REVIEW ARTICLE

Cyclin-Dependent Kinase 2 in Cellular Senescence and Cancer. A Structural and Functional Review

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Abstract: ***Background*:** Cyclin-dependent kinase 2 (CDK2) has been studied due to its role in the cell-cycle progression. The elucidation of the CDK2 structure paved the way to investigate the molecular basis for inhibition of this enzyme, with the coordinated efforts combining crystallography with functional studies.

***Objective*:** Our goal here is to review recent functional and structural studies directed to understanding the role of CDK2 in cancer and senescence.

***Methods*:** There are over four hundreds of crystallographic structures available for CDK2, many of them with binding affinity information. We use this abundance of data to analyze the essential features responsible for the inhibition of CDK2 and its function in cancer and senescence.

***Results*:** The structural and affinity data available CDK2 makes it possible to have a clear view of the vital CDK2 residues involved in molecular recognition. A detailed description of the structural basis for ligand binding is of pivotal importance in the design of CDK2 inhibitors. Our analysis shows the relevance of the residues Leu 83 and Asp 86 for binding affinity. The recent findings revealing the participation of CDK2 inhibition in senescence open the possibility to explore the richness of structural and affinity data for a new era in the development of CDK2 inhibitors, targeting cellular senescence.

***Conclusion*:** Here, we analyzed structural information for CDK2 in combination with inhibitors and mapped the molecular aspects behind the strongest CDK2 inhibitors for which structures and ligand-binding affinity data were available. From this analysis, we identified the significant intermolecular interactions responsible for binding affinity. This knowledge may guide the future development of CDK2 inhibitors targeting cancer and cellular senescence.

Keywords: CDK2, cyclin, cellular senescence, protein-ligand interactions, drug design, cancer.

1. INTRODUCTION

Cyclin-dependent kinases (CDK) have a central role in regulating the cell-cycle progression by activating expression of cyclins and endogenous CDK inhibitors at checkpoints [1-3]. Cyclin-dependent kinase 2 (p33 Protein Kinase or Cell Division Protein Kinase 2 or CDK2) is an enzyme encoded by the CDK2 gene and has a cyclin partner that regulates cell-cycle progression like centrosome duplication, DNA synthesis, G1-S transition, and modulation of G2 progression. During the G1 and S phases, the CDK2 function is imperative for transitioning from one stage to another [4].

Since CDK2 should be active for the transition from G1 to S phases, drugs that inhibit its action have the potential to treat cancer [5]. When there is damage to genome integrity, the signaling cascade machinery of the cell is activated to block cell-cycle progression. Proteins p53 and p21 are involved in halting this process. This intricate contrivance is part of the so-called DDR or DNA damage response [6-8].

On the other hand, while DDR interrupts specific cell activities and represses CDK activity to prevent mitotic entry of damaged cells, findings show that a reduced level of the same activities continues to go on even after damage. This mechanism drives damaged cells to accumulate cyclins and Polo-like kinase 1 (Plk1), affecting the new cells and contributing to senescence [9-12].

Minor levels of CDK2 can function as a crucial point to accumulate mitotic inducers to advance or not in the cell

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cycle through S and G2 phases, acquiescing the expression of G2 specific proteins and repair the damaged DNA. It can also induce the expression of other pathways such as the p21 expression and lead the cycle to an exit and senescence after G2 phase [10].

Seeing the importance of CDK2, information about the structural and ligand-binding affinity of this protein should be crucial to developing new inhibitors for this enzyme. Methods such as X-ray diffraction crystallography, cheminformatics as well as bioinformatics techniques could contribute to the design of more active inhibitors. Also, the application of such techniques can increase the chances of progress in the evaluation of the interaction with specific proteins [13, 14].

In this review, we examine the significance of CDK2 for cancer and the implication of CDK2 inhibition for cellular senescence. Also, we carried out an analysis of the crystallographic data available for CDK2 in complex with inhibitors. Our study reveals the critical structural features responsible for binding affinity and the potential use of the CDK2 inhibitors to address cellular senescence.

2. CDK FAMILY AND CDK2

Among 518 putative protein kinase genes identified in the human genome, 20 of these are CDKs [15]. Specifically, five CDKs are involved directly in the operation of the cell-cycle regulation known to be common to all eukaryotes: CDK1, CDK2, CDK3, CDK4, and CDK6. This group of proteins is of essential importance to the structure and workings of the animal cell [5, 16-18]. A previous study of the sequence alignment of cell-cycle-related CDKs [18] shows the conservation of the residues involved in the active site of CDKs. This conservation highlights the challenge in the design of specific inhibitors for CDK since an inhibitor designed to address one CDK might interfere with another CDK.

Considering the cell-cycle-related CDKs, all CDKs connect to a regulatory protein called cyclin, and, without the formation of the cyclin-CDK complex, there would be no high activity of this protein kinase [19, 20]. Specifically, CDK2 has as cyclin partners the cyclins A and E which have the primary function to regulate cell-cycle progression during phases S and G2 [19].

Structural analysis of CDK2 indicated that rotation of N- and C- terminal domains caused by the cyclin partner [21-23] is necessary for its activation. The CDK2 remains inactive when associated with cyclin A and the subsequent phosphorylation of Th160 results in active CDK2-cyclin A complex [21-23]. This active complex can also be responsible for the inactivation of some proteins that are engaged in regulating the G1 and S phases. When inhibited, CDK2 can diminish those activities and consequently stop cell-cycle progression [23-27].

A previously published study identified that CDK2 is superexpressed in a few tumor lines together with the increase in the presence of cyclin E [26]. Also, the inhibition of CDK2 by specific ligand and blockage of its function can reduce the growth of carcinogenic cells significantly in melanoma line [27].

Discovering ligands that could work on binding specifically to the ATP-binding pocket of CDK2 could induce apoptosis and lead to an impairment of cell-cycle progression, being sufficient as a therapy for some types of cancer. The findings and development of selective CDK2 inhibitors could be further explored as antitumor strategies and have other targets such as cellular senescence.

3. CDK2, CANCER, AND SENESCENCE

Being CDK2 intrinsically connected to the regulation and balance of cell-cycle progression, when activated or dysregulated, immediately an aberration in the proliferating machinery occurs, often leading to a severe uncontrolled mutation of cells [28]. Growth-promoting oncogenes when activate stimulate pathways can also trigger the activation of CDK2, culminating in the appearance of several abnormalities in cell cycle as mentioned before. Such mutations can cause the development of malignancies like cancer [29, 30].

Prior studies focusing on quantifying levels of CDK2 in different types of cancer, detected overexpressed numbers of this specific protein. In colon adenoma and focal carcinoma in adenomatous tissue, there were significantly higher levels of this enzyme [31]. For gene amplification, Western blot analysis reported higher levels of CDK2 for patients with colorectal cancer compared to levels of normal mucosa. Also, the ratio of the hyperphosphorylated form of pRb was higher for these types of malignancies as well [32]. The protein expression of cyclin (D1, D3, E, and A) and CDKs (CDK4, CDK2, and CDC2) was again higher for this type of tissue than in healthy tissue. In a group of eight patients, seven of them had increased CDK2 activity in cancer tissues [31]. Analysis of the expression of CDK2 and associated cyclins in human lung cancer showed higher levels of these proteins lung cancer when compared to normal tissues [33].

As part of cell-cycle progression, inhibition of CDK2 naturally occurs in the cell. Natural inhibitors, such as Cip/Kip and INK4, help to constrain CDK2 activity within the cell during checkpoints. Knowing that CIP/KIP and INK4 proteins have regulatory CDK2 functions, it is understood that CDK inhibitors are of great interest in cancer therapy [4-8, 14].

