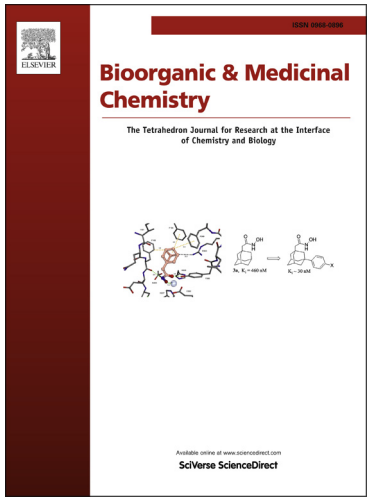

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Synthesis and Evaluation of Heteroaryl Substituted Diazaspirocycles as Scaffolds to Probe the ATP-binding site of Protein Kinases

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Keywords

spirocycle, protein kinase, kinase inhibitor, selectivity

Graphical Abstract

Synthesis and Evaluation of Heteroaryl Substituted Diazaspirocycles as Scaffolds to Probe the ATP-binding site of Protein Kinases

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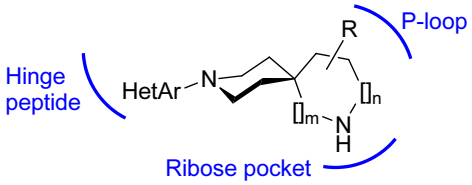

R = OH, CH_2OH, Ph, CH_2Ph, CO_2R, CONHR

m, n = 0, 1

Abstract

With the success of protein kinase inhibitors as drugs to target cancer, there is a continued need for new kinase inhibitor scaffolds. We have investigated the synthesis and kinase inhibition of new heteroaryl-substituted diazaspirocyclic compounds that mimic ATP. Versatile syntheses of substituted diazaspirocycles through ring-closing metathesis were demonstrated. Diazaspirocycles directly linked to heteroaromatic hinge binder groups provided ligand efficient inhibitors of multiple kinases, suitable as starting points for further optimization. The binding modes of representative diazaspirocyclic motifs were confirmed by protein crystallography. Selectivity profiles were influenced by the hinge binder group and the interactions of basic nitrogen atoms in the scaffold with acidic side-chains of residues in the ATP pocket. The introduction of more complex substitution to the diazaspirocycles increased potency and varied the selectivity profiles of these initial hits through engagement of the P-loop and changes to the spirocycle conformation, demonstrating the potential of these core scaffolds for future application to kinase inhibitor discovery.

Abbreviations

AGC, protein kinases A, G and C; ATP, adenosine triphosphate; 9-BBN, 9-borabicyclo[3.1.1.]nonane Boc, *tert*-butyloxycarbonyl; CAMK, calcium/calmodulin-dependent kinases; Cbz, benzyloxycarbonyl; DFG, aspartate-phenylalanine-glycine sequence; DIBAL-H diisobutylaluminium hydride; HA, heavy atom; LE, ligand efficiency; PKA, protein kinase A; PKB, protein kinase B; RCM, ring-closing metathesis; S_NAr, nucleophilic aromatic substitution; TK, tyrosine kinase.

PDB Codes: **12**-PKA, 3ZO1; **14**-PKA, 3ZO2; **16**-PKA, 3ZO3; **56**-PKA, 3ZO4.

1. Introduction

Protein kinases play a vital role in signal transduction pathways involved in cellular proliferation and survival, and are often implicated in deregulated signaling leading to cancer.[1-3] The development of protein kinase inhibitors to target the underlying mechanisms of cancer cell growth and survival has resulted in several licensed treatments.[4,5] The design of specific targeted inhibitors is important, but preclinical research and clinical experience also shows that inhibitors with multi-target profiles can be effective through the ability to inhibit multiple signaling events simultaneously.[6-8] There is a continued need for new protein kinase inhibitor scaffolds, particularly those that can act as platforms for modification to vary selectivity profiles.[9] Many inhibitors have been designed to target the conserved ATP cofactor binding site, and both highly specific and multi-targeted ligands can arise from this approach. Type I ATP-competitive inhibitors recognize the active conformation of kinases, and typically possess a core heteroaromatic ring system that forms hydrogen bonds to the hinge-peptide of the kinase in an analogous fashion to ATP. Interactions in other regions of the kinase active site can be introduced through extension of the core scaffold along appropriate vectors.[4, 10-12]

<Figure 1>

We have previously described compounds such as 4-(4-chlorobenzyl)-1-(7*H*-pyrrolo[2,3-d]pyrimidin-4-yl)piperidin-4-amine (**1**, Figure 1) which selectively inhibits PKB over other kinases within the AGC family.[13,14] Compound **1** closely resembles the pharmacophore defined by ATP bound to the kinase (Figure 1A). The selectivity of **1** was dependent upon the positioning of a basic primary amine in the ribose pocket, where it could interact with a glutamic acidic residue in PKB and backbone carbonyl functionality, as well as exploiting a close contact

with a methionine side chain.[13] 3-Aminopyrrolidine-based PKB inhibitors targeting these structural features have also been reported.[15]

We speculated that incorporation of the pendant primary amine into a second, spirocyclic ring could provide new scaffolds for ATP-competitive kinase inhibition, and that the inhibition profiles could be varied by modification of P-loop targeting substituents.[12,16] Diazaspirocycles have been proposed as generally useful scaffolds for drug design,[17] and these scaffolds incorporate a potentially favourable solubilising element into the core of the inhibitors.[18] Compared to more typical, planar kinase inhibitor motifs, the rigid spirocyclic structures increase the three-dimensional shape of molecules where they are present, which may be associated with better physicochemical properties.[19,20] Simple, unsubstituted aliphatic diazaspirocycles have been incorporated into kinase inhibitors as peripheral substituents in the patent literature, but these are most often seen as minor examples in much broader surveys of substituent amines.[21] The use of diazaspirocycles as core scaffolds to explore binding within the ATP-pocket, and the effect of substitution of these scaffolds on the selectivity profile of kinase inhibitors, has not been extensively detailed,[15,22] or has involved the embedding of the spirocycles in non-aliphatic fused structures, such as spiroindolines.[15,23]

We therefore investigated the synthesis and kinase inhibition of heteroaryl-substituted diazaspirocycles following the general design **2** (Figure 1B) capable of delivering a nitrogen atom into the ribose pocket of the ATP binding site, while orienting a heteroaromatic group to interact with the hinge peptide, and providing a platform for elaboration towards the P-loop. Unsubstituted diazaspirocyclic scaffolds were chosen to vary the position of the basic nitrogen relative to the point of attachment to the hinge-binding group. To each of the scaffolds, purin-6-yl[13] and isoquinolin-5-sulfonamide[24] kinase heteroaromatic hinge binders previously exemplified in AGC kinase inhibitors were attached, chosen to vary the degree and orientation of contact

between the ligand and the hinge-peptide, and the position of the spirocycles. Following assessment of these compounds against a representative panel of protein kinases, versatile synthetic routes were developed to introduce aromatic or polar functionality at two positions of a [5.5]-diazaspirocycle purine derivative with inhibitory activity, and thus to study the effect on the inhibition profiles with these new substituted diazaspirocycles.

2. Synthetic Chemistry

The 4-amino-4-benzylpiperidine scaffold **3** was made as a non-spirocyclic comparator, following our previously reported synthesis[25] (Scheme 1). Jenkins *et al*. have described robust syntheses of the [5.5]-azaspirocycle **4**, through ring-closing metathesis (RCM), and [4.5]-azaspirocycle **5**, through a bromine-mediated cyclisation, both starting from 1-benzylpiperidine-4-one.[17] Commercially available [5.5]-azaspirocycles **6**, **7** and [4.5]-azaspirocycle **8** provided variations in the positions of the nitrogen atoms. Compounds **5** and **6** were subjected to a protecting group exchange to generate scaffolds **9** and **10** with the basic nitrogen translocated.

<Scheme 1>

<Table 1>

Heteroaromatic groups were attached to the scaffolds **3**-**10** through S_NAr reactions with 6-chloropurine[13] and sulfonylation with isoquinoline-5-sulfonyl chloride.[24] *N*-Deprotection under acidic conditions gave compounds **11-26** (Table 1). The unprotected 4-amino-4-benzylpiperidine **3** reacted regioselectively at the more nucleophilic piperidine nitrogen with 6-chloropurine and isoquinoline-5-sulfonyl chloride to give **11** and **19**, respectively, as confirmed by NMR and MS-fragmentation analysis. With the exception of **21**, diazaspirocycles were introduced with mono-

N-protection to avoid poor regioselectivity in the couplings. 3-Azaspiro[5.5]undecane, 1-oxa- and 2-oxa-9-azaspiro[5.5]undecane purine derivatives **27**-**29** were prepared using similar methods (Scheme 2).

<Scheme 2>

The purine [5.5]-diazaspirocycle **12** was chosen for synthetic modification to introduce a selection of hydrophobic and polar functional groups to the C-3 and C-4 positions of the 1,9-diazaspiro[5.5]undecane. Compound **30**[17], an intermediate in the synthesis of **4**, was hydroborated with 9-BBN to give a chromatographically separable 1:1 mixture of alcohols **31** and **32** after oxidation (Scheme 3). Transfer hydrogenation was found to be optimum for the *N*-benzyl deprotection, as **33** and **34** failed to react under standard hydrogenation conditions. Introduction of the purine and isoquinoline sulfonamide substituents followed by *N*-Boc deprotection gave compounds **33-36**.

<Scheme 3>

Racemic syntheses were developed to introduce aromatic substituents to the 1,9-diazaspiro[5.5]undecane scaffold (Scheme 4). 4-Amino-4-allylpiperidine **37**[17] was acylated with acryloyl chloride to give a bis alkene, which underwent RCM with Grubbs I catalyst and $Ti(^{i}PrO)_4$ as an additive[26] to form the unsaturated lactam **38**. 1,4-Addition of cuprates introduced phenyl and benzyl groups to the C-4 position of **38**.[27] The success of these reactions depended on the copper (I) source and the concentration of the cuprate, with $CuBr.SMe_2$ and 1M concentration in THF identified as optimal for the formation of the 1,4-addition products **39** and

40. Compounds **39** and **40** were reduced using an excess of $LiAlH_4$, followed by *N*-benzyl deprotection to give the diamine intermediates in good yields. S_NAr and sulfonylation reactions of the diamines were regioselective for reaction at the less hindered piperidine nitrogen, but were inefficient. While purines **41** and **42** were isolated after chromatography, the corresponding isoquinoline compounds were not obtained.

<Scheme 4>

An alternative RCM strategy was investigated to install substituents to position C-3 of the 1,9-diazaspiro[5.5]undecane.[26,28] Amine **37** was acylated with freshly prepared 2-benzylacryloyl chloride (Scheme 4).[29] The RCM using Grubbs I catalyst was poorly reproducible, but Hoveyda-Grubbs II catalyst gave the tri-substituted lactam **43** reliably in good yield (66%). Filtration through amine-functionalised ion-exchange resin was found to be an effective method for the removal of ruthenium residues following the RCM.[30] Hydrogenation of **43** reduced the alkene while keeping the benzyl protecting groups intact. The lactam was reduced using excess $LiAlH_4$, followed by *N*-debenzylation to give **44** in good yield. A regioselective S_NAr reaction with 6-chloropurine gave compound **45**.

When applying the above route to introduce phenyl substitution at C-3, the RCM reaction using Hoveyda-Grubbs II catalyst with $Ti(^iPrO)_4$ generated **46** in low yields with unpredictable reaction times of between 24-72 hours. This was surprising, as phenyl-substituted dienes are reported to be metathesised efficiently.[31] The RCM was attempted using the less sterically hindered Grubbs catalyst dichloro[1,3-bis(2-methylphenyl)-2-imidazolidinylidene](2-isopropoxyphenylmethylene)ruthenium (II), which has been reported to give increased activity in the formation of trisubstituted olefins,[32] and **46** was isolated in good yield (76%). Hydrogenation

of **46** removed the double bond, but in contrast to lactam **43**, the *N*-benzyl protecting group of the piperidine ring was also cleaved. Reduction of the lactam was difficult, with $LiAlH_4$, DIBAL-H, BH_3.THF or 9-BBN giving either unreacted starting material or mixtures of starting material and the partially reduced hemiaminal. We speculated that the 3-phenyl substituent adjacent to the lactam carbonyl was sterically impeding nucleophilic attack. Therefore, the smaller and more reactive reducing agent AlH_3 was prepared *in-situ* from $LiAlH_4$ and $AlCl_3$,[33] and gratifyingly gave full reduction of the amide. Removal of the remaining *N*-benzyl group was achieved by transfer hydrogenolysis to give the diamine **47**. The regioselective S_NAr reaction of **47** with 6-chloropurine yielded **48**.

To prepare the C-3 ester derivative of the 1,9-diazaspiro[5.5]undecane, intermediate **37** was alkylated using methyl 2-(bromomethyl)acrylate to give bis-alkene **49** in good yield (Scheme 4). The RCM of **49** was first attempted with Grubbs I catalyst in the presence of *p*TsOH, according to a method outlined by Prusov and Maier[28] but no conversion of the starting material was observed with this catalyst, nor with Hoveyda-Grubbs II catalyst and $Ti(^iPrO)_4$. However, dichloro[1,3-bis(2-methylphenyl)-2-imidazolidinylidene](2-isopropoxyphenylmethylene) ruthenium (II) gave full conversion of the bis-alkene to the cyclised product **50** in good yield. The spirocycle **50** was hydrogenated to reduce the alkene and remove the *N*-benzyl protecting groups. A subsequent S_NAr reaction with 6-chloropurine gave **51**.

The ester **51** was converted to the carboxylic acid **52** and hydroxymethyl derivative **53** (Scheme 4). The methyl, dimethyl and morpholine amides **54**-**56** were prepared by hydrolysis of the ester **51** to the lithium salt of the acid followed directly by amine couplings. Exhaustive hydrogenation removed the *N*-benzyl protecting groups and reduced the alkenes, prior to S_NAr reactions to give **57-59**.

3. Results and Discussion

Compounds were tested for protein kinase inhibition in a panel of 24 enzymes containing representative members of the major kinome family subclasses, using a commercially available microfluidic assay format.[34,35] Given the small size of most of the compounds **11-27** (MW range 258-381, median MW = 308), the inhibitors were tested at a concentration of 30 μM, so that significant activity (>50% inhibition) would be indicative of ligand efficient binding (>0.3 kcal^{-1}HA^{-1}).[36] The ATP concentration used was approximately the $K_{m,[ATP]}$ for each enzyme tested.[37] To determine the variability and reproducibility of the assay, the pan-AGC kinase inhibitor H-89[38] was included as an internal control in each assay plate. Over multiple assay runs during compound screening, this control gave high reproducibility with low standard errors in the mean % inhibition (Figure 2). This gave confidence in the robustness of the assay for single-point screening. The determination of % inhibition at a single fixed concentration, rather than full IC_{50} or K_d values for each compound against each kinase, imposes limitations on the interpretation of the data. The non-linear relationship between % inhibition and concentration does not permit differentiation between compounds with similar high % inhibitions (e.g. between 95-100%), even though there may be significant differences in the IC_{50}, and thus selectivity cannot be accurately quantified. Nevertheless, the method provides an efficient means to identify potentially useful activity and distinguish major changes in selectivity patterns that we and others have previously used to assess kinase inhibition by new screening sets.[39-42]

<Figure 2>

The simple heteroaryl-substituted diazaspirocycles **12-18** and **20-26** (Table 1) showed activity profiles that were apparently dominated by the hinge-binder component (Figure 2). The purines

12-**18** were most promiscuous, inhibiting enzymes in the AGC, TK, CAMK, and CMGC families, with the highest activities against PKA and other AGC kinases. This was expected since these compounds were closest in structure to the PKA/PKB inhibitors such as **1**,[13] and compounds **12**-**18** generally showed similar activity patterns to the 4-amino-4-benzylpiperidin-1-yl purine **11**. The isoquinolines **20**-**26** showed a reduced spread of activity, inhibiting only certain AGC and some CAMK kinases. The 4-amino-4-benzylpiperidinyl isoquinoline **19** also showed a decrease in the spread of activity relative to the comparator purine **11**. The strong inhibition of PKA may reflect the similarity of the general design **2** to known isoquinoline-5-sulfonamide PKA inhibitors.[24] 5-(2,8-Diazaspiro[5.5]undecan-2-ylsulfonyl)isoquinoline (**25**) showed inhibition of CHK1 as well as PKA in the screen, and this was confirmed by IC_{50} determination[43] (**25**; CHK1 IC_{50} = 12.8 μM; PKA IC_{50} = 3.6 μM).

We had speculated that the protonated amine in the diazaspirocycles might provide a binding interaction to the kinase ribose pocket even with suboptimal hinge binding elements. However, our screening data indicates that the presence of an appropriate and correctly oriented hinge binder is critical if the binding contribution of the amine is to be realised, and that the presence of the protonated amine does not automatically confer promiscuous inhibition. This is consistent with a Free-Wilson analysis of the binding contributions of the functional groups in an ATP-competitive 2-(4-(1*H*-pyrazol-4-yl)phenyl)ethanamine PKA/PKB inhibitor that adopted a similar binding pose to **1**.[44]

The diazaspirocyclic substituent did modulate the activity of compounds within the hinge-binder sets. For the purine analogs, piperidin-4-yl derived scaffolds with a nitrogen atom proximal to the spirocentre were less promiscuous than those with the more distal nitrogen (compare **12**, **13** to **14**, **15**). The purines bearing piperidin-3-yl substituted scaffolds (**16**, **17**) were less active still. For the isoquinoline analogues the reverse was observed, whereby the 4-

piperidinyl scaffolds with proximal nitrogens (**20**, **21**) were active at more enzymes in the screen than the compounds with the nitrogen displaced one atom further around the second ring (**22**, **23**). Derivatives of the [4.5]-azaspirocycle **5** (e.g. **18**, **26**), which had the most extreme difference in positioning of the basic nitrogen compared to the comparator 4-amino-4-benzylpiperidines **11** and **19**, showed very limited activity.

As the purines **12** and **14** showed high activity in the initial screen, IC_{50} determinations were carried out against the 24 kinases using the microfluidic assay format and were found to be consistent with values estimated from the single-point percentage inhibition data using the Hill equation, validating the use of the single concentration screen to assess the library (Supplementary Material, Table S1). Compound **14** was more potent across the panel than **12**. Both compounds exhibited highest activity against PKA (IC_{50} = 80 nM **14**, 1 μM **12**), with high ligand efficiencies (LE = 0.48 **14**, 0.41 **12**). The IC_{50} values for **14** in the small panel spanned more than 3 orders of magnitude (0.08 – 183 μM), confirming meaningful selectivity between the most and least inhibited kinases tested. Although the inhibitory activity of these relatively low MW compounds is weak, it is efficient and comparable to hits of similar size that have been successfully progressed to potent inhibitors.[13,14,44] Thus the screening set **12**-**27** based on two points of the simple pharmacophore **2** generated novel hits with potentially useful differences in their activity profiles.

<Figure 3>

High resolution crystal structures were obtained of PKA in complex with the purines **12**, **14** and **16**. The use of PKA as a surrogate of other AGC family kinases is well established.[24,45] The binding mode for **12** (PDB 3Z01; Figure 3A) showed the purine hydrogen bonding to the hinge

peptide as expected, and the nitrogen of the azaspirocycle interacting with the side chain carboxylate of Glu127, a residue located in the ribose pocket and postulated to be involved in communicating ATP-binding to substrate-peptide binding residues.[46] Compounds **14** and **16** (PDB 3Z02, 3ZO3; Figure 3B, C) bound similarly to the hinge region but displayed an alternative interaction in the ribose pocket, with the azaspirocycle nitrogen interacting with the side chain carboxylate of Asp184 from the DFG motif, and close to Gln171 and the backbone carbonyl of Glu170. As the DFG aspartate residue is highly conserved across the kinome due to its role in the enzyme catalytic function, the different interactions of the nitrogen atoms are consistent with the increased promiscuity of **14** in the panel compared to **12**.

