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# Extra precision docking, free energy calculation and molecular dynamics simulation studies of CDK2 inhibitors

Sunil Kumar Tripathi [a], Ravikumar Muttineni [b], Sanjeev Kumar Singh [a,*]

[a] Computer Aided Drug Designing and Molecular Modeling Lab, Department of Bioinformatics, Alagappa University, Karaikudi-630 003, Tamil Nadu, India
[b] Schrodinger, Bangalore 560079, India

## HIGHLIGHTS

- Computational approach was applied to gain insight into selectivity for CDK2 inhibitors.
- These theoretical approaches reproduced the crystal structure precisely.
- The modification with substituents can show improved inhibitory activity against CDK2.

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## ABSTRACT

Molecular docking, free energy calculation and molecular dynamics (MD) simulation studies have been performed, to explore the putative binding modes of 3,5-diaminoindazoles, imidazo(1,2-*b*)pyridazines and triazolo(1,5-*a*) pyridazines series of Cyclin-dependent kinase (CDK2) inhibitors. To evaluate the effectiveness of docking protocol in flexible docking, we have selected crystallographic bound compound to validate our docking procedure as evident from root mean square deviations (RMSDs). We found different binding sites namely catalytic, inhibitory phosphorylation, cyclin binding and CKS-binding site of the CDK2 contributing towards the binding of these compounds. Moreover, correlation between free energy of binding and biological activity yielded a statistically significant correlation coefficient. Finally, three representative protein–ligand complexes were subjected to molecular dynamics simulation to determine the stability of the predicted conformations. The low value of the RMSDs between the initial complex structure and the energy minimized final average complex structure suggests that the derived docked complexes are close to equilibrium. We suggest that the phenylacetyl type of substituents and cyclohexyl moiety make the favorable interactions with a number of residues in the active site, and show better inhibitory activity to improve the pharmacokinetic profile of compounds against CDK2. The structure-based drug design strategy described in this study will be highly useful for the development of new inhibitors with high potency and selectivity.



## 1. Introduction

Cyclin-dependent kinases (CDKs) are serine/threonine kinases which control the proliferation of eukaryotic cell. Due to their crucial role in the regulation of the cell division cycle, CDKs have emerged as important therapeutic targets in anti-cancer drug research. The Cyclin-dependent kinase 2 (CDK2) is one of the prominent cell cycle regulators, which is dominantly active during the G1 phase and G1/S transition. The deregulation of CDKs is known to be associated with many serious diseases, such as cancer (Morgan, 1997; Malumbres and Barbacid, 2009; Child et al., 2010; Singh et al., 2012). This fact has attracted attention in the long term to the development of efficient inhibitors of CDKs (Besson et al., 2008). Many government organizations and pharmaceutical companies have engaged in programs aiming at the discovery of potent small molecule inhibitors of these enzymes which are firmly established targets in oncology. A relentless effort in this field has succeeded in bringing some CDK inhibitors to clinical trials (Meijer and Raymond, 2003; Johnson, 2009). Even though the targeting of CDK2 does not have to be an optimal strategy for cancer treatment because of the redundancy of CDKs in cell cycle regulation (Tetsu and McCormick, 2003), the CDK2 has remained a paradigm for rational drug design, because it is the best characterized CDK in terms of structure and biochemistry (Echalier et al., 2010).

As the ATP-binding pocket is present in all kinases, it is usually the site targeted by kinase inhibitors. Although the structure of the ATP binding site is conserved between the kinases, there are subtle differences between them, enabling drugs to specifically target one subclass without affecting the others. Small molecule

* Corresponding author. Tel.: +91 4565 223342 650/365; fax: +91 4565 225202.
*E-mail address:* skysanjeev@gmail.com (S.K. Singh).

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inhibitors competitively occupy the ATP binding pocket, often mimicking the hydrogen bonds made by the alanine moiety of ATP (Blagden and de Bono, 2005). Competition between an inhibitor and the native ATP substrate is an effective strategy to inhibit CDK2. An inhibitor binds to a deep cleft between two CDK2 lobes and, despite the numerous structurally varied CDK2 inhibitors known today, some common features can be identified. The discovery of the CDK2-inhibitor structure (De Azevedo et al., 1996, 1997) provided a useful starting point for the rational design of CDK2 inhibitors.

It is generally recognized that drug discovery and development are time and resource consuming process, requires various stages of screening. There is an ever growing effort to apply computational power to the combined chemical and biological space in order to streamline drug discovery, design, development and optimization (Kumar et al., 2006). Commonly used computational approaches include ligand-based and structure based drug design (Dror et al., 2004; Schneidman-Duhovny et al., 2004). An effective way to predict the binding of substrate with its receptor is docking simulation, which is successfully implemented in many applications (Dessalew and Singh, 2008; Otyepka et al., 2000; Vadivelan et al., 2007). Docking procedures basically aim to identify the correct conformation of ligands in the binding pocket of a protein and to predict the affinity between the ligand and protein (Dixon and Blaney, 1998). Several studies which provide independent benchmarks for widely used docking programs and among them the Glide was considered most accurate docking tools, which has been thoroughly reviewed in the literature over the years and has produced some notable successes (Perola et al., 2004; Friesner et al., 2004, Englebienne et al., 2007; Zhou et al., 2007). To test the molecular docking in this study, we selected Glide as they employ significantly different docking methodologies (Friesner et al.; 2004; Halgren et al., 2004; Friesner et al.; 2006) and have employed different collections of crystal complexes and binding data to weight their optimization algorithms.

