Articles

N^2-Substituted O^6-Cyclohexylmethylguanine Derivatives: Potent Inhibitors of Cyclin-Dependent Kinases 1 and 2

Ian R. Hardcastle,[†,*] Christine E. Arris,[‡] Johanne Bentley,[‡] F. Thomas Boyle,[§] Yuzhu Chen,[‡] Nicola J. Curtin,[‡] Jane A. Endicott,[▽] Ashleigh E. Gibson,[†] Bernard T. Golding,[†] Roger J. Griffin,[†] Philip Jewsbury,[§] Jerome Menyerol,[†] Veronique Mesguiche,[†] David R. Newell,[‡] Martin E. M. Noble,[▽] David J. Pratt,[▽] Lan-Zhen Wang,[‡] and Hayley J. Whitfield[†]

Northern Institute for Cancer Research, Bedson Building, School of Natural Sciences, University of Newcastle, Newcastle upon Tyne, NE1 7RU, UK, Northern Institute for Cancer Research, Paul O'Gorman Building, Medical School, Framlington Place, Newcastle Upon Tyne, NE2 4HH, UK, Laboratory of Molecular Biophysics and Department of Biochemistry, University of Oxford, Oxford, OX1 3QU, UK, and AstraZeneca Pharmaceuticals, Alderley Park, Cheshire, SK10 4TG, UK

Received December 16, 2003

The adenosine 5′-triphosphate (ATP) competitive cyclin-dependent kinase inhibitor O^6-cyclohexylmethylguanine (NU2058, **1**) has been employed as the lead in a structure-based drug discovery program resulting in the discovery of the potent CDK1 and -2 inhibitor NU6102 (**3**, IC_{50} = 9.5 nM and 5.4 nM vs CDK1/cyclinB and CDK2/cyclinA3, respectively). The SAR for this series have been explored further by the synthesis and evaluation of 45 N^2-substituted analogues of NU2058. These studies have confirmed the requirement for the hydrogen bonding N^2-NH group and the requirement for an aromatic N^2-substituent to confer potency in the series. Additional potency is conferred by the presence of a group capable of donating a hydrogen bond at the 4′-position, for example, the 4′-hydroxy derivative (**25**, IC_{50} = 94 nM and 69 nM vs CDK1/cyclinB and CDK2/cyclinA3, respectively), 4′-monomethylsulfonamide derivative (**28**, IC_{50} = 9 nM and 7.0 nM vs CDK1/cyclinB and CDK2/cyclinA3, respectively), and 4′-carboxamide derivative (**34**, IC_{50} = 67 nM and 64 nM vs CDK1/cyclinB and CDK2/cyclinA3, respectively). X-ray crystal structures have been obtained for key compounds and have been used to explain the observed trends in activity.

Introduction

The cyclin-dependent kinases (CDKs) play an essential role in regulating eukaryotic cell-cycle progression.[1,2] Sequential activation of CDK4/6 and CDK2 controls progression from G1 into S phase, and activation of CDK1 is essential for progression from G2 into M phase. Many cell-cycle regulatory mechanisms are altered in tumors. For example, mutation, deletion, or epigenetic silencing of the $p16^{INK4a}$ CDK inhibitor protein, loss of *p53* and hence reduced $p21^{Cip1}$ expression, lowered expression of $p27^{Kip1}$, overexpression of cyclin D, and mutation of CDK4 have all been reported.[3−5] These alterations can result in the loss of checkpoint control and so give rise to unregulated cell growth. Additionally, the cell cycle machinery may be overdriven in response to many oncogenic signaling pathways.

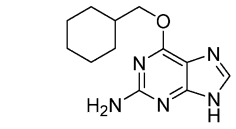
A large number of ATP-competitive CDK inhibitors from a variety of chemical classes have been identified.[6−9] Examples include staurosporine derivatives including UCN01,[10] flavonoids exemplified by flavopiridol,[11,12] and flavopiridol mimics such as 2-benzylidenebenzofuran-3-ones.[13] The 6-benzylamino- and 6-anilino-substituted purines form a large class of inhibitors,[14−19] a group that includes olomoucine, roscovitine, and the purvalanols. Other bicyclic nitrogen heterocycle-based inhibitors have also been described, of which oxindoles,[20,21] indolinones,[22] pyrrolopyrazines,[23] pyridopyrimidines,[24] and quinazolines[25,26] are well studied examples. Other notable inhibitors include pyrimidine-based compounds,[27−30] as well as tricyclic and more complex molecules,[31−39] some of which are natural products. First generation CDK inhibitors have entered clinical trials,[40] and it is already apparent that significant complexities will be encountered in the development of this drug class.[41] In particular, inhibitor specificity for CDKs over other kinases and selectivity for a specific target CDK within the CDK family of enzymes remain major challenges.

We have previously reported that compounds based on O^6-cyclohexylmethylpurine (NU2058, **1**) are competitive inhibitors of both CDK1 and CDK2 with respect to ATP, which also display good selectivity over CDK4.[27] Analysis of the X-ray structure of **1** bound to monomeric CDK2[27] revealed that **1** forms a triplet of hydrogen bonds within the CDK2 ATP binding site. NH-9 acts as a hydrogen bond donor to the backbone carbonyl group

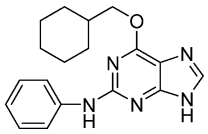
* Corresponding author. UK. Tel: +44 191 222 6645; fax +44 191 222 8591; e-mail I.R.Hardcastle@ncl.ac.uk.
† School of Natural Sciences, Northern Institute for Cancer Research.
‡ Medical School, Northern Institute for Cancer Research.
▽ University of Oxford.
§ AstraZeneca Pharmaceuticals.

10.1021/jm0311442 CCC: $27.50
Published on Web 06/10/2004

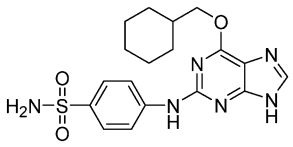
of Glu81, and N-3 and 2-NH_2 accept and donate a hydrogen bond to the backbone carbonyl and amide groups, respectively, of Leu83. The O^6-cyclohexylmethyl group of **1** occupies the CDK2 ribose-binding pocket.[27,42] Comparison of the structures of **1** and olomoucine bound to monomeric CDK2 revealed that **1** represents a different class of inhibitor to olomoucine as the two compounds bind in different orientations.[27] Extending this observation, the X-ray structure of **1** bound to fully active Thr160 phosphorylated CDK2/cyclinA (T160pCDK2/cyclin A) has also been determined.[43] This structure has been used to guide the design of more potent inhibitors that exploit the "specificity surface" of the CDK2 C-terminal domain beyond the ATP binding cleft. Examples of this compound series include the 2-anilinopurine derivative (NU6094, **2**) which displays IC_{50} values of 1.6 ± 0.1 and 0.97 ± 0.03 μM against CDK1/cyclin B1 and CDK2/cyclin A3, respectively, and the 2-(4-sulfanilyl)purine (NU6102, **3**) which exhibits nanomolar potency against CDK1 and CDK2 (IC_{50} values of 9.5 ± 0.1 and 5.4 ± 1.0 nM).[43] The greatly increased potency of **3** arises primarily from the formation of two additional hydrogen bonds to Asp86 of CDK2, which facilitate optimum hydrophobic packing of the anilino group with the specificity surface of CDK2. Cellular studies with **3** demonstrated inhibition of MCF-7 cell growth and target protein phosphorylation, consistent with CDK1 and CDK2 inhibition.[43]



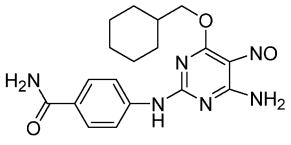
NU2058 (**1**)



NU6094 (**2**)



NU6102 (**3**)



(**35**)

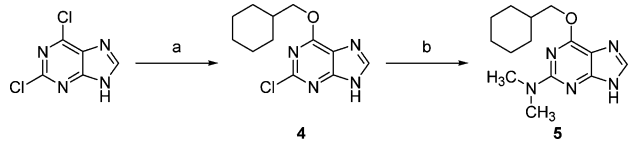
The studies described in this paper elaborate structure−activity relationships within the 2-substituted O^6-cyclohexylmethylpurine series. Compounds were designed with a knowledge of the details of the binding of **3** to T160pCDK2/cyclinA. In particular, the effect of modulating the potential to form hydrogen bonds with Asp86 was explored.

Chemistry

The N^2-substituted 6-cyclohexylmethoxypurines used in this study were prepared by following the routes described in Schemes 1−4. As illustrated in Scheme 1, compound **4** was prepared in good yield by the reaction of 2,6-dichloropurine with sodium cyclohexylmethoxide. Reaction of **4** with ethanolamine in DMF gave the dimethylamino compound **5** in good yield.[44]

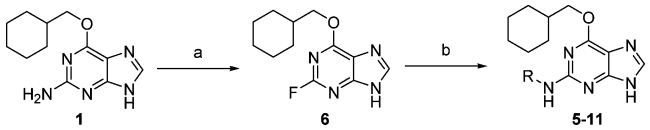
The key fluoro intermediate **6** was prepared from 2-amino-6-cyclohexylmethoxypurine (**1**) via a Balz−Schiemann reaction employing fluoroboric acid, to give the product in 44% yield (Scheme 2). The 2-fluoro group of **6** was then substituted by reaction with a selection

Scheme 1[a]



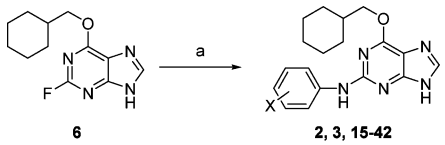
[a] Reagents and conditions: (a) cyclohexylmethanol, sodium, 90 °C; (b) ethanolamine, DMF, 90 °C.

Scheme 2[a]



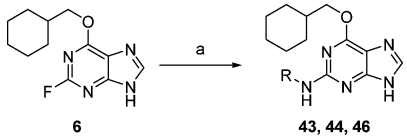
[a] Reagents and conditions: (a) HBF_4, $NaNO_2$, H_2O, −12 °C; (b) RNH_2, EtOH, reflux.

Scheme 3[a]



[a] Reagents and conditions: (a) either $ArNH_2$, glycerol, n-BuOH, reflux; $ArNH_2$, TFA, n-BuOH, reflux; $ArNH_2$, TFA, DMSO, 60 °C; or $ArNH_2$, TFA, TFE, reflux.

Scheme 4[a]



[a] Reagents and conditions: (a) RNH_2, n-BuOH, reflux.

of primary and secondary aliphatic amines and amino alcohols in refluxing ethanol to give the N^2-substituted purines **7**−**14** in modest to poor yields.

Initial attemps to substitute the 2-fluoro group of **6** with anilines under the same conditions met with failure (Scheme 3). However, the use of higher temperatures (120 °C; *n*-butanol) resulted in some reaction at prolonged reaction times. Optimization of these conditions resulted in the observation that the reaction was assisted by the addition of trifluoroacetic acid (TFA).[45] In many cases the poor solubility of the aniline was problematic. To overcome this problem, 2,2,2-trifluoroethanol (TFE) with TFA was employed as the solvent resulting in a significant improvement in the reaction yields. The use of TFE as solvent is considered to be optimal for substitution reactions of this type.[45]

The thiophenemethylamino and hydrazinosulfanilyl derivatives (**43**, **44**, and **46**) were prepared by reaction of the corresponding amines with fluoropurine **6** in *n*-butanol at 120 °C (Scheme 4). The 4-methoxyphenoxy compound (**45**) was synthesized by heating 4-methoxyphenol with **6** in DMSO at 140 °C in the presence of potassium fluoride on alumina and catalytic 18-crown-6 (Scheme 5).

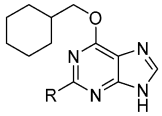
Results and Discussion

SAR for CDK1 and CDK2 Inhibition. Previous studies in which 58 O^6-substituted guanines were

Scheme 5[a]

[a] Reagents and conditions: (a) 4-methoxyphenol, KF on alumina, DMSO, 140 °C.

Table 1. Inhibition of CDKs 1 and 2 by N^2-Substituted O^6-Cyclohexymethylpurines



Compd #	2-Substituent (R)	Enzyme Inhibition: IC_{50} (μM) or *%inh at conc. indicated*	
		CDK1/Cyclin B	CDK2/Cyclin A3
1	NH_2-	7 ± 1	17 ± 2
4	Cl	*6 ± 4% at 10 μM*	*5 ± 3% at 10 μM*
5	Me_2N	*27 ± 6% at 10 μM*	*31 ± 3% at 10 μM*
6	F	*7 ± 4% at 10 μM*	*4 ± 4% at 10 μM*
7	Me, N, H	5.3 ± 1	5.0 ± 0.3
8	N, H	3.3 ± 0.1	2.8 ± 0.4
9	N, H	2.0 ± 0.1	1.2 ± 0.2
10	HO, N, H	3.2 ± 0.5	2.8 ± 0.9
11	Et, HO, N, H	*29 ± 5% at 10 μM*	*52 ± 1% at 10 μM*
12	Et, HO, N, H	*43 ± 2% at 10 μM*	*62 ± 1% at 10 μM*
13	HO, N, H	*1 ± 2% at 10 μM*	*19 ± 8% at 10 μM*
14	HO, N, H	*2 ± 2% at 10 μM*	*20 ± 7% at 10 μM*

synthesized and evaluated identified the O^6-cyclohexylmethoxy group as the optimal substituent for binding in the ribose pocket of CDK2.[42] Therefore this group was retained in all the compounds described here.

