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Article

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The design of a Janus Kinase 3 (JAK3) specific inhibitor 1-((2S,5R)-5-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-2-methylpiperidin-1-yl)prop-2-en-1-one (PF-06651600) allowing for the interrogation of JAK3 signaling in humans

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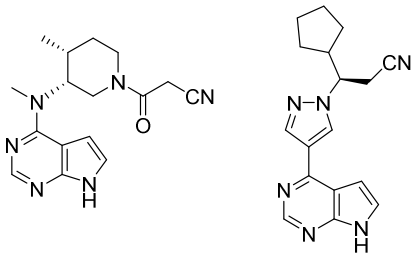
Abstract: Significant work has been dedicated to the discovery of JAK kinase inhibitors resulting in several compounds entering clinical development and two FDA approved NMEs. However, despite significant effort during the past two decades, identification of highly selective JAK3 inhibitors has eluded the scientific community. A significant effort within our research organization has resulted in the identification of the first orally active JAK3 specific inhibitor, which achieves JAK isoform specificity through covalent interaction with a unique JAK3 residue Cys-909. The relatively rapid resynthesis rate of the JAK3 enzyme presented a unique challenge in the design of covalent inhibitors with appropriate pharmacodynamics properties coupled with limited unwanted off-target reactivity. This effort resulted in the identification of **11** (PF-06651600), a potent and low clearance compound with demonstrated *in vivo* efficacy. The favorable efficacy and safety profile of this specific JAK3 inhibitor **11** led to its evaluation in several human clinical studies.

Introduction

Janus kinases (JAK) are non-receptor tyrosine kinases required for signaling through type I/II cytokine receptors.[1] There are four JAK family members: JAK1, JAK2, JAK3 and TYK2. JAK1, JAK2 and TYK2 are ubiquitously expressed while JAK3 expression is largely restricted to leukocytes. All four isoforms are involved in immune related functions while JAK1 and JAK2 also play critical roles related to hematopoiesis, growth and neuronal functions amongst others. A notable feature of JAK signaling is the requirement for a dimer of JAK kinases within the cytokine receptor complex, and cytokine receptors signaling through JAK1/JAK3, JAK1/JAK2, JAK1/TYK2, JAK2/TYK2 and JAK2/JAK2 have been described.

The importance of the individual JAKs in cytokine signaling has resulted in significant efforts to identify isoform selective inhibitors.[2,3] Several inhibitors have entered clinical evaluation for autoimmune diseases as well as various cancers and myeloproliferative disorders, resulting in two FDA approvals to date (Tofacitinib (**1**) and Ruxolitinib (**2**), (Figure 1).[4] JAK3 was among the first of the JAKs targeted for therapeutic intervention due to the strong validation provided by human SCID patients displaying JAK3 deficiencies.[5] That said, the majority of inhibitors reported to date lack selectivity within the JAK family. Therefore, the therapeutic potential of JAK3 selective inhibitors remains to be addressed. Indeed, in recent years there has been a debate on the role of JAK3 versus JAK1 for the common gamma-chain cytokines utilizing this heterodimeric kinase pair.[6] Recently, we illustrated that a selective JAK3 inhibitor could effectively inhibit IL-15 mediated signaling *in vitro*, reaffirming the therapeutic potential of selective JAK3 inhibition.[7]



Tofacitinib, 1 Ruxolitinib, **2**

Figure 1. FDA approved JAK inhibitors

Sequence alignment of the ATP binding site for the four JAK family members illustrates that JAK3 has only two unique residues that could be utilized to design selective inhibitors (4). In addition, available crystal structures for each JAK isoform highlight very minor conformational differences amongst the set.[8] This structural information, combined with the higher ATP affinity of JAK3 versus other family members, illustrates the extraordinary challenge associated with the design of highly selective, ATP competitive JAK3 inhibitors.[7] The unique residue Cys-909 in JAK3 provides an opportunity to engineer selectivity for JAK3 in a covalent manner to overcome the high sequence and shape similarity within the ATP binding cavity. This idea has been reported in the literature, most notably by the disclosure of highly selective JAK3 covalent inhibitors with supporting in vitro characterization.[9,10] Targeting JAK3 in a covalent manner provides alternative dimensions in drug design, such as the potential for an extended pharmacodynamic (PD) effect following target inactivation. Covalent modification also presents unique liabilities, such as off-target reactivity which could lead to undesired pharmacology and non-traditional metabolic clearance. We recently disclosed the selective covalent JAK3 inhibitor **11** (PF-06651600) and summarized efficacy results from multiple pre-clinical *in vivo* models of inflammatory diseases.[20] This highly optimized covalent inhibitor of JAK3 provides rapid kinase inactivation while possessing minimal chemical reactivity, resulting in an inhibitor with good ADME properties and overall selectivity. In this paper, we describe the research efforts which culminated in the discovery of **11**.

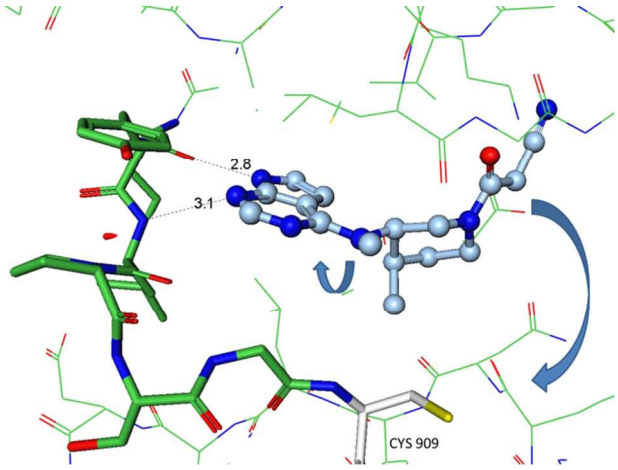
Results and discussion

Initial design and impact of reactivity on translation. The crystal structure of **1** in complex with the kinase domain of JAK3 provided the inspiration for the design of inhibitors that would interact in a covalent manner with Cys-909.[11] Rotation around the pyrimidyl amine N-C bond would potentially place the amide substituent in close proximity with Cys-909, where an appropriately designed electrophile could capture the nucleophilic cysteine (Figure 2a). Placing an acrylamide on the tofacitinib core resulted in compound **3,** which had modest inhibition of JAK3, but poor JAK family selectivity, indicating that the affinity was mainly due to reversible (Ki) protein interactions (Table 1). This hypothesis was supported by X-ray crystal structure analysis, which indicated that the pyrolopyrimidine hinge binder scaffold was well-ordered in the active site, while the acrylamide substituted piperidine was highly disordered (data not shown). Since JAK3 has the highest affinity for ATP among its family members, the Ki component of target inhibition must not be the major driver for potency as that would only provide modest selectivity gains through covalent interaction with Cys-909. In order to increase the selectivity for JAK3, the inactivation (Kinact) rate had to be improved. Modeling studies indicated that removal of the N-methyl and piperidine cis methyl groups would result in an analog with a low energy conformation, placing the electrophilic warhead in close proximity to Cys-909, Figure 2b.[12] Indeed, preparation of compound **4** revealed a significant improvement in potency (JAK3 IC_{50} = 56 nM, [ATP] = 1mM). An IC_{50} can be an excellent measure of potency of irreversible inhibitors when produced under conditions of relatively long assay reaction times, as this enables a good correlation with the time independent potency measurement Kinact/Ki.[13,14] In fact, the greater ease associated with IC_{50} determination and the logical translation to functional responses in both in vitro cell-based and in vivo assays renders IC_{50} the parameter of choice for SAR determination. The high JAK3 selectivity for **4** relative to **3** indicated that a significant improvement in the Kinact parameter had been achieved. The

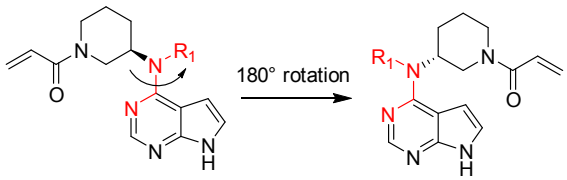
covalent bond forming event of **4** with Cys-909 was further verified in a follow on study where the compound was shown to have a dissociation rate of > 8 h.

The concept of high JAK3 specificity utilizing a covalent handle was extended via a range of electrophiles and motifs that would place the electrophile appropriately in the vicinity of Cys-909 resulting in analogs such as the anilinoacrylamide **6**, which demonstrated potent JAK3 inhibition (IC_{50} = 29 nM). Compounds **4** and **6** represent two different series (i.e. aliphatic and aryl aminoacrylamides) that were explored in parallel. While the majority of the potency for each series was obtained through rapid covalent inactivation (*i.e.* K_{inact}), each achieved this via significantly different principles. Our quantification of electrophilicity utilizing glutathione (GSH) as a trapping reagent illustrated that the two series have very different inherent chemical reactivities (aryl-aminoacrylamides: $T_{1/2}$ = 0.4 - 4h; aliphatic aminoacrylamides: 4 – 40 h).[15] Aryl aminoacrylamides have been successfully utilized in the design of irreversible inhibitors for JAK3[9]) EGFR[16] and BTK[17] resulting in potent inhibitors, therefore, it was unclear how differing relative reactivities would translate to off-target effects. The impact of reactivity became quite apparent when evaluating the translation from a cell-based PBMC assay to one conducted in human whole blood, a medium that is rich in proteins (Figure 3a). While the aliphatic aminoacrylamides demonstrated a solid correlation between assays, the aryl aminoacrylamides provided a poor translation. The reason for the differential translation for aliphatic vs. aryl aminoacrylamides has not been fully elucidated, however, the enhanced reactivity of aryl aminoacrylamides described above was likely a major contributor. In addition, blood stability studies conducted with representatives from both series demonstrated a similar trend, (Figure 3b). Therefore, while the aryl aminoacrylamide class of compounds generated some of our most potent analogs in enzymatic and PBMC cell-based assays, it was clear that the level of chemical reactivity inherent to this series needed to be attenuated to provide optimal potency in a whole blood environment.

2a)



2b)

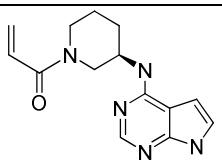
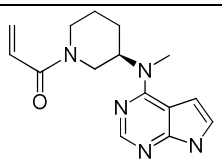
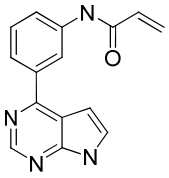
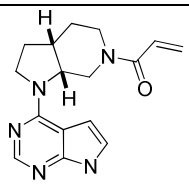
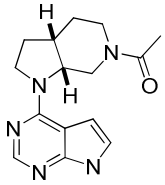
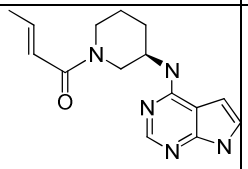
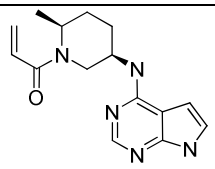


Cmpd#	R1	Conformer A (Kcal/mol)	Conformer B (Kcal/mol)
4	H	0.0	0.6
5	Me	0.0	0.02

Figure 2. 2a) Crystal structure of tofacitinib in JAK3 illustrating key movement around the N-C axis that would place the amide in close proximity to Cys-909. 2b) Illustrates the dependency of the NMe group on conformational energetics with conformer A being favored by 70:30 for **4**.

Table 1. Design of covalent inhibitors of JAK3 with JAK enzyme inhibition data

Cmpd #	Structure	JAK3 IC_{50} (nM) Km♦	JAK3 IC50 (nM)*	JAK1 IC50 (nM) Km#	JAK1 IC50 (nM)*	JAK2 IC50 (nM)*	TYK2 IC50 (nM)*	Il-15 PBMC (cell) IC50 (nM)	IL-15 HWB (cell) IC50 (nM)
3		75	1930	274	988	2030	>10000	ND	ND

Cmpd #	Structure	JAK3 IC_{50} (nM) Km♦	JAK3 IC50 (nM)*	JAK1 IC50 (nM) Km#	JAK1 IC50 (nM)*	JAK2 IC50 (nM)*	TYK2 IC50 (nM)*	Il-15 PBMC (cell) IC50 (nM)	IL-15 HWB (cell) IC50 (nM)
4		0.7	56	1400	>10000	>10000	>10000	107	580
5		18	3840	1150	7820	> 10000	>10000	ND	ND
6		0.3	29	966	8710	>10000	>10000	32	2280
7		0.4	47	984	>10000	>10000	>10000	116	399
8		372	>10000	863	>10000	>10000	>10000	ND	ND
9		1610	>10000	2290	>10000	>10000	>10000	ND	ND
11		0.3	33	1640	>10000	>10000	>10000	51	197

The enzyme assay were performed using the following description: ♦ATP concentration for JAK3 is 4 uM at Km, # ATP concentration for JAK1 is 40 uM at Km, * these assays utilized 1 mM ATP.

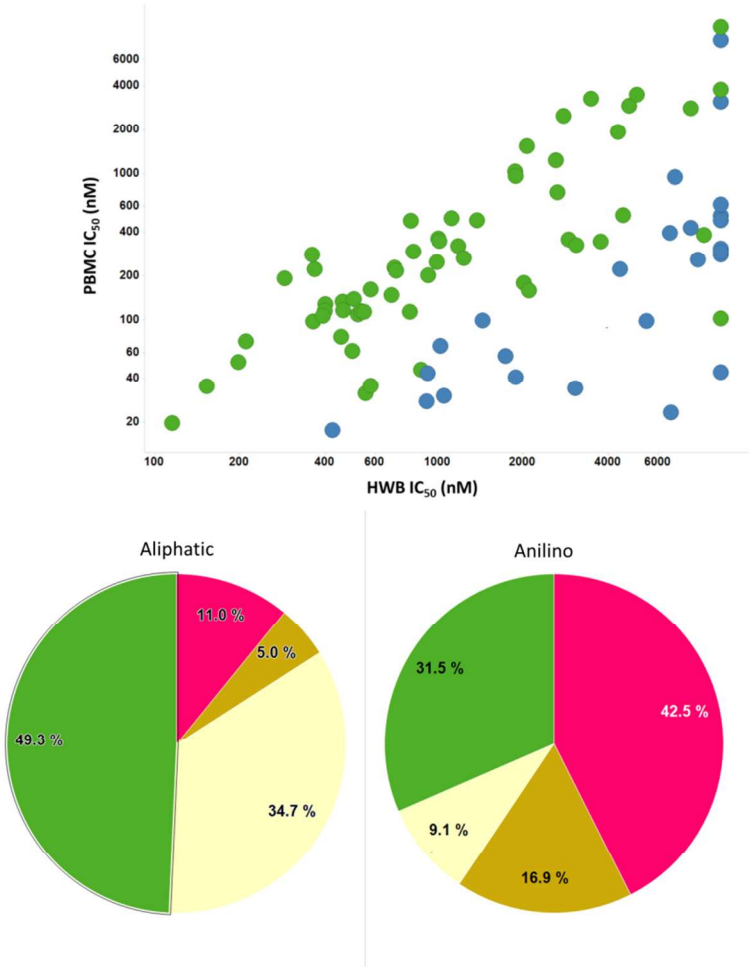


Figure 3. 3a) Correlation between our IL-15 PBMC assay and the corresponding HWB assay. Anilinoacrylamide = blue, aliphaticacrylamide = green. 3b) The stability of the compounds in HWB. Legend $T_{1/2}$ <100 min magenta, 100-200 min tan, 200-300 min yellow, >300 min green (n=107)

Identification of key parameters leading to the discovery of orally active JAK3 specific inhibitors. The identification of compound **4** illustrated an important guiding principle for future design. The analogs

would need to have a sufficient Ki component to effectively compete with ATP and initiate binding to the protein, but that this could only be a minor component of enzyme inhibition. Rapid inactivation (Kinact) continued to be the major driver for optimization as it was determined to be the key for achieving sufficient selectivity. Simply increasing chemical reactivity was deemed an unproductive strategy for Kinact optimization given the results discussed above for the aryl aminoacrylamides. When considering the individual events involved in covalent adduct formation, the initial bound complex EI would not necessarily represent the reactive complex EI* (Figure 4). Therefore, increasing the concentration of the reversibly bound EI* complex should result in acceleration of covalent capture. The increase in chemical reactivity needed to obtain potent compounds in the series represented by **6** likely indicated that a low concentration of the EI* complex was present.[18] The design principle of increased EI* complex concentration can be illustrated with compound **4**. The crystal structures of **4** and **6** bound to JAK3 provided significant guidance for additional design with a focus on stabilization of the reactive conformation (Figure 5a-b). Both of the compounds are Cys-909 adducts, but their conformations are significantly different with regard to acrylamide alignment. For **4**, the acrylamide is observed making two hydrogen bonds; one to a conserved water molecule that is interacting with the ligand pyrimidine nitrogen and the second to the NH of the Cys-909 amide. While these observations are post covalent bond formation, our assumption is that they exist in the EI* complex as well, stabilizing the reactive conformation and providing activation of the acrylamide for addition to the Cys-909. In contrast, the acrylamide in **6** does not retain any of these interactions, thus requiring increased inherent chemical reactivity to capture the Cys-909. These insights were applied to the design of a more ring constrained compound **7** providing a potent inhibitor of JAK3. The X-ray crystal structure of **7** bound to Jak3 illustrates that the cyclization of the piperidine ring back to the linker amine constrains the rotation around the piperidine-N bond, and stabilizes the piperidine in the appropriate chair conformation to present the acrylamide for nucleophilic attack by Cys-909, Figure 5c.

JAK1, JAK2, and TYK2 resynthesis rates are fairly rapid and in the range of 2 - 4 h.[19] The lack of information for JAK3 prompted measurement of the resynthesis rate in PBMC cells, which was determined to be 3.5 h.[20] This relatively rapid resynthesis rate indicated that the potential for an extended pharmacodynamic (PD) effect due to covalent inhibition was out of scope. This is contrary to our experience with slowly resynthesized biological targets such as FAAH[21] and BTK[22]. In these cases, relatively short-term drug exposure could provide long-term target knockdown in vivo. For JAK3, excellent pharmacokinetics (PK) with low clearance would be required in order to achieve sustained target inactivation and a high level efficacy. Compound **4** (data not shown) and **7** suffered from moderate to poor PK in rat despite very reasonable oxidative stability as judged by liver microsomes, (Table 2). A set of PK experiments with compound **7** and the corresponding acetate derivative **8** provided key insights into drug clearance mechanisms. For both compounds, very high clearance was observed in an *in vivo* rat PK experiment (Table 3). In order to gauge the contribution of oxidative metabolism to overall clearance, the same experiment was performed following the administration of the broad cytochrome P450 (CYP450) inhibitor aminobenzotriazole (ABT). In this instance, the acetate **8** illustrated a significant reduction in clearance while acrylamide **7** was still highly cleared, suggesting the presence of non-CYP450 clearance pathways. Indeed, glutathione-S-transferase (GST)-mediated glutathione (GSH) addition to the acrylamide was found to account for this discrepancy.[23] In order to build an SAR understanding of GST-mediated clearance, a whole blood stability assay was developed to provide an *in vitro* surrogate measure of whole body GST mediated clearance. The validation of this assay as a predictive tool has been described recently.[24]

Despite the poor PK of compound **7**, it was evaluated in an in vivo study utilizing the mouse Delayed Type Hypersensitivity (DTH) which is a T-cell dependent preclinical model that was utilized to

characterize **7**. In the prophylactic mouse DTH model, a Th1 response was induced by priming mice with sheep red blood cells (sRBC) subcutaneously on Day 0 and a challenge of sRBC was injected to the right hind footpads on Day 5 to elicit a measurable inflammatory response. Compound was dosed once daily throughout the study. **7** significantly inhibited the DTH reaction in the right hind footpad in a dosage-dependent manner, and efficacy was observed with dosages as low as 3 mg/kg twice daily. **1** was also efficacious with twice-daily dosing at 30 mg/kg in this DTH model (Figure 6). This provided our preliminary support that selective inhibition of JAK3 would result in in vivo functional consequences.

