DDT Vol. 9, No. 21 November 2004

Targeting JNK3 for the treatment of neurodegenerative disorders

Lynn Resnick and Myles Fennell

c-Jun N-terminal kinases (JNKs) have been recognized as important enzymes in cellular function. JNK3, which is predominantly found in CNS neurons, has been implicated in several neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease and stroke. In particular, JNK3 has been found to have an upstream role in neuronal ischemic apoptosis. JNK3 is highly expressed and activated in postmortem brains of individuals that suffered from Alzheimer's disease. Furthermore, mice that are deficient in JNK3 are more resistant to 1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine (a neurotoxin that mimics the neuropathological characteristics of Parkinson's disease) than their wild-type littermates. Because of the involvement of JNK3 in neuronal diseases, the inhibition of this enzyme is an attractive therapeutic target.

Lynn Resnick*
Myles Fennell
Wyeth Research
CN 8000
Princeton
NJ 08543 8000, USA
*e-mail:resnicl@wyeth.com

c-Jun N-terminal kinase (JNK) is a member of the family of serine and threonine mitogen-activated protein kinases (MAPK) and is involved with numerous physiological processes, including tissue differentiation and pathogenesis. Three genes encoding for JNKs have been identified in humans (*JNK1*, *JNK2* and *JNK3*) and ten isoforms resulting from the alternative splicing of these genes have been characterized. Whereas JNK1 and JNK2 have a broad tissue distribution, JNK3 is primarily localized in CNS neurons, making it an attractive CNS drug target; JNK3 is also found at low levels in heart and testis. Upstream activator kinases, such as mitogen-activated protein kinase kinase 4 (MKK4) and MKK7, catalyze the bis-phosphorylation of Thr and Tyr residues in the activation loop of JNKs, and it is this modification that activates the JNK family of kinases. Activator kinases are themselves activated by MAP3K kinases such as mixed lineage kinase (MLK) or apoptosis signal-regulating kinase 1 (ASK1) (Figure 1). Maximum activation of JNK requires the phosphorylation of both Thr and Tyr residues in the activation loop. Whereas phosphorylation of Thr by MKK7 results in significant activation of JNK3, phosphorylation of Thr by MKK4 alone triggers only low levels of JNK3 activation; however, MKK4 does increase the activity of JNK that has already undergone Thr phosphorylation [1]. Activation of JNK results in the phosphorylation of multiple targets, including transcription factors that belong to the activator protein-1 (AP-1) class, for example, c-Jun, anti-activating transcription factor-2 (ATF-2), JunB and JunD [2]. Protein-scaffold proteins and other protein–protein interactions are crucial to the function and regulation of JNK in the cell. Examples of these scaffold proteins are JNK-interacting proteins (JIP) and β-arrestins [3] (Figure 1).

JNK3 activation is thought to have a key role in triggering apoptosis and pathogenesis in several human neurodegenerative disorders. In the adult brain, JNK3 expression is primarily localized to pyramidal neurons in the CA1, CA4 and subiculum regions of the hippocampus, and to layers three and four of the neocortex [4]; these areas are affected by neurodegenerative disorders such as Alzheimer's disease (AD). Because of their potential therapeutic utility, small molecule inhibitors of JNK3 are currently being pursued by several companies for CNS indications such as AD, Parkinson's disease (PD) and stroke.

One of the major issues facing the development of JNK inhibitors for the treatment of human disease is isoform selectivity. Transgenic knockouts of JNK isoforms have provided crucial insights into the role played by each isoform. JNK1 and JNK2 have been shown to have important roles in the modulation of immune cell function, with JNK1 and JNK2 mediating alternative T-cell lineages [5,6]. Although it is unclear how chronic inhibition of JNK1 and JNK2 will affect the immune system in humans, there is clearly a risk of increased susceptibility to infection or cellular hyperproliferation. It has also been shown that JNK1 and JNK2

PII: S1359-6446(04)03251-9

are important in the development of the embryonic nervous system. Although single knockouts of JNK1, JNK2 or JNK3 show no early structural abnormalities, a double knockout of JNK1 and JNK2 leads to a severe dysregulation of apoptosis in the brain and is embryonically lethal [7]. Additionally, a study using *Jnk1* knockout mice demonstrated that JNK1 has an important regulatory and maintenance function in the CNS [8]; *Jnk1* knockout mice exhibit a progressive loss of microtubules in their axons and dendrites, which leads to progressive neuronal degeneration [8]. However, *Jnk2* knockout mice do not exhibit loss of microtubules.

The high systemic expression levels of JNK1 and JNK2 and their key regulatory roles make the *in vivo* chronic inhibition of these enzymes potentially detrimental. Therefore, the development of a selective JNK3 inhibitor is preferable for the treatment of neurodegenerative disorders. Furthermore, an inhibitor that is selective for JNK2 and JNK3 over JNK1 might also be tolerated and result in a more favorable side-effect profile than a pan-JNK inhibitor because the important microtubule-stabilizing role of JNK1 would be maintained [8].

