Research Article

Insilico Molecular Docking Studies of 1-substituted-2-((methyl) substituted)-1H-benzo[d]imidazole derivates as Cyclin Dependent Kinase-2 Inhibitors

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ABSTRACT

The cyclin-dependent kinase2 (cdk2) is a key enzyme in the cell cycle machinery of all eukaryotic organisms belongs to the family of conserved serine/threonine protein kinases. Their enzymatic role is activated by binding to regulatory cyclin partners. These cyclin/cdk2 complex control cell proliferation by regulating entry into and passage through the cell cycle, as well as their deregulation in several human cancers, makes them as attractive therapeutic targets in oncology. Since the activity of cdk is high in cell proliferative disease cancer associated with tumor development which is by over expression of positive regulator cyclin, so the therapeutic agents that block CDK activity may act as important targets in cancer therapy. In our present study, the X-ray structures of the CDK2 (PDB ID: 1DI8) was retrieved from protein data bank based on good Resolution (1.90) and Ramachandran's plot analysis. Novel compounds were synthesized and evaluated as inhibitors of CDK2 by docking analysis of these synthetic ligands on binding to Cyclin-dependent kinase 2 using Accelrys Discovery Studio2.5. These findings suggest that these novel compounds may provide potent leads for the research and development of suitable for the treatment of cancer.

Keywords: Anti-leukemic agents, Cancer therapy, Cyclin-dependent kinase 2 (CDK2), Synthetic ligands.

INTRODUCTION

The targeting of the cell cycle presents unique opportunities for drug development in cancer therapy.[1] Deregulated cell-cycle control mechanism, resulting in the uncontrolled growth and abnormal proliferation (neoplasia) of the cell is a characteristic of all cancers. Cell proliferation is the ability of cells to traverse the cell cycle, is intricately regulated in normal cells by the coordinate activity of both positive and negative regulating proteins, cyclin-dependent kinases (CDKs).[2-4] CDKs are serine/threonine kinases that play pivotal roles in cell cycle progression.[5] Their timely activation and deactivation drives the cell through different stages of the cell cycle. The positive regulation of CDK activity is achieved by their association with cyclins (A, B, D and E), and are negatively regulated by specific CDK inhibitors, their state of phosphorylation and ubiquitin mediated degradation.[6,7] A large number of human neoplasias show over expression of positive regulators of CDKs and/or decrease in negative regulators.[8] which has been observed in many human cancers including breast, brain, endometrial, and lung cancers, as well as lymphomas and leukemias. These abnormal activities by CDKs contribute to tumor genesis, often through interaction with pathways regulated by oncogenes and tumor suppressors, they have become valid targets for developing chemical inhibitors for cancer therapies.[9-11]

Taking all the above considerations, our search for potent and selective CDK inhibitors, In our present study novel compounds were synthesized and drawn using ChemSketch. The crystal structure of cyclin dependent kinase 2(PDB ID: 1DI8) was taken from PDB database and docking studies were performed on these synthetic ligands and CDK2. Docking analysis reveals that these novel compounds act as potent targets for the development of anti-leukemic agents in the treatment of cancer.

Chemistry

Synthesis of 2-(chloromethyl)-1H-benzo[d]imidazole3 (1)

A mixture of 4gm orthophenylene diamine, 36 ml of 4 N HCL and 3.4gm of Chloro acetic acid was taken in a round bottom flask and the solution was boiled under reflux for 3 hours until reaction completes which is checked by T.L.C analysis. Further the solution was cooled on ice and made alkaline by the addition of 30% NH3 solution. The precipitate formed was filtered, dried and recrystallized from suitable solvents. M.P; 140°C, Yield; 80 %.

