



## Amide-based inhibitors of p38 $\alpha$ MAP kinase. Part 2: Design, synthesis and SAR of potent *N*-pyrimidyl amides

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### ABSTRACT

Optimization of a tri-substituted *N*-pyridyl amide led to the discovery of a new class of potent *N*-pyrimidyl amide based p38 $\alpha$  MAP kinase inhibitors. Initial SAR studies led to the identification of 5-dihydrofuran as an optimal hydrophobic group. Additional side chain modifications resulted in the introduction of hydrogen bond interactions. Through extensive SAR studies, analogs bearing free amino groups and alternatives to the parent (*S*)- $\alpha$ -methyl benzyl moiety were identified. These compounds exhibited improved cellular activities and maintained balance between p38 $\alpha$  and CYP3A4 inhibition.

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p38 Mitogen-activated protein kinase (MAPK) is a serine–threonine protein kinase and one of the best characterized enzymes involved in the inflammatory process.<sup>1,2</sup> Of the four isoforms ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ), p38 $\alpha$  is the most studied and perhaps the most physiologically relevant kinase involved in inflammatory responses.

p38 $\alpha$  MAP kinase is activated in response to cellular stress, growth factors and cytokines such as interleukin-1 (IL-1) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Once activated, p38 $\alpha$  stimulates cytokine production through activation of additional kinases leading to phosphorylation of heat shock proteins and transcription factors. Since modulation of cytokines has shown clinical efficacy in treating inflammatory disorders such as rheumatoid arthritis,<sup>3–5</sup> inflammatory bowel disease,<sup>6</sup> congestive heart failure<sup>7</sup> and psoriasis,<sup>8</sup> the ability of p38 $\alpha$  to modulate cytokine production has led to its implication in inflammation-based pathologies.<sup>2,9–13</sup>

Driven by the central role of p38 $\alpha$  in settings of inflammation, this enzyme has been an active target for drug discovery efforts over the past decade.<sup>14–18</sup> Contributing to these efforts, we previously described initial studies focusing on a novel class of *N*-pyridyl amides (Fig. 1).<sup>19</sup>

Subsequent SAR studies involving incorporation of a (*S*)- $\alpha$ -methyl benzyl moiety at the 2-position of the pyridine accompanied by additional structural modifications led to the discovery of compound **2**.<sup>19</sup> Later conversion of the pyridine to a pyrimidine resulted in identification of compound **3** (Fig. 2) possessing

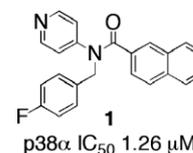


Figure 1. Structure and potency of *N*-pyridyl amide lead **1**.

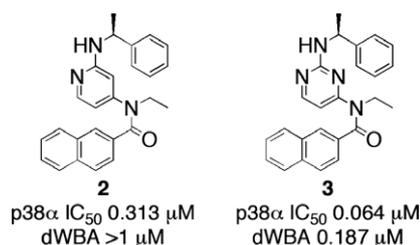


Figure 2. Structure and potency comparison between *N*-pyridyl amide **2** and *N*-pyrimidyl amide **3**.

significant improvements in potency against p38 $\alpha$  in both enzymatic and cell-based assays. Herein we describe our subsequent SAR activities focused on the pyrimidine scaffold.

As illustrated in Figure 3, the proposed binding mode of the *cis*-conformer of **3** in the ATP binding site of p38 $\alpha$  MAP kinase involves a hydrogen bond between a pyrimidine nitrogen and the amide NH of Met-109. An additional hydrogen bond is noted between the