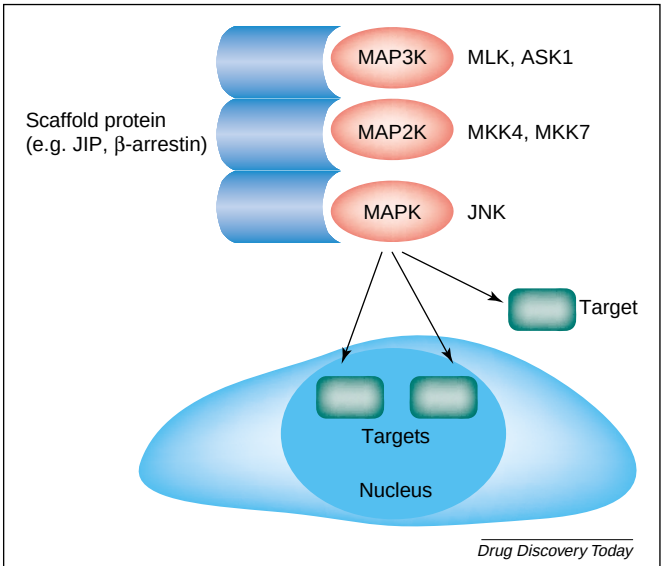

Figure 1. Mechanism of c-Jun N-terminal kinase activation by upstream kinases. The MAPK, JNK, is activated by a series of activator kinases belonging to the MAP2K and MAP3K families. This activation and subsequent phosphorylation of target protein is enhanced by scaffold proteins. Abbreviations: ASK1, apoptosis signal-regulating kinase 1; JIP, JNK-interacting proteins; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinases; MKK, mitogen-activated protein kinase kinase; MLK, mixed lineage kinase.

Alzheimer's disease and c-Jun N-terminal kinase

AD is the most common form of dementia in the elderly and is characterized by progressive neurodegeneration. Two hallmark lesions characterize the disease: (i) diffuse plaques, of which the main component is β-amyloid peptide (Aβ); and (ii) neurofibrillary tangles, which are comprised of aggregated, hyper-phosphorylated Tau protein [9]. Neurons derived (*ex vivo*) from mice lacking JNK3 are resistant to Aβ-induced apoptosis when compared with neurons from normal mice. The activation of JNK3, which is induced by Aβ, activates c-Jun and leads to Fas-ligand expression; it is proposed that this cascade leads to Aβ-induced apoptosis [10]. In human postmortem brains from AD patients, JNK phosphorylation is markedly increased and there is a pronounced redistribution of JNK compared with age-matched controls. JNK2 and JNK3 are associated with neurofibrillary pathology, and JNK upregulation has a complete overlap with phosphorylated Tau [11], a protein that is phosphorylated by JNK *in vitro* [12]. Additionally, aberrant phosphorylation of Tau by JNK3 induces the formation of oligomeric Tau fibrils in Cos-7 cells [13].

Amyloid precursor protein (APP) is a membrane-bound peptide that has been implicated in the pathology of AD; APP functions as a cargo receptor for fast axonal transport. The sequential cleavage of APP by β- and γ-secretases results in the production of toxic forms of Aβ (Aβ40 and Aβ42), which form amyloid plaques in the brain [14]. APP is an excellent substrate for JNK3, and phosphorylation of this peptide is thought to be one of the factors mediating its cleavage by secretases [15]. Therefore, it is possible that JNK3 is involved in the breakdown of synaptic transmission and the production of the toxic amyloid peptides observed in AD.

A mouse model of AD that incorporates the Swedish APP mutation and a mutant presenilin-1 (PS1) – Tg2576/PS1 – has provided crucial information about the link between JNK and AD [15]. Research using this model has demonstrated that JNK activation is associated with amyloid deposits and phospho-Tau staining. Increased activation and phosphorylation of JNK in the brain was observed in a time-dependent manner after three months of age, with a greater than fivefold increase in JNK activity by 12 months, which correlates with increased amyloid deposition. Although this mouse model of AD does not lead to a loss of neurons as a result of amyloid deposition, as would be expected in human AD, there is a significant decrease in synaptophysin levels, indicating a loss of functional synapses [15].

Parkinson's disease and c-Jun N-terminal kinase

PD is a neurodegenerative disease that is characterized by the progressive loss of dopamine-containing neurons in the midbrain. An important tool in the investigation of the pathogenesis of PD is the neurotoxin 1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine (MPTP), which induces a

neuropathology in animals that resembles the pathological features of PD in humans, primates and other mammalian species, including mice [16]. In mice treated with MPTP, and in human postmortem brains from PD patients, an increased activation of the downstream target of JNK, c-Jun, is detected [17]. Using gene knockout studies, it has been shown that mice deficient in JNK2 and JNK3 are more resistant to MPTP injury than their wild-type littermates, whereas JNK1 knockout mice are as susceptible to MPTP as control animals. A double gene deletion of *Jnk2* and *Jnk3* produced an even more pronounced resistance to MPTP injury than the knockout of *Jnk2* or *Jnk3* alone [17]. This study also demonstrated that expression of the proinflammatory gene *Cox2* resulted from MPTP injury. This *Cox2* induction is a direct result of *Jnk2* and *Jnk3* activity and can be decreased in single knockouts, or completely blocked in double knockouts of *Jnk2* and *Jnk3* [17].

Stroke and c-Jun N-terminal kinase

Stroke is currently the third largest cause of death in the USA and there is a significant unmet clinical need for effective treatments. The only approved therapy is tissue plasminogen activator (tPA), which is only effective if administered within 3 h of the initiation of the ischemic episode. Future neuroprotective treatments are needed, and JNK3 is a considerably attractive potential target. Neuronal death in cerebral ischemia is predominantly caused by excitotoxic mechanisms. JNK is activated in ischemic neurons downstream of glutamate-receptor activation, and thus mediates subsequent cell death [18]. Disruption of the gene that encodes JNK3 in mice causes them to be resistant to the excitotoxic glutamate-receptor agonist kainic acid [19]. These JNK*3* knockout mice show a reduction in seizure activity and hippocampal neuronal apoptosis associated with this injury was prevented. Phosphorylation of c-Jun and the transcriptional activity of AP-1 were markedly reduced in the JNK3 knockout mice. This indicates that the observed neuroprotection is caused by the blockade of a JNK3-mediated signaling pathway [19]. The targeted deletion of JNK3 also protects mice from brain injury after cerebral ischemia–hypoxia [20]. In hypoxic injury, the majority of the injury appears to be mediated by JNK3, with JNK2 only having a minor role, because the JNK3 knockout completely eliminates the population of JNK that is activated by ischemia–hypoxia [20].

