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Structure-based design of isoquinoline-5-sulfonamide inhibitors of protein kinase B

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Abstract—Structure-based drug design of novel isoquinoline-5-sulfonamide inhibitors of PKB as potential antitumour agents was investigated. Constrained pyrrolidine analogues that mimicked the bound conformation of linear prototypes were identified and investigated by co-crystal structure determinations with the related protein PKA. Detailed variation in the binding modes between inhibitors with similar overall conformations was observed. Potent PKB inhibitors from this series inhibited GSK3β phosphorylation in cellular assays, consistent with inhibition of PKB kinase activity in cells.

1. Introduction

Protein kinase B (PKB, or Akt) is an important protein involved in the control of cell proliferation and survival, and is a key downstream component of the phosphatidylinositol-3 kinase (PI3K) signalling pathway.[1–5] In response to receptor tyrosine kinase activation by extracellular hormones, PI3K activation in turn produces phosphatidylinositols anchored to the inner side of the plasma membrane. Cytosolic PKB binds to these through a pleckstrin homology (PH) domain, translocating the protein to the plasma membrane, where it is activated by phosphorylations on Ser473 and Thr308. Activated PKB phosphorylates a variety of protein substrates, including, for example, GSK-3β, FKHRL1, BAD and mTOR, and signals to pathways controlling cell proliferation, protein translation, cell survival and apoptosis, and progression through the cell cycle. There are 3 known isoforms of PKB (α, β and γ). Recent in vitro research suggests that dual inhibitors of PKB-α and PKB-β may be more effective than inhibitors of either isoform alone.[6,7]

There is substantial evidence to suggest that deregulation of signalling on the PI3K pathway, and in particular through PKB, is intimate to the development of some human cancers.[2,3] Loss of function of the lipid phosphatase PTEN, which dephosphorylates 3′-phosphatidylinositols, results in overactivation of signalling through PKB. PTEN is frequently deleted or mutated in human tumours, notably glioblastoma, endometrial and prostate cancers. Amplification and mutation of the gene encoding the PI3K 110α catalytic subunit are also frequently observed in human cancers, particularly ovarian and cervical tumours, resulting in overactivation of the pathway. The overexpression of Erb2 also leads to constitutive PKB activity in breast and other tumours. For these reasons, inhibitors that prevent the phosphorylation of substrates by PKB offer potential as therapies for human malignancies.[8]

Blockade of signalling via PKB might be achieved by preventing the phosphorylation, and hence the activation, of the enzyme by inhibition of upstream kinases such as PDK1 or through inhibition of the association of the PH domain of the enzyme with PIs.[6,7] Alternatively,

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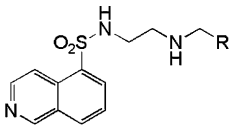


H-8 R = H
H-89 R = *trans*-CH=CH(4-Br-Ph)
NL-71-101 R = CH=C(Ph)$_2$

Figure 1. Known isoquinoline-5-sulfonamide inhibitors of PKA/PKB.

direct inhibition of the kinase activity of PKB is possible, and a number of ATP-competitive inhibitors of PKB have been identified.[9–14] Most recently, preliminary reports of in vivo efficacy in rodent models have been made for a series of pyridine-isoquinolines.[12,15]

As part of a programme to discover and optimise new, small-molecule, ATP-competitive inhibitors of PKB as potential cancer therapeutics,[16] we have explored compounds based on the isoquinoline-5-sulfonamide kinase inhibitor template.[17] Prior to this study, a combinatorial chemistry approach was adopted by Levitski et al.[11] to convert the PKA-selective inhibitor H-89 (Fig. 1) to the PKB/PKA inhibitor NL-71-101. We anticipated that alternative modifications to the structure of H-89 would reveal other scaffolds capable of generating PKB inhibitors with improved physicochemical properties. In particular, we sought replacements for the potentially metabolically labile alkenyl functionalities of H-89 and NL-71-101, and to reduce the lipophilicities of these ligands (*C*log*P* 4.4 and 5.0, respectively). Selectivity for PKB over other AGC kinases was also a consideration, although this was anticipated to be a challenge for PKA since the enzymes have a close sequence (~68%) homology in the kinase domain,[18] and within the adenine binding site itself only three residues differ between PKBβ and PKAα.[19] The availability of the X-ray crystal structure of PKBβ[20] offered the opportunity to use structure-based design techniques in pursuit of novel inhibitors. Such an approach has been taken previously using a PKA-PKB chimeric protein, to gain insight into the differences between the two enzymes.[19] As a result, ATP-competitive inhibitors with selectivity for PKB over PKA have been developed from templates derived from the natural product balanol, that exploit selectivity features outside the adenine and ribose binding regions.[9,10] A structure-based approach, again using balanol derivatives as the starting point, was used to delineate the determinants of selectivity between PKA and PKC.[21] The PKB structure has also been used in a virtual screen to identify inhibitors of the enzyme.[22]

2. PKB modelling and docking studies

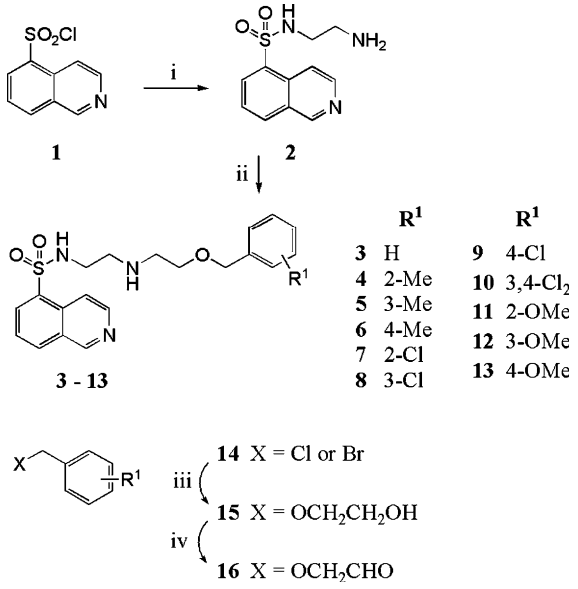
Compounds were docked into a binding pocket consisting of all the protein atoms within a 6 Å shell around the AMP–PNP ligand of the available PKB structure[20] (PDB 106K). Docking was performed with the program GOLD,[23] using the 'Default 3' Genetic Algorithm settings,[24] and docked conformations were scored using the Chemscore scoring function.[25] In order to reduce the number of unacceptable docking solutions, a pharmacophoric restraint was constructed[26] based on the available crystal structures of isoquinoline sulfonamides (H-series) complexed with PKA[27] (PDB 1YDR, 1YDS and 1YDT). The pharmacophoric restraint contained an acceptor term representing the binding of the isoquinoline nitrogen to the hinge region of the protein, a hydrophobic term centred on the isoquinoline bicycle and a term, composed of two acceptors and one donor, to represent the position of the sulfonamide group. The radius used for individual atoms in the pharmacophore was 0.7 Å.[26] To define the most favourable rotational conformation of the sulfonamide, an extensive search of crystal structures from the PDB and CCDC revealed the conformation observed for H-8 (PDB 1YDS) to be the global minimum, in which one S=O bond lies parallel to the plane of the aromatic group. The alternative arrangement seen for H-7 [1-(5-isoquinolinylsulfonyl)-2-methylpiperazine] (PDB 1YDR), in which the sulfonyl group is twisted out of the plane of the aromatic ring, was determined as a local minimum. Once identified, the global minimum conformation was used to select preferred docking solutions and for the design of new molecules.

A proprietary virtual screening platform[28] was used to generate suggested elaborations of the isoquinoline sulfonamide template. Virtual libraries were constructed from the coupling of isoquinolin-5-yl sulfonyl chloride and commercially available amines, and the libraries were virtually screened using the docking methodology described above with 'Default 4' Genetic Algorithm settings.[24] The selection of compounds for synthesis was made by analysing the binding mode, the conformation, the number of hydrogen bonds formed to key residues of PKB and the value of the scoring function.

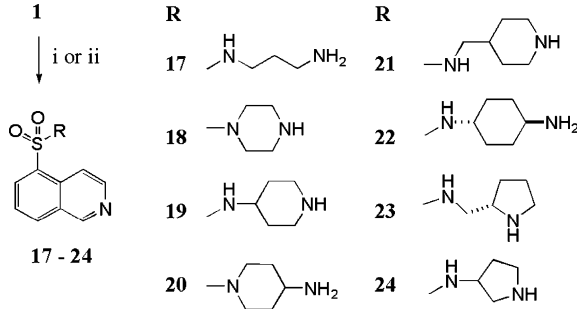
3. Synthetic chemistry

Direct mono-sulfonylation of ethane-1,2-diamine with freshly prepared isoquinoline-5-sulfonyl chloride **1**[29] gave the sulfonamide **2**,[29,30] which was further elaborated by reductive amination with 2-benzyloxyacetaldehyde to provide the benzyloxyethyl substitution product **3** (Scheme 1).[31] To prepare aryl-substituted analogues **4-13**, ethylene glycol was mono-alkylated with the appropriate benzyl halides **14** and the resulting alcohols **15** were oxidised to the aldehydes **16** using Dess–Martin reagent.[32,33] Reductive amination of the crude aldehydes **16** with **2** furnished the analogues **4**–**13**.

The unsubstituted analogues **17**[29] and **18** were prepared straightforwardly by mono-sulfonylation of symmetrical diamines. Various cyclic diamines were also mono-sulfonylated with **1** (Scheme 2). As previously demonstrated,[34] the reaction of unprotected 4-aminopiperidine led exclusively to sulfonylation of the primary amine to give **19**. Similar regioselectivity was seen in the sulfonylations giving **21**,[34] **23**[34] and **24**.[34] The regioisomeric piperidine sulfonamide **20** was prepared from commercially available Boc-protected 4-aminopiperidine followed by deprotection. The unambiguous synthesis of **20**



Scheme 1. Reagents and conditions: (i) ethylenediamine, CH_2Cl_2, rt; (ii) **17**, $NaB(OAc)_3H$, $ClCH_2CH_2Cl$, AcOH, 4 Å molecular sieves, rt; (iii) ethylene glycol, NaH, THF, reflux; (iv) Dess–Martin periodinane, CH_2Cl_2, rt. R^1 **3** H, **4** 2-Me, **5** 3-Me, **6** 4-Me, **7** 2-Cl, **8** 3-Cl, **9** 4-Cl, **10** 3,4-Cl_2, **11** 2-OMe, **12** 3-OMe, **13** 4-OMe.

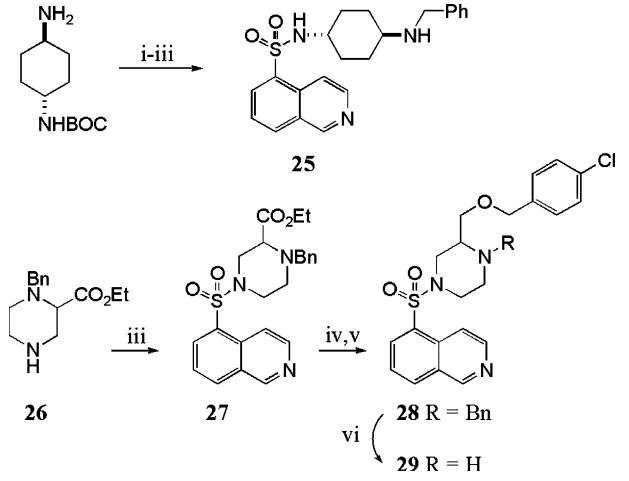


Scheme 2. Reagents and conditions: (i) diamine, Et_3N, CH_2Cl_2, rt; (ii) (a) mono-Boc-diamine, Et_3N, CH_2Cl_2, rt; (b) TFA, CH_2Cl_2, rt.

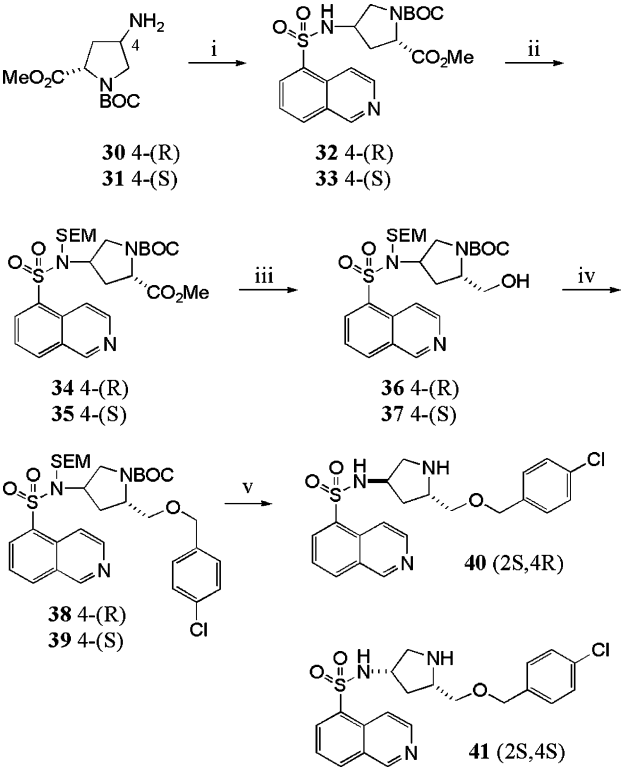
confirmed the regiochemistry of the sulfonylation leading to **19**. The *trans*-cyclohexyl-1,4-diamine analogue **22** was also synthesised from the mono-Boc-protected diamine since attempted mono-sulfonylation of the unprotected diamine gave an intractable mixture of products.

The most efficient route for elaboration of the analogue **22** was found to be reductive amination of benzaldehyde with the mono-Boc diamine, followed by deprotection and sulfonylation to give **25** (Scheme 3). The preparation of the 3-substituted piperazine **29** required a new approach from the known *N*-benzyl-protected piperazine ester **26**.[35] Sulfonylation of **26** and complete reduction of the ester **27** were followed by alkylation of the alcohol to give **28**. Selective removal of the *N*-benzyl-protecting group of **28** in the presence of the 4-chlorobenzyloxy substituent was achieved through N-activation using chloroethyl chloroformate.[36]

Single diastereoisomer, substituted aminopyrrolidines related to **23** were prepared separately from commercially available (2*S*,4*R*)- and (2*S*,4*S*)-*N*-Boc-4-aminopyrrolidine-2-carboxylate methyl esters **30** and **31** (Scheme 4). Sulfonylation was followed by sulfonamide protection with the SEM group,[37] to permit selective O-benzylation of the alcohols **36** and **37** later in the sequence. Acidic deprotection of the N-SEM and *N*-Boc-protecting groups was carried out in a single step to yield **40** and **41**.



