Bioorganic & Medicinal Chemistry 20 (2012) 6770–6789

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Targeting conserved water molecules: Design of 4-aryl-5-cyanopyrrolo[2,3-*d*]pyrimidine Hsp90 inhibitors using fragment-based screening and structure-based optimization

Nicholas G. M. Davies [a], Helen Browne [a], Ben Davis [a], Martin J. Drysdale [a], Nicolas Foloppe [a], Stephanie Geoffrey [a], Ben Gibbons [a], Terance Hart [a], Roderick Hubbard [a], Michael Rugaard Jensen [b], Howard Mansell [a], Andrew Massey [a], Natalia Matassova [a], Jonathan D. Moore [a], James Murray [a], Robert Pratt [a], Stuart Ray [a], Alan Robertson [a], Stephen D. Roughley [a], Joseph Schoepfer [b], Kirsten Scriven [a], Heather Simmonite [a], Stephen Stokes [a], Allan Surgenor [a], Paul Webb [a], Mike Wood [a], Lisa Wright [a], Paul Brough [a,*]

[a] *Vernalis Ltd, Granta Park, Great Abington, Cambridge CB21 6GB, UK*
[b] *Novartis Institutes for Biomedical Research, Novartis Pharma AG, CH-4002 Basel, Switzerland*

ARTICLE INFO

Article history:
Available online 4 September 2012

Keywords:
Hsp90
Fragment-based screening
Cancer
Structure-based design
Conserved water molecules

ABSTRACT

Inhibitors of the Hsp90 molecular chaperone are showing promise as anti-cancer agents. Here we describe a series of 4-aryl-5-cyanopyrrolo[2,3-*d*]pyrimidine ATP competitive Hsp90 inhibitors that were identified following structure-driven optimization of purine hits revealed by NMR based screening of a proprietary fragment library. Ligand-Hsp90 X-ray structures combined with molecular modeling led to the rational displacement of a conserved water molecule leading to enhanced affinity for Hsp90 as measured by fluorescence polarization, isothermal titration calorimetry and surface plasmon resonance assays. This displacement was achieved with a nitrile group, presenting an example of efficient gain in binding affinity with minimal increase in molecular weight. Some compounds in this chemical series inhibit the proliferation of human cancer cell lines in vitro and cause depletion of oncogenic Hsp90 client proteins and concomitant elevation of the co-chaperone Hsp70. In addition, one compound was demonstrated to be orally bioavailable in the mouse. This work demonstrates the power of structure-based design for the rapid evolution of potent Hsp90 inhibitors and the importance of considering conserved water molecules in drug design.

1. Introduction

Heat shock protein 90 (Hsp90) is a member of a class of proteins known as molecular chaperones that, via a complex ATP-driven mechanism, play a critical role in the maturation, stability and function of so called 'client' proteins within the cell.[1–5] Heat shock proteins are over-expressed under conditions of cellular stress such as an increase in temperature or oxidation potential, but also have a critical role within non-stressed cells in maintaining homeostasis. Chaperone proteins are abundant in cells, comprising between 5% and 10% of cellular proteins and Hsp90 may represent 1% of the total even under non stressed conditions. Well over 100 clients of Hsp90 have been identified[6] including many proteins that are involved in pathways associated with cancer pathology[7–10] and the hallmark traits of cancer.[11] Thus inhibition of Hsp90 has potential to disrupt multiple oncogenic pathways[12] by an indirect attack on critical proteins and has become an attractive target for cancer therapy. The abundance and role of chaperones in homeostasis contributed to early concerns as to the suitability of Hsp90 as a drug target. However, the inherent selectivity of Hsp90 inhibitors for cancer cells versus normal cells appears to provide a therapeutic window for these agents. Rationalization for this selectivity includes the prevalence of a high affinity form of Hsp90 in tumor cells,[13] and the 'addiction' or greater reliance of cancer cells on oncogenic client proteins (especially the activated mutated forms).[14] More recently Hsp90 inhibition has attracted research interest in non-oncology therapeutic areas such as inflammation and neurodegeneration. Pioneering studies of Hsp90 inhibition characterized the natural products geldanamycin[15–17] and radicicol[18,19] (Fig. 1) as competitive binders to an ATP site on the N-terminal domain of Hsp90.[20] These studies paved the way for the identification of semi-synthetic inhibitors such as 17-AAG[21] and 17-DMAG[22] (**5a** and **5b**; Fig. 2) which progressed into clinical evaluation. Identification of

* Corresponding author.
E-mail address: p.brough@vernalis.com (P. Brough).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.08.050

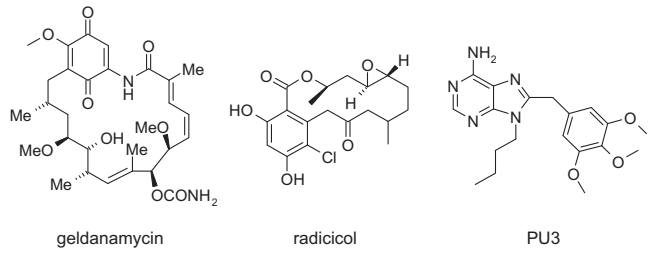


Figure 1. Structures of the natural product Hsp90 inhibitors geldanamycin and radicicol and the synthetic inhibitor PU3.

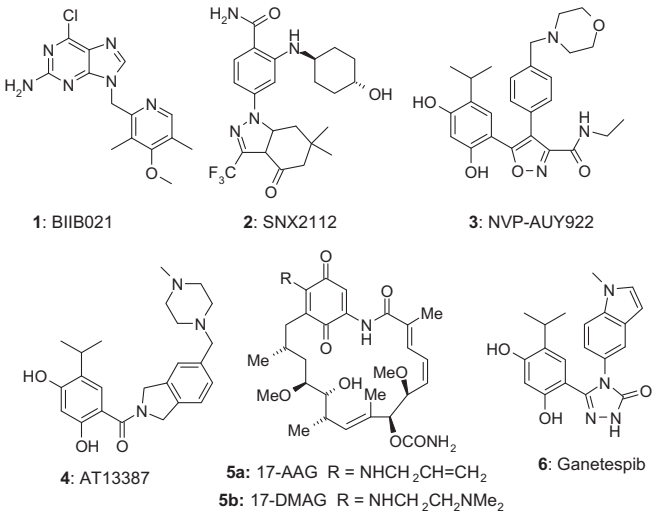


Figure 2. Disclosed structures of Hsp90 inhibitors in clinical trials.

synthetic inhibitors of Hsp90 function, such as the purine derivative PU3[23] (Fig. 1) has been followed by extensive research in the field[24–28] and led to a number of synthetic small molecule inhibitors entering the clinic[29,30] (Fig. 2).

We have previously disclosed a novel series of Hsp90 inhibitors based on a 4-aryl-5-cyanopyrrolopyrimidine scaffold.[31] The relevance of this core for Hsp90 inhibition has since been independently confirmed.[32] Here, we provide a full account of the structure-based design and detailed biological characterization of compounds from this chemotype.

2. Hit identification, fragment evolution and compound design

Hit identification remains a significant challenge in drug discovery and fragment-based screening has emerged as an efficient way to sample chemical space that can complement or replace other Hit ID techniques such as high throughput and in silico screening.[33–36] Combining fragment screening with structure-based design can provide a powerful strategy for drug discovery[37] and indeed fragment-based approaches have been extensively used in Hsp90 discovery programmes.[38]

We recently described a series of 2-aminothieno[2,3-*d*]pyrimidine inhibitors (exemplified by **9**; Figs. 3 and 6) that were designed by combining structural information from hits identified from fragment-based screening campaigns (e.g., **7** and **8**; Fig. 3) with that derived from in silico screening hits.[39]

Other hits identified from the fragment screening included purine compounds **10**–**12** (Fig. 3). Interestingly, X-ray structures revealed that these purine fragments bound to Hsp90 in an orientation different from that previously seen with amino-purines and

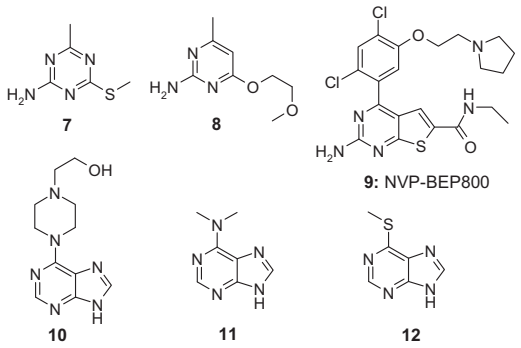


Figure 3. Fragment hits (**7**, **8** and **10**–**12**) identified by ^{1}H NMR screening, and the pre-clinical Hsp90 inhibitor NVP-BEP800 (**9**). Fragments **10**–**12** were starting points for Hsp90 inhibitors reported here.

amino-pyrimidine such as **1**, **PU3**, **8** and **9** and the natural co-factor analogue ADP-NP.[40] Figure 4A shows the different orientations of the purine moieties in fragment **11** and **PU3** when they bind the ATP site of Human Hsp90α. Such differences probably arise because compounds **10**–**12** do not have an exocyclic N–H amino group available to hydrogen-bond to Asp93, contrary to compounds **1**, **8** and **9**. Importantly, the new binding mode presented by the purine in fragments **10**–**12**, and the associated vectors for derivatisation, suggested that they could be exploited in the design of novel Hsp90 inhibitors. This has been achieved (see below), illustrating the great versatility of fragments as starting points for medicinal chemistry.

These structural data combined with in-house experience with earlier series led to the design and synthesis of simple 4-aryl derivatives (**13a**–**c**) that displayed moderate affinity to Hsp90 (Table 1).

The imidazole moiety in compounds **10**–**12** and **13a**–**c** donates a key hydrogen-bond to the carboxylate of Asp93 (Fig. 4A). This requires that the purine adopts a favourable tautomer on the imidazole nitrogens, while the alternative tautomer removes this interaction. To force location of the proton on the nitrogen hydrogen-bonding to Asp93, we synthesized an analogous series of 4-aryl-[2,3-*d*]pyrrolopyrimidines (**14a**–**c**, Table 1), since these compounds necessarily contain the required N7–H hydrogen bond donor. Yet, the replacement of N with C–H in the purine to pyrrolopyrimidine modification was clearly detrimental to binding affinity (Table 1). This was attributed to a change in the hydrogen-bond interactions between the ligand cores (purine or pyrrolopyrimidine) and the structurally conserved water molecules (Fig. 4A) that have been characterized in many ligand-Hsp90 structures.

The purine to pyrrolopyrimidine modification replaces an O–H···N hydrogen bond between water 2 and purine with an O–H···H–C5 steric clash between water and pyrrolopyrimidine, probably explaining the loss of affinity for compounds **14a**–**c**. However, this analysis raised the possibility that water 2 may be replaced to positive effect with respect to affinity by substituting the pyrrolopyrimidine core at position 5. This reasoning was based on the realization that (a) waters 2 and 3 are in a semi-hydrophobic environment (Leu48, Ile91 and Val186), a priori not the most favorable environment for water molecules; (b) displacing 'trapped' water molecules may sometimes have an entropic advantage;[41] (c) replacing a water may enable additional favorable direct interactions between ligand and protein. Computational chemistry identified a number of small groups that had potential for the investigation of this SAR with the cyano group being prioritized from the outset. The cyano group was preferred because it was predicted that it would maintain a key hydrogen-bond with the Asn51 side chain (Figs. 4B and 5). Indeed, analysis of the hydrogen-bond network formed by the conserved waters strongly suggested that

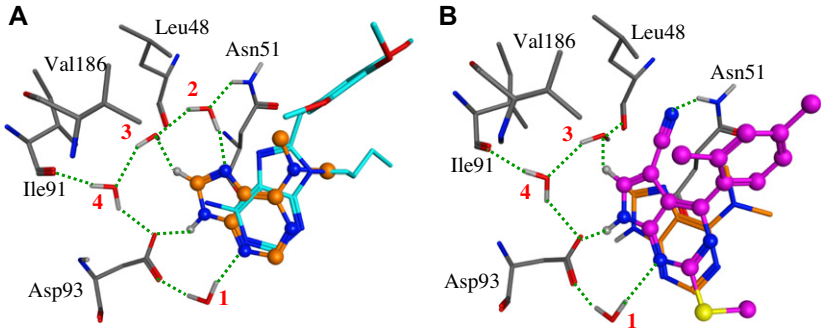
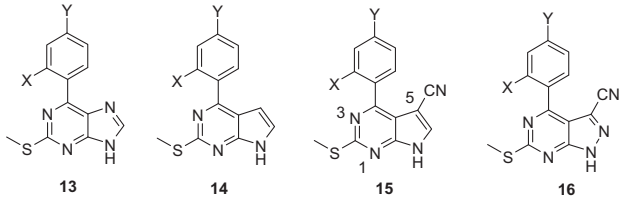


Figure 4. Design of the cyanopyrrolopyrimidine core from the initial amino-purine fragment hit, illustrated with X-ray crystal structures of the N-terminal domain of human Hsp90α in complex with representative compounds. The hydrogens were modeled on the X-ray coordinates so as to maximize the number of hydrogen-bonds between compound, conserved waters and protein. Hydrogen bonds are depicted as green dotted lines. For consistency, the water molecules are numbered as previously assigned.[32] **Panel A**: The initial fragment hit **11** [PDB code: 4FCP] (orange carbons, ball and stick) bound to the ATPase site of Hsp90, overlaid with the Hsp90 inhibitor PU3 [PDB code: 1UYM] (light blue carbons, sticks). The adenine moiety of **11** adopts an orientation different from the orientation of the adenine in PU3. The four conserved water molecules are shown and numbered with red labels. Residues Leu48, Ile91 and Val186 line the cavity which contains conserved water molecules 2–4. Water 2 accepts a hydrogen-bond from the side-chain amide of Asn51 and donates a hydrogen-bond to a nitrogen of **11**. **Panel B**: The cyanopyrrolopyrimidine **15a** [PDB code: 4FCQ] (magenta carbons, ball and stick), overlaid with the initial purine fragment **11** [PDB code: 4FCP] (orange carbons, sticks). Conserved water 2 was displaced by the nitrile group of **15a**, which accepts a hydrogen-bond from the side-chain amide of Asn51. Note also the plausible unconventional hydrogen-bond donated by the C6–H group of **15a** to water 3.

Table 1
Binding affinities of selected purines, pyrrolopyrimidines and pyrazolopyrimidines by fluorescence polarization (FP), surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) assays[a]



ID	X	Y	FP K_i[b] (μM)	SPR K_d (μM)	ITC K_d (μM)
13a	Me	Cl	0.661	>20	–
13b	Me	F	12.8	2.30	2.73
13c	H	CN	24.5	>20	–
14a	Me	Me	>200	>100	–
14b	Me	F	>200	>100	–
14c	H	CN	>200	>100	–
15a	Me	Me	0.195	0.101	0.156
15b	Me	F	0.385	0.430	0.251
15c	H	CN	0.204	1.22	–
16	Me	Me	5.23	38	–

[a] All results mean of at least two independent determinations.
[b] K_i calculated from IC_{50} values.

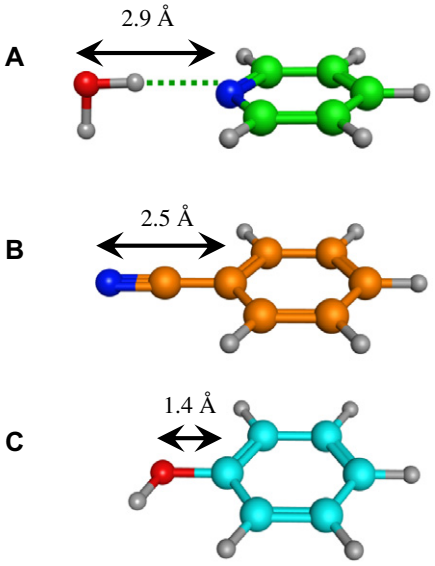


Figure 5. Simple geometric considerations regarding the displacement of water molecules by substituents of aryl groups. Three model systems are shown, after geometry optimization with the Merck molecular force-field[44]: (A) hydrogen-bond (green dotted line) between pyridine and a water molecule; the O···N distance is 2.9 Å; (B) benzonitrile, with a N≡C–C covalently bonded length of 2.5 Å; (C) phenol, with a O–C bond length of 1.4 Å. The aromatic rings are aligned vertically across the three systems, to facilitate the visual comparison of the noted lengths.

the oxygen of water 2 accepted a hydrogen-bond from the side-chain amide group of Asn51. Modeling indicated that the nitrile would project its nitrogen to a position similar to that of the oxygen of water 2. Other, shorter groups such as methyl, chlorine or hydroxyl would displace the water molecule, but without replacing its interaction with Asn51 (Fig. 5). In addition, a cyano substituent had the potential to further polarize the N7–H bond, enhancing the N7–H···Asp93 hydrogen-bond.

The present compound evolution suggests some generally interesting geometrical insights regarding the practical displacement of a water molecule which donates a hydrogen bond to a heteroatom acceptor in an aryl ring. This refers to water 2 donating a hydrogen bond to an imidazole nitrogen in compound **11** (Fig. 4A), and the replacement of this interaction with a nitrile group as in compound **15a** (Fig. 4B). This situation can be represented more generally with the simplified model compounds shown in Fig. 5. Figure 5A shows a water molecule donating a hydrogen-bond to a pyridine nitrogen; as expected, the O···N hydrogen-bonding distance is about 2.9 Å. Importantly, this distance is similar to the corresponding distance of 2.5 Å obtained with a cyano group (Fig. 5B). Thus, a cyano substituent puts its nitrogen in a position similar to that of the displaced water oxygen. Hence, the cyano substituent has the right length to provide an equivalent of the O···N interaction, especially if the water oxygen is also accepting a hydrogen-bond from a third group (e.g., Asn51 in Fig. 4A). In contrast, a hydroxyl substituent (Fig. 5C) is too short to bring its oxygen in a position equivalent to that of the water oxygen. In other words, a hydroxyl substituent is not expected to be a very good substitute for the initial hydrogen-bonded water, either sterically or electronically. This is worth noting since, based on the obvious analogy between a water and a hydroxyl, chemical intuition might suggest a hydroxyl group to displace the hydrogen-bonded water molecule. However, simple geometric considerations indicate that

a nitrile group may frequently be a more suitable choice to displace/replace a neighbouring water molecule. Indeed, there are other examples where a nitrile group added to a ligand was found to be very efficient way to displace water molecules.[42] In addition, the nitrile is a generally biocompatible functional group, incorporated in many drugs.[43]

We selected compounds **15a–c** (Table 1) as initial target compounds, based on their structural analogy to **13a–c** and **14a–c**. The conservation of substituents X and Y across the sub-series **13–16** enabled interpretation of SAR pertaining to the variation in the fused bicyclic core. Data presented in Table 1 showed that, compared to analogous purines, 5-cyanopyrrolo[2,3-*d*]pyrimidines displayed a significant and consistent enhancement of affinity, by three independent assay techniques.

We also considered a number of other 5-position substituents on the pyrrolo[2,3-*d*]pyrimidine core. As expected, data presented in Tables 1 and 2 show that no analogue matched the exquisite affinity of the cyano compounds **15a–c**, for reasons that can be understood by simple molecular recognition considerations. It is important to note that the nitrile provides a significant affinity gain with minimal addition of molecular weight to the compound and thus contributes to the high ligand efficiency observed (LE for **15b** = 0.41 kcal/mol/heavy atom).

