Structure-based design of 2-arylamino-4-cyclohexylmethoxy-5-nitroso-6-aminopyrimidine inhibitors of cyclin-dependent kinase 2

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An efficient synthesis of 2-substituted O^4-cyclohexylmethyl-5-nitroso-6-aminopyrimidines from 6-amino-2-mercaptopyrimidin-4-ol has been developed and used to prepare a range of derivatives for evaluation as inhibitors of cyclin-dependent kinase 2 (CDK2). The structure–activity relationships (SARs) are similar to those observed for the corresponding O^6-cyclohexylmethoxypurine series with the 2-arylsulfonamide and 2-arylcarboxamide derivatives showing excellent potency. Two compounds, 4-(6-amino-4-cyclohexylmethoxy-5-nitrosopyrimidin-2-ylamino)-*N*-(2-hydroxyethyl)benzenesulfonamide (**7q**) and 4-(6-amino-4-cyclohexylmethoxy-5-nitrosopyrimidin-2-ylamino)-*N*-(2,3-dihydroxypropyl)-benzenesulfonamide (**7s**), were the most potent with IC_{50} values of 0.7 ± 0.1 and 0.8 ± 0.0 nM against CDK2, respectively. The SARs determined in this study are discussed with reference to the crystal structure of 4-(6-amino-4-cyclohexylmethoxy-5-nitrosopyrimidin-2-ylamino)-*N*-(2,3-dihydroxy-propyl)benzenesulfonamide (**7j**) bound to phosphorylated CDK2/cyclin A.

Introduction

The progression of cells through the cell-cycle is a highly ordered process, which is strictly controlled by the cyclin-dependent kinase (CDK) family of enzymes and their cyclin partners. Regulation of the serine-threonine kinase activity of specific CDKs, necessary to allow the cells to pass through cell-cycle checkpoints, is achieved by the binding of the cyclin partner and phosphorylation to produce the fully activated protein kinase complex.[1] In cancer, the presence of oncogenic signalling pathways, or the absence of control resulting from the loss or mutation of tumour suppressor genes, inevitably results in abnormal cell-cycle control and increased CDK/cyclin activity.[2,3] For this reason, the development of potent and selective CDK inhibitors has become an important therapeutic goal.[4–6]

The development of potent and selective small-molecule ATP-competitive kinase inhibitors has been successful for a number of therapeutic targets. New drugs have entered clinical use, notably imatinib that inhibits Bcr-Abl kinase and c-Kit kinase, gefitinib and erlotinib which target the EGFR tyrosine kinase, and lapatinib, a dual EGFR, Her2 tyrosine kinase inhibitor.[7] A large number of small-molecule inhibitors of CDK2 have been reported based on a structurally diverse range of scaffolds. These include derivatives of the monoheterocycles—pyrimidine,[8] [1,2,4]triazole,[9] pyrazole,[10] pyridine;[11] biheterocycles—purine,[12–15] [1,3,5]triazine-pyridine,[16] 1,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazole,[17] pyrazolo[3,4-*c*]pyridazine,[18] triazolo[1,5-a]pyrimidine,[19] pyrazolo[1,5a]pyrimidine;[20] triheterocycles—benzodipyrazole,[21] aminoimidazo[1,2a]pyridine.[22] All the inhibitors are competitive with ATP, and vary in their potency and selectivity among the other members of the CDK family and also other unrelated kinases.

The biological effects of inhibition of CDK2 have been studied using molecular genetic approaches in cell lines and knockout animals. Experiments in cell lines have produced conflicting results, suggesting that CDK2 is not essential for proliferation in all situations.[23,24] Additionally, CDK2-knockout mice have been generated which are viable and apparently normal, other than being infertile.[25,26] These results cast doubt over the inhibition of CDK2 as a valuable therapeutic target. However, it should be noted that the absence or attenuation of a protein through genetic knockdown or knockout does not exactly mirror the situation where a protein is present at its usual cellular concentration, but is inactivated through a small-molecule inhibitor. Furthermore, a number of studies to date have demonstrated anti-tumour activity in tumour models with CDK2 inhibitors that have significant activity against other CDKs, including CDK4, CDK7 and CDK9,[27–29] and the first generation of CDK inhibitors has entered clinical trials as anti-tumour agents.[30]

We have previously reported the purine NU6102 (**1**) as a highly potent and selective ATP-competitive inhibitor of CDK2 (K_i = 6 nM), developed from the lead compound O^6-cyclohexylmethoxyguanine NU2058 (CDK2 K_i = 12 μM).[31,32] The X-ray crystal structure of **1** bound to CDK2 shows the purine core

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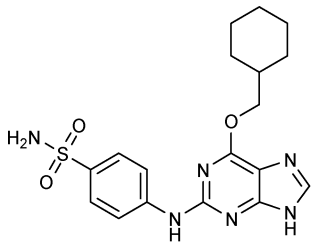
making a triplet of hydrogen bonds within the ATP binding site and two hydrogen bonds between the sulfonamide group and Asp86 of CDK2. We have also demonstrated previously that a series of nitrosopyrimidines are CDK2 inhibitors, *e.g.* NU6027 (**2**; CDK2 K_i = 1.3 μM). The nitroso group induces a 'purine-mimetic' conformation by hydrogen bonding to the adjacent NH_2 group. The X-ray structure of **2** in complex with CDK2 shows the pyrimidine binding in a similar orientation to NU2058 and **1**.[12] A limited series of analogues of **2** that explored similar structural modifications to those leading to the development of **1** has been communicated.[8] In this paper we report the synthesis of an expanded range of 2-arylamino-4-cyclohexylmethyl-5-nitroso-6-aminopyrimidines, based on **2**. The structure–activity relationships for the inhibition of CDK2 are discussed with reference to an X-ray crystal structure of a key inhibitor bound to the CDK2/cyclinA protein complex.



