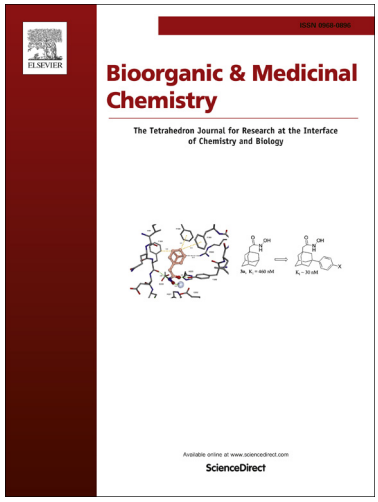

Synthesis and biological evaluation of substituted 3-anilino-quinolin-2(1*H*)-ones as PDK1 inhibitors

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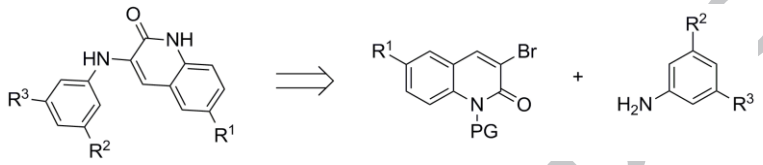

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Synthesis and biological evaluation of substituted 3-anilino-quinolin-2(1*H*)-ones as PDK1 inhibitors

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ABSTRACT

PDK1 is an important regulator of the PI3K/Akt pathway, which has been found frequently activated in a large number of human cancers. Herein we described the preparation of novel substituted 3-anilino-quinolin-2(1*H*)-ones as PDK1 inhibitors. The synthesis is based around a Buchwald-Hartwig cross-coupling of various 3-bromo-6-substituted-quinolin-2(1*H*)-ones with three different functionalised anilines. The modular nature of the designed synthesis allowed access to a series of novel inhibitors through derivatisation of a late-stage intermediate. All compounds were screened against isolated PDK1 enzyme, with modest inhibition observed.

1. Introduction

3-Phosphoinositide-dependent kinase 1 (PDK1) is the master regulator of the AGC signal transduction and is responsible for the activity of as many as 23 different agonist-stimulated AGC kinases, including Akt,[1,2] PKC isoforms,[3] P70 S6K,[4] p90 SGK[5] and RSK[6] by phosphorylation at its own activation loop.[7,8] These AGC kinases are members of the PI3K/Akt signalling pathway and are constitutively activated in a large number of human cancers in response to abnormally high levels of the secondary messenger, phosphatidylinositol 3,4,5-triphosphate (PIP3).[9,10] PDK1 plays a vital role in the positive regulation of the important cellular effects triggered by the PI3K/Akt signalling pathway and subsequent PIP3 production.[8,11] Activation of phosphatidylinositol 3-kinase (PI3K) stimulates the production of PIP3, which interacts with several downstream effector proteins to control the activity and subcellular localisation of a diverse range of signal transducers that are fundamental to cell growth, metabolism, proliferation and survival.[8,12,13]

Disruption or dysregulation of this pathway, through gene mutation, leads to the promotion of tumour proliferation, reduced apoptosis and angiogenesis.[14,15] Recent experimental evidence has shown that specific inhibitors of this pathway are able to promote apoptosis in cells and reduce tumour growth.[16] As PDK1 is the critical regulator within the PI3K/Akt pathway, targeted inhibition of this downstream enzyme represents a promising biological target for the development of anti-cancer drugs.[14,17]

A key area of the ATP binding site is the hinge region, where an inhibitor can lock in through hydrogen bonding interaction with the Ser-89, Tyr-90 and Ala-91 residues. Binding potency and selectivity can then be enhanced by additional interactions with other areas in or near the active site. We recently reported the synthesis of novel compounds containing 4-(1*H*-indol-6-yl)-1*H*-indazoles[18] as potential PDK1 inhibitors. These compounds were designed to have a heterocyclic core bound to the hinge region, with additional functionality to target various binding site pockets. In this contemporary study, again guided by molecular modelling of ligand interactions, a 3-anilino-quinolin-2(1*H*)-one core **1** was identified to also possess an appropriate geometry and electronic properties for the binding site (Figure 1).

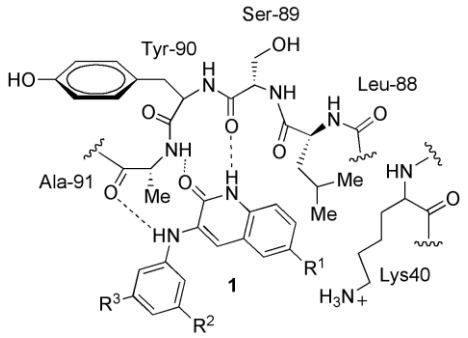

Fig. 1. Schematic representation of the 3-anilino-quinolin-2(1*H*)-one **1** core bound to the hinge region (Ser-89, Tyr-90, Ala-91) of PDK1 binding site.

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The nitrogens and the carbonyl of the quinolin-2(1*H*)-one backbone and 3-position aniline provide important interactions with the hinge region, locking the inhibitor into the binding site. As suggested by molecular modelling, additional functionality was added to the inhibitor core: access to the solvent exposed entrance regions (E_0 and E_1) and the ribose pocket (R) was targeted by adding functionality to the phenyl ring of the 3-position aniline (R^2 and R^3). Specific targeting of the catalytic residues (Cat.) of the phosphate pocket (P) and hydrophobic region (BP-I) was attempted by appending an appropriate functional group to the 6-position of the quinolin-2(1*H*)-one scaffold (R^1).

Compound **2** and **3** (Figure 2), potent PDK1 inhibitors developed by Berlex Biosciences,[19,20] were identified from recent literature, both with X-ray crystal structures available in complex with PDK1 (PDB codes: 1Z5M and 2PE2). Compound **2** contains a pyrrolidine urea moiety that binds to the ribose pocket of PDK1, whilst compound **3** contains the *N*-(2-(piperidin-1-yl)ethyl)acetamide chain that binds to the E_0 region and provides further interactions with the E_1 region.

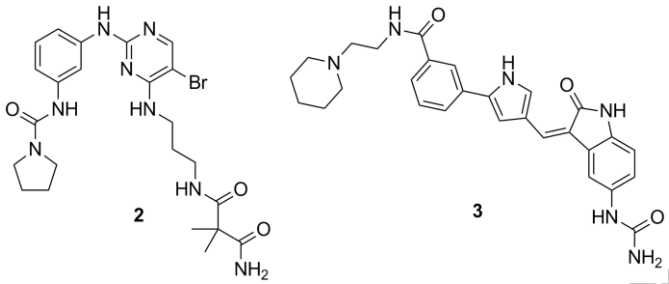


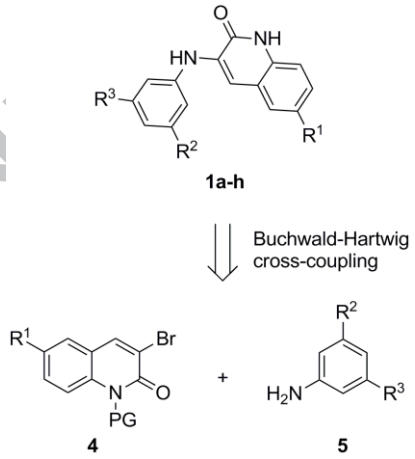
Fig. 2. Known PDK1 inhibitors developed by Berlex Biosciences.

A methylene linker was suggested by our modelling to give the appropriate chain length to reach the E_0 region from the 3-aniline ring. The pyrrolidine urea was incorporated onto the phenyl ring of the 3-position aniline (R^2 position) of target compound **1a** whilst both side chains were included (R^2 and R^3 positions) for target compound **1b** (Figure 3a and 3b, respectively, and Table 2). Compound **3** also contains a primary urea group, which provides key hydrogen bond interactions with the catalytic residues of the phosphate pocket, and this was incorporated at the 6-position (R^1 position) of the quinolinone scaffold for target compounds **1a** and **b**. For comparison, the primary urea at the R^1 position of the scaffold without any substitutions at either the R^2 and R^3 positions would also be undertaken. Replacement of the primary urea with a diverse group of R^1 substituents would enable the effect of such changes on biological activity to be further probed.

Herein, the synthetic route to this novel series of heterocyclic compounds is reported, along with results for inhibition of activity towards isolated PDK1 enzyme.

2. Results and Discussion

The synthetic strategy for the 3-anilino-quinolin-2(1*H*)-one derivatives **1a-h** was designed on the independent synthesis of two key fragments and then linking them together by a Buchwald-Hartwig cross-coupling reaction to form a new C-N bond (Scheme 1 and Table 2). The functionalised quinolin-2(1*H*)-one intermediate **4** was an appropriate heterocyclic core for the planned cross-coupling reaction with various substituted anilines **5**. Incorporation of a protecting group (PG) for this fragment was deemed to be a requirement for quinolin-2(1*H*)-ones when undertaking palladium catalysed cross-coupling reactions.[21,22] Following literature precedence,[23-25] a *p*-methoxybenzyl (PMB) group was chosen due to its ease of formation and cleavage from amides of quinolinones.



Scheme 1. Retrosynthetic strategy towards the novel substituted 3-anilino-quinolin-2(1*H*)-ones.

