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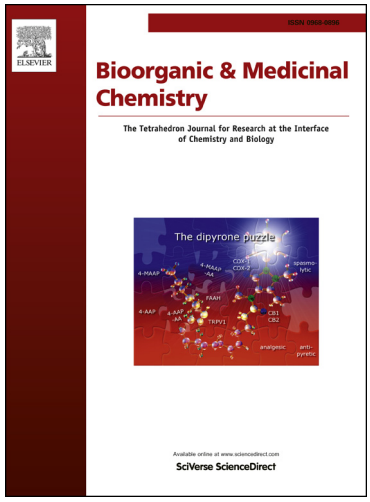

Discovery and SAR of novel pyrazolo[1,5-*a*]pyrimidines as inhibitors of CDK9

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Discovery and SAR of novel pyrazolo[1,5-*a*]pyrimidines as inhibitors of CDK9

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Keywords

pyrazolo[1,5-*a*]pyrimidine, CDK9 selectivity, CDK7, PI3Kα, FLT3, molecular modelling

Abstract

The serine-threonine kinase CDK9 is a target of emerging interest for the development of anti-cancer drugs. There are multiple lines of evidence linking CDK9 activity to cancer, including the essential role this kinase plays in transcriptional regulation through phosphorylation of the C-terminal domain (CTD) of RNA polymerase II. Indeed, inhibition of CDK9 has been shown to result in a reduction of short-lived proteins such as the pro-survival protein Mcl-1 in malignant cells leading to the induction of apoptosis. In this work we report our initial studies towards the discovery of selective CDK9 inhibitors, starting from the known multi-kinase inhibitor PIK-75 which possesses potent CDK9 activity. Our series is based on a pyrazolo[1,5-a]pyrimidine nucleus and, importantly, the resultant

lead compound **18b** is devoid of the structural liabilities present in PIK-75 and possesses greater selectivity.

1. Introduction

The Cyclin-Dependant Kinases (CDKs) are a family of serine-threonine kinases comprising more than 13 members. A number of CDKs (subtypes 1-4, 6 and 11) play a direct role in the regulation of the cell cycle,[1-4] while CDK subtypes 7-9 have been shown to control RNA polymerase II mediated transcription.[1] CDK9 is expressed in a range of human tissues and is highly expressed in terminally differentiated cells.[4] CDK9 is stabilised by the formation of heterodimers; these are either i) transient complexes with chaperones such as HSP70, HSP90 and Cdc37 or ii) active heterodimers with the T and K cyclins. The active heterodimers form the complex p-TEFb (positive transcription elongation factor b) where the cyclin forms the regulatory subunit and CDK9 the active enzyme component.[4] The p-TEFb complex plays two roles in the successful initiation and elongation of RNA transcription: phosphorylation of the RNA polymerase II C-terminus domain and usurping N-TEF (negative transcription elongation factor) leading to stable elongation of the RNA transcript.[5]

Inhibition of specific CDKs or CDK subsets has been the focus of considerable research and drug development activity over the last two decades with a number of inhibitors of varying selectivity profiles progressing to clinical trials, predominantly for the treatment of cancer.[3, 6] While a number of pan-specific CDK inhibitors (e.g. flavopiridol (**1**), SNS-032 (**2**), dinaciclib (**3**) and AT7519 (**4**)) have been examined in clinical trials, dose-limiting toxicities have been reported most likely due to their broad target inhibition.[7-9] More recently selective targeting of CDK9 for the treatment of various cancers has gained interest, since blocking RNA synthesis by the inhibition of CDK9 leads to a reduction in the levels of short-lived pro-survival proteins such as Mcl-1 and the induction of apoptosis.[3, 4] Of the many CDK inhibitors reported to possess potent activity against the CDK9 subtype [2, 3, 6, 10] few have been reported as CDK9 selective,[11, 12] one of which is the recently disclosed

LY2857785 (**5**).[13] The structures for some of the inhibitors with potent CDK9 activity are shown in *Figure 1*.

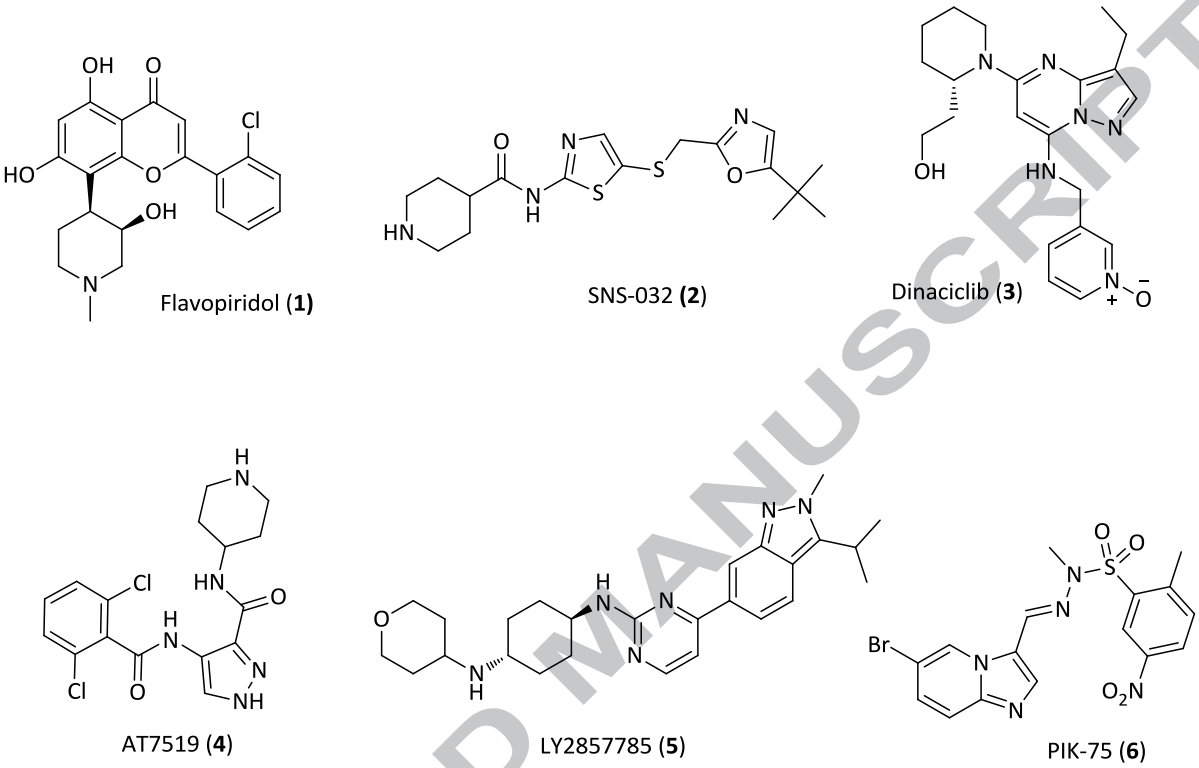


Figure 1. Structures of known CDK9 inhibitors (**1**-**5**) and the PI3Kα inhibitor PIK-75 (**6**)

As part of a phenotypic screening study looking for drugs targeting Mcl-1 in acute myeloid leukaemia (AML), we identified the literature standard PI3K p110α (PI3Kα) inhibitor PIK-75 (**6**)[14, 15] as being particularly active against a panel of AML cell lines and primary samples.[16] Further studies demonstrated that this compound also possessed significant CDK7 and CDK9 inhibitory activity and that this combination of activities was driving much of the PIK-75 mediated cytotoxicity in AML. Studies from other laboratories have also demonstrated that PIK-75 possesses significant CDK inhibitory activity resulting in the induction of apoptosis in diverse cancer cell lines.[17-19] In addition, our group showed in AML that the direct inhibition of CDK9 by PIK-75 causes blockade of the transcription of the *MCL-1* gene thus reducing levels of the short-lived pro-survival protein, Mcl-1,

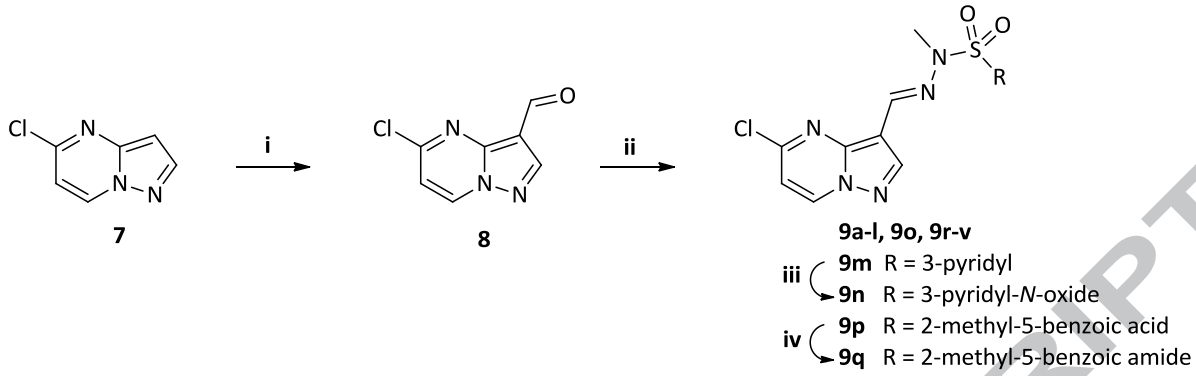
resulting in rapid cell death, given the critical survival role of Mcl-1 in AML.[20,21] Similarly, Lemke *et al* showed that PIK-75 leads to a reduction of the short-lived proteins Mcl-1 and c-FLIP, and subsequently a re-sensitisation of TRAIL resistant cancer cells to TRAIL.[18]

These data encouraged us to initiate a drug discovery program focused on the development of selective CDK9 inhibitors using PIK-75 as a chemical starting point. This compound, originally described by Hayakawa *et al* as a selective inhibitor of the kinase PI3Kα, shows significant activity against a number of kinases including CDK7, CDK9 and FLT3.[14-16] Furthermore, the compound possesses two non-drug-like structural motifs – a hydrazone linkage and aryl nitro moiety - that are perceived as developmental liabilities due to potential toxicity concerns.[22,23] Presumably as a consequence of these issues there has been little work published exploring variations on the PIK-75 structure [24-28] which offered the possibility to rapidly develop novel chemical matter. Below, we describe our preliminary work on a series of novel pyrazolo[1,5-*a*]pyrimidines that show an improved selectivity profile compared to PIK-75 and that are devoid of the structural liabilities of the original lead compound.

2. Results and Discussion

2.1 Synthesis

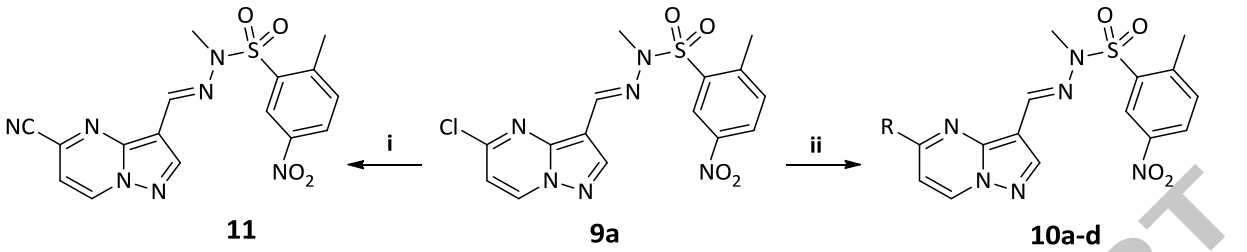
A series of sulfonylhydrazones were synthesised (*Scheme 1*) starting from commercially available 5-chloropyrazolo[1,5-*a*]pyrimidine (**7**) which was converted to the carbaldehyde (**8**) under Vilsmeier-Haack conditions.[25] A one pot, two-step procedure was then used to convert **8** to the desired aryl sulfonylhydrazones **9a-u** *via* condensation of **8** with methyl hydrazine, followed by sulfonylation with various sulfonyl chlorides. Use of 2,6-lutidine as base allowed isolation of the desired product in high purity *via* filtration, removing the need for aqueous workup and chromatography.



Scheme 1 Reagents: (i) $POCl_3$, DMF, RT; (ii) $MeHNNH_2.H_2SO_4$, $NaHCO_3$ or 2,6-lutidine then 2-arylsulfonyl chloride, RT; (iii) *m*CPBA, CH_2Cl_2, RT; (iv) HATU, DIPEA, $(NH_4)_2CO_3$, DMF, RT.

The pyridine N-oxide (**9n**) was synthesised by oxidation of the corresponding pyridine **9m** using *m*CPBA. Synthesis of the primary amide **9q** from the corresponding acid **9p** via an amide coupling reaction with ammonium carbonate proved problematic. Extended reaction times were necessary to effect complete conversion however these conditions also led to amination at the 5-chloro position of the pyrazolo[1,5-*a*]pyrimidine core. Shortening the reaction time resulted in a mixture of compounds from which **9q** could be isolated.

Alterations to the 5-position of the pyrazolo[1,5-*a*]pyrimidine core were achieved by modification of the sulfonylhydrazone **9a** (*Scheme 2*). A series of compounds with a C-C linkage in the 5-position were synthesised using palladium mediated chemistry. First, Suzuki-Miyaura coupling was used to install aromatic or heteroaromatic substituents in this position giving compounds **10b-e**. Synthesis of **10a** utilised trimethylboroxane as a methyl source[29] under conditions analogous to a Suzuki coupling, whilst palladium catalysed cyanation with zinc cyanide[30] was employed to furnish **11**.