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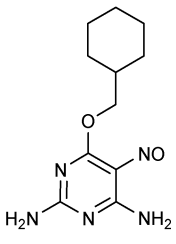

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Results and discussion

Chemical synthesis

The initial route to the target 2,4-disubstituted pyrimidines used 2,4,6-trichloropyrimidine as the starting material. Substitution with bis-*p*-methoxybenzylamine in refluxing ethanol, gave both the 2- and 4-substituted pyrimidines (**3a** and **3b**), which were separable by chromatography (Scheme 1). Unambiguous structure determination for **3a** and **3b** was not possible from their ^{1}H and ^{13}C NMR spectra, so an X-ray crystal structure was obtained for **3a** that confirmed it as the desired isomer (Fig. 1). A second substitution of the 2-substituted pyrimidine **3a** with *p*-methoxyaniline was achieved under more forcing conditions, in refluxing *n*-butanol with triethylamine as base, giving the 2,4-disubstituted pyrimidine **4a**, accompanied by the 2,6-disubstituted product **4b**, in a 2 : 3 ratio. Again, X-ray crystallography was used to unambiguously assign the structure of **4a** (Fig. 1). The final substitution was carried out using sodium cyclohexylmethoxide in cyclohexylmethanol, giving the trisubstituted pyrimidine **5**. Removal of the *p*-methoxybenzyl protecting groups with TFA, followed by nitrosation, gave 6-amino-2-anilino-4-cyclohexylmethoxy-5-nitrosopyrimidine (**7a**). An attempt to follow the same reaction scheme using 4-amino-*N*,*N*-dimethylbenzenesulfonamide, however, met with failure. The lack of regioselectivity in the initial substitution, and the formation of unwanted substitution products in the second step of Scheme 1, rendered this route unsuitable for the synthesis of a series of analogues.

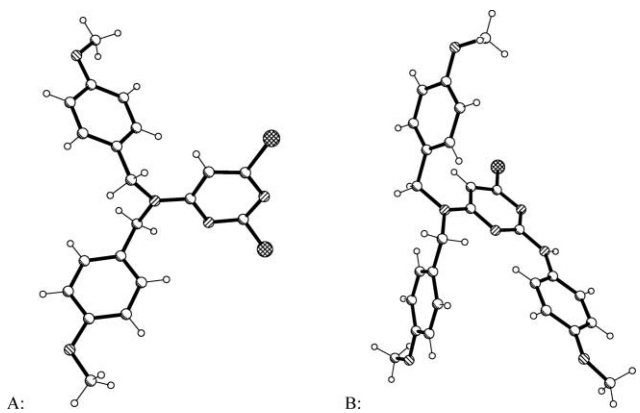


Fig. 1 Molecular structures of A: **3a**; B: **4a** from X-ray crystallography.

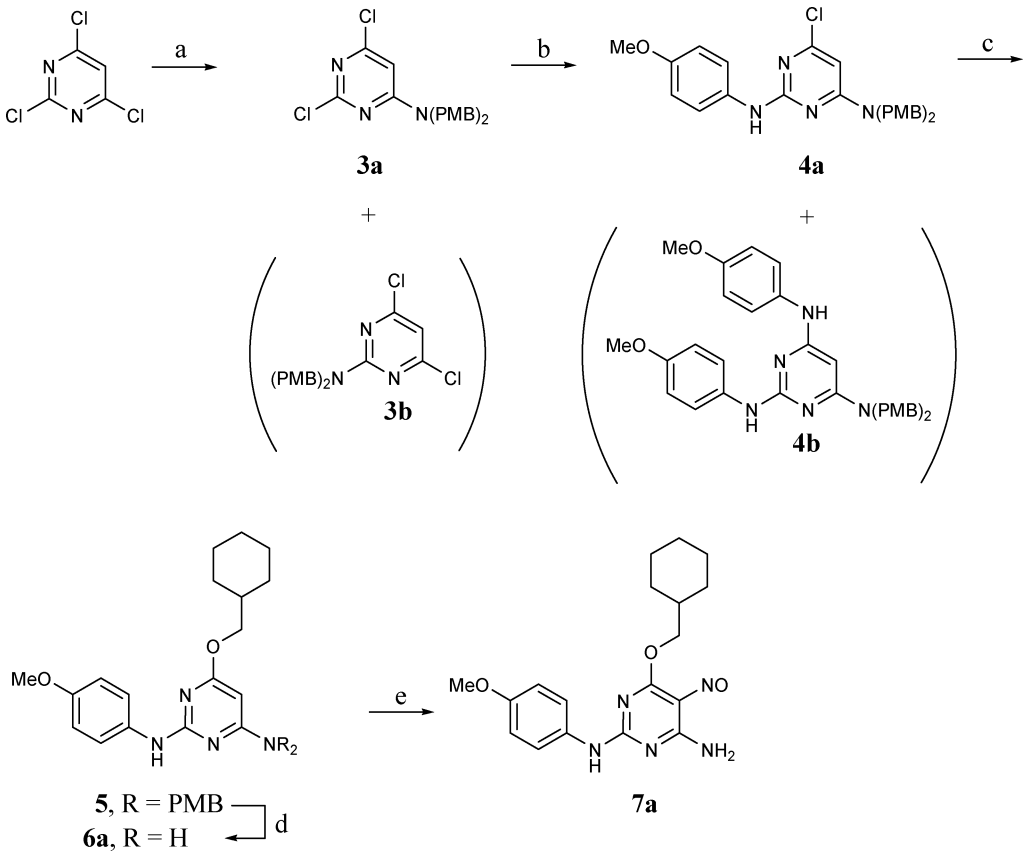
We considered that a more efficient synthesis could be achieved from 6-amino-2-mercaptopyrimidin-4-ol, which would allow the introduction of the anilino and cyclohexylmethoxy substituents and avoid the complications of regioselectivity seen in Scheme 1. The mercaptopyrimidine was alkylated to give the *n*-butyl sulfide (**8**) in excellent yield (Scheme 2), and then the cyclohexylmethoxy group was introduced under Mitsonubu conditions to give **9** in good yield. Direct displacement of the butylsulfide group from **9** with anilines proved unsuccessful, so the leaving group ability was improved by oxidation to the sulfone (**10**) using *m*CPBA, prior to displacement with the appropriate anilines to give the 2-arylaminopyrimidines **6b–s**. The optimum conditions for the sulfone displacement were found to be with trifluoroethanol as solvent and five equivalents of TFA as catalyst, as described previously.[33] Under these conditions, the displacements with various anilines proceeded in moderate to good yields. For the final step, nitrosation under standard conditions gave the desired 5-nitrosopyrimidines (**7b–s**).[12]