The inhibitory activities of the purines **12** and **13** across the small panel correlated to an extent with the identity of the residue equivalent to Glu127 in PKA (Figure 2). This was determined by sequence alignment and overlay of publically available crystal structures of the kinase domains. For most of the 24 kinases the relevant residue corresponded to the gatekeeper + 7 position in the kinase domain sequence, which occupies a position at the start of the first α -helix in the C-terminal subdomain. However for the four members of the CMGC family tested and the tyrosine kinase CK1d, there are differences in the conformation of the peptide loop between the gatekeeper residue and the start of the α-helix, such that the gatekeeper + 6 residues occupy this space. Activity was generally higher for kinases with glutamate at this position (**12** and **13**, >50% inhibition for 6/8 kinases with Glu at this position), with less likelihood of inhibition when the glutamate was replaced by aspartate or non-acidic residues (**12**, <50% inhibition for 12/16; **13** <50% inhibition for 11/16 kinases with non-glutamate residues at this position). Targeting the presence of the glutamate residue at the gatekeeper + 7 position is thus potentially useful to direct the activity profiles of the purine-diazaspirocyclic inhibitors towards a sub-set of kinases. These results confirm that appropriately positioned basic amines within the core pharmacophore

can contribute positively to potency and selectivity. No similar correlation of activity was seen for the isoquinoline-diazaspirocyclic compounds, which is consistent with the different vector expected for the spirocycle relative to the hinge when attached to this topologically distinct hinge-binder.[24]

For all three purine compounds **12**, **14** and **16** refinement of the ligand electron density indicated the [5.5]-spirocyclic scaffolds to adopt chair-chair conformations on binding to PKA. Predicted conformations of purines **13**, **15**, **17** and **18** bound to PKA were generated through structural modification of the most closely related ligand structure bound to PKA, followed by energy minimization of the ligand structure within the PKA protein, using the MMFF94X force field in the Molecular Operating Environment (MOE) program.[47] This modelling suggested that the size of the distal heterocyclic ring (piperidine vs. pyrrolidine), would not significantly affect the binding modes, so that [4.5]-azaspirocycles **13** and **15** closely mimicked the binding of **12** and **14**, respectively. The [4.5]-azaspirocycle **18** was predicted to be unable to simultaneously interact with an acidic residue in the main ribose pocket region and maintain key hinge-binding interactions, consistent with the poor activity in the screen. The amine of **16** was shown to bind to the DFG aspartate and this compound showed a more promiscuous profile than **17** which was predicted to contact the non-conserved glutamate residue.

To confirm the importance of the basic piperidine nitrogen, the *N*-Cbz-protected precursors of **14** and **22** were profiled and showed reduced activity compared to the corresponding unprotected piperidines (data not shown). This is potentially due to unfavorable steric clashes of the bulky substituent in the relatively tight ATP binding site of many kinases, as well as the removal of interactions to the protonated amines. Interestingly, in this screening panel Aurora A kinase was best able to tolerate the *N*-substitution. In the purine series, the 3-azaspiro[5.5]undecane **27**, and 1-oxa- and 2-oxa-9-azaspiro[5.5]undecanes **28** and **29** were also prepared (Scheme 2 and Figure

2). Removal of the basic amine (**27**) reduced activities in the panel with the exception of Aurora A kinase, emphasizing the importance of the basic functionality in the binding of this set of compounds. Replacement of the nitrogen proximal to the spirocentre with oxygen to give **28** recovered some of the AGC and CAMK kinase inhibitory activity, with potent inhibition of FYN and Aurora A, thus heteroaryl-substituted oxazaspirocycles may be of future interest as scaffolds for targeting the ribose-pocket of a different spectrum of kinases.

Based on its inhibitory profile and binding mode in PKA, purine analogue **12** was chosen to assess the effects of adding P-loop interacting substituents at position C-3 and C-4 of the 1,9-diazaspiro[5.5]undecane scaffold. It was expected that the activity profile of **12** could be sensitive to different functionality in these positions and therefore hydroxyl, phenyl, benzyl, carboxylate and carboxamide groups were targeted. The purines **33**, **35**, **41**, **42**, **45**, **48**, **51**-**53**, **57**-**59** and two related isoquinoline analogs (**34**, **36**) were tested for kinase inhibition at 30 μM for consistency with previous data (Figure 2), since the increase in size was limited (MW range 288-385).

The introduction of hydroxyl groups in **33** and **35** maintained the activities of **12**, except there was a reduction in TK inhibition (Figure 2). The hydroxymethyl substituted analogue **53** also maintained the original AGC kinase activity, with a reduction in activity across the other kinases in the panel. Therefore, the addition of hydroxyl substituents to the purine [5.5]-azaspirocycle **12**, led to a slight increase in specificity towards the AGC kinases in the small panel tested. The presence of a glutamate at the gatekeeper +7 position continued to be associated with generally higher activity. The C-3 and C-4 hydroxyl elaborated scaffolds were also attached to the isoquinoline-5-sulfonamide hinge-binder, to give **34** and **36**. Compared to the parent isoquinoline derivative **20**, compounds **34** and **36** showed increased inhibition of AGC kinases, in particular for RSK1, PKA and MSK1. In contrast to the purine analogues, isoquinolines **20**, **49** and **51**

showed no inhibition of PKB isoforms (AKT1 and AKT2), despite the high sequence similarity of these proteins with PKA.[45]

Phenyl and benzyl substituents were introduced at the C-4 position of the 1,9-diazaspiro[5.5]undecane, to give **41** and **42**, Compounds **41** and **42** showed increased activity towards the AGC sub-family compared to **12**. This was consistent with the activity profile of the 4-benzyl-4-aminopiperidin-1-yl purine inhibitor **11**. There was an appreciable increase in TK activity for the phenyl substituted analogue **41**, but this was less marked for the benzyl substituted compound **42** (Figure 2). For compounds **11**, **41** and **42** the addition of the P-loop targeting aromatic group reduced the influence on the activity pattern of the presence of glutamate at the gatekeeper + 7 residue, so that non-polar residues in this position became more frequently associated with higher activities.

Elaboration at the C-3 position of **12** to give **45** and **48** resulted in a reduction in overall activity, but maintained some selectivity towards the AGC kinases. The C-3 position of **12** was also functionalised to give the methyl ester **51** and the amides **57**-**58**. The ester and amide analogues showed reduced inhibitory activity compared to the parent purine [5.5]-azaspirocycle **12** (Figure 2) but enhanced selectivity for the AGC kinases. The acid **52** also had less activity against the kinases in the panel, but altered the profile of the undecorated [5.5]-azaspirocycle **12** towards AurA and RSK1 (AurA IC_{50} = 6.1 μM).[43]

The single-concentration inhibitory activities of **41**, **42** and **45** were determined against a 96-member kinase panel distributed across eight of the kinome sub-families, with the majority of kinases representing the four main sub-families (AGC, CAMK, CMGC and TK) (Supplementary Material, Table S2). This broader analysis confirmed that the C-4 elaborated phenyl **41** and benzyl **42** purine [5.5]-azaspirocycles possessed a degree of selectivity towards the AGC kinases. However, there was still significant inhibition of the kinases in the TK and CAMK sub-

families by **41** and in the CAMK sub-family by **42**. A reduction in overall activity was confirmed for the C-3 benzyl substituted compound **45**, particularly in the AGC kinase sub-family compared to **41** and **42**. Although **12**, **41**, **42** and **45** have a broad range of inhibitory activities, these screening data show that variation of the positioning of a lipophilic group interacting with the P-loop can vary the pattern of inhibition.

<Figure 4>

The substituted inhibitors **41**, **42**, **45**, **48** were modelled in the ATP-binding site of PKA starting from the bound conformation of **12** in PKA chimera. This suggested that the aryl rings of **41** and **42** could project along a vector towards the P-loop, however the extra carbon of the benzyl group of **42** would enable a closer interaction (Figure 4A), consistent with the increased inhibition of AGC and other kinases within the panels by **42**. Modelling of compounds **45** and **48** also showed the substituents approaching the P-loop, however they would likely require additional substitution to efficiently bind without compromising hinge peptide and ribose pocket binding interactions (Figure 4B). A crystal structure of **41** bound to PKA was determined (PDB 3ZO4; Figure 3D) and showed interesting differences between the binding of **12** and **41**, and with the prediction of **41** binding. A key variation was in the conformation of the spirocyclic core scaffold. Whereas the electron density for **12** bound to PKA was consistent with a chair-chair conformation of the spirocycle, the first piperidine ring of **41** adopted a twist-boat conformation. This led to the projection of the equatorial phenyl substituent along a more direct vector to the P-loop than was predicted from the model based on the chair-chair conformation of **12** (Figure 4A). The phenyl substituent of **41** in PKA occupied a similar space to the terminal 4-chloro substituent of **1** in PKA-PKB chimeric protein (Figure 1) and sat within a hydrophobic

environment created by the P-loop backbone and the side chain of Val57. The spirocyclic amine **41** formed hydrogen bonds directly to the side chain of Glu127 and the backbone carbonyl of Glu170, with a water-mediated contact to the side chain of Gln171. The distortion of the azaspirocycle conformation from a chair-chair (**12**) to twist-boat-chair (**41**) would suggest that any additional energy of binding obtained by **41** from contacting the P-loop could be offset by induced strain in the ligand. This was supported by the measured IC_{50} for inhibition of PKA[43] by **41** (IC_{50} = 149 nM) which, although substantially increased over **12** (IC_{50} = 1000 nM), was less improved than that of the C-4 benzylated analogue **42** (IC_{50} = 31 nM) where binding to the P-loop was expected to be compatible with maintaining a lower energy chair-chair conformation of the spirocycle.

In the above analysis, the changes in activity profile could be related in part to specific interactions of the ligands in the ATP site. However, the degree to which the distinct activity profiles of fragments or low-to-moderate molecular weight hit compounds such as those described here can be maintained and exploited during elaboration to larger molecular weight species is not yet fully defined.[39,40] While the results of this study are encouraging for the identification of starting points with different spectra of kinase inhibitory activities, to realize the controlled design of ATP-competitive kinase inhibitors with specific polypharmacology[8,9] would require more information on the inherent stability, or otherwise, of kinase selectivity profiles during optimization of other important molecular properties.

4. Conclusions

Heteroaryl-substituted diazaspirocyclic compounds were prepared as potential ATP-competitive kinase inhibitors based on the pharmacophore defined by the 4-aminopiperidin-1-yl purine PKB inhibitor **1** and ATP, and in particular to exploit the interactions of basic nitrogen

atoms in the ribose pocket. Several compounds showing ligand efficient inhibition of multiple kinases were identified, validating the design. The nature of the adenine-mimic largely determined the inhibitory activities shown by simpler spirocyclic compounds, with structural similarity to **1** conferring a similar activity profile, particularly inhibition of AGC kinases. However, varying the hinge-binding and diazaspirocyclic groups to produce compounds more structurally distinct to **1** also produced potentially useful variation in the inhibition profiles. Altering the position of the basic nitrogen in the diazaspirocycles was expected to change the interactions to the proteins. This was confirmed by X-ray crystallography in the case of purine-substituted examples, and was associated with changes in the activity profiles. Interaction of the basic amine with a non-conserved glutamate residue at the gatekeeper + 7 position in the ribose pocket conferred activity to purines substituted with diazaspirocycles containing an embedded 4-aminopiperidine motif.

Ring-closing metathesis was used to enable versatile syntheses of 1,9-diazaspiro[5.5]undecanes substituted at two positions with polar and hydrophobic groups to target the P-loop of the kinase active site within a series of purine derivatives. These routes should be readily extendable to prepare other substituted diazaspirocycles. The introduction of aryl substituents at C-4 of the *N*-purinyl 1,9-diazaspiro[5.5]undecane scaffold led to the greatest increases in activity, which was consistent with modeling that proposed this substitution pattern would most closely resemble the original PKB inhibitor **1**. Importantly, substitution at C-3 with corresponding aryl groups led to different activity profiles, suggesting that the addition of the P-loop targeting elements could change the intrinsic activity profile of the purine-diazaspirocyclic scaffolds. The C-4 phenyl substituted analogue **41** in PKA showed a change in the conformation of the core spirocycle from chair-chair to twist boat-chair to enable binding of the P-loop

targeting group, showing that a range of conformations are accessible to the diazaspirocyclic parts of the scaffolds.

Overall these results show that diazaspirocyclic motifs can be usefully incorporated into the core binding scaffolds of kinase inhibitors and that these motifs adopt well-defined conformations within the ATP-site leading to productive interactions. Different kinase inhibitory profiles can be associated with specific changes to the substitution pattern of the spirocycle and its orientation with respect to the hinge-binding heteroaryl group. The straightforward synthetic routes we have demonstrated to make novel substituted diazaspirocycles increase the potential application of these scaffolds for future inhibitor discovery.

5. Experimental

5.1 General Synthetic Chemistry

Reactions were carried out under N2. Organic solutions were dried over MgSO4 or Na2SO4. Starting materials and solvents were purchased from commercial suppliers and were used without further purification. Microwave reactions were carried out using a Biotage Initiator microwave reactor. Flash column chromatography was performed using Merck silica gel 60 (0.040-0.063 mm). Gradient silica column chromatography was performed with a Biotage SP1 medium pressure chromatography system, using prepacked silica gel cartridges. Preparative HPLC was performed using a Gilson GX-281 Liquid Handler system and Gilson 322 HPLC pump, with a 15 minute gradient elution of 10-90% MeOH/0.1% aqueous formic acid at a flow rate of 20 mL min-1. Ion exchange chromatography was performed using Isolute Flash SCX-II acidic resin cartridges. Melting points were determined on a Leica Gallen III or a SRS EZ-Melt Automated melting point apparatus. 1H NMR spectra were recorded on a Bruker AMX500 instrument at 500 MHz using internal deuterium locks. Chemical shifts ( ) are reported relative

to TMS (=0) and referenced to the solvent in which they were measured. 13C-nuclear magnetic resonance spectra were recorded at 126 MHz on a Bruker AMX500 spectrometer using an internal deuterium lock. Combined HPLC-MS analyses were performed on a Waters Alliance 2795 separations module and a Waters 2487 dual wavelength absorbance detector coupled to a Waters/Micromass LCt time of flight mass spectrometer with ESI source, or on an Agilent 1200 series HPLC and diode array detector coupled to a 6210 time of flight mass spectrometer with dual multimode APCI/ESI source. HPLC was performed using Supelco DISCOVERY C18, 50 mm x 4.6 mm or 30 mm x 4.6 mm i.d. columns, or using an Agilent 6210 TOF HPLC-MS with a Phenomenex Gemini 3 μm C18 (3 cm x 4.6 mm i.d.) column. Both HPLC systems were run at a temperature of 22 °C with gradient elution of 10-90% MeOH/0.1% aqueous formic acid at a flow rate of 1 mL min-1. UV detection was at 254 nm and ionisation was by positive or negative ion electrospray as indicated. The molecular weight scan range was 50-1000 amu. All tested compounds gave >95% purity as determined by these methods. All purified synthetic intermediates gave >95% purity as determined by these methods except where indicated in the text.

5.1.1 Benzyl 1,8-diazaspiro[4.5]decane-1-carboxylate (9)

A solution of Et_3N (1.1 mL, 0.80 mmol), **5**[17] (50 mg, 0.20 mmol) and CbzCl (37 μL, 0.26 mmol) in CH_2Cl_2 (2 mL) was stirred at r.t. for 48 h. Additional CbzCl (15 μL, 0.10 mmol) and Et_3N (0.2 mL, 0.20 mmol) were added and the mixture was stirred for a further 24 h. The mixture was washed with sat. aq. $NaHCO_3$ (3 x 5 mL), dried and concentrated. Flash column chromatography, eluting with 30% EtOAc/hexane, gave 1-benzyl 8-*tert*-butyl 1,8-diazaspiro[4.5]decane-1,8-dicarboxylate as colourless oil (61 mg, 0.15 mmol, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) 1.23-1.36 (2H, m), 1.46 (9H, s), 1.76-1.83 (2H, m), 1.90-1.99 (2H, m), 2.66-2.81 (4H, m), 3.49-3.58 (2H, m), 4.02-4.22 (2H, m), 5.09 (2H, s), 7.20-7.37 (5H, m); ^{13}C

NMR (126 MHz, $CDCl_3$) 22.1, 28.5, 33.0, 35.0, 41.3, 42.3, 47.7, 62.8, 66.1, 79.4, 127.7, 127.8, 128.4, 137.2, 153.6, 154.6; LC-MS (ESI+) *m/z* 397 (M+Na); t_R = 2.77 min, purity = 90%; HRMS *m/z* calcd. for $C_{21}H_{30}N_2O_4Na$ (M+Na) 397.2098, found 397.2104. A solution of 4 M HCl in dioxane (2.37 mL, 9.50 mmol) and 1-benzyl 8-*tert*-butyl 1,8-diazaspiro[4.5]decane-1,8-dicarboxylate (358 mg, 0.95 mmol) in MeOH (5 mL) was stirred at 0 °C for 1 h, then at 4 °C for 16 h. The reaction mixture was concentrated and purified by ion exchange chromatography on acidic resin, eluting with 2M NH_3 in MeOH, to give **9** as a colourless oil (261 mg, 0.95 mmol, 100%); ^{1}H NMR (500 MHz, $CDCl_3$) 1.31-1.38 (2H, m), 1.75-1.81 (2H, m), 1.92-2.01 (2H, m), 2.60-2.69 (4H, m), 3.02-3.13 (2H, m), 3.49-3.57 (2H, m), 5.04-5.25 (2H, m), 7.29-7.45 (5H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 22.3, 34.7, 35.8, 44.7, 47.6, 62.9, 66.0, 127.7, 127.7, 128.4, 137.3, 153.6; LC-MS (ESI+) *m/z* 275 (M+H); HRMS *m/z* calcd. for $C_{16}H_{23}N_2O_2$ (M+H) 275.1754, found 275.1752.

5.1.2 Benzyl 2,9-diazaspiro[5.5]undecane-2-carboxylate (10)

A solution of Et_3N (0.1 mL, 0.68 mmol), **6** (50 mg, 0.17 mmol) and CbzCl (38 mg, 0.22 mmol) in CH_2Cl_2 (1.7 mL) was stirred at r.t. for 48 h. Additional CbzCl (29 mg, 0.16 mmol) was added and the mixture was stirred for a further 24 h. The mixture was washed with sat. aq. $NaHCO_3$ (3 x 10 mL), dried and concentrated to give 2-benzyl 9-*tert*-butyl 2,9-diazaspiro[5.5]undecane-2,9-dicarboxylate as a colourless oil (55 mg, 0.13 mmol, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) 1.30-1.38 (2H, m), 1.38-1.50 (13H, m), 1.54-1.62 (2H, m), 3.16-3.61 (8H, m), 5.15 (2H, s), 7.30-7.41 (5H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 20.8, 21.0, 28.5, 32.1, 33.4, 35.5, 35.8, 39.5, 44.9, 51.4, 51.8, 67.1, 79.3, 127.0, 127.6, 127.9, 128.1, 128.5, 128.6, 136.9, 154.9, 155.4, 155.5, multiple peaks due to rotamers; LC-MS (ESI+) *m/z* 411 (M+Na); t_R = 2.85 min, purity = 90%; HRMS *m/z* calcd. for $C_{22}H_{32}N_2O_4Na$ (M+Na) 411.2254, found 411.2252. A solution of 4M HCl in dioxane (0.35 mL, 1.41 mmol) and 2-benzyl 9-*tert*-butyl 2,9-diazaspiro[5.5]undecane-2,9-dicarboxylate

(55 mg, 0.14 mmol) in MeOH (3 mL) was mixed at 0 °C then stirred at 4 °C for 16 h. The mixture was concentrated and purified by ion exchange chromatography on acidic resin, eluting with 2M NH_3 in MeOH, to give **10** as a colourless oil (32 mg, 0.11 mmol, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) 1.29-1.50 (6H, m), 1.51-1.59 (2H, m), 2.25 (1H, s), 2.68-2.89 (4H, m), 3.29-3.32 (1H, s), 3.32-3.36 (1H, s), 3.41-3.45 (2H, m), 5.12 (2H, s), 7.29-7.37 (5H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 20.6, 21.0, 32.2, 34.7, 36.2, 36.5, 42.0, 44.9, 51.8, 52.4, 67.0, 127.8, 128.0, 128.5, 136.9, 155.5, multiple peaks due to rotamers; LC-MS (ESI+) *m/z* 289 (M+H); HRMS *m/z* calcd. for $C_{17}H_{25}N_2O_2$ (M+H) 289.1911, found 289.1921.

