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Article

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Cyclin-Dependent Kinase (CDK) Inhibitors; Structure-Activity Relationships and Insights into the CDK-2 Selectivity of 6-Substituted 2-Arylaminopurines

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Abstract

Purines and related heterocycles substituted at C-2 with 4'-sulfamoylanilino and at C-6 with a variety of groups have been synthesized with the aim of achieving selectivity of binding to CDK2 over CDK1. 6-Substituents that favour competitive inhibition at the ATP binding site of CDK2 were identified and typically exhibited 10-80-fold greater inhibition of CDK2 compared to CDK1. Most impressive was 4-((6-([1,1'-biphenyl]-3-yl)-9*H*-purin-2-yl)amino) benzenesulfonamide (**73**) that exhibited high potency towards CDK2 (IC_{50} 0.044 μM), but was ~ 2000-fold less active towards CDK1 (IC_{50} 86 μM). This compound is therefore a useful tool for studies of cell cycle regulation. Crystal structures of inhibitor-kinase complexes showed that the inhibitor stabilizes a glycine-rich loop conformation that shapes the ATP ribose binding pocket, and that is preferred in CDK2, but has not been observed in CDK1. This aspect of the active site may be exploited for the design of inhibitors that distinguish between CDK1 and CDK2.

Key words

CDK / cyclin / X-ray crystallography / structure-aided inhibitor design / protein kinase

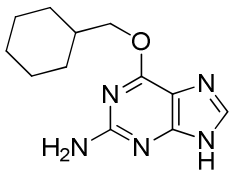
Introduction

The cyclin-dependent kinase (CDK) family of serine/threonine kinases plays an integral role in the regulation of the eukaryotic cell cycle, as well as having key functions in apoptosis, transcription, differentiation and neuronal function. [1, 2] Deregulation of the cell cycle, a hallmark of human cancer, is frequently associated with aberrant CDK activity through mechanisms that may include mutation/overexpression of CDKs, or mutations to genes encoding proteins that directly or indirectly modulate CDK activity. [3-5] CDK2 activation entails association with partner cyclins A and E, whereas the endogenous proteins $p21^{Cip1}$ and $p27^{Kip1}$ are inhibitory. Cyclin E overexpression and/or $p27^{Kip1}$ suppression are a common feature of many human tumors. [6, 7]

Pharmacological inhibition of CDK family members is a potential therapeutic approach for the treatment of cancer. In this context, a large number of ATP-competitive inhibitors have been evaluated. [5-9] The clinical development of CDK inhibitors as antitumour agents was originally hampered by poor kinase selectivity, particularly within the CDK family, and uncertainty as to which CDK constitutes the most appropriate therapeutic target. [3] However, an appreciation of the roles played by specific CDKs in sustaining signalling through dysregulated pathways that drive particular cancers is leading to more stringent patient selection and correspondingly improved efficacy. For example, the highly selective CDK4/6 inhibitor palbociclib has been approved for the treatment of ER-positive, HER2-negative breast cancer, while abemaciclib, palbociclib and ribociclib are in Phase III clinical trials to treat advanced breast cancer in which signalling through the CDK4/6-retinoblastoma axis is critical. [10] Similarly, agents that target a subset of CDKs are being evaluated in a range of other clinically defined segments. [11]

The validity of CDK2 as a cancer therapeutic target was originally called into question both by CDK2 knockdown experiments in which loss of CDK2 failed to induce cell cycle arrest in a number of tumour cell lines, [12] and by mouse knockout experiments where the animals were viable. [13, 14]

However, applying the rationale outlined above, CDK2 inhibitors are also expected to have utility in settings in which the cancer is addicted to enhanced CDK2 activity or where synthetic lethalities can be identified. Recent studies employing a chemical genetic approach in which CDK2 expression was maintained, but kinase activity was inhibited, provides compelling evidence that CDK2 is a valid cancer target. In a panel of human cancer cells transformed with various oncogenes, highly selective small-molecule CDK2 inhibition resulted in marked growth inhibition. [15] CDK2-selective inhibitors may also have applications in combination therapies in appropriate clinical settings. For example, a recent study demonstrated that a combination of phosphatidylinositol-3-kinase and CDK2 inhibitors induced apoptosis in malignant glioma xenografts via a synthetic-lethal interaction. [16] Accumulating evidence also implicates CDK2 as a prospective therapeutic target in BRCA-deficient cancers, [17] neuroblastoma [18] and ovarian cancer. [19] These observations, taken together with supportive clinical data, [20] have led to a resurgence of interest in CDK2 inhibitors as cancer therapeutic agents. [21, 22] Ideally, such an inhibitor would discriminate between CDK2 and CDK1 to avoid cell toxicity as a result of inhibiting CDK1 which is an essential member of the CDK family.



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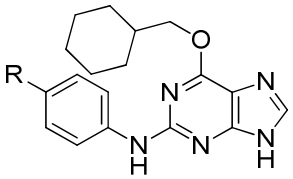
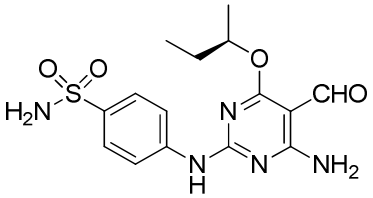


2; R = H
3; R = SO_2NH_2



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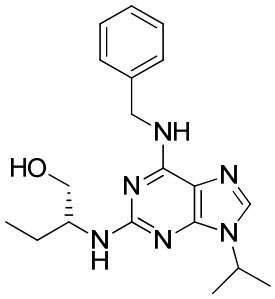
Our previous studies employing a structure-lead approach resulted in the development of selective ATP-competitive CDK2 inhibitors based on 2-amino-6-alkoxy- (**1**; NU2058) and 2-arylamino-6-alkoxy- (**2**) purine derivatives. [23, 24] These efforts were rewarded by the identification of potent purine-based inhibitors, exemplified by 6-cyclohexylmethoxy-2-(4'-sulfamoylanilino)purine (**3**; NU6102, CDK2 IC_{50} = 5.0 nM), [25] as well as alkoxypyrimidine inhibitors (*e.g.* **4**, CDK2 IC_{50} = 0.8

nM). [26] Importantly, **3** exhibits selectivity for CDK2 over other CDK-family members, proving some 50-fold selective for CDK2 over CDK1 (IC_{50} = 250 nM), a kinase with close structural homology to CDK2. In addition, **3** is only weakly active against CDK7 and CDK9 (IC_{50} = 4.4 μM and 1.1 μM, respectively). These CDKs play a key role in transcriptional regulation through phosphorylation of RNA polymerase II. [2, 27] Modulation of protein synthesis by off-target inhibition of CDK7/CDK9 has obfuscated previous studies to elucidate the pharmacological effects of clinically evaluated compounds initially identified as selective CDK2 inhibitors such as seliciclib (**5**), dinaciclib (**6**) and SNS-032 (**7**). [3, 28, 29]



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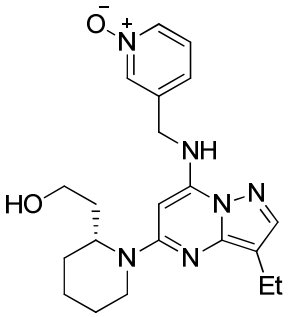

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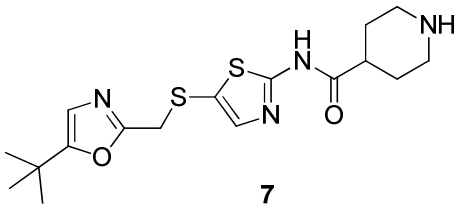

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Crystal structures of purines **1**-**3** in complex with T160-phosphorylated CDK2-cyclin A (CDK2-cyclin A) provided valuable details of inhibitor interactions within the ATP-binding site. [23, 24] The purine heterocycle anchors the inhibitor through a triplet of hydrogen bonds between N-9, N-3, and the 2-amino group, and the backbone carbonyl moiety of Glu81 and amide and carbonyl moieties of Leu83, respectively, located in the hinge region of the kinase (Figure 2). The greater potency and selectivity of **3** compared with **2** and the parent 2-amino-6-cyclohexylmethoxypurine **1**, has been attributed, at least in part, to additional interactions of the 2-sulfanilyl substituent of **3** with the specificity surface of CDK2. [24, 30] Notably, two hydrogen bond interactions between the sulfonamide group of **3** and Asp86 facilitate optimal hydrophobic packing of the arylamino ring.

Extensive structure-activity relationship studies (SARs) at the purine 2-position of **3** have further established the importance of these inhibitor-kinase interactions. [25] By contrast, although the 6-

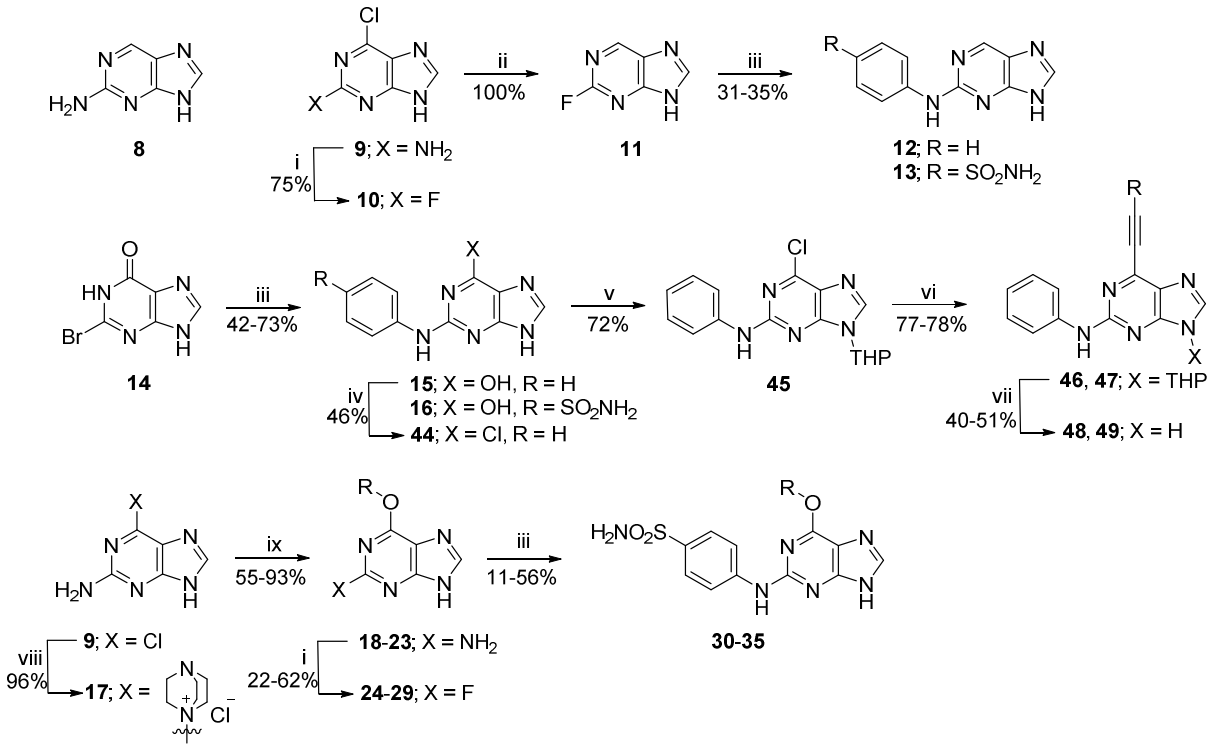
cyclohexylmethyl group of **3** occupies the ribose-binding pocket, the precise nature of the interactions made by substituents at the purine 6-position with the CDK2 active site remains uncertain, notwithstanding that a large number of derivatives have previously been evaluated empirically. [31]

In this paper, we report the results of studies to characterise interactions between substituents at the purine 6-position and the CDK2 ATP-binding site. The effect upon biological activity of modifying the core purine heterocycle of **3** is also reported. These studies have resulted in the identification of 4-((6-([1,1'-biphenyl]-3-yl)-9*H*-purin-2-yl)amino) benzenesulfonamide **73** as a potent and selective CDK2 inhibitor (IC_{50} = 44 nM), exhibiting some 2,000-fold-selectivity over CDK1 (IC_{50} = 86 μM).

Chemistry

With a view to establishing the overall contribution of the 6-cyclohexylmethoxy substituent of **1** and **3** to inhibitor binding, the simple 6-unsubstituted purines **8**, **12** and **13** were required. 2-aminopurine (**8**) was commercially available, while the 2-phenylaminopurine (**12**) and 2-sulfanilylpurine (**13**) derivatives were synthesised as outlined in Scheme 1. Thus, selective reductive dehalogenation of 6-chloro-2-fluoropurine (**10**), prepared from 2-amino-6-chloropurine (**9**) following a literature procedure, [32] afforded 2-fluoropurine (**11**). Treatment of **11** with aniline or 4-aminobenzenesulfonamide (sulfanilamide) in 2,2,2-trifluoroethanol (TFE) with catalysis by trifluoroacetic acid (TFA) as described previously, [33] gave the respective 2-arylaminopurines **12** and **13** in good yield. The required 2-arylaminoguanine derivatives **15** and **16** were also readily accessible from commercially available 2-bromohypoxanthine (**14**) under similar conditions. The 6-alkoxypurine derivatives (**30**-**35**) were synthesised utilising previously optimised methodology. [23, 25, 31] Briefly, introduction of the 6-alkoxy substituent was achieved either by direct reaction of the appropriate sodium alkoxide with 2-amino-6-chloropurine (**9**) employing the corresponding alcohol as solvent, or, where necessary, following conversion into the 'DABCO-purine' intermediate (**17**).[34]

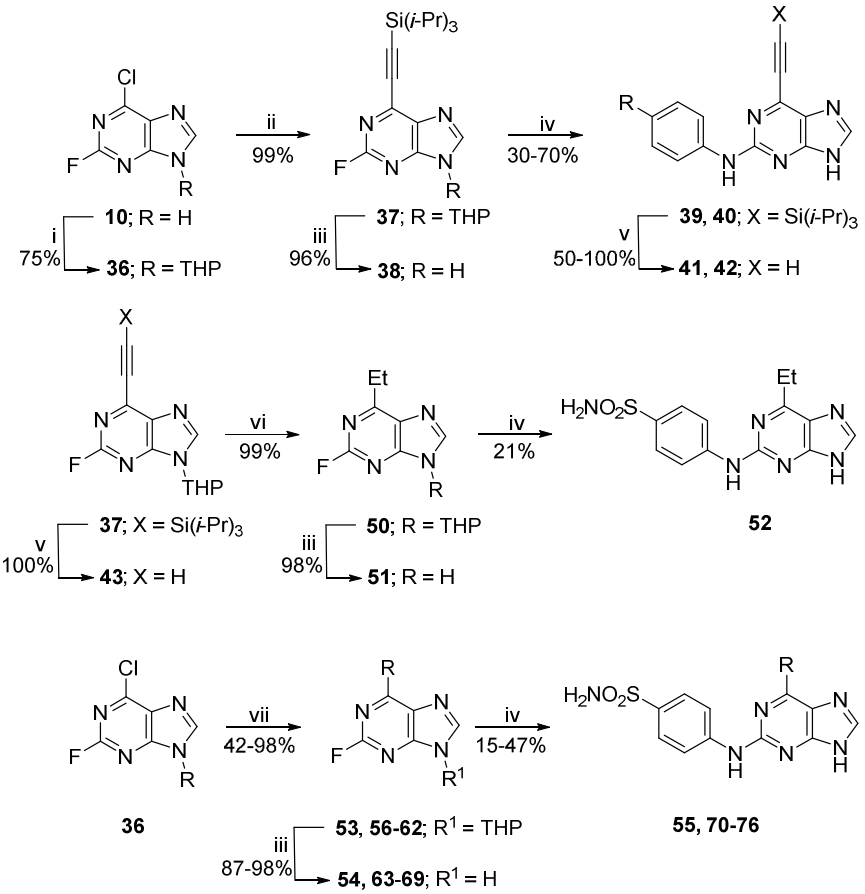
Elaboration of the 6-alkoxy-2-aminopurines (**18-23**) into the corresponding 2-fluoropurines (**24-29**) under Balz-Schiemann conditions, enabled subsequent introduction of the 2-(4-aminobenzenesulfonamide) group using the TFA-TFE procedure, to furnish the target purines (**30-35**) in good overall yields (Scheme 1).



Scheme 1. Reagents and conditions – (i) aq. HBF_4, $NaNO_2$, -15-0 °C; (ii) $Pd(OH)_2$, HCO_2NH_4, MeOH, reflux; (iii) $ArNH_2$, TFA, TFE, 90 ºC; (iv) $POCl_3$, $PhNMe_2$, reflux; (v) 3,4-dihydropyran, (*rac*)-camphorsulfonic acid, EtOAc, 75 °C; (vi) R-CCH, $PdCl_2(MeCN)_2$, dicyclohexyl-(2',4',6'-triisopropyl-[1,1'-biphenyl]-2-yl)phosphine, Cs_2CO_3, MeCN; (vii) TFA, *i*-PrOH, H_2O, reflux; (viii) DABCO, DMSO, 25 °C; (ix) For **18, 19, 22:** ROH, Na, reflux, For **20, 21, 23**: ROH, NaH, DMSO, 25 ºC. For definition of R groups see Table 1.

The 6-ethynylpurine derivatives (**41** and **42**) were synthesised from the THP-protected 6-chloro-2-fluoropurine (**36**) [35] via a Sonogashira alkynylation approach (Scheme 2). Coupling of triisopropylacetylene with **36**, employing $Pd(PPh_3)_2Cl_2$/CuI, afforded the protected 6-ethynylpurine (**37**) in excellent yield, and removal of the N^9-THP group to give **38** and introduction of the 2-

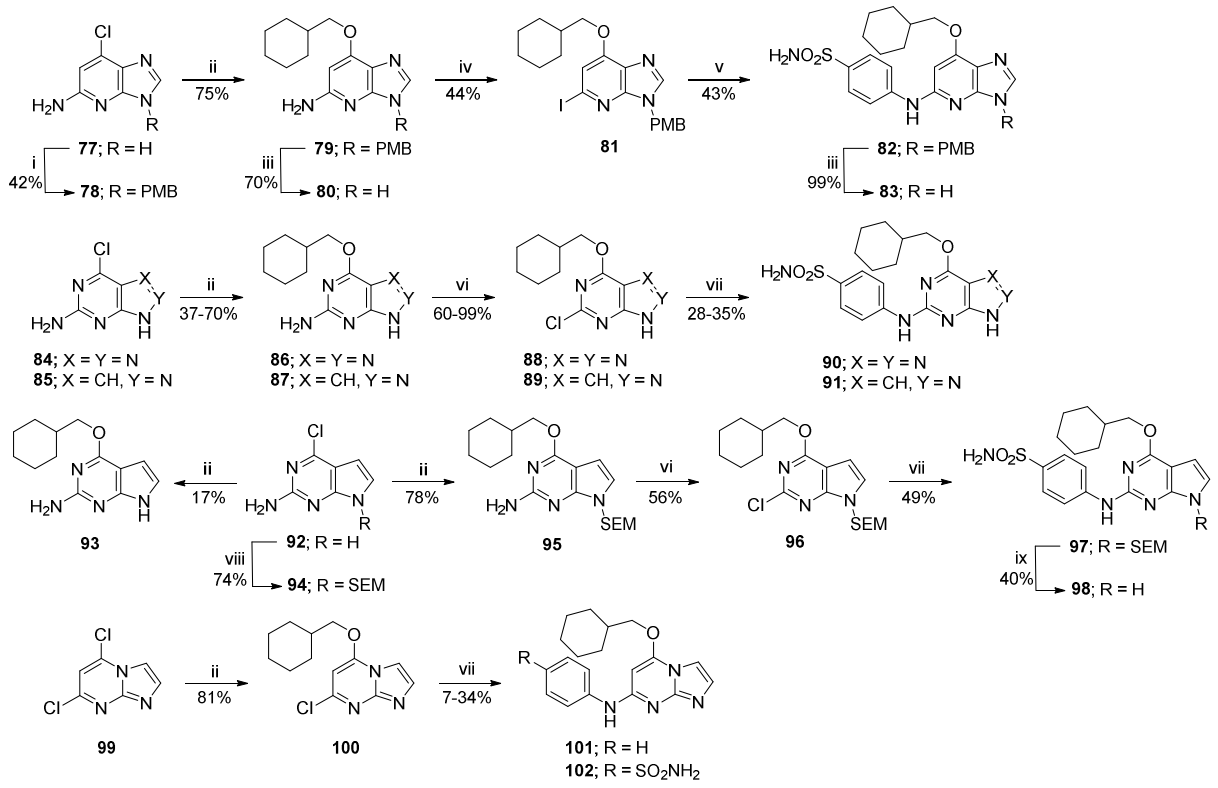
arylamino substituent, proceeded in the manner described above to give **39** and **40**. Removal of the TIPS group to furnish the target 2-arylamino-6-ethynylpurines **41** and **42** was achieved under standard conditions. The 6-propynyl- and 6-phenylethynyl-purine derivatives **48** and **49** were accessible from the guanine derivative **15** by sequential chlorination at the purine 6-position (**44**), THP protection (**45**), introduction of the appropriately substituted ethynyl group under Sonogashira conditions (**46**, **47**) and final N^9-deprotection to furnish the target purines **48** and **49** (Scheme 1). The 6-ethylpurine derivative (**52**) was readily obtained by reduction of the 6-ethynyl group of the THP derivative **43** with Lindlar's catalyst-quinoline to afford **50**, with N^9-deprotection (**51**) and final introduction of the 2-(4-aminobenzenesulfonamide) group giving **52** in modest overall yield.



Scheme 2. Reagents and conditions – (i) 3,4-dihydropyran, (*rac*)-camphorsulfonic acid, EtOAc, 75 °C; (ii) $(i\text{-Pr})_3SiCCH$, $Pd(PPh_3)Cl_2$, CuI, Et_3N, THF, 25 ºC; (iii) TFA, *i*-PrOH, H_2O, reflux; (iv) $PhNH_2$ or $4\text{-}NH_2C_6H_4SO_2NH_2$, TFA, TFE, 90 ºC; (v) $(n\text{-Bu})_4NF$, THF, 25 ºC; (vi) Lindlar's catalyst, quinoline, H_2, EtOAc, RT, 2 h; (vii) $RB(OH)_2$ or RBF_3K, $Pd(OAc)_2$, dicyclohexyl(2',6'-

dimethoxybiphenyl-2-yl)phosphine, K_3PO_4, PhMe, H_2O, 100 ºC. For definition of R groups see Table 1.

Efforts to synthesise the 6-cyclopropylpurine (**55**) employing Suzuki-Miyaura conditions were unsuccessful, likely due to competing protodeboronation of the cyclopropylboronic acid under the reaction conditions employed. This problem was successfully addressed as reported in the literature, by converting the cyclopropylboronic acid into the corresponding potassium trifluoroborate, [36] whereby coupling to the THP-protected 6-chloro-2-fluoropurine (**36**) occurred to furnish **53** in acceptable yield. Subsequent transformation into the target 6-cyclopropylpurine (**55**) was achieved readily. The introduction of aryl substituents at the purine 6-position was achieved using Suzuki-Miyaura cross-coupling chemistry with 6-chloro-2-fluoropurine derivative **36** (Scheme 2). Optimisation of the reagents and reaction conditions employed for the cross-coupling step, enabled the preparation of a defined series of 2-fluoro-6-arylpurine derivatives (**56**-**62**) in excellent yields, and conversion into the requisite 6-aryl-2-(4-sulfonamidophenyl)aminopurines (**70**-**76**) proceeded smoothly under the previously described conditions. [25, 33]



Scheme 3. Reagents and conditions – (i) PMB-Cl, K_2CO_3, DMF, 80 ºC; (ii) NaH, cyclohexylmethanol, DMSO, 25 ºC; (iii) TFA, 80 ºC; (iv) CH_2I_2, *iso*-amyl nitrite, CuI, THF, 80 ºC; (v) sulfanilamide, $Pd_2(dba)_3$, XPhos, K_2CO_3, MeCN, 80 ºC; (vi) LiCl, *iso*-amyl nitrite, $SOCl_2$, DMA or THF, 0 ºC - 25 ºC; (vii) $ArNH_2$, TFA, TFE, 90 ºC; (viii) SEM-Cl, NaH, MeCN, 0 ºC; (ix) TBAF, THF, 25 ºC.

