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Article

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Highly Potent, Selective and Orally Bioavailable 4-Thiazol-*N*-(pyridin-2-yl)pyrimidin-2-amine Cyclin-Dependent Kinase 4 and 6 Inhibitors as Anticancer Drug Candidates: Design, Synthesis and Evaluation

Solomon Tadesse[†], Mingfeng Yu[†], Laychiluh B. Mekonnen[†], Frankie Lam[†], Saiful Islam[†], Khamis Tomusange[†], Muhammed H. Rahaman[†], Benjamin Noll[†], Sunita K. C. Basnet[†], Theodosia Teo[†], Hugo Albrecht[†], Robert Milne[†] and Shudong Wang[†]*

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ABSTRACT

Cyclin D dependent kinases (CDK4 and CDK6) regulate entry into S phase of the cell cycle, and are validated targets for anti-cancer drug discovery. Herein we detail the discovery of a novel series of 4-thiazol-*N*-(pyridin-2-yl)pyrimidin-2-amine derivatives as highly potent and selective inhibitors of CDK4 and CDK6. Medicinal chemistry optimization resulted in **83**, an orally bioavailable inhibitor molecule with remarkable selectivity. Repeated oral administration of **83** caused marked inhibition of tumour growth in MV4-11 acute myeloid leukemia mouse xenografts without having a negative effect on body weight and showing any sign of clinical toxicity. The data merit **83** as a clinical development candidate.

INTRODUCTION

Cell cycle is an ordered sequence of events that leads to the transition from quiescence or cytokinesis to cell proliferation, and through its checkpoints, ensures genome stability.[1] It comprises four sequential phases *viz.* S phase, when DNA synthesis occurs; M phase, when the cell divides into two daughter cells; G1 phase after mitosis and before S phase, when cellular biosynthetic events take place at high rate and cells grow in size; G2 phase occurring between S and M phases, during which cells prepare for mitosis. Cells decide to enter S phase or remain quiescent using signaling pathways that link extracellular cues (e.g. growth factors) to G1 phase of the cell cycle.[2] Progression through G1 phase is regulated by retinoblastoma tumor suppressor proteins (Rbs), which repress the activity of E2 promoter binding factor (E2F) transcription factors whose functions are required for transition from G1 to S phase. Phosphorylation of Rb by G1-phase CDKs releases E2F transcription factors, promoting the transcription of genes that encode proteins necessary for DNA replication (e.g. *cyclin A* and *cyclin E*). The primary kinases that phosphorylate Rb proteins during G1 phase in mammalian cells are CDK4 and CDK6, hereafter referred to as CDK4/6.[3]

Previous studies have shown that the CDK4/6-Rb-E2F pathway is disrupted in 90% of cancers.[4-7] Besides, genetic studies have established that CDK4/6 are dispensable for the mitotic cell cycle, evidenced by the facts that mice lacking these kinases are viable and that inactivation of the *CDK4/6* genes only affects the proliferation of specific cell types .[8,9] Thus, CDK4/6 represent logical targets for the development of small-molecules for therapeutic intervention in cancers.[10] Palbociclib (Figure 1), the first and only approved CDK4/6 inhibitor for the treatment of metastatic breast cancer, has rejuvenated the field of selective CDK inhibitors.[11] In addition,

CDK4/6 inhibitors are now becoming recognized as important research tools to answer complex biological questions.[12,13]

As most inhibitors bind to the highly conserved ATP binding site, kinase selectivity remains unconquered. For instance, the *in vitro* kinase binding profile of the advanced clinical CDK4/6 inhibitors *i.e.* palbociclib, ribociclib and abemaciclib (Figure 1) against a near kinome-wide panel disclosed several additional targets.[14,15] The off-targets can impair drug safety and contribute to differential cellular potencies and dissimilar clinical responses. For example, palbociclib and ribociclib have principally bone marrow toxicities with little toxic effect on gastrointestinal tract. In contrast, abemaciclib causes abundant gastrointestinal adverse effects. Palbociclib and ribociclib are dosed intermittently (three weeks on and one week off) while abemaciclib is administered continuously due to a relatively low incidence of neutropenia.[14,15] Jointly, these lines of evidence suggest that new pharmacophores might afford the desired kinase selectivity profiles and thus offer an effective way to minimize undesirable side effects.

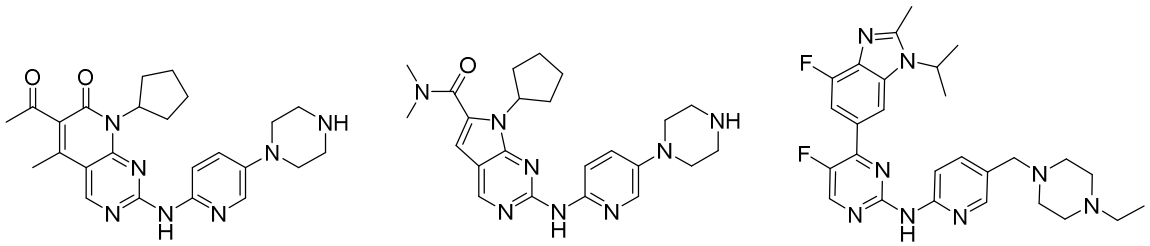


Figure 1. Structures of the advanced clinical CDK4/6 inhibitors.

In continuation of our search for new and selective CDK-targeting drugs,[16-18] herein we present the discovery of potent, highly selective, and orally bioavailable CDK4/6 inhibitor **83**. We demonstrate **83** exhibits significant anti-cancer efficacy in an MV4-11 acute myeloid leukemia (AML) subcutaneous xenograft mouse model.

RESULTS AND DISCUSSION

Rational Drug Design. Due to evolutionary conservation of the ATP-binding site, the discovery of highly selective small-molecule ATP-antagonistic CDK inhibitors is a daunting task.[10,19-21] In a bid to identify both potent and selective ATP competitive CDK2 or CDK9 inhibitors, we have previously reported the discovery of the 2-anilino-4-(thiazol-5-yl)pyrimidine pharmacophore (Figure 2).[16-18]

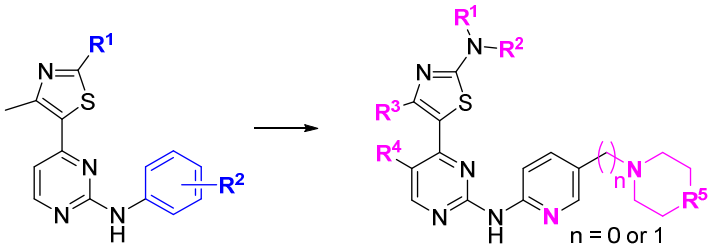


Figure 2. Structural modifications to 2-anilino-4-(thiazol-5-yl)pyrimidine pharmacophore.

Our previous studies on exploration of structure-activity relationships (SARs) of 2-anilino-4-(thiazol-5-yl)pyrimidines focused on modifications to the aniline and thiazole moieties of the pharmacophore. A large number of 2-anilino-4-(thiazol-5-yl)pyrimidine analogues were synthesized and screened against a wide panel of CDKs, and the results thus obtained disclosed several compounds with modest CDK4 inhibitory potency.[16-18] We also proposed that incorporation of a geometrically fitting, positively ionizable group into the 2-anilino-4-(thiazol-5-yl)pyrimidine scaffold would result in favorable interaction(s) with the acidic residue(s) Asp99 and/or Glu144 in the ATP binding site of CDK4 (Figure 3).[22] As hypothesized, amine-substituted anilines positioned at C2 of the pyrimidine ring afforded moderately potent and relatively selective CDK4 inhibitors. In addition, methylation of the bridging NH between the phenyl and pyrimidine moieties led to an inactive compound. This was not surprising because this bridging NH and pyrimidinyl-N1 act as a key hydrogen bond donor and acceptor to interact

with the backbone carbonyl and the NH groups of Val in the kinase hinge region, respectively. In another relevant study, it was also speculated that the interactions with the relatively less conserved hinge regions of CDK4/6 would be enhanced by the introduction of aromatic sp^2 nitrogen atom near to the rarely conserved His95 in CDK4 (His100 in CDK6).[23] In fact, Toogood *et al*. have shown that amino-pyridinyl compounds possess exquisite selectivity towards CDK4/6 over CDK2 than do their anilino counterparts.[24] More recently, the pyridinyl nitrogen atom of abemaciclib has been shown to interact with the imidazole ring of His100 of CDK6 *via* a water bridge, and such an interaction is also possible in the cases of palbociclib and ribociclib due to the three molecules sharing the common pyrimidine-NH-pyridine motif.[15]

Based on this knowledge, we explored whether structural modifications to the 2-anilino-4-(thiazol-5-yl)pyrimidine pharmacophore would lead to the generation of potent CDK4/6-specific inhibitors. To this end, we replaced the phenyl moiety with a pyridine ring, incorporated a variety of ionizable groups, and introduced an electron withdrawing group at C5 position of the pyrimidine ring. On the other hand, we retained some structural features that were shown to contribute towards the potency of the pharmacophore. The pyrimidine ring, which by virtue of its N1 and N3 is expected to make a hydrogen bond with Val of the hinge region of CDK4/6 and to ensure co-planarity of the pyrimidine and pyridine rings (an essential feature for ATP mimetic kinase inhibitors), respectively, was retained. Our previous studies showed that the thiazole C2-amino site interacted strongly with the Asp145 residue of the DFG motif of CDK2 (Asp167 in CDK9), enhancing the hydrophobic interactions of the thiazol-4-yl methyl group with the Phe80 gatekeeper residues of CDK2 (Phe103 in CDK9),[16-18,22] and these interactions are expected with CDK4/6 because the DFG motif and the gatekeeper Phe are highly conserved in the CDK family. As a result, the thiazole C2-amino moiety was not significantly altered. A total of 37 derivatives

of 4-(4-methylthiazol-5-yl)-*N*-(pyridin-2-yl)pyrimidin-2-amine (Figure 2) were synthesized accordingly and their CDK inhibitory activities and anti-proliferative effects are summarized in Tables 1-4.

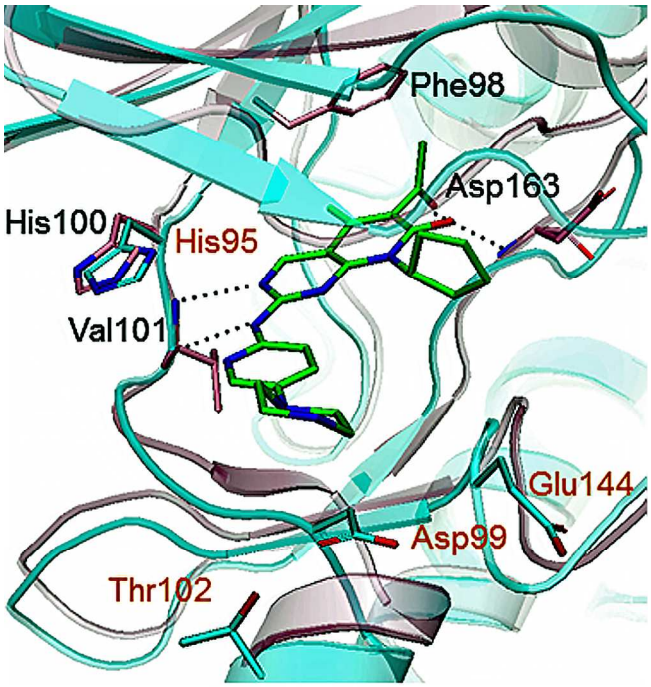
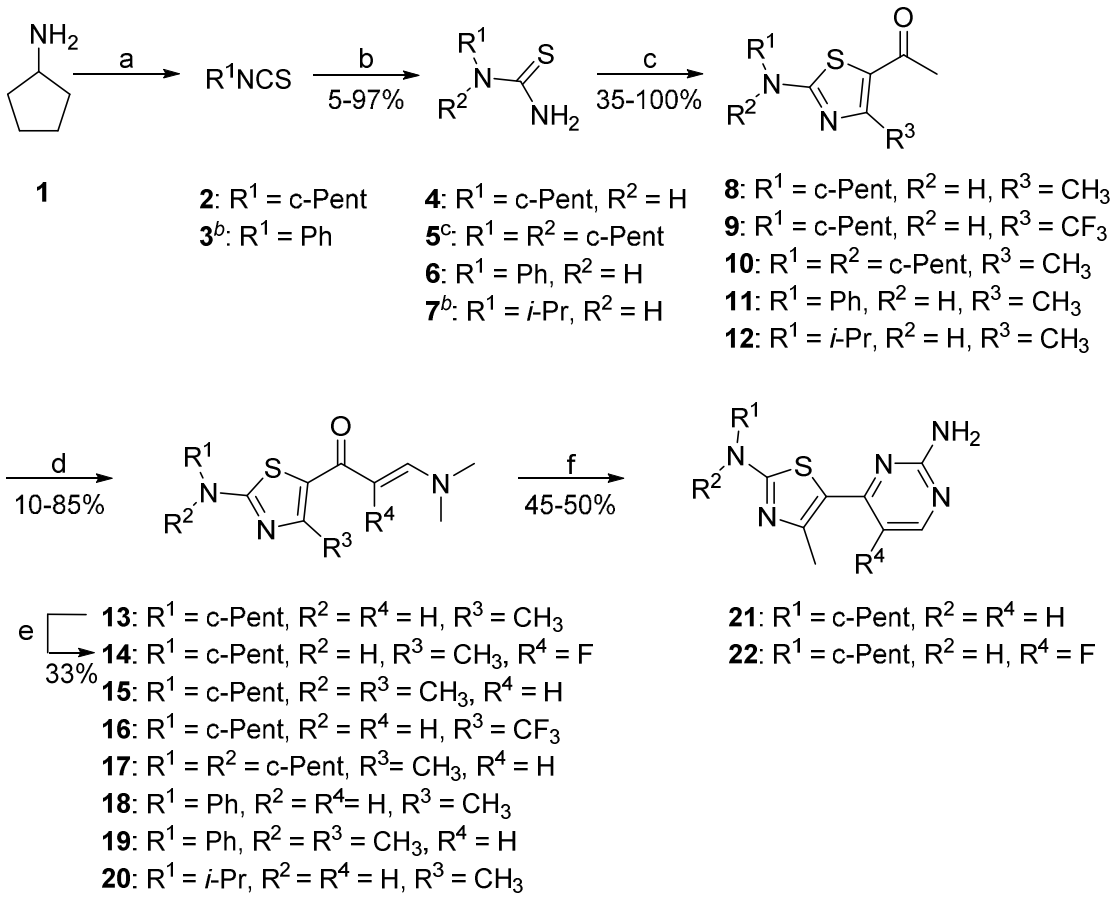


Figure 3. ATP binding site of CDK6 in complex with the inhibitor palbociclib (PDB ID: 2EUF) aligned with that of CDK4 (PDB ID: 2W96). CDK6 is shown in brown and CDK4 in cyan. Palbociclib, shown in green and bound in the ATP binding pocket of the kinases, forms two hydrogen bonds with conserved Val101 and one hydrogen bond with conserved Asp163. The hydrogen bonds are shown in black dashed lines. His95, Asp99, Thr102 and Glu144 of CDK4 are shown in cyan sticks. The figure was prepared using PyMOL1.3 (Schrödinger Inc., 2013).

Synthetic Chemistry. The synthesis of 4-thiazol-*N*-(pyridin-2-yl)pyrimidin-2-amine derivatives **78-112**, **115** and **116** requires the preparation of two pairs of building blocks, *i.e.,* enaminones/guanidines and pyrimidin-2-amines/aryl bromides. The synthetic route to a variety of enaminones and two pyrimidin-2-amines is described in Scheme 1.

Isothiocyanatocyclopentane **2** was synthesized from the commercially available cyclopentylamine **1** using a procedure reported by Munch *et al*.[25] Treatment of isothiocyanates **2** or **3** (obtained from commercial source) with ammonia afforded *N*-substituted thioureas **4**-**6**, with *N*,*N*-disubstituted thiourea **5** isolated as a by-product in the synthesis of **2**.[26] We speculate that **5** is formed from dicyclopentylcarbamodithioate, which is formed by the reaction between cyclopentyl(dithiocarboxy)amide intermediate and a halocyclopentane (minor contaminant of cyclopentylamine starting material). The dicyclopentylcarbamodithioate can then be desulfurylated by reacting with Boc_2O and ammonia to yield 5. 5-Acetylthiazoles **8**-**12** were prepared by the reaction of 3-chloro-2,4-pentanedione with the prepared thioureas **4**-**6** or the commercially available isopropyl thiourea **7** using Hantzsch-Traumann thiazole synthesis method[27] with the single exception of analogue **9** which was made from a two-step reaction of 1,1,1-trifluoropentane-2,4-dione with [hydroxy(tosyloxy)iodo]benzene and 1-cyclopentylthiourea **4**, sequentially.[28] 5-Acetylthiazoles **8**-**12** were readily converted to the corresponding enaminones **13** and **15**-**20** either by refluxing with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA)[29] or using *tert*-butoxybis(dimethylamino)methane (Bredereck's reagent) as previously reported.[18,29] Enaminone **13** was fluorinated with SelectFluor in methanol on an ice bath to give fluoride **14**.[17] The synthesis of pyrimidin-2-amines **21** and **22** was achieved by the coupling of enaminones **13** and **14** with guanidine hydrochloride, a classical route to construct pyrimidine ring systems.[30]

Scheme 1. Synthesis of enaminones **13**-**20** and pyrimidin-2-amines **21** and **22**[a]



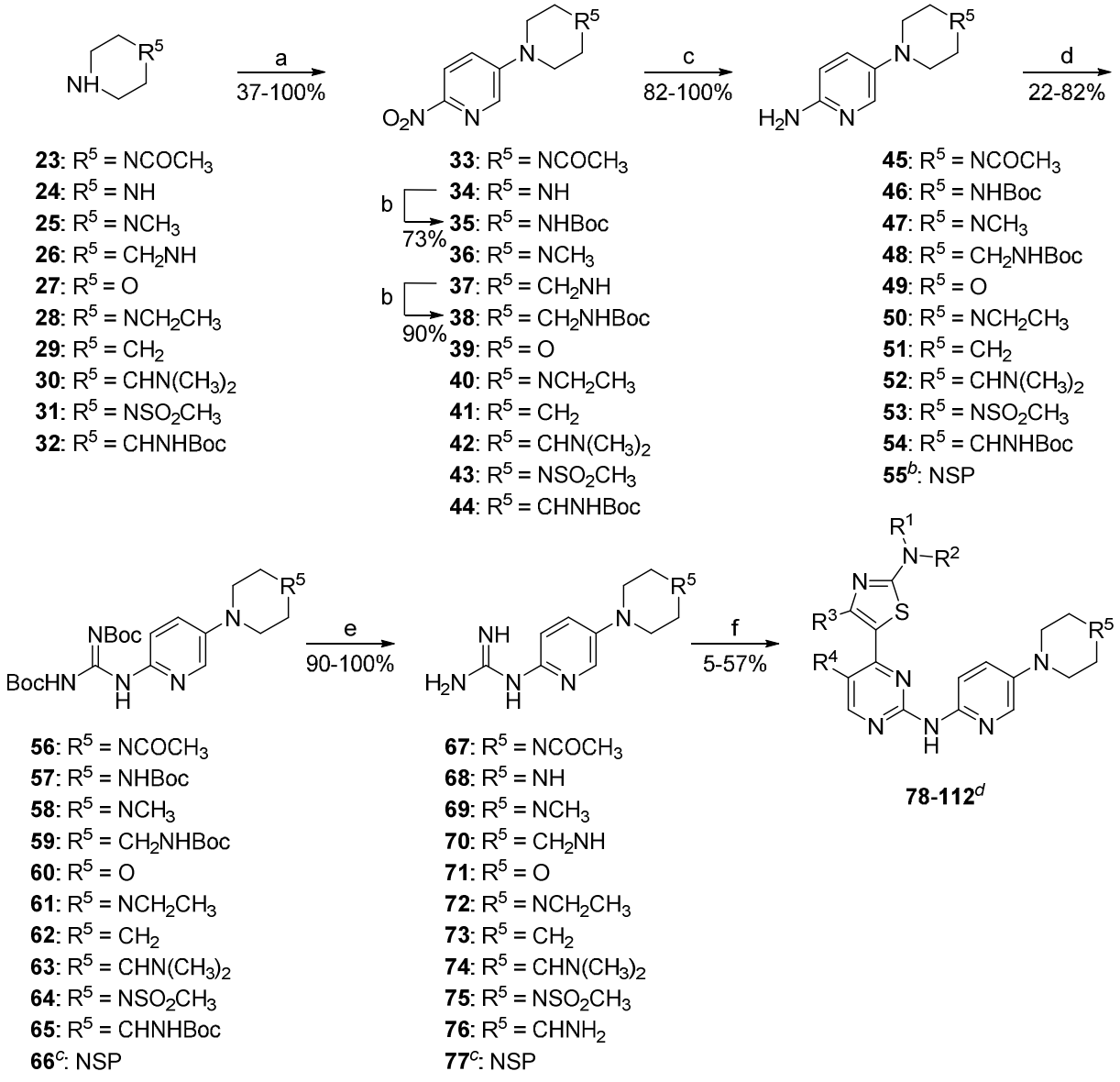
[a]Reagents and conditions: (a) CS_2, NEt_3, Boc_2O, DMAP, EtOH, rt, 30 min, ice bath, 15 min, rt, 30 min; (b) NH_3, H_2O, MeOH, rt, 24 h; (c) 3-chloro-2,4-pentadione, pyridine, MeOH, rt, 4-48 h; or 1,1,1-trifluoropentane-2,4-dione, hydroxyl(tosyloxyl)iodobenzene, MeCN, reflux, 1 h; (d) DMF-DMA, reflux, 3-36 h; or microwave, 150 °C, 30 min; or Bredereck's reagent, reflux, 6 h; (e) SelectFluor, MeOH, ice bath, 4 h; (f) **13** or **14**, guanidine hydrochloride, NaOH, 2-methoxyethanol, reflux, 6 h. [b]Intermediates **3** and **7** were obtained from commercial sources. [c] most likely from dicyclopentylcarbamodithioate, which is formed by the reaction between cyclopentyl(dithiocarboxy)amide intermediate and a halocyclopentane (minor contaminant of cyclopentylamine starting material) .

The synthesis of a wide range of guanidines is depicted in Scheme 2. Nucleophilic substitution of 5-bromo-2-nitropyridine with various *N*-heterocycles **23**-**32** in dimethylsulfoxide furnished

nitro compounds **33**, **34**, **36**, **37**, and **39-44** in moderate to excellent yields.[31] The secondary amino group of **34** and **37** was found to interfere with the subsequent guanidine formation with *N*,*N'*-bis-Boc-*S*-methylisothiourea, and therefore was masked by the *tert*-butyloxycarbonyl (Boc) group to give intermediates **35** and **38**, respectively. Nitro compounds **33**, **35**, **36** and **38-44** were reduced with gaseous hydrogen in the presence of 10% Pd/C in methanol to give respective amines **45-54** in good to excellent yields (82-100%), each of the amines **45-54** and the commercially available pyridine-2-amine **55** were subsequently coupled with *N*,*N*'-bis-Boc-*S*-methylisothiourea in the presence of Et_3N and $HgCl_2$ to afford bis-Boc-protected guanidine derivatives **56-66**.[32] Removal of Boc groups from **56-66** was carried out in a mixture of TFA and DCM (1:1) at reflux, giving the corresponding guanidines **67-77** in quantitative yields. The last building block 1-((6-bromopyridin-3-yl)methyl)-4-ethylpiperazine **114** was prepared in 25% yield by reductive amination of 6-bromopyridine-3-carbaldehyde **113** with ethylpiperazine using sodium triacetoxyborohydride (Scheme 3).

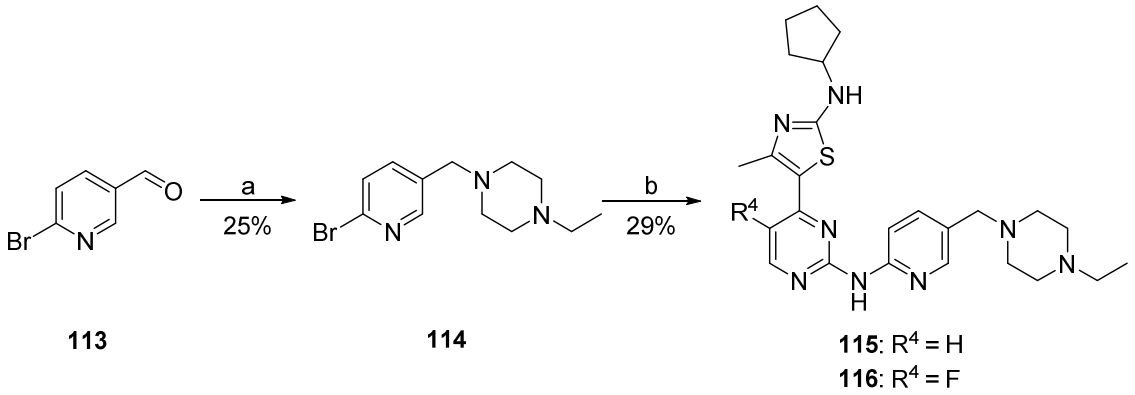
Finally, all desired 4-thiazol-*N*-(pyridin-2-yl)pyrimidin-2-amine derivatives were obtained through either pyrimidine ring cyclization[16] between an enaminone and a guanidine (**78-112**, Scheme 2) or Buchwald−Hartwig amination of a bromide and an amine[33] (**115** and **116**, Scheme 3). Each enaminone (**13-20**) was condensed with an appropriate pyridinyl guanidine (**67-77**) under our previously reported microwave-assisted conditions,[17] giving **78-112** in poor to moderate yields (5-57%) (Scheme 2). In parallel, **115** and **116** were prepared by the palladium-catalyzed coupling of pyrimidinyl-2-amine **21** or **22** with bromide **114** (Scheme 3).

