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4-Phenyl-7-azaindoles as potent and selective IKK2 inhibitors

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ABSTRACT

The synthesis and SAR of a novel series of IKK2 inhibitors are described. Modification around the hinge binding region of the 7-azaindole led to a series of potent and selective inhibitors with good cellular activity.

The transcription factor nuclear factor kappa B (NF-κB) is a ubiquitously expressed, major regulator of inflammatory genes including cytokines, chemokines and inflammatory enzymes.[1–3] In unstimulated cells, NF-κB resides in the cytoplasm as a silent complex with the IkB proteins. Upon cellular stimulation, IκB proteins are phosphorylated by IκB kinase (IKK) leading to degradation of the complex and translocation of NF-κB to the nucleus and the induction of gene transcription.[4] The IκB kinase complex contains two catalytic subunits, IKK1 and IKK2, and a regulatory subunit NF-κB essential modulator (NEMO).[5]

Although IKK1 and IKK2 are highly homologous serine-threonine protein kinases (61% of amino acids are identical in their aligned kinase domains) and contain similar structural domains, studies in knockout mice and derived mouse embryonic fibroblasts suggest that IKK2 is the predominant kinase involved in IkB phosphorylation and hence NF-κB activation.[6,7] IKK-1 deficient mice presented an unexpected phenotype including skeletal and skin abnormalities.[7] Given the attractiveness of NF-κB as a therapeutic target in inflammatory and autoimmume disorders, the development of selective IKK2 inhibitors has been an active area of pharmaceutical research for several years.[8,9] Recent publications[10–12] have suggested that IKK family members may be involved in cell cycle control through regulation of the mitotic kinase Aurora A. The activity[10] and cellular level[11] of Aurora A have been proposed to be regulated by IKK1 and 2, respectively. Such effects, if confirmed, will need to be taken into account when considering clinical utility of IKK inhibitors.

A series of 4-aryl-7-azaindole sulfonamides exemplified by **1** were identified as potent, ATP competitive, IKK2 inhibitors during a kinase cross screening campaign (Table 1). Although **1** demonstrated good IKK2 enzyme inhibition, it was less than 10-fold selective over IKK1 and offered poor selectivity over a broad kinase panel. The high inhibition observed at ROCK1 was of particular concern, given the involvement of this kinase in multiple biological processes.[13] Herein we describe our initial lead optimisation efforts which led to potent, selective and cellular active IKK2 inhibitors.

3,5-Disubstituted 7-azaindoles have been shown to bind to ABL and to CHK1 kinases with the N1 of the azaindole (pyrrole NH) located towards the inside of the hinge region of the ATP site ('N1-in'), as has the unsubstituted 7-azaindole fragment in a PKA/PKB chimera.[15] However, other 7-azaindole kinase crystal structures have shown that an alternative flipped 'N1-out' mode is also possible, in which the azaindole binds with a 180° flip, with the N1 atom towards the outside of the hinge region (unpublished results). Docking studies of **1** into an IKK2 homology model[16] were ambiguous, but slightly favoured this N1-out binding mode, as shown in Figure 1.

The IKK2 and IKK1 ATP binding sites differ by only one amino acid residue (Gln100 of IKK2 is equivalent to Ser99 of IKK1). However, the sidechain of this residue points away from the ATP site

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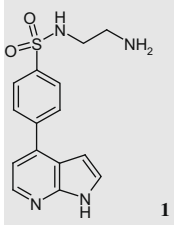
Table 1
Profile of lead azaindole **1**



Kinase [a]	pIC_{50}
IKK2	7.4
IKK1	6.6
ROCK1	7.7

1

pIC_{50} values are reported as mean of ≥2 experiments.
[a] Enzyme inhibition assays using recombinant human protein.[14]