Synthesis of (1H-benzo[d]imidazol-2-yl)-N,N-dimethyl methanamine (2)

2-(chloromethyl)-1H-benzo[d]imidazole (0.005 mol) was added to suspension of the appropriate secondary amine (dimethyl amine) (0.005 mol) and anhydrous potassium carbonate (0.005 mol) in dry acetone (15 ml). The reaction mixture was stirred for 6 – 8 hrs at ambient temperature and acetone was then evaporated. Distilled water was added to the residue and the formed precipitate was filtered, washed with water, dried and

recrystallized from appropriate solvent. The purity of the compound was checked by TLC and spectral data. M.P; 160o C, Yield; 68 %. IR (KBr)(cm-1): 3075(N-H str.), 3155 (Ar-H str.), 1650-1540 (C=C & C=N str.). H1 NMR (DMSO-d6): δ 5.2, (s, 1H, NH), 8.1-7.2 (m, 4H, Ar-H), 3.4 (s, 2H, -CH2-), 2.3 (s, 6H, -N(CH3)2). EI-MS: m/z = 175(M+), 176 (M+1).

Synthesis of (4-chloropyridin-2-yl)(2-((dimethylamino) methyl)-1H-benzo[d]imidazol-1-yl) methanone (3)

A solution of 2 (0.005 mol) in dry N,N-dimethyl formamide was treated with potassium tert-butoxide and the reddish brown mixture was stirred at room temperature for 2 hr. The contents were treated with 4-chloropyridine-2-carbonyl chloride (0.005 mol) and potassium carbonate and then heated to 80oC for 6 hr. The mixture was cooled to room temperature and poured into ethyl acetate. The combined organics were washed with brine, dried over sodium sulphate and concentrated to give (4-chloropyridin-2-yl) (2-((dimethylamino) methyl)-1H-benzo[d]imidazol-1-yl) methanone. M.P; 220o C, Yield; 63 %. IR (KBr) (cm-1): 3155 (Ar-H str.), 1610-1530 (C=C & C=N str.), 1240 (C-N), 1648 (C=O), 745 (C-Cl). H1NMR (DMSO-d6): δ 9.4 -7.2 (m, 7H, Ar-H), 3.8 (s, 2H, -CH2-), 2.5 (s, 6H, -N(CH3)2). EI-MS: m/z = 314(M+), 315(M+1).

Methodology

Protein preparation

The high resolution x-ray crystal structure of cyclin dependent kinase 2 was obtained from the protein databank. After evaluating number of entries, the best protein was selected based on high resolution, Ramachandran plot analysis, and Procheck using SAVS server based on number of residues in disallowed regions (Laskowaski et al., 1993). Using Accelrys Discovery studio 3.0 protein preparation was carried out by correcting the missing residues and the removing the complexes bound to receptor molecule. The structure was then refined by energy minimization with appropriate charges and parameters carried out using steepest descent gradient until the convergence gradient satisfied.

Ligand preparation and optimization

Using ACD/ ChemSketch (12.0)[12] Software the structures of the 34 synthetic ligand compounds i.e B-3 to B-36 compounds, were drawn and saved in mol2 format. The saved ligand compounds were later imported and minimized in Argus Lab after adding hydrogen bonds. The molecules thus obtained were saved in mol format.

Molecular Docking using Discovery Studio 2.5

Docking of ligands in to a receptor biding site was done by CDOCKER in Accelrys Discovery Studio which uses a CHARMm-based molecular dynamics (MD) scheme. Random ligand conformations are generated using high-temperature MD which is then translated into the binding site. Candidate poses are then created using random rigid-body rotations followed by simulated annealing. A final minimization is then used to refine the ligand poses.[13] The binding mode for all 29 ligands to Cyclin-dependent kinase 2 (CDK2) (PDB ID: 1DI8) was investigated by CDOCKER protocol which had been incorporated into Discovery Studio 2.5. The Binding-Site module (Accelrys Inc.) is a suite of programs for identifying and characterizing protein active sites, binding sites, and functional residues from protein structures and multiple sequence alignments. Two site finding routines are used to automatically locate binding sites. One identifies cavities within the receptor, the other builds a binding site based on a ligand molecule already in a known location. The algorithm for both is based on a grid search and "eraser" algorithm. The results can be used to guide the protein–ligand docking experiment.

