Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3101–3104

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Kinetics of Binding to p38 MAP Kinase by Analogues of BIRB 796

John Regan,[a,*] Christopher A. Pargellis,[b] Pier F. Cirillo,[a] Thomas Gilmore,[a] Eugene R. Hickey,[a] Gregory W. Peet,[b] Alfred Proto,[b] Alan Swinamer[a] and Neil Moss[a]

[a]*Departments of Medicinal Chemistry, Boehringer Ingelheim Pharmaceuticals, 900 Ridgebury Road, Ridgefield, CT 06877, USA*
[b]*Immunology and Inflammation, Boehringer Ingelheim Pharmaceuticals, 900 Ridgebury Road, Ridgefield, CT 06877, USA*

Received 14 April 2003; accepted 20 April 2003

Abstract—BIRB 796, a member of the *N*-pyrazole-*N*′-naphthly urea class of p38 MAPK inhibitors, binds to the kinase with both slow association and dissociation rates. Prior to binding, the kinase undergoes a reorganization of the activation loop exposing a critical binding domain. We demonstrate that, independent of the loop movement, association rates are governed by low energy conformations of the inhibitor and polar functionality on the tolyl ring. As anticipated, the dissociation rates of the inhibitors from the kinase are slowed by lipophilic and hydrogen bond interactions. The value of structure-kinetic relationships (SKR) in drug design is discussed.

A number of autoimmune diseases are associated with elevated levels of the pro-inflammatory cytokines TNFα and IL-1β. The observed pathophysiology in patients with diseases such as rheumatoid arthritis (RA), osteoarthritis, diabetes and inflammatory bowel disease is often the result of chronic inflammation.[1,2] Cytokines regulate the communication between many cell types associated with RA, including endothelial cells, infiltrating leukocytes, resident macrophages, mast cells, epithelial cells, and osteoclasts. Targeting the extracellular reduction of pro-inflammatory cytokine levels is an important advance in treating these diseases. Several biological agents (adalimumab, anakinra, etanercept, infliximab) have received FDA approval as anti-inflammatory therapies.[3–6] The production of cytokines is regulated, in part, by the mitogen-activated protein kinase (MAPK) signaling pathway. A critical kinase in this cascade is p38 MAPK which, upon activation, undergoes dual phosphorylation on the Thr-Gly-Tyr segment of the activation loop. Once activated, p38 phosphorylates other kinases and activates transcription factors leading to the regulation of target genes.[7–11] Potent and specific inhibitors of p38 MAPK, such as SB203580 (1) (Chart 1), have established that p38 mediates multiple cellular responses including the production of inflammatory cytokines.[12] In response to this discovery, numerous synthetic efforts produced a chemically diverse collection of p38 inhibitors.[13,14]

We reported the discovery of p38 inhibitor BIRB 796 (**2**) (Chart 1) which recently entered Phase II human clinical trials for the treatment of autoimmune disorders.[15] The identification of **2** from our lead compound **3** included appending a tolyl group to the pyrazole nucleus and replacing the chlorophenyl ring with naphthalene containing an ethoxy morpholine group. The X-ray crystallographic complex of **2** with human p38α reveals lipophilic binding of the *t*-butyl, tolyl, and naphthalene groups with the kinase. Hydrogen bonding interactions of **2** with p38 include a urea hydrogen with Glu71, the urea oxygen with Asp168, and the morpholine oxygen with Met109 (Fig. 1). In addition, we described slow binding kinetics for the urea class of inhibitors. In what we hypothesize to be the rate-determining step, a segment of the activation loop common to most kinases (Asp168, Phe169, Gly170), undergoes a substantial reorganization.[16] Before the loop movement (DFG-in conformation), the phenyl group of Phe169 resides in a lipophilic pocket between the two lobes of the kinase. As part of the movement, the phenyl group vacates the pocket (Phe pocket), moves approximately 10Å, and participates in a lipophilic interaction with the aryl portion of the inhibitor

*Corresponding author: Tel.: +1-203-798-4768; fax: +1-203-791-6072; e-mail: jregan@rdg.boehringer-ingelheim.com

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00656-5

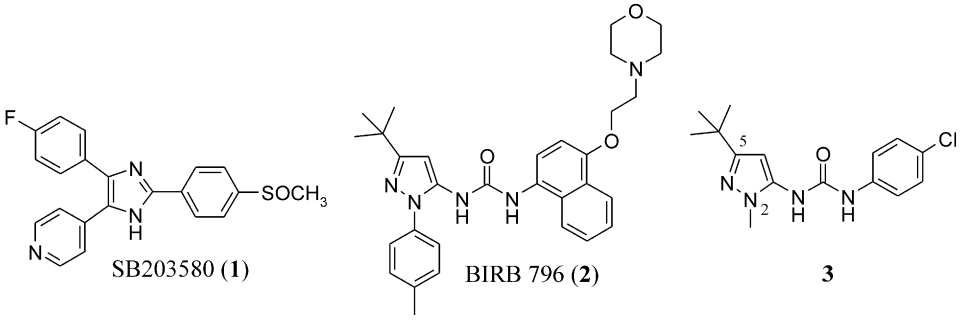


Chart 1.

