95.10, 69.85, 66.67, 49.85, 43.85, 43.71, 29.47, 22.39. m.p.: 221.0 – 223.7 ^oC. HPLC purity: 98.62%, retention time = 20.451 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{28}H_{35}N_7O_3$, 518.2874; found, 518.2891.

4.1.7.2.

*4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)-N-phenylpiperazine-1-carboxamide (**14b**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.15 g (0.72 mmol) of **13b** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14b** as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (s, 1H), 8.61 (s, 1H), 7.95 (d, *J* = 6.0 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 2H), 7.48 (d, *J* = 7.7 Hz, 2H), 7.24 (t, *J* = 7.9 Hz, 2H), 6.94 (t, *J* = 7.3 Hz, 1H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.24 (d, *J* = 6.1 Hz, 1H), 3.81 – 3.69 (m, 4H), 3.68 – 3.61 (m, 4H), 3.59 – 3.52 (m, 4H), 3.07 – 2.96 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.45, 159.90, 156.86, 155.46, 146.18, 140.90, 134.01, 128.78, 122.28, 120.49, 120.12, 116.10, 95.07, 66.67, 49.88, 43.82, 43.78. m.p.: 211.1 – 212.7 ^{o}C. HPLC purity: 96.99%, retention time = 23.209 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{25}H_{29}N_7O_2$, 460.2455; found, 460.2463.

4.1.7.3.

*N-(4-fluorophenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14c**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.17 g (0.72 mmol) of **13c** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14c** as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.66 (s, 1H), 7.95 (d, *J* = 6.0 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 2H), 7.49 (dd, *J* = 9.0, 5.0 Hz, 2H), 7.08 (t, *J* =

9.0 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.24 (d, *J* = 6.0 Hz, 1H), 3.76 – 3.70 (m, 4H), 3.68 – 3.60 (m, 4H), 3.59 – 3.52 (m, 4H), 3.09 – 2.93 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.45, 160.01, 158.67, 157.09, 155.48, 146.12, 137.20, 134.08, 121.87, 121.82, 120.42, 116.09, 115.34, 115.19, 95.04, 66.66, 49.88, 43.76, 43.71. m.p.: 221.7 – 223.1 ^{o}C. HPLC purity: 99.16%, retention time = 21.470 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{25}H_{28}FN_7O_2$, 478.2361; found, 478.2370.

4.1.7.4.

*N-(4-ethylphenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14d**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.17 g (0.72 mmol) of **13d** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14d** as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.52 (s, 1H), 7.95 (d, *J* = 6.0 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 6.25 (d, *J* = 6.0 Hz, 1H), 3.78 – 3.70 (m, 4H), 3.64 (s, 4H), 3.55 (d, *J* = 5.0 Hz, 4H), 3.08 – 2.95 (m, 4H), 2.57 – 2.52 (m, 2H), 1.15 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.45, 159.83, 156.69, 155.56, 149.28, 146.26, 138.48, 137.63, 134.01, 130.05, 127.99, 120.62, 120.36, 116.10, 95.10, 95.10, 66.67, 49.86, 43.83, 43.74, 27.79, 16.23. m.p.: 204.2 – 206.4 ^{o}C. HPLC purity: 97.32%, retention time = 22.769 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{27}H_{33}N_7O_2$, 488.2768; found, 488.2773.

4.1.7.5.

*N-(4-methoxyphenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14e**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.18 g (0.72 mmol) of **13e** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14e** as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.45 (s, 1H), 7.95 (d, *J* = 6.0 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 2H), 7.36 (d, *J* = 9.0 Hz, 2H), 6.85 (dd, *J* = 12.4, 9.0 Hz, 4H), 6.24 (d, *J* = 6.0 Hz, 1H), 3.75 – 3.71 (m, 4H), 3.71 (s, 3H), 3.68 – 3.59 (m, 4H), 3.58 – 3.49 (m, 4H), 3.04 – 2.98 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.45, 159.92, 156.90, 155.71, 155.00, 146.15, 134.03, 133.82, 122.07, 120.46, 116.09, 114.00, 95.05, 66.66, 55.58, 49.87, 43.80, 43.70. m.p.: 231.3 – 233.8 ^{o}C. HPLC purity: 96.93%, retention time = 21.910 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{26}H_{31}N_7O_3$, 490.2561; found, 490.2588.

4.1.7.6.

*N-(4-(diethylamino)phenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14f**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.20 g (0.72 mmol) of **13f** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14f** as a

grey solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (s, 1H), 8.26 (s, 1H), 7.95 (d, *J* = 6.0 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 2H), 7.20 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.59 (d, *J* = 9.0 Hz, 2H), 6.24 (d, *J* = 6.0 Hz, 1H), 3.81 – 3.69 (m, 4H), 3.63 (s, 4H), 3.52 (d, *J* = 5.0 Hz, 4H), 3.26 (q, *J* = 7.0 Hz, 4H), 3.06 – 2.96 (m, 4H), 1.05 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.45, 160.02, 157.05, 155.97, 146.09, 143.99, 134.12, 129.45, 122.89, 120.38, 116.09, 112.65, 95.04, 66.67, 49.88, 44.33, 43.80, 43.71, 12.88. m.p.: 246.3 – 247.9 ^{o}C. HPLC purity: 96.69%, retention time = 21.452 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{29}H_{38}N_8O_2$, 530.3190; found, 531.3172.

4.1.7.7.