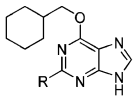
Nonaromatic C^2-Substitituted-O^6-cyclohexylmethylpurines (Table 1). To confirm that the increased potency of **2** and **3** was due in part to hydrophobic packing of the anilino group with the specificity surface of CDK2, compounds lacking an aromatic substituent at the 2-position were prepared. In comparison to **1**, compounds lacking an N−H at the 2-position (**4**−**6**) were less active against CDK1, although the dimethylamino derivative **5** retained activity against CDK2, as previously reported for the C^2-unsubstituted purine.[27] In contrast, the N^2-monosubstituted (methyl, **7**; ethyl, **8**; isopropyl, **9**; and 2-hydroxyethyl, **10**) derivatives were more potent as both CDK1 and CDK2 inhibitors than the parent **1**. This increase in potency was most marked against CDK2 where the compounds were ca. 10-fold more potent than **1**. However, larger alkyl groups such as *R*- or *S*-(1′-hydroxymethyl)propyl or *R*- or *S*-(1′-hydroxymethyl-2′-methyl)propyl (**11**−**14**) were not tolerated. The latter compounds were essentially devoid of kinase inhibitory activity at the maximum concentration that could be tested, due to solubility constraints (10 μM). Together, these data indicate that the hydrogen bond donated from the 2-NH to the backbone carbonyl moiety of Leu83 confers activity, particularly against CDK1, and that small hydrophobic substituents at N^2 can increase activity against CDK1 and more dramatically against CDK2.

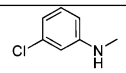
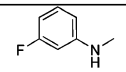
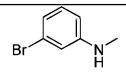
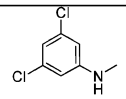
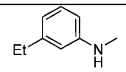
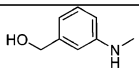
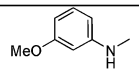
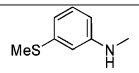
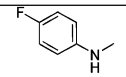
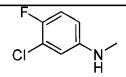
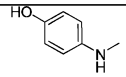
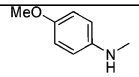
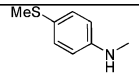
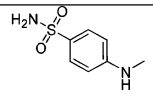
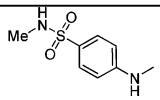
***C*^{2}-Anilino-*O*^{6}-cyclohexylmethylpurines** (Table 2). As described previously,[43] the 2-anilino compound **2** inhibited CDK1 and CDK2 with IC_{50} values of 1.6 ± 0.1 and 0.97 ± 0.03 μM, respectively. The crystal structure of **2** in complex with T160pCDK2/cyclin A3[43] revealed an extensive hydrophobic interaction between the phenyl ring of the anilino substituent and the hydrophobic surface of the enzyme. Taking **2** as a starting point, the effect of substitution at the 3′- and 4′-positions on the aniline ring was investigated.

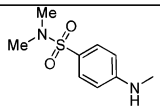
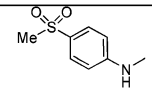
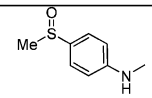
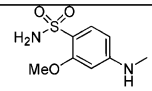
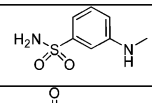
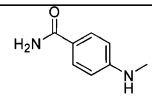
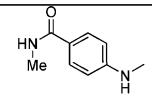
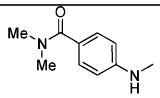
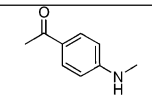
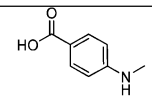
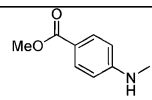
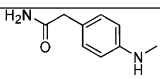
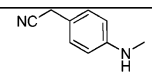
3′-Substituted *C*^{2}-anilino-*O*^{6}-cyclohexylmethylpurines. The X-ray structure of purvanalol B bound to CDK2[14] suggests that a favorable interaction between the inhibitor's 3′-chloro group and the enzyme is formed. The analogous anilino compound **15** was prepared but its activity was not greater than that of the parent **2**.[43] Similarly, the 3′-fluoro (**16**), 3′-bromo (**17**), and 3′,5′-dichloro (**18**) derivatives were no more active that **2**, with **15** being the most active of the compounds. The 3′-ethyl derivative **19** was again markedly less active than **2**. However, the 3′-hydroxymethyl derivative **20** was more potent than **2** against both CDK 1 and 2. Given the poor activity of **19**, the most persuasive explanation for the enhanced activity of **20** is that it is able to form a hydrogen bond with CDK2, presumably at a site conserved in CDK1 and CDK2, and potentially Asp86 as in the case of **3**. Consistent with the need for a hydrogen bond donor at the 3′-position for enhanced activity, both the 3′-methoxyanilino and 3′-methylmercaptoanilino compounds, **21** and **22**, displayed similar activity to the 3′-chloro compound **15** and the parent aniline **2**.

4′-Substituted *C*^{2}-Anilino-*O*^{6}-cyclohexylmethylpurines. As observed at the 3′-position, the introduction of a 4′-fluoro substituent (**23**) reduced activity relative to the parent aniline **2**. 3′-Cl-4′-F-substitution (**24**) improved activity over **23** but not to the level seen with **2**. In contrast, 4′-hydroxy substitution (**25**) generated a compound which was substantially more potent than the parent aniline **2**, with IC_{50} values of ≤100 nM for both CDK1 and CDK2. As with **20**, the result with **25** strongly suggests that there is a hydrogen bond acceptor in the region of the protein accessed by the 2-anilino group. Asp86, a residue that is conserved in CDK 1 and CDK2, is an obvious candidate.

Table 2. Inhibition of CDKs 1 and 2 by N^2-anilino-O^6-cyclohexymethylguanines



Compd #	2-Substituent (R)	Method	Enzyme Inhibition: IC_{50} (μM or **nM**) or *%inh at conc. indicated*	
			CDK1/Cyclin B	CDK2/Cyclin A3
2		B	1.6 ± 0.1	0.97 ± 0.03
15		B	2.2 ± 0.4	2.3 ± 0.3
16		B	*69 ± 16% at 10 μM*	*62 ± 16% at 10 μM*
17		B	5.3 ± 0.8	6.8 ± 4.8
18		C	13 ± 3	12 ± 6
19		B	*37, 32% at 10 μM*	*52, 37% at 10 μM*
20		C	0.30 ± 0.01	0.4 ± 0.1
21		B	2.2 ± 0.2	1.8 ± 0.2
22		B	2.4 ± 0.2	1.7 ± 0.1
23		B	*20 ± 12% at 10 μM*	*27 ± 8% at 10 μM*
24		B	*73 ± 3% at 10 μM*	*68 ± 7% at 10 μM*
25		B	**94 ± 12 nM**	**69 ± 1 nM**
26		B	0.79 ± 0.01	0.65 ± 0.03
27		B	*10 ± 5% at 10 μM*	*18 ± 7% at 10 μM*
3		C	**9.5 ± 0.1 nM**	**5.4 ± 1.0 nM**
28		C	**9 ± 2 nM**	**7.0 ± 0.1 nM**

Compd #	2-Substituent (R)	Method	Enzyme Inhibition: IC_{50} (μM or **nM**) or *%inh at conc. indicated*	
			CDK1/Cyclin B	CDK2/Cyclin A3
29		C	**77 ± 5 nM**	**56 ± 20 nM**
30		D	**80 ± 5 nM**	**63 ± 7 nM**
31		C	0.2 ± 0.1	0.10 ± 0.01
32		-	**103 ± 4 nM**	**70 ± 8 nM**
33		C	0.24 ± 0.01	0.21 ± 0.04
34		C	**67 ± 35 nM**	**64 ± 33 nM**
36		D	0.4 ± 0.2	0.20 ± 0.03
37		C	0.60 ± 0.03	0.20 ± 0.04
38		C	0.5 ± 0.2	0.30 ± 0.04
39		-	1 ± 1	0.8 ± 0.2
40		B	*66 ± 6% at 10 μM*	*25 ± 16% at 10 μM*
41		B	0.14 ± 0.02	0.13 ± 0.02
42		B	0.3 ± 0.1	0.30 ± 0.03

The structure of the **25**/T160pCDK2/cyclin A complex confirmed this prediction and showed that the binding mode of **25** to T160pCDK2/cyclin A is similar to that of **3** (Figure 1).[43] The lengths of the hydrogen bonds between the purine ring and CDK2 backbone residues within the hinge region, and the values of the purine/anilino twist angle and the anilino/backbone angle, are all very similar for each inhibitor in the two complexes. However, in the **25**/T160pCDK2/cyclin A structure the $\chi 2$ angle of Asp86 changes by 44° so that the carboxylate moiety forms a weak interaction with the 4-hydroxy group of the inhibitor (**25** O23−Asp86 OD2 = 3.3 Å). The hydroxy group of **25** also interacts with Asp86 via a water-mediated hydrogen bond (water molecules included in the atomic coordinates file as the Z chain) (**25** O23-Z143-Asp86 OD2 = 2.8 Å/3.0 Å, Figure 2).

To probe the requirement for a hydrogen bond donor at the 4′-position, the 4′-methoxyanilino derivative **26** was synthesized, and, as predicted, this compound was markedly less active than **25**, showing approximately 10-fold less activity against both CDK1 and CDK2. The corresponding 4′-methylmercaptoanilino derivative **27** was essentially inactive at 10 μM. The corresponding 3′-methoxyanilino and 3′-methylmercaptoanilino deriva-

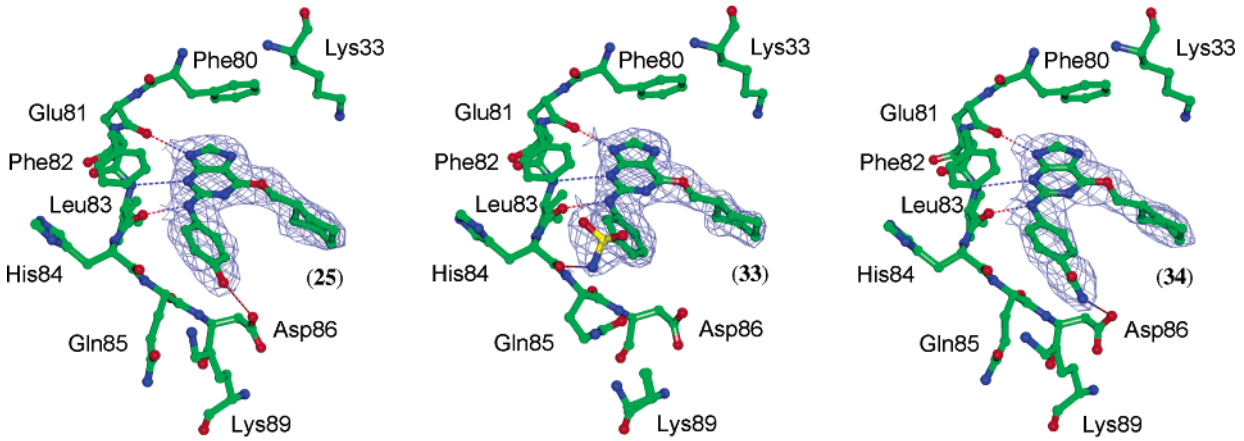


Figure 1. Binding of **25**, **34**, and **33** to T160pCDK2/cyclinA3. Selected CDK2 residues are labeled and drawn in ball-and-stick representation. The final $2F_o - F_c$ density for each of the inhibitors is shown as a blue grid. Hydrogen bonds are indicated by dashed lines.

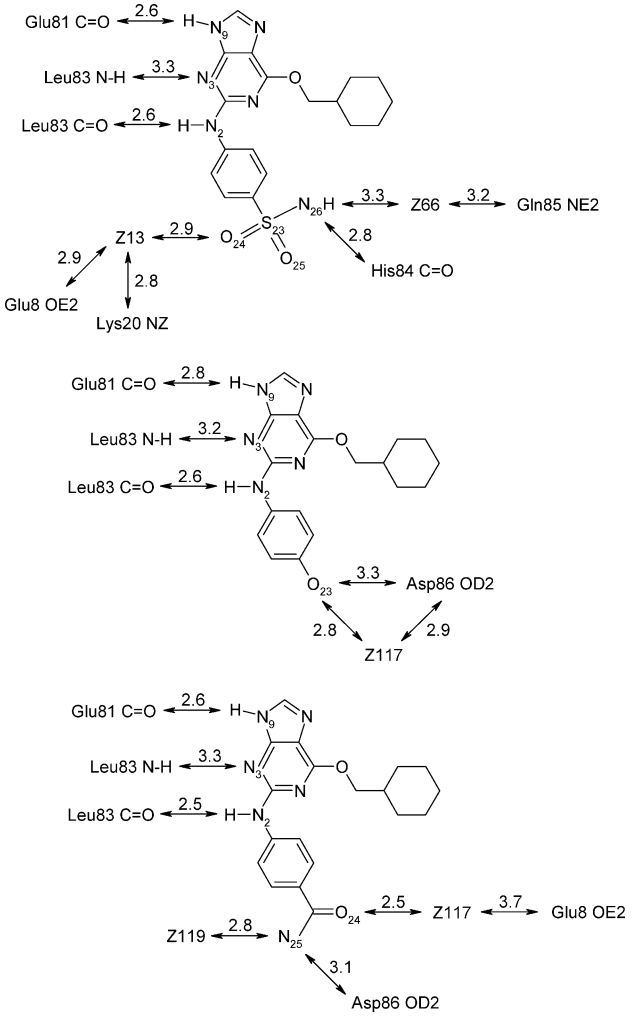


Figure 2. Hydrogen bonds between the inhibitors, CDK2, and crystallographic waters. All distances are in angstroms.

tives (**21** and **22**) were equipotent but less active than the 4′methoxy derivative **26**.

