Inhibitors of c-jun-N-Terminal Kinase (JNK)

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Abstract: Inhibitors of c-jun-N-Terminal Kinase (JNK) have many potential therapeutic indications ranging from neurodegenerative disease, to metabolic disorders, inflammation, cardiovascular disease, and cancer. This overview will highlight biological inhibitors such as JNK-interacting protein (JIP) as well as small molecule inhibitors from various structural classes including, aminopyrimidines and indazoles.

Key Words: c-jun-N-Terminal Kinase (JNK), mitogen activated protein (MAP), benzothiazole pyrimidines, benzothien-2-yl amides, indazoles, aminopyrimidines, aminopyridines, quinolines.

INTRODUCTION

The c-jun NH_2-terminal kinases (JNKs) are members of the mitogen activated protein (MAP) kinase family and are activated by a number of stress stimuli including cytokines [1, 2], UV light [3], hypoxia [4, 5], endoplasmic reticulum (ER) stress (including protein misfolding) [6-8], and reactive oxygen species [9]. As such, JNK has been termed a stress-activated kinase along with its MAP kinase family member p38. JNK and p38 belong to the broader class of CMGC kinases [cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAPKs), glycogen synthase kinases (GSKs), and CDK-like kinases (CLKs)] which are one of the three major classes of kinases (including classical Ser/Thr and Tyr kinases). These stress activators have been associated with a number of cell death scenarios and accordingly with numerous diseases. For example, IL-1 β-induced activation of the JNK pathway has been shown to induce apoptosis in rat INS-1 cells [2]. Similarly Kim *et al*. utilizing a pancreatic β-cell line showed that TNF-α and IFN-γ activated JNK thus promoting pancreatic β-cell apoptosis [1]. Numerous reports have also detailed the role of JNK in ER-stress related cell death scenarios. In 2000 Ron and colleagues showed that rat pancreatic cells treated with thapsigarin (an agent that induces ER stress) activated the JNK pathway through a signaling mechanism coupled to the unfolded protein response sensor, inositol-requiring enzyme 1 (IRE-1) [8]. More recently, Kerkelä *et al*. demonstrated that the cardiotoxicity of imatinib mesylate seen in man and rodents was due to the ER-stress induced activation of the JNK pathway by imatinib mesylate [6]. As a final example of the myriad of stress stimuli that can activate the JNK pathway and hence cause cell apoptosis, it is illustrative to include the oxidative stress mechanisms. One example is the dopamine-induced activation of the JNK pathway and subsequent cell death seen in HEK-293 cells and primary neonatal rat striatal neuronal cultures [9]. A second example is the 1-methyl-4-phenylpyridinium (MPP^+), induced apoptotic cell death of dopaminergic neurons from mesencephalic cultures reported by Gearan *et al*. In these studies MPP^+ treatment of E-17 ventral mesencephalon caused activation of JNK as measured by phospho-c-jun immunoreactivity [10]. These examples are by no means exhaustive of the literature in this area, but merely examples of cell based systems that are used to study apoptosis and have *in vivo* disease models which have also been shown to be dependent on the JNK pathway for cell death and disease. The *in vivo* data will be discussed shortly.

There are three genes (*Jnk1* [3], *Jnk2* [11, 12], and *Jnk3* [13]) that encode for human JNK, and from these, ten splice variants have been described [14]. There are four JNK1 splice variants, four JNK2 splice variants, and two JNK3 splice variants. While varying function has been ascribed to the JNK isoforms (i.e JNK1, JNK2, and JNK3), there have been no reports detailing any specific function to the splice variants. JNK1 and JNK2 are ubiquitously expressed, whereas JNK3 has much more limited expression being confined primarily to the nervous system with only low level expression in the heart and testis [13]. This tissue specific expression of JNK3 has led to the suggestion that inhibition of JNK3 may be a good target for central nervous system (CNS) diseases.

Validation for JNK as a compelling target for a variety of diseases including neurodegeneration, metabolic disorders, inflammation, cardiovascular disease, and cancer come in the form of data from knock out (KO) mouse studies, peptide inhibitors of JNK, and small molecule inhibitors of JNK. Numerous gene deletion studies in mice provide evidence that the inhibition of JNK may be very valuable as a therapeutic approach for a variety of disease. In 1997, Yang *et al*. [15] showed that mice lacking the *Jnk3* gene were resistant to kainic acid induced seizures. Not only were seizure scores for the mice dramatically reduced for the *Jnk3* treatment group compared to wild-type controls, but there was also a significant decrease in excitotoxicity-induced hippocampal apoptosis as measured by TUNEL assay in the *Jnk3* KO group as compared to wild type. *Jnk1* and *Jnk2* KO mice were not resistant to the kainic acid-induced seizure effects suggesting a unique role for *Jnk3* as a potential therapy for seizure. Similarly, deletion of *Jnk3* protects neonatal mice

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1389-5575/08 $55.00+.00

against cerebral hypoxic-ischemic injury suggesting that JNK3 inhibitors may be effective in the treatment of stroke [5]. Indeed, in this study significant attenuation of injury was observed in the cerebral cortex, hippocampus, striatum, and thalamus in *Jnk3* KO mice compared to control mice [5]. A third compelling knockout mouse study linking JNK3 and JNK2 to neurodegenerative disease was reported in 2004 by Flavell and colleagues. In this study the authors showed that *Jnk3* KOs, *Jnk2* KOs, and compound *Jnk3/Jnk2* KOs were resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurodegeneration and motor deficits in this mouse model of Parkinson's disease [16]. Measurements of striatal dopamine, survival of tyrosine hydroxylase(TH)-immunoreactive dopaminergic neurons in the substantia nigra pars compacta (SNpc), and motor function on a rotarod all showed statistically significant improvement compared to wild-type MPTP-lesioned mice [16]. This dramatic result further validated the potential for JNK inhibitors in the treatment of neurodegenerative disease.

