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Structure-Based Design of 2-Arylamino-4-cyclohexylmethyl-5-nitroso-6-aminopyrimidine Inhibitors of Cyclin-Dependent Kinases 1 and 2

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Abstract—A series of O^4-cyclohexylmethyl-5-nitroso-6-aminopyrimidines bearing 2-arylamino substituents was synthesised and evaluated for CDK1 and CDK2 inhibitory activity. Consistent with analogous studies with O^6-cyclohexylmethylpurines, 2-arylaminopyrimidines with a sulfonamide or carboxamide group at the 4′-position were potent inhibitors, with IC_{50} values against CDK2 of 1.1 ± 0.3 and 34 ± 8 nM, respectively. The crystal structure of the 4′-carboxamide derivative, in complex with phospho-Thr160 CDK2/cyclin A, confirmed the expected binding mode of the inhibitor, and revealed an additional interaction between the carboxamide function and an aspartate residue.

Introduction

The cyclin-dependent kinase (CDK) family of enzymes play a pivotal role in the control of cell-cycle progression, particularly at cell-cycle checkpoints. Aberrant cell cycle control, arising from tumour suppressor gene malfunction or oncogene activation, is associated with increased CDK/cyclin activity in human tumours.[1,2] CDKs are thus recognised as important therapeutic targets in the treatment of cancer and non-neoplastic proliferative diseases, and inhibitors of CDKs have recently entered clinical trials as prospective antitumour agents.[3,4] Studies to date with small molecule CDK inhibitors, which invariably target the ATP-binding domain of the enzymes, have demonstrated single-agent antitumour activity in preclinical tumour models, as well as synergistic activity in combination with conventional cytotoxic treatments. However, currently available inhibitors are sub-optimal with regard to one or more of the following properties: selectivity for CDKs over other kinases, specificity for individual CDKs, and potency against tumour cells both in vitro and in vivo.[5–8] The challenge in the area of CDK inhibitor development is thus to identify agents with improved kinase-specificity, coupled with greater potency against tumour cell lines. We have previously reported the purine NU2058 (**1**) as an ATP-competitive CDK inhibitor that exhibits reasonable inhibitory activity against CDK1 ($K_i = 5$ μM) and CDK2 ($K_i = 12$ μM), but which is essentially inactive against CDK4.[9] Elucidation of the crystal structure of **1** in complex with both monomeric CDK2, and fully active CDK2-cyclin A, revealed a binding orientation distinct from that of ATP and the benchmark 6-aminopurine CDK inhibitors, including roscovitine and purvalanol. Structure–activity relationship (SAR) studies conducted with **1** have demonstrated that a six-membered alicyclic group at the purine O^6-position affords maximum inhibitory potency, whereas

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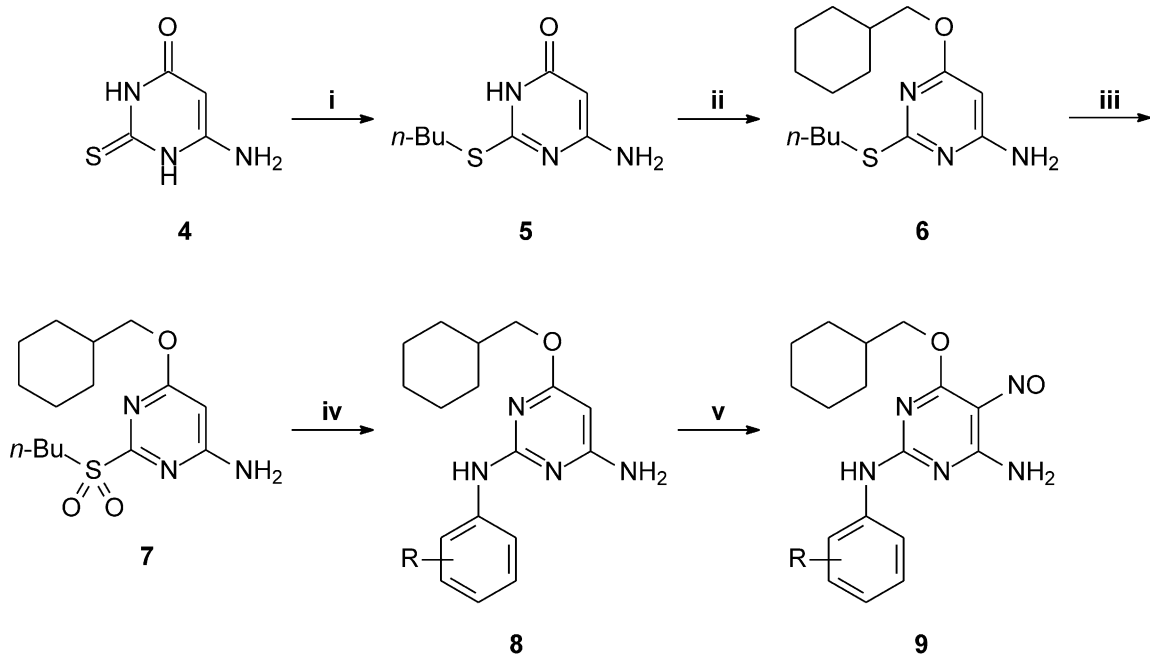