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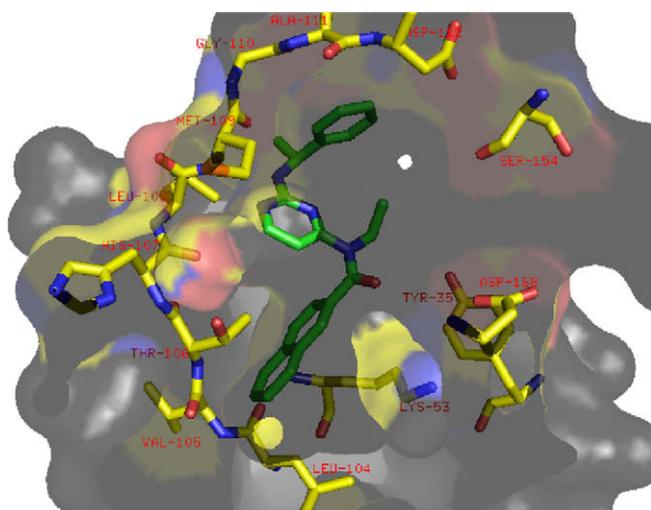
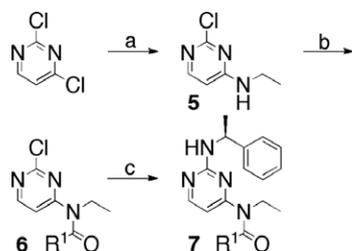


Figure 3. Proposed binding mode of **3** in the p38 $\alpha$  ATP binding site.<sup>20</sup>

bridging NH and the Met-109 carbonyl. The naphthyl group occupies the Thr-106 hydrophobic pocket known to be responsible for p38 $\alpha$  kinase specificity.<sup>23–25</sup> The  $\alpha$ -methyl benzyl moiety occupies an additional hydrophobic pocket. The model also identifies a distal hydrogen bonding formed between the amide carbonyl of **3** and the Lys-53 side chain. Based on this binding mode, we hypothesize that potency enhancements may be achieved through incorporation of additional hydrogen bonds with neighboring amino acids, such as Asp-112, Ser-154, Asp-168 and Tyr-35.

The compounds of the present study were prepared as illustrated in Scheme 1. As shown, reaction of 2,4-dichloro-pyrimidine with ethylamine gave 2-chloro-4-ethylaminopyrimidine, **5**. Deprotonation with NaH followed by quenching with various acid chlorides allowed development of the R<sup>1</sup> SAR. Final treatment with (*S*)- $\alpha$ -methyl benzyl amine via palladium catalysis gave the desired final products, compounds **7**.<sup>26</sup> The p38 activities for this series are summarized in Table 1.

As shown in Table 1, compared to the 2-naphthyl analog, **3**, compounds bearing bicyclic groups such as 5-dihydrobenzofuryl, **7a**, and 5-indanyl, **7e**, showed improved enzyme activity. In contrast, analogs incorporating groups such as 3,4-(methylenedioxy)phenyl, **7b**, 7-dihydrobenzofuryl, **7d**, 3-benzo-thienyl, **7g**, *N*-methyl-3-indolyl, **7h**, and 2-benzofuryl, **7i**, were several fold less active. Regarding compound **7f**, the fourfold loss in potency compared to **7e** may be caused by steric effects due to a six-membered ring versus a five-membered ring. Due to this hypothesis, a series of analogs bearing monocyclic groups at R<sup>1</sup> was prepared. This hypothesis was validated through the observation that substituted phenyl groups were compatible with potent p38 $\alpha$  inhibition. Of particular interest were *meta*-substituted compounds **7l**, **7o**, **7r**, and **7t**—all showing activity in the enzyme assay at approximately 80 nM or better. The two most



Scheme 1. General synthesis of *N*-pyrimidyl amides **7**. Reagents and conditions: (a) ethyl amine, K<sub>2</sub>CO<sub>3</sub>, DMF, 45%; (b) NaH, DMF then acid chloride, 70–95%; (c) (*S*)- $\alpha$ -methyl benzyl amine, Pd(OAc)<sub>2</sub>, BINAP, Cs<sub>2</sub>CO<sub>3</sub>, dioxane, 110 °C, 30–80%.