Scheme 3. Reagents and conditions: (i) PhCHO, $NaB(OAc)_3H$, $ClCH_2CH_2Cl$, AcOH, 4 Å molecular sieves, rt; (ii) TFA, CH_2Cl_2, rt; (iii) **1**, Et_3N, CH_2Cl_2, rt; (iv) DIBAL-H, CH_2Cl_2, −78 °C; (v) 1-bromomethyl-4-chlorobenzene, NaH, DMF, rt; (vi) 1-chloroethyl chloroformate, $ClCH_2CH_2Cl$, reflux.

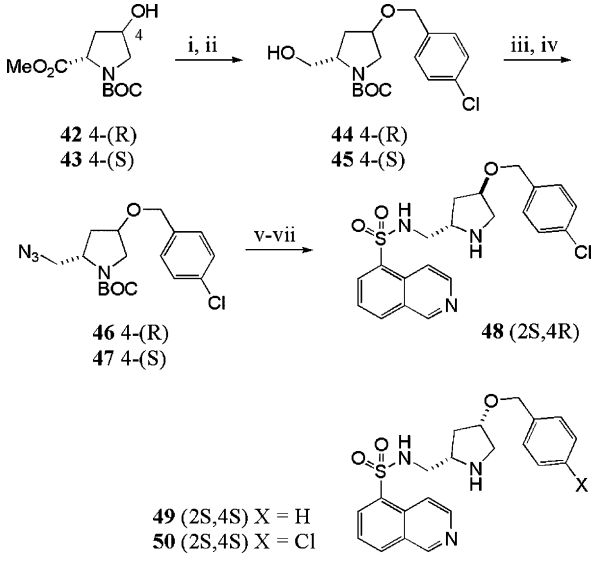


Scheme 4. Reagents and conditions: (i) **1**, Et_3N, DMAP, CH_2Cl_2, rt; (ii) NaH, SEMCl, DMF, 0 °C; (iii) $LiAlH_4$, THF, 0 °C; (iv) NaH, 4-chlorobenzyl bromide, DMF, 0 °C; (v) 6 M HCl, MeOH, reflux.

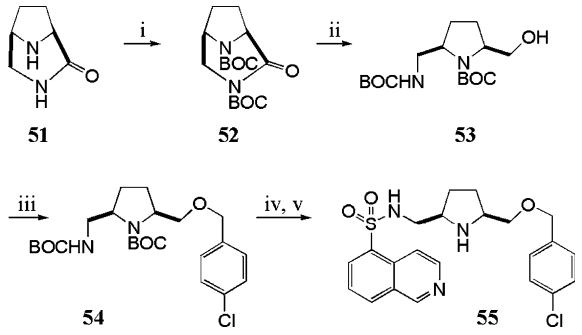
For substituted analogues of the aminomethylpyrrolidine **24**, the single diastereoisomer hydroxyproline esters[38] **42** and **43** were separately O-alkylated and reduced to the alcohols **44** and **45**, respectively (Scheme 5). The alcohols were activated as the mesylates and displaced with sodium azide to give the azides **46** and **47**. Hydrogenation of **46**, sulfonylation and deprotection were carried out without isolation of intermediates to give the analogue **48**. When this sequence was performed on the azide **47**, however, some reduction of the 4-chlorobenzyl substituent was seen in the hydrogenation step. The mixture of 4-chloro and des-chloro compounds was carried through the sulfonylation and deprotection, and was separated by preparative HPLC to give compounds **49** and **50**.

To prepare a related 2,5-*syn*-pyrrolidine **55**, the known racemic bicyclic amide **51**[39] was doubly protected with the *N*-*tert*-butyloxycarbonyl group (Scheme 6). On treatment of **52** with sodium hydroxide, the activated bicyclic amide was ring-opened to generate the racemic 2,5-*syn*-substituted pyrrolidine scaffold.[39] Without isolation, the liberated carboxylic acid was reduced with sodium borohydride to the alcohol **53**, which was alkylated on oxygen to give **54**. Complete N-deprotection of **54** gave a diamine that was sulfonylated selectively on the primary amine to yield the required analogue **55**.

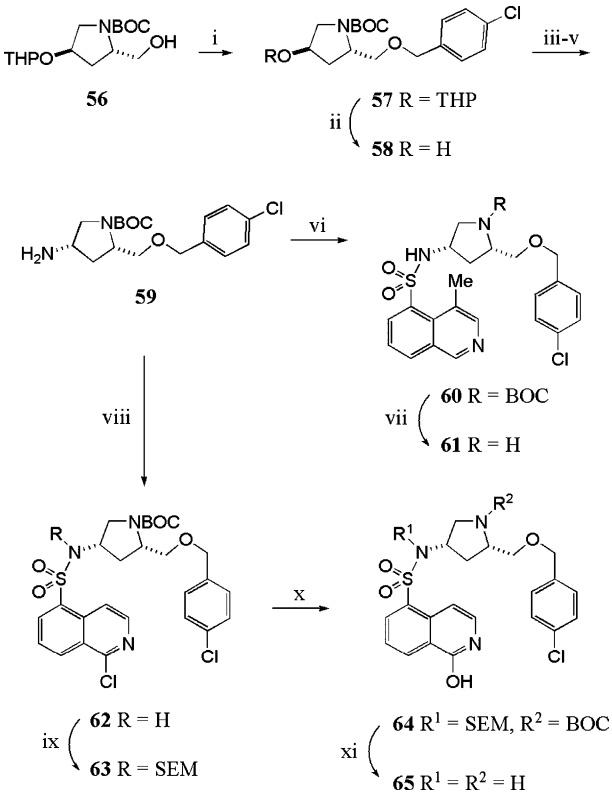
To allow the investigation of replacements of the parent isoquinoline-5-sulfonamide in the analogue **41**, the relevant single diastereoisomer diamine side-chain **59** was made (Scheme 7). The protected proline derivative **56** was prepared from (2*S*,4*R*)-4-hydroxyproline following literature procedures.[40] Alkylation of **56** was followed by selective O-deprotection using mild acid. The secondary alcohol **58** was activated as the mesylate and displaced, with inversion, by sodium azide.[41] Reduction of the intermediate azide gave



Scheme 5. Reagents and conditions: (i) NaH, 4-chlorobenzyl bromide, DMF, 0 °C; (ii) $LiAlH_4$, THF, 0 °C; (iii) Ms_2O, Et_3N, CH_2Cl_2, 0 °C; (iv) NaN_3, DMF, 80 °C; (v) 10% Pd/C, 1 atm. H_2, EtOAc, rt; (vi) **1**, Et_3N, DMAP, CH_2Cl_2, rt; (vii) 1 M HCl in Et_2O, MeOH, rt.



Scheme 6. Reagents and conditions: (i) $(Boc)_2O$, Et_3N, DMAP, CH_2Cl_2, rt; (ii) $NaBH_4$, MeOH, 0 °C; (iii) NaH, 4-chlorobenzyl bromide, DMF, 0 °C; (iv) 1 M HCl in Et_2O, MeOH, rt; (v) **1**, Et_3N, CH_2Cl_2, rt.



Scheme 7. Reagents and conditions: (i) KH, 4-chlorobenzyl chloride, THF, 0 °C; (ii) PPTS, EtOH, 55 °C; (iii) methanesulfonic anhydride, Et_3N, DCM, 0 °C; (iv) NaN_3, DMF, 80 °C; (v) PPh_3, H_2O, THF, rt; (vi) 4-methylisoquinoline-5-sulfonyl chloride, Et_3N, DMAP, CH_2Cl_2, rt; (vii) 4M HCl, dioxane, rt; (viii) 1-chloroisoquinoline-5-sulfonyl chloride, Et_3N, DMAP, CH_2Cl_2, rt; (ix) NaH, SEMCl, THF, 0 °C; (x) $KOSiMe_3$, THF, 80 °C; (xi) 4 M HCl, dioxane, rt.

the desired amine **59**, which was subsequently coupled to 4-methylisoquinoline-5-sulfonyl chloride[42] and deprotected to give **61**. Sulfonylation of **59** with 1-chloroisoquinoline-5-sulfonyl chloride[43] and sulfonamide N–H protection with the SEM group gave the sulfonamide **63**. Displacement of the iminoyl chloride from **63** through S_NAr reaction with potassium trimethylsilanolate[44] and acidic deprotection of the pyrrolidine and sulfonamide led to the 1-hydroxyisoquinoline analogue **65**.

4. PKA—ligand co-crystallography

As part of the structure-based design approach, the co-crystal structures of selected inhibitors with PKA were determined. The α catalytic subunit of bovine PKA was expressed and purified with reference to previously described protocols.[27,45] Hanging drops containing 17 mg/ml of tri-phospho PKA, 25 mM Mes–BisTris, pH 6.5, 75 mM LiCl, 0.1 mM EDTA, 1 mM DTT, 1.5 mM octanoyl-*N*-methylglucamide and 1 mM PKI(5–24) were equilibrated at 4 °C against 15% (v/v) methanol. Crystals of apo PKA appeared overnight and were soaked for approximately 18 h at 4 °C in a solution containing 10% PEG 400, 25 mM Mes–BisTris, pH 6.5, 0.1 mM EDTA, 1 mM DTT, and 10 mM of inhibitor. The crystals were briefly immersed in a cryo-protectant containing 22.5% MPD, 25 mM Mes–Bis-Tris, pH 6.5, 0.1 mM EDTA, and 1 mM DTT, before plunge-freezing into liquid nitrogen.

X-ray diffraction data were collected using a Rigaku-MSC Jupiter CCD mounted on an RU-H3R rotating anode generator. Data were integrated using MOSFLM[46] before input into a proprietary procedure for automated scaling, refinement and ligand fitting.[47] The starting model used for refinement was the previously solved PKA structure PDB 1YDS,[48] with ligand and waters removed. After ligand fitting, subsequent cycles of model adjustment and refinement were carried out using QUANTA (Accelrys Inc.) and REFMAC5.[49] Data processing and refinement statistics are given in the Supplementary Material.

5. Results and discussion

Following the observation that the benzhydryl group of NL-71-101 imparted a degree of selectivity for inhibition of PKB over PKA,[11] we initially attempted to prepare benzhydryl and close derivatives where the isoquinoline-5-sulfonamide was replaced by various aromatic and heteroaromatic groups linked through an amide. However, this generally led to compounds with poor solubility that confounded accurate testing in vitro (data not shown). We sought to improve the solubility of the ligands by restricting the terminal substituent to a single aromatic group, as in H-89, while incorporating a polar heteroatom into the linker. As well as potentially improving the physicochemical properties, this strategy also addressed the replacement of the potential metabolic site of the alkene in the linker.

Thus, a novel series of 2-benzyloxyethyl-substituted analogues **3**–**13** were prepared[16] (Table 1). The parent phenyl analogue **3** showed good in vitro PKB inhibitory activity in the radiometric enzyme inhibition assay[50] but no activity in the SRB (sulforhodamine B) cellular anti-proliferation assay.[51] The effects of simple aromatic substitution were examined and, in general, lipophilic electron-withdrawing or neutral substituents were optimal, a structure–activity relationship that parallels previous observations with analogues leading to NL-71-101.[11] Methyl substitution, as in compounds **4**–**6**, was tolerated at all positions, and the most active 2-methyl isomer **4** now showed measurable activity in cells. Chloro substitution, as in compounds **7**–**9**, was beneficial for both in vitro PKB inhibition and cellular antiproliferative activity. In contrast, electron-donating methoxy substituents **11**–**13** were not advantageous.

Table 1. Kinase inhibitory activity and cell growth inhibition of **3**–**13** and **17**–**24**

Compound[a]	R[1a]	PKB IC_{50} (μM)[b]	SRB IC_{50}(μM)[c]
3	H	1.17 (±0.18)	>50[d]
4	2-Me	0.57	37 (±3)
5	3-Me	1.1	[e]
6	4-Me	1.53	[e]
7	2-Cl	0.96	[e]
8	3-Cl	0.37	[e]
9	4-Cl	0.26 (±0.02)	25 (±1)
10	3,4-Cl_2	0.21	13
11	2-MeO	6.6	[e]
12	3-MeO	2.2	>50
13	4-MeO	2.9	>50
17	—	4.8	[e]
18	—	23	[e]
19	—	8.7	[e]
20	—	>30	[e]
21	—	57	[e]
22	—	8.1	[e]
23	—	3.9	[e]
24	—	39	[e]
H-8		7.6 (±0.40)	[e]
H-89		0.59 (±0.07)	18 (±1.4)

[a] See structures in Schemes 1 and 2.
[b] Mean of two independent IC_{50} determinations or mean (±SEM) for $n > 2$ determinations. Standards H-8 and H-89 gave SD ± 24% ($n = 20$) and ±37% ($n = 10$), respectively, in this assay.
[c] Mean of two independent IC_{50} determinations or mean (±SEM) for $n > 2$ determinations. Standard H-89 gave SD ± 34% ($n = 20$) in this assay.
[d] Single determination.
[e] Not determined

The selectivity of the more active compounds **7**–**9** against PKA and PKCα was measured (Table 3). The compounds were 10- to 20-fold selective against PKCα, but showed equipotent inhibition of PKA. This compares with the 8-fold selectivity for PKA shown by H-89, the change from the rigid all-carbon 3-atom linker to the more polar, and more flexible, 4-atom linker having overturned this.

Although the 4-chlorophenyl analogue **9** had sufficient potency to permit further investigations in cellular assays (vide infra), improvements in the activity and selectivity of the ligands were sought. The large number of rotatable bonds (*n*Rot = 10) in these structures was considered undesirable, since inverse correlations of flexibility and membrane permeability have been reported,[52–54] and this feature might account in part for the low cellular activity of the compounds relative to their in vitro enzyme inhibition. In addition, conformational restriction of linear chains can lead to increased potency by lowering the entropic penalty on binding and may also provide a potential means of increasing ligand selectivity.[55] To be successful in this series of compounds,

conformational restriction of the diamine linker must optimally deliver the terminal lipophilic aryl group, whilst not disrupting the important hydrogen-bonding interaction of the central amine. There is evidence from X-ray structural determinations of isoquinoline ligands bound to PKA that this interaction has some flexibility,[27] and it is reasonable to expect that PKB might exhibit similar tolerance in the positioning of the amine.

Simple cyclic diamines were introduced onto the isoquinoline-5-sulfonamide in an attempt to find conformationally restricted scaffolds of equal, or better, potency than the parent ethylenediamine (H-8) or the propylenediamine homologue **17** (Table 1). Virtual docking studies were undertaken to further select replacements for the linear diamines, and additionally to determine the positions on the cyclic diamine scaffolds from which elaboration with a 4-chlorobenzyloxy group, or similar, would be most likely to allow occupation of appropriate space in the enzyme.