As previously described for **3**, the NH_2 group of the sulfonamide donates a hydrogen bond to a side-chain oxygen of Asp86 (**3** N26 to Asp86 OD2 = 2.9 Å) and one sulfonamide oxygen accepts a hydrogen bond from the backbone nitrogen of Asp86 (**3** O24 to Asp86 NH = 3.1 Å). Hence compounds were synthesized to define in more detail the contributions of these two hydrogen bonds to activity against CDK1 and CDK2. The *N*-monomethyl derivative **28** displayed similar activity to **3**, whereas the *N*,*N*-dimethylsulfonamide (**29**) was an order of magnitude less potent. These results strongly suggest that a single sulfonamide N−H is sufficient for optimal binding to the enzyme. The 4-methyl sulfone derivative **30**, which would also be unable to donate a hydrogen bond to a side-chain oxygen of Asp86, displayed similar activity to **29**, again consistent with the hypothesis that the activity of **3** is due to the two H-bonds formed with Asp86. The 4′-methyl sulfoxide **31** was less active again than **30**, suggesting that in addition to the loss of the NH hydrogen bond, the remaining sulfonyl oxygen can no longer adopt an optimal geometry for binding to the backbone NH of Asp86. Lack of optimal H-bonding geometry presumably also underlies the greatly reduced activities of **32** and **33** against both CDKs, relative to **3**.

The structure of **15** in complex with T160pCDK2/cyclin A revealed that both conformations which the 3-chlorophenyl group would be predicted to adopt, one directed toward Asp86, the other directed toward His84, were present in the complex, each with approximately 50% occupancy.[43] However, the sulfonamide group of **33** bound to T160pCDK2/cyclin A is directed toward His84, and there is no indication that the compound binds in the alternative conformation with the sulfonamide directed toward Asp86 (Figure 1). The increased bulk of the sulfonamide moiety compared to that of the chlorine atom probably accounts for the lack of this second conformation. The sulfonamide group in **33** forms only one direct hydrogen bond with the protein (**33** N26−His84 CO = 2.8 Å). However, three potential water-mediated hydrogen bonds between **33** N26-Z66-Gln85 NE2, **33** O24-Z13-Glu8 OE2, and **33** O24-Z13-Lys20 NZ, of lengths 3.3 Å/3.2 Å, 2.9 Å/2.9, and 2.9 Å/2.8 Å, respectively, are also observed. Notably, CDK2 Glu8 has not been previously targeted in this series, and it is one of the sequence positions at which CDK2 and CDK4 differ; the equivalent residue in CDK4 is Ala10. The conformation of Gln85 is also different to that observed in all other structures for this series of compounds bound to T160pCDK2/cyclin A determined to date. The twist angle between the purine and anilino rings of compound **33** is between that of **2** and **3** at 41°,

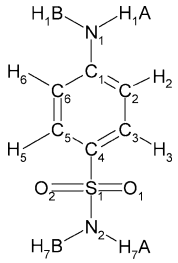


Figure 3. The structure of the 4-sulfamoylaniline used as a test compound to model sulfamoyl conformational energy.

and the angle between the anilino ring and plane of the peptide backbone between His84 and Gln85 is 27°. This observation suggests that the stacking interactions between the anilino ring of **33** and CDK2 are more favorable than those between **3** and CDK2.

To probe these results further, the preferred conformation of a sulfamoyl group attached to an aniline ring was analyzed. The Cambridge Structural Database (CSD) contains only 20 unique entries for this substructure. To derive a more complete picture of the sulfamoyl group conformational energy profile, quantum mechanical calculations were carried out using 4-sulfamoylaniline as a model compound for **3**. An all atom model was built that included hydrogen atoms (Figure 3) and minimized with the 6-31G** basis set in Gaussian.[44] All distances, angles, and torsions were allowed to be refined, except the C3−C4−S1−N2 torsion angle, which was fixed at a range of values. The relative energies of the various conformations, with respect to the C3−C4−S1−N2 and C3−C4−S1−O1 torsion angles, are shown in Figure 4. The overall minimized structure of 4′-sulfamoylaniline with the 6-31G** basis set shows that the preferred C3−C4−S1−O1 torsion angle is 8.6°. Assuming that 4-sulfamoylaniline is a valid model for **3** and that 3-sulfamoyl groups show similar behavior to 4-sulfamoyl groups, then the conformation of the **33** sulfamoyl group is energetically very favorable, with a torsion angle C21−C20−S23−O24 of 3°.

The above studies defined SARs for the anilinosulfonamide group in detail and provided a strong structural rational for the results of the enzyme inhibition assays. Analogous studies were therefore undertaken with 4′-carboxamide derivatives, the carboxamide substituent being potentially capable of forming a similar pattern of hydrogen bonds with CDK2 as the sulfonamide **3**. The 4′-carboxamide derivative **34** had CDK inhibitory activity similar to that of **29** and **30**, suggesting that **34** was forming only a single hydrogen bond with either CDK1 or CDK2.

The geometry of the 4′-carboxamide group precludes the possibility of it making both the interactions observed between Asp86 and the 4′-sulfonamide moiety of **3**, although the formation either one is possible. The structure of benzamide **35**, the pyrimidine analogue of compound **34**, bound to T160pCDK2/cyclin A has been solved recently.[47] The resolution of this latter structure was not sufficient to determine unambiguously whether the amide carbonyl was interacting with the backbone nitrogen of Asp86, or alternatively if the amide nitrogen was interacting with the carboxylate moiety of Asp86. Taking into consideration the predicted hydrogen bond lengths associated with each binding mode, compound **35** was modeled with the carboxamide moiety twisted 36° relative to the anilino ring so that a hydrogen bond of 3.3 Å results between **35** N25 and Asp86 OD2.

The resolution of the structure of the **34**/T160pCDK2/cyclin A complex was slightly higher at 2.4 Å than that of **35**/T160pCDK2/cyclin A, but still does not provide sufficient detail to make an unambiguous determination. The carboxamide in the **34**/T160pCDK2/cyclin A structure has been modeled in the same conformation as the carboxamide of **35**, with a hydrogen bond between **34** N25 and Asp86 OD2 of 3.1 Å and a twist of the carboxamide of 35° relative to the anilino ring. The mean twist angle of a carboxamide group bound to a phenyl ring is 18° (std dev = 10°; CSD), a result that implies that the conformations of the carboxamide groups in both the CDK2-bound **34** and **35** structures lead to a slight energy penalty. The twist of the anilino ring of **34** relative to the purine and to the peptide backbone between His84 and Gln85 is very similar to that of **3**, while the purine ring is shifted slightly closer to the hinge backbone. Finally, the carbonyl moiety of **34** forms a weak water-mediated hydrogen bond with Glu8 (Figure 2) that is not seen in the **35**/T160pCDK2/cyclin A complex structure.

The *N*-monomethyl derivative **36** was less active than the parent **34**, and of similar potency to **37**, the *N*,*N*-dimethylcarboxamide, whereas the acetophenone derivative **38** had equivalent activity to **37**. Together these data strongly suggest that the 4′-carboxamide derivatives form only a single H-bond with CDK1 and CDK2,

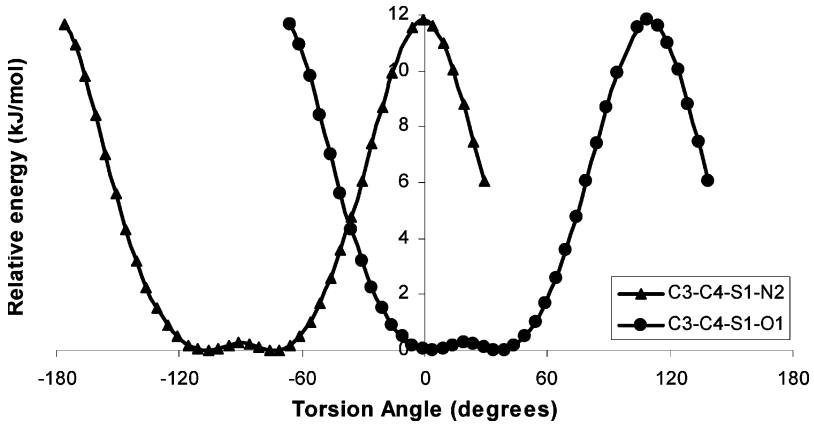
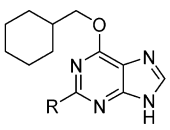
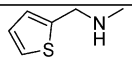
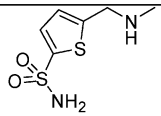
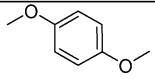
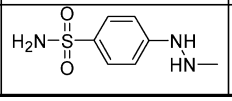


Figure 4. The energy profile of the 4-sulfamoylaniline compound with respect to the C3−C4−S1−N2 and C3−C4−S1−O1 torsion angles.

Table 3. Inhibition of CDKs 1 and 2 by O^6-Cyclohexymethylguanines with Non-anilino Aromatic and Heterocyclic N^2-Substituents



Compd #	2-Substituent (R)	Enzyme Inhibition: IC_{50} (μM) or *%inh at conc. indicated*	
		CDK1/Cyclin B	CDK2/Cyclin A3
43		0.7 ± 0.4	1.7 ± 0.9
44		5.7 ± 2.8	5.0± 0.2
45		*10 ± 9% at 100 μM*	*42 ± 16% at 100 μM*
46		8.9 ± 1.7	4.3 ± 1.1

and that this bond is donated by one of the NH hydrogens, presumably to the side-chain oxygen of Asp86.

Compared to the acetophenone derivative, the corresponding benzoic acid **39** has 2-fold reduced activity, while methylation of the benzoic acid to yield the methyl ester **40** produced a further 10-fold decrease in potency. The carboxamide homologue **41** was 2-fold less active than the 4′-carboxamide **34**, and the corresponding 4′-cyanomethyl derivative **42** was 4-fold less active.

***O*^{6}-Cyclohexylmethylpurines with Non-anilino Aromatic and Heterocyclic C^2 Substituents** (Table 3). As shown in Table 3, a small series of derivatives were prepared in which the C^2-anilino group was replaced with an aralkyl or heteroaralkyl group. The only compound of note was the thiophene derivative **43** that was of similar potency to the parent aniline **2**. However, the sulfonamide **44** was 1000-fold less potent than **3**, presumably because the sulfonamide group was not correctly oriented toward Asp 86. The inactivity of the phenoxy derivative **45** presumably reflects the importance of the hydrogen bond donated by the N2-amino group, and the poor activity of the hydrazine **46** again presumably reflects the importance of the interaction with Leu83.

The data presented suggest that the 2-anilino substituent can have a modest effect on CDK2 versus CDK1 selectivity, for example, the sulfone **30** and 3-methoxysulfonamide **32** display some selectivity for CDK2 over CDK1. Further studies with human CDK1, as opposed to the starfish CDK1 used in the current experiments, have shown that 2-anilinoguanines are selective CDK2 inhibitors with, for example, **3** being 46-fold more active against CDK2 than CDK1 (data not shown).

To confirm the selectivity of **3**, the compound has been independently tested as an inhibitor of 28 kinases.[43] In addition to CDK2 (IC_{50} = 30 nM), other kinases inhibited by **3** were Rho-dependent protein kinase II (ROCKII, IC_{50} = 600 nM), phospholipid dependent kinase 1 (PDK1, IC_{50} = 800 nM), and dual-specificity tyrosine phosphorylated and regulated kinase 1A (DYRK1A, IC_{50} = 900 nM) at an assay ATP concentration of 100 μM. These results indicate that **3** has at least 10-fold selectivity for the target kinases, CDK1 and -2.

Conclusions

The targeting of Asp86 in CDK2 by hydrogen bonding groups has been observed in a number of other series of CDK inhibitors. In the quinazoline series H-bonding between a 3-anilino group and the side-chain carboxyl group of Asp 86 was observed.[26] Interestingly, the oxindoles show a different pattern of activity to the anilino-purines.[20] In this case, the unsubstituted sulfonamide and mono- and disubstituted sulfonamides were found to be equipotent while cyclic sulfone derivatives suffered a small drop in activity, suggesting that a single H-bond formed between an aryl sulfonamide S═O to the backbone NH of Asp 86 was responsible for the gain in potency observed. The Asp86 residue has also been targeted in the flavopiridol mimicking benzilidinebenzofuran-3-one series, where a 10-fold gain in potency was observed on the introduction of a sulfonamide group at the position suggested by molecular modeling.[13]

In general, the gains in potency observed for the anilino-purine series described here results from favorable hydrophobic packing between the aromatic aniline ring and an area outside the ATP binding site of the CDK enzyme. Additional gains in potency are dependent on the formation of hydrogen bonds between carboxamide and sulfonamide groups, and Asp86, resulting in the identification of inhibitors with nanomolar potency. No direct interactions with Lys89, the residue originally targeted in the design of compound **3**, have yet been seen. However, interactions with other areas of the protein that show sequence differences between CDK2 and CDK4 have been observed (e.g., **15** and **33**), offering further avenues to explore for gains in potency and selectivity beyond the often-targeted Asp86/Lys89 pair.

