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Amide-based inhibitors of p38α MAP kinase. Part 1: Discovery of novel *N*-pyridyl amide lead molecules

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ABSTRACT

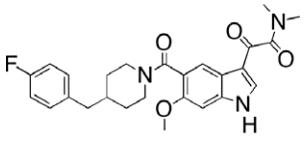
A novel series of *N*-pyridyl amides as potent p38α kinase inhibitors is described. Based on the structural similarities between the initial hit and a well-known imidazole pyrimidine series of p38α inhibitors, potencies within the newly discovered series were quickly improved by installation of an (*S*)-α-methylbenzyl moiety at the 2-position of the pyridine ring. The proposed binding modes of the new series to p38α were evaluated against SAR findings and provided rationale for further development of this series of molecules.

Modulation of cytokines has recently shown promise in the treatment of diseases involving chronic inflammation. Support for this approach is found in the utility of anti-TNFα therapies (Enbrel, Remicade) and anti-IL-1 treatment (Kineret).[1–3] In attempts to develop small molecule inhibitors with similar effects, several kinase signaling pathways were examined over recent years—including the p38 MAP kinase pathway.[4–9]

The α isoform of p38 MAP kinase has been identified as a control point which, when activated, translates multiple stimuli to multiple responses. The end result is the release of a pro-inflammatory cassette of cytokines which includes interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor α (TNFα).[10] As p38α activation is not involved in normal physiology, the downstream effects of activation are thought to play pathophysiological roles in inflammatory processes associated with diseases, such as rheumatoid arthritis,[10–12] inflammatory bowel disease,[13] congestive heart failure[14] and psoriasis.[15] Furthermore, since p38α activation leads to cytokine expression, it is postulated that inhibition of p38α can normalize this aberrant physiology and play a key role in the treatment of inflammation-related diseases.

Driven primarily by the central role of p38α in several settings of inflammation, this enzyme has been an active target for drug discovery efforts in the past decade.[16–18] Numerous structural classes of p38α kinase inhibitors have been reported in the literature, of which several have been evaluated in clinical trials (i.e., VX-745, AMG-548, BIRB-796 and SCIO-469).[19–22] Recently, we described our work surrounding the indole carboxamide-based lead **1** (Fig. 1).[23] Herein, we wish to report our initial findings on a novel class of heterocyclic amide inhibitors of p38α.

High throughput screening resulted in the identification of the *N*-pyridyl amide **2** (Fig. 2), as an inhibitor of p38α. Recognizing structural similarities with the well-known imidazole pyrimidine series of p38α inhibitors, exemplified by **3** (Fig. 2),[24] we developed a working model in which the *trans* conformer of compound **2** binds to the p38α enzyme as shown in Figure 3.[25] As illustrated, the pyridine ring makes the requisite hinge contact, while the 4-fluorobenzyl moiety occupies the hydrophobic pocket. Both hydrogen bond interactions to the hinge amino acid Met-109 and occupation of adjacent hydrophobic pocket are known to be critical for p38α activity.[26,27] Furthermore, a docking overlay of **2** and **3** within the p38α ATP binding site (Fig. 3) suggests that appropriate substituents on the pyridine ring should reveal SAR similar to what is known for the imidazole pyrimidine class of inhibitors.[24]



1
p38α IC_{50} 0.01 μM

Figure 1. Structure and potency of indole carboxamide-based p38α kinase inhibitor, (**1**).

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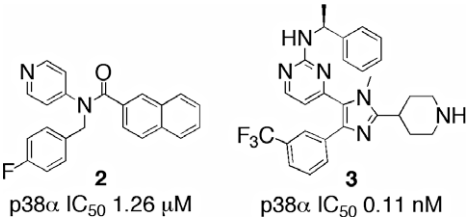


Figure 2. p38α screening hit (**2**) and imidazole pyrimidine p38α inhibitor (**3**).

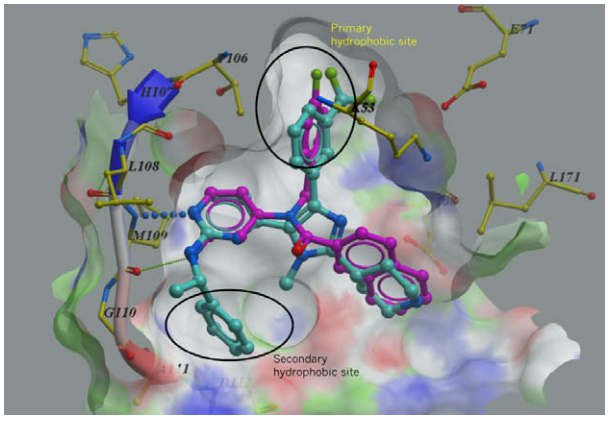
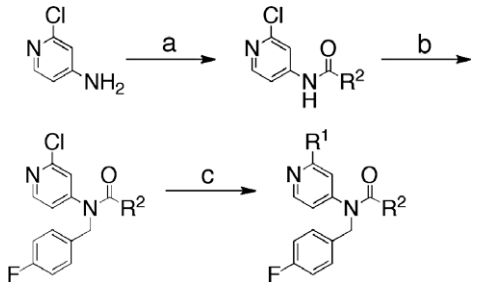


Figure 3. Initial proposed binding modes of **2** (magenta) and Merck imidazole pyrimidine p38α inhibitor **3** (blue) within the ATP binding pocket of p38α kinase.