Physiological regulators

The formation of stable complexes between JNK and upstream kinases (MAP2K and MAP3K) is thought to contribute to signal transmission and signal specificity. The N-terminal extension of MKK4, or the D-site, is a conserved sequence among MKKs that is essential for the binding of JNK; these D-sites are present on other JNK substrates, such as c-Jun [21]. As well as proteins that directly activate JNK, there are several scaffold proteins that allosterically modulate JNK activation. The first of these scaffold proteins to be identified was JIP-1 [22], which interacts with members of the JNK family and their upstream activators MKK7 and MLK [23], but not with p38 or extracellular-signal-related kinase (ERK). Subsequently, other JIPs have been identified with high-homology to JIP-1 (JIP-2 and JIP-3). The heat shock protein Hsp72, which also binds JNK and has cytoprotective properties, has been reported to be a negative regulator of JNK [24]. β-Arrestin-2 selectively binds to and activates JNK3, in addition to forming a scaffold for the JNK3-activating kinases MKK4 and ASK1 [25,26]. G-protein-coupled receptor (GPCR) activation by numerous ligands leads to the recruitment of arrestin molecules, resulting in receptor internalization and downregulation of GPCR signaling [27]. Thus, it appears that ligand-induced GPCR-signaling system is a physiological mechanism for JNK3 activation and could be a potential target for mechanism-specific and tissue-specific inhibition of JNK3.

Peptide inhibitors

The interaction between JNK and JIP-1, and JNK and other scaffold proteins, is highly selective and could therefore regulate the target specificity of JNK isoforms [28] and some of the tissue-specific isoform responses, making proteins like JIP-1 potential templates for the design of isoform-selective JNK inhibitors.

Overexpression of the JNK-binding domain of JIP-1 blocks activation of the JNK pathway and cell death in several physiological models. A sequence of 21 amino acids in the domain of JIP-1 that interacts with JNK was characterized as a peptide inhibitor, and subsequently an 11-amino acid truncated version of this peptide that also has peptide inhibitory activity was identified [29]. In the absence of JIP-1, this 11-amino acid inhibitor blocks the phosphorylation of the JNK substrates c-Jun and ATF-2 *in vitro*. A cell-permeable peptide inhibitor with a sequence based on the 21-amino acid sequence of the peptide from the JIP-1 domain potently protects against cerebral ischemic injury in rodent models [30]. This peptide inhibitor has an unusually long therapeutic window of between 6 and 12 h in a rotarod test and affects lesion size and functional outcome [30].

Recently, the crystal structure of JNK1 complexed with the 11-amino acid peptide inhibitor from JIP-1 was published [31]. This study demonstrated the binding interactions and the mode of allosteric inhibition of this peptide. The bound peptide induces a rotation between the N- and C-terminal lobes of JNK1, which results in a change in the

Table 1. Properties of examples of c-Jun N-terminal kinase inhibitors

Chemical class	Examples of compounds[a]	IC_{50} values for JNK3	Selectivity	*In vivo* activity	Refs
2-amino-4-imidazole pyrimidines	1	1–7 nM	No selectivity over JNK1 and JNK2. Up to 100-fold increased activity for p38	Active in animal models for neurodegeneration. X-ray co-crystal structure with JNK3	[33,36]
2-aminopyrimidinyls and aminopyridinyls	2,4 and 5	<500 nM	Moderate selectivity over JNK1 and JNK2 and comparable activity in p38, Lck and Src	NA	[41–52]
2-aminopyrimidinyls	3	NA	Moderate selectivity over JNK1and JNK2. Selective over p38, but not Src or Lck	NA	[42]
Pyrimidinyl acetonitriles	6	70 nM	No selectivity over JNK1 and JNK2. Selective over a range of kinases, including p38, ERK1 and Src	Demonstrated neuroprotection in *in vivo* models of stroke	[37–40]
Anthrapyrazoles	7	190 nM	No selectivity over JNK1 and JNK2, but exhibits selectivity over other kinases, including p38	Some effect in animal models of Parkinson's disease. X-ray co-crystal structure with JNK3	[53–57]
Thiophenyl and benzyl sulfonamides	8	150 nM	No selectivity over JNK1 and JNK2. Inactive against p38 and ERK	Active in neuronal survival assays and in *in vivo* stroke models	[58–65]
7-azaindoles, indazoles, isoindolones and indole-2,3-dione-3-oximes	9,10,11 and 12	≤500 nM	Selectivity not reported	NA	[66–78]

[a]Structures of compounds are given in Figure 2.
Abbreviations: ERK, extracellular-signal-related kinase; JNK, c-Jun N-terminal kinase; Lck, lymphocyte-specific protein tyrosine kinase p56; NA, not available.

conformation of the active site that leads to a reduction in the binding affinity of ATP.

Small molecule inhibitors

The structure homology among kinases, and particularly among the JNK isoforms, makes the development of a selective JNK3 inhibitor challenging. Most small molecule kinase inhibitors operate by competitive interaction at the ATP-binding site, a region that is structurally similar in all kinases. Nevertheless, among different kinases, minor residue differences in and near the ATP-binding pocket enable a degree of selectivity [32]. X-ray crystallography, in conjunction with homology modeling, enabled the characterization of the differences within the vicinity of the ATP-binding site between JNK1, JNK2 and JNK3 as conservative amino-acid substitutions that do not result in major structural variations [33,34]. In addition, small variations exist in the substrate-binding site [35].

