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Synthesis and SAR of aminopyrimidines as novel c-Jun N-terminal kinase (JNK) inhibitors

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Abstract—The development of a series of novel aminopyrimidines as inhibitors of c-Jun N-terminal kinases is described. The synthesis, in vitro inhibitory values for JNK1, JNK2 and CDK2, and the in vitro inhibitory value for a c-Jun cellular assay are discussed.

Inflammation is a key area of research for many pharmaceutical companies. Patients suffering from inflammatory disorders such as rheumatoid arthritis (RA) require therapeutic agents that not only demonstrate anti-inflammatory properties but also protect against cartilage degradation.

The c-Jun N-terminal protein kinases (JNKs) are a family of serine/threonine protein kinases and members of the mitogen-activated protein kinase (MAPK) family. JNKs activate proteins such as c-Jun by phosphorylation; these proteins then dimerise to form transcription factors, including AP-1, which mediate pro-inflammatory effects via induction of inflammatory factors including cytokines (TNF-α and IL-2) and matrix metalloproteinases (MMPs).[1] Mammals have three JNK genes *Jnk1*, *Jnk2* and *Jnk3*.[2] Gene knockout studies of *Jnk1* and *2* suggest a role for JNK in T cell proliferation.[3] Further studies also indicate that JNK inhibition may afford protection against cartilage and bone erosion.[4] Current evidence suggests that selective JNK1 and 2 inhibition would be beneficial for treating patients with RA.[3a,4,5]

In this communication, we report our efforts towards a series of aminopyrimidines as novel ATP competitive JNK1 and JNK2 inhibitors for the treatment of inflammatory disorders.

SP600125 (**1**) is a reversible ATP competitive inhibitor of all three JNK isoforms, identified from a high throughput screen (HTS) of Celgene's chemical library.[6] Another JNK inhibitor (**2**) has subsequently been published by Serono[7] which has shown efficacy via oral dosing in a model of RA. Further recent publications from Astra-Zeneca[8] and Abbott[9] emphasise the growing interest in JNK as a target (Fig. 1).

Screening of the UCB compound collection identified several hits with varying potencies against JNK1, 2 and 3. One of the most promising hits was the aminopyrimidine **5**, which showed good JNK potency and some selectivity against other protein kinases such as p38 (Fig. 2).

In order to use computer-aided drug design an X-ray crystal structure of an early lead, compound **6**, bound into the ATP binding site of JNK3 was obtained (Fig. 3). JNK3 protein was used as a surrogate in the absence of in-house JNK1 and JNK2 protein crystals and all modelling work discussed here is from the JNK3 protein. There is about 92% sequence identity

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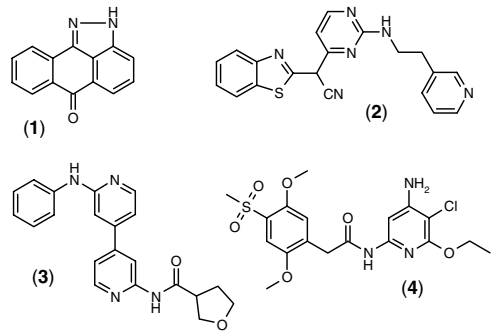


Figure 1. JNK inhibitors in the public domain. (**1**) SP600125, IC_{50} values: JNK1 110, JNK2 110, JNK3 150 nM. (**2**) AS601245, IC_{50} values: JNK1 150, JNK2 220, JNK3 70 nM.[7] (**3**) Astra-Zeneca compound, IC_{50} values: JNK1 384, JNK3 7 nM.[9b] (**4**) Abbott compound, IC_{50} values: JNK1 36, JNK2 70 nM.[9b]

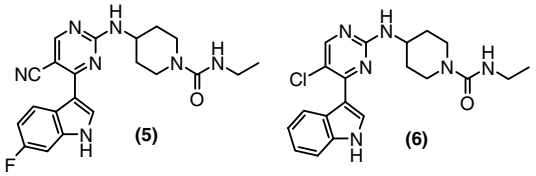


Figure 2. Hit from HTS, compound **5**, and early lead, compound **6**. IC_{50} values **5**: JNK1 92, JNK2 67, JNK3 412, p38 4863 nM. IC_{50} values **6**: JNK1 13, JNK2 25, JNK3 57, p38 5423 nM.

