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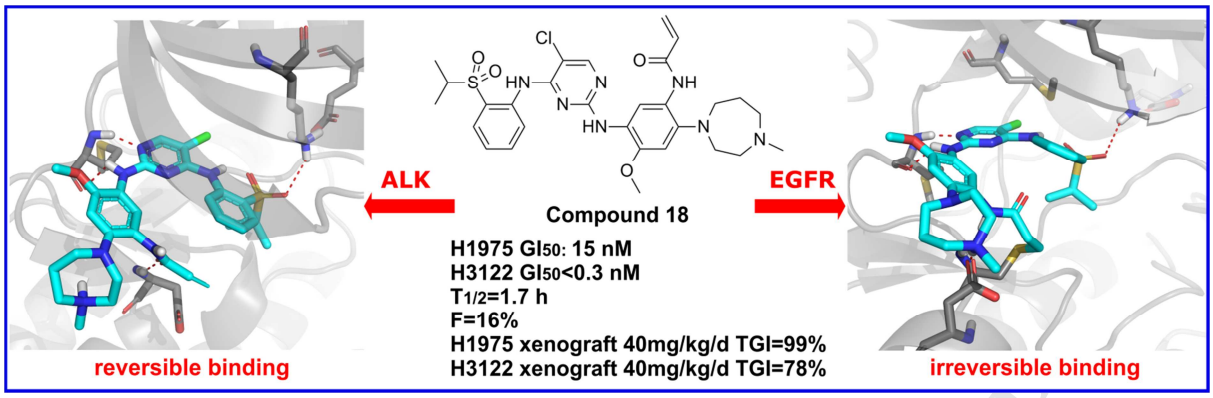

Discovery of *N*-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-methyl-1,4-diazepan-1-yl)phenyl)acrylamide (CHMFL-ALK/EGFR-050) as a Potent ALK/EGFR Dual Kinase Inhibitor Capable of Overcoming a Variety of ALK/EGFR Associated Drug Resistant Mutants in NSCLC

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ABSTRACT

Recently, more and more concomitant EGFR mutations and ALK rearrangement are observed from the clinic, which still lacks effective single-agent therapy. Starting from ALK inhibitor **14** (TAE684), we have developed a highly potent EGFR/ALK dual kinase inhibitor compound **18** (CHMFL-ALK/EGFR-050), which potently inhibited EGFR L858R, del 19 and T790M mutants as well as EML4-ALK, R1275Q, L1196M, F1174L and C1156Y mutants biochemically. Compound **18** significantly inhibited the proliferation of EGFR mutant and EML4-ALK driven NSCLC cell lines. In the cellular context it strongly affected EGFR and ALK mediated signaling pathways, induced apoptosis and arrested cell cycle at G0/G1 phase. In the *in vivo* studies, **18** significantly suppressed the tumor growth in H1975 cell inoculated xenograft model (40 mg/kg/d, TGI: 99%) and H3122 cell inoculated xenograft model (40 mg/kg/d, TGI: 78%). Compound **18** might be a potential drug candidate for EGFR- or ALK-individual as well as concomitant EGFR/ALK NSCLC.

Keywords

EGFR; ALK; Dual kinase inhibitor; Non-small cell lung cell cancer

Abbreviations used

NSCLC, non-small cell lung cell cancer; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; EML4, echinoderm microtubule-associated protein like 4; NPM, nucleophosmin; SAR, structure-activity relationship; CHL, Chinese hamster Lung; CHO, Chinese hamster ovary; eIF4E, eukaryotic initiation factor 4E; 4EBP1, 4E-binding protein 1; P70S6k, p70S6 kinase 1; STAT3, signal transducer and activator of transcription 3; ERK, extracellular regulated protein kinases; PARP, poly ADP-ribose polymerase; PK, pharmacokinetics; IHC, immunohistochemistry; HE, hematoxylin-eosin; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; TGI, tumor growth inhibition.

1. INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers. Currently a number of driving oncogenes are identified in NSCLC and among them EGFR activating mutations and ALK rearrangements are two prevalent ones. EGFR activating mutations are found in approximately 10–30% of the patients with NSCLC [1-3]. Two frequent and mutually exclusive primary mutations occur either in the activation loop as a point mutation (EGFR L858R) or by short deletion in exon 19 (EGFR-del19), which together account for approximately 85% of all cases.[4] First generation EGFR tyrosine kinase inhibitors (TKIs) such as compounds **1** (Erlotinib) [1,5] and **2** (Gefitinib) [6] are now

established therapies for NSCLC patients with oncogenic EGFR primary mutations (Figure 1). However, upon continuous treatment, patients become resistant and approximate 60% cases will develop a secondary T790M mutation at the gatekeeper position of EGFR [7-9]. To overcome the drug resistance caused by T790M mutation, several second generation irreversible EGFR inhibitors such as compounds **3** (Afatinib) [10], **4** (Dacomitinib) [11] and more selective third generation inhibitors such as compounds **5** (WZ4002) [12], **6** (Rociletinib) [13], **7** (Osimertinib) [14,15] and **8** (Olmutinib) [16] have been developed.

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase of the insulin receptor superfamily. Various ALK-fused oncogenes such as echinoderm microtubule associated protein like 4 (EML4)-ALK have been identified in 2-7% NSCLC [17]. Validation of ALK as an important therapeutic target has led to the development of ALK inhibitors such as compounds **9** (Crizotinib) [18], **10** (Ceritinib) [19], **11** (Alectinib) [20], **12** (Brigatinib) [21] and **13** (ASP3026) [22], which have been approved for clinical use except **13**. It is noteworthy is that compound **12** has been reported to be able to inhibit both ALK and EGFR kinases [21].

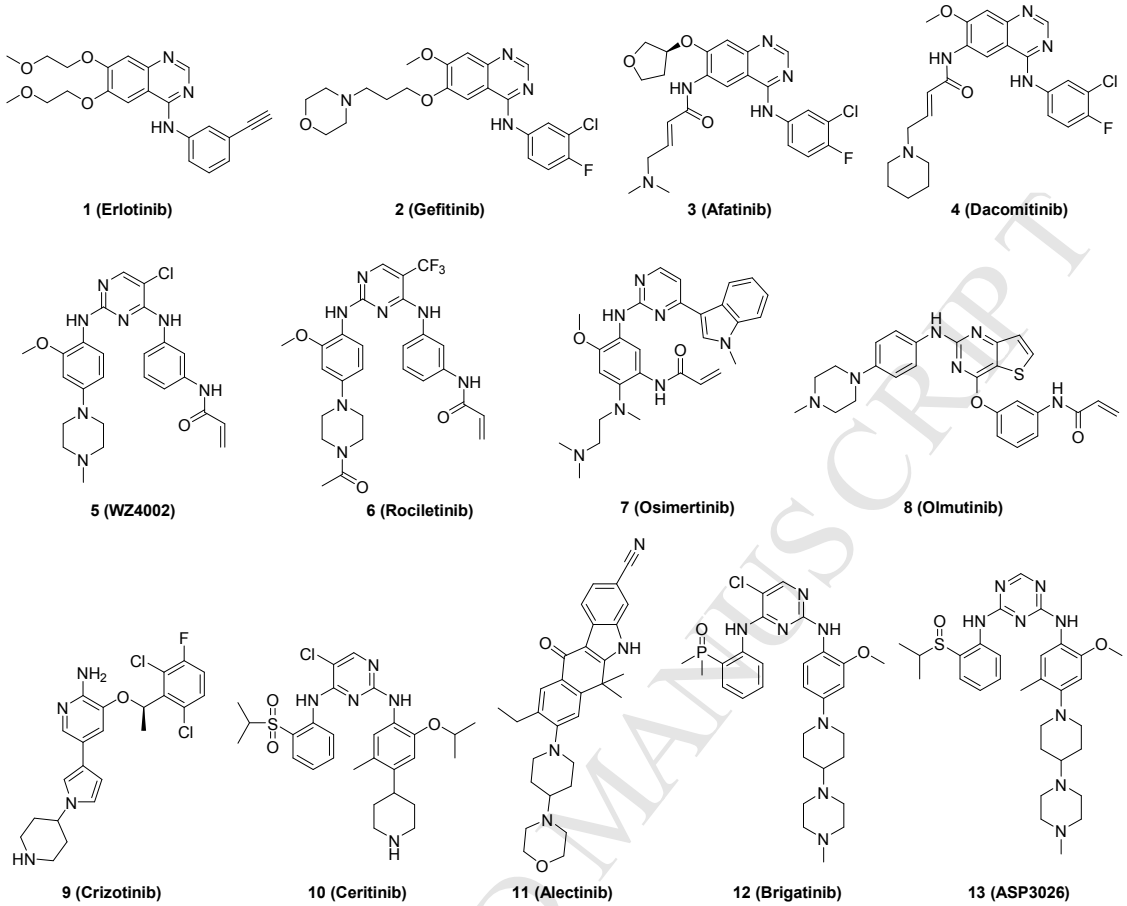


Figure 1. Chemical structures of representative EGFR and ALK kinase inhibitors.

EGFR mutants and ALK rearrangements have been once considered as mutually exclusive [23,24]. However, with the development of high throughput sequencing and deep sequencing technologies, more and more concomitant EGFR mutants and ALK rearrangements are identified from the single colony of the tumor tissue in patients [25-30], in which case single EGFR or ALK inhibitor could not display a good therapeutic effect. In addition, in ALK driven NSCLC cells H3122, an EGFR reactivation adaptive drug resistance mechanism has been identified [31]. Although theoretically combination of EGFR and ALK inhibitors would enhance the antitumor efficacy against the EGFR/ALK co-expression or co-activation, drug-drug interaction problems would make

the dual-target-single-agent strategy more preferred. Based on this concept, via structure based drug design approach, we have discovered a potent ALK/EGFR dual kinase inhibitor compound **18 (**CHMFL-ALK/EGFR-050), which displayed strong activities against EGFR mutants such as L858R, del 19, T790M and ALK mutants such as F1174L, C1156Y, L1196M, etc (Figure 2). During the preparation of this manuscript, Gray team has also reported a similar compound developed from compound **10** (Ceritinib) which could inhibit both ALK and EGFR T790M mutant [32].

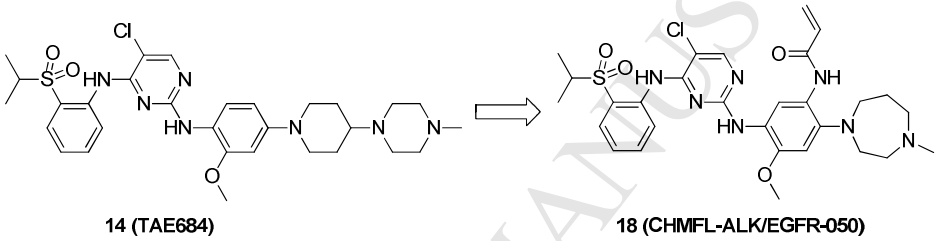


Figure 2. Schematic illustration of discovery of compound **18** (CHMFL-ALK/EGFR-050).

2. Results and discussion

2.1 Design and synthesis

In order to seek the proper core pharmacophore for medicinal chemistry exploration, we started from screening a panel of kinase inhibitors that are clinically approved or under clinical investiation against H1975 cells. We found that compound **14** (TAE-684) [33], which was a well-established ALK inhibitor, exhibited moderate antiproliferative efficacy (GI_{50} = 0.72 μM). Further testing in EGFR transformed BaF3 cells showed that **14** could moderately inhibit the proliferation of BaF3-TEL-EGFR (GI_{50}: 0.56 μM), BaF3-TEL-EGFR-T790M (GI_{50}: 0.42 μM) and displayed proper selectivity window against

parental BaF3 cells (GI_{50}: 1.1 μM), which indicated that it did bear the on-target activity against EGFR. We then docked **14** into the EGFR T790M X-ray structure (PDB ID: 3IKA) and found that the distance from R_1 position to Cys797 is about 5 Å and it would be approachable for an electrophilic group. Therefore we postulated that this scaffold might be amenable for developing an irreversible inhibitor to enhance the inhibitory effect (Figure 3A). In addition, installment of the irreversible warhead would probably not affect the ALK kinase binding (PDB ID: 2XB7) because there is no amino acid residue that can block the binding around this position (Figure 3B). On the basis of the analysis, we decided to introduce an acrylamide at the R_1 position and explore the R_2, R_3, R_4, R_5 and R_6 positions to obtain a highly potent ALK/EGFR dual kinase inhibitor (Figure 3C).

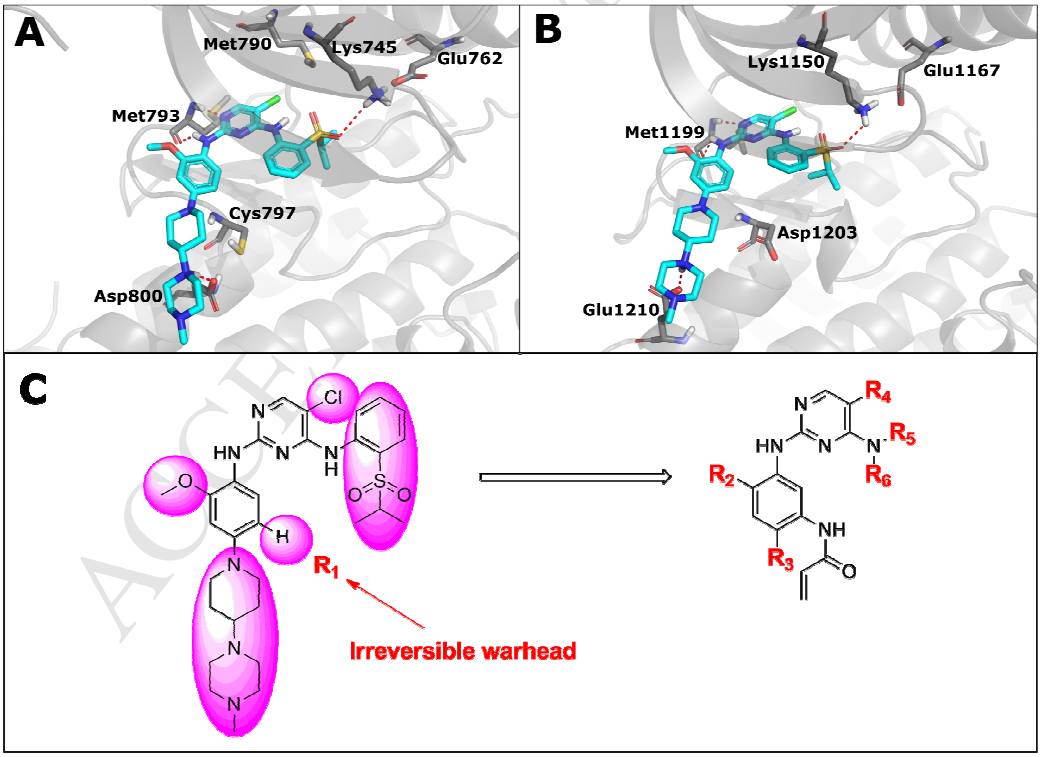
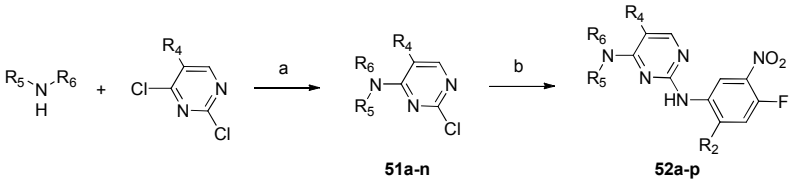


Figure 3. Design rationale based on compound **14**'s core pharmacophore. (A) Docking of **14** into EGFR T790M mutant protein (PDB ID: 3IKA). (B) Docking of **14**'s into ALK kinase (PDB ID: 2XB7). (C) Schematic illustration of SAR exploration starting from **14**'s core pharmacophore.

Compounds **15-49** were prepared according to the general synthetic route depicted in scheme 1. Substitution reaction between substituted amines and 2,4-dichloropyrimidine analogues afforded intermediates **51a-n**. Another substitution with 4-fluoro-3-nitroaniline analogues under acidic condition provided corresponding products **52a-p**. Compounds **53a-i'** were generated by substitution with piperazine or piperidine derivatives, or morpholine. The nitro group was then reduced with Fe or $SnCl_2$ to yield intermediates **54a-i'**. Finally acylation reaction with acryloyl chloride furnished the desired compounds **15-49**. Compound **50**, which was compound **18**'s reversible version, was prepared following the same synthetic route except that propionyl chloride was used instead in the last acylation step to react with compound **54d** (Scheme 2).

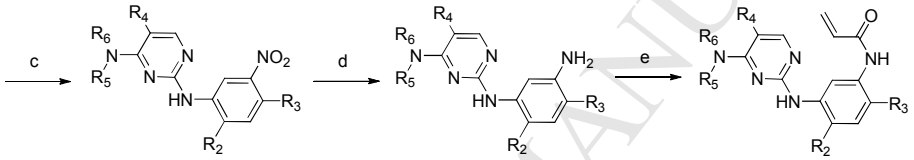
Scheme 1. Synthesis of compounds **15-49**[a]



51a-n **52a-p**

51a, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H
51b, R_4=H, R_5=2-(isopropylsulfonyl)phenyl, R_6=H
51c, R_4=CH_3, R_5=2-(isopropylsulfonyl)phenyl, R_6=H
51d, R_4=Cl, R_5=3-(trifluoromethyl)phenyl, R_6=H
51e, R_4=Cl, R_5=3-chloro-4-fluorophenyll, R_6=H
51f, R_4=Cl, R_5=2,6-difluorophenyl, R_6=H
51g, R_4=Cl, R_5=thiophen-2-ylmethyl, R_6=H
51h, R_4=Cl, R_5=cyclopropyl, R_6=H
51i, R_4=Cl, R_5=2-pivalamidophenyl, R_6=H
51j, R_4=Cl, R_5=3-(trifluoromethyl)phenyl, R_6=CH_3
51k, R_4=Cl, R_5=2-(*N*,*N*-dimethylsulfamoyl)phenyl, R_6=H
51l, R_4=Cl, R_5=2-(dimethylphosphoryl, R_6=H
51m, R_4=Cl, R_5=2-(methylcarbamoyl, R_6=H
51n, R_4=Cl, R_5=2-cyanophenyl, R_6=H

52a, R_2=OMe, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H
52b, R_2=OMe, R_4=H, R_5=2-(isopropylsulfonyl)phenyl, R_6=H
52c, R_2=OMe, R_4=CH_3, R_5=2-(isopropylsulfonyl)phenyl, R_6=H
52d, R_2=H,R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H
52e, R_2=$OCH(CH_3)_2$, R_4=CH_3, R_5=2-(isopropylsulfonyl)phenyl, R_6=H
52f, R_2=OMe, R_4=Cl, R_5=3-(trifluoromethyl)phenyl, R_6=H
52g, R_2=OMe, R_4=Cl, R_5=3-chloro-4-fluorophenyll, R_6=H
52h, R_2=OMe, R_4=Cl, R_5=2,6-difluorophenyl, R_6=H
52i, R_2=OMe, R_4=Cl, R_5=thiophen-2-ylmethyl, R_6=H
52j, R_2=OMe, R_4=Cl, R_5=cyclopropyl, R_6=H
52k, R_2=OMe, R_4=Cl, R_5=2-pivalamidophenyl, R_6=H
52l, R_2=OMe, R_4=Cl, R_5=3-(trifluoromethyl)phenyl, R_6=CH_3
52m, R_2=OMe, R_4=Cl, R_5=2-(*N*,*N*-dimethylsulfamoyl)phenyl, R_6=H
52n, R_2=OMe, R_4=Cl, R_5=2-(dimethylphosphoryl, R_6=H
52o, R_2=OMe, R_4=Cl, R_5=2-(methylcarbamoyl, R_6=H
52p, R_2=OMe, R_4=Cl, R_5=2-cyanophenyl, R_6=H