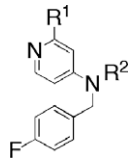
Scheme 1. General procedure for the synthesis of *N*-pyridyl amides. Reagents and conditions: (a) acid chloride, Et_3N, DCM, 38–59%; (b) NaH, DMF then 4-fluorobenzyl bromide, 27–60%; (c) amine, $Pd(OAc)_2$, BINAP, Cs_2CO_3, dioxane, 110 °C, 20–74%.

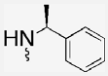
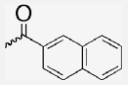
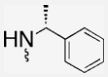
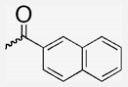
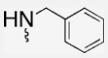
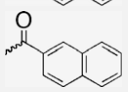
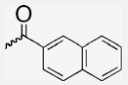
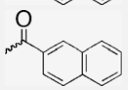
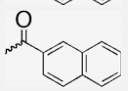
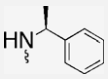
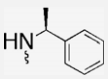
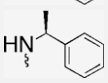
Based on our modeling studies, a number of 2-amino substituted pyridine analogs were prepared according to Scheme 1. As illustrated, acylation of 4-amino-2-chloro pyridine followed by NaH deprotonation and treatment with 4-fluorobenzyl bromide provided the desired amide. A Buchwald coupling was then used to couple an amine to the pyridine 2-position giving the desired products. Data for all analogs are shown in Table 1.

As shown in Table 1, incorporation of an amino (*S*)-α-methylbenzyl group on the pyridyl 2-position of compound **2** resulted in a significant increase in potency (**4**; IC_{50} = 0.345 μM). Furthermore, the stereochemical configuration at the benzylic position was found to be important as the opposite enantiomer **5**, showed a significant drop in potency. In addition, removal of the α-methyl, removal of the benzyl, or replacement of the benzyl with a cyclohexyl group (compounds **6**, **7** and **8**, respectively) significantly reduced potency. However, incorporation of an isopropyl group, **9**, maintained most of the activity associated with **4** illustrating the importance of the α-methyl group.

Regarding the p38α binding mode of this series of structures, compounds such as analog **4** are expected to bind similarly to

Table 1
p38α enzymatic potencies of *N*-pyridyl amides



Compd	R^1	R^2	p38 IC_{50}[23] (μM, n = 3)
4			0.345
5			7.8
6			1.22
7			5.08
8			1.11
9			0.671
10			0.589
11			0.408
12		H	0% @ 1 μM

the structures illustrated in Figure 3. Specifically, besides having the same binding interactions as compound **2**, the (*S*)-α-methylbenzyl group of **4** should reside in the same region occupied by the same moiety of compound **3**.

To understand the role of the naphthyl region, diverse structural alternatives to the naphthoyl group were studied. As shown in Table 1, benzoyl and acetyl groups were well tolerated

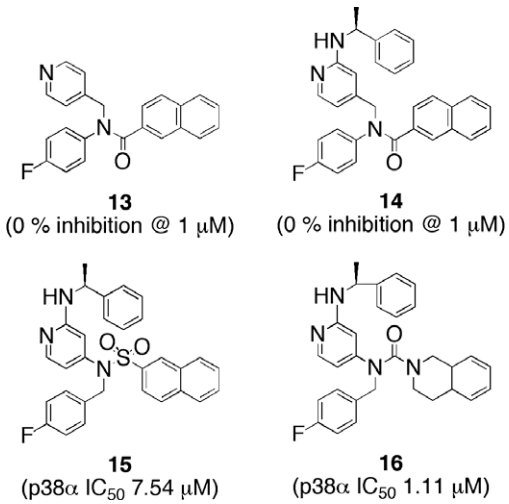


Figure 4. Additional analogs with associated p38α enzyme activities.

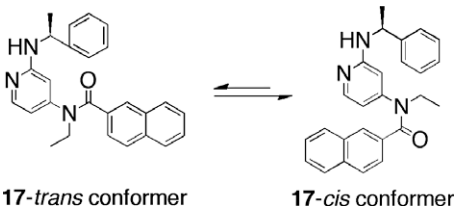


Figure 5. *Cis* and *trans* rotational isomers of **17**. Calculations show that the *cis* isomer is 7.54 kcal/mol lower in energy than the *trans* isomer.[30]

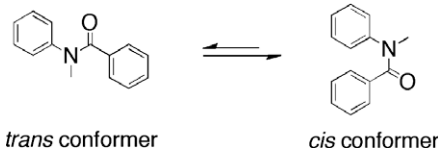


Figure 6. *Cis* and *trans* rotational isomers of *N*-acetyl-*N*-methylaniline. The *cis* isomer is the dominant structure as demonstrated by X-ray crystallography.