*4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)-N-(4-nitrophenyl)piperazine-1-carboxamide (**14g**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.19 g (0.72 mmol) of **13g** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14g** as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.32 (s, 1H), 8.85 (s, 1H), 8.17 (d, *J* = 9.0 Hz, 2H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.75 (d, *J* = 9.0 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 6.25 (d, *J* = 6.0 Hz, 1H), 3.76 – 3.71 (m, 4H), 3.69 – 3.64 (m, 4H), 3.61 (d, *J* = 3.6 Hz, 4H), 3.04 – 2.98 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.41, 160.05, 157.16, 154.54, 147.83, 146.10, 141.35, 134.09, 125.19, 120.40, 118.81, 116.08, 95.02, 66.66, 49.87, 43.87, 43.71. m.p.: 205.0 – 207.1 ^{o}C.

HPLC purity: 99.65%, retention time = 21.135 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{25}H_{28}N_8O_4$, 505.2306; found, 505.2324.

4.1.7.8.

*N-(4-cyanophenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14h**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.17 g (0.72 mmol) of **13h** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14h** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (s, 1H), 8.82 (s, 1H), 7.96 (d, J = 6.0 Hz, 1H), 7.69 (s, 4H), 7.56 (d, J = 9.0 Hz, 2H), 6.86 (d, J = 9.1 Hz, 2H), 6.24 (d, J = 6.1 Hz, 1H), 3.79 – 3.70 (m, 4H), 3.66 (m, 4H), 3.59 (m, 4H), 3.06 – 2.95 (m, 4H). MS (ESI) m/z: 485.44 $[M+H]^+$, 483.48 $[M-H]^-$. ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.43, 160.01, 157.07, 154.72, 146.14, 145.60, 134.07, 133.33, 120.45, 119.86, 119.48, 116.10, 103.56, 95.05, 66.67, 49.88, 43.82, 43.73. m.p.: 232.7 – 235.0 ^{o}C. HPLC purity: 99.07%, retention time = 18.412 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{26}H_{28}N_8O_2$, 485.2408; found, 485.2417.

4.1.7.9.

*4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)-N-(4-(trifluoromethyl)phenyl)piperazine-1-carboxamide (**14i**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.20 g (0.72 mmol) of **13i** and 0.22 g (1.8 mmol) of DIPEA were

mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14i** as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.86 (s, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.71 (d, *J* = 8.6 Hz, 2H), 7.61 (d, *J* = 8.7 Hz, 2H), 7.56 (d, *J* = 9.0 Hz, 2H), 6.87 (d, *J* = 9.1 Hz, 2H), 6.25 (d, *J* = 6.1 Hz, 1H), 3.74 – 3.72 (m, 4H), 3.66 (m, 4H), 3.60 – 3.57 (m, 4H), 3.03 – 3.00 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.45, 160.07, 157.17, 154.98, 146.10, 144.80, 134.11, 128.98, 126.11, 120.40, 119.39, 116.10, 95.30, 66.66, 49.88, 43.78, 43.74. m.p.: 262.5 – 265.3 ^{o}C. HPLC purity: 99.58%, retention time = 21.069 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{26}H_{28}F_3N_7O_2$, 528.2329; found, 528.2341.

4.1.7.10.

*N-(4-acetylphenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14j**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.18 g (0.72 mmol) of **13j** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14j** as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.00 (s, 1H), 8.84 (s, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.56 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.25 (d, *J* = 6.0 Hz, 1H), 3.75 – 3.72 (m, 4H), 3.66 (m, 4H), 3.59 (m, 4H), 3.03 – 3.00 (m, 4H), 2.51 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 196.76, 162.37, 159.98, 157.07, 154.81, 146.04, 145.67, 134.03, 130.71, 129.62,

120.33, 118.59, 116.03, 94.96, 66.59, 49.81, 43.72, 26.73 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δm.p.: 244.1 – 246.0 ^{o}C. HPLC purity: 99.41%, retention time = 17.765 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{27}H_{31}N_7O_3$, 502.2561; found, 502.2578.

4.1.7.11.

*Methyl-4-(4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamido)benzoate (**14k**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.20 g (0.72 mmol) of **13k** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14k** as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.86 (d, *J* = 9.0 Hz, 2H), 7.65 (d, *J* = 9.0 Hz, 2H), 7.56 (d, *J* = 9.0 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 6.26 (d, *J* = 6.0 Hz, 1H), 4.66 (s, 1H), 3.81 (s, 3H), 3.74 – 3.72 (m, 4H), 3.66 (s, 4H), 3.60 – 3.57 (m, 4H), 3.04 – 2.99 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.47, 162.42, 160.05, 157.14, 154.88, 146.10, 145.77, 134.10, 130.45, 122.75, 118.82, 116.09, 95.03, 66.66, 52.19, 49.88, 43.83, 43.75. m.p.: 182.7 – 184.3 ^{o}C. HPLC purity: 98.49%, retention time = 20.693 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{27}H_{31}N_7O_4$, 518.2510; found, 518.2517.

4.1.7.12.

N-(4-carbamoylphenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-

*1-carboxamide (**14l**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.18 g (0.72 mmol) of **13l** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14l** as a pale yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.85 (s, 1H), 8.26 (q, *J* = 4.5 Hz, 1H), 7.96 (d, *J* = 6.1 Hz, 1H), 7.74 (d, *J* = 8.9 Hz, 1H), 7.57 (d, *J* = 2.8 Hz, 2H), 7.55 (d, *J* = 2.8 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 6.25 (d, *J* = 6.1 Hz, 2H), 3.74 – 3.72 (m, 4H), 3.65 (m, 4H), 3.58 – 3.56 (m, 4H), 3.03 – 3.00 (m, 4H), 2.76 (d, *J* = 4.5 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.04, 161.44, 154.99, 144.26, 143.74, 128.54, 127.81, 118.74, 115.93, 95.99, 66.54, 49.03, 43.54; m.p.: 228.0 – 230.4 ^{o}C. HPLC purity: 98.77%, retention time = 16.926 min. HRMS (ESI) (m/z): [M + H]$^{+}$ calcd for $C_{26}H_{30}N_8O_3$, 503.2514; found, 503.2519.

