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8-Substituted *O*⁶-Cyclohexylmethylguanine CDK2 Inhibitors; Using Structure-Based Inhibitor Design to Optimise an Alternative Binding Mode.

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Abbreviations: CDK, cyclin-dependent kinase; pRb, ; PI3-K, phosphatidylinositol-3-kinase; DMAP, 4-dimethylaminopyridine; PMB, 4-methoxybenzyl; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Abstract

Evaluation of the effects of purine C-8 substitution within a series of CDK1/2-selective *O*⁶-cyclohexylmethylguanine derivatives, revealed that potency decreases initially with increasing size of the alkyl substituent. Structural analysis showed that C-8 substitution is poorly tolerated, and to avoid unacceptable steric interactions, these compounds adopt novel binding modes. Thus, 2-amino-6-cyclohexylmethoxy-8-isopropyl-9*H*-purine adopts a 'reverse' binding mode where the purine backbone has flipped 180°. This provided a novel lead chemotype from which we have designed more potent CDK2 inhibitors using, in the first instance, quantum mechanical energy calculations. Introduction of an *ortho*-tolyl or *ortho*-chlorophenyl group at the purine C-8 position restored the potency of these 'reverse' binding mode inhibitors to that of the parent 2-amino-6-cyclohexylmethoxy-9*H*-purine. By contrast, the corresponding 8-(2-methyl-3-sulfamoylphenyl)-purine derivative exhibited sub-micromolar CDK2-inhibitory activity by virtue of engineered additional interactions with Asp86 and Lys89 in the reversed binding mode, as confirmed by X-ray crystallography.

Introduction

Deregulation of the cell cycle is a vital motif in most, if not all, human malignancies.^{1,2}

Phosphorylation of pRb, the product of the retinoblastoma gene, by cyclin-dependent kinase (CDK) family members is required for G1 progression, and mutation of the pRb gene or abnormalities in the pRb signalling pathway are a common feature of cancer. For example, over-expression and/or mutation of CDK4 and cyclin D has been reported in various tumour types, and mutation of the *p16^{INK4A}* gene that results in its deletion or silencing, is one of the most frequent genetic alterations found in human tumors.^{3,4} Over-expression of cyclin E and reduced levels of p27^{Kip1} have also been observed to correlate with disease progression,^{5,6} suggesting that deregulation at various checkpoints throughout the cell cycle is a key contributor to the process of tumorigenesis. As a result of these studies, the CDK family is considered an important target for therapeutic intervention in cancer and other diseases, and a wide range of ATP-competitive inhibitors have been developed.⁷⁻⁹ However, although a number of small-molecule CDK inhibitors have progressed to clinical trials (*e.g.* **1-4**), a kinase-specific CDK inhibitor has yet to be approved by the FDA for clinical use.^{7,10,11}

Initial enthusiasm for CDK2 as a cancer therapeutic target was tempered by subsequent studies demonstrating that CDK2 siRNA or antisense constructs failed to induce cell cycle arrest in a number of human tumor cell lines,¹² and that CDK2 knockout mice are viable.^{13,14} However, more recent evidence suggests that CDK2 inhibition may elicit antitumor activity in a subset of tumors with defined genetic characteristics. For example, CDK2 knockdown or treatment with roscovitine

(1) is selectively toxic to *MYCN*-amplified neuroblastoma cells compared with their non-amplified counterparts.¹⁵ Compelling evidence to support a therapeutic role for pharmacological CDK2 inhibition in cancer treatment has also been adduced very recently, employing a chemical genetic approach.¹⁶ Thus, a comparison of selective small-molecule CDK2 inhibition with that imposed by siRNA knockdown in normal and tumor cells highlighted important differences between these approaches, with pharmacological inhibition resulting in growth inhibition of a panel of human cancer cells transformed with various oncogenes. A recent study demonstrating that a combination of phosphatidylinositol-3-kinase (PI3-K) and CDK2 inhibitors induced apoptosis in malignant glioma xenografts via a synthetic-lethal interaction, also highlights the potential of CDK2-selective inhibitors.¹⁷

We have previously reported the structure-based design of a series of potent CDK2-selective inhibitors derived from the lead compound *O*⁶-cyclohexylmethylguanine (NU2058, **5**; CDK2 IC₅₀ = 16.0 μM), as exemplified by **6** and **7** (CDK2; IC₅₀ = 1.0 μM and 5.0 nM, respectively).¹⁸⁻²² The X-ray crystal structure of **5** in complex with monomeric unphosphorylated CDK2, revealed that the purine heterocycle binds within the ATP-binding pocket in a different orientation from that previously reported for ATP, isopentenyladenine, and the prototypic purine CDK inhibitor olomoucine (**8**).²³ The 9-NH group of **5** forms a hydrogen-bond with the backbone carbonyl of Glu81, while the purine *N*-3 and 2-amino group make hydrogen-bonds with the backbone nitrogen NH and carbonyl of Leu83, respectively. In this orientation, the *C*-8 position of **5** is directed

towards the side-chain of Phe80 in the gatekeeper pocket (Figure 1). A number of purine-based CDK inhibitors derive potency from interacting with this residue; for example, purvalanol B (**9**)²⁴ and H717 (**10**)²⁵ both pack hydrophobic aliphatic substituents against the Phe80 side chain. In contrast, a series of bisanilinopyrimidines (*e.g.* **11**) abut polar and polarisable groups against the phenyl ring of Phe80, and the observed gain in potency for this compound series is attributed to induced dipole-dipole interactions.^{26, 27}

Figure 1. (A) Purine **5** bound to the CDK2 ATP-binding domain. The kinase is presented in dark green in ribbon representation, and selected CDK2 residues and **5** are drawn in ball and stick mode. Carbon atoms of **5** are coloured light green and hydrogen bonds are denoted by dashed lines. (B) Schematic representation of the bonds made between CDK2 and **5**. Hydrogen bonds are drawn as arrows. The double-headed arrow denotes the distance between the purine C8 atom and the side-chain of Phe80.

The close proximity of the imidazole ring of **5** to the Phe80 side-chain observed in the structure of the inhibitor in complex with CDK2, implied that substitution at the purine C-8 position would not be tolerated. To confirm this prediction, and as part of SAR studies for this chemotype, three derivatives of **5** bearing alkyl groups at the 8-position (**24-26**) were initially synthesised, and the crystal structure of the 8-isopropylpurine (**26**) bound to CDK2 was determined. Purine **26** was

found to adopt a different binding orientation from that observed for **5** within the ATP-binding domain, offering the opportunity to exploit alternative inhibitor-protein interactions through judicious structural modifications to the parent purine **5**. In this paper, we describe the elaboration of this binding mode through iterative compound synthesis, molecular modelling and X-ray crystallographic analysis of CDK2/cyclin A/inhibitor complexes. These studies have achieved improved CDK2-inhibitory activity by virtue of an engineered additional CDK2-inhibitor interaction.

Chemistry

The initial target 8-substituted *O*⁶-cyclohexylmethylguanine derivatives (**24–29**, **32**) were readily synthesised following the procedures illustrated in Scheme 1. Thus, acylation of the 6-amino group of 2,6-diamino-4-cyclohexylmethoxy-5-nitrosopyrimidine (**17**)¹⁸ afforded the 6-acylaminopyrimidines (**18–23**), which were readily cyclised to the required purines (**24–29**) in good overall yields on reduction of the 5-nitroso substituent by catalytic hydrogenation. Efforts to prepare the 8-trifluoromethylpurine **32** by this approach were unsuccessful, with **17** proving refractory to acylation with TFA-anhydride or ethyl trifluoroacetate. However, direct acylation of triaminopyrimidine **30** with TFA gave the required trifluoroacetylaminopyrimidine **31**, which slowly cyclised to the requisite purine **32** on prolonged heating in pyridine-DMAP.

Scheme 1^a

^a **Reagents and conditions:** (i) **18-21**, (RCO)₂O, DMF, 80 °C, **22** and **23**, ArCOCl, pyridine, DMAP, 25 °C; (ii) H₂, Pd/C, AcOH, THF, 20 °C; (iii) TFA, reflux; (iv) DMAP, pyridine, reflux.

The preparation of *O*⁶-cyclohexylmethylguanine derivatives bearing substituted aryl groups at the purine 8-position required palladium-catalyzed cross-coupling methodology (Scheme 2). Direct 8-arylation of the *N*⁹-benzylpurine **33** with the appropriate aryl bromides, employing CuI-Pd(OAc)₂ under microwave irradiation, furnished benzyl-protected intermediates **34** and **35**, and subsequent conversion into the requisite phenol **36** and benzoic acid **37** was achieved in moderate yield by catalytic hydrogenation under acidic conditions. An alternative approach utilized the 8-bromopurine **39**, readily synthesized by treatment of the *t*-BOC-protected purine derivative **38** with *N*-bromosuccinimide, which concomitantly removed the protecting group, or *via* direct bromination of **5** with pyridinium tribromide. Suzuki-Miyaura cross coupling of **39** with the appropriate boronate derivatives (**15**, **16**), prepared from the corresponding aryl halides (**12**, **14**) by standard methods, or generated *in situ* from **13**, gave the PMB-protected compounds (**40-42**), which were smoothly deprotected to the required 8-substituted purines **43-45** on treatment with TFA.

Scheme 2^a

^a **Reagents and conditions:** (i) (a) TsCl, pyridine, 0-25 °C, (b) PMB-Cl, DMAP, 25 °C; (ii) bis(pinacolato)diboron, PdCl₂[(C₄H₉)₂PC₅H₄]₂Fe, [(C₄H₉)₂PC₅H₄]₂Fe, KOAc, dioxane, MW 150 °C; (iii) PMB-Cl, NaH, DMF, 80 °C (**13**) 25 °C (**14**); (iv) BnBr, K₂CO₃, DMF, 25 °C; (v) Pd(OAc)₂, CuI, CsCO₃, DMF, MW 200 °C; (vi) H₂, Pd/C, AcOH, aq. HCl, 20 °C; (vii) (tBuO)₂CO, K₂CO₃, DMF, 20 °C; (viii) pyridinium tribromide, DCM, 70 °C; (ix) NBS, MeCN, 20 °C; (x) Appropriate arylboronate, PdCl₂[(C₄H₉)₂PC₅H₄]₂Fe, Cs₂CO₃, aq. dioxane, 110 °C; (xi) TFA, 20-110 °C.

Results and Discussion

The inhibitory activity of the purine derivatives against CDK1 and CDK2 is shown in Table 1. Our previous studies with *O*⁶-alkylguanines were founded on the benchmark *O*⁶-alkylguanine NU2058 (**5**) and culminated in the identification of NU6102 (**7**), a highly selective CDK2 inhibitor that was designed by a structure-based inhibitor design approach. The markedly increased potency of **7** (CDK2; IC₅₀ = 5 nM) compared with **5** (CDK2; IC₅₀ = 16 μM) is attributed to additional favourable

interactions between the sulfonamide function of **7** and the specificity surface of the ATP-binding domain of the kinase.²⁰ The primary sulfonamide function of **7** also provided an opportunity to develop a water-soluble prodrug, by virtue of *N*-acetylation and formulation as the potassium salt, and *in vivo* studies are in progress.²⁸ With a view to further delineating SARs for this interesting class of kinase inhibitor, the effect of substitution at the C-8 position of **5** was investigated.