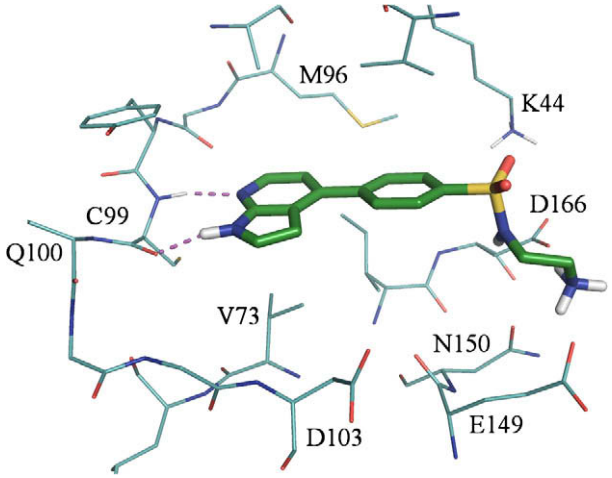


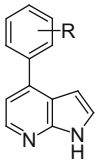
Figure 1. Predicted binding mode of compound **1** in IKK2.

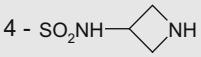
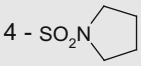
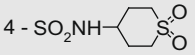
and hence would have minimal effect on inhibitor binding. Any influence from this substitution would be secondary, mediated through conformational effects or an indirect influence on other residues. Since there were no obvious structural differences between IKK1 and IKK2, it was felt that any selectivity for IKK2 over IKK1 would have to be a result of cumulative effects on the ATP site's conformation arising from relatively remote sequence differences, and that this would be impossible to predict.

Early SAR suggested that the ethylamine fragment of the sulfonamide **1** enhanced IKK2 potency whilst having little effect on IKK1 potency; the primary sulfonamide **2** was less potent and selective (Table 2). While models of **2** docked into the IKK2 homology model[16] did not position the sulfonamide oxygens within hydrogen-bonding distance of the catalytic Lys44, it was felt that the distance was close enough for the flexibility of the lysine sidechain to make this likely. The sulfonamide was found to be the optimal linker; the primary amide **3** was a marginally less potent at IKK2. This finding is consistent with that recently reported for a series of aminopyrimidine IKK2 inhibitors which share the same pharmacophore as the azaindoles.[17] Interestingly, removal of the amide or sulfonamide led only to a modest drop in potency; 4-phenyl-7-azaindole **4** retained some potency at IKK2 ($IC_{50} \sim 30\ \mu M$).

Initial medicinal chemistry efforts focussed on modification of the sulfonamide substituent with the aim of increasing IKK1 selectivity. Mono-methylation of the terminal amino group of **1** was tolerated at both IKK2 and IKK1 whereas dimethylation had a deleterious effect on potency at both isoforms as illustrated by compounds **5** and **6**, respectively. Several constrained amine ana-

Table 2
SAR of 4-phenyl-7-azaindoles



Compound	R	IKK2 pIC_{50} [a]	IKK1 pIC_{50} [a]
1	4-$SO_2NHCH_2CH_2NH_2$	7.4	6.6
2	4-SO_2NH_2	6.5	6.8
3	4-$CONH_2$	6.2	6.4
4	H	5.5	5.4
5	4-$SO_2NHCH_2CH_2NHCH_3$	7.5	6.5
6	4-$SO_2NHCH_2CH_2N(CH_3)_2$	6.7	6.1
7		7.4	7.5
8		6.6	5.9
9	4-$NHSO_2CH_3$	6.8	6.1
10	3-SO_2NH_2	5.3	5.9
11		7.1	7.3

pIC_{50} values are reported as mean of ≥2 experiments.
[a] Enzyme inhibition assays using recombinant human protein.[14]

logues of **1** were prepared, as exemplified by the azetidine **7**, but none offered any advantage in terms of potency or improved IKK1 selectivity.