5.1.3 4-Benzyl-1-(9H-purin-6-yl)piperidin-4-amine (11)

A solution of Et_3N (0.3 mL, 2.30 mmol) **3**[23] (89 mg, 0.46 mmol) and 6-chloropurine (72 mg, 0.46 mmol) in ^{n}BuOH (4.6 mL) was heated at 100 °C for 18 h. The reaction mixture was cooled and concentrated to dryness. The solid was washed with MeOH to give **11** as an off-white solid (101 mg, 0.35 mmol, 72%). mp 232 °C (dec); ^{1}H NMR (500 MHz, d_6-DMSO) 1.31-1.38 (2H, m), 1.53 (2H, ddd, *J* = 13.5, 12.5, 4.0 Hz), 2.64 (2H, s), 3.14-3.36 (2H, br m), 3.55-3.72 (2H, br m), 4.76-5.09 (2H, br m), 7.17-7.22 (3H, m), 7.25-7.29 (2H, m), 8.06 (1H, s), 8.16 (1H, s); ^{13}C NMR (126 MHz, d_6-DMSO) 37.0, 40.8, 49.8, 50.0, 118.6, 126.0, 127.7, 130.6, 137.4, 137.6, 151.3, 151.8, 153.0; LC-MS (ESI+) *m/z* 292 (M-NH_2); HRMS *m/z* calcd. for $C_{17}H_{21}N_6$ (M+H) 309.1822, found 309.1825.

5.1.4 6-(1,9-Diazaspiro[5.5]undecan-9-yl)-9H-purine (12)

A solution of Et_3N (0.24 mL, 1.70 mmol), **4**[17] (100 mg, 0.34 mmol) and 6-chloropurine (55 mg, 0.34 mmol) in ^{n}BuOH (3.4 mL) was stirred at 100 °C for 24 h. The reaction mixture was cooled, concentrated and triturated with MeOH (2.5 mL). The resulting solid was collected and dried to give *tert*-butyl 9-(9*H*-purin-6-yl)-1,9-diazaspiro[5.5]undecane-1-carboxylate as a yellow solid (107 mg, 0.29 mmol, 84%). A mixture of 4M HCl in dioxane (0.35 mL, 2.80 mmol) and *tert*-butyl 9-(9*H*-purin-6-yl)-1,9-diazaspiro[5.5]undecane-1-carboxylate (107 mg, 0.28 mmol) in

MeOH (2 mL) was stirred at r.t. for 48 h. The mixture was concentrated and purified by ion exchange chromatography on acidic resin, eluting with 2M NH_3 in MeOH to give **12** as an off-white solid (66 mg, 0.24 mmol, 84%). mp 250-256 °C; ^{1}H NMR (500 MHz, d_6-DMSO) 1.34-1.48 (6H, m), 1.51-1.57 (2H, m), 1.64-1.77 (2H, m), 2.69-2.71 (2H, m), 3.79-3.91 (2H, br m), 4.49-4.70 (2H, brd m), 8.07 (1H, s), 8.15 (1H, s); ^{13}C NMR (126 MHz, d_6-DMSO) 19.9, 26.9, 35.1, 36.7, 40.3, 40.7, 48.8, 118.7, 137.7, 151.4, 151.8, 153.1; LC-MS (ESI+) *m/z* 273 (M+H); HRMS *m/z* calcd. for $C_{14}H_{21}N_6$ (M+H) 273.1822, found 273.1822.

5.1.5 6-(1,8-Diazaspiro[4.5]decan-8-yl)-9H-purine (13)

A mixture of Et_3N (0.21 mL, 1.55 mmol), **9** (87 mg, 0.31 mmol) and 6-chloropurine (49 mg, 0.31 mmol) in ^{n}BuOH (1 mL) was heated to 100 °C for 16 h. The mixture was cooled to room temperature and concentrated to dryness. The solid was washed with MeOH (2 mL) to give benzyl 8-(9*H*-purin-6-yl)-1,8-diazaspiro[4.5]decane-1-carboxylate as an off-white solid (84 mg, 0.21 mmol, 69%). A mixture of 6 M HCl (1 mL) and benzyl 8-(9*H*-purin-6-yl)-1,8-diazaspiro[4.5]decane-1-carboxylate (40 mg, 0.10 mmol) was heated in a microwave reactor to 100 °C for 15 min. The mixture was concentrated and purified by column chromatography, eluting with 10% 2 M NH_3 in MeOH/CH_2Cl_2 to give **13** as an off-white solid (26 mg, 0.10 mmol, 99%). mp 223-235 °C; ^{1}H NMR (500 MHz, d_6-DMSO) 1.56-1.63 (6H, m), 1.76 (2H, dddd, *J* = 7.0, 7.0 Hz), 2.93 (2H, dd, *J* = 7.0, 7.0 Hz), 4.10-4.29 (4H, br m), 8.09 (1H, s), 8.19 (1H, s); ^{13}C NMR (126 MHz, d_6-DMSO) 24.9, 36.7, 36.8, 42.8, 45.1, 61.2, 119.2, 138.4, 151.9, 152.3, 153.5; LC-MS (ESI+) *m/z* 259 (M+H); HRMS *m/z* calcd. for $C_{13}H_{19}N_6$ (M+H) 259.1666, found 259.1658.

5.1.6 6-(2,9-Diazaspiro[5.5]undecan-9-yl)-9H-purine (14)

A mixture of Et_3N (0.19 mL, 1.35 mmol), **10** (78 mg, 0.27 mmol) and 6-chloropurine (42 mg, 0.27 mmol) in ^{n}BuOH (2.7 mL) was heated to 100 °C for 16 h. The mixture was cooled and concentrated to dryness. Flash column chromatography eluting with 5% MeOH/CH_2Cl_2 gave

benzyl 9-(9*H*-purin-6-yl)-2,9-diazaspiro[5.5]undecane-2-carboxylate as a white solid (67 mg, 0.17 mmol, 61%). A mixture of CF_3CO_2H (4 mL) and benzyl 9-(9*H*-purin-6-yl)-2,9-diazaspiro[5.5]undecane-2-carboxylate (53 mg, 0.13 mmol) was heated to 50 °C for 24 h. The mixture was concentrated and purified by ion exchange chromatography, eluting with 2M NH_3 in MeOH, to give **14** as a pale yellow oil (35 mg, 0.13 mmol, 100%). mp = 246-247 °C; ^{1}H NMR (500 MHz, $CDCl_3$) 1.54-1.58 (4H, m), 1.62-1.67 (4H, m), 2.73 (2H, s), 2.80-2.83 (2H, m), 4.15-4.43 (2H, br m), 5.02-5.69 (2H, br m), 7.93 (1H, s), 8.35 (1H, s); ^{13}C NMR (126 MHz, $CDCl_3$) 22.2, 31.2, 34.7, 34.7, 41.0, 47.2, 55.8, 119.6, 136.7, 151.2, 151.5, 153.9; LC-MS (ESI+) *m/z* 273.14 (M+H); HRMS *m/z* calcd. for $C_{14}H_{21}N_6$ (M+H) 273.1822, found 273.1828.

5.1.7 6-(2,8-Diazaspiro[4.5]decan-8-yl)-9H-purine (15)

Prepared as described for **12** starting from **8** and 6-chloropurine to give **15** as a colourless oil (53% over 2 steps); ^{1}H NMR (500 MHz, $CDCl_3$) 1.47-1.63 (6H, m), 2.66 (2H, s), 2.87 (2H, dd, *J* = 7.5, 7.5 Hz), 4.00-4.43 (4H, br m), 8.09 (1H, s), 8.19 (1H, s); ^{13}C NMR (126 MHz, $CDCl_3$) 36.1, 37.7, 42.0, 43.2, 45.6, 57.7, 119.2, 138.4, 151.9, 152.2, 153.6; LC-MS (ESI+) *m/z* 259 (M+H); HRMS *m/z* calcd. for $C_{13}H_{19}N_6$ (M+H) 259.1666, found 259.1669.

5.1.8 6-(2,9-Diazaspiro[5.5]undecan-2-yl)-9H-purine (16)

Prepared as described for **12** starting from **6** and 6-chloropurine to give **16** as a white solid (60% over 2 steps). mp 183-187 °C; ^{1}H NMR (500 MHz, $CDCl_3$) 1.49 (2H, ddd, J = 13.5, 9.5, 4.0 Hz, C7/10-Hax), 1.61-1.68 (4H, m, C7/10-H, C4-H), 1.74-1.80 (2H, m, C3-H), 2.90-2.96 (2H, m, C8/9-H), 3.06-3.13 (2H, m, C8/9-H), 4.14-4.44 (4H, brd m, C2/6-H), 7.95 (1H, s, C14-H), 8.34 (1H, m, C12-H); 13C NMR (126 MHz, CDCl3) 21.2 (C3), 33.2 (C5), 33.9 (C7/10), 36.8 (C4), 41.4 (C8/9), 119.1 (CAr), 136.6 (C14), 151.2 (CAr), 151.7 (C12), 154.24 (CAr), (C2 and C6 Missing); HRMS [M+H+] calcd for $C_{14}H_{21}N_6$ 274.1848; found 274.1849.

5.1.9 6-(2,8-Diazaspiro[5.5]undecan-2-yl)-9H-purine (17)

Prepared as described for **12** starting from **7** and 6-chloropurine to give **17** as a yellow oil (48% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) 1.40-1.76 (8H, m), 2.46 (1H, d, *J* = 13.0 Hz), 2.67 (1H, dd, *J* = 11.5, 11.5 Hz), 2.98-3.10 (1H, br s), 3.11-3.14 (1H, m), 3.41 (1H, d, *J* = 13.0 Hz), 4.77-5.10 (2H, br m), 5.65-6.07 (2H, m), 7.88 (1H, s), 8.33 (1H, s); ^{13}C NMR (126 MHz, $CDCl_3$) 21.1, 21.8, 33.5, 34.2, 35.7, 45.0 (br), 46.1, 51.6, 117.0, 137.3, 151.4, 152.2, 153.4; LC-MS (ESI+) *m/z* 273 (M+H); HRMS *m/z* calcd. for $C_{14}H_{21}N_6$ (M+H) 273.1822, found 273.1835.

5.1.10 6-(1,8-Diazaspiro[4.5]decan-1-yl)-9H-purine (18)

Prepared as described for **12** starting from **5** and 6-chloropurine to give **18** as a colourless oil (7% over 2 steps). ^{1}H NMR (500 MHz, d_6-DMSO) 1.45-1.52 (2H, m), 1.88-1.96 (2H, m), 2.06-2.12 (2H, m), 2.87-2.99 (2H, m), 3.19-3.25 (2H, m), 3.44-3.56 (2H, m), 4.22-4.27 (2H, m), 8.07 (1H, s), 8.19 (1H, s); ^{13}C NMR (126 MHz, d_6-DMSO) 22.5, 29.7, 30.5, 36.1, 40.0, 51.2; 150.9, 4 quaternary carbons not observed. LC-MS (ESI+) *m/z* 260 (M+H); HRMS *m/z* calcd. for $C_{13}H_{19}N_6$ 259.1666, found 259.1663.

5.1.11 4-Benzyl-1-(isoquinolin-5-ylsulfonyl)piperidin-4-amine (19)

A solution of Et_3N (0.3 mL, 2.19 mmol), **3**[23] (100 mg, 0.53 mmol) and 5-isoquinoline sulfonylchloride (120 mg, 0.44 mmol) in CH_2Cl_2 (5 mL) was stirred at r.t. for 16 h. The mixture was diluted with H_2O (5 mL) and washed with H_2O (3 x 10 mL). The organic layer was dried and concentrated. Flash column chromatography eluting with 1-5% MeOH/CH_2Cl_2 gave **19** as a pale yellow oil (65 mg, 0.17 mmol, 33%). ^{1}H NMR (500 MHz, $CDCl_3$) 1.35-1.41 (2H, m), 1.73 (2H, ddd, *J* = 12.0, 12.0, 4.0 Hz), 2.61 (2H, s), 2.99 (2H, ddd, *J* = 12.0, 12.0, 4.0 Hz), 3.63 (2H, ddd, *J* = 12.0, 4.0, 4.0 Hz), 7.08-7.10 (2H, m), 7.31-7.22 (3H, m), 7.71 (1H, dd, *J* = 7.5, 7.5 Hz), 8.21 (1H, dd, *J* = 7.5, 1.0 Hz), 8.38 (1H, dd, *J* = 7.5, 1.0 Hz), 8.52 (1H, d, *J* = 6.0 Hz), 8.67 (1H, d, *J* = 6.0 Hz), 9.35 (1H, s); ^{13}C NMR (126 MHz, $CDCl_3$) 37.0, 41.8, 49.3, 51.5, 117.8, 125.9, 126.7, 128.2, 129.1, 130.5, 132.0, 133.0, 133.6, 133.9, 136.2, 145.0, 153.2; LC-MS (ESI+) *m/z* 382 (M+H); HRMS *m/z* calcd. for $C_{21}H_{24}N_3O_2S$ (M+H) 382.1584, found 382.1601.

5.1.12 5-(1,9-Diazaspiro[5.5]undecan-9-ylsulfonyl)isoquinoline (20)

A mixture of Et_3N (0.12 mL, 0.85 mmol), **4**[17] (50 mg, 0.17 mmol) and 5-isoquinoline sulfonyl chloride (89 mg, 0.34 mmol) in CH_2Cl_2 (1.7 mL) was stirred at r.t. for 16 h. The reaction was quenched with H_2O (5 mL) and washed with H_2O (3 x 5 mL). The organic layer was dried and concentrated. Flash column chromatography eluting with 0-5% MeOH/CH_2Cl_2 (0.1% NEt3) gave *tert*-butyl 9-(isoquinolin-5-ylsulfonyl)-1,9-diazaspiro[5.5]undecane-1-carboxylate as a light brown oil (50 mg, 0.11 mmol, 66%). A mixture of 4M HCl in dioxane (0.26 mL, 1.05 mmol) and *tert*-butyl 9-(isoquinolin-5-ylsulfonyl)-1,9-diazaspiro[5.5]undecane-1-carboxylate (47 mg, 0.11 mmol) in MeOH (1 mL) was stirred at r.t. for 16 h. The mixture was concentrated and purified by ion exchange chromatography on acidic resin eluting with 2M NH_3 in MeOH to give **20** as a dark orange oil (33 mg, 0.10 mmol, 91%). 1H NMR (500 MHz, $CDCl_3$) 1.34-1.38 (2H, m), 1.40-1.47 (2H, m), 1.52-1.58 (2H, m), 1.62 (2H, ddd, *J* = 10.0, 12.0, 4.0 Hz), 1.67-1.74 (2H, m), 2.69 (2H, t, *J* = 7.0 Hz), 3.11-3.20 (2H, m), 3.35-3.43 (2H, m), 7.72 (1H, dd, *J* = 7.5, 7.5 Hz), 8.22 (1H, d, *J* = 7.5 Hz), 8.40 (1H, d, *J* = 7.5 Hz), 8.54 (1H, d, *J* = 6.0 Hz), 8.70 (1H, d, *J* = 6.0 Hz), 9.36 (1H, s); ^{13}C NMR (126 MHz, $CDCl_3$) 19.8, 26.6, 35.0, 36.8, 40.3, 41.4, 48.0, 117.7, 125.9, 129.1, 132.0, 126.9, 133.1, 133.8, 145.1, 153.3; LC-MS (ESI+) *m/z* 346 (M+H); HRMS *m/z* calcd. for $C_{18}H_{24}N_3O_2S$ (M+H) 346.1584, found 346.1587.

5.1.13 5-(1,8-Diazaspiro[4.5]decan-8-ylsulfonyl)isoquinoline (21)

4M HCl in dioxane (1.77 mL, 7.10 mmol) was added to a solution of **5**[17] (171 mg, 0.71 mmol) in MeOH (4 mL) and stirred at r.t. for 16 h. The reaction mixture was concentrated to give crude 1,8-diazaspiro[4.5]decane dihydrochloride salt (150 mg). Et_3N (0.79 mL, 5.68 mmol) and 5-isoquinoline sulfonyl chloride (188 mg, 0.71mmol) were added to the crude 1,8-diazaspiro[4.5]decane dihydrochloride salt in CH_2Cl_2 (6 mL) and the mixture was stirred at r.t. for 16 h. The reaction was quenched with H_2O (5 mL) and washed with H_2O (3 x 5 mL). The organic layer was dried and concentrated. Flash column chromatography eluting with 5%

MeOH/CH_2Cl_2 gave **21** as an orange oil (41 mg, 0.12 mmol, 17% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) 1.71 (2H, dd, *J* = 7.5, 7.5 Hz), 1.81 (2H, ddd, *J* = 13.0, 9.0, 3.0 Hz), 1.88-1.96 (4H, m), 3.10 (2H, dd, *J* = 7.0, 7.0 Hz), 3.25-3.32 (2H, m), 3.38-3.46 (2H, m), 7.71 (1H, dd, *J* = 7.5, 7.5 Hz), 8.22 (1H, d, *J* = 7.5 Hz), 8.38 (1H, d, *J* = 7.5 Hz), 8.45 (1H, d, *J* = 6.0 Hz), 8.68 (1H, d, *J* = 6.0 Hz), 9.34 (1H, s); ^{13}C NMR (126 MHz, $CDCl_3$) 23.3, 34.6, 36.1, 42.8, 44.6, 62.9, 117.6, 126.0, 129.2, 131.9, 132.7, 133.9, 134.0, 145.2, 153.3; LC-MS (ESI+) *m/z* 332 (M+H); HRMS *m/z* calcd. for $C_{17}H_{22}N_3O_2S$ (M+H) 332.1427, found 332.1432.

5.1.14 5-(2,9-Diazaspiro[5.5]undecan-9-ylsulfonyl)isoquinoline (22)

A solution of Et_3N (0.16 mL, 1.20 mmol), **10** (70 mg, 0.24 mmol) and 5-isoquinoline sulfonyl chloride (126 mg, 0.48 mmol) in CH_2Cl_2 (2 mL) was stirred at r.t. for 16 h then quenched with H_2O (5 mL). The organic layer was washed with H_2O (3 x 5 mL), dried and concentrated. Flash column chromatography eluting with 50% EtOAc/hexane gave benzyl 9-(isoquinolin-5-ylsulfonyl)-2,9-diazaspiro[5.5]undecane-2-carboxylate as a clear oil (68 mg, 0.14 mmol, 59%). A mixture of CF_3CO_2H (4 mL) and benzyl 9-(isoquinolin-5-ylsulfonyl)-2,9-diazaspiro[5.5]undecane-2-carboxylate (58 mg, 0.13 mmol) was heated to 45 °C for 16 h. The mixture was cooled and concentrated. Column chromatography on a Biotage SNAP KP-NH column, eluting with 1% EtOH/CH_2Cl_2, gave **22** as a light yellow oil (10 mg, 0.03 mmol, 23%). ^{1}H NMR (500 MHz, $CDCl_3$) 1.40-1.71 (6H, m), 1.74-1.79 (2H, m), 2.80 (2H, s), 2.95-3.06 (4H, m), 3.19-3.23 (2H, m), 7.74 (1H, dd, *J* = 8.0, 7.5 Hz), 8.20-8.25 (1H, m), 8.39-8.43 (1H, m), 8.45 (1H, d, *J* = 6.0 Hz), 8.69 (1H, d, *J* = 6.0 Hz), 9.38 (1H, s); ^{13}C NMR (126 MHz, $CDCl_3$) 18.1, 30.4, 31.9, 33.4, 40.8, 44.1, 51.0, 117.5, 125.9, 129.1, 131.8, 132.5, 134.0, 134.3, 145.1, 153.3; LC-MS (ESI+) *m/z* 346 (M+H); HRMS *m/z* calcd. for $C_{18}H_{24}N_3O_2S$ (M+H) 346.1584, found 346.1593.

