Article

An *in silico* evaluation of CDK Inhibitors targeting BCL2, TS and mTOR

Sharmin Ahmed Rakhi[1], Muhammad Asaduzzaman[2,*], Nishat Nasin[3] and Abul Bashar Mir Md. Khademul Islam[4]

[1]Department of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh
[2]Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Dhaka-1000, Bangladesh
[3]Department of Pharmacy, East West University, Jahurul Islam City Gate, A/2 Jahurul Islam Avenue, Dhaka-1212, Bangladesh
[4]Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka-1000, Bangladesh

*Corresponding author: Muhammad Asaduzzaman, PhD, Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Dhaka-1000, Bangladesh. Phone: +8801914701708; E-mail: asaduzzaman@du.ac.bd

Abstract: The cyclin dependent kinase (CDK) inhibitors have recently been found to be of potential use as anticancer drugs. The present research work focuses on screening of compounds targeting multiple pathways involved in human cancers along with CDK-regulated cell cycle for prospective anticancer potential. Molecular docking study of selected compounds were performed to determine the binding affinity of selected compounds towards respective targets of cancer cells to verify if there is any physical interaction of these inhibitors with their reported target proteins as claimed in the existing literatures. Prior to docking, molecular pathway prediction and gene set enrichment analyses were performed to identify the target molecules by using appropriate bioinformatics tools. Interestingly, the results of *in silico* molecular docking have been found to be in line with the laboratory findings that are obtained from the literatures. Specifically, few of our selected CDK inhibitors, namely Abemaciclib, Palbociclib, AMG 925 and RGB 286638 showed good binding scores against BCL2, TS, mTOR in addition to CDKs (4, 6 and 9). On the basis of scientific evidence based on published scholarly articles and according to molecular docking results, it can be inferred that these CDK inhibitors as anticancer agents may play a very promising role in cancer treatment and can be used as potential lead compounds for the development of target therapy against human cancers. However, more intensive research is needed to confirm the feasibility of these compounds to be used in treating cancer and it is expected that this work will provide a stimulating impetus for the development of chemotherapeutic agents in future.

Keywords: Cyclin dependent kinases; cell cycle; BCL2; TS; mTOR; amebaciclib; palbociclib

1. Introduction

Cyclin dependent kinase (CDK) enzymes belongs to a group of related proteins that control the cell cycle progression. CDKs are heteromeric serine/threonine protein kinases and among the 12 different isoforms of CDKs, only 5 (CDK1-CDK7) have been shown to drive the cell cycle directly (Ding *et al.*, 2020). CDK deregulation, either through direct or indirect means, is found in most cancer cells. Pharmacologically, CDK inhibition has become an attractive strategy towards mechanism-based and non-genotoxic therapies in oncology. Over the last decade, many potential drug candidate molecules are discovered which are capable of inhibiting CDKs, blocking cell-cycle progression, modulating transcription and inducing apoptosis selectively in cancer cells. A CDK (cyclin-dependent kinase) inhibitor potentially inhibits the cellular functions of CDKs. They are employed to treat cancers and hey act by preventing overproliferation of cancer cells (Law *et al*., 2015). CDK inhibitors are considered as target therapy. Majority of the currently available anticancer drugs are to some extent have some serious limitations including toxicity and importantly therapy failure. Anticancer drugs fail to

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provide efficacy in most of the cases when they target only single, selective pathway (Wang *et al.*, 2019). Scientists all over the world are therefore in quest of some super molecules that will be able to produce effective anticancer activity by targeting more than one pathways. Herein, we present the evidence of physical interaction *in silico* between few selected investigational molecules along with approved CDKIs and some putative targets as a baseline study for development of anticancer agents.

2. Materials and Methods
Docking methodology is used to predict the new binding modes and affinities of small molecules within the binding site of particular receptor targets and is currently used as a standard computational tool in drug design for lead compound optimization and in virtual screening studies to find novel biologically active molecules (Meng *et al.*, 2011).

