Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 16 (2006) 6102–6106
Bioorganic & Medicinal Chemistry Letters

# Inhibitors of unactivated p38 MAP kinase

James Bullington,[a,*] Dennis Argentieri,[a] Kristin Averill,[a] Demetrius Carter,[a] Druie Cavender,[a] Bohumila Fahmy,[a] Xiaodong Fan,[a] Daniel Hall,[a] Geoffrey Heintzelman,[a] Paul Jackson,[a] Wai-Ping Leung,[b] Xun Li,[a] Ping Ling,[b] Gilbert Olini,[a] Thomas Razler,[a] Michael Reuman,[a] Kenneth Rupert,[a] Ronald Russell,[a] John Siekierka,[a] Scott Wadsworth,[a] Russell Wolff,[a] Bangping Xiang[a] and Yue-Mei Zhang[a]

[a]*Johnson and Johnson Pharmaceutical Research and Development, L.L.C., 1000 Route 202, Raritan, NJ 08869, USA*
[b]*Johnson and Johnson Pharmaceutical Research and Development, L.L.C., 3210 Merryfield Row, LoJolla, CA 92121, USA*

Received 26 June 2006; revised 23 August 2006; accepted 28 August 2006
Available online 12 September 2006

**Abstract**—Inhibition of the p38 map kinase pathway has been shown to be beneficial in the treatment of inflammatory diseases. The first class of potent p38 kinase inhibitors was the pyridinylimidazole compounds from SKB. Since then several pyridinylimidazole-based compounds have been shown to inhibit activated p38 kinase in vitro and in vivo. We have developed a novel series of pyridinylimidazole-based compounds, which potently inhibit the p38 pathway by binding to unactivated p38 kinase and only weakly inhibiting activated p38 kinase activity in vitro.


With the success of anti-TNF-α biologics like Remicade, Enbrel, and Humira the desire for small molecule inhibitors of tumor necrosis factor-α (TNF) production has increased.[1] These drugs have demonstrated that blocking TNF production has a beneficial effect on rheumatoid arthritis (RA), ankylosing, spondylitis and systemic lupus erythematosus.

Since secretion of TNF is activated through the p38 MAP kinase pathway, many series of p38 MAP kinase inhibitors have been explored.[2] To date, no p38 inhibitor has advanced to the marketplace although several inhibitors have been clinically tested in humans and have advanced as far as Phase II.[3] Recent work has investigated new structural types and different binding modes.[2e] We now report potent pyridinylimidazole-based inhibitors of the p38 pathway which, unlike previous structures, have little effect on activated p38 catalytic activity in vitro. Instead, they potently bind to unphosphorylated p38 enzyme, consequently inhibiting its activation.

Previous work in our laboratories on pyridylpyrroles as p38 kinase inhibitors was impeded by cytochrome p450 inhibition.[4] (Fig. 1) The most promising structures of these early pyrroles potently inhibited both activated p38 kinase activity and LPS-induced TNF production in human peripheral blood mononuclear cells (PBMCs) in the single digit nanomolar range. They also inhibited TNF production orally in mice with $ED_{50}$s less than 10 mg/kg. However, this series potently inhibited multiple cytochrome p450 enzymes in the 100–300 nM range. Our goal was to reduce CYP450 inhibition while maintaining these biological profiles against p38.

It has been previously suggested that CYP inhibition can be reduced by introducing polarity on the side chain *ortho* to the pyridine ring.[5] We proposed applying this method to our pyrroles by replacing the propylphenyl

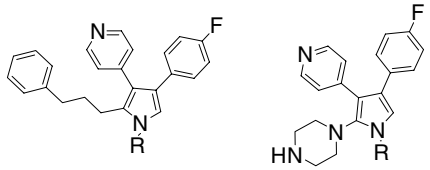



**Figure 1.** Changing the propylphenyl to piperazine.

*Keywords*: p38; Kinase; TNF; Pyrrole; Toxicity.
* Corresponding author. Tel.: +1 609 586 8123; e-mail: jbullington@ruxtonrx.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.08.101

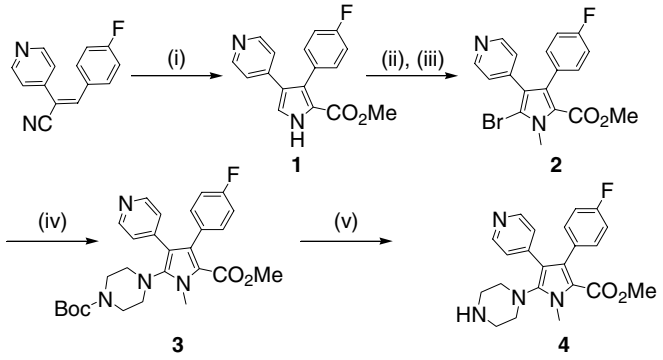
group of our earlier series with a piperazine (Fig. 1) to address CYP450 inhibition and increase solubility.

