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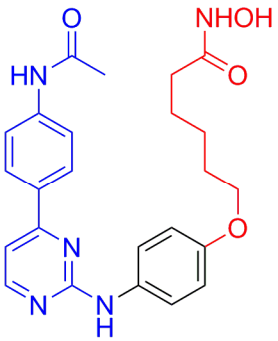
Design A



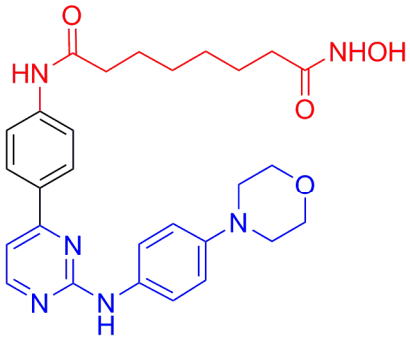
45h

KMS-12-BM cell IC_{50} = 70nM

JAK2
HDAC
Merged

JAK2 IC_{50} <10nM
HDAC6 IC_{50} <10nM

Design B



69c

PC-3 cell IC_{50} = 0.64μM

Design and Synthesis of Potent Dual Inhibitors of JAK2 and HDAC Based on Fusing the Pharmacophores of XL019 and Vorinostat

Yu-yi Chu-Farseeva,[a] Nurulhuda Mustafa,[b] Anders Poulsen,[c] Eng Chong Tan,[d] Jeffrey J. Y. Yen,[d] Wee Joo Chng,[b,e,f] Brian W. Dymock,[a]#

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Running Head: Synthesis and SAR of Novel Dual inhibitors of JAK2/HDAC

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Supporting information for this article includes additional synthesis of compounds **26**, **30**-**32**, **37**-**39**, **S1**-**S5**, **63b**-**f**; selectivity indices for inhibition of cell proliferation; additional JAK-STAT Western blots; and ^{1}H and ^{13}C NMR spectra for all key compounds.

ABSTRACT

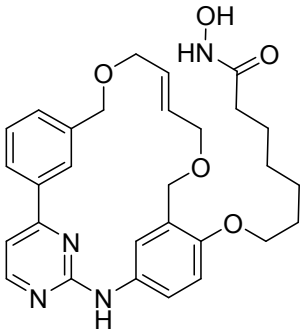
Specifically blocking more than one oncogenic pathway simultaneously in a cancer cell with a combination of different drugs is the mainstay of the majority of cancer treatments. Being able to do this via two targeted pathways without inducing side effects through a general mechanism, such as chemotherapy, could bring benefit to patients. In this work we describe new dual inhibitor of the JAK-STAT and HDAC pathways through designing and developing two types of molecule based on the JAK2 selective inhibitor XL019 and the pan-HDAC inhibitor, vorinostat. Both series of compounds had examples with low nanomolar JAK2 and HDAC1/6 inhibition. In some cases good HDAC1 selectivity was achieved while retaining HDAC6 activity. The observed potency is explained through molecular docking studies of all three enzymes. One example, **69c** had 16-25 fold selectivity against the three other JAK-family proteins JAK1, JAK3 and TYK2. A number of compounds had sub-micromolar potencies against a panel of 4 solid tumor cell lines and 4 hematological cell lines with the most potent compound, **45h**, having a cellular IC_{50} of 70 nM against the multiple myeloma cell line KMS-12-BM. Evidence of both JAK and HDAC pathway inhibition is presented in Hela cells showing that both pathways are modulated. Evidence of apoptosis with two compounds in 4 sold tumor cell lines is also presented.

KEYWORDS: multicomponent ligand, JAK2 inhibitor, HDAC inhibitor, JAK/HDAC dual inhibitor

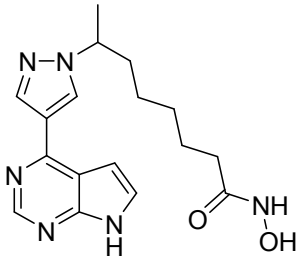
INTRODUCTION

Target therapies have achieved significant gains in the fight against cancer, however, they are still a long way from providing generally curative treatments for the majority of cancers. Targeted agents can be used with each other or with traditional chemo- or radiotherapy.[1] However more research is required to develop new combinations of targeted agents by finding pathways which can be inhibited

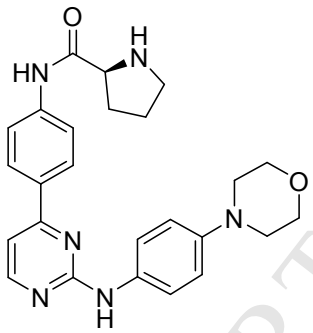
together and lead to better and more durable responses in patients.[2] Targeting the JAK-STAT pathway with JAK kinase inhibitors, such as ruxolitinib,[3] has provided value for patients with myeloproliferative neoplasms, conditions which can lead to leukemia.[4] Likewise, inhibitors of the histone deacetylase (HDAC) pathway have found value in T-cell lymphomas.[5] In an exciting new way forward, we propose to combine the targeting of JAK kinases and HDACs with a single, dual inhibitor. Clinical trials of ruxolitinib with panobinostat support the notion that blockade of these pathways may be useful in treating solid tumors or hematological malignancies.[6] Recently we have reported new dual JAK-HDAC inhibitors, such as **1** and **2** (Figure 1), based on the JAK inhibitor templates pacritinib and ruxolitinib, respectively.[7, 8] In this latest study we report the discovery of dual JAK-HDAC agents based on XL019 (**3**). Key discoveries with this series are the very potent JAK and HDAC1/6 activities which have led to very potent cellular activites. Compound **3** is a potent and selective JAK2 inhibitor with IC_{50} of 2.2 nM, exhibiting >50-fold selectivity over JAK1, JAK3 and TYK2. It has been studied in a phase 1 clinical trial but was forced to terminate due to CNS side effects.[9] In our study, CNS penetration is not expected due to the higher molecular weight and polarity of the compounds. Importantly, compound **3** has inherent selectivity for JAK2 over the other JAK family enzymes, unlike ruxolitinib which has a JAK1/2 profile, possibly a factor in its safety profile where patients with low platelet counts cannot be treated.[10] In this report we demonstrate the design and synthesis of novel JAK-HDAC dual inhibitors based on **3** and the marketed HDAC inhibitor vorinostat (**4**).



EY3238 (**1**)
JAK2 IC_{50} = 1.4 nM
HDAC6 IC_{50} = 2.1 nM



YLB343B (**2**)
JAK1 IC_{50} = 11 nM
HDAC1 IC_{50} = 6 nM
HDAC6 IC_{50} = 1 nM



XL019 (**3**)
JAK2 IC_{50} = 5.7 nM
JAK3 IC_{50} = 368.1 nM

Figure 1 Structures of JAK-HDAC dual inhibitors EY3238 (**1**),[7] YLB243B (**2**)[8] and the JAK2 selective XL019 (**3**).[9]

RESULTS AND DISCUSSION

In silico analysis and design of compounds

Designing a dual JAK-HDAC inhibitor by merging of the pharmacophores of **3** and **4** may be achieved with two designs (Figure 2). Both designs employ the core aniline substituted biarylpyrimidine as the JAK2 binding template. Both designs also take advantage of solvent channels in JAK2 where the hydroxamate bearing side chain, necessary for binding in the deep HDAC pocket, can be attached. By varying this chain length control over HDAC potency is expected to be achieved. In Design A the hydroxamate side chain is attached to the aniline at either the *meta* or *para* position with variations possible in the R group attached to the acetamide. In Design B, the hydroxamate side chain is attached to the acetamide, taking advantage of the solvent channel in JAK2, and the R group is again variable attached to the aniline phenyl ring. A range of compounds based on Designs A and B were then prepared varying the length of the hydroxamate bearing side chain and with different R groups.

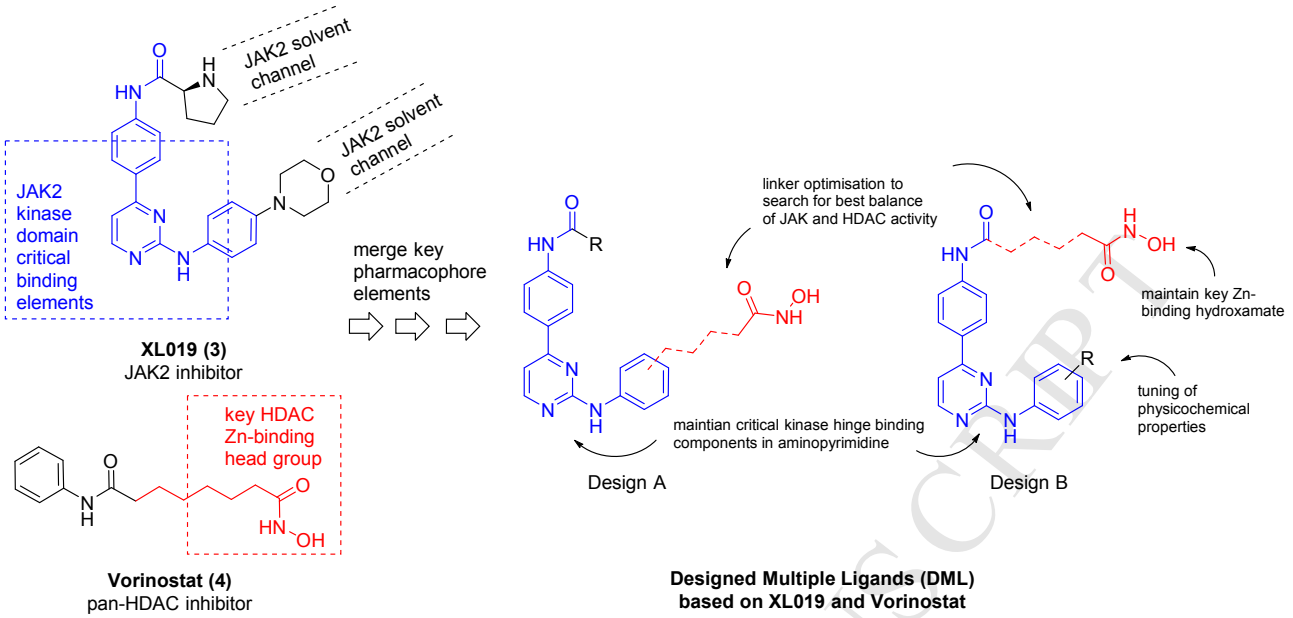
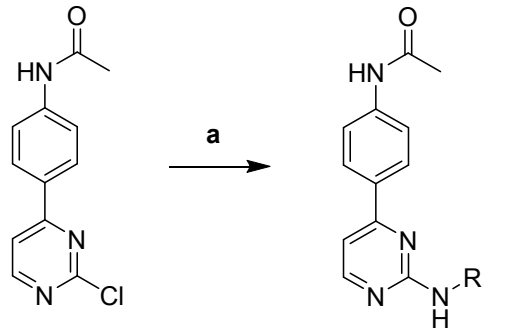


Figure 2 Design options for construction of a dual JAK-HDAC inhibitor from XL019 (**3**) and vorinostat (**4**). Designs A and B indicate attachment of the HDAC binding hydroxamate chain at two different positions of the core JAK binding biaryl-aminopyrimidine. Physical properties could be tuned by varying the 'R' group.

Chemistry

Simplified analogues of **3** were prepared to assess the 'base' levels of JAK2 activity in the scaffold (Figure 3). Replacement of the basic pyrrolidine of **3** with a methyl, giving an acetamide substitution, enabled isolation of reaction products without problems of protonation of the basic centre. Three substitutions of the pyrimidine: aniline (**6**), benzylamine (**7**) and phenethylamine (**8**) were prepared from **5** in hot butanol catalysed by *para*-toluene sulfonic acid (PTSA). Chloropyrimidine **5** was prepared by a palladium coupling between dichloropyrimidine and the boronate ester of acetanilide.[9]



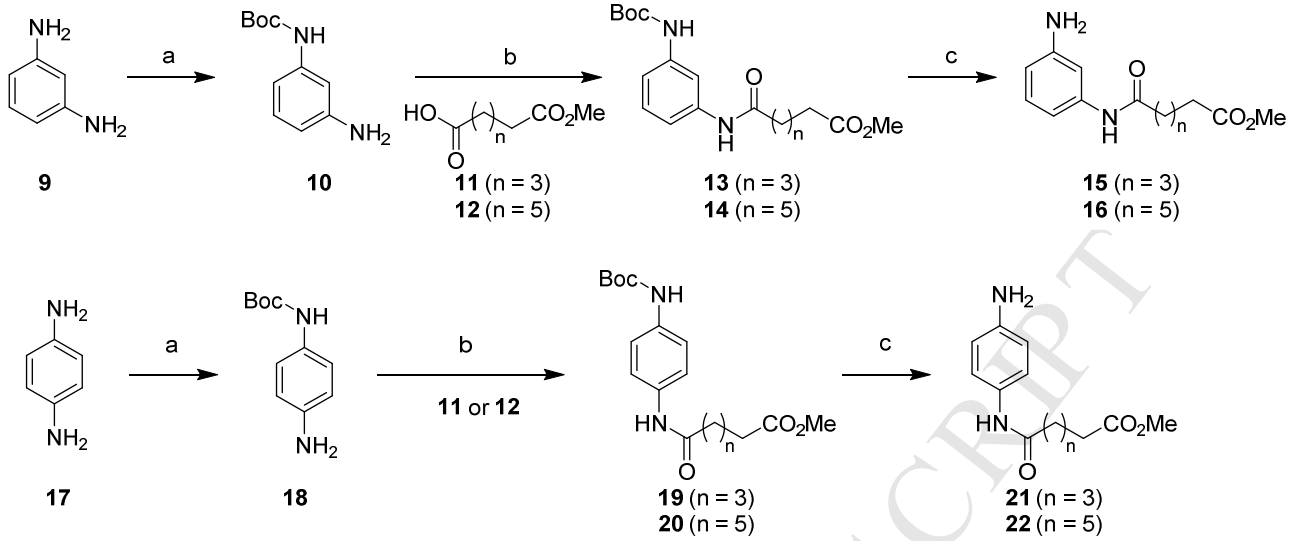
5

6 (R=Ph)
7 (R=Bn)
8 (R=$(CH_2)_2$Ph)

Reagents and Conditions: (a) aniline, benzylamine or phenethylamine, PTSA, nBuOH, 100°C, 2h.

Figure 3 Synthesis of compounds **6-8**.

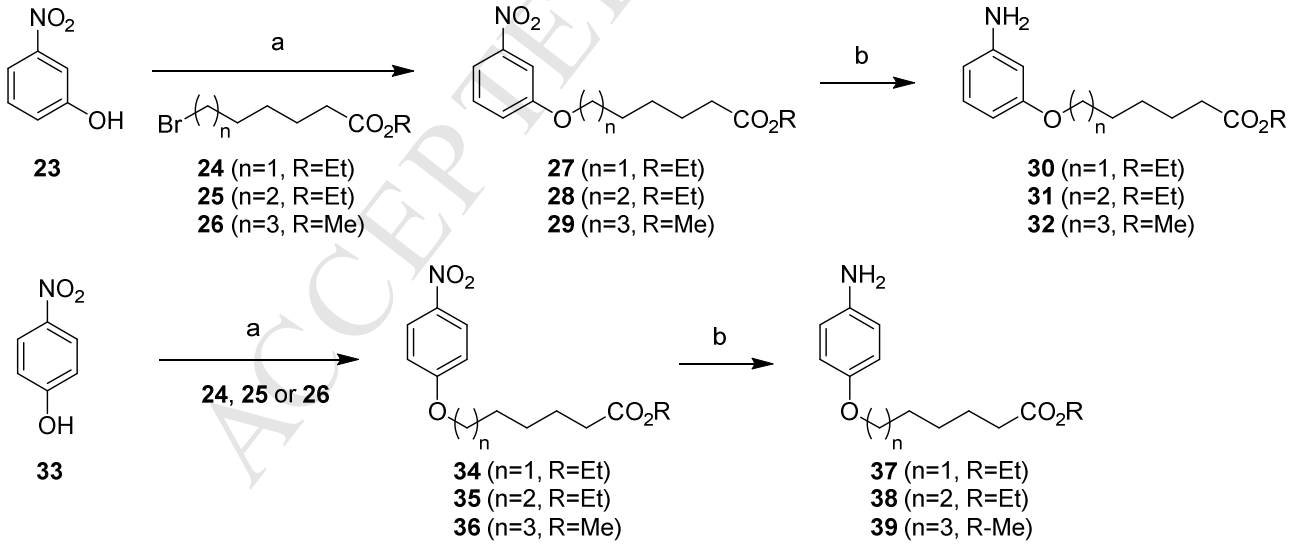
To enable exploration of amide-linked Design A (Figure 2) specific aniline building blocks had to be prepared with variation of chain length (Figure 4). Mono-Boc protection of diaminobenzene (*meta*- (**9**) and *para*-(**17**)) was achieved employing excess diamine to minimize unwanted bis-protection while ensuring practical isolation of **10** and **18**. Amide formation *via* acid chlorides of **11** (4 methylene chain) and **12** (6 methylene chain) gave protected esters **13**/**14** and **19**/**20**. Deprotection using concentrated sulfuric acid furnished the desired anilines **15**/**16** and **21**/**22** in overall good yield.



(a) Boc_2O, DCM, RT, 18h; (b) $ClCO_2Et$, NMM, DCM, 0°C to RT, 18h; (c) H_2SO_4, EA, RT, 18h

Figure 4 Synthesis of *meta* and *para* substituted aniline building blocks **21**-**22**.

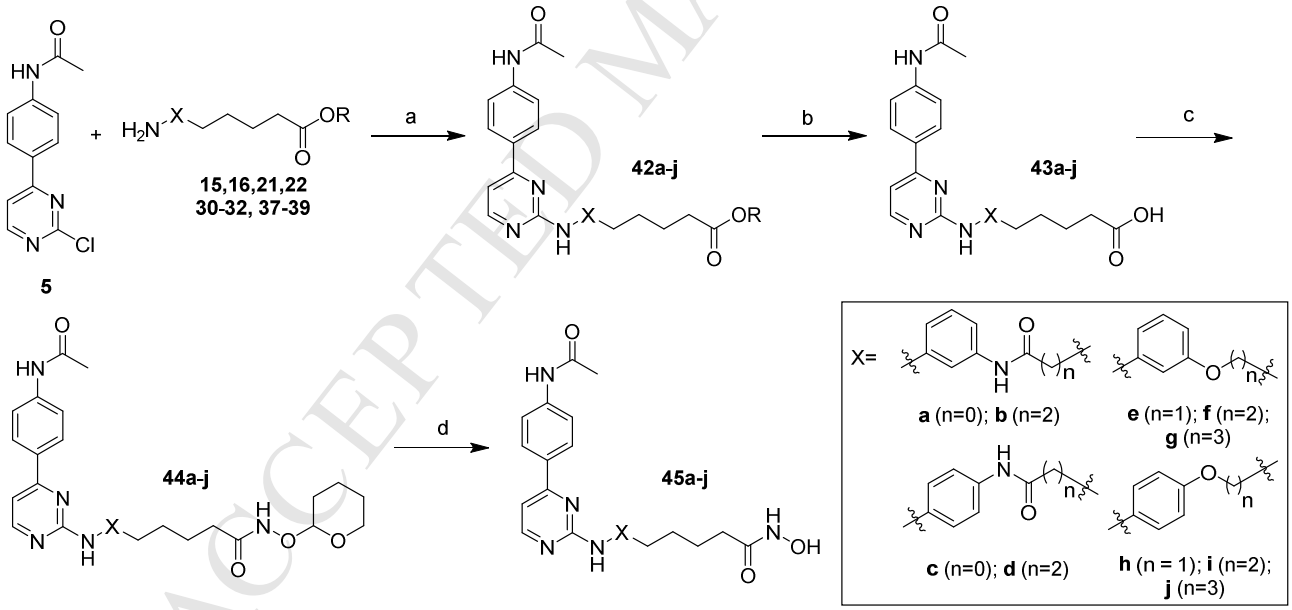
In addition to the amide linked series, we wanted to explore a flexible ether linked side chain (Figure 5). Hence *meta*- (**23**) or *para*-nitrophenols (**33**) were alkylated with bromo-esters **24**-**26** in hot acetonitrile. The resulting nitro-ethers **27**-**29** and **34**-**36** were reduced to the desired anilines **30**-**32** and **37**-**39** using iron and ammonium chloride in aqueous methanol.



(a) K_2CO_3, MeCN, 80°C, 18h; (b) Fe, NH_4Cl, $MeOH/H_2O$ (2;1), reflux, 3-18 h.

Figure 5 Synthesis of *meta* and *para* substituted ether building blocks **30**-**32** and **37**-**39**.

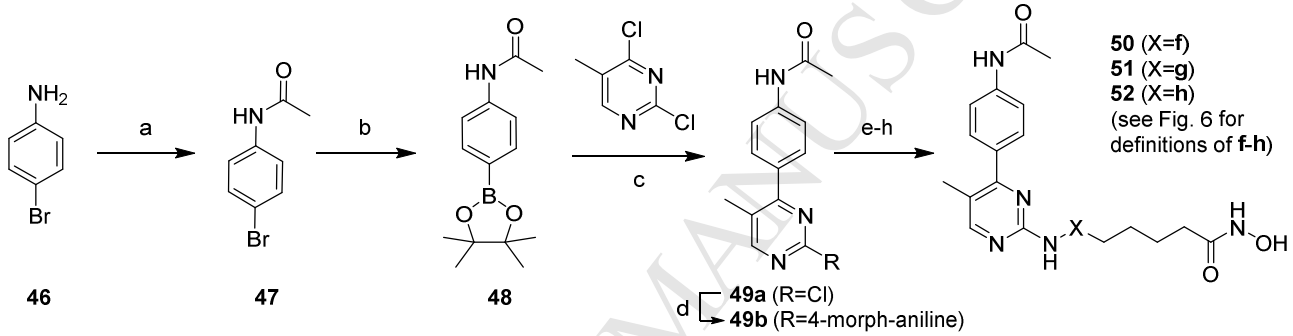
Ester intermediates **42a-j** were formed with a nucleophilic substitution reaction between biaryl **5** and anilines (Figures 4 and 5), under similar conditions to **6-8** (Figure 3). A mixture of the desired methyl and the transesterified butyl ester were obtained in moderate yield, however this was inconsequential since ester intermediates were saponified using lithium hydroxide in THF/water to yield the desired acids **43a-j**. The crude product was directly used for the next step without further purification. Formation of THP-protected hydroxamates **44a-j** was achieved in a HATU-mediated amide formation with THP-protected hydroxylamine in DMF overnight. Final THP deprotection was accomplished under mild conditions with *in situ* HCl generated from acetyl chloride in cold MeOH/DCM to afford **45a-j**. Target compounds which precipitated were washed with DCM or MeOH. Preparative reverse phase HPLC purification was necessary for target compounds which did not satisfactorily precipitate from the reaction mixture.



(a) PTSA, nBuOH, 100°C, 2h (**41a-41d**) OR $Pd_2(dba)_3$, X-Phos, K_2CO_3, t-BuOH, 100°C, 18h (**41e-41j**);(b) LiOH, THF/H_2O (9:1), RT, 18h; (c) $THPONH_2$, HATU, Et_3N, DMF,RT, 18h; (d) AcCl, MeOH/ DCM (1:1), RT, 3h

Figure 6 Synthesis of amide and ether linked JAK-HDAC dual inhibitors **45a-j**.

Improvement in JAK2 potency has been shown to be possible by adding small hydrophobic groups at the 5' position of the pyrimidine. Acetylation of *para*-bromoaniline (**46**) gave **47** followed by palladium coupling gave boronate ester **48**. Coupling of **48** with dichloropyrimidine furnished biaryl chloropyrimidine **49** which was then coupled in a third palladium catalyzed reaction followed by saponification, THP-protected hydroxamate formation and final deprotection to give three test compounds, **50-52**, exploring *meta/para* substitution and chain length (Figure 7 and Table **3**). Model compound **49b** was also prepared via aniline **63c** (see Figure 9).



(a) AcCl, K_2CO_3, DCM, 3h, 83%; (b) $(PinB)_2$, AcOK, $Pd(dppf)Cl_2$, Dioxane, 100°C, overnight, 89%; (c) $Pd(PPh_3)_4$, Na_2CO_3, MeCN, reflux, 18h, 64.7%; (d) **63c**, $Pd_2(dba)_3$, X-Phos, K_2CO_3, t-BuOH, 100°C, 18h; (e) **41f-h**, $Pd_2(dba)_3$, X-Phos, K_2CO_3, t-BuOH, 100°C, 18h; (f) 2 M LiOH, THF-H_2O, RT; (g) $THPONH_2$, HATU, Et_3N, DMF,RT, 18h; (h)AcCl, MeOH/ DCM (1:1), RT

Figure 7 Synthesis of 5'-methylpyrimidine intermediate **49**.

To explore the compatibility of a basic side chain with the hydroxamate we chose to install a methylpiperazine appended to a methylene, to increase flexibility, attached to the aromatic amide (Figure 8). Starting from intermediate **53** and **54** the acetamide was cleaved in 4N HCl to reveal anilines **55** and **56**. Amide formation with methylpiperazine acetic acid was achieved with 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) as coupling agent to give **57** and **58**. THP-protected hydroxamates **59** and **60** were then formed from THP protected hydroxylamine and deprotected as described previously to afford target compounds **61** and **62**.