Small molecule inhibitors of JNK have been pursued by several research laboratories for a variety of therapeutic indications. At least 40 structurally distinct chemical scaffolds have been described as JNK inhibitors in the patent literature. Most of these inhibitors are either not isoform selective or show modest selectivity (up to ~tenfold) for the different JNK isoforms, and many of these molecules inhibit other kinases besides JNK.

A common structural feature among small molecule JNK inhibitors is the 2-aminopyrimidine moiety, as exemplified by compounds **1**–**6** (Figure 2, Table 1). In this class of inhibitors, the amine group of the 2-aminopyrimidine core is usually substituted with a hydrophobic group (compounds **1**–**5**), whereas the 4-position of the pyrimidine incorporates an acetonitrile functionality (**6**) or a five-membered heterocycle that is fused (**4**,**5**) or unfused (**1**–**3**). In compounds **1**, **2**, **4** and **5**, the substituent on the five-membered ring adjacent to the pyrimidine is a halogen-substituted

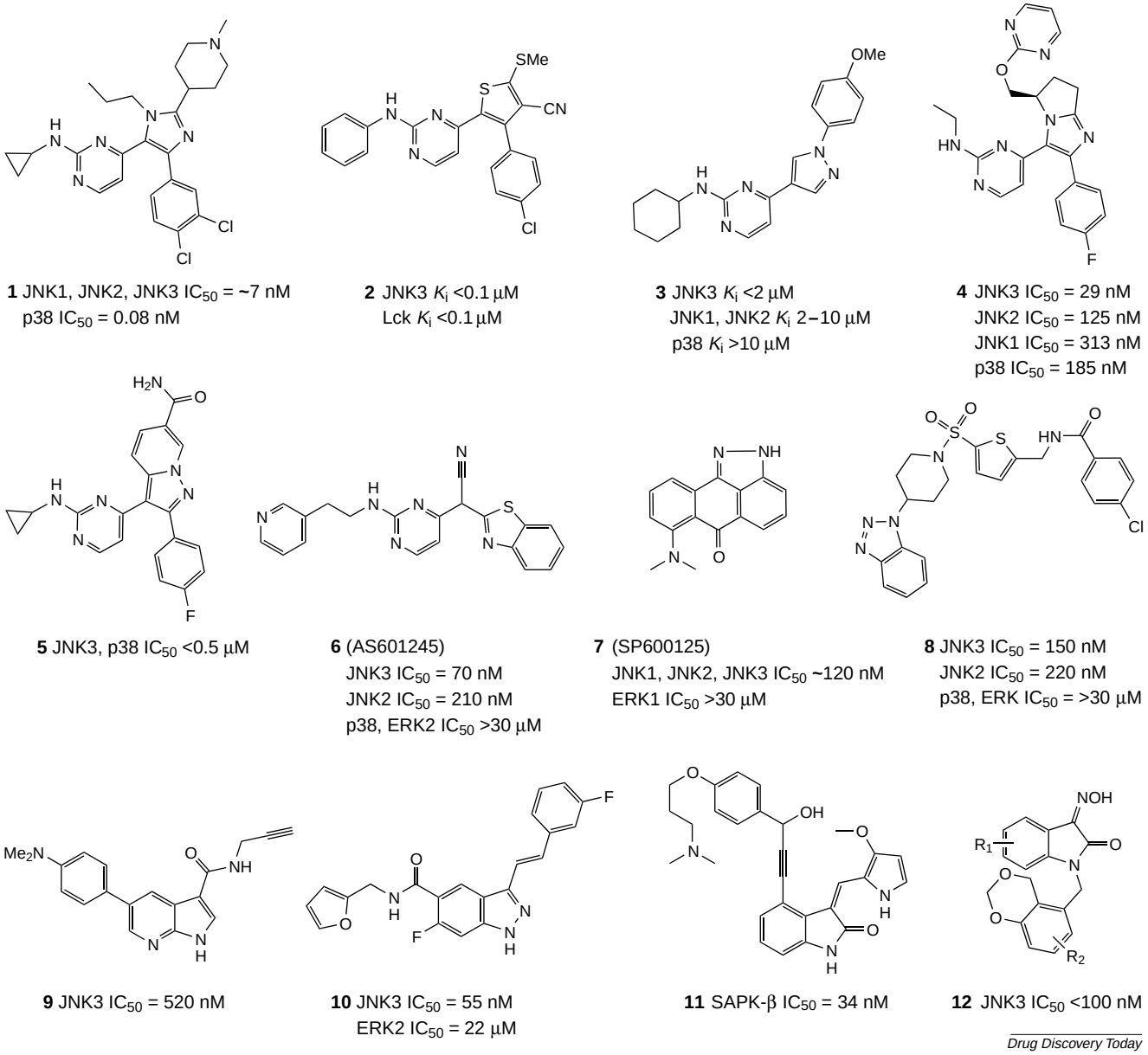

Figure 2. Examples of c-Jun N-terminal kinase inhibitors with enzyme activity data. Abbreviations: ERK, extracellular-signal-related kinase; JNK, c-Jun N-terminal kinase; Lck, including lymphocyte-specific protein tyrosine kinase p56; SAPK-β, stress-activated protein kinase-β.

phenyl. This substituted phenyl ring could influence selectivity. Compound **3** lacks this phenyl ring and exhibits higher selectivity for JNK over the closely related MAPK, p38. The considerable diversity among the other substitutions on the five-membered rings accounts for the differences in activities between the inhibitors and could be responsible for the selectivity observed among the JNK isoforms (e.g. compounds **3** and **4**, Figure 2). Crystal structures of JNK3 in complex with two analogs from the 2-aminopyrimidine class, including compound **1**, have been reported [33]. Compound **1** (Figure 2) has an IC_{50} value of 7.1 nM against JNK3, and similar activities against JNK1 and JNK2; **1** is also 100-fold more active against p38. The X-ray crystal structure reveals that this inhibitor binds in the ATP-binding pocket of the enzyme and hydrogen-bonding interactions are made between the 2-aminopyrimidine moiety and main chain nitrogen and oxygen atoms of Met149 in the adenine-binding region (Figure 3). Because of the structural similarities between 2-aminopyrimidine JNK inhibitors **1–6**, it can be postulated that each of these compounds bind in the JNK active site in an analogous orientation.