On the one hand, these essential occurring proteins are present in healthy cells and for cancer cell lines they are mutated or deleted, not being able to suppress CDK2 levels in malignancy cases. Compounds that can inhibit CDK2 activity are of interest for cancer research due to their potential in regulating cell-cycle progression [13].

Differently, the removal of CDK2 in p27Kipl knockout mice showed no significate changes in tumor progression or growth, indicating that its mechanisms are not affecting tumorigenesis. The inactivation of Cip-Kip endogenous inhibitors can lead to an intervention in the cell regulatory mechanism, being considered a good research strategy towards understanding more about arresting cell cycle [34].

Cellular senescence is the programmed mechanism of complete cell cycle arrest that occurs in viable cells and is activated when several stresses that can modify fundamental characteristics of this system occur [35, 36]. Shortening of telomeres associated with replicative senescence, genotoxic

agents that eventually cause DNA damage activating cell cycle checkpoints, and premature senescence stimulated by oncogenic signalization are factors that can trigger its machinery [37]. Oncogenes can be the cause of extreme stress on cellular levels and disseminate the awakening of a system that can work to prevent potential malignancies as previously observed. Such pathways can be the precursors of cellular senescence [29].

DDR pathways are known for their functions to regulate cell-cycle progression. This network of signaling and processing factors can be the target of selective therapies related to proteins that can monitor cell growth [38]. CDK2 is part of this intricate map, and when inhibited through DDR, it is still present in small levels and capable of maintaining their role as DNA replicators [39]. Functions like cell-cycle progression, DNA replication, and repair during S phase, all occur due to CDK2. Moreover, in G2 phase, CDK2 activity still allows p21 production. This never-ending involution remains an open loophole, thereby driving cell proliferation, and inducing DNA replication stress. The hypothesis to find a way to inhibit this protein, making this an exit of the degenerative cell cycle is of great importance [40].

Müllers and collaborators (2017) [41] found that when inhibiting CDK 1/2, p21 mRNA levels diminished, suggesting that CDK activity promotes senescence by establishing higher levels of p21; a strategy to suppress tumor growth [41, 42]. Contrarily, Zalzali and collaborators (2015) [43] tested a normal cell line of unstressed fibroblasts for CDK2 inhibition and concluded that there was a complete exit of the cell cycle and no traces of senescence.

Zalzali and collaborators (2015) [43] showed that p53-dependent repression of CDK2 could be a powerful tool and a key mechanism for making the cell exit its proliferative cycle entering a senescent state. Another result was that when tested clinically, the inhibition of CDK2 could drive cells that were protumorigenic into a senescent state, making this another critical finding towards exploring a cancer therapy.

Senescence induction as a therapeutic tool can be a way to combat tumor progression, but when triggered, there is an inevitable secretion of inflammatory cytokines and growth factors (senescence-associated secretory phenotype or SASP) from the senescent cells, which is responsible for potential stimulation of tumor cells and negatively affecting healthy cells and tissues surrounding the area [44, 45]. Riggelen and Felsher (2010) [46] inquired about the study that Myc can act both as an inducer and a repressor of senescence [47, 48].

To be able to suppress senescence, Campaner and collaborators [47] observed that the deficiency of or reduction in Myc in BRAFV600E-expressing melanoma cell leads to cellular senescence in several experiments when there is a programmed turn off of Myc in mice models, Myc being responsible for the stimulation of WRN expression and CDK2 activity [46, 49, 50]. For this scenario, Myc should start the suppression of its senescence-promoting induction [48].

A previous study showed that Myc and Ras are two oncogenes that not only stimulate cell proliferation but also induce tumor suppressive failsafe mechanisms [48]. In agreement with these findings, cellular senescence has been identified in several types of premalignant human tumors, which gives further evidence that this mechanism is an essential tumor-suppressive process [29, 44, 45]. Especially interesting is the evidence of the involvement of cell-cycle regulators, CDKN2a (p16Ink4a) and CDKN1A (p21Cip1), in the senescence response [51-53]. These proteins are naturally occurring CDK inhibitors. Furthermore, it has been demonstrated that inhibition of CDK2 and subsequent induction of senescence could be achieved through ligands selectively targeting CDK2 ATP-binding pocket [48].

In this line of thought, Campaner, Doni, and colleagues indicated that chemical inhibition of CDK2 is responsible for senescence in Myc-overexpressing cells by activating transcription of hTERT [54]. This study also reported that pharmacological inhibition of CDK2 provokes Myc-dependent senescence in several cell types, including a p53-null human cancer cell line. In Myc-driven tumors, the use of CDK2 inhibition suggests that cellular senescence might be a valid therapeutic mechanism [50, 55, 56]. This report indicated that pharmacological inhibition of CDK2 could trigger cellular senescence, and another study showed that inhibition of CDK1 causes apoptosis in Myc-expressing cells [57, 58].

Taken together, we may say that these studies suggested that dual inhibitors of CDK1 and CDK2 might be particularly advantageous for the treatment of Myc-driven tumors. Therefore, the establishment of the structural basis for inhibition of CDK is pivotal for the designing of a new generation of CDK inhibitors driven by the potential beneficial impact of dual inhibition of CDK1 and CDK2 in the treatment of Myc-driven tumors. Considering that CDK1 and CDK2 show high sequence identity and the active-site residues are conserved, the dual inhibition is most likely to be a common occurrence for these enzymes.

4. MATERIALS AND METHODS

In this work, our focus was on the crystallographic structures of human CDK2. Although CDK2 has a structural complexity that can be handled by other techniques such as nuclear magnetic resonance (NMR) spectroscopy [59], all structural data available for CDK2 was obtained using X-ray diffraction crystallography [60]. X-ray diffraction crystallography is a powerful technique to determine atomic coordinates of protein-ligand complexes and has been successfully applied for over four hundred of CDK structures (search carried out on the Protein Data Bank (PDB) [61-63] on June 6, 2018).

We have selected three-dimensional structures of human CDK2 solved by X-ray diffraction crystallography for which half-maximal inhibitory concentration (IC_{50}) information was available (search carried out on the PDB on May 29, 2018). PDB gathers experimental binding affinity data from three other databases: BindingDB [64, 65], MOAD [66, 67], and PDBbind [68, 69]. This search returned 98 CDK2 complex structures. We further filtered CDK2 structural information to eliminate entries without crystallographic water molecules or repeated ligands. After data filtering, we ended up with a dataset comprising of 87 unique (no repeated ligands) CDK2 complex structures. We included the presence of water molecules as search criteria due to the importance

of water-mediated hydrogen bonds for binding affinity, as previously reported for crystal structures of CDK2 with competitive inhibitors [70].

We show the complete list of the PDB access codes in Table **1**. The ensemble of CDK2 structures (Table **1**) will be referred to as CDK2 dataset. We used the program SAn-DReS [71] to download the PDB structures, binding affinity information, and to carry out data filtering for the CDK2 dataset. Ligand information and binding affinity data are on supplementary material 1.

Table 1.　PDB access codes for the CDK2 dataset.