SAR Discussion

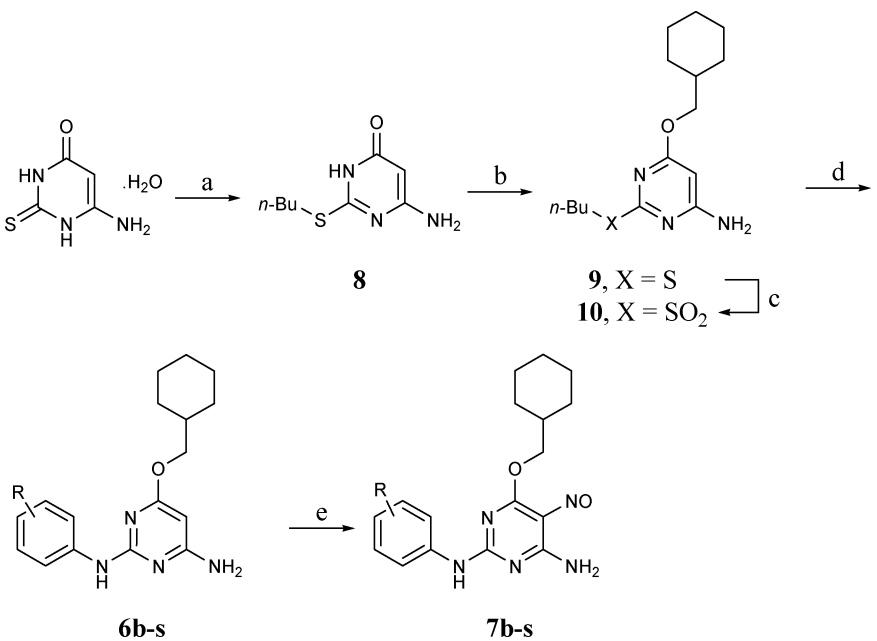
A series of 2-arylamino-5-nitrosopyrimidines was prepared and evaluated for CDK2 inhibitory activity and the results are shown in Table 1. Compounds lacking the 5-nitroso group (**6b**, **6e**, **6g**, **6h**, and **6j**) are at least 1×10^3 times less potent as CDK2 inhibitors compared with their 5-nitroso counterparts (**7b**, **7e**, **7g**, **7h**, and **7j**). These results are consistent with previous findings which showed that the 5-nitroso group forms an intramolecular hydrogen bond with the 6-amino group and orientates one of the amino NH bonds correctly to interact with the backbone carbonyl of Glu 81 of CDK2.[12,34]

As predicted from the results in the comparable purine series,[32] the nature and position of the substituent on the N^2-aryl moiety has a profound effect on the CDK inhibitory activity. Small-lipophilic substituents at the 3-position (**7b**, **7d**, **7e**) produced modest improvements in activity compared with the parent compound (**2**, IC_{50} = 2.2 ± 0.6 μM for CDK2), whereas polar substituents attached by a methylene group (**7g** and **7i**) resulted in up to 60-fold improvements in potency. In line with the purine series, the introduction of polar substituents at the 4-aryl position proved favourable. Compounds bearing 4-hydroxy or 4-carboxamido substituents (**7c** and **7j**) showed a 100-fold improvement in activity, whereas the 4-sulfonamido substituent **7m** gave a 2000-fold improvement in activity over the parent **2**.

We have previously reported the X-ray crystal structure of carboxamide **7j** bound to the CDK2/cyclin A phosphorylated on Thr160 (T160pCDK2/cyclin A) (Fig. 2, PDB accession code 1OGU).[8] Compounds **7j** and **2** bind in a similar orientation within

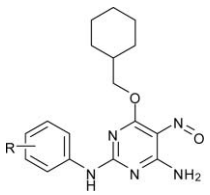


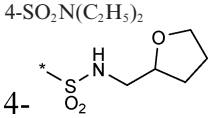
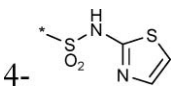
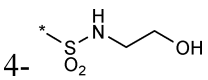
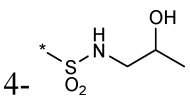
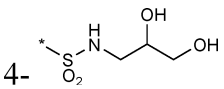
Scheme 1 *Reagents and conditions*: (a) bis-(4-methoxybenzyl)amine, Et_3N, *n*-BuOH, 75 °C; (b) *p*-anisidine, Et_3N, *n*-BuOH, DMSO, 95 °C; (c) cyclohexylmethanol, Na, 170 °C; (d) TFA, 60 °C; (e) AcOH, H_2O, $NaNO_2$.



Scheme 2 *Reagents and conditions*: (a) EtOH, NaOH, *n*-BuBr; (b) cyclohexylmethanol, PPh_3, DEAD, THF, 0 °C; (c) *m*CPBA, DCM; (d) appropriate aniline, TFE, TFA, Δ; (e) AcOH, H_2O, $NaNO_2$.

Table 1 Inhibition of CDK2 by 4-cyclohexylmethoxypyrimidines



Compound	R	X	CDK2 IC_{50} values (nM) or % inhibition at stated concentration
2[a]	—	NO	2.2 ± 0.6
7a[b]	4-OMe	NO	215 ± 50
6b	3-Br	H	26 000 ± 6000
7b[b]	3-Br	NO	500 ± 200
7c	4-OH	NO	16 ± 3
7d	3-OMe	NO	340 ± 70
6e	3-SMe	H	60 000 ± 5000
7e	3-SMe	NO	400 ± 100
7f[b]	4-SMe	NO	120 ± 30
6g	3-CH_2CN	H	41 ± 4% (10 μM)
7g	3-CH_2CN	NO	33 ± 3
6h	4-CH_2CN	H	24 000 ± 6000
7h	4-CH_2CN	NO	64 ± 3
7i	3-CH_2OH	NO	45 ± 21
6j[b]	4-$CONH_2$	H	59 000 ± 31 000
7j[b]	4-$CONH_2$	NO	34 ± 8
6k	4-$CON(CH_3)_2$	H	49% (100 μM)
7k	4-$CON(CH_3)_2$	NO	80 ± 10
6l	4-$CON(C_2H_5)_2$	H	29 ± 9% (10 μM)
7l	4-$CON(C_2H_5)_2$	NO	200 ± 100
6m[b]	4-SO_2NH_2	H	2930 ± 400
7m[b]	4-SO_2NH_2	NO	1.0 ± 0.3
6n	4-$SO_2N(C_2H_5)_2$	H	33 ± 9% (10 μM)
7n	4-$SO_2N(C_2H_5)_2$	NO	86 ± 8
7o		NO	8.1 ± 0.25
7p		NO	19 ± 1
7q		NO	0.7 ± 0.1
7r		NO	1.3 ± 0.2
7s		NO	0.8 ± 0.0