2-Hydroxyquinoline **6** was treated under standard nitration conditions to give 6-nitroquinolinone **7** in 95% yield (Scheme 2). Acid catalysed bromination, through *in situ* generation of hypobromous acid, furnished the desired 3-bromo-6-nitroquinolinone **8** in a yield of 87%.[26] Synthesis of compound **8** using these conditions had been previously reported by Lee et al.[27], but no experimental or characterisation detail was reported. Benzylation of **8** with *p*-methoxybenzyl chloride and potassium carbonate gave the *N*-PMB protected quinolinone **9**.[28,29]

a) b)

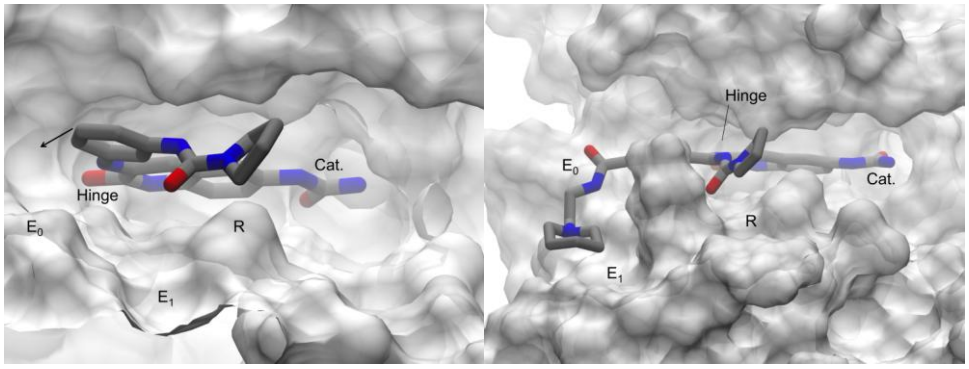
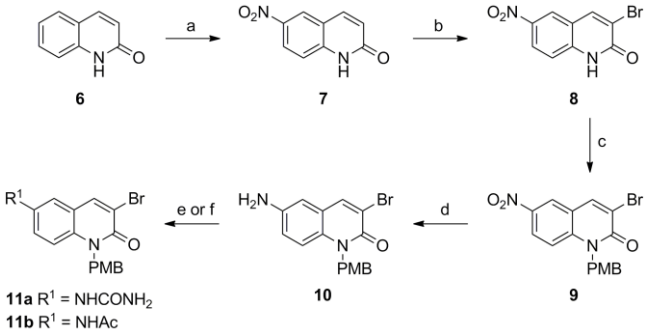


Fig. 3. Molecular model of target 3-anilino-quinolin-2(1*H*)-ones **1a** (a) and **1b** (b) bound to PDK1 illustrating access to the hinge region (Hinge), catalytic residues (Cat.), ribose pocket (R), and entrance regions (E_0 and E_1) from the designed scaffold. The vector in a) indicates extension of the scaffold at C3′ by addition of the R^3 side chain.

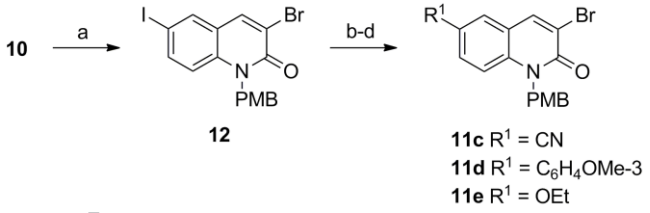
Metal catalysed reduction of compound **9** to the corresponding amine **10** was achieved in 80% yield with 10 equivalents of zinc powder in a 1:3 mixture of acetic acid and methanol at room temperature.[30]

Amine **10** provided access to the 6-ureido compound **11a** in 80% yield, by treatment with isocyanuric acid produced *in situ*. Acetanilide **11b** was observed in the ^{1}H-NMR spectrum of the crude material, but this minor side-product was successfully removed by recrystallisation. Generation of a reactive mixed anhydride ($H_2NCOOCOCH_3$) from the isocyanuric acid and acetic acid was a plausible explanation for the unwanted acetanilide formation. Formation of an acetanilide side-product was also observed by Silvestri et al.[31] when forming ureas with isocyanuric acid. Following conditions previously utilised by our laboratory,[32] amine **10** was also able to be acetylated to give **11b** in a quantitative yield.



Scheme 2. Reagents and Conditions: (a) $cH_2SO_4/cHNO_3$, 0 ˚C, 30 min, 95%; (b) $NaBrO_3$ (1.5 eq.), 48% aq. HBr, H_2O, 100 ˚C, 16 h, 87%; c) PMB-Cl (1.5 eq.), K_2CO_3 (1.5 eq), DMF, rt, 5 h; then rt → 50 ˚C, 16 h, 90%; (d) Zn (10 eq.), 1:3 AcOH/MeOH, rt, 30 min, 80%; (e) NaOCN (3 eq.), 4:1 AcOH/H_2O, rt, 1 h, 80%; (f) AcCl, Et_3N, THF, 0 ˚C, 30 min; then 0 ˚C → rt, 30 min, 98%.

Installation of an iodine moiety at the 6-position provided a handle for the introduction of a more diverse range of functional groups *via* cross-coupling chemistry. Insight into the relative reactivity of the 6-iodo versus the 3-bromo position was also obtained. While 3-bromo-6-iodoquinolin-2(1*H*)-one has been prepared, starting with 4-iodoaniline,[33] amine **10** provided access to this scaffold without starting a new synthetic pathway. Diazotization of amine **10** with sodium nitrite and then treatment with potassium iodide provided the iodo compound **12** in a 90% yield (Scheme 3).[34]



Scheme 3. Reagents and Conditions: (a) $NaNO_2$ (2 eq.), cHCl, -5 ˚C, 1 h; KI (25 eq.), H_2O, -5 ˚C → rt, 4 h, 91%; (b) $Zn(CN)_2$ (0.65 eq.), $Pd(PPh_3)_4$ (7.5 mol%), DMF, 80 ˚C, 4 h, 82%; (c) 3-$MeOC_6H_4B(OH)_2$ (1.2 eq.), $Pd(PPh_3)_4$ (5 mol%), 4:1 1,4-dioxane/H_2O, 90 ˚C, 16 h, 52%; (d) CuI (10 mol%), 1,10-phenanthroline (20 mol%), Cs_2CO_3 (2 eq.), EtOH, reflux, 16 h, 35%.

Nitrile installation using palladium catalysed cyanation conditions[35] with iodo **12** and zinc cyanide gave nitrile **11c** with high selectivity to the 6-position. A small amount (circa 5-10%) of the 3,6-disubstituted side-product was observed by ^{1}H-NMR, but this was easily removed by flash chromatography. Modified Suzuki cross-coupling[36] of 3-methoxyphenylboronic acid to iodo **12** afforded the 6-(3-methoxyphenyl) analogue **11d**, with a small amount (circa 10-15%) of the 3,6-disubstituted side-product which could only be removed by flash chromatography on a small scale (approx. 200 mg) due to the insolubility of the mixture. Installation of an ethoxy group was achieved by using a copper iodide/1,10-phenanthroline catalysed alkoxylation method reported by Buchwald et al.[37] to give a 1:1:1 mixture of the desired 6-ethoxy analogue **11e**, along with the 3-ethoxy-6-iodo **13a** and 3,6-diethoxy **13b** side-products which were all isolated by flash chromatography and characterised. The lack of regioselectivity between 3- and 6-positions was a noteworthy difference from the previous cyanation and Suzuki reactions. Attempts to install an alkoxy group by modified Suzuki cross-coupling conditions[38-41] with potassium methoxymethyltrifluoroborate were all unsuccessful.

The 3-anilino-quinolin-2(1*H*)-ones **1a-g** were synthesised by Buchwald-Hartwig cross-coupling of bromo compounds **11a**-**11e** with anilines **14** and **15** (Figure 3), that were synthesised by our laboratory,[42] as well as aniline, followed by an acid catalysed debenzylation.

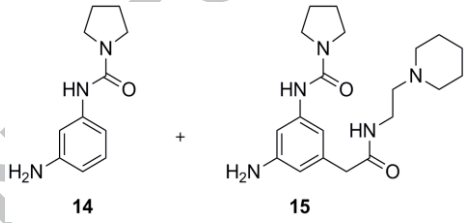
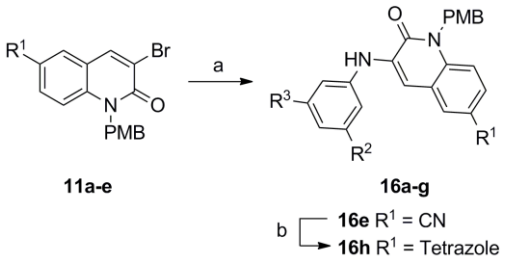


Fig. 3. Custom anilines **14** and **15**.

Buchwald-Hartwig cross-coupling conditions[43] yielded the *N*-PMB protected quinolinones **16a**-**16g** in yields ranging from 44-92% (Table 2). These conditions provided a highly efficient and reliable alternative for synthesising 3-anilino-quinolin-2(1*H*)-ones over conventional methods[44-52] which require multi-step procedures that suffer from low yields and limited substrate scope. Due to the insolubility of urea **11a**, the yields for intermediates **16a**-**16c** were initially lower than desired but were improved when the amount of reaction solvent was doubled. Tetrazole compound **16h** was synthesised in 98% yield by a [3 + 2] cycloaddition[53] of nitrile intermediate **16e** with trimethylsilyl azide in the presence of tetrabutylammonium fluoride.