In order to selectively substitute the pyrrole ring we used an ester to block one side of the pyrrole, which later could be easily removed to give our target structures. The synthesis of a key intermediate pyrrole (**4**) was initiated by a base condensation of 4-pyridylacetonitrile with a substituted benzaldehyde using known methods to form the α,β-unsaturated nitrile (Scheme 1). The pyrrole ring (**1**) was formed by treating the unsaturated nitrile with methyl isocyanoacetate in base.[6] Alkylation of the pyrrole nitrogen was achieved in high yield by deprotonation with sodium hydride in DMSO at 20 °C and rapid addition of methyl iodide in one portion. Slower addition of MeI resulted in alkylation of the pyridine nitrogen to give the quaternary salt as a by-product. Next, bromination with NBS proceeded easily in moderate yield to give compound **2**. The bromide was converted to structure **3** via a palladium catalyzed amination utilizing $Pd_2(dba)_3$, BINAP, and $Cs_2CO_3$ in toluene. Many different palladium conditions were used in an attempt to increase the yield, but all results were inferior to the conditions stated above. Removal of the Boc group with TFA produced the unprotected piperazine (**4**), which although was an intermediate was screened for TNF inhibition.

Surprisingly, we found that this intermediate (**4**) potently inhibited TNF production in the PBMC assay ($IC_{50}$ = 33 nM). In addition, this compound had no effect on cytochrome p450 enzymes below 10 μM with the exception of the BFC substrate of 3A4 (1.3 μM). It was also orally potent in our in vivo mouse model ($ED_{50}$ = 7.5 mg/kg). We were again surprised to discover that compound **4** did not inhibit activated p38 kinase activity in vitro at concentrations up to 10 μM. Substitutions *ortho* to the phenyl ring typically reduce the potency of inhibitors of activated p38.[2d] Since our compounds were not inhibiting the activated p38 enzyme, the ester substitution was tolerated. The selectivity profile of compound **4** was excellent with $IC_{50}$s over 10 μM against 21 in-house targets and greater than 1 μM in a Cerep panel with the exception of 5-$HT_3$ receptor ($IC_{50}$ = 275 nM). Unfortunately, when we attempted a 5-day hepatomegaly study, this compound was found to be acutely toxic in mice ($n$ = 5) at 200 mg/kg.

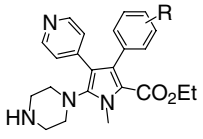
This prompted us to further explore the SAR in order to eliminate toxicity while maintaining the biological profile of this new series. We rapidly made some simple modifications to identify which regions of the molecule were responsible for the observed toxicity. Changing the substituents on the phenyl ring did not affect toxicity as shown by compounds **5**–**7** (Table 1). All compounds were still potent in the cellular and in vivo assays, but were also toxic.

Further modification of compound **4** was accomplished by methylation of the piperazine nitrogen via reductive amination using sodium triacetoxyborohydride and formaldehyde to give compound **8** (Table 2). This molecule was active in PBMCs and in the mouse model, inhibiting TNF production by 89 percent at 10 mg/kg. Although compound **8** was not toxic at 200 mg/kg, some lethargy was observed in the first few hours which



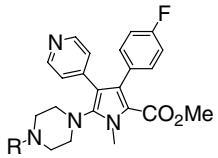

**Scheme 1.** Synthesis of compound **4**. Reagents and conditions: (i) $CNCH_2CO_2Me$, *t*-BuOK, THF, 0 °C, 89%; (ii) NaH, MeI, DMSO, 25 °C, 92%; (iii) NBS, $CH_2Cl_2$, 46%; (iv) $Pd_2$ $(dba)_3$, Boc-piperazine, BINAP, $Cs_2CO_3$, toluene, 100 °C, 40% (63% based on recovered SM); (v) TFA, $CH_2Cl_2$.

