

## Structural Basis for Interaction of Inhibitors with Cyclin-Dependent Kinase 2

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**Abstract:** Cell cycle progression is tightly controlled by the activity of cyclin-dependent kinases (CDKs). CDKs are inactive as monomers, and activation requires binding to cyclins, a diverse family of proteins whose levels oscillate during the cell cycle, and phosphorylation by CDK-activating kinase (CAK) on a specific threonine residue. The central role of CDKs in cell cycle regulation makes them a promising target for studying inhibitory molecules that can modify the degree of cell proliferation, the discovery of specific inhibitors of CDKs such as polyhydroxylated flavones has opened the way to investigation and design of antimitotic compounds. A chlorinated form, flavopiridol, is currently in phase II clinical trials as a drug against breast tumors. The aromatic portion of the inhibitor binds to the adenine-binding pocket of CDK2, and the position of the phenyl group of the inhibitor enables the inhibitor to make contacts with the enzyme not observed in the ATP complex structure, the analysis of the position of this phenyl ring not only explains the great differences of kinase inhibition among the flavonoid inhibitors but also explains the specificity of roscovitine and olomoucine to inhibit CDK2. There is strong interest in CDKs inhibitors that could play an important role in the discovery of a new family of antitumor agents. The crystallographic analysis together with bioinformatics studies of CDKs are generating new information about the structural basis for inhibition of CDKs. The relevant structural features that may guide the structure based-design of a new generation of CDK inhibitors are discussed in this review.

**Keywords:** CDK2, drug design, flavopiridol, roscovitine, inhibitor, indirubin.

### INTRODUCTION

Cell cycle progression is tightly controlled by the activity of cyclin-dependent kinases (CDKs) [1]. CDKs are inactive as monomers, and activation requires binding to cyclins, a diverse family of proteins whose levels oscillate during the cell cycle, and phosphorylation by CDK-activating kinase (CAK) on a specific threonine residue [2]. CAK is also known as CDK7. Figure 1 shows the activation

be the main region through which cyclins interact with CDKs. Since there are many CDKs and cyclins the possibilities of combinatorial control appears endless [4,5]. However, the number of complexes that actually form is limited. CDK2 forms complexes with cyclin A or cyclin E, with the activity of the CDK2:cyclin E complex peaking at the onset of S phase and generally preceding that of the CDK2:cyclin A complex. CDK4 and CDK6 form complexes

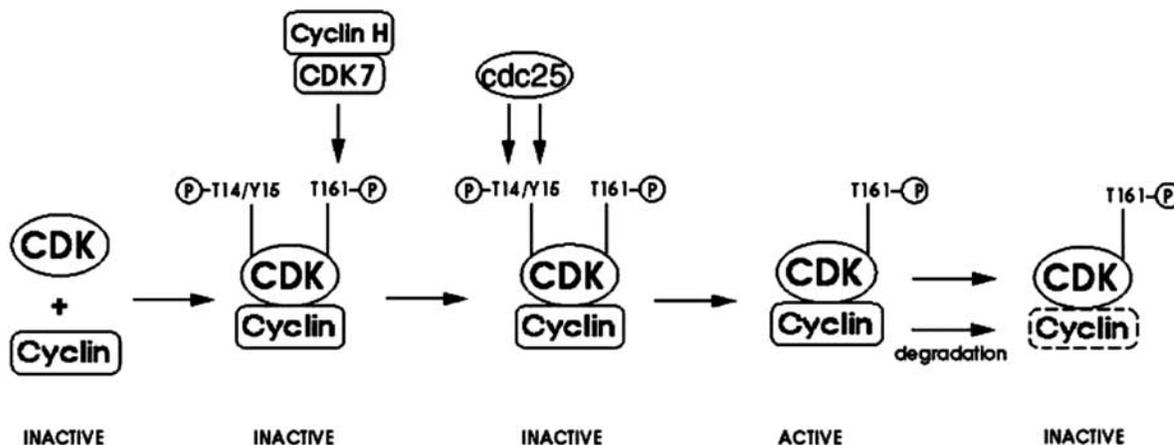


Fig. (1). Schematic diagram showing the model for activation of CDKs.

of CDKs. All cyclins contain a conserved region of approximately 150 residues, which has been termed the cyclin box [3]. Not surprisingly, the cyclin box appears to

with cyclin D and their activity peak earlier in G<sub>1</sub> phase. Figure 2 shows the participation of CDKs in different phases of the cell-cycle progression. The CDK1:cyclin B complex acts as the primary promoter of mitosis. CDK2 complexes are primarily responsible for triggering S phase, and CDK4 complex serves to integrate extracellular signals and directs the cell-cycle engine according the cell's environment [6].

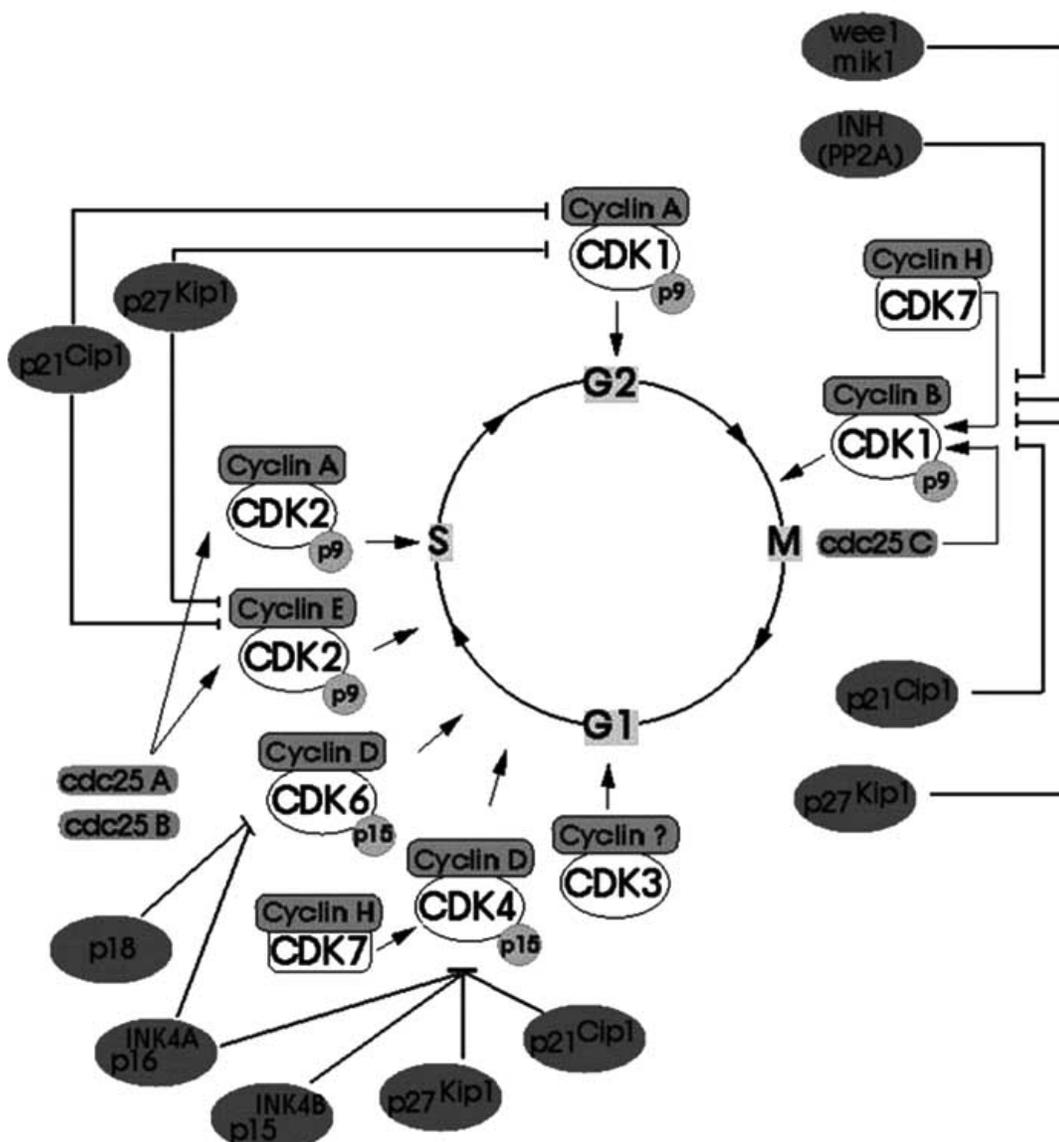
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In addition to the positive regulatory role of cyclins and CDK7, many negative regulatory proteins (CDK inhibitors, CKIs) have been discovered [7]. CKIs fall into two broad categories based upon whether they bind solely to the catalytic subunit (CDK) or whether they bind to CDK complexes. The former category has been identified only in higher eukaryotic cells and is termed the INK family. The most studied INKs are p15, p16, p18, and p19. These inhibitory proteins bind to CDK4 and 6 and prevent binding of cyclin D [8]. Inhibitors of the second category bind to CDK complexes. In mammalian cells, this family includes p21, p27, and p57 [8]. Besides positive and negative regulation by phosphorylation and binding of cyclins and CKIs, CDKs interact with another class of regulatory proteins known as CskHs. This protein is also known as p9. It was identified two homologues in humans (CskHs1 and CskHs2) [9]. Despite many studies investigating its role in the cell cycle, the function of p9 is not yet understood. The structure of the complex CDK2-p9 has been determined and based on this structure it was proposed that p9 acts in

