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Synthesis and biological evaluation of substituted 2-anilino-7*H*-pyrrolopyrimidines as PDK1 inhibitors

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ABSTRACT

An efficient and scalable route for a series of novel substituted 2-anilino-7*H*-pyrrolopyrimidine compounds as potential inhibitors of PDK1, an important regulator of the PI3K/Akt pathway that is dysregulated in many cancers, was developed and is described. The synthetic strategy was designed around Suzuki and Buchwald–Hartwig cross-couplings of a boronate fragment and various customised anilines sequentially with 2,4-dichloro-7-tosyl-7*H*-pyrrolopyrimidine. All fragments were constructed separately and cross-coupled to provide access to a range of novel compounds. Biological evaluation of these was undertaken, with modest inhibition observed.

1. Introduction

3-Phosphoinositide-dependent kinase 1 (PDK1) is the master regulator of the AGC kinase superfamily and plays a crucial role in the positive regulation of the important cellular effects triggered by the PI3K/Akt signalling pathway and subsequent phosphatidylinositol 3,4,5-triphosphate (PIP3) production.[1,2] PIP3 is activated by phosphatidylinositol 3-kinase (PI3K) and acts as a second messenger by interacting with several downstream effector proteins. These proteins control the activity and subcellular localisation of a diverse range of signal transducers that are fundamental to cell growth, proliferation and survival.[1,3,4] The physiological effects that PIP3 raises in cells are mediated by a particular set of AGC protein kinase family members. PDK1 is known to be responsible for the regulation of as many as 23 different agonist-stimulated AGC kinases, including Akt,[5,6] PKC isoforms,[7] P70 S6K,[8] p90 SGK[9] and RSK[10] by phosphorylation at its activation loop.

A large number of human cancers possess mutations in genes that lead to abnormally high levels of PIP3, resulting in constitutive activation of members within the PI3K/Akt pathway.[11,12] Disruption or dysregulation of this pathway promotes tumour proliferation, reduced apoptosis and angiogenesis.[13,14] Recent experimental evidence has shown that specific inhibitors of this pathway are able to promote apoptosis in cells and reduce tumour growth.[15] As PDK1 plays an important role in regulating the PI3K/Akt pathway, targeted inhibition of this enzyme represents a promising biological target for the treatment of cancer.[13,16]

Recently we reported the development of a series of novel substituted 4-(1*H*-indol-6-yl)-1*H*-indazoles as potential PDK1 inhibitors.[17] This set of compounds was designed with the heterocyclic core bound to the PDK1 hinge region, allowing for incorporation of additional functionality to target various pockets within the ATP binding site. Using the same molecular modelling-guided approach, we identified that a 2-anilino-7*H*-pyrrolopyrimidine core also possessed the correct geometry to potentially meet the requirements of a PDK1 inhibitor (Fig. 1).

The hydrogen bond donor, acceptor, donor backbone from the nitrogens of the 7*H*-pyrrolopyrimidine heterocyclic core and 2-position aniline provide strong interactions with Ala-91 and Ser-89 residues of the hinge region, which potentially locks the inhibitor into the binding site. An appropriate functional group at the 4-position of the 7*H*-pyrrolopyrimidine ring would allow binding to the catalytic residues of the phosphate pocket (P) and the hydrophobic region (BP-I). Compound **1** (Fig. 2), a potent PDK1 inhibitor developed by Vernalis,[18] contains the 3-(pyridin-3-ylmethoxy)phenyl side chain that is seen as a linker to the back cleft of the PDK1 binding site, interacting with important catalytic

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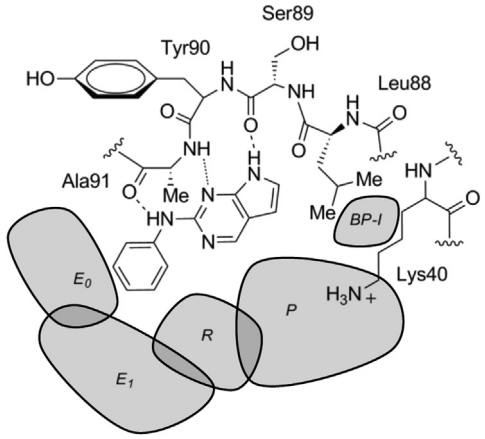


Fig. 1. Schematic illustration of the PDK1 binding site, which displays how the 2-anilino-7*H*-pyrrolopyrimidine core interacts with the residues of the hinge region and the potential to target various pockets within.

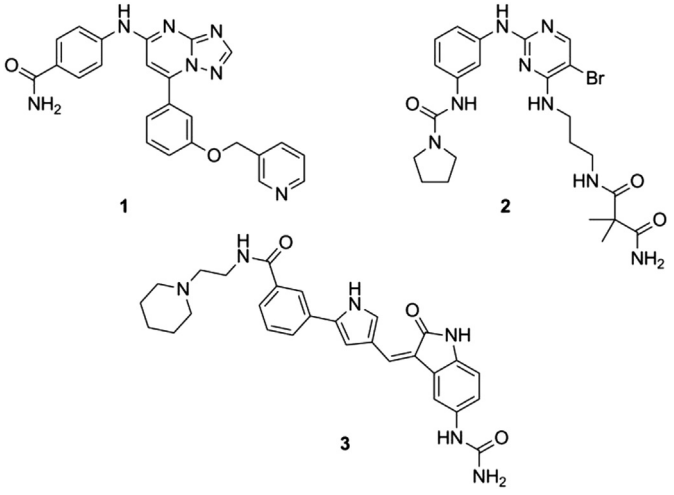


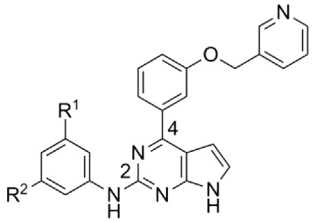
Fig. 2. Known PDK1 inhibitors developed by Vernalis and Berlex Biosciences.

residues in the phosphate pocket. As a result, all of the designed inhibitors retained this moiety at the 4-position of the pyrrolopyrimidine core, so the effects of changing the side chains at the 2-position could be observed.

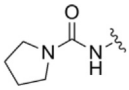
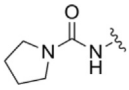
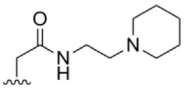
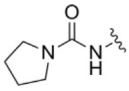
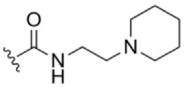
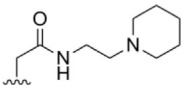
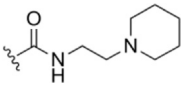
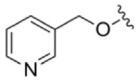
We were particularly interested in changes to the 2-anilino functional group in order to study differences in PDK1 binding according to the ability of the compound to access the solvent exposed regions (E_0 and E_1) and the ribose pocket (R). Compounds **2** and **3** (Fig. 2) produced by Berlex Biosciences[19,20] were identified with X-ray crystal structures available in complex with PDK1 (PDB codes: 1Z5M and 2PE2). The pyrrolidine urea of compound **2** binds to the ribose pocket of PDK1 while compound **3** contains the *N*-(2-(piperidin-1-yl)ethyl)acetamide chain that binds in the E_0 region and provides further interactions with the E_1 region. A methylene linker was suggested by our modelling to give the appropriate chain length to reach the E_0 region from the 7*H*-pyrrolopyrimidine heterocyclic core. Both piperidinyl and the pyrrolidine urea side chains (R^1 and R^2 positions) were incorporated into our work in various permutations to produce a series of compounds for synthesis and biological evaluation (Table 1).

The development of a convergent synthetic route, using the preparation of various anilines, to this novel class of heterocyclic compounds is described.

Table 1
2-Anilino-7*H*-pyrrolopyrimidines designed as potential PDK1 inhibitors

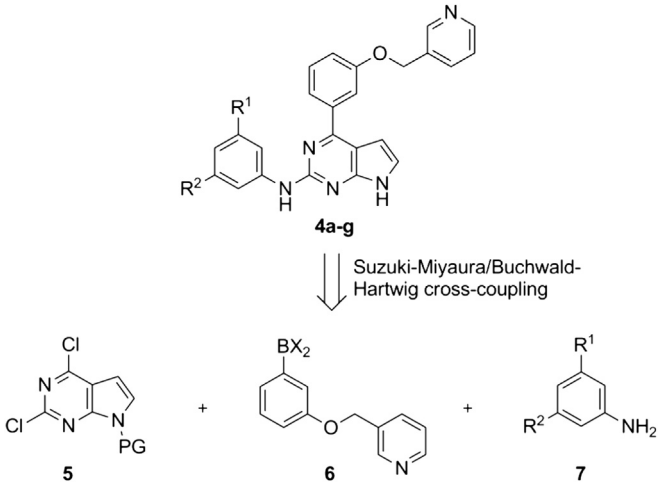


4a-g

Compound	R^1	R^2
4a		H
4b		
4c		
4d	H	
4e	H	
4f	H	H
4g		H

2. Results and discussion

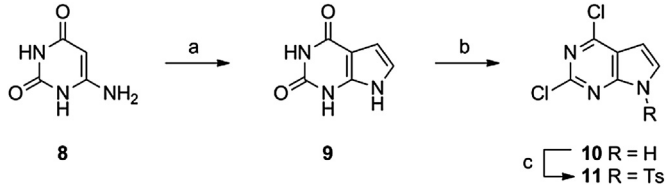
The synthetic strategy was designed around the separate synthesis of three key fragments before linking them together by sequential Suzuki and Buchwald–Hartwig cross-coupling reactions (Scheme 1). The 2,4-dichloro-7*H*-pyrrolopyrimidine fragment **5** was an appropriate heterocyclic core for the planned cross-coupling



Scheme 1. Designed synthetic strategy for the novel 2-anilino-7*H*-pyrrolopyrimidine series.

reactions as selectivity between the 2- and 4-positions could be achieved.[21] Incorporation of a protecting group for this fragment was thought to be a requirement for the proceeding palladium-catalysed cross-coupling reactions, as nitrogen based heteroaryls and free NH moieties are known to be notoriously difficult substrates in these reactions.[22,23]

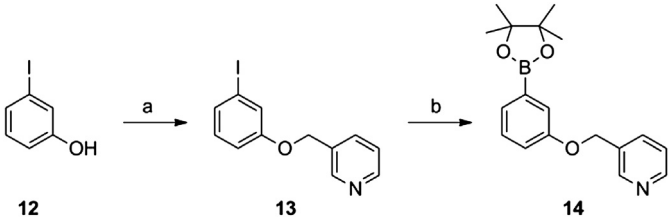
Following literature precedence,[24–26] a *p*-toluenesulfonyl (Ts) group was chosen due to its ease of formation and cleavage from pyrrolopyrimidine and pyrrole systems. 2,4-Dichloro-*N*-tosylpyrrolopyrimidine **11** was prepared by literature methods[25,27,28] (Scheme 2), with modifications made to the purification of intermediate **10**. Partial purification by flash chromatography (30% EtOAc/hexanes) and subsequent recrystallisation from chloroform was required to obtain product of sufficient purity for the tosylation reaction.



