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A novel series of potent and selective IKK2 inhibitors

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Abstract—A novel series of aminopyrimidine IKK2 inhibitors have been developed which show excellent in vitro inhibition of this enzyme and good selectivity over the IKK1 isoform. The relative potency and selectivity of these compounds has been rationalized using QSAR and structure-based modelling.

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. NF-κB induces the production of proteins,[3] for example TNF, that are themselves able to stimulate NF-κB, hence leading to an amplification on 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 acting via the NF–κB pathway, could lead to novel treatments for autoimmune and inflammatory diseases, such as rheumatoid arthritis.[12] Celgene has reported recently[13] that **SPC839** (Fig. 1), a selective IKK2 inhibitor, is undergoing pre-clinical development for the treatment of rheumatoid arthritis.

In our laboratories, we have identified a novel series of aminopyrimidines that show inhibitory action against IKK2. In this communication, we disclose the results of a study into the structure–activity relationship around ring A of our lead structure **1** (Fig. 1).

The lead compound **1** was found to have an IKK2 IC_{50} of 146 nM, with no selectivity for IKK2 over IKK1 (IKK1 IC_{50} 154 nM). No IKK crystal structures were available so a homology model was created.[14] Crystal structures of analogues of **1** in complex with two other kinases were available in-house (unpublished data). 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, and manually docked the compound as shown in Figure 2. This predicted binding mode is also shown in Figure 2. In this configuration, the aminopyrimidine-N forms a hydrogen bond with the backbone NH of Cys^{99} (IKK2) and the aminopyrimidine NH forms a hydrogen bond with the backbone carbonyl of the same residue.

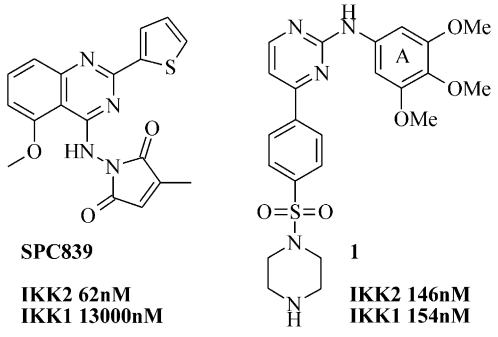

Figure 1. The Celgene IKK2 inhibitor SPC839 and our initial lead compound **1**.

Keywords: Potent; Selective; IKK2; Inhibitors.
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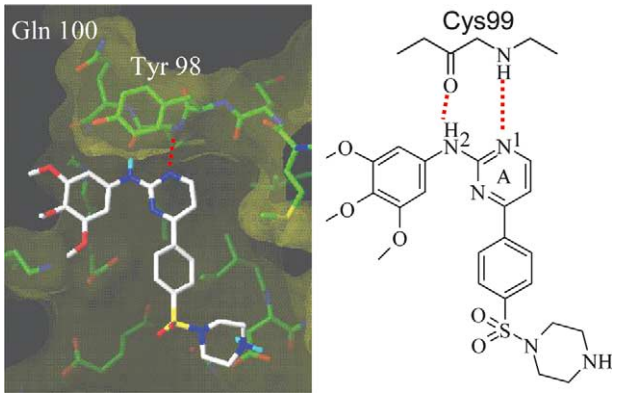
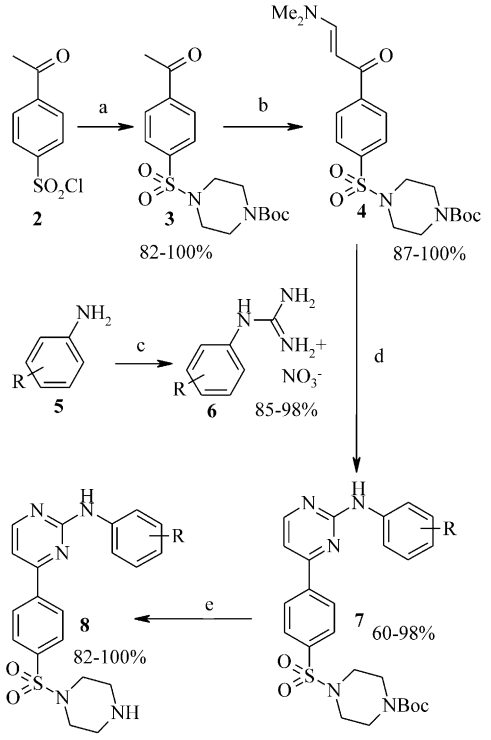


Figure 2. Predicted binding mode of compound **1** in IKK2 homology model.

