

PROTEIN KINASE INHIBITORS BY DESIGN

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Inhibiting the catalytic activity of protein kinases has become one of the major therapeutic concepts in contemporary drug discovery. The first protein kinase inhibitors were identified by screening more than a decade ago. From that time, the intense activity of structural biologists in the field, has given us access to hundreds of crystal structures of protein kinases (apoenzymes or ligand complexes). Concomitantly, a lot of experience has been gained in the structure-activity relationships of protein kinase inhibitors. The combined information has provided us with a deep insight into the structural determinants of kinase inhibition by small molecules binding to the ATP (cofactor) pocket. We present and illustrate here how this knowledge can be exploited to design, in a very efficient manner, new kinase inhibitors.

Structural Determinants of Protein Kinase Inhibition

The ATP binding site of a protein kinase can be divided in five regions defined by their spatial relationships with the different chemical groups in ATP. The four most important regions in terms of inhibitor design are briefly described below.

Adenine region. The adenine binding region comprises two hydrophobic amino acids Ade1 and Ade2 that hold the adenine moiety of ATP in a "hydrophobic sandwich"). With rare exceptions (casein kinase 2), Ade1 is an alanine throughout the protein kinase family. Ade2 is most frequently a leucine or methionine. In addition, the C6 amino group and N1 atom of adenine form bidentate hydrogen bonds with the backbone of the first (Hin1) and third (Hin3) residues of the hinge segment. The hinge segment is the extended coil stretch of six to seven amino acids that connects the N- and C-terminal domains of the kinase. This binding mode has been observed in all X-ray structures of kinases complexed with ATP or an ATP analogue yet determined. Remarkably, nearly all available X-ray structures of kinase-inhibitor complexes, show the existence of at least one hydrogen bond between the inhibitor and the hinge segment. The conserved hydrogen bond interaction occurs between an acceptor atom of the ligand and the backbone NH of Hin3. However, hydrogen bonding between the backbone carbonyl of Hin1 or that of Hin3 is, in addition, frequently observed when the inhibitor contains a donor-acceptor system in its structure. Satisfying the structural requirements of the adenine region in terms of shape and hydrogen bond complementarity appears to be the major factor to kinase inhibition since the core structure of most kinase inhibitors is an aromatic moiety bearing hydrogen bonding functionalities. An aromatic moiety has the required flat shape to fit between the side chains of Ade1 and Ade2 while offering substituent positions to engage the hinge loop in hydrogen bond interactions.

Sugar region. Three residues form the environment of the ribose ring of ATP in the cleft. Sug1 is the last residue of the hinge segment. It has a side chain proximal to the ribose 2'-OH group which allows formation of a hydrogen bond when the amino acid is polar. Variability exists at this position. In many kinases Sug1 is indeed a polar amino acid like aspartic acid or asparagine, however in some members of the family it is a more hydrophobic residue such as cysteine. The variability of Sug1 can be exploited to design selective inhibitors. The available

X-ray structures show that the second residue of the ribose region, Sug2, interacts with ligands (ATP or inhibitors) mainly through its backbone carbonyl. In most cases, its side chain projects outside the cleft. Thus, this amino acid is not a critical determinant of ligand binding. The last residue, Sug3, is located in the glycine-rich loop. Some exceptions exist but in almost all known kinases Sug3 is a valine. The proximity of Sug3 to the adenine binding region makes this residue a natural target for design of hydrophobic substituents that extend the structures of small ligand scaffolds fitting the adenine binding region.