Table 1  
p38 $\alpha$  enzyme and cellular activity for compounds **7a–v**

Compd	R <sup>1</sup>	p38 $\alpha$ IC <sub>50</sub> ( $\mu$ M, <i>n</i> = 3) <sup>21</sup>	dWBA ( $\mu$ M, <i>n</i> = 3) <sup>22</sup>
<b>3</b>		0.064	0.187
<b>7a</b>		0.017	0.387
<b>7b</b>		0.164	>1
<b>7c</b>		0.071	0.313
<b>7d</b>		0.09	0.829
<b>7e</b>		0.045	0.3
<b>7f</b>		0.180	>1
<b>7g</b>		0.188	>1
<b>7h</b>		0.154	>10
<b>7i</b>		0.179	>10
<b>7j</b>		0.193	>10
<b>7k</b>		0.267	>1
<b>7l</b>		0.081	0.533
<b>7m</b>		0.313	>10
<b>7o</b>		0.013	0.14
<b>7p</b>		0.091	0.143
<b>7q</b>		0.081	0.4
<b>7r</b>		0.065	0.152
<b>7s</b>		0.104	0.349
<b>7t</b>		0.064	>1
<b>7u</b>		0.039	0.178
<b>7v</b>		0.026	0.058

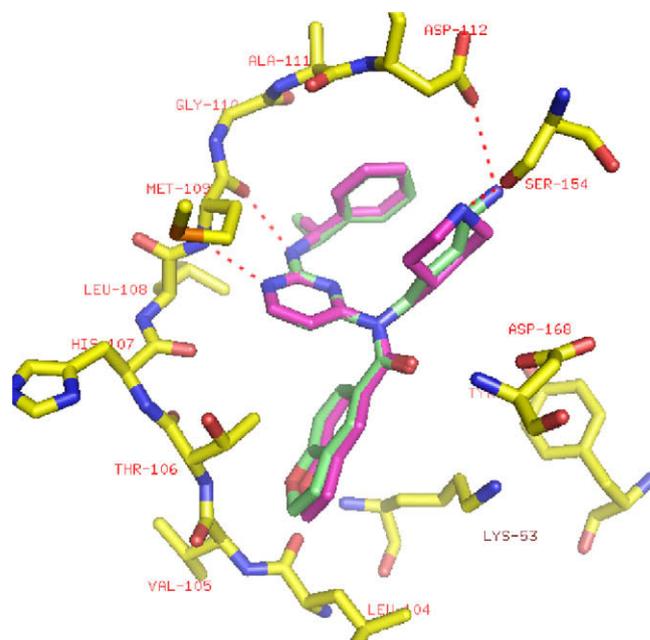
potent compounds (**7u** and **7v**) were identified as the more metabolically stable analogs of **7o**. In reconciling the enzyme inhibitory activity with the proposed binding mode in Figure 3, it appears that the hydrophobic pocket formed by Thr-106, Val-105, and Leu-104 can tolerate both polar and non-polar substituents. However, when polar groups reside in this pocket, activity is dependent upon the orientation of the group.

Also shown in Table 1, enzyme inhibitory activity did not necessarily track with cell-based data. While our most potent analogs bearing bicyclic groups at R<sup>1</sup> inhibited at approximately 400–900 nM, our best monocyclic derivative, compound **7o**, **7u**, and **7v** inhibited at 140 nM, 178 nM, and 58 nM, respectively. While these results showed promise, we felt that the benzofuran group of **7a** was able to optimally fill its respective hydrophobic pocket. Consequently, this compound was chosen as a new baseline for additional SAR studies targeting the amide side chain.

The compounds shown in Table 2 were prepared as illustrated in Scheme 1 by substituting ethylamine with alternative amines. In cases where final products contained free amino groups, these amines were incorporated into Scheme 1 as Boc derivatives. Boc cleavage was incorporated into the final step using standard TFA conditions.<sup>26</sup>

As shown in Table 2, modifications at R<sup>2</sup> generally resulted in weaker inhibition compared to compound **7a**. This observation was independent of the presence of hydrogen bond donors or acceptors. However, when R<sup>2</sup> was modified to incorporate a chain of four carbons, both enzymatic and cell-based potency improved. In this series, the best analogs (compounds **8e** and **8l**) showed low nM activity in both assays. As illustrated in Figure 4, the improved enzymatic activity is explained through the introduction of hydrogen bonds with Ser-154 and the side chain of Asp-112.