targeting the CDKs to other proteins or macromolecular assemblies [10].

It is clear that phosphorylation of serine, threonine and tyrosine residues is of major importance in all aspects of cell life and protein kinases are pharmacological targets [11]. Deregulation of cyclins and/or alteration or absence of CKIs have been associated with many cancers and there is strong interest in chemical CDKs inhibitors that could play an important role in the discovery of a new family of antitumor agents [12]. Since ATP is the authentic cofactor of CDK2 it can be considered as a "lead compound" for discovery of CDK2 inhibitors. However, there are two major concerns: adenine containing compounds are common ligands for many enzymes in cells, thus, and adenine derivatives may inhibit other enzymes in the cells; second, any highly charged groups such as phosphates in ATP will prevent uptake by cells [13].

More than 50 CDK2 inhibitors have now been described, some presents  $IC_{50}$  at nanomolar concentrations [11]. The structures of CDK2 in complexes with several different



**Fig. (2).** An overview of some essential steps in cell-cycle progression. CDKs (CDK1-7), their activators (cyclin, cdc25, CDK7:cyclin H), their regulators (p9 and p15), and their inhibitors (wee1, INH, p15, p16, p18, p21, and p27).

inhibitors have been described using biocrystallography and structural bioinformatics. We may divide the chemical inhibitors of CDK in two broad classes considering their specificities. Class I CDK inhibitors are those that are selective for CDK2 (and also CDK1, 5 and probably 9), such as olomoucine, roscovitine, purvalanol B, aminopurvalanol, hymenialdisine, indirubin-3'-monoxime, indirubin-5-sulfonate, SU9516 and alsterpaullone. Class II CDK inhibitors are those that are not selective for any specific CDK, such as deschloroflavopiridol and flavopiridol. Class II inhibitors are not specific for CDK2, although some of them present  $IC_{50}$  in nanomolar concentrations, such as flavopiridol. The effects of CDK inhibitors on the cell cycle progression and their potential value for the treatment of cancer have been extensively studied [11,14]. There are three main features that make CDK inhibitors potential anti-tumor agents: 1) They arrest cells in G1 or G2/M. 2) They trigger apoptosis, alone or in

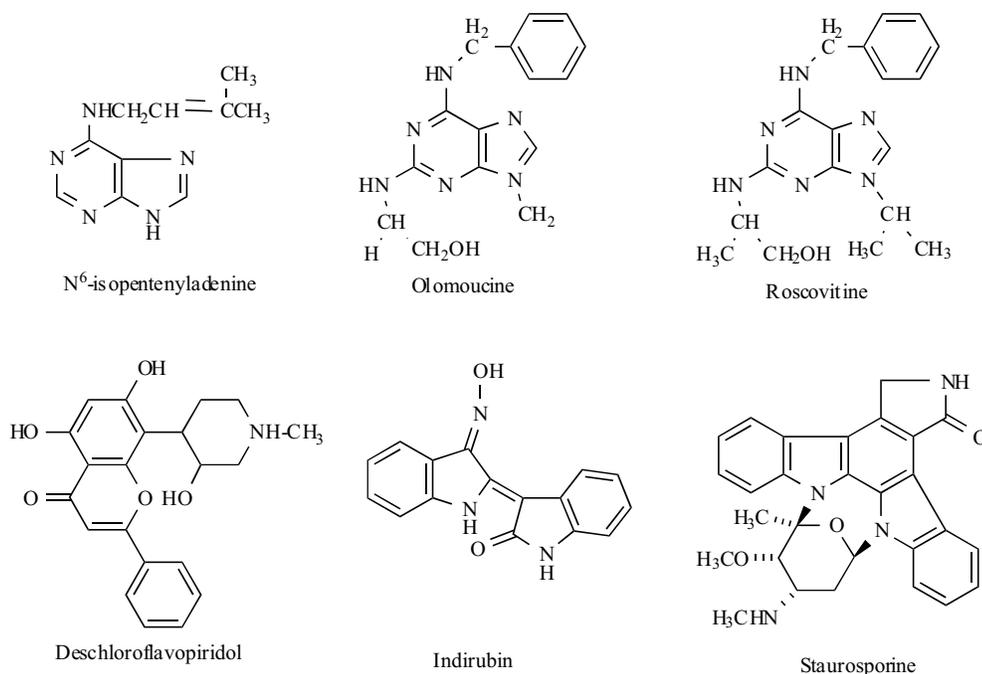
combination with other treatments. 3) In some case, inhibition of CDKs contributes to cell differentiation [15-18]. In this review, we focus on the inhibitors derivate from purine (such as olomoucine and roscovitine), flavone, oxindole, and staurosporine (figure 3 and table 1). These inhibitors are representative CDK inhibitors that have their structures determined in complex with CDK2 by X-ray diffraction crystallography and have at least one member of the inhibitor family in clinical trials.