5.1.15 5-(2,8-Diazaspiro[4.5]decan-8-ylsulfonyl)isoquinoline (23)

Prepared as described for **20** starting from **8** and 5-isoquinoline sulfonyl chloride to give **23** as a colourless oil (29% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) 1.54 (2H, dd, *J* = 7.0, 7.0 Hz), 1.61-1.64 (4H, m), 2.12 (1H, br s), 2.69 (2H, s), 2.99 (2H, dd, *J* = 7.0, 7.0 Hz), 3.15-3.21 (2H, m), 3.22-3.28 (2H, m), 7.74 (1H, dd, *J* = 7.5, 7.5 Hz), 8.23-8.26 (1H, m), 8.41 (1H, dd, *J* = 7.5, 1.5 Hz), 8.49-8.52 (1H, m), 8.71 (1H, d, *J* = 6.5 Hz), 9.38 (1H, d, *J* = 1.0 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) 35.5, 37.0, 40.9, 43.5, 45.7, 57.4, 117.7, 125.8, 129.1, 131.9, 132.8, 133.8, 134.1, 145.1, 153.3; LC-MS (ESI+) *m/z* 332 (M+H); HRMS *m/z* calcd. for $C_{17}H_{22}N_3O_2S$ (M+H) 332.1427, found 332.1437.

5.1.16 5-(2,9-Diazaspiro[5.5]undecan-2-ylsulfonyl)isoquinoline (24)

Prepared as described for **20** starting from **6** and 5-isoquinoline sulfonyl chloride to give **24** as a colourless oil (52% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) 1.26-1.40 (6H, m), 1.60-1.66 (2H, m), 2.57-2.63 (2H, ddd, *J* = 12.5, 8.5, 3.5 Hz), 2.65-2.71 (2H, m), 2.96 (2H, s), 3.14-3.18 (2H, m), 7.69 (1H, dd, *J* = 7.5, 7.5 Hz), 8.19 (1H, d, *J* = 7.5 Hz), 8.36 (1H, d, *J* = 7.5 Hz), 8.46 (1H, d, *J* = 6.0 Hz), 8.65 (1H, d, *J* = 6.0 Hz), 9.32 (1H, s); ^{13}C NMR (126 MHz, $CDCl_3$) 20.7, 32.0, 34.8, 35.3, 41.7, 46.3, 53.8, 117.7, 125.9, 129.1, 131.9, 133.0, 133.6, 133.9, 144.9, 153.2; LC-MS (ESI+) *m/z* 346 (M+H); HRMS *m/z* calcd. for $C_{18}H_{24}N_3O_2S$ (M+H) 346.1584, found 346.1588.

5.1.17 5-(2,8-Diazaspiro[5.5]undecan-2-ylsulfonyl)isoquinoline (25)

Prepared as described for **20** starting from **7** and 5-isoquinoline sulfonyl chloride to give **25** as a colourless oil (71% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) 1.19-1.26 (2H, m), 1.37-1.41 (3H, m), 1.47-1.53 (1H, m), 1.61-1.68 (2H, m), 1.75-1.88 (1H, br s), 2.50 (1H, d, *J* = 12.5 Hz), 2.60-2.67 (2H, m), 2.79-2.85 (1H, m), 3.02 (1H, d, *J* = 12.0 Hz), 3.11 (1H, d, *J* = 12.0 Hz), 3.13-3.24 (2H, m), 7.64-7.77 (1H, m), 8.20 (1H, d, *J* = 8.2 Hz), 8.40 (1H, dd, *J* = 7.5, 1.0 Hz), 8.52 (1H, d, *J* = 6.0 Hz), 8.69 (1H, d, *J* = 6.0 Hz), 9.35 (1H, d, *J* = 1.0 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) 20.8, 22.1, 32.2, 32.8, 33.4, 46.3, 46.5, 52.5, 53.9, 117.8, 126.0, 129.1, 131.9, 133.0,

133.5, 134.1, 145.1, 153.2; LC-MS (ESI+) *m/z* 346 (M+H); HRMS *m/z* calcd. for $C_{18}H_{24}N_3O_2S$, 346.1584, found 346.1597.

5.1.18 5-(1,8-Diazaspiro[4.5]decan-1-ylsulfonyl)isoquinoline (26)

Prepared as described for **20** starting from **5** and 5-isoquinoline sulfonyl chloride to give **26** as a yellow oil (2% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) 1.67-1.72 (2H, m), 1.80-1.93 (2H, m), 2.05 (2H, dd, *J* = 13.0, 7.0 Hz), 2.58-2.70 (4H, m), 3.11-3.16 (2H, m), 3.38 (2H, dd, *J* = 7.0, 7.0 Hz), 7.71 (1H, dd, *J* = 7.5, 7.5 Hz), 8.20-8.21 (1H, m), 8.41 (1H, dd, *J* = 7.5, 1.0 Hz), 8.64 (1H, d, *J* = 6.0 Hz), 8.71 (1H, d, *J* = 6.0 Hz), 9.36 (1H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 22.4, 35.7, 37.3, 44.7, 49.4, 68.5, 117.5, 125.8, 127.7, 129.2, 131.9, 132.7, 133.3, 144.8, 153.1; LC-MS (ESI+) *m/z* 332 (M+H); HRMS *m/z* calcd. for $C_{17}H_{22}N_3O_2S$ (M+H) 332.1427, found 332.1439.

5.1.19 6-(3-Azaspiro[5.5]undecan-3-yl)-9H-purine (27)

Prepared as described for **11** starting from 3-azaspiro[5.5]undecane and 6-chloropurine to give **27** as a white solid (65%). mp 266-268 °C; ^{1}H (500 MHz, d_6-DMSO) 1.40-1.46 (14H, m), 4.09-4.18 (4H, br m), 8.07 (1H, s), 8.16 (1H, s), 12.91 (1H, s); ^{13}C (126 MHz, d_6-DMSO) 21.5, 26.8, 31.6, 36.3, 36.4, 40.4, 119.3, 138.1, 151.7, 152.3, 153.6; LC-MS (ESI+) *m/z* 272 (M+H); HRMS *m/z* calcd. for $C_{15}H_{22}N_5$ (M+H) 272.1870, found 272.1875.

5.1.20 9-(9H-Purin-6-yl)-1-oxa-9-azaspiro[5.5]undecane (28)

Prepared as described for **11** starting from 1-oxa-9-azaspiro[5.5]undecane and 6-chloropurine to give **28** as an off-white solid (14%). mp 267 °C (dec); ^{1}H NMR (500 MHz, d_6-DMSO) 1.37-1.48 (6H, m), 1.55-1.62 (2H, m), 1.87-1.92 (2H, m), 3.41-3.55 (2H, m), 3.59-3.65 (2H, m), 4.76-5.07 (2H, m), 8.08 (1H, s), 8.17 (1H, s), 12.94 (1H, s); ^{13}C (126 MHz, d_6-DMSO) 18.8, 26.3, 34.3, 35.5, 40.8, 60.5, 70.1, 119.2, 138.2, 151.8, 152.3, 153.6; LC-MS (ESI+) *m/z* 274 (M+H); HRMS *m/z* calcd. for $C_{14}H_{20}N_5O$ (M+H) 274.1662, found 274.1660.

5.1.21 9-(9H-Purin-6-yl)-2-oxa-9-azaspiro[5.5]undecane (29)

Prepared as described for **11** starting from 2-oxa-9-azaspiro[5.5]undecane and 6-chloropurine to give **29** as a white solid (60%). mp 268-269 °C; ^{1}H NMR (500 MHz, d_6-DMSO) 1.44-1.54 (8H, m), 3.41 (2H, s), 3.53-3.55 (2H, m), 4.10-4.19 (4H, br m), 8.08 (1H, s), 8.17 (1H, s), 12.97 (1H, s); ^{13}C (126 MHz, d_6-DMSO) 22.0, 31.7, 33.4, 33.5, 40.4, 68.5, 75.4, 119.2, 138.2, 151.8, 152.3, 153.6; LC-MS (ESI+) *m/z* 274 (M+H); HRMS *m/z* calcd. for $C_{14}H_{20}N_5O$ (M+H) 274.1662, found 274.1669.

5.1.22 tert-Butyl 9-benzyl-3-hydroxy-1,9-diazaspiro[5.5]undecane-1-carboxylate (31) and tert-butyl 9-benzyl-4-hydroxy-1,9-diazaspiro[5.5]undecane-1-carboxylate (32)

A mixture of 0.5 M 9-BBN in THF (2.6 mL, 1.31 mmol) and *tert*-butyl 9-benzyl-1,9-diazaspiro[5.5]undec-3-ene-1-carboxylate **30**[17] (130 mg, 0.43 mmol) was heated to reflux for 3 h. The reaction mixture was cooled to 0 °C and 1 M NaOH aq. (1.3 mL) was added followed by 15 % w/v H_2O_2 (65 μL) ensuring that the temperature did not exceed 10 °C. The reaction mixture was stirred at 0 °C for 30 min then extracted with Et_2O (3 x 10 mL). The combined organic extracts were dried and concentrated. Flash column chromatography eluting with 3% 2 M NH_3 in MeOH/CH_2Cl_2 gave **31** as a yellow oil (48 mg, 31%) and **32** as a yellow oil (50 mg, 32%). **31**: ^{1}H NMR (500 MHz, $CDCl_3$) 1.49 (9H, s), 1.52-1.69 (4H, m), 1.83-1.96 (3H, m), 2.40-2.47 (2H, m), 2.48-2.55 (2H, m), 2.65-2.74 (2H, m), 3.48 (1H, dd, *J* = 14.5, 6.5 Hz), 3.52 (2H, s), 3.68 (1H, dd, *J* = 14.0, 4.0 Hz), 3.82-3.87 (1H, m), 7.24-7.28 (1H, m), 7.30-7.36 (4H, m,); ^{13}C NMR (126 MHz, $CDCl_3$) 27.7, 28.5, 30.4, 33.7, 47.5, 50.2, 50.3, 56.9, 63.0, 66.4, 79.9, 126.9, 128.2, 129.1, 156.2, one quaternary carbon not detected; LC-MS (ESI^+) *m/z* 361 (M+H); HRMS *m/z* calcd. for $C_{21}H_{33}N_2O_3$ (M+H) 361.2486, found 361.2496. **32**: ^{1}H NMR (500 MHz, $CDCl_3$) 1.38-1.46 (2H, m), 1.49 (9H, s), 1.55-1.61 (1H, m), 1.65-1.71 (1H, m), 1.94-2.01 (1H, m), 2.09 (1H, dd, *J* = 13.0, 4.5 Hz), 2.32-2.39 (1H, m), 2.43-2.47 (2H, m), 2.52-2.56 (2H, m), 3.02 (1H, ddd, *J* = 13.0, 11.0, 2.0 Hz), 3.21 (1H, ddd, *J* = 14.0, 10.0, 5.0 Hz), 3.52 (2H, d, *J* = 6.0 Hz), 3.94 (1H, ddd, *J* = 14.0, 5.0, 5.0 Hz), 4.04 (1H, dddd, *J* = 10.0, 10.0, 5.0, 5.0 Hz), 7.24-7.28 (1H, m), 7.30-

7.35 (4H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 28.5, 33.3, 34.7, 36.5, 40.1, 42.7, 49.5, 50.5, 57.4, 63.0, 65.1, 79.7, 127.0, 128.2, 129.2, 138.8, 155.5; LC-MS (ESI^+) *m/z* 361 (M+H); HRMS *m/z* calcd. for $C_{21}H_{33}N_2O_3$ (M+H) 361.2486, found 361.2492.

5.1.23 9-(9H-Purin-6-yl)-1,9-diazaspiro[5.5]undecan-3-ol (33)

A mixture of $(HCO_2)NH_4$ (175 mg, 2.78 mmol), **31** (200 mg, 0.57 mmol) and Pd/C (20 mg, 10 wt%) in MeOH (3 mL) under N_2 was stirred at reflux for 5 h. The mixture was cooled to r.t. and passed through celite to remove palladium. Purification by ion exchange chromatography on acidic resin eluting with 2M NH_3 in MeOH gave *tert*-butyl 3-hydroxy-1,9-diazaspiro[5.5]undecane-1-carboxylate as a clear oil (120 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) 1.47 (9H, s), 1.54-1.64 (4H, m), 1.87-1.97 (2H, m), 2.13 (2H, br s), 2.59-2.68 (2H, m), 2.76-2.80 (2H, m), 2.90-2.97 (2H, m), 3.41 (1H, dd, *J* = 14.0, 7.0 Hz), 3.70 (1H, dd, *J* = 14.0, 4.0 Hz), 3.85-3.89 (1H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 27.1, 28.1, 29.6, 33.9, 34.3, 42.7, 42.8, 46.9, 56.7, 65.6, 79.4, 155.5; LC-MS (ESI^+) *m/z* 271 (M+H); HRMS *m/z* calcd. for $C_{10}H_{19}N_2O_3$ (M-^{t}Bu) 215.1390, found 215.1398. A mixture of Et_3N (0.16 mL, 1.55 mmol), *tert*-butyl 3-hydroxy-1,9-diazaspiro[5.5]undecane-1-carboxylate (61 mg, 0.23 mmol) and 6-chloropurine (35 mg, 0.23 mmol) in ^{n}BuOH (1 mL) was heated to 100 °C for 16 h. The mixture was cooled and concentrated to dryness. Preparative TLC, eluting with 5% 2 M NH_3 in MeOH/CH_2Cl_2 gave *tert*-butyl 3-hydroxy-9-(9*H*-purin-6-yl)-1,9-diazaspiro[5.5]undecane-1-carboxylate as an off-white solid (41 mg, 46%). A portion of this material (20 mg, 0.052 mmol) was dissolved in MeOH (1 mL) and 4 M HCl in dioxane (0.13 mL, 0.52 mmol) was added, followed by stirring at r.t. for 16 h. The mixture was concentrated and purified by ion exchange chromatography on acidic resin eluting with 2M NH_3 in MeOH to give **33** as a white solid (12 mg, 0.04 mmol, 80%). mp = 189 ^{o}C (dec); ^{1}H NMR (500 MHz, d_6-DMSO) 1.21-1.29 (1H, m), 1.40-1.48 (3H, m), 1.52-1.63 (2H, m), 1.66-1.74 (1H, m), 1.75-1.82 (1H, m), 2.39-2.49 (1H, dd, *J* = 13.0, 8.0 Hz), 2.78 (1H, dd, *J*

= 13.0, 3.5 Hz), 3.26-3.44 (1H, m), 3.74-3.92 (2H, br m), 4.45-4.75 (2H, br m), 8.08 (1H, s), 8.17 (1H, s); ^{13}C NMR (126 MHz, d_6-DMSO) 29.1, 33.1, 34.8, 37.0, 41.7, 47.6, 49.1, 66.8, 119.2, 138.2, 151.8 , 152.3, 153.5; LC-MS (ESI$^+$) *m/z* 289 (M+H); R_t = 0.71, Purity = 90%; HRMS *m/z* calcd. for $C_{14}H_{21}N_60$ (M+H) 289.1761, found 289.1771

5.1.24 9-(Isoquinolin-5-ylsulfonyl)-1,9-diazaspiro[5.5]undecan-3-ol (34)

A mixture of $(HCO_2)NH_4$ (175 mg, 2.78 mmol), **31** (200 mg, 0.57 mmol) and Pd/C (20 mg, 10 wt%) in MeOH (3 mL) was stirred at reflux for 5 h. The mixture was cooled to r.t. and passed through celite to remove palladium. Purification by ion exchange chromatography on acidic resin eluting with 2M NH_3 in MeOH gave *tert*-butyl 3-hydroxy-1,9-diazaspiro[5.5]undecane-1-carboxylate as a clear oil. A mixture of Et_3N (0.41 mL, 2.95 mmol), *tert*-butyl 3-hydroxy-1,9-diazaspiro[5.5]undecane-1-carboxylate (160 mg, 0.59 mmol) and 5-isoquinoline sulfonyl chloride (231 mg, 0.88 mmol) in CH_2Cl_2 (3 mL) was stirred at r.t. for 4 h. The reaction was quenched with H_2O (5 mL) and washed with H_2O (3 x 5 mL). The organic layer was dried and concentrated. Preparative TLC eluting with 5% MeOH/ CH_2Cl_2 gave *tert*-butyl 3-hydroxy-9-(isoquinolin-5-ylsulfonyl)-1,9-diazaspiro[5.5]undecane-1-carboxylate as a yellow oil (100 mg). 4M HCl in dioxane (0.50 mL, 2.0 mmol) was added to a solution of this material in MeOH (2 mL) and stirred at r. t. for 48 h. The mixture was concentrated and purified by ion exchange chromatography on acidic resin eluting with 2M NH_3 in MeOH, followed by preparative TLC eluting with 5% MeOH/CH_2Cl_2, to give **34** as a colourless oil (30 mg, 14% over 2 steps); ^{1}H NMR (500 MHz, $CDCl_3$) 1.25-1.31 (1H, m), 1.56-1.72 (6H, m), 1.74-1.81 (1H, m), 2.58 (1H, dd, *J* = 13.5, 6.5 Hz) 2.83 (1H, dd, *J* = 13.5, 3.0 Hz), 3.06-3.14 (2H, m), 3.40-3.45 (2H, m), 3.65 (1H, dddd, *J* = 6.5, 6.5, 3.0, 3.0 Hz), 7.73 (1H, dd, *J* = 7.5, 7.5 Hz), 8.23 (1H, d, *J* = 7.5 Hz), 8.40 (1H, dd, *J* = 7.5, 1.0 Hz), 8.53 (1H, d, *J* = 6.0 Hz), 8.69 (1H, d, *J* = 6.0 Hz), 9.37 (1H, s); ^{13}C NMR (126 MHz, $CDCl_3$) 27.5, 32.5, 33.2, 34.8, 40.9, 41.1, 46.1, 47.8, 65.8, 117.3, 125.4,

128.7, 131.5, 132.4, 133.2, 133.6, 144.5, 152.8; LC-MS (ESI^+) *m/z* 362 (M+H); HRMS *m/z* calcd. for $C_{18}H_{24}N_3O_3S$ (M+H) 362.1533, found 362.1522.

5.1.25 9-(9H-Purin-6-yl)-1,9-diazaspiro[5.5]undecan-4-ol (35)

Prepared as described for **33** starting from **32** and 6-chloropurine to give **35** as a colourless oil (36% over 3 steps). mp = 198 ^{0}C (dec); ^{1}H NMR (500 MHz, CD_3OD) 1.25 (1H, dd, *J* = 12.5, 10.5 Hz), 1.40 (1H, dddd, *J* = 12.0, 12.0, 12.0, 4.5 Hz), 1.66-1.70 (1H, m), 1.72-1.79 (1H, m), 1.80-1.82 (2H, m), 1.94-1.97 (1H, m), 2.09 (1H, dd, *J* = 12.5, 3.5 Hz), 2.83-2.88 (1H, m), 2.95-2.98 (1H, m), 3.87-3.93 (1H, m), 4.21-4.33 (4H, m), 7.99 (1H, s), 8.20 (1H, s); ^{13}C NMR (126 MHz, CD_3OD) 31.9, 35.0, 38.2, 38.9, 40.8, 40.9, 43.3, 51.7, 64.4, 118.8, 137.8, 150.9, 151.7, 153.6; LC-MS (ESI^+) *m/z* 289 (M+H); HRMS *m/z* calcd. for $C_{14}H_{21}N_6O$ (M+H) 289.1771, found 289.1778.