In conclusion, we have identified highly potent CDK1 and CDK2 inhibitors based on a 6-cyclohexylmethoxypurine scaffold by the introduction of substituted 2-anilino groups. Further studies, including investigation of the cellular activies, broader kinase specificities, and additional structure−activity relationships are ongoing.

Experimental Section

Melting points were obtained on a Stuart Scientific SMP3 apparatus and are uncorrected. Infrared spectra (IR) were recorded as KBr disks on a Nicolet 20 PC Fourier Transform spectrometer or neat on an Excalibur series BioRad Spectrophotometer. Ultraviolet (UV) spectra were recorded in MeOH or EtOH on a U-2001 Hitachi Spectrophotometer. LC-MS analysis was conducted on a Waters/Micromass Platform LC instrument, LC chromatography used a Waters Symmetry column (50 × 4.6 mm) with a 10 min methanol/0.05% formic acid gradient, UV detection was achieved with a Waters 996 detector scanning from 240 to 400 nM, and MS was measured under electrospray mode in positive and negative ion mode. Electron impact (EI) mode mass spectra were determined on a Kratos MS80 spectrometer. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded at 300 and 75.5 MHz, respectively, on a Bruker Avance 300 spectrometer, using the deuterated solvent as internal standard. Unless indicated otherwise, spectra were recorded in

DMSO-d_6 as solvent. NH signals appeared as broad singlets (br s) exchangeable with D_2O. Chemical shift values are quoted in parts per million (ppm) and coupling constants (J) in hertz (Hz). Key: t = triplet, s = singlet, q = quartet, d = doublet, dd = double doublet, m = multiplet. The TLC systems employed Merck 1.05554 aluminum sheets precoated with Kieselgel 60F_{254} (0.2 mm) as the adsorbent and were visualized with UV light at 254 and 365 nm. Column chromatography was conducted under medium pressure on silica (Kieselgel 60, 240−400 mesh). Elemental analyses were performed in house on a Carlo-Erba Instrumentazione 1106 analyzer, or by Butterworth Laboratories, Middlesex, UK, and are within ±0.4% of theory unless otherwise specified. Reagents were purchased from Aldrich Chemical Co., Gillingham, UK, or Lancaster Synthesis and used as received unless otherwise stated. Ethanol and methanol were dried using Mg/I_2 and stored over 4 Å molecular sieves. Diethyl ether (predried over $CaCl_2$) and tetrahydrofuran (predried over KOH) were distilled from sodium/benzophenone. Acetonitrile was predried over potassium carbonate and distilled from CaH_2. Petroleum ether refers to that fraction in the boiling range 40−60 °C. Organic solvents from separations were dried using anhydrous $MgSO_4$. Reactions were routinely performed under an atmosphere of N_2 or Ar.

2-Chloro-6-cyclohexylmethoxypurine (4). Sodium (0.18 g, 7.94 mmol) was disolved in cyclohexylmethanol (10 mL, 79.0 mmol) at 90 °C. 2,6-Dichloropurine (0.50 g, 2.64 mmol) was added and heating continued at 90 °C for 1.5 h. The mixture was cooled to room temperature, neutralized (AcOH), and concentrated in vacuo. The residue was triturated with water and filtered giving **4** as a white solid (0.60 g, 85%), mp 234−240 °C. υ_{max}/cm^{-1} 3420 (NH_2), 3114 (NH), 2929 (CH_2), 1602 (C=C); ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.26 (5H, m, cyclohexyl), 1.83 (6H, m, cyclohexyl), 4.33 (2H, d, OCH_2, J = 6.12 Hz), 7.99 (1H, s, C(8)H); MS (EI) m/z 266 (M^+).

6-Cyclohexylmethoxy-2-dimethylaminopurine (5). A mixture of **4** (0.15 g, 0.56 mmol), ethanolamine (0.12 mL, 1.95 mmol), and DMF (3.0 mL) was heated at 90 °C for 3 days and then concentrated in vacuo. The residues were purified by chromatography (10% MeOH, DCM) and recrystallized (EtOAc) to give **5** as a white solid (0.10 g, 63%). ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.27 (5H, m, cyclohexyl), 1.99 (6H, m, cyclohexyl), 3.20 (6H, s, N(CH_3)$_2$), 4.36 (2H, d, OCH_2, J = 6.12 Hz), 7.95 (1H, s, C(8)H); MS (EI) m/z 275 (M^+). C, H, N. $C_{14}H_{21}N_5O$ requires C 59.15, H 7.75, N 24.65; found C 60.02, H 7.40, N 24.29.

***O*^{6}-Cyclohexylmethyl-2-fluoropurine (6).** To a stirred solution of 50% aqueous hydrofluoroboric acid (13 mL, 210 mmol) at 0 °C was added O^6-cyclohexylmethylguanine (2.6 g, 10.5 mmol) in one portion. To this was added a solution of sodium nitrite (1.45 g, 21.0 mmol) in water (20 mL) dropwise, ensuring that the reaction temperature did not exceed 10 °C. The reaction mixture was allowed to warm to room temperature and stirred for 18 h before being neutralized with 15% (w/v) sodium carbonate solution. The resulting precipitate was collected via filtration and stirred in ethyl acetate. The filtrate was concentrated under reduced pressure to yield a white crystalline solid (1.41 g, 53.6%): mp 172−173 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (5 H, m), 1.79 (6 H, m), 4.32 (2 H, d, J = 6.25 Hz), 6.57 (1 H, s), 8.39 (1 H, s). 13C NMR (300 MHz, DMSO-d_6) δ_{ppm} 25.4, 26.2, 29.3, 37.0, 72.6, 156.0, 158.8.

General Procedure A. A mixture of **6** and the appropriate amine in ethanol (10 mL) was heated to reflux for the specified time and then concentrated in vacuo. The residues were suspended in water (5 mL) and extracted (EtOAc, 3 × 10 mL). The combined organic extracts were dried ($MgSO_4$) and dried in vacuo. Column chromatography and/or recrystallization from the appropriate solvent gave the desired product.

General Procedure B. A mixture of **6**, the appropriate aniline (7 equiv), and trifluoroacetic acid (0.4 equiv), and either EtOH or *n*-BuOH was heated to reflux for the specified time and then cooled, and the desired product precipitated by addition of either MeOH or DCM. Recrystallization from the appropriate solvent or column chromatography gave the desired product.

General Procedure C. A mixture of **6**, the appropriate aniline (4 equiv) and trifluoroacetic acid (10 equiv) and either *n*-BuOH or a mixture of *n*-BuOH and glycerol was heated to reflux for the specified time, cooled, and concentrated in vacuo. The residue was diluted with water, neutralized ($NaHCO_3$), and extracted (EtOAc). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Recrystallization from the appropriate solvent gave the desired product.

General Procedure D. A mixture of **6**, the appropriate aniline (2 equiv), TFA (5 equiv), glycerol, and *n*-BuOH was heated to reflux for the specified time, cooled, and concentrated in vacuo. The residue was diluted with water, neutralized ($NaHCO_3$), and extracted (EtOAc). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Column chromatography gave the desired product.

General Procedure E. A mixture of **6**, the appropriate aniline derivative (7 equiv), TFA (0.4 equiv), glycerol, and either *n*-BuOH or EtOH, was heated to reflux for the specified time, cooled, and concentrated in vacuo. The residue was diluted with water, neutralized ($NaHCO_3$), and extracted (EtOAc). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Column chromatography gave the desired product.

General Procedure F. A mixture of **6**, the appropriate aniline (2 equiv), TFA (5 equiv), and 2,2,2-trifluoroethanol (4 mL) was heated to 100 °C for the specified time, cooled, and concentrated in vacuo. The residue was diluted with water, neutralized ($NaHCO_3$), and extracted (EtOAc). The combined organic layers were dried ($MgSO_4$), and concentrated in vacuo. Column chromatography gave the desired product.

2-Anilino-6-cyclohexylmethoxypurine (2). A mixture of **6** (0.20 g, 0.8 mmol), aniline (0.37 g, 4.0 mmol, 5 equiv), and n-BuOH (3.5 mL) was heated to 120 °C for 16 h and then cooled, and the solvent was removed in vacuo. The residues were disolved in DCM and filtered, and the filtrate was purified by chromatography (5% MeOH, DCM) giving **2** as a white solid (0.12 g, 46%). mp 196−199 °C (MeOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.26 (m, 5H, cyclohexyl), 1.93 (m, 6H, cyclohexyl), 4.34 (d, 2H, OCH_2), 7.02 (t, 1H, ArHC), 7.35 (d, 2H, ArH), 7.81 (d, 2H, ArH), 8.04 (s, 1H, H8), 9.50 (s, 1H, D_2O exch. N^9H); HRMS (EI) 323.175270 (M^+); Anal. ($C_{18}H_{21}N_5O$) C, H, N.

6-Cyclohexylmethoxy-2-(4′-sulfamoylanilino)purine (3). General Procedure C: a mixture of *n*-BuOH, glycerol, and sulfanilamide, reflux 4.5 days, washing (Et_2O, petroleum ether), followed by recrystallization (H_2O) gave NU6102, white solid (60%), mp 152−154 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.23 (5H, m), 1.91 (6H, m), 4.46 (2H, d), 7.27 (s, 2H), 7.81 (d, 2H), 8.08 (d, 2H), 8.19 (s, 1H), 9.82 (s, 1H). MS (EI) m/z 402 (M^+); Anal. ($C_{18}H_{22}N_6O_3S$ + 0.5 CF_3CO_2H) C, H, N.

2-Methylamino-6-cyclohexylmethoxypurine (7). General Procedure A: **6** (0.107 g, 0.43 mmol) and methylamine (10 mL, 8.03 M in ethanol), 18 h, gave **7** as a pale yellow solid (62 mg, 70%), mp 159−160 °C. ^{1}H NMR (200 MHz, D_2O) δ_{ppm} 1.2−1.5 (m, 5H, cyclohexyl) 1.7−2.1 (m, 6H), 2.9 (d, 3H, CH_3NH), 4.3 (2H, d, OCH_2), 6.8 (1H, m, NHCH$_3$, ex), 7.9 (1H, s, C^{8}H), 12.5 (1H, brs, N^9H, ex); HRMS (EI) m/z 261.158669 (M^+). C, H, N ($C_{13}H_{16}N_5O$) C, H; N 24.65; found 24.08.

6-Cyclohexylmethoxy-2-(ethylamino)purine (8). General Procedure A: **6** (0.49 g, 1.95 mmol) and ethylamine (10 mL; 2.0 M in EtOH), 20 h. Chromatography (10% MeOH, DCM) followed by recrystallization (petroleum ether, EtOAc, MeOH) gave **8** as a cream solid (0.0171 g, 3%), mp 165−166 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.0−2.0 (17H, m, cyclohexyl and CH(CH_3)$_2$), 4.1 (1H, m, CH(CH$_3$)$_2$), 4.4 (2H, d, OCH_2), 6.65 (1H ex, br t, NHCH$_2$), 7.9 (1H, s, C^8H), 12.6 (1H ex, br s, N^9H). HRMS (EI) m/z 289.191032 [M]$^+$. C, H, N ($C_{14}H_{21}N_5O$) C, H; N calcd N 25.45; found N 24.74.

6-Cyclohexylmethoxy-2-(1-methylethylamino)purine (9). General Procedure A: **6** (0.18 g, 0.72 mmol) and isopropylamine (0.21 g, 3.59 mmol), 28 h. Chromatography (EtOAc, petroleum ether; 1:1) followed by recrystallization (petroleum

ether, EtOAc, MeOH) gave **9** as a cream solid (0.096 g, 42%), mp 165−166 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.0−2.0 (17H, m, cyclohexyl and CH(CH_3)$_2$), 4.1 (1H, m, CH(CH$_3$)$_2$), 4.4 (2H, d, OCH_2), 6.65 (1H ex, br t, NHCH$_2$), 7.9 (1H, s, C^8H), 12.6 (1H ex, br s, N^9H). HRMS (EI) *m/z* 289.191032 [M]$^+$. Anal. ($C_{15}H_{23}N_5O$ + 0.25 CH_3OH) C, H, N.

6-Cyclohexylmethoxy-2-(2-hydroxyethylamino)purine (10). General Procedure A: **6** (0.10 g, 0.4 mmol) and ethanolamine (0.122 g, 2.0 mmol), 16 h, gave **10** as a cream solid (55 mg, 47%), mp 108−109 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.2−1.6 (5H, m, cyclohexyl), 1.8−2.1 (6H, m, cyclohexyl), 3.45 (2H, m, CH_2OH), 3.6 (2H, m, CH_2N), 4.3 (2H, d, OCH_2), 4.75 (1H ex, t, OH), 6.7 (1H ex, br t, NHCH$_2$), 7.9 (1H, s, C^8H), 12.4 (1H ex, br s, N^9H). HRMS (EI) *m/z* 291.170555 (M$^+$). Anal. ($C_{14}H_{21}N_5O_2$) C, H, N.