4.1.7.13.

*N-(4-(methylcarbamoyl)phenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14m**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.19 g (0.72 mmol) of **13m** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14m** as a pale yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.85 (s, 1H), 8.26 (q, *J* = 4.4 Hz, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* = 2.9 Hz, 2H), 7.55 (d, *J* = 2.7 Hz, 2H), 6.87 (d, *J* = 9.1 Hz, 2H), 6.25

(d, *J* = 6.1 Hz, 1H), 3.75 – 3.71 (m, 4H), 3.65 (m, 4H), 3.57 (m, , 4H), 3.03 – 2.99 (m, 4H), 2.76 (d, *J* = 4.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.75, 162.45, 160.02, 157.06, 155.11, 146.14, 143.60, 134.08, 128.03, 120.44, 118.81, 117.76, 116.10, 95.05, 66.67, 49.89, 43.79, 43.63. m.p.: 267.1 – 268.8 ^{o}C. HPLC purity: 97.87%, retention time = 19.813 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{27}H_{32}N_8O_3$, 517.2670; found, 517.2693.

4.1.7.14.

*Methyl-(4-(4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamido)benzoyl)glycinate (**14n**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.24 g (0.72 mmol) of **13n** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14n** as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.82 (s, 1H), 8.75 (t, *J* = 5.7 Hz, 1H), 7.96 (d, *J* = 5.9 Hz, 1H), 7.78 (d, *J* = 8.7 Hz, 2H), 7.59 (d, *J* = 8.8 Hz, 2H), 7.56 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.25 (d, *J* = 5.9 Hz, 1H), 3.99 (d, *J* = 5.7 Hz, 2H), 3.77 – 3.71 (m, 4H), 3.65 (m, 8H), 3.58 (s, 3H), 3.06 – 2.96 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.04, 166.69, 162.43, 160.05, 157.14, 155.05, 146.10, 144.10, 134.11, 128.34, 127.00, 120.40, 118.81, 116.09, 95.04, 66.67, 52.16, 49.88, 43.80, 43.66, 41.64. m.p.: 150.6 – 153.2 ^{o}C. HPLC purity: 97.58%, retention time = 15.666 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{29}H_{34}N_8O_5$, 575.2725; found, 575.2741.

4.1.7.15.

*N-(4-(morpholine-4-carbonyl)phenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14o**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.23 g (0.72 mmol) of **13o** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14o** as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.82 (s, 1H), 7.95 (d, *J* = 5.9 Hz, 1H), 7.56 (dd, *J* = 8.7, 1.5 Hz, 4H), 7.33 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.24 (d, *J* = 6.1 Hz, 1H), 3.76 – 3.69 (m, 4H), 3.65 (m, 4H), 3.58 (m, 8H), 3.49 (m, 4H), 3.05 – 2.97 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.64, 162.46, 160.04, 157.09, 155.21, 146.13, 142.45, 134.10, 128.79, 128.38, 120.44, 119.13, 116.11, 95.06, 66.67, 66.61, 49.90, 43.81. m.p.: 130.6 – 132.5 ^{o}C. HPLC purity: 98.99%, retention time = 17.016 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{30}H_{36}N_8O_4$, 573.2932; found, 573.2946.

4.1.7.16.

*N-(4-(methylsulfonyl)phenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14p**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.21 g (0.72 mmol) of **13p** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v)

to furnish **14p** as a pale yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.12 (s, 1H), 8.86 (s, 1H), 7.96 (d, *J* = 5.9 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.56 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 8.9 Hz, 2H), 6.25 (d, *J* = 6.0 Hz, 1H), 3.75 – 3.71 (m, 4H), 3.66 (s, 4H), 3.59 (m, 4H), 3.15 (s, 3H), 3.05 – 2.98 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.45, 160.08, 157.18, 154.85, 146.11, 145.90, 134.12, 133.43, 128.31, 120.41, 119.16, 116.10, 95.04, 66.67, 49.89, 44.42, 43.82, 43.73. m.p.: 261.4 – 262.6 ^{o}C. HPLC purity: 99.85%, retention time = 21.401 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{26}H_{31}N_7O_4S$, 538.2231; found, 538.2225.

4.1.7.17.

*4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)-N-(4-sulfamoylphenyl)piperazine-1-carboxamide (**14q**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.21 g (0.72 mmol) of **13q** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14q** as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (s, 1H), 8.93 (s, 1H), 7.95 (d, *J* = 5.7 Hz, 1H), 7.70 (d, *J* = 8.6 Hz, 2H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.19 (s, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 6.27 (d, *J* = 5.8 Hz, 1H), 3.73 (m, 4H), 3.67 (m, 4H), 3.59 (m, 4H), 3.02 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.41, 159.71, 156.57, 155.00, 146.27, 144.18, 137.24, 133.84, 130.12, 126.82, 120.61, 119.10, 116.10, 95.09, 66.66, 49.85, 43.75. m.p.: 236.0 – 238.2 ^{o}C. HPLC purity: 99.76%, retention time = 19.679 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for

$C_{25}H_{30}N_8O_4S$, 539.2183; found, 539.2202.

4.1.7.18.