8 9

Scheme 2. Reagents and conditions: (a) chloroacetaldehyde (1.5 equiv), NaOAc (1.5 equiv), H_2O, 80 °C, 30 min, 88%; (b) $POCl_3$ (3 equiv), DIPEA (1.5 equiv), PhMe, 75 °C, 2 h; then 105 °C, 16 h, 49%; (c) Ts-Cl (1.1 equiv), NaH (1.1 equiv), DMF, rt, 1 h, 92%.

Boronate fragment **6** was envisioned to be accessible through alkylation of a commercially available 3-halophenol, followed by Miyaura borylation. Alkylation of 3-iodophenol **12** with 3-picolyl chloride hydrochloride produced the iodoether **13** in a yield of 85% (Scheme 3). Borylation of iodoether **13** with bis(pinacolato) diboron under standard Miyaura conditions[27] gave the desired boronate fragment **14** in a respectable yield. Initial attempts to synthesise this fragment with the more economical 3-bromophenol were unsuccessful upon borylation, giving an 8:2 inseparable mixture of starting material to product.

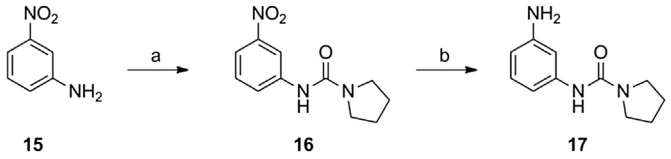


12 13 14

Scheme 3. Reagents and conditions: (a) 3-picolyl chloride HCl (1.15 equiv), Cs_2CO_3 (2.8 equiv), DMF, rt, 24 h; then 80 °C, 24 h, 85%; (b) B_2pin_2 (1.2 equiv), KOAc (3 equiv), $Pd(dppf)Cl_2$ (2 mol %), DMSO, 80 °C, 16 h, 70%.

Attachment of the third fragment **7** at the 2-position of the pyrrolopyrimidine scaffold required the synthesis of six customised anilines. *N*,*N*-Disubstituted urea **16** was prepared by reacting 3-nitroaniline **15** with triphosgene and then pyrrolidine (Scheme 4).[28] Palladium-catalysed hydrogenation afforded the desired aniline **17** in an overall yield of 82% over two steps, which is an improvement on the procedure reported by McIver et al.[29] and is comparable to the method reported by Perrior et al.[30]

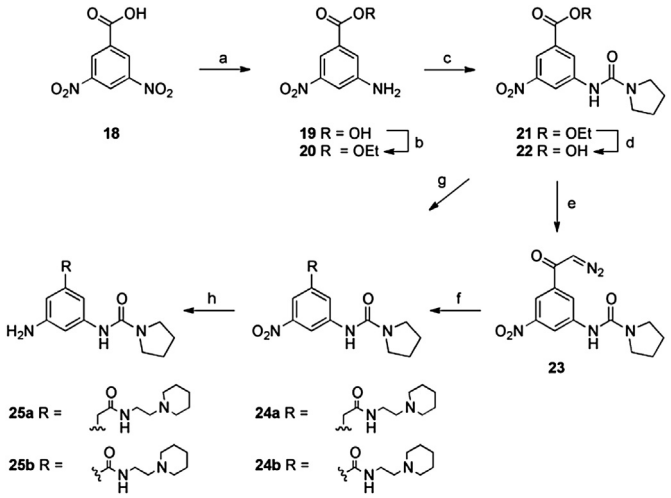
Ethyl 3-amino-5-nitrobenzoate **20** was prepared from 3,5-dinitrobenzoic acid **18** in good yield by literature methods[19,20] (Scheme 5). *N*,*N*-Disubstituted urea **21** was formed in 82% yield by reacting aniline **21** with triphosgene and then pyrrolidine.[28] Addition of pyrrolidine after 30 min at room temperature was required to reduce the formation of a symmetrical urea dimer. Ester



15 16 17

Scheme 4. Reagents and conditions: (a) triphosgene (0.4 equiv), C_5H_5N, CH_2Cl_2, 0 °C→rt, 1 h; then pyrrolidine (2 equiv), 16 h, 85%; (b) 10% Pd/C (5% w/w), H_2, MeOH, rt, 4 h, 96%.

hydrolysis with aqueous sodium hydroxide afforded the free acid **22** in high yield.



18 23

Scheme 5. Reagents and conditions: (a) Fe (3 equiv), AcOH, 120 °C, 2 h, 46%; (b) cat. concd H_2SO_4, 4 Å MS, EtOH, reflux, 16 h, 90%; (c) triphosgene (0.4 equiv), C_5H_5N, CH_2Cl_2, 0 °C→rt, 30 min; then pyrrolidine (2 equiv), 16 h, 82%; (d) 1 M NaOH, EtOH, THF, rt, 1 h, 86%; (e) *i*-BuOCOCl (1.05 equiv), Et_3N (1.05 equiv), THF, 0 °C, 30 min; then CH_2N_2 (5.02 equiv), CH_2Cl_2, 0 °C→rt, 2 h, 73%; (f) 1-(2-aminoethyl)piperidine (1.05 equiv), AgOAc (36 mol %), 1,4-dioxane, 70 °C, rt, 1 h, 90%; (g) *i*-BuOCOCl (1.05 equiv), Et_3N (1.05 equiv), THF, 0 °C, 30 min; then 1-(2-aminoethyl)piperidine (1.4 equiv), 0 °C→rt, 2 h, 80%; (h) 10% Pd/C (5% w/w), H_2, MeOH, rt, 4 h, 95–99%.

Diazo-alkylation of acid **22** with a freshly distilled dichloromethane solution of diazomethane afforded the desired diazoketone **23**, with a small amount of the methyl ester side-product, which could only be removed by flash chromatography on a small scale (~200 mg). Efforts to use the less hazardous TMS-diazomethane led to an unacceptable reduction in yield, or decomposition, with difficult and time-consuming purifications.[31–36] Silver-catalysed Wolff rearrangement of diazoketone **23** with 1-(2-aminoethyl)piperidine gave the homologated amide **24a** directly in a yield of 90%.[37,38] Alternatively, acid **22** underwent amide coupling with 1-(2-aminoethyl)piperidine, using isobutyl chloroformate and triethylamine, to give benzamide **24b**. Palladium-catalysed hydrogenation of **24a** and **24b**, as before, then furnished the desired anilines **25a** and **25b**.

Synthesis of anilines **28a** and **28b** followed similar chemistry to anilines previously described above (Scheme 6). 3-Nitrophenylacetic acid **26a** and 3-nitrobenzoic acid **26b** underwent amide coupling with 1-(2-aminoethyl)piperidine to give the corresponding amides **27a** and **27b** in yields of 82 and 80%, respectively. The amide coupling conditions for 3-nitrophenylacetic acid **26a** required activation with thionyl chloride instead of the mixed anhydride method due to the acidic α-protons. Palladium-catalysed hydrogenation gave the two anilines **28a** and **28b**. Synthesis of aniline **28b** was completed in a yield of 66% over two steps, which is comparable to the method of Kapuriya et al.[39]

Finally, aniline **32** was synthesised in high yield from 3-aminophenol according to literature methods[22,23] (Scheme 7).

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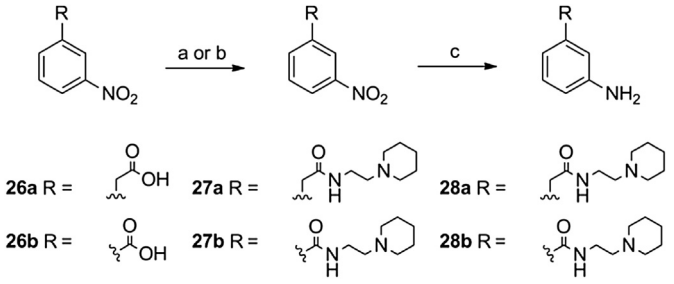
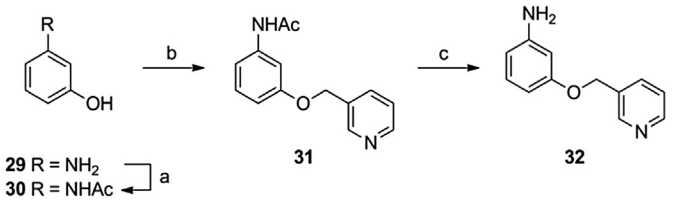


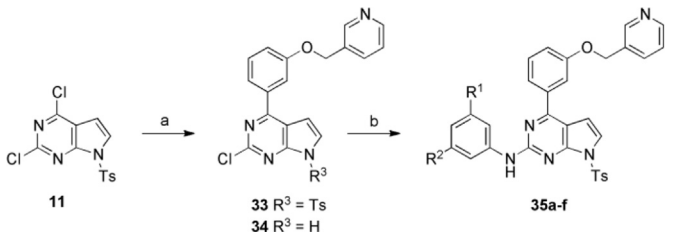
Scheme 6. Reagents and conditions: (a) $SOCl_2$, reflux, 1 h; then 1-(2-aminoethyl)piperidine (1.3 equiv), THF, rt, 2 h, 82%; (b) *i*-BuOCOCl, Et_3N, THF, 0 °C→rt, 30 min; then 1-(2-aminoethyl)piperidine (1.4 equiv), THF, rt, 2 h, 80%; (c) 10% Pd/C (5% w/w), H_2, MeOH, rt, 4 h, 83–94%.



Scheme 7. Reagents and conditions: (a) Ac_2O, H_2O, reflux, 15 min, 65%; (b) 3-picolyl chloride HCl (1.15 equiv), K_2CO_3 (2.8 equiv), DMF, rt, 24 h; then 80 °C, 24 h, 95%; (c) 2 M HCl, reflux, 3 h, 92%.

The 2-anilino-7*H*-pyrrolopyrimidines **4a**–**g** were synthesised by first employing a Suzuki cross-coupling of 2,4-dichloro-*N*-tosylpyrrolopyrimidine **11** with boronate **14**, followed by Buchwald–Hartwig cross-coupling with the synthesised anilines and base promoted detosylation. Suzuki cross-coupling, using standard conditions,[40] occurred exclusively at the 4-position and successfully yielded the desired Buchwald–Hartwig precursor **33**, along with some detosylated side-product **34**. Reduction of the initial reaction time from 4 h to 1 h substantially decreased the amount of detosylation and improved the yield of the product. The employed Buchwald–Hartwig cross-coupling conditions[26] fully converted precursor **33** to the desired 2-anilino-*N*-tosylpyrrolopyrimidines **35a**–**f** (Table 2). These conditions provided greatly enhanced coupling efficiency for pyrrolopyrimidines compared to conventional amination procedures with aromatic amines,[41–45] with only the desired product observed by TLC analysis and very good yields obtained.

