Bioorganic & Medicinal Chemistry 20 (2012) 58–68

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel pyrazolo[1,5-*a*]pyridines as p110α-selective PI3 kinase inhibitors: Exploring the benzenesulfonohydrazide SAR

Jackie D. Kendall [a,b,*], Anna C. Giddens [a], Kit Yee Tsang [a,†], Raphaël Frédérick [a,‡], Elaine S. Marshall [a], Ripudaman Singh [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

ARTICLE INFO

Article history:
Received 31 August 2011
Revised 9 November 2011
Accepted 16 November 2011
Available online 25 November 2011

Keywords:
PI3 kinase
PI3K
PIK3CA
p110α
Pyrazolo[1,5-*a*]pyridine
Sulfonohydrazide

ABSTRACT

Structure–activity relationship studies of the pyrazolo[1,5-*a*]pyridine class of PI3 kinase inhibitors show that substitution off the hydrazone nitrogen and replacement of the sulfonyl both gave a loss of p110α selectivity, with the exception of an *N*-hydroxyethyl analogue. Limited substitutions were tolerated around the phenyl ring; in particular the 2,5-substitution pattern was important for PI3 kinase activity. The *N*-hydroxyethyl compound also showed good inhibition of cell proliferation and inhibition of phosphorylation of Akt/PKB, a downstream marker of PI3 kinase activity. It had suitable pharmacokinetics for evaluation in vivo, and showed tumour growth inhibition in two human tumour cell lines in xenograft studies. This work has provided suggestions for the design of more soluble analogues.

1. Introduction

Phosphoinositide-3-kinases (PI3Ks) 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] *PIK3CA*, the gene encoding for the p110α isoform, is often over-expressed and mutated in many cancer types.[2,3]

Inhibition of PI3 kinase, and in particular, selective inhibition 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 only a handful of 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 molecule PI3 kinase inhibitors currently going through clinical trials,[9,10] however few of these are selective for p110α.

In our earlier work, we found that pyrazolo[1,5-*a*]pyridine sulfonohydrazides provided a potent template for p110α selective PI3 kinase inhibitors.[11] We investigated the structure–activity relationship (SAR) around the pyrazolo[1,5-*a*]pyridine ring and found that only the 5-position was tolerant of substitution. Furthermore, only small substituents were tolerated with 5-cyano (**1**, Table 1) the most active of the analogues made. Although **1** showed good

Abbreviations: ATP, adenosine triphosphate; AUC_{inf}, area under the curve to time infinity; C_{max}, maximum concentration; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; i.p., intraperitoneal; MEM, minimum essential medium; MTD, maximum tolerated dose; PDB, protein data bank; PEG-400, polyethylene glycol with molecular weight 380–420 g mol^{-1}; PH domain, pleckstrin homology domain; PIP2, phosphatidylinositol 4,5-diphosphate; PIP3, phosphatidylinositol 3,4,5-triphosphate; PI3 kinase, phosphoinositide-3-kinase; PKB, protein kinase B; q.d., daily; SAR, structure–activity relationships; $t_{1/2}$, biological half-life; TLC, thin layer chromatography; Tris, tris(hydroxymethyl)aminomethane.

* Corresponding author.
E-mail address: j.kendall@auckland.ac.nz (J.D. Kendall).
† Present address: Department of Chemistry, University of Hong Kong, Pokfulam Road, Hong Kong.
‡ 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.

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.11.031

in vitro activity, as demonstrated by its inhibition of phosphorylation of PKB, a downstream marker of PI3 kinase activity, it had only modest in vivo activity in a human tumour xenograft model. This was attributed to its poor aqueous solubility limiting the dose level that could be administered. In this study, we investigate further the SAR around **1** to understand better the mechanisms which control potency and selectivity, and to identify potential ways of preparing more soluble analogues.

2. Results and discussion

2.1. Synthesis

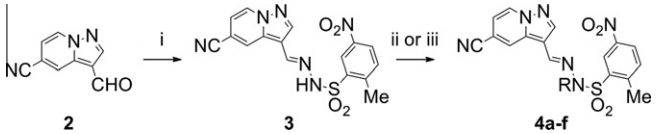
All the hydrazides were made starting from aldehyde **2**.[11] Condensation of **2** with 2-methyl-5-nitrobenzenesulfonohydrazide[12] gave sulfonohydrazide **3** (Scheme 1). N-alkylation was then carried out with either NaH and iodoethane or benzyl bromide to form compounds **4a**–**b**, or with an appropriate bromoalkyl amine and Cs_2CO_3 to form **4c**–**f** (Table 1).

Hydroxyethyl compound **5** was made from **2** by condensation with 2-hydroxyethylhydrazine followed by sulfonylation (Scheme 2). Sulfonohydrazides **6a**–**f**, **h**–**w** with a range of substituents around the phenyl ring (Table 2) were then made from **2** by condensation with methylhydrazine sulfate followed by sulfonylation with the appropriate sulfonyl chloride in a one-pot procedure. This reaction was carried out using either $NaHCO_3$ or 2,6-lutidine. The use of $NaHCO_3$ necessitated an aqueous work-up to remove inorganic impurities, whereas we later found that the procedure could be simplified by using 2,6-lutidine where the product precipitated out of the reaction mixture and could be filtered off without usually requiring further purification. Amino compound **6g** was prepared by cleaving the trifluoroacetamide group of **6f** with Cs_2CO_3. Acyl hydrazides **6x**,**y** (Table 2) were made similarly but with using an acyl chloride instead of a sulfonyl chloride.

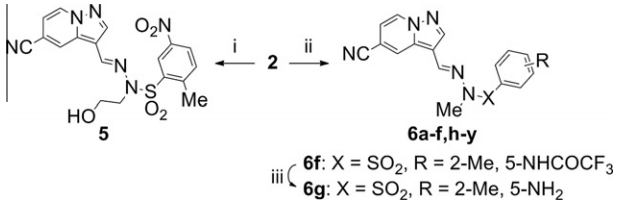
Finally, hydrazone **9** was made by reaction of benzylic chloride **7** with methylhydrazine using the method of Butler et al.,[13] followed by condensation with aldehyde **2** (Scheme 3).

2.2. In vitro biology

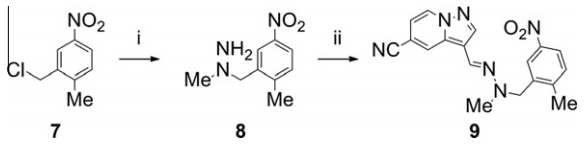
In our previous paper, we found that **1** was a potent and selective inhibitor of the p110α isoform of PI3 kinase.[11] This encouraged us to look further into the SAR around these compounds, in particular around the hydrazone and aryl sulfonyl moieties.



Scheme 1. Reagents and conditions: (i) 2-methyl-5-nitrobenzenesulfonohydrazide, MeOH; (ii) NaH, RX, DMF; (iii) $Br(CH_2)_2NR_2{\cdot}HBr$, Cs_2CO_3, DMF.

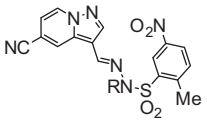


Scheme 2. Reagents and conditions: (i) $H_2N(CH_2)_2OH$, MeOH then 2,6-lutidine, 2-methyl-5-nitrobenzenesulfonyl chloride; (ii) $MeHNNH_2{\cdot}H_2SO_4$, $NaHCO_3$ or 2,6-lutidine, MeOH then sulfonyl chloride or acyl chloride; (iii) Cs_2CO_3, MeOH, H_2O.



Scheme 3. Reagents and conditions: (i) $MeHNNH_2$, EtOH; (ii) **2**, MeOH.

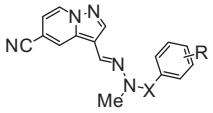
Table 1
Inhibition of PI3 kinase isoforms and cell proliferation for **1**, **4a**–**f** and **5**



Cmpd	R	IC_{50} (nM)[a]			IC_{50} (μM)[a]	
		p110α	p110β	p110δ	NZB5	NZOV9
1	Me	0.9	46	49	0.06	1.4
4a	Et	0.5	62	9.3	0.03	0.09
4b	CH_2Ph	27	>1000	32	n.d.	2.5
4c	$(CH_2)_2NMe_2$	77	490	180	0.36	0.39
4d	$(CH_2)_2NEt_2$	82	>1000	110	3.5	1.7
4e	$(CH_2)_2$N(morpholine)	4.5	230	10	0.50	0.54
4f	$(CH_2)_2$N(piperidine)	140	>1000	89	1.1	0.49
5	$(CH_2)_2OH$	1.3	51	54	0.06	0.14

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

Table 2
Inhibition of PI3 kinase isoforms and cell proliferation for **6a**–**y** and **9**



Cmpd	X	R	IC_{50} (nM)[a]			IC_{50} (μM)[a]	
			p110α	p110β	p110δ	NZB5	NZOV9
6a	SO_2	2-Me	700	>1000	2000	>20	12
6b	SO_2	2-Me, 3-NO_2	>1000	>1000	>1000	>20	>20
6c	SO_2	2-Me, 4-NO_2	65	>1000	>1000	>20	>20
6d	SO_2	2-Me, 6-NO_2	14	340	100	3.7	7.6
6e	SO_2	2-Me, 5-NHCOMe	>1000	>1000	>1000	>20	>20
6f	SO_2	2-Me, 5-$NHCOCF_3$	>1000	>1000	>1000	2.3	9.1
6g	SO_2	2-Me, 5-NH_2	>1000	>1000	>1000	n.d.	n.d.
6h	SO_2	2-Me, 5-CO_2H	>1000	>1000	>1000	>20	>20
6i	SO_2	2-Me, 5-CO_2Me	990	>1000	400	2.4	1.9
6j	SO_2	2-Me, 5-CN	2.4	130	59	0.28	0.21
6k	SO_2	2-Me, 5-SO_2Me	350	830	200	0.50	0.62
6l	SO_2	2-Me, 5-CF_3	21	60	35	0.98	1.3
6m	SO_2	2,5-Me_2	100	>1000	>1000	2.1	0.88
6n	SO_2	2-Me, 5-F	55	550	310	>20	14
6o	SO_2	2-Me, 5-Cl	13	360	230	0.67	0.54
6p	SO_2	2-Me, 5-Br	13	620	180	0.70	0.57
6q	SO_2	3-NO_2[b]	36	1200	410	0.38	0.69
6r	SO_2	2-Et, 5-NO_2	1.5	280	13	0.17	0.08
6s	SO_2	2-$CHMe_2$, 5-NO_2	1.8	300	39	0.18	0.26
6t	SO_2	2-F, 5-NO_2	4.7	44	120	0.48	0.40
6u	SO_2	2-Cl, 5-NO_2	0.5	64	24	0.88	0.35
6v	SO_2	2-OMe, 5-NO_2	0.8	63	22	0.09	0.05
6w	SO_2	2-NMe_2, 5-NO_2	2.2	44	24	0.04	0.10
6x	CO	2-Me, 5-NO_2	17	68	8.6	>20	>20
6y	CO	3-NO_2[b]	11	67	16	1.8	6.0
9	CH_2	2-Me, 5-NO_2	40	79	37	1.7	1.9

[a] All IC_{50} values are the mean of duplicate or triplicate measurements.
[b] 3-NO_2 is the equivalent of 5-NO_2 when the phenyl ring does not bear another substituent.

