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Substituting c-Jun N-terminal kinase-3 (JNK3) ATP-binding site amino acid residues with their p38 counterparts affects binding of JNK- and p38-selective inhibitors

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Abstract

c-Jun N-terminal kinase (JNK) activation is linked to the aberrant cell death in several neurodegenerative disorders, including Parkinson's and Alzheimer's disease. The sequence similarity among the JNK isoforms and fellow MAP kinase family member p38 has rendered the challenge of producing JNK3-specific inhibitors difficult. Using the crystal structure of JNK3 complexed with JNK inhibitors, potential compound-interacting amino acid residues were mutated to the corresponding residues in p38. The effects of these mutations on the kinetic parameters with three compounds were examined: a JNK3- (vs. p38-) selective inhibitor (SP 600125); a p38-selective inhibitor (Merck Z); and a potent combined JNK3 and p38 inhibitor (Merck Y). The data confirm the role of the JNK3 residues Ile-70 and Val-196 in both inhibitor and ATP-binding. Remarkably, the Ile-70-Val and Val-196-Ala mutations caused an increase and decrease, respectively, in the binding affinity of the p38-specific compound, Merck Z, of 10-fold. The Ile-70-Val effect may be due to the increased capacity of the active site to accommodate Merck Z, whereas the Val-196-Ala mutant may induce an unfavourable conformational change. Conservative mutations of the Asn-152 and Gln-155 residues inactivated the JNK3 enzyme, possibly interfering with protein folding in a critical hinge region of the protein.

Keywords: c-Jun N-terminal kinase; p38; Stress-activated protein kinase; Mitogen-activated protein kinase; Neuroprotection

Apoptosis, commonly referred to as a form of programmed cell death, plays a vital role in the normal development of the nervous system and is also thought to contribute largely to the aberrant neuronal cell death that characterizes many neurodegenerative diseases. The blocking of neuronal apoptosis could therefore be a possible therapeutic approach to treating neurodegenerative diseases, as inhibiting cell death could slow down or halt the progression of disease and onset of associated symptoms. One of the major path-

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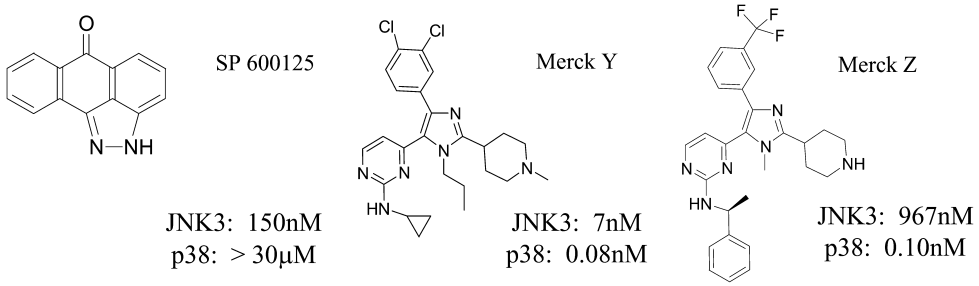

Fig. 1. Structures and previously established IC_{50} data for compounds used in the study.

ways implicated in determining neuronal cell death and survival is the mitogen-activated protein kinase (MAPK)[4] pathway, which controls cell proliferation and cell death in response to a plethora of extracellular stimuli.

Three sub-families of MAPK have been identified in mammals, the ERKs (extracellular regulated protein kinases), p38, and JNKs (c-Jun N-terminal kinases). In contrast to the ERK pathway, which is linked primarily to initiation of cellular growth and differentiation, JNK and p38, also known as the stress-activated protein kinases (SAPKs), are generally associated with apoptotic and inflammatory responses induced by a variety of chemical and physical stresses including oxidative stress, UV light, inflammatory cytokines, and osmotic shock.

There are 10 isoforms of JNK encoded by three genes, *jnk1*, *jnk2*, and *jnk3*. While *jnk1* and *jnk2* are widely expressed, *jnk3* is almost exclusively expressed in the brain, with very low levels expressed in the kidneys and the testis [1,2]. The selective expression of JNK3 in the brain, along with findings that JNK3 knockout mice are resistant to excitotoxic stress induced by kainic acid [3], make inhibiting this isoform a particularly promising therapeutic target for neurodegenerative diseases.

Once activated via an upstream kinase signalling cascade, JNK can translocate to the nucleus, where it phosphorylates several transcription factors involved in the apoptotic response, including c-Jun, activating transcription factor 2 (ATF-2), Elk1, nuclear factor of activated T cells (NFAT), and tumour suppressor p53 [4–9]. In addition to the activation of transcription factors, JNK has also been shown to phosphorylate MADD, a death domain protein [10]. JNK activation and c-Jun phosphorylation have been shown to play a key role in promoting apoptosis in response to various cellular stresses in a variety of different neuronal and other cell culture models of apoptotic cell death [4,5,11–15].

Recent studies have linked JNK activity with the cell death associated with Alzheimer's disease [16–20] and Parkinson's disease [21–24], the two most prevalent forms of neurodegenerative disease. However, the development of highly specific small molecule inhibitors for JNK3 is a difficult task, as many inhibitors of JNK3 would also be expected to inhibit JNK1, JNK2, and p38, due to their high degree of similarity. Inhibition of these additional MAPKs could therefore lead to potential side effect profiles on immune and inflammatory systems.

One approach to improving specificity of small molecule inhibitors towards JNK3 over p38 is through rational structure-based drug design. Xie et al. [25] first reported the crystal structure of unphosphorylated JNK3 complexed with an ATP analogue (adenylyl imidodiphosphate) in 1998. A truncated version of JNK3 [which produced comparable kinase activity to the wild-type (WT) protein after activation with mitogen-activated protein kinase (MKK7)] was used for ease of producing large, well-ordered crystals that diffracted at 2.3 Å resolution. JNK3 has a typical kinase fold, with the well-ordered ATP-binding site situated within a cleft between the N- and C-terminal domains. Further studies using a similar truncated version of JNK3 (38 N-terminal residues deleted) have elucidated the crystal structure of activated JNK3 complexed with two compounds known to inhibit JNK3 activity: Merck Y (potent against both JNK3 and p38) and SP 600125, an inhibitor selective for JNK3 vs. p38, though not selective vs. other kinases (Fig. 1) [26,27]. Merck Y has been shown to be a potent inhibitor of both dopaminergic and sympathetic neuronal cell death [28,29]. In 2004, Ruckle et al. [30] reported the synthesis of 2-(benzoylaminomethyl)thiophene sulphonamide benzotriazole (AS600292) that was a selective JNK inhibitor compared to 80 other serine/threonine and tyrosine kinases. With an IC_{50} for JNK3 = 150 nM, AS600292 also showed a fourfold selectivity against JNK2. In the present study, using this structural information together with modelling, potential compound-interacting residues in the active site of JNK were identified. Single amino acid substituted mutants of JNK3 at the ATP-binding site were gener-

[4] *Abbreviations used:* ATF2, activating transcription factor 2; ERK, extracellular signal-regulated kinase; EuK, europium cryptate; FRET, fluorescence resonance energy transfer; GST, glutathione *S*-transferase; HTRF, homogeneous time-resolved fluorescence; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MKK, MAPK kinase; SAPK, stress-activated protein kinase; WT, wild-type.