Of the unsubstituted analogues prepared, the 4-aminopiperidine **19**, *trans*-1,4-diaminocyclohexane **22** and 3-aminopyrrolidine **23** showed activity equivalent to the linear scaffolds of H-8 and **17** (Table 1). There was some divergence between the observed activity of the unsubstituted diamines, and the scaffolds predicted by modelling to be more promising for substituent addition. For example, although the parent amine had good activity against PKB, substitution of the *trans*-1,4-diaminocyclohexane **22** from the distal amine was predicted to occur along unfavourable vectors for reaching the lipophilic binding pocket. A similar problem was anticipated for the 4-aminopiperidine **20**. For the piperizine **18** and 4-aminopiperidine **19**, an apparent need to preserve a free N–H for optimum hydrogen bonding to the enzyme in the docking studies suggested that substitution from the α-carbon would be more productive. In contrast, the 2-(*S*)-aminomethylpyrrolidine **24**, although of relatively low activity, was predicted to be a competent scaffold for delivering the terminal lipophilic group when appropriately substituted. 3-Aminopyrrolidine was identified as a highly suitable scaffold, both from the modelling and the observation of the inhibitory activity of compound **23**. For the pyrrolidine templates, accurate prediction of the best relative stereochemistry of substitution was difficult, and both *cis* and *trans*-substitution patterns were considered for synthesis. Although in general no distinct preference for the absolute stereochemistry of the pyrrolidine fragments was discernible from the modelling, for the aminomethylpyrrolidine **24** the 2-(*S*) enantiomer gave consistently better docking predictions for both the parent and elaborated derivatives. Only the (*S*)-enantiomer of **24** was synthesised and tested.

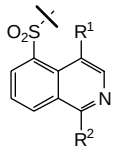
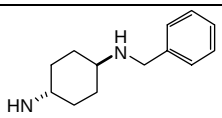
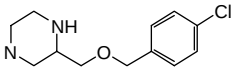
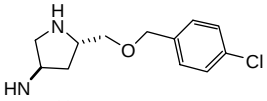
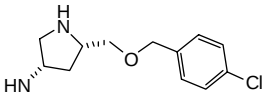
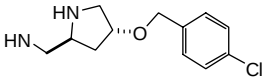
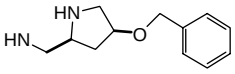
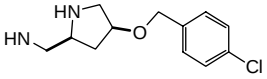
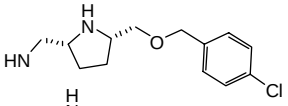
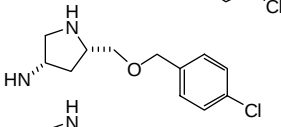
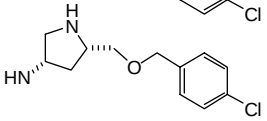
For the selected scaffolds, addition of appropriately linked 4-chlorophenyl groups was made (Table 2). As anticipated from the modelling studies, N-substitution of the *trans*-1,4-diaminocyclohexane **22** to give compound **25** did not improve the activity. Substitution α to nitrogen of the piperazine **18** was investigated to give compound **29**. The marginal increase in activity observed was not encouraging for pursuing this substitution pattern on this scaffold, or on the more synthetically demanding 4-aminopiperidine **19**. Instead, attention was focussed on the pyrrolidines **23** and **24**.

For the 3-aminopyrrolidine constraint, PKB docking studies and comparison to the structure of H-89 docked to PKA (PDB code 1YDT) indicated that the 5-position would provide the best vector for further substitution (Fig. 2). The calculated free energy difference between cis and trans isomers using the Chemscore scoring function was <0.5 kcal mol^{-1}, too low to allow any prediction to be drawn. In the event, substitution at this position was favourable for the (2*S*,4*S*) diastereomer **41**, which proved significantly more active than either the unsubstituted parent **23** or the (2*S*,4*R*) isomer **40**.

For the (*S*)-2-aminomethylpyrrolidine constraint **24**, docking indicated the 4-position as suitable for substitution (Fig. 3). Alternative orientations of the pyrrolidine ring were identified that suggested a (2*R*,5*R*) substitution pattern could also be accommodated, provided adjustments were made in the length of the linker at the 5-position. Some indication of preferred relative stereochemistry was obtained from the modelling of the 4-substituted compounds, in favour of the (*S*,*S*) diastereomer **50** over the (2*S*,4*R*) diastereisomer **48** (Chemscore $\Delta\Delta G$ 4.8 kcal mol^{-1}). This observation was not used in a proscriptive sense, however, and both isomers were prepared. In practice, a large improvement in activity over the unsubsituted parent **24** was observed upon attachment of the side chain at either the 4-position of the (*S*)-2-aminomethylpyrrolidine, **48**–**50**, or at the 5-position in the alternative enantiomeric series **55**. For the 4-substituted compounds, the (2*S*,4*S*) isomer **50** showed 3.5-fold more potent enzyme inhibition than the (2*S*,4*R*) isomer **48**. The importance of the lipophilic chlorine substituent for high activity, as observed in the linear diamines (cf. **9** vs **3**), was emphasised by the 7-fold drop in activity between **50** and the des-chloro analogue **49**.

Inhibition of cell growth was investigated for the more active pyrrolidine derivatives. Arguably, a limited general improvement in cellular penetration was achieved through the reduction in the number of rotatable bonds in these analogues (*n*Rot = 7–8), since compounds with biochemical activities of 1–5 μM (**40**, **41**, **48**, **50**, and **55**) showed measurable cellular activity. This contrasts with the ethylenediamine analogues **3**–**13** (Table 1), where biochemical activity below 1 μM was apparently necessary before measurable cellular activity was achieved. A caveat to this interpretation is that the cellular assay is not necessarily a simple integration of PKB/PKA activity and membrane permeability, and could reflect any other activities of the compounds. The selectivities of selected constrained analogues versus PKA and/or PKCα were determined (Table 3). In general, no significant selectivity was seen between PKB and PKA activities regardless of the presence or absence of the lipophilic side chain. In contrast, selectivity (ca. 10- to 100-fold) was observed for PKB/PKA versus PKCα for the fully elaborated pyrrolidines **41** and **50**. In these respects, constraint of

Table 2. Kinase inhibitory activity and cell growth inhibition of constrained analogues

Compound		R^1	R^2	PKB IC_{50} (μM)[a]	SRB IC_{50} (μM)[b]
25		H	H	6.6	[c]
29		H	H	12	[c]
40		H	H	5.2 (±0.41)	25
41		H	H	0.78 (±0.12)	18
48		H	H	1.7	23
49		H	H	3.4	[c]
50		H	H	0.48	28
55		H	H	1.7	21
61		Me	H	2.6	9.8
65		H	OH	0.52	40

[a] Mean of two independent IC_{50} determinations or mean (±SEM) for $n > 2$ determinations, see footnotes to Table 1.
[b] Single determination.
[c] Not determined.

the diamine linker did not provide a biochemical advantage over the linear scaffolds, for which similar selectivity profiles were observed.

For one of the most active pyrrolidines **41**, a limited study of conservative changes to the isoquinoline group was made, inspired by the known structure–activity relationships of isoquinoline-5-sulfonamide inhibitors of rho-kinase[56] (Table 2). Methylation of the rho-kinase inhibitor fasudil at C-4 of the isoquinoline has been shown to increase the affinity of the analogue for rho-kinase, while leaving PKA activity unchanged. For the compound **61**, introduction of the C-4 methyl group was detrimental to PKB activity, while PKA activity was unaffected, leading to 8-fold selectivity for PKA over PKB (Table 3). The metabolism of fasudil by oxidation at C-1 of the isoquinoline is known to lead to a more potent in vivo inhibitor of the rho-kinase enzyme.[57] However, the 1-hydroxyisoquinoline analogue **65** showed equivalent activity against PKB to the parent **41**. No significant selectivity was observed for PKB or PKA with compound **65**.

The binding mode of selected ligands (**9** and **48**) was evaluated crystallographically with PKA. PKA provides a useful surrogate for PKB as the active sites are approximately 80% identical,[18,19] and PKA is a robust crystallographic system amenable to high-throughput data collection. Given the general equipotency of this series of compounds for PKB and PKA, a correspondence between the binding modes to the two proteins would be expected. The constrained ligands **41**, **48** and

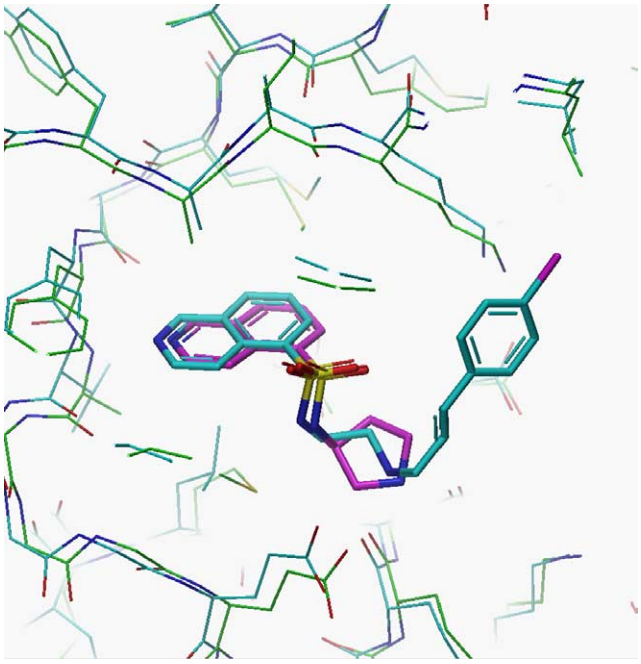


Figure 2. Overlay of H-89–PKA complex (1YDT)[26] and the modelled structure of **23** docked to PKB (1O6K)[19] (cyan, H-89–PKA; magenta, **23**; green, PKB).

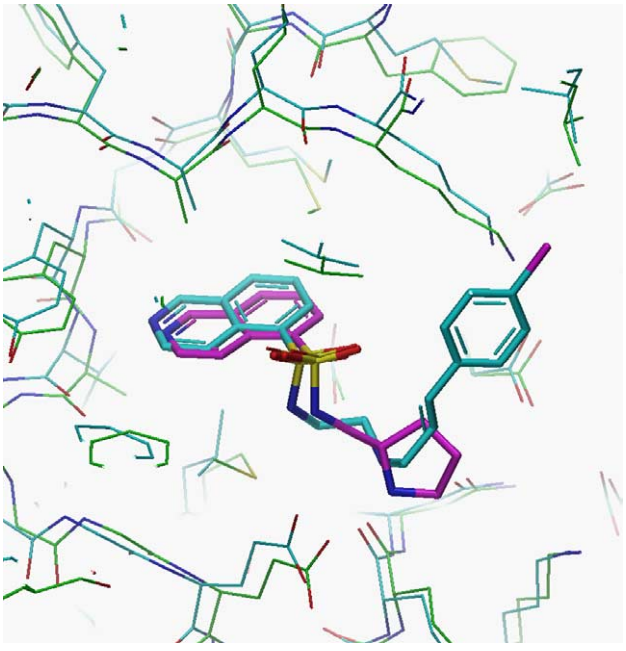


Figure 3. Overlay of H-89–PKA complex (1YDT)[26] and the modelled structure of **24** docked to PKB (1O6K)[19] (cyan, H-89–PKA; magenta, **24**; green, PKB).

50 showed equivalent inhibition of PKA activity to the linear inhibitor **9** (Table 3).

The co-crystal structure of **9** bound to PKA (PDB 2C1A) reveals an overall ligand orientation as expected from previous crystallographic studies with isoquinoline-5-sulfonamide ligands[27,58] (Fig. 4). The isoquinoline binds in the adenine site of the ATP cleft, sandwiched between the hydrophobic side chains of Val57 and Leu173, and forming a single hydrogen bond between its heteroaromatic nitrogen and the backbone amide of Val123 on the kinase hinge. A 'CHO' type interaction between the isoquinoline C-1 hydrogen and the backbone carbonyl of Glu121 is also observed, and the fused phenyl ring of the heterocycle is orientated towards the 'gatekeeper' Met120. No close protein contacts are observed with the sulfonamide group of the ligand, which adopts a rotational conformation placing the S–N bond orthogonal to the plane of the aromatic ring.

The flexible, 7-atom chain between the sulfonamide and the terminal aromatic ring adopts a pronounced U-shaped fold which is similar to that observed for H-89 complexed with PKA.[27] The protonated nitrogen occupies the PKA ribose binding site and is involved in salt-bridging and hydrogen-bonding interactions with

Table 3. Kinase inhibitory activites of selected compounds for PKB, PKA and PKCα

Compound	PKB IC_{50} (μM)[a]	PKA IC_{50} (μM)[b]	PKCα IC_{50} (μM)[c]
7	0.96	1.0	16
8	0.37	0.41	8.3
9	0.26 (±0.02)	0.17	1.9
19	8.7	3.9	[d]
22	8.1	5.7	54
23	3.9	[d]	19
41	0.78 (±0.12)	0.42	9.9
48	1.7	0.40	[d]
50	0.48	0.17	28
61	2.6	0.32	[d]
65	0.52	1.3	[d]
H-8	7.6 (±0.40)	5.3 (±0.53)	29 (±4.9)
H-89	0.59 (±0.07)	0.073	2.7

[a] Mean of two independent IC_{50} determinations or mean (±SEM) for $n > 2$ determinations, see footnotes to Table 1.
[b] Single determinations from duplicate points. Standard H8 gave SD ± 38% ($n = 14$) in this assay.
[c] Single determinations from duplicate points. Standard H8 gave SD ± 41% ($n = 6$) in this assay.
[d] Not determined.

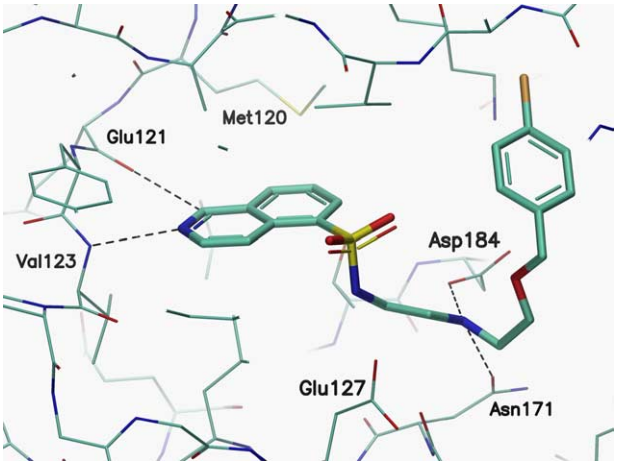


Figure 4. Crystal structure of the PKA–**9** complex (PDB 2C1A).

the carboxylate of Asp184 and the side-chain carboxamide of Asn171. The other potential salt-bridging partner in this region of the protein, Glu127, does not participate in direct interactions to the ligand. The ligand's terminal 4-chlorophenyl ring is directed towards the glycine-rich loop and occupies a hydrophobic pocket defined primarily by the side chains of Thr51, Val53, Lys72, Leu74 and the backbone carbonyls of Gly52 and Gly55.