The analogues were synthesized from commercially available starting materials. Sulfonyl chloride **2** was reacted with Boc-piperazine (Scheme 1), to yield the sulfonamide **3** (82–100%). The acetyl group was then elaborated to the enaminone **4** by refluxing in dimethylformamide-dimethylacetamide (87–100%). This was then reacted under basic conditions with a variety of aryl guanidines **6** [which were derived from reaction of commercially available anilines **5** with cyanamide and nitric acid (85–98%)] to yield the Boc-protected aminopyrimidines **7** (60–98%). Finally, the Boc group was removed using 10% trifluoroacteic acid in dichloromethane to yield the compounds **8** (82–100%).

One of the first compounds to be made in this series was the 4-cyano analogue (**8q**, Table 1, compounds ordered



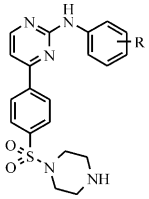
Scheme 1. Synthesis of arylaminopyrimidine analogues. Reagents: (a) BocPiperazine, Et_3N, DCM; (b) DMF–DMA; (c) NC–NH_2, HNO_3; (d) DMF, NaOH; (e) 10% TFA/DCM.

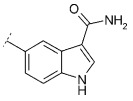
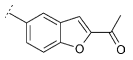
by absolute IKK2 activity). This compound is 3 fold more potent against IKK2 and 24 fold less potent against IKK1 than **1**. In our model structure of **1** bound to IKK2, the 4 position of the A ring is solvent exposed. This fact led to the hypothesis that the improvement in potency against IKK2 seen with **8q** compared to **1** was a consequence of electronic effects on the conjugated anilinopyrimidine core and not to changes in direct interactions of the 4-substituent with the protein. To test this hypothesis, analogues with a range of electronic properties, shapes and hydrophobicity were selected. A Craig plot[15] was used to help select the compounds that best matched our criteria with all four Craig plot quadrants being sampled. These compounds were then synthesized in the manner described earlier and tested in vitro to ascertain whether these properties could be a factor affecting enzyme inhibition.

The results of this study are represented in Table 1. In general, it was found that the compounds with the greatest IKK2 activity had substituents found in the top left-hand quadrant of the Craig Plot.[15] These substituents had high Hammett σ electronic substituent[16] values and low Hansch π hydrophobicity[17] values. However, multiple linear regression[18] using these two terms failed to produce an equation that could be used to predict IKK2 inhibition for related molecules (cross validated correlation coefficient, $q^2 = 0.08$, $n = 19$). Two related molecular descriptors were then calculated and used instead of σ and π, these were hydrogen bonding strength of N1 (Fig. 2) and ClogP, respectively. Hydrogen bonding strengths were calculated using the semi-empirical method of Gancia et al.[19] and ClogP was calculated using BioByte software.[20] This method enabled us to include three molecules for which no σ and π were available (**8a**, **8n** and **8t**) and resulted in a more predictive equation ($q^2 = 0.32$, $n = 22$). When an insoluble outlier was removed (**8b**) from the dataset the predictivity improved significantly (Correlation coefficient, $r^2 = 0.65$, $q^2 = 0.47$, $n = 21$). This final equation was IKK2 $pIC_{50} = -1.2$*H-Bond-0.1 *ClogP$+9.8$ ($pIC_{50} = -0.3$*H-Bond-0.1*ClogP$+7.0$ for standardized descriptors). It can be seen from these equations that the hydrogen bonding strength of N1 has the larger influence on IKK2 activity. The weaker the potential hydrogen bond to this atom, the greater the activity. In fact, the calculated hydrogen bond acceptor strength of the two nitrogens in the pyrimidine ring were effectively the same ($r = 0.99$, $n = 22$) whilst the hydrogen bond donating strength of the anilino NH was inversely correlated with the acceptor strength of these two atoms ($r = -0.97$ and -0.98). This means that greater activity against IKK2 tends to occur for molecules with a greater N2 hydrogen bonding potential (Fig. 3). Fortunately, the changes made to the A ring described above did not increase the activity against IKK1 by the same amount. As a result, the most active compounds against IKK2 tend to show an increase in selectivity over IKK1.

Replacing σ and π with calculated hydrogen bonding strength and logP gave a more predictive equation but this may only be because this allowed the inclusion of two or three extra data points as the two pairs of