**Table 1.** Changing the aromatic substitution does not affect toxicity




| Compound | R | p38 enzyme $IC_{50}$ (nM) | TNF % inhibition (cell) $IC_{50}$ (nM) | TNF % inhibition (mouse) 10 mg/kg |
|---|---|---|---|---|
| **5** | 3-$CF_3$ | >10,000 | 21 | 83 |
| **6** | 4-Me | 3939 | 60 | 79 |
| **7** | 3,5-F | >10,000 | 93 | 79 |

**Table 2.** *N*-Alkyl derivatives of the piperazine reduce toxicity



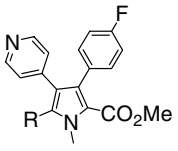
| Compound | R | TNF % inhibition (cell) $IC_{50}$ (nM) |
|---|---|---|
| **8** | Me | 95 |
| **9** | Et | 135 |
| **10** | *n*-Pr | 901 |
| **11** | *i*-Pr | 1981 |

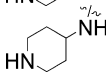
diminished with time. Since methylation reduced toxicity, reductive aminations were carried out to prepare the ethyl, *n*-propyl, and *i*-propyl piperazine derivatives (compounds **9**–**11**). We observed a decrease of TNF inhibition in the cell assay as the alkyl group increased in size. Similar to compound **8**, compound **9** demonstrated lethargy in the mouse at 200 mg/kg.

The reduced toxicity of compounds **8** and **9** prompted us to further investigate other functional groups at the 5 position of the pyrrole. We initially kept the methyl ester constant while replacing the piperazine with other heterocyclic rings (Table 3). Although pyrrolidine **12** and morpholine **13** were potent in the cell assay they did not show potency in vivo. Compound **14** was potent in the mouse model, inhibiting TNF production by 96% at 10 mg/kg, but again we observed lethargy in mice when dosed at 200 mg/kg. Compound **15** which introduced an amino linker was not potent in the cell assay and did not warrant further testing.

Additionally, we looked at acyclic analogs of the piperazine ring. These compounds were good inhibitors of TNF production in human PBMCs (Table 4). However compounds **16**–**18** did not potently inhibit TNF in vivo. Compound **19**, which is the methylated analog of compound **18**, inhibited TNF 78% and had an $ED_{50}$ of

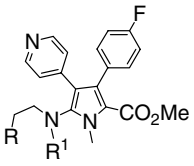
**Table 3.** Replacing the piperazine ring with other heterocycles



| Compound | R | TNF % inhibition (cell) $IC_{50}$ (nM) | TNF % inhibition (mouse) 10 mg/kg |
|---|---|---|---|
| **12** |  | 17 | ns |
| **13** |  | 47 | ns |
| **14** |  | 72 | 96 |
| **15** |  | 315 | nt |

ns, not significant; nt, not tested.

**Table 4.** Acyclic analogs of the piperazine

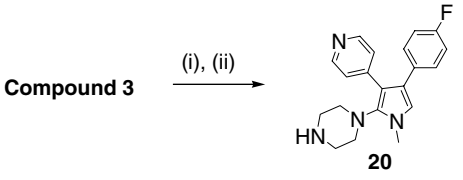


| Compound | R | $R^1$ | TNF % inhibition (cell) $IC_{50}$ (nM) |
|---|---|---|---|
| **16** |  | H | 98 |
| **17** |  | H | 92 |
| **18** |  | H | 50 |
| **19** |  | Me | 38 |

3 mg/kg in the in vivo mouse model. Unfortunately, this compound was found to be rapidly metabolized in human liver microsomes. In order to account for metabolism issues we injected LPS 2 h after dosing mice instead of our original 0.5 h. This helped us to filter out potential metabolically unstable analogs through in vivo potency in mice. For example, compound **19** was ineffective with this new time course.

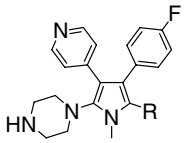
Examining the importance of the ester with respect to toxicity, we saponified compound **3** to the acid, which spontaneously decarboxylated at room temperature. This structure was deprotected with TFA to produce compound **20** (Scheme 2), which lacked the ester of our lead structure **4** (Scheme 1). Compound **20** had an $IC_{50}$ of 15 nM in the cell assay and inhibited TNF production by 75 percent in the in vivo mouse model at 10 mg/kg. Mice treated at 200 mg/kg survived, showing that the ester also plays a role in the toxicity of compound **4**.