A small set of analogous 5-cyanopyrazolopyrimidines were also made (e.g., **16**, Table 1) but these compounds had considerably lower affinity for Hsp90 than the analogous pyrrolopyrimidines. This reduction in binding affinity may be explained by an electrostatic clash between the oxygen of conserved water molecule 3 and the introduced N-6 atom in the pyrazolopyrimidines. Instead, in the pyrrolopyrimidines there is a favourable interaction with hydrogen-bond character between the pyrrole C6-H and the water oxygen (Fig. 4). Modeling of small aliphatic substituents on C6 was attempted, with the intention to displace conserved water 3. However, this exercise suggested that these substituents may clash with the protein and therefore these compounds were not made. A recent publication reporting an independent discovery of pyrrolo[2,3-*d*]pyrimidines has confirmed the reduced interest of such substituents.[32]

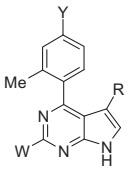
Following the successful strategy of targeting water 2 with cyanopyrrolopyrimidines **15a–c**, we initiated a programme of lead optimization on this series. Encouragingly, the high affinity of the new series led to moderate anti-proliferative activity by in vitro cancer cell proliferation assays (**15a** GI_{50} = 11.6 μM, BT474 cell line). Subsequent activity focused on enhancing cellular activity and achieving a compound profile suitable for oral dosing.

3. Compound optimisation and in vitro characterisation

Inspection of the crystal structures of Hsp90-bound cyanopyrrolopyrimidines and overlays with ligands from other series such as thienopyrimidine **9** (Fig. 6) indicated there were two main vectors for the elaboration of the cyanopyrrolopyrimidine series; (a) the 5′ position of the 4-aryl group (as used to effect in the programme leading to identification of **9**) and (b) the 2-position of the pyrrolopyrimidine core, via sulphur or other linking atom. Substitution at the para position of the 4-aryl group was limited to small moieties (Table 2) due to steric constraints imposed by the protein. Figure 6 shows the overlay of **15a** with **9**, illustrating these points. Subsequent elaboration via either or both the preferred vectors afforded a number of compounds with high affinity for Hsp90 and greatly improved anti-proliferative properties (Table 3).

These initial modifications however, generated many compounds with poor metabolic stability and an undesirable hERG binding profile (Table 4) which was possibly due to the incorporation of an alkyl amino functionality. However the S-acetamide compounds such as **33** (Table 3) had a somewhat more promising metabolic profile and further optimization identified the S-acetamide series **36a–36r** (Table 5). The data in Table 5 demonstrates that excellent anti-proliferative activity could be achieved in compounds that did not contain an amino solubilising group. This significantly reduced the propensity for hERG binding as seen for compounds **36q** and **36r** in Table 4. Compound **36r** was further characterized by isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR). The ITC-derived K_d for **36r** was <3 nM

Table 2
Binding affinities of selected 5-substituted pyrrolo[2,3-*d*]pyrimidines by fluorescence polarization (FP), surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) assays[a]



ID	W	Y	R	FP K_i[b] (μM)	SPR K_d (μM)	ITC K_d (μM)
14a	SMe	Me	H	>200	>100	—
14b	SMe	F	H	>200	>100	—
17	SMe	Me	Br	>200	>20	—
18	SMe	Me	Me	>200	19.3	—
19	SMe	Me	CF_3	2.64	55	—
20	SMe	Me	Cyclo-propyl	>50	>100	—
21	SMe	F	$CONH_2$	>200	>100	—
22	H	Me	CN	2.83	1.88	1.62
15b	SMe	F	CN	0.358	0.430	0.251
23	OMe	F	CN	0.628	2.81	0.725
24	NHMe	F	CN	4.07	4.26	3.69
25	Et	F	CN	1.72	2.22	1.83

[a] All results mean of at least two independent determinations.
[b] K_i calculated from IC_{50} values.

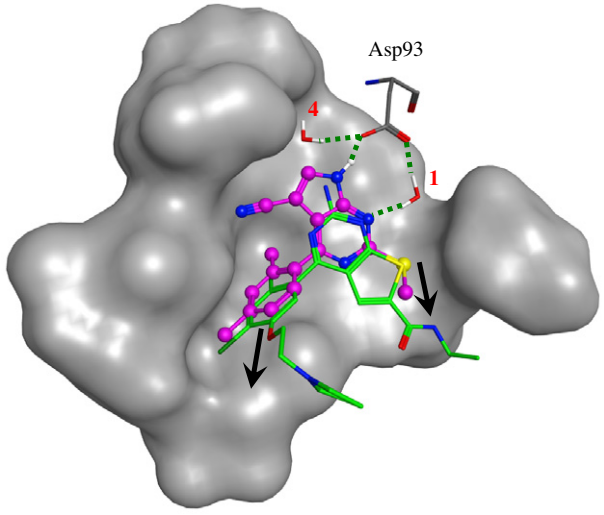
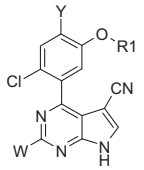
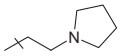
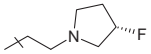
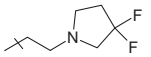
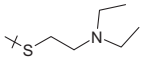
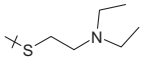
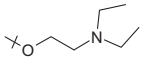
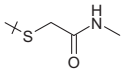
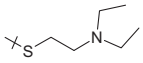
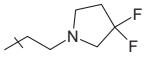


Figure 6. Vectors available for elaboration of the cyanopyrrolopyrimidine series when bound to Hsp90α (grey surface). The cyanopyrrolopyrimidine series is represented by **15a** [PDB code: 4FCQ] (magenta carbons, ball and stick). It is overlaid on compound **9** [PDB code: 2WI7] (green carbons, sticks), a representative of a previously reported 2-aminothieno[2,3-*d*]pyrimidine series of extensively elaborated Hsp90 inhibitors.[39] The overlay is based on X-ray structures, on which hydrogens were modeled and shown selectively (white). The protein is represented as a grey molecular surface around the biding site, to emphasize the tight structural fit between Hsp90 and the cyanopyrrolopyrimidine chemotype. In this context, the two positions (indicated with black vectors) particularly amenable for derivatisation of the cyanopyrrolopyrimidine were the 2-position of the pyrrolopyrimidine core and the 5′-position of the 4-aryl group. Asp93 is shown for orientation, with selected hydrogen bonds (green dotted lines). Conserved water molecules 1 and 4 are labeled.

Table 3
Affinity (FP) and anti-proliferative data (BT474 cells) for elaborated cyanopyrrolo[2,3-*d*]pyrimidines[a]



ID	W	Y	R1	FP K_i[b] (nM)	GI_{50} (BT474) (nM)
26	SMe	Cl		4	48
27	SMe	Cl	$CH_2CH_2NEt_2$	2	31
28	SMe	OMe		3	31
29	SMe	OMe		12	47
30		Cl	Me	<1	72
31		OMe	Me	7	26
32		Cl	Me	62	2010
33		Cl	$CH_2CH_2NEt_2$	8	78
34		CN	Me	<1	80
35		OMe		172	388

[a] All results mean of at least two independent determinations.
[b] K_i calculated from IC_{50} values.

Table 4
hERG binding[a] and patch clamp[b] data for selected compounds

ID	hERG binding IC_{50} (μM)	hERG patch clamp IC_{50} (μM)
26	3.64	—
27	1.71	—
30	1.40	—
33	3.14	17.8
34	1.07	—
36q	>30	>30
36r	>30	>30

[a] hERG binding IC_{50} determined in a ^{3}H-dofetilide assay.
[b] Effect on ionic currents in voltage-clamped HEK293 cells that stably express the hERG channel.

and the enthalpy of binding (ΔH) was −10.3 kcal/mol versus −5.5 kcal/mol for the less elaborated compound **15b**. The SPR-derived K_d for **36r** was 0.4 nM with a measured dissociation rate (K_{off}) of 0.0006 s^{-1} (vs 0.20 s^{-1} for **15b**).

One compound that showed potent anti-proliferative effects across a range of cell lines was the *N*,*N*-dimethyl amide derivative **36r**. Figure 7 shows the X-ray crystal structure for compound **36r** bound to Hsp90α. This demonstrates that the same binding pose of the core is maintained through series elaboration and highlights further interactions such as a water-mediated interaction from methoxy substituents to Phe138. Interestingly there is a similar interaction made from a phenolic oxygen in thienopyrimidine **9**.[39]

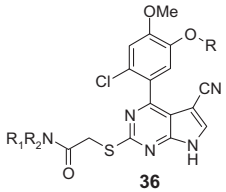
4. Synthetic chemistry

The pyrrolo[2,3-*d*]pyrimidine scaffold was constructed via a modified literature route[45] (Scheme 1) generating compound **41**, which was readily converted to the SEM protected halide **42**. At this point Suzuki cross coupling reactions could be used to introduce the 4-aryl moieties, followed by 3-bromination and conversion to 3-cyano compounds via Pd-mediated cyanation (**42** to **15** via **47**; Scheme 1). However this reaction proved capricious and we routinely used the longer, but more robust route shown, via the carboxylic acid **44**.

The compounds shown in Table 5 and compounds **30**–**35** in Table 3 were synthesized by a similar general route (Scheme 2) which utilized the SMe moiety as a versatile chemical handle by converting it to the electrophilic sulphone. Suzuki cross-coupling of **46** (Scheme 1) with MOM protected boronic acid **48a** (formed in situ from the corresponding bromide), afforded the 4-aryl derivative **49**. Oxidation of the sulphide to corresponding sulphone **50** enabled displacement by ethyl thioglycolate followed by elaboration to the desired amides **53**. Intermediate **50** could also be reacted with oxygen, carbon and nitrogen based nucleophiles, though these compounds were not as potent as the analogous sulphides. Removal of the MOM protecting group followed by either alkylation with alkyl iodides then TBAF mediated SEM removal or sole SEM removal (as appropriate) provided compounds **36(a–r)**. Several compounds from this series used the MEM protected compound **48b** as a starting point, with the subsequent transformation as described in scheme 2 affording similar chemical yields.

Pyrrolopyrimidine compounds substituted with other groups at the 5-position made use of key intermediates **47a** and **44** (Scheme 3). Thus the 5-bromo compound **47a** was a versatile intermediate for coupling and lithiation chemistry to furnish compounds **18**–**20**, whilst **55** was employed via Suzuki coupling to afford **21.** Intermediate **57** enabled the preparation of the 2-OMe,

Table 5
Binding affinity (FP) and anti-proliferative data (three cancer cell lines) for S-acetimido-4-aryl-5-cyanopyrrolo[2,3-*d*]pyrimidines[a]



36

ID	R	NR_1R_2	FP K_i[b] (nM)	BT474 GI_{50} (nM)	HCT116 GI_{50} (nM)	MDA-MB-468 GI_{50} (nM)
36a	Me		<1	69	60	62
36b	H		<1	102	1160	236
36c	Et		<1	25	20	23
36d	H	*i*-PrNH	3	161	238	178
36e	Me	EtNH	2	141	42	59
36f	Et	EtNH	<1	44	40	64
36g	H	*t*-BuNH	<1	128	117	152
36h	Me	*t*-BuNH	<1	121	50	84
36i	Et	*i*-PrNH	<1	84	65	77
36j	Me	*i*-PrNH	<1	81	81	102
36k	Me	$F(CH_2)_2NH$	5	136	98	178
36l	H	$F(CH_2)_2NH$	2	85	728	183
36m	Me	CF_3CH_2NH	<1	45	153	124
36n	H	Azetidine	<1	21	172	28
36o	Me	Azetidine	<1	31	28	35
36p	Me	MeNH	<1	56	42	33
36q	H	Me_2N	<1	20	131	23
36r	Me	Me_2N	<1	34	32	30

[a] All results mean of at least two independent determinations.
[b] K_i calculated from IC_{50} values.

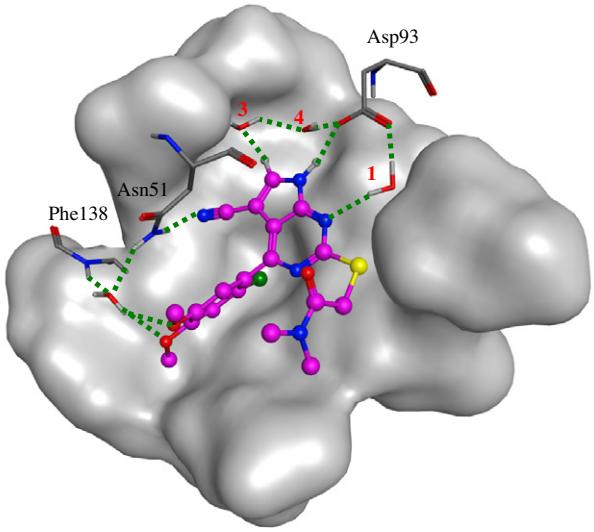


Figure 7. X-ray structure of cyanopyrrolopyrimidine **36r** [PDB code: 4FCR] (magenta carbons, ball and stick) bound to Hsp90α (grey surface). Hydrogen atoms were modeled on the X-ray structure and are shown selectively. The surface rendering of the protein emphasizes the excellent structural fit between Hsp90 and compound **36r**. Selected hydrogen-bonds (green dotted lines) are shown, in particular between **36r**, Asp93, Asn51, and water molecules of particular interest. Structurally conserved water molecules 1, 3 and 4 are labeled. A water molecule is also shown, which hydrogen-bonds the two methoxy groups of **36r**, the backbone of Phe138 and the side-chain of Asn51. The Asn51 side-chain also hydrogen-bonds the nitrile of **36r**.

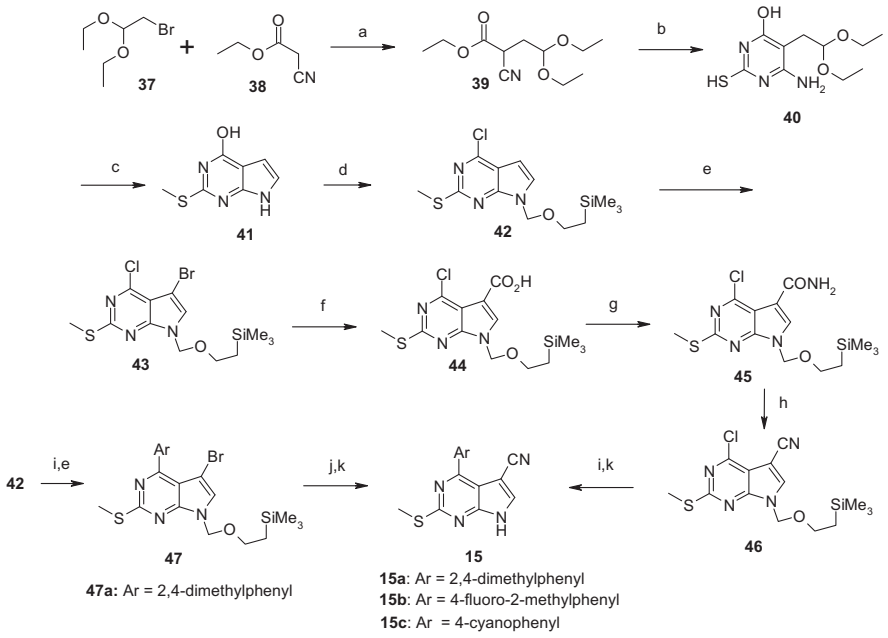
NHMe and ethyl analogues of the 2-*S*-methyl derivative **15b** (**23**–**25**, Table 2), by again making use of the sulphone moiety for nucleophilic displacement reactions (Scheme 3).

Other elaborated compounds from Table 3 were prepared as shown in Scheme 4. The procedures and transformations are similar to those already described but with some variety in the sequence of the compound construction. Mitsunobu reactions or phenolic alkylations with amino-alkyl halides were used to prepare the 5′ aryl phenolic substituents, either prior to or post coupling to the cyano-pyrrolopyrimidine scaffold. Scheme 5 illustrates how the analogous purines, 5-H pyrrolopyrimidines and pyrazolo-pyrimidines from Table 1 were prepared. Compounds **14a**–**c** used the same coupling and de-protection strategy as already described for **15a**–**c**, but using the intermediate **42**. Cross coupling reactions were also used to construct purines **13a**–**c** and pyrazolo derivative **16**.

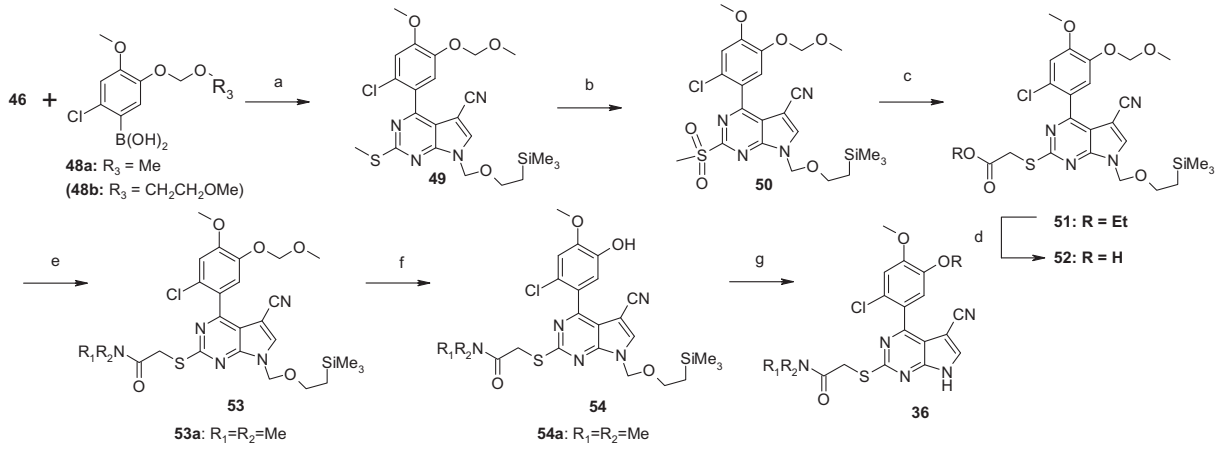
5. Pharmacodynamic and pharmacokinetic studies

To establish that the cyanopyrrolo[2,3-*d*]pyrimidine class of compounds retained the molecular signature of known Hsp90 inhibitors, we studied the effect of selected compounds **36q**, **36r** and **28** on cellular pharmacodynamic biomarkers compared to the thienopyrimidine compound **9**. Figure 8 shows that the representative compounds gave the expected depletion of the client proteins Her2, pHer2, pAKT and AKT and induction of the Hsp70 co-chaperone.

The pharmacokinetic (PK) profile following oral dosing was evaluated for cell active compounds **36q** and **36r** in the mouse (Table 6). In summary **36r** was bioavailable via the oral route of administration. The exposure of **36r** in plasma was moderate and the half life (>1 h) can be considered reasonable for the mouse. In contrast however **36q** was very poorly bioavailable via the oral route of administration (Table 7). The exposure of **36q** in plasma and kidney was very low and the half life could not be determined; **36q** was detected also in muscle, but at levels below the limit of quantification. The compounds differ by only one methyl group (methoxy in **36r** to hydroxyl in **36q**) and glucuronidation of the free phenolic OH in **36q** during first pass metabolism may explain



Scheme 1. Synthesis of 4-aryl-5-cyanopyrrolopyrimidines. Reagents and conditions: (a) Bu_4NBr, K_2CO_3, DMF, 90 °C, 40–55%; (b) thiourea, NaOEt, EtOH, reflux, 50%; (c) (i) NaOH (aq), dimethylsulphate, rt, (ii) HCl (aq), rt, 92%; (d) (i) $POCl_3$, 100 °C, 81%, (ii) SEM-chloride, NaH, DMF, 0 °C to rt, 86%; (e) NBS, DMF, rt, 70%; (f) *n*-BuLi, THF, CO_2, −78 °C to rt, 91%; (g) (i) CO_2Cl_2, CH_2Cl_2, DMF (cat), (ii) NH_3 (aq), rt, 77%; (h) TFAA, Et_3N, CH_2Cl_2, 0 °C to rt, 86%; (i) $ArB(OH)_2$, $NaHCO_3$, $Pd_2Cl_2(PPh_3)_2$, DMF, 80 °C; (j) CuCN, dppf, $Pd_2(dba)_3$, 1,4-dioxane, 100 °C; (k) TBAF, $H_2N(CH_2)_2NH_2$, THF, 50 °C.