Hydrophobic channel. In most kinases (PKA is an exception), a channel that opens the ATP binding cleft to solvent space extends along an axis coinciding approximately with the direction of the lone pair of the adenine N3 atom of ATP. The channel is formed by the α carbon of residue Hyc1 and the side chain of Hyc2. Hyc1 is in the hinge segment and is usually a glycine when there is no deletion in this part of the segment (as in the cyclin-dependent kinases or in members of the MAP kinase family). Hyc2 is a residue of the glycine-rich loop and is most often a leucine or an isoleucine. This part of the binding site is not filled by ATP. However, as revealed by several X-ray structures of kinase-inhibitor complexes, inhibitors can insert hydrophobic moieties into the channel to gain binding affinity. An aryl moiety appears to be optimal in terms of shape complementarity with the channel. The opening to solvent can be exploited to introduce substituents on the aryl moiety that do not interact with the kinase in order to modulate the physico-chemical properties of the inhibitor without compromising its inhibitory potency.

Hydrophobic pocket. In all kinases, a hydrophobic pocket faces the C6-C5-N7 edge of the ATP adenine ring. This pocket, not filled by ATP, provides opportunities to increase the binding affinity of ligands. However, its size is variable and critically depends on the nature of Hyp1 (also known as the “gate keeper”), the residue just preceding the first amino acid of the hinge segment in the kinase amino acid sequence. As confirmed by mutation studies, Hyp1 is a key residue in controlling inhibitor selectivity. Typically, in some kinases Hyp1 is a rather small amino acid such as valine or threonine while in others it is a bulkier methionine or phenylalanine. In the latter case, exemplified by the cyclin-dependent kinase family, the pocket is not deep. Thus, it offers less opportunities for building productive interactions between the ligand and the protein. For kinases with a small Hyp1 amino acid, inhibitors can possess large hydrophobic moieties extending in the direction of the lone pair of the ATP adenine N7 atom. These can reach the bottom of the pocket formed by residue Hyp5 (usually a methionine or a leucine). With the exception of Hyp7 and Hyp8, respectively a lysine and a glutamic acid absolutely conserved in the protein kinase family, all the amino acids of the pocket are variable. In certain types of inactive conformations of kinases, an enlargement of the hydrophobic pocket is observed. It can be caused by a movement of helix α C with the consequence that the side chain of Hyp8 is oriented towards the exterior of the protein (“Src or CDK -like” inactive conformations) or by a different conformation of the DFG motif forming the beginning of the activation loop (“DFG out” conformations). Many recently reported kinase inhibitors exploit additional interactions made possible by this enlargement of the hydrophobic pocket in particular with “DFG out” conformations.

General strategy for the design of new kinase inhibitors

Based on the structural determinants of kinase inhibition above described, a four-step strategy to design new kinase inhibitors can be proposed:

- I. Start by designing a mimic of the adenine ring of ATP (warhead). The mimic should form at least one hydrogen bond with the hinge backbone (possible partners : carbonyl of Hin1, carbonyl and NH of Hin3). In addition, it should present a ring that can be "sandwiched" between the side chains of Ade1 and Ade2. The reason for beginning

the process by designing an adenine mimic is that precisely positioning the molecular fragment in the ATP pocket is possible due to the directionality of the hydrogen bonds with the hinge backbone and the high rigidity of this part of the binding site. In addition, the adenine region appears to be the most productive for creating binding affinity.

- II. Design lipophilic moieties to attach to the adenine mimic scaffold in order to fill the hydrophobic channel, the hydrophobic pocket or interact with residue Sug3 of the sugar region. The creation of additional hydrophobic contacts is usually the most effective approach to gain binding affinity. The moiety filling the hydrophobic pocket can be designed to target either the active conformation or an inactive conformation.
- III. Synthesize or search for the designed compounds in a chemical database.
- IV. In a second optimization phase target other hydrogen bond interactions, in particular with Sug1, Sug2, Hyp7 (conserved lysine) or with the conserved aspartic acid (DFG motif) and asparagine amino acids of the phosphate binding region.

We routinely apply this strategy to design new kinase inhibitors. We will illustrate it by two detailed examples: one in which we designed a 3,5- diaryl pyrazole molecular scaffold to inhibit the CDK1/2 kinases, the other where a 2-amino, 5-aryl thiazole prototype inhibitor was designed to target the FLT3 kinase in a very selective manner.