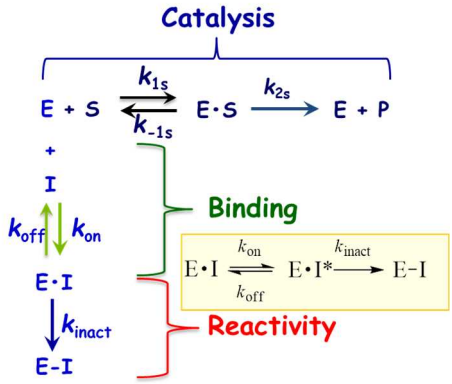
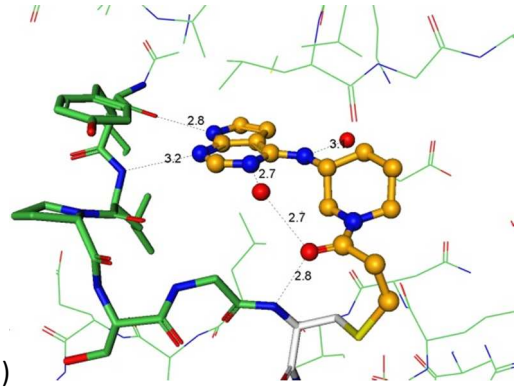
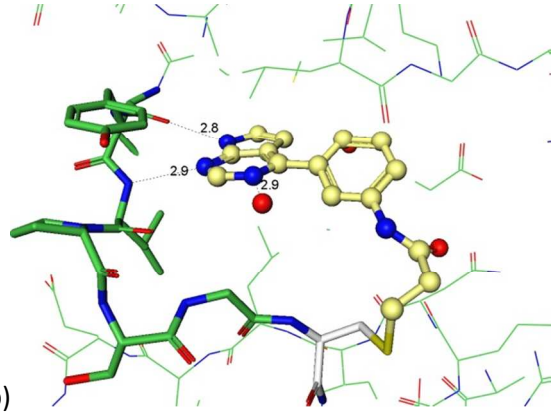


Figure 4. Illustration of individual binding and reactive events required for covalent modification of Cys-909 in JAK3. E = enzyme, S = substrate, I = inhibitor



5a)

5b)



5c)

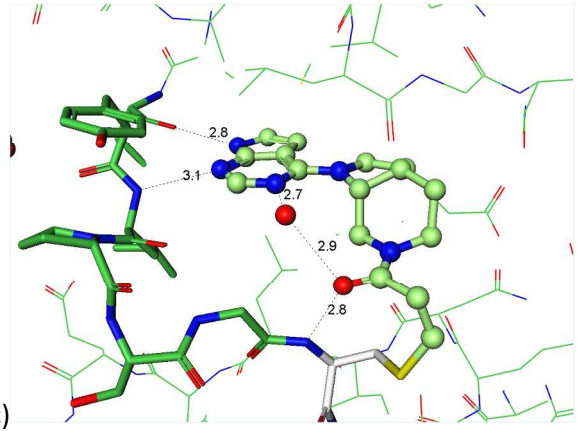


Figure 5. Crystal structures of compounds bound to JAK3 with a covalent bound to Cys-909 5a) **4** PDB code 5TTS, 5b) **6** PDB code 5TTV. 5c) 7 PDB code 5TTU

Table 2. ADME properties of JAK3 covalent inhibitors

Cmpd#	HLM (uL/min/mg)	RLM (uL/min/mg)	RRCK (10-6 cm/sec)	Human blood $T_{1/2}$ (min)	Rat blood $T_{1/2}$ (min)
4	8	<15	8	~332*	98
7	12.2	70	20	192	164
8	<8	25	16	NA	NA
11	8	<15	15	>360	161

*A broad range of stability was observed over multiple experiments (n=3 $T_{1/2}$ = >260, >360, 303

Table 3. In vivo rat pharmacoketic clearance of compound **7** and **8**, in the absence and presence of the broad based CYP450 inhibitor ABT

Cmpd#	7	8
CL (mL/min/kg) - ABT	109	76
CL (mL/min/kg) + ABT	81	10

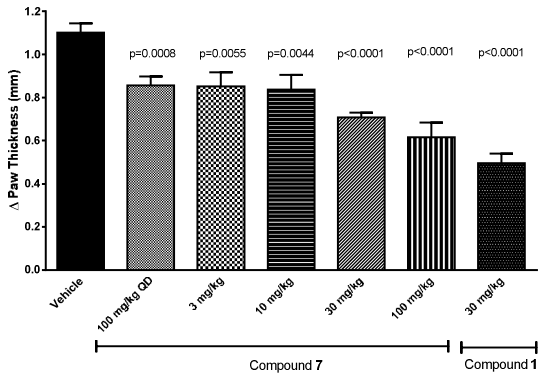


Figure 6. Efficacy of **7** Eight week old female BALB/c mice (Taconic) were sensitized with 2 x 10^7 sheep red blood cells (sRBC) subcutaneously on day 0. Oral dosing began on day 0 prior to the sensitization and continuing through day 6. The mice were challenged with 1 x 10^8 sRBC in the right hind foot pad and the left hind footpad received an injection of sterile PBS. Paw thickness was measured prior to the injection for a baseline measurement and then again at 24 hours via caliper. Paw thickness was significantly reduced in a dose-dependent manner compared to the mice that were dosed with the vehicle. All compounds were dosed twice daily except where otherwise noted.

Design of orally active JAK3 specific inhibitors. There is very limited knowledge regarding design strategies to modulate GST mediated clearance, and generally speaking, the GSTs are a class of enzymes that can tolerate a wide range of substrates.[25] Early on, we attempted to inhibit chemical reactivity by adding substituents to the acrylamide, as steric hindrance around the electrophile should reduce the ability of GSTs to catalyze GSH addition. This approach provided a significant reduction in GST-mediated clearance, but unfortunately, was not compatible with JAK3 affinity. For example, addition of a beta-methyl to the acrylamide moiety of **4** resulted in a nearly inactive analog **9** (IC_{50} > 1000 nM). An alternative approach was the incorporation of a remote steric handle to modulate GST binding; a strategy utilized in a related series of cyanamide JAK3 inhibitors.[26] This strategy was attempted with

analog **4** wherein methyl substitution was explored at various piperidine ring positions. A key initial analog was **10** (Table 5)**,** which showed enhanced blood stability relative to the desmethyl analog **4**, but unfortunately was poorly active against JAK3. Our rationale for the poor activity involved destabilization of the reactive conformation needed to capture the Cys-909 due to a potential steric clash with sidechain residue Leu-956 in the ATP pocket. Our modeling efforts indicated that moving the aminopyrrolopyrimidine group from the 2- to the 4-position would result in a highly favorable ground state conformation for interaction with Cys-909 without a steric clash to any residue in the ATP pocket of JAK3. In this conformation, we expected interaction of the acrylamide carbonyl with the NH of Cys-909 further stabilizing the reactive conformation and potentially activating the acrylamide for nucleophilic addition. This concept was realized in compound **11** which was found to be a highly specific inhibitor of JAK3 (IC_{50} 33 nM), devoid of relevant activity vs. other JAK enzymes at 1 mM ATP concentrations, and demonstrated by X-ray crystallography to be covalently attached to Cys-909.[27] The high *in vitro* potency of **11** translated to excellent activity illustrated in both cellular (IL-15 stimulated PBMCs, IC_{50} = 51 nM) and human whole blood assays (IL-15 stimulated HWB assay IC_{50} = 197 nM) (Table 1). The importance of free drug concentration of **11** driving JAK3 pharmacology was apparent following conversion of the whole blood IC_{50} to a free IC_{50} of 106 nM, which is within 2 fold of the PBMC IC_{50}. A comparable profile of inhibition for other common γ-chain cytokine signaling was observed, while exquisitely sparing the inhibition of cytokines which signal through other JAK kinase pairs.[20] The observed cellular functional potency correlated well with occupancy of the JAK3 enzyme by **11.**[28] Importantly, the placement of the 2-methyl moiety resulted in a stable compound as judged by human liver microsomes, hepatocytes and blood stability assays. *In vivo* pharmacokinetic studies demonstrated low clearance of compound **11** in rat and dog, but rapid clearance in monkey (relative to liver blood flow) and lower oral availability (Table 4). The pharmacokinetic profiles across species had a direct relationship to both stability in hepatocytes and blood, illustrating the value of applying both *in vitro*

tools to predict *in vivo* pharmacokinetic behavior. The high oral bioavailability of **11** in a number of species (rat/dog) was consistent with its high in vitro passive permeability (RRCK 15 x 10-6 cm/s), high aqueous solubility > 2 mg/mL), and low hepatic clearance. The metabolic clearance of **11** was determined to be mediated through both oxidative metabolism (acrylamide and ring oxidation primarily through CYP3A4) and glutathione conjugation (on the acrylamide). Renal and biliary clearance of **11** was low in rat. Through an understanding of clearance mechanisms and *in vitro* to *in vivo* correlations, human blood clearance of **11** was predicted to be approximately 5.6 mL/min/kg.[21] Human oral bioavailability was likewise predicted to be approximately 90% with a half-life of approximately 2 hrs. These results prompted a systematic evaluation of the impact of methyl substitution at other positions on the piperidine ring (Table 5). Analogs with ring substitution favoring the chair conformation observed in the **4**-JAK3 crystal structure typically place the electrophile in close proximity to Cys-909 and provide potency (**15** and **19**). The impact on GST-mediated clearance was more subtle as both enantiomer configuration (**14** vs. **12**) and relative stereochemical configuration (**11** vs **12**) had a dramatic impact on blood stability. This exercise enabled the identification of an additional analog (**19**) with both excellent potency (IC_{50} = 56 nM) and blood stability via a surprisingly remote steric effect.

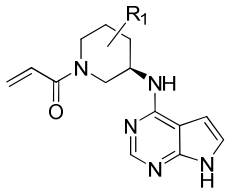
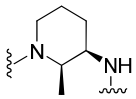
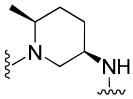
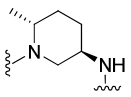
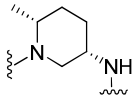
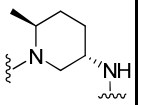
Table 4. In vitro and in vivo pharmacokinetic properties of **11** in various species

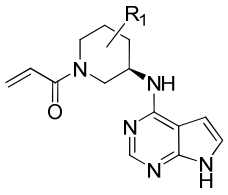
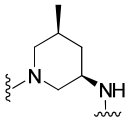
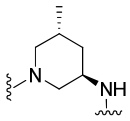
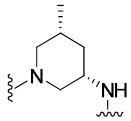
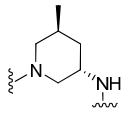
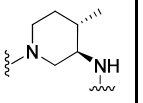
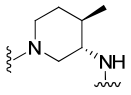
Species CL Assay	Mouse	Rat	Monkey	Dog	Human
Hepatocyte $CL_{int,u}$ (µl/min/million cells)	53	14	7.6	2.1	2.8
Blood $t_{1/2}$ (min)	>360	161	37	>360	>360
Observed In Vivo CL_b (mL/min/kg)	48	53	42	8.6	-
V_{ss} (L/kg)	0.8	1.4	2.6	1.1	1.3[a]
In Vivo Plasma $t_{1/2}$ (h)	1.3	0.3	0.7	1.1	1.8[b]
PO Bioavailability (%)	62	85	56	109	-

Species	Mouse	Rat	Monkey	Dog	Human
CL Assay					

CLint,u = Unbound intrinsic clearance; CLb = Blood clearance
[a] predicted from allometric scaling of unbound Vss
[b] predicted human half-life

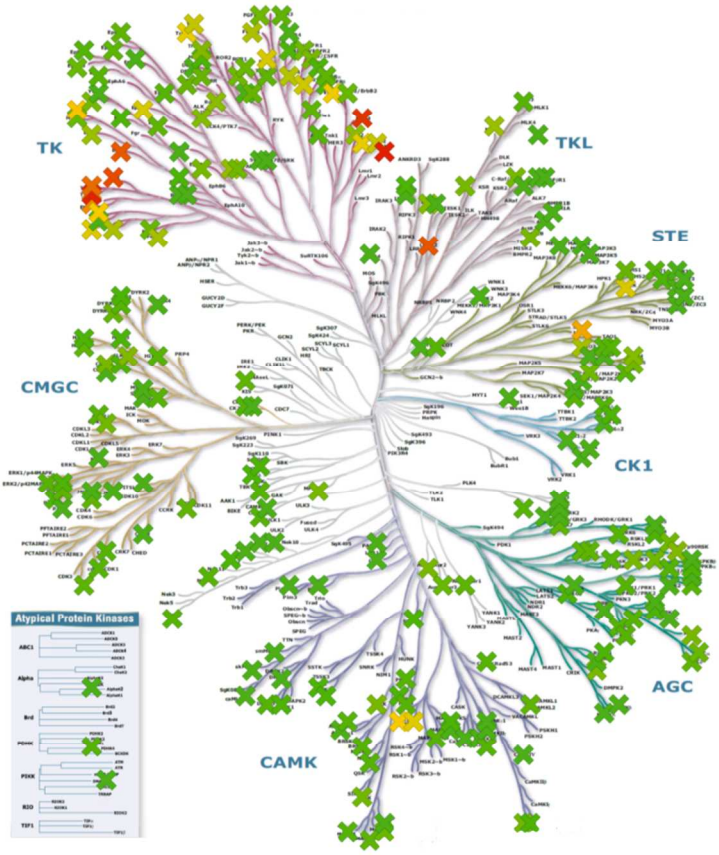
Table 5. Evaluation of remote small steric effect on JAK3 potency and GST stability

					
Cmpd#	**10**	**11**	**12**	**13**	**14**
J3 1mM IC50 (nM)	**>1000**	**33**	**>10,000**	**>2478**	**>10,000**
Hblood stab t1/2 (min)	**>360**	**>331**	**185**	**>360**	**>360**

						
Cmpd#	**15**	**16**	**17**	**18**	**19**	**20**
J3 1mM IC50 (nM)	**60**	**2021**	**>10,000**	**>10,000**	**56**	**7570**
Hblood stab t1/2 (min)	**>250**	**103**	**304**	**>360**	**>300**	**142**

Compound **11** demonstrated a high degree of selectivity vs. a panel of 305 kinases (Invitrogen, Figure 7). Approximately 200 kinases are known to contain a CYS residue within the ATP binding site.[29]

Within this set, 11 kinases contain a CYS at the same position as that found in JAK3. When evaluated against this subset, compound **11** demonstrated measurable activity against 8 of the targets. (Table 6). IC_{50} data generated for a subset of these targets utilizing 1mM ATP levels showed generally good selectivity vs. JAK3. The contribution of Ki and Kinact to activity was also determined for a subset of these targets. It is interesting to note that the Kinact contribution is the dominant feature with regard to JAK3 inhibition, illustrating how well-optimized the compound is for alignment of the electrophile, with rapid kinase inactivation by Cys-909 addition. In contrast, the inactivation of ITK is very slow and the majority of its affinity is associated with the Ki contribution. The very slow inactivation rate would require significant residence time for capture in a cellular environment with high ATP levels suggesting that **11** would be an ineffective inhibitor of ITK. In addition the utilization of strictly K_{inact}/K_i as a measure of selectivity ignores the differential impact competition with ATP has on functional outcome as seen when comparing BMX and TXK. We observed very weak affinity for three other kinases (SLK, FGR, and FLT3) containing CYS in the ATP site, and none of those kinases were inhibited in a covalent manner.



b)

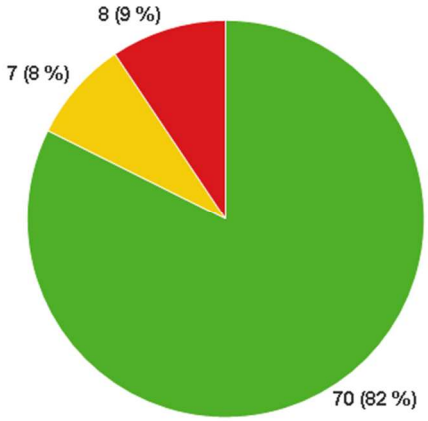


Figure 7. a) Kinome selectivity of **11** determined at 1 μM for 305 kinases determined at Invitrogen. The percent inhibition is color coded and ranges from 0% inhibition (darker green) to 100 % inhibition (darker red) with 50% inhibition displayed in yellow. The selectivity determined by Gini coefficient is 0.88 b) Kinases tested that contained CYS in the ATP site.

Table 6. Inactivation rates of **11** towards kinases with CYS at the same location as JAK3. IC_{50}'s were determined at Carna Bioscience.

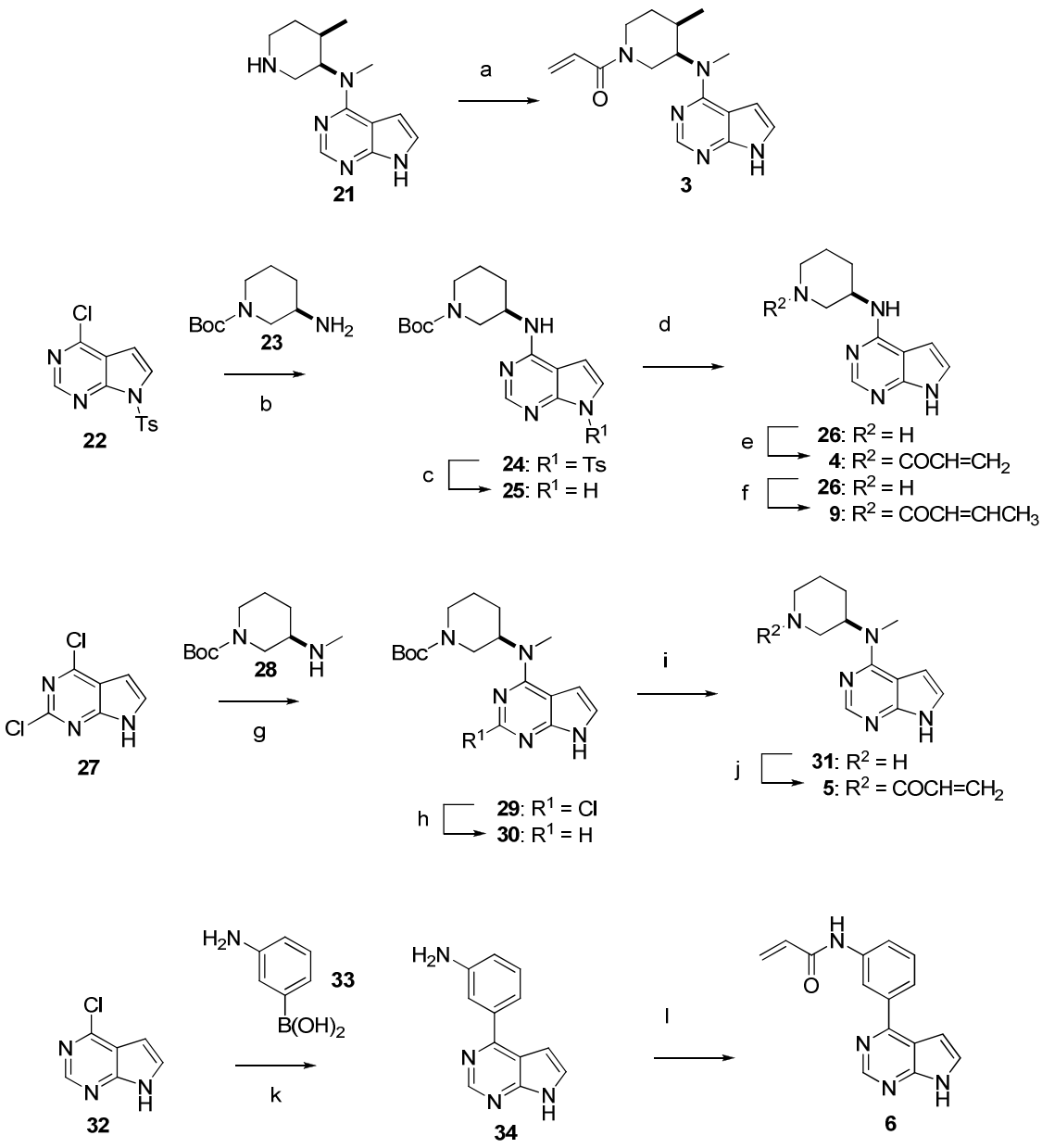
kinase	k_{inact} (s^{-1})	K_i (μM)	k_{inact}/K_i ($M^{-1}\cdot s^{-1}$)	IC50 (1mM ATP) units = nM
JAK3	2.32	6.31	**3.68E+05**	90
BMX	0.00564	0.545	**1.03E+04**	606
ITK	0.000144	0.0269	**5.35E+03**	8510
TXK	0.000487	0.131	**3.79E+03**	193
TEC	0.00156	0.679	**2.30E+03**	592
BTK	0.124	62.3	**1.99E+03**	607
BLK	0.0278	32.4	**8.58E+02**	19000
HER4	ND	ND	ND	25200
EGFR	ND	ND	ND	>50000
HER2	ND	ND	ND	>50000
MAP2K7	ND	ND	ND	>50000

Note: Given the experimental design [see Methods], exact solutions for individual kinetic parameters, K_i and k_{inact}, were obtained only for BMX, ITK, and TXK; the solutions reported for all other kinases are lower limits. For all kinases tested, the ratio (k_{inact}/K_i) is accurately determined.

The potent inhibition of *in vitro* cellular signaling demonstrated with **11** clearly illustrates that selective inhibition of JAK3 can effectively modulate signaling via the common γ-chain cytokine receptor which utilizes the heterodimeric JAK1/JAK3 pair. Furthermore, the high degree of efficacy reported for **11** and **7** in a variety of *in vivo* inflammatory disease models (AIA, DTH, CIA, and EAE) driven by gamma-chain signaling unambiguously demonstrates the therapeutic potential for selective JAK3 inhibition in a number of human diseases.[20] Based on these results, **11** has been selected for clinical evaluation.

CHEMISTRY. The synthesis of compounds **3, 4, 5, 6** and **9** are described in Scheme 1. Compound **3**, the acrylamide analog of tofactinib, was readily synthesized from the known amine, N-methyl-N-((3R,4R)-4-methylpiperidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine **21** (30) and acryloyl chloride in dichloromethane/DIPEA in 32% yield. Compound **4** was synthesized beginning from the known 4-chloro-7-tosyl-7*H*-pyrrolo[2,3-d]pyrimidine **22**[31] followed by S_{nAr} displacement of the chlorine with tert-butyl (R)-3-aminopiperidine-1-carboxylate **23** to give **24** in 42% yield.[32] The tosyl group at N-1 was subsequently removed under basic conditions ($LiOH*H_2O$, $THF:MeOH:H_2O$) to give **25** in 70% yield. Removal of the Boc group of **25** with 4N HCl/dioxane to give **26** and installation of the acrylamide (acryloyl chloride, TEA/DCM/EtOH) provided **4** in 33% yield. Similarly, compound **9** was synthesized using (E)-but-2-enoyl chloride in 32% yield. Compound **5** was prepared from 2,4-dichloro-7H-pyrrolo[2,3-d]pyrimidine **27** followed by S_{nAr} displacement of the 4-Cl with (R)-tert-butyl 3-(methylamino)piperidine-1-carboxylate to give compound **29** in 60% yield. Subsequent reduction of the 2-Cl gave **29**, which was treated with acid to give 30. The secondary amine of **30** was then acylated with acryloyl chloride to give compound **5**. Finally, compound **6** was synthesized via Suzuki coupling (33) of 4-chloro-7H-pyrrolo[2,3-d]pyrimidine **32** with (3-aminophenyl)boronic acid **33** under standard conditions ($Pd(PPh_3)_4$, Na_2CO_3, $THF:H_2O$) to provide intermediate **34** in 50% yield, followed by acylation of the anilino nitrogen with acryloyl chloride (THF/TEA) to give **6** in 20% isolated yield.

