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Synthesis and structure–activity relationship of aminopyrimidine IKK2 inhibitors

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Abstract—The synthesis and structure–activity relationship of a novel series of aminopyrimidines are exemplified. Results of key compounds from within this series in the E-selectin reporter cell assay are also reported.

The NF-κB pathway is important in regulating the expression of cellular genes that are involved in the control of the immune and inflammatory response.[1] The activation of NF-κB induces the expression of more than 150 genes[2] such as cytokines (TNF, IL-1, IL-6), chemokines (IL-8, MCP-1), cell adhesion molecules (ICAM-1, VCAM-1) and proteases. Remarkably, NF-κB induces the production of proteins,[3] for example, TNF, that are themselves able to stimulate NF-κB, hence leading to an amplification of any physiological effect of the NF-κB pathway.

Activation of NF-κB is mediated by the increase in the activation of two kinases, IKK1 and IKK2.[4–7] IKK2 (−/−) knockout mice data[8–11] have shown that IKK2 is more critical than IKK1 in activating the NF-κB pathway for the inflammatory response, whilst data from IKK1 (−/−) knockout mice have indicated a role for IKK1 in skin and skeletal development. Hence small molecule inhibition of IKK2 with selectivity over IKK1 could lead to novel treatments for some cancers, and autoimmune inflammatory diseases, such as rheumatoid arthritis.[12] In 2004, it was reported that **SPC839** (see Fig. 1), a selective IKK2 inhibitor, was undergoing pre-clinical development after showing good efficacy in cancer and rheumatoid arthritis animal models.[13,14] Recent reports suggest that this compound has now progressed into Phase I clinical trials targeting an ultimate endpoint of haematological malignancies.[15] This long time frame required to progress **SPC839** exemplifies the challenges of finding novel IKK2 inhibitor. Within our laboratories we have investigated a novel series of aminopyrimidines that show inhibitory action against IKK2. In a previous communication,[14] we disclosed a structure–activity study of variations in the aminobenzothiazole group of structure **1**. In this letter we disclose the results of a study into the structure–activity relationships of the 4-phenyl substituents of the aminopyrimidine structure **1** (Fig. 1).

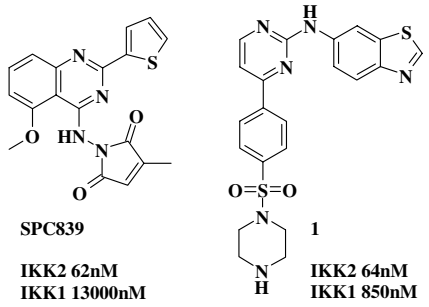

Figure 1. The IKK2 inhibitor **SPC839** and our initial lead compound **1**.

The lead compound **1** was found to have an IKK2 IC_{50} of 64 nM, with reasonable selectivity for IKK2 over IKK1 (IKK1 IC_{50} 850 nM). Unfortunately, these promising IKK2 primary data did not translate well into the E-selectin reporter cell assay[16] giving a very modest IC_{50}

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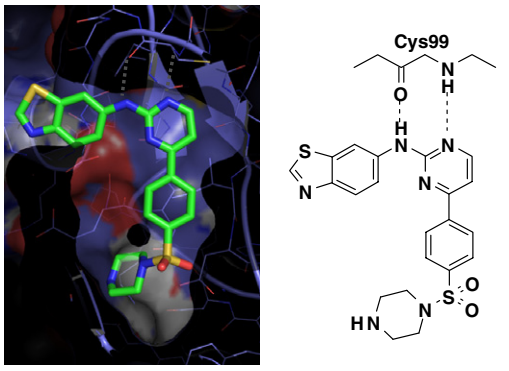
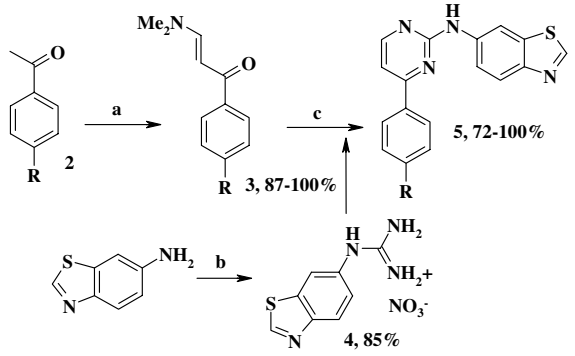


Figure 2. Predicted binding mode of compound **1** in IKK2.



