Discovery of [4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](2,3-difluoro-6-methoxyphenyl)methanone (R547), A Potent and Selective Cyclin-Dependent Kinase Inhibitor with Significant in Vivo Antitumor Activity

Xin-Jie Chu,*,† Wanda DePinto,‡ David Bartkovitz,† Sung-Sau So,† Binh T. Vu,† Kathryn Packman,‡ Christine Lukacs,† Qingjie Ding,† Nan Jiang,† Ka Wang,§ Petra Goelzer,§ Xuefeng Yin,‡ Melissa A. Smith,‡ Brian X. Higgins,‡ Yingsi Chen,|| Qing Xiang,|| John Moliterni,† Gerald Kaplan,† Bradford Graves,† Allen Lovey,*,† and Nader Fotouhi†

Departments of Discovery Chemistry, Discovery Oncology, Non-Clinical Drug Safety and Discovery Technologies, Hoffmann-La Roche Inc., 340 Kingsland Street, Nutley, New Jersey 07110

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The cyclin-dependent kinases (CDKs) and their cyclin partners are key regulators of the cell cycle. Since deregulation of CDKs is found with high frequency in many human cancer cells, pharmacological inhibition of CDKs with small molecules has the potential to provide an effective strategy for the treatment of cancer. The 2,4-diamino-5-ketopyrimidines **6** reported here represent a novel class of potent and ATP-competitive inhibitors that selectively target the cyclin-dependent kinase family. This diaminopyrimidine core with a substituted 4-piperidine moiety on the C2-amino position and 2-methoxybenzoyl at the C5 position has been identified as the critical structure responsible for the CDK inhibitory activity. Further optimization has led to a good number of analogues that show potent inhibitory activities against CDK1, CDK2, and CDK4 but are inactive against a large panel of serine/threonine and tyrosine kinases ($K_i > 10\ \mu M$). As one of these representative analogues, compound **39** (R547) has the best CDK inhibitory activities ($K_i = 0.001$, 0.003, and 0.001 μM for CDK1, CDK2, and CDK4, respectively) and excellent in vitro cellular potency, inhibiting the growth of various human tumor cell lines including an HCT116 cell line ($IC_{50} = 0.08\ \mu M$). An X-ray crystal structure of **39** bound to CDK2 has been determined in this study, revealing a binding mode that is consistent with our SAR. Compound **39** demonstrates significant in vivo efficacy in the HCT116 human colorectal tumor xenograft model in nude mice with up to 95% tumor growth inhibition. On the basis of its superior overall profile, **39** was chosen for further evaluation and has progressed into Phase I clinical trial for the treatment of cancer.

Introduction

Cyclin-dependent kinases (CDKs) are a family of serine-threonine protein kinases that govern the initiation, progression, and completion of the cell cycle. Activity of the CDKs allows the orderly transition between phases of the cell cycle. CDK activity is controlled by association with regulatory subunits (cyclins) and CDK inhibitor proteins, by their phosphorylation state and by ubiquitin-mediated proteolysis.[1−4] Since the loss of cell cycle control leading to deregulated cell proliferation is one of the hallmarks of cancer, it is anticipated that the inhibition of CDKs will provide an effective approach to control tumor growth and therefore have an impact on cancer therapy. Inhibition of CDKs has been studied by many organizations and has been achieved using a variety of structural templates with varying degrees of selectivity and activities. Many of these discoveries, including extensive patent literature, have been reviewed.[5−12]

CDK inhibitors such as flavopiridol (L868275) (**1**, Figure 1) and 7-hydroxystaurosporine (UCN-01) have been evaluated in clinical trials for some time.[13] Recently, more selective CDK inhibitors such as roscovitine (**2**) (CYC-202)[14] and BMS-387032 (**3**),[15] targeting CDK2, and PD0332991 (**4**),[16,17] targeting CDK4, have entered clinical trials. However, to date no small molecule inhibitor of any CDK or combination of CDKs has reached Phase III clinical trials as a single agent.

Recent siRNA experiments in which inhibiting CDK2 failed to halt the proliferation of osteosarcomas and Rb-negative cervical cancers suggested that CDK2 may not be a good target for cancer therapy.[18] CDK2 has also been found to be dispensable for regulating the inhibitory effects of $p27^{Kip1}$ and $p21^{Cip1}$ during the G_1 phase of the cell cycle.[19] Additionally, CDK2 knockout mouse experiments have implied the ability of other kinases to circumvent CDK2 and allow other CDKs and other cyclin partners to substitute for certain stages of the cell cycle that are essential for cells to progress toward mitosis.[20,21] Complex interactions within the cell cycle and data indicating that mice lacking all three D cyclins or both CDK4 or CDK6 survive until relatively late in the various stages of embryogenesis or even up to birth further cloud the overall picture.[22,23] These and other studies suggest a need to rethink existing models of cell cycle control in development and tumorigenesis.[24] The ability of CDKs and their cyclin partners to adjust, substitute, and compensate for one another leads to a clearer notion that the inhibition of more than one or two CDKs might be necessary to sustain the suppression of tumor growth in a clinical setting. The success of imatinib (Gleevec), initially thought to target only Abl tyrosine kinase but proved to be a somewhat less than targeted therapy, has suggested that inhibition of a number of targets by a single molecule could provide a useful therapy.[25]

Our goal was to identify small molecule inhibitors of CDKs with low nanomolar activities that were selective for the CDK family of kinases. We recently identified a series of diamino-

* To whom correspondence should be addressed. Phone, 973-235-5183; Fax, 973-235-7239; E-mail: xin-jie.chu@roche.com.
† Department of Discovery Chemistry.
‡ Department of Discovery Oncology.
§ Department of Non-Clinical Drug Safety.
|| Department of Discovery Technologies.

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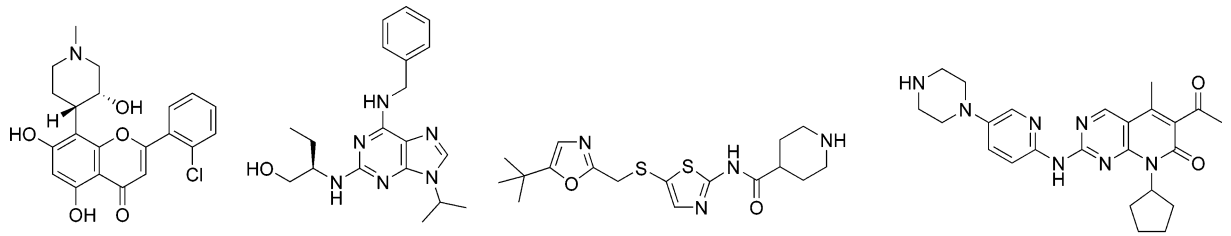


Figure 1. Selected CDK inhibitors flavopiridol (**1**), roscovitine (**2**), BMS-387032 (**3**), and PD0332991 (**4**).

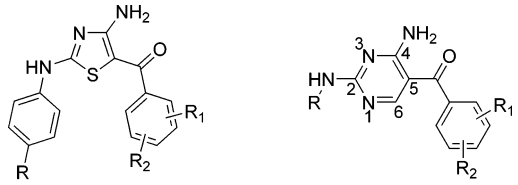


Figure 2. Generic structures of diaminothiazole **5** and diaminopyrimidine **6** as CDK inhibitors.

thiazole-based CDK4 inhibitors **5** (Figure 2) that were selective against CDK1 and CDK2.[26,27] One of these diaminothiazole analogues showed modest in vivo efficacy.[28] In this report, we describe the discovery of a novel series of 2,4-diamino-5-ketopyrimidines **6** that provide highly potent inhibitors of the CDK family and block the cell cycle at both G_1 and G_2 check points. Optimization of this series resulted in the selection of a lead compound for advancement into clinical trials.[29]

Chemistry

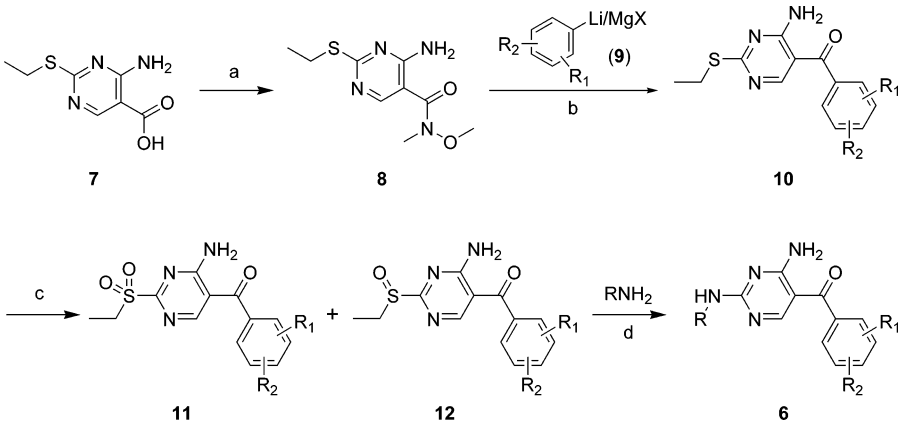
Most 2,4-diamino-5-ketopyrimidine compounds described in this report were prepared following a four-step synthetic route outlined in Scheme 1. Starting from commercially available 4-amino-2-ethylsulfanylpyrimidine-5-carboxylic acid (**7**), standard amide bond coupling with *N*,*O*-dimethylhydroxylamine provided *N*-methoxy-*N*-methylamide **8** as the key intermediate. Treatment of amide **8** with appropriately functionalized aryllithium or Grignard reagents **9** afforded ketones **10**. Oxidation of **10** using 2 equiv of *m*-chloroperoxybenzoic acid (*m*CPBA) gave ethyl sulfones **11**; alternatively, sulfoxides **12** were obtained as the major product when 1 equiv of *m*CPBA was used. Displacement of the sulfone or sulfoxide in **11** or **12** with various aromatic or aliphatic amines under microwave conditions furnished 2,4-diamino-5-ketopyrimidine derivatives **6**. For the piperidinyl substituted 2,4-diamino-5-ketopyrimidine series **16**, some analogues were directly prepared with this four-step sequence when the appropriate amines were available, whereas the other derivatives were synthesized from intermediate **15** as shown in Scheme 2. Reaction of 4-amino-*N*-*tert*-(butoxycarbonyl)piperidine (**13**) with sulfone **11** or sulfoxide **12** provided **14** which, upon treatment with trifluoroacetic acid, gave piperidine **15**. Selective *N*-alkylation, acylation, or sulfonylation of the secondary amine in the piperidine ring of **15** led to the desired 2,4-diamino-5-ketopyrimidines **16**.

Results and Discussion

Molecular modeling studies revealed that the 2,4-diamino-5-ketopyrimidine core has similar electrostatic characteristics in its donor−acceptor−donor (DAD) region as the diaminothiazole core. However, the replacement of the S atom by the C═N bond makes the new scaffold considerably more hydrophilic (Figure 3). Moreover, the expansion of ring size alters the geometry of the substituent vectors from the two scaffolds such that the distance between the two substituents attached to the diaminopyrimidine is significantly larger than that of the corresponding diaminothiazole (Figure 4). This subtle variation in hydrophilicity and geometric parameters suggested that we may find a different SAR between the two series. With this consideration in mind, a series of diaminopyrimidine analogues with small substituents on the A ring and 4-methylpiperazinyl on the B ring were prepared (Table 1). Pyrimidine **17** with R_1 as 3-methoxy was inactive, while compound **18** having R_1 as 3-fluoro showed modest CDK4 inhibitory activity (K_i = 0.450 μM). The 3-trifluoromethyl analogue **19** was inactive while **20**, having the trifluoromethyl at the C2 position, exhibited modest inhibition against CDK4 (K_i = 0.821 μM). The 2-methyl and 2-methoxy analogues **21** and **22**, respectively, demonstrated increasing CDK4 inhibition, suggesting that electron-donating substituents were preferred at the C2 position. The 2-methoxy derivative **22** with CDK4 inhibition of 0.159 μM (K_i) was 5-fold more potent than **20** and inhibited CDK1 and CDK2 as well.

