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Synthesis and Molecular Docking Studies of Novel 1-Substituted-2-((Methyl) Substituted)-1H-Benzo[D] Imidazole Derivatives

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ABSTRACT

We report here the synthesis and preliminary evaluation of novel synthetic compounds *in vivo* and investigation of their anticancer activities by binding to cyclin dependent kinase 2. Cyclin dependent kinases (CDKs) are a family of proteins involved in the regulation of cell cycle progression and attractive targets in oncology. Cyclin-dependent kinase 2 (CDK2) is a member of a highly conserved family of protein kinases that regulate the eukaryotic cell cycle. Tumour-associated cell cycle defects are often mediated by alterations in cyclin-dependent kinase (CDK) activity. According to current models, mammalian CDKs are essential for driving each cell cycle phase, so therapeutic strategies that block CDK activity are unlikely to selectively target tumour cells. Emerging evidence suggests that tumor cells may also require specific interphase CDKs for proliferation. Thus, selective CDK inhibition may provide therapeutic benefit against certain human neoplasias. The X-ray structures of the CDK2 (PDB ID: 1DI8) were retrieved from protein data bank based on good Resolution (1.90) and Ramachandran's plot analysis. We have studied the influence of synthetic ligands on the binding of Cyclin-dependent kinase 2 with the help of docking studies by using Accelrys Discovery Studio2.5. The findings obtained in these studies indicate that these compounds could be a potent anti – leukemic agent.

Keywords: Cyclin-dependent kinase 2 (CDK2), synthetic compounds, Leukemia, Anticancer activity

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INTRODUCTION

Cancer is the worldwide health problem and the most frightening disease of human.[1] Among different types of cancers, leukemia is one of the major causes of cancer related deaths.[2, 3] Leukemia originates from hematopoietic stem cells or cells at different stages of myeloid or erythroid differentiation which spread throughout the body. Although, the success of clinical trials in identifying new agents and treatment modalities has been significant, current treatments suffer from many limitations such as side effect of the drugs and drug resistance. Hence, the identification of novel, efficient and less toxic anticancer agents remains an important and challenging task in cancer biology. Cyclin-dependent kinases (CDKs) are regulatory proteins of the eukaryotic cell cycle. They act after association with different cyclins, the concentrations of which vary throughout the progression of the cell cycle. As central mediators of cell growth, CDKs are potential targets for inhibitory molecules that would allow disruption of the cell cycle in order to evoke an anti-proliferative effect and may therefore be useful as cancer therapeutics [4]. Cyclin-dependent kinases (CDKs) have long been known to be the main facilitators of the cell proliferation cycle. However, they also play important roles in the regulation of the RNA polymerase II transcription cycle. Cancer cells display aberrant cell cycle regulation to gain proliferative advantages and they also appear to have an exaggerated dependence on RNA polymerase II transcriptional activity to sustain pro-survival and anti-apoptotic signaling. A picture is now starting to emerge that both the cell-cycle and transcriptional functions of CDKs can be exploited pharmacologically with CDK inhibitors that possess appropriate selectivity profiles. In this article, recent advances into these mechanistic insights and how they can guide clinical development in terms of choice of indication are reviewed[5]. Since it is possible to selectively interrupt the cell cycle regulation in cancer cells by interfering with CDK action, the cell will die. Hence, CDKs are considered a potential target for anti-cancer medication[6].