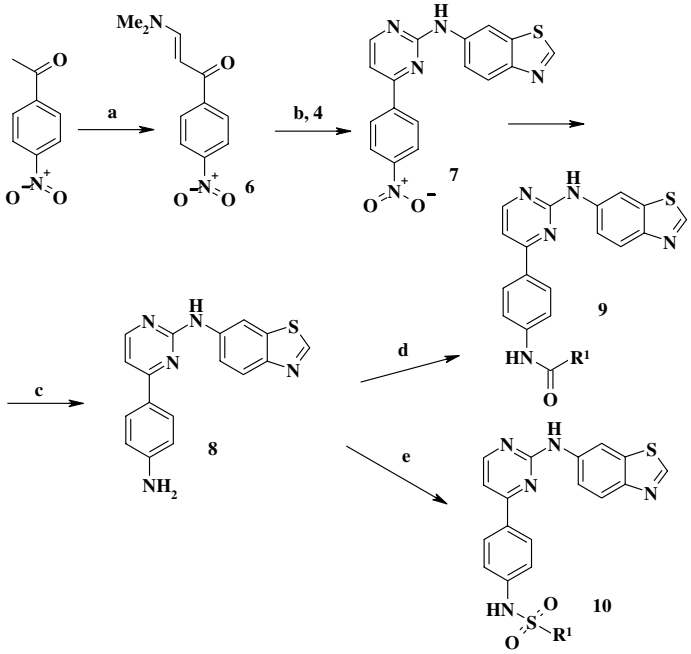
Scheme 1. Synthesis of arylaminopyrimidine analogues. Reagents: (a) DMF–DMA; (b) NC–NH_2, HNO_3; (c) DMF, NaOH.

value of 5700 nM. No IKK2 crystal structure is available, so a previously published homology model[17] was used to assist analogue design. This predicted binding mode is shown in Figure 2 and shows analogue **1** in complex with two other similar kinases. Since these analogues showed a consistent binding mode in all these crystal structures, we made the assumption that the binding of **1** in IKK2 would be equivalent. In this conformation, the aminopyrimidine-nitrogen forms a hydrogen bond with the backbone NH of Cys^{99} and the aminopyrimidine NH forms a hydrogen bond with the backbone carbonyl of the same residue. It is also believed that the sulfonylpiperazine portion of our lead structure overlays with the ribose-phosphate portion of the natural ligand ATP.

The purpose of this investigation was to probe the sulfonamide portion of our lead compound to optimize binding potency, which should also in turn increase cellular potency. We were also interested to see if other functional groups, such as amides, reverse amides/sulfonamides, or just a bond could act as sulfonamide bioisoteres, potentially offering different IKK2 activity, selectivity and cellular profiles.

The analogues were synthesized by several routes. The directly-linked compounds **5** were synthesized in a convergent manner. Firstly, the corresponding arylketone **2** (Scheme 1) was elaborated to the enone **3** using DMF–DMA. Secondly, commercially available amino benzothiazole was converted to benzothiazole guanidine **4** using cyanamide and nitric acid (85–98%). The enone **3** was combined with benzothiazole guanidine **4** under basic conditions to yield the desired aminopyrimidines **5**.

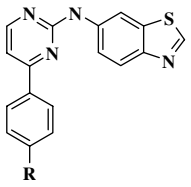
Amides and reverse sulfonamides were synthesized from commercially available 4-nitroacetophenone (Scheme 2). Elaboration to the enaminone **6** was performed using DMF–DMA, followed by cyclisation to the aminopyrimidine **7** under basic conditions. The nitro group