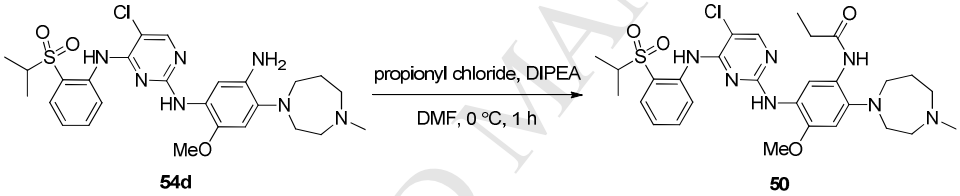


53a-i' **54a-i'** **15-49**

53a-54a,15; R_2=OCH_3, R_3=4-(4-methylpiperazin-1-yl)piperidin-1-yl, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53b-54b,16; R_2=OCH_3, R_3=4-(methylsulfonyl)piperazin-1-yl, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53c-54c,17; R_2=OCH_3, R_3=4-methylpiperazin-1-yl, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53d-54d,18; R_2=OCH_3, R_3=4-methyl-1,4-diazepan-1-yl, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53e-54e,19; R_2=OCH_3, R_3=4-isopropylpiperazin-1-yl, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53f-54f, 20; R_2=OCH_3, R_3=4-(2-methoxyethyl)piperazin-1-yl, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53g-54g, 21; R_2=OCH_3, R_3=4-cyclohexylpiperazin-1-yl, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53h-54h, 22; R_2=OCH_3, R_3=4-morpholino, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53i-54i, 23; R_2=OCH_3, R_3=piperidin-1-yl, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53j-54j, 24; R_2=H, R_3=4-(4-methylpiperazin-1-yl)piperidin-1-yl, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53k-54k, 25; R_2=$OCH(CH_3)_2$, R_3=4-ethylpiperazin-1-yl, R_4=Cl, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53l-54l, 26; R_2=OCH_3, R_3=4-(4-methylpiperazin-1-yl)piperidin-1-yl, R_4=CH_3, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53m-54m, 27; R_2=OCH_3, R_3=4-ethylpiperazin-1-yl, R_4=CH_3, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53n-54n, 28; R_2=OCH_3, R_3=4-ethylpiperazin-1-yl, R_4=H, R_5=2-(isopropylsulfonyl)phenyl, R_6=H;
53o-54o, 29; R_2=OCH_3, R_3=4-methylpiperazin-1-yl, R_4=Cl, R_5=3-(trifluoromethyl)phenyl, R_6=H;
53p-54p, 30; R_2=OCH_3, R_3=4-methylpiperazin-1-yl, R_4=Cl, R_5=3-chloro-4-fluorophenyl, R_6=H;
53q-54q, 31; R 2=OCH_3, R_3=4-methylpiperazin-1-yl, R_4=Cl, R_5=2,6-difluorophenyl, R_6=H;
53r-54r, 32; R_2=OCH_3, R_3=4-methylpiperazin-1-yl, R_4=Cl, R_5=thiophen-2-ylmethyl, R_6=H;
53s-54s, 33; R_2=OCH_3, R_3=4-methylpiperazin-1-yl, R_4=Cl, R_5=cyclopropyl, R_6=H;
53t-54t, 34; R_2=OCH_3, R_3=4-methylpiperazin-1-yl, R_4=Cl, R_5=2-pivalamidophenyl, R_6=H;
53u-54u, 35; R_2=OCH_3, R_3=4-methylpiperazin-1-yl, R_4=Cl, R_5=3-(trifluoromethyl)phenyl, R_6=CH_3;
53v-54v, 36; R_2=OCH_3, R_3=4-methylpiperazin-1-yl, R_4=Cl, R_5=2-(*N*,*N*-dimethylsulfamoyl)phenyl, R_6=H;
53w-54w, 37; R_2=OCH_3, R_3=4-ethylpiperazin-1-yl, R_4=Cl, R_5=2-(dimethylphosphoryl, R_6=H;
53x-54x, 38; R_2=OCH_3, R_3=4-4-(dimethylamino)piperidin-1-yl, R_4=Cl, R_5=2-(dimethylphosphoryl, R_6=H;
53y-54y, 39; R_2=OCH_3, R_3=4-morpholino, R_4=Cl, R_5=2-(dimethylphosphoryl, R_6=H;
53z-54z, 40; R_2=OCH_3, R_3=4-(methylsulfonyl)piperazin-1-yl, R_4=Cl, R_5=2-(dimethylphosphoryl, R_6=H;
53a'-54a', 41; R_2=OCH_3, R_3=1,4'-bipiperidin]-1'-yl, R_4=Cl, R_5=2-(dimethylphosphoryl, R_6=H;
53b'-54b', 42; R_2=OCH_3, R_3=4-methylpiperazin-1-yl, R_4=Cl, R_5=2-(methylcarbamoyl, R_6=H;
53c'-54c', 43; R_2=OCH_3, R_3=4-isopropylpiperazin-1-yl, R_4=Cl, R_5=2-(methylcarbamoyl, R_6=H;
53d'-54d', 44; R_2=OCH_3, R_3=4-acetylpiperidin-1-yl, R_4=Cl, R_5=2-(methylcarbamoyl, R_6=H;
53e'-54e', 45; R_2=OCH_3, R_3=4-(1-methylpiperidin-4-yl)piperazin-1-yl, R_4=Cl, R_5=2-(methylcarbamoyl R_6=H;
53f'-54f', 46; R_2=OCH_3, R_3=4-ethylpiperazin-1-yl, R_4=Cl, R_5=2-cyanophenyl, R_6=H;
53g'-54g', 47; R_2=OCH_3, R_3=4-isopropylpiperazin-1-yl, R_4=Cl, R_5=2-cyanophenyl, R_6=H;
53h'-54h', 48; R_2=OCH_3, R_3=4-morpholino, R_4=Cl, R_5=2-cyanophenyl, R_6=H;
53i'-54i', 49; R_2=OCH_3, R_3=4-(4-methylpiperazin-1-yl)piperidin-1-yl, R_4=Cl, R_5=2-cyanophenyl, R_6=H;

[a]Reagents and conditions: (a) for **51a**, NaH, DMF, 0 ^{o}C-rt, 12 h; for **51b-n**, DIPEA, propan-2-ol, reflux, 12 h; (b) for **52a-c** and **52f-p**, 4-fluoro-2-methoxy-5-nitroaniline; for **52d**, 4-fluoro-3-nitroaniline; for **52e**, 4-fluoro-2-isopropoxy-5-nitroaniline, PTSA, 2-pentanol, 115 ^{o}C, 5 h; (c) for **53a**, **53i-j**, **53l**, **53x**, **53a'**, **53i'**, piperidine derivatives; for **53b-c**, **53e-g**, **53k**, **53m-w**, **53z**, **53b'-g'**, piperazine derivatives; for **53d**, 1-methyl-1,4-diazepane; for **53h**, **53y**, **53h'**, morpholine, DMF, 140 ^{o}C, 2 h; (d) for **54a-v**, Fe, NH_4Cl, EtOH/H_2O, reflux, 1 h; for **54w-i'**, $SnCl_2$, EtOH, reflux, 2 h; (e) acryloyl chloride, DIPEA, DMF, 0 ^{o}C, 0.5 h.

Scheme 2. Synthesis of compound **50**[a]



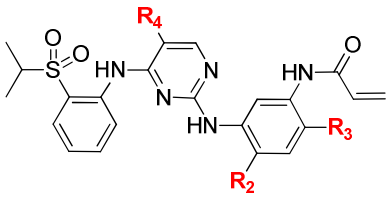
2.2 Biological evaluation

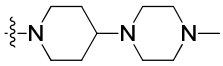
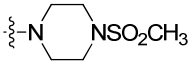
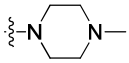
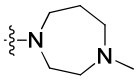
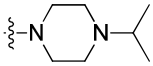
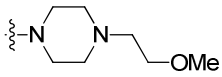
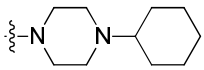
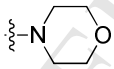
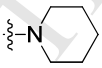
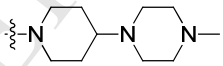
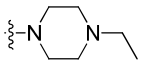
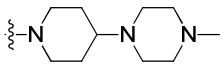
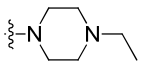
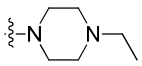
2.2.1 Structure-activity relationship (SAR) investigation

To test our design rationale, we first prepared compound **15** which bears an acrylamide moiety at R_1 to explore the antiproliferative potency against EGFR mutant T790M driven H1975 cells. Compared with compound **14**, **15** significantly increased the activity against H1975 cells (GI_{50} = 0.023 μM) (Table 1). Moreover, **15** retained the activity against EML4-ALK rearrangement driven NSCLC cell line H3122 (GI_{50} <0.0003 μM), which indicated that the molecular scaffold of **14** was suitable for developing ALK/ EGFR dual targets inhibitor.

We then prepared a series of modifications at different positions of skeleton of **14** to explore the SAR based on the anti-proliferative activity against EGFR and ALK driven cell lines. We first focused on the R_2, R_3 and R_4 moieties by fixing the R_5 and R_6 moieties. Inhibitory activities (GI_{50}) of these compounds against H1975 and H3122 cells were shown in Table 1. The results indicated that when keeping R_2 as methoxy group and R_4 as –Cl, variation of R_3 group as different piperazine-based derivatives (**15-21**) all led to potent inhibitory activities against H1975 (GI_{50}: 0.015-0.077 μM) and retained high potency against H3122 cells (GI_{50}s <0.0003 μM except 0.033 μM for **17**). In particular, the methylated 1,4-diazepane at R_3 (**18**) exhibited the best activity to H1975 and H3122 cells (GI_{50}: 0.015 μM and <0.0003 μM respectively). Interestingly, installment of morpholine (**22**) or piperidine (**23**) at R_3 resulted in significant activity loss to H1975 cells. Keeping compound **14**'s R_3 group and removing the methoxy group at R_2 (**24**) led to significant activity loss against H3122 cells (GI_{50}: 4.9 μM). Switching R_2 to a much larger group (isopropoxyl) (**25**) also caused obvious activity loss to H1975 (GI_{50} = 0.30 μM) although it retained the inhibitory activity against H3122 (GI_{50} < 0.0003 μM). Replacement of the -Cl atom with a methyl group (**26**, **27**) remained good inhibitory activities to both H1975 (GI_{50} = 0.027 μM, 0.043 μM) and H3122 (GI_{50} < 0.0003 μM, 0.002 μM). However, removal of the -Cl atom (**28**) resulted in activity loss to both H1975 (GI_{50} = 0.19 μM) and H3122 cells (GI_{50} = 0.041 μM).

Table 1. SAR Exploration Focused on the R_2/R_3/R_4 Moieties[a]



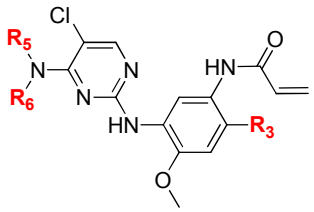
Compd.	R_2	R_3	R_4	H1975 (GI_{50}: μM)	H3122 (GI_{50}: μM)
15	$-OCH_3$		-Cl	0.023±0.0036	＜0.0003
16	$-OCH_3$		-Cl	0.040±0.0012	＜0.0003
17	$-OCH_3$		-Cl	0.043±0.0013	0.033±0.0052
18	$-OCH_3$		-Cl	0.015±0.0004	＜0.0003
19	$-OCH_3$		-Cl	0.025±0.0003	＜0.0003
20	$-OCH_3$		-Cl	0.041±0.04	＜0.0003
21	$-OCH_3$		-Cl	0.077±0.0047	＜0.0003
22	$-OCH_3$		-Cl	1.2±0.03	＜0.0003
23	$-OCH_3$		-Cl	0.24±0.0053	0.029±0.01
24	-H		-Cl	7.1±0.63	4.9±0.072
25	$-OCH(CH_3)_2$		-Cl	0.30±0.011	＜0.0003
26	$-OCH_3$		$-CH_3$	0.027±0.0021	＜0.0003
27	$-OCH_3$		$-CH_3$	0.043±0.0053	0.0020±0.0001
28	$-OCH_3$		-H	0.19±0.0058	0.041±0.0043

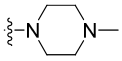
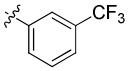
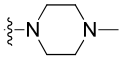
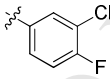
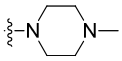
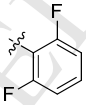
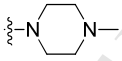
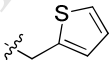
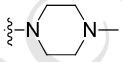
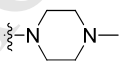
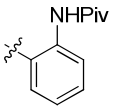
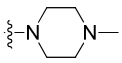
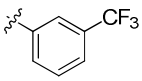
[a]All GI_{50} values were obtained by triplet testing.

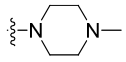
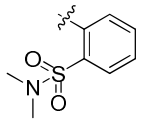
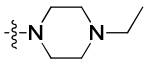
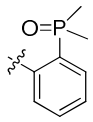
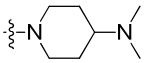
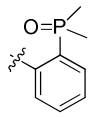
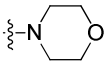
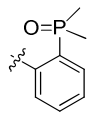
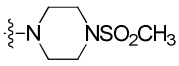
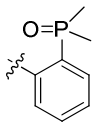
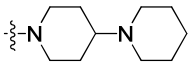
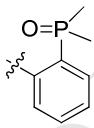
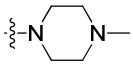
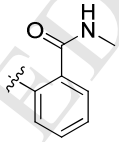
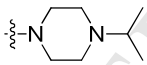
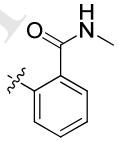
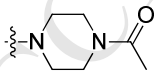
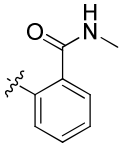
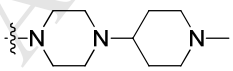
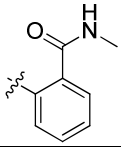
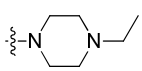
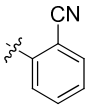
On the basis of these results, we decided to keep the R_2 (-CH_3O) and R_4 (-Cl) moieties and explore the R_3, R_5 and R_6 moieties (Table 2). When R_3 was set as methyl piperazine and R_6 was the -H atom or -CH_3 group, replacement of the isopropylsulfonyl benzene with other aromatic rings or cyclopropane which lack the hydrogen bond acceptor (**29-35**) all led to significant activity loss against both H1975 and H3122 cells. However, when switching the R_5 moiety back to isopropylsulfonyl benzene (**36**) started to gain back the activity against H1975 (GI_{50}: 0.031 μM) and H3122 cells (GI_{50}: 0.011 μM), although compared to compound **15**, it still lost 30-fold activity to H3122 cells. This indicated that the hydrogen bond acceptor at R_5 position is required for the EGFR and ALK kinase activity. We then replaced the isopropylsulfonyl substituent with dimethyl phosphine oxide and kept the –H atom at R_6 to explore the R_3 moiety. The results demonstrated that with ethyl piperazine at R_3, **37** exhibited good anti-proliferative effect against H1975 (GI_{50}: 0.026 μM) and H3122 (GI_{50}: 0.037 μM). However, switching this moiety to *N*,*N*-dimethylpiperidin-4-amine (**38**), morpholine (**39**) or methylsulfonylpiperazine (**40**) all led to significant activity loss to both H1975 and H3122 cells. Interestingly, with the 1,4'-bipiperidine at R_3, **41** started to gain back the activity to H1975 (GI_{50}: 0.041 μM) and H3122 (GI_{50}: 0.078 μM). We then changed the isopropylsulfonyl substituent to *N*-methylacetamide to further explore the R_3 moiety. The data demonstrated that methyl piperazine (**42**) and isopropyl piperazine (**43**) exhibited good activity to H1975 and H3122 cells. But acyl piperazine (**44**) and 1-(1-methylpiperidin-4-yl)piperazine (**45**) both caused significant activity loss compared to

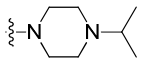
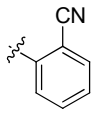
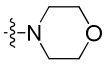
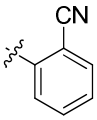
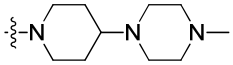
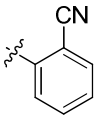
compound **15**. In addition, the efforts to switch the isopropylsulfonyl substituent to nitrile group at R_5 position (**46**-**49**) all resulted in obvious activity loss to both cell lines.

Table 2. SAR Exploration Focused on the $R_3/R_5/R_6$ Moieties[a]



Compd.	R_3	R_5	R_6	H1975 (GI_{50}: μM)	H3122 (GI_{50}: μM)
29			H	0.26±0.0012	0.77±0.0006
30			H	0.10±0.0058	0.30±0.017
31			H	0.13±0.02	2.9±0.052
32			H	0.22±0.025	0.27±0.02
33			H	>10	3.2±0.0023
34			H	0.95±0.032	6.5±0.75
35			CH_3	1.8±0.11	3.6±0.24

36			H	0.031±0.0012	0.011±0.0078
37			H	0.026±0.0004	0.037±0.0023
38			H	0.22±0.03	0.14±0.052
39			H	0.78±0.0051	0.36±0.032
40			H	0.89±0.0022	0.80±0.034
41			H	0.041±0.0016	0.078±0.0031
42			H	0.050±0.006	0.0035±0.0001
43			H	0.079±0.006	0.0045±0.0003
44			H	0.25±0.0093	0.012±0.0023
45			H	0.12±0.003	0.018±0.0003
46			H	0.44±0.07	0.11±0.019

47			H	0.43±0.052	0.11±0.029
48			H	1.2±0.056	0.37±0.021
49			H	0.20±0.0024	0.098±0.0004

[a]All GI_{50} values were obtained by triplet testing.

2.2.2 EGFR and ALK on-target activity examination of compound 18

Since compound **18** exhibited the best anti-proliferative effects against H1975 and H3122 cells, we then tested it in the enzymatic assays against purified proteins with Invitrogen's SelectScreen technology to confirm its on-target effect (Table 3). The results showed that **18** was highly potent against EGFR drug resistant mutants T790M (IC_{50}: 3.9 nM) and L858R/T790M (IC_{50}: 3.6 nM) meanwhile displayed 20-30 fold selectivity over EGFR gain-of-function mutant L858R (IC_{50}: 82.5 nM) and EGFR wt (IC_{50}: 108 nM). In addition, it exhibited IC_{50} values less than 1 nM against ALK mutants including ALK R1275Q, L1196M, F1174L and C1156Y and an IC_{50} of 9.8 nM against ALK wt. In order to further confirm these on-target effects, we then tested **18** in a panel of EGFR/ALK transformed BaF3 cells which grow dependently on the EGFR or ALK mutants. It showed that **18** was highly potent to EGFR wt (GI_{50}: 0.016 μM) and exhibited about 15-fold selectivity against EGFR-wt-C797S (GI_{50}: 0.23 μM) (Table 4). Similar trends were observed for EGFR L858R/L858R-T790M/L858R-T790M-C797S and EGFR del19 transformed BaF3 cells. In addition, compound **50** in which the acrylamide at R_1 was

replaced with propyl amide that lacks the covalent bond formation capability lost the activities against EGFR wt, L858R and del19 significantly compared to compound **18**. These results indicated that **18** inhibited EGFR and mutants with an irreversible binding mode through formation of a covalent bond with C797 residue, like the canonical EGFR irreversible inhibitor compound **5**. Furthermore, **18** could inhibit the proliferation of EGFR T790M and L858R-T790M mutants transformed BaF3 cells, which further confirmed its capability to overcome the drug-resistant gatekeeper mutations as the strong anti-proliferative efficacy observed in H1975 cells. Compound **18** also displayed potent inhibitory activities against NPM and EML4 rearranged ALK transformed BaF3 cells (Table 5). More importantly, it could overcome compound **9**'s drug-resistant mutants including ALK F1174L, C1156Y and L1196M. However, for the ALK G1202R mutant, neither **18** nor **9** exhibited good inhibitory activity.

Table 3. Compound **18**'s Enzymatic Activity against EGFR/ALK and Associated Mutants[a]

Target	Compd. **18** (IC_{50}: nM)
EGFR wt	108±1.8
EGFR L858R	82.5±1.5
EGFR T790M	3.9±0.2
EGFR L858R/T790M	3.6±0.2
ALK wt	9.8±0.3
ALK R1275Q	0.82±0.04
ALKL 1196M	0.59±0.02
ALK F1174L	0.92±0.22

ALK C1156Y	1.0±0.04

[a]All GI_{50} values were obtained by triple testing.

Table 4. Compound **18**'s Antiproliferative Effect against a Panel of EGFR/mutants Transformed BaF3 Cell Lines[a]

Cell Line	Compd. **18** (GI_{50}: μM)	Compd. **50** (GI_{50}: μM)	Compd. **5** (GI_{50}: μM)
Parental BaF3	1.2±0.056	7.1±0.077	2.2±0.047
BaF3-TEL-EGFR	0.016±0.0003	1.5±0.033	1.3±0.034
BaF3-TEL-EGFR-C797S	0.23±0.026	0.36±0.0057	0.89±0.032
BaF3-TEL-EGFR-T790M	0.0080±0.0004	0.80±0.049	0.0021±0.0002
BaF3-FL-EGFR-del19	0.0085±0.0005	0.27±0.0042	＜0.0003
BaF3-FL-EGFR-L858R	＜0.0003	0.37±0.04	＜0.0003
BaF3-FL-EGFR-L858R-T790M	＜0.0003	0.29±0.042	＜0.0003
BaF3-FL-EGFR-L858R-T790M-C797S	1.1±0.048	1.7±0.12	2.2±0.12

[a]All GI_{50} values were obtained by triple testing.

Table 5. Compound **18**'s Antiproliferative Effect against a Panel of ALK/mutants Transformed BaF3 Cell Lines[a]

Cell Line	Compd. **18** (GI_{50}: μM)	Compd. **9** (GI_{50}: μM)
Parental BaF3	1.2±0.056	1.1±0.05
BaF3-NPM-ALK	0.001±0.0001	＜0.0003
BaF3-EML4-ALK	＜0.0003	＜0.0003

BaF3-TEL-ALK	＜0.0003	<0.0003
BaF3-FL-ALK-F1174L	0.029±0.0031	0.32±0.026
BaF3-TEL-ALK-C1156Y	0.0069±0.0009	0.15±0.0086
BaF3-TEL-ALK-L1196M	＜0.0003	0.59±0.042
BaF3-TEL-ALK-G1202R	0.46±0.04	0.43±0.03

[a]All GI_{50} values were obtained by triple testing.

2.2.3 Compound 18's binding mode examination

In order to better understand compound **18**'s binding mechanism we then docked it into EGFR T790M (PDB ID: 3IKA) and ALK (PDB ID: 2XB7). The modeling results showed that in EGFR kinase, **18** formed two hydrogen bonds in the hinge binding area with Met793 (Figure 4A). The acrylamide at R_1 formed a covalent bond with Cys797 residue, and the diazepane at R_3 formed a hydrogen bond with Asp800 residue adjacent to the hinge binding area. Meanwhile, the –Cl at R_4 formed a halogen bond with Met790, which strengthened the binding. This could explain that **18** was much more potent against EGFR T790M mutant than EGFR wt and EGFR L858R in the biochemical enzymatic examination (IC_{50}) because the latter two kinases lacked this halogen bond. This is similar to the canonical EGFR T790M mutant selective inhibitor **7**.[12] In addition, the isopropylsulfonyl group formed a hydrogen bond with Lys745, which could explain why compounds **29-35** lost activities significantly and the hydrogen bond acceptor at this position was required. Similarly, in ALK kinase **18** formed two hydrogen bonds with Met1199 in the hinge binding area (Figure 4B). The acrylamide at R_1 formed a hydrogen bond with Asp1203, which further strengthened the binding. Also, the isopropylsulfonyl

group formed a hydrogen bond with Lys1150, which explained why compounds **29-35** lost activities to ALK.

In addition, we performed the washing out experiment in H1975 and H3122 cells to examine the phosphorylation of EGFR and ALK kinases. The results showed that **18** exhibited time- and dose-dependent inhibition of the pEGFR Y1068 site (Figure 4C). At the concentration of 300 nM, even after 24 h washing out the inhibitor, the phosphorylation level still remained inhibited, which indicated that **18** indeed worked with irreversible binding mode in the cellular context. However, in H3122 cell, after the washing out, pALK Y1604 recovered immediately, which indicated that it adopted a reversible binding mode (Figure 4D). This is consistent with the structure analysis because there is no approachable cysteine residue by the acrylamide.

