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Discovery of Novel 2,4-Diarylaminopyrimidine Analogues (DAAPalogues) Showing Potent Inhibitory Activities against Both Wild-type and Mutant ALK Kinases

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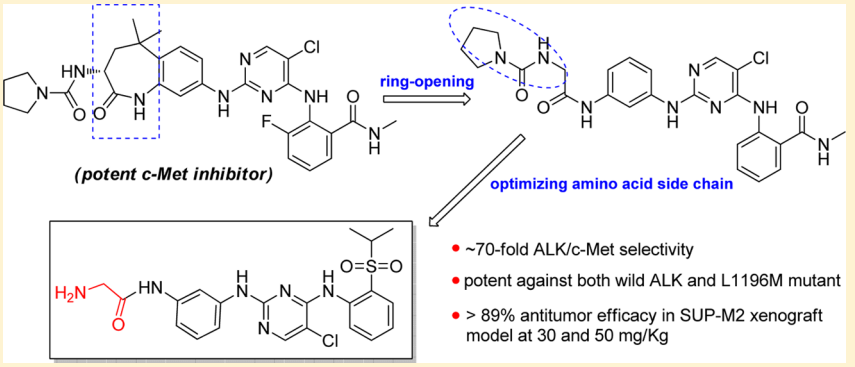
Supporting Information



ABSTRACT: We have developed a series of new 2,4-diarylaminopyrimidine analogues (DAAPalogues) bearing a flexible amino acid side chain, different from the majority of the literature reported ALK inhibitors that often possess a structurally constrained arylpiperazine fragment or its equivalents in the solvent-interaction region. Extensive structural elaboration led to compound **15** possessing IC_{50} values of 2.7 and 15.3 nM, respectively, in the ALK wild-type and gate-keeper mutant L1196M enzymatic assays. This compound not only showed high proliferative inhibition against ALK-addicted cells across different oncogenic forms but also effectively suppressed several ALK secondary mutant cells, including the gate-keeper L1196M and F1174L. Significant antitumor efficacy was achieved in the ALK-driven SUP-M2 xenograft model.

INTRODUCTION

Anaplastic lymphoma kinase (ALK) is an orphan receptor tyrosine kinase (RTK), structurally belonging to the insulin receptor family.[1] Implication in brain development and regulation of specific neurons of the central nervous system are the general proposed functions of ALK.[2−4] In 1994, the oncogenic nucleophosmin (NPM)−ALK fusion gene was discovered as an ALK translocation product occurring in 80% of anaplastic large-cell non-Hodgkin's lymphoma (ALCL) cases.[5] Subsequently, constitutive activations of ALK due to chromosomal translocations, amplifications, or point mutations have been found in other cancers.[4] The echinoderm microtubule-associated protein-like 4 (EML4)−ALK fusion gene has characteristics of "oncogene-addicted tumors" and is implicated in the largest non-small-cell lung cancer (NSCLC) patient populations.[6−8] The proof-of-concept of the "tumor-addicted oncogene" EML4−ALK as a novel therapeutic target[9−16] has been validated by Pfizer's first-generation ALK inhibitor crizotinib (PF2341066, Xalkori),[17−20] which was approved by FDA in 2011 as the standard treatment for ALK-positive NSCLCs. Unfortunately, despite the appreciable objective response rates and impressive progression-free survival times on crizotinib-treatment,[21] resistance to this drug was reported within a year of starting therapy, which led to the majority of patients experiencing relapse and tumor regrowth. Although activation of alternative signaling pathways that bypass ALK may be one of the resistance mechanisms, analyses of the resistant patient samples indicated that dozens of ALK gene secondary mutations[22−25] have developed following crizotinib-treatment, including the gate-keeper mutation L1196M, F1174L, and others.[26,27] Since the gatekeeper mutation L1196M is believed to cause steric hindrance of crizotinib binding and is similar to the gatekeeper mutations in the EGFR-T790M[28] and ABL-T3151[29] kinase domains, a new

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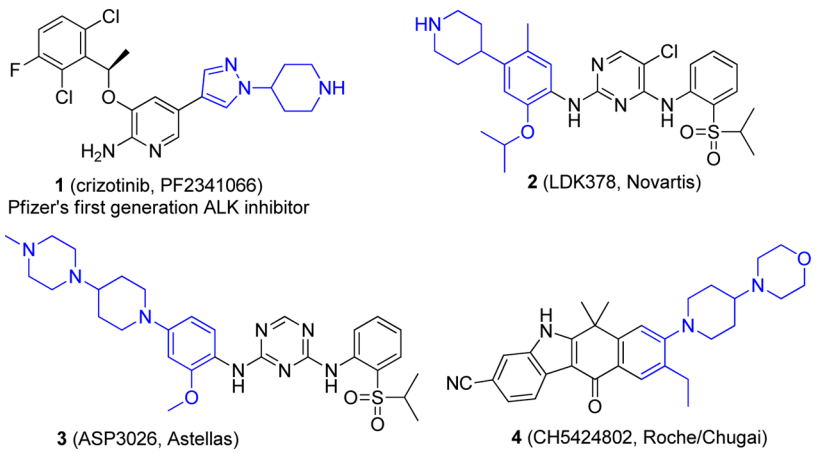


Figure 1. Marketed ALK inhibitor **1** and new clinical inhibitors **2**–**4** (blue color denotes solvent region).

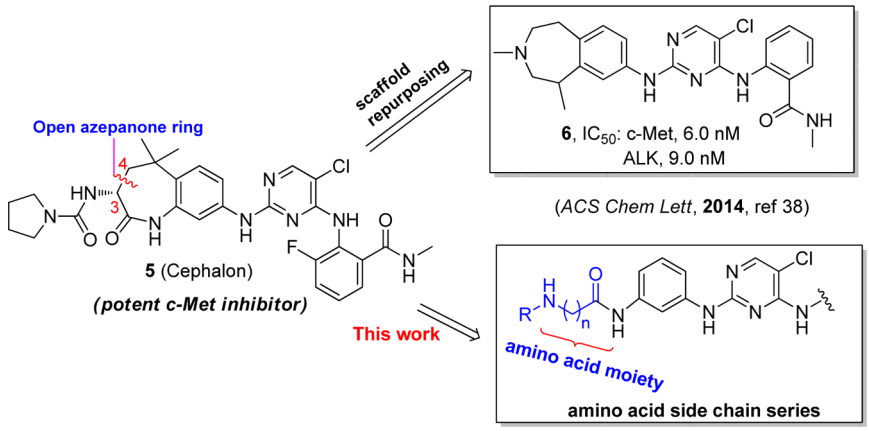
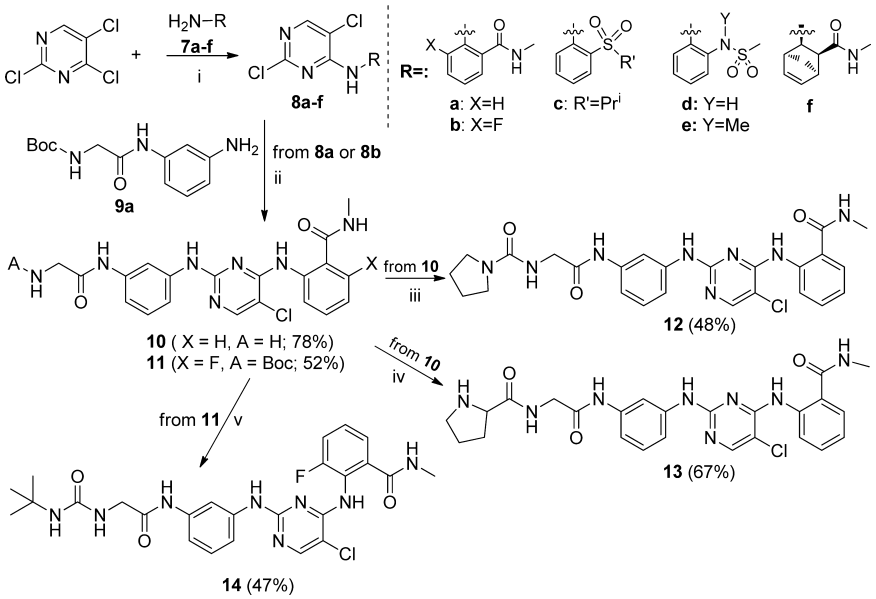


Figure 2. Proposed new DAAPalogues containing an amino acid side chain.

Scheme 1. Synthesis of Compounds 10–14[a]



[a]Reagents and conditions: (i) for **8a**, **8b**, **8d**, **8e**, and **8f**, DIPEA, IPA; for **8c**, NaH, DMF; (ii) for **10**, CSA, IPA, MW, 80 °C; for **11**, $Pd(OAc)_2$, X-Phos, Cs_2CO_3, 1,4-dioxane, MW, 100 °C; (iii) 1-pyrrolidinecarbonyl chloride, Et_3N, CH_2Cl_2; (iv) (a) Boc-L-Proline, TBTU, DIPEA, DMF; (b) TFA, CH_2Cl_2; (v) (a) TFA, CH_2Cl_2; (b) *tert*-butylisocyanate, CH_2Cl_2.

generation[30] of ALK inhibitors able to overcome the resistant mutant L1196M is greatly needed. Currently, a number of second-generation ALK inhibitors have progressed to clinical trials including 2,4-diarylaminopyrimidine **2**,[31,32] triazene **3**,[33]

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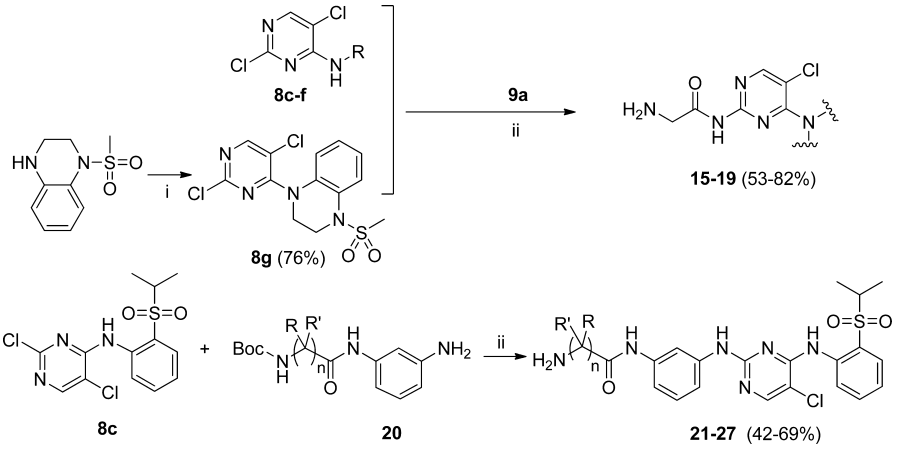
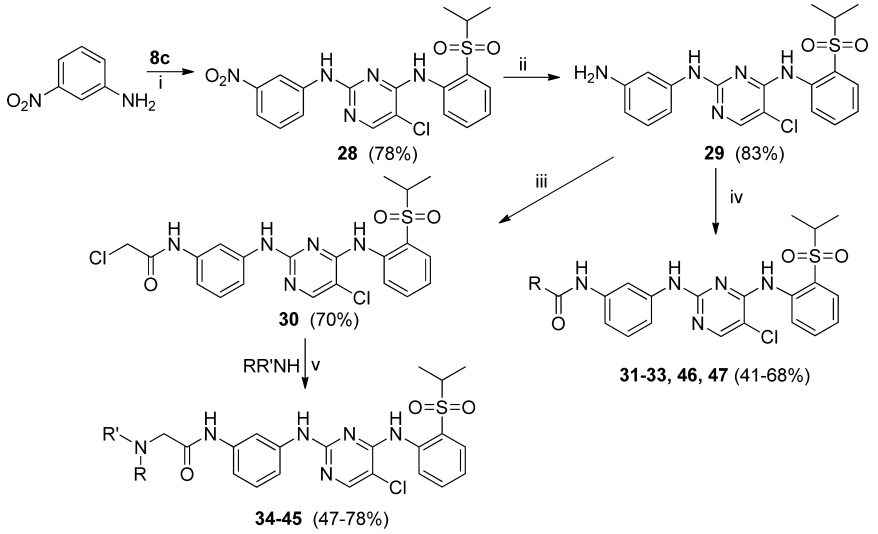
Scheme 2. Synthesis of Compounds 15−27[a]



[a]Reagents and conditions: (i) DIPEA, IPA; (ii) CSA, IPA, MW,80 °C.

