Journal of Medicinal Chemistry

Article

Discovery of 2-((3-amino-4-methylphenyl)amino)-N-(2-methyl-5-(3-(trifluoromethyl)benzamido)phenyl)-4-(methylamino)pyrimidine-5-carboxamide (CHMFL-ABL-053) as a potent, selective and orally available BCR-ABL/SRC/p38 kinase inhibitor for Chronic Myeloid Leukemia

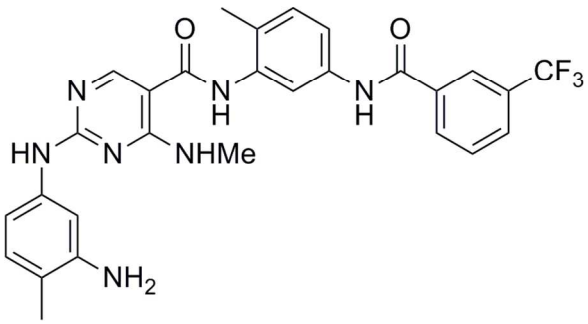
Xiaofei Liang, Xiaochuan Liu, Beilei Wang, Fengming Zou, Aoli Wang, Shuang Qi, Cheng Chen, Zheng Zhao, Wenchao Wang, Ziping Qi, Fengchao Lv, Zhenquan Hu, Li Wang, Shanchun Zhang, Qingsong Liu, and Jing Liu

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.5b01618 • Publication Date (Web): 20 Jan 2016

Just Accepted

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036



K562: GI_{50}: 14 nM
KU812: GI_{50}: 25 nM
MEG-01: GI_{50}: 16 nM
pABL1: EC_{50}: about 100 nM
ABL1: IC_{50}: 70 nM
S Score(1)=0.02
$T_{1/2}$: 4.3 h
Bioavailablity: 24%

121x40mm (300 x 300 DPI)

Discovery of 2-((3-amino-4-methylphenyl)amino)-N-(2-methyl-5-(3-(trifluoromethyl)benzamido)phenyl)-4-(methylamino)pyrimidine-5-carboxamide (CHMFL-ABL-053) as a potent, selective and orally available BCR-ABL/SRC/p38 kinase inhibitor for Chronic Myeloid Leukemia

Xiaofei Liang[1,2,7], Xiaochuan Liu[1,3,7], Beilei Wang[1,2,7], Fengming Zou[1,2,7], Aoli Wang[1,4], Shuang Qi[1,2], Cheng Chen[1,2], Zheng Zhao[1,2], Wenchao Wang[1,2], Ziping Qi[1,2], Fengchao Lv[1,4], Zhenquan Hu[1,2], Li Wang[1,2], Shanchun Zhang[2,5], Qingsong Liu[1,2,4,6], Jing Liu[1,2*]*

1. High Magnetic Field Laboratory, Chinese Academy of Sciences, Mailbox 1110, 350 Shushanhu Road, Hefei, Anhui, P. R. China, 230031
2. CHMFL-HCMTC Target Therapy Joint Laboratory, 350 Shushanhu Road, Hefei, Anhui, P. R. China, 230031
3. Department of Chemistry, University of Science and Technology of China, Hefei, Anhui, P. R. China, 230036
4. University of Science and Technology of China, P. R. China, Anhui, Hefei, P. R. China, 230036

5. Hefei Cosource Medicine Technology Co. LTD., 358 Ganquan Road, Hefei, Anhui, P. R. China, 230031

6. Hefei Science Center, Chinese Academy of Sciences, 350 Shushanhu Road, Hefei, Anhui, P. R. China, 230031

7. These authors contribute equally

ABSTRACT

Starting from a dihydropyrimidopyrimidine core scaffold based compound **27** (GNF-7), we discovered a highly potent (ABL1: IC_{50} of 70 nM) and selective (S score (1) = 0.02) BCR-ABL inhibitor **18a** (CHMFL-ABL-053). **18a** did not exhibit apparent inhibitory activity against c-KIT kinase, which is the common target of currently clinically used BCR-ABL inhibitors. Through significant suppression of the BCR-ABL auto-phosphorylation (EC_{50} about 100 nM) and downstream mediators such as STAT5, Crkl and ERK's phosphorylation, **18a** inhibited the proliferation of CML cell lines K562 (GI_{50} = 14 nM), KU812 (GI_{50} = 25 nM) and MEG-01 (GI_{50} = 16 nM). Pharmacokinetic study revealed that **18a** had over 4 hours half-life and 24% bioavailability in rats. 50mg/kg/day dosage treatment could almost completely suppress the tumor progression in the K562 cells inoculated xenograft mouse model. As a potential useful drug candidate for CML, **18a** is under extensive preclinical safety evaluation now.

INTRODUCTION

Chronic myeloid leukemia (CML), a hematological cancer of bone marrow white blood cells, constitutes about 15% of adult leukemia and usually 1-2 patient is diagnosed with CML per 100,000 people/per year in US.[1] It is characterized by a reciprocal chromosomal translocation between chromosome 9 and 22 of the break point cluster region (BCR) gene with the Abelson (ABL) gene for ABL1 kinase, which leads to a shortened chromosome 22 (*i.e.* Philadelphia chromosome).[2, 3] The fusion tyrosine kinase BCR-ABL is constitutively active and leads to

uncontrolled myeloid cell proliferation through downstream mediators such as Stat5 and ERK[4]. The seminal discovery of small molecule inhibitor Imatinib[5, 6] has validated BCR-ABL as the drug discovery target for CML. Since then, several ABL kinase inhibitors have been approved for clinical use such as Nilotinib[7], Dasatinib[8], Bosutinib[9] and Ponatinib[10] and a few are in the clinical trials now including Bafetinib[11], Danusertib[12] and Rebastinib[13], etc. In addition, a number of newly discovered inhibitors are in the extensive preclinical study such as GZD824[14] and allosteric inhibitor GNF2, GNF5[15] etc. Despite of the great clinical success, the FDA approved drugs such as Imatinib, Nilotinib, Bosutinib and Dasatinib all potently inhibit other targets such as DDR1/2, c-KIT and so on besides ABL1 kinase. Although the role of off-targets inhibition is not very clear in the clinical aspect, the highly selective BCR-ABL inhibitor is still highly demanded from both the preclinical pathological and clinical side effects mechanistic study point of view. Here we describe our medicinal chemistry effort from a dihydropyrimidopyrimidine scaffold based multiple targets BCR-ABL inhibitor GNF-7[16, 17] (compound **27**) to a pyrimidine scaffold based highly potent and selective BCR-ABL inhibitor compound **18a** (CHMFL-ABL-053), which completely abolished the c-KIT kinase activity (Figure 1).

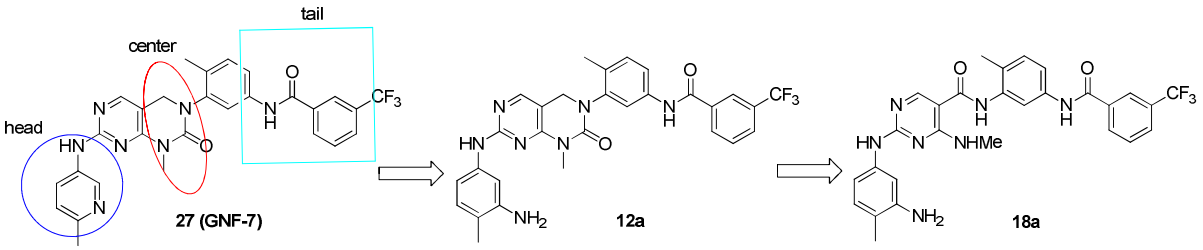


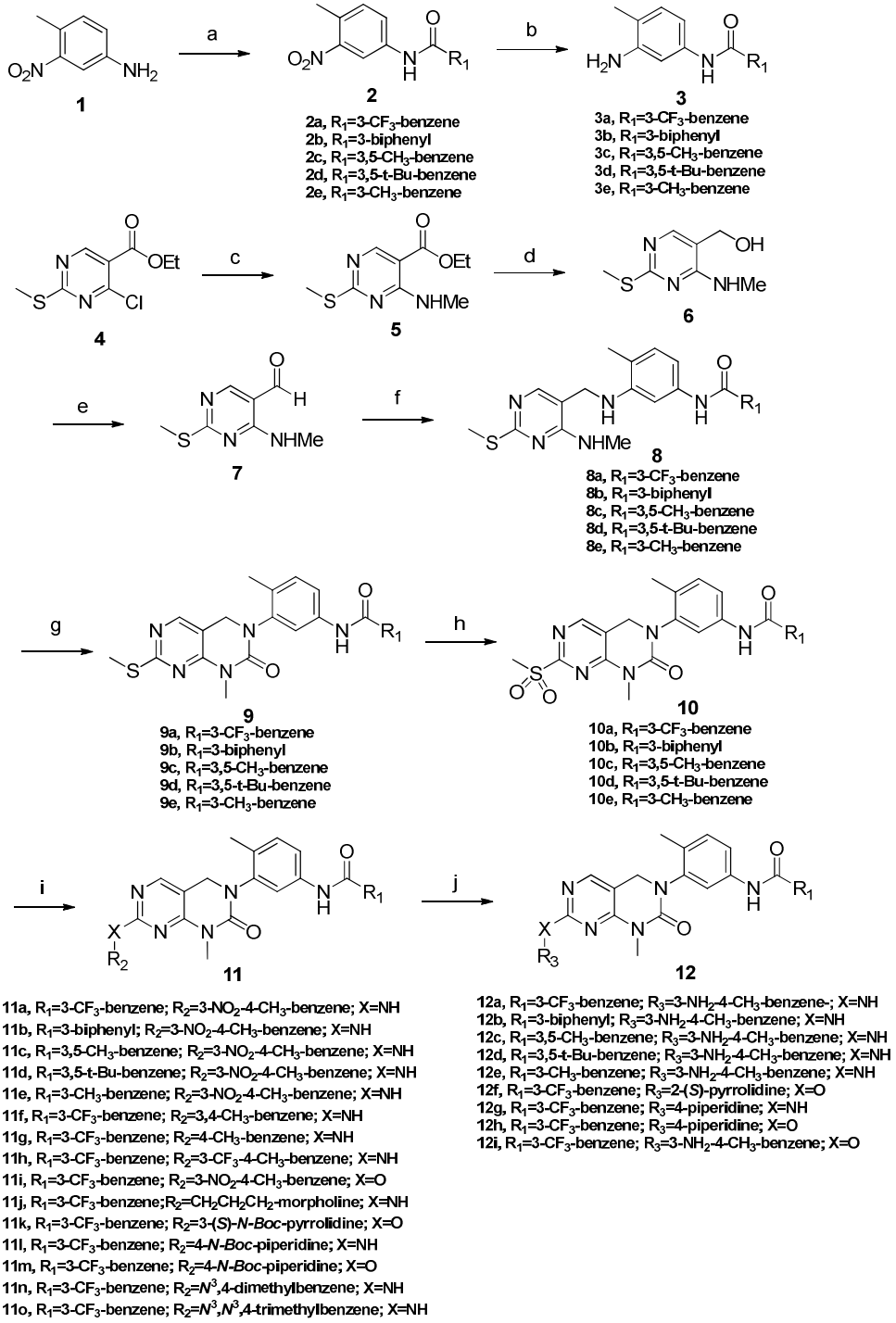
Figure 1. Schematic illustration of discovery of **18a**.

RESULTS AND DISCUSSTION

Chemistry

The synthesis of **12** (Scheme 1) began with amide coupling of 4-methyl-3-nitroaniline with appropriate carbonyl chloride. The nitrobenzene was then converted to the corresponding aniline **3** by reduction with hydrogen under 10% Pd/C condition. Intermediate pyrimidine aldehyde **7** was obtained from ethyl 4-chloro-2-(methylthio)pyrimidine-5-carboxylate **4**. Methylamine pyrimidine **5** was prepared with methylamine in almost quantitative yield. Subsequent reduction of **5** in the presence of LAH in anhydrous THF afforded desired alcohol **6,** which was then oxidized into aldehyde **7**. Reductive amination with **7** and **3** afforded diamine derivative **8**. The diamine was then converted into the dihydropyrimidopyrimidine **9** with triphosgene, which was subsequently oxidized to the corresponding sulfone **10**. Alcohol or aniline derivatives were readily reacted with this sulfone under etherification with base or simple thermal amination condition to provide the compound **11**. Finally, $SnCl_2$ mediated reduction of the nitrobenzene or N-Boc deprotection furnished compound **12**.

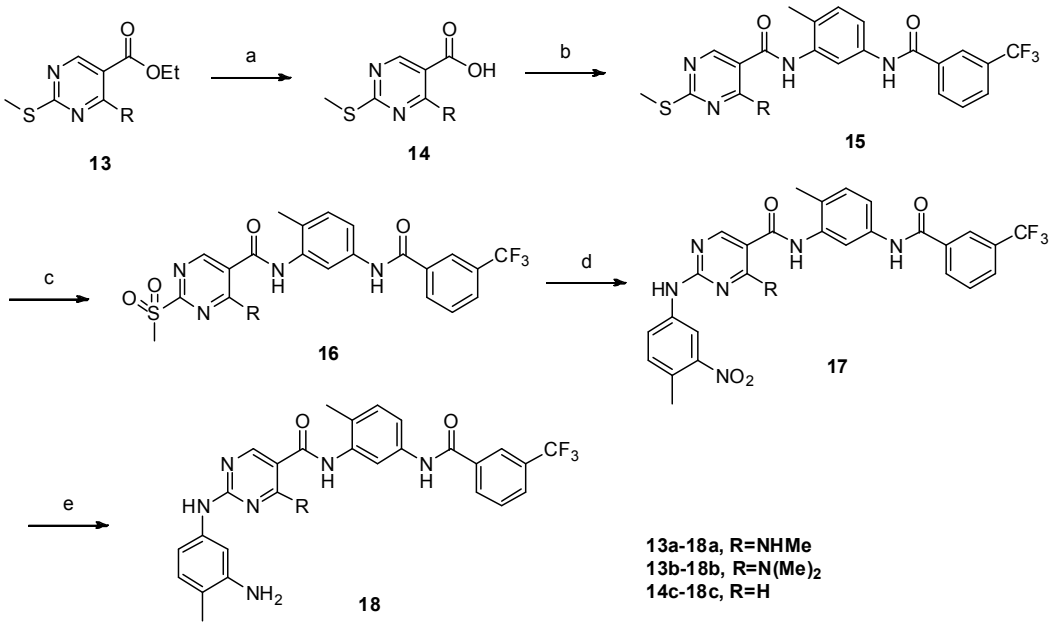
Scheme 1. Synthetic route to dihydropyrimidopyrimidine **12**[a]



[a]Reagents and conditions: (a) R_1COCl, THF, DIPEA, 0 °C; (b) H_2, 10% Pd/C, MeOH, rt; (c) THF, $MeNH_2$, TEA, 0 °C; (d) LAH, THF, 0 °C to reflux; (e) DCM, MnO_2, rt; (f) **3**, $Na(CN)BH_3$, MeOH, AcOH, rt; (g) triphosgene, DCM, DIPEA, 0 °C to rt; (h) m-CPBA, DCM, 0 °C; (i) R_2NH_2, dioxane, TFA, 120 °C or R_2OH, dioxane, K_2CO_3, rt; (j) $SnCl_2.2H_2O$, MeOH, reflux or 4 M HCl, dioxane, rt.

Hydrolysis of ethyl ester of pyrimidine **13** followed by amide-coupling conditions furnished amide **15** (Scheme 2). The sulfide of **15** was then subjected to oxidation, amination and subsequent reduction to provide aniline compounds **18**.

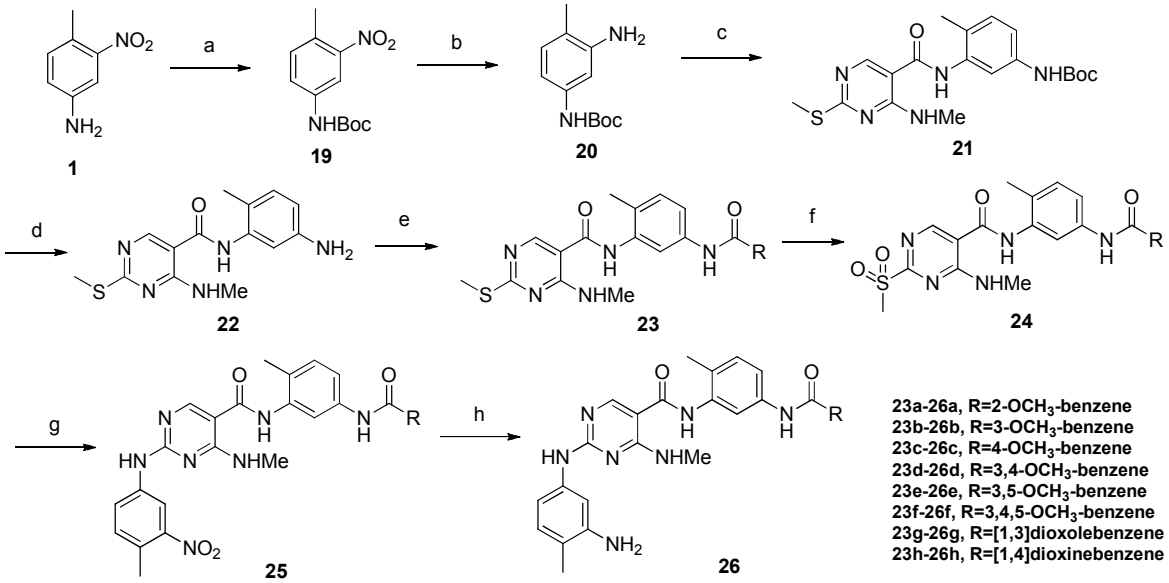
Scheme 2. Synthetic route to compound **18**[a]



[a]Reagents and conditions: (a) for synthesis of **14a-b**: NaOH, MeOH, H_2O, rt; (b) **3a**, HATU, DIPEA, DMF, rt; (c) m-CPBA, DCM, 0 °C; (d) 4-methyl-3-nitroaniline, dioxane, TFA, 120 °C; (e) $SnCl_2.2H_2O$, MeOH, reflux.

As shown in Scheme 3, compound **26** was prepared starting from 4-methyl-3-nitroaniline **1**. After Boc protection (**19**) and hydrogenation (**20**), amide **21** was obtained under the standard amide coupling condition with compound **14a**. Removal of the Boc protection (**22**) followed by acylation with appropriate acyl chloride formed the amide **23**, which was then subjected to oxidation (**24**), amination (**25**) and reduction to provide aniline compound **26**.

Scheme 3. Synthetic route to compound **26**[a]



[a]Reagents and conditions: (a) $(Boc)_2O$, THF, DMAP, reflux; (b) H_2, 10% Pd/C, MeOH, rt; (c) **14a**, HATU, DMF, DIPEA, rt; (d) 4 M HCl, MeOH, rt; (e) RCOCl, THF, DIPEA, 0 °C; (f) m-CPBA, DCM, 0 °C; (g) 4-methyl-3-nitroaniline, dioxane, TFA, 120 °C; (h) $SnCl_2.2H_2O$, MeOH, reflux.

Structure-Activity Relationship

The dihydropyrimidopyrimidine compound **27** (GNF-7) has been reported as a type II BCR-ABL inhibitor which could also overcome "gatekeeper" T315I mutation. However, it also potently inhibited other kinases such as JAK1, 2, 3, FGFR3, FLT3, PDGFR, TRKC, etc[17]. After careful analysis of the molecular modeling results, we envisioned that the methyl pyridine occupied hinge binding area (so called "tail' part), the dihydropyrimidopyrimidine moiety occupied "gatekeeper" residue Thr315 adjacent area (so called "center" part) and the trifluoromethylbenzene occupied DFG shifting created hydrophobic area (so called "tail" part) still have medicinal exploration space for better selectivity (Figure 1). We then chose to systematically optimize the "head", "center" and "tail" parts of **27** to obtain a full spectrum of structure-activity relationship (SAR) as illustrated in Figure 2 with cell based assays using ABL transfused isogenic BaF3 cells and intact CML cancer cell line K562 as primary readout.

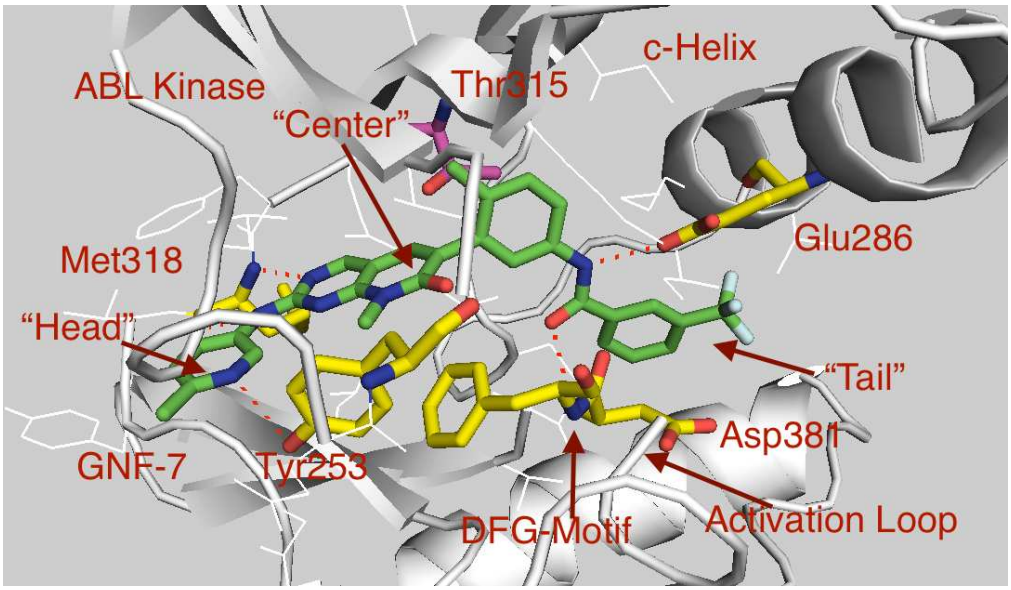


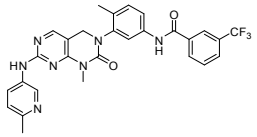
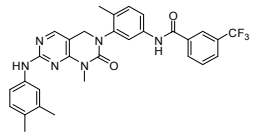
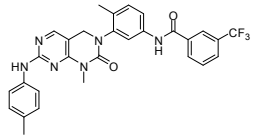
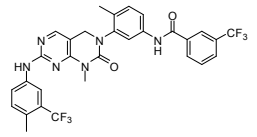
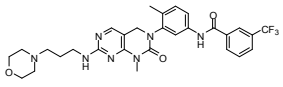
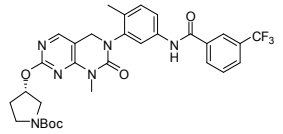
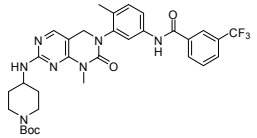
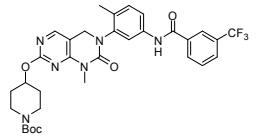
Figure 2. Illustration of SAR exploration rationale. ABL kinase was shown in white. GNF-7 was labeled in color by atoms (Carbon in green, Nitrogen in blue, Oxygen in red). The hydrogen bonds were labeled as red dashed lines. The key amino acid residues for the binding were labeled as Carbon in yellow, Nitrogen in blue and Oxygen in red. The gatekeeper residue Thr315 was labeled as Carbon in magenta, Nitrogen in blue and Oxygen in red. The "head", "Center", "Tail", DFG-motif, Activation loop were pointed out with red arrows.

