

## Mapping the Similarities/Dissimilarities of the active sites of CDK2 and CDK4

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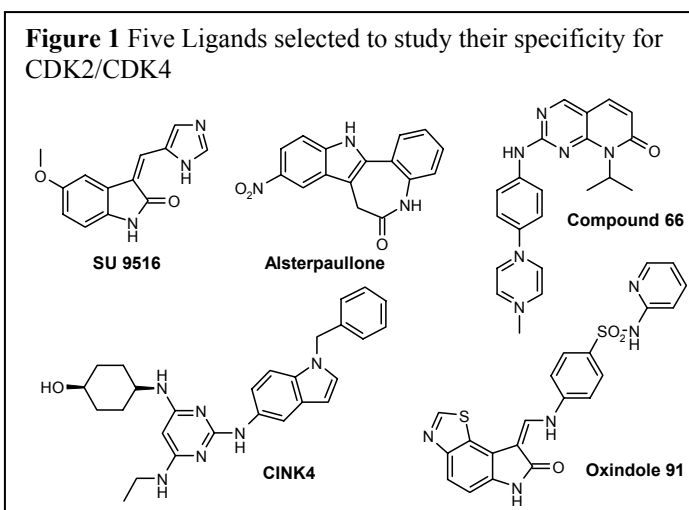
Designing of subtype specific inhibitors for the cyclin-dependent kinases (CDK) has become a great challenge. A study of the complex between CDKs and the known inhibitors that show some preference towards either CDK2 or CDK4 could lead towards achieving higher selectivity. Five such ligands were chosen and their binding modes with CDK2 and CDK4 were studied using molecular modeling and docking studies. Our studies have revealed that certain residues in both CDK2 and CDK4 which could be targeted for establishing selectivity. The results from our studies could be used in designing novel CDK subtype specific inhibitors.

Cyclin-dependent kinases are Ser/Thr kinases (1), which play a central role in the control of mammalian cell cycle responsible for cell growth and proliferation. Mutations in tumor associated genes result in the targeting of the basic regulatory mechanisms that control mammalian cell cycle (2, 3). Most abnormalities occur due to hyperphosphorylation of the tumor suppressor gene Rb by the CDKs. Of the 10 CDKs currently known, only CDKs1, 2, 3, 4 and 6 intervene directly in cell cycle (4). CDK4/6-D cyclin complexes are involved in the G1 phase (5, 6), while CDK2 in complex with cyclin E and cyclin A regulates the G1/S transition and S phase reselectively(7, 8). CDK4-cyclin D/INK4/Rb pathway is one of the most frequently mutated in human cancers. CDK4 is amplified and overexpressed in a wide variety of human tumors such as gliomas, sarcomas, breast tumors and carcinomas of the uterine cervix.(9) CDK2 associates either with Cyclin A and Cyclin E and it has been shown that these two complexes are both required and rate limiting for progression into S phase. Inhibition of CDK2/cyclin A results in elevated E2F concentrations leading to S phase arrest and apoptosis (10).

In principle, CDK activity can be therapeutically inhibited by a variety of approaches. These include overexpression of exogenous INK4 or Cip peptidomimetics, antisense technology, blocking cyclin binding to their cognate CDK catalytic subunit, modulation of the ubiquitination machinery responsible for the degradation of cyclins and modulation of upstream kinases. Among these, the discovery of small molecule inhibitors of the kinase activity, which target the ATP binding site of the kinases is one of

the most attractive one. Despite the striking diversity of functions displayed by the different CDKs, the catalytic residues are well conserved across eukaryotic protein kinases, making specificity a great challenge. However, three-dimensional structural models of CDKs should provide useful information for the design of novel CDK-selective inhibitors. There are some inhibitors known that are moderately selective for either CDK2 or CDK4.(11) Five of these inhibitors were selected based on their selectivity data (Figure 1). The binding modes of these inhibitors to CDK2 and CDK4 were studied to understand their selectivity. This could provide us with key differences in amino acids of CDK2 and CDK4 that can be taken advantage of to address the issue of specificity.