Table 2
Synthesis of 2-anilino-*N*-tosylpyrrolopyrimidines **34a**–**f** by Suzuki cross-coupling of **11** with boronate **14**, followed by Buchwald–Hartwig cross-coupling reaction

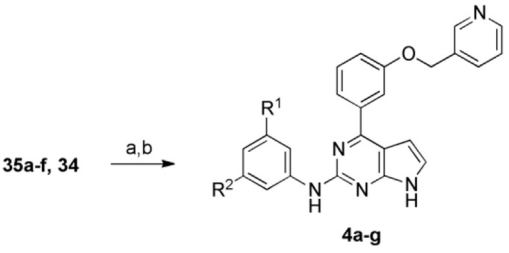


Entry	Aniline	Product	Yield (%)
1	**17**	**35a**	87
2	**25a**	**35b**	75
3	**25b**	**35c**	79
4	**28a**	**35d**	72
5	**28b**	**35e**	73
6	**PhNH$_2$**	**35f**	85

Reagents and conditions: (a) boronate **15** (1.2 equiv), Na_2CO_3 (2 equiv), $Pd(PPh_3)_4$ (6 mol %), 4:1 1,4-dioxane/H_2O, reflux, 2 h, 56%; (b) aniline (1.2 equiv), K_2CO_3 (2.2 equiv), $Pd_2(dba)_3$ (5 mol %), XPhos (10 mol %), *t*-BuOH, reflux, 16 h.

Treatment of 2-anilino-*N*-tosylpyrrolopyrimidines **34a**–**f** with base promoted detosylation conditions[26] gave the desired 2-anilino-7*H*-pyrrolopyrimidines **4a**–**f** in good yields and reproducibility (Table 3). Finally, detosylated side-product **34** was utilised to cross-couple aniline **32** to the 2-position of the pyrrolopyrimidine scaffold giving the *N*,4-bis(3-(pyridin-3-ylmethoxy) phenyl) substituted compound **4g** in an acceptable yield.

Table 3
Synthesis of 2-anilino-7*H*-pyrrolopyrimidines **4a**–**f** by base promoted detosylation and *N*,4-bis(3-(pyridin-3-ylmethoxy)phenyl) substituted compound **4g** by Buchwald–Hartwig cross-coupling of detosylated side-product **34** with aniline **32** as well as their percentage inhibitory activity against PDK1



Product	Yield%	% Inhibition at 10 μM[a]
4a	58	46±6
4b	85	36±2
4c	89	29±3
4d	93	7±3
4e	92	9±6
4f	95	32±5
4g	63	41±5

Reagents and conditions: (a) K_2CO_3 (3 equiv), 4:1 MeOH/H_2O, reflux, 3 h; (b) aniline **32** (1.2 equiv), K_2CO_3 (2.2 equiv), $Pd_2(dba)_3$ (5 mol %), XPhos (10 mol %), *t*-BuOH, reflux, 16 h.

[a] The mean±SD of at least three independent experiments (radiometric assay).

Compounds **4a**–**g** were screened against the isolated PDK1 enzyme at a concentration of 10 μM (Table 3). Unfortunately, only modest inhibitory activity was observed. Target compound **4a** was the best of the series, showing inhibition of 46±6%. Incorporation of the homologated *N*-(2-(piperidin-1-yl)ethyl)acetamide side chain (**4b**) decreased the inhibitory activity, whilst deduction of the methylene linker (**4c**) brought about a further drop in activity. Removal of the pyrrolidine urea moiety whilst maintaining the *N*-(2-(piperidin-1-yl)ethyl)acetamide side chain, in both homologated **4d** and primary **4e** forms, significantly reduced the inhibitory activity towards PDK1. Elimination of both the pyrrolidine urea and *N*-(2-(piperidin-1-yl)ethyl)acetamide side chains (**4f**) restored the inhibitory activity to 32±5%.

Overall, these results illustrate that the *N*-(2-(piperidin-1-yl) ethyl)acetamide side chain has little effect on the binding affinity, whilst the pyrrolidine urea was critical in maintaining inhibitory activity. In a more radical structure variation, we decided to include the *N*,4-bis(3-(pyridin-3-ylmethoxy)phenyl) substituted analogue **4g** and, unexpectedly, this compound showed the second best inhibition of the series, displaying inhibitory activity of 41±5%.

3. Conclusion

In summary, we have developed a practical and versatile synthetic route for the preparation of novel substituted 2-anilino-7*H*-dezapurines as potential inhibitors of PDK1. Synthesis of the 2,4-dichloro-*N*-tosylpyrrolopyrimidine core and boronate fragment gave access to the Buchwald–Hartwig cross-coupling precursor through a conventional Suzuki cross-coupling. Buchwald–Hartwig cross-coupling of various customised anilines was achieved in high efficiency and yields using this monoligated palladium-catalysed

system. The use of Buchwald–Hartwig cross-coupling to install functionality at the 2-position was found to greatly improve the efficiency of synthesising these novel pyrrolopyrimidine inhibitors over conventional S_NAr procedures. The modular nature of this synthesis allows for late stage derivatisation to be produced whilst maintaining synthetic efficiency and versatility. Biological screening against isolated PDK1 was undertaken, but only modest inhibition was observed. Investigation into the binding interactions with the catalytic residues of the phosphate pocket (P) and the hydrophobic region (BP-I) is required to further explore the inhibitory activity of these compounds and is currently underway.

4. Experimental section

4.1. General methods

Melting points were recorded on a Reichert 'Thermopan' microscope hot stage apparatus and are uncorrected. NMR spectra were recorded on a Bruker AV-500 at 500.19 MHz for ^{1}H nuclei and at 125.78 MHz for ^{13}C nuclei at 300 K unless otherwise stated. All of the chemical shifts were recorded as δ values in parts per million (ppm) and coupling constants (*J*) were recorded in Hertz (Hz) to the nearest 0.5 Hz. The following abbreviations were used when reporting ^{1}H NMR data: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; app, apparent; and br, broad. Low-resolution electrospray ionisation (ESI) mass spectra were recorded on a Bruker Daltronics Esquire 6000 ion trap mass spectrometer at 300 °C with a scan rate of 5500 *m*/*z*/s, a 40 eV cone voltage and in positive mode, unless otherwise stated. Methanol or acetonitrile with 0.1% formic acid was used as the mobile phase. High-resolution electrospray were recorded on an Agilent 1290 Infinity. All data were acquired and reference mass corrected via a dual-spray electrospray ionisation (ESI) source, in positive mode, unless otherwise stated. Reactions and chromatography fractions were monitored by thin layer chromatography (TLC) using Merck Kieselgel 60 F_{254} aluminium backed plates and visualised using a 254 nm UV lamp and/or treatment with a suitable stain (phosphomolybdic acid, ninhydrin or bromocresol green), followed by heating. Flash chromatography was performed using silica gel (Davisil® LC60 40–63 μm) as the stationary phase according to the method of Still et al.[46] All solvents used for flash chromatography, including triethylamine, were distilled prior to use except for acetic acid, which was analytical grade. Eluent systems containing dichloromethane satd with ammonia were freshly prepared as follows: Dichloromethane (400 mL) and ammonium hydroxide (28%, 80 mL) were shaken in a separating funnel and the dichloromethane layer was separated, to which the required volume of methanol was added if needed. All glassware used in moisture sensitive reactions was oven dried, flame dried under vacuum and then cooled under argon prior to use. All reactions were completed at room temperature unless otherwise stated. Reaction progress was monitored by thin layer chromatography (TLC) unless otherwise noted. Purification of solvents and reagents was carried out by the procedures described by Chai and Armarego.[47] All other solvents, reagents and starting materials were purchased as reagent-grade from commercial sources and used without further purification. All organic extracts were dried over magnesium sulfate unless otherwise stated.

4.2. Synthesis

*4.2.1. 3-((3-Iodophenoxy)methyl)pyridine (**13**).* To a stirred solution of 3-iodophenol **12** (3.00 g, 13.6 mmol) in DMF (100 mL) was added Cs_2CO_3 (12.43 g, 38.1 mmol). After 5 min, 3-picolyl chloride hydrochloride (2.57 g, 15.7 mmol) was added and the mixture was stirred at room temperature for 24 h, then heated at 80 °C for a further 24 h. The mixture was diluted with 1 M NaOH and extracted with EtOAc (×2). The combined organics were washed with brine (×6), dried, filtered and reduced in vacuo to give a brown coloured oil. The oil was purified by flash chromatography (30–40% EtOAc/hexanes) to give iodoether **13** (3.61 g, 85%) as a colourless oil; δ_H ($CDCl_3$): 8.65 (1H, d, *J* 1.5 Hz), 8.57 (1H, dd, *J* 4.5, 1.5 Hz), 7.96 (1H, ddd, *J* 8.0, 2.0, 1.5 Hz), 7.33–7.29 (3H, m), 6.99 (1H, t, *J* 8.0 Hz), 6.92 (1H, ddd, *J* 8.0, 2.5, 1.0 Hz), 5.02 (2H, s); δ_C ($CDCl_3$): 157.9, 149.3, 148.7, 135.4, 132.1, 130.9, 130.6, 124.0, 123.6, 114.4, 94.4, 67.6; *m*/*z* (ESI): 312.0 (MH^+). HRMS (ESI): MH^+, found 311.9877. $C_{12}H_{11}INO^+$ requires 311.9880.

*4.2.2. 3-((3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)methyl)pyridine (**14**).* Bis(pinacolato)diboron (2.05 g, 8.00 mmol), KOAc (1.98 g, 20.2 mmol) and iodoether **13** (2.10 g, 6.70 mmol) were dissolved in anhydrous DMSO (40 mL), which was purged under argon. Pd(dppf)Cl_2 (100 mg, 2 mol %) was added, and the mixture was heated at 80 °C for 16 h, then cooled, diluted with H_2O and extracted with EtOAc (×3). The combined organic extracts were washed with H_2O (×6), brine (×3), dried, filtered and the solvent was removed in vacuo to give a red/brown oil. The oil was purified by flash chromatography (50% EtOAc/hexanes) to give the boronate **14** (1.47 g, 70%) as a white solid; mp 65–68 °C; δ_H ($CDCl_3$): 8.80 (1H, s), 8.65 (1H, d, *J* 4.5 Hz), 8.18 (1H, d, *J* 8.0 Hz), 7.67 (1H, dd, *J* 7.5, 5.5 Hz), 7.46 (1H, d, *J* 7.5 Hz), 7.39 (1H, d, *J* 2.5 Hz), 7.32 (1H, t, *J* 3.0 Hz), 7.06 (1H, ddd, *J* 8.0, 2.5, 1.0 Hz), 5.20 (2H, s), 1.28 (12H, s); δ_C ($CDCl_3$): 157.9, 149.0, 148.6, 135.6, 132.9, 129.1, 127.9, 123.6, 119.8, 118.5, 83.9, 67.4, 24.9; *m*/*z* (ESI): 312.1 (MH^+); HRMS (ESI): MH^+, found 312.1767. $C_{18}H_{23}BNO_3^+$ requires 312.1766.