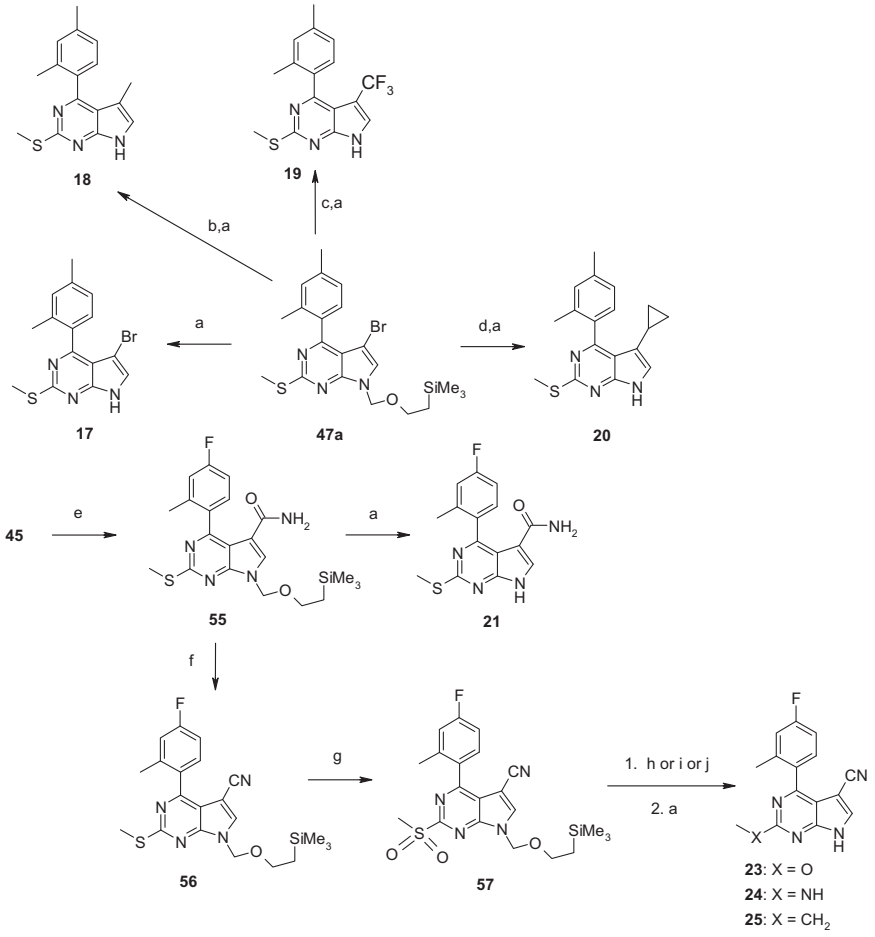


Scheme 2. Synthesis of 4-aryl-2-acetamido-5-cyanopyrrolo[2,3-*d*]pyrimidines. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, $NaHCO_3$, DMF, 80 °C, 1.5 h, 98%; (b) *m*CPBA, CH_2Cl_2, 0 °C to rt, 1 h, 80%; (c) ethyl thioglycolate, NaH, THF, 0 °C to rt, 1 h, 96%; (d) NaOH (aq), rt, 2 h; (e) HNR_1R_2, HBTU, MeCN, rt; (f) PPTS, *i*-PrOH, 85 °C, 16 h; (g) (i) RI, Cs_2CO_3, DMF, rt, 2 h and/or (ii) TBAF, $H_2N(CH_2)_2NH_2$, THF, 40 °C.

the significant difference in PK profiles. This hypothesis is supported by UGT intrinsic clearance (UGT CL_{int}) data from a microsomal incubation assay formatted to measure clearance via glucuronidation by UGT.[46] In this assay the UGT intrinsic clearance for **36r** was 4 μL/min/mg. This contrasts with a much higher value (UGT CL_{int} = 73 μL/min/mg) for **36q** (corresponding to a high extraction ratio of 0.8).

6. Conclusion

In summary we have identified a series of 4-aryl-5-cyanopyrrolo[2,3-*d*]pyrimidine ATP competitive Hsp90 inhibitors, using start points from a ^{1}H NMR screen of a proprietary fragment library. Ligand-Hsp90 X-ray crystallography in conjunction with molecular modeling studies led to rational displacement of a selected conserved water molecule by the inhibitors, which increased their binding affinity efficiently with the addition of a single nitrile group. Importantly, this affinity gain was obtained with minimal addition of molecular weight, and this decisively influenced the choice of core for subsequent elaboration by medicinal chemistry. Further structure-based optimization led to a series of high affinity compounds, examples of which displayed sub 100 nM GI_{50} values in anti-proliferative assays in several human cancer cell lines in vitro. One compound was additionally established to be bioavailable in the mouse following oral dosing. The study highlights the importance of precise consideration of water molecules in



Scheme 3. Synthesis of 5- and 2-substituted pyrrolo[2,3-*d*]pyrimidines. Reagents and conditions: (a) TBAF, $H_2N(CH_2)_2NH_2$, THF, 40 °C; (b) (i) *n*-BuLi, THF, −78 °C, (ii) MeI, −78 °C to rt; (c) CF_3CO_2Na, CuI, DMF, toluene, 170 °C, 16 h; (d) cyclopropyl boronic acid, $Pd(OAc)_2$, $P(Cy)_3$, K_3PO_4, toluene, H_2O, 100 °C, 2 h; (e) 2-methyl-4-fluoro-phenyl boronic acid, $NaHCO_3$, $Pd_2Cl_2(PPh_3)_2$, DMF, 80 °C; (f) TFAA, Et_3N, CH_2Cl_2, 0 °C to rt; (g) *m*CPBA, CH_2Cl_2, 0 °C to rt, 1 h; (h) KO^tBu, MeOH, THF, 0 °C; (i) $MeNH_2$, THF, DMF, 100 °C, 5 h; (j) EtMgBr, Et_2O, 0 °C.

ligand–protein binding and the potentially special role of the nitrile group in displacing and replacing such molecules.

7. Experimental

7.1. Fluorescence polarization assay

The ability of compounds to compete with a fluorescently labeled probe for binding to full length human Hsp90β was determined essentially as described previously[47] but with the following modifications: firstly, a modified probe (VER-51001) was used that binds to Hsp90 with a greater affinity that that described previously, allowing the accurate determination of IC50 for higher affinity compounds; secondly, a N-terminally truncated form of human Hsp90-alpha was used due to its more robust performance in FP assays. Synthetic procedures for VER-51001 and details relating o the FP assay can be found in the Supplementary data section.

7.2. Cell growth and client protein degradation

Growth inhibition assays using the SRB method were undertaken as previously described.[48] Her2-postive BT474 breast cancer cells were treated with the indicated concentrations of Hsp90 inhibitors for 24 h and client protein degradation determined by western blotting. Her2 (Abcam), pHer2 (Y1248, Abcam), AKT (Cell Signalling Technologies), pAKT (S473, Cell Signalling Technologies), HSP70 (Stressgen) and GAPDH (Cell Signalling Technologies) levels were detected using chemiluminescence and imaged on an LAS-4000 (Fuji Instruments). GAPDH levels were determined to show equal protein loading.

7.3. Pharmacokinetics

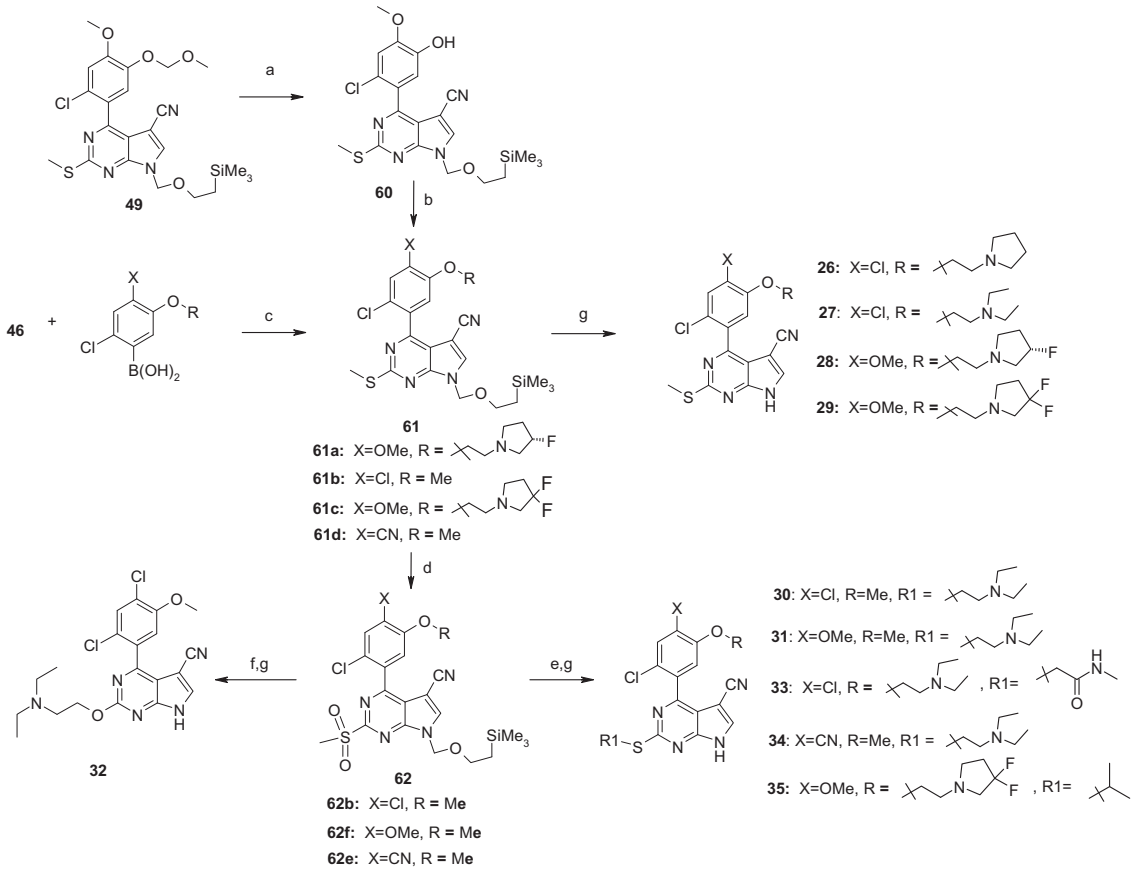
Male Balb-C mice received a single 5 mg/Kg dose of compound via oral gavage in 1% methyl cellulose (w/v). Plasma was prepared from terminal blood samples for analysis. LC–MS/MS was used for quantitative bioanalysis and pharmacokinetic analysis was performed using WinNonLin (Pharsight).

7.4. Glucuronidation assay

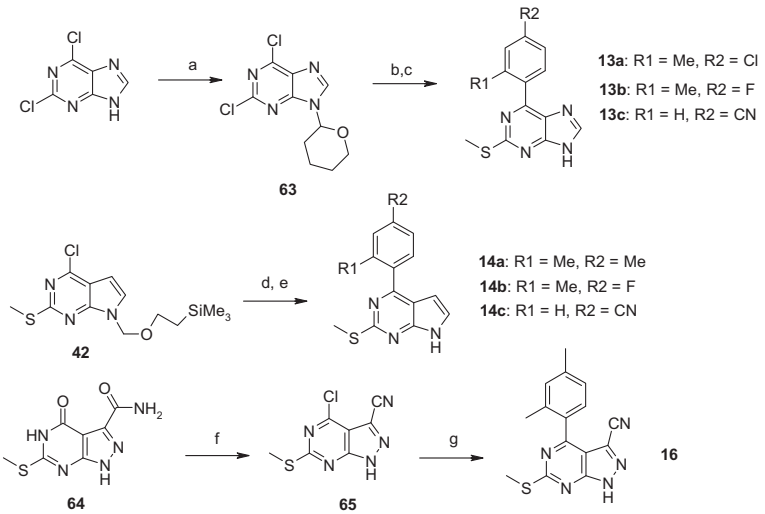
Rate of glucuronidation was measured by incubating test compounds in human liver microsomes supplemented with $MgCl_2$, alamethicin and UDPGA. A time course was run over 30 min. Individual wells for each time point were stopped by the addition of acetonitrile, samples were centrifuged and the supernatant analyzed by LC–MS/MS. The rate of compound disappearance was scaled to intrinsic clearance (UGT CL_{int}) and a UGT extraction ratio was generated for rank-ordering.

7.5. Isothermal titration calorimetry (ITC)

The ITC measurements were performed using an iTC200 instrument (Microcal, GE Healthcare). The feedback mode was 'low' with



Scheme 4. Synthesis of elaborated 5-cyano-pyrrolo[2,3-*d*]pyrimidines. Reagents and conditions: (a) PPTS, *i*-PrOH, 85 °C, 3 h, 82%; (b) DIAD, PPh_3, THF, ROH, 0 °C to rt; (c) $NaHCO_3$, $Pd_2Cl_2(PPh_3)_2$, DMF, 80 °C; (d) *m*CPBA, CH_2Cl_2, 0 °C to rt; (e) R1SH, Et_3N, 100 °C, 30 min.; (f) $Et_2N(CH_2)_2OH$, Et_3N, DMF, 100 °C, 30 min.; (g) TBAF, $H_2N(CH_2)_2NH_2$, THF, 40 °C.



Scheme 5. Synthesis of scaffold analogues of 5-cyano-pyrrolo[2,3-*d*]pyrimidines. Reagents and conditions: (a) (i) PTSA, EtOAc, (ii) 3,4-dihydro-2*H*-pyran, 50 °C, NH_3 (aq), 73%; (b) $ArB(OH)_2$, K_2CO_3, DMF, $(Ph_3P)_4Pd$, 110 °C, microwave; (c) NaSMe, DMF, 120°C, microwave, 15 min; (d) $ArB(OH)_2$, $NaHCO_3$, $Pd_2Cl_2(PPh_3)_2$, DMF, 80 °C; (e) TBAF, $H_2N(CH_2)_2NH_2$, THF, 40 °C; (f) $POCl_3$, *N*,*N*-dimethylaniline, Δ, 4 h; (g) 2,4-dimethylphenylboronic acid, K_3PO_4 (aq), $Pd(dppf)_2Cl_2$, 1,4-dioxane, 90 °C, 6 h.

reference power setting of 4 μCals^{-1}. The cell was stirred at 1000 rpm and thermostated at 25 °C. All experiments were performed using the dialysis buffer (see below) with 1% DMSO (v/v). All experiments were conducted with 10 μM ligand in the cell and 100–200 μM protein in the syringe. The experiments were conducted with 8–16 injections, with volumes of 4.75–2.37 μL, respectively, and 300 s spacing. The first 'waste' injection of 1.2 μL was discarded in all cases. All data were fitted to a one site model using the provided software.

Protein (6 mg/ml) was dialysed with stirring overnight at 4 °C, in 12.5 mM phosphate pH 7.4, 37.5 mM NaCl, 0.5 mM EDTA, 0.05% Triton-100. Upon recovery the protein was filtered

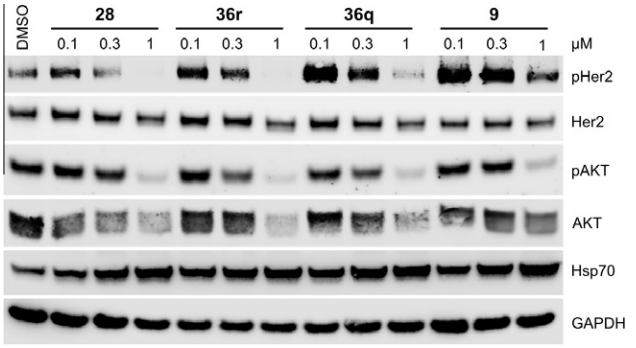


Figure 8. Western blot showing effects on PD biomarkers in BT474 cells of Hsp90 inhibitors **9**, **36q**, **36r** and **28** at 0.1, 0.3 and 1.0 μM compound concentration after 24 h incubation. GAPDH was used as a loading control.

Table 6
PK profile for **36r** following dosing at 5 mg/Kg po in Balb-C mouse

Parameter	Plasma	Muscle	Kidney
AUC (0–∞, ng/mL h)	297	231	6915
Half life (h)	1.4	2.4	1.2
C_{max} (ng/mL)	239	68	5389
C_{max} (μM)	0.553	0.158	12.48
T_{max} (h)	0.25	0.25	0.25
Tissue/plasma ratio	—	0.8	23.3

Table 7
PK profile for **36q** following dosing at 5 mg/Kg po in Balb-C mouse

Parameter	Plasma	Kidney
AUC (0–2 h, ng/mL h)	16	41
Half life (h)	ND	ND
C_{max} (ng/mL)	11	31
C_{max} (μM)	0.025	0.072
T_{max} (h)	0.25	0.50
Tissue/plasma ratio	—	2.6

ND = not determined.

(0.22 μM spin filter). The protein concentration was determined by UV absorbance spectroscopy at 280 nM using an extinction coefficient of 15900. The dialysis buffer was degassed; this was then used for subsequent preparation of protein and ligand solutions for the titration experiments. For each experiment the ligands were freshly prepared from a 20 mM DMSO stock solution.

7.6. Surface plasmon resonance (SPR)

SPR measurements were performed on a BIAcore T200 instrument (BIAcore GE Healthcare). All experiments were performed on Series S NTA chips (certified) according to provider's protocols with 10 mM HEPES pH 7.4, 150 mM NaCl, 25 M EDTA, 0.05% Tween-20 and 1% DMSO as a running buffer. The surface was generated using HSP90 aa9-236 construct containing on its N-terminus three consecutive hexa-Histidine tags (mhhhhhhhhgatgstagsgtagstgasgastggtgathhhhhhhhhddddkspmghhhhhhhhhhssghiddddk). Introduction of the multiple oligo-Histidine tag led to robust generation of a stable Hsp90 surface with no detectable drift of a protein from a chip for at least 30 min. The use of double his-tagged proteins to generate stable surfaces has been previously described in Ref. 49. First, 0.5 mM Ni^{2+} was been injected into the experimental channel till 60RU. Subsequently 100 nM HSP90 was injected over the sensor at 10 μL min^{-1} till saturation level of 1500 RU stably bound on the surface. Reference surfaces without immobilized Ni^{2+} were included on the chip to serve as controls for non-specific binding and refractive index changes. The sensor surface was regenerated between experiments as advised by manufacture with additional injections of 0.1 mg/ml Trypsin, 0.5 M Imidazole and 45% DMSO (all at a flow rate of 15 μL min^{-1} 60 s) to eliminate any carry-over of protein and/or analyte. For each concentration in the titration series the surface was prepared as described above. The concentration series was formed using doubling dilutions from the top concentration of at least 5–10 fold above the K_d. All sample measurements were performed at a flow rate of 35 μL min^{-1}. Injection times ranged from 60 to 480 s, dissociations times ranged from 60 to 480 s. Data processing was performed using BIAevaluation 1.1 software (BIAcore GE Healthcare Bio-Sciences Corp.). Sensorgrams were double referenced prior to global fitting of the concentration series to a single step kinetic model.