Similarly, KO studies have helped define the role of JNK in peripheral diseases such as type II diabetes mellitus, and obesity. In 2002, Hirosumi *et al*. showed that *Jnk1* -/- mice had decreased adiposity, significantly improved insulin sensitivity, and enhanced insulin receptor signaling capacity in both a diet-induced obesity (DIO) model and a genetic obesity model (using *ob/ob* mice). In addition, the DIO *Jnk1* -/- mice had decreased body weights compared to DIO wild type mice and also showed decreased blood glucose levels [17]. These data are strong evidence that a JNK1 inhibitor may be efficacious in the treatment of type II diabetes mellitus as well as in obesity.

Preclinical validation for JNK as a target for therapeutic intervention also comes in the form of biological inhibitors such as the JIP peptide as well as small molecule inhibitors of JNK. The JIP peptide comes from the JNK-interacting protein-1 (JIP-1), an anchor protein, that was first described as a cytoplasmic inhibitor of the JNK signaling pathway [18]. Indeed, three of the KO efficacy studies (cerebral ischemia, Parkinson's disease, and type II diabetes) have been corroborated by inhibition of JNK utilizing varying lengths of the peptide derived from the JNK-binding domain of JIP-1 [18]. In 2003 Borsello *et al*. [19] utilized a 20-amino-acid sequence of JIP made cell penetrant by fusing the HIV Tat (48-57) transporter sequence to the N-terminus of the JIP sequence, to show that intraventricular administration of this peptide reduced lesion volume in two models of middle cerebral artery occlusion (MACO) by 78% and 90%. This protection correlated with a decrease in c-jun phosphorylation suggesting inhibition of JNK in the JIP-binding domain may have significant therapeutic benefit in stroke. Small molecule validation for the JNK pathway as a therapeutic strategy in stroke came from the Serono group where they showed that a benzothiazole pyrimidine (AS601245) ($C_{25}H_{23}N_5O_2S$) produced a neuroprotective effect in rats at (1mg/kg i.v. bolus followed by 0.6 mg.kg/h i.v infusion) after focal ischemia [20]. This same compound was also shown to decrease infarct size after myocardial ischemia and reperfusion in rats [21].

Kaneto *et al*. [22] also utilized the 20-amino-acid sequence (RPKRPTTLNLFPQVPRSQDT) of JIP fused to HIV-Tat to show that intraperitoneal administration markedly improved insulin resistance and improved glucose tolerance in diabetic mice. A similar approach was utilized by Xia *et al*. [23] where they generated adenoviral constructs expressing JIP-1 residues 127-281 which contained the JNK binding domain (JBD). This peptide was shown to protect dopaminergic neurons in the MPTP model of Parkinson's disease in mice as well as reduce phospho c-jun levels. The big question that remains of course is: can a small molecule mimetic of the JBD of JIP be generated?

Several possibilities exist for small molecule binding pockets within JNK that may be used for inhibition of JNK. The aforementioned JIP peptides are selective inhibitors of JNK which do not inhibit other MAP kinase family members making that pocket possibly a very specific site for JNK inhibition. To date however there are no reports of small molecule inhibitors that mimic JIP and bind in the JBD utilized by JIP. Indeed the work cited above utilized either very large JIP peptides [23], or at the smallest only 20 amino acids [19, 22]. JIP peptides composed of 11-amino acids have been shown to inhibit both JNK 1 [24] and JNK2 [25] *in vitro*, and this may be a reasonable starting point for small molecule development although 11 amino acid peptides are still rather large compared to most small molecule drug-like molecules.

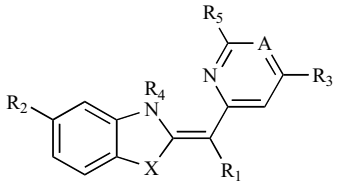
Another theoretical possibility for a structural motif within JNK that may yield selective development of small molecule inhibitors is the so-called "DFG out" pocket. The "DFG" pocket is found in the activation loop of many kinases and is so named due to the presence of the conserved aspartic acid, phenylalanine, and glycine residues found in this loop. Inhibitors that bind in this pocket of kinases are often referred to as type II kinase inhibitors because these inhibitors bind preferentially to inactive kinase conformations and do not exclusively utilize the ATP biding pocket. Many type II inhibitors have been described for many kinases and the literature and structural classes of compounds fitting this description have been extensively reviewed [26]. It should be stressed however, that like the JIP peptide site, there are no published reports of type II inhibitors for JNK.

The third structural motif within JNK that may be exploited for small molecule inhibitor design is the ATP binding pocket. To date, all published reports for JNK inhibitors show compounds that are targeted to this site. The compound that has been most widely utilized in many biological studies is the anthrapyrazolone, SP-600125 [27-34] ($C_{14}H_8N_2O$). This owes to the early report of this molecule [27], the modest biochemical potency (IC_{50} ~ 100 nM –*vs*- JNK3) [27], the commercial availability, and reported efficacy in a number of cell-based and animal models [28-34]. Indeed, like the JNK KO studies and the JIP inhibition study, SP-600125 was shown to be efficacious in the chronic MPTP model in mice [34]. These results in our view should be interpreted with caution given the poor selectivity of SP-600125 especially with regard to monoamine oxidase B (MAO-B) inhibition. Despite the vast biological literature on SP-600125, there are not really any structure activity relationship (SAR) reports around this molecule, and the general consensus is that the poor selectivity profile and weak pharmacokinetic properties

make this compound undesirable for further development. Since 2004, the medicinal chemistry literature has seen a substantial increase in the number of structural classes reported as JNK inhibitors. In the remaining sections of this review, we will discuss the SAR that has been established in the primary literature for the following structural classes: benzothiazole pyrimidines, indazoles, aminopyridines, benzothien-2-yl amides, aminopyrimidines, and quinolines.