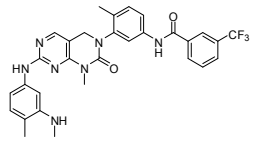
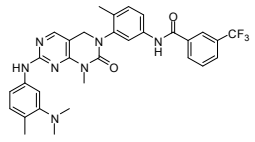
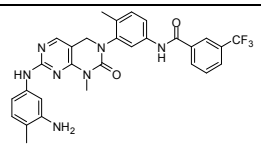
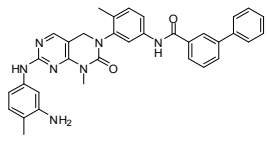
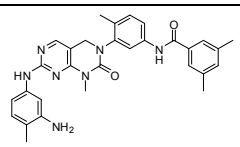
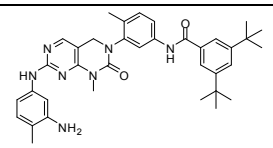
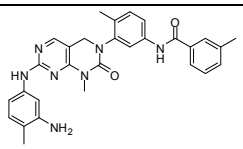
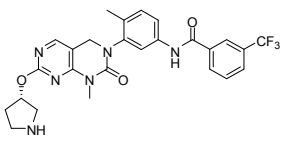
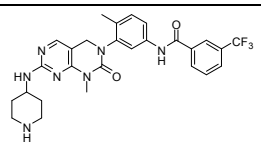
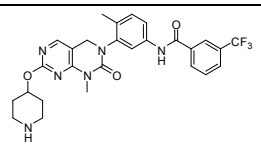
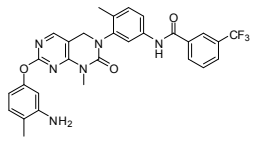
The "head" moiety occupied the hydrophobic pocket located proximal to the hinge binding region in Abl kinase. Removal of the hydrogen bond acceptor nitrogen atom in 2-methylpyridine of **27** by replacement with a 2-methylbenzene (**11g**) led to significant loss of activity against parental BaF3 cells (GI_{50}: 8.0 versus 0.12 μM) but not in K562 cells (GI_{50}: 0.018 μM versus 0.009 μM) (Table 1) which suggested a better selectivity profile and potent on-target (ABL) anti-proliferation effect in the TEL and BCR transfused isogenic BaF3 cells whose growth were dependent on the constitutively activated ABL kinase (GI_{50}: 0.050 μM and 0.019 μM, respectively). Increasing the size of this moiety (**11f**, **11h**, **11n**, and **11o**) retained the potency and selectivity suggesting a large size pocket residing in this region. However, when aniline analog was introduced (**12a)** it started to potently inhibit parental BaF3 cells again (GI_{50}: 0.44 μM). Interestingly, replacement of the aromatic substituents with cyclic aliphatic rings such as

piperidine (**12g**), N-Boc piperidine (**11l**) or the aliphatic chain (**11j**) did not affect the potency. This suggested that the hydrophobic pocket in this area was flexible. Since the modeling study revealed that the 2-aminopyrimidine part of **27** formed two hydrogen bonds in the hinge-binding region with Met318, we then tried to replace the –NH to oxygen atom to see if the activity was remained. Interestingly, compound **12i** still retained the activities against the K562 cells, Tel-ABL-BaF3 cells and P210-BaF3 cells (GI_{50}: 0.013 μM, 0.015 μM and 0.032 μM, respectively) meanwhile still exhibited good selectivity over parental BaF3 cells (GI_{50}: >10 μM). Unfortunately, when the "head" moiety was replaced by aliphatic rings with the O-bridged hinge binding such as (S)-N-Boc 3-hydroxypyrrolidine (**11k**), N-Boc 4-hydroxypiperidine (**11m**), (S)-pyrrolidin-3-ol (**12f**) and piperidin-4-ol (**12h**), significant activity loss were observed in K562 cells, Tel-ABL-BaF3 cells and P210-BaF3 cells. This suggested that the hinge binding factors and "head" hydrophobic binding factors are dependent on each other to contribute to the binding. We next explored the "tail" moiety, which occupied the hydrophobic pocket formed by the "DFG" motif shift in the inactive conformation. Keeping the "head" and "center" moiety as in **12a**, while switching the trifluoromethyl group in the "tail" to a methyl group (**12e)** resulted in about 23-fold potency loss against K562 cells (GI_{50}: 0.069 μM versus 0.003 μM), though the activities against TEL-Abl-BaF3 cells (GI_{50}: 0.006 μM) and P210-BaF3 cells (GI_{50}: 0.014 μM) and selectivity against parental BaF3 cells (GI_{50}: >10 μM) were remained. In addition, replacement of the trifluoromethyl group with a bulky aromatic group (**12b, 12c**) did not result in significant activity loss in K562 cells (GI_{50}: 0.023 μM and 0.026 μM), Tel-Abl-BaF3 cells (GI_{50}: 0.039 μM and 0.031 μM) and P210-BaF3 cells (GI_{50}: 0.046 μM and 0.042 μM). However, modification of the 3, 5-position of the tail group with two tert-butyl groups (**12d**) significantly lowered the activity against K562 cells (GI_{50}: 1.3 μM versus 0.003 μM), which suggested that

the "DFG" motif shifting created hydrophobic pocket in Abl kinase could only tolerate medium size of hydrophobic moiety.

Table 1. Anti-proliferation efficacies against intact and isogenic cancer cell lines of dihydropyrimidopyrimidine derivatives[a]

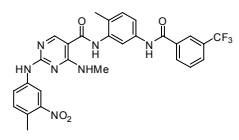
Compd	Structure	BaF3 (μM)	Tel-Abl-BaF3 (μM)	P210-BaF3 (μM)	K562 (μM)
27		0.12±0.0004	0.005±0.0003	0.014±0.0005	0.009±0.0006
11f		3.76±0.781	0.061±0.001	0.066±0.013	0.017±0.014
11g		8.00±1.136	0.050±0.007	0.019±0.004	0.018±0.005
11h		>10	0.185±0.001	0.067±0.032	0.029±0.006
11j		5.60±0.0002	0.009±0.0004	0.015±0.002	0.007±0.0002
11k		8.38±2.569	0.292±0.0014	0.126±0.003	0.030±0.0009
11l		7.52±0.778	0.105±0.000	0.055±0.044	0.018±0.003
11m		>10	0.288±0.0027	0.132±0.009	0.029±0.010

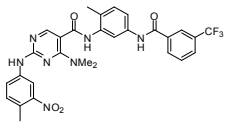
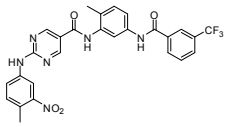
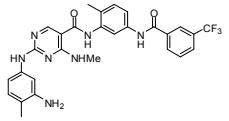
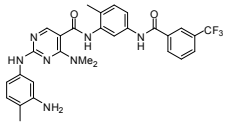
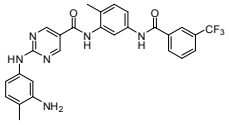
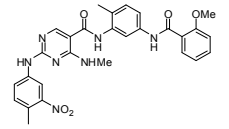
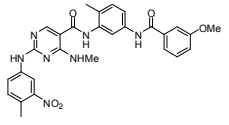
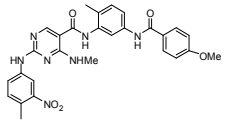
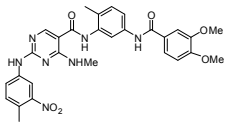
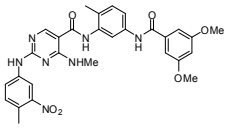
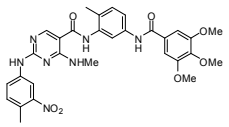
11n		>10	0.020±0.001	0.014±0.006	0.019±0.001
11o		>10	0.042±0.005	0.030±0.004	0.028±0.008
12a		0.44±0.093	0.005±0.000	0.005±±0.0002	0.003±0.000
12b		>10	0.039±0.007	0.046±0.013	0.023±0.001
12c		>10	0.031±0.0062	0.042±0.006	0.026±0.0021
12d		>10	0.096±0.0062	0.12±0.006	1.3±0.236
12e		>10	0.006±0.0001	0.014±0.005	0.0688±0.0012
12f		>10	1.380±0.021	1.26±0.03	0.45±0.0022
12g		3-10	0.100±0.1	0.15±0.005	0.019±0.0007
12h		>10	2.043±0.035	2.705±0.327	0.220±0.038
12i		>10	0.015±0.0003	0.032±0.003	0.013±0.001

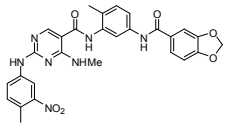
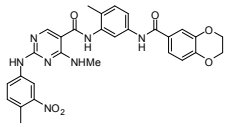
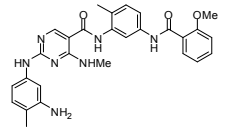
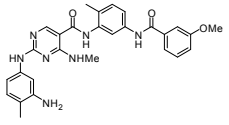
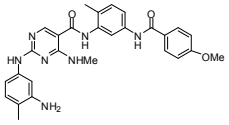
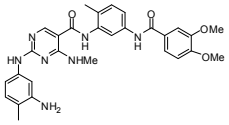
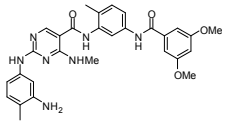
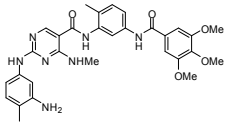
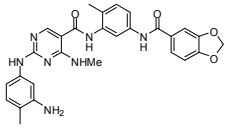
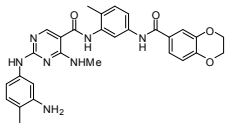
[a] all GI_{50} values are presented as mean±SEM (n=3).

Despite the similar potencies of **12a** against the model cell lines as **27**, the narrow selectivity window between the parental BaF3 and isogenic BaF3 cell lines led us to explore more of the "center" moiety by opening the cyclic urea ring of **12a**, which presumably would increase the flexibility and improve the solubility. Removal of the carbonyl group offered **18a,** which displayed potent anti-proliferation efficacy against K562 cells (GI_{50}: 0.014 μM) and P210-BaF3 cells (GI_{50}: 0.007 μM) meanwhile exhibited good selectivity over parental BaF3 cells (GI_{50}: >10 μM) (Table 2). Either introduction of one more methyl group at this position (**18b**) or removal of the 4-methylamino group (**18c**) resulted in significant activity loss against K562 cells (GI_{50}: 1.211 μM and 0.33 μM). Comparing to **18a-c**, the nitro group bearing compounds **17a-c** all resulted in losing their potency slightly or significantly. We then turned our attention to the "tail" part of **18a** scaffold. Replacement of the 3-trifluoromethyl group with 3-methoxy group in the tail phenyl ring (**25b** and **26b**) dramatically decreased their potency against K562 cells (GI_{50}: 0.48 μM and 0.33 μM, respectively). Shifting the 3-methoxy group to 2-postion (**25a**, **26a**), 4-position (**25c**, **26c**) or bearing dimethoxy group (**25d**, **25e**, **26d**, **26e**), trimethoxy group (**25f**, **26f**), [1,3]dioxo (**25g**, **26g**) and [1,4]dioxine (**25h**, **26h**) substituents in the tail phenyl ring all led to the significant activity loss.

Table 2. Anti-proliferation efficacies against intact and isogenic cancer cell lines of compound **18a** and its analogs[a]

Compd	Structure	BaF3 (μM)	Tel-ABL -BaF3 (μM)	P210-BaF3 (μM)	K562 (μM)
17a		>10	0.087±0.006	0.036±0.006	0.052±0.011

17b		>10	>10	>10	>10
17c		8.70±3.655	1.400±0.059	1.20±0.096	0.38±0.027
18a		>10	0.053±0.0012	0.007±0.002	0.014±0.001
18b		>10	1.030±0.200	1.849±0.196	1.211±0.031
18c		>10	0.590±0.247	0.47±0.055	0.33±0.025
25a		>10	>10	>10	>10
25b		6.31±0.1	0.983±0.067	1.1±0.154	0.48±0.021
25c		>10	2.900±0.188	3.6±0.399	1.53±0.110
25d		7.30±1.292	1.400±0.176	1.6±0.100	0.68±0.045
25e		>10	2.034±0.112	0.294±0.083	1.07±0.061
25f		>10	0.931±0.08	1.5±0.171	0.43±0.063

25g		>10	1.290±0.040	0.214±0.095	0.499±0.030
25h		>10	4.240±0.0763	4.1±0.617	0.32±0.017
26a		>10	5.20±0.202	6.4±0.212	1.98±0.351
26b		>10	0.524±0.003	0.57±0.097	0.33±0.155
26c		>10	1.300±0.017	2.1±0.181	0.61±0.079
26d		>10	1.100±0.259	1.6±0.174	0.38±0.078
26e		>10	0.628±0.004	0.311±0.084	0.230±0.151
26f		>10	0.445±0.111	0.67±0.117	0.25±0.047
26g		>10	0.570±0.010	0.124±0.020	0.353±0.006
26h		>10	0.787±0.129	1.1±0.153	0.32±0.086

[a] all GI_{50} values are presented as mean±SEM (n=3).

Since compound **18a** exhibited the best activity and selectivity profile, we then moved forward to study the binding mode of **18a** with ABL kinase via molecular modeling. The model

illustrated that it did prefer to adopt a type II binding mode as designed. The aminopyrimidine formed two hydrogen bonds with Met318 in the hinge area (Figure 3A). The Glu286 residue in the c-Helix and "DFG" residue Asp381 formed two typical hydrogen bonds with amide linkage between the "center" moiety and the "tail" moiety. The gatekeeper residue Thr315 formed a hydrogen bond with the carbonyl group linking the aminopyrimidine moiety and the "tail" part. Furthermore, Tyr253 also formed a hydrogen bond with the amino group in the "head" moiety, which provided the explanation for the activity difference between **17a-c** and **18a-c**. The hydrophobic pocket in the hinge binding area is shallow and flat, which explained that why both aromatic and aliphatic rings and chains could be tolerated (Figure 3B). 3-Trifluoromethylbenzene in the "tail" occupied the hydrophobic pocket formed by "DFG" motif through Van der Waals interactions (Figure 3C). In addition, Leu248, Val256 and Tyr253 formed a small hydrophobic pocket, which could accommodate the aminomethyl group in the aminopyrimidine "center" moiety. And this could explain the reason that compound **18b** and **18c** lost the activity (Figure 3D).

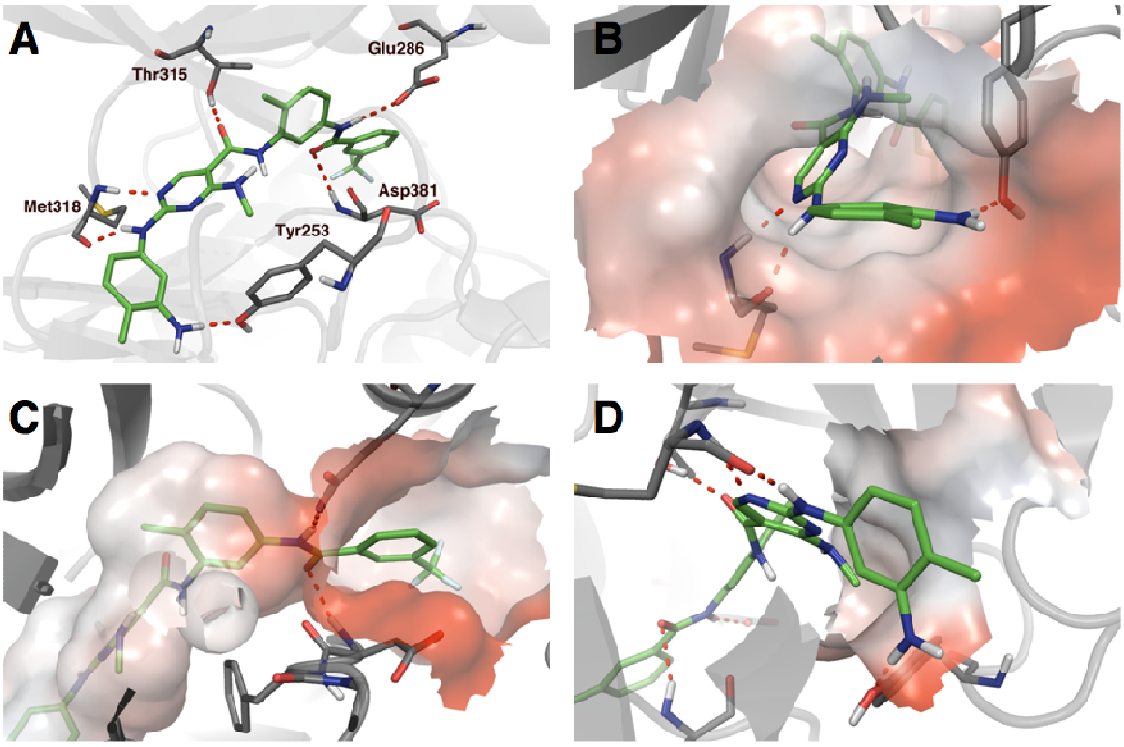
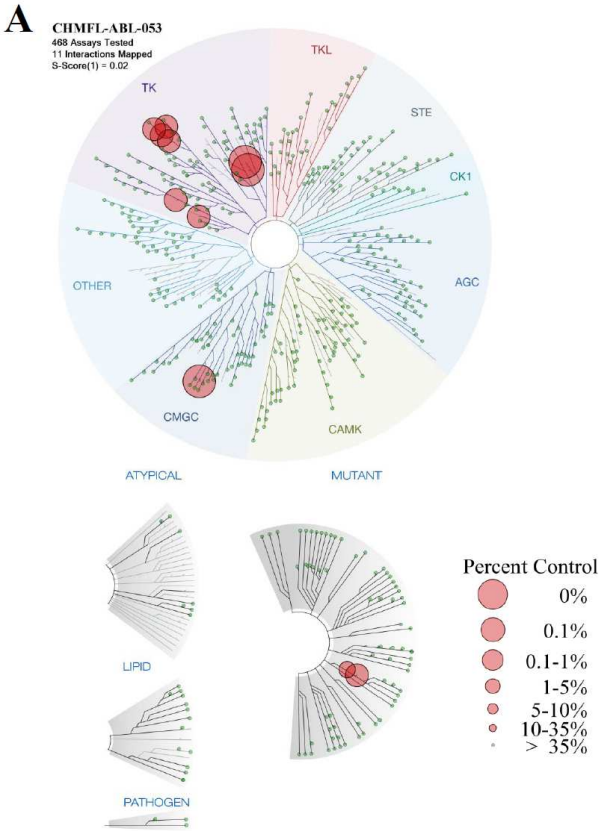


Figure 3. Compound **18a** was docked into Abl kinase (PDB ID: 2HYY). Hydrogen bonds are indicated by red dashed lines to key amino acid residues. (A) Cartoon view of the binding mode of **18a** with ABL1 kinase. (B) Solid surface view of the shallow hydrophobic pocket located adjacent to hinge binding area. (C) Solid surface view of the hydrophobic pocket formed by D(out)FG inactive conformation. (D) Solid surface view of the small hydrophobic pocket formed by Leu248, Val256 and Try253 to accommodate the methylamine moiety.

We next examined the kinome wide selectivity profile of **18a** (1 μM) on the DiscoveRx's KinomeScan profiling platform. The results demonstrated that **18a** was highly selective among 468 kinases tested and exhibited an S score (1)=0.02 (Figure 4 and Supplemental Table 1). The data also indicated that **18a** might had strong binding (with percent control number less than 1) against BLK, DDR1, DDR2, EPHA8, EphB6, HCK, LCK, p38α and SRC kinases. Further confirmation with Invitrogen SelectScreen biochemical assay revealed that **18a** exhibited an IC_{50} of 70 nM against ABL1 kinase, meanwhile also strongly inhibited p38α (IC_{50}: 62 nM) and SRC kinase (IC_{50}: 90 nM) (Table 3). However it was less potent to DDR1 (IC_{50}: 292 nM) and DDR2 (IC_{50}: 457 nM). Previous reports also showed that Dasatinib and Nilotinib had strong binding to

p38α (Kd: 27 nM and 460 nM respectively) (Supplemental Table 2). Bosutinib and Dasatinib showed strong binding to SRC (Kd: 1 nM and 0.21 nM respectively). In addition, **18a** did not exhibit apparent potency against c-KIT kinase (IC_{50}: over 10000 nM), which is the common off-target for clinically used BCR-ABL inhibitors Imatinib, Nilotinib, Botutinib and Dasatinib (Table 3 and Supplemental Table 2). In the TEL-fused isogenic BaF3 cells, **18a** displayed great selectivity between the BCR-ABL and other potential off-targets including SRC, DDR1, DDR2, LCK, BLK and HCK (Table 4). We also tested **18a** against a variety of clinically important mutations of ABL in the p210 fused BaF3 assay system. Interestingly, it was sensitive against ABL F317L, F317I and M351T mutants but was relatively resistant against E255K, Q252H, Y253F, H369P mutants and completely lost the activity to the gatekeeper mutant T315I (Table 4).

A



B

Assay Label	Assay Group	% Ctrl
BLK	TK	0.55
DDR1	TK	0
DDR2	TK	0
EPHA8	TK	0.8
EPHB6	TK	0.95
HCK	TK	0.7
KIT(L576P)	MUTANT	0.8
KIT(V559D)	MUTANT	1
LCK	TK	0.3
P38-α	CMGC	0
SRC	TK	0.5

Figure 4. Kinome wide selectivity profiling of **18a** with DiscoveRx KinomeScan technology. Measurements were performed at a concentration of 1 μM of the inhibitor. The affinity was defined with respect to a DMSO control. (A). Treespot demonstration of **18a**'s selectivity in 468 kinase targets. (B). Other targets that demonstrated strong binding to **18a** with a percent control number less than 1.

Table 3. Invitrogen SelectScreen biochemical characterization of **18a** (values=mean±SEM, n=2)

Kinase	**18a** (IC_{50}: nM)
ABL1	70±5
DDR1	292±53
DDR2	457±23
c-KIT	>10000
P38	62±6
SRC	90±3

Table 4. Confirmation of target inhibition revealed in the KinomeScan with isogenic BaF3 cell lines[a]

Cell line	**18a** (μM)	Imatinib (μM)	Nilotinib (μM)	Dasatinib (μM)
Parental BaF3	>10	6.7±0.2	2.1±0.05	>10
BaF3/p210	0.007±0.004	0.38±0.03	0.004±0.0005	0.003±0.0001
TEL-SRC	0.2±0.005	2.1±0.05	0.47±0.02	<0.0003
TEL-DDR1-BaF3	3.3±0.08	3-10	1.1±0.02	10-3
TEL-DDR2-BaF3	>10	7.7±0.5	1.4±0.05	10-3
TEL-LCK-BaF3	0.6±0.01	0.5±0.06	0.87±0.21	0.001±0.00009
TEL-BLK-BaF3	1.1±0.17	4.1±1.6	1.3±0.03	0.005±0.00002
TEL-HCK-BaF3	0.98±0.004	9.7±2	4.1±0.2	0.039±0.0001

p210-E255K-BaF3	0.313±0.032	1.93±0.253	0.021±0.006	0.017±0.0004
p210-F317L-BaF3	0.045±0.002	2.169±0.039	0.202±0.01	0.014±0.0009
p210-F317I-BaF3	0.073±0.008	0.855±0.081	0.0546±0.004	0.01±0.001
p210-M351T-BaF3	0.045±0.002	0.625±0.253	0.017±0.001	0.003±0.0005
p210-Q252H-BaF3	0.14±0.033	0.659±0.072	0.023±0.001	0.008±0.001
p210-Y253F-BaF3	0.363±0.001	>10	1.093±0.029	0.001±0.0006
p210-H369P-BaF3	0.440±0.029	1.69±0.177	0.025±0.0044	0.004±0.0002
p210-T315I-BaF3	9.25±0.01	>10	>10	9.94±4.0

[a] all GI_{50} values are presented as mean±SEM (n=3).

It is intriguing that compound **18a** uniquely abolished c-KIT kinase activity in comparison to other ABL inhibitors. In order to understand this from the structure point of view, we then docked compound **18a** into c-KIT kinase (PDB ID: 1T46) (Figure 5). The modeling results suggested that **18a** might adopt a similar type II binding mode as it binds to ABL kinase (Figure 5A). However, when c-KIT and ABL kinase were superimposed, we found that in ABL kinase the Tyr253 located in the p-loop which could provide a key hydrogen bond with the methyl aniline moiety in the "head" part of **18a** was replaced by Gly596 residue in c-KIT kinase (Figure 5B). Lacking of this key hydrogen bond may result in the loss of inhibitory potency of **18a** against c-KIT kinase.