RESULTS AND DISCUSSION

Selection of PDB Structure

Based on good resolution and Ramachandran's plot analysis the X-ray crystal structure of the protein Cyclin-dependent kinase 2 (PDB ID: 1DI8) was retrieved from protein data bank. The crystal structures of the human cyclin-dependent kinase 2(CDK2) in complex with 4-[3-hydroxyanilino]-6,7-dimethoxyquinazoline [14] have been determined to 2.20 Å resolutions with sequence length of 298 base pairs. The structure is bi-lobate, like that of the cyclic AMP-dependent protein kinase, but contains a unique helix-loop segment that interferes with ATP and protein substrate binding and probably plays a key part in the regulation of all cyclin-dependent kinases.

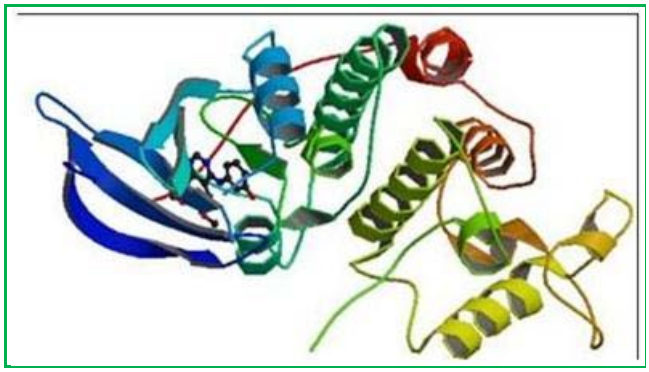


Figure 1: The structure of cyclin-dependent kinase 2 (cdk2) in complex with 4-[3-hydroxyanilino]-6,7-dimethoxyquinazoline.

Active Site Analysis of 1DI8 Structure

The structure was further analyzed for identifying the possible binding sites of Cyclin-dependent kinase 2 (CDK2) (Figure 3). Using Accelrys Discovery Studio software package docking was performed to find out the appropriate binding orientations and conformations of ligands on 1DI8.[15] The receptor molecule is first defined by using binding site tools of Discovery Studio. In this study, Active Site-Search was used to identify protein active sites and binding sites by locating cavity in the 1DI8 structure. When the search was completed, the largest

site was automatically displayed on the structure. And then, by using Asite-Display, other sites were also obtained.

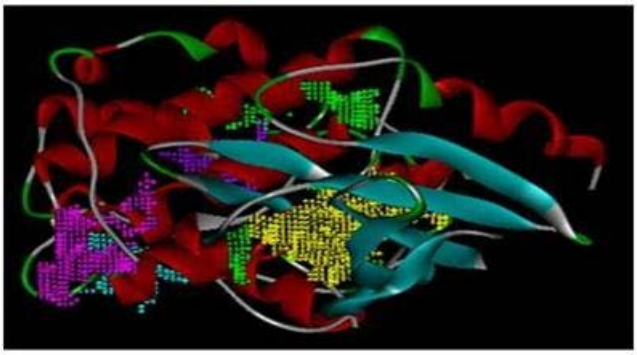


Figure 2: Identification of active site pocket in receptor molecule

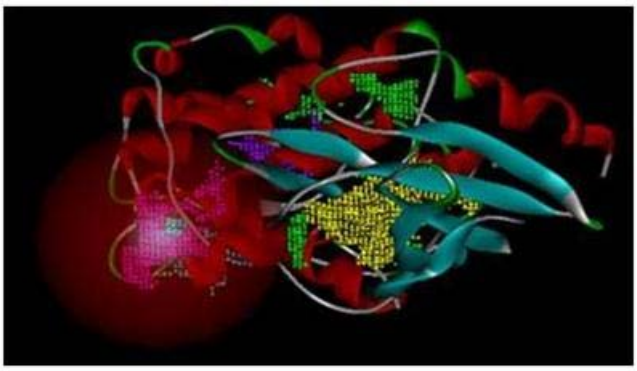


Figure 3: Defining of sphere around active site pocket 2

DOCKING

Docking of Synthetic ligands with 1DI8 structure:

CDOCKER is a grid-based molecular docking method that employs CHARMm (Brooks et al., 1983). The receptor was held rigid while the ligand was allowed to flex during the refinement. By the search mentioned above, prior knowledge of the binding site had been acquired. Hence it was possible to specify the ligand placement in the active site using a binding site sphere with the radius of 12A˚ (Figure 4). The CDOCKER interaction energy between the compounds and 1DI8 (E-binding) was finally computed. From the docking analysis, insights into the interactions between the ligands and the receptor were gained, which facilitated the selection of top 10 poses which were saved for comparison and analysis. Finally, the pose with the lowest CDOCKER energy was used for further study.

The default parameters were used in the docking simulations with CDOCKER. Different Poses of protein-ligand complex is obtained after docking process with their specific CDOCKER energy and CDOCKER interaction scores displayed in output file. The best ligand was chosen on the basis of their highly interacting amino acid residues (Table 1). The ligand poses were analyzed and interaction of ligand molecule with the 1DI8 protein structure was studied on the basis of H-bonding made by the poses to the receptor molecule and close contacts (Vander Waals clashes) between the poses and receptor molecule. As it is well known, H bonds play an important role for the structure and function of biological molecules, especially for the enzyme catalysis.

The H bonds present in the protein-ligand complex are shown in (Figure 4). In this study, it was found that ARG297 of receptor molecule (i.e. 1DI8) forms one H-bonds with oxygen atom of 7th synthetic ligand. Similarly GLY98, PRO100, HIS295 forms non-bonding interactions at active site pocket 2 of the receptor molecule.

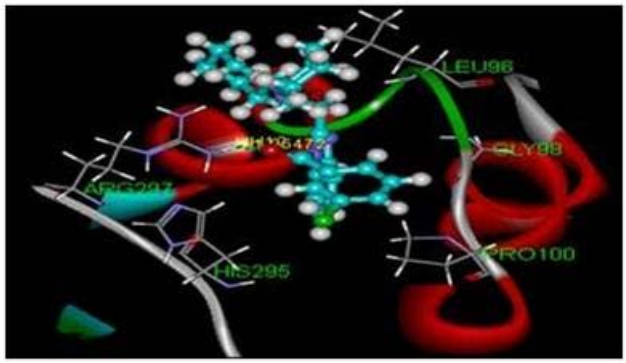
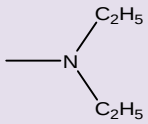
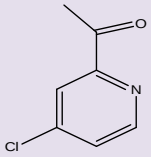
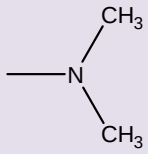
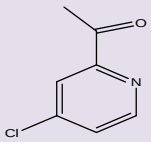
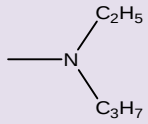
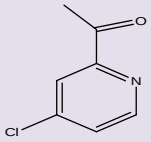
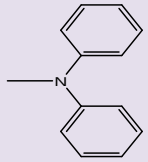
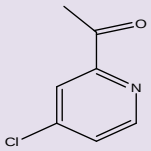
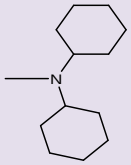
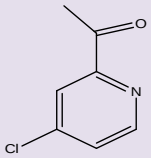
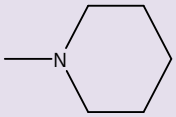
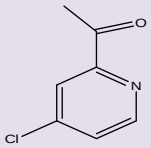
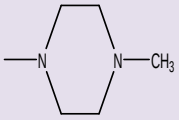
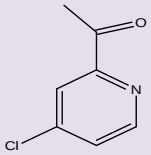
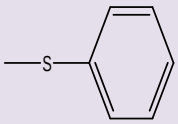
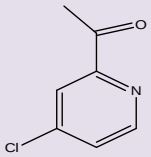
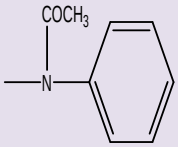
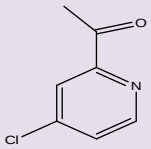
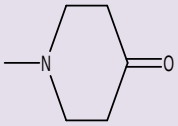
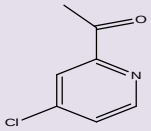
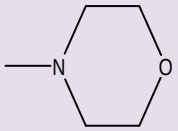
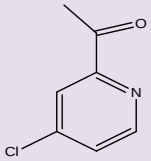