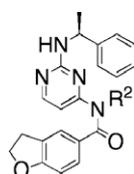
While the SAR thus far resulted in improvements in both enzymatic and cellular inhibition, the broader set of compounds studied, of which only a subset is presented herein, were consistently identified as potent inhibitors of CYP3A4. This CYP activity was traced to the (*S*)- $\alpha$ -methylbenzyl group. Thus, in order to proactively design structures with minimal potential for drug–drug interactions, structures bearing alternatives to this group were pre-



**Figure 4.** Proposed binding modes of **8e** and **8l** in the ATP binding site of p38 $\alpha$  enzyme.<sup>20</sup> For analog **8e**, the methyl piperidine nitrogen is able to form a hydrogen bond interaction with backbone carbonyl oxygen of Ser-154. For analog **8l**, the butyl amine nitrogen could potentially form two hydrogen bond interactions with Ser-154 and the side chain of Asp-112.

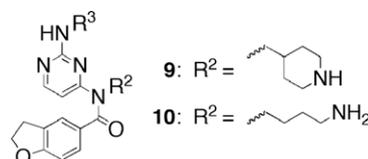
pared. These analogs, summarized in Table 3, were prepared according to Scheme 1 by replacing (*S*)- $\alpha$ -methyl benzylamine

**Table 2**  
p38 $\alpha$  enzyme and cellular activity for compounds **7a**, **8a–n**



Compd	R <sub>2</sub>	p38 $\alpha$ IC <sub>50</sub> ( $\mu$ M, <i>n</i> = 3) <sup>21</sup>	dWBA ( $\mu$ M, <i>n</i> = 3) <sup>22</sup>
<b>7a</b>		0.017	0.387
<b>8a</b>		0.462	2.088
<b>8b</b>		0.165	0.704
<b>8c</b>		0.345	>10
<b>8d</b>		0.158	>10
<b>8e</b>		0.054	0.015
<b>8f</b>		>10	–
<b>8g</b>		>10	–
<b>8h</b>		0.711	0.524
<b>8i</b>		0.673	>10
<b>8k</b>		>10	–
<b>8l</b>		0.004	0.009
<b>8m</b>		0.096	0.13
<b>8n</b>		0.072	0.564

**Table 3**  
p38 $\alpha$  activity and CYP3A4 profile for compounds **9a–10d**



Compd	R <sub>3</sub>	p38 $\alpha$ IC <sub>50</sub> ( $\mu$ M, <i>n</i> = 3) <sup>21</sup>	dWBA ( $\mu$ M, <i>n</i> = 3) <sup>22</sup>	CYP3A4 ( $\mu$ M, <i>n</i> = 3) <sup>27</sup>
<b>8e</b>		0.054	0.015	0.094
<b>9a</b>		0.024	0.088	2.9
<b>9b</b>		0.36	3.29	>20
<b>9c</b>		0.40	>10	>20
<b>9d</b>		0.316	0.46	2.02
<b>9e</b>		3.04	>10	1.54
<b>9f</b>		0.53	0.112	0.367
<b>9g</b>		0.60	0.05	0.34
<b>8l</b>		0.004	0.009	0.18
<b>10a</b>		0.017	0.09	3.115
<b>10b</b>		0.011	0.865	>20
<b>10c</b>		0.025	0.048	2.648
<b>10d</b>		0.31	2.94	11.72

with different amines.<sup>26</sup> The parent structures for this SAR were compounds **8e** and **8l**.

As shown in Table 3, replacing the (*S*)- $\alpha$ -methyl benzyl group impacted not only CYP3A4 activity, but also enzymatic and cellular activity. Of the modifications shown, only a 2-propyl alternative maintained p38 $\alpha$  activity while reducing CYP3A4 activity by up to two orders of magnitude compared to the enzymatic activity. In the case of parent compound **8l**, (*S*)-2-(1-methoxy)propyl was also well tolerated.

In summary, high throughput screening identified *N*-pyridyl amides as novel inhibitors of p38 $\alpha$ . Through subsequent SAR studies, both enzymatic and cellular activities were optimized (compounds **8e** and **8l**). Improved enzymatic activity is believed due to optimal occupation of hydrophobic pockets and introduction of additional hydrogen bonding interactions. Additional SAR produced structures with improved CYP profiles. These compounds (analogs **9a**, **10a**, and **10c**) were targeted as candidates for further development.

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