(*R*)-6-Cyclohexylmethoxy-2-(1-ethyl-2-hydroxyethylamino)purine (11). General Procedure A: **6** (0.44 g, 1.75 mmol) and (*R*)-2-aminobutanol (0.78 g, 8.76 mmol), 3 days. Chromatography (10% MeOH, DCM) followed by recrystallization (petroleum ether, EtOAc) gave **11** as a cream solid (0.129 g, 23%), mp 164−165 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.0 (3H, t, CH$_2$CH_3), 1.05−2.0 (13H, m, cyclohexyl and CH_2CH$_3$), 3.6 (2H, m, CH_2OH), 3.9 (2H, m, CH_2N), 4.3 (2H, d, OCH_2), 4.7 (1H ex, t, OH), 6.4 (1H ex, br t, NHCH$_2$), 7.95 (1H, s, C^8H), 12.6 (1H ex, br s, N^9H). HRMS (EI) *m/z* 319.201538 (M$^+$). Anal. ($C_{15}H_{25}N_5O_2$) C, H, N.

(*S*)-6-Cyclohexylmethoxy-2-(1-ethyl-2-hydroxyethylamino)purine (12). A mixture of **6** (0.23 g, 0.92 mmol), (*S*)-2-aminobutanol (0.41 g, 4.6 mmol), and *n*-butanol (10 mL) was heated to reflux for 24 h and then concentrated in vacuo. The residues were suspended in water (10 mL) and extracted (EtOAc, 3 × 10 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. Chromatography (10% MeOH, DCM) followed by recrystallization (petroleum ether, EtOAc) gave **12** as a cream solid (0.010 g, 4%), mp 165−166 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.0 (3H, t, CH$_2$CH_3), 1.1−2.0 (13H, m, cyclohexyl and CH_2CH$_3$), 3.5 (2H, m, CH_2OH), 3.9 (2H, m, CH_2N), 4.3 (2H, d, OCH_2), 4.7 (1H ex, t, OH), 6.4 (1H ex, br t, NHCH$_2$), 7.9 (1H, s, C^8H), 12.6 (1H ex, br s, N^9H). HRMS (EI) *m/z* 319.200279 (M$^+$). Anal. ($C_{16}H_{25}N_5O_2$) C, H, N.

(*R*)-6-Cyclohexylmethoxy-2-(1-hydroxymethyl-2-methylpropylamino)purine (13). General Procedure A: **6** (0.42 g, 1.67 mmol) and (*R*)-2-amino-3-methylbutanol (0.86 g, 8.37 mmol), 3 days. Chromatography (10% MeOH, DCM) followed by recrystallization (petroleum ether, EtOAc, MeOH) gave **13** as a cream solid (0.231 g, 42%), mp 161−163 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.0 (6H, d, CH(CH_3)$_2$), 1.05−1.5 and 1.6−2.0 (11H, m, cyclohexyl), 2.1 (1H, m, CH(CH$_3$)$_2$), 3.6 (2H, m, CH_2OH), 3.9 (2H, m, CH_2N), 4.4 (2H, d, OCH_2), 4.6 (1H ex, t, OH), 6.4 (1H ex, br t, NHCH$_2$), 7.95 (1H, s, C^8H), 12.5 (1H ex, br s, N^9H). HRMS (EI) *m/z* 333.217804 (M$^+$). Anal. ($C_{17}H_{27}N_5O_2$) C, H, N.

(*S*)-6-Cyclohexylmethoxy-2-(1-hydroxymethyl-2-methylpropylamino)purine (14). General Procedure A: **6** (0.25 g, 1.00 mmol) and (*S*)-2-amino-3-methylbutanol (0.52 g, 8.37 mmol), 3 days. Chromatography (10% MeOH, DCM) followed by recrystallization (hexane, EtOAc) gave **14** as a cream solid (0.0135 g, 4%), mp 157−158 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.0 (6H, d, CH(CH_3)$_2$), 1.1−1.5 and 1.7−2.0 (11H, m, cyclohexyl), 2.1 (1H, m, CH(CH$_3$)$_2$), 3.6 (2H, m, CH_2OH), 3.9 (2H, m, CH_2N), 4.3 (2H, d, OCH_2), 4.6 (1H ex, t, OH), 6.4 (1H ex, br t, NHCH$_2$), 7.9 (1H, s, C^8H), 12.5 (1H ex, br s, N^9H). HRMS (EI) *m/z* 333.216911 [M]$^+$. Anal. ($C_{17}H_{27}N_5O_2$) C, H, N.

2-(3′-Chloroanilino)-6-cyclohexylmethoxypurine (15). General Procedure B: 3-chloroaniline and *n*-BuOH, reflux 24 h, precipitation (MeOH). Recrystallization (MeOH) gave **15**, white solid (28%), mp 196−199 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.26 (5H, m), 1.93 (6H, m), 4.45 (2H, d), 7.07 (1H, d), 7.39 (1H, t), 7.72 (1H, d), 8.22 (1H, s), 8.56, (1H, s), 9.50 (1H, s). MS (EI) *m/z* 357 (M$^+$).

6-Cyclohexylmethoxy-2-(3′-fluoroanilino)purine (16). General Procedure B: 3-fluoroaniline and *n*-BuOH, reflux 1 h, precipitation (MeOH), gave **16**, white solid (50%), mp 224−227 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.23 (5H, m), 1.91 (6H, m), 4.46 (2H, d), 6.82 (1H, d.t), 7.38 (1H, q), 7.59 (1H, d), 8.04 (1H, d), 8.25 (1H, s), 9.70 (1H, s). ^{19}F NMR (500 MHz, DMSO-d_6) δ_{ppm} 81.03. MS (EI) *m/z* 341 (M$^+$). Anal. ($C_{18}H_{20}N_5OF$ + 0.25 CF_3CO_2H) C, H, N.

2-(3′-Bromoanilino)-6-cyclohexylmethoxypurine (17). General Procedure B: 3-bromoaniline and *n*-BuOH, reflux 18 h, precipitation (MeOH). Chromatography (silica; DCM:MeOH; 95:5), gave **17**, white solid (35%), mp 204−207 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.26 (5H, m), 1.93 (6H, m), 4.45 (2H, d), 7.18 (1H, d), 7.32 (1H, t), 7.77 (1H, d, 8.16 (1H, s), 8.40, (1H, s), 9.50 (1H, s). MS (EI) *m/z* 401 (M$^+$); Anal. ($C_{18}H_{20}BrN_5O$ + 0.5 MeOH) C, H, N.

2-(3,5-Dichloroanilino)-6-cyclohexylmethyloxypurine (18). General Procedure C: **6** (0.20 g, 0.8 mmol), 3,5-dichloroaniline (0.52 g, 3.2 mmol), reflux 6.5 h. Chromatography (silica; 5% MeOH, DCM) gave **18**, white solid (0.100 g, 32%), 218−220 °C (ex MeOH). ν (cm^{-1}), 3418 (NH stretch), 1435 (OCH$_2$), 947 (cyclohexane), 650, 560 (purine); ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.23 (5H, m, cyclohexyl), 1.86 (6H, m, cyclohexyl), 4.41 (2H, d, OCH_2), 7.12 (1H, s, ArH), 8.01 (2H, s, ArH), 8.16 (1H, s, H^8), 9.82 (1H, s, N^9H); MS (ESI) *m/z* = 392 [M + H]$^+$; Anal. ($C_{18}H_{19}N_5OCl_2$) C, H; N requires 17.89; found 16.90.

6-Cyclohexylmethoxy-2-(3′-ethylanilino)purine (19). General Procedure B: 3-ethylaniline and EtOH, reflux 1 h, precipitation on cooling. Recrystallization (MeOH) gave **19**, white solid (34%), mp 180−183 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.23 (5H, m), 1.22 (3H, t), 1.91 (6H, m), 2.63 (2H, q), 6.75 (1H, d), 7.14 (1H, t), 7.55 (1H, d), 7.68 (1H, s), 8.25 (1H, s), 9.70 (1H, br s). MS (EI) *m/z* 351 (M$^+$).

[3-(6-Cyclohexylmethoxy-9*H*-purin-2-ylamino)phenyl]methanol (20). General Procedure C: a mixture of *n*-BuOH/glycerol and (3-aminophenyl)methanol, reflux 48 h, trituration (Et$_2$O) gave **20** as a white solid (59%), 174−177 °C. λ_{max} (EtOH) 237.0 and 308.0 nm; ν (cm^{-1}) 3292 (OH), 3107 (NH), 1490 (OCH$_2$), 1038, 1009 (cyclohexane), 650, 560 (purine); ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.01 (5H, m, cyclohexyl), 1.74 (6H, m, cyclohexyl), 4.27 (2H, d, OCH_2), 4.39 (2H, s, CH_2OH), 6.80 (1H, d, ArH), 7.13 (1H, t, ArH), 7.57 (1H, d, ArH), 7.73 (1H, s, ArH), 8.04 (1H, s, H^8), 9.26 (1H, s, N^9H); HRMS (EI) M$^+$ 353.185555 ($C_{19}H_{23}N_5O_2$ calcd as 353.185175); Anal. ($C_{19}H_{23}N_5O_2$ + 0.2 EtOAc, 0.8 CF_3CO_2H) C, H, N.

6-Cyclohexylmethoxy-2-(3′-methoxyanilino)purine (21). General Procedure B: 3-methoxyaniline and *n*-BuOH, reflux 2 h, precipitation (DCM). Recrystallization (MeOH) gave **21**, white solid (40%), mp 186−189 C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.23 (5H, m), 1.91 (6H, m), 3.86 (3H, s), 4 46 (2H, d), 6.62 (1H, d.t), (7.28 (1H, q), 7.41 (1H, d), 7.73 (1H, d), 8.40 (1H, s), 9.70 (1H, s). MS (EI) *m/z* 353 (M$^+$); Anal. ($C_{19}H_{23}N_5O_2$ + 1.0 CF_3COOH, 0.5 H_2O) C, H, N.

6-Cyclohexylmethoxy-2-(3′-methylmercaptoanilino)purine (22). General Procedure B: 3-methylmercaptoaniline and EtOH, reflux 16 h, precipitation on cooling. Recrystallization (MeOH) gave **22**, white solid (44%), mp 190−192 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.23 (5H, m), 1.91 (6H, m), 2.60 (3H, s), 4 45 (2H, d), 6.92 (1H, d), 7.32 (1H, t), 7.63 (1H, d), 7.98 (1H, s), 8.42 (1H, s), 9.70 (1H, s). MS (EI) *m/z* 369 (M$^+$).

6-Cyclohexylmethoxy-2-(4′-fluoroanilino)purine (23). General Procedure B: 4-fluoroaniline and *n*-BuOH, reflux 16 h, precipitation (MeOH). Recrystallization (MeOH) gave **23**, off-white solid (25%), mp 207−209 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.33 (5H, m), 1.90 (6H, m), 4.43 (2H, d), 7.22 (2H, d), 7.93 (2H, d), 8.23 (1H, s), 9.44 (s, 1H). MS (EI) 341.165825 ($C_{18}H_{20}FN_5O$ calcd as 341.165189) M$^+$ 341 (15%), 57 (100%); Anal. ($C_{18}H_{20}FN_5O$ + 1.25 CF_3COOH) C, H, N.

6-Cyclohexylmethoxy-2-(3′-chloro-4′-fluoroanilino)purine (24). General Procedure B: 3-chloro-4-fluoroaniline and *n*-BuOH, reflux 16 h, precipitation (MeOH). Recrystallization (MeOH) gave **24**, off-white solid (21%), mp 225−226 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.31 (5H, m), 1.92 (6H, m), 4.44 (2H, d), 7.43 (1H, t), 7.70 (1H, m), 8.33 (2H, m), 9.67 (s, 1H). MS (EI) 375.126259 ($C_{20}H_{23}N_5O_3$ calcd as 375.126216)

M^+ 375 (50%), 279 (100%), Anal. ($C_{20}H_{23}N_5O_3 + 0.8\ CF_3CO_2H$, 0.2 H_2O) C, H, N.

6-Cyclohexylmethoxy-2-(4′-hydroxyanilino)purine (25). General Procedure B: 4-aminophenol and *n*-BuOH, reflux 16 h, precipitation (MeOH). Recrystallization (MeOH) gave **25**, off-white solid (51%). ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.31 (5H, m), 1.92 (6H, m), 4.40 (2H, d), 6.76 (2H, d), 7.62 (2H, d), 8.02 (1H, s), 9.04 (2H, s). MS (EI) 339.170807 (calcd as 339.169525) M^+ 339 (53%), 243 (100%), Anal. ($C_{18}H_{21}N_5O_2$ + 0.1 CF_3COOH, 0.1 M MeOH) C, H, N.

6-Cyclohexylmethoxy-2-(4′-methoxyanilino)purine (26). General Procedure B: 4-methoxyaniline and EtOH, reflux 16 h, precipitation on cooling. Recrystallization (MeOH) gave **26**, white solid (41%), mp 189−192 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.23 (5H, m), 1.22 (3H, t), 1.91 (6H, m), 2.63 (2H), 6.75 (1H, d), 7.14 (1H, t), 7.55 (1H, d), 7.68 (1H, s), 8.25 (1H, s), 9.70 (1H, s). MS (EI) *m*/*z* 353 (M^+).

6-Cyclohexylmethoxy-2-(4′-methylmercaptoanilino)-purine (27). General Procedure B: 4-methylmercaptoaniline and *n*-BuOH, reflux 16 h, precipitation on cooling. Recrystallizations (EtOH and MeOH), gave **27**, off-white solid (18%), mp 159−160 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.35 (5H, m), 1.86 (6H, m), 2.60 (3H, s), 4.43 (2H, d), 7.33 (2H, d), 7.89 (2H, d), 8.18 (1H, s), 9.70 (1H, s). MS (EI) 369.161194 (calcd as 369.162332) M^+ 369 (29%), 124 (100%); Anal. ($C_{19}H_{23}N_5OS$ + 0.9 CF_3CO_2H) C, H, N.