## CDK2 STRUCTURE

The CDK2 is folded into the bilobal structure typical for most of protein kinases, with smaller N-terminal domain consisting of a sheet of five antiparallel  $\beta$ -stands and a single large  $\alpha$ -helix. The larger C-terminal domain consists primarily of  $\alpha$ -helices. It contains a pseudo-4-helical bundle, a small  $\beta$ -ribbon and two additional  $\alpha$ -helices. Figure 4

**Table 1. Inhibitors Studied in the Present Review**

Inhibitor	Chemical class	Specificity class	$IC_{50}(\mu M)$
$N^6$ -Isopentenyladenine (IPA)	Purine	Class I	50
Olomoucine	Purine	Class I	7
(R)-roscovitine	Purine	Class I	0.7
DFP	Flavone	Class II	?
Flavopiridol	Flavone	Class II	0.4
Indirubin-5-sulfonate	Oxindole	Class I	0.04
Staurosporine	Indololocarbazole	Class II	0.007
UCN-01	Indololocarbazole	Class II	0.007



**Fig. (3).** Structures of CDK chemical inhibitors discussed in this review.

shows the structure of CDK2 solved at 1.8 Å resolution [13, 19, 20] (PDB access code: 1HCL). The ATP-binding pocket is found in the cleft between the two lobes. The adenine base is positioned in a hydrophobic pocket between the  $\beta$ -sheet of the small domain. The ATP phosphates are held in position by ionic and hydrogen-bonding interactions with several residues, including Lys33, Asp145, and the backbone amides of the glycine-rich loop (residues 10-17). It was observed that ATP binding to CDK2 appears to induce a slight closure of the cleft by a 2.1° hinge movement around an axis parallel to the longitudinal axis of the ATP molecule [20]. In all cases, except for CDK2-cyclin A complex, electron density is weak and poorly defined in two regions in the structure, spanning residues 36-47 which links the N-terminal domain and "PSTAIR" or cyclin recognition helix and residues 150-164 of the "T loop" containing the activating phosphorylation site. All inhibitors and ATP bind in the deep cleft between the two domains.

Sequence alignment of CDK1, 2, 5 and 9 (figure 5) indicates that these CDKs share several conserved structures, such as: Glycine loop, T loop, and P loop. The high sequence identity among these CDKs allows that molecular modeling studies may be used to predict structures of CDKs which have no crystallographic structures determined, such as CDK1 [21] and CDK9 [22].

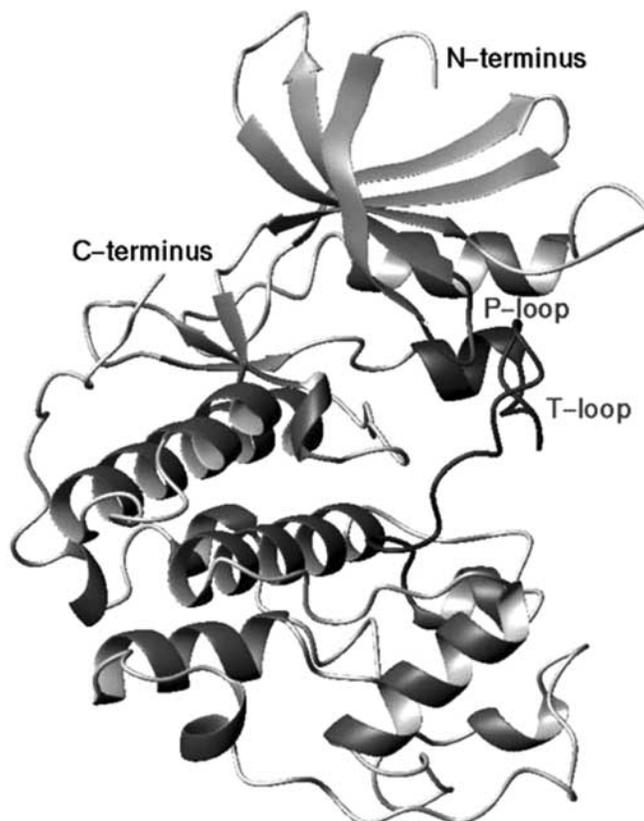
## INHIBITION OF CDK2

In addition to the classification of CDK inhibitor based on their specificity we may also classify these inhibitors using their chemical structure. Table 1 summarizes both

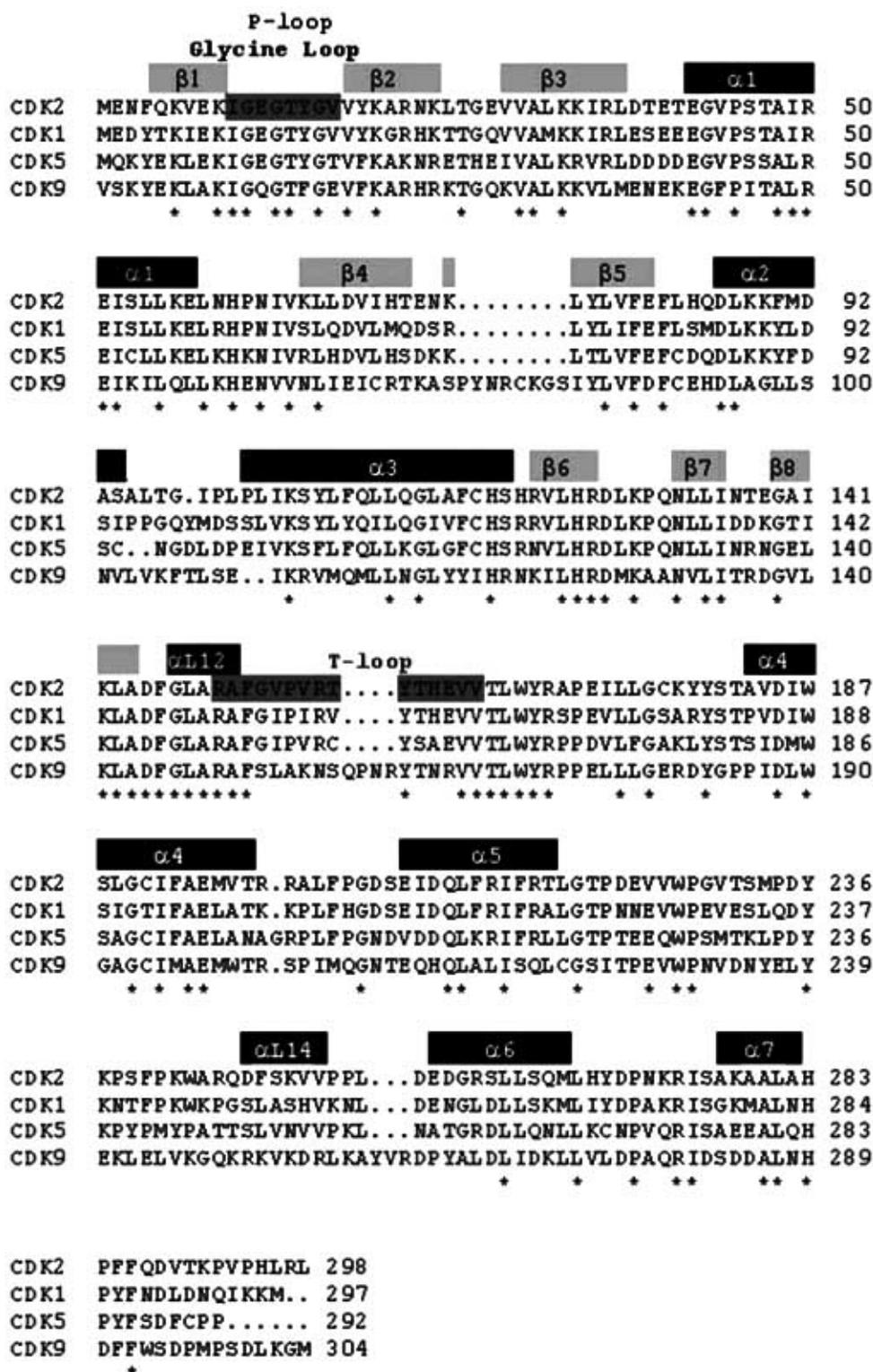
classifications and inhibitory constants as well. The CDK inhibitors studied in this review present  $IC_{50}$  ranging from 0.007 to 50  $\mu$ M [11]. They present low molecular weight, below 600, and they are all flat and hydrophobic heterocycles. Furthermore, all CDK inhibitors studied so far act by competing with ATP for binding in the CDK ATP-binding pocket [23]. Figure 6a-h show the interaction of the inhibitors with ATP-binding pocket. Most of the contacts are hydrophobic and the complexes present few intermolecular interactions. The variation of the charge distribution is due to different side chain positions in the complexes. Analysis of the contact area between inhibitor and CDK2 indicates that inhibitors with low  $IC_{50}$  values (higher affinity for CDK2) present higher contact areas and higher number intermolecular hydrogen bonds. The overall analysis of the intermolecular interaction between CDK2 and inhibitors strongly indicates that we would expect higher intermolecular contact area and higher number of intermolecular hydrogen bonds for inhibitors with low  $IC_{50}$ . This qualitative observation may help the guidance on exploring large chemical libraries, selecting potential new CDK inhibitor based on the intermolecular hydrogen bond pattern and contact area.