5.1.26 9-(Isoquinolin-5-ylsulfonyl)-1,9-diazaspiro[5.5]undecan-4-ol (36)

Prepared as described for **34** starting from **32** and 5-isoquinoline sulfonyl chloride to give **36** as a colourless oil (36% over 3 steps). ^{1}H NMR (500 MHz, $CDCl_3$) 1.00-1.08 (1H, m), 1.14-1.21 (1H, m), 1.44-1.60 (2H, m), 1.68-1.82 (3H, m), 1.87-1.91 (1H, m), 2.56-2.61 (1H, m), 2.86-2.88 (1H, m), 3.04-3.12 (2H, m), 3.40-3.45 (2H, m), 3.80-3.84 (1H, m), 7.71 (1H, dd, *J* = 7.5, 7.5 Hz), 8.21 (1H, d, *J* = 7.5 Hz), 8.37 (1H, d, *J* = 7.5 Hz), 8.52 (1H, d, *J* = 6.0 Hz), 8.67 (1H, d, *J* = 6.0 Hz), 9.35 (1H, s); ^{13}C NMR (126 MHz, $CDCl_3$) 32.1, 36.9, 38.9, 39.3, 41.3, 41.4, 46.4, 50.7, 65.4, 117.8, 125.9, 129.2, 132.0, 133.0, 133.6, 133.9, 145.0, 153.2; LC-MS (ESI^+) *m/z* 362 (M+H); HRMS *m/z* calcd. for $C_{18}H_{24}N_3O_3S$ (M+H) 362.1533, found 362.1528.

5.1.27 1,9-Dibenzyl-1,9-diazaspiro[5.5]undec-3-en-2-one (38)

Acryloyl chloride (1.33 mL, 16.6 mmol) was added to a solution of 4-allyl-*N*,1-dibenzylpiperidin-4-amine **37**[17] (3.80 g, 11.9 mmol), Et_3N (4.95 mL, 35.6 mmol) and DMAP (108 mg, 0.89 mmol) in CH_2Cl_2 (30 mL) at 0 °C. The reaction mixture was stirred for 16 h. Further acryloyl chloride (0.48 mL, 5.94 mmol) was added. After stirring for 16 h, the reaction mixture was diluted with H_2O (25 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The extracts were dried and concentrated. Ion exchange chromatography on acidic resin, eluting with 2M NH_3 in MeOH, gave *N*-(4-allyl-1-benzylpiperidin-4-yl)-*N*-benzylacrylamide as an orange oil (4.05 g, 10.8 mmol, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) 1.83-1.91 (2H, m), 2.19-2.24 (2H, m), 2.52-2.54 (2H, m), 2.69-2.71 (2H, m), 2.96 (2H, d, *J* = 12.0 Hz), 3.47 (2H, s), 4.57 (2H, s), 5.09-5.17 (2H, m), 5.54 (1H, dd, *J* = 8.0, 4.5 Hz), 5.75-5.84 (1H, m), 6.31-6.34 (2H, m), 7.22-7.32 (8H, m), 7.37-7.42 (2H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 33.0, 34.8, 48.9, 49.6, 61.3, 62.5, 118.1, 125.2, 126.5, 126.6, 126.9, 127.7, 128.4, 128.6, 131.1, 133.5, 137.7, 138.9, 168.5; LC-MS (ESI^+) *m/z* 375 (M+H); HRMS *m/z* calcd. for $C_{25}H_{31}N_2O$ (M+H) 375.2431, found 375.2441. $Ti(O^iPr)_4$ (3.00 mL, 11.9 mmol) was added to *N*-(4-allyl-1-benzylpiperidin-4-yl)-*N*-benzylacrylamide (4.45 g, 11.9 mmol) in CH_2Cl_2 (200 mL) and heated to reflux for 1 h. Grubbs I catalyst (389 mg, 0.474 mmol) was added and reflux was continued for 3 h. DMSO (0.84 mL) was added and the mixture was stirred for 16 h, then concentrated. Flash column chromatography, eluting with 50% EtOAc-hexanes, gave **38** (3.11 g, 9.01 mmol, 76%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$) 1.62-1.65 (2H, m,), 1.98-2.08 (4H, m), 2.54 (2H, dd, *J* = 4.5, 1.5 Hz), 2.73-2.75 (2H, m), 3.49 (2H, s), 4.81 (2H, s), 6.11 (1H, dt, *J* = 9.5, 1.5 Hz), 6.49 (1H, dt, *J* = 9.5, 4.5 Hz), 7.19-7.34 (10H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 31.6, 33.5, 43.1, 49.8, 58.1, 62.9, 125.4, 126.5, 126.6, 127.2, 128.3, 128.4, 129.1, 136.5, 137.8, 140.0, 165.5; LC-MS (ESI^+) *m/z* 347 (M+H); HRMS *m/z* calcd. for $C_{23}H_{27}N_2O$ (M+H) 347.2118, found 347.2112.

5.1.28 1,9-Dibenzyl-4-phenyl-1,9-diazaspiro[5.5]undecan-2-one (39)

Freshly prepared PhMgBr (5.9 mL, 1 M in THF) was added slowly to a suspension of $CuBr.SMe_2$ (607 mg, 2.96 mmol) in THF (12 mL) at -40 °C. The bright yellow solution was stirred for 30 min at -40 °C followed by addition of **38** (205 mg, 0.59 mmol) in THF (3 mL) and TMSCl (1.12 mL, 8.85 mmol) at -78 °C. The mixture was stirred at -78 °C for 1 h, then diluted with NH_4Cl (15 mL) and extracted with Et_2O (3 x 20 mL). The combined extracts were dried and concentrated. Ion exchange chromatography on acidic resin, eluting with 2 M NH_3 in MeOH, gave **39** (210 mg, 0.50 mmol, 84%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) 1.34-1.36 (1H, m), 1.69 (1H, dd, J = 13.5, 13.5 Hz), 1.82-1.85 (1H, m), 2.12-2.26 (4H, m), 2.69-2.73 (3H, m), 2.85-2.90 (2H, m), 3.15-3.22 (1H, m), 3.48 (2H, s), 4.45 (1H, d, *J* = 16.0 Hz), 5.18 (1H, d, *J* = 16.0 Hz), 7.20-7.34 (13H, m), 7.39-7.42 (2H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 32.2, 33.9, 38.0, 38.3, 40.2, 44.6, 49.6, 50.2, 59.1, 62.9, 126.3, 126.4, 126.7, 127.0, 127.2, 128.3, 128.3, 128.9, 129.0, 138.1, 139.4, 143.7, 170.8; LC-MS (ESI^+) *m/z* 425 (M+H); HRMS *m/z* calcd. for $C_{29}H_{33}N_2O$ (M+H) 425.2587, found 425.2608.

5.1.29 1,4,9-Tribenzyl-1,9-diazaspiro[5.5]undecan-2-one (40)

1M BnMgCl in Et_2O (7.23 mL, 7.23 mmol) was added slowly to a suspension of $CuBr.SMe_2$ (740 mg, 3.61 mmol) in THF (20 mL) under N_2 at -40 °C to give a bright yellow solution. After stirring for 30 min at -40 °C, **38** (250 mg, 0.72 mmol) in THF (5 mL) was added. The mixture

was stirred at r.t. for 16 h then quenched with NH_4Cl (25 mL). The mixture was extracted with Et_2O (3 x 20 mL), and the extracts were dried and concentrated. Ion exchange chromatography on acidic resin eluting with 2M NH_3 in MeOH gave **40** as a yellow oil (302 mg, 0.68 mmol, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) 1.10-1.20 (1H, m), 1.24 (1H, d, *J* = 11.0 Hz), 1.59-1.65 (1H, m), 1.88-1.96 (1H, m), 2.00-2.25 (6H, m), 2.43-2.53 (1H, m), 2.58-2.70 (4H, m), 2.71-2.76 (1H, m), 3.44 (2H, s), 4.34 (1H, d, *J* = 16.0 Hz), 5.12 (1H, d, *J* = 16.0 Hz), 7.13-7.23 (5H, m), 7.23-7.38 (10 H, m);. ^{13}C NMR (126 MHz, $CDCl_3$) 29.7, 32.1, 36.6, 37.8, 39.1, 42.5, 44.5, 49.6, 50.1, 58.9, 62.8, 126.2, 126.4, 126.4, 126.6, 127.2, 128.3, 128.5, 129.0, 129.0, 138.0, 139.0, 139.4, 171.1; LC-MS (ESI^+) *m/z* 439 (M+H); HRMS *m/z* calcd. for $C_{30}H_{35}N_2O$ (M+H) 439.2744, found 439.2727.

5.1.30 6-(4-Phenyl-1,9-diazaspiro[5.5]undecan-9-yl)-9H-purine (41)

$LiAlH_4$ (1 M in toluene, 1.15 mL) was added to a solution of **39** (122 mg, 0.290 mmol) in THF (2 mL) at 0 °C. The reaction mixture was heated to 50 °C for 4 h then cooled to r.t. H_2O (0.21 mL) and 20% NaOH aq. (0.21 mL) were added and the reaction mixture was stirred for 30 mins, followed by a second addition of H_2O (0.63 mL). The mixture was filtered and the filtrate was absorbed on to silica gel. Flash column chromatography, eluting with 30% EtOAc-hexanes, gave 1,9-dibenzyl-4-phenyl-1,9-diazaspiro[5.5]undecane as a clear oil (74 mg, 0.18 mmol, 63%). ^{1}H NMR (500 MHz, $CDCl_3$) 1.37-1.50 (2H, m), 1.63-1.66 (1H, m), 1.69-1.78 (1H, m), 1.88-1.96 (1H, m), 1.97-2.01 (1H, m), 2.21-2.38 (4H, m), 2.67-2.73 (3H, m), 2.75-2.90 (2H, m), 3.33 (1H, d, *J* = 14.0 Hz), 3.55 (2H, s), 4.19 (1H, d, *J* = 14.0 Hz), 7.21–7.29 (5H, m), 7.30–7.37 (8H, m), 7.37-7.41 (2H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 26.1, 31.7, 36.9, 37.8, 39.6, 46.2, 49.6, 50.0, 51.7, 54.5, 63.2, 126.1, 126.4, 126.9, 126.9, 128.1, 128.2, 128.2, 128.4, 129.1, 138.5, 141.8, 146.9; LC-MS (ESI^+) *m/z* 411 (M+H); HRMS *m/z* calcd. for $C_{29}H_{35}N_2$ (M+H) 411.2795, found 411.2779. A mixture of 1,9-dibenzyl-4-phenyl-1,9-diazaspiro[5.5]undecane (167 mg, 0.40

mmol) and Pd/C (17 mg) in MeOH (5 mL) was heated to reflux under H_2 (1 atm) for 48 h. The mixture was filtered and the filtrate was concentrated *in vacuo* to give crude 4-phenyl-1,9-diazaspiro[5.5]undecane as a yellow oil (76 mg, 0.33 mmol, 82%). A portion of the crude material (37 mg, 0.16 mmol), 6-chloropurine (24 mg, 0.16 mmol) and Et_3N (0.11 mL, 0.80 mmol) in ^{n}BuOH (1 mL) was stirred at 100 °C for 2 h. The mixture was cooled to r.t. and concentrated. Preparative HPLC gave **41** as a pale yellow solid (15 mg, 0.04 mmol, 27%). mp 217-219 °C; ^{1}H NMR (500 MHz, d_6-DMSO) 1.29-1.35 (1H, m), 1.40-1.59 (4H, m), 1.70 (2H, dd, J = 12.5, 3.0 Hz), 2.01-2.10 (1H, m), 2.85-2.89 (3H, m) 3.72-3.97 (2H, brd m), 4.55-4.81 (2H, brd m), 7.15-7.19 (1H, m), 7.21-7.24 (2H, m), 7.26-7.31 (2H, m), 8.08 (1H, s), 8.18 (1H, s); ^{13}C NMR (126 MHz, d_6-DMSO) 30.5, 34.4, 37.1, 39.0, 39.8, 40.1, 44.6, 49.8, 118.7, 125.8, 126.7, 128.3, 137.7, 146.9, 151.3, 151.8, 153.1; LC-MS (ESI$^+$) *m/z* 349 (M+H); HRMS *m/z* calcd. for $C_{20}H_{25}N_6$ (M+H) 349.2135, found 349.2130.

5.1.31 6-(4-Benzyl-1,9-diazaspiro[5.5]undecan-9-yl)-9H-purine (42)

1M $LiAlH_4$ in toluene (0.88 mL) was added to a solution of **40** (100 mg, 0.22 mmol) in THF (3 mL) at 0 °C. The mixture was heated to 50 °C for 3 h then cooled to r.t. H_2O (120 μL) followed by 20% NaOH aq (120 μL) were added and stirred for 30 min, followed by a second addition of H_2O (360 μL). The mixture was filtered and the filtrate was absorbed onto silica gel and purified by flash column chromatography, eluting with 20% EtOAc/hexane, to give 1,4,9-tribenzyl-1,9-diazaspiro[5.5]undecane as a yellow oil (75 mg, 0.18 mmol, 80%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.97-1.03 (1H, m), 1.16-1.28 (1H, m), 1.31-1.43 (2H, m), 1.74-1.85 (3H, m), 1.95-2.03 (1H, m), 2.09-2.17 (2H, m), 2.27-2.34 (1H, m), 2.44-2.59 (4H, m), 2.63-2.71 (1H, m), 2.72-2.81 (1H, m), 3.29 (1H, d, J = 14.0 Hz), 3.52 (2H, s), 4.08 (1H, d, J = 14.0 Hz), 7.14-7.18 (2H, m), 7.19-7.24 (2H, m), 7.27-7.37 (11H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 26.1, 29.9, 32.8, 36.3, 38.2, 43.5, 45.3, 49.0, 49.4, 50.9, 53.7, 62.7, 125.3, 125.8, 126.4, 127.6, 127.6, 128.4, 128.6, 128.6, 138.0,

140.1, 141.4; LC-MS (ESI$^+$) *m/z* 425 (M+H); HRMS *m/z* calcd. for $C_{30}H_{37}N_2$ (M+H) 425.2951, found 425.2971. Pd/C (17 mg) was added to 1,4,9-tribenzyl-1,9-diazaspiro[5.5]undecane (105 mg, 0.24 mmol) in MeOH (3 mL) and the flask was evacuated with air and refilled with 1 atm H_2 (x3). The mixture was heated to 50 ^oC for 4 days. The cooled mixture was filtered and the filtrate was concentrated to give a yellow oil that was used directly in next reaction. Et_3N (0.17 mL, 1.25 mmol) and 6-chloropurine (38 mg, 0.25 mmol) were added to a solution of the crude material from the previous step (61 mg, 0.24 mmol) in ^{n}BuOH (1 mL) and the mixture was heated to 100 ^oC for 16 h. The mixture was cooled to r.t. and concentrated. Flash column chromatography eluting with 10% MeOH/ CH_2Cl_2, followed by preparative TLC eluting with 10% MeOH/CH_2Cl_2, gave **42** as a pale yellow solid (30 mg, 33% over 2 steps); mp = 203 ^oC (dec); ^{1}H NMR (500 MHz, $CDCl_3$) 0.93-1.01 (1H, m), 1.08 (1H, dddd, *J* = 12.5, 12.5, 12.5, 4.5 Hz), 1.50-1.56 (1H, m), 1.58-1.74 (4H, m), 1.85-2.02 (2H, m), 2.44-2.57 (2H, m), 2.81 (1H, ddd, *J* = 13.0, 13.0, 3.5 Hz), 2.91-2.96 (1H, m), 3.90-4.10 (2H, br m), 4.48-4.72 (2H, br m), 7.08-7.23 (3H, m), 7.23-7.30 (2H, m), 7.93 (1H, s), 8.35 (1H, s); ^{13}C NMR (126 MHz, $CDCl_3$) 31.2, 33.4, 33.9, 40.6, 40.9, 40.9, 43.9, 44.1, 50.3, 119.6, 125.9, 128.2, 129.1, 136.6, 140.2, 151.2, 151.5, 153.9; LC-MS (ESI$^+$) *m/z* 363 (M+H); HRMS *m/z* calcd. for $C_{21}H_{26}N_6Na$ (M+H) 385.2111, found 385.2099.

5.1.32 1,3,9-Tribenzyl-1,9-diazaspiro[5.5]undec-3-en-2-one (43)

Oxalyl chloride (0.070 mL, 0.87 mmol), was added to a solution of 2-benzylacrylic acid (170 mg, 0.79 mmol) in CH_2Cl_2 (2 mL) followed by one drop of DMF. The mixture was stirred at r.t. for 2 h and then concentrated to give 2-benzylacryloyl chloride. Et_3N (0.36 mL, 2.65 mmol) was added to a solution of **37**[17] (170 mg, 0.53 mmol), 2-benzylacryloyl chloride (171 mg, 0.80 mmol) and DMAP (5 mg, 0.04 mmol) in CH_2Cl_2 (3 mL). The mixture was stirred at r.t. for 16 h, then diluted with H_2O (5 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The combined extracts were

dried and concentrated. Flash column chromatography, eluting with 30% EtOAc-hexanes, gave *N*-(4-allyl-1-benzylpiperidin-4-yl)-*N*,2-dibenzylacrylamide as a yellow oil (120 mg, 0.26 mmol, 49%). ^{1}H NMR (500 MHz, $CDCl_3$) 1.97-2.01 (2H, m), 2.25-2.37 (4H, m), 2.59-2.60 (2H, m), 2.87 (2H, d, *J* = 7.5 Hz), 3.46 (2H, s), 3.52 (2H, s), 4.49 (2H, s), 4.72 (1H, s), 5.04-5.07 (3H, m), 5.62 (1H, ddt, *J* = 17.5, 10.5, 7.5 Hz), 7.13-7.35 (15H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 33.2, 36.0, 40.7, 50.0, 50.7, 61.7, 62.9, 113.7, 118.3, 126.2, 126.6, 126.8, 127.0, 128.1, 128.5, 128.5, 129.1, 126.9, 134.1, 137.6, 138.2, 140.4, 146.7, 174.6; LC-MS (ESI$^+$) *m/z* 465.30 (M+H); HRMS *m/z* calcd. for $C_{32}H_{36}N_2O$ (M+H) 465.2900; found 465.2883. $Ti(O^iPr)_4$ (0.13 mL, 0.42 mmol), was added to *N*-(4-allyl-1-benzylpiperidin-4-yl)-*N*,2-dibenzylacrylamide (100 mg, 0.21 mmol) in CH_2Cl_2 (10 mL) and heated to reflux for 1 h. Hoveyda-Grubbs II catalyst (389 mg, 0.47 mmol) was added and reflux was continued for 30 h. DMSO (0.11 mL) was added and the mixture was stirred for 16 h, then concentrated. Flash column chromatography, eluting with 50% EtOAc-hexanes, gave **43** (60 mg, 0.14 mmol, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) 1.58-1.61 (2H, m), 1.96 (2H, ddd, *J* = 12.5, 12.5, 4.0 Hz), 2.05 (2H, ddd, *J* = 12.5, 12.5, 2.0 Hz), 2.46-2.53 (2H, m), 2.71-2.73 (2H, m), 3.47 (2H, s), 3.70-3.71 (2H, s), 4.80 (2H, s), 6.01 (1H, dd, *J* = 4.5, 4.5 Hz), 7.19-7.35 (15H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 31.4, 33.6, 36.8, 43.7, 49.8, 57.9, 62.9, 126.1, 126.5, 126.6, 127.1, 128.2, 128.3, 128.4, 129.1, 129.4, 131.4, 135.5, 137.19, 139.6, 140.1, 166.0; LC-MS (ESI$^+$) *m/z* 437.17 (M+H); HRMS *m/z* calcd. for $C_{30}H_{33}N_2O$ (M+H) 437.2587, found 437.2600.

