Covalent Inhibitors for Treatment of Inflammation and Neurodegeneration

(No. T4-1880)

Principal investigator

Nir London

Faculty of Chemistry
Department of Department of Chemical and Structural Biology

Overview

C-Jun NH2-terminal kinase (JNK) plays an integral role in many cellular processes and is associated with the pathophysiology of a number of neurological, inflammatory, and autoimmune diseases. Due to its ubiquity and its extensive activities, direct inhibition is likely and indeed was shown to have toxic effects. At the same time, inhibition of its upstream activators (MKK4/MKK7) is challenging due to their preserved ATP binding sites. The covalent inhibitor developed by the London group at WIS selectively targets a unique cysteine residue in the MKK7 protein. The inhibitor proved potent, specific to MKK7 and nontoxic in cultured cells, and is currently being optimized for in vivo testing.

Background and Unmet Need

The JNK signaling cascade plays an integral role in the pathology of several neurodegenerative diseases, including Parkinson’s and Alzheimer’s disease, as well as in inflammatory and autoimmune responses. Due to its extensive activity profile, its direct inhibition would likely have toxic effects. At the same time, inhibition of its activating kinases, MKK7 and MKK4, via ATP binding site antagonists, is challenging due to the close homology between its ATP binding and that of other kinases. To date, no selective MKK7/4-specific inhibitors have been reported.

The Solution

A selective MKK7 inhibitor which covalently targets a native, non-conserved cysteine residue.

Technology Essence

The designed inhibitor, identified using a proprietary covalent virtual screening platform, targets cysteine 218, found in an identical context in only 10 other protein kinases. The MKK7 inhibitor led to reduced JNK phosphorylation (Figure 1A) and subsequently to reduce phosphorylation of its downstream targets c-Jun and ATF2 in cultured cells in the single-digit micromolar range. In addition, it demonstrated a dose-dependent effect on B cell response to lipopolysaccharide challenge1 (Figure 1B). The inhibitor proved highly selective, with no inhibitory effect on p38 or ERK, two protein kinases involved in a broad range of cellular processes. Similarly, it had no effect on phosphorylated JNK levels in MKK7-knockout cell lines.

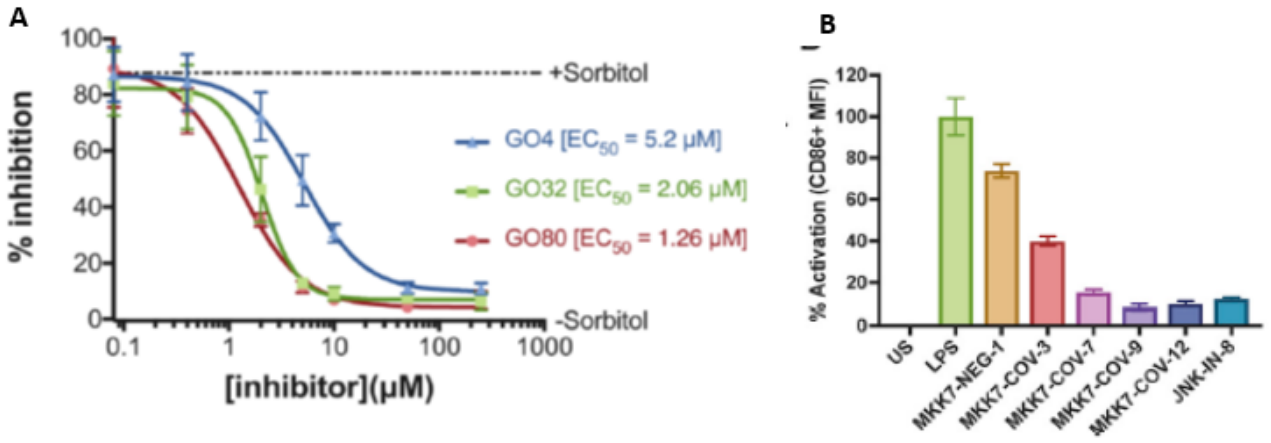

Figure 1 – (A) In-cell western assay demonstrating inhibition of JNK phosphorylation in HEK293 cells after 2 hr pre-incubation with MKK7 inhibitors and sorbitol. (B) Inhibition of Primary mouse B cells activation with MKK7 inhibitors (as assessed by CD86 staining). Except for the negative control MKK7-NEG-1, all compounds show >60% inhibition with MKK7-COV-9 and MKK7-COV-12 showing similar levels of inhibition to the positive control (JNK-IN-8)

Applications and Advantages

- Selective and context-specific inhibition
- Prolonged, potent inhibitory effect with therapeutic potential
- Metabolically stable
- Suitability for a number of inflammatory and neurological pathologies
- Likely blood brain barrier-penetrant
- Unique chemical tool, e.g., to define the role of MKK7 versus MKK4

Development Status

Cellular proof of inhibition has been achieved. The phenotype associated with its activity is currently being assessed, as well as its effect in disease-relevant models. In parallel, optimization of inhibitor potency, selectivity and metabolic stability is being pursued to enhance its suitability for in vivo applications.

References

Shraga A, Olshvang E, Davidzohn N, et al. Covalent Docking Identifies a Potent and Selective MKK7 Inhibitor. Cell Chem Biol. 2019;26(1):98-108.e5. doi:10.1016/j.chembiol.2018.10.011 [1]

Patent Status

USA Published: Publication Number: 2021-0238173-A1