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Fragment-based drug discovery of potent and selective MKK3/6 inhibitors

Mark Adams [a], Toshitake Kobayashi [e], J. David Lawson [b], Morihisa Saitoh [e], Kenichiro Shimokawa [e], Simone V. Bigi [a], Mark S. Hixon [c], Christopher R. Smith [a], Takayuki Tatamiya [e], Masayuki Goto [e], Joseph Russo [d], Charles E. Grimshaw [b], Steven Swann [a,*]

[a] Medicinal Chemistry, Takeda California Inc., 10410 Science Center Drive, San Diego, CA 92121, United States
[b] Computational Sciences and Crystallography, Takeda California Inc., 10410 Science Center Drive, San Diego, CA 92121, United States
[c] Global DMPK, Takeda California Inc., 10410 Science Center Drive, San Diego, CA 92121, United States
[d] CNS & Novel Target ID, Takeda California Inc., 10410 Science Center Drive, San Diego, CA 92121, United States
[e] Pharmaceutical Research Division, Takeda Pharmaceutical Company Ltd, 26-1, Muraoka-higashi 2-chome, Fujisawa, Kanagawa 251-8555, Japan

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ABSTRACT

The MAPK signaling cascade, comprised of several linear and intersecting pathways, propagates signaling into the nucleus resulting in cytokine and chemokine release. The Map Kinase Kinase isoforms 3 and 6 (MKK3 and MKK6) are responsible for the phosphorylation and activation of p38, and are hypothesized to play a key role in regulating this pathway without the redundancy seen in downstream effectors. Using FBDD, we have discovered efficient and selective inhibitors of MKK3 and MKK6 that can serve as tool molecules to help further understand the role of these kinases in MAPK signaling, and the potential impact of inhibiting kinases upstream of p38.

Treatment of chronic inflammatory diseases such as rheumatoid arthritis (RA), psoriasis, and Crohn's disease remains a significant unmet medical need. The mitogen-activated protein kinase (MAPK) family is implicated in perpetuating inflammation and tissue injury in RA, psoriasis, and Crohn's disease and therefore is an attractive target for small molecule drug intervention.[1–3] The MAPK family is comprised of several linear and intersecting pathways, connecting Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) and p38 MAPK, which propagate signaling in the nucleus and are essential components in a number of critical cellular processes.[4]

Over the past two decades, a large number of p38 MAPK inhibitors have demonstrated efficacy in multiple rodent models of arthritis and several of these molecules (VX-702,[5] VX-745, pamapimod,[6] iosmapimod, dilmapimod, doramapimod, BMS-582949, ARRY-797, PH797804, SCIO-469) have been investigated in Phase II for RA or Crohn's disease.[7,8] Despite the compelling rodent efficacy, the clinical results revealed modest to nominal efficacy. Explanations include p38a activation itself having anti-inflammatory effects, and/or the presence of significant redundancy in the intersecting MAPK pathways.

An emerging proposal in the literature, supported by rodent MKK3$^{-/-}$ and MKK6$^{-/-}$ knockout data,[9–11] is to explore protein targets upstream of p38 kinase. MKK3 and MKK6 are phosphorylated by upstream MKKKs such as TAK1 and ASK1, which in turn propagate the signal through p38 to the nucleus resulting in cytokine and chemokine release.[12]

Further study of the role of MKK3 and MKK6 in p38 MAPK signaling is hampered by the lack of potent, selective, and well-characterized inhibitors. This Letter describes the discovery of a chemical probe using fragment-based drug design (FBDD). We envisaged the probe should inhibit MKK3/6 with an IC_{50} <10 nM and selective (>50-fold) over other members of the MAPK signaling cascade.

An internal library of 11,012 fragments (heavy atom count between 11 and 19 atoms) was screened at 100 μM against phosphorylated MKK3 using a Caliper-based biochemical probe.[13] From the primary screen, single-point inhibition values were used to calculate approximate pIC_{50} ($ApIC_{50}$)[14] values for each fragment. These values were then used to estimate ligand efficiency (LE) and lipophilic ligand efficiency (LLE) values for each compound that exhibited >30% inhibition. Using this criteria, 93 fragments were identified with LE $\geqslant$0.35 and an LLE $\geqslant$4. A subset of these fragments was then evaluated by full dose response curves against both MKK3 and MKK6, and direct binding was measured using surface plasmon resonance.[15]

* Corresponding author.

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One fragment of interest, compound **1**, was a highly efficient inhibitor of both MKK3 (37 μM; LE = 0.44, LLE = 4.21) and MKK6 (16 μM; LE = 0.47, LLE = 4.8) and demonstrated direct binding to MKK3 with a K_d of 2 μM (shown in Fig. 1).

A putative binding mode for **1** was determined by molecular modeling using the MOE software package.[16] The compound was hand-docked into the protein and solvent of the MKK6/AMPPNP co-complex (PDB code: 3VN9)[17] using the in-house co-crystal structure of **1** in the kinase domain of BTK kinase (PDB code: 4ZLY)[18] as a guide. The binding pose suggested the primary amide and the free amine on the bicyclic ring formed a tridentate interaction with the hinge of the protein (Fig. 2).

The process of 'hit expansion' was initiated by testing a series of compounds structurally related to **1**, from either internal or commercial sources. The goal of this exercise was to establish structural activity relationships (SAR) and refine our docking studies to validate the putative binding mode.

From this exercise **2,** containing a 3-amino picolinamide core, was identified as an efficient inhibitor (IC_{50} = 4.2 μM; LE = 0.45) of MKK3 (Shown in Scheme 1). A docked pose of **2** in MKK6 suggested it bound similarly to **1** as described above (Fig. 3). Using this binding mode, further optimization of **2** was undertaken.

