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Discovery of pyrazolo[1,5-*a*]pyridines as p110α-selective PI3 kinase inhibitors

Jackie D. Kendall[a,b,*], Patrick D. O'Connor[a], Andrew J. Marshall[a], Raphaël Frédérick[a,†], Elaine S. Marshall[a], Claire L. Lill[c], Woo-Jeong Lee[c], Sharada Kolekar[c], Mindy Chao[c], Alisha Malik[c], Shuqiao Yu[c], Claire Chaussade[b,c,‡], Christina Buchanan[b,c], Gordon W. Rewcastle[a,b], Bruce C. Baguley[a,b], Jack U. Flanagan[a,b], Stephen M.F. Jamieson[a,b], William A. Denny[a,b], Peter R. Shepherd[b,c]

[a] *Auckland Cancer Society Research Centre, School of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[b] *Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[c] *Department of Molecular Medicine and Pathology, School of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*

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ABSTRACT

We have made a novel series of pyrazolo[1,5-*a*]pyridines as PI3 kinase inhibitors, and demonstrated their selectivity for the p110α isoform over the other Class Ia PI3 kinases. We investigated the SAR around the pyrazolo[1,5-*a*]pyridine ring system, and found compound **5x** to be a particularly potent example (p110α IC_{50} 0.9 nM). This compound inhibits cell proliferation and phosphorylation of Akt/PKB, a downstream marker of PI3 kinase activity, and showed in vivo activity in an HCT-116 human xenograft model.

1. Introduction

Phosphoinositide-3-kinases (PI3 kinases) are lipid kinases which phosphorylate the 3′-hydroxyl group of phosphatidylinositol 4,5-diphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 then recruits PH domain containing proteins such as protein kinase B (PKB, also known as Akt) to the cell membrane. Once recruited, PKB is phosphorylated and activated, leading to a cascade of cell signalling which control a range of cellular processes like cell proliferation, growth and survival.[1]

Furthermore, lipid phosphatase PTEN which dephosphorylates PIP3, is often deleted or inactivated in many cancer types, leading to increased levels of PIP3 and increased tumour survival.[2]

The PI3 kinases are split into three sub-families (class I, II and III), and class I is further split into class Ia and Ib based upon their mechanism of activation. The class Ia PI3 kinases are heterodimeric, consisting of a catalytic subunit (p110α, p110β or p110δ) in complex with a regulatory subunit.[1] *PIK3CA*, the gene encoding for p110α, is often over-expressed and mutated in many cancer types. Two of the most common of these mutations (E545K and H1047R) have been confirmed as activating mutations and hence increase levels of PIP3. Mutations in p110β and p110δ have not yet been reported.[3]

Inhibitors of PI3 kinase, and in particular selective inhibitors of p110α, could prove to be an important new strategy in cancer treatment. A recent report indicates that inhibiting p110α alone can block tumour progression[4] however there are few reports of p110α selective PI3 kinase inhibitors in the literature.[4–8] Many groups are now trying to exploit PI3 kinase inhibitors as an approach to cancer therapy, as exemplified by the number of small

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Abbreviations: ATP, adenosine triphosphate; Boc, *tert*-butoxycarbonyl; CDI, 1,1′-carbonyldiimidazole; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; DNPH, *O*-(2,4-dinitrophenyl)hydroxylamine; EDTA, ethylenediaminetetraacetic acid; ip, intraperitoneal; MEM, minimum essential medium; MSH, *O*-(mesitylsulfonyl)hydroxylamine; PDB, protein data bank; PEG-400, polyethylene glycol with molecular weight 380–420 g mol^{-1}; PH domain, pleckstrin homology domain; PIK-75, *N*′-((6-bromoimidazo[1,2-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide; PIP2, phosphatidylinositol 4,5-diphosphate; PIP3, phosphatidylinositol 3,4,5-triphosphate; PI3 kinase, phosphoinositide-3-kinase; PKB, protein kinase B; PTEN, phosphatase and tensin homologue; *p*-TsOH, *p*-toluenesulfonic acid monohydrate; q.d., daily; SAR, structure–activity relationship; THF, tetrahydrofuran; TFA, trifluoroacetic acid; tlc, thin layer chromatography; Tris, tris(hydroxymethyl)aminomethane.

* Corresponding author.
E-mail address: j.kendall@auckland.ac.nz (J.D. Kendall).

† Present address: Drug Design and Discovery Centre (D^3C), University of Namur, FUNDP, 61 rue de Bruxelles, 5000 Namur, Belgium.

‡ Present address: Centre for Cell Signalling, Barts Cancer Institute, Queen Mary University of London, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, UK.

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molecule PI3 kinase inhibitors currently going through clinical trials,[3,9] however few of these are selective for p110α.

PIK-75 (Fig. 1) is an experimental compound that shows good selectivity for p110α over the other class Ia PI3 kinases and has shown activity in a human cancer xenograft model.[6] However, there has been minimal literature around the structure–activity relationships (SARs) of PIK-75 beyond the original papers from Hayakawa et al.[5,6] Schmidt-Kittler et al.[8] have published analogues of PIK-75 with extra substituents on the benzene ring and showed that two of these compounds inhibit the formation of tumour metastases in mice, although they only had a minor growth delay effect in an HCT-116 cancer xenograft model. We reported on replacing the imidazo[1,2-*a*]pyridine ring with other heterocycles, and found that a suitably positioned nitrogen available for hydrogen bonding was essential for activity.[10] In this paper, we continue with this work and investigate the SAR around the novel pyrazolo[1,5-*a*]pyridine ring system.

2. Results and discussion

2.1. Synthesis

The sulfonohydrazides were assembled (Scheme 1) by conversion of an appropriately substituted pyridine (**2**) through to pyrazolo[1,5-*a*]pyridines containing a 3-ester group (**3b**–**j**) by initial N-amination with either *O*-(mesitylsulfonyl)hydroxylamine (MSH)[11] or *O*-(2,4-dinitrophenyl)hydroxylamine (DNPH),[12] followed by a 1,3-dipolar cycloaddition with ethyl propiolate.[13] Although DNPH was more stable and easier to handle than MSH, it was also less reactive and did not react well with pyridines containing an electron-withdrawing group. MSH proved far superior for these pyridines. In the case of 3-substituted pyridines (3-bromopyridine and 3-methylpyridine) both possible isomeric products were formed. These were easily separated by silica gel chromatography.

The ester functionality was then converted to an aldehyde (**4b**–**j**) by one of two methods. A reduction of the ester to the primary alcohol followed by reoxidation with MnO_2 worked in many cases, but we found that a one-pot ester hydrolysis and decarboxylation by refluxing in 40% aqueous H_2SO_4, followed by a Vilsmeier reaction to install the aldehyde was more efficient. Finally, conversion to sulfonohydrazides **5a**–**j** was achieved by condensation with methylhydrazine sulfate under weakly basic conditions followed by sulfonylation without isolation of the intermediate *N*-methylhydrazone.

The sulfonohydrazides containing functional groups which would not tolerate these reaction conditions were made by alternative methods. Compounds **5m**–**r** were made starting from *tert*-butyl pyridin-4-ylcarbamate (**6**) (Scheme 2). N-Amination and cyclisation to ester **3k** was carried out as before. Removal of the Boc protecting group with trifluoroacetic acid followed by diazotisation introduced a 5-halo substituent. Decarboxylation and Vilsmeier reaction afforded aldehydes **4m**–**o**. Additionally, bromo compound **4m** underwent Stille reactions to make cyclopropane **4p** and alkene **4q**, and a Sonogashira reaction to form alkyne **4r**. Finally, condensation and sulfonylation as before afforded sulfonohydrazides **5m**–**r**.

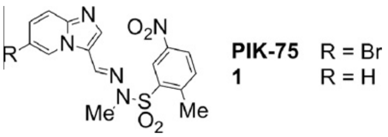


Figure 1. Structures of PIK-75 and **1**.

Compounds **5t**–**x** were made from methyl isonicotinate (**8**) by *N*-amination with MSH and cyclisation as before (Scheme 3). Diester **3t** was converted to mono-carboxylic acid **7s** by refluxing in 40% aqueous H_2SO_4, and was then re-esterified to ester **7t** with HCl in MeOH. A Vilsmeier reaction followed by ester hydrolysis afforded carboxylic acid **4s**, and then further elaboration was carried out by activation with thionyl chloride, followed by reaction with amines to form carboxamides **4u**–**w**. Alternatively, carboxylic acid **7s** was converted to carboxamide **7u** by activation with ^{i}BuOCOCl followed by reaction with ammonia gas. Vilsmeier conditions then introduced the formyl group and concomitantly dehydrated the carboxamide to form nitrile **4x**. Condensation and sulfonylation afforded sulfonohydrazides **5t**–**x**.

Disubstituted compound **5z** was made starting from 3-fluoroisonicotinaldehyde (**9**) by protecting the aldehyde as an acetal followed by N-amination and cyclisation to **3y** as before (Scheme 4). The aldehyde was then unmasked and oxidised to carboxylic acid **3z**. The ethyl ester was then decarboxylated with 40% aqueous H_2SO_4, and the remaining carboxylic acid converted to primary carboxamide **7z**. Vilsmeier conditions then firstly dehydrated the amide and then installed the required aldehyde group. Condensation and sulfonylation afforded sulfonohydrazide **5z**.

Compounds **5aa**–**ac** were made from 4-methoxypyridine (**10**) by N-amination and cyclisation as before (Scheme 5). Decarboxylation of ester **3aa** with 40% aqueous H_2SO_4 also cleaved the methyl ether; this was then reintroduced with iodomethane to afford ether **7aa**. A Vilsmeier reaction of pyrazolo[1,5-*a*]pyridines **7aa** and **7ab** introduced the formyl groups, and then subsequent hydroxyl acetylation of **4ab** afforded ester **4ac**. Aldehydes **4aa** and **4ac** did not react satisfactorily under the usual methylhydrazine sulfate reaction conditions. Reaction was slow for these aldehydes and decomposition was observed. However, **4aa** and **4ac** condensed in good yield with 2-methyl-5-nitrobenzenesulfonohydrazide,[14] and then subsequent N-methylation without isolation using diazomethane afforded **5aa** and **5ac**. Acetate **5ac** was then cleaved to phenol **5ab** with $NaHCO_3$.

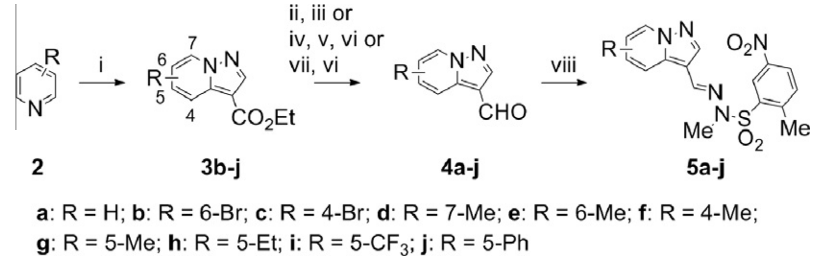
Sulfonohydrazides **13b**–**d** and **14b**–**d** were made by N-amination of isonicotinonitrile (**11**) with MSH, followed by cyclisation with an alkynyl ketone (Scheme 6). 3-Butyn-2-one gave methyl ketone **12b** in reasonable yield. We chose however, to make ethyl and propyl ketones **12c** and **12d** using the trimethylsilyl alkynes due to ease of handling of the reagents. The addition of potassium fluoride was found to significantly improve the yields in these instances. Condensation of **4x** and **12b**–**d** with 2-methyl-5-nitrobenzenesulfonohydrazide gave compounds **13a**–**d**, and then N-methylation proceeded smoothly with diazomethane to afford **14b**–**d**.

Finally, compounds **13e** and **14e** were made starting from *tert*-butyl pyridin-4-ylcarbamate (**6**) (Scheme 7). N-Amination and cyclisation with 3-butyn-2-one gave ketone **12e**, which on cleavage of the *tert*-butoxycarbonyl group and diazotisation, gave bromo compound **12f**. As before, condensation gave compound **13e** which was N-methylated with diazomethane to afford **14e**.

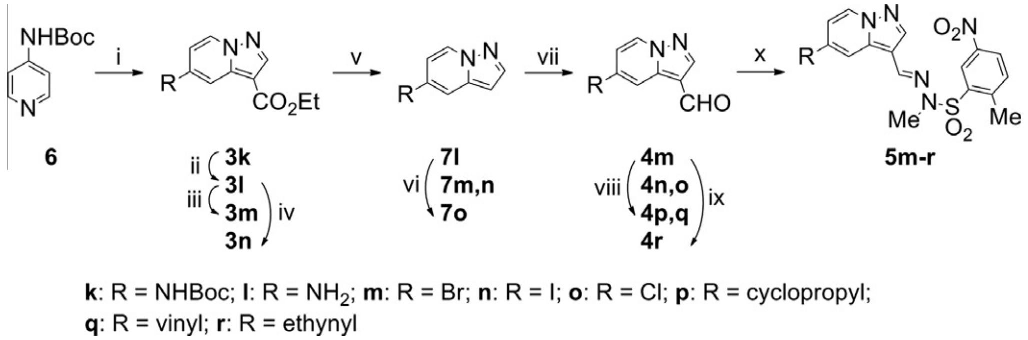
2.2. In vitro biology

In a previous paper, we described modifications to the heterocyclic core of PIK-75.[10] Subsequently, we found that the pyrazolo[1,5-*a*]pyridine ring system (**5a**) gave a similar potency towards p110α in comparison with the corresponding imidazo[1,2-*a*]pyridine (**1**) which did not contain the critical bromo substituent, whilst retaining good selectivity over p110β and p110δ (Table 1). This encouraging result led us to the present investigation of the SAR around this novel class of PI3 kinase inhibitors.

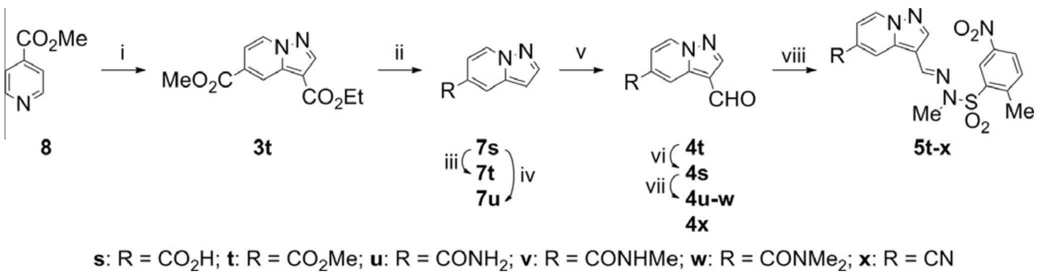
Firstly we looked at the effect of substituents around the 6-membered ring of the pyrazolo[1,5-*a*]pyridine ring system. Of the bromo isomers **5b**, **5c** and **5m**, only the 5-bromo compound **5m** showed any significant activity against p110α, with a 250-fold



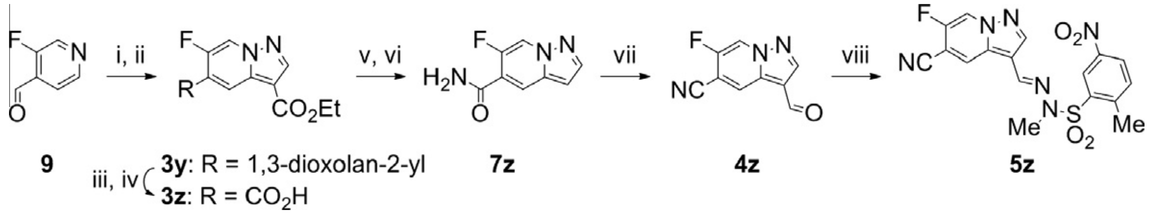
Scheme 1. Reagents: (i) MSH, CH_2Cl_2 or DNPH, MeCN then ethyl propiolate, K_2CO_3, DMF; (ii) 40% H_2SO_4, reflux; (iii) $POCl_3$, DMF; (iv) NaOH, H_2O, EtOH; (v) CDI, THF then $NaBH_4$; (vi) MnO_2, CH_2Cl_2; (vii) $LiAlH_4$, THF; (viii) $MeHNNH_2.H_2SO_4$, $NaHCO_3$, MeOH then 2-methyl-5-nitrobenzenesulfonyl chloride.



Scheme 2. Reagents: (i) DNPH, CH_2Cl_2 then ethyl propiolate, K_2CO_3, DMF; (ii) TFA, CH_2Cl_2; (iii) $NaNO_2$, H_2O, HBr then CuBr, HBr; (iv) $NaNO_2$, HCl, H_2SO_4, H_2O then urea then KI, H_2O; (v) 40% H_2SO_4, reflux; (vi) $NaNO_2$, CuCl, H_2O, HCl; (vii) $POCl_3$, DMF; (viii) $RSnBu_3$, $Pd(PPh_3)_4$, PhMe, reflux; (ix) Me_3SiCCH, $(Ph_3P)_2PdCl_2$, CuI, NEt_3, DMF then K_2CO_3, MeOH; (x) $MeHNNH_2.H_2SO_4$, $NaHCO_3$, MeOH then 2-methyl-5-nitrobenzenesulfonyl chloride.



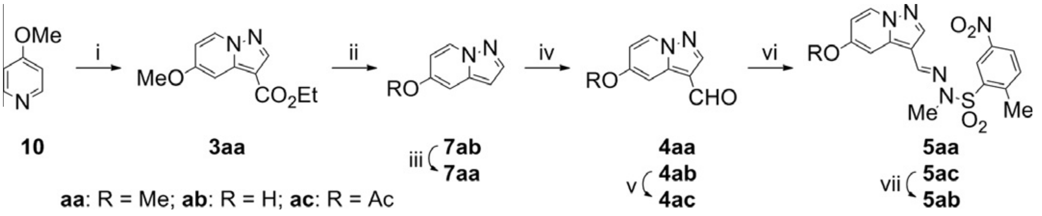
Scheme 3. Reagents: (i) MSH, CH_2Cl_2 then ethyl propiolate, K_2CO_3, DMF; (ii) 40% H_2SO_4, reflux; (iii) MeOH, HCl; (iv) ^{i}BuOCOCl, NEt_3, CH_2Cl_2 then $NH_{3(g)}$; (v) $POCl_3$, DMF; (vi) NaOH, H_2O, EtOH; (vii) $SOCl_2$, reflux then amine, CH_2Cl_2; (viii) $MeHNNH_2.H_2SO_4$, $NaHCO_3$, MeOH then 2-methyl-5-nitrobenzenesulfonyl chloride.