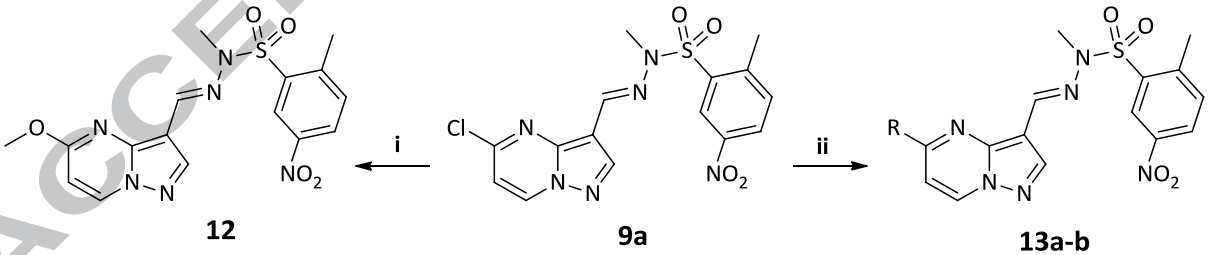


11 **9a** **10a-d**

a: R = Me
b: R = 3,4-dimethoxyphenyl
c: R = 3-pyridyl
d: R = 4-methoxy-3-pyridyl

Scheme 2 Reagents: (i) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 °C; (ii) R-pinacolatoboronate, K_2CO_3 or Na_2CO_3, $Pd(PPh_3)_4$, DME or 1,4-dioxane, 80-100 °C.

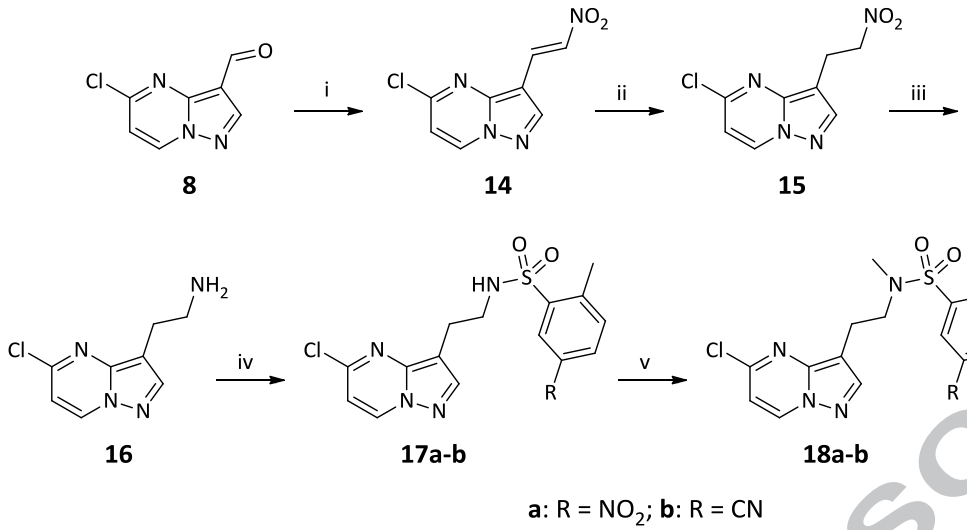
A second series of compounds were synthesised with a heteroatom linked directly to the 5-position of the pyrazolo[1,5-*a*]pyrimidine core (*Scheme 3*). Sodium methoxide was employed to give compound **12**. Compound **13b** was derived from reaction with morpholine under basic conditions while compound **13a** was the unexpected result of attempts to install a primary amine in the 5-position of **9a**, under identical conditions where this addition had been observed (as a side-reaction) in the synthesis of the primary amide **9q** (*vide supra*).



12 **9a** **13a-b**

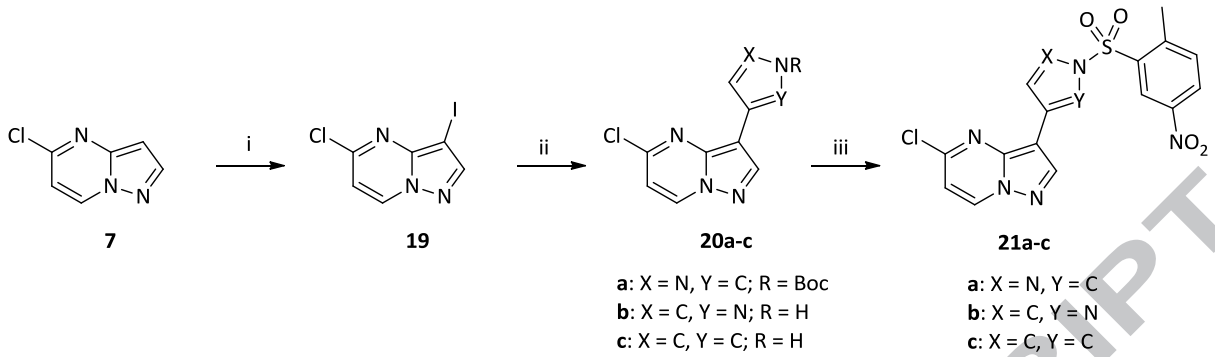
a: R = $N(Me)_2$
b: R = morpholine

Scheme 3 Reagents: (i) NaOMe, MeOH, 65 °C; (ii) $(NH_4)_2CO_3$, DIPEA, DMF, RT; or Morpholine, K_2CO_3, DMF, 70 °C.



Scheme 4 Reagents: (i) $MeNO_2$, $MeNH_2$.HCl, KOAc, MeOH, RT; (ii) $NaCNBH_3$, THF, 0 °C – RT; (iii) RaNi, H_2, MeOH, 40 °C; (iv) $ArSO_2Cl$, NEt_3, CH_2Cl_2, 0 °C – RT; (v) MeI, K_2CO_3, Acetone, RT.

Compounds **18a** and **18b** were synthesised from the aldehyde **8** (*Scheme 4*). The aldehyde was first subjected to a Henry reaction to give the nitroalkene **14**. Simultaneous reduction of the alkene and nitro groups could not be achieved under various conditions (e.g. $LiAlH_4$, BH_3) with dimerised products and partial nitro reduction being amongst the mixture of products obtained. Consequently, compound **16** was prepared in a two-step procedure. Reduction of the alkene was achieved using sodium cyanoborohydride; other reducing agents such as sodium borohydride resulted in compound degradation. Nitro group reduction was achieved with Raney nickel although some dechlorinated material, which could be removed by chromatography, was always observed. Treatment of compound **16** with the requisite sulfonyl chlorides, yielded compounds **17a-b** and alkylation of these using iodomethane furnished the desired compounds **18a-b** (respectively).



Scheme 5 Reagents: (i) NIS, DMF, RT; (ii) Boronic acid/pinacol ester, $PdCl_2(dppf)\cdot CH_2Cl_2$, K_3PO_4, H_2O, dioxane, 80 °C; (iii) 4 M HCl in dioxane (for Boc protected heterocycles); $ArSO_2Cl$, base (see text), CH_2Cl_2, RT.

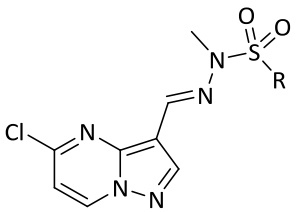
A series of compounds with a heterocyclic linker from the bicycle were synthesised (*Scheme 5*). Initial attempts using 5-chloro-3-bromopyrazolo[1,5-*a*]pyrimidine in a Suzuki-Miyaura coupling procedure using $Pd(dba)_2$ as a catalyst gave a mixture of the 3 and 5 regioisomers, whereas $Pd(PPh_3)_4$ gave exclusively reaction at the C-5 position. Consequently, the 3-iodo compound **19** was prepared from **7** using *N*-iodosuccinimide. Suzuki-Miyaura coupling between **19** and the desired heterocyclic boronic acids, or pinacol esters, in the presence of $PdCl_2(dppf)\cdot CH_2Cl_2$ gave reaction exclusively at the C-3 position (**20a-c**). Compound **20a** was treated with 4 M HCl in 1,4-dioxane to remove the Boc protecting group prior to reaction with the arylsulfonyl chloride under basic conditions to give **21a**. Compounds **20b** and **20c**, which had no Boc group in place, were treated with base and the aryl sulfonyl chloride to give **21b-c**. For the synthesis of **21c** it was necessary to use the stronger base sodium hydride to fully deprotonate the heterocycle and drive reaction to completion.

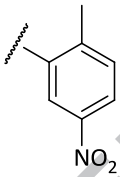
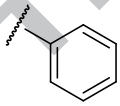
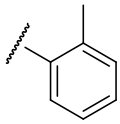
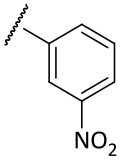
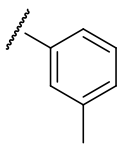
2.2 *In vitro* biology and structure-activity relationships

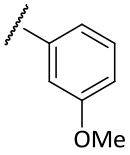
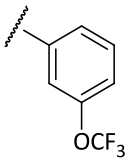
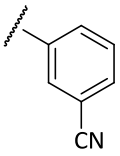
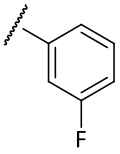
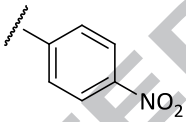
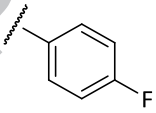
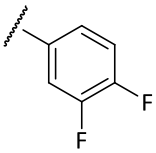
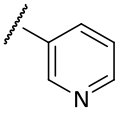
Preliminary work focused on transitioning from the imidazo[1,2-*a*]pyridine core of PIK-75 (**6**) to a pyrazolo[1,5-*a*]pyrimidine core, which we reasoned would increase rotational freedom for the 3-substituent by removing the hydrogen atom from the carbon (C4). Further, the presence of the bridgehead nitrogen (N7*a*) in the pyrazolo[1,5-*a*]pyrimidine should increase the acidity of the adjacent CH atom[31] to aid a putative hydrogen bond with the hinge region of the kinase. The direct pyrazolo[1,5-*a*]pyrimidine analogue (**9a**) of PIK-75 (**6**) was prepared to determine the effect these changes had against CDK9, and the PIK-75 target kinases (CDK7, PI3Kα and FLT3). Although compound **9a** showed a moderate reduction in potency against all enzymes (*Table 1*), we deemed the potency level suitable for further structural elaboration.

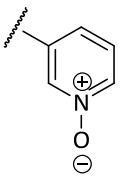
We next explored the structure-activity relationships (SAR) around the aryl sulfonamide. Substitution of this ring appears to be important for the activity of this series as shown by the general loss of activity for **9b** (*Table 1*). Introduction of a 2-methyl substituent (**9c**) did not restore activity to **9b**, whereas a 3-nitro substituent (**9d**) resulted in a compound with moderate levels of activity for both CDK9 and FLT3. To identify replacements for the nitro group we focused initially on mono substituted phenyl sulfonamides (*Table 1*). Substitution at the 3-position of the phenyl ring (equivalent to the 5-position of the 2,5-disubstituted ring) was largely unsuccessful with the 3-cyano compound (**9h)** being the one notable exception. This compound had moderate activity on CDK9 and FLT3 and importantly had lost measurable activity against CDK7 and PI3Kα. Moving the substituent to the 4-position (**9i**) had a detrimental effect on activity, as did disubstitution in the 3- and 4-positions (**9l**). Substitution of the phenyl ring for a pyridyl (**9m**) or the *N*-oxide (**9n**) also resulted in a loss of activity. The data for compounds **9c**, **9d** and **9h** suggests that the 2,5-substitution pattern plays an important role in the activity of this series by maintaining the optimum orientation of the phenyl sulfonamide ring to enable the 5-nitro, or 5-cyano, moieties to better interact with the kinase.

Table 1 *Single substitutions replacing nitro*



Compound	***R***	***IC_{50} (nM)***			
		CDK9	***CDK7***	***PI3Kα***	***FLT3***
PIK-75 (6)		1.37	NT[a]	0.5	5.15
9a		96	841	271	32
9b		>10000	>10000	>10000	4628
9c		>10000	>10000	>10000	>10000
9d		291	9244	4350	154
9e		>10000	>10000	>10000	>10000

9f		>10000	>10000	>10000	>10000
9g		>10000	>10000	>10000	7791
9h		201	>10000	>10000	90
9i		>10000	NT	4544	8855
9j		>10000	>10000	5373	>10000
9k		>10000	>10000	>10000	3301
9l		>10000	NT	7840	>10000
9m		>10000	>10000	>10000	1913

9n		>10000	>10000	>10000	8882

[a]NT = Not tested

In the absence of a crystal structure, molecular modelling and compound docking were used to examine potential binding modes of PIK-75 (**6**) and the pyrazolo[1,5-*a*]pyrimidine compounds in the ATP pocket of human CDK9. A putative binding mode for PIK-75 in CDK9 is illustrated in *Figure 2a, b*. PIK-75 (**6**) consistently docked into the CDK9 ATP binding site with the 6-bromo moiety pointing towards the back pocket (also known as the kinase specific affinity pocket; the region beyond the gatekeeper residue Phe103, near Asp167 of the DFG motif and residues Lys48, Val79 and Ala166). The PIK-75 imidazole N (N1) interacts with the backbone amide of Cys106 on the CDK9 hinge region and the imidazo[1,5-*a*]pyridine ring system forms edge-to-face π-π interactions with Phe103 (gatekeeper residue) and Phe105 (hinge residue). Leu156 forms hydrophobic contacts with the PIK-75 bicyclic ring system from below. The sulfonamide *N*-methyl group points up underneath the Gly-rich loop (also known as the P-loop) and makes hydrophobic interactions with Ile25, Phe30 and Val33. The sulfonamide oxygen atoms are solvent exposed and make no direct interaction with CDK9; however, an interaction with Asp109 may be possible through a water molecule. Phe30 on the Gly-rich loop flips down and becomes part of the ATP binding site. The PIK-75 phenylsulfonamide ring is located under the Gly-rich loop and makes π-π interactions with Phe30 and the 2-methyl phenyl substituent makes hydrophobic contacts with Phe30, Val33 and Lys48. Lys151 is within reach of the 5-nitro phenyl substituent and likely makes a charge-charge interaction. The 2-methyl, 5-nitro phenyl substituents and the *N*-methyl of the sulfonamide work in concert to orientate the phenylsulfonamide ring and maintain a relatively elongated PIK-75 conformation that can access the ATP binding site.