The sulfonamide NH is not required for potency, evident from the pyrollidine sulfonamide **8** retaining activity at IKK2. The reverse sulfonamide **9** maintained potency and gained marginal selectivity over IKK1. Both the *meta*-sulfonamide **10** and the corresponding *meta*-reverse sulfonamide were less potent at IKK2. The sulfone **11** was one of the most potent sulfonamides found within this sub-series but again lacked IKK1 selectivity.

Since all the 4-phenyl-7-azaindoles lacked the desired selectivity over IKK1 and the broader kinase panel (data not presented here) our attention turned to substitution around the azaindole core to address this potential liability. It was proposed that 2-substituted 7-azaindoles would be unable to bind in the 'N1-in' mode due to a lack of sufficient space at the back of the pocket causing a steric clash, especially with the gatekeeper residue Met96. Instead, in the 'N1-out' mode, the 2-substituent would point towards solvent and hence lend itself well as a handle to optimise the physicochemical properties of the molecule. AGC family kinases including ROCK1 have a C-terminal extension to the kinase domain, in which a hydrophobic motif is found. This motif packs against the N-terminal lobe and is involved in dimerisation of ROCK1.[18,19] Between the end of the kinase domain and the hydrophobic motif, the peptide chain passes close to the solvent exposed edge of the ATP site. In particular, the sidechain of Phe368 (Fig. 2) would block access to the solvent side of the site from the 2-position of the azaindole. Hence, it was hypothesised that 2-substitution would improve selectivity for the series over ROCK1.

Several 4-arylsulfonamide-7-azaindoles were chosen for subsequent substitution in the 2-position of the azaindole core. The data for the aminoethyl sulfonamide is shown in Table 3. As expected,

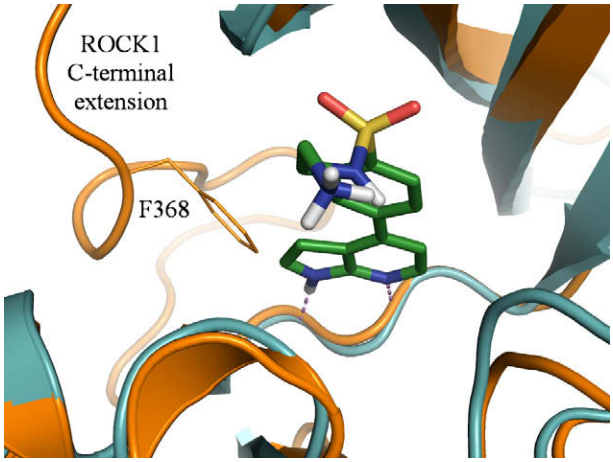
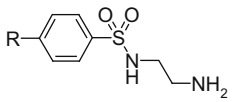
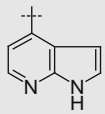
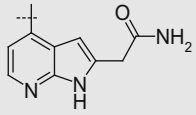
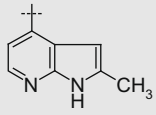


Figure 2. Predicted binding mode of compound **1** in IKK2 (cyan). The X-ray structure of ROCK1 (orange) is superimposed, showing the C-terminal extension and Phe368 that occludes the front of the ATP site.

polar substituents were tolerated in the 2-position. The key finding was that the introduction of a 2-substituent had a dramatic effect on the broad kinase selectivity profile of this series. The carboxamide **12** retained good potency at IKK2 and had similar selectivity to **1** over IKK1. Importantly, **12** had a far superior selectivity profile against a panel of 31 kinases, including ROCK1. However, **12** had poor activity in the IKK2 NF-κB mechanistic cellular assay (inhibition of TNFα induced NF-κB reporter activation in A549 cells),[14] with a drop of over a 100-fold in potency, a reflection of poor permeability caused by high polar surface area (PSA). Encouragingly, the 2-methyl-7-azaindole **13** retained both the good enzyme potency and broad kinase selectivity profile observed with carboxamide **12.** Moreover**,** due to a reduction in PSA, **13** had very good cellular activity (pIC_{50} 6.5) with approximately 10-fold drop-off in potency between the enzyme and the cellular assays.