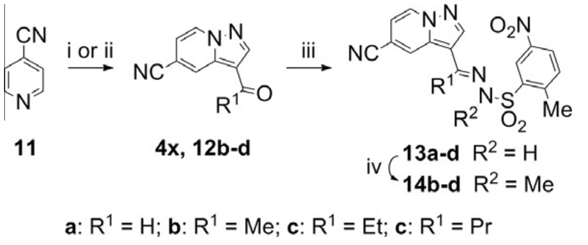
Scheme 4. Reagents: (i) ethylene glycol, *p*-TsOH, benzene, Dean–Stark, reflux; (ii) DNPH, MeCN then ethyl propiolate, K_2CO_3, DMF; (iii) $HClO_4$, dioxane, acetone; (iv) $NaClO_2$, H_2O_2, NaH_2PO_4, H_2O, MeCN; (v) 40% H_2SO_4, reflux; (vi) CDI, CH_2Cl_2, then NH_3, MeOH; (vii) $POCl_3$, DMF; (viii) $MeHNNH_2.H_2SO_4$, $NaHCO_3$, MeOH then 2-methyl-5-nitrobenzenesulfonyl chloride.

improvement in potency over **5a**, and good selectivity over p110β and p110δ (60-fold and 40-fold, respectively). A similar pattern was observed for the methyl isomers **5d**–**g**, although **5g** was less active than **5m**.

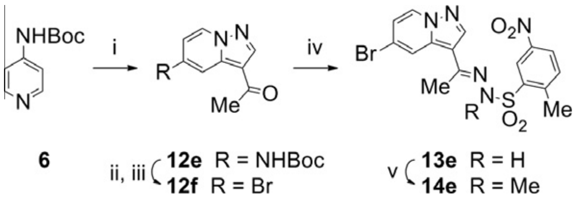
We further investigated 5-substitution with a range of large and small, and electron-donating and electron-withdrawing substituents. Ethyl **5h** and phenyl **5j** substituents gave a decrease in p110α activity, however trifluoromethyl **5i** and vinyl **5q** analogues still retained some p110α activity, and ethynyl **5r** was particularly active at 2.4 nM for p110α. Iodo compound **5n** was slightly less potent than bromo compound **5m**, whereas chloro compound **5o** had similar potency. The methyl ester **5t** and primary carboxamide **5u** retained weak activity, but adding further substitution to the amide (**5v** and **5w**) further decreased the p110α activity. Nitrile **5x** was the most potent of the 5-substituents (IC_{50} 0.9 nM for p110α) with 50-fold selectivity over both p110β and p110δ. Of



Scheme 5. Reagents: (i) DNPH, MeCN then ethyl propiolate, K_2CO_3, DMF; (ii) 40% H_2SO_4, reflux; (iii) MeI, K_2CO_3, DMF; (iv) $POCl_3$, DMF; (v) Ac_2O, NEt_3, CH_2Cl_2; (vi) 2-methyl-5-nitrobenzenesulfonohydrazide, MeOH then CH_2N_2, Et_2O, THF; (vii) $NaHCO_3$, H_2O, MeOH.



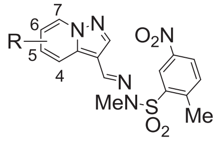
Scheme 6. Reagents: (i) MSH, CH_2Cl_2, then 3-butyn-2-one, K_2CO_3, DMF; (ii) MSH, CH_2Cl_2, then $R^1COC{\equiv}CSiMe_3$, KF, K_2CO_3, DMF; (iii) 2-methyl-5-nitrobenzenesulfonohydrazide, MeOH; (iv) CH_2N_2, Et_2O, THF.



Scheme 7. Reagents: (i) DNPH, MeCN then 3-butyn-2-one, K_2CO_3, DMF; (ii) TFA, CH_2Cl_2; (iii) $NaNO_2$, 48% HBr, H_2O, CuBr; (iv) 2-methyl-5-nitrobenzenesulfonohydrazide, MeOH, reflux; (v) CH_2N_2, Et_2O, THF.

Table 1

Inhibition of PI3 kinase isoforms and cell proliferation for PIK-75, **1** and compounds **5**



Compd	R	IC_{50}[a] (nM)			IC_{50}[a] (μM)	
		p110α	p110β	p110δ	NZB5	NZOV9
PIK-75[b]		7.8	340	910	0.069	0.066
1[b]		860	>10000	5400	0.35	0.68
5a	H	960	>10000	3400	0.47	0.66
5b	6-Br	>1000	>1000	>1000	>20	4.5
5c	4-Br	>1000	>1000	>1000	>20	6.5
5m	5-Br	3.8	230	150	0.31	0.05
5d	7-Me	1600	>1000	>1000	6.0	1.2
5e	6-Me	>1000	>1000	>1000	10	8.0
5f	4-Me	>1000	>1000	>1000	18	>20
5g	5-Me	110	>1000	700	0.05	0.07
5h	5-Et	>1000	>1000	>1000	0.41	0.56
5i	5-CF_3	150	>1000	>1000	0.07	0.09
5j	5-Ph	>1000	>1000	>1000	10	10
5n	5-I	16	370	170	0.04	0.05
5o	5-Cl	2.9	390	90	n.d.	0.55
5p	5-cyclopropyl	1100	>1000	>1000	0.74	0.67
5q	5-$CH{=}CH_2$	130	>1000	>1000	0.99	0.58
5r	5-$C{\equiv}CH$	2.4	30	41	0.16	0.11
5t	5-CO_2Me	360	>1000	690	1.8	1.5
5u	5-$CONH_2$	530	>1000	>1000	0.60	10
5v	5-CONHMe	>1000	>1000	>1000	10	10
5w	5-$CONMe_2$	>1000	>1000	>1000	10	10
5x	5-CN	0.9	46	49	0.06	1.4
5z	5-CN, 6-F	45	1800	2500	n.d.	n.d.
5aa	5-OMe	190	>1000	>1000	0.94	0.52
5ab	5-OH	2600	>1000	>1000	>20	>20
5ac	5-OCOMe	620	>1000	>1000	8.2	8.9

[a] All IC_{50} values are the mean of duplicate or triplicate measurements.

[b] Data from Ref. 10.

the *O*-linked compounds **5aa**–**ac**, the methoxy **5aa** was the most potent at 190 nM for p110α. Overall, it appears that small groups are tolerated best at the 5-position, and we selected the 5-bromo and 5-cyano substituents to carry out further SAR analysis on compounds carrying modifications elsewhere on the molecule.

We also investigated substitution on the hydrazone linker (Table 2). Removal of the R^3 methyl group (**13a**) gave a 14-fold decrease in p110α potency compared to **5x**. In comparison, ketone-derived hydrazones **13b**–**e** where R^3 was hydrogen were at least 10-fold more potent than their methylated counterparts **14b**–**e**, suggesting that the presence of substituents at both R^2 and R^3 were causing a change in conformation and having a detrimental effect on PI3 kinase activity. The size of the R^2 substituent was also critical; the p110α potency decreased as R^2 increased in size from methyl to propyl (**13b**–**d**, **14b**–**d**). Finally, 5-bromo **13e** and **14e** were less active than their 5-cyano analogues **13b** and **14b**.

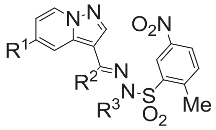
The compounds were also assayed for their effect on cell proliferation in a thymidine depletion assay in two early passage cell lines (Tables 1 and 2). The NZB5 cell line was derived from a brain tumour (medulloblastoma) and has the wild-type gene for p110α. The NZOV9 cell line was derived from an ovarian tumour (poorly differentiated endometrioid adenocarcinoma), and contains a mutant p110α enzyme with a single amino acid substitution in the kinase domain (Y1021C).

In general, the most active compounds in the p110α assay were also effective inhibitors of cell proliferation, particularly for the NZB5 cell line. Compounds **13a** and **13b** with no hydrazone N-substituents, were notable by their large drop in activity between p110α and NZB5, possibly due to a lack of cell penetration. The compounds bearing either no substituent at the 5-position of the pyrazolo[1,5-*a*]pyridine ring (**5a**), or an alkyl substituent (**5g**–**i** and **5p**) demonstrated greater potency in the NZB5 cells than for p110α. This may be driven by off-target activity, and additionally the divergences in IC_{50} values for the two cell lines may also point to the existence of off-target effects that reflect individual differences in the cell lines.

2.3. Molecular modelling

The PI3 kinase ATP binding site can be divided into three constitutive sites important in ligand binding, the adenine binding pocket (containing the hinge region backbone amide acceptor site that interacts with the nucleotide), an affinity pocket (extending from the adenine binding pocket), and a hydrophobic site on the C-terminal lobe of the catalytic domain. To identify potential interactions between the pyrazolo[1,5-*a*]pyridine based inhibitors and the p110α active site, docking studies were performed using

Table 2
Inhibition of PI3 kinase isoforms and cell proliferation for compounds **13a**–**e** and **14b**–**e**



Compd	R^1	R^2	R^3	IC_{50}[a] (nM)			IC_{50}[a] (μM)	
				p110α	p110β	p110δ	NZB5	NZOV9
13a	CN	H	H	13	620	580	2.6	4.9
13b	CN	Me	H	7	410	900	1.1	2.1
13c	CN	Et	H	10	>1000	440	0.088	0.36
13d	CN	Pr	H	39	>1000	310	0.37	0.64
13e	Br	Me	H	30	480	220	0.42	0.35
14b	CN	Me	Me	80	600	510	4.4	7.5
14c	CN	Et	Me	390	>1000	>1000	0.41	3.7
14d	CN	Pr	Me	610	>1000	>1000	0.68	2.3
14e	Br	Me	Me	1200	>1000	>1000	>20	>20

[a] All IC_{50} values are the mean of duplicate or triplicate measurements.

a model based on the apo p110α X-ray crystal structure (PDB code 2RD0).[15]

Most binding modes predicted for compounds **5a**, **5m**, **5g** and **5x** buried the pyrazolo[1,5-*a*]pyridine core in the adenine binding pocket, with a potential hydrogen bond interaction between the pyrazolo N atom and the backbone amide NH group of Val851. This is consistent with the characteristic hydrogen bond found in other PI3 kinase inhibitor complexes.[16] In this mode, the pyridyl ring of **5x** with its substituent cyano group extends into the affinity pocket where it forms a T-shape interaction with Tyr836 (Fig. 2a), an interaction also predicted for the related imidazo[1,2-*a*]pyridine core of PIK75.[10,17–19] The occupation of this pocket by the 5 position substituent is consistent with the substitution data reported in Table 1 that suggest smaller groups at the 5 position are better tolerated, and furthermore the cyano group may interact with the side chain hydroxyl of Tyr836 improving affinity over the bromine substituted compounds (**13b** vs **13e** and **14b** vs **14e**).

A range of possible interaction sites were predicted between the rest of the inhibitor and the p110α active site. Where the substituted pyrazolo[1,5-*a*]pyridine core accessed the affinity pocket these included a possible interaction between the sulfonyl moiety and the side chain carboxamide NH_2 group of the p110α specific residue Gln859 (Fig. 2a), while aryl nitro group interactions included the Lys802 side chain donor group in the catalytic centre, the backbone amide NH groups of Asn853 and Ser854 at the C-terminal end of the hinge region, and the side chains of Ser774 or the p110α specific Arg770 on the N-terminal wall of the catalytic cleft. Other modes also contacted the side chains of Gln859, Thr856 and Ser919 on the C-terminal wall of the active site. Those interactions which identified regions with p110α specific sequence differences were preferred. In these models, Gln859 is potentially involved in ligand binding and the aryl nitro group may interact with the p110α specific Arg770 or Ser774, with the alternative conformations illustrated in Figure 2b. Although Ser774 is found in other PI3 kinase isoforms, the surrounding residues are variable.[10] A binding site in this region is consistent with GDC0941 binding to both p110γ[20] and p110δ,[16] as well as AS15 binding to p110δ.[16] The central hydrazone moiety transitions from the pyrazolo[1,5-*a*]pyridine core buried at the base of the active site through to the aryl nitro binding site higher on the N-terminal lobe wall, and is adjacent to residues Thr856, Met922 and Gln859 on the C-terminal lobe, and N-terminal lobe residues Trp780 and Met772. Some flexibility around the sulfonohydrazide may be required to for optimal binding through this region as indicated by the data presented in Table 2.

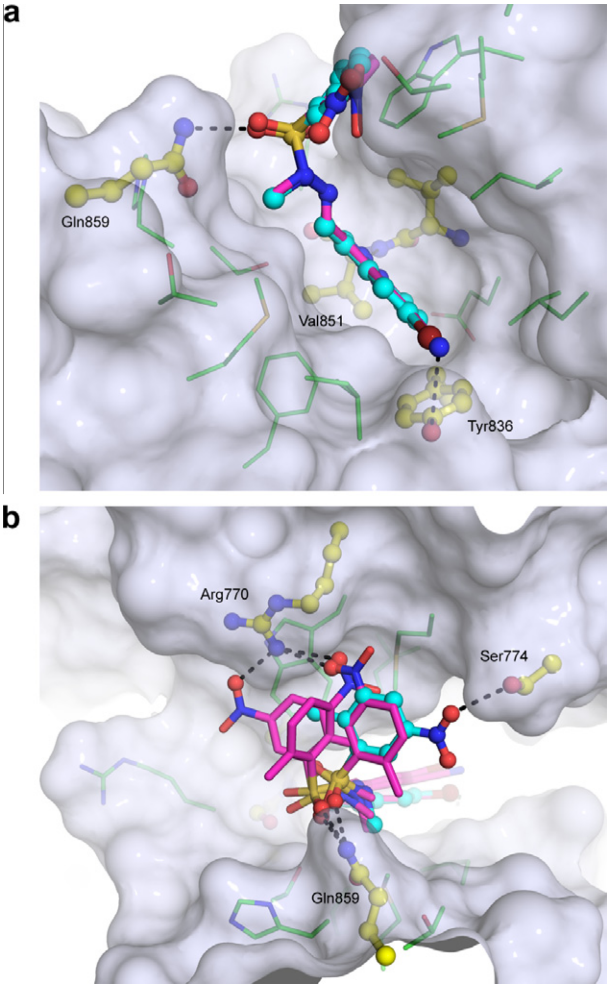


Figure 2. (a) Predicted binding mode for **5m** (cyan ball and stick) and **5x** (magenta stick) in the p110α ATP-binding site. Amino acids within 5 Å of **5m** are illustrated, and those likely to interact with the pyrazolo[1,5-*a*]pyridine core and sulfonohydrazide moiety are represented as yellow ball and stick with the interaction illustrated by black dashed lines. (b) Possible interactions predicted for the aryl nitro group of **5m** and **5x**. Compound **5m** is represented as cyan ball and stick, with alternative aryl nitro orientations including **5x** represented as magenta sticks. Ligand atoms potentially involved in p110α specific interaction are shown as balls. p110α ATP-binding site residues within 5 Å of **5m** are illustrated and those predicted to interact with the ligand are represented as yellow ball and stick with the interactions illustrated by black dashed lines. (Images generated using PyMol.)

2.4. Further in vitro screening of 5x

As the most active compound against PI3 kinase p110α that also showed good inhibition of cell proliferation, **5x** was selected for other investigation by screening against other kinases (Table 3). Compound **5x** showed similar potency against the two most common p110α mutations (E545K and H1047R) to that of wild-type p110α, and 15-fold selectivity over the class Ib PI3 kinase p110γ.

PI3 kinase inhibitors also commonly inhibit the closely related kinases DNA-PK (DNA-dependent protein kinase) and mTOR (mammalian target of rapamycin).[21] Compound **5x** is a potent inhibitor of DNA-PK but has 300-fold selectivity over mTOR (Table 3). PIK-75 has activity against a range of other kinases (<10% activity remaining at 10 μM),[4] and a representative seven of these were chosen for screening at 1 and 10 μM (Table 3). Compound **5x** has a similar level of potency to PIK-75 against five of these kinases, but weaker activity for TrkA (neurotrophic tyrosine kinase receptor, type 1) and IR (insulin receptor).

Table 3
Inhibition of PI3 kinase p110α mutations, p110γ, and other selected kinases by **5x**

Kinase	% Activity remaining	
	1 μM	10 μM
p110α E545K	(0.7 nM)[a]	
p110α H1047R	(0.5 nM)[a]	
p110γ	(14 nM)[a]	
DNA-PK[b]	(1.1 nM)[a]	
mTOR[b]	(300 nM)[a]	
MKK1[c]	10 ± 2	8 ± 2
p38γ MAPK[c]	18 ± 4	9 ± 1
RSK1[c]	4 ± 1	2 ± 1
TrkA[c]	19 ± 1	13 ± 1
PKA[c]	1 ± 0	1 ± 0
IR[c]	55 ± 2	42 ± 3
MINK1[c]	5 ± 1	2 ± 1

[a] Values in parentheses represent IC$_{50}$'s.
[b] Assays were performed by Invitrogen Drug Discovery Services (Madison, WI, USA).
[c] Assays were performed by The National Centre for Protein Kinase Profiling (Dundee, UK).

Since benzoyl hydrazones have previously been reported as irreversible inhibitors of Cathepsin S,[22] **5x** was tested for its ability to be washed off p110α in an in vitro assay using wortmannin[1] as a control for irreversible binding and LY294002[1] as a control for reversible ATP competitive binding. In this assay 70% of the inhibitory activity of **5x** at a concentration of 100 nM was washed off.

Compound **5x** was assayed for influence on cell signalling by measuring its effect on the phosphorylation of PKB, a downstream marker of PI3 kinase activity. This was carried out in HCT-116 cells which contain the p110α mutation H1047R (Fig. 3). Compound **5x** inhibited the phosphorylation at both Ser473 and Thr308 with IC$_{50}$s of 23 and 26 nM, respectively.