4-(6-Cyclohexylmethoxy-2-ylamino)-*N*-methylbenzene-sulfonamide (28). General Procedure C: *n*-BuOH and 4-amino-*N*-methylbenzenesulfonamide, reflux 48 h, chromatography (silica; EtOAc:petroleum ether; 1:1) gave **28**, white solid (27%), mp > 250 °C (decomp). ^{1}H NMR (200 MHz, MeOH-d_4) δ_{ppm} 1.37 (5H, m, cyclohexyl), 2.11 (6H, m, cyclohexyl), 2.71 (3H, s, CH_3), 4.57 (2H, d, OCH_2), 7.92 (2H, d, Ar*H*), 8.21 (1H, s, H^8), 8.21 (2H, d, Ar*H*). MS (EI) 416.163048 (calcd as 416.163061), 416 (M, 41%), 320 (M − C_7H_{12}, 100%).

4-(6-Cyclohexylmethoxy-2-ylamino)-*N*,*N*-dimethylben-zenesulfonamide (29). General Procedure C: a mixture of *n*-BuOH and glycerol as the solvents and 4-amino-*N*,*N*-dimethylbenzenesulfonamide, reflux 3 days, chromatography (silica; 1−4% MeOH:DCM) gave **29**, brown solid (12%), mp 151−153 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.18 (5H, m, cyclohexyl), 1.82 (6H, m, cyclohexyl), 2.82 (3H, s, CH_3), 3.93 (2H, d, OCH_2), 7.21 (2H, d, Ar*H*), 7.64 (2H, d, Ar*H*), 7.67 (1H, s, H^8), 9.47 (1H, s, N^9*H*). MS (EI) 430.180489 (calcd as 430.178711) 430 (M, 100%), 334 (M − C_7H_{13}, 94%), 226 (M − C_7H_{13} − $C_2H_6NSO_2$, 73%).

4-(6-Cyclohexylmethoxy-9*H*-purin-2-yl)−(4-methane-sulfonylphenyl)amine (30). General Procedure D: mixture of *n*-BuOH/glycerol and 4-methanesulfonylphenylamine, reflux for 3 days, chromatography (silica; 5% MeOH, DCM) gave **30**, beige solid (4.5%), mp > 270 °C. λ_{max} (MeOH) 210.5, 310.5 nm; ν (cm^{-1}) 3376 (sulfone), 2924, 2852 (CH), 640 (purine), 665 (imidazole); ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.06 (5H, m, cyclohexyl), 1.94 (6H, m, cyclohexyl), 3.22 (3H, s, CH_3), 4.55 (2H, d, OCH_2), 7.89 (2H, d, Ar*H*), 8.08 (2H, d, Ar*H*), 8.36 (1H, s, H^8), 10.20 (1H, br s, N^9*H*, exch in D_2O); MS (EI) *m*/*z* 402 (100% M^+); Anal. ($C_{19}H_{23}N_5SO_3$ + 0.6 CF_3CO_2H) C, H, N.

Methyl 4-[(6′-Cyclohexylmethylpurin-2-yl)amino]phenyl Sulfoxide (31). General Procedure C: *n*-BuOH and 4-(methanesulfinyl)phenylamine, reflux for 48 h. Chromatography (silica; EtOAc:petroleum ether; 95:5; plus DCM:MeOH; 9:1) gave **31**, brown solid (46%), mp 150−152 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.22 (5H, m, cyclohexyl), 1.86 (6H, m, cyclohexyl), 2.80 (3H, s, CH_3), 4.42 (2H, d, OCH_2), 7.70 (2H, d, Ar*H*), 8.15 (3H, d, 2 × Ar*H* + H^8), 9.90 (1H, s, N^9*H* exch. in D_2O). MS (EI) M^+ 385.158508 (calcd as 385.157247) 370 (M^+ − CH_3, 54%), 274 (M^+ − $C_7H_{11}O$, 100%); Anal. ($C_{19}H_{23}N_5SO_2$ + 0.2 CF_3CO_2H) C, H, N.

4-[6-Cyclohexylmethoxy-9*H*-purin-2-ylamino]benzene-(3′-methoxy)sulfonamide (32). A mixture of **6** (0.15 g, 0.60 mmol), 2-methoxysulfanilamide (0.37 g, 1.84 mmol), trifluoroacetic acid (0.46 mL, 6.02 mmol), and DMSO (2 mL) was heated to 60 °C for 16 h, cooled, neutralized (aq $NaHCO_3$), and extracted (EtOAc 3 × 20 mL). Chromatography (silica; 10% MeOH, DCM) gave **32** as a yellow solid (0.102 g, 39%), mp 170−173 °C. ν (cm^{-1}) 2924 (NH stretch), 2849, 1315.449 (OMe), 640 (purine ring), 665 (4,5 disubstituted imidazole); ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 0.97 (5H, m, cyclohexyl), 1.61 (6H, m, cyclohexyl), 3.82 (3H, s, OCH_3), 4.26 (2H, d, OCH_2), 6.79 (2H, s, NH_2), 7.31 (1H, d, Ar*H*), 7.50 (1H, d, Ar*H*), 7.80 (1H, s, Ar*H*), 8.01 (1H, s, H^8), 9.52 (1H ex, s, N^9*H*). Anal. ($C_{19}H_{24}N_6O_4S$ + 0.2 CF_3CO_2H) C, H, N.

3-(6-Cyclohexylmethoxy-9*H*-purin-2-ylamino)benzene-sulfonamide (33). General Procedure C: 3-aminobenzene-sulfonamide (0.55 g, 3.2 mmol). Chromatography (EtOAc) gave **33** (0.130 g, 40%), mp 175−176 °C. λ_{max} (EtOH) 289, 251, 240 nm; ν (cm^{-1}) 3441 (NH_2), 3350 (NH_2), 2921, 1533, 1391; ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm} 1.18 (5H, m, cyclohexyl) 1.77 (6H, m, cyclohexyl) 4.37 (2H, d, OCH_2) 7.37 (d, 1H, Ar*H*) 7.45 (dd, 1H, Ar*H*) 7.88 (d, 1H, Ar*H*) 8.01 (s, 1H, H^8) 8.50 (s, 1H, ex N*H*) 9.69 (s, 1H, ex N*H*) 12.86 (s, 1H, ex N*H*); MS (ESI+) *m*/*z* 403 (M^+, 100%); Anal. ($C_{18}H_{22}N_6O_3S$) H; calcd C 53.72; N 20.88; found C 53.26; N 21.59.

4-(6-Cyclohexylmethoxy-9*H*-purin-2-ylamino)benz-amide (34). General Procedure C: *n*-BuOH and 4-aminobenzamide, reflux 16 h. Washing (Et_2O, petroleum ether) and recrystallization (Et_2O, EtOH) gave **34**, white solid (46%), mp 156−158 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ ppm 1.24 (5H, m, cyclohexyl), 1.93 (6H, m, cyclohexyl), 4.46 (2H, d, OCH_2), 7.27 (1H, s, CON*H*), 7.88 (1H, s, CON*H*), 7.90 (2H, d, Ar*H*), 7.99 (2H, d, Ar*H*), 8.10 (1H, s, H^8), 9.70 (1H, s, N^9*H*). MS (ESI+) *m*/*z* 367 [M + H]$^+$; Anal. ($C_{19}H_{22}N_6O_2$ + 0.4 EtOAc, 0.1 CF_3CO_2H) C, H, N.

4-(6-Cyclohexylmethoxy-9*H*-purin-2-ylamino)-*N*-methyl-benzamide (36). General Procedure D: 4-amino-*N*-methylbenzamide, reflux 24 h, chromatography (silica; 1−10% MeOH: DCM) gave **36**, pink solid (29%), mp 139−142 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.09 (5H, m, cyclohexyl), 1.89 (6H, m, cyclohexyl), 2.44 (3H, t, CH_3), 4.56 (2H, d, OCH_2), 7.83 (4H, q, Ar*H*), 8.35 (1H, s, H^8), 9.8 (1H, s, N^9*H*). MS (ESI+) *m*/*z* 381 (M + 1); Anal. ($C_{20}H_{24}N_6O_2$) C, H, N.

4-(6-Cyclohexylmethoxy-9*H*-purin-2-ylamino)-*N*,*N*-di-methylbenzamide (37). General Procedure C: *n*-BuOH and 4-amino-*N*,*N*-dimethylbenzamide, reflux 48 h, chromatography (silica; EtOAc:petroleum ether; 2:3) gave **37**, beige solid (35%), mp 184−186 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.16 (5H, m, cyclohexyl), 1.90 (6H, m, cyclohexyl), 3.07 (6H, s, 3 × CH_3), 4.44 (2H, d, OCH_2), 7.45 (2H, d, Ar*H*), 7.98 (2H, d, Ar*H*), 8.13 (1H, s, H^8), 9.85 (1H, s, N^9*H*). MS (ESI+) *m*/*z* 395.4 (M + 1); Anal. ($C_{21}H_{26}N_6O_2$ 0.3 EtOAc) C, H, N.

4-(6′-Cyclohexylmethylpurin-2-yl)aminoacetophe-none (38). General Procedure C: *n*-BuOH and 1-(4-aminophenyl)ethanone, reflux 12 h. Washing (hot H_2O) gave **38**, yellow powder (61%), mp 236−238 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.28 (5H, m, cyclohexyl), 1.90 (6H, m, cyclohexyl), 2.63 (3H, s, CH_3), 4.46 (2H, d, OCH_2), 8.02 (4H, q, Ar*H*), 8.39 (1H, s, H^8), 9.99 (1H, s, N^9*H*). MS (EI) 365.184105 (calcd as 365.185175) M^+ 365 (80%) 269 (M^+ − C_7H_{12}) 100%; Anal. ($C_{20}H_{23}N_5O_2$ + 0.5 CF_3CO_2H, 0.1 H_2O) C, H, N.

4-(6-Cyclohexylmethoxypurin-2-ylamino)benzoic Acid (39). An ethanolic solution of KOH (2 equiv) was added dropwise to **40** in THF at 0 °C and the mixture stirred 24 h. Water was added and the mixture neutralized (HCl; 1 M) and extracted (DCM). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to give **39** (41%). ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.31 (5H, m), 1.93 (6H, m), 4 45 (2H, d), 8.02 (4H, q), 8.19 (1H, s), 9.92 (s, 1H), 13.05 (1H, s). MS (ESI+) *m*/*z* 368 (M + 1).

Methyl 4-*N*-(6′-Cyclohexylmethyloxypurin-2′-yl)amino-benzoate (40). General Procedure B: methyl 4-aminobenzoate and *n*-BuOH, reflux for 16 h, precipitation (MeOH). Chromatography (silica; DCM:MeOH; 9:1) and recrystallization (MeOH) gave **40**, off-white solid (8%), mp 244−246 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.31 (5H, m), 1.93 (6H, m), 3.92 (3H, s), 4 45 (2H, d), 8.02 (4H, q), 8.19 (1H, s), 9.92 (s, 1H). MS (EI) 381.181458 ($C_{20}H_{23}N_5O_3$ calcd as 381.180090) M^+ 381 (56%), 185 (100%); Anal. ($C_{20}H_{23}N_5O_3$ + 0.2 CF_3CO_2H, 0.1 MeOH) C, H, N.

2-[4-(6-Cyclohexylmethoxy-9*H*-purin-2-ylamino)phenyl]-acetamide (41). General procedure B: 4-aminophenylacetamide (0.53 g, 3.5 mmol), n-BuOH. Recrystallization (Et_2O, EtOAc, EtOH) gave **41** as a beige powder (0.017 g, 5.2%), 233.5−233.8 °C. ν (cm^{-1}) 3346.4, 3200.0 (NH amide stretch), 2918.2 (CH), 2855.3 (Ar*H*), 1676.7 (CO), 1606.5 (Ar), 1128.36 (C−O−C), 633.5 (purine); λ_{max} (EtOH) = 273, 396 nm; ^{1}H NMR (200 HMz, DMSO-d_6) δ_{ppm} 1.20−1.08 (5H, m, cyclohexyl), 1.80−1.60 (6H, m, cyclohexyl), 3.20 (2H, d, CH_2), 4.28 (2H, d, OCH_2), 6.80 (1H, s, N*H*), 7.09 (2H, d, Ar*H*), 7.36 (1H, s, N*H*), 7.65 (2H, d, Ar*H*), 7.93 (1H, s, H^8). LCMS (ESI-) m/z = 380 $[M]^+$, 379 $[M - H]^-$; Anal. ($C_{20}H_{24}N_6O_2$ + 0.1 CH_3OH) C, H, N.

6-Cyclohexylmethoxy-2-(4′-cyanomethylanilino)purine (42). General Procedure B: 4-aminophenylacetonitrile and n-BuOH, reflux 16 h, precipitation (MeOH). Recrystallization (MeOH) gave **42**, off-white solid (17%). ^{1}H NMR (200 MHz, DMSO-d_6) δ_{ppm} 1.31 (5H, m), 1.92 (6H, m), 4.04 (2H, s), 4 42 (2H, d), 7.34 (2H, d), 7.92 (2H, d), 8.11 (1H, s). MS (EI) 362.186272 (calcd as 362.185510) M^+ 362 (55%), 266 (100%); Anal. ($C_{20}H_{22}N_6O$) C, H, N.