Firstly we looked at the hydrazone nitrogen substituent (Table 1). We showed previously that a substituent on this nitrogen was essential for good activity.[11] Replacement of the methyl with ethyl **4a** gave similar activity for p110α, but the selectivity over p110δ dropped from 50-fold for **1** to only 20-fold. The larger benzyl **4b** and aminoalkyl **4c**–**f** substituents carried on this trend with a decrease in p110δ selectivity, and additionally they also gave a drop in p110α activity. Morpholine **4e** was the most active of these compounds with IC_{50} 4.5 nM for p110α; this is discussed further in Section 2.3. Hydroxyethyl **5** however, retained excellent p110α potency and selectivity.

We next investigated the aryl nitro group (Table 2). Removal of the nitro group (**6a**) gave a large loss of p110α activity compared with **1**, as did moving it to the 3-position (**6b**). 4-Nitro compound **6c** was 70-fold less potent than **1**, and 6-nitro compound **6d** was only 15-fold less potent. Several nitro group replacements were also investigated at the 5-position. Acetamide **6e**, trifluoroacetamide **6f** and amino **6g** all gave a large loss of activity, as did carboxylic acid **6h** and methyl ester **6i**. However, nitrile **6j** only lost twofold activity for p110α whilst retaining good selectivity over p110β and p110δ. In contrast, while methyl sulfone **6k** and trifluoromethyl **6l** retained some (albeit less) p110α activity, they lost virtually all selectivity against p110δ. Methyl **6m** and halo substituents **6n**–**p** showed moderate potency and selectivity for p110α.

The 2-position of the aryl ring was much more tolerant of substituent size. A substituent here was required, as evidenced by its removal in compound **6q** with a 40-fold loss of p110α activity. Such a large loss of potency suggests that this methyl group contributes to the relative orientation of the phenyl ring and hence location of the nitro group. Other small substituents (**6r**–**w**) were all well-tolerated with negligible loss of potency or selectivity.

Finally, replacement of the sulfonyl group (**6x**, **6y**, **9**) gave a slight decrease in p110α potency, but had negligible selectivity over p110δ. Interestingly when the sulfonyl group was replaced by carbonyl (**6x**, **y**), the aryl methyl group was now not necessary for retaining p110α potency since both **6x** and **6y** had similar activity against p110α. Methylene-linked compound **9** was slightly less potent for p110α. This suggests that the sulfonyl group is important for p110α selectivity, but that the aryl nitro and the substituents on the pyrazolo[1,5-*a*]pyridine ring are more important for overall PI3 kinase potency.

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 good inhibitors of cell proliferation, and in addition, inhibition in the two cell lines tracked reasonably well with all values being within fourfold of each other. Notably, two of the compounds where the nitro group was moved around the aryl ring (**6c**,**d**), and the two acylhydrazides (**6x**,**y**) were rather less active at inhibiting cell proliferation than they were for p110α. This suggests that they may not have good cell penetration.

2.3. Molecular modelling

Compounds with large hydrazone nitrogen substitutions such as those in Table 1 were not expected to be potent PI3 kinase inhibitors based on our model of compound **1** bound to p110α,[11] where the substituted hydrazone was adjacent to the side chains of Gln859 and Met772. Using compound **4e** as an example of good potency and reduced selectivity between the p110α and p110δ isoforms, a binding mode was predicted in the apo p110α structure where the pyrazolo[1,5-*a*]pyridine core was buried in the ATP-binding site (Fig. 1). This identified potential interactions between the heterocyclic core and the backbone amide NH group of Val851, and its cyano group with the sidechain of Tyr836. The aryl nitro group may also interact with either the p110α specific Arg770 or Ser774 on the N-terminal lobe wall, or even the side chain hydroxyl groups of Thr856 and Ser919 on the C-terminal lobe wall. Maintaining an interaction between the aryl nitro group and the active site is clearly important for potency as demonstrated by the data for compound **6a**. The absence of an interaction between the sulfonyl moiety and the p110α specific Gln859, as observed in compound **1**,[11] provides some support for the observed loss in isoform selectivity for **4e** and related compounds, and is consistent with reduced selectivity in absence of the sulfonyl group (compound **9**). The predicted binding mode with the highest Chemscore value after rescoring and minimisation had the hydrazone morpholine side chain directed toward the basic residue Lys802 at the catalytic centre (Fig. 1). In this orientation, the morpholine oxygen may interact with this residue, while the more basic side chains of **4c**, **4d** and **4f** are less preferred. This interaction along with the lower pK_a of morpholine may provide some basis for the difference in activity between **4e** and **4f**.

2.4. Aqueous solubility

Compounds **4a**, **5**, **6v** and **6w** were selected for further investigation since they exhibited the best potency for PI3 kinase p110α, selectivity over p110β and p110δ, and inhibition of cell proliferation in the two cell lines tested. Measurement of aqueous solubility showed that **5** was the most soluble and was 12-fold more soluble than **1** (Table 3). Thus **5** was selected for further investigation.

2.5. Further in vitro screening of 5

Compound **5** was then screened against additional kinases (Table 4). Compound **5** had similar potency against the two most common p110α mutations (E545K and H1047R) to that of wild-type

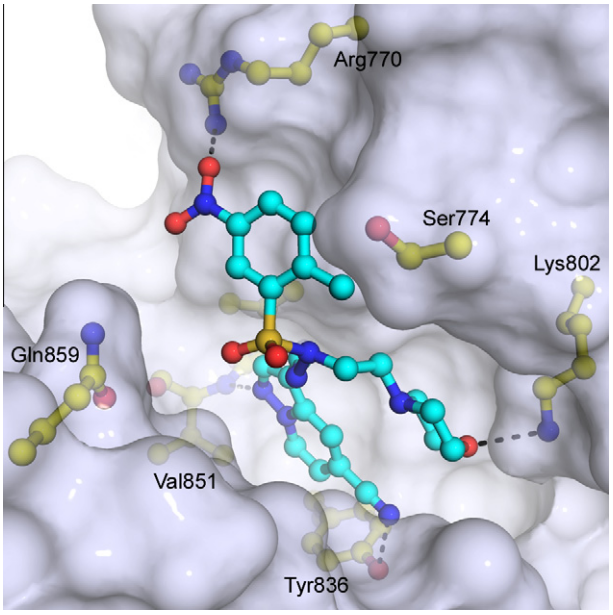


Figure 1. Predicted binding mode for **4e** (cyan ball and stick) in the p110α ATP-binding site. Amino acids likely to interact with the inhibitor are represented as yellow ball and stick with possible interactions illustrated by black dashed lines. (Image generated with PyMol).

Table 3
Aqueous solubility of selected compounds

Compound	Solubility (μg/mL)
1	0.1
4a	0.2
5	1.2
6v	0.2
6w	0.5

Table 4
Inhibition of PI3 kinase p110α mutations, p110γ, and other selected kinases by **5**

Kinase	% Activity remaining	
	1 μM	10 μM
p110α E545K	(0.8 nM)[a]	
p110α H1047R	(0.8 nM)[a]	
p110γ	(11 nM)[a]	
DNA-PK[b]	(0.3 nM)[a]	
mTOR[b]	(234 nM)[a]	
MKK1[c]	65 ± 8	5 ± 1
p38γ MAPK[c]	59 ± 6	13 ± 1
RSK1[c]	18 ± 2	5 ± 2
TrkA[c]	73 ± 2	15 ± 2
PKA[c]	59 ± 9	8 ± 1
IR[c]	76 ± 2	14 ± 2
MINK1[c]	16 ± 6	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).

p110α, and eightfold 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).[14] Compound **5** is a potent inhibitor of DNA-PK but has 200-fold selectivity over mTOR. Previous lead compound **1** showed activity against a selection of kinases at 1 and 10 μM,[11] hence **5** was screened against these same kinases at two concentrations (Table 4). Compound **5** showed weaker activity for all seven kinases than **1** at 1 μM, and furthermore inhibited only two of these at >50% at this concentration: RSK1 (p90 ribosomal S6 kinase 1) and MINK1 (misshapen-like kinase 1). Compound **5** has less off-target kinase activity than **1**.

The effect of **5** on a downstream marker of PI3 kinase, the phosphorylation of PKB, was also assessed in HCT-116 cells with the H1047R mutation in p110α (Fig. 2). Compound **5** showed good inhibition of phosphorylation at both Ser473 and Thr308 sites with IC_{50}s of 82 and 49 nM, respectively.

2.6. Pharmacokinetics

The pharmacokinetics of compound **5** was then assessed in CD-1 mice. It was administered as a single dose at 10 mg/kg by i.p. injection. The plasma concentration peaked at 5 min with a C_{max} of 7575 nM. Despite a relatively short terminal $t_{1/2}$ of 1.13 h, plasma AUC_{inf} was 3163 nM h.

2.7. In vivo antitumour efficacy

Compound **5** was evaluated alongside compound **1** in an HCT-116 human tumour xenograft model. Both compounds were dosed at MTD (**5**: 40 mg/kg, **1**: 20 mg/kg) daily by i.p. injection. Compound **5** demonstrated antitumour activity, causing a tumour growth delay on day 7 of 43.8 ± 5.5% (P <0.001) relative to controls and 37.0 ± 6.2% (P <0.005) relative to **1** (Fig. 3A). However, its treatment was associated with toxicity in 1/7 mice and moderate bodyweight loss in the remaining mice requiring treatment discontinuation on day 7, when mean bodyweight loss exceeded 15% (Fig. 3B). Dosing was also suspended early due to bodyweight loss for compound **1**, prior to its final tumour measurement on day 10, when it had a tumour growth delay of 21.2 ± 4.9% (P <0.01) versus controls. The improved solubility of **5** over **1** was evident by the presence of drug precipitation in the i.p. cavity on post-mortem examination in mice treated with **1**, but not in mice dosed with **5**.