2.5. In vivo antitumour efficacy

Compound **5x** was then evaluated in an HCT-116 human xenograft model in female Rag1$^{-/-}$ mice. The control vehicle, PIK-75 and **5x** were dosed ip at MTD (20 mg/kg) daily for 14 days (Fig. 4). Compound **5x** reduced tumour growth by 39.1 ± 4.3% (P <0.005) compared to controls by the completion of the study. PIK-75 also reduced tumour growth compared to controls, but this difference (23.2 ± 3.7%) did not reach statistical significance. Both drugs were similarly tolerated throughout the duration of the study. After an initial reduction following the first dose, animal bodyweight was stable until day 10, then steadily declined so that significant bodyweight loss (15–20% loss of initial weight) was observed in 3/7 mice treated with **5x** and 1/7 mice with PIK-75. Although the toxicity of **5x** may have resulted from its improved potency against p110α or through off-target activity against other protein or lipid kinases, the late decline in bodyweight loss and presence of drug precipitate in the peritoneal cavity of all treated mice on post-mortem examination suggests that poor solubility may at least be partially responsible for drug toxicity. Therefore, the design of soluble analogues of **5x** may help improve drug tolerance to provide a substantial improvement in therapeutic index over PIK-75.

3. Conclusions

We have found that the pyrazolo[1,5-*a*]pyridines are a new series of p110α selective PI3 kinase inhibitors and have explored the SAR around the ring system. Compound **5x** showed good selectivity for PI3 kinase p110α over p110β and p110δ, substantial inhibition of cell proliferation, and inhibition of phosphorylation of PKB, a downstream marker of PI3 kinase activity. It also showed modest tumour growth inhibition in an HCT-116 human xenograft study. However, the poor solubility of **5x** prevented all the drug from being absorbed and may have limited its in vivo tolerance, indicating that further investigation of the SAR around these compounds is required with a view to designing more soluble analogues.

4. Experimental

4.1. Chemistry

NMR spectra were recorded on a Bruker Avance 400 spectrometer; chemical shifts are reported in δ using SiMe$_4$ as the internal standard when measured in CDCl$_3$, and the residual DMSO as internal standard when measured in d_6-DMSO. Low resolution mass spectra were recorded on a Thermo Finnigan MSQ single quadrupole mass spectrometer. High resolution mass spectra were obtained on a Bruker micrOTOF-QII mass spectrometer using electrospray ionisation (ESI). Analyses were carried out in The Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand. Tested compounds were assessed as $\geqslant$95% purity, as determined by combustion analysis, or the purity was measured by HPLC conducted on an Agilent 1100 system using a reverse-phase C8 column with diode array detection. Silica gel chromatography was performed using 200–320 mesh silica gel obtained from APS Finechem Ltd. Yields have not been optimised.

4.1.1. Synthesis of ethyl pyrazolo[1,5-*a*]pyridine-3-carboxylates 3

These were made using MSH or DNPH as detailed below, unless otherwise stated. A fresh solution of MSH[23] in CH$_2$Cl$_2$ (1 equiv) was added to the substituted pyridine (1 equiv) in CH$_2$Cl$_2$ (10 mL) at 0 °C. After 2 h, the solvent was removed in vacuo. Alternatively, a solution of DNPH[12] (1 equiv) and the substituted pyridine (1 equiv) in MeCN (40 mL) was heated at 40 °C for 18 h. The solvent was removed in vacuo. The method continues by taking the residue from either method in dry DMF (8 mL), and then ethyl propiolate (1 equiv) and K$_2$CO$_3$ (2 equiv) were added, and the suspension stirred at room temperature for 18 h. The reaction mixture was diluted with water and extracted twice with EtOAc. The combined organic phases were washed three times with water then with brine, dried (Na$_2$SO$_4$) and the solvent removed in vacuo. Chromatography (eluting with a hexanes: EtOAc gradient, unless otherwise stated) gave the pyrazolo[1,5-*a*]pyridine.

4.1.1.1. Ethyl 6-bromopyrazolo[1,5-*a*]pyridine-3-carboxylate (3b) and ethyl 4-bromopyrazolo[1,5-*a*]pyridine-3-carboxylate (3c). Reaction of 3-bromopyridine (843 mg, 5.34 mmol) with MSH, after chromatography (eluting with hexanes: EtOAc 98:2 to 97:3 to 19:1 to 9:1) gave firstly **3b** as a pale brown solid (255 mg, 29%). ^{1}H NMR δ (400 MHz, CDCl$_3$) 8.67 (dd, J = 1.6, 0.8 Hz, 1H), 8.36 (s, 1H), 8.07 (dd, J = 9.4, 0.8 Hz, 1H), 7.47 (dd, J = 9.4, 1.6 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H). LC–MS (APCI$^+$) 269 (MH$^+$ with ^{79}Br, 100%), 271 (MH$^+$ with ^{81}Br, 80%). Followed by **3c** as a pale yellow solid (255 mg, 29%). ^{1}H NMR δ (400 MHz, CDCl$_3$) 8.52 (dd, J = 6.9, 1.0 Hz, 1H), 8.43 (s, 1H), 7.64 (dd, J = 7.4, 1.0 Hz, 1H), 6.80 (t, J = 7.2 Hz, 1H), 4.38 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H). LC–MS (APCI$^+$) 269 (MH$^+$ with ^{79}Br, 80%), 271 (MH$^+$ with ^{81}Br, 100%).

4.1.1.2. Ethyl 7-methylpyrazolo[1,5-*a*]pyridine-3-carboxylate (3d). Reaction of 2-methylpyridine (251 mg, 2.7 mmol) with MSH, after chromatography (eluting with hexanes: EtOAc 97:3 to 19:1) gave **3d** as a yellow solid (205 mg, 37%). ^{1}H NMR δ (400 MHz, CDCl$_3$) 8.44 (s, 1H), 8.08 (d, J = 8.9 Hz, 1H), 7.35 (dd, J = 8.9, 7.0 Hz, 1H), 6.81 (d, J = 7.0 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 2.80 (s, 3H), 1.42 (t, J = 7.1 Hz, 3H). LC–MS (APCI$^+$) 205 (MH$^+$, 100%).

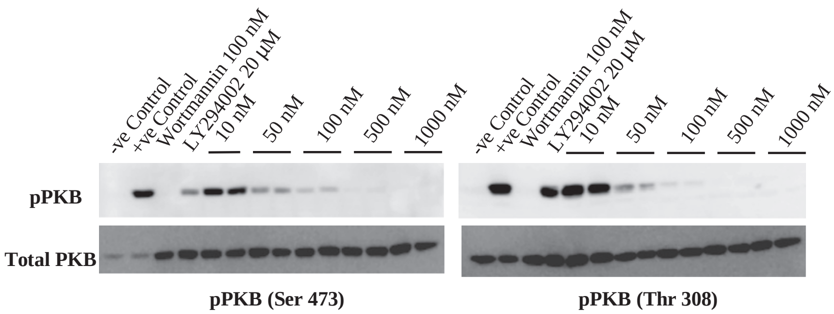


Figure 3. The effect of **5x** on the phosphorylation of PKB in HCT-116 cells at Ser473 (IC_{50} 23 nM) and Thr308 (IC_{50} 26 nM).

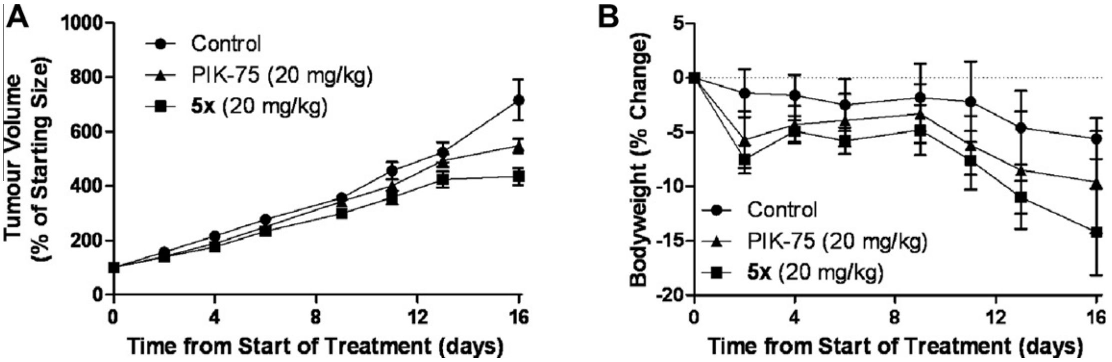


Figure 4. Average tumour volume (A) and bodyweight change (B) in $Rag1^{-/-}$ mice with HCT-116 tumours treated with **5x**, PIK-75 and the control vehicle (*n* = 7 per group). All treatments were administered by ip injection daily for 14 days.

4.1.1.3. Ethyl 6-methylpyrazolo[1,5-*a*]pyridine-3-carboxylate (3e) and ethyl 4-methylpyrazolo[1,5-*a*]pyridine-3-carboxylate (3f). Reaction of 3-methylpyridine (497 mg, 5.34 mmol) with MSH, after chromatography (eluting with hexanes: EtOAc 98:2 to 97:3 to 19:1 to 9:1) gave firstly **3f** as a pale yellow solid (152 mg, 21%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.41 (s, 1H), 8.38 (d, *J* = 6.9 Hz, 1H), 7.12 (d, *J* = 6.9 Hz, 1H), 6.83 (t, *J* = 6.9 Hz, 1H), 4.34 (q, *J* = 7.1 Hz, 2H), 2.84 (s, 3H), 1.40 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 205 (MH$^+$, 100%). Followed by **3e** as a yellow solid (160 mg, 23%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.33 (s, 1H), 8.32 (dd, *J* = 2.3, 1.1 Hz, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.26 (dd, *J* = 9.0, 1.5 Hz, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 2.39 (s, 1H), 1.41 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 205 (MH$^+$, 100%).

4.1.1.4. Ethyl 5-methylpyrazolo[1,5-*a*]pyridine-3-carboxylate (3g). Reaction of 4-methylpyridine (248 mg, 2.66 mmol) with MSH, after chromatography (eluting with hexanes: EtOAc 97:3 to 95:5 to 9:1) gave **3g** as a pale brown solid (175 mg, 53%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.39 (d, *J* = 7.1 Hz, 1H), 8.34 (s, 1H), 7.93 (s, 1H), 6.76 (dd, *J* = 7.1, 1.9 Hz, 1H), 4.38 (q, *J* = 7.1 Hz, 2H), 2.47 (s, 3H), 1.41 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 205 (MH$^+$, 100%).

4.1.1.5. Ethyl 5-ethylpyrazolo[1,5-*a*]pyridine-3-carboxylate (3h). Reaction of 4-ethylpyridine (215 mg, 2.01 mmol) with DNPH, after chromatography (eluting with hexanes: EtOAc 19:1 to 9:1) gave **3h** as a pale yellow solid (310 mg, 71%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.41 (d, *J* = 7.1 Hz, 1H), 8.34 (s, 1H), 7.95 (m, 1H), 6.80 (dd, *J* = 7.1, 1.9 Hz, 1H), 4.38 (q, *J* = 7.1 Hz, 2H), 2.76 (q, *J* = 7.6 Hz, 2H), 1.41 (t, *J* = 7.1 Hz, 3H), 1.32 (t, *J* = 7.6 Hz, 3H). LC–MS (APCI$^+$) 219 (MH$^+$, 100%).

4.1.1.6. Ethyl 5-(trifluoromethyl)pyrazolo[1,5-*a*]pyridine-3-carboxylate (3i). Reaction of 4-(trifluoromethyl)pyridine (441 mg, 3.00 mmol) with MSH, after chromatography (eluting with hexanes: EtOAc 19:1) gave **3i** as an off-white solid (276 mg, 36%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.62 (d, *J* = 7.3 Hz, 1H), 8.47–8.50 (m, 2H), 7.11 (dd, *J* = 7.3, 2.0 Hz, 1H), 4.42 (q, *J* = 7.1 Hz, 2H), 1.43 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 259 (MH$^+$, 100%).

4.1.1.7. Ethyl 5-phenylpyrazolo[1,5-*a*]pyridine-3-carboxylate (3j). Reaction of 4-phenylpyridine (388 mg, 2.50 mmol) with MSH, after chromatography (eluting with hexanes: EtOAc 97:3 to 19:1 to 9:1) gave **3j** as a yellow solid (192 mg, 29%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.56 (dd, *J* = 7.2, 0.8 Hz, 1H), 8.41 (s, 1H), 8.37 (dd, *J* = 2.0, 0.8 Hz, 1H), 7.73–7.68 (m, 2H), 7.54–7.48 (m, 2H), 7.47–7.42 (m, 1H), 7.22 (dd, *J* = 7.2, 2.0 Hz, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 1.43 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 267 (MH$^+$, 100%).

4.1.1.8. Ethyl 5-(*tert*-butoxycarbonylamino)pyrazolo[1,5-*a*]pyridine-3-carboxylate (3k). Reaction of *tert*-butyl pyridin-4-ylcarbamate (**6**) (1.77 g, 8.89 mmol) with DNPH, after chromatography (eluting with CH_2Cl_2: MeOH 99.5:0.5) gave **3k** as a yellow solid (1.25 g, 46%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.38 (d, *J* = 7.6 Hz, 1H), 8.32 (s, 1H), 7.95 (d, *J* = 1.9 Hz, 1H), 7.28 (m, 1H), 6.71 (s, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 1.55 (s, 9H), 1.41 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 306 (MH$^+$, 100%).

4.1.1.9. Ethyl 5-aminopyrazolo[1,5-*a*]pyridine-3-carboxylate trifluoroacetate (3l). A solution of **3k** (1.25 g, 4.10 mmol) and trifluoroacetic acid (3.2 mL, 41.5 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature for 18 h. The solvents were removed in vacuo to leave **3l** as a pale brown solid (1.72 g, 97%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.37 (dd, *J* = 7.4, 0.6 Hz, 1H), 8.25 (s, 1H), 7.22 (dd, *J* = 2.6, 0.6 Hz, 1H), 6.40 (dd, *J* = 7.4, 2.6 Hz, 1H), 4.35 (q, *J* = 7.1 Hz, 2H), 1.39 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 206 (MH$^+$, 100%).

4.1.1.10. Ethyl 5-bromopyrazolo[1,5-*a*]pyridine-3-carboxylate (3m). A solution of $NaNO_2$ (411 mg, 5.96 mmol) in water (6 mL) was added dropwise to a solution of **3l** (1.72 g, 3.97 mmol) in concentrated HBr (4 mL) at 0 °C over 5 min. After 10 min, a solution of CuBr (1.14 g, 7.95 mmol) in concentrated HBr (4 mL) was added, then the reaction mixture heated to 50 °C for 15 min until gas evolution ceased. Then the reaction mixture was basified to pH 5 with 6 M NaOH and extracted twice with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with hexanes: EtOAc 19:1 to 9:1) gave **3m** as a pale yellow solid (695 mg, 65%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.53–8.20 (m, 3H), 7.03 (dd, *J* = 7.3, 2.0 Hz, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 1.42 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 269 (MH$^+$ with ^{79}Br, 100%), 271 (MH$^+$ with ^{81}Br, 90%).

4.1.1.11. Ethyl 5-iodopyrazolo[1,5-*a*]pyridine-3-carboxylate (3n). $NaNO_2$ (36 mg, 0.52 mmol) was added to a solution of **3l** (150 mg, 0.40 mmol) in concentrated HCl (3 mL), H_2SO_4 (1 mL) and water (3 mL) at 0 °C. After 1 h, urea (2.4 mg, 0.04 mmol) was added, then after a further 15 min, a solution of KI (132 mg, 0.80 mmol) in water (3 mL) was added. After 1 h at room temperature, the reaction mixture was basified to pH 3 with 1 M NaOH and extracted twice with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with hexanes: EtOAc 5:1) gave **3n** as a pale yellow solid (57 mg, 45%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.59 (dd, *J* = 1.9, 0.7 Hz, 1H), 8.33 (s, 1H), 8.23 (dd, *J* = 7.2, 0.7 Hz, 1H), 7.18 (dd, *J* = 7.2, 1.9 Hz, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 1.41 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 317 (MH$^+$, 100%).

4.1.1.12. 3-Ethyl 5-methyl pyrazolo[1,5-*a*]pyridine-3,5-dicarboxylate (3t). Reaction of methyl isonicotinate (**8**) (2.45 g, 17.9 mmol) with MSH, after chromatography (eluting with hexanes: EtOAc 19:1 to 9:1) gave **3t** as a pale brown solid (1.88 g, 42%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.86 (dd, *J* = 1.8, 0.9 Hz, 1H), 8.55 (dd, *J* = 7.2, 0.9 Hz, 1H), 8.47 (s, 1H), 7.52 (dd, *J* = 7.2, 1.8 Hz, 1H), 4.42 (q, *J* = 7.1 Hz, 2H), 3.99 (s, 3H), 1.44 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 249 (MH$^+$, 100%).

4.1.1.13. Ethyl 5-(1,3-dioxolan-2-yl)-6-fluoropyrazolo[1,5-*a*]pyridine-3-carboxylate (3y). To a solution of 3-fluoroisonicotinaldehyde (**9**) (9.83 g, 78.5 mmol) and ethylene glycol (9.74 g, 157 mmol) in benzene (100 mL) was added *p*-TsOH·H_2O (14.2 g, 82.5 mmol) and the mixture was refluxed under a Dean–Stark apparatus. After 12 h, the reaction was cooled and quenched with 20% w/w NaOH. The benzene layer was removed and the remaining aqueous layer was extracted with EtOAc. The combined organic fractions were washed with brine, dried ($MgSO_4$) and the solvents removed in vacuo. Chromatography (eluting with hexanes: EtOAc 5:1) gave 4-(1,3-dioxolan-2-yl)-3-fluoropyridine as a colourless oil (11.4 g, 86%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.48 (d, *J* = 1.8 Hz, 1H), 8.45 (dd, *J* = 4.8, 0.8 Hz, 1H), 7.45 (dd, *J* = 6.0, 4.8 Hz, 1H), 6.09 (s, 1H), 4.16–4.04 (m, 4H). LC–MS (APCI$^+$) 170 (MH$^+$, 100%). Reaction of the above pyridine (6.87 g, 40.7 mmol) with DNPH, after chromatography (eluting with CH_2Cl_2: MeOH 98:2) gave a mixture of pyridine starting material and isomerically pure product (**3y**) (462 mg, 4.7%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.47 (dd, *J* = 4.8, 0.4 Hz, 1H), 8.40 (s, 1H), 8.31 (d, *J* = 7.1 Hz, 1H), 6.14 (s, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 4.20–4.08 (m, 4H), 1.42 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 281 (MH$^+$, 100%).