*4.2.3. N-(3-Nitrophenyl)pyrrolidine-1-carboxamide (**16**).* A heterogeneous solution of 3-nitroaniline **15** (4.00 g, 29.0 mmol) and anhydrous CH_2Cl_2 (100 mL) was placed under argon and cooled to −5 °C. To this solution was carefully added triphosgene (3.44 g, 11.6 mmol), then pyridine (12 mL). After 30 min, the reaction was allowed to warm to room temperature and stirred for 1 h. Then, pyrrolidine (4.76 mL, 58.0 mmol) was added drop wise and the reaction was stirred overnight. The reaction mixture was then diluted with H_2O and the aqueous layer was separated and extracted with CH_2Cl_2 (×2). The combined organic extracts were washed with 2 M HCl (×5), brine (×2), dried, filtered and concentrated in vacuo to give an orange residue. The residue was recrystallised from toluene to give the urea **16** (5.80 g, 85%) as a yellow solid; mp 140–143 °C; δ_H (DMSO-d_6): 8.62 (1H, s), 8.54 (1H, dd app t, *J* 2.0 Hz), 7.96 (1H, dd, *J* 8.0, 1.0 Hz), 7.75 (1H, dd, *J* 8.0, 1.5 Hz), 7.49 (1H, dd app t, *J* 8.0 Hz), 3.40–3.38 (4H, m), 1.87–1.85 (4H, m); δ_C (DMSO-d_6): 153.4, 147.8, 142.1, 129.4, 124.9, 115.7, 113.0, 45.7, 24.9; *m*/*z* (ESI): 236.1 (MH^+); HRMS (ESI): MH^+, found 236.1026. $C_{11}H_{14}N_3O_3^+$ requires 236.1030.

*4.2.4. General procedure A: Preparation of anilines via palladium-catalysed hydrogenation. N-(3-Aminophenyl)pyrrolidine-1-carboxamide (**17**).* A solution of nitrobenzene **16** (2.50 g, 10.6 mmol) in MeOH (150 mL) with 10% Pd on charcoal (5% w/w, 125 mg) was hydrogenated at atmospheric pressure at room temperature for 4 h. The catalyst was removed by filtration through a pad of Celite®, the filter cake rinsed with MeOH and the solvent removed in vacuo to give the aniline **17** (2.10 g, 96%) as a pink solid; mp 134–137 °C (from toluene); δ_H (DMSO-d_6): 7.72 (1H, s), 6.82 (1H, dd app t, *J* 8.0 Hz), 6.79 (1H, dd app t, *J* 2.0 Hz), 6.61 (1H, dd, *J* 8.0, 1.0 Hz), 6.14 (1H, dd, *J* 8.0, 1.0 Hz), 4.85 (2H, s), 3.34–3.32 (4H, m), 1.84–1.81 (4H, m); δ_C (DMSO-d_6): 153.9, 148.5, 141.1, 128.4, 107.9, 107.8, 105.5, 45.7, 24.9; *m*/*z* (ESI): 206.1 (MH^+); HRMS (ESI): MH^+, found 206.1287. $C_{11}H_{16}N_3O^+$ requires 206.1287.

*4.2.5. Ethyl 3-nitro-5-(pyrrolidine-1-carboxamido)benzoate (**21**).* Ethyl 3-amino-5-nitrobenzoate **20** (5.00 g, 23.8 mmol) was treated

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according to the procedure for synthesising compound **16** except pyrrolidine was added after 30 min of stirring at room temperature. The crude product was recrystallised from EtOH to give the urea **22** (5.24 g, 82%) as a tan solid; mp 178–181 °C; δ_H (DMSO-d_6): 8.90 (1H, s), 8.86 (1H, dd app t, *J* 2.0 Hz), 8.59 (1H, dd, *J* 2.0, 1.5 Hz), 8.19 (1H, dd, *J* 2.0, 1.5 Hz), 4.39 (2H, q, *J* 7.0 Hz), 3.41–3.38 (4H, m), 1.88–1.85 (4H, m), 1.36 (3H, t, *J* 7.0 Hz); δ_C (DMSO-d_6): 164.1, 153.2, 147.9, 142.6, 131.1, 124.6, 116.5, 115.6, 61.5, 45.7, 24.9, 14.1; *m*/*z* (ESI): 308.1 $(MH)^+$; HRMS (ESI): MH^+, found 308.1238. $C_{14}H_{18}N_3O_5^+$ requires 308.1241.

*4.2.6. 3-Nitro-5-(pyrrolidine-1-carboxamido)benzoic acid (**22**).* To a solution of urea **21** (6.35 g, 20.7 mmol) in THF (200 mL) and EtOH (100 mL), was slowly added 1 M NaOH (100 mL). Once TLC had indicated the reaction had finished (approx. 1 h), the reaction mixture was acidified with 1 M HCl until strongly acidic (pH ~1–2). The solid that separated was filtered and washed with H_2O to give acid **22** (5.0 g, 86%) as a light yellow solid; mp 229–231 °C; δ_H (DMSO-d_6): 13.52 (1H, br s), 8.81 (1H, s), 8.79 (1H, dd app t, *J* 2.0 Hz), 8.55 (1H, dd app t, *J* 2.0 Hz), 8.16 (1H, dd app t, *J* 2.0 Hz), 3.40–3.38 (4H, m), 1.88–1.85 (4H, m); δ_C (DMSO-d_6): 165.7, 153.3, 147.9, 142.5, 132.3, 125.0, 116.3, 115.9, 45.7, 24.9; *m*/*z* (ESI): 278.1 (MH^-); HRMS (ESI): MH^+, found 280.0925. $C_{12}H_{14}N_3O_5^+$ requires 280.0928.

*4.2.7. N-(3-(2-Diazoacetyl)-5-nitrophenyl)pyrrolidine-1-carboxamide (**23**).* A stirred solution of acid **22** (3.00 g, 10.7 mmol) in anhydrous THF (75 mL) was placed under argon and cooled to −15 °C. Isobutyl chloroformate (1.46 mL, 11.3 mmol) and Et_3N (1.57 mL, 11.3 mmol) were added successively and the mixture was stirred for 30 min. The resulting salt was removed by filtration before an anhydrous CH_2Cl_2 solution of CH_2N_2 (prepared from Diazald© (11.5 g, 53.7 mmol) and KOH (3.41 g, 60.9 mmol)) was added slowly to the reaction. The yellow solution was allowed to warm to room temperature and was further stirred for 2 h. The excess CH_2N_2 was quenched with AcOH (60 mL) and the solution was concentrated in vacuo to give an orange oil. The oil was taken up in EtOAc and washed with satd $NaHCO_3$ (×2), 10% citric acid (×2), brine (×2), dried, filtered and reduced in vacuo to give a light orange solid. The solid was recrystallised from MeOH to give diazoketone **23** containing 5% methyl ester side-product. On a small scale the impurity was removed by flash chromatography (50% EtOAc/hexanes) to give diazoketone **23** (2.40 g, 73%) as a light yellow solid; mp 164–167 °C (from MeOH); δ_H (DMSO-d_6): 8.87 (1H, s), 8.80 (1H, dd app t, *J* 2.0 Hz), 8.43 (1H, dd app t, *J* 2.0 Hz), 8.14 (1H, dd app t, *J* 2.0 Hz), 7.08 (1H, s), 3.42–3.39 (4H, m), 1.88–1.86 (4H, m); δ_C (DMSO-d_6): 183.9, 153.3, 148.1, 142.6, 137.2, 122.4, 116.3, 113.6, 55.2, 45.7, 24.9; *m*/*z* (ESI): 304.1 (MH^+); HRMS (ESI): MH^+, found 304.1038. $C_{13}H_{14}N_5O_4^+$ requires 304.1041.

*4.2.8. N-(3-Nitro-5-(2-oxo-2-((2-(piperidin-1-yl)ethyl)amino)ethyl)phenyl)pyrrolidine-1-carboxamide (**24a**).* Diazoketone **23** (1.00 g, 3.30 mmol) was suspended in anhydrous 1,4-dioxane (30 mL) and heated to 70 °C with a drying tube fitted to the reaction vessel. Once the starting material was fully dissolved, 1-(2-aminoethyl)piperidine (490 μL, 3.50 mmol) and AgOAc (200 mg, 36 mol %) were added successively. The reaction mixture was maintained at 70 °C for 1 h, then cooled and concentrated in vacuo to afford a black residue. The residue was purified by flash chromatography (2% MeOH/CH_2Cl_2 satd with NH_3) and recrystallised from *i*-PrOH to give the homologated amide **24a** (1.19 g, 90%) as a light yellow solid; mp 200–205 °C; δ_H (DMSO-d_6): 8.62 (1H, s), 8.43 (1H, dd app t, *J* 2.0 Hz), 8.01 (1H, t, *J* 6.0 Hz), 7.85 (1H, dd app t, *J* 2.0 Hz), 7.70 (1H, dd app t, *J* 2.0 Hz), 3.50 (2H, s), 3.40–3.37 (4H, m), 3.17–3.13 (2H, m), 2.31–2.28 (6H, m), 1.87–1.84 (4H, m), 1.46–1.42 (4H, m), 1.36–1.31 (2H, m); δ_C (DMSO-d_6): 169.1, 153.4, 147.7, 141.8, 138.3, 125.7, 116.4, 111.3, 57.6, 54.0, 45.7, 41.9, 36.4, 25.5, 24.9, 24.0; *m*/*z* (ESI): 404.2 (MH^+); HRMS (ESI): MH^+, found 404.2289. $C_{20}H_{30}N_5O_4^+$ requires 404.2293.