PDB Access Codes
1JVP, 1OIR, 1OIT, 1PXI, 1URW, 1YKR, 2A0C, 2B52, 2B54, 2B55, 2BHE, 2BTR, 2BTS, 2C68, 2C6I, 2C6K, 2C6M, 2CLX, 2R3F, 2R3G, 2R3H, 2R3I, 2R3J, 2R3K, 2R3L, 2R3M, 2R3N, 2R3O, 2R3P, 2R3R, 2VTH, 2VTQ, 2VTR, 2VTS, 2VTT, 2VV3, 2VV9, 2W05, 3DDQ, 3EZR, 3EZV, 3FZ1, 3IG7, 3IGG, 3NS9, 3PJ8, 3PXY, 3PXZ, 3PY0. 3QQK, 3QTQ, 3QTR, 3QTS, 3QTU, 3QTW, 3QTX, 3QU0, 3QXP, 3R8V, 3R8Z, 3R9D, 3R9N, 3R9O, 3RAH, 3RAL, 3RJC, 3RK7, 3RK9, 3RMF, 3RNI, 3RPR, 3RPV, 3RPY, 3RZB, 3S00, 3S1H, 3SQQ, 3TI1, 3TIY, 3UNJ, 4BGH, 4FKI, 4FKL, 4GCJ, 4NJ3, 4RJ3, 5D1J

To analyze protein-ligand interactions for CDK2-ligand complexes, we employed the program LigPlot+ [72, 73]. We focused on the intermolecular interaction analysis on the active ligands available for all structures in the CDK2 dataset. We could say that the specificity and affinity between a small-molecule ligand and its protein target depend on several physical-chemical parameters. The main features responsible for binding affinity are the following: electrostatic interactions, intermolecular hydrogen bonds, and van der Waals contacts as well as on geometric fit of the contact surfaces of both molecules [74-78].

We may say that the application of a robust computational tool to evaluate intermolecular interactions has a beneficial impact in the analysis of the main structural features responsible for binding affinity, which is even more critical when we consider two extra elements: the abundance of structural data for CDK2 and the existence of experimental binding information for these crystallographic structures.

5. RESULTS AND DISCUSSION

5.1. CDK2 Structure

The first crystallographic structure of human CDK2 was determined in 1993 at the University of California Berkeley [79]. Analysis of the high-resolution crystallographic structure revealed the typical bilobal molecular architecture of serine/threonine protein kinase (EC 2.7.11.1). Figure (**1**) shows the structure of CDK2 in complex with ATP (PDB access code: 1HCK) [22]. The N-terminal domain is mainly composed of a distorted beta-sheet and a short alpha helix. A helix bundle forms the C-terminal. The two lobes allow the binding of the ATP molecule, as we can see in Fig. (**1**).

Analysis of the CDK2-ATP interactions is shown in Fig. (**2**) [22]. The adenine ring shows two intermolecular hydrogen bonds involving main-chain atoms of Glu 81 and Leu 83. The phosphate groups exhibit an intricate network of electrostatic interactions and hydrogen bonds involving Lys 33, Lys 129, and Thr 14. The identification of the main residues responsible for binding of the ATP strongly indicate the pivotal intermolecular interactions to be explored in the design and development of competitive inhibitors for CDK2. In the next section, we describe the main aspects of CDK2-inhibitor interactions.

5.2. CDK2-ligand Interactions

Based upon an analysis of protein-ligand interactions carried out with the programs LigPlot [72, 73] and SAn-DReS [71]; we identified that the residues Ile 10, Phe 82, Leu 83, Leu 134, and Asp 145 are the most common intermolecular interactions involving CDK2 and inhibitors for the
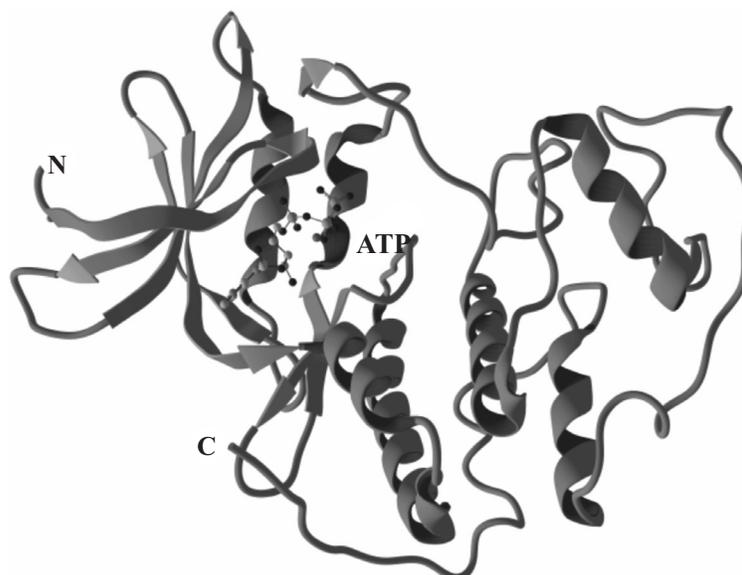
Fig. (1). Crystallographic structure of human CDK2 in complex with ATP (PDB access code: 1HCK). We indicate N- and C- terminus in the figure. The figure above was generated using Molegro Virtual Docker (MVD) [80].

1hck
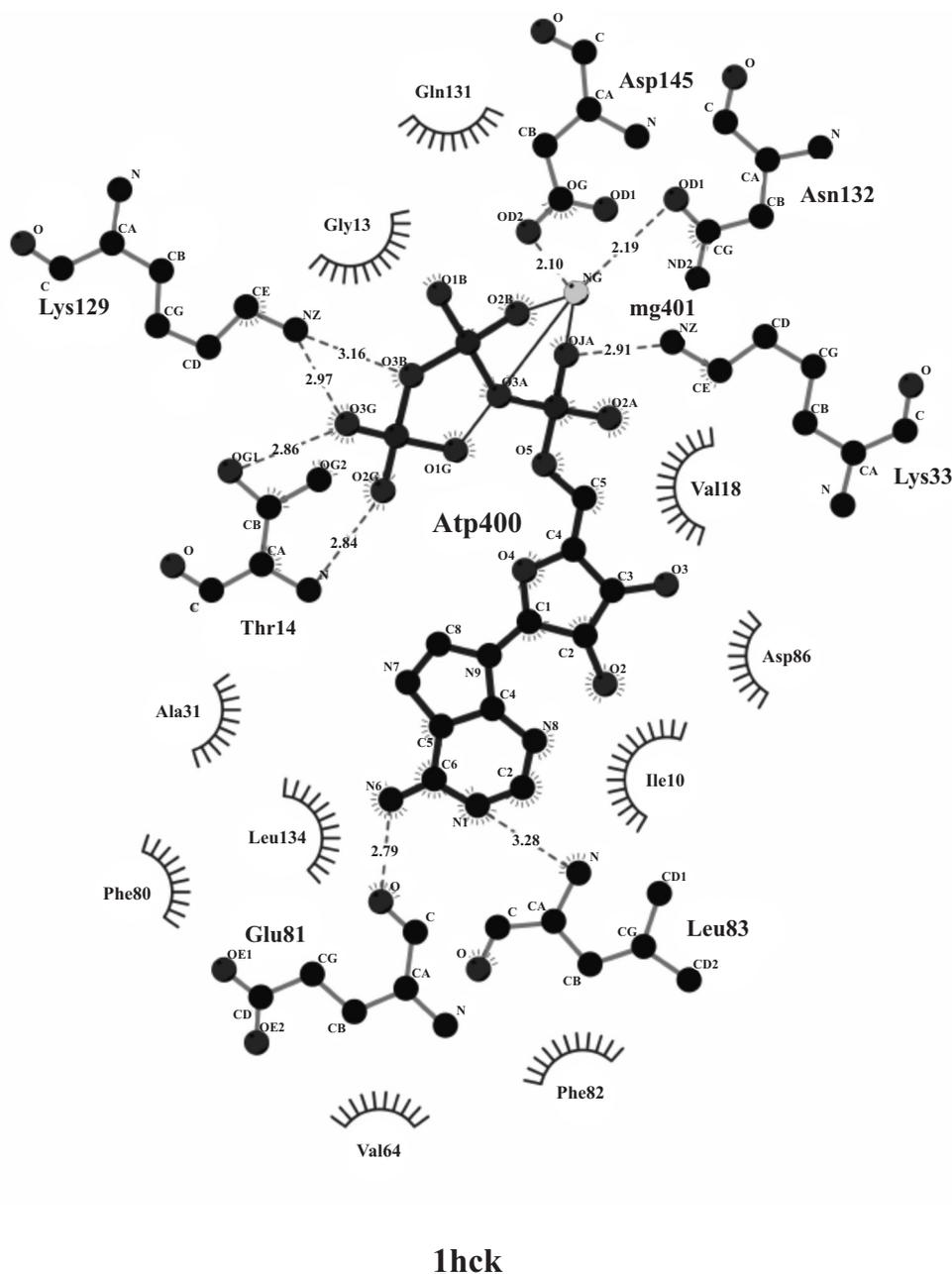
Fig. (2). Protein-ligand interactions of the CDK2 with ATP. The figure above was generated using LigPlot+ [72, 73] where we represent the hydrogen bonds as dashed lines. All distances are in Å.