7.7. Chemistry

7.7.1. 4-Chloro-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxy methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (42)

To a mixture of sodium hydride (60% dispersion in mineral oil; 276 mg; 6.89 mmol) in DMF (10 mL) at 0 °C was added drop-wise a solution of 4-chloro-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine [prepared as detailed in Ref. 45] (1.145 g; 5.74 mmol) in anhydrous DMF (20 mL). When the addition was complete, 2-(trimethylsilyl)ethoxymethyl chloride (1.32 ml; 7.46 mmol) was added drop-wise and the reaction mixture was stirred at 0 °C for 1.5 h then allowed to warm to ambient temperature. The reaction mixture was partitioned between water (100 mL) and ethyl acetate (100 mL). The organic phase was separated, dried over Na_2SO_4 and then filtered and the filtrate solvents evaporated in vacuo. The crude pro0duct was purified by flash chromatography on silica gel (70 g) eluting with a solvent gradient of 0–5% ethyl acetate in hexane to afford the title compound **42** (1.73 g, 91%) as a colourless oil: LC–MS (method A) t_R = 2.88 min; m/z = 332, 330 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6): δ −0.10 (s, 9H), 0.84 (t, 2H, J = 8.1 Hz), 2.53 (s, 3H), 3.52 (t, 2H, J = 8.1 Hz), 5.58 (s, 2H), 6.62 (d, 1H, J = 3.6 Hz), 7.69 (d, 1H, J = 3.6 Hz).

7.7.2. 5-Bromo-4-chloro-2-methylsulfanyl-7-(2-trimethylsila nyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (43)

To a solution of 4-chloro-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**42**, 0.5 g, 1.516 mmol) in DMF (14 mL) at −78 °C was added drop-wise a solution of N-bromosuccinimide (270 mg, 1.52 mmol) in DMF (6 mL). After 5 min the reaction was allowed to warm to ambient temperature. The solution was partitioned between EtOAc (2 × 40 mL) and water (40 mL). The combined organics were passed through a hydrophobic frit and evaporated in vacuo. The crude product was purified by flash chromatography on SiO_2 (50 g) eluting with hexane-5% EtOAc/hexane (gradient) to afford the title compound **43** (433 mg, 70%) as a colourless solid: LC–MS (method A) t_R = 3.11 min; m/z = 410, 408 $[M+H]^+$.

7.7.3. 4-Chloro-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxy methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid (44)

To a solution of *n*-butyl lithium (2.5 M in hexanes, 0.24 ml, 0.59 mmol) in THF (0.5 ml) at −78 °C was added slowly drop-wise a solution of 5-bromo-4-chloro-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**43**, 200 mg, 0.489 mmol) in THF (2 mL). After 2 min, crushed solid CO_2 (excess) was added and the mixture was left to warm to ambient temperature. Acetic acid (2 mL) was added, then water (20 mL) and the mixture extracted with EtOAc (2 × 20 mL). The combined organics were passed through a hydrophobic frit and evaporated in vacuo to

afford the title compound **44** (167 mg, 91%) as a colourless solid: LC–MS (method A) t_R = 2.66 min; m/z = 374 $[M+H]^+$.

7.7.4. 4-Chloro-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid amide (45)

To a solution of 4-chloro-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid (**44**, 100 mg, 0.268 mmol) in CH_2Cl_2 (1.5 mL) was added oxalyl chloride (2 M in CH_2Cl_2, 0.17 mL, 0.349 mmol) followed by a few drops of DMF. After 10 min the reaction mixture was evaporated in vacuo, then re-dissolved in CH_2Cl_2 (3 mL). Aqueous ammonia solution (2 mL) was added and the mixture was stirred vigorously for 15 min. Water (10 mL), and CH_2Cl_2 (10 mL) were added and the resultant phases separated. The aqueous phase was extracted with further CH_2Cl_2 (15 mL). The combined organics were passed through a hydrophobic frit and evaporated in vacuo. The crude product was purified by flash chromatography on SiO_2 (20 g) eluting with CH_2Cl_2-5% MeOH/CH_2Cl_2 (gradient) to afford the title compound **45** (77 mg, 77%) as a yellow solid: LC–MS (method A) t_R = 2.47 min; m/z = 375, 373 $[M+H]^+$.

7.7.5. 4-Chloro-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (46)

To a solution of 4-chloro-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid amide (**45**, 73 mg, 0.196 mmol) in CH_2Cl_2 at 0 °C was added Et_3N followed by TFAA (0.03 mL, 0.21 mmol) slowly drop-wise. The stirred reaction mixture was then allowed to warm to ambient temperature. Further CH_2Cl_2 (5 mL) was then added and the organic phase washed with sat. $NaHCO_3$ (aq) solution (15 mL). The organic layer was passed through a hydrophobic frit and evaporated in vacuo. The crude product was purified by flash chromatography on SiO_2 (20 g) eluting with hexane-20% EtOAc/hexane (gradient) to afford the title compound **46** (60 mg, 86%) as a colourless solid: LC–MS (method A) t_R = 2.84 min; m/z = 357, 355 $[M+H]^+$.

7.7.6. 5-Bromo-4-(2,4-dimethyl-phenyl)-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (47a)

To a solution of 4-(2,4-dimethyl-phenyl)-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**42**) (100 mg, 0.25 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added drop-wise a solution of N-Bromosuccinimide in CH_2Cl_2 (45 mg, 0.25 mmol) in DCM (3 mL). After 5 min the reaction was allowed to warm to ambient temperature. The solution was evaporated in vacuo and the residue was partitioned between EtOAc (2 × 20 mL) and sat. sodium thiosulfate solution (20 mL). The combined organics were passed through a hydrophobic frit and evaporated in vacuo. The crude was applied to a column of SiO_2 (20 g) eluting with hexane-5% EtOAc/hexane (gradient) to afford title compound (100 mg, 84%) as a colourless oil: LC–MS (method B) t_R = 5.92 min; m/z = 480, 478 $[M+H]^+$.

7.7.7. 4-(2,4-dimethyl-phenyl)-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (15a)

5-Bromo-4-(2,4-dimethyl-phenyl)-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**47a**, 90 mg, 0.188 mmol), CuCN (67 mg, 0.753 mmol), 1,1'-bis(diphenylphosphino)ferrocene (dppf) (17 mg, 0.03 mmol), tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$) (7 mg, 0.04 mmol) and 1,4-dioxane (1.5 mL) were combined and heated at 100 °C overnight. The reaction had not gone to completion so further amounts of CuCN (67 mg), dppf (17 mg) and $Pd_2(dba)_3$ (7 mg) were added and the reaction heated for a further 2 h. The reaction mixture was allowed to cool to ambient temperature, and partitioned between EtOAc (2 × 20 mL) and sat. $NaHCO_3$ (aq) solution (20 mL). The combined organics were passed through a hydrophobic frit and evaporated in vacuo to give a crude solid (100 mg). The crude product was purified by flash chromatography on SiO_2 (20 g) eluting with hexane to 10% EtOAc/hexane (gradient) to afford the SEM protected intermediate, 4-(2,4-dimethyl-phenyl)-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (10 mg, 0.024 mmol). This product was dissolved in THF (0.4 mL), then ethylenediamine (0.005 ml, 0.071 mmol) and tetrabutylammonium fluoride (TBAF, 1.0 M solution in THF, 0.15 mL, 0.142 mmol) were added sequentially. The reaction mixture was heated at 50 °C overnight, allowed to cool to ambient temperature and then was partitioned between EtOAc (2 × 10 mL) and water (10 mL). The combined organics were passed through a hydrophobic frit and evaporated in vacuo. The resultant crude product was purified by flash chromatography on SiO_2 (5 g) eluting with hexane to 40% EtOAc/hexane (gradient) to afford the title compound **15a** (5 mg, 72%) as an off-white solid: LC–MS (method A) t_R = 2.44 min; m/z = 295 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 2.26 (s, 3H), 2.43 (s, 3H), 2.65 (s, 3H), 7.19 (d, 1H, J = 7.7 Hz), 7.23 (s, 1H), 7.30 (d, 1H, J = 7.7 Hz), 8.11 (s, 1H) NH not obsd; HRMS, Calcd for $C_{16}H_{13}N_4S$ $[M-H]^-$. Found 293.0875 requires 293.0861; HPLC (from Method A) 100% (t_R = 2.41 min).

7.7.8. 4-(2-Methyl-4-fluoro-phenyl)-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (15b)

A mixture of 4-chloro-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**46**, 54 mg, 0.152 mmol), 2-methyl-4-fluorophenylboronic acid (30 mg, 0.198 mmol), $Pd_2Cl_2(PPh_3)_2$ (5 mg, 0.0076 mmol), $NaHCO_3$ aqueous solution (1 M, 0.46 ml, 0.456 mmol) and DMF (3 mL) was degassed by bubbling N_2 through the mixture for 5 min. The reaction was then heated under a nitrogen atmosphere at 80 °C for 3 h. The mixture was allowed to cool and was then partitioned between EtOAc (2 × 15 mL) and brine (15 mL). The combined organics were passed through a hydrophobic frit and evaporated in vacuo. The crude product was purified by flash chromatography on SiO_2 (20 g) eluting with hexane-20% EtOAc/hexane (gradient) to afford the title compound **15b** (54 mg, 83%) as a colourless solid. (LC–MS (method A) t_R = 2.91 min; m/z = 429 $[M+H]^+$. To the cross coupling product (50 mg, 0.126 mmol) in THF (1 mL) was added ethylenediamine (0.025 ml, 0.378 mmol) followed by TBAF (1.0 M solution in THF, 0.76 mL, 0.756 mmol). The reaction mixture was heated at 50 °C overnight. The reaction was allowed to cool to ambient temperature and was then partitioned between EtOAc (2 × 15 mL) and water (15 mL). The combined organics were passed through a hydrophobic frit and evaporated in vacuo. The resultant crude product was purified by flash chromatography on SiO_2 (20 g) eluting with 10% EtOAc/hexane to 40% EtOAc/hexane (gradient) to afford the title compound **15b** (19 mg, 51%) as a colourless solid: LC–MS (method A) t_R = 2.40 min; m/z = 299 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.22 (s, 3H); 2.57 (s, 3H), 7.15–7.20 (m, 1H), 7.26 (dd, 1H, J = 10.1, 2.2 Hz), 7.46 (dd, 1H, J = 8.6, 6.1 Hz); 8.44 (s, 1H), 13.19 (br s, 1H); HRMS, Calcd for $C_{15}H_{12}N_4SF$ $[M+H]^+$. Found 299.0760 requires 299.0767; HPLC 97.5% (t_R = 1.21 min).

7.7.9. 4-(4-Cyano-phenyl)-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (15c)

The title compound was prepared by the method outlined for **15b**, using 4-cyanophenyl boronic acid and **46** to afford **15c** (9 mg, 35%) as a pale yellow solid: LC–MS (method A) t_R = 3.56 min; m/z = 290 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.61 (s, 3H); 8.05 (d, 2H, J = 8.2 Hz), 8.07 (d, 2H, J = 8.2 Hz);

8.56 (s, 1H), 13.32 (br s, 1H); HRMS, Calcd for $C_{15}H_{10}N_5S$ $[M+H]^+$. Found 292.0654 requires 292.0657; HPLC 95.4% (t_R = 1.15 min).

7.7.10. 4-(2-Chloro-4-methoxy-5-methoxymethoxy-phenyl)-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (49)

To a solution of 1-bromo-2-chloro-4-methoxy-5-methoxymethoxy-benzene **48a** (prepared as outlined in SI section; 2.27 g, 8.06 mmol) in THF (50 mL) at −78 °C under N_2, was added tri-iso-propylborate (3.72 mL, 16.12 mmol) followed by ^{n}BuLi (2.5 M solution in hexanes, 4.2 mL, 10.48 mmol,) drop-wise. The reaction was then allowed to warm to room temperature and then evaporated in vacuo. DMF (20 mL), 4-chloro-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**46**, 2.0 g, 5.64 mmol), $NaHCO_3$ (1 N aq solution, 24.2 mL, 24.2 mmol), and $PdCl_2(PPh_3)_2$ (280 mg, 0.4 mmol) were added sequentially and the resultant mixture was degassed by bubbling N_2 through the mixture for 5 min. The reaction was then heated at 80 °C for 1.5 h and the resultant mixture partitioned between EtOAc (250 mL) and brine (250 mL). The organic phase was dried (Na_2SO_4) and evaporated in vacuo to afford a crude oil. This was purified by flash chromatography on SiO_2 (100 g) eluting with hexane to 30% EtOAc/hexane (gradient) to afford the title compound **49** (3.04 g, 98%) as a yellow oil: LC–MS (method A) t_R = 2.84 min; m/z = 523, 521 $[M+H]^+$.

7.7.11. 4-(2-Chloro-4-methoxy-5-methoxymethoxy-phenyl)-2-methanesulfonyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (50)

To an ice cooled solution of 4-(2-chloro-4-methoxy-5-methoxymethoxy-phenyl)-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**49**, 3.04 g, 5.5 mmol) in CH_2Cl_2 (100 mL) was added *m*CPBA (4.93 g, 22 mmol) dissolved in CH_2Cl_2 (70 mL) drop-wise. After the addition was complete the reaction was allowed to warm to ambient temperature and was then washed sequentially with aq sodium thiosulfate solution (150 mL) and saturated aq $NaHCO_3$ sol. (170 mL). The organic layer was dried (Na_2SO_4) filtered and the filtrates evaporated in vacuo to afford a crude oil, which was purified by flash chromatography on SiO_2 (50 g) eluting with hexane to 40% EtOAc/hexane (gradient) to afford the title compound **50** (2.5 g, 80%) as a yellow foam: LC–MS (method A) t_R = 2.62 min; m/z = 555, 553 $[M+H]^+$.

7.7.12. [4-(2-Chloro-4-methoxy-5-methoxymethoxy-phenyl)-5-cyano-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-acetic acid ethyl ester (51)

Ethyl thioglycolate (0.31 mL, 2.78 mmol) was added to a mixture of NaH (46 mg, 1.15 mmol, 60% dispersion in mineral oil) in THF (10 mL) at 0 °C under an N_2 atmosphere. A solution of 4-(2-chloro-4-methoxy-5-methoxymethoxy-phenyl)-2-methanesulfonyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**50**, 1.28 g, 2.32 mmol) in THF (10 mL) was then added drop-wise. The reaction was then allowed to warm to ambient temperature before being partitioned between EtOAc (2 × 100 mL) and aq NH_4Cl sol. (100 mL). The organic layer was dried (Na_2SO_4) and evaporated in vacuo to afford a crude oil, which was purified by flash chromatography on SiO_2 (70 g) eluting with hexane-40% EtOAc/hexane (gradient) to afford the title compound (1.32 g, 96%) as a yellow oil: LC–MS (method A) t_R = 2.84 min; m/z = 595, 593 $[M+H]^+$.

7.7.13. [4-(2-Chloro-4-methoxy-5-methoxymethoxy-phenyl)-5-cyano-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-acetic acid (52)

1 N aq NaOH (4.46 mL, 4.46 mmol) was added to a solution of [4-(2-chloro-4-methoxy-5-methoxymethoxy-phenyl)-5-cyano-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-acetic acid ethyl ester (**51**, 1.32 g, 2.23 mmol) in MeOH (20 mL) and stirred at ambient temperature for 2 h. The mixture was then evaporated in vacuo, water (100 mL) was added and the resultant solution acidified to pH 5 by cautious addition of 1.2 N aq HCl solution. This mixture was then extracted with EtOAc (2 × 100 mL). The combined organics were dried (Na_2SO_4) and evaporated in vacuo to afford the title compound **52** (1.27 g, 100%) as a yellow foam: LC–MS (method A) t_R = 2.63 Min; m/z = 567, 565 $[M+H]^+$.

7.7.14. 2-[4-(2-Chloro-4-methoxy-5-methoxymethoxy-phenyl)-5-cyano-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*,*N*-dimethyl-acetamide (53a)

To a solution of [4-(2-chloro-4-methoxy-5-methoxymethoxy-phenyl)-5-cyano-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo [2,3-*d*]pyrimidin-2-ylsulfanyl]-acetic acid (**52**, 500 mg, 0.885 mmol) in anhydrous MeCN (10 mL), were added sequentially triethylamine (0.19 mL, 1.33 mmol), dimethylamine (2.0 M solution in THF, 0.67 mL, 1.33 mmol,) and HBTU (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, 504 mg, 1.33 mmol). After 1 h the reaction mixture was partitioned between EtOAc (2 × 50 mL) and aqueous NH_4Cl solution (50 mL). The combined organics were dried (Na_2SO_4) and evaporated in vacuo. The resultant crude oil purified by flash chromatography on SiO_2 (50 g) eluting with hexane to 80% EtOAc/hexane (gradient) to afford the title compound **53** (470 mg, 89%) as a white foam: LC–MS (method A) t_R = 2.67 min; m/z = 594, 592 $[M+H]^+$.

7.7.15. 2-[4-(2-Chloro-5-hydroxy-4-methoxy-phenyl)-5-cyano-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*,*N*-dimethyl-acetamide (54a)

To a solution of 2-[4-(2-chloro-4-methoxy-5-methoxymethoxy-phenyl)-5-cyano-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo [2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*,*N*-dimethyl-acetamide (**53**, 628 mg, 1.06 mmol) in ^{i}PrOH (10 mL) PPTS (pyridinium *p*-toluenesulfonate) (53 mg, 0.212 mmol) was added and the mixture heated at 85 °C under N_2 overnight. The reaction was allowed to cool and partitioned between EtOAc (2 × 20 mL) and NH_4Cl solution (20 mL). The dried (Na_2SO_4) organics were evaporated in vacuo to give a crude oil. This was applied to a cartridge of SiO_2 (50 g) eluting with CH_2Cl_2-5% MeOH/CH_2Cl_2 (gradient) to afford the title compound **54** (411 mg, 71%) as a white foam: LC–MS (method A) t_R = 2.53 min; m/z = 548, 550 $[M+H]^+$.

7.7.16. 2-[4-(2-Chloro-5-hydroxy-4-methoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*,*N*-dimethyl-acetamide (36q)

To 2-[4-(2-chloro-5-hydroxy-4-methoxy-phenyl)-5-cyano-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*,*N*-dimethyl-acetamide (60 mg, 0.109 mmol) under N_2, anhydrous THF (2 mL), ethylenediamine (0.022 mL, 0.327 mmol) and 1.0 M TBAF in THF solution (0.65 mL, 0.65 mmol) were added sequentially. The mixture was heated at 40 °C overnight. The reaction mixture was allowed to cool and was then partitioned between EtOAc (30 mL) and water (30 mL). The organic layer was dried (Na_2SO_4), filtered and the filtrates evaporated in vacuo to give a crude oil (ca 60 mg). This was applied to a cartridge of SiO_2 (10 g) eluting with hexane-100% EtOAc (gradient) to afford the title compound **36q** (16 mg, 35%) as a colourless solid: LC–MS (method A) t_R = 1.86 min; m/z = 418, 420 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 2.97 (s, 3H), 3.18 (s, 3H), 3.93 (s, 3H), 4.19 (s, 2H), 6.89 (s, 1H), 7.09 (s, 1H), 8.08 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 34.4 (CH2), 36.5 (CH3), 38.2 (CH3), 56.7 (CH3), 86.8 (C), 113.7 (CH), 114.3 (C), 115.1 (C), 118.1 (CH), 124.0 (C), 128.4 (C), 136.9 (CH), 146.9 (C), 151.2 (C), 153.9 (C), 159.4

(C), 166.4 (C), 171.0 (C); HRMS, Calcd for $C_{18}H_{17}ClN_5O_3S$ $[M+H]^+$. Found 418.0723 requires 418.0741; HPLC 97.2% (t_R = 0.98 min).