[a] Ref. 12. [b] Ref. 8.

the CDK2 ATP binding site. Compound **7j** forms three hydrogen bonds with the hinge region of the enzyme, namely N6 to the peptide carbonyl of Glu81, and N1 and N2 to the backbone amide and carbonyl groups of Leu83, respectively. The 5-nitroso group forms the anticipated intramolecular hydrogen bond to N6, locking the molecule into a purine-like conformation. The N2 aryl ring stacks against the peptide backbone between His84 and Gln85. The orientation of the carboxamide substituent could not be determined unambiguously. However, the formation of an additional hydrogen bond from a carboxamide NH to the side chain of Asp86 is a reasonable interpretation of the data and is consistent both with the SARs and the observed structure of the sulfonamide **2**. The interaction of at least one carboxamide or sulfonamide NH with CDK2 can be inferred from the loss of activity observed for the dialkylcarboxamides (**7k** and **7l**) and the diethylsulfonamide (**7n**), consistent with the structural data for **7j**.

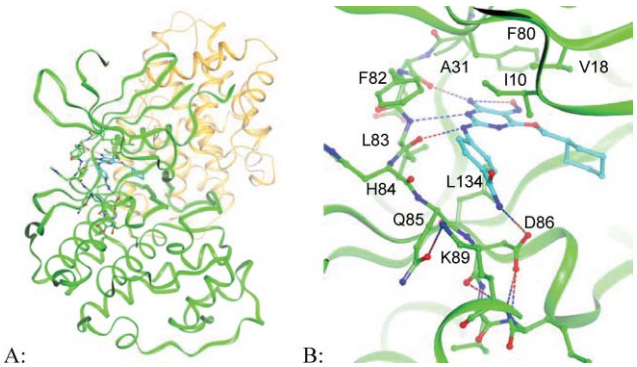
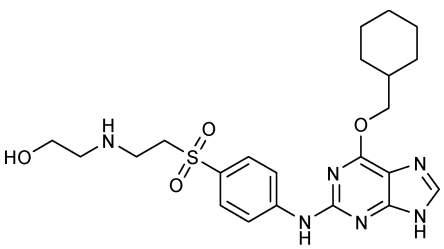


Fig. 2 A: Crystal structure of **7j** bound to Thr160pCDK2/cyclin A, overall fold. CDK2 and cyclin A are rendered in green and gold ribbons, respectively. Compound **7j** occupies the ATP binding site and is drawn in ball and stick mode with carbon, nitrogen, and oxygen atoms coloured green, red, and blue, respectively. B: Compound **7j** bound to the CDK2 active site. The side chains of selected residues that line the CDK2 active site are included. Hydrogen bonds between **7j** and CDK2 are drawn as dashed lines.

The X-ray crystal structure of the T160pCDK2/cyclin A/purine **2** complex shows that only one of the sulfonamide protons makes a hydrogen bond to Asp68, suggesting that substitution of the other position may be favourable.[32] It was anticipated that the pyrimidines would behave similarly. The monoalkylsulfonamides (**7o–s**) retained potent activity, in particular against CDK2. The 2-hydroxyethyl- and 2,3-dihydroxypropylsulfonamides (**7q** and **7s**) displayed a modest improvement in activity compared with the parent **7m**, and are amongst the most potent CDK2 inhibitors reported to date. The improvement in activity suggests that additional interactions may be formed between the additional hydroxyl group(s) and the enzyme. The formation of additional favourable hydrogen bonding interactions, in the region accessed by the sulfonamide substituents, has been observed in the purine series. For example, the X-ray structure of sulfoxide **8** bound to CDK2 shows the amine and hydroxyl groups bound to the side chain carboxyl group of Asp86.[35]



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The CDK selectivity of two of the most potent CDK2 inhibitors, the sulfonamide **7m** and the hydroxyethylsulfonamide **7q**, was evaluated using a panel of CDK1/cyclinB, CDK4/cyclinD, CDK7/cyclinH, and CDK9/cyclinT (Table 2). Both inhibitors showed excellent selectivity for CDK2, with around 100-fold selectivity observed against CDK1/cyclinB, and around 1000-fold selectivity against the other CDK/cyclin complexes.

Conclusions

Synthesis of 2-substituted O^4-cyclohexylmethyl-5-nitroso-6-aminopyrimidines from 2,4,6-trichloropyrimidine required the separation of mixtures of regioisomers at the first two steps and was not amenable to the introduction of a range of substituents. The improved route, from 6-amino-2-mercaptopyrimidin-4-ol,[8] has allowed the preparation of a range of derivatives for evaluation as inhibitors of CDK2. The structure activity relationships observed follow similar trends to those for the corresponding O^6-cyclohexylmethoxypurine series, with the 2-arylsulfonamide and 2-arylcarboxamide derivatives showing excellent potency. Two compounds, 4-(6-amino-4-cyclohexylmethoxy-5-nitrosopyrimidin-2-ylamino)-*N*-(2-hydroxyethyl)benzenesulfonamide (**7q**) and 4-(6-amino-4-cyclohexylmethoxy-5-nitrosopyrimidin-2-ylamino)-*N*-(2,3-dihydroxypropyl)benzenesulfonamide (**7s**), showed excellent potency against CDK2, with IC_{50} values of 0.7 ± 0.1 and 0.8 ± 0.0 nM, respectively. Excellent selectivity within the CDK family was found for **7m** and **7q**. These compounds are among the most potent and selective CDK2 inhibitors reported to date.

Experimental

Reagents were purchased from fine chemical vendors, and used as received unless otherwise stated. Solvents were purified and stored according to standard procedures. Petrol refers to that fraction in the boiling range 40–60 °C. Melting points were obtained on a Stuart Scientific SMP3 apparatus and are uncorrected. Thin layer chromatography was performed using silica gel plates (Kieselgel $60F_{254}$; 0.2 mm), and visualized with UV light or potassium permanganate. Chromatography was conducted under medium pressure on silica (BDH silica gel 40–63 μm). Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 spectrometer at 300 MHz or 75 MHz, repectively, employing TMS or the solvent as internal standard. NH signals appeared as broad singlets (br s) exchangeable with D_2O. Mass spectra were determined on a Micromass Autospec M spectrometer in electron impact (EI) mode. Liquid Chromatography-Mass Spectrometry (LCMS) was carried out on a Micromass Platform instrument operating in positive and negative ion electrospray mode, employing a 50 × 4.6 mm C18 column (Supelco Discovery or Waters Symmetry) and a 15 min gradient elution of 0.05% formic acid and methanol (10–90%). IR spectra were recorded on a Bio-Rad FTS 3000MX diamond ATR. Elemental analyses were performed by Butterworth

Table 2 Selectivity of inhibition of CDKs by **7m** and **7q**

	IC_{50} values (nM)				
Compound	CDK1/B	CDK2/A	CDK4/D	CDK7/H	CDK9/T
7m	100 ± 10	1 ± 0.3	1500 ± 500	2800 ± 1300	740 ± 100
7q	56 ± 20	0.7 ± 0.1	1300 ± 500	4800 ± 2600	2630 ± 200

Laboratories, Middlesex, UK. High-resolution mass spectra were recorded at the EPSRC Mass Spectrometry Service, Swansea, UK.