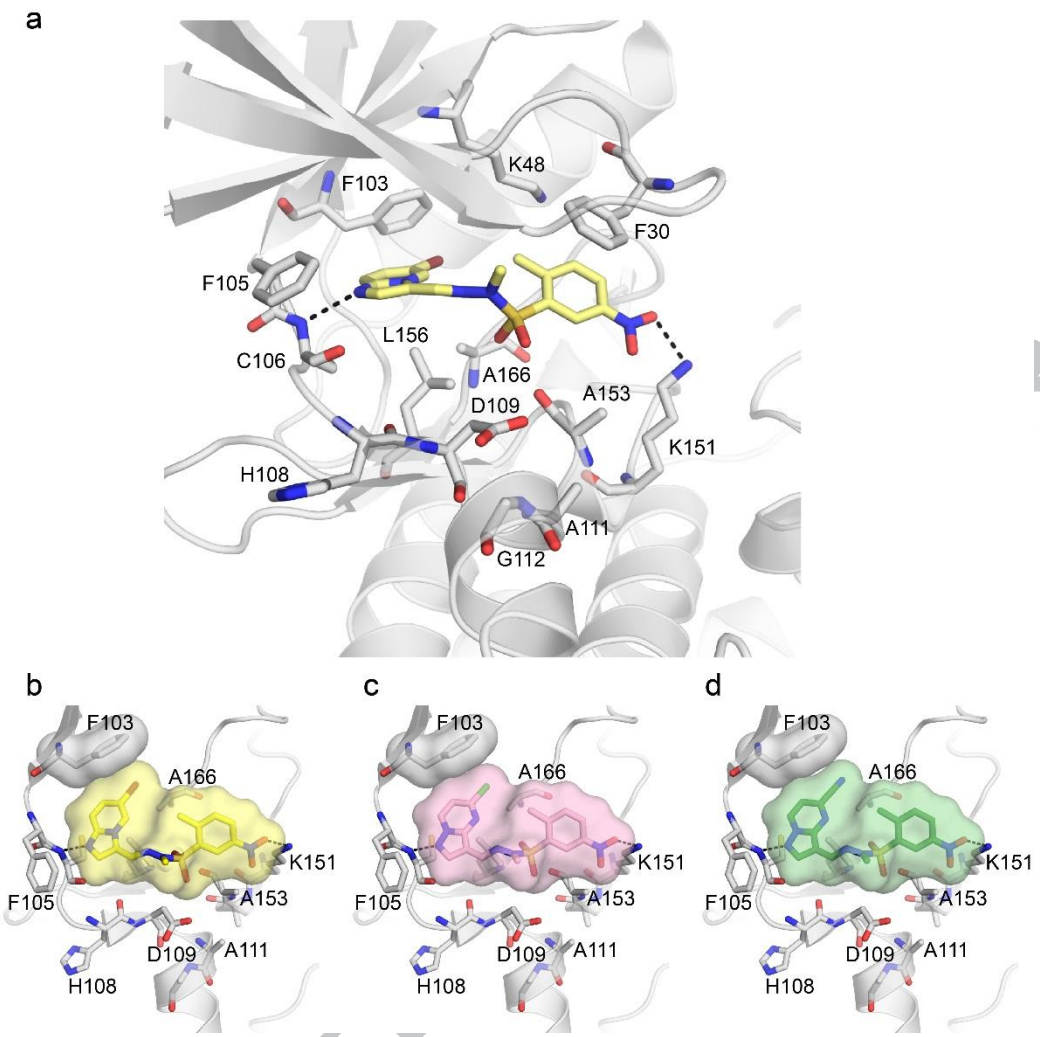


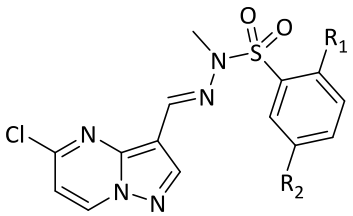
Figure 2. Putative binding modes of PIK-75 (**6**) (a) and (b), compound **9a** (c) and compound **11** (d) in the human CDK9 ATP binding site. See text for discussion.

Compound **9a** can adopt a similar binding mode to that of PIK-75 (**6**) in the CDK9 ATP pocket (*Figure 2c*). The decreased hydrophobicity of the 5-substituent (Br for PIK-75 (**6**); Cl for **9a**) may, in part, account for the difference in their activity profiles. The 3-nitro (**9d**) and 3-cyano (**9h**) compounds suffer only a ~5-fold reduction in CDK9 and FLT3 activity compared with PIK-75, presumably due to their ability to adopt low energy binding modes where the phenylsulfonamide substituent can interact with Lys151. Moving the nitro to the 4-position on the phenylsulfonamide (**9j**) would result in a steric clash with the Gly-rich loop, consistent with the loss of kinase activity for this compound.

For compounds **9b**, **9c, 9e**-**g**, **9i** and **9k-n** the aryl substituents allow the pyrazolo[1,5-*a*]pyrimidine compounds to adopt a wider range of conformations, including those where the aryl sulfonamide undergoes hydrophobic collapse with the bicycle to generate more compact conformers that would have difficulty accessing the kinase ATP pocket.

Given the promising CDK9 activity of **9h**, and a working understanding of the binding mode, we set out to explore the effect of different 2,5-substituents on the phenylsulfonamide (*Table 2*). Replacing the 5-nitro with the physically larger 5-bromo (**9o**) or 5-SO_2Me (**9t**) substituents reduced activity for all four kinases, possibly due to steric clashes with the Gly-rich loop, Lys151 and Ala153. By contrast, the absence of any measurable CDK9 activity for compounds **9p** and **9q** does not appear to be a steric issue but may be related to a decrease in the hydrophobicity of the 5-substituents for these two compounds compared to PIK-75 (**6**) and **9a** (clogP 1.5, 0.3, 3.5 and 2.4, respectively) limiting access to the hydrophobic ATP-binding site. Introduction of a 2-methyl substituent into **9h**, to furnish **9r**, gave a 10-fold increase in potency over **9h** and the compound retained 100-fold selectivity over PI3Kα. Exchange of the 2-methyl group for 2-chloro (**9u**) modestly improved potency against CDK9 (and FLT3) but significantly reduced selectivity over PI3Kα. Similarly, a 2-methoxy substituent (**9v**) led to an increase in the potency against PI3Kα but a slight reduction in activity against CDK9 and FLT3.

Table 2 *Disubstituted ring replacements*



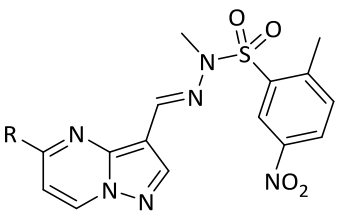
Compound	*R^1*	*R^2*	*IC_{50} (nM)*			
			CDK9	*CDK7*	*PI3Kα*	*FLT3*
9o	Me	Br	1960	>10000	>10000	5047
9p	Me	CO_2H	>10000	>10000	>10000	>10000
9q	Me	$C(O)NH_2$	>10000	NT[a]	>10000	6264
9r	Me	CN	22	3590	2960	33
9s	Me	F	>10000	NT	2963	>10000
9t	Me	SO_2Me	1064	NT	9430	6456
9u	Cl	NO_2	79	NT	29	21
9v	OMe	NO_2	174	NT	81	97

[a]NT = Not tested

Next we sought to explore the SAR at the 5-position of the pyrazolo[1,5-*a*]pyrimidine ring (*Table 3*). In the modelled binding mode of PIK-75 (**6**) and compound **9a** (*Figure 2a-c*), the 5-position is located at the back of the ATP pocket surrounded by Lys48, Val79, Phe103 (gatekeeper residue) and Ala166. Substitution of the 5-chloro for a 5-methyl (**10a**) resulted in moderate losses in activity across CDK9,

PI3Kα and FLT3. Introduction of the 5-methoxy substituent (**12**) appeared to reduce both CDK9 and FLT3 activity with a minimal impact of the PI3Kα activity whilst the 5-*N,N*-dimethylamino substituent (**13a**) resulted in a significant loss in activity. The introduction of larger substituents to the 5-position appeared to obliterate all measurable activity with the notable exceptions of the 3-pyridyl substituent (**10c**), which exhibited moderate CDK9 activity and selectivity over PI3Kα and FLT3. The size and planarity of the 5-substituent influences whether it can be accommodated in the back pocket and, in addition, how readily the compound can access the CDK9 ATP binding site. In the hinge region CDK9 has a preference for planar compound features and therefore conformations where the 5-substituent is out of the plane of the pyrazolo[1,5-*a*]pyrimidine ring will limit access to the ATP binding site. Thus, the methoxy substituent of **12**, the *N,N*-dimethyl amino substituent of **13a,** and the morpholino of **13b** are each predicted to have difficulty gaining access to the CDK9 ATP binding site. Thus, the 3-pyridyl of **10c** is at the physical limit of the pocket size, explaining the loss of CDK9 activity experienced with **10b** and **10d**. The 5-methyl group of **10a** is easily accommodated in the CDK9 back pocket, however, the CH_3-π interaction with Phe103 is weaker than the Cl-π interaction of **9a**[32, 33] and this likely explains the reduced CDK9 activity. Interestingly, the 5-cyano substituent of **11** gave a compound equipotent to **9a** across the four kinases profiled. The 5-cyano substituent is accommodated in the back pocket and extends the bicyclic ring π-π interaction with Phe103 (*Figure 2d*).

Table 3 *Substitutions for chloride in **9a***

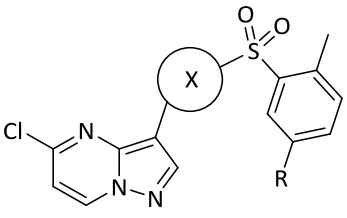


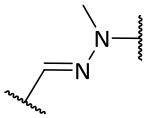
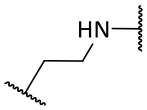
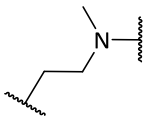
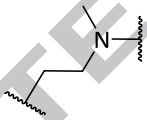
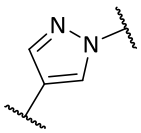
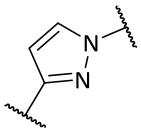
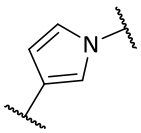
Compound	*R*	*IC_{50} (nM)*				*IC_{50} (μM)*
		CDK9	*CDK7*	*PI3Kα*	*FLT3*	*MV4;11*
PIK-75 (6)		1.37	NT[a]	0.5	5.15	0.003
9a	Cl	96	841	271	32	0.326
10a	Me	379	8750	902	222	0.762
10b	3,4-dimethoxyphenyl	>10000	>10000	233	3986	3.12
10c	3-Pyridyl	209	>10000	1240	874	7.48
10d	4-Methoxy-3-pyridyl	>10000	>10000	1030	>10000	>10
11	CN	43	1811	198	82	0.212
12	OMe	1165	NT	327	512	1.19
13a	NMe_2	2518	NT	>10000	4448	>10
13b	Morpholine	6555	NT	>10000	2545	15.1

[a]NT = Not tested

Finally we attempted to replace the metabolically labile hydrazone linking group present in these compounds (*Table 4*). Introducing an ethylamino linker (compounds **17a**, **18a** and **18b**) removes the hydrazone moiety at the expense of some CDK9 activity, though this can be improved through N-methylation of the sulfonamide. Interestingly, we found that replacement of the linker with a heterocycle (**21a**-**c**) removed all measurable activity in CDK9; however, **21a** and **21c** both showed good activity for PI3Kα and are thus selective for PI3Kα over CDK9. The pyrrole **21c** also exhibited inhibitory activity for FLT3 at a similar level as compounds **9a**, **9r**, **9u**, **9v** and **11**. The reason for the reduced CDK9 activity of these heterocycle-linked compounds is not immediately clear as modelling indicates that they can all be accommodated in the CDK9 ATP binding site (*Figure 3a*). We postulate that the loss of CDK9 activity arises from their reduced flexibility, which makes it difficult for these compounds to access the ATP site. Thus, in compounds **21a** and **21c**, the heterocyclic linker prefers to be planar with the pyrazolo[1,5-*a*]pyrimidine ring, resulting in a conformation like that shown in *Figure 3b,c* where the sulfonamide substituent blocks access to the CDK9 ATP binding site. Compound **21b** has the additional conformational restriction in that the unsubstituted *N* of the pyrazole linker has to be orientated away from the bicycle's pyrimidine nitrogen (N7α) to avoid electronic repulsion. The ATP binding sites of PI3Kα and FLT3 are more open (i.e. the Gly-rich loop generally sits higher than in CDK9) and the heterocyclic linker compounds **21a**-**c** have easier access, and this likely explains the selectivity profile of these compounds. These data are consistent with the early work of Waterfield and co-workers who demonstrated that the pyrazole analogue of PIK-75 (**6**) displayed excellent potency for PI3Kα.[14]

Table 4 *Linker group substitutions*



Compound	*R*	*X*	*IC_{50} (nM)*				*IC_{50} (μM)*
			CDK9	*CDK7*	*PI3Kα*[a]	*FLT3*	*MV4;11*
9r	CN		22	3590	2960	33	0.364
17a	NO_2		1144	NT[a]	900	184	0.166
18a	NO_2		265	NT	1870	516	0.374
18b	CN		203	>10000	>10000	219	0.177
21a	NO_2		>10000	1680	194	361	1.90
21b	NO_2		>10000	NT	1070	346	1.28
21c	NO_2		>10000	NT	164	58	0.123

[a]NT = Not tested

The ethylamino compounds **18a** and **18b** represent a significant development in this series. Both compounds display promising CDK9 affinity (~200nM), and in particular compound **18b** possesses excellent selectivity over PI3Kα and CDK7. Profiling of this compound against kinases known to be potently inhibited by PIK-75 (**6**) [18,19] indicates that compound **18b** shows improved selectivity (*Table 5*). Significantly, compound **18b** is devoid of both the problematic hydrazone and aryl nitro moieties present in PIK-75 (**6**) and is therefore a more drug-like lead for further optimisation.