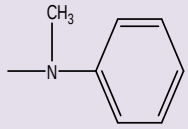
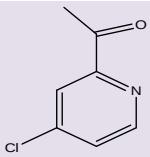
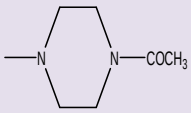
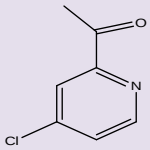
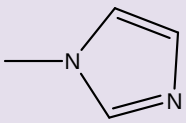
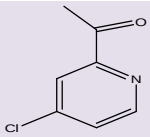
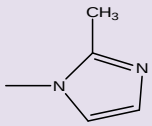
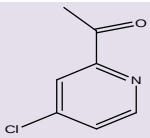
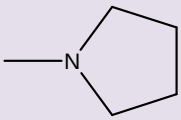
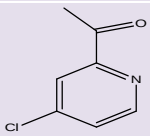
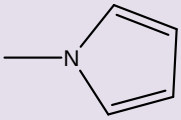
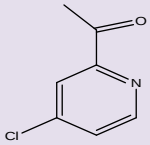
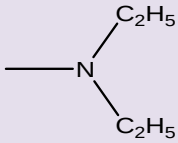
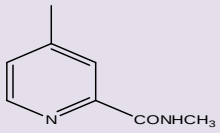
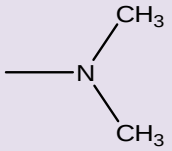
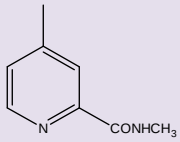
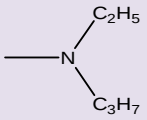
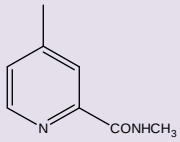
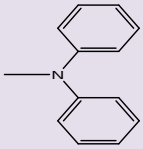
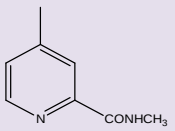
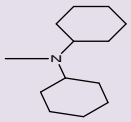
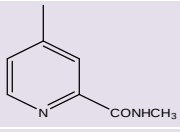
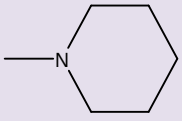
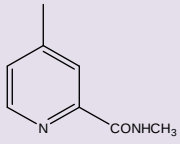
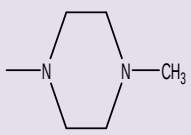
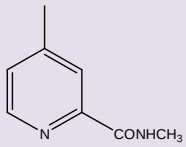
Figure 4: Docking model of active site residues of CDK2 with 7 mol ligand