4.1.1.14. 3-(Ethoxycarbonyl)-6-fluoropyrazolo[1,5-*a*]pyridine-5-carboxylic acid (3z). 70% w/w Perchloric acid (30 mL) was added to a stirred solution of **3y** (400 mg, 1.42 mmol) in dioxane (30 mL) and acetone (2 mL), and stirred for 3 h. The reaction mixture was then extracted with EtOAc, washed with brine, dried ($MgSO_4$) and the solvent removed in vacuo. The solid residue was taken up in CH_3CN (70 mL) and 0.16 M NaH_2PO_4 (10 mL), then cooled to 0 °C and 30% v/v H_2O_2 added in a single portion. A solution of $NaClO_2$ (750 mg, 8.29 mmol) in water (5 mL) was added dropwise over 5 min and the reaction was stirred at room temperature for 3 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$, and then the CH_3CN removed in vacuo. The remaining aqueous layer was diluted with 0.5 M NaOH and extracted with CH_2Cl_2. The aqueous layer was acidified to pH 4 with 1 M HCl and extracted with CH_2Cl_2. This last extract was dried ($MgSO_4$) and the solvent removed in vacuo to afford **3z** as a colourless white solid (320 mg, 77%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.89 (d, *J* = 7.2 Hz, 1H), 8.57 (d, *J* = 5.2 Hz, 1H), 8.48 (s, 1H), 4.43 (q, *J* = 7.1 Hz, 2H), 1.44 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 253 (MH$^+$, 100%).

4.1.1.15. Ethyl 5-methoxypyrazolo[1,5-*a*]pyridine-3-carboxylate (3aa). Reaction of 4-methoxypyridine (**10**) (244 mg, 2.24 mmol) with DNPH, after chromatography (eluting with hexanes: EtOAc 9:1 to 4:1) gave **3aa** as a yellow solid (114 mg, 23%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.31 (d, *J* = 7.5 Hz, 1H), 8.28 (s, 1H), 7.43 (d, *J* = 2.8 Hz, 1H), 6.61 (dd, *J* = 7.5, 2.8 Hz, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 3.93 (s, 3H), 1.41 (t, *J* = 7.1 Hz, 3H). LC–MS (APCI$^+$) 221 (MH$^+$, 100%).

4.1.2. Synthesis of pyrazolo[1,5-*a*]pyridine-3-carbaldehydes 4

These were made by decarboxylation/Vilsmeier or hydrolysis/reduction/reoxidation as detailed below, unless otherwise stated.

Decarboxylation was carried out by refluxing a solution of the ester (1 equiv) in 40% aqueous H_2SO_4 (3 mL) for 18 h. The solution was then cooled in ice and neutralised to pH 7 with 6 M NaOH, then extracted twice with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo to leave the decarboxylated material. The pyrazolo[1,5-*a*]pyridine was then reacted under Vilsmeier conditions in dry DMF (2 mL) with $POCl_3$ (3 equiv) at 0 °C under an atmosphere of N_2. The reaction mixture was then warmed to room temperature and stirred for 2 h. The solution was poured onto ice, basified to pH 10 with 1 M NaOH, stirred for 1 h then extracted twice with CH_2Cl_2. The combined extracts were washed twice with water, dried (Na_2SO_4) and the solvent removed in vacuo to leave the aldehyde.

Alternatively, the ester was hydrolysed by refluxing a solution of the ester (1 equiv) in 1 M NaOH (3 equiv) and EtOH (5 mL) for 6 h. The EtOH was removed in vacuo, and then the aqueous residue acidified to pH 1 with 1 M HCl. The precipitated carboxylic acid was filtered off, washed with water and dried. The carboxylic acid was reduced by adding CDI (1.5 equiv) to a suspension of carboxylic acid (1 equiv) in dry THF (10 mL) under an atmosphere of N_2. After stirring for 18 h, the resulting solution was added dropwise to a solution of $NaBH_4$ (5 equiv) in H_2O (10 mL) and stirred for 30 min. The reaction was then quenched by the addition of 1 M HCl and stirred for a further 30 min. The solution was neutralised with saturated aqueous $NaHCO_3$ and extracted twice with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with a hexanes: EtOAc gradient) gave the alcohol. Reoxidation was carried out by stirring a suspension of the pyrazolo[1,5-*a*]pyridine-3-methanol (1 equiv) and MnO_2 (10 equiv) in CH_2Cl_2 (2 mL) at room temperature for 4 days. The reaction mixture was then filtered through celite, washed with CH_2Cl_2, and the solvent removed from the filtrate in vacuo to leave the aldehyde.

4.1.2.1. 6-Bromopyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4b). Decarboxylation of ester **3b** (253 mg, 0.94 mmol) gave 6-bromopyrazolo[1,5-*a*]pyridine as a pale yellow oil (93 mg, 50%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.63 (m, 1H), 7.92 (d, *J* = 2.3 Hz, 1H), 7.44 (d, *J* = 9.4 Hz, 1H), 7.17 (dd, *J* = 9.4, 1.6 Hz, 1H), 6.54 (d, *J* = 2.3 Hz, 1H). LC–MS (APCI$^+$) 196 (MH$^+$ with ^{79}Br, 90%), 198 (MH$^+$ with ^{81}Br, 100%).

Vilsmeier reaction of the above compound (92 mg, 0.47 mmol) gave **4b** as a pale pink solid (87 mg, 83%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.05 (s, 1H), 8.73 (dd, *J* = 1.6, 0.8 Hz, 1H), 8.36 (s, 1H), 8.21 (dd, *J* = 9.3, 0.8 Hz, 1H), 7.60 (dd, *J* = 9.3, 1.6 Hz, 1H). LC–MS (APCI$^+$) 224 (MH$^+$ with ^{79}Br, 80%), 226 (MH$^+$ with ^{81}Br, 100%).

4.1.2.2. 4-Bromopyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4c). Hydrolysis of ester **3c** (253 mg, 0.94 mmol) gave 4-bromopyrazolo[1,5-*a*]pyridine-3-carboxylic acid as a white solid (212 mg, 93%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 12.50 (br s, 1H), 8.88 (d, *J* = 6.9 Hz, 1H), 8.44 (s, 1H), 7.82 (d, *J* = 7.4 Hz, 1H), 7.02 (t, *J* = 7.2 Hz, 1H). Reduction of the above acid (210 mg, 0.87 mmol) after chromatography (eluting with hexanes: EtOAc 3:1 to 2:1 to 1:1), gave (4-bromopyrazolo[1,5-*a*]pyridin-3-yl)methanol as a yellow solid (165 mg, 83%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.44 (d, *J* = 7.0 Hz, 1H), 8.02 (s, 1H), 7.38 (d, *J* = 7.2 Hz, 1H), 6.65 (t, *J* = 7.2 Hz, 1H), 5.03 (d, *J* = 6.2 Hz, 2H), 1.77 (t, *J* = 6.2 Hz, 1H). LC–MS (APCI$^+$) 226 (MH$^+$ with ^{79}Br, 80%), 228 (MH$^+$ with ^{81}Br, 100%). Reoxidation of the above alcohol (165 mg, 0.73 mmol) gave **4c** as a white solid (146 mg, 89%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.77 (s, 1H), 8.61–8.58 (m, 2H), 7.74 (dd, *J* = 7.5, 0.8 Hz, 1H), 6.89 (t, *J* = 7.3 Hz, 1H). LC–MS (APCI$^+$) 224 (MH$^+$ with ^{79}Br, 100%), 226 (MH$^+$ with ^{81}Br, 80%).

4.1.2.3. 7-Methylpyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4d). Decarboxylation of ester **3d** (97 mg, 0.55 mmol) gave 7-methylpyrazolo[1,5-*a*]pyridine as a pale brown oil (62 mg, 85%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.00 (d, *J* = 2.2 Hz, 1H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.06 (dd, *J* = 8.8, 6.8 Hz, 1H), 6.62 (d, *J* = 6.8 Hz, 1H), 6.56 (d, *J* = 2.2 Hz, 1H), 2.76 (s, 3H). LC–MS (APCI$^+$) 133 (MH$^+$, 100%). Vilsmeier reaction of the above compound (62 mg, 0.47 mmol) gave **4d** as a white solid (48 mg, 64%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.06 (s, 1H), 8.42 (s, 1H), 8.21 (d, *J* = 8.7 Hz, 1H), 7.47 (dd, *J* = 8.7, 7.0 Hz, 1H), 6.93 (d, *J* = 7.0 Hz, 1H), 2.83 (s, 3H). LC–MS (APCI$^+$) 161 (MH$^+$, 100%).

4.1.2.4. 6-Methylpyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4e). Hydrolysis of ester **3e** (158 mg, 0.77 mmol) gave 6-methylpyrazolo[1,5-*a*]pyridine-3-carboxylic acid as a pale yellow solid (109 mg, 80%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 12.30 (br s, 1H), 8.68 (s, 1H), 8.30 (m, 1H), 7.98 (d, *J* = 9.0 Hz, 1H), 7.42 (dd, *J* = 9.0, 0.9 Hz, 1H), 2.35 (s, 3H). Reduction of the above acid (107 mg, 0.61 mmol), after chromatography (eluting with hexanes: EtOAc 2:1 to 1:1 to EtOAc) gave (6-methylpyrazolo[1,5-*a*]pyridin-3-yl)methanol as a colourless oil (55 mg, 56%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.25 (m, 1H), 7.87 (s, 1H), 7.53 (d, *J* = 9.0 Hz, 1H), 7.01 (dd, *J* = 9.0, 1.4 Hz, 1H), 4.84 (s, 2H), 2.34 (s, 3H), 1.43 (br s, 1H). LC–MS (APCI$^+$) 163 (MH$^+$, 100%). Reoxidation of the above alcohol (55 mg, 0.34 mmol) gave **4e** as a white solid (48 mg, 89%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.01 (s, 1H), 8.38 (m, 1H), 8.32 (s, 1H), 8.19 (d, *J* = 8.9 Hz, 1H), 7.39 (dd, *J* = 8.9, 1.4 Hz, 1H), 2.43 (s, 3H). LC–MS (APCI$^+$) 161 (MH$^+$, 100%).

4.1.2.5. 4-Methylpyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4f). Hydrolysis of ester **3f** (158 mg, 0.77 mmol) gave 4-methylpyrazolo[1,5-*a*]pyridine-3-carboxylic acid as a white solid (118 mg, 91%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 12.20 (br s, 1H), 8.64 (d, *J* = 6.9 Hz, 1H), 8.36 (s, 1H), 7.27 (d, *J* = 6.9 Hz, 1H), 7.01 (t, *J* = 6.9 Hz, 1H), 2.75 (s, 3H). Reduction of the above acid (116 mg, 0.66 mmol), after chromatography (eluting with hexanes: EtOAc 2:1 to 1:1 to EtOAc) gave (4-methylpyrazolo[1,5-*a*]pyridin-3-yl)methanol as a white solid (58 mg, 54%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.32 (d, *J* = 6.9 Hz, 1H), 7.90 (s, 1H), 6.89 (d, *J* = 6.9 Hz, 1H), 6.68 (t, *J* = 6.9 Hz, 1H), 4.90 (d, *J* = 4.6 Hz, 2H), 2.70 (s, 3H), 1.46 (t, *J* = 4.6 Hz, 1H). LC–MS (APCI$^+$) 163 (MH$^+$, 100%). Reoxidation of the above alcohol (58 mg, 0.36 mmol) gave **4f** as a white solid (56 mg, 98%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.17 (s, 1H), 8.49 (s, 1H), 8.45 (d, *J* = 6.9 Hz, 1H), 7.27 (m, 1H), 6.94 (t, *J* = 6.9 Hz, 1H), 2.79 (s, 3H). LC–MS (APCI$^+$) 161 (MH$^+$, 100%).

4.1.2.6. 5-Methylpyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4g). Hydrolysis of ester **3g** (173 mg, 0.85 mmol) gave 5-methylpyrazolo[1,5-*a*]pyridine-3-carboxylic acid as a white solid (136 mg, 91%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 12.3 (br s, 1H), 8.70 (d, *J* = 7.0 Hz, 1H), 8.30 (s, 1H), 7.86 (s, 1H), 6.96 (dd, *J* = 7.0, 1.7 Hz, 1H), 2.44 (s, 3H). Reduction of the above acid (134 mg, 0.76 mmol), after chromatography (eluting with hexanes: EtOAc 2:1 to 1:1 to EtOAc) gave (5-methylpyrazolo[1,5-*a*]pyridin-3-yl)methanol as a colourless oil (18 mg, 15%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.33 (d, *J* = 7.1 Hz, 1H), 7.89 (s, 1H), 7.38 (s, 1H), 6.60 (dd, *J* = 7.1, 1.8 Hz, 1H), 4.83 (d, *J* = 5.4 Hz, 2H), 2.36 (s, 3H), 1.41 (t, *J* = 5.4 Hz, 1H). LC–MS (APCI$^+$) 163 (MH$^+$, 100%). Reoxidation of the above alcohol (18 mg, 0.11 mmol) gave **4g** as a white solid (14 mg, 78%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.00 (s, 1H), 8.44 (d, *J* = 7.0 Hz, 1H), 8.32 (s, 1H), 8.09 (s, 1H), 6.89 (dd, *J* = 7.0, 1.7 Hz, 1H), 2.50 (s, 3H). LC–MS (APCI$^+$) 161 (MH$^+$, 100%).

4.1.2.7. 5-Ethylpyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4h). Decarboxylation of ester **3h** (310 mg, 1.42 mmol) gave 5-ethylpyrazolo[1,5-*a*]pyridine as a pale brown oil (171 mg, 82%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.37 (d, *J* = 7.2 Hz, 1H), 7.89 (d, *J* = 2.1 Hz, 1H), 7.29 (m, 1H), 6.60 (dd, *J* = 7.2, 1.7 Hz, 1H), 6.39 (d, *J* = 2.1 Hz, 1H), 2.67 (q, *J* = 7.6 Hz, 2H), 1.28 (t, *J* = 7.6 Hz, 3H). LC–MS (APCI$^+$) 147 (MH$^+$, 100%). Vilsmeier reaction of the above compound (170 mg, 1.16 mmol) gave **4h** as a brown oil (203 mg, 100%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.01 (s, 1H), 8.46 (d, *J* = 7.1 Hz, 1H), 8.33 (s, 1H), 8.10 (m, 1H), 6.93 (dd, *J* = 7.1, 1.9 Hz, 1H), 2.79 (q, *J* = 7.5 Hz, 2H), 1.33 (t, *J* = 7.5 Hz, 3H). LC–MS (APCI$^+$) 175 (MH$^+$, 100%).

4.1.2.8. 5-(Trifluoromethyl)pyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4i). $LiAlH_4$ (4.3 mL, 1.0 mol L^{-1} in THF, 4.3 mmol) was added to a solution of ester **3i** (276 mg, 1.07 mmol) in dry THF (10 mL) at 0 °C under an atmosphere of N_2. The reaction mixture was then warmed to room temperature and stirred for 1 h. The reaction was quenched by the dropwise addition of water, then filtered through a plug of celite and washed with CH_2Cl_2. The solvent was removed from the filtrate in vacuo. Chromatography (eluting with hexanes: EtOAc 2:1 to 1:1) gave (5-(trifluoromethyl)pyrazolo[1,5-*a*]pyridin-3-yl)methanol as a white solid (28 mg, 12%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.54 (d, *J* = 7.3 Hz, 1H), 8.05 (s, 1H), 8.00 (d, *J* = 1.9 Hz, 1H), 6.94 (dd, *J* = 7.3, 1.9 Hz, 1H), 4.93 (d, *J* = 4.2 Hz, 2H), 3.48 (br s, 1H). LC–MS (APCI$^+$) 217 (MH$^+$, 100%). Reoxidation of the above alcohol (28 mg, 0.13 mmol) gave **4i** as an off-white solid (25 mg, 89%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.12 (s, 1H), 8.69 (d, *J* = 7.2 Hz, 1H), 8.63 (m, 1H), 8.50 (s, 1H), 7.24 (dd, *J* = 7.2, 2.0 Hz, 1H). LC–MS (APCI$^+$) 215 (MH$^+$, 100%).

4.1.2.9. 5-Phenylpyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4j). Decarboxylation of ester **3j** (192 mg, 0.72 mmol) gave 5-phenylpyrazolo[1,5-*a*]pyridine as a pale brown oil (80 mg, 57%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.52 (d, *J* = 7.3 Hz, 1H), 7.97 (d, *J* = 2.2 Hz, 1H), 7.72 (dd, *J* = 2.0, 0.7 Hz, 1H), 7.67–7.62 (m, 2H), 7.51–7.45 (m, 2H), 7.42–7.37 (m, 1H), 7.03 (dd, *J* = 7.3, 2.0 Hz, 1H), 6.56 (dd, *J* = 2.2, 0.7 Hz, 1H). LC–MS (APCI$^+$) 195 (MH$^+$, 100%). Vilsmeier reaction of the above compound (80 mg, 0.41 mmol) gave **4j** as a pale brown oil (91 mg, 99%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.07 (s, 1H), 8.61 (dd, *J* = 7.2, 0.8 Hz, 1H), 8.51 (dd, *J* = 2.0, 0.8 Hz, 1H), 8.39 (s, 1H), 7.75–7.70 (m, 2H), 7.55–7.43 (m, 3H), 7.34 (dd, *J* = 7.2, 2.0 Hz, 1H). LC–MS (APCI$^+$) 223 (MH$^+$, 100%).