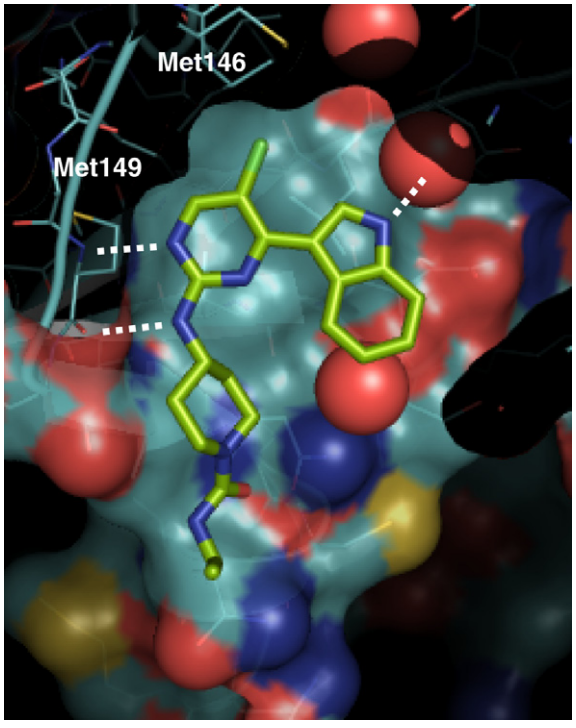
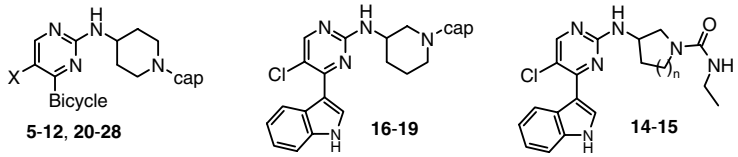


Figure 3. X-ray structure of compound **6** (PDB code 2P33).

between JNK1 and JNK3 and about 85% between JNK2 and JNK3. JNK3 enzyme activity was routinely checked to aid with our modelling and is shown in Table 1. No further discussion will be given in this paper as our biological results were unaffected by JNK3 activity. The X-ray crystal structure of **6** (Fig. 3) shows the aminopyrimidine nitrogens interacting with the hinge region of the active site via Met 149, the indole sitting in the pocket normally occupied by the ribose of the natural ligand ATP and the 5′-chloro group sterically interacting with the sulfur of Met 146. Met 146 is also known as the 'gatekeeper', a term used to describe the residue of a kinase that blocks the classical hydrophobic pocket. All protein kinases have a gatekeeper residue. A feature so far found only in JNKs[10] is the presence of a moveable gatekeeper, which allows access to the classical hydrophobic pocket. All JNKs have a methionine gatekeeper residue, p38 has a threonine residue and CDK2 has a phenylalanine residue, which is not capable of moving to expose the hydrophobic pocket. Modelling suggested that this difference might be used to improve selectivity. The aminopyrimidines described were synthesized as shown in Scheme 1. Appropriately substituted 2,4-dichloro pyrimidines (**31**) were reacted via a Suzuki coupling with 1-phenylsulfonyl indole-3-boronic acid, followed by a basic deprotected to give intermediates (**32**). Intermediates (**32**) could be reacted with substituted piperidines to give final compounds via deprotection and further reaction with appropriate capping groups. In order to synthesize compound **10**, indole was deprotonated using sodium hydride and reacted with 2,4,5-trichloropyrimidine (**33**). The product was then reacted as before with substituted piperidines, followed by deprotection and reaction with ethylisocyanate. It can be noted that the chlorine of compound **10** can be removed via hydrogenation under basic conditions to give compound **12**. 3-Bromoimidazopyridine (**34**) was reacted with trichloropyrimidine (**33**) via a Negishi coupling. The intermediate dichloro compounds were reacted with substituted piperidines as before to give compound **9**. Hydrogenation was used again to synthesize compound **11**. Finally the pyrazolopyridines were synthesized via a coupling of dichloropyridine (**35**) with a substituted acetylene followed by a cyclisation reaction to give intermediates **36**. Compound **5** was screened against a panel of protein kinases including cyclin-dependent protein kinase 2 (CDK2) which had been highlighted as a potential selectivity issue. A further screen of other 5′-cyano aminopyrimidines showed that CDK2 activity was indeed an issue that needed to be addressed.

