Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 4242–4247
Bioorganic & Medicinal Chemistry Letters

New modifications to the area of pyrazole-naphthyl urea based p38 MAP kinase inhibitors that bind to the adenine/ATP site

Neil Moss,[a,*] Steffen Breitfelder,[b] Raj Betageri,[a] Pier F. Cirillo,[a] Tazmeen Fadra,[a] Eugene R. Hickey,[a] Thomas Kirrane,[a] Rachel R. Kroe,[a] Jeffrey Madwed,[a] Richard M. Nelson,[a] Christopher A. Pargellis,[a] Kevin C. Qian,[a] John Regan,[a] Alan Swinamer[a] and Carol Torcellini[a]

[a]*Departments of Medicinal Chemistry, Immunology and Inflammation, Cardiovascular Disease, Biologics and Biomolecular Sciences, or Drug Discovery Support, Boehringer Ingelheim Pharmaceutical, Inc., 900 Ridgebury Road, Ridgefield, CT 0687, USA*
[b]*Department of Medicinal Chemistry, Boehringer Ingelheim Pharma GmbH and Co. KG, Birkendorfer Strasse 65, D-88397 Biberach an der Riss, Germany*

Received 12 March 2007; revised 10 May 2007; accepted 11 May 2007
Available online 18 May 2007

Abstract—Discovery of the pyrazole-naphthyl urea class of p38 MAP kinase inhibitors typified by the clinical candidate **BIRB 796** has encouraged further exploration of this particular scaffold. Modification to the part of the inhibitor that occupies the adenine/ATP binding site has resulted in a new way to obtain potent inhibitors that possess favorable in vitro and in vivo properties.

Considerable research over the past several years has been devoted to the identification of inhibitors of p38 MAP kinase.[1] This enzyme acts as a key regulator in the signaling pathways leading to the production of several proinflammatory cytokines such as TNFα and IL-1.[2] The clinical and commercial success of anti-TNFα biologics such as Enbrel®, Remicade®, and Humira® for arthritis alone highlights the therapeutic benefit of proinflammatory cytokine inhibition.[3] Consequently, researchers anticipate that p38 inhibitors will display at least similar therapeutic benefit as the anti-cytokine biologics with the convenience of oral dosage.

We previously disclosed a class of p38 inhibitors based on a pyrazole-naphthyl urea scaffold as typified by the clinical candidate **BIRB 796**.[4,5] A distinguishing feature of this class of compounds is a binding mode to p38 that involves interactions beyond those typically found with many ATP site kinase inhibitors.[6] In addition to commonly observed interactions in the adenine binding site and the adjacent kinase specificity pocket, this class of compounds also takes advantage of binding sites made available by movement of a phenylalanine found in the conserved DFG motif of the kinase activation loop.[7] Figure 1 graphically depicts key interactions made between **BIRB 796** and p38. Upon considering additional ways to expand on the potential of this pyrazole-naphthyl urea scaffold, we directed attention to the role and

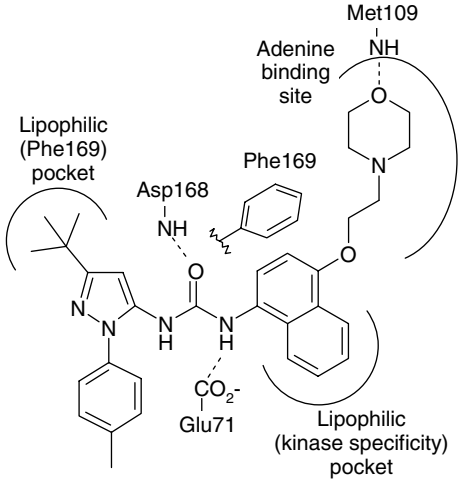
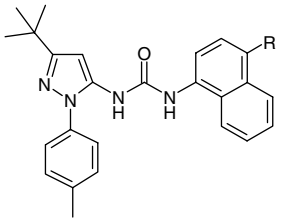