Making use of the similarity between MKK3 and MKK6, we drove our optimization process using MKK3 IC_{50} data. Once a compound was identified with a MKK3 IC_{50} <10 nM, a selectivity profile was established by screening against a wider panel of MAPK enzymes.

Highlighted in Scheme 2, further optimization of **2** was achieved by substituting the phenyl ring of **2** with a pyridin-4-yl (**3**), which had a MKK3 IC_{50} of 30 nM (LE = 0.64). Based on the docked pose of **2**, this boost in potency is likely a result of the pyridine nitrogen lone pair interacting favorably with the binding pocket Lysine-82. Adding a methyl group to **3** led to **4**, which gave an approximate 10-fold increase in potency and met the initial goal of discovering a MKK3 inhibitor with an IC_{50} <10 nM.

Modifications to the core structure of **4** were subsequently undertaken to potentially identify another series of MKK3 inhibitors that were structurally differentiated from the 3-aminopicolinamide chemotype. One design strategy involved cyclization of the picolinamide ring into other fused heterocycles (Scheme 3). Through this process a potent and efficient inhibitor of MKK3 (IC_{50} = 150 nM; LE = 0.51) was identified that contained an imidazolopyridine (**5**). Further optimization resulted in **6**, which contained a unique isothiazole ring and resulted in a 30-fold boost in potency.

A docked pose of **6** suggests a similar binding mode to **4** (Fig. 4). In this model, the isothiazole is within an acceptable distance for a favorable interaction with Lysine-82. Additionally, the sulfur likely maintains the planarity of the system, as well as the preferred conformation of the ring. The boost in potency could also result from a potential sulfur-sulfur interaction between Methionine-129 and the sulfur atom of the isothiazole ring.

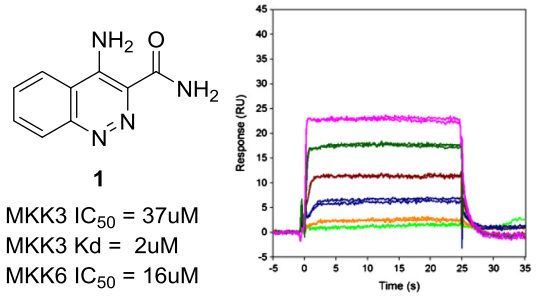


Figure 1. Structure and SPR isotherm of **1** binding to MKK3.

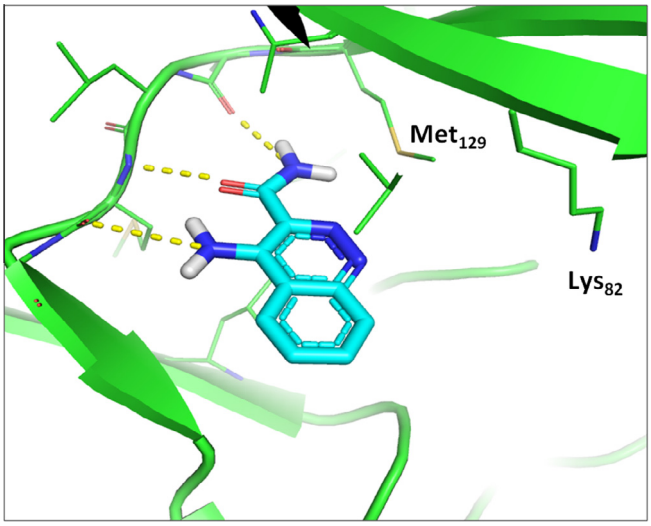
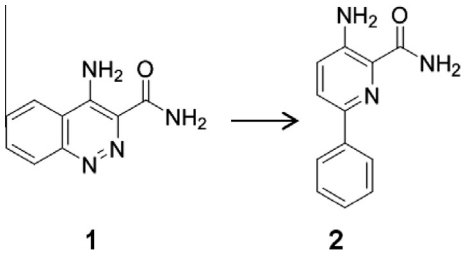


Figure 2. Model of **1** bound to MKK6.



Scheme 1. Identification of **2** from fragment hit **1.**

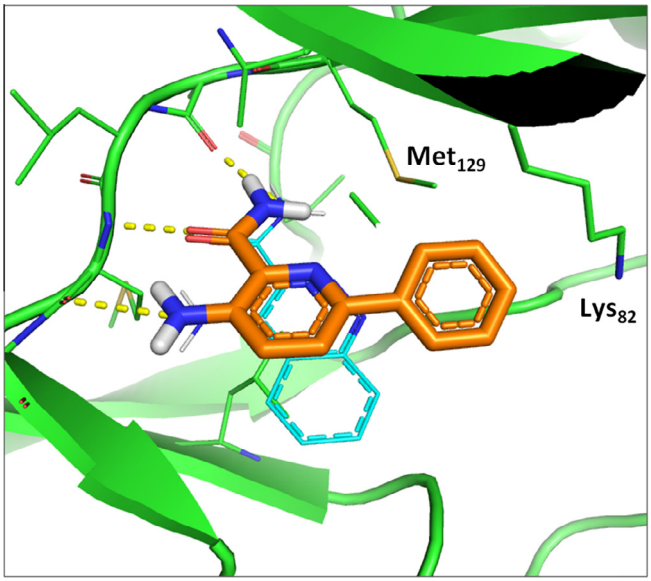


Figure 3. Model of **2** (orange) overlaid with 1 (blue) bound to MKK6.

The syntheses of **3** and **4** are shown in Scheme 4A. Picolyl nitrile **A**, a key starting material for this series, was converted into carboxamide **B** under oxidative conditions. Introduction of the pyridine ring was accomplished by employing a Suzuki–Miyaura coupling reaction providing **3**. With regard to **4**, N-acylation of **A** with TFAA followed by N-methylation gave **C** whose TFA group was cleaved