Table 3
SAR of 2-position of 7-azaindole



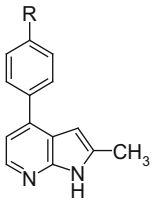
	R	IKK2[a] pIC_{50}	IKK1[a] pIC_{50}	A549 cell[b] pIC_{50}	ROCK1[a] pIC_{50}	PSA Å^2
1		7.4	6.6	6.2	7.7	101
12		7.7	6.8	5.4	5.0	144
13		7.7	6.9	6.5	6.5	101

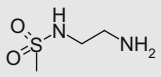
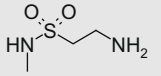
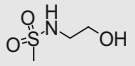
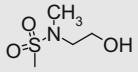
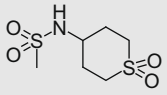
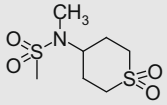
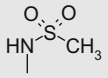
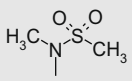
pIC_{50} values are reported as mean of ≥2 experiments.
[a] Enzyme inhibition assay using recombinant human protein.[14]
[b] Inhibition of TNFα induced NF-κB reporter activation in A549 cells.[14]

In comparison, the lead 7-azaindole **1** had a poor overall selectivity profile against a panel of 31 kinases. **1** inhibited 4 kinases within 10-fold of IKK2 (fold selectivity based on comparative IC_{50} values) whereas **13** inhibited only IKK1 within 30-fold of IKK2. Such kinase selectivity may be rationalised by the inability of these 2-substituted azaindoles to adopt the 'N1-in' binding mode, which may be the preferred mode for some kinases, because of a steric clash with their gatekeeper residues. It is believed that the dramatic increase in selectivity over ROCK1 for 2-substituted azaindoles compared to the 2-H azaindoles is a result of the hypothesis outlined earlier, and is due to a steric clash with Phe368.

With the improved broad kinase selectivity of the 2-substituted azaindoles in hand, we chose to re-optimise the sulfonamide moiety, to deliver good cellular potency and greater IKK1 selectivity. Since there was evidence that the azaindole 4-position SAR did not necessarily parallel across varying 2-position substituents, we decided to investigate the sulfonamide SAR with several 2-substituted azaindoles. The data for the 2-methyl azaindole compounds is shown in Table 4.

Table 4
SAR of 2-methyl-7-azaindoles



	R	IKK2[a] pIC_{50}	IKK1[a] pIC_{50}	A549 cell[b] pIC_{50}	PSA Å^2
13		7.7	6.9	6.5	101
14		7.8	6.9	5.7	101
15		7.7	6.5	6.3	96
16		7.4	5.5	6.1	86
17		7.7	7.9	7.3	109
18		7.4	6.0	6.3	100
19		7.2	5.4	6.0	75
20		6.5	5.1	5.7	66

pIC_{50} values are reported as mean of ⩾2 experiments.
[a] Enzyme inhibition assay using recombinant human protein.[14]
[b] Inhibition of TNFα induced NF-κB reporter activation in A549 cells.[14]

Table 5
Kinase selectivity profile of **16**

Kinase	pIC_{50}	Fold selectivity
IKK2	7.4	–
IKK1	5.5	79
Aurora B	5.8	40
Aurora A	5.1	200
ROCK1	<5	>250
GSK-3β	<4.6	>600

The reverse sulfonamide **14** had similar IKK2 potency and IKK1 selectivity compared to **13** but had a far greater drop-off in cellular potency. A number of reverse 2-ethylamino sulfonamides of this type were prepared and all had a significant drop-off in cellular potency of greater than a 100-fold. Such poor cellular permeability is possibly due to the combination of the acidic reverse sulfonamide (measured $pK_a \sim 7$) and basic primary amine (measured $pK_a \sim 9$) and hence a high proportion (>95%) of the compound being ionised at pH 7.4.