(6-Cyclohexylmethoxy-9*H*-purin-2-yl)thiophen-2-ylmethylamine (43). A mixture of **6** (0.20 g, 0.8 mmol), thiophene-2-methylamine (0.45 g, 4 mmol), and *n*-butanol (2 mL) was heated to 120 °C for 24 h, diluted with water (1 mL), neutralized with HCl (1 M), and extracted (EtOAc, 3 × 10 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. Chromatography (5% MeOH, DCM) gave **43** (0.070 g, 25%), mp 170−171 °C. ν (cm^{-1}) 3246 (NH), 2922 (CH_2), 2848 (OCH_2), 1625, 1589 (Ar*H*), 1499 (NH); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm} 1.04 (2H, m, cyclohexyl), 1.20 (3H, m, cyclohexyl), 1.80 (6H, m, cyclohexyl), 4.29 (2H, s, OCH_2), 4.54 (2H, s, NCH_2), 6.97 (1H, m, thiophene H-3′), 7.03 (1H, m, thiophene H-4′), 7.30 (1H, m, thiophene H-5′), 8.14 (1H ex, s, N*H*), 8.97 (1H, s, H^8); MS (EI+) m/z 343 (M^+ +1).

5-[(6-Cyclohexylmethoxy-9*H*-purin-2-ylamino)methyl]-thiophene-2-sulfonamide (44). A mixture of **6** (0.13 g, 0.53 mmol), 5-aminomethyl-thiophene-2-sulfonamide (0.22 g, 1.06 mmol), and *n*-butanol (3.5 mL) was heated to 120 °C for 24 h and then concentrated in vacuo. Chromatography (80% EtOAc, petroleum ether) gave **44**, mp 174 °C. λ_{max} (EtOH) 211, 244, 285; ν (cm^{-1}) 3414, 3267 (NH), 2923 (CH_2), 2850 (OCH_2), 1635, 1589, 1539, 1338, 1147; ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm} 1.12 (5H, m, cyclohexyl), 1.73 (6H, m, cyclohexyl), 4.24 (2H, s, OCH_2), 4.62 (2H, s, NCH_2), 6.98 (1H, d, thiophene), 7.36 (1H, d, thiophene), 7.51 (2H, s, NH_2),7.55 (1H, s), 7.85 (1H, s), 12.60 (1H, s, H^8); MS (ESI+) m/z 423 $[M + H]^+$.

6-Cyclohexylmethoxy-2-(4-methoxyphenoxy)-9*H*-purine (45). A mixture of **6** (0.20 g, 0.8 mmol), 4-methoxyphenol (0.10 g, 0.8 mmol), KF on alumina (0.5 g), 18-crown-6 (0.08 mmol), and DMSO (5 mL) was heated at 140 °C for 48 h, cooled, diluted with water (5 mL), and extracted (EtOAc 3 × 10 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. RP-HPLC (Jones C18; MeOH, H_2O) gave **45**, mp 167 °C. λ_{max} (EtOH); 205, 225, 266 nm; ν (cm^{-1}) 3445, 3082, 2922, 2852, 1590, 1500, 1448, 1362, 1333, 1197, 1125, 1036; ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm} 1.11 (5H, m, cyclohexyl), 1.72 (6H, m, cyclohexyl), 3.77 (3H, s, OCH_3), 4.24 (2H, d, OCH_2), 6.97 (2H, d, Ar*H*), 7.13 (2H, d, Ar*H*), 8.16 (1H, s), 12.83 (s, 1H, D_2O exch. N*H*); MS (ES+) m/z 355; Anal. ($C_{19}H_{22}N_4O_3$ + 0.5 H_2O) C, H, N.

4-[*N*′-(6-Cyclohexylmethoxy-9*H*-purin-2-yl)hydrazino]-benzenesulfonamide (46). A mixture of **6** (0.30 g, 1.2 mmol), 4-hydrazinobenzenesulfonamide (0.53 g, 2.4 mmol), and *n*-butanol (3 mL) was heated at 100 °C for 24 h and then concentrated in vacuo. The residues were partitioned between saturated sodium bicarbonate solution (10 mL) and EtOAc (10 mL), and then the organic phase was dried ($MgSO_4$) and concentrated in vacuo. Chromatography (75% EtOAc, petroleum ether) gave **46** (0.009 g, 1%), 249−250 °C. λ_{max} (EtOH) 286, 239 nm; ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm} 1.12 (5H, m, cyclohexyl), 1.73 (6H, m, cyclohexyl), 4.22 (2H, d, OCH_2), 6.77 (2H, d, Ar*H*), 6.97 (2H, d, NH_2), 7.54 (2H, d, Ar*H*), 7.87 (1H, s), 8.35 (s, 1H, D_2O exch. N*H*), 8.79 (s, 1H, D_2O exch. N*H*), 12.83 (s, 1H, D_2O exch. N*H*); MS (ESI+) m/z 418 $[M + H]^+$.

Enzyme inhibition studies. Inhibition of starfish oocyte (*Marthasterias glacialis*) CDK1/cyclin B1 and human CDK2/cyclinA was assayed as previously described.[27]

Expression and Purification of T160pCDK2/cyclinA3 and Crystallization of T160pCDK2/CyclinA3−Inhibitor Complexes. T160pCDK2/cyclin A was purified as described previously.[48] The protein was concentrated to 10 mg/mL in 0.2 M NaCl, 0.04 M HEPES, pH 7.0, 0.01% monothioglycerol, flash frozen in 25 μL aliquots in liquid nitrogen, and stored at −80 °C until required. Aliquots were rapidly thawed at room temperature and then incubated on ice. Prior to setting up crystallization trials, inhibitor stocks in 100% DMSO (Sigma) were added to the protein solution to a final concentration of 1 mM or 5 mM, (final DMSO 5% (v/v)). The mixtures were incubated on ice for 1 h and then spun through a 0.22 μm filter. Crystals were obtained by sitting drop vapor diffusion, with the mother liquor consisting of 0.7−0.85 M potassium chloride (Riedel-de Haën), 1.1−1.25 M ammonium sulfate (Riedel-de Haën), 40 mM HEPES, pH 7.0 (Sigma), and 5 mM dithiothreitol (Sigma). Crystal drops were typically composed of 1.5 μL of protein solution and 1 μL of mother liquor. Trays were set up at room temperature (ca. 21 °C) and then incubated at 4 °C. Crystals took between two weeks and three months to grow. A variety of morphologies were observed, with dimensions between 100 μm to 1000 μm on the longest edge.

X-ray Crystallography Data Collection and Processing. Crystals were warmed slowly overnight from 4 °C to room temperature (ca. 21 °C) before manipulation. Crystals were briefly immersed in cryoprotectant, 8 M sodium formate, and then plunge-frozen in liquid nitrogen. All datasets were collected at 100 K. Data were collected at the ESRF using MarCCDs on either ID14-EH4 (**25**) or ID14-EH1 (**34** and **33**). Data processing was carried out using MOSFLM,[49] SCALA,[50] and other programs of the CCP4 suite.[51]

Structure Solution and Refinement. The starting model in all cases was the structure of NU6102−T160pCDK2/cyclin A (PDB code 1H1S) with all nonprotein atoms removed. Rigid body refinement in REFMAC5,[52] with data to 3.5 Å, was applied, first with whole chains as individual rigid bodies and then with each protein domain as an individual rigid body (CDK2 residues 0−80, 81−84, 85−296, cyclinA residues 175−302 and 303−432). Unambiguous electron density for the inhibitors was visible after rigid body refinement. Inhibitor models were built using the Monomer Library Sketcher of the CCP4 suite[51] and with reference to the CSD[53]). Restrained refinement was then applied to the maximal resolution with alternative rounds of manual rebuilding in O.[54] Toward the end of refinement, waters were added using ARP[55] with reference to the NU6102 (**3**) structure.

Acknowledgment. The authors thank Tim Hunt for the human CDK2 and cyclin A constructs, Carl Mann for the gift of the Civ1 DNA clone, the beamline scientists at ID14-EH1 and ID14-EH4, ESRF, Grenoble, for providing excellent facilities during data collection, EPSRC Chemical Database Service at Daresbury, and R. Davison, P. Mackley, I. Taylor, E. F. Garman, and R. Bryan for technical support. This research was supported by grants from the Cancer Research UK, The Royal Society UK, Medical Research Council UK, BBSRC, Oxford University, and AstraZeneca PLC UK.

Supporting Information Available: Elemental analyses data. Refinement statistics for published structures. The three structures solved in this study of T160pCDK2/cyclin A in complex with the compounds **25**, **33** and **34** have been deposited with the PDB and assigned the codes 1OI9, 1OIU, 1OIY, respectively. The structure of NU2058 (**1**) solved previously has been assigned the pdb code 1e1v.[27] This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Norbury, C.; Nurse, P. A. Animal cell-cycles and their control. *Annu. Rev. Biochem.* **1992**, *61*, 441−470.