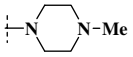
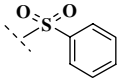
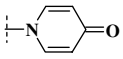
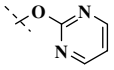
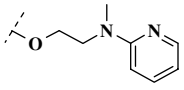
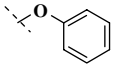
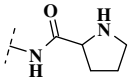
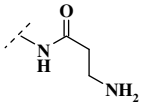
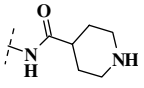
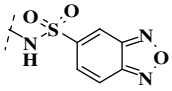
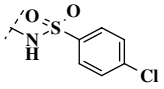
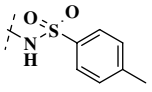
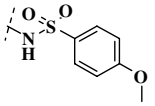
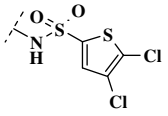


Scheme 2. Synthesis of arylaminopyrimidine analogues. Reagents: (a) DMF–DMA; (b) DMF, NaOH; (c) H_2, Pd–C; (d) RCO_2H, EDC, HOBt; (e) RSO_2Cl, TEA, DCM.

Table 1. Structure–activity relationship of substituents (R)

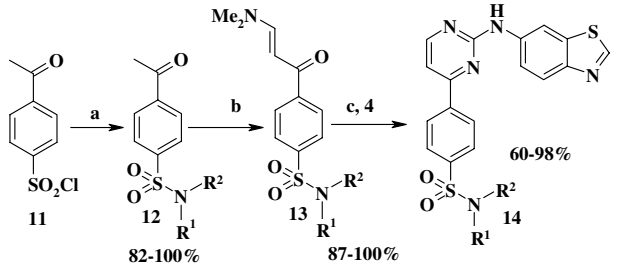
Structure



Number	R-group	IKK2[18]	IKK1[18]	IKK1/IKK2
5a		410	2630	6
5b		440	24% at 10 μM	>22
5c		700	34% at 10 μM	>14
5d		2500	14% at 10 μM	>4
5e		3600	NT	ND
5f		4000	NT	ND
5g		6600	NT	ND
9a		130	146	1
9b		270	731	3
9c		400	1910	5
10a		230	4840	21
10b		990	2990	3
10c		1500	NT	ND
10d		8900	NT	ND
10e		8900	NT	ND

was then reduced by hydrogenation to yield the anilino compound **8**. This was then reacted either with acids and EDC to form amides **9** or sulfonyl chlorides in DCM with triethylamine to yield sulfonamides **10**.

The structure–activity relationship of all analogues was explored against both the IKK1 and IKK2 enzymes.

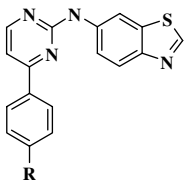


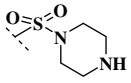
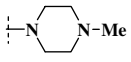
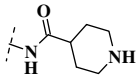
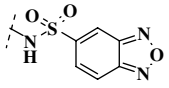
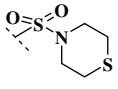
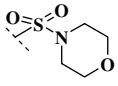
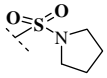
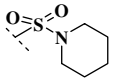
Scheme 3. Synthesis of arylaminopyrimidine analogues. Reagents: (a) RNH_2, TEA, DCM; (b) DMF–DMA; (c) DMF, NaOH.

Initially, the generality of our original hit sulfonamide linker was investigated (Table 1) with analogues (**5a**–**5g**), which had groups attached directly to the aryl ring. The most active structures in this series were compounds **5a** and **5b**, with both the compounds having IKK2 activities in the 400 nM range. As neither of these compounds had activities comparable to the starting lead **1**, it appeared that a linker or spacer was required. This concept was explored further with analogues which had either an amide (**9a**–**9c**, Table 1) or a reverse sulfonamide (**10a**–**10e**, Table 1) as a replacement for the sulfonamide portion. In general, we found that an amide linker could be used to yield some potent IKK2 inhibitors especially when used in conjunction with a group bearing a basic centre (cf **1** to **9a** and **9b**). Unfortunately, in the case of these amides the IKK2/IKK1 selectivity profile was less favourable, with a 5-fold selectivity for IKK2 over IKK1 being the best we could achieve in amide **9c** (compared to 13-fold for our lead structure **1**). In the case of the reverse sulfonamides their activities

