Identification of potential CDK 8 inhibitor from pyrimidine derivatives via In-Silico approach

Pramod Baburao Patil1,2, Subhash Trimbakrao Kumbhar1

1 School of Pharmaceutical Sciences, Sanjay Ghodawat University, Atigre, Kolhapur, Maharashtra, India
2 Ashokrao Mane College of Pharmacy, Pethadgaon, Kolhapur, Maharashtra, India

Corresponding author: Pramod Baburao Patil pramodbpatiresearch@gmail.com, Orcid Id: https://orcid.org/0000-0003-2308-5994

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Received - 18-01-2023, Revised - 10-08-2023, Accepted - 30-08-2023 (DD-MM-YYYY)

ABSTRACT

Pyrimidinesare six-membered heterocyclic scaffold present naturally in nucleic acid components and are promising leads for the synthesis of medicinally important compounds. Cyclin-dependent kinases (CDKs) with a serine/threonine catalytic core are important druggable targets for cancer therapy and the binding of regulatory subunits controls them. In the present study series of virtually designed pyrimidine derivatives were screened using molecular docking techniques against the cyclin-dependent kinase-8 (CDK8) as a targeted protein. The density functional theory calculation of compounds having good binding affinity was done to estimate the orbital energy. The molecular dynamics simulation of the best-docked compound with the CDK8 was simulated to estimate the effect of mobility on the interactions. The molecular docking provided insights regarding the binding ability of the designed compounds with the targeted CSK8 structure. As a result, the docked compounds exerted good interactions with the CDK8, and the compound PB129 showed the highest negative binding affinity of -12.4 kcal/mol with the formation of two hydrogen bonds. The results of the simulation study indicated that the complex of CDK-8 and PB129 hasa tight binding with constant hydrogen contacts. Moreover, the density functional theory indicated that PB129 has strong orbital energy and this compound will show tight interactions by either donating or accepting the electron with protein structure. Studied compounds showed good results for the docking study by exerting tight binding with the CDK-8 (PDB 6T41). Compound PB129 showed stable confirmation over the simulation run and has good orbital energies. Compound PB129 may act as a lead against the CDK8.

Keywords: Cancer, Cyclin-dependent kinases-8, In-silico approach, Pyrimidine derivatives.

INTRODUCTION

Cancer is the second main cause of mortality in the world and is a significant diagnostic challenge, followed by therapeutic effectiveness [1]. According to Global Cancer Observatory (GLOBOCAN), cancer death and prevalence are predicted to increase to 29.5 million and 16.3 million, respectively, by the year 2040 [2]. Variations in both the incidence rates and death rates of cancers have an impact on the future burden of these diseases, in addition to increases in cancer deaths caused on by demographic changes [3]. The occurrence of obesity, alcohol addiction, cigarette smoking, the influence of hereditary factors, physical inactivity, and poor nutrition are all risk factors for cancer [4]. India comes under the nations with a National Cancer Control Program, which is supported by the World Health Organization (WHO). WHO primarily contributes to the fight against the use of tobacco [5]. The most prevalent cancers produced by persistent infections are those of the uterine cervix, stomach, and liver caused by HPV, Helicobacter pylori, HBV, and Hepatitis C (HCV) virus, respectively [6]. The selective therapies are based on a better understanding of the biology and molecular genetics in the tumor progression used for the prospective treatments, in addition to common cancer treatments like surgery, radiation therapy, chemotherapy,
combination therapy, and laser therapy [7]. The primary focus of research is on cancer therapy strategies that target the different aspects of the disease [8]. The concept that targeted therapies specifically inhibit the growth of cancer cells or kill them gives them an advantage over both chemotherapy and radiation treatments [9]. The discovery of new cancer treatments and their development is considered to be an extremely time and money-consuming process [10].

The cell cycle cyclin-dependent kinases (CDKs) are essential for regulating both cellular transcription and the change between cell cycle stages [11]. Based on respective homologous sequences, 21 CDKs, and 5 CDK-like genes have been found in the human genome [12]. Some members of the CDK family have not played a direct role in controlling the cell cycle and those are CDK7, CDK8, and CDK9, which are involved in the control of transcription [13]. Since it was established that CDK-8 plays crucial roles in oncogenesis, it has received considerable interest recently [14]. Numerous substrates involved in transcription, DNA repair, and metabolic activities were revealed to be phosphorylated by CDK-8 [15]. A crucial aspect of the mediator complex is CDK-8 and specifically, CDK-8 is closely linked to the transcription of genes involved in the oncogenesis of several cancers, including colorectal, breast, prostate, and hematological malignancies [16]. Utilizing CDK8 inhibitors may have two main effects: first, directly targeting cancer cells; and second, indirectly stimulating natural killer (NK) cells to more effectively lyse cancer cells [17]. CDK8 was discovered to be an oncogene that frequently amplifies or is over expressed in colorectal cancer (CRC) and plays a significant role in colorectal carcinogenesis [18]. CDK8 binds to cyclin C (CCNC), which regulates several signaling pathways for the formation and progression of cancer and it plays a role in the regulation of tumor stress, energy supply, and drug resistance mechanisms [19]. Epithelial-to-mesenchymal transition (EMT) is essential for the invasion and metastasis of breast cancer cells, which is promoted by CDK-8 [20].