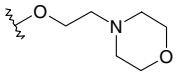
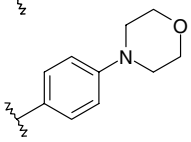
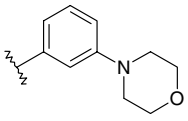
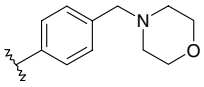
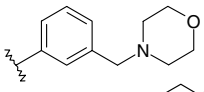
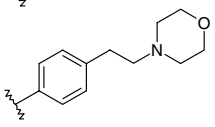
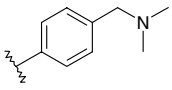
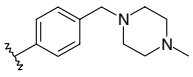
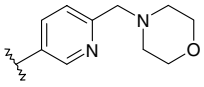
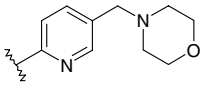
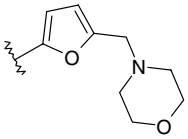
Figure 1. Graphical representation of key interactions between **BIRB 796** and p38.

Keywords: p38 MAP kinase; Inhibitor.
* Corresponding author. Tel.: +1 203 798 5101; fax: +1 203 791 6072; e-mail: nmoss@rdg.boehringeringleheim

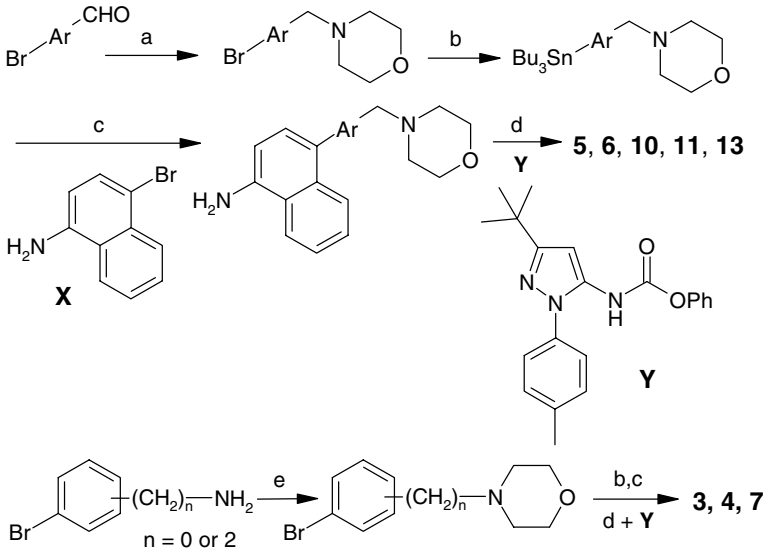
0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.05.042

Table 1. Effect of R group on potency



Compound	R	T_m (°C)[a]	Inhibition of TNFα from THP-1 cells IC_{50} (nM)[b]	Inhibition of TNFα from human whole blood IC_{50} (nM)[c]
BIRB 796		63.5	18	780
1		55.5	320	>15,000
2		55.5	1430	>15,000
3		55.5	470	>15,000
4		54.9	1350	>15,000
5		59.9	36	1100
6		57.1	56	3900
7		57.7	56	1700
8		60.1	104	9200
9		61.0	46	3600
10		63.6	9	230
11		62.3	26	590
12		55.3	390	—
13		59.4	70	6400

[a] Values are means of three experiments, standard deviation 0.1–0.5 °C.
[b] Values are means of 2–5 experiments, standard deviations for $n > 2$ experiments typically ±50% of reported value.
[c] Values are geometric means from 8–12 donor experiments, standard deviation typically ±50% of reported value.



Scheme 1. Reagents and conditions: (a) Morpholine, $NaHB(OAc)_3$, HOAc, THF, rt, 3 h, 31–91%; (b) *t*-BuLi, THF, Bu_3SnCl, −78 °C, 42–77%; (c) $Pd(PPh_3)_4$, dioxane, 100 °C, 24 h, 29–76%; (d) DMSO, 45 °C 12–16 h, 49–81%; (e) $(BrCH_2CH_2)_2O$, *i*-$PrNEt_2$, DMF, 31–90%.

contribution of the ethoxy morpholino group of **BIRB 796** that occupies the adenine binding site of p38. The perceived flexible nature and relatively small size of this group compared to the size and flexibility of groups from other classes of inhibitors that occupy the adenine/ATP site encouraged us to look for other ways to utilize the pyrazole-naphthyl urea scaffold.