An alternative human tumour xenograft model, SK-OV-3, featuring the same H1047R mutation in p110α that is present in HCT-116 cells, was used to test **5** at a reduced dose level (30 mg/kg) i.p. daily for 14 days (Fig. 3C and D). At the completion of dosing, **5** demonstrated tumour growth inhibition of 49.9 ± 9.1% (P <0.05) relative to controls. Compound **5** was tolerated throughout the 14-day treatment duration, except in one mouse which reached 20% bodyweight loss.

3. Conclusions

This investigation of the SAR around the pyrazolo[1,5-*a*]pyridine PI3 kinase inhibitors shows that substitution off the hydrazone nitrogen and replacement of the sulfonyl both reduced the p110α selectivity, with the exception of hydroxyethyl compound **5**. Limited substitutions were tolerated around the phenyl ring, and in particular the 2,5-substitution pattern was important for PI3 kinase activity. Compound **5** also showed good inhibition of cell proliferation and inhibition of phosphorylation of PKB, a downstream marker of PI3 kinase activity. It had suitable aqueous solubility and pharmacokinetics for evaluation in vivo, and showed tumour growth inhibition in two human xenograft models.

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

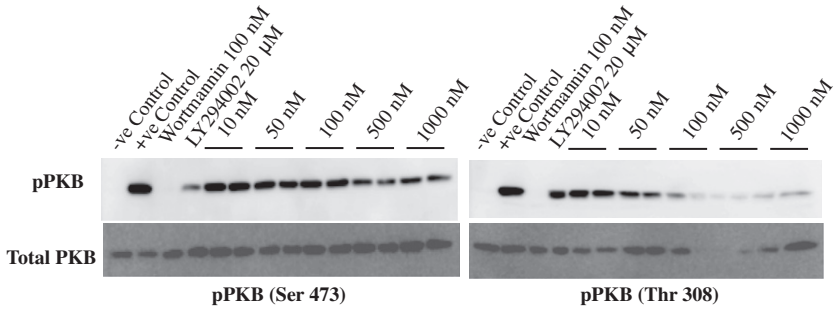


Figure 2. The effect of **5** on the phosphorylation of PKB in HCT-116 cells at Ser473 (IC_{50} 82 nM) and Thr308 (IC_{50} 49 nM).

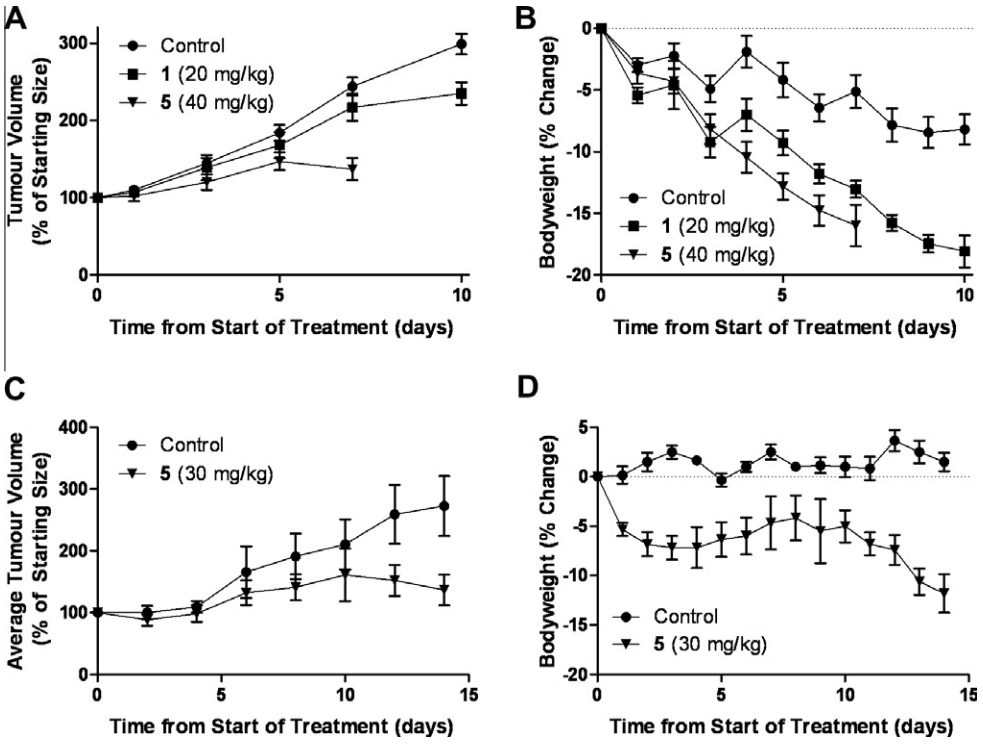


Figure 3. In vivo antitumour efficacy (A, C) and bodyweight change (B, D) of **1** and **5** in mice with HCT-116 tumours (n = 6–7) (A, B), or **5** in mice with SK-OV-3 tumours (n = 5–6) (C, D). All treatments were administered daily by i.p. injection.

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 ⩾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. Compounds **2** and **3** were prepared as described earlier.[11]

4.1.1. Synthesis of *N*-substituted sulfonohydrazides 4a–f

Unless otherwise stated, N-substituted sulfonohydrazides were made by the following method. A suspension of *N′*-((5-cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-2-methyl-5-nitrobenzenesulfonohydrazide (**3**) (20 mg, 0.052 mmol), $Br(CH_2)_2NR_2{\cdot}HBr$ (2 equiv) and Cs_2CO_3 (5 equiv) in DMF (3 mL) was stirred at room temperature for 2 h. The solution was diluted with water, extracted with CH_2Cl_2, the extracts were dried (Na_2SO_4) and the solvents removed in vacuo. Chromatography (eluting with a CH_2Cl_2:MeOH gradient) gave the substituted hydrazide.

4.1.1.1. *N′*-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*-ethyl-2-methyl-5-nitrobenzenesulfonohydrazide (4a). NaH (6.9 mg, 60% in oil, 0.17 mmol) was added to a solution of **3** (60 mg, 0.16 mmol) in dry DMF (5 mL) at room temperature. After 1 h, iodoethane (25 mg, 0.16 mmol) in DMF (0.5 mL) was added. After a further 2 h, the reaction mixture was diluted with water and extracted twice with EtOAc. The combined extracts were washed twice with water then with brine, dried (Na_2SO_4), and the solvent removed in vacuo. Chromatography (eluting with hexanes:EtOAc 2:1 to 1:1) gave **4a** as a yellow solid (23 mg, 36%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.85 (d, J = 2.4 Hz, 1H), 8.55 (dd, J = 7.2, 0.9 Hz, 1H), 8.36 (dd, J = 8.4, 2.4 Hz, 1H), 8.23 (s, 1H), 8.21 (s, 1H), 8.13 (dd, J = 1.8, 0.9 Hz, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.01 (dd, J = 7.2, 1.8 Hz, 1H), 3.89 (q, J = 7.1 Hz, 2H), 2.76 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H). LC–MS (APCI$^+$) 413 (MH^+, 100%). Anal. Calcd for $C_{18}H_{16}N_6O_4S$: C, 52.42; H, 3.91; N, 20.38. Found C, 52.66; H, 4.08; N, 20.10.

4.1.1.2. *N*-Benzyl-*N′*-((5-cyanopyrazolo[1,5-*a*]pyridin-3-yl)-methylene)-2-methyl-5-nitrobenzenesulfonohydrazide (4b). NaH (6.9 mg, 60% in oil, 0.17 mmol) was added to a solution of **3** (60 mg, 0.16 mmol) in dry DMF (5 mL) at room temperature. After 1 h, benzyl bromide (27 mg, 0.16 mmol) in DMF (0.5 mL) was added. After a further 1 h, the reaction mixture was diluted with water and extracted twice with EtOAc. The combined extracts were washed twice with water then with brine, dried (Na_2SO_4), and the solvent removed in vacuo. Chromatography (eluting with hexanes:EtOAc 4:1 to 2:1) gave **4b** as a yellow solid (36 mg, 49%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.92 (d, J = 2.4 Hz, 1H), 8.49 (dd, J = 7.2, 0.9 Hz, 1H), 8.38 (dd, J = 8.4, 2.4 Hz, 1H), 8.07 (dd, J = 1.8, 0.9 Hz, 1H), 8.04 (s, 1H), 7.89 (s, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.38 (m, 4H), 7.31 (m, 1H), 6.97 (dd, J = 7.2, 1.8 Hz, 1H), 5.09 (s, 2H), 2.84 (s, 3H). LC–MS (APCI$^+$) 475 (MH^+, 100%). Anal. Calcd for $C_{23}H_{18}N_6O_4S$: C, 58.22; H, 3.82; N, 17.71. Found C, 58.15; H, 3.97; N, 17.45.

4.1.1.3. *N′*-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*-(2-(dimethylamino)ethyl)-2-methyl-5-nitrobenzenesulfonohydrazide (4c). Reaction of **3** (40 mg, 0.10 mmol) and 2-bromo-*N*,*N*-dimethylethylamine hydrobromide (36 mg, 0.15 mmol), after chromatography (eluting with CH_2Cl_2:MeOH 99:1 to 98:2 to 97:3) gave **4c** as a yellow solid (22 mg, 47%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.88 (d, J = 2.4 Hz, 1H), 8.54 (dd, J = 7.2, 1.0 Hz, 1H), 8.35 (dd, J = 8.4, 2.4 Hz, 1H), 8.33 (s, 1H), 8.23 (s, 1H),

8.12 (dd, J = 1.9, 1.0 Hz, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.00 (dd, J = 7.2, 1.9 Hz, 1H), 3.92 (t, J = 6.8 Hz, 2H), 2.78 (s, 3H), 2.61 (t, J = 6.8 Hz, 2H), 2.33 (s, 6H). LC–MS (APCI$^+$) 456 (MH$^+$, 100%). Anal. Calcd for $C_{20}H_{21}N_7O_4S$.0.67 MeOH: C, 52.06; H, 5.00; N, 20.57. Found C, 51.99; H, 4.76; N, 20.45.

4.1.1.4. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*-(2-(diethylamino)ethyl)-2-methyl-5-nitrobenzenesulfonohydrazide (4d).