2,6-Dichloro-*N*,*N*-bis(4-methoxybenzyl)pyrimidin-4-amine (3a)

A mixture of 2,4,6-trichloropyrimidine (2.2 mL, 19 mmol) and bis-(4-methoxybenzyl)amine (4.8 g, 19 mmol) and triethylamine (3.2 mL, 22.7 mmol) in *n*-butanol (20 mL) was heated to 75 °C for 3 h, then allowed to cool and concentrated *in vacuo.* Chromatography (silica; 10% ethyl acetate, petrol) gave **3a** as a white solid (2.5 g, 33%) mp 129–131 °C; υ_{max}/cm^{-1} 3000, 2838 1610, 1568, 1487, 1127. δ_H (300 MHz, d_6-DMSO) 3.77 (3H, s, OCH_3), 4.64 (2H, br s, NCH_2), 4.82 (2H, br s, NCH_2), 6.89 (1H, s, H^5), 6.94 (4H, d, $J = 8.5$ Hz, ArH), 6.94 (4H, d, ArH), 7.17 (2H, br s, ArH), 7.26 (2H, br s, ArH); δ_C (125 MHz, d_6-DMSO; DEPT) 50.58 (sp^2), 55.82 (sp^3), 101.59 (sp), 114.78 (sp), 128.87 (sp), 129.94 (sp), 159.18 (q), 159.42 (q), 159.74 (q), 164.28 (q). *m*/*z* (ESI^+) = 404 $[M + H]^+$. HRMS (EI) *m*/*z* Calcd for $C_{20}H_{19}Cl_2N_3O_2$: 404.0927 $[M + H]^+$. Found 404.0932 $[M + H]^+$. $C_{20}H_{19}Cl_2N_3O_2$ requires C, 59.42; H, 4.74; N, 10.39; found C, 59.41; H, 4.73 N, 10.33%.

6-Chloro-N^4,N^4-bis(4-methoxybenzyl)-N^2-(4-methoxyphenyl)pyrimidine-2,4-diamine (4)

A mixture of **3a** (1.2 g, 2.9 mmol), *p*-anisidine (0.44 g, 3.6 mmol), anhydrous triethylamine (0.50 mL, 3.6 mmol), *n*-butanol (8 mL) and anhydrous DMSO (2 mL) was heated to 95 °C for 3 d, then allowed to cool and concentrated *in vacuo.* Water (50 mL) was added and the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried ($NaSO_4$) and concentrated *in vacuo.* Chromatography (silica; 30% ethyl acetate, petrol) gave **4** as a red solid (0.43 g, 30%) mp 142–145 °C. υ_{max}/cm^{-1}: 3263, 3110, 2954-2930, 2831, 1605, 1553, 1500, 1224. λ_{max} (EtOH): 274, 227 nm. δ_H (300 MHz, $CDCl_3$): 3.66 (6H, s, 2 × OCH_3), 3.69 (3H, s, OCH_3), 4.52 (4H, br, 2 × N–CH_2–), 5.89 (1H, s, CH), 6.66 (2H, d, $J = 8.9$ Hz), 6. 76 (4H, d, $J = 8.6$ Hz), 7.01 (4H, d, $J = 8.6$ Hz), 7.28 (2H, d, $J = 8.9$ Hz) ppm. δ_C (75 MHz, $CDCl_3$): 50.9 (N–CH_2), 55.6 (OCH_3), 55.8(OCH_3), 93.2, 114.5, 114.7, 122.4, 128.8, 129.3, 133.1, 156.0, 159.6, 159.8, 160.5, 164.4 ppm. *m*/*z* (ESI^+) 491.24 $[M + H]^+$. HRMS (EI) *m*/*z* Calcd for $C_{27}H_{17}ClN_4O_3$: 491.1844 $[M + H]^+$. Found 491.1841 $[M + H]^+$. $C_{27}H_{27}ClN_4O_3$ requires C, 66.05; H, 5.54; N, 11.41; found C, 65.83; H, 5.57; N, 11.34%.

N^4,N^4-bis(4-methoxybenzyl)-N^2,N^6-bis(4-methoxyphenyl)pyrimidine-2,4,6-triamine

(0.64 g, 45%) mp 130–133 °C. υ_{max}/cm^{-1}: 3330 (NH), 3132, 2992 (CH arom), 2897 (CH_2), 2835 (OCH), 1579, 1537 (C=C, C=N), 1502 (NR_3), 1230 (CH_30). λ_{max} (EtOH): 275, 227 nm. δ_H (300 MHz, $CDCl_3$): 3.60 (3H, s, OCH_3), 3.63(3H, s, OCH_3), 3.65 (6H, s, 2 × OCH_3), 4.46 (4H, br, 2 × N–CH_2–), 5.13 (1H, s, CH), 6.60–6.66 (4H, dd, $J = 9.1$. Hz), 6. 71 (4H, d, $J = 8.6$ Hz), 6,85 (2H, d, $J = 8.9$), 6.98 (4H, d, $J = 8.6$ Hz), 7.28 (2H, d, $J = 9.0$ Hz) ppm. δ_C (75 MHz, $CDCl_3$): 50.7 (N–CH_2), 55.7, 55.8, 55.9 (OCH_3), 75.9, 114.4, 114.5, 114.9, 121.8, 124.9, 128.9, 130.7, 132.9, 134.3, 155.3, 156.9, 159.3, 159.9, 162.8, 164.3 ppm. *m*/*z* (ESI^+) 578.34 $[M + H]^+$.