An internal drug discovery program at Serono Pharmaceutical Research Institute was launched with the goal of discovering small-molecule JNK inhibitors. High throughput screening of an in-house compound collection identified **1** (benzothioazole pyrimidine series) as a valid lead and was the starting point for medicinal chemistry [35]. As shown in Table **1**, among the combinations tested on the lead, the only transformation tolerated was removal of the bromine atom from C-6 (R_3) as in compound **3**. Even replacement of the bromine atom with a methyl group led to complete loss of activity (**4**). A benzimidazole (**2**) ring was also not a suitable replacement for the benzothiazole ring in the core structure of **1** nor was an ester for cyano replacement as in **7**. Addi-

Table 1. Modifications of Screening Hit 1, JNK3 Inhibition [35]



Cmpd.	A	X	R_1	R_2	R_3	R_4	R_5	rJNK3 IC_{50}, nM[a]
1	N	S	CN	H	Br	H	Cl	350
2	N	NH	CN	H	Br	H	Cl	>5000
3	N	S	CN	H	H	H	Cl	250
4	N	S	CN	H	Me	H	Cl	>5000
5	C	S	CN	H	H	H	Cl	>5000
6	N	S	CN	CF_3	H	H	Cl	993
7	N	S	CO_2Et	H	H	H	Cl	>5000
8	N	S	CN	H	H	Me	Cl	>5000
9	N	S	CN	H	H	H	H	>5000
10	N	S	CN	H	H	H	NH_2	7500
11	N	S	CN	H	H	H	$NHNH_2$	500
12	N	S	CN	H	H	H	NHMe	950
13	N	S	CN	H	H	H	NMe_2	9400
14	N	S	CN	H	H	H	N-morpholinyl	2100
15	N	S	CN	H	H	H	$NH(CH_2)_2Me$	>5000
16	N	S	CN	H	H	H	$NH(CH_2)_3$N-morpholinyl	407
17	N	S	CN	H	H	H	$NHCH_2$-tetrazol-2-yl	>5000
18	N	S	CN	H	H	H	$NH(CH_2)_2Ph$	>5000
19	N	S	CN	H	H	H	$NH(CH_2)_2$-Ph-2-F	273
20	N	S	CN	H	H	H	$NH(CH_2)_2Ph$-4-NH_2	80
21	N	S	CN	H	H	H	$NH(CH_2)_2Ph$-4-SO_2NH_2	41
22	N	S	CN	H	H	H	$NH(CH_2)_2$-pyridin-3-yl	120
23	N	S	CN	H	H	H	$NH(CH_2)_3$-N-imidazolyl	147

[a]All values in triplicate.

tionally, methylation of the benzothiazole nitrogen atom (**8**) kills potency.

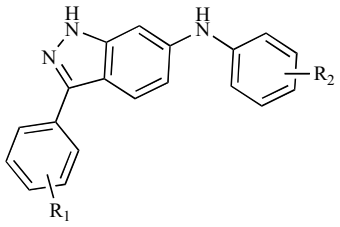
Simple modifications to the pyrimidine ring itself also proved detrimental to potency. Replacement of one of the pyrimidine nitrogen atoms with carbon (**5**) as well as substituting a hydrogen atom for chlorine (**9**) led to inactive compounds. All further modifications maintained the core found in **3** and looked at replacing the group attached to the carbon atom between the pyrimidine nitrogen atoms. Tertiary amines were less active than secondary amines (**13**-**14** *vs*. **12**) suggesting that the pyrimidine "NH" is critical for activity. This optimized atom arrangement is in agreement with the well-described bidentate interaction detailed in many publications on ATP-competitive inhibitors [36].

Simple aliphatic substitutions (**15**, **18**) at R_5 did little for potency, unless substituted with a polar functionality capable of picking up an additional interaction (**16**, **20**-**23**). The group is most likely acting as a hydrogen bond acceptor (HBA) given the lack of potency for tetrazole **17** as well as the difference observed between **18** and **19**. Fluorine has long been known to have HBA properties.

The introduction of polar side chains also allowed access to potent JNK3 inhibitors with improved physicochemical properties thereby improving the drug-like properties of the compounds in this series. The introduction of an additional protonable center provided for a dramatic increase in aqueous solubility as seen for **22**, which is soluble at over 100 mg/ml in saline as its bis-mesylate salt. It also exhibited acceptable bioavailibility in rats (F=38%), and hence was chosen as a proof of concept compound in several *in vivo* animal models of disease [35]. Although no additional data was given, the authors reported that none of the compounds shown demonstrated any specificity when tested against the rat or human versions of JNK1, -2, or -3. However, when compound **22** was profiled against a small panel of kinases, it exhibited a 10- to 20-fold selectivity over c-SRC, c-Raf, CDK2/CycA, and p38, and above 50- to 100-fold selectivity over a range of Ser/Thr and Tyr protein kinases [35].

Astra-Zeneca also identified several lead hits for their JNK3 program using a high throughput screening campaign [37]. Some of these compounds were co-crystallized with JNK3 and JNK1. This structural and activity information was used to design compounds with increased selectivity for JNK3 *vs* JNK1 and the closely related kinase p38. The X-ray crystal structure of **24** (Table **2**) revealed that the compound bound in an induced fit manner in the ATP-pocket of JNK3 [37]. The aniline portion of **24** fits into the selectivity pocket of JNK3 as the side chain of the gatekeeper methionine (Met146) has moved to accommodate the ligand. The indazole nitrogens of **24** displayed hydrogen bonds to the backbone amino acids in the hinge region (Met149 and Glu147), and the aniline NH hydrogen bonds to Lys93 *via* a water molecule. Compound **24** was equipotent against p38, but surprisingly had greater than 200-fold selectivity over JNK1. Interestingly, while substitutions on the aniline phenyl ring (R_2) led to no significant potency changes on JNK3 or p38, a loss in JNK1 selectivity was noticed (**25**-**26**). Substitution on

Table 2. IC_{50} Values for Compounds 24-34 Against JNK3, JNK1, and p38 [37]