4.1.2.10. 5-Bromopyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4m). Decarboxylation of ester **3m** (695 mg, 2.58 mmol) gave 5-bromopyrazolo[1,5-*a*]pyridine (**7m**) as a pale brown solid (489 mg, 96%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.34 (d, J = 7.4 Hz, 1H), 7.95 (d, J = 2.1 Hz, 1H), 7.73 (d, J = 1.8 Hz, 1H), 6.84 (dd, J = 7.4, 2.1 Hz, 1H), 6.48 (d, J = 1.8 Hz, 1H). LC–MS (APCI$^+$) 197 (MH$^+$ with ^{79}Br, 100%), 199 (MH$^+$ with ^{81}Br, 90%). Vilsmeier reaction of **7m** (438 mg, 2.22 mmol) gave **4m** as a pale brown solid (482 mg, 96%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.02 (s, 1H), 8.51 (d, J = 2.1 Hz, 1H), 8.42 (d, J = 7.3 Hz, 1H), 8.37 (s, 1H), 7.16 (dd, J = 7.3, 2.1 Hz, 1H). LC–MS (APCI$^+$) 225 (MH$^+$ with ^{79}Br, 100%), 227 (MH$^+$ with ^{81}Br, 95%).

4.1.2.11. 5-Iodopyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4n). Decarboxylation of ester **3n** (57 mg, 0.18 mmol) gave 5-iodopyrazolo[1,5-*a*]pyridine (**7n**) as a pale brown solid (42 mg, 95%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.21 (d, J = 7.3 Hz, 1H), 7.95 (d, J = 1.7 Hz, 1H), 7.90 (d, J = 2.2 Hz, 1H), 6.95 (dd, J = 7.3, 1.7 Hz, 1H), 6.44 (d, J = 2.2 Hz, 1H). LC–MS (APCI$^+$) 245 (MH$^+$, 100%). Vilsmeier reaction of **7n** (42 mg, 0.17 mmol) gave **4n** as a pale brown solid (45 mg, 96%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.02 (s, 1H), 8.74 (d, J = 1.7 Hz, 1H), 8.33 (s, 1H), 8.28 (d, J = 7.2 Hz, 1H), 7.31 (dd, J = 7.2, 1.7 Hz, 1H). LC–MS (APCI$^+$) 273 (MH$^+$, 100%).

4.1.2.12. 5-Chloropyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4o). Decarboxylation of ester **3l** (1.29 g, 2.98 mmol), except with carrying out the aqueous extraction from pH 12, gave pyrazolo[1,5-*a*]pyridin-5-amine (**7l**) as a pale brown solid (310 mg, 78%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.23 (d, J = 7.4 Hz, 1H), 7.79 (d, J = 2.0 Hz, 1H), 6.58 (d, J = 2.4 Hz, 1H), 6.22 (dd, J = 7.4, 2.4 Hz, 1H), 6.13 (d, J = 2.0 Hz, 1H), 3.81 (s, 2H). LC–MS (APCI$^+$) 134 (MH$^+$, 100%). A solution of $NaNO_2$ (27 mg, 0.39 mmol) in water (1 mL) was added dropwise to a solution of **7l** (40 mg, 0.30 mmol) and CuCl (74 mg, 0.75 mmol) in concentrated HCl (1 mL) at 0 °C over 2 min. After 30 min, the reaction mixture was heated to 80 °C for 15 min, and then cooled to room temperature, basified to pH 10 with 1 M NaOH, filtered through a plug of celite and washed with CH_2Cl_2. The layers of the filtrate were separated and the aqueous layer extracted with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with hexanes: EtOAc 3:1) gave 5-chloropyrazolo[1,5-*a*]pyridine (**7o**) as a white solid (6 mg, 13%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.38 (d, J = 7.4 Hz, 1H), 7.95 (d, J = 2.2 Hz, 1H), 7.53 (d, J = 1.8 Hz, 1H), 6.71 (dd, J = 7.4, 2.2 Hz, 1H), 6.47 (d, J = 1.8 Hz, 1H). LC–MS (APCI$^+$) 153 (MH$^+$ with ^{35}Cl, 100%), 155 (MH$^+$ with ^{37}Cl, 30%). Vilsmeier reaction of **7o** (6 mg, 0.039 mmol) gave **4o** as a white solid (7 mg, 100%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.02 (s, 1H), 8.48 (d, J = 7.3 Hz, 1H), 8.38 (s, 1H), 8.33 (d, J = 2.3 Hz, 1H), 7.04 (dd, J = 7.3, 2.3 Hz, 1H). LC–MS (APCI$^+$) 181 (MH$^+$ with ^{35}Cl, 100%), 183 (MH$^+$ with ^{37}Cl, 30%).

4.1.2.13. 5-Cyclopropylpyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4p). $Pd(PPh_3)_4$ (31 mg, 0.027 mmol) was added to a solution of **4m** (60 mg, 0.27 mmol) and tributyl(cyclopropyl)stannane (115 mg, 0.35 mmol) in toluene (10 mL) which had been deoxygenated by bubbling N_2 through it. After refluxing for 18 h, the solvent was removed in vacuo. Chromatography (eluting with hexanes: EtOAc 9:1 to 4:1) gave **4p** as a pale yellow solid (37 mg, 74%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.98 (s, 1H), 8.41 (dd, J = 7.2, 0.7 Hz, 1H), 8.30 (s, 1H), 7.97 (d, J = 1.9 Hz, 1H), 6.74 (dd, J = 7.2, 1.9 Hz, 1H), 2.02 (m, 1H), 1.15 (m, 2H), 0.89 (m, 2H). LC–MS (APCI$^+$) 187 (MH$^+$, 100%).

4.1.2.14. 5-Vinylpyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4q). $Pd(PPh_3)_4$ (26 mg, 0.022 mmol) was added to a solution of **4m** (50 mg, 0.22 mmol) and tributyl(vinyl)tin (84 µL, 0.29 mmol) in toluene (10 mL) which had been deoxygenated by bubbling N_2 through it. After refluxing for 2 h, the solvent was removed in vacuo. Chromatography (eluting with hexanes: EtOAc 9:1 to 85:15) gave **4q** as a pale yellow solid (35 mg, 92%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.03 (s, 1H), 8.49 (d, J = 7.2 Hz, 1H), 8.34 (s, 1H), 8.19 (d, J = 1.9 Hz, 1H), 7.18 (dd, J = 7.2, 1.9 Hz, 1H), 6.80 (dd, J = 17.5, 10.9 Hz, 1H), 5.97 (d, J = 17.5 Hz, 1H), 5.55 (d, J = 10.9 Hz, 1H). LC–MS (APCI$^+$) 173 (MH$^+$, 100%).

4.1.2.15. 5-Ethynylpyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4r). Ethynyltrimethylsilane (75 µL, 0.53 mmol) was added to a solution of **4m** (60 mg, 0.27 mmol), CuI (5.1 mg, 27 µmol) and $(Ph_3P)_2PdCl_2$ (9.4 mg, 13 µmol) in DMF (3 mL) and NEt_3 (3 mL) which had been deoxygenated by bubbling N_2 through it. After heating to 60 °C for 2 h the solvents were removed in vacuo, then K_2CO_3 (111 mg, 0.80 mmol) and MeOH (10 mL) were added and the reaction stirred for a further 2 h. The solvent was removed in vacuo, and the residue taken up in water and extracted twice with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with hexanes: EtOAc 4:1) gave **4r** as a white solid (14 mg, 31%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.05 (s, 1H), 8.50 (dd, J = 7.1, 0.9 Hz, 1H), 8.43 (m, 1H), 8.40 (s, 1H), 7.07 (dd, J = 7.1, 1.8 Hz, 1H), 3.36 (s, 1H). LC–MS (APCI$^+$) 171 (MH$^+$, 100%).

4.1.2.16. Methyl 3-formylpyrazolo[1,5-*a*]pyridine-5-carboxylate (4t). Decarboxylation of **3t** (2.65 g, 10.7 mmol), except for basifying the aqueous reaction mixture to pH 2 with 6 M NaOH, then the precipitated solid was filtered off, washed with water and dried to leave pyrazolo[1,5-*a*]pyridine-5-carboxylic acid (**7s**) as an off-white solid (1.60 g, 92%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 12.5 (br s, 1H), 8.74 (d, J = 7.3 Hz, 1H), 8.34 (m, 1H), 8.11 (d, J = 2.3 Hz, 1H), 7.25 (dd, J = 7.3, 1.9 Hz, 1H), 6.89 (dd, J = 2.3, 0.8 Hz, 1H). LC–MS (APCI$^-$) 161 (M−H, 100%). A solution of **7s** (31 mg, 0.19 mmol) and concentrated HCl (three drops) in MeOH (10 mL) was refluxed for 4 h. The solvent was removed in vacuo, saturated aqueous $NaHCO_3$ was added to the residue, and then it was extracted twice with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo to leave methyl pyrazolo[1,5-*a*]pyridine-5-carboxylate (**7t**) as an off-white solid (25 mg, 74%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.52 (dt, J = 7.3, 0.9 Hz, 1H), 8.33 (dd, J = 1.8, 0.9 Hz, 1H), 8.04 (d, J = 2.3 Hz, 1H), 7.34 (dd, J = 7.3, 1.8 Hz, 1H), 6.75 (dd, J = 2.3, 0.9 Hz, 1H), 3.96 (s, 3H). LC–MS (APCI$^+$) 177 (MH$^+$, 100%). Vilsmeier reaction of **7t** (25 mg, 0.14 mmol) gave **4t** as a yellow solid (22 mg, 76%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.12 (s, 1H), 8.95 (dd, J = 1.8, 0.9 Hz, 1H), 8.61 (dd, J = 7.2, 0.9 Hz, 1H), 8.47 (s, 1H), 7.64 (dd, J = 7.2, 1.8 Hz, 1H), 4.01 (s, 3H). LC–MS (APCI$^+$) 205 (MH$^+$, 100%).

4.1.2.17. 3-Formylpyrazolo[1,5-*a*]pyridine-5-carboxylic acid (4s). Hydrolysis of **4t** (1.23 g, 6.0 mmol) gave **4s** as a white solid (0.98 g, 85%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 13.8 (br s, 1H), 10.10 (s, 1H), 9.01 (dd, J = 7.1, 0.8 Hz, 1H), 8.76 (s, 1H), 8.74 (m, 1H), 7.58 (dd, J = 7.1, 1.9 Hz, 1H). LC–MS (APCI$^+$) 191 (MH$^+$, 100%).

4.1.2.18. 3-Formylpyrazolo[1,5-*a*]pyridine-5-carboxamide (4u). A solution of **4s** (60 mg, 0.32 mmol) in $SOCl_2$ (1 mL) was refluxed for 1 h. The solvent was removed in vacuo, and then the residue was taken up in CH_2Cl_2 (5 mL) and added to concentrated aqueous NH_3 (5 mL). After 30 min the reaction mixture was acidified to pH 1 with 1 M HCl, saturated with NaCl and extracted four times with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo to leave **4u** as a white solid (29 mg, 48%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 10.09 (s, 1H), 9.04 (dd, J = 7.2, 0.9 Hz, 1H), 8.75 (s, 1H), 8.73 (dd, J = 1.9, 0.9 Hz, 1H), 8.41 (br s, 1H), 7.77 (br s, 1H), 7.62 (dd, J = 7.2, 1.9 Hz, 1H). LC–MS (APCI$^-$) 188 (M−H, 100%).

4.1.2.19. 3-Formyl-*N*-methylpyrazolo[1,5-*a*]pyridine-5-carboxamide (4v). A solution of **4s** (60 mg, 0.32 mmol) in $SOCl_2$ (1 mL) was refluxed for 1 h. The solvent was removed in vacuo, then the residue taken up in CH_2Cl_2 (5 mL) and added to a suspension of $MeNH_2{\cdot}HCl$ (64 mg, 0.95 mmol) and NEt_3 (0.22 mL, 1.6 mmol) in CH_2Cl_2 (5 mL). After 3 h, water was added, the reaction mixture was acidified to pH 1 with 1 M HCl and stirred for 30 min. The reaction mixture was then basified to pH 10 with 1 M NaOH, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with EtOAc) gave **4v** as an off-white solid (51 mg, 80%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.08 (s, 1H), 8.62 (dd, *J* = 7.1, 0.9 Hz, 1H), 8.56 (dd, *J* = 2.0, 0.9 Hz, 1H), 8.45 (s, 1H), 7.60 (dd, *J* = 7.1, 2.0 Hz, 1H), 6.39 (br s, 1H), 3.08 (d, *J* = 4.9 Hz, 3H). LC–MS ($APCI^+$) 204 (MH^+, 100%).

4.1.2.20. 3-Formyl-*N*,*N*-dimethylpyrazolo[1,5-*a*]pyridine-5-carboxamide (4w). A solution of **4s** (100 mg, 0.53 mmol) in $SOCl_2$ (1.5 mL) was refluxed for 1 h. The solvent was removed in vacuo, then the residue taken up in CH_2Cl_2 (5 mL) and added to a suspension of $Me_2NH{\cdot}HCl$ (86 mg, 1.05 mmol) and NEt_3 (0.37 mL, 2.7 mmol) in CH_2Cl_2 (5 mL). After 3 h, water was added, the reaction mixture was acidified to pH 1 with 1 M HCl and stirred for 30 min. The reaction mixture was then basified to pH 10 with 1 M NaOH, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with EtOAc) gave **4w** as a pale yellow solid (86 mg, 75%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.06 (s, 1H), 8.61 (d, *J* = 7.1 Hz, 1H), 8.43 (s, 1H), 8.32 (s, 1H), 7.15 (dd, *J* = 7.1, 1.8 Hz, 1H), 3.16 (s, 3H), 3.08 (s, 3H). LC–MS ($APCI^+$) 218 (MH^+, 100%).

4.1.2.21. 3-Formylpyrazolo[1,5-*a*]pyridine-5-carbonitrile (4x). NEt_3 (2.08 mL, 14.9 mmol) and isobutyl chloroformate (1.94 mL, 15.0 mmol) were added to a solution of **7s** (2.20 g, 13.6 mmol) in dry THF (100 mL) at 0 °C. After 1 h, NH_3 was bubbled through the solution for ca. 2 min. The reaction mixture was warmed to room temperature and stirred for a further 1 h before diluting with CH_2Cl_2 and water. It was then basified to pH 12 with 1 M NaOH, saturated with NaCl, and extracted four times with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and the solvents removed. The resulting solid was triturated with hexanes-CH_2Cl_2 to leave pyrazolo[1,5-*a*]pyridine-5-carboxamide (**7u**) as a pale brown solid (1.78 g, 81%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.72 (d, *J* = 7.3 Hz, 1H), 8.25 (dd, *J* = 1.9, 0.8 Hz, 1H), 8.11 (br s, 1H), 8.07 (d, *J* = 2.3 Hz, 1H), 7.52 (br s, 1H), 7.28 (dd, *J* = 7.3, 1.9 Hz, 1H), 6.80 (dd, *J* = 2.3, 0.8 Hz, 1H). LC–MS ($APCI^+$) 162 (MH^+, 100%). Vilsmeier reaction of **7u** (2.36 g, 14.7 mmol), except for using 5 equiv of $POCl_3$, and filtration of the product from the basified aqueous reaction mixture, gave **4x** as a pale brown solid (1.94 g, 77%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.12 (s, 1H), 8.70 (m, 1H), 8.66 (d, *J* = 7.1 Hz, 1H), 8.52 (s, 1H), 7.19 (dd, *J* = 7.1, 1.7 Hz, 1H). LC–MS ($APCI^+$) 172 (MH^+, 100%).

4.1.2.22. 6-Fluoro-3-formylpyrazolo[1,5-*a*]pyridine-5-carbonitrile (4z). Decarboxylation of **3z** (320 mg, 1.27 mmol) gave 6-fluoropyrazolo[1,5-*a*]pyridine-5-carboxylic acid as a white solid (208 mg, 91%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.52 (d, *J* = 5.8 Hz, 1H), 8.36 (d, *J* = 7.2 Hz, 1H), 8.04 (d, *J* = 2.4 Hz, 1H), 6.81 (d, *J* = 2.4 Hz, 1H). LC–MS ($APCI^+$) 181 (MH^+, 100%). CDI (561 mg, 3.46 mmol) was added to a stirred suspension of the above acid (208 mg, 1.15 mmol) in CH_2Cl_2 (25 mL). After 30 min the starting material had dissolved and a solution of NH_3 (2 mL of a 6 M solution in MeOH, 12 mmol) was added in a single portion. The reaction mixture was stirred for an additional 30 min before the solvent was removed in vacuo. Chromatography (eluting with hexanes: EtOAc 1:1) gave 6-fluoropyrazolo[1,5-*a*]pyridine-5-carboxamide (**7z**) as a colourless white solid (113 mg, 55%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.51 (d, *J* = 6.8 Hz, 1H), 8.45 (d, *J* = 7.8 Hz, 1H), 8.03 (d, *J* = 2.4 Hz, 1H), 6.78 (dd, *J* = 2.4, 1.4 Hz, 1H), 6.60 (br s, 1H), 5.90 (br s, 1H). LC–MS ($APCI^+$) 180 (MH^+, 100%). Vilsmeier reaction of **7z** (113 mg, 179 mmol), after chromatography (eluting with hexanes: EtOAc 7:1) gave 6-fluoropyrazolo[1,5-*a*]pyridine-5-carbonitrile as a colourless solid (101 mg, 88%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.55 (d, *J* = 4.4 Hz, 1H), 8.07 (d, *J* = 2.4 Hz, 1H), 7.98 (d, *J* = 6.3 Hz, 1H), 6.81 (dd, *J* = 2.4, 0.7 Hz, 1H). LC–MS ($APCI^+$) 162 (MH^+, 100%). Vilsmeier reaction of the above compound (89 mg, 0.55 mmol), after chromatography (eluting with hexanes: EtOAc 3:1) gave **4z** as a colourless solid (55 mg, 52%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.10 (s, 1H), 8.73 (d, *J* = 6.4 Hz, 1H), 8.67 (d, *J* = 3.8 Hz, 1H), 8.52 (s, 1H).