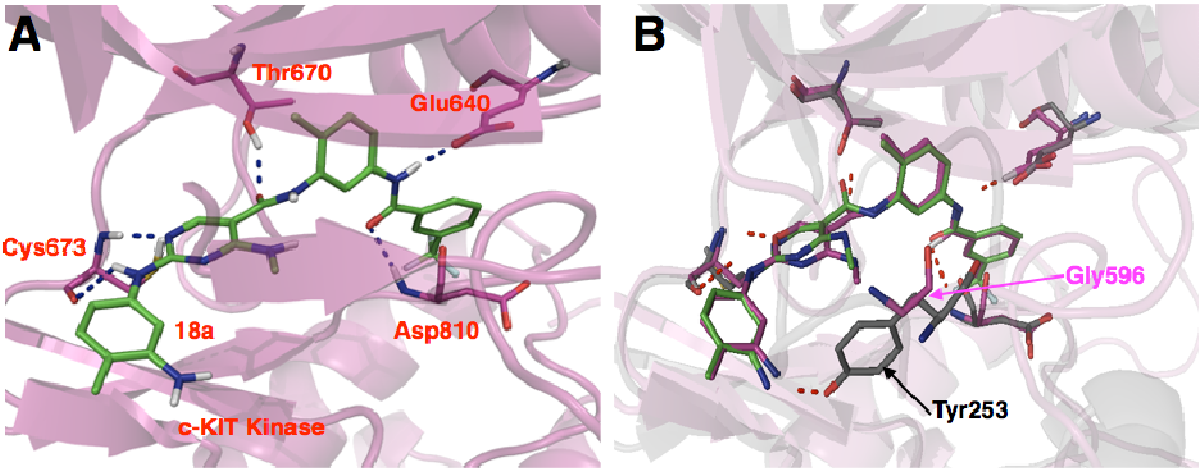


Figure 5. Comparison of the binding modes of compound **18a** between ABL and c-KIT kinase. (A). Compound **18a** was docked into c-KIT kinase (PDB ID: 1T46). C-KIT protein was shown in magenta. Compound **18a** was displayed as Carbon in green, Nitrogen in blue and Oxygen in red. The key binding amino acid residues from the protein were displayed as Carbon in magenta, Nitrogen in blue and Oxygen in red. (B). Superimposition of c-KIT (in magenta) and ABL (in gray, PDB ID: 2HYY) kinase in complex with compound **18a**.

We then compared **18a** with Imatinib and Nilotinib in a panel of intact leukemia cancer cell lines including the CML and AML (Table 5). **18a** exhibited potent anti-proliferation efficacies against all of the three BCR-ABL driven CML cell lines K562 (GI_{50}: 14 nM), KU812 (GI_{50}: 25 nM) and MEG-01 (GI_{50}: 16 nM) but not other AML cell lines, implying strong and selective on-target effects. In addition, **18a** did not display any apparent activity against CHL cell line, indicating a good non-specific toxicity profile.

Table 5. Anti-proliferation effects of **18a** against varieties of intact cancer cell lines[a]

Cell line	Cell type	**18a** (μM)	Imatinib (μM)	Nilotinib (μM)	Dasatinib (μM)
K562	CML	0.014±0.006	0.14±0.001	0.002±0.0002	<0.0003
KU812	CML	0.025±0.002	0.16±0.005	0.001±0.0002	<0.0003
MEG-01	CML	0.016±0.0056	0.24±0.0267	0.016±0.0014	<0.0003

MV4-11	AML	8±0.4	>10	>10	3.6±0.1
MOLM14	AML	>10	>10	>10	2.3±0.09
U937	AML	>10	>10	>10	>10
HEL	AML	>10	5.3±0.2	3.9±0.05	5.3±0.4
CHL	Hamster lung cell	>10	>10	4.2±0.6	0.27±0.1

[a] all GI_{50} values are presented as mean±SEM (n=3).

We then investigated **18a**'s effects on the BCR-ABL mediated signals in BCR-ABL driven CML cell lines K562, KU812 and MEG-01 (Figure 6). Compound **18a** almost completely suppressed BCR-ABL kinase auto-phosphorylation at Y245 site in K562, KU812 and MEG-01 at the concentration of 300 nM. BCR-ABL kinase downstream mediator Stat5, CrkL and ERK phosphorylation was also significantly inhibited in a concentration-dependent manner. Interestingly, unlike Imatinib which had no effect on CrkL's phosphorylation, both **18a** and Dasatinib could affect CrkL's phosphorylation, which indicated a different pharmacology profile among them. The results also showed that phosphorylation of SRC kinase was much less potently inhibited than phosphorylation of BCR-ABL by **18a**, though they showed similar sensitivity to **18a** in the biochemical assay. This indicated that the BCR-ABL inhibitory activity of **18a** majorly contributed to the anti-proliferative effect in these CML cell lines. P38α phosphorylation was potently inhibited which is in accordance to its biochemical inhibitory activities. In addition, even in the early 12 h, **18a** could dose-dependently arrest the cell cycle progression in the G0/G1 phase in these cells (Figure 7A). Upon 24 or 48 hours drug treatment, 100 nM concentration of **18a** could significantly induce the apoptotic cell death (Figure 7B).

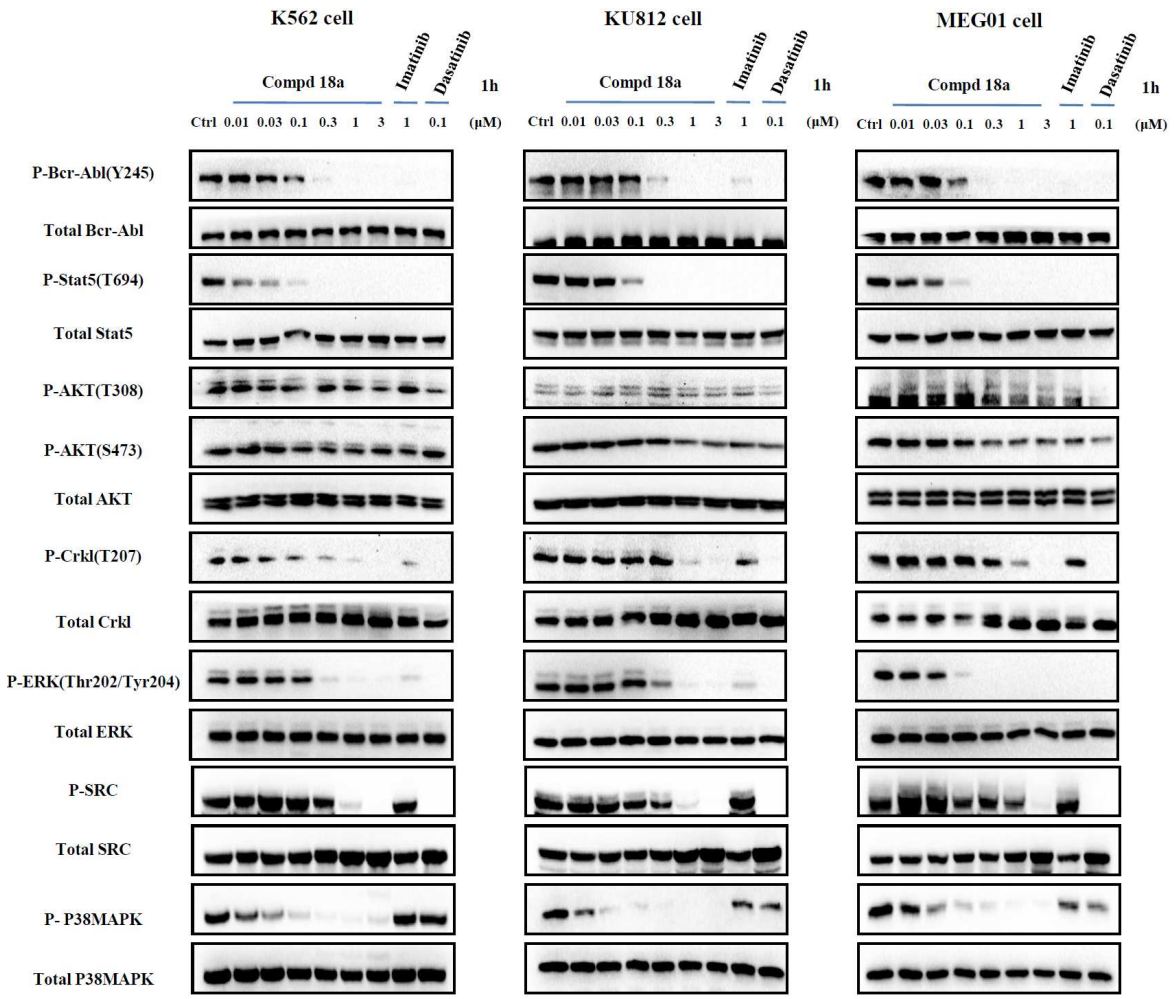


Figure 6. Compound **18a**'s effects on BCR-Abl kinase mediated signaling pathway in KU812, K562 and MEG-01 cancer cell lines. Cells were treated with **18a** at the indicated concentrations for 1 h, and whole cell lysates were then subjected to Western blot analyses.

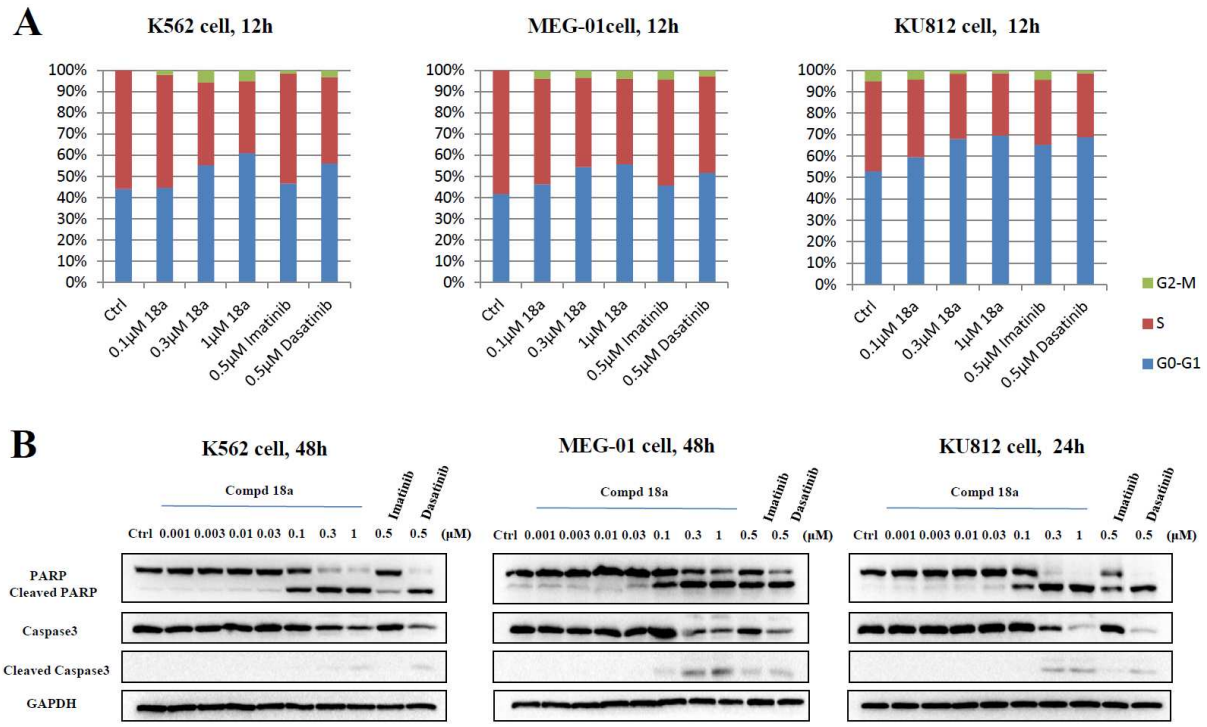


Figure 7. (A) **18a** arrested the cell cycle progression. (B) **18a** induced apoptosis in K562, KU812 and MEG-01 cell lines.

In the study of PK profiling in rats, compound **18a** exhibited good systemic exposure (AUC=1715.51 ng/mL*hr, C_{max}=367.61 ng/mL), favorable oral bioavailability (F=24.19%) and acceptable half-life ($T_{1/2}$=4.33 hr) following oral administration of a single dose of 10 mg/kg (Table 6).

Table 6. Pharmacokinetic study of **18a** on Sprague Dawley rats[a]

	$t_{1/2}$ hr	T_{max} hr	C_{max} ng/mL	$AUC_{(0-t)}$ ng/mL*hr	$AUC_{(0-\infty)}$ ng/mL*hr	Vz mL/kg	CLz mL/hr/kg	$MRT_{(0-\infty)}$ hr	F%
IV 1 mg/kg Mean	2.82	0.02	2395.22	635.98	720.69	6025.13	1538.39	3.1	NA
SD (n=3)	0.53	0.00	449.56	208.84	256.99	1632.22	642.39	0.69	NA

PO 10 mg/kg Mean	4.33	1.00	367.61	1715.51	1743.43	NA	NA	5.51	24.19
SD (n=3)	1.11	0.87	202.21	1083.42	1091.08	NA	NA	0.50	NA

[a]Compound **18a** was formulated as a clear solution in 5% DMSO, 40% PEG400 and 55% of 20% HP-β-CD in water for intravenous and oral administration.

The in vivo anti-tumor study of **18a** was performed in the K562 cell inoculated xenograft mouse model. After 16 days continuous treatment, compound **18a** dose-dependently inhibited the growth of the K562 tumor and a 50mg/kg/day dosage could almost completely suppress the tumor progression (Figure 8A). All doses of **18a** were well tolerated, with no mortality and no significant body weight loss observed (Figure 8B). Compound **18a** displayed obvious antitumor efficacy (TGI=48.3%) at 50 mg/kg/day dosage (Figure 8C, D). In addition, the immunohistochemistry (IHC) stain revealed that the proliferation was effectively inhibited (Ki-67 lane) and significant apoptosis was induced (TUNEL lane) in the tumor (Figure 8E).

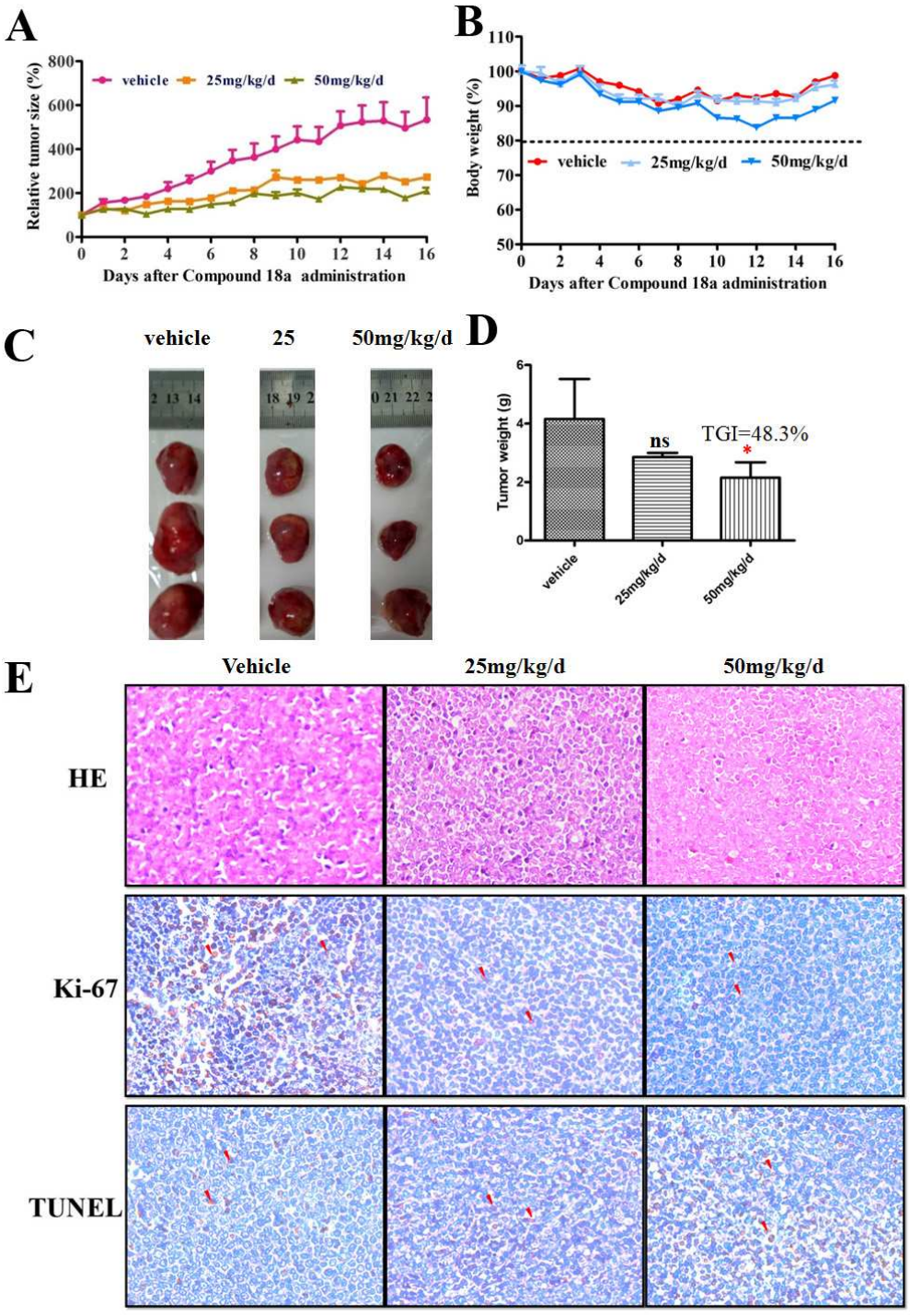


Figure 8. Compound **18a**'s anti-tumor efficacy in K562 xenograft model. Female nu/nu mice bearing established control group, K562 tumor xenografts were treated with **18a** at 25.0, 50.0 mg/kg/d, or vehicle. Daily oral administration was initiated when K562 tumors had reached a size of 200 to 400 mm^3. Each group contained 5 animals. Data=mean ± SEM. (A) Body weight measurement from K562 xenograft mice after **18a** administration. Initial body weight was set as

100%. (B) Tumor size measurements from K562 xenograft mice after **18a** administration. Initial tumor size was set as 100%. (C) Representative photographs of tumors in each group after 25.0 or 50.0 mg/kg/d **18a** or vehicle treatment. (D) Comparison of the final tumor weight in each group after 16-day treatment period. (E) Representative micrographs of hematoxylin and eosin (HE), Ki-67 and TUNEL staining of tumor tissues with **18a** treatment compared to the vehicle group. Note the specific nuclear staining of cells with morphology consistent with proliferation and apoptosis (E, red arrow).

CONCLUSION

Starting from a multiple targets dihydropyrimidopyrimidine scaffold based compound **27** (GNF-7) bearing high BCR-ABL potency, we used a focused medicinal chemistry approach guided by computer-aided drug design to obtain an aminopyrimidine scaffold based compound **18a** (CHMFL-ABL-053) via 7 steps of chemical syntheses (11% overall yield), which possessed highly potent anti-proliferative efficacy against BCR-ABL driven CML cell lines and exhibited good safety window against other leukemia cell lines such as AML. Compound **18a** displayed a high selectivity profile. Besides the ABL kinase, it also inhibited structurally related SRC kinase and p38α kinase, which is desired and might contribute positively to exert synergistic anti-leukemic effect of **18a** since SRC kinase is downstream of BCR-ABL and contributes to the proliferation and survival of myeloid cell line and p38α kinase is involved in the BCR-ABL inhibitor induced apoptosis pathway.[18, 19] Comparing to clinically used BCR-ABL inhibitors Imatinb, Nilotinib, Bosutinib and Dasatinib, compound **18a** completely abolished the c-KIT kinase inhibitory activity and exhibited better selectivity against DDR1/2 kinases. In addition, **18a** also showed suitable PK profile and potent in vivo anti-tumor efficacy. It is worthy to note that although compound **18a** exhibits a good selectivity among kinome, we cannot exclude the possibility that it may also affect other effective targets that may also contribute to its anti-leukemia effect, like Imatinib has been reported to inhibit the quinone oxidoreductase2

(NQO2).[20] Currently **18a** is under extensive preclinical safety evaluation and it might be a potential useful pharmacological candidate supplementary to the current BCR-ABL target therapies for the treatment of CML.

Experimental Procedures

Chemical Synthesis. All reagents and solvents were purchased from commercial sources and were used as received, unless specified otherwise, or prepared as described in the literature. All moisture-sensitive reactions were carried out using dry solvents under ultra pure argon protection. Glassware was dried in an oven at 140 °C for at least 12 h prior to use, and then assembled quickly while hot, sealed with rubber septa, and allowed to cool under a stream of argon. Reactions were stirred magnetically using Teflon-coated magnetic stirring bars. Commercially available disposable syringes were used for transferring the reagents and solvents. LC/MS 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 XDB-C18 column (4.6×50 mm, 1.8 μm) using a water/acetonitrile (each with 0.2% (v/v) formic acid) gradient at a flow rate at 0.4 mL/min. ^{1}H and ^{13}C spectra were recorded on a Bruker 400 MHz NMR spectrometer. Chemical shifts are expressed in ppm. In the NMR tabulation, s indicates singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. Flash column chromatography was conducted using silica gel (Silicycle 40−64 μm). The purities of all compounds were determined to be >95% by HPLC.

General Method A:

N-(3-amino-4-methylphenyl)-3-(trifluoromethyl)benzamide (3a) To a solution of 4-methyl-3-nitroaniline (2.82 g, 18.5 mmol, 1.00 equiv) in DCM (30 mL) was added TEA (2.84 mL, 20.35 mmol, 1.10 equiv). Then a solution of 3-(trifluoromethyl)benzoyl chloride (5.0 g, 19.5 mmol, 1.05 equiv) in DCM (15 mL) was dropwise added in 30 min at 0 °C under argon. The reaction mixture was stirred at 0 °C for 2 hours, then was allowed to warm to room temperature for overnight (10 h). The resulting solution was diluted with DCM (100 mL) and EtOAc (20 mL), washed with 1 M HCl (2×100 mL), 1 M NaOH (2×100 mL), brine (100 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded the crude product **2a**, which was used in the next step without further purification. To a solution of **2a** (18.5 mmol, 1.00 equiv) in

methanol (20 mL) was added 10% Pd/C (0.2 g) at room temperature under argon. Then the reaction mixture was stirred under a balloon of hydrogen for 20 h. The resulting mixture was filtered through a pad of celite and washed with methanol. Evaporation of the filtrate provided the crude product, which was purified by silica gel flash chromatography (eluting with MeOH in DCM 0-4%) to give **3a** as a white solid (2.83 g, two steps yield 54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.30 (d, J = 14.1 Hz, 2H), 7.95 (d, J = 7.1 Hz, 1H), 7.78 (t, J = 7.2 Hz, 1H), 6.93 (d, J = 7.7 Hz, 1H), 6.64 (s, 1H), 6.47 (d, J = 7.7 Hz, 1H), 4.97 (s, 2H), 2.09 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.12, 147.36, 136.66, 136.15, 132.09, 130.95, 130.15, 129.90, 129.58, 128.39, 124.68, 120.93, 112.92, 112.85, 17.39. LC-MS (ESI, m/z): 295.0992 $[M+H]^+$.

N-(3-amino-4-methylphenyl)biphenyl-3-carboxamide (3b) (Method A) yield 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.24 (s, 1H), 7.96 (d, J = 7.4 Hz, 1H), 7.85 (d, J = 7.4 Hz, 1H), 7.78 (d, J = 7.1 Hz, 2H), 7.62 (d, J = 7.5 Hz, 1H), 7.43 (d, J = 7.1 Hz, 1H), 7.22 (s, 1H), 6.92 (s, 2H), 4.92 (s, 2H), 2.08 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.48, 147.02, 140.69, 140.10, 138.07, 136.52, 130.20, 129.92, 129.47, 128.26, 127.40, 127.28, 126.23, 117.41, 109.47, 107.08, 17.50. LC-MS (ESI, m/z): 303.1425 $[M+H]^+$.

N-(3-amino-4-methylphenyl)-3,5-dimethylbenzamide (3c) (Method A) yield 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 7.57 (s, 2H), 7.20 (s, 1H), 7.17 (s, 1H), 6.88 (s, 2H), 4.86 (s, 2H), 2.36 (s, 6H), 2.06 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.84, 146.92, 138.20, 137.86, 135.90, 132.95, 130.14, 125.78, 117.20, 109.37, 106.95, 21.34, 17.46. LC-MS (ESI, m/z): 255.1425 $[M+H]^+$.