The synthesis of prospective CDK2 inhibitors encompassing alternative heterocyclic systems was undertaken as summarised in Scheme 3. In each case, the parent chloroheterocycles (**77**, **84**, **85**, **92**, **99**) were synthesised by adapting literature procedures [37-41] and the cyclohexylmethyl substituent introduced by treatment with the corresponding alkoxide, with N^9 protected where necessary, as described previously. Iodination (**81**) [42] or chlorination (**88**, **89**, **96**) of the respective 2-aminoheterocycles under Sandmeyer conditions facilitated introduction of the 2-arylamino group under the standard TFA-TFE conditions (**88**, **89**), or employing a Buchwald amination procedure (**81**, **96**), followed by deprotection as necessary to afford the target compounds **83**, **90**, **91**, and **98**.

The imidazo[1,2-*a*]pyrimidine derivatives (**101**, **102**) were prepared from 5,7-dichloroimidazo[1,2-*a*]pyrimidine (**99**) [43, 44] by regioselective 5-alkoxylation to give **100**, followed by final introduction of the arylamino group at the 7-position under TFA-TFE conditions. Given the possibility of alkoxide attack at the 7-position of **99** to furnish the alternative regioisomer, the structure of **102** was unambiguously confirmed by X-ray crystallography (Supporting Information).

Results and Discussion

The chemical structures, CDK-inhibitory activity, and *in vitro* antitumor activity for the purine derivatives and alternative heterocycles are summarised in Tables 1-3, with the values for compounds **1**-**3** included for comparative purposes.

Structure-Activity Relationships – The importance of a substituent at the purine 6-position was reaffirmed by the simple 2-amino- and 2-phenylamino-purines (**8** and **12**), where a dramatic reduction in potency was observed compared with the parent 6-cyclohexylmethoxy derivatives **1** and **2**, respectively. A similar effect was also evident for the sulfanilylpurine **13**, which was some 300-fold less active than **3**. These results are consistent with the putative interaction of this inhibitor class with the ATP-binding site of CDK2, which requires that the 6-substituent occupies a lipophilic pocket close to the ribose binding site, thereby orienting the purine to make the triplet of hydrogen bonds with the hinge region. [24] The low-micromolar potency of the 2-sulfanilyl derivative **13** likely reflects the contribution to binding arising through additional interactions between the arylsulfonamide group of **13** with the CDK2 surface adjacent to the ATP binding site on the C-terminal lobe (termed the "specificity surface"), despite the absence of a 6-substituent. Perhaps not surprisingly, the guanine derivatives **15** and **16** were only weakly active, attributable to unfavourable interactions between the 6-oxo functionality and the ribose-binding pocket. These derivatives also exhibited the very poor aqueous solubility characteristic of many guanine derivatives.

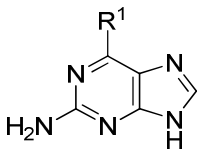
Replacement of the 6-cyclohexylmethoxy group of **3** by smaller alkoxy substituents proved informative, and was broadly consistent with our earlier investigations with derivatives of **1.** [31] In that study, 2-amino-6-methoxypurine (O^6-methylguanine) demonstrated negligible activity (CDK2; IC_{50} > 100 μM), and the relatively poor chemical stability of this purine militated against preparing the corresponding 2-sulfanilyl analogue. The 6-ethoxypurine **30** exhibited high CDK2-inhibitory activity (IC_{50} = 26 nM), with an approximately three-fold increase in potency being observed for the *n*-propoxy- (**31**; IC_{50} = 8 nM) and *iso*-propoxy (**32**; IC_{50} = 10 nM) derivatives. Potency was improved further by increasing the size of the 6-alkoxy group, as demonstrated by the activity of the isomeric butoxy analogues **33** and **34** with IC_{50} values of 3 nM and 1 nM, respectively. The relationship between the bulk of the 6-alkoxy group and the CDK2-inhibitory activity generally parallels that previously observed for the corresponding 2-amino-6-alkoxypurines, albeit that the sulfanilylpurines **30**-**34** are several orders of magnitude more potent. For example, **34** (IC_{50} = 1 nM) is approximately 50,000-fold more potent than the corresponding 2-amino-6-*sec*-butoxypurine (CDK2; IC_{50} = 49 ± 7 μM), [31] again corroborating the crucial binding contribution made by the arylsulfonamide function. A propargyloxy group at the purine O^6-position conferred activity comparable to the ethoxy derivative (compare **35** and **30**), in keeping with the trend reported previously for the 2-amino-6-alkoxypurine series. [31]

The introduction of an ethyl substituent at the purine 6-position (**52**) reduced CDK2-inhibitory activity some 10-fold compared with the 6-ethoxypurine (**30**), and very modest potency was also observed for the 6-ethynyl derivatives **41** and **42**. Although 6-alkoxy and 6-alkyl groups differ with regard to both their electronic and steric character, as well as lipophilicity, the high potency of the 6-triisopropylsilyethynylpurine (**40**; IC_{50} = 17 nM) shows that steric bulk, as well as electronic factors, is an important feature for binding in the ribose pocket of CDK2. The modest improvement in potency observed for the prop-1-ynyl (**48**) and phenylethynyl (**49**) derivatives compared with the parent 6-ethynylpurine **41**, also suggests that the overall shape of the 6-substituent is an important

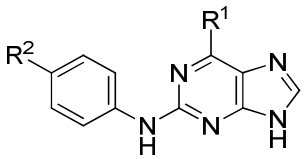
factor for occupancy of the ribose-binding pocket, supported by the activity of the 6-cyclopropylpurine **55** (IC_{50} = 19 nM). These data also suggest that the oxygen atom of the 6-alkoxypurines, which would act as a weak hydrogen bond acceptor, does not make a significant contribution to binding affinity.

The potency of the 6-phenylpurine (**70**; IC_{50} = 24 nM) prompted further elaboration, with comparable activity residing in the 3-methoxyphenyl (**71**), 4-methoxyphenyl (**72**), and 6-(3-phenylphenyl)purine (**73**) derivatives. By contrast, the piperonyl derivative (**74**) was some 20-fold less potent than **70**, and the introduction of still larger groups was detrimental, as evident from the very weak CDK2-inhibitory activity of the dibenzofuran-1-yl (**75**) and thianthren-1-yl (**76**) analogues. The bulkier bicyclic and tricyclic heteroaromatic rings of these purines are presumably poorly accommodated within the ribose-binding pocket. It is of interest to note that the CDK2-inhibitory activity of the 6-unsubstituted derivative **13** is equipotent with **75** and superior to that of **76**, implying that the 6-substituents of these purines make a negligible binding contribution.

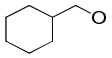
Table 1. Chemical structures and CDK2-inhibitory activity of purine derivatives

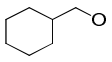
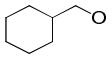
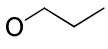
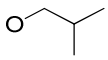
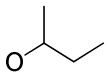
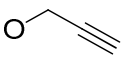


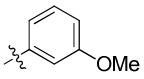
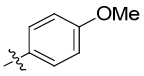
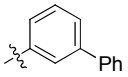
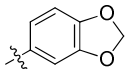
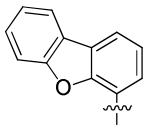
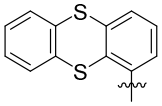
A



B

No.	Structure	R^1	R^2	CDK2 Inhibition IC_{50} (μM)[a] or Inhibition (%) at 100 μM
1	A		–	17 ± 2[b]

2	B		H	0.97 ± 0.03
3	B		SO_2NH_2	0.005 ± 0.001
8	A	H	–	(4 ± 3%)[c]
12	B	H	H	61 ± 0.1
13	B	H	SO_2NH_2	1.5 ± 0.1
15	B	OH (C=O)[d]	H	(34 ± 1%)
16	B	OH (C=O)[d]	SO_2NH_2	(52 ± 4%)
30	B	OEt	SO_2NH_2	0.026 ± 0.001
31	B		SO_2NH_2	0.008 ± 0.002
32	B		SO_2NH_2	0.01 ± 0.001
33	B		SO_2NH_2	0.003 ± 0.0003
34	B	 (rac)	SO_2NH_2	0.001 ± 0.0005
35	B		SO_2NH_2	0.019 ± 0.001
40	B	C≡CSi(*i*-Pr)$_3$	SO_2NH_2	0.017 ± 0.001
41	B	C≡CH	H	21 ± 9

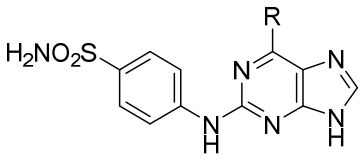
42	B	C≡CH	SO_2NH_2	0.83 ± 0.03
48	B	C≡CMe	H	6.4 ± 0.4
49	B	C≡CPh	H	8.9 ± 0.7
52	B	Et	SO_2NH_2	0.22 ± 0.003
55	B		SO_2NH_2	0.019 ± 0.001
70	B	Ph	SO_2NH_2	0.024 ± 0.002
71	B		SO_2NH_2	0.039 ± 0.013
72	B		SO_2NH_2	0.052 ± 0.004
73	B		SO_2NH_2	0.044 ± 0.012
74	B		SO_2NH_2	0.58 ± 0.02
75	B		SO_2NH_2	1.4 ± 0.2
76	B		SO_2NH_2	6.7 ± 0.5

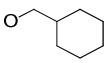
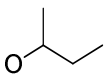
[a]IC_{50} values were determined in accordance with previously described methods. [20, 23] [b]Data shown are the mean of at least two independent experiments ± standard deviation. [c]% inhibition values are in brackets. [d]The oxo-tautomers predominate.

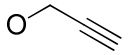
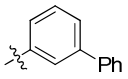
With a view to assessing selectivity for CDK2, selected purine derivatives were evaluated against a panel of CDKs (Table 2). As expected, removal of the 6-cyclohexylmethyl substituent of **3,** which makes a number of interactions with the conserved ATP ribose binding site, to furnish **13** compromised potency against all the CDKs evaluated, although the effect was less pronounced against CDK2.

The 6-alkoxypurines **34** and **35** exhibited good selectivity for CDK2 over the closely related CDK1 (80-fold and 50-fold, respectively), with **34** retaining selectivity over CDKs 4, 7 and 9 comparable with **3**. A similar profile was observed for the 6-phenylpurine **70**, which was some 30-fold selective for CDK2 over CDK1. By contrast, the 6-([1,1'-biphenyl]-3-yl)purine derivative **73** exhibited a very interesting CDK selectivity profile, proving some 2,000-fold selective for CDK2 over CDK1, whilst exhibiting only weak inhibitory activity against CDKs 4, 7, and 9. To the best of our knowledge this level of selectivity for CDK2 over CDK1 is unprecedented.

Table 2. CDK selectivity and cellular activity for selected compounds



Compd.	R	IC$_{50}$ (μM) or % inhibition at 100 μM[a]				
		CDK1/B	CDK2/A	CDK4/D	CDK7/H	CDK9/T
3		0.25 ± 0.05[b]	0.005 ± 0.001	1.45 ± 0.70	4.40 ± 0.90	1.07 ± 0.02
13	H	15.8 ± 1.2	1.5 ± 0.09	(12 ± 4%)[c]	75 ± 3	38 ± 11
34		0.097 ± 0.040	0.0012 ± 0.0005	1.30 ± 0.30	2.8 ± 0.1	0.57 ± 0.05

35		0.94 ± 0.1	0.019 ± 0.001	8.8 ± 1.2	–	–
70	Ph	0.73 ± 0.003	0.024 ± 0.002	3.8 ± 1.4	6.8 ± 0.4	0.57 ± 0.04
73		86 ± 4	0.044 ± 0.012	(26 ± 2%)	(28 ± 6%)	25 ± 8

[a]IC_{50} values were determined in accordance with previously described methods. [20, 23] [b]Data shown are the mean of at least two independent experiments ± standard deviation. [c]% inhibition values are in brackets.

Modification of the core purine heterocycle of **1** and **3** afforded further interesting results (Table 3). A comparison of 2-amino-6-cyclohexylmethylpurine **1** with the alternative heterocycles **86**, **87** and **93** revealed that although these compounds exhibited comparable potency against CDK2, activity against other CDK family members was markedly attenuated. The very weak activity of the imidazopyrimidine derivatives **101** and **102** is not surprising given the absence of the requisite donor-acceptor-donor motif, and presumably reflects the combined influence arising from the absence of the N^1 and N^7 functions found in the corresponding purine derivatives **1** and **3**. The modest CDK inhibition profile of imidazopyridine **83** indicates that removal of the N^1 nitrogen is detrimental to CDK-inhibitory activity. However, with the exception of the imidazopyridine (**83**) and imidazopyrimidine (**102**) derivatives, modification of the core purine heterocycle of **3** was generally tolerated without a marked loss of CDK2-inhibitory activity. Thus, triazolopyrimidine (**90**) analogue is 2.5-fold more potent than **3**, while pyrazolopyrimidine (**91**) and pyrrolopyrimidine (**98**) analogues proved only some 4-5-fold less potent than **3** against CDK2, and retained good selectivity over all other CDK family members examined. Notably, derivatives **91** and **98** were approximately 100-fold selective for CDK2 over CDK1.

Table 3. Chemical structures and CDK-inhibitory activity of other heterocyclic derivatives

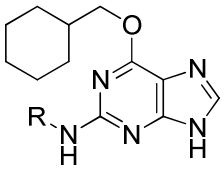
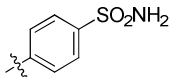
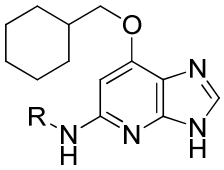
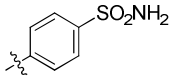
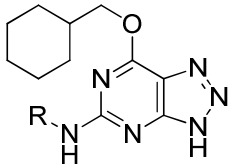
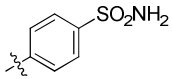
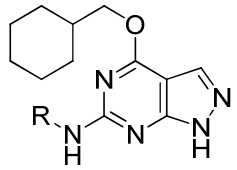
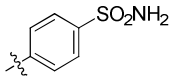
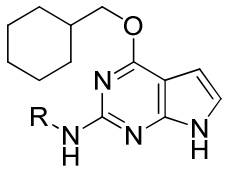
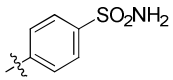
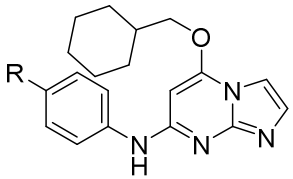
No.	Structure	R	CDK Inhibitory Activity IC_{50} (μM)[a] or Inhibition at 10 μM (%)					
			CDK1	CDK2	CDK4	CDK5	CDK7	CDK9
1		H	26 ± 4[b]	17 ± 2	(33 ± 9%)[c]	97 ± 3	(44 ± 6%)	(42 ± 11%)
3			0.25 ± 0.05	0.005 ± 0.001	1.5 ± 0.7	0.48 ± 0.07	4.4 ± 0.9	1.1 ± 0.2
80		H	-	28.4 ± 0.8	-	-	-	-
83			11 ± 2	0.29 ± 0.01	23 ± 1%	(37 ± 2%)	(37 ± 4%)	(42 ± 5%)
86		H	(21 ± 1%)	18 ± 1	(6.2 ± 3.6%)	(13 ± 1%)	(13 ± 6%)	(16 ± 8%)
90			0.75 ± 0.06	0.002 ± 0.0001	9.6 ± 0.5	3.3 ± 0.2	6.8 ± 0.7	0.75 ± 0.03

Table 3 (continued). Chemical structures and CDK-inhibitory activity of other heterocyclic derivatives

No.	Structure	R	CDK Inhibitory Activity IC_{50} (μM)[a] or Inhibition at 10 μM (%)					
			CDK1	CDK2	CDK4	CDK5	CDK7	CDK9
87		H	(32 ± 1%)	16 ± 4	(0%)	(14 ± 9%)	(16 ± 7%)	(16 ± 2%)
91			1.6 ± 0.2	0.018 ± 0.001	2.8 ± 0.3	5.9 ± 0.1	7.0 ± 0.3	4.6 ± 0.2
93		H	(11 ± 3%)	(41 ± 10%)[d]	(5.6 ± 7.9%)	(12 ± 1%)	(5.1 ± 5.5%)	(0%)
98			2.7 ± 0.2	0.026 ± 0.001	1.9 ± 0.1	4.4 ± 0.6	4.1 ± 0.2	1.0 ± 0.2
101		H	-	(10 ± 6%)	-	-	-	-
102		SO_2NH_2	-	(36 ± 3%)	-	-	-	-

[a] IC_{50} values were determined in accordance with previously described methods. [20, 23] [b]Data shown are the mean of at least two independent experiments ± standard deviation. [c]% inhibition values are in brackets. [d]Value determined at 100 μM.

Cellular studies

The ability of compounds to inhibit cellular proliferation was examined in a 5-day growth assay using five histologically distinct retinoblastoma protein (Rb)-proficient human tumour lines (A375 melanoma, Calu-6 lung carcinoma, MDA-MB-231 breast adenocarcinoma, SJSA1 osteosarcoma and SKUT-1B uterine corpus leiomyosarcoma). Whilst compound **73** demonstrated appreciable nanomolar potency versus CDK2 in isolated kinase assays, the maximal concentration tested in cellular assays (30 μM) had no or limited effects on the growth of cells, thereby preventing the concentration that induced a 50% growth inhibitory (GI_{50}) effect to be determined (data not shown). In contrast, compounds **91** and **98**, which were only 2.4 and 1.7 fold more potent respectively in CDK2 kinase assays, dose-dependently inhibited the growth of each tumour cell line with GI_{50} values ranging from 1 – 22 μM. The cellular activity of these compounds compared favourably to that of compound **3**.

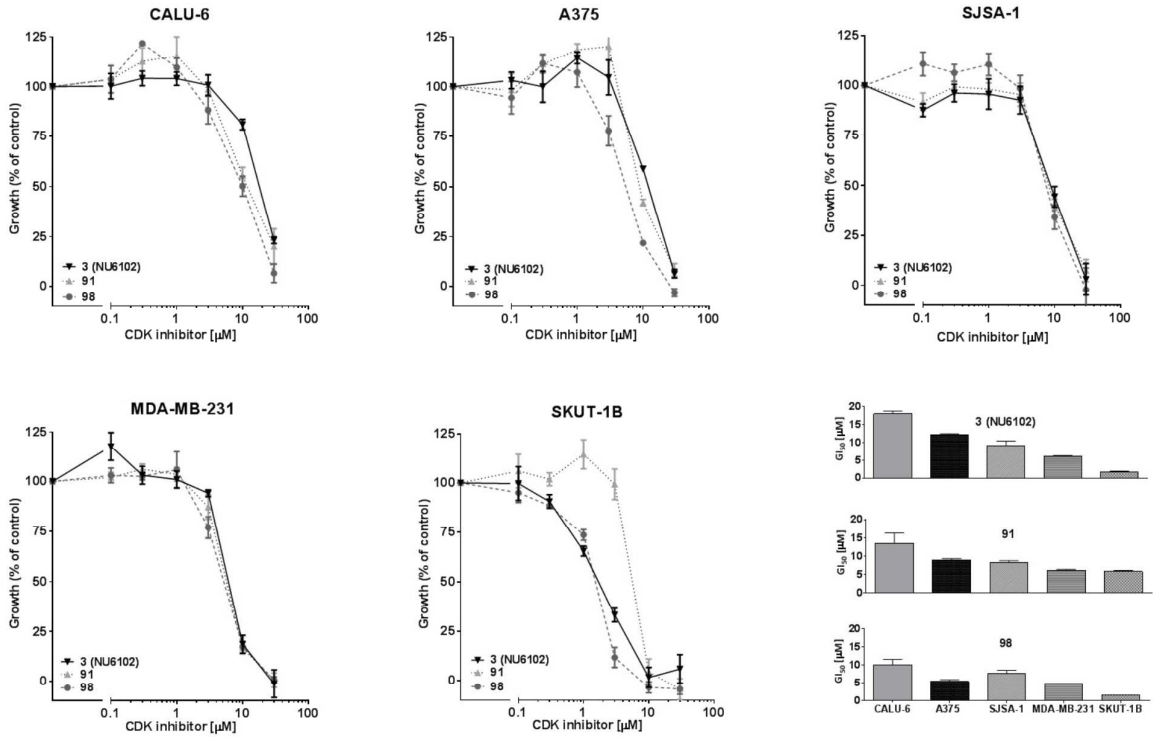


Figure 1. Activity of compounds 3, 91 and 98 against tumour cell growth.[45] Rb-proficient human tumour cell lines were incubated for 120h with compounds and cellular protein determined

by SRB assay. Dose-response curves and GI_{50} bar charts represent the mean ± SEM from 3 or 4 independent experiments.

Structure determination- Compounds within this series show greater activity towards CDK2 than CDK1, generally varying between 10 and 80-fold (Table 2). However, **73** exhibits exceptional discrimination being a *circa* 2000-fold more potent inhibitor of CDK2. In order to confirm the binding mode and to identify potential interactions that might explain the selectivity of **73** for CDK2 over CDK1, **73** and **3** were co-crystallised with CDK2-cyclin A and CDK1-cyclin B- cyclin-dependent kinases regulatory subunit 2 (CKS2) respectively. The poor potency of **73** towards CDK1 appears to preclude determination of a CDK1-cyclin B-CKS2-**73** co-complex structure, as despite repeated attempts we were unable to visualise the compound in the active site. The statistics for the datasets and for the crystallographic refinements are presented in Table 4. Compound **73** emulates the interactions made by **3** within the CDK2 active site (Figure 2A). [24] Indeed, the purine and aniline rings of each inhibitor superimpose very well. The purine rings occupy the CDK2 ATP binding site and each makes a triplet of hydrogen bonds with the backbone carbonyl of Glu81 and the amide NH and carbonyl moieties of Leu83 within the hinge sequence (Figure 2A). The respective anilino rings project out of the CDK2 catalytic site so that the sulfonamide NH_2 group and one of the oxygens in each make hydrogen bonds with the side chain and backbone nitrogen of Asp86, respectively.

The structures of **73** and **3** differ in the substitution present at the purine C-6 position. The O^6-cyclohexylmethyl substituent of **3** occupies the ATP ribose binding pocket and is complementary in shape and forms favourable hydrophobic interactions with an apolar pocket created by the conformation of the CDK2 glycine-rich loop (residues 9-19). [24] This loop adopts an identical backbone structure when bound to **73,** so that the purine-proximal phenyl ring emulates the position of the cyclohexylmethyl substitutent of **3**. The distal phenyl ring substituted at the *meta* position can

then be comfortably accommodated as it twists towards the aniline moiety (Figure 2A). In contrast, *para* substitutions of the purine-proximal phenyl ring exemplified by **72**, **74**, **75** and **76** (Table 1) would be directed out of the CDK2 active site towards the tip of the glycine-rich loop. Smaller substitutions, for example a methoxy in **72** can be accommodated but larger ring systems as exemplified by **74** and **76** lead to considerable drops in potency suggesting they may sterically clash with the CDK2 structure in this region (Table 1).

To probe further the binding mode of the series, CDK1-cyclin B-CKS2 was co-crystallised with **3** (Figure 2B). This structure shows that the purine backbone emulates the interactions made by this inhibitor within the CDK2 binding site and that the O^6-cyclohexylmethyl substituent occupies the ribose binding pocket. As previously reported, [46] inhibitor binding within the ATP binding site is accompanied by minor remodelling of the CDK1-cyclin B into a conformation compatible with catalysis.