structures in the CDK2 dataset. Figure (**3**) shows the plot of intermolecular interactions identified for binary complexes in the CDK2 dataset. All inhibitors analyzed in this dataset are composed of competitive inhibitors with ATP. Considering these most common residues in the intermolecular interactions, we see the predominance of hydrophobic interactions involving residues Ile 10, Phe 82, Leu 83, and Leu 134. Only one residue has charged side chain amongst the most prominent residues found in the intermolecular interactions, the residue Asp 145.

The importance of these residues for CDK2 inhibition has been highlighted since the determination of the first X-ray diffraction crystal structure of CDK2 [79]. The residue Leu 83 has been previously proposed as a member of the

molecular fork [81-86], which mediates most of the intermolecular contacts. This part of the CDK2 structure is composed of a pattern of the acceptor, donor, and acceptor involving main-chain atoms, as shown in Fig. (**4**). The first acceptor is C=O group present in the Glu 81. In the sequence, we have the N-H and C=O groups found in the Leu 83. Furthermore, analysis of intermolecular hydrogen bonds indicates that the main-chain atoms of Leu 83 are the most common intermolecular interactions in the CDK2 dataset. The residue Leu 83 shows hydrogen-bond interactions in 84 complexes out of 87 structures in the CDK2 dataset. Also, we see that Leu 83 is not involved in intermolecular hydrogen bonds for the structures 1PXI (inhibitor CK1) [87], 2R3G (inhibitor SC9) [88], 3FZ1 (inhibitor B98) [89], and 4FKL (inhibitor CK2).
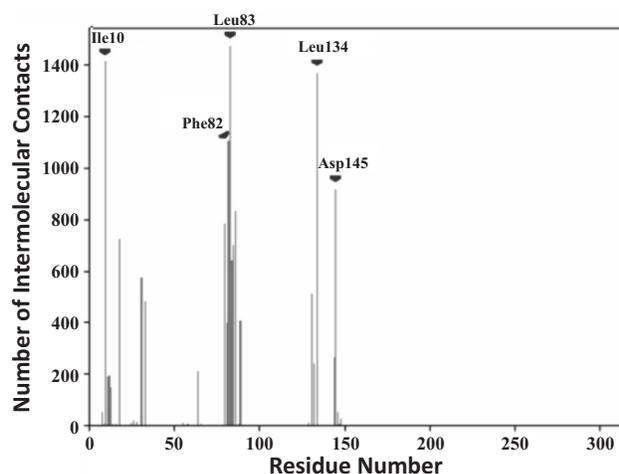
Fig. (3). Plot of the number of intermolecular contacts for all structures in the CDK2 dataset. The figure above was generated using SAnDReS [71].
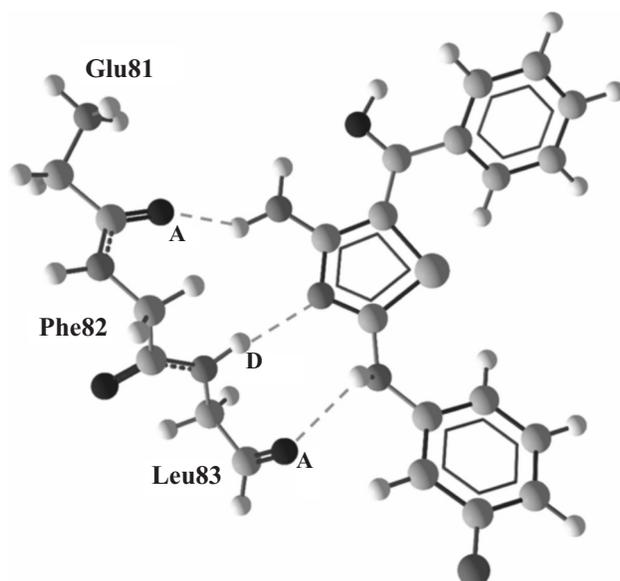
Fig. (4). Representation of the molecular fork of the cyclin-dependent kinase (PDB access code: 3RJC). In the figure, A represents the acceptor and D represents the donor of intermolecular hydrogen bonds. The figure above was generated using MVD [80]. Details about the intermolecular interactions for all ligands in the CDK2 dataset are shown in the supplementary materials 2 and 3.

The intermolecular interactions for structures of CDK2 complexes where the residue Leu 83 is not participating in intermolecular interactions are shown in Fig. (**5A-5D**). These inhibitors show IC_{50} values ranging from 12 to 17,000 nM. Only one inhibitor shows $IC_{50} < 20$ nM, the ligand B98 ($IC_{50} = 12$ nM) in the structure 3FZ1. As we can see in the intermolecular interactions for the ligand B98 (Fig. **5C**), it is tempting to attribute the low IC_{50} value of B98 for CDK2 to the hydrogen bonds involving the NZ atom from Lys 33. This interatomic distance is less than 3.05 Å for the NZ of Lys 33 and the O10 of the inhibitor B98. Also, we see a strong intermolecular hydrogen bond

involving the OD1 atom of Asn 132 and N14 atom of B98, with an interatomic distance shorter than 2.6 Å. We did not see these pattern of intermolecular hydrogen bonds in the other complex structures (PDB access codes: 1PXI, 2R3G, and 4FKL).

Analysis of intermolecular hydrogen bonds for ligands CK1 ($IC_{50} = 17,000$ nM), SC9 ($IC_{50} = 800$ nM), and CK2 ($IC_{50} = 6,500$ nM) also indicates the participation of NZ of lysine residues, the Lys 33 for structures 1PXI and 4FKL; and the Lys 89 for structure 2R3G. Nevertheless, all these complex structures show intermolecular distances higher than 3.1 Å. Also, no intermolecular hydrogen bonds are observed with a length shorter than 2.6 Å, as found for an OD1 atom of Asn 132 and N14 of B98 (Fig. **5C**) in the 3FZ1 structure.

Considering all structures in the CDK2 dataset, we see that the IC_{50} ranges from 1.0 to 120,000 nM (supplementary material 1). There are six CDK2 inhibitors (PDB access codes: 1OIT (inhibitor HDT) [90], 1URW (inhibitor I1P) [91], 2B52 (inhibitor D42) [92], 2W05 (inhibitor FRT) [93], 3NS9 (inhibitor NS9) [94], and 4BGH (inhibitor 3I6) [95] with $IC_{50} < 10$ nM. Fig. (**6A-6F**) show intermolecular interactions for all CDK2 inhibitors with $IC_{50} < 10$ nM. Analysis of the intermolecular interactions for these strong CDK2 inhibitors highlights some common structural features that could be explored in the design of novel CDK2 inhibitors.