(compounds **10** and **11**, respectively). However, elimination of this group altogether (compound **12**[28]) resulted in a complete loss of activity. Finally, proper positioning of the amide bond seemed key to binding as, in **13**[29] and **14**,[29] relocation of this group relative to compounds **2** and **4** resulted in no inhibition at 1 μM (Fig. 4). Additional support for the importance of the amide is found in the decrease in activity noted when alternative functional groups were used (Fig. 4, compounds **15**[28] and **16**[28]).

Moving beyond the SAR trends relative to pyridine substituents and acyl groups, a surprising observation was noted while investigating the significance of the 4-fluorobenzyl group. Specifically, it was found that this group could be replaced by an ethyl group (**17**[28]; IC_{50} 0.313 μM) with no deleterious impact on potency relative to compound **4**. Since this result seemed to contradict predictions by our initial binding model, we carried out more detailed modeling experiments on compound **17**. Subsequent conformational analysis of **17** demonstrated that this compound could exist as a mixture of *cis* and *trans* conformers with the *cis* conformer as the dominating lower energy rotamer (Fig. 5).[30] Furthermore, the structurally similar *N*-acyl-*N*-methylanilines are known to adopt lower energy *cis* conformations as demonstrated through X-ray crystallography, NMR and molecular orbital calculations (Fig. 6).[31,32]

Docking of the *cis* conformer into the p38α binding site (Fig. 7)[25] demonstrated that the pyridine nitrogen and 2-amino nitrogen could still form two hydrogen bond interactions with Met-109 in the hinge region. The naphthyl group is shown able to occupy the adjacent p38α hydrophobic pocket attributed to kinase specificity. While the α-methylbenzyl moiety sits in another hydrophobic pocket, a distal hydrogen bond interaction is seemingly formed between the amide carbonyl oxygen and Lys-53. In fact, there is precedence for such a binding mode involving Lys-53 and N-3 of imidazole-based inhibitors.[33] Furthermore, this binding mode can be used to explain the potencies obtained for all analogs shown in Table 1. The only exception is the activity of the acetyl analog **11** which is more appropriate explained by the originally proposed binding mode. Finally, the new model suggests that the amide substituent could potentially be modified to pick up additional interactions with neighboring amino acid such as Asp-112, Ser-154, Asp-168 and Tyr-35.

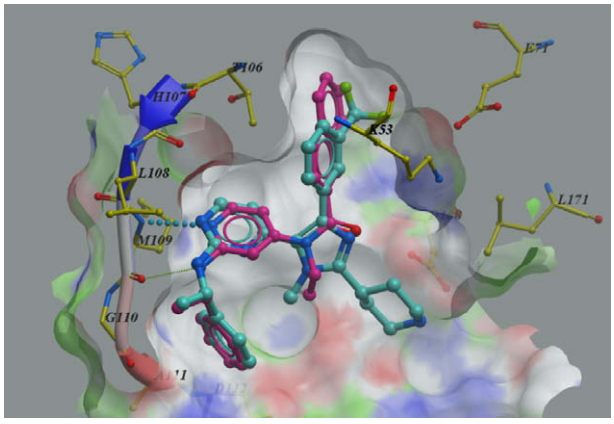


Figure 7. Docking overlay of **17**-*cis* conformer with Merck imidazole pyrimidine compound **3** within the ATP binding pocket of p38α kinase.

In summary, a novel series of *N*-pyridyl amides was identified as potent inhibitors of p38α kinase. Based on structural similarities between the initial hit **2** and known p38α inhibitors, sub-micromolar activity was quickly achieved by applying SAR known for imidazole pyrimidine-based inhibitors. However, the results of these SAR studies, were inconsistent with the initial binding mode proposed based on the structural similarities between the two series. The newly proposed binding mode of amide **17** indicated that, in this series, an energetically more favorable *cis* conformer may be the actual bioactive conformation.

References and notes

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25. Docking studies were conducted using a high-resolution p38α X-ray structure of p38α (PDB ID: 1ove). The extra-precision mode of Glide, a ligand–receptor docking tool from Schrodinger, LLC, was used to generate a number of poses for analogs **2**, **3** and **17**-*cis*, which were further filtered by visual inspection.
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29. **13** and **14** were prepared similar to Scheme 1, except 4-fluoroaniline was used.
30. Conformational searches for compound **17** were run with MacroModel and MMFFs force field with water as the default solvent and utilizing systemic torsional sampling. The calculation shows that lowest energy *cis* conformer is lower in energy than lowest energy *trans* conformer for compound **17** by 7.54 kcal/mol.
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