MATERIALS AND METHOD

Swissprot

The Swiss-Prot group is part of the Swiss Institute of Bioinformatics (SIB). It is in charge of research and development in the fields of bioinformatics, and in particular of protein databases. Its main activity, in collaboration with the European Bioinformatics Institute (EBI), is the development of Swiss-Prot. The UniProt Consortium comprises the European Bioinformatics Institute (EBI), the Swiss Institute of Bioinformatics (SIB), and the Protein Information Resource (PIR). EBI located at the Welcome Trust Genome Campus in Hinxton, UK, hosts a large

resource of bioinformatics databases and services. SIB, located in Geneva, Switzerland, maintains the ExPASy (Expert Protein Analysis System) servers that are a central resource for proteomics tools and databases. PIR, hosted by the National Biomedical Research Foundation (NBRF) at the Georgetown University Medical Center in Washington, DC, USA, is heir to the oldest protein sequence database, Margaret Dayhoff's Atlas of Protein Sequence and Structure, first published in 1965[7]. In 2002, EBI, SIB, and PIR joined forces as the UniProt Consortium. UniProt is a comprehensive, high-quality and freely accessible database of protein sequence and functional information, many of which are derived from genome sequencing projects. It contains a large amount of information about the biological function of proteins derived from literature.

3D Structure Database

Selection of PDB Structure

The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organizations (PDBe, PDBj, and RCSB). The PDB is overseen by an organization called the Worldwide Protein Data Bank, wwPDB[8]. The PDB is a key resource in areas of structural biology, such as structural genomics. The X-ray structures of the proteins Cyclin-dependent kinase 2 were retrieved from protein data bank[9] based on good resolution (2.20) Ramachandran plot analysis.

Ligand generation and Optimization

Total 34 synthetic ligand compounds i.e **Va-Vq** and **VIa-VIq** compounds of Scheme-1, were drawn using ACD/ ChemSketch (12.0)[10] 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 PDB format.

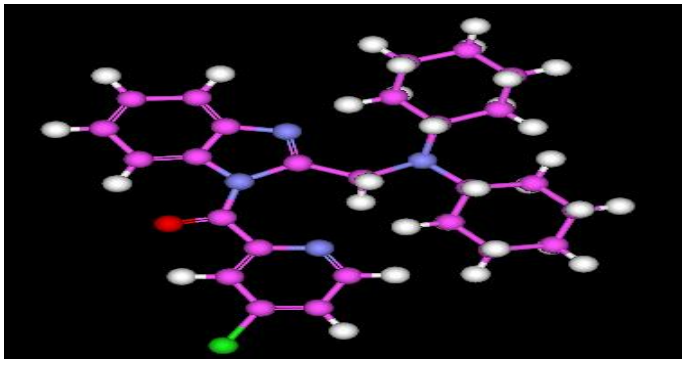


Figure 1: (2-((dicyclohexylamino) methyl)-1H-benzo [d] imidazol-1-yl) (4-chloropyridin-2-yl) methanone (VIe)

Molecular Docking using Discovery Studio 2.5

CDOCKER in Accelrys Discovery Studio uses a CHARMm-based molecular dynamics (MD) scheme to dock ligands into a receptor binding site. Random ligand conformations are generated using high-temperature MD. The conformations are 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[11]. The binding mode for all 34 ligands to Cyclin-dependent kinase 2 (CDK2) (PDB ID: 1DI8) was investigated by CDOCKER protocol which had been incorporated into Discovery Studio 2.5. TheBinding-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.

Selection of PDB Structure

The X-ray crystal structure of the protein Cyclin-dependent kinase 2 (PDB ID: 1DI8) were retrieved from protein data bank based on good resolution and Ramachandran's plot analysis. The crystal structures of the human cyclin-dependent kinase 2(CDK2) in complex with 4-[3-hydroxyanilino]-6, 7-dimethoxyquinazoline[12] 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.

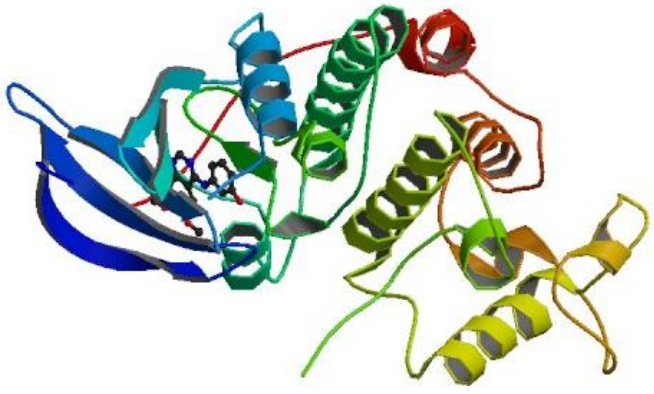


Figure 2: The structure of cyclin-dependent kinase 2 (cdk2) in complex with 4-[3-hydroxyanilino]-6,7-dimethoxyquinazoline.