In order to attain better CDK2 selectivity, while retaining JNK potency, we investigated the SAR around four distinct areas of the original hit (**5**), the 5′-substituent, the bicycle, the heterocycle and the piperidine substituent, referred to as the cap. The first two areas of investigation were alternative 5′-substituents and bicycle replacements (Table 1, **5**–**13**). From Table 1 it can be seen that there is a subtle interplay between 5′-substituents and the bicycles affecting aminopyrimidine SAR. When 5′ is chloro the 3-indole (**6**) has greater potency against the JNKs than the imidazopyridine (**9**), followed by the 1-indole (**10**), which shows only moderate activity. Modelling suggests that the shorter carbon–nitrogen of the 1-indoles results in a steric clash with the 5′-chlorine which in turn leads to a loss in

Table 1. Enzymatic and cellular activity of aminopyrimidine analogues

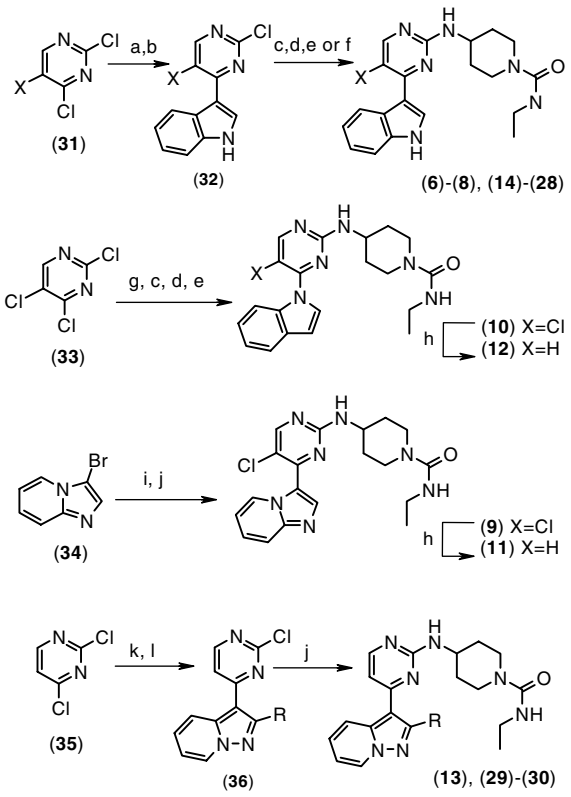


Compound	5′ X	Bicycle	Heterocycle	Cap	JNK1[11] IC_{50} (nM)	JNK2[11] IC_{50} (nM)	JNK3[11] IC_{50} (nM)	CDK2[12] IC_{50} (nM)	c-Jun[13] IC_{50} (nM)
5	CN	6-F-3-Indole	4-Piperidine	CONHEt	92	67	412	412	3700
6	Cl	3-Indole	4-Piperidine	CONHEt	13	25	57	1517	704
7	Me	3-Indole	4-Piperidine	CONHEt	320	250	410	IA	10,000
8	H	3-Indole	4-Piperidine	CONHEt	74	245	na	10,000	8091
9	Cl	3-Imidazopyridine	4-Piperidine	CONHEt	41	55	na	605	7723
10	Cl	1-Indole	4-Piperidine	CONHEt	457	709	na	4443	>10,000
11	H	3-Imidazopyridine	4-Piperidine	CONHEt	59	281	708	4219	19,331
12	H	1-Indole	4-Piperidine	CONHEt	71	512	na	10,000	29,891
13	H	Pyrazolopyridine	4-Piperidine	CONHEt	69	194	na	8663	4000
14	Cl	3-Indole	3-Pyrrolidine	CONHEt	360	177	582	2483	6268
15	Cl	3-Indole	Azetidine	CONHEt	1340	1551	5000	na	4272
16	Cl	3-Indole	3-(*S*)-Piperidine	CONHEt	29	15	32	555	6995
17	Cl	3-Indole	3-(*S*)-Piperidine	$CH_2CONHMe$	139	267	na	>10,000	14,667
18	Cl	3-Indole	3-(*R*)-Piperidine	CONHEt	60	88	107	1264	3883
19	Cl	3-Indole	3-(*R*)-Piperidine	$CH_2CONHMe$	15	31	31	612	2807
20	Cl	3-Indole	4-Piperidine	$CH_2CONHMe$	13	22	14	123	1769
21	Cl	3-Indole	4-Piperidine	COOEt	37	49	82	na	na
22	Cl	3-Indole	4-Piperidine	$CONMe_2$	15	37	na	4358	741
23	Cl	3-Indole	4-Piperidine	CO-(4Me piperazine)	18	26	na	5281	2770
24	Cl	3-Indole	4-Piperidine	$COCH_2NHCOMe$	28	46	na	551	1938
25	Cl	3-Indole	4-Piperidine	$COCH_2NHMe$	67	85	179	2672	2028
26	Cl	3-Indole	4-Piperidine	$COCH_2NMe_2$	57	45	120	1126	2497
27	Cl	3-Indole	4-Piperidine	CO-(4Me piperidine)	15	14	48	1895	813
28	Cl	3-Indole	4-Piperidine	CONH-(4-Me piperidine)	47	62	na	6652	807

na, not available.