All of the sidechains contacted by **73** in the co-complex with CDK2-cyclin A are conserved in CDK1, so that selectivity must derive from indirect readout of remote sequence differences. One mechanism by which such differences can impact inhibitor binding is where they shape the conformational energy landscape, permitting conformations in one kinase that are precluded in another. As described above, the glycine-rich loop makes a significant contribution to the catalytic cleft, shaping the binding site that accommodates the purine C-6 substituents. We note that the glycine-rich loop in the CDK2-cyclin A-**73** structure adopts a conformation in which Tyr15 is folded into the active site, contacting residues of the C-helix (Figure 2C). This position for the sidechain of Tyr15 is seen in a number of other CDK2-cyclin A-inhibitor complexes, suggesting that it reflects a preferred conformation of CDK2. However, in available structures of apo CDK1-cyclin B, and CDK1-cyclin bound to compound **23** [46] or **3** (this paper), a comparable location for the side chain of Tyr15 has not been observed (Figure 2C). We speculate, therefore, that **73** binds

more tightly to CDK2 than to CDK1 because in doing so, it stabilises a glycine-rich loop conformation that is preferred in CDK2 but not in CDK1.

Table 4. X-ray data collection and refinement statistics

	CDK2-cyclin A-**73**[1]	CDK1-cyclin B-CKS2-**3**[1]
Data collection		
Space group	$P2_12_12_1$	P1
Unit cell (Å)	a=73.5, b=132.1, c=149.2	a=65.0, b=67.8, c=85.1, α=103.9, β=90.9, γ=90.4
Resolution (Å) (highest resolution shell)	66.03-2.97 (3.13-2.97)	65.76-2.06 (2.10-2.06)
Total observations	226,370 (7244)	169709 (8389)
Unique	30,773 (1098)	84841 (4161)
R_{merge}[a]	0.086	0.092
Mean I/σ(I)	6.7 (1.6)	6.2 (1.2)
Completeness %	100 (99.7)	97.3 (95.5)
Refinement		
Total number of atoms protein other waters	 8,927 64 18	 20,449 100 291
R (highest resolution shell)	0.213	0.198
R_{free} (highest resolution shell)	0.266	0.254
Rmsd bonds (Å)	0.0122	0.0217
Rmsd angles (°)	1.770	2.233

[1] The structures have been deposited in the PDB with accession codes 5LQE (CDK2-cyclin A-**73**) and 5LQF (CDK1-cyclin B-CKS2-**3**).

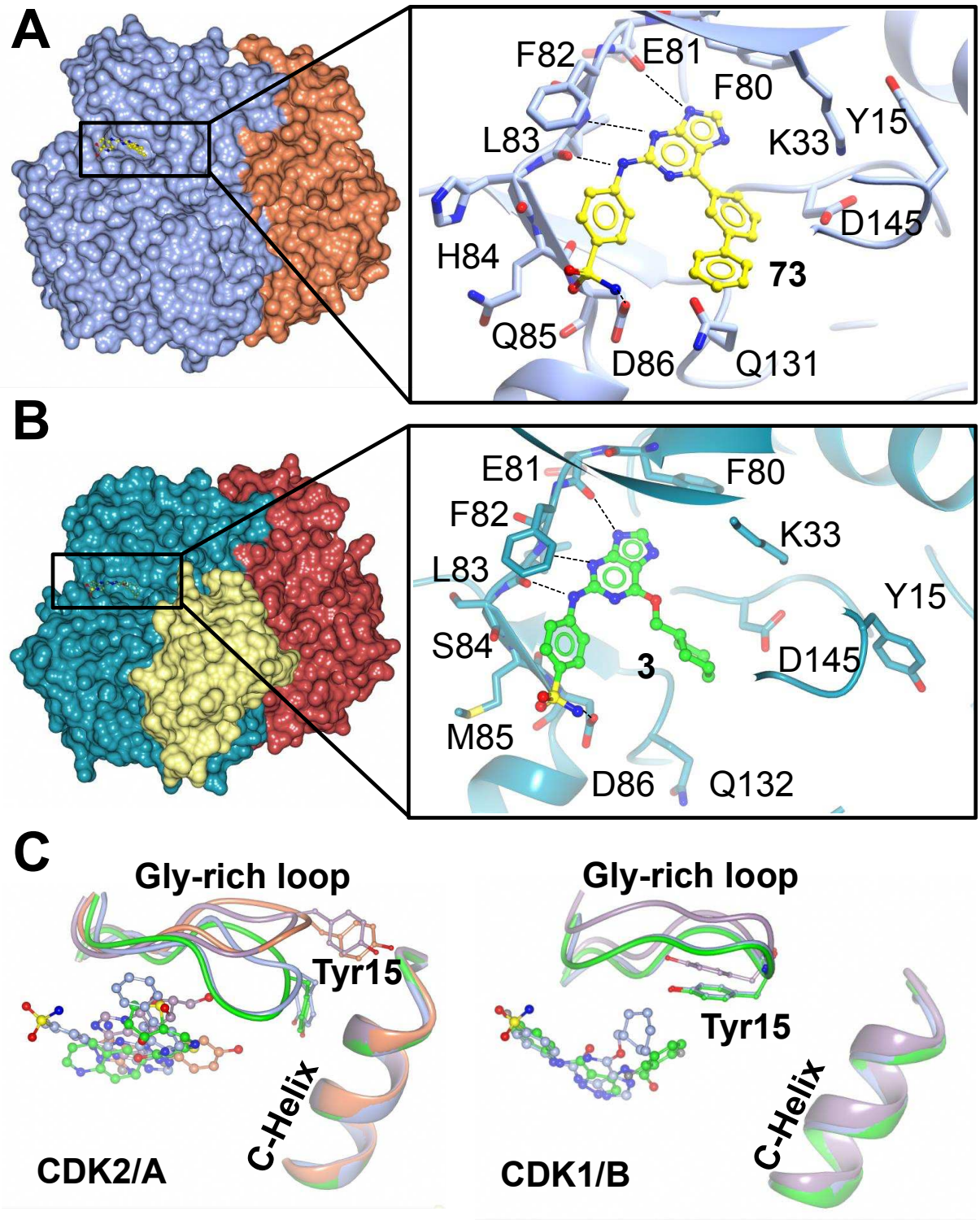


Figure 2. Structures of CDK2-cyclin A-73 and CDK1-cyclin B-CKS2-3. (A) Surface representation of CDK2 (ice blue) bound to cyclin A (coral) in complex with **73** (yellow). The inset shows the interactions of **73** within the ATP site of CDK2-cyclin A. (B) Surface representation of

CDK1 (dark cyan) bound to cyclin B (pale crimson) and CKS2 (lemon) in complex with **3** (green). The inset shows the interactions of **3** within the ATP site of CDK1-cyclin B-CKS2. In (A) and (B) the protein backbone is rendered in ribbon representation and selected CDK2 and CDK1 residues are drawn respectively with carbon atoms coloured ice blue and dark cyan. The hydrogen bonds are shown as black dotted lines. (C) The structural conformations of the CDK2-cyclin A and CDK1-cyclin B-CKS2 glycine-rich loops to illustrate the alternative poses of catalytic residue Tyr15. The left hand side panel compares CDK2-cyclin A in complex with **73** (ice blue) to other inhibitor bound CDK2-cyclin A complexes (PDB entries 4EOS (green), 3TNW (coral), and 3MY5 (lilac)). The right hand panel overlays CDK1-cyclin B-CKS2 bound to **3** (ice blue) with apo CDK1-cyclin B-CKS2 (PDB entry 4YC3, lilac) and CDK1-cyclin B-CKS2 in complex with compound 23 (PDB entry 5HQ0, green). The conformations of the loop when CDK1 is bound to **3** or Compound 23 cannot be distinguished. Gly-rich loop, glycine-rich loop.

Conclusions

Novel 6-substituted 2-(4'-sulfamoylanilino)purines have been designed as competitive inhibitors acting at the ATP binding site of CDK2 with particular attention being given to abrogating activity against CDK1. A variety of substituents were explored, either attached directly to C-6 or via an oxygen link, in the context of their possible interaction with a lipophilic pocket close to the ATP ribose binding site. The relationship between the size of a 6-alkoxy group and CDK2-inhibitory activity was found to parallel that previously observed for the corresponding 2-amino-6-alkoxypurines. In general, the new compounds were significantly less potent (typically 10-80×) against CDK1 than CDK2. Most impressive was 4-((6-([1,1'-biphenyl]-3-yl)-9*H*-purin-2-yl)amino) benzenesulfonamide that was ~ 2000-fold less active towards CDK1 (IC_{50} 86 μM), whilst retaining high potency against CDK2 (0.044 μM). Compounds substituted with relatively large conformationally constrained groups, e.g. bicyclic and tricyclic aromatic systems, showed greatly reduced inhibitory activity, indicating poorly accommodation of these substituents in the lipophilic

binding site. Analogues of 6-cyclohexylmethoxy-2-(4'-sulfamoylanilino)purine, in which the purine ring was replaced by a triazolopyrimidine, pyrazolopyrimidine or pyrrolopyrimidine were only marginally less active against CDK2 than the parent purine. However, replacement with an imidazopyridine or imidazopyrimidine gave much less potent derivatives. Co-crystal structures of inhibitors bound to CDK2 and CDK1 revealed that the binding mode of the purine-based inhibitor series is conserved. We show that inhibitor binding to CDK2 stabilises a glycine-rich loop conformation that shapes the ATP ribose binding pocket, resulting in effective inhibition of CDK2. We propose that this region of the active site might be the basis of the design of further inhibitors differentiating between CDK1 and CDK2.

Experimental Section

General Synthetic Procedures

Chemicals and solvents were obtained from standard suppliers. Solvents were either dried by standard techniques or purchased as anhydrous. Reactions needing microwave irradiation were carried out in an Initiator^{TM} Sixty Biotage apparatus. Petrol refers to petroleum ether (bp 40–60 °C, reagent grade, Fisher Scientific). All reactions that required inert or dry atmosphere were carried out under a blanket of nitrogen, which was dried by passage through a column of phosphorus pentoxide. Glassware was dried in an oven prior to use. Column chromatography was carried out using 40-60 μm mesh silica in glass columns under medium pressure or with a Biotage SP4 flash purification system using KP-Si. Thin layer chromatography (TLC) was performed on 20 mm precoated plates of silica gel (Merck, silica gel 60F254); visualisation was achieved using ultraviolet light (254 nm). NMR spectra were recorded on a Bruker Spectrospin AC 300E (300 MHz) NMR Spectrometer or Bruker BioSpin UltraShield Plus 500 MHz using deuterated solvent as a lock. IR spectra were recorded on a Bio-Rad FTS 3000MX diamond ATR, and UV analysis was performed using a Hitachi U-2000 spectrophotometer. LC-MS analysis was carried out on a Micromass Platform instrument operating in positive and negative ion electrospray mode,

employing a 50 × 4.6 mm C18 column (Supelco Discovery or Waters Symmetry) and a 15 min gradient elution of 0.05% formic acid and methanol (10–90%). HRMS were measured using a Finnigan MAT 95 XP or a Finnigan MAT 900 XLT by the EPSRC National Mass Spectrometry Service Centre (Swansea). The purity of final compounds was assessed by reversed-phase HPLC; all tested compounds were >95% purity. HPLC instrument, Agilent 1200 equipped with a photodiode array detector (190-400 nm). Sample temperature, ambient; injection volume, 5 μL; flow rate, 1mL/min. 5% to 100% MeCN gradient over 9 min and an isocratic hold at 100% MeCN for 2.5 min, before returning to initial conditions. Mobile phase A = 0.1% ammonia in water or 0.1% formic acid in water, mobile phase B = MeCN. Column: Waters XSELECT CSH C18, 3.5 μm, 4.6 mm × 150 mm or Waters XTerra RP18, 5 μm, 4.6 mm × 150 mm. Column maintained at ambient temperature.

General procedure A. To a stirred suspension of the appropriate haloheterocycle (1.0 mol. equiv.) and 4-aminobenzenesulfonamide (2.0 mol. equiv.) in TFE (25 mL/g of haloheterocycle) was added TFA (2.5 mL/g of haloheterocycle) dropwise. The resulting solution was heated under reflux for 12-48 h under a nitrogen atmosphere. The solvent was removed *in vacuo* and the residue was redissolved in EtOAc (10-20 mL). The solution was washed with saturated aqueous sodium bicarbonate solution (3 × 10 mL), and the aqueous extracts were combined and washed with EtOAc (2 × 15 mL). The combined organic layers were dried (Na_2SO_4) and the solvent removed under reduced pressure to give a residue that was purified in the manner indicated.

6-Chloro-2-fluoro-9*H*-purine (10). [47] To a stirred solution of HBF_4 (48% aqueous, 120 mL) at 0 ºC, was added 2-amino-6-chloropurine (**9**) (6.0 g, 35.0 mmol). Over 20 min, a solution of $NaNO_2$ (4.9 g, 70.0 mmol) in water (200 mL) was added dropwise, ensuring the temperature remained close to 0 ºC. The pale yellow solution was raised to room temperature and stirred for 18 h. The resulting solution was neutralised to pH 7 in an ice bath at 0 °C, by addition of Na_2CO_3 (6.00 g) in water (200 mL). Solvents were removed *in vacuo* and the residual solid was redissolved in MeOH (100 mL) and adsorbed onto silica (250 mL). The crude material was purified by chromatography (silica; 10%

MeOH:DCM) to afford **10** as a white crystalline solid (4.52 g, 75%); mp 171-173 ºC (lit., [47] mp 174 ºC); UV λ_{max} (EtOH) 393 nm; IR (cm^{-1}) 2964, 2785, 1735, 1581; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.60 (1H, s, H-8), 13.9 (1H, s, N*H*); LRMS (ES^+) *m/z* 172.6 $[M+H]^+$.

2-Fluoro-9*H*-purine (11). [48, 49] To a stirred suspension of 6-chloro-2-fluoropurine (**10**) (0.30 g, 1.74 mmol) and palladium hydroxide on carbon (20% w/w, 0.30 g) in methanol (15 mL) was added ammonium formate (0.34 g, 5.35 mmol). The suspension was heated under reflux for 1h before filtering through a pad of Celite, eluting with methanol (20 mL). Removal of volatiles under reduced pressure afforded **11** as a white solid (240 mg, 100%); mp 219 °C (dec.) (lit., [48] decomposed at 216 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.60 (1H, s, H-8), 9.01 (1H, s, H-6), 13.9 (1H, s, N*H*); LRMS (ES^+) *m/z* 139.2 $[M+H]^+$.

Phenyl-(9*H*-purin-2-yl)amine (12). Prepared from aniline (0.182 mL, 2 mmol) and 2-fluoro-9*H*-purine (**11**, 0.138 g, 1.0 mmol) in accordance with **general procedure A**. The reaction mixture was stirred under reflux for overnight. After removal of the solvent *in vacuo* the residue was dissolved in EtOAc (10-30 mL), washed with saturated aqueous $NaHCO_3$ (3 × 30 mL) and dried ($MgSO_4$). The crude material was purified by chromatography (silica; 0-20% MeOH:DCM), followed by recrystallization from DCM to afford **12** as an off-white solid (65 mg, 31%); R_f 0.20 (5% MeOH:DCM); mp 200-201 ^{o}C; UV λ_{max} (EtOH) 328, 270, 239, 206 nm; IR (cm^{-1}) 3234, 3103, 2804, 1624, 1602, 1539, 1402, 1292, 1217; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.91 (1H, t, J = 7.5 Hz, H-4'), 7.28 (2H, t , J = 7.5 Hz, H-3' and H-5'), 7.83 (2H, d, J = 9.0 Hz, H-2' and H-6'), 8.82 (1H, s, H-6), 8.24 (1H, s, H-8), 9.53 (1H, s, N*H*), 12.9 (1H, s, N*H*); LRMS (ES^+) *m/z* 212.0 $[M+H]^+$; HRMS calcd for $C_{11}H_{10}N_5$ $[M+H]^+$ 212.0931, found 212.0933.

4-(9*H*-Purin-2-ylamino)benzenesulfonamide (13). Prepared following **general procedure A** from 2-fluoro-9*H*-purine (**11**, 0.10 g, 0.725 mmol). Recrystallization from MeOH:H_2O gave **13** as a pale white solid (74 mg, 35%); R_f 0.40 (20% MeOH:DCM); mp > 320 ^{o}C (dec); UV λ_{max} (EtOH) 287, 213 nm; IR (cm^{-1}) 3223, 1585, 1481, 1307, 1251, 1148, 1091; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.17 (2H, s, SO_2NH_2), 7.73 (2H, d, J = 9.0 Hz, H-3' and H-5'); 7.99 (2H, d, J = 9.0 Hz, H-2' and

H-6'), 8.33 (1H, s, H-6), 8.00 (1H, s, H-8), 10.00 (1H, s, N*H*); LRMS (ES^+) *m/z* 291.0 $[M+H]^+$; HRMS calcd for $C_{11}H_{11}N_6O_2S$ $[M+H]^+$ 291.0659, found 291.0659.

***N*^{2}-Phenylguanine 2,2,2-trifluoroacetate (15).** The title compound was prepared following **general procedure A** using 2-bromohypoxanthine (**14,** 1.00 g, 4.7 mmol), aniline (0.9 mL, 9.40 mmol) to yield **15** as a white solid (1.17 g, 73%). The isolated compound was pure by analytical HPLC without the need for further purification; mp 229-231 ºC; UV λ_{max} (EtOH) 273 nm; IR (cm^{-1}) 3332, 3128, 2943, 2756, 2555, 2387, 1678, 1572; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.07 (1H, t, J = 7.5 Hz, H-4'), 7.36 (2H, dd, J = 7.5, 8.0 Hz, H-3' and H-5'), 7.62 (2 H, d, J = 8.0 Hz, H-2' and H-6'), 7.94 (1H, s, H-8), 8.46 (1H, br s, N*H*), 9.00 (1H, br s, N*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 113, 120, 123, 129, 138, 139, 150, 152, 155; LRMS (ES^+) *m/z* 228.3 $[M+H]^+$; HRMS calcd for $C_{11}H_{10}N_5O$ $[M+H]^+$ 228.0881, found 228.0880.

6-Oxo-2-((4-sulfamoylphenyl)amino)-6,9-dihydro-1*H*-purine 2,2,2-trifluoroacetate (16). Synthesised in accordance with **general procedure A** from 2-bromohypoxanthine (**14,** 0.10 g, 0.47 mmol) to yield **16** as an amorphous white solid (60 mg, 42%); mp > 300 ºC; UV λ_{max} (EtOH) 275 nm; IR (cm^{-1}) 3348, 3113, 2990, 2877, 2792, 2333, 1672, 1575, 1365, 1153; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.28 (2H, s, SO_2NH_2), 7.79 (4H, m, H-2', H-3', H-5' and H-6'), 8.31 (1H, s, H-8), 9.25 (1H, br s, N*H*), 10.84 (1H, br s, N*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 113, 120, 123, 127, 138, 139, 150, 152, 156; LRMS (ES^+) *m/z* 307.3 $[M+H]^+$.

1-(2-Amino-9*H*-purin-6-yl)-4-aza-1-azoniabicyclo[2.2.2]octane (17). [34] 1,4-Diazabicyclo[2.2.2]octane (9.90 g, 88.4 mmol) was added to a solution of 2-amino-6-chloropurine **9** (5.00 g, 29.5 mmol) in DMSO (100 mL) over 1 h and the mixture was stirred at room temperature for 24 h. The resulting white precipitate was filtered and washed with diethyl ether. The solid was suspended in DCM (200 mL) and stirred for 1 h. After filtration and washing with DCM several times, **9** was obtained as a white solid, which was dried *in vacuo* (7.95 g, 96%) and used without further purification; mp 230 ºC (dec) (lit., [34] decomposed at 230 ºC); ^{1}H NMR (300 MHz, D_2O) δ

3.31 (6H, t, J = 7.5 Hz, $(N(CH_2)_3)$, 4.07 (6H, t, J = 7.5 Hz, $(N^+(CH_2)_3)$, 8.13 (1H, s, H-8); ^{13}C NMR (75 MHz, D_2O) δ 38.7, 53.4, 116.0, 143.7, 151.3, 158.4.

General procedure B. 2-Amino-6-chloropurine (**9**, 1.0 mol. equiv.) was added to a solution prepared from metallic sodium (5.0 mol. equiv.) dissolved in the appropriate alcohol (3.4 mL/mmol). The mixture was stirred at reflux until LCMS analysis indicated the absence of starting materials (3-24 h). After cooling, the reaction mixture was neutralised with glacial AcOH and the volatile material was removed *in vacuo*. Unless otherwise indicated, purification was achieved either by recrystallisation from H_2O or by adding H_2O to the reaction mixture and extracting the product into EtOAc (3 × 100 mL), followed by drying ($MgSO_4$) and removal of the solvent *in vacuo*.

General Procedure C. The appropriate alcohol (4.0 mol. equiv.) was added dropwise to a stirred suspension of NaH (3.0 mol. equiv.) in DMSO (2.5-3.0 mL/mmol) and the resulting mixture was stirred for 1-2 h. To this was added DABCO-purine (**17,** 1.0 mol. equiv.) or the appropriate haloheterocycle (1.0 mol. equiv), and the mixture was stirred for 24 h with heating as specified. Water (20-200 mL) was added and the basic emulsion was neutralised with glacial acetic acid. The aqueous phase was extracted with EtOAc (3 × 50-100 mL) and the organic layers were washed with saturated aqueous NaCl (100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to yield the crude product, which was purified by chromatography on silica and/or recrystallisation from an appropriate solvent.

General Procedure D. To a stirred solution of hydrofluoroboric acid (50%, aq., 20.0 mol. equiv.) cooled below -20 ºC, was added the appropriate 2-amino-6-alkoxypurine (1.0 mol. equiv.). Whilst maintaining the temperature at –15 ºC, a solution of $NaNO_2$ (2.0 mol. equiv.) in H_2O (1-3 mL/mmol $NaNO_2$) was added dropwise over 10 min. The mixture was stirred at room temperature for 3 h, neutralised at -15 ºC by the dropwise addition of 15% (w/v) aqueous Na_2CO_3 solution, and the precipitated solid was collected by filtration and washed with H_2O. The residual solid was triturated

with EtOAc (3 × 100 mL) and filtered. The combined filtrates were concentrated under reduced pressure to furnish the product, which was purified as indicated.

General procedure E. The appropriate 9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (1.0 mol. equiv.) was dissolved in 2-propanol (60 mL/g) and deionised water (20 mL/g). Trifluoroacetic acid (10.0 mol. equiv.) was added, and the reaction mixture was stirred at 100 ºC for 2 h, cooled to room temperature, and the solution was adjusted to pH 8 by addition of conc. aqueous ammonia solution. The volume of solvent was reduced by 50% under reduced pressure; if a precipitate resulted, this was collected and washed with 2-propanol (2 × 10 mL). If precipitation was not observed following neutralisation, the reaction mixture was partitioned between EtOAc (20 mL) and saturated aqueous NaCl solution (20 mL) The organic layer was dried (Na_2SO_4), evaporated under reduced pressure, and the product was purified by chromatography as specified.

General Procedure F. Tetrabutylammonium fluoride solution (1.0 M in THF, 1.5 mol. equiv.) was added dropwise to a stirred solution of the appropriate 6-triisopropylsilylethynylpurine (1.0 mol. equiv.) in anhydrous THF (10-20 mL), and the mixture was stirred for 5-15 min at ambient temperature under N_2. Volatiles were evaporated under reduced pressure, and the residue was redissolved in EtOAc (100 mL/g), washed with saturated aqueous NaCl (100 mL/g), and the organic fraction was removed *in vacuo*. The product was purified as described.