The structure of PKA with the pyrrolidine ligand **48** (PDB 2C1B) allows a comparison with the linear compound **9** and shows that the isoquinoline sulfonamide moieties adopt identical positions and conformations within the adenine site and form essentially equivalent interactions with the hinge (Fig. 5). Likewise, the terminal 4-chlorophenyl groups occupy very similar regions of space, although there are subtle differences in the exact positioning and orientation of the chlorophenyl due to additional constraints on the cyclised ligand. The largest difference, however, is observed for the pyrrolidine linker, the basic amine being displaced by ca. 2.4 Å, relative to the position in the unconstrained molecule, and having a significant effect on the binding interactions of this functional group (Fig. 6). The salt bridge between the basic group and Asp184 is lost and is replaced by an interaction with Glu127. Asp184, which has been observed to be flexible in other structures, now adopts an alternative rotamer in which it forms a salt bridge with the basic amine of Lys72. In addition, a water mediated hydrogen bond is formed between the basic pyrrolidine amine and the phenol of Tyr330. The positions of other residues in the ATP-site of PKA for both inhibitor complexes are essentially equivalent.

Thus, although the constrained pyrrolidine **48** is a very effective mimic of the overall fold of the linear molecule **9**, there is a change in the detailed binding mode between the two inhibitors. This may reflect the presence of multiple potential binding sites (principally Asp184 and Glu127) for a basic group within the ribose binding region of the PKA (and by homology, PKB) kinase domain. The redundancy of potential interactions to the basic amine and flexibility of the protein in this region would imply that the other constrained inhibitors **40**, **41**, **49**, **50** and **55** may adopt slightly different contacts to the protein, whilst maintaining a high degree of overall conformational similarity. These changes in detailed binding mode, and the associated small changes in protein conformation and water structure, may make the effects of ligand conformational restriction on binding affinity harder to predict exactly.

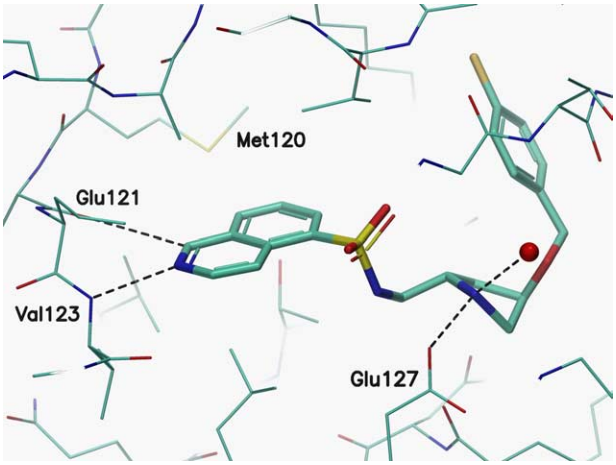


Figure 6. Crystal structure of the PKA–**48** complex (PDB 2C1B).

A comparison of the co-crystal structure of **48** and PKA with the structure predicted from docking of **48** to PKB (PDB Code 1O6K) (Fig. 7) shows that there is a good correspondence in the overall conformation of the ligand. The glycine-rich loop of PKB is displaced by ca. 1 Å relative to its position in PKA, and this is reflected

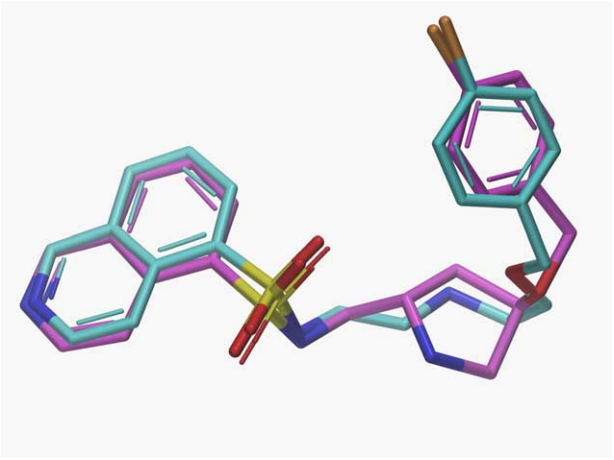


Figure 5. Overlay of the ligands **9** (cyan) and **48** (magenta) bound to PKA (not shown), as determined by crystallography.

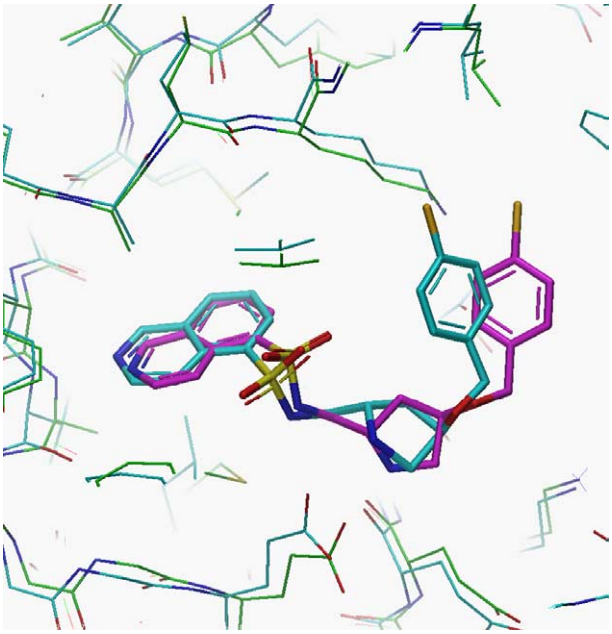


Figure 7. Overlay of the crystal structure of the PKA–**48** complex (cyan) with the model of **48** (magenta) docked to PKB (green).

by a shift in position of the 4-chlorophenyl ring by a similar amount. This loop is known to be a highly flexible region of kinases and can adopt a variety of conformations depending upon the ligand bound and the protein crystal system.[59,60] The contacts and positions of the isoquinoline ring are identical in the PKB model and the PKA determined structure. The model also correctly predicts the interaction of the basic amine of the ligand **48** with Glu127 rather than Asp184.

Although suitable conformational constraints of the linear inhibitor **9** were identified by modelling, and shown by ligand–protein co-crystallography to adopt favourable binding conformations, no improvement in the in vitro inhibition of PKB was seen. The expected entropic gain in affinity through rotatable bond restriction is difficult to quantify, but has been estimated to be approximately 2- to 3-fold/rotor.[61] For these compounds, it is possible that any relatively small affinity gain achieved by constraining the molecule is offset by less favourable protein–ligand interactions in the new complex. Although Glu127 and Asp184 may be equivalent sites for binding to ligand basic groups, predicting the precise energetic consequences associated with changing these interactions is difficult, particularly when protein movements and changes in hydration are involved. In addition, it should be noted that the subtle changes in the presentation of the chlorophenyl group to the hydrophobic pocket in the glycine loop may affect the free-energy of binding.

The constrained analogues offered no advantage in selectivity for PKB versus PKA, although it was noted that PKB/PKA selectivity did show some response to changes in the spacer between the basic amine and terminal lipophilic group (cf. H-89 vs **9**, **41**). Recent studies have shown that high selectivity for PKB versus PKA can be achieved when ATP-competitive inhibitors access a region of the enzyme beyond the glycine-rich loop.[9,10] Significant structural extension of the inhibitors described here might be needed to occupy similar sites in the enzyme, and although the current compounds fall within the accepted ranges of 'drug-like' physicochemical properties[62] (e.g., **9** MW 420, *C*log *P* 4.0; **41** MW 432, *C*log *P* 3.9), significant additional functionality might lead to a less favourable physicochemical profile.

The effects of the inhibitors **9** and **41** on the PI3K-PKB pathway in cells were studied in more detail. In particular, the ability of the inhibitors to block GSK3β phosphorylation on Ser9 by PKB was examined by Western blotting in PC-3 cells (Fig. 8). This cell line is expected to show increased signalling activity through PI3K-PKB due to the deletion of the negative regulator PTEN.[5,63] At concentrations equivalent to or greater than the IC_{50} for cell growth inhibition, both compounds showed inhibition of GSK3β phosphorylation, indicating that the compounds inhibit PKB kinase activity towards cells. No effects on total levels of GSK3β or PKB protein were observed. Additionally, **9** and **41** were assayed for their ability to inhibit PDK1 kinase activity in vitro, and found to have no activity at concentrations up to 30 μM.[64] Quantification of the cellular effects of

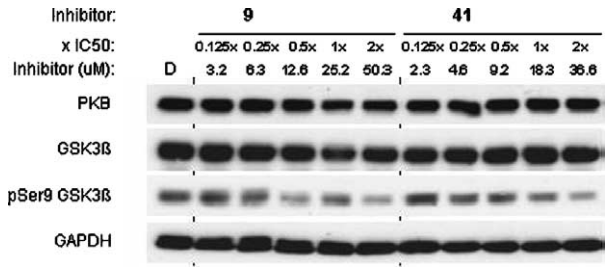


Figure 8. Western blot showing titration of inhibitors **9** and **41** for inhibition of pSer9 GSK3β phosphorylation in PC-3 cells following 1 h treatment, D, DMSO vehicle control.

the inhibitors was achieved using a Ser9 GSK3β cellular ELISA protocol to measure the extent of GSK3β phosphorylation in PC-3 cells.[65] The inhibitors **9** and **41** inhibited Ser9 GSK3β phosphorylation in cells with IC_{50} values of 15 and 17 μM, respectively, consistent with both the Western blot data, and their cell growth inhibition in the SRB assay. Since ligands from the isoquinolinesulfonamide class have been shown to be ATP-competitive inhibitors of a range of kinases, particularly serine/threonine kinases,[11,66] the correlation of PKB enzyme inhibition with antiproliferative effects on cells would be enhanced by further kinase counterscreening data.[67]

6. Conclusions

A series of isoquinoline-5-sulfonamide inhibitors of PKB was developed, with sub-micromolar activity and structurally distinct from previous ligands in this class. Structure-based design using the PKB crystal structure was successful in guiding the preparation of constrained analogues that mimic the bound conformation of the linear prototypes, which was confirmed by co-crystal structure determinations using PKA. Detailed variation in the binding mode between inhibitors with very similar overall conformations was observed. Although effective in this manner, the conformational constraints did not significantly change the in vitro activity of the compounds. Selectivity for in vitro inhibition of PKB over PDK1 and PKCα was observed in this series of ligands, while the compounds were equipotent at PKA. In cellular assays, the most potent inhibitors in this series showed the characteristic inhibition of GSK3β phosphorylation expected for PKB inhibitors. These molecules should prove useful tools for further exploration of the biological effects of inhibition of PKB.

7. Experimental

7.1. PKB in vitro enzyme assay

Recombinant, purified PKBβ enzyme was provided by Professor David Barford, Structural Biology, Institute of Cancer Research, Chester Beatty Labs, Fulham Road. Calbiochem, Merck Biosciences, UK, supplied the peptide substrate, AKTide-2T (ARKRERTYSFGHHA), and assays were run in polypropylene 96-well plates. Assay volume was 25 μL, with a

buffer consisting of 20 mM Mops, pH 7.2, 25 mM β-glycerophosphate, 5 mM EDTA, 15 mM $MgCl_2$ and 1 mM DTT. The final peptide concentration was 25 μM, with 30 μM ATP and 0.35 μCi [^{33}P-γ]ATP. In the enzyme reaction, the ^{33}P-γ-labelled phosphate from ATP was transferred to the serine residue of the peptide. Incubations were stopped with 30 μL of 2% orthophosphoric acid. The reaction mixture was transferred to a phosphocellulose filter plate (MAPHNOB from Millipore, UK), where the labelled peptide binds, and the unused ATP was washed away with 0.5% orthophosphoric acid. The filter plate was dried, scintillant was added to the wells and the incorporated activity was measured on a TopCount™ (Perkin-Elmer). Under these conditions, enzyme activity was linear with time (up to 30 min) and enzyme concentration (up to 1.5 nM). Compounds were diluted in 12.5% DMSO and added to the assay such that the final DMSO concentration was 2.5%. Inhibitor IC_{50} values were determined from an eight-point concentration range run in duplicate and calculated in GraphPad Prism 4.00. The standard H-89 gave IC_{50} 0.59 (±0.07) μM under these conditions (lit. value[11] IC_{50} 2.5 μM, assay [ATP] 10 μM).

7.2. General synthetic chemistry experimental

Reaction was carried out under N_2. Organic solutions were dried over $MgSO_4$ or Na_2SO_4. Starting materials and solvents were purchased from commercial suppliers and were used without further purification. Flash silica chromatography was performed using Merck silica gel 60 (0.025–0.04 mm). Ion exchange chromatography was performed using Isolute Flash SCX-II (acidic) or Flash NH2 (basic) resin cartridges. ^{1}H NMR spectra were recorded on a Bruker AC250 instrument and chemical shifts (δ) are reported relative to TMS and/or referenced to the solvent in which they were measured. Combined HPLC–MS analyses were recorded using a Waters Alliance 2795 Separations Module and Waters/Micromass LCT mass detector with electrospray ionisation (+ve or −ve ion mode as indicated). Analytical HPLC was performed using a Supelco DISCOVERY C_{18} 5 cm × 4.6 mm i.d., 5 μm column, with a gradient elution of 10–90% MeOH/0.1% aqueous formic acid at a flow rate of 1 mL min^{-1} and a running time of 10 min. Compounds were detected at 254 nm using a Waters 2487 Dual λ Absorbance Detector. High resolution mass spectra were measured at the Department of Chemistry, Cambridge University, UK, on a Bruker Bio Apex II 4.7e FTICR instrument, or a Waters LCT Premier ESI-ToF instrument, and are within ±5 ppm.

Synthetic intermediates were characterised by ^{1}H NMR spectra and MS (where electrospray ionisation permitted observation of a relevant ion) for identity and were assessed by ^{1}H NMR for homogeneity. Compounds for biological testing were additionally characterised by HRMS for identity and were assessed by HPLC (UV detection) for homogeneity.

Compounds **2**[11,29,30] and **17**[29] were prepared according to the literature procedures.

7.3. Compound preparation and characterisation

7.3.1. Isoquinoline-5-sulfonic acid (2-(2-(4-chlorobenzyloxy)ethylamino)ethyl)amide (9)

7.3.1.1. General method A. Ethylene glycol (1.67 mL, 30 mmol) was added over 10 min to a slurry of NaH (60% dispersion in oil, 0.246 g, 10.3 mmol) and dry THF (15 mL) at rt. The mixture was warmed to 50 °C, and a solution of 4-chlorobenzyl bromide (2.0 g, 9.8 mmol) in dry THF (10 mL) was added during 45 min. The mixture was refluxed for 18 h, cooled to rt and partitioned between Et_2O (20 mL) and water (20 mL). The organic layer was washed with brine, dried, filtered and concentrated. Flash silica chromatography (10% MeOH–CH_2Cl_2) gave the alcohol **15** (R = 4-Cl) as a pale yellow oil (1.5 g, 8.0 mmol, 82%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.02 (1H, br s), 3.60–3.63 (2H, m), 3.77–3.80 (2H, m), 4.55 (2H, s), 7.31 (2H, dd, J = 6, 3 Hz), 7.35 (2H, dd, J = 6, 3 Hz).