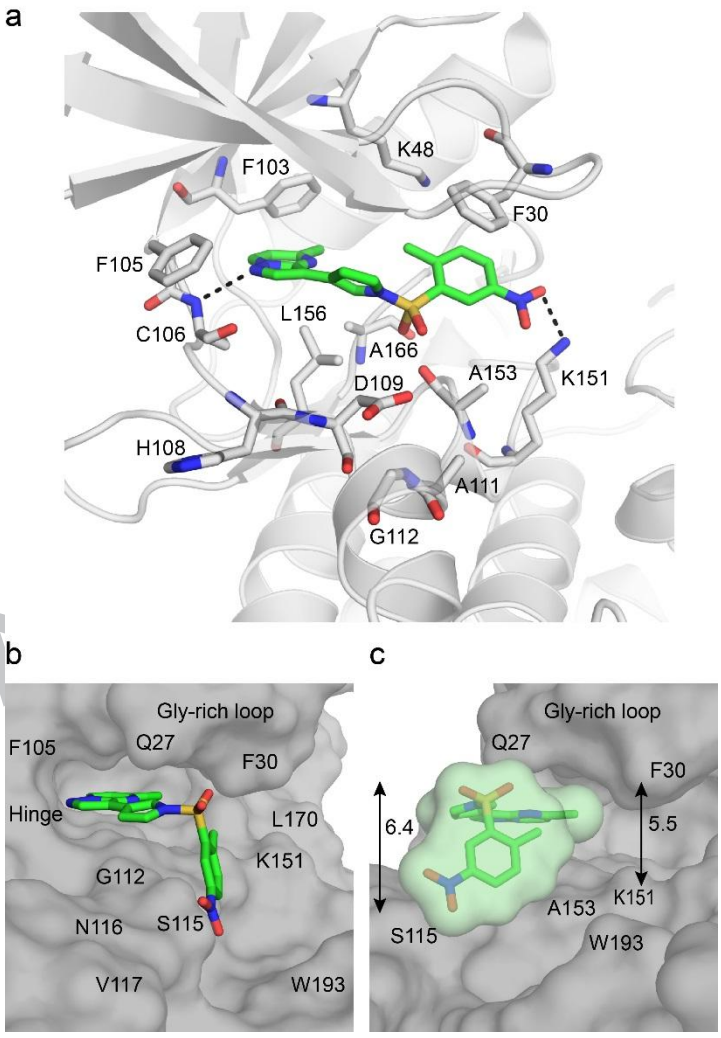


Figure 3. Modelled CDK9 binding mode (a) and minimum energy conformation (b) and (c) of the inactive compound **21c**. In (b) and (c) CDK9 is depicted as a grey molecular surface and in (c) a semi-transparent molecular surface for compound **21c** is also shown. The views in (b) and (c) differ by a rotation of -90° about the X-axis. The minimum energy conformation has difficulty gaining access to the CDK9 ATP binding site and would require the compound to adopt a less favourable non-planar conformation, as in panel (a), and/or CDK9 to adopt a more open conformation with greater access to the ATP pocket. In (c) arrows indicate the distance between CDK9 residues Phe30 and Lys151 (5.5 Å), and also the height of the compound (6.4 Å).

Table 5 *% Enzyme activity remaining,* ***18b*** *@ 200 nM*

Kinase	***% Activity***
CDK1/cyclin A	90.7
CDK2/cyclin E	101.1
CLK1	20.5
c-MET	102.3
DNA-PK	95.1
DYRK1A	52.6
GSK3β	34.0
JAK2	93.7
PKCε	15.9

2.3 Cellular Activity

The cellular activity of selected compounds from this work was determined in a proliferation assay using the leukaemia cell line MV4;11 (*Tables 3* and *4*). This cell line possesses an activating mutation in FLT3 and thus FLT3 inhibitors potently inhibit the proliferation of these cells.[34] Nonetheless, compounds from our study show variable activity against this cell line, independent of their FLT3 activity. Thus, for example, while PIK-75 (**6**) (FLT3 IC_{50} 5 nM) inhibits cell proliferation with an IC_{50} of 3 nM, **9r** (FLT3 IC_{50} 33 nM) is 100-fold less active against cells; the potency of PIK-75 arising from its multi-kinase activity.[16] Conversely, potent cellular activity of other compounds with reduced FLT3 activity must result from activities other than FLT3 inhibition. Our previous work has shown that MV4;11 AML cells require CDK9 activity for their survival[16] and the significant anti-proliferative activity of **18b** is consistent with its potent inhibition of CDK9. While 200nM **18b** inhibits PKCε and CLK1 by >80% (Table 5), these kinases demonstrate low expression in human AML[35] and so are unlikely to be key targets. Nonetheless, further studies are required to delineate the role of CDK9 inhibition in the activity of our compound series against leukaemia cell lines and this, along with *in vivo* studies, will be the subject of future publications.

3. Conclusions

The development of selective inhibitors of CDK9 represents a novel approach for the treatment of cancer by targeting the transcriptional machinery. In this work we have demonstrated that the prototypical PI3Kα inhibitor PIK-75 (**6**), which is known to also inhibit CDK9, can be used for the design of novel CDK9 inhibitors with improved selectivity over PI3Kα. Importantly we have shown that the two major structural liabilities with PIK-75 – the hydrazone and aryl nitro moieties – can be substituted with more drug-like functionality whilst retaining appreciable CDK9 affinity. Furthermore, using molecular modelling and compound docking into published crystal structures of CDK9, we have identified a putative binding mode of the pyrazolo[1,5-*a*]pyrimidine compounds which is predictive of their activity. While our current studies define a path for the synthesis and

development of novel potent and selective inhibitors of CDK9, future studies assessing their *in vivo* pharmacokinetics, pharmacodynamics and toxicity, as well as efficacy using *in vivo* models of AML, will be important for determining their therapeutic potential.

4. Experimental

4.1 Chemistry

NMR spectra were recorded on a Bruker AV-II DRX 300 NMR spectrometer at 300 K; chemical shifts are reported in δ ppm using residual solvent as the internal standard. Liquid chromatography mass spectroscopy (LCMS) was carried out on a Waters ZQ 3100 using reverse phase HPLC (column: XBridgeTM C18 5 µm 4.6 x 100 mm), Solvent A: Water 0.1% Formic Acid, Solvent B: Acetonitrile 0.1% Formic Acid, Gradient: 10-100% B over 10 min, Flow rate: 1.5 mL/min, detection: 100-600 nm and ESI in positive mode with source temperature 150 °C. All compounds submitted for biochemical assay were assessed to have purity ≥95% as measured by HPLC analysis at 254 nm UV absorbance. High Resolution Mass Spectrometry (HRMS) was conducted on an Agilent Q-TOF 6200 using positive mode electrospray ionisation (ESI). Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 aluminium backed plates and visualised with short wavelength UV (254 nm) absorbance. Chromatography was performed using either the CombiFlash® Rf purification system (Teledyne, ISCO, Lincoln, NE, USA) with pre-packed silica gel columns (particle size 40-63 µm). Anhydrous solvents were dried using an automated solvent purification system (MBraun SPS, Garching, Germany) based upon a technology originally described by Grubbs *et al*.[36] Reagents were purchased from a range of chemical suppliers and used without further purification.

Synthesis of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (8)

5-Chloropyrazolo[1,5-*a*]pyrimidine (1 g, 6.51 mmol) was dissolved in anhydrous DMF (10 mL) and cooled to -5 °C. Phosphorous oxychloride (1.82 mL, 19.5 mmol) was added and the reaction was allowed to warm slowly to RT and stirred for 16 h. The reaction was poured onto ice (~200 g) and the pH was adjusted to pH 10 with 1 M NaOH. The aqueous suspension was extracted with CH_2CL_2 (100 mL) and the aqueous layer was washed with CH_2Cl_2 (2 x 50 mL). The combined organic layers were washed with water (2 x 70 mL), brine (50 mL), dried (Whatman® PS1 filter paper) and the solvent was removed *in vacuo*. The crude product was adsorbed onto silica and purified by automated flash chromatography on silica gel (0-50% EtOAc – cyclohexane) to yield the title compound (**8**) (864 mg, 73%) as a colourless solid. ^{1}H NMR δ_H (d_6-DMSO, 300 MHz) 10.09 (s, 1H), 9.39 (d, *J* 7.2, 1H), 8.77 (s, 1H), 7.49 (d, *J* 7.2, 1H); LC-MS t_R 4.08 min; LC_{254} 99.0%; *m/z* 182.2/184.1 [M+H].

Preparation of 5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl sulfonohydrazones

These were made using one of either method A or method B:

General method A: To a solution of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (1 eq) in anhydrous MeOH (0.1 M) was added $NaHCO_3$ (5 eq) followed by methylhydrazine sulfate (1.2 eq). After stirring for 2 h at RT the sulfonyl chloride was added (1.3 eq) and the reaction was stirred for a further hour. The MeOH was removed *in vacuo* and the orange residue was partitioned between CH_2Cl_2 and water. The aqueous phase was washed with CH_2Cl_2, the organic phases were combined, dried (Whatman® PS1 filter paper) and the solvent was removed *in vacuo*. The crude material was adsorbed onto silica and purified by automated flash chromatography on silica gel (generally 0-50% EtOAc – cyclohexane) to yield the desired product.

General method B: Using the same procedure as Method A $NaHCO_3$ was replaced with 2,6-lutidine. Following stirring for up to 16 h the precipitate was isolated by filtration, dried (Whatman® PS1 filter paper) *in vacuo* and purified as for Method A.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (9a)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (168 mg, 0.93 mmol) with 2-methyl-5-nitrobenzenesulfonyl chloride using method A gave, after chromatography (0-50% EtOAc – cyclohexane) **9a** as a yellow solid (123 mg, 32%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.87 (d, *J* 2.5, 1H), 8.55 (d, *J* 7.3, 1H), 8.37 (s, 1H), 8.28 (dd, *J* 8.4, 2.5, 1H), 7.92 (s, 1H), 7.49 (d, *J* 8.4, 1H), 6.85 (d, *J* 7.2, 1H), 3.42 (s, 3H), 2.85 (s, 3H). LC-MS t_R 7.28 min; LC_{254} 98.6%; *m/z* 409.2/411.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{15}H_{13}ClN_6O_4S$ $[M+H]^+$: 409.0480; found 409.0487.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*-methylbenzenesulfonohydrazide (9b)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with benzenesulfonyl chloride using method A gave after chromatography (0-50% EtOAc – cyclohexane) **9b** as a yellow solid (118 mg, 77%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.58 (d, *J* 7.2, 1H), 8.57 (s, 1H), 7.98-7.93 (m, 3H), 7.63-7.50 (m, 3H), 6.87 (d, *J* 7.3, 1H), 3.28 (s, 3H). LC-MS t_R 6.75 min; LC_{254} 99.0%; *m/z* 350.1/352.1 [M+H]; HRMS (ESI^+) Calc'd for $C_{14}H_{12}ClN_5O_2S$ $[M+H]^+$: 350.0473; found 350.0481.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethylbenzenesulfonohydrazide (9c)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with *o*-toluenesulfonyl chloride using method A gave after chromatography (0-50% EtOAc – cyclohexane) **9c** as a yellow solid (88 mg, 55%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.56 (d, *J* 7.2, 1H), 8.43 (s, 1H), 8.02 (dd,