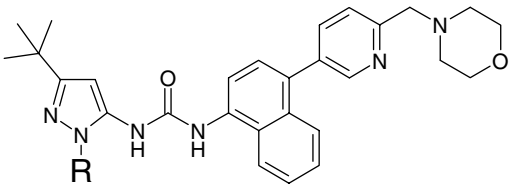
The key molecules in this paper originated from the idea of using an aromatic ring appended to the naphthyl group of compound **1** as a template for adding functionality that could engage in interactions with the adenine/ATP binding site. Compound **2** was prepared as the initial test case. Cross coupling of naphthyl bromide **X** with phenylboronic acid provided a phenylnaphthyl amine product that upon treatment with pyrazole carbamate **Y** gave compound **2**. The equivalent molecular potency of compounds **1** and **2** (Table 1) as determined by a thermal denaturation assay (3.0–3.5 °C change in T_m roughly equivalent to 10-fold change in binding potency)[8] showed that a phenyl ring was tolerated and could potentially serve as a useful template. We previously demonstrated that the presence of the morpholine group in **BIRB 796** not only improved molecular potency but also improved potency in cell assays of LPS-induced TNFα production.[5] Consequently, the morpholine group became a logical first choice to append to the phenyl ring of compound **2** using a 0–2 carbon linker (compounds, **3**–**7**). Scheme 1 outlines general methodology applicable to most of the compounds in Table 1 and relies on the Stille coupling of a morpholine-containing aryl tin derivative to naphthyl bromide **X**.[9] Completion of the final urea molecule involves reacting the resultant arylnaphthyl amine with pyrazole carbamate **Y**.

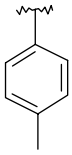
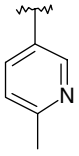
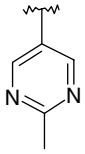
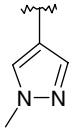
As can be seen from a comparison of compounds **2**–**7** in Table 1, appending a morpholine group to the phenyl ring of compound **2** via one or two methylene groups results in an increase of both molecular and cellular potency, while direct attachment has little positive or negative effect. We obtained a similar positive result through appending a morpholine group to a furan (c.f. compounds **12** and **13**). Since the overall potency values of compound **5** trended to be best of the morpholine analogues, it became a frame of reference for additional modifications. Replacement of the morpholine oxygen atom in **BIRB 796** with a CH_2 or NH resulted in an 8 °C drop in T_m (>250-fold drop in calculated K_d).[5] This structure–activity result supported the X-ray co-crystal observation that the morpholine oxygen of **BIRB 796** engages in a key hydrogen bond with the NH of Met 109, the same amino acid that interacts with the adenine group of ATP. Consequently, it was initially surprising that the dimethyl amino and the *N*-methyl piperidinyl analogues of compound **5** (compounds **8** and **9**) possessed essentially equal binding potency to compound **5**. These results clearly indicated that the appended morpholine group in compound **5** was improving binding potency in a manner distinct from the morpholine group in **BIRB 796**. While compounds **5**, **8**, and **9** have very similar molecular potency, the morpholine derivative **5** possessed the best potency in an assay of LPS-induced TNFα production in human whole blood.

Compound **5** is significantly more lipophilic (could not determine a $\log D_{7.4}$) than **BIRB 796** ($\log D_{7.4} = 4.3$). One way we explored to reduce lipophilicity involved replacement of the right hand side phenyl group in compound **5** with a pyridyl (compounds **10** [$\log D_{7.4} = 5.2$] and **11**). This modification resulted in a modest increase in molecular potency, to the level of **BIRB 796**. More significantly, the cellular potency, particularly of compound **10** in human whole blood, proved better than **BIRB 796**.