N-(3-amino-4-methylphenyl)-3,5-di-tert-butylbenzamide (3d) (Method A) yield 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.92 (s, 1H), 7.77 (s, 2H), 7.59 (s, 1H), 7.11 (s, 1H), 6.89 (s, 2H), 2.07 (s, 3H), 1.34 (d, J = 13.6 Hz, 18H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.31, 150.80, 146.92, 138.04, 135.41, 130.08, 125.26, 122.13, 117.31, 109.82, 107.46, 35.16, 31.68, 17.48. LC-MS (ESI, m/z): 339.2365 $[M+H]^+$.

N-(3-amino-4-methylphenyl)-3-methylbenzamide (3e) (Method A) yield 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 8.07 (s, 1H), 7.79 (dd, J = 8.2, 4.8 Hz, 2H), 7.65 (d, J = 8.2 Hz, 1H), 7.41 (d, J = 4.5 Hz, 2H), 7.29 (d, J = 8.3 Hz, 1H), 2.40 (s, 3H), 2.36 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.22, 138.45, 138.17, 135.05, 132.76, 131.70, 130.93, 128.75, 128.70, 127.37, 125.41, 120.43, 115.90, 21.43, 17.20. LC-MS (ESI, m/z): 241.1272 $[M+H]^+$.

ethyl 4-(methylamino)-2-(methylthio)pyrimidine-5-carboxylate (5) To a solution of ethyl 4-chloro-2 (methylthio)pyrimidine -5- carboxylate (5.00 g, 21.5 mol, 1.0 equiv) in THF (100 mL) was added TEA (6.9 mL, 49.45 mmol, 2.3 equiv) and methylamine hydrochloride (2.70 g, 49.45 mmol, 2.3 equiv) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 1 hour, then it was allowed to warm to room temperature for overnight (14 h). The reaction mixture was concentrated to remove the THF. The residue was diluted with water (100 mL), extracted with EtOAc (3×80 mL). The combined organic layers were washed with water (2×80 mL), brine (80 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded the crude **5** as a white solid (4.6 g, 94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (d, *J* = 1.7 Hz, 1H), 8.23 (s, 1H), 4.37 – 4.20 (m, 2H), 2.97 (dd, *J* = 4.8, 1.6 Hz, 3H), 2.48 (d, *J* = 1.7 Hz, 3H), 1.30 (td, *J* = 7.1, 1.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 175.41, 166.23, 160.16, 158.09, 101.25, 60.98, 40.65, 40.44, 40.23, 40.02, 39.82, 39.61, 39.40, 27.75, 14.50, 14.01. LC-MS (ESI, m/z): 228.0730 $[M+H]^+$.

4-(methylamino)-2-(methylthio)pyrimidine-5-carbaldehyde (**7**) To a solution of ethyl 4-(methylamino)-2-(methylthio)pyrimidine-5-carboxylate (3.30 g, 14.5 mmol, 1.00 equiv) in anhydrous THF (30 mL) was added LAH (2.4 M in THF, 8.7 mL, 17.45 mmol, 1.20 equiv) at 0 °C under argon. The reaction mixture was then stirred at 0 °C for 1h, and was slowly to warm to room temperature for 14h. The resulting mixture was concentrated to remove the solvent. The residue was quenched by ice-cold water, extracted with DCM (3×50 mL). The combined organic layers were washed with water (2×50 mL), brine (50 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded the crude **6**. To a solution of **6** (14.5 mmol, 1.00 equiv) in anhydrous DCM (60 mL) was added activated MnO_2 (12.6 g, 145.0 mmol, 10 equiv) at room temperature under argon. Then the reaction mixture was stirred at room temperature for 6h. The resulting mixture was filtered and washed with DCM (2×30 mL). Evaporation of the solvent afforded the crude product, which was purified by flash column chromatography (eluting with MeOH in DCM 0-2%) to give **7** as a yellow solid (1.95 g, two steps yield 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.68 (s, 1H), 8.27 (s, 1H), 3.09 (d, *J* = 5.0 Hz, 3H), 2.54 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.79, 177.48, 162.76, 159.43, 109.43, 27.10, 14.27. LC-MS (ESI, m/z): 184.0470 $[M+H]^+$.

General Method B:

N-(4-methyl-3-((4-(methylamino)-2-(methylthio)pyrimidin-5-yl)methylamino)phenyl)

-3-(trifluoromethyl)benzamide (8a) To a solution of 4-(methylamino)-2-(methylthio)pyrimidine-5-carbaldehyde (1.10 g, 6.0 mmol, 1.0 equiv) and N-(3-amino-4-methylphenyl)-3-(trifluoromethyl)benzamide (1.76 g, 6.0 mmol, 1.0 equiv) in methanol (30 mL) was added acetic acid (0.7 mL, 12.0 mmol, 2.00 eqiv) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 30 min, then $NaBH_3(CN)$ was portion added. The reaction mixture was stirred at 0 °C for 1h, then was allowed to warm to room temperature for 24 h. The resulting mixture was concentrated to remove the solvent. The residue was diluted with water (50 mL), extracted with EtOAc (3×50 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and dried over (anhydrous $MgSO_4$). Evaporation of the solvent afforded the crude product, which was purified by flash column chromatography (eluting with MeOH in DCM 0-2%) to yield **8a** as an off white solid (2.53 g, 92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.22 (s, 1H), 8.25 (s, 3H), 7.93 (s, 1H), 7.88 (s, 1H), 7.76 (s, 1H), 7.14 (s, 1H), 7.06 (s, 1H), 6.99 (s, 2H), 4.13 (s, 2H), 2.93 (s, 3H), 2.42 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.82, 164.25, 160.60, 152.67, 146.42, 138.25, 136.72, 132.20, 130.17, 130.02, 128.30, 124.69, 118.37, 110.91, 108.88, 102.51, 41.02, 28.01, 17.76, 13.76. LC-MS (ESI, m/z): 462.1453 $[M+H]^+$.

N-(4-methyl-3-((4-(methylamino)-2-(methylthio)pyrimidin-5-yl)methylamino)phenyl) biphenyl l-3-carboxamide (8b) (Method B) yield 74%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.16 (s, 1H), 7.87 (dd, *J* = 14.4, 7.4 Hz, 3H), 7.77 (d, *J* = 7.3 Hz, 2H), 7.62 – 7.57 (m, 1H), 7.56 – 7.48 (m, 3H), 7.43 (d, *J* = 7.0 Hz, 1H), 7.14 (s, 1H), 7.06 (d, *J* = 7.8 Hz, 1H), 7.01 (s, 1H), 6.96 (d, *J* = 7.9 Hz, 1H), 5.44 (s, 1H), 4.11 (s, 2H), 2.91 (d, *J* = 4.0 Hz, 3H), 2.41 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.79, 165.67, 160.57, 152.69, 146.37, 140.70, 140.07, 138.59, 136.57, 130.14, 129.95, 129.48, 128.27, 127.40, 127.22, 126.25, 118.03, 110.95, 108.76, 102.45, 41.10, 27.81, 17.75, 13.78. LC-MS (ESI, m/z): 470.1943 $[M+H]^+$.

3,5-dimethyl-N-(4-methyl-3-((4-(methylamino)-2-(methylthio)pyrimidin-5-yl)methylamino) phenyl)benzamide (8c) (Method B) yield 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 7.88 (s, 1H), 7.51 (s, 2H), 7.19 (s, 1H), 7.14 (s, 1H), 7.06 (d, *J* = 7.8 Hz, 1H), 6.99 (s, 1H), 6.93 (d, *J* = 7.8 Hz, 1H), 5.42 (s, 1H), 4.10 (s, 2H), 2.92 (d, *J* = 2.9 Hz, 3H), 2.42 (s, 3H), 2.35 (s, 6H), 2.10 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.78, 165.99, 160.58, 152.73, 146.30, 138.71, 137.88, 135.93, 132.97, 130.08, 125.74, 117.78, 110.96, 108.61, 102.30, 41.11, 27.80, 21.33, 17.73, 13.88. LC-MS (ESI, m/z): 422.1947 $[M+H]^+$.

3,5-di-tert-butyl-N-(4-methyl-3-((4-(methylamino)-2-(methylthio)pyrimidin-5-yl) methylamino)phenyl)benzamide (8d) (Method B) yield 59%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 7.88 (s, 1H), 7.69 (s, 2H), 7.59 (s, 1H), 7.14 (s, 1H), 7.04 (s, 1H), 6.95 (s, 2H), 5.42 (s, 1H), 4.12 (s, 2H), 2.92 (s, 3H), 2.42 (s, 3H), 2.12 (s, 3H), 1.35 (s, 18H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.82, 166.72, 160.60, 152.81, 150.84, 146.33, 138.61, 135.61, 130.06, 125.36, 122.08, 117.94, 110.98, 109.07, 102.81, 41.12, 35.16, 31.67, 27.81, 17.77, 13.79. LC-MS (ESI, m/z): 506.2882 $[M+H]^+$.

3-methyl-N-(4-methyl-3-((4-(methylamino)-2-(methylthio)pyrimidin-5-yl) methylamino)phenyl)benzamide (8e) (Method B) 69%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 7.87 (s, 1H), 7.72 (s, 2H), 7.38 (s, 2H), 7.13 (s, 1H), 7.07 (d, *J* = 7.1 Hz, 1H), 6.99 (s, 1H), 6.94 (d, *J* = 7.7 Hz, 1H), 5.43 (s, 1H), 4.10 (s, 2H), 2.92 (s, 3H), 2.42 (s, 3H), 2.40 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.78, 165.88, 160.57, 152.71, 146.32, 138.67, 138.04, 135.91, 132.27, 130.10, 128.64, 128.52, 125.19, 117.86, 110.95, 108.62, 102.32, 41.10, 27.81, 21.43, 17.73, 13.79. LC-MS (ESI, m/z): 408.1791 $[M+H]^+$.

General Method C:

N-(4-methyl-3-(1-methyl-7-(methylthio)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin -3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (9a) To a solution of N-(4-methyl-3-((4-(methylamino)-2-(methylthio)pyrimidin-5-yl)methylamino)phenyl)-3-(trifluoromethyl)benzamide (2.40 g, 5.21 mmol, 1.00 equiv) in anhydrous dioxane (26 mL) was added DIPEA (2.6 mL, 15.63 mmol, 3.00 equiv) at 0 °C under argon. Then a solution of triphosgene (0.53 g, 1.77 mmol, 0.34 equiv) in dioxane (10 mL) was added. The reaction mixture was stirred at 0 °C for 1h, then was allowed to warm to room temperature for 24h. The resulting mixture was concentrated to remove the solvent. The residue was diluted with water (50 mL), extracted with EtOAc (3×50 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded the crude product, which was purified by flash column chromatography (eluting with MeOH in DCM 0-2%) to yield **9a** as a yellow solid (1.05 g, 42%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.59 (s, 1H), 8.33 (s, 1H), 8.30 (d, *J* = 7.7 Hz, 1H), 8.26 (s, 1H), 7.96 (d, *J* = 7.3 Hz, 1H), 7.86 (s, 1H), 7.79 (t, *J* = 7.5 Hz, 1H), 7.67 (d, *J* = 8.1 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 4.78 (d, *J* = 14.5 Hz, 1H), 4.59 (d, *J* = 14.6 Hz, 1H), 3.40 (s, 3H), 2.53 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (101 MHz,

DMSO-d_6) δ 170.39, 164.32, 156.84, 152.73, 152.23, 141.37, 138.13, 136.06, 132.26, 131.37, 131.21, 130.16, 128.56, 124.68, 120.41, 119.79, 108.22, 60.20, 28.48, 17.29, 14.36. LC-MS (ESI, m/z): 488.1235 $[M+H]^+$.

N-(4-methyl-3-(1-methyl-7-(methylthio)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)biphenyl-3-carboxamide (9b) (Method C) yield 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 8.26 (d, *J* = 11.7 Hz, 2H), 7.96 (d, *J* = 7.0 Hz, 1H), 7.90 (d, *J* = 7.0 Hz, 1H), 7.86 (s, 1H), 7.79 (d, *J* = 7.0 Hz, 2H), 7.65 (dd, *J* = 16.8, 8.1 Hz, 2H), 7.52 (d, *J* = 7.1 Hz, 2H), 7.44 (d, *J* = 6.6 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 4.79 (d, *J* = 14.5 Hz, 1H), 4.60 (d, *J* = 14.5 Hz, 1H), 3.34 (s, 3H), 2.55 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.38, 165.75, 156.90, 152.77, 152.24, 141.38, 140.81, 139.99, 138.45, 135.93, 131.19, 131.05, 130.27, 129.63, 129.50, 128.33, 127.40, 127.28, 126.22, 120.31, 119.69, 108.31, 46.93, 28.52, 17.21, 14.07. LC-MS (ESI, m/z): 496.1730 $[M+H]^+$.

3,5-dimethyl-N-(4-methyl-3-(1-methyl-7-(methylthio)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)benzamide (9c) (Method C) yield 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H), 8.26 (s, 1H), 7.86 (s, 1H), 7.66 (d, *J* = 7.9 Hz, 1H), 7.58 (s, 2H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.21 (s, 1H), 4.77 (d, *J* = 14.5 Hz, 1H), 4.58 (d, *J* = 14.6 Hz, 1H), 3.32 (s, 3H), 2.53 (s, 3H), 2.36 (s, 6H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.38, 166.08, 156.83, 152.72, 152.20, 141.31, 138.63, 138.00, 135.29, 133.30, 131.12, 130.78, 125.78, 120.10, 119.39, 108.23, 60.22, 46.92, 28.50, 21.33, 17.19, 14.06. LC-MS (ESI, m/z): 448.1735 $[M+H]^+$.

3,5-di-tert-butyl-N-(4-methyl-3-(1-methyl-7-(methylthio)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)benzamide (9d) (Method C) yield 80%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H), 8.12 (s, 1H), 7.63 (s, 3H), 7.52 (d, *J* = 8.2 Hz, 1H), 7.47 (s, 1H), 7.16 (d, *J* = 8.1 Hz, 1H), 4.62 (d, *J* = 14.4 Hz, 1H), 4.45 (d, *J* = 14.7 Hz, 1H), 3.18 (s, 3H), 2.38 (s, 3H), 1.99 (s, 3H), 1.20 (s, 18H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.41, 166.66, 156.84, 152.75, 152.27, 151.03, 141.27, 138.43, 134.72, 131.13, 130.99, 125.73, 122.13, 120.62, 120.01, 108.21, 46.61, 35.05, 31.51, 28.23, 17.28, 14.00. LC-MS (ESI, m/z): 532.2672 $[M+H]^+$.

3-methyl-N-(4-methyl-3-(1-methyl-7-(methylthio)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)benzamide (9e) (Method C) yield 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1H), 8.26 (s, 1H), 7.85 (s, 1H), 7.78 (s, 1H), 7.77 – 7.74 (m, 1H), 7.64 (d, *J* = 7.9 Hz, 1H), 7.41 (s, 2H), 7.29 (d, *J* = 8.0 Hz, 1H), 4.77 (d, *J* = 14.3 Hz, 1H), 4.58 (d, *J* = 14.6 Hz, 1H), 3.32 (s, 3H), 2.53 (s, 3H), 2.40 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-

d_6) δ 170.37, 166.00, 156.85, 152.74, 152.22, 141.32, 138.54, 138.17, 135.27, 132.62, 131.15, 130.88, 128.77, 128.52, 125.24, 120.18, 119.47, 108.26, 46.91, 28.51, 21.43, 17.20, 14.06. LC-MS (ESI, m/z): 434.1581 $[M+H]^+$.

General Method D :

N-(4-methyl-3-(1-methyl-7-(methylsulfonyl)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (10a) To a solution of N-(4-methyl-3-(1-methyl-7-(methylthio)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (480 mg, 1.00 mmol, 1.00 equiv) in DCM (20 mL) was added m-CBPA (362 mg, 2.10 mmol, 2.10 equiv) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 2h, then was allowed to warm to room temperature for 20 h. The resulting mixture was diluted with DCM (30 mL), washed with water (30 mL), brine (30 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded the crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-2%) to give the title compound **10a** as a white solid (0.36 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (s, 1H), 8.30 (s, 1H), 8.15 (s, 1H), 8.11 (d, *J* = 7.9 Hz, 1H), 7.81 (d, *J* = 1.7 Hz, 1H), 7.78 (d, *J* = 7.8 Hz, 1H), 7.60 (t, *J* = 7.8 Hz, 1H), 7.29 (s, 1H), 7.06 (d, *J* = 8.4 Hz, 1H), 4.85 (d, *J* = 16.0 Hz, 1H), 4.61 (d, *J* = 15.9 Hz, 1H), 3.54 (s, 3H), 3.37 (s, 3H), 1.74 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.13, 164.66, 158.22, 153.02, 151.52, 140.93, 138.63, 138.04, 135.30, 133.34, 131.21, 130.85, 125.77, 120.34, 119.41, 115.96, 47.17, 28.94, 21.34, 17.06. LC-MS (ESI, m/z): 520.1123 $[M+H]^+$.

N-(4-methyl-3-(1-methyl-7-(methylsulfonyl)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)biphenyl-3-carboxamide (10b) (Method D) yield 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (d, *J* = 4.2 Hz, 1H), 8.26 (d, *J* = 4.3 Hz, 1H), 8.04 – 7.88 (m, 3H), 7.86 – 7.75 (m, 2H), 7.74 – 7.62 (m, 2H), 7.60 – 7.50 (m, 2H), 7.48 – 7.40 (m, 1H), 7.40 – 7.29 (m, 1H), 5.06 – 4.91 (m, 1H), 4.79 (dd, *J* = 15.2, 4.4 Hz, 1H), 3.44 (d, *J* = 4.2 Hz, 3H), 3.38 (t, *J* = 7.2 Hz, 3H), 2.17 (d, *J* = 4.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.70, 164.68, 158.24, 153.03, 151.55, 141.00, 140.82, 140.00, 138.41, 135.88, 131.29, 131.10, 130.31, 129.66, 129.51, 128.35, 127.41, 127.30, 126.24, 120.42, 119.57, 115.98, 47.19, 28.97, 17.21. LC-MS (ESI, m/z): 528.1630 $[M+H]^+$.

3,5-dimethyl-N-(4-methyl-3-(1-methyl-7-(methylsulfonyl)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)benzamide (10c) (Method D) yield 83%. ^{1}H NMR (400 MHz,

DMSO-d_6) δ 10.24 (s, 1H), 8.60 (s, 1H), 7.89 (s, 1H), 7.63 (d, *J* = 8.2 Hz, 1H), 7.57 (s, 2H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.23 (s, 1H), 4.95 (d, *J* = 15.3 Hz, 1H), 4.77 (d, *J* = 15.6 Hz, 1H), 3.42 (s, 3H), 3.38 (s, 3H), 2.37 (s, 6H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.13, 164.66, 158.22, 153.02, 151.52, 140.93, 138.63, 138.04, 135.30, 133.34, 131.21, 130.85, 125.77, 120.34, 119.41, 115.96, 47.17, 39.48, 28.94, 21.34, 17.17. LC-MS (ESI, m/z): 480.1630 $[M+H]^+$.

3,5-di-tert-butyl-N-(4-methyl-3-(1-methyl-7-(methylsulfonyl)-2-oxo-1,2-dihydropyrimido [4,5-d]pyrimidin-3(4H)-yl)phenyl)benzamide (10d) (Method D) yield 76%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 8.61 (s, 1H), 7.82 (s, 1H), 7.78 (s, 2H), 7.67 (d, *J* = 8.3 Hz, 1H), 7.62 (s, 1H), 7.33 (d, *J* = 8.2 Hz, 1H), 4.96 (d, *J* = 15.4 Hz, 1H), 4.79 (d, *J* = 15.4 Hz, 1H), 3.43 (d, *J* = 1.2 Hz, 3H), 3.38 (s, 3H), 2.16 (s, 3H), 1.36 (d, *J* = 1.1 Hz, 18H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.60, 164.68, 158.23, 153.02, 151.55, 151.01, 140.93, 138.52, 134.77, 131.18, 130.99, 125.75, 122.13, 120.82, 120.01, 115.97, 59.99, 46.74, 35.02, 31.51, 29.03, 17.30. LC-MS (ESI, m/z): 564.2570 $[M+H]^+$.

3-methyl-N-(4-methyl-3-(1-methyl-7-(methylsulfonyl)-2-oxo-1,2-dihydropyrimido[4,5-d] pyrimidin-3(4H)-yl)phenyl)benzamide (10e) (Method D) yield 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.82 (s, 1H), 7.73 (s, 1H), 7.69 (d, *J* = 2.8 Hz, 1H), 7.49 (dd, *J* = 6.0, 2.0 Hz, 1H), 7.37 (d, *J* = 4.3 Hz, 2H), 7.27 (d, *J* = 8.3 Hz, 1H), 4.96 (d, *J* = 15.8 Hz, 1H), 4.72 (d, *J* = 15.8 Hz, 1H), 3.51 (s, 3H), 3.39 (s, 3H), 2.43 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.27, 167.56, 161.26, 155.42, 154.84, 142.74, 141.31, 140.71, 137.51, 135.51, 134.35, 134.26, 131.33, 130.92, 127.38, 123.91, 122.30, 118.07, 50.10, 41.84, 31.71, 23.95, 19.71. LC-MS (ESI, m/z): 466.1480 $[M+H]^+$.

General Method E:

N-(4-methyl-3-(1-methyl-7-(4-methyl-3-nitrophenylamino)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (11a) To a solution of N-(4-methyl-3-(1-methyl-7-(methylsulfonyl)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (450 mg, 0.88 mmol, 1.0 equiv) in ahydrous dioxane (2 mL) was added 4-methyl-3-nitroaniline (1.3 g, 8.80 mmol, 10.0 equiv), TFA (0.32 mL, 8.80 mmol, 10.0 equiv) at room temperature under argon. The reaction mixture was then heated to 120 °C for 2 hours. The resulting mixture was diluted with DCM (30 mL), washed with water (30 mL), brine (30 mL) and dried over (anhydrous $MgSO_4$). Evaporation of the solvent afforded

the crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-2%) to give the title compound **11a** as a white solid (320 mg, 61%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 10.01 (s, 1H), 8.78 (s, 1H), 8.32 (s, 1H), 8.28 (d, *J* = 7.2 Hz, 1H), 8.21 (s, 1H), 7.98 (d, *J* = 6.8 Hz, 1H), 7.81 (d, *J* = 13.0 Hz, 3H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.32 (d, *J* = 7.9 Hz, 1H), 4.74 (d, *J* = 13.8 Hz, 1H), 4.56 (d, *J* = 13.9 Hz, 1H), 3.39 (s, 3H), 2.47 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.32, 159.06, 157.47, 153.67, 152.51, 149.09, 141.60, 140.11, 138.09, 136.09, 133.21, 132.28, 131.40, 131.23, 130.22, 128.64, 125.80, 125.31, 124.65, 123.79, 120.31, 119.82, 113.97, 103.95, 47.12, 28.72, 19.62, 17.25. LC-MS (ESI, m/z): 592.1849 $[M+H]^+$.

Etherification method of **11i**, **11k**, **11m**: To a solution of compound **10** (0.88 mmol, 1.0 equiv) in anhydrous dioxane (5 mL) was added ROH (8.8 mmol, 10.0 equiv) and K_2CO_3 (8.8 mmol, 10.0 equiv) at room temperature under argon. The reaction mixture was stirred at room temperature for 24 h. Then the resulting mixture was concentrated to dryness. The residue was diluted with water, extracted with EtOAc. The organic layers were washed with water, brine and dried. Evaporation of the solvent afforded the crude product **11**, which was purified by flash chromatography.