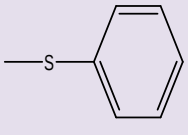
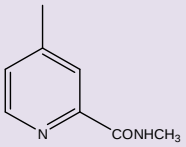
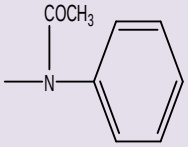
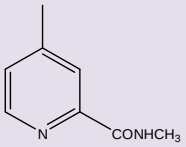
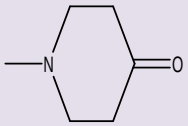
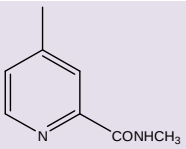
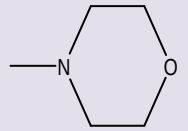
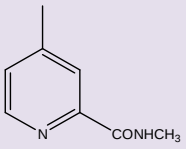
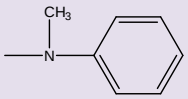
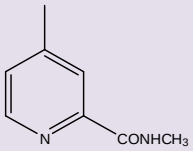
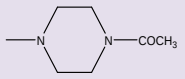
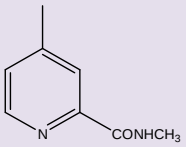
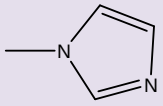
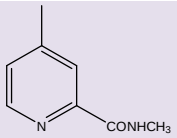
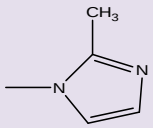
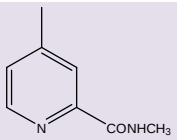
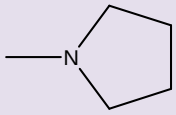
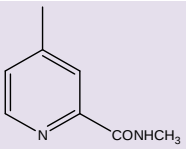
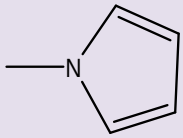
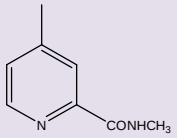
DISCUSSION

Our compounds are likely inhibiting CDK as one of the important drug target in the cancer therapy. The X-ray crystal structure of the protein human Cyclin-dependent kinase 2 (PDB ID: 1DI8) in complex with 4-[3-hydroxyanilino]-6,7-dimethoxyquinazoline were retrieved from protein data bank based on good resolution of 2.20 Å with sequence length of 298 base pairs and The structure was analyzed for identifying the possible binding sites of Cyclin-dependent kinase 2 (CDK2) (Figure 2). 2D structures of the synthetic ligand compounds (B-3 to B-36) were generated using ChemSketch and subjected to energy minimization using Argus lab. Docking of these optimized compounds against Cyclin-dependent kinase 2 (1DI8) structures at the catalytic active site residues were performed by Accelrys Discovery Studio 2.5. To locate the appropriate binding orientations and conformations of ligands on 1DI8, molecular docking was performed by using CDOCKER. The CDOCKER interaction energy between the synthetic ligand compounds and protein receptor 1DI8 (E-binding) was finally computed. Out of 34 docked complexes, we got 1 best docked synthetic compound showing highest H-bond interactions & lowest CDOCKER energy with the amino acid residues of the receptor molecule (Table 1). The H-bond interaction of best docked complex is shown in Figure 4. It is evident from this analysis that the best inhibitors are located in the center of the active site and is stabilized by hydrogen bonding interactions. As it's well known, hydrogen bonding plays an important role for the structure and function of biological molecules, especially for inhibition in a complex.

Table 1: Physical data & CDOCKER energy interaction of synthesized compounds (B-3 – B-36) with CDK2 protein (1DI8) at active site pocket 2

Compound	R_1	R_2	Mol. Formula	Mol. Wt.	CDOCKER Energy	CDOCKER Interaction Energy
B-3			$C_{18}H_{19}ClN_4O$	342.82	-3.29681	30.3914
B-4			$C_{16}H_{15}ClN_4O$	314.77	-0.399646	32.3641
B-5			$C_{19}H_{21}ClN_4O$	356.85	-5.10712	29.2814
B-6			$C_{26}H_{19}ClN_4O$	438.91	-4.90609	30.9103
B-7			$C_{26}H_{31}ClN_4O$	451	-43.288	22.0734
B-8			$C_{19}H_{19}ClN_4O$	354.83	-11.0752	30.7617
B-9			$C_{19}H_{20}ClN_5O$	369.85	-11.1349	34.2819
B-10			$C_{20}H_{14}ClN_3OS$	379.86	-5.81713	29.5668
B-11			$C_{22}H_{17}ClN_4O_2$	404.85	-4.26403	32.5233
B-12			$C_{19}H_{17}ClN_4O_2$	368.82	-6.986	30.1902
B-13			$C_{18}H_{17}ClN_4O_2$	356.81	-9.58124	30.7904