Active Site Analysis of 1DI8 Structure

The structure was analyzed for identifying the possible binding sites of Cyclin-dependent kinase 2 (CDK2) (Figure 3). To locate the appropriate binding orientations and conformations of ligands on 1DI8, docking was performed by using Accelrys Discovery Studio software package. 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.

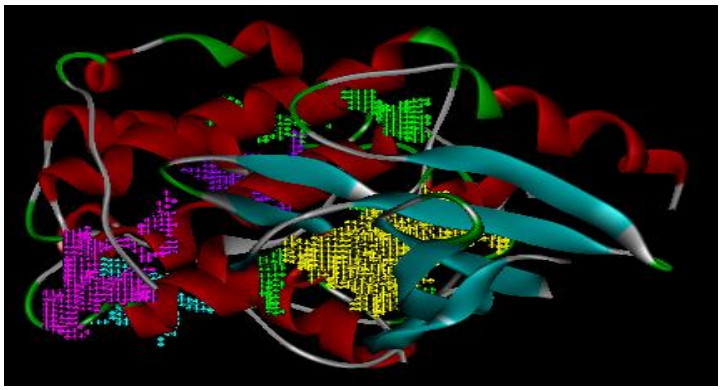


Figure 3: Identification of active site pocket in receptor molecule.

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.