Scheme 1. General synthesis of N^2-arylaminopyrimidines. Reagents and conditions: (i) *n*-BuBr, NaOH, EtOH, 50 °C; (ii) $C_6H_{11}CH_2OH$, DIAD, PPh_3, THF, 25 °C; (iii) *m*-CPBA, DCM, 25 °C; (iv) $RC_6H_4NH_2$, TFE, TFA, reflux; (v) $NaNO_2$, AcOH (30% aq), 80 °C.

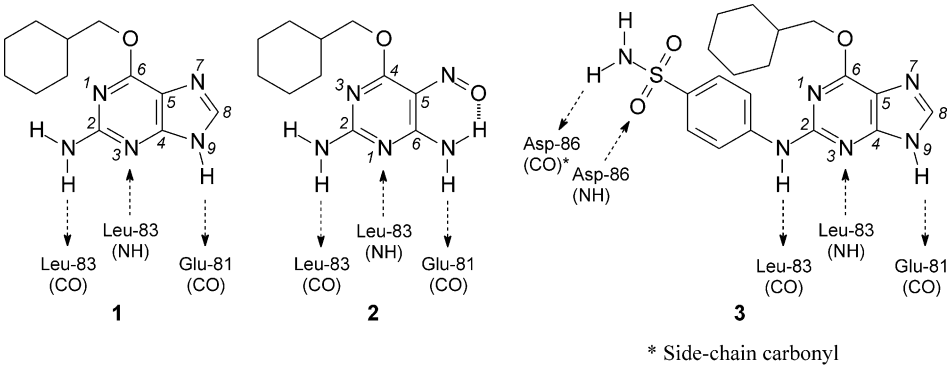


Figure 1. Structures of NU2058 (**1**), NU6027 (**2**) and NU6102 (**3**), indicating hydrogen bond interactions with the backbone of amino acid residues within the ATP-binding site of CDK2.

substitution at the *N*-9 position essentially abolishes activity.[10] Interestingly, the purine core structure is not a prerequisite for CDK inhibitory activity and comparable potency was observed with the corresponding 'purine-mimetic' nitrosopyrimidine derivative NU6027 (**2**; $K_i = 2.5$ and 1.3 μM for CDK1 and CDK2, respectively).[9] The crystal structure of **2** in complex with CDK2 showed that the binding mode of the pyrimidine was nearly identical to that of the purine **1**, both inhibitors making a triplet of hydrogen bond interactions within the ATP-binding site as represented in Figure 1. Consistent with this observation, SARs for the O^4-position of **2** closely parallel those observed for the O^6-position of **1**, with cyclohexylmethyl again proving optimal.[11]

A comparison of the binding mode of **1** with that of the 6-aminopurine CDK inhibitors olomoucine and purvalanol A, suggested that an aryl substituent at the purine 2-position of **1** would make favourable interactions with the ATP-binding domain and improve potency. This structure-based design approach enabled the eventual identification of NU6102 (**3**), which proved 1000-fold more potent than the parent O^6-alkylguanine ($K_i = 9$ and 6 nM for CDK1 and CDK2, respectively). Importantly, the increased potency of **3** could be fully rationalised on the basis of additional protein–ligand interactions observed in the inhibitor–CDK2 complex, most notably the formation of two additional hydrogen bonds between the sulfonamide group and Asp 86 (Fig. 1).[12] The close similarity of binding between **1** and **2**, and the remarkable increase in potency observed for **3** compared with **1**, clearly offered the opportunity to improve activity in the pyrimidine series through analogous modifications at the pyrimidine 2-position. In this communication, we report the results of these studies.