Reaction of **3** (20 mg, 0.052 mmol) and 2-bromo-*N*,*N*-diethylethylamine hydrobromide (27 mg, 0.10 mmol), after chromatography (eluting with CH_2Cl_2:MeOH 99:1 to 98:2) gave **4d** as a yellow solid (10 mg, 40%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.88 (d, J = 2.4 Hz, 1H), 8.53 (dd, J = 7.2, 1.0 Hz, 1H), 8.38 (s, 1H), 8.35 (dd, J = 8.4, 2.4 Hz, 1H), 8.21 (s, 1H), 8.10 (s, 1H), 7.56 (d, J = 8.4 Hz, 1H), 6.99 (dd, J = 7.2, 1.8 Hz, 1H), 3.93 (m, 2H), 2.78 (m, 5H), 2.64 (m, 4H), 1.07 (m, 6H). LC–MS (APCI$^+$) 484 (MH$^+$, 100%). Anal. Calcd for $C_{22}H_{25}N_7O_4S$.0.5 MeOH: C, 53.99; H, 5.49; N, 19.50. Found C, 54.27; H, 5.51; N, 19.26.

4.1.1.5. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-2-methyl-*N*-(2-morpholinoethyl)-5-nitrobenzenesulfonohydrazide (4e).

Reaction of **3** (40 mg, 0.10 mmol) and 4-(2-bromoethyl)morpholine hydrobromide (43 mg, 0.16 mmol), after chromatography (eluting with CH_2Cl_2:MeOH 99:1 to 98:2) gave **4e** as a yellow solid (41 mg, 79%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.85 (d, J = 2.4 Hz, 1H), 8.55 (dd, J = 7.2, 1.0 Hz, 1H), 8.39–8.33 (m, 2H), 8.23 (s, 1H), 8.10 (dd, J = 1.8, 1.0 Hz, 1H), 7.57 (d, J = 8.5 Hz, 1H), 7.01 (dd, J = 7.2, 1.8 Hz, 1H), 3.93 (t, J = 6.6 Hz, 2H), 3.70 (m, 4H), 2.78 (s, 3H), 2.67 (t, J = 6.6 Hz, 2H), 2.55 (m, 4H). LC–MS (APCI$^+$) 498 (MH$^+$, 100%). Anal. Calcd for $C_{22}H_{23}N_7O_5S$.0.25 H_2O: C, 52.63; H, 4.72; N, 19.53. Found C, 52.61; H, 4.71; N, 19.24.

4.1.1.6. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-2-methyl-5-nitro-*N*-(2-(piperidin-1-yl)ethyl)benzenesulfonohydrazide (4f).

Reaction of **3** (40 mg, 0.10 mmol) and 1-(2-bromoethyl)piperidine hydrobromide (43 mg, 0.16 mmol), after chromatography (eluting with CH_2Cl_2:MeOH 99:1 to 98:2) gave **4f** as a yellow solid (43 mg, 83%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.86 (d, J = 2.4 Hz, 1H), 8.53 (dd, J = 7.2, 0.9 Hz, 1H), 8.39–8.33 (m, 2H), 8.21 (s, 1H), 8.08 (m, 1H), 7.56 (d, J = 8.4 Hz, 1H), 6.99 (dd, J = 7.2, 1.9 Hz, 1H), 3.96 (t, J = 6.5 Hz, 2H), 2.78 (s, 3H), 2.64 (t, J = 6.5 Hz, 2H), 2.48 (m, 4H), 1.59 (m, 4H), 1.46 (m, 2H). LC–MS (APCI$^+$) 496 (MH$^+$, 100%). Anal. Calcd for $C_{23}H_{25}N_7O_4S$: C, 55.74; H, 5.08; N, 19.79. Found C, 56.13; H, 5.39; N, 19.56.

4.1.2. Synthesis of *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)-methylene)-*N*-(2-hydroxyethyl)-2-methyl-5-nitrobenzenesulfonohydrazide (5)

2-Hydroxyethylhydrazine (134 mg, 1.76 mmol) was added to a solution of aldehyde **2** (200 mg, 1.17 mmol) in MeOH (30 mL). After 1 h, 2,6-lutidine (0.27 mL, 2.3 mmol) and 2-methyl-5-nitrobenzenesulfonyl chloride (413 mg, 1.75 mmol) were added and the reaction mixture stirred for a further 1.5 h. The precipitated product was filtered off, washed with a little MeOH and dried to leave **5** as a yellow solid (302 mg, 60%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.96 (dd, J = 7.2, 1.0 Hz, 1H), 8.73 (d, J = 2.5 Hz, 1H), 8.45–8.38 (m, 3H), 8.08 (dd, J = 1.9, 1.0 Hz, 1H), 7.77 (d, J = 8.4 Hz, 1H), 7.32 (dd, J = 7.2, 1.9 Hz, 1H), 5.05 (t, J = 5.8 Hz, 1H), 3.99 (t, J = 5.8 Hz, 2H), 3.68 (q, J = 5.8 Hz, 2H), 2.69 (s, 3H). LC–MS (APCI$^+$) 429 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{16}N_6O_5S$: C, 50.46; H, 3.76; N, 19.62. Found C, 50.09; H, 3.86; N, 19.27.

4.1.3. Synthesis of hydrazides 6a–y

These were made using $NaHCO_3$ or 2,6-lutidine as detailed below, unless otherwise stated. Methylhydrazine sulfate (1.2 equiv) and $NaHCO_3$ (5 equiv) were added to a solution of 3-formylpyrazolo[1,5-*a*]pyridine-5-carbonitrile (**2**) (1 equiv) in MeOH (5 mL). After all of the aldehyde was consumed, sulfonyl chloride or acyl 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 or trituration then afforded the hydrazides. Alternatively, methylhydrazine sulfate (1.2 equiv) and 2,6-lutidine (5 equiv) were added to a solution of **2** (1 equiv) in MeOH (5 mL). After all of the aldehyde was consumed, sulfonyl chloride or acyl chloride (1.3 equiv) was added and the reaction mixture stirred for a further 30 min. The hydrazide was then filtered off, washed with MeOH and dried.

4.1.3.1. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethylbenzenesulfonohydrazide (6a).

Reaction of **2** (30 mg, 0.18 mmol) and 2-methylbenzenesulfonyl chloride (51 μL, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with hexanes:EtOAc 3:1 to 2:1 to 1:1) gave **6a** as a yellow solid (50 mg, 81%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.46 (dd, J = 7.2, 0.9 Hz, 1H), 8.24 (m, 1H), 8.12 (s, 1H), 7.86 (dd, J = 1.8, 0.9 Hz, 1H), 7.76 (s, 1H), 7.57–7.62 (m, 2H), 7.34 (m, 1H), 6.92 (dd, J = 7.2, 1.8 Hz, 1H), 3.45 (s, 3H), 2.60 (s, 3H). LC–MS (APCI$^+$) 354 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{15}N_5O_2S$: C, 57.78; H, 4.28; N, 19.82. Found C, 57.89; H, 4.29; N, 20.02.

4.1.3.2. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-3-nitrobenzenesulfonohydrazide (6b).

Reaction of **2** (30 mg, 0.18 mmol) and 2-methyl-3-nitrobenzenesulfonyl chloride (83 mg, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with hexanes:EtOAc 3:1 to 2:1 to 1:1) gave **6b** as a yellow solid (42 mg, 60%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.96 (dd, J = 7.2, 1.0 Hz, 1H), 8.41 (s, 1H), 8.36 (dd, J = 8.0, 1.1 Hz, 1H), 8.22 - 8.15 (m, 2H), 8.13 (dd, J = 1.9, 1.0 Hz, 1H), 7.76 (t, J = 8.0 Hz, 1H), 7.32 (dd, J = 7.2, 1.9 Hz, 1H), 3.41 (s, 3H), 2.61 (s, 3H). LC–MS (APCI$^+$) 399 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{14}N_6O_4S$.0.1 hexanes: C, 51.82; H, 3.78; N, 20.72. Found C, 52.08; H, 3.71; N, 21.05.

4.1.3.3. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-4-nitrobenzenesulfonohydrazide (6c).

Reaction of **2** (30 mg, 0.18 mmol) and 2-methyl-4-nitrobenzenesulfonyl chloride[15] (92 mg, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with CH_2Cl_2:MeOH 99.5:0.5) gave **6c** as a yellow solid (41 mg, 59%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.95 (dd, J = 7.2, 1.0 Hz, 1H), 8.40 (s, 1H), 8.32 (s, 1H), 8.27 (m, 2H), 8.17 (s, 1H), 8.13 (dd, J = 1.9, 1.0 Hz, 1H), 7.31 (dd, J = 7.2, 1.9 Hz, 1H), 3.41 (s, 3H), 2.71 (s, 3H). LC–MS (APCI$^+$) 399 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{14}N_6O_4S$: C, 51.24; H, 3.54; N, 21.09. Found C, 50.95; H, 3.55; N, 21.10.

4.1.3.4. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-6-nitrobenzenesulfonohydrazide (6d).

2-Methyl-6-nitroaniline (1.01 g, 6.62 mmol) was suspended in concentrated HCl (5 mL) at 0 °C. A solution of $NaNO_2$ (690 mg, 10 mmol) in water (2 mL) was added dropwise over 5 min, and the solution stirred for 45 min. Meanwhile, AcOH (6 mL) was saturated with SO_2, then $CuCl_2{\cdot}2H_2O$ (338 mg, 1.98 mmol) was added and SO_2 bubbled through for a further 10 min. The AcOH mixture was cooled to 5 °C, then the diazonium solution added over 5 min. The resulting mixture was stirred for a further 2 h at 0 °C then 1 h at room temperature. The solution was diluted with water and extracted four times with CH_2Cl_2. The combined organic extracts were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (hexanes:EtOAc 95:5 to 4:1) gave 2-methyl-6-nitrobenzenesulfonyl chloride as a yellow powder (1.107 g, 71%). ^{1}H NMR δ (400 MHz,

$CDCl_3$) 7.71 (t, J = 7.8 Hz, 1H), 7.60 (dm, J = 7.8 Hz, 1H), 7.48 (dm, J = 7.8 Hz, 1H), 2.87 (s, 3H). LC–MS ($APCI^-$) 216 (M−Cl+O, 100%). Reaction of **2** (201 mg, 1.18 mmol) and the above sulfonyl chloride (587 mg, 2.49 mmol) using 2,6-lutidine, after recrystallisation from CH_2Cl_2–MeOH, gave **6d** as a yellow solid (213 mg, 45%). ^1H NMR δ (400 MHz, d_6-DMSO) 8.95 (dd, J = 7.2, 0.8 Hz, 1H), 8.41 (s, 1H), 8.21 (dd, J = 1.8, 0.8 Hz, 1H), 8.15 (s, 1H), 7.82 (t, J = 7.7 Hz, 1H), 7.76 (d, J = 7.7 Hz, 1H), 7.71 (d, J = 7.7 Hz, 1H), 7.31 (dd, J = 7.2, 1.8 Hz, 1H), 3.44 (s, 3H), 2.63 (s, 3H). LC–MS ($APCI^+$) 399 (MH^+, 100%). Anal. Calcd for $C_{17}H_{14}N_6O_4S.0.55\ H_2O$: C, 50.01; H, 3.73; N, 20.58. Found C, 51.25; H, 3.54; N, 21.09.