The successful outcome of introducing a pyridyl group encouraged an investigation of nitrogen heterocycles

Table 2. Effect of R group on potency



Compound	R	log $D_{7.4}$	T_m (°C)[a]	Inhibition of TNF-α from		
				THP-1 cells IC_{50} (nM)[b]	Human whole blood IC_{50} (nM)[c]	LPS treated mice at dose (mg/kg)
10		5.2	63.6	9	230	74% at 10 NS[d] at 3
14		3.9	59.6	32	950	NS at 10
15		4.2	65.1	11	200	86% at 10 50% at 3
16		4.0	63.7	14	160	95% at 10 85% at 3
17		3.9	64.3	11	90	73% at 3

[a] Values are means of three experiments, standard deviation 0.1–0.5 °C.
[b] Values are means of 2–5 experiments, standard deviations for $n > 2$ experiments typically ±50% of reported value.
[c] Values are geometric means from 8–12 donor experiments, standard deviation typically ±50% of reported value.
[d] Not statistically significant inhibition.

on the other end of the molecule. Given the still suboptimal physicochemical properties of compound **10**, one intention was to investigate the consequences of further decreasing overall lipophilicity (Table 2). The left hand side fragments of compounds **14** and **15** were prepared as previously reported.[5] The pyrimidine and pyrazole containing left hand sides required for the preparation of analogues **16** and **17** relied on the vinamidinium chemistry outlined in Scheme 2.

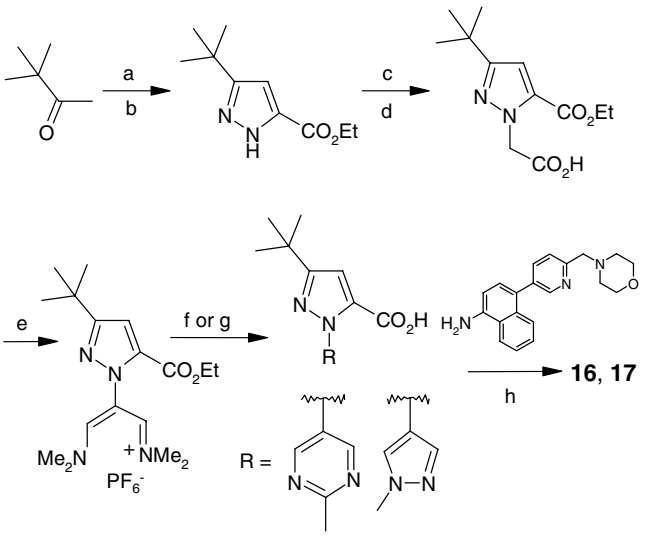
The decrease in potency observed by replacing the lipophilic tolyl group in **10** with a methyl group (**14**) confirms the importance of a bulkier moiety at this position as previously observed with **BIRB 796**.[5] Curiously the drop in T_m in this series is significantly less (4.0 °C) than for **BIRB 796** (9.0 °C). In contrast, decreasing lipophilicity through introduction of nitrogen heterocycles at this position has little impact on molecular potency while trending to improve potency in the human whole blood assay. The impact of these heterocycles becomes more substantial when comparing the potency in a mouse model of LPS-induced TNFα production. In this well-established model, mice are dosed 30 min prior to LPS challenge, and plasma TNFα levels are determined 1 h after LPS challenge.[10] While **BIRB 796** inhibits 63% of TNFα at a dose of 10 mg/kg,[5] the heterocyclic compounds **16** and **17** in particular trend to better efficacy at 3-fold lower dose. The reason for this improvement in in vivo potency is not immediately clear. The protein binding for **BIRB 796**, **10**, and **16** are all greater than 97% as determined by equilibrium dialysis. A determination of compound plasma concentrations at 90 min post dosing reveals levels of compounds **16** and **17** that are at least 10-fold lower than those observed for either **BIRB 796** or compound **10**. One might assume the concentrations of these compounds in the tissues most responsible for TNFα

production to be significantly different than those seen in plasma.

The kinase selectivity profile of compound **10** appears similar to **BIRB 796**.[11] While compound **10** does not inhibit over 40 kinases at a test concentration of 3 μM, it does inhibit Jnk 2 (IC_{50} 2–5 nM), cRaf (IC_{50} 400 nM), and Src, Lyn, and Abl (IC_{50} 1–2 μM).