4.1.2.23. 5-Methoxypyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4aa)[24]. Decarboxylation of **3aa** (114 mg, 0.52 mmol) gave pyrazolo[1,5-*a*]pyridin-5-ol (**7ab**) as a pale brown solid (61 mg, 88%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 10.06 (s, 1H), 8.45 (d, *J* = 7.5 Hz, 1H), 7.79 (d, *J* = 2.2 Hz, 1H), 6.77 (d, *J* = 2.4 Hz, 1H), 6.46 (dd, *J* = 7.5, 2.4 Hz, 1H), 6.23 (d, *J* = 2.2 Hz, 1H). LC–MS ($APCI^+$) 135 (MH^+, 100%). Iodomethane (23 µL, 0.37 mmol) was added to a suspension of **7ab** (25 mg, 0.19 mmol) and K_2CO_3 (52 mg, 0.38 mmol) in DMF (2 mL). After 3 h, the reaction mixture was diluted with water and extracted twice with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo to leave 5-methoxypyrazolo[1,5-*a*]pyridine (**7aa**) as a pale brown oil (24 mg, 86%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.28 (d, *J* = 7.6 Hz, 1H), 7.85 (d, *J* = 2.2 Hz, 1H), 6.74 (d, *J* = 2.7 Hz, 1H), 6.44 (dd, *J* = 7.6, 2.7 Hz, 1H), 6.32 (d, *J* = 2.2 Hz, 1H), 3.84 (s, 3H). LC–MS ($APCI^+$) 149 (MH^+, 100%). Vilsmeier reaction of **7aa** (24 mg, 0.16 mmol) gave **4aa** as a pale yellow solid (27 mg, 93%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.96 (s, 1H), 8.37 (d, *J* = 7.5 Hz, 1H), 8.27 (s, 1H), 7.60 (d, *J* = 2.7 Hz, 1H), 6.72 (dd, *J* = 7.5, 2.7 Hz, 1H), 3.96 (s, 3H). LC–MS ($APCI^+$) 177 (MH^+, 100%)

4.1.2.24. 5-Hydroxypyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4ab). Vilsmeier reaction of **7ab** (36 mg, 0.27 mmol), except for reacidifying the aqueous mixture to pH 3 before extracting, gave **4ab** as a red-brown solid (42 mg, 95%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 11.10 (s, 1H), 9.83 (s, 1H), 8.71 (d, *J* = 7.4 Hz, 1H), 8.44 (s, 1H), 7.42 (d, *J* = 2.6 Hz, 1H), 6.77 (dd, *J* = 7.4, 2.6 Hz, 1H). LC–MS ($APCI^+$) 163 (MH^+, 100%).

4.1.2.25. 3-Formylpyrazolo[1,5-*a*]pyridin-5-yl acetate (4ac). A solution of **4ab** (42 mg, 0.26 mmol), Ac_2O (37 µL, 0.39 mmol) and NEt_3 (54 µL, 0.39 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature for 3 days. The reaction mixture was diluted with CH_2Cl_2 and washed with water, dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with hexanes: EtOAc 3:1 to 2:1) gave **4ac** as a pale brown solid (48 mg, 91%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.02 (s, 1H), 8.54 (dd, *J* = 7.4 Hz, 1H), 8.38 (s, 1H), 8.05 (d, *J* = 2.5 Hz, 1H), 6.91 (dd, *J* = 7.4, 2.5 Hz, 1H), 2.37 (s, 3H). LC–MS ($APCI^+$) 205 (MH^+, 100%).

4.1.3. Synthesis of sulfonohydrazides 5

Unless otherwise stated, sulfonohydrazides **5** were made by the following method. Methylhydrazine sulfate (1.2 equiv) and $NaHCO_3$ (5 equiv) were added to a solution of aldehyde (1 equiv) in MeOH (5 mL). After all of the aldehyde was consumed, 2-methyl-5-nitrobenzenesulfonyl chloride (1.3 equiv) was added and the reaction mixture stirred for a further 30 min. The solvent was removed in vacuo and the residue taken up in CH_2Cl_2 and water. The layers were separated and the aqueous phase extracted with CH_2Cl_2, then the combined organic layers were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with a hexanes: EtOAc gradient, unless otherwise stated) afforded **5**.

4.1.3.1. *N*,2-Dimethyl-5-nitro-*N′*-(pyrazolo[1,5-*a*]pyridin-3-ylmethylene)benzenesulfonohydrazide (5a). Reaction of pyrazolo[1,5-*a*]pyridine-3-carbaldehyde[25] (**4a**) (43 mg, 0.29 mmol), after chromatography (eluting with hexanes: EtOAc 3:1 to 2:1 to 1:1) gave **5a** as a yellow solid (63 mg, 57%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.01 (d, *J* = 2.4 Hz, 1H), 8.46 (d, *J* = 7.0 Hz, 1H), 8.28 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.04 (s, 1H), 7.95 (s, 1H), 7.87 (d, *J* = 8.9 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.28 (m, 1H), 6.90 (td, *J* = 7.0, 1.3 Hz, 1H), 3.41 (s, 3H), 2.76 (s, 3H). LC–MS (APCI$^+$) 374 (MH$^+$, 100%). Anal. Calcd for $C_{16}H_{15}N_5O_4S$: C, 51.47; H, 4.05; N, 18.76. Found: C, 51.72; H, 4.13; N, 18.98.

4.1.3.2. *N*′-((6-Bromopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (5b). Reaction of **4b** (85 mg, 0.38 mmol) after chromatography (eluting with hexanes: EtOAc 2:1 to 1:1 to 1:2) gave **5b** as a yellow solid (116 mg, 68%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.99 (d, *J* = 2.4 Hz, 1H), 8.61 (dd, *J* = 1.7, 0.8 Hz, 1H), 8.29 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.01 (s, 1H), 7.88 (s, 1H), 7.80 (dd, *J* = 9.4, 0.8 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.34 (dd, *J* = 9.4, 1.7 Hz, 1H), 3.41 (s, 3H), 2.75 (s, 3H). LC–MS (APCI$^+$) 452 (MH$^+$ with ^{79}Br, 95%), 454 (MH$^+$ with ^{81}Br, 100%). Anal. Calcd for $C_{16}H_{14}BrN_5O_4S$: C, 42.49; H, 3.12; N, 15.48. Found: C, 42.71; H, 3.07; N, 15.45.

4.1.3.3. *N*′-((4-Bromopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (5c). Reaction of **4c** (90 mg, 0.40 mmol) after chromatography (eluting with hexanes: EtOAc 5:1 to 3:1 to 2:1) gave **5c** as a yellow solid (27 mg, 15%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.86 (d, *J* = 2.4 Hz, 1H), 8.65 (s, 1H), 8.45 (d, *J* = 6.9 Hz, 1H), 8.30 (s, 1H), 8.29 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.45–7.52 (m, 2H), 6.69 (t, *J* = 7.1 Hz, 1H), 3.39 (s, 3H), 2.86 (s, 3H). LC–MS (APCI$^+$) 452 (MH$^+$ with ^{79}Br, 100%), 454 (MH$^+$ with ^{81}Br, 90%). Anal. Calcd for $C_{16}H_{14}BrN_5O_4S$. 0.1 hexanes: C, 43.26; H, 3.37; N, 15.19. Found: C, 43.53; H, 3.37; N, 15.10.

4.1.3.4. *N*,2-Dimethyl-*N*′-((7-methylpyrazolo[1,5-*a*]pyridin-3-yl)methylene)-5-nitrobenzenesulfonohydrazide (5d). Reaction of **4d** (48 mg, 0.30 mmol) after chromatography (eluting with hexanes: EtOAc 3:1 to 2:1) gave **5d** as a yellow solid (78 mg, 67%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.01 (d, *J* = 2.4 Hz, 1H), 8.28 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.09 (s, 1H), 7.99 (s, 1H), 7.79 (d, *J* = 8.9 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.23 (dd, *J* = 8.9, 7.0 Hz, 1H), 6.77 (d, *J* = 7.0 Hz, 1H), 3.40 (s, 3H), 2.75 (s, 6H). LC–MS (APCI$^+$) 388 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{17}N_5O_4S$: C, 52.70; H, 4.42; N, 18.08. Found: C, 52.90; H, 4.61; N, 17.93.

4.1.3.5. *N*,2-Dimethyl-*N*′-((6-methylpyrazolo[1,5-*a*]pyridin-3-yl)methylene)-5-nitrobenzenesulfonohydrazide (5e). Reaction of **4e** (30 mg, 0.19 mmol) after chromatography (eluting with hexanes: EtOAc 3:1 to 2:1 to 1:1) gave **5e** as a yellow solid (44 mg, 60%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.01 (d, *J* = 2.4 Hz, 1H), 8.28 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.26 (m, 1H), 7.97 (s, 1H), 7.94 (s, 1H), 7.77 (d, *J* = 9.0 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.13 (dd, *J* = 9.0, 1.4 Hz, 1H), 3.39 (s, 3H), 2.75 (s, 3H), 2.36 (s, 3H). LC–MS (APCI$^+$) 388 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{17}N_5O_4S$: C, 52.70; H, 4.42; N, 18.08. Found: C, 52.85; H, 4.55; N, 17.78.

4.1.3.6. *N*,2-Dimethyl-*N*′-((4-methylpyrazolo[1,5-*a*]pyridin-3-yl)methylene)-5-nitrobenzenesulfonohydrazide (5f). Reaction of **4f** (54 mg, 0.34 mmol) after chromatography (eluting with hexanes: EtOAc 3:1) gave **5f** as a yellow solid (80 mg, 61%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.83 (d, *J* = 2.4 Hz, 1H), 8.36 (s, 1H), 8.34 (d, *J* = 6.9 Hz, 1H), 8.29 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.25 (s, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.02 (m, 1H), 6.75 (t, *J* = 6.9 Hz, 1H), 3.32 (s, 3H), 2.83 (s, 3H), 2.60 (s, 3H). LC–MS (APCI$^+$) 388 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{17}N_5O_4S$: C, 52.70; H, 4.42; N, 18.08. Found: C, 52.95; H, 4.51; N, 17.96.

4.1.3.7. *N*,2-Dimethyl-*N*′-((5-methylpyrazolo[1,5-*a*]pyridin-3-yl)methylene)-5-nitrobenzenesulfonohydrazide (5g). Reaction of **4g** (14 mg, 0.09 mmol) after chromatography (eluting with CH_2Cl_2 to CH_2Cl_2: MeOH 99.5:0.5) gave **5g** as a yellow solid (14 mg, 41%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.00 (d, *J* = 2.4 Hz, 1H), 8.34 (d, *J* = 7.1 Hz, 1H), 8.30 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.00 (s, 1H), 7.99 (s, 1H), 7.61 (s, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 6.72 (dd, *J* = 7.1, 1.9 Hz, 1H), 3.39 (s, 3H), 2.76 (s, 3H), 2.38 (s, 3H). LC–MS (APCI$^+$) 388 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{17}N_5O_4S$.0.1 EtOAc: C, 52.75; H, 4.53; N, 17.67. Found: C, 52.66; H, 4.48; N, 17.49.

4.1.3.8. *N*′-((5-Ethylpyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (5h). Reaction of **4h** (50 mg, 0.29 mmol) after chromatography (eluting with hexanes: EtOAc 3:1 to 2:1) gave **5h** as a yellow solid (65 mg, 57%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.98 (d, *J* = 2.4 Hz, 1H), 8.36 (d, *J* = 7.1 Hz, 1H), 8.29 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.01 (s, 1H), 8.00 (s, 1H), 7.66 (m, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 6.76 (dd, *J* = 7.1, 1.9 Hz, 1H), 3.39 (s, 3H), 2.76 (s, 3H), 2.66 (q, *J* = 7.6 Hz, 2H), 1.25 (t, *J* = 7.6 Hz, 3H). LC–MS (APCI$^+$) 402 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{19}N_5O_4S$: C, 53.85; H, 4.77; N, 17.45. Found: C, 54.11; H, 4.83; N, 17.24.

4.1.3.9. *N*,2-Dimethyl-5-nitro-*N*′-((5-(trifluoromethyl)pyrazolo[1,5-*a*]pyridin-3-yl)methylene)benzenesulfonohydrazide (5i). Reaction of **4i** (25 mg, 0.12 mmol) after chromatography (eluting with hexanes: EtOAc 3:1 to 2:1) gave **5i** as a yellow solid (45 mg, 87%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.92 (d, *J* = 2.4 Hz, 1H), 8.55 (d, *J* = 7.3 Hz, 1H), 8.32 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.17 (s, 1H), 8.10 (m, 1H), 7.94 (s, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.03 (dd, *J* = 7.3, 2.0 Hz, 1H), 3.45 (s, 3H), 2.76 (s, 3H). LC–MS (APCI$^+$) 442 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{14}F_3N_5O_4S$.0.1 hexanes: C, 46.98; H, 3.45; N, 15.56. Found: C, 46.99; H, 3.35; N, 15.84.

4.1.3.10. *N*,2-Dimethyl-5-nitro-*N*′-((5-phenylpyrazolo[1,5-*a*]pyridin-3-yl)methylene)benzenesulfonohydrazide (5j). Reaction of **4j** (45 mg, 0.20 mmol) after chromatography (eluting with hexanes: EtOAc 3:1 to 2:1 to 1:1) gave **5j** as a yellow solid (83 mg, 91%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.86 (d, *J* = 2.4 Hz, 1H), 8.51 (dd, *J* = 7.2, 0.9 Hz, 1H), 8.20 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.12 (dd, *J* = 2.0, 0.9 Hz, 1H), 8.09 (s, 1H), 8.07 (s, 1H), 7.57 (m, 2H), 7.54–7.44 (m, 3H), 7.42 (d, *J* = 8.4 Hz, 1H), 7.17 (dd, *J* = 7.2, 2.0 Hz, 1H), 3.39 (s, 3H), 2.76 (s, 3H). LC–MS (APCI$^+$) 450 (MH$^+$, 100%). Anal. Calcd for $C_{22}H_{19}N_5O_4S$.0.1 EtOAc: C, 58.71; H, 4.35; N, 15.28. Found: C, 58.98; H, 4.49; N, 15.15.

4.1.3.11. *N*′-((5-Bromopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (5m). Reaction of **4m** (22 mg, 0.10 mmol) after chromatography (eluting with hexanes: EtOAc 3:1 to 2:1 to 1:1) gave **5m** as a yellow solid (43 mg, 98%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.01 (d, *J* = 2.4 Hz, 1H), 8.36 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.28 (d, *J* = 7.3 Hz, 1H), 8.03 (s, 1H), 7.89 (s, 1H), 7.76 (d, *J* = 2.2 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 6.94 (dd, *J* = 7.3, 2.2 Hz, 1H), 3.43 (s, 3H), 2.74 (s, 3H). LC–MS (APCI$^+$) 452 (MH$^+$ with ^{79}Br, 80%), 454 (MH$^+$ with ^{81}Br, 100%). Anal. Calcd for $C_{16}H_{14}BrN_5O_4S$: C, 42.49; H, 3.12; N, 15.48. Found: C, 42.77; H, 3.07; N, 15.47.

4.1.3.12. *N*′-((5-Iodopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (5n). Reaction of **4n** (45 mg, 0.17 mmol) after chromatography (eluting with hexanes: EtOAc 3:1 to 2:1 to 1:1) gave **5n** as a yellow solid (60 mg, 72%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.99 (d, *J* = 2.4 Hz, 1H), 8.36 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.16 (dd, *J* = 7.2, 0.7 Hz, 1H), 8.05 (m, 1H), 8.00 (s, 1H), 7.90 (s, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.09 (dd, *J* = 7.2, 1.9 Hz, 1H), 3.43 (s, 3H), 2.75 (s, 3H). LC–MS (APCI$^+$) 500 (MH$^+$, 100%). Anal.

Calcd for $C_{16}H_{14}IN_5O_4S$.0.15 hexanes: C, 39.63; H, 3.17; N, 13.67. Found: C, 39.63; H, 2.95; N, 13.69.

4.1.3.13. *N′*-((5-Chloropyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (5o). Reaction of **4o** (7 mg, 0.039 mmol) after chromatography (eluting with hexanes: EtOAc 3:1 to 1:1) gave **5o** as a yellow solid (13 mg, 81%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.01 (d, *J* = 2.4 Hz, 1H), 8.32–8.38 (m, 2H), 8.04 (s, 1H), 7.89 (s, 1H), 7.56 (d, *J* = 1.9 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 6.83 (dd, *J* = 7.3, 2.3 Hz, 1H), 3.42 (s, 3H), 2.75 (s, 3H). LC–MS (APCI$^+$) 408 (MH$^+$ with ^{35}Cl, 100%), 410 (MH$^+$ with ^{37}Cl, 25%). Anal. Calcd for $C_{16}H_{14}ClN_5O_4S$: C, 47.12; H, 3.46; N, 17.17. Found: C, 47.06; H, 3.53; N, 16.97.

4.1.3.14. *N′*-((5-Cyclopropylpyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (5p). Reaction of **4p** (37 mg, 0.20 mmol) after chromatography (eluting with hexanes: EtOAc 3:1 to 2:1 to 1:1) gave **5p** as a yellow solid (37 mg, 45%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.98 (d, *J* = 2.4 Hz, 1H), 8.32-8.24 (m, 2H), 8.00-7.96 (m, 2H), 7.62 (d, *J* = 1.9 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 6.53 (dd, *J* = 7.2, 2.0 Hz, 1H), 3.39 (s, 3H), 2.75 (s, 3H), 1.90 (m, 1H), 1.10 (m, 2H), 0.73 (m, 2H). LC–MS (APCI$^+$) 414 (MH$^+$, 100%). Anal. Calcd for $C_{19}H_{19}N_5O_4S$: C, 55.20; H, 4.63; N, 16.94. Found: C, 54.80; H, 4.57; N, 16.65.

4.1.3.15. *N*,2-Dimethyl-5-nitro-*N′*-((5-vinylpyrazolo[1,5-*a*]pyridin-3-yl)methylene)benzenesulfonohydrazide (5q). Reaction of **4q** (35 mg, 0.20 mmol) after chromatography (eluting with hexanes: EtOAc 4:1 to 2:1 to 1:1) gave **5q** as a yellow solid (36 mg, 44%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.98 (d, *J* = 2.4 Hz, 1H), 8.37 (d, *J* = 7.3 Hz, 1H), 8.29 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.02 (s, 1H), 7.97 (s, 1H), 7.74 (d, *J* = 1.9 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.03 (dd, *J* = 7.3, 1.9 Hz, 1H), 6.67 (dd, *J* = 17.5, 10.9 Hz, 1H), 5.82 (d, *J* = 17.5 Hz, 1H), 5.48 (d, *J* = 10.9 Hz, 1H), 3.41 (s, 3H), 2.75 (s, 3H). LC–MS (APCI$^+$) 400 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{17}N_5O_4S$: C, 54.13; H, 4.29; N, 17.53. Found: C, 54.42; H, 4.29; N, 17.38.