Furthermore, we replaced the methyl ester with ketones and nitriles (**21**–**24**) which maintained good inhibition in the cellular assay. Unlike ketones **21**–**23** the nitrile showed good in vivo inhibition in mice (Table 5). Compound **21** showed lethargy in the mouse toxicity screen, but compound **22** did not show any signs of toxicity at 200 mg/kg. The bulkier aryl ketone and amides (**25**–**27**) possessed significantly reduced potency of TNF inhibition in the cell assays.




**Scheme 2.** Reagents: (i) KOH, MeOH, $NH_4Cl$; (ii) TFA, $CH_2Cl_2$.

**Table 5.** Ester replacements



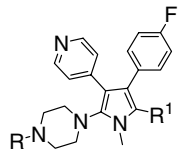
| Compound | R | TNF % inhibition (cell) $IC_{50}$ (nM) | TNF % inhibition (mouse) 10 mg/kg |
|---|---|---|---|
| **21** | COCOMe | 28 | 52 |
| **22** | COMe | 40 | 39 |
| **23** | COEt | 67 | 32 |
| **24** | CN | 18 | [a] |
| **25** | COPh | 660 | nt |
| **26** | CONHMe | 779 | nt |
| **27** | CONH-*i*Pr | 10,000 | nt |

[a] $ED_{50}$, 5 mg/kg; nt, not tested.

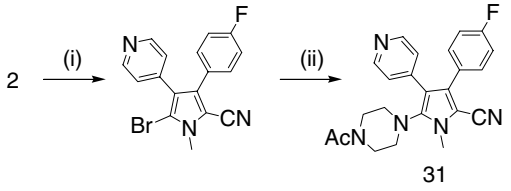
Since the ketones and nitriles looked promising, we examined modifications to the piperazine along with these ester replacements. We hoped to achieve an additive effect from lowering toxicity on two regions of the molecule (Table 6). Compound **28** shows good inhibition in the cell assay and also in the in vivo model when tested in the original 0.5 h assay, but when tested in the 2 h assay the potency was reduced indicating a possible short half-life. This compound was non-toxic at 200 mg/kg, but with the potential metabolism issue the compound may not be present in high enough concentrations to show toxicity in this model. Unlike the ester derivative (**8**) methylation of the piperazine when a methyl ketone is present (**29**) decreased potency dramatically when compared to its unsubstituted analog (compound **22**). Methylation of the nitrile derivative (**30**) follows similar SAR to the ester series.

We were pleased to find that compound **31** was potent in the TNF mouse model with an $ED_{50}$ of 0.5 mg/kg and also non-toxic at 200 mg/kg. It weakly inhibited activated p38 catalytic activity ($IC_{50}$ = 2 μM) and bound with high affinity to unphosphorylated p38α ($K_d$ = 100 nM). Furthermore, compound **31** inhibited p38 phosphorylation by the upstream kinases.

**Table 6.** Combining the R and $R^1$ effects of reducing toxicity



| Compound | R | $R^1$ | TNF % inhibition (cell) $IC_{50}$ (nM) | TNF % inhibition (mouse) 10 mg/kg | |
|---|---|---|---|---|---|
| | | | | 0.5 h | 2.0 h |
| **28** | COMe | COMe | 43 | 61 | 28 |
| **29** | Me | COMe | 529 | | 40 |
| **30** | Me | CN | 80 | | 47 |
| **31** | COMe | CN | 123 | | 81 |



**Scheme 3.** Synthesis of compound **31**. Reagents and conditions: (i) compound **2**, $NH_3$, $(CH_3)_3Al$, 125 °C, 68%; (ii) $Pd(OAc)_2$, acetylpiperazine, BINAP, $Cs_2CO_3$, toluene, 100 °C, 93%.

The synthesis of compound **31** is shown in Scheme 3. Starting with intermediate **2** (Scheme 1), a one-step conversion of the ester to a nitrile was accomplished by heating to 125 °C with trimethylaluminum in ammonia. This nitrile was used in a palladium amination reaction using acetylpiperazine in conditions similar to reaction (iv) in Scheme 1 to give structure **31**.