The first striking common feature to all these inhibitors is the type of ligand atoms in intermolecular hydrogen bonds with Leu 83. They all exhibit nitrogen atoms of the inhibitors participating in intermolecular hydrogen bonds involving the main-chain atoms of Leu 83. This is the only residue that participates in the intermolecular hydrogen bonds for all six structures. Furthermore, Leu 83 participates with two hydrogen bonds with all strong inhibitors. Analysis of the ligand atoms involved in intermolecular hydrogen bonds with Leu 83 indicates that they all show the participation of nitrogen atoms of the inhibitors. Also, we found two additional hydrogen bonds involving the Asp 86 and the NZ from the side chain of Lys 89. Taken together, we could say interactions with Leu 83 are mandatory for binding of the strongest CDK2 inhibitors. Based on this pattern of intermolecular hydrogen bonds, we could say that improvement of binding affinity may be achieved with additional hydrogen bonds involving main chain N-H and OD side chain groups of Asp 86. This interaction is found in complex with inhibitors HDT ($IC_{50} = 2.25$ nM), I1P ($IC_{50} = 3.0$ nM), FRT ($IC_{50} = 1.0$ nM), and 3I6 ($IC_{50} = 4.0$ nM).

6. AUTHORS INSIGHT ON THE TOPIC

In our view, the richness of structural and ligand-binding affinity data that is available due to the initial development of CDK inhibitors directed to control cell-cycle progression could be beneficial on the research for the treatment of Myc-driven tumors. In the last case, we target the inhibition of CDK2 to cause senescence in Myc-overexpressing cells [54]. In this context, the role of dual inhibition of CDK1 and
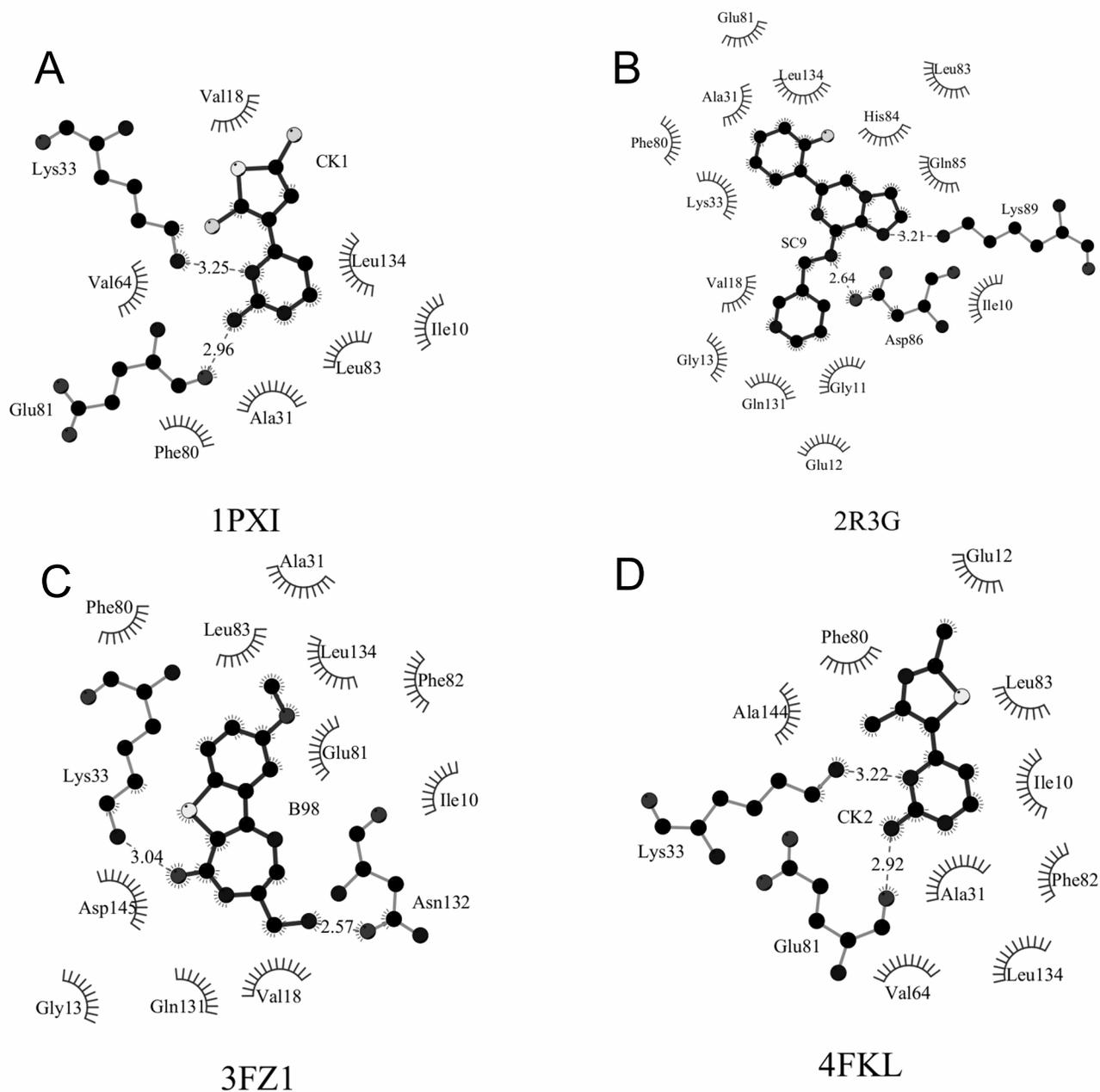
Fig. (5). Protein-ligand interactions of the structures of the CDK2 dataset without the hydrogen bond between the residue Leu83 and the ligands A) CK1, B) SC9, C) B98, D) CK2. The figures above were generated using LigPlot+ [72, 73] where we represent the hydrogen bonds as dashed lines. All distances are in Å.

CDK2 is especially interesting as a promising therapy to treat Myc-driven tumors. The therapeutical potential of dual inhibition of CDK1 and CDK2 emphasizes the importance of an integrated study of these enzymes [54, 57]. The use of CDK1 and CDK2 dual inhibitors have the potential to benefit from nearly three decades of research on CDK inhibition. Also, the use of CDK2 inhibitors in the study of cellular senescence mechanism has a positive impact on the understanding of the molecular mechanism of this crucial biological mechanism.

