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STI571 (IMATINIB): STRUCTURAL AND ENZYMATIC STUDIES OF INTERACTIONS WITH ABL KINASE AND RESISTANCE MUTANTS

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In chronic myelogenous leukemia (CML), a reciprocal balanced chromosomal translocation in haematopoietic stem cells produces the BCR-ABL hybrid gene, which encodes a constitutively activated kinase. STI571, marketed as imatinib, is a potent ATP-competitive inhibitor of c-Abl (IC50 188 nM). Despite the efficacy of the drug in stable-phase CML, in advanced leukemias, where there is a high frequency of DNA mutation, many patients relapse with point mutations in the kinase domain. We have determined the crystal structure of STI571 in complex with human Abl kinase (spacegroup $C222_1$) and determined the inhibitry activity of STI571 towards many of the Abl kinase mutants. The structure shows that STI571 stabilises an inactive conformation of the protein, thus comfirming Schindlers findings (Science 2000, 289:1938-1942) for murine Abl-kinase in complex with a structurally-related inhibitor (spacegroup *F*222). These studies allow us to rationalise the decreased sensitivity of mutant Bcr-Abl to STI571. Point mutations such as T315I in the ATP-binding site result in loss of a hydrogen bond and steric hindrance to the binding of STI571 ($IC50_{T315I}$ (STI571) > 10 μM). Mutations in the glycine-rich loop such as E255V (IC50 (STI571) 3.5 μM), E255K and Y253H (IC50 (STI571) >10 μM), destabilise the unusual conformation that this loop adopts in the inactive state, as does H396P in the activation loop. The variety and spread of these and other mutants makes it a challenge to design drugs to overcome resistance.

Keywords: KINASES DRUG DESIGN ONCOLOGY

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DISCOVERY OF A NEW BINDING POCKET OF FKBP52-N AND DESIGN OF TWO NOVEL NEUROTROPHIC LIGANDS OF FKBP12

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FKBP (FK506 binding protein) family is a kind of immunophilin possessing peptidylprolyl isomerase (PPIase) activity, which can be inhibited by the immunosuppressant drug FK506. FKBP52 is a high molecular mass immunophilin belonging to FKBP family. It can be divided into two PPIase domains, one tetratricopeptide repeats (TPR) domain, and one calmodulin binding domain. The first PPIase domain (FKBP52-I), which is homologous with FKBP12 is mainly responsible for the FK506 binding and the PPIase activity of the whole protein. Recent studies have showed that FKBP52, rather than FKBP12, can mediate the neurotrophic activity of FK506 and its analogues. The 2.5 Å structure of the FKBP52-I has been solved and given us more detailed insight into the function mechanism of the FKBP52-I and FKBP52 full-length protein. Most interestingly, besides the FK506 binding pocket, a new hydrophobic pocket is detected at the N-terminus, which does not exist in FKBP12. This pocket is a potential new binding site of small molecules and other proteins related with FKBP52 for drug design. FKBP12 is now well known to be a target protein related with the nerve regeneration. Ligands that bind to the protein FKBP12 have attracted attention in recent years due to their neuroregenerative abilities. Aided by the structure-based drug design, two novel FK506 analogues have been synthesized. Both of them showed higher affinity to FKBP12 and much better neurotrophic effection than GPI-1046, which is undergoing second phase clinical test as a neurotrophic drug. Furthermore, two 1.8 Å crystal structures of human FKBP12 complexed with the two ligands respectively have been determined, which gave a strong structure proof at atomic level to our neurotrophic ligands design.

Keywords: FKBP NEUROTROPHIC DRUG

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CRYSTAL ENGINEERING YIELDS CRYSTALS OF CYCLOPHILIN D DIFFRACTING TO 1.5 Å RESOLUTION: A SOUND BASIS FOR THE SUPPORT OF DRUG DISCOVERY

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Mitochondrial Cyclophilin D (CypD) belongs to a family of cyclosporin-A binding proteins, which catalyse rotation about prolyl peptide bonds. Structures of the three other types of mammalian cyclophilins (A, B and C) are known, but crystallization of CypD has not been reported yet. A truncated version of CypD with the same length as CypA was expressed in *Escherichia coli* and purified to homogeneity. The protein shows a monodisperse size distribution in dynamic light scattering, but crystallization trials gave no crystals. Therefore, site-directed mutagenesis was used to obtain a variant of CypD suitable for crystallization and structure determination. A series of mutants was selected such that crystal contacts seen in the X-ray structures of CypA would be restored or the unusually high solubility and isoelectric point (pI) of 10.3 reduced. The crystals of the mutant K133I were most promising and yielded readily crystals diffracting to 1.5 Å resolution using in house data collection facilities - an excellent basis for structure based drug design. This encouraging result shows that surface mutations have a dramatic effect on the protein and are a powerful tool for the improvement of protein crystallisation properties. This crystallographic system was successfully used to confirm hits from screening by biophysical methods (NMR, SPR and fluorescence studies) and to guide improvements of small molecule binding to cyclophilin D by structure based drug design.

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THE STRUCTURE OF AN ALDOSE-REDUCTASE INHIBITOR COMPLEX SHOWS FLEXIBILITY OF THE INHIBITORS CARBOXYLATE AS A FUNCTION OF pH

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Aldose Reductase, an enzyme involved in diabetes complications, is the target of an extended effort in inhibitor development. Previous work has showed the existence of an anchoring point in the active site to which the carboxylate head of inhibitors can be bound. The carboxylate head mimics the transient enolate state of the substrate, and binds the two catalytic residues, Tyr 48 and His 110. Different orientations of the carboxylate have been observed, depending on the pH of the crystallisation conditions and on the inhibitor. In each previous case, the carboxylate has been well ordered, especially in the complex that was crystallized at pH 5 and that led to a subatomic resolution (0.66 Å) structure. We have obtained crystals of the same complex at pH 8 as well, and solved the structure at 1.5 Å resolution. The conformations of most of the inhibitor are very close to each other at pH 5 and pH 8 but clearly shows two alternative conformations of the carboxylate head. These conformations correspond to optimising the hydrogen bond geometry with the His 110 and the Tyr 48 respectively, and could correspond to alternative catalytic mechanisms in which the proton donor is either His110 or Tyr48.

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