General Procedure G. An oxygen-free solution of the required boronic acid or potassium trifluoroborate salt (1.09 mmol), the appropriately 2-substituted 6-chloro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (0.78 mmol), dicyclohexyl(2',6'-dimethoxybiphenyl-2-yl)phosphine (SPhos) (2.5 mol%) and palladium acetate (1.0 mol%) in toluene (4 mL) was degassed by bubbling nitrogen through the solution in a sealed vial for 5 min. To the pale yellow solution was added K_3PO_4 (1.56 mmol) and water (approx. 50 µL). The solution was again degassed for 15 min and subsequently heated to 100 ºC for 18 h. The black-brown suspension was filtered through Celite, eluting with MeOH (3 × 10 mL), and the product was isolated by chromatography as indicated.

2-Amino-6-ethoxypurine (18). [50, 51] Treatment of EtOH (100 mL) with Na (3.38 g, 147.5 mmol), followed by addition of 2-amino-6-chloropurine **9** (5.0 g, 29.5 mmol) according to **general procedure B**, afforded the crude product. Recrystallisation from H_2O gave **18** as a white powder (4.75 g, 90%); R_f 0.26 (10% MeOH:DCM), mp 247 °C (dec) (lit. [51] mp 230 °C (dec), lit. [50] mp 293 °C (dec)); IR (cm^{-1}) 3321, 2981, 2363, 2143, 1619, 1584, 1508, 1458, 1427, 1396, 1375, 1342, 1285, 1223, 1161, 1118 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.1 Hz, CH_3), 4.44 (2H, q, J = 7.1 Hz, CH_2), 6.21 (2H, s, NH_2), 7.81 (1H, s, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.9, 61.6, 160.1; LRMS (ES^+) *m/z* 180.3 $[M+H]^+$; Anal. Found C 47.11 H 4.97 N 38.97; $C_7H_9N_5O$ requires C 46.92 H 5.06 N 39.09.

2-Amino-6-*n*-propoxypurine (19). [50, 51] Treatment of 1-propanol (20 mL) with Na (0.68 g, 29.5 mmol), followed by addition of 2-amino-6-chloropurine **9** (1.0 g, 5.9 mmol) according to **general procedure B**, gave the crude product. Recrystallisation from H_2O afforded **19** (1.06 g, 93%) as a yellow solid, R_f 0.21 (10% MeOH:DCM), mp 196-198 ºC (lit. [51] mp 199-201 ºC, lit. [50] mp 208 ºC); IR (cm-1) 3484, 3389, 3318, 3203, 2965, 2935, 2882, 2522, 2361, 2338, 1578, 1507, 1449, 1398, 1362, 1331, 1276, 1225, 1165, 1119; ^1H NMR (300 MHz, DMSO-d_6) δ 0.97 (3H, t, J = 7.4 Hz, CH_3) 1.76 (2H, tq, J = 7.2, 7.2 Hz, $CH_3CH_2CH_2O$), 4.34 (2H, t, J = 6.8 Hz, OCH_2), 6.20 (2H, s, NH_2), 7.82 (1H, s, H-8), 12.37 (1H, br s, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 10.7, 22.2, 67.3, 138.5, 160.1, 160.3, 162.2; LRMS (ES^+) *m/z* 194.17 $[M+H]^+$; Anal. Found C 46.81 H 5.95 N 33.78; $C_8H_{11}N_5O.0.7\ H_2O$ requires C 46.69 H 6.07 N 34.03.

2-Amino-6-isopropoxypurine (20). [31] Treatment of isopropanol (0.85 mL, 14.2 mmol) with NaH (0.26 mg, 10.7 mmol.) in DMSO (10 mL), followed by addition of **17** (1.0 g, 3.6 mmol) was performed according to **general procedure C**, to afford the crude product. Purification by chromatography (silica; 5-10% MeOH:DCM) afforded **20** as a yellow oil. Trituration of the oil with diethyl ether afforded **20** (0.38 g, 55%) as an off-white solid; R_f 0.28 (10% MeOH:DCM), mp 205-207 ºC (lit. [31] mp 209-210 ºC); IR (cm^{-1}) 3491, 3331, 3195, 2976, 2773, 1618, 1580, 1504,

1445, 1391, 1372, 1321, 1272, 1224, 1180, 1144; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (6H, d, J = 6.2 Hz, 2 × CH_3), 5.47 (1H, septet, J = 6.2 Hz, OCH), 6.17 (2H, s, NH_2), 7.81 (1H, s, H-8), 12.39 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.3, 68.2, 160.1; LRMS (ES$^+$) *m/z* 194.15 [M+H]$^+$; Anal. Found C 49.48 H 5.64 N 35.71; $C_8H_{11}N_5O$.0.1 H_2O requires C 49.27 H 5.79 N 35.91

2-Amino-6-(2-methyl-1-propoxy)purine (21). [31] 2-Methyl-1-propanol (1.31 mL, 14.2 mmol) was added to NaH (0.25 mg, 10.7 mmol) in DMSO (10 mL), followed by addition of **17** (1.0 g, 3.6 mmol) according to **general procedure C**, to afford the crude product. Trituration of the residual yellow oil with petrol furnished a cream solid. Purification by chromatography (silica; 5-10% MeOH:DCM) gave **21** (0.42 g, 57%) as pale yellow crystals; R_f 0.41 (10% MeOH/DCM), mp 180-182 ºC (lit. [31] mp 89-92 ºC); IR (cm^{-1}) 3512, 3473, 3359, 2958, 2875, 1620, 1575, 1507, 1451, 1386, 1363, 1332, 1272, 1223, 1159, 1121; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.98 (6H, d, J = 6.7 Hz, 2 × CH_3), 2.07 (1H, septet, J = 6.7 Hz, OCH$_2$CH), 4.17 (2H, d, J = 6.8 Hz, OCH_2), 6.21 (2H, s, NH_2), 7.82 (1H, s, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.4, 27.8, 71.9, 160.1, 162.2; LRMS (ES$^-$) *m/z* 206.81 [M-H]$^-$.

2-Amino-6-(1-methylpropoxy)purine (22). [31] Treatment of 2-butanol (20 mL) with Na (0.68 g, 29.5 mmol), followed by addition of 2-amino-6-chloropurine **9** (1.0 g, 5.9 mmol) was performed according to **general procedure B**, to give a pale yellow solid. H_2O was added and the product was extracted into EtOAc (3 × 100 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield **22** (0.90 g, 74%) as off-white crystals, R_f 0.3 (10% MeOH:DCM); mp 77 ºC (dec) (lit. [31] mp 88-90 ºC); IR (cm^{-1}) 3322, 3190, 2971, 2936, 2876, 2772, 1616, 1575, 1504, 1451, 1393, 1375, 1326, 1273, 1222; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91 (3H, t, J = 7.4 Hz, CH_3CH$_2$CHCH$_3$), 1.29 (3H, d, J = 6.2 Hz, CH$_3$CH$_2$CHCH_3), 1.57-1.79 (2H, m, CH$_3$CH_2CHCH$_3$), 5.27-5.38 (1H, m, CH$_3$CH$_2$CHCH$_3$), 6.19 (2H, s, NH_2), 7.79 (1H, s, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 10.0,

19.8, 28.9, 72.7, 137.7, 160.1; LRMS (ES^-) *m/z* 206.08 $[M-H]^-$; Anal. Found C 51.52 H 6.43 N 32.64; $C_9H_{13}N_5O.0.25\ H_2O$ requires C 51.05 H 6.43 N 33.08.

2-Amino-6-(prop-2-ynyloxy)purine (23). [51] Treatment of propargyl alcohol (0.83 mL, 14.2 mmol) with NaH (0.25 mg, 10.7 mmol) in DMSO (10 mL), followed by addition of **17** (1.0 g, 3.6 mmol) was performed according to **general procedure C**, affording the product **23** (0.54 g, 80%) as a beige solid, R_f 0.26 (10% MeOH:DCM); mp 181 ºC (dec) (lit. [51] mp 230 ºC (dec)); IR (cm^{-1}) 3319, 3190, 2777, 2126, 1651, 1621, 1589, 1510, 1460, 1438, 1400, 1339, 1277, 1221, 1184, 1142; ^1H NMR (300 MHz, DMSO-d_6) δ 3.58 (1H, t, J = 2.4 Hz, OCH_2CCH), 5.10 (2H, d, J = 2.4 Hz, OCH_2CCH), 6.35 (2H, s, NH_2), 7.85 (1H, s, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 53.0, 78.0, 79.6, 113.7, 138.5, 155.7, 159.1, 159.9; LRMS (ES^+) *m/z* 190.00 $[M+H]^+$.

2-Fluoro-6-ethoxypurine (24). Prepared following **general procedure D** from **18** (1.0 g, 5.6 mmol) to give **24** as a white solid (0.22 g, 22%); R_f 0.39 (10% MeOH/DCM); mp 225-227 ºC; IR (cm^{-1}) 3117, 2997, 2740, 2669, 2506, 2363, 2339, 1836, 1760, 1605, 1487, 1425, 1373, 1343, 1281, 1219, 1151, 1111, 1038, 1007; ^1H NMR (300 MHz, DMSO-d_6) δ 1.41 (3H, t, J = 7.1 Hz, CH_3), 4.57 (2H, q, J = 7.1 Hz, CH_2), 8.39 (1H, s, H-8), 13.55 (1H, br s, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.6 (CH_3), 64.0 (CH_2), 144.0 (C^8); LRMS (ES^+) *m/z* 183.1 $[M+H]^+$; Anal. Found C 44.80 H 3.67 N 29.48; $C_7H_7N_4OF.0.3\ H_2O$ requires C 44.83 H 4.08 N 29.87.

2-Fluoro-6-*n*-propoxypurine (25). Synthesised in accordance with **general procedure D** from **19** (1.0 g, 5.2 mmol) to yield **25** (0.58 g, 57%) as a white solid; R_f 0.18 (70% EtOAc:petrol); mp 197-199 ºC; IR (cm^{-1}) 3105, 2973, 2944, 2886, 2741, 2680, 2523, 2362, 2338, 1772, 1607, 1422, 1368, 1347, 1269, 1213, 1149, 1038; ^1H NMR (300 MHz, DMSO-d_6) δ 1.00 (3H, t, J = 7.4 Hz, CH_3) 1.82 (2H, tq, J = 7.1, 7.1 Hz, $CH_3CH_2CH_2O$), 4.47 (2H, t, J = 6.7 Hz, $CH_3CH_2CH_2O$), 8.39 (1H, s, H-8), 13.55 (1H, br s, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 10.5 (CH_3), 22.0 ($CH_3CH_2CH_2O$), 69.4 (O^6CH_2), 144.0 (Ar-C), 156.0 (C^8), 158.8 (Ar-C); LRMS (ES^+) *m/z* 154.0 $[M-C_3H_7]^+$; HRMS calcd for $C_8H_9FN_4O$ $[M^+]$ 196.0760, found 196.0757.

2-Fluoro-6-isopropoxypurine (26). Synthesised by **general prodecure D** from **20** (0.30 g, 1.6 mmol) to yield **26** as a white solid (0.16 g, 53%); R_f 0.22 (70% EtOAc:petrol); mp 184 ºC (dec); IR (cm^{-1}) 2990, 2536, 1605, 1472, 1424, 1371, 1275, 1034, 1017; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40 (6H, d, J = 6.2 Hz, 2 × CH_3), 5.49 (1H, septet, J = 6.2 Hz, OCH), 8.37 (1H, s, H-8), 13.52 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.0 (CH_3), 71.5 (O^6CH), 162.2 (C^8); LRMS (ES^+) *m/z* 154.0 $[M\text{-}C_3H_7]^+$; HRMS calcd for $C_8H_9FN_4O$ $[M^+]$ 196.0760, found 196.0760.

2-Fluoro-6-(2-methyl-1-propoxy)purine (27). From **21** (0.40 g, 1.9 mmol) in accordance with **general procedure D** to yield **27** (0.25 g, 62%) as a white solid; R_f 0.25 (70% EtOAc:petrol); mp 202-204 ºC; IR (cm^{-1}) 3130, 3059, 2968, 2937, 2884, 2804, 2693, 2528, 1760, 1700, 1603, 1476, 1451, 1418, 1364, 1341, 1267, 1211, 1140, 1113, 1040; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.01 (6H, d, J = 6.7 Hz, 2 × CH_3), 2.13 (1H, septet, J = 6.7 Hz, OCH_2CH), 4.30 (2H, d, J = 6.7 Hz, OCH_2), 8.40 (1H, s, H-8), 13.55 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.2 (CH_3), 27.7 (O^6CH_2CH), 73.7 (O^6CH_2); LRMS (ES^+) *m/z* 154.0 $[M\text{-}C_3H_7]^+$; HRMS calcd for $C_9H_{11}FN_4O$ $[M^+]$ 210.0917, found 210.0922.

2-Fluoro-6-(1-methylpropoxy)purine (28). Prepared according to **general procedure D** from **22** (0.66 g, 3.2 mmol) as a white solid (0.29 g, 43%); R_f 0.21 (70% EtOAc:petrol); mp 187-189 ºC; IR (cm^{-1}) 2975, 2940, 1604, 1468, 1421, 1372, 1344, 1277, 1211, 1148, 1038; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (3H, t, J = 7.4 Hz, $CH_3CH_2CHCH_3$), 1.36 (3H, d, J = 6.2 Hz, $CH_3CH_2CHCH_3$), 1.66-1.83 (2H, m, $CH_3CH_2CHCH_3$), 5.29-5.37 (1H, m, $CH_3CH_2CHCH_3$), 8.37 (1H, s, H-8), 13.55 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 9.8 ($CH_3CH_2CHCH_3$), 19.5 ($CH_3CH_2CHCH_3$), 28.6 ($CH_3CH_2CHCH_3$), 75.9 ($CH_3CH_2CHCH_3$), 158.8 (C^8); LRMS (ES^-) *m/z* 209.1 $[M\text{-}H]^-$; Anal. Found C 51.71 H 5.27 N 26.33; $C_9H_{11}N_4OF$ requires C 51.42 H 5.27 N 26.65.

2-Fluoro-6-(prop-2-ynyloxy)purine (29). Synthesised from **23** (0.28 g, 1.5 mmol) in accordance with **general procedure D** as a pale yellow solid (77 mg, 27%); R_f 0.18 (70% EtOAc:petrol); mp 201 ºC (dec); IR (cm^{-1}) 3261, 3123, 2970, 2864, 2803, 2363, 2336, 2140, 1651, 1607, 1460, 1379, 1341, 1275, 1217, 1142, 1107, 1048; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.70 (1H, t, J = 2.4 Hz,

OCH$_2$CC*H*), 5.23 (2H, d, *J* = 2.2 Hz, OC*H*$_2$CCH), 8.45 (1H, s, H-8); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 55.4 (*O*^{6}CH$_2$C*C*H), 78.5 (*O*^{6}*C*H$_2$CCH), 79.1 (*O*^{6}CH$_2$*C*CH), 162.2 (C^8); LRMS (ES$^+$) *m/z* 192.0 [M$^+$]; HRMS calcd for $C_8H_5FN_4O$ [M$^+$] 192.0447, found 192.0448.

2-Sulfanilyl-6-ethoxypurine (30). Prepared from **24** (0.20 g, 1.1 mmol) following **general procedure A**. Purification by chromatography (silica; 5-20% MeOH:DCM) afforded **30** (39 mg, 11%) as an off-white solid; R$_f$ 0.20 (10% MeOH:DCM); mp 265 ºC (dec); UV λ_{max} (EtOH) 257, 241 nm; IR (cm^{-1}) 3473, 3317, 3223, 2984, 2787, 2363, 2338, 1628, 1578, 1540, 1499, 1442, 1376, 1312, 1254, 1142, 1013; ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 1.43 (3H, t, *J* = 7.1 Hz, C*H*$_3$), 4.59 (2H, q, *J* = 7.1 Hz, C*H*$_2$), 7.16 (2H, s, N*H*$_2$), 7.71 (2H, d, *J* = 8.9 Hz, H-3' and H-5'), 7.97 (2H, d, *J* = 8.9 Hz, H-2' and H-6'), 8.08 (1H, s, H-8), 9.76 (1H, s, N*H*), 12.69 (1H, s, N*H*); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 14.9 (CH$_3$), 62.6 (CH$_2$), 117.6 (Ar-C), 126.8 (Ar-C), 155.1 (C^8); LRMS (ES$^+$) *m/z* 335.0 [M+H]$^+$; HRMS calcd for $C_{13}H_{14}N_6O_3S$ [M$^+$] 334.0848, found 334.0835.

2-Sulfanilyl-6-propoxypurine (31). Prepared from **25** (0.25 g, 1.3 mmol) according to **general procedure A**. Purification by chromatography (silica; 50-100% EtOAc:petrol followed by 0-10% MeOH:DCM), afforded **31** (0.25 g, 56%) as a beige solid; R$_f$ 0.10 (70% EtOAc:petrol); mp 207 ºC (dec); UV λ_{max} (EtOH) 310 nm; IR (cm^{-1}) 3251, 3115, 2971, 2934, 2878, 2361, 2340, 1700, 1596, 1580, 1528, 1498, 1453, 1389, 1321, 1306, 1243, 1146, 1094; ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 1.03 (3H, t, *J* = 7.4 Hz, C*H*$_3$) 1.85 (2H, tq, *J* = 6.9, 6.9 Hz, CH$_3$C*H*$_2$CH$_2$O), 4.50 (2H, t, *J* = 6.8 Hz, CH$_3$CH$_2$C*H*$_2$O), 7.17 (2H, s, N*H*$_2$), 7.72 (2H, d, *J* = 8.8 Hz, H-2' and H-6'), 7.97 (2H, d, *J* = 8.8 Hz, H-3' and H-5'), 8.09 (1H, s, H-8), 9.77 (1H, s, NH), 12.96 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 10.7 (CH$_3$), 22.2 (CH$_3$*C*H$_2$CH$_2$O), 68.2 (*O*^{6}CH$_2$), 117.6 (*C*HCNH), 126.8 (*C*HCSO$_2$NH$_2$), 135.7 (Ar-CNH), 144.6 (CSO$_2$NH$_2$), 155.1 (C^8); LRMS (ES$^+$) *m/z* 349.8 [M+H]$^+$; HRMS calcd for $C_{14}H_{16}N_6O_3S$ [M$^+$] 348.1005, found 348.0994; Anal. Found C 45.41 H 4.75 N 22.40; $C_{14}H_{16}N_6O_3S.0.3\ CF_3CO_2H$ requires C 45.83 H 4.29 N 21.97.

2-Sulfanilyl-6-isopropoxypurine (32). Synthesised in accordance with **general procedure A** from **26** (0.07 g, 0.4 mmol). Purification by chromatography (silica; 50-100% EtOAc:petrol followed by

0-10% MeOH:DCM), afforded **32** (38 mg, 31%) as a beige solid; R_f 0.03 (70% EtOAc:petrol); mp 177 ºC (dec); UV λ_{max} (EtOH) 307 nm; IR (cm^{-1}) 3472, 3312, 3210, 3117, 2982, 2793, 2691, 2643, 2362, 2342, 1715, 1685, 1617, 1597, 1578, 1538, 1500, 1440, 1381, 1306, 1255, 1140, 1117, 1096; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.42 (6H, d, J = 6.2 Hz, 2 × CH_3), 5.59 (1H, septet, J = 6.2 Hz, OC*H*), 7.16 (2H, s, NH_2), 7.72 (2H, d, J = 8.8 Hz, H-2' and H-6'), 7.96 (2H, d, J = 8.8 Hz, 2 × H-3' and H-5'), 8.07 (1H, s, H-8), 9.74 (1H, s, N*H*), 12.91 (1H, br s, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.9 (CH_3), 69.0 (O^6CH), 117.1 (*C*HCNH), 126.5 (*C*HCSO$_2$NH$_2$), 135.2 (Ar-CNH), 144.3 (CSO_2NH_2), 154.7 (C^8); LRMS (ES^+) *m/z* 349.8 $[M+H]^+$; HRMS calcd for $C_{14}H_{16}N_6O_3S$ $[M^+]$ 348.1005, found 348.1007.

2-Sulfanilyl-6-(2-methyl-1-propoxy)purine (33). Prepared following **general procedure A** from **27** (0.12 g, 0.6 mmol). Purification by chromatography (silica, 50-100% EtOAc:petrol followed by 0-10% MeOH:DCM), afforded **33** (0.11 g, 53%) as a light brown solid; R_f 0.21 (70% EtOAc:petrol); mp 133 ºC (dec); UV λ_{max} (EtOH) 308 nm; IR (cm^{-1}) 3285, 3108, 2962, 2360, 2337, 1595, 1576, 1543, 1499, 1438, 1396, 1321, 1249, 1221, 1144, 1096, 1016; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (6H, d, J = 6.7 Hz, 2 × CH_3), 2.16 (1H, septet, J = 6.7 Hz, OCH$_2$C*H*), 4.33 (2H, d, J = 6.8 Hz, OCH_2CH), 7.17 (2H, s, NH_2), 7.71 (2H, d, J = 8.9 Hz, H-2' and H-6'), 7.97 (2H, d, J = 8.9 Hz, H-3' and H-5'), 8.09 (1H, s, H-8), 9.77 (1H, s, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.4 (CH_3), 27.9 (O^6CH$_2$*C*H), 72.7 (O^6CH$_2$), 117.6 (*C*HCNH), 126.8 (*C*HCSO$_2$NH$_2$), 135.7 (Ar-CNH), 144.6 (CSO_2NH_2), 155.1 (C^8); LRMS (ES^+) *m/z* 363.8 $[M+H]^+$; HRMS calcd for $C_{15}H_{18}N_6O_3S$ $[M^+]$ 362.1161, found 362.1145.

(*R*/*S*)-6-(1-Methylpropoxy)-2-sulfanilylpurine (34). Synthesised from **28** (0.1 g, 0.5 mmol) following **general procedure A**. Purification by chromatography (silica; 50-100% EtOAc:petrol followed by 0-10% MeOH:DCM), afforded **34** (41 mg, 24%) as a light brown solid; R_f 0.10 (70% EtOAc:petrol); mp 162 ºC (dec); UV (EtOH) λ_{max} 308 nm; IR (cm^{-1}) 3649, 3317, 3219, 3106, 2972, 2938, 2874, 2363, 2340, 1701, 1596, 1579, 1528, 1498, 1453, 1389, 1306, 1243, 1145, 1094; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.97 (3H, t, J = 7.4 Hz, CH_3CH$_2$CHCH$_3$), 1.39 (3H, d, J = 6.2 Hz,

$CH_3CH_2CHCH_3$), 1.68-1.87 (2H, m, $CH_3CH_2CHCH_3$), 5.40-5.46 (1H, m, $CH_3CH_2CHCH_3$), 7.17 (2H, s, NH_2), 7.72 (2H, d, J = 8.8 Hz, H-2' and H-6'), 7.96 (2H, d, J = 8.9 Hz, H-3' and H-5'), 8.13 (1H, s, H-8), 9.76 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 10.0 ($CH_3CH_2CHCH_3$), 19.7 ($CH_3CH_2CHCH_3$), 28.8 ($CH_3CH_2CHCH_3$), 74.2 ($CH_3CH_2CHCH_3$), 117.6 (CHCNH), 126.8 ($CHCSO_2NH_2$), 135.8 (Ar-CNH), 144.6 (CSO_2NH_2), 155.2 (C^8); LRMS (ES^+) *m/z* 363.8 $[M+H]^+$; HRMS calcd for $C_{15}H_{18}N_6O_3S$ $[M^+]$ 362.1161, found 362.1166; Anal. Found C 48.32 H 5.15 N 22.08; $C_{15}H_{18}N_6O_3S.0.2\ CF_3CO_2H$ requires C 48.02 H 4.76 N 21.82.