## MOLECULAR FORK

It has been observed in several structures of CDK2 complexed with inhibitors the participation of a molecular fork (Figure 7), composed by a C=O group of Glu81 and the N-H and C=O group of Leu83, in intermolecular hydrogen bonds between CDK2 and the inhibitors. There are sixty



**Fig. (4).** Ribbon diagram of CDK2 apoenzyme refined to 1.8 Å resolution (PDB access code: 1HCL).

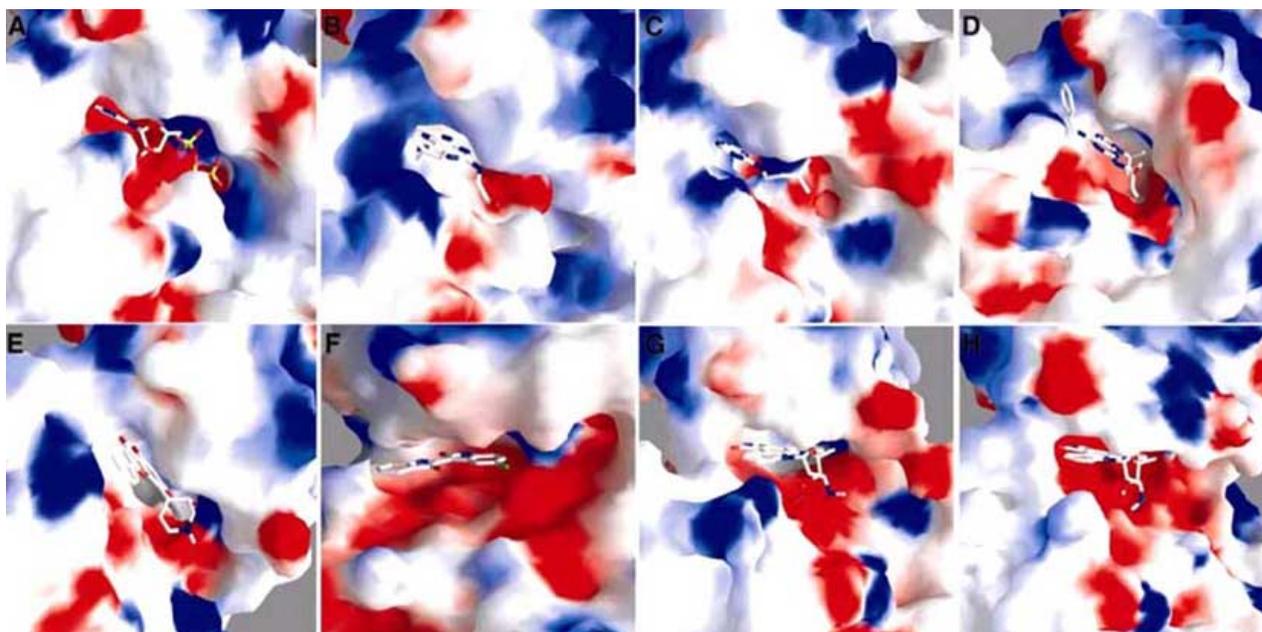


**Fig. (5).** Sequence alignment of human CDK2 with CDK9, CDK1, and CDK5. There are 39.1% of identity between CDK2 and CDK9 sequences, 65.1% between CDK2 and CDK1, and 58.3% between CDK2 and CDK5 sequences. The multiple alignment was performed with the program MultAlin [54].

structures of CDK2 complexed with inhibitors (search performed on the PDB [24] on August 2004). Analysis of the intermolecular hydrogen bond distances between CDK2 and all inhibitors that have atomic coordinates deposited in the PDB and also for the complexes of CDK2 with IPA, olomoucine, roscovitine, and DFP strongly indicates the

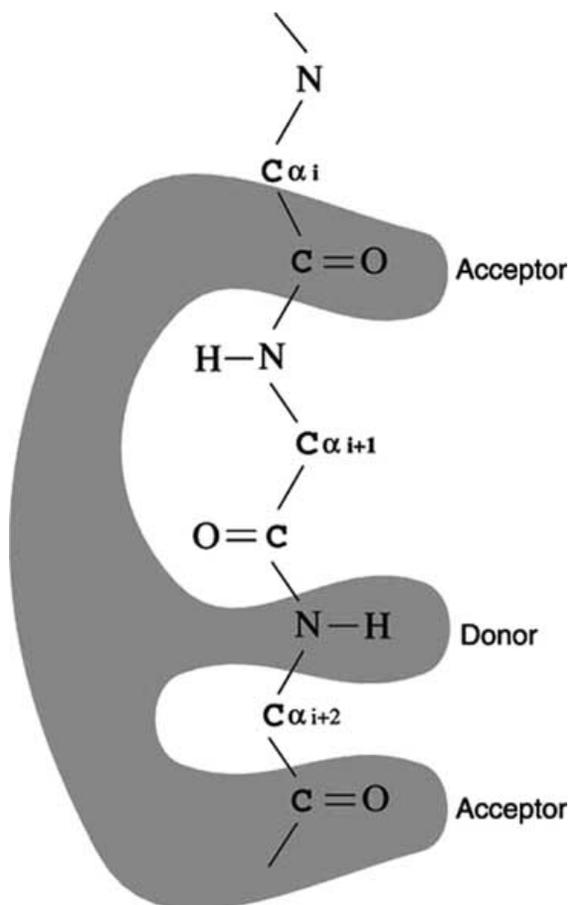
conservation of at least one member of the molecular fork in hydrogen bonding.