Furthermore, our analysis of the crystallographic structures of CDK2 for which ligand-binding affinity data is available clearly shows the importance of the molecular fork for ligand-binding interactions. Here, we described the identification of the pivotal role of main-chain atoms of Leu 83 and main-chain N-H and OD side-chain groups of Asp 86 found in the strongest CDK2 inhibitors, which may serve as a guide to direct the development of inhibitors. This structural evidence is a good starting point for the development of CDK1 and CDK2 dual inhibitors targeted to the treatment of Myc-driven tumors.
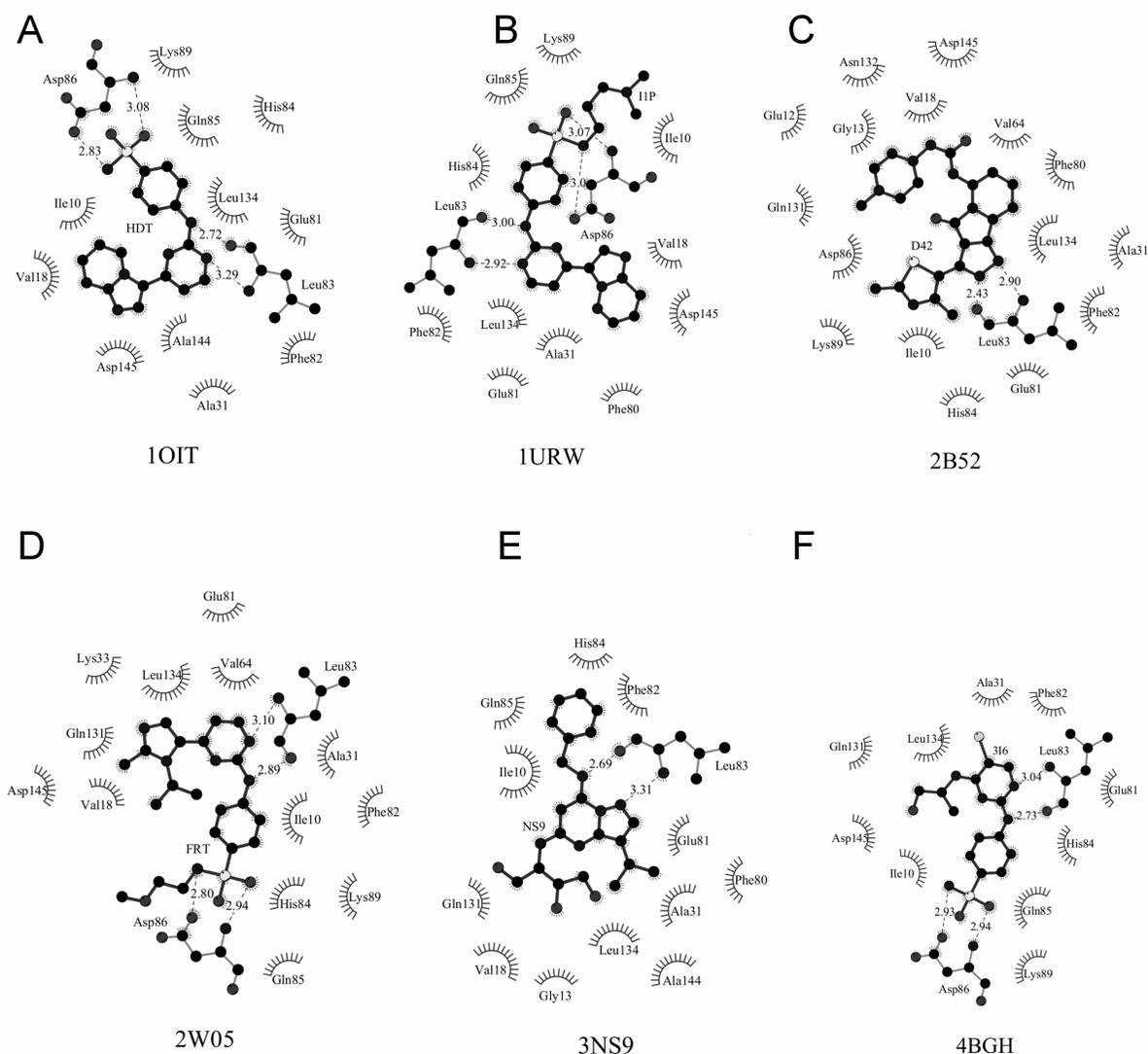
Fig. (6). Protein-ligand interactions of the structures of the CDK2 dataset with the hydrogen bond between the residue Leu83 and the ligands ($IC_{50} < 10$ nM)) A) HDT, B) I1P, C) D42, D) FRT, E) NS9, F) 3I6. The figures above were generated using LigPlot+ [72, 73]. Plots for the intermolecular interactions for all ligands are on supplementary material 4.

CONCLUSION

Their potential use in cancer therapy mostly drove the development of CDK2 inhibitors. The intensive efforts combining structural and activity studies were able to clarify the structural basis for inhibition of CDK2, allowing us to explore the most promising moieties for the development of inhibitors with K_i and IC_{50} in the nanomolar range. The recent discoveries relating the pivotal role of CDK2 inhibition on cellular senescence highlight the importance of CDK2 also on the design of potential new drugs for the treatment of Myc-driven tumors.

LIST OF ABBREVIATIONS

ATP   =   Adenosine-tri-phosphate
CDK   =   Cyclin-dependent kinase
DDR   =   DNA damage response
DNA   =   Deoxyribonucleic acid
EC   =   Enzyme classification

IC_{50} =   Half-maximal inhibitory concentration
K_i =   Inhibition constant
MVD   =   Molegro Virtual Docker
NMR   =   Nuclear magnetic resonance
PDB   =   Protein data bank
Plk1   =   Polo-like kinase 1
RNA   =   Ribonucleic acid
SAnDReS   =   Statistical analysis of Docking results and Scoring function
SASP   =   Senescence-associated secretory phenotype

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

REFERENCES

[1] Sausville EA, Johnson J, Alley M, Zaharevitz D, Senderowicz AM. Inhibition of CDKs as a therapeutic modality. Ann N Y Acad Sci 2000; 910(1): 207-22.

[2] Babu PJ, Narasub ML, Srinivasc K. Pyridines, pyridazines and guanines as CDK2 inhibitors: a review. Arkivoc 2007; 2007(2): 247-65.

[3] Malumbres M. Cyclin-dependent kinases. Genome Biol 2014; 15(6): 122.

[4] Chohan TA, Qian H, Pan Y, Chen JZ. Cyclin-dependent kinase-2 as a target for cancer therapy: progress in the development of CDK2 inhibitors as anti-cancer agents. Curr Med Chem 2015; 22(2): 237-63.

[5] Malumbres, M, Barbacid M. Cell cycle kinases in cancer. Curr Opin Genet Dev 2007; 17(1): 60-5.

[6] Canavese M, Santo L, Raje N. Cyclin dependent kinases in cancer: potential for therapeutic intervention. Cancer Biol Ther 2012; 13(7): 451-7.

[7] Criscitiello C, Viale G, Esposito A, Curigliano G. Dinaciclib for the treatment of breast cancer. Expert Opin Investig Drugs 2014; 23(9): 1305-12.

[8] Kumar SK, LaPlant B, Chng WJ, et al. Dinaciclib, a novel CDK inhibitor, demonstrates encouraging single-agent activity in patients with relapsed multiple myeloma. Blood 2015; 125(3): 443-8.

[9] Krenning L, Feringa FM, Shaltiel IA, van den Berg J, Medema RH. Transient activation of p53 in G2 phase is sufficient to induce senescence. Mol Cell 2014; 55(1): 59-72.

[10] Müllers E, Silva Cascales H, Jaiswal H, Saurin AT, Lindqvist A. Nuclear translocation of Cyclin B1 marks the restriction point for terminal cell cycle exit in G2 phase. Cell Cycle 2014; 13: 2733-43.

[11] Cascales HS, Müllers E, Lindqvist A. How the cell cycle enforces senescence. Aging 2017; 9(10): 2022-23.

[12] Jaiswal H, Benada J, Müllers E, et al. ATM/Wip1 activities at chromatin control Plk1 re-activation to determine G2 checkpoint duration. EMBO J 2017; 36(14): 2161-76.

[13] Wadler S. Perspectives for cancer therapies with cdk2 inhibitors. Drug Resist Updat 2001; 4(6): 347-67.

[14] Levin NBM, Pintro VO, de Àvila MB, de Mattos BB, De Azevedo WF Jr. Understanding the Structural Basis for Inhibition of Cyclin-Dependent Kinases. New Pieces in the Molecular Puzzle. Curr Drug Targets 2017; 18(9): 1104-11.

[15] Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science 2002; 298(5600): 1912-34.

[16] Bettayeb K, Oumata N, Echalier A, et al. CR8, a potent and selective, roscovitine-derived inhibitor of cyclin-dependent kinases. Oncogene 2008; 27(44): 5797-807.

[17] Idowu MA. Cyclin-dependent kinases as drug targets for cell growth and proliferation disorders. A role for systems biology approach in drug development. Part I-cyclin-dependent kinases as

[18] Tanaka S, Tak YS, Araki H. The role of CDK in the initiation step of DNA replication in eukaryotes. Cell division 2007; 2(1): 16.