7.7.17. 2-[4-(2-Chloro-4,5-dimethoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*,*N*-dimethyl-acetamide (36r)

To a mixture of 2-[4-(2-chloro-5-hydroxy-4-methoxy-phenyl)-5-cyano-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-2-yl-sulfanyl]-*N*,*N*-dimethyl-acetamide (**54:** NR_1R_2 = NMe_2, 80 mg, 0.15 mmol), Cs_2CO_3 (71 mg, 0.22 mmol) and anhydrous DMF (2 mL) under N_2 was added methyl iodide (0.01 mL, 0.15 mmol). The reaction mixture was stirred at ambient temperature for 2 h, then partitioned between EtOAc (20 mL) and aq NH_4Cl solution (20 mL). The organic layer was washed with brine (20 mL) then dried (Na_2SO_4) filtered and filtrate solvents evaporated in vacuo to afford a yellow oil, (96 mg, 100%) which was de-protected with TBAF and ethylene diamine by the method utilized for **36q** above. Purification by flash chromatography (hexanes/EtOAc) afforded title compound **36r** as a colourless solid: LC–MS (method A) t_R = 1.99 min; m/z = 434, 432 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 2.96 (s, 3H), 3.20 (s, 3H), 3.86 (s, 3H), 3.91 (s, 3H), 4.22 (s, 2H), 7.09 (s, 1H), 7.13 (s, 1H), 8.10 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 34.4 (CH2), 36.5 (CH3), 38.2 (CH3), 56.8 (CH3), 56.8 (CH3), 86.5 (C), 114.0 (CH), 114.4 (C), 115.1 (CH), 115.4 (C), 125.8 (C), 128.0 (C), 137.6 (CH), 149.4 (C), 152.5 (C), 154.4 (C), 159.2 (C), 166.1 (C), 170.9 (C); HRMS, Calcd for $C_{19}H_{19}ClN_5O_3S$ $[M+H]^+$. Found 432.0887 requires 432.0879; HPLC 100% (t_R = 1.05 min).

7.7.18. 2-[4-(2-Chloro-4,5-dimethoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*-cyclopropyl-acetamide (36a)

The title compound was prepared by the methods outlined for **36r**, via the MEM protected intermediate **48b.** Purification by flash chromatography, eluting with DCM to 5% MeOH in DCM (gradient) afforded **36a** (30 mg; 51%) as a yellow solid: LC–MS (method A) t_R = 2.02 min; m/z = 444, 446 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.36 (m, 2H), 0.58 (m, 2H), 2.60 (m, 1H), 3.81 (s, 3H), 3.87 (s, 3H), 3.88 (s, 2H); 7.13 (s, 1H); 7.19 (s, 1H); 8.17 (d, 1H, J = 4.0 Hz); 8.45 (s, 1H); 13.14 (br s, 1H); HRMS, Calcd for $C_{20}H_{19}ClN_5O_3S$ $[M+H]^+$. Found 444.0879 requires 444.0897; HPLC 100% (t_R = 1.06 min).

7.7.19. 2-[4-(2-Chloro-5-hydroxy-4-methoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*-cyclopropyl-acetamide (36b)

The title compound was prepared by the methods outlined for **36q**, via the MEM protected intermediate **48b.** Purification by flash chromatography, eluting with DCM to 4% MeOH in DCM (gradient) afforded **36b** (30 mg, 42%) as a colourless solid: LC–MS (method A) t_R = 1.90 min; m/z = 430, 432 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 0.37–0.40 (m, 2H), 0.63–0.68 (m, 2H), 2.60–2.66 (m, 1H), 3.87 (s, 2H), 3.94 (s, 3H), 6.93 (s, 1H), 7.10 (s, 1H), 8.10 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 6.5 (CH2), 23.7 (CH), 35.6 (CH2), 56.8 (CH3), 86.8 (C), 113.8 (CH), 114.5 (C), 115.1 (C), 118.1 (CH), 124.1 (C), 128.3 (C), 137.2 (CH), 146.9 (C), 151.2 (C), 153.9 (C), 159.5 (C), 166.1 (C), 173.0 (C); HRMS, Calcd for $C_{19}H_{17}ClN_5O_3S$ $[M+H]^+$. Found 430.0730 requires 430.0741; HPLC 100% (t_R = 0.99 min).

7.7.20. 2-[4-(2-Chloro-5-ethoxy-4-methoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*-cyclopropyl-acetamide (36c)

The title compound was prepared by the methods outlined for **36r**, via the MEM protected intermediate **48b**. Purification by flash chromatography, eluting with heaxane to 80% EtOAc in hexane (gradient) afforded the title compound **36c** (27 mg, 41%) as a pale-yellow solid: LC–MS (method A) t_R = 2.01 min; m/z = 458, 460 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 0.36–0.40 (m, 2H), 0.63–0.68 (m, 2H), 1.40 (t, 3H, J = 7.0 Hz), 2.59–2.65 (m, 1H), 3.88 (s, 2H), 3.91 (s, 3H), 4.09 (q, 2H, J = 7.0 Hz), 7.08 (s, 1H), 7.13 (s, 1H), 8.11 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 6.5 (CH2), 15.0 (CH3), 23.7 (CH), 35.6 (CH2), 56.8 (CH3), 66.1 (CH2), 86.8 (C), 114.1 (CH), 114.4 (C), 115.2 (C), 116.3 (CH), 125.8 (C), 127.9 (C), 137.2 (CH), 148.7 (C), 152.8 (C), 154.0 (C), 159.4 (C), 166.1 (C), 172.9 (C); HRMS, Calcd for $C_{21}H_{21}ClN_5O_3S$ $[M+H]^+$. Found 458.1042 requires 458.1054; HPLC 96.9% (t_R = 1.11 min).

7.7.21. 2-[4-(2-Chloro-5-hydroxy-4-methoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*-isopropyl-acetamide (36d)

The title compound was prepared by the methods outlined for **36q**. Purification by flash chromatography, eluting with DCM/MeOH (9:1) afforded **36d** (52 mg, 50%) as a pale-brown solid: LC–MS (method A) t_R = 1.66 min, m/z = 432 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 1.03 (d, 6H, J = 6.6 Hz), 3.88 (s, 2H), 3.93 (sept, 1H, J = 6.6 Hz), 3.93 (s, 3H), 6.94 (s, 1H), 7.10 (s, 1H), 8.10 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 22.4 (CH3), 35.8 (CH2), 43.0 (CH), 56.8 (CH3), 86.9 (C), 113.8 (CH), 114.5 (C), 115.0 (C), 118.1 (CH), 124.1 (C), 128.3 (C), 137.2 (CH), 146.9 (C), 151.2 (C), 153.9 (C), 159.5 (C), 166.2 (C), 170.5 (C); HRMS, Calcd for $C_{19}H_{19}ClN_5O_3S$ $[M+H]^+$. Found 432.0878 requires 432.0897; HPLC 91.0% (t_R = 1.03 min).

7.7.22. 2-[4-(2-Chloro-4,5-dimethoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*-ethyl-acetamide (36e)

The title compound was prepared by the methods outlined for **36r**. Purification by flash chromatography, eluting with EtOAc afforded **36e** (15 mg, 14%) as a pale yellow solid: LC–MS (method A) t_R = 1.70 min, m/z = 432 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97 (t, 3H, J = 7.3 Hz), 3.08 (m, 2H), 3.80 (s, 3H), 3.87 (s, 3H), 3.91 (s, 2H), 7.13 (s, 1H), 7.19 (s, 1H), 8.08 (t, 1H, J = 5.0 Hz), 8.44 (s, 1H), 13.08 (br s, 1H); HRMS, Calcd for $C_{19}H_{19}ClN_5O_3S$ $[M+H]^+$. Found 432.0879 requires 432.0897; HPLC 97.1% (t_R = 1.05 min).

7.7.23. 2-[4-(2-Chloro-5-ethoxy-4-methoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*-ethyl-acetamide (36f)

The title compound was prepared by the methods outlined for **36r**. Purification by flash chromatography, eluting with EtOAc followed by trituration with diethyl ether afforded **36f** (30 mg, 26%) as a pale-yellow solid: LC–MS (method A) t_R = 1.80 min, m/z = 448, 446 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97 (t, 3H, J = 7.3 Hz), 1.33 (t, 3H, J = 6.7 Hz), 3.07 (m, 2H), 3.87 (s, 3H), 3.91 (s, 2H), 4.05 (q, 2H, J = 6.7 Hz), 7.10 (s, 1H), 7.18 (s, 1H), 8.07 (t, 1H, J = 5.0 Hz), 8.43 (s, 1H), 13.10 (br s, 1H); HRMS, Calcd for $C_{20}H_{21}ClN_5O_3S$ $[M+H]^+$. Found 446.1040 requires 446.1054; HPLC 100% (t_R = 1.03 min).

7.7.24. N-tert-Butyl-2-[4-(2-chloro-5-hydroxy-4-methoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-acetamide (36g)

The title compound was prepared by the methods outlined for **36q**. Purification by flash chromatography, eluting with 5% MeOH in DCM afforded **36g** (36 mg, 62%) as an off-white solid: LC–MS (method A) t_R = 3.00 min, m/z = 446 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 1.22 (s, 9H), 3.83 (s, 2H), 3.93 (s, 3H), 6.96 (s, 1H), 7.10 (s, 1H), 8.11 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 28.7 (CH3), 36.6 (CH2), 52.3 (C), 56.8 (CH3), 86.9 (C), 113.8 (CH), 114.4 (C), 115.0 (C), 118.2 (CH), 124.2 (C), 128.3 (C), 137.1 (CH), 147.0 (C), 151.3 (C), 153.8 (C), 159.5 (C), 166.3 (C), 170.7 (C); HRMS, Calcd for $C_{20}H_{21}ClN_5O_3S$ $[M+H]^+$. Found 446.1035 requires 446.1054; HPLC 100% (t_R = 1.10 min).

7.7.25. N-tert-Butyl-2-[4-(2-Chloro-4,5-dimethoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-acetamide (36h)

The title compound was prepared by the methods outlined for **36r**. Purification by flash chromatography, eluting with 5% MeOH in DCM afforded **36h** (9 mg, 21%) as an off-white solid: LC–MS (method A) t_R = 3.13 min, m/z = 460 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 1.22 (s, 9H), 3.85 (s, 2H), 3.87 (s, 3H), 3.92 (s, 3H), 7.14 (s, 1H), 7.67 (s, 1H), 8.13 (s, 1H); HRMS, Calcd for $C_{21}H_{23}ClN_5O_3S$ $[M+H]^+$. Found 460.1195 requires 460.1210; HPLC 95.3% (t_R = 1.17 min).

7.7.26. 2-[4-(2-Chloro-5-ethoxy-4-methoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*-isopropyl-acetamide (36i)

The title compound was prepared by the methods outlined for **36r**. Purification by flash chromatography, eluting with EtOAc afforded **36i** (51 mg, 39%) as a pale-brown solid: LC–MS (method A) t_R = 2.09 min, m/z = 462, 460 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 1.03 (d, 6H, J = 6.6 Hz), 1.40 (t, 3H, J = 7.0 Hz), 3.90 (s, 2H), 3.91 (s, 3H), 3.93 (sept, 1H, J = 6.6 Hz), 4.09 (q, 2H, J = 7.0 Hz), 7.09 (s, 1H), 7.13 (s, 1H), 8.11 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 15.0 (CH3), 22.4 (CH3), 35.8 (CH2), 43.0 (CH), 56.8 (CH3), 66.1 (CH2), 86.8 (C), 114.2 (CH), 114.4 (C), 115.2 (C), 116.3 (CH), 125.8 (C), 127.9 (C), 137.3 (CH), 148.7 (C), 152.8 (C), 154.0 (C), 159.4 (C), 166.1 (C), 170.4 (C); HRMS, Calcd for $C_{21}H_{23}ClN_5O_3S$ $[M+H]^+$. Found 460.1215 requires 460.1210; HPLC 100% (t_R = 1.15 min).

7.7.27. 2-[4-(2-Chloro-4,5-dimethoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*-isopropyl-acetamide (36j)

The title compound was prepared by the methods outlined for **36r**. Purification by flash chromatography, eluting with EtOAc followed by trituration with diethyl ether afforded **36j** (53 mg; 38%) as a pale-brown solid: LC–MS (method A) t_R = 2.00 min, m/z = 448, 446 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 1.03 (d, 6H, J = 6.6 Hz), 3.87 (s, 3H), 3.90 (s, 2H), 3.91 (s, 3H), 3.93 (sept, 1H, J = 6.6 Hz), 7.11 (s, 1H), 7.13 (s, 1H), 8.12 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 22.4 (CH3), 35.8 (CH2), 43.0 (CH), 56.8 (CH3), 86.7 (C), 114.0 (CH), 114.5 (C), 115.1 (CH), 115.3 (C), 125.8 (C), 127.9 (C), 137.5 (CH), 149.5 (C), 152.6 (C), 154.1 (C), 159.3 (C), 166.1 (C), 170.4 (C); HRMS, Calcd for $C_{20}H_{21}ClN_5O_3S$ $[M+H]^+$. Found 446.1039 requires 446.1054; HPLC 98.5% (t_R = 1.10 min).

7.7.28. 2-[4-(2-Chloro-4,5-dimethoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*-(2-fluoro-ethyl)-acetamide (36k)

The title compound was prepared by the methods outlined for **36r**. Purification by flash chromatography, eluting with EtOAc/hexane (2:1) afforded **36k (**25 mg, 30%) as a brown solid: LC–MS (method A) t_R = 2.94 min, m/z = 450 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.51-3.60 (m, 2H,), 3.90-3.98 (m, 8H), 4.32–4.47 (m, 2H), 7.00 (s, 1H,), 7.05 (s, 1H,), 7.38 (m, 1H), 7.81 (s, 1H), 10.60-11.00 (br s, 1H); HRMS, Calcd for $C_{19}H_{18}ClFN_5O_3S$ $[M+H]^+$. Found 450.0775 requires 450.0803; HPLC 95.5% (t_R = 1.04 min).

7.7.29. 2-[4-(2-Chloro-5-hydroxy-4-methoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*-(2-fluoro-ethyl)-acetamide (36l)

The title compound was prepared by the methods outlined for **36q**. Purification by flash chromatography, eluting with EtOAc/hexane (2:1) to EtOAc (gradient) afforded **36l** (25 mg, 53%) as a colourless solid: LC–MS (method A) t_R = 2.85 min, m/z = 436 $[M+H]^+$; ^{1}H NMR (400 MHz DMSO-d_6) δ 3.35 (m, 1H), 3.42 (m, 1H), 3.86 (s, 3H), 3.95 (s, 2H), 4.32–4.37(m, 2H), 6.90 (s, 1H), 7.15 (s, 1H), 8.35 (m, 1H), 8.42 (s, 1H), 9.56 (s, 1H), 13.0–13.20 (br s, 1H); HRMS, Calcd for $C_{18}H_{16}ClFN_5O_3S$ $[M+H]^+$. Found 436.0632 requires 436.0646; HPLC 100% (t_R = 0.97 min).

7.7.30. 2-[4-(2-Chloro-5-hydroxy-4-methoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*-(2,2,2-trifluoro-ethyl)-acetamide (36m)

The title compound was prepared by the methods outlined for **36r**. Purification by flash chromatography, eluting with 1% MeOH in DCM afforded **36m** (51 mg, 81%)) as a colourless solid: LC–MS (method B) t_R = 3.23 min, m/z = 472 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 3.89 (q, 2H, J_{H-F} = 9.4 Hz), 3.93 (s, 3H), 4.00 (s, 2H), 6.90 (s, 1H), 7.09 (s, 1H), 8.10 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 35.5 (CH2), 41.7 (CH2, q, J_{C-F} = 34.6 Hz), 56.8 (CH3), 86.9 (C), 113.8 (CH), 114.5 (C), 115.0 (C), 118.1 (CH), 124.1 (C), 125.7 (C, q, J_{C-F} = 278.2 Hz), 128.3 (C), 137.1 (CH), 146.9 (C), 151.2 (C), 153.8 (C), 159.6 (C), 165.8 (C), 172.1 (C); HRMS, Calcd for $C_{18}H_{14}ClF_3N_5O_3S$ $[M+H]^+$. Found 472.0431 requires 472.0458; HPLC 97.3% (t_R = 1.05 min).

7.7.31. 2-(2-Azetidin-1-yl-2-oxo-ethylsulfanyl)-4-(2-Chloro-5-hydroxy-4-methoxy-phenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (36n)

The title compound was prepared by the methods outlined for **36q**. Purification by flash chromatography, eluting with Et_3N/EtOH/EtOAc (0:0:1–1:5:44 gradient) afforded **36n** (48 mg, 39%)) as a yellow solid: LC–MS (method B) t_R = 2.97 min, m/z = 430, 432 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 2.08–2.16 (m, 2H), 3.77 (s, 2H), 3.92 (s, 3H), 3.95–3.99 (m, 2H), 4.29–4.33 (m, 2H), 6.94 (s, 1H), 7.10 (s, 1H), 8.06 (s, 1H); HRMS, Calcd for $C_{19}H_{17}ClN_5O_3S$ $[M+H]^+$. Found 430.0730 requires 430.0741; HPLC 95.6% (t_R = 0.98 min).

7.7.32. 2-(2-Azetidin-1-yl-2-oxo-ethylsulfanyl)-4-(2-Chloro-4,5-dimethoxy-phenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (36o)

The title compound was prepared by the methods outlined for **36r**. Purification by flash chromatography, eluting with Et_3N/EtOH/EtOAc (0:0:1–1:5:44 gradient) afforded **36n** (60 mg, 49%) as a yellow solid LC–MS (method B) t_R = 3.10 min, m/z = 444, 446 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 2.09–2.17 (m, 2H), 3.81 (s, 2H), 3.84 (s, 3H), 3.89 (s, 3H), 3.93–3.97 (m, 2H), 4.32–4.36 (m, 2H), 7.10 (s, 1H), 7.12 (s, 1H), 8.07 (s, 1H); HRMS, Calcd for $C_{20}H_{19}ClN_5O_3S$ $[M+H]^+$. Found 444.0878 requires 444.0897; HPLC 97.3% (t_R = 1.04 min).