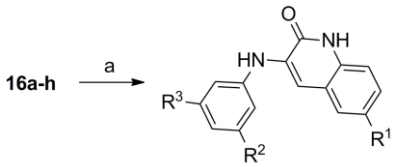
Table 2. Synthesis of *N*-PMB protected quinolinones **16a-g** by Buchwald-Hartwig cross-coupling and tetrazole compound **16h** by [3 + 2] cycloaddition with TMS-N_3.



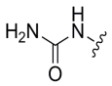
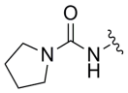
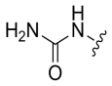
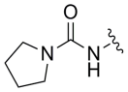
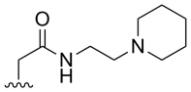
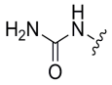
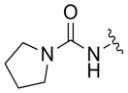
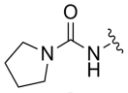
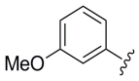
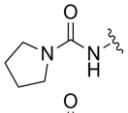
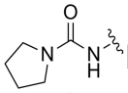
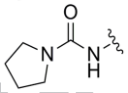
Bromo cmpd	Aniline	Product	Yield %
11a	**14**	**16a**	51
11a	**15**	**16b**	58
11a	**$PhNH_2$**	**16c**	44
11b	**14**	**16d**	81
11c	**14**	**16e**	92
11d	**14**	**16f**	73
11e	**14**	**16g**	78

Reagents and Conditions: (a) aniline (1.2 eq.), K_2CO_3 (2.2 eq.), $Pd_2(dba)_3$ (5 mol%), XPhos (10 mol%), *t*-BuOH, reflux, 16 h; (b) TMS-N_3 (10 eq.), TBAF (1.2 eq.), 80 ˚C, 48 h, 98%.

Table 3. Synthesis of 3-anilino-quinolin-2(1*H*)-ones **1a-h** by acid catalysed debenzylation, and their percentage inhibitory activity against PDK1.



1a-h

Compound	R^1	R^2	R^3	Yield %	% Inhibition at 10 μM[a]
1a			H	65	40±5
1b				26[b]	40±6
1c		H	H	47	36±8
1d			H	62	20±6
1e			H	29	30±7
1f			H	55	29±8
1g			H	60	23±8
1h			H	34	14±7

Reagents and Conditions: (a) TfOH (3.5 eq.), TFA, rt, 3 h. [a]The mean ± SD of at least 3 independent experiments (radiometric assay). [b]Required purification by RP-HPLC.

Treatment of *N*-PMB protected quinolinones **16a-g** with triflic acid in trifluoroacetic acid[54] furnished the desired novel 3-anilino-quinolin-2(1*H*)-ones **1a-g** in moderate yields (Table 3). Trifluoroacetic acid alone, commonly used for this type of debenzylations,[55-61] failed though, for example, **9** was debenzylated successfully. Thus, the new 3-aniline linker was the problem. It was reasoned that trifluoroacetic acid was monoprotonating compounds **16a-h** at the exocyclic nitrogen rather than the ring nitrogen, the latter being a necessary condition for debenzylation. The stronger triflic acid seemingly achieved this outcome through diprotonation. The use of the Buchwald-Hartwig cross-coupling to install aniline functionality at the 3-position of quinolin-2(1*H*)-ones has been previously reported,[21,22,62] but this is the first example to the best of our knowledge where the protecting group has been subsequently removed to furnish the *N*-unprotected product. The highly insoluble nature of products **1e** and **1h** in organic solvents made purification difficult and is the reason for the lower yields obtained from these debenzylation reactions.

Target compounds **1a-h** were screened against isolated PDK1 using a radiometric biological assay at a concentration of 10 μM (Table 3). Unfortunately, only modest inhibitory activity was observed. The initial target compounds **1a** and **1b** were the best inhibitors of the series, both possessing inhibition of 40±5-6%, which illustrates the homologated *N*-(2-(piperidin-1-yl)ethyl)acetamide side chain of **1b** is not a necessity for activity. Elimination of both the pyrrolidine urea and *N*-(2-(piperidin-1-yl)ethyl)acetamide side chains only slightly decreased the activity of **1c**. However, changes to the 6-position of the quinolin-2(1*H*)-one scaffold had a significant impact on the activity of this class of compounds and reduced the inhibition of compounds **1d-h** towards PDK1. Direct derivatisation to an acetanilide **1d** decreased activity to 20±6%, a 2-fold decrease in comparison to the designed target compounds **1a** and **1b**. This shows the importance of the hydrogen bond donors from the NH_2 moiety of the urea to activity. Addition of a 3-methoxyphenyl moiety **1f** decreased the inhibition to 29±8%, whilst an ethoxy group **1g** gave a similar result of 23±8%. Installation of a nitrile moiety **1e** also showed a reduction of inhibition (30±7%), whilst further transformation to a tetrazole **1h** gave the poorest result (14±7%). Even though the tetrazole is a potential hydrogen bond donor, its high insolubility profile was a plausible explanation behind its lack of activity.

3. Conclusion

We have developed an efficient and reliable route for the preparation of novel substituted 3-anilino-quinolin-2(1*H*)-ones. Synthesis of a 6-aminoquinolinone intermediate gave access to a range of 3-bromo-6-substituted-quinolin-2(1*H*)-ones, which were cross-coupled at the 3-position with three different anilines. Use of Buchwald-Hartwig cross-coupling over conventional methods was highly effective. In addition, utilisation of the PMB protecting group provided a versatile system for the synthesis of various *N*-unprotected 3-anilino-quinolin-2(1*H*)-ones. Selectivity for the 6-iodo over the 3-

bromo was shown for both palladium cross-coupling reactions, with only small amounts of the 3,6-disubstituted side-products observed. Unfortunately, the assessed compounds displayed only modest inhibitory activity towards PDK1. The primary urea moiety was found to be beneficial for binding interactions with the catalytic residues over other 6-position substituents. Further investigation into replacing the primary urea group at the 6-position with various hydrogen bond donor groups could enhance the binding potential of these compounds.

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 ionization (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/sec, 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 ionization (ESI) source, in positive mode, unless otherwise stated. Preparatory RP-HPLC was performed on a Beckman system (125 Solvent Module and 166 Detector) fitted with a Phenomenex® Jupiter C18 300 Å column (250 mm x 10 mm, 10 µm) at a flow rate of 5 mL/min, monitored at 220 nm. 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.[63] 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 saturated 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.[64] 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 sulphate unless otherwise stated.

4.2 Synthesis

*4.2.1 6-Nitroquinolin-2(1H)-one (**7**).* To a stirring solution of 2-hydroxyquinoline **6** (3.50 g, 24.1 mmol) in conc. H_2SO_4 (15 mL) at -5 ºC, was added conc. HNO_3 (500 µL) dropwise ensuring the reaction temperature was < 20 ºC. The solution was stirred at -5 °C for 30 min and then poured onto ice. The precipitate that formed was filtered and washed with H_2O to give the 6-nitroquinolinone **7** (4.35 g, 95%) as a light green solid; mp 279-281 ºC (from *i*-PrOH) (lit.[65] mp 280 °C); δ_H (DMSO-d_6): 12.26 (1H, s), 8.65 (1H, d, *J* 2.5 Hz), 8.29 (1H, dd, *J* 9.0, 2.5 Hz), 8.09 (1H, d, *J* 9.5 Hz), 7.40 (1H, d, *J* 9.0 Hz), 6.65 (1H, d, *J* 9.5 Hz); δ_C (DMSO-d_6): 161.93, 143.2, 141.4, 140.1, 125.0, 124.2, 123.8, 118.5, 116.5. *m/z* (ESI): 191.0 (MH^+).

*4.2.2 3-Bromo-6-nitroquinolin-2(1H)-one (**8**).* To a suspension of the 6-nitroquinolinone **7** (4.35 g, 22.8 mmol), $NaBrO_3$ (4.14 g, 27.4 mmol) and H_2O (15 mL) was added 48% aq. HBr (80 mL) and the reaction mixture was heated at 100 °C for 4 h, then cooled and poured onto ice. The precipitate that formed was filtered and washed with H_2O. The solid was recrystallised from AcOH and EtOH to give the 3-bromo-6-nitroquinolinone **8** (5.35 g, 87%) as a white solid; mp >300 ºC; δ_H (DMSO-d_6): 12.76 (1H, s), 8.72 (1H, d, *J* 4.5 Hz), 8.69 (1H, dd, *J* 4.5, 2.5 Hz), 8.35 (1H, ddd, *J* 9.0, 3.5, 2.5 Hz), 7.46 (1H, dd, *J* 9.0, 3.5 Hz); δ_C (DMSO-d_6): 157.7, 142.3, 141.8, 141.4, 125.1, 123.6, 119.3, 118.8, 116.3; *m/z* (ESI): 269.0 ($M[^{79}Br]H^+$), 271.0 ($M[^{81}Br]H^+$); HRMS (ESI): $M[^{79}Br]H^+$, found 268.9555. $C_9H_6BrN_2O_3^+$ requires 268.9557.

