

**STRUCTURE-BASED OPTIMIZATION OF CDK2 INHIBITORS**

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The timing and co-ordination of the cell cycle progression is governed by the cyclin-dependent protein kinases (CDKs). Inhibitors of these enzymes block the progression of the cell cycle and are of interest as antiproliferative agents in anticancer drug research. The potent low-molecular weight inhibitors prevent binding of the co-factor ATP to the enzyme [1-3].

At Schering we have evaluated three diverse lead series of CDK2-inhibitory compounds: aminopyrazoles, aminopyrimidines and indirubines. Although a large number of crystal structures of CDK2 in complex with a variety of ligands are known, the correct binding mode of new inhibitors is often difficult to predict. This fact is mostly based on the relatively high flexibility of the active site. Thereupon our aim was to identify the exact binding modes of our three lead series within the ATP-site and to further on optimize these leads by structure-based design. [4-6].

CDK2 features a typical kinase fold: The N- and C-terminal domains are linked by the hinge region. ATP or its competitors bind in the cleft between the two domains by forming either two or three hydrogen bonds to backbone atoms of the enzyme.

Compounds from all three lead series were successfully co-crystallized with CDK2 and their binding modes could be determined. In the case of the indirubines, the lactam amide nitrogen donates a hydrogen bond to the backbone carbonyl oxygen of Glu81 whereas the lactam amide oxygen acts as a hydrogen bond acceptor for the backbone nitrogen of Leu83. The cyclic nitrogen is acting as a hydrogen bond donor to the backbone carbonyl oxygen of Leu83. The lead structure exhibits *in vivo* efficacy, is moderately selective against other kinases, but shows very poor solubility and absorption. Using the binding mode as a starting point for structure-based drug design, we were able to improve the solubility of the indirubines by replacement of the planar carbonyl group by tetrahedral moieties.

The aminopyrazoles interact by forming the same three hydrogen bonds to the hinge region of the enzyme as observed in the indirubines. They exhibit a preferred tautomeric form which can be explained by its binding mode. Unlike the above mentioned inhibitors, the 2,4-diaminopyrimidines form only two hydrogen bonds to the hinge region of CDK2. Interestingly these two hydrogen bonds are different from the ones which are known from ATP. The amine nitrogen in the 2-position donates a hydrogen bond to the backbone carbonyl

oxygen of Leu83, and one of the pyrimidine nitrogens acts as a hydrogen bond acceptor for the backbone amine of Leu83. A similar hydrogen bonding pattern is observed in the structures of CDK2 with olomoucine, roscovitine, purvalanol B, OL567 and H717. To improve the binding as well as the cellular activity, introduction of a para-sulfonamide moiety at the aniline in the 2-position was successful. Further computational docking and X-ray structures of those compounds gave an explanation for this increased affinity.

#### REFERENCES

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