2.1. Selection of compounds for docking
By a systematic literature search, we found 49 compounds with CDK inhibitory property. Among these compounds include molecules already approved by the USFDA and those in various phases of clinical trial. These compounds target different type of CDK such as CDK1, CDK2, CDK4, CDK6, CDK7 and CDK9 etc. From these compounds, we screened only seven compounds (Table1) as these have been found in the literature to target multiple pathway components along with CDK especially CDK4, CDK6, CD7 and CDK9.

Table 1. List of CDK inhibitors (PubChem, 2021 and The Drug Repurposing Hub, 2021).

Compound name	PubChem CID	MOA	Target CDK	Developmental stage
RGB286638	11285002	CDK inhibitor	CDK1, CDK2, CDK3, CDK4, CDK5, CDK6, CDK7, CDK9	Phase 1
AT7519	11338033	CDK inhibitor	CDK1, CDK2, CDK4, CDK5, CDK6, CDK9	Phase 2
AMG925		CDK inhibitor, FLT3 inhibitor	CDK4, CDK6	Phase 1
Alvocidib	46220502	CDK inhibitor	CDK1, CDK2, CDK4, CDK5, CDK6, CDK7, CDK8, CDK9	Phase 2
Ribociclib	44631912	CDK inhibitor	CDK4, CDK6	Launched
Palbociclib	5330286	CDK inhibitor	CDK4, CDK6	Launched
Abemaciclib	46220502	CDK inhibitor	CDK4, CDK6	Launched

2.2. Obtaining 3D structures of target proteins and molecules for docking
From our review, we obtained 49 compounds and selected 7 of them for docking study which are: Ribociclib (Spring & Bardia, 2018), Palbociclib (Carlson, 2014), Abemaciclib (de Lartigue, 2017), RGB-286638 (van der Biessen *et al.*, 2014), AT-7519 (Thomas *et al.*, 2012), AMG-925 (Li *et al.*, 2014), and Alvocidib (Zocchi *et al.*, 2018) (Table 1). These compounds were chosen as these were reported to target mainly cyclin dependent kinases (CDKs) along with other target proteins (Table 2).

Table 2. List of target proteins for docking.

Target protein	PDB ID
BCL 2	1GJH
CDK 4	3G33
CDK 6	4TTH
CDK 7	1UA2
CDK9	3BLH
DHFR	5DXV
MAPK8	3O17
MAPK9	3E7O
mTOR	4JSV
TS	6B2F

The 3D structures of the target proteins in PDB format were obtained from RCSB Protein Data Bank (RCSB-PDB) (Bank, 2021, Berman *et al.*, 2000), a reservoir for three dimensional structures of proteins identified by X-ray crystallography, NMR etc. The 3D structures of the selected target proteins were retrieved from RCSB-PDB

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in PDB format. The 3D structures of the selected drug molecules were retrieved from PubChem in SDF (Structure data file) format.

2.3. Active pocket site identification

Active sites of the target proteins were predicted using the tools CASTp (Dundas *et al*., 2006). This provides a resource for locating, delineating and measuring concave surface regions on 3D structures of proteins. After analyzing the pockets suggested by the above tools, a consensus active site was chosen for docking analyses. The choice of active site was also done keeping the conserved region of protein 3D structure under consideration.

2.4. Preparation of Target Proteins and Ligands

For docking, the structure of the target proteins (Table 2) were converted into PDBQT format after performing necessary modifications in Auto Doc Tools (ADT) (version 1.5.6) (Morris *et al.*, 1998). The modification involves stripping all heteroatoms (including waters), re-protonating all atoms, and re-assigning Gasteiger charges to all atoms (Morris *et al.*, 1998). The ligands were converted to PDBQT with Open Bable tool (O'Boyle *et al*., 2011). Open Bable basically purifies and modifies the ligand and convert them into format of interest.