Compound **1** and similar analogs [36] protect against neurodegeneration, as determined by assays where the compounds promoted neuronal survival of dopaminergic neurons exposed to 1-methyl-4-phenylpyridium ion, the neurotoxic agent derived from MPTP. These compounds also promoted the survival of sympathetic neurons deprived of nerve growth factor [33]. Unfortunately, the poor kinase-selectivity profile exhibited by the compounds renders them unlikely therapeutic agents.

AS601245 [2-aminopyrimidine (**6**)] exhibits 10–100-fold selectivity for JNK over a range of kinases, including p38, ERK1 and Src [37]. This compound is representative of a series of JNK inhibitors discovered by researchers from Applied Research Systems that incorporates a benzothiazole [38,39] or thiazole [40] and acetonitrile moieties on the pyrimidine core. AS601245 inhibits JNK1, JNK2 and JNK3 in an ATP-competitive manner with IC_{50} values of 150, 220 and 70 nM, respectively. The compound was neuroprotective in models of focal cerebral ischemia in rat and global ischemia in gerbil [37].

Compounds **2** and **3** are among the many 2-aminopyrimidinyl or 2-aminopyridinyl JNK inhibitors discovered by Vertex (http://www.vrtx.com) [41–48]. Most of the compounds described in the patents filed by Vertex were shown to inhibit other protein kinases in addition to JNK, including lymphocyte-specific protein tyrosine kinase p56 (Lck) and Src. For example, compound **2** (Figure 2) inhibits JNK3 and Lck with K_i values of <0.1 μM [41], whereas the pyrazole **3** inhibits JNK3 (K_i <2 μM), JNK1 and JNK2 (K_i of 2–10 μM) and Src and Lck (values not disclosed). Compound **3** was shown to be selective for JNK1, JNK2 and JNK3 over p38, with a K_i of >10 μM. [42].

Compound **4**, a dihydropyrrolo-imidazole discovered at Eisai (http://www.eisai.com), has K_i values of 29 nM, 125 nM and 313 nM for JNK3, JNK2 and JNK1, respectively, and is one of the few JNK inhibitors that display greater than tenfold selectivity for JNK3 over JNK1 [49]. Such a selectivity profile would make this compound particularly interesting for the treatment of neurodegenerative disorders. However, compound **4** also inhibits p38 with a K_i of 185 nM.

Compound **5** inhibits JNK3 and p38 with IC_{50} values of <0.5 μM; activity against other JNK isoforms was not disclosed [50]. Other 2-aminopyrimidine JNK inhibitors have been synthesized by Takeda (http://www.takeda.co.jp) (these compounds comprise thiazoles or imidazoles at the C-4 position [51]) and by Signal (http://www.signalpharm.com) [52].

Some of the first identified JNK inhibitors are the anthrapyrazolones and related structures that are manufactured by Celgene (http://www.celgene.com) [53,54]. One compound, SP600125 (**7**), was found to inhibit JNK1, JNK2 and JNK3 with K_i values of ~190 nM [55]. SP600125 was initially reported to be selective for JNK over several other kinases, including p38. The recent testing of SP600125 against a panel of 28 protein kinases demonstrated that this compound inhibited 13 of the 28 protein kinases tested (including p38) with a similar or greater potency than JNK [56]. This study used a ~tenfold higher concentration of ATP compared with the initial study (100 μM versus 11 μM), which explains the discrepancy observed in the results produced from the two studies: SP600125 is an ATP-competitive inhibitor. SP600125 was tested in the MPTP model of PD and partially restored dopamine levels [57]. However, this result cannot necessarily be attributed solely to inhibition of JNK.

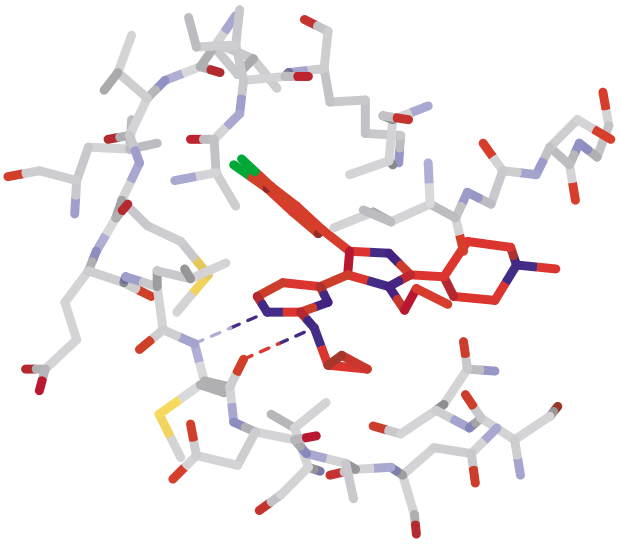

Figure 3. A c-Jun N-terminal kinase inhibitor in complex with the ATP binding site of c-Jun N-terminal kinase 3. The inhibitor in complex with c-Jun N-terminal kinase 3 is compound **1** (Figure 2). Hydrogen-bonding interactions between the ligand and Met149 are shown as broken lines.