7.7.33. 2-[4-(2-Chloro-4,5-dimethoxy-phenyl)-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl]-*N*-methyl-acetamide (36p)

The title compound was prepared by the methods outlined for **36r**. Purification by flash chromatography, eluting with DCM to 4% MeOH in DCM (gradient) afforded **36p** (10 mg, 18%) as a brown solid: LC–MS (method A) t_R = 1.59 min, m/z = 418, 420 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 2.72 (s, 3H), 3.86 (s, 3H), 3.91 (s, 3H), 3.93 (s, 2H), 7.08 (s, 1H), 7.13 (s, 1H), 8.12 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 26.8 (CH3), 35.5 (CH2), 56.8 (CH3), 86.8 (C), 114.0 (CH), 114.4 (C), 115.0 (CH), 115.2 (C), 125.8 (C), 127.9 (C), 137.2 (CH), 149.4 (C), 152.6 (C), 154.0 (C), 159.5 (C), 165.9 (C), 171.9 (C); HRMS, Calcd for $C_{18}H_{17}ClN_5O_3S$ $[M+H]^+$. Found 418.0727 requires 418.0741; HPLC 100% (t_R = 1.00 min).

7.7.34. 5-Bromo-4-(2,4-dimethyl-phenyl)-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (17)

To a solution of 5-bromo-4-(2,4-dimethyl-phenyl)-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **(47a**, 500 mg, 1.05 mmol) in THF (4 mL) was added a

solution of tetrabutylammonium fluoride (1.0 M in THF, 6.3 mL, 6.3 mmol), followed by ethylene diamine (0.21 mL, 3.14 mmol). The mixture was heated under a nitrogen atmosphere for 24 h, allowed to cool and partitioned between EtOAc (50 mL) and water (50 mL). The organic phase was separated and passed through a hydrophobic frit and filtrates solvent removed in vacuo. The crude product was purified by flash chromatography on silica gel (50 g), eluting with 30–40% EtOAc in hexane gradient to afford the title compound **17** (288 mg, 79%) as a colourless solid: LC–MS (method B) t_R = 4.44 min, m/z = 350, 348 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δ 2.08 (s, 3H), 2.38 (s, 3H), 2.59 (s, 3H), 7.09–7.14 (m, 3H), 7.33 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 14.2 (CH3), 19.9 (CH3), 21.4 (CH3), 89.5 (C), 113.1 (C), 126.4 (CH), 126.9 (CH), 130.5 (CH), 131.6 (CH), 134.3 (C), 137.4 (C), 140.2 (C), 153.3 (C), 162.0 (C), 166.2 (C); HRMS, Calcd for $C_{15}H_{15}BrN_3S$ $[M+H]^+$. Found 348.0160 requires 348.0170; HPLC 97.4% (t_R = 1.38 min).

7.7.35. 4-(2,4-dimethyl-phenyl)-5-methyl-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (18)

To a solution of *n*-butyl lithium (2.5 M, 0.10 mL, 0.253 mmol) in anhydrous THF (2 mL) cooled in a CO_2-acetone bath under a nitrogen atmosphere was added a solution of 5-bromo-4-(2,4-dimethyl-phenyl)-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**47a**,110 mg, 0.23 mmol) in anhydrous THF (1.4 mL) drop-wise. When addition was complete, methyl iodide (72 μL, 1.15 mmol) was added and the reaction mixture stirred for 5 min, the cooling bath was removed and reaction mixture allowed to warm to ambient temperature. The reaction mixture was partitioned between sat. (aq) NH_4Cl solution and EtOAc and the organic phase was passed through a hydrophobic frit and solvents removed in vacuo to give an oil which was purified by flash chromatography on SiO_2 eluting with a 0 to 10% gradient of EtOAc in hexane to afford intermediate product as a colourless oil (80 mg; 84%). The SEM group was removed by the procedure described for **17** above and resulting the crude product was purified by flash chromatography on silica gel eluting with a gradient of o to to 25% EtOAc in hexane followed by trituration with diethyl ether to afford the title compound **18** (36 mg, 56%) as a colourless solid: LC–MS (method A) t_R = 2.84 min, m/z = 284 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δ 1.70 (d, 3H, J = 1.1 Hz), 2.07 (s, 3H), 2.38 (s, 3H), 2.59 (s, 3H), 6.98 (q, 1H, J = 1.1 Hz), 7.10–7.15 (m, 3H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 11.2 (CH3), 14.1 (CH3), 19.6 (CH3), 21.3 (CH3), 111.9 (C), 114.8 (C), 123.8 (CH), 127.1 (CH), 129.9 (CH), 131.7 (CH), 136.0 (C), 136.9 (C), 140.1 (C), 154.4 (C), 161.7 (C), 164.6 (C); HRMS, Calcd for $C_{16}H_{18}N_3S$ $[M+H]^+$. Found 284.1221 requires 284.1221; HPLC 94.4% (t_R = 1.36 min).

7.7.36. 4-(2,4-Dimethyl-phenyl)-2-methylsulfanyl-5-trifluoro methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (19)

5-Bromo-4-(2,4-dimethyl-phenyl)-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**47a**, 100 mg, 0.209 mmol), CuI (80 mg, 0.418 mmol), sodium trifluoroacetate (57 mg, 0.418 mmol), toluene (0.5 ml) and DMF (1 mL) were combined under N_2 atmosphere and heated to 170 °C overnight. The reaction mixture was allowed to cool to RT and was then partitioned between EtOAc (2 × 15 mL) and water (15 mL). The organics were passed through a hydrophobic frit and evaporated in vacuo. The resultant crude product was purified by flash chromatography on silica gel (20 g) eluting with hexane-6% EtOAc/hexane (gradient) to afford the desired protected product together with small quantity of dehalogenated product. The SEM group was removed by the procedure described for **17** above and the resulting crude product was purified by preparative HPLC (pH 4) to afford title compound **19** (7 mg, 10%) as an off-white solid: LC–MS (method A) t_R = 2.68 min, m/z = 338 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 1.98 (s, 3H); 2.34 (s, 3H); 2.55 (s, 3H); 7.04–7.15 (m, 3H); 8.08 (s, 1H). 10.80 (br s, 1H); HRMS, Calcd for $C_{16}H_{15}N_3F_3S$ $[M+H]^+$. Found 338.0938 requires 338.0939; HPLC 96.7% (t_R = 1.39 min).

7.7.37. 5-Cyclopropyl-4-(2,4-dimethyl-phenyl)-2-methylsulfa nyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (20)

5-Bromo-4-(2,4-dimethyl-phenyl)-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**47a**, 100 mg, 0.209 mmol), $Pd(OAc)_2$ (3 mg, 0.01 mmol), $P(Cy)_3$ (57 mg, 0.418 mmol), K_3PO_4 (170 mg, 0.80 mmol), cyclopropylboronic acid (25 mg, 0.30 mmol), toluene (1.0 ml) and water (0.05 ml) were combined under N_2 atmosphere and the mixture was degassed by bubbling N_2 through the mixture for 5 min. The reaction mixture was then heated at 100 °C for 2 h, allowed to cool to RT and was then partitioned between EtOAc (2 × 15 mL) and water (15 mL). The organics were dried (Na_2SO_4) and passed through a hydrophobic frit and evaporated in vacuo. The resultant crude product was purified by flash chromatography on SiO_2 (10 g) eluting with hexane-5% EtOAc/hexane (gradient) to afford the desired product and some dehalogenated product (17 mg). This compound mixture was de-protected using the method outlined for **17** and the crude product was purified by preparative HPLC (performed at pH 4) to afford the title compound **20** (4 mg, 6%) as an off-white solid: LC–MS (method A) t_R = 2.70 min, m/z = 310 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 0.37–0.39 (m, 4H), 1.03–1.10 (m, 1H), 2.10 (s, 3H), 2.34 (s, 3H), 2.52 (s, 3H), 7.00–7.01 (m, 1H), 7.10 (d, 1H, J = 8.0 Hz), 7.14 (s, 1H), 7.21 (d, 1H, J = 8.0 Hz), 11.73 (s, 1H); HRMS, Calcd for $C_{18}H_{20}N_3S$ $[M+H]^+$. Found 310.1368 requires 310.1378; HPLC 99.4% (t_R = 1.41 min).

7.7.38. 4-(4-Fluoro-2-methy-phenyl)-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid amide (21)

The title compound was prepared by the methods outlined for **17**. Thus **55** (65 mg, 0.15 mmol) was reacted with TBAF and ethylenediamine in THF and the product purified by flash chromatography on silica gel eluting with DCM to 4% MeOH/DCM (gradient) to afford the title compound **21** (17 mg, 36%) as a colourless solid: LC–MS (method A) t_R = 1.92 min, m/z = 317 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δ 2.19 (s, 3H), 2.61 (s, 3H), 6.98–7.03 (m, 1H), 7.05 (dd, 1H, J_{H-H} = 2.5 Hz, J_{H-F} = 10.0 Hz), 7.32 (dd, 1H, J_{H-H} = 8.4 Hz, J_{H-F} = 5.9 Hz), 7.82 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 14.2 (CH3), 20.0 (CH3, d, J_{C-F} = 1.2 Hz), 112.2 (C), 112.9 (C), 113.3 (CH, d, J_{C-F} = 21.8 Hz), 117.7 (CH, d, J_{C-F} = 21.7 Hz), 130.4 (CH), 132.2 (CH, d, J_{C-F} = 8.8 Hz), 135.8 (C, d, J_{C-F} = 3.0 Hz), 140.6 (C, d, J_{C-F} = 8.2 Hz), 155.1 (C), 161.1 (C), 164.5 (C, d, J_{C-F} = 246.0 Hz), 166.4 (C), 168.4 (C); HRMS, Calcd for $C_{15}H_{14}N_4OFS$ $[M+H]^+$. Found 317.0860 requires 317.0872; HPLC 97.9% (t_R = 1.00 min).

7.7.39. 4-(2,4-Dimethyl-phenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (22)

The title compound was prepared as outlined in the SI section, to afford the title compound **22** (75 mg, 59%) as a colourless solid: LC–MS (method A) t_R = 2.03 min, m/z = 249 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δ 2.19 (s, 3H), 2.40 (s, 3H), 7.17 (d, 1H, J = 7.8 Hz), 7.21 (s, 1H), 7.27 (d, 1H, J = 7.8 Hz), 8.27 (s, 1H), 8.91 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 19.7 (CH3), 21.4 (CH3), 86.4 (C), 114.9 (C), 117.3 (C), 127.4 (CH), 130.6 (CH), 132.2 (CH), 133.9 (C), 137.3 (C), 138.7 (CH), 141.1 (C), 153.2 (C), 153.5 (CH), 162.1 (C); HRMS, Calcd for $C_{15}H_{13}N_4$ $[M+H]^+$. Found 249.1146 requires 249.1140; HPLC 100% (t_R = 1.05 min).

7.7.40. 4-(4-Fluoro-2-methyl-phenyl)-2-methanesulfonyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (57)

To a solution of 4-[(4-fluoro-2-methyl-phenyl]-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **56** (189 mg, 0.44 mmol) prepared as outlined

in **15b** (part 1) in CH_2Cl_2 (8.5 mL) at 0 °C was added drop-wise a solution of *m*CPBA (396 mg, 1.76 mmol) in CH_2Cl_2 (8.5 mL). After addition was complete the reaction was allowed to warm to ambient temperature. After 1 h the reaction mixture was washed with 5% sodium thiosulphate (aq) solution (20 mL) and the aqueous layer was extracted with further CH_2Cl_2 (20 mL). The combined organics were then washed with sat. $NaHCO_3$ (aq) solution. (40 mL) then passed through a hydrophobic frit and the filtrate evaporated in vacuo. The resultant crude product was purified by flash chromatography on SiO_2 (25 g) eluting with 20–40% EtOAc in hexane (gradient) to afford the title compound **57** (187 mg, 92%) as a colourless oil: LC–MS (method A) t_R = 2.65 min, m/z = 461 $[M+H]^+$.

7.7.41. 4-(4-Fluoro-2-methyl-phenyl)-2-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (23)

To a mixture of KO^{t}Bu (20 mg, 0.17 mmol) in THF (1 mL) at 0 °C under N_2, was added MeOH (0.007 mL, 0.17 mmol) followed by drop-wise addition of a solution of 4-(4-fluoro-2-methyl-phenyl)-2-methanesulfonyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo [2,3-*d*]pyrimidine-5-carbonitrile (**57**, 40 mg, 0.087 mmol) in THF (0.5 mL). After 30 min. the reaction mixture was partitioned between EtOAc (2 × 10 ml) and sat. $NaHCO_3$ solution (15 mL). The organics were then passed through a hydrophobic frit and evaporated in vacuo to give the crude protected product, which was de-protected with tetrabutylammonium fluoride using the method outlined for compound **17**. Purification was by flash chromatography on silica gel (10 g) eluting with 10 to 50% EtOAc in hexane (gradient) to afford the title compound **23** (12 mg, 48%) as a white solid: LC–MS (method A) t_R = 2.16 min, m/z = 283 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 2.26 (s, 3H), 4.06 (s, 3H), 7.04–7.10 (m, 1H), 7.13 (dd, 1H, $J_{H\text{-}H}$ = 2.5 Hz, J_{H-F} = 9.9 Hz), 7.40 (dd, 1H, $J_{H\text{-}H}$ = 8.4 Hz, J_{H-F} = 5.8 Hz), 8.04 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 19.9 (CH3, d, J_{C-F} = 1.1 Hz), 55.5 (CH3), 86.3 (C), 112.4 (C), 113.6 (CH, d, J_{C-F} = 21.9 Hz), 115.2 (C), 118.1 (CH, d, J_{C-F} = 21.9 Hz), 132.6 (CH, d, J_{C-F} = 9.0 Hz), 133.0 (C, d, J_{C-F} = 3.1 Hz), 136.7 (CH), 140.6 (C, d, J_{C-F} = 8.3 Hz), 155.5 (C), 163.0 (C), 164.2 (C), 164.9 (C, d, J_{C-F} = 247.0 Hz); HRMS, Calcd for $C_{15}H_{12}N_4OF$ $[M+H]^+$. Found 283.0994 requires 283.0995; HPLC 100% (t_R = 1.11 min).

7.7.42. 4-(4-Fluoro-2-methyl-phenyl)-2-methylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (24)

To a solution of 4-(4-fluoro-2-methyl-phenyl)-2-methanesulfonyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**57**) (59 mg, 0.109 mmol) in DMF (2 mL) was added a solution of methylamine (2.0 M in THF, 0.11 mL, 0.218 mmol). The reaction mixture was heated at 100 °C for 2 h, a further 1.0 mL of the methylamine solution was added and heating continued for 3 h, the reaction mixture was then partitioned between EtOAc (20 mL) and sat. $NaHCO_3$ (aq) solution (15 mL). The phases were separated and organics were then passed through a hydrophobic frit and evaporated in vacuo to give the crude protected product which was purified by flash chromatography on silica gel (20 g) eluting with 0–35% EtOAc in hexane (gradient) to afford 40 mg of SEM protected product. De-protection of the SEM group was as outlined for **17** above, followed by purification by flash chromatography on silica gel (10 g) eluting with 0–60% EtOAc in hexane (gradient) to afford title compound **24** (18 mg, 60%) as a colourless solid: LC–MS (method A) t_R = 2.08 min, m/z = 282 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 2.25 (s, 3H), 2.96 (s, 3H), 7.01–7.06 (m, 1H), 7.09 (dd, 1H, $J_{H\text{-}H}$ = 2.6 Hz, J_{H-F} = 9.9 Hz), 7.35 (dd, 1H, $J_{H\text{-}H}$ = 8.4 Hz, J_{H-F} = 5.9 Hz), 7.75 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 19.9 (CH3), 28.6 (CH3), 86.0 (C), 108.9 (C), 113.4 (CH, d, J_{C-F} = 21.9 Hz), 115.7 (C), 117.9 (CH, d, J_{C-F} = 21.8 Hz), 132.2 (CH, d, J_{C-F} = 9.0 Hz), 133.8 (C, d, J_{C-F} = 3.0 Hz), 134.1 (CH), 140.4 (C, d, J_{C-F} = 8.2 Hz), 155.7 (C), 162.0 (C), 162.3 (C), 164.6 (C, d, J_{C-F} = 246.5 Hz); HRMS, Calcd for $C_{15}H_{13}N_5F$ $[M+H]^+$. Found 282.1148 requires 282.1155; HPLC 100% (t_R = 1.08 min).

7.7.43. 2-Ethyl-4-(4-fluoro-2-methyl-phenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (25)

EtMgBr (3.0 M solution in Et_2O, 0.04 ml, 0.11 mmol,) was added to a stirred ice-bath cooled solution of 4-[(4-fluoro-2-methyl-phenyl]-2-methanelsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **57** (50 mg, 0.11 mmol). After 20 min. the reaction was partitioned between EtOAc (2 × 15 ml) and water (15 ml). The combined organic phase was then passed through a hydrophobic frit and evaporated in vacuo to give the crude protected product which was de-protected with tetrabutylammonium fluoride using the method outlined for **17**. Purification was by flash chromatography on silica gel (10 g) eluting with CH_2Cl_2 to 6% MeOH/CH_2Cl_2 (gradient) to afford the title compound (24 mg, 79%) as a beige solid: LC–MS (method A) t_R = 2.20 min, m/z = 281 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 1.39 (t, 3H, J = 7.6 Hz), 2.23 (s, 3H), 3.04 (q, 2H, J = 7.6 Hz), 7.06–7.11 (m, 1H), 7.14 (dd, 1H, $J_{H\text{-}H}$ = 2.6 Hz, J_{H-F} = 9.9 Hz), 7.41 (dd, 1H, $J_{H\text{-}H}$ = 8.4 Hz, J_{H-F} = 5.8 Hz), 8.18 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 13.5 (CH3), 19.9 (CH3, d, J_{C-F} = 1.2 Hz), 33.0 (CH2), 86.0 (C), 113.6 (CH, d, J_{C-F} = 21.9 Hz), 114.8 (C), 115.0 (C), 118.1 (CH, d, J_{C-F} = 21.8 Hz), 132.6 (CH, d, J_{C-F} = 8.9 Hz), 133.3 (C, d, J_{C-F} = 3.1 Hz), 137.8 (CH), 140.7 (C, d, J_{C-F} = 8.6 Hz), 154.0 (C), 160.9 (C), 164.8 (C, d, J_{C-F} = 247.2 Hz), 168.2 (C); HRMS, Calcd for $C_{16}H_{14}N_4F$ $[M+H]^+$. Found 281.1199 requires 281.1202; HPLC 98.7% (t_R = 1.14 min).

7.7.44. 4-[2,4-Dichloro-5-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (26)

To a solution of 1-[2-(2,4-Dichloro-5-iodo-phenoxy)-ethyl]-pyrrolidine (**58**) [preparation method in SI] (200 mg, 0.518 mmol) in THF at −78 °C under an N_2 atmosphere was added triisopropyl borate (0.24 mL, 1.04 mmol) followed by ^{n}BuLi (2.5 M in hexanes, 0.27 ml, 0.67 mmol) drop-wise. The reaction was allowed to warm to RT and was then evaporated in vacuo to give the crude boronic acid. This was combined with 4-chloro-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**46**) (92 mg, 0.26 mmol), 1.0 M $NaHCO_3$ sol. (0.8 mL, 0.78 mmol), $PdCl_2(PPh_3)_2$ (9 mg, 0.01 mmol) and DMF (6 mL). The mixture was degassed by bubbling N_2 through the mixture for 5 min. and was subsequently heated at 80 °C for 2 h under an N_2 atmosphere. The reaction was allowed to cool before being partitioned between EtOAc (2 × 60 ml) and aqueous NH_3 solution (60 mL). The combined organics were dried (Na_2SO_4) and evaporated in vacuo to give a crude oil. This was purified by flash chromatography on silica gel (50 g) eluting with CH_2Cl_2 to 5% MeOH/CH_2Cl_2 (gradient) to afford the protected product as yellow oil, 160 mg (still impure). This was de-protected using the method outlined for **17** and purified by flash chromatography on silica gel (20 g) to afford the title compound **26** (49 mg, 42%) as a yellow solid: LC–MS (method A) t_R = 1.83 min, m/z = 450, 448 $[M+H]^+$; ^{1}H NMR (DMSO-d_6) δ 1.68 (m, 4H), 2.60 (m, 7H), 2.89 (t, 2H, J = 5.5 Hz), 4.22 (t, 2H, J = 5.5 Hz), 7.18 (s, 1H), 7.42 (s, 1H), 8.46 (s, 1H); HRMS, Calcd for $C_{20}H_{20}N_5OSCl_2$ $[M+H]^+$. Found 448.0764 requires 448.0766; HPLC 98.6% (t_R = 1.7 min).