The differential SAR observed between the portions of **BIRB 796** and compound **10** that occupy the adenine binding site of p38 encouraged us to obtain an X-ray co-crystal structure of compound **10**.[12] Unlike the morpholine group of **BIRB 796**, the morpholine group of compound **10** does not appear to be involved in any obvious hydrogen bonding interactions in the adenine binding site (see Fig. 2). This is consistent with the similar T_{ms} observed for compounds **5**, **8**, and **9**. However, the combination of the pyridyl and the morpholine together does occupy more of the adenine/ATP binding site than does the ethoxy morpholine group of **BIRB 796**. Since the morpholine and other dialkyl amino groups in this new series of compounds do appear important for good binding potency, this may be a consequence of van der Waals interactions and/or desolvation within the adenine/ATP binding site. An additional notable feature is a water mediated hydrogen bond between the pyridyl nitrogen and the NH of Met 109 and carbonyl of His 107. This indirect interaction may contribute to the increased potency of compound **10** over compound **5**.

The results discussed herein highlight an extension to our previously reported class of potent p38 kinase inhibitors. These new compounds take advantage of interactions in the adenine binding site of p38 unique from **BIRB 796**. Investigations to decrease the overall lipophilicity of this class of compounds resulted in compounds with substantially improved potency in the mouse model of LPS-induced TNFα production, and thus further highlight the therapeutic potential of this class of compounds.



Scheme 2. Reagents and condition: (a) LiHMDS, THF, diethyl oxalate; (b) H_2NNH_2, HOAc, 90 °C, 66% two steps; (c) KO^{t}Bu, DMSO, *t*-butyl bromoacetate, 70%; (d) TFA, 94%; (e) $POCl_3$, DMF, $NaPF_6$, H_2O, 78%; (f) acetamidine hydrochloride, NaOEt, EtOH, 60%; (g) *N*-methylhydrazine, EtOH, LiOH, MeOH, 78%; (h) NEt_3, PhH, DPPA, naphthylamine X, 50–75%.

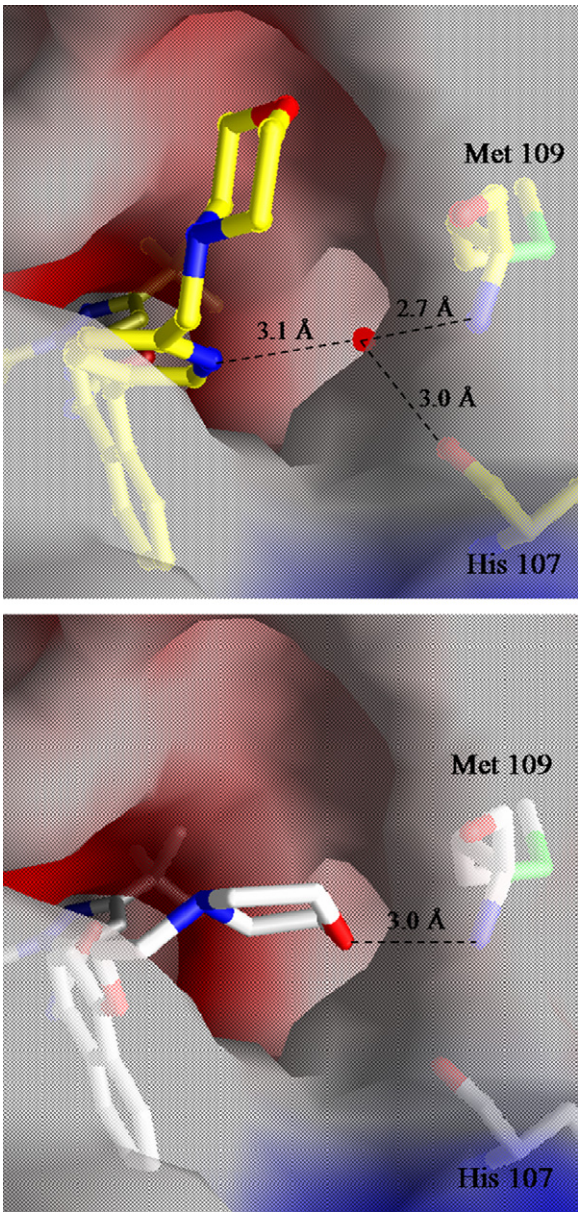


Figure 2. Comparison of compound **10** (top, yellow) and **BIRB 796** (bottom, white) in the adenine binding site of p38.