This molecular fork, composed of two hydrogen bond acceptors (C=O) and one hydrogen bond donor (N-H), allows a wide range of different molecules to dock on to the ATP binding pocket, such as: olomoucine,



**Fig. (6).** Interaction of CDK2 with ligands. a) CDK2-ATP; b) CDK2-IPA; c) CDK2-olomoucine; d) CDK2-roscovitine; e) CDK2-DFP; f) CDK2-indirubin; g) CDK2-staurosporine; h) CDK2-UCN01. All images are taken with the molecule in the same orientation. Electrostatic potential surface calculated with GRASP [55] shown from -10kT (red) to +10kT (blue). Uncharged regions are white.

isopentenyladenine, and roscovitine [19,25], staurosporine [26], purvalanols [27], indirubins [28], hymenialdisine [29],



**Fig. (7).** Schematic diagram of the molecular fork. Residues from  $i$  to  $i+2$  are Glu81, Phe82, Leu83 for CDK2.

NU2058 [12]. All these inhibitors have pairs of hydrogen bond partners that show complementarity to the molecular fork on CDK2, most of them involving at least two hydrogen bonds with the molecular fork. The relative orientation of the inhibitor in the ATP-binding pocket of CDK2 locates one hydrogen bond donor close to C=O in Glu81 and/or Leu83, and an acceptor close to N-H in Leu83. Such simple paradigm is conserved in all CDK2:inhibitor complex structures solved so far. In all binary models obtained by homology modeling this molecular fork is also conserved [22,30].

#### CONFORMATION DIFFERENCES BETWEEN ACTIVE AND INACTIVE FORM OF CDK2 AND A MECHANISM OF ACTIVATION

Comparison of the structure of CDK2 in the ATP complex [19-20] and the ternary complex of CDK2 with ATP and cyclin A [31] clearly shows that there are five significant conformational changes: (a) the two domains are more open in the ternary complex, thus, pushing the phosphate-loop (P loop) toward the N-terminal domain; (b) the conformation of the triphosphate of ATP and surrounding residues are different; (c) a short  $\alpha$ -helix becomes a  $\beta$ -strand in the " $\alpha/\beta$  transition box" on cyclin A binding; (d) the T loop containing Thr160, which becomes phosphorylated for CDK2 to be fully activated, reorients completely; and (e) the cyclin binding helix (PSTAIR) changes its location and orientation. Figure 8 shows a diagram for these differences. The conformational changes of the residues surrounding ATP, in turn, change the conformation of the triphosphate of ATP so that the scissile bond between  $\beta$ - and  $\gamma$ - phosphates now becomes aligned with the direction of attacking hydroxyl oxygen of the substrates of CDK2, the necessary alignment for ATP hydrolysis [13].

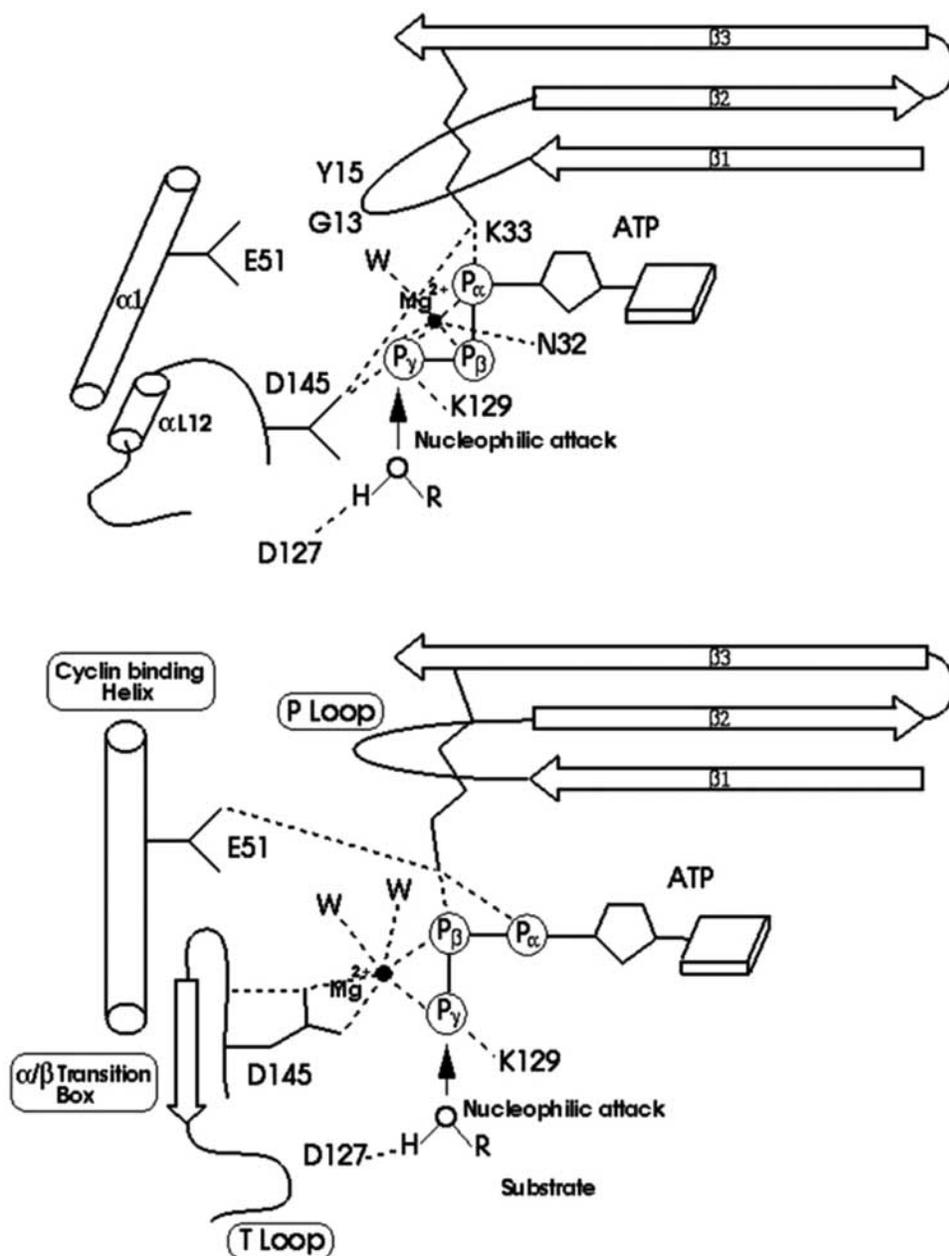


Fig. (8). Schematic drawing of the five regions of CDK2 that have different conformations.