Scheme 3. Synthesis of Compounds 28−48[a]



[a]Reagents and conditions: (i) CPA, IPA, MW, 80 °C; (ii) 10% Pd/C, H_2, MeOH; (iii) chloroacetyl chloride, Et_3N, CH_2Cl_2; (iv) TBTU, DIPEA, DMF; (v) K_2CO_3, Et_3N, MeCN.

and tetracyclic **4**[34−36] (Figure 1). All these new ALK inhibitors showed high potency against both wild-type and mutant ALK and exerted significant *in vivo* antitumor efficacy in crizotinib-resistant ALK-positive NSCLC patients.

By using a scaffold repurposing strategy,[37] we recently developed[38] a series of 2,4-diarylaminopyrimidine analogues (DAAPalogues) from Cephalon's tyrosine kinase c-Met inhibitor **5**[39] (Figure 2). These compounds structurally featured a 1-methyl/aryl-N^3-benzazepine framework and showed high potency against both c-Met and ALK kinases. As a continuation of this work,[40] we conducted an alternative structural modification approach by opening the azepanone ring of **5** at the C3−C4 position, thus leading to a series of new DAAPalogues bearing a flexible amino acid side chain. These new compounds differed from most of the reported ALK inhibitors that often contained a structurally constrained arylpiperazine fragment or its equivalent in the solvent-interaction region (as that in Figure 1, blue color). Structural elaboration indicated that subtle change within the amino acid component led to significant difference in the ALK potency and selectivity. Compound **15** was found to be the most potent and selective ALK inhibitor with efficacy against both wild-type ALK and L1196M mutant. Herein, we present the synthesis and pharmacological results.

CHEMISTRY

As outlined in Scheme 1, 4-amine-2,5-dichloropyrimidines **8a−f**[32,38,39] were used as the key intermediates, which were prepared from 2,4,5-trichloropyrimidine and commercially available amines **7a−f** by following literature procedures. Substitution of **8a** with *tert*-butyl (2-((3-aminophenyl)-amino)-2-oxoethyl) carbamate (**9a**) in the presence of CSA provided compound **10** in 78% yield.[39] Meanwhile, compound **11** was prepared by treating chloride **8b** with **9a** in the presence of $Pd(OAc)_2$, X-Phos, and Cs_2CO_3 in 52% yield.[38] Urea derivative **12** was obtained by treating amine **10** with 1-pyrrolidinecarbonyl chloride in 48% yield. Condensation of **10** with Boc-L-proline followed by deprotection led to amino amide **13** in 67% yield.[38] Deprotection of compound **11** followed by reacting with 2-isocyanato-2-methylpropane provided compound **14** in 47% yield.

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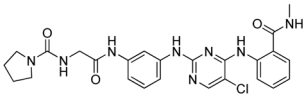
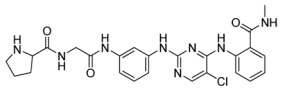
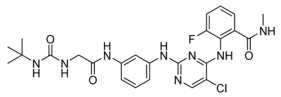
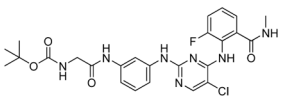
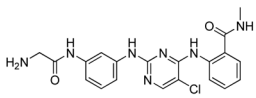
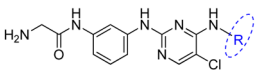
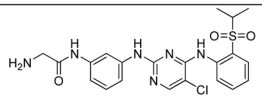
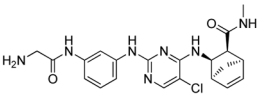
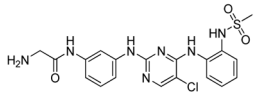
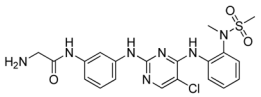
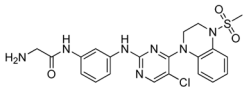
Table 1. Inhibitory Effects of New Compounds against c-Met and ALK Kinases[a]

Compound		c-Met (IC_{50}, nM)	ALK (IC_{50}, nM)
12		52.5 ± 0.5	280 ± 34.8
13		16.4 ± 0.4	80.3 ± 9.2
14		7.5 ± 0.4	>1000
11		7.1 ± 1.0	>1000
10		6.5 ± 1.0	32.8 ± 2.1
Compound		c-Met (IC_{50}, nM)	ALK (IC_{50}, nM)
15		153.0 ± 3.5	2.7 ± 0.8
16		33.6 ± 5.6	111 ± 8.0
17		119.8 ± 32.2	94.5 ± 0.7
18		51.4 ± 4.1	7.7 ± 4.8
19		>1000	>1000
5[39]	-	24	-
2	-	-	3.4 ± 1.1
Crizotinib	-	2.0 ± 0.4	13.7 ± 1.6

[a]IC_{50}'s were calculated by Logit method from the results of at least two independent tests with eight concentrations each and expressed as means ± SD.

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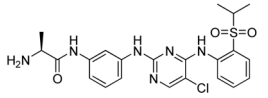
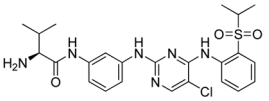
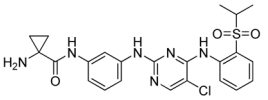
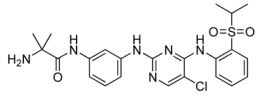
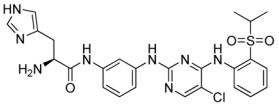
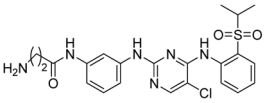
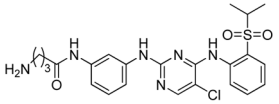
Table 2. Inhibitory Effects of New Compounds against c-Met and ALK Kinases[a]

Compound		c-Met (IC_{50}, nM)	ALK (IC_{50}, nM)
21		6.9 ± 1.8	1.8 ± 0.3
22		137 ± 7.2	14.9 ± 6.0
23		>1000	50.8 ± 7.8
24		26.8 ± 5.0	5.5 ± 2.1
25		143 ± 19.2	28.0 ± 2.9
26		43.1 ± 12.5	3.1 ± 0.1
27		292 ± 57.5	19.1 ±2.6

[a]IC_{50}'s were calculated by Logit method from the results of at least two independent tests with eight concentrations each and expressed as means ± SD.

Meanwhile, dichloropyrimidine **8g** was obtained in 76% yield by treating commercially available 1-(methylsulfonyl)-1,2,3,4-tetrahydro-quinoxaline with 2,4,5-trichloropyrimidine. Subsequent condensation of **9a** with pyrimidines **8c−g** in the presence of CSA yielded amino amides **15−19** in 53−82% yields. Meanwhile, treating **8c** with various amino acid derivatives **20** under a similar condensation conditions provided DAAPalogues **21−27** in 42−69% yields (Scheme 2).

In addition, *N*-(3-aminophenyl)-5-chloro-*N*′-[2-(isopropylsulfonyl)phenyl]pyrimidine-2,4-diamine (**29**) was prepared from commercially available 3-nitroaniline by following a literature procedure.[41] Subsequent condensation[38] of **29** with various amino acids provided compounds **31−33**, **46**, and **47** in 41−68% yields.

Amidation of **29** with 2-chloroacetyl chloride yielded compound **30**. Compounds **34−43** bearing a cyclic amine functionality were prepared in 47−78% yields by treating intermediate **30** with corresponding cyclic amine substrates in the presence of K_2CO_3 and Et_3N.[42] Similarly, compounds **44** and **45** were obtained in 48−56% yields by condensation of **30** with corresponding *N*-Boc amines followed by deprotection (Scheme 3).

RESULTS AND DISCUSSION

Enzymatic Assay. The 2,4-diarylaminopyrimidine (DAAP) scaffold has long been recognized as a classical kinase inhibitor motif;[43,44] our recent study also confirmed that such compounds displayed high potency against several kinase targets, especially c-Met and ALK.[38] Therefore, all the new synthetic compounds were initially assayed for their inhibitory effects against both c-Met and ALK, and compounds with high potency and selectivity for ALK over c-Met will be selected for further profiling. As shown in Table 1, opening the azepanone ring of Cephalon's c-Met inhibitor **5** by deletion of the C4−C5 ethylene unit generated urea **12**, which retained good inhibitory effect against c-Met, but the potency at ALK was moderate (280 nM). Isomerization of **12** to dipeptide **13** led to slightly enhanced potencies at both c-Met and ALK kinases, with IC_{50} values of 16 and 80 nM, respectively. *N*-*t*-Butyl urea **14** showed significantly reduced potency at ALK, whereas higher potency against c-Met was retained. Similar high c-Met potency and selectivity were observed as well for compound **11** bearing a *t*-butoxycarbonyl moiety as the terminal capping group. Quite interestingly, simple 2-amino-*N*-ethylacetamide **10** bearing a naked primary amino group showed high potency at both c-

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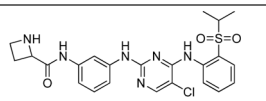
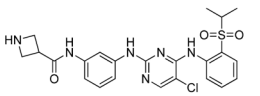
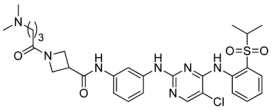
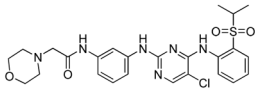
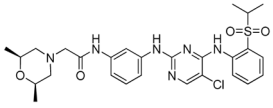
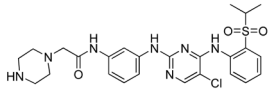
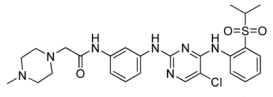
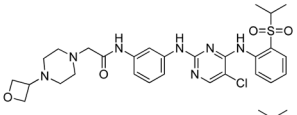
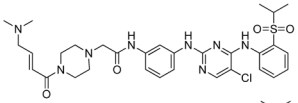
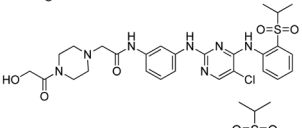
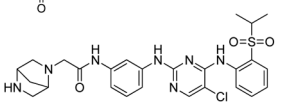
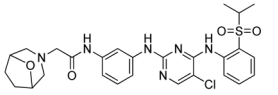
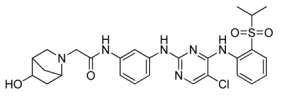
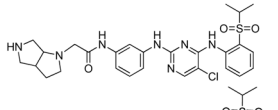
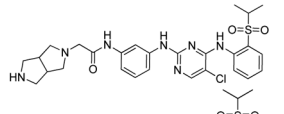
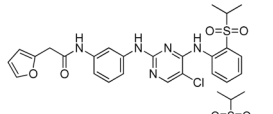
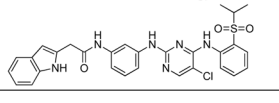
Table 3. Inhibitory Effects of New Compounds against c-Met and ALK Kinases[a]

Compound		c-Met (IC_{50}, nM)	ALK (IC_{50}, nM)
31		35.2 ± 10.0	44.2 ± 6.2
32		>1000	20.7 ± 10.8
33		>1000	0.8 ± 0.2
34		389 ± 27.8	17.8 ± 0.8
35		148 ± 42.5	25.5 ± 4.8
36		31.7 ± 7.9	2.5 ± 1.5
37		549 ± 3.8	1.7 ± 0.2
38		130 ± 37.7	4.9 ± 0.2
39		102 ± 22.5	0.7 ± 0.4
40		338 ± 10.9	9.0 ± 2.7
41		164 ± 77.1	2.3 ± 0.4
42		>1000	51.3 ± 5.4
43		112 ± 18.8	3.0 ± 0.6
44		448 ± 79.5	2.3 ± 0.2
45		244 ± 69.0	5.5 ± 3.2
46		634 ± 63.7	31.9 ± 4.0
47		>1000	95.3 ± 34.3

[a]IC_{50}'s were calculated by Logit method from the results of at least two independent tests with eight concentrations each and expressed as means ± SD.