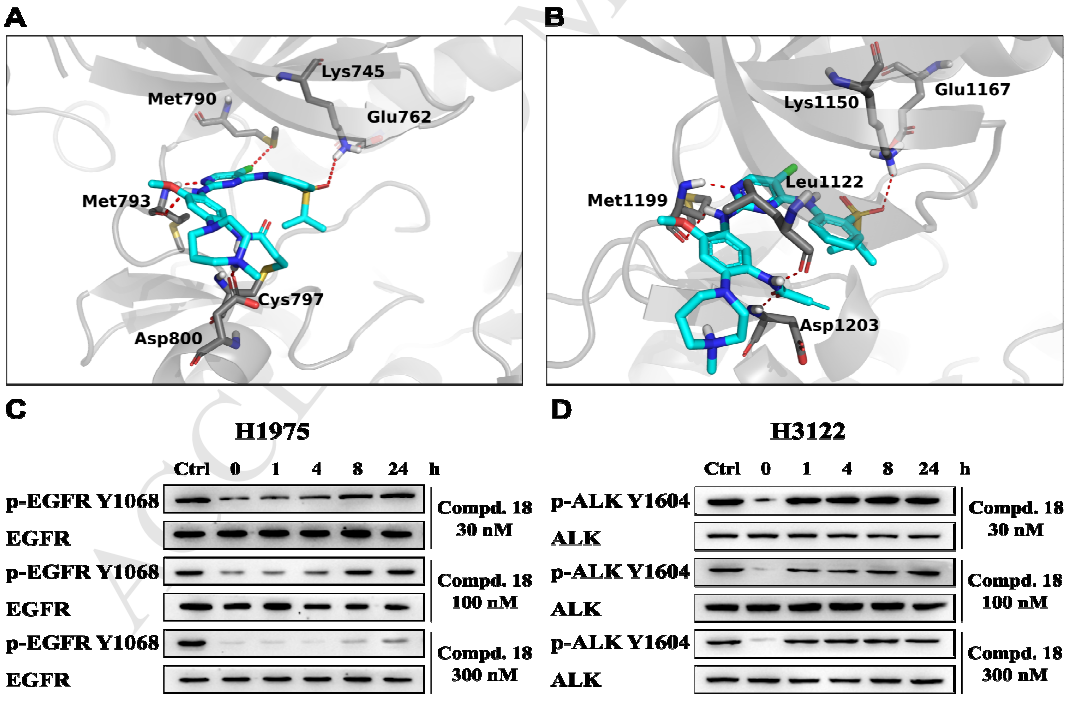


Figure 4. Binding mode characterization of compound **18** on EGFR and ALK kinases. (A) Docking of **18** into X-ray crystal structure of EGFR T790M (PDB ID: 3IKA). (B)

Docking of **18** into X-ray crystal structure of ALK kinase (PDB ID: 2XB7). (C) Washing out experiment of **18** in H1975 cell for the phosphorylation of EGFR kinase at different concentrations and time points. (D) Washing out experiment of **18** in H3122 cell for the phosphorylation of ALK kinase at different concentrations and time points.

2.2.4 Compound 18's antiproliferative effects on a panel of EGFR and ALK driven NSCLC cell lines

We next evaluated compound **18**'s anti-proliferative effects in the EGFR and ALK driven NSCLC cell lines (Table 6). The data showed that besides H1975 cell (EGFR L858R/T790M) and H3122 cell (EML4-ALK), **18** also potently inhibited the proliferation of PC-9 (EGFR del 19) (GI_{50}: 0.015 μM), HCC827 (EGFR del 19) (GI_{50}: 0.0062 μM), H3255 (EGFR L858R) (GI_{50}: 0.031 μM) as well as H2228 (EML4-ALK) cells (GI_{50}: 0.028 μM). It displayed much less anti-proliferative inhibitory activities against EGFR wt expressing cells including A549, H2122 and H1355 cells. In addition, **18** exhibited good selectivity against Chinese hamster ovarian cells CHO (GI_{50}: 1.8 μM) and CHL (GI_{50}: 3.5 μM), which indicated no apparent general cytotoxicity. EGFR inhibitor compound **5** and ALK inhibitor compound **9** displayed similar trends in the EGFR driven and ALK driven cell lines respectively. Furthermore, **18** exhibited better antiproliferative efficacy than **5** in the EGFR mutant driven cell lines and **9** in the ALK rearrangement driven cell lines. Meanwhile, in the EGFR mutant driven cell lines, compound **18** was much more potent than its reversible version compound **50**, which further confirmed that the irreversible binding effect of **18** was biologically relevant.

Table 6. Compound **18**'s Antiproliferative Effects against a Panel of EGFR/ALK mutants Driven Cell Lines[a]

Cell Line	Compd. **18** (GI_{50}: μM)	Compd. **50** (GI_{50}: μM)	Compd. **5** (GI_{50}: μM)	Compd. **9** (GI_{50}: μM)
H1975 (EGFR L858R/T790M)	0.015±0.0003	0.7±0.0055	0.056±0.003	/
PC-9 (EGFR del19)	0.015±0.003	0.34±0.042	0.027±0.001	/
HCC827 (EGFR del19)	0.0062±0.0005	2.6±0.51	0.0074±0.002	/
H3255 (EGFR L858R)	0.031±0.0059	0.83±0.0072	0.39±0.034	/
A549 (EGFR wt)	5.6±0.59	1.2±0.024	>10	/
H2122 (EGFR wt)	1.8±0.02	3.3±0.28	5.7±0.35	/
H1355 (EGFR wt)	1.4±0.035	2.2±0.0064	5.3±0.19	/
H3122(EML4-ALK varian1)	＜0.0003	＜0.0003	/	0.037±0.003
H2228 (EML4-ALK varian3)	0.028±0.005	0.1±0.0065	/	0.073±0.002
CHO	1.8±0.032	3.6±0.19	3.9±0.0041	3.2±0.065
CHL	3.5±0.41	1.3±0.1	4.4±0.0046	0.45±0.019

[a]All GI_{50} values were obtained by triple testing.

2.2.5 Compound 18's cellular effects on the EGFR/ALK mutants driven NSCLC cell lines

We next examined the effects of compound **18** on the EGFR/ALK mediated signaling pathways. In H1975 and PC9 cells, at the concentration of 30 nM compound **18** could completely inhibit the phosphorylation of EGFR Y1068 and subsequently block the downstream mediator ERK Thr202/204 phosphorylation (Figure 5A). As expected, in the EGFR wt expressing NSCLC cell line A549, only pEGFR started to be affected at the concentration of 300 nM and none of the downstream mediators was inhibited. In EML4-ALK rearranged H3122 cells, **18** almost completely blocked the phosphorylation of ALK

Y1064 at the concentration of 100 nM, and also affected the phosphorylation of downstream mediators such as AKT and ERK. In addition, compound **18** could effectively arrest H1975, PC9 and H3122 cell cycle into G0/G1 phase at 24 h but did not affect A549 cell cycle progression (Figure 5B). In H1975, PC9 and H3122 cells, compound **18** could start to induce the apoptosis by examining the cleaved PARP and Caspase-3 from 300 nM concentration upon 24 h treatment. But in A549 cells, it did not exhibited apoptosis induction effect up to 1 μM concentration (Figure 5C). These results indicated that **18**'s biological effect was indeed from EGFR/ALK on-target inhibition.

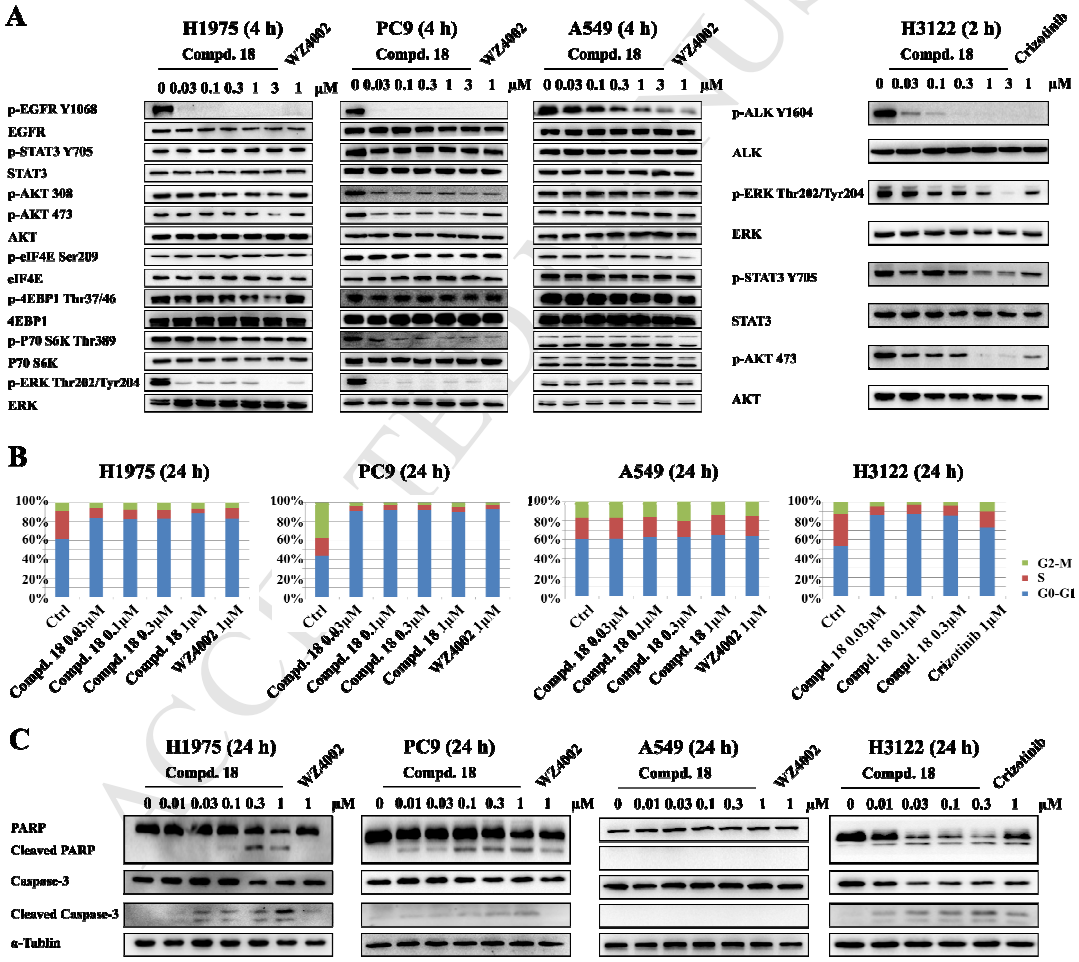


Figure 5. Compound **18**'s effects in the cellular context in the EGFR/ALK driven NSCLC cell lines. (A) **18**'s effects on the EGFR and ALK mediated signaling pathways in H1975, PC9, A549 and H3122 cells. (B) **18**'s effects on cell cycle progression in H1975, PC9, A549 and H3122 cells. (C) **18**'s effects on apoptosis in H1975, PC9, A549 and H3122 cells.

2.2.6 *In vivo* pharmacokinetic properties of compound 18

We then evaluated compound **18**'s PK properties in rats following intravenous injection (I.V.) and oral administration (P.O.) (Table 7). The half-life of **18** was about 1 h through I.V. and 1.75 h through P.O.. The AUC_{0-t} was about 1140 ng/mL*h via P.O.. These data indicated that **18** was acceptable for oral treatment in the animal model study.

Table 7. *In Vivo* PK Parameters of Compound **18**

Parameter	I.V. (1 mg/kg)	P.O. (10 mg/kg)
AUC_{0-t} (ng/mL*h)	707.336±49.93	1141.84±146.199
$AUC_{0-\infty}$ (ng/mL*h)	720.424±42.218	1296.403±223.56
$MRT_{(0-t)}$ (h)	0.947±0.143	4.118±0.33
C_{max} (ng/mL)	1488.219±377.84	282.805±28.356
T_{max} (h)	0.017±0	4±0
$T_{1/2}$ (h)	0.991±0.14	1.747±0.211
F (%)	—	16.14%

2.2.7 *In vivo* antitumor efficacy of compound 18

We next tested compound **18**'s antitumor efficacy in EGFR mutant driven H1975 cell inoculated mouse xenograft model. The results showed that at dosages of 10 mg/kg/day, 20 mg/kg/day and 40 mg/kg/day, **18** did not affect the body weights which indicated that it has no obvious cytotoxicity (Figure 6A). **18** could dose dependently suppress tumor progression and almost completely suppress tumor growth at 20 mg/kg QD oral dosage with the tumor growth inhibition (TGI) of 96% (Figure 6B-D). At 40 mg/kg/day dosage a TGI of 99% was achieved. Immunohistochemistry (IHC) stain showed that **18** could dose dependently inhibit the proliferation of tumor cells (*K*i-67 stain) and induce tumor cell apoptosis (TUNEL stain) in tumor tissues (Figure 6E). Similarly, in the ALK rearrangement driven H3122 cell inoculated mouse xenograft model, **18** did not affect the animal body weights and it could dose dependently suppress tumor progression (Figure 7A-D). At 40 mg/kg/day oral dosage **18** achieved the TGI of 78.4%. IHC stain also demonstrated the dose dependent anti-proliferation and apoptosis induction in tumor tissues (Figure 7E).

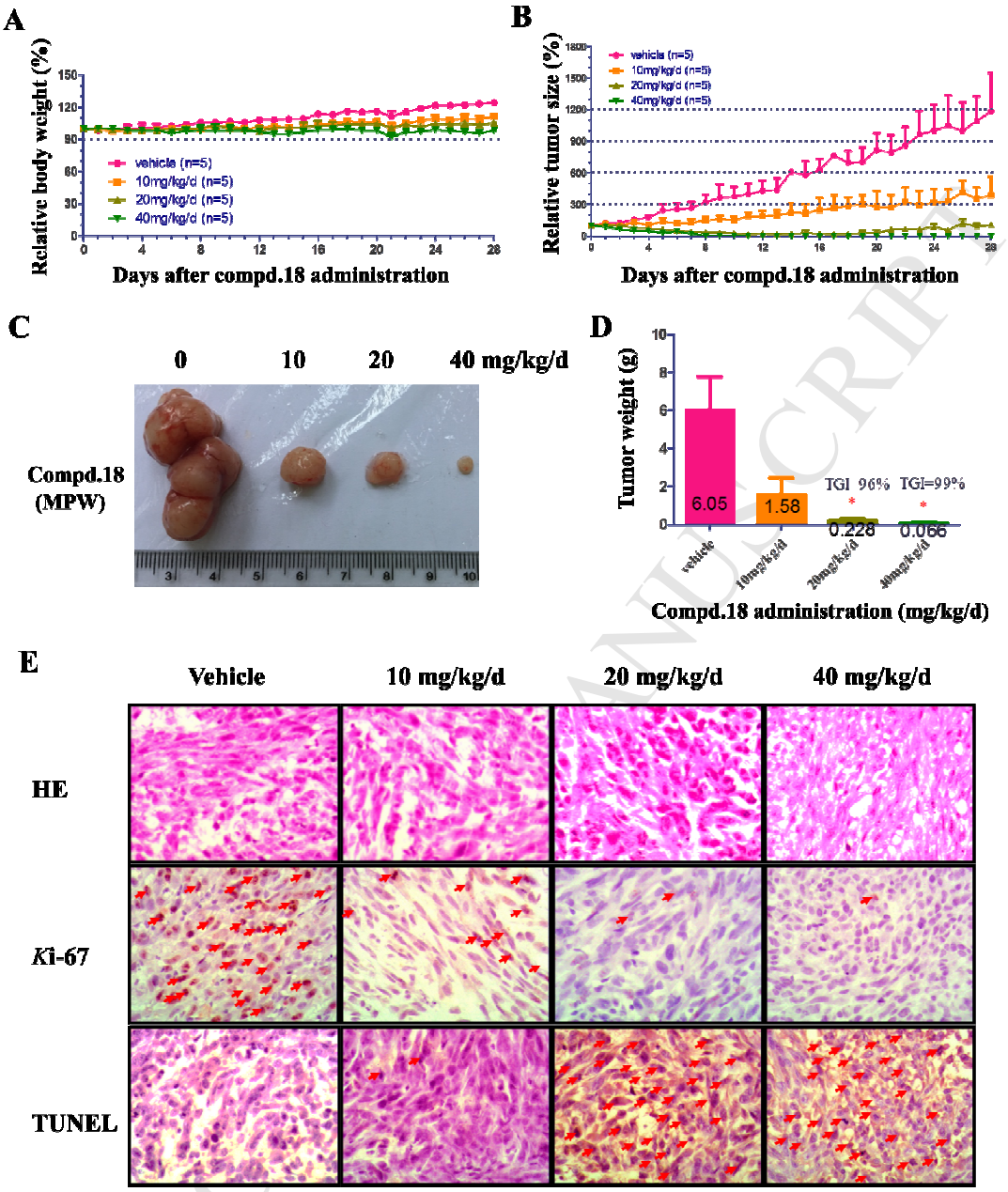


Figure 6. Compound **18**'s antitumor efficacy in H1975 xenograft mouse model. Female nu/nu mice bearing established H1975 tumor xenografts were treated with **18** at 10.0, 20.0 and 40.0 mg/kg/d dosage or vehicle. Daily oral administration was initiated when H1975 tumors had reached a size of 200 to 400 mm^3. Each group contained seven animals. Data, mean ± SEM. (A) Body weight and (B) Tumor size measurements from

H1975 xenograft mice after 21 days **18** administration. Initial body weight and tumor size were set as 100%. (C) Representative photographs of tumors in each group after 0, 10.0, 20.0, 40.0 mg/kg/d **18** or vehicle treatment. (D) Comparison of the final tumor weights in each group after 21-day treatment period of **18**. Numbers in columns indicate the mean tumor weight in each group. ns, $p>0.05$, (∗) $p<0.05$, (∗∗) $p<0.01$. (E) Representative micrographs of hematoxylin and eosin (HE), *K*i-67, and TUNEL staining of tumor tissues with **18** treatment groups in comparison with the vehicle treatment group. Note the specific nuclear staining of cells with morphology consistent with proliferation and apoptosis (E, red arrows).

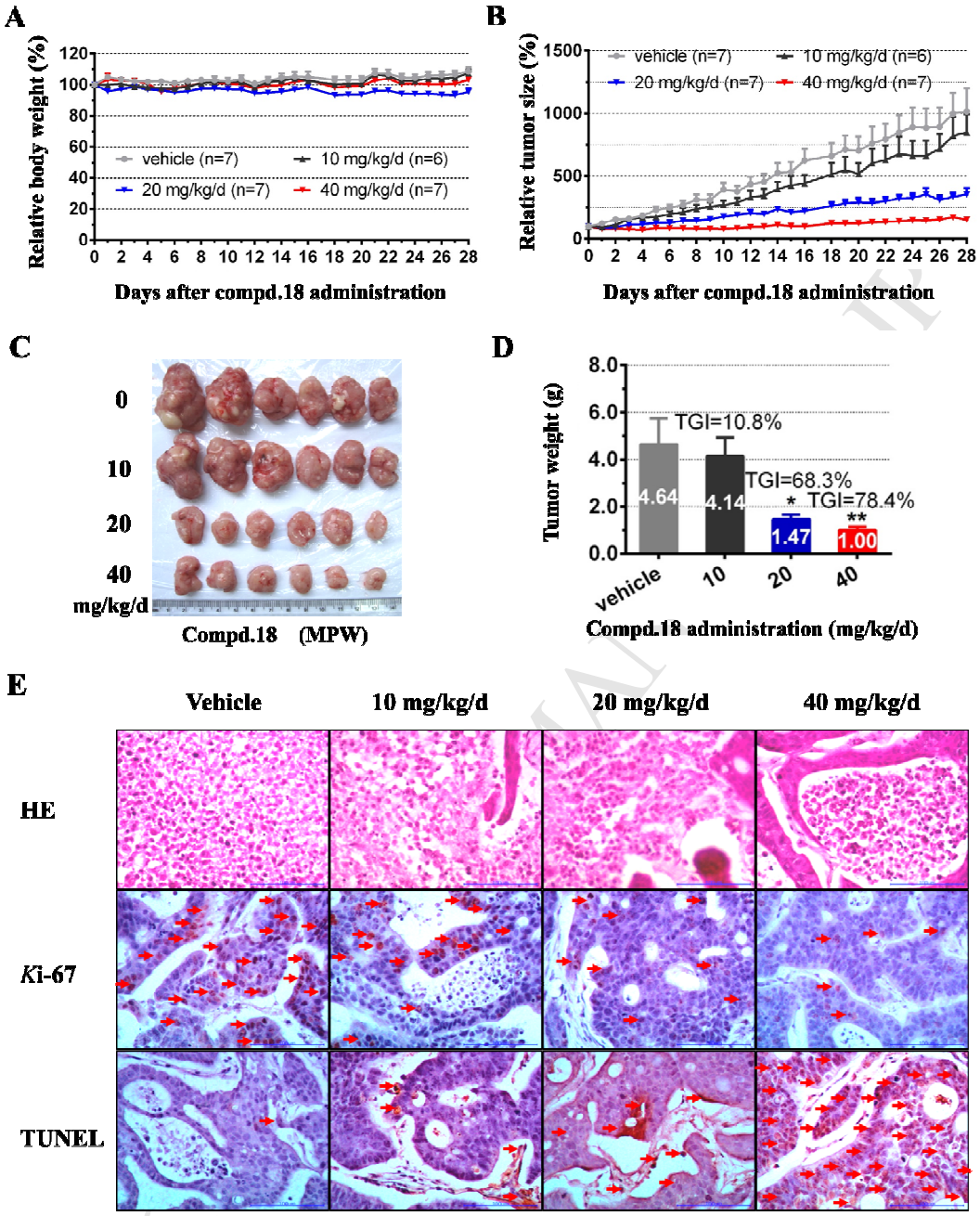


Figure 7. Compound **18**'s antitumor efficacy in H3122 xenograft mouse model. Female nu/nu mice bearing established H3122 tumor xenografts were treated with **18** at 10.0, 20.0 and 40.0 mg/kg/d dosage or vehicle. Daily oral administration was initiated when H3122 tumors had reached a size of 200 to 400 mm^3. Each group contained seven

animals. Data, mean ± SEM. (A) Body weights and (B) Tumor size measurements from H3122 xenograft mice after 21 days **18** administration. Initial body weight and tumor size were set as 100%. (C) Representative photographs of tumors in each group after 10.0, 20.0 and 40.0 mg/kg/d **18** or vehicle treatment. (D) Comparison of the final tumor weights in each group after 21-day treatment period of **18**. Numbers in columns indicate the mean tumor weight in each group. ns, $p>0.05$, (∗) $p<0.05$, (∗∗) $p<0.01$. (E) Representative micrographs of hematoxylin and eosin (HE), *K*i-67, and TUNEL staining of tumor tissues with **18** treatment groups in comparison with the vehicle treatment group. Note the specific nuclear staining of cells with morphology consistent with proliferation and apoptosis (E, red arrows).