Cmpd.	R_1	R_2	JNK3 IC_{50}, nM	JNK1 IC_{50}, nM	p38 IC_{50}, nM
24	H	H	48	>10,000	30
25	H	2-Cl	30	5350	18
26	H	2-OMe	202	1600	46
27	4-CO_2H	2-Cl	32	246	13
28	4-$CONH(CH_2)_2N(CH_3)_2$	2-Cl	1.9	45	8.7
29	3-$CONH_2$	2-Cl	3.3	81	3.2
30	3-CO_2H	2-Cl	5.3	61	24
31	3-$CONH(CH_2)_3$-4-morpholinyl	2-Cl	3.4	228	3.7
32	3-$CONH(CH_2)_2N(CH_3)_3$	2-Cl	21	698	16
33	3-CONH-4-piperidinyl	2-Cl	1.4	71	4.4
34	3-CO_2H,5-$NHCO(CH_2)_3N(CH_3)_2$	2-Cl	3	101	903

the phenyl ring at C-3 of the indazole provided improvements in potency against JNK3 with modest selectivity *vs* JNK1, but little to no selectivity against p38.

When comparing the crystal structures of **30** and **31** in JNK3, the acid and amide groups appeared to bind in different directions [37]. The 3-carboxylic acid hydrogen bonds to Asn152, whereas the 3-amide substituent, bearing a basic amine side chain, extends out to interact with Asp150. It was anticipated that incorporation of these two groups into the same molecule might provide some selectivity against p38. In p38, the corresponding amino acid to Asn152 is Asp112, an acidic amino acid, which might repel the carboxylic acid on the ligand and hence reduce affinity. Therefore, compound **34** was synthesized. In agreement with the prediction, **34** showed excellent selectivity against p38 (300-fold), but JNK1 selectivity dropped to only 30-fold. While installation of an acidic group into the molecule helps dial out p38, it may worsen physiochemical properties and CNS penetration. Nonetheless, this work shows the power of structure based drug design and the potential it has to fashion molecules that bind the ATP pocket of structurally related kinases.

Compound **35** (Table **3**) was another JNK inhibitor from the amino pyridine structural class identified in the high throughput screen run at AstraZeneca [38]. The SAR of lead structure **35** was not successful in improving potency against JNK3 (data not shown), but more importantly, compounds in this class had poor solubility properties. Hence, a search for more soluble bipyridine analogues was conducted by synthesizing libraries. Interesting results were obtained in the series encompassed by compounds shown in Table **3** (**37**-**48**).

Most amide group substitutions, both saturated and heterocyclic systems (**37**-**42**), were tolerated and gave potent compounds against JNK3; however, they exhibited less than 10-fold selectivity over JNK1. Compared to the solubility of **36**, compounds **37**-**42** were 40- to 1000-fold more soluble in phosphate buffer at pH=7.4. This was also achieved without sacrificing potency against JNK3 or selectivity against JNK1. Amongst the isomeric N-Acetyl piperidines synthesized (**40**, **43**-**44**), the meta-analog (**43**) was the most active. Compound **43** was also screened against a collection of kinases (~30) and exhibited no significant activity at 10μM for the majority of the kinases in the panel [36].

During the course of their SAR, compound **45** was identified, which was 50-fold selective over JNK1 and 26-fold selective over p38. This was the most selective analog found against JNK1, though more selectivity against p38 was still desired. While compounds related to **35** were ultimately abandoned, SAR revealed that a 2'-*p*-fluoro substitution increased selectivity against p38, and so the 2'-*p*-fluoro analog of **45** was synthesized (not shown) and separated into its enantiomers (**46**-**47**). Unfortunately, p38 selectivity was increased at the expense of JNK1 activity. Compound **48** was a good compromise between potency and selectivity as it contains no chiral centers, however **45**, with slightly better selectivity, was chosen for further profiling. This compound displayed a good level of *in vitro* metabolic stability (rat microsomes Cl_{int} = 12μL/min/mg) as well as good Caco-2 permeability (29.9 x 10^{-6} cm/s) and the IC_{50} was above 10μM for the 5 CYP isoforms tested. In rats, the Cl_p = 30 mL/min/kg, $t_{1/2}$ = 4.7h (po), and F% = 16 [38].

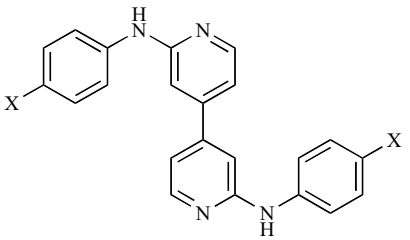
To help explain the observed selectivity in this series, **35** was co-crystallized with JNK3 and JNK1 [38]. In JNK3, the 2'-anilino part of the molecule of **35** binds into hydrophobic pocket II, the so-called selectivity pocket. The side chain of the gatekeeper amino acid (Met146) has moved more than 2Å to accommodate the phenyl group of the ligand in JNK3 compared to the corresponding amino acid Met108 in the JNK1 ATP binding pocket. This anomaly has not been observed when ATP is bound to JNK3 or when compounds in this series are bound to JNK1. The pyridine nitrogen and the 2-anilino NH display a bidentate hydrogen bonding interaction to the backbone amino acid Met149 in the hinge in JNK3. The induced fit binding into JNK3 appears to be favored for JNK3 over JNK1. Nonetheless, despite the differences in the sequence of amino acids which line the selectivity pocket (especially Leu144 in JNK3 compared to Ileu106 in JNK1), and the induced manner in which compounds bind to JNK3, enough interactions are possible to achieve good JNK1 potency. Hence, the modest 4-fold difference in binding to JNK3 *vs* JNK1. However, the crystal structure of **35** in JNK3 and JNK1 alone cannot account for the observed selectivities for some of the compounds in Table **3** (**38**, **45**) where simple substitutions not affecting the selectivity pocket are encountered. Obviously, more subtle effects are in play here (*in vitro*) that cannot be simply explained with a static model (X-ray).

Scientists at Abbott discovered the pyridine carboxamide structure **49** (Table **4**) *via* a high throughput screen of their sample collection [39, 40]. This lead compound is quite unique when compared to other JNK kinase inhibitors described in both the primary and patent literature. Competition experiments confirmed that this inhibitor binds competitively and reversibly to the ATP site of JNK1. It also showed some inhibition in a phospho-c-jun cell assay [39, 40].