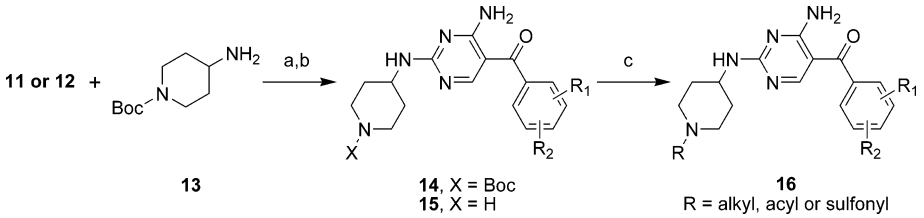
After the establishment of 2,4-diaminopyrimidines as novel CDK inhibitors, the focus was then placed on optimizing the B ring to improve the CDK inhibitory activity while the 2-methoxy group was maintained on the A ring (Table 2). Systematic SAR exploration revealed that the non-aromatic 4-aminopiperidine group instead of an aromatic B ring (as in compounds **17**−**22**) was critical for improving potency of the diaminopyrimidine series. While the tertiary amine **24** was inactive, the secondary amine **23** showed modest enzyme inhibitory activity against CDK4 and weak inhibition against CDK1 and CDK2. Urea **25** with a non-basic NH improved both CDK1 and CDK2 inhibitory activities by 4-fold (K_i = 1.5 and 1.7 μM, respectively) while maintaining modest potency against CDK4 (K_i = 0.409 μM). Without the potential hydrogen-bond donor as in urea **25**, carbamate **26** exhibited a further 3−6-fold improvement in potency against CDK1, CDK2, and CDK4, resulting in a multi-targeted, submicromolar CDK inhibitor (K_i = 0.246, 0.597, and 0.110 μM, respectively). In an effort to improve both the potency and the physicochemical properties (data not shown) of the diaminopyrimidine series, we focused our attention on modifying the carbamate moiety of compound **26**. Amides **27** and **28** were found to have equal to slightly more potent kinase inhibition against CDK1, CDK2, and CDK4 compared to carbamate **26**. However, with the introduction of a strongly polarized sulfonyl group, sulfonamides (**29** and **30**), demonstrated a significant increase in kinase inhibitory activity (CDK1, CDK2, and CDK4) and modest improvement in cellular potency against HCT116 human colon cell line. For example, methyl sulfonamide **29** was nearly 1 order of magnitude more potent than carbamate **26** in the kinase assays (K_i = 0.028, 0.024, and 0.014 μM for CDK1, CDK2, and CDK4, respectively) and resulted in favorable cell growth inhibition (HCT116, IC_{50} = 1.6 μM). It was also noted that extending the alkyl group in

Scheme 1. General Synthesis of 2,4-Diamino-5-ketopyrimidines[a]



[a] Reagents and conditions: (a) MeONHMe·HCl, HOBt/HBTU, DiPEA, DMF, rt, 98%; (b) 3−4 equiv of ArLi or ArMgX, THF, −78 °C, 65∼90%; (c) *m*CPBA, $CHCl_3$, 0 °C, 90−98%; (d) i-PrOH, 100−110 °C, microwave, 20 min, >90%.

Scheme 2. Synthesis of 4-Piperidinyl Substituted 2,4-Diamino-5-ketopyrimidines[a]



[a] Reagents and conditions: (a) i-PrOH, 100∼110 °C, microwave, 20 min, >90%; (b) TFA, CH_2Cl_2, 0 °C, >95%; (c) R_3X, R_3COCl or R_3SO_2Cl, DiPEA, CH_2Cl_2, rt, 90−98%.

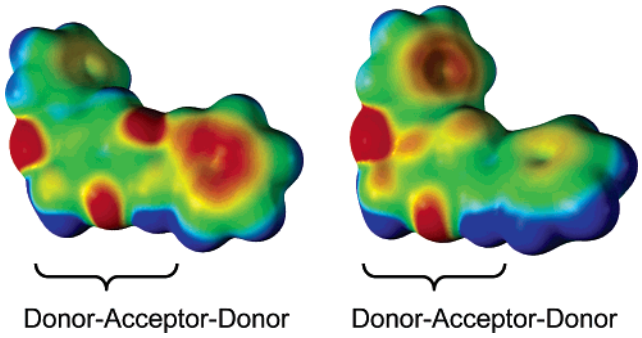


Figure 3. Electrostatic potential maps of diaminopyrimidine (on the left) and diaminothiazole (on the right). An isodensity surface is first obtained for the two molecules, and the surface is then assigned different colors according to their electrostatic potential. The red area denotes a region with the most negative potential and blue areas the most positive. The maps are calculated using Spartan′02.

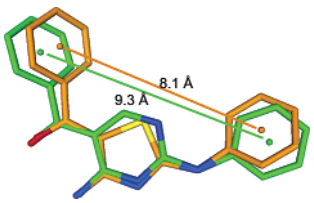
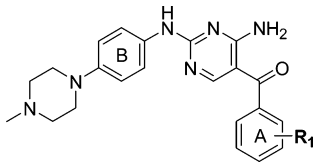


Figure 4. Overlay of diaminopyrimidine (green) vs diaminothiazole (orange). Distances in angstrom (Å) between the centroids of the two phenyl substituents for each core structure are shown.

the amides (e.g. **27**, **28**) or sulfonamides (e.g. **29**, **30**) had a very limited impact on kinase inhibition, consistent with the observation from the crystal structure that the R_2 group points toward solvent (vide infra). With the substituted 4-piperidine identified as the most preferred B ring, a re-examination of the simple monosubstitution on the aromatic A ring was carried

Table 1. Identification of 2,4-Diamino-5-ketopyrimidines as Inhibitors of CDKs[a]

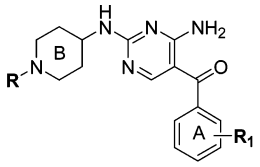


compd	R_1	K_i (μM)[b] CDK4	CDK1	CDK2	IC_{50} (μM)[c] HCT116
17	3-MeO	>1	>10	>10	ND[d]
18	3-F	0.450	>10	>10	ND
19	3-CF_3	>1	>10	>10	ND
20	2-CF_3	0.821	>10	>10	ND
21	2-Me	0.584	>10	>10	16.5
22	2-MeO	0.159	1.259	5.141	11.0

[a] See ref 32 for a description of assay conditions. [b] K_i values are reported as the mean of several determinations. The variability around the mean value was less than 50%. [c] Assay were run as singlets. [d] ND = not determined.

out through the preparation of a small number of analogues with variation at the C2 position (**31**−**33**). 2-Fluoro- and 2-methyl-substituted compounds (**31** and **32**, respectively) substantially reduced CDK inhibition compared with 2-methoxy analogue **26**. We believe that preorganization of the binding conformation of the ligand to CDK is promoted by the 2-methoxy group, which enforces non-coplanarity of the A ring with respect to the pyrimidine core. Surprisingly, the 2-ethoxy derivative **33** resulted in greater than 10-fold loss in CDK1, CDK2, and CDK4 inhibitory activity (vs amide **27**), suggesting that this binding pocket is not big enough to accommodate larger substitutions on the A ring. This was consistent with our earlier observation outlined in Table 1 and confirmed that the 2-methoxy was the optimal group for the C2 position of the aromatic A ring.

Table 2. Optimization of R_1 on Ring A and R on Piperidine Ring B in 2,4-Diamino-5-ketopyrimidines[a]



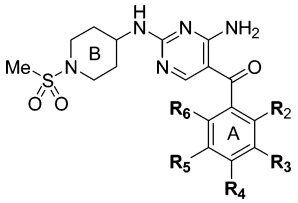
			Ki (μM)[b]			IC_{50} (μM)[c]
compound	R_1	R	CDK4	CDK1	CDK2	HCT116
23	2-MeO	H	0.481	5.841	6.266	2.1
24	2-MeO	Me	>1	6.615	>10	ND[d]
25	2-MeO		0.409	1.53	1.756	3.6
26	2-MeO		0.110	0.246	0.597	4.2
27	2-MeO		0.066	0.181	0.327	5.0
28	2-MeO		0.098	0.278	0.886	5.6
29	2-MeO		0.014	0.028	0.024	1.6
30	2-MeO		0.016	0.043	0.051	2.6
31	2-F		0.734	1.638	3.137	ND
32	2-Me		0.665	1.108	2.166	>30
33	2-EtO		0.986	2.500	2.030	ND

[a] See ref 32 for a description of assay conditions. [b,c] K_i and IC_{50} values are reported as the mean of several determinations. The variability around the mean value was less than 50%. [d] ND = not determined.

With the identification of 1-methanesulfonyl-4-piperidine as the optimal B ring and 2-methoxy as the most preferred group for the C2 position of the aromatic A ring, further exploration of other substitutions on the A ring was carried out (Table 3). Introduction of a fluorine to the C5 position of compound **29** yielded a more potent CDK inhibitor (**35**) with K_i values of less than 10 nM with respect to CDK2 and CDK4, while the 4-fluoro analogue **34** reduced the inhibition against CDK1, CDK2, and CDK4. Compound **35** also proved to be more potent in our cell-based assay (HCT116 cell line) with an IC_{50} of 0.66 μM. A notable difference for the effect of substitution at the C5 position was that the chloro analogue **36** was 10-fold less active in the cell-based assay than the corresponding fluoride **35**, suggesting the size of the substituent at this position may affect potency. Similar to the 5-fluoro analogue **35**, compound **37** with the fluorine at the C6 position also significantly increased CDK inhibitory activity and cellular potency (IC_{50} = 0.30 μM, HCT116 cell line). Previous reports have indicated that 2,6-difluorophenyl substitution provides a beneficial effect on the potency of the CDK inhibiton.[30,31] Interestingly, substitution of the 2-methoxy group in **37** with a fluorine atom resulting in the 2,6-difluoro analogue **38** proved to be inconsequential with respect to CDK inhibition and yet resulted in more than 10-fold loss in the cellular potency. This distinct difference confirmed the early observation that the 2-methoxy group is unique for this diaminopyrimidine series. Compound **39** possessing both 5- and 6-fluoro substitution culminated in an inhibitor with low, single-digit nanomolar potency against the CDKs (K_i = 0.001, 0.003, and 0.001 μM for CDK1, CDK2, and CDK4, respectively) and excellent cellular potency (IC_{50} = 0.08 μM, HCT116 cell line). Another difluoro-substituted analogue **40**, with fluorines at both the C4 and C5 positions, had a potency equal to that of monofluoro derivative **35** but 6-fold less than that of compound **39**. A 4,5,6-trifluoro substitution was still tolerated, as seen in derivative **41**, with a slight loss of the cellular potency compared to **39**. However, when the third fluorine substitution was at the C3 position (**42**), the CDK inhibitory activities dropped considerably. From these data we concluded that the 5,6-difluoro-2-methoxy substitution on the A ring is the most preferred pattern, resulting in excellent CDK inhibitory activities and in vitro cellular potency for the diaminopyrimidine series.

Kinase Selectivity Profile. The most promising 2,4-diamino-5-ketopyrimidine analogues were examined in both our in-house kinase selectivity panel and the Upstate kinase selectivity screen.[32] As shown in Table 4, compound **39** was found to be a potent and ATP-competitive inhibitor of the CDK family of protein kinases. It strongly inhibits CDK1/cyclin B, CDK2/cyclin E, and CDK4/cyclin D and shows little or no inhibitory activity against a panel of 18 other serine/threonine kinases. Compound **39** was also effective in inhibiting the phosphorylation of retinoblastoma protein (pRb) in human tumor cells in a time dependent manner. Treatment of HCT116 cells with compound **39** inhibited phosphorylation at both the serine 795 and 780 sites on pRb, consistent with the two phosphorylation sites specifically phosphorylated by CDK4. Cell cycle block in

Table 3. Further Substitution Patterns on Aromatic Ring A of 2,4-Diamino-5-ketopyrimidines[a]



compd	R_2	R_3	R_4	R_5	R_6	K_i (μM)[b] CDK4	CDK1	CDK2	IC_{50} (μM)[c] HCT116
34	OMe	H	F	H	H	0.021	0.193	0.084	3.6
35	OMe	H	H	F	H	0.007	0.014	0.007	0.65
36	OMe	H	H	Cl	H	0.036	0.133	0.451	7.0
37	OMe	H	H	H	F	0.003	0.017	0.013	0.30
38	F	H	H	H	F	0.017	0.038	0.015	3.4
39	OMe	H	H	F	F	0.001	0.001	0.003	0.08
40	OMe	H	F	F	H	0.006	0.014	0.006	0.52
41	OMe	H	F	F	F	0.001	0.005	0.002	0.22
42	OMe	F	F	F	H	0.267	1.667	0.495	ND[d]

[a] See ref 32 for a description of assay conditions. [b,c] K_i and IC_{50} values are reported as the mean of several determinations. The variability around the mean value was less than 50%. [d] ND = not determined.