3. Conclusions

Rational design of multi-target inhibitor is challenging because multiple factors need to be considered at one time for different targets. Starting from an inhibitor with well-defined target and selectively incorporating another target's binding element would be an efficient approach to achieve the multi-target inhibition rationally. Based upon the analysis of EGFR and ALK kinase structures, we realized that the cysteine residue 797 in EGFR kinase would be a good selective element for the rational design of EGFR/ALK dual inhibitors. Not only due to more and more concomitant EGFR mutant and ALK rearrangement being observed in clinic, but also previously identified EGFR reactivation-mediated drug resistance in ALK rearrangement driven NSCLC, the single-agent-dual-targets inhibitor is highly desired. Therefore, we seek to apply this multi-target inhibitor rational design approach to develop EGFR/ALK dual inhibitor. Starting from well-

established ALK inhibitor **14**, which showed moderate activity to EGFR kinase, we have developed a highly potent EGFR/ALK dual kinase inhibitor compound **18**, which displayed potent antitumor efficacy both *in vitro* and *in vivo* in the EGFR mutants and ALK rearrangement driven NSCLC models. In addition, compound **18** exhibited potent inhibitory activities against a variety of EGFR/ALK gain-of-function and drug-resistant mutants. With an acceptable PK profile and potent antitumor efficacy at well-tolerated dosages, compound **18** might be a good drug candidate for contaminant EGFR/ALK NSCLC as well as EGFR reactivation-mediated ALK inhibitor resistant NSCLC.

4. Experimental section

4.1 Chemistry.

All solvents and reagents were used as obtained. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded with a Bruker 400 MHz NMR spectrometer and referenced to deuterium dimethyl sulfoxide (DMSO-d_6) or deuterium chloroform ($CDCl_3$). Chemical shifts are expressed in ppm. In the NMR tabulation, s indicates singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad peak. LC/MS experiments were performed on an Agilent 6224 TOF using an ESI source coupled to an Agilent 1260 Infinity HPLC system operating in reverse mode with an Agilent Eclipse Plus C18 1.8 μm, 3.0 mm × 50 mm column. Flash column chromatography was conducted using silica gel (Silicycle 40−64 μm). The purities of all final compounds were determined to be above 95% by HPLC.

4.1.1 Compounds **51a-c** were prepared following the synthetic procedure of **51a**. Compounds **51d-n** were prepared following the synthetic procedure of **51l**.

4.1.1.1 *2,5-Dichloro-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine (**51a**).* To a suspension of NaH (1.0 g, 25 mmol) in a mixture of DMF (30 mL) was added dropwise 2-(isopropylsulfonyl)benzenamine (1.99 g, 10 mmol) in DMF (10 mL) at 0 °C. The solution was stirred for 30 min, and then 2,4,5-trichloropyrimidine (1.82 g, 10 mmol) in DMF (10 mL) was added slowly. The solution was warmed to room temperature and stirred for 12 h. Water was added and the mixture was extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (20% EtOAc in petroleum ether) to afford compound **51a** (1.38 g, 40%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H), 8.55 (s, 1H), 8.32 (d, J = 7.2 Hz, 1H), 7.90-7.85 (m, 2H), 7.47 (d, J = 6.4 Hz, 1H), 3.50-3.30 (m, 1H), 1.16 (d, J = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.15, 157.09, 156.34, , 156.34, 137.14, 135.66, 131.62, 126.63, 125.60, 124.75, 115.29, 55.11, 15.27; HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{13}H_{14}Cl_2N_3O_2S$: 346.0184, found: 346.0186.

4.1.1.2 *2-Chloro-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine (**51b**).* Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.21 (s, 1H), 7.92 (s, 1H), 7.82 (s, 2H), 7.53 (s, 1H), 6.85 (s, 1H), 3.41 (s, 1H), 1.12 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.92, 159.84, 158.54, 137.36, 135.53, 131.52, 130.53, 128.33, 126.71, 106.38, 54.53, 15.24. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{13}H_{15}ClN_3O_2S$: 312.0574, found: 312.0577.

4.1.1.3 *2-Chloro-N-(2-(isopropylsulfonyl)phenyl)-5-methylpyrimidin-4-amine (**51c**).* Yield = 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (s, 1H), 8.40 (d, J = 6.8 Hz, 1H), 8.23 (s, 1H), 7.87-7.83 (m, 2H), 7.42 (d, J = 6.4 Hz, 1H), , 3.50 (s, 1H), 2.17 (s, 3H), 1.15 (d, J = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.10, 157.47, 157.11, 138.31,

135.64, 131.59, 125.59, 124.60, 124.15, 115.78, 55.20, 15.26, 13.16. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{14}H_{17}ClN_3O_2S$: 326.0730, found: 326.0729.

4.1.1.4 *2,5-Dichloro-N-(3-(trifluoromethyl)phenyl)pyrimidin-4-amine (**51d**).* Yield = 70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H), 8.46 (s, 1H), 8.06 (s, 1H), 7.96 (d, J = 5.6 Hz, 1H), 7.64 (s, 1H), 7.53 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.52, 157.14, 156.39, 138.90, 130.17, 129.90, 129.58, 127.13, 121.50, 119.88, 114.43. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{11}H_7Cl_2F_3N_3$: 307.9969, found: 307.9968.

4.1.1.5 *2,5-Dichloro-N-(3-chloro-4-fluorophenyl)pyrimidin-4-amine (**51e**)* . Yield = 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H), 8.43 (s, 1H), 7.86 (s, 1H), 7.61 (s, 1H), 7.46 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.59, 157.20, 156.18, 135.14, 125.63, 124.46, 124.40, 119.42 (d, J = 8.0 Hz), 117.14 (d, J = 11.0 Hz), 114.21. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{10}H_6Cl_3FN_3$: 291.9611, found: 291.9614.

4.1.1.6 *2,5-Dichloro-N-(2,6-difluorophenyl)pyrimidin-4-amine (**51f**).* Yield = 63%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (s, 1H), 8.45 (s, 1H), 7.46 (d, J = 6.0 Hz, 1H), 7.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.03, 158.72, 157.70, 156.32, 129.71, 114.51, 113.75, 112.71, 112.48, 99.99. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{10}H_6Cl_2F_2N_3$: 275.9907, found: 275.9911.

4.1.1.7 *2,5-Dichloro-N-(thiophen-2-ylmethyl)pyrimidin-4-amine (**51g**).* Yield = 40%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, J = 7.2 Hz, 1H), 8.23 (s, 1H), 7.38 (s, 1H), 7.04 (d, J = 6.4 Hz, 1H), 6.97 (s, 1H), 4.72 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.82, 157.72, 154.61, 141.42, 127.11, 126.71, 125.85, 113.40, 39.35. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_9H_8Cl_2N_3S$: 259.9816, found: 259.9820.

4.1.1.8 *2,5-Dichloro-N-cyclopropylpyrimidin-4-amine (**51h**).* Yield = 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.95 (s, 1H), 2.85 (s, 1H), 0.75 (d, J = 2.4 Hz, 2H), 0.66 (s, 2H); ^{13}C NMR(100 MHz, DMSO-d_6) δ 160.45, 157.89, 154.13, 113.38, 24.71, 6.54. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_7H_8Cl_2N_3$: 204.0095, found: 204.0097.

4.1.1.9 *N-(2-((2,5-dichloropyrimidin-4-yl)amino)phenyl)pivalamide (**51i**).* Yield = 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (s, 1H), 8.88 (s, 1H), 8.39 (s, 1H), 7.64 (s, 1H), 7.37 (s, 1H), 7.30 (s, 2H), 1.21 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.10, 157.59, 155.63, 132.43, 131.44, 126.99, 126.70, 126.45, 126.20, 114.14, 38.17, 27.69. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{15}H_{17}Cl_2N_4O$: 339.0779, found: 339.0783.

4.1.1.10 *2,5-Dichloro-N-methyl-N-(3-(trifluoromethyl)phenyl)pyrimidin-4-amine (**51j**).* Yield = 35%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 7.75 (s, 1H), 7.64 (s, 3H), 3.48 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.56, 159.06, 157.29, 145.52, 130.91, 130.64, 129.98, 123.64, 122.76, 116.04, 41.84. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{12}H_9Cl_2F_3N_3$: 322.0126, found: 322.0125.

4.1.1.11 *2-((2,5-Dichloropyrimidin-4-yl)amino)-N,N-dimethylbenzenesulfonamide (**51k**).* Yield = 64%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.67 (s, 1H), 8.56 (s, 1H), 8.28 (d, J = 5.2 Hz, 1H), 7.86 (s, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.46 (s, 1H), 2.64 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.26, 157.06, 156.41, 135.81, 134.66, 130.71, 126.51, 125.83, 125.32, 114.95, 37.57. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{12}H_{13}Cl_2N_4O_2S$: 347.0136, found: 347.0133.

4.1.1.12 *(2-((2,5-Dichloropyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**51l**).* A solution of 2,4,5-trichloropyrimidine (1.82 g, 10 mmol), (2-aminophenyl)dimethylphosphine oxide (1.69 g, 10 mmol) and *N*,*N*-

diisopropylethylamine (1.94 g, 15 mmol) in propan-2-ol (25 mL) was heated under reflux for 12 hours. The solvent was removed by evaporation and the residue was dissolved in CH_2Cl_2 (100 mL). The solution was washed with water and saturated sodium chloride solution and dried, filtered and concentrated. The residue was purified by flash chromatography on silica gel (0−2% MeOH in DCM) to afford compound **51l** (1.60 g, 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.84 (s, 1H), 8.43 (s, 2H), 7.62 (s, 2H), 7.25 (s, 1H), 1.83 (s, 3H), 1.80 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.01, 155.99, 142.48, 132.73, 131.47 (d, J = 10 Hz), 124.17 (d, J = 6.0 Hz), 122.64, 122.06 (d, J = 3.0 Hz), 121.73, 115.30, 18.98, 18.28. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{12}H_{13}Cl_2N_3OP$: 316.0173, found: 316.0175.

4.1.1.13 *2-((2,5-Dichloropyrimidin-4-yl)amino)-N-methylbenzamide (**51m**).* Yield = 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.68 (s, 1H), 8.69 (s, 1H), 8.21 (s, 1H), 7.55 (s, 1H), 7.50 (s, 1H), 7.14 (s, 1H), 6.30 (s, 1H), 3.03 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.06, 156.38, 135.78, 134.66, 130.70, 126.52, 125.85, 125.33, 114.94, 37.56. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{12}H_{11}Cl_2N_4O$: 297.0310, found: 297.0315.

4.1.1.14 *2-((2,5-Dichloropyrimidin-4-yl)amino)benzonitrile (**51n**).* Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.48 (s, 1H), 7.93 (s, 1H), 7.79 (s, 1H), 7.58 (s, 1H), 7.50 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.43, 157.40, 156.54, 140.19, 134.52, 133.74, 128.45, 127.75, 117.24, 114.01, 110.78. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{11}H_7Cl_2N_4$: 265.0048, found: 265.0050.

4.1.2 Compounds **52a**-**p** were prepared following the synthetic procedure of **52a**.

4.1.2.1 *5-Chloro-N^2-(4-fluoro-2-methoxy-5-nitrophenyl)-N^4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (**52a**).* 4-Methylbenzenesulfonic acid

hydrate (77.4 mg, 0.45 mmol) was added to compound **51a** (103.5 mg, 0.3 mmol) and 4-fluoro-2-methoxy-5-nitroaniline (67.0 mg, 0.36 mmol) in 2-pentanol (3 mL). The resulting mixture was stirred at 115 °C for 5 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (0−5% MeOH in DCM) to afford compound **52a** (74 mg, 50%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.73 (s, 1H), 8.56 (s, 1 H), 8.45 (s, 1H), 8.33 (s, 1 H), 7.82 (d, J = 6.8 Hz, 1H), 7.59 (s, 1H), 7.35 (s, 1H), 7.34 (s, 1H), 3.95 (s, 3H), 3.50-3.33 (m, 1H), 1.16 (d, J = 5.2 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.90, 157.19, 155.68, 155.31, 154.36, 138.14, 135.23, 131.39, 129.09, 125.41, 124.66, 124.15, 123.81, 118.88, 106.33, 101.85 (d, J = 13 Hz), 57.79, 55.39, 15.30. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{20}H_{20}ClFN_5O_5S$: 496.0858, found: 496.0854.

4.1.2.2 *N^2-(4-Fluoro-2-methoxy-5-nitrophenyl)-N^4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (**52b**).* Yield = 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.75 (s, 1H), 8.16 (s, 1 H), 8.13 (s, 1H), 8.00 (s, 1 H), 7.84 (s, 1H), 7.62 (s, 1H), 7.37-7.29 (m, 2H), 6.51 (s, 1H), 3.97 (s, 3H), 3.51 (s, 1H), 1.13 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.89, 159.31, 157.81, 155.51, 153.41, 150.83, 138.66, 135.10, 131.25, 127.32, 126.15, 125.95, 124.72, 116.37, 101.44 (d, J = 26 Hz), 100.46, 57.82, 54.82, 15.22. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{20}H_{21}FN_5O_5S$: 462.1247, found: 462.1252.

4.1.2.3 *N^2-(4-Fluoro-2-methoxy-5-nitrophenyl)-N^4-(2-(isopropylsulfonyl)phenyl)-5-methylpyrimidine-2,4-diamine (**52c**).* Yield = 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ

9.05 (s, 1H), 8.81 (s, 1H), 8.56 (s, 1H), 8.19 (s, 1H), 8.10 (s, 1 H), 7.80 (s, 1H), 7.62 (s, 1H), 7.33 (s, 1H), 7.30 (s, 1H), 3.98 (s, 3H), 3.45 (s, 1H), 2.12 (s, 3H), 1.16 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.58, 157.86, 156.78, 155.62, 150.83, 139.23, 135.34, 131.34, 129.01, 126.25, 123.87, 123.83, 123.39 (d, J = 22 Hz), 116.19, 108.16, 101.45 (d, J = 26 Hz), 57.79, 55.36, 15.27, 13.10. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{21}H_{23}FN_5O_5S$: 476.1404, found: 476.1407.

4.1.2.4 *5-Chloro-N^2-(4-fluoro-3-nitrophenyl)-N^4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (**52d**)* . Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H), 9.47 (s, 1H), 8.47 (s, 2H), 8.37 (s, 1H), 7.86 (s, 2H), 7.74 (s, 1H), 7.48 (s, 1H), 7.41 (s, 1H), 3.45 (s, 1H), 1.16 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.41, 155.52, 151.00, 148.45, 138.07, 137.53, 136.82, 135.37, 131.43, 126.71, 126.63, 125.40, 124.58, 118.79 (d, J = 22.0 Hz), 115.35, 106.51, 55.35, 15.27. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{19}H_{18}ClFN_5O_4S$: 466.0752, found: 466.0751.

4.1.2.5 *5-Chloro-N^2-(4-fluoro-2-isopropoxy-5-nitrophenyl)-N^4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (**52e**).* Yield = 63%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.55 (s, 1H), 8.52 (s, 1H), 8.38 (s, 1H), 8.34 (s, 1H), 7.83 (s, 1H), 7.57 (s, 1H), 7.38 (s, 1H), 7.35 (s, 1H), 4.86 (s, 1H), 3.44 (s, 1H), 1.29 (s, 6H), 1.17 (s, 6H); ^{13}C NMR(100 MHz, DMSO-d_6) δ 157.96, 155.83, 155.53, 155.34, 151.87, 138.11, 135.17, 131.43, 128.74, 125.85, 124.83, 124.27, 123.88, 119.06, 106.13, 102.78 (d, J = 13 Hz), 73.06, 55.35, 21.80, 15.30. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{22}H_{24}ClFN_5O_5S$: 524.1171, found: 524.1174.

4.1.2.6 *5-Chloro-N^2-(4-fluoro-2-methoxy-5-nitrophenyl)-N^4-(3-(trifluoromethyl)phenyl)pyrimidine-2,4-diamine (**52f**).* Yield = 50%. ^{1}H NMR (400 MHz,

DMSO-d_6) δ 9.18 (s, 1H), 8.53 (s, 1H), 8.40 (s, 1H), 8.25 (s, 1H), 7.99 (s, 1H), 7.93 (s, 1H), 7.46 (s, 1H), 7.39 (s, 1H), 7.31 (d, J = 12.8 Hz, 1H), 3.94 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ δ 157.80, 156.75 (d, J = 9.0 Hz), 156.08, 155.61 , 154.19, 151.61, 139.69, 129.76, 129.39, 129.05 (d, J = 3.5 Hz), 126.26, 125.54, 120.34, 118.97, 118.27, 105.74, 101.73 (d, J = 13 Hz), 57.77. ESI-HRMS (m/z) exact mass calculated for $C_{18}H_{13}ClF_4N_5O_3$ [M + H]+: 458.0643, found: 458.0648.

4.1.2.7 *5-Chloro-N4-(3-chloro-4-fluorophenyl)-N2-(4-fluoro-2-methoxy-5-nitrophenyl)pyrimidine-2,4-diamine (**52g**).* Yield = 53%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.51 (s, 1H), 8.41 (s, 1H), 8.21 (s, 1H), 7.81 (s, 1 H), 7.59 (d, J = 6.4 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.30 (s, 1H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.74, 156.61, 156.04, 155.40, 152.88, 151.53, 135.98, 129.12, 125.57, 124.47, 123.37 (d, J = 3.0 Hz), 119.29 (d, J = 9.0 Hz), 117.98, 116.68 (d, J = 22 Hz), 105.47, 101.72 (d, J = 13 Hz), 57.77. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{17}H_{12}Cl_2F_2N_5O_3$: 442.0285, found: 442.0290.

4.1.2.8 *5-Chloro-N4-(2,6-difluorophenyl)-N2-(4-fluoro-2-methoxy-5-nitrophenyl)pyrimidine-2,4-diamine (**52h**).* Yield = 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 (s, 1H), 8.33 (s, 1H), 8.21 (s, 1 H), 8.08 (s, 1H), 7.35 (s, 1 H), 7.22 (d, J = 10.0 Hz, 1H), 7.12 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.20, 157.85, 157.74 (d, J = 2.5 Hz), 157.53, 155.87 (d, J = 5.0 Hz), 155.24, 153.52, 150.96, 128.97 (d, J = 5.0 Hz), 128.90 (d, J = 7.0 Hz), 125.53, 116.85, 115.33, 112.23 (d, J = 12 Hz), 105.29, 101.43 (d, J = 13 Hz), 57.70. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{17}H_{12}ClF_3N_5O_3$: 426.0581, found: 426.0586.

4.1.2.9 *5-Chloro-N2-(4-fluoro-2-methoxy-5-nitrophenyl)-N4-(thiophen-2-ylmethyl)pyrimidine-2,4-diamine (**52i**).* Yield = 61%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H), 8.05 (s, 2H), 7.98 (s, 1 H), 7.32 (s, 2H), 6.98 (s, 1H), 6.91 (s, 1H), 4.80 (s, 2H), 4.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ δ157.42, 157.12, 153.36, 152.94, 152.76 (d, J = 4.0 Hz), 140.38, 129.62, 126.97, 126.28, 125.99, 125.34, 114.29, 105.82 99.56 (d, J = 13 Hz), 56.78, 40.15. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{16}H_{14}ClFN_5O_3S$: 410.0490, found: 410.0493.

4.1.2.10 *5-Chloro-N4-cyclopropyl-N2-(4-fluoro-2-methoxy-5-nitrophenyl)pyrimidine-2,4-diamine (**52j**).* Yield = 48%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 7.99 (s, 1H), 7.85 (s, 1H), 7.49 (s, 1H), 7.30 (d, J = 12 Hz, 1H), 4.02 (s, 3H), 2.90 (s, 1H), 0.80 (s, 2H), 0.65 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.32, 157.44, 153.51, 150.47, 129.10, 126.35, 114.20, 105.27, 101.25 (d, J = 14 Hz), 57.94, 24.69, 6.65. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{14}H_{14}ClFN_5O_3$: 354.0769, found: 354.0765.

4.1.2.11 *N-(2-((5-chloro-2-((4-fluoro-2-methoxy-5-nitrophenyl)amino)pyrimidin-4-yl)amino)phenyl)pivalamide (**52k**).* Yield = 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 8.52 (s, 1H), 8.38 (s, 1H), 8.20 (d, J = 4.8 Hz, 2H), 7.68 (s, 1H), 7.27 (s, 2H), 7.16 (s, 2H), 3.94 (s, 3H), 1.20 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.11, 157.85, 156.12, 154.79, 153.81, 151.24, 132.57, 131.58, 129.10, 126.51, 126.21, 125.97, 125.63, 117.72, 105.72, 101.57 (d, J = 13 Hz), 57.76, 55.33, 27.70. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{22}H_{23}ClFN_6O_4$: 489.1453, found: 489.1455.

4.1.2.12 *5-Chloro-N^2-(4-fluoro-2-methoxy-5-nitrophenyl)-N^4-methyl-N^4-(3-(trifluoromethyl)phenyl)pyrimidine-2,4-diamine (**52l**).* Yield = 40%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (d, J = 7.6 Hz, 1H), 8.46 (s, 1H), 8.18 (s, 1H), 7.59-7.54 (m, 4H), 7.34

(d, *J* = 8.8 Hz, 1H), 4.01 (s, 3H), 3.53 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.81, 158.16, 157.51, 155.34, 153.64, 151.06, 146.52, 130.81, 130.44 (d, *J* = 16 Hz), 129.20, 128.92, 125.76, 122.49, 121.82, 115.90, 108.29, 101.50 (d, *J* = 14 Hz), 57.90, 41.50. ESI-HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{19}H_{15}ClF_4N_5O_3$: 472.0800, found: 472.0805.