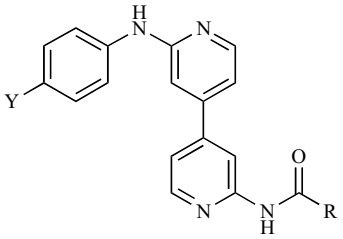
Exploration of the R_1-amide position revealed that larger groups than the original acetamide could improve potency (**50**). Substituted phenacetyl analogs, in particular, methoxy substituted ones (**54**-**55**), gave the greatest boosts in potency with 2-,5-dimethoxy analog **56** as the most potent analog (IC_{50}= 45 nM JNK1) in this series. Substitution was also tolerated at the 4-position on the dimethoxy aryl ring (**57**-**58**), and while no improvement in potency was observed, these substitutions did improve microsome stability and rat pharmacokinetics [39]. Aliphatic amine containing side chains off the amide group were also acceptable (**62**-**64**), but offered no improvements in biochemical or cell potency.

In regards to the core pyridine ring, attempts to modify the ethoxy group at R_2 were unsuccessful with the exception of other small ether groups (**65**). Amines and alkyl or aryl groups diminished activity (**67**-**69**). Changes elsewhere on the diaminopyridine core were generally not tolerated. Replacement of the 4-amine group with a methyl substituent led to an inactive compound as did simultaneous deletion of both the C-3 nitrile and C-4 amino groups (not shown). Installation of a chlorine atom at C-5, the only unsubstituted posi-

Table 3. IC_{50} Values for Compounds 35-48 Against JNK3, JNK1, and p38 [38]



35 X = H
36 X = F



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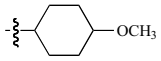
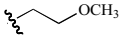
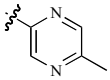
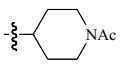
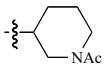
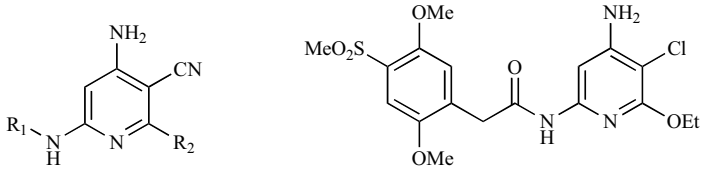
Cmpd.	Y	R	Solubility (μM)	JNK3 IC_{50}, nM[a]	JNK1 IC_{50}, nM[a]	p38α IC_{50}, nM[a]
35	-	-	-	30	117	-
36	-	-	<0.1	17	-	-
37	H		8	32	106	-
38	H		20	20	451	-
39	H		40	33	261	-
40	H		94	44	219	-
41	H		17	175	780	-
42	H		4	18	42	-
43	H		-	9	82	37
44	H		-	207	-	-
45	H		14	7	384	180
46	F		-	3	48	561
47	F		-	9	55	421
48	H		-	8	122	251

[a]Values are means of n ≥ 2 determinations, standard deviation ≤±10%.

tion on the ring was also not tolerated (not shown). Lastly, N-methylation of the amide side chain at C-6 abolished activity as well (not shown).

In general, the compounds showed EC_{50} values in a cell-based assay monitoring c-jun phosphorylation about 15-fold less than their biochemical IC_{50} values. There appeared to be

Table 4. Pyridine Carboxamide SAR [39]



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Cmpd.	R_1	R_2	JNK1 IC_{50}, nM	JNK2 IC_{50}, nM	c-jun EC_{50}, nM
49	CH_3CO	OEt	750	1100	6800
50	*n*-BuCO	OEt	350	690	
51	$PhCH_2CO$	OEt	1400	3500	
52	2-(CH_3)-$PhCH_2CO$	OEt	400	740	
53	3-(Cl)-$PhCH_2CO$	OEt	300	900	
54	2-(MeO)-$PhCH_2CO$	OEt	120	260	1400
55	4-(MeO)-$PhCH_2CO$	OEt	180	274	1400
56	2,5-(diMeO)-$PhCH_2CO$	OEt	45	160	920
57	4-$(MeSO_2)$-2,5-(diMeO)-$PhCH_2CO$	OEt	38	150	1300
58	4-Br-2,5-(diMeO)-$PhCH_2CO$	OEt	77	160	890
59	CH_3OCO	OEt	>10,000	>10,000	
60	EtNHCO	OEt	>10,000	>10,000	
61	2,5-(diMeO)-$PhCH_2CH_2$	OEt	>10,000	>10,000	
62	(n-propyl)(2-hydroxyethyl)NCH_2CO	OEt	160	740	5500
63	N-morpholinyl-CH_2CO	OEt	270	1200	
64	4-(*n*-butylaminocarbonyl)-1-piperidinyl-CH_2CO	OEt	69	180	>10,000
65	CH_3CO	O-*i*-Pr	310	470	
66	CH_3CO	S-*n*-Bu	8900	>10,000	
67	CH_3CO	NHEt	2400	>10,000	
68	CH_3CO	Ph	1900	7700	
69	CH_3CO	*n*-Pr	>10,000	>10,000	
70	2,5-(diMeO)-$PhCH_2CO$	Br	1700	>10,000	
71	2,5-(diMeO)-$PhCH_2CO$	$O(CH_2)_2OH$	74	240	1600
72	2,5-(diMeO)-$PhCH_2CO$	H	2600	5100	
73	See above	-	36	70	1750

no correlation between inhibitor cell permeability (data not given) and the EC_{50} to IC_{50} ratio. Hence, cell penetration alone does not explain the shift in cellular activity. The higher ATP concentration in the cell (presumed 10mM) may also be a contributing factor.