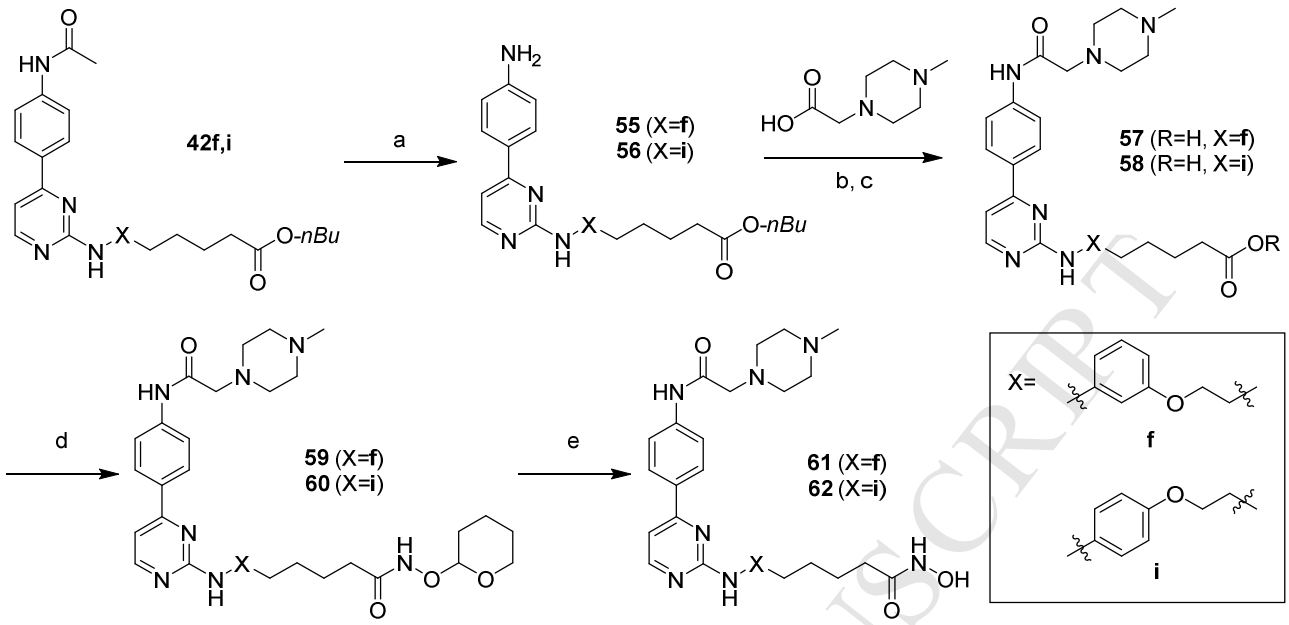
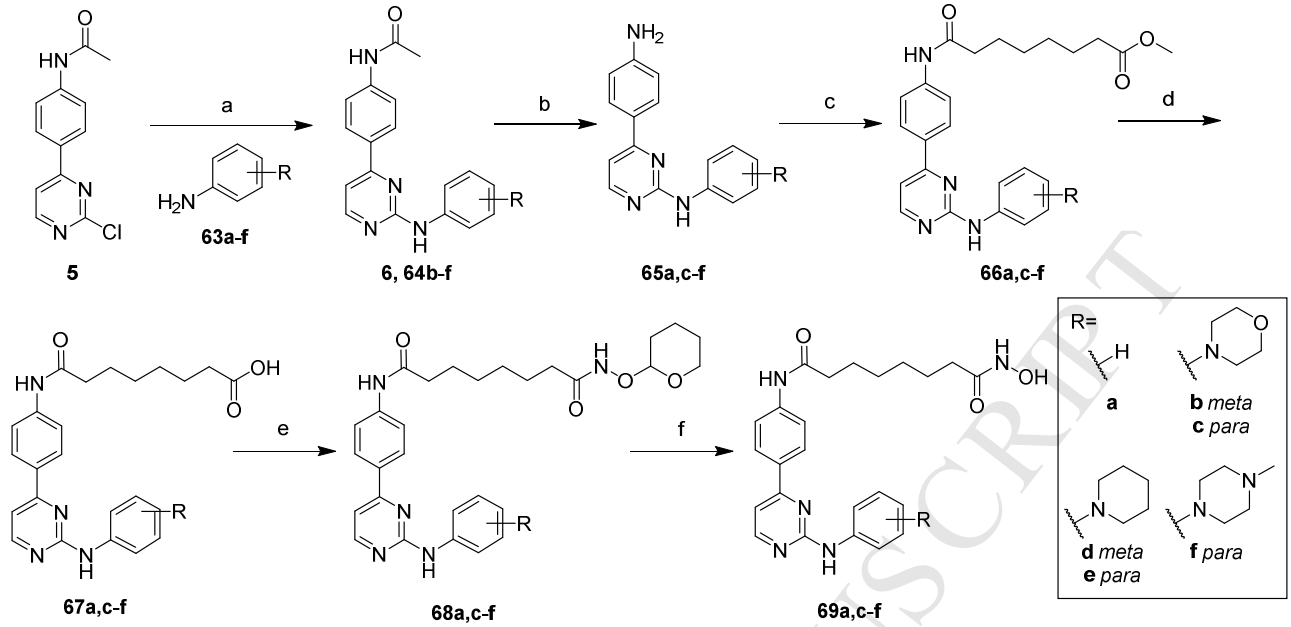


Figure 8 Synthesis of basic methylpiperazine hydroxamates **61** and **62**.

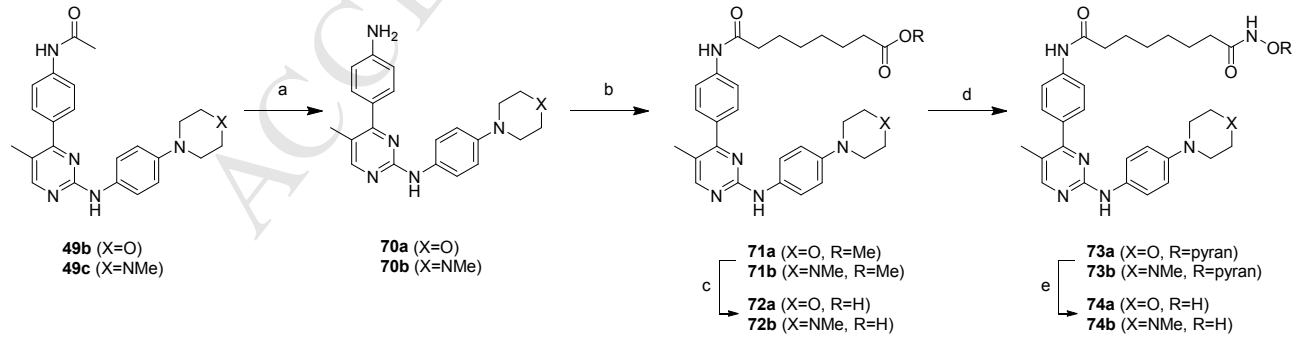
To prepare analogues with the hydroxamate chain appended to the upper aryl group of the structure chloropyrimidine intermediate **5** underwent Buchwald coupling with aniline (**63a**) and a range of *meta*- and *para*- substituted anilines **63b-f** (Figure 9). The acetamides **64a-f** of the resulting anilinopyrimidines were cleaved to give anilines **65a,c-f** then amides formed with methyloctanoic acid affording esters **66a,c-f**. Saponification of the esters formed **67a,c-f** and was followed by hydroxamate formation (**68a,c-f**) *via* the THP protected hydroxamate and deprotection as described above to give target compounds **69a,c-f**.



(a) Anilines **63a-f**, $Pd_2(dba)_3$, X-Phos, K_2CO_3, t-BuOH, 100°C, 18h, 44-77%; (b) 4N HCl/Dioxane, MeOH, 50°C, ST, 18h; (c) Methyl-1,8-dioctanoic acid, HATU, Et_3N, DMF, RT, 18h; (d) LiOH, THF/H_2O (3:2), RT, 18ht; (e) $THPONH_2$, HATU, Et_3N, DMF,RT, 18h; (f) 4N HCl/Dioxane, DCM, RT, 3-18h

Figure 9 Synthesis of hydroxamates **69a, c-f** linked through the aromatic amide.

Appending a methyl group to the 5' position of the pyrimidine of **69b**,**f** was achieved from 5'-methylpyrimidine intermediate **49**. Initial hydrolysis of **49** with HCl/dioxane to give anilines **70** followed by amide formation to install the methylene chain (**71**), hydrolysis to acids (**72**), capping with THP-protected hydroxylamine and final deprotection with either acetyl chloride in methanol (**74a**) or HCl/dioxane (**74b**).



(a) 4N HCl/Dioxane, MeOH, 50°C, 18h; (b) Methyl-1,8-dioctanoic acid, HATU, Et_3N, DMF, RT, 18h; (c) LiOH, THF/H_2O (3:2), RT, 18ht; (d) $THPONH_2$, HATU, Et_3N, DMF,RT, 18h; (e) AcCl, MeOH/ DCM (1:10), RT, 18h (**74a**) or 4N HCl/Dioxane, DCM, RT, 18h (**74b**)

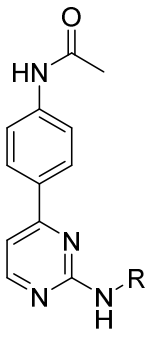
Figure 10 Synthesis of 5'-methyl substituted hydroxamates **74a,b**.

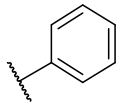
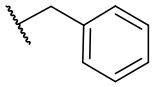
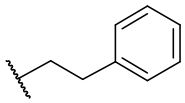
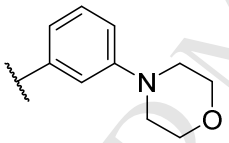
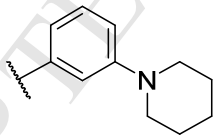
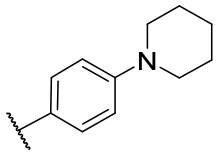
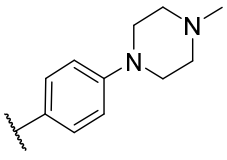
In Vitro Enzyme Assay Assessments

Synthesised compounds were tested in single concentration or dose response assays against JAK2, HDAC1 and HDAC6. A biochemical assay measuring the rate of ATPase activity of JAKs was used for all kinase data and a fluorescence assay measuring the rate of HDAC cleavage of an acetylated peptide substrate was used for quantification of HDAC inhibition.[7]

Model JAK2 compounds **6-8** were tested in single concentration assays to ascertain the best template for connection to the aminopyrimdine (Table 1). Not surprisingly, the aniline **6** gave over 96% inhibition of JAK2 activity at 10 μM (IC_{50} of 27.4 nM, see Table 2). However benzyl **7** and phenethyl **8** had only weak inhibition of the kinase. Based on this result further studies were carried out with **6** as the core template substituting various 6-membered rings to the *meta*- and *para*- positions of the aryl ring. These compounds were prepared using a Pd-coupling protocol as intermediates for dual inhibitors (see Figure 9). *Meta*-substituted morpholine **64b** and piperidine **64d** both had JAK2 IC_{50} values in the 10-20 nM range. Furthermore, *para*-substituted piperidine **64e** and methylpiperazine **64f** had IC_{50} values less than 10 nM. Hence both *meta*- and *para*-substituted aryls were selected for linking to hydroxamates.

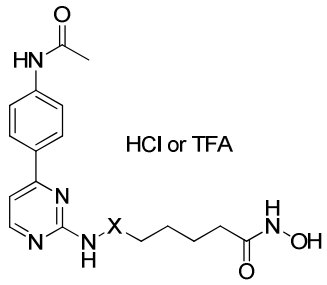
Table 1. JAK2 Kinase Inhibition for model compounds



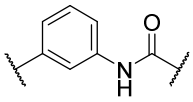
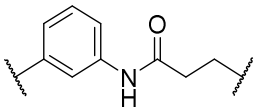
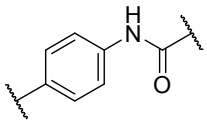
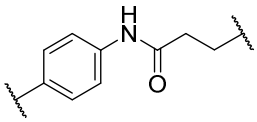
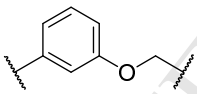
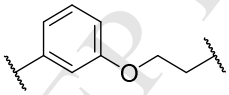
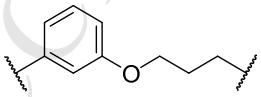
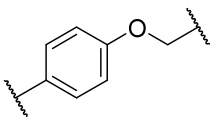
Compound	R	JAK2 IC_{50} (nM) or % Inhibition @ 10 μM
3 (XL019)[9]	-	2.2
6		96.42%
7		3.85%
8		6.22%
64b		19.6
64d		13.8
64e		2.9
64f		7.9

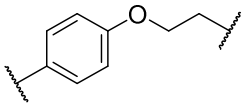
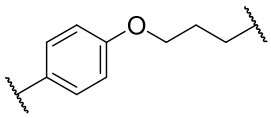
Testing of amide and ether linked hydroxamates was carried out in duplicate 10-point dose responses against JAK2, HDAC1 and HDAC6 (Table 2). Surprisingly, the *meta*-substituted 4 methylene **45a** had an IC_{50} of 2.6 nM for JAK2 and 4.6 nM for HDAC6 with selectivity of nearly 100 fold over HDAC1. Extending the number of methylenes in the linker to 6 (**45b**) resulted in a boost of HDAC1 activity by over an order of magnitude and of HDAC6 potency by more than 5 fold with only a small loss of JAK2 activity. This level of potency was not replicated with *para*-derivative **45c**, bearing 4 methylenes, which had reduced potency against all targets. However extending this compound to 6 methylenes (**45d**) resulted in sub-nanomolar potency against both JAK2 (0.9 nM) and HDAC6 (0.1 nM) with over 7,000 fold selectivity for HDAC6 over HDAC1. For confirmation the methyl/butyl ester **42i** was tested against all targets and confirmed to show negligible HDAC activity while retaining moderate JAK2 activity. A similar trend continued into the ether series with *meta*-substituted **45e/45f** having low nanomolar activity for JAK2 and HDAC6 with moderate HDAC1 activity. A further extension to 7 methylenes (**45g**) did not substantially affect the profile. *Para*-substituted analogues **45h-j** exhibited a similar overall profile to the *meta*-substituted analogues with the most selective having the longer linker.

Table 2. JAK2 and HDAC Inhibition for Compounds **45a-j**



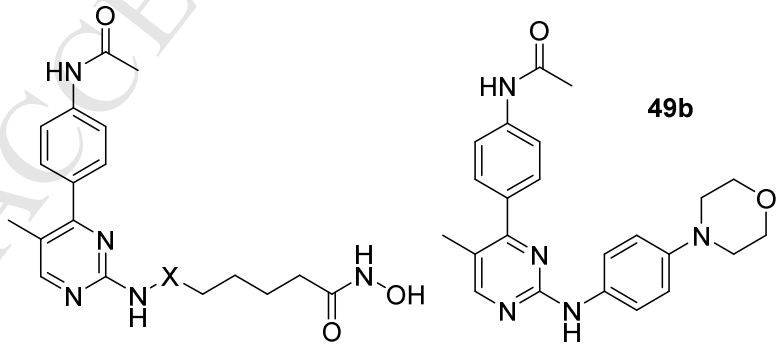
Compound	X	JAK2 IC_{50} (nM)	HDAC1 IC_{50} (nM)	HDAC6 IC_{50} (nM)

4 (Vorinostat)[29]	-	-	306	20
6	-	27.4 ± 1.2		
45a		2.6	384	4.6
45b		9.2 ± 1.5	32.4 ± 4.2	0.81 ± 0.05
45c		58.3 ± 12.8	3350 ± 2774	117 ± 138
45d		0.9 ± 0.4 nM	774 ± 30	0.10 ± 0.05
45e		3.4 ± 3.1	637 ± 214	2.4 ± 0.4
45f		3.6 ± 0.1	83 ± 29	1.8 ± 1.2
45g		17 ± 1.5	1043 ± 95	6.7 ± 5.7
45h		33 ± 12	89 ± 31	1.3 ± 0.6
42i	Ester	239	>10,000	3150

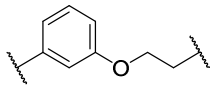
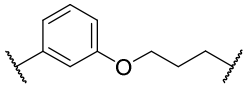
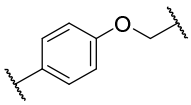
45i		23 ± 6.4	145 ± 8	3.0 ± 0.7
45j		46 ± 12	1701 ± 1227	14 ± 3.5

JAK2 inhibitors with a biaryl pyrimidine have been reported to enjoy an increase in potency when a methyl group is installed at the 5'position.[11, 12] This is due to hydrophobic interactions in a small pocket at the base of the ATP site.[13] We prepared one reference compound to confirm that potency was present in our template (**49b**). This benefit does not transfer to ethers **52** and **50** (Table 3), which have a very similar profile to their corresponding non-methyl counterparts (see compounds **45h** and **45i**, Table 2). On the other hand, **51** has gained just over 5 fold JAK2 activity, compared to **45g**, while increasing selectivity over HDAC1 with maintenance of potency against HDAC6.

Table 3. JAK2 and HDAC Inhibition for Compounds **49b**, **50**-**52**

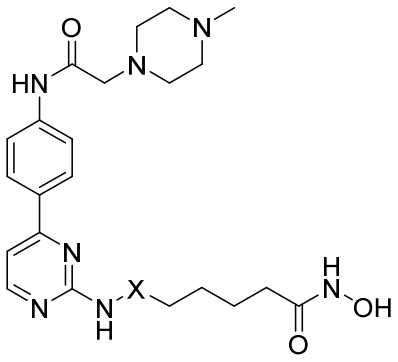


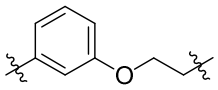
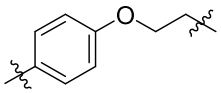
Compound	X	JAK2 IC_{50}	HDAC1 IC_{50}	HDAC6 IC_{50}

		(nM)	(nM)	(nM)
49b	-	28.7	-	-
50		3.9 ± 2.7	155 ± 75	6.1 ± 0.7
51		2.93 ± 1.21	4581 ± 2367	9.7 ± 6.3
52		25.6 ± 4.2	260 ± 116	3.5 ± 1.7

Compound **3** possesses a basic pyrrolidine appended to its aromatic amide. This pyrrolidine is somewhat rigid and could restrict optimal binding to either JAK2 or HDAC. Hence to explore whether basicity in our series was compatible with a hydroxamate side chain we prepared two analogues bearing a flexible methylpiperazine side chain, **61** and **62**. Generally, this change reduced potency against all targets but particularly against JAK2 with a 30 fold loss of activity for **61** (compared with **45f**) but only a 4 fold loss for the para-linked derivative **62** (compared with **45i**). However HDAC1 and HDAC6 activities were not strongly affected.

Table 4. JAK2 and HDAC Inhibition for Compounds **61** and **62**

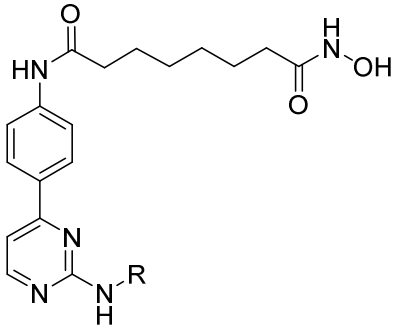


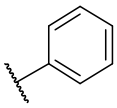
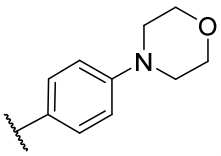
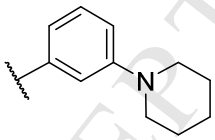
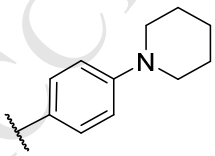
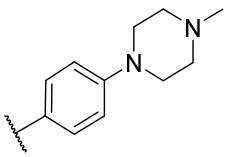
Compound	X	JAK2 IC_{50} (nM)	HDAC1 IC_{50} (nM)	HDAC6 IC_{50} (nM)
61		109 ± 4.7	348 ± 43	6.8 ± 0.9
62		95.5 ± 7.39	142 ± 89	6.1 ± 0.7

Having explored connection of the hydroxamate *via* the pyrimidine ring we next turned to investigation of Design B (Figure 2) with the hydroxamate being connected to the phenyl ring of the biaryl system. A linker of 6 methylenes, generally optimal for HDAC potency, was maintained with variation of the pyrimidine substituent (Table 5). With a simple aniline (**69a**), very good potency was achieved for both HDACs 1 and 6, however the JAK2 IC_{50} dropped to 41 nM. Despite the drop in JAK2 potency, this template is clearly worthy of exploration as a potency dual JAK/HDAC inhibitor. Exploring *para*-substituted analogues with 3 rings known to be potent against JAK2 in the XL019 series led to interesting results. Morpholine **69c** had low nanomolar activity with IC_{50}s of 3.1 nM and 1.2 nM for JAK2 and HDAC6, respectively, whereas potency against HDAC1 was 56 nM. This data indicates that **69c** is one of the most potent HDAC1 inhibitors of the series along with **45b**. Piperidine

215 **69d** generally loses potency, especially against HDAC1, but piperazine **69f** generally loses potency with similar IC_{50}s to **69c**. *Meta*-substituted **69d** was more potent than its *para*-substituted analogue with a similar selectivity profile.

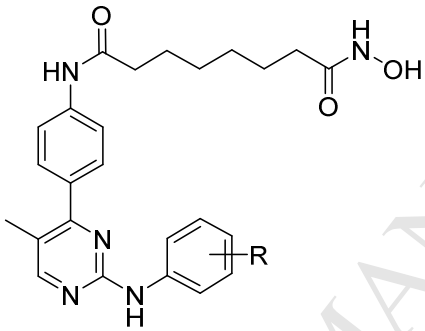
Table 5. *In vitro* enzyme inhibition activities (IC_{50}) of target compounds (**69a,c-f**)

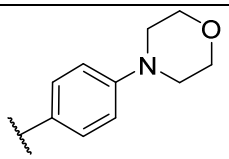
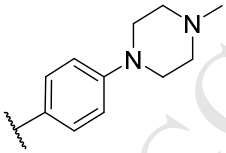


Compound	R	JAK2 IC_{50} (nM)	HDAC1 IC_{50} (nM)	HDAC6 IC_{50} (nM)
69a		41.3	3.8	1.44
69c		3.1 ± 2.4	56 ± 23	1.2 ± 0.1
69d		6.6 ± 0.3	179 ± 27	9.0 ± 0.7
69e		18 ± 0.1	934 ± 9.6	23 ± 4.6
69f		4.6 ± 0.4	77 ± 15	3.1 ± 0.3

220 Methylation of the 5'-position of the pyrimidine (**74a**, Table 6) retained potency against JAK2 and HDAC6 while potency against HDAC1 was reduced about 5 fold (compare **74a** with **69c**). With methylpiperazine derivative **74b** the inhibitory profile did not change upon methylation (compare **74b** with **69f**).

Table 6. Enzyme inhibition activities (IC_{50}) of compounds with 5'methyl pyrimidine substitution



#	R	JAK2 IC_{50} (nM)	HDAC1 IC_{50} (nM)	HDAC6 IC_{50} (nM)
74a		1.8 ± 0.1	289 ± 15	3.7 ± 1.1
74b		4.3 ± 0.3	139 ± 23	2.1 ± 1.2

225

Inhibition of JAK Family Kinases

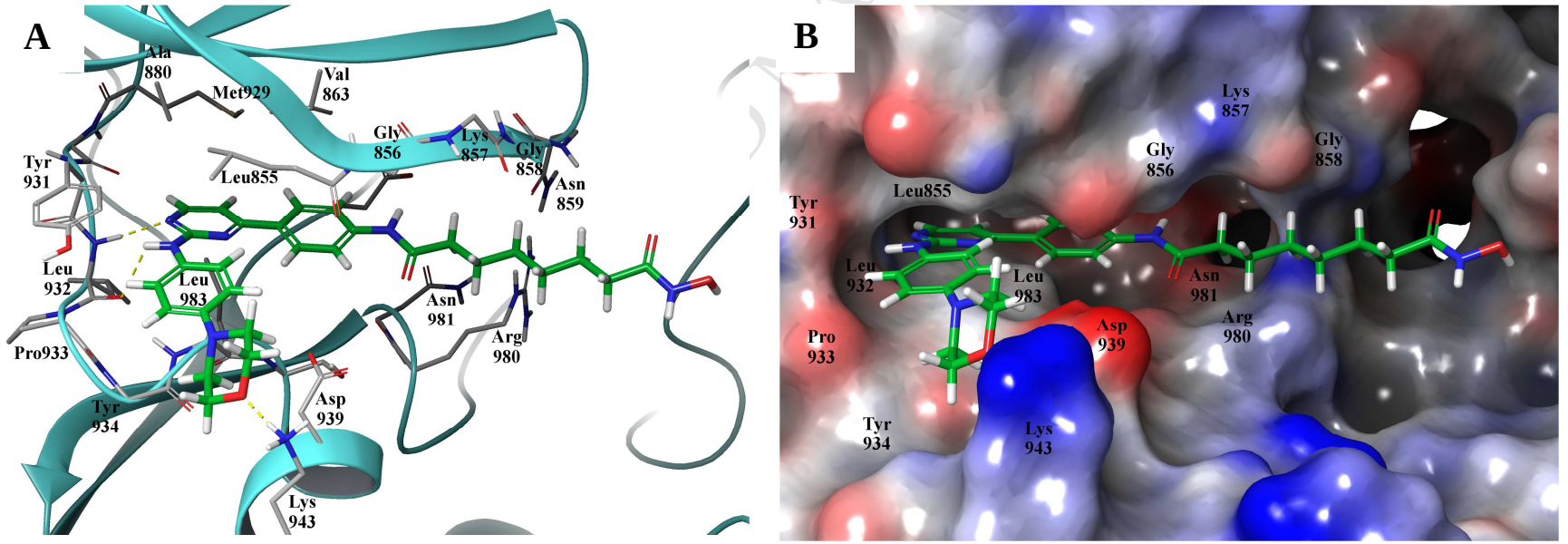
Compound **69c**, with strong JAK2 potency, was selected for assessment against the JAK3 family kinases comprising JAK1, JAK3 and TYK2. **69c** had an IC_{50} of 52.1 Nm against JAK1, a selectivity of 16.8 fold. For JAK3 and TYK2 the values were very similar, around 80 nM, with selectivities of 25-26 fold (Table 7).