4.1.2.13 *2-((5-Chloro-2-((4-fluoro-2-methoxy-5-nitrophenyl)amino)pyrimidin-4-yl)amino)-N,N-dimethylbenzenesulfonamide (**52m**).* Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 8.74 (s, 1H), 8.55 (s, 1H), 8.42-8.38 (m, 1H), 8.33 (s, 1H), 7.79 (d, *J* = 4.8 Hz, 1H), 7.53 (d, *J* = 7.2 Hz, 1H), 7.37-7.29 (m, 2H), 3.95 (s, 3H), 2.64 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.99, 157.35, 157.26, 155.73, 155.28, 151.81, 136.77, 134.21, 130.49, 129.07, 125.42, 124.25, 119.09, 106.03, 101.88 (d, *J* = 13 Hz), 57.79, 37.61. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{19}H_{19}ClFN_6O_5S$: 497.0810, found: 497.0811.

4.1.2.14 *(2-((5-Chloro-2-((4-fluoro-2-methoxy-5-nitrophenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**52n**).* Yield = 70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.31 (s, 1H), 8.60 (s, 1H), 8.49 (s, 1H), 8.45 (s, 1H), 8.21 (s, 1H), 7.57 (s, 1H), 7.34 (d, *J* = 7.2 Hz, 2H), 7.13 (s, 1H), 3.96 (s, 3H), 1.80 (s, 3H), 1.78 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.86, 157.00, 155.84, 155.15, 143.29, 132.35, 131.21, 129.11, 125.70, 125.67, 123.13, 122.07, 118.68, 106.66, 101.72 (d, *J* = 12 Hz), 57.78, 18.96, 18.26. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{19}H_{19}ClFN_5O_4P$: 466.0847., found: 466.0850.

4.1.2.15 *2-((5-Chloro-2-((4-fluoro-2-methoxy-5-nitrophenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide (**52o**).* Yield = 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.74 (s, 1H), 8.79 (s, 1H), 8.63 (s, 2H), 8.57 (s, 1H), 8.26 (s, 1H), 7.74 (s, 1H), 7.39-7.30

(m, 2H), 7.09 (d, *J* = 9.6 Hz, 1H),3.96 (s, 3H), 2.80 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.36, 157.86, 156.94, 156.84, 155.46, 154.88, 139.55, 131.89, 128.39, 125.63, 122.42, 121.23, 120.95, 118.46, 106.75, 101.69 (d, *J* = 26 Hz), 57.77, 26.76. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{19}H_{17}ClFN_6O_4$: 447.0984, found: 447.0980.

4.1.2.16 *2-((5-Chloro-2-((4-fluoro-2-methoxy-5-nitrophenyl)amino)pyrimidin-4-yl)amino)benzonitrile (**52p**).* Yield = 62%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H), 8.37 (d, *J* = 7.6 Hz, 1H), 8.26 (s, 2H), 7.75 (s, 1H), 7.65 (s, 1H), 7.58 (d, *J* = 10.0 Hz, 1H), 7.36 (s, 1H), 7.26 (d, *J* = 9.6 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.64, 156.96, 156.22, 155.65, 141.08, 134.12, 133.36, 129.05, 128.98, 127.82, 126.72, 125.53, 117.54, 117.37, 110.19, 105.53, 101.59 (d, *J* = 13 Hz), 57.77. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{18}H_{13}ClFN_6O_3$: 415.0722, found: 415.0725.

4.1.3 Compounds **53a-i'** were prepared following the synthetic procedure of **53d**.

4.1.3.1 *5-Chloro-N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(2-methoxy-4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)-5-nitrophenyl)pyrimidine-2,4-diamine (**53a**).* Yield = 40%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.59 (s, 1H), 8.46 (s, 1H), 8.27 (s, 1H), 8.24 (s, 1H), 7.82 (s, 1H), 7.54 (s, 1H), 7.32 (s, 1H), 6.78 (s, 1H), 3.91 (s, 3H), 3.50-2.31 (m, 17H), 1.86 (s, 2H), 1.54 (s, 2H), 1.16 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.49, 156.45, 155.76, 155.22, 145.37, 138.33, 135.15, 134.00, 131.38, 124.38, 123.93, 123.58, 122.22, 121.52, 105.50, 103.53, 60.92, 56.81, 55.39, 54.87, 51.63, 48.25, 45.25, 28.45, 15.31. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{30}H_{40}ClN_8O_5S$: 659.2531, found: 659.2535.

4.1.3.2 *5-Chloro-N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(2-methoxy-4-(4-(methylsulfonyl)piperazin-1-yl)-5-nitrophenyl)pyrimidine-2,4-diamine (**53b**).* Yield =

55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.56 (s, 1H), 8.91 (s, 1H), 8.39 (s, 1H), 8.22 (s, 2H), 7.96 (s, 1H), 7.61 (s, 1H), 7.52 (s, 1H), 7.28 (s, 1H), 6.65 (s, 1H), 4.02 (s, 3H), 3.43 (s, 4H), 3.25 (s, 5H), 2.86 (s, 3H), 1.33 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.30, 156.08, 155.74, 155.28, 144.25, 138.26, 135.37, 135.20, 131.36, 124.54, 124.06, 123.74, 123.65, 120.73, 105.72, 104.74, 57.00, 55.37, 51.74, 46.06, 34.55, 15.30. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{25}H_{31}ClN_7O_7S_2$: 640.1415, found: 640.1411.

4.1.3.3 *5-Chloro-N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(2-methoxy-4-(4-methylpiperazin-1-yl)-5-nitrophenyl)pyrimidine-2,4-diamine(methylsulfonyl)piperazin-1-yl)-5-nitrophenyl)pyrimidine-2,4-diamine (**53c**).* Yield = 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.59 (s, 1H), 8.46 (s, 1H), 8.28 (s, 2H), 7.81 (s, 1H), 7.55 (s, 1H), 7.32 (s, 1H), 6.80 (s, 1H), 3.92 (s, 3H), 3.45 (s, 1H), 3.12 (s, 4H), 2.64 (s, 4H), 2.37 (s, 3H), 1.16 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.93, 155.51, 155.25, 151.91, 141.68, 137.83, 136.92, 134.74, 131.34, 125.05, 124.48, 123.86, 123.58, 115.88, 107.03, 102.56, , 56.34, 55.65, 54.78, 51.37, 45.30, 15.33. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{25}H_{31}ClN_7O_5S$: 576.1796, found: 576.1800.

4.1.3.4 *5-Chloro-N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(2-methoxy-4-(4-methyl-1,4-diazepan-1-yl)-5-nitrophenyl)pyrimidine-2,4-diamine (**53d**).*Compound **52a** (49.5 mg, 0.1 mmol), 1-methyl-1,4-diazepane (13.7 mg, 0.12 mmol) and K_2CO_3 (28.0 mg, 0.2 mmol) in DMF (2 mL) were stirred for 2 h at 140 ^{o}C. The mixture was diluted with water, and then extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (0−10% MeOH in DCM) to afford compound **53d** (47.0 mg, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (s, 1H), 8.65 (s, 1H), 8.30 (s, 1H), 8.19 (s,

1 H), 7.80 (s, 1H), 7.66 (s, 1H), 7.30 (s, 1H), 6.99 (s, 1H), 6.75 (s, 1H), 3.66 (s, 3H), 3.48-3.30 (m, 9H), 2.75 (s, 3H), 2.08 (s, 2H), 1.16 (s, 6H); ^{13}C NMR(100 MHz, DMSO-d_6) δ 158.95, 156.98, 155.81, 155.14, 145.34, 138.46, 135.19, 131.39, 130.52, 124.08, 123.71, 123.26, 122.85, 119.45, 105.10, 100.81, 58.63, 56.49, 56.39, 55.41, 52.42, 52.30, 46.37, 27.39, 15.31. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{26}H_{33}ClN_7O_5S$: 590.1952, found: 590.1954.

4.1.3.5 *5-Chloro-N^2-(4-(4-isopropylpiperazin-1-yl)-2-methoxy-5-nitrophenyl)-N^4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (**53e**).* Yield = 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.64 (s, 1H), 8.47 (s, 1H), 8.28 (s, 2H), 7.81 (d, J = 5.2 Hz, 1H), 7.56 (s, 1H), 7.33 (s, 1H), 6.82 (s, 1H), 3.93 (s, 3H), 3.52-2.50 (m, 9H), 1.37-1.00 (m, 12H); ^{13}C NMR(100 MHz, DMSO-d_6) δ 156.88 , 155.45, 155.20, 151.93, 141.36, 137.81, 137.18, 134.73, 131.32, 125.02, 124.82, 123.82, 123.55, 115.65, 107.08, 102.91, 56.43, 55.64, 50.92, 48.50, 17.65, 15.31. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{27}H_{35}ClN_7O_5S$: 604.2109, found: 604.2107.

4.1.3.6 *5-Chloro-N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(2-methoxy-4-(4-(2-methoxyethyl)piperazin-1-yl)-5-nitrophenyl)pyrimidine-2,4-diamine (**53f**).* Yield = 70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.60 (s, 1H), 8.47 (s, 1H), 8.28 (s, 2H), 7.80 (s, 1H), 7.55 (s, 1H), 7.32 (s, 1H), 6.81 (s, 1H), 3.91 (s, 3H), 3.48 (s, 4H), 3.26 (s, 4H), 3.07 (s, 4H), 2.59 (s, 4H), 1.16 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.43, 156.36, 155.74, 155.21, 144.96, 138.30, 135.15, 134.41, 131.36, 124.39, 123.93, 123.58, 122.56, 121.35, 105.54, 103.64, 70.21, 58.48, 57.34, 56.84, 55.37, 53.45, 51.74, 15.31. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{27}H_{35}ClN_7O_6S$: 620.2058, found: 620.2058.

4.1.3.7 *5-Chloro-N^2-(4-(4-cyclohexylpiperazin-1-yl)-2-methoxy-5-nitrophenyl)-N^4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (**53g**).* Yield = 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.64 (s, 1H), 8.47 (s, 1H), 8.33 (s, 1H), 8.29 (s, 1H), 7.81 (s, 1H), 7.56 (s, 1H), 6.81 (s, 1H), 3.93 (s, 3H), 3.34-2.50 (m, 10H), 2.00-1.16 (m, 16H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.88, 155.49, 155.22, 151.92, 141.28, 137.83, 137.28, 134.73, 131.33, 125.00, 124.96, 123.83, 123.57, 115.61, 107.12, 102.94, , 65.02, 56.45, 55.64, 50.85, 48.90, 27.70 25.54, 25.40, 15.32. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{30}H_{39}ClN_7O_5S$: 644.2422, found: 644.2418.

4.1.3.8 *5-Chloro-N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(2-methoxy-4-morpholino-5-nitrophenyl)pyrimidine-2,4-diamine (**53h**).* Yield = 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.60 (s, 1H), 8.47 (s, 1H), 8.28 (s, 2H), 7.82 (s, 1H), 7.55 (s, 1H), 7.32 (s, 1H), 6.84 (s, 1H), 3.92 (s, 3H), 3.74 (s, 4H), 3.44 (s, 1H), 3.06 (s, 4H), ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.40, 156.33, 155.75, 155.26, 144.78, 138.29, 135.16, 134.68, 131.37, 124.48, 124.00, 123.66, 122.93, 121.19, 105.63, 103.76, 66.65, 56.90, 55.37, 52.34, 15.31. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{24}H_{28}ClN_6O_6S$: 563.1480, found: 563.1483.

4.1.3.9 *5-Chloro-N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(2-methoxy-5-nitro-4-(piperidin-1-yl)phenyl)pyrimidine-2,4-diamine (**53i**).* Yield = 83%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.56 (s, 1H), 8.47 (s, 1H), 8.27 (s, 1H), 8.23(s, 1H), 7.81 (s, 1H), 7.53 (d, J = 7.2Hz, 1H), 7.32 (d, J = 6.0Hz, 1H), 6.77 (s, 1H), 3.90 (s, 3H), 3.45 (s, 1H), 3.02 (s, 4H), 1.66 (s, 4H), 1.57 (s, 2H), 1.16(s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.53, 156.46, 155.77, 155.23, 146.04, 138.35, 135.14, 134.13, 131.36, 124.40, 123.92, 123.57,

122.08, 121.56, 121.52, 103.54, 56.77, 55.37, 53.21, 26.06, 24.05, 15.31. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{25}H_{30}ClN_6O_5S$: 561.1687, found: 561.1682

4.1.3.10 *5-Chloro-N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)-3-nitrophenyl)pyrimidine-2,4-diamine (**53j**).* Yield = 40%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s, 1H), 9.44 (s, 1H), 8.46 (s, 1H), 8.34 (s, 1H), 8.14(s, 1H), 7.85 (d, J = 4.8 Hz, 1H), 7.70 (s, 2H), 7.40 (s, 1H), 7.27 (d, J = 7.2 Hz, 1H), 3.23-2.27 (m, 17H), 1.81 (s, 2H), 1.48 (s, 2H), 1.15 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.68, 155.62, 155.46, 144.27, 140.26, 138.21, 135.62, 135.28, 131.41, 125. 27, 124.74, 124.45, 122.72, 115.06, 105.85, 60.87, 55.35, 54.96, 52.19, 48.32, 45.39, 28.63, 15.29. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{29}H_{38}ClN_8O_4S$: 629.2425, found: 629.2421.

4.1.3.11 *5-Chloro-N^2-(4-(4-ethylpiperazin-1-yl)-2-isopropoxy-5-nitrophenyl)-N^4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (**53k**).* Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.43 (s, 2H), 8.30 (s, 2H), 7.81 (d, J = 6.0 Hz, 1H), 7.54 (s, 1H), 7.33 (s, 1H), 6.82 (s, 2H), 4.89 (s, 1H), 3.45-2.50 (m, 11H), 1.36-1.07 (s, 15H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.32, 155.82, 155.30, 154.33, 144.15, 138.25, 135.08, 134.58, 131.40, 124.69, 124.13, 123.86, 123.76, 120.90, 105.56, 105.36, 71.90, 55.37, 52.02, 51.64, 50.73, 21.99, 15.30, 11.08. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{28}H_{37}ClN_7O_5S$: 618.2265, found: 618.2263.

4.1.3.12 *N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(2-methoxy-4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)-5-nitrophenyl)-5-methylpyrimidine-2,4-diamine (**53l**).* Yield = 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H), 8.57 (s, 1H), 8.49 (s, 1H), 8.03 (s, 2H), 7.79(s, 1H), 7.56 (s, 1H), 7.27 (s, 1H), 6.78 (s, 1H), 3.93 (s, 3H), 3.42-3.36 (m, 5H), 2.83-2.35 (m, 12H), 2.10 (s, 3H), 1.86 (s, 2H), 1.59 (s, 2H), 1.15(s, 6H); ^{13}C NMR (100 MHz,

DMSO-d_6) δ 158.58, 157.65, 156.71, 151.74, 142.04, 139.02, 136.31, 134.86, 131.18, 124.11, 123.75, 123.32, 122.94, 115.67, 107.51, 102.21, 61.63, 56.12, 55.71, 54.58, 52.17, 48.01, 45.15, 28.33, 15.32, 13.29. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{31}H_{43}N_8O_5S$: 639.3077, found: 639.3078.

4.1.3.13 *N^2-(4-(4-ethylpiperazin-1-yl)-2-methoxy-5-nitrophenyl)-N^4-(2-(isopropylsulfonyl)phenyl)-5-methylpyrimidine-2,4-diamine (**53m**).* Yield = 80%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (s, 1H), 8.56 (s, 2H), 8.06 (s, 2H), 7.79 (d, J = 4.8 Hz, 1H), 7.57 (s, 1H), 7.27 (d, J = 6.8 Hz, 1H), 6.82 (s, 1H), 3.95 (s, 3H), 3.45-3.40 (m, 1H), 3.18-2.35 (m, 10H), 2.10 (s, 3H), 1.56 (s, 6H), 1.10 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.58, 158.33, 156.93, 154.69, 143.22, 139.36, 135.24, 135.05, 131.32, 124.09, 123.76, 123.45, 123.16, 118.54, 107.44, 103.70, 56.94, 55.35, 52.32, 51.79, 51.19, 15.29, 13.10, 11.55. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{27}H_{36}N_7O_5S$: 570.2499, found: 570.2502.

4.1.3.14 *N^2-(4-(4-ethylpiperazin-1-yl)-2-methoxy-5-nitrophenyl)-N^4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (**53n**).* Yield = 78%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (s, 1H), 8.45 (s, 1H), 8.11 (s, 1H), 8.03 (s, 1H), 7.99 (s, 1H), 7.82 (d, J = 6.8 Hz, 1H), 7.61 (d, J = 5.6 Hz, 1H), 7.34 (s, 1H), 6.80 (s, 1H), 6.44 (s, 1H), 3.94 (s, 3H), 3.35 (s, 1H), 3.06 (s, 5H), 2.64-2.50 (m, 5H), 1.13 (s, 6H), 1.06 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.83, 159.69, 157.87, 154.34, 143.28, 138.82, 135.18, 135.04, 131.22, 127.04, 125.97, 124.54, 123.65, 118.37, 103.62, 99.86, 56.94, 54.85, 52.57, 51.92, 51.61, 15.22, 12.00. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{26}H_{34}N_7O_5S$: 556.2342, found: 556.2341.

4.1.3.15 *5-Chloro-N^2-(2-methoxy-4-(4-methylpiperazin-1-yl)-5-nitrophenyl)-N^4-(3-*

*(trifluoromethyl)phenyl)pyrimidine-2,4-diamine (**53o**).* Yield = 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H), 8.28 (s, 2H), 8.20 (s, 1H), 8.05 (s, 1 H), 7.93 (s, 1H), 7.42 (s, 1H), 7.38 (s, 1H), 6.78 (s, 1H), 3.91 (s, 3H), 3.09 (s, 4H), 2.61 (s, 4H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.24, 156.05, 155.71, 155.59, 144.35, 139.88, 134.60, 129.66, 126.09 122.97, 120.31, 120.16, 118.81, 105.14, 103.67, 56.89, 54.75, 51.31, 45.62. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{23}H_{24}ClF_3N_7O_3$: 538.1581, found: 538.15782.

4.1.3.16 *5-Chloro-N^4-(3-chloro-4-fluorophenyl)-N^2-(2-methoxy-4-(4-methylpiperazin-1-yl)-5-nitrophenyl)pyrimidine-2,4-diamine (**53p**).* Yield = 80%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1H), 8.28 (s, 1H), 8.24 (s, 1H), 8.16 (s, 1 H), 7.82 (s, 1H), 7.58 (d, J = 7.2 Hz, 1H), 7.25 (d, J = 5.6 Hz, 1H), 6.78 (s, 1H), 3.91 (s, 3H), 3.07 (s, 4H), 2.50 (s, 4H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.26, 156.07, 155.70, 155.44, 144.40, 136.14, 134.57, 124.39, 123.26, 122.92, 120.25, 119.20, 116.71, 116.49, 104.78, 103.68, 56.88, 54.86, 51.46, 45.82. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{22}H_{23}Cl_2FN_7O_3$: 522.1223, found: 522.1220.

4.1.3.17 *5-Chloro-N^4-(2,6-difluorophenyl)-N^2-(2-methoxy-4-(4-methylpiperazin-1-yl)-5-nitrophenyl)pyrimidine-2,4-diamine (**53q**).* Yield = 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (s, 1H), 8.16 (s, 1H), 8.07 (s, 1H), 7.92 (s, 1 H), 7.34 (s, 1H), 7.11 (s, 2H), 6.72 (s, 1H), 3.89 (s, 3H), 3.02 (s, 4H), 2.58 (s, 4H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.17, 158.10, 157.71, 157.51, 155.26, 154.27, 143.11, 135.38, 128.95, 123.36, 118.06, 115.40, 112.29, 112.06, 104.69, 103.71, 56.85, 54.79, 51.43, 45.64. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{22}H_{23}ClF_2N_7O_3$: 506.1519, found: 506.1522.

4.1.3.18 *5-Chloro-N^2-(2-methoxy-4-(4-methylpiperazin-1-yl)-5-nitrophenyl)-N^4-*

*(thiophen-3-ylmethyl)pyrimidine-2,4-diamine (**53r**).* Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (s, 1H), 8.00 (s, 2H), 7.98 (s, 1H), 7.83 (s, 1 H), 6.94 (s, 1H), 6.90 (s, 1H), 6.80 (s, 1H), 4.77 (s, 2H), 3.97 (s, 3H), 3.06 (s, 4H), 2.56 (s, 4H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.88, 157.62, 153.93, 153.83, 143.51, 142.71, 134.65, 126.92, 126.06, 125.40, 123.71, 117.55, 104.86, 103.44, 57.03, 54.91, 51.60, 45.86, 39.08. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{21}H_{25}ClN_7O_3S$: 490.1428, found: 490.1423.

4.1.3.19 *5-Chloro-N^4-cyclopropyl-N^2-(2-methoxy-4-(4-methylpiperazin-1-yl)-5-nitrophenyl)pyrimidine-2,4-diamine (**53s**).* Yield = 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H), 7.96 (s, 1H), 7.74 (s, 1H), 7.45 (s, 1 H), 6.82 (s, 1H), 4.00 (s, 3H), 3.07 (s, 4H), 2.86 (s, 1H), 2.61 (s, 4H) , 2.33 (s, 3H) , 0.76 (s, 2H) , 0.63 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.30, 157.69, 153.50, 152.94, 142.77, 135.17, 124.18, 116.02, 104.74, 103.48, 57.08, 54.91, 51.59, 45.79, 24.64, 6.65. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{19}H_{25}ClN_7O_3$: 434.1707, found: 434.1709.