Scheme 2. Synthesis of guanidine derivatives **67**-**77** and 4-thiazol-*N*-(pyridin-2-yl)pyrimidin-2-amines **78**-**112**[a]



[a]Reagents and conditions: (a) 5-bromo-2-nitropyridine, Et_3N, DMSO, 120 °C, 16 h; (b) Boc_2O, DMAP, Et_3N, DCM, o/n, rt; (c) H_2, 10% Pd/C, MeOH, o/n, rt; (d) *N*,*N*′-bis-Boc-*S*-methylisothiourea, $HgCl_2$, Et_3N, DCM, 0 °C to rt, 14 h; (e) TFA/DCM (1:1), reflux, 16 h; (f) **13**-**20**, NaOH, 2-methoxyethanol, microwave, 180 °C, 1 h. [b]No substitution on carbon 5 of the pyridine ring and obtained from commercial source. [c]No substitution on carbon 5 of the pyridine ring. [d]See tables 1, 2 and 4 for the structures of **78**-**112**.

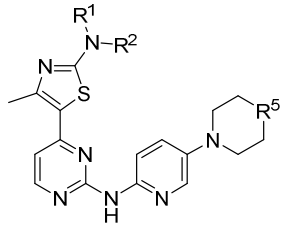
Scheme 3. Synthesis of **115** and **116** *via* Buchwald−Hartwig amination[a]



113 **114** **115**: R^4 = H; **116**: R^4 = F

[a]Reagents and conditions: (a) ethylpiperazine, sodium triacetoxyborohydride, DCM, 0 °C to rt, 12 h; (b) **21** or **22**, $Pd_2(dba)_3$, xantphos, *t*-BuONa, 1,4-dioxane, microwave, 150 °C, 1 h.

Table 1. Structures and *in vitro* biological activities of 4-(4-methylthiazol-5-yl)-*N*-(pyridin-2-yl)pyrimidin-2-amine derivatives **78-99**



Compd	Substituent			CDK inhibition, K_i (μM)[a]						Growth inhibition, GI_{50} (μM)[b]	
No.	R^1	R^2	R^5	4D[c]	6D3[c]	1B[c]	2A[c]	7H[c]	9T1[c]	MV4-11	MDA-MB-453
78	c-Pent	H	NH	0.001	0.034	> 5	> 5	1.108	0.220	0.023 ± 0.024	0.070 ± 0.013
79	c-Pent	H	CH_2NH	0.001	0.008	4.070	0.278	0.282	0.508	0.029 ± 0.002	0.102 ± 0.117
80	c-Pent	H	NCH_3	0.002	0.009	2.675	0.206	0.865	0.180	0.009 ± 0.000	0.130 ± 0.011
81	c-Pent	H	NCH_2CH_3	0.002	0.011	3.020	0.355	0.780	0.141	0.009 ± 0.001	0.287 ± 0.070
82	c-Pent	H	$NCOCH_3$	0.006	0.093	3.252	0.776	3.453	0.286	0.024 ± 0.028	0.591 ± 0.256
83	c-Pent	H	O	0.004	0.030	NA	4.683	NA	> 5	0.209 ± 0.030	3.683 ± 0.285
84	c-Pent	H	$CHNH_2$	0.003	0.133	1.230	0.181	1.187	0.173	0.013 ± 0.005	0.055 ± 0.012
85	c-Pent	H	CH_2	0.070	0.257	NA	> 5	NA	> 5	0.191 ± 0.029	7.035 ± 0.710
86	c-Pent	H	NSO_2CH_3	0.007	0.055	1.100	0.077	2.640	1.321	0.176 ± 0.009	0.215 ± 0.052

87	c-Pent	H	NSP[d]	0.570	-	-	-	-	> 5	0.300 ± 0.035	4.379 ± 0.691
88	c-Pent	CH_3	NH	0.002	0.010	1.39	0.174	3.20	1.801	0.285 ± 0.041	0.402 ± 0.006
89	c-Pent	CH_3	NCH_3	0.002	0.010	> 5	0.476	> 5	1.800	0.020 ± 0.015	3.360 ± 0.286
90	c-Pent	CH_3	$NCOCH_3$	0.010	0.031	3.17	0.121	NA	> 5	0.328 ± 0.007	6.864 ± 0.798
91	c-Pent	c-Pent	NH	0.071	0.539	> 5	> 5	> 5	> 5	0.714 ± 0.179	0.373 ± 0.117
92	Ph	H	NH	0.005	0.066	2.04	> 5	1.66	0.436	0.056 ± 0.011	0.279 ± 0.044
93	Ph	H	NCH_3	0.019	0.485	NA	NA	NA	> 5	0.508 ± 0.042	0.494 ± 0.081
94	Ph	CH_3	NCH_3	0.026	0.100	> 5	1.04	> 5	2.00	0.421 ± 0.044	0.150 ± 0.029
95	*i*-Pr	H	NH	0.005	0.011	3.140	0.240	0.775	2.420	0.093 ± 0.010	0.031 ± 0.002
96	*i*-Pr	H	CH_2NH	0.016	0.028	> 5	0.800	1.16	0.925	0.255 ± 0.085	0.938 ± 0.068
97	*i*-Pr	H	NCH_3	0.003	0.015	3.815	0.399	0.760	0.773	0.075 ± 0.005	0.618 ± 0.193
98	*i*-Pr	H	$NCOCH_3$	0.021	0.105	2.695	0.200	4.385	3.717	2.071 ± 0.321	0.344 ± 0.126
99	*i*-Pr	H	O	0.041	0.082	> 5	0.127	> 5	> 5	0.032 ± 0.003	0.115 ± 0.024
Palbociclib				0.003	0.027	> 5	> 5	> 5	0.364	0.050 ± 0.004	0.326 ± 0.047

[a] K_m values were used as ATP concentrations. Apparent inhibition constants (K_i) were calculated using the half maximal inhibition (IC_{50}) and the appropriate K_m(ATP) of each kinase as described in the experimental section. NA indicates no inhibition or compound

activity that could not be fit to an IC_{50} curve. [b]Anti-proliferative activity was determined by 72 h resazurin and MTT assays using MV4-11 and MDA-MB-453 cell lines, respectively. The data given are derived from at least two replicates. [c]4D1, 6D3, 1B, 2A, 7H, and 9T1 represent CDK4-cyclin D1, CDK6-cyclin D3, CDK1-cyclin B, CDK2-cyclin A, CDK7-cyclin H and CDK9-cyclinT1, respectively. [d]NSP indicates no substitution at C5 of the pyridine ring.

Structure-Activity Relationship Analysis. The very first 4-(4-methylthiazol-5-yl)-*N*-(pyridin-2-yl)pyrimidin-2-amine prepared with an ionizable piperazine group on the pyridine ring was **78**. This molecule was found to exhibit excellent potency (K_i = 1 and 34 nM for CDK4 and CDK6, respectively) and a relatively high selectivity for CDK4/6 over CDK1, CDK2, CDK7 and CDK9. Compound **78** is also a highly effective anti-proliferative agent with a GI_{50} (concentration for 50% inhibition of cell proliferation) value of 23 nM against MV4-11. As expected, inhibition of CDK4/6 by **78** resulted in accumulation of MV4-11 cells in the G1 phase of cell cycle in a concentration dependent manner (Figure 4). At the highest concentration (0.40 μM) tested, **78** accumulated 85% G1 population compared to the 61% in untreated cells. Similar cell cycle effects of palbociclib were also observed. This observation encouraged us to further explore the utility and versatility of the 4-(4-methylthiazol-5-yl)-*N*-(pyridin-2-yl)pyrimidin-2-amine scaffold as CDK4/6 inhibitors.

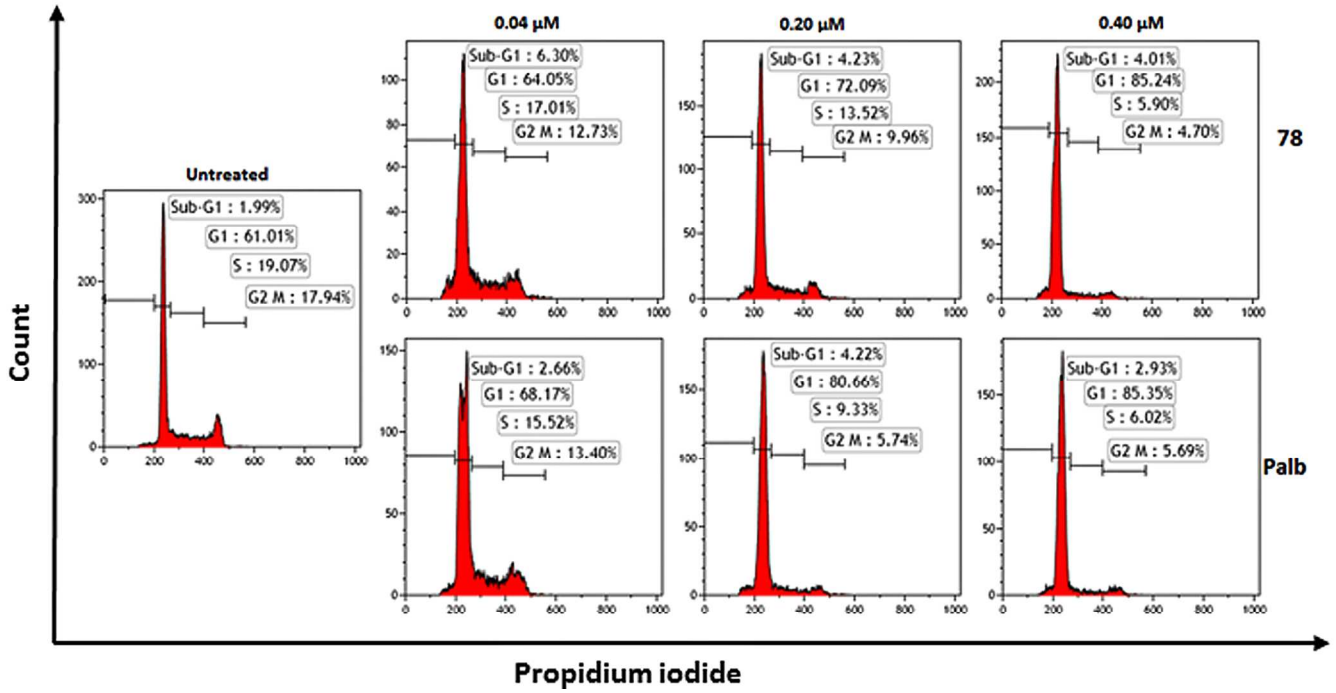
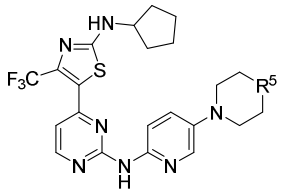


Figure 4. Cell cycle analysis of **78**. Compound **78** induced G1 cell cycle accumulation in MV4-11 cells. The cells were treated with increasing concentrations of **78** or palbociclib (Palb) for 24 h, and analyzed by flow cytometry. The experiments were repeated twice and representative data were presented.

Several structural alterations in relation to the substitution at the amino site of the thiazole moiety were made to examine whether the nature of the substituent would affect enzymatic and cellular efficacies (Table 1). Replacement of the cyclopentyl group (**78** and **80**) with the bulkier and more rigid aromatic phenyl group (**92** and **93**) resulted in a moderate reduction in both CDK4/6 and cellular activity. Similarly, substitution with the smaller and more flexible isopropyl group (**78**-**80** and **82** *versus* **95**-**98**) slightly decreased both CDK inhibitory activity and anti-proliferative effect. However, despite its weaker CDK4/6 inhibitory activity (K_i = 41 and 82 nM for CDK4 and CDK6, respectively), **99** with an isopropyl substituent was more potent than the corresponding cyclopentyl analog **83** (K_i = 4 and 30 nM for CDK4 and CDK6, respectively) in terms of anti-proliferative activity (GI_{50} = 32 and 115 nM for **99** *versus* GI_{50} = 209 nM and > 3 μM for **83** against MV4-11 and MDA-MB-453, respectively), suggesting off-target effect(s) by **99**. Increasing the steric bulk at the amino site by introduction of a second alkyl group, *i.e.,* methyl or cyclopentyl, moderately to greatly diminished the potency against both cell lines, which could be at least partially attributed to the reduced CDK7/9 inhibitory activity observed (**78**, **80**, and **82** *versus* **88**-**90**, and **78** *versus* **91**). Collectively, structural modifications made to the amino site of the thiazole ring indicated that mono-substitution of a cyclopentyl group afforded the optimal combination of both enzymatic and cellular potencies as well as the selectivity for CDK4/6. As a result, this functionality was retained and further alterations at C4 position of the thiazole ring were carried out (Table 2). Substitution of the methyl group with the

bulkier electron withdrawing trifluoromethyl (**78**, **80**, **82** and **83** *versus* **100-103**) markedly increased the potency against MV4-11 cells with GI_{50} values ranging from 1 to 69 nM while the trend was not conclusive for MDA-MD-453 cells. In addition to the increased CDK2/7 inhibitory activity, the inhibition of CDK1, prevalently considered to induce toxicity against both malignant and untransformed cells,[34] was found to be significantly enhanced, thus severely jeopardizing the CDK4/6 selectivity profile. Taken together, the data show that the cyclopentylamino and methyl group are optimal at C2 and C4 positions of the thiazole ring, respectively.

Table 2. Structures and biological activities of *N*-cyclopentyl-5-(2-(pyridin-2-ylamino)pyrimidin-4-yl)-4-(trifluoromethyl)thiazol-2-amine derivatives **100-104**



Compd.	Substituent	CDK inhibition, K_i (μM)[a]						Growth inhibition, GI_{50} (μM)[b]	
No.	R^5	4D1[c]	6D3[c]	1B[c]	2A[c]	7H[c]	9T1[c]	MV4-11	MDA-MB-453
100	NH	0.008	0.002	0.220	0.022	0.194	0.258	0.002 ± 0.001	0.081 ± 0.039
101	NCH_3	0.001	0.003	0.241	0.022	0.189	0.831	0.001 ± 0.001	0.420 ± 0.120
102	$NCOCH_3$	0.004	0.006	0.297	0.014	2.615	> 5	0.012 ± 0.001	0.077 ± 0.001
103	O	0.008	0.011	NA	0.154	NA	> 5	0.069 ± 0.013	0.415 ± 0.103
104	NCHO	0.011	0.007	0.345	0.015	1.900	> 5	0.004 ± 0.001	0.336 ± 0.188

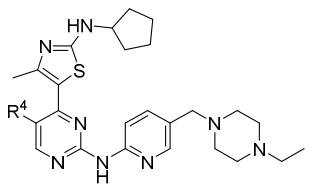
[a] K_m values were used as ATP concentrations. Apparent inhibition constants (K_i) were calculated using the half maximal inhibition (IC_{50}) and the appropriate K_m(ATP) of each kinase as described in the experimental section. NA indicates no inhibition or compound activity that

could not be fit to an IC_{50} curve. [b]Anti-proliferative activity was determined by 72 h resazurin and MTT assays using MV4-11 and MDA-MB-453 cell lines, respectively. The data given are derived from at least two replicates. [c]4D1, 6D3, 1B, 2A, 7H, and 9T1 represent CDK4-cyclin D1, CDK6-cyclin D3, CDK1-cyclin B, CDK2-cyclin A, CDK7-cyclin H and CDK9-cyclinT1, respectively.

With the optimal thiazole moiety in hand, we next explored a variety of substituents at C5 position of the pyridine ring. Given that a basic amino substituent at this position was previously shown to improve both kinase selectivity and pharmaceutical properties,[24] our effort mainly focused on introduction of various nitrogen-containing cyclic substituents. In fact, analysis of the crystal structure of palbociclib bound to CDK6 revealed that the piperazine ring sits in the kinase channel near the solvent accessible region, and thus is capable of accommodating a wide variety of substituents (Figure 3).[35] The secondary amino site of the piperazine ring is expected to be protonated at physiological pH and consequently interact with Thr102 in CDK4 (Thr107 of CDK6) which corresponds to the unfavorable (due to electrostatic repulsion) positively charged Lys residues of CDK1/2, rendering the selectivity towards CDK4/6 over these kinases. In contrast, in CDK9, the corresponding residue is Gly which can be in contact with the protonated amino site, and thus contribute to CDK9 inhibition, albeit with a higher inhibition constant. In our current study, extending the cyclic secondary amino site of **78** further into the solvent channel gave rise to the exocyclic primary amino compound **84**, which exhibited decreased potency for CDK6 and selectivity for CDK4 over CDK2, and a marginally increased activity against both cell lines (Table 1). The piperidine analogue **85**, lacking the secondary amino site, was 70- and > 7-fold less potent against CDK4 and CDK6, respectively, than **78** (K_i = 70 and 257 nM for **85** *versus* K_i = 1 and 34 nM for **78** towards CDK4 and CDK6, respectively). The

absence of a heterocyclic substituent on the pyridine ring (**87**) was not tolerated with a K_i value of 570 nM for CDK4. The significant loss of the CDK4 inhibitory potency in the cases of **85** and **87** confirmed the requirement of an appropriate heterocyclic substituent at the C5 position of the pyridine ring for CDK4/6 inhibition. Nevertheless, these two compounds exhibited good anti-proliferative activity against MV4-11 cells, indicating potential off-targets. Elongation of the linker by insertion of a methylene spacer between the pyridine ring and the piperazine moiety (**115** and **116** *versus* **81** and **108**) did not significantly impact the CDK4/6 selectivity and cellular potency (Tables 1, 3 and 4).

Table 3. Structures and *in vitro* biological activities of *N*-cyclopentyl-5-(2-((5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)amino)pyrimidin-4-yl)-4-methylthiazol-2-amine derivatives **115** and **116**



Compd.	Substituent	CDK inhibition, K_i (μM)[a]						Growth inhibition, GI_{50} (μM)[b]	
No.	R^4	4D1[c]	6D3[c]	1B[c]	2A[c]	7H[c]	9T1[c]	MV4-11	MDA-MB-453
115	H	0.006	0.009	> 5	0.416	0.211	1.98	0.019 ± 0.003	0.069 ± 0.023
116	F	0.003	0.014	NA	0.620	0.630	3.57	0.010 ± 0.002	0.622 ± 0.208

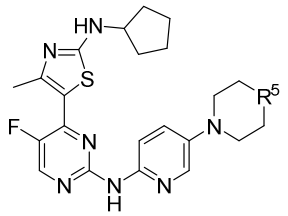
[a] K_m values were used as ATP concentrations. Apparent inhibition constants (K_i) were calculated using the half maximal inhibition (IC_{50}) and the appropriate K_m(ATP) value of each kinase as described in the experimental section. NA indicates no inhibition or compound activity that could not be fit to an IC_{50} curve. [b] Anti-proliferative activity was determined by 72 h resazurin and MTT assays using MV4-11 and MDA-MB-453 cell lines, respectively. The data

given are derived from at least two replicates. [c]4D1, 6D3, 1B, 2A, 7H, and 9T1 represent CDK4-cyclin D1, CDK6-cyclin D3, CDK1-cyclin B, CDK2-cyclin A, CDK7-cyclin H and CDK9-cyclinT1, respectively.

Recently, we successfully optimized the pharmacokinetic (PK) properties of CDK9 inhibitors by introduction of a fluorine atom at C5 position of the pyrimidine ring.[36,37] This strategy was also deployed in the current study to seek potent and selective CDK4/6 inhibitors with improved bioavailability. In addition, we envisioned that the electronegative fluorine atom would acidify the bridging NH between the pyrimidine and pyridine rings, thereby increasing the strength of the hydrogen bonding with the carbonyl group of Val in the hinge region.[38] Regrettably, all the attempts thus made led to little success in further improving CDK4/6 inhibitory activity and selectivity, anti-proliferative potency (Table 4), and PK properties (data not shown). Nonetheless, probably due to its bulkier ionizable amino site in the vicinity of Thr102, the 4-(dimethylamino)piperidine analogue **112** possesses an increased selectivity towards CDK4.

Interestingly, all morpholine-containing compounds **83**, **99**, **103** and **110** displayed excellent selectivity towards CDK4/6 over the other CDKs tested (Tables 1, 2 and 4). Particularly, **83** showed > 1,200-fold selectivity over CDK2 and was inactive against the rest of CDKs tested. Setting a maximum K_i value of 10 nM and a minimum selectivity of 100-fold for CDK4 as criteria, we have identified 9 compounds, *i.e.*, **78**, **79**, **83**, **89**, **97**, **107**, **108, 112** and **116**, as highly selective CDK4/6 inhibitors, all of which exhibited potent cellular anti-proliferative activities in cancer cell lines.

Table 4. Structures and *in vitro* biological activities of *N*-cyclopentyl-5-(5-fluoro-2-(pyridin-2-ylamino)pyrimidin-4-yl)-4-methylthiazol-2-amine derivatives **105-112**



Compd.	Substituent	CDK inhibition, K_i (μM)[a]						Growth inhibition, GI_{50} (μM)[b]	
No.	R^5	4D1[c]	6D3[c]	1B[c]	2A[c]	7H[c]	9T1[c]	MV4-11	MDA-MB-453
105	NH	0.003	0.007	2.235	0.256	0.790	0.787	0.053 ± 0.004	0.780 ± 0.598
106	CH_2NH	0.014	0.010	> 5	0.417	0.815	0.679	0.029 ± 0.002	0.083 ± 0.009
107	NCH_3	0.001	0.003	2.330	0.103	2.020	0.505	0.073 ± 0.028	0.202 ± 0.030
108	NCH_2CH_3	0.002	0.006	NA	0.349	0.685	> 5	0.013 ± 0.002	0.066 ± 0.019
119	$NCOCH_3$	0.034	0.023	> 5	0.228	> 5	4.990	0.015 ± 0.002	8.107 ± 1.147
110	O	0.006	0.020	4.350	0.104	> 5	> 5	0.290 ± 0.062	1.437 ± 0.304
111	NSO_2CH_3	0.039	0.101	NA	0.348	NA	> 5	2.628 ± 0.582	2.813 ± 0.089
112	$CHN(CH_3)_2$	0.001	0.031	NA	1.150	NA	1.091	0.454 ± 0.040	0.356 ± 0.024

[a] K_m values were used as ATP concentrations. Apparent inhibition constants (K_i) were calculated using the half maximal inhibition (IC_{50}) and the appropriate K_m(ATP) of each kinase as described in the experimental section. NA indicates no inhibition or compound activity that could not be fit to an IC_{50} curve. [b] Anti-proliferative activity was determined by 72 h resazurin and MTT assays using MV4-11 and MDA-MB-453 cell lines, respectively. The data given are derived from at least two replicates. [c] 4D1, 6D3, 1B, 2A, 7H, and 9T1 represent CDK4-cyclin D1, CDK6-cyclin D3, CDK1-cyclin B, CDK2-cyclin A, CDK7-cyclin H and CDK9-cyclinT1, respectively.

Kinase Selectivity Profiling of 83. Upon identification of 9 potent compounds with high selectivity for CDK4/6 over the other CDK family members tested, a selection of compounds, *i.e.*, **78**, **83**, **107** and **112**, were further screened against a panel of 50 different kinases (Table S1). Compounds **78** and **107** potently inhibited (< 10% kinase residual activity at 10 μM) a number of kinases including CK1δ, DAPK1, DYRK1A/B, FGFR1, Flt1, Flt3, Flt4, HIPK2, JNK3, Mnk2, Pim-1 and SGK. In contrast, DYRK1A/B and HIPK2 were the only two other kinases significantly affected by **112**. Strikingly, **83** was the most selective CDK4/6 inhibitor with no appreciable effect on other kinases tested. The subsequent profiling of **83** against a wider panel of 369 kinases at the concentration of 1 μM (~ 250 × K_i) (Figure 5, Table S2) revealed that **83** is exceptionally selective for CDK4/6 within the human kinome; DYRK2/3, LIMK1 and STK16, all of which are not commonly associated with cancer, were the only other kinases significantly inhibited by this compound. To the best of our knowledge, the kinase selectivity of **83** is superior to those of CDK4/6 inhibitors previously reported.[14,15]

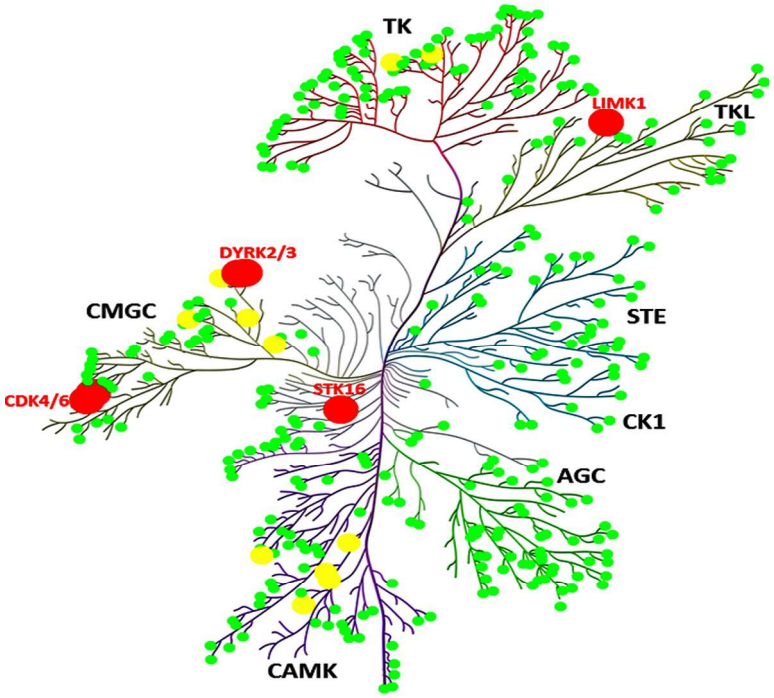


Figure 5. Kinome-wide selectivity of **83** at 1 μM. Red, yellow and green colors indicate kinase residual activity of < 10%, 10-20% and > 20%, respectively. Individual kinase residual activity values are given in Table S2. The human kinome map is adapted with permission from Reaction Biology Corp.