Pharmacokinetic profiles were determined for several inhibitors given in Table **4**. Some showed short half lives (1-2h) and high clearance rates (1-3 L/hr/kg) and little to no bioavailability (3-5 %F), while others such as **57** and **73** had better bioavailability (31% and 49% respectively), but still had high clearances (1.5 and 1.7 L/hr/kg) [39]. It's not clear however, if this is due to improved first pass metabolism or better oral absorption.

An X-ray crystal structure of **58** and JNK1 helped identify some of the key binding interactions between ligand and enzyme. The inhibitor lies in the ATP binding site, with the

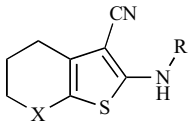
pyridine ring positioned deep within the adenosine binding region [39]. The C-4 amine makes a weak hydrogen bond with the gatekeeper amino acid Glu109, but is suboptimal because the amine and Glu109 are not in the same plane. The cyano group forms another weak hydrogen bond with Lys55, while the ethyl ether moiety appears to have no clear hydrogen bonding interactions. The amide oxygen atom of **58** forms another weak, out of plane hydrogen bond with Met111 in the hinge region. The bromo- and methoxy substituents point out towards solvent and may have interactions, but these were not evident in the crystal structure.

Compounds in this series exhibited a remarkable selectivity profile, showing little cross-reactivity with any of 74 other non-c-Jun-N-terminal kinases tested. Where off-target activity was found, the compounds were well over 100-fold selective. Examination of the crystal structure of the enzyme-inhibitor complex offers a potential explanation for this selectivity. Most kinase inhibitors typically form strong, unambiguous hydrogen bonds to the hinge region [41-43]. These inhibitors are held in the active site by the additive effect of a series of weak hydrogen bonds and hydrophobic interactions within the hinge and elsewhere. Such weak hinge binding may offer selectivity with respect to binding to other kinases, and this selectivity may be helpful for delineating the role of JNK inhibition *in vivo*.

A screening exercise at GlaxoSmithKline led to the identification of a series of (tetrahydro-1-benzothien-2-yl)amides typified by compound **74** (Table **5**) as potent JNK3 inhibitors [44]. Initial simple amide modifications revealed a clear SAR. Amides with aryl substituents (**75-80**) were tolerated whereas benzyl, alkyl, or cycloalkyl substituents (**81-84**) were not. Sulfonamide (**85**) and urea (**86**) (direct analogs of **75**) as well as other examples (**87**) were inactive.

Appreciable selectivity for JNK3 over JNK1 and p38 were observed, a significant feature not common in the literature. In order to understand the nature of this selectivity and to help guide future medicinal chemistry efforts, a crystal structure of **79** with JNK3 was obtained. The conserved hydrogen bond from the hinge Met149 in JNK3 is accepted by the 3-cyano substituent of **79**, an unusual interaction. An-

Table 5. JNK1, JNK3, and p38α Inhibition, Values in pIC_{50} [44]



Cmpd.	X	R	JNK1	JNK3	p38α
74	CH_2	-C(O)(3-thienyl)	-	6.2	<4.8
75	CH_2	-C(O)(1-naphthyl)	<5.0	6.7	<4.8
76	CH_2	-C(O)(4-F-1-naphthyl)	<5.0	6.0	<4.8
77	CH_2	-C(O)phenyl	<5.0	5.8	<4.8
78	CH_2	-C(O)(4-F-phenyl)	<5.0	5.5	<4.8
79	CH_2	-C(O)(2-F-phenyl)	<5.0	6.4	<4.8
80	CH_2	-C(O)(2-Br-phenyl)	<5.5	5.5	<4.8
81	CH_2	-C(O)CH_2phenyl	<5.0	<4.8	<4.8
82	CH_2	-C(O)CH_2(2-Br-phenyl)	ND[a]	<4.8	<4.8
83	CH_2	-C(O)CH_2(4-morpholine)	ND	<4.8	<4.8
84	CH_2	-C(O)cyclohexyl	ND	<4.8	<4.8
85	CH_2	-SO_2(1-naphthyl)	ND	<4.8	<4.8
86	CH_2	-C(O)NH(1-naphthyl)	ND	<4.8	<4.8
87	CH_2	-SO_2(3-Me-phenyl)	ND	<4.8	<4.8
88	-NC(O)cyclopropyl	-C(O)(1-naphthyl)	<5.0	6.6	<4.8
89	-NC(O)(1*H*-pyrazol-3-yl)	-C(O)(1-naphthyl)	<5.0	6.6	<4.8
90	-NCH_2(4-SO_2Me-phenyl)	-C(O)(1-naphthyl)	<5.0	6.0	<4.8
91	-NCH_2(3-pyridyl)	-C(O)(1-naphthyl)	<5.0	6.3	<4.8
92	-NSO_2phenyl	-C(O)(1-naphthyl)	<5.0	5.8	<4.8

[a]ND, not determined.

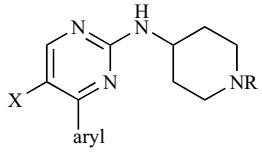
other unusual feature of the structure is the hydrogen bond between the inhibitor amide nitrogen atom and the sulfur atom of Met146. The inhibitor amide oxygen is hydrogen-bound *via* a bridging water molecule to the conserved Lys93. The 2-(2-fluoro) benzamide group occupies the 'induced-fit' selectivity pocket at the back of the active site, after movement of the gatekeeper Met146. The hydrophobic selectivity pocket contains the only active-site residue that differs between JNK3 (Leu144) and JNK1 (Ile106). As previously proposed, this small change may alter the shape of the pocket, hence, the observed selectivity [38]. Analogs with structurally related side chains (**81**, **84**) to that of **77** were inactive, indicating the limited extent of the pocket. Additionally, the differentially substituted phenyl analogs (**77-80**) show subtle differences in potency.

Based on the crystal structure of **79**, it appeared likely that space was available within the active site to allow substitution at the 6- or 7- positions of the tetrahydrobenzothiophene ring. This could be beneficial as additional, favorable contacts within the active site might be found, and could allow for attenuation of physicochemical properties (solubility, lipophilicity, etc). Introduction of substituents at C-6 (not shown) decreased potency against JNK3 although selectivity against JNK1 and p38 was maintained [44]. Substitution at the C-7 (X) position was more productive, with a variety of amides (**88-89**), amines (**90-91**), and sulfonamides (**92**) being tolerated.