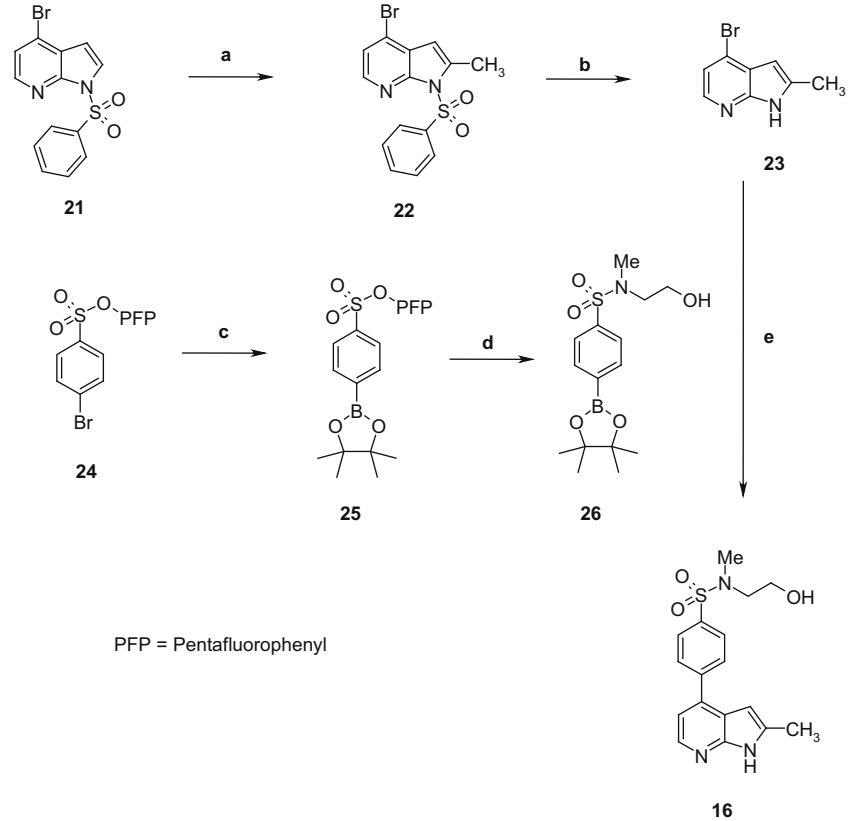
The primary alcohol **15** maintained IKK2 potency and offered 10-fold selectivity over IKK1. Secondary sulfonamides were often marginally more potent than tertiary sulfonamides at IKK2 due to a postulated H-bond interaction between the sulfonamide NH and the conserved Asp166 residue sidechain, or at least a close contact with that residue that disfavours substitution. However, tertiary sulfonamides offered greater selectivity over IKK1. For example, sulfonamide **15** was marginally more potent than the corresponding tertiary sulfonamide **16** but considerably less selective over IKK1. Both compounds showed approximately 10-fold drop-off in cellular potency. A similar trend was observed with the cyclic sulfones **17** and **18**. **17** had excellent potency at IKK2 (pIC_{50} 8.0) which translated into excellent cellular activity (pIC_{50} 7.3) but **17** was essentially equipotent at IKK1. **18** however, was threefold less potent at IKK2 and suffered a greater drop-off in cellular potency but was approximately 25-fold selective over IKK1. It is noteworthy that **17** has a relatively high PSA of 109 and this had minimal effect on the drop-off in cellular potency. Importantly, the poor selectivity of **17** and hence the high inhibition of IKK1 should have no effect on the NF-kB cellular potency. The reasons for the improved selectivity of the tertiary sulfonamides for IKK2 over IKK1 are unclear. There are no amino acid differences in this region of the ATP site. From the docked model, the sidechains of Asn150 and Asp166 are nearby. Whether the NH of the primary sulfonamide forms a hydrogen bond to these sidechains or not is ambiguous. However, the most likely explanation for the selectivity difference between IKK1 and IKK2 is that these sidechains can adopt different conformations in the two kinases.