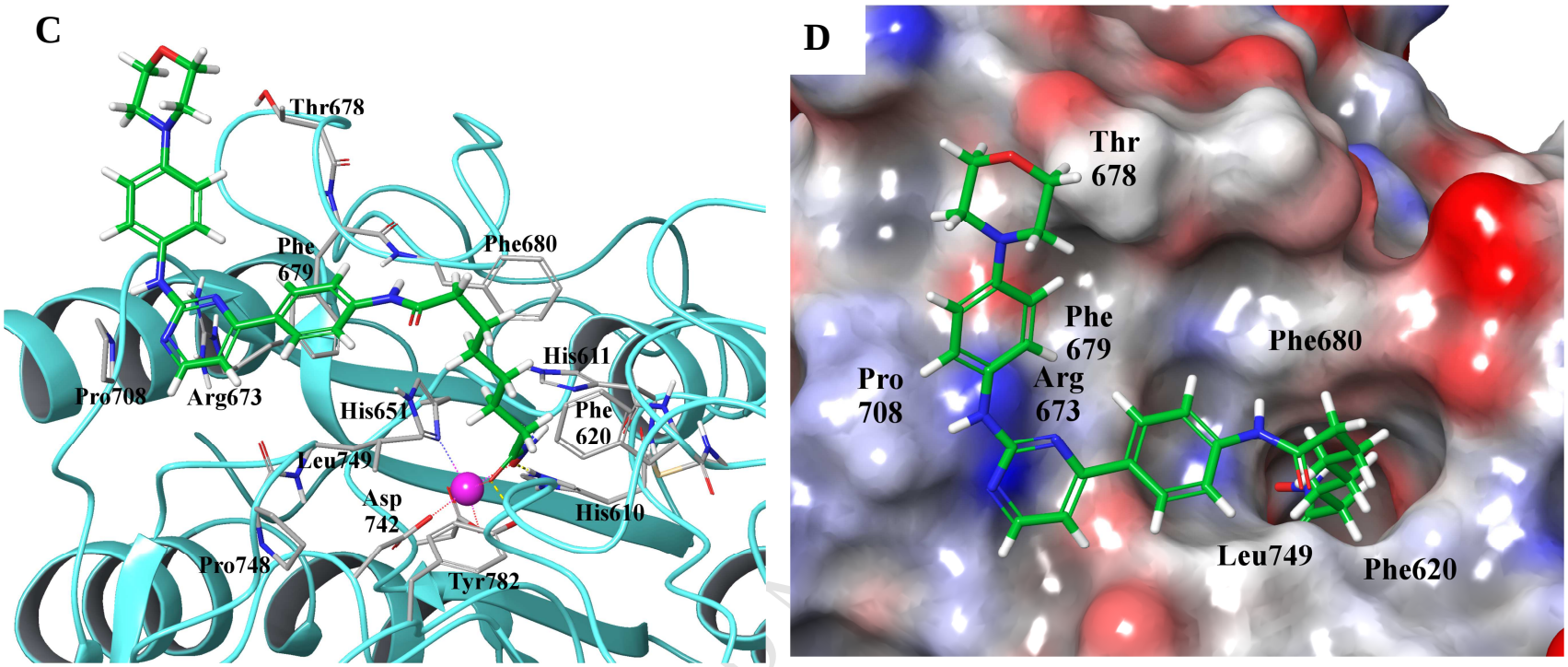
Table 7. Enzyme inhibition activities (IC_{50}) and fold selectivities of compound **69c** against the JAK family kinases

	JAK1	**JAK3**	**TYK2**
IC_{50} (nM)	52.1	80.1	79.4
Selectivity over JAK2	16.8	25.8	25.6

Docking Studies Explain the Observed Potency

When docked into JAK2 **69c** forms 2 hydrogen bonds with the hinge residue Leu932 (Figure 11A) utilising both the carbonyl and NH. Furthermore, the phenyl and elongated side chain is buried in a pocket formed from the glycine rich loop (Figure 11B) with the hydroxamate extending out into solvent. In HDAC6, the hydroxamate of **69c** chelates the Zn at the base of the substrate pocket with the extended elongated chain exiting the binding site at the amide linkage (Figure 11C/D). The hinge-binding motif interacts on the surface of HDAC6 with residues Arg673, Phe679 and Thr678. Taken together, modelling studies indicate that **69c** can bind to both JAK2 and HDAC6 explaining the observed potency.





240 **Figure 11** Compound **69c** docked into JAK2 and the second catalytic domain of HDAC6. Compound **69c** is shown with green carbon in stick representation. JAK2 is shown as a cyan ribbon with selected residues in thin stick with grey carbon (A) and with an electrostatic surface (B). The aminopyrimidine forms 2 hydrogen bonds (yellow dashed lines) to the backbone of the kinase hinge residue Leu932 (NH and C=O). The phenyl ring bearing the long side chain extends under the glycine-rich loop with the hydroxamic acid extending out of the binding site into solvent. HDAC6 is shown as a cyan ribbon (C) and with an electrostatic surface (D). The hydroxamic acid forms a
245 cluster of hydrogen bonds/ionic interactions to the His611, Tyr782 and the catalytic Zinc. Compound **69c** is shown in stick with green carbon and the catalytic Zinc in magenta CPK representation. The hydroxamic acid coordinates to the catalytic Zinc in a bidentate manner. The kinase hinge-binding motif binds on the surface of HDAC6 while the morpholine-aniline may interact with a shallow hydrophobic pocket around Thr678 and Met682.

Inhibition of Cell Proliferation

All dual inhibitors were tested against a panel of 4 solid tumor cell lines selected as representative of hard-to-treat solid tumors while being known to be sensitive to HDAC inhibitors: colorectal HCT116, breast MDA-MB-231 and MCF-7, and prostate PC-3 (Table 8). Reported JAK-HDAC dual inhibitors, **1** and **2**, compared similarly to the pan-HDAC inhibitor **4**, whereas JAK2 inhibiting **3** was less antiproliferative in these cell lines. Of the new compounds from Design A, HCT116 cells were sensitive to some compounds (most potent **45f** had IC_{50} = 0.66 μM) but not others (least active **45c** IC_{50} = 65.6 μM). Compound **45c** was the least active against all cell lines and was also the least active over the three isolated enzymes as well. It may at first appear that the cellular activity is driven by HDAC1 activity (**45c** has the lowest HDAC1 IC_{50} and **45b** is 10 fold less active for HDAC1 then **45a** with a commensurate reduction in cell activity). However, this does not hold for **45d** and **45e** which both have lower HDAC1 activity but good cell activity. This result could be due to a strong JAK2/HDAC6 dual potency which these two compounds from different series have in common. This trend appears to hold for the remainder of the compounds, which have strong JAK2/HDAC6 activity with moderate HDAC1 inhibition. The exception appears to be **51** which has a very weak HDAC1 potency with commensurately lower cellular activity. It is possible that a balance of activity across the enzyme targets is required for broad cell activity.

To assess whether general toxicity contributed to the antiproliferative action of the dual inhibitors we assessed their effects on two normal cells lines, the mouse cell line TAMH[14] and the cardiomyocyte cell line AC10[15] (see Supporting Information, Table S1). As an example, one of the more potent compounds, **45d** has IC_{50} values of 19.5 and 12.5 µM for AC10 and TAMH, respectively, was up to 18 fold less active against AC10 and nearly 12-fold for TAMH. In general virtually all the data against AC10 and TAMH cells indicates higher potency towards cancer cells. These levels of selectivity

suggest that the antiproliferative actions of the compounds is not due a generally toxic mechanism of action.

For compounds from Design B, **69a**, highly potent against HDAC1 and 6 and moderately potent against JAK2, was the most potent across all cell lines with sub-micromolar IC_{50}s. This compound has SI values in AC10 and TAMH cells ranging from 9 fold to over 38 fold indicating that this compound is not simply toxic (see Supporting Information, Table S1). Other compounds in this series were potent in the low micromolar range with significantly positive SI values indicating that dual JAK-HDAC inhibitors have broad cellular efficacy without affecting normal cells in the same concentration range.

Table 8. *In vitro* cell proliferation activities (IC_{50}) of selected compounds in solid tumor cell lines

Compound	IC_{50} (μM) ± SD[a]			
	HCT-116[b]	MDA-MB-231[c]	MCF-7[c]	PC-3[d]
1	2.23 ± 0.63	1.43 ± 0.53	1.47 ± 0.37	1.7 ± 0.3
2	2.32 ± 1.03	0.79 ± 0.18	0.84 ± 0.07	2.41 ± 0.1
3 (XL019)	7.34 ± 0.32	9.67 ± 3.09	27.2 ± 6.87	14.9 ± 3.12
4 (Vorinostat)	2.20 ± 0.73	1.49 ± 0.11	0.81 ± 0.01	1.00 ± 0.15
45a	34.1 ± 6.16	14.6 ± 3.31	26.7 ± 9.45	18.6 ± 4.00
45b	1.15 ± 0.31	2.48 ± 0.74	0.32 ± 0.12	0.34 ± 0.04
45c	65.6 ± 9.92	32.1 ± 0.69	43.4 ± 3.88	47.3 ± 0.83
45d	1.97 ± 0.16	1.54 ± 0.20	1.09 ± 0.56	3.84 ± 0.38
45e	0.79 ± 0.08	1.24 ± 0.18	2.55 ± 0.38	1.66 ± 0.14
45f	0.66 ± 0.11	1.76 ± 0.08	2.42 ± 0.43	1.89 ± 0.15
45g	1.51 ± 0.40	3.61 ± 0.64	1.22 ± 0.09	1.64 ± 0.99
45h	1.15 ± 0.32	1.02 ± 0.07	2.01 ± 0.12	1.51 ± 0.12

45i	0.99 ± 0.04	1.56 ± 0.13	0.81 ± 0.08	1.47 ± 0.30
45j	1.80 ± 0.35	5.33 ± 1.51	5.53 ± 0.45	4.73 ± 1.25
50	1.19 ± 0.14	1.40 ± 0.16	1.71 ± 0.12	2.76 ± 0.06
51	18.4 ± 6.71	11.44 ± 1.12	30.8 ± 3.70	32.8 ± 1.37
52	0.92 ± 0.34	1.06 ± 0.21	2.37 ± 0.48	2.41 ± 0.29
61	0.77 ± 0.11	0.65 ± 0.07	1.32 ± 0.48	1.15 ± 0.30
62	1.69 ± 0.19	3.88 ± 0.26	4.84 ± 0.03	4.09 ± 0.34
69a	0.19 ± 0.1	0.27 ± 0.09	0.33 ± 0.09	0.21 ± 0.03
69c	1.05 ± 0.14	0.99 ± 0.16	0.70 ± 0.18	0.64 ± 0.04
69e	1.07 ± 0.4	2.83 ± 0.29	1.89 ± 0.56	5.58 ± 0.34
69f	0.99± 0.6	0.91 ± 0.03	1.61 ± 0.080	1.02 ± 0.14
74a	2.53 ± 0.7	1.23 ± 0.23	2.47 ± 0.74	1.74 ± 0.23
74b	1.05 ± 0.42	0.97 ± 0.05	1.30 ± 0.26	1.04 ± 0.08

[a]: 10 dose response carried out at least in triplicate; [b]: colorectal cancer; [c]: breast cancer; [d]: prostate cancer.

Both JAK2 and HDAC inhibitors have been found to be effective in hematological cancers and have progressed to the later stages of clinical trials.[16, 17] We therefore selected compounds for testing in a small panel of hematological cell lines, the erythroleukemia cell line HEL92.1.7 (bearing mutant $JAK2^{V617F}$), the acute T-cell leukemia cell line Jurkat and two multiple myeloma cell lines KMS-12-BM and XG-6 (Table 9). These cells were sensitive to reference compounds **1**, **2** and **4** in the 0.5-2.0 µM range. The tested compounds had broadly similar activities to the reference compounds but with some notable exceptions. The multiple myeloma cell line KMS-12-BM was the most sensitive to the compounds, but not the reference compounds, with IC_{50} values as low as 70 and 100 nM for **45h** and **45i**, respectively. Interestingly, even **51** was quite potent against KMS-12-BM cells despite being of low potency against the other cell lines. On the other hand, IL-6 driven XG-6 cells were somewhat

insensitive to the compounds with IC_{50} values ranging from 1.07 to 10.2 µM. Surprisingly, *meta*-substituted amides **45a** and **45b** were not potent at all at the top concentration tested of 10 µM whereas the *para*-substituted amide **45d** had IC_{50}s in the low micromolar range for 3 cell lines tested. We surmised that this could be due to unfavourable properties of the *meta*-substituted amide, perhaps poor permeability. This was not investigated further since good activity was noted with the ether linked series of compounds. HDAC1 potency did not appear to be a driver of activity in all cases, for example **45d** and **45e**, with HDAC1 IC_{50}s of around 1 µM were quite potent in all cell lines whereas **45g**, with a similar level of HDAC1 potency was only active against KMS-12-BM cells. Similar to the pattern of activity against solid tumor cells, it appears that a good balance of activity between the JAK and HDAC targets is required for optimal inhibition of proliferation.

When the IC_{50}s against the normal cells AC10 and TAMH were assessed (see Supporting Information, Table S2) some very high SI values were apparent for the KMS-12-BM cell line particularly for the very potent compound **45h**. Other SI values for other cell lines were also significantly positive. Compound **45g** was notable due to its lack of sensitivity in AC10 cells but its lower IC_{50} value for TAMH resulted in less favourable SI values. In general, this group of JAK-HDAC dual inhibitors have good potency in hematological cell lines with many examples of lower toxicity as measured in the AC10 and TAMH cell lines.

Table 9. *In vitro* cell proliferation activities (IC_{50}) of selected compounds in hematological cell lines

Compound	IC_{50} (μM) ± SD[a]			
	HEL92.1.7[b]	Jurkat[c]	KMS-12-BM[d]	XG-6[d]
1	0.94 ± 0.22	1.19 ± 0.22	2.11 ± 0.91	-
2	1.32 ± 0.94	0.47 ± 0.15	2.02 ± 1.07	2.01 ± 0.99
4 (Vorinostat)	0.49 ± 0.10	0.59 ± 0.17	0.94 ± 0.24	-
45a	>10	>10	-	-
45b	>10	>10	-	-
45d	2.53 ± 0.18	-	1.16 ± 0.48	3.17 ± 0.37
45e	1.83	1.19	0.20 ± 0.09	3.70 ± 2.30
45f	0.97	1.06	0.35 ± 0.15	4.69 ± 4.57
45g	3.34	>10	0.68 ± 0.34	7.22 ± 6.46
45h	1.36	1.03	**0.07 ± 0.05**	1.07 ± 0.50
45i	1.51	1.77	**0.10 ± 0.04**	2.08 ± 0.67
45j	3.92	3.48	1.96 ± 0.50	10.2 ± 4.91
51	8.96	9.99	0.54 ± 0.19	8.30 ± 1.85

[a]: 10 dose response carried out at least in triplicate; [b]: Erythroleukemia ($JAK2^{V617F}$); [c]: acute T-cell leukemia; [d]: multiple myeloma.

Assessment of Cellular Mechanism of Action

Increase in levels of acetylated histone 3 (Ac-H3) is indictive of HDAC1, 2 or 3 blockade in cells (Figure 12).[18] Similarly, acetylated tubulin (Ac-Tub) is increased upon inhibition of HDAC6.[19] A selected compound, **45d**, was studied in HEL92.1.6 cells at a range of concentrations. Clear increases in Ac-Tub were observed at 1 µM and mild but visible increases in Ac-H3 were discernible compared to the vehicle control. This data indicated that both pathways are being inhibited with stronger HDAC6

activity as suggested by the enzyme inhibition profile of this compound. The JAK2 pathway was assessed in Hela cells following incubation of **45d** for 1 hour. A decrease in phosphorylated STAT5 was observed at the top two concentrations tested. Although weak, much more sensitive effects were seen in the increase of phosphorylated JAK2 (p-JAK2) which is known to be correlated with JAK2 inhibition.[20] This is supported by effects with the JAK2 inhibitor Ruxolitinib at a 20 fold lower concentration, in agreement with the differences in JAK2 enzyme inhibition between these two compounds (see Supporting Information Figure S1).

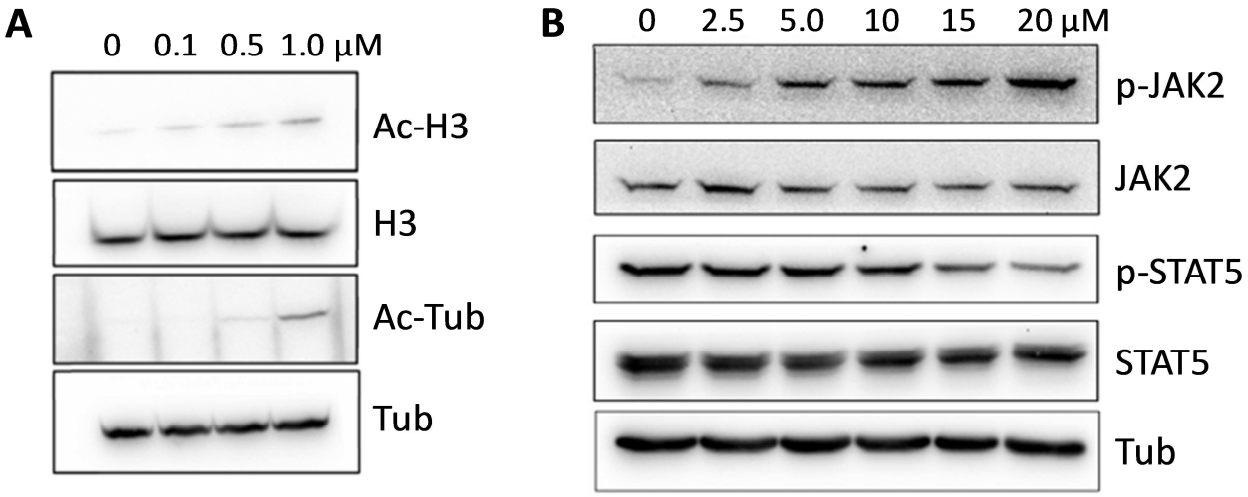


Figure 12 Western blot analysis of HDAC and JAK2 pathway proteins upon treatment with compound **45d**. (A) Treatment of HEL92.1.7 cells with increasing doses of **45d** indicates an increase in acetylated H3 (Ac-H3) and acetylated tubulin (Ac-Tub). (B) Treatment of Hela cells with increasing doses of **45d** indicates an increase in phosphorylated JAK2 (p-JAK2) and a decrease in phosphorylated STAT5 (p-STAT5).

We further wanted to understand the mechanism of cell death and selected compounds **69c** and **69f** for apoptosis[21] studies in a panel of panel of 4 solid tumor cell lines PC-3 (prostate), HCT-116 (colorectal), MCF-7 and MDA-MB-231 (breast cancer) (Figure 13). Annexin V, a Ca^{2+}-dependent phospholipid-binding protein, has high affinity for membrane phosphatidylserine (PS), and fluorochrome-labeled Annexin V can be used for the detection of exposed PS in the early stages of

apoptosis using flow cytometry.[22] In the later stages of apoptosis the cell membrane begins to break down and propidium iodide (PI) can enter hence indicating a readout for cells which are nearly dead. We studied **69c** and **69f** at their IC_{50} concentrations for cell proliferation in each cell line (see Table 8). Compound **69c** showed a significant increase in Annexin V stained cells (annexin V+PI−) for all cell lines (30-45% range compared to 1-2% for untreated controls) whereas compound **69f** had a very strong response in PC-3 cells (73%), a good response in MDA-MB-231 cells (39%) but only a small effect in HCT-116 and MCF-7 cells. In the annexin V+PI+ quadrant, indicating cells that are nearly dead, **69c** treated cells had significant populations in all cell lines with MDA-MB-231 being the most pronounced. A similar result was seen for **69f** except for MCF-7 where the result was not significant. Taken together, this data on 4 cell lines and two compounds supports apoptosis as being the mechanism of cell death.

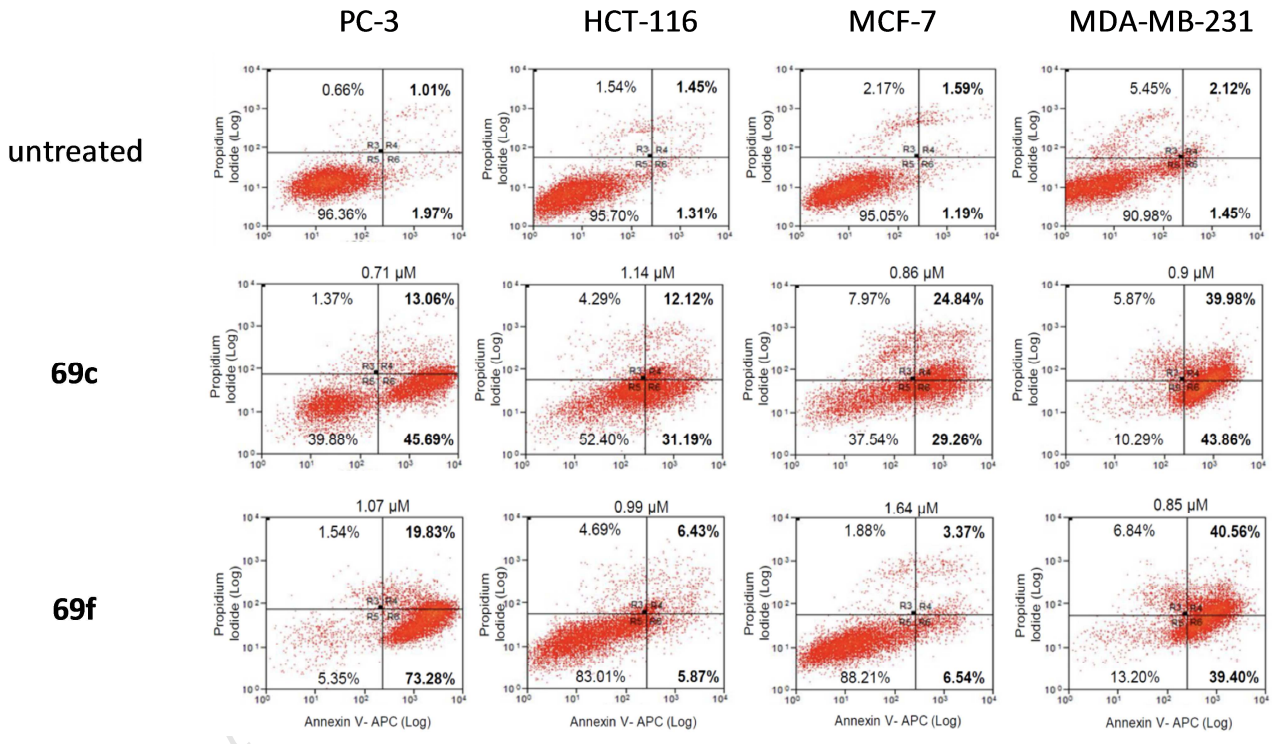


Figure 13 Analysis of apoptosis mechanism in a panel of 4 solid tumor cell lines PC-3 (prostate), HCT-116 (colorectal), MCF-7 and MDA-MB-231 (breast cancer). Annexin V FITC vs propidium iodide plots from the gated cells show the populations corresponding to viable and non-apoptotic (annexin V−PI−), early (annexin V+PI−), and late (annexin V+PI+) apoptotic cells following 72 h

treatment of compounds **69c** and **69f** at their corresponding IC_{50}s in the respective cell lines (see Table 8).

Concluding Remarks

Two series of dual JAK/HDAC inhibitors were designed utilizing a merging strategy to combine the core JAK-inhibiting template of **3** with the long chain hydroxamate of **2**. Synthesis and testing of a range of compounds based on the two designs produced a range of highly potent JAK2 inhibitors with very potent HDAC6 activity. Depending on the specific design, HDAC1 activity could be varied, suggesting control is possible over HDAC selectivity while maintaining JAK2 activity. An example compound indicated that good JAK family selectivity was retained in the new compounds. Broad potency against a panel of solid tumor cell lines and also against a panel of hematological cell lines without potency against two normal cell lines indicated the utility of the compounds. Confirmation that both the JAK-STAT and HDAC pathways were affected in cells was demonstrated with a selected compound. This work expands on previous work demonstrating the range of control that is possible with the design of a multiple ligand which inhibits two different classes of enzyme.

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EXPERIMENTAL SECTION

All reagents purchased from commercial sources (Sigma-Aldrich, Combi-blocks, Merck and Alpha chemical) were of the highest purity grade available and were used without further purification. Commercially available AR grade solvents or anhydrous solvents packed in resealable bottles were used as received. Only ethyl acetate was distilled before use. All reaction temperatures stated in the procedures are external bath temperatures. Non-aqueous reactions were performed under a positive pressure of nitrogen in oven-dried glassware. Yields refer to chromatographically and spectroscopically homogeneous materials, unless otherwise stated. Reaction progress was monitored by analytical thin layer chromatography (TLC) with 0.25 mm Merck pre-coated silica gel plates (60F-254) using UV light (254 nm) as visualizing agent, or potassium permanganate solutions as a developing stain. All products were purified by flash chromatography using silica gel (Merck 60-200 mesh, purchased from SiliCycle or Merck) as the stationary phase or purified by crystallization. The structures of synthesized compounds were verified by ^{1}H NMR, ^{13}C NMR, and mass spectrometry. ^{1}H (400 MHz) and ^{13}C (101 MHz) NMR spectra were recorded in deuterated solvents (purchased from Cambridge Isotopes and used without further purification) on a Bruker Avance III 400 (Ultrashield Plus) spectrometer. Chemical shifts are assigned and reported in ppm (δ); corresponding to reference standard solvents $CDCl_3$ (7.26 ppm), DMSO-d6 (2.50 ppm) and CD_3OD (3.31 ppm). Multiplicities are reported as singlet (s), doublet (d), triplet (t), multiplet (m), and broad singlet (bs). Coupling constants (*J*) are reported in Hz. Mass spectra were recorded on a Liquid Chromatography-Mass Spectrometry (LCMS) system (Agilent 1260 Infinity quaternary LC system with 150mm column and Agilent 6130

quadrupole mass spectrometer). The unit of molecular weight reported is g/mol. Analytical HPLC purity was determined using an Agilent technologies 1200 series instrument with Zorbax SB-C18 4.6 x 250 mm 5 μm column at 254 nM, using gradient MeCN/H_2O 5:95 to 95:5 over 20 minutes. Preparative HPLC was performed using a Gilson GX281 with 21.2 x 250 mm 7 μm, Hypersil^{TM} BDS C18 column to purify polar compounds. All purity profiles were recorded with gradient MeCN/H_2O 5:95 to 95:5 at 0.5 mL per minute over 20 minutes. All the structures and their IUPAC names have been generated using ChemBioDraw Ultra 12.0.