Chemical Synthesis

The required N^2-substituted pyrimidines were synthesised from the 2-*n*-butylsulfonylpyrimidine (**7**), which

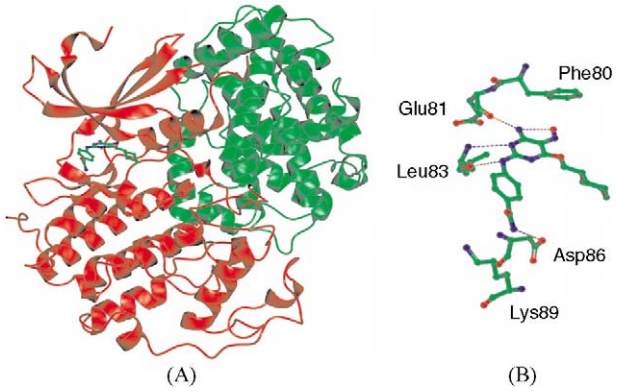
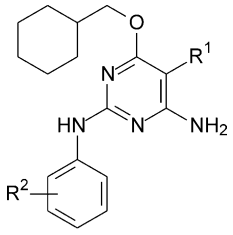


Figure 2. (A) Crystal structure of compound **9d** bound to phospho-Thr160 CDK2/cyclin A. Phospho-CDK2 is shown in red and cyclinA in green. Compound **9d** is drawn in ball and stick mode with carbon, nitrogen and oxygen atoms coloured green, blue and red, respectively; (B) interaction of compound **9d** with the active-site, showing hydrogen-bonds between the inhibitor and the protein.

Table 1. Inhibition of CDK1 and CDK2 by selected 2-arylaminopyrimidine derivatives[9]



No.	R^1	R^2	IC_{50} (μM)[a]	
			CDK1	CDK2
1 (NU2058)[b]	—	—	7.0±0.7[c]	17±2
2 (NU6027)[b]	—	—	2.9±0.1	2.2±0.6
3 (NU6102)[d]	—	—	0.0095±0.0013	0.0054±0.001
8a	H	4-$CONH_2$	63±3	59±31
8b	H	4-SO_2NH_2	5.3±0.9	2.9±0.4
9a	NO	3-Br	0.8±0.1	0.5±0.2
9b	NO	4-OMe	0.23±0.023	0.22±0.05
9c	NO	4-SMe	0.22±0.03	0.12±0.03
9d	NO	4-$CONH_2$	0.07±0.005	0.034±0.008
9e	NO	4-SO_2NH_2	0.005±0.0015	0.0011±0.0003

[a]12.5 μM ATP concentration.
[b]Ref 9.
[c]Data are mean±standard deviation of three or more individual experimental determinations.
[d]Ref 12.

was prepared in a three-step procedure from commercially available 4-amino-6-hydroxy-2-mercaptopyrimidine **4** (Scheme 1). Reaction of **4** with *n*-butyl bromide in EtOH–NaOH gave the alkyl sulfide **5** in excellent yield, and subsequent introduction of the 4-cyclohexylmethyl group was readily achieved under Mitsunobu conditions, affording pyrimidine **6**. Attempts to effect direct displacement of the *n*-butyl sulfide group of **6** with substituted anilines were unsatisfactory, giving only intractable mixtures. However, the corresponding sulfone (**7**), prepared by oxidation of **6** with *m*-CPBA, was susceptible to nucleophilic attack at the pyrimidine 2-position. Accordingly, the required 2-arylaminopyrimidines **8** were prepared by reaction of **7** with the appropriate aniline in 2,2,2-trifluoroethanol (TFE), in the presence of trifluoroacetic acid (TFA). Final nitrosation at the pyrimidine C^5-position proceeded smoothly on treatment with $NaNO_2$ in AcOH, to furnish the target pyrimidines **9** in good yield.[13]

Results and Discussion

A structure-based inhibitor design approach, utilising the O^6-alkylguanine NU2058 (**1**), has previously enabled identification of the 2-arylamino derivative NU6102 (**3**) as a nanomolar CDK1/CDK2 inhibitor. SAR studies conducted on the 2-arylamino ring of **3**, indicate that substituents possessing dual hydrogen bond donor-acceptor functions, for example SO_2NH_2 or $CONH_2$, confer potent inhibitory activity,[14] consistent with additional interactions observed within the ATP-binding site of inhibitor–CDK2 complexes. Although NU6027 (**2**) has a very similar binding mode to **1**, the O^4-alkylpyrimidine is a more potent inhibitor, and preliminary results suggest that **2** may have favourable cellular properties compared with **1**. The introduction of appropriately substituted 2-arylamino groups onto the pyrimidine scaffold was investigated in the expectation that such compounds would combine nanomolar potency with enhanced cellular penetration.