2.5. Docking and post-docking visualization for binding actions

For this computational study, we used various available software and online databases as outlined in Table 3. The docking of the ligands with the target proteins was done by using YASARA (Krieger and Vriend, 2014). Then, the prepared PDBQT format of the proteins and the ligands were imported for docking. Next, a Grid box was generated into the predicted active sites of the target proteins. The docking parameters were set using the software Autodock Tools (Morris *et al.*, 1998). Finally, the output files were saved in PDBQT format. The output files were opened in Autodock Tools (Morris *et al.*, 1998) for visualizing the interaction of the specific active sites of target proteins with the ligands of interest.

**Table 3. Software and databases used in current computational study.**

No.	Name of the software databases	Reference
1.	CASTp	Dundas *et al*., 2006
2.	DoGSiteScorer	Volkamer *et al*., 2012
3.	AutoDoc Tools	Morris *et al.*, 1998
4.	Open Bable	O'Boyle *et al*., 2011
5.	YASARA	Krieger and Vriend, 2014
6.	RCSB-PDB database	Berman *et al*., 1999
7.	PubChem project database	Kim *et al*., 2015
8.	Drug bank database	Knox *et al.,*2010

3. Results

Almost all of our selected molecules showed remarkable binding affinities for most of the target proteins. RGB286638 showed excellent binding affinity with BCL2 (8.124kcal/mol), CDK4 (8.413kcal/mol), CDK6 (8.528kcal/mol), CDK9 (8.007kcal/mol), mTOR (9.674kcal/mol) and TS (10.063kcal/mol). Evidently, this molecule holds a good potential as a multi-target anticancer drug candidates. Along with the classical pathway inhibition like thymidylate synthase (TS) and mTOR, inhibition of cell cycle and anti-apoptotic protein might be a good strategy to combat therapy-resistant cancers. Likewise, abemaciclib, a launched drug which have activity against CDK also showed excellent binding affinity with BCL2 (7.982kcal/mol), CDK4 (8.525kcal/mol), CDK6 (7.717kcal/mol), CDK9 (8.472kcal/mol), mTOR (8.748kcal/mol) and TS (8.551kcal/mol) thereby showing similar anticancer activity like RGB286638. These two molecules thus demonstrated strong binding against the selected targets (Table 4 and Figure 1). Other molecules showing strong binding interactions against the selected targets are presented in Table 4. Among the other putative targets include DHFR, one of the classical anticancer targets as well as MAPK8 and MAPK9, which are often implicated in various types of human cancers (Gkouveris and Nikitakis, 2017).

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Table 4. Result of molecular docking of the compounds against target proteins.