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Met and ALK, with IC_{50} values of 6.5 and 32.8 nM, respectively.

With the moderate ALK inhibitor **10** as our new lead, we optimized the pyrimidin-2-yl aminobenzamide moiety. It was found that replacing the *N*-methylcarbonyl substituent in the aminobenzamide component of **10** with the isopropylsulfonyl group as that in clinical ALK inhibitors **2** and **3** significantly increased ALK potency and repositioned the selectivity from c-Met to ALK. Compound **15** showed an IC_{50} value of 2.7 nM for ALK and was 56-fold more potent against ALK than against c-Met kinase. Incorporation of the 3-aminobicyclo[2.2.1]hept-5-ene-2-carboxylic amide fragment, a more lipophilic group used by Cephalon[45] for generation of new ALK inhibitors led to compound **16**, which retained good potency and selectivity at c-Met, but the potency at ALK was moderate. Compared with benzamide **10**, methanesufonylamine **17** was 3-fold less potent against the ALK kinase, but much higher potency was observed when an *N*-methyl substituent was introduced. *N*-Methylmethanesufonylamine **18** showed an IC_{50} value of 7.7 nM and was 7-fold more potent against ALK than against c-Met. Tetrahydroquinoxaline **19**, formed by connecting the two *N*-atoms of the benzene-1,2-diamino component with an ethylene unit lost potency at both ALK and c-Met. The results indicated that the free NH connecting to central pyrimidine core, rather than the NH in the methanesufonylamino moiety, was critical to the interactions with both kinases.

Since compound **15** displayed high potency and selectivity for ALK kinase, our further optimization was focused on exploring the steric and electronic capacities of the amino acetamide side chain based on the structure **15**. As shown in Table 2, we first tested various amino acids bearing a primary amino group. Insertion of a methyl group yielded 2-aminopropanamide **21**, which retained high potency against ALK (1.8 nM), but the selectivity over c-Met was only 4-fold. More bulky groups such as those in **22**–**25** led to slightly increased selectivity, whereas the potency against ALK was sacrificed. Compound **26** with a one-carbon longer alkyl chain retained high potency against ALK with an IC_{50} value of 3.1 nM, which was 14-fold higher than that against c-Met. However, further enlongating the alkyl chain to 4-amino butaneamide **27** caused reduction in ALK potency.

Meanwhile, to elucidate the necessity of the primary amino group for the high ALK potency of compound **15**, a large number of 2-amino acetamido fragments bearing diversified substitutions on either the amino group or the alkyl chain or both were investigated. As shown in Table 3, substitution on the primary amino group by forming an azetidine ring led to compound **31**, which showed an IC_{50} value of 44 nM against ALK. Notably, the 3-azetidine regiomer **32** was 2-fold more potent against ALK than **31** and nearly inactive against c-Met, thus providing a 50-fold ALK/c-Met selectivity. Extremely high potency was observed for the *N*-4-(dimethylamino)butanoyl substituted azetidine analogue **33**, which showed an IC_{50} value of 0.8 nM at ALK and was over 1000-fold selective versus c-Met. Conversion of the primary amino group in **15** to morpholines **34** and **35** also provided relatively good potency and selectivity for ALK, but they were much less potent than either **15** or **33**. Interestingly, replacement of the primary amino group in **15** by a substituted piperazinyl moiety generated highly potent and selective ALK inhibitors **36**–**40**. All these compounds displayed IC_{50} values of less than 10 nM at ALK, but the selectivity over c-Met differed. The alkyl substituted piperazine analogues **37** and **38** were more selective for ALK over c-Met than the nonsubstituted piperazine **36**. High potency was observed on *N*-acyl substituted piperazine analogue **39**, which showed an IC_{50} value of 0.7 nM and an ALK/c-Met selectivity of 145. This compound is superior to compound **15** in both potency and selectivity for ALK. The *N*-containing bicyclic analogues **41**–**43** generally had moderate selectivity for ALK over c-Met, but compounds **41** and **43** still retained high ALK potency, indicating that a remote H-bonding donor is beneficial for interaction with ALK kinase. High potency and selectivity against ALK were also obtained from compounds **44** and **45** bearing an octahydropyrrolo[3,4-*b*]pyrrole terminal group. Replacement of the primary amino group in **15** with a furan (**46**) or an indole (**47**) moiety led to reduction in both potency and selectivity at the ALK kinase.

Cellular Inhibition Study. On the basis of the enzymatic assays, potent and selective ALK inhibitors were selected for cellular assay. The antiproliferative effects[38] of the compounds in ALK-addicted lymphoma cell line SUP-M2 harboring NPM–ALK are listed in Table 4. Meanwhile, tumor cell line

Table 4. Inhibitory Effects of Potent Compounds on Cell Proliferation

		IC_{50}	
compd	ALK enzyme IC_{50} (nM)	ALK SUP-M2 (nM)	A549 (μM)
15	2.7	15.3 ± 3.7	1.1 ± 0.4
21	1.8	<0.1	0.2 ± 0.01
26	3.1	79.0 ± 18.6	2.3 ± 0.08
33	0.8	365 ± 3.9	3.9 ± 0.2
37	1.7	0.1 ± 0.04	0.2 ± 0.02
39	0.7	7.7 ± 2.2	0.2 ± 0.02
43	3.0	0.2 ± 0.09	0.1 ± 0.02
44	2.3	5.5 ± 1.4	0.8 ± 0.1
2[a]	3.4	167 ± 35.9	>1
crizotinib	13.7	174 ± 20.8	>1

[a]Compound **2** (LDK378) was reported[32] to be more sensitive against BaF3/NPM–ALK cells with an IC_{50} value of 26.0 nM.

A549 whose growth was not dependent on ALK was used to test the potential off-target effects of the selected ALK inhibitors. In parallel with the enzymatic results, all the selected compounds displayed high antiproliferative effects in the SUP-M2 cell lines. Compound **15** bearing a primary amino group showed an IC_{50} value of 15.3 nM and 70-fold selectivity over A549 cells. Compound **21** bearing a methyl group in the side chain, though equally potent to **15** in the enzymatic assay, was much more potent (>150-fold) in the cellular assay with an IC_{50} value of less than 0.1 nM. Quite disappointingly, this compound also showed high potency in the A549 cells indicating that a general cytotoxicity might exist. Although the exact reason was not clear, the higher cellular potency and cytotoxicity of **21** might be partially related to the increased lipophilicity by the methyl group. Compound **26** with a longer side chain and compound **33** bearing an *N*-acyl substituted azetidine moiety displayed moderate potency in the SUP-M2 cells, despite their high enzymatic potencies. Although equally potent to compound **15** in the ALK enzymatic assay, the piperazinyl DAAPalogue **37** displayed 150-fold higher cellular potency with an IC_{50} value of 0.1 nM. However, this compound was also highly potent in the A549 cell lines. In comparison to their high enzymatic potency, *N*-acyl substituted piperazine analogue **39** and the octahydropyrrolo[3,4-*b*]pyrrole **44** were less potent in the cell and showed higher general cytotoxicity in

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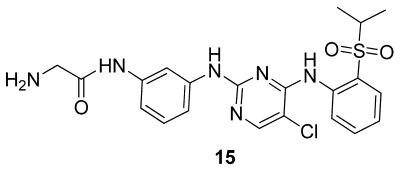

15

ALK(IC_{50}): enzyme, 2.7 nM; cell, 15.3 nM

MW: 474.96; tPSA: 138; CLogP: 3.4

PK (2.5 mg/kg, i.g.):
AUC: 454 ± 10.1 ng.h/mL; $t_{1/2}$: 0.5 h

Aqueous solubility: < 0.1 mg/mL (free base)
~ 4.0 mg/mL (HCl salt)

Figure 3. Preliminary PK results.

the A549 cell lines. The bicyclic compound **43** showed 15-fold higher cellular potency than its enzymatic potency, whereas significant cytotoxicity was observed as well in A549 cells. All these new DAAPalogues were more potent than the marketed drug crizotinib (**1**) and clinical compound **2** in both ALK kinase and SUP-M2 cells.

Preliminary Pharmacokinetic Study. From the results above, compound **15** stood out having high potency against both ALK enzyme and cells and showed less potential for cytotoxicity. This compound was further evaluated in our preliminary pharmacokinetic study[37,46] at a dose of 2.5 mg/kg (i.g., intragastric) in rats. As shown in Figure 3, compound **15** showed a moderate plasma exposure ($AUC_{0-\infty}$ = 454 ng·h/mL) and a short half-life. It has a moderate lipophilicity but with poor aqueous solubility (less than 0.1 mg/mL). However, the solubility was much improved by forming the corresponding hydrochloride salt (~4 mg/mL).

Overcoming ALK Resistant Gatekeeper Mutant Study. To determine the potential of compound **15** in overcoming crizotinib's resistance, it was evaluated against the ALK gatekeeper mutant L1196M.[30] An IC_{50} value of 15.3 nM was observed indicating that compound **15** was potent against both wild-type and mutant ALK (Table 5). Meanwhile, compound **15** was further evaluated for the inhibitory activities on a panel of our in-house[47] tyrosine kinases. Compound **15** was found also to show high potency against LTK kinase, a family member of ALK; otherwise much weaker inhibitory effects were observed on the remaining 17 tested kinases (IC_{50} > 1 μM or 0.1 μM) (Table 5). Therefore, compound **15** was a potent ALK family inhibitor with against the resistant gatekeeper mutant.

Table 5. Kinase Selectivity Profile of Compound 15

	% or IC_{50}	
kinase[a]	**15**	**1** (crizotinib)
ALK	2.7 ± 0.8 nM	13.7 ± 1.6 nM
ALK-L1196M	15.3 ± 0.1 nM	122 ± 20.4 nM
LTK	93% @ 0.1 μM	
RON	32.2% @ 0.1 μM	
AXL	>1 μM	
Tyto3	>1 μM	
Mer	>1 μM	
PDGFR-α	>1 μM	
PDGFR-β	>1 μM	
EGFR	>1 μM	
$EGFR^{T790M-L858R}$	35% @ 0.1 μM	
FGFR1	50% @ 0.1 μM	
c-Src	>1 μM	
RET	43% @ 0.1 μM	
KDR	>1 μM	
Flt-1	>1 μM	
c-Kit	>1 μM	
Abl	>1 μM	
ErbB4	12% @ 0.1 μM	

[a]IGF1R, insulin-like growth factor 1 receptor; PDGFR, platelet derived growth factor receptor; EGFR, epidermal growth factor receptor; KDR, kinase insert domain receptor; FGFR1, fibroblast growth factor receptor 1; Flt-1: Fms-like tyrosine kinase.

Antiproliferative Effects against Both ALK-Addicted and Crizotinib-Resistant Cells. Since activated ALK is known to trigger cancer cell proliferation, we then set out to investigate the inhibitory effects of compound **15** on cell proliferation in a panel of human cancer cell lines harboring different levels of ALK expression and activation. As shown in Table 6, compound **15** significantly inhibited cell proliferation of ALK constitutively activated SUP-M2, SU-DHL-1, NB-1, H3122, and BaF3/EML4–ALK (V3) cells that cover the frequently occurring oncogenic forms of ALK (ALK amplification, ALK chromosomal rearrangement), with IC_{50} values of 15.3, 15.0, 28.6, 96.8, and 41.9 nM, respectively. These results suggested that compound **15** inhibited ALK-dependent cell proliferation across different oncogenic forms.

Table 6. Effects of Compound 15 on Cell Proliferation in ALK-Addicted Cell Lines[a]

cell line	IC_{50} (nM)
SUP-M2 (NPM-ALK)	15.3 ± 3.7
SU-DHL-1 (NPM-ALK)	15.0 ± 0.3
NB-1 (ALK Amplification)	28.6 ± 2.3
H3122 (EML4-ALK variant1)	96.8 ± 15.7
BaF3/EML4-ALK (V3)	41.9 ± 9.7

[a]The IC_{50} values are shown as the mean ± SD (nM) from three separate experiments.