*4.2.9. N-(3-Nitro-5-((2-(piperidin-1-yl)ethyl)carbamoyl)phenyl)pyrrolidine-1-carboxamide (**24b**).* A solution of acid **22** (2.00 g, 7.16 mmol) in anhydrous THF (40 mL) was placed under argon and cooled to −15 °C. Isobutyl chloroformate (975 μL, 7.52 mmol) and Et_3N (1.05 mL, 7.52 mmol) were added successively and the mixture was stirred for 30 min. The salt was removed by vacuum filtration before 1-(2-aminoethyl)piperidine (1.42 mL, 10.0 mmol) was added and the mixture was stirred at room temperature for 2 h. The solvent was removed in vacuo and the resulting solid obtained was recrystallised from *i*-PrOH to give the benzamide **24b** (2.25 g, 80%) as a white solid; mp 210–213 °C; δ_H (DMSO-d_6): 8.80 (1H, s), 8.73 (1H, dd app t, *J* 2.0 Hz), 8.68 (1H, t, *J* 6.0 Hz), 8.40 (1H, dd app t, *J* 2.0 Hz), 8.23 (1H, dd app t, *J* 2.0 Hz), 3.41–3.36 (6H, m), 2.44 (2H, t, *J* 7.0 Hz), 2.38 (4H, br s), 1.88–1.86 (4H, m), 1.51–1.47 (4H, m), 1.39–1.36 (2H, m); δ_C (DMSO-d_6): 164.3, 153.3, 147.7, 142.2, 135.9, 124.1, 115.1, 113.9, 57.5, 54.0, 45.7, 37.1, 25.5, 24.9, 24.0; *m*/*z* (ESI): 390.2 (MH^+); HRMS (ESI): MH^+, found 390.2135. $C_{19}H_{28}N_5O_4^+$ requires 390.2136.

*4.2.10. N-(3-Amino-5-(2-oxo-2-((2-(piperidin-1-yl)ethyl)amino)ethyl)phenyl)pyrrolidine-1-carboxamide (**25a**).* Nitrobenzene **24a** (650 mg, 1.61 mmol) was treated according to General procedure A to give the aniline **25b** (570 mg, 95%) as a white solid; mp 122–124 °C (from benzene); δ_H ($CDCl_3$): 7.06 (1H, s), 6.54 (1H, br s), 6.51 (1H, s), 6.24 (1H, s), 6.20 (1H, br s), 3.66 (2H, br s), 3.43–3.41 (4H, m), 3.38 (2H, s), 3.33–3.29 (2H, m), 2.45 (2H, br s), 2.41 (4H, br s), 1.95–1.93 (4H, m), 1.55 (4H, br s), 1.40 (2H, br s); δ_C ($CDCl_3$): 171.0, 153.8, 147.7, 140.8, 136.4, 110.3, 110.2, 104.9, 56.8, 54.1, 45.7, 43.8, 36.2, 25.8, 25.5, 24.2; *m*/*z* (ESI): 374.2 (MH^+); HRMS (ESI): MH^+, found 374.2537. $C_{20}H_{32}N_5O_2^+$ requires 374.2551.

*4.2.11. N-(3-Amino-5-((2-(piperidin-1-yl)ethyl)carbamoyl)phenyl)pyrrolidine-1-carboxamide (**25b**).* Nitrobenzene **24b** (1.00 g, 2.56 mmol) was treated according to General procedure A. The crude product was recrystallised from toluene to afford the title compound **25b** (920 mg, 99%) as a light brown candy; mp 94–97 °C; δ_H ($CDCl_3$): 7.31 (1H, dd app t, *J* 2.0 Hz), 7.05 (1H, br s), 6.99 (1H, dd app t, *J* 2.0 Hz), 6.71 (1H, dd app t, *J* 2.0 Hz), 6.29 (1H, s), 3.80 (2H, br s), 3.53–3.49 (2H, m), 3.44–3.41 (4H, m), 2.59 (2H, t, *J* 6.0 Hz), 2.50 (4H, br s), 1.95–1.93 (4H, m), 1.67–1.61 (4H, m), 1.48–1.44 (2H, m); δ_C ($CDCl_3$): 167.6, 153.8, 147.4, 140.6, 136.2, 108.6, 108.1, 107.7, 57.2, 54.3, 45.8, 36.5, 25.8, 25.5, 24.2; *m*/*z* (ESI): 360.2 (MH^+); HRMS (ESI): MH^+, found 360.2391. $C_{19}H_{30}N_5O_2^+$ requires 360.2395.

*4.2.12. 2-(3-Nitrophenyl)-N-(2-(piperidin-1-yl)ethyl)acetamide (**27a**).* A mixture of 3-nitrophenylacetic acid **26a** (700 mg, 3.86 mmol) and freshly distilled $SOCl_2$ (15 mL) was heated at reflux for 1 h. The excess $SOCl_2$ was removed in vacuo and the residue was taken up in anhydrous THF (15 mL), to which 1-(2-aminoethyl)piperidine (713 μL, 5.02 mmol) was added and the mixture was stirred for 2 h. The solvent was removed in vacuo and the resulting residue was purified by flash chromatography (1% Et_3N, EtOAc) to give the homologated amide **27a** (920 mg, 82%) as a white solid; mp 92–94 °C; δ_H ($CDCl_3$): 8.13–8.10 (2H, m), 7.64–7.63 (1H, m), 7.49 (1H, dd app t, *J* 8.0 Hz), 6.34 (1H, br s), 3.62 (2H, s), 3.30–3.27 (2H, m), 2.37 (2H, t, *J* 6.0 Hz), 2.29 (4H, br s), 1.47–1.42 (4H, m), 1.40–1.36 (2H, m); δ_C ($CDCl_3$): 169.2, 148.4, 137.2, 135.6, 129.5, 124.2, 122.1, 56.6, 54.1, 43.0, 36.1, 25.8, 24.2; *m*/*z* (ESI): 292.1 (MH^+); HRMS (ESI): MH^+, found 292.1653. $C_{15}H_{22}N_3O_3^+$ requires 292.1656.

*4.2.13. 3-Nitro-N-(2-(piperidin-1-yl)ethyl)benzamide (**27b**).* 3-Nitrobenzoic acid **26b** (600 mg, 3.59 mmol) was treated

according to the procedure for synthesising compound **24b**. Purification by flash chromatography (1% Et_3N, EtOAc) afforded the benzamide **27b** (790 mg, 80%) as a white solid; mp 201–204 °C (lit.[39] 203–204 °C); δ_H ($CDCl_3$): 8.62 (1H, t, *J* 2.0 Hz), 8.33 (1H, ddd, *J* 8.0, 2.0, 1.0 Hz), 8.14 (1H, ddd, *J* 8.0, 2.0, 1.0 Hz), 7.62 (1H, t, *J* 8.0 Hz), 7.22 (1H, br s), 3.55–3.52 (2H, m), 2.55 (2H, t, *J* 6.0 Hz), 2.43 (4H, br s), 1.61–1.57 (4H, m), 1.48–1.45 (2H, m); δ_C ($CDCl_3$): 164.8, 148.2, 136.4, 133.0, 129.7, 125.8, 121.9, 56.7, 54.2, 36.6, 26.0, 24.2; *m*/*z* (ESI): 278.1 (MH^+).

*4.2.14. 2-(3-Aminophenyl)-N-(2-(piperidin-1-yl)ethyl)acetamide (**28a**).* Nitrobenzene **27a** (720 mg, 2.47 mmol) was treated according to General procedure A to give the aniline **28a** (600 mg, 94%) as a white solid; mp 101–104 °C; δ_H ($CDCl_3$): 7.08 (1H, dd app t, *J* 8.0 Hz), 6.58–6.53 (3H, m), 6.21 (1H, br s), 3.66 (2H, br s), 3.41 (2H, s), 3.23–3.20 (2H, m), 2.30 (2H, t, *J* 6.0 Hz), 2.23 (4H, br s), 1.43–1.38 (4H, m), 1.36–1.32 (2H, m); δ_C ($CDCl_3$): 171.0, 147.0, 136.2, 129.7, 119.4, 115.9, 113.8, 56.6, 54.0, 43.8, 36.1, 25.8, 24.2; *m*/*z* (ESI): 262.1 (MH^+); HRMS (ESI): MH^+, found 262.1914. $C_{15}H_{24}N_3O^+$ requires 262.1914.

*4.2.15. 3-Amino-N-(2-(piperidin-1-yl)ethyl)benzamide (**28b**).* Nitrobenzene **27b** (590 mg, 2.13 mmol) was treated according to General procedure A to give the aniline **28b** (0.44 g, 83%) as a beige solid; mp 91–94 °C; δ_H ($CDCl_3$): 7.18 (1H, t, *J* 8.0 Hz), 7.15 (1H, t, *J* 2.0 Hz), 7.06 (1H, d, *J* 8.0 Hz), 6.96 (1H, br s), 6.76 (1H, ddd, *J* 8.0, 2.0, 1.0 Hz), 3.78 (2H, br s), 3.52–3.49 (2H, m), 2.55 (2H, t, *J* 6.0 Hz), 2.44 (4H, br s), 1.62–1.58 (4H, m), 1.47–1.43 (2H, m); δ_C ($CDCl_3$): 167.5, 146.8, 135.9, 129.3, 117.6, 116.3, 113.8, 57.0, 54.2, 36.4, 26.0, 24.3. *m*/*z* (ESI): 248.1 (MH^+).

*4.2.16. 2-Chloro-4-(3-(pyridin-3-ylmethoxy)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidine (**33**).* To a solution of the 2,4-dichloro-*N*-tosylpyrrolopyrimidine **11** (1.50 g, 4.38 mmol) in 1,4-dioxane (60 mL) and H_2O (15 mL) was added boronate **14** (1.64 g, 5.26 mmol) and Na_2CO_3 (929 mg, 8.76 mmol), and the mixture was degassed under argon. To this mixture was added $Pd(PPh_3)_4$ (304 mg, 6 mol %) and the reaction mixture was heated at reflux for 1 h, then cooled to room temperature and the solvent was removed in vacuo. The residue was dissolved in EtOAc and washed with H_2O (×2), brine (×2), dried, filtered and the solvent was removed in vacuo to give a yellow residue. The residue was purified by flash chromatography (50% EtOAc/hexanes) and recrystallised from EtOH to give precursor **33** (1.20 g, 56%) as a white solid; mp 167–170 °C; δ_H (DMSO-d_6): 8.71 (1H, s), 8.56 (1H, d, *J* 3.5 Hz), 8.09 (1H, d, *J* 4.0 Hz), 8.06 (2H, d, *J* 8.0 Hz), 7.91 (1H, ddd app dt, *J* 8.0, 2.0 Hz), 7.63 (1H, t, *J* 8.0 Hz), 7.61 (1H, dd app t, *J* 2.0 Hz), 7.53 (1H, t, *J* 8.0 Hz), 7.51 (2H, d, *J* 8.0 Hz), 7.44 (1H, dd, *J* 7.5, 5.0 Hz), 7.29 (1H, dd, *J* 7.5, 2.0 Hz), 7.17 (1H, d, *J* 4.0 Hz), 5.27 (2H, s), 2.38 (3H, s); δ_C (DMSO-d_6): 159.7, 158.5, 153.7, 152.5, 149.2, 149.1, 146.7, 136.6, 135.7, 133.6, 132.4, 130.4, 130.3, 128.5, 127.9, 123.6, 121.8, 118.3, 116.2, 114.7, 104.9, 67.2, 21.2; *m*/*z* (ESI): 491.1 ($M[^{35}Cl]H^+$), 493.1 ($M[^{37}Cl]H^+$); HRMS (ESI): $M[^{35}Cl]H^+$, found 491.0934. $C_{25}H_{20}ClN_4O_3S^+$ requires 491.0940.