Compound Name	Binding Energy(kcal/mol)									
	BCL2	CDK4	CDK6	CDK7	CDK9	DHFR	MAPK8	MAPK9	mTOR	TS
RGB286638	8.1	8.4	8.5	7.4	8.1	7.5	7.9	8.6	9.6	10.1
AT7519	6.4	6.9	6.1	5.6	6.7	7.2	6.1	6.7	6.5	6.2
Ribociclib	8.2	7.4	7.7	6.7	7.7	7.1	7.5	7.6	7.9	7.6
Abemaciclib	7.9	8.5	7.7	7.5	8.4	7.8	8.8	7.9	8.7	8.5
Alvocidib	7.9	8.3	7.8	7.3	7.9	8.5	7.5	7.3	7.2	9.1
Palbociclib	8.4	8.2	7.5	7.1	7.8	7.5	6.7	7.3	8.4	8.3
AMG 925	8.3	8.7	8.1	7.5	9.4	8.8	7.8	8.2	9.7	9.4
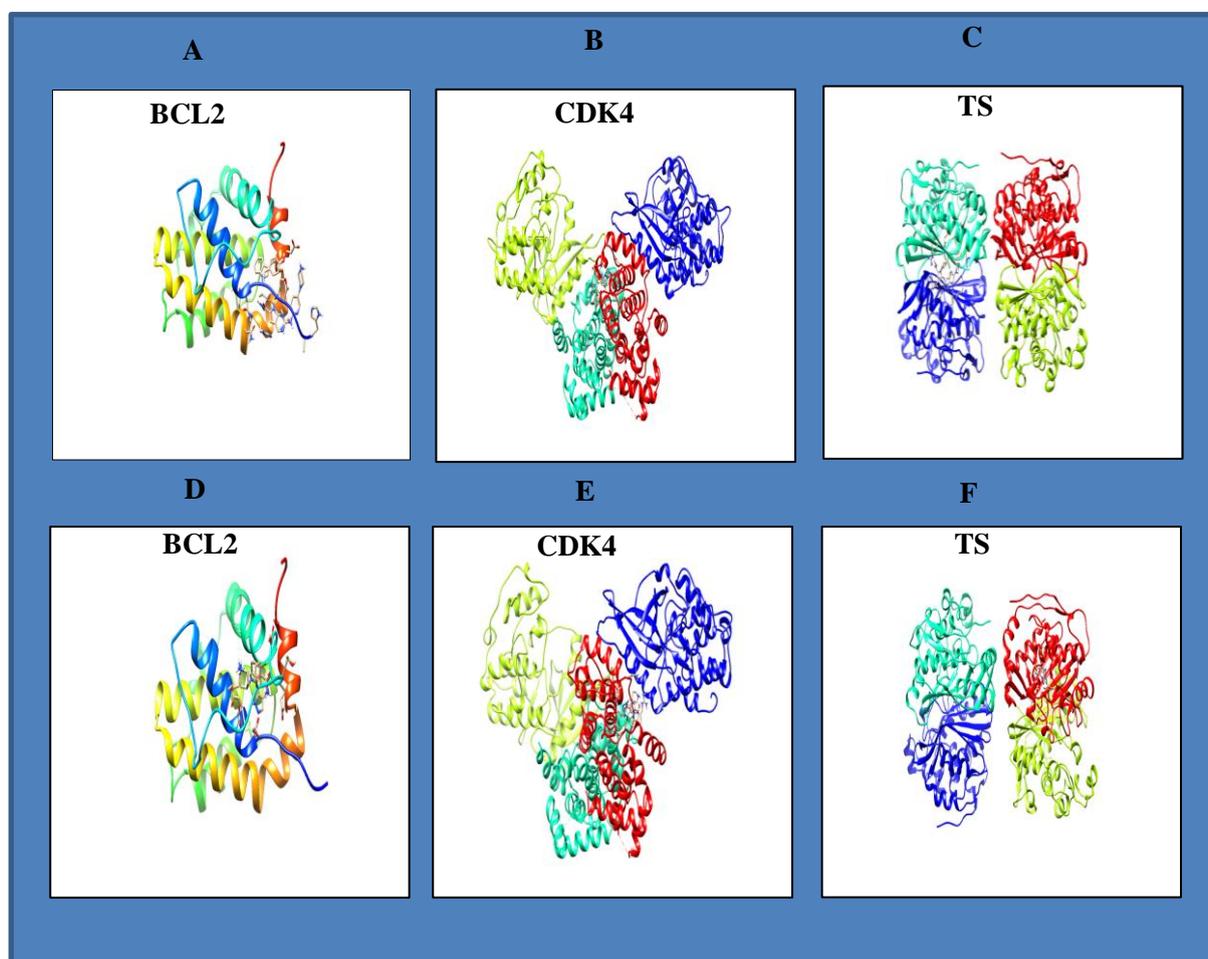
Figure 1. Molecular interactions of RGB286638 (A, B and C) and Abemaciclib (D, E and F) with BCL2, CDK4 and TS.