Table 1. Inhibition of CDK1 and CDK2 by C8-substituted *O*⁶-cyclohexylmethylguanines

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>A</p> </div> <div style="text-align: center;"> <p>B</p> </div> <div style="text-align: center;"> <p>C</p> </div> </div>						
Number	Structure	R	R'	IC ₅₀ (μM) ^a or % inhibition		
				CDK2/Cyclin A	CDK1/Cyclin B	
5	A	H	-	16 ± 2.0 ^b	26 ± 3.5	
6	B	H	-	0.97 ± 0.03	10.0 ± 1.6	
7	B	SO ₂ NH ₂	-	0.005 ± 0.001	0.25 ± 0.05	
24	A	Me	-	44.7 ± 3.2	7%, 11% ^c	
25	A	Et	-	30.3 ± 1.5	77.5	
26	A	<i>i</i> -Pr	-	27.7 ± 3.2	92.3 ± 4.3	
27	C	H	H	10% ^c	35% ^c	
28	C	Cl	H	17 ± 1.8	> 100	
29	C	Me	H	18.2 ± 4.3	93.6 ^d	
32	A	CF ₃	-	49 ± 2.6% ^e	> 100	
36	C	Me	OH	23.5 ± 4.8	> 100	
37	C	Me	CO ₂ H	> 100	> 100	
43	C	Me	CONH ₂	39.8 ± 13.9	62% ^d	

44	C	H	SO ₂ NH ₂	8.7 ± 3.4	> 100
45	C	Me	SO ₂ NH ₂	0.51 ± 0.05	2.8 ^d

^aIC₅₀ values determined as described in reference 20. ^bStandard deviations obtained from three separate experiments. ^cActivity determined at 10 µM owing to limiting solubility. ^dSingle determination. ^eActivity measured at 100 µM.

The crystal structure of **5** bound to CDK2/cyclin A indicated that 8-substitution would not be well tolerated, as a result of a steric clash between the C-8 moiety and the side-chain of Phe80 ($r_{C8-F80C\beta} = 3.7\text{\AA}$ in the CDK2/**5** structure).²⁰ This observation appeared to be confirmed by the CDK2-inhibitory activity of the three initial derivatives, which showed a modest decrease in potency compared with **5** on addition of a methyl (**24**), ethyl, (**25**) or isopropyl (**26**) group at the purine 8-position (IC₅₀ values of 45, 30, and 28 µM, respectively), albeit that **25** and **26** were essentially equipotent with **24** despite steric differences (Table 1). Where measurable, activity against CDK1 compared with CDK2 also generally tracked that observed for **5**, with **25** and **26** proving some 2-3 times less potent against CDK1. Notably, a dramatic reduction in potency against both kinases was observed upon introduction of the bulky C-8 phenyl group, although a precise determination of the activity of **27** was limited by very poor aqueous solubility.

Crystal structure analysis - To establish the binding mode of this inhibitor series within the ATP pocket, X-ray crystallographic analysis of the 8-isopropyl- and 8-trifluoromethyl-purines (**26** and **32**) in complex with CDK2 was undertaken, following crystal soaking with each inhibitor. Interestingly, **26** was found to adopt a novel orientation within the ATP-binding site distinct from that observed for the parent CDK2 inhibitor **5** (Figure 2). By comparison with the ‘conventional’ binding mode of **5** (Figure 1), the new orientation adopted by **26** can be described by a 180° rotation of the purine ring about a vector coincident with the C-6 to N-3 bond. In this orientation C-8 of **26** is directed towards the ‘selectivity surface’ of the kinase,^{20, 29} with the purine 2-amino group being

projected towards Phe80. Thus, both **5** and **26** form a conserved triplet of hydrogen bonds with the CDK2 hinge region, and the *O*⁶-cyclohexylmethyl substituent is retained in the ATP ribose-binding pocket. In both cases, the conformation and location of the *O*⁶-cyclohexylmethyl group is analogous to that previously observed for the structure of **7** bound to CDK2/cyclin A.²⁰ (Supplementary Figure S1).

Figure 2. (A) Stereoview of **26** in complex with CDK2. The kinase is presented in dark green in ribbon representation, and selected CDK2 residues and **26** are drawn in ball and stick mode. Carbon atoms of **26** are coloured light green and hydrogen bonds are denoted by dashed lines. Water molecules are drawn as red spheres. (B) Schematic representation of the bonds made between

CDK2 and **26**. Hydrogen bonds are drawn as arrows. The double-headed arrow denotes the distance between the purine C2 substituent and the side-chain of Phe80.

Additional interactions of **26** with the glycine-rich loop of CDK2 contribute to stabilisation of the activation segment, as previously observed for the structure of **5** bound to CDK2.¹⁸ These interactions are predominantly lipophilic in nature, arising between the *O*⁶-cyclohexyl ring of **5** and **26** and a hydrophobic patch on the glycine loop contributed by the side chain of residue Val18 and the peptide backbone around residue Gly11. The binding conformation adopted by **26** has not been reported previously, despite extensive structural analyses of various substituted purine-based CDK ATP-competitive ligands.^{18-20, 22-24, 30,31}

To assess whether smaller C-8 substituents would also promote this 'reverse' binding mode, the 8-trifluoromethylpurine **32** was co-crystallised with CDK2/cyclin A phosphorylated on CDK2 Thr160 (CDK2/cyclin A). Previous comparative crystallographic studies of inhibitor binding to CDK2 and to CDK2/cyclin A have shown that the interactions made between inhibitors within this series, and the two alternative forms of CDK2 within the adenine binding pocket, are conserved. Surprisingly, **32** did not share the same binding mode as **26**, but rather adopted another that is reminiscent of, but distinct from, that of **5** (Figure 3). In contrast to the 8-isopropyl group of **26**, the 8-trifluoromethyl substituent of **32** was better accommodated, although this group is regarded as having a similar size to isopropyl.³² The purine of **32** was found to bind in a near identical orientation to that of **5**, albeit less deeply within the CDK2 ATP-binding pocket. As a consequence, both the structure of the glycine-rich loop and the nature of the hydrogen bond interactions between **32** and the residues within the CDK2 hinge are modified. Most notably, the characteristic hydrogen bond interaction between the purine 9-NH and the backbone carbonyl moiety of Glu81 observed for **5** and **7**, was absent with **32**. N-2 is positioned to enable hydrogen bonding with the carbonyl group of Leu83 or, for this inhibitor series, to form a novel interaction with the backbone carbonyl of His84. The 9-NH of **32** forms two indirect hydrogen bonds with CDK2 *via* a water molecule, which is also engaged in a polar contact with one of the fluorine atoms of the C-8 trifluoromethyl group

that is packed against the Phe80 aromatic ring ($r(\text{CF}_3\text{-aromF80}) = 4.2 \text{ \AA}$). A second fluorine atom mediates a network of polar contacts with two water molecules that in turn interact with the side chains of Glu51 and Lys33, and the amide group of Asp145.

Figure 3. (A) Stereoview of **32** in complex with CDK2/cyclin A. The kinase is presented in dark green in ribbon representation, with selected CDK2 residues and **32** drawn in ball and stick mode. Carbon atoms of **32** are coloured light green and hydrogen bonds are denoted by dashed lines. Water molecules are drawn as red spheres. (B) Schematic representation of the bonds made between CDK2 and **32**. Hydrogen bonds and polar interactions are drawn as arrows. The double-headed arrow denotes a potential hydrophobic interaction between the purine C8 substituent and the

side-chain of Phe80.

The CDK-inhibitory activity (Table 1) of the C-8 substituted purines (**24-27**, **32**) was reassessed in light of the structural biology results described above. The changes in potency observed against both CDK1 and CDK2 that accompany C-8 alkyl substitution, compared with the parent purine **5**, can be modelled as occurring in two phases as the size of the alkyl substituent increases. Firstly, the initial decrease in potency is consistent with the formation of sub-optimal interactions imposed to accommodate small alkyl groups such as methyl (**24**) at the purine C8 position. As illustrated by the structure of **32** in complex with CDK2/cyclin A (Figure 3), the inhibitor is forced away from Phe80 and, consequently, the requisite triplet of hydrogen bonds with the hinge cannot be maintained. Further small adjustments of the ATP site residues are also necessary to accommodate the inhibitor, and taken together, these changes lead to a decrease in binding affinity compared with **5** as reflected by CDK1/CDK2 inhibitory activity in the 100 μ M range for **32**. Increasing the size of the C-8 substituent further results in an unacceptable steric clash with Phe80, and the inhibitor is forced to adopt an alternative binding mode where the C-8 group faces the selectivity surface, as observed with **26** (Figure 2).

*Replicating the N-2 aryl group interactions of **6** and **7** with CDK2 for C-8 substituted purines*

The unique binding orientation adopted by the 8-isopropylpurine (**26**) offered an opportunity to design novel CDK2 inhibitors based on the 6-alkoxypurine template. Accordingly, we sought to exploit this finding by applying the structure-aided design principles employed previously for the optimisation of the 2-arylaminopurines **6** and **7**,²⁰ for the identification of more potent derivatives of **26**. A key feature of the binding of **6** and **7** to CDK2, is the packing of the N-2 aryl ring against the CDK2 surface to form a π - π stacking interaction with the peptide backbone between Gln85 and Asp86.²⁰ Notably, the aryl ring appears to have a low barrier to rotation about the N-2 to C-1' bond, as demonstrated by the different purine-aryl interplane torsion angles of these and other derivatives.^{20,22}

A structural superposition of **7** and **26** in complex with CDK2 and CDK2/cyclin A,²⁰ respectively, (Supplementary Figure S1) indicated that to replicate the aryl-backbone interactions made between **7** and CDK2, the aryl ring would need to be attached directly to the C-8 position, a compound that is most closely modelled in this series by the 8-phenylpurine **27**. However, the weak CDK2-inhibitory activity of **27** suggested that if, as predicted, the compound binds to CDK2 in the reverse binding mode, the requisite additional interactions are not arising between the 8-phenyl ring and the kinase. One possible explanation for this observation is that the purine and phenyl rings of **27** are essentially co-planar as a consequence of a favourable interaction of their π -systems, which would preclude the conformation necessary to facilitate an optimal interaction between the 8-phenyl ring and CDK2. Indeed, evidence in support of this is provided by the crystal structure of the aloisine derivative RP90 (6-phenyl[5H]pyrrolo[2,3-*b*]pyrazine) in complex with CDK2, where the pyrrole and 6-phenyl rings are found to adopt a coplanar conformation.³³

With a view to optimising the interaction of the 8-phenyl ring of **27** with the backbone of the ATP-binding domain of CDK2, the possibility of imposing a favourable conformational twist between the imidazole and phenyl rings, by virtue of *ortho*-substitution on the 8-phenyl ring, was considered. To this end, quantum mechanical calculations were employed to elucidate the conformation-energy profiles for nine derivatives of **27**, to establish whether a non-coplanar conformation was energetically favourable, and to identify substituents with the potential to confer a non-planar arrangement between the two ring systems. The ideal *ortho*-substituent on the 8-phenyl ring of **27** would induce an inter-plane twist of approximately 40°, with relatively free rotation arising about the optimum conformation of $\pm 20^\circ$. This latter aspect would allow for optimisation of aryl ring packing against the kinase. Such a twist was predicted to be tolerated based on literature evidence, where relatively large variations of torsion angles between non-conjugated ring systems have been observed for a number of purine-based inhibitors.^{20, 22, 24}

Quantum mechanical calculations on model compounds derived from 27

A simplified model of **27** was used to investigate the possibility of inducing a phenyl-purine twist with C-8 phenyl *ortho*-substituents. As a first approximation, it was assumed that the *O*⁶-cyclohexylmethoxy substituent of **27** would have little effect on the phenyl-purine twist angle, and this group was not included in the calculations. Nine model compounds (**A-I**) encompassing combinations of fluoro, chloro and methyl substituents were investigated in comparison with **27**. Each model was constructed and energy minimised, with the purine and aryl rings constrained to a co-planar orientation. A rigid potential energy scan was then performed at a range of inter-planar angles between the two co-planar conformations (A full description of the results of this analysis for each compound can be found in the Supplementary Results and Supplementary Figure S2).