The three-dimensional (3D) structure of CDK4 is presently unknown. A homology model of CDK4 was developed using MODELLER (12) based on the Blast alignment with the crystal structure of CDK2 (1FIN.pdb) (13). ATP was docked into the ATP binding site of the homology

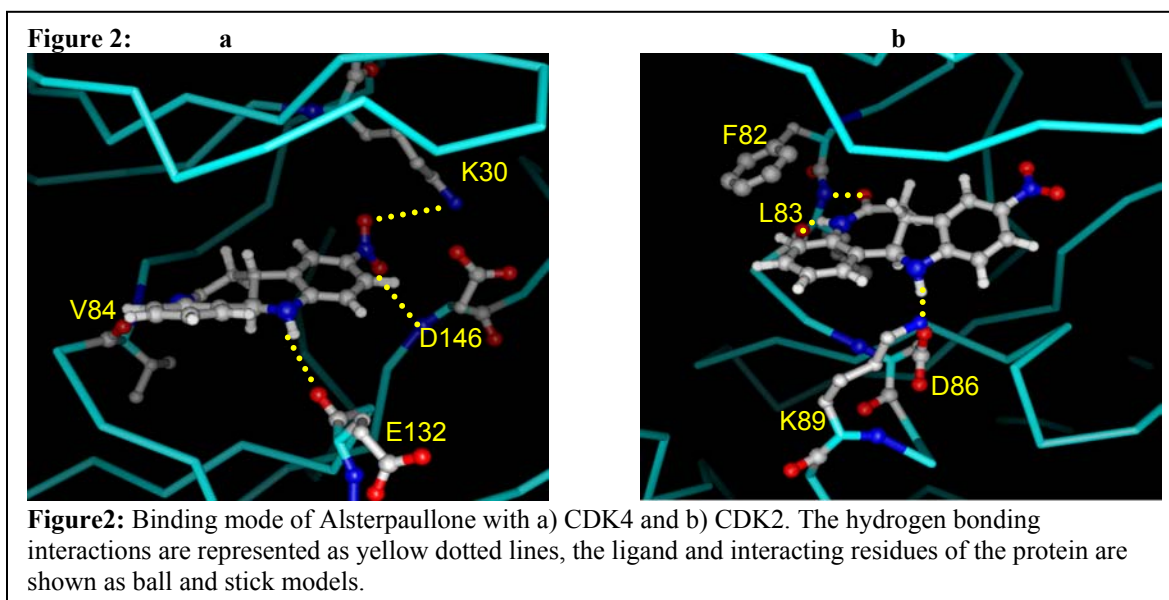


model of CDK4 using FlexX and the complex was minimized to generate a model with an optimized binding pocket. The chosen five molecules shown in Table 1 were docked into the binding site of the crystal structure of CDK2 and the homology model of CDK4 using FlexiDock. The Binding energy of each of the ligands was then calculated based on the difference between the total energy of the ligand-protein complex and the sum of the ligand and protein energies for the uncomplexed structure.

Initial docking experiments revealed a wealth of information regarding the different modes of binding of the five inhibitors to CDK2 and CDK4. The binding energy of each of the ligands to CDK2 and CDK4 were calculated (Table 1). It can be clearly deduced that the higher the binding energy potency is also higher. A correlation of the binding energies to their inhibitory potency (IC<sub>50</sub>) was 0.87 for CDK2 and 0.899 for CDK4. Such a correlation of the binding energies with their *in-vitro* inhibitory activity can help us in developing a model for predicting the potency of the ligands for either CDK2 or CDK4.