B-14			$C_{21}H_{17}ClN_4O$	376.84	-4.98781	27.6649
B-15			$C_{20}H_{20}ClN_5O_2$	397.86	-3.69185	35.8494
B-16			$C_{17}H_{12}ClN_5O$	337.76	-2.282	30.3436
B-17			$C_{18}H_{14}ClN_5O$	351.79	1.7189	30.6319
B-18			$C_{18}H_{17}ClN_4O$	340.81	-17.0205	33.3479
B-19			$C_{18}H_{13}ClN_4O$	336.78	-2.44068	30.7775
B-20			$C_{19}H_{23}N_5O$	337.42	1.18617	34.553
B-21			$C_{17}H_{19}N_5O$	309.37	2.52818	31.5818
B-22			$C_{20}H_{25}N_5O$	351.45	1.25762	31.575
B-23			$C_{27}H_{23}N_5O$	433.5	-5.23282	29.3869
B-24			$C_{19}H_{35}N_5O$	445.6	-44.7338	28.1144
B-25			$C_{20}H_{23}N_5O$	349.43	-6.33888	28.5135
B-26			$C_{20}H_{24}N_6O$	364.44	-7.04899	31.949

B-27			$C_{21}H_{18}N_4OS$	374.46	1.97034	29.131
B-28			$C_{23}H_{21}N_5O_2$	399.45	-0.618252	30.5434
B-29			$C_{20}H_{21}N_5O_2$	363.41	-1.17912	30.3505
B-30			$C_{19}H_{21}N_5O_2$	351.4	-3.72093	26.6278
B-31			$C_{22}H_{21}N_5O$	371.44	1.15408	27.2038
B-32			$C_{21}H_{24}N_6O_2$	392.45	-3.01997	25.4988
B-33			$C_{18}H_{16}N_6O$	332.36	1.40656	25.7671
B-34			$C_{19}H_{18}N_6O$	346.39	5.76038	29.0931
B-35			$C_{19}H_{21}N_5O$	335.4	-13.9	27.8714
B-36			$C_{19}H_{17}N_5O$	331.37	2.44898	28.1617

CONCLUSION

The present study demonstrated the selection of X-ray crystal structure of human Cyclin-dependent kinase 2 (PDB ID: 1DI8) from Protein Data Bank. Among 80 different structures, 1DI8 is chosen as it was having maximum residues (89.4%) lie in the most favoured and no residues in disallowed region of Ramachandran plot. Further the structure was used to find the best inhibitors by studying the interaction between 1DI8 protein receptor and twenty-nine synthetic ligand compounds. Previous studies have shown that loss of CDK1 activity or the aberrant expression of CDK1 involved in G2 phase arrest and many tumor types, thereby validating CDK1 as a therapeutic target. Therefore, a surge of interest has been devoted to searching for potent CDK1 inhibitors as effective chemotherapeutic agents. Herein we focus, in this review, mainly on the studies about the structure, different structure classes & binding activity of potent synthetic ligand compounds as CDK1 inhibitors. Based on the docking score, 7th ligand has shown lowest CDOCKER energy & best binding activity with our protein receptor.

Thus our study confirms that, out of 34 compounds, 7th ligand compound based on bonding & non-bonding interaction could be potentially act as a drug candidate's yet pharmacological study will yet confirm it to be promising. This study could be utilized for the designing of effective drug for the treatment of cancer.

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