Scapin *et al.* [33] reported the co-crystal structure of SP600125 with JNK3. This inhibitor forms two hydrogen-bonding interactions in the linker region of the enzyme and hydrophobic interactions in the upper portion of the adenine-binding pocket. The selectivity for JNK over p38 could arise because the p38-binding pocket is larger and, as such, this inhibitor is too far from the hydrophobic residues to form Van der Waals contacts that are equivalent strength to those formed by JNK. An X-ray crystal structure of a ternary complex of JNK1 with SP600125 and the allosteric 11-amino acid peptide originating from JIP-1 (pepJIP-1) has been reported [31]. Because this ternary complex could closely resemble the state of JNK *in vivo*, this complex could be used as a preferred model for the design of JNK inhibitors.

A novel class of JNK inhibitors that is exemplified by compound **8** contains either a benzene sulfonamide or, more typically, a thiophene sulfonamide [58–65]. Analogs from this series were reported to rescue neurons from cell death in a sympathetic neuron culture model. Furthermore, some compounds from this series have been reported to protect cells from neuronal apoptosis during induced global ischemia in gerbils [58–65]. Many of the sulfonamide analogs reported in the patents filed by Applied Research Systems are selective for JNK over p38 and ERK. Although compound **8** (Figure 2) inhibits JNK3 and JNK2 with IC_{50} values of 150 nM and 220 nM, respectively, it is inactive against p38 and ERK (IC_{50} >30 μM) [58]. Within this series, greater structural diversity seems to be tolerated in the substituents that are attached to the sulfonamide portion of the thiophene core. However, this is not the case with substitution in the 5-position of the thiophene core; this position is generally occupied by a 4-chloro-benzamido-methyl group. In many cases, the sulfonamide attachment comprises a basic nitrogen.

Several other series of JNK inhibitors have been reported in the patent literature. Researchers from Eisai disclosed a series of 7-azaindoles that is exemplified by compound **9**; this compound was reported to inhibit JNK3 with an IC_{50} value of 520 nM [66,67]. Eisai has also described a series of indazoles, some of which have IC_{50} values against JNK3 in the range 50–100 nM [68,69]. An example of this series is compound **10**, which inhibits JNK3 with an IC_{50} value of 55 nM and is ~1000-fold selective for JNK3 over ERK. Additional indazole JNK inhibitors have been described in the patent literature [70–74]. Hoffman-La Roche (http://www.roche.com) has reported three novel isoindolone scaffolds that demonstrate activity as inhibitors of JNK. The first two series have aryl [75] or alkynyl [76] C-4 substitutions, respectively, and the third series incorporates a fused pyrazine functionality [77]. Alkynyl analog **11**, which is representative of the reported classes, inhibits rat stress-activated protein kinase-β, an enzyme with high homology to human JNK, with an IC_{50} value of 34 nM. Indole-2,3-dione-3-oximes that act as inhibitors of JNK have been reported by Vertex. Many of the disclosed compounds inhibit the JNKs with K_i values of <1 μM (e.g. compound **12**, Figure 2) [78].

Summary

JNK3 is involved in key mechanisms that effect neurological diseases including PD, AD and stroke. Inhibition of JNK3 is a potential route to treating these diseases. Non-selective JNK3 inhibitors could lead to drugs with adverse side effects, including a compromised cellular immune response and cellular hyperproliferation. Small molecule inhibitors of JNK have been pursued by several research laboratories with the hope of finding safe and effective drugs for a variety of indications. Recent advances demonstrate that it could be possible to identify molecular scaffolds that selectively inhibit JNK3 over other JNK isoforms and protein kinases. However, the ongoing challenge in the development of clinically useful JNK3 inhibitors will depend heavily on widening that margin of selectivity.

Acknowledgements

We thank Alan Katz for creating the image of the JNK inhibitor in complex with the ATP-binding site and for providing insight into the structural differences between the JNK isoforms. We also thank John Butera and John Dunlop for their thoroughness in reviewing the manuscript.

References

1 Lisnock, J. *et al.* (2000) Activation of JNK3α1 requires both MKK4 and MKK7: kinetic characterization of *in vitro* phosphorylated JNK3α1. *Biochemistry* 39, 3141–3148
2 Ip, Y.T. and Davis, R.J. (1998) Signal transduction by the c-Jun N-terminal kinase (JNK)-from inflammation to development. *Curr. Opin. Cell Biol.* 10, 205–219
3 Morrison, D.K. and Davis, R.J. (2003) Regulation of MAP kinase signaling modules by scaffold proteins in mammals. *Annu. Rev. Cell Dev. Biol.* 19, 91–118
4 Mohit, A.A. *et al.* (1995) P493F12 Kinase: a novel MAP kinase expressed in a subset of neurons in the human nervous system. *Neuron* 14, 67–78
5 Dong, C. *et al.* (1998) Defective T cell differentiation in the absence of JNK1. *Science* 282, 2092–2095
6 Yang, D. *et al.* (1998) Differentiation of CD4+ T cells to Th1 cells requires MAP kinase JNK2. *Immunity* 9, 575–585
7 Kuan, C.Y. *et al.* (1999) The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron* 22, 667–676
8 Chang, L. *et al.* (2003) JNK1 is required for maintenance of neuronal microtubules and controls phosphorylation of microtubule-associated proteins. *Dev. Cell* 4, 521–533
9 Selkoe, D.J. (2001) Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.* 81, 741–766
10 Morishima, Y. *et al.* (2001) β-Amyloid induces neuronal apoptosis via a mechanism that involves the c-Jun N-terminal kinase pathway and the induction of Fas ligand. *J. Neurosci.* 21, 7551–7560
11 Zhu, X. *et al.* (2001) Activation and redistribution of c-Jun N-terminal kinase/stress activated protein kinase in degenerating neurons in Alzheimer's disease. *J. Neurochem.* 76, 435–441
12 Reynolds, C.H. *et al.* (1997) Stress activated protein kinase/c-Jun N-terminal kinase phosphorylates τ protein. *J. Neurochem.* 68, 1736–1744
13 Sato, S. *et al.* (2002) Aberrant tau phosphorylation by glycogen synthase kinase-3β and JNK3 induces oligomeric tau fibrils in COS-7 cells. *J. Biol. Chem.* 277, 42060–42065
14 Citron, M. (2004) β-Secretase inhibition for the treatment of Alzheimer's disease – promise and challenge. *Trends Pharmacol. Sci.* 25, 92–97
15 Savage, M.J. *et al.* (2002) Activation of c-Jun N-terminal kinase and p38 in an Alzheimer's disease model is associated with amyloid deposition. *J. Neurosci.* 22, 3376–3385
16 Van Dam, H. *et al.* (1995) ATF-2 is preferentially activated by stress-activated protein kinases to mediate c-Jun induction in response to genotoxic agents. *EMBO J.* 14, 1798–1811
17 Hunot, S. *et al.* (2004) JNK-mediated induction of cyclooxygenase 2 is required for neurodegeneration in a mouse model of Parkinson's disease. *Proc. Natl. Acad. Sci. U. S. A.* 101, 665–670
18 Wu, D.C. *et al.* (2000) Activation of mitogen-activated protein kinases after permanent cerebral artery occlusion in mouse brain. *J. Cereb. Blood*