N-(4-methyl-3-(1-methyl-7-(4-methyl-3-nitrophenylamino)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)biphenyl-3-carboxamide (11b) (Method E) yield 79%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 9.99 (s, 1H), 8.77 (s, 1H), 8.25 (s, 1H), 8.21 (s, 1H), 7.96 (d, *J* = 7.7 Hz, 1H), 7.87 (dd, *J* = 15.4, 9.7 Hz, 3H), 7.78 (d, *J* = 7.6 Hz, 2H), 7.69 (d, *J* = 8.3 Hz, 1H), 7.63 (t, *J* = 7.7 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 2H), 7.43 (d, *J* = 7.3 Hz, 1H), 7.39 (d, *J* = 8.6 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 4.74 (d, *J* = 14.1 Hz, 1H), 4.56 (d, *J* = 14.2 Hz, 1H), 3.39 (d, *J* = 5.2 Hz, 3H), 2.47 (s, 3H), 2.16 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.75, 159.07, 157.47, 153.68, 152.52, 149.11, 141.59, 140.81, 140.13, 139.99, 138.47, 135.93, 133.21, 131.18, 130.26, 129.62, 129.48, 128.31, 127.39, 127.30, 126.23, 125.31, 123.81, 120.23, 119.70, 114.00, 103.95, 47.16, 28.73, 19.61, 17.25. LC-MS (ESI, m/z): 600.2690 $[M+H]^+$.

3,5-dimethyl-N-(4-methyl-3-(1-methyl-7-(4-methyl-3-nitrophenylamino)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)benzamide (11c) (Method E) yield 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 9.99 (s, 1H), 8.77 (s, 1H), 8.21 (s, 1H), 7.84 (s, 2H), 7.65 (d, *J* = 6.1 Hz, 1H), 7.58 (s, 2H), 7.40 (s, 1H), 7.29 (d, *J* = 7.2 Hz, 1H), 7.22 (s, 1H),

4.73 (d, *J* = 13.5 Hz, 1H), 4.55 (d, *J* = 13.7 Hz, 1H), 3.39 (s, 3H), 2.47 (s, 3H), 2.36 (s, 6H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.10, 159.06, 157.47, 153.67, 152.50, 149.12, 141.54, 140.13, 138.60, 138.02, 135.32, 133.32, 133.22, 131.12, 130.84, 125.78, 125.33, 123.80, 120.05, 119.47, 113.99, 103.95, 47.15, 28.72, 21.33, 19.60, 17.23. LC-MS (ESI, m/z): 552.2290$[M+H]^+$.

3,5-di-tert-butyl-N-(4-methyl-3-(1-methyl-7-(4-methyl-3-nitrophenylamino)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)benzamide (11d) (Method E) yield 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H), 9.99 (s, 1H), 8.78 (s, 1H), 8.22 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.78 (s, 3H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.62 (s, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 4.74 (d, *J* = 14.0 Hz, 1H), 4.57 (d, *J* = 14.0 Hz, 1H), 3.40 (s, 3H), 2.47 (s, 3H), 2.16 (s, 3H), 1.35 (s, 18H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.59, 159.07, 157.49, 153.67, 152.53, 151.00, 149.12, 141.53, 140.13, 138.48, 134.80, 133.21, 131.09, 130.94, 125.30, 123.80, 122.13, 120.51, 120.03, 113.99, 103.96, 99.99, 49.24, 46.98, 34.81, 31.74, 28.68, 19.59, 17.08. LC-MS (ESI, m/z): 636.3230 $[M+H]^+$.

3-methyl-N-(4-methyl-3-(1-methyl-7-(4-methyl-3-nitrophenylamino)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)benzamide (11e) (Method E) yield 79%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1H), 9.99 (s, 1H), 8.77 (s, 1H), 8.21 (s, 1H), 7.85 (s, 2H), 7.79 (s, 2H), 7.69 – 7.61 (m, 1H), 7.41 (d, *J* = 3.1 Hz, 3H), 7.30 (d, *J* = 7.7 Hz, 1H), 4.73 (d, *J* = 14.0 Hz, 1H), 4.55 (d, *J* = 14.0 Hz, 1H), 3.39 (s, 3H), 2.47 (s, 3H), 2.41 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.99, 159.07, 157.48, 153.67, 152.50, 149.12, 141.55, 140.13, 138.55, 138.17, 135.30, 133.21, 132.61, 131.14, 130.88, 128.76, 128.53, 125.25, 123.81, 120.09, 119.52, 113.99, 103.96, 55.36, 28.72, 21.43, 19.60, 17.24. LC-MS (ESI, m/z): 538.2125 $[M+H]^+$.

N-(3-(7-(3,4-dimethylphenylamino)-1-methyl-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)-4-methylphenyl)-3-(trifluoromethyl)benzamide (11f) (Method E) yield 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (d, *J* = 12.6 Hz, 1H), 9.40 (s, 1H), 8.33 (s, 1H), 8.29 (d, *J* = 7.4 Hz, 1H), 8.13 (s, 1H), 7.97 (d, *J* = 7.0 Hz, 1H), 7.86 – 7.76 (m, 2H), 7.67 (d, *J* = 6.7 Hz, 1H), 7.58 (s, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 7.5 Hz, 1H), 7.04 (d, *J* = 7.7 Hz, 1H), 4.70 (d, *J* = 13.7 Hz, 1H), 4.52 (d, *J* = 14.0 Hz, 1H), 3.65 (s, 3H), 3.38 (s, 3H), 2.21 (s, 3H), 2.17 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.32, 159.61, 157.32, 153.67, 153.01, 152.71, 141.72, 138.72, 138.09, 136.31, 136.10, 132.27, 131.42, 131.31, 130.21, 129.84, 129.54, 129.38, 128.62,

124.66, 120.75, 119.85, 116.97, 102.63, 72.56, 28.24, 20.16, 19.12, 17.49. LC-MS (ESI, m/z): 561.2153 $[M+H]^+$.

N-(4-methyl-3-(1-methyl-2-oxo-7-(p-tolylamino)-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (11g) (Method E) yield 89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 9.48 (s, 1H), 8.33 (s, 1H), 8.29 (d, *J* = 7.3 Hz, 1H), 8.14 (s, 1H), 7.97 (d, *J* = 7.1 Hz, 1H), 7.85 – 7.77 (m, 2H), 7.67 (d, *J* = 7.6 Hz, 2H), 7.32 (d, *J* = 7.6 Hz, 1H), 7.10 (d, *J* = 7.3 Hz, 2H), 4.70 (d, *J* = 13.7 Hz, 1H), 4.52 (d, *J* = 13.8 Hz, 1H), 3.65 (s, 3H), 2.26 (s, 3H), 2.16 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.33, 159.55, 157.38, 153.60, 152.69, 141.70, 138.46, 138.09, 136.10, 132.27, 131.42, 131.21, 130.59, 130.21, 129.85, 129.65, 129.54, 129.34, 128.63, 124.66, 120.28, 119.85, 119.58, 119.47, 102.77, 72.14, 28.81, 21.02, 17.25. LC-MS (ESI, m/z): 547.1200 $[M+H]^+$.

N-(4-methyl-3-(1-methyl-7-(4-methyl-3-(trifluoromethyl)phenylamino)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (11h) (Method E) yield 78%. ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.33, 159.23, 157.43, 153.73, 152.55, 141.63, 139.33, 138.08, 136.09, 132.86, 132.29, 131.41, 131.23, 130.25, 129.82, 129.51, 128.64, 128.27, 127.91, 127.62, 126.53, 125.81, 124.62, 123.81, 123.10, 122.39, 120.30, 119.82, 115.93, 115.87, 103.59, 47.12, 28.60, 18.54, 17.26. ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.33, 159.23, 157.43, 153.73, 152.55, 141.63, 139.33, 138.08, 136.09, 132.86, 132.29, 131.41, 131.23, 130.25, 129.82, 129.51, 128.64. LC-MS (ESI, m/z): 615.1901 $[M+H]^+$.

N-(4-methyl-3-(1-methyl-7-(4-methyl-3-nitrophenoxy)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (11i) (etherification method) yield 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H), 8.16 (s, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 8.03 (s, 1H), 7.97 (s, 1H), 7.80 – 7.74 (m, 2H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.44 (d, *J* = 1.4 Hz, 2H), 7.23 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 4.72 (d, *J* = 15.2 Hz, 1H), 4.50 (d, *J* = 15.0 Hz, 1H), 3.48 (s, 3H), 2.66 (s, 3H), 1.66 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.43, 164.01, 159.24, 155.18, 153.74, 153.09, 150.91, 149.14, 139.55, 137.59, 135.37, 133.65, 133.48, 131.44, 131.11, 130.85, 129.16, 128.24, 126.66, 124.64, 124.35, 120.99, 120.07, 118.39, 111.24, 107.00, 46.97, 28.75, 20.26, 16.23. LC-MS (ESI, m/z): 593.1680 $[M+H]^+$.

N-(4-methyl-3-(1-methyl-7-(3-morpholinopropylamino)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (11j) (Method E) yield 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (s, -1H), 8.33 (s, 1H), 8.29 (d, *J* = 7.4 Hz, 1H), 7.95 (d, *J* =

10.8 Hz, 2H), 7.78 (dd, *J* = 15.1, 7.1 Hz, 2H), 7.67 (d, *J* = 8.1 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.16 (s, 1H), 4.62 (d, *J* = 13.6 Hz, 1H), 4.42 (d, *J* = 13.6 Hz, 1H), 3.62 (s, 6H), 3.34 (d, *J* = 5.6 Hz, 2H), 3.19 (s, 3H), 2.48 (s, 6H), 2.13 (s, 3H), 1.75 (d, *J* = 5.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.31, 162.05, 158.74, 157.32, 152.90, 141.80, 138.11, 136.08, 132.21, 131.34, 131.11, 130.12, 129.88, 129.56, 128.55, 125.78, 124.67, 123.07, 120.16, 119.77, 56.35, 53.83, 53.48, 49.02, 47.14, 42.13, 28.17, 25.96, 17.18, 12.74. LC-MS (ESI, m/z): 584.2524 $[M+H]^+$.

(S)-tert-butyl 3-(8-methyl-6-(2-methyl-5-(3-(trifluoromethyl)benzamido)phenyl)-7-oxo-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidin-2-yloxy)pyrrolidine-1-carboxylate (11k) (etherification method) yield 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.17 (s, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 8.01 (s, 1H), 7.75 (d, *J* = 6.9 Hz, 1H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.27 (d, *J* = 10.4 Hz, 1H), 7.02 – 7.00 (m, 1H), 4.68 (dd, *J* = 14.3, 3.5 Hz, 1H), 4.45 (d, *J* = 14.3 Hz, 1H), 4.27 (dt, *J* = 11.7, 6.4 Hz, 1H), 3.72 (s, 2H), 3.66 – 3.57 (m, 2H), 3.52 (d, *J* = 6.6 Hz, 3H), 2.27 (t, *J* = 16.8 Hz, 2H), 1.64 (d, *J* = 6.3 Hz, 3H), 1.49 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.31, 162.06, 151.57, 151.29, 137.66, 135.76, 133.66, 129.51, 129.05, 128.57, 126.97, 125.97, 123.19, 122.51, 120.55, 118.87, 118.06, 103.39, 77.36, 74.17, 66.23, 63.49, 44.96, 36.45, 27.76, 26.37, 14.31. LC-MS (ESI, m/z): 627.2470 $[M+H]^+$.

tert-butyl 4-(8-methyl-6-(2-methyl-5-(3-(trifluoromethyl)benzamido)phenyl)-7-oxo-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidin-2-ylamino)piperidine-1-carboxylate (11l) (Method E) yield 79%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.19 (s, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 7.83 (s, 1H), 7.74 (d, *J* = 7.7 Hz, 1H), 7.65 (s, 1H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 8.3 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 4.58 (d, *J* = 13.9 Hz, 1H), 4.34 (d, *J* = 14.0 Hz, 1H), 4.22 – 3.84 (m, 4H), 3.47 (s, 3H), 3.00 (t, *J* = 11.5 Hz, 2H), 2.12 – 2.04 (m, 3H), 1.63 (s, 3H), 1.49 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.25, 161.02, 157.39, 154.82, 153.81, 152.98, 140.07, 137.71, 135.70, 131.46, 130.88, 130.73, 128.93, 124.46, 120.67, 120.12, 79.67, 48.42, 47.29, 32.12, 28.45, 28.19, 16.20. LC-MS (ESI, m/z): 640.2490 $[M+H]^+$.

tert-butyl 4-(8-methyl-6-(2-methyl-5-(3-(trifluoromethyl)benzamido)phenyl)-7-oxo-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidin-2-yloxy)piperidine-1-carboxylate (11m) (etherification method) yield 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.17 (s, 1H), 8.13 (d, *J* = 7.9 Hz, 1H), 8.00 (s, 1H), 7.75 (d, *J* = 7.2 Hz, 2H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.25 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 4.67 (d, *J* = 14.5 Hz, 1H), 4.44 (d, *J* = 14.5 Hz, 1H), 3.92 – 3.83 (m, 2H), 3.53 (s, 3H), 3.33 – 3.23 (m, 2H), 2.12 – 2.04 (m, 2H), 1.90 – 1.82 (m, 2H), 1.62 (s, 3H),

1.50 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.22, 164.01, 158.81, 154.87, 153.65, 153.39, 139.76, 137.78, 135.59, 131.47, 130.93, 130.63, 128.99, 127.99, 124.37, 120.79, 120.04, 105.00, 99.99, 79.56, 72.79, 46.97, 29.71, 28.60, 28.34, 16.18. LC-MS (ESI, m/z): 641.2633 $[M+H]^+$.

N-(4-methyl-3-(1-methyl-7-(4-methyl-3-(methylamino)phenylamino)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (11n) (Method E) yield 79%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 9.26 (s, 1H), 8.32 (s, 1H), 8.28 (d, *J* = 7.7 Hz, 1H), 8.13 (s, 1H), 7.98 (d, *J* = 7.5 Hz, 1H), 7.80 (t, *J* = 7.6 Hz, 2H), 7.67 (d, *J* = 8.1 Hz, 1H), 7.33 (d, *J* = 8.3 Hz, 1H), 7.11 (s, 1H), 6.94 – 6.83 (m, 2H), 4.70 (d, *J* = 13.7 Hz, 1H), 4.51 (d, *J* = 13.8 Hz, 1H), 3.38 (s, 3H), 2.75 (d, *J* = 4.6 Hz, 3H), 2.15 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.34, 159.70, 157.29, 153.75, 152.74, 148.10, 141.74, 139.94, 138.06, 136.12, 132.29, 131.44, 131.22, 130.24, 129.71, 128.64, 124.62, 120.27, 119.88, 115.47, 111.29, 106.89, 102.28, 100.67, 47.19, 30.71, 28.70, 17.44, 17.26. LC-MS (ESI, m/z): 576.2260 $[M+H]^+$.

N-(3-(7-(3-(dimethylamino)-4-methylphenylamino)-1-methyl-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)-4-methylphenyl)-3-(trifluoromethyl)benzamide (11o) (Method E) yield 65%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (s, 1H), 8.21 (s, 1H), 8.15 (d, *J* = 7.6 Hz, 1H), 7.96 (s, 1H), 7.77 (d, *J* = 7.5 Hz, 1H), 7.68 (s, 1H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.44 (s, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.29 (s, 1H), 7.16 (s, 2H), 7.03 (d, *J* = 8.1 Hz, 1H), 4.65 (d, *J* = 13.9 Hz, 1H), 4.41 (d, *J* = 14.0 Hz, 1H), 3.58 (s, 3H), 2.77 (s, 6H), 2.34 (s, 3H), 1.67 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.25, 159.53, 157.30, 153.07, 137.70, 135.69, 131.33, 131.05, 129.03, 128.01, 126.47, 124.39, 120.71, 120.06, 114.84, 113.79, 110.27, 101.95, 101.61, 96.94, 47.38, 44.23, 29.71, 28.71, 17.93. LC-MS (ESI, m/z): 590.2421$[M+H]^+$.

General Method F:

N-(3-(7-(3-amino-4-methylphenylamino)-1-methyl-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)-4-methylphenyl)-3-(trifluoromethyl)benzamide (12a) To a solution of N-(4-methyl-3-(1-methyl-7-(4-methyl-3-nitrophenylamino)-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (50 mg, 0.085 mmol, 1.00 equiv) in methanol (5 mL) was added $SnCl_2.2H_2O$ (191 mg, 0.85 mg, 10.0 equiv) at room temperature under argon. The reaction mixture was then heated to reflux for14h. The resulting mixture was the concentrated to dryness. The residue was diluted with water (50 mL), and added 1 N NaOH

to pH about 10. The mixture was extracted with EtOAc (3×30 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded the crude product, which was purified by flash column chromatography (eluting with MeOH in DCM 0-5%) to yield **12a** as a white solid (40 mg, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 1H), 10.72 (s, 1H), 8.34 (s, 2H), 8.32 (s, 1H), 8.27 (s, 1H), 7.97 (d, *J* = 7.5 Hz, 1H), 7.91 (s, 1H), 7.86 (s, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 7.69 (d, *J* = 8.1 Hz, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.33 (dd, *J* = 8.1, 3.8 Hz, 2H), 4.76 (d, *J* = 14.4 Hz, 1H), 4.60 (d, *J* = 14.5 Hz, 1H), 2.37 (s, 3H), 2.15 (s, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 164.37, 159.62, 151.49, 141.00, 138.19, 137.11, 136.00, 132.37, 132.01, 131.35, 131.28, 130.21, 129.79, 129.47, 128.64, 127.64, 125.80, 124.76, 124.73, 123.09, 120.77, 120.64, 119.71, 116.23, 104.28, 46.54, 29.59, 17.22, 17.17. LC-MS (ESI, m/z): 562.1109 $[M+H]^+$.

N-Boc deprotection method of **12f** and **12h**: To a solution of compound **11** (0.085 mmol, 1.0 equiv) in anhydroup dioxane (1 mL) was added 1 mL of 4 M HCl in dioxane at room temperature. The reaction mixture was stirred at room temperature for 30 min. The resulting mixture was concentrated to afford the title compound **12**.

N-(3-(7-(3-amino-4-methylphenylamino)-1-methyl-2-oxo-1,2-dihydropyrimido[4,5-d] pyrimidin-3(4H)-yl)-4-methylphenyl)biphenyl-3-carboxamide (12b) (Method F) yield 85%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.17 (s, 1H), 7.96 (s, 1H), 7.90 (d, *J* = 7.7 Hz, 1H), 7.83 – 7.77 (m, 2H), 7.71 – 7.66 (m, 2H), 7.57 (t, *J* = 7.6 Hz, 2H), 7.46 (t, *J* = 7.5 Hz, 2H), 7.38 (d, *J* = 7.4 Hz, 1H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.15 (s, 1H), 7.01 (q, *J* = 8.1 Hz, 2H), 4.51 (d, *J* = 14.1 Hz, 1H), 3.44 (s, 3H), 2.21 (s, 3H), 2.17 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 167.37, 158.81, 157.70, 153.39, 151.66, 141.58, 140.65, 140.12, 138.14, 137.64, 135.30, 131.60, 131.12, 130.35, 130.16, 128.86, 128.70, 127.56, 126.87, 126.11, 126.02, 122.56, 120.72, 119.67, 118.40, 111.61, 102.16, 48.90, 47.08, 28.01, 16.43. LC-MS (ESI, m/z): 570.2540 $[M+H]^+$.

N-(3-(7-(3-amino-4-methylphenylamino)-1-methyl-2-oxo-1,2-dihydropyrimido[4,5-d] pyrimidin-3(4H)-yl)-4-methylphenyl)-3,5-dimethylbenzamide (12c) (Method F) yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.91 (s, 1H), 7.73 (s, 1H), 7.49 (s, 2H), 7.45 (s, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.14 (d, *J* = 7.6 Hz, 2H), 7.08 (s, 1H), 7.02 (d, *J* = 8.1 Hz, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 4.65 (d, *J* = 14.0 Hz, 1H), 4.40 (d, *J* = 14.1 Hz, 1H), 3.49 (s, 3H), 2.37 (s, 6H), 2.16 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.11, 159.29, 157.47, 153.10,

152.45, 144.93, 140.58, 138.22, 137.48, 134.69, 133.31, 131.21, 131.12, 130.65, 125.08, 120.22, 119.44, 116.98, 109.97, 106.26, 102.09, 47.17, 29.88, 28.67, 21.01, 16.65. LC-MS (ESI, m/z): 522.2546 $[M+H]^+$.

N-(3-(7-(3-amino-4-methylphenylamino)-1-methyl-2-oxo-1,2-dihydropyrimido[4,5-d] pyrimidin-3(4H)-yl)-4-methylphenyl)-3,5-di-tert-butylbenzamide (12d) (Method F) yield 75%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.91 (s, 1H), 7.80 (s, 2H), 7.72 (s, 1H), 7.67 (s, 1H), 7.63 (d, *J* = 6.8 Hz, 1H), 7.22 (d, *J* = 8.3 Hz, 1H), 7.11 (s, 1H), 6.91 (s, 2H), 4.60 (d, *J* = 14.0 Hz, 1H), 4.40 (d, *J* = 14.0 Hz, 1H), 3.38 (d, *J* = 5.9 Hz, 3H), 2.11 (s, 6H), 1.37 (s, 18H). ^{13}C NMR (101 MHz, CD_3OD) δ 168.26, 159.44, 157.14, 153.52, 152.68, 151.09, 145.12, 140.82, 138.54, 137.79, 134.29, 131.43, 130.86, 129.88, 125.72, 121.59, 120.59, 119.83, 117.04, 110.03, 106.83, 101.91, 99.99, 34.56, 30.48, 27.78, 15.87, 15.62. LC-MS (ESI, m/z): 606.3485 $[M+H]^+$.

N-(3-(7-(3-amino-4-methylphenylamino)-1-methyl-2-oxo-1,2-dihydropyrimido[4,5-d] pyrimidin-3(4H)-yl)-4-methylphenyl)-3-methylbenzamide (12e) (Method F) yield 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.69 (s, 2H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.42 – 7.35 (m, 3H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.14 – 6.90 (m, 3H), 4.75 (d, *J* = 13.9 Hz, 1H), 4.49 (d, *J* = 13.5 Hz, 1H), 3.46 (s, 3H), 2.44 (s, 3H), 2.21 (s, 3H), 2.18 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.83, 161.72, 157.13, 144.44, 142.29, 141.24, 138.56, 136.41, 135.38, 135.25, 134.46, 132.32, 131.83, 128.30, 128.25, 124.73, 123.53, 114.91, 100.04, 51.12, 33.38, 32.49, 25.04, 20.79. LC-MS (ESI, m/z): 508.2390 $[M+H]^+$.

(S)-N-(4-methyl-3-(1-methyl-2-oxo-7-(pyrrolidin-3-yloxy)-1,2-dihydropyrimido[4,5-d] pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (12f) (deprotection method) yield 95%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.31 (s, 1H), 8.27 (s, 1H), 8.23 (d, *J* = 7.8 Hz, 1H), 7.96 – 7.88 (m, 2H), 7.74 (dd, *J* = 10.2, 5.1 Hz, 1H), 7.64 – 7.59 (m, 1H), 7.35 (d, *J* = 8.3 Hz, 1H), 4.93 – 4.91 (m, 1H), 4.74 (d, *J* = 14.7 Hz, 1H), 3.80 (s, 2H), 3.59 (d, *J* = 5.6 Hz, 1H), 3.52 (s, 3H), 2.54 (s, 2H), 2.24 (s, 3H), 2.06 – 2.00 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 165.47, 161.47, 158.68, 150.95, 144.49, 140.02, 137.64, 135.66, 131.80, 131.14, 130.97, 129.34, 128.48, 128.03, 124.22, 120.93, 119.48, 107.32, 78.97, 50.38, 46.15, 43.90, 30.30, 28.67, 15.64. LC-MS (ESI, m/z): 527.1950 $[M+H]^+$.

N-(4-methyl-3-(1-methyl-2-oxo-7-(piperidin-4-ylamino)-1,2-dihydropyrimido[4,5-d] pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (12g) (deprotection method) yield 96%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.28 (m, 2H), 7.83 (m, 3H), 7.67 (s, 1H), 7.56 (s, 1H), 7.24

(s, 1H), 4.53 (m, 2H), 3.44 (s, 5H), 3.23 (br, 3H), 2.25 (br, 2H), 1.94 (m, 5H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.79, 171.60, 165.31, 152.87, 151.31, 140.10, 137.56, 135.53, 131.85, 131.14, 131.08, 130.36, 129.43, 128.08, 125.27, 124.25, 122.63, 120.94, 119.64, 46.65, 46.03, 42.64, 27.58, 19.34, 15.86. LC-MS (ESI, m/z): 540.2260 $[M+H]^+$.