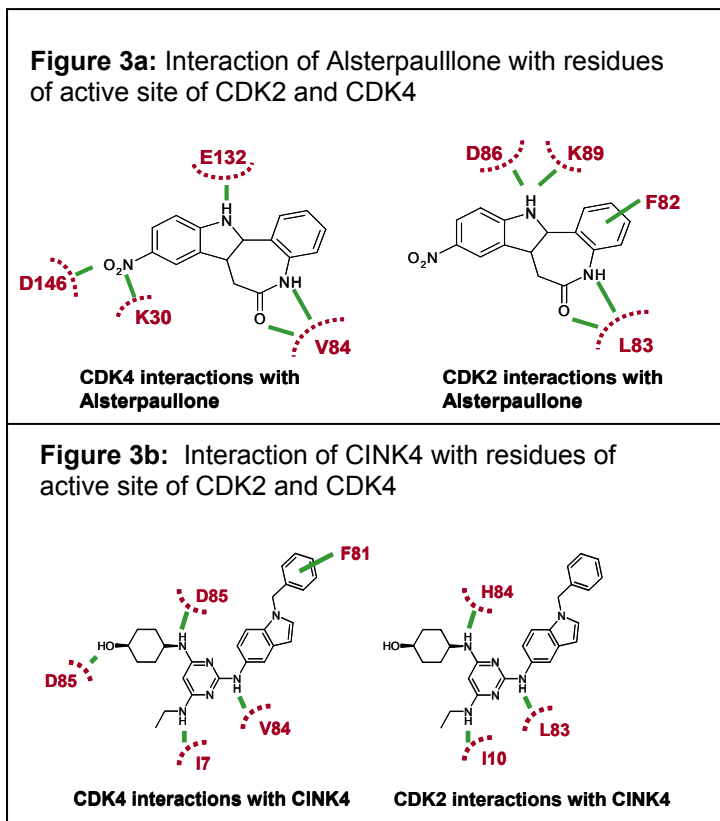
**Table 1** Binding Energy of inhibitors with CDK2 and CDK4.

Compound Name	CDK2 (IC <sub>50</sub> )	B.E CDK2	CDK4 (IC <sub>50</sub> )	B.E CDK4
Alsterpauillone	0.035	-75	>10	24
CINK4	>50	-1	1.5	-1.6
SU 9516	0.022	-45	0.2	-34
Oxindole 91	0.01	-66	0.13	-53
Compound 66	0.675	-15	0.032	-34



The orientation of the ligands was similar for both CDK2 and CDK4, but they had different interactions with the amino acid residues surrounding them. In all cases hydrogen bonds were made with the carbonyl and the amido group of the hinge region residues such as V84 in the case of CDK4 and L83 in the case of CDK2. The selectivity of the ligands is primarily dictated by their interactions with other residues surrounding the binding pocket. For example, Alsterpauillone on binding to CDK4 (Figure 2a) exhibits the two hydrogen bonds at the hinge region with V84. In addition, another hydrogen bond interaction of –NH with E132 along with weak interaction of the –NO<sub>2</sub> group with K30 and D146 are seen. In the case of CDK2 (Figure 2b), alsterpauillone has got the required hydrogen bonds with the hinge region residue L83. It has also got three strong additional hydrogen bonding interactions of –NH with D86 and K89 along with ring-ring interaction of the phenyl ring with its counterpart of F82. These additional interactions of alsterpauillone with CDK2 seem to be fortifying a strong binding to the protein and more than that seen with CDK4 (Figure 3a). In the case of binding of CINK4 with CDK4, additional strong interactions of two –NH groups with D85 and I7, –OH with D85 and the ring-ring interaction of the phenyl group with a

similar group of F81. Binding of CINK4 with CDK2 shows only two additional interactions of the –NH groups with I10 (Figure 3b).



Our studies will aid in identifying the residues surrounding the binding pocket which can be targeted for improving the selectivity of the ligands to subtypes of CDK family. Design of CDK selective inhibitors will help us to study the effect of a specific pathways influenced by these proteins.

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