The comparably fast and inexpensive docking protocols can be combined with accurate but more expensive molecular dynamics (MD) simulation techniques to predict more reliable protein–ligand complex structures (Karplus and McCammon, 2002; Norberg and Nilsson, 2003). On one hand docking techniques are used to search massive conformational space in a short time, allowing the analysis of a large library of drug compounds at a sensible cost (Kitchen et al., 2004). On another hand, MD simulation accounts for both ligand and protein in a flexible way, allowing for an induced fit into the receptor-binding site around the newly introduced ligand (Lin et al., 2002). MD simulation can be used: during the preparation of protein receptor before docking, to optimize its structure and account for protein flexibility (Schames et al., 2004); for the refinement of the docked complex, to include solvent effects and account for induced fit (Huo et al., 2002). This also calculates binding-free energies (Brandsdal et al., 2003), as well as providing an accurate ranking of the potential ligands (Wang et al., 1999).

In this work, we bring further information to understand the binding modes of known imidazo(1,2-*b*)pyridazines, 3,5-diaminoindazoles and triazolo(1,5-*a*) pyridazines series of CDK2 inhibitors using molecular docking, MD simulation and free-energy calculation. MD simulations were carried out to determine the stability and dynamical changes of predicted binding conformations. An MM-GB/SA (Molecular Mechanics-Generalized Born/Surface Area) analysis was carried out to calculate the binding free energies of the proteins with CDK2 inhibitors. We also examined in detail the role of H bonding with ligand. We show that the origin of selectivity with these inhibitors with key sites of the CDK2 contributing to the binding of these inhibitors. The information from this study will be highly useful to design or optimize CDK2 inhibitors by these molecular modeling approaches.

## 2. Materials and method

All computational analyses were carried out on Red Hat 5.1 Linux platform in IBM System x 3200 M2 server on Intel Xeon quad-core 2.83 GHz.

### 2.1. Biological data

Significantly, the biological activity of a compound against a receptor relies on its binding, which primarily depends on the structurally steric orientation and the electrostatic property. It is usual that small structure difference may give rise to a great biological diversity. Therefore, 27 compounds from 3,5-diaminoindazoles, imidazo(1,2-*b*)pyridazines, and triazolo(1,5-*a*)pyridazines series of CDK2 based on their wide range biological activity and structural diversity were taken from literature (Lee et al., 2008; Byth et al., 2004; Richardson et al., 2006). The structure of these inhibitors along with free energy of binding and their biological activity ($pIC_{50}$ value) are shown in Table 1.

### 2.2. Preparation of protein target structure

In the present study, the X-ray crystal structure of CDK2 in complex with compound **14** (PDB ID: 1URW) was obtained from Protein Data Bank (Berman et al., 2000) and further prepared by protein preparation wizard, which is available in Glide (2011). The protein preparation wizard facility has two components namely, preparation and refinement. After ensuring chemical accuracy, the preparation component adds hydrogen and neutralizes side chain that is neither close to binding cavity nor involve in formation of salt bridges. OPLS-AA force field was used for this purpose and then active site of protein was defined. Glide uses full OPLS-AA force field at an intermediate docking stage and is claimed to be more sensitive to geometrical detail compare to other docking algorithms. In the next step, water molecules were removed and H atoms were added to structure, most likely positions of hydroxyl and thiol hydrogen atoms, protonation states and tautomers of His residue and Chi 'flip' assignment for Asn, Gln and His residue were selected by protein assignment script provided by Schrödinger. Minimization was performed until the average root mean square deviation of the nonhydrogen atoms reached 0.3 Å.

### 2.3. Ligand preparation

All the compounds were constructed using the fragment library of Maestro 9.2, and all compounds were prepared by using the LigPrep 2.4 (LigPrep, 2011), which can produce a number of structures from each input structure with various ionization states, tautomers, stereochemistries and ring conformations to eliminate molecules using various criteria including molecular weight or specified numbers and types of functional groups present with correct chiralities for each successfully processed input structure. The OPLS-2005 force field was used for optimization, which produces the low-energy conformer of the ligand (Hayes et al., 2004).

### 2.4. Molecular docking simulation

To test the docking parameters all compounds were docked into the binding site of the CDK2 protein (PDB ID: 1URW) using Grid-Based Ligand Docking With Energetics (Glide) software from Schrodinger (Halgren et al., 2004; Friesner et al., 2004). To soften the potential for nonpolar parts of the receptor, the scaling factor for protein van der Waals radii was 1.0 in the receptor grid