Detosylated side-product **34** was isolated from the crude mixture using flash chromatography (50% EtOAc/hexanes) and recrystallised from EtOH as a white solid (500 mg); mp 187–191 °C; δ_H (DMSO-d_6): 12.49 (1H, s), 8.73 (1H, d, *J* 2.0 Hz), 8.56 (1H, dd, *J* 5.0, 1.5 Hz), 7.93 (1H, ddd, *J* 8.0, 2.0, 1.5 Hz), 7.75 (1H, ddd, *J* 8.0, 2.0, 1.5 Hz), 7.71 (1H, dd, *J* 2.5, 1.5 Hz), 7.70 (1H, d, *J* 3.5 Hz), 7.54 (1H, t, *J* 8.0 Hz), 7.45 (1H, ddd, *J* 8.0, 5.0, 1.0 Hz), 7.27 (1H, ddd, *J* 8.0, 2.5, 1.0 Hz), 6.88 (1H, d, *J* 3.5 Hz), 5.30 (2H, s); δ_C (DMSO-d_6): 158.4, 157.3, 154.0, 152.1, 149.1, 149.0, 138.0, 135.6, 132.5, 130.2, 128.8, 123.6, 121.5, 117.5, 114.4, 113.8, 100.5, 67.1; *m*/*z* (ESI): 337.1 ($M[^{35}Cl]H^+$), 339.0 ($M[^{37}Cl]H^+$); HRMS (ESI): $M[^{35}Cl]H^+$, found 337.0848. $C_{18}H_{14}ClN_4O^+$ requires 337.0851.

*4.2.17. General procedure B: Preparation of 2-anilino-N-tosylpyrrolopyrimidines via palladium-catalysed Buchwald–Hartwig cross-coupling. N-(3-((4-(3-(Pyridin-3-ylmethoxy)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)phenyl)pyrrolidine-1-carboxamide (**35a**).* A mixture of precursor **33** (200 mg, 0.41 mmol), aniline **17** (100 mg, 0.49 mmol) and K_2CO_3 (124 mg, 0.89 mmol) in anhydrous *t*-BuOH (10 mL) was degassed under argon. To this mixture was added XPhos (19 mg, 10 mol %) and $Pd_2(dba)_3$ (19 mg, 5 mol %) and the reaction mixture was heated at reflux overnight, then allowed to cool. To this mixture was added H_2O and the whole mixture was extracted with CH_2Cl_2 (×2). The combined organic extracts were washed with brine (×2), dried, filtered and concentrated in vacuo to give a dark red residue. This was purified by flash chromatography (EtOAc) and recrystallised from *i*-PrOH to give the 2-anilino-*N*-tosylpyrrolopyrimidine **35a** (235 mg, 87%) as a yellow solid; mp 115–118 °C; δ_H (DMSO-d_6): 9.75 (1H, s), 8.70 (1H, s), 8.56 (1H, d, *J* 4.0 Hz), 8.08 (2H, d, *J* 8.5 Hz), 8.03 (1H, s), 7.91–7.89 (2H, m), 7.67–7.65 (4H, m), 7.49 (1H, t, *J* 8.0 Hz), 7.45 (1H, dd, *J* 8.0, 5.0 Hz), 7.39 (2H, d, *J* 8.5 Hz), 7.24 (2H, m), 7.12 (1H, d, *J* 8.0 Hz), 6.92 (1H, d, *J* 4.0 Hz), 5.25 (2H, s), 3.37–3.34 (4H, m), 2.33 (3H, s), 1.83–1.80 (4H, m); δ_C (DMSO-d_6): 158.4, 156.7, 154.0, 153.5, 149.2, 149.1, 146.0, 140.7, 140.4, 138.2, 135.7, 134.2, 132.5, 130.1, 130.0, 128.1, 127.9, 123.8, 123.7, 121.5, 117.4, 114.4, 114.0, 113.1, 111.6, 109.8, 105.3, 67.1, 45.7, 25.0, 21.1; *m*/*z* (ESI): 660.2 (MH^+); HRMS (ESI): MH^+, found 660.2382. $C_{36}H_{34}N_7O_4S^+$ requires 660.2388.

*4.2.18. N-(3-(2-Oxo-2-((2-(piperidin-1-yl)ethyl)amino)ethyl)-5-((4-(3-(pyridin-3-ylmethoxy)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)phenyl)pyrrolidine-1-carboxamide (**35b**).* Precursor **33** (200 mg, 0.41 mmol) was coupled with aniline **25a** according to General procedure B. Purification by flash chromatography (1% MeOH/CH_2Cl_2 satd with NH_3) and recrystallisation from toluene afforded the 2-anilino-*N*-tosylpyrrolopyrimidine **35b** (248 mg, 75%) as a yellow solid; mp 115–118 °C; δ_H (DMSO-d_6): 9.71 (1H, s), 8.69 (1H, d, *J* 2.0 Hz), 8.56 (1H, dd, *J* 5.0, 1.5 Hz), 8.10 (2H, d, *J* 8.5 Hz), 8.02 (1H, s), 7.90 (1H, dt, *J* 8.0, 2.0 Hz), 7.82 (1H, br s), 7.73 (1H, t, *J* 5.5 Hz), 7.68–7.67 (3H, m), 7.63 (1H, d, *J* 4.0 Hz), 7.48 (1H, t, *J* 8.0 Hz), 7.44 (1H, dd, *J* 8.0, 4.0 Hz), 7.37 (2H, d, *J* 8.5 Hz), 7.22 (1H, dd, *J* 8.0, 1.5 Hz), 7.00 (1H, s), 6.92 (1H, d, *J* 4.0 Hz), 5.25 (2H, s), 3.41 (2H, s), 3.36–3.33 (4H, m), 3.15–3.11 (2H, m), 2.33 (3H, s), 2.26 (2H, t, *J* 7.0 Hz), 2.25 (4H, br s), 1.82–1.79 (4H, m), 1.42–1.38 (4H, m), 1.30–1.26 (2H, m); δ_C (DMSO-d_6): 169.9, 158.4, 158.3, 156.7, 154.0, 153.5, 149.2, 149.0, 145.8, 140.4, 140.3, 138.2, 136.2, 135.6, 134.2, 132.5, 130.0$_3$, 130.0$_0$, 127.9, 123.7, 123.6, 121.6, 117.4, 114.7, 114.3, 114.2, 109.9, 109.7, 105.3, 67.1, 57.5, 54.0, 45.6, 42.9, 36.4, 25.4, 25.0, 24.0, 21.1; *m*/*z* (ESI): 828.3 (MH^+); HRMS (ESI): MH^+, found 828.3646. $C_{45}H_{50}N_9O_5S^+$ requires 828.3651.

*4.2.19. N-(3-((2-(Piperidin-1-yl)ethyl)carbamoyl)-5-((4-(3-(pyridin-3-ylmethoxy)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)phenyl)pyrrolidine-1-carboxamide (**35c**).* Precursor **33** (200 mg, 0.41 mmol) was coupled to aniline **25b** according to General procedure B. Purification by flash chromatography (10% MeOH/CH_2Cl_2) and recrystallisation from toluene afforded the tosyl aniline **35c** (262 mg, 79%) as a yellow solid; mp 115–119 °C; δ_H (DMSO-d_6): 9.87 (1H, s), 8.69 (1H, d, *J* 1.0 Hz), 8.56 (1H, dd, *J* 5.0, 1.0 Hz), 8.24 (1H, s), 8.19 (1H, s), 8.11 (2H, d, *J* 8.0 Hz), 8.04 (2H, m), 7.90 (1H, d, *J* 8.0 Hz), 7.69–7.65 (3H, m), 7.52 (1H, br s), 7.48 (1H, t, *J* 8.0 Hz), 7.44 (1H, dd, *J* 8.0, 5.0 Hz), 7.37 (2H, d, *J* 8.0 Hz), 7.23 (1H, dd, *J* 8.0, 2.0 Hz), 6.94 (1H, d, *J* 4.0 Hz), 5.25 (2H, s), 3.38–3.36 (4H, m), 2.41 (2H, t, *J* 6.5 Hz), 2.27 (7H, br s), 1.84–1.81 (4H, m), 1.46–1.42 (4H, m), 1.35–1.31 (2H, m); δ_C (DMSO-d_6): 167.1, 158.6, 158.6, 156.8, 154.1, 153.7, 149.4, 149.2, 146.1, 140.8, 140.6, 138.4, 135.8, 135.6, 134.3, 132.7, 130.2, 128.4, 128.1, 124.1, 123.8, 121.8, 117.8, 114.4, 114.1, 113.3, 111.9, 110.2, 105.5, 67.2, 57.8, 54.1, 45.8, 37.0, 25.6, 25.2, 24.1,

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21.2; *m*/*z* (ESI): 814.3 (MH^+); HRMS (ESI): MH^+, found 814.3490. $C_{44}H_{48}N_9O_5S^+$ requires 814.3494.

*4.2.20. N-(2-(Piperidin-1-yl)ethyl)-2-(3-((4-(3-(pyridin-3-ylmethoxy)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino) phenyl)acetamide (**35d**).* Precursor **33** (230 mg, 0.47 mmol) was coupled with aniline **28a** according to General procedure B. Purification by flash chromatography (1% MeOH/CH_2Cl_2 satd with NH_3) and recrystallisation from CCl_4 afforded the 2-anilino-*N*-tosylpyrrolopyrimidine **35d** (230 mg, 72%) as a yellow solid; mp 94–97 °C; δ_H (DMSO-d_6): 9.80 (1H, s), 8.72 (1H, d, *J* 2.0 Hz), 8.57 (1H, dd, *J* 5.0, 2.0 Hz), 8.07 (2H, d, *J* 8.0 Hz), 7.92–7.90 (2H, m), 7.82 (2H, dd app t, *J* 8.0 Hz), 7.67 (1H, d, *J* 4.0 Hz), 7.65–7.64 (2H, m), 7.52 (1H, t, *J* 8.0 Hz), 7.46 (1H, ddd, *J* 8.0, 5.0, 1.0 Hz), 7.39 (2H, d, *J* 8.0 Hz), 7.30 (1H, t, *J* 8.0 Hz), 7.26–7.23 (1H, m), 6.94 (1H, s), 6.93 (1H, d, *J* 4.0 Hz), 5.27 (2H, s), 3.46 (2H, s), 3.16 (2H, br s), 2.34 (3H, s), 2.21 (4H, br s), 1.44 (4H, br s), 1.32 (2H, br s); δ_C (DMSO-d_6): 169.9, 158.5, 158.4, 156.5, 153.3, 149.2, 149.0, 145.9, 140.4, 138.2, 136.7, 135.6, 134.3, 132.4, 130.1, 130.0, 128.3, 127.7, 123.8, 123.6, 122.2, 121.4, 119.7, 117.3, 116.8, 114.5, 109.8, 105.3, 67.1, 57.4, 53.9, 42.7, 36.2, 25.3, 23.8, 21.0; *m*/*z* (ESI): 716.2 (MH^+); HRMS (ESI): MH^+, found 716.3009. $C_{40}H_{42}N_7O_4S^+$ requires 716.3014.