Table 4. Kinase Selectivity Profile for Compound 39[a]

kinases	K_i (μM)[b]	kinases	K_i (μM)[b]
CDK4/cyclin D	0.001 ± 0.0001	GSK3β	8
CDK2/cyclin E	0.003 ± 0.0008	KDR	>5
CDK1/cyclin B	0.002 ± 0.0008	FGFR	>5
PKA	>5	EGFR	>5
PKB	>5	PDGF	>5
PKCα	>5	MAPK2	>50
PKCβ	>5	IGFR	>5
FYN	>5	SRC	>5
EPHB3	>5	FAK	>5
SGK	>5	AURORA	>5
p38	>50		

[a] See ref 32 for a description of assay conditions and inhibitory activity data against additional kinases. [b] $K_i = IC_{50}/(1 + S/K_m)$ where S is the substrate ATP concentration and K_m is the Michaelis−Menten constant for ATP; mean of at least two separate determinations.

$G_1 + G_2$ inducing apoptosis in a dose-dependent manner in the same cell line has also been observed.[32]

Structural Study of Inhibitors bound to CDK2. The crystal structure of compound **39** bound to CDK2, shown in Figure 5, revealed a binding motif whereby the hinge region (residues Phe80-Glu81-Phe82-Leu83) makes key hydrogen-bond interactions with the two donors in the donor−acceptor−donor (DAD) portion of the diaminopyrimidine core. The central nitrogen acceptor is 3.7 Å away from the backbone NH of Leu83. The B ring piperidine makes hydrophobic contacts similar to those seen in many kinase inhibitors,[34] with the sulfonamide making both direct and water-mediated hydrogen bonds to Lys89, Asp86, and the backbone carbonyl of His84. The 2,6-disubstitution pattern on the A ring forces it out of the plane of the pyrimidine core and allows the difluoro to pack into the hydrophobic pocket near Phe80 and the alkyl chain of Lys33.

Pharmacokinetics Study. To assess the preliminary pharmacokinetic (PK) properties of this class of 2,4-diamino-5-ketopyrimidines, several representative lead analogues including compounds **29**, **35**, and **39** were administered orally and intravenously (iv) to female nude mice. The mean plasma concentration versus time profiles after an oral dose of 100 mg/kg are shown in Figure 6. All three compounds were rapidly absorbed and their C_{max} was reached within 1 h. Over the 4 h period, the observed drug concentration of compound **29** decreased substantially. But the concentrations of the 5-fluoro analogue **35** remained above or close to its IC_{90} level (IC_{90} = 1.8 μM or 762 ng/mL in HCT116 cell line). The plasma exposure of the 5,6-difluoro analogue **39** greatly exceeded (about 10-fold at 8 h post dose) its IC_{90} values (IC_{90} = 0.17 μM or 75 ng/mL in HCT116 cell line). Sufficient drug exposure can be expected from a dose regiment of 50 and 75 mg/kg three times per week in the experimental animals. The clearance (CL) and the volume of distribution (V_{ss}) of these three compounds also improved as the number of substitutions on the aromatic A ring increased (Table 5).

As summarized in Table 6, the systemic plasma clearance of compound **39** in female athymic non-tumor bearing mice was 38.6 mL/min/kg. A 100 mg/kg oral dose resulted in a half-life ($t_{1/2}$) of 2.8 h, and the measured oral bioavailability (F_{po}) of compound **39** in this study was approximately 21%. The observed large volume of distribution (1070 mL/kg) suggests deep tissue penetration. The pharmacokinetic profile of com-

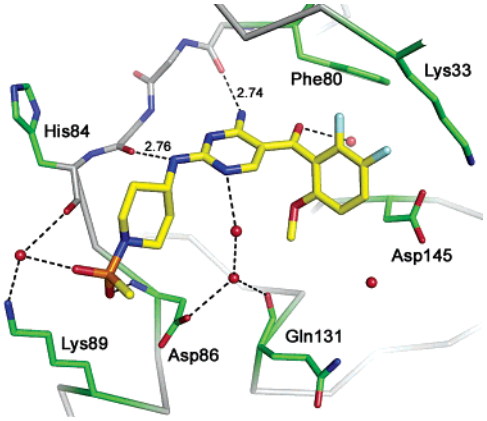
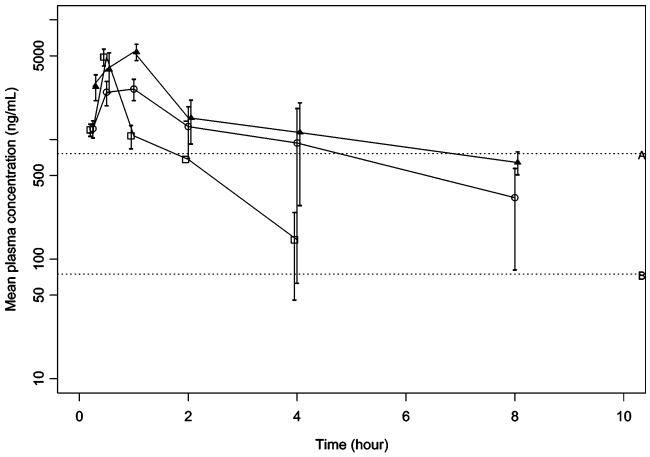


Figure 5. The 1.85 Å crystal structure of compound **39** bound in the ATP binding pocket of CDK2 (no cyclin). Nitrogen atoms are in blue, oxygen atoms in red, sulfur in orange, and fluorine in green, and key hydrogen bonds are depicted. Bridging waters play a crucial role in the enhanced binding of this compound to the enzyme. The figure was made with program PyMOL.[33]



compound **29** (open square), compound **35** (open circle), compound **39** (filled triangle)

Figure 6. Mean plasma concentration versus time profiles of selected compounds **29**, **35**, and **39** following oral administration (100 mg/kg) in female athymic nontumor bearing mice. The error bars represent the percent coefficient of variation. The dashed lines A (for **35**) and B (for **39**) represent the in vitro IC_{90} values of the cell inhibition in the HCT116 cell line (IC_{90} = 762 ng/mL and 75 ng/mL for compounds **35** and **39**, respectively): compound **29** (open square), compound **35** (open circle), compound **39** (filled triangle).

Table 5. Mouse PK Data Following Single IV Administration (10 mg/kg) of Selected Compounds[a]

compd	CL (mL/min/kg)	V_{ss} (mL/kg)
29	47.1	401
35	48.3	885
39	38.6	1070

[a] Based on mean data from three mice/time point.

Table 6. Pharmacokinetic Properties of Compound **39** in Mice[a]

parameter	unit	iv administration	po administration
dose	mg/kg	10	100
C_{max}	ng/mL	7310	2700
T_{max}	h	0.083	0.5
$AUC_{(0-t)}$	ng h/mL	4152	8169
$T_{1/2}$	h	0.39	2.8
CL	mL/min/kg	38.6	−
V_{ss}	mL/kg	1070	−
F_{po}	%	−	21

[a] Based on mean data from three mice/time point.

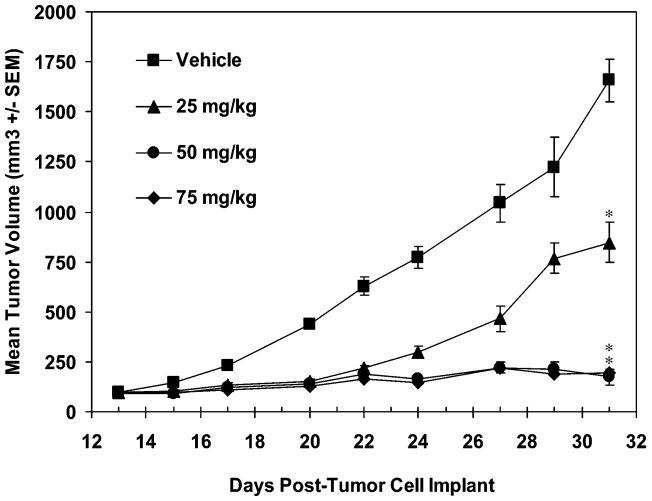


Figure 7. Effect of compound **39** on HCT116 human colorectal tumor xenograft growth. Statistical analysis was determined by the rank sum test and one-way Anova and a post-hoc Bonferroni *t*-test. * = Statistically significant as compared to the vehicle-treated group, where $p < 0.001$.

pound **39** along with the excellent in vitro cellular potency warranted the advancement of these 2,4-diamino-5-ketopyrimidine analogues into in vivo efficacy studies.

In Vivo Antitumor Activity. Compound **39** was evaluated for its in vivo antitumor activity in female nude mice bearing established HCT116 human colorectal xenografts with a mean starting volume of about 100 mm^3 (Figure 7). In this study, compound **39** was administered orally three times per week on Monday, Wednesday, and Friday (M, W, F) as a suspension in 1% Klucel LF in water with 0.1% Tween 80 for 18 days (a total of eight doses). At the end of the two and a half week study, compound **39** demonstrated significant in vivo antitumor activity in the absence of gross or histological indications of toxicity. A dose of 25 mg/kg three times per week inhibited HCT116 colorectal tumor growth by 52%, while doses of 50 and 75 mg/kg three times per week inhibited tumor growth by 95% and 94%, respectively, as compared to vehicle-treated mice. A more extensive biochemical and biological characterization of compound **39**, including a comprehensive in vivo efficacy evaluation, will be published separately.[32]

Conclusions

In summary, we have reported the discovery of a novel class of 2,4-diamino-5-ketopyrimidines (**6**) as potent, selective, and ATP-competitive CDK inhibitors. The SAR of the diaminopyrimidine core proved to be unique in that it favors a 2-methoxyphenyl substitution on the A ring rather than a 2,6-difluoro substitution. Further lead optimization resulted in the identification of compound **39** as a potent inhibitor of CDK1, CDK2, and CDK4 ($K_i = 0.001-0.003\ \mu M$) which was selective against a large panel of related and unrelated serine/threonine and tyrosine kinases ($K_i > 10\ \mu M$). It exhibited excellent in vitro cellular potency, inhibiting the growth of HCT116 human colorectal tumor cell line. Furthermore, compound **39** demonstrated significant in vivo efficacy against the HCT116 human colorectal tumor xenograft model. On the basis of its superior overall profile, compound **39** (R547)[29] was chosen as the clinical candidate and is currently in Phase I clinical trial for the treatment of cancer. An expanded SAR study of this novel series of 2,4-diamino-5-ketopyrimidine CDK inhibitors and the related biological evaluation of further lead compounds will be reported soon.