4. Discussion

After analyzing the docking results, we observed that, all the compounds showed excellent binding interactions with our selected target molecules. Our computational findings indicate that these compounds have significant potential to become good anticancer leads to target multiple pathways including cell cycle regulatory network. In particular, our findings clearly demonstrate that both abemaciclib (launched) and RGB286638 (in clinical trial) can potentially target the anti-apoptotic protein BCL2, TS as well as mTOR. Among these, the anti-apoptotic BCL2 has been one of the potential drug able targets that led the scientists to the development of target therapy as anti-BCL2 anticancer agents, more popularly known as BCL2 inhibitors (Thomas *et al*., 2013; Huang, 2000). Since anti-apoptotic BCL2 play a major role in tumor cell survival, BCL2 inhibitors have been developed as direct inducers of apoptosis. One such molecule, ABT-199 (venetoclax) received breakthrough

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therapy designation from the FDA due to its remarkable efficacy in chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML) (Soderquist RS and Eastman A, 2016). TS and mTOR are two of the most popular targets against conventional anticancer drugs that have been in the focal points of the scientists for many decades. Besides, mitogen-activated protein kinases (MAPKs) including MAPK8 and MAPK9 have found to be overexpressed and hence implicated in various human cancers. Also, they mediate resistance against chemotherapy and apoptosis (Gkouveris and Nikitakis, 2017). Since our docking study identifies all these targets along with the CDKs (CDK4, 6 and 9) for abemaciclib and RGB286638, it can be suggested that these two compounds can produce promising anticancer activities by inhibiting these target proteins (Figure 2).
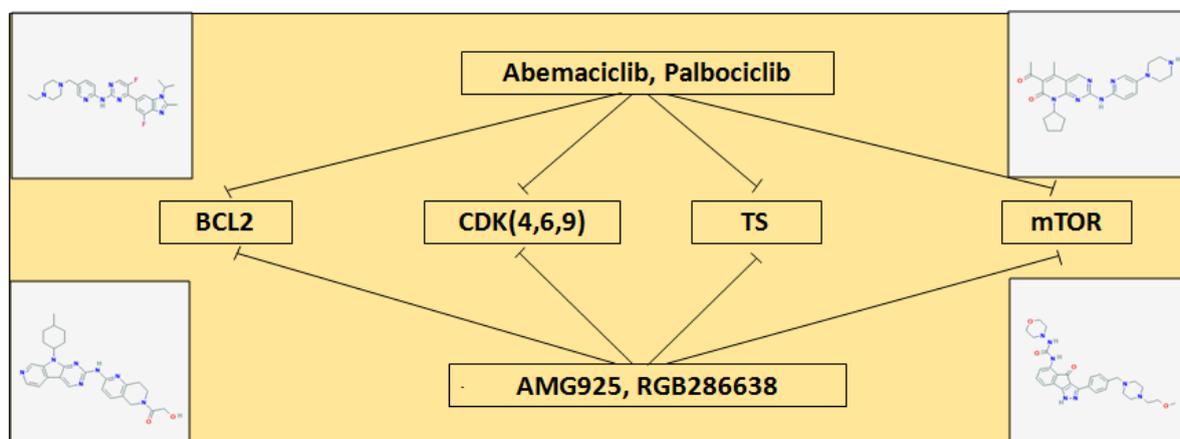
Figure 2. Proposed model of anticancer mechanism for selected CDK inhibitors against putative targets.

5. Conclusions

In this computational study, we summarized the binding interaction of seven inhibitors against putative targets that provide us an insight for rational drug development that require extensive structural modifications and medicinal investigations. After molecular modification of structures, these compounds need to be tested on cell lines overexpressing their respective targets to confirm their better interaction and improved potency to develop them as potential anticancer drugs or drug leads. In short, more detailed and precisely designed pharmacological, biochemical and toxicological investigations in relevant *in vivo* and *in vitro* models are required to confirm the effectiveness, usefulness and toxicity to validate the feasibility of these promising molecules as lead compounds.

Acknowledgements

Conflict of interest

None to declare.

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