Anti-proliferative Activity of 83. The most selective CDK4/6 inhibitor **83** was further tested against a panel of 12 cancerous and two non-transformed cell lines to examine whether the anti-proliferative effect was cell-type specific (Table 5). Compound **83** potently inhibited the proliferation of seven cell lines (MOLM13, NB4, M229, M249, M249R, A2780 and PC-3) with GI_{50} values between 0.038 and 0.681 μM, and moderately restrained the growth of two cell lines (MCF-7 and M238) with a GI_{50} value of 1.887 and 2.200 μM, respectively. The remaining three cancer cell lines were not sensitive to **83** with a GI_{50} value of > 10 μM. In comparison, palbociclib was more effective against the cancer cell lines except in the cases of melanoma cancer cells M229, M249 and M247R. Both **83** and palbociclib exerted little toxic effects on non-transformed human prostatic epithelial cell BPH-1 and the normal lung fibroblast MRC-5.

Table 5. Anti-proliferative activity of **83** against a panel of human cell lines

Origin	Cell lines	Growth inhibition, GI_{50} (μM)[a]	
		83	Palbociclib
Breast	MCF-7	1.887 ± 0.130	0.557 ± 0.057
	MDA-MB-468	> 10	5.960 ± 2.400
Leukemia	MOLM13	0.385 ± 0.098	0.062 ± 0.012
	NB4	0.681 ± 0.018	0.066 ± 0.012
Melanoma	M229	0.150 ± 0.007	1.220 ± 0.610
	M238	2.200 ± 0.430	1.970 ± 0.260
	M249	0.082 ± 0.002	1.990 ± 0.520
	M249R	0.038 ± 0.005	2.960 ± 0.220
Ovarian	A2780	0.058 ± 0.017	0.032 ± 0.008
Prostate	Du-145	> 10	5.792 ± 0.801
	C4-2B	> 10	4.150 ± 1.100
	PC-3	0.150 ± 0.007	0.071 ± 0.009
Non-transformed	BPH-1	> 10	7.442 ± 0.552
	MRC-5	> 10	> 10

[a]Anti-proliferative activity was determined by 72 h resazurin or MTT assays. The data given are derived from two replicates.

Cellular Mechanism of Action of 83. To evaluate whether the observed anti-proliferative activity of **83** is derived from the inhibition of cellular CDK4/6, we examined the status of Rb phosphorylation at the CDK4/6-specific site Ser780, cell cycle distribution, and apoptosis induction. Western blot analysis showed that **83** inhibited phosphorylation of the Rb protein (at

Ser780) in MV4-11 cells in a dose-dependent manner upon 12 h and 24 h treatments (Figure 6). In comparison, much faster and stronger inhibition of both total Rb and Rb phosphorylation was observed upon treatment with palbociclib. This finding is consistent with a previous study, where plabociclib was shown not only to decrease the protein levels of Rb and phosphorylated Rb but also the transcription of *Rb1* gene.[39] As phosphorylated Rb protein is required for G1 to S transition of the cell cycle, selective inhibition of Rb phosphorylation at CDK4/6-specific site should cause G1 cell cycle arrest. As expected, a dose-dependent accumulation of cells in G1 phase was observed following 24 h treatment with **83** or palbociclib (Figure 7). This G1 phase arrest was maintained with 10 x GI_{50} μM (and higher concentrations, data not shown) for both compounds signifying no off-target cell-cycle activity. The lack of an increase in the population of sub-G1 cells (Figure 7) in both cases is in agreement with the absence of noticeable apoptosis (Figure 8). This was not unexpected as previous studies have demonstrated that CDK4/6 inhibitors induced either cytostasis or apoptosis depending on the type of cells, concentration of drug and duration of treatement.[40,41] Taken together, these results indicate that **83** effectively triggers G1 cell cycle arrest by inhibiting the CDK4/6-mediated phosphorylation of Rb without causing significant cell death in MV4-11 cells.

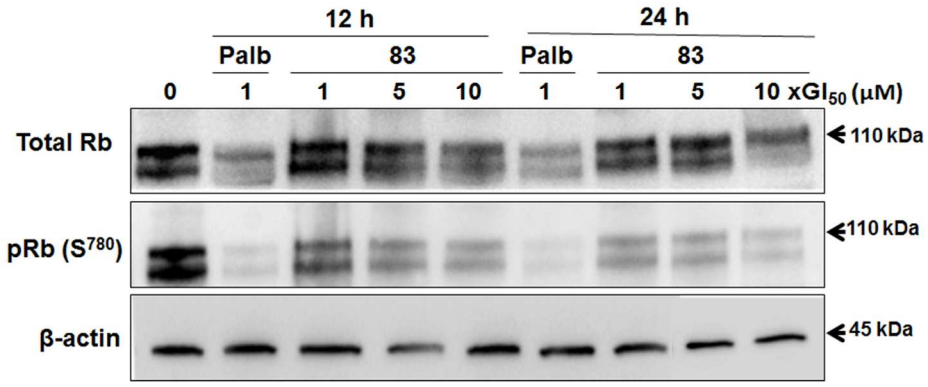


Figure 6. Western blot analysis of MV4-11 cancer cells following treatment with **83** or palbociclib for 12 or 24 h. Both **83** and palbociclib (palb) inhibited the phosphorylation of Rb at

Ser780. β-Actin was used as a loading control. The data are representative of two independent experiments.

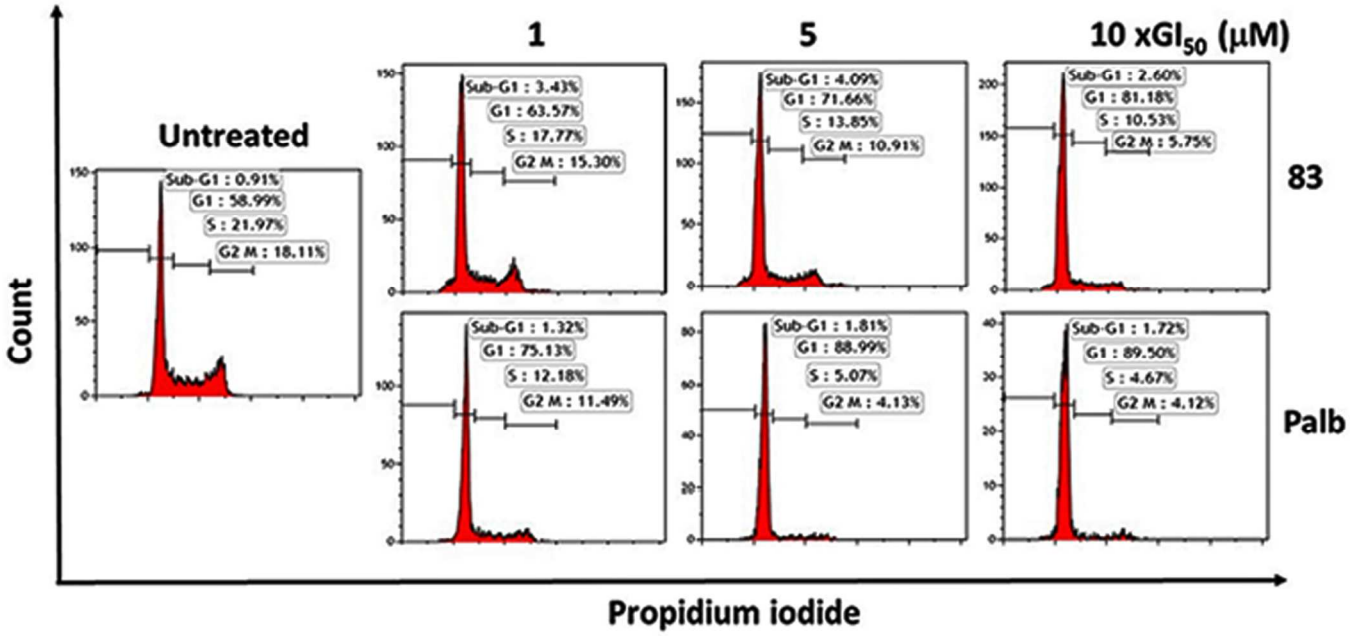


Figure 7. Cell cycle effects of **83** and palbociclib on MV4-11 cells. Cells were treated with increasing concentrations of **83** or palbociclib (palb) for 24 h, harvested, fixed, and stained with propidium iodide prior to flow cytometric analysis. The experiments were repeated twice. Representative data were selected to generate this figure.

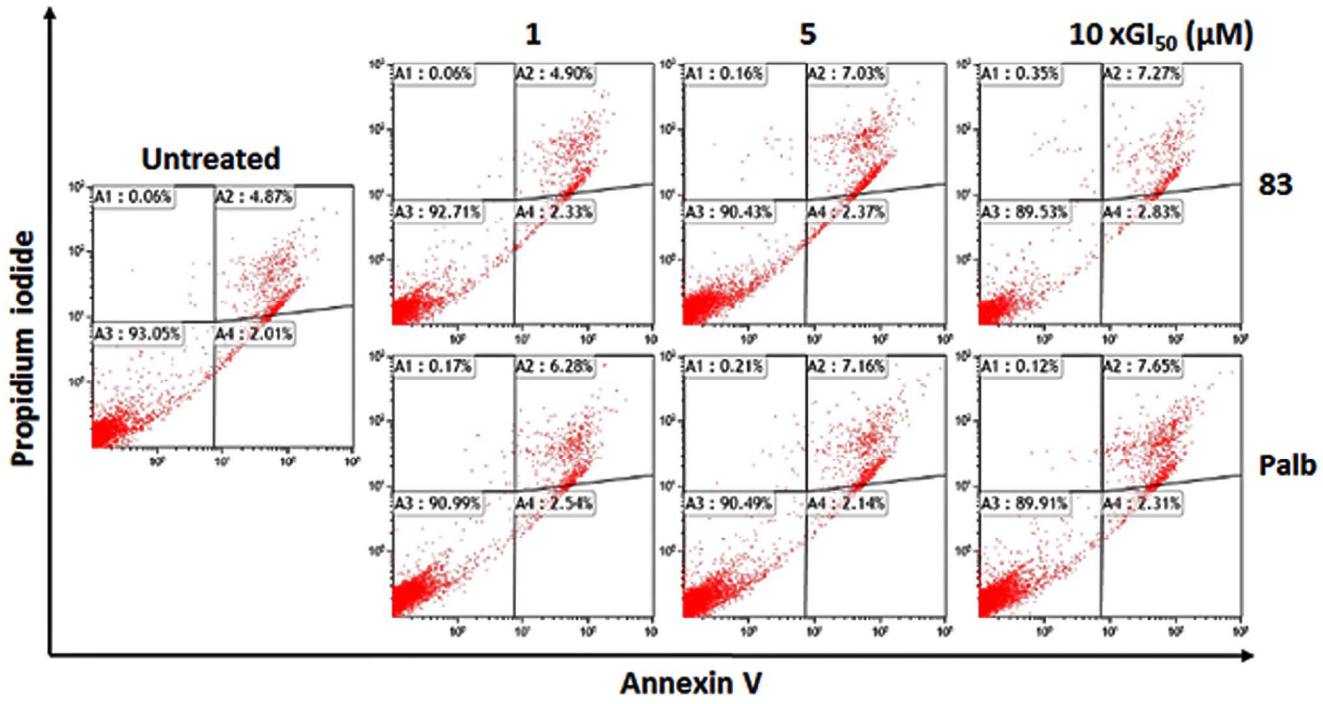


Figure 8. Effects of **83** and palbociclib on the induction of apoptosis. Treatment with **83** or palbociclib (palb) showed little effect on the induction of apoptosis in MV4-11 cells. The cells were exposed to increasing concentrations of each compound for 24 h, and analyzed by annexin V/propidium iodide double staining. The data are representative of two independent experiments.

Biopharmaceutical Profiling of 83. In light of its excellent *in vitro* biological profile, **83** was further subjected to *in vitro* biopharmaceutical and *in vivo* pharmacokinetic evaluations. To assess the safety and potential oral bioavailability of **83**, the *in vitro* ADME properties of **83** were determined. CYP450 and hERG (human ether-a-go-go related gene) channel inhibition screens were used to evaluate the potential of **83** towards drug-drug interactions and cardiac liability, respectively. As shown in Table 6, **83** exhibited a very good safety profile with IC_{50} > 25 μM against five common CYP450 isoforms, and with IC_{50} > 10 μM against hERG. **83** was found to be highly permeable with apparent permeability coefficient (P_{app}) of 10.1 × 10^{-6} cm/s, suggesting high intestinal absorption. The efflux ratio (ER) of **83** was 0.4, demonstrating that **83**

was not subjected to active efflux that is usually indicated by a value > 2. **83** has high lipophilicity with a $logD_{7.4}$ value of 4.4 (Table 6).

Table 6. Biopharmaceutical profiles of **83**

Inhibition of CYP450 (IC_{50}, μM)[a]	CYP3A4	>25
	CYP2D6	>25
	CYP1A	>25
	CYP2C9	>25
	CYP2C19	>25
Inhibition of hERG (IC_{50})[b]	μM	>10
Partition coefficient[c]	$LogD_{7.4}$	4.4
Dissociation constant[d]	pKa	3.98, 5.68
Caco2 permeability[e]	A−B P_{app} (10^{-6} cm/s)	10.1
	B−A P_{app} (10^{-6} cm/s)	3.98
	efflux ratio	0.396

[a]Human liver microsomes and NADPH in the presence of a cytochrome P450 isoform-specific probe substrate: midazolam for CYP3A4, dextromethorphan for CYP2D6, ethoxyresorufin for CYP1A, tolbutamide for CYP2C9 and mephenytoin for CYP2C19. [b]CHO-hERG cells were used for the cardiac toxicity assay. The data is usually categorized into the following classification bands: highly potent IC_{50} < 0.1 μM; potent IC_{50} = 0.1 – 1 μM; moderately potent IC_{50} = 1 – 10 μM; weak or no inhibition IC_{50} > 10 μM. [c]Determined using a pH-metric method. [d]by Shake Flask. [e]Apparent permeability coefficient measured by Caco-2 assay. Efflux Ratio = (mean P_{app} B-A / mean P_{app} A-B).

Pharmacokinetic Properties of 83. Encouraged by the *in vitro* DMPK profile of **83**, single-dose PK studies were then performed with male albino Wistar rats at 5 mg/kg by IV and 20 mg/kg by PO and with male BALB/c mice at 2 mg/kg by IV and 10 mg/kg by PO, and the results

are summarized in table 7. When **83** was administered *via* intravenous route, its systemic plasma clearance (CL) of 3.5 L/h·kg in mice or 0.71 L/h·kg in rats was low relative to the hepatic blood flow rate of the respective murine species. The large volume of distribution observed (V_{ss} = 5.6 and 1.5 L/kg in mice and rats, respectively) suggested extravascular distribution. Maximal plasma concentrations (C_{max}) of 1.6 μM in mouse and 1.4 μM in rat were achieved at 2.0 and 3.7 h respectively after oral dosing. **83** exhibited good oral half-life ($t_{1/2}$) in both species, with $t_{1/2}$ = 5 h in mouse and $t_{1/2}$ = 2.8 h in rat. Its oral bioavailability was moderate (F = 27%) in rats and excellent (F = 100%) in mice, achieving the $AUC_{0\text{-}t}$ = 5.9 μM·h in mice and $AUC_{0\text{-}t}$ = 15.9 μM·h in rats. The plasma concentration-time profile of **83** following IV and PO administration to rats and mice are shown in Figures S1 and S2.

Table 7. Pharmacokinetic parameters of **83** in rats and mice

Parameter	Rat		Mouse	
	IV	PO	IV	PO
Dose (mg/kg)	5	20	2	10
CL (L/h·kg)	0.71	-	3.5	-
V_{ss} (L/kg)	1.5	-	5.6	-
$AUC_{0\text{-}t}$ (μM·h)	15.9	15.9	1.2	5.9
C_{max} (μM)	38.8	1.4	2.3	1.6
T_{max} (h)	-	3.7	-	2.0
$t_{1/2}$ (h)	4.2	2.8	1.7	5.0
F (%)	-	25.1	-	100

Pharmacological Safety and *In vivo* Anti-cancer Activity of 83. The favourable pharmacokinetic profile of **83** along with its highly desirable cellular activity and CDK4/6 inhibitory potency and selectivity warranted its advancement into *in vivo* safety and efficacy studies. First, we intended to determine the maximum tolerated dose of **83** in mice, but the compound did not cause any adverse effect on body weight or any other signs of overt toxicity at daily doses up to 100 mg/kg. Therefore, we assessed the *in vivo* anti-cancer activity of **83** in a subcutaneous human MV4-11 MLL-AML xenograft murine model. Tumor-bearing mice were treated orally with **83** (100 mg/kg, QD) for 33 consecutive days and left untreated for another 50 days. The treatment resulted in the marked inhibition of tumor growth (Figure 9A and B) and a prolongation of animal life span (Figure 9C). Tumor volume compared to control (T/C) significantly diminished to 30% on day 31 ($p < 0.00001$), and on day 33 of treatment the tumor growth inhibition (TGI) was 76%. Strikingly, **83** induced a complete and sustained tumor regression in three out of nine mice, and no tumour regrowth was observed during the 50 days post-treatment (Figures 9B and 9C). The regression and delay in tumor growth were translated to the increased survival rate of the treated group (median survival period = 55 days) when compared to vehicle-treated mice (median survival time = 33 days) (Figure 9C), suggesting that **83** was highly efficacious against MV4-11 xenograft. Compound **83** exhibited excellent safety characteristics without having a negative effect on body weight and any signs of overt toxicity (Figure 9D).

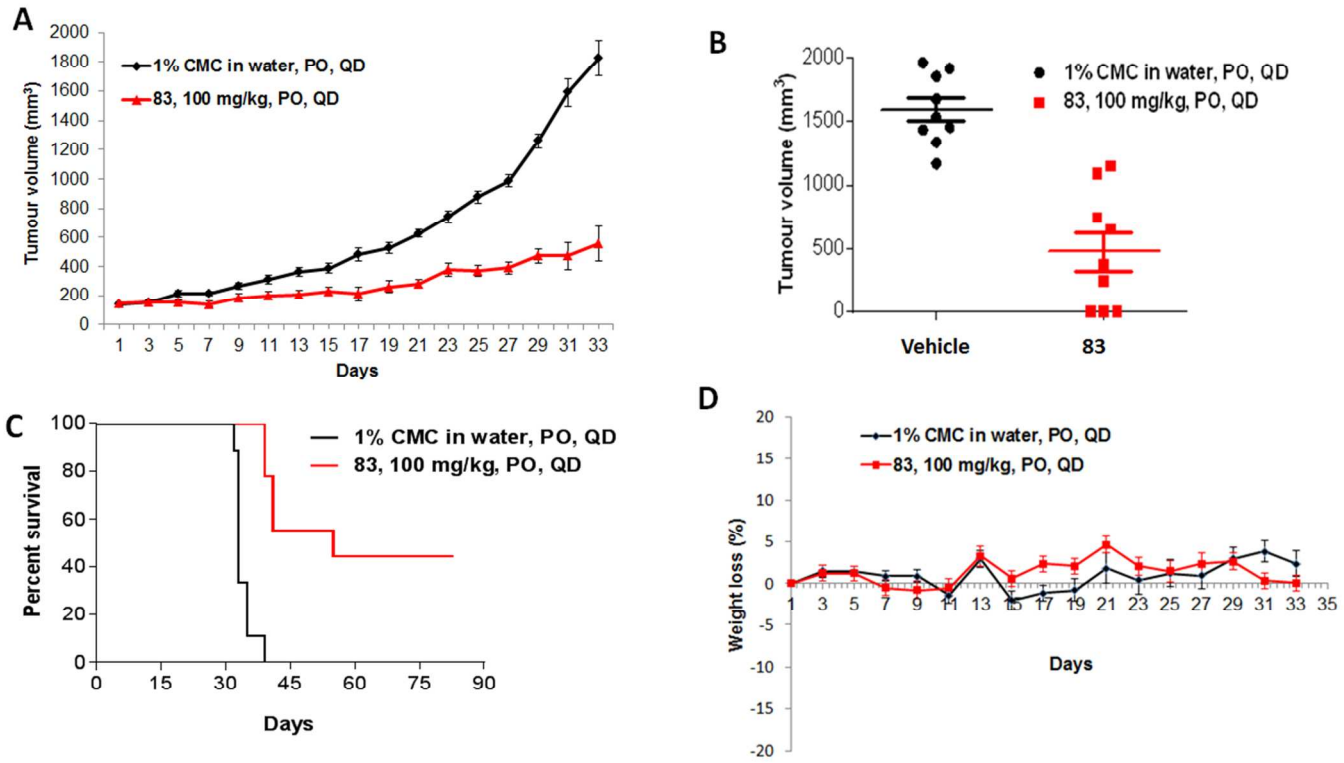


Figure 9. *In vivo* anti-cancer efficacy of **83**. (A) Female nude (nu/nu) BALB/c mice at 6-8 weeks of age with subcutaneously implanted MV4-11 leukemia xenograft were orally treated with 100 mg/kg of **83** (n = 9) or vehicle (1% CMC, n = 9) daily for 33 days. Treatment with **83** significantly reduced tumor burden in comparison to vehicle-treated controls. Significant reduction in tumor volume ($p < 0.05$) was observed in drug treated group from day 5 till the end of experiment when compared with vehicle treated group. (B) Tumor volumes of each mouse on day 31. (C) Kaplan−Meier analysis of animal survival following treatment with **83** or vehicle. (D) **83** was well tolerated without causing a negative effect on body weight in MV4-11 leukemia xenograft bearing mice.

CONCLUSION

In summary, we have discovered a new series of potent inhibitors of CDK4/6, and provided rationale for the observed biological activities. One of the lead compounds of this class, **83** was

identified as a CDK4/6-specific inhibitor with little or no effect on a large panel of kinases. Compound **83** exhibited cellular CDK4/6 targeted mechanism of action, favorable pharmacokinetics and significant anti-cancer efficacy in animal models. With its high oral activity along with excellent safety profile, **83** offers a very exciting prospect as a clinical development candidate.

EXPERIMENTAL SECTION

Chemistry. Chemical reagents and solvents were purchased from commercial sources, and were used as received. Microwave-assisted synthesis was performed in an Asynt DrySyn insert (10 mL, Isleham, UK) using a CEM Discover SP and Explorer 48/72/96 microwave system (Matthews, NC, USA) controlled by Synergy software (Firmware version DSCA02.17). Reaction yields were not optimized and refer to pure, isolated products of a single experiment.

Thin-layer chromatography was performed on Merck silica gel 60 F254 pre-coated aluminum plates (0.2 mm) and visualized under UV light (254 nm). Flash column chromatography was carried out using fritted solid loaders packed with Scharlau silica gel (0.04–0.06 mm) on Biotage FlashMaster Personal^{+} flash chromatography or using glass columns. High-resolution mass spectra were recorded using an AB SCIEX TripleTOF 5600 mass spectrometer (Concord, ON, Canada), and ionization of all samples was carried out using ESI. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker Avance III HD spectrometer (Faellanden, Switzerland) at 500 and 125 MHz respectively, and were analyzed using a Bruker Topspin 3.2 program. Chemical shifts are reported in parts per million (ppm), and are referenced to ^{1}H signals of residual non deuterated solvents and to ^{13}C signals of the deuterated solvents. Multiplicity of ^{1}H NMR signals is reported as: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m =

multiplet, and br = broad. Coupling constants *J* (Hz) are quoted to the nearest 0.1 Hz. Melting points were determined using an open capillary on a Stuart SMP10 melting point apparatus and are uncorrected.

The purity of compounds used for biological evaluation was determined to be greater than 95% using Shimadzu Prominence UFLC system (UltraFast Liquid Chromatograph, Kyoto, Japan) equipped with a CBM-20A communications bus module, a DGU-20A5R degassing unit, an LC-20AD liquid chromatograph pump, an SIL-20AHT auto-sampler, an SPD-M20A photo diode array detector, a CTO-20A column oven and a Phenomenex Kinetex 5u C_{18} 100A 250 mm × 4.60 mm column. Method A (gradient 5% to 95% MeOH containing 0.1% formic acid (FA) over 7 min at a flow rate of 1 mL/min, followed by 95% MeOH containing 0.1% FA over 13 min) and method B (gradient 5% to 95% MeCN containing 0.1% FA over 7 min at a flow rate of 1 mL/min, followed by 95% MeCN containing 0.1% FA over 13 min) were used for analytical RP-HPLC. Data acquired were processed using LabSolutions Analysis Data System. In addition, all inhibitor compounds were screened for potential PAINS using PAINS filters: FAF-Drugs3 and cbligand. No hit was recorded to contain PAINS substructures.

General Synthetic Procedure 1: 5-Acetylthiazole Formation. To a solution of 3-chloro-2,4-pentadione (1.0 equiv.) in MeOH were added the appropriate thiourea (1.0 equiv.) and pyridine (1.0 equiv.). The reaction mixture was stirred at room temperature for 4-48 h, washed with saturated aqueous $NaHCO_3$ and concentrated under reduced pressure, and the residue was purified by FlashMaster Personal$^+$ chromatography (silica gel, DCM ramping to DCM:MeOH = 95:5) or filtered to give 5-acetylthiazole derivatives.

General Synthetic Procedure 2: Enaminone Formation. A solution of 5-acetylthiazole derivative (1.0 equiv.) in DMF-DMA (2.0-7.0 equiv.) was heated at reflux for 6-36 h and

concentrated under reduced pressure. The residue was triturated with diethyl ether and filtered. The solid obtained was purified by flash column chromatography (silica gel, solvent system I: PE ramping to PE:EtOAc = 50:50, II: DCM ramping to EtOAc, or III: DCM ramping to DCM:MeOH = 94:6) to afford the desired enaminone.

General Synthetic Procedure 3: Pyrimidine Amine Formation. A mixture of enaminone (1.0 equiv.), NaOH (2.0 equiv.) and guanidine hydrochloride (2.0 equiv.) in 2-methoxethanol was heated at reflux for 6 h and concentrated under reduced pressure. The residue was purified by FlashMaster Personal$^+$ chromatography (silica gel, DCM ramping to DCM:MeOH = 96:4) to give the desired pyrimidine amine derivative.