Flow Metab. 20, 1320–1330
19 Yang, D.D. *et al*. (1997) Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. *Nature* 389, 865–870
20 Kuan, C-Y. *et al*. (2003) A critical role of neural-specific JNK3 for ischemic apoptosis. *Proc. Natl. Acad. Sci. U. S. A*. 100, 15184–15189
21 Ho, D.T. *et al*. (2003) A docking site in MKK4 mediates high affinity binding to JNK MAPKs and competes with similar docking sites in JNK substrates. *J. Biol. Chem*. 278, 32662–32672
22 Dickens, M. *et al*. (1997) A cytoplasmic inhibitor of the JNK signal transduction pathway. *Science* 277, 693–696
23 Whitmarsh, A.J. *et al*. (1998) A mammalian scaffold complex that selectively mediates MAP kinase activation. *Science* 281, 1671–1674
24 Park, H.S. *et al*. (2001) Hsp72 functions as a natural inhibitory protein of c-Jun N-terminal kinase. *EMBO J*. 20, 446–456
25 McDonald, P.H. *et al*. (2000) Beta-arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK3. *Science* 290, 1574–1577
26 Miller, W.E. *et al*. (2001) Identification of a motif in the carboxyl terminus of beta-arrestin 2 responsible for activation of JNK3. *J. Biol. Chem*. 276, 27770–27777
27 Miller, W.E. and Lefkowitz, R.J. (2001) Expanding roles for beta-arrestins as scaffolds and adapters in GPCR signaling and trafficking. *Curr. Opin. Cell Biol*. 13, 139–145
28 Gupta, S. *et al*. (1996) Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J*. 15, 2760–2770
29 Barr, R.K. *et al*. (2002) Identification of the critical features of a small peptide inhibitor of JNK activity. *J. Biol. Chem*. 277, 10987–10997
30 Borsello, T. *et al*. (2003) A peptide inhibitor of c-Jun N-terminal kinase protects against excitotoxicity and cerebral ischemia. *Nat. Med*. 9, 1180–1186
31 Heo, Y.S. *et al*. (2004) Structural basis for the selective inhibition of JNK1 by scaffolding protein JIP1 and SP600125. *EMBO J*. 23, 2185–2195
32 Scapin, G. (2002) Structural biology in drug design: selective protein kinase inhibitors. *Drug Discov. Today* 7, 601–611
33 Scapin, G. *et al*. (2003) The structure of JNK3 in complex with small molecule inhibitors: structural basis for potency and selectivity. *Chem. Biol*. 10, 705–712
34 Xie, X. *et al*. (1999) Vertex Pharmaceuticals. Crystallizable JNK complexes. WO 9957253 A2
35 Kallunki, T. *et al*. (1994) JNK2 contains a specificity-determining region responsible for efficient c-Jun binding and phosphorylation. *Genes Dev*. 8, 2996–3007
36 Lograsso, P. *et al*. (2001) Merck. Use of (di-substituted-phenyl)-pyrimidinyl-imidazole derivatives as JNK-inhibitors. WO 2001091749 A1
37 Carboni, S. *et al*. (2004) AS601245: A JNK inhibitor with neuroprotective properties. *J. Pharmacol. Exp. Ther*. 310, 25–32
38 Halazy, S. *et al*. (2001) Applied Research Systems. Benzazole derivatives and their use as JNK modulators. EP 1110957 A1
39 Gaillard, P. *et al*. (2003) Applied Research Systems. Piperazine benzothiazoles as agents for the treatment of cerebral ischemic disorders or CNS disorders. WO 03091249 A1
40 Gaillard, P. *et al*. (2003) Applied Research Systems. Azole methylidene cyanide derivatives and their use as protein kinase modulators. WO 2003106455
41 Cao, J. *et al*. (2002) Vertex Pharmaceuticals. Preparation of thienyl-substituted pyrimidinyl, pyridinyl and triazinyl amines as inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases. WO 2002083667
42 Ledeboer, M. *et al*. (2002) Vertex Pharmaceuticals. Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases. WO 0246184 A1
43 Harrington, E. (2003) Vertex Pharmaceuticals. Preparation of isoxazole derivatives as inhibitors of Src and other protein kinases. US 2003207873 A1
44 Moon, Y-C. (2002) Vertex Pharmaceuticals. Preparation of pyrazolyl pyridinamines and pyrimidinamines as inhibitors of Src and other protein kinases. WO 2002092573 A2
45 Harrington, E. (2002) Vertex Pharmaceuticals. Preparation of isoxazole derivatives as inhibitors of Src and other protein kinases. WO 2002083668
46 Ledeboer, M. *et al*. (2004) Vertex Pharmaceuticals. Imidazoles, oxazoles and thiazoles with protein kinase inhibiting activities. WO 2004005283 A1
47 Green, J. *et al*. (2001) Vertex Pharmaceuticals. Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases. WO 0112621 A1