Meanwhile, antiproliferative effects of compound **15** in a number of crizotinib-resistant cell lines were tested as well (Table 7). Notably, compound **15** showed an equally high potency against both native EML4–ALK and EML4–ALK L1196M-mediated NIH/3T3 cell proliferation with IC_{50} values of 142 and 149 nM, respectively. Moreover, compound **15** significantly inhibited the proliferation of Kelly cells (IC_{50} =

Table 7. Antiproliferative Effects of Compound 15 in Crizotinib-Resistant Cells[a]

	IC_{50} (nM)	
cell line	**15**	crizotinib
NIH/3T3/EML4–ALK (V1)	142 ± 26.6	95.4 ± 0.3
NIH/3T3/EML4–ALK (V1)-L1196M	149 ± 7.8	606 ± 51.5
Kelly (ALK F1174L)	28.1 ± 6.9	>1000
LAN-5 (ALK R1275Q)	31.3 ± 2.3	>1000

[a]The IC_{50} values are shown as the mean ± SD (nM) from three separate experiments.

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28.1 nM), which harbor crizotinib-induced resistant mutant F1174L. In addition, compound **15** significantly inhibited the proliferation of cell line LAN-5 (IC_{50} = 31.3 nM) harboring another hot spot-activating mutation (R1275Q) in neuroblastoma and a mutant much less sensitive to crizotinib (Table 7).[23] These data further confirmed that compound **15** was capable of blocking the ALK resistant mutants.

***In Vivo* Antitumor Efficacy Study.** Since compound **15** possessed high ALK enzymatic and cellular potencies and was also effective in blocking the ALK resistant mutants, we proceeded to evaluate its antitumor efficacy *in vivo*. The SUP-M2 xenograft model,[47,48] which was specifically driven by constitutively active ALK, was used (Figure 4). Compound **15**

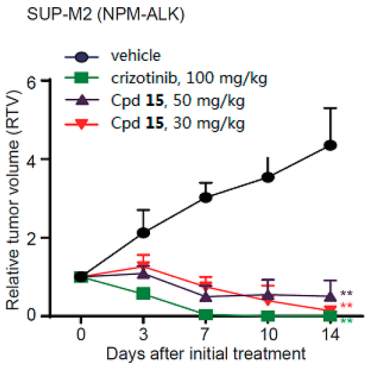

Figure 4. Antitumor activity of compound **15** in SUP-M2 xenograft model. Mice bearing SUP-M2 were orally administered compound **15** in the HCl salt form with 0.5% CMC-Na (sodium carboxymethyl cellulose) or crizotinib in water once daily for 14 days after the tumor volume reached 80 to 100 mm^3 at the indicated doses. The results were expressed as the mean ± standard error (drug-treated group, n = 5; vehicle group, n = 10) $^{**}p < 0.01$ vs vehicle group on final day, using Student's t test.

was administered orally as its hydrochloride salt with 0.5% CMC-Na (sodium carboxymethyl cellulose) at doses of 50 or 30 mg/kg once daily for 14 consecutive days. Compared with the vehicle treatment, compound **15** significantly inhibited tumor growth at both doses with tumor growth inhibitory rates of >89% ($p < 0.01$) and with no significant body weight loss.

CONCLUSION

In summary, we have developed a series of new DAAPalogues bearing a flexible amino acid side chain. These new compounds differed from most of the literature reported ALK inhibitors, which often contain a structurally constrained arylpiperazine fragment or its equivalents in the solvent-interaction region. Structural elaboration indicated that subtle change within the amino acid fragment led to significant difference in both the ALK potency and selectivity. Compound **15** was identified as a potent and selective ALK inhibitor possessing IC_{50} values of 2.7 and 15.3 nM against the ALK wild-type and L1196 mutant enzymes, respectively. It showed high antiproliferative effects toward a number of ALK-dependent cells across different oncogenic forms and was potent as well against several ALK resistant mutant cells, including the gate-keeper L1196M and L1174M. Significant antitumor efficacy was achieved for compound **15** in the ALK-driven SUP-M2 xenograft model.

EXPERIMENTAL SECTION

Chemistry. ^{1}H NMR spectral data were recorded in $CDCl_3$, $CDCl_3/CD_3OD$, or DMSO-d_6 on Varian Mercury 300 or 400 NMR spectrometer, and ^{13}C NMR was recorded in $CDCl_3$ on Varian Mercury 400 or 500 NMR spectrometer. Low-resolution mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded at anionizing voltage of 70 eV on a Finnigan/MAT95 spectrometer. Column chromatography was carried out on silica gel (200−300 mesh). All reactions were monitored using thin layer chromatography (TLC) on silica gel plates. Compounds **8a**−**f**[32,38,39] were prepared according to the literature procedures.

*General Procedure for Synthesis of Compounds **10**, **15**−**19**, **21**−**27**, and **28**.* A microwave tube (10 mL) was charged with 4-amine-2,5-dichloropyrimidines (**8a**−**g**) (0.2 mmol), an appropriate aniline (**9a** or **20**) bearing an amino acid side chain (0.28 mmol), (1*S*)-(+)-10-camphorsulfonic acid (CSA, 0.4 mmol), and isopropanol (3 mL). The mixture was heated to 80 °C under microwave irradiation (80 W) for 1 h. The solution was concentrated under vacuum, washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and purified by chromatography ($CHCl_3$/MeOH = 10:1) to afford corresponding target compound.

*2-((2-((3-(2-Aminoacetamido)phenyl)amino)-5-chloropyrimidin-4-yl)amino)-N-methylbenzamide (**10**).* White solid (78%). ^{1}H NMR (300 MHz, DMSO) δ 11.68 (s, 1H), 9.47 (d, J = 3.4 Hz, 1H), 8.85−8.68 (m, 2H), 8.25−8.17 (m, 1H), 7.85 (s, 1H), 7.79−7.68 (m, 1H), 7.49−7.33 (m, 2H), 7.31−7.06 (m, 3H), 3.28 (s, 2H), 3.25 (d, J = 3.1 Hz, 2H), 2.79 (t, J = 3.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 171.6, 168.9, 157.7, 154.9, 154.4, 140.5, 139.3, 138.8, 131.5, 128.5, 127.9, 121.8, 121.2, 120.4, 115.0, 113.0, 110.8, 105.2, 45.3, 26.3. MS (ESI) m/z 426 $[M + H]^+$. HRMS: calcd for $C_{22}H_{21}ClN_7O_2$ $[M + H]^+$, 426.1445; found 426.1446.

*2-Amino-N-(3-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)acetamide (**15**).* Off-white solid (82%). ^{1}H NMR (300 MHz, DMSO) δ 10.13 (s, 1H), 9.57 (s, 1H), 8.65 (d, J = 7.7 Hz, 1H), 8.26 (s, 1H), 7.91−7.76 (m, 4H), 7.68 (t, J = 7.5 Hz, 1H), 7.39−7.06 (m, 5H), 6.19 (s, 1H), 3.65 (s, 1H), 3.23 (s, 2H), 1.15 (d, J = 6.7 Hz, 6H).^{13}C NMR (126 MHz, CD_3OD) δ 164.2, 158.1, 151.8, 141.9, 138.8, 136.2, 135.6, 134.7, 131.3, 129.4, 128.1, 126.4, 126.1, 119.5, 117.4, 114.9, 106.2, 55.5, 40.8, 13.9. MS (ESI) m/z 475 $[M + H]^+$. The free base was converted to its HCl salt. Anal. Calcd for $C_{21}H_{23}N_6O_3S.2HCl.0.$ $5H_2O$: C, 45.29, H, 4.71, N, 15.09; found C: 45.17, H: 4.96, N: 15.08.

*(1*S*,2*S*,3*R*,4*R*)-3-((2-((3-(2-Aminoacetamido)phenyl)amino)-5-chloropyrimidin-4-yl)amino)-N-methylbicyclo[2.2.1]hept-5-ene-2-carboxamide (**16**).* Off-white solid (78%). ^{1}H NMR (400 MHz, DMSO) δ 9.32 (s, 1H), 8.31 (d, J = 4.6 Hz, 1H), 7.96 (d, J = 7.4 Hz, 2H), 7.68 (d, J = 7.7 Hz, 1H), 7.51−7.43 (m, 1H), 7.18 (d, J = 5.9 Hz, 2H), 6.31 (dd, J = 5.6, 2.8 Hz, 1H), 6.22 (dd, J = 5.4, 3.0 Hz, 1H), 4.17 (t, J = 7.9 Hz, 1H), 2.83 (s, 1H), 2.76 (s, 1H), 2.62 (d, J = 4.5 Hz, 3H), 2.53 (s, 1H), 2.12 (d, J = 8.6 Hz, 1H), 1.39 (d, J = 8.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 174.4, 172.1, 158.5, 156.8, 153.3, 141.5, 139.1, 137.1, 128.9, 114.5, 112.8, 110.5, 104.1, 52.4, 48.1, 47.3, 45.8, 44.8, 44.2, 26.1. MS (ESI) m/z 442 $[M + H]^+$. HRMS: calcd for $C_{21}H_{25}ClN_7O_2$ $[M + H]$, 442.1758; found 442.1754.

*2-Amino-N-(3-((5-chloro-4-((2-(methylsulfonamido)phenyl)-amino)pyrimidin-2-yl)amino)phenyl)acetamide (**17**).* Off-white solid (62%). ^{1}H NMR (400 MHz, $CDCl_3$/MeOD) δ 7.98 (s, 1H), 7.90 (d, J = 7.9 Hz, 1H), 7.55 (s, 1H), 7.33 (d, J = 7.7 Hz, 1H), 7.29−7.25 (m, 1H), 7.18 (d, J = 8.2 Hz, 3H), 7.11 (d, J = 8.0 Hz, 1H), 3.35 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.3, 157.5, 156.2, 154.2, 139.8, 137.7, 134.3, 129.1, 128.9, 127.6, 126.7, 125.8, 125.6, 115.6, 113.9, 110.8, 105.5, 44.6, 38.9. MS (ESI) m/z 462 $[M + H]^+$. HRMS: calcd for $C_{19}H_{20}ClN_7O_3S$ $[M + H]$, 462.1115; found 462.1123.

*2-Amino-N-(3-((5-chloro-4-((2-(N-methylmethylsulfonamido)-phenyl)amino)pyrimidin-2-yl)amino)phenyl)acetamide (**18**).* Off-white solid (72%). ^{1}H NMR (400 MHz, DMSO) δ 10.44 (s, 1H), 9.55 (s, 1H), 8.41 (s, 2H), 8.22 (s, 1H), 7.83 (s, 1H), 7.64 (d, J = 7.9 Hz, 1H), 7.44−7.38 (m, 2H), 7.23 (dd, J = 19.7, 8.5 Hz, 3H), 3.68 (s, 2H), 3.21 (s, 3H), 3.12 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ

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166.4, 158.2, 155.3, 154.7, 141.1, 138.8, 137.0, 132.9, 129.1, 127.4, 124.8, 123.4, 115.8, 113.5, 111.2, 105.1, 42.2, 39.3, 35.5. MS (ESI) m/z 476 $[M + H]^+$. HRMS: calcd for $C_{20}H_{22}ClN_7O_3S$ [M + H], 476.1272; found 476.1268.

*2-Amino-N-(3-((5-chloro-4-(4-(methylsulfonyl)-3,4-dihydroquinoxalin-1(2H)-yl)pyrimidin-2-yl)amino)phenyl)acetamide (**19**).* Off-white solid (53%). ^1H NMR (400 MHz, $CDCl_3$/MeOD) δ 8.13 (s, 1H), 8.09 (s, 1H), 7.67 (dd, J = 7.7, 1.9 Hz, 1H), 7.22 (dd, J = 12.5, 5.0 Hz, 2H), 7.11−7.02 (m, 3H), 6.78 (dd, J = 7.6, 1.8 Hz, 1H), 4.16 (t, J = 6.1 Hz, 2H), 3.99 (t, J = 6.2 Hz, 2H), 3.40 (s, 2H), 2.85 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ171.1, 158.2, 158.0, 157.9, 139.7, 138.1, 135.4, 129.2, 128.9, 125.6, 125.2, 124.1, 120.5, 115.0, 113.7, 110.3, 109.7, 48.0, 47.2, 44.6, 37.3. MS (ESI) m/z 488 $[M + H]^+$. HRMS: calcd for $C_{21}H_{22}ClN_7O_3S$ [M + H], 488.1272; found 488.1280.