5.1.33 6-(3-Benzyl-1,9-diazaspiro[5.5]undecan-9-yl)-9H-purine (45)

A mixture of **43** (40 mg, 0.09 mmol) and Pd/C (4 mg) in EtOH (1 mL) and THF (1 mL) with a drop of 1 M HCl was stirred at 30 °C under H_2 (1 atm) for 48 h. The mixture was filtered and the filtrate was concentrated to give 1,3,9-tribenzyl-1,9-diazaspiro[5.5]undecan-2-one HCl salt as a yellow oil (40 mg, 0.09 mmol). The material was dissolved in THF (1.0 mL), $LiAlH_4$ (1 M in

THF, 0.36 mL, 0.36 mmol) was added and the mixture was heated to 50 °C for 1 h, then cooled to r.t. The mixture was diluted with H_2O (14 μL) and 20% NaOH aq. (14 μL) and stirred for 30 min, followed by addition of H_2O (41 μL). The mixture was filtered and the filtrate was concentrated to give 1,3,9-tribenzyl-1,9-diazaspiro[5.5]undecane as a yellow oil (26 mg, 0.06 mmol, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) 1.21 (1H, ddd, *J* = 12.5, 3.5, 3.5 Hz), 1.29-1.38 (1H, m), 1.39-1.47 (1H, m), 1.50-1.58 (1H, m), 1.74-1.96 (4H, m), 2.07 (1H, ddd, *J* = 14.0, 10.5, 4.0 Hz), 2.23-2.40 (4H, m), 2.51 (1H, dd, *J* = 13.5, 6.0 Hz), 2.57 (1H, ddd, *J* = 12.5, 3.5, 1.5 Hz), 2.66-2.74 (1H, m), 2.79-2.85 (1H, m), 3.42 (1H, d, *J* = 14.0 Hz), 3.54 (2H, s), 3.94 (1H, d, *J* = 14.0 Hz), 7.05-7.08 (2H, m), 7.14-7.18 (1H, m), 7.20-7.25 (3H, m), 7.27-7.36 (9H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 26.0, 27.1, 31.0, 35.1, 35.8, 40.6, 49.6, 49.9, 51.9, 51.9, 53.6, 63.1, 125.6, 126.3, 127.0, 128.1, 128.2, 128.2, 129.0, 129.3, 129.3, 138.1, 140.6, 141.7; LC-MS (ESI^+) *m/z* 425.21 (M+H); HRMS *m/z* calcd. for $C_{30}H_{37}N_2$ (M+H) 425.2951; found 425.2970. A mixture of 1,3,9-tribenzyl-1,9-diazaspiro[5.5]undecane (42 mg, 0.10 mmol) and Pd/C (4 mg) in MeOH (4 mL) with 1 drop of 1 M HCl was stirred at 40 °C under H_2 (1 atm) for 24 h. The mixture was filtered and the filtrate was concentrated to give crude 3-benzyl-1,9-diazaspiro[5.5]undecane HCl salt (**44**) as a yellow oil (30 mg, 0.094 mmol). A mixture of crude **44** HCl salt (30 mg, 0.094 mmol), Et_3N (60 μL, 0.47 mmol) and 6-chloropurine (14 mg, 0.094 mmol) in ^{n}BuOH (0.5 mL) was stirred at 100 °C for 2 h. The mixture was cooled to r.t. and concentrated. The crude material was triturated with MeOH (1 mL) and the solid product was collected and washed with further MeOH (2 mL). The white solid was dried to give **45** (13 mg, 0.04 mmol, 38%). mp 265 °C (dec); ^{1}H NMR (500 MHz, d_6-DMSO) 1.15 (1H, ddd, *J* = 12.5, 12.5, 3.5 Hz), 1.23-1.30 (1H, m), 1.40-1.56 (6H, m), 1.87 (1H, ddd, *J* = 13.5, 4.0, 4.0 Hz), 2.36-2.49 (3H, m), 2.64 (1H, ddd, *J* = 13.0, 4.0, 2.0 Hz), 3.68-3.94 (2H, br m), 4.40-4.75 (2H, m), 7.13-7.18 (3H, m), 7.26 (2H, dd, *J* = 8.5, 6.5 Hz), 8.07 (1H, s), 8.16 (1H, s); ^{13}C NMR (126 MHz, d_6-DMSO) 26.7, 31.3, 36.7, 36.2, 39.4,

40.4, 40.7, 46.1, 49.2, 119.1, 126.1, 128.6, 129.3, 138.2, 141.1, 151.8, 152.3, 153.5; LC-MS (ESI^+) *m/z* 363.20 (M+H); HRMS *m/z* calcd. for $C_{21}H_{26}N_6$ (M+H) 363.2291, found 363.2294.

5.1.34 1,9-Dibenzyl-3-phenyl-1,9-diazaspiro[5.5]undec-3-en-2-one (46)

$(COCl)_2$ (0.34 mL, 4.03 mmol) was added to a solution of 2-phenyl acrylic acid (541 mg, 3.66 mmol) in CH_2Cl_2 (5 mL) under N_2, followed by the addition of one drop of DMF. The mixture was stirred for 2 h then concentrated to give crude 2-phenyl acryloyl chloride. EtN^iPr_2 (1.54 mL, 9.15 mmol) was added to a solution of **37**[17] (588 mg, 1.83 mmol), 2-phenyl acryloyl chloride (739 mg, 3.66 mmol) and DMAP (16 mg, 0.14 mmol) in CH_2Cl_2 (8 mL) under N_2. The mixture was stirred at r.t. for 16 h, then diluted with H_2O (15 mL). The mixture was extracted with CH_2Cl_2 (3 x 10 mL), and the combined extracts were dried and concentrated. Gradient column chromatography using a Biotage® KPNH column, eluting with 0-8% EtOAc/cyclohexane, gave *N*-(4-allyl-1-benzylpiperidin-4-yl)-*N*-benzyl-2-phenylacrylamide as a yellow oil (689 mg, 1.53 mmol, 84%); ^1H NMR (500 MHz, $CDCl_3$) 1.99 (2H, ddd, *J* = 12.5, 12.5, 4.0 Hz), 2.24 (2H, ddd, *J* = 12.0, 12.0, 2.5 Hz), 2.44-2.55 (2H, m), 2.68 (2H, ddd, *J* = 11.0, 3.5, 3.5 Hz), 3.05 (2H, d, *J* = 7.5 Hz), 3.46 (2H, s), 4.48 (2H, s), 5.09-5.18 (2H, m), 5.23 (1H, s), 5.38 (1H, s), 5.77 (1H, ddt, *J* = 17.5, 10.0, 7.5 Hz), 7.15-7.19 (2H, m), 7.20-7.37 (11H, m), 7.41-7.47 (2H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 33.3, 35.3, 50.0, 50.6, 61.9, 62.9, 113.2, 118.6, 125.8, 126.0, 126.7, 127.0, 128.2, 128.4, 128.4, 128.7, 129.1, 133.9, 136.0, 138.2, 139.8, 146.7, 173.0; LC-MS (ESI^+) *m/z* 451 (M+H); HRMS *m/z* calcd. for $C_{31}H_{34}N_2ONa$ (M+H) 473.2563, found 473.2550. A solution of *N*-(4-allyl-1-benzylpiperidin-4-yl)-*N*-benzyl-2-phenylacrylamide (700 mg, 1.50 mmol) in toluene (10 mL) was flushed with N_2 for 20 min. Dichloro[1,3-bis(2-methylphenyl)-2-imidazolidinylidene](2-isopropoxyphenyl-methylene) ruthenium (II) (30 mg, 0.05 mmol) was added and the mixture was heated to 80 °C for 16 h. A further 2 portions of ruthenium catalyst (2 x 30mg, 0.05 mmol) were added to the reaction over the 16 h period. The mixture was cooled to

r.t. and filtered through basic ion exchange resin washing with CH_2Cl_2 to remove the majority of the ruthenium. The filtrate was concentrated and purified by gradient chromatography, using a Biotage® KPNH column eluting with 0-12% EtOAc/cyclohexane, to give **46** as a yellow oil (481 mg, 1.13 mmol, 76%); ^{1}H NMR (500 MHz, $CDCl_3$) 1.71-1.74 (2H, m), 2.01-2.17 (4H, m), 2.69 (2H, d, *J* = 4.5 Hz), 2.76-2.78 (2H, m). 3.51 (2H, s), 4.88 (2H, s), 6.60 (1H, t, *J* = 4.5 Hz), 7.18-7.41 (14H, m), 7.49-7.51 (1H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 31.8, 33.8, 43.9, 49.9, 58.1, 62.9, 126.5, 126.9, 127.2, 127.6, 127.9, 128.3, 128.4, 128.7, 129.1, 133.1, 136.3, 137.0, 137.9, 140.2, 165.2; LC-MS (ESI^+) *m/z* 423.28 (M+H); HRMS *m/z* calcd. for $C_{29}H_{31}N_2O$ (M+H) 423.4321, found 423.2436.

5.1.35 3-Phenyl-1,9-diazaspiro[5.5]undecane (47)

Pd/C (40 mg) and one drop of 1 M HCl were added to a solution of **46** (405 mg, 0.95 mmol) in MeOH (10 mL) under N_2. The flask was evacuated of air and refilled with H_2 to 1 atm (x3). The mixture was heated to 40 °C for 5 d. The mixture was filtered and the filtrate was concentrated. Ion exchange chromatography on acidic resin eluting with 2M NH_3 in MeOH gave 1,9-dibenzyl-3-phenyl-1,9-diazaspiro[5.5]undecan-2-one as a yellow oil (245 mg) which was used directly in the next reaction. 1 M $LiAlH_4$ in THF (2.93 mL, 2.93 mmol) was added to a solution of crude 1,9-dibenzyl-3-phenyl-1,9-diazaspiro[5.5]undecan-2-one in THF (8 mL) under N_2 at r.t., followed by $AlCl_3$ (330 mg, 2.48 mmol). The mixture was stirred at 50 ^{0}C for 3 h then cooled to r.t. H_2O (0.5 mL) and 20% NaOH aq. (0.5 mL) were added. The suspension was stirred for 30 min followed by further addition of H_2O (1 mL). The mixture was filtered and the solids washed

with Et_2O (5 mL). The filtrates were concentrated to give 1,9-dibenzyl-3-phenyl-1,9-diazaspiro[5.5]undecane as a yellow oil (137 mg) which was used directly in the next reaction. $(HCO_2)NH_4$ (134 mg, 2.14 mmol) was added to a mixture of the crude 1,9-dibenzyl-3-phenyl-1,9-diazaspiro[5.5]undecane (137 mg) and Pd/C (13 mg) in 1,4-dimethoxyglycol (5 mL). The mixture was stirred at 60 ^{0}C for 16 h. The mixture was cooled and filtered, and the filtrate was concentrated. Preparative HPLC, followed by column chromatography eluting with 10-20% 2M NH_3 in MeOH/CH_2Cl_2, gave **47** as a colourless oil (16 mg, 0.07 mmol, 7% over 3 steps); ^{1}H NMR (500 MHz, $CDCl_3$) 1.44-1.52 (1H, m), 1.54-1.59 (1H, m), 1.79-1.91 (4H, m), 1.92-2.03 (1H, m), 2.17-2.20 (1H, m), 2.62-2.65 (1H, m), 2.79 (1H, dd, *J* = 13.5, 11.5 Hz), 2.98-3.09 (3H, m), 3.18-3.28 (2H, m), 3.42 (1H), 3.89 (1H), 7.20-7.26 (3H, m), 7.31-7.35 (2H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 26.8, 28.0, 37.0, 37.6, 39.5, 39.9, 44.4, 47.2, 48.4, 126.5, 127.0, 128.5, 143.9; LC-MS (ESI+) *m/z* 231 (M+H); HRMS *m/z* calcd. for $C_{15}H_{22}N_2Na$ (M+Na) 253.1675, found 253.1668.

5.1.36 6-(3-Phenyl-1,9-diazaspiro[5.5]undecan-9-yl)-9H-purine (48)

A mixture of Et_3N (50 µL, 0.35 mmol), **47** (16 mg, 0.07 mmol) and 6-chloropurine (10 mg, 0.07 mmol) in ^{n}BuOH (1 mL) was heated to 100 ^{o}C for 4 h. The mixture was cooled to r.t. and concentrated. The resulting solid was washed with MeOH (2 mL) to give the desired product (3 mg). The washings were concentrated and purified by column chromatography, eluting with 10% MeOH/CH_2Cl_2. This material was combined with the previously recovered solids to give **48** as a white solid (6 mg, 0.17 µmol, 25%). mp = 298 ^{o}C (dec); ^{1}H NMR (500 MHz, d_6-DMSO) 1.41

(1H, ddd, *J* = 13.0, 13.0, 3.5 Hz), 1.50-1.66 (3H, m), 1.69-1.75 (1H, m), 1.77-1.90 (2H, m), 2.03-2.10 (1H, m), 2.59-2.68 (1H, m), 2.81-2.88 (2H, m), 3.87-4.14 (2H, br m), 4.35-4.77 (2H, br m), 7.19-7.22 (1H, m), 7.27-7.34 (4H, m), 8.11 (1H, s), 8.20 (1H, s); ^{13}C NMR (126 MHz, d_6-DMSO) 27.4, 30.3, 36.3, 40.0, 40.3, 43.9, 47.1, 49.8, 119.2, 126.7, 127.6, 128.8, 138.3, 145.0, 151.8, 152.3, 153.5; LC-MS (ESI$^+$) *m/z* 349 (M+H); HRMS *m/z* calcd. for $C_{20}H_{25}N_6$ (M+H) 349.2135, found 349.2143.

5.1.37 Methyl 2-(((4-allyl-1-benzylpiperidin-4-yl)(benzyl)amino)methyl) acrylate (49)

A mixture of methyl 2-(bromomethyl)acrylate (0.49 mL, 4.29 mmol), K_2CO_3 (790 mg, 5.73 mmol) and **37**[17] (916 mg, 2.86 mmol) in MeCN (8 mL) was stirred at r.t. under N_2 for 18 h. The mixture was filtered and the filtrate was concentrated. Gradient chromatography on a Biotage® KPNH column, eluting with 0-8% EtOAc/cyclohexane, gave **49** as a light yellow oil, (1.14 g, 2.72 mmol, 95%); ^{1}H NMR (500 MHz, $CDCl_3$) 1.66-1.73 (2H, m), 1.84 (2H, ddd, *J* = 13.5, 10.0, 4.0 Hz), 2.19-2.27 (2H, m), 2.46 (2H, dt, *J* = 7.5, 1.5 Hz), 2.71 (2H, ddd, *J* = 10.5, 4.5, 4.5 Hz), 3.49-3.57 (4H, m), 3.66 (3H, s), 3.81 (2H, s), 5.07-5.16 (2H, m), 5.73-5.75 (1H, m), 5.92-5.95 (1H, m), 6.04 (1H, ddt, *J* = 17.5, 10.0, 7.5 Hz), 7.14-7.18 (1H, m), 7.20-7.27 (5H, m), 7.31-7.34 (4H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 32.9, 37.5, 49.7, 49.9, 51.5, 53.8, 58.4, 63.2, 117.3, 126.4, 126.4, 126.9, 127.9, 128.1, 128.7, 129.2, 135.2, 138.3, 139.8, 141.5, 167.4; LC-MS (ESI$^+$) *m/z* 419 (M+H); HRMS *m/z* calcd. for $C_{27}H_{35}N_2O_2$ (M+H) 419.2693, found 419.2699.

5.1.38 Methyl 1,9-dibenzyl-1,9-diazaspiro[5.5]undec-3-ene-3-carboxylate (50)

A solution of **49** (324 mg, 0.78 mmol) in toluene (8 mL) was flushed with N_2 for 20 min. Dichloro-[1,3-bis(2-methylphenyl)-2-imidazolidinylidene]-(2-isopropoxyphenylmethylene)-ruthenium (II), (15 mg, 0.026 mmol) was added and the mixture was heated to 80 °C for 16 h. A further 2 portions of ruthenium catalyst (2 x 15mg, 0.026 mmol) were added over the 16 h period. The reaction was cooled and filtered through basic ion exchange resin, washing with

CH_2Cl_2. The filtrate was concentrated and purified by gradient chromatography on a Biotage® KPNH column, eluting with 0-12% EtOAc/cyclohexane, to give **50** as a yellow oil (249 mg, 0.64 mmol, 82%); ^{1}H NMR (500 MHz, $CDCl_3$) 1.61 (2H, ddd, *J* = 13.0, 9.0, 3.0 Hz), 1.91-1.95 (2H, m), 2.17-2.18 (2H, m), 2.42-2.45 (2H, m), 2.72 (2H, ddd, *J* = 11.5, 9.0, 3.0 Hz), 3.31-3.33 (2H, m), 3.58-3.61 (4H, m), 3.69 (3H, s), 7.08 (1H, tt, *J* = 4.0, 1.5 Hz), 7.23-7.37 (10H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 31.7, 34.2, 44.2, 48.8, 50.4, 51.5, 51.8, 63.2, 126.6, 127.0, 127.5, 128.2, 128.2, 128.3, 129.3, 137.6, 138.3, 140.6, 166.9; LC-MS (ESI$^+$) *m/z* 391 (M+H), HRMS *m/z* calcd. for $C_{25}H_{31}N_2O_2$ (M+H) 391.2380, found 391.2381.

5.1.39 Methyl 9-(9H-purin-6-yl)-1,9-diazaspiro[5.5]undecane-3-carboxylate (51)

Pd/C (17 mg) and 1 drop of 1M HCl were added to **50** (177 mg, 0.45 mmol) in MeOH (5 mL) and the flask was evacuated of air and refilled with 1 atm H_2 (x3). The mixture was heated to 40 °C for 16 h. The mixture was filtered and the filtrate was concentrated. Ion exchange chromatography on acidic resin eluting with 2 M NH_3 in MeOH gave methyl 1,9-diazaspiro[5.5]undecane-3-carboxylate as a yellow oil (90 mg, 0.42 mmol, 93%). A mixture of Et_3N (0.3 mL, 2.10 mmol), methyl 1,9-diazaspiro[5.5]undecane-3-carboxylate (90 mg, 0.42 mmol) and 6-chloropurine (65 mg, 0.42 mmol) in ^{n}BuOH (4 mL) was heated to 100 ^{o}C for 4 h. The mixture was cooled to r.t. and concentrated. Gradient column chromatography using a Biotage SNAP KPNH column, eluting with 0-10% EtOH/CH_2Cl_2, gave **51** as a white solid (60 mg, 0.18 mmol, 43%). mp = 189-192 ^{o}C; ^{1}H NMR (500 MHz, d_6-DMSO) 1.21-1.26 (1H, m), 1.37 (1H, ddd, *J* = 14.0, 10.5, 4.0 Hz), 1.49-1.54 (3H, m), 1.72-1.83 (3H, m), 2.36 (1H, dddd, *J* = 13.0, 13.0, 10.0, 10.0 Hz), 2.76 (1H, dd, *J* = 13.0, 10.0 Hz), 2.90-2.94 (1H, m), 3.60 (3H, s), 3.64-3.90 (2H, br m), 4.43-4.85 (2H, br m), 8.07 (1H, s), 8.16 (1H, s); ^{13}C NMR (126 MHz, d_6-DMSO) 22.9, 32.0, 35.7, 38.4, 40.6, 42.5, 42.6, 48.8, 51.7, 119.1, 138.2, 151.8, 152.3, 153.5,

174.9; LC-MS (ESI+) *m/z* 331 (M+H); HRMS *m/z* calcd. for C16H22N6O2Na (M+Na) 353.1696, found 353.1693.