[19] Lim S, Kaldis P. Cdks, cyclins and CKIs: Roles beyond cell cycle regulation. Development 2013; 140(15): 3079-93.

[20] Tsai LH, Harlow E, Meyerson M. Isolation of the human CDK2 gene that encodes the cyclin A-and adenovirus E1Aassociated p33 kinase. Nature 1991; 353(6340): 174-7.

[21] Eom EM, Cho JK, Lim SO, Byun YJ, Lee DH. Molecular cloning and expression of a small GTP-binding protein of the Rop family from mung bean. Plant Science 2006; 171(1): 41-51.

[22] Schulze-Gahmen U, De Bondt HL, Kim SH. High-resolution crystal structures of human cyclin-dependent kinase 2 with and without ATP: bound waters and natural ligand as guides for inhibitor design. J Med Chem 1996; 39(23): 4540-6.

[23] Davies TG, Pratt DJ, Endicott JA, Johnson LN, Noble ME. Structure based design of cyclin-dependent kinase inhibitors. Pharmacol Thera 2002; 93(2-3): 125-33.

[24] Sherr CJ. The Pezcoller Lecture: Cancer Cell Cycles Revisited. Cancer Res 2000; 60(14): 3689-95.

[25] Noble MEM, Endicott JA. Chemical inhibitors of cyclindependent kinases: insights into design from X-ray crystallographic studies. Pharmacol Ther 1999; 82(2-3): 269-78.

[26] Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer 2009; 9(3): 153-66.

[27] Du J, Widlund HR, Horstmann MA, et al. Critical role of CDK2 for melanoma growth linked to its melanocyte-specific transcriptional regulation by MITF. Cancer Cell 2004; 6(6): 565-576.

[28] Peyressatre M, Prével C, Pellerano M, Morris M. Targeting cyclin-dependent kinases in human cancers: From small molecules to peptide inhibitors. Cancers 2015; 7(1): 179-237.

[29] Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. Nature 2004; 432(7015): 307-15.

[30] Ahmed D, Sharma M. Cyclin-dependent kinase 5/p35/p39: a novel and imminent therapeutic target for diabetes mellitus. Int J Endocrinol 2011; 2011: 530274.

[31] Kim JH, Kang MJ, Park CU, Kwak HJ, Hwang Y, Koh GY. Amplified CDK2 and cdc2 activities in primary colorectal carcinoma. Cancer 1999; 85(3): 546-53.

[32] Yamamoto H, Monden T, Ikeda K, et al. Coexpression of cdk2/cdc2 and retinoblastoma gene products in colorectal cancer. Br J Cancer 1995; 71(6): 1231-6.

[33] Dobashi Y, Shoji M, Jiang SX, Kobayashi M, Kawakubo Y, Kameya T. Active cyclin a-CDK2 complex, a possible critical factor for cell proliferation in human primary lung carcinomas. Am J Pathol 1998; 153(3): 963-72.

[34] Lapenna S, Giordano A. Cell cycle kinases as therapeutic targets for cancer. Nat Rev Drug Discov 2009; 8(7): 547-66.

[35] Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. Genes Dev 2010; 24(22): 2463-79.

[36] Salama R, Sadaie M, Hoare M, Narita M. Cellular senescence and its effector programs. Genes Dev 2014; 28(2): 99-114.

[37] Gire V, Dulić V. Senescence from G2 arrest, revisited. Cell Cycle 2015; 14(3): 297-304.

[38] O'Connor MJ. Targeting the DNA Damage Response in Cancer. Molecular Cell 2015; 60(4): 547-60.

[39] Bartek J, Lukas C, Lukas J. Checking on DNA damage in S phase. Nat Rev Mol Cell Biol 2004; 5(10): 792-804.

[40] Macheret M, Halazonetis TD. DNA replication stress as a hallmark of cancer. Annu Rev Pathol Mech Dis 2015; 10: 425-48.

[41] Müllers E, Silva Cascales H, Burdova K, Macurek L, Lindqvist A. Residual Cdk1/2 activity after DNA damage promotes senescence. Aging Cell 2017; 16(3): 575-84.

[42] Zhang C, Wang F, Xie Z, et al. Dysregulation of YAP by the Hippo pathway is involved in intervertebral disc degeneration, cell contact inhibition, and cell senescence. Oncotarget 2017; 9(2): 2175-92.

[43] Zalzali H, Nasr B, Harajly M, et al. Cell cycle and senescence CDK2 transcriptional repression is an essential effector in p53-dependent cellular Senescence— implications for therapeutic intervention. Mol Cancer Res 2015; 13(1): 29-40.

drug targets in cancer. Biotechnol Biotec 2011; 25(4): 2583- 6.

[44]   Collado M, Serrano M. Senescence in tumours: evidence from mice and humans. Nat Rev Cancer 2010; 10(1): 51-7.

[45]   Campisi J, d'Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol 2007; 8(9): 729-40.

[46]   Riggelen VJ, Felsher DW. Myc and a Cdk2 senescence switch. Nat Cell Biol 2010; 12(1): 7-9.

[47]   Campaner S, Doni M, Verrecchia A, Fagà G, Bianchi L, Amati B. Myc, Cdk2 and cellular senescence: Old players, new game. Cell Cycle 2010; 9(18): 3679-85.

[48]   Hydbring P, Larsson LG. Tipping the Balance: Cdk2 Enables Myc to Suppress Senescence. Cancer Res 2010; 70(17): 6687-91.

[49]   Zhuang D, Mannava S, Grachtchouk V, *et al.* C-MYC overexpression is required for continuous suppression of oncogene-induced senescence in melanoma cells. Oncogene 2008; 27(53): 6623-34.

[50]   Wu CH, Van Riggelen J, Yetil A, Fan AC, Bachireddy P, Felsher DW. Cellular senescence is an important mechanism of tumor regression upon c-Myc inactivation. Proc Natl Acad Sci USA 2007; 104(32): 13028-33.

[51]   Beauséjour CM, Krtolica A, Galimi F, *et al.* Reversal of human cellular senescence: roles of the p53 and p16 pathways. EMBO J 2003; 22: 4212-22.

[52]   Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 1997;88:593–602.

[53]   Zhu J, Woods D, McMahon M, Bishop JM. Senescence of human fibroblasts induced by oncogenic Raf. Genes Dev 1998;12:2997–3007.

[54]   Campaner S, Doni M, Hydbring P, *et al.* Cdk2 suppresses cellular senescence induced by the c-myc oncogene. Nat Cell Biol 2010;12(1): 54-9

[55]   Schmitt, C. A. Cellular senescence and cancer treatment. Biochim Biophys. Acta 2006; 1775: 5-20.

[56]   Schmitt, C. A. *et al.* A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. Cell 2002; 109; 335-346.

[57]   Goga, A, Yang D, Tward AD, Morgan DO, Bishop JM. Inhibition of CDK1 as a potential therapy for tumors over-expressing MYC. Nature Med 2007; 13: 820-7.

[58]   Hydbring P, Bahram F, Su Y, Tronnersjo S, Hogstrand K, *et al.* Phosphorylation by Cdk2 is required for Myc to repress Ras-induced senescence in cotransformation. Proc Natl Acad Sci U S A. 2010; 107: 58-63.

[59]   Fadel V, Bettendorff P, Herrmann T, *et al.* Automated NMR structure determination and disulfide bond identification of the myotoxin crotamine from Crotalus durissus terrificus. Toxicon 2005; 46(7): 759-67.

[60]   Canduri F, de Azevedo WF. Protein crystallography in drug discovery. Curr Drug Targets 2008; 9(12): 1048-53.

[61]   Berman HM, Westbrook J, Feng Z, *et al.* The Protein Data Bank. Nucleic Acids Res 2000; 28(1): 235-42.