4.1.3.16. *N′*-((5-Ethynylpyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (5r). Reaction of **4r** (14 mg, 0.08 mmol) after chromatography (eluting with hexanes: EtOAc 3:1 to 2:1 to 1:1) gave **5r** as a yellow solid (24 mg, 73%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.04 (d, *J* = 2.4 Hz, 1H), 8.39-8.29 (m, 2H), 8.05 (s, 1H), 7.90 (s, 1H), 7.64 (m, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 6.86 (dd, *J* = 7.2, 1.8 Hz, 1H), 3.44 (s, 3H), 3.29 (s, 1H), 2.73 (s, 3H). LC–MS (APCI$^+$) 398 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{15}N_5O_4S$: C, 54.40; H, 3.80; N, 17.62. Found: C, 54.37; H, 3.87; N, 17.26.

4.1.3.17. Methyl 3-((2-methyl-2-(2-methyl-5-nitrophenylsulfonyl)-hydrazono)methyl)pyrazolo[1,5-*a*]pyridine-5-carboxylate (5t). Reaction of **4t** (22 mg, 0.11 mmol) after chromatography (eluting with hexanes: EtOAc 3:1 to 2:1 to 1:1) gave **5t** as a yellow solid (43 mg, 93%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.95 (d, *J* = 2.4 Hz, 1H), 8.51 (dd, *J* = 1.9, 0.9 Hz, 1H), 8.47 (dd, *J* = 7.2, 0.9 Hz, 1H), 8.32 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.13 (s, 1H), 7.96 (s, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.42 (dd, *J* = 7.2, 1.9 Hz, 1H), 3.98 (s, 3H), 3.44 (s, 3H), 2.78 (s, 3H). LC–MS (APCI$^+$) 432 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{17}N_5O_6S$.0.2 H_2O: C, 49.70; H, 4.03; N, 16.10. Found: C, 49.65; H, 4.08; N, 15.79.

4.1.3.18. 3-((2-Methyl-2-(2-methyl-5-nitrophenylsulfonyl)-hydrazono)methyl)pyrazolo[1,5-*a*]pyridine-5-carboxamide (5u). Reaction of **4u** (28 mg, 0.15 mmol) except that after the reaction solvent was removed in vacuo, the residue was taken up in CH_2Cl_2 and water. The solid was filtered off, washed with water and CH_2Cl_2 then dried to leave **5u** as a yellow solid (30 mg, 48%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.84 (dd, *J* = 7.3, 0.8 Hz, 1H), 8.68 (d, *J* = 2.5 Hz, 1H), 8.40 (dd, *J* = 8.4, 2.5 Hz, 1H), 8.34 (dd, *J* = 1.9, 0.8 Hz, 1H), 8.31 (s, 1H), 8 = 24 (s, 1H), 8.07 (br s, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.58 (br s, 1H), 7.40 (dd, *J* = 7.3, 1.9 Hz, 1H), 3.39 (s, 3H), 2.74 (s, 3H). LC–MS (APCI$^+$) 417 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{16}N_6O_5S$: C, 49.03; H, 3.87; N, 20.18. Found: C, 49.29; H, 3.92; N, 20.30.

4.1.3.19. *N*-Methyl-3-((2-methyl-2-(2-methyl-5-nitrophenylsulfonyl)hydrazono)methyl)pyrazolo[1,5-*a*]pyridine-5-carboxamide (5v). Reaction of **4v** (51 mg, 0.25 mmol) after chromatography (eluting with hexanes: EtOAc 1:1 to EtOAc) gave **5v** as a yellow solid (80 mg, 74%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.91 (d, *J* = 2.4 Hz, 1H), 8.49 (dd, *J* = 7.2, 0.9 Hz, 1H), 8.35 (dd, *J* = 1.9, 0.9 Hz, 1H), 8.31 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.11 (s, 1H), 7.97 (s, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.33 (dd, *J* = 7.2, 1.9 Hz, 1H), 6.36 (br s, 1H), 3.42 (s, 3H), 3.09 (d, *J* = 4.9 Hz, 3H), 2.77 (s, 3H). LC–MS (APCI$^+$) 431 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{18}N_6O_5S$.0.33 H_2O: C, 49.54; H, 4.31; N, 19.26. Found: C, 49.58; H, 4.25; N, 18.97.

4.1.3.20. *N*,*N*-Dimethyl-3-((2-methyl-2-(2-methyl-5-nitrophenylsulfonyl)hydrazono)methyl)pyrazolo[1,5-*a*]pyridine-5-carboxamide (5w). Reaction of **4w** (43 mg, 0.20 mmol) after chromatography (eluting with hexanes: EtOAc 1:1 to EtOAc) gave **5w** as a yellow solid (83 mg, 94%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.93 (d, *J* = 2.4 Hz, 1H), 8.45 (dd, *J* = 7.1, 0.9 Hz, 1H), 8.34 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.09 (s, 1H), 7.93 (s, 1H), 7.75 (dd, *J* = 1.8, 0.9 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 6.89 (dd, *J* = 7.1, 1.8 Hz, 1H), 3.42 (s, 3H), 3.15 (s, 3H), 3.04 (s, 3H), 2.73 (s, 3H). LC–MS (APCI$^+$) 445 (MH$^+$, 100%). Anal. Calcd for $C_{19}H_{20}N_6O_5S$: C, 51.34; H, 4.54; N, 18.91. Found: C, 51.20; H, 4.66; N, 19.09.

4.1.3.21. *N′*-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (5x). Reaction of **4x** (49 mg, 0.29 mmol) after chromatography (eluting with CH_2Cl_2: MeOH 99.5:0.5) gave **5x** as a yellow solid (59 mg, 52%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.91 (d, *J* = 2.4 Hz, 1H), 8.52 (d, *J* = 7.2 Hz, 1H), 8.37 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.18 (s, 1H), 8.04 (m, 1H), 7.90 (s, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 6.98 (dd, *J* = 7.2, 1.7 Hz, 1H), 3.45 (s, 3H), 2.79 (s, 3H). LC–MS (APCI$^+$) 399 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{14}N_6O_4S$: C, 51.25; H, 3.54; N, 21.09. Found: C, 50.95; H, 3.54; N, 20.90.

4.1.3.22. *N′*-((5-Cyano-6-fluoropyrazolo[1,5-*a*]pyridin-3-yl)-methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (5z). Reaction of **4z** (55 mg, 0.29 mmol) after chromatography (eluting with CH_2Cl_2: ^{i}PrOH 99:1) and trituration with Et_2O, gave **5z** as a yellow solid (107 mg, 85%). HPLC purity 97%. ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.88 (d, *J* = 2.4 Hz, 1H), 8.52 (dd, *J* = 4.1, 0.7 Hz, 1H), 8.37 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.16 (s, 1H), 8.11 (d, *J* = 6.4 Hz, 1H), 7.85 (s, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 3.45 (s, 3H), 2.78 (s, 3H). LC–MS (APCI$^+$) 417 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{13}FN_6O_4S$: C, 49.04; H, 3.15; N, 20.18. Found: C, 48.81; H, 2.89; N, 20.04. HRMS (ESI$^+$) Calcd for $C_{17}H_{13}FN_6O_4SNa$: 439.0595; found (M+Na$^+$) 439.0604.

4.1.3.23. *N′*-((5-Methoxypyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (5aa). A solution of **4aa** (27 mg, 0.15 mmol) and 2-methyl-5-nitrobenzenesulfonohydrazide[14] (39 mg, 0.17 mmol) in MeOH (5 mL) was stirred at room temperature for 4 h. THF (2 mL) was then added, followed by the dropwise addition of an ethereal solution of CH_2N_2 solution until reaction was complete by tlc analysis. The solvents were removed in vacuo. Chromatography (eluting with CH_2Cl_2: MeOH 99.5:0.5) gave **5aa** as a yellow solid (36 mg, 58%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.67 (d, *J* = 2.5 Hz, 1H), 8.62 (d, *J* = 7.6 Hz, 1H), 8.39 (dd, *J* = 8.4, 2.5 Hz, 1H), 8.15 (s, 1H), 8.12 (s, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.26 (d, *J* = 2.8 Hz, 1H), 6.74 (dd, *J* = 7.6, 2.8 Hz, 1H), 3.81 (s, 3H), 3.29 (s, 3H), 2.71 (s, 3H). LC–MS (APCI$^+$) 404 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{17}N_5O_5S$: C, 50.61; H, 4.25; N, 17.36. Found: C, 50.85; H, 4.29; N, 17.51.

4.1.3.24. 3-((2-Methyl-2-(2-methyl-5-nitrophenylsulfonyl)-hydrazono)methyl)pyrazolo[1,5-*a*]pyridin-5-yl acetate (5ac). A solution of **4ac** (48 mg, 0.24 mmol) and 2-methyl-5-nitrobenzenesulfonohydrazide[14] (60 mg, 0.26 mmol) in MeOH (5 mL) was stirred at room temperature for 4 h. THF (2 mL) was then added, followed by the dropwise addition of an ethereal solution of CH_2N_2 solution until reaction was complete by tlc analysis. The solvents were removed in vacuo. Chromatography (eluting with CH_2Cl_2: MeOH 99.5:0.5) gave **5ac** as a yellow solid (72 mg, 71%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.02 (d, *J* = 2.4 Hz, 1H), 8.41 (dd, *J* = 7.5, 0.7 Hz, 1H), 8.30 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.03 (s, 1H), 7.92 (s, 1H), 7.51 (d, *J* = 2.5 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 6.71 (dd, *J* = 7.5, 2.5 Hz, 1H), 3.42 (s, 3H), 2.72 (s, 3H), 2.39 (s, 3H). LC–MS (APCI$^+$) 432 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{17}N_5O_6S.0.5\ H_2O$: C, 49.09; H, 4.12; N, 15.90. Found: C, 49.31; H, 4.00; N, 15.91.

4.1.3.25. *N*′-((5-Hydroxypyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (5ab). Saturated aqueous $NaHCO_3$ (5 mL) was added to a suspension of **5ac** (52 mg, 0.12 mmol) in MeOH (10 mL) and stirred for 2 h. The MeOH was removed in vacuo, and the resulting solid filtered off and washed with water. Chromatography (eluting with CH_2Cl_2: MeOH 99:1 to 19:1) gave **5ab** as a yellow solid (26 mg, 55%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 10.58 (br s, 1H), 8.69 (d, *J* = 2.5 Hz, 1H), 8.54 (d, *J* = 7.5 Hz, 1H), 8.41 (dd, *J* = 8.4, 2.5 Hz, 1H), 8.12 (s, 1H), 8.06 (s, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 6.88 (d, *J* = 2.6 Hz, 1H), 6.60 (dd, *J* = 7.5, 2.6 Hz, 1H), 3.27 (s, 3H), 2.69 (s, 3H). LC–MS (APCI$^+$) 390 (MH$^+$, 100%). Anal. Calcd for $C_{16}H_{15}N_5O_5S$: C, 49.35; H, 3.88; N, 17.99. Found: C, 49.51; H, 3.94; N, 17.72.

4.1.4. Synthesis of pyrazolo[1,5-*a*]pyridine-3-ketones 12b–f

Unless otherwise stated, the following method was used. A fresh solution of MSH[23] in CH_2Cl_2 (1 equiv) was added to the pyridine (1 equiv) in CH_2Cl_2 (10 mL) at 0 °C. After 2 h, the solvent was removed in vacuo. The residue was taken up in dry DMF (8 mL), then the alkynyl ketone (1 equiv) and K_2CO_3 (2 equiv) were added, and the suspension stirred at room temperature for 18 h. The reaction mixture was diluted with water and extracted twice with EtOAc. The combined organic phases were washed three times with water then with brine, dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with a hexanes: EtOAc gradient, unless otherwise stated) gave the pyrazolo[1,5-*a*]pyridine.

4.1.4.1. 3-Acetylpyrazolo[1,5-*a*]pyridine-5-carbonitrile (12b). Reaction of **11** (1.19 g, 11.4 mmol) and 3-butyn-2-one (0.89 mL, 11.4 mmol), after chromatography (eluting with hexanes: EtOAc 4:1 to 3:1 to 2:1 to 1:1) gave **12b** as a pale yellow solid (746 mg, 35%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.80 (dd, *J* = 1.8, 1.0 Hz, 1H), 8.61 (dd, *J* = 7.2, 1.0 Hz, 1H), 8.45 (s, 1H), 7.13 (dd, *J* = 7.2, 1.9 Hz, 1H), 2.60 (s, 3H). LC–MS (APCI$^+$) 186 (MH$^+$, 100%).

4.1.4.2. 3-Propionylpyrazolo[1,5-*a*]pyridine-5-carbonitrile (12c). $AlCl_3$ (921 mg, 6.91 mmol) was added to a solution of propionic anhydride (0.74 mL, 5.76 mmol) and bis(trimethylsilyl)acetylene (1.43 mL, 6.31 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C. After 30 min, the reaction mixture was warmed to room temperature, stirred for a further 1 h, and then poured onto ice-1 M HCl (1:1, 80 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with hexanes: Et_2O 98:2) gave 1-(trimethylsilyl)pent-1-yn-3-one as a brown oil (739 mg, 83%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 2.58 (q, *J* = 7.4 Hz, 2H), 1.14 (t, *J* = 7.4 Hz, 3H), 0.24 (s, 9H). LC–MS (APCI$^+$) 155 (MH$^+$, 100%). Reaction of **11** (529 mg, 5.08 mmol) and the above ketone (520 mg, 3.38 mmol) with the addition of KF (589 mg, 10.1 mmol), after chromatography (eluting with CH_2Cl_2) gave **12c** as an off-white solid (479 mg, 71%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.81 (dd, *J* = 1.8, 1.0 Hz, 1H), 8.61 (dd, *J* = 7.2, 1.0 Hz, 1H), 8.46 (s, 1H), 7.11 (dd, *J* = 7.2, 1.8 Hz, 1H), 2.96 (q, *J* = 7.4 Hz, 2H), 1.27 (t, *J* = 7.4 Hz, 3H). LC–MS (APCI$^+$) 200 (MH$^+$, 100%).

4.1.4.3. 3-Butyrylpyrazolo[1,5-*a*]pyridine-5-carbonitrile (12d). $AlCl_3$ (3.08 g, 23.1 mmol) was added to a solution of butyryl chloride (2.00 mL, 19.3 mmol) and bis(trimethylsilyl)acetylene (4.80 mL, 21.2 mmol) in dry CH_2Cl_2 (50 mL) at 0 °C. After 30 min, the reaction mixture was warmed to room temperature, stirred for a further 2 h, and then poured onto ice-1 M HCl (1:1, 200 mL). The layers were separated, and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with hexanes: Et_2O 98:2) gave 1-(trimethylsilyl)hex-1-yn-3-one as a brown oil (2.98 g, 92%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 2.54 (t, *J* = 7.3 Hz, 2H), 1.70 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H), 0.24 (s, 9H). LC–MS (APCI$^+$) 169 (MH$^+$, 100%). Reaction of **11** (137 mg, 1.32 mmol) and the above ketone (147 mg, 0.88 mmol) with the addition of KF (153 mg, 2.63 mmol), after chromatography (eluting with hexanes: EtOAc 85:15 to 4:1) gave **12d** as an off-white solid (153 mg, 82%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.82 (dd, *J* = 1.9, 1.0 Hz, 1H), 8.61 (dd, *J* = 7.2, 1.0 Hz, 1H), 8.46 (s, 1H), 7.12 (dd, *J* = 7.2, 1.9 Hz, 1H), 2.89 (t, *J* = 7.3 Hz, 2H), 1.82 (m, 2H), 1.04 (t, *J* = 7.4 Hz, 3H). LC–MS (APCI$^+$) 214 (MH$^+$, 100%).

4.1.4.4. *tert*-Butyl 3-acetylpyrazolo[1,5-*a*]pyridin-5-ylcarbamate (12e). A solution of **6** (4.94 g, 25.4 mmol) and DNPH (5.05 g, 25.4 mmol) in MeCN (60 mL) was heated at 40 °C for 18 h. The solvent was removed in vacuo. The residue taken up in DMSO (150 mL), then K_2CO_3 (7.02 g, 50.8 mmol) followed by 3-butyn-2-one (0.87 mL, 37.9 mmol) were then added. The suspension stirred at room temperature for 18 h, then diluted with cold water. The aqueous layer was extracted three times with ethyl acetate, and the combined organic extracts were washed with 1 M HCl, followed by water then brine. The organic layer was dried (Na_2SO_4) and the solvent removed in vacuo. Filtration through a plug of neutral Al_2O_3 (eluting with EtOAc) gave **12e** as a pale yellow solid (4.20 g, 60%). 1H NMR δ (400 MHz, CDCl3) 8.41 (d, *J* = 7.6 Hz, 1H), 8.26 (s, 1H), 8.08 (d, *J* = 2.3 Hz, 1H), 7.58 (m, 1H), 6.96 (s, 1H), 2.71 (s, 3H), 1.55 (s, 9H). LC–MS (APCI+) 276 (MH+, 100%).