*N-(3-cyanophenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14r**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.17 g (0.72 mmol) of **13r** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14r** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.82 (s, 1H), 7.95 (d, *J* = 5.9 Hz, 1H), 7.56 (dd, *J* = 8.7, 1.5 Hz, 4H), 7.33 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.24 (d, *J* = 6.1 Hz, 1H), 3.76 – 3.69 (m, 4H), 3.65 (m, 4H), 3.58 (m, 8H), 3.49 (m, 4H), 3.05 – 2.97 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.43, 160.05, 157.16, 155.00, 146.10, 141.93, 134.10, 130.28, 125.62, 124.36, 122.44, 119.43, 116.09, 111.64, 95.03, 66.66, 49.88, 43.72. m.p.: 204.3 – 206.7 ^{o}C. HPLC purity: 97.19%, retention time = 19.537 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{26}H_{28}N_8O_2$, 485.2408; found, 485.2420.

4.1.7.19.

*4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)-N-(3-(trifluoromethyl)phenyl)piperazine-1-carboxamide (**14s**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.20 g (0.72 mmol) of **13s** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column

chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14s** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (s, 1H), 8.82 (s, 1H), 7.96 (m, 2H), 7.77 (d, *J* = 8.6 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 2H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 7.7 Hz, 1H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.25 (d, *J* = 6.1 Hz, 1H), 3.76 – 3.71 (m, 4H), 3.66 (m, 4H), 3.59 (m, 4H), 3.06 – 2.96 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.45, 160.02, 157.05, 155.11, 146.15, 141.85, 134.07, 129.97, 129.76, 123.42, 123.27, 120.45, 118.41, 116.10, 115.87, 95.06, 66.67, 49.89, 43.73. m.p.: 223.3 – 225.7 ^{o}C. HPLC purity: 96.70%, retention time = 19.668 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{26}H_{28}F_3N_7O_2$, 528.2329; found, 528.2333.

4.1.7.20.

*4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)-N-(2-(trifluoromethyl)phenyl)piperazine-1-carboxamide (**14t**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.20 g (0.72 mmol) of **13t** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14t** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.33 (s, 1H), 7.95 (d, *J* = 6.0 Hz, 1H), 7.73 – 7.60 (m, 2H), 7.56 (d, *J* = 9.0 Hz, 2H), 7.49 – 7.37 (m, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.24 (d, *J* = 6.0 Hz, 1H), 3.77 – 3.69 (m, 4H), 3.63 (m, 4H), 3.55 (m, 4H), 3.07 – 2.95 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.53, 160.06, 157.13, 156.36, 146.12, 142.87, 137.77, 133.18, 131.03, 126.76, 126.50, 120.40, 116.12, 95.11, 66.6, 49.90, 43.86, 43.74. m.p.: 218.1 – 220.1 ^{o}C.

HPLC purity: 96.78%, retention time = 18.714 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{26}H_{28}F_3N_7O_2$, 528.2329; found, 528.2343.

4.1.7.21.

*N-(3-acetylphenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14u**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.18 g (0.72 mmol) of **13u** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14u** as a white solid. ^{1}H NMR (600 MHz, DMSO) δ 8.86 (s, 1H), 8.85 (s, 1H), 8.07 (s, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.57 (d, *J* = 8.7 Hz, 3H), 7.41 (t, *J* = 7.9 Hz, 1H), 6.87 (d, *J* = 8.9 Hz, 2H), 6.25 (d, *J* = 6.0 Hz, 1H), 3.76 – 3.70 (m, 4H), 3.66 (m, 4H), 3.58 (m, 4H), 3.05 – 2.96 (m, 4H), 2.55 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 198.26, 162.47, 160.06, 157.14, 155.29, 146.12, 141.40, 137.56, 134.12, 129.20, 124.51, 120.42, 119.25, 116.10, 95.05, 66.67, 65.38, 49.90, 43.74, 27.19. m.p.: 232.7 – 235.1 ^{o}C. HPLC purity: 99.46%, retention time = 17.923 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{27}H_{30}N_7O_3$, 502.2561; found, 502.2567.

4.1.7.22.

*N-(2-acetylphenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14v**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.18 g (0.72 mmol) of **13v** and 0.22 g (1.8 mmol) of DIPEA were mixed and

heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14v** as a white solid. ^{1}H NMR (600 MHz, DMSO) δ 11.34 (s, 1H), 8.89 (s, 1H), 8.43 (d, *J* = 8.5 Hz, 1H), 8.04 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.68 – 7.50 (m, 3H), 7.14 – 7.03 (m, 1H), 6.88 (d, *J* = 8.9 Hz, 2H), 6.24 (d, *J* = 6.0 Hz, 1H), 3.80 – 3.67 (m, 8H), 3.66 – 3.53 (m, 4H), 3.08 – 2.95 (m, 4H), 2.67 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 203.90, 162.37, 160.04, 157.12, 154.42, 146.09, 142.50, 135.16, 134.11, 132.74, 121.93, 120.34, 119.58, 116.13, 95.00, 66.67, 49.89, 43.45, 43.13, 29.05. m.p.: 230.1 – 233.7 ^{o}C. HPLC purity: 99.34%, retention time = 19.059 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{27}H_{30}N_7O_3$, 502.2561; found, 502.2579.

4.1.7.23.