References and notes

1. (a) Dominguez, C.; Powers, D. A.; Tamayo, N. *Curr. Opin. Drug Disc. Dev.* **2005**, *8*, 421; (b) Nikas, S. N.; Drosos, A. A. *Curr. Opin. Invest. Drugs* **2004**, *5*, 1205; (c) Cirillo, P. F.; Pargellis, C.; Regan, J. *Curr. Top. Med. Chem.* **2002**, *2*, 1021.
2. (a) Saklatvala, J. *Curr. Opin. Pharm.* **2004**, *4*, 372; (b) Kumar, S.; Boehm, J.; Lee, J. C. *Nat. Rev. Drug Disc.* **2003**, *2*, 717; (c) Ono, K.; Han, J. *Cell. Signal.* **2000**, *12*, 1.

3. (a) Klinkhoff, A. *Drugs* **2004**, *64*, 1267; (b) Barry, J.; Kirby, B. *Expert Opin. Biol. Ther.* **2004**, *4*, 975.
4. Regan, J.; Breitfelder, S.; Cirillo, P.; Gilmore, T.; Graham, A. G.; Hickey, E. R.; Klaus, B.; Madwed, J.; Moriak, M.; Moss, N.; Pargellis, C. A.; Pav, S.; Proto, A.; Swinamer, A.; Tong, L.; Torcellini, C. *J. Med. Chem.* **2002**, *45*, 2994.
5. Regan, J.; Capolino, A.; Cirillo, P. F.; Gilmore, T.; Graham, A. G.; Hickey, E.; Kroe, R. R.; Madwed, J.; Moriak, M.; Nelson, R.; Pargellis, C. A.; Swinamer, A.; Torcellini, C.; Tsang, M.; Moss, N. *J. Med. Chem.* **2003**, *46*, 4676.
6. Pargellis, C. A.; Tong, L.; Churchill, L.; Cirillo, P.; Gilmore, T.; Graham, A. G.; Grob, P. M.; Hickey, E. R.; Moss, N.; Pav, S.; Regan, J. *Nat. Struct. Biol.* **2002**, *9*, 272.
7. Mol, C. D.; Fabbro, D.; Hosfield, D. J. *Curr. Opin. Drug Disc. Dev.* **2004**, *7*, 648.
8. Kroe, R. R.; Regan, J.; Proto, A.; Peet, G. W.; Roy, T.; Dickert, L.; Fuschetto, N.; Pargellis, C. A.; Ingraham, R. H. *J. Med. Chem.* **2003**, *46*, 4675, T_m values can be converted to an approximate K_a using the formula $T_m = 3.08(\log K_a) + 31.2$.
9. Certain experimental details can be found in: Betageri, R.; Breitfelder, S.; Cirillo, P. F.; Gilmore, T. A.; Hickey, E. R.; Kirrane, T. M.; Moriak, M. H.; Moss, N.; Patel, U. R.; Proudfoot, J. R.; Regan, J. R.; Sharma, R.; Sun, S.; Swinamer, A.; Takahashi, H. U.S. Patent 6,660,732, 2003.
10. Experimental details for this model can be found in Ref. 5.
11. Additional perspective on the kinase selectivity profile of **BIRB 796** can be found in the supplementary information of the following publication. Compound **10** was not screened against many of the kinases in this publication so the provided selectivity information should be regarded as a limited indication of overall selectivity. Fabian, M. A.; Biggs, W. H.; Treiber, D. K.; Atteridge, C. E.; Azimioara, M. D.; Benedetti, M. G.; Carter, T. A.; Ciceri, P.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Galvin, M.; Gerlach, J. L.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Insko, M. A.; Lai, A. G.; Lelias, J.-M.; Mehta, S. A.; Milanov, Z. V.; Velasco, A.; Wodicka, L. M.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J. *Nat. Biotechnol.* **2005**, *23*, 329.
12. The coordinates of compound **10** bound to p38 have been deposited with the RCSB Protein Data Bank (access code 2PUU).