4.1.3.20 *N-(2-((5-chloro-2-((2-methoxy-4-(4-methylpiperazin-1-yl)-5-nitrophenyl)amino)pyrimidin-4-yl)amino)phenyl)pivalamide (**53t**).* Yield = 70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 8.31 (s, 1H), 8.25 (s, 1H), 8.14 (s, 1 H), 8.10 (s, 1 H), 7.67 (s, 1 H), 7.25 (s, 1 H), 7.14 (s, 2H), 6.77 (s, 1H), 3.90 (s, 3H), 3.04 (s, 4H), 2.55 (s, 4H), 2.31 (s, 3H) , 1.20 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.11, 158.25, 156.08, 155.07, 154.90, 143.82, 135.08, 132.69, 131.48, 126.52, 126.14, 125.97, 125.53, 123.17, 119.46, 105.01, 103.69, 56.87, 54.90, 51.57, 45.86, 39.25, 27.71. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{27}H_{34}ClN_8O_4$: 569.2392, found: 569.2395.

4.1.3.21 *5-Chloro-N^2-(2-methoxy-4-(4-methylpiperazin-1-yl)-5-nitrophenyl)-N^4-methyl-N4-(3-(trifluoromethyl)phenyl)pyrimidine-2,4-diamine (**53u**)* . Yield = 50%. ^{1}H NMR

(400 MHz, DMSO-d_6) δ 8.84 (s, 1H), 8.27 (s, 1H), 8.14 (s, 1H), 7.56 (s, 3H), 7.52 (s, 1 H), 6.82 (s, 1 H), 4.00 (s, 3H), 3.12 (s, 5H), 2.66 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.88, 158.17, 157.85, 154.40, 146.53, 143.78, 134.42, 130.78, 130.25, 129.02, 125.70, 123.35, 122.34, 121.60, 118.10, 107.85, 103.52, 57.06, 54.66, 51.23, 45.43, 41.37. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{24}H_{26}ClF_3N_7O_3$: 552.1738, found: 552.1738.

4.1.3.22 *5-Chloro-N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(2-methoxy-4-(4-methylpiperazin-1-yl)-5-nitrophenyl)pyrimidine-2,4-diamine* ***(53v)*** . Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 8.57 (s, 1H), 8.44 (s, 1H), 8.27 (s, 2H), 7.78 (s, 1 H), 7.50 (s, 1 H), 7.30 (s, 1 H), 6.80 (s, 1H), 3.93 (s, 3H), 3.11 (s, 5H), 2.64 (s, 9H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.01, 155.56, 155.19, 151.87, 142.01, 136.69, 136.47, 133.68, 130.48, 124.89, 124.26, 124.11, 123.90, 115.97, 106.72, 102.28 , 56.24, 54.94, 51.81, 45.72, 37.62. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{24}H_{30}ClN_8O_5S$: 577.1748, found: 577.1749.

4.1.3.23 *(2-((5-Chloro-2-((4-(4-ethylpiperazin-1-yl)-2-methoxy-5-nitrophenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**53w**).* Yield = 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.26 (s, 1H), 8.41 (s, 2H), 8.32 (s, 1H), 8.19 (s, 1 H), 7.56 (s, 1H), 7.32 (s, 1H), 7.12 (s, 1H), 6.84 (s, 1H), 3.94 (s, 3H), 3.17-2.72 (m, 10H), 1.80 (s, 3H), 1.76 (s, 3H), 1.12 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.43, 156.42, 155.79, 155.29, 144.45, 143.43, 134.60, 132.26 , 131.20, 131.10, 122.90,123.26, 121.98, 121.28, 105.90, 103.80, 56.92, 52.15, 51.75, 50.92, , 19.00, 18.30, 11.35. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{25}H_{32}ClN_7O_4P$: 560.1942, found: 560.1939.

4.1.3.24 *(2-((5-Chloro-2-((4-(4-(dimethylamino)piperidin-1-yl)-2-methoxy-5-nitrophenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**53x**).* Yield = 40%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.26 (s, 1H), 8.43 (s, 1H), 8.40 (s, 1H), 8.32 (s, 1 H), 8.17 (s, 1 H), 7.56 (s, 1H), 7.32 (s, 1H), 7.12 (s, 1H), 6.83 (s, 1H), 3.94 (s, 3H), 3.34-2.90 (m, 5H), 2.63 (s, 6H), 2.07 (s, 2H), 1.79 (s, 4H), 1.76 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.46, 156.41, 155.80, 155.29, 144.77, 143.48, 134.55, 132.25, 131.22, 123.12, 122.98, 12.1.94 , 121.31, 120.81, 105.87, 104.07, 62.18, 56.89, 51.05, 27.10 (s, 2H), 19.04, 18.33. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{26}H_{34}ClN_7O_4P$: 574.2098, found: 574.2103.

4.1.3.25 *(2-((5-Chloro-2-((2-methoxy-4-morpholino-5-nitrophenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**53y**).* Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.26 (s, 1H), 8.43 (s, 2H), 8.31 (s, 1 H), 8.18 (s, 1 H), 7.56 (m, 1H), 7.33 (s, 1H), 7.12 (s, 1H), 6.86 (s, 1H), 3.94 (s, 3H), 3.75 (s, 4H), 3.07 (s, 4H), 1.80 (s, 3H), 1.76 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.47, 158.45, 156.42, 155.81, 155.31, 144.73, 143.46, 134.82, 131.20, 123.25, 123.00, 122.03, 121.27, 120.85, 105.88, 103.80, 66.67, 56.90, 52.38, 19.02, 18.32. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{23}H_{27}ClN_6O_5P$: 533.1469, found: 533.1465.

4.1.3.26 *(2-((5-Chloro-2-((2-methoxy-4-(4-(methylsulfonyl)piperazin-1-yl)-5-nitrophenyl)amino)pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**53z**).* Yield = 52%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.26 (s, 1H), 8.44 (s, 1H), 8.40 (s, 1H), 8.32 (s, 1H), 8.18 (s, 1 H), 7.56 (s, 1H), 7.32 (s, 1H), 7.12 (s, 1H), 6.83 (s, 1H), 3.94 (s, 3H), 2.91 (s, 4H), 2.12 (s, 2H), 1.79 (s, 8H), 1.76 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.54, 156.79, 156.09, 154.78, 151.68, 143.24, 140.99, 137.90, 132.70, 129.79, 125.59,

123.14, 122.66, 115.43, 107.69, 102.93, 56.29, 52.30, 46.17, 34.37, 18.86, 18.15. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{24}H_{30}ClN_7O_6PS$: 610.1404, found: 610.1408.

4.1.3.27 *(2-((2-((4-([1,4'-bipiperidin]-1'-yl)-2-methoxy-5-nitrophenyl)amino)-5-chloropyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide (**53a'**)*. Yield = 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.27 (s, 1H), 8.43 (s, 2H), 8.32 (s, 1H), 8.17 (s, 1 H), 7.58-7.54 (M, 1 H), 7.32 (s, 1H), 7.13 (s, 1H), 6.83 (s, 1H), 3.93 (s, 3H), 3.46-2.85 (m, 9H), 2.17-1.44 (m, 16H); ^{13}C NMR(100 MHz, DMSO-d_6) δ 158.44, 156.42, 155.80, 155.29, 144.68, 143.45, 134.50, 132.26, 131.11, 123.16, 121.96, 121.74, 121.30, 120.83, 105.88, 104.05, 62.60, 56.89, 51.17, 49.55, 26.72, 23.50, 22.48, 19.01, 18.31. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{29}H_{38}ClN_7O_4P$: 614.2411, found: 614.2413.

4.1.3.28 *2-((5-Chloro-2-((2-methoxy-4-(4-methylpiperazin-1-yl)-5-nitrophenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide (**53b'**)* . Yield = 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H), 8.75 (s, 1H), 8.55 (s, 1H), 8.43 (s, 1 H), 8.32 (s, 1 H), 8.20 (s, 1H), 7.73 (s, 1H), 7.30 (s, 1H), 7.10 (s, 1H), 6.82 (s, 1H), 3.94 (s, 3H), 3.10 (s, 4H), 2.80 (s, 3H), 2.51 (s, 4H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.39, 158.58, 156.45, 155.46, 155.10, 144.93, 139.70, 134.37, 131.82, 128.42, 122.78, 122.31, 121.45, 121.20, 120.89, 105.87, 103.59, 56.85, 54.99, 51.65, 46.08, 26.77. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{24}H_{28}ClN_8O_4$: 527.1922, found: 527.1922.

4.1.3.29 *2-((5-Chloro-2-((4-(4-isopropylpiperazin-1-yl)-2-methoxy-5-nitrophenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide (**53c'**).* Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.69 (s, 1H), 8.75 (s, 1H), 8.56 (s, 1H), 8.46 (s, 1H), 8.36-8.28 (m, 1 H), 8.20 (s, 1 H), 7.74 (s, 1H), 7.31 (s, 1H), 7.10 (s, 1H), 6.84 (s, 1H), 3.94 (s, 3H), 3.33-2.50 (m, 12H), 1.20-0.98 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ

169.38, 158.55, 156.42, 155.47, 155.09, 144.74, 139.70, 134.54, 131.82, 128.42, 122.99, 122.32, 121.36, 121.22, 120.90, 105.91, 103.68, 56.86, 54.77, 51.72, 48.46, 26.77, 18.35. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{26}H_{32}ClN_8O_4$: 555.2235, found: 555.2234

4.1.3.30 *2-((2-((4-(4-Acetylpiperazin-1-yl)-2-methoxy-5-nitrophenyl)amino)-5-chloropyrimidin-4-yl)amino)-N-methylbenzamide (**53d'**).* Yield = 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.69 (s, 1H), 8.75 (s, 1H), 8.55 (s, 1H), 8.44 (s, 1 H), 8.37 (s, 1 H), 8.21 (s, 1 H), 7.74 (s, 1H), 7.32 (s, 1H), 7.10 (s, 1H), 6.89 (s, 1H), 3.94 (s, 3H), 3.61 (s, 4H), 3.09 (s, 2H), 3.05 (s, 2H), 2.80 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.38，168.90, 158.44, 156.16, 155.48，155.09, 144.53，139.66, 135.12, 131.86, 128.41, 123.57, 122.37, 121.25, 120.94, 118.25，106.03, 104.32, 56.92, 52.44, 51.87, 46.29, 41.46, 26.77, 21.69. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{25}H_{28}ClN_8O_5$: 555.1871, found: 555.1874.

4.1.3.31 *2-((5-Chloro-2-((2-methoxy-4-(4-(1-methylpiperidin-4-yl)piperazin-1-yl)-5-nitrophenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide (**53e'**).* Yield = 40%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (s, 1H), 8.76 (s, 1H), 8.55 (s, 1H), 8.43 (s, 1H), 8.30 (s, 1 H), 8.20 (s, 1H), 7.72 (s, 1H), 7.31 (s, 1H), 7.10 (s, 1H), 6.83 (s, 1H), 3.93 (s, 3H), 3.08 (s, 4H), 3.00-2.35 (m, 15H), 1.84 (s, 2H), 1.53 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.38, 158.56, 156.46, 155.46, 155.08, 144.92, 139.69, 134.49, 131.82, 128.44, 122.84, 122.33, 121.44, 121.20, 120.87, 105.87, 103.58, 56.84, 54.10, 53.95, 53.41, 52.18, 49.18, 26.99, 26.76. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{29}H_{37}ClN_9O_4$: 610.2657, found: 610.2659.

4.1.3.32 *2-((5-Chloro-2-((4-(4-ethylpiperazin-1-yl)-2-methoxy-5-nitrophenyl)amino)pyrimidin-4-yl)amino)benzonitrile (**53f'**).* Yield = 70%. ^{1}H NMR (400

MHz, DMSO-d_6) δ 9.30 (s, 1H), 8.21 (s, 1H), 8.14 (s, 1H), 8.04 (s, 1 H), 7.75 (s, 1 H), 7.67-7.59 (m, 2H), 7.36 (s, 1H), 6.75 (s, 1H), 3.89 (s, 3H), 3.03 (s, 4H), 2.56-2.37 (m, 6H), 1.08 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.92, 156.90, 155.65, 154.59, 143.50, 141.17, 135.16, 134.04, 133.30, 127.65, 126.61, 123.15, 118.69, 117.54, 110.03, 104.93, 103.63, 56.86, 52.59, 51.94, 51.63, 12.08. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{24}H_{26}ClN_8O_3$: 509.1816, found: 509.1818.

4.1.3.33 *2-((5-Chloro-2-((4-(4-isopropylpiperazin-1-yl)-2-methoxy-5-nitrophenyl)amino)pyrimidin-4-yl)amino)benzonitrile (**53g'**)* . Yield = 61%.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (s, 1H), 8.21 (s, 1H), 8.12 (s, 1H), 8.03 (s, 1 H), 7.74 (s, 1 H), 7.63 (s, 1H), 7.60 (s, 1H), 7.36 (s, 1H), 6.75 (s, 1H), 3.89 (s, 3H), 3.01 (s, 4H), 2.74-2.50 (m, 5H), 1.04 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.92, 156.90, 155.65, 154.54, 143.65, 141.16, 135.18, 134.04, 133.28, 127.63, 126.62, 123.02, 118.65, 117.55, 110.02, 104.89, 103.57, 56.83, 54.21, 52.24, 48.49, 18.59. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{25}H_{28}ClN_8O_3$: 523.1973, found: 523.1975.

4.1.3.34 *2-((5-Chloro-2-((2-methoxy-4-morpholino-5-nitrophenyl)amino)pyrimidin-4-yl)amino)benzonitrile (**53h'**).* Yield = 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.30 (s, 1H), 8.22 (s, 1H), 8.14 (s, 1H), 8.04 (s, 1 H), 7.75 (s, 1H), 7.63 (s, 1H), 7.60 (s, 1H), 7.36 (s, 1H), 6.78 (s, 1H), 3.90 (s, 3H), 3.71 (S, 4H), 2.99 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.87, 156.92, 155.66, 154.53, 143.37, 141.17, 135.44, 134.05, 133.30, 127.68, 126.65, 123.45, 118.50, 117.55, 110.07, 104.97, 103.75, 66.67, 56.90, 52.41. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{22}H_{21}ClN_7O_4$: 482.1344, found: 482.1340.

4.1.3.35 *2-((5-Chloro-2-((2-methoxy-4-(4-(1-methylpiperidin-4-yl)piperazin-1-yl)-5-nitrophenyl)amino)pyrimidin-4-yl)amino)benzonitrile (**53i'**).* Yield = 45%. ^{1}H NMR (400

MHz, DMSO-d_6) δ 9.28 (s, 1H), 8.21 (s, 1H), 8.12 (s, 1H), 8.01 (s, 1 H), 7.74 (s, 1 H), 7.63 (s, 1H), 7.60 (s, 1H), 7.36 (s, 1H), 6.71 (s, 1H), 3.88 (s, 3H), 3.18-2.29 (m, 16H), 1.82 (s, 2H), 1.56 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.96, 156.88, 155.65, 154.66, 144.07, 141.17, 134.75, 134.04, 133.30, 127.62, 126.59, 122.76, 118.87, 117.55, 110.01, 104.87, 103.50, 60.93, 56.80, 54.94, 51.75, 48.31, 45.36, 28.46. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{28}H_{33}ClN_9O_3$: 578.2395, found: 578.2395.

4.1.4 The target compounds **15−36** were prepared following the synthetic procedure of **18**. The target compounds **37−50** were prepared following the synthetic procedure of **37**.

4.1.4.1 *N-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)acrylamide (**15**)*. Yield = 48%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1 H), 8.97 (s, 1 H), 8.52 (s, 1H), 8.49 (s, 1 H), 8.23(s, 1H), 8.13 (s, 1 H), 7.78 (d, J = 8.0 Hz, 1H), 7.53 (s, 1H), 7.26 (t, J = 8.0 Hz, 1H), 6.84 (s, 1H), 6.67-6.63 (m, 1H), 6.16 (d, J = 16.0 Hz, 1H), 5.71 (d, J = 12.0 Hz, 1H), 3.77 (s, 3H), 3.43 (heptet, J = 4.0 Hz, 1H), 3.07 (d, J = 8.0 Hz,, 2H), 3.08-2.41 (m, 14H), 1.87 (s, 2H), 1.76 (s, 2H), 1.17 (d, J = 4.0 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.27, 159.00, 155.85, 155.02, 149.58, 141.96, 138.49, 135.20, 132.76, 131.25, 126.57, 124.88, 123.80, 123.48, 123.34, 123.24, 119.80, 104.62, 103.99, 61.29, 56.18, 55.42, 54.73, 51.80, 48.22, 45.13, 28.59, 15.34. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{33}H_{44}ClN_8O_4S$: 683.2895, found: 683.2897.

4.1.4.2 *N-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-(methylsulfonyl)piperazin-1-yl)phenyl)acrylamide (**16**)*. Yield = 46%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 9.03 (s, 1H), 8.52 (s, 2H), 8.22 (d, J = 8.0 Hz, 2H), 7.78 (d, J = 8.0 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.27 (dd, J = 8.0, 4.0 Hz, 1H),

6.94 (s, 1H), 6.70-6.63 (m, 1H), 6.17 (d, *J* = 16.0 Hz, 1H), 5.77-5.71 (m, 1H), 3.79 (s, 3H), 3.46-3.30 (m, 6H), 2.98 (s, 6H), 1.17 (d, *J* = 4.0 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.29, 158.91, 155.84, 155.03, 149.52, 140.59, 138.46, 135.20, 132.72, 131.24, 126.75, 125.29, 124.14, 123.88, 123.53, 123.29, 119.48, 104.79, 56.29, 55.43, 51.37, 46.12, , 34.68, 15.33. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{28}H_{35}ClN_7O_6S_2$: 664.1779, found: 664.1776.

4.1.4.3 *N-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide (**17**).* Yield = 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.97 (s, 1H), 8.53-8.47 (m, 2H), 8.23 (s, 1H), 8.11 (s, 1 H), 7.77 (d, *J* = 7.6 Hz, 1H), 7.56-7.54 (m, 1H), 7.26 (t, *J* = 7.6 Hz, 1H), 6.86 (s, 1 H), 6.63-6.58 (m, 1H), 6.16 (d, *J* = 14.6 Hz, 1H), 5.72 (d, *J* = 10.0 Hz, 1H), 3.79 (s, 3H), 3.46 (heptet, *J* = 6.0 Hz, 1H), 2.90 (s, 3H), 3.18-2.60 (m, 8H), 1.17 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.34, 158.90, 155.83, 155.03, 149.53 , 140.78, 138.45, 135.24, 132.73, 131.24, 126.59, 125.03, 123.86, 123.53, 123.27, 119.81, 104.73, 104.17, 56.27, 55.43, 54.29, 50.32, 44.68, 15.33. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{28}H_{35}ClN_7O_4S$: 600.2160, found: 600.2156.

4.1.4.4 *N-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-methyl-1,4-diazepan-1-yl)phenyl)acrylamide (**18**).* Compound **53d** (29.5 mg, 0.05 mmol), iron (28.0 mg, 0.5 mmol) and ammonium chloride (16.0 mg, 0.3 mmol) were heated in ethanol (3 mL) and water (1 mL) at reflux for 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate and filtered. The solvents were removed to afford the crude compound **54d**, which was used

in the next step without further purification. Acryloyl chloride (2.0 mg, 0.02 mmol) in CH_2Cl_2 (0.2 mL) was added dropwise to a solution of compound **54d** (11.0 mg, 0.02 mmol) and DIPEA (5.2 mg, 0.04 mmol) in CH_2Cl_2 (1 mL) in an ice/water bath. The mixture was stirred for 30 min, then diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution. The organic layer were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (0−10% MeOH in DCM) to afford the desired product **18** (8.5 mg, 28%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 9.12 (s, 1H), 8.53 (s, 1H), 8.46 (s, 1H), 8.21 (s, 1H), 7.94 (s, 1H), 7.77 (s, 1H), 7.55 (s, 1H), 7.26 (s, 1H), 6.84 (s, 1H), 6.56 (s, 1H), 6.15 (d, J = 16.0 Hz, 1H), 5.70 (s, 1H), 3.76 (s, 3H), 3.41 (br, 1H), 3.15 (s, 4H), 2.68 (s, 4H), 2.33 (s, 3H), 1.87 (s, 2H), 1.16 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.37, 159.02, 155.83, 154.99, 149.82, 144.24, 138.51, 135.25, 132.63, 131.26, 126.49, 123.85, 123.73, 123.44, 123.19, 122.50, 120.93, 104.86, 104.53, 58.31, 57.28, 56.08, 55.37, 54.28, 53.53, 47.08, 28.17, 15.33; HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{29}H_{37}ClN_7O_4S$: 614.2316, found: 614.2317.

4.1.4.5 *N-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-2-(4-isopropylpiperazin-1-yl)-4-methoxyphenyl)acrylamide (**19**).* Yield = 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 9.30 (s, 1H), 8.55-8.51 (m, 2H), 8.23 (s, 1H), 7.78-7.27 (m, 3H), 6.94-6.87 (m, 1H), 6.80 (s, 1H), 6.63-6.58 (m, 1H), 6.16 (d, J = 16.0 Hz, 1H), 5.70 (d, J = 10.0 Hz, 1H), 3.79 (s, 3H), 3.43-2.74 (m, 10H), 1.36-1.17 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.38, 158.91, 155.84, 155.02, 149.51, 140.03, 138.45, 135.20, 132.74, 131.24, 126.73, 125.11, 124.07, 123.69, 119.78, 104.75, 103.99, 56.73,

56.25, 55.44, 49.36, 48.01, 17.29, 15.33. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{30}H_{39}ClN_7O_4S$: 628.2473, found: 628.2471.

4.1.4.6 *N-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-(2-methoxyethyl)piperazin-1-yl)phenyl)acrylamide (**20**).* Yield = 49%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.98 (s, 1H), 8.52-8.47 (m, 2H), 8.22 (s, 1H), 8.11 (s, 1H), 7.77 (s, 1H), 7.54 (s, 1H), 7.26 (s, 1H), 6.88 (s, 1H), 6.60 (s, 1H), 6.15 (d, *J* = 16.0 Hz, 1H), 5.70 (s, 1H), 3.78 (s, 3H), 3.49-2.57 (m, 16H), 1.17 (s, 6H); ^{13}C NMR(100 MHz, DMSO-d_6) δ 163.27, 158.98, 155.85, 155.02, 149.70, 141.72, 138.49, 135.22, 132.72, 131.25, 126.54, 124.84, 123.79, 123.49, 123.24, 119.94, 104.55, 104.13, 70.51, 58.50, 57.57, 56.20, 55.41, 53.77, 51.86, 15.33. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{30}H_{39}ClN_7O_5S$: 644.2422, found: 644.2420.