Scheme 1. Synthesis of Acrylamides **3, 4, 5, 6** and **9**

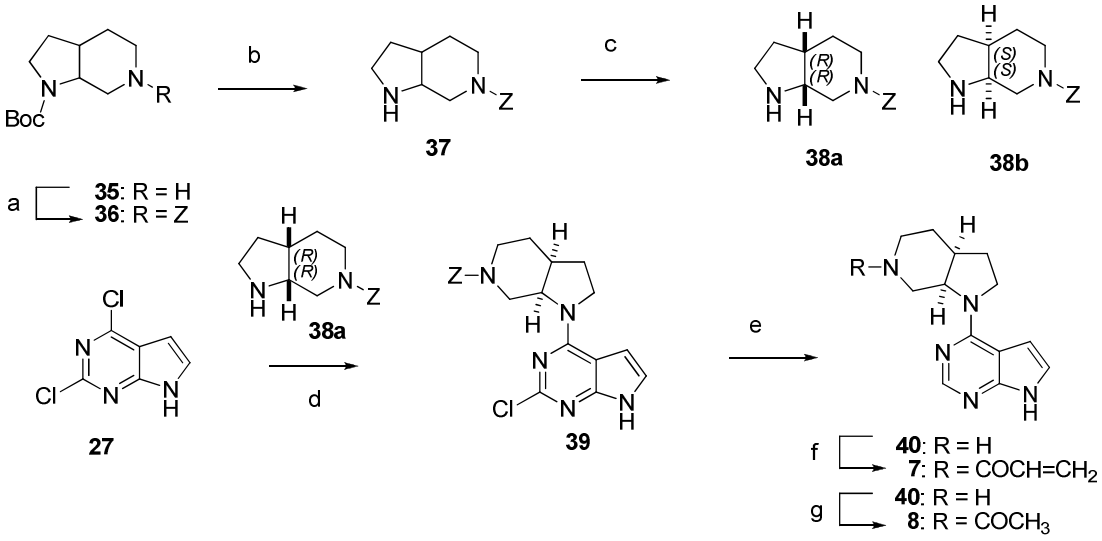


[a]Reagents and conditions: (a) 1.1 equiv of acryloyl chloride, 1.3 equiv TEA, DCM, 0 °C, 1 hr, 32%;(b) 1.2 equiv **23**, 1.2 equiv DIPEA, ^{n}BuOH, 70 °C, 14 hr, 42%; (c) 2 equiv LiOH*H_2O, THF:MeOH:H_2O (1:1:1), 60 °C, 1 hr, 70%; (d) 4N HCl/dioxane, rt, 92%; (e) 1.0 equiv acryloyl chloride, DCM:EtOH (10:1), 4.4 equiv TEA, 0 °C, 2 hr, 33%; (f) 1.0 equiv (E)-but-2-enoyl chloride, DCM:EtOH (10:1), 4.4 equiv TEA, 0 °C, 2 hr, 38%; (g) 1.0 equiv of **28**, 5.0 equiv of DIPEA, ^{n}BuOH, 130 °C, 60%; (h) 10% Pd/C, MeOH:THF (~2:1), 50 psi, H_2, 48 hrs, 100%; (i) TFA, CH_2Cl_2, 0 °C, 2 hrs, 76%; (j) 0.86 equiv of acrylogyl chloride, THF:H_2O, $NaHCO_3$, 5 °C, 1 hr, 14%; (k) 1.2 equiv **32**, 3.0 equiv K_2CO_3, dioxane:H_2O (5:1), 0.1 equiv $Pd(PPh_3)_4$, 110 °C, 16 hr, 50%; (l) 1.0 equiv of acryloyl chloride, THF, 4.0 equiv TEA, 0 °C, 2 hr, 20%

The synthesis of compounds **7** and **8** are described in Scheme 2. The racemic tert-butyl octahydro-1H-pyrrolo[2,3-c]pyridine-1-carboxylate amine **35** was treated with Cbz-Cl to give **36** in 90% yield followed

by removal of the Boc group to give intermediate **37** in 85% yield. The racemic (cis-fused) compound **37** was separated by chiral super critical fluid chromatography (SFC) to give the enantiomeric pair of (**38a** and **38b**). The absolute stereochemistry of **38b** was determined by X-ray crystallography (see Supporting Information). Nucleophilic aromatic substitution of the more activated chlorine at the 4-position of 2,4-dichloro-7H-pyrrolo[2,3-d]pyrimidine **27** with compound **38a** (R,R) provided **39** in 80% yield. Reduction of the Cbz group and 2-chloro with 10% Pd/C-H_2 provided compound **40** in 90% yield. Final installation of the acrylamide was accomplished using aqueous basic conditions and acryloyl chloride to give analog **7** in 81% yield. Compound **8** was prepared in a similar manner, except using acetyl chloride.

Scheme 2. Synthesis of compound **7** and **8**

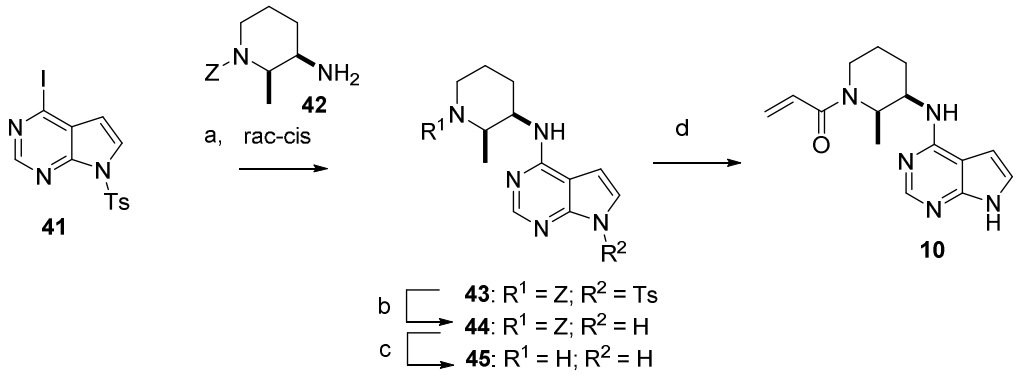


Reagents and conditions: (a) 2.2 equiv DIPEA, 1.2 equiv CbzCl, DCM, 45 min, 90%; (b) 4N HCl/dioxane, rt, 15 hr, 85%; (c) SFC purification, AS 250x50, 10 μm, CO_2/EtOH (0.05%DEA); (d) 1.1 equiv **38a**, 4.5 equiv DIPEA, ^{n}BuOH, 80 ^{o}C, 3 hr, 80%; (e) 10 mol% 10%Pd/C, EtOH, 50 psi H_2, 65 ^{o}C, 48 hr, 90%; (f) 1.1 equiv of acryloyl chloride, 3.3 equiv of $NaHCO_3$, H_2O, CH_3CN, 0 ^{o}C, 81%; (g) 1.1 equiv of AcCl, 5.1 equiv of DIPEA, DCM, 0 ^{o}C, 74%.

For compound **10**, the synthesis was accomplished as described in Scheme 3. 4-iodo-7-tosyl-7H-pyrrolo[2,3-d]pyrimidine **41** and racemic benzyl (2R,3R)-3-amino-2-methylpiperidine-1-carboxylate **42**

were reacted under S_{nAr} conditions to give **43,** which was then treated with base to effect tosyl group removal to give **44** in 91% yield. The Cbz group was removed under transfer hydrogenation conditions ($Pd(OH)_2$/cyclohexadiene) to give the free piperidine **45**, which was subsequently acylated (acryloyl chloride/DCM/DIPEA) to give **10** in 71% yield.

Scheme 3. Synthesis of compound **10**

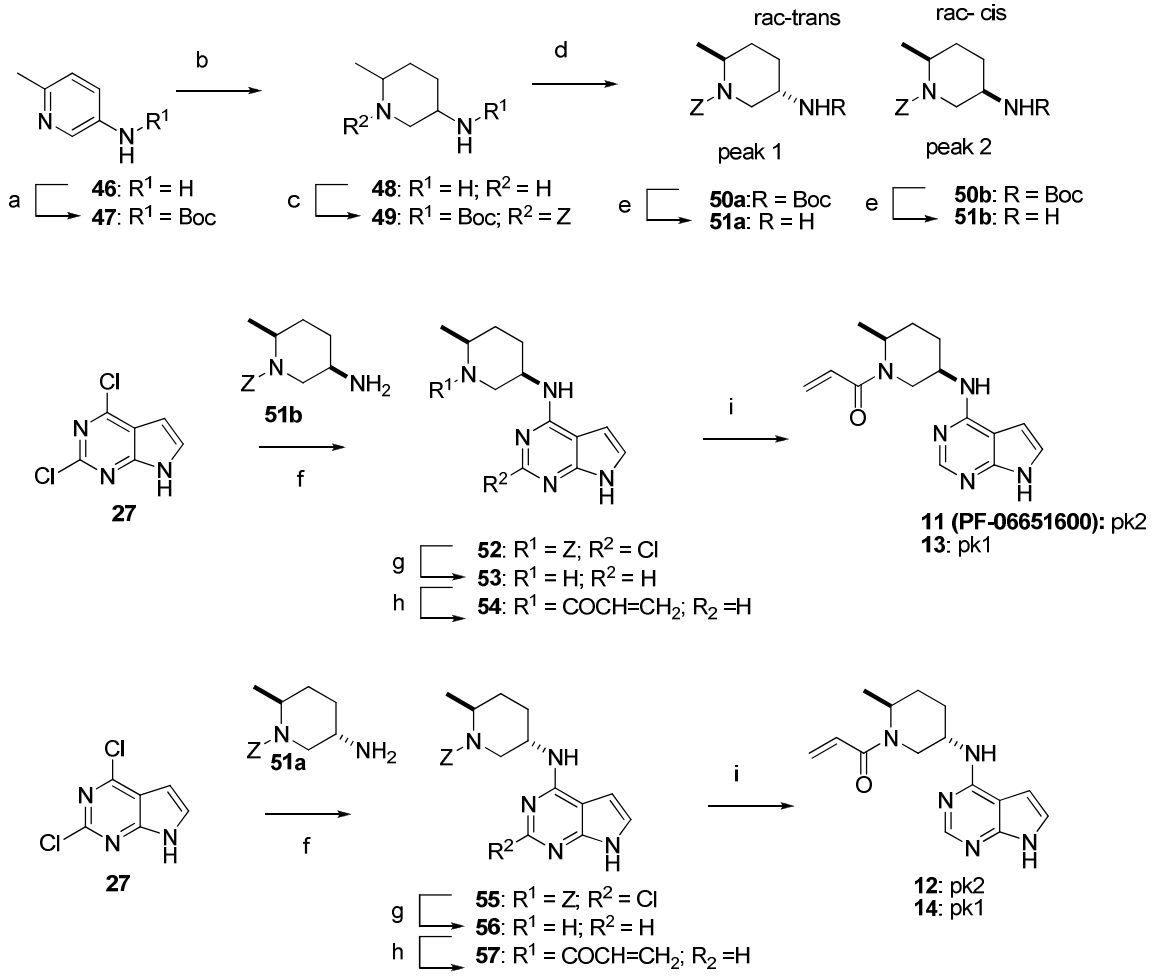


Reagents and conditions: (a) 1.1 equiv **42**, 4.0 equiv DIPEA, ^{n}BuOH, 85 ^{o}C, 18 hr, 36%; (b) 3.0 equiv LiOH*H_2O, MeOH:H_2O (3:1), 60 ^{o}C, 1.5 h, 91%; (c) 1.0 equiv $Pd(OH)_2$, MeOH, 40 equiv cyclohexadiene, 14 hr, 52%; (d) 1.1 equiv acryloyl chloride, DCM, 5.0 equiv DIPEA, DCM, 0 ^{o}C, 2 hr, 71%

The synthesis of examples **11**-**14** are described in Scheme 4. Beginning from 6-methylpyridin-3-amine **46** the free amino group was protected as the Boc derivative to give pyridine **47**, which was then reduced using PtO_2/H_2/HOAc at 50 ^{o}C to give piperidine **48** as a mixture of diastereomers (~2:1 cis/trans) . The piperidine nitrogen of the diastereomeric mixture **48** was subsequently protected as the Cbz carbamate to give **49** as a diastereomeric mixture. Compound **49** was separated into its diasteromeric pair using chiral SFC to give compound **50a** (rac-trans, pk1) and compound **50b** (rac-cis, pk2). The Boc groups of **50a** and **50b** were removed under standard conditions to give **51a** (rac-trans) and **51b** (rac-cis). For compounds **11** and **12**, the cis isomer **51b** was reacted with **38** to give intermediate **52** (rac-cis), which was then subjected to reduction conditions to give **53**. Compound **53** was then acylated to provide compound **54** as a rac-cis mixture. The racemic mixture was separated by chiral SFC to provide

the enantiomeric pair **11** and **13**. The preparation of compounds **12** and **14** was similarly accomplished, except **51b** (rac-trans) was utilized via intermediates **55**-**57** with chiral SFC of **57** (rac-trans) to give the enantiomeric pair **12** and **14**.

Scheme 4. Synthesis of compounds **11-14**

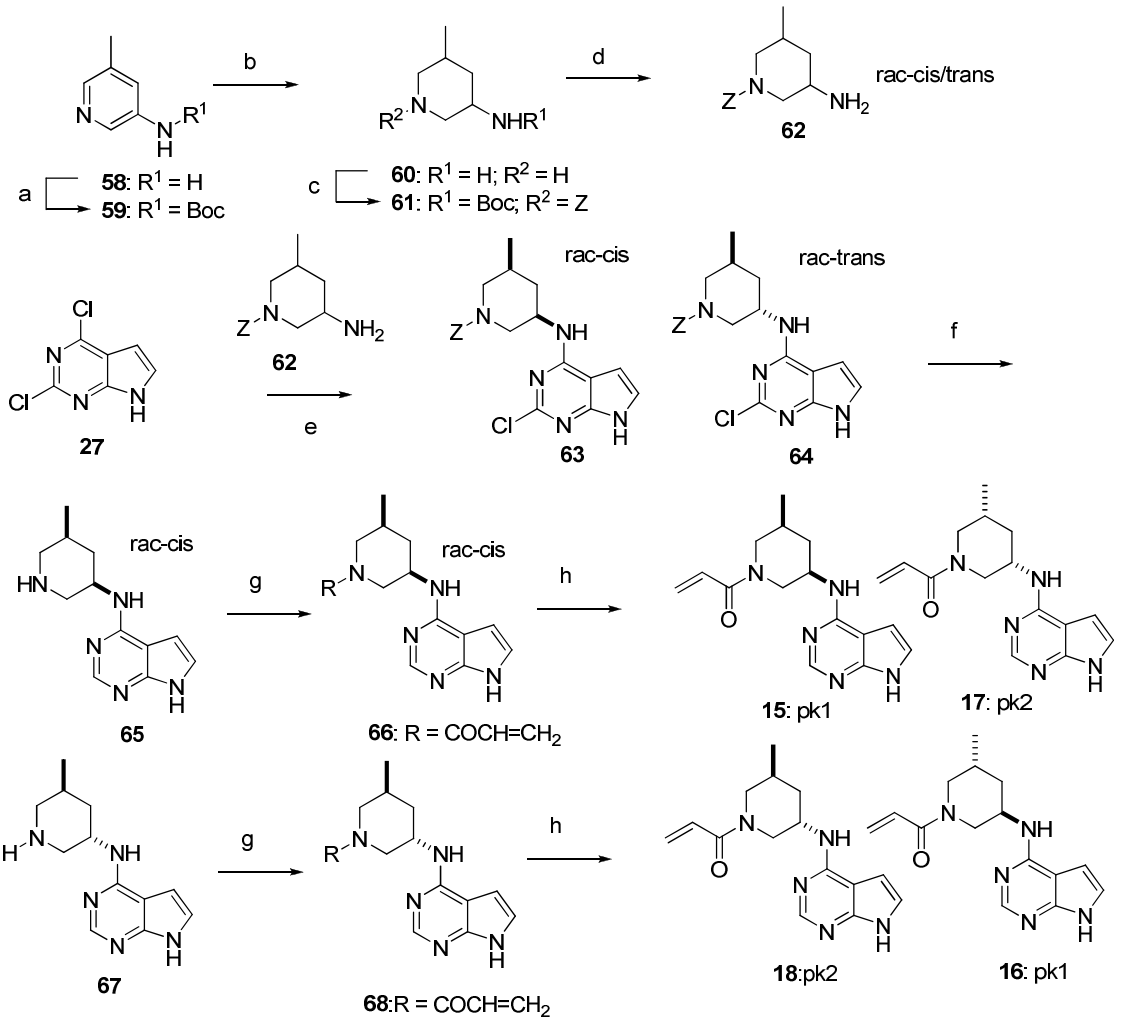


Reagents and conditions: (a) 1.3 equiv Boc_2O, EtOH, 0 °C, 14 hr, 67%; (b) 10 mol% PtO_2, HOAc, 55 psi H_2, 50 °C, 100%, ~ 2:1 cis/trans; (c) 1.5 eqiv CbzCl, 7.0 equiv $NaHCO_3$, THF/H_2O (1:1), 100%; (d) Chiral SFC, ChiralCel OD 300 mm x 50 mm, 10 um, CO_2/IPA (0.1%NH_3); (e) 4N HCl/dioxane, DCM, rt; (f) 1.1 equiv **51b** or **51a**, 5.0 equiv DIPEA, ^{n}BuOH, 140 °C, 14 hr, 86%; (g) 10 mol% 10%Pd/C, MeOH/THF (6:1), 45 psi H_2, 25 °C, 48 hr, 95%; (h) 1.2 equiv of acryloyl chloride, satd. $NaHCO_3$, THF, 0 °C, 38%; (i) Chiral SFC, ChiralPak IC 300 mm*50mm, 10 μm, CO_2/EtOH (0.05% NH_3), 45%

For compounds **15**-**18** the headpiece amine was synthesized as described in Scheme 5, similar to the piperidine amine **49** utilized for compounds **11**-**14** (Scheme 4), but utilizing 5-methylpyridin-3-amine as the pyridine precursor. Unlike the 2-methyl amines, the 3-methyl amine derivatives **61** and **62** were inseparable by chromatographic methods and therefore were carried on as a rac-cis/trans mixture **62**, with the potential for separation further along the synthesis. Indeed SnAR reaction of (**62**, rac-cis/trans) with **38** provided a separable mixture, providing **63** and **64**. Intermediate **63** (rac-cis) was then taken

forward and the Cbz group and Cl removed to give **65** and the acrylamide installed to give **66** (rac- cis). Chiral SFC of **66** provided the enantiomeric pair **15** and **17**. Similarly, **64** (rac-trans) was taken forward via intermediates **67** and **68** and final chiral SFC to provide the enantiomeric pair **18** and **16**.

Scheme 5. Synthesis of compounds **15**-**18**

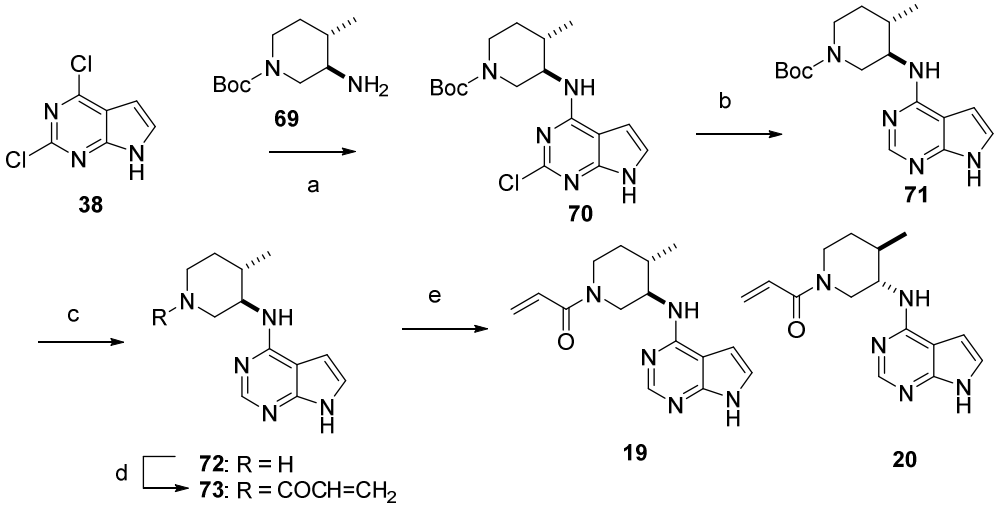


Reagents and conditions: (a) 1.1 equiv Boc_2O, THF, rt, 5 hr, 69%; (b) 10 mol% PtO_2, HOAc, 5 d, 55 psi H_2, 50 °C, 100%, mixture cis/trans; (c) 1.2 equiv CbzCl, 3.8 equiv $NaHCO_3$, rt, THF/H_2O (2:1), 8 h, 45%; (d) 4N HCl/dioxane, DCM, rt, 43%; (e) 1.1 equiv **61ab**, 4.0 equiv DIPEA, ^{n}BuOH, 140 °C, 30 hr, 34%(cis) and 10% (trans); (f) 10 mol% 10%Pd/C, MeOH:THF (3:1), 50 psi H_2, 40 °C, 3 d, 100%; (g) 1.1 equiv of acryloyl chloride, satd. $NaHCO_3$, THF, 0 °C, 2 hr, 71% (for **64**); 50% (for **66**); (h) Chiral SFC, AD 250 mm*30 mm, 20 μm, CO_2/MeOH (0.05% NH_3), 45% (for **15** and **17**); 40% (for **18** and **16**)

Finally, the synthesis of compounds **19** and **20** is described in Scheme 6. The pyrrolopyrimidine **38** and racemic tert-butyl (3R,4S)-3-amino-4-methylpiperidine-1-carboxylate **69** were reacted under SnAR

conditions to give compound **70** in 66% yield. The chlorine of **70** was reduced under catalytic hydrogenation conditions to give **71** (93% yield), which was then subjected to acidic conditions (4N HCl/dioxane) to effect removal of the Boc group and provide **72**. Treatment of **72** with acryloyl chloride under aqueous basic conditions gave acrylamide **73** in 34% yield. The racemic acrylamide **73** was separated by chiral SFC to provide the enantiomeric pair, **19** and **20**.

Scheme 6. Synthesis of Compounds **19** and **20**



Reagents and conditions: (a) 1.1 equiv **69**, 3.0 equiv DIPEA, ^{n}BuOH, 140 ^{o}C, 24 hr, 66%; (b) 10 mol% 10%Pd/C, MeOH:THF (3:1), 50 psi H_2, 40 ^{o}C, 2 d, 93%; (c) 4N HCl/dioxane, 0 ^{o}C, 100%; (d) 1.1 equiv of acryloyl chloride, satd. $NaHCO_3$, THF, 0 ^{o}C, 2 hr, 34%; (e) Chiral SFC, AD 250 mm*30 mm, 5 μm, CO_2/EtOH (0.05% NH_3), 45% (for **19** and **20**).