General Synthetic Procedure 4: Substitution of Bromide with Secondary Amines. To a solution of 5-bromo-2-nitropyridine (1.0 equiv.) in DMSO were added heterocyclic secondary amine derivatives (1.0-2.5 equiv.) and triethylamine (3.0-6.0 equiv.). The reaction mixture was heated at 120 °C overnight and cooled down to room temperature; triethylamine was removed under reduced pressure. The residue was triturated with EtOAc and filtered. The solid was purified by FlashMaster Personal$^+$ chromatography (silica gel, solvent system I: PE ramping to EtOAc, II: DCM ramping to EtOAc, or III: DCM ramping to DCM:MeOH = 90:10) to give the desired tertiary amine derivative.

General Synthetic Procedure 5: Reduction of Nitro Compounds to Amines. To a suspension of a nitro compound (1.0 equiv.) in MeOH was added 10% Pd/C (0.01 equiv.). The reaction mixture was stirred at room temperature under H_2 overnight, and filtered through a pad of Celite. The solids were washed with methanol. The filtrate and washing were combined and concentrated under reduced pressure to give the desired amine which was used in the next step without purification.

General Synthetic Procedure 6: Mercury-promoted Guanylation Reaction. To a solution of an amine (1.0 equiv.), *N*,*N*′-bis-Boc-*S*-methylisothiourea (1.1-1.5 equiv.) and triethylamine (3.5 equiv.) in DCM on an ice bath was added $HgCl_2$ (1.1-2.0 equiv.). The reaction mixture was stirred on the ice bath for 30 min and at room temperature overnight, and filtered through a pad of Celite. The solids were washed with DCM (250 mL), and the filtrate and washing were combined and concentrated under reduced pressure and *in vacuo*. The residue was dissolved in DCM (300 mL), washed with brine (3 × 200 mL) and H_2O (3 × 200 mL), and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, solvent system I: PE ramping to EtOAc or II: DCM ramping to DCM:MeOH = 95:5) to give the desired bis-Boc-protected guanidine.

General Synthetic Procedure 7: TFA-mediated Boc Removal. Bis-Boc-protected guanidine (1.0 equiv.) was dissolved in a mixture of TFA (10 equiv.)/DCM (1:1). The reaction mixture was heated at reflux overnight, concentrated under reduced pressure, triturated with diethyl ether and filtered to give the desired guanidine which was directly used in the next step without further purification.

General Synthetic Procedure 8: Pyrimidine Cyclization. To a mixture of a guanidine trifluoroacetate derivative (2.0 eq.) and an appropriate enaminone (1.0 eq.) in 2-methoxyethanol was added NaOH (2.0 eq.). The reaction mixture was heated at 180 °C under microwave irradiation for 1-2 h, cooled to room temperature and concentrated under reduced pressure. The residue was purified by FlashMaster Personal$^+$ chromatography (silica gel, solvent system I: PE ramping to EtOAc, II: DCM ramping to DCM:MeOH = 90:10, or III: DCM ramping to DCM:MeOH:NH_4OH = 90:10:1) to give the desired product.

1,1-Dicyclopentylthiourea (5). Thiourea **5** was isolated as a by-product in the preparation of **4.** White solid (1.00 g, 5%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.33-1.38 (m, 4H), 1.48-1.52 (m, 4H), 1.58-1.63 (m, 4H), 1.82-1.88 (m, 4H), 4.37 (s, 2H), 7.15 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.9, 33.2, 55.8, 180.2 (seven carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 213.1425 $[M+H]^+$; calcd. for $C_{11}H_{21}N_2S^+$ $[M+H]^+$ 213.1420.

1-(2-(Cyclopentylamino)-4-methylthiazol-5-yl)ethan-1-one (8). Acetylthiazole **8** was prepared from **4** (2.00 g, 13.9 mmol) according to general synthetic procedure 1. White solid (1.20 g, 35%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.67-1.87 (m, 6H), 2.04-2.10 (m, 2H), 2.50 (s, 3H), 2.64 (s, 3H), 3.71 (s, 1H), 10.21 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.5, 23.4, 29.2, 31.9, 57.3 (six carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 225.1032 $[M+H]^+$; calcd. for $C_{11}H_{17}N_2OS^+$ $[M+H]^+$ 225.1056.

1-(2-(Cyclopentylamino)-4-(trifluoromethyl)thiazol-5-yl)ethan-1-one (9). To a solution of 1,1,1-trifluoropentane-2,4-dione (2.00 g, 12.8 mmol) in MeCN (30 mL) were added [hydroxyl(tosyloxyl)iodo]benzene (6.00 g, 15.4 mmol). The reaction mixture was stirred at reflux for 1 h, and cooled. **4** (2.20 g, 15.4 mmol) was added and the mixture refluxed for 4 h, cooled to room temperature and concentrated under reduced pressure. The solid obtained was purified by FlashMaster Personal$^+$ chromatography (silica gel, PE ramping to PE:EtOAc = 8:2) to give **9** as a yellow solid (2.10 g, 58%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.50-1.56 (m, 4H), 1.65-1.67 (m, 2H), 1.91-1.94 (m, 2H), 2.40 (s, 3H), 3.86 (s, 1H), 9.03 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.3, 29.3, 31.8, 56.7, 116.8, 119.0, 121.2, 123.3, 124.9, 141.5 (q, J = 37.5, 169.3, 186.9. HRMS (ESI-TOF): *m/z* 279.0782 $[M+H]^+$; calcd. for $C_{11}H_{14}F_3N_2OS^+$ $[M+H]^+$ 279.0773.

1-(2-(Dicyclopentylamino)-4-methylthiazol-5-yl)ethan-1-one (10). Acetylthiazole **10** was prepared from **5** (1.00 g, 4.70 mmol) according to general synthetic procedure 1. White solid (1.40 g, 100%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.59-1.63 (m, 2H), 1.80-1.88 (m, 8H), 1.97-2.00 (m, 2H), 2.09-2.13 (m, 2H), 2.36-2.37 (m, 2H), 2.50 (s, 3H), 2.72 (s, 3H), 3.72 (m, 1H), 5.57 (m, 1H,). ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.1, 23.6, 24.2, 25.2, 30.3, 31.4, 33.1, 52.8, 60.2, 62.6, 114.6, 116.9, 119.6, 146.0, 167.6, 188.3. MS (ESI-TOF): *m/z* 293.2 $[M+H]^+$.

1-(4-Methyl-2-(phenylamino)thiazol-5-yl)ethan-1-one (11). Acetylthiazole **11** was prepared from **6** (5.00 g, 33.0 mmol) according to general synthetic procedure 1. White solid (6.49 g, 85%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.42 (s, 3H), 2.54 (s, 3H), 7.03-7.06 (m, 1H), 7.34-7.37 (m, 2H), 7.60 (d, 2H, *J* 7.5), 10.74 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.5, 29.8, 118.1, 122.2, 129.2, 140.0, 156.7, 165.2, 189.2 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 233.0750 $[M+H]^+$; calcd. for $C_{12}H_{13}N_2OS^+$ $[M+H]^+$ 233.0743.

1-(2-(Isopropylamino)-4-methylthiazol-5-yl)ethan-1-one (12). Acetylthiazole **12** was prepared from **7** (5.00 g, 42.3 mmol) according to general synthetic procedure 1. Yellow solid (5.40 g, 64%). ^{1}H NMR (500 MHz, DMSO-d_6) δ. 1.15 (s, 3H), 1.16 (s, 3H), 2.31 (s, 3H), 2.42 (s, 3H), 3.75 (br s, 1H), 8.35 (d, 1H, *J* 7.5). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.5, 22.0, 29.4, 46.5, 120.6, 157.8, 169.0, 188.0 (one carbon signal overlapping or obscured). HRMS (ESI-TOF): *m/z* 199.0901 $[M+H]^+$; calcd. for $C_9H_{15}N_2OS^+$ $[M+H]^+$ 199.0900.

(*E*)-1-(2-(Cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (13). Enaminone **13** was prepared from **8** (1.00 g, 4.46 mmol) according to general synthetic procedure 2 and purified by solvent system II. Light yellow solid (1.00 g, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.50-1.73 (m, 6H), 2.00-2.06 (m, 2H), 2.54 (s, 3H), 2.94 (br s, 6H), 3.75 (app s, 1H), 5.29 (d, 1H, *J* 12.0), 5.84 (s, 1H), 7.65 (d, 1H, *J* 12.0). ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.5,

23.7, 33.1, 57.7, 94.5, 122.7, 152.7, 154.5, 169.2, 181.1 (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 280.1507 $[M+H]^+$; calcd. for $C_{14}H_{22}N_3OS^+$ $[M+H]^+$ 280.1478.

(*E*)-1-(2-(Cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)-2-fluoroprop-2-en-1-one (14). To a well-stirred solution of **13** (559 mg, 2.00 mmol) in MeOH (15 mL) on an ice bath was added SelectFluor (1.06g, 3.00 mmol), and the mixture was stirred for 4 h. The mixture was concentrated and purified by FlashMaster Personal$^+$ chromatography (silica gel, PE ramping to EtOAc) to give **14** as a yellow solid (350 mg, 33%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.55-1.74 (m, 6H), δ 2.02-2.08 (m, 2H), 2.54 (s, 3H), 3.08 (s, 6H), 3.75 (m, 1H), 6.88 (d, 1H, *J* 25.0). MS (ESI-TOF): *m/z* 298.1 $[M+H]^+$.

(*E*)-1-(2-(Cyclopentyl(methyl)amino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (15). A solution of **8** (1.70 g, 7.58 mmol) in DMF-DMA (6.00 mL, 45.2 mmol) was heated at 150 °C under microwave irradiation for 30 minutes, cooled to room temperature and concentrated under reduced pressure. The solid obtained was purified by FlashMaster Personal$^+$ chromatography (silica gel, DCM ramping to EtOAc) to give **15** as a light yellow solid (900 mg, 41%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.58-1.74 (m, 6H), 1.92-1.97 (m, 2H), 2.58 (s, 3H), 2.96 (br s, 9H), 4.47 (m, 1H), 5.31 (d, 1H, *J* 12.0), 7.65 (d, 1H, *J* 12.0). ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.5, 23.7, 33.1, 57.7, 94.5, 122.7, 152.7, 154.5, 169.2, 181.1 (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 294.1638 $[M+H]^+$; calcd. for $C_{15}H_{24}N_3OS^+$ $[M+H]^+$ 294.1635.

(*E*)-1-(2-(Cyclopentylamino)-4-(trifluoromethyl)thiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (16). Enaminone **16** was prepared from **9** (2.00 g, 3.59 mmol) according to general synthetic procedure 2 and purified by solvent system III. Yellow solid (1.40 g, 58%). ^{1}H NMR

(500 MHz, DMSO-d_6) δ 1.49-1.55 (m, 4H), 1.64-1.66 (m, 2H), 1.89-1.91 (m, 2H), 2.82 (s, 3H), 3.12 (s, 3H), 3.83 (m, 1H), 5.25 (d, 1H, *J* 12.0), 7.60 (d, 1H, *J* 12.0), 8.41 (d, 1H, *J* 6.5). ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.3, 32.0, 37.0, 44.5, 56.4, 93.9, 117.5, 119.6, 121.8, 124.0, 128.3, 136.7 (q, *J* = 25.0) 154.2, 167.2, 176.9. HRMS (ESI-TOF): *m/z* 334.1200 $[M+H]^+$; calcd. for $C_{14}H_{19}F_3N_3OS^+$ $[M+H]^+$ 334.1195.

(*E*)-1-(2-(Dicyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (17). Enaminone **17** was prepared from **10** (1.80 g, 6.16 mmol) according to general synthetic procedure 2 and purified by solvent system I. Brown solid (275 mg, 10%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.46-1.51 (m, 4H), 1.52-1.55 (m, 4H), 1.64-1.70 (m, 4H), 1.76-1.80 (m, 2H), 1.85-1.87 (m, 2H), 2.32-2.39 (m, 2H), 2.54 (s, 3H), 2.83 (br s, 3H), 3.02 (br s, 3H), 3.30 (m, 1H), 4.42 (m, 1H), 5.10 (d, 2H, *J* 12.0), 7.57 (d, 2H, *J* 12.5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 24.2, 25.9, 27.8, 33.8, 56.8, 65.8, 94.9, 108.7, 144.2, 151.6, 152.5, 181.1 (six carbon signals overlapping or obscured). MS (ESI-TOF): *m/z* 248.2 $[M+H]^+$.

(*E*)-3-(Dimethylamino)-1-(4-methyl-2-(phenylamino)thiazol-5-yl)prop-2-en-1-one (18). A mixture of **11** (1.00 g, 4.30 mmol) and Bredereck's reagent (1.70 mL, 8.60 mmol) was heated at reflux for 6 h. The reaction mixture was cooled and triturated with DCM (5 mL) to give a yellowish solid. The solid was then purified by FlashMaster Personal$^+$ chromatography (silica gel, PE ramping to PE:EtOAc = 50:50) to give **18** as a brown solid (927 mg, 75%).^{1}H NMR (500 MHz, $CDCl_3$) δ 2.60 (s, 3H), 2.88 (br s, 6H), 5.32 (d, 1H, *J* 12.0), 7.09-7.13 (m, 1H), 7.34-7.39 (m, 4H), 7.70 (d, 1H, *J* 12.0). 8.75 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.5, 37.4, 45.1, 94.6, 119.5, 122.8, 124.0, 129.6, 139.9, 153.2, 165.4, 181.1 (three carbon signals overlapping or obscured). MS (ESI-TOF): *m/z* 288.2 $[M+H]^+$.

(*E*)-3-(Dimethylamino)-1-(4-methyl-2-(methyl(phenyl)amino)thiazol-5-yl)prop-2-en-1-one (19). Enaminone **19** was prepared from **11** (2.50 g, 11.0 mmol) according to general synthetic procedure 2 and purified by solvent system II. Reddish brown solid (649 mg, 20%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.63 (s, 3H), 2.82 (br s, 3H), 3.02 (br s, 3H), 3.53 (s, 3H), 5.19 (d, 1H, *J* 12.5), 7.26-7.30 (m, 1H), 7.38-7.45 (m, 4H), 7.62 (d, 1H, *J* 12.5). HRMS (ESI-TOF): *m/z* 302.1322 $[M+H]^+$; calcd. for $C_{16}H_{20}N_3OS^+$ $[M+H]^+$ 302.1322.

(*E*)-3-(Dimethylamino)-1-(2-(isopropylamino)-4-methylthiazol-5-yl)prop-2-en-1-one (20). Enaminone **20** was prepared from **12** (5.00 g, 25.2 mmol) according to general synthetic procedure 2 and purified by solvent system I. Yellow solid (5.43 g, 85%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.15 (s, 3H), 1.16 (s, 3H), 2.41 (s, 3H), 2.81 (s, 3H), 3.06 (s, 3H), 3.71 (m, 1H,), 5.20 (d, 1H, *J* 12.5), 7.49 (d, 1H, *J* 12.0), 7.88 (d, 1H, *J* 7.5). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.3, 22.2, 46.2, 93.7, 121.0, 152.2, 153.0, 167.0, 179.2 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 254.1322 $[M+H]^+$; calcd. for $C_{12}H_{20}N_3OS^+$ $[M+H]^+$ 254.1322.

5-(2-Aminopyrimidin-4-yl)-*N*-cyclopentyl-4-methylthiazol-2-amine (21). Pyrimidine amine **21** was prepared from **13** (500 mg, 3.58 mmol) according to general synthetic procedure 3. Brown solid (222 mg, 45%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.51-1.53 (m, 4H), 1.65-1.67 (m, 2H), 1.90-1.93 (m, 2H), 2.50 (s, 3H), 3.87 (m, 1H), 6.45 (s, 2H), 6.64 (d, 1H, *J* 5.5), 8.05 (d, 1H, *J* 7.0), 8.10 (d, 1H, *J* 5.5). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.7, 23.8, 33.2, 57.7, 106.9, 128.0, 139.4, 150.8, 152.0, 157.6, 160.1, 162.7, 169.5. HRMS (ESI-TOF): *m/z* 276.1288 $[M+H]^+$; calcd. for $C_{13}H_{18}N_5S^+$ $[M+H]^+$ 276.1277.

5-(2-Amino-5-fluoropyrimidin-4-yl)-*N*-cyclopentyl-4-methylthiazol-2-amine (22). Pyrimidine amine **22** was prepared from **14** (2.00 g, 6.72 mmol) according to general synthetic

procedure 3. Yellow solid (1.00 g, 50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.54-1.75 (m, 6H), 2.04-2.11 (m, 2H), 2.52 (d, 3H, *J* 2.5), 3.80 (m, 1H), 5.00 (s, 2H), 5.75 (d, 1H, *J* 6.0), 8.07 (d, 1H, *J* 3.5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.6 (d, *J* 5.0), 18.7, 30.7, 109.7 (d, *J* 8.0), 145.5, 145.7, 147.0, 147.0, 147.7, 153.7, 159.8, 170.3 (d, *J* 4.5) (one carbon signal overlapping or obscured). HRMS (ESI-TOF): *m/z* 294.1188 $[M+H]^+$; calcd. for $C_{13}H_{17}FN_5S^+$ $[M+H]^+$ 294.1183.

1-(4-(6-Nitropyridin-3-yl)piperazin-1-yl)ethan-1-one (33). Nitro compound **33** was prepared from acetylpiperazine **23** (5.00 g, 39.0 mmol) according to general synthetic procedure 4 and purified by solvent system III. Yellow solid (5.66 g, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.16 (s, 3H), 3.47 (t, 2H, *J* 5.5), 3.52 (t, 2H, *J* 5.5), 3.71 (t, 2H, *J* 5.5), 3.83 (t, 2H, *J* 5.5), 7.23 (dd, 1H, *J* 9.5 & 3.0), 8.14 (d, 1H, *J* 3.0), 8.20 (d, 1H, *J* 9.0). ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.3, 40.5, 45.3, 46.6, 46.7, 119.7, 121.1, 134.0, 148.3, 149.5, 169.2. HRMS (ESI-TOF): 251.1130 $[M+H]^+$; calcd. for $C_{11}H_{15}N_4O_3^+$ $[M+H]^+$ 251.1139.

4-(6-Nitropyridin-3-yl)piperazin-1-ium bromide (34). Nitro compound **34** was prepared from piperazine **24** (2.40 g, 27.8 mmol) according to general synthetic procedure 4 to give **34** as a light yellow solid (2.05 g, 58%), which was directly used in the next step without further purification. MS (ESI-TOF): *m/z* 209.1 $[M-Br]^+$.

***Tert*-butyl 4-(6-nitropyridin-3-yl)piperazine-1-carboxylate (35).** To a solution of **34** (2.05 g, 7.09 mmol) and Boc_2O (2.50 g, 11.4 mmol) in DCM (15 mL) was added Et_3N (4.00 mL, 28.7 mmol). The reaction mixture was stirred at room temperature for 8 h and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, PE ramping to PE:EtOAc = 1:1) to give **35** as a yellow solid (1.60 g, 73%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.48 (s, 9H), 3.45 (t, 4H, *J* 5.5), 3.63 (t, 4H, *J* 5.0), 7.20 (dd, 1H, *J* 9.5 & 3.5), 8.12 (d, 1H, *J* 3.0), 8.17 (d, 1H, *J* 9.0). ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.4, 46.6, 80.6, 119.8, 120.9,

133.9, 148.0, 149.7, 154.4 (five carbon signals overlapping or obscured). MS (ESI-TOF): *m/z* 309.2 $[M+H]^+$.

1-Methyl-4-(6-nitropyridin-3-yl)piperazine (36). Nitro compound **36** was prepared from piperazine **25** (6.66 mL, 60.0 mmol) according to general synthetic procedure 4. Dark brown solid (8.09 g, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.35 (s, 3H), 2.56 (t, 4H, *J* 5.0), 3.45 (t, 4H, *J* 5.0), 7.18 (dd, 1H, *J* 9.0 & 3.0), 8.11 (d, 1H, *J* 3.0), 8.14 (d, 1H, *J* 9.5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 46.2, 46.7, 54.4, 119.9, 120.6, 133.8, 147.8, 149.9 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 223.1192 $[M+H]^+$; calcd. for $C_{10}H_{14}N_4O_2^+$ $[M+H]^+$ 223.1190.

1-(6-Nitropyridin-3-yl)-1,4-diazepane (37). Nitro compound **37** was prepared from homopiperazine **26** (5.03 g, 50.2 mmol) according to general synthetic procedure 4. The residue was diluted with saturated aqueous Na_2CO_3 solution (100 mL), basified to pH 14 with 2.0 M NaOH and extracted with DCM (6 × 100 mL). The organic extracts were combined and concentrated under reduced pressure to give **37** as an orange solid (5.67 g, 51%). $\boldsymbol{R_F}$ (DCM:MeOH = 9:1) 0.10. mp 127-128 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.90-1.96 (m, 2H), 2.85 (t, 2H, *J* 6.0), 3.07 (t, 2H, *J* 5.5), 3.64 (t, 2H, *J* 5.5), 3.71 (t, 2H, *J* 6.0), 7.01 (dd, 1H, *J* 9.5 & 3.0), 7.98 (d, 1H, *J* 3.0), 8.14 (d, 1H, *J* 9.0) (one proton signal (NH) not observed). ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.6, 48.1, 48.2, 48.4, 52.5, 117.8, 120.4, 131.5, 146.6, 148.4. HRMS (ESI-TOF-TOF) *m/z* 223.1190 $[M+H]^+$; calcd. for $C_{10}H_{15}N_4O_2^+$ 223.1190 $[M+H]^+$.

***Tert*-butyl 4-(6-nitropyridin-3-yl)-1,4-diazepane-1-carboxylate (38).** To a solution of **37** (4.17 g, 20.0 mmol) and triethylamine (5.58 mL, 40.0 mmol) in DCM (250 mL) on an ice bath were added DMAP (489 mg, 4.00 mmol) and Boc_2O (6.55 g, 30.0 mmol). The reaction mixture was stirred on the ice bath for 1 h and at room temperature overnight, washed with 0.5 M HCl (100 mL), H_2O (100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated under

reduced pressure. The residue was purified by flash column chromatography (silica gel, DCM ramping to DCM:MeOH = 96:4) to give **38** as a yellow solid (5.84 g, 90%). $\boldsymbol{R_F}$ (DCM:MeOH = 95:5) 0.45**.** mp 146-147 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.33 (s, 3H), 1.37 (s, 6H), 1.94-1.98 (m, 2H), 3.28 (t, 1H, *J* 6.0), 3.37 (t, 1H, *J* 5.5), 3.61-3.70 (m, 6H), 7.03 (dd, 1H, *J* 9.0 & 3.5), 7.97 (d, 1H, *J* 3.0), 8.13 (d, 1H, *J* 9.0). ^{13}C NMR (125 MHz, $CDCl_3$) (*tert*-butyl 4-(6-nitropyridin-3-yl)-1,4-diazepane-1-carboxylate exists as two rotamers in approximately 4.5:5.5 ratio.) δ 24.2, 24.4 (one carbon), 28.1 (three carbons), 45.5, 45.6, 45.7, 46.2, 48.6, 48.9, 49.9, 50.0 (four carbons), 79.9, 80.0 (one carbon), 118.0, 118.1 (one carbon), 120.1, 120.2 (one carbon), 131.1, 131.2 (one carbon), 146.5, 147.3, 147.4 (one carbon), 154.4, 154.9 (one carbon). HRMS (ESI-TOF) *m/z* 323.1718 $[M+H]^+$; calcd. for $C_{15}H_{23}N_4O_4^+$ 323.1714 $[M+H]^+$.

4-(6-Nitropyridin-3-yl)morpholine (39). Nitro compound **39** was prepared from morpholine **27** (1.30 mL, 14.8 mmol) according to general synthetic procedure 4 and purified by solvent system III. Yellow solid (2.29 g, 100%). ^{1}HNMR (500 MHz, $CDCl_3$) δ 3.41 (t, 4H, *J* 5.0), 3.89 (t, 4H, *J* 5.0), 7.23 (dd, 1H, *J* 9.0 & 3.0), 8.14 (d, 1H, *J* 3.0), 1.18 (d, 1H, *J* 9.0). ^{13}C NMR (125 MHz, $CDCl_3$) δ 46.8, 66.3, 119.8, 120.8, 133.8, 148.3, 150.1 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 210.0858 $[M+H]^+$; calcd. for $C_9H_{11}N_3O_3^+$ $[M+H]^+$ 210.0873.

1-Ethyl-4-(6-nitropyridin-3-yl)piperazine (40). Nitro compound **40** was prepared from 1-ethylylpiperazine **28** (7.00 mL, 55.2 mmol) according to general synthetic procedure 4. Yellow solid (9.80 g, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (t, *J* 7.5), 2.63 (t, 4H, *J* 5.5), 3.13 (q, 2H, *J* 7.5), 3.48 (t, 4H, *J* 5.5), 7.19 (dd, 1H, *J* 9.0 & 3.0), 8.13 (d, 1H, *J* 3.0), 8.15 (d, 1H, *J* 9.5). MS (ESI-TOF): *m/z* 237.1 $[M+H]^+$.

2-Nitro-5-(piperidin-1-yl)pyridine (41). Nitro compound **41** was prepared from piperidine **29** (2.40 g, 27.8 mmol) according to general synthetic procedure 4. The solid was washed with H_2O

(100 mL) and purified by solvent system III. Yellow solid (4.06 g, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.69 (s, 6H), 3.45 (t, 4H), 7.13 (dd, 1H, *J* 9.0 & 2.5), 8.07 (d, 1H, *J* 2.5), 8.10 (d, 1H, *J* 9.0). ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.0, 25.2, 48.1, 120.0, 133.6, 146.9, 150.0 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 208.1081 $[M+H]^+$. calcd. for $C_{10}H_{14}N_3O_2^+$ $[M+H]^+$ 208.1077.