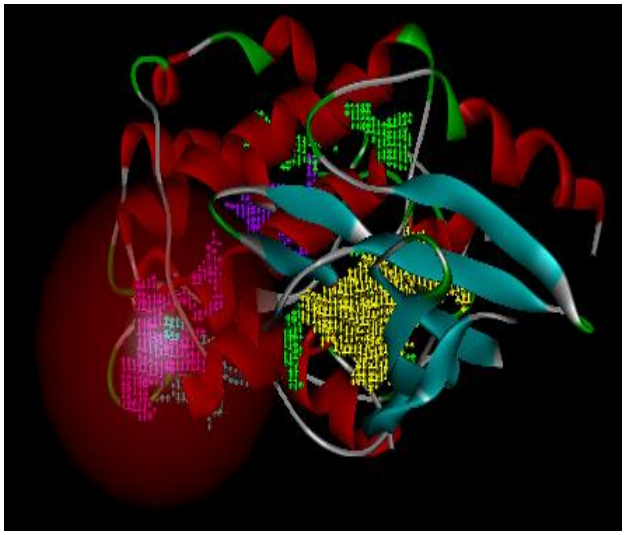


Figure 4: Defining of sphere around active site pocket 2

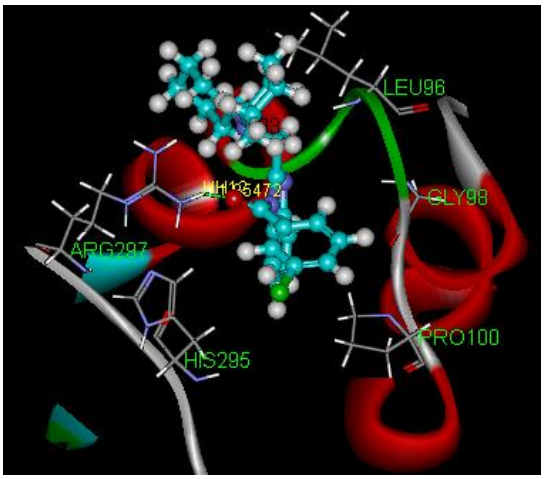


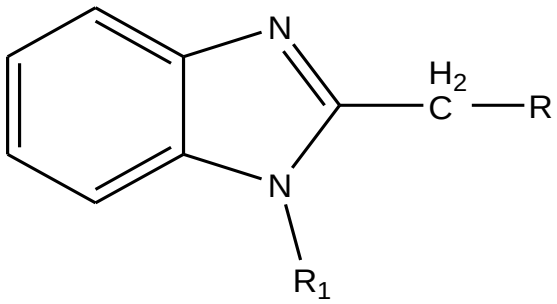
Figure 5: Docking model of active site residues of CDK2 with 15 mol ligand

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 5). In this study, it was found that ILE135 of VIe synthetic ligand forms two H-bonds with oxygen atom of receptor molecule (i.e. 1DI8). Similarly THR97 forms H-bond with hydrogen atom of the

receptor protein and MET91, LEU87, LYS89, ASP86 & ARG237 forms non-bonding interactions at active site pocket 2 of the receptor molecule.

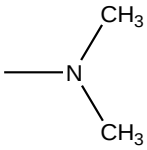
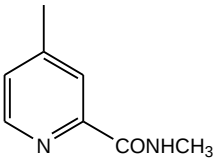
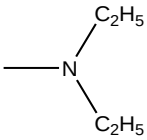
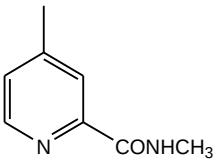
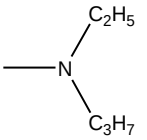
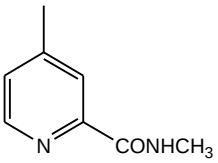
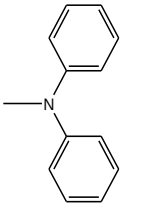
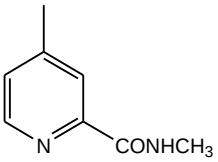
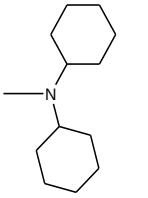
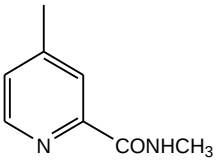
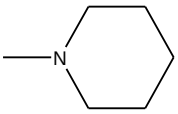
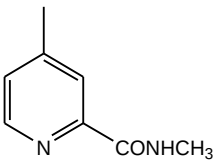
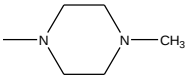
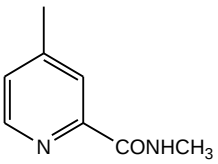
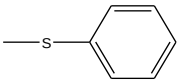
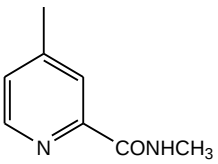
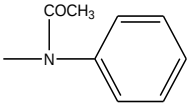
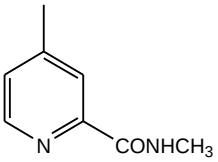
RESULTS AND DISCUSSION