4.1.3.5. *N*-(3-(2-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-1-methylhydrazinylsulfonyl)-4-methylphenyl)acetamide (6e). *N*-*p*-Tolylacetamide (500 mg, 3.35 mmol) was added portionwise to $ClSO_3H$ (1.3 mL, 20 mmol) at 0 °C. After 10 min, the reaction mixture was heated to 100 °C for 2 h. The solution was dripped slowly onto ice and stood until all of the ice had melted. Then the oily product was extracted twice with CH_2Cl_2, the combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo to leave 5-acetamido-2-methylbenzenesulfonyl chloride as an off-white solid (636 mg, 77%). ^1H NMR δ (400 MHz, d_6-DMSO) 9.86 (br s, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.59 (dd, J = 8.1, 2.3 Hz, 1H), 7.02 (d, J = 8.1 Hz, 1H), 2.44 (s, 3H), 2.00 (s, 3H). NOESY's were observed from the signal at 9.86 to 7.79, 9.86 to 7.59, and 2.44 to 7.02. LC–MS ($APCI^+$) 248 (MH^+ with ^{35}Cl, 100%), 250 (MH^+ with ^{37}Cl, 30%). Reaction of **2** (30 mg, 0.18 mmol) and the above sulfonyl chloride (87 mg, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with CH_2Cl_2:MeOH 99:1 to 98.5:1.5) gave **6e** as a yellow solid (48 mg, 67%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.47 (dd, J = 7.2, 0.9 Hz, 1H), 8.18 (d, J = 2.2 Hz, 1H), 8.12 (s, 1H), 8.11 (br s, 1H), 7.96 (dd, J = 8.3, 2.2 Hz, 1H), 7.75 (s, 1H), 7.63 (br s, 1H), 7.28 (d, J = 8.3 Hz, 1H), 6.92 (dd, J = 7.2, 1.9 Hz, 1H), 3.49 (s, 3H), 2.52 (s, 3H), 2.20 (s, 3H). LC–MS ($APCI^+$) 411 (MH^+, 100%). Anal. Calcd for $C_{19}H_{18}N_6O_3S.0.5\ H_2O$: C, 54.41; H, 4.57; N, 20.04. Found C, 54.30; H, 4.61; N, 20.02.

4.1.3.6. *N*-(3-(2-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-1-methylhydrazinylsulfonyl)-4-methylphenyl)-2,2,2-trifluoroacetamide (6f). 2,2,2-Trifluoro-*N*-*p*-tolylacetamide (316 mg, 1.56 mmol) was added portionwise to $ClSO_3H$ (0.62 mL, 9.3 mmol) at 0 °C. After 10 min, the reaction mixture was heated to 100 °C for 2 h. The solution was dripped slowly onto ice and stood until all of the ice had melted. Then the oily product was 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 98:2 to 9:1) gave firstly 5-methyl-2-(2,2,2-trifluoroacetamido)-benzenesulfonyl chloride as a white solid (166 mg, 35%). ^1H NMR δ (400 MHz, $CDCl_3$) 9.82 (br s, 1H), 8.40 (d, J = 8.5 Hz, 1H), 7.88 (d, J = 1.9 Hz, 1H), 7.61 (dd, J = 8.5, 1.9 Hz, 1H), 2.47 (s, 3H). NOESY's were observed from the signal at 2.47 to 7.61, and 2.47 to 7.88. ^{13}C NMR δ 155.1 (q, J = 39 Hz), 137.8, 137.0, 132.8, 130.7, 129.0, 123.5, 115.4 (q, J = 289 Hz), 20.9. Followed by 2-methyl-5-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride as a white solid (51 mg, 11%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.15 (d, J = 2.3 Hz, 1H), 8.06 (br s, 1H), 8.02 (dd, J = 8.4, 2.3 Hz, 1H), 7.48 (d, J = 8.4 Hz, 1H), 2.78 (s, 3H). NOESY was observed from the signal at 2.78 to 7.48. ^{13}C NMR δ 155.1 (q, J = 38 Hz), 143.4, 135.8, 134.5, 133.8, 126.8, 120.4, 115.5 (q, J = 289 Hz), 19.8. LC–MS ($APCI^-$) 300 ($M-H^+$ with ^{35}Cl, 100%), 302 ($M-H^+$ with ^{37}Cl, 30%). Reaction of **2** (29 mg, 0.17 mmol) and 2-methyl-5-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride (51 mg, 0.17 mmol) using $NaHCO_3$, after chromatography (eluting with CH_2Cl_2:MeOH 99.5:0.5) gave **6f** as a yellow solid (54 mg, 69%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.47 (dd, J = 7.2, 0.9 Hz, 1H), 8.42 (d, J = 2.4 Hz, 1H), 8.35 (br s, 1H), 8.13 (s, 1H), 7.82–7.77 (m, 3H), 7.40 (d, J = 8.4 Hz, 1H), 6.89 (dd, J = 7.2, 1.9 Hz, 1H), 3.48 (s, 3H), 2.57 (s, 3H). LC–MS ($APCI^+$) 465 (MH^+, 100%). Anal. Calcd for $C_{19}H_{15}F_3N_6O_3S$: C, 49.14; H, 3.26; N, 18.10. Found C, 48.81; H, 3.26; N, 18.45.

4.1.3.7. 5-Amino-*N*′-((5-cyanopyrazolo[1,5-*a*]pyridin-3-yl)-methylene)-*N*,2-dimethylbenzenesulfonohydrazide (6g). Cs_2CO_3 (178 mg, 0.55 mmol) was added to a solution of **6f** (39 mg, 84 μmol) in MeOH (5 mL) and H_2O (2 mL), and at room temperature for 3 days. The MeOH was removed in vacuo, and the aqueous residue extracted twice with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo. Chromatography (eluting with CH_2Cl_2:MeOH 99.5:0.5) gave **6g** as a yellow solid (2.0 mg, 6%). HPLC purity 83%. ^1H NMR δ (400 MHz, $CDCl_3$) 8.47 (dd, J = 7.2, 0.9 Hz, 1H), 8.12 (s, 1H), 7.92 (dd, J = 1.8, 0.9 Hz, 1H), 7.73 (s, 1H), 7.57 (d, J = 2.6 Hz, 1H), 7.11 (d, J = 8.1 Hz, 1H), 6.92 (dd, J = 7.2, 1.8 Hz, 1H), 6.88 (dd, J = 8.1, 2.6 Hz, 1H), 3.44 (s, 3H), 2.43 (s, 3H). LC–MS ($APCI^+$) 369 (MH^+, 100%). HRMS (ESI^+) Calcd for $C_{17}H_{17}N_6O_2S$: 369.1128; found (MH^+) 369.1122.

4.1.3.8. 3-(2-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-1-methylhydrazinylsulfonyl)-4-methylbenzoic acid (6h). *p*-Toluic acid (500 mg, 3.67 mmol) was added portionwise to $ClSO_3H$ (1.5 mL, 23 mmol) at 0 °C. After 10 min, the reaction mixture was heated to 100 °C for 2 h. The solution was dripped slowly onto ice and stood until all of the ice had melted. The precipitated product was then filtered off, washed with water and dried to leave 3-(chlorosulfonyl)-4-methylbenzoic acid as a pale brown solid (746 mg, 87%). ^1H NMR δ (400 MHz, d_6-DMSO) 8.32 (d, J = 1.9 Hz, 1H), 7.76 (dd, J = 7.8, 1.9 Hz, 1H), 7.25 (d, J = 7.8 Hz, 1H), 5.55 (br s, 1H), 2.58 (s, 3H). LC–MS ($APCI^-$) 233 ($M-H^+$ with ^{35}Cl, 100%), 235 ($M-H^+$ with ^{37}Cl, 40%). Reaction of **2** (30 mg, 0.18 mmol) and the above sulfonyl chloride (82 mg, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with CH_2Cl_2:MeOH 98:2 to 19:1 to 9:1) gave **6h** as a yellow solid (42 mg, 60%). ^1H NMR δ (400 MHz, d_6-DMSO) 13.30 (br s, 1H), 8.95 (dd, J = 7.2, 0.9 Hz, 1H), 8.49 (d, J = 1.6 Hz, 1H), 8.37 (s, 1H), 8.22 (s, 1H), 8.14 (s, 1H), 8.09 (dd, J = 7.9, 1.6 Hz, 1H), 7.58 (d, J = 7.9 Hz, 1H), 7.30 (dd, J = 7.2, 1.9 Hz, 1H), 3.34 (s, 3H), 2.66 (s, 3H). LC–MS ($APCI^+$) 398 (MH^+, 100%). Anal. Calcd for $C_{18}H_{15}N_5O_4S.0.66$ MeOH: C, 53.55; H, 4.25; N, 16.73. Found C, 53.37; H, 4.01; N, 16.76.

4.1.3.9. Methyl 3-(2-((5-cyanopyrazolo[1,5-*a*]pyridin-3-yl) methylene)-1-methylhydrazinylsulfonyl)-4-methylbenzoate (6i). 3-(Chlorosulfonyl)-4-methylbenzoic acid (200 mg, 0.85 mmol) was refluxed in $SOCl_2$ (1.0 mL) for 1 h. The solvent was removed in vacuo, then MeOH (5 mL) added and the solution stirred for 1 h. The solvent was removed in vacuo to leave methyl 3-(chlorosulfonyl)-4-methylbenzoate as a pale brown solid (127 mg, 60%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.72 (d, J = 1.7 Hz, 1H), 8.25 (dd, J = 7.9, 1.7 Hz, 1H), 7.52 (d, J = 7.9 Hz, 1H), 3.97 (s, 3H), 2.86 (s, 3H). LC–MS ($APCI^-$) 229 (M−Cl+O, 100%). Reaction of **2** (30 mg, 0.18 mmol) and the above sulfonyl chloride (87 mg, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with hexanes:EtOAc 2:1 to 1:1 to EtOAc) gave **6i** as a yellow solid (53 mg, 74%). ^1H NMR δ (400 MHz, $CDCl_3$) 8.78 (d, J = 1.5 Hz, 1H), 8.49 (d, J = 7.2 Hz, 1H), 8.21 (dd, J = 7.9, 1.5 Hz, 1H), 8.15 (s, 1H), 7.98 (dd, J = 1.8, 0.8 Hz, 1H), 7.84 (s, 1H), 7.45 (d, J = 7.9 Hz, 1H), 6.94 (dd, J = 7.2, 1.8 Hz, 1H), 3.95 (s, 3H), 3.44 (s, 3H), 2.69 (s, 3H). LC–MS ($APCI^+$) 412 (MH^+, 100%). Anal. Calcd for $C_{19}H_{17}N_5O_4S$: C, 55.47; H, 4.16; N, 17.02. Found C, 55.72; H, 4.26; N, 17.18.