*N-(3-carbamoylphenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14w**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.18 g (0.72 mmol) of **13w** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14w** as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.85 (s, 1H), 8.07 (s, 1H), 7.96 (d, *J* = 5.9 Hz, 1H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 8.8 Hz, 3H), 7.41 (t, *J* = 8.0 Hz, 1H), 6.87 (d, *J* = 8.8 Hz, 2H), 6.25 (d, *J* = 6.0 Hz, 1H), 3.74 – 3.72 (m, 4H), 3.66 (s, 4H), 3.58 (m, 4H), 3.04 – 3.00 (m, 4H), 2.55 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.52, 162.42, 156.55, 155.34, 146.21, 140.97, 135.16, 130.11, 128.57,

122.77, 120.41, 119.80, 116.09, 95.09, 66.66, 49.85, 43.83, 43.74. m.p.: 245.5 – 247.5 ^{o}C. HPLC purity: 96.51%, retention time = 15.982 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{26}H_{30}N_8O_3$, 503.2514; found, 503.2523.

4.1.7.24.

*N-(2-carbamoylphenyl)-4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**14x**).* Synthesized using the procedure for **14a**, 0.2 g (0.59 mmol) of **11a** and 0.18 g (0.72 mmol) of **13x** and 0.22 g (1.8 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **14x** as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.34 (s, 1H), 8.89 (s, 1H), 8.43 (d, *J* = 8.5 Hz, 1H), 8.04 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.59 (d, *J* = 7.3 Hz, 1H), 7.58 – 7.53 (m, 2H), 7.11 – 7.07 (m, 1H), 6.88 (d, *J* = 8.9 Hz, 2H), 6.24 (d, *J* = 6.0 Hz, 1H), 3.76 – 3.69 (m, 8H), 3.64 – 3.59 (m, 4H), 3.05 – 2.99 (m, 4H), 2.67 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ171.94, 162.40, 159.99, 157.05, 154.46, 146.12, 142.26, 134.08, 132.76, 128.95, 120.98, 120.37, 119.74, 118.21, 116.14, 95.02, 66.66, 49.89, 43.52, 43.12. m.p.: 191.4 – 192.3 ^{o}C. HPLC purity: 96.36%, retention time = 18.562 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{26}H_{30}N_8O_3$, 503.2514; found, 503.2533.

4.1.7.25.

N-(4-acetylphenyl)-4-(2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)pip

*erazine-1-carboxamide (**15a**).* Synthesized using the procedure for **14a**, 0.2 g (0.57 mmol) of **11b** and 0.17 g (0.68 mmol) of **13j** and 0.22 g (1.71 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (20:1, v/v) to furnish **15a** as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.84 (s, 1H), 7.95 (d, *J* = 6.0 Hz, 1H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.24 (d, *J* = 6.0 Hz, 1H), 3.66 (s, 4H), 3.60 – 3.57 (m, 4H), 3.07 – 3.02 (m, 4H), 2.51 (m, 4H), 2.48 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 196.83, 162.44, 160.07, 157.16, 154.88, 146.07, 145.74, 133.83, 130.78, 129.68, 120.40, 118.67, 116.36, 94.99, 55.14, 49.39, 46.13, 43.82, 43.75, 26.79. m.p.: 238.1 – 240.8 ^{o}C. HPLC purity: 97.51%, retention time = 19.632 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{28}H_{34}N_8O_2$, 515.2877; found, 515.2894.

4.1.7.26.

*N-(4-carbamoylphenyl)-4-(2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**15b**).* Synthesized using the procedure for **14a**, 0.2 g (0.57 mmol) of **11b** and 0.17 g (0.68 mmol) of **13l** and 0.22 g (1.71 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (20:1, v/v) to furnish **15b** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (d, *J* = 11.7 Hz, 2H), 7.95 (d, *J* = 5.9 Hz, 1H), 7.87 – 7.81 (m, 1H), 7.78 (d, *J* = 8.7 Hz, 2H), 7.58 –

7.52 (m, 4H), 7.16 (s, 1H), 6.85 (d, *J* = 8.7 Hz, 2H), 6.24 (d, *J* = 6.0 Hz, 1H), 3.62 (m, 8H), 3.04 (s, 4H), 2.46 (s, 4H), 2.22 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.03, 162.45, 160.07, 157.15, 155.10, 146.01, 143.80, 133.87, 128.99, 128.53, 127.72, 120.40, 120.26, 118.70, 117.64, 116.80, 116.39, 95.00, 60.84, 55.07, 50.84, 49.32, 46.01, 43.38. m.p.: 250.3 – 252.3 ^{o}C. HPLC purity: 99.70%, retention time = 16.850 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{27}H_{33}N_9O_2$, 516.2830; found, 516.2852.

4.1.7.27.

*4-(2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)-N-(4-sulfamoylphenyl)piperazine-1-carboxamide (**15c**).* Synthesized using the procedure for **14a**, 0.2 g (0.57 mmol) of **11b** and 0.20 g (0.68 mmol) of **13q** and 0.22 g (1.71 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (20:1, v/v) to furnish **15c** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1H), 8.82 (s, 1H), 7.95 (d, *J* = 6.0 Hz, 1H), 7.70 (d, *J* = 8.7 Hz, 2H), 7.65 (d, *J* = 8.9 Hz, 2H), 7.54 (d, *J* = 8.7 Hz, 2H), 7.18 (s, 2H), 6.85 (d, *J* = 8.9 Hz, 2H), 6.24 (d, *J* = 6.0 Hz, 1H), 3.62 (m,8H), 3.04 (s, 4H), 2.47 (s, 4H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.45, 162.46, 160.08, 157.16, 155.00, 146.01, 144.19, 137.24, 137.24, 132.05, 129.12, 126.82, 120.42, 119.09, 116.40, 95.01, 67.89, 63.56, 55.05, 49.29, 45.96, 43.79. m.p.: 182.9 – 184.9 ^{o}C. HPLC purity: 98.14%, retention time = 16.898 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{26}H_{33}N_9O_3S$, 552.2500; found, 552.2507.