*4.2.3 3-Bromo-1-(4-methoxybenzyl)-6-nitroquinolin-2(1H)-one (**9**).* To a solution of the 3-bromo-6-nitroquinolinone **8** (4.00 g, 14.8 mmol) in anhydrous DMF (40 mL) was added K_2CO_3 (3.08 g, 22.3 mmol) and *p*-methoxybenzyl chloride (3.02 mL, 22.3 mmol). The reaction was stirred at room temperature for 5 h and later heated to 50 °C overnight, then cooled and poured onto ice. The precipitate that formed was filtered, washed with H_2O and recrystallised from EtOH to give the *N*-PMB protected quinolinone **9** (5.20 g, 90%) as a fluffy white solid; mp 208-209 °C; δ_H (DMSO-d_6): 8.82 (1H, s), 8.74 (1H, d, *J* 2.5 Hz), 8.33 (1H, dd, *J* 9.5, 2.5 Hz), 7.65 (1H, d, *J* 9.5 Hz), 7.17 (2H, d, *J* 8.5 Hz), 6.87 (2H, d, *J* 8.5 Hz), 5.54 (2H, s), 3.69 (3H, s); δ_C (DMSO-d_6): 158.5, 157.7, 142.3, 141.9, 141.2, 128.0, 127.4, 125.1, 124.3, 120.0, 118.4, 116.6, 114.1, 55.0, 46.5; *m/z* (ESI): 801.0 ($M_2[^{79,81}Br]Na^+$), 411.0 ($M[^{79}Br]Na^+$), 413.0 ($M[^{81}Br]Na^+$); HRMS (ESI): $M_2[^{79,81}Br]Na^+$, found 800.9980 (100%). $C_{34}H_{26}Br_2N_4NaO_8^+$ requires 800.9990.

*4.2.4 6-Amino-3-bromo-1-(4-methoxybenzyl)quinolin-2(1H)-one (**10**).* To a heterogeneous solution of the *N*-PMB protected quinolinone **9** (1.50 g, 3.85 mmol) in MeOH (60 mL) and AcOH (15 mL), was added Zn powder (2.51 g, 38.5 mmol) and the reaction mixture was stirred for 30 min. The excess Zn was filtered off and the filtrate was concentrated *in vacuo*. The resulting residue was triturated with CH_2Cl_2, filtered again and the filtrate washed with satd $NaHCO_3$ (×2), brine (×2), dried, filtered and the solvent was removed *in vacuo* to give a yellow solid. The solid was purified by flash chromatography (60% EtOAc/hexanes) and recrystallised from *i*-PrOH to give the amine **10** (1.10 g, 80%) as a yellow solid; mp 168-170 °C; δ_H (DMSO-d_6): 8.35 (1H, s), 7.20 (1H, d, *J* 9.0 Hz), 7.14 (2H, d, *J* 9.0 Hz), 6.87 (1H, dd, *J* 9.0, 2.5 Hz), 6.86 (2H, d, *J* 9.0 Hz), 6.78 (1H, d, *J* 2.5 Hz), 5.43 (2H, s), 5.18 (2H, s), 3.71 (3H, s); δ_C (DMSO-d_6): 158.3, 156.8, 144.2, 140.8, 129.8, 128.6, 128.0, 121.3, 119.4, 116.2, 116.1, 114.0, 109.8, 55.0, 45.6; *m/z* (ESI):

359.0 (M[^{79}Br]H$^+$), 360.9 (M[^{81}Br]H$^+$); HRMS (ESI): M[^{79}Br]H$^+$, found 359.0397. $C_{17}H_{16}BrN_2O_2^+$ requires 359.0390.

*4.2.5 1-(3-Bromo-1-(4-methoxybenzyl)-2-oxo-1,2-dihydroquinolin-6-yl)urea (**11a**).* To a solution of amine **10** (1.00 g, 2.78 mmol) in AcOH (40 mL) was added NaOCN (543 mg, 8.35 mmol) in H_2O (10 mL) and the solution was stirred at room temperature for 1 h. The reaction was poured onto ice and the solid that separated was filtered and washed with H_2O. The solid was recrystallised from cyclohexanone and *n*-PrOH to give the urea **11a** (0.89 g, 80%) as a white solid; mp 234-237 °C; δ_H (DMSO-d_6): 8.69 (1H, br s), 8.53 (1H, s), 7.79 (1H, d, *J* 2.5 Hz), 7.53 (1H, dd, *J* 9.5, 2.5 Hz), 7.37 (1H, d, *J* 9.5 Hz), 7.16 (2H, d, *J* 8.5 Hz), 6.88 (2H, d, *J* 8.5 Hz), 5.89 (2H, s), 5.47 (2H, s), 3.70 (3H, s); δ_C (DMSO-d_6): 158.4, 157.2, 156.0, 141.3, 135.7, 133.1, 128.3, 128.0, 122.3, 120.6, 116.6, 115.7$_2$, 155.7$_0$, 114.0, 55.0, 45.8; *m/z* (ESI): 402.0 (M[^{79}Br]H$^+$), 403.9 (M[^{81}Br]H$^+$); HRMS (ESI): M[^{79}Br]H$^+$, found 402.0440. $C_{18}H_{17}BrN_3O_3^+$ requires 402.0448.

*4.2.6 N-(3-Bromo-1-(4-methoxybenzyl)-2-oxo-1,2-dihydroquinolin-6-yl)acetamide (**11b**).* To a solution of amine **10** (250 mg, 0.70 mmol) in anhydrous THF (10 mL) at 0 °C was added Et_3N (156 µL, 1.04 mmol) and AcCl (78 µL, 1.04 mmol), and the mixture was stirred for 5 min. The reaction mixture was allowed to warm to room temperature and was stirred for a further 30 min. The solvent was removed *in vacuo*, H_2O was added and the resulting solid was filtered to give the acetanilide **11b** (280 mg, 98%) as a tan solid; mp 133-136 °C; δ_H (DMSO-d_6): 10.11 (1H, s), 8.58 (1H, s), 8.01 (1H, d, *J* 2.5 Hz), 7.64 (1H, dd, *J* 9.0, 2.5 Hz), 7.44 (1H, d, *J* 9.0 Hz), 7.16 (2H, d, *J* 8.5 Hz), 6.87 (2H, d, *J* 8.5 Hz), 5.48 (2H, s), 3.69 (3H, s), 2.05 (3H, s); δ_C (DMSO-d_6): 168.3, 158.4, 157.3, 141.3, 134.3, 134.2, 128.2, 128.1, 123.1, 120.4, 117.3, 116.8, 115.8, 114.1, 55.0, 45.8, 23.8; *m/z* (ESI): 423.0 (M[^{79}Br]Na$^+$), 425.0 (M[^{81}Br]Na$^+$); HRMS (ESI): M[^{79}Br]H$^+$, found 401.0483. $C_{19}H_{18}BrN_2O_3^+$ requires 401.0496.

*4.2.7 3-Bromo-6-iodo-1-(4-methoxybenzyl)quinolin-2(1H)-one (**12**).* To a suspension of amine **10** (2.20 g, 6.12 mmol) in conc. HCl (60 mL) at -5°C, was added $NaNO_2$ (845 mg, 12.2 mmol) in H_2O (40 mL) dropwise over 5 min and the reaction was stirred for 1 h. The resulting yellow solution was added dropwise to a -5°C solution of KI (50.0 g, 306.0 mmol) in H_2O (250 mL) and the reaction was stirred for 15 min at -5°C and for a further 4 h at room temperature. The reaction mixture was diluted with H_2O and extracted with EtOAc (3×). The combined organic extracts were washed with 2.5 M NaOH (×2), 10% $Na_2S_2O_3$ (×2), 2.5 M NaOH (×1), H_2O (×1), brine (×1), dried, filtered and the solvent was removed *in vacuo* to give a light brown solid. The solid was recrystallised from *n*-PrOH to give the iodo **12** (2.60 g, 91%) as a metallic brown coloured solid; mp 202-205 °C; δ_H (DMSO-d_6): 8.54 (1H, s), 8.14 (1H, d, *J* 2.0 Hz), 7.83 (1H, dd, *J* 9.0, 2.0 Hz), 7.28 (1H, d, *J* 9.0 Hz), 7.13 (2H, d, *J* 8.5 Hz), 6.86 (2H, d, *J* 8.5 Hz), 5.47 (2H, s), 3.70 (3H, s). δ_C (DMSO-d_6): 158.4, 157.4, 140.3, 138.9, 137.8, 136.2, 127.9, 127.8, 122.4, 117.7, 117.3, 114.1, 86.4, 55.0, 45.8; *m/z* (ESI): 507.9 (M[^{79}Br]K$^+$), 509.9 (M[^{81}Br]K$^+$); HRMS (ESI): M[^{79}Br]H$^+$, found 469.9251. $C_{17}H_{15}BrINO_2^+$ requires 469.9248.