4.1.3.10. 5-Cyano-*N*′-((5-cyanopyrazolo[1,5-*a*]pyridin-3-yl)-methylene)-*N*,2-dimethylbenzenesulfonohydrazide (6j). 3-Amino-4-methylbenzonitrile (407 mg, 3.09 mmol) was suspended in concentrated HCl (3 mL) at 0 °C. A solution of $NaNO_2$ (320 mg, 4.64 mmol) in water (1 mL) was added dropwise over 5 min, and

the solution stirred for 45 min. Meanwhile, AcOH (3 mL) was saturated with SO_2, then $CuCl_2{\cdot}2H_2O$ (158 mg, 0.93 mmol) was added and SO_2 bubbled through for a further 5 min. The AcOH mixture was cooled to 5 °C, then the diazonium solution added over 5 min. The resulting mixture was stirred for a further 1.5 h, then the precipitate filtered off, washed with a little water and dried to leave 5-cyano-2-methylbenzenesulfonyl chloride as a yellow solid (167 mg, 25%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.36 (d, J = 1.7 Hz, 1H), 7.87 (dd, J = 7.9, 1.7 Hz, 1H), 7.58 (d, J = 7.9 Hz, 1H), 2.88 (s, 3H). LC–MS (APCI$^-$) 196 (M−Cl+O, 100%). Reaction of **2** (30 mg, 0.18 mmol) and the above sulfonyl chloride (45 mg, 0.21 mmol) using $NaHCO_3$, after chromatography (eluting with CH_2Cl_2:MeOH 99.75:0.25) gave **6j** as a yellow solid (32 mg, 48%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.96 (dd, J = 7.2, 1.0 Hz, 1H), 8.41 (s, 1H), 8.32 (d, J = 1.8 Hz, 1H), 8.21 (dd, J = 1.9, 1.0 Hz, 1H), 8.16 (s, 1H), 8.05 (dd, J = 8.0, 1.8 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.32 (dd, J = 7.2, 1.9 Hz, 1H), 3.37 (s, 3H), 2.68 (s, 3H). LC–MS (APCI$^+$) 379 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{14}N_6O_2S.0.2\ H_2O$: C, 56.59; H, 3.80; N, 22.00. Found C, 56.57; H, 3.86; N, 22.02.

4.1.3.11. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-(methylsulfonyl)benzenesulfonohydrazide (6k).

4-(Methylsulfonyl)toluene (250 mg, 1.47 mmol) was added portionwise to $ClSO_3H$ (0.6 mL, 9.0 mmol) at 0 °C. After 10 min, the reaction mixture was heated to 100 °C for 2 h. The solution was dripped slowly onto ice and stood until all of the ice had melted. The precipitated product was then filtered off, washed with water and dried, to leave 2-methyl-5-(methylsulfonyl)benzenesulfonyl chloride as a white solid (327 mg, 83%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.62 (d, J = 1.9 Hz, 1H), 8.17 (dd, J = 8.0, 1.9 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H), 3.12 (s, 3H), 2.91 (s, 3H). LC–MS (APCI$^-$) 249 (M−Cl+O, 100%). Reaction of **2** (30 mg, 0.18 mmol) and the above sulfonyl chloride (94 mg, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with hexanes:EtOAc 2:1 to 1:1 to EtOAc) gave **6k** as a yellow solid (66 mg, 87%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.54 (d, J = 2.0 Hz, 1H), 8.52 (dd, J = 7.2, 1.0 Hz, 1H), 8.21 (dd, J = 1.8, 1.0 Hz, 1H), 8.18 (s, 1H), 8.05 (dd, J = 8.0, 2.0 Hz, 1H), 7.89 (s, 1H), 7.58 (d, J = 8.0 Hz, 1H), 6.99 (dd, J = 7.2, 1.8 Hz, 1H), 3.41 (s, 3H), 3.09 (s, 3H), 2.80 (s, 3H). LC–MS (APCI$^+$) 432 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{17}N_5O_4S_2$: C, 50.10; H, 3.97; N, 16.23. Found C, 50.35; H, 3.97; N, 16.26.

4.1.3.12. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-(trifluoromethyl)benzenesulfonohydrazide (6l).

4-Methylbenzotrifluoride (250 mg, 1.56 mmol) was added portionwise to $ClSO_3H$ (0.6 mL, 9.0 mmol) at 0 °C. After 10 min, the reaction mixture was heated to 100 °C for 2 h. The solution was dripped slowly onto ice and stood until all of the ice had melted. The oily product was 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 98:2) gave 2-methyl-5-(trifluoromethyl)benzenesulfonyl chloride as a colourless oil (150 mg, 37%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.33 (m, 1H), 7.86 (dd, J = 8.0, 1.4 Hz, 1H), 7.58 (d, J = 8.0 Hz, 1H), 2.87 (s, 3H). LC–MS (APCI$^-$) 239 (M−Cl+O, 100%). Reaction of **2** (30 mg, 0.18 mmol) and the above sulfonyl chloride (54 mg, 0.21 mmol) using $NaHCO_3$, after chromatography (eluting with CH_2Cl_2:MeOH 99.75:0.25) gave **6l** as a yellow solid (36 mg, 49%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.51 (dd, J = 7.2, 0.9 Hz, 1H), 8.33 (m, 1H), 8.16 (s, 1H), 8.02 (dd, J = 1.8, 0.9 Hz, 1H), 7.87 (s, 1H), 7.79 (dd, J = 8.0, 1.6 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 6.97 (dd, J = 7.2, 1.8 Hz, 1H), 3.41 (s, 3H), 2.73 (s, 3H). LC–MS (APCI$^+$) 422 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{14}F_3N_5O_2S$: C, 51.30; H, 3.35; N, 16.62. Found C, 51.15; H, 3.34; N, 16.48.

4.1.3.13. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2,5-trimethylbenzenesulfonohydrazide (6m).

Reaction of **2** (30 mg, 0.18 mmol) and 2,5-dimethylbenzenesulfonyl chloride (43 mg, 0.21 mmol) using 2,6-lutidine, gave **6m** as a yellow solid (45 mg, 70%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.47 (dd, J = 7.2, 0.9 Hz, 1H), 8.12 (s, 1H), 8.00 (s, 1H), 7.86 (s, 1H), 7.77 (s, 1H), 7.39 (dd, J = 7.7, 1.2 Hz, 1H), 7.24 (d, J = 7.7 Hz, 1H), 6.93 (dd, J = 7.2, 1.9 Hz, 1H), 3.43 (s, 3H), 2.55 (s, 3H), 2.49 (s, 3H). LC–MS (APCI$^+$) 368 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{17}N_5O_2S$: C, 58.84; H, 4.66; N, 19.06. Found C, 58.73; H, 4.51; N, 19.03.

4.1.3.14. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-5-fluoro-*N*,2-dimethylbenzenesulfonohydrazide (6n).

Reaction of **2** (30 mg, 0.18 mmol) and 5-fluoro-2-methylbenzenesulfonyl chloride (73 mg, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with hexanes:EtOAc 3:1 to 2:1 to 1:1) gave **6n** as a yellow solid (51 mg, 78%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.50 (dd, J = 7.2, 0.9 Hz, 1H), 8.15 (s, 1H), 7.98 (dd, J = 1.8, 0.9 Hz, 1H), 7.85 (dd, J = 8.4, 2.7 Hz, 1H), 7.82 (s, 1H), 7.26–7.37 (m, 2H), 6.96 (dd, J = 7.2, 1.8 Hz, 1H), 3.42 (s, 3H), 2.59 (s, 3H). LC–MS (APCI$^+$) 372 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{14}FN_5O_2S$: C, 54.98; H, 3.80; N, 18.86. Found C, 55.28; H, 3.80; N, 19.18.

4.1.3.15. 5-Chloro-*N*′-((5-cyanopyrazolo[1,5-*a*]pyridin-3-yl)-methylene)-*N*,2-dimethylbenzenesulfonohydrazide (6o).

Reaction of **2** (30 mg, 0.18 mmol) and 5-chloro-2-methylbenzenesulfonyl chloride (47 mg, 0.21 mmol) using 2,6-lutidine, after recrystallisation from CH_2Cl_2-hexanes gave **6o** as a yellow solid (31 mg, 46%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.51 (dd, J = 7.2, 0.9 Hz, 1H), 8.16 (s, 1H), 8.10 (d, J = 2.3 Hz, 1H), 8.01 (dd, J = 1.8, 0.9 Hz, 1H), 7.84 (s, 1H), 7.52 (dd, J = 8.2, 2.3 Hz, 1H), 7.30 (d, J = 8.2 Hz, 1H), 6.97 (dd, J = 7.2, 1.8 Hz, 1H), 3.41 (s, 3H), 2.61 (s, 3H). LC–MS (APCI$^+$) 388 (MH$^+$ with ^{35}Cl, 100%), 390 (MH$^+$ with ^{37}Cl, 40%). Anal. Calcd for $C_{17}H_{14}ClN_5O_2S$: C, 52.65; H, 3.64; N, 18.06. Found C, 52.86; H, 3.67; N, 18.15.

4.1.3.16. 5-Bromo-*N*′-((5-cyanopyrazolo[1,5-*a*]pyridin-3-yl)-methylene)-N,2-dimethylbenzenesulfonohydrazide (6p).

Reaction of **2** (30 mg, 0.18 mmol) and 5-bromo-2-methylbenzenesulfonyl chloride[16] (57 mg, 0.21 mmol) using 2,6-lutidine, gave **6p** as a yellow solid (47 mg, 62%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.51 (dd, J = 7.2, 1.0 Hz, 1H), 8.23 (d, J = 2.1 Hz, 1H), 8.16 (s, 1H), 8.03 (dd, J = 1.8, 1.0 Hz, 1H), 7.84 (s, 1H), 7.67 (dd, J = 8.2, 2.1 Hz, 1H), 7.24 (d, J = 8.2 Hz, 1H), 6.98 (dd, J = 7.2, 1.8 Hz, 1H), 3.40 (s, 3H), 2.59 (s, 3H). LC–MS (APCI$^+$) 432 (MH$^+$ with ^{79}Br, 100%), 434 (MH$^+$ with ^{81}Br, 100%). Anal. Calcd for $C_{17}H_{14}BrN_5O_2S.0.05$ hexanes: C, 47.59; H, 3.39; N, 16.04. Found C, 47.72; H, 3.42; N, 16.32.