4.1.7.28.

*N-(4-acetylphenyl)-4-(2-((4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**16a**).* Synthesized using the procedure for **14a**, 0.2 g (0.54 mmol) of **11e** and 0.17 g (0.65 mmol) of **13a** and 0.21 g (1.62 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (15:1, v/v) to furnish **16a** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H), 8.91 (s, 1H), 7.97 (d, *J* = 6.0 Hz, 1H), 7.87 (d, *J* = 8.6 Hz, 2H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.60 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 6.27 (d, *J* = 6.0 Hz, 1H), 4.11 (t, *J* = 5.2 Hz, 2H), 3.65 (s, 4H), 3.60 (d, *J* = 4.0 Hz, 4H), 3.03 (s, 2H), 2.80 (s, 4H), 2.51 (s, 3H), 1.78 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 196.85, 162.45, 160.03, 157.13, 154.94, 152.88, 145.81, 135.21, 130.77, 129.64, 120.73, 118.71, 114.96, 95.28, 72.98, 65.55, 63.55, 54.27, 54.00, 43.85, 43.78, 26.79, 23.31. m.p.: 125.2 – 127.3 ^{o}C. HPLC purity: 97.01%, retention time = 19.953 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{29}H_{35}N_7O_3$, 530.2874; found, 530.2870.

4.1.7.29.

*N-(4-carbamoylphenyl)-4-(2-((4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**16b**).* Synthesized using the procedure for **14a**, 0.2 g (0.54 mmol) of **11e** and 0.17 g (0.65 mmol) of **13l** and 0.21 g (1.62 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column

chromatography on silica using mobile phase dichloromethane−methanol (15:1, v/v) to furnish **16b** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.91 (s, 1H), 8.90 (s, 1H), 7.97 (d, *J* = 5.6 Hz, 1H), 7.84 (s, 1H), 7.78 (d, *J* = 8.6 Hz, 2H), 7.57 (d, *J* = 8.3 Hz, 2H), 7.18 (d, *J* = 9.5 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 6.27 (d, *J* = 5.6 Hz, 1H), 4.12 (s, 2H), 3.65 (s, 4H), 3.59 (s, 4H), 3.09 (s, 2H), 2.86 (s, 4H), 1.79 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.00, 163.01, 161.66, 155.01, 152.65, 147.60, 143.69, 135.09, 128.99, 128.55, 128.02, 127.86, 118.73, 117.60, 115.58, 96.16, 64.05, 54.32, 53.35, 51.15, 50.98, 43.61, 43.59, 23.00. m.p.: 211.2 – 213.4 ^{o}C. HPLC purity: 99.35%, retention time = 18.574 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{28}H_{34}N_8O_3$, 531.2827; found, 531.2829.

4.1.7.30.

*4-(2-((4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)amino)pyrimidin-4-yl)-N-(4-sulfamoylphenyl)piperazine-1-carboxamide (**16c**).* Synthesized using the procedure for **14a**, 0.2 g (0.54 mmol) of **11e** and 0.19 g (0.65 mmol) of **13q** and 0.21 g (1.62 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (15:1, v/v) to furnish **16c** as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 9.03 (s, 1H), 8.90 (s, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.66 (d, *J* = 8.9 Hz, 2H), 7.59 (d, *J* = 8.8 Hz, 2H), 7.19 (s, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.26 (d, *J* = 6.0 Hz, 1H), 4.06 (t, *J* = 5.5 Hz, 2H), 3.66 (s, 4H), 3.59 (s, 4H), 2.90 (s, 2H), 2.66 (s, 4H), 1.73 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.45, 160.05, 157.15, 153.17, 150.16,

144.19, 137.23, 135.00, 134.98, 126.82, 120.77, 119.08, 114.84, 95.24, 54.59, 54.44, 43.84, 43.78, 23.47. m.p.: 155.2 – 157.1 ^{o}C. HPLC purity: 99.09%, retention time = 13.393 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{27}H_{34}N_8O_4S$, 567.2496; found, 567.2510.

4.1.7.31.

*N-(4-acetylphenyl)-4-(2-((4-thiomorpholinophenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**17a**).* Synthesized using the procedure for **14a**, 0.2 g (0.56 mmol) of **11d** and 0.17 g (0.67 mmol) of **13j** and 0.22 g (1.68 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17a** as a pale yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.91 (s, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.88 (d, *J* = 8.6 Hz, 2H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.55 (d, *J* = 8.8 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.26 (d, *J* = 6.0 Hz, 1H), 3.66 (s, 4H), 3.59 (s, 4H), 3.38 (m, 4H), 3.35 (s, 3H), 2.73 – 2.66 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 196.84, 162.42, 159.82, 156.79, 154.89, 146.30, 145.75, 134.01, 130.80, 129.68, 120.59, 118.68, 117.81, 95.10, 65.97, 52.66, 43.83, 43.73, 26.65. m.p.: 273.6 – 276.2 ^{o}C. HPLC purity: 98.45%, retention time = 20.589 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{27}H_{31}N_7O_2S$, 518.2333; found, 518.2316.

4.1.7.32.