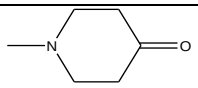
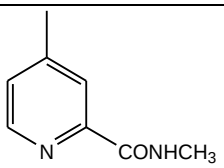
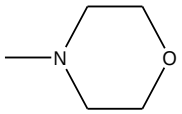
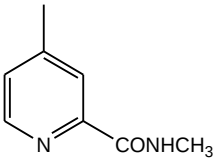
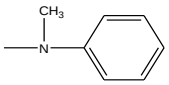
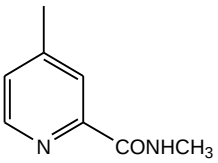
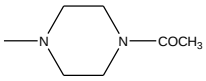
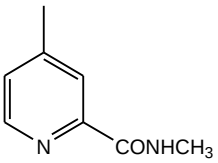
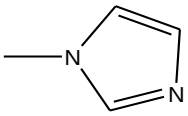
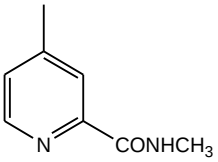
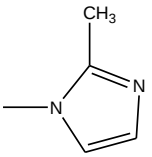
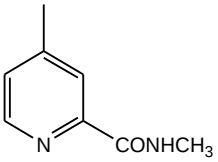
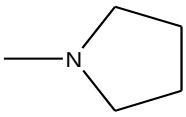
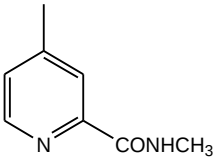
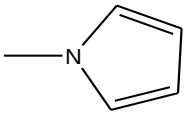
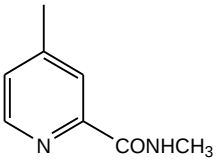
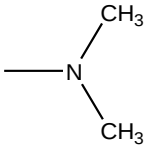
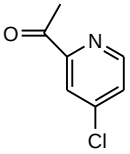
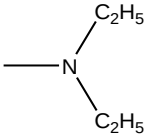
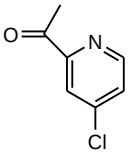
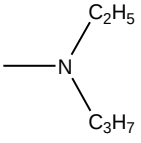
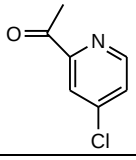
The X-ray crystal structure of the protein human Cyclin-dependent kinase 2 (PDB ID: 1DI8) in complex with 4-[3-hydroxyanilino]-6,7-dimethoxyquinazoline were retrieved from protein data bank based on good resolution of 2.20 Å with sequence length of 298 base pairs and The structure was analyzed for identifying the possible binding sites of Cyclin-dependent kinase 2 (CDK2) (Figure 3). 2D structures of the synthetic ligand compounds **(Va– Vq and VIa - VIq)** were generated using ChemSketch and subjected to energy minimization using Argus lab. Docking of these optimized compounds against Cyclin-dependent kinase 2 (1DI8) structure 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 thirty four docked complexes, we got two best docked synthetic compounds 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 5. 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.

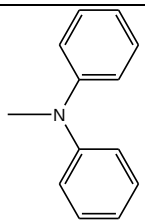
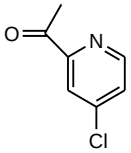
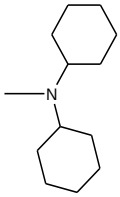
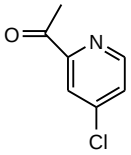
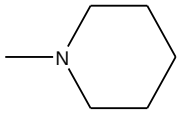
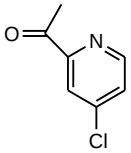
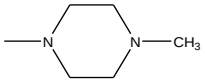
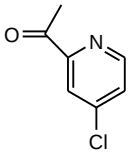
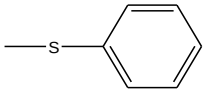
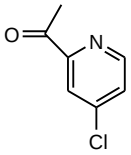
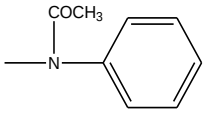
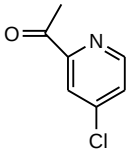
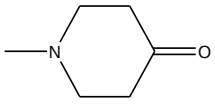
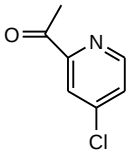
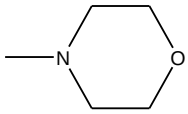
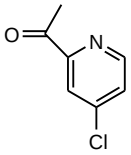
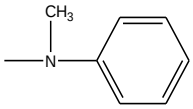
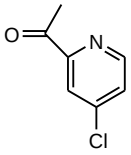
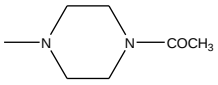
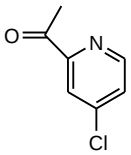