This molecule did not inhibit cytochrome p450 enzymes below 15 μM for CYP1A2, 2D6, and 3A4 (BQ, RBE, DBF, and BFC substrates). It weakly inhibited CYP2C9 and CYP2C19 with an $IC_{50}$ of 4, 8 μM, respectively. The hydrochloride salt of this molecule easily dissolved in water at 100 mg/mL and had good bioavailability of 70–100% in mice, rats, dogs, and monkeys. When tested in vivo, compound **31** showed a 50–60% reduction of the clinical score in the collagen-induced arthritis (CIA) mouse at 30 mg/kg (po). It also reduced swelling 91% in the adjuvant-induced arthritis rat at 30 mg/kg (po). Compound **31** also had an $ED_{50}$ of 3 mg/kg in the carrageenan hind paw edema model. These data supported the advancement of compound **31** to our preclinical development pipeline.

## References and notes


1. (a) Revesz, L.; Blum, E.; Di Padova, F. E.; Buhl, T.; Feifel, R.; Gram, H.; Hiestand, P.; Manning, U.; Neumann, U.; Rucklin, G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 262; (b) Bao, J.; Hunt, J. A.; Miao, S.; Rupprecht, K. M.; Stelmach, J. E.; Liu, L.; Ruzek, R. D.; Sinclair, P. J.; Pivnichny, J. V.; Hop, C. E. C. A.; Kumar, S.; Zaller, D. M.; Shoop, W. L.; O'Neill, E. A.; O'Keefe, S. J.; Thompson, C. M.; Cubbon, R. M.; Wang, R.; Zhang, W. X.; Thompson, J. E.; Doherty, J. B. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 64; (c) Michelotti, E. L.; Moffett, K. K.; Nguyen, D.; Kelly, M. J.; Shetty, R.; Chai, X.; Northrop, K.; Namboodiri, V.; Campbell, B.; Flynn, G. A.; Fujimoto, T.; Hollinger, F. P.; Bukhtiyarova, M.; Springman, E. B.; Karpusas, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5274; (d) Liu, C.; Wrobleski, S. T.; Lin, J.; Ahmed, G.; Metzger, A.; Wityak, J.; Gillooly, K. M.; Shuster, D. J.; McIntyre, K. W.; Pitt, S.; Shen, D. R.; Zhang, R. F.; Zhang, H.; Doweyko, A. M.; Diller, D.; Henderson, I.; Barrish, J. C.; Dodd, J. H.; Schieven, G. L.; Leftheris, K. *J. Med. Chem.* **2005**, *48*, 6261.
2. (a) Miwatashi, S.; Arikawa, Y.; Kotani, E.; Miyamoto, M.; Naruo, K.; Kimura, H.; Tanaka, T.; Asahi, S.; Ohkawa, S. *J. Med. Chem.* **2005**, *48*, 5966; (b) Miwatashi, S.; Arikawa, Y.; Naruo, K.; Igaki, K.; Watanabe, Y.; Kimura, H.; Kawamoto, T.; Ohkawa, S. *Chem. Pharm. Bull.* **2005**, *53*, 410; (c) Tamayo, N.; Liao, L.; Goldberg, M.; Powers, D.; Tudor, Y. Y.; Yu, V.; Wong, L. M.; Henkle, B.; Middleton, S.; Syed, R.; Harvey, T.; Jang, G.; Hungate, R.; Domin-

guez, C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2409; (d) Jackson, P. F.; Bullington, J. L. *Curr. Top. Med.* **2002**, *2*, 1011; (e) Cirillo, P. F.; Pargellis, C.; Regan, J. *Curr. Top. Med.* **2002**, *2*, 1021.
3. Pargellis, C.; Regan, J. *Curr. Opin. Invest. Drugs* **2003**, *4*, 566.
4. Bullington, J. L. U.S. Patent 6,562,843, 2003.
5. (a) Laufer, S. A.; Zimmermann, W.; Ruff, K. J. *J. Med. Chem.* **2004**, *47*, 6311; (b) Adams, J. l.; Boehm, J. C.; Kassis, S.; Gorycki, P. D.; Webb, E. F.; Hall, R.; Sorenson, M.; Lee, J. C.; Ayrton, A.; Griswold, D. E.; Gallagher, T. F. *Bioorg. Med. Chem. Lett.* **1998**, 3111.
6. Bullington, J. L.; Wolff, R. R.; Jackson, P. F. *J. Org. Chem.* **2002**, *67*, 9439.