planarity and fit with the binding site. When 5′ is hydrogen (**8**) (**11**) (**12**) there is an overall drop in JNK activity. However in this case the 5′-substituent has less effect, with all bicycles having comparable potencies. This interplay also has an effect on CDK2 activity. When chloro, JNK1 and JNK2 activities are comparable, but more potent than CDK2. When hydrogen, JNK1 activity is greater than JNK2, which in turn is more potent than CDK2. 5′-Chloro analogues have far greater JNK potency, however selectivity will need to be addressed as exemplified by high activity against CDK2. Alternatively, 5′-hydrogen analogues offer a better JNK/CDK selectivity profile and so have potential if JNK potency can be improved. Our next area of interest was to look for heterocycles as replacements for the 4-piperidine of the original hit (**5**) (**14**–**19**). Changing the ring size from six (**6**) to five (**14**) to four (**15**) gave a reduction in JNK activity and CDK2 activity, suggesting a less optimal fit of the smaller rings in the active site of both JNKs and CDK2. The 4-piperidine ring seems to be occupying space that is key to potency. To test this several 3-piperidine analogues (**16**) to (**19**) were designed. Overall, these compounds were either equipotent or less active than the 4-piperidine compound (**6**), with the (*S*)-3-piperidine compounds **16** and **17** having an undesirable effect on CDK2 potency. Comparison of enantiomers suggests a stereochemical preference that is dependent on the nature of the cap. Compounds **20**–**28** exemplify the final area of diversification, which involved varying groups attached to the nitrogen of the 4-piperidine. Many groups were well tolerated and it can be seen from Table 1 that different groups can improve selectivity against CDK2, whilst retaining JNK potencies. This area has huge scope and is under investigation with the aim of improving physical properties, such as solubility. As discussed earlier, modelling suggested that the gatekeeper area could be used to induce selectivity for JNKs over CDK2 by having R groups that move the gatekeeper and access the classical hydrophobic pocket (Table 2, Fig. 4). Compound **13** showed good JNK1 activity, moderate JNK2 potency, with good selectivity over CDK2, providing confidence that this would be a good template to work from. Two further compounds were made (**29**–**30**) and interestingly it seems that R can give rise to potent and selective compounds.

When R is phenyl (**30**) JNK1 and JNK2 potency is 22 and 5 nM, respectively, with excellent selectivity over CDK2. While this work needs further investigation the hypothesis proposed by modelling has been supported by experimental evidence.



Scheme 1. Synthesis of aminopyrimidines. Reagents and conditions: (a) 1-phenylsulfonyl indole-3-boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, MeCN, H_2O, reflux, 90 min, 50–90%; (b) KOH, MeOH, reflux 30 min, 50–60%; (c) 1-bocaminopiperidine, DMF, 120 °C, 6 h, 50–70%; (d) 2 N HCl in ether, MeOH, rt, 40–60%; (e) EtNCO, Et_3N, Na_2CO_3, DCM, rt, 10–40%; (f) chloromethylacetamide, Na_2CO_3, DMF, 60 °C, 2 h, 10–40%; (g) indole, NaH, DMF, rt, 60–70%; (h) Pd/C, H_2, NaOH, MeOH, 80–85%; (i) *n*-BuLi, ZnBr, THF, then 2,4,5-trichloropyrimidine, $Pd(PPh_3)_4$, 32%; (j) 1-ethylurea-4-aminopiperidine, Et_3N, DMF, 50%; (k) R-acetylene, $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, rt, 18 h, 70–80%; (l) 1-aminopyridinium iodide, DBU, MeCN, 50 °C, 18 h, 20–50%.

JNK inhibitors need to be cell permeable to reach their intracellular targets; therefore a phospho c-Jun cell based assay[13] was utilized to test cellular potencies (Table 1). Compound **4** gave moderate cellular activity and seemed to be a good starting point for further development, although only a few analogues showed submicromolar cellular potency (**22**, **27**, **28**). Further work is underway to improve our understanding of cellular potency; however at present other bicycles have not demonstrated significant cellular activity.