*4.2.21. N-(2-(Piperidin-1-yl)ethyl)-3-((4-(3-(pyridin-3-ylmethoxy) phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)benzamide (**35e**).* Precursor **33** (250 mg, 0.51 mmol) was coupled with aniline **28b** according to General procedure B. Purification by flash chromatography (1% MeOH/CH_2Cl_2 satd with NH_3) and recrystallisation from toluene afforded the 2-anilino-*N*-tosylpyrrolopyrimidine **35e** (260 mg, 73%) as a white solid; mp 200–202 °C; δ_H (DMSO-d_6): 9.95 (1H, s), 8.71 (1H, d, *J* 2.0 Hz), 8.56 (1H, dd, *J* 5.0, 1.5 Hz), 8.40 (1H, s), 8.21 (1H, t, *J* 5.5 Hz), 8.09 (2H, d, *J* 8.5 Hz), 8.04 (1H, dt, *J* 7.0, 2.0 Hz), 7.91 (1H, dt, *J* 8.0, 2.0 Hz), 7.69 (1H, d, *J* 4.0 Hz), 7.67–7.64 (2H, m), 7.51 (1H, t, *J* 8.0 Hz), 7.46–7.43 (3H, m), 7.38 (2H, d, *J* 8.0 Hz), 7.24 (1H, ddd, *J* 8.0, 2.5, 1.0 Hz), 6.94 (1H, d, *J* 4.0 Hz), 5.27 (2H, s), 3.38–3.35 (2H, m), 2.41 (2H, t, *J* 7.5 Hz), 2.33 (4H, br s), 2.32 (3H, s), 1.48–1.43 (4H, m), 1.37–1.32 (2H, m); δ_C (DMSO-d_6): 166.6, 158.6, 158.4, 156.4, 153.3, 149.2, 149.1, 146.0, 140.6, 138.1, 135.7, 135.5, 134.2, 132.5, 130.1$_2$, 130.0$_6$, 128.4, 127.8, 124.1, 123.6, 121.5, 121.3, 120.0, 117.8, 117.5, 114.3, 110.2, 105.3, 67.1, 57.7, 54.1, 37.0, 25.6, 24.0, 21.1; *m*/*z* (ESI): 702.3 (MH^+); HRMS (ESI): MH^+, found 702.2852. $C_{39}H_{40}N_7O_4S^+$ requires 702.2857.

*4.2.22. N-Phenyl-4-(3-(pyridin-3-ylmethoxy)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-2-amine (**35f**).* Precursor **33** (250 mg, 0.51 mmol) was coupled with aniline (56 μL, 0.61 mmol) according to General procedure B. Purification by flash chromatography (70% EtOAc/hexanes) and recrystallisation from CCl_4 afforded the 2-anilino-*N*-tosylpyrrolopyrimidine **35f** (240 mg, 85%) as a light yellow solid; mp 129–131 °C; δ_H (DMSO-d_6): 9.81 (1H, s), 8.72 (1H, s), 8.58 (1H, d, *J* 3.5 Hz), 8.05 (2H, d, *J* 8.5 Hz), 7.94–7.91 (3H, m), 7.68 (1H, d, *J* 4.0 Hz), 7.66–7.63 (2H, m), 7.52 (1H, t, *J* 8.0 Hz), 7.46 (1H, dd, *J* 8.0, 4.5 Hz), 7.41–7.37 (4H, m), 7.25 (1H, ddd, *J* 8.0, 2.5, 1.0 Hz), 7.02 (1H, tt, *J* 7.5, 1.0 Hz), 6.93 (1H, d, *J* 4.0 Hz), 5.27 (2H, s), 2.33 (3H, s); δ_C (DMSO-d_6): 158.6, 158.4, 156.5, 153.3, 149.2, 149.0, 145.9, 140.4, 138.1, 135.6, 134.3, 132.4, 130.1, 130.0, 128.5, 127.6, 123.8, 123.6, 121.5, 121.4, 118.7, 117.3, 114.5, 109.9, 105.2, 67.1, 21.0; *m*/*z* (ESI): 548.2 (MH^+); HRMS (ESI): MH^+, found 548.1746. $C_{31}H_{26}N_5O_3S^+$ requires 548.1751.

*4.2.23. General procedure C: Preparation of 2-anilino-7H-pyrrolopyrimidines via base promoted detosylation. N-(3-((4-(3-(Pyridin-3-ylmethoxy)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)phenyl) pyrrolidine-1-carboxamide (**4a**).* To a solution of 2-anilino-*N*-tosylpyrrolopyrimidine **35a** (140 mg, 0.21 mmol) in MeOH (5 mL) and H_2O (3 mL) was added K_2CO_3 (88 mg, 0.63 mmol) and the mixture was refluxed for 3 h. To the cooled solution was added H_2O and the whole mixture was extracted with EtOAc (×2). The combined organic extracts were washed with brine (×2), dried, filtered and concentrated in vacuo to give a yellow residue. The residue was purified by flash chromatography (EtOAc) and recrystallised from EtOAc/hexanes to give the title compound **4a** (62 mg, 58%) as a yellow solid; mp 141–143 °C; δ_H ($CDCl_3$): 9.90 (1H, s), 8.71 (1H, d, *J* 1.5 Hz), 8.58 (1H, dd, *J* 5.0, 1.5 Hz), 8.17 (1H, s), 7.80 (1H, dt, *J* 8.0, 2.0 Hz), 7.73 (1H, dd app t, *J* 2.0 Hz), 7.71 (1H, d, *J* 8.0 Hz), 7.44 (1H, t, *J* 8.0 Hz), 7.34 (1H, s), 7.32 (1H, dd, *J* 8.0, 5.0 Hz), 7.21–7.17 (2H, m), 7.10 (1H, dd, *J* 8.0, 2.0 Hz), 6.98 (1H, dt, *J* 7.0, 2.0 Hz), 6.81 (1H, dd, *J* 3.5, 2.0 Hz), 6.55 (1H, dd, *J* 3.5, 2.0 Hz), 6.28 (1H, s), 5.16 (2H, s), 3.36–3.33 (4H, m), 1.87–1.84 (4H, m); δ_C ($CDCl_3$): 158.7, 158.1, 156.0, 154.3, 153.9, 149.5, 149.0, 140.9, 140.0, 139.9, 135.3, 132.5, 129.8, 129.2, 123.5, 123.0, 121.9, 116.9, 114.5, 113.2, 112.7, 109.9, 109.7, 101.2, 67.7, 45.8, 25.5; *m*/*z* (ESI): 506.3 (MH^+); HRMS (ESI): MH^+, found 506.2296. $C_{29}H_{28}N_7O_2^+$ requires 506.2299.

*4.2.24. N-(3-(2-Oxo-2-((2-(piperidin-1-yl)ethyl)amino)ethyl)-5-((4-(3-(pyridin-3-ylmethoxy)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl) amino)phenyl)pyrrolidine-1-carboxamide (**4b**).* 2-Anilino-*N*-tosylpyrrolopyrimidine **35b** (146 mg, 0.18 mmol) was treated according to General procedure C. Purification by flash chromatography (2.5% MeOH/CH_2Cl_2 satd with NH_3) and recrystallisation from EtOAc/hexanes afforded the title compound **4b** (101 mg, 85%) as a yellow solid; mp 118–121 °C; δ_H (DMSO-d_6): 11.60 (1H, s), 9.11 (1H, s), 8.71 (1H, d, *J* 2.0 Hz), 8.56 (1H, dd, *J* 5.0, 2.0 Hz), 8.01 (1H, s), 7.91 (1H, ddd, *J* 8.0, 2.0 Hz), 7.87 (1H, dd app t, *J* 2.0 Hz), 7.82–7.78 (2H, m), 7.78 (1H, t, *J* 5.0 Hz), 7.49 (1H, t, *J* 8.0 Hz), 7.45 (1H, ddd, *J* 8.0, 5.0, 1.0 Hz), 7.31 (1H, s), 7.26 (1H, dd, *J* 3.5, 2.0 Hz), 7.21–7.19 (1H, m), 6.93 (1H, s), 6.64 (1H, dd, *J* 3.5, 2.0 Hz), 5.27 (2H, s), 3.15–3.12 (2H, m), 2.29 (6H, br t, *J* 6.5 Hz), 1.81–1.78 (4H, m), 1.45–1.40 (4H, m), 1.33–1.29 (2H, m); δ_C (DMSO-d_6): 170.0, 158.3, 155.9$_6$, 155.9$_5$ 154.5, 154.0, 149.1, 149.0, 141.3, 140.4, 139.7, 135.9, 135.6, 132.6, 129.8, 124.4, 123.6, 121.5, 116.8, 114.2, 113.9, 113.8, 109.4, 108.8, 100.2, 67.0, 57.5, 53.9, 45.6, 43.0, 36.3, 25.4, 25.0, 24.0; *m*/*z* (ESI): 674.3 (MH^+); HRMS (ESI): MH^+, found 674.3561. $C_{38}H_{44}N_9O_3^+$ requires 674.3562.