7.7.45. 4-[2,4-Dichloro-5-(2-diethylamino-ethoxy)-phenyl]-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (27)

The title compound was prepared by the methods outlined for **26** using 2-(2,4-dichloro-5-iodo-phenoxy)-ethyl]-diethyl-amine. Purification by flash chromatography, eluting with 0–15% MeOH in DCM (gradient) afforded **27** (31 mg, 63%) as a yellow solid:

LC–MS (method A) t_R = 1.86 min, m/z = 452, 450 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 0.99 (t, 6H, J = 7.0 Hz), 2.58 (s, 3H), 2.62 (q, 4H, J = 7.0 Hz), 2.90 (br t, 2H, J = 5.4 Hz), 4.17 (br t, 2H, J = 5.4 Hz), 7.42 (s, 1H), 8.47 (s, 1H), 8.47 (s, 1H), 12.83 (br s, 1H); HRMS, Calcd for $C_{20}H_{22}N_5OSCl_2$ $[M+H]^+$. Found 450.0920 requires 450.0922; HPLC 95.6% (t_R = 1.04 min).

7.7.46. 4-(2-Chloro-5-hydroxy-4-methoxy-phenyl)-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (60)

A solution of **49** (510 mg, 0.98 mmol) and PPTS (50 mg 0.196 mmol) in isopropanol (7 mL) was heated to 85 °C for 3 h then allowed to cool. The reaction mixture was then diluted with dichloromethane (30 mL) and washed with sat NaCl (aq) solution (2 × 20 mL) then the organic phase was dried over magnesium sulfate and filtered. The filtrate solvents were evaporated and the residual product was purified by flash chromatography on silica gel (50 g) eluting with 0 to 30% EtOAc in hexane (gradient) to afford title compound **60** (382 mg, 82%) as a colourless solid: LC–MS (method A) t_R = 2.76 min, m/z = 479, 477 $[M+H]^+$;

7.7.47. 4-{2-Chloro-5-[2-((*S*)-3-fluoro-pyrrolidin-1-yl)-ethoxy]-4-methoxy-phenyl}-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (61a)

A mixture of **60** (75 mg, 0.157 mmol), 2-((*S*)-3-fluoro-pyrrolidin-1-yl)-ethanol (31 mg, 0.236 mmol) and triphenyl phosphine (62 mg, 0.236 mmol) in THF (2 mL) was stirred at ambient temperature for 10 min. The mixture was cooled with an ice-water bath and a solution of DIAD (46 μL, 0.236 mmol) in anhydrous THF (2 mL) was added drop-wise. The mixture was then stirred at ambient temperature until the reaction was complete, and then diluted with EtOAc (20 mL) and water (20 mL). The organic phase was washed with sat Na_2CO_3 (aq) solution, sat. NaCl (aq) then dried over $MgSO_4$. The crude product was purified by flash chromatography on silica gel (25 g) eluting with 20% EtOAc in DCM. Further purification was via an SCX-II ion exchange column (Biotage, eluting with DCM, then 7 N NH_3 in methanol). This afforded the title compound **61a** (36 mg, 39%) as a solid: LC–MS (method B) t_R = 3.75 min, m/z = 592, 594 $[M+H]^+$.

7.7.48. 4-{2-Chloro-5-[2-((*S*)-3-fluoro-pyrrolidin-1-yl)-ethoxy]-4-methoxy-phenyl}-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (28)

Compound **61a** (36 mg, 0.06 mmol) was de-protected by the method used for **17**. Purification was by prep HPLC (pH 4) affording the title compound **28** (4.4 mg, 16%) as a colourless solid: LC–MS (method A) t_R = 1.72 min, m/z = 464, 462 $[M+H]^+$; ^1H NMR (CD_3OD) δ 1.97-2.12 (m, 1H), 2.14-2.31 (m, 1H), 2.62 (s, 3H), 2.70-2.77 (m, 1H), 2.94 (ddd, 1H, J = 31.6, 12.4, 4.8 Hz), 3.03-3.27 (m, 4H), 3.92 (s, 3H), 4.22 (t, 2H, J = 5.6 Hz), 5.21 (dm, 1H, J = 55 Hz), 7.15 (s, 1H), 7.16 (s, 1H), 8.09 (s, 1H); HRMS, Calcd for $C_{21}H_{22}N_5O_2SClF$ $[M+H]^+$. Found 462.1176 requires 462.1167; HPLC 95.3% (t_R = 0.96 min).

7.7.49. 4-{2-Chloro-5-[2-(3,3-difluoro-pyrrolidin-1-yl)-ethoxy]-4-methoxy-phenyl}-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (29)

The title compound was prepared by the methods outlined for **61a** using 2-(3,3-difluoro-pyrrolidin-1-yl)-ethanol to afford **61c**, followed by de-protection with TBAF as per compound **17** and purification by flash chromatography, eluting with 0–4% MeOH in DCM (gradient) to afford **29** (36 mg, 74%) as a colourless solid: LC–MS (method A) t_R = 2.07 min, m/z = 482, 480 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δ 2.19–2.30 (m, 2H), 2.62 (s, 3H), 2.88 (t, 2H, J = 7.1 Hz), 2.92 (t, 2H, J = 5.3 Hz), 3.07 (t, 2H, J_{H-F} = 13.4 Hz), 3.92 (s, 3H), 4.17 (t, 2H, J = 5.3 Hz), 7.12 (s, 1H), 7.15 (s, 1H), 8.08 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 14.3 (CH3), 36.6 (CH2, t, J_{C-F} = 24.9 Hz), 54.0 (CH2, t, J_{C-F} = 3.7 Hz), 55.3 (CH2), 56.8 (CH3), 63.4 (CH2, t, J_{C-F} = 29.4 Hz), 69.3 (CH2), 86.6 (C), 113.8 (C), 114.2 (CH), 115.4 (C), 116.9 (CH), 126.4 (C), 127.9 (C), 131.3 (C, t, J_{C-F} = 247.2 Hz), 136.8 (CH), 148.4 (C), 153.0 (C), 154.2 (C), 159.1 (C), 168.2 (C); HRMS, Calcd for $C_{21}H_{21}N_5O_2SClF_2$ $[M+H]^+$. Found 480.1056 requires 480.1073; HPLC 100% (t_R = 1.12 min).

7.7.50. 4-(2,4-dichloro-5-methoxy-phenyl)-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (61b)

The title compound was prepared by the methods outlined for **15b.** Thus **46** (390 mg, 1.1 mmol) was reacted with 2-chloro-4,5-dimethoxyphenyl boronic acid [compound **59**, see Supplementary data for preparation] (1.37 mmol) and the intermediate product purified by flash chromatography on silica gel (70 g) eluting with hexane-20% EtOAc/hexane (gradient) to afford the title compound **61e** (260 mg, 48%) as a yellow oil: LC–MS (method A) t_R = 2.96 min, m/z = 497, 495 $[M+H]^+$.

7.7.51. 4-(2,4-Dichloro-5-methoxy-phenyl)-2-methanesulfonyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (62b)

To a solution of **61b** (230 mg, 0.468 mmol) in DCM (8 mL) cooled with an ice-water bath was added drop-wise a solution of *m*-chloroperoxybenzoic acid (70%, 420 mg, 1.87 mmol) in DCM (8 mL). The reaction mixture was then allowed to warm to room temperature and after 1 h diluted with DCM (20 mL) and then washed with 5% (w/v) aq sodium thiosulphate solution. The phases were separated and the aqueous phase was re-extracted with DCM (20 mL). The combined organics were washed with sat Na_2CO_3(aq) solution, dried over sodium sulphate and filtered. The filtrate was evaporated in vacuo to afford desired product as a yellow solid (230 mg, 90%): LC–MS (method A) t_R = 2.73 min, m/z = 529, 527 $[M+H]^+$.

7.7.52. 4-(2,4-Dichloro-5-methoxy-phenyl)-2-(2-diethylamino-ethylsulfanyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (30)

A mixture of **62b** (50 mg, 0.095 mmol), 2-diethylaminoethanethiol hydrochloride (64 mg, 0.38 mol) and triethylamine in DMF (2 mL) was heated at 100 °C for 30 min. then allowed to cool. The mixture was partitioned between EtOAc (40 mL) and aqueous ammonia solution (40 mL), and phases separated. The organic phase dried over Na_2SO_4 and solvent was removed in vacuo to afford a brown oil (120 mg). This product was de-protected with TBAF according to methods utilized for compound **17**, and purified by flash chromatography on silica gel (20 g) eluting with 0 to 20% MeOH in DCM (gradient) to afford the title compound **30** (26 mg, 61%) as a yellow solid: LC–MS (method A) t_R = 1.84 min, m/z = 450, 452 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δ 1.12 (t, 6H, J = 7.2 Hz), 2.83 (q, 4H, J = 7.2 Hz), 3.05–3.09 (m, 2H), 3.36–3.39 (m, 2H), 3.93 (s, 3H), 7.26 (s, 1H), 7.61 (s, 1H), 8.14 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 11.1 (CH3), 27.4 (CH2), 48.3 (CH2), 53.1 (CH2), 57.2 (CH3), 86.1 (C), 114.2 (C), 115.3 (C), 115.7 (CH), 125.2 (C), 125.9 (C), 131.8 (CH), 135.4 (C), 138.2 (CH), 154.7 (C), 155.5 (C), 158.3 (C), 166.5 (C); HRMS, Calcd for $C_{20}H_{22}N_5SOCl_2$ $[M+H]^+$. Found 450.0920 requires 450.0922; HPLC 97.5% (t_R = 1.05 min).

7.7.53. 4-(2-Chloro-4,5-dimethoxy-phenyl)-2-(2-diethylamino-ethylsulfanyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (31)

The title compound was prepared by the methods outlined for preparation of compound **30** (Scheme 4). Final purification by flash chromatography on silica gel (10 g) eluting with 0 to 8% MeOH in DCM (gradient) afforded the title compound **31** (22 mg, 60%) as a yellow solid: LC–MS (method A) t_R = 1.68 min, m/z = 446 $[M+H]^+$;

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97 (t, 6H, J = 7.0 Hz), 2.63 (m, 4H), 2.86 (m, 2H), 3.28 (m, 2H), 3.80 (s, 3H), 3.86 (s, 3H), 7.16 (s, 1H), 7.19 (s, 1H), 8.44 (s, 1H), 12.60 (br s, 1H); HRMS, Calcd for $C_{21}H_{23}N_5O_2SCl$ $[M-H]^-$. Found 444.1271 requires 444.1261; HPLC 99.1% (t_R = 0.93 min).

7.7.54. 4-(2,4-Dichloro-5-methoxy-phenyl)-2-(2-diethylamino-ethoxy)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (32)

The title compound was prepared by the methods outlined for preparation of compound **30** (Scheme 4) utilizing 2-diethylamino-ethanol at the appropriate step. Final purification by flash chromatography on silica gel (10 g) eluting with 1:10 Et_3N/DCM to 1:10:89 Et_3N/MeOH/DCM (gradient) afforded the title compound **32** (16 mg, 28%) as a cream-coloured solid: LC–MS (method A) t_R = 1.75 min, m/z = 436, 434 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 1.11 (t, 6H, J = 7.2 Hz), 2.75 (q, 4H, J = 7.2 Hz), 3.03 (t, 2H, J = 6.1 Hz), 3.93 (s, 3H), 4.60 (t, 2H, J = 6.1 Hz), 7.25 (s, 1H), 7.61 (s, 1H), 8.08 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 11.3 (CH3), 48.8 (CH2), 52.1 (CH2), 57.2 (CH3), 66.3 (CH2), 86.4 (C), 112.7 (C), 115.3 (C), 115.5 (CH), 125.2 (C), 125.9 (C), 131.8 (CH), 135.3 (C), 137.5 (CH), 155.5 (C), 155.7 (C), 159.8 (C), 163.4 (C); HRMS, Calcd for $C_{20}H_{20}N_5O_2Cl_2$ $[M-H]^-$. Found 432.0987 requires 432.0994; HPLC 100% (t_R = 1.00 min).

7.7.55. 2-{5-Cyano-4-[2,4-dichloro-5-(2-diethylamino-ethoxy)-phenyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ylsulfanyl}-*N*-methyl-acetamide (33)

The title compound was prepared by the methods outlined for preparation of compound **30** (Scheme 4) utilizing 2-diethylamino-ethanol and 2-mercapto-*N*-methylacetamide for the appropriate steps. Final purification by prep HPLC (pH 9) afforded the title compound **33** (7 mg, 30%) as a colourless solid (formate salt): LC–MS (method A) t_R = 1.70 min, m/z = 509, 507 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 1.31 (t, 6H, J = 7.3 Hz); 2.73 (s, 3H); 3.20 (q, 4H, J = 7.3 Hz); 3.49 (t, 2H, J = 5.0 Hz), 3.95 (s, 2H), 4.43 (t, 2H, J = 5.0 Hz); 7.34 (s, 1H); 7.69 (s, 1H); 8.18 (s, 1H); 8.53 (s, 1H); HRMS, Calcd for $C_{22}H_{23}N_6O_2SCl_2$ $[M-H]^-$. Found 505.0967 requires 505.0980; HPLC 94.8% (t_R = 0.95 min).

7.7.56. 4-(2-Chloro-4-cyano-5-methoxy-phenyl)-2-(2-diethylamino-ethylsulfanyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (34)

The title compound was prepared by the methods outlined for preparation of compound **30** (Scheme 4) utilizing 2-chloro-4-cyano-5-methoxyphenylboronic acid **66** [see Supplementary data section for synthesis] and diethylaminoethanethiol for the appropriate steps. Final purification by flash chromatography on silica gel (20 g) eluting with 0–10% MeOH in DCM (gradient) afforded the title compound **34** (25 mg, 66%) as yellow solid: LC–MS (method A) t_R = 1.75 min, m/z = 441 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 1.16 (t, 6H, J = 7.2 Hz), 2.93 (q, 4H, J = 7.2 Hz), 3.16–3.20 (m, 2H), 3.40–3.43 (m, 2H), 3.99 (s, 3H), 7.42 (s, 1H), 7.90 (s, 1H), 8.18 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 10.8 (CH3), 27.1 (CH2), 48.5 (CH2), 52.9 (CH2), 57.6 (CH3), 85.9 (C), 105.2 (C), 114.1 (C), 115.1 (C), 115.5 (C), 115.8 (CH), 125.5 (C), 135.5 (CH), 138.5 (CH), 141.6 (C), 154.8 (C), 157.5 (C), 161.3 (C), 166.3 (C); HRMS, Calcd for $C_{21}H_{22}N_6OSCl$ $[M+H]^+$. Found 441.1251 requires 441.1264; HPLC 94.9% (t_R = 0.97 min).

7.7.57. 4-{2-Chloro-5-[2-(3,3-difluoro-pyrrolodin-1-yl)-ethoxy]-4-methoxy-phenyl}-2-isopropylsulfanyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (35)

The title compound was prepared by the methods outlined for preparation of compound **30** (Scheme 4) utilizing 2-(3,3-difluoro-pyrrolidin-1-yl)-ethanol and isopropylthiol at the appropriate steps. Final purification by flash chromatography on silica gel (25 g) eluting with 0–60% EtOAC in hexane followed by purification by prep HPLC, afforded the title compound **35** as a colourless solid: LC–MS (method A) t_R = 2.33 min, m/z = 510, 508 $[M+H]^+$; ^{1}H NMR (CD_3OD) δ 0.65 (d, 6H, J = 7.1 Hz), 1.39-1.51 (m, 2H), 2.06-2.14 (m, 4H), 2.27 (t, 2H, J = 13.4 Hz), 3.12 (s, 3H), 3.20-3.28 (m, 1H), 3.36 (t, 2H, J = 5.3 Hz), 6.31 (s, 1H), 6.35 (s, 1H), 7.29 (s, 1H); HRMS, Calcd for $C_{23}H_{25}N_5O_2SClF_2$ $[M+H]^+$. Found 508.1390 requires 508.1386; HPLC 100% (t_R = 1.26 min).

7.7.58. 4-[(4-Fluoro-2-methyl-phenyl]-2-methylsulfanyl-7-(2-trimethylsilanyl-ethoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid amide (55)

The title compound was prepared by the methods outlined for **15b** (step 1). Thus **45** (60 mg, 0.161 mmol) was reacted with 2-methyl-4-fluorophenyl boronic acid (32 mg, 0.209 mmol) and the product purified by flash chromatography on silica gel eluting with 20–65% EtOAc in hexane (gradient) to afford the title compound **55** (65 mg, 90%) as a colourless oil: LC–MS (method A) t_R = 2.67 min, m/z = 447 $[M+H]^+$.

7.7.59. 2,6-Dichloro-9-(tetrahydro-pyran-2-yl)-9*H*-purine (63)

To anhydrous ethyl acetate (25 mL) under nitrogen atmosphere was added 2,6-dichloropurine (3 g, 16 mmol) and *p*-toluenesulphonic acid (48 mg, 0.22 mmol), this mixture was warmed to 50 °C and stirred as a suspension. 3,4-Dihydro-2H-pyran (1.63 mL, 17.9 mmol) was added drop-wise over 10 min and the resulting mixture stirred at 50 °C for 1 h, then cooled to ambient temperature. Aqueous ammonia (0.880, 2 mL) was added and the solution stirred for 5 min. The reaction mixture was diluted with ethyl acetate and washed twice with water. The phases were separated and the organic phase dried over $MgSO_4$ and filtered. The filtrate solvents were removed in vacuo to give a pale-yellow solid, which was suspended in refluxing hexane for 15 min, allowed to cool for 1 h and filtered and dried to afford title compound (**63**) (3.19 g, 73%) as a colourless solid: R_f 0.76 CH_2Cl_2/MeOH (10:1) SiO_2; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.55 (m, 2H), 1.75 (m, 1H), 2 (m, 2H), 2.25 (m, 1H), 3.75 (m, 1H), 4.00 (m, 1H), 5.80 (dd, 1H), 8.95 (s, 1H).

7.7.60. General procedure A: cross-coupling reaction with 63

To a solution of 2,6-dichloro-9-(tetrahydro-pyran-2-yl)-9H-purine (**63**, 350 mg, 1.28 mmol) in DMF (10 mL) was added the appropriate boronic acid (1.41 mmol) and potassium carbonate (532 mg, 3.85 mmol) and the resulting solution then sparged with nitrogen gas. tetrakis(triphenylphosphine)palladium (74 mg, 0.06 mmol) was added and the mixture heated to 110 °C for 1 h in a microwave synthesizer. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate and washed with saturated brine solution. Organics were dried over $MgSO_4$ then purified via silica gel chromatography, eluting with a gradient of 0–50% EtOAc in hexane.