J 7.9, 1.5, 1H), 7.89 (s, 1H), 7.47 (ddd, *J* 7.5, 7.4, 1.4, 1H), 7.38-7.29 (m, 2H), 6.85 (d, *J* 7.2, 1H), 3.38 (s, 3H), 2.75 (s, 3H). LC-MS t_R 7.13 min; LC_{254} 99.0%; *m/z* 364.2/366.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{15}H_{14}ClN_5O_2S$ $[M+H]^+$: 364.0629; found 364.0633.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*-methyl-3-nitrobenzenesulfonohydrazide (9d)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with 3-nitrobenzenesulfonyl chloride using method A gave after chromatography (0-50% EtOAc – cyclohexane) **9d** as a yellow solid (101 mg, 58%). ^1H NMR δ_H ($CDCl_3$, 300 MHz) 8.80 (dd, *J* 1.8, 1.8, 1H), 8.57 (d, *J* 7.2, 1H), 8.52 (s, 1H), 8.43 (ddd, *J* 8.2, 2.3, 1.1, 1H), 8.31 (ddd, *J* 7.8, 1.7, 1.1, 1H), 7.97 (s, 1H), 7.75 (dd, *J* 8.2, 7.8, 1H), 6.87 (d, *J* 7.3, 1H), 3.30 (s, 3H). LC-MS t_R 6.98 min; LC_{254} 99.0%; *m/z* 395.1/397.1 [M+H]; HRMS (ESI^+) Calc'd for $C_{14}H_{11}ClN_6O_4S$ $[M+H]^+$: 395.0324; found 395.0329.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,3-dimethylbenzenesulfonohydrazide (9e)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with *m*-toluenesulfonyl chloride using method A gave after chromatography (0-50% EtOAc – cyclohexane) **9e** as a yellow solid (122 mg, 76%). ^1H NMR δ_H ($CDCl_3$, 300 MHz) 8.58 (d, *J* 7.2, 1H), 8.58 (s, 1H), 7.94 (s, 1H), 7.76-7.74 (m, 2H), 7.41-7.39 (m, 2H), 6.87 (d, *J* 7.2, 1H), 3.27 (s, 3H), 2.43 (s, 3H). LC-MS t_R 7.12 min; LC_{254} 99.0%; *m/z* 364.2/366.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{15}H_{14}ClN_5O_2S$ $[M+H]^+$: 364.0629; found 364.0633.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-3-methoxy-*N*-methylbenzenesulfonohydrazide (9f)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with 3-methoxybenzenesulfonyl chloride using method A gave after chromatography (0-50% EtOAc – cyclohexane) **9f** as a yellow solid (93 mg, 56%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.57 (s, 1H), 8.56 (d, *J* 7.3, 1H), 7.92 (s, 1H), 7.50 (m, 1H), 7.43-7.37 (m, 2H), 7.09 (ddd, *J* 8.1, 2.6, 1.0, 1H), 6.85 (d, *J* 7.2, 1H), 3.83 (s, 3H), 3.25 (s, 3H). LC-MS t_R 6.92 min; LC_{254} 99.0%; *m/z* 380.2/382.1 [M+H]; HRMS (ESI^+) Calc'd for $C_{15}H_{14}ClN_5O_3S$ $[M+H]^+$: 380.0579; found 380.0581.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*-methyl-3-(trifluoromethoxy)benzenesulfonohydrazide (9g)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with 3-(trifluoromethoxy)benzenesulfonyl chloride using method A gave after chromatography (0-50% EtOAc – cyclohexane) **9g** as a yellow solid (67 mg, 35%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.59 (d, *J* 7.2, 1H), 8.53 (s, 1H), 7.97 (m, 1H), 7.90 (ddd, *J* 7.8, 1.6, 1.1, 1H), 7.84 (m, 1H), 7.59 (dd, *J* 8.2, 8.2, 1H), 7.44 (m, 1H), 6.88 (d, *J* 7.2, 1H), 3.29 (s, 3H). LC-MS t_R 7.67 min; LC_{254} 99.0%; *m/z* 434.1/436.1 [M+H]; HRMS (ESI^+) Calc'd for $C_{15}H_{11}ClF_3N_5O_3S$ $[M+H]^+$: 434.0296; found 434.0302.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-3-cyano-*N*-methylbenzenesulfonohydrazide (9h)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with 3-cyanobenzenesulfonyl chloride using method A gave after chromatography (0-50% EtOAc – cyclohexane) **9h** as a yellow solid (96 mg, 58%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.60 (d, *J* 7.3, 1H), 8.52

(s, 1H), 8.26-8.21 (m, 2H), 7.99 (s, 1H), 7.87 (ddd, *J* 7.8, 1.4, 1.3, 1H), 7.69 (dd, *J* 7.8, 7.8, 1H), 6.91 (d, *J* 7.2, 1H), 3.31 (s, 3H). LC-MS t_R 6.68 min; LC_{254} 99.0%; *m/z* 375.1/377.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{15}H_{11}ClN_6O_2S$ $[M+H]^+$: 375.0425; found 375.0436.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-3-fluoro-*N*-methylbenzenesulfonohydrazide (9i)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with 3-fluorobenzenesulfonyl chloride using method A gave after chromatography (0-50% EtOAc – cyclohexane) **9i** as a yellow solid (82 mg, 51%). ^1H NMR δ_H ($CDCl_3$, 300 MHz) 8.59 (d, *J* 7.2, 1H), 8.54 (s, 1H), 7.95 (s, 1H), 7.76 (ddd, *J* 7.8, 1.6, 1.0, 1H), 7.67 (ddd, *J* 8.2, 2.6, 1.7, 1H), 7.52 (ddd, *J* 8.2, 8.0, 5.3, 1H), 7.30 (dddd, *J* 8.3, 8.3, 2.6, 1.1, 1H), 6.88 (d, *J* 7.2, 1H), 3.29 (s, 3H). LC-MS t_R 6.13 min; LC_{254} 99.0%; *m/z* 368.1/370.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{14}H_{11}ClFN_5O_2S$ $[M+H]^+$: 368.0379; found 368.0390.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*-methyl-4-nitrobenzenesulfonohydrazide (9j)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with 4-nitrobenzenesulfonyl chloride using method A gave after chromatography (0-50% EtOAc – cyclohexane) **9j** as a yellow solid (59 mg, 34%). ^1H NMR δ_H ($CDCl_3$, 300 MHz) 8.60 (d, *J* 7.2, 1H), 8.50 (s, 1H), 8.39 (d, *J* 8.8, 2H), 8.19 (d, *J* 8.8, 2H), 7.97 (s, 1H), 6.91 (d, *J* 7.2, 1H), 3.32 (s, 3H). LC-MS t_R 7.03 min; LC_{254} 99.0%; *m/z* 395.2/397.1; HRMS (ESI^+) Calc'd for $C_{14}H_{11}ClN_6O_4S$ $[M+H]^+$: 395.0324; found 395.0330.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-4-fluoro-*N*-methylbenzenesulfonohydrazide (9k)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with 4-fluorobenzenesulfonyl chloride using method A gave after chromatography (0-60% EtOAc – cyclohexane) **9k** as a yellow solid (108 mg, 68%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.59 (d, *J* 7.2, 1H), 8.54 (s, 1H), 7.99 (dd, *J* 9.0, 5.1, 2H), 7.95 (s, 1H), 7.21 (dd, *J* 8.8, 8.5, 2H), 6.89 (d, *J* 7.2, 1H), 3.29 (s, 3H). LC-MS t_R 7.00 min; LC_{254} 98%; *m/z* 368.1/370.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{14}H_{11}ClFN_5O_2S$ $[M+H]^+$: 368.0379; found 368.0383.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-3,4-difluoro-*N*-methylbenzenesulfonohydrazide (9l)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with 3,4-difluorobenzenesulfonyl chloride using method A gave after chromatography (0-60% EtOAc – cyclohexane) **9l** as a yellow solid (106 mg, 62%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.59 (d, *J* 7.3, 1H), 8.52 (s, 1H), 7.97 (s, 1H), 7.83 (ddd, *J* 9.4, 7.3, 2.2, 1H), 7.77 (m, 1H), 7.33 (ddd, *J* 9.6, 8.6, 7.4, 1H), 6.89 (d, *J* 7.2, 1H), 3.28 (s, 3H); LC-MS t_R 6.28 min; LC_{254} 99.0%; *m/z* 386.1/388.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{14}H_{10}ClF_2N_5O_2S$ $[M+H]^+$: 386.0285; found 386.0272.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*-methylpyridine-3-sulfonohydrazide (9m)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with pyridine-3-sulfonyl chloride using method A gave after chromatography (0-90% EtOAc – cyclohexane) **9m** as a yellow solid (112 mg, 73%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 9.17 (d, *J* 2.2, 1H), 8.82 (dd, *J* 4.8, 1.6, 1H),

8.59 (d, *J* 7.2, 1H), 8.52 (s, 1H), 8.31 (ddd, *J* 8.1, 2.1, 1.8, 1H), 7.97 (s, 1H), 7.51 (dd, *J* 8.1, 4.9, 1H), 6.89 (d, *J* 7.2, 1H), 3.31 (s, 3H); LC-MS t_R 5.88 min; LC_{254} 99.0%; *m/z* 351.1/353.1 [M+H]; HRMS (ESI^+) Calc'd for $C_{13}H_{11}ClN_6O_2S$ $[M+H]^+$: 351.0425; found 351.0429.

(*E*)-5-Bromo-*N*'-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethylbenzenesulfonohydrazide (9o)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (100 mg, 0.55 mmol) with 2-methyl-5-bromobenzenesulfonyl chloride using method A gave after chromatography (0-50% EtOAc – cyclohexane) **9o** as a yellow solid (150 mg, 62%). ^1H NMR δ_H ($CDCl_3$, 300 MHz) 8.57 (d, *J* 7.2, 1H), 8.43 (s, 1H), 8.17 (d, *J* 2.1, 1H), 7.91 (s, 1H), 7.57 (dd, *J* 8.2, 2.1, 1H), 7.18 (d, *J* 8.2, 1H), 6.85 (d, *J* 7.2, 1H), 3.39 (s, 3H), 2.70 (s, 3H). LC-MS t_R 7.83 min; LC_{254} 99.0%; *m/z* 442.1/444.1/446.1 [M+H]; HRMS (ESI^+) Calc'd for $C_{15}H_{13}BrClN_5O_2S$ $[M+H]^+$: 441.9735; found 441.9751.

(*E*)-3-((2-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-1-methylhydrazinyl)sulfonyl)-4-methylbenzoic acid (9p)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (300 mg, 1.65 mmol) 3-chlorosulfonyl-4-methyl benzoic acid using method B gave **9p** as a yellow solid (507 mg, 75%). ^1H NMR δ_H (d_6-DMSO, 300 MHz) 9.22 (d, *J* 7.3, 1H), 8.44 (d, *J* 1.8, 1H), 8.35 (s, 1H), 8.06 (dd, *J* 7.9, 1H), 7.95 (s, 1H), 7.56 (d, *J* 8.1, 1H), 7.24 (d, *J* 7.3, 1H), 3.32 (s, 3H), 2.75 (s, 3H); LC-MS t_R 6.37 min; LC_{254} 98.6%; *m/z* 408.2/410.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{16}H_{14}ClN_5O_4S$ $[M+H]^+$: 408.0528; found 408.0536.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-5-cyano-*N*,2-dimethylbenzenesulfonohydrazide (9r)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) 2-methyl-5-cyanobenzenesulfonyl chloride using method B gave **9r** as a yellow solid (116 mg, 68%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.58 (d, *J* 7.2, 1H), 8.38 (s, 1H), 8.33 (d, *J* 1.8, 1H), 7.93 (s, 1H), 7.73 (dd, *J* 7.9, 1.7, 1H), 7.44 (d, *J* 7.9, 1H), 6.88 (d, *J* 7.2, 1H), 3.43 (s, 3H), 2.83 (s, 3H); LC-MS t_R 6.98 min; LC_{254} 99.0%; *m/z* 389.2/391.1; HRMS (ESI$^+$) Calc'd for $C_{16}H_{13}ClN_6O_2S$ $[M+H]^+$: 389.0582; found 389.0591.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-5-fluoro-*N*,2-dimethylbenzenesulfonohydrazide (9s)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with 2-methyl-5-fluorobenzenesulfonyl chloride using method A gave, after chromatography (0-50% EtOAc – cyclohexane) **9s** as a yellow solid (82 mg, 49%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.57 (d, *J* 7.2, 1H) 8.42 (s, 1H), 7.90 (s, 1H), 7.75 (dd, *J* 8.6, 2.8, 1H), 7.30-7.25 (m, 1H), 7.17 (ddd, *J* 8.3, 8.3, 2.7, 1H), 6.86 (d, *J* 7.2, 1H), 3.40 (s, 3H), 2.70 (s, 3H). LC-MS t_R 6.40 min; LC_{254} 99.0%; *m/z* 382.1/384.1 [M+H]; HRMS (ESI$^+$) Calc'd for $C_{15}H_{13}ClFN_5O_2S$ $[M+H]^+$: 382.0535; found 382.0547.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-(methylsulfonyl)benzenesulfonohydrazide (9t)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with 5-methanesulfonyl-2-methylbenzenesulfonyl chloride using method A gave, after chromatography (0-70% EtOAc – cyclohexane) **9t** as a yellow solid (73 mg, 37%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.59 (d, *J* 2.0, 1H), 8.57 (d, *J* 7.2, 1H), 8.43 (s, 1H), 8.02 (dd, *J* 8.0, 2.0, 1H), 7.94 (s, 1H), 7.53 (d, *J* 8.0, 1H), 6.87

(d, *J* 7.3, 1H), 3.42 (s, 3H), 3.11 (s, 3H), 2.87 (s, 3H); LC-MS t_R 5.80 min; LC_{254} 99.0%; *m/z* 442.17/444.12 [M+H]; HRMS (ESI^+) Calc'd for $C_{16}H_{16}ClN_5O_4S_2$ $[M+H]^+$: 442.0405; found 442.0412.

(*E*)-2-Chloro-*N*'-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*-methyl-5-nitrobenzenesulfonohydrazide (9u)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with 2-chloro-5-nitrobenzenesulfonyl chloride using method A gave, after chromatography (0-50% EtOAc – cyclohexane) **9u** as a yellow solid (76 mg, 40%). ^1H NMR δ_H ($CDCl_3$, 300 MHz) 9.14 (d, *J* 2.6, 1H), 8.55 (d, *J* 7.3, 1H), 8.35 (dd, *J* 8.7, 2.7 1H), 8.32 (s, 1H), 7.93 (s, 1H), 7.69 (d, *J* 8.7, 1H), 6.86 (d, *J* 7.2, 1H), 3.59 (s, 3H); LC-MS t_R 6.33 min; LC_{254} 99.0%; *m/z* 429.0/431.1/433.1 [M+H]; HRMS (ESI^+) Calc'd for $C_{14}H_{10}Cl_2N_6O_4S$ $[M+H]^+$: 428.9934; found 428.9953.