N-(4-(2-chloropyrimidin-4-yl)phenyl)acetamide (**5**)

A RBF was charged with 2, 4-dichloropyrimindine (784 mg, 5.2 mmol, 1 equiv.), compound **48** (1.5 g, 5.74 mmol, 1.1 equiv.), $Pd(PPh_3)_4$ (250 mg, 0.226 mmol,0.05 equiv.) and sodium carbonate (991 mg, 9.36 mmol, 1.8 equiv.) MeCN: H_2O (9 : 1, 40 mL) were added into the RBF and the reaction mixture was degassed under N_2, stirred and refluxed overnight under N_2. The reaction was cooled, and the reaction mixture was concentrated under vacuum. EA and water were added for extraction. By collecting the organic layer and evaporating solvent, a light yellow crude product was obtained, which was purified using flash chromatography (elution system - EA/Hexane = 1 : 1 to MeOH/EA = 2 : 98) to give **5** a light yellow solid (1.25 g, 97%).

R_f = 0.43 (EA/Hexane = 1 : 1); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.27 (s, 1H), 8.75 (d, *J* = 5.4 Hz, 1H), 8.16 (d, *J* = 8.9 Hz, 2H), 8.06 (d, *J* = 5.4 Hz, 1H), 7.77 (d, *J* = 8.9 Hz, 2H), 2.09 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.33, 166.20, 161.29, 160.98, 143.40, 129.14, 128.72, 119.39, 115.70, 24.64; **MS** (ESI) 248.0 $[M+H]^+$.

N-(4-(2-(phenylamino)pyrimidin-4-yl)phenyl)acetamide (**6**)

The general procedure according to **49a** was followed using compound **5** (100 mg, 0.4 mmol, 1 equiv.), aniline (70 μL, 0.77 mmol, 1.9 equiv.) and PTSA (78 mg, 0.41 mmol, 1 equiv.) in a 0.5-2 mL microwave in *n*-BuOH (1.5 mL). The reaction vial was capped and heated on an oil bath for 1 h at 100 °C. The reaction progress was monitored by LC-MS. The reaction mixture was filtered and the residue washed with methanol and diethyl ether, and the final product was dried under vacuum to give **6** as a light yellow solid (34 mg, 28%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.19 (s), 9.59 (s, 1H), 8.50 (d, *J* = 5.2 Hz, 1H), 8.12 (d, *J* = 8.8 Hz, 2H), 7.83 (dd, *J* = 8.6, 1.0 Hz, 2H), 7.75 (d, *J* = 8.8 Hz, 2H), 7.39 – 7.26 (m, 3H), 6.96 (t, *J* = 7.3 Hz, 1H), 2.09 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.24, 163.57, 160.60, 159.21, 142.24, 141.09, 131.48, 128.98,128.05, 125.97, 121.78, 119.34, 107.80, 24.57; **MS** (ESI) 305.1 $[M+H]^+$.

N-(4-(2-(benzylamino)pyrimidin-4-yl)phenyl)acetamide (**7**)

The general procedure was followed according to **49a**. Compound **5** (50 mg, 0.2 mmol, 1 equiv.), benzylamine (42 μL, 0.39 mmol, 1.9 equiv.), and PTSA (31 mg, 0.16 mmol, 0.8 equiv.) in *n*-BuOH (2 mL) were used. The reaction was heated on an oil bath overnight at 120°C. The reaction was monitored using TLC, and purified using flash chromatography (elution system- EA/Hexane from 7: 3 to 100% EA) to give **7** as a white solid (65.5 mg, 98%).

^{1}H NMR(400 MHz, $CDCl_3$) δ 10.14 (s, 1H), 8.29 (d, *J* = 5.2 Hz, 1H), 8.03 (d, *J* = 8.8 Hz, 2H), 7.73 – 7.62 (m, 3H), 7.36 (d, *J* = 7.2 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 2H), 7.20 (t, *J* = 7.2 Hz, 1H, H-17), 7.09 (d, *J* = 5.2 Hz, 1H),4.57 (d, *J* = 6.2 Hz, 2H), 2.07 (s, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.04, 162.86,

159.20, 141.98, 141.17, 131.74, 128.59, 127.80, 127.62, 126.91, 119.11, 105.62, 44.54, 24.56; **MS** (ESI) 319.1 $[M+H]^+$.

N-(4-(2-(phenethylamino)pyrimidin-4-yl)phenyl)acetamide (**8**)

The general procedure was followed according to **49a** using compound **5** (50 mg, 0.2 mmol, 1 equiv.) in *n*-BuOH (2 mL), 2-phenylethanamine (47 μL, 0.37 mmol, 1.9 equiv.) and PTSA (31 mg, 0.16 mmol, 0.8 equiv.). The reaction was heated on an oil bath overnight at 100 °C. On completion, the reaction was monitored by LCMS, and purified by flash chromatography (elution system- EA/Hex from 7:3 to 100% EA). The obtained compound was further purified by prep HPLC (elution system - H_2O/MeCN = 95 : 5 to 5 : 95) to give **8** as a white solid (25 mg, 38%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.23 (s, 1H), 8.34 (d, J = 5.6 Hz, 1H),8.11 (s, 2H), 7.74 (d, J = 8.6 Hz, 3H), 7.35 – 7.26 (m, 4H), 7.24 – 7.13 (m, 2H), 3.63 (s, 2H), 2.91 (t, J = 7.3 Hz, 2H), 2.09 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.27, 158.97, 158.63, 142.92, 139.92, 130.80, 129.19, 128.82, 128.59, 126.58, 119.22, 105.40, 42.94, 35.35, 24.60; **MS** (ESI) 333.2 $[M+H]^+$.

Tert-butyl (3-aminophenyl)carbamate (**10**)

To a solution of 1,3-phenylenediamine (**9**) (5 g, 46 mmol, 4 equiv.), in DCM (30 mL) on ice, was added a solution of Boc_2O (2.4 mL, 11.5 mmol, 1 equiv.) in DCM (10 mL) dropwise. The mixture was stirred overnight at room temperature. The solvent was evaporated and the residue was purified by column chromatography using silica gel as stationary phase (elution system - EA/Hexane = 4 : 6) to give the desired product as a light pink solid (2.7 g, 99%).

R_f = 0.26 (EA/Hexane = 2 : 3); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.03 (t, *J* = 8.0 Hz, 1H), 6.97 (s, 1H), 6.54 (ddd, *J* = 8.0, 2.0, 0.8 Hz, 1H), 6.40 (s, 1H), 6.36 (ddd, *J* = 7.9, 2.2, 0.8 Hz, 1H), 1.51 (s, 9H); **MS** (ESI) 209.1 $[M+H]^+$.

Methyl 6-((3-((tert-butoxycarbonyl)amino)phenyl)amino)-6-oxohexanoate (**13**)

To a solution of 6-methoxy-6-oxooctanoic acid (**11**) (643 μL, 4.01 mmol, 1.4 equiv.) and *N*-methyl morpholine (595 μL, 5.41 mmol, 1.9 equiv.) in 45 mL DCM was added ethyl chloroformate (413 mL, 4.34 mmol, 1.5 equiv.). The mixture was stirred for 15 min on ice. Compound **10** (600 mg, 2.88 mmol, 1 equiv.) was added to the solution and the reaction mixture was stirred at room temperature overnight. Reaction progress was checked by TLC under UV and also stained with $KMnO_4$. Crude compound was obtained after evaporation and was purified by flash chromatography (elution system - EA/Hexane = 4 : 6 to 8 : 2) to give **13** as a light pink solid (809 mg, 80%).

R_f = 0.43 (EA/Hexane = 1 : 1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.59 (s, 2H), 7.19 (d, *J* = 8.3 Hz, 1H), 7.19 (d, *J* = 8.3 Hz, 1H), 7.12 (t, *J* = 8.0 Hz, 1H), 7.00 (d, *J* = 7.7 Hz, 1H), 6.61 (s, 1H), 2.28 (q, *J* = 6.9 Hz, 4H), 1.73 – 1.57 (m, 4H), 1.43 (s, 10H), 1.43 (s, 10H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 174.05, 171.00, 152.77, 138.99, 138.68, 129.41, 114.38, 114.19, 109.90, 80.58, 51.59, 37.15, 33.65, 28.32, 24.90, 24.31; **MS** (ESI) 351.2 $[M+H]^+$.

Methyl 8-((3-((tert-butoxycarbonyl)amino)phenyl)amino)-8-oxooctanoate (**14**)

The title compound was synthesized following a procedure similar to **13** by using 8-methoxy-8-oxooctanoic acid (**12**) (1.62 mL, 9.08 mmol, 2 equiv.), *N*-methyl morpholine (1.25 mL, 11.37 mmol, 2.5 equiv.), ethyl chloroformate (0.86 mL, 9 mmol, 2 equiv.), and compound **10** (945 mg, 4.54 mmol, 1 equiv.) in 20 mL DCM to give **14** as a light pink solid (1.6 g, quant).

R_f = 0.58 (EA/Hexane = 1 : 1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.52 (s, 1H), 7.25 (d, *J* = 5.8 Hz, 1H), 7.18 (t, *J* = 8.0 Hz, 1H), 7.03 (d, *J* = 7.7 Hz, 1H), 6.70 (s, 1H), 3.65 (s, 3H), 2.31 (dd, *J* = 16.0, 8.6 Hz, 4H), 1.73 – 1.57 (m, 4H), 1.49 (s, 9H), 1.35 – 1.33 (m, 4H); **MS** (ESI) 379.2 $[M+H]^+$.

Methyl 6-((3-aminophenyl)amino)-6-oxohexanoate (**15**)

To a solution of compound **13** (400 mg, 1.14 mmol, 1 equiv.) in EA/DCM (1 : 1, 40 mL), concentrated sulfuric acid (186 μL, 3.42 mmol, 3 equiv.) was added dropwise and stirred at ambient temperature. The end point of the reaction was monitored by TLC. The reaction mixture was neutralized by addition of $NaHCO_3$ solution and the aqueous phase was extracted with DCM three times. The combined organic extract was washed with brine and dried with anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to obtain **15** without further purification as a black solid (263 mg, 92%); **MS** (ESI) 279.2 $[M+H]^+$.

Methyl 8-((3-aminophenyl)amino)-8-oxooctanoate (**16**)

The title compound was synthesized following a procedure similar to **15** by using compound **14** (1.56 g, 4.12 mmol, 1 equiv.), and concentrated sulfuric acid (0.66 mL, 12.36 mmol, 3 equiv.) in EA (30 mL). Compound **16** was isolated as a mixture of methyl:ethyl esters (4:3) as a light pink solid (1.06 g, 91%).

R_f = 0.48 (EA/Hexane = 1 : 1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.31 (s, 1H), 7.17 (s, 1H), 7.05 (t, *J* = 8.0 Hz, 1H), 6.65 (d, *J* = 7.2 Hz, 1H), 6.40 (dd, *J* = 7.9, 1.4 Hz, 1H), 4.11 (q, *J* = 7.1 Hz, 1H), 3.66 (s, 3H), 2.30 (t, *J* = 7.4 Hz, 4H), 1.70 (dt, *J* = 14.9, 7.4 Hz, 2H), 1.66 – 1.56 (m, 3H), 1.40 – 1.29 (m, 6H), 1.24 (t, *J* = 7.1 Hz, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 174.26, 171.28, 147.25, 139.05, 129.64,

110.92, 109.60, 106.55, 60.21, 60.24, 51.48, 34.11 (dd, *J* = 28.0, 3.8 Hz), 28.74, 25.34, 24.69, 14.24; **MS** (ESI) 279.0 $[M+H]^+$.

Tert-butyl (4-aminophenyl)carbamate (**18**)

The title compound was synthesized following a procedure similar to **10** using 1, 4-phenylenediamine (**17**) (6 g, 55 mmol, 4 equiv.) and Boc_2O (3.2 mL, 13.8 mmol, 1 equiv.) to give **18** as a light pink solid (2.78 g, 97%).

R_f = 0.27 (EA/Hexane = 2 : 3); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.11 (d, *J* = 8.0 Hz, 2H), 6.65 – 6.57 (m, 2H), 6.38 (s, 1H), 3.54 (s, 2H), 1.49 (s, 9H) ; **MS** (ESI) 209.1 $[M+H]^+$.

Methyl 6-((4-((tert-butoxycarbonyl)amino)phenyl)amino)-6-oxohexanoate (**19**)

The title compound was synthesized following a procedure similar to **13** by using 6-methoxy-6-oxooctanoic acid (**11**) (5.15 μL, 3.60 mmol, 1.25 equiv.), *N*-methyl morpholine (475 μL, 4.32 mmol, 1.5 equiv.), ethyl chloroformate (330 mL, 3.46 mmol, 1.2 equiv.), and compound **18** (600 mg, 2.88 mmol, 1 equiv.) in 40 mL DCM. Compound **19** was obtained as a light pink solid (933 mg, 92%).

R_f = 0.31 (EA/Hexane = 1 : 1); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 9.51 (s, 1H), 7.25 (d, *J* = 8.7 Hz, 2H), 6.57 (d, *J* = 8.7 Hz, 2H), 3.58 (s, 3H), 2.32 (t, *J* = 6.8 Hz, 2H), 2.23 (t, *J* = 6.8 Hz, 2H), 1.63 – 1.47 (m, 5H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.72, 170.54, 143.05, 130.17, 121.26, 115.31, 51.65, 36.29, 33.54, 25.19, 24.57; **MS** (ESI) 351.4 $[M+H]^+$.

Methyl 8-((4-((tert-butoxycarbonyl)amino)phenyl)amino)-8-oxooctanoate (**20**)

The title compound was synthesized following a procedure similar to **19** by using 8-methoxy-8-oxooctanoic acid (**12**) (1.4 mL, 7.6 mmol, 1 equiv.), *N*-methyl morpholine (1 mL, 9.1 mmol, 1.2 equiv.) ethyl chloroformate (0.69 mL, 7.2 mmol, 1 equiv.) and **18** (1.5 g, 7.2 mmol, 0.95 equiv.) in 20 mL DCM to give **20** as a light pink solid (1.87 g, 63%).

R_f = 0.5 (EA/Hexane = 2 : 3).

Methyl 6-((4-aminophenyl)amino)-6-oxohexanoate (**21**)

The title compound was synthesized following a procedure similar to **15** by using compound **19** (400 mg, 1.14 mmol, 1 equiv.), and concentrated sulfuric acid (182 μL, 3.42 mmol, 3 equiv.) in EA/DCM (1 : 1, 40 mL) to give **21** as a light pink solid (245 mg, 86%).

R_f = 0.15 (EA/Hexane = 1 : 1); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 9.53 (s, 1H), 6.92 (t, *J* = 1.9 Hz, 1H), 6.88 (t, *J* = 8.0 Hz, 1H), 6.66 (dd, *J* = 8.0, 1.4 Hz, 1H), 6.23 (ddd, *J* = 8.0, 2.1, 0.8 Hz, 1H), 4.99 (s, 2H), 3.58 (s, 3H), 2.33 (t, *J* = 7.0 Hz, 2H), 2.26 (t, *J* = 6.9 Hz, 2H), 1.54 (m, 4H); **MS** (ESI) 251.1 $[M+H]^+$.

Methyl 8-((4-aminophenyl)amino)-8-oxooctanoate (**22**)

The title compound was synthesized following a procedure similar to **21** using **20** (1.78 g, 4.7 mmol, 1 equiv.) and concentrated sulfuric acid (0.77 ml, 14.11 mmol, 3 equiv.) in EA (30 mL). Compound **22** was isolated as a mixture of methyl:ethyl esters (2:1) as a light pink solid (768 mg, 59%).

R_f = 0.48 (EA/Hexane = 1 : 1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.38 (s, 1H, H-4), 7.17 (d, *J* = 8.7 Hz, 2H), 6.57 (d, *J* = 8.7 Hz, 2H), 4.08 (dd, *J* = 14.2, 7.1 Hz, 1H), 3.57 (s, 3H), 2.21 (q, *J* = 7.5 Hz, 4H),

1.63 – 1.57 (m, 2H), 1.56 – 1.45 (m, 2H), 1.26 (dd, *J* = 6.9, 3.3 Hz, 4H,, 1.19 (t, *J* = 7.1 Hz, 1H); **MS** (ESI) 279.1 $[M+H]^+$.

Methyl 6-((3-((4-(4-acetamidopheny l)pyrimidin-2-yl)amino)phenyl)amino)-6-oxohexanoate (**42a**)

41a (220 mg, 0.88 mmol, 1.1 equiv.) was added to the solution of compound **5** (198 mg, 0.80 mmol) in 3 mL *n*-BuOH in a microwave vial. PTSA (91 mg, 0.48 mmol, 0.6 equiv.) was added and the reaction vial was capped and heated on an oil bath for 2 hr at 100 °C. On completion, the reaction was cooled and analysed by LCMS. The reaction mixture was evaporated under vacuum and the obtained crude compound was purified by flash chromatography (elution system - MeOH/DCM = 3 : 97 to 5 : 95) to give **42a** as a mixture of methyl : *n*-butyl esters (1:1) as a brown solid (188 mg, 49%).

^{1}H NMR (400 MHz, DMF) δ 10.16 (s, 1H), 9.82 (s, 1H), 9.56 (s, 1H), 8.49 (d, *J* = 5.2 Hz, 1H), 8.28 – 8.12 (m, 3H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 5.3 Hz, 1H), 7.20 (t, *J* = 7.9 Hz, 1H), 7.16 (d, *J* = 8.2 Hz, 1H), 4.00 (t, *J* = 6.6 Hz, 1H), 3.58 (s, 1H), 2.46 – 2.24 (m, 4H), 2.09 (s, 3H), 1.62 (dt, *J* = 9.7, 5.3 Hz, 4H), 1.54 (dd, *J* = 14.4, 7.4 Hz, 1H), 1.31 (d, *J* = 7.5 Hz, 1H), 0.87 (t, *J* = 7.4 Hz, 1H); **MS** (ESI) 462.2 (Me ester) and 504.3 (*n*-Bu ester) $[M+H]^+$.

Methyl 8-((3((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenyl)amino)-8-oxooctanoate (**42b**)

The title compound was synthesized following a procedure similar to **42a** using compound **5** (100 mg, 0.40 mmol, 1 equiv.), **41b** (344 mg, 1.22mmol, 1.5 equiv), and PSTA (123 mg, 0.65 mmol, 0.8 equiv.) in 2 mL *n*-BuOH. Compound **42b** was isolated as a mixture of methyl : *n*-butyl esters (1:4) as a light brown solid (94 mg, 21%).

R_f = 0.32 (EA/DCM/MeOH = 1 : 1 : 1); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.17 (s, 1H), 9.81 (s, 1H), 9.56 (s, 1H), 8.48 (d, *J* = 5.3 Hz, 1H), 8.18 (d, *J* = 8.8 Hz, 3H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.41 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.34 (d, *J* = 5.3 Hz, 1H), 7.19 (dd, *J* = 14.6, 6.8 Hz, 2H), 3.99 (t, *J* = 6.6 Hz, 2H), 3.57 (s, 1H), 2.29 (dt, *J* = 14.4, 7.3 Hz, 4H), 2.08 (s, 3H), 1.66 – 1.58 (m, 2H), 1.51 (dd, *J* = 14.6, 6.7 Hz, 4H), 1.37 – 1.24 (m, 6H), 0.87 (t, *J* = 7.4 Hz, 3H); **MS** (ESI) 490.2 $[M+H]^+$ (Methyl-), 532.3 $[M+H]^+$ (Butyl-); **MS** (ESI) 490.3 (Me ester) and 532.3 (*n*-Bu ester) $[M+H]^+$.

Methyl 6-((4-((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenyl)amino)-6-oxohexanoate (**42c**)

The title compound was synthesized following a procedure similar to **42a** using compound **5** (100 mg, 0.404 mmol, 1 equiv.), **41c** (111 mg, 0. 44 mmol, 1.1 equiv), and PSTA (46 mg, 0. 24 mmol, 0.6 equiv.) in 2 mL *n*-BuOH. Compound **42c** was isolated as a mixture of methyl : *n*-butyl esters (1:1) as a light brown solid (59 mg, 30%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.19 (s, 1H), 9.76 (s, 1H), 9.49 (s, 1H), 8.47 (d, *J* = 5.2 Hz, 1H), 8.12 (d, *J* = 8.8 Hz, 2H), 7.75 (d, *J* = 8.7 Hz, 2H), 7.72 (d, *J* = 9.0 Hz, 2H), 7.52 (d, *J* = 8.9 Hz, 2H), 7.31 (d, *J* = 5.3 Hz, 1H), 4.02 (t, *J* = 6.6 Hz, 1H), 3.60 (s, 2H), 2.33 (dt, *J* = 14.3, 6.8 Hz, 5H), 2.10 (s, 3H), 1.67 – 1.48 (m, 5H), 1.33 (dq, *J* = 14.5, 7.4 Hz, 1H), 0.89 (t, *J* = 7.4 Hz, 2H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.72, 173.30, 170.96, 169.14, 163.56, 160.62, 159.17, 142.23, 136.46, 133.74, 131.53, 128.02, 120.05, 119.74, 119.30, 107.49, 63.89, 51.67, 36.39, 33.79, 33.55, 30.68, 25.12, 24.64, 24.58, 19.09, 14.00; **MS** (ESI) 462.2 (Me ester) and 504.3 (*n*-Bu ester) $[M+H]^+$.

Methyl 8-((4-((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenyl)amino)-8 oxooctanoate (**42d**)

41d (140 mg, 0.5 mmol, 1.25 equiv.) was added to the solution of compound **5** (100 mg, 0.4 mmol) in 2 mL *n*-BuOH in a microwave vial. PTSA (78 mg, 0.41 mmol, 1 equiv.) was added and the reaction vial was capped and heated on an oil bath for 2 hr at 100 °C. On completion, the reaction was cooled and analysed by LCMS. The reaction mixture was evaporated under vacuum and the obtained crude compound was purified by flash chromatography (elution system - EA/Hexane = 2 : 3 to 4 : 1) to give **42d** as a mixture of methyl : *n*-butyl esters (1:3) as a light brown solid (53 mg, 28%).

R_f = 0.33 (EA/Hexane = 4 : 1); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.18 (s, 1H), 9.73 (s, 1H), 9.49 (s, 1H), 8.46 (d, *J* = 5.2 Hz, 1H), 8.11 (d, *J* = 8.8 Hz, 2H), 7.73 (dd, *J* = 12.5, 8.9 Hz, 4H), 7.51 (d, *J* = 9.0 Hz, 2H), 7.30 (d, *J* = 5.3 Hz, 1H), 4.00 (t, *J* = 6.6 Hz, 2H), 3.58 (s, 1H), 2.35 – 2.22 (m, 4H), 2.09 (s, 3H), 1.70 – 1.43 (m, 4H), 1.31 (dd, *J* = 13.9, 6.3 Hz, 4H), 0.88 (t, *J* = 7.4 Hz, 3H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.40, 171.19, 169.14, 163.54, 160.62, 159.16, 142.24, 136.42, 133.79, 131.52, 128.02, 120.03, 119.71, 119.28, 107.47, 63.82, 36.72, 33.96, 30.68, 28.81, 28.70, 25.51, 24.86, 24.59, 19.08, 13.99; **MS** (ESI) 490.3 $[M+H]^+$ (Methyl-), 532.3 $[M+H]^+$ (Butyl-).

Ethyl 6-(3-((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenoxy)hexanoate (**42e**)

Compound **5** (153 mg, 0.62 mmol, 1 equiv.), **30** (1.2 equiv.), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (X-Phos, 0.042 mmol, 20 mg, 0.1 equiv.), $Pd_2(dba_3)$, $CHCl_3$ (0.019 mmol, 20 mg, 0.05 equiv.) and K_2CO_3 (1.24 mmol, 171 mg, 2 equiv.) were mixed in anhydrous tBuOH (2 mL) in a sealed tube. The mixture was degassed under N_2 by sonicator for 10 mins. The tube was sealed and heated in an oil bath at 100°C overnight. The reaction mixture was extracted with DCM, washed with brine, and dried with sodium sulphate. After removing the solvent by vacuum evaporator, the obtained residue was purified by flash chromatography (elution system - MeOH/DCM = 0 : 100 to 2 : 98 + 1%

NH_3 aqueous solution) to give the crude compound. The crude product was washed with 1 : 1 diethyl ether/hexane to obtain **42e** as a light yellow solid (176 mg, 61%).

***R*$_f$** = 0.23 (MeOH/DCM = 5 : 95)

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.18 (s, 1H), 9.57 (s, 1H), 8.51 (d, *J* = 5.2 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 2H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.65 (t, *J* = 2.1 Hz, 1H), 7.35 (d, *J* = 5.3 Hz, 1H), 7.31 (d, *J* = 1.2 Hz, 1H), 7.18 (t, *J* = 8.1 Hz, 1H), 6.52 (dd, *J* = 7.8, 2.1 Hz, 1H), 4.04 (q, *J* = 7.1 Hz, 2H), 3.97 (t, *J* = 6.4 Hz, 2H), 2.32 (t, *J* = 7.3 Hz, 2H), 2.09 (s, 3H), 1.80 – 1.69 (m, 2H), 1.64 – 1.56 (m, 2H), 1.48 – 1.42(m, 2H), 1.16 (t, *J* = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.26, 169.10, 163.49, 160.52, 159.39, 159.18, 142.33, 142.29, 131.43, 129.60, 127.99, 119.20, 111.60, 107.85, 107.80, 105.40, 67.58, 60.07, 33.91, 28.90, 25.55, 24.69, 24.55, 14.54; **MS** (ESI) 463.0 $[M+H]^+$.

Methyl 7-(3-((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenoxy)heptanoate (**42f**)

The title compound was synthesized following a procedure similar to **42e** using compound **5** (171 mg, 0.69 mmol), **31** (187 mg, 1.37 mmol, 2 equiv.), X-Phos (20 mg, 0.042 mmol, 0.061 equiv.), $Pd_2(dba)_3{\cdot}CHCl_3$(0.03 equiv., 0.028 mmol, 20 mg) and K_2CO_3 (191 mg, 1.38 mmol, 2 equiv.). Compound **42f** was isolated as a yellow solid (146 mg, 44%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.18 (s, 1H), 9.57 (s, 1H), 8.51 (d, *J* = 5.2 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 2H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.66 (t, *J* = 2.1 Hz, 1H), 7.35 (d, *J* = 5.3 Hz, 1H), 7.32 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.18 (t, *J* = 8.1 Hz, 1H), 6.52 (dd, *J* = 8.1, 1.6 Hz, 1H), 3.97 (t, *J* = 6.5 Hz, 2H), 3.57 (s, 3H), 2.30 (t, *J* = 7.4 Hz, 2H), 2.08 (s, 3H), 1.78 – 1.69 (m, 2H), 1.59 – 1.52 (m, 2H), 1.48 – 1.39 (m, 2H), 1.37 – 1.31 (m, 2H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.81, 169.14, 163.53, 160.57, 159.45,

159.22, 142.36, 142.34, 131.48, 129.63, 128.02, 119.24, 111.63, 107.91, 107.82, 105.44, 67.68, 51.59, 33.69, 29.10, 28.70, 25.71, 24.85, 24.58; **MS** (ESI) 463.0 $[M+H]^+$.