*(S)-2-Amino-N-(3-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)propanamide (**21**).* Off-white solid (69%). ^1H NMR (400 MHz, $CDCl_3$) δ 9.62 (s, 1H), 9.49 (s, 1H), 8.61 (d, J = 8.2 Hz, 1H), 8.18 (s, 1H), 7.94−7.84 (m, 2H), 7.64−7.56 (m, 1H), 7.47−7.39 (m, 2H), 7.24 (ddd, J = 8.1, 7.5, 3.0 Hz, 3H), 3.63 (q, J = 7.0 Hz, 1H), 3.31−3.21 (m, 1H), 1.95 (s, 2H), 1.43 (d, J = 7.0 Hz, 3H), 1.32 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.7, 157.5, 155.2, 155.1, 139.8, 138.3, 134.5, 131.1, 129.2, 124.3, 123.4, 123.0, 115.5, 113.7, 110.8, 106.4, 55.5, 51.1, 21.5, 15.3. MS (ESI) m/z 489 $[M + H]^+$. HRMS: calcd for $C_{22}H_{26}ClN_6O_3S$ $[M + H]^+$, 489.1476; found 489.1487.

*(S)-2-Amino-N-(3-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)-3-methylbutanamide (**22**).* Off-white solid (65%). ^1H NMR (400 MHz, DMSO) δ 9.59 (s, 1H), 8.72 (d, J = 8.1 Hz, 1H), 8.30 (s, 1H), 7.84 (dd, J = 7.8, 1.5 Hz, 2H), 7.72 (t, J = 7.9 Hz, 1H), 7.37 (dd, J = 14.3, 7.2 Hz, 2H), 7.27 (d, J = 8.2 Hz, 1H), 7.18 (t, J = 8.0 Hz, 1H), 3.50−3.43 (m, 1H), 3.11 (d, J = 5.3 Hz, 1H), 1.94 (dd, J = 12.5, 6.6 Hz, 1H), 1.17 (d, J = 6.8 Hz, 6H), 0.92 (d, J = 6.8 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 174.1, 158.1, 155.6, 140.7, 139.4, 138.5, 135.4, 131.3, 129.0, 124.3, 123.9, 115.5, 113.8, 111.4, 105.4, 61.0, 55.3, 32.1, 20.1, 17.6, 15.3. MS (ESI) m/z 517 $[M + H]^+$. HRMS: calcd for $C_{24}H_{30}ClN_6O_3S$ [M + H], 517.1789; found 517.1790.

*1-Amino-N-(3-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (**23**).* Off-white solid (62%). ^1H NMR (400 MHz, DMSO) δ 9.95 (s, 1H), 9.59 (s, 1H), 9.53 (s, 1H), 8.68 (d, J = 8.1 Hz, 1H), 8.31 (s, 1H), 7.90 (s, 1H), 7.84 (dd, J = 8.0, 1.4 Hz, 1H), 7.72 (t, J = 7.8 Hz, 1H), 7.40−7.29 (m, 2H), 7.26 (d, J = 8.2 Hz, 1H), 7.18 (t, J = 8.0 Hz, 1H), 3.50−3.41 (m, 1H), 1.39 (s, 2H), 1.17 (d, J = 6.8 Hz, 6H), 1.16−1.13 (m, 2H), 0.89 (dd, J = 6.9, 3.6 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ173.6, 157.6, 155.2, 154.7, 140.2, 138.7, 137.9, 134.9, 130.9, 128.5, 124.0, 123.5, 115.0, 113.1, 110.7, 104.8, 54.8, 36.0, 26.3, 18.1, 14.8. MS (ESI) m/z 501 $[M + H]^+$. HRMS: calcd for $C_{23}H_{26}ClN_6O_3S$ [M + H], 501.1476; found 501.1479.

*2-Amino-N-(3-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)-2-methylpropanamide (**24**).* White solid (65%). ^1H NMR (400 MHz, $CDCl_3$) δ 9.88 (s, 1H), 9.63 (s, 1H), 8.62 (d, J = 8.3 Hz, 1H), 8.20 (s, 1H), 7.93−7.87 (m, 2H), 7.61 (t, J = 7.2 Hz, 1H), 7.41 (d, J = 7.6 Hz, 1H), 7.34 (s, 1H), 7.24 (t, J = 5.1 Hz, 2H), 3.26 (dt, J = 13.8, 6.8 Hz, 1H), 1.45 (s, 6H), 1.33 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ175.7, 157.6, 155.3, 155.2, 139.8, 138.7, 138.3, 134.5, 131.1, 129.2, 124.4, 123.4, 123.0, 115.4, 113.7, 110.7, 106.5, 55.6, 55.3, 29.2, 15.3. MS (ESI) m/z 503 $[M + H]^+$. HRMS: calcd for $C_{23}H_{27}ClN_6O_3S$ [M + H], 503.1632; found 503.1642.

*(S)-2-Amino-N-(3-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)-3-(1H-imidazol-4-yl)propanamide (**25**).* Off-white solid (42%). ^1H NMR (300 MHz, $CDCl_3$) δ 9.62 (s, 2H), 8.58 (d, J = 8.2 Hz, 1H), 8.15 (s, 1H), 7.92−7.77 (m, 2H), 7.56 (s, 2H), 7.38 (d, J = 7.3 Hz, 1H), 7.20 (s, 3H), 6.87 (s, 1H), 3.74 (s, 1H), 3.49 (s, 1 H) 3.25 (m, 2H), 3.07 (m, 2H), 1.31 (d, J = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.9, 157.5, 155.2, 155.0, 139.9, 138.3, 138.2, 135.2,134.5, 131.2, 129.2, 124.2, 123.3, 123.1, 115.8, 113.9, 110.0, 106.5, 55.6, 55.5, 29.7, 15.3. MS (ESI) m/z 555$[M + H]^+$. HRMS: calcd for $C_{25}H_{28}ClN_8O_3S$ [M + H],: 555.1688; found 555.1688.

*3-Amino-N-(3-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)propanamide (**26**).* Off-white solid (45%). ^1H NMR (400 MHz, $CDCl_3$) δ 10.02 (s, 1H), 9.62 (s, 1H), 8.62 (d, J = 7.8 Hz, 1H), 8.16 (s, 1H), 7.89 (dd, J = 8.0, 1.5 Hz, 1H), 7.79 (s, 1H), 7.63−7.55 (m, 2H), 7.41 (d, J = 8.0 Hz, 1H), 7.23 (ddd, J = 8.0, 3.2, 1.8 Hz, 2H), 7.16 (d, J = 8.3 Hz, 1H), 3.25 (dt, J = 13.7, 6.9 Hz, 1H), 3.14−3.04 (m, 2H), 2.51−2.44 (m, 2H), 1.89 (s, 2H), 1.32 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 157.7, 154.8, 154.6, 139.3, 138.5, 137.9, 134.0, 130.7, 128.7, 123.9, 123.0, 122.6, 115.0, 113.7, 110.9, 105.9, 55.1, 38.3, 37.4, 14.8. MS (ESI) m/z 489 $[M + H]^+$. HRMS: calcd for $C_{22}H_{25}ClN_6O_3S$ [M + H], 489.1476; found 489.1476.

*4-Amino-N-(3-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)butanamide (**27**).* White solid (48%). ^1H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.60 (d, J = 8.3 Hz, 1H), 8.11 (s, 1H), 7.87 (dd, J = 8.0, 1.4 Hz, 1H), 7.73 (s, 1H), 7.67 (s, 1H), 7.56 (t, J = 7.2 Hz, 1H), 7.43 (d, J = 7.3 Hz, 1H), 7.24−7.12 (m, 3H), 3.66 (s, 2H), 3.27−3.19 (m, 1H), 2.89 (t, J = 6.5 Hz, 2H), 2.49 (t, J = 6.9 Hz, 2H), 1.95−1.88 (m, 2H), 1.30 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 157.1, 154.7, 154.5, 139.3, 138.4, 137.8, 134.0, 130.7, 128.6, 123.8, 122.9, 122.6, 115.3, 113.7, 110.9, 105.9, 55.2, 40.0, 34.3, 25.9, 14.8. MS (ESI) m/z 503 $[M + H]^+$. HRMS: calcd for $C_{23}H_{27}ClN_6O_3S$ [M + H], 503.1632; found 503.1629.

*Synthesis of N-(2-((3-((5-Chloro-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)amino)-2-oxoethyl)-pyrrolidine-1-carboxamide (**12**).* A solution of pyrrolidine-1-carbonyl chloride (27 mg, 0.2 mmol) in CH_2Cl_2 was added to the solution of compound **10** (42 mg, 0.1 mmol) and Et_3N (40 mg, 0.4 mmol) in CH_2Cl_2 at 0 °C. The mixture was stirred for 5 h, poured into water, and then extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, concentrated in vacuo, and purified by chromatography to afford the corresponding product **12** as a white solid in 48% yield. ^1H NMR (300 MHz, $CDCl_3$+CD_3OD) δ 8.74 (d, J = 8.4 Hz, 1H), 8.08 (s, 1H), 7.68 (s, 1H), 7.54 (s, 1H), 7.44 (d, J = 7.5 Hz, 1H), 7.25 (m, 2H), 7.13 (m, 2H), 6.95 (t, J = 7.3 Hz, 1H), 5.57 (s, 1H), 3.81 (s, 2H), 3.25 (s, 4H), 2.86 (d, J = 3.9 Hz, 3H), 1.81 (s, 4H). ^{13}C NMR (126 MHz, DMSO) δ 169.1, 168.9, 157.7, 156.5, 154.9, 154.4, 140.4, 139.3, 139.0, 131.6, 128.4, 127.9, 121.8, 121.2, 120.4, 115.0, 113.2, 111.1, 105.2, 45.3, 44.1, 26.3, 25.0. MS (ESI) m/z 545 $[M + Na]^+$. HRMS: calcd for $C_{25}H_{27}ClN_8O_3$ [M + Na], 545.1792; found 545.1799.

*Synthesis of Pyrimidin-2-yl(amino)-N-(2-((3-(5-chloro-4-(2-(methylcarbamoyl)phenyl)amino)phenyl)amino)-2-oxoethyl)-pyrrolidine-2-carboxamide (**13**).* TBTU (128 mg, 0.4 mmol) and DIPEA (103 mg, 0.8 mmol) were added successively to a solution Boc-L-proline (43 mg, 0.3 mmol) and compound **10** (85 mg, 0.2 mmol) in DMF (3 mL). The reaction was stirred overnight at rt and then diluted with water (8 mL). After filtration, the filter cake was dried to provide *tert*-butyl 2-((2-((3-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)-amino)-2-oxoethyl)carbamoyl)pyrrolidine-1-carboxylate as a crude product. The crude compound was then dissolved in a mixed solvent system (CH_2Cl_2/TFA = 1:1, 5 mL), and the mixture was stirred at rt for 2 h. After removal of the solvent, the residue was taken up in water (5 mL), alkalified with $NaHCO_3$ to pH > 7, and then extracted with $CHCl_3$.The combined organic layer was washed with brine (10 mL), dried, filtered, and then evaporated. The residue was subjected to column chromatography on silica gel ($CHCl_3$/MeOH, 20/1) to give compound **13** as a white solid in 67% yield. ^1H NMR (400 MHz, DMSO) δ 11.71 (s, 1H), 9.98 (s, 1H), 9.50 (s, 1H), 8.80 (dd, J = 18.6, 6.3 Hz, 2H), 8.30 (t, J = 5.5 Hz, 1H), 8.22 (s, 1H), 7.83 (s, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.44 (dd, J = 19.7, 7.3 Hz, 2H), 7.21 (d, J = 7.6 Hz, 2H), 7.13 (t, J = 7.5 Hz, 1H), 3.92 (d, J = 5.8 Hz, 2H), 3.63 (dd, J = 8.8, 5.4 Hz, 1H), 2.89 (dd, J = 11.6, 5.0 Hz, 1H), 2.81 (d, J = 4.3 Hz, 3H), 2.04−1.92 (m, 2H), 1.73 (dd, J = 12.5, 5.7 Hz, 1H), 1.63 (dd, J = 6.7, 3.7 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.4, 168.9, 167.4, 157.7, 154.9, 154.4, 140.5, 139.3, 138.8, 131.5, 128.5, 127.8, 121.8, 121.2, 120.4, 115.1, 113.1, 111.0, 105.2, 60.0, 46.6, 42.3, 30.2, 26.2,

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25.6. MS (ESI) m/z 523 $[M + H]^+$. HRMS: calcd for $C_{25}H_{28}ClN_8O_3$ [M + H], 523.1973; found 523.1969.