7.7.61. General procedure B: preparation of 13a–c

The 4-aryl purine products from **63** (general procedure A) (0.20 mmol) in dimethylformamide (2 mL) were treated with sodium thiomethoxide (18 mg, 0.26 mmol) and then heated to 120 °C for 15 min in a microwave synthesizer. The reaction mixture was diluted with ethyl acetate and washed with saturated sodium bicarbonate solution followed by saturated brine solution. Organics were dried over $MgSO_4$ and concentrated in vacuo. The residue was applied to a Biotage SCX-2 (silica bound sulphonic acid) ion exchange column, and washed with methanol and product eluted off with 7 N NH_3 in methanol solution. Products were purified by preparative HPLC at pH 4.

7.7.62. 6-(4-Chloro-2-methyl-phenyl)-2-methylsulfanyl-9*H*-purine (13a)

Prepared by general procedures A then B to afford title compound **13a** (12 mg, 20%) as a colourless solid: LC–MS (method A) t_R = 2.33 min, m/z = 291 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD + DMSO-d_6) δ 2.36 (s, 3H), 2.63 (s, 3H), 7.38 (dd, 1H, J = 8.3, 1.9 Hz), 7.44 (d, 1H, J = 1.9 Hz), 7.59 (d, 1H, J = 8.3 Hz), 8.35 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD + DMSO-d_6) δ 14.6 (CH3), 20.4 (CH3), 127.0 (CH), 127.4 (C, br), 131.8 (CH), 133.0 (CH), 134.9 (C), 136.5 (C), 140.6 (C), 145.8 (CH), 156.9 (C, br), 156.9 (C, br), 166.9 (C); HRMS, Calcd for $C_{13}H_{12}N_4SCl$ $[M+H]^+$. Found 291.0467 requires 291.0471; HPLC 96.2% (t_R = 1.19 min).

7.7.63. 6-(4-Fluoro-2-methyl-phenyl)-2-methylsulfanyl-9*H*-purine (13b)

Prepared by general procedures A then B to afford title compound **13b** (11 mg, 20%) as a colourless solid: LC–MS (method A) t_R = 2.19 min, m/z = 275 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 2.36 (s, 3H), 2.62 (s, 3H), 7.06–7.11 (m, 1H), 7.14 (dd, 1H, J_{H-H} = 2.5 Hz, J_{H-F} = 9.9 Hz), 7.59 (dd, 1H, J_{H-H} = 8.4 Hz, J_{H-F} = 5.9 Hz), 8.33 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 14.5 (CH3), 20.5 (CH3, d, J_{C-F} = 1.5 Hz), 113.7 (CH, d, J_{C-F} = 21.7 Hz), 118.5 (CH, d, J_{C-F} = 21.6 Hz), 127.4 (C, br), 132.4 (C, d, J_{C-F} = 2.9 Hz), 133.4 (CH, d, J_{C-F} = 8.9 Hz), 141.4 (C, d, J_{C-F} = 8.4 Hz), 145.7 (CH), 157.0 (C, br), 157.2 (C), 164.9 (C, d, J_{C-F} = 247.8 Hz), 167.1 (C); HRMS, Calcd for $C_{13}H_{12}N_4SF$ $[M+H]^+$. Found 275.0763 requires 275.0767; HPLC 100% (t_R = 1.11 min).

7.7.64. 4-(2-Methylsulfanyl-9*H*-purin-6-yl)-benzonitrile (13c)

Prepared by general procedures A then B to afford title compound **13c** (8 mg, 15%) as an off-white solid: LC–MS (method A) t_R = 2.22 min, m/z = 268 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD + DMSO-d_6) δ 2.71 (s, 3H), 7.99 (d, 2H, J = 8.3 Hz), 8.44 (s, 1H), 8.98 (d, 2H, J = 8.3 Hz); ^{13}C NMR (100.6 MHz, CD_3OD + DMSO-d_6) δ 14.8 (CH3), 115.0 (C), 119.7 (C), 128.4 (C, br), 131.3 (CH), 133.6 (CH), 141.2 (C), 145.7 (CH), 152.0 (C), 157.0 (C, br), 166.5 (C); HRMS, Calcd for $C_{13}H_{10}N_5S$ $[M+H]^+$. Found 268.0663 requires 268.0657; HPLC 96.9% (t_R = 1.12 min).

7.7.65. 4-(2,4-Dimethyl-phenyl)-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (14a)

The title compound was prepared by the methods outlined for **15b.** Thus **42** (100 mg, 0.303 mmol) was reacted with 2,4-dimethyl-phenyl boronic acid (55 mg, 0.364 mmol), followed by de-protection (TBAF) and final product purification by flash chromatography on silica gel (20 g) eluting with 0–20% EtOAc in hexane (gradient) to afford the title compound **14a** (25 mg, 32%) as a colourless solid: LC–MS (method B) t_R = 4.18 min, m/z = 270 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 2.26 (s, 3H), 2.38 (s, 3H), 2.60 (s, 3H), 6.26 (d, 1H, J = 3.6 Hz), 7.13 (d, 1H, J = 7.7 Hz), 7.18 (s, 1H), 7.26 (d, 1H, J = 3.6 Hz), 7.32 (d, 1H, J = 7.7 Hz); ^{13}C NMR (100.6 MHz, CD_3OD) δ 14.2 (CH3), 20.1 (CH3), 21.3 (CH3), 101.7 (CH), 115.3 (C), 126.3 (CH), 127.4 (CH), 130.5 (CH), 132.6 (CH), 135.4 (C), 137.4 (C), 140.4 (C), 154.4 (C), 161.4 (C), 164.9 (C); HRMS, Calcd for $C_{15}H_{16}N_3S$ $[M+H]^+$. Found 270.1065 requires 270.1065; HPLC 96.9% (t_R = 1.33 min).

7.7.66. 4-(4-Fluoro-2-methyl-phenyl)-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (14b)

The title compound was prepared by the methods outlined for **15b**. Thus **42** (200 mg, 0.61 mmol) was reacted with 2-fluoro-4-methyl-phenyl boronic acid (112 mg, 0.72 mmol), followed by de-protection (TBAF) and final product purification by flash chromatography on silica gel (20 g) eluting with 0 to 20% EtOAc in hexane (gradient) to afford the title compound **14b** (17 mg, 50%) as a colourless solid: LC–MS (method A) t_R = 2.49 min, m/z = 274 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD + DMSO-d_6) δ 2.33 (s, 3H), 2.62 (s, 3H), 6.30 (d, 1H, J = 3.5 Hz), 7.06–7.11 (m, 1H), 7.15 (dd, 1H, J_{H-H} = 2.5 Hz, J_{H-F} = 10.0 Hz), 7.31 (d, 1H, J = 3.5 Hz), 7.50 (dd, 1H, J_{H-H} = 8.4 Hz, J_{H-F} = 6.0 Hz); ^{13}C NMR (100.6 MHz, CD_3OD + DMSO-d_6) δ 14.3 (CH3), 20.4 (CH3, d, J_{C-F} = 1.5 Hz), 101.4 (CH), 113.6 (CH, d, J_{C-F} = 21.7 Hz), 115.3 (C), 118.5 (CH, d, J_{C-F} = 21.4 Hz), 126.7 (CH), 132.7 (CH, d, J_{C-F} = 8.9 Hz), 134.6 (C, d, J_{C-F} = 2.9 Hz), 140.8 (C, d, J_{C-F} = 8.3 Hz), 154.5 (C), 160.0 (C), 164.4 (C, d, J_{C-F} = 246.7 Hz), 164.9 (C); HRMS, Calcd for $C_{14}H_{13}N_3SF$ $[M+H]^+$. Found 274.0819 requires 274.0814; HPLC 98.6% (t_R = 1.28 min).

7.7.67. 4-(2-Methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl-benzonitrile (14c)

The title compound was prepared by the methods outlined for **15b**. Thus **42** (100 mg, 0.303 mmol) was reacted with 4-cyanophenyl boronic acid (58 mg, 0.394 mmol), followed by de-protection (TBAF) and final product purification by flash chromatography on silica gel (20 g) eluting with 0–50% EtOAc in hexane (gradient) to afford the title compound **14c** (20 mg, 25%) as a yellow solid: LC–MS (method A) t_R = 2.37 min, m/z = 267 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD + DMSO-d_6) δ 2.68 (s, 3H), 6.86 (d, 1H, J = 3.7 Hz), 7.48 (d, 1H, J = 3.7 Hz), 7.98 (d, 2H, J = 8.4 Hz), 8.37 (d, 2H, J = 8.4 Hz); ^{13}C NMR (100.6 MHz, CD_3OD + DMSO-d_6) δ 14.5 (CH3), 101.2 (CH), 113.6 (C), 114.4 (C), 119.7 (C), 127.9 (CH), 130.7 (CH), 133.8 (CH), 143.5 (C), 155.6 (C), 155.7 (C), 165.2 (C); HRMS, Calcd for $C_{14}H_{11}N_4S$ $[M+H]^+$. Found 267.0699 requires 267.0704; HPLC 100% (t_R = 1.21 min).

7.7.68. 4-Chloro-6-methylsulfanyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (65)

6-Methylsulfanyl-4-oxo-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-carboxylic acid amide (prepared according to the literature procedure described in Ref. 50; 380 mg, 1.69 mmol) was added to a stirring mixture of phosphorus oxychloride (15 mL) and *N*,*N*-dimethylaniline (1.5 mL). The reaction mixture was heated to reflux where it was held for 4 h and then allowed to cool to RT. The excess $POCl_3$ was removed in vacuo and the residue was added in portions to rapidly stirring ice/H_2O, and the pH then adjusted to two by the careful addition of ammonia solution. The acidic aqueous mixture was extracted with ethyl acetate (3 × 25 mL) and the combined extracts were washed with water (30 mL), brine (2 × 30 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (25 g) and the desired product eluted using 30% ethyl acetate/iso-hexane. The pure fractions containing product were combined and concentrated in vacuo to yield the title compound **65** (185 mg, 49%) as a pale-yellow solid.

7.7.69. 4-(2,4-Dimethyl-phenyl)-6-methylsulfanyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (16)

4-Chloro-6-methylsulfanyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (**65**, 75 mg, 0.33 mmol), 2,4-dimethylphenylboronic acid (65 mg, 0.43 mmol), 2 M K_3PO_4 solution (1 mmol, 0.5 mL) and 1,4-dioxane (3 mL) were charged into a flask and thoroughly degassed. [1,1′-bis(diphenylphosphino)ferrocene]dichloro-palladium(II) (13.5 mg, 0.0167 mmol,) was added and the reaction mixture was heated at 90 °C for 6 h. After cooling, the reaction mixture was partitioned between ethyl acetate and brine. The organic layer was separated and the aqueous layer extracted with a further portion of ethyl acetate. The combined ethyl acetate extracts were washed with saturated sodium hydrogen carbonate solution (2 × 30 mL) then brine (2 × 30 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (10 g) and the product eluted using 20% ethyl acetate in iso-hexane. The fractions containing product were combined and concentrated in vacuo and the residue further purified by

preparative HPLC at pH 4 to afford the title compound **16** (4.3 mg, 4%) as a colourless solid: LC–MS (method A) t_R = 2.55 min, m/z = 296 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δ 2.31 (s, 3H), 2.42 (s, 3H), 2.65 (s, 3H), 7.20 (d, 1H), 7.24 (s, 1H), 7.37 (d, 1H); HRMS, Calcd for $C_{15}H_{14}N_5S$ $[M+H]^+$. Found 296.0965 requires 296.0970; HPLC (from method A) 97.3% (t_R = 2.51 min).

Acknowledgment

We thank Loic Le Strat for compound purification and analysis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.08.050.

References and notes

1. Mosser, D. D.; Morimoto, R. I. *Oncogene* **2004**, *23*, 2907.
2. Pearl, L. H.; Prodromou, C. *Annu. Rev. Biochem.* **2006**, *75*, 271.
3. Obermann, W. M.; Sondermann, H.; Russo, A. A.; Pavletich, N. P.; Hartl, F. U. *J. Cell Biol.* **1998**, *143*, 901.
4. Panaretou, B.; Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *EMBO J.* **1998**, *17*, 4829.
5. Pearl, L. H.; Prodromou, C.; Workman, P. *Biochem. J.* **2008**, *410*, 439.
6. For a list, see the web page maintained by D. Picard: http://www.picard.ch/downloads/Hsp90interactors.pdf (accessed June 2012).
7. Whitesell, L.; Lindquist, S. L. *Nat. Rev. Cancer* **2005**, *5*, 761.
8. Maloney, A.; Workman, P. *Expert Opin. Biol. Ther.* **2002**, *2*, 3.
9. Kamal, A.; Boehm, M. F.; Burrows, F. J. *Trends Mol. Med.* **2004**, *10*, 283.
10. Chiosis, G.; Vilenchik, M.; Kim, J.; Solit, D. *Drug Discovery Today* **2004**, *9*, 881.
11. Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57.
12. Workman, P. *Cancer Lett.* **2004**, *206*, 149.
13. Kamal, A.; Thao, L.; Sensintaffar, J.; Zhang, L.; Boehm, M. F.; Fritz, L. C.; Burrows, F. J. *Nature* **2003**, *425*, 407.
14. da Rocha, D. S.; Friedlos, F.; Light, Y.; Springer, C.; Workman, P.; Marais, R. *Cancer Res.* **2005**, *65*, 10686.
15. DeBoer, C.; Meulman, P. A.; Wnuk, R. J.; Peterson, D. H. *J. Antibiot. (Tokyo)* **1970**, *23*, 442.
16. Supko, J. G.; Hickman, R. L.; Grever, M. R.; Malspeis, L. *Cancer Chemother. Pharmacol.* **1995**, *36*, 305.
17. Neckers, L.; Schulte, T. W.; Mimnaugh, E. *Invest. New Drugs* **1999**, *17*, 361.
18. Delmotte, P.; Delmotte-Plaque, J. *Nature* **1953**, *171*, 344.
19. Soga, S.; Shiotsu, Y.; Akinaga, S.; Sharma, S. V. *Curr. Cancer Drug Targets* **2003**, *3*, 359.
20. Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *J. Med. Chem.* **1999**, *42*, 260.
21. Schulte, T. W.; Neckers, L. M. *Cancer Chemother. Pharmacol.* **1998**, *42*, 273.
22. Kaur, G.; Belotti, D.; Burger, A. M.; Fisher-Nielson, K.; Borsotti, P.; Riccardi, E.; Thillainathan, J.; Hollingshead, M.; Sausville, E. A.; Giavazzi, R. *Clin. Cancer Res.* **2004**, *10*, 4813.
23. Chiosis, G.; Timaul, M. N.; Lucas, B.; Munster, P. N.; Zheng, F. F.; Sepp-Lorenzino, L.; Rosen, N. *Chem. Biol.* **2001**, *8*, 289.
24. Patel, H. J.; Modi, S.; Chiosis, G.; Taldone, T. *Expert Opin. Drug Discovery* **2011**, *6*, 559.
25. Taldone, T.; Sun, W.; Chiosis, G. *Bioorg. Med. Chem.* **2009**, *17*, 2225.
26. Drysdale, M. J.; Brough, P. A. *Curr. Top. Med. Chem. (Sharjah, United Arab Emirates)* **2008**, *8*, 859.
27. Messaoudi, S.; Peyrat, J. F.; Brion, J. D.; Alami, M. *Anticancer Agents Med. Chem.* **2008**, *8*, 761.
28. Janin, Y. L. *Drug Discovery Today* **2010**, *15*, 342.
29. Biamonte, M. A.; Van de Water, R.; Arndt, J. W.; Scannevin, R. H.; Perret, D.; Lee, W. C. *J. Med. Chem.* **2010**, *53*, 3.
30. Gao, Z.; Garcia-Echeverria, C.; Jensen, M. R. *Curr. Opin. Drug Discov. Dev.* **2010**, *13*, 193.
31. Brough, P. A.; Drysdale, M. J.; Davies, N. G. M.; Foloppe, N. N.; Stokes, S. PCT Int. Appl. WO2007104944, 2007.
32. Kung, P. P.; Sinnema, P. J.; Richardson, P.; Hickey, M. J.; Gajiwala, K. S.; Wang, F.; Huang, B.; McClellan, G.; Wang, J.; Maegley, K.; Bergqvist, S.; Mehta, P. P.; Kania, R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3557.
33. Hajduk, P. J.; Greer, J. *Nat. Rev. Drug Discovery* **2007**, *6*, 211.
34. Congreve, M.; Chessari, G.; Tisi, D.; Woodhead, A. J. *J. Med. Chem.* **2008**, *51*, 3661.
35. Jhoti, H.; Leach, A. R. *Structure-Based Drug Discovery*; Springer: Dordretcht, The Netherlands, 2007.
36. Hubbard, R. E.; Davis, B.; Chen, I.; Drysdale, M. J. *Curr. Top. Med. Chem.* **2007**, *7*, 1568.
37. Foloppe, N. *Future Med. Chem.* **2011**, *3*, 1111.
38. Roughley, S. D.; Hubbard, R. E. *J. Med. Chem.* **2011**, *54*, 3989.
39. Brough, P. A.; Barril, X.; Borgognoni, J.; Chene, P.; Davies, N. G. M.; Davis, B.; Drysdale, M. J.; Dymock, B.; Eccles, S. A.; Garcia-Echeverria, C.; Fromont, C.; Hayes, A.; Hubbard, R. E.; Jordan, A. M.; Jensen, M. R.; Massey, A.; Merrett, A.; Padfield, A.; Parsons, R.; Radimerski, T.; Raynaud, F. I.; Robertson, A.; Roughley, S. D.; Schoepfer, J.; Simmonite, H.; Sharp, S. Y.; Surgenor, A.; Valenti, M.; Walls, S.; Webb, P.; Wood, M.; Workman, P.; Wright, L. *J. Med. Chem.* **2009**, *52*, 4794.
40. PDB codes 1: 3060; PU3: 1UY6; 8: 2WI1; 9: 2WI7.
41. Dunitz, J. D. *Science (Washington, DC, 1883)* **1994**, *264*, 670.
42. Chen, J. M.; Xu, S. L.; Wawrzak, Z.; Basarab, G. S.; Jordan, D. B. *Biochemistry* **1998**, *37*, 17735.
43. Fleming, F. F.; Yao, L.; Ravikumar, P. C.; Funk, L.; Shook, B. C. *J. Med. Chem.* **2010**, *53*, 7902.
44. Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490.
45. Davoll, J. *J. Chem. Soc.* **1960**, 131.
46. Fisher, M. B.; Campanale, K.; Ackermann, B. L.; VandenBranden, M.; Wrighton, S. A. *Drug Metab. Dispos.* **2000**, *28*, 560.
47. Howes, R.; Barril, X.; Dymock, B. W.; Grant, K.; Northfield, C. J.; Robertson, A. G.; Surgenor, A.; Wayne, J.; Wright, L.; James, K.; Matthews, T.; Cheung, K. M.; McDonald, E.; Workman, P.; Drysdale, M. J. *Anal. Biochem.* **2006**, *350*, 202.
48. Kelland, L. R.; Sharp, S. Y.; Rogers, P. M.; Myers, T. G.; Workman, P. *J. Natl. Cancer Inst.* **1999**, *91*, 1940.
49. Potter et al *Bioorg. Med. Chem. Lett.* **2010**, *20*, 586.
50. Petrie, C. R.; Cottam, H. B.; Mckernan, P. A.; Robins, R. K.; Revankar, G. R. *J. Med. Chem.* **1985**, *28*, 1010.