4.1.3.17. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*-methyl-3-nitrobenzenesulfonohydrazide (6q).

Reaction of **2** (30 mg, 0.18 mmol) and 3-nitrobenzenesulfonyl chloride (78 mg, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with hexanes:EtOAc 3:1 to 2:1 to 1:1) gave **6q** as a yellow solid (53 mg, 79%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.71 (t, J = 1.9 Hz, 1H), 8.57 (d, J = 7.2 Hz, 1H), 8.48 (m, 1H), 8.40 (s, 1H), 8.29 (d, J = 7.8 Hz, 1H), 8.21 (s, 1H), 8.02 (s, 1H), 7.83 (t, J = 8.0 Hz, 1H), 7.05 (dd, J = 7.2, 1.9 Hz, 1H), 3.32 (s, 3H). LC–MS (APCI$^+$) 385 (MH$^+$, 100%). Anal. Calcd for $C_{16}H_{12}N_6O_4S.0.33\ CH_2Cl_2$: C, 47.56; H, 3.09; N, 20.38. Found C, 47.72; H, 3.13; N, 20.40.

4.1.3.18. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-2-ethyl-*N*-methyl-5-nitrobenzenesulfonohydrazide (6r).

Reaction of **2** (30 mg, 0.18 mmol) and 2-ethyl-5-nitrobenzenesulfonyl chloride[17] (88 mg, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with hexanes:EtOAc 4:1 to 1:1 to EtOAc) gave **6r** as a yellow solid (66 mg, 92%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.89 (d, J = 2.4 Hz, 1H), 8.52 (dd, J = 7.2, 1.0 Hz,

1H), 8.41 (dd, J = 8.5, 2.4 Hz, 1H), 8.18 (s, 1H), 8.07 (dd, J = 1.8, 1.0 Hz, 1H), 7.89 (s, 1H), 7.62 (d, J = 8.5 Hz, 1H), 6.98 (dd, J = 7.2, 1.8 Hz, 1H), 3.44 (s, 3H), 3.21 (q, J = 7.5 Hz, 2H), 1.33 (t, J = 7.5 Hz, 3H). LC–MS (APCI$^+$) 413 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{16}N_6O_4S$: C, 52.42; H, 3.91; N, 20.38. Found C, 52.41; H, 3.93; N, 20.21.

4.1.3.19. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-2-isopropyl-*N*-methyl-5-nitrobenzenesulfonohydrazide (6s).

1-Isopropyl-4-nitrobenzene (500 mg, 3.03 mmol) was added portionwise to $ClSO_3H$ (1.2 mL, 18 mmol) at 0 °C. After 10 min, the reaction mixture was heated to 100 °C for 2 h. The solution was dripped slowly onto ice and stood until all of the ice had melted. The oily product was 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 9:1) gave 2-isopropyl-5-nitrobenzenesulfonyl chloride as a pale yellow solid (127 mg, 16%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.92 (d, J = 2.4 Hz, 1H), 8.50 (dd, J = 8.7, 2.4 Hz, 1H), 7.81 (d, J = 8.7 Hz, 1H), 4.15 (septet, J = 6.8 Hz, 1H), 1.40 (d, J = 6.8 Hz, 6H). LC–MS (APCI$^-$) 244 (M−Cl+O, 100%). Reaction of **2** (30 mg, 0.18 mmol) and the above sulfonyl chloride (55 mg, 0.21 mmol) using $NaHCO_3$, after chromatography (eluting with hexanes:EtOAc 3:1 to 2:1) gave **6s** as a yellow solid (41 mg, 55%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.91 (d, J = 2.4 Hz, 1H), 8.51 (dd, J = 7.2, 1.0 Hz, 1H), 8.44 (dd, J = 8.7, 2.4 Hz, 1H), 8.17 (s, 1H), 8.05 (dd, J = 1.8, 1.0 Hz, 1H), 7.87 (s, 1H), 7.72 (d, J = 8.7 Hz, 1H), 6.97 (dd, J = 7.2, 1.8 Hz, 1H), 4.09 (septet, J = 6.8 Hz, 1H), 3.44 (s, 3H), 1.25 (d, J = 6.8 Hz, 6H). 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, 53.56; H, 4.42; N, 19.84.

4.1.3.20. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-2-fluoro-*N*-methyl-5-nitrobenzenesulfonohydrazide (6t).

Reaction of **2** (250 mg, 1.46 mmol) and 2-fluoro-5-nitrobenzenesulfonyl chloride[18] (455 mg, 1.90 mmol) using 2,6-lutidine, gave **6t** as a yellow solid (485 mg, 82%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.93 (dd, J = 5.8, 2.9 Hz, 1H), 8.53 (dd, J = 7.2, 1.0 Hz, 1H), 8.49 (ddd, J = 9.0, 4.0, 2.9 Hz, 1H), 8.30 (dd, J = 1.8, 1.0 Hz, 1H), 8.19 (s, 1H), 7.98 (s, 1H), 7.40 (t, J = 8.9 Hz, 1H), 7.01 (dd, J = 7.2, 1.8 Hz, 1H), 3.46 (d, J = 1.6 Hz, 3H). LC–MS (APCI$^+$) 403 (MH$^+$, 100%). Anal. Calcd for $C_{16}H_{11}FN_6O_4S$: C, 47.76; H, 2.76; N, 20.89. Found C, 48.04; H, 2.97; N, 20.69.

4.1.3.21. 2-Chloro-*N*′-((5-cyanopyrazolo[1,5-*a*]pyridin-3-yl)-methylene)-*N*-methyl-5-nitrobenzenesulfonohydrazide (6u).

Reaction of **2** (30 mg, 0.18 mmol) and 2-chloro-5-nitrobenzenesulfonyl chloride (90 mg, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with hexanes:EtOAc 3:1 to 1:1) gave **6u** as a yellow solid (20 mg, 27%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.16 (d, J = 2.7 Hz, 1H), 8.50 (dd, J = 7.2, 0.9 Hz, 1H), 8.40 (dd, J = 8.7, 2.7 Hz, 1H), 8.16 (s, 1H), 8.00 (dd, J = 1.8, 0.9 Hz, 1H), 7.86 (s, 1H), 7.72 (d, J = 8.7 Hz, 1H), 6.96 (dd, J = 7.2, 1.8 Hz, 1H), 3.57 (s, 3H). LC–MS (APCI$^+$) 419 (MH$^+$ with ^{35}Cl, 100%), 421 (MH$^+$ with ^{37}Cl, 30%). Anal. Calcd for $C_{16}H_{11}ClN_6O_4S.0.33\ H_2O$: C, 45.24; H, 2.77; N, 19.79. Found C, 45.33; H, 2.79; N, 20.08.

4.1.3.22. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-2-methoxy-*N*-methyl-5-nitrobenzenesulfonohydrazide (6v).

Reaction of **2** (30 mg, 0.18 mmol) and 2-methoxy-5-nitrobenzenesulfonyl chloride (88 mg, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with CH_2Cl_2:MeOH 99.5:0.5) gave **6v** as a yellow solid (56 mg, 77%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.93 (dd, J = 7.2, 0.9 Hz, 1H), 8.72 (d, J = 2.9 Hz, 1H), 8.51 (dd, J = 9.2, 2.9 Hz, 1H), 8.37 (s, 1H), 8.12 (s, 1H), 8.07 (dd, J = 1.9, 0.9 Hz, 1H), 7.46 (d, J = 9.2 Hz, 1H), 7.29 (dd, J = 7.2, 1.9 Hz, 1H), 4.02 (s, 3H), 3.45 (s, 3H). LC–MS (APCI$^+$) 415 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{14}N_6O_5S$: C, 49.27; H, 3.41; N, 20.28. Found C, 49.31; H, 3.41; N, 20.09.

4.1.3.23. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-2-(dimethylamino)-*N*-methyl-5-nitrobenzenesulfonohydrazide (6w).

Reaction of **2** (30 mg, 0.18 mmol) and 2-(dimethylamino)-5-nitrobenzenesulfonyl chloride[19] (93 mg, 0.35 mmol) using $NaHCO_3$, after chromatography (eluting with hexanes:EtOAc 2:1 to 1:1 to EtOAc) gave **6w** as a yellow solid (22 mg, 29%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.95 (dd, J = 7.2, 1.0 Hz, 1H), 8.66 (d, J = 2.8 Hz, 1H), 8.39 (s, 1H), 8.28 (dd, J = 9.3, 2.8 Hz, 1H), 8.15 (s, 1H), 8.05 (dd, J = 1.9, 1.0 Hz, 1H), 7.38 (d, J = 9.3 Hz, 1H), 7.30 (dd, J = 7.2, 1.9 Hz, 1H), 3.39 (s, 3H), 2.99 (s, 6H). LC–MS (APCI$^+$) 428 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{17}N_7O_4S.0.05$ hexanes: C, 50.91; H, 4.13; N, 22.71. Found C, 51.06; H, 4.03; N, 22.91.

4.1.3.24. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzohydrazide (6x).

Reaction of **2** (30 mg, 0.18 mmol) and 2-methyl-5-nitrobenzoyl chloride (70 mg, 0.35 mmol) using $NaHCO_3$, after trituration with MeOH gave **6x** as a yellow solid (40 mg, 63%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.91 (dd, J = 7.2, 0.9 Hz, 1H), 8.42 (s, 1H), 8.28 (s, 1H), 8.24 (dd, J = 8.4, 2.5 Hz, 1H), 8.19 (d, J = 2.5 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.23 (dd, J = 7.2, 1.9 Hz, 1H), 7.09 (dd, J = 1.9, 0.9 Hz, 1H), 3.54 (s, 3H), 2.30 (s, 3H). LC–MS (APCI$^+$) 363 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{14}N_6O_3$: C, 59.67; H, 3.89; N, 23.19. Found C, 59.46; H, 3.94; N, 23.11.

4.1.3.25. *N*′-((5-Cyanopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*-methyl-3-nitrobenzohydrazide (6y).