## INTERACTIONS BETWEEN CDK2 AND LIGANDS

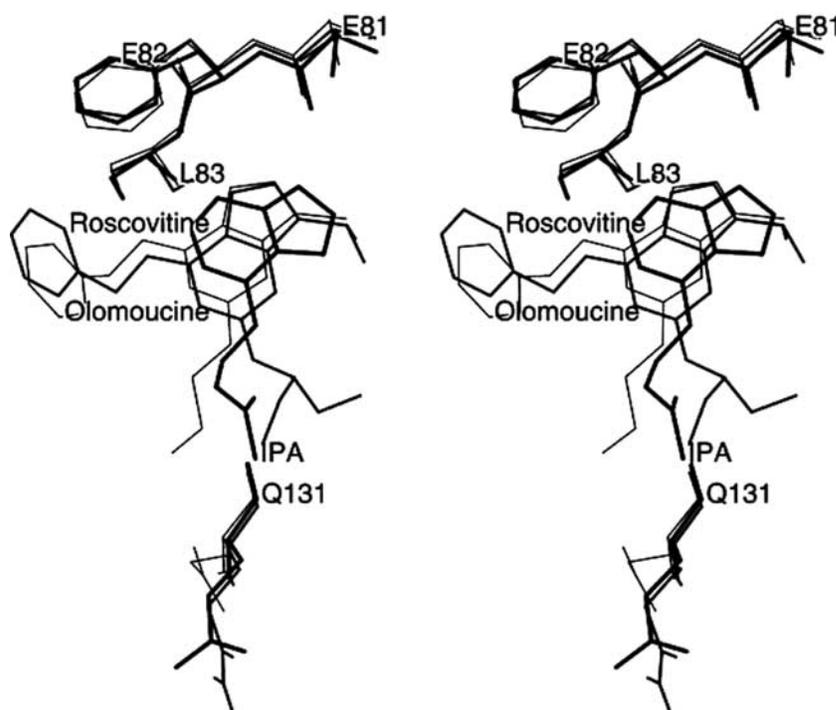
### ATP

The binding site for ATP as well as all the inhibitors which have their crystallographic structure determined in complex with CDK2 is located between the N- and C-terminal domains, and the binding of these ligands are associated with conformational changes of surrounding residues [13]. The refinement of CDK2 apoenzyme and ATP complex to 1.8 Å and 1.9 Å resolution, respectively, resulted in very accurate protein structural models as indicated by low R-values and good overall stereochemical quality [13, 19, 20]. The electron density for the refined structure of CDK2-ATP complex shows clear electron density for the ATP molecule with and 3 phosphates, a  $Mg^{2+}$  ion, and few water molecules in the binding pocket. The five-membered ribose ring is puckered into a C2'-endo

envelope [13, 19, 20]. As can be expected from the relative hydrophobicity of the ATP molecule, most hydrogen bonds and salt bridges are formed with the phosphate moieties, while the adenine base and ribose are involved in only 3 hydrogen bonds each. Three water molecules in the binding pocket are also mediating CDK2-ATP interactions. The  $Mg^{2+}$  ion is bound by one oxygen from each phosphate, and one from Asn132 and Asp145 side-chains and one water molecule [13].

### PURINE DERIVATIVES

Kinase assay of purine derivatives revealed that purine derivatives have inhibitory activity against CDK2, with  $IC_{50}$  of up to 0.4  $\mu M$ . There are crystallographic structures for CDK2 complexed with isopentenyladenine (IPA), olomoucine, and roscovitine solved to a resolution up to 2.0



**Fig. (9).** Relative orientation of purine derivative inhibitors in the ATP-binding pocket of CDK2. In thick line is the CDK2-roscovitine complex, in medium line is the CDK2-isopentenyladenine complex, and in thin line is the CDK2-olomoucine complex.

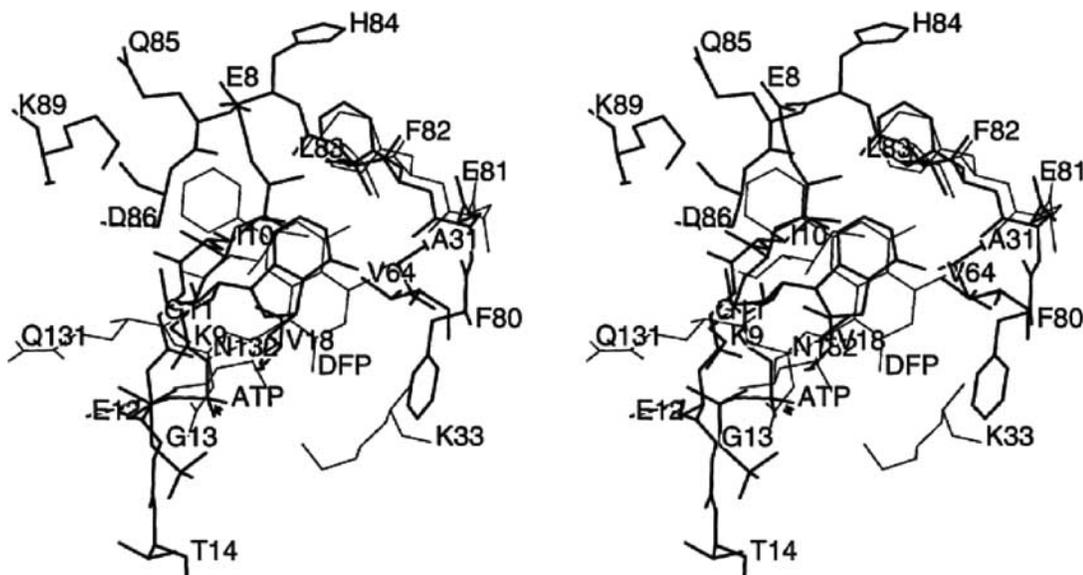
Å. Among these inhibitors (*R*)-roscovitine is the most promising drug candidate. Roscovitine was developed through a series of structure-lead structure-activity relation studies from 6-dimethylaminopurine [25]. *R*-roscovitine is currently undergoing phase II clinical trials in combination with standard chemotherapy regimes for advanced breast cancer and stage IIB/IV non-small cell lung cancer [32]. These inhibitors belong to class I of CDK inhibitors. In the structures of the complexes of CDK2 with purine derivatives the purine rings bind roughly in the same area of the ATP-binding pocket, the relative orientation of IPA purine ring with respect to the protein is different of the other adenine derivative inhibitors. Figure 9 shows the relative orientation of these CDK2 inhibitors in the ATP-binding pocket. This is probably due to the different size of the substituents groups of the purine in all inhibitors; the N6 amino group of the purine ring is replaced by isopentenylamino group in IPA and by bulky substituent groups in the other inhibitors.