Experimental Section

Chemistry. All nonaqueous reactions were carried out under an argon or nitrogen atmosphere at room temperature, unless otherwise noted. All reagents and anhydrous solvents were used as obtained commercially without further purification or distillation, unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed on EMD Chemicals silica gel 60 F_{254} precoated plates (0.25 mm). Compounds were visualized by UV light and/or stained with either *p*-anisaldehyde, iodine, or phosphomolybdic acid solutions followed by heating. Analytical high-pressure liquid chromatography (HPLC) and LC−MS analyses were conducted using the following two instruments and conditions. Method 1: Hewlett-Packard HP-1090 pump and HP-1090 PDA detector set at 215 nm with the MS detection performed with a Micromass Platform II mass spectrometer with electrospray ionization (ESI); Chromegabond WR C18 3 μm, 120 Å, 3.2 mm × 30 mm column; solvent A, H_2O−0.02% TFA; solvent B, MeCN−0.02% TFA; flow rate = 2 mL/min; start 2% B, final 98% B in 4 min, linear gradient. Method 2*:* Waters 2795 pump and Waters 2996 photodiode array detector set at 214 nm with the MS detection performed with a Waters ZQ mass spectrometer (ESI); Epic Polar Hydrophilic 3 μm, 120 Å, 3.2 mm × 30 mm column; solvent A, H_2O−0.03% HCO_2H; solvent B, MeCN−0.03% HCO_2H; flow rate = 2 mL/min; start 10% B, final 100% B in 3 min linear gradient, remaining for 1 min. Compounds were purified using either of the following methods. Flash column chromatography was performed on EM Science silica gel 60 (particle size of 32−63 μm, 60 Å). Preparative reverse-phase high-pressure liquid chromatography (RP HPLC) was performed using a Waters Delta prep 4000 pump/controller, a 486 detector set at 215 nm, and a LKB Ultrorac fraction collector. The sample was dissolved in a mixture of MeCN/20 mM aqueous NH_4Ac, applied on a C-18 20 × 100 mm column and eluted at 30 mL/min with a 20 min linear gradient of 10%−90% B, where solvent A = H_2O with 20 mM NH_4Ac and solvent B = MeCN. The pooled fractions were concentrated under reduced pressure and lyophilized to afford the desired compounds. ^1H NMR spectra were recorded using a Varian Mercury 300 MHz or Varian Inova 400 MHz spectrometer and calibrated using an internal reference. The chemical shifts are in parts per million (δ) referenced to Me_4Si (0.00 ppm) or $CHCl_3$ (7.26 ppm). High-resolution mass spectra (HMRS) were recorded on a Bruker Apex II FTICR mass spectrometers with a 4.7 T magnet (ES) or Micromass AutoSpec (EI) mass spectrometers.

Crystallization, Data Collection, and Refinement. Crystals of CDK2 were grown at 4 °C by the vapor diffusion method. CDK2-(1−298) at 12 mg/mL was mixed with and equilibrated against

10% PEG 3350, 0.2 M ammonium phosphate, 0.1 M Bicine, pH 9.0. β-Mercaptoethanol (2%) was added to the reservoir after mixing of the drop. Crystals appeared overnight, after which **39** at 100 mM in DMSO was added to the drop. After soaking for 24 h, crystals were transferred to cryoprotectant containing 20% PEG 3350, 15% ethylene glycol, 0.2 M ammonium phosphate, 0.1 M Bicine, pH 9.0, and then cooled to liquid nitrogen temperature. Data were collected at beamline ×8C at the National Synchrotron Light Source at Brookhaven National Laboratories. The crystals belonged space group $P2_12_12_1$ with unit cell dimensions $a = 53.5$ Å, $b = 71.65$ Å, $c = 71.7$ Å. Data were processed to 1.85 Å with the HKL package[35] to an R-sym of 0.039. The structure was determined by the difference Fourier method and was refined with CNX (CNX v.2000.1, Molecular Simulations Inc.), with 0.05% of reflections in the test set, to a final R-factor/R-free of 0.208/0.237. Coordinates have been deposited to the PDB under accession code 2FVD.

Pharmacokinetic Study Methods. Female immunodeficient nude mice (13−14 weeks old) obtained from Charles River Laboratories (Wilmington, DE) were allowed to acclimate for a minimum of 72 h prior to study start. Mice received a single oral (100 mg/kg) or intravenous (10 mg/kg) dose of the test compound, and blood samples from three mice/time point were collected over a 24-h period in EDTA vials. The oral dose was administered as a suspension in 1% Klucel and 0.1% Tween 80, and the iv dose was administered as a solution in 10% PEG 400 and 90% hydroxypropyl-β-cylcodextrin (28%). Plasma samples were analyzed by LC/MS for the test compound, and noncompartmental PK analysis was performed on the mean plasma concentration−time profiles using WinNonlin (Pharsight) or Watson.

4-Amino-2-ethylsulfanylpyrimidine-5-carboxylic Acid Methoxymethylamide (8). To a solution of 4-amino-2-ethylsulfanylpyrimidine-5-carboxylic acid (**7**) (1.00 g, 5.02 mmol) and diisopropylethylamine (2.79 g, 21.58 mmol, 4 equiv) in DMF (20 mL) were added 1-hydroxybenzotriazole (1.09 g, 8.07 mmol, 1.5 equiv) and *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (3.06 g, 8.07 mmol, 1.5 equiv) at 0 °C. After stirring for 15 min, *N*,*O*-dimethylhydroxylamine hydrochloride (790 mg, 8.10 mmol) was added and the reaction was allowed to stir at 0−20 °C for 2 h. The resulting reaction mixture was treated with H_2O and extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified on silica gel with hexane/EtOAc (1:1) to give **8** as a white solid (1.19 g, 98% yield): Anal. RP-HPLC $t_R = 1.15$ min (method 1, >95% purity), $t_R = 0.89$ min (method 2, >95% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (t, 3H, $J = 7.5$ Hz), 3.11 (q, 2H, $J = 7.5$ Hz), 3.34 (s, 3H), 3.62 (s, 3H), 8.62 (s, 1H); HRMS for $C_9H_{14}N_4O_2S$ (M^+) calcd 242.0837, observed 242.0836.

(4-Amino-2-ethylsulfanylpyrimidin-5-yl)(2-methoxyphenyl)-methanone (10c). To a solution of 2-iodoanisole (20.0 g, 85.47 mmol) in anhydrous THF (130 mL) was added over 30 min a 1.6 M solution of *n*-butyllithium in hexane (51.0 mL, 81.6 mmol), and the mixture was stirred for a further 40 min at −78 °C to give a clear solution. A portion of this freshly prepared 2-methoxyphenyllithium reagent (70.0 mL, ∼30 mmol, ∼3.5 equiv) was added slowly to a solution of amide **8** (2.07 g, 8.54 mmol) in anhydrous THF (30 mL) and the mixture was stirred at −78 °C for 1−2 h until the complete consumption of amide **8**. The resulting mixture was quenched with aqueous NH_4Cl solution and extracted with EtOAc (3×). The combined organic extracts were washed with brine (2×), dried over Na_2SO_4, and evaporated. The residue was purified on silica gel with hexane/EtOAc (80/20 → 60/40) to give **10c** as a light yellow solid (1.85 g, 75% yield): Anal. RP-HPLC $t_R = 1.93$ min (method 1, > 95% purity), $t_R = 1.72$ min (method 2, >95% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (t, 3H, $J = 7.4$ Hz, CH_3), 3.13 (q, 2H, $J = 7.4$ Hz, SCH_2), 3.78 (s, 3H, OCH_3), 5.74 (br, 1H, NH), 6.98 (d, 1H, $J = 8.6$ Hz, aromatic), 7.04 (dt, 1H, $J = 7.8$ and 0.6 Hz, aromatic), 7.26 (m, 1H, aromatic), 7.46 (m, 1H, aromatic), 8.18 (s, 1H, aromatic), 8.77 (br, 1H, NH); HRMS for $C_{14}H_{16}N_3O_2S$ $(M + H)^+$ calcd 290.0958, observed 290.0961.

(4-Amino-2-ethylsulfanylpyrimidin-5-yl)(3-fluorophenyl)methanone (10a) was prepared with the same procedure as described for **10c**: Anal. RP-HPLC $t_R = 2.06$ min (method 1, 94% purity), $t_R = 1.81$ min (method 2, 95% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (t, 3H, $J = 7.3$ Hz, CH_3), 3.14 (q, 2H, $J = 7.3$ Hz, SCH_2), 5.75 (br, 1 H, NH), 7.23−7.52 (m, 4 H, aromatic), 8.41 (s, 1 H, aromatic), 8.58 (br, 1 H, NH); HRMS for $C_{13}H_{13}FN_3OS$ $(M + H^+)$ calcd 278.0761, observed 278.0758.

(4-Amino-2-ethylsulfanylpyrimidin-5-yl)-*o*-tolylmethanone (10b) was prepared with the same procedure as described for **10c**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (t, 3H, $J = 7.2$ Hz), 2.29 (s, 3H), 3.12 (q, 2H, $J = 7.2$ Hz), 5.76 (brd, 1H, NH), 7.17−7.29 (m, 3H, aromatic), 7.34−7.41 (m, 1H, aromatic), 8.14 (s, 1H, aromatic), 8.79 (brd, 1H, NH); HRMS for $C_{14}H_{15}N_3OS$ (M+) calcd 273.0936, observed 273.0933.

(4-Amino-2-ethylsulfanylpyrimidin-5-yl)(2-fluorophenyl)methanone (10d) was prepared with the same procedure as described for **10c**: Anal. RP-HPLC $t_R = 2.00$ min (method 1, >99% purity), $t_R = 1.78$ min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (t, 3H, $J = 7.4$ Hz, CH_3), 3.14 (q, 2H, $J = 7.4$ Hz, SCH_2), 5.80 (br, 1 H, NH), 7.18 (td, 1 H, $J_{ortho} = {}^3J_{HF} = 8.4$ Hz, $J_{meta} = 1.1$ Hz, aromatic), 7.29 (td, 1H, $J_{ortho} = 7.6$ Hz, $J_{meta} = 1.1$ Hz, aromatic), 7.43 (ddd, 1H, $J_{ortho} = 7.6$ Hz, $^4J_{HF} = 6.8$ Hz, $J_{meta} = 1.8$ Hz, aromatic), 7.52 (m, 1H, aromatic), 8.27 (d, $^6J_{HF} = 2.9$ Hz, 1 H, aromatic), 8.70 (br, 1 H, NH); HRMS for $C_{13}H_{13}FN_3OS$ $(M + H^+)$ calcd 278.0758, observed 278.0761.

(4-Amino-2-ethylsulfanylpyrimidin-5-yl)(2-ethoxyphenyl)-methanone (10e) was prepared with the same procedure as described for **10c**: white solid; Anal. RP-HPLC $t_R = 2.12$ min (method 1, 98% purity), $t_R = 1.86$ min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, 3H, $J = 7.1$ Hz, CH_3), 1.38 (t, 3H, $J = 7.4$ Hz, CH_3), 3.12 (q, 2H, $J = 7.4$ Hz, SCH_2), 4.03 (q, 2H, $J = 7.1$ Hz, 2 H, OCH_2), 5.70 (br, 1 H, NH), 6.95 (d, 1H, $J = 8.4$ Hz, aromatic), 7.03 (dt, 1H, $J_{ortho} = 7.5$ Hz, $J_{meta} = 0.8$ Hz, aromatic), 7.28 (dd, 1H, $J_{ortho} = 7.5$ Hz, $J_{meta} = 1.8$ Hz, aromatic), 7.34 (ddd, 1H, $J_{ortho} = 8.4$ Hz, $J_{ortho} = 7.5$ Hz, $J_{meta} = 1.8$ Hz, aromatic), 8.21 (s, 1 H, aromatic), 8.72 (br, 1 H, NH); HRMS for $C_{15}H_{18}N_3O_2S$ $(M + H^+)$ calcd 304.1114, observed 304.1117.

(4-Amino-2-ethanesulfanylpyrimidin-5-yl)(4-fluoro-2-methoxyphenyl)methanone (10f) was prepared with the same procedure as described for **10c**: white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (t, 3H, $J = 7.4$ Hz, CH_3), 3.13 (q, 2H, $J = 7.4$ Hz, SCH_2), 3.77 (s, 3H, OCH_3), 5.78 (br, 1H, NH), 6.58−6.88 (m, 2H, aromatic), 7.18−7.39 (m, 1 H, aromatic), 8.17 (s, 1H, aromatic), 8.73 (br, 1 H, NH); LR-MS $(M + H^+)$ 308.

(4-Amino-2-ethylsulfanylpyrimidin-5-yl)(5-fluoro-2-methoxyphenyl)methanone (10g) was prepared with the same procedure as described for **10c**: white solid; Anal. RP-HPLC $t_R = 2.06$ min (method 1, >99% purity), $t_R = 1.82$ min (method 2, > 99% purity); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.27 (t, 3H, $J = 7.3$ Hz, CH_3), 3.06 (q, 2H, $J = 7.3$ Hz, SCH_2), 3.71 (s, 3 H, OCH_3), 7.16 (dd, 1H, $J_{ortho} = 9.2$, $^4J_{HF} = 4.4$ Hz, aromatic), 7.20 (dd, 1H, $^3J_{HF} = 8.4$, $J_{meta} = 3.2$ Hz, aromatic), 7.34 (ddd, 1H, $J_{ortho} = 9.2$, $^3J_{HF} = 8.7$, $J_{meta} = 3.2$ Hz, aromatic), 7.97 (s, 1H, aromatic), 8.33 (br, 1 H, NH), 8.44 (br, 1 H,NH); HRMS for $C_{14}H_{15}FN_3O_2S$ $(M + H^+)$ calcd 308.0864, observed 308.0863.