N-(4-methyl-3-(1-methyl-2-oxo-7-(piperidin-4-yloxy)-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)phenyl)-3-(trifluoromethyl)benzamide (12h) (deprotection method) yield 95%. 1H NMR (400 MHz, DMSO-d6) δ 10.67 (s, 1H), 9.29 (s, 1H), 8.33 (s, 1H), 8.26 (s, 1H), 7.97 (d, J = 6.1 Hz, 1H), 7.86 (s, 1H), 7.79 (s, 1H), 7.68 (d, J = 6.4 Hz, 1H), 7.32 (d, J = 7.2 Hz, 1H), 4.76 (d, J = 13.6 Hz, 1H), 4.60 (d, J = 14.0 Hz, 1H), 3.31 (s, 3H), 3.23 (s, 2H), 3.13 (s, 2H), 2.20 (s, 2H), 2.13 (s, 3H), 2.01 (s, 2H). 13C NMR (101 MHz, CD3OD) δ 163.77, 160.03, 158.58, 150.71, 148.91, 142.69, 139.21, 136.22, 134.31, 130.41, 129.71, 127.99, 126.66, 122.81, 119.38, 118.27, 99.97, 68.49, 45.19, 39.39, 26.79, 25.30, 14.35.
LC-MS (ESI, m/z): 541.2301 $[M+H]^+$.

N-(3-(7-(3-amino-4-methylphenoxy)-1-methyl-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)-4-methylphenyl)-3-(trifluoromethyl)benzamide (12i) (Method F) yield 79%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.42 (s, 1H), 8.26 (s, 1H), 8.23 (d, J = 7.4 Hz, 1H), 7.91 (d, J = 6.8 Hz, 2H), 7.75 (t, J = 7.4 Hz, 1H), 7.61 (d, J = 8.2 Hz, 3H), 7.47 (s, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.18 (s, 1H), 4.80 (s, 2H), 3.37 (s, 3H), 2.50 (s, 3H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 165.55, 161.53, 150.95, 150.03, 139.99, 137.62, 135.71, 132.76, 132.17, 131.84, 131.15, 130.79, 130.71, 130.19, 129.33, 128.01, 124.21, 122.29, 121.58, 120.95, 119.56, 116.96, 109.88, 108.37, 48.48, 46.51, 28.44, 15.41. LC-MS (ESI, m/z): 563.1900 $[M+H]^+$.

General Method G:

4-(methylamino)-2-(methylthio)pyrimidine-5-carboxylic acid (14a) To a solution of ethyl 4-(methylamino)-2-(methylthio)pyrimidine-5-carboxylate (0.976g, 4.3 mmol, 1,0 equiv) in methanol (10 mL) and water (2 mL) was added NaOH (0.18 g, 4.4 mmol, 1.02 equiv) at room temperature under argon. The reaction mixture was stirred at room temperature for 20 h. The resulting mixture was concentrated to dryness. The residue was diluted with water (50 mL), acidified by 1N HCl to pH = 3. The white precipitate was filtered and dried to provide **14a** as a white solid (0.70 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H), 8.35 (s, 1H), 2.96 (d, J

= 3.9 Hz, 3H), 2.47 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 175.07, 168.31, 160.62, 158.32, 101.76, 27.59, 14.00. TOF LCMS(m/z):200.0423[M+H]$^+$.

4-(dimethylamino)-2-(methylthio)pyrimidine-5-carboxylic acid (14b) (Method G) yield 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (s, 1H), 3.15 (s, 6H), 2.57 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.66, 159.23, 154.08, 137.14, 106.24, 40.50, 14.00. LC-MS (ESI, m/z): 214.0585 [M+H]$^+$.

General Method H:

N-(2-methyl-5-(3-(trifluoromethyl)benzamido)phenyl)-4-(methylamino)-2-(methylthio) pyrimidine-5-carboxamide (15a) To a solution of the 4-(methylamino)-2-(methylthio)pyrimidine-5-carboxylic acid (0. 6 g, 3.0 mmol, 1.00 equiv) in anhydrous DMF (5 mL) was added N-(3-amino-4-methylphenyl)-3-(trifluoromethyl)benzamide (1.06 g, 3.6 mmol, 1.20 equiv), HATU (1.36 g, 3.6 mmol, 1.2 equiv) and DIPEA (1.6 mL, 6.0 mmol, 2.0 equiv) at room temperature under argon. The reaction mixture was stirred at room temperature for 20 h. The resulting mixture was concentrated to dryness. The residue was diluted with water (100 mL), extracted with EtOAc (3×50 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded the crude product, which was purified by flash column chromatography (eluting with MeOH in DCM 0-5%) to yield **15a** as a white solid (1.14g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 9.56 (s, 1H), 8.83 (s, 1H), 8.41 – 8.23 (m, 3H), 7.97 (d, *J* = 6.6 Hz, 1H), 7.80 (d, *J* = 6.8 Hz, 1H), 7.64 (d, *J* = 7.0 Hz, 1H), 7.26 (d, *J* = 7.3 Hz, 1H), 4.14 (s, 3H), 2.60 (s, 3H), 2.30 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.28, 160.68, 159.45, 137.46, 136.52, 136.19, 132.32, 130.67, 130.12, 129.79, 128.52, 124.69, 117.75, 116.03, 111.46, 55.28, 17.29, 13.99. LC-MS (ESI, m/z): 476.1235 [M+H]$^+$.

4-(dimethylamino)-N-(2-methyl-5-(3-(trifluoromethyl)benzamido)phenyl)-2-(methylthio) pyrimidine-5-carboxamide(15b) (Method H) yield 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 10.01 (s, 1H), 8.28 (d, *J* = 16.4 Hz, 3H), 7.97 (s, 1H), 7.89 (s, 1H), 7.79 (d, *J* = 6.1 Hz, 1H), 7.64 (s, 1H), 7.26 (s, 1H), 3.12 (s, 6H), 2.50 (d, *J* = 5.9 Hz, 3H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.61, 166.09, 164.41, 158.95, 155.95, 137.26, 136.37, 136.17, 132.27, 130.85, 130.17, 129.12, 128.67, 128.55, 124.67, 118.83, 117.98, 111.19, 17.86, 13.92. LC-MS (ESI, m/z): 490.1452 [M+H]$^+$.

N-(2-methyl-5-(3-(trifluoromethyl)benzamido)phenyl)-2-(methylthio)pyrimidine-5-carboxamide (15c) (Method H) yield 90%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 9.95 (s, 1H), 8.77 (s, 1H), 8.33 (s, 1H), 8.29 (d, *J* = 7.3 Hz, 1H), 7.98 – 7.92 (m, 2H), 7.84 (s, 1H), 7.80 (d, *J* = 7.4 Hz, 1H), 7.63 (d, *J* = 7.7 Hz, 1H), 7.28 (d, *J* = 8.1 Hz, 1H), 2.50 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.52, 165.66, 164.33, 162.11, 156.16, 137.30, 136.31, 136.19, 132.28, 130.75, 130.14, 129.98, 128.54, 125.80, 124.66, 119.39, 118.95, 104.54, 17.86, 13.84. LC-MS (ESI, m/z): 447.1042 $[M+H]^+$.

N-(2-methyl-5-(3-(trifluoromethyl)benzamido)phenyl)-4-(methylamino)-2-(methylsulfonyl) pyrimidine-5-carboxamide (16a) (Method D) yield 62%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 10.26 (s, 1H), 8.91 (s, 1H), 8.82 (s, 1H), 8.32 (s, 1H), 8.28 (d, *J* = 7.4 Hz, 1H), 7.98 (d, *J* = 7.3 Hz, 1H), 7.88 (s, 1H), 7.84 – 7.77 (m, 1H), 7.61 (d, *J* = 7.4 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 3.37 (s, 3H), 3.01 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.68, 164.38, 161.39, 155.60, 137.33, 136.18, 135.81, 132.30, 130.85, 130.20, 129.92, 128.60, 124.68, 119.28, 111.37, 39.16, 28.17, 17.87. LC-MS (ESI, m/z): 508.1190 $[M+H]^+$.

4-(dimethylamino)-N-(2-methyl-5-(3-(trifluoromethyl)benzamido)phenyl)-2-(methylsulfonyl)pyrimidine-5-carboxamide (16b) (Method D) yield 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (s, 1H), 10.23 (s, 1H), 8.56 (s, 1H), 8.30 (d, *J* = 14.1 Hz, 2H), 7.96 (s, 2H), 7.80 (s, 1H), 7.65 (d, *J* = 7.7 Hz, 1H), 7.28 (d, *J* = 6.5 Hz, 1H), 3.22 (s, 6H), 2.52 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.91, 164.71, 164.43, 159.16, 156.24, 137.36, 136.18, 135.94, 132.31, 130.97, 130.19, 129.48, 128.63, 128.38, 124.73, 118.87, 117.80, 117.09, 39.23, 17.88. LC-MS (ESI, m/z): 522.1355 $[M+H]^+$.

N-(2-methyl-5-(3-(trifluoromethyl)benzamido)phenyl)-2-(methylsulfonyl)pyrimidine-5-carboxamide (16c) (Method D) yield 74%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H), 10.24 (s, 1H), 8.96 (s, 1H), 8.32 (s, 3H), 7.97 (s, 1H), 7.91 (s, 1H), 7.80 (s, 1H), 7.62 (d, *J* = 6.9 Hz, 1H), 7.30 (d, *J* = 7.3 Hz, 1H), 3.35 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.55, 164.40, 163.12, 156.67, 137.33, 136.15, 135.86, 133.82, 133.14, 132.28, 131.07, 130.87, 130.18, 129.91, 129.29, 128.55, 128.36, 125.77, 124.66, 123.10, 119.30, 66.42, 17.84. LC-MS (ESI, m/z): 479.0935 $[M+H]^+$.

2-(4-methyl-3-nitrophenylamino)-N-(2-methyl-5-(3-(trifluoromethyl)benzamido) phenyl)-4-(methylamino)pyrimidine-5-carboxamide (17a) (Method E) yield 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1H), 10.48 (s, 1H), 9.93 (s, 1H), 9.13 (s, 1H), 8.86 (s, 1H),

8.77 (s, 1H), 8.35 – 8.26 (m, 2H), 7.97 (d, *J* = 7.1 Hz, 1H), 7.86 (s, 1H), 7.80 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J* = 7.3 Hz, 1H), 7.46 (d, *J* = 8.3 Hz, 1H), 7.28 (d, *J* = 8.1 Hz, 1H), 3.07 (s, 3H), 2.50 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.03, 164.33, 161.96, 149.06, 138.72, 137.29, 136.23, 133.39, 132.27, 130.72, 130.16, 129.98, 128.42, 127.01, 124.90, 124.67, 123.04, 119.39, 118.94, 115.55, 102.03, 99.99, 28.29, 19.65, 17.90. LC-MS (ESI, m/z): 580.1850 $[M+H]^+$.

4-(dimethylamino)-2-(4-methyl-3-nitrophenylamino)-N-(2-methyl-5-(3-(trifluoromethyl) benzamido)phenyl)pyrimidine-5-carboxamide (17b) (Method E) yield 65%.^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H), 10.38 (s, 1H), 10.02 (s, 1H), 8.77 (s, 1H), 8.36 (s, 1H), 8.32 (s, 1H), 8.28 (s, 1H), 7.98 (d, *J* = 7.2 Hz, 1H), 7.93 (s, 1H), 7.80 (d, *J* = 7.5 Hz, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 7.3 Hz, 1H), 7.45 (s, 1H), 7.26 (d, *J* = 7.9 Hz, 2H), 3.21 (s, 6H), 2.51 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.36, 160.30, 149.06, 138.87, 137.31, 136.42, 136.20, 133.41, 132.30, 130.83, 130.17, 129.80, 129.48, 128.62, 128.55, 126.70, 125.82, 124.74, 124.69, 123.11, 118.67, 118.14, 115.16, 108.39, 19.66, 17.93. LC-MS (ESI, m/z): 594.2013 $[M+H]^+$.

2-(4-methyl-3-nitrophenylamino)-N-(2-methyl-5-(3-(trifluoromethyl)benzamido) phenyl)pyrimidine-5-carboxamide (17c) (Method E) yield 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (s, 1H), 10.52 (s, 1H), 9.96 (s, 1H), 8.85 (s, 1H), 8.49 (s, 2H), 8.38 – 8.26 (m, 2H), 8.01 – 7.92 (m, 2H), 7.88 (s, 1H), 7.79 (d, *J* = 6.8 Hz, 1H), 7.60 (d, *J* = 7.3 Hz, 1H), 7.44 (d, *J* = 7.9 Hz, 1H), 7.27 (d, *J* = 7.7 Hz, 1H), 2.49 (d, *J* = 14.5 Hz, 3H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.66, 164.34, 163.37, 149.43, 138.32, 137.31, 136.20, 133.25, 132.25, 130.75, 130.10, 129.90, 128.51, 126.84, 125.06, 124.69, 119.34, 118.96, 115.69, 101.80, 19.36, 17.87. LC-MS (ESI, m/z): 551.1583$[M+H]^+$.

2-(3-amino-4-methylphenylamino)-N-(2-methyl-5-(3-(trifluoromethyl)benzamido) phenyl)-4-(methylamino)pyrimidine-5-carboxamide (18a) (Method F) yield 63%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (s, 1H), 9.68 (s, 1H), 9.27 (s, 1H), 8.69 (s, 2H), 8.30 (d, *J* = 10.0 Hz, 2H), 7.97 (s, 1H), 7.81 (s, 2H), 7.59 (d, *J* = 6.2 Hz, 1H), 7.26 (d, *J* = 7.2 Hz, 1H), 7.14 (s, 1H), 6.93 (s, 1H), 6.83 (s, 1H), 4.74 (s, 2H), 3.18 (s, 1H), 3.00 (s, 3H), 2.22 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.27, 164.29, 162.49, 160.61, 157.36, 146.74, 139.21, 137.20, 136.81, 136.23, 132.30, 130.62, 130.19, 130.07, 128.54, 124.68, 119.46, 118.55, 115.52, 108.72, 106.10,

40.61, 40.41, 40.20, 39.99, 39.78, 39.58, 39.36, 27.85, 17.96, 17.38. LC-MS (ESI, m/z): 550.2110 $[M+H]^+$.

2-(3-amino-4-methylphenylamino)-4-(dimethylamino)-N-(2-methyl-5-(3-(trifluoromethyl) benzamido)phenyl)pyrimidine-5-carboxamide (18b) (Method F) yield 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 9.78 (s, 1H), 9.02 (s, 1H), 8.32 (s, 1H), 8.29 (d, *J* = 7.9 Hz, 1H), 8.23 (s, 1H), 7.97 (d, *J* = 7.2 Hz, 1H), 7.89 (s, 1H), 7.79 (s, 1H), 7.73 (s, 1H), 7.68 (s, 1H), 7.63 (d, *J* = 8.3 Hz, 1H), 7.24 (d, *J* = 8.2 Hz, 1H), 7.08 (s, 1H), 6.87 (d, *J* = 7.9 Hz, 1H), 6.80 (d, *J* = 8.0 Hz, 1H), 3.11 (s, 6H), 2.26 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.42, 166.58, 164.31, 160.96, 159.40, 157.81, 146.71, 139.51, 137.25, 136.24, 132.31, 132.19, 131.98, 130.71, 130.15, 130.04, 129.13, 128.59, 125.83, 124.68, 118.22, 115.10, 108.40, 105.78, 30.48, 18.94. LC-MS (ESI, m/z): 564.2263 $[M+H]^+$.

2-(3-amino-4-methylphenylamino)-N-(2-methyl-5-(3-(trifluoromethyl)benzamido) phenyl) pyrimidine-5-carboxamide (18c) (Method F) yield 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H), 9.67 (s, 1H), 9.18 (s, 1H), 8.77 (s, 1H), 8.34 (s, 1H), 8.30 (d, *J* = 7.3 Hz, 1H), 7.96 (d, *J* = 7.0 Hz, 1H), 7.84 (s, 1H), 7.80 (d, *J* = 7.3 Hz, 1H), 7.62 (d, *J* = 7.4 Hz, 2H), 7.27 (d, *J* = 7.5 Hz, 2H), 6.94 (d, *J* = 7.5 Hz, 1H), 6.83 (d, *J* = 7.9 Hz, 1H), 2.24 (s, 3H), 2.06 (d, *J* = 15.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.12, 164.32, 163.51, 160.81, 158.08, 146.70, 139.18, 137.23, 136.86, 136.24, 132.28, 130.64, 130.14, 130.05, 129.96, 128.52, 125.82, 124.66, 123.11, 119.48, 118.59, 115.64, 109.02, 106.52, 60.22, 17.94, 17.32, 14.51. LC-MS (ESI, m/z): 521.1843 $[M+H]^+$.

tert-butyl 3-amino-4-methylphenylcarbamate (20) To a solution of 4-methyl-3-nitroaniline (5.00 g, 32.9 mmol, 1.00 equiv) in anhydrous THF (50 mL) was added $(Boc)_2O$ (7.88g, 36.1 mmol, 1.10 equiv) and DMAP (0.3 g) at 0 °C under argon. Then the reaction mixture was allowed to warm to room temperature for 1h. After that, the reaction mixture was heated to reflux for 20h. The resulting mixture was the concentrated to dryness. The residue was diluted with water (100 mL), extracted with EtOAc (3×100 mL). The combined organic layers were washed with 1N HCl (100 mL), water (100 mL), brine (100 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded the tert-butyl 4-methyl-3-nitrophenylcarbamate **19**, which was used in the next step without further purification. To a solution of crude **19** (6.90 g, 27.38 mmol, 1.00 equiv) in methanol (50 mL) was added 10% Pd/C (0.69g, 10%) at room temperature under argon. Then the reaction mixture was stirred a balloon of hydrogen for 2h.

The resulting mixture was filtered and washed with methanol. The filtrate was concentrated to afford the crude product **20**, which was crystallized from EtOAc/hexanes as a needle solid (5 g, 83%). ^{1}H NMR (400 MHz, DMSO-d_6 δ 8.92 (s, 1H), 6.87 (s, 1H), 6.76 (d, J = 7.7 Hz, 1H), 6.53 (d, J = 7.6 Hz, 1H), 4.73 (s, 2H), 1.99 (s, 3H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 153.22, 146.94, 138.40, 130.13, 115.54, 107.28, 104.82, 78.81, 28.67, 17.27. LC-MS (ESI, m/z): 223.1375 $[M+H]^+$.

tert-butyl 4-methyl-3-(4-(methylamino)-2-(methylthio)pyrimidine-5-carboxamido) phenylcarbamate (21) To a solution of 4-(methylamino)-2-(methylthio)pyrimidine-5-carboxylic acid (4.0 g, 20.0 mmol, 1.00 equiv) in anhydrous DMF (20 mL) was added tert-butyl 3-amino-4-methylphenylcarbamate (4.98 g, 3.6 mmol, 1.10 equiv), HATU (9.88 g, 26.0 mmol, 1.2 equiv) and DIPEA (12 mL, 70.0mmol, 3.5 equiv) at room temperature under argon. The reaction mixture was stirred at room temperature for 20 h. The resulting mixture was concentrated to dryness. The residue was diluted with water (100 mL), extracted with EtOAc (3×100 mL). The combined organic layers were washed with water (100 mL), brine (100 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded the crude product, which was purified by flash column chromatography (eluting with MeOH in DCM 0-5%) to offer **21** as a white solid (6.60g, 82%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 9.33 (s, 1H), 8.67 (s, 2H), 7.51 (s, 1H), 7.22 (d, J = 8.0 Hz, 1H), 7.13 (d, J = 8.1 Hz, 1H), 2.96 (d, J = 3.1 Hz, 3H), 2.52 (s, 3H), 2.13 (s, 3H), 1.48 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.60, 165.68, 160.55, 155.22, 153.24, 138.04, 136.26, 130.63, 127.94, 117.00, 116.72, 104.82, 79.40, 28.60, 27.58, 17.72, 13.98. LC-MS (ESI, m/z): 404.1685 $[M+H]^+$.

N-(5-amino-2-methylphenyl)-4-(methylamino)-2-(methylthio)pyrimidine-5-carboxamide (22) To a solution of tert-butyl 4-methyl-3-(4-(methylamino)-2-(methylthio)pyrimidine-5-carboxamido)phenylcarbamate (4.03 g, 10.0 mmol, 1.00 equiv) in methanol (15 mL) was added 10 mL of 4 M HCl (in methanol) at room temperature under argon. The reaction mixture was stirred at room temperature for 2h. The resulting mixture was concentrated to the provide **22** as a off white solid (4.2g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (s, 1H), 9.51 (s, 1H), 9.00 (s, 1H), 7.41 (s, 1H), 7.26 (s, 1H), 3.08 (s, 3H), 2.64 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.07, 163.84, 159.40, 147.58, 136.68, 134.31, 132.62, 131.88, 131.10, 129.92, 121.65, 104.86, 28.66, 18.17, 14.02. LC-MS (ESI, m/z): 304.1163 $[M+H]^+$.

General Method I:

N-(5-(2-methoxybenzamido)-2-methylphenyl)-4-(methylamino)-2-(methylthio)pyrimidine-5-carboxamide (23a) To a solution of N-(5-amino-2-methylphenyl)-4-(methylamino)-2-(methylthio)pyrimidine-5-carboxamide (0.40 g, 0.9 mmol, 1.00 equiv) in anhydrous THF (10 mL) was added a solution of 2-methylbenzoyl chloride (0.13 mL, 0.93 mmol, 1.05 equiv) and DIPEA (0.96 mL, 5.4 mmol, 6.0 equiv) at 0 °C under argon. The reaction mixture was stirred at 0 °C under argon for 2h. The resulting mixture was concentrated to dryness. The residue was diluted with water (50 mL), extracted with EtOAc (3×50 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded the crude product, which was purified by flash column chromatography (eluting with MeOH in DCM 0-5%) to offer **23a** as a white solid (0.34 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H), 8.13 (d, *J* = 7.2 Hz, 1H), 7.75 (s, 1H), 7.45 (dd, *J* = 19.1, 12.3 Hz, 1H), 7.37 (d, *J* = 7.5 Hz, 1H), 7.15 (d, *J* = 8.0 Hz, 1H), 7.09 – 7.01 (m, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 3.99 (s, 3H), 3.00 (s, 3H), 2.51 (s, 3H), 2.24 (d, *J* = 28.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.33, 166.08, 163.51, 160.55, 157.25, 153.78, 136.32, 135.46, 133.34, 132.19, 130.86, 129.33, 121.51, 121.41, 118.79, 118.16, 111.53, 104.18, 56.16, 27.08, 17.44, 14.00. LC-MS (ESI, m/z): 438.1560 $[M+H]^+$.

N-(5-(2-methoxybenzamido)-2-methylphenyl)-4-(methylamino)-2-(methylthio)pyrimidine-5-carboxamide (23b) (Method I) ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 9.98 (s, 1H), 8.70 (s, 1H), 8.66 (s, 1H), 7.84 (s, 1H), 7.60 (d, *J* = 8.3 Hz, 1H), 7.56 (d, *J* = 6.8 Hz, 1H), 7.52 (s, 1H), 7.46 (d, *J* = 6.6 Hz, 1H), 7.25 (d, *J* = 8.1 Hz, 1H), 7.17 (t, *J* = 8.9 Hz, 2H), 3.85 (s, 3H), 2.96 (d, *J* = 4.2 Hz, 3H), 2.21 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.63, 165.77, 165.56, 160.56, 159.66, 155.25, 137.60, 136.76, 136.18, 130.62, 130.13, 129.99, 129.72, 122.02, 120.33, 119.38, 118.90, 117.74, 114.41, 113.36, 104.88, 55.80, 27.38, 17.72, 13.99. LC-MS (ESI, m/z): 438.1537 $[M+H]^+$.

N-(5-(4-methoxybenzamido)-2-methylphenyl)-4-(methylamino)-2-(methylthio)pyrimidine-5-carboxamide (23c) (Method I)yield 79%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.74 (d, *J* = 7.3 Hz, 2H), 7.63 (s, 1H), 7.28 (d, *J* = 6.4 Hz, 1H), 7.03 (d, *J* = 7.5 Hz, 1H), 6.82 (d, *J* = 7.4 Hz, 2H), 3.78 (s, 3H), 2.96 (s, 3H), 2.49 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.27, 166.21, 166.08, 162.32, 160.46, 153.83, 136.45, 135.09, 130.69, 129.43, 129.17, 126.75,

119.13, 118.52, 113.61, 104.06, 55.32, 27.05, 17.30, 13.97. LC-MS (ESI, m/z): 438.1522 $[M+H]^+$.