## FLAVONE INHIBITORS

Previous studies have shown that flavopiridol, a flavonoid, and not a purine derivative, can inhibit growth of breast and lung carcinoma cell lines and can inhibit CDK activity at nanomolar concentration range of the inhibitor [33]. Flavopiridol is a synthetic flavonoid analogue of a natural alkaloid extract from the Indian plant, *Dysoxylum binactariferum* [34]. Flavopiridol is being tested in phase I and II clinical trials, because of its antiproliferative properties. Treatment with flavopiridol resulted in blocking cell cycle progression, promoting differentiation and inducing apoptosis in various types of cancerous cells [35]. Flavopiridol and deschloroflavopiridol (DFP) are class II CDK inhibitors, and the crystallographic structure of CDK2

in complex with deschloroflavopiridol (DFP) was determined to a resolution of 2.3 Å [36]. Analysis of the complex structure indicated that DFP binds in the ATP-binding pocket, with the benzopyran ring of DFP occupying approximately the same position as the purine ring of ATP. The two ring systems overlap in the same plane, but the benzopyran ring in DFP is rotated about 60° relative to the adenine in ATP, measured as the angle between the carbon-carbon bonds joining the cycles in benzopyran ring and adenine ring, respectively (Fig. 10). In this orientation, the O5 hydroxyl and the O4 of DFP are close to the positions of N7 amino group and N1 in adenine, respectively. In the DFP the phenyl ring is pointing toward the outside of the ATP-binding pocket and occupies a region not occupied any parts of ATP in the CDK2-ATP complex. The piperidinyl ring partially occupies the  $\alpha$ -phosphate pocket and is assigned to a chair conformation.

The DFP inhibitor molecule is quite hydrophobic. Hence, binding to CDK2 is characterized by predominantly hydrophobic and van der Waals interactions with the same hydrophobic enzyme residues that form the pocket for the adenine base in the CDK2-ATP complex structure. However, there are more contacts between the enzyme and the benzopyran ring of DFP (34 contacts) than were observed between CDK2 and the adenine ring of ATP (26 contacts). It was observed 5 hydrogen bonds between DFP and CDK2, involving residues Lys33, Glu81, Leu83, and Asp145, residues that also form intermolecular hydrogen bonds in the CDK2-ATP complex. There are 7 van der Waals contacts with hydroxymethyl piperidinyl ring, and 10 with the phenyl ring, which increases the total number of contacts between DFP and CDK2 to 56. Many of the contacts between DFP and CDK2 are made by only three residues, Ile10, Leu83, and Leu134, which form a total of 24



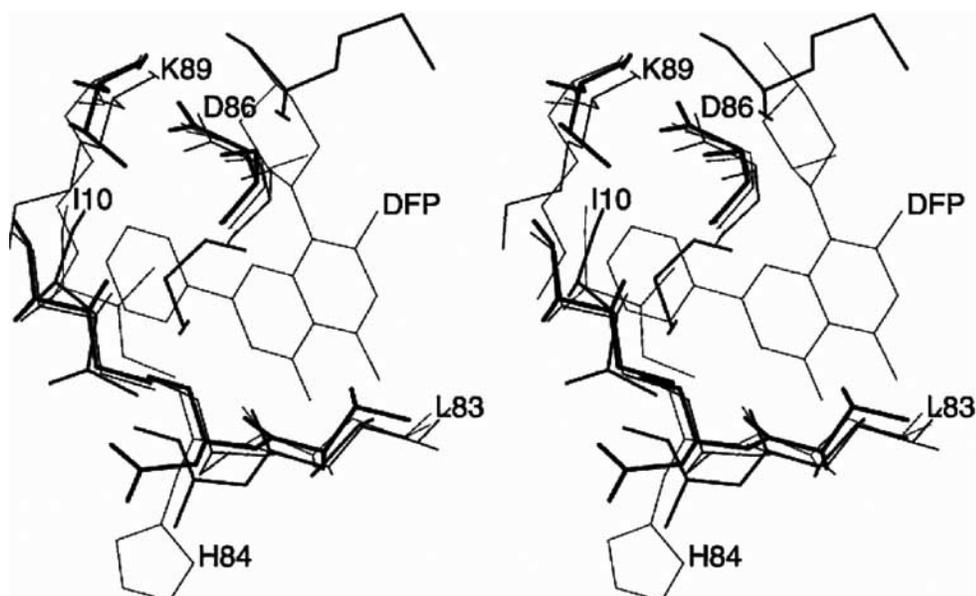
**Fig. (10).** Relative orientation of ATP (thick line) and DFP (thin line) after superposition of the complex structures on C $\alpha$  atoms of CDK2.

contacts, corresponding to 43% of the observed intermolecular contacts.

The flavopiridol molecule has a chlorophenyl instead of the phenyl in the DFP molecule, and this modification increases the kinase inhibition by a factor of 6 [36]. This is probably due to the new possible intermolecular contacts that the chlorine makes with CDK2, involving residues Leu10, Phe82, and Leu83, which increase the total number of contacts between flavopiridol and CDK2 to 61. The observation that when the phenyl group is replaced by an ethyl or propyl group, the kinase inhibition goes down by a factor of approximately 6 [35] can also be understood from the poor interaction of these groups with the protein at this region. The crystal structure of the complex CDK2-DFP suggests few positions of DFP for potential modifications to improve kinase inhibition, such as: a) Change the

chlorophenyl ring of flavopiridol by a bulky group, for instance additional chlorine in the phenyl group. b) Increase the number of hydrogen bond partners to rise the number of intermolecular hydrogen bonds, especially in the benzopyran ring of flavopiridol.

CDK1, CDK2 and CDK4 kinase activity is potently inhibited by flavopiridol ( $IC_{50}$  up to 0.1  $\mu M$ ) [11]. In contrast, flavopiridol has not been shown to inhibit as potently other protein kinases, such as cyclic AMP-dependent protein kinase (PKA) ( $IC_{50}$  = 145  $\mu M$ ) [33]. This can be understood by comparison of the residues that make intermolecular contact with DFP in the complex CDK2-DFP with equivalent residues in the other kinases. The major differences between, CDK1, CDK2, CDK4 and PKA occur in the residues of CDKs that make intermolecular contacts with the phenyl ring of DFP inhibitor (Ile10, Leu83, His84,



**Fig. (11).** Superposition of the active site of CDK2-deschloroflavopiridol complex (CDK2-DFP) with CDK1, CDK4, and PKA (thick line). It can be observed the orientation of the residues of PKA.

Asp86, and Lys89) (Figure 11). In CDKs, which are potently inhibited by flavopiridol, these residues are almost completely conserved as Ile10, Leu83, Ser84, Asp86, and Lys89. In contrast, in PKA, which is not potently inhibited, no residue is conserved.

## INDIRUBINS

Indirubins are CDK inhibitors which belong to the chemical class of oxindole. Indirubin is a minor constituent of a chinese antileukaemia medicine [37]. It has undergone clinical trials in China to treat CML with promising results, although its mechanism of action remains unclear [38]. Indirubin proved to be an inhibitor of CDK2 and also a potent inhibitor of CDK5. The crystallographic study of the complex CDK2- Indirubin-5-sulfonate [39] indicates that Indirubin-5-sulfonate binds in the deep cleft between the two domains of CDK2, as observed for all CDK inhibitors (Figure 6f). There are total of five hydrogen bonds. All members of the molecular fork of CDK2 participate in intermolecular hydrogen bonds with the inhibitor.

