Structure-based drug design case study: p38

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INTRODUCTION

The overproduction of cytokines has been implicated in a wide variety of inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, psoriasis, multiple sclerosis, osteoporosis, Alzheimer's disease, and congestive heart failure. The ability of p38 mitogen-activated protein kinase (p38 MAPK) to regulate the release and activity of multiple pro-inflammatory cytokines has attracted the interest of numerous pharmaceutical companies and independent researchers during the past decade or so. Since its initial discovery in 1994 as a potential molecular target for a novel class of cytokine suppressive inhibitors (SB-203580),[1] more than 150 patent applications from at least thirty pharmaceutical companies have been published, all claiming novel p38 inhibitors. Four distinct isoforms of p38 MAPK are known: p38α and p38β are widely expressed in eukaryotic cells, including endothelial and inflammatory cells; p38γ is found in skeletal muscle; and p38δ is predominantly found in the small intestine, kidneys, and lung tissue.[2,3] Of these four isoforms, p38α has been the most studied and is believed to be the most physiologically relevant. Numerous reviews have been published that focus on both the biology[4–7] and chemistry of p38 inhibitors.[8–17] The focus of this chapter is an illustration of p38 inhibitor design guided by structural information obtained both from modeling and actual x-ray crystallographic data. Structure references with a ".pdb" suffix refer to those obtained from the Research Collaboratory for Structural Bioinformatics.[18]

TRIAZINES AND PYRIMIDINES

We begin with a collaborative venture by Bristol-Myers Squibb and Pharmacopeia aimed at the development of a novel series of trisubstituted triazines. High-throughput screening applied to a collection of 2.1 million compounds derived from a combinatorial library based on the template shown in Scheme 13.1 yielded the 1,3,5-triaminotriazine aniline amide PS200981, having a p38α IC_{50} of 1 μM.[19] Further analyses identified PS166276 with a p38α IC_{50} of 28 nM having 10× less cytotoxity and superior inhibition of lipopolysaccharide-(LPS) induced TNFα production in THP-1 monocytes (170 nM). These inhibitors were found to compete for the ATP binding site in p38α. Additionally, statistical analysis of the combinatorial data indicated a significant contribution to activity in this series correlated to the presence of the 4-methyl-3-benzamido aniline moiety. When the x-ray crystal structure of the protein-inhibitor complex for a member of this triaminotriazine aniline amide series was determined, the structure/activity relationship (SAR) for the series was quickly rationalized. Specifically, *N*-methoxy-4-methyl-3-(4-(methyl(neopentyl)amino)-6-(4-methyl-1,4-diazepan-1-yl)-1,3,5-triazin-2-ylamino)benzamide (**1**) was cocrystallized with unactivated p38α protein (Figure 13.1), confirming that the series binds to the ATP pocket.[20] In a manner similar to ATP, **1** binds to the hinge region of p38α (characterized by residues 106–110), forming an anchoring H-bond interaction with Met109. Unlike ATP, **1** makes use of an intervening water molecule to form the interaction between Met109 backbone NH and the triazine N3. Also characteristic of other kinases is the presence of a deep hydrophobic pocket near the so-called gatekeeper residue (Thr106, not shown) which provides for one of the more interesting features of kinase inhibitor design in that it represents a space not occupied by ATP and, thus, of potential value in the search for inhibitor selectivity against off-target kinases. In the present case, the 4-methyl-3-benzamido aniline moiety occupies that space. Further interactions in the binding site include H-bonds among Lys53, Glu71, and amido NH, between Asp168 backbone NH and amido C=O, and between the protonated diazepan nitrogen and Asp168 carboxylate. Interestingly, the distance between triazine N1 and Lys53 is suggestive of an intervening water molecule; however, none was evident from the crystallography. The potential for H-bonding between Lys53 and an acceptor atom in other ligands was eventually realized with subsequent inhibitors. Synthetic efforts targeted variation at all three positions (2,4,6) of the triazine core, and the emergent SAR dovetailed nicely with the crystallographically determined binding mode. For example, the methylhydroxamate ester was found to have superior binding affinity compared to amides in general, a consequence of both its small size (complementing the relatively narrow pocket at that position) and its electron-deficient NH proton H-bond donor. The use of branched alkyl amines at the