N-(4-acetylphenyl)-4-(2-((4-(4-methylpiperidin-1-yl)phenyl)amino)pyrimidin-4-yl)pip

*erazine-1-carboxamide (**18a**).* Synthesized using the procedure for **14a**, 0.2 g (0.57 mmol) of **11c** and 0.17 g (0.68 mmol) of **13j** and 0.22 g (1.71 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **18a** as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (s, 1H), 8.80 (s, 1H), 7.95 (d, *J* = 6.0 Hz, 1H), 7.88 (d, *J* = 8.6 Hz, 2H), 7.64 (d, *J* = 8.6 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 6.23 (d, *J* = 6.0 Hz, 1H), 3.65 (s, 4H), 3.59 (s, 4H), 3.52 (d, *J* = 11.9 Hz, 2H), 2.55 (d, *J* = 11.6 Hz, 2H), 2.51 (s, 3H), 1.68 (d, *J* = 12.0 Hz, 2H), 1.51 – 1.39 (m, 1H), 1.27 – 1.18 (m, 2H), 0.94 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 196.83, 162.44, 160.02, 157.02, 154.89, 146.74, 145.74, 133.48, 130.80, 129.68, 120.51, 118.68, 116.92, 94.96, 50.41, 43.83, 43.77, 34.26, 30.63, 26.79, 22.29. m.p.: 244.6 – 246.9 ^{o}C. HPLC purity: 99.10%, retention time = 19.474 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{29}H_{35}N_7O_2$, 514.2925; found, 514.2938.

4.1.7.33.

*N-(4-acetylphenyl)-4-(2-((4-(3-(pyrrolidin-1-yl)propoxy)phenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**19a**).* Synthesized using the procedure for **14a**, 0.2 g (0.52 mmol) of **11f** and 0.16 g (0.63 mmol) of **13j** and 0.20 g (1.56 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (15:1, v/v) to furnish **19a** as a pale yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.01 (s, 1H),

8.91 (s, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.88 (d, *J* = 8.9 Hz, 2H), 7.64 (d, *J* = 8.9 Hz, 2H), 7.59 (d, *J* = 9.0 Hz, 2H), 6.85 (d, *J* = 9.0 Hz, 2H), 6.27 (d, *J* = 6.0 Hz, 1H), 4.04 (m, 2H), 3.59 (m, 4H), 3.58 (m, 4H), 3.34 (m, 4H), 2.51 (m, 5H), 1.53 (m, 4H), 1.40 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 196.82, 162.42, 159.97, 157.08, 154.88, 153.32, 147.55, 145.73, 134.82, 130.79, 129.68, 119.78, 118.66, 114.81, 95.42, 66.65, 54.04, 43.81, 43.75, 26.79. m.p.: 150.6 – 153.3 ^{o}C. HPLC purity: 97.58%, retention time = 14.654 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{24}H_{27}N_8O_3S$, 544.3031; found, 544.3048.

4.1.7.34.

*N-(4-acetylphenyl)-4-(2-((4-(2-morpholinoethoxy)phenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**20a**).* Synthesized using the procedure for **14a**, 0.2 g (0.52 mmol) of **11g** and 0.16 g (0.62 mmol) of **13j** and 0.20 g (1.56 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (15:1, v/v) to furnish **20a** as a pale yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.92 (s, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.27 (d, *J* = 6.0 Hz, 1H), 4.04 (t, *J* = 5.7 Hz, 2H), 3.66 (m, 4H), 3.59 (m, 8H), 3.35 (m, 2H), 2.71 (m, 2H), 2.51 (m, 5H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 196.82, 162.42, 159.97, 157.08, 154.88, 153.32, 147.25, 145.73, 134.82, 130.79, 129.68, 120.78, 118.66, 114.81, 95.22, 66.55, 54.04, 43.81, 43.75, 26.79. m.p.: 201.7 – 203.9 ^{o}C. HPLC purity: 98.78%, retention

time = 13.826 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{29}H_{35}N_7O_4$, 546.2823; found, 546.2832.

4.1.7.35.

*N-(4-acetylphenyl)-4-(2-((4-(3-morpholinopropoxy)phenyl)amino)pyrimidin-4-yl)piperazine-1-carboxamide (**21a**).* Synthesized using the procedure for **14a**, 0.2 g (0.50 mmol) of **11f** and 0.15 g (0.60 mmol) of **13j** and 0.19 g (1.50 mmol) of DIPEA were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (15:1, v/v) to furnish **21a** as a pale yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.12 (s, 1H), 8.94 (s, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 9.0 Hz, 2H), 6.85 (d, *J* = 9.0 Hz, 2H), 6.27 (d, *J* = 6.0 Hz, 1H), 3.97 (t, *J* = 5.7 Hz, 2H), 3.67 (m, 8H), 3.60 (m, 4H), 3.35 (m, 2H), 2.68 (m, 2H), 2.51 (m, 5H), 1.96 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 196.82, 162.42, 160.05, 157.15, 154.88, 153.52, 145.74, 134.71, 130.77, 129.68, 120.76, 118.66, 114.71, 95.17, 66.67, 66.37, 55.43, 53.86, 43.82, 43.75, 26.80, 26.48. m.p.: 139.6 – 141.7 ^{o}C. HPLC purity: 94.42%, retention time = 14.295 min. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{30}H_{37}N_7O_4$, 560.2980; found, 560.2988.

4.2. Biological section

4.2.1. In vitro enzyme assay

Enzymatic activity assay against JAK2 (Carna) and FLT3 (Carna) was carried out by a well-established mobility shift assay (Shanghai, ChemPartner). The kinase

base buffer was consist of 50mM HEPES (pH 7.5), 0.0015% Brij-35. The stop buffer contained a mixture of 100mM HEPES (pH 7.5), 0.015% Brij-35, 0.2% Coating Reagent #3 and 50mM EDTA.