2- Sulfanilyl-6-(prop-2-ynyloxy)purine (35). Synthesised in accordance with **general procedure A** from **29** (0.045 g, 0.2 mmol). Purification by chromatography (silica; 50-100% EtOAc:petrol followed by 0-10% MeOH:DCM), yielded **35** (27 mg, 34%) as a beige solid; R_f 0.1 (70% EtOAc:petrol); mp 161 ºC (dec); UV (EtOH) λ_{max} 307 nm; IR (cm^{-1}) 3370, 3245, 2971, 2795, 2361, 2340, 1715, 1699, 1619, 1585, 1575, 1540, 1499, 1472, 1454, 1443, 1396, 1321, 1307, 1271, 1143, 1095; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.68 (1H, t, J = 2.3 Hz, OCH_2CCH), 5.24 (2H, d, J = 2.3 Hz, OCH_2CCH), 7.18 (2H, s, NH_2), 7.72 (2H, d, J = 8.8 Hz, H-2' and H-6'), 7.98 (2H, d, J = 8.8 Hz, H-3' and H-5'), 8.13 (1H, s, H-8), 9.87 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 53.6 (O^6CH_2CCH), 78.0 (O^6CH_2CCH), 79.1 (O^6CH_2CCH), 115.1 (Ar-C), 117.5 (CHCNH), 126.5 ($CHCSO_2NH_2$), 135.5 (Ar-CNH), 139.9 (C^2NH), 143.9 (CSO_2NH_2), 154.3 (C^8), 158.6 (Ar-C); LRMS (ES^+) *m/z* 345.8 $[M+H]^+$; HRMS calcd for $C_{14}H_{12}N_6O_3S$ $[M^+]$ 344.0692, found 344.0700.

6-Chloro-2-fluoro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (36). 3,4-Dihydropyran (60 µL, 0.58 mmol) was added dropwise over 10 min to a vigorously stirred solution of **10** (0.10 g, 0.58 mmol) and (*rac*)-camphorsulfonic acid (5 mg, 0.02 mmol) in EtOAc (50 mL) at 65 ºC. The temperature was maintained at 65 ºC for 18 h. The resulting bright yellow solution was neutralised to pH 7 by careful addition of aqueous NH_3 solution, until a cloudy suspension persisted. The crude mixture was washed with brine (2 × 30 mL) and the aqueous phase was re-extracted with EtOAc (2 × 30 mL). The combined organic extracts were dried (Na_2SO_4) and purified by chromatography (silica; 30% EtOAc:petrol) to afford **36** as a pale yellow oil which became a white waxy solid on

refrigeration (110 mg, 75%); mp 92-94 °C; UV λ_{max} (EtOH) 269 nm; IR (cm^{-1}) 3132, 2955, 2876, 1574; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.57-1.64 (2H, m, CH_2), 1.70-1.80 (1H, m, C*H*), 1.94-2.02 (2H, m, CH_2), 2.23-2.32 (1H, m, C*H*), 3.70-3.77 (1H, m, C*H*), 4.00-4.06 (1H, m, C*H*), 5.71 (1H, dd, J = 2.3, 10.9 Hz, NC*H*), 8.92 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.0 (N^9-CH*C*H_2CH_2), 24.3 (N^9-CHOCH$_2$*C*H_2), 29.6 (N^9-CHCH_2*C*H_2), 67.7 (N^9-CHO*C*H_2CH_2), 81.7 (N^9-*C*H), 130.1 (d, J_{CF} = 4.8 Hz, Ar-C), 146.4 (d, J_{CF} = 2.8 Hz, Ar-C), 150.6 (d, J_{CF} = 18.5 Hz, Ar-C), 153.2 (d, J_{CF} = 17.6 Hz, Ar-C), 156.2 (d, J_{CF} = 213.7 Hz, Ar-C^2); LRMS (ES^+) *m/z* 257.2 $[M+H]^+$.

2-Fluoro-9-(tetrahydro-2*H*-pyran-2-yl)-6-((triisopropylsilyl)ethynyl)-9*H*-purine (37). An oxygen-free solution of **36** (0.050 g, 1.95 mmol), bis(triphenylphosphine)palladium (II) chloride (41 mg, 3.0 mol%) and copper iodide (7 mg, 2.0 mol%) in THF (10 mL) was degassed by bubbling nitrogen through the solution in a sealed vial for 5 min. Triisopropylsilylacetylene (0.50 mL, 2.2 mmol) and triethylamine (0.70 mL, 4.9 mmol) were added to the mixture, which was again degassed for 15 min. After stirring at room temperature for 18 h, the black-brown suspension was filtered through Celite, eluting with MeOH (3 × 20 mL). Purification by chromatography (silica; 10% EtOAc:petrol) furnished **37** as a viscous yellow oil (78 mg, 99%); R_f 0.30 (10% EtOAc:petrol); UV λ_{max} (EtOH) 303 nm; IR (cm^{-1}) 3433, 2946, 2866, 2706, 2361, 1703; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.12-1.25 (21H, m, Si(C*H*(CH_3)$_2$)$_3$), 1.56-1.63 (2H, m, CH_2), 1.71-1.80 (1H, m, CH), 1.94-2.03 (2H, m, CH_2), 2.20-2.30 (1H, m, C*H*), 3.70-3.78 (1H, m, CH), 4.01-4.07 (1H, m, C*H*), 5.70 (1H, dd, J = 2.2, 10.8 Hz, NC*H*), 8.89 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 10.5 (Si(*C*H(CH$_3$)$_2$)$_3$), 18.4 (Si(CH(*C*H$_3$)$_2$)$_3$), 22.0 (N^9-CH*C*H_2CH_2), 24.4 (N^9-CHOCH$_2$*C*H_2), 29.7 (N^9-CHCH_2*C*H_2), 67.7 (N^9-CHO*C*H_2CH_2), 81.4 (N^9-*C*H), 100.2 (C≡C), 102.8 (C≡C), 133.9 (d, J_{CF} = 4.6 Hz, Ar-C), 140.8 (d, J_{CF} = 17.9 Hz, Ar-C), 146.6 (d, J_{CF} = 2.8 Hz, Ar-C), 153.8 (d, J_{CF} = 17.8 Hz, Ar-C), 157.4 (d, J_{CF} = 210.6 Hz, Ar-C^2); HRMS calcd for $C_{21}H_{32}FN_4OSi$ $[M+H]^+$ 403.2324, found 403.2324.

2-Fluoro-6-((triisopropylsilyl)ethynyl)-9*H*-purine (38). The title compound was prepared according to **general procedure E** from **37** (0.29 g, 0.72 mmol). Purification by chromatography

(silica; 30% EtOAc:petrol) gave **38** as a pale yellow oil (22 mg, 96%); R_f 0.25 (30% EtOAc:petrol); UV λ_{max} (EtOH) 302 nm; IR (cm^{-1}) 2945, 2866, 2361, 2000, 1584; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.12-1.21 (21H, m, Si(C*H*(C*H*$_3$)$_2$)$_3$), 8.68 (1H, s, H-8), 13.89 (1H, br, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.1 (Si(*C*H(CH$_3$)$_2$)$_3$), 18.5 (Si(CH(*C*H$_3$)$_2$)$_3$), 99.5 (C≡C), 106.1 (C≡C), 131.2 (m, Ar-C), 141.6 (m, Ar-C), 145.3 (m, Ar-C), 156.6 (m, Ar-C), 158.6 (d, J_{CF} = 217.4 Hz, Ar-C^2); HRMS calcd for $C_{16}H_{24}FN_4Si$ $[M+H]^+$ 319.1749, found 319.1752.

6-(2-(Triisopropylsilyl)ethynyl)-*N*-phenyl-9*H*-purin-2-amine (39). Prepared in accordance with **general procedure A** from **38** (0.455 g, 1.43 mmol). Purified by chromatography (silica; 5% MeOH:DCM) to give the title compound as a yellow gum (0.39 g, 1.00 mmol, 70%); R_f 0.39 (5% MeOH:DCM); UV λ_{max} (EtOH) 276 nm; IR (cm^{-1}) 3389, 2361, 2021; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.09-1.22 (21H, m, Si(C*H*(C*H*$_3$)$_2$)$_3$), 7.00-7.04 (1H, m, H-4'), 7.22 (1H, s, N*H*), 7.29 (2H, dd, *J* = 7.7, 8.1 Hz, H-3' and H-5'), 7.46 (1H, s, H-8), 7.52 (2H, dd, *J* = 1.7, 7.7 Hz, H-2' and H-6'), 10.42 (1H, s, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.3 (Si(*C*H(CH$_3$)$_2$)$_3$), 18.7 (Si(CH(*C*H$_3$)$_2$)$_3$), 100.8 (C≡C), 101.8 (C≡C), 120.7 (Ar-C), 123.7 (Ar-C), 129.4 (Ar-C), 139.2 (Ar-C), 141.0 (Ar-C), 142.6 (Ar-C), 153.4 (Ar-C), 156.5 (Ar-C); LRMS (ES^+) *m/z* 392.0 $[M+H]^+$.

4-(6-((Triisopropylsilyl)ethynyl)-9*H*-purin-2-ylamino)benzenesulfonamide (40). Following **general procedure A** employing **38** (0.156 g, 0.49 mmol). Purification by reversed phase column chromatography (C18 silica; 25% to 95% MeCN/water + 0.1% HCOOH), afforded **40** as a yellow solid (70 mg, 30%); mp 163-165 ºC; UV λ_{max} (EtOH) 361 nm; IR (cm^{-1}) 3327, 2944, 2867, 1569, 1531, 1368, 1149; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.15 (21H, m, Si(C*H*(C*H*$_3$)$_2$)$_3$), 7.17 (2H, s, SO_2NH_2), 7.70 (2H, d, *J* = 9.0 Hz, H-2' and H-6'), 7.95 (2H, d, *J* = 9.0 Hz, H-3' and H-5'), 8.33 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 10.6, 18.4, 90.6, 98.4, 117.1, 123.4, 126.5, 135.6, 144.0, 155.5, 165.0, 166.3, 166.5; 291.0, 286.5, 215.5; LRMS (ES^+) *m/z* 471.4 $[M+H]^+$; HRMS calcd for $C_{22}H_{30}N_6O_2SSi$ $[M+H]^+$ 471.1987, found 471.1942.

6-Ethynyl-*N*-phenyl-9*H*-purin-2-amine (41). Treatment of **39** (0.330 g, 0.84 mmol) according to general procedure F, followed by purification by chromatography (silica; EtOAc), yielded **41** as a

yellow solid (201 mg, 100%); R_f 0.27 (100% EtOAc); mp 140-160 °C (dec); UV λ_{max} (EtOH) 243 nm; IR (cm^{-1}) 3414, 3111, 3072, 2920, 2110, 1704; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.86 (1H, s, C≡C*H*), 6.91-6.96 (1H, m, H-4'), 7.29 (2H, dd, *J* = 7.6, 8.0 Hz, H-3' and H-5'), 7.80 (2H, dd, *J* = 2.0, 8.0 Hz, H-2' and H-6'), 8.30 (1H, s, H-8), 9.70 (1H, s, N*H*), 13.17 (1H, br, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 87.0 (C≡*C*H), 118.3 (Ar-C), 120.0 (Ar-C), 128.4 (Ar-C), 140.8 (Ar-C), 156.2 (Ar-C); HRMS calcd for $C_{13}H_{10}N_5$ $[M+H]^+$ 236.0931, found 236.0934.

4-(6-Ethynyl-9*H*-purin-2-ylamino)benzenesulfonamide (42). Deprotection of **40** (0.050 g, 0.11 mmol) in accordance with **general procedure F**, followed by purification by chromatography (silica; 10% MeOH:DCM), afforded **42** as a beige solid (18 mg, 50%); mp 156-158 ºC; IR (cm^{-1}) 3347, 3255, 2920, 2848, 2118, 1568, 1529, 1477, 1128; UV λ_{max} (EtOH) 356, 292, 215 nm; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.90 (1H, s, C≡C*H*), 7.16 (2H, s, SO_2NH_2), 7.17 (2H, d, *J* = 9.0 Hz, H-2' and H-6'), 7.93 (2H, d, *J* = 9.0 Hz, H-3' and H-5'), 8.36 (1H, s, H-8); LRMS (ES^+) *m/z* 315.1 $[M+H]^+$; HRMS calcd for $C_{13}H_{10}N_6O_2S$ $[M+H]^+$ 315.0664, found 315.0687. Note: Insufficient material for ^{13}C NMR analysis.

6-Ethynyl-2-fluoro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (43). Deprotection of **37** (68 mg, 0.169 mmol) in accordance with **general procedure F**, but using 1.2 mol equiv. of TBAF, followed by purification by chromatography (silica; 15-100% EtOAc:petrol), afforded **43** as an off-white solid (41 mg, 100%); R_f 0.32 (50% EtOAc:petrol); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.58-1.63 (1H, m, C*H*), 1.63-1.78 (2H, m, CH_2), 1.90-1.99 (1H, m, C*H*), 1.99-2.06 (1H, m, C*H*), 2.07-2.12 (1H, m, C*H*), 3.68-3.74 (1H, m, C*H*), 3.71 (1H, s, C≡C*H*), 4.09-4.14 (1H, m, C*H*), 5.65 (1H, dd, *J* = 2.6, 10.8 Hz, NC*H*), 8.26 (1H, s, H-8); LRMS (ES^+) *m/z* 247.0 $[M+H]^+$. Note: Insufficient material for ^{13}C NMR analysis.

6-Chloro-*N*-phenyl-9*H*-purin-2-amine (44). A mixture of N^2-phenylguanine trifluoroacetate (**15**) (2.00 g, 5.87 mmol) and *N*,*N*-diethylaniline (1.9 mL, 11.73 mmol) in $POCl_3$ (30 mL) was heated at 115 °C for 60 min. The resultant yellow solution was carefully added dropwise to crushed ice-water with stirring, and the aqueous solution was neutralised by cautious addition of aqueous NaOH

solution (1.0 M). The aqueous mixture was extracted with EtOAc (2 × 20 mL), and the combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure. Purification by chromatography (silica; 50% EtOAc:petrol) gave **44** as a white powder (670 mg, 46%); mp 172-174 °C; UV λ_{max} (EtOH) 329, 272 nm; IR (cm^{-1}) 3399, 3289, 1627, 1601, 1571, 1540; ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.89-6.92 (1H, t, J = 7.5 Hz, H-4'), 7.23-7.26 (2H, dd, J = 7.4, 7.5 Hz, H-3' and H-5'), 7.71-7.73 (2H, d, J = 7.4 Hz, H-2' and H-6'), 8.20 (1H, s, H-8), 9.81 (1H, br s, N*H*), 13.20 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 118.6, 121.5, 128.5, 140.3, 142.4, 155.5; LRMS (ES^+) *m/z* 246.1 $[M+H]^+$.

6-Chloro-*N*-phenyl-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (45). To a solution of **44** (0.2 g, 0.82 mmol) and CSA (0.01 g, 0.041 mmol) in EtOAc (25 mL) was added DHP (83 μL, 0.90 mmol), and the mixture was heated at 75 °C for 17 h. After cooling, the pH was adjusted to 8-9 with concentrated aqueous ammonia, and the volatiles were removed *in vacuo*. Purification by chromatography (silica; 1/2 to 2/1 EtOAc:petrol) furnished **45** as a waxy solid (194 mg, 72%); mp 194-196 °C; UV λ_{max} (EtOH) 330, 275 nm; IR (cm^{-1}) 3274, 3106, 2934, 2849, 1579, 1573, 1535; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.38-1.39 (2H, m, CH_2), 1.49-1.54 (1H, m, C*H*), 1.76-1.80 (2H, m, CH_2), 2.09-2.17 (1H, m, C*H*), 3.43-3.48 (1H, m, C*H*), 3.81-3.83 (1H, m, C*H*), 5.38-5.40 (1H, dd, J = 2.0, 11.0 Hz, NC*H*), 6.74-6.77 (1H, t, J = 8.5 Hz, H-4'), 7.07-7.11 (2H, dd, J = 7.5, 8.5 Hz, H-3' and H-5'), 7.56-7.58 (2H, d, J = 7.5 Hz, H-2' and H-6'), 8.26 (1H, s, H-8), 9.79 (1H, br s, N*H*), 13.21 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 24.5, 29.4, 54.9, 67.7, 81.6, 118.7, 121.7, 124.7, 128.6, 140.1, 142.4, 149.3, 152.7, 155.3; LRMS (ES^+) *m/z* 329.7 $[M+H]^+$.

***N*-Phenyl-6-(prop-1-yn-1-yl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (46)**. A suspension of **45** (0.1 g, 0.30 mmol), $PdCl_2(CH_3CN)_2$ (0.8 mg, 0.003 mmol), dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (4.3 mg, 0.009 mmol) and Cs_2CO_3 (0.26 g, 0.79 mmol) in acetonitrile (1 mL) was degassed for 10 min under a nitrogen atmosphere at room temperature. While degassing, an excess of methylacetylene was condensed and added to the reaction mixture via a cannula, and the reaction mixture was heated and stirred at 40 °C for 2.5 h.

After cooling to room temperature, water (5 mL) was added and the mixture was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with brine (10 mL), dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. Purification by chromatography (silica; 30%-80% EtOAc:petrol) gave **46** as a colourless glassy compound (78 mg, 77%); R_f 0.27 (30% EtOAc:petrol); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.62-1.85 (3H, m, CH_2 and C*H*), 2.06-2.15 (3H, m, CH_2 and C*H*), 2.23 (3H, s, C≡CCH_3), 3.74-3.81 (1H, m, C*H*), 4.15-4.21 (1H, m, C*H*), 5.60-5.67 (1H, m, C*H*), 7.04 (1H, dd, *J* = 7.4, 7.3 Hz, H-4'), 7.34 (2H, dd, *J* = 8.5, 7.4 Hz, H-2' and H-6'), 7.47 (1H, s, N*H*), 7.34 (2H, dd, *J* = 8.6, 0.9 Hz, H-3' and H-5'), 8.03 (1H, s, H-8); LRMS (ES^+) *m/z* 334.0 $[M+H]^+$. Note: Insufficient material for ^{13}C NMR analysis.

***N*-Phenyl-6-(phenylethynyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (47).** To a degassed suspension of **45** (0.1 g, 0.30 mmol), $PdCl_2(CH_3CN)_2$ (0.8 mg, 0.003 mmol), dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (4.3 mg, 0.009 mmol) and Cs_2CO_3 (0.26 g, 0.79 mmol) in anhydrous acetonitrile (1 mL) was added an excess of phenylacetylene, and the mixture was heated at 80 °C for 2 h. After cooling, water (5 mL) was added and the suspension was extracted with EtOAc (3 × 15 mL). The combined organic extract was washed with brine (10 mL), dried ($MgSO_4$), and the solvent was removed *in vacuo*. Purification by chromatography (silica; 0-80% EtOAc:petrol) gave **47** as a colourless glassy compound (94 mg, 78%); R_f 0.53 (65% EtOAc:petrol); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.64-1.88 (3H, m, CH_2 and C*H*), 2.07-2.19 (3H, m, CH_2 and C*H*), 3.74-3.84 (1H, m, C*H*), 4.16-4.24 (1H, m, C*H*), 5.63-5.71 (1H, m, C*H*), 7.03-7.08 (1H, m, H-4'), 7.32-7.46 (5H, m, phenyl), 7.50 (1H, s, N*H*), 7.70 (2H, d, *J* = 8.3 Hz, H-2' and H-6'), 7.72-7.75 (2H, m, H-3' and H-5'), 8.08 (1H, s, H-8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.9, 25.0, 31.5, 68.8, 82.2, 84.1, 97.6, 118.9, 121.5, 122.5, 128.5, 129.0, 129.4, 130.0, 132.9, 139.8, 141.0, 142.3, 152.5, 156.2; LRMS (ES^+) *m/z* 396.4 $[M+H]^+$.

***N*-Phenyl-6-(prop-1-yn-1-yl)-9*H*-purin-2-amine (48)**. Prepared in accordance with **general procedure E** from **46** (0.061 g, 0.18 mmol). Recrystallisation from EtOAc-petrol gave **48** as a pale yellow solid (18 mg, 40%); mp 155-158 °C (dec); UV λ_{max} (EtOH) 272 nm; IR (cm^{-1}) 1529, 1577,

2236, 2919; ^{1}H NMR (500 MHz, MeOD-d_4) δ 2.12 (3H, s, C≡CCH_3), 6.87 (1H, t, J = 7.3 Hz, H-4'), 7.18 (2H, t, J = 7.8 Hz, H-2' and H-6'), 7.63 (2H, d, J = 7.9 Hz, H-3' and H-5'), 8.04 (1H, s, H-8); ^{13}C NMR (125 MHz, MeOD-d_4) δ 4.3, 116.9, 119.2, 120.1, 122.9, 129.6, 163.2, 163.5; LRMS (ES$^+$) *m/z* 250.1 $[M+H]^+$; HRMS calcd for $C_{14}H_{12}N_5$ $[M+H]^+$ 250.1087, found 250.1085.

***N*-Phenyl-6-(phenylethynyl)-9*H*-purin-2-amine (49)**. Prepared in accordance with **general procedure E** from **47** (0.055 g, 0.14 mmol). Recrystallisation from EtOAc-petrol gave **49** as a yellow solid (22 mg, 51%); mp 206-208 °C (dec); UV λ_{max} (EtOH) 281 nm; IR (cm^{-1}) 1532, 1574, 2208, 3226; ^{1}H NMR (500 MHz, MeOD-d_4) δ 6.79 (1H, t, J = 7.3 Hz, H-4'), 7.11 (2H, dd, J = 7.8, 8.1 Hz, H-2' and H-6'), 7.23-7.32 (3H, m, H-3', H-5' and Ar-H), 7.54-7.58 (4H, m, 4 × ArH), 8.02 (1H, s, H-8); ^{13}C NMR (125 MHz, MeOD-d_4) δ 97.3, 120.2, 122.8, 122.9, 129.6, 129.8, 131.2, 133.5, 141.8, 158.6; LRMS (ES$^+$) *m/z* 312.2 $[M+H]^+$; HRMS calculated for $C_{19}H_{14}N_5$ $[M+H]^+$ 312.1244, found 312.1242.

6-Ethyl-2-fluoro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (50). To a solution of **43** (0.05 g, 0.20 mmol) and quinoline (20 μL) in EtOAc (5 mL) was added Lindlar's catalyst (10 mg, 20% w/w), and the mixture was stirred for 2 h under an atmosphere of H_2. The suspension was filtered through Celite, eluting with methanol (30 mL), and volatiles were removed *in vacuo*. Purification by chromatography (silica; 50% EtOAc:petrol) yielded **50** as a colourless oil (50 mg, 99%); UV λ_{max} (EtOH) 264 nm; IR (cm^{-1}) 2946, 2860, 2364, 2338, 1604; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.5 Hz, CH_3CH$_2$), 1.59 (2H, m, CH_2), 1.97 (1H, m, CH), 1.99 (2H, d, J = 10.5 Hz, CH_2), 2.50 (1H, m, CH), 3.01 (2H, q, J = 7.5 Hz, CH$_3$CH_2), 3.73 (1H, m, CH), 4.04 (1H, d, J = 10.5 Hz, CH), 5.69 (1H, d, J = 10.5 Hz, CH), 8.74 (1H, s, H-8); ^{13}C NMR (500 MHz, DMSO-d_6) δ 12.3, 22.7, 24.8, 26.5, 31.8, 68.9, 82.1, 142.1, 157.9, 159.6, 167.4, 167.5; LRMS (ES$^+$) *m/z* 251.0 $[M+H]^+$.

6-Ethyl-2-fluoro-9*H*-purine (51). Prepared in accordance with **general procedure E** from **50** (0.18 g, 0.72 mmol). Purification by chromatography (silica; 25% EtOAc:petrol) yielded **51** as a white solid (0.117 g, 98%); mp 146-148 °C; UV λ_{max} (EtOH) 269 nm; IR (cm^{-1}) 1676, 1616, 1573;

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (3H, t, J = 7.5 Hz, CH_3CH$_2$), 3.00 (2H, q, J = 7.5 Hz, CH$_3$CH_2), 8.17 (1 H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 11.7, 25.9, 68.3, 116.0, 118.4, 146.4, 158.0 (d, $J_{C\text{-}F}$ = 206.0 Hz), 158.3 (d, $J_{C\text{-}F}$ = 31.5 Hz); LRMS (ES$^+$) *m/z* 167.7 [M+H]$^+$.