Table 2. Structure–activity relationship of sulfonamides

Structure



Number	R^1	IKK2[18]	IKK1[18]	IKK1/IKK2	Cell[16]	Cell/IKK2	PSA
1		64	850	13	5700	89	104
5a		410	2630	6	1270	3.1	85
9c		400	1910	5	3820	9.5	93
10a		230	4840	21	17,800	77	135
14a		250	5000	20	289	1.2	88
14b		380	6700	18	1810	4.8	99
14c		410	28% at 10 μM	>24	1920	4.7	88
14d		540	48% at 10 μM	>18	1500	2.8	90
14e		600	30% at 10 μM	>17	2080	3.5	86

tended to be lower than the original lead (**10a**, 230 nM being the best observed), although it should be noted that none of the groups posses the basic nitrogen which seems to be preferred (although not essential) for activity. However, the most potent reverse sulfonamide **10a** did display reasonable selectivity (21-fold) for the desired IKK2 isoform.

Although removing and replacing the sulfonamide linker in our original lead compound yielded some compounds with interesting selectivity profiles, no overall improvement in IKK2 potency was achieved.

Attention was now turned to retaining the sulfonamide linker in the same orientation as **1** and exploring the piperazine ring. The sulfonamide series was synthesized from commercially available starting materials (Scheme 3). Sulfonyl chloride **11** was reacted with a series of amines to yield the sulfonamides **12** (82–100%). The acetyl group was then elaborated to the enaminones **13** by refluxing in DMF–DMA (87–100%). This was then reacted under basic conditions with **4** to yield the aminopyrimidines **14** (60–98%). If a Boc-protecting group was present on R^1R^2NH this was removed using 10% trifluoroacetic acid in dichloromethane. The results from this series are displayed in Table 2.

The direct oxygen and sulfur analogues (**14a** and **14b**) were both found to be less active than **1**. This again suggested that a basic nitrogen was preferred for activity. When the sulfide of **14a** was oxidized to the sulfoxide (data not shown) this also led to a loss of activity. The need for a saturated ring was also examined using the dimethyl analogue **14c**, and although not more active than the original hit, it was more active than the 5- and 6-membered saturated ring **14d** and **14e**. When the free piperazine NH of **1** was methylated it led to a large loss of activity (data not shown), suggesting that there is limited space in this region of the protein.

Having established that maintaining a sulfonamide linker group in the lower portion of the molecule was optimal based on primary protein in vitro activity. The original hit **1**, some closely related compounds and representative examples of other linkers (**5a**, **9c**, **10a**) were evaluated further in the E-selectin reporter cell assay[16] (Table 2). Despite showing a very encouraging protein IKK2 activity, **1** demonstrated a significant drop-off in activity when tested in a cellular system. Conversely compound **14a** which had a more modest protein IKK2 activity, translated very favourably into the cellular system, with essentially no drop-off. The relative cell penetrating abilities of the compounds may explain this. A number of physicochemical factors are known to affect cell penetration, with one being polar surface area (PSA). Comparing the PSA of this small set of compounds it does appear that compounds with a PSA < 100 show very little drop-off when tested in the cellular system, whereas a PSA > 100 in the case of **1** and **10a** may lead to a dramatic drop-off. A larger set of compounds would need to be tested to thoroughly investigate this observation, although it is quite probable that PSA could be a good descriptor to use in designing further analogues with optimized activity and cell-penetration potential.[19]

In conclusion, a comprehensive strategy has been employed to explore the 4-phenyl substituents region of our lead **1**. Various novel analogues have been synthesized using different synthetic routes. This has enabled a number of alternative linkers to be accessed and their activity explored with respect to the type of linker and the actual substituents. The sulfonamide linker appears to be optimal, with the original hit **1** having very good activity in the IKK2 protein assay. This activity does not translate well into the E-selectin reporter cell assay, and one reason for this may be its high PSA. Compound **14a**, however, despite having a lower IKK2 protein assay result, showed very favourable activity in the E-selectin reporter cell assay, and represented a considerable improvement on the original lead molecule **1**. These improvements mean compound **14a** is deemed suitable for pharmacokinetic analysis.

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15. Merck Kga, A., Company Pipeline, 2007.
16. A549 cells were stably transfected with a segment of the E-selectin promoter upstream of a luciferase reporter gene. Cells activated with TNFα (R&D systems) and treated with test compound for 6 h. Luminescence detected using commercially available Luciferase reporter gene reagent (Luclite, Perkin-Elmer). Values are the mean of at least two experiments.

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