*Synthesis of tert-Butyl (2-((3-((5-Chloro-4-((2-fluoro-6-(methylcarbamoyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)-amino)-2-oxoethyl)carbamate (**11**).* A microwave tube was charged with **8b** (66 mg, 0.22 mmol), **9a** (53 mg, 0.2 mmol), Pd $(OAc)_2$ (7 mg, 0.03 mmol), X-Phos (35 mg, 0.06 mmol), Cs_2CO_3 (160 mg, 0.5 mmol), and 1,4-dioxane (3 mL) under N_2 atmosphere. The mixture was heated to 100 °C under 80 W for 1.5 h and then concentrated in vacuum. The residue was taken up in water and $CHCl_3$. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated in vacuo, and the residue was purified by chromatography to afford compound **11** as an off-white solid in 52% yield. ^{1}H NMR (300 MHz, DMSO) δ 9.73 (s, 1H), 9.29 (s, 2H), 8.52 (d, J = 4.6 Hz, 1H), 8.15 (s, 1H), 7.53−7.42 (m, 3H), 7.38 (dd, J = 7.3, 4.5 Hz, 1H), 7.29 (d, J = 8.3 Hz, 1H), 7.11 (d, J = 8.1 Hz, 1H), 7.06−6.93 (m, 2H), 3.68 (d, J = 5.9 Hz, 2H), 3.33 (s, 1H), 2.71 (d, J = 4.5 Hz, 3H), 1.37 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 168.4, 167.6, 158.3, 158.2, 156.5, 156.4, 156.3, 154.9, 140.9, 139.2, 132.2, 128.7, 126.9, 126.8, 125.8, 125.7, 124.1, 118.7, 118.6, 114.5, 113.3, 110.6, 105.0, 78.4, 44.1, 28.6, 26.6. MS (ESI) m/z 566 $[M + Na]^+$. HRMS: calcd for $C_{25}H_{27}ClN_7O_4F$ [M + Na], 566.1695; found 566.1696.

*Synthesis of tert-Butyl 2-((2-((3-(2-(3-(tert-Butyl)ureido)-acetamido)phenyl)amino)-5-chloropyrimidin-4-yl)amino)-3-fluoro-N-methylbenzamide (**14**).* Amine **15** (54 mg, 0.1 mmol) was dissolved in a mixed solvent system (CH_2Cl_2/TFA = 1:1, 5 mL) and stirred at rt for 2 h. After removal of the solvent, the residue was taken up in water (5 mL), alkalified with $NaHCO_3$ to pH > 7, and then extracted with $CHCl_3$. The combined organic layer was washed with brine, dried, filtered, and then evaporated. The residue was dissolved in CH_2Cl_2 (5 mL), and 2-isocyanato-2-methylpropane (30 mg, 0.3 mmol) was added. The mixture was stirred for 24 h at rt and extracted with water and CH_2Cl_2. The combined organic layer was washed with brine, dried, filtered, and then evaporated. The residue was purified by chromatography to afford compound **14** as an off-white solid in 47% yield. ^{1}H NMR (300 MHz, DMSO) δ 9.77 (s, 1H), 9.29 (d, J = 4.9 Hz, 2H), 8.52 (d, J = 4.6 Hz, 1H), 8.15 (s, 1H), 7.56−7.41 (m, 3H), 7.37 (dd, J = 7.4, 4.5 Hz, 1H), 7.28 (d, J = 8.2 Hz, 1H), 7.11 (d, J = 8.2 Hz, 1H), 6.97 (t, J = 8.1 Hz, 1H), 6.04 (s, 1H), 5.93 (t, J = 5.4 Hz, 1H), 3.76 (d, J = 5.4 Hz, 2H), 2.71 (d, J = 4.5 Hz, 3H), 1.20 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 169.3, 167.6, 158.3, 157.7, 156.4, 154.9, 140.9, 139.1, 132.2, 128.7, 126.9, 125.8, 125.7, 124.1, 118.7, 114.6, 113.4, 110.8, 105.0, 49.5, 43.5, 29.7, 26.6. MS (ESI) m/z 543 $[M + H]^+$. HRMS: calcd for $C_{25}H_{29}ClN_8O_3F$ [M + H] 543.1957; found 543.2045.

*Synthesis of 2-Chloro-N-(3-((5-chloro-4-((2-(isopropylsulfonyl)-phenyl)amino)pyrimidin-2-yl)amino)phenyl)acetamide (**30**).* To a crude compound **29** (417 mg, 1 mmol) and Et_3N (404 mg, 4 mmol) in CH_2Cl_2 (20 mL) was added dropwise a solution of 2-chloroacetyl chloride (224 mg, 2 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred at rt for 1 h. After being quenched with aqueous sodium bicarbonate, the mixture was extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was used directly without further purification.

*General Procedure for Synthesis of Compounds **31**, **32**, **46**, and **47**.* TBTU (64 mg, 0.2 mmol) and DIPEA (52 mg, 0.4 mmol) were added successively to a solution of an appropriate acid (0.15 mmol) and compound **30** (42 mg, 0.1 mmol) in DMF (2 mL). The reaction was stirred overnight at rt, diluted with water (8 mL), and extracted with $CHCl_3$. The combined organic layers were washed with brine (10 mL) and dried over Na_2SO_4. After filtration, the solvents were evaporated. The residue was subjected to column chromatography on silica gel ($CHCl_3$/MeOH, 20/1) to give the expected compound.

*N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)azetidine-2-carboxamide (**31**).* White solid (68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (s, 1H), 9.58 (s, 1H), 8.62 (d, J = 8.5 Hz, 1H), 8.19 (s, 1H), 7.92 (dd, J = 8.0, 1.6 Hz, 2H), 7.64 (t, J = 7.1 Hz, 1H), 7.44 (dd, J = 7.6, 3.5 Hz, 1H), 7.28−7.18 (m, 2H), 4.46 (t, J = 8.5 Hz, 1H), 3.86 (q, J = 8.5 Hz, 1H), 3.37 (td, J = 8.3, 3.9 Hz, 1H), 3.27 (dt, J = 13.6, 6.8 Hz, 1H), 2.73 (d, J = 8.0 Hz, 1H), 2.51−2.40 (m, 1H), 1.34 (d, J = 6.4 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 157.5, 155.3, 155.1, 139.8, 138.3, 138.2, 134.5, 131.2, 129.3, 124.5, 123.4, 123.1, 115.6, 113.8, 110.6, 106.6, 59.5, 55.6, 43.4, 26.5, 15.3, 15.3. MS (ESI) m/z 501 $[M + H]^+$. HRMS: calcd for $C_{23}H_{25}ClN_6O_3S$ $[M + H]^+$, 501.1476; found 501.1476.

*N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)azetidine-3-carboxamide (**32**).* White solid (63%). ^{1}H NMR (400 MHz, $CDCl_3$+MeOD) δ 8.58 (s, 1H), 8.06 (d, J = 3.9 Hz, 1H), 7.83 (s, 1H), 7.66 (s, 1H), 7.55 (s, 1H), 7.42 (s, 1H), 7.20 (s, 3H), 3.75 (s, 1H), 3.62 (d, J = 6.3 Hz, 1H), 3.21 (s, 1H), 3.10−2.92 (m, 2H), 2.84 (s, 1H), 1.26 (d, J = 4.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.2, 157.5, 155.2, 154.8, 139.6, 138.4, 138.1, 134.6, 131.1, 129.1, 124.0, 123.4, 123.2, 116.3, 114.4, 111.6, 106.1, 55.6, 50.4, 43.1, 41.2, 15.1. MS (ESI) m/z 501$[M + H]^+$. HRMS: calcd for $C_{23}H_{26}ClN_6O_3S$ [M + H], 501.1471; found 501.1470.

*N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)-2-(furan-2-yl)acetamide (**46**).* White solid (78%). ^{1}H NMR (400 MHz, DMSO) δ 10.13 (s, 1H), 9.57 (d, J = 16.3 Hz, 2H), 8.69 (d, J = 7.9 Hz, 1H), 8.30 (s, 1H), 7.90−7.80 (m, 2H), 7.69 (s, 1H), 7.57 (s, 1H), 7.36 (t, J = 6.0 Hz, 2H), 7.19 (d, J = 7.8 Hz, 2H), 6.40 (s, 1H), 6.26 (d, J = 3.0 Hz, 1H), 3.72 (s, 2H), 3.50−3.40 (m, 1H), 1.17 (d, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 158.0, 155.5, 155.1, 149.9, 142.4, 139.7, 138.3, 135.2, 131.4, 128.9, 124.3, 124.1, 123.8, 115.7, 113.7, 111.4, 110.9, 108.0, 105.3, 55.3, 36.5, 15.3. MS (ESI) m/z 526 $[M + H]^+$. HRMS: calcd for $C_{25}H_{24}ClN_5O_4S$ [M +H], 526.1316; found 526.1315.

*N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)-2-(1H-indol-2-yl)acetamide (**47**).* White solid (72%). ^{1}H NMR (300 MHz, $CDCl_3$+MeOD) δ 8.48 (d, J = 8.4 Hz, 1H), 8.02 (s, 1H), 7.78 (dd, J = 7.9, 1.5 Hz, 1H), 7.49 (dd, J = 16.2, 9.8 Hz, 3H), 7.33 (dd, J = 12.5, 4.6 Hz, 2H), 7.15−7.02 (m, 5H), 6.93 (d, J = 8.0 Hz, 1H), 3.78 (s, 2H), 3.15 (dd, J = 13.7, 6.8 Hz, 1H), 1.22 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6, 157.4, 155.1, 154.7, 139.5, 138.0, 136.4, 134.5, 131.0, 129.0, 126.7, 124.1, 123.4, 123.2, 122.3, 119.8, 118.3, 116.3, 114.5, 111.7, 111.6, 107.6, 55.6, 34.3, 15.2. MS (ESI) m/z 573 [M − H]. HRMS: calcd for ($C_{29}H_{26}N_6O_3ClS$), 573.1470; found 573.1480.

*Synthesis of N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)-amino)pyrimidin-2-yl)amino)phenyl)-1-(4-(dimethylamino)-butanoyl)azetidine-3-carboxamide (**33**).* TBTU (128 mg, 0.4 mmol) and DIPEA (103 mg, 0.8 mmol) were added successively to a solution (*E*)-4-(dimethylamino)but-2-enoic acid (39 mg, 0.3 mmol) and compound **29** (100 mg, 0.2 mmol) in DMF (3 mL). The reaction was stirred overnight at rt and then diluted with water (8 mL). After filtration, the filter cake was dried. The residue was dissolved in MeOH (10 mL), and 10% Pd/C (15%) was added. The mixture was stirred for 2 h under H_2 atmosphere and then filtered. The filtrate was concentrated in vacuum, and the residue was subjected to column chromatography on silica gel ($CHCl_3$/MeOH, 20/1) to give compound **33** (white solid, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.61 (s, 1H), 8.59 (d, J = 12.7 Hz, 2H), 8.12 (s, 1H), 7.86 (d, J = 7.2 Hz, 1H), 7.75 (s, 1H), 7.58 (d, J = 18.1 Hz, 2H), 7.41 (s, 1H), 7.20 (s, 3H), 4.42 (d, J = 5.3 Hz, 1H), 4.18 (d, J = 28.9 Hz, 3H), 3.45 (s, 1H), 3.31−3.12 (m, 1H), 2.30 (d, J = 6.6 Hz, 2H), 2.21 (s, 6H), 2.11 (d, J = 6.7 Hz, 2H), 1.76 (d, J = 6.2 Hz, 2H), 1.29 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.2, 169.9, 157.5, 155.2, 155.0, 139.9, 138.4, 134.5, 131.2, 129.2, 124.1, 123.3, 123.1, 116.1, 114.3, 111.4, 106.5, 58.7, 55.6, 52.2, 50.8, 45.1, 33.7, 28.7, 22.3, 15.3. MS (ESI) m/z 612 [M − H]. HRMS: calcd for $C_{29}H_{35}N_4O_7ClS$ [M − H], 612.2154; found 612.2160.