The inhibitory activity of the 2-arylamino-5-nitrosopyrimidines (**9a**–**9e**) against CDK1 and CDK2 was determined as described previously,[9] and the results are shown in Table 1. Pyrimidine intermediates (**8a** and **8b**), which lack a 5-nitroso substituent, are included for comparative purposes, together with NU6027 (**2**) and the purines NU2058 (**1**) and NU6102 (**3**). In all cases, the introduction of a substituted 2-arylamino group onto the pyrimidine template improved potency against both kinases compared with NU6027, activity varying widely with the nature of the aryl substituent. Thus, whereas a 3-bromophenyl group (**9a**) gave only a modest improvement in activity, the introduction of 4-methoxyphenyl (**9b**) or 4-methylthiophenyl (**9c**) resulted in an approximate ten-fold increase in potency compared with **2**. These results closely parallel those observed for the 2-arylaminopurine series, where comparable increases in potency were observed relative to the parent inhibitor **1** (results not shown).

As predicted, and consistent with the results obtained in the purine series, the introduction of a carboxamide (**9d**) or sulfonamide (**9e**) group at the 4-position of the pyrimidine 2-arylamino ring resulted in a dramatic increase in activity over the parent nitrosopyrimidine **2**. Indeed, in keeping with the relative potencies of **1** and **2**, the sulfonamide derivative (**9e**) proved to be approximately 2-fold and 5-fold more active than the corresponding purine (**3**) against CDK1 and CDK2, respectively. Pyrimidines lacking a 5-nitroso function (**8a** and **8b**) were markedly less potent than their counterparts (**9d** and **9e**), with a 1000-fold reduction in activity being observed for **8b** compared with **9e**. This is consistent with previous SARs in the nitrosopyrimidine series,[11]

and supports the putative role of the nitroso group in constraining the 6-amino group of NU6027 into an optimal binding orientation, by virtue of an intramolecular hydrogen bond with the pyrimidine 6-amino group.[9] Nevertheless, the 2-sulfanilylpyrimidine (**8b**) exhibited potency comparable with the parent nitrosopyrimidine **2**.

The interaction of the 2-arylaminopyrimidines with CDK2 was investigated by determining the crystal structure of the carboxamide derivative (**9d**) bound to phospho-Thr160 CDK2/cyclin A complex at 2.6 Å (Fig. 2).[15] Compound **9d** is bound in the ATP-binding site of the kinase in the expected orientation for this series of compounds. The pyrimidine moiety binds analogously to the purine of NU6102, forming three hydrogen bonds with the hinge region of the protein ($r_{\text{Glu81(CO)-N6}} = 2.7$ Å; $r_{\text{Leu83(NH)-N1}} = 3.5$ Å; $r_{\text{Leu83(CO)-N2}} = 2.7$ Å). As expected, the 5-nitroso group forms an intramolecular hydrogen bond with N6 to stabilise the purine-mimetic conformation. The N2-aryl substituent also binds similarly to that of NU6102, packing against the peptide backbone of CDK2 between residues His84 and Gln85. The Cambridge Structural Database (CSD) for small molecule crystal structures[16] was consulted to determine the preferred orientation of a carboxamide substituent on a phenyl ring. The preferred torsion angle between the plane of the ring and the plane of the carboxamide is $18 \pm 10°$. This result was used as a restraint in the refinement of the crystal structure. Possible interactions close to the carboxamide include the Asp86 side chain and backbone nitrogen. Although electron density cannot, at this resolution, unambiguously distinguish the carboxamide nitrogen from the carboxamide oxygen, the orientation can be inferred from the surrounding protein groups. In the most chemically reasonable conformation, the carboxamide nitrogen interacts with the Asp86 side chain with $r_{\text{N-Asp86(OD2)}} = 3.3$ Å and a torsion angle of 41°. Thus, the carboxamide function of **9d** mimics one of the two hydrogen bonding interactions previously observed for the NU6102 sulfonamide group.

In summary, an expedient route for the synthesis of 2-arylamino-4-cyclohexylmethyl-5-nitrosopyrimidine CDK inhibitors has been developed, and utilised for the preparation of a small series of compounds. The results are consistent with the known binding mode of NU6027 within the ATP-binding site of CDK2, and with SARs observed for the analogous purine series. The crystal structure of **9d** in complex with CDK2–cyclinA, reveals additional protein–ligand interactions compared with **2**, which account for the potent inhibitory activity of the pyrimidine.

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