4-(6-Ethyl-9*H*-purin-2-ylamino)benzenesulfonamide (52). Prepared by **general procedure A** from **51** (0.081 g, 0.49 mmol), following purification by chromatography (silica; 50% EtOAc:petrol), as a white solid (33 mg, 21%); mp 291-293 ºC; UV λ_{max} (EtOH) 318, 287, 212 nm; IR (cm^{-1}) 3377, 3060, 2852, 1388, 1158; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 6.0 Hz, CH_3CH$_2$), 3.00 (2H, q, J = 6.0 Hz, CH$_3$CH_2), 7.14 (2H, br s, SO$_2$NH_2), 7.69 (2H, d, J = 7.5 Hz, H-2' and H-6'), 7.99 (2H, d, J = 7.5 Hz, H-3' and H-5'), 8.17 (1H, s, H-8), 9.89 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 12.3, 26.0, 117.0, 118.9, 125.4, 126.4, 135.2, 141.2, 144.4, 154.0, 155.5; LRMS (ES$^+$) *m/z* 318.6 [M+H]$^+$; HRMS calcd for $C_{13}H_{14}N_6O_2S$ [M+H]$^+$ 319.097076, found 319.09788.

6-Cyclopropyl-2-fluoro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (53). The title compound was prepared from **36** (0.04 g, 0.27 mmol) and potassium cyclopropyltrifluoroborate (50 mg, 0.19 mmol) following **general procedure G**. Purification by chromatography (silica; 50% EtOAc:petrol) afforded **53** as a colourless oil (21 mg, 42%); UV λ_{max} (EtOH) 211, 325, 391 nm; IR (cm^{-1}) 2986; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25 (4H, d, J = 6.5 Hz, cyclopropyl 2 × CH_2), 1.55 (2H, m, CH_2), 1.72 (1H, m, C*H*), 1.95 (2H, d, J = 10.5 Hz, CH_2), 2.27 (1H, m, C*H*), 2.67 (1H, m, cyclopropyl C*H*), 3.70 (1H, m, C*H*), 3.99 (1H, d, J = 10.5 Hz, C*H*), 5.63 (1H, d, J = 10.5 Hz, C*H*), 8.70 (1H, s, H-8); ^{13}C NMR (500 MHz, DMSO-d_6) δ 11.9, 13.2, 22.2, 24.4, 29.7, 67.7, 81.3, 130.9, 144.1, 151.8, 157.6, 159.8, 176.9; LRMS (ES$^+$) *m/z* 262.4 [M+H]$^+$.

6-Cyclopropyl-2-fluoro-9*H*-purine (54). The title compound was prepared from **53** (0.19 g, 0.72 mmol) employing **general procedure E**. Purification by chromatography (silica; EtOAc) afforded **54** as a white solid (0.12 g, 95%); mp 137-139 °C; UV λ_{max} (EtOH) 323, 213 nm; IR (cm^{-1}) 3062; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (4H, d, J = 6.5 Hz, cyclopropyl 2 × CH_2), 2.51 (1H, m,

cyclopropyl C*H*), 8.40 (1H, s, H-8); ^{13}C NMR (400 MHz, DMSO-d_6) δ 11.6, 13.1, 131.2, 144.2, 151.8, 157.6, 159.8; LRMS (ES$^+$) *m/z* 179.0 $[M+H]^+$.

4-(6-Cyclopropyl-9*H*-purin-2-ylamino)benzenesulfonamide (55). Synthesised following **general procedure A** from **54** (0.086 g, 0.49 mmol). Purification by chromatography (silica; EtOAc) afforded **55** as a pale yellow solid (24 mg, 15%); mp 291-293 ºC; UV λ_{max} (EtOH) 315.5, 289, 213 nm; IR (cm^{-1}) 3389, 3062, 2921, 2851, 1727, 1604, 1579, 1531, 1379, 1149; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.23 (4H, d, J = 6.5 Hz, cyclopropyl 2 × CH_2), 1.35 (1H, m, cyclopropyl C*H*), 7.18 (2H, br s, SO_2NH_2), 7.74 (2H, d, J = 8.0 Hz, H-2' and H-6'), 7.99 (2H, d, J = 8.0 Hz, H-3' and H-5'), 8.22 (1H, s, H-8), 9.75 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 10.5, 13.0, 117.1, 126.4, 126.6, 141.1, 144.4, 151.8, 155.5, 162.9; LRMS (ES$^+$) *m/z* 331.1 $[M+H]^+$; HRMS calcd for $C_{14}H_{15}N_6O_2S$ $[M+H]^+$ 331.0977, found 331.0980.

2-Fluoro-6-phenyl-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (56). Synthesised in accordance with **general procedure G** from **36** (0.20 g, 0.78 mmol) and phenylboronic acid (0.133 g, 1.09 mmol). Purification by chromatography (silica; 20% EtOAc:petrol) gave **56** as a white solid (0.30 g, 95%); mp 138-140 ºC; UV λ_{max} (EtOH) 306, 269, 203 nm; IR (cm^{-1}) 3117, 2956, 2858, 1586; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60 (2H, m, CH_2), 1.76 (1H, m, C*H*), 2.02 (2H, m, CH_2), 2.29 (1H, s, C*H*), 3.77 (1H, t, J = 12.0 Hz, C*H*), 4.04 (1H, d, J = 12.0 Hz, C*H*), 5.74 (1H, d, J = 12.0 Hz, C*H*), 7.64, (3H, m, H-3", H-4" and H-5"), 8.79 (2H, dd, J = 3.0, 7.5 Hz, H-2" and H-6"), 8.25 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.2, 24.4, 29.7, 67.7, 81.3, 128.9, 129.3, 129.5, 132.0, 134.0, 145.4, 154.3 (d, $J_{C\text{-}F}$ = 17.5 Hz), 155.5 (d, $J_{C\text{-}F}$ = 144.0 Hz), 157.0, 158.7; LRMS (ES$^+$) *m/z* 299.3 $[M+H]^+$.

2-Fluoro-6-(3-methoxyphenyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (57). Prepared according to **general procedure G** from **36** (0.20 g, 0.78 mmol) and 4-methoxyphenylboronic acid (0.16 g, 1.09 mmol). Purification by chromatography (silica; 30% EtOAc:petrol) gave **57** as a white solid (0.29 g, 80%); mp 133-135 ºC; UV λ_{max} (EtOH) 306, 271, 204 nm; IR (cm^{-1}) 3089, 2938, 2857, 1761, 1586, 1516; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60 (2H, m, CH_2), 1.76 (1H, m, C*H*),

2.02 (2H, m, CH_2), 2.29 (1H, s, C*H*), 3.77 (1H, t, *J* = 12.0 Hz, C*H*), 3.89 (3H, s, OCH_3), 4.04 (1H, d, *J* = 12.0 Hz, C*H*), 5.74 (1H, d, *J* = 12.0 Hz, C*H*), 7.18 (1H, m, H-2''), 7.50-7.55 (1H, m, H-5''), 8.45-8.49 (2H, m, H-4'', H-6''), 8.78 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.2, 24.4, 29.7, 55.3, 67.7, 81.3, 114.4, 117.9, 122.0, 129.4, 130.0, 135.3, 145.4, 154.3 (d, $J_{C\text{-}F}$ = 17.5 Hz), 155.0 (d, $J_{C\text{-}F}$ = 160.0 Hz), 156.9, 158.6, 159.4; LRMS (ES$^+$) *m/z* 334.2 $[M+H]^+$.

2-Fluoro-6-(4-methoxyphenyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (58). Synthesised in accordance with **general procedure G** from **36** (0.20 g, 0.78 mmol) and 4-methoxyphenylboronic acid (0.16 g, 1.09 mmol). Purification by chromatography (silica; 30% EtOAc:petrol) afforded **58** as a white solid (0.35 g, 98%); mp 131-133 ºC; UV λ_{max} (EtOH) 306, 271, 204 nm; IR (cm^{-1}) 3089, 2938, 2857, 1761, 1586, 1516; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60 (2H, m, CH_2), 1.76 (1H, m, C*H*), 2.02 (2H, m, CH_2), 2.29 (1H, s, C*H*), 3.77 (1H, t, *J* = 12.0 Hz, C*H*), 3.88 (3H, s, OCH_3), 4.04 (1H, d, *J* = 12.0 Hz, C*H*), 5.74 (1H, d, *J* = 12.0 Hz, C*H*), 7.15-7.18 (2H, d, *J* = 9.0 Hz, H-3'' and H-5''), 8.79-8.81 (2H, d, *J* = 9.0 Hz, H-2'' and H-6''), 8.85 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.2, 24.4, 29.7, 55.5, 67.7, 81.2, 114.3, 126.5, 128.5, 131.5, 144.7, 153.9 (d, $J_{C\text{-}F}$ = 17.5 Hz), 155.2 (d, $J_{C\text{-}F}$ = 160.0 Hz), 157.0, 158.7, 162.4; LRMS (ES$^+$) *m/z* 329.2 $[M+H]^+$.

6-(3-Biphenyl)-2-fluoro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (59). Prepared following **general procedure G** from **36** (0.20 g, 0.78 mmol) and 3-biphenylboronic acid (0.22 g, 1.09 mmol). Purification by chromatography (silica; 20% EtOAc:petrol) furnished **59** as a pale yellow solid (0.33 g, 80%); mp 221-223 ºC; UV λ_{max} (EtOH) 346, 274 nm; IR (cm^{-1}) 2992, 2844, 2601, 1589; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60 (2H, m, CH_2), 1.76 (1H, m, C*H*), 2.02 (2H, m, CH_2), 2.29 (1H, s, C*H*), 3.77 (1H, t, *J* = 12.0 Hz, C*H*), 4.04 (1H, d, *J* = 12.0 Hz, C*H*), 5.79 (1H, d, *J* = 12.0 Hz, C*H*), 7.45 (1H, d, *J* = 7.0 Hz, H-6''), 7.56 (1H, dd, *J* = 7.0, 7.1 Hz, H-5''), 7.78 (5H, m, Ph), 8.40 (1H, s, H-2''), 8.84 (1H, d, *J* = 7.1 Hz, H-4''), 9.18 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.2, 24.4, 29.7, 67.7, 81.3, 126.8, 127.8, 127.9, 128.4, 129.1, 129.5, 129.6, 130.3, 134.7, 139.6, 140.8, 145.6, 154.4 (d, $J_{C\text{-}F}$ = 17.5 Hz), 155.2 (d, $J_{C\text{-}F}$ = 116.0 Hz), 157.0, 158.5; LRMS (ES$^+$) *m/z* 375.1 $[M+H]^+$.

2-Fluoro-6-(3-piperonyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (60). Synthesised in accordance with **general procedure G** from **36** (0.20 g, 0.78 mmol) and 3,4-(methylenedioxy)phenylboronic acid (0.18 g, 1.09 mmol). Purification by chromatography (silica; 30% EtOAc:petrol) yielded **60** as a white solid (0.21 g, 56%); mp 151-153 °C; UV λ_{max} (EtOH) 351, 284 nm; IR (cm^{-1}) 3011, 2917, 1721, 1616, 1511; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60 (2H, m, CH_2), 1.76 (1H, m, C*H*), 2.02 (2H, m, CH_2), 2.29 (1H, s, C*H*), 3.73 (1H, t, *J* = 12.0 Hz, C*H*), 4.04 (1H, d, *J* = 12.0 Hz, C*H*), 5.73 (1H, d, *J* = 12.0 Hz, C*H*), 6.17 (2H, s, OCH_2O), 7.14-7.17 (1H, d, *J* = 8.0 Hz, H-5"), 8.28 (1H, s, H-2"), 8.48-8.51 (1H, d, *J* = 8.0 Hz, H-6"), 8.82 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.2, 24.4, 29.7, 67.7, 81.2, 101.9, 108.6, 125.4, 128.0, 128.6, 144.9, 147.8, 150.6, 154.1, 154.8, 156.9, 158.7; LRMS (ES^+) *m/z* 343.1 $[M+H]^+$.

6-(4-Dibenzofuryl)-2-fluoro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (61). Prepared according to **general procedure G** from **36** (0.20 g, 0.78 mmol) and 4-dibenzofuranboronic acid (0.23 g, 1.09 mmol). Purification by chromatography (silica; 30% EtOAc:petrol) yielded **61** as a cream solid (0.39 g, 92%); mp 168-170 ºC; UV λ_{max} (EtOH) 398, 256, 202 nm; IR (cm^{-1}) 3092, 2589, 1579; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60 (2H, m, CH_2), 1.76 (1H, m, C*H*), 2.02 (2H, m, CH_2), 2.29 (1H, s, C*H*), 3.77 (1H, t, *J* = 12 Hz, C*H*), 4.04 (1H, d, *J* = 12.0 Hz, C*H*), 5.79 (1H, d, *J* = 12.0 Hz, C*H*), 7.45-7.47 (1H, dd, *J* = 7.0, 7.1 Hz, H-2"), 7.52-7.62 (1H, dd, *J* = 8.0, 8.1 Hz, H-7"), 7.61-7.71 (1H, dd, *J* = 8.0, 8.1 Hz, H-8"), 7.76-7.79 (1H, d, *J* = 8.0 Hz, H-9"), 8.25-8.27 (1H, d, *J* = 7.1 Hz, H-3"), 8.42-8.44 (1H, d, *J* = 8.0 Hz, H-6"), 8.55-8.58 (1H, d, *J* = 7.5 Hz, H-1"), 8.93 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.2, 24.5, 29.7, 67.8, 68.3, 81.4, 111.9, 119.3, 121.3, 122.9, 123.1, 123.4, 124.3, 125.2, 128.1, 130.1, 145.6, 153.1, 154.7 (d, $J_{C\text{-}F}$ = 18.0 Hz), 155.7 (d, $J_{C\text{-}F}$ = 160.0 Hz), 156.9, 158.5; LRMS (ES^+) *m/z* 389.4 $[M+H]^+$.

2-Fluoro-6-(1-thianthrenyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (62). Prepared in accordance with **general procedure G** from **36** (0.20 g, 0.78 mmol) and 1-thianthreneboronic acid (0.28 g, 1.09 mmol). Purification by chromatography (silica; 30% EtOAc:petrol) afforded **62** as an orange solid (0.43 g, 90%); mp 164-166 ºC; UV λ_{max} (EtOH) 396, 224 nm; IR (cm^{-1}) 3092, 2814,

2589, 1611, 1497; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60 (2H, m, CH_2), 1.76 (1H, m, C*H*), 2.02 (2H, m, CH_2), 2.29 (1H, s, C*H*), 3.77 (1H, t, *J* = 12.0 Hz, C*H*), 4.04 (1H, d, *J* = 12.0 Hz, C*H*), 5.79 (1H, d, *J* = 12.0 Hz, C*H*), 7.21-7.30 (1H, dd, *J* = 7.4, 7.5 Hz, H-3''), 7.31-7.39 (1H, dd, *J* = 7.5, 7.6 Hz, H-7''), 7.37-7.45 (1H, d, *J* = 7.5 Hz, H-9''), 7.51-7.58 (1H, dd, *J* = 7.5, 7.6 Hz, H-8''), 7.59-7.65 (1H, d, *J* = 7.5 Hz, H-6''), 7.79 (1H, d, *J* = 7.4 Hz, H-2''), 7.89 (1H, d, *J* = 7.5 Hz, H-4''), 8.93 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.2, 24.4, 29.5, 67.7, 68.3, 81.5 127.5, 128.2, 128.5, 128.7, 128.9, 130.6, 130.9, 131.4, 134.5, 134.7, 135.1, 135.5, 136.4, 146.2, 153.8 (d, $J_{C\text{-}F}$ = 17.5 Hz), 156.2, 155.0 (d, $J_{C\text{-}F}$ = 150.0 Hz), 157.2; LRMS (ES$^+$) *m/z* 437.2 [M+H]$^+$.

2-Fluoro-6-phenyl-9*H*-purine (63). Prepared from **56** (0.22 g, 0.72 mmol) following **general procedure E**. Repeated trituration of the precipitated solid with water gave **63** (0.14 g, 90%); mp 273-275 °C; UV λ_{max} (EtOH) 349 nm; IR (cm^{-1}) 2820, 1599, 1560; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.62 (3H, m, H-3'', H-4'' and H-5''), 8.68 (1 H, s, H-8), 8.81 (2H, m, H-2'' and H-6''); ^{13}C NMR (125 MHz, DMSO-d_6) δ 128.8, 129.4, 131.7, 134.4, 145.7, 154.4, 156.0, 157.1, 158.7; LRMS (ES$^+$) *m/z* 214.6 [M+H]$^+$.

2-Fluoro-6-(3-methoxyphenyl)-9*H*-purine (64). Deprotection of **57** (0.24 g, 0.72 mmol) in accordance with **general procedure E** afforded the title compound **64** (0.17 g, 98%) following repeated trituration of the precipitated solid with water; mp 237-238 °C; UV λ_{max} (EtOH) 251 nm; IR (cm^{-1}) 2837, 1864, 1587, 1565; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.89 (3H, s, OCH$_3$), 7.18 (1 H, s, H-2''), 7.50-7.55 (1H, m, H-5''), 8.37 (1H, s, H-8), 8.45-8.49 (2H, m, H-4'', H-6''); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.3, 114.2, 117.6, 121.8, 130.0, 135.7, 146.3, 154.3, 157.2, 158.6, 159.4; LRMS (ES$^+$) *m/z* 245.5 [M+H]$^+$.

2-Fluoro-6-(4-methoxyphenyl)-9*H*-purine (65). Synthesised in accordance with **general procedure E** from **58** (0.24 g, 0.72 mmol). Trituration of the precipitated solid with water furnished **65** as a pale yellow solid (0.17 g, 95%); mp 212-214 °C; UV λ_{max} (EtOH) 204 nm; IR (cm^{-1}) 2845, 1586; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.88 (3H, s, OCH_3), 7.15-7.18 (2H, d, *J* = 9.0 Hz, H-3'' and H-5''), 8.60 (1H, s, H-8), 8.79-8.81 (2H, d, *J* = 9.0 Hz, H-2'' and H-6''); ^{13}C NMR (500 MHz,

DMSO-d_6) δ 55.4, 114.1, 114.3, 126.8, 127.5, 131.3, 145.3, 157.1, 158.8, 162.2; LRMS (ES^+) *m/z* 245.2 $[M+H]^+$.

6-(Biphenyl-3-yl)-2-fluoro-9*H*-purine (66). The title compound was prepared according to **general procedure E** from **59** (0.27 g, 0.72 mmol). Repeated washing of the precipitated solid with water gave **66** (0.20 g, 97%); mp 245-247 °C; UV λ_{max} (EtOH) 349 nm; IR (cm^{-1}) 1607, 1568; ^1H NMR (300 MHz, DMSO-d_6) δ 7.43-7.45 (1H, d, *J* = 7.0 Hz, H-6"), 7.54-7.58 (1H, dd, *J* = 6.9, 7.0 Hz, H-5"), 7.74-7.81 (5H, m, Ph), 8.40 (1H, s, H-2"), 8.80-8.85 (1H, d, *J* = 6.9 Hz, H-4"), 8.91 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 126.8, 127.7, 127.9, 128.2, 129.1, 129.5, 130.0, 135.0, 139.7, 140.7, 146.1 (m, $J_{C\text{-}F}$), 157.1, 158.7; LRMS (ES^+) *m/z* 291.1 $[M+H]^+$.

6-(Benzo[*d*][1,3]dioxol-5-yl)-2-fluoro-9*H*-purine (67). Deprotection of **60** (0.25 g, 0.72 mmol) was achieved following **general procedure E**. Trituration of the white solid that precipitated yielded **67** (0.16 g, 87%); mp 165-167 °C; UV λ_{max} (EtOH) 351, 292 nm; IR (cm^{-1}) 2955, 2859, 1601, 1570, 1504; ^1H NMR (300 MHz, DMSO-d_6) δ 6.17 (2H, s, OCH_2O), 7.18-7.18 (1H, d, *J* = 8.0 Hz, H-5"), 8.28 (1H, s, H-2"), 8.48-8.51 (1H, d, *J* = 8.0 Hz, H-6"), 8.63 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 101.9, 108.6, 125.1, 128.4, 145.3, 147.8, 150.3, 153.9 (d, $J_{C\text{-}F}$ = 17.5 Hz), 156.0 (d, $J_{C\text{-}F}$ = 150.0 Hz), 157.0, 158.6; LRMS (ES^+) *m/z* 259.2 $[M+H]^+$.

6-(4-Dibenzofuranyl)-2-fluoro-9*H*-purine (68). The title compound was prepared in accordance with **general procedure E** from **61** (0.28 g, 0.72 mmol), and purified by repeated washing with water to afford **68** as an off-white solid (0.20 g, 94%); mp 266-268 °C; UV λ_{max} (EtOH) 250 nm; IR (cm^{-1}) 3020, 2923, 2832, 1867, 1611, 1568; ^1H NMR (300 MHz, DMSO-d_6) δ 7.45-7.48 (1H, dd, *J* = 7.0, 7.1 Hz, H-2"), 7.55-7.63 (1H, dd, *J* = 7.9, 8.0 Hz, H-7"), 7.61-7.68 (1H, dd, *J* = 7.9, 8.0 Hz, H-8"), 7.76-7.79 (1H, d, *J* = 8.0 Hz, H-9"), 8.26-8.29 (1H, d, *J* = 7.1 Hz, H-3"), 8.43-8.45 (1H, d, *J* = 8.0 Hz, H-6"), 8.55-8.58 (1H, d, *J* = 7.0 Hz, H-1"), 8.78 (1H, s, H-8); ^{13}C NMR (500 MHz, DMSO-d_6) δ 112.1, 121.3, 123.0, 123.5, 124.0, 125.0, 128.0, 130.0, 155.7; LRMS (ES^+) *m/z* 305.1 $[M+H]^+$.

2-Fluoro-6-(thianthren-1-yl)-9*H*-purine (69). Synthesised following **general procedure E** from **62** (0.32 g, 0.72 mmol), and purified by repeated trituration with water to give **69** (0.36 g, 93%); mp 166-168 °C; UV λ_{max} (EtOH) 250 nm; IR (cm^{-1}) 3068, 2817, 2572, 1609, 1511; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.21-7.29 (1H, dd, J = 7.4, 7.5 Hz, H-3''), 7.31-7.38 (1H, dd, J = 7.5, 7.6 Hz, H-7''), 7.39-7.46 (1H, d, J = 7.5 Hz, H-9''), 7.49-7.55 (1H, dd, J = 7.5, 7.6 Hz, H-8''), 7.58-7.64 (1H, d, J = 7.5 Hz, H-6''), 7.79 (1H, d, J = 7.5 Hz, H-2''), 7.88-7.91 (1H, d, J = 7.4 Hz, H-4''), 8.31 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 124.6, 127.5, 128.2, 128.4, 128.7, 128.9, 130.5, 131.1, 134.7, 134.9, 156.4, 159.9, 160.5; LRMS (ES^+) *m/z* 353.1 $[M+H]^+$.

4-(6-Phenyl-9*H*-purin-2-ylamino)benzenesulfonamide (70). The title compound was synthesised according to **general procedure A** from **63** (0.10 g, 0.49 mmol). Purified by chromatography (silica; 50% EtOAc:petrol) to give **70** as a white solid (80 mg, 45%); mp 153-155 ºC; UV λ_{max} (EtOH) 349, 294 nm; IR (cm^{-1}) 3313, 2922, 2852, 1706, 1589, 1569, 1524, 1373, 1144; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.17 (2 H, br s, SO_2NH_2), 7.60 (3H, m, H-3'', H-4'' and H-5''), 7.75-7.78 (2H, d, J = 8.5 Hz, H-2' and H-6'), 8.03-8.06 (2H, d, J = 8.5 Hz, H-3' and H-5') 8.37 (1H, s, H-8), 8.94 (2H, m, H-2'' and H-6''), 9.91 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 117.2, 125.4, 126.5, 128.5, 129.3, 130.8, 135.4, 135.8, 142.6, 144.3, 153.0, 154.6, 155.3; LRMS (ES^+) *m/z* 367.2 $[M+H]^+$; HRMS calcd for $C_{17}H_{14}N_6O_2S$ $[M+H]^+$ 367.0972, found 367.0974.