6-(Cyclohexylmethoxy)-N^4,N^4-bis(4-methoxybenzyl)-N^2-(4-methoxyphenyl)pyrimidine-2,4-diamine (5)

Sodium (0.036 g, 1.55 mmol) was heated in cyclohexylmethanol (1.5 mL) at 120 °C under N_2 for 1 h. **4** (0.38 g, 0.77 mmol) was added and the mixture heated at 160 °C for 3 h, then allowed to cool, diluted with petrol (60 mL), and washed with water (3 × 30 mL). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo.* Chromatography (silica; 10% ethyl acetate, petrol) gave **5** as a pale yellow oil containing residual cyclohexylmethanol, which was used without further purification. (0.88 g). δ_H (300 MHz, d_6-DMSO) 0.84–1.38 (m, C_6H_{11}), 1.68–1.77 (m, C_6H_{11}), 3.71 (3H, s, OCH_3), 3.76 (6H, s, 2 × OCH_3), 4.03 (2H, d, $J = 6.7$ Hz, OCH_2), 4.66 (4H, br s, 2 × NCH_2), 5.34 (1H, s, H^5), 6.77 (2H, d, $J = 9.0$ Hz, ArH), 6.92 (4H, d, $J = 8.5$ Hz, ArH), 7.19 (4H, d, $J = 8.0$ Hz, ArH), 7.58 (2H, d, $J = 9.0$ Hz, ArH), 8.86 (1H, s, D_2O ex, NH). *m*/*z* (ESI^+) = 569 $[M + H]^+$.

6-(Cyclohexylmethoxy)-N^2-(4-methoxyphenyl)pyrimidine-2,4-diamine (6a)

A solution of **5** (0.44 g, corrected to 0.2 mmol) in trifluoroacetic acid (5 mL) was heated to 60 °C for 5 h, then allowed to cool, concentrated *in vacuo*, then diluted with water (20 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo.* Chromatography (silica; 30% ethyl acetate, petrol) gave **6a** as a pale brown solid (0.06 g, 91%) mp 102–105 °C. UV λ_{max} (EtOH): 276, 205 nm. υ_{max}/cm^{-1}: 3341, 3221, 3070, 2966, 2823, 1520, 1496, 1219. δ_H (300 MHz, $CDCl_3$): 0.96–1.19 (5H, m, C_6H_{11}), 1.57–1.68 (6H, m, C_6H_{11}), 3.66 (3H, s, OCH_3), 3.93 (2 H, d, $J = 6.3$ Hz), 4.84 (2H, br, NH_2), 5.19 (1H, s, CH), 6.72 (2H, d, $J = 9.3$ Hz), 7.38 (2H, d, $J = 9.3$ Hz) ppm. δ_C (75 MHz, $CDCl_3$): 26.1, 26.8, 30.1, 37.8, 55.8, 71.6, 79.2, 114.3, 121.7, 133.7, 155.6, 159.9, 165.3, 171.7 ppm. *m*/*z* (ESI^+) 329.28 $[M + H]^+$. HRMS (EI) *m*/*z* calcd for $C_{18}H_{24}N_4O_2$: 329.1972 $[M + H]^+$; found 329.1973 $[M + H]^+$.

6-(Cyclohexylmethoxy)-N^2-(4-methoxyphenyl)-5-nitrosopyrimidine-2,4-diamine (7a)

To a solution of **6a** (0.05 g, 0.15 mmol) in 30% acetic acid and water (5 mL) at 80 °C was added sodium nitrite (0.014 g, 0.2 mmol) in water (0.2 mL) giving a brown precipitate. Heating was continued for 2 h then the mixture was allowed to cool to room temperature, and concentrated *in vacuo.* The residues were dissolved in ethyl acetate (40 mL) and washed with Na_2CO_3 solution (3 × 20 mL) and water (2 × 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo.* Chromatography (silica; 5% methanol, ethyl acetate) followed by HPLC (C_{18}, acetonitrile, water) gave **7a** as a dark green solid (0.044 g, 26%) mp 168–171 °C. υ_{max}/cm^{-1}: 3288, 3070, 2923, 2851, 1557, 1496, 1446, 1240. λ_{max} (EtOH): 368, 292, 244 nm. δ_H (300 MHz, d_6-acetone) 0.74–1.79 (11H, m, C_6H_{11}), 3.67 (3H, s, OCH_3), 4.24 (2H, s, OCH_2), 6.76 (2H, br s, ArH), 7.32 (1H, br s, D_2O ex, NH), 7.66(2H, br s, ArH), 9.07 (1H, s, D_2O ex, NH), 10.33 (1H, s, D_2O ex, NH). *m*/*z* (ESI^+) = 358 $[M + H]^+$. δ_C (75 MHz, DMSO): 25.4, 26.3, 29.5, 37.2, 55.7, 72.3, 114.2, 123.5, 131.9, 140.4, 150.3, 156.5, 160.2, 171.6 ppm. *m*/*z* (ESI^+) 358.26 $[M + H]^+$. HRMS (ESI^+) *m*/*z* calcd for $C_{18}H_{23}N_5O_3$: 358.1874 $[M + H]^+$; found 358.1878 $[M + H]^+$.

$C_{18}H_{23}N_5O_3$ requires C, 60.49; H, 6.49; N, 19.59; found C, 60.87; H, 6.49, N, 18.95%

6-Amino-2-*n*-butylsulfanyl-*H*^{3}-pyrimidin-4-one (8)

6-Amino-2-mercaptopyrimidin-4-ol monohydrate (5.0 g, 31 mmol) was slurried in EtOH (30 mL) at 50 °C, and treated with NaOH (3.25 M, 10 mL, 32.1 mmol) and the mixture stirred for 30 min. 1-Bromobutane (3.45 mL, 32.1 mmol) was added dropwise to the slurry and stirring continued for 18 h. Water (10 mL) was added and the mixture stirred for 30 min. After cooling to room temperature, the mixture was concentrated *in vacuo* giving **8** as an off-white solid (5.70 g, 92%): mp 183–186 °C, lit. 175 °C[36]; ν_{max}/cm^{-1} 1570, (C=N), 1601 (C=C), 2858–2928 (CH_3, CH_2), 3271, 3464 (OH, NH_2). δ_H (300 MHz, d_6-DMSO) 0.89 (3H, t, J = 7 Hz, CH_3), 1.31–1.43 (2H, m, CH_3CH_2), 1.53–1.63 (2H, m, $CH_3CH_2CH_2$), 3.06 (2H, t, J = 7 Hz, SCH_2), 4.87 (1H, s, H5), 6.43 (2H, br s, NH_2, exchangeable with D_2O); δ_C (125 MHz, d_6-DMSO) 13.5 (CH_3), 21.3 (CH_3CH_2), 29.0 ($CH_3CH_2CH_2CH_2S$), 31.0 ($CH_3CH_2CH_2CH_2S$), 81.1 (C5), 163.5 (C6). m/z (ESI$^+$) = 200 [M + H]$^+$.