***N*,*N*-Dimethyl-1-(6-nitropyridin-3-yl)piperidin-4-amine (42).** Nitro compound **42** was prepared from dimethylaminopiperidine **30** (2.50 g, 19.5 mmol) according to general synthetic procedure 4 and purified by solvent system III. Yellow solid (1.5 g, 37%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.51 (m, 2H), 1.94 (app d, 2H, *J* 12.0), 2.39 (s, 6H), 2.78 (app br s, H), 3.02 (m, 2H), 1.94 (app d, 2H, *J* 13.5), 7.49 (dd, 1H, *J* 9.0 & 3.0), 8.13 (d, 1H, *J* 9.0), 8.26 (d, 1H, *J* 9.5). ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.3, 45.0, 61.9, 119.9, 120.9, 133.8, 146.6, 149.1 (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 251.1506 $[M+H]^+$; calcd. for $C_{12}H_{19}N_4O_2^+$ $[M+H]^+$ 251.1503.

1-(Methylsulfonyl)-4-(6-nitropyridin-3-yl)piperazine (43). Nitro compound **43** was prepared from 1-methylsulfonylpiperazine **31** (4.2 g, 25.4 mmol) according to general synthetic procedure 4 and purified by solvent system II. Yellow solid (3.00 g, 41%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.93 (s, 3H), 3.26 (t, 4H, *J* 5.5), 3.65 (t, 4H, *J* 5.0), 7.54 (dd, 1H, *J* 9.5 & 3.0), 8.18 (d, 1H, *J* 9.0), 8.30 (d, 1H, *J* 3.0). ^{13}C NMR (125 MHz, DMSO-d_6) δ 34.2, 44.7, 45.8, 119.8, 121.3, 134.1, 147.1, 149.4 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 287.0815 $[M+H]^+$; calcd. for $C_{10}H_{15}N_4O_4S^+$ $[M+H]^+$ 287.0809.

***Tert*-butyl (1-(6-nitropyridin-3-yl)piperidin-4-yl)carbamate (44).** Nitro compound **44** was prepared from 4-Boc-aminopiperazine **32** (5.92 g, 29.6 mmol) according to general synthetic procedure 4 and purified by solvent system I. Yellow solid (5.42 g, 75%). ^{1}H NMR (500 MHz,

$CDCl_3$) δ 1.43 (s, 9H), 1.49-1.52 (m, 2H), 2.10 (app dd, 2H), 3.08-3.14 (m, 2H), 3.73 (app s, H), 3.89 (app d, 2H), 4.53 (s, H), 7.18 (dd, 1H, *J* 9.5 & 3.5), 8.10 (d, 1H, *J* 3.0), 8.12 (d, 1H, *J* 9.5). MS (ESI-TOF): *m/z* 323.1696 $[M+H]^+$.

1-(4-(6-Aminopyridin-3-yl)piperazin-1-yl)ethan-1-one (45). Nitro compound **33** (2.51 g, 10.0 mmol) was reduced according to general synthetic procedure 5 to give **45** as a brown solid (2.20 g, 100%). HRMS (ESI-TOF): *m/z* 221.1390 $[M+H]^+$; calcd. for $C_{11}H_{17}N_4O^+$ $[M+H]^+$ 221.1397.

***Tert*-butyl 4-(6-aminopyridin-3-yl)piperazine-1-carboxylate (46).** Nitro compound **35** (1.60 g, 5.19 mmol) in MeOH (15 mL) was reduced according to general synthetic procedure 5 to give **46** as a grey solid (1.20 g, 83%). HRMS (ESI-TOF): *m/z* 279.1836 $[M+H]^+$; calcd. for $C_{14}H_{23}N_4O_2^+$ $[M+H]^+$ 279.1816.

5-(4-Methylpiperazin-1-yl)pyridin-2-amine (47). Nitro compound **36** (1.80 g, 8.10 mmol) was reduced according to general synthetic procedure 5 to give **47** as a brown solid (1.66 g, 100%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.34 (s, 3H), 2.58 (t, 4H, *J* 5.0), 3.05 (t, 4H, *J* 5.0), 3.83 (br s, 2H), 6.47 (d, 1H, *J* 9.0), 7.17 (dd, 1H, *J* 9.0 & 3.0), 7.76 (d, 1H, *J* 3.0). ^{13}C NMR (125 MHz, $CDCl_3$) δ 46.3, 50.8, 55.2, 109.2, 128.9, 137.2, 140.8, 153.1 (two carbon signals overlapping or obscured). HRMS (ESI-TOF) *m/z* 193.1466 $[M+H]^+$; calcd for $C_{10}H_{17}N_4^+$ $[M+H]^+$ 193.1448.

***Tert*-butyl 4-(6-aminopyridin-3-yl)-1,4-diazepane-1-carboxylate (48).** Nitro compound **37** (5.80 g, 18.0 mmol) was reduced according to general synthetic procedure 5 to give **48** as a dark purple glue (5.26 g, 100%). $\boldsymbol{R_F}$ (DCM:MeOH = 9:1) 0.56. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37 (s, 3H), 1.42 (s, 6H), 1.90-1.95 (m, 2H), 3.21 (t, 1H, *J* 6.0), 3.32 (t, 1H, *J* 5.5), 3.41-3.46 (m, 4H), 3.52-3.56 (m, 2H), 3.96 (br s, 2H), 6.47 (d, 1H, *J* 8.5), 6.96 (dd, 1H, *J* 9.0 & 3.0), 7.61 (app s,

1H). ^{13}C NMR (125 MHz, $CDCl_3$) (*tert*-butyl 4-(6-aminopyridin-3-yl)-1,4-diazepane-1-carboxylate exists as two rotamers in approximately 4.5:5.5 ratio.) δ 25.5, 25.6 (one carbon), 28.4, 28.5 (three carbons), 45.7, 46.1, 46.4, 46.5, 48.7, 49.3, 50.7, 50.9 (four carbons), 79.6, 79.7 (one carbon), 109.9, 110.0 (one carbon), 123.6, 123.9 (one carbon), 131.9, 132.2 (one carbon), 137.4, 137.7 (one carbon), 150.1, 150.2 (one carbon), 155.2, 155.5 (one carbon). HRMS (ESI-TOF) *m/z* 293.1972 $[M+H]^+$; calcd. for $C_{15}H_{25}N_4O_2^+$ 293.1972 $[M+H]^+$.

5-Morpholinopyridin-2-amine (49). Nitro compound **39** (1.46 g, 6.96 mmol) in MeOH (50 mL) was reduced according to general synthetic procedure 5 to give **49** as a brown solid (1.24 g, 99 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.89 (t, 4H, *J* 4.5), 3.69 (t, 4H, *J* 4.5), 5.42 (s, 2H), 6.42 (d, 1H, *J* 9.0), 7.15 (dd, 1H, *J* 9.0 & 3.0), 7.61 (d, 1H, *J* 3.0). ^{13}C NMR (125 MHz, DMSO-d_6) δ 50.4, 66.2, 108.4, 127.9, 135.8, 138.9, 154.5 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 180.1159 $[M+H]^+$; calcd. for $C_9H_{13}N_3O^+$ $[M+H]^+$ 180.1131.

5-(4-Ethylpiperazin-1-yl)pyridin-2-amine (50). Nitro compound **40** (9.80 g, 41.5 mmol) was reduced according to general synthetic procedure 5 to give **50** as a brown solid (9.00 g, 100%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.45 (t, *J* 7.5, 3H), 2.81 (app br s, 4H), 3.13 (q, 2H, *J* 7.5), 3.29 (app br s, 4H), 3.36 (br s, 2H), 6.51 (d, 1H, *J* 9.0), 7.21 (dd, 1H, *J* 9.0 & 3.0), 7.77 (d, 1H, *J* 3.0). MS (ESI-TOF): *m/z* 207.1 $[M+H]^+$.

5-(Piperidin-1-yl)pyridin-2-amine (51). Nitro compound **41** (3.70 g, 15.9 mmol) was reduced according to general synthetic procedure 5 to give **51** as a dark brown solid (3.10 g, 97%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.53 (m, 2H), 1.71 (m, 4H), 2.97 (t, 4H, *J* 5.5), 4.15 (s, 2H), 6.47 (d, 1H, *J* 9.0), 7.18 (dd, 1H, *J* 9.0 & 3.0), 7.77 (d, 1H, *J* 3.0). ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.1, 26.1, 52.5, 109.3, 129.7, 137.2, 142.0, 152.7 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 178.1347 $[M+H]^+$; calcd. for $C_{10}H_{16}N_3^+$ $[M+H]^+$ 178.1339.

5-(4-(Dimethylamino)piperidin-1-yl)pyridin-2-amine (52). Nitro compound **42 (**1.50 g, 5.99 mmol) was reduced according to general synthetic procedure 5 to give **52** as a white solid (1.20 g, 91%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.55 (m, 2H), 1.88 (app d, 2H, *J* 12.0), 2.37 (s, 6H), 2.45 (app br s, H), 2.51 (m, 2H), 3.39 (app d, 2H, *J* 12.0), 5.40 (s, 2H), 6.40 (d, 1H, *J* 9.0), 7.17 (dd, 1H, *J* 9.0 & 3.0), 7.62 (d, 1H, *J* 3.0). ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.5, 50.0, 61.5, 108.3, 128.8, 136.6, 138.9, 154.4, (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 221.1756 $[M+H]^+$; calcd for $C_{12}H_{21}N_4^+$ $[M+H]^+$ 221.1761.

5-(4-(Methylsulfonyl)piperazin-1-yl)pyridin-2-amine (53). Nitro compound **43** (2.50 g, 8.71 mmol) was reduced according to general synthetic procedure 5 to give **53** as a brown solid (1.80 g, 82%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.91 (s, 3H), 3.00 (t, 4H, *J* 5.0), 3.22 (t, 4H, *J* 4.5), 6.41 (d, 1H, *J* 8.5), 7.19 (dd, 1H, *J* 9.5 & 3.0), 7.64 (d, 1H, *J* 3.0). ^{13}C NMR (125 MHz, DMSO-d_6) δ 33.8, 33.9, 50.1, 108.2, 128.8, 137.0, 138.3, 154.9 (two carbon signals overlapping or obscured). HRMS **(**ESI-TOF): *m/z* 257.1068 $[M+H]^+$; calcd for $C_{10}H_{17}N_4O_2S^+$ $[M+H]^+$ 257.1067.

***Tert*-butyl(1-(6-aminopyridin-3-yl)piperidin-4-yl)carbamate (54).** Nitro compound **44** (4.00 g, 12.4 mmol) was reduced according to general synthetic procedure 5 to give **54** as a grey solid (3.27g, 90%).^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.50-1.57 (m, 2H), 2.02 (app d, 2H), 2.72 (app t, 2H), 3.31 (app d, 2H), 3.55 (app s, H), 3.88 (br, 2H), 4.55 (s, H), 6.47 (d, 1H, *J* 9.0), 7.16 (dd, 1H, *J* 9.5 & 3.5), 7.73 (d, 1H, *J* 2.5), HRMS (ESI-TOF): *m/z* 293.3830 $[M+H]^+$; calcd. for $C_{14}H_{23}N_4O_2^+$ $[M+H]^+$ 293.3831.

1-Acetyl-4-(6-(2,3-bis(*tert*-butoxycarbonyl)guanidino)pyridin-3-yl)piperazine (56). Compound **56** was prepared from **45** (2.21 g, 10.0 mmol) according to general synthetic procedure 6 and purified by solvent system II. Light yellow solid (3.82 g, 82%). ^{1}H NMR (500

MHz, $CDCl_3$) δ 1.53 (s, 18 H), 2.14 (s, 3H), 3.13 (t, 2H, *J* 5.5), 3.18 (t, 2H, *J* 5.5), 3.63 (t, 2H, *J* 5.5), 3.78 (t, 2H, *J* 5.5), 7.29 (dd, 1H, *J* 9.0 & 3.0), 7.87 (d, 1H, *J* 7.5), 7.99 (d, 1H, *J* 2.5), 10.90 (br s, 1H), 11.58 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.5, 28.2, 28.3, 28.4, 41.2, 46.1, 49.3, 49.6, 53.6, 81.1, 84.5, 116.9, 125.2, 126.8, 136.7, 143.7, 144.3, 153.1, 153.4, 162.6, 169.1 (one carbon signal overlapping or obscured). HRMS (ESI-TOF): *m/z* 463.2668 $[M+H]^+$; calcd. for $C_{22}H_{35}N_6O_5^+$ $[M+H]^+$ 463.2663.

***Tert*-butyl 4-(6-(2,3-bis(*tert*-butoxycarbonyl)guanidino)pyridin-3-yl)piperazine-1-carboxylate (57).** Compound **57** was prepared from **46** (1.30 g, 4.67 mmol) according to general synthetic procedure 6 and purified by solvent system I. White solid (1.00 g, 41%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.52 (s, 18H), 3.10 (t, 4H, *J* 5.0), 3.57 (t, 4H, *J* 5.0), 7.27 (dd, 1H, *J* 9.0 & 3.0), 7.92 (app br s, 1H), 7.96 (d, 1H, *J* 3.0), 10.85 (s, 1H), 11.54 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 28.4, 49.3, 80.0, 126.4, 144.3, 154.6 (nineteen carbon signals overlapping or obscured). MS (ESI-TOF): *m/z* 521.3.

1-Methyl-4-(6-(2,3-bis(*tert*-butoxycarbonyl)guanidino)pyridin-3-yl)piperazine (58). Compound **58** was prepared from **47** (1.64 g, 8.53 mmol) according to general synthetic procedure 6 purified by solvent system II. Beige solid (2.23g, 60%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.53 (s, 18H), 2.35 (s, 3H), 2.58 (t, 4H, *J* 5.0), 3.18 (t, 4H, *J* 5.0), 7.27 (dd, 1H, *J* 9.0 & 3.0), 7.97 (d, 1H, *J* 2.0), 8.19 (d, 1H, *J* 6.5), 10.7 (br s, 1H), 11.52 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.2, 28.3, 46.3, 49.3, 54.9, 79.8, 83.8, 116.6, 125.6, 136.5, 143.3, 144.5, 152.9, 163.5 (seven carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 435.2712 $[M+H]^+$; calcd. for $C_{21}H_{35}N_6O_4^+$ $[M+H]^+$ 435.2714.

***Tert*-butyl 4-(6-(2,3-bis(*tert*-butoxycarbonyl)guanidino)pyridin-3-yl)-1,4-diazepane-1-carboxylate (59).** Compound **59** was prepared from **48** according to general synthetic procedure

6 and purified by solvent system I. Yellow foam (5.70 g, 59%). R_F (DCM:MeOH = 98:2) 0.24. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40 (s), 1.43 (s), 1.51(s), 1.52 (s) (total 27H), 1.94-1.98 (m, 2H), 3.18 (t, 1H, *J* 6.0), 3.29 (t, 1H, *J* 5.5), 3.52-3.55 (m, 6H), 7.05 (dd, 1H, *J* 9.0 & 2.5), 7.78 (app s, 1H), 8.15 (d, 1H, *J* 9.0), 10.63 (br s, 1H), 11.50 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) (*tert*-butyl 4-(6-(2,3-bis(*tert*-butoxycarbonyl)guanidino)pyridin-3-yl)-1,4-diazepane-1-carboxylate exists as two rotamers in approximately 4.5:5.5 ratio.) δ 24.8, 24.9 (one carbon), 28.2, 28.3, 28.4, 28.5 (nine carbons), 45.8, 46.0, 46.4, 46.7, 47.9, 48.4, 50.4, 50.6 (four carbons), 79.7, 79.8, 79.9 (one carbon), 83.7, 117.2, 117.3 (one carbon), 120.7, 120.8 (one carbon), 131.5, 131.6 (one carbon), 140.5, 140.6, 140.7, 140.9 (two carbons), 152.8, 152.9 (one carbon), 155.0, 155.4, 163.5. HRMS (ESI-TOF) *m/z* 535.3246 $[M+H]^+$; calcd. for $C_{26}H_{43}N_6O_6^+$ 535.3239 $[M+H]^+$.

4-(6-(2,3-Bis(*tert*-butoxycarbonyl)guanidino)pyridin-3-yl-)morpholine (60). Compound **60** was prepared from **49** (1.65 g, 9.21 mmol) according to general synthetic procedure 6 and purified by solvent system I. Yellow solid (1.48 g, 38%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.56 (s, 18H), 3.21 (t, 4H, *J* 5.0), 3.87 (t, 4H, *J* 5.0), 7.09 (d, 1H, *J* 5.0), 7.29 (d, 1H, *J* 9.0), 7.29 (dd, 1H, *J* 9.0 & 3.0), 8.02 (d, 1H, *J* 2.0), 11.25 (br s, 1H), 11.74 (broad s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.2 (*J* 25.0), 49.4, 66.8, 79.8, 83.8, 116.6, 123.4, 136.1, 143.7, 144.5, 153.0 (*J* 12.5), 163.4 (eight carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 422.2370 $[M+H]^+$; calcd. for $C_{20}H_{32}N_5O_5^+$ $[M+H]^+$ 422.2398.

1-Ethyl-4-(6-(2,3-bis(tert-butoxycarbonyl)guanidino)pyridin-3-yl)piperazine (61). Compound **61** was prepared from **50** (9.00 g, 43.6 mmol) according to general synthetic procedure 6 and purified by solvent system I. White solid (9.98 g, 51%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.11 (t, 3H, *J* 7.0), 1.51 (app d, 18H, *J* 2.0), 2.46 (q, 2H, *J* 7.0), 2.60 (t, 4H, *J* 5.0), 3.17 (t, 4H, *J* 5.0), 7.27 (dd, 1H, *J* 9.0 & 3.0), 7.97 (d, 1H, *J* 3.0), 8.20 (d, 1H, *J* 8.5), 10.70 (s, 1H),

11.51 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.1, 28.1, 28.3, 49.3, 52.4, 52.6, 79.1, 83.8, 116.6, 125.5, 136.4, 143.3, 144.6, 152.9, 163.5 (seven carbon signals overlapping or obscured). HRMS (ESI-TOF) *m/z* 449.2870 $[M+H]^+$; calcd. for $C_{22}H_{37}N_6O_4^+$ $[M+H]^+$ 449.2871.

***N*-(6-(2,3-Bis(tert-butoxycarbonyl)guanidino)pyridin-3-yl-)piperidine (62).** Compound **62** was prepared from **51** (3.10 g, 17.5 mmol) according to general synthetic procedure 6 and purified by solvent system I. White solid (4.00 g, 55%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.51 (d, 18H, *J* 4.0), 1.57 (m, 2H), 1.70 (t, 4H, *J* 5.5), 3.11 (t, 4H, *J* 5.5), 7.26 (dd, 1H, *J* 9.0 & 3.0), 7.96 (d, 1H, *J* 3.0), 8.17 (d, 1H, *J* 8.0), 10.68 (s, 1H), 11.51 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.1, 25.7, 28.1, 28.3, 50.8, 79.7, 82.1, 83.7, 116.5, 126.0, 136.8, 142.8, 145.5, 149.8, 152.9, 163.5 (five carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 420.2604 $[M+H]^+$; calcd. for $C_{21}H_{34}N_5O_4^+$ $[M+H]^+$ 420.2605.

4-Dimethylamino-1-(6-(2,3-bis(tert-butoxycarbonyl)guanidino)pyridin-3-yl)piperidine (63). Compound **63** was prepared from **52** (1.20 g, 5.45 mmol) according to general synthetic procedure 6 and purified by solvent system II. White solid (1.20 g, 48%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.51 (s, 18H), 1.72 (m, 2H), 2.06 (app d, 2H, *J* 12.0), 2.50 (s, 6H), 2.70 (m, 3H), 3.62 (app d, 2H, *J* 12.5), 7.21 (dd, 1H, *J* 9.0 & 3.0), 7.89 (d, 1H, *J* 2.5), 8.12 (s, 1H), 10.63 (s, 1H), 11.45 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.7, 28.0, 28.1, 31.2, 40.4, 48.8, 62.4, 116.5, 126.2, 136.7, 143.4, 143.9, 152.8, 163.2 (nine carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 463.3027 $[M+H]^+$; calcd. for $C_{23}H_{39}N_6O_4^+$ $[M+H]^+$ 463.3027.

1-Methylsulfonyl-4-(6-(2,3-bis(tert-butoxycarbonyl)guanidino)pyridin-3-yl)piperazine (64). Compound **64** was prepared from **53** (1.80 g, 7.02 mmol) according to general synthetic procedure 6 and purified by solvent system II. Beige solid (1.20 g, 22%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.52 (s, 18H), 2.83 (s, 3H), 3.25 (t, 4H, *J* 5.0), 3.39 (t, 4H, *J* 4.5), 7.29 (dd, 1H, *J* 9.0 &

3.0), 7.98 (d, 1H, *J* 2.0), 8.23 (s, 1H), 10.76 (s, 1H), 11.52 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.2, 28.3, 34.7, 45.7, 49.6, 53.6, 80.0, 84.0, 116.8, 126.8, 137.4, 143.8, 144.4, 153.1, 163.3 (six carbon signals overlapping or obscured). HRMS (ESI): *m/z* 499.2336 $[M+H]^+$; calcd. for $C_{21}H_{35}N_6O_6S^+[M+H]^+$ 499.2333.

***Tert*-butyl (1-(6-(2,3-bis(tert-butoxycarbonyl)guanidino)pyridin-3-yl)piperidin-4-yl)carbamate (65).** Compound **59** was prepared from **54** (3.70 g, 12.7 mmol) according to general synthetic procedure 6 and purified by solvent system I. Yellow solid (4.00 g, 57%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.50 (app d, 29H), 2.03 (app d, 2H), 2.81 (app t, 2H), 3.51 (app d, 2H), 3.59 (br s, 1H), 4.51 (app br s, 1H), 7.24 (app br s, 1H), 7.95 (app s, 1H), 8.18 (app s, 1H, *J* 2.5), 10.69 (s, 1H), 11.50 (s, 1H). **MS** (ESI-TOF): *m/z* 535.3485 $[M+H]^+$.

2-(2,3-Bis(*tert*-butoxycarbonyl)guanidino)pyridine (66). Compound **66** was prepared from pyridin-2-amine **55** (2.78 g, 29.5 mmol) according to general synthetic procedure 6 and purified by solvent system I and recrystallized with DCM and hexane. White solid (4.18 g, 42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.52 (s, 18H), 7.01 (dd, 1H, *J* 7.0 & 5.0), 7.70 (t, 1H, *J* 7.0), 8.29 (d, 1H, *J* 4.0), 8.37 (app br s, 1H), 10.89 (br s, 1H), 11.53 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.2, 28.3, 80.1, 84.0, 116.2, 120.0, 138.3, 148.2, 150.7, 152.8, 153.2, 163.3 (four carbon signals overlapping or obscured). HRMS (ESI-TOF) *m/z* 337.1885 $[M+H]^+$; calcd. for $C_{16}H_{25}N_4O_4^+$ 337.1870 $[M+H]^+$.

1-(5-(4-Acetylpiperazin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (67). Guanidine **67** was prepared from **56** (724 mg, 1.56 mmol) according to general synthetic procedure 7. Beige solid (410 mg, 100%). **MS** (ESI-TOF): *m/z* 263.2 $[M-TFA+H]^+$.

1-(5-(Piperazin-1-yl)pyridin-2-yl)guanidine (68). Guanidine **68** was prepared from **57** (1.00 g, 1.92 mmol) according to general synthetic procedure 7. The residue was re-dissolved in

MeOH (50 mL), and a suspension of excess Ambersep 900 resin (hydroxide form, pre-swelled with H_2O for 30 min and MeOH for 30 min) in MeOH (50 mL) was added. The mixture was stirred at room temperature overnight and filtered, and the solid was washed with MeOH (50 mL). The filtrate and washing were combined and concentrated under reduced pressure to give **68** as a beige solid (423 mg, 100%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.01 (t, 4H, *J* 5.0), 3.17 (t, 4H, *J* 5.0), 6.97 (d, 1H, *J* 9.0), 7.57 (dd, 1H, *J* 9.0 & 3.0), 7.95 (d, 1H, *J* 2.5), 8.33 (br s, 3H) (two NH proton signals not observed). ^{13}C NMR (125 MHz, $CDCl_3$) δ 44.2, 47.6, 113.7, 127.6, 133.3, 143.6, 144.6, 155.1 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 221.1526 $[M+H]^+$; calcd. for $C_{10}H_{17}N_6^+$ $[M+H]^+$ 221.1509.

1-(5-(4-Methylpiperazin-1-yl)pyridine-2-yl)guanidine (69). Guanidine **69** was prepared from **58** (2.20 g, 5.06 mmol) according to general synthetic procedure 7. The residue was redissolved in MeOH (50 mL), and a suspension of excess Ambersep 900 resin (hydroxide form, pre-swelled with H_2O for 30 min and MeOH for 30 min) in MeOH (50 mL) was added. The mixture was stirred at room temperature overnight and filtered, and the solid was washed with MeOH (50 mL). The filtrate and washing were combined and concentrated under reduced pressure to give **69** as a beige solid (1.19 g, 100%). ^{1}H NMR (500 MHz, CD_3OD) δ 2.34 (s, 3H), 2.61 (t, 4H, *J* 5.0), 3.11 (t, 4H, *J* 5.0), 6.74 (d, 1H, *J* 9.0), 7.32 (dd, 1H, *J* 9.0 & 3.0), 7.85 (d, 1H, *J* 3.0) (four NH proton signals not observed due to H/D exchange). ^{13}C NMR (125 MHz, CD_3OD) δ 46.1, 50.8, 55.9, 119.2, 129.1, 135.3, 142.5, 157.3, 158.7 (two carbon signals overlapping or obscured). MS (ESI-TOF): *m/z* 235.2 $[M+H]^+$.