Methyl 8-(3-((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenoxy)octanoate (**42g**)

The title compound was synthesized following a procedure similar to **42e** using compound **5** (113 mg, 0.45 mmol), **32** (160 mg, 0.545 mmol, 1.2 equiv.), X-Phos (20 mg, 0.042 mmol, 0.093 equiv.), $Pd_2(dba)_3 \cdot CHCl_3$ (0.043 equiv., 0.019 mmol, 20 mg) and K_2CO_3 (124 mg, 0.9 mmol, 2 equiv.). Compound **42g** was isolated as a pale yellow solid (118 mg, 55%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.18 (s, 1H), 9.57 (s, 1H), 8.51 (d, *J* = 5.2 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 2H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.66 (t, *J* = 2.1 Hz, 1H), 7.35 (d, *J* = 5.3 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.18 (t, *J* = 8.1 Hz, 1H), 6.52 (dd, *J* = 8.1, 1.9 Hz, 1H), 3.97 (t, *J* = 6.5 Hz, 2H), 3.57 (s, 3H), 2.28 (d, *J* = 7.4 Hz, 2H), 2.09 (s, 3H), 1.79 – 1.67 (m, 2H), 1.59 – 1.48 (m, 2H), 1.47 – 1.37 (m, 2H), 1.37 – 1.21 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.82, 169.13, 163.52, 160.56, 159.46, 159.22, 142.35, 142.34, 131.47, 129.63, 128.02, 119.23, 111.61, 107.89, 107.82, 105.44, 67.72, 51.58, 33.72, 29.18, 28.88, 25.88, 24.85, 24.58; **MS** (ESI) 477.1 $[M+H]^+$.

Ethyl 6-(4-((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenoxy)hexanoate (**42h**)

The title compound was synthesized following a procedure similar to that of **42e** using compound **5** (162 mg, 0.65 mmol), **37** (214 mg, 0.85 mmol, 1.3 equiv.), X-Phos (20 mg, 0.042 mmol, 0.064 equiv.), $Pd_2(dba_3)\cdot CHCl_3$ (0.03 equiv., 0.019 mmol, 20 mg) and K_2CO_3 (179 mg, 1.3 mmol, 2 equiv.). Compound **42h** was isolated as a beige solid (191 mg, 64%).

R_f = 0.32 (MeOH/DCM = 5 : 95)

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.17 (s, 1H), 9.36 (s, 1H), 8.44 (d, *J* = 5.0 Hz, 1H), 8.10 (d, *J* = 8.3 Hz, 2H), 7.74 (d, *J* = 8.3 Hz, 2H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.27 (d, *J* = 5.1 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 2H), 4.05 (dd, *J* = 13.9, 6.9 Hz, 2H), 3.92 (t, *J* = 6.2 Hz, 2H), 2.30 (t, *J* = 7.3 Hz, 2H), 1.70 (dt, *J* = 13.1, 6.4 Hz, 2H), 1.59 (dt, *J* = 14.1, 7.1 Hz, 2H), 1.42 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.17 (t, *J* = 7.0 Hz, 3H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.30, 169.13, 163.51, 160.75, 159.12, 154.03, 142.20, 134.21, 131.60, 127.98, 121.06, 119.28, 114.88, 107.18, 67.97, 60.12, 33.97, 28.96, 25.58, 24.74, 24.58, 14.59; **MS** (ESI) 463.0 $[M+H]^+$.

Methyl 7-(4-((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenoxy)heptanoate (**42i**)

The title compound was synthesized following a procedure similar to that of **42e** using compound **5** (181 mg, 0.73 mmol), **38** (200 mg, 0.8 mmol, 1.1 equiv.), X-Phos (20 mg, 0.042 mmol, 0.058 equiv.), $Pd_2(dba)_3{\cdot}CHCl_3$ (0.03 equiv., 0.038 mmol, 20 mg) and K_2CO_3 (202 mg, 1.46 mmol, 2 equiv.). Compound **42i** was isolated as a yellow solid (218 mg, 64%).

R_f = 0.34 (MeOH/DCM = 5 : 95)

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.17 (s, 1H), 9.36 (s, 1H), 8.44 (d, *J* = 5.2 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 2H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 9.1 Hz, 2H), 7.27 (d, *J* = 5.3 Hz, 1H), 6.90 (d, *J* = 9.1 Hz, 2H), 3.93 (t, *J* = 6.5 Hz, 2H), 3.58 (s, 3H), 2.31 (t, *J* = 7.4 Hz, 2H), 2.09 (s, 3H), 1.75 – 1.64 (m, 2H), 1.59 – 1.52 (m, 2H), 1.47 – 1.37 (m, 2H), 1.31 – 1.36 (m, 2H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.82 , 169.13 , 163.50 , 160.75 , 159.14 , 154.05 , 142.20 , 134.19 , 131.59 , 127.99 , 121.06 , 119.27 , 114.88 , 107.19 , 68.02 , 51.62 , 33.71 , 29.11 , 28.69 , 25.70 , 24.86 , 24.59; **MS** (ESI) 463.0 $[M+H]^+$.

Methyl 8-(4-((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenoxy)octanoate (**42j**)

The title compound was synthesized following a procedure similar to **42e** using compound **5** (140 mg, 0.57 mmol), **39** (165 mg, 0.62 mmol, 1.1 equiv.), X-Phos (20 mg, 0.042 mmol, 0.074 equiv.), $Pd_2(dba)_3 \cdot CHCl_3$ (0.034 equiv., 0.019 mmol, 20 mg) and K_2CO_3 (158 mg, 1.14 mmol, 2 equiv.). Compound **42j** was isolated as a yellow solid (126 mg, 46%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.17 (s, 1H), 9.37 (s, 1H), 8.45 (d, *J* = 5.1 Hz, 1H), 8.11 (d, *J* = 8.4 Hz, 2H), 7.75 (d, *J* = 8.3 Hz, 2H), 7.70 (d, *J* = 8.5 Hz, 2H), 7.28 (d, *J* = 5.1 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 2H), 3.94 (t, *J* = 6.2 Hz, 2H), 3.59 (s, 4H), 2.31 (t, *J* = 7.4 Hz, 3H), 2.10 (s, 7H), 1.78 – 1.65 (m, 3H), 1.60 – 1.48 (m, 3H), 1.48 – 1.37 (m, 3H), 1.37 – 1.23 (m, 6H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.81, 169.11, 163.50, 160.75, 159.11, 154.05, 142.21, 134.19, 131.59, 127.97, 121.04, 119.26, 114.85, 107.17, 68.05, 51.59, 33.73, 29.21, 28.88, 25.87, 24.85, 24.59; **MS** (ESI) 477.1 $[M+H]^+$.

N^1-(3-((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenyl)-N^6-hydroxyadipamide trifluoroacetic acid (**45a**)

Step 1. To the solution of compound **42a** (92 mg, 0.19 mmol, 1 equiv.) in THF (4 mL) was added 2M LiOH aqueous solution (1.9 mL, 3.8 mmol, 20 equiv.). The reaction was stirred at ambient temperature overnight. The reaction progress was monitored by TLC and upon completion it was neutralized to pH 7 with 1M HCl aqueous solution. The solvent was removed under vacuum and the crude product (**43a**) was used for the next reaction without further purification. $\mathbf{R_f}$ = 0.09 (MeOH/DCM = 5 : 95); **MS** (ESI) 448.1 $[M+H]^+$.

Step 2. To the solution of the crude **43a** from step 1 in 10 mL DMSO was added *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (90 mg, 0.75 mmol, 4 equiv.), HATU (175 mg, 0.46 mmol, 2.4 equiv.), and triethylamine (160 μL, 1.14 mmol, 6 equiv.). The resulting reaction mixture was stirred at ambient temperature overnight. The reaction was extracted and reverse extracted with EA, washed with brine, dried with Na_2SO_4, and concentrated onto silica gel. The residue on silica was purified by column chromatography (elution system - MeOH/DCM = 5 : 95 to 1 : 9) using silica gel as stationary phase to furnish **44a** as a light orange solid (178mg, quant). $\mathbf{R_f}$ = 0.32 (MeOH/DCM = 1 : 9).

Step 3. To a solution of **44a** (65 mg, 0.12 mmol, 1 equiv.) in 40 mL MeOH : DCM 1 : 3 solution cooled in an ice bath acetyl chloride (25 μL, 0.36 mmol, 3 equiv.) was added dropwise. The mixture was stirred at ambient temperature for 3 h. The final product precipitated from the solution and was filtered and washed with DCM, and purified by pre-HPLC. Compound **45a** was isolated as a light yellow solid (6 mg, 10%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.39 (s, 1H), 10.20 (s, 1H), 9.82 (s, 1H), 9.55 (s, 1H), 8.48 (d, *J* = 5.3 Hz, 1H), 8.24 (s, 1H), 8.19 (d, *J* = 8.8 Hz, 2H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.38 (d, *J* = 7.7 Hz, 1H), 7.34 (d, *J* = 5.3 Hz, 1H), 7.20 (t, *J* = 8.0 Hz, 1H), 7.14 (d, *J* = 8.2 Hz, 1H), 2.32 (dt, *J* = 8.9, 4.6 Hz, 3H), 2.09 (s, 3H), 2.01 (t, *J* = 6.8 Hz, 2H), 1.58 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.79, 169.68, 169.66, 169.28, 160.52, 159.10, 142.27, 141.31, 139.87, 131.36, 128.30, 121.56, 119.28, 114.67, 110.82, 107.72, 36.68, 32.67, 25.41; **MS** (ESI) 463.1 $[M+H]^+$; **HPLC** purity 93.10%.

N^1-(3-((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenyl)-N^8-hydroxyoctanediamide hydrochloride (**45b**)

Step 1. To the solution of compound **42b** (40 mg, 0.082 mmol, 1 equiv.) in THF (5 mL) in 10 mL RBF, LiOH (39 mg, 1.64 mmol, 20 equiv.) and H_2O (2 mL) were added, and the reaction was stirred at ambient temperature overnight. The reaction progress was monitored by TLC and upon completion it was neutralized to pH 7 with 1M HCl aqueous solution. The solvent was removed under vacuum and the crude product **43b** was used for the next reaction without further purification (**MS** (ESI) 476.2 $[M+H]^+$).

Step 2. To the solution of the crude **43b** from step 1 in 10 mL DMSO, *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (19 mg, 0.16 mmol, 2 equiv.), HOBt (19 mg, 0.12 mmol, 1.5 equiv.), EDC (25 mg, 0.16 mmol, 2 equiv.) and triethylamine (114 μL, 0.82 mmol, 10 equiv.) were added. The resulting reaction mixture was stirred at ambient temperature overnight. The reaction was extracted and reverse extracted with EA, washed with brine, dried with Na_2SO_4, and concentrated onto silica gel. The residue on silica was purified by column chromatography (elution system - MeOH/DCM = 5 : 95 to 1 : 9) using silica gel as stationary phase to obtain compound **44b** as a light orange solid (44 mg, 93%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.34 (s, 1H), 9.40 (s, 1H), 8.90 (s, 1H), 8.28 (d, *J* = 5.3 Hz, 1H), 7.96 (d, *J* = 8.5 Hz, 2H), 7.90 (s, 1H), 7.74 (s, 1H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 7.7 Hz, 1H), 7.18 (t, *J* = 7.9 Hz, 1H), 7.12 (d, *J* = 7.7 Hz, 1H), 7.04 (d, *J* = 5.3 Hz, 1H), 2.26 (t, *J* = 7.4 Hz, 2H), 2.08 (s, 3H), 2.02 (t, *J* = 7.1 Hz, 2H), 1.46 – 1.70 (m, 10H), 1.29 (s, 4H); **MS** (ESI) 575.3 $[M+H]^+$.

Step 3. To a solution of **44b** (44 mg, 0.076 mmol, 1 equiv.) in 10 mL MeOH : DCM 1 : 1 cooled in an ice bath, was added acetyl chloride (27 μL, 0.39 mmol, 5 equiv.) in 5 mL DCM dropwise. The mixture was stirred at ambient temperature for 3 h. The final product precipitated from the solution and was filtered and washed with DCM, MeOH and diethyl ether then dried under vacuum. Compound **45b** was isolated as a yellow solid (13 mg, 33%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.33 (s, 1H), 10.18 (s, 1H), 9.81 (s, 1H), 9.56 (s, 1H), 8.65 (s, 1H), 8.49 (d, *J* = 5.2 Hz, 1H), 8.18 (d, *J* = 8.7 Hz, 3H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.41 (d, *J* = 7.4 Hz, 1H), 7.34 (d, *J* = 5.3 Hz, 1H), 7.22 – 7.17 (m, 2H), 2.31 (t, *J* = 7.3 Hz, 2H), 2.09 (s, 3H), 1.94 (t, *J* = 7.3 Hz, 2H), 1.64 – 1.57 (m, 2H), 1.54 – 1.44 (m, 2H), 1.36 – 1.22 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 171.56, 169.62, 169.14, 163.59, 160.62, 159.16, 142.27, 141.35, 139.92, 131.38, 128.91, 128.27, 119.25, 114.59, 113.23, 110.73, 107.74, 36.87, 32.74, 28.92, 28.88, 25.61, 25.51, 24.60; **MS** (ESI) 491.2 $[M+H]^+$; **HPLC** 96.6%.

N^1-(4-((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenyl)-*N*^{6}-hydroxyadipamide trifluoroacetic acid (**45c**)

Compound **44c** was synthesized following a procedure similar to **44a**.

Step 1. Using compound **42c** (55 mg, 0.11 mmol, 1 equiv.) in THF (3 mL), and 2M LiOH aqueous solution (0.57 mL, 1.1 mmol, 10 equiv.) gave crude **43c**.

R_f = 0 (base line) (MeOH/DCM = 5 : 95).

Step 2. Using crude compound from step 1, *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (26 mg, 0.22 mmol, 2 equiv.), HATU (50 mg, 0.13 mmol, 1.2 equiv.), and triethylamine (46 μL, 0.33 mmol, 3 equiv.) in DMF 5 mL. Compound **44c** was obtained as a light yellow solid (62 mg, 60%).

R_f = 0.23 (MeOH/DCM = 1 : 9); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.91 (s, 1H), 10.18 (s, 1H), 9.74 (s, 1H), 9.49 (s, 1H), 8.47 (d, *J* = 5.2 Hz, 1H), 8.11 (d, *J* = 8.8 Hz, 2H), 7.73 (dd, *J* = 11.5, 8.9 Hz, 4H), 7.51 (d, *J* = 8.9 Hz, 2H), 7.30 (d, *J* = 5.3 Hz, 1H), 4.81 (s, 1H), 3.91 (t, *J* = 9.7 Hz, 1H), 3.49 (d, *J* = 12.2 Hz, 1H), 2.28 (t, *J* = 6.7 Hz, 2H), 2.09 (s, 3H), 2.02 (t, *J* = 6.4 Hz, 2H), 1.64 (m, 3H), 1.53 (m, 7H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 171.04, 169.46, 169.13, 163.56, 160.63, 159.16, 142.24,

136.44, 133.76, 131.53, 128.02, 120.05, 119.73, 119.30, 107.49, 101.34, 61.79, 36.59, 32.61, 28.29, 25.32, 25.25, 25.16, 24.59, 18.79.

Step 3. The title compound was synthesized following a procedure similar to **45a** using compound **44c** (62 mg, 0.113 mmol, 1 equiv.) and acetyl chloride (50 μL, 0.57 mmol, 5 equiv.) in 4 mL THF. Compound **45c** was isolated as a yellow solid (21 mg, 33%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.35 (s, 1H), 10.20 (s, 1H), 9.76 (s, 1H), 9.52 (s, 1H), 8.46 (d, *J* = 5.3 Hz, 1H), 8.11 (d, *J* = 8.8 Hz, 2H), 7.75 (d, *J* = 8.7 Hz, 2H), 7.70 (d, *J* = 8.9 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H), 7.31 (d, *J* = 5.3 Hz, 1H), 2.28 (dd, *J* = 8.6, 4.4 Hz, 2H), 2.09 (s, 3H), 1.98 (t, *J* = 6.7 Hz, 2H), 1.55 (d, *J* = 3.7 Hz, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 170.95, 169.16, 163.74, 160.43, 158.88, 142.31, 136.30, 133.88, 131.46, 128.07, 120.11, 120.07, 119.87, 119.31, 107.48, 36.59, 32.67, 25.38, 24.58; **MS** (ESI) 463.1 $[M+H]^+$; **HPLC** 91.4%.

N^1-(4-((4-(4-acetamidophenyl)pyrimidin-2-yl)amino)phenyl)-*N*^{8}-hydroxyoctanediamide hydrochloride (**45d**)

Following a procedure similar to **44a**:

Step 1. Using compound **42d** (53 mg, 0.1 mmol, 1 equiv.) in THF (5 mL), and 10M LiOH aqueous solution (0.104 mL, 1 mmol, 10 equiv.) gave crude **43d**.

R$_f$ = Base line (MeOH/DCM = 5 : 95).

Step 2. Using crude **43d** from step 1, and *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (27 mg, 0.23 mmol, 2 equiv.), HATU (50 mg, 0.13 mmol, 1.2 equiv.), and triethylamine (46 μL, 0.33 mmol, 3 equiv.) in DMF 5 mL. Compound **44d** was obtained as a light orange solid (45mg, 72%).

R_f = 0.38 (MeOH/DCM = 1 : 9); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.99 (s, 1H), 10.25 (s, 1H), 9.81 (s, 1H), 9.40 (s, 1H), 8.44 (d, *J* = 5.3 Hz, 1H), 8.08 (d, *J* = 8.8 Hz, 2H), 7.69 (dd, *J* = 13.1, 8.9 Hz, 4H), 7.48 (d, *J* = 9.0 Hz, 2H), 7.29 (d, *J* = 5.3 Hz, 1H), 4.77 (s), 2.25 (t, *J* = 7.4 Hz, 2H), 2.07 (s, 3H), 2.00 – 1.91 (m, 2H), 1.68 – 1.40 (m,10H), 1.33 – 1.20 (m, 4H); **MS** (ESI) 575.3 $[M+H]^+$.

Step 3. To a solution of **44d** (45 mg, 0.078 mmol, 1 equiv.) in 10 mL MeOH : DCM 1 : 1, acetyl chloride (27 μL, 0.39 mmol, 5 equiv.) in 10 mL DCM solution was added dropwise. The mixture was stirred at ambient temperature for 3 h. The final product precipitated from the solution and was filtered and washed with DCM, MeOH and diethyl ether then dried under vacuum. Compound **45d** was isolated as a yellow solid (15 mg, 37%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.34 (s, 1H), 10.23 (s, 1H), 9.77 (s), 9.59 (s), 8.46 (d, *J* = 5.3 Hz, 1H, H-10), 8.11 (d, *J* = 8.8 Hz, 2H), 7.75 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 9.0 Hz, 2H), 7.53 (d, *J* = 9.0 Hz, 2H), 7.33 (d, *J* = 5.4 Hz, 1H), 2.27 (t, *J* = 7.4 Hz, 2H2.09 (s, 3H), 1.93 (dd, *J* = 14.6, 7.1 Hz Hz, 2H), 1.62 – 1.53 (m, 2H), 1.53 – 1.45 (m, 2H), 1.29– 1.28 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 171.33, 169.60, 169.26, 164.57, 159.36, 157.44, 142.69, 135.53, 134.46, 131.02, 128.38, 120.45, 120.08, 119.3, 107.39, 36.76, 32.72, 28.90, 28.88, 25.57, 25.51, 24.60; **MS** (ESI) 491.2 $[M+H]^+$/ **HPLC** 92%.

6-(3-((4-(4-Acetamidophenyl)pyrimidin-2-yl)amino)phenoxy)-*N*-hydroxyhexanamide (**45e**)

Step 1. To a solution of compound **42e** (76 mg, 0.164 mmol, 1 equiv.) in THF (5 mL), 2M LiOH aqueous solution (1.64 mL, 3.29 mmol, 20 equiv.) was added. The reaction was stirred at ambient temperature overnight. The reaction progress was monitored by TLC and upon completion, it was

neutralized to pH 7 with 1 M HCl and saturated Na_2HCO_3 aqueous solution. The solvent was removed under vacuum and the crude **43e** was used for the next reaction without further purification.

Step 2. To the solution of the crude **43e** from step 1 in DMF (12 mL), *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (38.4 mg, 0.328 mmol, 2 equiv.), HOBt (37.7 mg, 0.246 mmol, 1.5 equiv.), EDC (51 mg, 0.328 mmol, 2 equiv.) and N,N-DIPEA (224 µL, 1.312 mmol, 8 equiv.) were added. The resulting reaction mixture was stirred at ambient temperature overnight. The reaction was extracted and reverse extracted with EA, washed with brine, dried with Na_2SO_4, and all solvents were evaporated. The residue was purified by column chromatography (elution system - MeOH/DCM = 5 : 95 to 1 : 9 + 1% NH_3 aqueous solution) using silica gel as stationary phase to obtain crude **44e** (60 mg). The received compound was directly used for the next step without further purification. $\mathbf{R_f}$ = 0.15 (MeOH/DCM = 5 : 95); **MS** (ESI) 534.4 $[M+H]^+$.

Step 3. The crude **44e** (87 mg, 0.164 mmol) was suspended in DCM and few drops of MeOH was added to fully dissolve the compound. Acetyl chloride (35 µL, 0.492 mmol, 3 equiv.) was added dropwise and the reaction mixture was stirred at RT for 3 h. The solvent was removed by rotary evaporator and the solid was dried to provide **45e** as a yellow solid (71 mg, 89%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.38 (s, 1H), 10.27 (s, 1H), 9.68 (s, 1H), 8.51 (d, *J* = 5.4 Hz, 1H), 8.14 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.8 Hz, 2H), 7.64 (s, 1H), 7.38 (d, *J* = 5.4 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.19 (t, *J* = 8.1 Hz, 1H), 6.54 (dd, *J* = 8.1, 1.8 Hz, 1H), 3.96 (t, *J* = 6.4 Hz, 2H), 2.08 (s, 3H), 2.00 (t, *J* = 7.3 Hz, 2H), 1.74 – 1.69 (m, 2H), 1.61 – 1.54 (m, 2H), 1.48 – 1.36 (m, 3H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 170.81, 169.63, 169.35, 164.59, 159.49, 159.17, 157.40, 142.83, 141.58, 130.92, 129.81, 128.39, 119.27, 112.17, 108.67, 107.73, 105.98, 67.74, 32.70, 29.01, 25.70, 25.41, 24.60, 21.21; **MS** (ESI) 450.1 $[M+H]^+$; **HPLC** 97.55%.

7-(3-((4-(4-Acetamidophenyl)pyrimidin-2-yl)amino)phenoxy)-*N*-hydroxyheptanamide (**45f**)

Step 1. The reaction was carried out following the synthesis procedure for **44e** with 2M LiOH aqueous solution (1.3 mL, 2.6 mmol, 20 equiv.) in 5 mL THF. $\mathbf{R_f}$ = 0.05 (MeOH/DCM = 5 : 95).

Step 2. The crude **43f** from Step 1 was dissolved in 6 mL DMSO and HOBt (30 mg, 0.195 mmol, 1.5 equiv.), *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine (60 mg, 0.32 mmol, 4 equiv.), EDCI (40 mg, 0.26 mmol, 2 equiv.) and TEA (109 μL, 0.78 mmol, 6 equiv.) were used. Compound **44f** was isolated as a yellow solid (80 mg). $\mathbf{R_f}$ = 0.27 (MeOH/DCM = 5 : 95); **MS** (ESI) 549.0 $[M+H]^+$.

Step 3. The title compound was synthesized from **44f** (71 mg, 0.13 mmol) following the procedure of **45e** using acetyl chloride (27.8 μL, 0.39 mmol, 3 equiv.).The collected solid was washed with diethyl ether and DCM/MeOH mixed solvent (1 : 1) in sequence to obtain **45f** as a yellow solid (22 mg, 34%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.36 (s, 1H), 10.23 (d, *J* = 9.7 Hz, 1H), 9.63 (s, 1H), 8.51 (d, *J* = 5.3 Hz, 1H), 8.14 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.8 Hz, 2H), 7.66 (s, 1H), 7.37 (d, *J* = 5.3 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.20 (t, *J* = 8.1 Hz, 1H), 6.54 (dd, *J* = 8.1, 1.8 Hz, 1H), 3.97 (t, *J* = 6.4 Hz, 2H), 2.10 (s, 3H), 1.97 (t, *J* = 7.3 Hz, 2H), 1.81 – 1.66 (m, 2H), 1.54 (dt, *J* = 14.9, 7.4 Hz, 2H), 1.49 – 1.38 (m, 2H), 1.36 – 1.28(m, 2H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.67, 169.34, 164.66, 159.50, 159.09, 157.29, 142.86, 141.52, 130.89, 129.82, 128.41, 119.27, 112.19, 108.67, 107.73, 106.05, 67.77, 32.69, 29.15, 28.83, 25.77, 25.55, 24.60; **MS** (ESI) 464.2 $[M+H]^+$; **HPLC** 90.55%.

8-(3-((4-(4-Acetamidophenyl)pyrimidin-2-yl)amino)phenoxy)-*N*-hydroxyoctanamide hydrochloride (**45g**)

Step 1. The reaction was carried out following the synthesis procedure for **44e** with 2M LiOH aqueous solution (2.2 mL, 4.4 mmol, 20 equiv.) in 5 mL THF.

Step 2. The crude **43g** from Step 1 was dissolved in DMSO (6 mL) and HATU (100 mg, 0.264 mmol, 1.2 equiv.), *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine (47 mg, 0.44 mmol, 2 equiv.) and TEA (307 μL, 2.2 mmol, 10 equiv.) were used. Compound **44g** was isolated as an orange liquid (246 mg). $\mathbf{R_f}$ = 0.25 (MeOH/DCM = 5 : 95).