The *ortho*-fluorophenylpurine (**A**) (Figure 4) was found to have both the desired characteristics of inducing an inter-plane twist of approximately 40°, and allowing relatively free rotation about this optimum conformation. However, **A** possesses a significantly more stable conformation at 180°, presumably due to an intramolecular hydrogen bond. The 2-chlorophenyl derivative **B** has minima at approximately 40° and 140° that would, potentially, allow the formation of favourable aryl-backbone packing interactions. The 40° conformation is in a relatively shallow energy well, indicating some degree of rotation between the two ring systems. However, the more stable, and therefore preferred, conformation at 140° has a relatively steep energy well, implying a more restricted rotational freedom. By contrast, the *ortho*-methylphenylpurine **C** has a global minimum at around 40° in a relatively flat energy well. The *ortho*-disubstituted C8-phenylpurines **E**, **F**, **H** and **I** all show potential to form the required conformation, especially the 8-(2-chloro-6-methylphenyl)purine (**I**), which has a minimum in a relatively flat energy well at ~ 50°.

27 ; R ¹ = R ² = H	D ; R ¹ = R ² = F	H ; R ¹ = R ² = Cl
A ; R ¹ = F, R ² = H	E ; R ¹ = Me, R ² = F	I ; R ¹ = Me, R ² = Cl
B ; R ¹ = Cl, R ² = H	F ; R ¹ = Cl, R ² = F	
C ; R ¹ = Me, R ² = H	G ; R ¹ = R ² = Me

B

C

Figure 4. Chemical structures of **27** and modelled *ortho*-substituted 8-arylpurines (**A-I**), together with conformation-energy profiles of **27** and three representative model compounds.

To assess the validity of the modelling studies, the *ortho*-chlorophenyl and *ortho*-tolyl derivatives **B** and **C** were synthesised (**28** and **29**) and evaluated for CDK-inhibitory activity, with both compounds exhibiting potency comparable with the parent 2-amino-6-cyclohexylmethylpurine **5** (Table 1). Interestingly, **28** and **29** were essentially inactive against CDK1 (IC_{50} values of $> 100 \mu M$ and $94 \mu M$, respectively), although the poor aqueous solubility of **27** militated against a direct comparison with **28** and **29**. The crystal structure of **29** in complex with CDK2/cyclin A was determined. As anticipated from the analysis of **26**, the *ortho*-tolylpurine **29** binds in the same reverse binding mode and makes an identical triplet of hydrogen bonds with Glu81 and Leu83 (Figure 5A). However, the interaction with Lys33 is not maintained, as the residue adopts a conformation in which the side chain is directed away from the inhibitor. The *o*-tolyl group of **29** is in the same orientation as the isopropyl group of **26**, and projects towards the selectivity surface of

the ATP pocket such that the C-8 atoms of compounds **26** and **29** almost coincide with the C-17 atom of **7** (first carbon atom of the 2-arylamino group, Figure 5B).

Figure 5. (A) Binding of **29** to CDK2/cyclin A. (B) Comparative binding of **7** and **29**. A. CDK2 is shown in dark green. Selected residues from the CDK2 ATP binding pocket and **29** are shown as ball-and-stick representation and coloured dark green and light green, respectively. Water molecules in the vicinity of the inhibitor are included as red spheres. B. The inhibitors and selected residues within the CDK2 ATP binding site are shown in ball-and-stick representation. Carbon

atoms of CDK2 residues from **7** and **29** complex structures are coloured grey and dark green, respectively. Purine **7** is shown in coral and **29** in light green.

Importantly, the structure of **29** bound to CDK2/cyclin A supported the results of the quantum mechanical calculations. The *o*-tolyl group on the 8-aryl ring of the purine induces an inter-ring twist of approximately 38° which is close to the predicted value of ~ 40°. This value is also similar to the inhibitor inter-planar ring tilts observed for the CDK2/cyclin A/**6** and CDK2/cyclin A/**7** structures (50° and ~34°, respectively),²⁰ suggesting that an equivalent disposition of two ring systems can be accommodated in either binding mode. The introduction of an *ortho*-methyl (or *ortho*-chloro) substituent onto the C-8 phenyl ring of **27** restores the potency of the compounds that adopt this altered binding mode to that of **5**, the benchmark 6-alkoxypurine CDK2 inhibitor in the series (Table 1). However, there remained a 20-fold difference in potency between **29** (CDK2; IC₅₀ = 18.2 μM), and the 2-phenylaminopurine derivative **6** (CDK2; IC₅₀ = 1.0 μM) that does not adopt the alternative binding mode. The lower potency of **29** likely results from misalignment of the purine, resulting in weakened hydrogen bond interactions with the CDK2 backbone in the hinge region. A concomitant shift of the purine ring out of the cleft will also result in sub-optimal packing of the C-8 phenyl ring against the peptide backbone at residues Gln85 and Asp86.

Our previous studies with other purine- and pyrimidine-based CDK inhibitor series suggested the possibility of exploiting sequence differences between kinases on the 'selectivity surface,' through further elaboration of the 8-aryl ring of **29**. For example, a number of inhibitor series probe both the conserved aspartate residue (Asp86 in CDK2) and the sequence differences that exist between CDKs at the residue equivalent to CDK2 Lys89 (Thr102 and Val100 in CDK4 and CDK7, respectively), to derive considerable gains in potency and selectivity.^{20,24,26,34,35} The potent CDK2 inhibitor **7** evolved from **6** by the rational introduction of a sulfonamide group at the 4-position of the 2-phenylamino ring.²⁰ The resultant 200-fold increase in potency, is attributable, at least in part, to optimal H-bond interactions arising between the sulfonamide moiety and the backbone amide

and side-chain of Asp86. In an effort to emulate these interactions in the context of the reversed binding mode, compound **29** was initially further substituted with hydroxyl (**36**) and carboxylate (**37**) groups at the *meta*-position on the C-8 aryl ring. However, these substitutions did not lead to a significant increase in potency, and while **36** (CDK2; $IC_{50} = 28 \mu M$) retained activity approaching that of **29** (CDK2; $IC_{50} = 18.2 \mu M$), the carboxylic acid derivative **37** was essentially devoid of activity against both CDK1 and CDK2 ($IC_{50} > 100 \mu M$). Determination of the structures of compounds **36** and **37** bound to CDK2/cyclin A revealed the molecular details of the inhibitor binding modes (Supplementary Figure S3).

Figure 6. Crystal Structures of (A) **44** and (B) **45** bound to CDK2/cyclin A. CDK2 is shown in green, with selected residues from the ATP-binding pocket shown in ball and stick mode. **44** and **45** are also drawn in ball-and-stick representation with carbon atoms coloured light green.

The disappointing activity of **36** and **37** prompted the introduction of *meta*-substituents on the 8-aryl ring having both donor and acceptor properties, with a view to recapitulating the interactions with Asp86 observed for **7**. Although the potency of the 3-carboxamido derivative **43** (CDK2; $IC_{50} = 41/53 \mu M$) suggested sub-optimal binding, the introduction of a 3-sulfonamide group (**45**)

conferred sub-micromolar CDK2-inhibitory activity ($IC_{50} = 0.51 \pm 0.05 \mu M$). Thus, **45** proved approximately 50-fold more potent than **26**, and exhibited activity comparable with the 2-phenylamino purine **6**. Interestingly, the 8-aryl purine **44**, which differs from **45** only in lacking the *ortho*-methyl group, was some 17-fold less potent (CDK2; $IC_{50} = 8.7 \pm 3.4 \mu M$), supportive of the role of the *ortho*-methyl function in imposing the desired conformational twist of the 8-aryl ring of the purine.

The predicted binding modes of **44** and **45** were confirmed by determination of the structures of these purines bound to CDK2/cyclin A. Both inhibitors adopt the reverse binding mode, and, as predicted, the sulfonamide substituent makes hydrogen bonds to Asp86 and Lys89 (Figure 6). The presence of the 2-methyl group on the C-8 aryl ring of **45** imposes the requisite rotation of the entire group, and also precludes a steric clash between the methyl moiety and the N7 position. This rotation also results in a slight decrease in the distance between the sulfonamide substituent and Asp86 and Lys89 that may contribute to the observed increases in potency. The hydrogen bonds between the purine ring of **45** and the CDK2 backbone within the hinge sequence are maintained, but again a water molecule mediates an indirect network of interactions between N9 and the side chains of Lys33 and Glu81.

Conclusion

In the current study, the effect of substitutions at the C-8 position within a series of O^6 -cyclohexylmethylguanines, on their ability to inhibit CDK2, has been evaluated. The introduction of alkyl groups was poorly tolerated leading to decreases in potency, the origins of which were elucidated by crystallographic analysis of selected inhibitors bound to CDK2/cyclin A. The crystal structures of compounds **26** and **32** bound to CDK2/cyclin A revealed that they adopt novel binding modes within the CDK2 ATP-binding site. An increase in the size of the C-8 alkyl group from methyl to isopropyl induced a rotation of the binding mode so that the C-8 substituent was positioned to face the CDK2 specificity surface. Quantum mechanical calculations were employed

to identify **29**, a compound that adopts the novel, reverse binding mode and is equipotent with the parent purine **5** against CDK2.

Initial further elaboration of **29** by *meta*-substitution to exploit potential interactions with Asp86 failed to increase potency. Structure determination of compounds **36** and **37** bound to CDK2/cyclin A revealed that these compounds occupy a similar position to compound **29** within the CDK2 ATP-binding cleft. However, despite interacting with the same region as the sulfonamide group of **7**, the hydroxyl and carboxylate substituents of **36** and **37**, respectively, are not orientated to reproduce the favourable interactions observed in the structure of **7**. However, in derivative **45** a sulfonamide substituent at the *meta*-position of the C-8 arylpurine, in tandem with an *ortho*-methyl group, maintained the reversed binding mode and also emulated the interactions made by **7**, leading to the predicted increases in potency. Unfortunately, the relatively modest potency of **45** compared with the parent 2-arylamino-6-alkoxypurine CDK2 inhibitor **7**, combined with only limited opportunities for further elaboration, militate against further optimisation of this series.

Small-molecule CDK inhibitors have enjoyed a resurgence of interest, as evidenced by a very recent review of the patent literature,³⁶ and clinical studies with the CDK4/6 inhibitor palbociclib (PD-0332991) are at an advanced stage.³⁷ These clinical studies are underpinned by a greater appreciation of the importance of CDK activity to normal cells, and the more careful selection of patient populations to ensure that effective therapeutic windows can be achieved. Previous experience has highlighted the importance of achieving selectivity for the target CDK over other family members, and the availability of crystal structures for the majority of the CDKs to guide inhibitor design, has proven invaluable. In this paper, we have demonstrated the application of molecular modelling and structure-based design for the exploitation of an unorthodox binding orientation of a purine-based CDK2 inhibitor. The results of the study contribute to a further understanding of the possible interactions of this inhibitor class with the ATP-binding domain of CDK2, and will be of value both in the design of more potent CDK2 inhibitors and for abrogating CDK2-inhibitory activity where this is required.

Experimental Section

General Synthetic Procedures

Reagents were purchased from fine chemicals 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. THF refers to anhydrous tetrahydrofuran, either by distillation from sodium benzophenone, or from commercial sources. Melting points were obtained on a Stuart Scientific SMP3 apparatus and are uncorrected. Thin layer chromatography was performed using silica gel plates (Kieselgel 60F₂₅₄; 0.2 mm), and visualised with UV light or potassium permanganate. Chromatography was conducted under medium pressure in glass columns or using a Biotage SP4 instrument in prepacked columns (FLASH+ Silica columns [40-63 µm, 60Å]). Semi-prep HPLC was conducted with Varian ProStar HPLC system equipped with 2 ProStar 210 solvent delivery modules, a ProStar 320 UV-Vis detector, a ProStar 701 fraction collector and controlled by Varian Star Chromatography Workstation. Proton (¹H) and carbon (¹³C) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Spectrospin AC 300E (¹H at 300 MHz, ¹³C at 75 MHz), a Jeol JNM-LA500 spectrometer (¹H at 500 MHz, ¹³C at 125 MHz), or a Bruker Avance II 500 (¹H at 500 MHz, ¹³C at 125 MHz). Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), using known solvent peaks as internal standards. Coupling constants (J) are reported in hertz (Hz).