(4-Amino-2-ethylsulfanylpyrimidin-5-yl)(5-chloro-2-methoxyphenyl)methanone (10h) was prepared with the same procedure as described for **10c**: light yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.27 (t, 3H, $J = 7.2$ Hz, CH_3), 3.05 (q, 2H, $J = 7.2$ Hz, SCH_2), 3.72 (s, 3H, OCH_3), 7.17 (d, 1H, $J_{ortho} = 8.7$, aromatic), 7.36 (d, 1H, $J_{meta} = 2.7$ Hz, aromatic), 7.53 (dd, 1H, $J_{ortho} = 8.7$ Hz, $J_{meta} = 2.4$ Hz, aromatic), 7.97 (s, 1H, aromatic), 8.33 (br, 1 H, NH), 8.42 (br, 1 H,NH); HRMS for $C_{14}H_{15}ClN_3O_2S$ $(M + H^+)$ calcd 324.0568, observed 324.0568.

(4-Amino-2-methylsulfanylpyrimidin-5-yl)(2-fluoro-6-methoxyphenyl)methanone (10i). The aryllithium reagent was generated via deprotonation of 3-fluoroanisole in a similar manner as described for the preparation of **10c**, which was reacted with **8** to give **10i** as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.52 (s, 3H, SCH_3), 3.78 (s, 3H, OCH_3), 5.82 (br, 1H, NH), 6.73−6.83 (m, 2H, aromatic), 7.33−7.44 (m, 1H, aromatic), 8.17 (s, 1H, aromatic), 8.75 (br, 1H, NH); MS $(M + H^+)$ 307.

(4-Amino-2-ethylsulfanylpyrimidin-5-yl)(2,6-difluorophenyl)-methanone (10j) was prepared following a similar procedure as described for **10i**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (t, 3H, J = 7.3 Hz, CH_3), 3.13 (q, 2H, J = 7.3 Hz, CH_2), 5.83 (br, 1 H, NH), 7.01 (t, 2H, J = 7.9 Hz, 2 H, aromatic), 7.46 (tt, 1H, J = 8.4 and 6.4 Hz, aromatic), 8.21 (s, 1 H, aromatic), 8.68 (br, 1 H, NH); LR-MS (M + H$^+$) 296.

(4-Amino-2-ethylsulfanylpyrimidin-5-yl)(2,3-difluoro-6-methoxyphenyl)methanone (10k) and (4-Amino-2-ethylsulfanylpyrimidin-5-yl)(4,5-difluoro-2-methoxyphenyl)methanone (10l). Following a similar procedure as described for **10c**, the reaction of the aryllithium reagent, generated from 2-bromo-4,5-difluoroanisole and *n*-butyllithium, with amide **8** was allowed to slowly warm to −35 °C for 2 h. The crude material was purified on prep HPLC to give isomers **10k** and **10l** in an approximate 8:1 ratio.

For compound **10k**: Anal. RP-HPLC t_R = 2.12 min (method 1, >99% purity), t_R = 1.90 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (t, 3H, J = 7.4 Hz, CH_3), 3.14 (q, 2H, J = 7.4 Hz, SCH_2), 3.75 (s, 3H, OCH_3), 5.91 (br, 1 H, NH), 6.68 (ddd, 1H, J_{ortho} = 9.2 Hz, $^4J_{HF}$ = 3.2 Hz, $^5J_{HF}$ = 2.0 Hz, aromatic), 7.23 (q, 1H, $^3J_{HF}$ = $^4J_{HF}$ = J_{ortho} = 9.2 Hz, aromatic), 8.16 (s, 1 H, aromatic), 8.75 (br, 1 H, NH); HRMS for $C_{14}H_{14}F_2N_3O_2S$ (M + H$^+$) calcd 326.0770, observed 326.0771.

For compound **10l**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29 (t, 3H, J = 7.3 Hz, CH_3), 3.08 (q, 2H, J = 7.3 Hz, SCH_2), 3.74 (s, 3H, OCH_3), 7.34 (dd, 1H, $^3J_{HF}$ = 12.7 Hz, $^4J_{HF}$ = 6.3 Hz, aromatic), 7.50 (t, 1H, $^3J_{HF}$ = $^4J_{HF}$ = 9.7 Hz, aromatic), 8.06 (s, 1 H, aromatic), 8.34 (br, 1 H, NH), 8.43 (br, 1 H, NH); HRMS for $C_{14}H_{14}F_2N_3O_2S$ (M + H$^+$) calcd 326.0770, observed 326.0772

(4-Amino-2-ethylsulfanylpyrimidin-5-yl)(2,3,4-trifluoro-6-methoxyphenyl)methanone (10m) was prepared following a similar procedure as described for **10i**; the aryllithium reagent was generated from deprotonation of 3,4,5-trifluoroanisole and reacted with **8** to give **10m**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (t, 3H, J = 7.3 Hz, CH_3), 3.13 (q, 2H, J = 7.3 Hz, SCH_2), 3.75 (s, 3H, OCH_3), 5.85 (br, 1 H, NH), 6.61 (ddd, 1H, $^3J_{HF}$ = 11.5 Hz, $^4J_{HF}$ = 5.3 Hz, $^5J_{HF}$ = 2.2 Hz, aromatic), 8.15 (s, 1H, aromatic), 8.67 (br, 1 H, NH); LR-MS (M + H$^+$) 344.

(4-Amino-2-ethylsulfanylpyrimidin-5-yl)(3,4,5-trifluoro-2-methoxyphenyl)methanone (10n) was prepared following a similar procedure as described for **10i**; the aryllithium reagent was generated from deprotonation of 2,3,4-trifluoroanisole and reacted with **8** to give **10n**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (t, 3H, J = 7.4 Hz, CH_3), 3.14 (q, 2H, J = 7.4 Hz, SCH_2), 3.91 (s, 3H, OCH_3), 5.86 (br, 1H, NH), 6.78 (ddd, 1H, $^3J_{HF}$ = 8.0 Hz, $^4J_{HF}$ = 5.4 Hz, $^5J_{HF}$ = 2.4 Hz, aromatic), 8.26 (d, 1H, J = 3.7 Hz, aromatic), 8.61 (br, 1 H, NH); LR-MS (M + H$^+$) 344.

(4-Amino-2-ethanesulfonylpyrimidin-5-yl)(2-methoxyphenyl)-methanone (11c). To a cooled solution of **10c** (2.66 g, 9.19 mmol) in chloroform (120 mL) was added in three portions 3-chloroperoxybenzoic acid (~77% purity, 5.20 g, ~23 mmol, 2.5 equiv) and the mixture stirred at 0 °C for 1 h. The resulting mixture was diluted with CH_2Cl_2 (80 mL), washed with 10% aqueous sodium thiosulfate (2 × 30 mL) and brine (2 × 20 mL), dried over Na_2SO_4, and evaporated. The residue was purified on silica gel with hexane/EtOAc (60/40) to give **11c** as a white solid (2.30, 78% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (t, 3H, J = 7.5 Hz, CH_3), 3.50 (q, 2H, J = 7.4 Hz, SO_2CH_2), 3.78 (s, 3H, OCH_3), 6.30 (br, 1 H, NH), 7.01 (d, 1H, J = 8.3 Hz, aromatic), 7.10 (dt, 1H, J_{ortho} = 7.6 Hz, J_{meta} = 0.8 Hz, aromatic), 7.34 (dd, 1H, J_{ortho} = 7.6 Hz, J_{meta} = 1.8 Hz, aromatic), 7.53 (m, 1H, aromatic), 8.50 (s, 1 H, aromatic), 8.93 (br, 1 H, NH); HRMS for $C_{14}H_{15}N_3O_4SNa$ (M + Na)$^+$ calcd 344.0675, observed 344.0679.

(4-Amino-2-ethanesulfonylpyrimidin-5-yl)(3-fluorophenyl)-methanone (11a): Anal. RP-HPLC t_R = 1.70 min (method 1, 90% purity), t_R = 1.46 min (method 2, 92% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (t, 3H, J = 7.2 Hz, CH_3), 3.53 (q, 2H, J = 7.2 Hz, SO_2CH_2), 6.34 (br, 1H, NH), 7.30−7.58 (m, 4 H, aromatic), 8.67 (br, 1H, NH), 8.72 (s, 1H, aromatic); HRMS for $C_{13}H_{12}FN_3O_3S$ (M +) calcd 309.0583, observed 309.0582.

(4-Amino-2-ethanesulfonylpyrimidin-5-yl)-*o*-tolylmethanone (11b): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (t, 3H, J = 7.5 Hz), 2.25 (s, 3H), 3.44 (q, 2H, J = 7.5 Hz), 6.34 (brd, 1H, NH), 7.16−7.28 (m, 3H, aromatic), 7.35−7.41 (m, 1H, aromatic), 8.40 (s, 1H, aromatic), 8.93 (brd, 1H, NH); HRMS for $C_{14}H_{16}N_3O_3S$ (M + H) + calcd 306.0907, observed 306.0910.

(4-Amino-2-ethanesulfonylpyrimidin-5-yl)(2-ethoxyphenyl)-methanone (11e). The same procedure was used as described for **11a**: white solid; Anal. RP-HPLC t_R = 1.82 min (method 1, 95% purity), t_R = 1.60 min (method 2, 98% purity); ^{1}H NMR (300 MHz, methanol-d_4) δ 1.16 (t, 3H, J = 7.0 Hz, CH_3), 1.35 (t, 3H, J = 7.4 Hz, CH_3), 3.52 (q, 2H, J = 7.4 Hz, SO_2CH_2), 4.06 (q, 2H, J = 7.0 Hz, OCH_2), 7.10 (dt, 1H, J_{ortho} = 7.5 Hz, J_{meta} = 0.8 Hz, aromatic), 7.13 (d, 1 H, J = 8.6 Hz, aromatic), 7.40 (dd, 1H, J_{ortho} = 7.5 Hz, J_{meta} = 1.8 Hz, aromatic), 7.55 (ddd, 1H, J_{ortho} = 8.6 Hz, J_{ortho} = 7.5 Hz, J_{meta} = 1.8 Hz, aromatic), 8.38 (s, 1 H, aromatic); HRMS for $C_{15}H_{18}N_3O_4S$ (M + H$^+$) calcd 336.1013, observed 336.1016.

(4-Amino-2-ethanesulfonylpyrimidin-5-yl)(5-fluoro-2-methoxyphenyl)methanone (11g): white solid; Anal. RP-HPLC t_R = 1.71 min (method 1, 87% purity), t_R = 1.49 min (method 2, 92% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (t, 3H, J = 7.5 Hz, CH_3), 3.55 (q, 2H, J = 7.5 Hz, SO_2CH_2), 3.77 (s, 3H, OCH_3), 6.32 (br, 1 H, NH), 6.97 (dd, 1H, $^4J_{HF}$ = 3.5 Hz, J_{ortho} = 8.6 Hz, aromatic), 7.09 (dd, 1H, J_{meta} = 2.8 Hz, $^3J_{HF}$ = 7.9 Hz, aromatic), 7.22 (m, 1H, aromatic), 8.50 (s, 1 H, aromatic), 8.89 (br, 1 H, NH); HRMS for $C_{14}H_{15}FN_3O_4S$ (M + H$^+$) calcd 340.0762, observed 340.0762.

(4-Amino-2-ethanesulfonylpyrimidin-5-yl)(5-chloro-2-methoxyphenyl)methanone (11h): Anal. RP-HPLC t_R = 1.89 min (method 1, 95% purity), t_R = 1.67 min (method 2, 92% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (t, 3H, J = 7.4 Hz, CH_3), 3.51 (q, 2H, J = 7.4 Hz, SO_2CH_2), 3.77 (s, 3 H, OCH_3), 6.32 (br, 1 H, NH), 6.96 (d, 1H, J_{ortho} = 8.8 Hz, aromatic), 7.32 (d, 1H, J_{meta} = 2.6 Hz, aromatic), 7.48 (dd, 1H, J_{ortho} = 8.8, J_{meta} = 2.6 Hz, aromatic), 8.48 (s, 1 H, aromatic), 8.88 (br, 1 H, NH); HRMS for $C_{14}H_{15}ClN_3O_4S$ (M + H$^+$) calcd 356.0467, observed 356.0466.