(*E*)-*N*'-((5-Chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-2-methoxy-*N*-methyl-5-nitrobenzenesulfonohydrazide (9v)

Reaction of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (80 mg, 0.44 mmol) with 2-methoxy-5-nitrobenzenesulfonyl chloride using method A gave, after chromatography (0-70% EtOAc – cyclohexane) **9v** as a yellow solid (85 mg, 43%). ^1H NMR δ_H ($CDCl_3$, 300 MHz) 9.02 (d, *J* 2.9, 1H), 8.55 (d, *J* 7.2, 1H), 8.43 (dd, *J* 9.1, 2.8, 1H), 8.36 (s, 1H), 7.90 (s, 1H), 7.08 (d, *J* 9.1, 1H), 6.84 (d, *J* 7.3, 1H), 4.05 (s, 3H), 3.55 (s, 3H). LC-MS t_R 5.95 min; LC_{254} 98.0%; *m/z* 425.2/427.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{15}H_{13}ClN_6O_5S$ $[M+H]^+$: 425.0429; found 425.0442.

Synthesis of (*E*)-3-((2-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-1-methylhydrazinyl)sulfonyl)pyridine 1-oxide (9n)

(*E*)-*N*'-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*-methylpyridine-3-sulfonohydrazide (**9m**) (80 mg, 0.23 mmol) was added to a solution of *m*CPBA (43 mg, 0.25 mmol) in anhydrous CH_2Cl_2 (5 mL) and the reaction was stirred at RT for 16 h. The resulting precipitate was removed *via* filtration and the solution was diluted with CH_2Cl_2 (15 mL) washed with sat $NaHCO_3$ (3 x 10 mL), dried (Whatman® PS1 filter paper) and solvent was removed *in vacuo* to give **9n** as a yellow solid (44 mg, 52%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.76 (m, 1H), 8.62 (d, *J* 7.3, 1H), 8.52 (s, 1H), 8.38 (m, 1H), 8.04 (s, 1H), 7.88 (m, 1H), 7.48 (ap. t, *J* 7.4, 1H), 6.92 (d, *J* 7.2, 1H), 3.34 (s, 3H); LC-MS t_R 5.15 min; LC_{254} 85%; *m/z* 367.1/369.1 [M+H]; HRMS (ESI^+) Calc'd for $C_{13}H_{11}ClN_6O_3S$ $[M+H]^+$: 367.0375; found 367.0376.

Synthesis of (*E*)-3-((2-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-1-methylhydrazinyl)sulfonyl)-4-methylbenzamide (9q)

To a solution of (*E*)-3-((2-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-1-methylhydrazinyl)-sulfonyl)-4-methylbenzoic acid (**9p**) (75 mg, 0.184 mmol) in anhydrous DMF (5 mL), was added HATU (91 mg, 0.239 mmol) and DIPEA (112 µL, 0.644 mmol). After stirring at RT for 30 mins, ammonium carbonate (88 mg, 0.920 mmol) was added and the reaction was stirred at RT for a further 90 mins. The reaction mixture was quenched with sat. $NaHCO_3$ (50 mL) and the resulting precipitate was isolated *via* filtration. After chromatography (0-10% MeOH-CH_2Cl_2) this gave **9q** as a yellow solid (13 mg, 18%). ^{1}H NMR δ_H (d_6-DMSO, 300 MHz) 9.22 (d, *J* 7.3, 1H), 8.41 (d, *J* 1.8, 1H), 8.38 (s, 1H), 8.19 (bs, 1H), 8.03 (dd, *J* 8.0, 1.8, 1H), 7.93 (s, 1H), 7.55 (bs, 1H), 7.51 (d, *J* 8.3, 1H), 7.24 (d, *J* 7.3, 1H), 3.32 (s, 3H), 2.73 (s, 3H); LC-MS t_R 5.85 min; LC_{254} 99.0%; *m/z* 407.2/409.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{16}H_{15}ClN_6O_3S$ $[M+H]^+$: 407.0688; found 407.0695.

Synthesis of (*E*)-*N*,2-dimethyl-*N*'-((5-methylpyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-5-nitrobenzenesulfonohydrazide (10a)

(*E*)-*N*'-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (**9a**) (80 mg, 0.196 mmol) was dissolved in anhydrous dioxane under nitrogen and K_2CO_3 (81 mg, 0.588 mmol) was added. To this was added $Pd(PPh_3)_4$ (23 mg, 0.02 mmol) and trimethylboroxine (27 µL, 0.196 mmol) and the reaction was heated at 105 °C for 16 h. The reaction mixture was cooled and filtered through a Celite® pad and washed with THF, and the filtrate was concentrated *in vacuo*. Chromatography (0-100% EtOAc-cyclohexane) afforded **10a** as a yellow solid (20 mg, 26%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.88 (d, *J* 2.2, 1H), 8.53 (d, *J* 7.1, 1H), 8.35 (s, 1H), 8.29 (dd, *J* 8.3, 2.1, 1H), 8.08 (s, 1H), 7.50 (d, *J* 8.4, 1H), 6.76 (d, *J* 7.2, 1H), 3.43 (s, 3H), 2.89 (s, 3H), 2.64 (s, 3H); LC-MS t_R 5.93 min; LC_{254} 96.5%; *m/z* 389.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{16}H_{16}N_6O_4S$ $[M+H]^+$: 389.1027; found 389.1036.

Preparation of 5-aryl or heteroaryl pyrazolo[1,5-*a*]pyrimidin-3-yl sulfonohydrazones

General method C: (*E*)-*N*'-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (**9a**) (1 eq), boronic acid or boronic ester (1.5eq) and 2 M Na_2CO_3 (1.2 eq) were combined in anhydrous DME (0.05 M), tetrakis (triphenylphosphino) palladium (0) (0.02 eq) was added and the reaction was heated at 80 °C for 16 h. The solvent was removed *in vacuo* and the residue was taken up in either CH_2Cl_2 or EtOAc and filtered through a Celite® pad. The mother liquor was washed with water and brine, dried (Whatman® PS1 filter paper) and the solvent was removed *in vacuo*. The crude material was adsorbed onto silica and purified by automated flash chromatography on silica gel (generally 0-100% EtOAc – cyclohexane) to yield the desired product.

(*E*)-*N*'-((5-(3,4-Dimethoxyphenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (10b)

Reaction of (*E*)-*N*'-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzene-sulfonohydrazide (**9a**) (80 mg, 0.196 mmol) with 3,4-dimethoxyphenyl boronic acid using method C gave, after chromatography (0-90% EtOAc – cyclohexane) **10b** as a yellow solid (68 mg, 68%). ^{1}H NMR δ_H (d_6-DMSO, 300 MHz) 9.17 (d, *J* 7.5, 1H), 8.63 (d, *J* 2.5, 1H), 8.38 (dd, *J* 8.5, 2.6, 1H), 8.34 (s, 1H), 8.17 (s, 1H), 7.90 (m, 2H), 7.79 (d, *J* 7.6, 1H), 7.72 (d, *J* 8.5, 1H), 7.12 (d, *J* 9.2, 1H), 3.90 (s, 3H), 3.86 (s, 3H), 3.37 (s, 3H), 2.81 (s, 3H); LC-MS t_R 7.53 min; LC_{254} 99.0%; *m/z* 511.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{23}H_{22}N_6O_6S$ $[M+H]^+$: 511.1394; found 511.1406.

(*E*)-*N*,2-Dimethyl-5-nitro-*N*'-((5-(pyridin-3-yl)pyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)benzenesulfonohydrazide (10c)

Reaction of (*E*)-*N*'-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzene-sulfonohydrazide (**9a**) (80 mg, 0.196 mmol) with 3-pyridine boronic acid using method C gave, after chromatography (40-100% EtOAc – cyclohexane) **10c** as a yellow solid (36 mg, 41%). ^{1}H NMR δ_H (d_6-DMSO, 300 MHz) 9.48 (d, *J* 2.3, 1H), 9.32 (d, *J* 7.4, 1H), 8.75 (dd, *J* 4.8, 1.6, 1H), 8.66 (m, 2H), 8.41 (s, 1H), 8.38 (dd, *J* 8.5, 2.6, 1H), 8.19 (s, 1H), 7.87 (d, *J* 7.5, 1H), 7.74 (d, *J* 8.5, 1H), 7.60 (dd, *J* 8.0, 4.8, 1H), 3.41 (s, 3H), 2.80 (s, 3H); LC-MS t_R 6.45 min; LC_{254} 99.0%; *m/z* 452.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{20}H_{17}N_7O_4S$ $[M+H]^+$: 452.1136; found 452.1148.

(*E*)-*N*'-((5-(6-Methoxypyridin-3-yl)pyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (10d)

Reaction of (*E*)-*N*'-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzene-sulfonohydrazide (**9a**) (100 mg, 0.245 mmol) with 6-methoxy-3-pyridyl boronic acid using method C gave, after chromatography (0-100% EtOAc – cyclohexane) **10d** as a yellow solid (60 mg, 51%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.91 (d, *J* 2.3, 1H), 8.89 (d, *J* 2.5 1H), 8.69 (d, *J* 7.4, 1H), 8.43 (dd, *J* 8.8, 2.5, 1H), 8.40 (s, 1H), 8.30 (dd, *J* 8.3, 2.5, 1H), 8.14 (s, 1H), 7.51 (d, *J* 8.6, 1H), 7.30 (d, *J* 7.4, 1H), 6.93 (d, *J* 9.1, 1H), 4.07 (s, 3H), 3.46 (s, 3H), 2.90 (s, 3H); LC-MS t_R 7.63 min; LC_{254} 99.0%; *m/z* 482.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{21}H_{19}N_7O_5S$ $[M+H]^+$: 482.1241; found 482.1252.

Synthesis of (E)-*N*'-((5-cyanopyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (11)

Zinc cyanide (12 mg, 0.102 mmol) was added to a solution of (*E*)-*N*'-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (**9a**) (70 mg, 0.171 mmol) in anhydrous DMF. To this was added $Pd(PPh_3)_4$ (14 mg, 0.012 mmol) and the reaction was heated at 80 °C for 40 h. The reaction was cooled to RT and diluted with toluene (10 mL) and washed with NH_4OH (2 x15 mL). The aqueous layer was extracted with toluene (2 x 15 mL), the combined organic layers were washed with brine (20 mL), dried (Whatman® PS1 filter paper) and solvent was removed *in vacuo*. After chromatography (0-50% EtOAc-cyclohexane) this gave **11** as a yellow solid (25 mg, 37%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.90 (d, *J* 2.5, 1H), 8.83 (d, *J* 7.2, 1H), 8.55 (s, 1H), 8.31 (dd, *J* 8.4, 2.4, 1H), 7.96 (s, 1H), 7.53 (d, *J* 8.5, 1H), 7.15 (d, *J* 7.2, 1H), 3.50 (s, 3H), 2.88 (s, 3H); LC-MS t_R 7.10 min; LC_{254} 95%; *m/z* 400.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{16}H_{13}N_7O_4S$ $[M+H]^+$: 400.0822; found 400.0829.

Synthesis of (*E*)-*N*'-((5-methoxypyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (12)

Sodium methoxide (19 mg, 0.352 mmol) was added to a solution of (*E*)-*N*'-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (**9a**) (72 mg, 0.176 mmol) in anhydrous MeOH (5 mL). The reaction was heated at 65 °C for 4 days with further aliquots of sodium methoxide (19 mg, 0.352 mmol) added each day. The solvent was removed *in vacuo* and the resulting solid was partitioned between CH_2Cl_2 (20 mL) and water (20 mL). The organic layer was dried (Whatman® PS1 filter paper) and the solvent was removed *in vacuo*. After chromatography (0-30% EtOAc – cyclohexane) this gave **12** as a yellow solid (10.3 mg, 14%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.86 (d, *J* 2.3, 1H), 8.40 (d, *J* 7.5, 1H), 8.30 (dd, *J* 8.4, 2.4, 1H), 8.27 (s, 1H), 8.04 (s, 1H), 7.51 (d, *J* 8.4, 1H), 6.41 (d, *J* 7.4, 1H), 4.05 (s, 3H), 3.39 (s, 3H), 2.88 (s, 3H). LC-MS t_R 7.18 min; LC_{254} 95.0%; 405.3 [M+H]; HRMS (ESI^+) Calc'd for $C_{16}H_{16}N_6O_5S$ $[M+H]^+$: 405.0976; found 405.0985.

Synthesis of (*E*)-*N*'-((5-(dimethylamino)pyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (13a)

To a solution of (*E*)-*N*'-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (**9a**) (80 mg, 0.196 mmol) in anhydrous DMF (5 mL) was added DIPEA (119 µL, 0.685 mmol) followed by ammonium carbonate (94 mg, 0.975 mmol) and the reaction was stirred at RT for 40 h. A further aliquot of ammonium carbonate (94 mg, 0.975 mmol) was added and the reaction was stirred for an additional 72 h. The reaction was poured into saturated $NaHCO_3$ (20 mL) and the precipitate was isolated by filtration and dried (Whatman® PS1 filter paper) *in vacuo*. After chromatography (40-100% EtOAc – cyclohexane) this gave **13a** as a yellow solid (14.5 mg, 17%). ^{1}H NMR δ_H (d_6-DMSO, 300 MHz) 8.64 (d, *J* 7.9, 1H), 8.58 (d, *J* 2.3, 1H), 8.39 (dd, *J* 8.4, 2.3, 1H), 8.01 (s, 1H), 7.99 (s, 1H), 7.74 (d, *J* 8.7, 1H), 6.65 (d, *J* 7.9, 1H), 3.23 (s, 3H), 3.15 (s, 6H), 2.78 (s, 3H); LC-MS t_R 7.00 min; LC_{254} 98%; 418.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{17}H_{19}N_7O_4S$ $[M+H]^+$: 418.1292; found 418.1301.