IR spectra were recorded on a Bio-Rad FTS 3000MX diamond ATR. Liquid Chromatography-Mass Spectrometry (LCMS) was carried out on a Micromass Platform instrument operating in positive and negative ion electrospray mode, employing a 50 x 4.6 mm C18 column (Waters Symmetry or Waters Atlantis) 5 or 12 min gradient elution with 0.05% formic acid in methanol (10-90%). All compounds gave $\geq 95\%$ purity by ¹H NMR, HPLC or LCMS, unless stated otherwise. Elemental analyses were performed by The School of Pharmacy, Analytical Facility, University of London, WC1N 1AX. Accurate masses were measured using a Finnigan MAR 95 XP or a Finnigan MAR

900 XLT at the EPSRC National Mass Spectrometry Service Centre (Chemistry Department, University of Wales, Swansea, Wales, SA2 8PP).

3-Bromo-N-(4-methoxybenzyl)-2-methylbenzamide (12). *p*-Toluenesulfonyl chloride (1.06 g, 5.58 mmol) was added dropwise to a stirred solution of 3-bromo-2-methylbenzoic acid (1.0 g, 4.65 mmol) in anhydrous pyridine (5 mL) at 0 °C, and the mixture was allowed to warm to room temperature with stirring over 72 h. The solvent was removed *in vacuo* and the residue was redissolved in EtOAc (20 mL), washed sequentially with aqueous HCl solution (0.1 M, 20 mL) and brine (2 x 20 mL), dried (MgSO₄) and concentrated under reduced pressure to give 3-bromo-2-methylbenzoic 4-methylbenzenesulfonic anhydride as a white solid (1.72 g, 100%), which was used directly for the next reaction. To a solution of the tosylate ester (0.46 g, 1.24 mmol) in DCM (5 mL) was added DMAP (15 mg, 0.12 mmol), and the mixture was stirred for 15 min at room temperature, prior to addition of 4-methoxybenzylamine (0.8 mL, 6.3 mmol). After stirring for a further 30 min, DCM (10 mL) was added, and the reaction mixture was washed sequentially with aqueous HCl solution (0.1 M, 20 mL) and brine (2 x 20 mL), dried (MgSO₄) and the organic fraction was concentrated under reduced pressure. Purification by chromatography on silica, employing a gradient elution of Petrol:EtOAc (9:1) to Petrol:EtOAc (1:1), gave the title compound as a white powder (0.41 g, 100%); mp 121-122 °C; IR 3248, 1612, 1513 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 2.44 (0.74H, s, Ar-CH₃), 2.47 (2.26H, s, Ar-CH₃), 3.78 (0.74H, s, OCH₃), 3.80 (2.26, s, OCH₃), 4.05 (0.49H, d, *J* = 6.1 Hz, NCH₂), 4.50 (0.25H, t, *J* = 5.9 Hz, NH), 4.55 (1.51H, d, *J* = 5.7 Hz, NCH₂), 5.95 (0.75H, br s, NH), 6.78-6.82 (0.48H, m, *H*-3'), 6.86-6.91 (1.51H, m, *H*-3'), 7.02-7.07 (0.75H, m, *H*-5), 7.08-7.13 (0.5H, m, *H*-2'), 7.25-7.29 (2.1H, m, *H*-4, *H*-2'), 7.31 (0.5H, d, *J* = 8.3 Hz), 7.58 (0.74H, dd, *J* = 8.0, 1.1 Hz, *H*-6), 7.73-7.77 (0.5H, m); ¹³C NMR (CDCl₃, 125 MHz) δ 20.2, 21.7, 43.7, 46.9, 55.4, 55.5, 114.4, 114.2, 125.8, 126.8, 127.2, 127.3, 128.4, 129.4, 129.9, 130.0, 134.0, 135.7, 137.0, 138.8, 143.6, 159.5, 159.3, 169.2; HRMS [M+H]⁺ (C₁₆H₁₇BrNO₂) calcd 334.0437, obsd 334.0438.

3-Bromo-*N,N*-bis(4-methoxybenzyl)benzenesulfonamide (13). NaH (60% in mineral oil, 75 mg; 1.86 mmol) was added to a solution of 3-bromobenzenesulfonamide (0.2 g, 0.85 mmol) in DMF (2 mL) and the solution was stirred at room temperature for 5 min. 4-Methoxybenzyl chloride (0.25 mL, 1.86 mmol) was added dropwise, and the mixture was stirred at 80 °C for 2 h and saturated aqueous ammonium chloride solution (5 mL). The resulting solution was extracted with DCM (3 x 20 mL), and the combined organic phases were dried (MgSO₄), and concentrated *in vacuo*. Purification by chromatography on silica, using a gradient elution of 100% EtOAc to Petrol: EtOAc (5:1) gave the title compound as a crystalline white solid (0.34 g; 84%); mp 136-137 °C; IR 3009, 2837, 1608, 1509 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 3.79 (6H, s, OCH₃), 4.27 (4H, s, NCH₂), 6.78 (4H, d, *J* = 8.6 Hz, *H*-3'), 7.01 (4H, d, *J* = 8.6 Hz, *H*-2'), 7.35 (1H, dd, *J* = 7.9, 8.0 Hz, *H*-5), 7.67 (1H, br d, *J* = 7.9 Hz, *H*-4), 7.72 (1H, br d, *J* = 7.9 Hz, *H*-6), 7.83 (1H, m, *H*-2); ¹³C NMR (CDCl₃, 125 MHz) δ 49.8, 55.4, 114.0, 125.7, 127.3, 130.0, 130.1, 130.6, 135.4, 159.3; HRMS [M+H]⁺ (C₂₂H₂₃BrNO₄S) calcd 476.0526, obsd 476.0522.

3-Chloro-*N,N*-bis(4-methoxybenzyl)-2-methylbenzenesulfonamide (14). To a solution of 3-chloro-2-methylbenzenesulfonamide (0.8 g, 3.89 mmol) in DMF (5 mL) was added NaH (60% in mineral oil, 0.2 g, 8.56 mmol) followed by 4-methoxybenzyl chloride (1.16 mL, 8.56 mmol). After stirring at room temperature for 1 h, saturated aqueous NH₄Cl solution (5 mL) was added to the reaction mixture, followed by H₂O (10 mL). The resulting solution was extracted with EtOAc (3 x 20 mL), and the combined organic fractions were washed with brine, dried (MgSO₄), and the solvent was removed *in vacuo*. Purification by chromatography on silica using gradient elution of 100% Petrol to 30% Petrol:EtOAc (3:1) afforded the benzenesulfonamide as a colourless crystalline solid (1.52 g, 88%); mp 66-68 °C; IR 2838, 1611, 1511 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 2.63 (3H, s, Ar-CH₃), 3.80 (6H, s, OCH₃), 4.25 (4H, s, NCH₂), 6.81 (4H, d, *J* = 8.6 Hz, *H*-3'), 6.96 (4H, d, *J* = 8.6 Hz, *H*-2'), 7.23 (1H, dd, *J* = 7.9, 8.0 Hz, *H*-5), 7.59 (1H, dd, *J* = 8.0, 1.0 Hz, *H*-4), 7.89 (1H, dd, *J* = 7.9, 1.1 Hz, *H*-6); ¹³C NMR (500 MHz, CDCl₃) 17.0, 48.8, 55.4, 114.1, 126.6, 127.2,

128.8, 130.2, 133.6, 135.9, 137.2, 140.9, 159.4; HRMS $[M]^+$ ($C_{23}H_{24}ClNO_4S$) calcd 445.1108, obsd 445.1109.

***N*-(4-Methoxybenzyl)-2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide**

(15). A mixture of **12** (0.16 mg, 0.49 mmol), *bis*(pinacolato)diboron (0.19 g, 0.74 mmol), [1,1'-*Bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (17 mg, 0.024 mmol), 1,1'-*Bis*(diphenylphosphino)ferrocene (26 mg, 0.048 mmol) and KOAc (0.144 g, 1.47 mmol) in dioxane (2 mL) was purged with N_2 and heated under microwave irradiation at 150 °C for 90 min. After cooling, the reaction mixture was filtered through Celite, EtOAc (10 mL) was added, and the mixture was washed with brine (2 x 10 mL), dried ($MgSO_4$), and the solvents were evaporated under reduced pressure. Purification by chromatography on silica, employing a gradient eluent of Petrol:EtOAc (10:1) to Petrol:EtOAc (1:1), yielded the title compound as a colourless syrup (0.144 g, 77%); IR 3484, 3181, 2980, 2933, 1644, 1612, 1580, 1512 cm^{-1} ; 1H NMR ($CDCl_3$, 500 MHz) δ 1.34 (12H, s, CH_3), 2.44 (0.6H, s, Ar- CH_3), 2.60 (2.4H, s, Ar- CH_3), 3.78 (0.6H, s, OCH_3), 3.80 (2.4H, s, OCH_3), 4.05 (0.4H, d, J = 6.1 Hz, NCH_2), 4.51 (0.2H, t, J = 5.9 Hz, NH), 4.56 (1.6H, d, J = 5.6 Hz, NCH_2), 5.88 (0.8H, t, J = 4.9 Hz, NH), 6.78-6.82 (0.4H, m, H -3'), 6.85-6.90 (1.6H, m, H -3'), 7.08-7.12 (0.4H, m, H -2'), 7.17 (0.8H, dd, J = 7.5, 7.6 Hz, H -5), 7.26-7.29 (1.6H, m, H -2'), 7.31 (0.4H, d, J = 8.3 Hz), 7.38 (0.8H, dd, J = 7.6, 1.4 Hz, H -4), 7.74-7.80 (1H, m, H -6); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 19.1, 21.6, 24.9, 43.5, 46.9, 55.4, 55.4, 83.8, 114.2, 114.3, 124.9, 127.3, 128.3, 129.1, 129.3, 129.4, 129.8, 130.3, 137.0, 137.3, 137.4, 141.8, 143.6, 159.2, 159.5, 170.6; HRMS $[M]^+$ ($C_{22}H_{28}BNO_4$) calcd 381.2220, obsd 381.2222.

***N,N*-bis(4-Methoxybenzyl)-2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-**

yl)benzenesulfonamide (16). A mixture of **14** (0.18 mg, 0.41 mmol), *bis*(pinacolato)diboron (0.16 g, 0.62 mmol), [1,1'-*Bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (14 mg, 0.02 mmol), 1,1'-*Bis*(diphenylphosphino)ferrocene (22 mg, 0.04 mmol) and KOAc (0.119 g, 1.21 mmol) in dioxane (2 mL) was purged under N_2 for 10 min, and heated under microwave conditions 150 °C for 120 min. The cooled reaction mixture was filtered through Celite, diluted with EtOAc (10 mL),

and washed with brine (3 x 10 mL). The combined organic fractions were dried (MgSO₄) and the solvent was removed under reduced pressure. Purification by chromatography on silica using a gradient elution of Petrol:EtOAc (10:1) from Petrol:EtOAc (1:1) afforded the title compound as a white solid (87 mg, 40%); mp 126-128 °C; IR 2981, 2158, 1610, 1510 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 1.38 (12H, s, CH₃), 2.79 (3H, s, Ar-CH₃), 3.79 (6H, s, OCH₃), 4.23 (4H, s, NCH₂), 6.76-6.81 (4H, m, H-3'), 6.92-6.98 (4H, m, H-2'), 7.28 (1H, dd, *J* = 7.8, 7.4 Hz, H-5), 7.95 (1H, dd, *J* = 7.4, 1.3 Hz, H-4), 8.07 (1H, dd, *J* = 7.8, 1.4 Hz, H-6); ¹³C NMR (CDCl₃, 125 MHz): 19.1, 25.0, 48.6, 55.4, 84.1, 114.0, 125.2, 127.7, 130.2, 132.6, 139.0, 140.2, 144.6, 159.2; HRMS [M]⁺ (C₂₉H₃₆NO₆BS) calcd 537.2465, obsd 537.2465.