N-(5-(3,4-dimethoxybenzamido)-2-methylphenyl)-4-(methylamino)-2-(methylthio) pyrimidine-5-carboxamide (23d) (Method I) yield 85%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.95 (s, 1H), 8.88 (s, 1H), 8.83 (s, 1H), 8.74 (s, 1H), 8.16 (s, 1H), 7.72 (s, 2H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 1H), 4.21 (s, 3H), 4.17 (s, 3H), 3.32 (d, *J* = 4.2 Hz, 3H), 2.86 (s, 3H), 2.45 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 170.53, 162.15, 161.83, 156.59, 149.92, 147.99, 144.88, 132.48, 131.34, 126.76, 124.71, 123.08, 116.24, 114.80, 113.96, 106.69, 106.28, 100.14, 56.49, 52.02, 23.33, 13.46, 10.16. LC-MS (ESI, m/z): 468.26329 $[M+H]^+$.

N-(5-(3,5-dimethoxybenzamido)-2-methylphenyl)-4-(methylamino)-2-(methylthio) pyrimidine-5-carboxamide (23e) (Method I) yield 84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.19 (s, 1H), 6.96 (d, *J* = 7.2 Hz, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.55 (s, 2H), 6.11 (s, 1H), 4.10 (s, 6H), 2.56 (s, 3H), 2.06 (s, 3H), 1.73 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.97, 169.65, 169.21, 163.56, 163.32, 156.55, 139.54, 139.27, 137.96, 133.41, 133.24, 122.37, 122.11, 108.09, 106.77, 106.45, 57.96, 29.56, 19.83, 16.35. LC-MS (ESI, m/z): 468.1630 $[M+H]^+$.

N-(2-methyl-5-(3,4,5-trimethoxybenzamido)phenyl)-4-(methylamino)-2-(methylthio) pyrimidine-5-carboxamide (23f) (Method I) yield 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 – 8.45 (m, 1H), 7.81 – 7.72 (m, 1H), 7.51 – 7.41 (m, 1H), 7.29 – 7.15 (m, 3H), 3.93 – 3.88 (m, 6H), 3.87 – 3.84 (m, 3H), 3.07 – 3.02 (m, 3H), 2.58 – 2.53 (m, 3H), 2.25 – 2.19 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.83, 169.20, 169.08, 163.16, 156.46, 155.54, 143.37, 139.28, 137.90, 133.18, 132.99, 132.63, 122.17, 122.06, 107.61, 106.65, 62.70, 58.27, 29.24, 19.66, 15.93. LC-MS (ESI, m/z): 498.1782 $[M+H]^+$.

N-(5-(benzo[d][1,3]dioxole-5-carboxamido)-2-methylphenyl)-4-(methylamino)-2-(methylthio)pyrimidine-5-carboxamide (23g) (Method I) yield 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H), 7.92 (s, 1H), 7.76 – 7.65 (m, 2H), 7.64 (s, 1H), 7.45 (d, *J* = 5.1 Hz, 1H), 7.12 (d, *J* = 4.7 Hz, 1H), 6.31 (s, 2H), 3.10 (s, 2H), 2.85 (s, 3H), 2.49 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.26, 169.44, 169.23, 163.58, 156.83, 153.63, 150.92, 139.59, 138.17, 133.77, 133.18, 131.68, 125.45, 122.54, 122.11, 110.91, 110.79, 107.05, 104.81, 41.42, 30.04, 20.29, 16.89. LC-MS (ESI, m/z): 452.1319 $[M+H]^+$.

N-(5-(2,3-dihydrobenzo[b][1,4]dioxine-6-carboxamido)-2-methylphenyl)-4-(methylamino)-2-(methylthio)pyrimidine-5-carboxamide (23h) (Method I) yield 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 9.95 (s, 1H), 8.68 (d, *J* = 12.8 Hz, 2H), 7.83 (s, 1H), 7.56 (s, 3H), 7.23 (s, 1H), 7.00 (s, 1H), 4.32 (s, 4H), 2.97 (s, 3H), 2.52 (d, *J* = 4.6 Hz, 3H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.63, 165.75, 164.85, 160.55, 155.22, 146.83, 143.41, 137.78, 136.13, 130.58, 129.46, 128.16, 121.67, 119.23, 118.77, 117.30, 117.14, 104.89, 64.87, 64.50, 27.58, 17.86, 13.99. LC-MS (ESI, m/z): 467.1485 $[M+H]^+$.

N-(5-(2-methoxybenzamido)-2-methylphenyl)-4-(methylamino)-2-(methylsulfonyl) pyrimidine-5-carboxamide (24a) (Method D) yield 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 10.16 (s, 1H), 8.92 (s, 1H), 8.86 (s, 1H), 7.88 (s, 1H), 7.65 (s, 1H), 7.52 (s, 2H), 7.26 (d, *J* = 7.9 Hz, 1H), 7.18 (d, *J* = 6.8 Hz, 1H), 7.08 (s, 1H), 3.91 (s, 3H), 3.02 (s, 3H), 2.38 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.66, 164.85, 164.37, 161.41, 156.97, 155.64, 137.62, 135.85, 132.53, 130.85, 130.15, 129.52, 128.38, 125.29, 120.99, 118.49, 112.49, 111.25, 56.47, 39.17, 28.01, 17.88. LC-MS (ESI, m/z): 470.1735 $[M+H]^+$.

N-(5-(3-methoxybenzamido)-2-methylphenyl)-4-(methylamino)-2-(methylsulfonyl) pyrimidine-5-carboxamide (24b) (Method D) yield 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.64 (s, 1H), 8.38 (s, 1H), 8.28 (s, 1H), 8.05 (s, 1H), 7.33 (s, 2H), 7.25 (d, *J* = 6.9 Hz, 1H), 7.10 – 6.93 (m, 3H), 3.77 (s, 3H), 3.21 (s, 3H), 2.88 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.67, 165.98, 164.82, 160.86, 159.68, 154.38, 136.15, 135.78, 134.93, 130.80, 130.26, 129.58, 119.44, 118.87, 117.90, 117.08, 112.68, 111.01, 55.48, 38.56, 27.73, 17.64. LC-MS (ESI, m/z): 470.1426 $[M+H]^+$.

N-(5-(4-methoxybenzamido)-2-methylphenyl)-4-(methylamino)-2-(methylsulfonyl) pyrimidine-5-carboxamide (24c) (Method D) yield 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 10.13 (s, 1H), 8.91 (s, 1H), 8.84 (s, 1H), 7.98 (d, *J* = 7.4 Hz, 3H), 7.88 (s, 1H), 7.59 (d, *J* = 6.7 Hz, 1H), 7.26 (d, *J* = 7.5 Hz, 1H), 7.07 (d, *J* = 7.5 Hz, 3H), 3.85 (s, 3H), 3.38 (s, 3H), 3.01 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.65, 165.26, 164.34, 162.38, 161.39, 155.57, 137.91, 135.68, 130.72, 130.03, 129.30, 127.36, 119.09, 114.08, 111.34, 55.90, 39.16, 28.17, 17.85. LC-MS (ESI, m/z): 470.1430 $[M+H]^+$.

N-(5-(3,4-dimethoxybenzamido)-2-methylphenyl)-4-(methylamino)-2-(methylsulfonyl) pyrimidine-5-carboxamide (24d) (Method D) yield 90%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 10.10 (s, 1H), 8.90 (s, 1H), 8.83 (s, 1H), 7.85 (s, 1H), 7.64 (d, *J* = 7.5 Hz, 1H),

7.60 (d, *J* = 8.6 Hz, 1H), 7.56 (s, 1H), 7.27 (d, *J* = 8.1 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 1H), 3.86 (s, 6H), 3.38 (s, 3H), 3.01 (d, *J* = 3.6 Hz, 3H), 2.22 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.64, 165.25, 164.35, 161.38, 155.55, 152.13, 148.78, 137.83, 135.68, 133.17, 131.11, 130.72, 129.35, 128.38, 127.35, 121.53, 119.26, 111.50, 56.15, 39.15, 28.17, 17.83. LC-MS (ESI, m/z): 500.1535 $[M+H]^+$.

N-(5-(3,5-dimethoxybenzamido)-2-methylphenyl)-4-(methylamino)-2-(methylsulfonyl) pyrimidine-5-carboxamide (24e) (Method D) yield 76%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H), 9.66 (s, 1H), 9.24 (s, 1H), 8.70 (s, 2H), 7.79 (s, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.14 (d, *J* = 8.1 Hz, 3H), 6.94 (d, *J* = 7.8 Hz, 1H), 6.83 (d, *J* = 7.9 Hz, 1H), 6.72 (s, 1H), 4.73 (s, 2H), 3.84 (s, 6H), 3.39 (s, 5H), 3.00 (d, *J* = 3.1 Hz, 3H), 2.21 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.65, 165.38, 164.37, 161.39, 160.86, 155.58, 137.56, 137.33, 135.72, 131.09, 130.76, 129.67, 129.30, 119.28, 111.33, 106.08, 103.81, 55.96, 55.49, 28.44, 28.16, 17.85, 17.72. LC-MS (ESI, m/z): 500.1530 $[M+H]^+$.

N-(2-methyl-5-(3,4,5-trimethoxybenzamido)phenyl)-4-(methylamino)-2-(methylsulfonyl) pyrimidine-5-carboxamide (24f) (Method D) Yield 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 1H), 10.18 (s, 1H), 8.92 (s, 1H), 8.84 (s, 1H), 7.85 (s, 1H), 7.62 (d, *J* = 6.1 Hz, 1H), 7.32 (s, 3H), 3.89 (s, 6H), 3.75 (s, 3H), 3.39 (s, 3H), 3.02 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.65, 165.23, 164.40, 161.39, 155.57, 153.11, 140.81, 137.59, 135.73, 130.77, 130.36, 129.63, 119.42, 111.34, 105.75, 60.85, 56.13, 28.52, 18.00, 15.43. LC-MS (ESI, m/z): 530.1645 $[M+H]^+$.

N-(5-(benzo[d][1,3]dioxole-5-carboxamido)-2-methylphenyl)-4-(methylamino)-2-(methylsulfonyl)pyrimidine-5-carboxamide (24g) (Method D) yield 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 10.10 (s, 1H), 8.91 (s, 1H), 8.84 (s, 1H), 7.87 (s, 1H), 7.57 (dd, *J* = 18.1, 9.8 Hz, 3H), 7.26 (d, *J* = 7.8 Hz, 1H), 7.06 (d, *J* = 7.8 Hz, 1H), 6.14 (s, 2H), 3.38 (s, 3H), 3.01 (d, *J* = 3.1 Hz, 3H), 2.22 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.65, 164.86, 164.35, 161.39, 155.57, 150.52, 147.85, 137.79, 135.69, 130.73, 129.42, 129.11, 123.30, 119.10, 111.34, 108.39, 108.16, 102.29, 39.15, 28.16, 17.85. LC-MS (ESI, m/z): 484.1222 $[M+H]^+$.

N-(5-(2,3-dihydrobenzo[b][1,4]dioxine-6-carboxamido)-2-methylphenyl)-4-(methylamino)-2-(methylsulfonyl)pyrimidine-5-carboxamide (24h) (Method D) yield 79%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (d, *J* = 12.6 Hz, 2H), 9.85 (s, 1H), 9.17 (s, 1H), 8.41 (s, 1H), 7.80 (s, 1H), 7.28 – 7.18 (m, 2H), 6.99 (d, *J* = 7.3 Hz, 2H), 4.32 (s, 4H), 2.93 (s, 3H), 2.50 (s, 3H), 2.17

(s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.89, 164.62, 155.57, 153.77, 146.84, 146.58, 143.42, 137.84, 135.92, 130.62, 129.41, 128.17, 121.66, 119.14, 118.84, 117.31, 117.14, 64.89, 64.51, 46.12, 28.21, 17.84. LC-MS (ESI, m/z): 498.1375 $[M+H]^+$.

N-(5-(2-methoxybenzamido)-2-methylphenyl)-4-(methylamino)-2-(methylsulfonyl) pyrimidine-5-carboxamide (25a) (Method E) yield 76%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 10.12 (s, 1H), 9.95 (s, 1H), 9.17 (s, 1H), 8.86 (s, 1H), 8.76 (s, 1H), 7.82 (s, 2H), 7.64 (d, J = 6.8 Hz, 1H), 7.55 – 7.43 (m, 3H), 7.21 (dd, J = 18.5, 8.2 Hz, 2H), 7.08 (d, J = 6.7 Hz, 1H), 3.91 (s, 3H), 3.06 (s, 3H), 2.50 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.95, 164.81, 161.91, 156.95, 149.03, 138.65, 137.57, 136.26, 133.41, 132.46, 130.72, 130.12, 129.55, 127.06, 125.39, 124.91, 120.97, 118.57, 118.19, 115.54, 112.48, 101.98, 56.36, 28.31, 19.69, 17.91. LC-MS (ESI, m/z): 542.2080 $[M+H]^+$.

N-(5-(3-methoxybenzamido)-2-methylphenyl)-2-(4-methyl-3-nitrophenylamino)-4-(methylamino)pyrimidine-5-carboxamide (25b) (Method E) yield 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 10.24 (s, 1H), 9.95 (s, 1H), 9.18 (s, 1H), 8.86 (s, 1H), 8.77 (s, 1H), 7.85 (s, 1H), 7.79 (d, J = 8.0 Hz, 1H), 7.56 (s, 2H), 7.50 (s, 1H), 7.47 (s, 1H), 7.45 (s, 1H), 7.25 (d, J = 8.1 Hz, 1H), 7.16 (d, J = 7.7 Hz, 1H), 3.85 (s, 3H), 3.06 (s, 4H), 2.50 (s, 3H), 2.22 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.58, 164.89, 161.88, 159.66, 149.02, 137.60, 136.78, 136.12, 133.42, 130.65, 130.00, 129.66, 127.17, 124.95, 120.32, 119.29, 118.88, 117.71, 115.60, 113.37, 102.04, 55.79, 28.34, 19.70, 17.89. LC-MS (ESI, m/z): 542.2083 $[M+H]^+$.

N-(5-(4-methoxybenzamido)-2-methylphenyl)-2-(4-methyl-3-nitrophenylamino)-4-(methylamino)pyrimidine-5-carboxamide (25c) (Method E) yield 78% ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 10.10 (s, 1H), 9.89 (s, 1H), 9.03 (s, 1H), 8.87 (s, 1H), 8.74 (s, 1H), 7.97 (d, J = 8.5 Hz, 2H), 7.81 (d, J = 9.6 Hz, 2H), 7.54 (d, J = 8.2 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 8.1 Hz, 1H), 7.07 (d, J = 8.6 Hz, 2H), 3.85 (s, 3H), 3.04 (d, J = 3.7 Hz, 3H), 2.20 (s, 3H), 2.00 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.84, 165.26, 162.36, 162.04, 158.65, 153.87, 149.07, 138.96, 137.80, 136.19, 133.36, 130.59, 130.01, 129.41, 127.38, 126.69, 124.83, 119.25, 118.77, 115.37, 114.07, 60.41, 55.69, 21.44, 14.80. LC-MS (ESI, m/z): 542.2090 $[M+H]^+$.

N-(5-(3,4-dimethoxybenzamido)-2-methylphenyl)-2-(4-methyl-3-nitrophenylamino)-4-(methylamino)pyrimidine-5-carboxamide (25d) (Method E) yield 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (s, 1H), 10.09 (s, 1H), 9.92 (s, 1H), 9.14 (s, 1H), 8.86 (s, 1H), 8.76 (s, 1H),

7.80 (d, *J* = 13.2 Hz, 2H), 7.63 (d, *J* = 8.1 Hz, 1H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.2 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 1H), 3.85 (s, 6H), 3.06 (s, 3H), 2.50 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.24, 164.98, 161.93, 152.10, 149.03, 148.77, 137.77, 136.10, 133.41, 130.60, 129.40, 127.40, 127.05, 124.91, 121.51, 119.37, 118.91, 115.53, 111.48, 111.40, 102.01, 56.10, 28.30, 19.69, 17.91. LC-MS (ESI, m/z): 572.2185 $[M+H]^+$.

N-(5-(3,5-dimethoxybenzamido)-2-methylphenyl)-2-(4-methyl-3-nitrophenylamino)-4-(methylamino)pyrimidine-5-carboxamide (25e) (Method E) yield 70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 1H), 10.19 (s, 1H), 9.92 (s, 1H), 9.09 (s, 1H), 8.85 (s, 1H), 8.74 (s, 1H), 7.79 (d, *J* = 9.7 Hz, 2H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.46 (d, *J* = 8.1 Hz, 1H), 7.25 (d, *J* = 8.2 Hz, 1H), 7.10 (s, 2H), 6.71 (s, 1H), 3.82 (s, 9H), 2.51 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.40, 165.07, 161.95, 160.85, 149.04, 138.71, 137.46, 137.36, 136.16, 133.40, 130.66, 129.77, 127.00, 124.92, 119.42, 118.95, 115.51, 106.05, 103.78, 101.99, 55.96, 28.26, 19.66, 17.87. LC-MS (ESI, m/z): 572.2183 $[M+H]^+$.

2-(4-methyl-3-nitrophenylamino)-N-(2-methyl-5-(3,4,5-trimethoxybenzamido) phenyl)-4-(methylamino)pyrimidine-5-carboxamide (25f) (Method E) yield 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H), 10.15 (s, 1H), 9.95 (s, 1H), 9.22 (s, 1H), 8.86 (s, 1H), 8.78 (s, 1H), 7.80 (s, 1H), 7.77 (s, 1H), 7.57 (d, *J* = 7.5 Hz, 1H), 7.46 (d, *J* = 8.3 Hz, 1H), 7.30 (s, 2H), 7.26 (d, *J* = 8.1 Hz, 1H), 3.88 (s, 6H), 3.74 (s, 3H), 3.07 (s, 3H), 2.50 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.18, 164.82, 161.85, 153.09, 148.98, 140.78, 138.37, 137.55, 136.09, 133.42, 130.65, 130.42, 129.62, 127.25, 124.94, 119.49, 119.06, 115.61, 105.73, 102.03, 60.63, 56.13, 28.09, 20.07, 17.99. LC-MS (ESI, m/z): 602.2293 $[M+H]^+$.

N-(5-(benzo[d][1,3]dioxole-5-carboxamido)-2-methylphenyl)-2-(4-methyl-3-nitrophenylamino)-4-(methylamino)pyrimidine-5-carboxamide (25g) (Method E) yield 79%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 10.07 (s, 1H), 9.95 (s, 1H), 9.27 (s, 1H), 8.84 (s, 1H), 8.78 (s, 1H), 7.85 (s, 1H), 7.75 (d, *J* = 7.2 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.54 (d, *J* = 9.5 Hz, 2H), 7.44 (d, *J* = 7.9 Hz, 1H), 7.23 (d, *J* = 7.9 Hz, 1H), 7.04 (d, *J* = 7.8 Hz, 1H), 6.13 (s, 2H), 3.06 (s, 3H), 2.49 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.83, 161.76, 159.62, 150.49, 148.89, 147.89, 147.83, 137.74, 135.98, 133.43, 130.62, 129.44, 129.14, 124.94, 123.27, 119.18, 115.66, 108.34, 108.15, 102.26, 102.11, 28.23, 19.93, 17.85. LC-MS (ESI, m/z): 556.1870 $[M+H]^+$.

N-(5-(2,3-dihydrobenzo[b][1,4]dioxine-6-carboxamido)-2-methylphenyl)-2-(4-methyl-3-nitrophenylamino)-4-(methylamino)pyrimidine-5-carboxamide (25h) (Method E) yield 67%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (s, 1H), 10.06 (s, 1H), 9.92 (s, 1H), 9.16 (s, 1H), 8.86 (s, 1H), 8.77 (s, 1H), 7.83 (d, *J* = 12.8 Hz, 2H), 7.51 (d, *J* = 25.3 Hz, 3H), 7.24 (s, 1H), 7.00 (s, 1H), 4.32 (s, 4H), 3.06 (s, 3H), 2.51 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.86, 161.93, 152.17, 149.05, 146.85, 143.42, 138.57, 137.78, 136.07, 133.42, 130.60, 129.42, 128.16, 127.11, 124.97, 121.66, 119.19, 118.75, 117.31, 117.14, 115.62, 102.06, 64.89, 64.52, 28.33, 19.70, 17.88. LC-MS (ESI, m/z): 570.2030 $[M+H]^+$.

2-(3-amino-4-methylphenylamino)-N-(5-(2-methoxybenzamido)-2-methylphenyl)-4-(methylamino)pyrimidine-5-carboxamide (26a) (Method F) yield 63%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.22 (s, 1H), 9.67 (s, 1H), 9.25 (s, 1H), 8.71 (s, 2H), 7.82 (s, 1H), 7.58 (s, 2H), 7.55 – 7.41 (m, 2H), 7.24 (d, *J* = 7.6 Hz, 1H), 7.16 (s, 2H), 6.95 (d, *J* = 6.2 Hz, 1H), 6.84 (s, 1H), 3.85 (s, 3H), 3.01 (s, 3H), 2.22 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.27, 165.55, 162.51, 160.62, 159.66, 157.34, 146.73, 139.22, 137.52, 136.83, 136.73, 130.52, 130.07, 130.01, 129.71, 120.33, 119.43, 118.52, 117.72, 115.54, 113.35, 108.76, 106.13, 99.99, 55.80, 27.84, 17.95, 17.38. LC-MS (ESI, m/z): 512.2340 $[M+H]^+$.

2-(3-amino-4-methylphenylamino)-N-(5-(3-methoxybenzamido)-2-methylphenyl)-4-(methylamino)pyrimidine-5-carboxamide (26b) (Method F) yield 63%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.22 (s, 1H), 9.67 (s, 1H), 9.25 (s, 1H), 8.71 (s, 2H), 7.82 (s, 1H), 7.58 (s, 2H), 7.55 – 7.41 (m, 2H), 7.24 (d, *J* = 7.6 Hz, 1H), 7.16 (s, 2H), 6.95 (d, *J* = 6.2 Hz, 1H), 6.84 (s, 1H), 3.85 (s, 3H), 3.01 (s, 3H), 2.22 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.27, 165.55, 162.51, 160.62, 159.66, 157.34, 146.73, 139.22, 137.52, 136.83, 136.73, 130.52, 130.07, 130.01, 129.71, 120.33, 119.43, 118.52, 117.72, 115.54, 113.35, 108.76, 106.13, 99.99, 55.80, 27.84, 17.95, 17.38. LC-MS (ESI, m/z): 512.2340 $[M+H]^+$.

2-(3-amino-4-methylphenylamino)-N-(5-(4-methoxybenzamido)-2-methylphenyl)-4-(methylamino)pyrimidine-5-carboxamide (26c) (Method F) yield 83%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H), 9.81 (s, 1H), 9.38 (s, 1H), 8.85 (s, 2H), 8.12 (d, *J* = 8.4 Hz, 2H), 7.96 (s, 1H), 7.71 (d, *J* = 6.6 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.30 (s, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 6.6 Hz, 1H), 6.97 (d, *J* = 7.9 Hz, 1H), 3.97 (s, 3H), 3.57 (s, 3H), 2.34 (s, 3H), 2.17 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.30, 165.26, 162.52, 162.35, 160.63, 157.33, 146.71,

139.23, 137.78, 136.69, 130.49, 130.10, 130.03, 129.44, 127.46, 119.38, 118.48, 115.59, 114.06, 108.82, 106.17, 100.20, 55.87, 27.84, 17.93, 17.36. LC-MS (ESI, m/z): 512.2340 $[M+H]^+$.

2-(3-amino-4-methylphenylamino)-N-(5-(3,4-dimethoxybenzamido)-2-methylphenyl)-4-(methylamino)pyrimidine-5-carboxamide (26d) (Method F) yield 81%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (s, 1H), 9.65 (s, 1H), 9.23 (s, 1H), 8.70 (s, 2H), 7.78 (s, 1H), 7.64 (d, *J* = 8.3 Hz, 1H), 7.57 (d, *J* = 9.2 Hz, 2H), 7.23 (d, *J* = 8.1 Hz, 1H), 7.15 (s, 1H), 7.09 (d, *J* = 8.4 Hz, 1H), 6.94 (d, *J* = 7.8 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 3.86 (s, 3H), 3.40 (s, 3H), 3.00 (d, *J* = 4.3 Hz, 3H), 2.21 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.28, 165.22, 162.50, 160.61, 157.32, 152.09, 148.78, 146.72, 139.21, 137.69, 136.68, 130.48, 130.08, 129.45, 127.45, 121.51, 119.50, 118.57, 115.56, 111.49, 111.42, 108.77, 106.14, 100.17, 56.25, 27.83, 17.71, 17.07. LC-MS (ESI, m/z): 542.2445 $[M+H]^+$.