*4.2.25. N-(3-((2-(Piperidin-1-yl)ethyl)carbamoyl)-5-((4-(3-(pyridin-3-ylmethoxy)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)phenyl)pyrrolidine-1-carboxamide (**4c**).* 2-Anilino-*N*-tosylpyrrolopyrimidine **35c** (150 mg, 0.18 mmol) was treated according to General procedure C. Purification by flash chromatography (10% MeOH/CH_2Cl_2) and recrystallisation from EtOAc/hexanes afforded the title compound **4c** (100 mg, 89%) as a yellow solid; mp 138–141 °C; δ_H (DMSO-d_6): 11.65 (1H, s), 9.29 (1H, s), 8.72 (1H, d, *J* 1.5 Hz), 8.56 (1H, dd, *J* 5.0, 1.5 Hz), 8.19 (1H, s), 8.08 (1H, t, *J* 5.5 Hz), 8.03 (1H, dd app t, *J* 1.5 Hz), 7.92–7.91 (2H, m), 7.83–7.80 (2H, m), 7.49 (1H, t, *J* 8.0 Hz), 7.44 (1H, dd, *J* 8.0, 5.0 Hz), 7.42 (1H, s), 7.28 (1H, dd, *J* 3.5, 2.5 Hz), 7.20 (1H, dd, *J* 8.0, 2.0 Hz), 6.66 (1H, dd, *J* 3.5, 2.0 Hz), 5.27 (2H, s), 3.35–3.30 (2H, m), 2.40 (2H, t, *J* 7.0 Hz), 2.27 (4H, br s), 1.83–1.80 (4H, m), 1.49–1.45 (4H, m), 1.38–1.34 (2H, m); δ_C (DMSO-d_6): 167.1, 158.4, 156.0, 155.8, 154.5, 154.0, 149.1, 149.0, 141.4, 140.5, 139.7, 135.6, 135.3, 132.6, 129.8, 124.6, 123.6, 121.5, 116.9, 114.1, 113.5, 112.2, 111.9, 109.1, 100.2, 67.0, 57.6, 54.0, 45.6, 36.8, 25.5, 25.0, 24.0; *m*/*z* (ESI): 660.3 (MH^+); HRMS (ESI): MH^+, found 660.3403. $C_{37}H_{42}N_9O_3^+$ requires 660.3406.

*4.2.26. N-(2-(Piperidin-1-yl)ethyl)-2-(3-((4-(3-(pyridin-3-ylmethoxy)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)phenyl) acetamide (**4d**).* 2-Anilino-*N*-tosylpyrrolopyrimidine **35d** (125 mg, 0.17 mmol) was treated according to General procedure C. Purification by flash chromatography (1% MeOH/CH_2Cl_2 satd with NH_3) and recrystallisation from EtOAc/hexanes afforded the title compound **4d** (92 mg, 93%) as a yellow solid; mp 78–82 °C; δ_H ($CDCl_3$): 10.38 (1H, s), 8.67 (1H, d, *J* 1.0 Hz), 8.57 (1H, dd, *J* 5.0, 1.0 Hz),

7.77–7.74 (2H, m), 7.71 (1H, d, *J* 7.5 Hz), 7.59 (1H, s), 7.56 (1H, s), 7.47–7.42 (2H, m), 7.29 (1H, dd, *J* 7.5, 5.0 Hz), 7.19 (1H, t, *J* 8.0 Hz), 7.08 (1H, dd, *J* 8.0, 2.0 Hz), 6.95 (1H, d, *J* 3.5 Hz), 6.82 (1H, d, *J* 7.5 Hz), 6.59 (1H, d, *J* 3.5 Hz), 6.45 (1H, t, *J* 4.5 Hz), 5.15 (2H, s), 3.48 (2H, s), 3.27–3.24 (2H, m), 2.35 (2H, t, *J* 6.0 Hz), 2.30 (4H, br s), 1.41–1.36 (4H, m), 1.31–1.27 (2H, m); δ_C ($CDCl_3$): 171.2, 158.7, 158.1, 156.0, 154.4, 149.4, 149.0, 141.0, 139.8, 135.8, 135.3, 132.4, 129.8, 129.2, 123.5, 123.2, 122.5, 121.9, 119.6, 117.5, 116.9, 114.6, 109.9, 101.3, 67.6, 56.9, 54.0, 43.9, 36.2, 25.7, 24.1; *m*/*z* (ESI): 562.3 (MH^+); HRMS (ESI): MH^+, found 562.2923. $C_{33}H_{36}N_7O_2^+$ requires 562.2925.

*4.2.27. N-(2-(Piperidin-1-yl)ethyl)-3-((4-(3-(pyridin-3-ylmethoxy) phenyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)amino)benzamide (**4e**).* 2-Anilino-*N*-tosylpyrrolopyrimidine **35e** (150 mg, 0.21 mmol) was treated according to General procedure C. Purification by flash chromatography (2% MeOH/CH_2Cl_2 satd with NH_3) and recrystallisation from EtOAc/hexanes afforded the title compound **4e** (107 mg, 92%) as a light yellow solid; mp 203–205 °C; δ_H (DMSO-d_6): 11.72 (1H, s), 9.47 (1H, s), 8.74 (1H, d, *J* 1.5 Hz), 8.57 (1H, dd, *J* 5.0, 1.5 Hz), 8.39 (1H, s), 8.26 (1H, dd, *J* 4.5 Hz), 7.99 (1H, dt, *J* 8.0, 2.0 Hz), 7.94 (1H, dt, *J* 8.0, 2.0 Hz), 7.81 (1H, dd, *J* 2.5, 1.5 Hz), 7.78 (1H, d, *J* 8.0 Hz), 7.52 (1H, t, *J* 8.0 Hz), 7.46 (1H, ddd, *J* 8.0, 5.0, 1.0 Hz), 7.35 (1H, t, *J* 8.0 Hz), 7.32 (1H, dd app t, *J* 1.5 Hz), 7.30 (1H, dd, *J* 4.0, 2.0 Hz), 7.22 (1H, ddd, *J* 8.0, 2.5, 1.0 Hz), 6.66 (1H, dd, *J* 4.0, 2.0 Hz), 5.31 (2H, s), 2.45 (2H, br s), 2.40 (4H, br s), 1.51–1.46 (4H, m), 1.39–1.35 (2H, m); δ_C (DMSO-d_6): 166.7, 158.4, 156.1, 155.6, 154.3, 149.2, 149.1, 141.7, 139.6, 135.7, 135.3, 132.6, 129.9, 128.2, 124.7, 123.6, 121.4, 120.7, 118.7, 117.5, 116.9, 114.1, 109.2, 100.2, 67.0, 57.5, 54.0, 36.8, 25.4, 23.9; *m*/*z* (ESI): 548.3 (MH^+); HRMS (ESI): MH^+, found 548.2765. $C_{32}H_{34}N_7O_2^+$ requires 548.2769.

*4.2.28. N-Phenyl-4-(3-(pyridin-3-ylmethoxy)phenyl)-7H-pyrrolo [2,3-d]pyrimidin-2-amine (**4f**).* 2-Anilino-*N*-tosylpyrrolopyrimidine **35f** (140 mg, 0.26 mmol) was treated according to General procedure C. Purification by flash chromatography (70% EtOAc/hexanes) and recrystallisation from EtOAc/hexanes afforded the title compound **4f** (95 mg, 95%) as a yellow solid; mp 182–184 °C; δ_H ($CDCl_3$): 9.49 (1H, s), 8.71 (1H, s), 8.59 (1H, s), 7.79 (1H, d, *J* 8.0 Hz), 7.74–7.68 (4H, m), 7.45 (1H, t, *J* 8.0 Hz), 7.35–7.30 (4H, m), 7.10 (1H, dd, *J* 8.0, 2.0 Hz), 7.00 (1H, t, *J* 8.0 Hz), 6.76 (1H, dd, *J* 3.5, 2.0 Hz), 6.58 (1H, dd, *J* 3.5, 2.0 Hz), 5.15 (2H, s); δ_C ($CDCl_3$): 158.7, 158.3, 156.1, 154.3, 149.5, 149.0, 140.3, 139.8, 135.3, 132.5, 129.9, 129.0, 123.6, 122.8, 122.0, 121.9, 119.2, 117.0, 114.6, 109.8, 101.5, 67.7; *m*/*z* (ESI): 394.2 (MH^+); HRMS (ESI): MH^+, found 394.1659. $C_{24}H_{20}N_5O^+$ requires 394.1663.

*4.2.29. N,4-Bis(3-(pyridin-3-ylmethoxy)phenyl)-7H-pyrrolo[2,3-d] pyrimidin-2-amine (**4g**).* Detosylated side-product **34** (100 mg, 0.30 mmol) was coupled with aniline **32** according to General procedure B. Purification by flash chromatography (1% MeOH/CH_2Cl_2 satd with NH_3) and recrystallisation from EtOH afforded the title compound **4g** (94 mg, 63%) as a beige solid; mp 186–188 °C; δ_H (DMSO-d_6): 11.72 (1H, s), 9.38 (1H, s), 8.71 (2H, dd, *J* 10.0, 2.0 Hz), 8.55 (2H, m), 7.91–7.87 (3H, m), 7.78–7.60 (2H, m), 7.51 (1H, t, *J* 8.0 Hz), 7.45–7.37 (3H, m), 7.30 (1H, dd, *J* 3.0, 2.5 Hz), 7.22 (1H, dd, *J* 8.0, 2.0 Hz), 7.17 (1H, t, *J* 8.0 Hz), 6.64 (1H, d, *J* 2.0 Hz), 5.57 (1H, dd, *J* 8.0, 2.0 Hz), 5.26 (2H, s), 5.15 (2H, s); δ_C (DMSO-d_6): 158.5, 158.3, 156.2, 155.7, 154.1, 149.2, 149.1_2, 149.0_5, 142.9, 139.6, 135.8, 135.7, 132.8, 132.6, 129.9, 129.1, 124.5, 123.7, 123.6, 121.3, 116.6, 114.5, 111.0, 108.9, 106.3, 104.7, 100.2, 67.0, 66.8; *m*/*z* (ESI): 501.2 (MH^+); HRMS (ESI): MH^+, found 501.2031. $C_{30}H_{25}N_6O_2^+$ requires 501.2034.

4.3. Biological screening

All compounds were assayed using a radiometric in vitro kinase assay that was in accordance of Biondi et al.,[48] except magnesium acetate was used instead of magnesium chloride. Compounds tested using the biological assay were prepared in freshly distilled dimethyl sulfoxide to a stock concentration of 100 mM and then diluted as required for assays. The literature inhibitor compound RM1 was used as the positive control (IC_{50} of 200 nM).[16] PDK1 enzyme was purchased from Invitrogen at a purity of 70% and diluted to a concentration of 6.25 ng/μL using 50 mM Tris buffer (adjusted to pH 7.5) containing 0.1% β-mercaptoethanol and 1% bovine serum albumin. PDKtide was purchased from GL Biochem Pty Ltd at a purity of 95.3% by RP-HPLC analysis and used without further purification. P81 cellulose paper was purchased from Millipore as pre-labelled squares and stored in the freezer at −5 °C. [γ-^{32}P]-ATP was purchased from Perkin Elmer Easytides at a 10 mCi/mL concentration and was stored at 2–8 °C. ^{32}P radiation was counted using Pharmcia Wallac 1410 Liquid Scintillation Counter using 'easy count' mode. All other reagents used in the assay were purchased from Sigma–Aldrich and used without further purification.

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