Extensive profiling of this series (data not given) revealed that the selectivity observed for JNK3 over JNK1 and p38 is also mirrored in its selectivity when screened against a panel of more than 30 kinases. In addition, **77** was submitted to the Kinomescan assay panel of over 200 kinases (Ambit Biosciences) [45] and only showed significant activity at JNK3. Not surprising, however, this series of compounds did not show selectivity against JNK2. Only one residue differs in the active sites; Met115 in JNK3 and Leu77 in JNK2 and the residue is peripheral, so it should not impact inhibitor binding. The unique selectivity profile of this series parallels that seen for the Abbott series. In lieu of forming strong hydrogen bonds to the hinge region in the backbone of the enzyme to acquire potency, it is the cumulative effect of a multitude of weak hydrogen bonding interactions that hold the inhibitor in the active site.

High-throughput screening of the UCB sample collection uncovered several hits with varying activities against JNK1, 2, and 3 [46]. Aminopyrimidine **93** (Table **6**) was a promising lead and showed good potency against JNK, but also had

Table 6. Enzymatic and Cellular Activity of Aminopyrimidine Analogues [46]



Cmpd.	X	R	Aryl	JNK1 IC_{50}, nM	JNK2 IC_{50}, nM	JNK3 IC_{50}, nM	CDK2 IC_{50} nM	c-Jun IC_{50}, nM
93	CN	CONHEt	6-F-3-indole	92	67	412	412	3700
94	Cl	CONHEt	3-indole	13	25	57	1517	704
95	Me	CONHEt	3-indole	320	250	410	-	>10,000
96	H	CONHEt	3-indole	74	245	-	>10,000	8091
97	Cl	CONHEt	3-imidazopyridine	41	55	-	605	7723
98	Cl	CONHEt	1-indole	457	709	-	4443	>10,000
99	H	CONHEt	3-imidazopyridine	59	281	708	4219	19,331
100	H	CONHEt	1-indole	71	512	-	10,000	29,891
101	H	CONHEt	pyrazolopyridine	69	194	-	8663	4000
102	Cl	$CH_2CONHMe$	3-indole	13	22	14	123	1789
103	Cl	CO_2Et	3-indole	37	49	82	-	-
104	Cl	$CONMe_2$	3-indole	15	37	-	4358	741
105	Cl	$COCH_2NMe_2$	3-indole	57	45	120	1126	2497
106	Cl	CONH-(4-Me-piperidine)	3-indole	47	62	-	6652	807
107	H	CONHEt	2-*i*-Pr-pyrazolopyridine	520	698	-	>10,000	21,160
108	H	CONHEt	2-Ph-pyrazolopyridine	22	5	5	>10,000	3845

off-target activity against CDK2. To facilitate computer-assisted structure based drug design, a crystal structure of **94** was obtained in the active site of JNK3. The solved structure showed the aminopyrimidine nitrogens interacting with the hinge region of the active site *via* Met149, the indole sitting in the ribose pocket, and 5'-chloro group sterically interacting with the sulfur atom of Met146. Met146 is the 'gatekeeper' residue in JNK (*vs* Phe in CDK2) and is moveable allowing access to the classical hydrophobic selectivity pocket, a feature not privy to CDK2. Exploitation of this difference was used to garner selectivity against CDK2. As can be seen from Table **6**, there is a subtle interplay between the 5'-substituent (H *vs* Cl *vs* Me) and the aryl ring affecting aminopyrimidine SAR as well as CDK2 selectivity. When X=Cl, the choice of aryl group can affect JNK potency as well as CDK2 selectivity (**94**, **97**-**98**). If X=H (**99**-**101**), the choice of aryl substituent has little affect. Interestingly, keeping the 5'-substituent (Cl) and aryl ring (indole) constant and varying the amide group on the piperidine can provide CDK2 selectivity (>100-fold) without affecting JNK potency (**104**-**105**). This site of modification holds the most potential as many modifications are tolerated, and may allow one to attenuate the physicochemical properties of the molecules while maintaining potency and selectivity. Only two analogs were made wherein the aryl ring was substituted (**107**-**108**), and from the data, this is an obvious area that needs further investigation. Compound **108** is the most potent analog described and also displays excellent CDK2 selectivity.

JNK inhibitors need to be cell permeable to reach their intracellular targets and exhibit their directed function. Most inhibitors in the aminopyrimidine series outlined in Table **6** displayed modest to little activity in the cell. The most potent analogs against JNK1 (**94**, **102**, **104**, **108**) showed a 50- to 200-fold shift in potency in the cellular assay. This large shift in activity (biochemical to cell) has been noted in a number of reports [39, 40]. Most accounts suggest the higher cell ATP concentration and/or the lack of cell permeability as possible explanations for the decreased cellular activity. Further work is underway to better understand this observation.

A modestly potent phenanthroline **109** (Table **7**) inhibitor of JNK3 with a structure quite different from most previously described kinase inhibitors was found from screening the sample collection at Merck [47]. The X-ray crystal structure for this ligand complexed with JNK3 was solved at 2.1Å and revealed that the ligand was bound in a portion of the ATP binding site comprising the adenine binding pocket and hydrophobic pocket II. The inhibitor makes four hydrogen bonds: one with the linker region (main chain nitrogen of Met149 in the hinge), two with the side chain of Gln155, and one with the main chain nitrogen of Gln75. One proposed reason for the selectivity over p38 is that **109** is a small, flat, hydrophobic molecule that binds better to the smaller JNK3 ATP pocket than to the wider and more solvent-exposed p38 cavity.