4.1.4.7 *N-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-2-(4-cyclohexylpiperazin-1-yl)-4-methoxyphenyl)acrylamide (**21**).* Yield = 47%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 9.02 (s, 1H), 8.50 (s, 2H), 8.23 (s, 1H), 8.14 (s, 1H), 7.77 (d, *J* = 7.6 Hz, 1H), 7.56-7.54 (m, 1H), 7.26 (t, *J* = 7.6 Hz, 1H), 6.85 (s, 1H), 6.66-6.60 (m, 1H), 6.15 (d, *J* = 16.0 Hz, 1H), 5.71 (d, *J* = 9.6 Hz, 1H), 3.79 (s, 3H), 3.44-1.17 (m, 26H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.35, 158.91, 155.82, 155.02, 149.51, 140.28, 138.46, 135.21, 132.75, 131.23, 126.63, 125.02, 123.96, 123.84, 123.53, 123.26, 119.78, 104.72, 103.93, 64.10, 56.24, 55.44, 49.80, 48.52, 27.38, 25.60, 25.26, 15.33. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{33}H_{43}ClN_7O_4S$: 668.2786, found: 668.2783.

4.1.4.8 *N-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-morpholinophenyl)acrylamide (**22**).* Yield = 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 9.07 (s, 1H), 8.49 (s, 2H), 8.22 (s, 1H), 8.14 (s, 1H), 7.77 (s,

1H), 7.54 (s, 1H), 7.26 (s, 1H), 6.87 (s, 1H), 6.63 (s, 1H), 6.15(d, *J* = 16.0 Hz, 1H), 5.71 (s, 1H), 3.80 (s, 7 H), 3.43 (s, 1H), 2.86 (s, 4H), 1.17 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.31, 158.95, 155.85, 155.03, 149.69, 141.39, 138.47, 135.21, 132.70, 131.25, 126.63, 125.00, 123.84, 123.74, 123.51, 123.26, 119.96, 104.68, 104.24, 66.78, 56.24, 55.41, 52.26, 15.33. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{27}H_{32}ClN_6O_5S$: 587.1843, found: 587.1848.

4.1.4.9 *N-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(piperidin-1-yl)phenyl)acrylamide (**23**)*. Yield = 48%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.96 (s, 1H), 8.54 (s, 1H), 8.49 (s, 1H), 8.21 (s, 1H), 8.14 (s, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.53 (s, 1H), 7.25 (t, *J* = 8.0 Hz, 1H), 6.84 (s, 1H), 6.64-6.59 (m, 1H), 6.15 (d, *J* = 20 Hz, 1H), 5.71 (d, *J* = 8.0 Hz, 1H), 3.78 (s, 3H), 3.44 (heptet, *J* = 8.0 Hz, 1H), 2.82 (s, 4H), 1.73 (s, 4H), 1.56 (s, 2H), 1.17 (d, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.33, 157.53, 155.31, 145.34, 138.23, 137.70, 134.32, 132.09, 130.92, 126.44, 126.23, 125.24, 124.66, 123.96, 123.06, 112.25, 106.12, 103.32, 55.99, 55.62, 53.97, 27.00, 24.01, 15.33. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{28}H_{34}ClN_6O_4S$: 585.2051, found: 585.2051.

4.1.4.10 *N-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-2-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)acrylamide (**24**)*. Yield = 56%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H), 9.57 (s, 1H), 8.55 (s, 1H), 8.32 (s, 2H), 8.26 (s, 1H), 7.81(s, 1H,), 7.57 (s, 1H), 7.37 (s, 1H), 7.32 (s, 1H), 7.09 (s, 1H), 6.51 (s, 1H), 6.19 (d, *J* = 16.0 Hz, 1H), 5.72 (s, 1H), 3.45-2.44 (m, 17H), 1.85 (s, 1H), 1.54 (s, 1H), 1.19 (s, 6H); ^{13}C NMR(100 MHz, DMSO-d_6) δ 163.28, 157.94, 155.72, 155.28, 139.35, 138.32, 135.47, 135.07, 133.90, 132.55, 131.35, 126.75, 124.55, 123.97, 123.78,

120.37, 115.09, 113.10, 105.80, 60.87, 55.45, 54.13, 51.70, 47.45, 44.40, 28.82, 15.33. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{32}H_{42}ClN_8O_3S$: 653.2789, found: 653.2785.

4.1.4.11 *N-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-2-(4-ethylpiperazin-1-yl)-4-isopropoxyphenyl)acrylamide (**25**).* Yield = 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 1H), 9.06 (s, 1H), 8.47 (s, 1H), 8.32 (s, 1H), 8.23 (s, 2H), 7.76 (s, 1H), 7.52 (s, 1H), 7.24 (s, 1H), 6.83(s, 1H), 6.68 (s, 1H), 6.14 (d, J = 16.0 Hz, 1H), 5.71 (s, 1H), 4.58 (s, 1H), 3.50-2.80 (m, 11H), 1.40-1.10 (m, 15H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.33, 158.79, 155.88, 155.04, 147.18, 140.19, 138.37, 135.11, 132.72, 131.24, 130.02, 126.68, 125.50, 123.99, 123.65, 123.44, 119.39, 107.17, 104.72, 71.47, 55.44, 51.66, 51.42, 49.79, 22.34, 15.32, 10.43. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{31}H_{41}ClN_7O_4S$: 642.2629, found: 642.2630

4.1.4.12 *N-(5-((4-((2-(Isopropylsulfonyl)phenyl)amino)-5-methylpyrimidin-2-yl)amino)-4-methoxy-2-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)acrylamide (**26**).* Yield = 46%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H), 8.97 (s, 1H), 8.62 (s, 1H), 8.32 (s, 1H), 8.00 (s, 1H), 7.88 (s, 1H), 7.75 (s, 1H), 7.55 (s, 1H), 7.20 (s, 1H), 6.81 (s, 1H), 6.65 (s, 1H), 6.14 (d, J = 16.0 Hz, 1H), 5.71 (s, 1H), 3.80 (s, 3H), 3.65-2.21 (m, 14H), 2.09 (s, 3H), 1.92-1.70 (m, 4H), 1.16 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.23, 159.07, 158.39, 157.19, 148.20, 140.66, 139.57, 135.25, 132.82, 131.20, 126.46, 124.90, 124.41, 123.06, 122.63, 118.21, 106.33, 61.28, 56.27, 55.37, 54.22, 51.80, 47.73, 44.55, 28.50, 15.33, 13.09. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{34}H_{47}N_8O_4S$: 663.3441, found: 663.3443

4.1.4.13 *N-(2-(4-Ethylpiperazin-1-yl)-5-((4-((2-(isopropylsulfonyl)phenyl)amino)-5-methylpyrimidin-2-yl)amino)-4-methoxyphenyl)acrylamide (**27**).* Yield = 60%. ^{1}H NMR

(400 MHz, DMSO-d_6) δ 9.04 (s, 1H), 9.00 (s, 1H), 8.63 (s, 1H), 8.32 (s, 1H), 8.00 (s, 1H), 7.92 (s, 1H), 7.76 (s, 1H), 7.56 (s, 1H), 7.20 (s, 1H), 6.85 (s, 1H), 6.61 (s, 1H), 6.14 (d, J = 16.0 Hz, 1H), 5.71 (s, 1H), 3.81 (s, 3H), 3.60-2.00 (m, 11H), 2.09 (s, 3H), 1.20-1.00 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.73, 158.53, 157.64, 155.85, 145.50, 139.22, 134.82, 134.56, 131.82, 130.91, 126.84, 126.07, 123.44, 122.43, 112.66, 107.21, 103.45, 56.13, 55.74, 52.44, 52.28, 50.19, 15.30, 13.20, 10.10. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{30}H_{40}N_7O_4S$: 594.2862, found: 594.2863.

4.1.4.14 *N-(2-(4-Ethylpiperazin-1-yl)-5-((4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxyphenyl)acrylamide* ***(28)***. Yield = 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H), 8.89 (s, 1H), 8.37 (s, 1H), 8.11 (s, 1H), 7.80 (s, 1H), 7.60 (s, 1H), 7.53 (s, 1H), 7.14 (s, 1H), 6.74 (s, 1H), 6.42 (s, 1H), 6.29 (d, J = 16.0 Hz, 1H), 6.20 (s, 1H), 5.67 (s, 1H), 3.84 (s, 3H), 3.75-3.00 (m, 11H), 1.43 (s, 3H), 1.28(s, 6H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.00, 159.93, 158.96, 156.60, 145.93, 138.88, 134.70, 131.72, 131.15, 127.03, 126.41, 125.86, 124.32, 123.31, 122.95, 113.59, 103.47, 99.34, 56.21, 55.69, 52.28, 52.00, 49.37, 15.30, 9.45. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{29}H_{38}N_7O_4S$: 580.2706, found: 580.2707.

4.1.4.15 *N-(5-((5-Chloro-4-((3-(trifluoromethyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide* ***(29)***. Yield = 42%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (s, 2H), 8.18 (s, 2H), 8.14 (s, 2H), 7.93 (s, 1H), 7.40 (s, 1H), 7.30 (s, 1H), 6.82 (s, 1H), 6.65 (s, 1H), 6.16 (d, J = 16 Hz, 1H), 5.71 (s, 1H), 3.78 (s, 3H), 3.00-2.50 (m, 11H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.32, 158.83, 155.86, 155.61, 149.17, 140.48, 140.09, 132.74, 129.65, 126.55, 125.68, 125.08, 124.19, 119.67, 119.35,

118.36, 104.27, 104.18, 56.32, 54.35, 50.34, 44.70. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{26}H_{28}ClF_3N_7O_2$: 562.1945, found: 562.1948.

4.1.4.16 *N-(5-((5-Chloro-4-((3-chloro-4-fluorophenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide (**30**).* Yield = 51%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 (s, 1H), 8.84 (s, 1H), 8.21 (s, 1H), 8.15 (s, 1H), 8.10 (s, 1H), 7.84 (s, 1H), 7.68 (s, 1H), 7.20 (s, 1H), 6.83 (s, 1H), 6.63 (s, 1H), 6.16 (d, J = 16 Hz, 1H), 5.71 (s, 1H), 3.79 (s, 3H), 3.00-2.40 (m, 11H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.29, 158.88, 155.77, 155.48, 152.38, 149.19, 140.47, 136.48, 132.67, 126.51, 125.11, 124.12, 123.58, 122.56, 119.32, 119.03, 116.63, 104.32, 104.04, 56.31, 54.44, 50.47, 44.83. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{25}H_{27}Cl_2FN_7O_2$: 546.1587, found: 546.1587

4.1.4.17 *N-(5-((5-Chloro-4-((2,6-difluorophenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide (**31**).* Yield = 58%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.78 (s, 1H), 8.09 (s, 1H), 7.90 (s, 1H), 7.67 (s, 1H), 7.32-7.08 (m, 3H), 6.71 (s, 1H), 6.66-6.59 (m, 1H), 6.22 (d, J = 20 Hz, 1H), 5.75 (d, J = 8.0 Hz, 1H), 3.77 (s, 3H), 2.94-2.30 (m, 11H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.40, 160.25, 158.49, 157.81, 157.41, 155.11, 147.93, 140.16, 132.87, 128.68, 126.45, 124.32, 118.48, 115.57, 112.34, 112.09, 107.62, 104.12, 103.61, 56.31, 54.23, 50.07, 44.39. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{25}H_{27}ClF_2N_7O_2$: 530.1883, found: 530.1884.

4.1.4.18 *N-(5-((5-Chloro-4-((thiophen-2-ylmethyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide (**32**).* Yield = 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.84 (s, 1H), 7.94 (s, 1H), 7.82 (s, 1H), 7.66 (s, 1H), 7.27 (s, 1H), 6.89 (s, 2H), 6.81 (s, 1H), 6.73 (s, 1H), 6.21 (d, J = 16.0 Hz, 1H), 5.71 (s, 1H), 4.81 (s, 2H), 3.86 (s, 3H), 3.30-2.70 (m, 11H); ^{13}C NMR (100 MHz, DMSO-d_6) δ

163.38, 158.23, 157.63, 153.81, 146.40, 143.13, 137.59, 132.85, 126.84, 126.58, 125.93, 125.43, 125.20, 115.78, 104.20, 103.89, 56.60, 53.34, 49.09, 43.09, 38.97. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{24}H_{29}ClN_7O_2S$: 514.1792, found: 514.1794.

4.1.4.19 *N-(5-((5-Chloro-4-(cyclopropylamino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide (**33**).* Yield = 13%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H), 8.94 (s, 1H), 7.91 (s, 1H), 7.58 (s, 1H), 7.26 (s, 1H), 6.79 (s, 1H), 6.73 (s, 1H), 6.16 (d, J = 16.0 Hz, 1H), 5.70 (s, 1H), 3.89 (s, 3H), 3.30-2.70 (m, 12H), 0.61 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.47, 159.22, 158.00, 153.48, 145.85, 137.68, 132.72, 126.38, 125.66, 125.09, 115.76, 104.13, 103.54, 56.65, 53.31, 48.90, 42.99, 24.83, 6.53. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{22}H_{29}ClN_7O_2$: 458.2071, found: 458.2075.

4.1.4.20 *N-(5-((5-Chloro-4-((2-pivalamidophenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide (**34**).* Yield = 54%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 8.97 (s, 1H), 8.22 (s, 1H), 8.11-8.09 (m, 2H), 7.98 (s, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.24-7.09(m, 3H), 6.79 (s, 1H), 6.67-6.60 (m, 1H), 6.20 (d, J = 16.0 Hz, 1H), 5.74 (d, J = 8.0 Hz, 1H), 3.78 (s, 3H), 2.89-2.36 (m, 11H), 1.21(s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.07, 163.45, 158.72, 155.92, 154.89, 148.63, 140.28, 132.89, 132.81, 131.16, 126.59, 126.16, 125.90, 125.09, 124.87, 124.29, 118.94, 104.33, 103.96, 99.99, 56.32, 54.29, 50.26, 44.60, 39.24, 27.75. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{30}H_{38}ClN_8O_3$: 593.2755, found: 593.2753.

4.1.4.21 *N-(5-((5-Chloro-4-(methyl(3-(trifluoromethyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide (**35**).* Yield = 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1H), 8.60 (s, 1H), 8.07 (s, 2H), 7.60-7.46(m, 4H),

6.84 (s, 1H), 6.62 (s, 1H), 6.19 (d, *J* = 16.0 Hz, 1H), 5.70 (s, 1H), 3.87 (s, 3H), 3.48 (s, 3H), 3.00-2.30 (m, 11H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.30, 158.93, 158.40, 158.17, 147.33, 146.82, 139.63, 132.79, 130.72, 130.23, 128.68, 126.46, 125.07, 124.39, 121.96, 121.21, 116.80, 107.36, 103.91, 56.50, 54.84, 51.06, 45.47, 41.29. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{27}H_{30}ClF_3N_7O_2$: 576.2102, found: 576.2098.

4.1.4.22 *N-(5-((5-Chloro-4-((2-(N,N-dimethylsulfamoyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide (**36**).* Yield = 64%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.38 (s, 1H), 8.96 (s, 1H), 8.50-8.44 (m, 2H), 8.23 (s, 1H), 8.12 (s, 1H), 7.74 (d, *J* = 8.0 Hz, 1H),7.49 (s, 1H), 7.24 (s, 1H), 6.86 (s, 1H), 6.64-6.57 (m, 1H), 6.15 (d, *J* = 16.0 Hz, 1H), 5.71 (d, *J* = 12 Hz, 1H), 3.79 (s, 3H), 2.89 (s, 4H), 2.66-2.61 (m, 10H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.28, 158.95, 155.85, 154.98, 149.59, 141.68, 137.14, 134.23, 132.72, 130.34, 126.53, 124.77, 123.57, 123.53, 123.51, 123.25, 119.94, 104.41, 104.00, 56.21, 55.28, 51.67, 46.27, 37.62. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{27}H_{34}ClN_8O_4S$: 601.2112, found: 601.2110.

4.1.4.23 *N-(5-((5-chloro-4-((2-(dimethylphosphoryl)phenyl)amino)pyrimidin-2-yl)amino)-2-(4-ethylpiperazin-1-yl)-4-methoxyphenyl)acrylamide (**37**).* Compound **53w** (56 mg, 0.1 mmol) and $SnCl_2.2H_2O$ (226.0 mg, 1.0 mmol) were heated in ethanol (5 mL) at reflux for 2 h. The reaction was quenched with saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate and filtered. The solvents were removed to afford the crude compound **54w**, which was used in the next step without further purification. Acryloyl chloride (8.0 mg, 0.08 mmol) in CH_2Cl_2 (0.1 mL) was added dropwise to a solution of crude compound **54w** (42.0 mg, 0.08 mmol) and DIPEA (20.8 mg, 0.16

mmol) in DMF (5.0 mL) in an ice/water bath. The mixture was stirred for 30 min, then diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution. The organic layer were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (0−10% MeOH in DCM) to afford the desired product **37** as a pale yellow solid. Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 9.01 (s, 1H), 8.47 (s, 1H), 8.28 (s, 1H), 8.12 (s, 2H), 7.52 (s, 1H), 7.33 (s, 1H), 7.07 (s, 1H), 6.87 (s, 1H), 6.63 (s, 1H), 6.19 (d, J = 17.2 Hz, 1H), 5.73 (s, 1H), 3.80 (s, 3H), 2.91-2.50 (m, 10H), 1.79 (s, 3H), 1.76 (s, 3H), 1.02 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.43, 158.99, 155.70, 155.35, 149.68, 143.66, 132.74, 132.34, 131.04, 126.67, 125.05, 124.27, 122.65, 122.02, 121.35, 120.49, 120.02, 105.00, 104.14, 56.27, 51.88, 51.57, 50.09, 19.06, 18.36, 10.84. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{28}H_{36}ClN_7O_3P$: 584.2306, found: 584.2304.

4.1.4.24 *N-(5-((5-Chloro-4-((2-(dimethylphosphoryl)phenyl)amino)pyrimidin-2-yl)amino)-2-(4-(dimethylamino)piperidin-1-yl)-4-methoxyphenyl)acrylamide (**38**).* Yield = 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.21 (s, 1 H), 9.08 (s, 1 H), 8.46 (s, 1 H), 8.30 (s, 1 H), 8.15 (s, 1 H), 8.11 (s, 1 H), 7.52 (s, 1 H), 7.31 (s, 1 H), 7.05 (s, 1 H), 6.85 (s, 1 H), 6.71 (s, 1 H), 6.20 (d, J = 16.0 Hz, 1 H), 5.73 (s, 1 H), 3.78 (s, 3 H), 3.14 (s, 3 H), 2.70 (s, 8 H), 2.10-1.75 (m, 10 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.38, 159.03, 155.69, 155.36, 149.66, 143.66, 141.24, 136.13, 132.74, , 132.30, 131.07, 126.71, 124.97, 124.00, 121.95, 119.99, 111.63, 104.98, 104.28, 62.48, 56.24, 50.86, 50.01, 26.72, 19.07, 18.37. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{29}H_{38}ClN_7O_3P$: 598.2462, found: 598.2458.

4.1.4.25 *N-(5-((5-Chloro-4-((2-(dimethylphosphoryl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-morpholinophenyl)acrylamide (**39**).* Yield = 60%. ^{1}H NMR (400

MHz, DMSO-d_6) δ 11.21 (s, 1H), 9.10 (s, 1H), 8.46 (s, 1H), 8.29 (s, 1H), 8.14 (s, 1H), 8.11 (s, 1H), 7.52 (s, 1H), 7.33 (s, 1H), 7.06 (s, 1H), 6.88 (s, 1H), 6.65 (s, 1H), 6.18 (d, *J* = 16.0 Hz, 1H), 5.72 (s, 1H), 3.81 (s, 7H), 2.87 (s, 4H), 1.80 (s, 3H), 1.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.42, 159.03, 155.70, 155.37, 149.78, 143.68, 141.38, 132.72, 132.35, 131.06, 126.65 , 125.01, 124.07, 122.62, 121.99, 120.42, 120.14, 104.96, 104.20, 66.80, 56.24, 52.29, 19.06, 18.36. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{26}H_{31}ClN_6O_4P$: 557.1833, found: 557.1834.

4.1.4.26 *N-(5-((5-Chloro-4-((2-(dimethylphosphoryl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-(methylsulfonyl)piperazin-1-yl)phenyl)acrylamide (**40**).* Yield = 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (s, 1H), 9.06 (s, 1H), 8.45 (s, 1H), 8.31 (s, 1H), 8.20 (s, 1H), 8.11 (s, 1H), 7.53 (s, 1H), 7.32 (s, 1H), 7.06 (s, 1H), 6.94 (s, 1H), 6.67 (s, 1H), 6.20 (d, *J* = 16 Hz, 1H), 5.74 (s, 1H), 3.79 (s, 3H), 3.17 (s, 2H), 2.97 (s, 9H), 1.78 (s, 3H), 1.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.37, 158.99, 155.69, 155.37, 149.68, 143.64, 140.65, 132.71, 132.32, 131.02, 126.80, 125.24, 124.43, 122.64, 121.95, 121.35, 119.75, 104.99, 104.75, 56.28, 51.38, 46.13, 34.65, 19.05, 18.35. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{27}H_{34}ClN_7O_5PS$: 634.1768, found: 634.1767.

4.1.4.27 *N-(2-([1,4'-Bipiperidin]-1'-yl)-5-((5-chloro-4-((2-(dimethylphosphoryl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxyphenyl)acrylamide (**41**).* Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 10.00 (s, 1H), 9.03 (s, 1H), 8.48 (s, 1H), 8.27 (s, 1H), 8.15 (s, 1H), 8.12 (s, 1H), 7.53 (s, 1H), 7.33 (s, 1H), 7.07 (s, 1H), 6.85 (s, 1H), 6.68 (s, 1H), 6.21 (d, *J* = 15.6 Hz, 1H), 5.74 (s, 1H), 3.80 (s, 3H), 3.44-2.76 (m, 8H), 2.15-1.25 (m, 17H); ^{13}C NMR (100 MHz, DMSO) δ 163.39, 159.01, 155.69, 155.36, 149.64, 143.67, 141.17, 132.71, 132.32, 131.07, 126.75, 124.90,

123.99, 122.64, 121.97, 121.36, 119.99, 104.95, 104.20, 63.07, 56.21, 50.99, 49.44, 26.44, 23.20, 22.33, 19.06, 18.36. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{32}H_{42}ClN_7O_3P$: 638.2775, found: 638.2780.