Both the secondary and tertiary reverse sulfonamides **19** and **20** showed good selectivity over IKK1 of greater than 30-fold; again the secondary sulfonamide was more potent at IKK2.

Compound **16** offered the preferred overall profile with good IKK2 potency, excellent kinase selectivity including approximately 80-fold selectivity over IKK1, and cellular activity at micromolar concentrations. The selectivity profile of **16** was determined against a panel of 36 kinases. In this set only two kinases (IKK1 and AurB) were inhibited within 100-fold of IKK2 (fold selectivity based on comparative IC_{50} values) (Table 5).

The 4-aryl-7-azaindoles described herein were prepared via a number of alternative methods as previously reported.[14] A versatile route involved coupling the 4-bromo-7-azaindole **23** with the corresponding tetramethyl-1,3,2-dioxaborolan-2-yl **26** species under Suzuki cross coupling conditions (Scheme 1).

Modification of the 2-position was accomplished by lithiation of the N-protected 4-bromo-7-azaindole **21** and subsequent reaction



Scheme 1. Reagents and conditions: (a) LDA, THF, −35 °C 30 min then MeI, 2 h, 80%; (b) TBAF, THF, microwave 60 °C, 1 h, 89%; (c) pinacolato diboron, $PdCl_2$(dppf), dppf, NaOAc, 1,4-dioxane 105 °C, 16 h, 17%; (d) amine, Et_3N, DMF, microwave 120 °C, 10 min, not isolated; (e) K_3PO_4, 2′-(dimethylamino-biphenyl-palladium(II) chloride dinorbornylphosphine complex, 1,4-dioxane, water, microwave 130 °C, 30 min, 56% for steps d and e.

with a suitable electrophile. The 2-methyl-7-azaindoles were prepared by quenching with methyl iodide. Deprotection of the 7-azaindole **22** was carried out with TBAF in THF. The arylsulfonamide boronate esters were prepared in two steps from pentafluorophenyl 4-bromobenzenesulfonate **24**.[20] **24** was treated with pinacolato diboron in the presence of $PdCl_2$(dppf) catalyst and sodium acetate in refluxing 1,4-dioxane to yield the boronate ester **25**. **25** was then treated with the appropriate amine to yield the corresponding sulfonamide which was subsequently coupled with the azaindole core **23** under standard conditions to give the final compounds.

In conclusion, we have described the SAR around a series of ATP-competitive 4-aryl-7-azaindoles which represent a novel class of IKK2 inhibitors. The key discovery was that substitution close to the azaindole hinge binding region led to dramatic improvements in broad kinase selectivity. Further optimisation of the sulfonamide moiety, with a conscious effort to maintain moderate PSA, improved IKK1 selectivity and led to compounds with good cellular activity. Subsequent efforts are focussed on optimising the pharmacokinetic profile of this series and these studies will be reported in due course.