Synthesis of (*E*)-*N*,2-dimethyl-*N*'-((5-morpholinopyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-5-nitrobenzenesulfonohydrazide (13b)

To a solution of a (*E*)-*N*'-((5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (**9a**) (50 mg, 0.122 mmol) in anhydrous DMF (2 mL) was added K_2CO_3 (25 mg, 0.183 mmol) followed by morpholine (16 µL, 0.183 mmol) and the reaction was heated at 70 °C for 2 h. The reaction was diluted with EtOAc (20 mL) and washed with water (20 mL) and brine (20 mL), dried (Whatman® PS1 filter paper) and the solvent was removed *in vacuo*. After chromatography (0-100% EtOAc – cyclohexane) this gave **13b** as a yellow solid (43.3 mg, 77%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.82 (d, *J* 2.5, 1H), 8.39 (s, 1H), 8.32 (d, *J* 8.0, 1H), 8.31 (dd, *J* 8.5, 2.6, 1H), 8.17 (s, 1H), 7.53 (d, *J* 8.4, 1H), 6.41 (d, *J* 7.9, 1H), 3.86-3.83 (m, 4H), 3.79-3.75 (m, 4H), 3.34 (s, 3H), 2.87 (s, 3H). LC-MS t_R 6.80 min; LC_{254} 99.0%; *m/z* 460.3 [M+H]; HRMS (ESI^+) Calc'd for $C_{19}H_{21}N_7O_5S$ $[M+H]^+$: 460.1398; found 460.1413.

Synthesis of (*E*)-5-chloro-3-(2-nitrovinyl)pyrazolo[1,5-*a*]pyrimidine (14)

To a suspension of 5-chloropyrazolo[1,5-*a*]pyrimidine-3-carbaldehyde (**8**) (500 mg, 2.75 mmol) in MeOH (25 mL) was added Methylamine hydrochloride (511 mg, 7.57 mmol), KOAc (1.08 g, 11 mmol) and nitromethane (164 µL, 3.03 mmol). After stirring at RT for 72 h water (25 mL) was added and the precipitate was isolated by filtration. After chromatography (0-100% EtOAc-cyclohexane) this gave **14** as a bright yellow solid (205 mg, 33%). ^{1}H NMR δ_H (d_6-DMSO, 300 MHz) 9.37 (d, *J* 7.2, 1H), 8.83 (s, 1H), 8.24 (d, *J* 13.4 1H), 8.14 (d, *J* 13.4, 1H), 7.45 (d, *J* 7.2, 1H); LC-MS t_R 6.45 min; LC_{254} 99.0%; *m/z* 225.1/227.3 [M+H].

Synthesis of 5-chloro-3-(2-nitroethyl)pyrazolo[1,5-*a*]pyrimidine (15)

Sodium cyanoborohydride (34 mg, 0.536 mmol) was added portionwise to a solution of (*E*)-5-chloro-3-(2-nitrovinyl)pyrazolo[1,5-*a*]pyrimidine (**14**) (60 mg, 0.268 mmol) in THF (3 mL) at 0 °C. The reaction was allowed to warm slowly to RT and stirred for 16 h. THF was removed *in vacuo* and the residue was partitioned between water (15 mL) and EtOAc (15 mL). The aqueous phase was washed with EtOAc (2 x 15 mL) and the combined organic phases were washed with brine, dried (Whatman® PS1 filterpaper) and solvent was removed *in vacuo*. After chromatography (0-50% EtOAc-cyclohexane) this gave **15** as a yellow solid (9 mg, 15%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.55 (d, *J* 7.2, 1H), 8.04 (s, 1H), 6.82 (d, *J* 7.2, 1H), 4.75 (t, *J* 6.9, 2H), 3.51 (t, *J* 6.9, 2H).

Synthesis of *N*-(2-(5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)ethyl)-2-methyl-5-nitrobenzenesulfonamide (17a)

A solution of of 5-chloro-3-(2-nitroethyl)pyrazolo[1,5-*a*]pyrimidine (**15**) (30 mg, 0.132 mmol) in MeOH (5 mL) was treated with a RaNi cartridge on a Thales H-Cube® at 50 °C on full H_2 mode. Solvent was removed *in vacuo*. The resulting material was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. To this was added NEt_3 (34 µL, 0.244 mmol) followed by 2-methyl-5-nitrobenzenesulfonyl chloride. The reaction was allowed to warm slowly to RT and was stirred for 16 h. The reaction was diluted with CH_2Cl_2 (10 mL) and washed with sat. $NaHCO_3$ (10 mL) and water (2 x 10 mL) dried (Whatman® PS1 filterpaper) and solvent was removed *in vacuo*. After chromatography (40-100% EtOAc-cyclohexane) this gave **17a** as a yellow solid (2.9 mg, 6%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.77 (d, *J* 2.5, 1H), 8.53 (d, *J* 7.2, 1H), 8.27 (dd, *J* 8.3, 2.4, 1H), 7.96 (s, 1H), 7.45 (d, *J* 8.6, 1H), 6.80 (d, *J* 7.3, 1H), 5.74 (bm, 1H), 3.45 (dt, *J* 6.2, 5.6, 2H), 3.00 (t, *J* 6.4, 2H), 2.71 (s, 3H). LC-MS t_R 5.72 min; LC_{254} 97.3%; *m/z* 396.2/398.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{15}H_{14}ClN_5O_4S$ $[M+H]^+$: 396.0528; found 396.0539.

Synthesis of *N*-(2-(5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)ethyl)-*N*,2-dimethyl-5-nitrobenzenesulfonamide (18a)

K_2CO_3 (9 mg, 0.28 mmol) was added a solution of *N*-(2-(5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)ethyl)-2-methyl-5-nitrobenzenesulfonamide (**17a**) (11 mg, 0.028 mmol) and MeI (7 µL, 0.10 mmol) in acetone (1 mL) and the recation was stirred at RT for 16 h. The reaction was partitioned bewteen EtOAc (10 mL) and water (10 mL), the aqueous phase was washed with EtOAc (2 x 10 mL), the combined organic phases were dried (Whatman® PS1 filter paper) and solvent was removed *in vacuo*. After chromatography (0-100% EtOAc-cyclohexane) this gave **18a** as a yellow solid (5 mg, 44%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.59 (d, *J* 2.0, 1H), 8.57 (d, *J* 7.2, 1H), 8.43 (s, 1H), 8.02 (dd, *J* 8.0, 2.0, 1H), 7.94 (s, 1H), 7.53 (d, *J* 8.0, 1H), 6.87 (d, *J* 7.3, 1H), 3.42 (s, 3H), 3.11 (s, 3H), 2.87 (s, 3H). LC-MS t_R 5.80 min; LC_{254} 99.0%; *m/z* 410.2/412.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{16}H_{16}ClN_5O_4S$ $[M+H]^+$: 410.0684; found 410.0675.

Synthesis of *N*-(2-(5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)ethyl)-*N*,2-dimethyl-5-cyanobenzenesulfonamide (18b)

A solution of of 5-chloro-3-(2-nitroethyl)pyrazolo[1,5-*a*]pyrimidine (**15**) (45 mg, 0.199 mmol) in MeOH (10 mL) and treated with a spatula of RaNi slurrey under hydrogen at RT for 1 hr. The reaction mixture was filtered through a pad of Celite® and the solvent was removed *in vacuo*. The resulting material was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. To this was added NEt_3 (83 µL, 0.597 mmol) followed by 2-methyl-5-cyanobenzenesulfonyl chloride (47 mg, 0.218 mmol). The reaction was allowed to warm slowly to RT and was stirred for 16 h. The reaction was diluted with CH_2Cl_2 (10 mL) and washed with sat. $NaHCO_3$ (10 mL) and water (2 x 10 mL) dried (Whatman® PS1 filterpaper) and solvent was removed *in vacuo*. After chromatography (40-100% EtOAc-cyclohexane) this gave *N*-

(2-(5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)ethyl)-2-methyl-5-cyanobenzenesulfonamide (**17b**) as a yellow solid (27.7 mg, 37%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.54 (d, *J* 7.3, 1H), 8.24 (d, *J* 1.5, 1H), 7.96 (s, 1H), 7.70 (dd, *J* 7.9, 1.8, 1H), 7.39 (d, *J* 7.9, 1H), 6.81 (d, *J* 7.2, 1H), 5.64 (m, 1H), 3.41 (m, 2H), 2.98 (t, *J* 6.2, 2H), 2.67 (s, 3H); LC-MS t_R 5.88 min; LC_{254} 99.0%; *m/z* 390.1/392.2[M+H].

Using the conditions outlined for **18a**, **17b** (16 mg, 0.04 mmol) was treated with MeI (9 µL, 0.145 mmol) to yield **18b** as a yellow solid (12 mg, 78%). ^{1}H NMR δ_H (d_6-Acetone, 300 MHz) 8.82 (d, *J* 7.3, 1H), 8.09 (d, *J* 1.8, 1H), 8.07 (s, 1H), 7.82 (dd, *J* 7.9, 1.8, 1H), 7.42 (d, *J* 7.9, 1H), 6.97 (d, *J* 7.3, 1H), 3.57 (t, *J* 6.8, 2H), 3.07 (s, 3H), 3.03 (t, *J* 6.8, 2H), 2.43 (s, 3H). ^{13}C NMR δ_C (d_6-Acetone, 75 MHz) 145.71, 142.94, 139.21, 137.38, 135.44, 133.60, 132.44, 117.29, 110.13, 108.53, 106.61, 49.47, 32.81, 20.84, 19.76; LC-MS t_R 5.88 min; LC_{254} 99.0%; *m/z* 390.1/392.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{17}H_{16}ClN_5O_2S$ $[M+H]^+$: 390.0786; found 390.0791.

Synthesis of 5-chloro-3-iodopyrazolo[1,5-*a*]pyrimidine (19)

N-Iodosuccinimide (1.61 g, 7.16 mmol) was added to a solution of 5-chloropyrazolo[1,5-*a*]pyrimidine (**7**) (1 g, 6.51 mmol) in anhydrous DMF (10 mL). After stirring at RT for 4 h the reaction was poured into 10% sodium thiosulfate (75 mL). The resulting precipitate was isolated *via* filtration and dried (Whatman® PS1 filter paper) to give **19** as a pale brown solid (1.62 g, 89%). ^{1}H NMR δ_H (d_6-DMSO, 300 MHz) 9.19 (d, *J* 7.2, 1H), 8.38 (s, 1H), 7.18 (d, *J* 7.2, 1H). LC-MS t_R 6.37 min; LC_{254} 99%; *m/z* 280.1/282.0 [M+H]. This was used without further purification.

Preparation of 3-heteroaryl pyrazolo[1,5-*a*]pyrimidines

General Method D: A solution of K_3PO_4 (3.5 eq) in water (2M) was added to a solution of 5-chloro-3-iodopyrazolo[1,5-*a*]pyrimidine (**19**) (1 eq) and the desired boronic acid pinacol ester (1.2 eq) in

dioxane (0.1 M). To this was added $PdCl_2(dppf).CH_2Cl_2$ (0.5 eq) and the reaction was heated at 80 °C for 16 h. The crude material was adsorbed onto silica and purified by automated flash chromatography on silica gel (generally EtOAc – cyclohexane) to yield the desired product.

tert-Butyl 4-(5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)-1*H*-pyrazole-1-carboxylate (20a)

Reaction of 5-chloro-3-iodopyrazolo[1,5-*a*]pyrimidine (**19**) (129 mg, 0.462 mmol) with 1-(Boc)-1*H*-pyrazole-4-boronic acid pinacol ester using method D gave, after chromatography (0-60% EtOAc – cyclohexane) filtration through silica gave **20a** as a yellow solid which was used without further purification. LC-MS t_R 7.05 min; LC_{254} 97.0%; *m/z* 320.3/322.1 [M+H].

5-Chloro-3-(1*H*-pyrazol-3-yl)pyrazolo[1,5-*a*]pyrimidine (20b)

Reaction of 5-chloro-3-iodopyrazolo[1,5-*a*]pyrimidine (**19**) (250 mg, 0.895 mmol) with pyrazole-3-boronic acid pinacol ester using method D gave, after chromatography (0-100% EtOAc – cyclohexane) **20b** as a yellow solid (33 mg, 17%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.60 (d, *J* 7.2, 1H), 8.45 (s, 1H), 7.67 (bs, 1H), 6.87 (d, *J* 7.3, 1H), 6.78 (bs, 1H); LC-MS t_R 5.20 min; LC_{254} 93%; *m/z* 220.2/222.2 [M+H].

5-Chloro-3-(1*H*-pyrrol-3-yl)pyrazolo[1,5-*a*]pyrimidine (20c)

Reaction of 5-chloro-3-iodopyrazolo[1,5-*a*]pyrimidine (**19**) (250 mg, 0.895 mmol) with pyrrole-3-boronic acid pinacol ester using method D gave, after chromatography (0-40% EtOAc – cyclohexane) **20c** as a yellow solid (58 mg, 30%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.53 (d, *J* 7.3, 1H), 8.30 (s, 1H), 7.46

(bd, *J* 1.2, 1H), 6.90 (dd, *J* 4.5, 2.5, 1H), 6.74 (d, *J* 7.3, 1H), 6.70 (m, 1H); LC-MS t_R 5.97 min; LC_{254} 99%; *m/z* 219.2/221.2 [M+H].