(4-Amino-2-ethanesulfonylpyrimidin-5-yl)(2,6-difluorophenyl-)methanone (11j): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (t, 3H, J = 7.5 Hz, CH_3), 3.51 (q, 2H, J = 7.5 Hz, SO_2CH_2), 6.45 (br, 1 H, NH), 7.07 (t, 2H, J_{ortho} = $^3J_{HF}$ = 8.0 Hz, aromatic), 7.54 (m, 1H, aromatic), 8.55 (s, 1 H, aromatic), 8.93 (br, 1 H, NH); HRMS for $C_{13}H_{12}F_2N_3O_3S$ (M + H)$^+$ calcd 328.0562, observed 328.0566.

(4-Amino-2-ethanesulfonylpyrimidin-5-yl)(2,3-difluoro-6-methoxyphenyl)methanone (11k): light yellow solid; Anal. RP-HPLC t_R = 1.82 min (method 1, 95% purity), t_R = 1.57 min (method 2, 98% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (t, 3H, J = 7.4 Hz, CH_3), 3.51 (q, 2H, J = 7.4 Hz, SO_2CH_2), 3.77 (s, 3H, OCH_3), 6.37 (br, 1H, NH), 6.72 (ddd, 1H, J_{ortho} = 9.4 Hz, $^4J_{HF}$ = 3.1 Hz, $^5J_{HF}$ = 1.9 Hz, aromatic), 7.30 (q, 1H, $^3J_{HF}$ = $^4J_{HF}$ = J_{ortho} =9.4 Hz, aromatic), 8.48 (s, 1H, aromatic), 8.92 (br, 1 H, NH); HRMS for $C_{14}H_{14}F_2N_3O_4S$ (M + H$^+$) calcd 358.0668, observed 358.0671.

(4-Amino-2-ethanesulfonylpyrimidin-5-yl)(4,5-difluoro-2-methoxyphenyl)methanone (11l): off-white solid; Anal. RP-HPLC t_R = 1.82 min (method 1, 95% purity), t_R = 1.58 min (method 2, 93% purity); ^{1}H NMR (300 MHz, methanol-d_4) δ 1.65(t, 3H, J = 7.4 Hz, CH_3), 3.53 (q, 2H, J = 7.4 Hz, SO_2CH_2), 3.77 (s, 3H, OCH_3), 7.18 (dd, 1H, $^3J_{HF}$ = 12.1 Hz, $^4J_{HF}$ = 6.2 Hz, aromatic), 7.39 (dd, 1H, $^4J_{HF}$ = 8.8 Hz, $^3J_{HF}$ = 12.1 Hz, aromatic), 8.44 (s, 1 H, aromatic); HRMS for $C_{14}H_{14}F_2N_3O_4S$ (M + H$^+$) calcd 358.0668, observed 358.0672.

[4-Amino-2-[4-(4-methylpiperazin-1-yl)phenylamino]pyrimidin-5-yl](2-methoxyphenyl)methanone (22). A suspension of **11c** (20.0 mg, 0.062 mmol), 4-(4-methylpiperazino)aniline (15.5 mg, 0.081 mmol, 1.3 equiv), and *p*-TsOH hydrate (15 mg, 0.078 mmol, 1.2 equiv) in ^{i}PrOH (2.5 mL) was placed in a sealed vessel and heated at 100−110 °C under microwave irradiation for 1 h. The resulting mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution (2×) and brine (2×), dried, and concentrated. The crude product was purified on silica gel with CH_2Cl_2/MeOH (95:5) to give **22** as a light yellow solid (23.6 mg, 91% yield): Anal. RP-HPLC t_R = 1.35 min (method 1, 98% purity), t_R = 0.87 min (method 2, 99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37

(s, 3H, NCH_3), 2.60 (brd, 4H, 2×CH_2), 3.17 (m, 4H, 2×CH_2), 3.78 (s, 3H, OCH_3), 5.59 (brd, 1H, NH), 6.87−7.11 (m, 5H, 4 aromatic and 1 NH), 7.25 (m, 1H, aromatic), 7.37−7.49 (m, 3H, aromatic), 8.14 (s, 1H, aromatic), 8.84 (brd, 1H, NH); HRMS for $C_{23}H_{27}N_6O_2$ $(M + H)^+$ calcd 419.2195, observed 419.2190.

[4-Amino-2-[4-(4-methylpiperazin-1-yl)phenylamino]pyrimidin-5-yl](3-fluorophenyl)methanone (18): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (s, 3H, CH_3), 2.54 (brd, 4H, 2×CH_2), 3.13 (t, 4H, J = 4.8 Hz, 2×CH_2), 5.57 (brd, 1H, NH), 6.86 (d, 2H, J = 8.8 Hz), 7.04 (brd, 1H, NH), 7.10−7.44 (m, 6H), 8.31(s, 1H), 8.63 (brs, 1H, NH); HRMS for $C_{22}H_{24}FN_6O$ $(M + H)^+$ calcd 407.1990, observed 407.1994.

[4-Amino-2-[4-(4-methylpiperazin-1-yl)phenylamino]pyrimidin-5-yl]-*o*-tolylmethanone (21): Anal. RP-HPLC t_R = 1.39 min (method 1, >99% purity), t_R = 0.97 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.22 (s, 3H), 2.30 (s, 3H), 2.54 (m, 4H), 3.10−3.17 (m, 4H), 5.57 (brd, 1H, NH), 6.81−6.91 (m, 2H, aromatic), 7.07 (brd, 1H, NH), 7.12−7.21 (m, 3H, aromatic), 7.25−7.30 (m, 1H, aromatic), 7.36−7.43 (m, 2H, aromatic), 8.02 (s, 1H, aromatic), 8.82 (brd, 1H, NH); HRMS for $C_{23}H_{27}N_6O$ $(M + H^+)$ calcd 403.2241, observed 403.2247.

4-[4-Amino-5-(2-methoxybenzoyl)pyrimidin-2-ylamino]piperidine-1-carboxylic Acid *tert*-Butyl Ester (14c). A suspension of **11c** (1.88 g, 5.87 mmol) and 4-amino-*N*-*tert*-(butoxycarbonyl)-piperidine (**13**) (1.41 g, 7.04 mmol, 1.2 equiv) in ^{i}PrOH (50 mL) was placed in a sealed vessel and heated at 110 °C under microwave conditions for 1.5 h. The resulting mixture was concentrated and the crude product was purified on silica gel with CH_2Cl_2/MeOH (95/5) to give **14c** as a light yellow solid (2.23 g, 89% yield): Anal. RP-HPLC t_R = 1.76 min (method 1, >99% purity), t_R = 1.49 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (m, 2H, CH_2), 1.47 (s, 9H, t-Bu), 2.01 (m, 2H, CH_2), 2.93 (m, 2H, NCH_2), 3.80 (s, 3H, OCH_3), 4.03 (brm, 3H, NCH_2 & NCH), 5.00−5.70 (br, 2H, NH), 6.97 (d, 1H, J_{ortho} = 8.6 Hz), 7.02 (dt, 1H, J_{ortho} = 7.5 Hz, J_{meta} ~ 0.8 Hz), 7.24 (m, 1H), 7.42 (m, 1H), 7.94−8.22 (br, 1H,), 8.61−9.01 (br, 1H, NH); HRMS for $C_{22}H_{30}N_5O_4$ $(M + H)^+$ calcd 428.2293, observed 428.2296.

4-[4-Amino-5-(5-fluoro-2-methoxybenzoyl)pyrimidin-2-ylamino]piperidine-1-carboxylic Acid *tert*-Butyl Ester (14g): Anal. RP-HPLC t_R = 1.82 min (method 1, >99% purity), t_R = 1.65 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.41 (m, 2H, 2×CH of 2CH_2), 1.47 (s, 9H, t-Bu), 2.01(m, 2H, 2×CH of 2CH_2), 2.92 (m, 2H, 2×NCH of 2NCH_2), 3.77 (s, 3H, OCH_3), 4.02 (br, 3H, NCH and 2×NCH of 2NCH_2), 5.04−5.68 (br, 2H, NH's), 6.90 (dd, 1H, $^4J_{HF}$ = 4.0 Hz, J_{ortho} = 9.0 Hz, aromatic), 6.98 (dd, 1H, J_{meta} = 3.1 Hz, $^3J_{HF}$ = 7.9 Hz, aromatic), 7.11 (ddd, 1H, J_{meta} = 3.1 Hz, $^3J_{HF}$ = 7.9 Hz, J_{ortho} = 9.0 Hz, aromatic), 7.97−8.22 (br, 1H, aromatic), 8.56−8.93 (br, 1H, NH); HRMS for $C_{22}H_{29}FN_5O_4$ $(M + H^+)$ calcd 446.2198, observed 446.2196.

[4-Amino-2-(piperidin-4-ylamino)pyrimidin-5-yl](2-methoxyphenyl)methanone (23). TFA (15 mL) was added to a stirred solution of compound **14c** (1.96 g, 4.58 mmol) in CH_2Cl_2 (30 mL) at 0 °C. After the reaction mixture was stirred for 1 h, it was concentrated in vacuo to give **23** as a TFA salt (3.32 g, light yellow solid). A portion of this crude product was purified on HPLC to give **23** as a free base: Anal. RP-HPLC t_R = 1.11 min (method 1, 98% purity), t_R = 0.49 min (method 2, >99% purity); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.23−1.41 (m, 2H, CH_2), 1.88−2.05 (br, 2H, CH_2), 2.58−2.75 (br, 2H, CH_2), 2.94−3.11 (br, 2H, CH_2), 3.72 (s, 3H, OCH_3), 3.80−3.99 (m, 1H, CH), 4.99−5.57 (br, 2H, NH), 6.89 (d, 1H, J = 8.2 Hz), 6.94 (t, 1H, J = 7.5 Hz), 7.16 (dd, 1H, J_{ortho} = 7.5 Hz, J_{meta} = 1.8 Hz), 7.34 (dt, 1H, J_{ortho}= 8.2 Hz, J_{meta} = 1.8 Hz), 7.91−8.12 (br, 1H, aromatic), 8.53−8.88 (br, 1H, NH); HRMS for $C_{17}H_{22}N_5O_2$ $(M + H)^+$ calcd 328.1768, observed 328.1771.

[4-Amino-2-(piperidin-4-ylamino)pyrimidin-5-yl](5-fluoro-2-methoxyphenyl)methanone (15g): Anal. RP-HPLC t_R = 1.10 min (method 1, >99% purity), t_R = 0.59 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42−1.80 (m, 2H, 2×CH of 2CH_2), 2.00 (m, 1H, CH of 2CH_2), 2.47 (m, 1H, CH of 2CH_2), 2.82−3.09 (m, 2H, 2×NCH of 2NCH_2), 3.16−3.40 (m, 2H, 2×NCH of 2NCH_2), 3.71 (s, 3H, OCH_3), 3.84−4.15 (br, 1H, NCH), 7.14 (m, 1H, aromatic), 7.30 (m, 1H, aromatic), 7.51 and 8.00 (2 br, 1H, NH), 7.79 and 8.18 (br d, 1H, NH), 7.86 and 7.88 (2 s, 1H, aromatic), 8.30 (br, 1H, NH), 8.46 (br, 1H, NH), 8.61 (br, 1H, NH); HRMS for $C_{17}H_{21}FN_5O_2$ $(M + H^+)$ calcd 346.1674, observed 346.1673.