*General Procedure for Synthesis of Compounds **34**−**37** and **40**−**44**.* An appropriate amine (0.2 mmol), Et_3N (1 mmol), and K_2CO_3 (1.2 mmol) were added successively to a solution of compound **30** (0.1 mmol) in MeCN (10 mL). The reaction was heated to reflux overnight. After removal of the solvent, the residue was diluted with water and extracted with $CHCl_3$.The combined organic layers were washed with brine, dried, filtered, and then evaporated. The residue

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was subjected to column chromatography on silica gel ($CHCl_3$/MeOH, 20/1) to give the expected compound.

*N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)-2-morpholinoacetamide (**34**).* White solid (61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.66 (s, 1H), 9.07 (s, 1H), 8.62 (d, J = 8.4 Hz, 1H), 8.19 (s, 1H), 7.92 (dd, J = 8.0, 1.6 Hz, 1H), 7.87 (t, J = 1.9 Hz, 1H), 7.65−7.58 (m, 1H), 7.50 (dd, J = 8.1, 1.5 Hz, 1H), 7.29 (d, J = 8.8 Hz, 1H), 7.28−7.22 (m, 2H), 7.12 (dd, J = 8.0, 1.3 Hz, 1H), 3.82−3.73 (m, 4H), 3.31−3.21 (m, 1H), 3.15 (s, 2H), 2.67−2.60 (m, 4H), 1.33 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.9, 157.4, 155.2, 155.0, 139.9, 138.3, 137.9, 134.4, 131.2, 129.3, 124.4, 123.3, 123.0, 115.8, 113.7, 110.8, 106.7, 66.9, 62.4, 55.6, 53.7, 15.3. MS (ESI) m/z 545[M + H]$^+$. HRMS: calcd for $C_{25}H_{30}ClN_6O_4S$ [M + H], 545.1738; found 545.1732.

*N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)-2-((2S,6R)-2,6-dimethylmorpholino)-acetamide (**35**).* White solid (65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.67 (s, 1H), 9.08 (s, 1H), 8.63 (d, J = 8.4 Hz, 1H), 8.20 (s, 1H), 7.94−7.85 (m, 2H), 7.62 (t, J = 7.9 Hz, 1H), 7.50 (dd, J = 7.9, 1.7 Hz, 1H), 7.29−7.23 (m, 2H), 7.12 (dd, J = 8.0, 1.2 Hz, 1H), 3.72 (ddd, J = 10.0, 6.2, 2.0 Hz, 2H), 3.30−3.22 (m, 1H), 3.12 (s, 2H), 2.76 (d, J = 10.4 Hz, 2H), 2.08−2.01 (m, 2H), 1.33 (d, J = 6.9 Hz, 6H), 1.20 (d, J = 6.3 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.1, 157.5, 155.3, 155.1, 139.9, 138.3, 138.0, 134.5, 131.2, 129.3, 124.4, 123.3, 123.1, 115.7, 113.6, 110.8, 106.7, 71.9, 62.0, 59.4, 55.6, 18.9, 15.3. MS (ESI) m/z: 573 [M + H]$^+$. HRMS: calcd for $C_{27}H_{33}ClN_6O_4S$ [M + H], 573.2051; found 573.2052.

*N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)-2-(piperazin-1-yl)acetamide (**36**).* White solid (53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 9.16 (s, 1H), 8.62 (d, J = 8.4 Hz, 1H), 8.19 (s, 1H), 7.91 (dd, J = 7.9, 1.5 Hz, 1H), 7.86 (s, 1H), 7.61 (s, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.30 (s, 1H), 7.24 (d, J = 7.4 Hz, 1H), 7.12 (d, J = 7.9 Hz, 1H), 3.26 (dt, J = 13.8, 6.9 Hz, 1H), 3.12 (s, 2H), 2.99−2.92 (m, 4H), 2.60 (d, J = 4.3 Hz, 4H), 1.33 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.4, 157.4, 155.2, 155.1, 139.9, 138.3, 138.0, 134.4, 131.2, 129.3, 124.4, 123.4, 123.0, 115.7, 113.7, 110.8, 106.6, 62.6, 55.6, 54.6, 46.2, 15.3. MS (ESI) m/z 544 [M + H]$^+$. HRMS: calcd for $C_{25}H_{30}ClN_7O_3S$ [M + H], 544.1898; found 544.1889.

*N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)-2-(4-methylpiperazin-1-yl)acetamide (**37**).* Off-white solid (73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 9.12 (s, 1H), 8.62 (d, J = 8.2 Hz, 1H), 8.19 (s, 1H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H), 7.86 (t, J = 1.9 Hz, 1H), 7.63−7.56 (m, 1H), 7.50 (dd, J = 7.9, 1.9 Hz, 1H), 7.40 (s, 1H), 7.30−7.27 (m, 1H), 7.23 (dd, J = 10.1, 2.9 Hz, 1H), 7.13−7.09 (m, 1H), 3.26 (dt, J = 13.7, 6.8 Hz, 1H), 3.14 (s, 2H), 2.66 (s, 4H), 2.51 (s, 3H), 2.34 (s, 3H), 2.26 (s, 1H), 1.33 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.3, 157.5, 155.2, 155.0, 139.9, 138.3, 138.0, 134.4, 131.1, 129.2, 124.3, 123.4, 123.0, 115.8, 113.7, 110.9, 106.5, 61.8, 55.6, 55.1, 53.3, 45.9, 15.3. MS (ESI) m/z 558 [M + H]$^+$. HRMS: calcd for $C_{26}H_{33}ClN_7O_3S$ [M + H], 558.2054; found 558.2056.

*N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)-2-(4-(2-hydroxyacetyl)piperazin-1-yl)acetamide (**40**).* White solid (55%). ^{1}H NMR (300 MHz, $CDCl_3$/MeOD) δ 8.54 (d, J = 8.4 Hz, 1H), 8.05 (s, 1H), 7.80 (dd, J = 8.0, 1.5 Hz, 1H), 7.66 (s, 1H), 7.51 (t, J = 7.9 Hz, 1H), 7.40 (d, J = 8.2 Hz, 1H), 7.17 (td, J = 7.9, 4.9 Hz, 2H), 7.07 (d, J = 7.2 Hz, 1H), 4.10 (s, 2H), 3.64 (s, 2H), 3.30 (d, J = 5.1 Hz, 2H), 3.17 (dt, J = 13.7, 6.9 Hz, 1H), 3.10 (s, 2H), 2.58−2.52 (m, 4H), 1.23 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 167.9, 157.4, 155.2, 154.8, 139.9, 138.1, 137.5, 134.5, 129.2, 123.1, 116.4, 114.0, 111.3, 106.2, 61.7, 59.6, 55.6, 52.9, 52.6, 43.4, 42.0, 15.1. MS (ESI) m/z 602 [M + H]$^+$. HRMS: calcd for $C_{27}H_{32}ClN_7O_5S$ [M + H], 602.1952; found 602.1945.

*2-(2,5-Diazabicyclo[2.2.1]heptan-2-yl)-N-(3-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)-acetamide (**41**).* Off-white solid (47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (s, 1H), 9.20 (s, 1H), 8.62 (d, J = 8.4 Hz, 1H), 8.18 (s, 1H), 7.92−7.84 (m, 2H), 7.64−7.55 (m, 2H), 7.50−7.43 (m, 1H), 7.27−7.20 (m, 2H), 7.12 (d, J = 8.9 Hz, 1H), 3.65 (s, 1H), 3.40 (d, J = 5.9 Hz, 1H), 3.35 (s, 1H), 3.31−3.21 (m, 2H), 3.11 (d, J = 10.4 Hz, 1H), 2.99−2.91 (m, 2H), 2.68 (d, J = 9.6 Hz, 1H), 2.26 (s, 1H), 1.82 (d, J = 9.6 Hz, 1H), 1.66 (d, J = 9.8 Hz, 1H), 1.32 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.0, 157.5, 155.1, 155.0, 139.8, 138.2, 138.0, 134.4, 131.1, 129.1, 124.2, 123.3, 122.9, 115.7, 113.7, 110.9, 106.4, 63.3, 62.6, 59.6, 56.9, 55.5, 49.7, 36.2, 15.2. MS (ESI) m/z 556 [M + H]$^+$. HRMS: calcd for $C_{26}H_{30}ClN_7O_3S$ [M + Na], 578.1717; found 578.1719.

*2-(8-Oxa-3-azabicyclo[3.2.1]octan-3-yl)-N-(3-((5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenyl)-acetamide (**42**).* Off-white solid (52%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.64 (s, 1H), 9.14 (s, 1H), 8.60 (d, J = 8.4 Hz, 1H), 8.20−8.11 (m, 1H), 7.89 (dd, J = 5.7, 1.9 Hz, 2H), 7.59 (dd, J = 10.8, 4.9 Hz, 1H), 7.47 (d, J = 7.9 Hz, 1H), 7.29 (d, J = 2.6 Hz, 1H), 7.22 (d, J = 2.6 Hz, 1H), 7.08 (d, J = 7.9 Hz, 1H), 4.35 (s, 2H), 3.29−3.18 (m, 1H), 3.06 (d, J = 2.2 Hz, 2H), 2.63 (s, 4H), 2.01 (s, 4H), 1.30 (d, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7, 157.0, 154.8, 154.6, 139.6, 137.9, 137.6, 134.0, 130.8, 129.0, 123.9, 122.9, 122.6, 115.2, 112.8, 110.0, 106.2, 73.9, 60.9, 58.2, 55.2, 28.1, 14.9. MS (ESI) m/z 571 [M +H]$^+$. HRMS: calcd for $C_{27}H_{31}ClN_6O4S$ [M + Na], 593.1714; found 593.1713.

*N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)-2-(3-hydroxy-8-azabicyclo[3.2.1]-octan-8-yl)acetamide (**43**).* Off-white solid (41%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.64 (s, 2H), 8.61 (d, J = 8.4 Hz, 1H), 8.15 (s, 1H), 7.89 (d, J = 7.9 Hz, 1H), 7.82 (s, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.48 (d, J = 8.1 Hz, 1H), 7.33 (s, 1H), 7.21 (d, J = 8.1 Hz, 1H), 7.12 (d, J = 7.7 Hz, 1H), 4.09 (s, 1H), 3.19 (s, 3H), 3.10 (s, 2H), 2.20 (d, J = 7.5 Hz, 2H), 2.13 (s, 1H), 2.09 (s, 1H), 2.04 (s, 1H), 1.96 (s, 2H), 1.79 (d, J = 14.6 Hz, 2H), 1.31 (d, J = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.6, 157.1, 154.8, 154.5, 139.4, 137.9, 137.7, 134.0, 130.7, 128.8, 123.9, 122.9, 122.6, 115.4, 113.4, 110.5, 106.1, 63.5, 59.5, 55.2, 26.0, 14.8. MS (ESI) m/z 583 [M − H]. HRMS: calcd for $C_{28}H_{32}ClN_6O_4S$ [M − H], 583.1889; found 583.1897.