(DFG-out conformation). The vacated Phe pocket is filled with the *t*-butyl group of the inhibitor.

For the slow binding of the urea class of compounds to p38, we developed a kinetic assay based on paired progression curves.[15] This assay can dissect the contribution that the rates of association (k_{on}) and dissociation (k_{off}) have on K_d with the kinase ($K_d = k_{off}/k_{on}$). This method provides an opportunity to observe how differences in binding affinity originate by examining the changes to association and dissociation rates of the inhibitors with the kinase. In this report, we establish the structure–kinetic relationship (SKR) for analogues of **2** and show how this information can be useful to the drug design process. An SKR analysis of ATP-binding p38 inhibitors[17] and HIV-1 protease inhibitors[18] have recently been reported.

In the urea class of inhibitors, the *t*-butyl group occupies the Phe pocket following the DFG-out movement of the kinase (Fig. 1). The importance of this group to binding kinetics is seen with compounds containing changes to the size and polarity of this pharmacophore. Replacing the *t*-butyl group with hydrogen provides a compound (**4**) with an association rate (k_{on}) and dissociation rate (k_{off}) too weak to measure kinetically (Table 1). However, *iso*-propyl analogue **6** and *t*-butyl compound **5** show comparable association rates to the kinase. The faster dissociation of **6** compared to **5** is presumably a consequence of diminished lipophilic interactions that produce weaker binding affinity (K_d). For the polarity modification, no significant difference in association is seen for **2** and hydroxymethyl analogue **7**. The 50-fold faster dissociation of **7**, compared to **2**, results in a substantial decrease in binding affinity probably due to a decrease in lipophilic interactions. Thus, reducing the size of the *t*-butyl group or introducing polar functionality primarily increases the dissociation rate. The lack of effect on association rate is consistent with the pro-

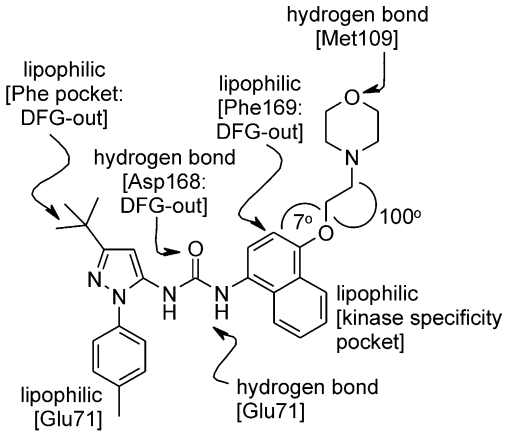
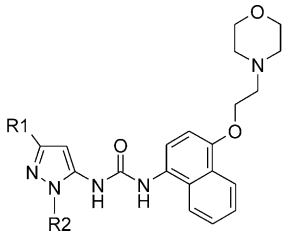
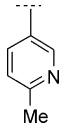
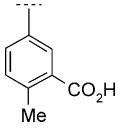
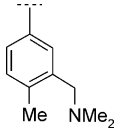
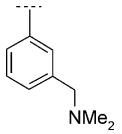


Figure 1. Summary of key interactions of BIRB 796 (**2**) with human p38α. The contacted amino acid residues and domains are in brackets.

Table 1. Effects of size and lipophilicity at C-5 and tolyl modifications at N-2 of the pyrazole ring



Compd	R1	R2	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	K_d (nM)
2	*t*-Butyl	*p*-Tolyl	84,900	8.3×10^{-6}	0.098
4	H	Phenyl	—	—	2000[a]
5	*t*-Butyl	Phenyl	154,000	1.5×10^{-5}	0.097
6	*iso*-Propyl	Phenyl	64,800	3.8×10^{-4}	5.9
7	$HOCH_2(Me)_2C$	*p*-Tolyl	25,400	4.0×10^{-4}	16
8	*t*-Butyl	Me	145,000	3.3×10^{-3}	23
9	*t*-Butyl		21,500	2.3×10^{-5}	1.1
10	*t*-Butyl		25,200	6.2×10^{-5}	2.5
11	*t*-Butyl		7900	3.8×10^{-5}	4.8
12	*t*-Butyl		1600	2.6×10^{-5}	16

[a] K_d value determined by thermal calorimetry.

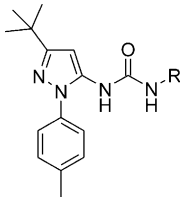
posed mechanism that the DFG-out movement is a primary factor for determining association between inhibitor and kinase.