2-(3-amino-4-methylphenylamino)-N-(5-(3,5-dimethoxybenzamido)-2-methylphenyl)-4-(methylamino)pyrimidine-5-carboxamide (26e) (Method F) yield 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H), 9.66 (s, 1H), 9.24 (s, 1H), 8.70 (s, 2H), 7.79 (s, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.14 (d, *J* = 8.1 Hz, 3H), 6.94 (d, *J* = 7.8 Hz, 1H), 6.83 (d, *J* = 7.9 Hz, 1H), 6.72 (s, 1H), 4.73 (s, 2H), 3.84 (s, 6H), 3.39 (s, 5H), 3.00 (d, *J* = 3.1 Hz, 3H), 2.21 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.28, 165.33, 162.50, 160.86, 160.62, 157.33, 146.73, 139.21, 137.43, 136.72, 130.53, 130.08, 129.75, 119.51, 118.58, 115.56, 108.76, 106.13, 106.06, 103.81, 100.15, 55.97, 27.83, 17.93, 17.36. LC-MS (ESI, m/z): 542.2440 $[M+H]^+$.

2-(3-amino-4-methylphenylamino)-N-(2-methyl-5-(3,4,5-trimethoxybenzamido) phenyl)-4-(methylamino)pyrimidine-5-carboxamide (26f) (Method F) yield 65%. 1H NMR (400 MHz, DMSO-d6) δ 10.13 (s, 1H), 9.66 (s, 1H), 9.24 (s, 1H), 8.71 (s, 2H), 7.77 (s, 1H), 7.59 (d, J = 7.4 Hz, 1H), 7.32 (s, 2H), 7.25 (d, J = 7.8 Hz, 1H), 7.16 (s, 1H), 6.95 (d, J = 7.1 Hz, 1H), 6.83 (d, J = 7.5 Hz, 1H), 4.74 (s, 2H), 3.89 (s, 6H), 3.75 (s, 3H), 3.01 (s, 3H), 2.22 (s, 3H), 2.03 (s, 3H). 13C NMR (101 MHz, DMSO-d6) δ 166.29, 165.15, 162.49, 160.60, 157.32, 153.09, 146.70, 140.77, 139.19, 137.45, 136.72, 130.53, 130.44, 130.07, 129.68, 119.64, 118.64, 115.57, 108.77, 106.14, 105.74, 60.59, 56.56, 27.83, 17.91, 17.35. LC-MS (ESI, m/z): 572.2550 [M+H]+.

2-(3-amino-4-methylphenylamino)-N-(5-(benzo[d][1,3]dioxole-5-carboxamido)-2-methylphenyl)-4-(methylamino)pyrimidine-5-carboxamide (26g) (Method F) yield 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 9.66 (s, 1H), 9.24 (s, 1H), 8.71 (s, 2H), 7.81 (s, 1H),

7.61 (d, *J* = 8.1 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.16 (s, 1H), 7.07 (d, *J* = 8.0 Hz, 1H), 6.95 (d, *J* = 7.6 Hz, 1H), 6.83 (d, *J* = 7.8 Hz, 1H), 6.14 (s, 2H), 4.73 (s, 2H), 3.01 (s, 3H), 2.21 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.28, 164.81, 162.51, 160.62, 157.34, 150.48, 147.85, 146.73, 139.23, 137.65, 136.70, 130.49, 130.09, 129.51, 129.21, 123.28, 119.36, 118.45, 115.56, 108.78, 108.39, 108.16, 106.14, 102.27, 27.84, 17.93, 17.36. LC-MS (ESI, m/z): 526.2130 $[M+H]^+$.

2-(3-amino-4-methylphenylamino)-N-(5-(2,3-dihydrobenzo[b][1,4]dioxine-6-carboxamido)-2-methylphenyl)-4-(methylamino)pyrimidine-5-carboxamide (26h) (Method F) yield 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (s, 1H), 7.84 (s, 1H), 7.55 (dd, *J* = 12.6, 3.9 Hz, 3H), 7.27 – 7.15 (m, 2H), 7.08 – 6.94 (m, 2H), 6.84 (d, *J* = 8.1 Hz, 1H), 4.31 (s, 4H), 3.01 (s, 3H), 2.21 (s, 3H), 2.06 (d, *J* = 17.7 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.17, 164.78, 162.42, 160.55, 157.34, 146.83, 146.64, 143.41, 139.13, 137.61, 136.57, 130.48, 130.10, 129.42, 128.17, 121.67, 119.19, 118.33, 117.31, 117.15, 115.56, 108.74, 106.01, 100.14, 64.87, 64.49, 27.69, 17.91, 17.32. LC-MS (ESI, m/z): 540.2290 $[M+H]^+$.

Cell lines and cell culture

BaF3, P210-BaF3, Tel-ABL-BaF3, K562 (CML), Ku812 (CML), MEG-01 (CML), MV4-11 (AML), MOLM14 (AML), REC-1 (human B-cell lymphoma cell), OCI-AML-3 (AML), U937 (AML), Kasumi-1 (AML), HEL (AML), CHL (Hamster lung cell), CHO (Hamster ovary cell) were used. All the cells were grown in a humidified incubator at 37 °C under 5% CO_2. CHO cells were maintained in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin. BaF3, P210/BaF3, Tel-ABL-BaF3, K562, Ku812, MEG-01, MV4-11, MOLM14, REC-1, OCI-AML-3, U937, Kasumi-1, HEL, CHL cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supported with 10% FBS, and 1% penicillin/streptomycin. Cells were grown in tissue culture flasks until they were 85-95% confluent prior to use. These non-adherent cells were collected by spin down at 700 rpm/min for 4 min.

General proliferation protocol for non-adherent cells

A density of 2 to 3×10^4 cells/mL cells were mixed with various concentrations of compounds then 100 μL was added to each well and incubated for 72 hours. Cell viability was determined using the CellTiter-Glo (Promega, USA) or CCK-8 (Beboy, China). Both assays were performed

according to the manufacturer instructions. For CellTiter-Glo assay, luminescence was determined in a multi-label reader (Envision, PerkinElmer, USA). For CCK-8 assay, absorbance was measured in a microplate reader (iMARK, Bio-Rad, USA) at 450 nm and 650 nm. Data were normalized to control group (DMSO). GI_{50}s were calculated using Prism 5.0 (GraphPad Software, San Diego, CA).

TEL-isogenic cell generation

Retroviral constructs for BaF3-FLT3 mutants were made based on the pMSCVpuro (Clontech) backbone. For TEL-FLT3 vector, 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 sequence of FLT3 was inserted in-frame between TEL and 3xFLAG sequences. For full-length expression vectors, the coding sequences of FLT3 variants were directly cloned in pMSCVpuro vector with a 3xFLAG tag at the C- terminal end. All mutagenesis were performed using the QuikChange Site-Directed Mutagenesis Kit (Stratagene) following the manufacturer's instructions. Retrovirus was packaged in HEK293T cells by transfecting FLT3-containing MSCV vectors together with two helper plasmids. Virus supernatants were harvested 48 hours after transfection and filtered before infection. Then BaF3 cells were infected with harvested virus supernatants using spinoculation protocol and stable cell lines were obtained by puromycin selection for 48 hours. The IL-3 concentrations in the culture medium were gradually withdrawn until cells were able to grow in the absence of IL-3.

Signaling pathway study

KU812, K562 and MEG-01 cells were treated with DMSO, serially diluted compound **18a**, 1 μM Imatinib, 0.1 μM Dasatinib for 1 h. Cells were then washed in PBS and lysed in cell lysis buffer. Phospho-c-Abl (Tyr245)(73E5) Rabbit mAb #2868, c-Abl antibody #2862, STAT5 (3H7) Rabbit mAb #9358, Phospho-STAT5 (Tyr694)(C71E5) Rabbit mAb #9314, Akt (pan)(C67E7) Rabbit mAb #4691, Phospho-Akt (Thr308) (244F9) Rabbit mAb #4056, Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060, Phospho-Crkl (Tyr207) antibody #3181, Crkl (32H4) Mouse mAb #3182, Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (197G2) Rabbit mAb #4377, p44/42

MAPK (Erk1/2) (137F5) Rabbit mAb #4695 antibody (Cell signaling Technology) were used for immunoblotting.

Apoptosis effect examination

KU812, K562, MEG-01 cells were treated with DMSO, serially diluted compound **18a**, 0.5 μM Imatinib, 0.5 μM Dasatinib for the indicated periods. Cells were then washed in PBS and lysed in cell lysis buffer. PARP (46D11) Rabbit mAb #9532, Caspase-3 (8G10) Rabbit mAb #9665, GAPDH (14C10) Rabbit mAb #2118 antibody (Cell signaling Technology) were used for immunoblotting.

Cell cycle analysis

KU812, K562, MEG-01 cells were treated with DMSO, serially diluted compound **18a**, 0.5 μM Imatinib, 0.5 μM Dasatinib for the indicated periods. 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.

In vivo pharmacodynamics studies

Compound **18a** 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-eight weeks 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 group 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 group 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 0.2 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).

K562 xenograft model

Six weeks old female nu/nu mice were purchased from the Shanghai Experimental Center, Chinese Academy of Sciences (Shanghai, China). All animals were maintained in a specific pathogen-free facility and used according to the animal care regulations of Hefei Institutes of Physical Science, Chinese Academy of Sciences (Hefei, China), and all efforts were made to minimize animal suffering. To obtain orthotopic xenograft of human mammary tumor in the mice, cells were harvested during exponential growth. Six million K562 cells in PBS were suspended in 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 K562 tumors had reached a size of 200 to 400 mm^3. Animals were then randomized into treatment groups of 5 mice each for efficacy studies. Compound **18a** was delivered daily in a PEG300 solution (30% PEG300/10% ethanol in ddH_2O) by orally gavages. A range of doses of **18a** or its vehicle was administered, as indicated in figure 7 legends. Body weight and tumor growth was measured daily after **18a** treatment. Tumor volumes were calculated as follows: tumor volume (mm^3)= [($W^2\times$ 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.

HE staining

HE staining was carried out according to the previous report[21]. First hydrate the sections and then dip the slide into a Coplin jar containing Mayer's hematoxylin and agitate for 30 sec. After rinsing the slide in H_2O for 1min, stain with 1% eosin Y solution for 10-30 sec with agitation. Subsequently, dehydrate the sections with two changes of 95% alcohol and two changes of 100% alcohol for 30 sec each. And then extract the alcohol with two changes of xylene. Finally, add one or two drops of mounting medium and cover with a cover slip.

Ki-67 staining

For IHC demonstration of Ki-67, tissue sections were quenched for endogenous peroxides and placed in an antigen retrieval solution (0.01M citrate buffer, PH 6.0) for 15 min in a microwave oven at 100℃ at 600W. After incubation in the casein block, mouse MAb anti-Ki-67 (ZSGB-BIO, China) was applied to the sections at dilutions of 1:50. Incubations with primary antibodies lasted overnight at 4℃. 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.

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 15min 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℃. Following washes, the slides were covered by converter-POD solution for 30 min at 37℃. 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.

Molecular modeling

Molecular docking of small molecules to ABL1 kinase was performed with software Autodock 4.0.[22] The ABL kinase structure (PDB ID: 2HYY) including the chain A of the kinase domain was used as the receptor, and polar hydrogen atoms were added to the receptor structure. All small molecules were constructed using the online-demo CORINA server. The grip map was adjusted as a dimension of 64×54×50 points with a spacing of 0.375 Å. The default parameters were used and a total of 50 runs were performed with Lamarckian genetic algorithm. The docked models were then clustered and sorted by binding energy.

ASSOCIATED CONTENT

Supporting Information.

Table S1 listing the DiscoveRx's KinomeScan selectivity profiling data of compound **18a**. Table S2 listing the binding Kd of several clinical inhibitors determined by DiscoveRx's binding assay

which was obtained from previous publication. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Qingsong Liu, PhD

High Magnetic Field Laboratory

Chinese Academy of Sciences, Mailbox 1110

350 Shushanhu Road, Hefei, Anhui, P. R. China, 230031

Email: qsliu97@hmfl.ac.cn

*Jing Liu, PhD,

High Magnetic Field Laboratory

Chinese Academy of Sciences, Mailbox 1110

350 Shushanhu Road, Hefei, Anhui, P. R. China, 230031

Email: jingliu@hmfl.ac.cn

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. [7]These authors contributed equally.

Notes

Dr. Shanchun Zhang is a shareholder of Hefei Cosource Medicine Technology Co. LTD.

ACKNOWLEDGMENT

We thank Scientific Research Grant of Hefei Science Center of CAS (SRG-HSC # 2015SRG-HSC022). J. Liu, Q. Liu and W. Wang are supported by the grant of "Cross-disciplinary Collaborative Teams Program for Science, Technology and Innovation (2014-2016)" from Chinese Academy of Sciences. Z. Zhao is supported by Anhui Province Natural Science Foundation Annual Key Program (grant number: 1301023011). We want to thank China "Thousand Talent Program" support for Prof Q. Liu and "Hundred Talent Program" of the Chinese Academy of Sciences support for Prof J. Liu and W. Wang. X. Liang was supported by the National Natural Science Foundation of China (grant number: 81402797).

ABBREVIATIONS

CML, chronic myeloid leukemia; AML, acute myeloid leukemia; MCL, mantel cell lymphoma; BCR, break point cluster region; ABL kinase, abelson kinase; HATU, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; DIPEA, N, N-diisopropylethylamine; DMF, dimethylformamide; DMAP, 4-dimethylaminopyridine; LAH, lithium aluminum hydride; TGI, tumor growth inhibition.

REFERENCES

(1) Sawyers, C. L.; Hochhaus, A.; Feldman, E.; Goldman, J. M.; Miller, C. B.; Ottmann, O. G.; Schiffer, C. A.; Talpaz, M.; Guilhot, F.; Deininger, M. W. N.; Fischer, T.; O'Brien, S. G.; Stone, R. M.; Gambacorti-Passerini, C. B.; Russell, N. H.; Reiffers, J. J.; Shea, T. C.; Chapuis, B.; Coutre, S.; Tura, S.; Morra, E.; Larson, R. A.; Saven, A.; Peschel, C.; Gratwohl, A.; Mandelli, F.; Ben-Am, M.; Gathmann, I.; Capdeville, R.; Paquette, R. L.; Druker, B. J. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. *Blood* **2002,** 99 (10), 3530-3539.

(2) Aamir Rana, Sabir Hussain Shah, Nazia Rehman, Shaukat Ali, Ghulam Muhammad Ali, Shahzad Bhatti, Ammad Ahmad Farooqi. Chronic Myeloid Leukemia: Attributes Of Break Point Cluster Region-Abelson (BCR-ABL). *J. Cancer Res. Exp. Oncol.* **2011,** 3 (6), 5.

(3) Greuber, E. K.; Smith-Pearson, P.; Wang, J.; Pendergast, A. M. Role of ABL family kinases in cancer: from leukaemia to solid tumours. *Nat. Rev. Cancer* **2013,** 13 (8), 559-571.

(4) Deininger, M. W. N.; Goldman, J. M.; Melo, J. V. The molecular biology of chronic myeloid leukemia. *Blood* **2000,** 96 (10), 3343-3356.

(5) Schindler, T.; Bornmann, W.; Pellicena, P.; Miller, W. T.; Clarkson, B.; Kuriyan, J. Structural mechanism for STI-571 inhibition of Abelson tyrosine kinase. *Science* **2000,** 289 (5486), 1938-1942.

(6) Deininger, M.; Buchdunger, E.; Druker, B. J. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. *Blood* **2005,** 105 (7), 2640-2653.

(7) Weisberg, E.; Manley, P. W.; Breitenstein, W.; Bruggen, J.; Cowan-Jacob, S. W.; Ray, A.; Huntly, B.; Fabbro, D.; Fendrich, G.; Hall-Meyers, E.; Kung, A. L.; Mestan, J.; Daley, G. Q.; Callahan, L.; Catley, L.; Cavazza, C.; Mohammed, A.; Neuberg, D.; Wright, R. D.; Gilliland, D. G.; Griffin, J. D. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell* **2005,** 7 (2), 129-141.

(8) Shah, N. P.; Tran, C.; Lee, F. Y.; Chen, P.; Norris, D.; Sawyers, C. L. Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* **2004,** 305 (5682), 399-401.

(9) Boschelli, D. H.; Ye, F.; Wang, Y. D.; Dutia, M.; Johnson, S. L.; Wu, B. Q.; Miller, K.; Powell, D. W.; Yaczko, D.; Young, M.; Tischler, M.; Arndt, K.; Discafani, C.; Etienne, C.; Gibbons, J.; Grod, J.; Lucas, J.; Weber, J. M.; Boschelli, F. Optimization of 4-phenylamino-3-quinolinecarbonitriles as potent inhibitors of Src kinase activity. *J. Med. Chem.* **2001,** 44 (23), 3965-3977.

(10) O'Hare, T.; Shakespeare, W. C.; Zhu, X. T.; Eide, C. A.; Rivera, V. M.; Wang, F.; Adrian, L. T.; Zhou, T. J.; Huang, W. S.; Xu, Q. H.; Metcalf, C. A.; Tyner, J. W.; Loriaux, M. M.; Corbin, A. S.; Wardwell, S.; Ning, Y. Y.; Keats, J. A.; Wang, Y. H.; Sundaramoorthi, R.; Thomas, M.; Zhou, D.; Snodgrass, J.; Commodore, L.; Sawyer, T. K.; Dalgarno, D. C.; Deininger, M. W. N.; Druker, B. J.; Clackson, T. AP24534, a Pan-BCR-ABL Inhibitor for Chronic Myeloid Leukemia, Potently Inhibits the T315I Mutant and Overcomes Mutation-Based Resistance. *Cancer Cell* **2009,** 16 (5), 401-412.

(11) Niwa Tomoko, A. T., Kimura Shinya. NS-187 (INNO-406), a Bcr-Abl/Lyn dual tyrosine kinase inhibitor. *Anal. Chem. Insights* **2007,** 2, 14.

(12) Carpinelli, P.; Ceruti, R.; Giorgini, M. L.; Cappella, P.; Gianellini, L.; Croci, V.; Degrassi, A.; Texido, G.; Rocchetti, M.; Vianello, P.; Rusconi, L.; Storici, P.; Zugnoni, P.; Arrigoni, C.; Soncini, C.; Alli, C.; Patton, V.; Marsiglio, A.; Ballinari, D.; Pesenti, E.; Fancelli, D.; Moll, J. PHA-739358, a potent inhibitor of Aurora kinases with a selective target inhibition profile relevant to cancer. *Mol. Cancer Ther.* **2007,** 6 (12), 3158-3168.

(13) Chan, W. W.; Wise, S. C.; Kaufman, M. D.; Ahn, Y. M.; Ensinger, C. L.; Haack, T.; Hood, M. M.; Jones, J.; Lord, J. W.; Lu, W. P.; Miller, D.; Patt, W. C.; Smith, B. D.; Petillo, P. A.; Rutkoski, T. J.; Telikepalli, H.; Vogeti, L.; Yao, T.; Chun, L.; Clark, R.; Evangelista, P.; Gavrilescu, L. C.; Lazarides, K.; Zaleskas, V. M.; Stewart, L. J.; Van Etten, R. A.; Flynn, D. L. Conformational Control Inhibition of the BCR-ABL1 Tyrosine Kinase, Including the Gatekeeper T315I Mutant, by the Switch-Control Inhibitor DCC-2036. *Cancer Cell* **2011,** 19 (4), 556-568.

(14) Ren, X. M.; Pan, X. F.; Zhang, Z.; Wang, D. P.; Lu, X. Y.; Li, Y. P.; Wen, D. H.; Long, H. Y.; Luo, J. F.; Feng, Y. B.; Zhuang, X. X.; Zhang, F. X.; Liu, J. Q.; Leng, F.; Lang, X. F.; Bai, Y.; She, M. Q.; Tu, Z. C.; Pan, J. X.; Ding, K. Identification of GZD824 as an Orally Bioavailable Inhibitor That Targets Phosphorylated and Nonphosphorylated Breakpoint Cluster Region-Abelson (Bcr-Abl) Kinase and Overcomes Clinically Acquired Mutation-Induced Resistance against Imatinib. *J. Med. Chem.* **2013,** 56 (3), 879-894.

(15) Zhang, J. M.; Adrian, F. J.; Jahnke, W.; Cowan-Jacob, S. W.; Li, A. G.; Iacob, R. E.; Sim, T.; Powers, J.; Dierks, C.; Sun, F. X.; Guo, G. R.; Ding, Q.; Okram, B.; Choi, Y.; Wojciechowski, A.; Deng, X. M.; Liu, G. X.; Fendrich, G.; Strauss, A.; Vajpai, N.; Grzesiek, S.; Tuntland, T.; Liu, Y.; Bursulaya, B.; Azam, M.; Manley, P. W.; Engen, J. R.; Daley, G. Q.; Warmuth, M.; Gray, N. S. Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. *Nature* **2010,** 463 (7280), 501-U116.

(16) Nonami, A.; Sattler, M.; Weisberg, E.; Liu, Q. S.; Zhang, J. M.; Patricelli, M. P.; Christie, A. L.; Saur, A. M.; Kohl, N. E.; Kung, A. L.; Yoon, H.; Sim, T.; Gray, N. S.; Griffin, J. D. Identification of novel therapeutic targets in acute leukemias with NRAS mutations using a pharmacologic approach. *Blood* **2015,** 125 (20), 3133-3143.

(17) Choi, H. G.; Ren, P. D.; Adrian, F.; Sun, F. X.; Lee, H. S.; Wang, X.; Ding, Q. A.; Zhang, G. B.; Xie, Y. P.; Zhang, J. M.; Liu, Y.; Tuntland, T.; Warmuth, M.; Manley, P. W.; Mestan, J.; Gray, N. S.; Sim, T. A Type-II Kinase Inhibitor Capable of Inhibiting the T315I "Gatekeeper" Mutant of Bcr-Abl. *J. Med. Chem.* **2010,** 53 (15), 5439-5448.

(18) Martinelli, G.; Soverini, S.; Rosti, G.; Baccarani, M. Dual tyrosine kinase inhibitors in chronic myeloid leukemia. *Leukemia* **2005,** 19 (11), 1872-1879.

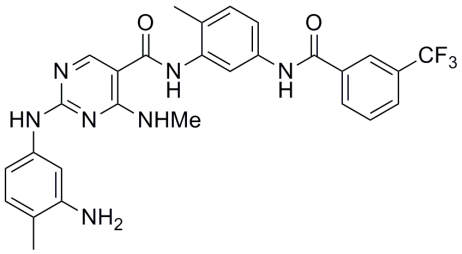
(19) Dong, Y.; Xiong, M.; Duan, L.; Liu, Z.; Niu, T.; Luo, Y.; Wu, X.; Xu, C.; Lu, C. H2AX phosphorylation regulated by p38 is involved in Bim expression and apoptosis in chronic myelogenous leukemia cells induced by imatinib. *Apoptosis* **2014,** 19 (8), 1281-1292.

(20) Winger, J. A.; Hantschel, O.; Superti-Furga, G.; Kuriyan, J. The structure of the leukemia drug imatinib bound to human quinone reductase 2 (NQO2). *BMC Struct. Biol.* **2009**, 9, 7.

(21) Fischer, A. H.; Jacobson, K. A.; Rose, J.; Zeller, R. Hematoxylin and eosin staining of tissue and cell sections. *CSH Protoc.* **2008,** 2008, pdb prot4986.

(22) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009,** 30 (16), 2785-2791.

Table of Contents



K562: GI_{50}: 14 nM
KU812: GI_{50}: 25 nM
MEG-01: GI_{50}: 16 nM
pABL1: EC_{50}: about 100 nM
ABL1: IC_{50}: 70 nM
S Score(1)=0.02
$T_{1/2}$: 4.3 h
Bioavailablity: 24%