4.1.4.28 *2-((2-((5-Acrylamido-2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-chloropyrimidin-4-yl)amino)-N-methylbenzamide (**42**)*. Yield = 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.66 (s, 1H), 9.02 (s, 1H), 8.73 (s, 1H), 8.59 (s, 1H), 8.33 (s, 1H), 8.15 (s, 2H), 7.72 (s, 1H), 7.32 (s, 1H), 7.04 (s, 1H), 6.86 (s, 1H), 6.63 (s, 1H), 6.18 (d, J = 16 Hz, 1H), 5.73 (s, 1H), 3.80 (s, 3H), 3.00-2.30 (m, 14H); ^{13}C NMR (100 MHz, DMSO) δ 169.42, 163.37, 159.07, 155.41, 155.14, 149.69, 141.55, 139.90, 132.70, 131.97, 128.30, 126.56, 124.78 123.84, 121.99, 121.27, 120.61, 120.11, 105.02, 104.00, 56.22, 55.15, 51.48, 46.02, 26.76. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{27}H_{32}ClN_8O_3$: 551.2286, found: 551.2282.

4.1.4.29 *2-((2-((5-Acrylamido-4-(4-isopropylpiperazin-1-yl)-2-methoxyphenyl)amino)-5-chloropyrimidin-4-yl)amino)-N-methylbenzamide (**43**).* Yield = 50%.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (s, 1H), 9.13 (s, 1H), 8.78 (s, 1H), 8.59 (s, 1H), 8.35 (s, 1H), 8.23 (s, 1H), 8.14 (s, 1H), 7.74 (s, 1H), 7.32 (s, 1H), 7.03 (s, 1H), 6.83 (s, 1H), 6.74 (s, 1H), 6.18 (d, J = 20 Hz, 1H), 5.72 (s, 1H), 3.79 (s, 3H), 3.30-2.80 (m, 12H), 1.23 (s, 6H); LC/MS (ESI, *m/z*) 551.2 $[M + H]^+$. ^{13}C NMR (100 MHz, DMSO) δ 169.41, 163.45, 159.08, 155.42, 155.14, 149.64, 139.91, 132.74, 131.94, 128.33, 126.77, 125.06, 124.29, 122.03, 121.28, 120.58, 120.02, 105.08, 103.94, 56.24, 49.64, 48.08, 26.76, 17.43. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{29}H_{36}ClN_8O_3$: 579.2599, found: 579.2597.

4.1.4.30 *2-((2-((4-(4-Acetylpiperazin-1-yl)-5-acrylamido-2-methoxyphenyl)amino)-5-chloropyrimidin-4-yl)amino)-N-methylbenzamide (**44**)*. Yield = 65%. ^{1}H NMR (400

MHz, DMSO-d_6) δ 11.67 (s, 1H), 9.14 (s, 1H), 8.73 (s, 1H), 8.59 (s, 1H), 8.35 (s, 1H), 8.23 (s, 1H), 8.16 (s, 1H), 7.71 (s, 1H), 7.31 (s, 1H), 7.06 (s, 1H), 6.90 (s, 1H), 6.68 (s, 1H), 6.20 (d, J = 20 Hz, 1H), 5.73 (s, 1H), 3.79 (s, 3H), 3.67 (s, 4H), 2.86-2.74 (m, 7H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.42, 168.76, 163.41, 161.58, 159.05, 155.42, 155.14, 149.56, 140.93, 139.90, 132.72, 131.94, 126.69, 125.25, 124.28, 122.02, 121.30, 120.62, 119.70, 105.08, 104.58, 56.22, 52.34, 51.75, 46.50, 41.58, 26.76, 21.71. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{28}H_{32}ClN_8O_4$: 579.2235, found: 579.2236

4.1.4.31 *2-((2-((5-Acrylamido-2-methoxy-4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)amino)-5-chloropyrimidin-4-yl)amino)-N-methylbenzamide (**45**).* Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H), 9.01 (s, 1H), 8.75 (s, 1H), 8.59 (s, 1H), 8.32 (s, 1H), 8.15 (s, 2H), 7.71 (s, 1H), 7.31 (s, 1H), 7.05 (s, 1H), 6.83 (s, 1H), 6.67 (s, 1H), 6.18 (d, J = 16 Hz, 1H), 5.72 (s, 1H), 3.79 (s, 3H), 3.10-2.30 (m, 19H), 1.92-1.73 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.41, 163.33, 159.11, 155.40, 155.14, 149.65, 141.87, 139.92, 132.74, 131.93, 128.32, 126.60, 124.84, 123.65, 121.98, 121.25, 120.55, 119.97, 104.96, 103.95, 61.25, 56.18, 54.31, 51.78, 47.71, 29.49, 28.50, 26.76. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{32}H_{41}ClN_9O_3$: 634.3021, found: 634.3018.

4.1.4.32 *N-(5-((5-Chloro-4-((2-cyanophenyl)amino)pyrimidin-2-yl)amino)-2-(4-ethylpiperazin-1-yl)-4-methoxyphenyl)acrylamide (**46**).* Yield = 70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H), 8.90 (s, 1H), 8.17 (s, 1H), 8.01 (s, 1H), 7.91 (s, 1H), 7.76 (s, 1H), 7.69 (s, 1H), 7.61 (s, 1H), 7.31 (s, 1H), 6.78 (s, 1H), 6.60 (s, 1H), 6.20 (d, J = 16.0 Hz, 1H), 5.74 (s, 1H), 3.78 (s, 3H), 3.35-2.50 (m, 10H), 1.06 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.37, 158.39, 156.64, 155.61, 148.25, 141.22, 140.13, 133.88, 133.29, 132.80, 127.07, 126.55, 126.03, 124.79, 124.30, 118.62, 117.54, 109.23, 104.36,

103.83, 56.36, 51.93, 51.57, 50.09, 10.76. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{27}H_{30}ClN_8O_2$: 533.2180, found: 533.2182.

4.1.4.33 *N-(5-((5-Chloro-4-((2-cyanophenyl)amino)pyrimidin-2-yl)amino)-2-(4-isopropylpiperazin-1-yl)-4-methoxyphenyl)acrylamide (**47**).* Yield = 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (s, 1H), 8.91 (s, 1H), 8.17 (s, 1 H), 8.03 (s, 1H), 7.91 (s, 1H), 7.76 (s, 1H), 7.69 (s, 1H), 7.61 (s, 1H), 7.31(s, 1H), 6.77 (s, 1H), 6.63 (s, 1H), 6.21 (d, J = 16.0 Hz, 1H), 5.76 (s, 1H), 3.78 (s, 3H), 3.00-2.50 (m, 9H), 1.04 (s, 6H); ^{13}C NMR (100 MHz, DMSO) δ 163.38, 158.41, 156.54, 155.62, 148.28, 141.22, 139.61, 133.87, 133.29, 132.82, 127.09, 126.65, 126.05, 124.82, 124.37, 118.67, 117.54, 109.23, 104.35, 103.67, 56.52, 56.34, 49.40, 48.04, 17.30. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{28}H_{32}ClN_8O_2$: 547.2337, found: 547.2339.

4.1.4.34 *N-(5-((5-Chloro-4-((2-cyanophenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-morpholinophenyl)acrylamide (**48**).* Yield = 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.98 (s, 1H), 8.16 (s, 1H), 8.03 (s, 1H), 7.90 (s, 1H), 7.74 (s, 1H), 7.68 (s, 1H), 7.59 (s, 1H), 7.29 (s, 1H), 6.78 (s, 1H), 6.64 (s, 1H), 6.20 (d, J = 20 Hz, 1H), 5.74 (s, 1H), 3.78 (s, 7H), 2.81 (s, 4H); ^{13}C NMR (100 MHz, DMSO) δ 163.37, 158.39, 156.55, 155.62, 148.30, 141.22, 140.89, 133.87, 133.29, 132.80，127.04, 126.46, 126.03, 124.75, 124.12, 118.70, 117.53, 109.17, 104.32, 103.88, 66.81, 56.34, 52.25. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{25}H_{25}ClN_7O_3$: 506.1707, found: 506.1708.

4.1.4.35 *N-(5-((5-Chloro-4-((2-cyanophenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)acrylamide (**49**).* Yield = 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.88 (s, 1H), 8.17 (s, 1H), 8.03 (s, 1H), 7.89 (s, 1H), 7.76 (s, 1H), 7.69 (s, 1H), 7.60 (s, 1H), 7.31(s, 1H), 6.75 (s, 1H), 6.65 (s, 1H), 6.21

(d, *J* = 12.0 Hz, 1H), 5.74 (s, 1H), 3.77 (s, 3H), 3.35-2.30 (m, 16H), 1.85-1.60 (m, 4H); ^{13}C NMR (100 MHz, DMSO) *δ* 163.31, 158.42, 156.53, 155.61, 148.18, 141.30, 141.22, 133.87, 133.29, 132.82, 127.03, 126.49, 126.03, 124.61, 123.75, 118.61, 117.53, 109.17, 104.26, 103.67, 61.22, 56.29, 53.49, 51.55, 47.01, 43.68, 28.30. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{31}H_{37}ClN_9O_2$: 602.2759, found: 602.2758.

4.1.4.36 *N-(5-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-4-methoxy-2-(4-methyl-1,4-diazepan-1-yl)phenyl)propionamide (**50**).* Yield = 65%. ^{1}H NMR (400 MHz, DMSO-d_6) *δ* 9.55 (s, 1H), 9.10 (s, 1H), 8.53 (s, 1H), 8.48 (s, 1H), 8.22 (s, 1H), 7.93 (s, 1H), 7.79 (d, *J* = 6.4 Hz, 1 H), 7.62 (s, 1H), 7.28 (s, 1H), 6.86 (s, 1H), 3.77 (s, 3H), 3.61-2.85 (m, 9H), 2.50-1.97 (m, 5H), 1.26-1.00 (m, 11H); ^{13}C NMR (100 MHz, DMSO) *δ* 172.27, 158.95, 155.84, 154.98, 149.33, 143.16, 138.53, 135.43, 131.26, 125.23, 123.68, 123.45, 123.12, 120.95 , 105.45 , 104.63, 56.73, 56.23, 55.43, 54.81, 54.62, 50.56, 44.67, 29.57, 25.75, 15.33, 10.22. HRMS (ESI, m/z) $[M+H]^+$ calcd for $C_{29}H_{39}ClN_7O_4S$: 616.2473, found: 616.2478.

4.2 Biology

4.2.1 Cell Lines, Antibodies and Chemicals. The human cancer cell lines H1975, HCC827, NCI-H2122, H1355, H3122, H2228, CHO, and CHL were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). PC9 was purchased from Sigma-Aldrich (St. Louis, MO, USA). A549, H3255 were purchased from Cobioer Biosciences CO., LTD (Nanjing, China). H1975, HCC827, PC9, NCI-H2122, H1355, H3122, H2228, CHL, and EGFR mutant isogenic BaF3 cells lines were cultured in RPMI 1640 media (Corning, USA) with 10% fetal bovine serum (FBS) and supplemented with 2% L-glutamine and 1% penicillin/streptomycin. CHO was cultured in DMEM media

(Corning, USA) with 10% fetal bovine serum (FBS) and supplemented with 2% L-glutamine and 1% penicillin/streptomycin. A549 was cultured in F-12K nutrient mixture (kaighn's modification) (Gibco, USA), and H3255 was cultured in BEGM media (LONZA, USA) with 10% FBS and supplemented with 2% L-glutamine and 1% pen/strep. All cell lines were maintained in culture media at 37 ºC with 5% CO_2.

The following antibodies were purchased from Cell Signaling Technology (Danvers, MA): ALK (C26G7) rabbit mAb (#3333), phospho-ALK (Tyr1604) (#3341), Akt (pan) (C67E7) rabbit mAb (#4691), phospho-Akt (Ser473) (D9E) XP rabbit mAb (#4060), phospho-Akt (Thr308) (D25E6) XP rabbit mAb (#13038), phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP rabbit mAb(#4370), GAPDH (D16H11) XP rabbit mAb, PARP (46D11) rabbit mAb (#9532), caspase-3 (8G10) rabbit mAb (#9665), 4E-BP1 (#9644), 4E-BP1 (53H11) rabbit mAb (#9644), eIF4E (C46H6) rabbit mAbv (#2067), phospho-eIF4E (Ser209) (#9741), EGF receptor (D38B1) XP rabbit mAb (#4267), phospho-EGF receptor (Tyr1068) (D7A5) XP rabbit mAb (#3777), Stat3 (#9132), phospho-Stat3 (Tyr705) (D3A7) XP® rabbit mAb (#9145). Antibodies were used at 1:1000. α-Tublin (Santa Cruz Biotechnology -Aldrich) served as a loading control.

WZ4002 and Crizotinib were purchased from Shanghai Haoyuan Chemexpress Inc. (Shanghai, China).

4.2.2 Antiproliferation Assays. Cells were grown in 96-well culture plates (2500-3000/well). The compounds of various concentrations were added into the plates. Cell proliferation was determined after treatment with compounds for 72 h. Cell viability was measured using the CellTiter-Glo assay (Promega, USA) according to the manufacturer’s

instructions, and luminescence was measured in a multilabel reader (Envision, PerkinElmer, USA). Data were normalized to control groups (DMSO) and represented by the mean of three independent measurements with standard error of < 20%. GI_{50} values were calculated using Prism 5.0 (GraphPad Software, San Diego, CA).

4.2.3 TEL-Isogenic Cell Generation. Retroviral constructs for Ba/F3-TEL-EGFR and Ba/F3-EGFR mutants were made based on the pMSCVpuro (Clontech) backbone as described in the literature.[34] For TEL-fusion vectors, the first 1 kb of human TEL gene with an artificial myristoylation sequence (MGCGCSSHPEDD) was cloned into pMSCVpuro retroviral vector, followed by a 3xFLAG tag sequence and a stop codon. Then the kinase domain coding sequences of EGFR variants were inserted in-frame between TEL and 3xFLAG sequences. For full-length expression vectors, the coding sequences of EGFR variants were directly cloned in pMSCVpuro vector with a 3xFLAG tag at the C-terminal end. All mutations were performed using the QuikChange Site-Directed Mutagenesis Kit (Stratagene) following the manufacturer's instructions. Retrovirus was made using the same method described above and was used to infect Ba/F3 cells. After puromycin selection, the IL-3 concentration in the medium was gradually withdrawn until cells were able to grow in the absence of IL-3.

4.2.4 Signaling Pathway Study. H1975, PC9 and A549 cells were treated with serially diluted compound **18**, 1 μM WZ4002 for 4 h. Cells were then washed in PBS and lysed in cell lysis buffer. Phospho-EGF receptor, phospho-EGF receptor (Tyr1068), Stat3, phospho-Stat3 (Tyr705), AKT, phospho-AKT (Ser473), phospho-AKT (Thr308), p44/42 MAPK (Erk1/2), phospho-p44/42 MAPK (Erk1/2) (Thr202/ Tyr204), p70 S6 kinase，

phospho-p70 S6 kinase (Thr389), eIF4E, phospho-eIF4E (Ser209), 4E-BP1, phospho-4E-BP1 (Thr37/46) antibody (Cell signaling Technology) were used for immunoblotting.

H3122 cells were treated with serially diluted compound **18**, 1 μM crizotinib for 2 h. Cells were then washed in PBS and lysed in cell lysis buffer. Phospho-ALK receptor, phospho-ALK receptor (Tyr1604)，Stat3, phospho-Stat3 (Tyr705), AKT, phospho-AKT (Ser473), p44/42 MAPK (Erk1/2), phospho-p44/42 MAPK (Erk1/2) (Thr202/ Tyr204) antibody (Cell signaling Technology) were used for immunoblotting.

4.2.5 Apoptosis Effect Examination. H1975, PC9, A549 cells were treated with serially diluted compound **18** and 1 μM WZ4002 for 24 h. H3122 cells were treated with serially diluted compound **18** and 1μM crizotinib for 24 h. Cells were then washed in PBS and lysed in cell lysis buffer. PARP, caspase-3, GAPDH antibody (Cell signaling Technology) were used for immunoblotting.

4.2.6 Cell Cycle Analysis. H1975, PC9, A549 cells were treated with DMSO, compound **18** (0.03 μM, 0.1 μM, 0.3 μM, 1 μM), WZ4002 (1 μM) for 24 h before cells were harvested by trypsin and washed with cold PBS. H3122 cells were treated with DMSO, compound **18** (0.01 μM, 0.03 μM, 0.1 μM, 0.3 μM), crizotinib (1 μM) for 24 h before cells were harvested by trypsin and washed with cold PBS. The cells were fixed in 70% cold ethanol and incubated at −20 °C overnight then stained with PI/RNase staining buffer (BD Pharmingen). Flow cytometry was performed using a FACS Calibur (BD), and results were analyzed by ModFit software.

4.2.7 *In Vivo* Pharmacokinetics Study. Compound **18** was dissolved in 55% saline containing 5% DMSO and 40% PEG400 by vortex. The final concentration of the stock solution was 1 mg/mL for administration. Six 8-week old male Sprague-Dawely rats

were fasted overnight before starting drug treatment via intravenous and oral administration. Animal blood collection time points were as follows: for groups 1, 3, 5 (intravenous): 1 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h before and after administration was selected; for groups 2, 4, 6 (oral): 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h before and after dosing. Each time about 0.3 mL blood was collected through the jugular vein adding heparin for anticoagulation and kept on ice. Then plasma was separated by centrifugation at 8000 rpm for 6 minutes at 2-8 °C. The obtained plasma was stored at -80 °C before analysis. After finishing the test, all surviving animals will be transferred to the repository or euthanasia (CO_2 asphyxiation).

4.2.8 H1975 and H3122 Xenograft Models. Four-week old female nu/nu mice were purchased from the Shanghai Experimental Center, Chinese Science Academy (Shanghai, China). All animals were housed in a specific pathogen-free facility and used according to the animal care regulations of Hefei Institutes of Physical Science, Chinese Academy of Sciences. Prior to implantation, cells were harvested during exponential growth. Five million H1975 or H3122 cells in PBS were formulated as a 1:1 mixture with Matrigel (BD Biosciences) and injected into the subcutaneous space on the right flank of nu/nu mice. Daily oral administration was initiated when H1975 or H3122 tumors had reached a size of 200 to 400 mm^3. Animals were then randomized into treatment groups of 5 or 6-7 mice each for efficacy study. Compound **18** was delivered daily in a HKI solution by orally gavage. A range of doses of compound **18** or its vehicle was administered, as indicated in figure legends. Body weights and tumor growth was measured daily after compound **18** treatment. Tumor volumes were calculated as follows: tumor volume

(mm^3) = [(W^2× L)/2] in which width (W) is defined as the smaller of the two measurements and length (L) is defined as the larger of the two measurements.

4.2.9 HE Staining. HE staining was carried out according to previous publication.[25] First, the sections were hydrated and then the slide was dipped into a Coplin jar containing Mayer's hematoxylin and agitated for 30 s. After rinsing the slide in H_2O for 1 min, it was stained with 1% eosin Y solution for 10−30 s with agitation. Subsequently, the sections were dehydrated with two changes of 95% alcohol and two changes of 100% alcohol for 30 s each, and then the alcohol was extracted with two changes of xylene. Finally, one or two drops of mounting medium was added and covered with a coverslip.

4.2.10 *K*$_i$-67 Staining. For IHC demonstration of *K*$_i$-67, tissue sections were quenched for endogenous peroxides and placed in an antigen retrieval solution (0.01 M citrate buffer, PH 6.0) for 15 min in a microwave oven at 100 °C at 600 W. After incubation in the casein block, mouse mAb anti-*K*$_i$-67 (ZSGB-BIO, China) was applied to the sections at dilutions of 1:50. Incubations with primary antibodies lasted overnight at 4 °C. The secondary detection system was used to visualize antibody binding. Staining was developed with DAB, and the slides were counterstained with hematoxylin, dehydrated, and mounted.

4.2.11 TUNEL Staining. TUNEL staining was performed using the POD in situ cell death detection kit (Roche, USA). Briefly, sections were deparaffinized in xylene, rehydrated in decreasing concentration of ethanol, and then treated by nuclease free proteinase K for 15 min at room temperature before endogenous peroxidase was blocked in 3% H_2O_2 in methanol. Terminal deoxynucleotidyl transferase (TdT) in reaction buffer was applied to sections for 1 h at 37 °C. Following washes, the slides were covered by

converter-POD solution for 30 min at 37 °C. Apoptotic cells were detected after incubation in 3,3'-diaminobenzidine (DAB) chromogen (Beyotime Biotechnology, China) for approximately 8 min, and the slides were counterstained with hematoxylin.

4.3 Molecular Modeling. All calculations were performed using the Schrödinger Suite. ALK and EGFR(T790M) crystal structures (PDB: 2XB7 and 3IKA, respectively) were used for docking studies. The crystal structures were prepared using the Protein Preparation Wizard (Protein Preparation Wizard, Schrödinger, LLC, New York, NY); protonation states were assigned using PROPKA[35], the optimized model structures were minimized until the average RMSD of the non-hydrogen atoms reached 0.18 Å. The ligand structures were built in Maestro and prepared for docking using LigPrep 3.4 (LigPrep, Schrödinger, LLC, New York, NY). The extended sampling IFD protocol (Induced Fit Docking protocol, Schrödinger, LLC, New York, NY) was used for the docking process of compound **18**/TAE-684 to ALK and EGFR(T790M) with default settings. The CovDock module[36] was used to perform covalent docking on the reactive residue Cys797 of EGFR(T790M) to the ligand compound **18** with default settings. The top 10 docking poses were visually inspected and the best results were further optimized by MacroModel with backbone constraint refinement.

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Highlights

- A highly potent EGFR/ALK dual kinase inhibitor compound **18**.
- Potently inhibited EGFR L858R, del 19 and T790M mutants.
- Potently inhibited EML4-ALK, R1275Q, L1196M, F1174L and C1156Y mutants.
- Suppressed the tumor growth in H1975 and H3122 cell inoculated xenograft models