4-(6-(3-Methoxyphenyl)-9*H*-purin-2-ylamino)benzenesulfonamide (71). Prepared in accordance with **general procedure A** from **64** (0.12 g, 0.49 mmol), and purified by chromatography (silica; 50% EtOAc:petrol) to furnish **71** as a white solid (64 mg, 33%); mp 288-290 ºC; UV λ_{max} (EtOH) 294 nm; IR (cm^{-1}) 3377, 3097, 3020, 2838, 1729, 1597, 1566, 1531, 1365, 1145; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.89 (3H, s, OCH_3), 7.17 (2H, s, SO_2NH_2), 7.18 (1H, s, H-2''), 7.50-7.55 (1H, m, H-5''), 7.74-7.77 (2H, d, J = 8.0 Hz, H-2' and H-6'), 8.03-8.06 (2H, d, J = 8.0 Hz, H-3' and H-5'), 8.37 (1H, s, H-8), 8.45-8.49 (2H, m, H-4'' and H-6''); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.2, 114.2, 116.6, 117.2, 121.8, 126.5, 129.6, 135.4, 137.1, 144.3, 146.3, 155.2, 115.6, 159.3; LRMS (ES^+) *m/z* 397.3 $[M+H]^+$; HRMS calcd for $C_{18}H_{16}N_6O_3S$ $[M+H]^+$ 397.1077, found 397.1071.

4-(6-(4-Methoxyphenyl)-9*H*-purin-2-ylamino)benzenesulfonamide (72). Synthesed following **general procedure A** from **65** (0.12 g, 0.49 mmol) and isolated by chromatography (silica; 50% EtOAc:petrol) to yield **72** as a white solid (90 mg, 46%); mp 172-174 ºC; UV λ_{max} (EtOH) 301, 203 nm; IR (cm^{-1}) 3322, 2133, 2005, 1594, 1533, 1514, 1352, 1147; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.88 (3H, s, OCH_3), 7.17 (2H, br s, SO_2NH_2), 7.18 (2H, d, J = 8.5 Hz, H-3" and H-5"), 7.74-7.77 (2H, d, J = 8.0 Hz, H-2' and H-6'), 8.02-8.05 (2H, d, J = 8.0 Hz, H-3' and H-5'), 8.32 (1H, s, H-8), 8.84-8.87 (2H, d, J = 8.5 Hz, H-2" and H-6"); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.4, 114.0, 117.2, 124.8, 126.5, 128.2, 131.0, 135.3, 142.0, 144.4, 152.8, 154.3, 155.3, 161.5; LRMS (ES^+) *m/z* 397.3 $[M+H]^+$; HRMS calcd for $C_{18}H_{16}N_6O_3S$ $[M+H]^+$ 397.1077, found 397.1071.

4-((6-([1,1'-biphenyl]-3-yl)-9*H*-purin-2-yl)amino)benzenesulfonamide (73). Treatment of **66** (0.14 g, 0.49 mmol) in accordance with **general procedure A**, with purification by chromatography (silica; EtOAc) afforded **73** as a white solid (46 mg, 21%); mp 153-155 ºC; UV λ_{max} (EtOH) 349, 294 nm; IR (cm^{-1}) 3313, 2922, 2852, 1706, 1589, 1569, 1524, 1373, 1144; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.18 (1H, br s, SO_2NH_2), 7.39-7.50 (1H, d, J = 7.0 Hz, H-6"), 7.50-7.62 (1H, dd, J = 7.0, 7.1 Hz, H-5"), 7.66-7.83 (5H, m, Ph), 7.83-7.93 (2H, d, J = 8.0 Hz, H-2' and H-6'), 8.00-8.13 (2H, d, J = 8.0 Hz, H-3' and H-5'), 8.35-8.40 (1H, s, H-2"), 8.78-8.93 (1H, d, J = 7.1 Hz, H-4"), 9.18 (1H, s, H-8), 10.00 (1H, br s, N*H*); LRMS (ES^+) *m/z* 367.2 $[M+H]^+$; HRMS calcd for $C_{23}H_{18}N_6O_2S$ $[M+H]^+$ 367.0972, found 367.0974; HPLC: >96%, XSELECT column, retention time 7.48 min, reverse phase, H_2O/MeCN/ HCOOH gradient run, 1 mL min^{-1}.

4-(6-(Benzo[*d*][1,3]dioxol-5-yl)-9*H*-purin-2-ylamino)benzenesulfonamide (74). The title compound was prepared following **general procedure A** from **67** (0.13 g, 0.49 mmol). Purification by chromatography (silica; EtOAc) gave **74** as a pale yellow solid (30 mg, 15%); mp 284-286 ºC; UV λ_{max} (EtOH) 352, 295 nm; IR (cm^{-1}) 3174, 1573, 1526, 1408, 1350, 1153; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.14 (2H, s, OCH_2O), 7.15 (2H, s, SO_2NH_2), 7.73-7.75 (2H, d, J = 8.0, H-2' and H-6'), 7.98-8.01 (2H, d, J = 8.0 Hz, H-3' and H-5'), 8.31 (1H, s, H-8), 8.37 (1H, s, H-2"), 8.55 (2H, m, H-5" and H-6"), 9.89 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 101.6, 108.4, 108.6, 117.2,

124.7, 126.5, 129.9, 133.2, 135.5, 139.8, 142.2, 144.3, 147.6, 149.6, 151.9, 152.4, 154.4, 155.2; LRMS (ES^+) *m/z* 443.3 $[M+H]^+$; HRMS calcd for $C_{18}H_{14}N_6O_4S$ $[M+H]^+$ 443.1285, found 443.1289.

4-(6-(4-Dibenzofuryl)-9*H*-purin-2-ylamino)benzenesulfonamide (75). Synthesised in accordance with **general procedure A** from **68** (0.15 g, 0.49 mmol). Purification by chromatography (silica; 50% EtOAc:petrol) gave **75** as a white solid (0.10 g, 47%); mp 213-215 ºC; UV λ_{max} (EtOH) 292, 250 nm; IR (cm^{-1}) 3275, 3199, 3104, 2921, 2852, 1714, 1593, 1574, 1535, 1500, 1355, 1146; ^1H NMR (300 MHz, DMSO-d_6) δ 7.16 (2H, br s, SO_2NH_2), 7.44-7.53 (1H, dd, *J* = 6.9, 7.0 Hz, H-2''), 7.54-7.68 (2H, m, H-7'' and H-8''), 7.68-7.74 (1H, d, *J* = 8.0 Hz, H-9''), 7.69-7.79 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 8.18-8.26 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 8.23-8.32 (1H, d, *J* = 6.9 Hz, H-3''), 8.38 (1H, s, H-8), 8.38-8.44 (1H, d, *J* = 7.5 Hz, H-6''), 8.49-8.58 (1H, d, *J* = 7.0 Hz, H-1''), 10.07 (1H, br s, N*H*), 13.22 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.5, 117.3, 121.0, 121.3, 122.9, 123.1, 123.2, 123.4, 124.9, 126.4, 128.0, 130.0, 135.4, 143.0, 144.4, 152.2, 153.0, 154.4, 155.3, 155.7; LRMS (ES^+) *m/z* 457.3 $[M+H]^+$; HRMS calcd for $C_{23}H_{16}N_6O_3S$ $[M+H]^+$ 457.1077, found 457.1075.

6-(4-Thianthren-1-yl)-9*H*-purin-2-ylamino)benzenesulfonamide (76). Synthesised following **general procedure A** from (**69**) (0.17 g, 0.49 mmol), and purified by chromatography (silica; 50% EtOAc:petrol) to furnish **76** as an orange solid (81 mg, 33%); mp 170-172 ºC; UV λ_{max} (EtOH) 339, 294, 250 nm; IR (cm^{-1}) 3317, 2922, 2851, 1706, 1588, 1524, 1371, 1146; ^1H NMR (300 MHz, DMSO-d_6) δ 7.16 (2H, s, SO_2NH_2), 7.21-7.28 (1H, dd, *J* = 7.4, 7.5 Hz, H-3''), 7.30-7.36 (1H, dd, *J* = 7.5, 7.6 Hz, H-7''), 7.38-7.43 (1H, d, *J* = 7.5 Hz, H-9''), 7.48-7.53 (1H, dd, *J* = 7.5, 7.6 Hz, H-8''), 7.58-7.63 (1H, d, *J* = 7.5 Hz, H-6''), 7.73 (2H, d, *J* = 8.5 Hz, H-2' and H-6'), 7.77-7.80 (1H, d, *J* = 7.4 Hz, H-2''), 7.88-7.91 (1H, d, *J* = 7.5 Hz, H-4''), 8.06 (2H, d, *J* = 8.5 Hz, H-3' and H-5'), 8.34 (1H, s, H-8); LRMS (ES^+) *m/z* 505.2 $[M+H]^+$; HRMS calcd for $C_{23}H_{16}N_6O_2S_3$ $[M+H]^+$ 505.0570, found 505.0573.

7-Chloro-3-(4-methoxybenzyl)-3*H*-imidazo[4,5-*b*]pyridin-5-amine (78). To a stirred solution of **77** (0.04 g, 0.24 mmol) in anhydrous DMF (1 mL) was added anhydrous K_2CO_3 (36 mg, 0.26

mmol) and 4-methoxybenzyl chloride (34 μL, 0.25 mmol). The reaction mixture was stirred under N_2 at 65 ^oC for 18 h, and the solvent was removed under reduced pressure. The residual solid was redissolved in EtOAc (30 mL), washed with brine (3 × 30 mL), and the combined organic fractions were dried (Na_2SO_4) and the solvent was removed under reduced pressure. Purification by chromatography (silica; 40% EtOAc:petrol) afforded **78** as a light yellow solid (29 mg, 42 %); R_f 0.21 (40% EtOAc:petrol); mp 105 ^oC; UV λ_{max} (EtOH) 311 nm; IR (cm^{-1}) 3431, 3312, 3198, 3022, 2940, 2798, 1571, 1513; ^1H NMR (500 MHz, DMSO-d_6) δ 3.71 (3H, s, OCH_3), 5.22 (2H, s, CH_2), 6.24 (2H, br s, NH_2), 6.48 (1H, s, H-5), 6.88 (2H, d, J = 8.7 Hz, H-2" and H-6"), 7.23 (2H, d, J = 8.8 Hz, H-3" and H-5"), 8.07 (1H, s, H-2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 45.4 (Ar-CH_2-Ar), 55.1 (OCH_3), 103.8 (Ar-C), 114.0 (Ar-C), 124.4 (Ar-C), 128.7 (Ar-C), 129.2 (Ar-C), 133.9 (Ar-C), 140.5 (Ar-C), 146.6 (Ar-C), 157.4 (Ar-C), 158.7 (Ar-C); HRMS calcd for $C_{14}H_{14}ClN_4O$ $[M+H]^+$ 289.0851, found 289.0855.

7-(Cyclohexylmethoxy)-3-(4-methoxybenzyl)-3*H*-imidazo[4,5-*b*]pyridin-5-amine (79). Synthesised following **general method C** from **78** (0.172 g, 0.60 mmol). The product was isolated by chromatography (silica; 80% EtOAc:petrol) to furnish **79** as a colourless oil (0.16 g, 0.45 mmol, 75%); R_f 0.47 (20% EtOAc:Petrol); UV λ_{max} (EtOH) 271 nm; IR (cm^{-1}) 3311, 2954, 2899, 2163, 1672, 1598, 1516; ^1H NMR (500 MHz, DMSO-d_6) δ 0.90-1.87 (11H, m, cyclohexyl), 3.71 (3H, s, OCH_3), 4.00 (2H, d, J = 6.7 Hz, OCH_2), 5.17 (2H, br s, NH_2), 5.24 (2H, s, CH_2), 5.90 (1H, s, H-5), 6.87 (2H, d, J = 8.7 Hz, H-2" and H-6"), 7.20 (2H, d, J = 8.8 Hz, H-3" and H-5"), 7.82 (1H, s, H-2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.2 (CH_2), 26.0 (CH_2), 29.1 (CH_2), 37.0 (CH), 45.0 (Ar-CH_2), 55.1 (CH_3), 73.3 (CH_2), 87.5 (Ar-C), 113.9 (Ar-C), 118.2 (Ar-C), 128.7 (Ar-C), 129.7 (Ar-C), 137.9 (Ar-C), 147.4 (Ar-C), 157.8 (Ar-C), 158.5 (Ar-C), 158.6 (Ar-C); HRMS calcd for $C_{21}H_{27}N_4O_2$ $[M+H]^+$ 367.2129, found 367.2132.

7-(Cyclohexylmethoxy)-3*H*-imidazo[4,5-*b*]pyridin-5-amine (80). A solution of **79** (0.035 g, 0.10 mmol) in TFA (1 mL) was heated under reflux for 2 h, and the cooled solution was neutralised with saturated aqueous $NaHCO_3$ (25 mL). The reaction mixture was extracted with EtOAc (3 × 30 mL),

and the combined organic fraction was dried ($MgSO_4$) and evaporated under reduced pressure. Purification by chromatography (silica; 10% MeOH:DCM) to furnish **80** as a white solid (16.5 mg, 70%); mp 99-102 ^oC; UV λ_{max} (EtOH) 293, 244 nm; IR (cm^{-1}) 3447, 3323, 3187, 2923, 2850, 2795, 2026, 1591, 1527; ^1H NMR (500 MHz, $CDCl_3$) δ 1.03-2.00 (11H, m, cyclohexyl), 4.03 (2H, d, J = 6.1 Hz, OCH_2), 4.37 (2H, br s, NH_2), 5.91 (1H, s, H-5), 7.77 (1H, s, H-2), 9.89 (1H, br s, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.7, 26.4, 29.8, 37.5, 74.4, 88.1, 157.3; HRMS calcd for $C_{13}H_{19}N_4O$ $[M+H]^+$ 247.1553, found 247.1556.

7-(Cyclohexylmethoxy)-5-iodo-3-(4-methoxybenzyl)-3*H*-imidazo[4,5-*b*]pyridine (81). A solution of **79** (0.112 g, 0.31 mmol) in THF (4 mL) containing diiodomethane (123 µL, 1.50 mmol), CuI (59 mg, 0.31 mmol), and isoamyl nitrite (187 µL, 0.92 mmol) was heated to reflux for 4 h, and the dark brown solution was filtered (Celite) and extracted with EtOAc (2 × 20 mL). The combined organic fraction was washed sequentially with aqueous sodium thiosulfate solution (10%, 2 × 40 mL) and brine (40 mL), dried ($MgSO_4$), and the solvent was removed *in vacuo*. Purification by chromatography (silica; 30% EtOAc:petrol) afforded **81** as a yellow oil (64 mg, 44%); R_f 0.19 (30% EtOAc:petrol); UV λ_{max} (EtOH) 262 nm; IR (cm^{-1}) 3333, 2951, 2891, 2122, 1672, 1584, 1521; ^1H NMR (500 MHz, DMSO-d_6) δ 0.98-1.89 (11H, m, cyclohexyl), 3.71 (3H, s, OCH_3), 4.19 (2H, d, J = 6.5 Hz, OCH_2), 5.17 (2H, s, CH_2), 6.90 (2H, d, J = 8.7 Hz, H-2" and H-6"), 7.18 (1H, s, H-5), 7.26 (2H, d, J = 8.8 Hz, H-3" and H-5"), 8.31 (1H, s, H-2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.1 (CH_2), 25.9 (CH_2), 28.9 (CH_2), 37.0 (CH), 45.8 (Ar-CH_2), 55.1 (CH_3), 74.4 (CH_2), 112.0 (Ar-C), 112.7 (Ar-C), 114.1 (Ar-C), 124.6 (Ar-C), 128.8 (Ar-C), 129.0 (Ar-C), 142.6 (Ar-C), 148.5 (Ar-C), 157.0 (Ar-C), 158.8 (Ar-C); HRMS calcd for $C_{21}H_{25}IN_3O_2$ $[M+H]^+$ 478.0986, found 478.0980.

4-((7-(Cyclohexylmethoxy)-3-(4-methoxybenzyl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl) amino)benzenesulfonamide (82). To a solution of **81** (0.048 g, 0.10 mmol) in anhydrous MeCN (2 mL) was added sulfanilamide (19 mg, 0.11 mmol), anhydrous K_2CO_3 (28 mg, 0.20 mmol), $Pd_2(dba)_3$ (2.3 mg, 0.004 mmol) and XPhos (1.9 mg, 0.004 mmol). The reaction mixture was degassed under N_2 for 30 min, and stirred at 80 ^oC for 2 h. The cooled solution was filtered (Celite)

extracted with EtOAc (2 × 20 mL), and the combined organic extract was washed sequentially with aqueous HCl (1 M, 20 mL), saturated aqeuous $NaHCO_3$ (20 mL), and brine (30 mL), dried (Na_2SO_4), and the volatiles were evaporated *in vacuo*. Purification by chromatography (silica; 70% EtOAc:petrol) yielded **82** as a white solid (23 mg, 43%); R_f 0.59 (70% EtOAc:petrol); mp 142 ^oC; UV λ_{max} (EtOH) 257 nm; IR (cm^{-1}) 3329, 2933, 2874, 2158, 1662, 1588, 1501; ^1H NMR (500 MHz, $CDCl_3$) δ 1.00-1.97 (11H, m, cyclohexyl), 3.79 (3H, s, OCH_3), 4.01 (2H, d, J = 6.1 Hz, OCH_2), 4.76 (2H, br s, NH_2), 5.28 (2H, s, CH_2), 6.17 (1H, s, H-5), 6.79 (1H, br s, NH), 6.88 (2H, d, J = 8.8 Hz, H-2" and H-6"), 7.24 (2H, d, J = 8.7 Hz, H-3" and H-5"), 7.61 (2H, J = 8.8 Hz, H-2' and H-6'), 7.81 (2H, J = 8.8 Hz, H-3' and H-5'), 8.11 (1H, s, H-2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.7 (CH_2), 26.4 (CH_2), 29.8 (CH_2), 37.6 (CH_2), 46.9 (CH), 55.4 (CH_2), 74.8 (OCH_3), 90.7 (OCH_2), 114.3 (Ar-C), 117.1 (Ar-C), 128.0 (Ar-C), 129.1 (Ar-C), 139.7 (Ar-C), 145.5 (Ar-C), 152.1 (Ar-C); HRMS calcd for $C_{27}H_{32}N_5O_4S$ $[M+H]^+$ 522.2170, found 522.2166.

4-((7-(Cyclohexylmethoxy)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)amino) benzenesulfonamide (83). A solution of **82** (0.022 g, 0.042 mmol) in TFA (1 mL) was heated under reflux for 2 h, and the cooled solution was neutralised with saturated aqueous $NaHCO_3$ (25 mL). The reaction mixture was extracted with EtOAc (3 × 30 mL), and the combined organic fraction was dried ($MgSO_4$) and evaporated under reduced pressure. The title compound was purified by preparative TLC on silica, using 5% MeOH:DCM as eluent, to afford **83** as a white solid (16.7 mg, 99%); R_f 0.25 (5% MeOH:DCM); mp 165-166 ^oC; UV λ_{max} (EtOH) 324, 274 nm; IR (cm^{-1}) 3332, 2925, 2852, 2164, 1677, 1583, 1500; ^1H NMR (500 MHz, MeOD-d_4) δ 1.07-2.05 (11H, m, cyclohexyl), 4.03 (2H, d, J = 6.2 Hz, OCH_2), 6.33 (1H, s, H-5), 7.77 (2H, d, J = 8.9 Hz, H-2' and H-6'), 7.92 (2H, d, J = 8.8 Hz, H-3' and H-5'), 7.96 (1H, s, H-2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.9 (CH_2), 27.6 (CH_2), 30.8 (CH_2), 38.9 (CH), 74.9 (CH_2O), 90.9 (Ar-C), 117.9 (Ar-C), 128.2 (Ar-C); HRMS calcd for $C_{19}H_{24}N_5O_3S$ $[M+H]^+$ 522.2170, found 522.2166; HPLC: >98%, XSELECT column, retention time 5.47 min, reverse phase, H_2O/MeCN/ HCOOH gradient run, 1 mL min^{-1}.

7-(Cyclohexylmethoxy)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-5-amine (86). Prepared in accordance with **general procedure C** from **84** (1.50 g, 8.82 mmol). Precipitation from aqueous AcOH, and repeated washing with water, gave **86** a pale orange solid (1.52 g, 70%); mp 214-216 ^{o}C; UV λ_{max} (EtOH) 285 nm; IR (cm^{-1}) 3494, 3378, 2925, 2780, 2651, 2362, 2329, 2115, 2085, 2013, 1906, 1628, 1608, 1578, 1488; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.03-1.89 (11H, m, cyclohexyl), 4.30 (2H, d, J = 6.5 Hz, OCH_2), 6.91 (2H, br s, NH_2), 15.28 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.1 (CH_2), 25.9 (CH_2), 29.1 (CH_2), 36.6 (CH), 71.2 (CH_2), 162.2 (Ar-C); HRMS calcd for $C_{11}H_{17}N_6O$ $[M+H]^+$ 249.1458, found 249.1461.

4-(Cyclohexylmethoxy)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (87). Prepared according to the **general procedure C** from **85** (0.125 g, 0.74 mmol). Purification by chromatography (silica; 10% MeOH:DCM) gave **87** as an off white crystalline solid (68 mg, 37%); R_f 0.70 (10% MeOH:DCM), mp 193-195 ^{o}C; UV λ_{max} (EtOH) 275 nm; IR (cm^{-1}) 3498, 3353, 3232, 3121, 2960, 2921, 2851, 2750, 2667, 2158, 2038, 1902, 1794, 1633, 1578, 1497 ,1469, 1441; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.98-1.82 (11H, m, cyclohexyl), 4.21 (2H, d, J = 6.5 Hz, OCH_2), 6.55 (2H, br s, NH_2), 7.77 (1H, s, CH), 12.78 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.2 (CH_2), 26.0 (CH_2), 29.1 (CH_2), 36.7 (CH), 70.5 (CH_2), 95.4 (Ar-C), 131.6 (Ar-C), 158.9 (Ar-C), 162.0 (Ar-C), 163.4 (Ar-C); HRMS calcd for $C_{12}H_{18}N_5O$ $[M+H]^+$ 248.1506, found 248.1508.

5-Chloro-7-(cyclohexylmethoxy)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidine (88). To a solution of LiCl (34 mg, 0.80 mmol) in DMA (0.5 mL) at 0 ^{o}C, was added **86** (0.05 g, 0.20 mmol), followed by isoamyl nitrite (61 µL, 0.30 mmol) and $SOCl_2$ (16 µL, 0.22 mmol). The reaction mixture was stirred for 1 h, and allowed to warm to ambient temperature over 18 h. After addition of saturated aqueous $NaHCO_3$ (15 mL), the reaction mixture was extracted with EtOAc (3 × 15 mL), and the combined organic layers were washed with brine (2 × 30 mL), dried (Na_2SO_4). Evaporation of the solvent under reduced pressure furnished **88** as a brown oil (54 mg, >99 %) which was used directly without further purification.