Reaction of **2** (30 mg, 0.18 mmol) and 3-nitrobenzoyl chloride (65 mg, 0.35 mmol) using $NaHCO_3$, after trituration with MeOH gave **6y** as a yellow solid (55 mg, 90%). ^{1}H NMR δ (400 MHz, d_6-DMSO) 8.93 (dd, J = 7.2, 0.9 Hz, 1H), 8.46–8.41 (m, 2H), 8.37 (ddd, J = 8.2, 2.4, 1.1 Hz, 1H), 8.30 (s, 1H), 8.06 (dt, J = 7.7, 1.3 Hz, 1H), 7.85 (m, 1H), 7.59 (s, 1H), 7.26 (dd, J = 7.2, 1.9 Hz, 1H), 3.54 (s, 3H). LC–MS (APCI$^+$) 349 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{12}N_6O_3$: C, 58.62; H, 3.47; N, 24.13. Found C, 58.46; H, 3.54; N, 24.38.

4.1.4. Synthesis of 3-((2-Methyl-2-(2-methyl-5-nitrobenzyl) hydrazono)methyl)pyrazolo[1,5-*a*]pyridine-5-carbonitrile (9)

A solution of 2-(chloromethyl)-1-methyl-4-nitrobenzene[20] (**7**) (200 mg, 1.08 mmol) and methylhydrazine (0.28 mL, 5.3 mmol) in EtOH (6 mL) was refluxed for 1 h. The solvent was removed in vacuo, then taken up in CH_2Cl_2, washed with 1 M aqueous NaOH, dried (Na_2SO_4) and the solvent removed in vacuo to leave 1-methyl-1-(2-methyl-5-nitrobenzyl)hydrazine (**8**) as a yellow oil (210 mg, 100%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.20 (d, J = 2.5 Hz, 1H), 8.04 (dd, J = 8.3, 2.5 Hz, 1H), 7.31 (d, J = 8.3 Hz, 1H), 3.65 (s, 2H), 3.00 (br s, 2H), 2.57 (s, 3H), 2.47 (s, 3H). LC–MS (APCI$^+$) 196 (MH$^+$, 100%). A suspension of **2** (30 mg, 0.18 mmol) and **8** (35 mg, 0.18 mmol) was stirred in MeOH (10 mL) for 3 days. The precipitate was filtered off and dried to leave **9** as a yellow solid (40 mg, 66%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.45 (dd, J = 7.2, 0.9 Hz, 1H), 8.27 (dd, J = 1.8, 0.9 Hz, 1H), 8.18 (d, J = 2.4 Hz, 1H), 8.12 (dd, J = 8.3, 2.4 Hz, 1H), 8.07 (s, 1H), 7.49 (s, 1H), 7.41 (d, J = 8.3 Hz, 1H), 6.86 (dd, J = 7.2, 1.8 Hz, 1H), 4.50 (s, 2H), 2.95 (s, 3H), 2.51 (s, 3H). LC–MS (APCI$^+$) 349 (MH$^+$, 100%). Anal. Calcd for $C_{18}H_{16}N_6O_2$: C, 62.06; H, 4.63; N, 24.12. Found C, 61.76; H, 4.57; N, 24.06.

4.2. Enzyme assays

The Class I PI3 kinase assays were performed using a basic thin layer chromatography technique, as described previously.[21] The PI3 kinase isoforms were prepared in-house, as described previ-

ously.[21] 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%), 2X 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 [γ^{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 (Storm-Imager, 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 as previously described as were the ligands.[11] Docking calculations were also performed as previously described.[11]

4.4. Cellular assay

The early passage cell lines used in this study were developed in this laboratory and cultured as previously described.[22] 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 analyzed 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. Aqueous solubility

The solid compound sample was mixed with water (to make a 2 mM solution), and the suspension was sonicated for 15 mins and then centrifuged at 13000 rpm for 6 min. An aliquot of the clear supernatant was diluted 2-fold with water and then centrifuged again at 13000 rpm for 6 min. A 100 μL aliquot of the clear supernatant was injected into the HPLC and the peak area measured. The solubility was calculated by comparing the peak area obtained with that from a standard solution of the compound in DMSO (after allowing for varying dilution factors and injection volumes).

4.6. 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).[21]

4.7. Pharmacokinetics

Age-matched specific pathogen-free male CD-1 mice were administered a single 10 mg/kg dose by i.p. injection of **5** in 5% DMSO, 45% PEG-400, 20% 2-hydroxypropyl-β-cyclodextrin, 30% water. Blood samples were collected at multiple time-points after dosing and the plasma supernatant was retained after centrifugation for 10 min at 6000 rpm at 20 °C. Quantitative analysis of plasma was carried out by LC-MS/MS as previously described.[4] Pharmacokinetic parameters were determined by non compartmental analysis using Phoenix WinNonlin 6.2 (Pharsight).

4.8. Xenograft studies

Age-matched specific pathogen-free male Rag1$^{-/-}$ mice and female NIH III nude mice were subcutaneously inoculated with 5×10^6 HCT-116 cells and 5×10^6 SK-OV-3 cells, respectively, in phosphate buffered saline. Tumour volume (mm^3) was calculated using the formula $(L \times w^2) \times \pi/6$ (where; L = longest tumor diameter and w = perpendicular diameter). Dosing began when tumours reached a volume of approximately 150 mm^3. Compounds **1** and **5** were administered q.d. in solution in 7.5% DMSO, 42.5% PEG-400, 20% 2-hydroxypropyl-β-cyclodextrin, 30% water by i.p. injection. Control animals were treated with the dosing vehicle alone. Animal bodyweight was measured daily, and tumour volume was measured three times per week. Treatment was discontinued if mean bodyweight loss exceeded 15% of starting weight and 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.

Acknowledgements

The authors would like to thank Sisira Kumara for the aqueous solubility measurements, Maruta Boyd for the NMR spectra, and Aaron Thompson for technical assistance. This work was funded by the Health Research Council of New Zealand, Maurice Wilkins Centre for Molecular Biodiscovery, and Pathway Therapeutics Inc.

References and notes

1. Shuttleworth, S.; Silva, F.; Tomassi, C.; Cecil, A.; Hill, T.; Rogers, H.; Townsend, P. *Prog. Med. Chem.* **2009**, *48*, 81.
2. Cleary, J. M.; Shapiro, G. I. *Curr. Oncol. Rep.* **2010**, *12*, 87.
3. Liu, P.; Cheng, H.; Roberts, T. M.; Zhao, J. J. *Nat. Rev. Drug Disc.* **2009**, *8*, 627.
4. Jamieson, S.; Flanagan, J. U.; Kolekar, S.; Buchanan, C.; Kendall, J. D.; Lee, W.-J.; Rewcastle, G. W.; Denny, W. A.; Singh, R.; Dickson, J.; Baguley, B. C.; Shepherd, P. R. *Biochem. J.* **2011**, *438*, 53.
5. Hayakawa, M.; Kaizawa, H.; Kawaguchi, K.; Ishikawa, N.; Koizumi, T.; Ohishi, T.; Yamano, M.; Okada, M.; Ohta, M.; Tsukamoto, S.; Raynaud, F. I.; Waterfield, M. D.; Parker, P.; Workman, P. *Bioorg. Med. Chem.* **2007**, *15*, 403.
6. Hayakawa, M.; Kawaguchi, K.; Kaizawa, H.; Koizumi, T.; Ohishi, T.; Yamano, M.; Okada, M.; Ohta, M.; Tsukamoto, S.; Raynaud, F. I.; Parker, P.; Workman, P.; Waterfield, M. D. *Bioorg. Med. Chem.* **2007**, *15*, 5837.
7. Sun, M.; Hillmann, P.; Hofmann, B. T.; Hart, J. R.; Vogt, P. K. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 15547.
8. Schmidt-Kittler, O.; Zhu, J.; Yang, J.; Liu, G.; Hendricks, W.; Lengauer, C.; Gabelli, S. B.; Kinzler, K. W.; Vogelstein, B.; Huso, D. L.; Zhou, S. *Oncotarget* **2010**, *1*, 339.

9. Ciraolo, E.; Morello, F.; Hirsch, E. *Curr. Med. Chem.* **2011**, *18*, 2674.
10. Dienstmann, R.; Rodon, J.; Markman, B.; Tabernero, J. *Recent Pat. Anti-Cancer Drug Disc.* **2011**, *6*, 210.
11. Kendall, J. D.; O'Connor, P. D.; Marshall, A. J.; Frédérick, R.; Marshall, E. S.; Lill, C. L.; Lee, W.-J.; Kolekar, S.; Chao, M.; Malik, A.; Yu, S.; Chaussade, C.; Buchanan, C.; Rewcastle, G. W.; Baguley, B. C.; Flanagan, J. U.; Jamieson, S. M. F.; Denny, W. A.; Shepherd, P. R. *Bioorg. Med. Chem.* **2011**, doi:10.1016/j.bmc.2011.11.029.
12. Fel'dman, I. K.; Mikhailova, V. N. *Zh. Obshch. Khim.* **1963**, *33*, 38.
13. Butler, D. E.; Alexander, S. M.; McLean, J. W.; Strand, L. B. *J. Med. Chem.* **1971**, *14*, 1052.
14. Knight, Z. A.; Gonzalez, B.; Feldman, M. E.; Zunder, E. R.; Goldenberg, D. D.; Williams, O.; Loewith, R.; Stokoe, D.; Balla, A.; Toth, B.; Balla, T.; Weiss, W. A.; Williams, R. L.; Shokat, K. M. *Cell* **2006**, *125*, 733.
15. Courtin, A. *Helv. Chim. Acta* **1976**, *59*, 379.
16. Chen, Q.-H.; Rao, P. N. P.; Knaus, E. E. *Bioorg. Med. Chem.* **2005**, *13*, 2459.
17. Hansch, C.; Schmidhalter, B.; Reiter, F.; Saltonstall, W. *J. Org. Chem.* **1956**, *21*, 265.
18. Courtin, A. *Helv. Chim. Acta* **1982**, *65*, 546.
19. Abramovitch, R. A.; Chellathurai, T.; Holcomb, W. D.; McMaster, I. T.; Vanderpool, D. P. *J. Org. Chem.* **1977**, *42*, 2920.
20. Maulding, D. R.; Lotts, K. D.; Robinson, S. A. *J. Org. Chem.* **1983**, *48*, 2938.
21. Chaussade, C.; Rewcastle, G. W.; Kendall, J. D.; Denny, W. A.; Cho, K.; Gronning, L. M.; Chong, M. L.; Anagnostou, S. H.; Jackson, S. P.; Daniele, N.; Shepherd, P. R. *Biochem. J.* **2007**, *404*, 449.
22. Marshall, E. S.; Baguley, B. C.; Matthews, J. H. L.; Jose, C. C.; Furneaux, C. E.; Shaw, J. H. F.; Kirker, J. A.; Morton, R. P.; White, J. B.; Rice, M. L.; Isaacs, R. J.; Coutts, R.; Whittaker, J. R. *Oncol. Res.* **2004**, *14*, 297.