Dess–Martin periodinane (0.670 g, 1.60 mmol) was added in one portion to a solution of **15** (R = 4-Cl) (0.273 g, 1.46 mmol) in wet CH_2Cl_2 (15 mL). After stirring at rt for 2 h, the mixture was diluted with saturated aq $NaHCO_3$ and sodium thiosulfate (50 mL). After the disappearance of the precipitate (10 min), the mixture was extracted with CH_2Cl_2 and the organic phase was washed with brine, dried and concentrated to give the aldehyde **16** (R = 4-Cl) as a colourless oil (0.253 g, 1.38 mmol, 94%) that was used without further purification. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 4.14 (2H, s), 4.62 (2H, s), 7.28–7.39 (4H, m), 9.75 (1H, s).

A solution of **16** (R = 4-Cl) (0.116 g, 0.71 mmol) in 1,2-dichloroethane (6 mL) was added to a stirred mixture of the amine **2** (0.136 g, 0.57 mmol), activated 4 Å molecular sieves (155 mg) and 1,2-dichloroethane (10 mL) at room temperature. The reaction mixture was stirred for 20 min followed by addition of $NaB(OAc)_3H$ (0.259 g, 1.18 mmol) in one portion and then AcOH (10 drops). The reaction mixture was stirred at rt until the reactants were consumed as determined by the TLC analysis. The reaction was quenched by addition of saturated aq $NaHCO_3$ and filtered through a layer of Celite. The product was extracted with EtOAc, and the combined organic extracts were dried and concentrated. Flash silica chromatography (10% MeOH–CH_2Cl_2) gave **9** (0.073 g, 0.17 mmol, 30%). ^{1}H NMR (CD_3OD, 250 MHz) δ 2.69–2.71 (4H, m), 3.03 (2H, t, J = 6 Hz), 3.51 (2H, t, J = 6 Hz), 4.49 (2H, s), 7.40 (4H, br s), 7.82 (1H, t, J = 8 Hz), 8.43 (1H, d, J = 8 Hz), 8.51 (1H, d, J = 8 Hz), 8.59 (1H, d, J = 7 Hz), 8.65 (1H, d, J = 6 Hz), 9.42 (1H, s); MS (ESI) m/z 420 [(M+H)$^+$]. HRMS M+H$^+$ calcd for $C_{20}H_{23}ClN_3O_3S$ 420.1143; found 420.1135. HPLC t_R 5.98 min; purity (AUC) > 99%.

7.3.2. Isoquinoline-5-sulfonic acid (2-(2-benzyloxyethylamino)ethyl)amide (3). Prepared from 2-benzyloxyacetaldehyde using General Method A. ^{1}H NMR (CD_3OD, 250 MHz) δ 2.72–2.81 (4H, m), 3.08 (2H, t, J = 6 Hz), 3.57 (2H, t, J = 6 Hz), 4.56 (2H, s), 7.36–7.45 (5H, m), 7.89 (1H, t, J = 8 Hz), 8.47 (1H, d, J = 7 Hz), 8.55 (1H, d, J = 7 Hz), 8.61 (1H, d, J = 7 Hz), 8.69 (1H, d,

J = 6 Hz), 9.46 (1H, s); MS (ESI) m/z 386 [(M+H)$^+$]. HRMS M+H$^+$ calcd for $C_{20}H_{24}N_3O_3S$ 386.1533; found 386.1537. HPLC t_R 5.62 min; purity (AUC) 97%.

7.3.3. Isoquinoline-5-sulfonic acid (2-(2-(2-methylbenzyloxy)ethylamino)ethyl)amide (4). Prepared from 2-methylbenzyl alcohol using General Method A. ^{1}H NMR (CD_3OD, 250 MHz) δ 2.34 (3H, s), 2.61–2.69 (4H, m), 3.00 (2H, t, J = 6 Hz), 3.51 (2H, t, J = 6 Hz), 4.51 (2H, s), 7.15–7.31 (4H, m), 7.83 (1H, t, J = 8 Hz), 8.41 (1H, d, J = 8 Hz), 8.49 (1H, d, J = 7 Hz), 8.56 (1H, d, J = 6 Hz), 8.62 (1H, d, J = 6 Hz), 9.40 (1H, s); MS (ESI) m/z 400 [(M+H)$^+$]. HRMS M+H$^+$ calcd for $C_{21}H_{26}N_3O_3S$ 400.1689; found 400.1690. HPLC t_R 5.80 min; purity (AUC) 95%.

7.3.4. Isoquinoline-5-sulfonic acid (2-(2-(3-methylbenzyloxy)ethylamino)ethyl)amide (5). Prepared from 3-methylbenzyl alcohol using General Method A. ^{1}H NMR (CD_3OD, 250 MHz) δ 2.36 (3H, s), 2.61–2.69 (4H, m), 3.01 (2H, t, J = 6 Hz), 3.48 (2H, t, J = 6 Hz), 4.46 (2H, s), 7.14–7.27 (4H, m), 7.84 (1H, t, J = 8 Hz), 8.41 (1H, d, J = 8 Hz), 8.50 (1H, d, J = 7, 1 Hz), 8.57 (1H, d, J = 6 Hz), 8.63 (1H, d, J = 6 Hz), 9.41 (1H, s); MS (ESI) m/z 400 [(M+H)$^+$]. HRMS M+H$^+$ calcd for $C_{21}H_{26}N_3O_3S$ 400.1689; found 400.1691. HPLC t_R 4.22 min; purity (AUC) > 99%.

7.3.5. Isoquinoline-5-sulfonic acid (2-(2-(4-methylbenzyloxy)ethylamino)ethyl)amide (6). Prepared from 4-methylbenzyl alcohol using General Method A. ^{1}H NMR (CD_3OD, 250 MHz) δ 2.35 (3H, s), 2.61–2.66 (4H, m), 3.01 (2H, t, J = 6 Hz), 3.46 (2H, t, J = 6 Hz), 4.44 (2H, s), 7.15–7.25 (4H, m), 7.84 (1H, t, J = 8 Hz), 8.41 (1H, d, J = 8 Hz), 8.49 (1H, d, J = 7, 1 Hz), 8.57 (1H, d, J = 6 Hz), 8.63 (1H, d, J = 6 Hz), 9.41 (1H, s); MS (ESI) m/z 400 [(M+H)$^+$]. HRMS M+H$^+$ calcd for $C_{21}H_{26}N_3O_3S$ 400.1689; found 400.1685. HPLC t_R 5.99 min; purity (AUC) 96%.

7.3.6. Isoquinoline-5-sulfonic acid (2-(2-(2-chlorobenzyloxy)ethylamino)ethyl)amide (7). Prepared from 2-chlorobenzyl alcohol using General Method A. ^{1}H NMR (CD_3OD, 250 MHz) δ 2.62–2.72 (4H, m), 3.02 (2H, t, J = 6 Hz), 3.56 (2H, t, J = 6 Hz), 4.60 (2H, s), 7.30–7.53 (4H, m), 7.84 (1H, t, J = 8 Hz), 8.41 (1H, d, J = 8 Hz), 8.50 (1H, d, J = 6 Hz), 8.57 (1H, d, J = 6 Hz), 8.63 (1H, d, J = 6 Hz), 9.41 (1H, s); MS (ESI) m/z 420 [(M+H)$^+$]. HRMS M+H$^+$ calcd for $C_{20}H_{23}ClN_3O_3S$ 420.1143; found 420.1138. HPLC t_R 5.79 min; purity (AUC) 95%.

7.3.7. Isoquinoline-5-sulfonic acid (2-(2-(3-chlorobenzyloxy)ethylamino)ethyl)amide (8). Prepared from 3-chlorobenzyl alcohol using General Method A. ^{1}H NMR (CD_3OD, 250 MHz) δ 2.61–2.69 (4H, m), 3.02 (2H, t, J = 6 Hz), 3.49 (2H, t, J = 6 Hz), 4.49 (2H, s), 7.29–7.37 (4H, m), 7.84 (1H, t, J = 8 Hz), 8.41 (1H, d, J = 8 Hz), 8.50 (1H, dd, J = 7, 1 Hz), 8.58 (1H, d, J = 6 Hz), 8.64 (1H, d, J = 6 Hz), 9.41 (1H, s); MS (ESI) m/z 420 [(M+H)$^+$]. HRMS M+H$^+$ calcd for $C_{20}H_{23}ClN_3O_3S$ 420.1143; found 420.1140. HPLC t_R 5.98 min; purity (AUC) > 99%.

7.3.8. Isoquinoline-5-sulfonic acid (2-(2-(3,4-dichlorobenzyloxy)ethylamino)ethyl)amide (10). Prepared from 3,4-dichlorobenzyl alcohol using General Method A. ^{1}H NMR (CD_3OD, 250 MHz) δ 2.70–2.78 (4H, m), 3.09 (2H, t, J = 6 Hz), 3.58 (2H, t, J = 5 Hz), 4.55 (2H, s), 7.36 (1H, dd, J = 10, 2 Hz), 7.57 (1H, d, J = 8 Hz), 7.59 (1H, s), 7.91 (1H, t, J = 8 Hz), 8.48 (1H, d, J = 8 Hz), 8.57 (1H, dd, J = 8, 2 Hz), 8.64 (1H, d, J = 8 Hz), 8.71 (1H, d, J = 5 Hz), 9.48 (1H, s). MS (ESI) m/z 454 [(M+H)$^+$]. HRMS M+H$^+$ calcd for $C_{20}H_{22}Cl_2N_3O_3S$ 454.0753; found 454.0745. HPLC t_R 4.67 min; purity (AUC) >99%.

7.3.9. Isoquinoline-5-sulfonic acid (2-(2-(2-methoxybenzyloxy)ethylamino)ethyl)amide (11). Prepared from 2-methoxybenzyl alcohol using General Method A. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.61–2.67 (4H, m), 3.00 (2H, t, J = 5 Hz), 3.50 (2H, t, J = 5 Hz), 3.83 (3H, s), 4.51 (2H, s), 6.92 (1H, d, J = 5 Hz), 6.98 (1H, t, J = 7 Hz) 7.29–7.35 (2H, m), 7.73 (1H, t, J = 7 Hz), 8.23 (1H, J = 7 Hz), 8.46–8.50 (2H, m), 8.71 (1H, d, J = 5 Hz), 9.39 (1H, s). MS (ESI) m/z 416 [(M+H)$^+$]. HRMS M+H$^+$ calcd for $C_{21}H_{26}N_3O_4S$ 416.1644; found 416.1649. HPLC t_R 3.77 min; purity (AUC) 97%.

7.3.10. Isoquinoline-5-sulfonic acid (2-(2-(3-methoxybenzyloxy)ethylamino)ethyl)amide (12). Prepared from 3-methoxybenzyl alcohol using General Method A. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.55–2.75 (2H, m), 2.89 (2H, t, J = 4 Hz), 2.97 (2H, t, J = 4 Hz), 3.30–3.50 (2H, m), 3.67 (3H, d, J = 5Hz), 4.36 (2H, s), 6.73 (1H, t, J = 8 Hz), 6.81 (1H, t, J = 6 Hz) 7.10–7.25 (2H, m), 7.52 (1H, dt, J = 4, 7 Hz), 8.02 (1H, dd, J = 2, 7 Hz), 8.24–8.40 (2H, m), 8.48 (1H, d, J = 5 Hz), 9.18 (1H, s). MS (ESI) m/z 416 [(M+H)$^+$]. HRMS M+H$^+$ calcd for $C_{21}H_{26}N_3O_4S$ 416.1644; found 416.1653. HPLC t_R 3.69 min; purity (AUC) 96%.

7.3.11. Isoquinoline-5-sulfonic acid (2-(2-(4-methoxybenzyloxy)ethylamino)ethyl)amide (13). Prepared from 4-methoxybenzyl alcohol using General Method A. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.37 (2H, t, J = 5 Hz), 2.47 (2H, t, J = 4 Hz), 2.81 (2H, t, J = 5 Hz), 3.23 (2H, t, J = 5 Hz), 3.66 (3H, s), 4.24 (2H, s), 6.72–6.77 (2H, m) 7.06–7.25 (2H, m), 7.55 (1H, t, J = 7 Hz), 8.05 (1H, d, J = 8 Hz), 8.28–8.31 (2H, *m*), 8.54 (1H, d, J = 6 Hz), 9.21 (1H, s). MS (ESI) m/z 416 [(M+H)$^+$]. HRMS M+H$^+$ calcd for $C_{21}H_{26}N_3O_4S$ 416.1644; found 416.1649. HPLC t_R 3.64 min; purity (AUC) 93%.

7.3.12. Isoquinoline-5-sulfonic acid piperazin-1 ylamide[11] (18)

7.3.12.1. General Method B. A solution of **1** (0.228 g, 1 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a stirred solution of piperazine (0.172 g, 2 mmol) and Et_3N (0.275 mL, 2 mmol) in CH_2Cl_2 (10 mL) cooled in an ice bath. The solution was warmed to rt. After 3 h, water (20 mL) was added and the two phases were separated. The organic layer was dried and concentrated to give **18**[11] (0.090 g, 0.324 mmol, 32%) as an oil, which crystallised on storage at −10 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.87 (1H, br s), 2.87–3.16 (4H, m), 3.12–3.16 (4H, m), 7.72 (1H, t, J = 8 Hz), 8.21–8.24 (1H,

m), 8.36–8.39 (1H, m), 8.52–8.55 (1H, m), 8.68 (1H, d, $J = 8$ Hz), 9.35 (1H, s). MS (ESI) *m/z* 278 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{13}H_{16}N_3O_2S$ 278.0958; found 278.0961. HPLC t_R 1.60 min; purity (AUC) 90%.

7.3.13. Isoquinoline-5-sulfonic acid piperidin-4-ylamide (19). Prepared from 4-aminopiperidine using General Method B. ^1H NMR (CD_3OD, 250 MHz) δ 1.30–1.46 (2H, m), 1.88 (2H, br d, $J = 12$ Hz), 2.63–2.74 (3H, m), 3.86 (2H, br d, $J = 12$ Hz), 7.88 (1H, t, $J = 8$ Hz), 8.46 (1H, d, $J = 8$ Hz), 8.46 (1H, dd, $J = 5$, 1 Hz), 8.60–8.66 (2H, m), 9.42 (1H, s). MS (ESI) *m/z* 292 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{14}H_{18}N_3O_2S$ 292.1114; found 292.1119. HPLC t_R 2.71 min; purity (AUC) 95%.