*4.2.8 3-Bromo-6-cyano-1-(4-methoxybenzyl)quinolin-2(1H)-one (**11c**).* To a solution of iodo **12** (340 mg, 0.72 mmol) in DMF (10 mL) was added $Zn(CN)_2$ (55 mg, 0.47 mmol) and the mixture was purged with argon. To this was added $Pd(PPh_3)_4$ (62 mg, 7.5 mol%) and the reaction mixture was heated to 90 °C for 4 h. The reaction mixture was cooled, diluted with 30% NH_4OH and extracted with EtOAc (×2). The combined organic extracts were washed with brine (×4), dried, filtered and the solvent was removed *in vacuo* to give a yellow solid. The solid was purified by flash chromatography (0-1% MeOH/CH_2Cl_2) and recrystallised from *n*-PrOH to give the nitrile **11c** (220 mg, 82%) as a white solid; mp 202-205 °C; δ_H (DMSO-d_6): 8.64 (1H, s), 8.29 (1H, d, *J* 2.0 Hz), 7.95 (1H, dd, *J* 9.0, 2.0 Hz), 7.62 (1H, d, *J* 9.0 Hz), 7.16 (2H, d, *J* 9.0 Hz), 6.87 (2H, d, *J* 9.0 Hz), 5.53 (2H, s), 3.70 (3H, s); δ_C (DMSO-d_6): 158.5, 157.7, 140.9, 140.6, 133.3, 133.2, 128.0, 127.5, 120.4, 118.2, 118.1, 116.7, 114.2, 105.0, 55.0, 46.1; *m/z* (ESI): 761.0 (M$_2$[^{79,81}Br]Na$^+$), 407.0 (M[^{79}Br]K$^+$), 409.0 (M[^{81}Br]K$^+$); HRMS (ESI): M$_2$[^{79,81}Br]Na$^+$, found 761.0206. $C_{36}H_{26}Br_2N_4NaO_4^+$ requires 761.0193.

*4.2.9 3-Bromo-1-(4-methoxybenzyl)-6-(3-methoxyphenyl)quinolin-2(1H)-one (**11d**).* To a solution of iodo **12** (400 mg, 0.85 mmol) in 1,4-dioxane (20 mL) and H_2O (2 mL) was added 3-methoxyphenylboronic acid (155 mg, 1.02 mmol), Na_2CO_3 (180 mg, 1.70 mmol) and LiCl (72 mg, 1.70 mmol), and the mixture was degassed under argon. To this was added $Pd(PPh_3)_4$ (50 mg, 5 mol%) and the reaction mixture was heated to 100 °C overnight, then cooled and the solvent was removed *in vacuo*. The resulting residue was dissolved in CH_2Cl_2, washed with H_2O (×2), brine (×2), dried, filtered and the solvent was removed *in vacuo* to give a brown residue. The residue was purified by flash chromatography (15-20% EtOAc/hexanes) to give an inseperable mixture (10:1) of the title compound **11d** and the *bis*(3-methoxyphenyl) substituted side-product, obtained as a light brown residue (199 mg, 52%). On a small scale (approx. 200 mg), the impurity was removed by flash chromatography (15% EtOAc/hexanes) to give the 6-(3-methoxyphenyl) **11d** as a white solid; mp 60-65 ºC; δ_H (DMSO-d_6): 8.65 (1H, s), 8.10 (1H, d, *J* 2.0 Hz), 7.90 (1H, dd, *J* 9.0, 2.0 Hz), 7.54 (1H, d, *J* 9.0 Hz), 7.39 (1H, t, *J* 8.0 Hz), 7.25 (1H, d, *J* 8.0 Hz), 7.22 (1H, dd app t, *J* 2.0 Hz), 7.19 (2H, d. *J* 8.5 Hz), 6.95 (1H, dd, *J* 8.0, 2.0 Hz), 6.88 (2H, d, *J* 8.5 Hz), 5.55 (2H, s), 3.82 (3H, s), 3.70 (3H, s); δ_C (DMSO-d_6): 159.8, 158.4, 157.6, 141.7, 140.0, 137.7, 134.3, 130.1, 129.6, 128.2, 128.0, 126.3, 120.7, 118.8, 116.6, 116.0, 114.1, 113.2, 112.1, 55.2, 55.1, 45.9; *m/z* (ESI): 472.1 (M[^{79}Br]Na$^+$), 474.1 (M[^{81}Br]Na$^+$); HRMS (ESI): M[^{79}Br]H$^+$, found 450.0680. $C_{24}H_{21}BrNO_3^+$ requires 450.0700.

*4.2.10 3-Bromo-6-ethoxy-1-(4-methoxybenzyl)quinolin-2(1H)-one (**11e**).* To a suspension of iodo **12** (600 mg, 1.28 mmol), Cs_2CO_3 (832 mg, 2.55 mmol) and 1,10-phenanthroline (46 mg, 20 mol%) in EtOH (20 mL) was added CuI (24 mg, 10 mol%) and the mixture was heated to 110 °C in a pressure tube overnight. The reaction mixture was cooled, passed through a silica pad eluting with EtOAc and the filtrate was concentrated *in vacuo* to give the crude material as a 1:1:1 mixture of the desired ethoxy **11e**, as well as two other cross-coupled side-products. The mixture was separated by flash chromatography (15% EtOAc/hexanes) to give 6-ethoxy **11e** (170 mg, 35%) as a tan solid; mp 118-121 °C; δ_H (DMSO-d_6): 8.51 (1H, s), 7.39 (1H, d, *J* 9.5 Hz), 7.30 (1H, d, *J* 3.0 Hz), 7.19 (1H, dd, *J* 9.5, 3.0 Hz), 7.15 (2H, d, *J* 9.0 Hz), 6.87 (2H, d, *J* 9.0 Hz), 5.48 (2H, s), 4.04 (2H, q, *J* 7.0 Hz), 3.69 (3H, s), 1.33 (3H, t, *J* 7.0 Hz); δ_C (DMSO-d_6): 158.4, 157.2, 153.7, 141.0, 132.7, 128.3, 128.0, 121.1, 119.9, 116.9, 116.7, 114.1, 110.8, 63.5, 55.0, 45.9, 14.5; *m/z* (ESI): 426.0 (M[^{79}Br]K$^+$), 428.0 (M[^{81}Br]K$^+$); HRMS (ESI): M[^{79}Br]H$^+$, found 388.0548. $C_{19}H_{19}BrNO_3^+$ requires 388.0543.

Side-products *3-Ethoxy-6-iodo-1-(4-methoxybenzyl)quinolin-2(1H)-one* ***(13a)*** and *3,6-diethoxy-1-(4-methoxybenzyl)quinolin-2(1H)-one* ***(13b)*** were obtained from the crude mixture using flash chromatography (60-80% EtOAc/hexanes) to give:

3-Ethoxy-6-iodo **13a** (70 mg) as a light brown solid; mp 184-190 °C; δ_H (DMSO-d_6): 8.12 (1H, d, *J* 2.0 Hz), 7.79 (1H, dd, *J* 9.0, 2.0 Hz), 7.19 (1H, d, *J* 9.0 Hz), 7.10 (2H, d, *J* 9.0 Hz), 6.85 (2H, d, *J* 9.0. Hz), 6.13 (1H, s), 5.35 (2H, br s), 4.22 (2H, q, *J* 7.0 Hz), 3.69 (3H, s), 1.44 (3H, t, *J* 7.0 Hz); δ_C (DMSO-d_6): 162.3, 159.9, 158.2, 139.2, 138.2, 130.8, 128.6, 127.7, 118.0, 117.6, 114.0, 97.3, 85.2, 64.7, 55.0, 43.5, 14.0; *m/z* (ESI): 436.0 (MH^+); HRMS (ESI): MH^+, found 436.0388. $C_{19}H_{19}INO_3^+$ requires 436.0405.

3,6-Diethoxy **13b** (45 mg) as a light yellow solid; mp 131-135 °C; δ_H (DMSO-d_6): 7.31 (1H, s), 7.30 (1H, d, *J* 9.0 Hz), 7.15 (1H, dd, *J* 9.0, 3.0 Hz), 7.12 (2H, d, *J* 9.0 Hz), 6.85 (2H, d, *J* 9.0 Hz), 6.10 (1H, s), 5.36 (2H, br s), 4.22 (2H, q, *J* 7.0 Hz), 4.21 (2H, q, *J* 7.0 Hz), 3.69 (3H, s), 1.44 (3H, t, *J* 7.0 Hz), 1.31 (3H, t, *J* 7.0 Hz). δ_C (DMSO-d_6): 162.2, 160.6, 158.2, 153.2, 133.0, 129.1, 127.8, 119.6, 166.6$_4$, 116.5$_9$, 113.9, 105.8, 96.9, 64.3, 63.5, 55.0, 43.5, 14.6, 14.0; *m/z* (ESI): 354.1 (MH^+); HRMS (ESI): MH^+, found 354.1688. $C_{21}H_{24}NO_4^+$ requires 354.1700.