Table 1. IKK2/IKK1 experimental inhibition data and calculated properties



Compd	R-group	IKK2 (nM)	IKK1 (nM)	IKK1/IKK2	σ	π	H-bond	ClogP[20]	Aqueous solubility (pH 6.5 in μg/mL)
8a		550	3090	6	nd	nd	2.75	2.26	nt
8b	4-CF_3	513	3981	8	0.54	0.88	1.90	4.59	Not detected
8c	4-$NHCOCH_3$	331	1622	5	0.00	−0.97	2.45	2.69	64.0
8d	4-N-Butyl	309	2692	9	−0.51	1.16	2.53	4.75	nt
8e	4-CH_3	269	2089	8	−0.17	0.56	2.42	4.17	0.1
8f	4-OCH_3	214	1230	6	−0.27	−0.02	2.38	3.60	27.8
8g	4-$NHCONH_2$	155	1479	10	−0.24	−1.30	2.45	2.37	nt
8h	4-Cl	148	2344	16	0.23	0.71	2.17	4.40	4.8
1	3,4,5-Trimethoxy	145	155	1	−0.03	−0.06	2.17	2.81	nt
8i	3,5-Difluoro	117	4074	35	0.68	0.24	1.97	3.98	5.0
8j	4-$SOCH_3$	91	2344	26	0.49	−1.58	2.08	2.13	nt
8k	H	91	1175	13	0.00	0.00	2.25	3.67	nt
8l	3-CN	83	5129	62	0.56	−0.57	2.02	3.16	nt
8m	4-NO_2	66	6918	105	0.78	−0.28	1.58	3.46	Not detected
8n		60	851	14	nd	nd	2.17	3.67	0.1
8o	4-$COCH_3$	59	1096	19	0.50	−0.55	2.11	3.15	0.5
8p	4-$CONH_2$	48	3981	83	0.36	−1.49	2.12	2.21	Not detected
8q	4-CN	44	3802	87	0.66	−0.57	1.89	3.16	0.2
8r	4-$NHSO_2CH_3$	43	331	8	0.03	−1.18	2.15	2.48	27.0
8s	4-SO_2CH_3	31	1202	39	0.72	−1.63	1.75	2.09	209.0
8t		26	1698	65	nd	nd	2.11	3.70	nt
8u	4-SO_2NH_2	11	245	21	0.57	−1.82	1.87	1.86	nt

nd refers to a value that was not determined; nt refers to a compound that was not tested; σ/π values were obtained using TSAR-3-D program (Accelrys Inc.);[18] H-bond values were calculated using the procedure described in ref 19 for N1 (see Fig. 2).

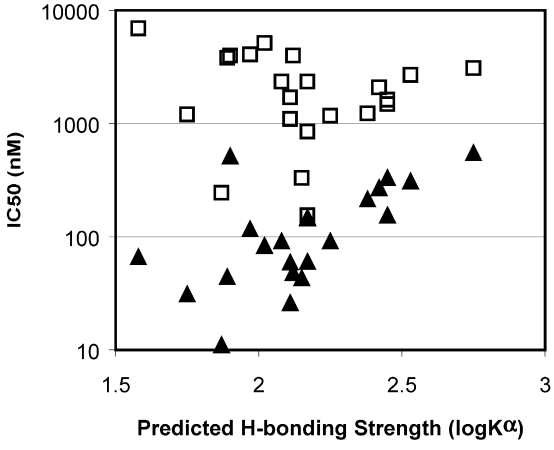


Figure 3. Hydrogen bonding strength of aminopyridimidine core N1 (see Fig. 2) plotted against IKK inhibition IKK1 ■ and IKK2 ▲.

descriptors are highly correlated ($r = -0.92$ and 0.98, respectively). An added benefit of using calculated properties such as hydrogen bonding strength and clogP is it enables groups that have no reported measured σ and π values to be investigated.

This observed relationship between the activity and the electron withdrawing properties of substituents attached to the A ring could be due to a number of factors. One possibility is that changes in intermolecular hydrogen bonding strengths result in changes in the affinity of the ligand for the target. This could be due to small changes in the binding orientation of the compounds. The fact that IKK1 activity is not correlated with that of IKK2 for this set of compounds suggests differences between the binding sites of the two enzymes. Our homology models show that the amino acid residues proximal to the ATP binding site are the same in both proteins with one exception (Ser^{99} in IKK1 equivalent to Gln^{100} in IKK2).

As these amino acid residues are found close to the predicted location of Tyr^{98} in IKK2 (equivalent to Tyr^{99} in IKK1, Fig. 2), it is not unreasonable to postulate that this Gln/Ser difference gives rise to the differences in activity observed. The amino acids at this position could interact differently with this tyrosine residue, resulting in differences in its orientation, which in turn could lead to a change in shape of the ATP binding pocket.

In conclusion, a large number of novel analogues has been synthesized with a variety of substituents on the aromatic ring A. The choice of substituents has been guided by a Craig plot to enhance the range of electronic and hydrophobic properties explored by our analogues. This has enabled a correlation between these properties and IKK2 activity to be investigated. A large increase in absolute IKK2 potency has been obtained, without increasing the IKK1 activity, thereby increasing the observed selectivity. Our starting compound **1** had an original IKK2 IC_{50} of 146 nM, and this series has been optimized to 11 nM in the case of analogue **8u**. Our starting lead also showed no selectivity for IKK2 over IKK1, whereas we have now achieved a selectivity of 105-fold in the case of **8m**.

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