*Synthesis of N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)-amino)pyrimidin-2-yl)amino)phenyl)-2-(4-(oxetan-3-yl)piperazin-1-yl)acetamide (**38**).* To a solution of **37** (54 mg, 0.1 mmol) in MeOH (5 mL), oxetan-3-one (14 mg, 0.2 mmol) was added. The mixture was stirred for 1 h, $NaHB(CN)_3$ (25 mg, 0.4 mmol) was added, and the reaction was stirred overnight. The reaction was quenched with saturated NH_4Cl. After the solvent was evaporated, the residue was diluted with water and then extracted with $CHCl_3$. The combined organic layers were washed with brine, dried, filtered, and then evaporated. The crude product was subjected to column chromatography on silica gel ($CHCl_3$/MeOH, 20/1) to give compound **38** (white solid, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.66 (s, 1H), 9.07 (s, 1H), 8.62 (d, J = 8.5 Hz, 1H), 8.19 (s, 1H), 7.92 (dd, J = 7.9, 1.4 Hz, 1H), 7.87 (s, 1H), 7.63 (t, J = 7.9 Hz, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.25 (d, J = 7.6 Hz, 1H), 7.16−7.05 (m, 2H), 4.67 (dt, J = 24.3, 6.3 Hz, 4H), 3.60−3.51 (m, 1H), 3.27 (dt, J = 13.5, 6.8 Hz, 1H), 3.17 (s, 2H), 2.70 (s, 4H), 2.43 (s, 4H), 1.34 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.2, 157.4, 155.3, 155.1, 139.9, 138.3, 138.0, 134.5, 131.2, 129.4, 124.4, 123.4, 123.1, 115.7, 113.7, 110.8, 106.7, 75.4, 61.9, 59.0, 55.6, 53.0, 49.7, 15.3. MS (ESI) m/z 600 [M +H]$^+$. HRMS: calcd for $C_{28}H_{34}ClN_7O_4S$ [M + Na], 622.1979; found 622.1980.

*Synthesis of (E)-N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)-amino)pyrimidin-2-yl)amino)phenyl)-2-(4-(4-(dimethylamino)but-2-enoyl)piperazin-1-yl)acetamide (**39**).* This compound was prepared from (*E*)-4-(dimethylamino)but-2-enoic acid (39 mg, 0.3 mmol) and compound **37** as an off-white solid in 78% yield by following a similar procedure as that for preparation of compound **33**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.66 (s, 1H), 8.99 (s, 1H), 8.62 (d, J = 8.4 Hz, 1H), 8.19 (s, 1H), 7.95−7.85 (m, 2H), 7.62 (t, J = 7.9 Hz, 1H), 7.50 (dd, J = 7.9, 1.7 Hz, 1H), 7.26 (dd, J = 10.0, 5.2 Hz, 2H), 7.12 (dd, J = 7.9, 1.2 Hz, 1H), 6.89 (dt, J = 15.2, 5.9 Hz, 1H), 6.45 (d, J = 15.2 Hz, 1H), 3.71 (d, J = 40.5 Hz, 4H), 3.26 (dt, J = 13.7, 6.9 Hz, 1H), 3.18 (s, 2H), 3.11 (dd, J = 5.9, 1.4 Hz, 2H), 2.67−2.60 (m, 4H), 2.29 (s, 6H), 1.33 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.6, 165.2, 157.4, 155.3, 155.1, 143.1, 140.0, 138.3, 137.9, 134.4, 131.2, 129.4, 124.4, 123.3, 123.1, 121.5, 115.8, 113.7, 110.8, 106.8, 61.9, 60.6, 55.6,

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45.4, 15.3. MS (ESI) m/z 655 $[M + H]^+$. HRMS: calcd for $C_{31}H_{40}ClN_8O_4S$ $[M + H]^+$, 655.2852; found 655.2853.

*General Procedure for Synthesis of Compounds **44** and **45**.* An appropriate amine (0.2 mmol), Et_3N (1 mmol), and K_2CO_3 (1.2 mmol) were added successively to a solution of compound **30** (0.1 mmol) in MeCN (10 mL). The reaction was heated to reflux overnight. After removal of the solvent, the residue was diluted with water and extracted with $CHCl_3$. The combined organic layers were washed with brine, dried, filtered, and then evaporated. The residue was then dissolved in a mixed solvent system (CH_2Cl_2/TFA = 1:1, 5 mL) and the mixture was stirred at rt for 2 h. After removal of the solvent, the residue was taken up in water (5 mL), alkalified with $NaHCO_3$ to pH > 7, and then extracted with $CHCl_3$.The combined organic layer was washed with brine, dried, filtered, and then evaporated. The residue was subjected to column chromatography on silica gel ($CHCl_3$/MeOH, 20/1) to give the corresponding product.

*N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)-2-(hexahydropyrrolo[3,4-b]pyrrol-1(2H)-yl)acetamide (**45**).* Off-white solid (48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (s, 1H), 9.26 (s, 1H), 8.62 (d, J = 8.3 Hz, 1H), 8.18 (s, 1H), 7.91 (dd, J = 8.0, 1.5 Hz, 1H), 7.83 (s, 1H), 7.62 (dd, J = 11.4, 4.3 Hz, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.27−7.17 (m, 4H), 3.48 (d, J = 16.7 Hz, 1H), 3.23 (ddd, J = 11.0, 10.5, 4.7 Hz, 3H), 3.14−3.05 (m, 1H), 2.98 (t, J = 7.8 Hz, 1H), 2.87 (d, J = 6.3 Hz, 2H), 2.75 (s, 1H), 2.62 (dd, J = 11.5, 4.7 Hz, 1H), 2.43−2.35 (m, 1H), 2.14 (d, J = 13.7 Hz, 1H), 1.62−1.53 (m, 1H), 1.33 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.4, 157.5, 155.3, 155.1, 139.7, 138.3, 134.5, 131.2, 129.2, 124.3, 123.4, 123.1, 115.8, 114.3, 111.3, 106.6, 70.3, 58.8, 55.7, 55.6, 54.0, 52.4, 42.6, 32.4, 15.3. MS (ESI) m/z 570 $[M + H]^+$. HRMS: calcd for $C_{27}H_{33}ClN_7O_3S$ $[M + H]$, 570.2054; found 570.2052.

*N-(3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)-pyrimidin-2-yl)amino)phenyl)-2-(hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)acetamide (**44**).* Off-white solid (56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 9.34 (s, 1H), 8.63 (d, J = 8.5 Hz, 1H), 8.16 (s, 1H), 7.91 (d, J = 6.4 Hz, 1H), 7.78 (s, 1H), 7.62 (t, J = 7.9 Hz, 1H), 7.54 (d, J = 8.1 Hz, 1H), 7.40 (s, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.25 (t, J = 7.8 Hz, 2H), 3.31−3.24 (m, 3H), 3.22 (s, 2H), 3.01 (d, J = 11.2 Hz, 2H), 2.87 (s, 2H), 2.80 (d, J = 9.6 Hz, 2H), 2.61 (d, J = 6.2 Hz, 2H), 1.33 (d, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.6, 157.5, 155.3, 155.0, 139.8, 138.4, 138.3, 134.6, 131.2, 129.2, 124.3, 123.5, 123.1, 115.8, 114.3, 111.3, 106.5, 60.4, 58.9, 55.6, 53.4, 42.4, 15.3. MS (ESI) m/z 570 $[M + H]^+$. HRMS: calcd for $C_{27}H_{32}ClN_7O_3S$ $[M + H]$, 570.2054; found 570.2051.

ELISA Kinase Assay. Kinase activity was evaluated according to the following procedure. Briefly, in enzyme-linked-immunosorbent assay (ELISA), 20 μg/mL poly(Glu, Tyr)$_{4:1}$ (Sigma) was precoated as a substrate in 96-well plates. ATP solution (50 μL of 10 μM) diluted in kinase reaction buffer (50 mM HEPES, pH 7.4, 50 mM $MgCl_2$, 0.5 mM $MnCl_2$, 0.2 mM Na_3VO_4, 1 mM DTT) was added to each well. One microliter of various concentrations of tested compounds diluted in 1% DMSO (v/v) (Sigma) was added to each reaction well. DMSO (1% v/v) was used as the negative control. The kinase reaction was initiated after the addition of purified tyrosine kinase proteins diluted in 49 μL of kinase reaction buffer solution. After incubation for 60 min at 37 °C, the plate was washed three times with phosphate buffered saline (PBS) containing 0.1% Tween 20 (T-PBS). Anti-phosphotyrosine (PY99) antibody (100 μL, 1:500 diluted in 5 mg/mL BSA T-PBS) was then added. After 30 min incubation at 37 °C, the plate was washed three times. Horseradish peroxidase-conjugated goat anti-mouse IgG (100 μL, 1:2000 diluted in 5 mg/mL BSA T-PBS) was added. The plate was then incubated at 37 °C for 30 min and washed 3 times. A solution (100 μL) containing 0.03% H_2O_2 and 2 mg/mL o-phenylenediamine in 0.1 mol/L citrate buffer, pH 5.5, was added. The reaction was terminated by the addition of 50 μL of 2 mol/L H_2SO_4 as color changed, and the plate was read using a multiwell spectrophotometer (SpectraMAX 190, Molecular Devices) at 490 nm. The inhibition rate (%) was calculated using the following equation: $[1 - A_{490}/(A_{490}\ \text{control})] \times 100\%$. IC_{50} values were calculated from the inhibition curves from two separate experiments.

Cell Proliferation Assay. Cells were seeded in 96-well tissue culture plates. On the next day, cells were exposed to various concentrations of compounds and further cultured for 72 h. Cell proliferation was then determined using sulforhodamine B (SRB, Sigma) or thiazolyl blue tetrazolium bromide (MTT, Sigma) assay. IC_{50} values were calculated by concentration−response curve fitting using the four-parameter method.

In Vivo Antitumor Activity Assay. Female nude mice (4−6 weeks) were housed five mice per cage in a specific pathogen-free room with a 12 h light/dark schedule at 25 ± 1 °C and were feed an autoclaved chow diet and water ad libitum. All animal experiments were performed according to institutional ethinical guidelines of animal care. The cells at a density of $(5-10) \times 10^6$ in 200 μL were first implanted sc into the right flank of each nude mouse and then allowed to grow to 700−800 mm^3, defined as a well-developed tumor. After that, the well-developed tumors were cut into 1 mm^3 fragments and transplanted sc into the right flank of nude mice using a trocar. When the tumor volume reached 80−100 mm^3, the mice were randomly assigned into control and treatment groups (n = 5 in treated group, n = 10 in vehicle group). Control groups were given vehicle alone, and treatment groups received compound **15** hydrochloride salt with indicated doses via oral administration (with 0.5% CMC-Na (sodium carboxymethyl cellulose)) or crizotinib hydrochloride salt with water via oral administration once daily for 2 weeks. The sizes of the tumors were measured twice per week using a microcaliper. The tumor volume (TV) was calculated as TV = (length × width^2)/2. Relative tumor volume (RTV) = $TV^{Day\text{-}N}/TV^{Day\text{-}0} \times 100\%$. Data was shown on indicated days as the median RTV ± SE. Percent (%) inhibition values were measured on the final day of study for drug-treated compared with vehicle-treated mice and are calculated as $100 \times (1 - [(\text{treated final day} - \text{treated day 1})/(\text{control final day} - \text{control day 1})])$. Significant differences between the treated versus control groups were determined using Student's t test.

■ ASSOCIATED CONTENT

Supporting Information

Copies of the ^{1}H and ^{13}C spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ALK, anaplastic lymphoma kinase; RTK, receptor tyrosine kinase; ALCL, anaplastic large-cell lymphoma; NSCLC, non-small-cell lung cancer; EML4, echinoderm microtubule-associated protein-like 4; NPM, nucleophosmin; c-Met, mesenchymal epithelial transition growth factor; DAAPalogues, 2,4-diarylaminopyrimidine analogues; crizotinib, 3-[(1*R*)-1-(2,6-dichloro-3-fluorophenyl)ethoxy]-5-(1-piperidin-4-ylpyrazol-4-yl)pyridine-2-amine; CSA, (1*S*)-(+)-10-camphorsulfonic acid; X-Phos, 2-(dicyclohexylphosphino)-2′,4′,6′-triisopropyl biphenyl; IGF1R, insulin-like growth factor 1 receptor; PDGFR, platelet derived growth factor receptor; EGFR, epidermal growth factor receptor; KDR, kinase insert domain receptor; FGFR1, fibroblast growth factor receptor 1; Flt-1, fms-like tyrosine kinase

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