## UCN-01

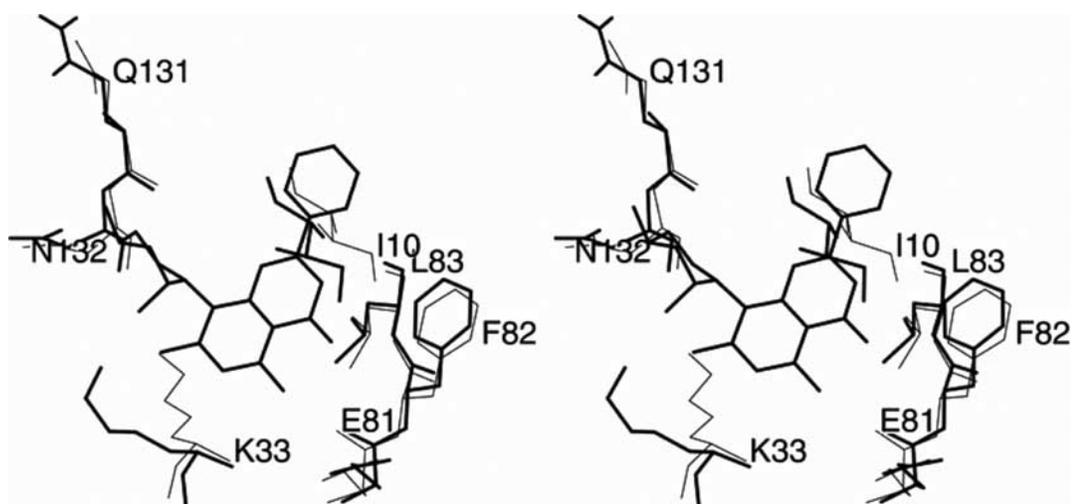
UCN-01 is another CDK2 inhibitor that is on clinical trials [23] and has its structure determined in complex with CDK2 [40]. UCN-01 is a hydroxylated form of staurosporine (7-hydroxystaurosporine) and both compounds are natural products, originally identified as potent protein kinase C inhibitors [40]. Staurosporine is non-selective and too toxic for use in therapy, but UCN-01 shows greater selectivity and is currently undergoing clinical trials in the United States and Japan, shown antiproliferative effects and cytostatic properties in several human tumor cell lines [23]. Analysis of the complex between CDK2 and UCN-01 [40] (PDB access code:1PKD) indicates that there are three intermolecular hydrogen bonds between the inhibitor and CDK2, surprisingly involving only the molecular fork. Analysis of the intermolecular contact between UCN-01 and CDK2 indicates that the inhibitor mimic the contacts made by the adenine moiety of ATP, and exploit the non-polar nature of the ATP-binding pocket.

## DIFFERENCES IN CDK2 SIDE-CHAIN CONFORMATIONS IN THE BINDING POCKET

In contrast to the finding that cyclin A changes little on CDK2 binding, the conformation of CDK2 appears to be much more adaptable as manifested by its changes on binding of ATP, inhibitors, as well as cyclin A [12,13,19, 20,24]. The crystal structure of the complex CDK2-cyclin A revealed the mechanism of CDK activation by cyclin binding. Specifically, cyclin-induced conformation changes in the T loop and the PSTAIRE helix expose the ATP-binding pocket and CDK2 Thr160 for phosphorylation [31].

Superposition of the CDK2 in complex with inhibitors and CDK2 apoenzyme based on their C $\alpha$  atoms revealed significant movement of few side chains. Figure 12 shows ATP-binding pocket of CDK2 after the superposition of the complex CDK2:DFP onto CDK2 apoenzyme to illustrate the main conformational changes. In addition to small changes in the backbone of the P loop, four residues change their side chain conformations most: Ile10, Lys33, Gln131, and Asn132. The side-chain of Gln131 is directed away from the binding pocket in complexes CDK2-ATP and CDK2-IPA. In the other purine derivatives complexed with CDK2, however, the side-chain points into the binding pocket and forms intermolecular hydrogen bonds and van der Waals contacts with the purine derivative inhibitors.

Lys33 and its equivalent Lys72 in PKA are important for ATP binding. In all inhibitor bound CDK2 complexes the side-chain of this residue has moved away to accommodate the inhibitors except in the CDK2-IPA complex, where the side chain conformation is the same as that of CDK2 apoenzyme, presumably because of the small size of the inhibitor (IPA). In the complex CDK2-ATP, the residue Lys33 makes intermolecular salt bridges with the  $\alpha$ -phosphate of ATP and Asp145, a residue involved in ATP binding [20]. The residues Ile10 and Asn132 undergo smaller conformational changes on the ligand binding. In summary, the observed side-chain differences in the ATP-binding pocket seem to improve the fit between CDK2 and inhibitor molecules.



**Fig. (12).** Stereo view of the ATP-binding pocket of CDK2 apoenzyme (thin line) and CDK2-DFP (thick line) after superposition of the complex structures of CDK2.

## CONCLUDING REMARKS

In recent years there was an explosion in the number of crystallographic structures of CDK2 in complex with inhibitors [41-48]. These recent structures were reviewed in several articles in the last two years [49-53]. However, only four CDK2 inhibitors reached clinical trials.

Several research groups have works on the discovery, optimization, and characterization of potent CDK inhibitors. Many have reached the nanomolar IC<sub>50</sub> level, and present good selectivity [11]. The comparison of the three-dimensional structures of CDK2 complexed with inhibitors described here explain the specificity and potency of the CDK inhibitors, and suggest structure-based design and combinatorial library design may enhance the chance of discovering improved inhibitors of CDK2. There are four CDK2 inhibitors in clinical trials: flavopiridol [22], (R)-roscovitine [32], indirubin-5-sulfonate [39], and UCN-01 [40]. Analysis of the structure of these inhibitors complexed with CDK2 revealed some common features that may guide future drug developments. We summarize the lessons learned from these studies in the following observations:

- 1) The specificity of each inhibitor for CDK2 over other kinases comes from the interaction between substitution groups outside the aromatic scaffold and the peripheral surface of the ATP-binding pocket, except for the UCN-01 [40]. This observation dispels the conventional wisdom that "the common base such as adenine can not be a good scaffold for inhibitor design because there are many enzymes in cells that bind adenine derivatives". Previous studies clearly indicate that specificity can be created by appropriate modification of a common scaffold molecule [13,25].
- 2) The ATP-binding pocket of CDK2 has a surprising ability to accommodate molecular structures that are completely different from adenine derivatives, such as flavones, oxindole derivatives and indirubin with some unpredictable conformational changes of the residues in the pocket. This may be related to the relatively weak binding of the ligands (Kd- $\mu$ M).
- 3) The knowledge of the orientation of adenine of ATP in binding pocket did not allow predicting how the adenine moiety of roscovitine, olomoucine and IPA would bind to CDK2. The crystallographic structures of these complexes strongly indicates that any "rational drug design" based on adenine position of ATP would have completely missed in predicting roscovitine, olomoucine and IPA as potential CDK2 inhibitors.
- 4) One striking common structural feature observed in all crystallographic structures of complexes between CDK2 and inhibitors is the participation of the molecular fork of CDK2 in intermolecular hydrogen bonds. Furthermore, analysis of the CDK inhibitor currently in clinical trials (flavopiridol, roscovitine, indirubin, and UCN-01) strongly indicate that at least two members of the molecular fork should participate in intermolecular hydrogen bonds, to ensure proper orientation in ATP-binding pocket. This structure may be exploit in the design of future CDK inhibitors.

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