Initially, the tested compounds were diluted to 50-fold of the desired highest concentration in reaction by 100% DMSO. The tested compound dilution (100 μL) was transferred into a well in 96-well plate. Then, the controls were formed by adding 100 mL of 100% DMSO to two empty wells, which was marked as source plate. The intermediate plate was prepared by transferring 10 μL of compound from source plate to a new 96-well plate. In the intermediate plate, additional 90 μL of kinase buffer was added to each well. The intermediate plate was swayed for 10 min. Then, 5 μL of each well from the 96-well intermediate plate were transferred to a 384-well plate in duplicates as the assay plate. In the each well of 384-well assay plate, the prepared enzyme solution (appropriate kinase in kinase base buffer) was added. The plate was then incubated at room temperature for 10 min. After that, the addition 10 μL of prepared peptide solution (FAM-labeled peptide and ATP in kinase base buffer) was added. The sample was incubated at 28 ^{o}C for 1h, then 25 μL of stop buffer was added. The conversion data was copied from Caliper program, and the values were converted to inhibition values. Percent inhibition = (max-conversion)/(max-min)×100. Data was presented in MS Excel and the curves fitted by XLfit excel add-in version 5.4.0.8. Equation is: Y=Bottom + (Top-Bottom)/(1+(IC_{50}/X)^HillSlope)

4.2.2. Cell viability assays

Cell proliferation was evaluated using a CCK-8 assay by the safety evaluation

center of Shenyang Research Institute of Chemical Industry (Shenyang, China). The cancer cell lines were cultured in RPMI 1640 (Corning) complete culture medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells (HEL 3000 cells/well, MV4-11 5000 cells/well and HL60 3000 cells/well) were grown in 96-well culture plates. Increasing concentrations of compounds were added to the plates. Cell proliferation was determined after treatment with the compounds for 72 h. Cell viability was measured using CCK-8 according to the manufacturer's instructions, 15 μL of CCK-8 (Solarbio) was added to each well and the cells were then incubated for an additional 3 h. The plates were read at 450 nm on the microplate spectrophotometer (Synergy HT, BioTek). The inhibition rate on cell proliferation was calculated as % inhibition rate = $(1–A_{Sample}/A_{Control})\times100$. The data were normalized to the control groups (DMSO) and represented as the means of three independent measurements with standard errors of <20%. IC_{50} values were calculated using Prism 5.0 (GraphPad Software).

4.2.3. Cell-Cycle Assay

The Cell-Cycle Assay was done by Shenyang Takara Biotech. Subconfluent HEL cells were treated with test compounds at different concentrations for 72 h. The cultures were pulse-labeled with 10 μM 5-bromo-2'-deoxyuridine (BrdU) for 30 min at 37 ^{o}C prior to harvest. The cells were subsequently washed in PBS, fixed with 70% ethanol, and denatured in 2 M HCl. Following neutralization, the cells were stained with anti-BrdU fluorescein-labeled antibodies, washed, stained with propidium iodide, and analyzed by flow cytometry with a 488 nm laser (Cell Lab Quanta SC, Beckman

Coulter, Brea, CA). Cell-cycle analyses were made with a FACScan cytometer (FACSCalibun, Becton Dickinson, Franklin Lakes, NJ).

4.2.4. Cell-Apoptosis Assays

The apoptosis of the HEL cells was determined by an Annexin V-FITC/PI assay.[21] Cells (3×10^5 cells/mL) were seeded in 6-well plate and were treated with varying concentrations of an inhibitor for 72 h. HEL cells were harvested and washed twice with cold PBS buffer. Cell cycle analysis follows the directions of the PI/RNase staining solution (Shenyang Takara Biotech). The collected cells were fixed in 70% ethanol for 1 h. Then cells were stained in propidium iodide (PI) solution at room temperature in the dark for 30 min. In the annexin-V apoptosis assay, cell samples were re-suspended in binding buffer (apoptosis analysis kit from Shenyang Takara Biotech) and incubated with annexin-V and propidium iodide solution protected from light. The samples in both assays were analyzed using a FACS Calibur Cytometer (Becton Dickinson, San Jose, CA, USA). The upper left corner of the quadrant represents debris, the lower left is live cells, the upper right is advanced-apoptotic or necrotic cells, and the lower right is apoptotic cells.

4.3. Molecular docking

The molecules were built using Maestro, version 8.0.308, or converted to 3D structures from the 2D structure using LigPrep, version 2.1.207. The JAK2 (PDB entry 4AQC), and FLT3 (PDB entry 4XUF) X-ray structures were downloaded from the Protein Data Bank (PDB, http://www.rcsb.org/). The protein structures were prepared using the protein preparation wizard in Maestro with standard settings. Grids

were generated using Glide, version 4.5.208, following the standard procedure recommended by Schrödinger. The conformational ensembles were docked flexibly using Glide with standard settings in both standard and extra precision mode. Only poses with low energy conformations and good hydrogen bond geometries were considered. Figures were drawn using PyMOL (version 1.7). The t-PSA values were calculated using Marvin Sketch, version 6.1.0, with standard settings.

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Highlights

1. A series of novel 4-piperazinyl-2-aminopyrimidine derivatives were firstly designed and synthesized as JAK2/FLT3 dual inhibitors.
2. Compound **14j** showed the most balanced antiproliferative activity against JAK2 and FLT3 with the IC_{50} value of 27 nM and 30 nM, respectively.
3. Compound **14j** showed good inhibition against HEL and moderate inhibition against MV4-11 and HL60.
4. **14j** arrested cell cycle in G_1/S phase and induced the apoptosis of HEL cells in a dose-dependent manner.