Step 3. The title compound was synthesized from **44g** (123 mg, 0.22 mmol) following the procedure of **44e** using acetyl chloride (47.1 μL, 0.66 mmol, 3 equiv.). The collected solid was washed with DCM and diethyl ether in sequence to obtain **45g** (58 mg. 51%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.34 (s, 1H), 10.27 (s, 1H), 9.70 (s, 1H), 8.51 (d, *J* = 5.4 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.8 Hz, 2H), 7.62 (t, *J* = 2.0 Hz, 1H), 7.38 (d, *J* = 5.4 Hz, 1H), 7.30 (dd, *J* = 8.1, 1.0 Hz, 1H), 7.20 (t, *J* = 8.1 Hz, 1H), 6.55 (dd, *J* = 8.1, 1.8 Hz, 1H), 3.97 (t, *J* = 6.5 Hz, 2H), 2.09 (s, 3H), 1.94 (t, *J* = 7.3 Hz, 2H), 1.79 – 1.65 (m, 3H), 1.49 (dt, *J* = 14.8, 7.5 Hz, 2H), 1.41 (dd, *J* = 14.7, 7.1 Hz, 2H), 1.37 – 1.21 (m, 4H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 174.42, 174.08, 169.59, 164.33, 163.63, 161.75, 147.69, 146.16, 135.56, 134.59, 133.22, 124.02, 117.00, 113.57, 112.47, 110.87, 72.58, 37.45, 33.94, 33.73, 33.69, 30.67, 30.27, 29.35; **MS** (ESI) 478.2 $[M+H]^+$; **HPLC** 95.11%.

6-(4-((4-(4-Acetamidophenyl)pyrimidin-2-yl)amino)phenoxy)-*N*-hydroxyhexanamide hydrochloride (**45h**)

Step 1. The reaction was carried out using the synthesis procedure for **44e** with 2M LiOH aqueous solution (1.5 mL, 3.02 mmol, 20 equiv.) in 5 mL THF.

Step 2. The crude **43h** from Step 1 was dissolved in DMSO (12 mL) and DI water (1 mL), and HATU (136 mg, 0.36 mmol, 2.4 equiv.), *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (76 mg, 0.6 mmol, 4

equiv.) and TEA (125 μL, 0.9 mmol, 6 equiv.) were used. Compound **44h** was isolated as a pale yellow solid (163 mg). $\mathbf{R_f}$ = 0.1 (MeOH/DCM = 5 : 95).

Step 3. The title compound was synthesized from **44h** (82mg, 0.15 mmol) by following the procedure of **45e** using acetyl chloride (32 μL, 0.45 mmol, 3 equiv.). Compound **45h** was isolated as a yellow solid (16 mg, 22%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.35 (s, 1H), 10.24 (s, 1H), 10.20 (s, 2H), 10.02 (s, 1H), 9.54 (s, 1H), 8.44 (d, *J* = 5.4 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 2H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.66 (d, *J* = 7.7 Hz, 2H), 7.32 (d, *J* = 5.6 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 2H), 3.93 (t, *J* = 6.2 Hz, 2H), 2.09 (s, 3H), 1.98 (t, *J* = 5.9 Hz, 2H), 1.63 – 1.48 (m, 2H), 1.46 – 1.32 (m, 2H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.56, 169.38, 165.58, 160.57, 158.18, 155.09, 143.14, 132.47, 130.53, 128.70, 122.40, 119.32, 115.10, 107.03, 68.06, 32.70, 28.98, 25.67, 25.38, 24.61; **MS** (ESI) 450.1 $[M+H]^+$; **HPLC** 95.99%.

7-(4-((4-(4-Acetamidophenyl)pyrimidin-2-yl)amino)phenoxy)-*N*-hydroxyheptanamide hydrochloride (**45i**)

Step 1. The reaction was carried out following the synthesis procedure for **44e** with 2M LiOH aqueous solution (2 mL, 2.6 mmol, 18.18 equiv.) in 5 mL THF. $\mathbf{R_f}$ = 0.18 (MeOH/DCM = 5 : 95) to give **43i** (**MS** (ESI) 449.0 $[M+H]^+$).

Step 2. The crude **43i** from Step 1 was dissolved in DMSO (13 mL) and HATU (100 mg, 0.26 mmol, 1.2 equiv.), *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine (52 mg, 0.44 mmol, 2 equiv.) and TEA (92 μL, 0.66 mmol, 6 equiv.) were used. Compound **44i** was isolated as a yellow solid (209 mg, 38%). $\mathbf{R_f}$ = 0.25 (MeOH/DCM = 5 : 95); **MS** (ESI) 547.0 $[M+H]^+$.

Step 3. The title compound was synthesized from **44i** (120 mg, 0.22 mmol) following the procedure of **45e** using acetyl chloride (47.1 μL, 0.66 mmol, 3 equiv.). The collected solid was washed with DCM to obtain **45i** as a yellow solid (100 mg, 98%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.34 (s, 1H), 10.26 (s, 1H), 9.71 (s, 1H), 9.61 (s, 1H), 8.43 (d, *J* = 5.4 Hz, 1H), 8.12 (d, *J* = 8.3 Hz, 2H), 7.75 (d, *J* = 8.1 Hz, 2H), 7.64 (d, *J* = 7.0 Hz, 2H), 7.34 (s, 1H), 6.93 (d, *J* = 8.4 Hz, 2H), 3.94 (t, *J* = 6.3 Hz, 3H), 2.09 (s, 3H), 1.96 (t, *J* = 7.3 Hz, 2H), 1.76 – 1.63 (m, 2H), 1.55 – 1.48 (m, 2H), 1.45 – 1.36 (m, 2H), 1.34 – 1.24 (m, 2H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.64, 169.39, 165.76, 158.00, 155.22, 143.18, 132.29, 130.47, 128.76, 122.59, 119.33, 115.13, 107.02, 68.11, 32.68, 29.12, 28.81, 25.74, 25.54, 24.61; **MS** (ESI) 464.2 $[M+H]^+$; **HPLC** 92.65%.

8-(4-((4-(4-Acetamidophenyl)pyrimidin-2-yl)amino)phenoxy)-*N*-hydroxyoctanamide hydrochloride (**45j**)

Step 1. The reaction was carried out following the synthesis procedure for **44e** with 2M LiOH aqueous solution (2.3 mL, 4.62 mmol, 20 equiv.) in 5 mL THF. $\mathbf{R_f}$ = 0.07 (MeOH/DCM = 5 : 95).

Step 2. The crude **43j** from Step 1 was dissolved in DMSO (10 mL) and HATU (104 mg, 0.276 mmol, 1.2 equiv.), *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine (54 mg, 0.46 mmol, 2 equiv.) and TEA (192 μl, 1.38 mmol, 6 equiv.) were used. Compound **44j** was isolated as an orange liquid (735 mg in DMSO). $\mathbf{R_f}$ = 0.25 (MeOH/DCM = 5 : 95); **MS** (ESI) 562.2 $[M+H]^+$.

Step 3. The title compound was synthesized from **44**j (129 mg, 0.23 mmol) following the procedure of **44e** using acetyl chloride (49 μL, 0.69 mmol, 3 equiv.). The collected solid was washed with DCM/diethyl ether mixed solvent (1 : 1) to obtain **45j** (88 mg, 74%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.33 (s, 1H), 9.83 (s, 1H), 8.45 (d, *J* = 5.6 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 2H), 7.77 (d, *J* = 8.8 Hz, 2H), 7.63 (d, *J* = 9.0 Hz, 2H), 7.38 (d, *J* = 5.7 Hz, 1H), 6.94 (d, *J* = 9.1 Hz, 2H), 3.95 (t, *J* = 6.5 Hz, 3H), 2.10 (s, 3H), 1.95 (t, *J* = 7.3 Hz, 2H), 1.74 – 1.65 (m, 2H), 1.50 (dt, *J* = 14.5, 7.4 Hz, 2H), 1.45 – 1.36 (m, 2H), 1.36 – 1.22 (m, 5H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.56, 169.26, 165.23, 158.53, 154.94, 142.95, 132.64, 132.42, 130.65, 128.56, 122.21, 119.25, 115.03, 107.00, 68.08, 32.68, 29.19, 28.97, 28.91, 25.88, 25.49, 24.57; **MS** (ESI) 478.2 $[M+H]^+$; **HPLC** 91.21%.

N-(4-bromophenyl)acetamide (**47**)

To the solution of 4-bromoaniline (**46**) (2.5 g, 14.5 mmol, 1 equiv.) and potassium carbonate (6.05 g, 43.78 mmol, 3 equiv.) in dichloromethane (50 mL) was added acetyl chloride (1.55 mL, 2 equiv.). The solution was stirred at ambient temperature until TLC showed the consumption of the starting material. Acetyl chloride and DCM were evaporated under reduced pressure at 45°C. Water was added to dissolve the salt in the residue. The residue mixture was filtered and washed with DCM. The product (**47**) was dried under vacuum to give a light grey solid (2.78 g, 89%) which was directly used in the next step without further purification.

R_f = 0.22 (EA/Hexane = 1 : 1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.49 – 7.34 (m, 4H), 7.17 (s, 1H), 2.17 (s, 3H).

N-(4-(4,4,5,5-tetramethyl-1, 3,2-dioxaborolan-2-yl)phenyl)acetamide (**48**)

To a solution of compound **47** (250 mg, 1.45 mmol) in dioxane (6 mL) at room temperature was added bis(pinacolato)diboron (554 g, 2.18 mmol, 1.5 equiv.), and KOAc (427mg, 4.35 mmol, 3 equiv.). The

reaction mixture was degassed under N_2. Pd(dppf)Cl_2 (90mg, 0.12 mmol, 0.1 equiv.) was added to the mixture and degassed under N_2. The reaction mixture was heated to 100°C and stirred overnight under nitrogen. The reaction mixture was monitored using TLC until completion, filtered through Celite and washed with EA. The reaction solvent was evaporated under reduced pressure to give a residue, which was purified via silica gel column chromatography (elution system - EA/Hexane = 1 : 1) to give compound **48** as a white solid (870 mg, 71%).

R_f = 0.43 (EA/Hexane = 1 : 1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.3 Hz, 2H), 7.51 (d, *J* = 7.9 Hz, 2H), 7.19 (s, 1H), 2.18 (s, 3H), 1.33 (s, 12H).

N-(4-(2-Chloro-5-methylpyrimidin-4-yl)phenyl)acetamide (**49a**)

The title compound was synthesized following a procedure similar to compound **5** by using 2,4-dichloro-5-methylpyrimidine (567 mg, 3.48 mmol, 1 equiv.), compound **48** (1 g, 3.8 mmol, 1.1 equiv.), $Pd(PPh_3)_4$ (192 mg, 0.17 mmol, 0.05 equiv.) and sodium carbonate (553 mg, 5.52 mmol, 1.5 equiv.) in MeCN: H_2O (2 : 1, 30 mL) to give the product as a yellow solid (983 mg, quant).

R_f = 0.34 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, MeOD-*d4*) δ 8.53 (s, 1H), 7.73 (d, *J* = 8.7 Hz, 2H), 7.65 (d, *J* = 8.7 Hz, 3H), 2.39 (s, 3H), 2.16 (s, 3H).

N-(4-(5-methyl-2-((4-morpholinophenyl)amino)pyrimidin-4-yl)phenyl)acetamide (**49b**)

The title compound was synthesized following a procedure similar to **S1** by using compound **49a** (150 mg, 0.57 mmol), 4-morpholinoaniline (**63c**) (112 mg, 0.63 mmol, 1.1 equiv.), X-Phos (25 mg,

0.057 mmol, 0.1 equiv.), $Pd_2(dba)_3{\cdot}CHCl_3$ (0.05 equiv., 0.028 mmol, 25 mg) and K_2CO_3 (165 mg, 1.2 mmol, 2.1 equiv.). Compound **49b** was isolated as a light brown solid (137 mg, 60%).

R_f = 0.38 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.12 (s, 1H), 9.21 (s, 1H), 8.29 (s, 1H), 7.70 (s, 2H), 7.63 (dd, *J* = 8.9, 3.0 Hz, 4H), 6.86 (d, *J* = 9.1 Hz, 2H), 3.78 – 3.66 (m, 4H), 3.05 – 2.97 (m, 4H), 2.20 (s, 3H), 2.08 (d, *J* = 1.7 Hz, 3H); **MS** (ESI) 404.2 $[M+H]^+$; **HPLC** 99.57%.

7-(3-((4-(4-Acetamidophenyl)-5-methylpyrimidin-2-yl)amino)phenoxy)-N-hydroxyheptanamide 2,2,2-trifluoroacetate (**50**)

Step 1. Following a procedure similar to **S1** using compound **49a** (100 mg, 0.38 mmol), **31** (111 mg, 0.42 mmol, 1.1 equiv.), X-Phos (20 mg, 0.042 mmol, 0.11 equiv.), $Pd_2(dba)_3{\cdot}CHCl_3$ (0.074 equiv., 0.028 mmol, 20 mg) and K_2CO_3 (110 mg, 0.8 mmol, 2.1 equiv.) to give a light brown solid (118 mg, 65%) used directly for the next step.

R_f = 0.55 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.12 (s, 1H), 9.47 (s, 1H), 8.38 (s, 1H), 7.75 – 7.65 (m, 5H), 7.24 (d, *J* = 9.2 Hz, 1H), 7.11 (t, *J* = 8.1 Hz, 1H), 6.45 (dd, *J* = 7.7, 2.0 Hz, 1H), 3.90 (t, *J* = 6.6 Hz, 2H), 3.57 (s, 3H), 2.29 (t, *J* = 7.4 Hz, 2H), 2.25 (s, 3H), 2.08 (s, 3H), 1.73 – 1.63 (m, 2H), 1.54 (dt, *J* = 15.0, 7.4 Hz, 2H), 1.35 (ddd, *J* = 24.9, 15.6, 8.1 Hz, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.81, 169.02, 164.28, 160.28, 159.43, 159.06, 142.68, 140.73, 133.17, 129.88, 129.53, 118.75, 118.19, 111.12, 107.47, 104.87, 67.58, 51.60, 33.70, 29.06, 28.70, 25.68, 24.85, 24.54, 16.60.

Step 2. Following the synthesis procedure for **45e** with 2 M LiOH aqueous solution (1 mL, 2 mmol, 10 equiv.) in THF/H_2O (4 mL + 2 mL). **R_f** = 0.075 (MeOH/DCM = 5 : 95).

Step 3. The crude product from Step 2 was dissolved in DMF (4 mL) and HATU (96 mg, 0.252 mmol, 1.2 equiv.), *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine (49 mg, 0.42 mmol, 2 equiv.) and DIPEA (357 μL, 2.1 mmol, 10 equiv.) were added to give a yellow solid (133 mg, quat.). **R_f** = 0.18 MeOH/DCM = 5 : 95).

Step 4. The title compound was synthesized from the crude product from Step 3 following the procedure of **45e** using acetyl chloride (51 μL, 0.71 mmol, 3 equiv.). The collected solid was washed with DCM and purified by prep HPLC to obtain **50** as a beige solid (5 mg, 3.5%).

R_f = 0.025 MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.35 (s, 1H), 10.16 (s, 1H), 9.47 (s, 1H), 8.66 (s, 1H), 8.39 (s, 1H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.9 Hz, 3H), 7.25 (d, *J* = 8.0 Hz, 1H), 7.13 (t, *J* = 8.1 Hz, 1H), 6.47 (dd, *J* = 8.1, 1.9 Hz, 1H), 3.91 (t, *J* = 6.4 Hz, 2H), 2.26 (s, 3H), 2.10 (s, 3H), 1.96 (t, *J* = 7.4 Hz, 2H), 1.75 – 1.64 (m, 2H), 1.52 (dt, *J* = 15.1, 7.6 Hz, 2H), 1.45 – 1.34 (m, 2H), 1.30 (dd, *J* = 13.4, 6.8 Hz, 2H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.63, 169.18, 165.04, 159.47, 159.06, 158.03, 142.08, 141.07, 132.69, 130.01, 129.67, 118.78, 118.33, 111.52, 108.02, 105.31, 67.67, 32.69, 29.12, 28.84, 25.75, 25.54, 24.55, 16.62; **MS** (ESI) 478.2 $[M+H]^+$; **HPLC** 96.00%.

8-(3-((4-(4-Acetamidophenyl)-5-methylpyrimidin-2-yl)amino)phenoxy)-*N*-hydroxyoctanamide trifluoroacetic acid (**51**)

Step 1. Following a procedure similar to **S1** using compound **49a** (118 mg, 0.45 mmol), **32** (161 mg, 0.545 mmol, 1.2 equiv.), X-Phos (20 mg, 0.042 mmol, 0.093 equiv.), $Pd_2(dba)_3{\cdot}CHCl_3$ (0.043 equiv., 0.019 mmol, 20 mg) and K_2CO_3 (124 mg, 0.9 mmol, 2 equiv.), a light brown solid was obtained (130 mg, 57%).

R_f = 0.21 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.13 (s, 1H), 9.47 (s, 1H), 8.39 (s, 1H), 7.70 (ddd, *J* = 6.8, 6.2, 2.1 Hz, 5H), 7.24 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.12 (t, *J* = 8.1 Hz, 1H), 6.46 (dd, *J* = 8.1, 1.7 Hz, 1H), 3.91 (t, *J* = 6.5 Hz, 2H), 3.58 (s, 3H), 2.30 (t, *J* = 7.4 Hz, 2H), 2.26 (s, 3H), 2.09 (s, 3H), 1.78 – 1.63 (m, 2H), 1.59 – 1.47 (m, 2H), 1.45 – 1.34 (m, 2H), 1.34 – 1.19 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.85, 169.06, 164.27, 160.30, 159.44, 159.05, 142.66, 140.71, 133.19, 129.88, 129.54, 118.78, 118.22, 111.13, 107.50, 104.89, 67.63, 51.60, 33.72, 29.12, 28.85, 25.83, 24.85, 24.53, 16.59.

Step 2. Following the synthesis procedure for **45e** with 2 M LiOH aqueous solution (2.4 mL, 4.9 mmol, 20 equiv.) in THF/H_2O (4 mL + 2 mL) gave a product that was used directly. **R_f** = 0.08 (MeOH/DCM = 5 : 95).

Step 3. The crude product from Step 2 was dissolved in DMF (4 mL) and HATU (96 mg, 0.252 mmol, 1.2 equiv.), *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine (49 mg, 0.42 mmol, 2 equiv.) and DIPEA (357 μL, 2.1 mmol, 10 equiv.) were added. A beige solid was obtained (200 mg, quant.) and used directly. **R_f** = 0.11(MeOH/DCM = 5 : 95).

Step 4. The title compound was synthesized following the procedure of **45e** using acetyl chloride (56 μL 0.78 mmol, 3 equiv.). The collected solid was washed with DCM to obtain the target compound as an orange hygroscopic solid (15 mg, 11%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.34 (s, 1H), 10.17 (s, 1H), 9.49 (s, 1H), 8.38 (s, 1H), 7.73 (d, *J* = 8.5 Hz, 2H), 7.68 (d, *J* = 8.7 Hz, 2H), 7.65 (t, *J* = 2.1 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.12 (t, *J* = 8.1 Hz, 1H), 6.47 (d, *J* = 8.2 Hz, 1H), 3.90 (t, *J* = 6.4 Hz, 2H), 2.25 (s, 3H), 2.09 (s, 3H), 2.09 – 1.82 (m, 2H), 1.75 – 1.60 (m, 2H), 1.49 (dt, *J* = 14.3, 7.3 Hz, 2H), 1.38 (dd, *J* = 18.4, 11.2 Hz, 2H), 1.32 – 1.11 (m, 6H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 174.97, 169.73, 169.24, 165.22, 159.48, 158.78, 141.92,

141.12, 132.63, 130.00, 129.71, 118.82, 118.39, 111.63, 108.27, 105.44, 67.73, 32.69, 29.13, 28.96, 28.92, 25.88, 25.52, 24.53, 16.60; **MS** (ESI) 492.2 $[M+H]^+$; **HPLC** 90.91%.

6-(4-((4-(4-Acetamidophenyl)-5-methylpyrimidin-2-yl)amino)phenoxy)-N-hydroxyhexanamide 2,2,2-trifluoroacetate (**52**)

Step 1. Following a procedure similar to **S1** using compound **49a** (100 mg, 0.38 mmol), **37** (120 mg, 0.46 mmol, 1.2 equiv.), X-Phos (20 mg, 0.042 mmol, 0.11 equiv.), $Pd_2(dba)_3{\cdot}CHCl_3$ (0.051 equiv., 0.019 mmol, 20 mg) and K_2CO_3 (110 mg, 0.8 mmol, 2.1 equiv.) gave a light brown solid (100 mg, 55%) used directly for the next step.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.62 (s, 4H), 7.49 (d, *J* = 8.9 Hz, 3H), 7.02 (s, 1H), 6.85 (d, *J* = 9.0 Hz, 2H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.94 (t, *J* = 6.4 Hz, 2H), 2.33 (t, *J* = 7.5 Hz, 3H), 2.24 (s, 3H), 2.20 (s, 3H), 1.83 – 1.74 (m, 3H), 1.70 (dt, *J* = 15.2, 7.4 Hz, 3H), 1.57 – 1.44 (m, 3H), 1.25 (t, *J* = 7.1 Hz, 4H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 173.70, 168.42, 164.86, 159.82, 159.08, 154.65, 138.82, 134.36, 133.07, 129.69, 121.11, 119.25, 117.91, 114.94, 68.06, 60.25, 34.29, 29.02, 25.68, 24.75, 16.46, 14.25.

Step 2. The reaction was carried out following the synthesis procedure for **45e** with 2 M LiOH aqueous solution (1 mL, 2 mmol, 11.11 equiv.) in THF/H_2O (4.5 mL + 1 mL). $\mathbf{R_f}$ = 0.14 (MeOH/DCM = 5 : 95).

Step 3. The crude product from Step 2 was dissolved in DMF (10 mL) and HATU (164 mg, 0.43 mmol, 2.4 equiv.), *O*-(tetrahydro-*2H*-pyran-2-yl) hydroxylamine (100 mg, 0.85 mmol, 4.74 equiv.) and DIPEA (300 μL, 1.76 mmol, 9.8 equiv.) were added. The unpurified product was used directly for the next step. $\mathbf{R_f}$ = 0.26 MeOH/DCM = 5 : 95).

Step 4. The title compound was synthesized from the crude product from Step 3 following the procedure of **45e** by using acetyl chloride (47.1 μL, 0.66 mmol, 3 equiv.). The collected solid was washed with DCM and purified by prep HPLC to obtain **52** as a brown liquid (25 mg, 25%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.36 (s, 1H), 10.21 (s, 1H), 9.50 (s, 1H), 8.34 (s, 1H), 7.74 (d, *J* = 8.7 Hz, 2H), 7.66 (d, *J* = 8.8 Hz, 2H), 7.62 (d, *J* = 9.1 Hz, 2H), 6.87 (d, *J* = 9.1 Hz, 2H), 3.91 (t, *J* = 6.4 Hz, 4H), 2.24 (s, 3H), 2.10 (s, 3H), 1.98 (t, *J* = 7.3 Hz, 2H), 1.75 – 1.64 (m, 2H), 1.56 (dt, *J* = 15.0, 7.3 Hz, 2H), 1.39 (dt, *J* = 14.9, 7.5 Hz, 2H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.50, 169.20, 166.16, 162.18, 157.15, 154.70, 141.28, 132.98, 132.25, 130.12, 121.80, 118.83, 117.78, 115.03, 68.02, 32.69, 28.98, 25.67, 25.38, 24.57, 16.58; **MS** (ESI) 464.2 $[M+H]^+$; **HPLC** 96.02%.

Methyl 7-(3-((4-(4-(2-(4-methylpiperazin-1-yl)acetamido)phenyl)pyrimidin-2-yl)amino) phenoxy) heptanoate (**57**)

Step 1. Compound **42f** was dissolved in 2 mL MeOH/THF (1 : 1) in a sealed tube, and 4N HCl/Dioxane (43 μL, 0.172 mmol, 4 equiv.) was added to the solution. The tube was sealed and heated in an oil bath at 50°C overnight. The tube was cooled and the reaction mixture extracted with DCM three times and washed with brine then dried with Na_2SO_4. The solvent was removed by rotary evaporator to obtain the crude **55**. $\mathbf{R_f}$ = 0.42 (MeOH/DCM = 5 : 95).

Step 2. Crude **55** from step 1 was dissolved in 2 mL DMF and THF 1 mL. 2-(4-methylpiperazin-1-yl)acetic acid (8.2 mg, 0.0516 mmol, 1.2 equiv.), HATU (20 mg, 0.516 mmol, 1.2 equiv.) and DIPEA (37 μL, 0.215 mmol, 5 equiv.) were added into the solution. The reaction mixture was stirred at RT overnight. The reaction mixture was extracted with DCM three times and washed with brine then dried

with Na_2SO_4, and the solvent was removed under vacuum to obtain the crude product. The crude product was purified by flash chromatography to obtain **57**-ester as a orange solid (20 mg, 83%).

R_f = 0.04 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.30 (s, 1H), 8.45 (d, *J* = 5.2 Hz, 1H), 8.09 (d, *J* = 8.7 Hz, 2H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.46 (t, *J* = 2.2 Hz, 1H), 7.23 (d, *J* = 7.7 Hz, 1H), 7.21 – 7.17 (m, 1H), 7.15 (s, 1H), 7.14 (d, *J* = 5.3 Hz, 1H), 6.63 – 6.54 (m, 1H), 4.01 (t, *J* = 6.5 Hz, 2H), 3.66 (s, 3H), 3.18 (s, 2H), 2.69 (s, 4H), 2.53 (s, 4H), 2.36 – 2.30 (m, 5H), 1.87 – 1.76 (m, 2H), 1.67 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.52 – 1.46 (m, 2H), 1.46 – 1.33 (m, 2H); **MS** (ESI) 561.3 $[M+H]^+$.

Methyl 7-(4-((4-(4-(2-(4-methylpiperazin-1-yl)acetamido)phenyl)pyrimidin-2-yl)amino)phenoxy)heptanoate (**58**)

Step 1. The reaction was carried out using **42i** by following the procedure of **57** in 4 mL MeOH, and 4N HCl/Dioxane (20μL, 0.88 mmol, 4 equiv.) to give **56**. **R_f** = 0.34 (MeOH/DCM = 5 : 95) ; **MS** (ESI) 421.2 $[M+H]^+$.