6-Acylamino-2-amino-4-cyclohexylmethoxy-5-nitrosopyrimidines: Method I. General

Procedure. To a solution of 2,6-diamino-4-cyclohexylmethoxy-5-nitrosopyrimidine¹⁸ (**17**) (1.0 mol. equiv.) in DMF (5-10 mL) was added the appropriate anhydride (1.1 mol. equiv.), and the mixture was heated at 80 °C for the specified time. After cooling, volatiles were removed under reduced pressure, water (10-20 mL) was added, and the reaction mixture was extracted with EtOAc (3 x 20 mL). The combined organic fractions were dried (Na₂SO₄), concentrated *in vacuo*, and the required acylaminopyrimidines (**18-23**) were isolated by chromatography on silica as described.

6-Acetyl-amino-2-amino-4-cyclohexylmethoxy-5-nitrosopyrimidine (18). Prepared from **17** (0.50 g, 2.0 mmol) and acetic anhydride (4 mL) in accordance with Method I, and purified by chromatography on silica (eluent DCM:MeOH, 10:1) to furnish **18** as an aquamarine crystalline solid (0.35 g; 60%); mp 149-150 °C; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 1.13-1.29 (5H, m, cyclohexyl), 1.79-1.94 (6H, m, cyclohexyl), 2.57 (3H, s, Me), 4.45 (2H, d, *J* = 6.3, CH₂O), 8.73 (2 H, br s, NH₂), 12.3 (1 H, br s, NH). HRMS [M]⁺ (C₁₃H₁₉N₅O₃) calcd 293.1488, obsd 293.1484. Anal. (C₁₃H₁₉N₅O₃ + 0.05 EtOAc) C, H, N.

2-Amino-4-cyclohexylmethoxy-5-nitroso-6-propionylaminopyrimidine (19). Synthesized from **17** (0.50 g, 2.0 mmol) and propionic anhydride (0.29 g, 2.2 mmol) in DMF (6.0 mL) following Method I; purified by chromatography on silica employing Petrol: EtOAc (3:2) as eluent, to give

the title compound as an aquamarine crystalline solid (0.28 g, 46%): mp 160-161 °C; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 1.11-1.18 (3H, t, *J* = 7.3 Hz, CH₂CH₃), 1.23-1.30 (5H, m, cyclohexyl), 1.78-1.94 (6H, m, cyclohexyl), 2.95 (2H, q, *J* = 7.3 Hz, CH₂CH₃), 4.44 (2H, d, *J* = 6.3 Hz, CH₂O), 8.72 (2H, br s, NH₂), 12.4 (1 H, br s, NH); HRMS [M]⁺ (C₁₄H₂₁N₅O₃) calcd 307.1644, obsd 307.1632. Anal. (C₁₄H₂₁N₅O₃ + 0.3 EtOAc) C, H, N.

2-Amino-4-cyclohexylmethoxy-6-isobutylamino-5-nitrosopyrimidine (20). From **17** (0.82 g, 3.25 mmol) and isobutyric anhydride (0.57 g 3.57 mmol) in DMF (12 mL) employing Method I; purification by chromatography on silica with Petrol:EtOAc (3:2) as eluent, followed by recrystallisation from EtOAc-Petrol to furnish **20** as a pale blue crystalline solid (0.63 g, 60%); mp 180-181 °C; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 0.92-1.39 (5H, m, cyclohexyl), 1.22 (6H, d, *J* = 6.8 Hz, CH(CH₃)₂), 1.78-1.93 (6 H, m, cyclohexyl), 3.26 (1H, quin, *J* = 6.8 Hz, CH(CH₃)₂), 4.44 (2 H, d, *J* = 6.8 Hz, CH₂CH), 8.74 (2 H, br s, NH₂), 12.6 (1 H, br s, NH). Anal. (C₁₅H₂₃N₅O₃) C, H, N.

2-Amino-6-benzoylamino-4-cyclohexylmethoxy-5-nitrosopyrimidine (21). Synthesized following Method I from **17** (0.50 g, 2.0 mmol) and benzoic anhydride (0.50 g; 2.2 mmol) in DMF (8 mL), and isolated by chromatography on silica using DCM:MeOH (95:5) as eluent. Recrystallisation from EtOAc-Petrol yielded **21** as a blue crystalline solid (0.24 g; 33.8%); mp 210-211 °C; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 1.16-1.43 (5H, m, cyclohexyl), 1.81-1.98 (6 H, m, cyclohexyl), 4.50 (2H, d, *J* = 6.3 Hz, OCH₂), 7.75-7.86 (3H, m, Ar-H), 8.08-8.12 (2H, m, Ar-H), 8.88 (1 H, br s, NH), 8.99 (1H, br s, NH), 13.8 (1H, br s, NH); HRMS [M]⁺ (C₁₈H₂₁N₅O₃) calcd 355.1644, obsd 355.1646. Anal. (C₁₈H₂₁N₅O₃) C, H, N.

2-Amino-6-(2-chlorobenzoyl)amino-4-cyclohexylmethoxy-5-nitrosopyrimidine (22). To a stirred solution of **17** (0.50 g, 2.00 mmol) and *N,N*-dimethyl-4-aminopyridine (24 mg, 0.20 mmol) in pyridine (12 mL), was added 2-chlorobenzoyl chloride (280 μL, 2.20 mmol). The solution was stirred at 25 °C under N₂ for 18h, concentrated under reduced pressure, and the residual solid was dissolved in DCM (40 mL) and washed with aqueous hydrochloric acid solution (5% v/v, 3 x 20 mL). The organic fraction was dried (Na₂SO₄) and the solvent was removed *in vacuo* to give a dark

green solid, which was recrystallized from EtOAc-Petrol to furnish the title compound as a blue-green crystalline solid (0.6 g; 78%); mp 184-185 °C; IR 3467, 3202, 2925, 1728, 1594, 1536 cm⁻¹; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 0.94-1.28 (5H, m, cyclohexyl), 1.60-1.97 (6H, m, cyclohexyl), 4.35 (2H, d, *J* = 6.6 Hz, OCH₂), 6.15 (2H, s, NH₂), 7.32-7.53 (3H, m, Ar-H), 7.54 (1H, d, Ar-H); ¹³C NMR, (*d*₆-DMSO, 75 MHz) δ 26.0, 26.7, 30.0, 37.5, 74.0, 127.7, 129.9, 131.3, 132.0, 132.8, 135.1, 139.6, 164.9, 166.6; MS (ES⁺) *m/z* 390 [M(³⁵Cl)+H]⁺, 392 [M(³⁷Cl)+H]⁺. Anal. (C₁₈H₂₀ClN₅O₃ + 0.1 EtOAc) C, H, N.

2-Amino-4-cyclohexylmethoxy-6-(2-methylbenzoyl)amino-5-nitrosopyrimidine (23). Prepared analogously to **22** above from **17** (0.51 g, 2.00 mmol) and 2-methylbenzoyl chloride (300 µL, 2.20 mmol) in pyridine (12 mL). Recrystallisation from EtOAc-Petrol gave a dark blue crystalline solid (0.57 g; 77%); mp 177-179 °C; IR 3418, 3233, 2916, 1713, 1598, 1520 cm⁻¹; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 0.94-1.28 (5H, m, cyclohexyl), 1.60-1.95 (6H, m, cyclohexyl), 2.47 (3H, s, ArCH₃), 4.35 (2H, d, *J* = 6.7 Hz, OCH₂), 6.17 (1H, s), 7.22-7.57 (3 H, m, Ar-H), 7.57 (1H, s), 7.59 (1H, d, *J* = 1.2 Hz); ¹³C NMR, (*d*₆-DMSO, 75 MHz) δ 21.0, 26.0, 26.7, 30.1, 37.5, 73.9, 126.8, 128.3, 132.2, 132.3, 134.5, 138.8, 139.8, 165.1, 168.9; MS (ES⁺) *m/z* 370 [M+H]⁺. Anal. (C₁₉H₂₃N₅O₃) C, H, N.

8-Substituted 2-amino-6-cyclohexylmethoxypurines: Method II. General Procedure. A stirred solution of the appropriate 6-acylamino-2-amino-4-cyclohexylmethoxy-5-nitrosopyrimidine (0.5-2.0 mmol) in THF (20-30 mL) containing AcOH (glacial, 1-2 mL) was cooled to -78 °C and purged with N₂, prior to addition of Pd (10% on carbon, 15% w/w). The reaction mixture was stirred under H₂ at atmospheric pressure for 24 h at ambient temperature, filtered through Celite, and the filtrate was neutralised with saturated NaHCO₃ solution (30-50 mL) and extracted with EtOAc (3 x 30 mL). The combined organic fractions were dried (Na₂SO₄) and evaporated under reduced pressure to give the required 8-substituted purine. Compound purification was achieved by chromatography on silica and recrystallisation as described.

2-Amino-6-cyclohexylmethoxy-8-methylpurine (24). Prepared in accordance with Method II from **18** (0.35 g, 1.20 mmol), and isolated by chromatography on silica employing DCM:MeOH

(10:1) as eluent. Recrystallisation from a mixture of EtOAc-MeOH gave **24** as a cream crystalline solid (0.19 g, 60%); mp 99-100 °C; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 1.08-1.45 (5 H, m, cyclohexyl), 1.78-1.92 (6 H, m, cyclohexyl), 2.42 (3H, s, Me), 4.26 (2 H, d, *J* = 6.2 Hz, OCH₂), 6.20 (2 H, br s, NH₂), 12.2 (1 H, br s, NH); HRMS [M]⁺ (C₁₃H₁₉N₅O) calcd 261.1590, obsd 261.1587. Anal. (C₁₃H₁₉N₅O + 1.15 MeOH) C, H, N.

2-Amino-6-cyclohexylmethoxy-8-ethylpurine (25). Synthesised by Method II from pyrimidine **19** (0.28 g, 0.91 mmol) and isolated by chromatography on silica employing Petrol:EtOAc (3:2) as eluent. Recrystallisation from a mixture of EtOAc-Et₂O gave **25** as a cream solid. (0.10 g, 39.9%); mp 137-138 °C; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 1.06-1.18 (5 H, m, cyclohexyl), 1.31 (3 H, t, *J* = 7.5 Hz, CH₂CH₃), 1.76-1.90 (6 H, m, cyclohexyl), 2.74 (2 H, q, *J* = 7.5 Hz, CH₂CH₃), 4.23 (2 H, d, *J* = 6.1 Hz, OCH₂), 6.18 (2 H, br s, NH₂), 12.2 (1 H, br s, NH); HRMS [M]⁺ (C₁₄H₂₁N₅O) calcd 275.1746, obsd 275.1743. Anal. (C₁₄H₂₁N₅O + 0.1 EtOAc) C, H, N.

2-Amino-6-cyclohexylmethoxy-8-isopropylpurine (26). In accordance with Method II from **20** (0.63 g; 2.0 mmol). Isolated by chromatography on silica with DCM:MeOH (10:1) as eluent, followed by recrystallisation from EtOAc-Et₂O to yield the title purine as a cream crystalline solid (0.39 g; 70%); mp 136-138 °C; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 1.08-1.19 (5 H, m, cyclohexyl), 1.36 (6 H, d, *J* = 6.9 Hz, CH(CH₃)₂) 1.79-1.92 (6 H, m, cyclohexyl), 3.07 (1 H, quin, *J* = 6.9 Hz, CH(CH₃)₂), 4.25 (2 H, d, *J* = 6.1 Hz, OCH₂), 6.20 (2 H, br s, NH₂), 12.6 (1 H, s, NH). (C₁₅H₂₃N₅O + 0.25 H₂O) C, H, N.