Conclusion

In summary we have disclosed the design of the first specific orally active JAK3 inhibitor in this report. The ultimate selection of **11** illustrated how to improve Kinact without an increase in chemical reactivity. Potency was coupled with minimizing GST mediated clearance by utilizing a remote steric effect through appropriate substitution of a methyl group on the piperdine ring. High metabolic stability resulted in an overall profile that made **11** suitable for oral dosing. The compound has exceptionally low reactivity for other proteins containing cysteine as judged by kinome, HSA and human liver hepatocyte profiling illustrating its specific design for inhibition of JAK3. This compound has been utilized to

illustrate the consequences on JAK3 inhibition in a range of *in vitro* and *in vivo* systems once for all settling the debate that it is sufficient to inhibit JAK3 only as the partner in the heterodimeric pair of JAK1/JAK3 kinase mediated cytokine signaling though the γ-common chain receptors. **11** is currently in clinical development for potential applications in inflammatory disease.

Experimental Section

The JAK enzyme data and the PBMC data was obtained as previously described.[7]

Stability of JAK3 Covalent Inhibitors in Rat and Human Whole Blood

All animal experiments were performed following protocols reviewed and approved by Pfizer Institutional Animal Care and Use Committee. Pfizer facilitites that supported this work are accredited by AAALAC International. Rat blood was collected from 3 male Sprague-Dawley rats (200-250g, Charles River Laboratories) and pooled for each study. Human blood was collected from one male and one female healthy subject at the Occupational Health & Wellness Center at Pfizer, Groton, CT and pooled for each study. Both rat and human blood was collected freshly into K2-EDTA tubes and kept on ice. An aliquot of the blood was transferred to microtubes and pre-warmed for 10 min at 370C using a heat block. The test compound was then added (1 µM final concentration) and the incubation was continued for 180 min at 37 0C in duplicates. An aliquot of the incubation mixture was removed at designated time points during the course of the incubation, mixed with an aliquot of acetonitrile containing an internal standard, vortexed and centrifuged. The resulting supernatants were removed and subjected to LC-MS/MS analyses to determine parent compound concentrations. Peak area ratios of the parent compound vs the internal standard were used to determine the % of parent compound remaining vs incubation time.

HWB IL-15 induced STAT5 phosphorylation Assay

After serial dilution of the test compounds 1:2 in DMSO at desired concentration (500X of final), the compounds were further diluted in PBS (by adding 4 µL compound/DMSO in 96 µL PBS, [DMSO]=4%, 20X final). To 96-well polypropylene plates were added 90 µl HWB (heparin treated Human Whole Blood)/well, followed by 5 µl/well 4% DMSO in D-PBS or various concentrations of 20X inhibitor in 4% DMSO in D-PBS (w/o Ca+2 or Mg+2) to give 1X in 0.2% DMSO. After mixing and incubating for 45 minutes at 37ºC, 5 µl D-PBS (unstimulated control) or 20X stocks of 5µl human IL-15 (final concentration is 50 ng/ml) were added, and mixed three times. After incubating 15 minutes at 37ºC, 1X Lyse/Fix Buffer (BD Phosflow 5x Lyse/Fix Buffer) was added to all wells at 1000 µl/well, then incubated for 20 minutes at 37 ºC and spun 5 mins at 1200 rpm. After washing in 1000 µl FACS buffer 1X and spinning for 5mins at 1200 rpm, 400 µl ice cold Perm Buffer III were added to each well. After mixing gently (1-2X) and incubating on ice for 30 minutes, spinning for 5 mins at 1200 rpm without interruption, and washing 1X in cold 1000 ml FACS buffer (D-PBS containing 0.1 % BSA and 0.1% sodium azide) 250 µl/well of the desired AlexaFluor647-conjugated anti-phospho STAT5 antibody at 1:125 dilution in FACS buffer was added. Following incubating at 4 ºC over night, all the samples were transferred to 96-well polypropylene U-bottom plate, and checked by flow cytometry gated on total lymphocytes. IC50 values obtained are listed in the Table.

TR-FRET Inhibitor Binding and Kinase Inactivation (k_{inact}/K_i) Assay.

The kinetics of **11** binding to and inactivation of JAK3 and a set of possible off-target kinases was measured using a time-resolved Förster resonance energy transfer (TR-FRET) assay based on the LanthaScreen® Kinase Binding Assay (Invitrogen/Life Technologies, Carlsbad, CA). The assay system employs three components: (1) a recombinant, epitope-tagged kinase, (2) a europium-labeled, anti-

epitope antibody (Eu-Ab), and (3) a kinase-specific Alexa Fluor®-conjugated probe. A TR-FRET signal is generated between the Eu-Ab·kinase conjugate pair and probe when the probe is bound to the kinase.

Assay buffer was 20 mM HEPES, pH 7.5, 10 mM $MgCl_2$, 0.01% BSA, 1 mM DTT, 0.0005% Tween 20, and 2% DMSO. Serial dilutions of **11** were prepared at 100X final concentration in 100% DMSO, from which 4X **11** solutions were prepared by dilution into assay buffer (at 4% DMSO, no DTT) immediately prior to the start of the experiment. Experiments were performed in a Corning, low volume NBS, black 384-well plate (Corning Cat. #3676). For each kinase, the standard procedure is to run multiple experiments at various compound concentrations and employing various pre-incubation times intended to measure the time-dependence of covalent adduct formation.

Two different procedures to measure the kinetics of **11** dissociation (off-rate assay) from JAK3 were employed: In brief, the kinase is first pre-incubated with compound for a measured time, after which an appropriate off-rate probe is added. In the first procedure, 5 µL of a 4X solution of **11** was combined with 10 µL of a 2X solution of (final concentrations): 2 nM Eu-Ab, and 2.85 nM of JAK3-GST. This solution was allowed to pre-incubate for a variable amount of time (between 30 s or 120 min), after which 5 µL of 4X probe (150 nM, final concentration) was added. In the second procedure, a 2X solution (10 µL) containing 28.9 nM JAK3-GST and 20 nM anti-GST Eu-Ab (both are final concentrations) was mixed with 10 µL of 2X **11**: 0 – 1.5 µM (final concentration during pre-incubation time). Samples were manually mixed in individual 1.5 mL tubes and allowed to pre-incubate for 10, 20 or 60 s, at which time, 2 µL aliquots were quenched by addition of 20 µL of 148 nM (final concentration) probe KT236 (Invitrogen PV5592) in 2% DMSO. The approximate 10-fold dilution of compound greatly reduces the amount of covalent adduct formation during the pre-steady state period after probe addition, which enables higher kinetic resolution of the assay. The quenched samples exhibited stable TR-FRET signals for well over an

hour due to the very slow dissociation kinetics of the off-rate probe. These samples were then transferred to a black 384-well plate (Corning Cat. #3676), and data was collected using an EnVision plate reader as above.

To measure the kinetics of **11** binding to and/or inactivation (on-rate assay) of a target kinase, a 1.33X solution of (final concentrations) 2 nM Eu-Ab, 1-8 nM kinase (detailed below) and 150 nM of the appropriate on-rate probe was prepared and allowed to incubate for 1 h before addition of 15 µL of this solution to 5 µL of a 4X solution of **11**.

The assays were read using an EnVision plate reader (Perkin Elmer, Waltham, MA). The excitation wavelength was 340 nm, and the output monitored was the emission ratio, calculated by dividing the signal from the emission peak of the probe (665 nm) by that of the europium (615 nm). Measurements were taken every 120 s for 1.5 h.

Kinase-Specific Inhibitor Binding and Kinase Inactivation Experimental Conditions.

JAK3

JAK3-GST: human protein, GST-tagged, catalytic domain (amino acids 781-1124; Life Technologies cat. no. PR7507B) was used at 2.14 nM final concentration in the standard assay, as defined above. The conjugate antibody used was Eu-anti-GST Ab (Life Technologies catalog no. PV5594). The on-rate probe used was KT178 (Life Technologies catalog no. PV5593), K_d = 150 nM. The off-rate probe used was KT236 (Life Technologies catalog no. PV5592), K_d = 0.1 nM. The following experiments were performed: (**A**) Standard on-rate assay using 2.85 nM JAK3, and [**11**] = 0, 0.49, 1.48, 4.44, 13.3, and 40 nM. (**B**) Standard off-rate assay using 2.85 nM JAK3, and [**11**] = 0, 0.66, 1.98, 5.93, 17.8, 53.3 nM. The following

experiments were performed following the standard protocol: [**11**] = 0, 0.66, 1.98, 5.93, 17.8, 53.3, 160, 480 nM; pre-incubation time was (**C**) 30 s, (**D**) 60 s, and (**E**) 1.5 h. The following experiments were performed using the variation of the off-rate protocol: During compound pre-incubation, [JAK3] was 28.9 nM, and [**11**] = 0, 0.0185, 0.056, 0.1667, 0.5, 1.5 μM; pre-incubation time was (**F**) 10 s, (**G**) 20 s, and (**H**) 60 s.

Possible off-target kinases

The kinases BTK, BLK, BMX, ITK, and TEC contain a Cys residue at the H10 position, and thus were tested as possible off-targets for **11** using the TR-FRET assay. The following combinations of enzyme, conjugate antibody, and probe were employed: *BLK*: His-tagged BLK, human full-length protein (Life Technologies cat. no. PV3683) was used at 5.75 nM final concentration in the standard assay, as defined above. The conjugate antibody system used was Eu-streptavidin (Life Technologies catalog no. PV5899) and biotin-anti-His Ab (Life Technologies catalog no. PV6089). The off-rate probe used was KT236 (Life Technologies catalog no. PV5592), K_d = 40 nM. *BTK*: human, His-tagged BTK, full length protein (Life Technologies cat. no. P3363) was used at 1.26 nM final concentration in the standard assay, as defined above. The conjugate antibody used was Eu-anti-His Ab (Life Technologies catalog no. PV6089). The off-rate probe used was KT236 (Life Technologies catalog no. PV5592), K_d = 30.5 nM. *BMX*: His-tagged, human full-length BMX protein (Life Technologies cat. no. PV3371) was used at 2.0 nM final concentration in the standard assay, as defined above. The conjugate antibody system used was Eu-streptavidin (Life Technologies catalog no. PV5899) and biotin-anti-His Ab (Life Technologies catalog no. PV6089). The off-rate probe used was KT236 (Life Technologies catalog no. PV5592), K_d = 74 nM. *ITK*: GST-tagged ITK, human full-length protein (Life Technologies cat. no. PV3875) was used at 1.82 nM final concentration in the standard assay, as defined above. The conjugate antibody used was Eu-anti-GST Ab (Life Technologies catalog no. PV5594). The off-rate probe used was KT236 (Life Technologies catalog

no. PV5592), K_d = 12.8 nM. *TEC*: His-tagged TEC, human full length protein (Life Technologies cat. no. P3041) was used at 7.4 nM final concentration in the standard assay, as defined above. The conjugate antibody used was Eu-anti-His Ab (Life Technologies catalog no. PV6089). The off-rate probe used was KT178 (Life Technologies catalog no. PV5593), K_d = 1.0 nM. In all cases, the experiments were performed following the standard protocol: (**A**) [**11**] = 0, 4.9, 14.8, 44.4, 133.3, and 400 nM; pre-incubation time = 2 h. (**B**) [**11**] = 0, 0.5, 1.0, 2.0, 4.0, and 8.0 μM; pre-incubation time = 120 s.

Irreversible Inhibitor Binding (k_{inact}/K_i) Data Analysis.

All data pertaining to a specific kinase were simultaneously analyzed by the global fitting software, KinTek Explorer (34). This analysis involves computationally fitting TR-FRET data as a function of both the concentrations of the reaction components (kinase, **11**, and probe) and time to a defined kinetic mechanism (steps 1 – 4, below). The computational process involves numerical integration of multiple differential equations that define the component processes to generate simulated data; the values of the kinetic rate constants are iteratively varied until a best fit between simulated and actual data are found.

Step 1. $E + I = E{\cdot}I$

Step 2. $E{\cdot}I \rightarrow E{\cdot}I^*$

Step 3. $E + probe1 = E{\cdot}probe1$

Step 4. $E + probe2 = E{\cdot}probe2$

The "observable" statement, used by the program to generate simulated data during data fitting, was:

$$scale1*(a*[\text{E·probe1}] + b*[\text{probe1}]) + bkg$$

The parameters, *a* and *b*, are the intrinsic molar TR-FRET signals of a particular enzyme-probe complex and free probe, respectively; *bkg* is the background signal; and *scale1* is a scale factor that is necessary for simultaneous fitting of data sets collected at different probe and/or Eu-Ab concentrations. The terms with square brackets indicate concentration of the indicated species.

Note: for the JAK3, TEC, BTK, and BLK data sets, even though the aggregate parameter (k_{inact}/K_i) was accurately determined, only lower limits for the *individual parameters* (k_{inact} and K_i) could be determined. This limitation is imposed by the kinetics of the system (k_{inact}) and the time scale over which the kinetic data were measured (the minimum "dead time" in our experiments was on the order of 10 – 60 s required to manually prepare the sample and start data collection). The extent to which the kinetic parameters are well determined by global fitting can be empirically defined by "confidence contour analysis" as described in reference.[35]

Biochemical Inactivation Kinetic Assay of TXK Kinase.

Recombinant TXK kinase (N-terminal GST-fusion protein, Carna Biosciences, Kobe, Japan) failed to produce a suitable TR-FRET signal in combination with anti-GST Eu-antibody (Life Technologies) and probe. Thus, an alternative approach was taken to measure k_{inact}/K_i, utilizing the classic pyruvate kinase/lactate dehydrogenase (PK/LDH) coupled enzyme assay. In the PK/LDH assay, ADP, which is a product of the kinase reaction, is measured by coupling its production first to the dephosphorylation of phosphoenolpyruvate (PEP) to form pyruvate, which is coupled to NADH-dependent reduction of

pyruvate to form lactate. The concomitant oxidation of NADH to form NAD^+ is monitored spectrophotometrically by loss of absorbance at 340 nm.

Assay Protocol: The TXK kinase buffer was: 50 mM HEPES, pH 7.5, 10 mM Mg_2Cl, 0.01% Triton X-100, 1 mM DTT, and 1% DMSO. Also included (PK/LDH assay reagents): 0.25 mM NADH (Sigma, N8129), 2.5 mM phosphoenolpyruvate (PEP) (Sigma, P0564), 12 U/mL pyruvate kinase (PK) and 18 U/mL lactate dehydrogenase (LDH) (Sigma P0294). Final substrate concentrations were 100 µM each ATP and Srctide peptide (sequence: GEPLYWSFPAKKK, AnaSpec, Inc., Fremont, CA). Serial dilutions of **11** were prepared at 100X final concentration in 100% DMSO, from which 4X **11** solutions were prepared by dilution into assay buffer (at 4% DMSO, no DTT) immediately prior to the start of the experiment. Experiments were performed in a Corning, 384-well black with clear bottom plate (Corning Cat. #3544). In each experiment, 10 µL of **11** solution (concentration during pre-incubation: 0, 10.4167, 20.83, 41.67, 83.3, 333.3, 666.7, 1333.3, 2666.7 nM) was combined with 20 µL of solution containing TXK kinase (34 nM concentration during pre-incubation) + PK/LDH solution reagents. After a variably timed pre-incubation period (15 min, 30 min, 1 h, 1.5 h, and 2 h), 10 µL of 4X ATP/peptide substrate was added to initiate the reaction. The reaction was monitored by absorbance (340 nm) every 60 s for 1 h with an EnVision plate reader (Perkin Elmer, Waltham, MA).

Data Analysis for TXK Inactivation Kinetics.

The absorbance data were converted to concentration data using the *apparent* difference molar absorptivity value of 0.00413/µM product formed, which was empirically determined using a calibration curve prepared from a standard solution of NADH, measured using an EnVision plate reader under identical condition as the enzyme assay. The primary absorbance (A_{340}) data were plotted versus time and were linear. The observed A_{340} change (ΔA_{340}) and y-intercept were determined in excel. The

concentration of product at each time point was calculated from the primary (A_{340}) data by the equation:

product concentration (in μM) = ((A_{340}) –(y-intercept))*(-1/0.00413 μM^{-1} s^{-1})

where *t* is the time in s.

The product vs. time data were analyzed by KinTek Explorer global fitting software (KinTek Corp., Austin, TX). The following kinetic steps for a general bi-substrate reaction were defined for global data analysis:

Step 1. E + I = E·I

Step 2. E·I → E·I*

Step 3. E + ATP = E·ATP

Step 4. E·ATP + peptide = E·ATP·peptide

Step 5. E + peptide = E·peptide

Step 6. E·peptide + ATP = E·ATP·peptide

Step 7. E·ATP·peptide → E + phosphopeptide + ADP

The rate constants describing steps 3-7 were determined in separate preliminary experiment and set to their determined values during data fitting.

Sheep red blood cell (SRBC)–induced delayed-type hypersensitivity (DTH) model. BALB/c mice (Taconic, Germantown, NY) were kept at the animal facility of Pfizer Research in accordance with the Guide for the Care and Use of Laboratory Animals. All study protocols were approved by the Pfizer Institutional Animal Care and Use Committee. BALB/c mice (n = 10) were treated with vehicle or compounds orally for 7 days (day –1 to day 5). On day 0, mice were primed with a subcutaneous injection of sheep red blood cells (sRBCs). On day 5, the right hind footpad was measured using a caliper. The right hind footpad was then challenged by injecting sSRBCs. Foot pad swelling (ventrodorsal thickness) was measured 24 hours later. The difference in thickness prior to and after challenge of the right hind footpad was reported as the change in right hind footpad thickness.

General

All chemicals, reagents, and solvents were purchased from commercial sources when available and used without further purification. Anhydrous solvents (dioxane, dichloromethane (CH_2Cl_2), toluene, acetonitrile) were purchased from Aldrich (SureSeal) and used in reactions without further purification. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) using precoated Whatman 250 μm silica gel plates and visualized by UV light and by GC/MS. Silica gel chromatography was performed utilizing ACS grade solvents and ISCO or Biotage purification systems and pre-packaged columns. Yields refer to purified compounds. ^{1}H NMR spectra (400 MHz field strength) and ^{13}C NMR spectra (101 MHz field strength) were obtained on a Bruker AV III spectrometer equipped with a BBFO probe. Alternatively, ^{1}H NMR spectra (500 MHz field strength) and ^{13}C NMR spectra (125 MHz field

strength) were obtained on a Bruker 500 Avance III HD using a 5 mm Prodigy BBO cryoprobe. Chemical shifts were referenced to the residual ^{1}H solvent signals ($CDCl_3$, δ 7.27) , (DMSO-d_6, δ 2.50), (MeOH-d_4, δ 3.31), (D_2O, δ 4.75) and solvent ^{13}C signals ($CDCl_3$, δ 77.00), (DMSO-d_6, δ 39.51), (MeOH-d_4, δ 49.15). Signals are listed as follows: chemical shift in ppm (multiplicity identified as s = singlet, br = broad, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constants in Hz; integration). High resolution mass spectrometry (HRMS) was performed via atmospheric pressure chemical ionization (APCI) or electron scatter ionization (ESI) sources.

Purity of final compounds was determined by RP-HPLC to be >95% (UV detection (=220 and 254 nM) and ELSD), prior to biological assay.

Synthesis of 1-((3R,4R)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-yl)prop-2-en-1-one (3). To a solution of N-methyl-N-((3R,4R)-4-methylpiperidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine **21** (77 mg, 0.31 mmol, WO 2000/2096909 A1, CAS: 477600-74-1) in CH_2Cl_2 (3.0 mL) was added DIPEA (0.1 uL, 1.3 eq). The reaction mixture was cooled to 0 ^{o}C and then a solution of acryloyl chloride in CH_2Cl_2 (27 uL in 1 mL) was added dropwise. After 1 hr, water was added and the mixture passed through a phase separator and the organic concentrated. The residue was purified by chromatography (silica, MeOH/DCM, 0 to 7.5%) to give the desired product (30 mg, 32%). LC/MS (M+H) = 300.1.^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.66 (br. s., 1 H) 8.10 (s, 1 H) 7.14 (br. s., 1 H) 6.71 - 6.96 (m, 1 H) 6.56 (br. s., 1 H) 6.12 (d, *J*=16.39 Hz, 1 H) 5.47 - 5.78 (m, 1 H) 4.86 (br. s., 1 H) 3.87 - 4.10 (m, 2 H) 3.34 - 3.82 (m, 4 H) 2.41 (br. s., 1 H) 1.43 - 1.86 (m, 2 H) 0.89 - 1.19 (m, 4 H);^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 164.4, 156.9, 151.7, 150.5, 128.4, 128.3, 127.3, 120.7, 102.2, 101.7, 53.6, 53.1, 45.6, 41.9, 41.5, 33.9, 31.8, 31.4, 30.3, 14.06, 13.7. HRMS (ESI) m/z: calculated for $C_{16}H_{21}N_5O$ $[M + H]^+$ 300.1824; observed 300.1815.

Synthesis of (R)-1-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-yl)prop-2-en-1-one (4).

*Step 1: (R)-tert-butyl 3-((7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidine-1-carboxylate (**24**).* To a stirred solution of 4-chloro-7-tosyl-7H-pyrrolo[2,3-d]pyrimidine **22** (8.73 g, 28.4 mmol) in n-Butanol (100 mL) was added DIPEA (6.0 mL, 1.2 eq) and (R)-3-amino piperidine-1-carboxylic acid tert-butyl ester **23** (6.82 g, 1.2 eq, AlfaAesar, cat no. H26937, CAS 188111-79-7). The reaction mixture was heated at 70°C overnight. The solvent was removed under reduced pressure and the crude residue was purified by column chromatography (100-200 mesh silica, 0-3% MeOH in DCM) to obtain **24** (5.6 g, 42%). LC-MS (ESI) m/z 472.2 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.25 (s, 1 H) 7.95 (d, *J*=8.31 Hz, 2 H) 7.55 (d, *J*=3.91 Hz, 2 H) 7.41 (d, *J*=8.07 Hz, 2 H) 6.95 (br. s., 1 H) 3.90 - 4.07 (m, 1 H) 3.43 - 3.69 (m, 1 H) 3.05 - 3.30 (m, 1 H) 2.34 (s, 3 H) 1.92 (br. s., 1 H) 1.77 (br. s., 1 H) 0.91 - 1.68 (m, 13 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 155.7, 153.8, 153.3, 149.9, 145.6, 134.6, 130.0, 127.5, 121.9, 104.78, 103.8, 78.2, 64.9, 47.4, 46.3, 27.8, 21.0, 15.1, 14.0 .; HRMS (ESI) m/z: calculated for $C_{23}H_{29}N_5O_4S$ $[M + Na]^+$ 494.1838; observed 494.1831.