48 Bethiel, R. *et al*. (2002) Vertex Pharmaceuticals. Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases. WO 2002079197 A1
49 Graczyk, P. *et al*. (2002) Eisai. Jun kinase inhibitors. WO 2002081475 A1
50 Alberti, M. J. *et al*. (2002) Glaxo Group. Preparation of pyrazolo[1,5-a]pyridines as protein kinase inhibitors for treatment of inflammatory and autoimmune diseases. WO 2002016359 A1
51 Ohkawa, S. *et al*. (2002) Takeda Chemical Industries. Preparation of azoles as JNK inhibitors. WO 2002062792 A1
52 Kois, A. *et al*. (2002) Signal Pharmaceuticals. Anilinopyrimidine derivatives as JNK pathway inhibitors and compositions and methods related thereto. WO 0246170 A2
53 Bennett, B.L. *et al*. (2001) Signal Pharmaceuticals. Pyrazoloanthrone and derivatives thereof as JNK inhibitors and their compositions. WO 0112609 A1
54 Sakata, S.T. and Raymon, H.K. (2002) Signal Pharmaceuticals. Anthrone derivatives and their use as JNK inhibitors. WO 2002066450 A2
55 Bennett, B. *et al*. (2001) SP600125, an anthrapyrazole inhibitor of Jun N-terminal kinase. *Proc. Natl. Acad. Sci. U. S. A*. 98, 13681–13686
56 Bain, J. *et al*. (2003) The specificities of protein kinase inhibitors: an update. *Biochem. J*. 371, 199–204
57 Wang, W. *et al*. (2004) SP600125, a new JNK inhibitor, protects dopaminergic neurons in the MPTP model of Parkinson's disease. *Neurosci. Res*. 48, 195–202
58 Arkinstall, S. (2001) Applied Research Systems. Pharmaceutically active sulfonamide derivatives. EP 1088821 A1
59 Halazy, S. *et al*. (2002) Applied Research Systems. Pharmaceutically active sulfonamide derivatives bearing both lipophilic and ionisable moieties as inhibitors of protein Jun kinases. EP 1193268 A1
60 Halazy, S. *et al*. (2002) Applied Research Systems. Pharmaceutically active sulfonamide derivatives as inhibitors of protein Jun kinases. EP 1193267 A1
61 Arkinstall, S. *et al*. (2001) Applied Research Systems. Pharmaceutically active sulfonyl hydrazide derivatives. WO 0123382 A1
62 Halazy, S. (2002) Applied Research Systems. Pharmaceutically active benzsulfonamide derivatives as inhibitors of JNK proteins. EP 1193256 A1
63 Rueckle, T. *et al*. (2002) Applied Research Systems. Arylsulfonamide derivatives as c-Jun N-terminal kinases (JNK's) inhibitors. WO 2003010164 A1
64 Arkinstall, S. (2001) Applied Research Systems. Pharmaceutically active sulfonyl amino acid derivatives. EP 1088815 A1
65 Arkinstall, S. *et al*. (2001) Applied Research Systems. Pharmaceutically active sulfonyl hydrazide derivatives. EP 1088822 A1
66 Graczyk, P. *et al*. (2003) Eisai. Preparation of 7-azaindoles as inhibitors of c-Jun N-terminal kinases. WO 2003082869 A1
67 Graczyk, P. *et al*. (2003) Eisai. Preparation of 7-azaindoles as inhibitors of c-Jun N-terminal kinases for treatment of neurodegenerative disorders. WO 2003082868 A1
68 Ohi, N. *et al*. (2003) Eisai. Pyrazole compound and medicinal composition containing the same. WO 2003101968 A1
69 Oinuma, H. *et al*. (2002) Eisai. Novel 1H-indazole compound. WO 2002083648 A1
70 Malmstroem, J. and Swahn, B-M. (2003) AstraZeneca AB. Preparation of indazoles as inhibitors of Jun N-terminal kinases (JNK), in particular JNK3. WO 2003068754 A1
71 Bhagwat, S.S. *et al*. (2002) Signal Pharmaceuticals. Indazole derivatives as JNK inhibitors and compositions and methods related thereto. WO 0210137 A2
72 Ohi, N. *et al*. (2003) Eisai. Preparation of novel fused ring indazole derivatives as JNK inhibitors. WO 2003072550 A1
73 Kanai, F. *et al*. (2004) Kyowa Hakko Kogto. JNK Inhibitor. WO 2004050088 A1
74 Bhagwat, S.S. *et al*. (2004) Signal Pharmaceuticals. Methods for treating an inflammatory condition or inhibiting JNK. US 20040127536 A1
75 Corbett, W. *et al*. (2000) Hoffmann-La Roche AG. 4-Aryloxindoles as inhibitors of JNK protein kinases. WO 0035909 A1
76 Luk, K-C. *et al*. (2000) Hoffmann-La Roche AG. 4- and 5-Alkynyloxindoles and 4- and 5-alkenyloxindoles. WO 0035906 A2
77 Luk, K-C. and Michoud, C. (2000) Hoffmann-La Roche AG. 4,5-Pyrazinoxindoles as protein kinase inhibitors. WO 0035921
78 Salituro, F. *et al*. (2000) Vertex Pharmaceuticals. Inhibitors of c-Jun N-terminal kinases (JNK). WO 0064872 A1