[4-Amino-2-(1-methylpiperidin-4-ylamino)pyrimidin-5-yl](2-methoxyphenyl)methanone (24). To a stirred mixture of compound **23** as the TFA salt (prepared above, 65.0 mg, ~0.090 mmol calculated from compound **14c**) and K_2CO_3 (75 mg, 0.54 mmol, 6 equiv) in anhydrous DMF (2 mL) was added iodomethane (23 mg, 0.16 mmol, 1.8 equiv). The reaction mixture was stirred at room temperature for 2.5 h before it was diluted with EtOAc (40 mL), washed with water and brine, dried, and concentrated. The residue was purified on HPLC to give amine **24** as a white solid (14.1 mg, 46% yield): Anal. RP-HPLC t_R = 1.10 min (method 1, >99% purity), t_R = 0.52 min (method 2, >99% purity); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.50 (m, 2H, CH_2), 1.95 (m, 2H, CH_2), 2.07 (m, 2H, NCH_2), 2.22 (s, 3H, NCH_3), 2.71 (m, 2H, NCH_2), 3.72 (s, 3H, OCH_3), 3.79 (m, 1H, NCH), 4.97−5.65 (m, 2H, NH), 6.97 (d, 1H, J_{ortho} = 8.4 Hz), 7.01 (t, 1H, J_{ortho} = 7.4 Hz), 7.24 (m, 1H), 7.42 (m, 1H), 7.85−8.18 (br, 1H), 8.51−8.95 (br, 1H, NH); HRMS for $C_{18}H_{24}N_5O_2$ $(M + H)^+$ calcd 342.1925, observed 342.1927.

4-[4-Amino-5-(2-methoxybenzoyl)pyrimidin-2-ylamino]piperidine-1-carboxylic Acid Ethylamide (25). To a stirred solution of compound **23** (65.0 mg, TFA salt prepared above, ~0.090 mmol) and triethylamine (0.10 mL, 72.6 mg, 0.71 mmol, 8 equiv) in CH_2Cl_2 (3 mL) was added ethyl isocyanate (8.0 mg, 0.11 mmol, 1.2 equiv) at 0 °C. The reaction mixture was stirred for 1 h before it was concentrated in vacuo. The residue was purified on silica gel with CH_2Cl_2/MeOH (95/5) to give **25** as a white solid (31.7 mg, 89% yield): Anal. RP-HPLC t_R = 1.30 min (method 1, >99% purity), t_R = 1.05 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (br, 3H, CH_3), 1.34 (m, 2H, CH_2), 1.95 (m, 2H, CH_2), 2.89 (m, 2H, NCH_2), 3.20 (m, 2H, NCH_2), 3.70 (s, 3H, OCH_3), 3.81 (m, 2H, NCH_2), 3.96 (m, 1H, NCH), 4.31 (br, 1H, NH), 4.87−5.68 (m, 2H, NH), 6.94−7.08 (m, 2H), 7.24 (m, 1H), 7.42 (m, 1H), 7.83−8.17 (br, 1H), 8.47−8.95 (br, 1H, NH); HRMS for $C_{20}H_{27}N_6O_3$ $(M + H)^+$ calcd 399.2139, observed 399.2143.

4-[4-Amino-5-(2-methoxybenzoyl)pyrimidin-2-ylamino]piperidine-1-carboxylic Acid Ethyl Ester (26). A suspension of **11c** (43.1 mg, 0.134 mmol) and ethyl 4-amino-1-piperidinecarboxylate (30.0 mg, 0.174 mmol, 1.3 equiv) in ^{i}PrOH (4 mL) was heated at 100−110 °C under microwave irradiation for 1 h. The resulting mixture was concentrated and the residue was purified on silica gel with CH_2Cl_2/MeOH (95/5) to give **26** as a light yellow solid (47.1 mg, 88% yield): Anal. RP-HPLC t_R = 1.52 min (method 1, >99% purity), t_R = 1.29 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, 3H, J = 7.1 Hz, CH_3), 1.31−1.49 (m, 2H, 2×CH of 2CH_2), 2.02 (m, 2H, 2×CH of 2CH_2), 2.88−3.07 (m, 2H, 2×NCH of 2NCH_2), 3.79 (s, 3H, OCH_3), 3.95−4.19 (m, 3H, NCH and 2×NCH of 2NCH2), 4.13 (q, 2H, J = 7.1 Hz, OCH_2), 5.02−5.67 (br, 2H, NH), 6.97 (d, 1H, J_{ortho} = 8.3 Hz, aromatic), 7.02 (t, 1H, J = 7.4 Hz, aromatic), 7.23 (d, 1H, J_{ortho} = 7.4 Hz, aromatic), 7.41 (t, 1H, J = 8.3 Hz, aromatic), 7.97−8.21 (br, 1H, aromatic), 8.60−9.00 (br, 1H, NH); HRMS for $C_{20}H_{26}N_5O_4$ $(M + H)^+$ calcd 400.1980, observed 400.1984.

1-[4-[4-Amino-5-(2-methoxybenzoyl)pyrimidin-2-ylamino]-piperidin-1-yl]ethanone (27). Following a similar procedure as described for compound **25**, the reaction of **23** with acetyl chloride gave **27** as a white solid: Anal. RP-HPLC t_R = 1.27 min (method 1, >99% purity), t_R = 0.99 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26−1.37 (m, 2H, CH_2), 1.92−2.09 (m, 2H, CH_2), 2.03 (s, 3H, CH_3), 2.74, 3.13, 3.72, 4.43 (4x m, 4H, 2×NCH_2), 3.72 (s, 3H, OCH_3), 4.01 (brm, 1H, NCH), 4.93−5.61 (br, 2H, NH), 6.97 (d, 1H, J_{ortho} = 8.3 Hz), 7.02 (dt, 1H, J_{ortho} = 7.4 Hz, J_{meta} = ~0.8 Hz), 7.24 (m, 1H), 7.34 (m, 1H), 7.96 (br, 1H), 8.79 (br, 1H, NH); HRMS for $C_{19}H_{24}N_5O_3$ $(M + H)^+$ calcd 370.1874, observed 370.1875.

1-[4-[4-Amino-5-(2-methoxybenzoyl)pyrimidin-2-ylamino]-piperidin-1-yl]butan-1-one (28): Anal. RP-HPLC t_R = 1.45 min

(method 1, 98% purity), t_R = 1.18 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, 3H, J = 7.4 Hz, CH_3), 1.39 (m, 2H, CH_2), 1.67 (m, 2H, CH_2), 2.09 (m, 2H, CH_2), 2.32 (m, 2H, CH_2), 2.82, 3.18, 3.83, 4.54 (4m, 4H, 2×NCH_2), 3.80 (s, 3H, OCH_3), 4.08 (br, 1H, NCH), 4.98−5.67 (br, 2H, NH), 6.97 (d, 1H, J = 8.1 Hz), 7.02 (dt, 1H, J_{ortho} = 7.5 Hz, J_{meta} = 0.9 Hz), 7.24 (m, 1H), 7.42 (m, 1H), 7.98−8.21 (br, 1H), 8.57−9.03 (br, 1H, NH); HRMS for $C_{21}H_{28}N_5O_3$ $(M + H)^+$ calcd 398.2187, observed 398.2189.

[4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](2-methoxyphenyl)methanone (29). Following a similar procedure as described for compound **25**, the reaction of **23** with methanesulfonyl chloride gave **29** as a white solid: Anal. RP-HPLC t_R = 1.35 min (method 1, >99% purity), t_R = 1.09 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55 (m, 2H, CH_2), 2.06 (m, 2H, CH_2), 2.73 (s, 3H, SCH_3), 2.82 (m, 2H, NCH_2), 3.68 (m, 2H, NCH_2), 3.71 (s, 3H, OCH_3), 3.93 (m, 1H, NCH), 4.89−5.64 (m, 2H, NH), 6.94−7.07 (m, 2H), 7.24 (dd, 1H, J_{ortho} = 7.5 Hz, J_{meta} = 1.7 Hz), 7.42 (m, 1H), 7.98 (br, 1H), 8.45−8.99 (br, 1H, NH); HRMS for $C_{18}H_{24}N_5O_4S$ $(M + H)^+$ calcd 406.1544, observed 406.1546.

[4-Amino-2-(1-ethanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](2-methoxyphenyl)methanone (30): white solid; Anal. RP-HPLC t_R = 1.40 min (method 1, >99% purity), t_R = 1.20 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (brt, 3H, CH_3), 1.60 (m, 2H, CH_2), 2.12 (m, 2H, CH_2), 2.97 (q, 2H, J = 7.2 Hz, SCH_2), 2.93−3.06 (m, 2H, NCH_2), 3.78 (m, 2H, NCH_2), 3.80 (s, 3H, OCH_3), 4.02 (m, 1H, NCH), 4.95−5.71 (m, 2H, NH), 6.97 (d, 1H, J_{ortho} = 8.4 Hz), 7.02 (t, 1H, J_{ortho}=7.4 Hz), 7.24 (dd, 1H, J_{ortho} = 7.4 Hz, J_{meta} = 1.8 Hz), 7.42 (dt, 1H, J_{ortho} = 8.4 Hz, J_{meta} = 1.8 Hz), 8.06 (brs, 1H), 8.61−9.05 (br, 1H, NH); HRMS for $C_{19}H_{26}N_5O_4S$ $(M + H)^+$ calcd 420.1700, observed 420.1704.

4-[4-Amino-5-(2-methylbenzoyl)pyrimidin-2-ylamino]piperidine-1-carboxylic Acid Ethyl Ester (32): white solid; Anal. RP-HPLC t_R = 1.63 min (method 1, >99% purity), t_R = 1.38 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, 3H, J = 7.4 Hz, CH_3), 1.40 (m, 2H, 2×CH of $2CH_2$), 2.03 (m, 2H, 2×CH of $2CH_2$), 2.29 (s, 3H, CH_3), 2.98 (m, 2H, 2×NCH of $2NCH_2$), 3.98−4.20 (m, 3H, NCH and 2×NCH of $2NCH_2$), 4.14 (q, 2H, J = 7.4 Hz, OCH_2), 5.04−5.73 (4br, 2H, NH and NH of NH2), 7.17−7.38 (m, 4H, aromatic), 7.94−8.15 (2br, 1H, aromatic), 8.67−9.03 (2br, 1H, NH of NH2); HRMS for $C_{20}H_{26}N_5O_3$ $(M + H)^+$ calcd 384.2030, observed 384.2035.

1-[4-[4-Amino-5-(2-ethoxybenzoyl)pyrimidin-2-ylamino]piperidin-1-yl]ethanone (33). Following a similar procedure as described for **14c**, the reaction of **11e** with 1-(4-aminopiperidin-1-yl)ethanone under microwave irradiation gave **33** as a white solid: Anal. RP-HPLC t_R = 1.15 min (method 1, >99% purity), t_R = 1.10 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (t, 3H, J = 7.0 Hz, CH_3), 1.46 (m, 2H, CH_2), 2.06 (m, 2H, CH_2), 2.11 (s, 3H, CH_3), 2.91, 3.23, 3.80, 4.46 (4m, 4H, 2×NCH_2), 4.05 (q, 2H, J = 7.0 Hz, OCH_2), 4.11 (m, 1H, NCH), 4.90−6.46 (m, 2H, NH), 6.95 (d, 1H, J = 8.4 Hz), 7.01 (t, 1H, J = 7.6 Hz), 7.16 (m, 1H), 7.33 (m, 1H), 7.90−8.29 (br, 1H), 8.57−9.17 (br, 1H, NH); HRMS for $C_{20}H_{26}N_5O_3$ $(M + H^+)$ calcd 384.2030, observed 384.2035.

[4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](5-fluoro-2-methoxyphenyl)methanone (35): Anal. RP-HPLC t_R = 1.46 min (method 1, >99% purity), t_R = 1.24 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.63 (m, 2H, CH_2), 2.15 (m, 2H, CH_2), 2.81 (s, 3H, SCH_3), 2.92 (m, 2H, NCH_2), 3.74 (m, 2H, NCH_2), 3.77 (s, 3H, OCH_3), 4.01 (m, 1H, NCH), 5.01−5.71 (m, 2H, NH), 6.91 (dd, 1H, J_{ortho} = 9.1 Hz, $^4J_{HF}$ = 4.0 Hz), 6.97 (dd, 1H, $^3J_{HF}$ = 7.9 Hz, J_{meta} = 3.2 Hz), 7.11 (ddd, 1H, J_{ortho} = 9.1 Hz, $^3J_{HF}$ = 7.9 Hz, J_{meta} = 3.2 Hz), 7.93−8.21 (br, 1H), 8.55−9.01 (br, 1H, NH); HRMS for $C_{18}H_{23}FN_5O_4S$ $(M + H)^+$ calcd 424.1450, observed 424.1455.