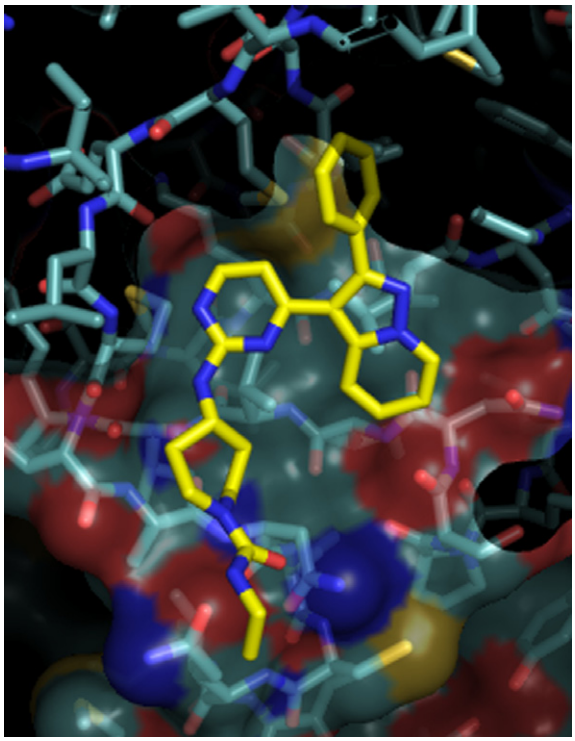
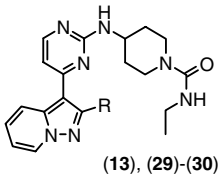


Figure 4. Docking of Compound **30** in JNK3 protein (GOLD, CCDC, UK) into 1 pmn.[10]

A large number of compounds have been synthesized and assayed against JNK1, JNK2 and CDK2 free enzymes and tested in a c-Jun cellular assay. The results presented highlight our success in synthesizing compounds that led to improvements in JNK potency and good selectivity over CDK2.

X-ray crystal structures and docking have been employed to understand the complex SAR of this series of molecules regarding their activity against JNK. Predictions for improved selectivity, such as accessing the classical hydrophobic pocket, have been tried

Table 2. Enzymatic and cellular activity of aminopyrimidine analogues



Compound	5′ X	Bicycle	R	JNK1[11] IC_{50} (nM)	JNK2[11] IC_{50} (nM)	JNK3[11] IC_{50} (nM)	CDK2[12] IC_{50} (nM)	c-Jun[13] IC_{50} (nM)
6	Cl	3-Indole	H	13	25	57	1517	704
13	H	Pyrazolopyridine	H	69	194	na	8663	4000
29	H	Pyrazolopyridine	^{i}Pr	520	698	na	>10,000	21,160
30	H	Pyrazolopyridine	Ph	22	5	5	>10,000	3845

na, not available.

experimentally and the evidence so far supports these predictions. Further work on this series of compounds and their selectivity profiles will be communicated in due course. Work is underway to improve our understanding of cellular potency; however at present other bicycles have not demonstrated significant cellular activity.

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11. The JNK-1/2/3 in vitro enzyme assay determines the effect of test compounds on phosphorylation of the GST-c-Jun (1–89) substrate at the Ser73 site. GST-c-Jun is pre-bound to glutathione plates. Plates are washed prior to addition of MKK4 activated JNK-1/2/3, test compound and ATP. Plates are washed and the level of phosphorylation detected with an anti-phospho Ser73 antibody.
12. The CDK2 in vitro enzyme assay determines the effect of test compounds on the phosphorylation of a fluorescein labelled Histone H1-derived peptide detected by IMAP technology. FAM-peptide, ATP, CDK2/Cyclin A and test compounds are added to a 384-well black plate. The reaction mixture is incubated for 60 min at RT and stopped by addition of 60 μl progressive binding buffer 'A' containing 1/400 IMAP beads.
13. A549 cells are plated out at 1.25×10^4/well in RPMI media containing 10% FBS, penicillin/streptomycin and glutamine and incubated overnight at 37 °C/5% CO_2. The cells are stimulated with 1 ng/ml recombinant human IL-1β and incubated for a further 30 min at 37 °C/5% CO_2. c-Jun translocation is then measured using a c-Jun Activation Screening Hitkit product (Cellomics).