7.3.14. Isoquinoline-5-sulfonic acid (piperidin-4-ylmethyl)amide (21). Prepared from 4-aminomethyl-piperidine using General Method B. ^1H NMR (CD_3OD, 250 MHz) δ 0.75–0.93 (2H, m), 1.24–1.51 (3H, m), 2.22–2.36 (2H, m), 2.62 (2H, d, $J = 7$ Hz), 2.77–2.81 (2H, m), 7.66–7.74 (1H, m), 8.22–8.36 (2H, m), 8.42–8.53 (2H, m), 9.27 (1H, s). MS (ESI) *m/z* 306 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{15}H_{20}N_3O_2S$ 306.1276; found 306.1267. HPLC t_R 1.32 min; purity (AUC) 95%.

7.3.15. Isoquinoline-5-sulfonic acid (4-aminopiperidin-1-yl)amide (20)

7.3.15.1. General Method C. 4-(*N*-Boc-amino)piperidine (891 mg, 4.45 mmol) was treated as described in General Method A followed by recrystallisation from CH_2Cl_2–hexane to afford [1-(isoquinoline-5-sulfonyl)piperidin-4-yl]carbamic acid *tert*-butyl ester as a white solid (398 mg, 1.02 mmol, 47%). ^1H NMR ($CDCl_3$, 250 MHz) δ 1.40 (9H, s), 1.86–1.98 (4H, m), 2.67–2.77 (2H, m), 3.40–3.49 (1H, br s), 3.75–3.80 (2H, m), 4.37–4.42 (1H, br s), 7.72 (1H, t, $J = 8$ Hz), 8.23 (1H, d, $J = 8$ Hz), 8.38 (1H, d, $J = 8$ Hz), 8.46 (1H, d, $J = 7$ Hz), 8.68 (1H, d, $J = 6$ Hz), 9.36 (1H, s). MS (ESI) *m/z* 336 [$(M+H-{}^tBu)^+$].

Trifluoroacetic acid (2 mL) was added dropwise to a stirred solution of [1-(isoquinoline-5-sulfonyl)piperidin-4-yl]carbamic acid *tert*-butyl ester (309 mg, 0.79 mmol) in CH_2Cl_2 (5 mL) cooled in an ice bath. After 3 h, the solvents were concentrated in vacuo. The crude mixture was dissolved in water (20 mL), basified to pH 10 by addition of 1 M aq NaOH and extracted with EtOAc. The organic layer was dried and concentrated. Flash silica chromatography (20% MeOH–CH_2Cl_2) gave **20** (61 mg, 0.21 mmol, 26%). ^1H NMR (CD_3OD, 250 MHz) δ 1.53–1.66 (2H, m), 2.01–2.06 (2H, m), 2.71–2.81 (2H, m), 3.05–3.14 (1H, m), 3.97–4.03 (2H, m), 7.91 (1H, t, $J = 8$ Hz), 8.48–8.52 (2H, m), 8.60 (1H, d, $J = 8$ Hz), 8.68 (1H, d, $J = 5$ Hz), 9.45 (1H, s). MS (ESI) *m/z* 292 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{14}H_{18}N_3O_2S$ 292.1114; found 292.1116. HPLC t_R 2.72 min; purity (AUC) 95%.

7.3.16. Isoquinoline-5-sulfonic acid *trans*-(4-aminocyclohexyl)amide (22). Prepared from *trans*-1,4-diaminocyclohexane using General Method C. ^1H NMR (CD_3OD, 250 MHz) δ 0.94–1.91 (8H, m), 2.52–2.61 (1H, m), 3.01–3.07 (1H, m), 7.84 (1H, t, $J = 8$ Hz), 8.42 (1H, d, $J = 8$ Hz), 8.52 (1H, d, $J = 8$ Hz), 8.57 (1H, d, $J = 5$ Hz), 8.64 (1H, d, $J = 8$ Hz), 9.42 (1H, s). MS (ESI) *m/z* 306 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{15}H_{20}N_3O_2S$ 306.1271; found 306.1273. HPLC t_R 1.13 min; purity (AUC) 93%.

7.3.17. Isoquinoline-5-sulfonic acid pyrrolidin-3-ylamide (23). Prepared from 3-aminopyrrolidine using General Method C. ^1H NMR (CD_3OD, 250 MHz) δ 1.60–1.73 (1H, m), 2.01–2.14 (1H, m), 3.04 (1H, dd, $J = 10$, 5 Hz), 3.33–3.58 (4H, m), 7.85 (1H, dd, $J = 8$, 8 Hz), 8.40–8.49 (2H, m), 8.62–8.68 (2H, m), 9.39 (1H, s); MS (ESI) *m/z* 278 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{13}H_{16}N_3O_2S$ 278.0963; found 278.0961. HPLC t_R 1.34 min; purity (AUC) 94%.

7.3.18. Isoquinoline-5-sulfonic acid ((2S)-pyrrolidin-2-ylmethyl)amide (24). Prepared from (2*S*)-2-aminomethylpyrrolidine using General Method C. ^1H NMR (CD_3OD, 250 MHz) δ 1.49–1.84 (4H, m), 3.02–3.09 (2H, m), 3.39–3.52 (2H, m), 4.00–4.10 (1H, m), 7.91 (1H, dd, $J = 8$, 8 Hz), 8.50 (1H, d, $J = 8$ Hz), 8.55 (1H, d, $J = 8$ Hz), 8.67 (1H, d, $J = 7$ Hz), 8.83 (1H, $J = 7$ Hz), 9.44 (1H, s); MS (ESI) *m/z* 292 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{14}H_{18}N_3O_2S$ 292.1120; found 292.1109. HPLC t_R 1.47 min; purity (AUC) 97%.

7.3.19. Isoquinoline-5-sulfonic acid *trans*-(4-benzylaminocyclohexyl)amide (25). A solution of benzaldehyde (0.044 mL, 0.43 mmol) in 1,2-dichloroethane (5 mL) was added to a vigorously stirred mixture of (*trans*-4-aminocyclohexyl)carbamic acid *tert*-butyl ester (98 mg, 0.45 mmol), activated 4 Å molecular sieves (164 mg) and 1,2-dichloroethane (20 mL) at 0 °C. After 20 min, $Na(OAc)_3H$ (179 mg, 0.8 mmol) was added in one portion, followed by AcOH (0.5 mL). The reaction mixture was stirred at rt until the reactants were consumed as determined by TLC. The reaction was quenched by addition of a saturated solution of $NaHCO_3$, followed by filtration through a layer of Celite. The filtrate was extracted with EtOAc and the organic extract was dried and concentrated. Flash silica chromatography (1% NH_3–9% MeOH–90% CH_2Cl_2) gave *trans*-(4-benzylaminocyclohexyl)carbamic acid *tert*-butyl ester (62 mg, 0.20 mmol, 45%). ^1H NMR ($CDCl_3$, 250 MHz) δ 1.06–1.31 (4H, m), 1.43 (9H, s), 1.99–2.02 (5H, m), 2.45–2.53 (1H, m), 3.39 (1H, br s), 3.81 (2H, s) 4.31 (1H, br s), 7.28–7.36 (5H, m). MS (ESI) *m/z* 306 [$(M+H)^+$].

CF_3CO_2H (1 mL) was added dropwise at 0 °C to a stirred solution of *trans*-(4-benzylaminocyclohexyl)carbamic acid *tert*-butyl ester (62 mg, 0.2 mmol) in CH_2Cl_2 (1 mL). After 3 h, the solvents were concentrated. The crude product was purified by ion exchange on basic resin (MeOH) to afford *N*-benzyl-*trans*-cyclohexane-1,4-diamine (37 mg, 0.18 mmol, 91%). ^1H NMR (CD_3OD, 250 MHz) δ 1.12–1.29 (4H, m), 1.92–2.07 (4H, m), 2.46–2.56 (1H, m), 2.65–2.75 (1H, m), 3.82 (2H, s) 7.28–7.39 (5H, m). MS (ESI) *m/z* 205 [$(M+H)^+$].

N-Benzyl-*trans*-cyclohexane-1,4-diamine (37 mg, 0.18 mmol) was treated as described in General Method A to give

25 (11 mg, 0.03 mmol, 15%) as a white solid. ^{1}H NMR (CD_3OD, 250 MHz) δ 1.05–1.33 (4H, m), 1.62–1.93 (4H, m), 2.42 (1H, m), 3.05 (1H, m), 3.75 (2H, s) 7.28–7.39 (5H, m), 7.86 (1H, t, J = 8 Hz), 8.42 (1H, d, J = 8 Hz), 8.53 (1H, d, J = 8 Hz), 8.58 (1H, d, J = 8 Hz), 8.66 (1H, d, J = 8 Hz), 9.43 (1H, s). MS (ESI) m/z 396 [$(M+H)^+$]. MS (ESI) m/z 292 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{22}H_{26}N_3O_2S$ 396.1740; found 396.1740. HPLC t_R 2.72 min; purity (AUC) 95%.

7.3.20. Isoquinoline-5-sulfonic acid (3-(4-chlorobenzyloxymethyl)piperazin-1-yl)amide (29). 1-Benzyl-piperazine-2-carboxylic acid ethyl ester[35] (**26**) (60 mg, 0.24 mmol) was treated as described in General Method A, followed by flash silica chromatography, (4% MeOH–CH_2Cl_2), to give 1-benzyl-4-(isoquinoline-5-sulfonyl)piperazine-2-carboxylic acid ethyl ester (**27**) (61 mg, 0.14 mmol, 57%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.20 (3H, t, J = 8 Hz), 2.48–2.56 (1H, m), 2.99–3.45 (5H, m), 3.61–3.70 (2H, m), 3.88–4.11 (3H, m), 7.23–7.32 (5H, m), 7.74 (1H, t, J = 8 Hz), 8.26 (1H, d, J = 8 Hz), 8.40 (1H, dd, J = 8, 1 Hz), 8.47 (1H, d, J = 6 Hz), 8.70 (1H, d, J = 6 Hz), 9.38 (1H, s). MS (ESI) m/z 440 [$(M+H)^+$].

A solution of **27** (193 mg, 0.44 mmol) in THF (2 mL) was added to a stirred suspension of $LiAlH_4$ (1 M in THF, 0.45 mL, 0.45 mmol) at 0 °C. After 1.5 h, water (30 μL) was added, followed by 1 M aq NaOH (40 μL). After stirring at rt for 18 h, further water (30 μL) was added and the suspension was filtered through a layer of Celite, washing the filter cake with Et_2O. The organic filtrate was concentrated. Flash silica chromatography (10% MeOH–CH_2Cl_2) gave [1-benzyl-4-(isoquinoline-5-sulfonyl)piperazin-2-yl]-methanol ((116 mg, 0.29 mmol, 66%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.20 (1H, s), 2.41–2.50 (1H, m), 2.72–2.73 (1H, m), 2.86–2.96 (2H, m), 3.04–3.12 (1H, m), 3.37–3.65 (3H, m), 3.63–3.84 (1H, m), 3.92–4.06 (2H, m), 7.24–7.35 (5H, m), 7.75 (1H, t, J = 8 Hz), 8.26 (1H, d, J = 8 Hz), 8.40 (1H, dd, J = 8, 1 Hz), 8.53 (1H, d, J = 6 Hz), 8.70 (1H, d, J = 8 Hz), 9.39 (1H, s). MS (ESI) m/z 398 [$(M+H)^+$].

7.3.20.1. General Method D. NaH (60% dispersion in oil, 27 mg, 0.67 mmol) was added at rt to a suspension of [1-benzyl-4-(isoquinoline-5-sulfonyl)piperazin-2-yl]-methanol (130 mg, 0.33 mmol) in DMF (5 mL). After 15 min, 4-chlorobenzyl bromide (64 mg, 0.31 mmol) was added. After 4.5 h, the solution was diluted with water (20 mL) and extracted with EtOAc. The organic layer was washed with water, dried and concentrated. Flash silica chromatography (10% MeOH–CH_2Cl_2) gave 5-[4-benzyl-3-(4-chloro-benzyloxymethyl)piperazine-1-sulfonyl]-isoquinoline (**28**) (84 mg, 0.16 mmol, 52%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.32–2.46 (1H, m), 2.67–2.84 (2H, m), 3.06–3.93 (8H, m), 4.31 (2H, br s), 7.16–7.33 (9H, m), 7.70 (1H, t, J = 8 Hz), 8.20 (1H, d, J = 8 Hz), 8.36 (1H, d, J = 7 Hz), 8.52 (1H, br s), 8.67 (1H, d, J = 6 Hz), 9.34 (1H, s). MS (ESI) m/z 522 [$(M+H)^+$].

A solution of 1-chloroethyl chloroformate (0.040 mL, 0.38 mmol) in 1,2-dichloroethane (2 mL) was added dropwise over 10 min to a stirred solution of **28** (84 mg, 0.16 mmol) in 1,2-dichloroethane (4 mL) at 0 °C. Stirring was continued at 0 °C for 10 min, after which the mixture was refluxed for 3 days. The mixture was cooled to rt and concentrated. The resulting oil was dissolved in MeOH (5 mL) and refluxed for 2 h. The solvent was evaporated and the residue was dissolved in water (10 mL). The solution was washed sequentially with Et_2O and CH_2Cl_2, then basified to pH 9 with $NaHCO_3$ (s) and extracted with CH_2Cl_2. The organic extract was dried and concentrated. Flash silica chromatography (10% MeOH–CH_2Cl_2) gave **29** (29 mg, 0.07 mmol, 42%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.35–2.46 (1H, m, 1H), 2.67–3.63 (9H, m), 4.41 (2H, s), 7.20 (2H, d, J = 8 Hz), 7.30 (2H, d, J = 8 Hz), 7.72 (1H, t, J = 8 Hz), 8.23 (1H, d, J = 7 Hz), 8.36 (1H, d, J = 8 Hz), 8.50 (1H, d, J = 6 Hz), 8.68 (1H, d, J = 6 Hz), 9.35 (1H, s). MS (ESI) m/z 432 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{21}H_{23}ClN_3O_3S$ 432.1143; found 432.1147. HPLC t_R 4.49 min; purity (AUC) 99%.

7.3.21. Isoquinoline-5-sulfonic acid (2*S*,4*R*)-[2-(4-chlorobenzyloxymethyl)pyrrolidin-4-yl]amide (40). (2*S*,4*R*)-4-Aminopyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester hydrochloride (**30**) (265 mg, 0.94 mmol) was treated according to General Method B followed by flash silica chromatography (5% MeOH–CH_2Cl_2) to give (2*S*,4*R*)-4-(isoquinoline-5-sulfonylamino)pyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (**32**) as an oil (309 mg, 0.595 mmol, 63%). ^{1}H NMR ($CDCl_3$) δ 1.27 (9H, s), 1.81–2.24 (2H, m), 2.90–3.12 (1H, m), 3.37–3.50 (1H, m), 3.62 (3H, s), 3.77–3.94 (1H, m), 4.10–4.27 (1H, m), 5.91–6.27 (1H, m), 7.60–7.68 (1H, m), 8.15–8.18 (1H, m), 8.31–8.41 (2H, m), 8.55–8.62 (1H, m), 9.30 (1H, s). MS (ESI) m/z 436 [$(M+H)^+$].