2-Amino-6-cyclohexylmethoxy-8-phenylpurine (27). Prepared following Method II from **21** (0.24 g, 0.67 mmol) and isolated by chromatography on silica employing DCM:MeOH (10:1) as eluent. Recrystallisation from MeOH-EtOAc gave **27** as pale pink crystals (0.16 g, 76%); mp 271-272 °C; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 1.12-1.31 (5H, m, cyclohexyl), 1.81-1.97 (6H, m, cyclohexyl), 4.33 (2H, d, *J* = 6.2 Hz, OCH₂), 6.43 (2H, br s, NH₂), 7.56-7.60 (3H, m, Ar-H), 8.16 (2 H, m, Ar-H), 13.0 (1H, br s, NH); HRMS [M]⁺ (C₁₈H₂₁N₅O) calcd 323.1746, obsd 323.1746. (C₁₈H₂₁N₅O + 0.4 MeOH) C, H, N.

2-Amino-8-(2-chlorophenyl)-6-cyclohexylmethoxypurine (28). Prepared from **22** (375 mg; 0.971 mmol) following Method II, and purified by chromatography on silica employing EtOAc: Petrol (2:1), to afford the title compound as an off-white powder (241 mg, 70%); mp 190-191 °C; IR 3487, 3289, 3171, 2021, 1578 cm⁻¹; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 1.02-1.33 (5H, m, cyclohexyl), 1.64-1.83 (6H, m, cyclohexyl), 4.23 (2H, d, *J* = 6.24 Hz, OCH₂), 6.34 (2H, br s, NH₂), 7.45-7.54 (2H, m, Ar-H), 7.61-7.64 (1H, m, Ar-H), 7.73-7.75 (1H, m, Ar-H), 12.69 (1 H, br s, NH); ¹³C NMR, (*d*₆-DMSO, 75 MHz) δ 25.6, 26.4, 29.6, 37.2, 70.9, 114.7, 127.6, 130.4, 130.5, 131.3, 132.2, 145.1, 156.2, 160.2, 160.8; MS (ES⁺) *m/z* 358 [M(³⁵Cl)+H]⁺, 360 [M(³⁷Cl)+H]⁺. Anal. (C₁₈H₂₀ClN₅O + 0.1 EtOAc) C, H, N.

2-Amino-6-cyclohexylmethoxy-8-(2-methylphenyl)purine (29). Synthesized in accordance with Method II from **23** (327 mg; 0.88 mmol) and purified by chromatography on silica with EtOAc:Petrol (2:1); pale yellow powder (302 mg, 100%); mp 187-188 °C; IR 3480, 3310, 3179, 3085, 2045, 1582 cm⁻¹; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 1.01-1.29 (5H, m, cyclohexyl), 1.64-1.84 (6H, m, cyclohexyl), 2.54 (3H, s, Ar-CH₃), 4.25 (2H, d, *J* = 6.4 Hz, OCH₂), 6.28 (2H, br s, NH₂), 7.28-7.38 (3H, m, Ar-H), 7.64 (1H, m, Ar-H), 12.60 (1H, br s, NH); ¹³C NMR, (*d*₆-DMSO, 75 MHz) δ 21.3, 25.5, 26.3, 29.6, 37.2, 70.9, 114.9, 126.2, 129.3, 129.6, 130.3, 131.4, 137.6, 147.8, 156.3, 160.0, 160.6; MS (ES⁺) *m/z* 338 [M+H]⁺; HRMS [M+H]⁺ (C₁₉H₂₄N₅O) calcd 338.1975 found 338.1973; [M-H]⁻ (C₁₉H₂₂N₅O) calcd 336.1830 found 336.1826. Anal. (C₁₉H₂₃N₅O + 0.2 EtOAc) C, H, N.

Purine **29** was also synthesised by the following alternative method: a mixture of 8-bromo-6-(cyclohexylmethoxy)-9H-purin-2-amine (83 mg; 0.25 mmol), *o*-tolyl boronic acid (69 mg; 0.51 mmol), Cs₂CO₃ (166 mg; 0.51 mmol) and dichloro [1,1' *bis*(*di-tert*-butylphosphino)]ferrocene palladium (II) (8 mg; 0.01 mmol) in toluene (15 mL) was degassed with nitrogen and heated under at 100 °C with stirring for 3 h. After cooling, the reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure to give a yellow solid. The crude material was purified by chromatography on silica, employing a gradient of Petrol:EtOAc (1:1 to 0:1) as eluent to

afford **29** as a beige-white solid (60 mg; 70%). All analytical data (mp, NMR, MS) were identical with **29** prepared previously.

2,4,6-Triamino-5-cyclohexylmethoxypyrimidine (30). Zinc powder (0.26 g, 3.9 mmol) was added to a solution of 2,6-diamino-4-cyclohexylmethoxy-5-nitrosopyrimidine (**17**, 0.20 g, 0.79 mmol) in AcOH (5 mL), and the solution was stirred for 12 h at room temperature. The reaction mixture was filtered through Celite and the filtrate was evaporated under reduced pressure to furnish a yellow solid, which was redissolved in water (20 mL) and the solution was adjusted to pH 7.0 with saturated aqueous sodium bicarbonate solution. The mixture was extracted with EtOAc (3 x 30 mL), the combined organic layers were dried (Na₂SO₄), and the solvent was removed *in vacuo* to give a pale yellow solid. Recrystallisation from MeOH afforded the title compound (0.14 g, 74%); mp 151-153 °C; IR 3446, 3390, 3309, 3178, 2918, 2847, 1613, 1577 cm⁻¹; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 0.92-1.29 (5H, m, cyclohexyl), 1.67-1.79 (6H, m, cyclohexyl), 3.39 (2H, br, NH₂), 3.93 (2H, d, *J* = 6.0 Hz, OCH₂), 5.27 (2H, br, NH₂), 5.66 (2H, br, NH₂); ¹³C NMR, (*d*₆-DMSO, 75 MHz) δ 24.9, 25.7, 28.9, 36.7, 69.6, 99.9, 155.2, 155.4, 157.8; HRMS [*M*]⁺ (C₁₁H₁₉N₅O) calcd 237.1587, obsd 237.1590. Anal. (C₁₁H₁₉N₅O + 0.1 H₂O) C, H, N.

2,5-Diamino-4-cyclohexylmethoxy-6-(2,2,2-trifluoroacetyl)aminopyrimidine (31). A solution of **30** (171 mg; 0.72 mmol) in TFA (5 mL) was heated under reflux for 6 h, and the solvent was evaporated *in vacuo*. The title compound was isolated as a yellow solid (213 mg; 91%) by chromatography on silica using DCM:MeOH, (9:1) as eluent, and used directly for the next reaction; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 0.95-1.17 (5H, m, cyclohexyl), 1.64-1.67 (6H, m, cyclohexyl), 3.98 (2H, d, *J* = 3.9, OCH₂), 6.98 (2H, br s, NH₂), 7.24 (2H, br s, NH₂), 9.97 (1H, s, NHCO); MS (ES⁺) *m/z* 334 [*M*+H]⁺.

2-Amino-6-cyclohexylmethoxy-8-trifluoromethylpurine (32). To a solution of **31** (213 mg; 0.64 mmol) in pyridine (6 mL) was added *N,N*-dimethyl-4-aminopyridine (21 mg; 0.17 mmol) and the mixture was heated under reflux for 5 days. The residual red oil remaining, following removal of the solvent under reduced pressure, was purified by chromatography on silica utilising using DCM:

MeOH (95:5) furnished the title compound as a pale orange crystalline solid (106 mg; 53%); mp 252-253 °C; IR 3496, 1632, 1585 cm⁻¹; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 0.97-1.32 (5 H, m, cyclohexyl), 1.63-1.81 (6 H, m, cyclohexyl), 4.23 (2H, d, *J* = 6.2 Hz, OCH₂), 6.76 (2H, br s, NH₂), 13.71 (1H, s, NH); ¹³C NMR (*d*₆-DMSO, 75 MHz) δ 25.5, 26.3, 29.5, 37.1, 71.3, 114.2, 117.7, 121.3, 124.8, 155.3, 160.5, 161.9; MS (ES⁺) *m/z* 316 [M+H]⁺. Anal. (C₁₃H₁₆F₃N₅O + 0.1 H₂O) C, H, N.

2-Amino-9-benzyl-6-cyclohexylmethoxypurine (33). To a solution of **5** (2.60 g, 10.5 mmol) in DMF (20 mL) was added K₂CO₃ (7.3 g, 52.6 mmol) and benzyl bromide (1.5 mL, 12.6 mmol), and the mixture was stirred at room temperature for 12 h. Volatiles were removed *in vacuo*, and the residual oil was redissolved in EtOAc (20 mL), washed with water (3 x 25 mL), and the organic fraction was dried (Na₂SO₄) and evaporated under reduced pressure. Purification by chromatography on silica, using EtOAc/Petrol (1:1) as eluent, gave the 9-benzylpurine (**33**) as a white solid (1.04 g, 29%); IR 3394, 3341, 3225, 3123, 2923, 2851, 1733, 1643, 1518 cm⁻¹; ¹H NMR (*d*₆-DMSO, 300 MHz) δ 0.95-1.27 (5H, m, cyclohexyl), 1.61-1.95 (6H, m, cyclohexyl), 4.21 (2H, d, *J* = 6.3 Hz, OCH₂), 5.25 (2H, s, CH₂Ph), 6.42 (2H, s, NH₂), 7.15-7.40 (5H, m, Ar-H), 7.95 (1H, s, purine C8-H); ¹³C NMR (*d*₆-DMSO, 75 MHz) δ 26.1, 26.8, 30.2, 37.8, 47.2, 72.4, 116.0, 128.0, 128.5, 129.3, 136.4, 139.3, 154.7, 159.9, 162.3.

9-Benzyl-8-(3-(benzyloxy)-2-methylphenyl)-6-(cyclohexylmethoxy)-9H-purin-2-amine (34) 1-Benzyloxy-3-bromo-2-methylbenzene (616 mg, 2.22 mmol) and caesium carbonate (604 mg, 1.85 mmol) were added to a solution of **33** (250 mg, 0.74 mmol) in DMF (15 mL). The mixture was purged with N₂ for 15 min, prior to addition of copper (I) iodide (423 mg, 2.22 mmol) and Pd(OAc)₂ (0.1M in DMF, 0.37 mL), and heated for 40 minutes under microwave irradiation at 200 °C. The solvent was removed under reduced pressure, and the residual oil was redissolved in EtOAc (25 mL) and washed with water (3 x 25 mL), dried (Na₂SO₄), and the organic fraction was evaporated *in vacuo*. The product was purified by chromatography on silica with Petrol:AcOEt (2:1) as eluent, to furnish the title compound as a pale yellow solid (190 mg, 48%); IR: 3376, 3346,

3223, 2923, 2848, 1648, 1605, 1578 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.95-1.15 (5H, m, cyclohexyl), 1.50-1.95 (6H, m, cyclohexyl), 1.85 (3H, s, Ar- CH_3), 4.23 (2H, d, $J = 6.0$ Hz, OCH_2), 4.84 (2H, br s, NH_2), 4.99 (2H, s, N- CH_2 -Ar), 5.04 (2H, s, O- CH_2 -Ar), 6.71-7.40 (13H, m, Ar-H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 13.5, 26.2, 26.9, 30.3, 37.9, 46.7, 70.9, 72.5, 113.8, 123.3, 126.6, 127.5, 127.9, 128.0, 128.2, 128.7, 128.9, 129.3, 131.9, 136.9, 137.7, 157.5, 159.7.

Benzyl 3-(2-amino-9-benzyl-6-(cyclohexylmethoxy)-9H-purin-8-yl)-2-methyl benzoate (35).