6-Chloro-4-(cyclohexylmethoxy)-1*H*-pyrazolo[3,4-*d*]pyrimidine (89). To a solution of LiCl (69 mg,1.62 mmol) in DMA (1 mL) at 0 ^{o}C was added **87** (0.100 g, 0.40 mmol), followed by isoamyl nitrite (123 µL, 0.61 mmol) and $SOCl_2$ (32 µL, 0.45mmol). The reaction mixture was stirred at room temperature for 18 h, cooled, and EtOAc (45 mL) was added. The resultant solution was washed sequentially with aqueous saturated $NaHCO_3$ solution (45 mL) and brine (2 × 45 mL), dried (Na_2SO_4) and the volatiles were evaporated under reduced pressure. The product was purified by chromatography (silica; 5% MeOH:DCM) to give **89** as a yellow solid (65 mg, 60%); R_f 0.32 (5% MeOH:DCM), mp 152-153 ^{o}C; UV λ_{max} (EtOH) 252 nm; IR (cm^{-1}) 3148, 2922, 2851, 1677, 1579, 1443; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.96-1.95 (11H, m, cyclohexyl), 4.39 (2H, d, J = 6.0 Hz, OCH_2), 8.09 (1H, s, C*H*), 11.19 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.2 (CH_2), 26.0 (CH_2), 29.1 (CH_2), 36.7 (CH), 70.5 (CH_2O), 95.4 (Ar-C), 131.6 (C^1-H), 158.9 (Ar-C), 162.0 (Ar-C), 163.4 (Ar-C); HRMS calcd for $C_{12}H_{16}ClN_4O$ $[M+H]^+$ 267.1007, found 267.1012.

4-((7-(Cyclohexylmethoxy)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-5-yl)amino) benzenesulfonamide (90). The title compound was prepared following **general procedure A** from **88** (0.054 g, 0.20 mmol) and isolated by chromatography (silica; 30% EtOAc:petrol) to give the title compound **90** as a white solid (28 mg, 35%); R_f 0.18 (70% EtOAc:petrol); mp 217-219 ^{o}C; UV λ_{max} (EtOH) 310 nm; IR (cm^{-1}) 3254, 3121, 2924, 2851, 2363, 2342, 2199, 2140, 1992, 19118, 1700, 1582, 1534, 1492, 1447; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.04-1.92 (11H, m, cyclohexyl), 4.36 (2H, d, J = 6.8 Hz, OCH_2), 7.18 (2H, br s, SO_2NH_2), 7.72 (2H, d, J = 8.8 Hz, H-2' and H-6'), 7.99 (2H, d, J = 9.0 Hz, H-3' and H-5'), 9.92 (1H, br s, N*H*) 13.34 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.1 (CH_2), 29.1 (CH_2), 35.9 (CH), 118.0 (Ar-C), 126.4 (Ar-C); HRMS calcd for $C_{17}H_{22}N_7O_3S$ $[M+H]^+$ 404.1499, found 404.1500; HPLC: >99.5%, XTerra column, retention time 11.64 min, reverse phase, H_2O/MeCN/ HCOOH gradient run, 1 mL min^{-1}.

4-((4-(Cyclohexylmethoxy)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)amino)benzenesulfonamide (91). The title compound was prepared by **general procedure A** from **89** (0.042 g, 0.18 mmol). Chromatography (silica; 70% EtOAc:petrol) afforded **91** as a white solid (20 mg, 28%); R_f 0.21

(70% EtOAc:petrol), mp 268-269 °C; UV λ_{max} (EtOH) 304 nm; IR (cm^{-1}) 3367, 3333, 3269, 2922, 2853, 2162, 2012, 1691, 1658, 1624, 1583, 1551, 1524; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.04-1.92 (11H, m, cyclohexyl), 4.36 (2H, d, J = 6.8 Hz, OCH_2), 7.18 (2H, br s, SO_2NH_2), 7.72 (2H, d, J = 8.8 Hz, H-2' and H-6'), 7.98 (1H, br s, CH), 7.99 (2H, d, J = 9.0 Hz, H-3' and H-5'), 9.92 (1H, br s, NH) 13.34 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.4 (CH_2), 26.5 (CH_2), 29.5 (CH_2), 37.1 (CH), 71.5 (CH_2) 118.6 (Ar-C), 136.6 (Ar-C), 144.0 (Ar-C); HRMS calcd for $C_{18}H_{23}N_6O_3S$ $[M+H]^+$ 403.1547, found 403.1547; HPLC: >98.5%, XTerra column, retention time 11.92 min, reverse phase, H_2O/MeCN/ HCOOH gradient run, 1 mL min^{-1}.

4-(Cyclohexylmethoxy)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (93). Prepared in accordance with **general method C** from **92** (0.15 g, 0.89 mmol), with heating under microwave irradiation (170 °C, 5 h). The product was isolated by chromatography (silica; 10% MeOH:DCM) to yield **93** as a white crystalline solid (37 mg, 17%); R_f 0.55 (10% MeOH:DCM); mp 216.3-217.3 °C; UV λ_{max} (EtOH) 221 nm; IR (cm^{-1}) 3494, 3362, 3117, 2922, 2851, 2164, 2045, 1968, 1617, 1585, 1499, 1478; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.98-1.83 (11H, m, cyclohexyl), 4.15 (2H, d, J = 6.5, OCH_2), 5.91 (2H, br s, NH_2), 6.17 (1H, dd, J = 1.9 and 3.5, CH), 6.79 (1H, dd, J = 2.2 and 3.5, HNCH), 10.97 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.2 (CH_2), 26.0 (CH_2), 29.3 (CH_2), 36.8 (CH), 69.9 (CH_2O), 96.9 (C^1-H), 97.9 (Ar-C), 119.1 (C^2-H), 155.0 (Ar-C), 159.3 (Ar-C), 162.7 (Ar-C); HRMS calcd for $C_{13}H_{19}N_4O$ $[M+H]^+$ 247.1553, found 247.1556.

4-Chloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (94). NaH (60% in mineral oil, 26 mg, 0.65 mmol) was added dropwise to a solution of **92** (0.10 g, 0.59 mmol) in anhydrous MeCN (3 mL) at -30 °C, and the reaction mixture was stirred for 45 min. 2-(Trimethylsilyl)ethoxymethyl chloride (90%, 122 µL, 0.62 mmol) was added dropwise, and the reaction mixture was stirred for a further 3 h at room temperature. After cooling and addition of water (20 mL), the resultant solution was extracted with EtOAc (20 mL), and the combined organic fractions were washed with brine (3 × 20 mL), dried (Na_2SO_4), and the solvent was removed *in vacuo*. Purification by chromatography (silica; 15% EtOAc:petrol) gave **94** as a yellow solid (130

mg, 74%); R_f 0.41 (15% EtOAc:petrol); mp 63-64 ^oC; UV λ_{max} (EtOH) 317, 234 nm; IR (cm^{-1}) 3423, 3318, 3212, 3096, 2952, 1631, 1608, 1546, 1493; ^1H NMR (500 MHz, $CDCl_3$) δ -0.04 (9H, s, $(CH_3)_3$), 0.91 (2H, t, J = 8.0 Hz, OCH_2CH_2Si), 3.81 (2H, t, J = 8.3 Hz, OCH_2CH_2Si), 4.96 (2H, br s, NH_2), 5.44 (2H, s, CH_2), 6.43 (1H, d, J = 3.7 Hz, CH), 6.99 (1H, d, J = 3.7 Hz, NCH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 0.0 ($Si(CH_3)_3$), 19.2 (CH_2CH_2Si), 67.7 (OCH_2CH_2), 74.3 (NCH_2O), 102.2 (C^5H), 112.2 (Ar-C), 127.0 (C^6H), 154.2 (Ar-C), 155.6 (Ar-C), 160.2 (Ar-C); HRMS calcd for $C_{12}H_{20}ClN_4OSi$ $[M+H]^+$ 299.1089, found 299.1090.

4-(Cyclohexylmethoxy)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (95). Synthesised following **general procedure C** from **94** (0.30 g, 1.01 mmol), except using THF as solvent. The title compound was isolated by chromatography (silica; 15% EtOAc:petrol) to furnish **95** as a yellow solid (296 mg, 78%); R_f 0.17 (15% EtOAc:petrol); mp 88-90 ^oC; UV λ_{max} (EtOH) 286, 260, 223 nm; IR (cm^{-1}) 3346, 3222, 2926, 2852, 1647, 1602; ^1H NMR (500 MHz, $CDCl_3$) δ -0.05 (9H, s, $(CH_3)_3$), 0.91 (2H, t, J = 8.3 Hz, OCH_2CH_2Si), 1.01-1.91 (11H, m cyclohexyl), 3.51 (2H, t, J = 8.3 Hz, OCH_2CH_2Si), 4.21 (2H, d, J = 6.5 Hz, OCH_2), 4.68 (2H, br s, NH_2), 5.41 (2H, s, CH_2), 6.40 (1H, d, J = 3.7 Hz, CH), 6.82 (1H, d, J = 3.7 Hz, NCH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 0.0 ($Si(CH_3)_3$), 19.2 (CH_2CH_2Si), 27.2 (CH_2), 28.0 (CH_2), 31.3 (CH_2), 38.8 (CH), 67.4 (OCH_2CH_2), 72.6 (CH_2), 74.1 (NCH_2O), 101.2 (C^5H), 123.6 (C^6H), 160.6 (Ar-C), 165.4 (Ar-C); HRMS calcd for $C_{19}H_{33}N_4O_2Si$ $[M+H]^+$ 377.2367, found 377.2367.

2-Chloro-4-(cyclohexylmethoxy)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (96). To a solution of LiCl in THF (0.5 M, 2.13 mL, 1.1 mmol) was added **95** (0.10 g, 0.27 mmol) and the reaction mixture was cooled to 0 ^oC. Isoamyl nitrite (81 μL, 0.40 mmol) and $SOCl_2$ (21.3 μL, 0.29 mmol) were added, and the solution was stirred at room temperature for 5 h. The reaction mixture was added to a solution of aqueous NH_4Cl (2 M, 30 mL), extracted with EtOAc (2 × 30 mL), and the combined organic layers were washed with water (3 × 30 mL), dried (Na_2SO_4), and evaporated *in vacuo*. Purification by chromatography (silica; 15% EtOAc:petrol) gave **96** as a clear oil (60 mg, 56%); R_f 0.45 (15% EtOAc:Petrol); UV λ_{max} (EtOH) 270 nm; IR (cm^-

^{1}) 2924, 2852, 1750, 1661, 1588, 1558, 1506; ^{1}H NMR (500 MHz, DMSO-d_6) δ -0.09 (9H, s, $(CH_3)_3$), 0.82 (2H, t, J = 8.1 Hz, OCH_2CH_2Si), 0.95-1.89 (11H, m cyclohexyl), 3.50 (2H, t, J = 8.1 Hz, OCH_2CH_2Si), 4.28 (2H, d, J = 6.0 Hz, OCH_2), 5.52 (2H, s, CH_2), 6.61 (1H, d, J = 3.6 Hz, C*H*), 7.55 (1H, d, J = 3.5 Hz, NC*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 0.0 ($Si(CH_3)_3$), 18.5 (CH_2CH_2Si), 26.6 (CH_2), 30.5 (CH_2) 31.8 (CH_2), 38.8 (CH), 67.1 (OCH_2CH_2), 73.3 (CH_2), 74.2 (NCH_2O), 100.4 (C^5H), 126.4 (C^6H), 129.7 (Ar-C); HRMS calcd for $C_{19}H_{31}ClN_3O_2Si$ $[M+H]^+$ 396.1869, found 396.1872.

4-((4-(Cyclohexylmethoxy)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)amino)benzenesulfonamide (97). To a degassed solution of **96** (0.050 g, 0.13 mmol) in MeCN (2 mL) was added sulfanilamide (24 mg, 0.14 mmol), anhydrous K_2CO_3 (35 mg, 0.25 mmol), $Pd_2(dba)_3$ (3 mg, 5.0 x 10^{-3} mmol) and XPhos (2.4 mg, 5.0 x 10^{-3} mmol), and the reaction mixture was stirred under N_2 at 80 ^{o}C for 2 h. The cooled mixture was filtered (Celite), diluted with EtOAc (20 mL), and washed sequentially with aqueous HCl solution (1 M, 20 mL), saturated aqueous $NaHCO_3$ solution (20 mL), and brine (30 mL). After drying (Na_2SO_4), volatiles were removed under reduced pressure, and the product was isolated by chromatography (silica; 50% EtOAc:petrol) to give **97** as a yellow oil (33 mg, 49%); R_f 0.63 (50% EtOAc:petrol); UV λ_{max} (EtOH) 269 nm; IR (cm^{-1}) 3644, 3342, 2924, 2853, 1586, 1528, 1488; ^{1}H NMR (500 MHz,$CDCl_3$) δ -0.08 (9H, s, $Si(CH_3)_3$), 0.88-0.95 (2H, t, J = 8.36 Hz, OCH_2CH_2Si) 1.04-1.93 (11H, m, cyclohexyl), 3.56 (2H, t, J = 3.4 Hz, OCH_2CH_2Si) 4.29 (2H, d, J = 6.5 Hz, OCH_2), 4.72 (2H, br s, NH_2), 5.52 (2H, s, CH_2), 6.49 (1H, d, J = 3.5 Hz, C*H*), 6.96 (1H, d, J = 3.6 Hz, NC*H*), 7.20 (1H, br s, NH), 7.87 (4H, ap s, H-2', H-3', H-5' and H-6'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 0.0 ($SiCH_3$), 15.6 (CH_2Si), 27.2 (CH_2), 27.9 (CH_2), 31.3 (CH_2), 38.8 (CH), 67.8 (CH_2), 73.2 (CH_2), 74.5 (CH_2), 101.5 (Ar-C), 101.9 (Ar-C), 118.8 (Ar-C), 125.0 (Ar-C), 129.3 (Ar-C), 146.2 (Ar-C); HRMS calcd for $C_{25}H_{38}N_5O_4SSi$ $[M+H]^+$ 532.2408, found 532.2406. Note: not all carbons are visible.

4-((4-(Cyclohexylmethoxy)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)amino)benzenesulfonamide (98). A solution of **97** (0.040 g, 7.5 x 10^{-2} mmol) in TFA (1.5 mL) was heated under reflux for 10 min.,

and the cooled solution was neutralised with saturated aqueous $NaHCO_3$ solution. The aqueous solution was extracted with EtOAc (2 × 30 mL), and the solvent was evaporated under reduced pressure. The residual yellow solid was redissolved in MeCN:H_2O (1:1, 8 mL), the pH of the solution was adjusted to 10 with dilute aqueous ammonia (28%), and the solution was stirred for 1 h at room temperature and evaporated *in vacuo*. Isolation by chromatography (silica; 70% EtOAc:petrol) to give **98** as a white solid (12 mg, 40%); R_f 0.39 (70% EtOAc:petrol); mp 251.2-252.0 ^oC; UV λ_{max} (EtOH) 312 nm; IR (cm^{-1}) 3374, 3322, 2058, 2924, 2856, 2012, 1615, 1595, 1579; ^1H NMR (500 MHz, DMSO-d_6) δ 1.07-1.95 (11H, m, cyclohexyl), 4.31 (2H, d, J = 6.3 Hz, OCH_2), 6.34 (1H, dd, J = 2.1 and 2.1 Hz, C*H*), 7.05 (1H, dd, J = 2.2 and 2.2 Hz, HNC*H*), 7.13 (2H, br s, SO_2NH_2), 7.68 (2H, d, J = 8.9 Hz, H-2' and H-6') 7.99 (2H, d, J = 8.8 Hz, H-3' and H-5'), 9.57 (1H, br s, N*H*), 11.54 (1H, br s, N*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.2 (CH_2), 26.0 (CH_2), 29.3 (CH_2), 36.8 (CH), 70.6 (CH_2), 98.2 (C^5H), 116.9 (Ar-C), 126.4 (C^6H), 134.9 (Ar-C), 144.6 (Ar-C), 146.4 (Ar-C), 153.6 (Ar-C), 154.4 (Ar-C), 162.4 (Ar-C); HRMS calcd for $C_{19}H_{24}N_5O_3S$ $[M+H]^+$ 402.1594, found 402.1596; HPLC: >99.5%, XSELECT column, retention time 8.45 min, reverse phase, H_2O/MeCN/ HCOOH gradient run, 1 mL min^{-1}.

7-Chloro-5-cyclohexylmethoxyimidazo[1,2-*a*]pyrimidine (100). Synthesised in accordance with **general procedure C** from **99 (**1.0 g, 5.4 mmol). The product was purified by precipitation with glacial acetic acid, and repeated washing with water, to afford **100** as a pale orange powder (1.16 g, 81%); R_f 0.23 (60% EtOAc:petrol); ^1H NMR (300 MHz, DMSO-d_6) δ 1.05-1.95 (11H, m, cyclohexyl), 4.30 (2H, d, J = 6.1 Hz, OCH_2), 6.63 (1H, s, H-6), 7.63 (2H, d, J = 1.2 Hz, H-3), 7.81 (2H, d, J = 1.3 Hz, H-2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 25.4, 26.2, 29.0, 36.9, 76.3, 88.8, 107.6, 134.2, 148.1, 152.1, 155.6; LRMS (ES^+) *m/z* 266.0 $[M+H]^+$.

5-Cyclohexylmethoxy-7-anilinoimidazo[1,2-*a*]pyrimidine (101). The title compound was synthesised according to **general procedure A** from **100** (0.075 mg, 0.28 mmol). Purification by chromatography (silica; EtOAc), followed by crystallisation from MeOH, gave **101** (31 mg, 34%); R_f 0.11 (100% EtOAc); mp 252-253 °C; UV λ_{max} (EtOH) 305, 257, 212 nm; IR (cm^{-1}) 3153, 2926,

2847, 2161, 1627, 1595, 1568, 1531, 1489, 1291, 1259, 1122; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.05–1.85 (11H, m, cyclohexyl), 4.35 (2H, d, J = 6.2 Hz, OCH_2), 5.98 (1H, s, H-6), 6.99 (1H, t, J = 7.3 Hz, Ph*H*), 7.26 (1H, d, J = 1.6 Hz, H-3), 7.33 (2H, dd, J = 7.6, 8.2 Hz, 2 × Ph*H*), 7.44 (1H, d, J = 1.6 Hz, H-2), 7.83 (2H, d, J = 7.7 Hz, 2 × Ph*H*), 9.45 (1H, s, N*H*); LRMS (ES$^+$) *m/z* 323.0 [M+H]$^+$; Anal. Found: C, 70.41; H, 6.59; N, 17.40. $C_{19}H_{22}N_4O$ requires C, 70.78; H, 6.88; N, 17.38%.

5-Cyclohexylmethoxy-7-(4'-sulfamoylanilino)imidazo[1,2-*a*]pyrimidine (102). Prepared in accordance with **general procedure A** from **100** (0.25 g, 0.94 mmol), and purified by chromatography (silica; 5% MeOH:EtOAc) to give **102** as a colourless solid (28 mg, 7%); R_f 0.47 (10% MeOH:EtOAc); mp 228-229 °C; IR (cm^{-1}) 3348, 3292, 2926, 2847, 1653, 1617, 1591, 1536, 1429, 1310, 1155; UV λ_{max} (EtOH) 315, 266, 216 nm; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15-1.90 (11H, m, cyclohexyl), 4.16 (2H, d, J = 6.1 Hz, OCH_2), 6.03 (1H, s, H-6), 7.24 (2H, s, SO_2NH_2), 7.32 (1H, d, J = 1.6 Hz, H-3), 7.51 (1H, d, J = 1.6 Hz, H-2), 7.78 (2H, d, J = 8.9 Hz, H-2' and H-6'), 7.99 (2H, d, J = 8.9 Hz, H-3' and H-5'), 9.85 (1H, s, N*H*). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 25.45, 26.19, 29.16, 36.85, 75.00, 78.91, 105.83, 118.42, 127.05, 131.95, 137.03, 144.01, 154.73, 155.68. LRMS (ES$^+$) *m/z* 402.0 [M+H]$^+$; Anal. Found: C, 57.06; H, 5.53; N, 17.09. $C_{19}H_{23}N_5O_3S$ requires C, 56.84; H, 5.77; N, 17.44%.

Protein kinase assays

Human CDK1/B was purchased from New England Biolabs or prepared as described, [46] CDK2/A2 was prepared as described, [46] CDK4/D was provided by AstraZeneca and CDK7/H and CDK9/T were purchased from Upstate. IC_{50} values for CDK1/B, CDK2/A and CDK4/D were determined as described. [23] IC_{50} values for CDK7/H and CDK9/H were determined according to the Supplier's instructions (Upstate). CDK1/B, CDK2/A and CDK4/D were assayed at an ATP concentration of 12.5 μM, CDK7/H and CDK9/T at an ATP concentration of 100 μM. Published K_m(ATP) values for CDK1-cyclin B, CDK2-cyclin A, CDK4-cyclin D2, CDK7-cyclin H-MAT and CDK9-cyclin T are 0.8 μM, 0.58 μM, 3.8 μM, 4.1 μM and 0.7 μM respectively (www.proqinase.com).

Growth inhibition assays

The ability of selected compounds to inhibit cell growth was assessed in a panel of human cancer cell lines using an SRB assay. [45] Briefly, cells in 96-well plates were exposed to inhibitor (0.1-30 μM, 3 replicates per drug concentration) or 0.5 % (v/v) DMSO (for compound **73** treatments) or 0.1% (v/v) DMSO (for all other inhibitors) for 120 h, stained with sulforhodamine B and the absorbance at 570 nm measured (SpectraMax®250; Molecular Devices, Wokingham, UK). At least three independent experiemnts were performed. Growth inhibitory GI_{50} values were calculated using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA).

Crystallisation and structure determination

Crystals of CDK2-cyclin A bound to **73** and CDK1–cyclin B-CKS2 bound to **3** were grown as previously described. [46] Briefly, the inhibitors were added in twofold molar excess (which was also a 10-fold higher concentration than the inhibitor IC_{50}) to a low concentration solution of the appropriate protein complex and then the samples were concentrated to 10–12 mg ml^{-1} for crystallization. CDK2-cyclin A-**73** crystals were grown from a well solution containing 0.6–0.8 M KCl, 0.9–1.2 M $(NH_4)_2SO_4$, and 100 mM HEPES (pH 7.0). CDK1-cyclin B-CKS2 was co-crystallised with **3** from a screen covering the conditions 0.1 M MES/imidazole buffer (pH 6.7),

6.5% MPD, 5% PEG4K, 10% PEG1K. Before data collection, crystals were cryoprotected by brief immersion in either 8 M sodium formate (CDK2-cyclin A) or 25% ethyleneglycol (CDK1-cyclin B-CKS2).

Data processing was carried out using XDS, MOSFLM, POINTLESS/AIMLESS [52] and other programs of the CCP4 suite, [53] run through the CCP4i2 GUI. [54] The structures of the different complexes were solved by molecular replacement using Phaser, [55] and search models drawn from PDB entries 1QMZ (cyclin A-bound CDK2) and 4Y72 (CDK1-cyclin B-CKS2). Models of **73** and **3** and ligand restraints were generated using ACEDRG within the CCP4i2 suite, [54] and built into the electron density using Coot. [56] Structures were refined using REFMAC, [57] interspersed with manual rebuilding in Coot, including TLS refinement.

Accession Codes

CDK2-cyclin A in complex with compound 73 (5LQE), and CDK1-cyclin B-CKS2 in complex with compound 3 (5LQF) have been deposited with the PDB and the atomic coordinates and experimental data will be released upon article publication.

Associated Content

Supporting Information

Structure of compound **102** and associated crystal data and refinement statistics

^{1}H NMR Spectra for Compounds **70**, **73**, **83**, **90**, **91** and **98**.

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Abbreviations

CDK, cyclin-dependent kinase

CKS, cyclin-dependent kinase subunit

GI_{50}, half-maximum growth inhibition

HEPES, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid

MES, 2-morpholin-4-ylethanesulfonic acid

MPD, 2-Methyl-2,4-pentanediol

SRB, sulforhodamine B

TFE, 2,2,2-trifluoroethanol

TLS, translation, libration and screw-rotation

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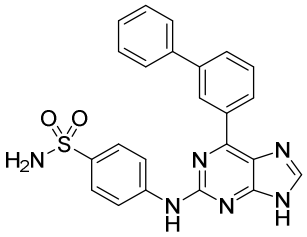
TOC Graphic



CDK2/A IC_{50} = 0.044 μM
CDK1/B IC_{50} = 86 μM