*Step 2: (R)-tert-butyl 3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidine-1-carboxylate (**25**).* To a stirred solution of **24** (29.4g, 62mmol) in MeOH (96 mL), THF (96 mL) and water (96mL) was added $LiOH{\cdot}H_2O$ (2.99 g, 125 mmol, 2 eq). The mixture was heated at 60°C for 1 hour. After the reaction mixture was cooled to room temperature, the organic solvent was evaporated *in vacuo*. The aqueous mixture was made slightly acidic and then extracted with ethyl acetate (4 x 150 mL). The organic fractions were combined and washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by column chromatography (100-200 mesh silica, 0-2% MeOH in DCM) to provide 8.5 g (70%) of **25** as an off white solid. LC-MS (ESI) m/z 318.2 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.48 (br. s., 1 H) 8.11 (s, 1 H) 7.14 (br. s., 1 H) 6.97 - 7.11 (m, 1 H) 6.59 (br. s., 1 H) 4.06 (br. s., 1 H) 3.54 - 3.97 (m, 1 H) 2.71 - 3.13 (m, 1 H) 1.99 (d, *J*=9.05 Hz, 1 H) 1.78 (br. s., 1 H) 1.11 - 1.68 (m, 13 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 155.4, 153.9, 151.2, 150.3, 120.8, 102.5, 98.7, 78.4,

46.2, 33.5, 28.1, 27.9, 21.7, 13.9; HRMS (ESI) m/z: calculated for $C_{16}H_{23}N_5O_2$ $[M + H]^+$ 318.1930; observed 318.1760.

*Step 3: (R)-N-(piperidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine HCl salt (**26**)*. To a stirred solution of **25** in dioxane (40 mL) was added 4M HCl in dioxane (60 mL) dropwise. The reaction was stirred for ~1 hr and then diluted with diethyl ether to form a solid, which was filtered and collected. The solid was dried on high vacuum to give amine HCl salt **26** (4.6 g, 92%). LC-MS (ESI) m/z 218.2 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.48 (br. s., 1 H) 8.09 (s, 1 H) 7.19 (d, *J*=7.83 Hz, 1 H) 7.07 (br. s., 1 H) 6.60 (d, *J*=2.20 Hz, 1 H) 4.20 (br. s., 1 H) 3.51 (br. s., 1 H) 3.21 (d, *J*=2.69 Hz, 1 H) 2.94 (d, *J*=11.98 Hz, 1 H) 2.54 - 2.65 (m, 2 H) 1.97 (br. s., 1 H) 1.68 - 1.84 (m, 1 H) 1.44 - 1.62 (m, 2 H) ; ^{13}C NMR (125 MHz, DMSO- d_6) δ ppm 155.3, 151.3, 150.2, 120.8, 102.4, 98.7, 50.1, 46.1, 45.0, 30.2, 24.0; *DMSO+D_2O* **:** ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.90 - 8.19 (m, 1 H) 7.07 (d, *J*=1.47 Hz, 1 H) 6.57 (d, *J*=2.20 Hz, 1 H) 4.17 (br. s., 1 H) 3.56 - 3.88 (m, 7 H) 3.17 (d, *J*=11.74 Hz, 1 H) 2.93 (d, *J*=12.23 Hz, 1 H) 2.55 - 2.63 (m, 1 H) 1.95 (d, *J*=9.78 Hz, 1 H) 1.67 - 1.81 (m, 1 H) 1.33 - 1.63 (m, 2 H); *D_2O*: ^{1}H NMR (500 MHz,D_2O) δ ppm 8.02 (s, 1 H) 7.11 (d, *J*=3.42 Hz, 1 H) 6.44 (d, *J*=3.67 Hz, 1 H) 3.95 - 4.22 (m, 1 H) 3.31 (dd, *J*=12.23, 3.18 Hz, 1 H) 2.95 - 3.17 (m, 1 H) 2.50 - 2.87 (m, 2 H) 1.98 - 2.21 (m, 1 H) 1.77 - 1.93 (m, 1 H) 1.42 - 1.74 (m, 2 H) ; HRMS (ESI) m/z: calculated for $C_{11}H_{15}N_5$ $[M + H]^+$ 218.1406; observed 218.1360.

*Step 4: (R)-1-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-yl)prop-2-en-1-one (**4**)* . To a round bottom flask containing (R)-N-(piperidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine HCl salt **26** (1.0 g, 3.44 mmol) was added DCM (30 mL), EtOH (3 mL) and TEA (2.11 mL, 4.4 eq). After 30 min, acrylolyl chloride in 20 ml of DCM was added dropwise and the reaction stirred at rt for 2 hrs. The mixture was poured into water and the layers separated. The organic layer was dried (Na_2SO_4) and the solvent removed to give crude product (~900 mg). The material was purified by chromatography (silica, DCM/MeOH) to give **4** (310 mg, 33%). LC-MS (ESI) m/z 272.1 $[M+H]^+$; rotamers observed. ^{1}H

NMR@300K: ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.51 (br. s., 1 H) 8.12 (d, *J*=18.34 Hz, 1 H) 7.16 - 7.32 (m, 1 H) 7.09 (t, *J*=2.69 Hz, 1 H) 6.67 - 6.91 (m, 1 H) 6.60 (d, *J*=8.07 Hz, 1 H) 6.09 (t, *J*=14.92 Hz, 1 H) 5.50 - 5.72 (m, 1 H) 4.55 (d, *J*=10.76 Hz, 0.5 H) 4.03 - 4.21 (m, 3 H) 4.00 (d, *J*=12.96 Hz, 0.5 H) 2.84 - 3.15 (m, 1.5H) 2.64 (t, *J*=11.25 Hz, 0.5 H) 2.04 (br. s., 1 H) 1.83 (d, *J*=4.40 Hz, 1 H) 1.37 - 1.75 (m, 2 H); ^{1}H NMR @313K: ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.46 (br. s., 1 H) 7.95 - 8.27 (m, 1 H) 7.19 (br. s., 1 H) 7.08 (d, *J*=5.38 Hz, 1 H) 6.66 - 6.90 (m, 1 H) 6.60 (br. s., 1 H) 6.08 (d, *J*=15.65 Hz, 1 H) 5.47 - 5.72 (m, 1 H) 4.55 (br.s., 0.5 H) 4.13 (br. s., 2 H) 3.98 (br.s., 0.5 H) 2.86 - 3.19 (m, 1.5 H) 2.66 (br.s., 0.5 H) 2.05 (d, *J*=8.80 Hz, 1 H) 1.83 (d, *J*=10.03 Hz, 1 H) 1.37 - 1.74 (m, 2 H);^{1}H NMR @353K: ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.31 (br. s., 1 H) 8.13 (s, 1 H) 7.06 (d, *J*=2.93 Hz, 1 H) 6.99 (d, *J*=6.60 Hz, 1 H) 6.73 (dd, *J*=15.04, 11.13 Hz, 1 H) 6.60 (d, *J*=2.93 Hz, 1 H) 6.07 (dd, *J*=16.75, 1.83 Hz, 1 H) 5.63 (d, *J*=10.27 Hz, 1 H) 4.19 - 4.53 (m, 1 H) 3.93 - 4.18 (m, 2 H) 3.03 (br. s., 2 H) 2.02 - 2.14 (m, 1 H) 1.85 (dt, *J*=13.33, 3.85 Hz, 1 H) 1.63-1.74 (m, 1 H) 1.44 - 1.57 (m, 1 H); ^{13}C NMR@353K: ^{13}C NMR(125 MHz, DMSO-d_6) δ ppm 164.4, 155.2, 150.9, 150.1, 128.5, 126.0, 120.5, 102.3, 98.3, 48.2, 48.2, 46.6, 29.9, 23.3.; HRMS (ESI) m/z: calculated for $C_{11}H_{15}N_5$ [M + H]$^{+}$ 272.1511; observed 272.1775.

Synthesis of (R)-1-(3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-yl)prop-2-en-1-one (5). *Step 1: tert-butyl (R)-3-((2-chloro-7H-pyrrolo[2,3-d]pyrimidin-4-yl)(methyl)amino)piperidine-1-carboxylate (**xx**).* To a solution of 2,4-dichloro-7H-pyrrolo[2,3-d]pyrimidine **27** (300 mg, 1.6 mmol) and DIPEA (1.03 g, 7.98 mmol) in ^{n}BuOH (14 mL) was added tert-butyl (R)-3-(methylamino)piperidine-1-carboxylate **28** (342 mg, 1.60 mmol). The reaction mixture was heated to 130 ^{o}C for 18 hrs and then the solvent removed in vacuo. The residue was dissolved into DCM and washed with water. The organic extract was dried (Na_2SO_4) and the solvent removed to give the crude material, which after chromatography (silica, Ethyl acetae: Pet Ether 50% to 75%) gave the desired product **29** (350 mg, 60%).

^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 7.09 (d, *J*=3.51 Hz, 1 H) 6.65 (d, *J*=3.51 Hz, 1 H) 4.52 - 4.80 (m, 1 H) 3.97 - 4.27 (m, 2 H) 3.37-3.29 (m, 3 H) 3.01 (t, *J*=11.80 Hz, 1 H) 2.75 (br. s., 1 H) 1.77 - 2.02 (m, 3 H) 1.60 - 1.72 (m, 1 H) 1.50 (s, 9 H),; LC/MS (M+H) expected 366.1687, observed 366.1.

*Step 2: tert-butyl (R)-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidine-1-carboxylate (**30**).* To a dry Parr hydrogenation bottle was added **29** (350 mg, 0.96 mmol), MeOH:THF (15 mL:8 mL) and 10% Pd/C (183 mg). The mixture was shaken for 48 hrs @ 50 psi H_2. The reaction mixture was then filtered thru celite and the solvent removed to give the crude product **30** (320 mg, 100%), which was used in the next step without further purification. LC/MS (M+H) expected 332.2087, observed 332.2.

*Step 3: (R)-N-methyl-N-(piperidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**31**).* To a solution of **30** (320 mg, 0.97 mmol) in CH_2Cl_2 (15.0 mL) at 0 ^{o}C was added TFA (5.0 mL) dropwise. After 2 hrs, the solvent was removed to give a yellow oil, which was dissolved in CH_2Cl_2. The mixture then washed with saturated Na_2CO_3 (pH ~ 8). The organic extract was collected, dried (Na_2SO_4) and the solvent removed to give the desired product (**31**, 170 mg, 76%). ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 8.12 (s, 1 H) 7.11 (d, *J*=3.51 Hz, 1 H) 6.70 (d, *J*=3.51 Hz, 1 H) 4.24-4.23 (m,, 1 H) 3.28 (s, 3 H) 2.69 - 3.13 (m, 4 H) 2.35 - 2.63 (m, 1 H) 1.65 - 2.05 (m, 3 H),; LC/MS (M+H) expected 232.1484, observed 232.1.

*Step 4: (R)-1-(3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-yl)prop-2-en-1-one **(5)**.* To a solution of **31** (100 mg, 0.43 mmol) in THF (8 mL) and sat. $NaHCO_3$ (5 mL) cooled to 5 ^{o}C was added slowly acryloyl chloride (33.8 mg, 0.37 mmol, in 2 mL THF) dropwise. After 1 hr at 0 ^{o}C, the reaction mixture was poured into water/ CH_2Cl_2 and the layers separated. The organic layer was collected, dried (Na_2SO_4) and the solvent removed to give a residue, which was purified by chromatography (silica, CH_2Cl_2:MeOH, 10:1) to give the desired product. The product was further purified by HPLC (Phenomenex, C-18, 25*50mm*10 um, H_2O:CH_3CN (+ 0.05% NH_4OH) 15 to 45%, 10 min) to give the final product **5** (15 mg, 14%). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.55 (br. s., 1 H) 8.32 (s, 1 H) 7.08

(d, *J*=3.01 Hz, 1 H) 6.50 - 6.79 (m, 2 H) 6.18 - 6.44 (m, 1 H) 5.49 - 5.88 (m, 1 H) 4.61 - 5.06 (m, 2 H) 3.93 - 4.31 (m, 1 H) 3.34 (br. s., 3 H) 2.44 - 3.20 (m, 2 H) 1.63 - 2.19 (m, 4 H); LC/MS (M+H) expected 286.1668, observed 285.9.

Synthesis of N-(3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)acrylamide (6). *Step 1: 3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)aniline (**33**).* To a solution of 4-chloro-7H-pyrrolo[2,3-d]pyrimidine **31** (15 g, 98 mmol), (3-aminophenyl)boronic acid **32** (18 g, 117 mmol) in dioxane/H_2O (450 mL:90 mL) was added K_2CO_3 (40.1 g, 294 mmol) and $Pd(PPh_3)_4$ (11.25 g, 9.8 mmol) under N_2. The mixture was degassed four times with N_2 and the mixture heated to 110 ^oC for 16 hours. TLC (Petroleum ether/ EtOAc = 1:3) indicated the starting material was consumed completely. The mixture was concentrated and water (500 mL) and CH_2Cl_2 (500 mL) added. The resulting mixture was filtered and the filtrate extracted with CH_2Cl_2 (500 mL x 3). The organic layer was washed with brine (700 mL x 2) and dried over anhydrous Na_2SO_4. The solvent was removed and the crude material purified by column chromatography (CH_2Cl_2 /methanol = 1%-5%) to give **33** (10 g, 50%) as yellow solid.; ^{1}H NMR (400 MHz, DMSO): δ 12.19 (s, 1H), 8.79 (s, 1H), 7.63-7.62 (m, 1H), 7.45 (s, 1H), 7.33-7.32 (d, 1H), 7.23-7.19 (m, 1H), 6.86-6.85 (m, 1H), 6.74-6.72 (m, 1H), 5.32 (s, 1H). LC-MS (ESI) m/z 211.1 $[M+H]^+$

*Step 2. N-(3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)acrylamide (**6**).* To a solution of **33** (200 mg, 0.95 mmol) in 10 mL of THF at 0 ^oC was added DIPEA (500 mg, 3.72 mmol). A solution of acryloyl chloride (86 mg, 0.95 mmol) in 0.5 mL of THF was added dropwise. After addition, the mixture was stirred at 0 ^oC for 30 minutes. The mixture was stirred at room temperature for 2h. TLC (PE/EA = 1/3) indicated the starting material was consumed completely. Methanol (5 mL) was added and reaction concentrated to give crude material, which was purified by HPLC to give **6** (50 mg, 20%) as a white solid.; ^{1}H NMR (400 MHz, DMSO): δ 12.29 (s, 1H), 10.40 (s, 1H), 8.84 (s, 1H), 8.65(s, 1H), 7.94-7.92 (d, 1H), 7.84-7.82 (d, 1H),

7.70-7.69 (d, 1H), 7.56-7.53 (m, 1H), 6.97-6.96 (d, 1H), 6.52-6.45 (m, 1H), 6.34-6.31 (d, 1H), 5.82-5.80 (d, 1H). LC-MS (ESI) m/z 264.9 $[M+H]^+$

Synthesis of 1-((3aS,7aS)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)octahydro-6H-pyrrolo[2,3-c]pyridin-6-yl)prop-2-en-1-one (7). *Step 1: (3aS,7aR)-tert-butyl octahydro-1H-pyrrolo[2, 3-c]pyridine-1-carboxylate (**36**)* . To a stirred solution of (3aS,7aR)-tert-butyl octahydro-1H-pyrrolo[2, 3-c]pyridine-1-carboxylate **35** (200 g, 0.885 mol) and DIPEA (251 g, 1.95 mol) in DCM (2L) at 0 ^oC was added dropwise Cbz-Cl (181 g, 1.06 mol) over a period of 45 min. After the addition, the resulting mixture was stirred at room temperature for 16 hours. TLC(DCM/MeOH, 10:1) showed the starting material was consumed completely. The reaction mixture was evaporated to dryness, and then partitioned between EtOAc (8L) and water (3 L); the organic layer was washed with water (3 L) and brine (3 L), dried over anhydrous Na_2SO_4 and concentrated to give **36** (1.1 kg, 90%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 1.15 —1.48 (m, 9 H) 1.51 —1.65 (m, 1 H) 1.68 —1.90 (m, 2 H) 2.32 (br s, 1 H) 2.72 (t, *J*=11.04 Hz, 1 H) 2.97 (br s, 1 H) 3.13 —3.56 (m, 3 H) 3.73 (s, 2 H) 3.85 —4.28 (m, 1 H) 4.91 —5.14 (m, 2 H) 7.12 —7.38 (m, 5 H).

*Steps 4-6: (3aR, 7aR)-Benzyl hexahydro-1H-pyrrolo[2, 3-c]pyridine-6(2H)-carboxylate and (3aS,7aS)-benzyl hexahydro-1H-pyrrolo[2, 3-c]pyridine-6(2H)-carboxylate (**37a** and **37b**).* To a 0 ^oC stirred solution of (3aS,7aR)-tert-butyl octahydro-1H-pyrrolo[2, 3-c]pyridine-1-carboxylate **36** (280 g, 0.68 mol) in DCM (600 mL) was added dropwise 4N HCl in dioxane (2.5 L) over a period of 1 hour. The reaction mixture was stirred at room temperature for 15 hours. TLC (petroleum ether/EtOAc, 2:1) showed the starting material was consumed completely. The reaction mixture was evaporated to dryness, and then partitioned between MTBE (6L) and water H_2O (4 L), the aqueous phase was then basified to pH 9-10 and extracted with DCM (3 Lx4). The combined organic layers were concentrated to give rac-(3aR, 7aR)-benzylhexahydro-1H-pyrrolo[2, 3-c]pyridine-6(2H)-carboxylate **37** (687 g, 85%), which was separated by SFC to give **38b**: (3aS,7aS)-benzyl hexahydro-1H-pyrrolo[2, 3-c]pyridine-6(2H)-carboxylate (280 g, 42.2%)

and **38a**: (3aR, 7aR)-benzyl hexahydro-1H-pyrrolo[2, 3-c]pyridine-6(2H)-carboxylate (270, 39.3%) as yellow oil. (**38a**: Peak 2 was (3aR, 7aR)-benzyl hexahydro-1H-pyrrolo[2, 3-c]pyridine-6(2H)-carboxylate, RT = 9.81 min, , $[\alpha]_D^{25}$ = +9.78; peak 1, **38b** was (3aS,7aS)-benzyl hexahydro-1H-pyrrolo[2, 3-c]pyridine-6(2H)-carboxylate, RT = 10.63 min, , $[\alpha]_D^{25}$ =-8.97). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 —1.63 (m, 3 H) 1.68 —1.90 (m, 2 H) 1.97—2.09 (m, 1 H) 2.71 —3.19 (m, 4 H) 3.26 —3.43 (m, 1 H) 3.55 —3.77 (m, 2 H) 5.02 (br s,2 H) 7.10 —7.35 (m, 5 H). Separation conditions: Instrument: SFC 350; Column: AS 250mmx50mm, 10 μm; Mobile phase: A: Supercritical CO_2, B: EtOH (0.05%DEA), A:B =65:35 at 240ml/min; Column Temp: 38 ^{o}C; Nozzle Pressure: 100 Bar; Nozzle Temp: 60 ^{o}C; Evaporator Temp: 20 ^{o}C; Trimmer Temp: 25 ^{o}C; Wavelength: 220 nm**.** Absolute stereochemistry determined by single X-ray (see Supporting Information)

*Step 7: (3aS,7aS)-Benzyl 1-(2-chloro-7H-pyrrolo[2, 3-d]pyrimidin-4-yl) hexahydro-1Hpyrrolo[2, 3-c]pyridine-6(2H)-carboxylate (**39**)*. A mixture of (3aR,7aR)-benzyl hexahydro-1H-pyrrolo[2, 3-c]pyridine-6(2H)-carboxylate, **38a**:peak 2 (135 g, 0.52 mol), DIPEA (268 g,2. 1 mol) and **27** (88.7 g, 0.47 mol) in n-BuOH (1L) was heated to 80 ^{o}C for 3 hours, TLC (Petroleum ether/ether, 2:1) showed **27** was consumed completely. The reaction mixture was cooled to room temperature and evaporated to dryness via oil pump at 45 ^{o}C. The residue was partitioned between DCM (2 L) and water (1.5 L); the organic layer was washed with water (1 L) and brine (1 L), dried over Na_2SO_4 and concentrated to give **39** (310 g, 80%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.58 —2.35 (m, 5 H) 2.90 —3.28 (m, 2 H) 3.58—4.07 (m, 3 H) 4.35 (br s, 2 H) 5.16 (br s, 2 H) 6.46 —6.85 (m, 1 H) 7.12 —7.57 (m, 6 H) 11.87 (br s, 1 H).

*Step 8: 4-((3aS,7aR)-octahydro-1H-pyrrolo[2,3-c]pyridin-1-yl)-7H-pyrrolo[2,3-d]pyrimidine (**40**)***.** To a dry Parr hydrogenation bottle, Pd/C (12 g) was added under Ar atmosphere. Then a solution of **39** (62 g, 0.15 mol) in EtOH (1.2 L) was added and the resulting mixture was hydrogenated under 50 psi of H_2 at 65 ^{o}C for 48 hours, TLC (Petroleum ether/EtOAc, 1:1) showed the starting material was consumed

completely; the reaction mixture was filtered and the filter cake was washed with warm MeOH and water (v/v 1:1,500 mL x 2). The combined filtrate was evaporated to give **40** (190 g, 90%) as a white solid.