Synthesised in a manner analogous to that for **34** above, from **33** (250 mg, 0.74 mmol) and benzyl 3-bromo-2-methylbenzoate (678 mg, 2.22 mmol); Purification by chromatography on silica with Petrol:AcOEt (2:1) as eluent, gave the title compound as a yellow powder (131 mg, 32%); IR 3349, 3225, 2923, 2851, 1721, 1653, 1603, 1580 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.92-1.17 (5H, m, cyclohexyl), 1.55-1.97 (6H, m, cyclohexyl), 2.18 (3H, s, Ar CH_3), 4.32 (2H, d, $J = 6.0$ Hz, OCH_2), 4.93 (2H, s, NH_2), 5.05 (2H, s, N- CH_2 -Ar), 5.37 (2H, s, O- CH_2 -Ar), 6.82-6.87 (2H, m, Ar-H), 7.10-7.50 (10H, m, Ar-H), 8.01 (1H, d, $J = 7.5$ Hz, Ar-H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 18.2, 26.1, 26.9, 30.3, 37.8, 46.7, 67.2, 72.5, 115.7, 125.6, 128.0, 128.5, 128.6, 128.8, 128.9, 131.8, 132.2, 132.6, 134.3, 136.4, 136.6, 140.6, 149.2, 155.6, 159.8, 162.1, 167.5.

3-(2-Amino-6-cyclohexylmethoxy-9H-purin-8-yl)-2-methylphenol (36). To a solution of **34** (125 mg, 0.23 mmol) in acetic acid (10 mL), containing hydrochloric acid (12M, 0.25 mL), was added Pd (10% on carbon, 125 mg), and the reaction mixture was stirred under H_2 at atmospheric pressure for 12 h. Water (10 mL) was added, and the mixture was heated under reflux for 1h, filtered through Celite, and the solvents were evaporated under reduced pressure. Purification by semi-preparative HPLC afforded the title compound as white crystals (11 mg, 13%); ^1H NMR (d_6 -DMSO, 300 MHz) δ 0.99-1.84 (m, 11H, cyclohexyl), 2.17 (3H, s, Ar- CH_3), 4.21 (2H, d, $J = 6.3$ Hz, OCH_2), 6.80 (1H, d, $J = 7.8$ Hz, Ar-H), 6.90 (1H, d, $J = 7.5$ Hz, Ar-H), 6.99-7.05 (1H, dd, $J = 7.8, 7.5$ Hz, Ar-H), 8.43 (2H, br s, NH_2); HRMS $[\text{M}+\text{H}]^+$ ($\text{C}_{19}\text{H}_{24}\text{N}_5\text{O}_2$) calcd 354.1925, obsd 354.1925.

3-(2-Amino-6-cyclohexylmethoxy-9H-purin-8-yl)-2-methylbenzoic acid (37). Prepared as described for **36** from **35** (105 mg, 0.19 mmol) and Pd (10% on carbon, 105 mg); purification by

semi-preparative HPLC gave the title compound as a white powder (17 mg, 24%); ^1H NMR (d_6 -DMSO, 300 MHz) δ 0.99-1.71 (m, 11H, cyclohexyl), 2.36 (3H, s, ArCH₃), 4.21 (2H, d, J = 6.3 Hz, CH₂), 7.15-7.20 (1H, m, Ar-H), 7.34 (1H, dd, J = 1.2, 7.5 Hz, Ar-H), 7.41 (1H, dd, J = 1.2, 7.5 Hz, Ar-H); ^{13}C NMR (d_6 -DMSO, 75 MHz) 18.3, 27.3, 27.9, 31.0, 31.3, 39.3, 41.1, 73.3, 126.7, 129.9, 130.7, 132.6, 135.2, 142.8, 145, 150.0, 161.8; HRMS $[\text{M}+\text{H}]^+$ (C₂₀H₂₄N₅O₃) calcd 382.1874, obsd 382.1872.

2-Amino-6-cyclohexylmethoxy-purine-9-carboxylic acid *t*-butyl ester (38). To a stirred suspension of **5** (98.4 mg; 4.0 mmol) and potassium carbonate (67.1 mg; 4.8 mmol) in DMF (12 mL) was added di-*t*-butyl dicarbonate (1.2 mL; 5.0 mmol), and the mixture was stirred at room temperature for 18 h. Water (20 mL) was added and the reaction mixture was stirred a further 1 h, whereupon the resulting white precipitate was collected and triturated with Et₂O to give the product as a white solid (1.16 g; 84%); R_f = 0.61 (F); ^1H NMR (CDCl₃, 300 MHz) δ 1.01-1.32 (5 H, m, cyclohexyl), 1.68 (9H, s, C(CH₃)₃), 1.74-1.92 (6H, m, cyclohexyl), 4.28 (2H, d, J = 6.3 Hz, OCH₂), 5.14 (2H, br s, NH₂), 8.00 (1H, s, NH); ^{13}C NMR (CDCl₃, 75 MHz) δ 26.1, 26.8, 28.4, 30.1, 37.6, 72.5, 86.5, 137.7, 146.6, 153.9, 161.1, 162.3. HRMS $[\text{M}]^+$ (C₁₇H₂₅N₅O₃) calcd 347.1948, obsd 347.1957.

2-Amino-8-bromo-6-cyclohexylmethoxypurine (39). To a stirred suspension of **38** (1.13 g; 3.26 mmol) in MeCN (36 mL) and water (8.6 mL) was added *N*-bromosuccinimide (87.4 mg; 4.89 mmol). The mixture was stirred at room temperature for 1 h, filtered, and the filtrate concentrated under reduced pressure to give a tan solid. Purification by chromatography on silica using gradient elution with EtOAc: Petrol (2:1) as eluent afforded the title compound as an off-white solid (442 mg, 41.6%); mp 228-229 °C; IR 3482, 3303, 3163, 2921, 2850, 1623, 1563 cm⁻¹; ^1H NMR (CDCl₃, 300 MHz) δ 1.04-1.31 (5H, m, cyclohexyl), 1.70-1.91 (6H, m, cyclohexyl), 4.28 (2H, d, J = 5.8 Hz, OCH₂), 5.10 (2 H, br s, NH₂); ^{13}C NMR (CDCl₃, 75 MHz): 26.1, 26.8, 30.1, 37.6, 72.8, 155.6, 158.8, 161.0; HRMS $[\text{M}]^+$ (C₁₂H₁₆BrN₅O) calcd 325.0536, obsd 325.0538.

Bromopurine **39** was also prepared directly from **5** as follows: A mixture of **5** (1.0 g; 4.0 mmol) and pyridinium tribromide (2.58 g; 8.1 mmol) in DCM (10 mL) was stirred at 70 °C for 96 h. Saturated aqueous sodium metabisulfite solution (10 mL) was added, followed by water (10 mL), and the mixture was extracted with EtOAc (3 x 20 mL). The organic phase was washed with brine (2 x 10 mL), dried (MgSO₄) and concentrated *in vacuo*. The residual solid was purified by chromatography on silica, employing EtOAc: MeOH (10:1) as eluent, to give **39** as an off-white solid (0.39 g; 29%), identical (mp, NMR, MS) to that prepared above.

PMB-protected 2-amino-8-aryl-6-cyclohexylmethoxypurines (40-42): Method III. General

Procedure. A mixture of **39** (1.0 mol. equiv.), the appropriate benzamide (**15**) or sulfonamide (**16**) (1.0 mol. equiv.) dichloro [1,1' *bis*(di-*tert*-butylphosphino)]ferrocene palladium (II) ('Pd-118') (0.5 mol. equiv.) and Cs₂CO₃ (2.0 mol. equiv.) in dioxane:H₂O (4:1, 2.5 mL), was purged under N₂ for 15 min and stirred for 4 h at 110 °C. The reaction mixture was filtered through Celite, EtOAc (10 mL) was added, and the organic fraction was washed sequentially with saturated aqueous NH₄Cl and brine, dried over MgSO₄, filtered and concentrated *in vacuo*. The target compound was isolated by chromatography on silica as described.

3-(2-Amino-6-(cyclohexylmethoxy)-9H-purin-8-yl)-N-(4-methoxybenzyl)-2-methylbenzamide (40). Prepared in accordance with Method III from **39** (98 mg, 0.26 mmol) and **15** (84 mg, 0.26 mmol). Purification by chromatography on silica using a gradient eluent of 100% DCM to DCM:MeOH (5:1) afforded the title compound as a beige solid (54 mg; 42%); mp 252-255 °C; IR 3485, 3185, 2926, 1580, 1512 cm⁻¹; ¹H NMR (*d*₆-DMSO, 500 MHz) δ 0.98-1.09 (2H, m, cyclohexyl), 1.13-1.32 (3H, m, cyclohexyl), 1.62-1.75 (3H, m, cyclohexyl), 1.77-1.87 (3H, m, cyclohexyl), 2.43 (3H, s, Ar-CH₃), 3.74 (3H, s, OCH₃), 4.24 (2H, d, *J* = 6.4 Hz, OCH₂), 4.39 (2H, d, *J* = 6.0 Hz, NCH₂), 6.28 (2H, s, NH₂), 6.91 (2H, d, *J* = 8.7 Hz, *H*-3'), 7.28 (2H, d, *J* = 8.7 Hz, *H*-2'), 7.32-7.41 (2H, m, *H*-4, *H*-5), 7.61 (1H, br d, *J* = 7.0 Hz, *H*-6), 8.87 (1H, t, *J* = 6.0 Hz, NH), 12.63 (1H, s, N⁹-H); ¹³C NMR (*d*₆-DMSO, 125 MHz) δ 17.3, 25.2, 26.0, 29.3, 36.8, 41.8, 55.0, 70.6,

113.7, 125.5, 127.6, 128.5, 130.4, 131.5, 139.2, 158.2, 159.7, 168.9; HRMS $[M+H]^+$ ($C_{28}H_{33}N_6O_3$) calcd 501.2609, obsd 501.2598.

3-(2-Amino-6-(cyclohexylmethoxy)-9H-purin-8-yl)-N,N-bis(4-methoxybenzyl)

benzenesulfonamide (41). Prepared following Method III from 8-bromo-6-(cyclohexylmethoxy)-9H-purin-2-amine **39** (32 mg, 0.098 mmol), except that the diisopropyl (3-(N,N-bis(4-methoxybenzyl)sulfamoyl)phenyl)boronate was generated *in situ* as follows: to a solution of 3-bromo-N,N-bis(4-methoxybenzyl)benzenesulfonamide **13** (62 mg, 0.13 mmol) in THF (2 mL), at -78 °C was added *n*-BuLi (2.5 M in hexane, 55 μ L, 0.14 mmol) dropwise. The mixture was stirred for 10 min, triisopropylborate (35 μ L, 0.15 mmol) was added, and stirring was continued for a further 15 min at -78 °C. The reaction mixture was allowed to warm to room temperature overnight, and the solvent was evaporated under reduced pressure to give diisopropyl (3-(N,N-bis(4-methoxybenzyl)sulfamoyl)phenyl)boronate (72 mg, 0.137 mmol) which was used directly.

Purification by chromatography on silica, using a gradient elution from EtOAc:Petrol (65:15) to 100% EtOAc, yielded the title compound as a white solid (18 mg; 29%); 1H NMR ($CDCl_3$, 500 MHz,) δ 0.91-1.05 (2H, m, cyclohexyl), 1.14-1.29 (3H, m, cyclohexyl), 1.63-1.87 (6H, m, cyclohexyl), 3.69 (6H, s, OCH_3), 4.03-4.23 (6H, m, NCH_2 , OCH_2), 4.94 (2H, s, NH_2), 6.66 (4H, d, $J = 8.1$ Hz, $H-3'$), 6.85 (4H, d, $J = 7.9$ Hz, $H-2'$), 7.48 (1H, dd, $J = 7.6, 7.9$ Hz, $H-3$), 7.72 (1H, d, $J = 7.6$ Hz, $H-4$), 8.29 (1H, br s, $H-6$), 8.40 (1H, s, $H-2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 25.8, 26.6, 29.9, 37.2, 49.8, 55.3, 72.3, 113.9, 116.6, 124.6, 127.5, 127.8, 129.8, 129.9, 130.4, 131.1, 141.9, 146.6, 155.5, 159.2, 159.4, 161.7; HRMS $[M+H]^+$ ($C_{34}H_{39}N_6O_5S$) calcd 643.2697, obsd 643.2693.