Step 2. The crude **56** from Step 1 was dissolved in 6 mL DMF and HATU (120 mg, 0.32 mmol, 1.5 equiv.), 2-(4-methylpiperazin-1-yl)acetic acid (40 mg, 0.252 mmol, 1.2 equiv.) and DIPEA (286 μL, 1.68 mmol, 8 equiv.) were used to give **58**-ester isolated as a yellow oil (76 mg, 64%).

R_f = 0.28 (MeOH/DCM = 10 : 90); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.28 (s, 1H), 8.39 (d, *J* = 5.2 Hz, 1H), 8.06 (d, *J* = 8.7 Hz, 2H), 7.70 (d, *J* = 8.7 Hz, 2H), 7.55 (d, *J* = 9.0 Hz, 2H), 7.14 (s, 1H), 7.08 (d, J = 5.3 Hz, 1H), 6.95 – 6.87 (m, 2H), 3.96 (t, *J* = 6.5 Hz, 2H), 3.67 (s, 3H), 3.17 (s, 2H), 2.69 (s, 5H), 2.56 (s, 5H), 2.35 (s, 3H), 2.35 – 2.30 (m, 3H), 1.88 – 1.74 (m, 2H), 1.67 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.55 – 1.45 (m, 2H), 1.45 – 1.33 (m, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 174.20, 168.49, 164.06,

160.59, 158.46, 154.99, 139.90, 132.79, 132.65, 128.01, 121.62, 119.25, 114.88, 107.41, 68.21, 61.89, 55.16, 53.33, 51.47, 45.85, 34.02, 29.17, 28.90, 25.76, 24.88; **MS** (ESI) 561.3 $[M+H]^+$.

N-Hydroxy-7-(3-((4-(4-(2-(4-methylpiperazin-1-yl)acetamido)phenyl)pyrimidin-2-yl)amino)phenoxy)heptanamide 2,2,2-trifluoroacetate (**61**)

Step 1. The reaction was carried out following the synthesis procedure of **45e** with 2 M LiOH aqueous solution (180 μL, 0.36 mmol, 10 equiv.) in 2 mL THF to give acid **58**. $\mathbf{R_f}$ = 0.155 (MeOH/DCM = 5 : 95); **MS** (ESI) 547.4 $[M+H]^+$.

Step 2. Crude **58** from Step 1 was dissolved in 2 mL DMF and HATU (21 mg, 0.108 mmol, 8 equiv.), *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine (17 mg, 0.144 mmol, 4 equiv.) and TEA (40 μL, 0.288 mmol, 8 equiv.) were added. After work-up **59** was isolated and used without further purification. $\mathbf{R_f}$ = 0.2 (MeOH/DCM = 10 : 90).

Step 3. The title compound was synthesized following the procedure of **45e** using acetyl chloride (20 μL, 0.08 mmol, 3 equiv.). The collected solid was washed with DCM and purified by prep HPLC to obtain the target compound as an orange solid (6 mg, 32%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.39 (s, 1H), 10.17 (s, 1H), 9.61 (s, 1H), 8.52 (d, *J* = 5.2 Hz, 1H), 8.17 (d, *J* = 8.8 Hz, 2H), 7.80 (d, *J* = 8.8 Hz, 2H), 7.69 (s, 1H), 7.38 (d, *J* = 5.3 Hz, 1H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.18 (t, *J* = 8.1 Hz, 1H), 6.53 (dd, *J* = 8.1, 1.9 Hz, 1H), 3.97 (t, *J* = 6.4 Hz, 2H), 3.44 (s, 2H), 3.20 – 2.99 (m, 4H), 2.81 (s, 3H), 1.97 (t, *J* = 7.4 Hz, 2H), 1.78 – 1.68 (m, 2H), 1.53 (dt, *J* = 14.8, 7.5 Hz, 2H), 1.48 – 1.38 (m, 2H), 1.32 (dd, *J* = 14.8, 8.1 Hz, 2H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.84, 164.22, 163.90, 159.47, 159.29, 157.79, 141.62, 141.45, 132.07, 129.80, 128.48, 119.81,

112.05, 108.58, 107.92, 105.93, 105.86, 67.77, 49.65, 48.85, 32.69, 29.17, 28.85, 25.81, 25.58; **MS** (ESI) 562.2 $[M+H]^+$; **HPLC** 97.02%.

N-Hydroxy-7-(4-((4-(4-(2-(4-methylpiperazin-1-yl)acetamido)phenyl)pyrimidin-2-yl)amino)phenoxy)heptanamide hydrochloride (**62**)

Step 1. The reaction was carried out using **57** by following the synthesis procedure for **59** with 2 M LiOH aqueous solution (680 μL, 1.36 mmol, 10 equiv.) in 6 mL THF (4 mL)/H_2O (2 mL) mixed solvent to give crude **60**. $\mathbf{R_f}$ = 0.05 (MeOH/DCM = 5 : 95); **MS** (ESI) 646.5 $[M+H]^+$.

Step 2. Crude **60** from Step 1 was dissolved in 5 mL DMF and HATU (62 mg, 0.163 mmol, 1.2 equiv.), *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine (24 mg, 0.204 mmol, 1.5 equiv.) and DIPEA (185 μL, 1.09 mmol, 8 equiv.) were used. $\mathbf{R_f}$ = 0.08 (MeOH/DCM = 10 : 90).

Step 3. The title compound was synthesized following the procedure of **61** by using acetyl chloride (26 μL, 0.36 mmol, 3 equiv.). The collected solid was recrystallized in DCM by adding excess diethyl ether to obtain target compound **62** as a yellow solid (74 mg, 99%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.19 (s, 1H), 9.84 (s, 1H), 8.48 (d, *J* = 5.4 Hz, 1H), 8.18 (d, *J* = 8.5 Hz, 2H), 7.84 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.9 Hz, 2H), 7.41 (d, *J* = 5.6 Hz, 1H), 6.94 (d, *J* = 8.7 Hz, 2H), 3.96 – 3.91 (m, 6H), 3.36 (dd, *J* = 13.4, 7.1 Hz, 2H), 2.84 (s, 4H), 1.95 (dd, *J* = 14.1, 6.7 Hz, 2H), 1.75 – 1.64 (m, 3H), 1.51 (dt, *J* = 14.8, 7.5 Hz, 3H), 1.41 (dt, *J* = 14.4, 7.3 Hz, 4H), 1.35 – 1.23 (m, 3H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.67, 165.80, 164.06, 155.32, 154.99, 141.92, 132.06, 131.43, 128.97, 122.67, 119.92, 115.14, 107.15, 68.10, 49.61, 48.84, 42.12, 32.67, 29.10, 28.81, 25.73, 25.54; **MS** (ESI) 562.4 $[M+H]^+$; **HPLC** 96.26%.

N-(4-(2-((3-Morpholinophenyl)amino)pyrimidin-4-yl)phenyl)acetamide (**64b**)

The title compound was synthesized following a procedure similar to compound **6** using **5** (100 mg, 0.4 mmol), compound **63b** (120 mg, 0.67 mmol, 1.1 equiv.), X-Phos (25 mg, 0.052 mmol, 0.86 equiv.), $Pd_2(dba)\cdot CHCl_3$ (25 mg, 0.04 equiv., 0.024 mmol) and K_2CO_3 (177 mg, 1.28 mmol, 2.1 equiv.). Compound **64b** was isolated as a brick coloured solid (72 mg, 30%).

R_f = 0.54 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.18 (s, 1H), 9.46 (s, 1H), 8.49 (d, *J* = 5.2 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 2H), 7.74 (d, *J* = 8.8 Hz, 3H), 7.33 (d, *J* = 5.2 Hz, 1H), 7.20 (d, *J* = 8.3 Hz, 1H), 7.15 (t, *J* = 8.0 Hz, 1H), 6.57 (d, *J* = 7.9 Hz, 1H), 3.81 – 3.74 (m, 4H), 3.15 – 3.10 (m, 4H), 2.09 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.18, 163.38, 160.65, 159.30, 151.98, 142.32, 141.93, 131.55, 129.34, 128.03, 119.17, 110.78, 109.16, 107.58, 106.31, 66.66, 49.30, 24.60.

N-(4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)phenyl)acetamide (**64c**)

The title compound was synthesized following a procedure similar to **6** using compound **5** (150 mg, 0.61 mmol), **63c** (140 mg, 0.79 mmol, 1.3 equiv.), X-Phos (30 mg, 0.063 mmol, 0.1 equiv.), $Pd_2(dba)_3\cdot CHCl_3$ (0.05 equiv., 0.03 mmol, 30 mg) and K_2CO_3 (177 mg, 1.28 mmol, 2.1 equiv.). Compound **64c** was isolated as a brown solid (124 mg, 52%).

R_f = 0.29 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, MeOD-*d4*) δ 8.35 (d, *J* = 5.3 Hz, 1H), 8.11 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.61 (d, *J* = 9.0 Hz, 2H), 7.21 (d, *J* = 5.4 Hz, 1H), 7.00 (d, *J* = 9.0 Hz, 2H), 3.90 – 3.82 (m, 4H), 3.16 – 3.07 (m, 4H), 2.16 (s, 3H); **MS** (ESI) 390.2 $[M+H]^+$.

N-(4-(2-((3-(piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)acetamide (**64d**)

The title compound was synthesized following a procedure similar to **6** using compound **5** (100 mg, 0.4 mmol), **63d** (85 mg, 0.48 mmol, 1.2 equiv.), X-Phos (20 mg, 0.042 mmol, 0.105 equiv.), $Pd_2(dba)_3 \cdot CHCl_3$ (20 mg, 0.019 mmol 0.048 equiv.) and K_2CO_3 (116 mg, 0.84 mmol, 2.1 equiv.). Compound **64d** was isolated as a brown solid (81 mg, 52%).

^{1}H NMR (400 MHz, MeOD-*d4*) δ 8.40 (d, *J* = 5.3 Hz, 1H), 8.14 (d, *J* = 8.7 Hz, 2H), 7.76 (t, *J* = 2.0 Hz, 1H), 7.72 (d, *J* = 8.7 Hz, 2H), 7.24 (d, *J* = 5.3 Hz, 1H), 7.18 (t, *J* = 8.1 Hz, 1H), 7.08 (dd, *J* = 8.0, 1.1 Hz, 1H), 6.67 (dd, *J* = 7.9, 2.0 Hz, 1H), 3.22 – 3.16 (m, 4H), 2.16 (s, 3H), 1.82 – 1.72 (m, 4H), 1.66 – 1.54 (m, 2H); **^{13}C NMR** (100 MHz, MeOD-*d4*) δ 170.44, 164.12, 160.41, 158.17, 152.85, 141.21, 141.01, 132.44, 128.64, 127.53, 119.31, 111.09, 110.96, 108.31, 106.92, 51.31, 25.60, 24.03, 22.61; **MS** (ESI) 388.2 $[M+H]^+$; **HPLC** 99.08%.

N-(4-(2-((4-(Piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)acetamide (**64e**)

The title compound was synthesized following a procedure similar to **6** using compound **5** (150 mg, 0.61 mmol), **63e** (128 mg, 0.73 mmol, 1.2 equiv.), X-Phos (30 mg, 0.063 mmol, 0.1 equiv.), $Pd_2(dba)_3 \cdot CHCl_3$ (0.05 equiv., 0.03 mmol, 30 mg) and K_2CO_3 (177 mg, 1.28 mmol, 2.1 equiv.) and further purified by prep-HPLC. Compound **64e** was isolated as a brown solid (152 mg, 65%).

R_f = 0.22 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.23 (s, 1H), 9.91 (s, 1H), 8.54 (d, *J* = 5.3 Hz, 1H), 8.14 (d, *J* = 8.7 Hz, 2H), 7.98 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.7 Hz, 2H), 7.60 (d, *J* = 7.2 Hz, 2H), 7.42 (d, *J* = 5.3 Hz, 1H), 3.51 (s, 4H), 2.09 (s, 3H), 1.90 (s, 4H), 1.67 (s, 2H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.24, 163.77, 160.24, 159.24, 142.45, 131.21, 128.14, 121.82, 119.86, 119.30, 108.48, 56.36, 31.13, 24.59, 23.97, 21.35; **MS** (ESI) 388.2 $[M+H]^+$; **HPLC** 97.40%.

N-(4-(2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)acetamide (**64f**)

The title compound was synthesized following a procedure similar to **6** using compound **5** (150 mg, 0.61 mmol), **63f** (93 mg, 0.48 mmol, 1.2 equiv.), X-Phos (20 mg, 0.042 mmol, 0.1 equiv.), $Pd_2(dba)_3 \cdot CHCl_3$ (20 mg, 0.019 mmol, 0.048 equiv.) and K_2CO_3 (116 mg, 0.84 mmol, 2.1 equiv.). Compound **64f** was isolated as a brown solid (126 mg, 78%).

R_f = 0.23 (MeOH/DCM = 1: 9); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.20 (s, 1H), 9.33 (s, 1H), 8.43 (d, *J* = 5.2 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 2H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.65 (d, *J* = 9.0 Hz, 2H), 7.25 (d, *J* = 5.3 Hz, 1H), 6.92 (d, *J* = 9.1 Hz, 2H), 3.09 – 3.03 (m, 4H), 2.48 – 2.42 (m, 4H), 2.21 (s, 3H), 2.09 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.13, 163.45, 160.76, 159.13, 146.57, 142.19, 133.24, 131.62, 127.97, 120.65, 119.24, 116.37, 106.99, 55.21, 49.39, 46.26, 24.61; **MS** (ESI) 403.2 $[M+H]^+$; **HPLC** 92.18%.

4-(4-Aaminophenyl)-N-(3-(piperidin-1-yl)phenyl)pyrimidin-2-amine (**65d**)

The reaction was carried out following the procedure of **55** using compound **64d** (75 mg, 0.19 mmol) in 4 mL MeOH, and 4N HCl/Dioxane (190 μL, 0.76 mmol, 4 equiv.). Compound **65d** was isolated as an orange brown solid (75 mg, 99%).

R_f = 0.4 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.35 (d, *J* = 5.3 Hz, 1H), 7.96 (d, *J* = 8.7 Hz, 2H), 7.60 (s, 1H), 7.31 (s, 1H), 7.07 (s, 1H), 7.04 (d, *J* = 5.3 Hz, 1H), 7.00 (dd, *J* = 7.6, 1.5 Hz, 1H), 6.74 (d, *J* = 8.7 Hz, 2H), 6.63 (dd, *J* = 7.9, 1.5 Hz, 1H), 3.96 (s, 2H), 3.25 – 3.19 (m, 4H), 1.77 – 1.70 (m, 4H), 1.63 – 1.58 (m, 2H); **MS** (ESI) 348.2 $[M+H]^+$.

4-(4-Aminophenyl)-N-(4-(piperidin-1-yl)phenyl)pyrimidin-2-amine (**65e**)

Following the procedure of **55** using compound **64e** (152 mg, 0.39 mmol) in 5 mL MeOH, and 4N HCl/Dioxane (390 μL, 1.24 mmol, 4 equiv.). Compound **64f** was isolated as a brown solid (166 mg, 99%).

R_f = 0.41 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 5.3 Hz, 1H), 7.92 (d, *J* = 8.7 Hz, 2H), 7.53 (d, *J* = 9.0 Hz, 2H), 6.99 (d, *J* = 5.3 Hz, 2H), 6.94 (d, *J* = 11.2 Hz, 1H), 6.74 (d, *J* = 8.7 Hz, 2H), 3.94 (s, 2H), 3.15 – 3.07 (m, 4H), 1.81 – 1.67 (m, 4H), 1.62 – 1.55 (m, 2H); **MS** (ESI) 346.2 $[M+H]^+$.

4-(4-Aminophenyl)-*N*-(4-(4-methylpiperazin-1-yl)phenyl)pyrimidin-2-amine (**65f**)

Following the procedure of **55** using compound **64f** (100 mg, 0.25 mmol) in 5 mL MeOH, and 4N HCl/Dioxane (500 μL, 2 mmol, 8 equiv.). Compound **65f** was isolated as a dark brown solid (64 mg, 71%).

R_f = 0.09 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 5.3 Hz, 1H), 7.91 (d, *J* = 8.7 Hz, 2H), 7.55 (d, *J* = 8.9 Hz, 2H), 7.00 (d, *J* = 5.3 Hz, 1H), 6.96 (d, *J* = 9.0 Hz, 3H), 6.74 (d, *J* = 8.7 Hz, 2H), 3.94 (s, 2H), 3.21 – 3.15 (m, 4H), 2.62 – 2.57 (m, 4H), 2.36 (s, 3H); **MS** (ESI) 361.2 $[M+H]^+$.

Methyl 8-oxo-8-((4-(2-(phenylamino)pyrimidin-4-yl)phenyl)amino)octanoate (**66a**)

Step 1. The reaction is followed by the procedure of **57**. Compound **6** was dissolved in MeOH (5 mL), and 4N HCl/Dioxane (280 μL, 1.13 mmol, 4 eq.) was added. Compound **65a** was used directly for the next step. **R_f** = 0.66 (MeOH/DCM = 5 : 95).

Step 2. The crude **65a** from Step 1 was dissolved in DMF (7 mL) and HATU (361 mg, 0.95 mmol, 2.5 eq.), 8-methoxy-8-oxooctanoic acid (136 μL, 0.76 mmol, 2 eq.) and DIPEA (517 μL, 3.04 mmol, 8 eq.) were added. Compound **66a** was used directly for the next step (**MS** (ESI) 433.2 $[M+H]^+$).

Methyl 8-((4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)phenyl)amino)-8-oxooctanoate (**66c**)

Following the procedure of **55**:

Step 1. Compound **64c** (120 mg, 0.31 mmol) was dissolved in 5 mL MeOH, and 4N HCl/Dioxane (310 μL, 1.24 mmol, 4 equiv.). Compound **65c** was used directly for the next step. $\mathbf{R_f}$ = 0.56 (MeOH/DCM = 5 : 95).

Step 2. The crude product from Step 1 was dissolved in 6 mL DMF and HATU (142 mg, 0.38 mmol, 1.5 equiv.), 8-methoxy-8-oxooctanoic acid (53 μL, 0.3 mmol, 1.2 equiv.) and DIPEA (340 μL, 2 mmol, 8 equiv.) were added. Compound **66c** was isolated as a light yellow solid (75 mg, 58%).

$\mathbf{R_f}$ = 0.35 (EA/Hexane = 1 : 1); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.10 (s, 1H), 9.33 (s, 1H), 8.43 (d, *J* = 5.2 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 2H), 7.75 (d, *J* = 8.8 Hz, 2H), 7.67 (d, *J* = 9.0 Hz, 2H), 7.26 (d, *J* = 5.3 Hz, 1H), 6.93 (d, *J* = 9.1 Hz, 2H), 3.83 – 3.69 (m, 4H), 3.58 (s, 3H), 3.13 – 2.99 (m, 4H), 2.41 – 2.23 (m, 4H), 1.60 (dt, *J* = 10.5, 5.3 Hz, 2H), 1.53 (dd, *J* = 14.4, 7.3 Hz, 2H), 1.39 – 1.28 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.81, 172.06, 163.48, 160.75, 159.12, 146.55, 142.20, 133.59, 131.57, 127.96, 120.65, 119.31, 116.14, 107.05, 66.67, 51.62, 49.81, 36.89, 33.71, 31.15, 28.77, 28.69, 25.32, 24.79; **MS** (ESI) 518.2 $[M+H]^+$.

Methyl 8-oxo-8-((4-(2-((3-(piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)amino)octanoate (**66d**)

The title compound was synthesized following the procedure of **57** step 2 using **65d** (75 mg, 0.22 mmol) in 5 mL DMF and HATU (251 mg, 0.66 mmol, 3 equiv.), 8-methoxy-8-oxooctanoic acid (80 μL, 0.434 mmol, 2 equiv .) and TEA (245 μL, 1.76 mmol, 8 equiv.). Compound **66d** was isolated as a pink solid (80 mg, 71%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.14 (s, 1H), 9.44 (s, 1H), 8.49 (d, *J* = 5.2 Hz, 1H), 8.15 (d, *J* = 8.8 Hz, 2H), 7.77 (d, *J* = 8.9 Hz, 3H), 7.33 (d, *J* = 5.3 Hz, 1H), 7.12 (d, *J* = 5.3 Hz, 2H), 6.60 – 6.50 (m, 1H), 3.59 (s, 3H), 3.20 – 3.14 (m, 4H), 2.38 – 2.27 (m, 4H), 1.73 – 1.64 (m, 4H), 1.62 – 1.48 (m, 6H), 1.36 – 1.27 (m, 4H).

Methyl 8-oxo-8-((4-(2-((4-(piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)amino)octanoate (**66e**)

The title compound was synthesized following the procedure of **57** step 2 using **65e** (166 mg, 0.32 mmol) in 10 mL DMF and HATU (548 mg, 1.44 mmol, 3 equiv.), 8-methoxy-8-oxooctanoic acid (185 μL, 0.96 mmol, 2 equiv.) and TEA (535 μL, 3.84 mmol, 8 equiv.). Compound **66e** was isolated as a yellow solid (130 mg, 53%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.11 (s, 1H), 9.31 (s, 1H), 8.42 (d, *J* = 5.2 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 2H), 7.75 (d, *J* = 8.8 Hz, 2H), 7.63 (d, *J* = 9.1 Hz, 2H), 7.25 (d, *J* = 5.3 Hz, 1H), 6.91 (d, *J* = 9.1 Hz, 2H), 3.58 (s, 3H), 3.08 – 3.02 (m, 4H), 2.32 (dt, *J* = 14.9, 7.4 Hz, 4H), 1.79 – 1.57 (m, 6H), 1.56 – 1.44 (m, 4H), 1.37 – 1.26 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.81, 172.06, 163.46, 160.78, 159.10, 147.45, 142.19, 133.06, 131.58, 127.95, 120.67, 119.31, 117.00, 106.96, 51.62, 51.03, 36.89, 33.71, 28.77, 28.70, 25.95, 25.33, 24.79, 24.37.

Methyl 8-((4-(2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)amino)-8-oxooctanoate (**66f**)

The title compound was synthesized following the procedure of **57** step 2 using **65f** (64 mg, 0.18 mmol) in DMF and HATU (205 mg, 0.54 mmol, 3 equiv.), 8-methoxy-8-oxooctanoic acid (70 μL, 0.36 mmol, 2 equiv.) and TEA (200 μL, 1.44 mmol, 8 equiv.). Compound **66f** was isolated as a yellow solid (58 mg, 61%).

R_f = 0.23 (MeOH/DCM = 1: 9); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 10.12 (s, 1H), 9.33 (s, 1H), 8.43 (s, 1H), 8.11 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.8 Hz, 2H), 7.65 (d, *J* = 9.0 Hz, 2H), 7.26 (d, *J* = 5.3 Hz, 1H), 6.93 (d, *J* = 9.1 Hz, 2H), 3.59 (s, 4H), 3.11 – 3.05 (m, 4H), 2.48 – 2.44 (m, 4H), 2.33 (dt, *J* = 14.9, 7.4 Hz, 4H), 2.23 (s, 3H), 1.62 (dd, *J* = 14.6, 7.2 Hz, 2H), 1.54 (dd, *J* = 14.4, 7.2 Hz, 2H), 1.35 – 1.27 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 173.81, 172.07, 163.46, 160.76, 159.12, 146.57, 142.20, 133.25, 131.57, 127.96, 120.65, 119.31, 116.37, 106.99, 55.22, 51.63, 49.39, 46.27, 36.89, 33.71, 28.77, 28.70, 25.33, 24.79.

N^1-(4-(2-(phenylamino)pyrimidin-4-yl)phenyl)-*N^8*-((tetrahydro-2H-pyran-2-yl)oxy)octanediamide **(68a**)

Step 1. Following the synthesis procedure of **59** with 2 M LiOH aqueous solution (850 μL, 1.7 mmol, 10 eq.) in 3 mL THF. The crude **67a** was used directly for the next step (**MS** (ESI) 419.2 $[M+H]^+$).

Step 2. The crude product from Step 1 was in 4 mL DMF and HATU (78 mg, 0.204 mmol, 1.2 eq.), *O*-(tetrahydro-*2H*-pyran-2-yl) hydroxylamine (40 mg, 0.34 mmol, 2 eq.) and TEA (190 μL, 1.36 mmol, 8 eq.) were used. Compound **68a** was isolated as a beige coloured oil (77 mg, 88%). **R_f** = 0.26 (MeOH/DCM = 5 : 95); **MS** (ESI) 518.2 $[M+H]^+$.

N^1-hydroxy-N^8-(4-(2-((4-morpholinophenyl)amino)pyrimidin-4-yl)phenyl)octanediamide 2,2,2-trifluoroacetate (**69c**)

Step 1. The reaction was carried out by following the synthesis procedure for **44e** with 2 M LiOH aqueous solution (676 μL, 1.35 mmol, 10 equiv.) in 6 ml THF/H_2O (5 : 1). The crude **67c** was used directly for the next step (**MS** (ESI) 504.3 $[M+H]^+$).

Step 2. The crude **67c** from Step 1 was dissolved in DMF (5 mL) and HATU (64 mg, 0.17 mmol, 1.2 equiv.), *O*-(tetrahydro-*2H*-pyran-2-yl) hydroxylamine (33 mg, 0.28 mmol, 2 equiv.) and DIPEA (119 μL, 0.7 mmol, 5 equiv.) were added. Compound **68c** was isolated as an amber liquid (82 mg, 97%). $\mathbf{R_f}$ = 0.2 (MeOH/DCM = 5 : 95).

Step 3. The title compound was synthesized following the procedure of **61** using acetyl chloride (47.1 μL, 0.66 mmol, 3 equiv.). The collected solid was washed with diethyl ether and purified by prep HPLC to obtain the **69c** as an orange brown solid (6 mg, 11%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.33 (s, 1H), 10.13 (s, 1H), 9.49 (s, 1H), 8.45 (d, *J* = 4.7 Hz, 1H), 8.12 (d, *J* = 8.6 Hz, 2H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 4.3 Hz, 1H), 7.05 (d, *J* = 8.1 Hz, 2H), 3.79 (s, 4H), 3.15 (s, 4H), 2.35 (t, *J* = 7.4 Hz, 2H), 1.95 (t, *J* = 7.3 Hz, 2H), 1.67 – 1.55 (m, 2H), 1.55 – 1.44 (m, 2H), 1.38 – 1.17 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 172.16, 169.59, 164.11, 159.99, 158.98, 158.62, 158.21, 142.49, 131.23, 128.18, 120.85, 119.36, 117.54, 107.38, 66.14, 50.88, 36.94, 32.72, 28.86, 25.44; **MS** (ESI) 519.3 $[M+H]^+$; **HPLC** 99.52%.