2-*n*-Butylsulfanyl-4-cyclohexylmethoxypyrimidin-6-ylamine (9)

To a mixture of **8** (1.50 g, 7.53 mmol), cyclohexylmethanol (1.39 mL, 11.30 mmol), and PPh_3 (2.96 g, 11.30 mmol) in THF (50 mL) was added DEAD (1.78 mL, 11.30) dropwise over 20 min at 0 °C, and stirring continued for 24 h. The mixture was concentrated *in vacuo* yielding a yellow oil which was stirred in diethyl ether at 0 °C, forming a white precipitate, which was removed by filtration. The filtrate was collected and concentrated *in vacuo*. Chromatography (silica; 20% ethyl acetate, petrol) followed by recrystallisation (MeOH) gave **9** as a white solid (1.60 g, 72%): mp 79–82 °C; ν_{max}/cm^{-1} 1547, 1583 (C=N), 1631 (C=C), 2850–3156 (CH_3, CH_2), 3294–3425 (NH_2). δ_H (200 MHz, d_6-DMSO) 1.08 (3H, t, J = 7 Hz, CH_3), 1.22–1.62 (9H, m, C_6H_{11} + $CH_3CH_2CH_2$), (6H, m, C_6H_{11}), 3.12 (2H, t, J = 7 Hz, SCH_2), 4.18 (2H, d, J = 6 Hz, OCH_2), 5.57 (1H, s, H5), 6.80 (2H, br s, NH_2, exchangeable with D_2O) ppm; δ_C (50 MHz, d_6-DMSO) 13.5 (CH_3), 21.5 (CH_3CH_2), 25.5 (C_6H_{11}), 26.3 (C_6H_{11}), 29.3 (C_6H_{11}), 31.6 (C_6H_{11}), 32.1 ($CH_3CH_2CH_2CH_2S$), 36.89 ($CH_3CH_2CH_2CH_2S$), 66.6, 70.3 (OCH_2), 81.57 (C5), 165.14 (C2), 168.79 (C6), 169.17 (C4) ppm. m/z (ESI$^+$) = 296 [M + H]$^+$. $C_{15}H_{25}N_3OS$ requires: C, 60.98; H, 8.53; N, 14.22; S, 10.85%; found: C, 61.01; H, 8.52; N, 13.85; S, 10.42%.

2-(*n*-Butane-1-sulfonyl)-4-cyclohexylmethoxypyrimidin-6-ylamine (10)

To a stirred solution of **9** (1.0 g, 3.38 mmol) in DCM (20 mL) was added *m*CPBA (2.34 g, 13.5 mmol) and stirring continued for 17 h. The mixture was concentrated *in vacuo* giving a yellow solid which was extracted into ethyl acetate (2 × 20 mL). The combined extracts were washed with saturated sodium sulfite solution (30 mL) and aqueous $NaHCO_3$ solution (30 mL), then dried (Na_2SO_4) and concentrated *in vacuo* yielding **10** as an off-white solid (0.99 g, 89%). Recrystallisation (ethyl acetate, petrol) gave a white solid: mp 141–143 °C; ν_{max}/cm^{-1} 1130 (SO_2 sym. str.), 1300 (SO_2 asym. str.) 1595 (C=N), 1635 (C=C), 2852–3212 (CH_3, CH_2), 3323, 3424 (NH_2). δ_H (300 MHz, $CDCl_3$) 0.89 (3H, t, J = 7 Hz, CH_3), 1.15–1.18 (5H, m, C_6H_{11}), 1.41 (2H, q, J = 7, 15 Hz, $CH_3CH_2CH_2$), 1.66–1.79 (8H, m, C_6H_{11} + $CH_3CH_2CH_2$), 3.32–3.38 (2H, m, SO_2CH_2), 4.05 (2H, d, J = 6 Hz, OCH_2), 5.48 (2H, br s, NH_2, exchangeable with D_2O), 5.75 (1H, s, H5) ppm; δ_C (75 MHz, $CDCl_3$) 14.0 (CH_3), 22.2 (CH_3CH_2), 24.5 ($CH_3CH_2CH_2$), 26.7 (C_6H_{11}), 27.0 (C_6H_{11}), 30.1 (C_6H_{11}), 37.7 (C_6H_{11}), 51.1 (CH_2SO_2), 73.0 (OCH_2), 89.1 (C5), 164.7 (C2), 165.5 (C6), 171.2 (C4). m/z (ESI$^+$) = 328 [M + H]$^+$. $C_{15}H_{25}N_3O_3S$ requires C, 55.02; H, 7.70; N, 12.83; found C, 54.88; H, 7.71; N, 12.63%.

General Procedure A

To a solution of **10** (0.20 g, 0.61 mmol) and the appropriate aniline (2 mol. eq.) in TFE (4 mL) was added TFA (0.24 mL, 3.05 mmol). The mixture was stirred for 10 min at room temperature, then heated under reflux for a further 2 h, and concentrated *in vacuo* yielding a white solid. The solid was extracted into ethyl acetate (2 × 20 mL). The combined extracts were washed with copious amounts of water (100 mL), then dried (Na_2SO_4), and concentrated *in vacuo*.

4-(6-Amino-4-cyclohexylmethoxypyrimidin-2-ylamino)-benzenesulfonamide (6m)

General Procedure A: 10 (0.30 g, 0.92 mmol), 4-aminobenzenesulfonamide (0.32 g, 1.84 mmol), TFE (3 mL), TFA (0.38 mL, 4.60 mmol). HPLC (C18; methanol, water) gave **6m** as a white solid (0.24 g, 69%): mp 177–179 °C; ν_{max}/cm^{-1} 1564 (C=C, C=N str.), 1620 (SO_2NH_2, NH_2 def.), 2853–2925 (CH_2), 3104–3216 (=C–H str.), 3346 (asym + sym N–H str.), 3471 (NH_2); λ_{max} = 210 and 296 nm. δ_H (300 MHz d_6-DMSO) 0.98–1.26 (5H, m, C_6H_{11}), 1.68–1.78 (6H, m, C_6H_{11}), 4.01 (2H, d, J = 6 Hz, CH_2O), 5.29 (1H, s, H5), 6.43 (2H, s, NH_2 exchangeable with D_2O), 7.13 (2H, s, NH_2 exchangeable with D_2O), 7.63 (2H, d, J = 9 Hz, ArH), 7.93 (2H, d, J = 9 Hz, ArH), 9.34 (NH, exchangeable with D_2O) ppm. m/z (ESI$^+$) = 378 [M + H]$^+$. $C_{17}H_{23}N_5O_3S{\cdot}0.33CH_3OH$ requires: C, 53.54; H, 6.32; N, 18.04; found: C, 53.50; H, 6.20; N, 18.04%.