1-(5-(1,4-Diazepan-1-yl)pyridin-2-yl)guanidine di(2,2,2-trifluoroacetate) (70). Guanidine **70** was prepared from **59** (802 mg, 1.50 mmol) according to general synthetic procedure 7. Yellow glue (494 mg, 100%). ^{1}H NMR (500 MHz, CD_3OD) δ 2.18-2.24 (m, 2H), 3.31 (t, 2H, *J*

7.0), 3.42 (t, 2H, *J* 5.0), 3.60 (t, 2H, *J* 7.0), 3.80 (t, 2H, *J* 5.0), 6.97 (d, 1H, *J* 9.0), 7.39 (dd, 1H, *J* 9.0 & 3.0), 7.91 (d, 1H, *J* 3.0) (seven proton signals (NH_2 & 3 × NH & 2 × COOH) not observed due to H/D exchange). ^{13}C NMR (125 MHz, CD_3OD) δ 26.4, 46.6, 46.8, 47.2, 48.3, 115.0, 117.9 (q, *J* 287.5), 124.6, 131.2, 143.0, 144.0, 156.8, 162.7 (q, *J* 37.5). HRMS (ESI-TOF) *m/z* 235.1674 $[M\text{-}2CF_3COOH+H]^+$; calcd. for $C_{11}H_{19}N_6^+$ 235.1666 $[M\text{-}2CF_3COOH+H]^+$.

1-(5-Morpholinopyridin-2-yl)guanidine (71). Guanidine **71** was prepared from **60** (857 mg, 2.03 mmol) according to general synthetic procedure 7. The residue was added to a suspension of Ambersep 900 resin (hydroxide form) and stirred for 70 min, filtered and washed with MeOH. The filtrate and washing were combined and dried under reduced pressure to give **71** as pale yellow solid (296 mg, 66%). **MS** (ESI-TOF): *m/z* 222.1419 $[M+H]^+$.

1-(5-(4-Ethylpiperazin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (72). Guanidine **72** was prepared from **61** (2.20 g, 4.46 mmol) according to general synthetic procedure 7. Yellow solid (1.70 g, 100%). **HRMS** (ESI-TOF): *m/z* 249.1803 $[M+H]^+$. calcd. for $C_{12}H_{21}N_6^+$ $[M+H]^+$ 249.1822.

1-(5-(Piperidin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (73). Guanidine **73** was prepared from **62** (4.00 g, 9.12 mmol) according to general synthetic procedure 7. White solid (2.30 g, 100%). **HRMS** (ESI-TOF): *m/z* 220.1558 $[M+H]^+$; calcd. for $C_{11}H_{18}N_5^+$ $[M+H]^+$ 220.1557.

1-(5-(4-(Dimethylamino)piperidin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (74).

Guanidine **74** was prepared from **63** (1.20 g, 2.59 mmol) according to general synthetic procedure 7. Yellow solid (1.80 g, 100%). HRMS (ESI-TOF): *m/z* 263.1979 $[M+H]^+$. calcd. for $C_{13}H_{23}N_6^+$ $[M+H]^+$ 263.1979.

1-(5-(4-(Methylsulfonyl)piperazin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (75). Guanidine **75** was prepared from **64** (1.80 g, 3.89 mmol) according to general synthetic procedure 7. Grey solid (1.52 g, 100%). HRMS (ESI-TOF): *m/z* 299.1286 $[M+H]^+$. calcd. for $C_{11}H_{19}N_6O_2S^+$ $[M+H]^+$ 299.1285.

1-(5-(4-Aminopiperidin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (76). Guanidine **76** was prepared from **65** (2.00 g, 3.74 mmol) according to general synthetic procedure 7. Yellow solid (568 mg, 65%). **MS** (ESI-TOF): *m/z* 235.1788 $[M+H]^+$.

1-(Pyridin-2-yl)guanidine 2,2,2-trifluoroacetate (77). Guanidine **77** was prepared from **66** (3.60 g, 10.7 mmol) according to general synthetic procedure 7. White solid (2.65 g, 99%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.04 (dt, 1H, *J* 7.5 & 0.5), 7.18 (ddd, 1H, *J* 7.5 & 5.0 & 1.0), 7.85 (ddd, 1H, *J* 9.5 & 7.5 & 2.0), 8.34 (ddd, 1H, *J* 5.0 & 2.0 & 1.0) (five proton signals (NH_2 & 2 × NH & COOH) not observed due to H/D exchange). ^{13}C NMR (125 MHz, CD_3OD) δ 114.3, 118.2 (q, *J* 287.5), 120.8, 140.6, 147.8, 153.5, 157.2, 163.3 (q, *J* 37.5). HRMS (ESI-TOF) *m/z* 137.0827 $[M\text{-}CF_3COOH+H]^+$; calcd. for $C_6H_9N_4^+$ 137.0822 $[M\text{-}CF_3COOH+H]^+$.

***N*-Cyclopentyl-4-methyl-5-(2-((5-(piperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl) thiazol-2-amine (78).** 1-(5-(Piperazin-1-yl)pyridin-2-yl)guanidine (**68**, 441 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**13**, 279 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system III and recrystallized with DCM and MeOH to give **78** as a dark yellow solid (70.0 mg, 16%). R_F (DCM:MeOH:NH_4OH = 9:1:0.5) 0.10. mp 210-212 °C. ^{1}H NMR (500 MHz, DMSO-d_6) 1.49-1.68 (m, 7H), 1.89-1.94 (m, 2H), 2.46 (s, 3H), 2.85 (t, 4H, *J* 4.5), 3.02 (t, 4H, *J* 5.0), 3.98 (m, 1H), 6.90 (d, 1H, *J* 5.5), 7.36 (dd, 1H, *J* 9.0 & 3.0), 7.98 (d, 1H, *J* 3.0), 8.07 (d, 1H, *J* 9.0), 8.18 (d, 1H, *J* 7.0), 8.33 (d, 1H, *J* 5.5), 9.33 (s, 1H). ^{13}C NMR (125 MHz, DMSO-

d_6) δ 19.2, 23.8, 32.7, 45.9, 50.2, 56.3, 107.6, 113.7, 117.8, 125.5, 135.9, 143.5, 146.1, 152.9, 158.0, 159.0, 159.2, 168.4 (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 437.2222 $[M+H]^+$; calcd. for $C_{22}H_{29}N_8S^+$ $[M+H]^+$ 437.2230. Anal. RP-HPLC Method A: t_R 10.10 min, purity > 99%; Method B: t_R 7.78 min, purity > 99%.

5-(2-((5-(1,4-Diazepan-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)-*N*-cyclopentyl-4-methylthiazol-2-amine (79). 1-(5-(1,4-Diazepan-1-yl)pyridin-2-yl)guanidine di(2,2,2-trifluoroacetate) (**70**, 469 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**13**, 279 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **79** as yellow solid (35 mg, 8%). R_F (DCM:MeOH = 9:1) 0.10. mp 211-213 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.56-1.79 (m, 6H), 1.89-1.94 (m, 2H), 2.06-2.12 (m, 2H), 2.54 (s, 3H), 2.84 (t, 2H, *J* 6.0), 3.06 (t, 2H, *J* 5.5), 3.54 (t, 2H, *J* 5.0), 3.59 (t, 2H, *J* 6.0), 3.86-3.92 (m, 1H), 5.43 (d, 1H, *J* 7.0), 6.81 (d, 1H, *J* 5.5), 7.09 (dd, 1H, *J* 9.0 & 3.0), 7.64 (s, 1H), 7.82 (d, 1H, *J* 3.0), 8.16 (d, 1H, *J* 9.0), 8.30 (d, 1H, *J* 5.5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.8, 23.9, 29.8, 33.3, 48.1, 48.4, 48.5, 52.3, 57.6 107.5, 113.9, 120.0, 121.1, 131.7, 140.7, 143.1, 152.2, 157.5, 159.1, 159.6, 169.3 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 451.2384 $[M+H]^+$; calcd. for $C_{23}H_{31}N_8S^+$ $[M+H]^+$ 451.2387. Anal. RP-HPLC Method A: t_R 9.91 min, purity > 97%; Method B: t_R 7.49 min, purity > 99%.

***N*-Cyclopentyl-4-methyl-5-(2-((5-(4-methylpiperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)thiazol-2-amine (80).** 1-(5-(4-Methylpiperazin-1-yl)pyridine-2-yl)guanidine (**69**, 468 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**13**, 279 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **80** as a yellow solid (100 mg, 22%). R_F

(DCM:MeOH = 9:1) 0.10. mp 203-205 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.51-1.71 (m, 6H), 1.99-2.05 (m, 2H), 2.35 (s, 3H), 2.48 (s, 3H), 2.62 (t, 4H, *J* 5.0), 3.14 (t, 4H, *J* 5.0), 3.79 (br, 1H),), 6.03 (br, 1H), 6.78 (d, 1H, *J* 5.0), 7.27 (dd, 1H, *J* 9.0 & 3.0), 7.96 (d, 1H, *J* 3.0), 8.10 (s, 1H), 8.21 (d, 1H, *J* 9.0), 8.29 (d, 1H, *J* 5.0). ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.5, 23.9, 33.2, 45.9, 49.7, 54.9, 57.7, 107.8, 113.2, 119.6, 126.8, 142.7, 146.7, 151.7, 157.6, 158.9, 159.4, 167.9, 169.5 (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 451.2396 $[M+H]^+$; calcd. for $C_{23}H_{31}N_8S^+$ $[M+H]^+$ 451.2387. Anal. RP-HPLC Method A: t_R 9.56 min, purity > 99%; Method B: t_R 9.50 min, purity > 98%.

***N*-Cyclopentyl-5-(2-((5-(4-ethylpiperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)-4-methylthiazol-2-amine (81).** 1-(5-(4-Ethylpiperazin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (**72**, 496 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**13**, 279 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II and recrystallized from MeOH to give **81** as a yellow solid (117 mg, 25%). R_F (DCM:MeOH = 9:1) 0.32. mp 210-213 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.14 (t, 3H, *J* 7.0), 1.56-1.76 (m, 6H), 2.06-2.12 (m, 2H), 2.49 (q, 2H, *J* 7.5), 2.54 (s, 3H), 2.64 (s, 3H), 3.19 (t, 4H, *J* 4.5), 3.14 (t, 4H, *J* 5.0), 3.86 (app s, 1H), 5.77 (s, 1H), 6.84 (d, 1H, *J* 5.0), 7.34 (dd, 1H, *J* 9.0 & 3.0), 7.94 (d, 1H, *J* 3.0), 7.94 (s, 1H), 8.01 (d, 1H, *J* 3.0), 8.26 (d, 1H, *J* 9.0), 8.33 (d, 1H, *J* 5.5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.1, 18.7, 23.9, 33.3, 50.0, 52.5, 52.8, 57.6, 107.9, 113.2, 119.7, 126.6, 136.7, 143.0, 146.5, 152.2, 157.5, 158.9, 159.5, 169.5 (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 465.2541 $[M+H]^+$; calcd. for $C_{24}H_{33}N_8S^+$ $[M+H]^+$ 465.2543. Anal. RP-HPLC Method A: t_R 13.24 min, purity > 98%; Method B: t_R 8.96 min, purity > 99%.

1-(4-(6-((4-(2-(Cyclopentylamino)-4-methylthiazol-5-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazin-1-yl) ethan-1-one (82). 1-(5-(4-Acetylpiperazin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (**67**, 524 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**13**, 279 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **82** as a light yellow solid (153 mg, 32%). R_F (DCM:MeOH = 9:1) 0.59. mp 207-210 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.57-1.75 (m, 6H), 2.05-2.11 (m, 2H), 2.14 (s, 3H), 2.54 (s, 3H), 3.08-3.14 (m, 4H), 3.63 (t, 2H, *J* 5.0), 3.79 (t, 2H, *J* 5.0), 3.87 (m, 1H), 5.70 (s, 1H), 6.86 (d, 1H, *J* 5.0), 7.33 (dd, 1H, *J* 9.0 & 3.0), 8.03 (d, 1H, *J* 2.0), 8.19 (br s, 1H,), 8.31 (d, 1H, *J* 9.0), 8.35 (d, 1H, *J* 5.0). ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.7, 21.5, 23.9, 33.3, 41.4, 46.4, 50.3, 50.7, 57.7, 108.0, 113.1, 119.7, 127.5, 137.5, 142.5, 147.3, 157.5, 158.8, 159.5, 169.2, 169.4 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 479.2340 $[M+H]^+$; calcd. for $C_{24}H_{31}N_8OS^+$ $[M+H]^+$ 479.2336 Anal. RP-HPLC Method A: t_R 10.86 min, purity > 99%; Method B: t_R 8.51 min, purity > 98%.

***N*-Cyclopentyl-4-methyl-5-(2-((5-morpholinopyridin-2-yl)amino)pyrimidin-4-yl)thiazol-2-amine (83).** 1-(5-Morpholinopyridin-2-yl)guanidine (**71**, 442 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**13**, 279 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II and recrystallized with Et_2O to give **83** as a dark brown solid (130 mg, 30%). R_F (DCM:MeOH = 9:1) 0.30. mp 262-263 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.57-1.74 (m, 6H), 2.06-2.12 (m, 2H), 2.55 (s, 3H), 3.13 (t, 4H, *J* 4.5), 3.88 (t, 4H, *J* 4.5), 5.67 (d, *J* 4.5, 1H), 6.85 (d, 1H, *J* 5.5), 7.32 (dd, 1H, *J* 9.0 & 3.0), 8.02 (d, 1H, *J* 3.0), 8.16 (s, 1H), 8.30 (d, 1H, *J* 9.5), 8.35 (d, 1H, *J* 5.5),. ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.8, 23.9, 33.3, 50.2, 57.6, 67.0, 107.9,

113.2, 119.8, 126.4, 136.5, 142.8, 146.8, 152.4, 157.5, 158.9, 159.5, 169.4 (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 438.2088 $[M+H]^+$; calcd. for $C_{22}H_{28}N_7OS^+$ $[M+H]^+$ 438.2071. Anal. RP-HPLC Method A: t_R 10.92 min, purity > 99%; Method B: t_R 9.51 min, purity > 99%.

5-(2-((5-(4-Aminopiperidin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)-*N*-cyclopentyl-4-methylthiazol-2-amine (84). 1-(5-(4-Aminopiperidin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (**76**, 702 mg, 3.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**13**, 558 mg, 2.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system III and recrystallized with n-hexane and DCM to give **84** as a dark yellow solid (90 mg, 10%). R_F (DCM:MeOH = 9:1) 0.10. mp 185-186 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.50-1.77 (m, 10H), 1.95 (d, 2H, *J* 10.5), 2.07-2.13 (m, 2H), 2.54 (s, 3H), 2.75-2.85 (m, 3H), 3.53-3.56 (m, 2H), 3.85-3.91 (m, 1H), 5.43 (d, 1H, *J* 5.0), 6.84 (d, 1H, *J* 5.5), 7.34 (dd, 1H, *J* 9.0 & 3.0), 7.75 (s, 1H), 8.00 (d, 1H, *J* 3.0), 8.25 (d, 1H, *J* 9.0), 8.32 (d, 1H, *J* 5.5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.8, 23.9, 33.3, 35.8, 48.6, 49.6, 57.6, 89.8, 107.9, 113.1, 119.9, 127.2, 137.1, 143.2, 146.2, 152.5, 157.5, 158.9, 159.5, 169.4 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 451.2415 $[M+H]^+$; calcd. for $C_{23}H_{31}N_8S^+$ $[M+H]^+$ 451.2387. Anal. RP-HPLC Method A: t_R 9.34 min, purity > 95%; Method B: t_R 8.06 min, purity > 95%.

***N*-Cyclopentyl-4-methyl-5-(2-((5-(piperidin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)thiazol-2-amine (85).** 1-(5-(Piperidin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (**73**, 439 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**13**, 279 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II and recrystallized with DCM and MeOH to give **85**

as yellow solid (250 mg, 57%). R_F (DCM:MeOH = 9:1) 0.63. mp 220-221 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.53 (br s, 6H), 1.64 (br s, 6H), 1.93 (br s, 2H), 2.46 (s, 3H), 3.07 (t, 4H, *J* 10.0), 3.98 (br s, H), 6.89 (d, 1H, *J* 5.0), 7.37 (app d, 1H, *J* 9.0), 7.99 (s, 1H), 8.06 (d, 1H, *J* 9.0), 8.18 (s, 1H), 8.33 (d, 1H, *J* 5.0), 9.26 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.7, 23.4, 25.2, 50.1, 55.8, 58.0, 60.0, 73.9, 107.1, 113.3, 117.3, 125.5, 136.0, 143.2, 145.4, 152.5, 157.6, 158.6, 158.8, 168.0 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 436.2280 $[M+H]^+$; calcd. for $C_{23}H_{30}N_7S^+$ $[M+H]^+$ 436.2278. Anal. RP-HPLC Method A: t_R 12.08 min, purity > 99%; Method B: t_R 9.36 min, purity > 99%.

***N*-Cyclopentyl-4-methyl-5-(2-((5-(4-(methylsulfonyl)piperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)thiazol-2-amine (86).** 1-(5-(4-(Methylsulfonyl)piperazin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (**75**, 596 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**13**, 279 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **86** as a brown solid (62 mg, 12%). R_F (DCM:MeOH = 9:1) 0.50. mp 197-198 °C. ^{1}H NMR (500 MHz, DMSO-d_6) 1.54-1.57 (m, 4H), 1.66-1.69 (m, 2H), 1.91-1.95 (m, 2H), 2.47 (s, 3H), 2.94 (s, 3H), 2.23 (t, 4H, *J* 3.5), 3.27 (t, 4H, *J* 3.5), 3.98 (m, 1H), 6.91 (d, 1H, *J* 5.5), 7.43 (dd, 1H, *J* 9.0 & 3.0), 8.05 (d, 1H, *J* 3.0), 8.12 (d, 1H, *J* 9.0), 8.19 (d, 1H, *J* 7.0), 8.34 (d, 1H, *J* 5.5), 9.36 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.7, 23.4, 32.2, 33.9, 45.3, 48.6, 55.8, 107.3, 113.2, 117.3, 125.9, 136.4, 141.9, 146.4, 152.6, 157.6, 158.7, 158.7, 168.0 (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 515.2004 $[M+H]^+$; calcd. for $C_{23}H_{31}N_8O_2S_2^+$ $[M+H]^+$ 515.2006. Anal. RP-HPLC Method A: t_R 10.48 min, purity > 97%; Method B: t_R 8.61 min, purity > 99%.

***N*-Cyclopentyl-4-methyl-5-(2-(pyridin-2-ylamino)pyrimidin-4-yl)thiazol-2-amine (87).** 1-(Pyridin-2-yl)guanidine 2,2,2-trifluoroacetate (**77**, 272 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**13**, 279 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **87** as an orange solid (150 mg, 43%). R_F (DCM:MeOH = 9:1) 0.52. mp 221-222 °C. ^{1}H NMR (500 MHz, DMSO-d_6) 1.50-1.57 (m, 4H), 1.66-1.69 (m, 2H), 1.91-1.95 (m, 2H), 2.48 (s, 3H), 3.98 (m, 1H), 6.99 (m, 2H), 7.74 (m, 1H), 8.23 (d, 1H, *J* 7.0), 8.26 (d, 1H, *J* 8.5), 8.29 (m, 1H), 8.39 (d, 1H, *J* 5.5), 9.59 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.7, 23.4, 32.2, 55.8, 107.9, 112.5, 117.1, 117.3, 137.5, 147.9, 152.9, 153.1, 157.6, 158.6, 158.7, 168.0 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 353.1555 $[M+H]^+$; calcd. for $C_{18}H_{21}N_6S^+$ $[M+H]^+$ 353.1543. Anal. RP-HPLC Method A: t_R 10.45 min, purity > 97%; Method B: t_R 9.24 min, purity > 98%.

***N*-Cyclopentyl-*N*,4-dimethyl-5-(2-((5-(piperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)thiazol-2-amine (88).** 1-(5-(Piperazin-1-yl)pyridin-2-yl)guanidine (**68**, 319 mg, 1.45 mmol) and (*E*)-1-(2-(cyclopentyl(methyl)amino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**15**, 250 mg, 0.85 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II and recrystallized with hexane to give **88** as a reddish brown solid (113 mg, 25%). R_F (DCM:MeOH = 9:1) 0.50. mp 166-169 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.58-1.79 (m, 6H), 1.96-2.02 (m, 2H), 2.11 (br, 1H), 2.56 (s, 3H), 3.01 (s, 3H), 3.06 (t, 4H, *J* 6), 3.10 (t, 4H, *J* 6.0), 4.55 (m, 1H), 6.82 (d, 1H, *J* 5.5), 7.32 (dd, 1H, *J* 9.0 & 3.0), 8.02 (d, 1H, *J* 3.0), 8.13 (br, 1H), 8.28 (d, 1H, *J* 9.0), 8.32 (d, 1H, *J* 5.5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.1, 24.3, 28.5, 32.2, 46.1, 51.2, 53.6, 61.5, 107.9, 113.0, 118.8, 126.9, 136.9, 143.2, 146.8, 153.1, 157.3, 158.9, 159.7, 170.6 (three carbon signals overlapping or obscured). HRMS (ESI-TOF):

m/z 451.2387 $[M+H]^+$; calcd. for $C_{23}H_{31}N_8S^+$ $[M+H]^+$ 451.2387. Anal. RP-HPLC Method A: t_R 10.28 min, purity > 95%; Method B: t_R 8.69 min, purity > 95%.

***N*-Cyclopentyl-*N*,4-dimethyl-5-(2-((5-(4-methylpiperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)thiazol-2-amine (89).** 1-(5-(4-Methylpiperazin-1-yl)pyridine-2-yl)guanidine (**69**, 468 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentyl(methyl)amino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**15**, 293 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system III and recrystallized with MeOH to give **89** as a yellow solid (149 mg, 32%). R_F (DCM:MeOH = 9:1) 0.38. mp 169-170 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.65-1.76 (m, 6H), 1.99-2.02 (m, 2H), 2.56 (s, 3H), 2.75 (s, 3H), 3.16 (br s, 4H), 3.47 (t, 4H, *J* 5.0), 4.58 (m, 1H), 6.86 (d, 1H, *J* 5.5), 7.36 (dd, 1H, *J* 9.0 & 3.0), 8.05 (d, 1H, *J* 3.0), 8.07 (s, 1H), 8.32 (d, 1H, *J* 5.5), 8.35 (d, 1H, *J* 9.0). ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.2, 24.4, 28.6, 32.3, 44.4, 48.6, 48.6, 54.2, 108.2, 112.9, 118.7, 127.9, 138.8, 141.3, 148.1, 153.4, 157.2, 158.7, 159.8, 170.7 (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 465.2530 $[M+H]^+$; calcd. for $C_{24}H_{33}N_8S^+$ $[M+H]^+$ 465.2543. Anal. RP-HPLC Method A: t_R 10.15 min, purity > 96%; Method B: t_R 8.47 min, purity > 96%.

1-(4-(6-((4-(2-(Cyclopentyl(methyl)amino)-4-methylthiazol-5-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)ethan-1-one (90). 1-(5-(4-Acetylpiperazin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (**67,** 525 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentyl(methyl)amino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**15**, 293 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II and recrystallized with Et_2O to give **90** as a yellow solid (300 mg, 61%). R_F (DCM:MeOH = 9:1) 0.53. mp 153-154 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.61-1.76 (m, 6H), 1.97-2.02 (m, 2H), 2.15 (s, 3H), 2.57 (s, 3H), 3.01 (s, 3H), 3.09 (t, 2H, *J* 5.0), 3.13 (t, 2H, *J* 5.0),

3.63 (t, 2H, *J* 5.0), 3.79 (t, 2H, *J* 5.0), 4.56 (m, 1H), 6.85 (d, 1H, *J* 5.5), 7.34 (dd, 1H, *J* 9.0 & 3.0), 7.93 (s, 1H), 8.00 (d, 1H, *J* 3.0), 8.31 (s, 1H), 8.32 (d, 1H, *J* 5.0). ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.2, 21.5, 24.4, 28.6, 32.3, 41.5, 46.4, 50.4, 50.7, 61.6, 108.1, 113.0, 118.7, 127.6, 137.6, 142.4, 147.4, 153.3, 157.2, 158.8, 159.8,169.1, 170.7 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 493.2482 $[M+H]^+$; calcd. for $C_{25}H_{33}N_8OS^+$ $[M+H]^+$ 493.2493. Anal. RP-HPLC Method A: t_R 11.55 min, purity > 96%; Method B: t_R 9.57 min, purity > 96%.

***N*,*N*-Dicyclopentyl-4-methyl-5-(2-((5-(piperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)thiazol-2-amine (91).** 1-(5-(Piperazin-1-yl)pyridin-2-yl)guanidine (**68**, 441 mg, 2.00 mmol) and (*E*)-1-(2-(dicyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**17**, 200 mg, 0.580 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II and recrystallized with DCM and MeOH to give **91** as a yellow solid (60 mg, 21%). R_F (DCM:MeOH:NH_4OH = 9:1:0.5) 0.1. mp 198-199 °C. ^{1}H NMR (500 MHz, $CDCl_3$) 1.53-1.59 (m, 8H), 1.74-1.76 (m, 4H), 1.85-1.89 (m, 2H), 1.91-1.98 (m, 2H), 2.42-2.47 (m, 1H), 2.58 (s, 3H), 3.05 (t, 4H, *J* 3.0), 3.10 (t, 4H, *J* 3.0), 3.41-3.44 (m, 1H), 4.47-4.54 (m, 1H), 6.61 (d, 1H, *J* 5.5), 7.32 (dd, 1H, *J* 9.0 & 3.0), 7.70 (s, 1H), 7.98 (d, 1H, *J* 3.0), 8.25 (d, 1H, *J* 9.0), 8.28 (d, 1H, *J* 5.5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.2, 24.4, 26.0, 28.1, 34.0, 46.2, 51.3, 57.5, 106.4, 109.4, 113.2, 126.7, 136.8, 140.9, 143.5, 146.5, 151.7, 157.4, 158.7, 159.9 (seven carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 505.2873 $[M+H]^+$; calcd. for $C_{27}H_{37}N_8S^+$ $[M+H]^+$ 505.2856. Anal. RP-HPLC Method A: t_R 8.57 min, purity > 98%; Method B: t_R 7.33 min, purity > 96%.