(2) Morgan, D. O. Principles of CDK regulation. *Nature* **1995**, *374*, 131−134.
(3) Sherr, C. J.; Roberts, J. M. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev.* **1999**, *13*, 1501−1512.
(4) Hall, M.; Peters, G. Genetic alterations of cyclins, cyclin-dependent kinases, and CDK inhibitors in human cancer. *Adv. Cancer Res. 68*, **1996**, 67−108.
(5) Stewart, Z. A.; Westfall, M. D.; Pietenpol, J. A. Cell-cycle dysregulation and anticancer therapy. *Trends Pharmacol. Sci.* **2003**, *24*, 139−145.
(6) Sielecki, T. M.; Boylan, J. F.; Benfield, P. A.; Trainor, G. L. Cyclin-dependent Kinase inhibitors: useful targets in cell cycle regulation. *J. Med. Chem.* **2000**, *43*, 1−18.
(7) Hardcastle, I. R.; Golding, B. T.; Griffin, R. J. Designing inhibitors of cyclin-dependent kinases. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 325−348.
(8) Knockaert, M.; Greegard, P.; Meijer, L. Pharmacological inhibitors of cyclin-dependent kinases. *Trends Pharmacol. Sci.* **2002**, *23*, 417−425.
(9) Toogood, P. L. Progress toward the development of agents to modulate the cell cycle. *Curr. Opin. Chem. Biol.* **2002**, *6*, 472−478.
(10) Akinaga, S.; Sugiyama, K.; Akiyama, T. UCN-01 (7-hydroxystaurosporine) and other indolocarbazole compounds: a new generation of anti-cancer agents for the new century? *Anti-Cancer Drug Des.* **2000**, *15*, 43−52.
(11) de Azevedo, W. F.; Mueller-Dieckmann, H.-J.; Schulze-Gahmen, U.; Worland, P. J.; Sausville, E.; Kim, S.-H. Structural basis for the specificity and potency of a flavonoid inhibitor of human CDK2, a cell cycle kinase. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2735−2740.
(12) Kim, K. S.; Sack, J. S.; Tokarski, J. S.; Qian, L. G.; Chao, S. T.; Leith, L.; Kelly, Y. F.; Misra, R. N.; Hunt, J. T.; Kimball, S. D.; Humphreys, W. G.; Wautlet, B. S.; Mulheron, J. G.; Webster, K. R. Thio- and oxoflavopiridols, cyclin-dependent kinase 1-selective inhibitors: Synthesis and biological effects. *J. Med. Chem.* **2000**, *43*, 4126−4134.
(13) Schoepfer, J.; Fretz, H.; Chaudhuri, B.; Muller, L.; Seeber, E.; Meijer, L.; Lozach, O.; Vangrevelinghe, E.; Furet, P. Structure-based design and synthesis of 2-benzylidene- benzofuran-3-ones as flavopiridol mimics. *J. Med. Chem.* **2002**, *45*, 1741−1747.
(14) Gray, N. S.; Wodicka, L.; Thunnissen, A.-M. W. H.; Norman, T. C.; Kwon, S.; Espinoza, F. H.; Morgan, D. O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S.-H.; Lockhart, D. J.; Schultz, P. G.. Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. *Science* **1998**, *281*, 533−538.
(15) Meijer, L.; Raymond, E. Roscovitine and other purines as kinase inhibitors. From starfish oocytes to clinical trials. *Acc. Chem. Res.* **2003**, in press.
(16) Schow, S. R.; Mackman, R. L.; Blum, C. L.; Brooks, E.; Horsma, A. G.; Joly, A.; Kerwar, S. S.; Lee, G.; Schiffman, D.; Nelson, M. G.; Wang, X.; Wick, M. M.; Zhang, X.; Lum, R. T. Synthesis and activity of 2,6,9-trisubstituted purines. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2697−2702.
(17) Imbach, P.; Capraro, H.-G.; Furet, P.; Mett, H.; Meyer, T.; Zimmermann, J. 2,6,9-Trisubstituted purines: Optimization towards highly potent and selective CDK1 inhibitors. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 91−96.
(18) Legraverend, M.; Tunnah, P.; Noble, M.; Ducrot, M.; Ludwig, O.; Grierson, D. S.; Leost, M.; Meijer, L.; Endicott, J. Cyclin-dependent kinase inhibition by new C-2 alkynylated purine derivatives and molecular structure of a CDK2−inhibitor complex. *J. Med. Chem.* **2000**, *43*, 1282−1292.
(19) Dreyer, M. K.; Borcherding, D. R.; Dumont, J. A.; Peet, N. P.; Tsay, J. T.; Wright, P. S.; Bitonti, A. J.; Shen, J.; Kim, S.-H. Crystal structure of human cyclin-dependent kinase 2 in complex with the adenine derivative H717. *J. Med. Chem.* **2001**, *44*, 524−530.
(20) Bramson, H. N.; Corona, J.; Davis, S. T.; Dickerson, S. H.; Edelstein, M.; Frye, S. V.; Gampe, R. T.; Harris, P. A.; Hassell, A.; Holmes, W. D.; Hunter, R. N.; Lackey, K. E.; Lovejoy, B.; Luzzio, M. J.; Montana, V.; Rocque, W. J.; Rusnak, D.; Shewchuk, L.; Veal, J. M.; Walker, D. H.; Kuyper, L. F. Oxindole-based inhibitors of cyclin-dependent kinase 2 (CDK2): Design, synthesis, enzymatic activities, and X-ray crystallographic analysis. *J. Med. Chem.* **2001**, *44*, 4339−4358.
(21) Dermatakis, A.; Luk, K.-C.; DePinto, W. Synthesis of potent oxindole CDK2 inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *11*, 1873−1881.
(22) Andreani, A.; Cavalli, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Recanatini, M.; Garnier, M.; Meijer, L. Imidazo 2,1-b thiazolylmethylene- and indolylmethylene-2-indolinones: a new class of cyclin-dependent kinase inhibitors. Design, synthesis, and CDK1/cyclin B inhibition. *Anti-Cancer Drug Des.* **2000**, *15*, 447−452.
(23) Mettey, Y.; Gompel, M.; Thomas, V.; Garnier, M.; Leost, M.; Ceballos-Picot, I.; Noble, M.; Endicott, J.; Vierfond, J.-M.; Meijer, L. Aloisines, a new family of CDK/GSK-3 inhibitors. SAR study, crystal structure in complex with CDK2, enzyme selectivity, and cellular effects. *J. Med. Chem.* **2003**, *46*, 222−236.
(24) Barvian, M.; Boschelli, D. H.; Cossrow, J.; Dobrusin, E.; Fattaey, A.; Fritsch, A.; Fry, D.; Harvey, P.; Keller, P.; Garrett, M.; La, F.; Leopold, W.; McNamara, D.; Quin, M.; Trumpp-Kallmeyer, S.; Toogood, P.; Wu, Z. P.; Zhang, E. L. Pyrido 2,3-d pyrimidin-7-one inhibitors of cyclin-dependent kinases. *J. Med. Chem.* **2000**, *43*, 4606−4616.
(25) Shewchuk, L.; Hassell, A.; Wisely, B.; Rocque, W.; Holmes, W.; Veal, J.; Kuyper, L. F. Binding mode of the 4-anilinoquinazoline class of protein kinase inhibitors: X-ray crystallographic studies of 4-anilinoquinazolinones bound to cyclin-dependent kinase 2 and p38 kinase. *J. Med. Chem.* **2000**, *43*, 133−138.
(26) Sielecki, T. M.; Johnson, T. L.; Liu, J.; Muckelbauer, J. K.; Grafstrom, R. H.; Cox, S.; Boylan, J.; Burton, C. R.; Chen, H.; Smallwood, A.; Chang, C.-H.; Boisclair, M.; Benfield, P. A.; Trainor, G. L.; Seitz, S. P. Quinazolines as cyclin-dependent kinase inhibitors. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1157−1160.
(27) Arris, C. E.; Boyle, F. T.; Calvert, A. H.; Curtin, N. J.; Endicott, J. A.; Garman, E. F.; Gibson, A. E.; Golding, B. T.; Grant, S.; Griffin, R. J.; Jewsbury, P.; Johnson, L. N.; Lawrie, A. M.; Newell, D. R.; Noble, M. E. M.; Sausville, E. A.; Schultz, R.; Yu, W. Identification of novel purine and pyrimidine cyclin-dependent kinase inhibitors with distinct molecular interactions and tumor cell growth inhibition profiles. *J. Med. Chem.* **2000**, *43*, 2797−2804.
(28) Furet, P.; Meyer, T.; Strauss, A.; Raccuglia, S.; Rondeau, J. M. Structure-based design and protein X-ray analysis of a protein kinase inhibitor. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1157−1160.
(29) Soni, R.; O'Reilly, T.; Furet, P.; Muller, L.; Stephan, C.; Zumstein-Mecker, S.; Fretz, H.; Fabbro, D.; Chaudhuri, B. Selective in vivo and in vitro effects of a small molecule inhibitor of cyclin-dependent kinase 4. *J. Natl. Cancer Inst.* **2001**, *93*, 436−446.
(30) Mesguiche, V.; Parsons, R. J.; Arris, C. E.; Bentley, J.; Boyle, F. T.; Curtin, N. J.; Davies, T. G.; Endicott, J. A.; Gibson, A. E.; Golding, B. T.; Griffin, R. J.; Jewsbury, P.; Johnson, L. N.; Newell, D. R.; Noble, M. E. M.; Wang, L.; Hardcastle, I. R. 4-Alkoxy-2,6-diaminopyrimidine Derivatives: Inhibitors of Cyclin Dependent Kinases 1 and 2. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 217−222.
(31) Honma, T.; Hayashi, K.; Aoyama, T.; Hashimoto, N.; Machida, T.; Fukasawa, K.; Iwama, T.; Ikeura, C.; Ikuta, M.; Suzuki-Takahashi, I.; Iwasawa, Y.; Hayama, T.; Nishimura, S.; Morishima, H. Structure-based generation of a new class of potent CDK4 inhibitors: New de novo design strategy and library design. *J. Med. Chem.* **2001**, *44*, 4615−4627.
(32) Yue, E. W.; Higley, C. A.; DiMeo, S. V.; Carini, D. J.; Nugiel, D. A.; Benware, C.; Benfield, P. A.; Burton, C. R.; Cox, S.; Grafstrom, R. H.; Sharp, D. M.; Sisk, L. M.; Boylan, J. F.; Muckelbauer, J. K.; Smallwood, A. M.; Chen, H. Y.; Chang, C. H.; Seitz, S. P.; Trainor, G. L. Synthesis and evaluation of indenopyrazoles as cyclin-dependent kinase inhibitors. 3. Structure activity relationships at C3. *J. Med. Chem.* **2002**, *45*, 5233−5248.
(33) Kim, K. S.; Kimball, S. D.; Misra, R. N.; Rawlins, D. B.; Hunt, J. T. et al. Discovery of aminothiazole inhibitors of cyclin-dependent kinase 2: Synthesis, X-ray crystallographic analysis, and biological activities. *J. Med. Chem.* **2002**, *45*, 3905−3927.
(34) Ortega, M. A.; Montoya, M. E.; Zarranz, B.; Jaso, A.; Aldana, I.; Leclerc, S.; Meijer, L.; Monge, A. Pyrazolo[3,4-*b*]quinoxalines. A new class of cyclin-dependent kinase inhibitors. *Bioorg. Med. Chem. Lett.* **2002**, *10*, 2177−2184.
(35) Brachwitz, K.; Voigt, B.; Meijer, L.; Lozach, O.; Schachtele, C.; Molnar, J.; Hilgeroth, A. Evaluation of the first cytostatically active 1-aza-9-oxofluorenes as novel selective CDK1 inhibitors with P-glycoprotein modulating properties. *J. Med. Chem.* **2003**, *46*, 876−879.
(36) Hoessel, R.; LeClerc, S.; Endicott, J. A.; Nobel, M. E. N.; Lawrie, A.; Tunnah, P.; Leost, M.; Damiens, E.; Marie, D.; Marko, D.; Niederberger, E.; Tang, W.; Eisenbrand, G.; Meijer, L. Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases. *Nature Cell Biol.* **1999**, *1*, 60−67.
(37) Meijer, L.; Thunnissen, A.-M. W. H.; White, A. W.; Garnier, M.; Nikolic, M.; Tsai, L.-H.; Walter, J.; Cleverley, K. E.; Salinas, P. C.; Wu, Y. Z.; Biernat, J.; Mandelkow, E.-M.; Kim, S.-H.; Pettit, G. R. Inhibition of cyclin-dependent kinases, GSK-3β and casein kinase I by hymenialdisine, a marine sponge constituent. *Chem. Biol.* **2000**, *7*, 51−63.
(38) Soni, R.; Muller, L.; Furet, P.; Schoepfer, J.; Stephan, C.; Zumstein-Mecker, S.; Fretz, H.; Chaudhuri, B. Inhibition of cyclin-dependent kinase 4 (Cdk4) by fascaplysin, a marine natural product. *Biochem. Biophys. Res. Commun.* **2000**, *275*, 877−884.

(39) Gussio, R. Structure-based design modifications of the paullone molecular scaffold for cyclin-dependent kinase inhibition. *Anticancer Drug. Des.* **2000**, *15*, 53−66.
(40) Senderowicz, A. M.; Sausville, E. A. Preclinical and clinical development of cyclin-dependent kinase modulators. *J. Natl. Cancer Inst.* **2000**, *92*, 376−387.
(41) Sausville, E. A. Complexities in the development of cyclin-dependent kinase inhibitor drugs. *Trends Mol. Med.* **2002**, *8* (Suppl. 4), S32−S37.
(42) Gibson, A. E.; Arris, C. E.; Bentley, J.; Boyle, F. T.; Curtin, N. J.; Davies, T. G.; Endicott, J. A.; Golding, B. T.; Grant, S.; Griffin, R. J.; Jewsbury, P.; Johnson, L. N.; Mesguiche, V.; Newell, D. R.; Noble, M. E. M.; Tucker, J. A.; Whitfield, H. J. Probing the ATP ribose-binding domain of cyclin-dependent kinases 1 and 2 with O^6-substituted guanine derivatives. *J. Med. Chem.* **2002**, *45*, 3381−3393.
(43) Davies, T. G.; Bentley, J.; Arris, C. E.; Boyle, F. T.; Curtin, N. J.; Endicott, J. A.; Gibson, A. E.; Golding, B. T.; Griffin, R. J.; Hardcastle, I. R.; Jewsbury, P.; Johnson, L. N.; Mesguiche, V.; Newell, D. R.; Noble, M. E. M.; Tucker, J. A.; Wang, L.; Whitfield, H. J. Structure-based design of a potent purine-based cyclin-dependent kinase inhibitor. *Nat. Struct. Biol.* **2002**, *9*, 745−749.
(44) Yamamoto, H. Anomalous halogen to dimethylamino replacement with *N*,*N*-dimethylformamide catalyzed by ethylenediamine or 2-aminoethanol. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2685−2686.
(45) Whitfield, H. J.; Griffin, R. J.; Hardcastle, I. R.; Henderson, A.; Menyerol, J.; Mesguiche, V.; Sayle, K. S.; Golding, B. T. *J. Chem. Soc. Chem. Commun.* **2003**, 2802−2803.
(46) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Bohnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian 1998, Version A.7. Gaussian, Inc., Pittsburgh, PA, 1998.
(47) Sayle, K. L.; Bentley, J.; Boyle, F. T.; Calvert, A. H.; Cheng, Y.; Curtin, N. J.; Endicott, J.; Golding, B. T.; Hardcastle, I. R.; Jewsbury, P.; Mesguiche, V.; Newell, D. R.; Noble, M. E. M.; Parsons, R. J.; Pratt, D. J.; Wang, L. Z.; Griffin, R. J. Structure-based design of 2-arylamino-4-cyclohexylmethyl-5-nitroso-6-aminopyrimidine inhibitors of cyclin dependent kinases 1 and 2. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3079−3082.
(48) Brown, N. R.; Noble, M. E.; Lawrie, A. M.; Morris, M. C.; Tunnah, P.; Divita, G.; Johnson, L. N.; Endicott, J. A. Effects of phosphorylation of threonine 160 on cyclin-dependent kinase 2 structure and activity. *J. Biol. Chem.* **1999**, *274*, 8746−8756.
(49) Leslie, A. G. W. Recent changes to the MOSFLM package for processing film and image plate data. *Joint CCP4 and ESF-EAMCB Newsletter on Protein Crystallography* **1992**, 26.
(50) Evans, P. R. *Data Reduction*; Science and Engineering Research Council UK: Daresbury Laboratory, Warrington, 1993; pp 114−122.
(51) CCP4 The CCP4 Suite: Programs for Protein Crystallography. *Acta Crystallogr., Sect. D*: *Biol. Crystallogr.* **1994**, *50*, 760−763.
(52) Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr., Sect. D*: *Biol. Crystallogr.* **1997**, *53*, 240−255.
(53) Fletcher, D. A.; McMeeking, R. F.; Parkin, D. The United Kingdom Chemical Database Service. *J. Chem. Inf. Comput. Sci.* **1996**, 36, 746−749.
(54) Jones, T. A.; Zou, J. Y.; Cowan, S. W. Kjeldgaard Improved methods for binding protein models in electron density maps and the location of errors in these models. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1991**, *47*, 110−119.
(55) Lamzin, V. S.; Wilson, K. S. Automated refinement of protein models. *Acta Crystallogr., Sect. D*: *Biol. Crystallogr.* **1993**, *49*, 129−147.

JM0311442