NaH (60% dispersion in oil, 43 mg, 1.07 mmol) was added in one portion to a stirred solution of **32** (357 mg, 0.82 mmol) in DMF (8 mL) at 0 °C. After 5 min, SEM-Cl (152 μL, 0.859 mmol) was added and stirring was continued for 5 min. The mixture was diluted with water (30 mL) and extracted with EtOAc (3 × 30 mL). The organic layers were dried and concentrated. Flash silica chromatography (70% EtOAc–petroleum ether) gave (2*S*,4*R*)-4-[isoquinoline-5-sulfonyl(2-trimethylsilanylethoxymethyl)amino]pyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (**34**) as an oil (372 mg, 0.658 mmol, 80%). ^{1}H NMR ($CDCl_3$) δ 0.00 (9H, s), 0.80–0.88 (2H, m), 1.37, 1.42 (9H, 2× s), 1.85–2.55 (2H, m), 3.26–3.37 (1H, m), 3.52–3.62 (3H, m), 3.69 (3H, s), 4.28–4.49 (2H, m), 4.87–5.04 (2H, m), 7.68–7.74 (1H, m), 8.22–8.46 (3H, m), 8.69–8.71 (1H, m), 9.37 (1H, s). MS (ESI) m/z 566 [$(M+H)^+$].

7.3.21.1. General Method E. To a solution of **34** (330 mg, 0.58 mmol) in THF (10 mL) at 0 °C was added $LiAlH_4$ (584 μL of a 1 M sol in THF, 0.58 mmol). After 20 min, further $LiAlH_4$ (300 μL, 0.30 mmol) was added. After 20 min, a third portion of $LiAlH_4$ (200 μL, 0.20 mmol) was added. The reaction mixture was diluted with Et_2O (20 mL) and quenched by dropwise addition of water (40 μL), followed by 10% aq NaOH (40 μL).

After stirring for 18 h, water (120 μL) was added dropwise and the resulting white precipitate was filtered through a pad of Celite. The filtrate was concentrated. Flash silica chromatography (EtOAc) gave (2*S*,4*R*)-2-hydroxymethyl-4-[isoquinoline-5-sulfonyl(2-trimethylsilanylethoxymethyl)amino]pyrrolidine-1-carboxylic acid *tert*-butyl ester (**36**) as an oil (227 mg, 0.422 mmol, 72%). ^{1}H NMR ($CDCl_3$) δ 0.00 (9H, s), 0.81–0.87 (2H, m), 1.44 (9H, s), 1.85–2.30 (2H, m), 3.30–4.50 (8H, m), 4.87–5.04 (2H, m), 7.69–7.75 (1H, m), 8.22–8.26 (1H, m), 8.35–8.37 (1H, m), 8.44–8.47 (1H, m), 8.70–8.72 (1H, m), 9.38 (1H, s). MS (ESI) *m*/*z* 538 [$(M+H)^+$], 560 [$(M+Na)^+$].

Compound **36** (113 mg, 0.21 mmol) was treated according to General Method D with flash silica chromatography (70% EtOAc–petroleum ether) to give (2*S*,4*R*)-2-(4-chlorobenzyloxymethyl)-4-[isoquinoline-5-sulfonyl(2-trimethylsilanylethoxymethyl)amino]pyrrolidine-1-carboxylic acid *tert*-butyl ester (**38**) as an oil (92 mg, 0.139 mmol, 66%). ^{1}H NMR ($CDCl_3$) δ 0.00 (9H, s), 0.82–0.88 (2H, m), 1.41 (9H, s), 1.80–2.35 (2H, m), 3.21–4.05 (7H, m), 4.38 (2H, s), 4.52–4.63 (1H, m), 4.90–5.05 (2H, m), 7.16–7.32 (4H, m), 7.52–7.58 (1H, m), 8.16–8.19 (1H, m), 8.30–8.36 (2H, m), 8.65–8.68 (1H, m), 9.35 (1H, s). MS (ESI) *m*/*z* 662 [$(M+H)^+$].

Six molar HCl (1 mL) was added to a solution of **38** (22 mg, 0.046 mmol) in MeOH (1 mL) at rt. After refluxing for 4 h, the solution was cooled, concentrated and purified by ion exchange on basic resin (MeOH) to give **40** as an oil (15 mg, 0.035 mmol, 76%). ^{1}H NMR ($CDCl_3$) δ 1.48–1.57 (2H, m), 2.51 (1H, dd, J = 11, 5 Hz), 2.91 (1H, dd, J = 11, 6 Hz), 3.11–3.34 (3H, m), 3.66–3.75 (1H, m), 4.33 (2H, s), 7.08–7.25 (4H, m), 7.44–7.47 (1H, m), 8.13–8.16 (1H, m), 8.31–8.40 (2H, m), 8.59–8.61 (1H, m), 9.30 (1H, s). MS (ESI) *m*/*z* 432 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{21}H_{23}ClN_3O_3S$ 432.1143; found 432.1146. HPLC t_R 6.00 min; purity (AUC) >99%.

7.3.22. Isoquinoline-5-sulfonic acid (2*S*,4*S*)-(2-(4-chlorobenzyloxymethyl)pyrrolidin-4-yl)amide (41). Prepared as described for **40** starting from (2*S*,4*S*)-4-aminopyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester hydrochloride (**31**). ^{1}H NMR ($CDCl_3$) δ 1.25–1.43 (1H, m), 1.97–2.10 (1H, m), 2.67–2.80 (2H, m), 3.27–3.56 (3H, m), 3.79–3.84 (1H, m), 4.51 (1H, d, J = 12 Hz), 4.58 (1H, d, J = 12 Hz), 7.29–7.40 (4H, m), 7.66–7.73 (1H, m), 8.15–8.22 (2H, m), 8.40–8.43 (1H, m), 8.58–8.60 (1H, m), 9.36 (1H, d, J = 1 Hz). MS (ESI) *m*/*z* 432 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{21}H_{23}ClN_3O_3S$ 432.1143; found 432.1143. HPLC t_R 4.57 min; purity (AUC) 95%.

7.3.23. Isoquinoline-5-sulfonic acid (2*S*,4*R*)-(4-(4-chlorobenzyloxy)pyrrolidin-2-yl-methyl)amide (48). (2*S*,4*R*)-4-Hydroxypyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester[38] (**42**) (2.00 g, 8.15 mmol) was treated according to General Method D with flash silica chromatography (40% EtOAc–petroleum ether) to give an oil (1.81 g, impure). The crude material was treated according to General Method E with flash silica chromatography (Et_2O) to give (2*S*,4*R*)-4-(4-chlorobenzyloxy)-2-hydroxymethylpyrrolidine-1-carboxylic acid *tert*-butyl ester (**44**) (1.23 g, 3.60 mmol, 44%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 1.6–2.3 (2H, m), 3.40–4.20 (6H, m), 4.45–4.55 (2H, m), 4.7–4.9 (1H, m), 7.25–7.36 (4H, m). MS (ESI) *m*/*z* 364 [$(M+Na)^+$].

7.3.23.1. General Method F. Ms_2O (2 M in CH_2Cl_2, 932 μL, 1.87 mmol) was added dropwise to a solution of **44** (425 mg, 1.24 mmol) and Et_3N (520 μL, 3.73 mmol) in CH_2Cl_2 (4 mL) at 0 °C and the resulting solution was stirred for 15 min. CH_2Cl_2 (10 mL) was added and the solution was washed with 1 M HCl (10 mL), saturated aq $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried and concentrated to give the crude mesylate as an oil (483 mg), which was used immediately without further purification. The oil (483 mg) was dissolved in DMF (6 mL) and NaN_3 was added (162 mg, 2.49 mmol). After heating at 80 °C for 3 h, the solution was cooled, water (30 mL) was added and the mixture was extracted with EtOAc (30 mL). The organic layer was washed with brine (30 mL), dried and concentrated to give (2*S*,4*R*)-2-azidomethyl-4-(4-chlorobenzyloxy)pyrrolidine-1-carboxylic acid *tert*-butyl ester (**46**) as an oil (400 mg, 1.09 mmol, 88%). ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 3.2–4.2 (8H, m), 4.50 (2H, br s), 7.25–7.37 (4H, m). MS (ESI) *m*/*z* 389 [$(M+Na)^+$].

A solution of **46** (50 mg, 0.14 mmol) in EtOAc (3 mL) with 10% Pd/C (10 mg) was hydrogenated at rt, 1 atm for 2 h. The mixture was filtered and concentrated. Ion exchange on acidic resin (MeOH, then 1 M NH_3 in MeOH) gave the crude amine as an oil (27 mg), which was treated according to General Method B. The crude sulfonamide thus obtained was dissolved in MeOH (1 mL) and 1 M HCl in Et_2O (2 mL). After 3.5 h, the mixture was concentrated. Ion exchange on acidic resin (MeOH, then 1 M NH_3 in MeOH) gave **48** (31 mg, 0.072 mmol, 53%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.44–1.52 (1H, m), 1.92–2.06 (1H, m), 2.67–2.75 (2H, m), 2.88–3.05 (2H, m), 3.45–3.52 (1H, m), 3.98–4.03 (1H, m), 4.37 (2H, s), 7.13–7.35 (4H, m), 7.67–7.74 (1H, m), 8.17–8.23 (1H, m), 8.43–8.46 (2H, m), 8.69–8.73 (1H, m), 9.37 (1H, s). MS (ESI) *m*/*z* 432 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{21}H_{23}ClN_3O_3S$ 432.1143; found 432.1146. HPLC t_R 4.87 min; purity (AUC) 96%.

7.3.24. Isoquinoline-5-sulfonic acid (2*S*,4*S*)-(4-(benzyloxy)pyrrolidin-2-yl-methyl)amide (49) and Isoquinoline-5-sulfonic acid (2*S*,4*S*)-(4-(4-chlorobenzyloxy)pyrrolidin-2-yl-methyl)amide (50). Prepared following the procedure described for **48** to give a mixture of **49** and **50**. A portion of the mixture was separated by preparative HPLC to give **49**: ^{1}H NMR ($CDCl_3$) δ 1.84–1.88 (1H, m), 2.26–2.38 (1H, m), 3.15–3.22 (1H, m), 3.33–3.45 (2H, m), 3.62–3.67 (1H, m), 4.05–4.12 (1H, m), 4.26 (1H, br s), 4.45 (1H, d, J = 12 Hz), 4.56 (1H, d, J = 12 Hz), 7.25–7.38 (4H, m), 7.61–7.68 (1H, m), 8.16–8.20 (1H, m), 8.35–8.38 (1H, m), 8.48–8.50 (1H, m), 8.63–8.66 (1H, m), 9.34 (1H, s). MS (ESI) *m*/*z* 398 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{21}H_{24}N_3O_3S$ 398.1533; found 398.1535. HPLC t_R 4.45 min; purity (AUC) >99%. **50**: ^{1}H NMR ($CDCl_3$) δ 1.83–1.89 (1H,

m), 2.26–2.38 (1H, m), 3.16–3.20 (1H, m), 3.32–3.41 (2H, m), 3.61–3.66 (1H, m), 3.95–4.15 (1H, m), 4.25 (1H, br s), 4.40 (1H, d, $J = 12$ Hz), 4.53 (1H, d, $J = 12$ Hz), 7.21–7.30 (4H, m), 7.63–7.69 (1H, m), 8.18–8.21 (1H, m), 8.34–8.37 (1H, m), 8.47–8.50 (1H, m), 8.64–8.68 (1H, m), 9.36 (1H, s). MS (ESI) *m/z* 432 $[(M+H)^+]$. HRMS $M+H^+$ calcd for $C_{21}H_{23}N_3O_3ClS$ 432.1143; found 432.1146. HPLC t_R 4.94 min; purity (AUC) >99%.

7.3.25. Isoquinoline-5-sulfonic acid (2*R,5*S**)-(5-(4-chlorobenzyloxymethyl)pyrrolidin-2-yl-methyl)amide (55).** Et_3N (3.5 mL, 24.8 mmol), DMAP (303 mg, 2.48 mmol) and Boc_2O (3.25 g, 14.9 mmol) were added to a stirred solution of (1*S**,5*R**)-3,8-diazabicyclo[3.2.1]octan-2-one[39] (**51**) (626 mg, 4.96 mmol) in CH_2Cl_2 (20 mL) at rt. After 15 h, the mixture was absorbed onto silica and purified by flash silica chromatography (50–80% Et_2O–hexane) to give (1*S**,5*R**)-2-oxo-3,8-diazabicyclo[3.2.1]octane-3,8-dicarboxylic acid di-*tert*-butyl ester (692 mg, 2.13 mmol, 43%) (**52**) as an oil. ^1H NMR ($CDCl_3$) δ 1.48 (9H, s), 1.54 (9H, s), 1.72–1.83 (1H, m), 2.05–2.23 (3H, m), 3.43 (1H, dd, $J = 1$ Hz), 3.87 (1H, dd, $J = 1$ Hz), 4.56 (2H, br s). MS (ESI) *m/z* 349 $[(M+Na)^+]$.

$NaBH_4$ (76 mg, 2.00 mmol) was added in one portion to a solution of **52** (327 mg, 1.00 mmol) in MeOH (10 mL) at 0 °C. After 1 h, water (20 mL) was added and the mixture was extracted with Et_2O (3× 20 mL). The organic extracts were dried and concentrated to give (2*R**,5*S**)-2-(*tert*-butoxycarbonylaminomethyl)-5-hydroxymethylpyrrolidine-1-carboxylic acid *tert*-butyl ester (**53**) (331 mg, 2.00 mmol, 100%) as an oil. ^1H NMR ($CDCl_3$) δ 1.36 (9H, s), 1.42 (9H, s), 1.6–2.0 (4H, m), 2.9–4.0 (6H, m). MS (ESI) *m/z* 353 $[(M+Na)^+]$.

Compound **53** (205 mg, 0.62 mmol) was treated according to General Method D followed by flash silica chromatography (50–100% EtOAc–hexane) to give (2*R**,5*S**)-2-(*tert*-butoxycarbonylaminomethyl)-5-(4-chlorobenzyloxymethyl)pyrrolidine-1-carboxylic acid *tert*-butyl ester (**54**) (200 mg, 0.44 mmol, 71%) as an oil. ^1H NMR ($CDCl_3$) δ 1.35 (18H, s), 1.6–2.0 (4H, m), 3.07–3.42 (4H, m), 3.87 (2H, br s), 4.36–4.51 (2H, m), 7.16–7.26 (4H, m). MS (ESI) *m/z* 477 $[(M+Na)^+]$.