N^1-hydroxy-N^8-(4-(2-((3-(piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)octanediamide hydrochloride (**69d**)

Step 1. The reaction was carried out following the synthesis procedure for **59** with 2 M LiOH aqueous solution (800 μL, 2 mmol, 10 equiv.) in 5 mL THF/H_2O (4 : 1). The crude **67d** was used directly for the next step. $\mathbf{R_f}$ = 0.08 (MeOH/DCM = 5 : 95).

Step 2. The crude **67d** from Step 1 was dissolved in 6 mL DMF and HATU (183 mg, 0.48 mmol, 3 equiv.), *O*-(tetrahydro-*2H*-pyran-2-yl) hydroxylamine (75 mg, 0.64 mmol, 4 equiv.) and TEA (180 μL, 1.28 mmol, 8 equiv.) were added. Compound **68d** was isolated as a yellow solid (98 mg, 99%). **MS** (ESI) 601.3 $[M+H]^+$.

Step 2. Compound **68d** was dissolved in DCM (5 mL) and 4N HCl/Dioxane (120 μL, 0.33 mmol, 3 equiv.) was added into the solution. The reaction mixture was stirred at RT overnight and filtered through filter paper to collect a precipitate. The collected residue was dissolved in MeOH and excess DCM was added to precipitate the title compound which was filtered and dried to give **68e** as a dark yellow solid (28 mg, 33%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.38 (s, 1H), 10.29 (s, 1H), 10.07 (s, 1H), 8.57 (d, *J* = 5.3 Hz, 1H), 8.48 (s, 1H), 8.18 (d, *J* = 8.7 Hz, 2H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.73 (d, *J* = 7.8 Hz, 1H), 7.51 (t, *J* = 8.1 Hz, 1H), 7.45 (t, *J* = 6.7 Hz, 2H), 3.65 – 3.45 (m, 4H), 2.36 (t, *J* = 7.3 Hz, 2H), 2.18 – 1.85 (m, 6H), 1.72 (s, 2H), 1.66 – 1.56 (m, 2H), 1.54 – 1.43 (m, 2H), 1.38 – 1.18 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 172.22, 169.56, 163.98, 159.96, 158.98, 142.67, 142.34, 131.01, 130.49, 128.34, 119.32, 114.34, 112.03, 108.57, 56.41, 36.93, 32.71, 28.86, 25.49, 25.40, 23.49; **MS** (ESI) 517.2 $[M+H]^+$; **HPLC** 90.53%.

N^1-Hydroxy-*N^8*-(4-(2-((4-(piperidin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)octanediamide 2,2,2-trifluoroacetate (**69e**)

Step 1. The reaction was carried out by following the synthesis procedure for **59** with 2 M LiOH aqueous solution (1 mL, 2 mmol, 10 equiv.) in 10 mL THF/H_2O (10 : 1). The crude **67e** was used directly for the next step (**MS** (ESI) 502.3 $[M+H]^+$).

Step 2. The crude **67e** from Step 1 was dissolved in 11 mL mixed solvent (10 mL DMF + 1 mL H_2O) and HATU (217 mg, 0.57 mmol, 3 equiv.), *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine (89 mg, 0.76 mmol, 4 equiv.) and TEA (215 μL, 1.52 mmol, 8 equiv.) were used. Compound **68e** was isolated as a brown solid (80 mg, 70%). $\mathbf{R_f}$ = 0.15 (MeOH/DCM = 5 : 95).

Step 3. The title compound was synthesized following the procedure of **61** using acetyl chloride (30 μL, 0.4 mmol, 3 equiv.). The collected solid was washed with diethyl ether and purified by column to obtain the target compound as a beige solid (20 mg, 60%).

$\mathbf{R_f}$ = 0.49 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, DMSO-*d6*) δ 12.61 (s, 1H), 10.38 (s, 1H), 10.11 (s, 1H), 8.54 (d, *J* = 5.4 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 9.1 Hz, 2H), 7.86 (d, *J* = 9.1 Hz, 2H), 7.82 (d, *J* = 8.8 Hz, 2H), 7.46 (d, *J* = 5.5 Hz, 1H), 3.51 (s, 4H), 2.36 (t, *J* = 7.4 Hz, 2H), 2.31 – 2.01 (m, 3H), 1.95 (t, *J* = 7.3 Hz, 3H), 1.88 – 1.71 (m, 2H), 1.67 – 1.54 (m, 3H), 1.54 – 1.42 (m, 2H), 1.40 – 1.15 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 172.30, 169.61, 164.42, 159.38, 158.12, 142.86, 141.66, 136.85, 130.79, 128.32, 122.46, 120.01, 119.39, 108.45, 56.62, 36.92, 32.69, 28.84, 25.49, 25.40, 23.38, 21.25; **MS** (ESI) 517.2 $[M+H]^+$; **HPLC** 99.52%.

N^1-Hydroxy-N^8-(4-(2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)octanediamide 2,2,2-trifluoroacetate (**69f**)

Step 1. The reaction was carried out by following the synthesis procedure for **59** with 2 M LiOH aqueous solution (500 μL, 1 mmol, 10 equiv.) in 8 mL THF/H_2O (3 : 1). The crude **67f** was used directly for the next step. $\mathbf{R_f}$ = 0.09 (MeOH/DCM = 1: 9).

Step 2. The crude **67f** from Step 1 was dissolved in DMF (7 mL) and HATU (114 mg, 0.3 mmol, 3 equiv.), *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine (47 mg, 0.4 mmol, 4 equiv.) and TEA (150 μL, 1 mmol, 8 equiv.) were used. Compound **68f** was isolated as a yellow solid (40 mg, 65%).

$\mathbf{R_f}$ = 0.125 (MeOH/DCM = 1: 9); **^{1}H NMR** (400 MHz, MeOD-*d4*) δ 8.31 (d, *J* = 5.3 Hz, 1H), 8.07 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 8.7 Hz, 2H), 7.58 (d, *J* = 9.0 Hz, 2H), 7.14 (d, *J* = 5.3 Hz, 1H), 6.94 (d, *J* = 9.1 Hz, 2H), 4.04 – 3.94 (m, 1H), 3.61 – 3.53 (m, 1H), 3.16 – 3.08 (m, 4H), 2.62 – 2.57 (m, 4H), 2.37 (t, *J* = 7.5 Hz, 2H), 2.11 (t, *J* = 7.4 Hz, 2H), 1.85 – 1.48 (m, 12H), 1.46 – 1.31 (m, 4H).

Step 3. The title compound was synthesized following the procedure of **61** using 4N HCl/Dioxane (50 μL, 0.33 mmol, 3 equiv.) in DCM (5 mL). The final purification was achieved by prep-HPLC to give **69f** a yellow solid (26 mg, 62%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.36 (s, 1H), 10.16 (s, 1H), 9.70 (s, 1H), 9.43 (s, 1H), 8.45 (d, *J* = 5.3 Hz, 1H), 8.11 (d, *J* = 8.7 Hz, 2H), 7.76 (d, *J* = 8.6 Hz, 2H), 7.71 (d, *J* = 9.0 Hz, 2H), 7.30 (d, *J* = 5.3 Hz, 1H), 7.01 (d, *J* = 9.0 Hz, 2H), 3.76 (d, *J* = 13.1 Hz, 4H), 3.19 (s, 4H), 2.88 (s, 4H), 2.35 (t, *J* = 7.3 Hz, 2H), 1.95 (t, *J* = 7.3 Hz, 2H), 1.60 (dt, *J* = 14.9, 7.6 Hz, 2H), 1.50 (dt, *J* = 14.6, 7.3 Hz, 2H), 1.30 (dd, *J* = 7.6, 4.2 Hz, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 172.19, 169.64, 163.65, 160.49, 158.91, 144.84, 142.29, 134.31, 131.44, 128.02, 120.69, 119.35, 117.17, 107.23, 52.93, 46.95, 42.61, 36.91, 32.70, 28.85, 25.48, 25.40; **MS** (ESI) 532.2 $[M+H]^+$; **HPLC** 92.56%.

Methyl 8-((4-(5-methyl-2-((4-morpholinophenyl)amino)pyrimidin-4-yl)phenyl)amino)-8-oxooctanoate (**71a**)

The reaction was carried out following the procedure of **49b**:

Step 1. Compound *N*-(4-(5-methyl-2-((4-morpholinophenyl)amino)pyrimidin-4-yl)phenyl) acetamide (**49b**) (137 mg, 0.34 mmol) was dissolved in MeOH (5 mL), and 4N HCl/Dioxane (340 μL, 1.36 mmol, 4 equiv.) was added. Compound **70a** was used directly for the next step. $\mathbf{R_f}$ = 0.39 (MeOH/DCM = 5 : 95).

Step 2. The crude **70a** from Step 1 was dissolved in DMF (6 mL) and HATU (194 mg, 0.51 mmol, 1.5 equiv.), 8-methoxy-8-oxooctanoic acid (73 μL, 0.41 mmol, 1.2 equiv.) and DIPEA (463 μL, 2.72 mmol, 8 equiv.) were added. Compound **71a** was isolated as a brown oil (229 mg, quant.).

$\mathbf{R_f}$ = 0.37 (MeOH/DCM = 5 : 95); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.02 (s, 1H), 7.64 (s, 3H), 7.53 (d, *J* = 8.9 Hz, 2H), 6.99 (s, 1H), 6.91 (d, *J* = 8.9 Hz, 2H), 3.89 – 3.85 (m, 4H), 3.13 – 3.09 (m, 4H), 2.96 (s, 3H), 2.88 (s, 3H), 2.40 (d, *J* = 7.3 Hz, 2H), 2.33 (t, *J* = 7.4 Hz, 3H), 1.83 – 1.72 (m, 3H), 1.67 (dd, *J* = 14.7, 7.2 Hz, 3H), 1.46 – 1.37 (m, 4H); **MS** (ESI) 532.3 $[M+H]^+$.

N^1-Hydroxy-N^8-(4-(5-methyl-2-((4-morpholinophenyl)amino)pyrimidin-4-yl)phenyl)octanediamide (**74a**)

Step 1. Following the synthesis procedure of **44e**, compound **71a** was dissolved in THF (6 mL) and 2 M LiOH aqueous solution (1.7 μL, 3.4 mmol, 10 equiv.) was added. Compound **72a** was used directly for the next step (**MS** (ESI) 518.3 $[M+H]^+$).

Step 2. The crude **72a** from Step 1 was dissolved in DMF (5 mL) and HATU (155 mg, 0.41 mmol, 1.2 equiv.), *O*-(tetrahydro-*2H*-pyran-2-yl) hydroxylamine (80 mg, 0.68 mmol, 2 equiv.) and DIPEA (289 μL, 1.7 mmol, 5 equiv.) were added. Compound **73a** was isolated as a brown oil (110 mg, 52%). $\mathbf{R_f}$ = 0.16 (MeOH/DCM = 5 : 95); **MS** (ESI) 617.3 $[M+H]^+$.

Step 3. The title compound was synthesized following the procedure of **45e** using **73a** in acetyl chloride (47.1 μL, 0.66 mmol, 3 equiv.). The collected solid was washed with diethyl ether and purified by prep HPLC to furnish **74a** as an orange solid (5 mg, 4%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.36 (s, 1H), 10.09 (s, 1H), 9.44 (s, 1H), 7.69 (dd, *J* = 35.1, 8.6 Hz, 6H), 7.04 (s, 2H), 3.78 (s, 4H), 3.16 (d, *J* = 7.5 Hz, 4H), 2.33 (t, *J* = 7.4 Hz, 2H), 2.22 (s, 3H), 1.94 (d, *J* = 7.4 Hz, 2H), 1.60 (s, 2H), 1.50 (s, 2H), 1.29 (s, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 172.05, 169.59, 158.84, 140.70, 133.13, 129.87, 118.89, 117.65, 66.19, 50.85, 36.92, 32.72, 28.87, 25.50, 25.47, 16.56; **MS** (ESI) 533.3 $[M+H]^+$; **HPLC** 90.7%.

N-(4-(5-Methyl-2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)acetamide (**49c**)

The title compound was synthesized following a procedure similar to **6** using compound **5** (100 mg, 0.38 mmol), **63f** (88 mg, 0.46 mmol, 1.2 equiv.), X-Phos (20 mg, 0.042 mmol, 0.11 equiv.), $Pd_2(dba)_3{\cdot}CHCl_3$ (0.051 equiv., 0.019 mmol, 20 mg) and K_2CO_3 (110 mg, 0.8 mmol, 2.1 equiv.). Compound **49c** was isolated as a brown oil (110 mg, 69%) and used directly.

Methyl 8-((4-(5-methyl-2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)amino)-8-oxooctanoate (**71b**)

Step 1. Compound **49c** (110 mg, 0.12 mmol) was dissolved in MeOH (5 mL), and 4 N HCl/Dioxane (135 μL, 0.48 mmol, 4 equiv.) was added. Compound **70b** was used directly for the next step. $\mathbf{R_f}$ = 0.24 (MeOH/DCM = 1: 9).

Step 2. The crude **70b** from Step 1 was dissolved in DMF (5 mL) and HATU (297 mg, 0.78 mmol, 3 equiv.), 8-methoxy-8-oxooctanoic acid (100 μL, 0.52 mmol, 2 equiv.) and TEA (290 μL, 0.08 mmol, 8 equiv.) were added. Compound **71b** was isolated as a brown oil (80 mg, 92%).

$\mathbf{R_f}$ = 0.2 (MeOH/DCM = 1: 9); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.63 (s, 4H), 7.51 (d, *J* = 8.9 Hz, 2H), 6.93 (d, *J* = 9.0 Hz, 2H), 6.90 (s, 1H), 3.67 (s, 3H), 3.18 – 3.14 (m, 4H), 2.62 – 2.56 (m, 4H), 2.41 – 2.36 (m, 2H), 2.36 (s, 3H), 2.35 – 2.30 (m, 2H), 2.25 (s, 3H), 1.77 (dt, *J* = 15.2, 7.5 Hz, 2H), 1.66 (dd, *J* = 14.7, 7.4 Hz, 2H), 1.44 – 1.35 (m, 4H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 174.35, 171.78, 164.89, 159.75, 159.08, 146.90, 139.10, 134.09, 132.76, 129.62, 120.79, 119.27, 117.77, 117.08, 55.12, 51.52, 49.88, 46.06, 33.94, 28.75, 28.70, 25.27, 24.66, 16.48; **MS** (ESI) 545.3 $[M+H]^+$.

N^1-(4-(5-Methyl-2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)-*N^8*-((tetrahydro-2H-pyran-2-yl)oxy)octanediamide (**73b**)

Step 1. Following the synthesis procedure for **59** with **71b** being dissolved in THF (5 mL) and adding 2 M LiOH aqueous solution (735 μL, 1.47 mmol, 10 equiv.). The crude **72b** was used directly in the next step (**MS** (ESI) 531.2 $[M+H]^+$).

Step 2. The crude **72b** from Step 1 was dissolved in DMF (7 mL) and HATU (168 mg, 0.44 mmol, 3 equiv.), *O*-(tetrahydro-*2H*-pyran-2-yl) hydroxylamine (69 mg, 0.59 mmol, 4 equiv.) and TEA (165 μL, 1.2 mmol, 8 equiv.) were added. Compound **73b** was isolated as a yellowish brown oil (58 mg, 63%)

R_f = 0.2 (MeOH/DCM = 1: 9); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.32 (s, 1H), 8.23 (s, 1H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.49 (d, *J* = 8.9 Hz, 2H), 7.19 (s, 1H), 6.90 (d, *J* = 9.0 Hz, 2H), 3.96 (t, *J* = 8.9 Hz, 1H), 3.66 – 3.56 (m, 1H), 3.17 – 3.11 (m, 4H), 2.61 – 2.54 (m, 4H), 2.34 (s, 4H), 2.23 (s, 3H), 2.16 – 2.09 (m, 3H), 2.09 – 2.02 (m, 2H), 1.85 – 1.74 (m, 2H), 1.74 – 1.66 (m, 2H), 1.66 – 1.57 (m, 2H), 1.58 – 1.49 (m, 2H), 1.38 – 1.29 (m, 4H); **MS** (ESI) 630.3 $[M+H]^+$.

N^1-Hydroxy-*N^8*-(4-(5-methyl-2-((4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)phenyl)octanediamide 2,2,2-trifluoroacetate (**74b**)

The title compound was synthesized following the procedure of **61** taking **73b** up in 4 N HCl/Dioxane (70 μL, 0.28 mmol, 3 equiv.) in DCM (5 mL). Compound **74b** was obtained by dissolving the obtained crude in MeOH and adding excess DCM to precipitate a solid which was filtered and dried to give a brown solid (30 mg, 60%).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.83 (s, 1H), 10.29 (s, 1H), 10.27 (s, 1H), 9.78 (s, 1H), 8.36 (s, 1H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.67 (d, *J* = 8.7 Hz, 2H), 7.61 (d, *J* = 9.0 Hz, 2H), 7.00 (d, *J* = 9.1 Hz, 2H), 3.48 (d, *J* = 11.4 Hz, 4H), 3.09 (dd, *J* = 23.0, 10.6 Hz, 4H), 2.80 (s, 3H), 2.35 (t, *J* = 7.4 Hz, 2H), 2.24 (s, 3H), 1.95 (t, *J* = 7.4 Hz, 2H), 1.65 – 1.53 (m, 2H), 1.53 – 1.42 (m, 2H), 1.38 – 1.20 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 172.24, 169.63, 166.14, 157.35, 156.99, 145.14, 141.32, 133.31, 132.20, 130.12, 121.27, 118.9, 117.88, 117.29, 52.57, 46.74, 42.44, 36.90, 32.70, 28.84, 25.45, 16.58; **MS** (ESI) 546.2 $[M+H]^+$; **HPLC** 91.56%.

Molecular Modelling Experimental

The human JAK2 X-ray structure (3FUP - second kinase domain),[23] human HDAC2 structure (4LXZ)[24] and human HDAC6 structures (5EDU - second HDAC domain)[25] was downloaded from the

Protein Data Bank (http://www.rcsb.org) and prepared using the protein preparation wizard in Maestro version 11.2[26] using standard settings. This included the addition of hydrogen atoms, bond assignments, removal of water molecules further than 5 Å from the ligand, protonation state assignment, optimization of the hydrogen bond network and restrained minimization using the OPLS3 force field.[27] The non-HDAC part of the HDAC structures were deleted. The HDAC proteins were then superimposed using structural alignment and the HDAC2 structure ligand SAHA was duplicated into HDAC6. The ligands from the HDAC2 and JAK2 structure were used as templates for manual docking. The remaining part of the inhibitors were built onto the ligands in a low energy conformation. The JAK2 and HDAC6 inhibitor-protein complexes were finally minimized using Macromodel 11.6. [4] In the HDAC complexes, distance constraints between both the hydroxamate oxygen atoms and Zinc (2.3 Å) as well as the 3 residues that hydrogen bond with the hydroxamate (2.8 Å) were included. All residues more than 9 Å from the ligand were constrained before the complex was subjected to 500 steps of Polak-Ribiere-Conjugate-Gradient[28] minimization using the OPLS3 force field and GB/SA continuum solvation model.[29]

Enzyme Assays

Enzyme inhibition assays for HDAC and kinase enzymes (kinase 'Hotspot' profiling) were carried out by Reaction Biology Corporation (RBC) as previously described.[30] For HDAC assays: HDAC enzyme (2x) was added into the reaction plate except for the control wells (no enzyme), where buffer (50 mM tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, and 1 mM $MgCl_2$) was added instead. Inhibitors were added in 100% DMSO into the enzyme mixture via acoustic technology (Echo550; nanoliter range). Plate was spinned down and preincubated. Substrate mixture (2x) was added: Fluorogenic HDAC General Substrate: 50 μM, ArgHis-Lys-Lys(Ac). HDAC8 only substrate: 50 μM, Arg-His-Lys(Ac)Lys(Ac). Class2A substrate: acetyl-Lys(trifluoroacetyl)-AMC) in all reaction wells to initiate

the reaction. Spin and shake. Incubate for 2 h at 30 °C with seal. Add developer with trichostatin A to stop the reaction and to generate fluorescent color. Kinetic measurements were carried out for 1.5 h with Envision with 15 min interval (Ex/Em = 360/460 nm). Take end point reading for analysis after the development reaches plateau. Kinase assays were carried out according to the published procedures.[31] Kinase pro filing was performed using the "HotSpot" assay platform. Briefly, specific kinase/substrate pairs along with required cofactors were prepared in reaction buffer: 20 mM Hepes pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA, 0.02% Brij35, 0.02 mg/mL BSA, 0.1 mM Na_3VO_4, 2 mM DTT, and 1% DMSO. Compounds were delivered into the reaction, followed ~20 min later by addition of a mixture of ATP (Sigma) and ^{33}P ATP (PerkinElmer) to a final concentration of 10 μM. Reactions were carried out at 25 °C for 120 min, followed by spotting of the reactions onto P81 ion exchange filter paper (Whatman). Unbound phosphate was removed by extensive washing of filters in 0.75% phosphoric acid. After subtraction of background derived from control reactions containing inactive enzyme, kinase activity data were expressed as the percent remaining kinase activity in test samples compared to vehicle (dimethylsulfoxide) reactions. IC_{50} values and curve fits were obtained using Prism (GraphPad Software).

Cell proliferation inhibition assays

Human breast cancer cell lines MCF-7 and MDA-MB231, prostate cancer cell line PC-3 and colon cell line HCT-116 were purchased from ATCC (Rockville, MD) and assays carried out as previously described.[7] Erythroleukemia HEL92.1.7, acute T-cell leukemia jurkat, multiple myeloma cell lines, KMS12BM and XG-6 were used as for assays previously described.[7] Human breast cancer MCF-7 cells, human breast cancer MDA-MB231 cells, prostate cancer cell line PC-3, and colon cell line HCT-116 cells were purchased from ATCC (Rockville, MD). The first three cell lines were grown in DMEM Media (Invitrogen, Singapore) and the last in McCoy's Media. They were supplemented with

10% fetal bovine serum, 50 μg/mL penicillin, and 50 μg/mL streptomycin at 37 °C with 5% CO_2. The cells were subcultured to 80−90% confluency and used within 15−20 passages for the assay. Cell viability was assessed with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) as follows: cells were seeded at 2500 cells per well in a 96-well plate for 24 h. The media was removed and aliquots of test compounds (initially prepared as 10 mM stock solutions in DMSO) were added to each well and the plates were incubated for 72 h. The final amount of DMSO per well was maintained at 0.5% v/v. At the end of the incubation period, the media was removed and replaced with FBS free media (150 μL) and MTT (50 μL of 2 mg/mL solution in phosphate buffer saline, pH 7.4). After incubation for 3 h at 37 °C with 5% CO_2, the supernatant was removed and a solution of 100 μL of DMSO was added to dissolve the formazan crystals. Absorbance was measured at 570 nm on a micro plate reader (Tecan Infinite 200). Cell viability was determined from readings of treated wells compared to control wells (absence of test compound) with correction of background absorbance. The IC_{50} (concentration required to reduce cell viability to 50% of control/untreated cells) was determined in triplicates on separate occasions, using two different stock solutions. Percent viability readings for each test compound were plotted against log concentration on GraphPad Prism (version 5.0, GraphPad Software, San Diego, CA), with constraints set at ≥0 and ≤100%. A sigmoidal curve was generated from which the IC_{50} was obtained.

Immunoblotting

Western blot analyses of HEL92.1.7 and HeLa cell lines were performed as previously described.[7] Cells (5 x 10^5/mL) were seeded into 6-well plates and treated with inhibitor (0, 0.1, 0.5, and 1 μM) for 1 h. Cells were collected and washed with PBS and the cell pellet was lysed in RIPA buffer supplemented with phosphatase inhibitor cocktail (Roche Life Science) and protease inhibitor cocktail (Sigma-Aldrich). The lysate was loaded into 10% (HEL cells) or 8% (HeLa stable clone)

polyacrylamide gel. Proteins were then transferred to PVDF membrane (Millipore) and detected through specific antibodies: anti-acetylated tubulin (6−11B-1) (Biolegend), anti-STAT5 (Santa Cruz Biotechnology), anti-H3 (9715S), anti-acetylated H3 (Lys9/Lys14) (9677S), anti-pJAK2 (Y1007/1008), anti-JAK2 (D2E12), anti-pSTAT5 (Tyr694) and anti-tubulin (Cell Signaling Technology).

Determination of Toxicity in Transforming growth factor-alpha mouse hepatocyte (TAMH) and AC10 Human cardiomyocyte Cells

TAMH cell assay was carried out as previously described.[7] AC-10 cells were seeded at a cell density of 5000 cells/well (75,000 cells/ml) in a 96-well plate (NUNC) a day before drug treatment. Cells were then treated across a wide concentration range starting from a top concentration of 100 µM (Final DMSO %: 0.5% - 0.5 μL of drug in 99.5 μL of media in every well). Stock concentrations used were 100 μM, 10 μM and 1 μM) and final dilute concentrations were 100, 50, 25, 12.5, 6.25, 3.125, 1.5, 0.78 and 0.315 μM. Drug-treated cells were then incubated at 37 °C for 24 h. After 24 h, cell viability was determined with CellTiter-Glo® Cell Viability Assay (Promega Corporation) as per manufacturer's instructions. The cell-reagent mixture was then transferred to a solid white flat-bottom 96-well plate (Greiner) for luminescence reading. Luminescence was then recorded with an integration time of 0.25 s with a Tecan Infinite® M200 Microplate reader. Based on the analyses from the above mentioned concentration range, cell viability assay was repeated with a more focused drug concentration range as required to obtained consistent IC_{50} data.

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Highlights

- Two designs of new agents with a dual mode of action targeting both JAK2 kinase and HDACs are reported.
- Structure-activity relationships revealed potent inhibition of JAK2, HDAC6 and HDAC1.
- Compound **69c** had 16-25 fold selectivity against three other JAK-family proteins JAK1, JAK3 and TYK2.
- Compound **45h** has a cellular IC_{50} of 70 nM against the multiple myeloma cell line KMS-12-BM.
- Both JAK and HDAC pathway inhibition is shown in Hela cells; mechanism of cell death is via apoptosis.