4.2.11 General Procedure A. Preparation of N-PMB protected quinolinones via Palladium Catalysed Buchwald-Hartwig Cross-Coupling. N-(3-((1-(4-Methoxybenzyl)-2-oxo-6-ureido-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide ***(16a)***. A mixture of bromo compound **11a** (400 mg, 0.99 mmol), custom aniline **14** (245 mg, 1.19 mmol) and K_2CO_3 (302 mg, 2.18 mmol) in anhydrous *t*-BuOH (40 mL) was degassed under argon. To this was added XPhos (47 mg, 10 mol%) and $Pd_2(dba)_3$ (46 mg, 5 mol%) and the reaction mixture was heated to reflux overnight, then allowed to cool to room temperature. To the reaction mixture was added H_2O and the mixture was extraced with CH_2Cl_2 (×2). The combined organic extracts were washed with brine (×2), dried, filtered and the solvent removed *in vacuo* to give a dark red residue. The residue was purified by flash chromatography (2-4% MeOH/CH_2Cl_2) and recrystallised from *n*-PrOH to give the *N*-PMB protected quinolinone **16a** (266 mg, 51%) as a beige solid; mp 214-217 °C; δ_H (DMSO-d_6): 8.50 (1H, s), 8.12 (1H, s), 7.87 (1H, s), 7.65 (1H, dd, *J* 2.5, 1.5 Hz), 7.63 (1H, d, *J* 2.5 Hz), 7.36 (1H, s), 7.28 (1H, d, *J* 9.5 Hz), 7.22-7.19 (5H, m), 6.94-6.92 (1H, m), 6.88 (2H, d, *J* 9.5 Hz), 5.81 (2H, s), 5.54 (2H, s), 3.71 (3H, s), 3.40-3.37 (4H, m), 1.87-1.84 (4H, m); δ_C (DMSO-d_6): 158.3, 157.6, 156.0, 153.9, 141.4, 140.9, 135.6, 132.4, 129.0, 128.7, 128.0, 127.7, 122.0, 117.1, 115.1, 114.2, 114.0, 113.5, 113.2, 110.2, 107.5, 55.0, 45.7, 45.1, 25.0; *m/z* (ESI): 527.2 (MH^+). HRMS (ESI): MH^+, found 527.2380. $C_{29}H_{31}N_6O_4^+$ requires 527.2402.

4.2.12 N-(3-((1-(4-Methoxybenzyl)-2-oxo-6-ureido-1,2-dihydroquinolin-3-yl)amino)-5-(2-oxo-2-((2-(piperidin-1-yl)ethyl)amino)ethyl)phenyl)pyrrolidine-1-carboxamide ***(16b)***. Bromo compound **11a** (350 mg, 0.87 mmol) was coupled with custom aniline **15** according to General Procedure A. Purification by flash chromatography (5-6% MeOH/CH_2Cl_2 satd with NH_3) and recrystallisation from EtOH afforded the *N*-PMB protected quinolinone **16b** (350 mg, 58%) as a white solid; mp 156-159 °C; δ_H (DMSO-d_6): 8.50 (1H, s), 8.12 (1H, s), 7.81 (1H, t, *J* 5.5 Hz), 7.76 (1H, s), 7.60 (1H, d, *J* 2.5 Hz), 7.50 (1H, dd app t, *J* 2.0 Hz), 7.32 (1H, s), 7.27 (1H, d, *J* 9.0 Hz), 7.21-7.17 (3H, m), 7.08 (1H, dd, *J* 1.5 Hz), 6.87 (2H, d, *J* 8.5 Hz), 6.83 (1H, dd app t, *J* 1.5 Hz), 5.79 (2H, s), 5.53 (2H, s), 3.70 (3H, s), 3.39-3.37 (4H, m), 3.34 (2H, s), 3.15 (2H, q, *J* 6.0 Hz), 2.30 (2H, t, *J* 6.5 Hz), 2.28 (4H, br s), 1.87-1.84 (4H, m), 1.44-1.40 (4H, m), 1.32-1.28 (2H, m); δ_C (DMSO-d_6): 169.8, 158.3, 157.6, 156.0, 153.9, 141.3, 140.6, 137.2, 135.6, 132.5, 128.7, 128.0, 127.6, 121.9, 117.0, 115.1, 114.2, 114.0, 108.8, 107.4, 57.6, 55.0, 54.0, 45.7, 45.1, 42.8, 36.4, 25.5, 25.0, 24.0; *m/z* (ESI): 695.4 (MH^+); HRMS (ESI): MH^+, found 695.3667. $C_{38}H_{47}N_8O_5^+$ requires 695.3664.

4.2.13 1-(1-(4-Methoxybenzyl)-2-oxo-3-(phenylamino)-1,2-dihydroquinolin-6-yl)urea ***(16c)***. Bromo compound **11a** (240 mg, 0.60 mmol) was coupled with aniline according to General Procedure A, except that once the reaction was complete, the solvent was removed *in vacuo* and H_2O was added. The resulting precipitate was filtered and purified by flash chromatography (2.5-4.0% MeOH/CH_2Cl_2) followed by recrystallisation from *n*-BuOH to give the *N*-PMB protected quinolinone **16c** (105 mg, 44%) as a grey solid; mp >300 °C; δ_H (DMSO-d_6): 8.46 (1H, s), 8.03 (1H, s), 7.67 (1H, d, *J* 2.5 Hz), 7.42-7.35 (5H, m), 7.41 (1H, d, *J* 9.0 Hz), 7.20 (2H, d, *J* 9.0 Hz), 7.18 (1H, dd, *J* 9.0, 2.0 Hz), 7.00 (1H, tt, *J* 7.0, 1.0 Hz), 6.88 (2H, d, *J* 9.0 Hz), 5.83 (2H, s), 5.53 (1H, s), 3.70 (3H, s); δ_C (DMSO-d_6): 158.3, 157.6, 156.1, 141.1, 135.6, 132.4, 129.2, 128.7, 128.1, 127.8, 121.9, 121.7, 119.6, 117.2, 115.0, 114.3, 114.0, 107.6, 55.0, 45.1; *m/z* (ESI): 437.2 (MNa^+); HRMS (ESI): MH^+, found 415.1753. $C_{24}H_{23}N_4O_3^+$ requires 415.1765.

4.2.14 N-(3-((6-Acetamido-1-(4-methoxybenzyl)-2-oxo-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide ***(16d)***. Bromo compound **11b** (250 mg, 0.62 mmol) was coupled with custom aniline **14** according to General Procedure A. Purification by flash chromatography (2.5% MeOH/CH_2Cl_2) and recrystallisation from *n*-PrOH afforded the *N*-PMB protected quinolinone **16d** (265 mg, 81%) as an off-white solid; mp 168-172 °C; δ_H (DMSO-d_6): 9.96 (1H, s), 8.13 (1H, s), 7.93 (1H, s), 7.81 (1H, dd app t, *J* 1.5 Hz), 7.66 (1H, dd app t, *J* 2.0 Hz), 7.36-7.34 (3H, m), 7.21-7.17 (4H, m), 6.94-6.92 (1H, m), 6.87 (2H, d, *J* 8.5 Hz), 5.54 (2H, s), 3.70 (3H, s), 3.40-3.37 (4H, m), 2.03 (3H, s), 1.87-1.85 (4H, m); δ_C (DMSO-d_6): 168.6, 158.8, 158.2, 154.4, 141.9, 141.3, 134.8, 133.1, 129.5, 129.2, 129.1, 128.5, 122.3, 118.3, 116.2, 115.6, 114.5, 114.2, 113.7, 110.8, 107.7, 55.5, 46.2, 45.6, 25.5, 24.4; *m/z* (ESI): 548.3 (MNa^+); HRMS (ESI): MH^+, found 526.2431. $C_{30}H_{32}N_5O_4^+$ requires 526.2449.

4.2.15 N-(3-((6-Cyano-1-(4-methoxybenzyl)-2-oxo-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide ***(16e)***. Bromo compound **11c** (175 mg, 0.47 mmol) was coupled with custom aniline **14** according to General Procedure A. Purification by flash chromatography (1-2% MeOH/CH_2Cl_2) and recrystallisation from *n*-PrOH afforded the *N*-PMB protected quinolinone **16e** (215 mg, 92%) as a yellow solid; mp 204-206 °C; δ_H (DMSO-d_6): 8.15 (1H, s), 8.11 (1H, s), 8.05 (1H, d, *J* 2.0 Hz), 7.63-7.60 (2H, m), 7.53 (1H, d, *J* 9.0 Hz), 7.45 (1H, s), 7.24-7.22 (2H, m), 7.19 (2H, d, *J* 8.5 Hz), 6.97-6.95 (1H, m), 6.89 (2H, d, *J* 8.5 Hz), 5.60 (2H, s), 3.70 (3H, s), 3.40-3.37 (4H, m), 1.87-1.84 (4H, m); δ_C (DMSO-d_6): 158.5, 158.2, 153.9, 141.4, 140.2, 135.3, 134.0, 130.5, 129.0, 128.0, 127.9$_3$, 127.9$_1$, 122.5, 118.9, 115.9, 114.4, 114.2, 114.1, 111.7, 105.6, 104.9, 55.0, 45.7, 45.3, 25.0; *m/z* (ESI): 516.3 (MNa^+); HRMS (ESI): MH^+, found 494.2164. $C_{29}H_{28}N_5O_3^+$ requires 494.2187.