One molar HCl in Et_2O (3 mL) was added to a stirred solution of **54** (116 mg, 0.255 mmol) in MeOH (3 mL) at rt After 22 h, the solution was concentrated and the residues were purified by ion exchange on acidic resin (MeOH, then 1 M NH_3 in MeOH). The basic fractions were concentrated to give the crude deprotected amine as an oil (59 mg). Treatment of the crude amine according to General Method B was followed by ion exchange on basic resin (MeOH). Flash silica chromatography (10% MeOH–CH_2Cl_2) gave **55** (45 mg, 0.101 mmol, 40%) as an oil. ^1H NMR ($CDCl_3$) δ 1.42–1.86 (4H, m), 2.76 (2H, d, $J = 6$ Hz), 3.41–3.54 (2H, m), 3.69–3.79 (1H, m), 3.85–3.94 (1H, m), 4.38 (1H, d, $J = 12$ Hz), 4.43 (1H, d, $J = 12$ Hz), 7.12–7.27 (4H, m), 7.60–7.66 (1H, m), 8.12–8.16 (1H, m), 8.35–8.38 (1H, m), 8.60–8.62 (2H, m), 9.27 (1H, s). MS (ESI) *m/z* 446 $[(M+H)^+]$. HRMS $M+H^+$ calcd for $C_{22}H_{25}ClN_3O_3S$ 446.1300; found 446.1297. HPLC t_R 5.30 min; purity (AUC) >99%.

7.3.26. 4-Methylisoquinoline-5-sulfonic acid (2*S*,4*S*)-(2-(4-chlorobenzyloxymethyl)pyrrolidin-2-yl)amide (61). KH (30% dispersion in oil, 8.19 g, 61 mmol) was washed with hexane (30 mL) and suspended in THF (50 mL). The suspension was cooled in an ice bath and a solution of (2*S*,4*R*)-2-hydroxymethyl-4-(tetrahydropyran-2-yloxy)-pyrrolidine-1-carboxylic acid *tert*-butyl ester[40] (**56**) (11.3 g, 37.5 mmol) in THF (50 mL) was added dropwise via cannula. The mixture was stirred for 5 min, followed by addition of a solution of 4-chlorobenzyl chloride (7.83 g, 48.6 mmol) in THF (20 mL) via cannula. The mixture was stirred for 48 h at rt and then poured into EtOAc (300 mL). The solution was washed with water (100 mL), saturated aq $NaHCO_3$ (50 mL) and brine (50 mL). The organic fraction was dried and concentrated. Flash silica chromatography (20% EtOAc–hexane) gave (2*S*,4*R*)-2-(4-chlorobenzyloxymethyl)-4-(tetrahydropyran-2-yloxy)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (**57**) (8.66 g, 20.4 mmol, 54%). ^1H NMR (250 MHz, $CDCl_3$) δ 1.40–2.00 (8H, m), 1.47 (9H, s), 3.35–3.75 (4H, m), 3.75–4.25 (2H, m), 4.40–4.85 (4H, m), 7.23–7.40 (4H, m). MS (ESI) *m/z* 448 $[(M+Na)^+]$.

PPTS (0.59 g, 2.3 mmol) was added to a stirred solution of **57** (8.66 g, 20.3 mmol) in EtOH (50 mL). The solution was heated at 55 °C for 4 h. The cooled solution was diluted with EtOAc (250 mL) and then washed with saturated aq $NaHCO_3$ (50 mL) and brine (50 mL). The organic fraction was dried and concentrated. Flash silica chromatography (30–60% EtOAc–hexane) gave (2*S*,4*R*)-2-(4-chlorobenzyloxymethyl)-4-hydroxy-pyrrolidine-1-carboxylic acid *tert*-butyl ester (**58**) (5.13 g, 15.0 mmol, 74%). ^1H NMR (250 MHz, $CDCl_3$) δ 1.47 (9H, s), 2.00–2.30 (2H, m), 3.40–3.80 (4H, m), 4.05–4.25 (1H, m), 4.45–4.60 (3H, m), 7.25 (2H, d, $J = 10$ Hz), 7.34 (2H, d, $J = 10$ Hz). MS (ESI) *m/z* 364 $[(M+Na)^+]$.

Compound **58** (1.86 g, 5.40 mmol) was treated according to General Method F to give the crude azide. The azide was dissolved in THF (20 mL) and PPh_3 (3.56 g, 13.6 mmol) and water (2 mL, 111 mmol) were added. The mixture was stirred at rt for 18 h. Ion exchange on acidic resin (MeOH, then 2 M NH_3 in MeOH) and flash silica chromatography (5–10% MeOH–CH_2Cl_2) gave (2*S*,4*S*)-4-amino-2-(4-chlorobenzyloxymethyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (**59**) (1.29 g, 3.78 mmol, 70%). ^1H NMR (250 MHz, CD_3OD) δ 1.45 (9H, s), 1.82 (1H, m), 2.42 (1H, m), 3.03 (1H, dd, $J = 6.3$, 11.0 Hz), 3.42 (1H, t, $J = 6.6$ Hz), 3.60 (1H, dd, $J = 2.5$, 9.4 Hz), 3.75 (1H, dd, $J = 6.9$, 10.6 Hz), 3.95 (1H, br s), 4.53 (1H, d, $J = 12$ Hz), 4.60 (1H, d, $J = 12$ Hz), 7.36 (4H, s). MS (ESI) *m/z* 341 $[(M+H)^+]$.

Et_3N (50 μL, 0.36 mmol), 4-methylisoquinoline-5-sulfonylchloride[42] (30 mg, 0.12 mmol) and DMAP (2 mg, 0.016 mmol) were added to **59** (40 mg, 0.12 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred for 72 h at rt.

Flash silica chromatography (5% MeOH–CH_2Cl_2) gave (2*S*,4*S*)-2-(4-chlorobenzyloxymethyl)-4-(4-methylisoquinoline-5-sulfonylamino)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (**60**) (67 mg, 0.12 mmol, 100%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.45 (9H, s), 1.60–1.80 (1H, m), 1.95–2.20 (1H, m), 2.52 (1H, br s), 3.05 (3H, s), 3.47–3.60 (3H, m), 3.90–4.30 (3H, m), 4.60–4.75 (2H, m), 6.80–7.50 (4H, m), 7.57 (1H, dd, J = 7.5, 7.5 Hz), 8.17 (1H, d, J = 10 Hz), 8.44 (1H, d, J = 7.5 Hz), 8.55 (1H, s), 9.16 (1H, s). MS (ESI −ve ion) m/z 544 [$(M-H)^-$].

Four molar HCl in dioxane (1 mL) was added to **60** (58 mg, 0.106 mmol). The mixture was stirred at rt for 1 h and then diluted with Et_2O (4 mL). The liquid was decanted and the solid residue was triturated and washed with a further portion of Et_2O (4 mL). The solid was dissolved in MeOH and purified by ion exchange on acidic resin (MeOH, then 2 M NH_3 in MeOH) to give **61** (38 mg, 0.085 mmol, 81%). ^{1}H NMR (250 MHz, $CDCl_3$) 1.72–1.85 (1H, m), 2.20–2.35 (1H, m), 2.93 (1H, dd, J = 5, 11 Hz), 3.06 (3H, s), 3.20 (1H, d, J = 11 Hz), 3.41–3.60 (2H, m), 3.65 (1H, dd, J = 3, 9 Hz), 3.90–3.97 (1H, m), 4.55 (1H, d, J = 12 Hz), 4.63 (1H, J = 12 Hz), 7.29–7.31 (4H, m), 7.60 (1H, dd, J = 8, 8 Hz), 8.17 (1H, dd, J = 1, 8 Hz), 8.51 (1H, dd, J = 1, 8 Hz), 8.56 (1H, d, J = 1 Hz), 9.17 (1H, s). MS (ESI) m/z 446 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{22}H_{25}ClN_3O_3S$ 446.1300; found 446.1298. HPLC t_R 5.04 min; purity (AUC) 95%.

7.3.27. 1-Hydroxyisoquinoline-5-sulfonic acid (2*S*,4*S*)-(2-(4-chlorobenzyloxymethyl)pyrrolidin-4-yl)amide (65). Chlorosulfonic acid (9 mL, 135 mmol) was added to 1-chloroisoquinoline (3.38 g, 20 mmol) and the solution was heated at 125 °C for 72 h. After cooling to rt, the solution was added dropwise to saturated aq $NaHCO_3$ (500 mL) and the resulting precipitate was collected by filtration. The solid was washed with water (100 mL) and dried to give 1-chloroisoquinoline-5-sulfonyl chloride (4.21 g, 16 mmol, 80%). ^{1}H NMR (250 MHz, d_6-acetone) δ 8.11 (1H, dd, J = 10, 8 Hz), 8.51 (1H, dd, J = 6, 1 Hz), 8.65 (1H, d, J = 6 Hz), 8.76 (1H, dd, J = 8, 1 Hz), 8.91 (1H, d, J = 10 Hz).

To a solution of **59** (0.32 g, 0.94 mmol) in CH_2Cl_2 (5 mL) were added Et_3N (300 μL, 2.1 mmol) and then 1-chloroisoquinoline-5-sulfonyl chloride (0.25 g, 0.94 mmol) and DMAP (11 mg, 0.09 mmol). The solution was stirred at rt for 72 h. Flash silica chromatography (5% MeOH–CH_2Cl_2) gave (2*S*,4*S*)-2-(4-chlorobenzyloxymethyl)-4-(1-chloroisoquinoline-5-sulfonylamino)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (**62**) (0.44 g, 0.78 mmol, 83%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.29 (9H, s), 1.52 (1H, m), 2.24 (1H, m), 3.03 (1H, d, J = 12 Hz), 3.15–3.40 (2H, m), 3.70–4.10 (3H, m), 4.51 (2H, s), 7.20–7.33 (4H, m), 7.65 (1H, dd, J = 8.5, 8 Hz), 7.96 (1H, d, J = 6 Hz), 8.21 (1H, d, J = 6 Hz), 8.32 (1H, dd, J = 1, 8 Hz), 8.54 (1H, d, J = 8.5 Hz). MS (ESI) m/z 588 [$(M+Na)^+$].

NaH (60% dispersion in oil, 0.128 g, 3.2 mmol) was added to a solution of **62** (0.34 g, 0.60 mmol) in THF (1 mL). The mixture was stirred for 5 min and then SEM-Cl (200 μL, 1.1 mmol) was added. The mixture was stirred for 90 min and then poured into CH_2Cl_2 (75 mL). The solution was washed with saturated aq NH_4Cl (25 mL). The organic layer was dried and concentrated. Flash silica chromatography (30% EtOAc–hexane) gave (2*S*,4*S*)-2-(4-chlorobenzyloxymethyl)-4-[(1-chloroisoquinoline-5-sulfonyl)-(2-trimethylsilanyleth-oxymethyl)amino]-pyrrolidine-1-carboxylic acid *tert*-butyl ester (**63**) (0.44 g, quantitative). ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.00 (9H, s), 0.82 (2H, t, J = 8 Hz), 1.44 (9H, s), 2.05–2.30 (2H, m), 3.20 (1H, dd, J = 10.4, 10.6 Hz), 3.40–4.00 (5H, m), 4.16 (1H, m), 4.48 (2H, s), 4.93 (1H, d, J = 11 Hz), 4.99 (1H, d, J = 11 Hz), 7.24 (2H, d, J = 8 Hz), 7.34 (2H, d, J = 8 Hz), 7.81 (1H, dd, J = 8, 7.5 Hz), 8.42 (1H, d, J = 6 Hz), 8.43 (1H, d, J = 6 Hz), 8.53 (1H, d, J = 7.5 Hz), 8.70 (1H, d, J = 8 Hz). MS (ESI) m/z 718 [$(M+Na)^+$].

$KOSiMe_3$ (0.16 g, 1.3 mmol) was added to a solution of **63** (0.102 g, 0.146 mmol) in THF (2 mL). The solution was stirred at 80 °C for 2 h, then cooled to rt and partitioned between CH_2Cl_2 (25 mL) and saturated aq NH_4Cl (250 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 25 mL) and the combined organic extracts were dried and concentrated. Flash silica chromatography (50–100% EtOAc–hexane) gave (2*S*,4*S*)-2-(4-chlorobenzyloxymethyl)-4-[(1-hydroxyisoquinoline-5-sulfonyl)-(2-trimethylsilanylethoxymethyl)amino]-pyrrolidine-1-carboxylic acid *tert*-butyl ester (**64**) (62 mg, 0.092 mmol, 63%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.00 (9H, s), 0.85 (2H, t, J = 8 Hz), 1.42 (9H, s), 2.04–2.35 (1H, m), 3.21 (1H, t, J = 11 Hz), 3.40–3.95 (6H, m), 4.47 (2H, s), 4.90 (1H, d, J = 11 Hz), 4.96 (1H, d, J = 11 Hz), 7.20–7.35 (6H, m), 7.60 (1H, t, J = 8 Hz), 8.39 (1H, dd, J = 1, 8 Hz), 8.70 (1H, dd, J = 1, 8 Hz), 11.32 (1H, br s). MS (ESI) m/z 700 [$(M+Na)^+$].

HCl (1 mL, 4 M solution in dioxane) was added to a solution of **64** (65 mg, 0.096 mmol) in MeOH (1 mL). The solution was stirred at rt for 24 h and then diluted with Et_2O (10 mL). The supernatant was decanted and the solid was washed with Et_2O (10 mL). The solid was purified by ion exchange on acidic resin (MeOH, then 0.5 M NH_3 in MeOH). Flash silica chromatography (12.5% MeOH–CH_2Cl_2) gave **65** (10 mg, 0.022 mmol, 24%). ^{1}H NMR (250 MHz, CD_3OD) δ 1.21 (1H, ddd, J = 8, 8, 13 Hz), 1.87 (1H, ddd, J = 8, 8, 13 Hz), 2.53 (1H, dd, J = 6, 11 Hz), 2.77 (1H, dd, J = 7, 11 Hz), 3.21 (1H, m), 3.31 (1H, dd, J = 2, 5 Hz), 3.60 (1H, m), 4.36 (2H, s), 7.15–7.27 (6H, m), 7.54 (1H, dd, J = 8, 8 Hz), 8.23 (1H, dd, J = 1, 8 Hz), 8.49 (1H, dd, J = 1, 8 Hz). MS (ESI) m/z 448 [$(M+H)^+$]. HRMS $M+H^+$ calcd for $C_{21}H_{23}ClN_3O_4S$ 448.1092; found 448.1097. HPLC t_R 4.70 min; purity (AUC) 95%.

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Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmc.2005.09.055.

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