4.1.4.5. 1-(5-Bromopyrazolo[1,5-*a*]pyridin-3-yl)ethanone (12f). A solution of **12e** (81 mg, 0.29 mmol) and TFA (0.43 mL, 5.8 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 18 h. The solvents were removed in vacuo to leave 1-(5-aminopyrazolo[1,5-*a*]pyridin-3-yl)ethanone trifluoroacetate as a brown solid (86 mg, 100%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.33 (d, *J* = 7.4 Hz, 1H), 8.22 (s, 1H), 7.51 (d, *J* = 2.6 Hz, 1H), 6.46 (dd, *J* = 7.4, 2.6 Hz, 1H), 2.52 (s, 3H). LC–MS (APCI$^+$) 176 (MH$^+$, 100%). A solution of $NaNO_2$ (0.378 g, 5.5 mmol) in H_2O (4 mL) was added dropwise to a suspension of the above amine (1.7 g, 4.2 mmol) in 48% HBr (5 mL) in an ice-salt bath at a rate so that the internal temperature of the reaction was maintained below −5 °C. The reaction was stirred for 10 min, then a solution of CuBr (1.51 g, 10.5 mmol) in 48% HBr (5 mL) was added dropwise to maintain the internal temperature of the reaction between −5 and 0 °C. After complete addition of the CuBr, the reaction was stirred at 0 °C for a further 30 min, and then at room temperature for 1 h. The reaction was cooled and carefully neutralised to pH 8 with 2 M NaOH. A solution of EDTA (3 g) in water (100 mL), followed by CH_2Cl_2 (100 mL) were added and the reaction was stirred vigorously for 15 min. The solution was filtered and the layers separated. The aqueous layer was extracted twice with EtOAc, washed with water and brine. The combined organic extracts were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with hexanes to hexanes: EtOAc 85:15) gave **12f** as a pale yellow solid (677 mg, 51%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.61 (dd, *J* = 2.2, 0.8 Hz, 1H), 8.37 (dd, *J* = 7.3, 0.8 Hz, 1H), 8.32 (s, 1H), 7.10 (dd, *J* = 7.3, 2.2 Hz, 1H), 2.55 (s, 3H). LC–MS (APCI$^+$) 239 (MH$^+$ with ^{79}Br, 100%), 241 (MH$^+$ with ^{81}Br, 80%).

4.1.5. Synthesis of sulfonohydrazides 13a–e

A solution of the aldehyde or ketone (1 equiv) and 2-methyl-5-nitrobenzenesulfonohydrazide (1.1 equiv) in MeOH (5 mL) was refluxed for 18 h unless otherwise stated. After cooling to room temperature, the precipitated solid was filtered off, washed with a little MeOH and dried.

4.1.5.1. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-2-methyl-5-nitrobenzenesulfonohydrazide (13a). Reaction of **4x** (150 mg, 0.88 mmol) at room temperature for 18 h gave **13a** as a yellow solid (305 mg, 91%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 11.99 (s, 1H), 8.94 (dd, *J* = 7.2, 0.9 Hz, 1H), 8.72 (d, *J* = 2.5 Hz, 1H), 8.42 (s, 1H), 8.38 (dd, *J* = 8.4, 2.5 Hz, 1H), 8.20 (s, 1H), 8.10 (dd, *J* = 1.9, 0.9 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.31 (dd, *J* = 7.2, 1.9 Hz, 1H), 2.77 (s, 3H). LC–MS (APCI$^+$) 385 (MH$^+$, 100%). Anal. Calcd for $C_{16}H_{12}N_6O_4S$: C, 50.00; H, 3.15; N, 21.86. Found: C, 50.21; H, 3.15; N, 21.80.

4.1.5.2. *N*′-(1-(5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)ethylidene)-2-methyl-5-nitrobenzenesulfonohydrazide (13b). Reaction of **12b** (50 mg, 0.27 mmol) gave **13b** as a yellow solid (82 mg, 76%). HPLC purity 93%. ^{1}H NMR δ (400 MHz, d_6-DMSO) 11.15 (s, 1H), 8.91 (dd, *J* = 7.2, 0.6 Hz, 1H), 8.77 (d, *J* = 2.5 Hz, 1H), 8.55 (s, 1H), 8.40 (dd, *J* = 8.4, 2.5 Hz, 1H), 7.93 (s, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.28 (dd, *J* = 7.2, 1.9 Hz, 1H), 2.78 (s, 3H), 2.35 (s, 3H). LC–MS (APCI$^+$) 399 (MH$^+$, 100%). HRMS (ESI$^+$) Calcd for $C_{17}H_{15}N_6O_4S$: 399.0870; found (MH$^+$) 399.0879.

4.1.5.3. *N*′-(1-(5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)propylidene)-2-methyl-5-nitrobenzenesulfonohydrazide (13c). Reaction of **12c** (71 mg, 0.36 mmol), after chromatography (eluting with CH_2Cl_2: MeOH 99.5:0.5) followed by trituration with MeOH gave **13c** as a yellow solid (105 mg, 71%). HPLC purity 96%. ^{1}H NMR δ (400 MHz, d_6-DMSO) 11.37 (s, 1H), 8.93 (dd, *J* = 7.2, 0.9 Hz, 1H), 8.76 (d, *J* = 2.4 Hz, 1H), 8.56 (s, 1H), 8.40 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.92 (dd, *J* = 1.8, 0.9 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.29 (dd, *J* = 7.2, 1.8 Hz, 1H), 2.82 (q, *J* = 7.6 Hz, 2H), 2.76 (s, 3H), 1.10 (t, *J* = 7.6 Hz, 3H). LC–MS (APCI$^+$) 413 (MH$^+$, 100%). HRMS (ESI$^+$) Calcd for $C_{18}H_{17}N_6O_4S$: 413.1027; found (MH$^+$) 413.1021.

4.1.5.4. *N*′-(1-(5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)butylidene)-2-methyl-5-nitrobenzenesulfonohydrazide (13d). Reaction of **12d** (57 mg, 0.27 mmol), after chromatography (eluting with CH_2Cl_2: MeOH 99.75:0.25) followed by trituration with MeOH gave **13d** as a yellow solid (68 mg, 60%). HPLC purity 97%. ^{1}H NMR δ (400 MHz, d_6-DMSO) 11.36 (s, 1H), 8.91 (dd, *J* = 7.2, 0.9 Hz, 1H), 8.76 (d, *J* = 2.5 Hz, 1H), 8.55 (s, 1H), 8.39 (dd, *J* = 8.4, 2.5 Hz, 1H), 7.96 (dd, *J* = 1.9, 0.9 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.27 (dd, *J* = 7.2, 1.9 Hz, 1H), 2.81–2.75 (m, 5H), 1.54 (m, 2H), 0.96 (t, *J* = 7.3 Hz, 3H). LC–MS (APCI$^+$) 427 (MH$^+$, 100%). HRMS (ESI$^+$) Calcd for $C_{19}H_{19}N_6O_4S$: 427.1183; found (MH$^+$) 427.1184.

4.1.5.5. *N*′-(1-(5-Bromopyrazolo[1,5-*a*]pyridin-3-yl)ethylidene)-2-methyl-5-nitrobenzenesulfonohydrazide (13e). Reaction of **12f** (60 mg, 0.25 mmol) gave **13e** as an orange solid (88 mg, 78%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 10.97 (br s, 1H), 8.79 (d, *J* = 2.5 Hz, 1H), 8.67 (d, *J* = 7.3 Hz, 1H), 8.44 (dd, *J* = 8.4, 2.5 Hz, 1H), 8.39 (s, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.70 (d, *J* = 2.2 Hz, 1H), 7.11 (dd, *J* = 7.3, 2.2 Hz, 1H), 2.77 (s, 3H), 2.32 (s, 3H). LC–MS (APCI$^+$) 452 (MH$^+$ with ^{79}Br, 100%), 454 (MH$^+$ with ^{81}Br, 100%). Anal. Calcd for $C_{16}H_{15}BrN_5O_4S$.0.04 hexanes: C, 43.16; H, 3.30; N, 15.74. Found: C, 42.80; H, 3.22; N, 15.37.

4.1.6. Synthesis of sulfonohydrazides (14b–e) by methylation

An ethereal solution of CH_2N_2 was added dropwise to a solution of the sulfonohydrazide (1 equiv) in THF (5 mL) until reaction was complete by tlc analysis. The solvents were removed in vacuo. Chromatography (eluting with a hexanes: EtOAc gradient, unless otherwise stated) gave the methylated hydrazide.

4.1.6.1. *N*′-(1-(5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)ethylidene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (14b). Reaction of hydrazide **13b** (50 mg, 0.13 mmol) after chromatography (eluting with CH_2Cl_2: MeOH 99.75:0.25 to 99.5:0.5) gave **14b** as a yellow solid (35 mg, 67%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.76 (d, *J* = 2.4 Hz, 1H), 8.59 (dd, *J* = 7.2, 1.0 Hz, 1H), 8.42 (dd, *J* = 8.4, 2.5 Hz, 1H), 8.38 (s, 1H), 8.30 (dd, *J* = 1.9, 1.0 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.04 (dd, *J* = 7.2, 1.9 Hz, 1H), 3.02 (s, 3H), 2.72 (s, 3H), 2.67 (s, 3H). LC–MS (APCI$^+$) 413 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{16}N_6O_4S$.0.33 H_2O: C, 51.68; H, 4.01; N, 20.09. Found: C, 51.69; H, 4.07; N, 19.81.

4.1.6.2. *N*′-(1-(5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)propylidene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (14c). Reaction of hydrazide **13c** (70 mg, 0.17 mmol) after chromatography (eluting with CH_2Cl_2: MeOH 99.75:0.25) gave **14c** as a yellow solid (40 mg, 56%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.77 (d, *J* = 2.4 Hz, 1H), 8.59 (dd, *J* = 7.2, 1.0 Hz, 1H), 8.44 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.37 (s, 1H), 8.26 (dd, *J* = 1.9, 1.0 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.04 (dd, *J* = 7.2, 1.9 Hz, 1H), 3.11 (q, *J* = 7.7 Hz, 2H), 3.01 (s, 3H), 2.71 (s, 3H), 1.37 (t, *J* = 7.7 Hz, 3H). LC–MS (APCI$^+$) 427 (MH$^+$, 100%). Anal. Calcd for $C_{19}H_{18}N_6O_4S$: C, 53.51; H, 4.25; N, 19.71. Found: C, 53.43; H, 4.35; N, 19.36.

4.1.6.3. *N*′-(1-(5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)butylidene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (14d). Reaction of hydrazide **13d** (44 mg, 0.10 mmol) after chromatography (eluting with hexanes: EtOAc 4:1 to 3:1) gave **14d** as a yellow solid (42 mg, 93%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.75 (d, *J* = 2.4 Hz, 1H), 8.58 (dd, *J* = 7.2, 1.0 Hz, 1H), 8.43 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.35 (s, 1H), 8.26 (dd, *J* = 1.9, 1.0 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.04 (dd, *J* = 7.2, 1.9 Hz, 1H), 3.06 (m, 2H), 3.00 (s, 3H), 2.70 (s, 3H), 1.78 (m, 2H), 1.11 (t, *J* = 7.4 Hz, 3H). LC–MS (APCI$^+$) 441 (MH$^+$, 100%). Anal. Calcd for $C_{20}H_{20}N_6O_4S$.0.25 EtOAc: C, 54.54; H, 4.79; N, 18.17. Found: C, 54.34; H, 4.73; N, 18.19.

4.1.6.4. *N*′-(1-(5-Bromopyrazolo[1,5-*a*]pyridin-3-yl)ethylidene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (14e). Reaction of hydrazide **13e** (49 mg, 0.11 mmol) after chromatography (eluting with hexanes: EtOAc 4:1 to 3:1) gave **14e** as a cream-coloured solid (27 mg, 53%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.78 (d, *J* = 2.4 Hz, 1H), 8.38 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.36 (dd, *J* = 7.3, 0.7 Hz, 1H), 8.24 (s, 1H), 8.06 (d, *J* = 2.2 Hz, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.02 (dd, *J* = 7.3, 2.2 Hz, 1H), 3.00 (s, 3H), 2.71 (s, 3H), 2.63 (s, 3H). LC–MS (APCI$^+$) 466 (MH$^+$ with ^{79}Br, 90%), 468 (MH$^+$ with ^{81}Br, 100%). Anal. Calcd for $C_{17}H_{16}BrN_5O_4S$. 0.33 EtOAc: C, 44.42; H, 3.79; N, 14.14. Found: C, 44.36; H, 3.77; N, 14.14.

4.2. Enzyme assays

The Class I PI3 kinase assays were performed using a basic thin layer chromatography technique, as described previously.[26] The

PI3 kinase isoforms were prepared in-house, as described previously.[26] Reactions were made containing 0.1 μg recombinant enzyme, 10 μg L-α-phosphatidylinositol, inhibitor (DMSO only or DMSO + inhibitor to a final concentration of 1%), 2× Lipid Kinase Buffer (40 mM Tris–HCl pH 7.4, 200 mM NaCl, 1 mM EDTA), and activated upon the addition of an ATP mix (5 mM $MgCl_2$, 100 μM ATP, 0.1 μL [$\gamma^{33}P$]ATP). Reactions were incubated at room temperature for 1 h following which the reactions were stopped by the addition of 1 M HCl. The lipids were then extracted using a two step procedure. Firstly, 200 μL of chloroform:methanol (1:1) was added, the biphasic reactions mixed and centrifuged briefly, and the inorganic phase was removed and discarded. Following this 80 μL of methanol:hydrochloric acid (1:1) was added and the same procedure followed. Next, 70 μL of the organic phase was transferred to a clean 1.6 mL tube and the reactions were dried using a speed vac, with no heating, for 30 min. The reactions were spotted onto TLC plates (Merck Ltd) and developed for 1 h in 1-propanol:2 M acetic acid (13:7). The TLC plates were then dried at room temperature and quantified using a phosphorimager (StormImager, Amersham). Nine inhibitor concentrations were used to determine the IC_{50}. Each experiment was performed twice and the average IC_{50} value used.

4.3. Molecular modelling

The p110α apo structure (PDB code 2RD0) was prepared by stripping all waters and assigning protons using SYBYL8.0.3 (Tripos, St. Louis). Prior to this, sidechain orientations were checked using Molprobity[27] and only those within the docking cavity were adjusted if there was clear evidence, and included residues His855 and His917. The side chain of Met772 was repositioned between the side chains of Trp780 and Pro778 as it was thought that its orientation in the native structure may be influenced by the Ras binding domain loop from a neighbouring p110α molecule located in the ATP-binding site.[15] The new orientation is more consistent with that observed in the wortmannin bound p110α structure (PDB code 3HHM). The side chain of Ser863 was also redirected toward the affinity pocket. All sidechain adjustments used the Lovell rotamer library as implemented in SYBYL8.0.3. As the neighbouring molecule may also affect the p110α C-terminal tail, predicted by PSI-PRED[28] to be helical, MODELLER[29] (9v3) was used to incorporate a C-terminal helix into the 2RD0 structure after αK12 based on that present in the p110γ structure 1EU7. The p110γ residues Lys1065 to Asp1070 were aligned to the p110α residues Leu1036 to Lys1041, with the p110α residues Gln1042 to His1065 modelled based on corresponding positions in 1EU7. All other 2RD0 amino acids were kept in their original position. A loop was also generated by Modeller for the activation residues Lys941 to Glu950 while the remaining structure was kept fixed. These additional structures were not included in the docking cavity.

Low energy ligand conformations were built by OMEGA2.2.1 (Openeyes, Santa Fe) using default conditions with the MMFF94s forcefield option and dielectric set to 80. Subsequent modifications were performed with the SKETCHER module of SYBYL8.0.3 with optimisation by MAXIMIN2 using the MMFF94s forcefield with MMFF94 charges and a distance dependent dielectric function with a dielectric constant of 80, and converged to 0.05 Kcal/mol * Å. Ligands were docked into an 18 Å cavity centred on Ile800 using GOLD[30] (v5.0.1) with search efficiency set at 200%. The Chemscore scoring function with kinase specific modification was used, and 20 dockings were performed with all poses kept. All ligand flexibility options were turned on with the exception of internal hydrogen bonds, the flip option was selected when available, and the solvate all option was also turned off as well. Poses were then mimimised and rescored in GOLD using the same scoring function.

4.4. Cellular assay

The early passage cell lines used in this study were developed in this laboratory and cultured as previously described.[31] Cell lines were grown in α-modified minimal essential growth medium supplemented insulin, transferrin, selenite and 5% foetal bovine serum. Individual wells of 96-well tissue culture plates contained 1000 cells in a volume of 150 μL. Drugs were added at 10-fold concentration steps to a maximum of 20 μM and plates were incubated under an atmosphere of 5% O_2, 5% CO_2 and 90% N_2 for five days, with ^{3}H-thymidine (0.04 μCi per well) being added over the last 6 h. Cells were harvested and the incorporated radioactivity was measured. Duplicate samples were analysed for each drug dose with multiple control samples and data were fitted to a least-squares regression of the form $y = y_0 + ae^{-bx}$, where y is the incorporated radioactivity, x is the drug concentration and y_0, a and b are variables. The IC_{50} value was defined as the drug concentration reducing ^{3}H-thymidine incorporation by 50%.

4.5. Phospho-PKB assay

HCT-116 cells were grown in MEM alpha supplemented with 10% (v/v) foetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin (all from Invitrogen). For inhibition studies, cells were seeded in 12-well plates and grown for 1 day before overnight starvation in serum-free media. Cells were then exposed to varying concentrations of inhibitor dissolved in DMSO (final concentration of DMSO in media 0.1%) for 15 min before stimulation with 500 nM insulin for 5 min. Protein isolation and immunoblotting for phospho-PKB was carried out according to the methods previously described, with antibodies from Cell Signaling Technology (Ser473 catalogue# 9271, Thr308 catalogue# 9275).[26]

4.6. Xenograft studies

Age-matched specific pathogen-free female Rag1$^{-/-}$ mice were subcutaneously inoculated with 5×10^6 HCT-116 cells in *phosphate buffered saline*. Tumour volume (mm^3) was calculated using the formula $(L \times w^2) \times \pi/6$ (where; L = longest tumour diameter and w = perpendicular diameter). Dosing began when tumours reached a volume of approximately 150 mm^3. Compound **5x** and PIK-75 were administered in solution in 7.5% DMSO, 42.5% PEG-400, 20% 2-hydroxypropyl-β-cyclodextrin, 30% water by ip injection at 20 mg/kg q.d. for 14 days. Control animals were administered the control vehicle alone. Animal bodyweight was measured daily, and tumour volume measured three times per week. Mice were culled if bodyweight loss exceeded 20% of starting weight. All animal experiments followed protocols approved by the Animal Ethics Committee of The University of Auckland. The statistical significance of mean tumour volume at study completion was determined by 1-way ANOVA with Holm-Sidak multiple comparison analysis using SigmaPlot 11.0.

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