4-Methyl-*N*-phenyl-5-(2-((5-(piperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)thiazol-2-amine (92). 1-(5-(Piperazin-1-yl)pyridin-2-yl)guanidine (**68**, 468 mg, 2.00 mmol) and (*E*)-3-

(dimethylamino)-1-(4-methyl-2-(phenylamino)thiazol-5-yl)prop-2-en-1-one (**18**, 287 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system III to give **92** as a light yellow solid (178 mg, 40%). R_F (DCM:MeOH = 9:1) 0.10. mp 228-230 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.58 (s, 4H), 2.86 (t, 4H, *J* 4.5), 3.03 (t, 4H, *J* 4.0), 7.00 (m, 2H), 7.35 (m, 3H), 7.65 (d, 2H, *J* 8.0), 8.00 (d, 1H, *J* 2.5), 8.06 (d, 1H, *J* 9.0), 8.41 (d, 1H, *J* 5.0), 9.46 (s, 1H), 10.53 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.7, 45.4, 49.7, 107.7, 113.5, 117.6, 119.6, 122.1, 124.9, 129.1, 135.5, 140.6, 143.2, 145.5, 151.3, 158.2, 158.3, 158.9, 163.5 (five carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 445.1918 $[M+H]^+$; calcd. for $C_{23}H_{25}N_8S^+$ $[M+H]^+$ 445.1917. Anal. RP-HPLC Method A: t_R 10.01 min, purity > 99%.; Method B: t_R 8.17 min, purity > 99%..

4-Methyl-5-(2-((5-(4-methylpiperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)-*N*-phenylthiazol-2-amine (93). 1-(5-(4-Methylpiperazin-1-yl)pyridine-2-yl)guanidine (**69**, 468 mg, 2.00 mmol) and (*E*)-3-(dimethylamino)-1-(4-methyl-2-(phenylamino)thiazol-5-yl)prop-2-en-1-one (**18**, 287 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II and recrystallized with DCM to give **93** as a light yellow solid (220 mg, 48%). R_F (DCM:MeOH = 9:1) 0.17. mp 210-211 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.23 (s, 3H), 2.58 (s, 3H), 3.12 (br, 4H), 3.38 (t, 4H), 7.00 (m, 2H), 7.37 (m, 3H), 7.65 (d, 2H, *J* 8.0), 8.01 (d, 1H, *J* 2.0), 8.07 (d, 1H, *J* 9.0), 8.41 (d, 1H, *J* 5.0), 9.46 (s, 1H), 10.54 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.7, 45.8, 48.5, 54.5, 107.8, 113.5, 117.7, 119.6, 122.1, 125.0, 129.1, 135.5, 140.6, 142.6, 145.6, 151.4, 158.2, 158.3, 158.9, 163.5 (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 459.2063 $[M+H]^+$; calcd. for $C_{24}H_{27}N_8S^+$ $[M+H]^+$ 459.2074. Anal. RP-HPLC Method A: t_R 9.93 min, purity > 99%; Method B: t_R 9.17 min, purity > 99%.

***N*,4-Dimethyl-5-(2-((5-(4-methylpiperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)-*N*-phenylthiazol-2-amine (94).** 1-(5-(4-Methylpiperazin-1-yl)pyridine-2-yl)guanidine (**69**, 468 mg, 2.00 mmol) and (*E*)-3-(dimethylamino)-1-(4-methyl-2-(methyl(phenyl)amino)thiazol-5-yl)prop-2-en-1-one (**19**, 301 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II and recrystallized with hexane to give **94** as a reddish brown solid (184 mg, 39%). R_F (DCM:MeOH = 9:1) 0.40. mp 212-215 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.36 (s, 3H), 2.59 (app br, 7H), 3.14 (t, 4H, *J* 5.0), 3.57 (s, 3H), 6.80 (d, 1H, *J* 5.5), 7.19 (dd, 1H, *J* 9.0 & 3.0), 7.32 (m, 1H), 7.44 (m, 4H), 8.00 (d, 1H, *J* 3.0), 8.04 (s, 1H), 8.16 (d, 1H, *J* 9.0), 8.32 (d, 1H, *J* 5.5) ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.9, 40.1, 46.3, 49.9, 55.1, 108.1, 113.2, 120.6, 125.6, 126.4, 127.2, 130.0, 136.9, 142.8, 145.9, 146.4, 152.3, 157.6, 158.8, 159.5, 169.7 (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 473.2220 $[M+H]^+$; calcd. for $C_{25}H_{29}N_8S^+$ $[M+H]^+$ 473.2230. Anal. RP-HPLC Method A: t_R 9.57 min, purity > 98%; Method B: t_R 7.90 min, purity > 98%.

***N*-Isopropyl-4-methyl-5-(2-((5-(piperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)thiazol-2-amine (95).** To a suspension of 1-(4-(6-((4-(2-(isopropylamino)-4-methylthiazol-5-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)ethan-1-one (**98**, 143 mg, 0.320 mmol) in methanol HCl (32%, 3 mL) was added and reflexed overnight. The reaction mixture was concentrated and purified by FlashMaster Personal$^+$ chromatography (silica gel, DCM ramping to DCM:MeOH:NH_4OH = 90:10:1) to give **95** as a yellow solid (120 mg, 92 %). R_F (DCM:MeOH = 9:1 + 3 drops of 32% aqueous ammonia) 0.12. mp 218-220 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19 (d, 6H, *J* 6.5), 2.46 (s, 3H), 2.87 (t, 4H, *J* 5.0), 3.03 (t, 4H, *J* 5.5), 3.80-3.87 (m, 1H), 6.90 (d, 1H, *J* 5.5), 7.37 (dd, 1H, *J* 9.0 & 3.0), 8.00 (d, 1H, *J* 3.0), 8.05 (d, 2H, *J* 7.5), 8.08 (d, 1H, *J* 9.0), 8.33 (d, 1H, *J* 5.5), 9.29 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ

18.7, 22.3, 45.2, 46.1, 49.5, 107.2, 113.3, 117.1, 125.0, 135.5, 142.9, 145.7, 152.4, 157.6, 158.6, 158.8, 167.5 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 411.2072 $[M+H]^+$; calcd. for $C_{20}H_{27}N_8S^+$ $[M+H]^+$ 411.2074.Anal. RP-HPLC Method A: t_R 8.43 min, purity > 96%; Method B: t_R 7.61 min, purity > 99%.

5-(2-((5-(1,4-Diazepan-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)-*N*-isopropyl-4-methylthiazol-2-amine (96). 1-(5-(1,4-Diazepan-1-yl)pyridin-2-yl)guanidine di(2,2,2-trifluoroacetate) (**70**, 469 mg, 2.00 mmol) and (*E*)-3-(dimethylamino)-1-(2-(isopropylamino)-4-methylthiazol-5-yl)prop-2-en-1-one (**20**, 253 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **96** as an orange solid (114 mg, 34%). R_F (DCM:MeOH = 9:1) 0.10. mp 163-164 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19 (d, 6H, *J* 6.5), 2.04-2.09 (m, 2H), 2.47 (s, 3H), 3.16 (s, 1H, *J* 5.5), 3.27 (s, 2H, *J* 5.0), 3.50 (d, 2H, *J* 6.0), 3.70 (t, 2H, *J* 5.0), 3.80-3.86 (m, 1H), 6.87 (d, 1H, *J* 5.5), 7.24 (dd, 1H, *J* 9.0 & 3.0), 7.89 (d, 1H, *J* 3.0), 8.03 (d, 2H, *J* 5.5), 8.05 (d, 1H, *J* 4.0), 8.31 (d, 1H, *J* 5.5), 8.75 (s, 1H). 9.15 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.7, 22.3, 24.7, 44.9, 45.3, 45.4, 46.0, 46.8, 107.0, 113.9, 116.1, 117.1, 118.5, 121.1, 131.7, 139.8, 143.7, 152.3, 157.6, 158.8, 167.5. HRMS (ESI-TOF): *m/z* 425.2231 $[M+H]^+$; calcd. for $C_{21}H_{29}N_8S^+$ $[M+H]^+$ 425.2230 Anal. RP-HPLC Method A: t_R 8.48 min, purity > 95%; Method B: t_R 7.69 min, purity > 98%.

***N*-Isopropyl-4-methyl-5-(2-((5-(4-methylpiperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)thiazol-2-amine (97).** 1-(5-(4-Methylpiperazin-1-yl)pyridine-2-yl)guanidine (**69**, 468 mg, 2.00 mmol) and (*E*)-3-(dimethylamino)-1-(2-(isopropylamino)-4-methylthiazol-5-yl)prop-2-en-1-one (**20**, 253 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **97** as a yellow solid (131 mg, 31%). R_F (DCM:MeOH = 9:1) 0.70. mp 175-177 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19 (d, 6H, *J* 6.5),

2.22 (s, 3H), 2.46 (br s, 7H), 3.11 (t, 4H, *J* 5.0), 3.81-3.85 (m, 1H), 6.90 (d, 1H, *J* 5.5), 7.38 (dd, 1H, *J* 9.0 & 3.0), 8.00 (d, 1H, *J* 3.0), 8.04 (d, 2H, *J* 7.5), 8.08 (d, 1H, *J* 9.0), 8.34 (d, 1H, *J* 5.5), 9.32 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.7, 22.3, 45.8, 46.0, 48.5, 54.5, 107.2, 113.2, 117.2, 125.0, 135.4, 142.4, 145.7, 152.4, 157.6, 158.6, 158.7, 167.5 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 425.2235 $[M+H]^+$; calcd. for $C_{21}H_{29}N_8S^+$ $[M+H]^+$ 425.2230.Anal. RP-HPLC Method A: t_R 8.56 min, purity > 99%; Method B: t_R 7.73 min, purity > 99%..

1-(4-(6-((4-(2-(Isopropylamino)-4-methylthiazol-5-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)ethan-1-one (98). 1-(5-(4-Acetylpiperazin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (**67**, 525 mg, 2.00 mmol) and (*E*)-3-(dimethylamino)-1-(2-(isopropylamino)-4-methylthiazol-5-yl)prop-2-en-1-one (**20**, 253 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **98** as an orange solid (80 mg, 18 %). R_F (DCM:MeOH = 9:1) 0.63. mp 232-234 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19 (d, 6H, *J* 6.5), 2.05 (s, 3H), 2.46 (s, 3H), 3.06 (t, 2H, *J* 5.0), 3.13 (t, 2H, *J* 5.0), 3.59 (q, 4H, *J* 5.5), 3.81–3.85 (m, 1H), 6.91 (d, 1H, *J* 5.5), 7.40 (dd, 1H, *J* 9.0 & 3.0), 8.02 (d, 1H, *J* 3.0), 8.05 (m, 2H, *J* 7.5), 8.10 (d, 1H, *J* 9.0), 8.33 (d, 1H, *J* 5.5), 9.31 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.7, 22.2, 22.3, 40.6, 45.4, 46.1, 48.8, 49.3, 107.3, 113.2, 117.1, 125.7, 136.1, 142.2, 146.2, 152.5, 157.6, 158.6, 158.7, 167.5, 168.3 (one carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 453.2187 $[M+H]^+$; calcd. for $C_{22}H_{29}N_8OS^+$ $[M+H]^+$ 453.2180. Anal. RP-HPLC Method A: t_R 10.03 min, purity > 99%; Method B: t_R 8.85 min, purity > 99%.

***N*-Isopropyl-4-methyl-5-(2-((5-morpholinopyridin-2-yl)amino)pyrimidin-4-yl)thiazol-2-amine (99).** 1-(5-Morpholinopyridin-2-yl)guanidine (**71,** 443 mg, 2.00 mmol) and (*E*)-3-(dimethylamino)-1-(2-(isopropylamino)-4-methylthiazol-5-yl)prop-2-en-1-one (**20**, 253 mg, 1.00

mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **99** pressure as a yellow solid (200 mg, 48 %). R_F (DCM:MeOH = 9:1) 0.63. mp 237-238 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19 (d, 6H, *J* 6.5), 2.45 (s, 3H), 3.09 (t, 4H, *J* 4.0), 3.76 (t, 4H, *J* 4.0), 3.81-3.85 (m, 1H), 6.90 (d, 1H, *J* 5.5), 7.39 (dd, 1H, *J* 9.0 & 3.0), 7.01 (d, 1H, *J* 2.5), 8.05 (d, 2H, *J* 7.5), 8.10 (d, 1H, *J* 9.0), 8.34 (d, 1H, *J* 5.5), 9.33 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.7, 22.3, 46.1, 48.9, 66.0, 107.2, 113.2, 117.1, 124.8, 135.3, 142.4, 146.0, 152.5, 157.6, 158.6, 158.7, 167.5 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 412.1912 $[M+H]^+$; calcd. for $C_{20}H_{26}N_7OS^+$ $[M+H]^+$ 412.1914. Anal. RP-HPLC Method A: t_R 10.21 min, purity > 99%; Method B: t_R 9.08 min, purity > 99%.

***N*-Cyclopentyl-5-(2-((5-(piperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)-4-(trifluoromethyl)thiazol-2-amine (100).** 1-(5-(Piperazin-1-yl)pyridin-2-yl)guanidine (**68**, 441 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-(trifluoromethyl)thiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**16**, 333 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system III to give **100** as an orange solid (260 mg, 53%). R_F (DCM:MeOH = 9:1) 0.35. mp 207-208 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.50-1.59 (m, 4H), 1.65-1.70 (m, 2H), 1.92-1.97 (m, 2H), 2.26 (s, 1H), 2.84 (t, 4H, *J* 5.0), 3.02 (t, 4H, *J* 5.0), 3.96 (m, 1H), 6.96 (d, 1H, *J* 6.0), 7.37 (dd, 1H, *J* 9.0 & 3.0), 7.98 (d, 1H, *J* 4.0), 7.99 (d, 1H, *J* 1.0), 8.50 (d, 1H, *J* 5.5), 8.59 (d, 1H, *J* 6.5), 9.59 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.3, 32.0, 45.5, 49.7, 56.1, 108.8, 113.7, 119.7, 121.9, 124.8, 125.0, 135.4, 136.8, 143.4, 145.0, 155.6, 158.9, 159.0, 168.2 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 491.1952 $[M+H]^+$; calcd. for $C_{22}H_{26}F_3N_8S^+$ $[M+H]^+$ 491.1948. Anal. RP-HPLC Method A: t_R 10.31 min, purity > 99%; Method B: t_R 8.30 min, purity > 98%.

***N*-Cyclopentyl-5-(2-((5-(4-methylpiperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)-4-(trifluoromethyl)thiazol-2-amine (101).** 1-(5-(4-Methylpiperazin-1-yl)pyridine-2-yl)guanidine (**69**, 468 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-(trifluoromethyl)thiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**16**, 148 mg, 0.50 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II and to give **101** as a brown solid (30.0 mg, 6%). R_F (DCM:MeOH = 9:1) 0.28. mp 209-210 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.56-1.59 (m, 4H), 1.67-1.69 (m, 2H), 1.93-1.97 (m, 2H), 2.21 (s, 1H), 2.46 (t, 4H, *J* 5.0), 3.12 (t, 4H, *J* 5.0), 3.96 (m, 1H), 6.96 (d, 1H, *J* 6.0), 7.39 (dd, 1H, *J* 9.0 & 3.0), 8.00 (d, 1H, *J* 9.0), 8.01 (d, 1H, *J* 3.0), 8.50 (d, 1H, *J* 5.5), 8.59 (d, 1H, *J* 7.0), 9.62(s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.3, 32.0, 45.8, 48.4, 54.5, 54.9, 56.1, 108.8, 113.6, 119.7, 124.8, 135.4, 136.8, 137.1, 142.8, 145.1, 155.6, 155.9, 159.1, 168.2 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 505.2103 $[M+H]^+$; calcd. for $C_{23}H_{28}F_3N_8S^+$ $[M+H]^+$ 505.2104. Anal. RP-HPLC Method A: t_R 10.49 min, purity > 96%; Method B: t_R 9.46 min, purity > 97%.

1-(4-(6-((4-(2-(Cyclopentylamino)-4-(trifluoromethyl)thiazol-5-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)ethan-1-one (102). 1-(5-(4-Acetylpiperazin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (**67,** 524 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-(trifluoromethyl)thiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**16**, 333 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **102** as a brown solid (50.0 mg, 9%). R_F (DCM:MeOH = 9:1) 0.40. mp 200-201 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.53-1.59 (m, 4H), 1.67-1.69 (m, 2H), 1.94-1.97 (m, 2H), 2.05 (s, 3H), 3.08 (t, 2H, *J* 4.5), 3.14 (t, 2H, *J* 4.5), 3.59 (app d, 4H, *J* 4.5), 3.95 (m, 1H), 6.97 (d, 1H, *J* 5.0), 7.43 (dd, 1H, *J* 9.0 & 3.0), 8.02 (s, 1H), 8.04 (d, 1H, *J* 3.0), 8.50 (d, 1H, *J* 5.5), 8.59 (d, 1H,

J 6.5), 9.66 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.2, 23.3, 32.0, 45.4, 48.7, 49.1, 56.1, 109.0, 113.6, 119.7, 121.9, 125.0, 125.6, 136.1, 136.9, 142.5, 145.7, 155.7, 158.9, 159.1, 168.2 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 533.2053 $[M+H]^+$; calcd. for $C_{24}H_{27}F_3N_8OS^+$ $[M+H]^+$ 533.2058. Anal. RP-HPLC Method A: t_R 12.56 min, purity > 97%; Method B: t_R 9.39 min, purity > 95%.

***N*-Cyclopentyl-5-(2-((5-morpholinopyridin-2-yl)amino)pyrimidin-4-yl)-4-(trifluoromethyl)thiazol-2-amine (103).** 1-(5-Morpholinopyridin-2-yl)guanidine (**71,** 442 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-(trifluoromethyl)thiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (**16**, 333 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system I to give **103** as an orange solid (200 mg, 41%). R_F (PE:EtOAc = 1:1) 0.40. mp 229-230 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.52-1.59 (m, 4H), 1.64-1.69 (m, 2H), 1.92-1.99 (m, 2H), 3.09 (t, 4H, *J* 4.5), 3.75 (t, 4H, *J* 4.5), 3.95 (m, 1H), 6.97 (d, 1H, *J* 5.0), 7.41 (dd, 1H, *J* 9.0 & 3.0), 8.01 (s, 1H), 8.02 (d, 1H, *J* 2.5), 8.51(d, 1H, *J* 5.5), 8.59 (d, 1H, *J* 6.5), 9.64 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.8, 33.2, 50.1, 57.9, 66.9, 109.8 (d, *J* 4.8), 113.1, 126.2, 136.5, 143.1, 146.3, 156.6, 157.7, 158.8, 169.2 (seven carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 492.1786 $[M+H]^+$; calcd. for $C_{22}H_{24}F_3N_7OS^+$ $[M+H]^+$ 498.1788. Anal. RP-HPLC Method A: t_R 12.90 min, purity > 97%; Method B: t_R 9.69 min, purity > 99%.

4-(6-((4-(2-(Cyclopentylamino)-4-(trifluoromethyl)thiazol-5-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazine-1-carbaldehyde (104). Compound **104** was obtained as beige solid (25.0 mg, 7%) by-product in the process of synthesizing and purifying **100.** R_F (DCM:MeOH = 9:1) 0.63. mp 238-239 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.53-1.59 (m, 4H), 1.67-1.69 (m, 2H), 1.94-1.97 (m, 2H), 3.08 (t, 2H, *J* 5.0), 3.14 (t, 2H, *J* 5.0), 3.59 (m, 4H), 3.96

(m, 1H), 6.97 (d, 1H, *J* 4.5), 7.45 (dd, 1H, *J* 9.0 & 3.0), 8.03 (d, 1H, *J* 9.0), 8.05 (d, 1H, *J* 3.0), 8.09 (s, 1H), 8.50 (d, 1H, *J* 5.5), 8.59 (d, 1H, *J* 7.0), 9.67 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.3, 32.0, 44.5, 48.6, 49.8, 56.1, 109.0, 113.5, 119.7, 121.9, 125.0, 125.6, 136.1, 136.9, 137.1, 142.5, 145.8, 155.7, 158.9, 159.1, 160.9, 168.2 (one carbon signal overlapping or obscured). HRMS (ESI-TOF): *m/z* 519.1897 $[M+H]^+$; calcd. for $C_{23}H_{26}F_3N_8OS^+$ $[M+H]^+$ 519.1906. Anal. RP-HPLC Method A: t_R 11.57 min, purity > 91%; Method B: t_R 9.39 min, purity > 95%.

***N*-Cyclopentyl-5-(5-fluoro-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)-4-methylthiazol-2-amine (105).** 1-(5-(Piperazin-1-yl)pyridin-2-yl)guanidine (**68**, 441 mg, 2.00 mmol) and ((*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)-2-fluoroprop-2-en-1-one (**14**, 297 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system III to give **105** as a yellow solid (101 mg, 22%). R_F (DCM:MeOH:NH_4OH = 9:1:0.5) 0.30. mp 207-208 °C. ^{1}H NMR (500 MHz, DMSO-d_6) 1.54-1.57 (m, 4H), 1.66-1.69 (m, 2H), 1.92-1.95 (m, 2H), 2.47 (s, 3H), 3.26 (t, 4H, *J* 2.5), 3.31 (t, 4H, *J* 2.5), 3.95 (m, 1H), 7.46 (dd, 1H, *J* 9.0 & 3.0), 8.00 (d, 1H, *J* 9.0), 8.05 (d, 1H, *J* 3.0), 8.25 (d, 1H, *J* 7.0), 8.42 (d, 1H, *J* 3.5), 8.84 (d, 1H, *J* 3.5), 9.57 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.0 (*J* 4.7), 23.3, 32.2, 42.7, 46.2, 56.0, 109.2, 113.0, 126.0, 136.2, 141.4, 145.3, 145.5, 146.5, 146.7, 147.1, 147.2, 148.5, 155.0, 155.2, 169.4 (*J* 4.7) (one carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 455.2139 $[M+H]^+$; calcd. for $C_{22}H_{28}FN_8S^+$ $[M+H]^+$ 455.2136. Anal. RP-HPLC Method A: t_R 9.55 min, purity > 99%; Method B: t_R 7.86 min, purity > 99%.

5-(2-((5-(1,4-Diazepan-1-yl)pyridin-2-yl)amino)-5-fluoropyrimidin-4-yl)-*N*-cyclopentyl-4-methylthiazol-2-amine (106). 1-(5-(1,4-Diazepan-1-yl)pyridin-2-yl)guanidine di(2,2,2-trifluoroacetate) (**70**, 469 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-

yl)-3-(dimethylamino)-2-fluoroprop-2-en-1-one (**14**, 297 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **106** as a yellow solid (100 mg, 21%). R_F (DCM:MeOH = 9:1) 0.15. mp 170-171 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.49-1.59 (m, 4H), 1.64-1.72 (m, 2H), 1.92-1.95 (m, 2H), 2.05-2.09 (m, 2H), 2.47 (d, 3H, *J* 2.0), 2.55 (s, 1H), 3.16 (br s, 2H), 3.27 (d, 2H, *J* 4.0), 3.70 (t, 2H, *J* 5.0), 3.94-3.98 (m, 1H), 7.32 (dd, 1H, *J* 9.0 & 3.0), 7.87 (d, 1H, *J* 2.5), 7.89 (d, 1H, *J* 3.0), 8.27 (d, 1H, *J* 7.0), 8.40 (d, 1H, *J* 3.5), 8.9 (br s, 1H), 9.54 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.0 (*J* 4.7), 23.3, 24.6, 32.2, 34.3, 44.9, 45.3 (*J* 8.6), 46.8, 56.0, 109.1 (*J* 8.1), 114.2, 116.1, 118.5, 140.0, 143.4, 145.3 (*J* 25.4), 146.4, 147.2 (*J* 11.6), 148.3, 155.0, 155.3, 158.2, 169.4 (*J* 4.5). HRMS (ESI-TOF): *m/z* 469.2297 $[M+H]^+$; calcd. for $C_{23}H_{30}FN_8S^+$ $[M+H]^+$ 469.2293. Anal. RP-HPLC Method A: t_R 9.53 min, purity > 97%; Method B: t_R 8.53 min, > purity 99%.

***N*-Cyclopentyl-5-(5-fluoro-2-((5-(4-methylpiperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)-4-methylthiazol-2-amine (107).** 1-(5-(4-Methylpiperazin-1-yl)pyridine-2-yl)guanidine (**69**, 234 mg, 1.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)-2-fluoroprop-2-en-1-one (**14**, 148 mg, 0.500 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system III to give **107** as a dark brown solid (100 mg, 5%). R_F (DCM:MeOH:NH_4OH = 9:1) 0.35. mp 207-209 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.52-1.79 (m, 6H), 1.06-2.12 (m, 2H), 2.38 (s, 3H), 2.55 (s, 3H), 2.63 (t, 4H, *J* 5.0), 3.18 (t, 4H, *J* 5.0), 3.84 (m, 1H), 5.56 (d, *J* 6.0, 1H), 7.31 (dd, 1H, *J* 9.0 & 3.0), 7.82 (s, 1H), 7.99 (d, 1H, *J* 3.0), 8.18 (d, 1H, *J* 9.0), 8.23 (d, 1H, *J* 3.5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.0, 19.1, 23.8, 33.3, 46.2, 49.8, 55.1, 57.6, 89.8, 111.9, 112.6, 126.7, 136.8, 142.9, 145.3, 146.5, 147.3, 148.2, 149.4, 155.2, 170.7 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z*

469.2287 $[M+H]^+$; calcd. for $C_{23}H_{30}FN_8S^+$ $[M+H]^+$ 469.2293. Anal. RP-HPLC Method A: t_R 10.37 min, purity > 97%; Method B: t_R 8.42 min, purity > 98%.