4-(6-Amino-4-cyclohexylmethoxypyrimidin-2-ylamino)-*N*-(2-hydroxyethyl)benzenesulfonamide (6q)

General Procedure A: 9 (0.11 g, 0.33 mmol), amino-*N*-(2-hydroxyethyl)benzenesulfonamide (0.14 g, 0.65 mmol) TFE (3 mL), TFA (0.13 mL, 1.63 mmol). HPLC (C18; methanol, water) gave **6q** as a white solid (0.09 g, 0.22 mmol, 66%). m/z (ESI$^+$) = 422 [M + H]$^+$.

General Procedure B: Nitrosation

The appropriate pyrimidine was dissolved in aqueous acetic acid (30%; 3 mL) and the solution was heated to 80 °C, then sodium nitrite (0.02 g, 0.35 mmol) in water (0.2 mL) was added dropwise. The mixture was stirred for a further 2 h, then extracted into ethyl acetate (30 mL). The extract was washed with water (30 mL), dried (Na_2SO_4) and concentrated *in vacuo*.

4-(6-Amino-4-cyclohexylmethoxy-5-nitrosopyrimidin-2-ylamino)-benzenesulfonamide (7m)

General Procedure B: 6m (0.08 g, 0.20 mmol). HPLC (C18; methanol, water) gave **7m** as a green solid (0.02 g, 25%): mp

159–162 °C; ν_{max}/cm^{-1} 1528 (N=O str.), 1633 (NH_2 def.), 2846–3040 (–CH_2–), 3280 (asym + sym N–H str.), 3364 (NH_2); λ_{max} = 362 nm. δ_H (300 MHz d_6-DMSO) 1.00–1.23 (5H, m, C_6H_{11}), 1.71–1.88 (6H, m, C_6H_{11}), 4.43 (2H, d, J = 6 Hz, CH_2O), 7.29 (2H, s, NH_2 exchangeable with D_2O), 7.73 (2H, d, J = 8 Hz, ArH), 8.00 (2H, s br, ArH), 8.60 (NH, exchangeable with D_2O), 10.25 (1H, s, NH, exchangeable with D_2O), 10.60 (NH, exchangeable with D_2O) ppm. m/z (ESI$^+$) = 407 [M + H]$^+$. $C_{17}H_{22}N_6O_4S{\cdot}0.5CH_3CO_2H$ requires C, 49.53; H, 5.54; N, 19.25; found: C, 49.44; H, 5.37; N, 19.53%.

4-(6-Amino-4-cyclohexylmethoxy-5-nitrosopyrimidin-2-ylamino)-*N*-(2-hydroxyethyl)benzenesulfonamide (7q)

General Procedure B: 6q (0.09 g, 0.22 mmol). Recrystallisation (MeOH) gave **7q** as a green solid: mp 193–195 °C; ν_{max}/cm^{-1} 1522 (N=O str.), 1576 (C=N, C=C), 2852–2926 (–CH_2–), 3100–3267 (NH_2, OH); λ_{max} = 362, 263, 239 and 208 nm. δ_H (300 MHz d_6-DMSO) 1.05–1.30 (5H, m, C_6H_{11}), 1.50–1.88 (6H, m, C_6H_{11}), 2.78 (2H, q, J = 6 Hz, $NHCH_2$), 3.36 (2H, t, CH_2OH), 4.39 (2H, d, J = 6 Hz, CH_2O), 4.68 (1H, t, OH exchangeable with D_2O), 7.40 (1H, t, $NHCH_2$ exchangeable with D_2O), 7.71 (2H, d, J = 8 Hz, ArH), 7.97 (2H, d, J = 8 Hz, ArH) 8.50 (1H, s, NH exchangeable with D_2O) ppm. m/z (ESI$^+$) = 451 [M + H]$^+$. $C_{19}H_{26}N_6O_5S{\cdot}0.5CH_3CO_2H$ requires C, 49.99; H, 5.87; N, 17.49; found: C, 49.67; H, 5.26; N, 17.60%.

Biological Evaluation

Compounds were assayed for the inhibition of human cyclin-dependent kinases 1 and 2, as described previously.[12] The final ATP concentration within the assay was 12.5 μM.

X-Ray Crystallography†

Crystals of **3a** and **4a** were small and weakly diffracting, and data were collected at 120 K with synchrotron radiation (λ = 0.8462 Å) at station 16.2SMX of the Synchrotron Radiation Source at Daresbury Laboratory, through the EPSRC National Crystallography Service. Crystal data for **3a**: $C_{20}H_{19}Cl_2N_3O_2$, M_r = 404.3, monoclinic, space group $P2_1/c$, a = 21.9143(10), b = 10.1032(5), c = 8.7088(4) Å, β = 98.099(1)°, U = 1908.94(16) Å^3, T = 120(2) K, Z = 4, μ = 0.36 mm^{-1}, 13 035 data measured, 3886 unique (R_{int} = 0.029), 246 refined parameters, R (F, $F^2 > 2\sigma$) = 0.038, R_w (F^2, all data) = 0.098, S = 1.03, final difference map within ± 0.29 e Å^{-3}. Crystal data for **4a**: $C_{27}H_{27}ClN_4O_3$, M_r = 491.0, monoclinic, space group $C2/c$, a = 16.9620(14), b = 10.7877(9), c = 27.228(2) Å, β = 98.375(1)°, U = 4929.0(7) Å^3, T = 120(2) K, Z = 8, μ = 0.19 mm^{-1}, 15 124 data measured, 5026 unique (R_{int} = 0.042), 323 refined parameters, R (F, $F^2 > 2\sigma$) = 0.045, R_w (F^2, all data) = 0.117, S = 1.06, final difference map within ± 0.31 e Å^{-3}.

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