*Step 9: 1-((3aR,7aR)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)tetrahydro-1H-pyrrolo[2,3-c]pyridin-6(2H,7H,7aH)-yl)prop-2-en-1-one (**7**).* To a solution of **40** (150 g, 0.54 mol) in aq $NaHCO_3$ (150 g, 1.79 mol) in H_2O (1.5 L) at 0 ^oC was added dropwise a solution of acryloyl chloride (53.3 g, 0.59 mol) in MeCN (150 mL) carefully. After the addition, the resulting mixture was stirred at room temperature for 2 hours. TLC (DCM/MeOH, 5:1) showed **40** was consumed completely. The reaction mixture was extracted with DCM (500 mL*4) and the combined organic layers were concentrated to give the crude product, which was purified by column chromatography to give **7** (130 g, 81%) as a white solid.; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.58 (s, 1H) 8.09-8.07 (d, *J*=9.2Hz, 1H) 7.115(s, 1H), 6.82-6.78 (m, 1H), 6.51 (m, 1H), 6.05-6.01 (m, 1H), 5.69-5.85 (m, 1H), 4.69-4.68 (m, 0.5H), 4.27 (s, 1H), 3.90-3.74 (m,3H), 3.13-3.24 (m, 2H), 2.74-2.71 (m, 0.5H), 2.19-1.74 (m, 4.5 H).

Synthesis of 1-((3aR,7aR)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)octahydro-6H-pyrrolo[2,3-c]pyridin-6-yl)ethan-1-one (8). *Step 1: 1-((3aR,7aR)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)octahydro-6H-pyrrolo[2,3-c]pyridin-6-yl)ethan-1-one (**8**)*. To a solution of **40** (70 mg, 0.22 mmol) in DCM at 0 ^oC was added DIPEA (0.20 mL, 1.1 mmol) followed by addition of a solution of acetyl chloride (16 uL in 1 .0 mL DCM) . The resulting mixture was stirred at room temperature for 2 hours. TLC (DCM/MeOH, 5:1) showed **40** was consumed completely. The reaction mixture was diluted with water (30 mL) and DCM (25 mL). The pH was adjusted to ~5 with 1N HCL. The layers were separated and the aqueous mixture extracted with DCM (10 mL*3). The combined organic layers were concentrated to give the crude product, which was purified by column chromatography to give **8** (46.6 mg, 74%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.59 (d, *J*=14.83 Hz, 1 H) 7.88 - 8.40 (m, 1 H) 7.13 (br. s., 1 H) 6.58 (br. s., 1 H) 4.66 (dd,

J=12.68, 5.66 Hz, 0.5 H) 4.17 - 4.47 (m, 1 H) 3.37 - 4.10 (m, 4 H) 3.26 (t, *J*=11.51 Hz, 1 H) 2.63 (t, *J*=11.71 Hz, 1 H) 1.49 - 2.30 (m, 7.5 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 168.0, 151.2, 151.0, 121.0, 120.9, 100.7, 59.7, 54.9, 47.0, 45.1, 41.8, 25.7, 24.8, 21.1, 20.9; LC-MS (ESI) m/z 286.1 $[M+H]^+$

Synthesis of (R,E)-1-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-yl)but-2-en-1-one (9). To a round bottom flask containing **26** (300 mg, 1.03 mmol) was added DCM (10 mL), EtOH (1 mL) and TEA (0.634 mL, 4.4 eq). After 30 min, (E)-but-2-enoyl chloride in 5 ml of DCM was added dropwise and the reaction stirred at rt for 2 hrs. The mixture was poured into water and the layers separated. The organic layer was dried (Na_2SO_4) and the solvent removed to give crude product (~295 mg). The material was purified by chromatography (silica, DCM/MeOH) to give **9** (38%). LC-MS (ESI) m/z 286.18 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.51 (br. s., 1 H) 8.14 (d, *J*=13.27 Hz, 1 H) 6.96 - 7.36 (m, 2 H) 6.29 - 6.84 (m, 3 H) 4.54 (br. s., 1 H) 4.10 (dd, *J*=10.15, 4.68 Hz, 4 H) 2.78 - 3.27 (m, 3 H) 2.62 (br. s., 1 H) 1.35 - 2.24 (m, 9 H).

Synthesis of rac-1-((2R,3R)-3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-2-methylpiperidin-1-yl)prop-2-en-1-one (10). *Step 1: rac- benzyl (2R,3R)-2-methyl-3-((7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidine-1-carboxylate (**43**).* To a round bottom flask containing 4-iodo-7-tosyl-7H-pyrrolo[2,3-d]pyrimidine **41** (510 mg, 2.05 mmol CAS: 906092-45-3, WO 2007/117494 A1 20071018) in ^{n}BuOH (6.8 mL) was added DIPEA (2.8 mL, 16 mmol) and rac-benzyl (2S,3S)-3-amino-2-methylpiperidine-1-carboxylate **42** (896 mg, 2.24 mmol, 1.1 eq, CAS:912451-60-6, WO 2008132500 A2 20081106). The reaction mixture was heated to 85 ^{o}C for 18 hrs and then allowed to cool to room temperature. The solvent was removed *in vacuo* and the residue purified by chromatography (silica, ethyl acetate/heptane, 10 to 60%) to give **43** as foam (382 mg, 36%). LC-MS (ESI) m/z 519.0 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.43 (s, 1 H) 8.07 (d, *J*=8.20 Hz, 2 H) 7.45 (d, *J*=3.12 Hz, 1 H) 7.28 - 7.41 (m, 8 H)

6.44 (d, *J*=3.51 Hz, 1 H) 5.08 - 5.29 (m, 2 H) 4.85 - 4.98 (m, 1 H) 4.78 (br. s., 1 H) 4.00 - 4.11 (m, 1 H) 2.74 - 2.95 (m, 1 H) 2.4 (s, 3 H) 1.82 - 1.92 (m, 1 H) 1.72 - 1.81 (m, 1 H) 1.57 - 1.69 (m, 1 H) 1.19 - 1.35 (m, 1 H) 1.09 (d, *J*=6.83 Hz, 3 H) .

*Step 2. Rac-benzyl (2R,3R)-3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-2-methylpiperidine-1-carboxylate (**44**)*. To a round bottom flask containing **43** (374 mg, 0.72 mmol) in MeOH:H_2O (5.5 mL:2.0 mL) was added LiOH*H_2O (51 mg, 2.1 mmol). The reaction mixture was stirred at 60 ^{o}C for 1.5 h and then cooled to room temperature. The reaction mixture was diluted with water (30 mL)/CH_2Cl_2 (25 mL) and the pH adjusted to ~5 with 1M HCl. The layers were separated and the aqueous solution extracted with DCM (15 mL x 3). The organic extracts were combined and washed with brine (50 mL), dried (Na_2SO_4) and the solvent removed to give the product **44** (241 mg, 91%). LC-MS (ESI) m/z 365.0 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 10.00 (br. s., 1 H) 8.34 (s, 1 H) 7.30 - 7.45 (m, 5 H) 7.05 (br. s., 1 H) 6.41 (br. s., 1 H) 5.11 - 5.26 (m, 2 H) 4.95 - 5.08 (m, 1 H) 4.11 (d, *J*=12.49 Hz, 1 H) 2.95-2.88 (m, 1 H) 1.89 - 2.00 (m, 1 H) 1.60 - 1.85 (m, 5 H) 1.16 (d, *J* = 8 Hz, 3 H)

*Step 3: N-((2R,3R)-2-methylpiperidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**45**).* To a solution of **44** (241 mg, 0.66 mmol) in MeOH (12 mL) was added $Pd(OH)_2$ (352 mg, 0.50 mmol) and cyclohexadiene (2.3 g, 2.65 mL, 28 mmol, 40 eq). The reaction mixture was heated to reflux for 14 hrs and then cooled to room temperature. The reaction was then filtered through Celite and the filtrate concentrated under reduced pressure to give an oil, which after chromatography (silica, CH_2Cl_2/MeOH/NH_4OH (2% in DCM)) gave the desired product **45** (79.6 mg, 52%). LC-MS (ESI) m/z 232.3 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.47 (br. s., 1 H) 8.08 (s, 1 H) 7.08 (br. s., 1 H) 6.35 - 6.82 (m, 2 H) 4.31 (br. s., 1 H) 2.80 - 3.02 (m, 2 H) 2.56 - 2.69 (m, 2 H) 1.88 (br. s., 1 H) 1.60 (br. s., 2 H) 1.35 (br. s., 1 H) 0.96 (d, *J*=6.63 Hz, 3 H).; ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 156.0, 151.4, 150.2, 120.7,

102.5, 98.5, 53.5, 47.9, 45.5, 29.3, 21.2, 18.1.; HRMS (ESI) m/z: calculated for $C_{12}H_{17}N_5$ $[M + H]^+$ 232.1562; observed 232.1556.

*Step 4: rac-1-((2R,3R)-3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-2-methylpiperidin-1-yl)prop-2-en-1-one (**10**).* To a vial containing **45** (35 mg, 0.15 mmol) was added DCM (1.5 mL) and DIPEA (0.13 mL, 0.74 mmol). The mixture was cooled to 0 ^{o}C and a solution of acryloyl chloride (15 uL (0.18 mmol) in 0.7 mL of DCM) was added dropwise. The mixture was stirred at 0 ^{o}C for 2 hr and then diluted with water (25 mL) and DCM (20 mL). The pH was adjusted to a pH~5 with 1M HCl and the layers separated. The aqueous solution was extracted with DCM (10 mL x 3). The organic extracts were combined, dried (Na_2SO_4) and the solvent removed to give an oil, which was treated with ethyl acetate/heptane to precipitate the desired product **10** as a solid (31 mg, 71%). LC-MS (ESI) m/z 286.4 $[M+H]^+$; Mixture of rotamers: ^{1}H NMR@300K: ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.56 (br. s., 1 H) 8.16 (d, *J*=10.51 Hz, 1 H) 7.23 - 7.49 (m, 1 H) 7.13 (br. s., 1 H) 6.76 - 6.91 (m, 1 H) 6.71 (br. s., 1 H) 6.00 - 6.27 (m, 1 H) 5.61 - 5.83 (m, 1 H) 5.15 (br. s., 0.5 H) 4.78 (br. s., 1 H) 4.15 - 4.45 (m, 2 H) 3.91-3.93 (m, 0.5 H) 3.06 - 3.23 (m, 0.5 H) 2.74 - 2.89 (m, 0.5 H) 1.71 - 2.00 (m, 3 H) 1.05 - 1.22 (m, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 164.8,164.6, 155.1, 151.3, 150.2, 129.34, 128.7, 126.9, 126.7, 120.9, 102.4, 98.8, 50.3, 49.7, 48.9, 45.7, 35.1, 25.2, 24.5, 24.4, 24.1, 11.0, 10.3, 9.9.; ^{1}H NMR@313K: ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.46 (br. s., 1 H) 8.11 (br. s., 1 H) 7.00 - 7.37 (m, 2 H) 6.58 - 6.90 (m, 2 H) 6.11 (br.s., 1 H) 5.68 (br. s., 1 H) 5.11 (br. s., 0.5 H) 4.47 - 4.84 (m, 0.5 H) 4.10 - 4.44 (m, 1.5 H) 3.75 - 3.97 (m, 0.5 H) 3.08 (m, 0.5 H) 2.76 (m, 0.5 H) 1.66 - 2.02 (m, 3 H) 1.39 - 1.60 (m, 1 H) 0.93 - 1.20 (m, 3 H).; ^{1}H NMR@353K (peaks coalesce): ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.31 (br. s., 1 H) 8.12 (s, 1 H) 6.92 - 7.15 (m, 2 H) 6.55 - 6.82 (m, 2 H) 6.08 (dd, *J*=16.87, 1.71 Hz, 1 H) 5.66 (dd, *J*=10.64, 2.08 Hz, 1 H) 4.93 (br. s., 1 H) 4.05 - 4.36 (m, 2 H) 2.80 -3.01 (m, 1 H) 1.65 - 2.03 (m, 3 H) 1.40 - 1.62 (m, 1 H) 0.93 - 1.22 (m, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 164.6, 155.0, 151.0, 150.2, 125.7, 120.4, 102.3, 98.4, 24.1.; HRMS (ESI) m/z: calculated for $C_{15}H_{19}N_5$ O $[M + H]^+$ 286.1668; observed 286.1665

Synthesis of 1-((2S,5R)-5-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-2-methylpiperidin-1-yl)prop-2-en-1-one (11) and 1-((2R,5S)-5-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-2-methylpiperidin-1-yl)prop-2-en-1-one (12). *Step 1: tert-butyl (6-methylpyridin-3-yl)carbamate (**47**).* To a solution of 6-methylpyridin-3-amine **46** (25 g, 231. mmol)) in EtOH (100 mL) at 0 ^{o}C was added $(Boc)_2O$ (55.5 g, 298 mmol) dropwise slowly. After the addition, the solution was stirred at room temperature overnight. TLC (petroleum ether/EtOAc, 2:1) showed 6-methylpyridin-3-amine was consumed completely. The reaction mixture was filtered and the filter cake was washed with EtOH (30 mL x 3). The combined filtrate was concentrated in vacuo to afford a yellow residual, which was purified by chromatography (petroleum ether/EtOAc, 4:1 to 1:1) to give **47** (32.5 g, 67.4%) as a white solid. ; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.41 (br. s., 1 H) 8.47 (s, 1 H) 7.74 (d, *J*=8.3 Hz, 1 H) 7.13 (d, *J*=8.3 Hz, 1 H) 2.37 (s, 3 H) 1.47 (s, 9 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 152.8, 151.1, 139.2, 133.6, 125.6, 122.6, 79.4, 28.0, 23.2; HRMS (ESI) m/z: calculated for $C_{11}H_{17}N_2O_2$ $[M + H]^+$ 209.1290; observed 209.1285.

*Step 2: tert-butyl (6-methylpiperidin-3-yl)carbamate (**48**).* To a dry hydrogenation bottle, PtO_2 (2.5 g) was added under Ar atmosphere. Then a solution of **47** (33 g, 158.5 mmol) in HOAc (300 mL) was added and the resulting mixture was hydrogenated under 55 psi of H_2 at 50 ^{o}C for 30 hours. TLC (petroleum ether/EtOAc, 2:1) showed the starting material was consumed completely. The reaction mixture was filtered and the filter cake was washed with MeOH (50 mL x 2). The combined filtrate was evaporated to give **48** (34 g, 100%) as a yellow oil (~2:1 cis/trans), which was used directly to next step without further purification. LC-MS (ESI) m/z 215.2 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 6.49 - 6.77 (m, 1 H) 3.48 (br. s., 1 H) 3.26 (br. s. 0.5 H) 2.98 (d, *J*=11.00 Hz, 0.5 H) 2.66 - 2.85 (m, 2 H) 2.40 - 2.51 (m, 0.5 H) 2.27 (t, *J*=11.00 Hz, 0.5 H) 1.49 - 1.85 (m, 3 H) 1.38 - 1.49 (m, 9 H) 1.20 - 1.35 (m, 2 H) 0.93 - 1.16 (m, 3 H)

*Step 3: rac-cis/trans benzyl 5-((tert-butoxycarbonyl)amino)-2-methylpiperidine-1-carboxylate (**49**)* . To a stirred solution of **48** (27.0 g, 126 mmol) and $NaHCO_3$ (74.2 g, 883 mmol) in THF (350 mL)/H_2O (350 mL) was added CbzCl (32.17 g, 189 mmol) dropwise at room temperature. After the addition, the resulting mixture was stirred at room temperature for 2 hours. TLC (CH_2Cl_2/MeOH, 10:1) showed the starting material was consumed completely. The reaction mixture was extracted with EtOAc (300 mL x 2). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated to give crude product. The crude product was purified by chromatography (petroleum ether/ethyl acetate, 30:1-10:1) to give **49** (44.0 g, 100%, ~2:1 cis/trans) as a colorless oil. ; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.29 - 7.44 (m, 7 H) 6.78 - 6.97 (m, 1 H) 5.00 - 5.12 (m, 2 H) 4.50 (d, *J*=5.62 Hz, 0.3 H) 4.22 - 4.36 (m, 1.2 H) 3.95 (d, *J*=12.96 Hz, 1.2 H) 3.57 (br. s., 0.2 H) 3.22 (br. s., 0.8 H) 3.02 (dd, *J*=13.82, 2.32 Hz, 0.3 H) 2.55 - 2.67 (m, 0.8 H) 1.95 - 2.06 (m, 0.3 H) 1.75 - 1.87 (m, 0.3 H) 1.45 - 1.68 (m, 4 H) 1.31 - 1.44 (m, 11 H) 1.18 - 1.29 (m, 0.5 H) 1.02 - 1.14 (m, 4 H)

*Step 4: rac-benzyl (2S,5R)-5-((tert-butoxycarbonyl)amino)-2-methylpiperidine-1-carboxylate(**50a**) and rac-benzyl (2S,5S)-5-((tert-butoxycarbonyl)amino)-2-methylpiperidine-1-carboxylate (**50b**)*. The rac-cis/trans benzyl 5-((tert-butoxycarbonyl)amino)-2-methylpiperidine-1-carboxylate **49** (44 g) was separated by chiral SFC to give rac-trans-benzyl (2R,5R)-5-((tert-butoxycarbonyl)amino)-2-methylpiperidine-1-carboxylate **50a** (Peak 1, 12.3 g, 27.95%) and rac-cis-benzyl (2S,5R)-5-((tert-butoxycarbonyl)amino)-2-methylpiperidine-1-carboxylate **50b** (Peak 2, 24.5 g, 55.68%). Peak 2, cis material **50b** (as determined by ^{1}H NMR spectra) was carried on to Boc removal. Prep SFC Column : ChiralCel OD 300mm x 50mm, 10 μm; Mobile phase: A: Supercritical CO_2 , B: IPA (0.1%NH_3H_2O), A:B =85:15 at 180ml/min; Column Temp: 38 ^{o}C; Nozzle Pressure: 100 Bar; Nozzle Temp: 60 ^{o}C; Evaporator Temp: 20 ^{o}C; Trimmer Temp: 25 ^{o}C; Wavelength: 220 nm .; **50a**: Trans-isomer (pk1): ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.29 - 7.40 (m, 5 H) 5.09 - 5.19 (m, 2 H) 4.88 (br. s., 1 H) 4.47 (br. s., 1 H) 4.05 (d, *J*=14.18 Hz, 1 H) 3.81 (br. s., 1 H) 3.11 (d, *J*=13.45 Hz, 1 H) 1.65 - 1.92 (m, 3 H) 1.34 - 1.48 (m, 10 H) 1.17

(d, *J*=6.85 Hz, 3 H).; ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 156.0, 155.1, 136.7, 128.5, 128.0, 127.7, 79.3, 67.1, 46.2, 44.7, 42.8, 28.4, 24.5, 23.1, 15.4.; HRMS (ESI) m/z: calculated for $C_{19}H_{28}N_2O_4$ $[M + Na]^+$ 371.1947; observed 371.1943.; **50b**: Cis-isomer (pk2): ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.30 - 7.44 (m, 5 H) 5.01 - 5.24 (m, 2 H) 4.43 - 4.58 (m, 1 H) 4.38 (br. s., 1 H) 4.26 (d, *J*=10.03 Hz, 1 H) 3.48 (br. s., 1 H) 2.58 (t, *J*=12.10 Hz, 1 H) 1.88 (d, *J*=10.03 Hz, 1 H) 1.77 (tt, *J*=13.66, 4.68 Hz, 1 H) 1.53 - 1.65 (m, 2 H) 1.44 (s, 9 H) 1.14 (d, *J*=7.09 Hz, 3 H); ^{13}C NMR (125 MHz,) δ ppm 155.1, 155.0, 136.8, 128.5, 127.9, 127.8, 79.5, 67.1, 45.3, 43.6, 28.9, 28.3, 26.0, 22.7, 15.4; HRMS (ESI) m/z: calculated for $C_{19}H_{28}N_2O_4$ $[M + Na]^+$ 371.1947; observed 371.1945.

*Step 5. Rac-benzyl (2S,5S)-5-amino-2-methylpiperidine-1-carboxylate (**51a**) and benzyl (2S,5R)-5-amino-2-methylpiperidine-1-carboxylate (**51b**).* To a solution of rac- **(**2S,5R)-benzyl 5-((tert-butoxycarbonyl)amino)-2-methylpiperidine-1-carboxylate **50b** (Pk 2, 40.0 g, 115.6 mmol) in CH_2Cl_2 (60 mL) at 0 ^{o}C was added (4M HCl (g)/dioxane (200 mL) dropwise. After the addition, the solution was stirred at room temperature for 4 hrs. TLC (petroleum ether/EtOAc, 2:1) showed the starting material was consumed completely. The reaction mixture was concentrated to give (31.0 g, 94.8%) **51b** as a white solid (HCl salt). ; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.21 (br. s., 3 H) 7.21 - 7.46 (m, 5 H) 4.96 - 5.19 (m, 2 H) 4.25 - 4.46 (m, 1 H) 4.15 (dd, *J*=12.35, 3.30 Hz, 1 H) 2.83 - 3.10 (m, 2 H) 1.87-1.80 (m, 1 H) 1.49 - 1.76 (m, 3 H) 1.11 (d, *J*=6.85 Hz, 3 H).; ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 154.2, 136.7, 128.4, 127.9, 127.6, 66.4, 46.7, 45.1, 40.2, 27.6, 22.6, 15.1.; HRMS (ESI) m/z: calculated for $C_{14}H_{20}N_2O_2$ $[M + Na]^+$ 271.1422; observed 271.1414 . Similarly the trans-isomer (**50a**) was treated with 4N HCl/diox to give **51a** (94%) . Trans (**51a):** ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.18 (br. s., 3 H) 7.04 - 7.54 (m, 5 H) 4.90 - 5.17 (m, 2 H) 4.36-4.30 (m,, 1 H) 3.99 (d, *J*=14.67 Hz, 1 H) 3.39 (br. s., 1 H) 3.23 (dd, *J*=14.55, 2.57 Hz, 1 H) 1.85 - 2.03 (m, 2 H) 1.77-1.70 (m, 1 H) 1.30 - 1.47 (m, 1 H) 1.11 (d, *J*=6.85 Hz, 3 H).; ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 154.9, 136.7, 128.4, 127.8, 127.5, 66.4, 45.8, 44.7, 22.8, 20.6, 15.3.; HRMS (ESI) m/z: calculated for $C_{14}H_{20}N_2O_2$ $[M + H]^+$ 249.1603; observed 249.1598

*Step 6: rac-(2S,5R)-benzyl 5-((2-chloro-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-2-methylpiperidine-1-carboxylate (**52**).* A mixture of **27** (21.8 g, 0.116 mol), DIPEA (67.7 g, 0.525 mol) and racemic cis (2S,5R)-benzyl 5-amino-2-methylpiperidine-1-carboxylate **51b** (30 g, 0.105 mol) in nBuOH (300 mL) was heated to 140 °C overnight. LC-MS indicated the reaction was completed. The reaction mixture was cooled to room temperature and evaporated to dryness; the residue was partitioned with EtOAc (500 mL) and water (500 mL). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to give crude product, which was triturated with MTBE to give (36 g, 86 %) **52** as a yellow solid. ; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.70 (br. s., 1 H) 7.71 (d, *J*=7.83 Hz, 1 H) 7.26 - 7.48 (m, 5 H) 7.10 (s, 1 H) 6.56 (s, 1 H) 4.93 - 5.20 (m, 2 H) 4.39 (br. s., 1 H) 3.91 - 4.26 (m, 2 H) 3.18 (d, *J*=5.14 Hz, 1 H) 2.77 (br. s., 1 H) 1.54 - 1.91 (m, 4 H) 1.17 (d, *J*=7.09 Hz, 3 H); ^{13}C NMR (125 MHZ, DMSO-d_6) δ ppm 156.1, 152.5, 150.8, 136.9, 128.4, 127.8, 127.4, 121.6, 101.2, 98.9, 66.2, 48.6, 42.5, 28.5, 24.5.; HRMS (ESI) m/z: calculated for $C_{20}H_{22}ClN_5O_2$ $[M + H]^+$ 400.1540; observed 400.1535.