The initial SAR strategy outlined by Jiang *et al*. [48] to improve activity was to maintain the core structure of **109** (a,b,c ring system) since two key hydrogen bonding interactions were identified between the two nitrogen atoms of **109** and the main chain of the JNK3 ATP binding site. Most of the attempts to modify the phenol as well as to simplify the core by removing ring b led to loss of activity (data not shown). Additionally, the phenanthroline scaffold had less than desirable physicochemical properties, so breaking up the three fused ring system was a goal. Ultimately, deconstructing ring c led to a series of inhibitors typified in Table **7** (**110**-**125**) which retained activity against JNK3 and maintained their selectivity over p38.

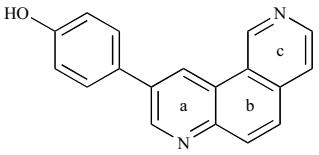
The SAR for this class of compounds revealed that a 2-pyridyl-amino group at C-5 (**110**) slightly improved potency compared to **109**. This was a significant breakthrough as this was the first non-phenanthroline ring system that maintained inhibitory activity against JNK3. Introduction of an extra nitrogen atom in the ring (**111**) is tolerable, but the 2-pyridinylamide (**112**) is not. Five-membered ring heterocycles are also allowed (**113**). The largest improvements in potency came with small meta-substituted 2-pyridylamine groups (**116**-**117**), whereas large substituents or substitution at other positions diminished activity (**114**-**115**, **118**). Conversion of the phenanthroline to a quinoline scaffold did not pick up p38 activity, as all analogs tested were essentially inactive [48].

Attempts were then made to eliminate the C-5 amino group and to attach the pyridyl ring directly to the quinoline core (**119**-**121**). The 2-pyridyl analog, **119**, could then place its nitrogen atom in the same position in space as phenanthroline **109** as observed in a 2-dimensional overlap. It was surprising that the **119** was completely inactive versus JNK3, whereas the 3- and 4-pyridyl analogs (**120**-**121**) though slightly better, were still considerably less active than the N-linked analogs **110**-**118**. At this time, it was unclear what role the NH-group played in conferring potency to analogs described [48]. Further modification of the initial sulfonamide **110** revealed that the inverse sulfonamide was 2- to 3-fold more potent (**122**) depending on whether it was a primary or secondary. Unfortunately, the 3-fold boost in potency the m-chloro group conferred in going from **110** to **116** was not noticed with the inverse sulfonamide series.

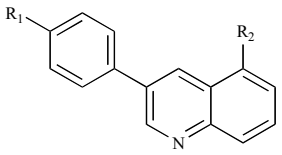
A crystal structure of **122** was pursued in an attempt to help explain its binding mode and further aid in structure based drug design [48]. Surprisingly, compound **122** bound in a completely unexpected manner, and quite differently from phenanthroline **109**. The structure revealed that the newly introduced 2-aminopyridine group binds to Met149 in the hinge region of JNK3 in a bidentate fashion, much like many other kinase inhibitors. However, this forces the rest of the molecule to wrap around the hinge region and extend into solvent exposed space. The sulfonamide group picks up additional hydrogen bonding interactions outside the active site. These compounds have a completely different binding mode from **109** and many other kinase inhibitors by not taking advantage of most of the ATP binding site, which, perhaps will lead to unanticipated selectivities against other closely related kinases. Synthesis and profiling of additional compounds is still ongoing.

The extensive SAR described in this review along with the myriad of cell- biological, peptide inhibitor, and knockout data, provide strong evidence that the JNKs are good pharmacological targets for therapeutic intervention in neu-

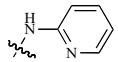
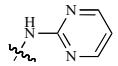
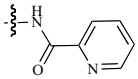
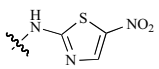
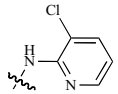
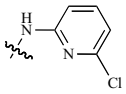
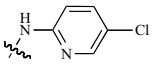
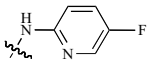
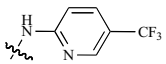
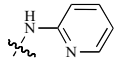
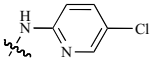
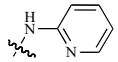
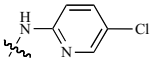
Table 7. Inhibition of JNK3 by Quinoline Derivatives 109-125 [48]



109



110-125

Cmpd	R_1	R_2	JNK3 IC_{50}, μM[a]	p38 IC_{50}, μM[a]
109	-	-	0.590	>20
110	-$NHSO_2Me$		0.44 ± 0.09	>20
111	-$NHSO_2Me$		0.48 ± 0.06	>20
112	-$NHSO_2Me$		3.6 ± 0.32	nt
113	-$NHSO_2Me$		0.76 ± 0.15	nt
114	-$NHSO_2Me$		15 ± 0.17	nt
115	-$NHSO_2Me$		6.5 ± 0.53	nt
116	-$NHSO_2Me$		0.12 ± 0.02	>20
117	-$NHSO_2Me$		0.14 ± 0.02	nt
118	-$NHSO_2Me$		0.49 ± 0.06	nt
119	-$NHSO_2Me$		>20	nt
120	-$NHSO_2Me$		5.2 ± 0.39	>20
121	-$NHSO_2Me$		5.0 ± 0.37	>20
122	-SO_2NHt-Bu		0.15 ± 0.02	>20
123	-SO_2NHt-Bu		0.25 ± 0.03	>20
124	-SO_2NH_2		0.20 ± 0.02	nt
125	-SO_2NH_2		0.24 ± 0.01	nt

[a]Values are means of three experiments, (nt = not tested).

rodegenerative disease, metabolic disorders, cardiovascular disease, and cancer. Moreover, given the early success of the medicinal chemistry efforts reported herein, it seems highly likely that potent, selective JNK inhibitors with good physiochemical, brain penetration, and pharmacokinetic properties will be developed that demonstrate efficacy in various animal models and will be good candidates for clinical evaluation.

ACKNOWLEDGEMENTS

We are grateful to Yamille Del Rosario for editorial assistance in preparation of this manuscript.

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Received: 19 December, 2007 Revised: 11 February, 2008 Accepted: 13 February, 2008