5.1.40 9-(9H-Purin-6-yl)-1,9-diazaspiro[5.5]undecane-3-carboxylic acid (52)

A mixture of LiOH (56 mg, 1.45 mmol) and **51** (60 mg, 0.18 mmol) in THF (2 mL), H_2O (0.8 mL) and MeOH (1.2 mL) was stirred at r.t. for 16 h and then concentrated. The solid was washed with MeOH (3 x 3 mL) to give **52** as white solid (15 mg, 0.05 mmol, 27%). mp = 261-263 °C; ^{1}H NMR (500 MHz, d_6-DMSO) 1.30 (1H, ddd, *J* = 14.0, 12.0, 4.0 Hz), 1.42 (1H, ddd, *J* = 14.0, 10.0, 4.0 Hz), 1.47-1.57 (3H, m), 1.61-1.71 (1H, m), 1.72-1.83 (2H, m), 2.18 (1H, dddd, *J* = 8.5, 8.5, 4.5, 4.5 Hz), 2.76 (1H, dd, *J* = 13.0, 9.0 Hz), 2.89 (1H, dd, *J* = 13.0, 4.0 Hz), 3.75-4.09 (2H, br m), 4.31-4.75 (2H, br m), 8.07 (1H, s), 8.16 (1H, s); ^{13}C NMR (126 MHz, d_6-DMSO) 23.3, 32.8, 35.2, 37.3, 40.4, 42.8, 43.2, 49.5, 119.0, 138.4, 151.9, 152.2, 153.5, 176.7; LC-MS (ESI$^+$) *m/z* 317 (M+H); HRMS *m/z* calcd. for $C_{15}H_{21}N_6O_2$ (M+H) 317.1721, found 317.1722.

5.1.41 (9-(9H-Purin-6-yl)-1,9-diazaspiro[5.5]undecan-3-yl)methanol (53)

1M $LiAlH_4$ in THF (0.14 mL, 0.14 mmol) was added to a solution of **51** (6 mg, 0.019) in THF (1 mL) under N_2 and the mixture was stirred at 50 °C for 4 h. The mixture was cooled to r.t. and H_2O (50 µL) and 20% w/w aq. NaOH (50 µL) were added. After stirring for 30 min, H_2O (150 µL) was added and the mixture was filtered, washing the solids with Et_2O (3 mL). The filtrate was concentrated and purified by ion exchange chromatography on acidic resin, eluting with 2M NH_3 in MeOH, to give **53** as a colourless oil (4 mg, 0.013 mmol, 68%); ^{1}H NMR (500 MHz, d_6-DMSO) 1.11-1.29 (2H, m), 1.33-1.47 (4H, m), 1.51-1.56 (2H, m), 1.67-1.75 (1H, m) 2.37 (1H, dd, *J* = 13.0, 10.0 Hz), 2.77 (1H, dd, *J* = 12.5, 3.5 Hz), 3.22-3.25 (2H, m), 3.75-3.86 (2H, m), 4.41-4.45 (2H, m), 7.47 (1H, s), 7.87 (1H, s); ^{13}C NMR (126 MHz, d_6-DMSO) 23.5, 31.5, 36.5, 39.9, 40.6, 43.5, 49.3, 64.8, 121.9, 148.4, 148.5, 153.2, one quaternary carbon signal not detected; LC-MS (ESI$^+$) *m/z* 303 (M+H); HRMS *m/z* calcd. for $C_{15}H_{23}N_6O$ (M+H) 303.1928, found 303.1928.

5.1.42 1,9-Dibenzyl-N-methyl-1,9-diazaspiro[5.5]undec-3-ene-3-carboxamide (54)

A mixture of LiOH (74 mg, 1.93 mmol) and **50** (94 mg, 0.24 mmol) in THF (4 mL), H_2O (1 mL) and MeOH (2 mL) was stirred at r.t. for 16 h and then concentrated to give the crude carboxylate salt. The material was dissolved in DMF (2 mL) under N_2 and HATU (119 mg, 0.31 mmol) and EtN^iPr2 (0.25 mL, 1.44 mmol) were added. The mixture was stirred for 30 min at r.t. 2M $MeNH_2$ in MeOH (0.48 mL, 0.96 mmol) was added and the mixture was stirred for a further 15 h. H_2O (5 mL) was added and the mixture was extracted with EtOAC (3x 5 mL). The extracts were dried and concentrated, using heptane as an azeotrope to remove traces of DMF. Gradient column chromatography using a Biotage® KPNH column, eluting with 30-60% EtOAc/cyclohexane, gave **54** as a yellow oil (48 mg, 0.12 mmol, 50% over 2 steps); ^{1}H NMR (500 MHz, $CDCl_3$) 1.61 (2H, ddd, *J* = 12.5, 9.0, 3.0 Hz), 1.90-1.92 (2H, m), 2.12-2.14 (2H, m), 2.40-2.43 (2H, m), 2.75 (2H, m), 2.81 (3H, d, *J* = 5.0 Hz), 3.28 (2H, s), 3.58-3.60 (4H, m), 5.47 (1H, d, *J* = 4.5 Hz), 6.70 (1H, dd, *J* = 5.5, 2.5 Hz), 6.69- 7.36 (10H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 26.3, 31.1, 34.2, 44.2, 48.8, 50.4, 51.9, 63.2, 126.7, 127.0, 128.2, 128.3, 128.4, 129.3, 130.2, 130.9, 138.3, 140.5, 167.7; LC-MS (ESI$^+$) *m/z* 390 (M+H); HRMS *m/z* calcd. for $C_{25}H_{32}N_3O$ (M+H) 390.2540, found 390.2542.

5.1.43 1,9-Dibenzyl-N,N-dimethyl-1,9-diazaspiro[5.5]undec-3-ene-3-carboxamide (55)

Prepared as described for **54** starting from **50** and Me_2NH (94% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) 1.65 (2H, ddd, *J* = 12.5, 9.0, 3.0 Hz), 1.92-2.03 (2H, m), 2.04-2.08 (2H, m), 2.42-2.45 (2H, m), 2.68-2.72 (2H, m), 2.90-3.02 (6H, m), 3.23 (2H, s), 3.60 (2H, s), 3.65 (2H, s), 5.90-5.94 (1H, m), 7.22-7.25 (1H, m), 7.25-7.37 (9H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 30.1, 34.4, 34.4, 38.6, 45.2, 48.8, 50.4, 51.9, 63.2, 125.2, 126.7, 126.9, 128.2, 128.3, 128.5, 129.3, 132.2, 138.4, 140.6, 171.2; LC-MS (ESI+) *m/z* 404 (M+H); HRMS *m/z* calcd. for $C_{26}H_{34}N_3O$ (M+H) 404.2696, found 404.2703.

5.1.44 (1,9-Dibenzyl-1,9-diazaspiro[5.5]undec-3-en-3-yl)(morpholino) methanone (56)

Prepared as described for **54** starting from **50** and morpholine (49% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) 1.65 (2H, ddd, *J* = 12.5, 9.0, 3.0 Hz), 1.95-1.98 (2H, m), 2.06-2.08 (2H, m), 2.43-2.45 (2H, m), 2.68-2.73 (2H, m), 3.22 (2H, s), 3.55-3.65 (12H, m), 5.92-5.93 (1H, m), 7.23-7.25 (10H, m); ^{13}C NMR (126 MHz, $CDCl_3$) 30.2, 34.4, 45.3, 48.8, 50.5, 51.9, 63.2, 63.2, 66.9, 125.7, 126.8, 127.0, 128.2, 128.4, 128.4, 129.3, 131.5, 138.3, 140.3, 170.2; LC-MS (ESI+) *m/z* 446 (M+H); HRMS *m/z* calcd. for $C_{28}H_{36}N_3O_2$ (M+H) 446.2802, found 446.2809.

5.1.45 N-Methyl-9-(9H-purin-6-yl)-1,9-diazaspiro[5.5]undecane-3-carboxamide (57)

Pd/C (7 mg) was added to **54** (63 mg, 0.16 mmol) in ^{i}PrOH (5 mL) with 1 drop of 1M CH_3CO_2H aq. The flask evacuated of air and refilled with 1 atm H_2 (x3). The mixture was heated to 40 °C for 24 h. Catalyst was removed by filtration, fresh catalyst was added and the reaction was repeated for a further 24 h at 40 °C. The mixture was filtered and the filtrate was concentrated. Ion exchange chromatography on acidic resin, eluting with 2 M NH_3 in MeOH gave *N*-methyl-1,9-diazaspiro[5.5]undecane-3-carboxamide as a yellow oil (36 mg). A mixture of Et_3N (0.12 mL, 0.85 mmol), *N*-methyl-1,9-diazaspiro[5.5]undecane-3-carboxamide (36 mg, 0.17 mmol) and 6-chloropurine (26 mg, 0.17 mmol) in ^{n}BuOH (1 mL) was heated to 100 ^{o}C for 4 h. The mixture was cooled to r.t. and concentrated. Flash column chromatography eluting with 10% 2 M NH_3 in MeOH/CH_2Cl_2, followed by gradient column chromatography using a Biotage® KPNH column and eluting with 0-15% EtOH/CH_2Cl_2, gave **57** as a colourless oil (13 mg, 0.04 mmol, 23% over 2 steps); ^{1}H NMR (500 MHz, CD_3OD) 1.37-1.42 (1H, m), 1.59-1.71 (2H, m), 1.76-1.95 (5H, m), 2.35 (1H, dddd, *J* = 11.0, 11.0, 4.5, 4.5 Hz), 2.74 (3H, s), 2.89 (1H, ddd, *J* = 13.0, 4.5, 1.5 Hz), 2.99 (1H, dd, *J* = 13.0, 10.5 Hz), 4.20-4.37 (4H, br m), 8.01 (1H, s), 8.20 (1H, s); ^{13}C NMR (126 MHz, CD_3OD) 23.0, 24.9, 31.0, 33.5, 38.7, 40.8, 41.0, 42.1, 43.9, 49.2, 118.9, 138.0, 151.0, 151.6, 153.7, 176.4; LC-MS (ESI^{+}) *m/z* 330 (M+H); HRMS *m/z* calcd. for $C_{16}H_{24}N_7O$ (M+H) 330.2037, found 330.2046.

5.1.46 N,N-Dimethyl-9-(9H-purin-6-yl)-1,9-diazaspiro[5.5]undecane-3-carboxamide (58)

Prepared as described for **57** starting from **55** and 6-chloropurine (24% over 2 steps). ^{1}H NMR (500 MHz, CD_3OD) 1.44-1.52 (1H, m), 1.67-1.88 (5H, m), 1.89-2.04 (2H, m), 2.73-2.80 (1H, m), 2.94-3.03 (4H, m), 3.06-3.17 (4H, m), 4.10-4.63 (4H, br m), 7.94 (1H, s), 8.33 (1H, s); ^{13}C NMR (126 MHz, CD_3OD) 22.8, 32.0, 34.6, 35.5, 37.2, 38.6, 39.5, 41.0, 42.4, 50.0, 119.6, 136.7, 151.1, 151.5, 153.8, 174.0; LC-MS (ESI+) *m/z* 344 (M+H); HRMS *m/z* calcd. for $C_{17}H_{26}N_7O$ (M+H) 344.2193, found 344.2192.

5.1.47 (9-(9H-Purin-6-yl)-1,9-diazaspiro[5.5]undecan-3-yl)(morpholino)methanone (59)

Prepared as described for **57** starting from **56** and 6-chloropurine (32% over 2 steps). ^{1}H NMR (500 MHz, CD_3OD) 1.39-1.48 (1H, m), 1.59-1.73 (2H, m), 1.76-1.81 (1H, m), 1.81-1.95 (4H, m), 2.77-2.86 (2H, m), 3.01 (1H, dd, J = 13.0, 10.5 Hz), 3.58-3.72 (8H, m), 4.21-4.40 (4H, br m), 8.02 (1H, s), 8.21 (1H, s); ^{13}C NMR (126 MHz, CD_3OD) 22.7, 30.9, 33., 38.7, 39.0, 40.7, 41.0, 41.9, 45.9, 49.2, 66.4, 66.6, 118.3, 137.7, 150.1, 151.6, 153.6, 173.7; LC-MS (ESI+) *m/z* 386 (M+H); HRMS *m/z* calcd. for $C_{19}H_{28}N_7O_2$ 386.2299, found 386.2307.

6. Supplementary Material

IC_{50} determinations for **12**, **14** (Table S1); Single concentration inhibition of 96 kinases by **41**, **42**, **45** (Table S2); Details of PKA protein expression, purification and crystallisation, and of data collection and refinement of the crystal structures of **12**-PKA, **14**-PKA, **16**-PKA, **41**-PKA; Experimental methods for the kinase inhibition assays.

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8. Declaration of conflict of interest

The authors are, or have been, employees of The Institute of Cancer Research, London which has a commercial interest in PKB, CHK1 and Aurora kinase inhibitors. Authors who are, or have been, employed by The Institute of Cancer Research are subject to a 'Rewards to Inventors Scheme' which may reward contributors to a programme that is subsequently licensed.

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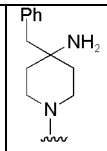
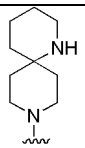
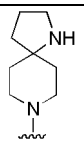
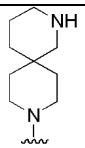
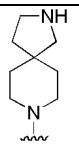
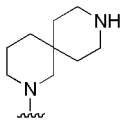
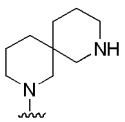
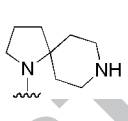
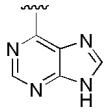
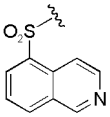
Table 1. Synthetic conditions and yields for the coupling and *N*-deprotection of the 4-benzyl-4-aminopiperidine **3** and spirocycles **4-10** (R^1) with the hinge binding heteroaromatic groups (R^2).

R^1 → R^2 ↓								
	**11** (72%)[a]	**12** (71%)[a, b]	**13** (68%)[a,c]	**14** (61%)[a,d]	**15** (53%)[a, b]	**16** (60%)[a, b]	**17** (48%)[a, b]	**18** (7%)[a, b]
	**19** (33%)[e]	**20** (60%)[e, b]	**21** (17%)[e, f]	**22** (14%)[e, d]	**23** (29%)[e, b]	**24** (52%)[e, b]	**25** (71%)[e, b]	**26** (2%)[e, b]

[a]6-Chloropurine, NEt_3, [n]BuOH, 100 °C.

[b]4 M HCl in dioxane, MeOH, r.t.

[c]6 M HCl, microwave irradiation, 100 °C; 15 min.

[d]CF_3CO_2H, 40 °C, 24 h.

[e]Isoquinolin-5-yl sulfonyl chloride, NEt_3, CH_2Cl_2.

[f]Prepared by sulfonylation of the unprotected diamine intermediate.

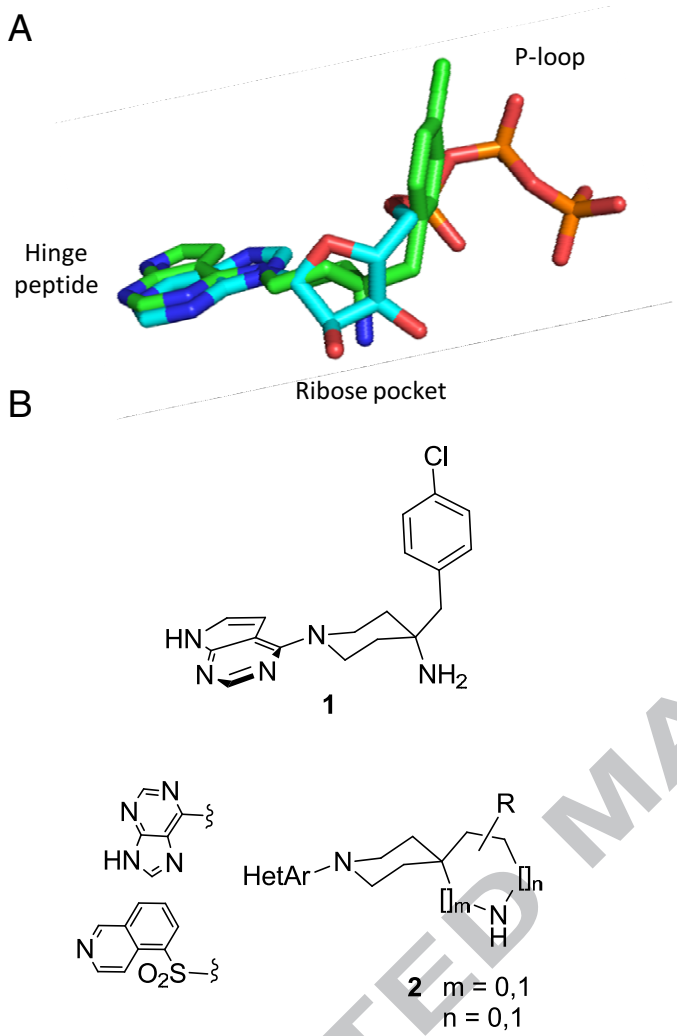


Figure 1 (A) Overlay of ATP (cyan carbons; PDB 1Q24) and **1** (green carbons, PDB 2VO6) in the bound conformations observed in the active sites of PKB–PKA chimeric proteins; (B) Design of diazaspirocyclic scaffolds to fulfil the pharmacophore defined by ATP and **1**.