[4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](5-chloro-2-methoxyphenyl)methanone (36). Following a similar procedure as described for **14c**, reaction of **11h** and 1-methanesulfonylpiperidin-4-ylamine TFA salt (prepared as follows) gave **36** as a white solid: Anal. RP-HPLC t_R = 1.63 min (method 1, >99% purity), t_R = 1.38 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.69 (m, 2H, CH_2), 2.14 (m, 2H, CH_2), 2.83 (s, 3H, SCH_3), 3.06 (m, 1H, NCH), 3.58−3.86 (m, 3H, 3×NCH), 3.78 (s, 3H, OCH_3), 4.06 (m, 1H, NCH), 5.02−6.79 (br, 2H, NH), 6.93 (m, 1H), 7.23 (m, 1H), 7.40 (m, 1H), 7.90−8.20 (br, 1H), 8.59−9.09 (br, 1H, NH); HRMS for $C_{18}H_{23}ClN_5O_4S$ $(M + H^+)$ calcd 440.1154, observed 440.1160.

Preparation of 1-Methanesulfonylpiperidin-4-ylamine. Methanesulfonyl chloride (1.0 g, 8.8 mmol) was added to a stirred solution of commercially available piperidin-4-ylcarbamic acid *tert*-butyl ester (1.0 g, 5.0 mmol) and diisopropylethylamine (4 mL) in THF (40 mL) at 5 °C. The reaction mixture was allowed to warm to room temperature over 1 h before it was poured into water and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were washed with 5% aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo to give a crude solid which was purified by trituration with ether/hexane to give (1-methanesulfonyl-piperidin-4-yl)carbamic acid *tert*-butyl ester as a white solid [MS $(M + H^+)$ 278]. This solid was suspended in CH_2Cl_2 (15 mL), treated with TFA (5.3 mL), and stirred for 2 h at room temperature. The resulting mixture was concentrated and the residue was triturated with ether. The solid was filtered off, washed with ether, and dried in a vacuum to give a quantitative yield of 1-methanesulfonyl-piperidin-4-ylamine trifluoroacetate. A portion of the material was dissolved in EtOAc, washed with saturated aqueous $NaHCO_3$ solution and brine, dried, and concentrated to give the free base 1-methanesulfonylpiperidin-4-ylamine as a white waxy solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24 (m, 2H, CH_2), 1.73 (m, 2H, CH_2), 1.88 (br, 1H, NH), 2.58−2.76 (m, 3H, NCH and NCH_2), 2.81 (s, 3H, SCH_3), 3.25−3.3.35 (br, 1H, NH), 3.40 (m, 2H, NCH_2).

General Procedure for Preparation of Sulfoxides 12. To a stirred solution of sulfide **10** (1.8 mmol) in $CHCl_3$ (16 mL) at −15 °C was added portionwise mCPBA (70% purity, 2.1 mmol, 1.2 equiv). The mixture was stirred at the same temperature for 1 h before it was diluted with CH_2Cl_2 (30 mL); washed successively with 10% aqueous sodium thiosulfate (2 × 5 mL), 10% aqueous Na_2CO_3 (2 × 5 mL), brine (2 × 5 mL); dried; and concentrated to give a quantitative yield of 9:1 ratio mixture (by NMR) of sulfoxide **12** and sulfone **11**. This mixture was used for next reaction without further purification while pure sulfoxide **12** in some examples was obtained by chromatographic purification of a small portion of the mixture.

4-[4-Amino-5-(2-fluorobenzoyl)pyrimidin-2-ylamino]piperidine-1-carboxylic Acid Ethyl Ester (31). Following a similar procedure as described for compound **26**, a mixture of sulfoxide **12d** (major) and sulfone **11d** (minor) was reacted with ethyl 4-amino-1-piperidinecarboxylate to give **31** as off-white soild: Anal. RP-HPLC t_R = 1.58 min (method 1, >99% purity), t_R = 1.40 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ1.19 (t, 3H, J = 7.2 Hz, CH_3), 1.34 (m, 2H, CH_2), 1.95 (m, 2H, CH_2), 2.91 (m, 2H, NCH_2), 3.88−4.11 (m, 3H, NCH & NCH_2), 4.06 (q, 2H, J = 7.2 Hz, OCH_2), 4.94−5.76 (br, 2H, NH), 7.07 (t, 1H, J = 8.9 Hz), 7.17 (m, 1H), 7.32 (m, 1H), 7.39 (m, 1H), 7.92−8.25 (br, 1H), 8.43−8.97 (br, 1H, NH); HRMS for $C_{19}H_{23}N_5O_3F$ $(M + H^+)$ calcd 388.1780, observed 388.1783.

[4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](4-fluoro-2-methoxyphenyl)methanone (34). Similar to the preparation of compound **31**, the reaction of a mixture of sulfoxide **12f** (major) and sulfone **11f** (minor) with 1-methanesulfonylpiperidin-4-ylamine TFA salt gave **34**: white solid; Anal. RP-HPLC t_R = 1.39 min (method 1, >99% purity), t_R = 1.20 min (method 2, >99% purity); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.53 (m, 2H, CH_2), 1.88 (m, 2H, CH_2), 2.66−2.90 (m, 5H, NCH_2 & SCH_3), 3.51 (m, 2H, NCH_2), 3.73 (s, 3H, OCH_3), 3.89 (m, 1H, NCH), 6.84 (dt, 1H, J_{ortho} = 8.6 Hz,$^3J_{HF}$ = 8.6 Hz, J_{meta} = 2.3 Hz), 7.03 (dd, 1H, $^3J_{HF}$ = 11.5 Hz, J_{meta} = 1.8 Hz), 7.24 (m, 1H), 7.40 and 7.68 (2brs, 1H, NH), 7.54 and 7.74 (2brd, 1H, NH), 7.79 and 7.86 (2s, 1H, aromatic), 8.29 and 8.49 (2brs, 1H, NH); HRMS for $C_{18}H_{23}FN_5O_4S$ $(M + H)^+$ calcd 424.1450, observed 424.1453.

[4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](2-fluoro-6-methoxyphenyl)methanone (37): Anal. RP-HPLC t_R = 1.46 min (method 1, >99% purity), t_R = 1.30 min (method 2, >99% purity); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.54 (m, 2H, CH_2), 1.89 (m, 2H, CH_2), 2.78 (m, 2H, NCH_2), 2.83 and 2.87 (2s, 3H, SCH_3), 3.52 (m, 2H, NCH_2), 3.74 (s, 3H, OCH_3), 3.90 (m, 1H, NCH), 6.90 (t, 1H, J = 8.5 Hz, aromatic), 6.97 (d, 1H, J = 8.0 Hz, aromatic), 7.46 (q, 1H, J = 8.0 Hz, aromatic), 7.52 and 7.75−7.90 (2br, 1H, NH), 7.64 and 7.75−7.90 (2br, 1H, NH), 7.79 and 7.86 (2s, 1H, aromatic), 8.25 and 8.44 (2br, 1H, NH); HRMS for $C_{18}H_{23}FN_5O_4S$ $(M + H)^+$ calcd 424.1450, observed 424.1451.

[4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](2,6-difluorophenyl)methanone (38): Anal. RP-HPLC t_R = 1.58 min (method 1, >99% purity), t_R = 1.40 min (method 2, >99% purity); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.57 (m, 2H, CH_2), 1.89 (m, 2H, CH_2), 2.79 (m, 2H, NCH_2), 2.84 and 2.87 (2s, 3H, SO_2CH_3), 3.52 (m, 2H, NCH_2), 3.92 (m, 1H, NCH), 7.23 (t, 2H, J_{ortho} = 8.4 Hz, $^3J_{HF}$ = 8.4 Hz, aromatic), 7.58 (m, 1H, aromatic), 7.65 and 7.94 (2br, 1H, NH), 7.78 and 8.00 (2br, 1H, NH), 7.72 and 7.99 (2br, 1H, aromatic), 8.82 and 8.43 (2br, 1H, NH); HRMS for $C_{17}H_{20}F_2N_5O_3S$ $(M + H)^+$ calcd 412.1250, observed 412.1248.

[4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](2,3-difluoro-6-methoxyphenyl)methanone (39): white solid; mp 218−219 °C; Anal. RP-HPLC t_R = 1.58 min (method 1, >99% purity), t_R = 1.44 min (method 2, >99% purity); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.68 (m, 2H, CH_2), 2.14 (m, 2H, CH_2), 2.82 (s, 3H, SCH_3), 3.02 (m, 1H, NCH), 3.64−3.82 (m, 3H, 3×NCH), 3.76 (s, 3H, OCH_3), 4.05 (m, 1H, NCH), 5.06−6.37 (br, 2H, NH), 6.68 (br, 1H, aromatic), 7.21 (m, 1H, aromatic), 7.99 and 8.10 (2s, 1H, aromatic), 8.60 and 8.88 (2br, 1H, NH); HRMS for $C_{18}H_{22}F_2N_5O_4S$ $(M + H)^+$ calcd 442.1355, observed 442.1358.

[4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](4,5-difluoro-2-methoxyphenyl)methanone (40): white solid; Anal. RP-HPLC t_R = 1.53 min (method 1, >99% purity), t_R = 1.30 min (method 2, >99% purity); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.54 (m, 2H, CH_2), 1.88 (m, 2H, CH_2), 2.79 (m, 2H, NCH_2), 2.84 and 2.86 (2s, 3H, SCH_3), 3.51 (m, 2H, NCH_2), 3.71 (s, 3H, OCH_3), 3.90 (m, 1H, NCH), 7.29 (dd, 1H, $^3J_{HF}$ = 13.0 Hz, $^4J_{HF}$ = 6.7 Hz, aromatic), 7.39 (t, 1H, $^3J_{HF}$ = 9.5 Hz, $^4J_{HF}$ = 9.5 Hz, aromatic), 7.43 and 7.72 (2br, 1H, NH), 7.57 and 7.77 (2br, 1H, NH), 7.82 and7.89 (2s, 1H, aromatic), 8.24 and 8.44 (2br, 1H, NH); HRMS for $C_{18}H_{22}F_2N_5O_4S$ $(M + H)^+$ calcd 442.1355, observed 442.1358.

[4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](2,3,4-trifluoro-6-methoxyphenyl)methanone (41): white solid; Anal. RP-HPLC t_R = 1.70 min (method 1, >99% purity), t_R = 1.51 min (method 2, >99% purity); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.55 (m, 2H, CH_2), 1.89 (m, 2H, CH_2), 2.78 (m, 2H, NCH_2), 2.84 and 2.89 (2s, 3H, SCH_3), 3.52 (m, 2H, NCH_2), 3.73 (s, 3H, OCH_3), 3.91 (br, 1H, NCH), 7.20 (m, 1H, aromatic), 7.56 and 7.84 (2br, 1H, NH), 7.71 and 7.90 (2br, 1H, NH), 7.95 and 8.02 (2s, 1H, aromatic), 8.20 and 8.39 (2br, 1H, NH); HRMS for $C_{18}H_{21}F_3N_5O_4S$ $(M + H)^+$ calcd 460.1261, observed 460.1267.

[4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](3,4,5-trifluoro-2-methoxyphenyl)methanone (42): white solid; Anal. RP-HPLC t_R = 1.75 min (method 1, >99% purity), t_R = 1.57 min (method 2, >99% purity); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.55 (m, 2H, CH_2), 1.90 (m, 2H, CH_2), 2.79 (m, 2H, NCH_2), 2.84 and 2.87 (2s, 3H, SCH_3), 3.53 (m, 2H, NCH_2), 3.86 (s, 3H, OCH_3), 3.93 (m, 1H, NCH), 7.08 (m, 1H, aromatic), 7.58 and 7.87 (2br, 1H, NH), 7.72 and 7.93 (2br, 1H, NH), 8.05 and 8.12 (2s, 1H, aromatic), 8.22 and 8.42 (2br, 1H, NH); HRMS for $C_{18}H_{21}N_5O_4SF_3$ $(M + H)^+$ calcd 460.1261, observed 460.1267.

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