***N*-Cyclopentyl-5-(2-((5-(4-ethylpiperazin-1-yl)pyridin-2-yl)amino)-5-fluoropyrimidin-4-yl)-4-methylthiazol-2-amine (108).** 1-(5-(4-Ethylpiperazin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (**72**, 497 mg, 2.00 mmol) and ((*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)-2-fluoroprop-2-en-1-one (**14**, 297 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **108** as a yellow solid (74 mg, 15%). R_F (DCM:MeOH = 9:1) 0.45. mp 169-170 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.03 (t, 3H, *J* 7.0), 1.50-1.57 (m, 4H), 1.66-1.69 (m, 2H), 1.90-1.95 (m, 2H), 2.37 (q, 2H, *J* 7.0), 2.46 (d, 3H, *J* 2.5), 3.11 (t, 4H, *J* 5.0), 3.32 (s, 4H), 3.96 (app s, 1H), 7.39 (dd, 1H, *J* 9.0 & 3.0), 7.94 (d, 1H, *J* 9.0), 7.97 (d, 1H, *J* 3.0), 8.23 (d, 1H, *J* 7.0), 8.40 (d, 1H, *J* 3.5), 9.44 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 12.0, 19.0 (*J* 5.1), 23.4, 32.2, 48.6, 51.7, 52.2, 56.0, 109.3, 113.2, 125.1, 135.3, 142.6, 145.4, 145.6, 145.7, 146.4, 147.0, 147.1, 148.4, 154.9, 155.3 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 483.2442 $[M+H]^+$; calcd. for $C_{24}H_{32}FN_8S^+$ $[M+H]^+$ 483.2449. Anal. RP-HPLC Method A: t_R 9.78 min, purity > 98%; Method B: t_R 7.88 min, purity > 99%.

1-(4-(6-((4-(2-(Cyclopentylamino)-4-methylthiazol-5-yl)-5-fluoropyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)ethan-1-one (109). 1-(5-(4-Acetylpiperazin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (**67,** 524 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)-2-fluoroprop-2-en-1-one (**14**, 297 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **109** as a light yellow solid (153 mg, 32%). R_F (DCM:MeOH = 9:1) 0.60. mp 206-209 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.51-1.75 (m, 4H), 1.66-1.68 (m, 2H), 1.92-1.95 (m, 2H), 2.04

(s, 3H), 2.47 (d, 3H, *J* 2.0), 3.06 (t, 2H, *J* 5.0), 3.12 (t, 2H, *J* 5.0), 3.58 (t, 4H, *J* 5.0), 3.96 (t, 1H), 7.43 (dd, 1H, *J* 9.0 & 3.0), 7.98 (d, 1H, *J* 9.0), 8.01 (d, 1H, *J* 3.0), 8.24 (d, 1H, *J* 7.0), 8.42 (d, 1H, *J* 3.5), 9.51 (br s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.0 (d, *J* 4.7), 23.3, 25.7, 32.2, 47.9, 56.0, 58.0, 60.0, 62.2, 73.9, 109.2 (d, *J* 8.3), 113.2, 118.5, 125.9, 136.1, 141.9, 145.3, 145.5, 146.4, 147.0, 147.1, 148.4, 154.9, 155.2 (d, *J* 2.4), 157.6, 157.9, 158.1, 169.4 (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 497.2245 $[M+H]^+$; calcd. for $C_{24}H_{30}FN_8OS^+$ $[M+H]^+$ 497.2242 Anal. RP-HPLC Method A: t_R 11.02 min, purity > 97%.; Method B: t_R 9.91 min, purity > 96%.

***N*-Cyclopentyl-5-(2-((5-morpholinopyridin-2-yl)amino)pyrimidin-4-yl)-4-(trifluoromethyl)thiazol-2-amine (110).** To a mixture of crude 1-(5-morpholinopyridin-2-yl)guanidine (**71,** 442 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)-2-fluoroprop-2-en-1-one (**14**, 297 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II and recrystallized with DCM and MeOH to give **110** as a brown solid (120 mg, 26%). R_F (DCM:MeOH = 9:1) 0.34. mp 199-200 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.50-1.57 (m, 4H), 1.66-1.69 (m, 2H), 1.90-1.95 (m, 2H), 2.47 (d, 1H, *J* 2.5), 3.09 (t, 4H, *J* 5.0), 3.75 (t, 4H, *J* 5.0), 3.96 (m, 1H), 7.42 (dd, 1H, *J* 9.0 & 3.0), 7.96 (d, 1H, *J* 9.0), 7.98 (d, 1H, *J* 3.0), 8.24 (d, 1H, *J* 7.0), 8.41 (d, 1H, *J* 7.0), 9.52 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.9 (d, *J* 5.1), 23.3, 32.2, 48.8, 48.4, 56.0, 66.0, 89.2, 109.2 (d, *J* 8.4), 113.2, 125.1, 134.9, 142.5, 145.3, 145.5, 145.9, 146.4, 147.0, 147.1, 148.4, 154.9, 155.2, 169.4. HRMS (ESI-TOF): *m/z* 456.1976 $[M+H]^+$; calcd. for $C_{22}H_{25}F_3N_7OS^+$ $[M+H]^+$ 456.1967. Anal. RP-HPLC Method A: t_R 11.28 min, purity > 96%; Method B: t_R 8.93 min, > 99%.

N*-Cyclopentyl-5-(5-fluoro-2-((5-(4-(methylsulfonyl)piperazin-1-yl)pyridin-2-yl)amino)pyrimidin-4-yl)-4-methylthiazol-2-amine (111).** 1-(5-(4-(Methylsulfonyl)piperazin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (**75**, 596 mg, 2.00 mmol) and **(E***)**-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)-2-fluoroprop-2-en-1-one (**14**, 297 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system II to give **111** as yellow solid (53 mg, 10%). R_F (DCM:MeOH = 9:1) 0.5. mp 168-169 °C. ^{1}H NMR (500 MHz, DMSO-d_6) 1.51-1.57 (m, 4H), 1.66-1.68 (m, 2H), 1.91-1.95 (m, 2H), 2.47 (s, 3H), 2.94 (s, 3H), 3.22 (t, 4H, *J* 5.0), 3.26 (t, 4H, *J* 5.0), 3.95-3.97 (m, 1H), 7.46 (dd, 1H, *J* 9.0 & 2.5), 7.98 (d, 1H, *J* 9.0), 8.02 (d, 1H, *J* 2.5), 8.24 (d, 1H, *J* 7.0), 8.42 (d, 1H, *J* 3.5), 9.57 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.0 (*J* 4.7), 23.3, 32.2, 33.9, 45.2, 48.6, 56.0, 109.2 (*J* 8.1), 113.2, 126.3, 141.9, 145.3, 145.5, 146.3, 146.4, 147.1, 147.2, 148.4, 155.0, 155.2, 169.4 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 533.1916 $[M+H]^+$; calcd. for $C_{23}H_{30}FN_8O_2S_2^+$ $[M+H]^+$ 533.1912. Anal. RP-HPLC Method A: t_R 10.96 min, purity > 99%; Method B: t_R 10.25 min, purity > 98%.

***N*-Cyclopentyl-5-(2-((5-(4-(dimethylamino)piperidin-1-yl)pyridin-2-yl)amino)-5-fluoropyrimidin-4-yl)-4-methylthiazol-2-amine (112).** 1-(5-(4-(Dimethylamino)piperidin-1-yl)pyridin-2-yl)guanidine trifluoroacetate (**74**, 524 mg, 2.00 mmol) and (*E*)-1-(2-(cyclopentylamino)-4-methylthiazol-5-yl)-3-(dimethylamino)-2-fluoroprop-2-en-1-one (**14**, 297 mg, 1.00 mmol) were coupled according to general procedure 8, and the residue was purified using solvent system III to give **112** as a yellow solid (134 mg, 27%). R_F (DCM:MeOH = 9:1) 0.15. mp 172-173 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.49-1.56 (m, 6H), 1.64-1.70 (m, 2H), 1.84 (d, 3H, *J* 11.5), 1.90-1.96 (m, 2H), 2.20 (s, 7H), 2.46 (s, 3H), 2.65 (t, 2H, *J* 11.0), 3.63 (d, 1H, *J* 12.0), 3.92-3.99 (m, 1H), 7.39 (dd, 1H, *J* 9.0 & 3.0), 7.93 (d, 1H, *J* 9.0), 7.98 (d, 1H, *J* 2.5),

8.23 (1H, *J* 7.0), 8.40 (d, 1H, *J* 3.0), 9.41 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.0 (*J* 5.4), 23.8, 28.4, 33.3, 41.9, 50.1, 57.6, 62.0, 111.9, 112.0, 112.6, 127.1, 137.1, 143.2, 145.1, 145.3, 146.3, 147.3, 148.1 (*J* 12.4), 149.3, 155.1 (*J* 2.5), 170.3 (*J* 4.9) (three carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 497.2608 $[M+H]^+$; calcd. for $C_{25}H_{34}FN_8S^+$ $[M+H]^+$ 497.2606. Anal. RP-HPLC Method A: t_R 9.81 min, purity > 95%; Method B: t_R 8.75 min, purity > 99%.

1-((6-Bromopyridin-3-yl)methyl)-4-ethylpiperazine (114). To a solution of 6-bromo-pyridine-3-carbaldehyde (**113**, 5.00 g, 26.9 mmol) in DCM (100 mL) 1-ethylpiperazine (3.80 mL, 26.9) was added. Then, sodium triacetoxyborohydride (6.30 g, 29.6 mmol) was added in portions and the mixture stirred at room temperature for 12 h. The residue was diluted with 5% brine (50 mL), basified with 2 M aqueous NaOH, and extracted with DCM (3 × 150 mL). The organic extracts were combined and concentrated under reduced pressure. The residue was purified by FlashMaster Personal$^+$ chromatography (silica gel, DCM ramping to DCM:MeOH = 96:4) to give **114** as a brown solid (1.91 g, 25%).^{1}H NMR (500 MHz, DMSO-d_6) δ 1.00 (t, 3H, *J* 7.0), 2.34 (q, 2H, *J* 7.0), 2.41 (br s, 8H), 3.44 (s, 2H), 7.36 (d, 1H, *J* 8.0), 7.48 (dd, 1H, *J* 8.0 & 2.5), 8.20 (d, 1H, *J* 2.5). ^{13}C NMR (125 MHz, DMSO-d_6) δ 12.1, 52.3, 52.7, 53.1, 59.4, 127.8, 133.3, 139.4, 140.8, 150.7 (two carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 284.0761 $[M(^{79}Br)+H]^+$; calcd. for $C_{12}H_{19}BrN_3^+$ $[M(^{79}Br)+H]^+$ 284.0757; *m/z* 286.0739 $[M(^{81}Br)+H]^+$; calcd. for $C_{12}H_{19}BrN_3^+$ $[M(^{81}Br)+H]^+$ 286.0736.

***N*-Cyclopentyl-5-(2-((5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)amino)pyrimidin-4-yl)-4-methylthiazol-2-amine (115).** To a solution of 5-(2-aminopyrimidin-4-yl)-*N*-cyclopentyl-4-methylthiazol-2-amine (**21**, 275 mg, 1.00 mmol) in 1,4-dioxane (3 mL) were added 1-((6-bromopyridin-3-yl)methyl)-4-ethylpiperazine (**114**, 341 mg, 1.20 mmol), Pd_2dba_3 (45.8 mg, 0.05

mmol), xantphose (58.0 mg, 0.100 mmol) and *t*- BuONa (144 mg, 1.50 mmol) and heated under microwave irradiation at 150 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified by FlashMaster Personal$^+$ chromatography (silica gel, DCM ramping to DCM:MeOH:NH4OH = 9:1:0.3) and recrystallized with DCM and MeOH to give **115** as a white solid (200 mg, 42%). R_F (DCM:MeOH = 9:1) 0.13. mp 226-228 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.09 (t, 3H, *J* 7.0), 1.58-1.76 (m, 6H), 2.08-2.14 (m, 2H), 2.43 (q, 2H, *J* 7.0), 2.55 (br s, 11H), 3.48 (s, 2H), 3.86-3.92 (m, 1H), 5.42 (d, 2H, *J* 7.0), 6.90 (d, 1H, *J* 5.5), 7.68 (dd, 1H, *J* 9.0 & 2.5), 7.89 (s, 1H), 8.19 (d, 1H, *J* 2.0), 8.35-8.38 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 1.1 (*J* 13.2), 12.1, 18.9, 23.9, 33.3, 52.4, 53.1, 57.6, 60.0, 108.3, 112.2, 119.8, 127.1, 139.1, 148.7, 152.2, 152.7, 157.5, 158.8, 159.6, 169.5 (four carbon signals overlapping or obscured). HRMS (ESI-TOF): *m/z* 479.2703 $[M+H]^+$; calcd. for $C_{25}H_{35}N_8S^+$ $[M+H]^+$ 479.2700. Anal. RP-HPLC Method A: t_R 9.33 min, purity > 98%; Method B: t_R 7.67 min, purity > 99%.

***N*-Cyclopentyl-5-(2-((5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)amino)-5-fluoropyrimidin-4-yl)-4-methylthiazol-2-amine** (**116**). To a solution of 5-(2-amino-5-fluoropyrimidin-4-yl)-*N*-cyclopentyl-4-methylthiazol-2-amine (**22**, 200 mg, 0.680 mmol) in 1,4-dioxane (3 mL) were added 1-((6-bromopyridin-3-yl)methyl)-4-ethylpiperazine (**144**, 233 mg, 0.820 mmol), Pd_2dba_3(31.0 mg, 0.034 mmol), xantphose (41.0 mg, 0.070 mmol) and *t*- BuONa (98.0 mg, 1.02 mmol) and heated under microwave irradiation at 150 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified by FlashMaster Personal$^+$ chromatography (silica gel, DCM ramping to DCM:MeOH = 93:7) to give **116** as an orange solid (100 mg, 29%). R_F (DCM:MeOH = 9:1) 0.40. mp 175-176 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.99 (t, 3H, *J* 7.0), 1.49-1.59 (m, 4H),

1.64-1.72 (m, 2H), 1.90-1.97 (m, 2H), 2.38 (br s, 10H), 2.48 (d, 3H, *J* 2.5), 3.42 (s, 2H), 3.95-3.98 (m, 1H), 7.64 (dd, 1H, *J* 8.5 & 2.0), 8.10 (d, 1H, *J* 8.5), 8.16 (d, 1H, *J* 2.0), 8.27 (d, 1H, *J* 7.0), 8.46 (d, 1H, *J* 3.5), 9.77 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.9 (*J* 5.1), 23.3, 32.2, 51.4, 52.1, 56.0, 58.7, 78.7, 79.0, 79.2, 109.2 (*J* 8.2), 111.8, 123.6, 138.3, 145.3, 145.5, 146.6, 147.1, 147.2, 148.1, 148.6, 152.2, 154.9, 155.1 (one carbon signal overlapping or obscured). HRMS (ESI-TOF): *m/z* 497.2601 $[M+H]^+$; calcd. for $C_{25}H_{34}FN_8S^+$ $[M+H]^+$ 497.2606. Anal. RP-HPLC Method A: t_R 9.89 min, purity > 96%; Method B: t_R 8.66 min, purity > 98%.

Kinase Assay. Half-maximal inhibition (Tables 1-4) and kinome-wide selectivity of **83** were measured by Kinase HotSpot radioisotope based assay platform using ^{33}P (Reaction Biology Corporation, PA, USA). The assay protocols are available at http://www.reactionbiology.com/webapps/site/KinaseProfiling.aspx. K_i values were calculated from half-maximal inhibition (IC_{50}) values using the Cheng-Prusoff equation:[42] $K_i = IC_{50}/[1 + ([ATP]/K_m \text{ (app) ATP})]$, where [ATP] is the ATP concentration used for the IC_{50} determination and K_m (app) (ATP) for each kinase is determined experimentally. Percentages of residual kinase activity upon treatment with **78, 83, 107** or **112** were measured using the Millipore KinaseProfiler services (Millipore UK Ltd, Dundee, UK) according to the detailed procedures described at http://www.millipore.com/drugdiscovery/dd3/, where ATP concentration for each kinase assay was set within 15 μM of the apparent K_m for ATP where determined.

Cell Culture. All cell lines were obtained from the cell bank at the Centre for Drug Discovery and Development, University of South Australia. The cell lines were maintained following ATCC recommendation either in RPMI-1640 (Roswell Park Memorial Institute), DMEM (Dulbecco's Modified Eagle's Medium), or MEM (Minimum Essential Media) with 10% fetal

bovine serum. All cell lines were cultured at 37 °C in a humidified incubator in the presence of 5% CO_2. All cells were mycoplasma tested.

Cell Viability Assays. MTT (Life Technologies, Mulgrave, VIC, Australia) and resazurine (Sigma Aldrich, Castle Hill, NSW, Australia) assays were performed on solid tumor cell lines and leukemia cell lines, respectively, as previously reported.[16,43] Compound concentrations required to inhibit 50% of cell growth (GI_{50}) were calculated using non-linear regression analysis.

Western Blot Assay. Western blotting was performed as described previously.[16,18,43] The following antibodies obtained from Cell Signaling Technology (Danvers, MA, USA) were used for protein detection: total Rb, phosphorylated Rb Ser780 and β-actin. Both anti-mouse and anti-rabbit immunoglobulin G (IgG) horseradish peroxidase (HRP)-conjugated antibodies were obtained from Dako, Kingsgrove, NSW, Australia.

Cell Cycle Analysis. Cell cycle analysis was performed as described previously.[44] Cells were seeded at 8×10^4 cells per well using 6 well plate and incubated overnight at 37 °C, 5% CO_2. After treatment with each compound the cells were incubated for 24 h. Cells were transferred to FACS tubes and centrifuged at 300 × *g* for 5 min. Cell pellets were collected and re-suspended in 1 mL PBS and centrifuged at 300 × *g* for 5 min. The supernatant PBS was removed and cell pellets were fixed by adding 500 µL ice-cold 70% EtOH drop wise on ice for 15 min and collected again after being centrifuged at 300 × *g* for 5 min. The supernatant ethanol was removed and collected pellets were incubated with propidium iodide cell cycle solution in PBS (50 µg/mL propidium iodide, 0.1 mg/mL RNase A, 0.05% Triton X-100) at room temperature for 1.5 h and analyzed with a Gallios flow cytometer (Beckman Coulter, Brea, CA, USA). Data were analyzed using Kaluza v1.2 (Beckman Coulter, Brea, CA, USA).

Detection of Apoptosis. Apoptosis study was performed as described previously.[44] Cells were seeded at 8×10^4 cells per well using a 6-well plate and incubated overnight at 37 °C, 5% CO_2. After treatment with each compound the cells were incubated for 24 h. Cells were transferred to FACS tubes and centrifuged at 300 × *g* for 5 min. Cell pellets were collected and re-suspended in 1 mL of warm PBS and centrifuged at 300 × *g* for 5 min. The supernatant PBS was removed and cell pellets were diluted to 1×10^5 cells/mL with warm PBS and centrifuged at 300 × *g* for 5 min. The supernatant PBS was removed and cell pellets were re-suspended with 1 mL of ice-cold PBS and centrifuged at 300 × *g* for 5 min. The supernatant PBS was removed and cell pellets were re-suspended with 100 µL of 1× binding buffer. 3 µL of Annexin V and 3 µL of propidium iodide was added to each sample with slight vortexing and cells were incubated in the dark for 15 min. After incubation 200 µL of 1× binding buffer was added to each sample and analyzed by the Gallios flow cytometer (Beckman Coulter, Brea, CA, USA). Data were analyzed using Kaluza v1.2 (Beckman Coulter, Brea, CA, USA).

Biopharmaceutical Profiling. The assays were performed at Cyprotex Ltd. (Macclesfield, UK). hERG inhibition was assessed using automated patch clamp electrophysiology measurement in CHO-hERG cells.[45] CYP450 inhibition assay was measured by incubating the compounds with human liver microsomes and NADPH in the presence of CYP450-isoform specific probe substrate.[46,47] Test compound partitioning between octanol and aqueous buffer was carried out using the shake-flask method.[48] Compound pKa values were determined using a pH-metric titration method (GLpKa; Sirius).[49] Apparent permeability coefficients were measured using a Caco-2 cell layer assay.[50]

PK Determination. For pharmacokinetic measurements, healthy male adult BALB/c mice (weighing 20–25 g) or male albino Wistar rats (weighing 250-350 g) were split into weight

matched groups of 3 per group. Compound **83** was administered IV (2 mg/kg for mice, 5 mg/kg for rats in a formulation of PEG400/NMP, 9/1, v/v) *via* the tail vein or by oral gavage (10 mg/kg for mice, 20 mg/kg for rats, compound suspended in 1% carboxy methyl cellulose (CMC), w/v). Blood samples were collected from animals by jugular vein cannula (rats) or under anaesthesia by cardiac puncture (mice) at time zero and at intervals up to 24 h. Harvested blood was centrifuged at 7000 × *g* for 3 minutes, and the plasma aspirated and frozen at -20 °C until analysis. Quantitative analysis of the compound in plasma was carried out using a validated LC-MS/MS method with a triple TOF-MS 5600 (AB SCIEX). Non-compartmental pharmacokinetics analysis was performed with Phoenix WinNonlin (Pharsight, St. Louis, US) for each concentration−time profile. Animal experiments were performed under the approval of the SA Pathology Animal Ethics Committee (Animal Ethics Number: 91C-12).

Maximum Tolerated Dose Determination. To determine the *in vivo* maximum tolerated dose (MTD), **83** (in 1% CMC) was administered PO to BALB/c mice (n = 3) at a dose of 20 mg/kg once every day for 7 days. To minimise exposure of the animals to any unexpected acute toxicity, the test compound was first administered to a mouse in the group which was then monitored closely for clinical signs of toxicity for 1 h; when no such signs were observed, the test compound was administered to the remaining 2 mice in the group. The mice in the test group were then monitored for signs of toxicity at 1, 8 and 24 h after administration. The weights and clinical signs of toxicity of the mice were monitored until the fourteenth day after administration. Since no significant signs of toxicities were observed in the group treated with 20 mg/kg, the MTD study was repeated at 40 mg/kg resulting in the same finding. Thus, we increased the dose to 100 mg/kg. Similar to the 20 and 40 mg/kg study, no significant changes in weight and signs of clinical toxicities were observed at 100 mg/kg, indicating the MTD was apparently not

reached in this study. Accordingly, 100 mg/kg was selected for *in vivo* efficacy study. All the animal experiments were performed according to the institutional ethical guidelines on animal care and under the approval by the SA Pathology Animal Ethics Committee (Animal Ethics Number: U03-14).

MV4-11 Xenograft. Female nude (nu/nu) BALB/c mice (6-8 weeks) were used for the *in vivo* anti-cancer activity. All the animal experiments were performed according to the institutional ethical guidelines on animal care and under the approval by the SA Pathology Animal Ethics Committee (Animal Ethics Number: U03-14). MV4-11 leukemia xenograft model was established by inoculating 5×10^6 MV4-11 cells subcutaneously on their hind flanks. The cells were suspended in a 1:1 mixture of RPMI-160 and Matrigel (BD Biosciences, NSW, Australia).[51] Tumors were allowed to develop and once the tumor size reached 100-200 mm^3, mice were allocated into a control or treatment group of nine. An independent-samples t-test was conducted to compare baseline tumor volumes of control and treatment groups and there was no significant difference in the scores for controls (mean tumor volume = 142.04 ± 28.46) and treatment groups (mean tumor volume = 142.77 ± 24.22) and $p = 0.911$, before treatment started. Compound **83** and vehicle control (1% CMC) were administered by oral gavage daily for 33 consecutive days. Mice were observed for any clinical signs of toxicity and weight loss every day whereas tumor volume measurements were taken every other day prior to dosing. Tumor volume was measured in cubic millimeters (length × width × height). Animals were euthanized if the tumor volume exceeded 2000 mm^3 or if weight loss exceeded 15% of their pre-treatment mass. Four of the nine mice from the **83**-treated group survived after the whole study period (83 days) and were humanely killed to terminate the study as the tumor failed to re-appear after 50 days of follow-up. Data was analyzed using GraphPad Prism version 6.03 for Windows

(GraphPad Software, La Jolla, California, USA). Tumor volume and body weight loss analysis was performed with 2-tailed t-test. Survival analysis was performed with Gehan-Breslow-Wilcoxon test. The results were considered significant when $p \leq 0.05$. Tumor growth inhibition (TGI) was calculated using the following formula; %TGI= [((cTVd-cTVi)-(tTVd-tTVi))/ (cTVt-cTVi)] × 100. Where: cTVd is tumor volume in control group on day 33 of treatment, cTVi is tumor volume in the control group at baseline, tTVd is tumor volume in the treatment group on day 33, and tTVi is tumor volume in the treatment group at baseline.

Statistical Analysis. All experiments were performed in triplicate and repeated at least twice; representative data were selected for generating figures. The statistical difference between treatments and controls was analyzed using Student's t-test. $p \leq 0.05$ was considered statistically significant.

ASSOCIATED CONTENT

Supporting Information

Percentages of residual kinase activities of **78**, **83**, **107** and **112**, kinome-wide selectivity of **83**, plasma concentration-time curves of **83** in mice and in rats, NMR spectra and HPLC analysis results for **83**, and molecular formula strings. This material is available free of charge via the internet at http://pubs.acs.org.

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Authors' Contributions

Conception and overall study design and supervision: S.W. Chemistry experiments and data analysis: S.T. and M.Y. Biological experiments: L.B., F.L., S.I., K.T., S.K.B. T.T. and H.A. *In*

vivo experiments: L.B., M.H.R., B.N. and R.M. Writing, review and/or revision of the manuscript: S.T., M.Y., S.W., L.B., M.H.R., B.N., S.I. and K.T.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

AGC, protein kinase A, protein kinase G, and protein kinase C containing families; AML, acute myeloid leukemia; CAMK, calcium/calmodulin-dependent protein kinase; CK1, casein kinase 1; CMC, sodium carboxymethyl cellulose; CMGC, cyclin-dependent kinase, mitogen-activated protein kinase, glycogen synthase kinase 3, and dual specificity protein kinase containing families; E2F, E2 promoter binding factor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Rb, retinoblastoma protein; SelectFluor, 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octanebis(tetrafluoroborate); PI, propidium iodide, STE, homologues of yeast sterile 7, sterile 11, sterile 20 kinases; T/C, relative tumor growth inhibition of the treated group *versus* the control group; TK, tyrosine kinase; TKL, tyrosine kinase-like.

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