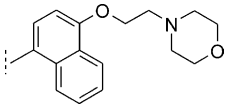
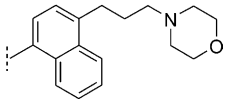
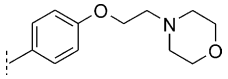
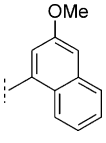
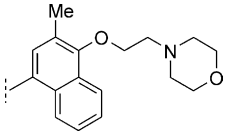
We previously demonstrated that aromatic rings attached to N-2 of the pyrazole nucleus bind tighter to p38 than alkyl groups (i.e. **3**).[19] Binding of the aromatic ring with the kinase occurs with a π–CH_2 lipophilic interaction with the alkyl portion of Glu71. As seen in Table 1, replacing the tolyl group of **2** with methyl (**8**) does not significantly alter the association rate between the two compounds. However, due to the diminished lipophilic binding, the dissociation of **8** from the kinase increases 400-fold. This result further supports the concept that the DFG-out movement of the kinase is a primary determinant of the association rate. In an attempt to address the physicochemical properties of **2**, pyridine (**9**) and phenyl rings with polar substituents, such as carboxylic acid (**10**) and dimethylaminomethyl (**11** and **12**) were prepared. Interestingly, these polar modifications tended towards decreasing association rates compared to **2** (Table 1). Compounds **5** and **12** are the most dramatic example of differences in association rates. However, despite polarity changes affecting association, the dissociation rates among **9**–**12** are similar. These results show that polar functionality in this region of the inhibitor slows the association to the kinase, but once bound, has negligible affects on dissociation due to a lack of significant productive interactions. Possible explanations to the slow association of the polar inhibitors include the kinase undergoes an additional reorganization, unproductive inhibitor–kinase interactions or desolvation becomes rate-limiting.

All together the modifications leading from the initial compound (**3**) to clinical candidate **2** improved binding affinity 10,000-fold. It is worth noting that the key individual changes (i.e. **3**→**13**→**14**→**2**, Table 2) affected exclusively the dissociation rate. We were initially surprised **2** has the same association rate as the other compounds because the conformational flexibility of the ethoxy linker might slow **2** from adopting the bioactive conformation. However, the X-ray crystal structure of **2** bound to p38 reveals the torsional angles adopted by the ethoxy linker are likely near the low energy minimum for alkoxy-aryl[20] and 1,2-di-heteroatom ethane[21] groups (Fig. 1). It stands to reason that structural modifications providing additional low energy conformations or disfavoring the bioactive conformation would result in slower association rates. Table 2 shows that replacing the ethoxy linker of **2** with propyl (**15**) reduces the rate of association 4-fold. To bind to the kinase, the propyl linker of **15** could maintain similar torsional angles observed for **2** but are higher in energy.[22] Lower energy conformations of the propyl linker are less likely to facilitate the productive binding interaction of the morpholine oxygen with the ATP-binding domain, primarily because of steric interactions with the kinase. Another modification that slows the association rate is replacing the naphthalene ring of **2** with phenyl (i.e. **16**). The ethoxy linker in **16** likely can adopt the bioactive torsional angles. However, the phenyl ring of **16** does not optimally bind deep in the kinase specificity pocket (cf. **13** vs **14**) to properly position the morpholine oxygen for hydrogen bonding with the kinase. These results suggest the association rate is slowed by modifications to the inhibitor which hinder access to the bioactive conformation. As would be expected for these types of changes, increases in the rate of dissociation from the kinase are also observed.

As mentioned previously, non-productive inhibitor-kinase interactions prior to binding could slow the association rate. Another example involves substituents attached at the 3-position of the naphthalene ring. Groups such as methoxy (**17**) or methyl (**18**) reduce the association rate 4- to 8-fold compared to **14** and **2** (Table 2). A possible explanation is that these groups sterically interact with the phenyl ring of Phe169 in the DFG-out conformation rendering slower association rates of the inhibitor to the kinase. In the case of **17**, the dissociation rate is unaffected compared to **14**. Another influence of the methyl group of **18** is that it forces the ethoxy linker to adopt out-of-plane conformations disfavoring the bioactive conformation.[23] For this reason, the morpholine oxygen may fail to undergo a hydrogen bonding interaction with the ATP-binding domain resulting in a dissociation rate similar to **14**.