*4.2.16 N-(3-((1-(4-Methoxybenzyl)-6-(3-methoxyphenyl)-2-oxo-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide (**16f**).* Bromo compound **11d** (150 mg, 0.33 mmol) was coupled with custom aniline **14** according to General Procedure A. Purification by flash chromatography (0-2% MeOH/CH_2Cl_2) afforded the *N*-PMB protected quinolinone **16f** (140 mg, 73%) as a yellow solid; mp 103-106 °C; δ_H (DMSO-d_6): 8.11 (1H, s), 7.94 (1H, s), 7.88 (1H, d, *J* 2.0 Hz), 7.63 (1H, s), 7.57 (1H, dd, *J* 9.0, 2.0 Hz), 7.56 (1H, s), 7.45 (1H, d, *J* 9.0 Hz), 7.35 (1H, t, *J* 8.0 Hz), 7.25 (1H, d, *J* 8.0 Hz), 7.23-7.21 (5H, m), 6.99-6.96 (1H, m), 6.93-6.89 (3H, m), 5.61 (2H, br s), 3.81 (3H, s), 3.70 (3H, s), 3.39-3.36 (4H, m), 1.86-1.84 (4H, m); δ_C (DMSO-d_6):159.8, 158.4, 158.1, 153.9, 141.4, 140.9, 140.8, 134.3, 132.8, 132.3, 129.9, 129.0, 128.5, 128.0, 124.29, 124.27, 122.2, 118.8, 115.3, 114.1, 113.8, 113.5, 112.9, 111.9, 111.0, 107.7, 55.1$_1$, 55.0$_5$, 45.7, 45.2, 25.0; *m/z* (ESI): 597.3 (MNa^+); HRMS (ESI): MH^+, found 575.2631. $C_{35}H_{35}N_4O_4^+$ requires 575.2653.

*4.2.17 N-(3-((6-Ethoxy-1-(4-methoxybenzyl)-2-oxo-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide (**16g**).* Bromo compound **11e** (142 mg, 0.37 mmol) was coupled with custom aniline **14** according to General Procedure A. Purification by flash chromatography (70% EtOAc/hexanes) afforded the *N*-PMB protected quinolinone **16g** (145 mg, 78%) as a white solid; mp 201-203 °C; δ_H (DMSO-d_6): 8.10 (1H, s), 7.88 (1H, s), 7.59 (1H, dd app t, *J* 2.0 Hz), 7.40 (1H, s), 7.29 (1H, d, *J* 9.5 Hz), 7.21-7.17 (4H, m), 7.08 (1H, d, *J* 3.0 Hz), 6.95-6.93 (1H, m), 6.88 (2H, d, *J* 9.0 Hz), 6.85 (1H, dd, *J* 9.0, 3.0 Hz), 5.54 (2H, s), 4.01 (2H, q, *J* 7.0 Hz), 3.70 (3H, s), 3.39-3.37 (4H, m), 1.87-1.84 (4H, m), 1.31 (3H, t, *J* 7.0 Hz); δ_C (DMSO-d_6): 158.3, 157.6, 154.0, 153.9, 141.4, 140.8, 132.8, 129.0, 128.7, 128.0, 127.1, 122.8, 116.0, 114.3, 114.0, 113.7, 113.5, 111.0, 109.3, 107.2, 65.2, 55.0, 45.7, 45.1, 25.0, 14.7; *m/z* (ESI): 535.3 (MNa^+); HRMS (ESI): MH^+, found 513.2483. $C_{30}H_{33}N_4O_4^+$ requires 513.2497.

*4.2.18 N-(3-((1-(4-methoxybenzyl)-2-oxo-6-(2H-tetrazol-5-yl)-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide (**16h**).* To a suspension of *N*-PMB protected quinolinone **16e** (220 mg, 0.44 mmol) and TMS-N_3 (586 µL, 4.46 mmol) under argon was added TBAF (1M in tetrahydrofuran containing 5 wt. % of H_2O, 534 µL, 0.53 mmol) and the mixture was heated to 85 °C for 48 h. The reaction was cooled, concentrated *in vacuo* and diluted out with 1M HCl. The solid that separated was filtered, washed with 1M HCl and H_2O to give the tetrazole intermediate **16h** (234 mg, 98%) as a brown solid; mp 156-162 °C; δ_H (DMSO-d_6): 8.23 (1H, d, *J* 2.0 Hz), 8.12 (1H, s), 8.08 (1H, br s), 7.86 (1H, dd, *J* 9.0, 2.0 Hz), 7.68 (1H, dd, *J* 2.0, 1.0 Hz), 7.63 (1H, d, *J* 9.0 Hz), 7.53 (1H, s), 7.24-7.21 (4H, m), 6.98 (1H, m), 6.89 (2H, d, *J* 9.0 Hz), 5.63 (2H, s), 3.70 (3H, s), 3.41-3.38 (4H, m), 1.88-1.85 (4H, m); δ_C (DMSO-d_6): 158.4, 158.1, 153.9, 141.4, 140.4, 134.3, 133.4, 129.0, 128.2, 128.0, 124.9, 123.7, 122.3, 115.8, 114.1$_3$, 114.0$_9$, 113.7, 111.0, 106.6, 55.0, 45.7, 45.3, 25.0; *m/z* (ESI): 535.4 (MH^-); HRMS (ESI): MH^+, found 537.2334. $C_{29}H_{29}N_8O_3^+$ requires 537.2358.

*4.2.19 General Procedure B. Preparation of 3-Anilino-quinolin-2-(1H)-ones via Acid Catalysed Debenzylation. N-(3-((2-Oxo-6-ureido-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide (**1a**).* To a solution of *N*-PMB protected quinolinone **16a** (140 mg, 0.27 mmol) in TFA (5 mL) was added TfOH (82 µL, 0.93 mmol). The reaction mixture was stirred at room temperature for 3h and then carefully poured onto ice-cold satd $NaHCO_3$. The solid that separated was filtered and the filtrate was extracted with CH_2Cl_2 (×3). The combined organic extracts were washed with brine (×2), dried, filtered and the solvent was removed *in vacuo* to give a beige solid. The resulting crude solid was combined with the previously isolated solid and purified by flash chromatography (5-6% MeOH/CH_2Cl_2 satd with NH_3) followed by recrystallisation from EtOH to give the title compound **1a** (65 mg, 65%) as a white solid; mp >300°C; δ_H (DMSO-d_6): 11.96 (1H, s), 8.47 (1H, s), 8.11 (1H, s), 7.71 (1H, s), 7.61 (1H, s), 7.59 (1H, d, *J* 2.0 Hz), 7.32 (1H, s), 7.21-7.14 (4H, m), 6.92-6.88 (1H, m), 5.79 (2H, s), 3.40-3.37 (4H, m), 1.87-1.84 (4H, m); δ_C (DMSO-d_6): 157.7, 156.1, 153.9, 141.4, 140.9, 135.3, 133.0, 128.9, 127.3, 121.1, 117.2, 114.8, 113.4, 113.2, 113.0, 109.9, 107.4, 45.7, 25.0; *m/z* (ESI): 407.2 (MH^+); HRMS (ESI): MH^+, found 407.1826. $C_{21}H_{23}N_6O_3^+$ requires 407.1827.

*4.2.20 N-(3-(2-Oxo-2-((2-(piperidin-1-yl)ethyl)amino)ethyl)-5-((2-oxo-6-ureido-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide (**1b**).* *N*-PMB protected quinolinone **16b** (160 mg, 0.23 mmol) was treated according to General Procedure B, except that the crude product was extracted using 15% *i*-PrOH in CH_2Cl_2. Purification by flash chromatography (7-8% MeOH/CH_2Cl_2 satd with NH_3) afforded an off-white solid (86 mg, 65%). Further purification by preparatory reverse phase HPLC (20-60% MeCN/10 mM aq NH_4CO_3) provided the title compound **1b** (35 mg, 26%) as a white solid; mp >300 °C; δ_H (DMSO-d_6): 11.88 (1H, s), 8.41 (1H, s), 8.04 (1H, s), 7.74 (1H, t, *J* 5.0 Hz), 7.56 (1H, d, *J* 2.5 Hz), 7.53 (1H, s), 7.46 (1H, dd app t, *J* 2.0 Hz), 7.27 (1H, s), 7.19 (1H, dd, *J* 9.0, 2.0 Hz), 7.15 (1H, d, *J* 9.0 Hz), 7.08 (1H, dd app t, *J* 2.0 Hz), 6.79 (1H, dd app t, *J* 2.0 Hz), 5.70 (2H, s), 3.40-3.37 (4H, m), 3.37 (2H, s), 3.21-3.17 (2H, m), 2.38 (6H, br s), 1.87-1.85 (4H, m), 1.48-1.43 (4H, m), 1.36-1.31 (2H, m); δ_C (DMSO-d_6): 169.9, 157.5, 156.0, 153.8, 141.2, 140.6, 137.0, 135.2, 133.0, 127.3, 121.0, 117.2, 114.8, 113.9, 113.8, 113.5, 108.3, 107.4, 57.2, 53.7, 45.6, 42.7, 36.1, 25.1, 24.9, 23.6; *m/z* (ESI): 575.3 (MH^+); HRMS (ESI): MH^+, found 575.3087. $C_{30}H_{39}N_8O_4^+$ requires 575.3089.