References and notes

1. Barnes, P. J.; Karin, M. *N. Eng. J. Med.* **1997**, *336*, 1066.
2. Li, Q.; Verma, I. M. *Nat. Rev. Immunol.* **2002**, *2*, 725.
3. Karin, M.; Yamamoto, Y.; Wang, Q. M. *Nat. Rev. Drug Disc.* **2004**, *3*, 17.
4. Hayden, M. S.; Ghosh, S. *Genes Dev.* **2004**, *18*, 2195.
5. Yamamoto, Y.; Gaynor, R. B. *Trends Biochem. Sci.* **2004**, *29*, 72.
6. Li, J.; Peet, G. W.; Pullen, S. S. *J. Biol. Chem.* **1998**, *273*, 30736.
7. Hu, Y.; Baud, V.; Delhase, M. *Science* **1999**, *284*, 316.
8. Strnad, J.; Burke, J. R. *Trends Pharm. Sci.* **2007**, *28*, 142.
9. Coish, P. D. G.; Wickens, P. L.; Lowinger, T. B. *Expert Opin. Ther. Patents* **2006**, *16*, 1.
10. Prajapati, S.; Tu, Z.; Yamamoto, Y.; Gaynor, R. B. *Cell Cycle* **2006**, *5*, 2371.
11. Irelan, J. T.; Murphy, T. J.; DeJesus, P. D.; Teo, H.; Xu, D.; Gomez-Ferreria, M. A.; Zhou, Y.; Miraglia, L. J.; Rines, D. R.; Verma, I. M.; Sharp, D. J.; Tergaonkar, V. *Proc. Natl. Acad. Sci.* **2007**, *104*, 16940.
12. Blazkova, H.; Von Schubert, C.; Mikule, K.; Schwab, R.; Angliker, N.; Schmuckli-Maurer, J.; Fernandez, P. C.; Doxsey, S.; Dobbelaere, D. A. *Cell Cycle* **2007**, *6*, 2531.
13. Olson, M. F. *Curr. Opin. Cell Biol.* **2008**, *20*, 242.
14. The IKK2 enzyme assay, NF-κB cellular mechanistic assay and azaindole syntheses are described in the following reference: Bamborough, P. A.; Barker, M. D.; Campos, S. A.; Cousins, R. P. C.; Faulder, P.; Hobbs, H.; Holmes, D. S.; Johnston, M. J.; Liddle, J.; Payne, J. J.; Pritchard, J. M.; Whitworth, C. WO2008/034860.
15. Kinase complexes of 3,5-disubsituted 7-azaindoles have been solved with c-Abl (PDB entry 2qoh: Zhou, T.; Parillon, L.; Li, F.; Wang, Y.; Keats, J.; Lamore, S.; Xu, Q.; Shakespeare, W.; Dalgarno, D.; Zhu, X. *Chem. Biol. Drug Des.* **2007**, *30*, 171) and CHK1 (PDB entry 1zys: Stavenger, R.; Zhao, B.; Zhou, B.; Brown, M.; Lee, D.; Holt, D. Unpublished results). The structure of a PKA-PKB chimera with 7-azaindole has also been solved (PDB entry 2uvx: Donald, A.; McHardy, T.; Rowlands, M.; Hunter, L.; Davies, T.; Berdini, V.; Boyle, R.; Aherne, G.; Garrett, M.; Collins, I. *J. Med. Chem.* **2007**, *50*, 2289.
16. A homology model of IKK2 was built as previously described: Christopher, J. A.; Avitabile, B. G.; Bamborough, P.; Champigny, A. C.; Cutler, G. J.; Dyos, S. L.; Grace, K. G.; Kerns, J. K.; Kitson, J. D.; Mellor, G. W.; Morey, J. V.; Morse, M. A.; O'Malley, C. F.; Patel, C. B.; Probst, N.; Rumsey, W.; Smith, C. A.; Wilson, M. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3972. A model of IKK1 was also built using the same template and refinement procedure.
17. Bingham, A. H.; Davenport, R. J.; Fosbeary, R.; Gower, L.; Knight, R. L.; Lowe, C.; Owen, D. A.; Parry, D. M.; Pitt, W. R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3622.
18. Doran, J.; Liu, X.; Taslimi, P.; Saadat, A.; Fox, T. *Biochem. J.* **2004**, *384*, 255–262.
19. Jacobs, M.; Hayakawa, K.; Swenson, L.; Bellon, S.; Fleming, M.; Taslimi, P.; Doran, J. *J. Biol. Chem.* **2006**, *281*, 260.
20. Avitabile, B. G.; Smith, C. A.; Judd, D. B. *Org. Lett.* **2005**, *7*, 843.