Table 2. Effect of ethoxy and naphthalene ring modifications



Compd	R	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	K_d (nM)
2		84,900	8.3×10^{-6}	0.098
3	—	118,000	1.4×10^{-1}	1190
13	phenyl	73,300	1.6×10^{-3}	22
14	1-naphthyl	113,000	1.2×10^{-4}	1.1
15		18,700	2.3×10^{-5}	1.2
16		13,000	3.9×10^{-3}	300
17		36,100	3.4×10^{-4}	9.4
18		10,700	1.5×10^{-4}	14

This class of p38 inhibitors offers a unique opportunity to study changes in association and dissociation rates and correlate them to binding affinity. This analysis confirms the widely held belief among medicinal chemists that increasing lipophilic or hydrogen bond interactions slows dissociation rates and improves binding affinity. Also highlighted is the fact that analogues with higher energy barriers to the bioactive conformation have slower rates of association and faster dissociations from the kinase. Thus, we have shown SKR can be an important tool for the drug design process. It is also noteworthy that, in the absence of kinetic data, an erroneous assumption could be made that structural modifications resulting in similar binding affinities have not influenced inhibitor–kinase interactions (cf. **14** and **15**). Therefore, a practical approach towards the goal of identifying tightly bound inhibitors would be to optimize compounds for a fast association rate and a slow dissociation rate from the kinase.

References and Notes

1. Dinarello, C. A. *Curr. Opin. Immunol.* **1991**, *3*, 941.
2. Feldmann, M.; Brennan, F. M.; Maini, R. N. *Annu. Rev. Immunol.* **1996**, *14*, 397.
3. Present, D. H.; Rutgeerts, P.; Targan, S.; Hanauer, S. B.; Mayer, L.; van Hogezand, R. A.; Podolsky, D. K.; Sands, B. E.; Braakman, T.; DeWoody, K. L.; Schaible, T. F.; van Deventer, S. J. *N. Engl. J. Med.* **1999**, *340*, 1398.
4. Jarvis, B.; Faulds, D. *Drugs* **1999**, *57*, 945.
5. Bresnihan, B. *BioDrugs* **2001**, *15*, 87.
6. Weinblatt, M. E.; Keystone, E. C.; Furst, D. E.; Moreland, L. W.; Weisman, M. H.; Birbara, C. A.; Teoh, L. A.; Fischkoff, S. A.; Chartash, E. K. *Arthritis Rheum.* **2003**, *48*, 35.
7. Herlaar, E.; Brown, Z. *Mol. Med. Today* **1999**, *5*, 439.
8. Tibbles, L. A.; Woodgett, J. R. *Cell. Mol. Life Sci.* **1999**, *55*, 1230.
9. Ono, K.; Han, J. *Cell. Signal* **2000**, *12*, 1.
10. Lee, J. C.; Kumar, S.; Griswold, D. E.; Underwood, D. C.; Votta, B. J.; Adams, J. L. *Immunopharmacology* **2000**, *47*, 185.
11. Salituro, F. G.; Germann, U. A.; Wilson, K. P.; Bemis, G. W.; Fox, T.; Su, M. S. *Curr. Med. Chem.* **1999**, *6*, 807.
12. Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallagher, T. F.; Kumar, S.; Green, D.; McNulty, D.; Blumenthal, M. J.; Heys, J. R.; Landvatter, S. W.; Strickler, J. E.; McLaughlin, M. M.; Siemens, I. R.; Fisher, S. M.; Livi, G. P.; White, J. R.; Adams, J. L.; Young, P. R. *Nature* **1994**, *372*, 739.
13. Jackson, P. F.; Bullington, J. L. *Curr. Topics Med. Chem.* **2002**, *2*, 1009.
14. Cirillo, P. F.; Pargellis, C. A.; Regan, J. *Curr. Topics Med. Chem.* **2002**, *2*, 1021.
15. 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*, 268.
16. The remainder of the activation loop, residues 171–184, is disordered in the X-ray crystal structure with this class of inhibitors.
17. Thurmond, R. L.; Wadsworth, S. A.; Schafer, P. H.; Zivin, R. A.; Siekierka, J. J. *Eur. J. Biochem.* **2001**, *268*, 5747.
18. Markgren, P. O.; Schaal, W.; Hamalainen, M.; Karlen, A.; Hallberg, A.; Samuelsson, B.; Danielson, U. H. *J. Med. Chem.* **2002**, *45*, 5430.
19. 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.
20. Spellmeyer, D. C.; Grootenhuis, P. D. J.; Miller, M. D.; Kuyper, L. F.; Kollman, P. A. *J. Phys. Chem.* **1990**, *94*, 4483.
21. Wolfe, S. *Acct. Chem. Res.* **1972**, *5*, 102.
22. Maxwell, D. S.; Tirado-Rives, J.; Jorgensen, W. L. *J. Comp. Chem.* **1995**, *16*, 984.
23. Ab-initio calculations of the alkoxy naphthalene portion of **18** indicates that, due to the methyl group, the torsional angle of the bioactive conformation is 5.8 kcal/mol higher in energy than the lowest energy torsional angle of 90°. The calculations were performed using Density Function Theory (B3LYP).