*Step 7: rac -N-((3R,6S)-6-methylpiperidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**53**).* To a dry hydrogenation bottle, 10% dry Pd/C (7 g) was added under Ar atmosphere. Subsequently, a solution of **52** (36 g, 0.09 mol) in MeOH (1500 mL) and THF (250 mL) was added and the resulting mixture was shaken on a Parr apparatus (45 psi of H_2 at 25 ^{o}C for 48 hours). LC-MS indicated the Cbz was removed completely, but ~30% of chloride remained. The reaction mixture was filtered and the filtrate was subjected to the reaction conditions again with 5 g of 10% dry Pd/C under 50 psi of H_2 at 45 ^{o}C for 12 h. LC-MS showed the reaction was completed. The reaction mixture was filtered through a pad of Celite® and the cake was washed with MeOH three times. The combined filtrates were concentrated to give **53** (23 g, 94.6%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.47 (br. s., 1 H) 8.08 (s, 1 H) 7.08 (d, *J*=2.69 Hz, 1 H) 6.85 (d, *J*=7.34 Hz, 1 H) 6.65 (br. s., 1 H) 4.03 - 4.25 (m, 1 H) 2.95 (d, *J*=12.47 Hz, 1 H) 2.82 (dd, *J*=12.72, 2.69 Hz, 1 H) 2.56 - 2.66 (m, 1 H) 2.04 (br. s., 1 H) 1.86 - 1.97 (m, 1 H) 1.63 (tt, *J*=12.72, 3.91 Hz, 1 H) 1.40 - 1.49 (m, 1 H) 1.24 - 1.39 (m, 1 H) 1.04 (d, *J*=6.36 Hz, 3 H).; ^{13}C NMR (125 MHz, DMSO-

d_6) δ ppm 155.4, 151.9, 150.2, 120.7, 102.4, 98.7, 50.7, 49.4, 44.3, 29.5, 28.2, 21.8.; HRMS (ESI) m/z: calculated for $C_{12}H_{17}N_5$ [M + H]+ 232.1562; observed 232.1558.

*Step 8: rac-1-((2S,5R)-5-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-2-methylpiperidin-1-yl)prop-2-en-1-one (**54**).* To a stirred solution of HCl salt **53** (5.00 g, 18.5 mmol) in THF (250 mL) and saturated aq. $NaHCO_3$ solution (250 mL) was added acryloyl chloride (2.02 g, 22.2 mmol) dropwise at 0 °C carefully. After addition, the resulting mixture was stirred at 0 °C for 4 hours. TLC (DCM/MeOH/NH_4OH, 10:1:1) showed **53** was consumed completely. The reaction mixture was diluted with H_2O (125 mL) and extracted with EtOAc (125 mL x 3); the combined organic layer was washed with brine, and dried over Na_2SO_4. The most volatile components were removed in vacuo. The crude product was purified by column chromatography on silica gel (DCM/MeOH, 10:1) to give pure product. The product was triturated with EtOAc (150 mL) and filtered to give **54** (2.0 g, 38% yield) as a white solid. Rotamers observed. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.52 (br. s., 1 H) 8.11 (d, *J*=15.65 Hz, 1 H) 7.22 - 7.37 (m, 1 H) 7.05 - 7.15 (m, 1 H) 6.72 - 6.90 (m, 1 H) 6.55 (br. s., 1 H) 6.10 (dd, *J*=16.75, 2.08 Hz, 1 H) 5.68 (dd, *J*=10.51, 1.71 Hz, 1 H) 4.81 (br. s., 0.5 H) 4.56 (d, *J*=10.03 Hz, 0.5 H) 4.38 (br. s., 0.5 H) 3.96 - 4.20 (m, 1.5 H) 2.96 (t, *J*=11.86 Hz, 0.5 H) 2.56 - 2.68 (m, 0.5 H) 1.60 - 1.91 (m, 4 H) 1.09 - 1.30 (m, 3 H); ^{13}C NMR (125 MHZ, DMSO-d_6) δ ppm 164.8, 164.5, 155.3, 151.4, 150.3, 129.0, 127.0, 121.0, 102.5, 98.5, 47.6, 46.9, 46.4, 44.4, 42.7, 40.4, 29.4, 28.5, 25.1, 16.5, 15.2. ^{1}H NMR@353K: ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.31 (br. s., 1 H) 8.12 (s, 1 H) 6.95 - 7.22 (m, 2 H) 6.76 (dd, *J*=16.75, 10.64 Hz, 1 H) 6.56 (dd, *J*=3.18, 1.71 Hz, 1 H) 6.08 (dd, *J*=16.87, 2.45 Hz, 1 H) 5.66 (dd, *J*=10.64, 2.32 Hz, 1 H) 3.93 - 4.21 (m, 1 H) 3.10 (s, 3 H) 1.56 - 2.00 (m, 4 H) 1.22 (d, *J*=6.85 Hz, 3 H) ;^{13}C NMR@353K : ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 164.5, 155.2, 151.0, 150.2, 128.9, 125.9, 120.5, 102.3, 98.1, 46.9, 28.7, 24.9, 15.4; HRMS (ESI) m/z: calculated for $C_{15}H_{19}N_5O$ [M + H]+ 286.1668; observed 286.1664.

*Step 9: (+)-1-((2R,5S)-5-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-2-methylpiperidin-1-yl)prop-2-en-1-one (**13**) and (-) 1-((2S,5R)-5-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-2-methylpiperidin-1-yl)prop-2-en-1-one (**11**)*. Compound **54** was purified by chiral SFC to give pure enantiomers. Peak 1, RT = 7.81 min, (4.63 g, +) **13** and peak 2, RT = 8.45 min, (4.42 g, -) **11** SFC conditions: Column: ChiralPak IC (300 mm*50 mm, 10 μm); Mobile phase: 40% ethanol (0.05% NH_3 in H_2O) in CO_2; Flow rate: 200 mL/min; wavelength: 220nm. The absolute stereochemistry was assigned based on X-ray crystallographic analysis with protein (see Supporting information). **13:** Peak 1**:**. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.52 (br. s., 1 H) 8.11 (d, *J*=15.65 Hz, 1 H) 7.22 - 7.37 (m, 1 H) 7.05 - 7.15 (m, 1 H) 6.72 - 6.90 (m, 1 H) 6.55 (br. s., 1 H) 6.10 (dd, *J*=16.75, 2.08 Hz, 1 H) 5.68 (dd, *J*=10.51, 1.71 Hz, 1 H) 4.81 (br. s., 0.5 H) 4.56 (d, *J*=10.03 Hz, 0.5 H) 4.38 (br. s., 0.5 H) 3.96 - 4.20 (m, 1.5 H) 2.96 (t, *J*=11.86 Hz, 0.5 H) 2.56 - 2.68 (m, 0.5 H) 1.60 - 1.91 (m, 4 H) 1.09 - 1.30 (m, 3 H); LC-MS (ESI) m/z 286.2 $[M+H]^+$; $[a]_D^{20}$ = +0.34 (c = 0.6, MeOH).; HRMS (ESI) m/z: calculated for $C_{15}H_{19}N_5O$ $[M + H]^+$ 286.1668; observed 286.1625. **11:** Peak 2**:** ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.52 (br. s., 1 H) 8.11 (d, *J*=15.65 Hz, 1 H) 7.22 - 7.37 (m, 1 H) 7.05 - 7.15 (m, 1 H) 6.72 - 6.90 (m, 1 H) 6.55 (br. s., 1 H) 6.10 (dd, *J*=16.75, 2.08 Hz, 1 H) 5.68 (dd, *J*=10.51, 1.71 Hz, 1 H) 4.81 (br. s., 0.5 H) 4.56 (d, *J*=10.03 Hz, 0.5 H) 4.38 (br. s., 0.5 H) 3.96 - 4.20 (m, 1.5 H) 2.96 (t, *J*=11.86 Hz, 0.5 H) 2.56 - 2.68 (m, 0.5 H) 1.60 - 1.91 (m, 4 H) 1.09 - 1.30 (m, 3 H); LC-MS (ESI) m/z 286.2 $[M+H]^+$; $[a]_D^{20}$ = -0.36 (c = 0.6, MeOH).; HRMS (ESI) m/z: calculated for $C_{15}H_{19}N_5O$ $[M + H]^+$ 286.1668; observed 286.1692.

Synthesis of 1-[(2R,5R)-2-methyl-5-(7H-pyrrolo[2, 3-d]pyrimidin-4-ylamino) piperidin-1-yl]prop-2-en-1-one (12) pk2 and 14: Pk1, 1-[(2S,5S)-2-methyl-5-(7H-pyrrolo[2, 3-d]pyrimidin-4-ylamino)piperidin-1-yl]prop-2-en-1-one (14) pk1 . *Step 1: rac-(2R, 5R)-benzyl 5-((2-chloro-7H-pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-2-methylpiperidine-1-carboxylate (**55**).* A mixture of **27** (266.5 mg, 1.425 mmol), DIPEA (613 mg, 4.75 mmol) and rac-(2R, 5R)-benzyl 5-amino-2-methylpiperidine-1-carboxylate **51a** (270 mg, 0.950 mmol) in nBuOH(10 mL) was heated to 130 ^{o}C overnight. LC-MS indicated **51b** was consumed

completely. The reaction mixture was cooled to room temperature and evaporated to dryness *in vacuo* and the residue purified by chromatography (silica, PE/EA, 12%-100%)to give **55** (290 mg, 76.5%) as a white solid. ^{1}H NMR (400MHz, DMSO-d_6) δ 11.70 (br s, 1H), 7.57 (d, *J*=5.8 Hz, 1H), 7.21 -6.98 (m, 6H), 6.78 (br s, 1H), 5.04-4.92 (m,1H), 4.92 -4.79 (m, 1H), 4.34 (br s, 1H), 4.29-4.14 (m, 2H), 3.19 (d, *J*=12.3 Hz,1H), 2.27 -2.12 (m, 1H), 2.08 -1.95 (m, 1H), 1.66 (d, *J*=11.5 Hz, 1H), 1.44 -1.30 (m,1H), 1.22 —1.10 (m, 3H).

*Step 2: rac-N-((3R, 6R)-6-methylpiperidin-3-yl)-7Hpyrrolo[2, 3-d]pyrimidin-4-amine (**56**)* . To a Parr bottle, 10% Pd/C (100 mg) was added under Ar atmosphere. Then a solution of(2R, 5R)-benzyl 5-((2-chloro-7H-pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-2-methylpiperidine-1-carboxylate **55** (290 mg, 0.727 mmol) in MeOH (20 mL) was added and the resulting mixture was hydrogenated under 45 psi of H~ at 25 'C for 18 hours. After TLC (DCM/MeOH, 10:1) indicated the starting material to be consumed, the reaction mixture was filtered and the filter cake was washed with MeOH. The combined filtrate was evaporated to give **56** (180 mg, 100%) as a white solid.

*Step 3: rac- 1-((2R,5R)-5-((7H pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-2-methylpiperidin-1-yl)prop-2-en-1-one (**57**)***.** To a stirred solution of **56** (130 mg, 0.563 mmol) in aq. $NaHCO_3$ solution (1mL) and THF (1 mL) at 0 ^{o}C was added acryloyl chloride (55.7 mg, 0.619 mmol) dropwise. After the addition, the resulting mixture was stirred at 0 ^{o}C for 3 hours. TLC (CH_2Cl_2/MeOH/NH_4OH = 10:1:1) indicated **56** was consumed completely. The reaction mixture was diluted with H_20 (5 mL) and extracted with EtOAc (5 mLx4), the combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated to give crude product, which was purified by prep TLC to give **57** (30 mg, 18.75%).

*Step 4. 1-[(2S,5S)-2-Methyl-5-(7H-pyrrolo[2, 3-d]pyrimidin-4-ylamino) piperidin-1-yl]prop-2-en-1-one(**14**) and 1-[(2R,5R)-2-methyl-5-(7H-pyrrolo[2, 3-d]pyrimidin-4-ylamino) piperidin-1-yl]prop-2-en-1-one (**12**)* . Compound **57** was purified by chiral SFC to give two peaks, stereochemistry arbitrarily

assigned: **14**: Pk1, (5.1 mg) and **12**: Pk2, (5.2 mg).; SFC Conditions: Column: ChiralPak IC 250x4.6mm I.D. , 5 pm; Mobile phase: ethanol (0.05% DEA) in CO_2 from 5% to 40%; Flow rate: 2.35mL/min; Wavelength: 215nm.; **14**: Pk1: LC-MS (ESI) m/z 286.2 $[M+H]^+$; ^{1}H NMR (400MHz, MeOH-d_4) δ 8.15 (s, 1H), 7.06 (d, *J*=3.5 Hz, 1H), 6.64 (d, *J*=3.3 Hz, 1H), 6.28 (br s, 1H), 5.95 (br s, 1H), 5.34 (br s, 1H), 4.59 (br s, 1H), 4.38 (br s, 2H), 3.53 —3.34 (m, 1H), 2.25 —2.09 (m, 2H), 1.80 (br s, 1H), 1.57 —1.45 (m,1H), 1.29 (d, *J*=6.8 Hz, 3H). ; **12:** Pk2: LC-MS (ESI) m/z 286.2 $[M+H]^+$; ^{1}H NMR (400MHz, MeOH-d_4) δ 8.16 (s, 1H), 7.07 (d, *J*=3.5 Hz, 1H), 6.65 (d, *J*=3.3 Hz, 1H), 6.30 (br s, 1H), 5.96 (br s, 1H), 5.35 (br s,1H), 4.59 (s, 1H), 4.50-4.22 (m, 2H), 3.52-3.34 (m, 1H), 2.23-2.17 (m, 2H), 1.89-1.76 (m, 1H), 1.57 —1.48 (m, 1H), 1.29 (s, 3H).

Synthesis of 1-((3R,5S)-3-((7H-Pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-5-methylpiperidin-1-yl) prop-2-en-1-one (15) and 1-((3S,5R)-3-((7H-pyrrolo[2, 3-d]pyrimidin-4-yl) amino)-5-methylpiperidin-1-yl)prop-2-en-1-one (17). *Step 1: tert-butyl (5-methylpyridin-3-yl)carbamate (**59**)*. A solution of 5-methylpyridin-3-amine **58** (20 g, 185 mmol) and $(Boc)_2O$ (44.4 g, 203.5 mmol) in THF (360 mL) was stirred at room temperature for 5 h. TLC (PE/ EtOAc, 1:1) indicated the reaction was complete. The reaction mixture was concentrated, and triturated with MTBE to give **59** (26.4 g, 69%) as a white solid. ^{1}H NMR(400MHz, $CDCl_3$) δ 8.21 (d, *J*=2.3 Hz, 1H), 8.15-8.10 (m, 1H), 7.88 (br s, 1H), 6.66 (br s, 1H), 2.33 (s, 3H), 1.53 (s, 9H).

*Step 2: rac-cis/trans- tert-Butyl (5-methylpiperidin-3-yl)carbamate (**60**)*. To a dry hydrogenation bottle, PtO_2 (3.0 g) was added under N_2 atmosphere. A solution of compound **59** (26.4 g, 127 mmol) in HOAc (300 mL) was added, and the resulting mixture was heated to 50 ^{o}C under 55 psi of H_2 for 5 days. ^{1}H NMR indicated starting material was consumed. The reaction mixture was filtered and the filter cake was washed with MeOH. The combined filtrate was evaporated under high vacuum to give **60** (27.3 g, 100%) as a yellow oil. LC-MS (ESI) m/z 214.2 $[M+H]^+$

*Step 3: rac-cis/trans-Benzyl 3-((tert-butoxycarbonyl)amino)-5-methylpiperidine-1-carboxylate (**61**)*. To a solution of **60** (27.3 g, 127 mmol) in THF (200 mL) and H_20 (100 mL) was added $NaHCO_3$ (40.53

g, 482 mmol, 3.8 eq.) at room temperature, and the reaction stirred at room temperature for 1 h. CbzCl (26 g, 152 mmol, 1.2 eq.) was added dropwise, and stirred at room temperature for 8 h. TLC (PE/EtOAc, 2:1) showed the reaction to be complete. The reaction mixture was extracted with EtOAc (200 mL x 3). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (PE/EtOAc, 8:1 - 4:1) to give **61** (20 g, 45 %, containing some benzyl alcohol) as a white solid. LC-MS (ESI) 348.2 $[M+H]^+$

*Step 4: rac-cis/trans-Benzyl 3-amino-5-methylpiperidine-1-carboxylate (**62**)*. To a solution of **61** (20 g, 57.4 mmol) in DCM (40 mL) was added 4 N HCl (g)/dioxane (50 mL) dropwise at room temperature, and the reaction stirred at room temperature for 6 hrs. LCMS showed the reaction to be complete. The reaction mixture was concentrated, filtered, and the then triturated with MTBE to give **62** (5.8 g, 43%, 0.817 mol HCl) as a gray solid. ^{1}H NMR (400MHz, MeOH-d_4) δ 7.43 -7.27 (m, 5H), 5.14 (s, 2H), 4.50 -4.39 (m, 1H), 4.12 (d, *J*=10.3 Hz, 1H), 4.04-3.90 (m, 1H), 3.74-3.43 (m, 1H), 3.23-3.10 (m, 1H), 2.82-2.59 (m, 1H), 2.40 (s, 1H), 2.26-2.05 (m, 1H), 1.92 (d, J=11.3 Hz, 1H), 1.78-1.58 (m, 1H), 1.30 (s, 1H), 1.25-1.05 (m, 2H), 1.01 -0.93 (m, 3H). LC-MS (ESI) m/z 248.9 $[M+H]^+$

*Step 5: rac-cis-(3R, 5S)-Benzyl 3-((2-chloro-7H-pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-5-methylpiperidine-1-carboxylate (**63**) and rac-trans-(3R, 5R)-benzyl 3-((2-chloro-7Hpyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-methylpiperidine-1-carboxylate (**64**)*. To a mixture of **62** (prepared similarly as described in WO201102904)) (4 g, 14.046 mmol) and 2, 4-dichloro-7H-pyrrolo[2, 3-d]pyrimidine (2.9 g, 15.451 mmol, 1.1 eq.) in n-BuOH (70 mL) at room temperature was added DIPEA (7.248 g, 56.184 mmol, 4.0 eq.). The reaction mixture was heated to 140 ^{o}C for 30 h. After LCMS showed the reaction to be complete, the reaction mixture was concentrated to dryness *in vacuo*. The residue was dissolved in EtOAc (150 mL), and diluted with water (150 mL) and the organic layer was separated. The aqueous layer was extracted with EtOAc (150 mLx2), and the combined organic layers were washed with brine,

dried with sodium sulfate. The solvent was removed under reduced pressure. The residue was purified by column chromatography (PE/EtOAc, 6:1 to 2:1)to give **63** (rac-cis, spot 2 on the TLC plate -high polarity, 1.934 g, 34 %) and **64** (rac-trans, spot 1 on the TLC plate-low polarity, 559 mg, 10 %) as a yellow solid. **63**: Pk2: (rac-cis): ^{1}H NMR (400MHz, DMSO-d_6) δ 11.70 (br s, 1H), 7.70 (d, *J*=7.5 Hz, 1H), 7.45 —7.24 (m, 5H), 7.09 (br s, 1H), 6.58 (br s, 1H), 5.21 -5.01 (m, 2H), 4.33 (br s, 1H), 4.07 -3.96 (m,2H), 3.17 (d, *J*=5.3 Hz, 1H), 2.61 -2.53 (m, 1H), 2.33 (br s, 1H), 2.06-1.94 (m, 1H),1.67 (br s, 1H), 1.29 —1.13 (m, 1H), 0.91 (d, *J*=6.5 Hz, 3H). **64**: Pk1: (rac-trans): ^{1}H NMR (400MHz, DMSO-d_6) δ 11.69 (br s, 1H), 7.63-6.59 (m, 8H), 5.05 (d, *J*=16.8 Hz, 1H), 4.87 (br s, 1H), 4.35-3.95 (m,2H), 3.86-3.51 (m, 2H), 3.11 -2.64 (m, 1H), 2.19 (br s, 1H), 1.90-1.72 (m, 2H),1.56 (br s, 1H), 0.91 (d, *J*=6.5 Hz, 3H),

*Step 6: rac-cis-N-((3R, 5S)-5-Methylpiperidin-3-yl)-7H-pyrrolo [2, 3-d]pyrimidin-4-amine (**65**)*. To a dry Parr hydrogenation bottle, dry Pd/C (500 mg) was added under N_2 atmosphere. Then, a solution of **63** (rac-cis, 1.934 g, 4.835 mmol) in CH_3OH/THF (60 mL/20 mL) was added. The resulting mixture was heated to 40 ^oC under 50 psi of H_2 for 3 days. After LCMS showed the reaction to be complete and Cl atom was removed, the reaction mixture was filtered, and the filter cake was washed with MeOH. The combined filtrate was evaporated to give **65** (rac-cis, 1.4 g, 100%) as a pink solid. LC-MS (ESI) m/z 231.2 $[M+H]^+$