*4.2.211-(2-Oxo-3-(phenylamino)-1,2-dihydroquinolin-6-yl)urea (**1c**).* *N*-PMB protected quinolinone **16c** (80 mg, 0.19 mmol) was treated according to General Procedure B, except once quenched with satd $NaHCO_3$ the mixture was refrigerated overnight and the resulting precipitate was collected by filtration. Purification by flash chromatography (5-20% MeOH/CH_2Cl_2) and washing with H_2O and acetone afforded the title compound **1c** (26 mg, 47%) as a light brown solid; mp >300 °C; δ_H (DMSO-d_6): 11.96 (1H, s), 8.41 (1H, s), 7.87 (1H, s), 7.61 (1H, d, *J* 2.0 Hz), 7.38-7.32 (5H, m), 7.17 (1H, dd, *J* 9.0, 2.0 Hz), 7.13 (1H, d, *J* 9.0 Hz), 6.97 (1H, tt, *J* 7.0, 1.0 Hz), 5.79 (2H, s); δ_C (DMSO-d_6): 157.7, 156.1, 141.1, 135.3, 133.0, 129.2, 127.5, 121.5, 121.1, 119.3, 117.3, 114.9, 113.6, 107.6; *m/z* (ESI): 293.1 (MH^-); HRMS (ESI): MH^+, found 295.1191. $C_{16}H_{15}N_4O_2^+$ requires 295.1190.

*4.2.22 N-(3-((6-Acetamido-2-oxo-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide (**1d**).* *N*-PMB protected quinolinone **16d** (115 mg, 0.22 mmol) was treated according to General Procedure B. Purification by flash chromatography (5-7.5% MeOH/CH_2Cl_2) and recrystallisation from *n*-PrOH afforded the title compound **1d** (54 mg, 62%) as a white solid; mp 231-234 °C; δ_H (DMSO-d_6): 12.10 (1H, s), 9.91 (1H, s), 8.11 (1H, s), 7.78 (1H, d, *J* 2.0 Hz), 7.75 (1H, s), 7.62 (1H, s), 7.34 (1H, dd, *J* 9.0, 2.0 Hz), 7.31 (1H, s), 7.19-7.16 (3H, m), 6.90-6.88 (1H, m), 3.40-3.37 (4H, m), 2.03 (3H,

s), 1.87-1.84 (4H, m); δ_C (DMSO-d_6): 168.0, 157.8, 153.9, 141.4, 140.9, 134.0, 133.2, 129.0, 128.4, 121.0, 117.9, 115.0, 114.9, 113.4, 113.1, 109.0, 107.2, 45.7, 25.0, 23.9; *m/z* (ESI): 428.2 (MNa^+); HRMS (ESI): MH^+, found 406.1879. $C_{22}H_{24}N_5O_3^+$ requires 406.1874.

*4.2.23 N-(3-((6-Cyano-2-oxo-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide (**1e**).* *N*-PMB protected quinolinone **16e** (200 mg, 0.41 mmol) was treated according to General Procedure B. Purification by flash chromatography (2.5-5% MeOH/CH_2Cl_2) and recrystallisation from EtOH afforded the title compound **1e** (44 mg, 29%) as a beige solid; mp 279-281 °C; δ_H (DMSO-d_6): 12.47 (1H, s), 8.00 (1H, s), 7.99 (1H, s), 7.98 (1H, d, *J* 2.0 Hz), 7.60 (1H, dd, *J* 8.0, 2.0 Hz), 7.58 (1H, dd app t, *J* 2.0 Hz), 7.37 (1H, s), 7.36 (1H, d, *J* 8.5 Hz), 7.22-7.18 (2H, m), 6.94-6.91 (1H, m), 3.39-3.37 (4H, m), 1.87-1.84 (4H, m); δ_C (DMSO-d_6): 158.3, 153.9, 141.4, 140.3, 135.1, 134.7, 129.9, 129.0, 127.8, 121.6, 119.3, 115.6, 114.2, 114.0, 111.5, 105.5, 104.3, 45.7, 25.0; *m/z* (ESI): 396.1 (MNa^+); HRMS (ESI): MH^+, found 374.1617. $C_{21}H_{20}N_5O_2^+$ requires 374.1612.

*4.2.24 N-(3-((6-(3-Methoxyphenyl)-2-oxo-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide (**1f**).* *N*-PMB protected quinolinone **16f** (120 mg, 0.21 mmol) was treated according to General Procedure B. Purification by flash chromatography (2.5% MeOH/CH_2Cl_2) and recrystallisation from *n*-PrOH afforded the title compound **1f** (51 mg, 55%) as a white solid; mp 203-206 °C; δ_H (DMSO-d_6): 12.18 (1H, s), 8.09 (1H, s), 7.81 (1H, d, *J* 2.0 Hz), 7.78 (1H, s), 7.59 (1H, s), 7.58 (1H, dd, *J* 8.5, 2.0 Hz), 7.48 (1H, s), 7.36 (1H, t, *J* 8.0 Hz), 7.33 (1H, d, *J* 8.5 Hz), 7.25 (1H, ddd, *J* 8.0, 2.0, 1.0 Hz), 7.22-7.21 (1H, m), 7.20-7.18 (2H, m), 6.95-6.92 (1H, m), 6.91 (1H, ddd, *J* 8.0, 2.0, 1.0 Hz), 3.83 (3H, s), 3.39-3.36 (4H, m), 1.86-1.84 (4H, m); δ_C (DMSO-d_6): 159.7, 158.1, 153.9, 141.4_1, 141.3_9, 140.8, 134.0, 133.4, 132.0, 129.9, 128.9, 124.2, 123.4, 121.4, 118.8, 115.2, 113.5, 113.4, 112.6, 111.9, 110.7, 107.6, 55.1, 45.7, 25.0; *m/z* (ESI): 477.2 (MNa^+); HRMS (ESI): MH^+, found 455.2086. $C_{27}H_{27}N_4O_3^+$ requires 455.2078.

*4.2.25 N-(3-((6-Ethoxy-2-oxo-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide (**1g**).* *N*-PMB protected quinolinone **16g** (120 mg, 0.23 mmol) was treated according to General Procedure B. Purification by flash chromatography (5% MeOH/CH_2Cl_2) and recrystallisation from EtOH afforded the title compound **1g** (55 mg, 60%) as a white solid; mp 225-229 °C; δ_H (DMSO-d_6): 11.98 (1H, s), 8.08 (1H, s), 7.72 (1H, s), 7.56 (1H, dd app t, *J* 1.5 Hz), 7.34 (1H, s), 7.18-7.16 (3H, m), 7.01 (1H, d, *J* 2.5 Hz), 6.92-6.89 (1H, m), 6.86 (1H, dd, *J* 9.0, 2.5 Hz), 4.01 (2H, q, *J* 7.0 Hz), 3.39-3.36 (4H, m), 1.87-1.84 (4H, m), 1.32 (3H, t, *J* 7.0 Hz); δ_C (DMSO-d_6): 157.5, 153.8, 141.4, 140.8, 133.4, 128.9, 126.6, 121.8, 115.8, 114.5, 113.4, 113.3, 110.6, 108.2, 107.2, 63.2, 45.6, 25.0, 14.7; *m/z* (ESI): 415.2 (MNa^+); HRMS (ESI): MH^+, found 393.1926. $C_{22}H_{25}N_4O_3^+$ requires 393.1922.

*4.2.26 N-(3-((2-Oxo-6-(2H-tetrazol-5-yl)-1,2-dihydroquinolin-3-yl)amino)phenyl)pyrrolidine-1-carboxamide (**1h**).* Tetrazole intermediate **16h** (150 mg, 0.28 mmol) was treated according to General Procedure B, except once quenched with satd $NaHCO_3$ the mixture was refrigerated overnight. The resulting precipitate was collected by filtration and purified by washing with acetone followed by recrystallisation from *n*-PrOH to afford the title compound **1h** (40 mg, 34%) as a brown solid; mp >300 °C; δ_H (DMSO-d_6): 12.11 (1H, s), 8.09 (1H, s), 8.07 (1H, d, *J* 1.5 Hz), 7.89 (1H, dd, *J* 8.5, 2.0 Hz), 7.72 (1H, s), 7.66 (1H, dd app t, *J* 2.0 Hz), 7.52 (1H, s), 7.27 (1H, d, *J* 8.5 Hz), 7.20 (1H, dt, *J* 7.5, 2.0 Hz), 7.16 (1H, t, *J* 8.0 Hz), 6.91 (1H, dt, *J* 7.5, 2.0 Hz), 3.42-3.39 (4H, m), 1.88-1.85 (4H, m); δ_C (DMSO-d_6): 160.4, 158.1, 153.9, 141.4, 141.0, 132.6, 131.5, 128.9, 127.2, 123.9, 122.2, 120.9, 114.6, 113.1, 112.7, 109.4, 108.1, 45.7, 25.0; *m/z* (ESI): 415.3 (MH^-); HRMS (ESI): MH^+, found 417.1786. $C_{21}H_{21}N_8O_2^+$ requires 417.1782.

4.3 Biological Screening

All compounds were assayed using a radiometric *in vitro* kinase assay that was in accordance of Biondi et al.[66], 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).[17] 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 ºC. [γ-^{32}P]-ATP was purchased from Perkin Elmer Easytides at a 10 mCi/mL concentration and was stored at 2-8 ºC. ^{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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