[62]   Berman HM, Battistuz T, Bhat TN, *et al.* The Protein Data Bank. Acta Crystallogr D Biol Crystallogr 2002; 58(Pt 6 No 1): 899-907.

[63]   Westbrook J, Feng Z, Chen L, Yang H, Berman HM. The Protein Data Bank and structural genomics. Nucleic Acids Res 2003; 31(1): 489-91.

[64]   Liu T, Lin Y, Wen X, Jorissen RN, Gilson MK. BindingDB: a web-accessible database of experimentally determined protein–ligand binding affinities. Nucleic Acids Res 2007; 35(Database issue): D198-201.

[65]   Gilson MK, Liu T, Baitaluk M, *et al.* BindingDB in 2015: A public database for medicinal chemistry, computational chemistry and systems pharmacology. Nucleic Acids Res 2015; 44(D1): D1045-53.

[66]   Benson ML, Smith RD, Khazanov NA, *et al.* Binding MOAD, a high-quality protein-ligand database. Nucleic Acids Res 2008; 36(Database issue): D674-8.

[67]   Ahmed A, Smith RD, Clark JJ, Dunbar Jr. JB, Carlson HA. Recent improvements to Binding MOAD: a resource for protein–ligand binding affinities and structures. Nucleic Acids Res 2015; 43(Database issue): D465-9.

[68]   Wang R, Fang X, Lu Y, Wang S. The PDBbind database: collection of binding affinities for protein-ligand complexes with known three-dimensional structures. J Med Chem 2004; 47(12): 2977-80.

[69]   Liu Z, Li Y, Han L, *et al.* PDB-wide collection of binding data: current status of the PDBbind database. Bioinformatics 2015; 31(3): 405-12.

[70]   Schulze-Gahmen U, Brandsen J, Jones HD, *et al.* Multiple modes of ligand recognition: crystal structures of cyclin-dependent protein kinase 2 in complex with ATP and two inhibitors, olomoucine and isopentenyladenine. Proteins 1995; 22(4): 378-91.

[71]   Xavier MM, Heck GS, de Avila MB, *et al.* SAnDReS a computational tool for statistical analysis of docking results and development of scoring functions. Comb Chem High Throughput Screen 2016; 19(10): 801-12.

[72]   Wallace AC, Laskowski RA, Thornton JM. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng 1995; 8(2): 127-34.

[73]   Laskowski RA, Swindells MB. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model 2011; 51(10): 2778-86.

[74]   De Azevedo WF Jr., Leclerc S, Meijer L, *et al.* Inhibition of cyclin-dependent kinases by purine analogues: crystal structure of human cdk2 complexed with roscovitine. Eur J Biochem 1997; 243(1-2): 518-26.

[75]   De Azevedo WF Jr., Mueller-Dieckmann HJ, Schulze-Gahmen U, *et al.* Structural basis for specificity and potency of a flavonoid inhibitor of human CDK2, a cell cycle kinase. Proc Natl Acad Sci U S A 1996; 93(7): 2735-40.

[76]   Canduri F, Teodoro LG, Fadel V, *et al.* Structure of human uropepsin at 2.45 A resolution. Acta Crystallogr D Biol Crystallogr 2001; 57(Pt 11): 1560-70.

[77]   De Azevedo WF Jr., Canduri F, da Silveira NJ. Structural basis for inhibition of cyclin-dependent kinase 9 by flavopiridol. Biochem Biophys Res Commun 2002; 293(1): 566-71.

[78]   De Azevedo WF Jr., Gaspar RT, Canduri F, Camera JC Jr., da Silveira NJF. Molecular model of cyclin-dependent kinase 5 complexed with roscovitine. Biochem Biophys Res Commun 2002; 297(5): 1154-8.

[79]   De Bondt HL, Rosenblatt J, Jancarik J, *et al.* Crystal structure of cyclin-dependent kinase 2. Nature 1993; 363(6430): 595-602.

[80]   Thomsen R, Christensen MH. MolDock: a new technique for high-accuracy molecular docking. J Med Chem 2006; 49(11): 3315-21.

[81]   Azevedo WF, Leclerc S, Meijer L, *et al.* Inhibition of cyclin-dependent kinases by purine analogues. FEBS J 1997; 243(1-2): 518-26.

[82]   Meijer L, Raymond E. Roscovitine and other purines as kinase inhibitors. From starfish oocytes to clinical trials. Acc Chem Res 2003; 36(6): 417-25.

[83]   Canduri F, Azevedo J. Structural basis for interaction of inhibitors with cyclin-dependent kinase 2. Curr Comput Aided Drug Des 2005; 1(1): 53-64.

[84]   Levin NM, Pintro VO, de Ávila MB, de Mattos BB, de Azevedo WF Jr. (2017). Understanding the structural basis for inhibition of cyclin-dependent kinases. New pieces in the molecular puzzle. Curr drug targets 2017; 18(9), 1104-11.

[85]   Paparidis NF, Durvale MC, Canduri F. The emerging picture of CDK9/P-TEFb: more than 20 years of advances since PITALRE. Mol Biosyst 2017; 13(2): 246-76.

[86]   Dos Santos PNF, Canduri F. The Emerging Picture of CDK11: Genetic, Functional and Medicinal Aspects. Curr Med Chem 2018; 25(8): 880-8.

[87]   Wu SY, McNae I, Kontopidis G, *et al.* Discovery of a novel family of CDK inhibitors with the program LIDAEUS: structural basis for ligand-induced disordering of the activation loop. Structure 2003; 11(4): 399-410.

[88]   Fischmann TO, Hruza A, Duca JS, *et al.* Structure-guided discovery of cyclin-dependent kinase inhibitors. Biopolymers 2008; 89(5): 372-9.

[89]   Anderson DR, Meyers MJ, Kurumbail RG, *et al.* Benzothiophene inhibitors of MK2. Part 2: improvements in kinase selectivity and cell potency. Bioorg Med Chem Lett 2009; 19(16): 4882-4.

[90]   Anderson M, Beattie JF, Breault GA, *et al.* Imidazo[1,2-a]pyridines: a potent and selective class of cyclin-dependent kinase inhibitors identified through structure-based hybridisation. Bioorg Med Chem Lett 2003; 13(18): 3021-6.

[91]   Byth KF, Cooper N, Culshaw JD, *et al.* Imidazo[1,2-b]pyridazines: a potent and selective class of cyclin-dependent kinase inhibitors. Bioorg Med Chem Lett 2004; 14(9): 2249-52.

[92]   Yue EW, DiMeo SV, Higley CA, *et al.* Synthesis and evaluation of indenopyrazoles as cyclin-dependent kinase inhibitors. Part 4: Heterocycles at C3. Bioorg Med Chem Lett 2004; 14(2): 343-6.

[93]   Anderson M, Andrews DM, Barker AJ, *et al.* Imidazoles: SAR and development of a potent class of cyclin-dependent kinase

inhibitors. Bioorg Med Chem Lett 2008; 18(20): 5487-92.

[94]   Heathcote DA, Patel H, Kroll SH, *et al.* A novel pyrazolo[1,5-a]pyrimidine is a potent inhibitor of cyclin-dependent protein kinases 1, 2, and 9, which demonstrates antitumor effects in human tumor xenografts following oral administration. J Med Chem 2010; 53(24): 8509-22.

[95]   Lücking U, Jautelat R, Krüger M, *et al.* The lab oddity prevails: discovery of pan-CDK inhibitor (R)-S-cyclopropyl-S-(4-{[4-{[(1R,2R)-2-hydroxy-1-methylpropyl]oxy}-5-(trifluoromethyl)pyrimidin-2-yl]amino}phenyl)sulfoximide (BAY 1000394) for the treatment of cancer. ChemMedChem 2013; 8(7): 1067-85.