*Step 7: rac-cis-1-((3R, 5S)-3-((7H-Pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-5-methylpiperidin-1-yl)prop-2-en-1-one (**66**)*. To a solution of **65** (400 mg, 1.49 mmol) in THF (20 mL) was added saturated aq. $NaHCO_3$ (15 mL) at 0 ^oC was added acryloyl chloride (149 mg, 1.64 mmol, 1.1 eq.) slowly. The reaction was stirred at 0 ^oC for 2 hours. After TLC (EtOAc/ MeOH, 10:1) showed the reaction to be complete, the reaction mixture was diluted with water (80 mL), and extracted with EtOAc (80 mLx2). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (EtOAc/MeOH, 10:1) to give **66** (300 mg, 71%) as a white solid. ^{1}H NMR

(400MHz, DMSO-d_6) δ 11.52 (br s, 1H), 8.10 (d, *J*=14.3 Hz, 1H), 7.39-7.22 (m, 1H), 7.07 (br s, 1H), 6.94 —6.78 (m, 1H), 6.56 (br s, 1H), 6.12 (dd, *J*=8.9, 16.2 Hz, 1H), 5.69 (t, *J*=10.4 Hz, 1H), 4.71 (d, *J*=10.0 Hz, 1H), 4.47 -4.29 (m, 1H), 4.03 (d, *J*=11.0 Hz, 2H), 2.73 (t, *J*=11.5 Hz, 1H), 2.58 (t, *J*=12.3 Hz, 1H), 2.40 —2.30 (m, 1H), 2.19 (t, *J*=11.5 Hz, 1H), 2.05 (d, *J*=11.8 Hz, 1H), 1.36 —1.17 (m, 1H), 0.97 —0.89 (m, 3H). LC-MS (ESI) m/z 285.9 $[M+H]^+$

*Step 8: 1-((3R,5S)-3-((7H-Pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-5-methylpiperidin-1-yl) prop-2-en-1-one (**17**) and 1-((3S,5R)-3-((7H-pyrrolo[2, 3-d]pyrimidin-4-yl) amino)-5-methylpiperidin-1-yl)prop-2-en-1-one (**15**)*. Compound **66** was separated by chiral SFC (AD, 250 mm x 30 mm, 20 um, 35% MeOH/NH_4OH, 80 ml/min) to give **17**: (pk1, RT =7.81) and **15**: (pk 2, RT =6.93 min). **17**: Peak 1: ^{1}H NMR (400MHz, DMSO-d_6) δ 11.53 (br s, 1H), 8.10 (d, *J*=14.3 Hz, 1H), 7.42 —7.23 (m, 1H), 7.08 (br s, 1H), 6.86 (td, *J*=11.4, 16.4 Hz, 1H), 6.57 (br s, 1H), 6.18-6.06 (m, 1H), 5.70 (t, *J*=10.2 Hz, 1H), 4.71 (d, *J*=9.8 Hz, 1H), 4.48-4.30 (m, 1H), 4.03 (d, *J*=11.8 Hz, 1H), 2.79-2.54 (m, 1H), 2.42 -2.14 (m, 1H), 2.06 (d, *J*=12.5 Hz, 1H), 1.63 (br s, 1H), 1.39-1.17 (m, 1H), 0.99-0.87 (m, 3H). LC-MS (ESI) m/z 285.9 $[M+H]^+$; **15**:Peak 2: ^{1}H NMR (400MHz, DMSO-d_6) δ 11.53 (br s, 1H), 8.10 (d, *J*=14.6 Hz, 1H), 7.38-7.23 (m, 1H), 7.08 (br s, 1H), 6.94-6.79 (m, 1H), 6.56 (br s,1H), 6.12 (dd, *J*=7.8, 16.8 Hz, 1H), 5.75-5.64 (m, 1H), 4.71 (d, *J*=11.8 Hz, 1H), 4.49-4.30 (m, 1H), 4.03 (d, *J*=11.5 Hz, 1H), 2.81 -2.54 (m, 1H), 2.42 -2.15 (m, 1H), 2.06 (d, *J*=12.3 Hz, 1H), 1.62 (br s, 1H), 1.38 —1.18 (m, 1H), 0.99 —0.88 (m, 3H). LC-MS (ESI) m/z 285.9 $[M+H]^+$

Synthesis of 1-[(3R,5R)-3-Methyl-5-(7H-pyrrolo[2, 3-d]pyrimidin-4-ylamino) piperidin-1-yl]prop-2-en-1-one (16) and 1-((3S,5S)-3-((7H-Pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-5-methylpiperidin-1-yl)prop-2-en-1-one (18). *Step 1: rac-N-((3S,5S)-5-methylpiperidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**67**)*. To a dry hydrogenation bottle, Pd/C (200 mg) was added under N_2 atmosphere. Then, a solution of compound **64** (rac-trans, 559 mg, 1.398 mmol) in CH_3OH/THF (30 mL/10 mL) was added, and the resulting mixture was heated to 40 ^{o}C under 50 psi of H_2 for 3 days. LC-MS showed the reaction was

completed. The reaction mixture was filtered and the filter cake was washed with MeOH. The combined filtrate was evaporated to give compound **67** (rac-trans, 413 mg, 100 %) as a pink solid.

*Step 2: Rac- 1-((3S,5S)-3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-methylpiperidin-1-yl)prop-2-en-1-one (**68**)*. To a solution of compound **67** (413 mg, 1.542 mmol) in THF (20 mL) was added saturated aq.$NaHCO_3$ (15 mL) and acryloyl chloride (154 mg, 1.70 mmol, 1.1 eq.) at 0 ^{o}C. The reaction was stirred at 0 ^{o}C for 2 hours. TLC (EtOAc: MeOH = 10:1) showed the reaction was completed. The reaction mixture was diluted with water (50 mL), and extracted with EtOAc (50 mL×2). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (EtOAc: MeOH = 10:1) to give **68** (221 mg, 50 %) as a white solid.; ^{1}H NMR (400MHz, DMSO-d_6) δ: 11.48 (br. s., 1H), 8.30 - 8.04 (m, 1H), 7.13 - 6.96 (m, 2H), 6.83 (dd, *J*=10.3, 16.8 Hz, 1H), 6.69 - 6.54 (m, 1H), 6.36 (dd, *J*=10.5, 16.6 Hz, 1H), 6.08 (d, *J*=17.8 Hz, 1H), 5.89 (d, *J*=17.1 Hz, 1H), 5.66 (d, *J*=8.8 Hz, 1H), 5.35 (d, *J*=10.5 Hz, 1H), 4.47 - 4.20 (m, 1H), 4.04 - 3.84 (m, 2H), 3.61 - 3.37 (m, 2H), 2.88 - 2.75 (m, 1H), 2.15 (br. s., 1H), 1.93 - 1.76 (m, 1H), 1.71 - 1.53 (m, 1H), 0.98 - 0.88 (m, 3H); LC-MS (ESI) m/z 285.9 [M+H]

*Step 3: 1-[(3R,5R)-3-Methyl-5-(7H-pyrrolo[2, 3-d]pyrimidin-4-ylamino) piperidin-1-yl]prop-2-en-1-one (**16**) and 1-((3S,5S)-3-((7H-Pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-5-methylpiperidin-1-yl)prop-2-en-1-one (**18**)*. Compound **68** (150 mg) was separated by chiral SFC to give two peaks arbitrarily assigned: **16**: (pk 1, RT = 5.81 min, 60 mg, 80 %) as a white solid and **18**: (RT = 6.29 min, pk 2, 60 mg, 80%) as a white solid. SFC conditions: ChiralPak AD(250mmx30mm, 5 μm); 20% EtOH, NH_4OH; 60mL/min. **16**: Pk 1: ^{1}H NMR(400MHz, DMSO-d_6) δ 11.51 (br s, 1H), 8.19 —8.07 (m, 1H), 7.06 (d, *J*=6.0 Hz, 2H),6.84 (dd, *J*=10.2, 16.4 Hz, 1H), 6.70-6.55 (m, 1H), 6.35 (dd, *J*=10.4, 16.7 Hz, 1H),6.08 (d, *J*=18.6 Hz, 1H), 5.88 (dd, *J*=2.3, 16.8 Hz, 1H), 5.66 (d, *J*=10.3 Hz, 1H), 5.35(dd, *J*=2.3, 10.5 Hz, 1H), 4.41 -4.21 (m, 1H), 4.06-3.84 (m, 2H), 3.61 -3.42 (m,1H), 2.85 —2.75 (m, 1H), 2.15 (br s, 1H), 1.99 —1.78 (m, 1H), 1.73 —1.50 (m, 1H),1.23 (s,

1H), 1.00 —0.86 (m, 3H). LC-MS (ESI) m/z 286.1 $[M+H]^+$; **18**: Pk 2: ^{1}H NMR (400MHz, DMSO- d_6) δ 11.51 (br s, 1H), 8.19-8.05 (m, 1H), 7.06 (br s, 1H),6.84 (dd, *J*=10.3, 16.8 Hz, 1H), 6.69-6.55 (m, 1H), 6.35 (dd, *J*=10.5, 16.6 Hz, 1H),6.08 (d, *J*=16.6 Hz, 1H), 5.88 (d, *J*=15.1 Hz, 1H), 5.66 (d, *J*=9.3 Hz, 1H), 5.35 (d, *J*=10.0 Hz, 1H), 4.42-4. 19 (m, 1H), 4.06-3.82 (m, 1H), 3.62-3.44 (m, 1H), 2.86-2.73 (m, 1H), 2.15 (br s, 1H), 1.96 —1.77 (m, 1H), 1.72 —1.53 (m, 1H), 1.23 (br s,1H), 1.01 —0.64 (m, 3H). LC-MS (ESI) m/z 286.1 $[M+H]^+$

Synthesis of 1-((3R,4S)-3-((7H-Pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-4-methylpiperidin-1-yl)prop-2-en-1-one (19) and 1-((3S,4R)-3-((7H-pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-4-methylpiperidin-1-yl) prop-2-en-1-one (20). *Step 1: rac-(3R, 4S)-tert-butyl 3-((2-chloro-7H-pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-4-methylpiperidine-1-carboxylate (**70**)* . To a solution of **69** (prepared as described in WO2011029046) (500 mg, 2.33 mmol) and 2, 4-dichloro-7H-pyrrolo[2, 3-d]pyrimidine **38** (483 mg, 2.56 mmol, 1.1eq.) in ^{n}BuOH (15 mL) was added DIPEA (903 mg, 6.99 mmol, 3.0 eq.) at room temperature, and heated to 140 ^{o}C overnight. After LCMS indicated the reaction was complete, the reaction mixture was concentrated to dryness *in vacuo*. The residue was dissolved in EtOAc (50 mL) and diluted with water (50 mL). The layers were separated and the aqueous layer was extracted with EtOAc (50 mLx2), and the combined organic layers were washed with brine, dried with sodium sulfate. The solvent was removed under reduced pressure. The residue was purified by column chromatography (EtOAc/PE = 8% - 50%) to give **70** (rac-trans, 563 mg, 66%) as a yellow solid. ^{1}H NMR (400MHz, $CDCl_3$) δ 11.92 (br s, 1H), 7.14 (br s, 1H), 6.46 (br s, 1H), 4.40 (d, *J*=9.3 Hz, 1H), 4.08-3.65 (m, 2H), 2.98-2.63 (m, 2H), 1.90 —1.60 (m, 3H), 1.52-1.38 (m, 1H), 1.48 (s, 9H), 1.11 —1.05 (m, 3H).

*Step 2: Rac-(3R, 4S)-tert-Butyl 3-((7H-pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-4-methylpiperidine-1-carboxylate (**71**)*. To a dry Parr hydrogenation bottle, dry Pd/C (100 mg) was added under N_2 atmosphere. A solution of **70** (560 mg, 1.531 mmol) in MeOH /THF (30 mL/10 mL) was added, and the resulting mixture was heated to 40 ^{o}C under 50 psi of H_2 for 2 days. After LCMS showed the reaction to

be complete, the reaction mixture was filtered and the filter cake was washed with MeOH. The combined filtrate was evaporated to give **71** (520 mg, 93 %) as a yellow solid. LC/MS (ESI) m/z 332.2 $[M+H]^+$

*Step 3: Rac-N-((3R, 4S)-4-Methylpiperidin-3-yl)-7H-pyrrolo[2, 3-d]pyrimidin-4-amine (**72**)*. To a solution of **71** (520 mg, 1.531 mmol) in DCM (15 mL) at 0 ^oC was added 4.0 M HCl/ dioxane (15 mL). The reaction mixture was allowed to warm to room temperature and stirred for 3 hours. After LCMS indicated the reaction to be complete, the reaction mixture was concentrated to give **72** (410 mg, 100 %) as a solid. LC/MS (ESI) m/z 232.2 $[M+H]^+$

*Step 4: rac-1-((3R, 4S)-3-((7H-Pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-4-methylpiperidin-1-yl) prop-2-en-1-one (**73**)*. To a solution of **72** (410 mg, 1.530 mmol) in THF (20 mL) and saturated. $NaHCO_3$ (15 mL) at 0 ^oC was added acryloyl chloride (152 mg, 1.683 mmol, 1.1 eq.). The reaction mixture was stirred at 0 ^oC for 2 hours. After TLC (EtOAc/MeOH, 10:1) indicated the reaction to be complete, the reaction mixture was diluted with water (50 mL), and extracted with EtOAc (50 mLx2). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (MeOH/EtOAc, 2%-10%)and lyophilized to give **73** (150 mg, 34 %) as a white solid. ^1H NMR (400MHz, DMSO-d_6) δ 11.53 (br s, 1H), 8.08 (d, *J*=15.1 Hz, 1H), 7.32 —7.20 (m, 1H), 7.08 (br s, 1H), 6.81 (dt, *J* =10.5, 17.3 Hz,1H), 6.59 (br s, 1H), 6.12 (d, *J* =14.8 Hz, 1H), 5.69 (d, *J* =10.3 Hz, 1H), 4.65 —4.39 (m, 1H), 4.27 —4.04 (m, 1H), 3.94-3.71 (m, 1H), 3.08 —2.96 (m, 0.5H), 2.89 —2.77 (m, 0.5H), 2.71 —2.60 (m, 0.5H), 2.46 —2.28 (m, 0.5H), 1.82 (d, *J* =12.3 Hz, 2H), 1.29 —1.12 (m, 1H), 0.94 (dd, *J* =6.0, 12.3 Hz, 3H). LC/MS (ESI) m/z 286.1 $[M+H]^+$

*Step 5: 1-((3R,4S)-3-((7H-Pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-4-methylpiperidin-1-yl)prop-2-en-1-one (**19**) and 1-((3S,4R)-3-((7H-pyrrolo[2, 3-d]pyrimidin-4-yl)amino)-4-methylpiperidin-1-yl) prop-2-en-1-one (**20**)*. Compound **73** (120 mg) was separated by chiral SFC (Chiral Pak-AD (250 x 30 mm, 5um), 30%

EtOH (0.05% NH_3 in H_20) in CO_2) to give a pair of enantiomers, (peak 1,RT = 4.93 min, 47.8 mg) and (peak 2, RT =5.64 min, 48.2 mg) as white solids, absolute stereochemistry arbitrarily assigned. **19**: Peak 1 data: ^{1}H NMR (400MHz, DMSO-d_6) δ 11.53 (br s, 1H), 8.08 (d, *J* =15.1 Hz, 1H), 7.32 -7.20 (m, 1H), 7.08 (br s, 1H), 6.81 (dt, *J* =10.5, 17.3 Hz, 1H), 6.59 (br s, 1H), 6.12 (d, *J* =14.8 Hz, 1H), 5.69 (d, *J* =10.3 Hz, 1H), 4.65-4.39 (m, 1H), 4.27—4.04 (m, 1H), 3.94 —3.71 (m, 1H), 3.08 —2.96 (m, 0.5H), 2.89 —2.77 (m, 0.5H), 2.71—2.60 (m, 0.5H), 2.46 —2.28 (m, 0.5H), 1.82 (d, *J*=12.3 Hz, 2H), 1.29-1.12 (m, 1H), 0.94 (dd, *J* =6.0, 12.3 Hz, 3H). LC/MS (ESI) m/z 285.9 $[M+H]^+$; **20**: Peak 2 data: ^{1}H NMR (400MHz, DMSO-d_6) δ 11.53 (br s, 1H), 8.08(d, *J* =15.1 Hz, 1H), 7.32-7.20 (m, 1H), 7.08 (br s, 1H), 6.81 (dt, *J* =10.5, 17.3 Hz, 1H), 6.59 (br s, 1H), 6.12 (d, *J* =14.8 Hz, 1H), 5.69 (d, *J* =10.3 Hz, 1H), 4.65-4.39 (m,1H), 4.27 -4.04 (m, 1H), 3.94-3.71 (m, 1H), 3.08-2.96 (m, 0.5H), 2.89-2.77 (m,0.5H), 2.71 -2.60 (m, 0.5H), 2.46 -2.28 (m, 0.5H), 1.82 (d, *J* =12.3 Hz, 2H), 1.29—1.12 (m, 1H), 0.94 (dd, *J* =6.0, 12.3 Hz, 3H). LC/MS (ESI) m/z 285.9 $[M+H]^+$

X-ray crystallography

Crystals of Jak3 were prepared as described previously (11). Briefly, the Jak3 kinase domain, encompassing residues 812-1124 and including a 6XHis tag, was purified in the presence of the pan-Jak inhibitor, CMP-6 (36), and crystallized as a complex under the reported conditions. To generate the desired complexes, Jak3/CMP-6 crystals were soaked for ~24 hours in the reservoir solution supplemented with 3 mM compound, resulting in full exchange of the bound CMP-6 for the compound of interest. Data for Jak3/Compound 2 were collected at 100 K on a Rigaku FRE X-ray generator outfitted with a Saturn 944 CCD detector and processed with HKL2000 (37). Data for Jak3/Compound 3 were collected at 100 K at the Life Sciences Collaborative Access Team Beamline 21-ID-D (Advanced Photon Source, Argonne National Laboratory, Argonne, IL, USA), and processed using HKL2000 (37). All other data manipulations utilized the CCP4 (38) suite of programs. For all data sets, a common test set of reflections corresponding to ~5% of the total were used throughout refinement. All structures were

solved by rigid body refinement in REFMAC (39) using a reference structure of Jak3 with all ligands and waters removed. Iterative rounds of refinement using AUTOBUSTER (40) were interspersed with manual model building in COOT (41). The covalent linkage between the acrylamide and the sulfur of Cys 909 was refined and maintained to ideal geometry throughout the refinement process. Crystallographic data statistics and refinement results are detailed in Table 7. Authors will release the atomic coordinates and experimental data upon article publication

Table 7.

Compound	**4**	**6**	**7**
PDB code	5TTS	5TTV	5TTU
A. Data collection			
Space Group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit Cell	a= 47.4	a= 46.6	a= 47.2
	b=75.8	b=75.7	b=75.8
	c=89.6	c=88.7	c=90.1
Resolution (Å)	50-2.34	20-1.95	90-1.72
(high res)	(2.38-2.34)	(2.02-1.95)	(1.73-1.72)
Completeness (%)	99.2 (89.3)	97.0 (97.9)	99.3 (75.4)
R_{sym} [a]	0.05 (0.39)	0.06 (0.33)	0.03 (0.37)
χ^2	1.65 (1.49)	1.05 (0.62)	
Redundancy	4.5 (3.1)	4.4 (4.1)	6.1 (3.6)
$\langle I \rangle / \langle \sigma(I) \rangle$	36.0 (3.9)	27.0 (3.9)	28.8 (2.6)
B. Refinement			
R_{work} [b]	0.202	0.192	0.197
R_{free} [b]	0.263	0.25	0.212
Amino Acid Residues (#)	274	281	275
Waters (#)	184	125	230
RMSD bond length (Å)	0.01	0.01	0.01
RMSD angles (degrees)	1.06	1.13	0.97
Average B ($Å^2$)	47.5	32.2	38.8
Ramachandran (%)	98.9	98.2	99.6
Ramachandran outliers(%)	1.1	1.8	0.4

[a] $R_{sym} = \Sigma_{hkl}(|I_{hkl} - \langle I_{hkl} \rangle|)/\Sigma_{hkl}\langle I_{hkl} \rangle$, where I_{hkl} is the intensity of reflection hkl, and $\langle I_{hkl} \rangle$ is the average intensity of multiple observations.

[b] $R_{work} = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes, respectively. R_{free} is the R-factor for a randomly selected 5% of reflections which were not used in the refinement.

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Supporting Information

Single Molecule X-Ray Crystallography for **38b.** Kinetics of covalent modification of on-target and off-target kinases by 11. Molecular Formula Strings.

Abbreviations Used

JAK3, Janus kinase 3; NMEs, new molecular entity; Cys, cysteine; JAK1, Janus kinase 1; JAK2, Janus kinase 2; TYK2, Tyrosine kinase 2; SCID, Severe combined immunodeficiency; ATP, Adenosine triphosphate; SAR, structure activity relationships; GSH, glutathione; PBMC, peripheral blood mononuclear cell; EGFR, epidermal growth factor receptor; BTK, Bruton's tyrosine kinase; FAAH, Fatty acid amide hydrolase; ABT, aminobenzotriazole; GST, glutathione-S-transferase; DTH, Delayed Type Hypersensitivity; sRBC, sheep red blood cells; ITK, interleukin-2-inducible T-cell kinase; SLK, STE20-like serine/threonine-protein kinase; FLT3, receptor-type tyrosine-protein kinase FLT3; BMX, Cytoplasmic tyrosine-protein kinase BMX

; TXK, Tyrosine-protein kinase TXK; TEC, Tyrosine-protein kinase Tec; BLK, B lymphocyte kinase; HER4, Receptor tyrosine-protein kinase erbB-4; HER2, Receptor tyrosine-protein kinase erbB-2; AIA, adjuvant-induced arthritis; CIA, collagen-induced arthritis; EAE, experimental autoimmune encephalomyelitis

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