Synthesis of 5-chloro-3-(1-((2-methyl-5-nitrophenyl)sulfonyl)-1*H*-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidine (21a)

tert-Butyl 4-(5-chloropyrazolo[1,5-*a*]pyrimidin-3-yl)-1H-pyrazole-1-carboxylate (**20a**) (57 mg, 0.178 mmol) was dissolved in 4 M HCl in 1,4-dioxane. The reaction was stirred at RT for 15 min until a precipitate formed. The solvent was removed *in vacuo* and the solid was suspended in CH_2Cl_2 (2 mL) and cooled to 0 °C, NEt_3 (99 µL, 0.712 mmol) was added and a solution was formed. To this was added 2-methy-5-nitrobenzene sulfonyl chloride (43 mg, 0.182 mmol) and the reaction was allowed to warm slowly to RT and stirred for 72 h. The reaction was diluted with CH_2Cl_2 (10 mL) and washed sequentially with saturated $NaHCO_3$ (15 mL), water (2 x 15 mL) and brine (15 mL). The organic layer was dried (Whatman® PS1 filter paper) and the solvent was removed *in vacuo*. After purification by preparative HPLC (Waters Alliance HT; XBridge™ Prep C18, 5 µm, 10 x 100 mm; Solvent A: H_2O-0.1% HCOOH, Solvent B: CH_3CN 0.1% HCOOH, Gradient: 30-100% B over 12min @ 4.0 mL/min) **21a** was obtained as a yellow solid (7.9 mg, 11%). ^1H NMR δ_H ($CDCl_3$, 300 MHz) 9.00 (d, *J* 2.4, 1H), 8.70 (d, *J* 0.7, 1H), 8.59 (d, *J* 7.2, 1H), 8.39 (dd, *J* 8.3, 2.4, 1H), 8.32 (s, 1H), 8.26 (d, *J* 0.7, 1H), 7.55 (d, *J* 8.7, 1H), 6.88 (d, *J* 7.2, 1H), 2.84 (s, 3H); LC-MS t_R 7.70 min; LC_{254} 98.8%; *m/z* 419.1/421.1 [M+H]; HRMS (ESI^+) Calc'd for $C_{16}H_{11}ClN_6O_4S$ $[M+H]^+$: 419.0324; found 419.0322.

Synthesis of 5-chloro-3-(1-(2-methyl-5-nitrophenylsulfonyl)-1*H*-pyrazol-3-yl)pyrazolo[1,5-*a*]pyrimidine (21b)

5-Chloro-3-(1*H*-pyrazol-3-yl)pyrazolo[1,5-*a*]pyrimidine (**20b**) (30 mg, 0.137 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. To this was added NEt_3 (76 µL, 0.548 mmol) followed by 2-methyl-5-nitrobenzenesulfonyl chloride (33 mg, 0.140 mmol). The reaction was allowed to warm slowly to RT and was stirred for 16 h. The reaction was diluted with CH_2Cl_2 (10 mL) and washed with sat. $NaHCO_3$ (10 mL) and water (2 x 10 mL) dried (Whatman® PS1 filter paper) and solvent was removed *in vacuo*. Purification by preparative HPLC (same conditions as for **21a**) gave **21b** as a yellow solid (9 mg, 16%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.98 (d, *J* 2.4, 1H), 8.60 (s, 1H), 8.59 (d, *J* 7.3, 1H), 8.37 (dd, *J* 8.4, 2.3, 1H), 8.26 (d, *J* 2.8, 1H), 7.54 (d, *J* 8.5, 1H), 7.30 (d, *J* 2.8, 1H), 6.88 (d, *J* 7.3, 1H), 2.87 (s, 3H). LC-MS t_R 7.72 min; LC_{254} 98%; *m/z* 419.2/421.2 [M+H]; HRMS (ESI^+) Calc'd for $C_{16}H_{11}ClN_6O_4S$ $[M+H]^+$: 419.0324; found 419.0341.

Synthesis of 5-chloro-3-(1-(2-methyl-5-nitrophenylsulfonyl)-1*H*-pyrrol-3-yl)pyrazolo[1,5-*a*]pyrimidine (21c)

5-Chloro-3-(1*H*-pyrrol-3-yl)pyrazolo[1,5-*a*]pyrimidine (**20c**) (60 mg, 0.274 mmol) was dissolved in THF (2 mL) and cooled to -10 °C and NaH (60 % dispersion, 16 mg, 0.412 mmol) was added. After stirring for 30 mins, 2-Methyl-5-nitrobenzenesulfonyl chloride (84 mg, 0.356 mmol) was added and the reaction was allowed to warm slowly to RT and was stirred for 72 h. The reaction was quenched with water (20 mL) and extracted with EtOAc (3 x 15 mL), the combine organic phases were dried (Whatman® PS1 filterpaper) and solvent was removed *in vacuo*. After chromatography (0-80% EtOAc-cyclohexane) this gave **21c** as a yellow solid (7.4 mg, 7%). ^{1}H NMR δ_H ($CDCl_3$, 300 MHz) 8.55 (d *J* 7.2, 1H), 8.54 (d, *J* 3.5, 1H), 8.33 (dd, *J* 8.3, 2.4, 1H), 8.29 (s, 1H), 7.79 (dd, *J* 1.9, 1.8, 1H), 7.53 (d, *J* 8.3, 1H), 7.30 (dd, *J* 3.3, 2.4, 1H), 6.94 (dd, *J* 3.3, 1.5, 1H), 6.82 (d, *J* 7.2, 1H), 2.81 (s, 3H); LC-MS t_R 7.98 min; LC_{254} 99.0%; *m/z* 418.10/420.10 [M+H]; HRMS (ESI^+) Calc'd for $C_{17}H_{12}ClN_5O_4S$ $[M+H]^+$: 418.0371; found 418.0385.

4.2 Biology

Biological screening of compounds against CDK9/T1 cyclin

This assay method is based on quantifying the inhibition of CDK9 using luminescent readout. Briefly, the assay measures ADP formed from ATP in a kinase reaction in which the luminescent signal is proportional to kinase activity.

Recombinant human CDK9/cyclin T1 (Life Technologies Cat# PR7541B) was diluted in the enzyme dilution buffer (20 mM Tris HCl (pH7.5), 0.02% Triton X-100, 0.01% BSA, 2 mM DTT, 0.5 mM Na_3VO_4, 10% Glycerol) to a working concentration of 10 ng/mL; an aliquot of 2.5 µL/well added to a 384 well plate (Corning white plate, #3673). Compounds were added to a final 2% DMSO by pin tool (Minitrak™, Perkin Elmer). The substrate CDK7/9-tide (H-YSPTSPSYSPTSPSYSPTSPS-KKKK-OH (Cat# 12-526, Millipore), and ATP were added to a final concentration of 100 µM and 10 µM, respectively, in assay buffer (25 mM Tris HCl (pH7.5), 10 mM $MgCl_2$, 0.5 mM EGTA (pH 8), 0.5 mM Na_3VO_4, 5 mM β-glycerol phosphate, 0.1 % Triton X-100, 2.5 mM DTT) at 2.5 µL/well. The plate was sealed and incubated for 90 min at 30°C. After the first incubation step, 5 µL/well ADP-Glo ™ (Promega ADP-Glo Kinase Assay kit, V9102) was added to all wells and the plate sealed and left at room temperature for 45 min. For the final incubation step, 10 µL/well of Kinase Detection Reagent was added to all wells and the plate sealed and left at room temperature for 30 mins. The plate was read on a luminescence plate reader (EnVision® Multilabel, Perkin Elmer). The control positive wells contain CDK9 and substrate mixture and control negative wells contain enzyme dilution buffer and substrate mixture. The reported IC_{50} values are means of at least two separate determinations with typical variations of less than ±20%.

Biological screening of compounds against FLT3

Kinase assays were performed in Greiner White Flat-bottom, 384-well, small volume plates (Cat no: 784075) using an Alphascreen Phosphotyrosine Assay Kit (P-Tyr-100) (Perkin Elmer 6760620M).

A biotin labelled peptide is used as substrate (amino acid sequence: Biotin-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2). The FLT3 enzyme was purchased from Carna Biosciences (cat no: 08-154). The 15 µL assay contained 10 mM HEPES pH 7.4, 25 mM NaCl, 10 mM $MgCl_2$, 0.01% (v/v) Tween-20, 50 µM Na_3VO_4, 0.01% (w/v) albumin from chicken egg white, 1 mM DTT, 110 nM peptide substrate, 94 µM ATP, and 0.045 ng/reaction FLT3 enzyme, with the enzyme being omitted from negative control reactions. 100 nL of compound was added to the reaction from a dilution series made up in DMSO. Positive and negative control reactions received the same volume of DMSO without compounds. The plates were sealed with adhesive seals and incubated for 90 min at 30 °C. The reactions were stopped with the detection reagents added at the same time. Product formation was quantified as amplified luminescence between PerkinElmer AlphaScreen™ beads, using Streptavidin-coated donor and anti-phosphotyrosine (P-Tyr-100) acceptor beads. To each reaction, 5 µL containing 10 mM HEPES pH 7.4, 25 mM NaCl, 100 mM EDTA, 0.01% (v/v) Tween-20, and 6.25 µg/ml of each bead type were added. Plates were incubated for 4 h before being read on a PerkinElmer EnVision™ plate reader in HTS AlphaSreen mode. IC_{50} values were obtained by calculating percent inhibition (% I) for each reaction relative to controls on the same plate (% I=(I-CN)/(CP-CN) where CN/CP are the averages of the negative/positive reactions, respectively), then fitting the % I data vs compound concentration [I] to % I=(A+((B-A)/(1+((C/[I]D)))) where A is the lower asymptote, B is the upper asymptote, C is the IC_{50} value, and D is the slope factor.

Biological screening of compounds against CDK7 and PI3Kα

Kinase activity against CDK7 and PI3Kα was determined at a commercial provider using a radiometric assay[37] with [ATP] at 10 μM (Reaction Biology, Malvern, PA, USA).

Cellular viability assays

Cell viability and proliferation assays were performed using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega). Briefly, MV4;11 cells (ATCC, VA) were seeded in opaque 96-well plates, treated with 11 point dilution of indicated compounds and incubated at 37 °C/10 % CO_2 for 72 h. After addition of the CellTiter-Glo reagent the plates were read using a luminescent plate reader (Envision, Perkin Elmer) and the data was analysed using nonlinear regression algorithms in Prism GraphPad software (La Jolla, CA) to calculate IC_{50} values. Data shown represents the mean of 2 independent experiments.

4.3 Molecular modelling and compound docking

There are fourteen CDK9-ligand crystal structures in the PDB (December 2014), all are in complex with cyclin T1: ten are CDK9-inhibitor complexes (PDB IDs: 3BLR, 3LQ5, 3MY1, 3TNH, 3TN8, 4BCF, 4BCG, 4BCH, 4BCI and 4BCJ)[38-43] and four contain CDK9-ATP (or adenosine analogues), in some cases the complexes include additional proteins (PDB IDs: 3BLQ, 3MIA, 4IMY and 4OGR).[38, 44-46] The fourteen CDK9-ligand crystal complexes were aligned via the kinase domain Cα atoms (root mean square deviation < 0.6 Å); the kinase domain conformations were very similar, with the greatest variation observed in the Gly-rich loop (also known as the P-loop). Three CDK9-ligand complexes with varying Gly-rich loop conformations were selected for the compound docking studies (PDB IDs: 3BLR, 3MY1 and 4BCI; with resolutions of 2.80 Å, 2.80 Å and 3.10 Å respectively); consistent docking

poses were obtained for PIK-75 (**6**) and the pyrazolo[1,5-*a*]pyrimidine compounds presented in *Tables 1-4*, therefore only the results for 3BLR are described.

The crystal structure ligands were extracted from the ATP binding sites of 3BLR, 3MY1 and 4BCI. PIK-75 (**6**) and the pyrazolo[1,5-*a*]pyrimidine compounds were constructed using standard bond lengths and bond angles within SYBYL-X 2.1.1 (Certara, L.P. Princeton, NJ, USA). The compound structures were geometrically optimised within SYBYL-X 2.1.1 using the MMFF94s forcefield and partial atomic charges, conjugate gradient convergence method, termination of the optimisation was achieved when the gradient difference of successive steps was < 0.05 kCal/mol·Å. Flexible protein and compound docking was performed using Surflex-Dock v2.7 (within SYBYL-X 2.1.1); the protomol was generated using the ligand method, a threshold of 0.50 and a bloat value of 2. The docking mode used was GeomX with protein flexibility enabled and all other parameters set to default values. The docked poses were ranked using C-Score and the top 50 ranked poses of each compound were retained for examination. The docked poses were all within 10 kCal/mol of the compound global energy minimum. As positive controls, the crystal structure ligands were included in the docking process and the crystal structure conformation was amongst the top 5 ranked poses for each ligand. The top 50 ranked poses for each compound were examined for potential interactions within the ATP binding site, in particular with the known CDK9 hinge interaction point Cys106. ATP and Type I kinase inhibitors all interact with a residue on the hinge region of the ATP binding pocket, therefore a hydrogen bond to Cys106 was considered essential when analysing the CDK9 docking results and any pose where the compound did not make a hydrogen bond to this residue was rejected.

ClogP was calculated using SYBYL-X 2.1.1. *Figures 2 and 3* were created using The PyMOL Molecular Graphics System, Version 1.6.0.0 (Schrödinger LLC, Cambridge, MA).

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Graphical abstract