3-(2-Amino-6-(cyclohexylmethoxy)-9H-purin-8-yl)-N,N-bis(4-methoxybenzyl)-2-

methylbenzenesulfonamide (42). Prepared in accordance with Method III from **39** (104 mg, 0.32 mmol) and **16** (171 mg, 0.32 mmol). Purification by chromatography on silica) with a gradient elution of 100% DCM to DCM:MeOH (10:1) gave the title compound as an-off white solid (24 mg; 24%); mp 209-210 °C; IR 3366, 2926, 1582, 1512 cm^{-1} ; 1H NMR (d_6 -DMSO, 500 MHz) δ 0.99-1.11 (2H, m, cyclohexyl), 1.13-1.32 (3H, m, cyclohexyl), 1.63-1.76 (3H, m, cyclohexyl), 1.78-1.88

(3H, m, cyclohexyl), 2.63 (3H, s, Ar-CH₃), 3.72 (6H, s, OCH₃), 4.21-4.30 (6H, m, OCH₂, NCH₂), 6.35 (2H, s, NH₂), 6.84 (4H, d, *J* = 8.7 Hz, *H*-3'), 6.98 (4H, d, *J* = 8.7 Hz, *H*-2'), 7.50 (1H, dd, *J* = 7.3, 7.9 Hz, *H*-5), 7.84 (1H, d, *J* = 7.3 Hz, *H*-4), 7.95 (1H, d, *J* = 7.9 Hz, *H*-6), 12.76 (1H, s, N⁹-H); ¹³C NMR (*d*₆-DMSO, 125 MHz) δ 16.9, 25.2, 25.9, 29.3, 36.8, 48.9, 55.0, 70.6, 113.8, 114.5, 126.0, 127.3, 129.7, 129.9, 133.7, 134.6, 136.3, 139.6, 146.0, 155.9, 158.7, 159.8, 160.4. HRMS [M+H]⁺ (C₃₅H₄₁N₆O₅S) calcd 657.2854, obsd 657.2840.

2-Amino-8-aryl-6-cyclohexylmethoxypurines (43-45): Method IV. General Procedure. A

solution of the appropriate PMB-protected 2-amino-8-aryl-6-cyclohexylmethoxypurine (0.04-0.2 mmol) in TFA (1-2 mL) was stirred at the specified temperature until the reaction was complete.

The reaction mixture was neutralised with saturated aqueous Na₂CO₃ solution, extracted with EtOAc (3 x 10 mL), and the combined organic fractions were dried (MgSO₄) filtered and evaporated *in vacuo*. The target compounds were isolated by chromatography on silica as described.

3-(2-Amino-6-(cyclohexylmethoxy)-9H-purin-8-yl)-2-methylbenzamide (43). Synthesised from **40** (90 mg, 0.18 mmol) and TFA (1 mL) (48 h, 110 °C) following Method IV. Purification by chromatography on silica, using a gradient elution of 100% EtOAc to EtOAc:MeOH (5:1), gave the required purine as a white solid (4 mg; 6 %); mp 271-272 °C; IR 3379, 3210, 2932, 2857, 1662, 1624, 1591, 1574, 1533 cm⁻¹; ¹H NMR (*d*₆-DMSO, 500 MHz) δ 1.02-0.14 (m, 2H, cyclohexyl), 1.16-1.36 (m, 3H, cyclohexyl), 1.65-1.79 (m, 3H, cyclohexyl), 1.80-1.92 (m, 3H, cyclohexyl), 2.52 (s, 3H, Ar-CH₃), 4.28 (d, 2H, *J* = 6.4 Hz, OCH₂), 6.32 (s, 2H, NH₂), 7.37 (dd, 1H, *J* = 7.4, 7.5 Hz, *H*-5), 7.43 (d, 1H, *J* = 7.4 Hz, *H*-4), 7.52 (s, 1H, NH), 7.63 (d, 1H, *J* = 7.5 Hz, *H*-6), 7.88 (s, 1H, NH), 12.67 (s, 1H, NH); ¹³C NMR (*d*₆-DMSO, 125 MHz) δ 17.5, 26.8, 27.5, 30.8, 38.7, 72.8, 126.9, 129.5, 132.4, 132.6, 135.7, 139.7, 161.6, 175.2; HRMS [M+H]⁺ (C₂₀H₂₅N₆O₂) calcd 381.2034, obsd 381.2030.

3-(2-Amino-6-(cyclohexylmethoxy)-9H-purin-8-yl)benzenesulfonamide (44). In accordance with Method IV from **41** (29 mg; 0.045 mmol) and TFA (1 mL) (3h at 20 °C). Purification by chromatography on silica, employing a gradient elution of EtOAc:Petrol (4:1) to 100% EtOAc,

furnished the title compound as a white solid (11 mg; 61%); mp 159-162 °C; IR 3323, 2920, 2851, 1651, 1625, 1530 cm^{-1} ; ^1H NMR ($\text{MeOD-}d_4$, 500 MHz,) δ 1.09-1.20 (2H, m, cyclohexyl), 1.24-1.40 (3H, m, cyclohexyl), 1.68-1.84 (3H, m, cyclohexyl), 1.90-1.97 (3H, m, cyclohexyl), 4.33 (2H, d, J = 6.2 Hz, OCH_2), 7.68 (1H, dd, J = 7.8, 7.9 Hz, H -5), 7.99 (1H, d, J = 7.8 Hz, H -4), 8.22 (1H, d, J = 7.9 Hz, H -6), 8.58 (1H, s, H -2); ^{13}C NMR ($\text{MeOD-}d_4$, 125 MHz) δ 26.9, 27.57, 30.81, 38.78, 73.0, 125.21, 128.4, 130.75, 130.88, 131.8, 146.22, 161.61; HRMS $[\text{M}+\text{H}]^+$ ($\text{C}_{18}\text{H}_{23}\text{N}_6\text{O}_3\text{S}$) calcd 403.1547, obsd 403.1546.

3-(2-Amino-6-(cyclohexylmethoxy)-9H-purin-8-yl)-2-methylbenzenesulfonamide (45).

Prepared in accordance with Method IV from **42** (60 mg; 0.092 mmol) and TFA (1 mL) (90 min at 80 °C. Isolated by chromatography on silica, using a gradient elution of 100% EtOAc to EtOAc: MeOH (5:1), to give the title compound as a white powder (30 mg; 78%); mp 258-259 °C; IR 3503, 3407, 2927, 2851, 1629, 1587 cm^{-1} ; ^1H NMR (d_6 -DMSO, 500 MHz) δ 0.94-1.10 (2H, m, cyclohexyl), 1.15-1.33 (3H, m, cyclohexyl), 1.61-1.75 (3H, m, cyclohexyl), 1.77-1.86 (3H, m, cyclohexyl), 2.68 (3H, s, Ar- CH_3), 4.24 (2H, d, J = 6.3 Hz, OCH_2), 6.32 (2H, s, NH_2), 7.49 (1H, dd, J = 7.6, 7.8 Hz, H -5), 7.55 (2H, s, NH_2), 7.73 (1H, d, J = 7.6 Hz, H -4), 8.00 (1H, d, J = 7.8 Hz, H -6), 12.70 (1H, s, NH); ^{13}C NMR (d_6 -DMSO, 125 MHz) δ 17.0, 25.2, 26.0, 29.2, 36.8, 70.6, 125.7, 143.4, 159.7, 160.3; HRMS $[\text{M}+\text{H}]^+$ ($\text{C}_{19}\text{H}_{25}\text{N}_6\text{O}_3\text{S}$) calcd 417.1703, obsd 417.1697.

Crystallography

Crystals of monomeric CDK2 (kindly supplied by C.A. Minshall, AstraZeneca, Macclesfield, UK) were soaked for 24 h in a 5 mM solution of compound **26** in mother liquor (50 mM ammonium acetate, 10% PEG-3350, 15 mM NaCl, 100 mM HEPES, pH = 7.4, 10% DMSO). The crystals were cryo-protected in 50 mM ammonium acetate, 10% PEG-3350, 25% glycerol, 100 mM HEPES, pH 7.4 and mounted into a 100 K nitrogen stream. Data collection was carried out at AstraZeneca, Macclesfield, UK, using a Bruker-Nonius FR591 rotating anode generator and an XrayResearch Mar345 image plate. Compounds **29**, **32**, **36**, **37**, **44**, and **45** were co-crystallised with CDK2/cyclin A as previously described.²⁰ Before data collection, crystals were briefly immersed in cryo-protectant (8 M sodium formate) before freezing.

Data processing was carried out using MOSFLM,³⁸ SCALA³⁹ and other programs of the CCP4 suite.⁴⁰ The structures of CDK2/compound **26** and of compounds **29**, **32**, **36** and **37** bound to CDK2/cyclin A were solved by molecular replacement using MOLREP⁴¹ taking, respectively, the protein atoms of an unpublished structure of CDK2 bound to a derivative of **5**, or CDK2/cyclin A/7 (PDB accession code 1H1S,²²) as the search model. Structures of **44** and **45** bound to CDK2/cyclin A were solved by Phaser,⁴² using as the search model a high-resolution structure of a recruitment peptide bound to CDK2/cyclin A (PDB accession code 2CCH). Each solution was then subjected to rigid body refinement in REFMAC,⁴³ revealing unambiguous electron density in the CDK2 ATP-binding site, consistent with the expected shapes of the inhibitors. Models of **26**, **29**, **32**, **36** and **37** were created using the CCP4 Sketcher,⁴⁰ and manually docked into the density using either O⁴³ (compounds **26** and **29**) or Coot⁴⁵ (compounds **32**, **36** and **37**). Models of **44** and **45** were created using the Elbow Builder utility, and built into the electron density using Coot. The inhibitor atoms were kept in all subsequent models during refinement. The structures were then refined further by rounds of manual rebuilding in O (for compounds **26** and **29**) or in Coot (compounds **32**, **36**, **37**, **44** and **45**) and restrained refinement in REFMAC. Towards the end of refinement, waters were added using ARP/wARP,⁴⁶ (compounds **26** and **29**) or the Coot water picking utility (**32**, **36**, **37**, **44** and

45) and manually verified. The statistics for the datasets and for the crystallographic refinement are presented in Table S1. The structures of CDK2/compound **26**, and compounds **32**, **29**, **36** **37**, **44** and **45** bound to CDK2/cyclin A have been deposited in the PDB with accession codes 1W8C, 4CFN, 4CFM, 4CFV, 4CFU, 4CFX and 4CFW respectively.

Modelling

The refined structure of mCDK2/compound **26** was aligned with CDK2/inhibitor structures using LSQKAB.⁴⁷ The kinases were aligned based on the alpha carbon positions of the residues Glu81, Phe82, Leu83, His84, Gln85 and Ala31. The hinge residues (Glu81-Gln85) were used as all the inhibitors investigated form hydrogen bonds with the backbone atoms in this region. Ala31 was used to allow the reliable alignment of the monomeric and the cyclin-bound CDK2 structures.⁴⁸ Conformation-energy profiles were calculated with Gaussian.⁴⁷ Models were created using the CCP4 monomer sketcher⁴⁰ and InsightII (Accelrys, CA, USA), and then converted into Z matrices. The purine and aryl rings were constrained to co-planarity, and the models energy minimised with the 6-31G** basis set. Rigid potential energy scans were carried out at 5° intervals between the two co-planar conformations using the 3-21G basis set.

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Supporting Information: Quantum mechanical calculations, structural data for the CDK2/cyclin A/17 and CDK2/cyclin A/18 complexes, and combustion analyses. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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