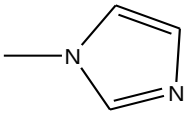
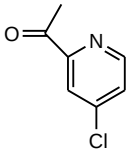
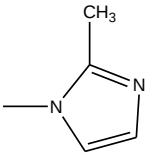
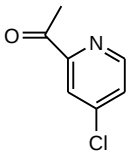
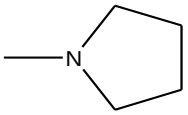
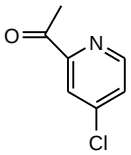
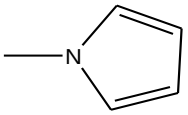
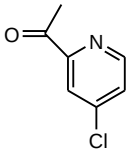
1-substituted-2-((methyl) substituted)-1H-benzo[d] imidazole derivatives

Table 1: Physical data & CDOCKER energy interaction of synthesized compounds (Va – Vq and VIa - VIq) with CDK2 protein (1DI8) at active site pocket 2

Comp.	R	R_1	Mol. Formula	C docker Energy	C docker interaction energy
Va			$C_{17}H_{19}N_5O$	1.18617	34.553
Vb			$C_{19}H_{23}N_5O$	2.52818	31.581
Vc			$C_{20}H_{25}N_5O$	1.25762	31.575
Vd			$C_{27}H_{23}N_5O$	-5.23282	29.386
Ve			$C_{19}H_{35}N_5O$	-44.7338	28.114
Vf			$C_{20}H_{23}N_5O$	-6.33888	28.513
Vg			$C_{20}H_{24}N_6O$	-7.04899	31.949
Vh			$C_{21}H_{18}N_4OS$	1.97034	29.131
Vi			$C_{23}H_{21}N_5O_2$	-0.618252	30.543

Vj			C_{20} $H_{21}N_5O_2$	-1.17912	30.350
Vk			C_{19} $H_{21}N_5O_2$	-3.72093	26.627
Vl			C_{22} $H_{21}N_5O$	1.15408	27.203
Vm			C_{21} $H_{24}N_6O_2$	-3.01997	25.498
Vn			C_{18} $H_{16}N_6O$	1.40656	25.767
Vo			C_{19} $H_{18}N_6O$	5.76038	29.093
Vp			C_{19} $H_{21}N_5O$	-13.9	27.871
Vq			C_{19} $H_{17}N_5O$	2.44898	28.161
VIa			C_{17} $H_{19}N_5O$	-3.29681	30.391
VIb			C_{19} $H_{23}N_5O$	-0.399646	32.364
VIc			C_{20} $H_{25}N_5O$	-5.10712	29.281

VId			$C_{27}H_{23}N_5O$	-4.90609	30.910
VIe			$C_{19}H_{35}N_5O$	-43.288	22.073
VIf			$C_{20}H_{23}N_5O$	-11.0752	30.761
VIg			$C_{20}H_{24}N_6O$	-11.1349	34.281
VIh			$C_{21}H_{18}N_4OS$	-5.81713	29.566
VIi			$C_{23}H_{21}N_5O_2$	-4.26403	32.523
VIj			$C_{20}H_{21}N_5O_2$	-6.986	30.190
VIk			$C_{19}H_{21}N_5O_2$	-9.58124	30.790
VIl			$C_{22}H_{21}N_5O$	-4.98781	27.664
VIm			$C_{21}H_{24}N_6O_2$	-3.69185	35.849

VIn			$C_{18}H_{16}N_6O$	-2.282	30.343
VIo			$C_{19}H_{18}N_6O$	1.7189	30.631
VIp			$C_{19}H_{21}N_5O$	-17.0205	33.347
VIq			$C_{19}H_{17}N_5O$	-2.44068	30.777

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 favored 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 thirty four 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 our research work, 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, VIe ((2-((dicyclohexylamino) methyl)-1H-benzo [d] imidazol-1-yl) (4-chloropyridin-2-yl) methanone) ligand has shown lowest CDOCKER energy & best binding activity with our protein receptor. Thus our study confirms that, out of thirty four compounds, VIe 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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