

Abstract

Structure-assisted drug discovery is a powerful approach that utilizes detailed knowledge on 3D structure to design and optimize new inhibitors targeting medically relevant enzymes. X-ray crystallography is a widely used structural biology technique since it provides detailed snapshot of protein-inhibitor complex, which is used to analyze protein-inhibitor interactions.

PNP plays an important role in salvage pathway of purine metabolism, it is a target in treatment of T-cell malignancies and/or parasitic infections. Our effort focused on human and *M. tuberculosis* PNP, and our aim was to develop new inhibitors with high selectivity and specificity. Our inhibitors are acyclic nucleoside phosphates with 9-deazahypoxanthine nucleobase that contain three moieties binding to all three regions of the active site: purine, phenyl and phosphonate moieties. The best inhibitors have IC50 values as low as 19 nM (human) and 4 nM (*M. tuberculosis*). The presence of short substituents at central phenyl moiety, such as methoxy and bromide group, decreases inhibitor's affinity towards human PNP, but does not affect affinity towards mycobacterial PNP. At the same time, bulky substituents, such as fluorinated phenyl ring, decrease inhibitor's affinity towards human PNP but increase affinity towards mycobacterial enzyme. Active site comparison did not provide a clear structural explanation for these changes, but comparison of subunit-subunit interaction region revealed that this region is smaller but more flexible in mycobacterial PNP compared to human PNP. CDK2 is a cell cycle regulator and is a target in the treatment of different types of cancer. As a member of the CDK family, belonging to kinases, development of selective inhibitors targeting a specific CDK presents the biggest challenge in drug development. A large number of inhibitors, targeting different parts of CDKs, were developed over the years, but most of them failed due to low selectivity and specificity. In this thesis, we report three novel series of ATP-competitive inhibitors, based on pyrazolo-pyridine core and/or pyrazolo-quinoline core. Crystal structures of series representatives were used to optimize inhibitor's affinity towards CDK2, as well as development of novel inhibitors targeting other members of kinase family such as FLT3, CDK12 and CDK7.