			Kinase Family	AGC						CAMK					CMGC				TK								
			Kinase	PKA	AKT1	AKT2	MSK1	RSK1	PKC	MK2	MAPKAPK5	PKD2	CHK1	CHK2	Erk1	Erk2	GSK3β	p38a	ABL	FYN	INSR	LYN	LCK	MET	SRC	CK1d	AurA
Compounds	Purines		11	96	93	74	73	94	0	23	40	53	62	74	41	67	53	11	45	39	0	72	71	8	63	22	96
		Diazaspirocycles	12	98	87	57	88	60	0	24	11	42	85	58	46	50	28	3	33	34	4	52	25	7	25	21	81
			13	98	91	72	95	72	0	48	29	82	85	81	53	50	19	2	26	31	2	20	21	10	24	2	55
			14	100	98	87	98	81	41	74	51	91	97	72	55	50	49	0	82	63	4	70	64	18	70	23	67
			15	99	94	70	94	77	26	66	41	80	84	64	39	38	39	3	80	84	6	65	86	17	61	21	59
			16	98	85	26	89	71	21	19	3	55	71	35	16	28	32	2	32	33	3	33	15	1	19	25	70
			17	89	77	37	71	39	3	11	8	7	37	26	19	15	15	5	5	8	4	2	7	6	5	0	34
			18	39	12	0	13	13	0	17	8	8	8	26	13	10	43	5	8	14	9	5	9	4	6	21	18
	Isoquinolines		19	80	15	13	58	42	12	0	0	16	4	8	10	17	19	10	7	5	4	10	15	7	6	7	43
		Diazaspirocycles	20	89	27	10	76	66	0	14	25	24	0	26	0	9	28	7	12	9	3	18	15	3	12	11	71
			21	92	28	11	85	82	22	27	15	81	10	50	6	7	20	0	9	5	0	11	6	8	10	16	43
			22	77	0	14	36	29	9	8	11	15	8	65	0	13	5	9	0	11	6	5	9	21	35	3	24
			23	76	18	17	41	33	0	13	18	19	46	59	14	4	1	9	0	1	4	3	10	7	10	0	51
			24	83	24	17	39	29	4	0	0	21	2	13	0	2	3	2	0	2	4	9	11	14	5	8	36
			25	79	30	12	38	20	0	4	5	18	94	23	6	8	14	7	7	14	5	19	17	8	21	0	37
			26	77	27	13	47	16	5	10	7	2	15	12	7	0	9	0	0	1	3	3	6	4	5	9	27
	Carbo- and oxaza-cycles		27	55	16	6	35	45	2	10	6	5	17	25	12	18	40	6	20	33	8	21	35	11	32	11	71
			28	77	26	10	49	64	0	20	24	40	22	47	31	28	56	0	36	97	12	35	52	5	49	16	92
			29	67	25	6	45	46	7	15	9	17	26	28	41	35	66	3	33	36	2	34	37	4	41	21	67
	C-3 and C-4 substituted 1,9-diazaspiro[5.5]undecanes		33	99	93	59	89	67	0	11	30	48	71	53	46	42	31	3	25	18	4	26	21	15	38	15	80
			34	90	21	14	89	95	0	19	49	64	0	59	17	8	80	2	17	17	0	23	19	13	29	25	87
			35	98	94	59	93	40	0	8	32	23	80	44	28	32	19	4	23	28	0	39	14	17	22	11	63
			36	92	36	13	86	74	0	1	18	38	60	50	20	9	51	1	12	9	0	25	26	24	18	10	39
			41	100	99	89	97	86	0	3	18	72	77	78	44	56	20	0	45	62	2	75	75	11	77	25	82
			42	101	101	99	101	91	0	3	8	84	86	98	67	75	21	10	20	28	0	53	60	6	67	16	64
			45	95	80	37	75	50	1	15	7	32	69	58	31	41	14	6	31	20	18	38	44	4	33	18	89
			48	96	85	41	78	64	0	17	23	33	69	44	26	35	21	10	51	18	31	44	62	9	33	17	90
			51	87	61	19	48	35	0	6	21	36	36	16	14	19	12	8	18	6	10	16	16	11	26	4	64
			52	78	40	17	51	97	0	21	0	44	0	2	36	37	28	21	2	5	44	16	15	0	20	0	95
			53	98	80	38	88	74	0	22	43	45	27	56	31	32	21	14	15	1	17	16	15	81	34	12	84
			57	95	83	41	80	42	0	10	21	35	70	34	30	30	17	16	23	8	16	26	31	61	24	6	74
			58	86	69	27	93	28	6	10	18	30	54	27	20	25	7	14	17	0	17	14	19	7	16	9	69
			59	85	66	25	62	27	18	11	14	27	65	29	18	20	9	9	28	0	16	17	16	8	15	1	72
	H-89[a] (± SEM)			100 (±1.7)	97 (±0.4)	85 (±0.7)	99 (±0.2)	91 (±0.8)	64 (±0.8)	46 (±1.7)	41 (±1.9)	85 (±1,1)	90 (±0.8)	82 (±0.7)	48 (±1.9)	45 (±0.9)	11 (±1.2)	7 (±1.1)	12 (±1.2)	14 (±1.0)	29 (±1.2)	50 (±1.5)	22 (±1.5)	22 (±1.2)	4 (±2.6)	54 (±1.1)	75 (±1.5)
	Residue coresponding to Glu127 PKA[b]			Glu	Glu	Glu	Glu	Asp	Asp	Glu	Glu[c]	Met[c]	Glu	Glu	Asp[d]	Asp[d]	Thr[d]	Thr[d]	Asn	Ser	Asp	Ser	Ser	Asp	Ser	Ser[d]	Thr

[a]Mean (±sem) for n=19 independent determinations.
[b]Determined from sequence alignment and overlay of kinase domain crystal structures; corresponds to GK+7 residue unless indicated.
[c]Predicted by sequence alignment only. [d] GK+6 residue.

Figure 2. Heat map showing the % inhibition at 30 μM of 24 kinases by selected compounds from Table 1, Schemes 2, 3 and 4, grouped by *N*-substituent (n=1). Deeper colour corresponds to greater % inhibition. Data for the control compound H-89 is compiled from multiple assay determinations. The identity of the residue corresponding to Glu127 in PKA, is shown, determined by sequence alignment and overlay of kinase domain crystal structures.

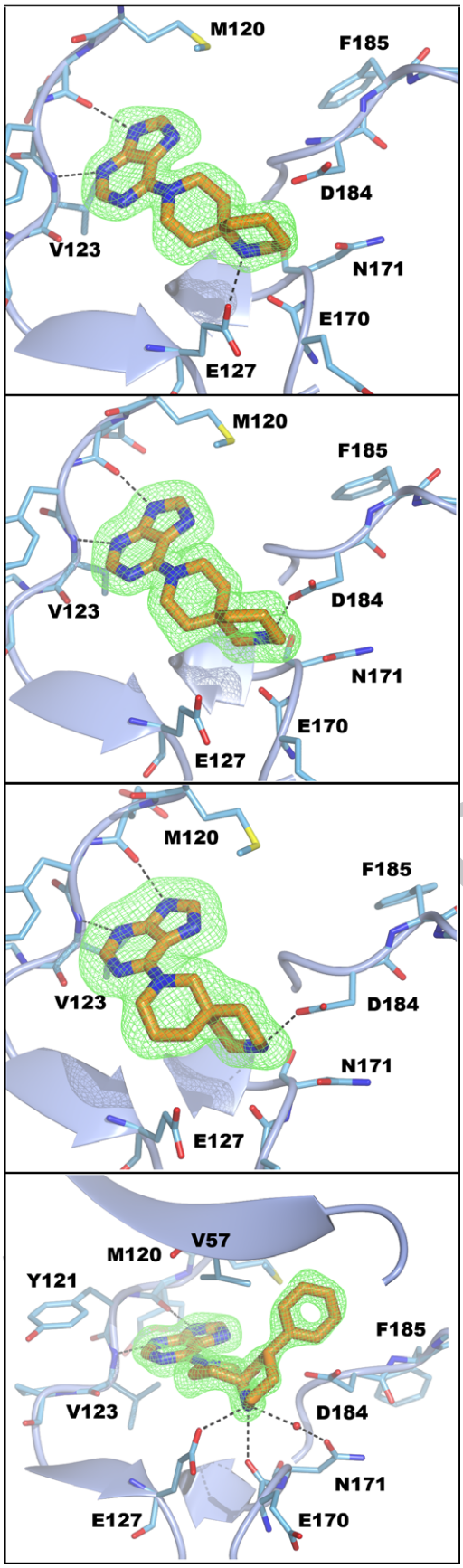


Figure 3. Crystal structures of PKA in complex with a) **12** (PDB 3ZO1); b) **14** (PDB 3ZO2); c) **16** (PDB 3ZO3); d) **41** (PDB 3ZO4). The ligands are shown with orange carbon atoms and key amino acid residues from the gatekeeper (M120), hinge peptide (E121 – P124), ribose binding pocket (E127) and DFG-motif (D184 – G186) are shown in light blue. Water molecules are shown as red spheres. Hydrogen bonding interactions are shown as dashed lines. The electron density shown in green is from an Fo-Fc omit map contoured at 3σ. The figures were prepared with CCP4MG.[48]

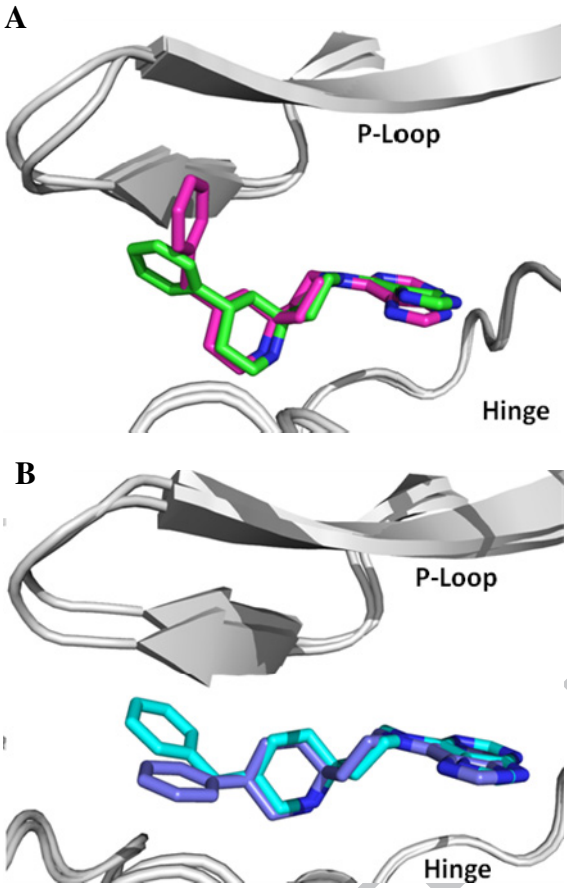
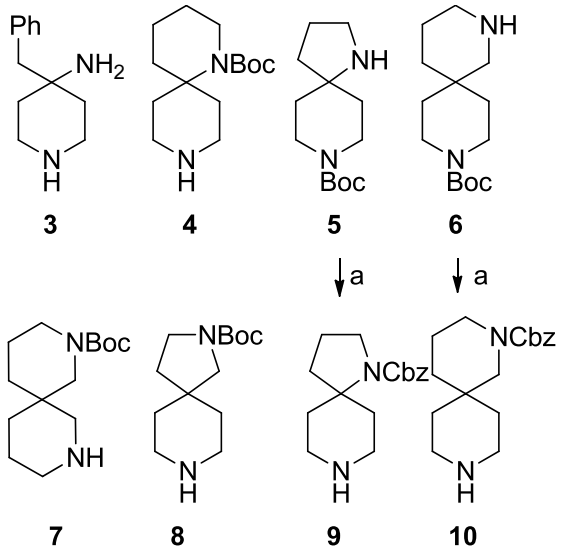


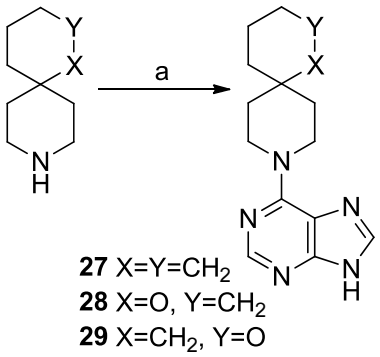
Figure 4. Model of 1,9-diazaspiro[5.5]undecanes bound to PKA, generated by energy minimization of the elaborated structure of **12** within the PKA protein using the MMF94X force field within MOE;[47] A) Comparison of 4-substituted 1,9-diazaspiro[5.5]undecanes **41** (green) and **42** (magenta); B) Comparison of 3-substituted 1,9-diazaspiro[5.5]undecanes **45** (cyan) and **48** (blue). The P-loop and hinge peptide of PKA are shown as cartoons (grey).

Scheme 1.[a] 4-Benzyl-4-aminopiperidine **3** and *N*-protected, unsubstituted [4.5]- and [5.5]-diazaspirocyclic scaffolds **4-10**.



[a] Reagents and conditions: a) i) Cbz-Cl, NEt_3, CH_2Cl_2, 65-82% then ii) 4 M HCl in dioxane, MeOH, 0-4°C, 80-100%.

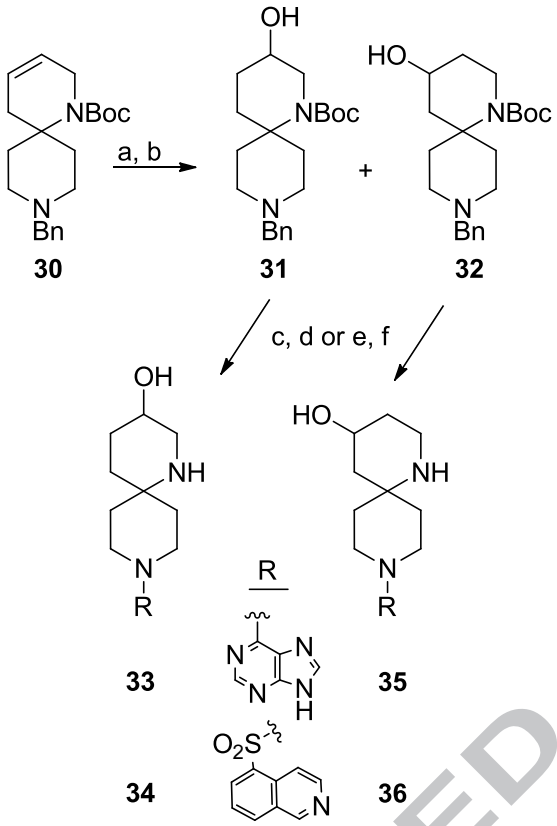
Scheme 2.[a] Preparation of 3-azaspiro[5.5]undecane, 1-oxa- and 2-oxa-9-azaspiro[5.5]undecane derivatives **27-29**.



27 X=Y=CH_2
28 X=O, Y=CH_2
29 X=CH_2, Y=O

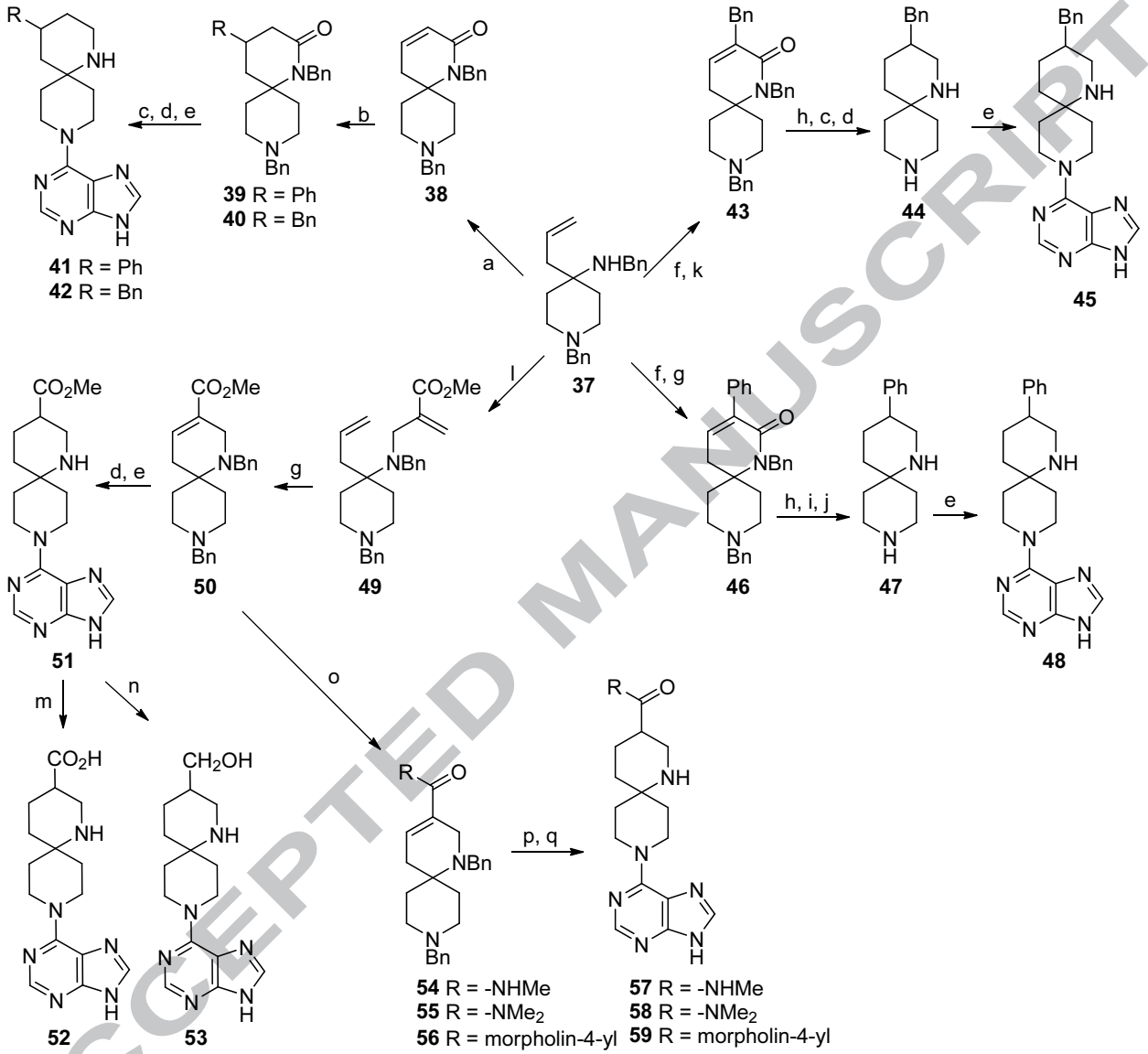
[a] Reagents and conditions: a) 6-chloropurine, NEt_3, ^{n}BuOH, 100 ^{o}C, 14-65%.

Scheme 3. Synthesis of compounds **33-36** containing racemic 1,9-diazaspiro[5.5]undecan-3-ol and 1,9-diazaspiro[5.5]undecan-4-ol scaffolds.[a]



[a]Reagents and conditions: a) i) 0.5 M 9-BBN, THF, 0 °C-reflux, ii) 1 M NaOH, 15% H_2O_2; b) separation by chromatography, **46** 31%, **47** 32%; c) HCO_2NH_4, 10% Pd/C, MeOH, reflux, 86-100%; d), 6-chloropurine, NEt_3, ^{n}BuOH, 100°C, reflux, 45-46%; e), isoquinolin-5-yl sulfonyl chloride, NEt_3, CH_2Cl_2, 37-42%; f) 4 M HCl in dioxane, MeOH, 80-86%.

Scheme 4.[a] Preparation of phenyl-, benzyl-, carboxylate- and carboxamide-substituted racemic 1,9-diazaspiro[5.5]undecane derivatives **41**, **42**, **45**, **51**-**53** and **57**-**59**.



[a] Reagents and conditions: a) i) Acryloyl chloride, DMAP, Et_3N, CH_2Cl_2, 91%; ii) Grubbs I catalyst, $Ti(^iPrO)_4$, CH_2Cl_2, reflux, 76%; b) CuBr.SMe_2, PhMgBr or BnMgBr, TMSCl, THF, -40 °C to -78 °C, 84-95%; c) $LiAlH_4$, THF, 50 °C, 63-80%; d) 10% Pd/C, H_2, MeOH, 50 °C, 82-100%; e) 6-chloropurine, Et_3N, ^nBuOH, 100 °C, 23-43%; f) 2-phenyl- or 2-benzylacryloyl chloride, DMAP, Et_3N or EtN^iPr_2, CH_2Cl_2 49-84%; g) Hoveyda-Grubbs II catalyst, $Ti(^iPrO)_4$, CH_2Cl_2, 40 °C, 66%; h) 10% Pd/C, H_2, EtOH, HCl, 30 °C, 100%; i) 10% Pd/C, H_2, MeOH, HCl, 40 °C, 60-94%; j) Dichloro[1,3-bis(2-methylphenyl)-2-imidazolidinylidene](2-isopropoxyphenylmethylene)ruthenium (II), toluene, 80 °C, 76-82%; k) 1 M $LiAlH_4$, $AlCl_3$, THF, 50 °C, 55%; l) 10% Pd/C, $(HCO_2)NH_4$ 1,2-dimethoxyglycol, 16%; m) methyl 2-(bromomethyl)acrylate, K_2CO_3, MeCN, 95%; n) LiOH, THF:MeOH:H_2O (3:2:1), 27%; o) i) LiOH, THF:MeOH:H_2O

(3:2:1); ii) HATU, DIPEA, DMF, R^1R^2NH, 49-94% (2 steps); p) 10% Pd/C, H_2, ^{i}PrOH, AcOH, 100%.

Graphical Abstract

Synthesis and Evaluation of Heteroaryl Substituted Diazaspirocycles as Scaffolds to Probe the ATP-binding site of Protein Kinases

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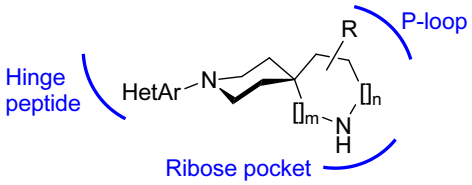

R = OH, CH_2OH, Ph, CH_2Ph, CO_2R, CONHR

m, n = 0, 1