MATERIALS AND METHODS
Computational methods
Ligand preparation

ACD/ChemSketch software was used to draw the chemical structures and SMILES of designed compounds and saved them in mol2 file format as shown in Table 1. Hydrogen atoms were added to designed chemical structures via BIOVIA Discovery Studio to correct the ionization and tautomeric state [21]. Further, the protonated chemical structures were energy minimized using the Open Babel plugin of the PyRx 0.8 program [22]. The MMFF94 force field with the steepest descent algorithm was applied to minimize the energy of newly designed compounds. Energy-minimized compounds were then converted to pdbqt file format for docking study.

Protein preparation

The previously reported 3D crystal structure of CDK8 (PDB 6T41) having a resolution of 2.45 Å was acquired from the online RCSB Protein data bank (Available at https://www.rcsb.org/) [23,24]. The downloaded protein was refined for docking study by removing all the HETATM. Further, polar hydrogen atoms were added to protonate and correct ionization as well as tautomeric states of amino acids of the refined protein structures. The protein refinement step was performed using BIOVIA Discovery Studio [21].

Molecular docking

A molecular docking study of a virtually designed ligand library with CDK8 (PDB 6T41) was achieved using various modules of the PyRx 0.8 program [25,26]. Initially, energy-minimized ligands and structure of CDK8 were imported in PyRx and selected in the AutoDOck Vina wizard module of PyRx 0.8 [27,28]. The blind docking protocol was used to explore the entire protein surface for binding ability with the docked compounds. The exhaustiveness was set to default at 8 [29]. The best-docked pose with the highest negative binding affinity was saved and binding interactions were analyzed with the help of BIOVIA Discovery Studio [21].

Density functional theory assessment

The DFT study of the designed compounds was done with the help of the Orca 4.2.1 package [30]. The input files for the orca were generated via the orca-enhanced version of Avogadro and the same tool was used to visualize the frontier molecular orbitals (FMO) [31]. The B3LYP functional was implemented to optimize the compound structure [32-34]. The def2-SVP basis set was used to perform the final DFT calculation [35]. The FMO analysis and global chemical reactivity descriptors of synthesized compounds were estimated according to the previously reported equations of Koopmans’ theory [36,37].

Molecular dynamics simulation

Molecular dynamics (MD) simulation study was carried out using GROMACS with the GROMOS96 43a1 force field [38-40]. The PRODRG2 server was utilized to generate the ligand topologies of the entire protein-ligand complex was done using a simple point charge (SPC) water model with a triclinic box [42]. The energy minimization (EM) of the complex systems was achieved with 10,000 steps of the steepest descent algorithm. The present MD simulation study was carried out in the presence of 0.15 M NaCl [28,43]. Equilibration of the simulated complex systems was performed with canonical (NVT) and isothermal–isobaric (NPT) ensembles after the completion of each step of EM [44-46]. The temperature was kept constant at 310K using the Nosé–Hoover thermostat approach while pressure on the system was kept constant at 1.0 bar using the Parrinello–Rahmanbaro stat approach to control the simulated complex systems [47-49]. The prepared complex systems were simulated for 50 ns and the output MD trajectory was used further for statistical analysis of deviation, fluctuation, and formation of the number of hydrogen contacts [50].
Table 1: List of Pyrimidine derivatives studied

<table>
<thead>
<tr>
<th>Code</th>
<th>SMILES</th>
</tr>
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<tbody>
<tr>
<td>PB1</td>
<td>Clc1nc2ccccc2nc1cc(n(n1)N1N=C(C)CC1=O)c1ccccc1</td>
</tr>
<tr>
<td>PB2</td>
<td>Nc1ccccc1cc(n(n1)N1N=C(C)CC1=O)c1ccccc2nc1Cl</td>
</tr>
<tr>
<td>PB3</td>
<td>Nc1ccccc1cc(n(n1)N1N=C(C)CC1=O)c1ccccc2nc1Cl</td>
</tr>
<tr>
<td>PB4</td>
<td>Nc1ccccc1cc(n(n1)N1N=C(C)CC1=O)c1ccccc2nc1Cl</td>
</tr>
<tr>
<td>PB5</td>
<td>Bre1cc(c)c1cc(n(n1)N1N=C(C)CC1=O)c1ccccc2nc1Cl</td>
</tr>
<tr>
<td>PB6</td>
<td>Oc1cccc(c)c1cc(n(n1)N1N=C(C)CC1=O)c1ccccc2nc1Cl</td>
</tr>
<tr>
<td>PB7</td>
<td>Oc1cccc(c)c1cc(n(n1)N1N=C(C)CC1=O)c1ccccc2nc1Cl</td>
</tr>
<tr>
<td>PB8</td>
<td>Oc1cccc(c)c1cc(n(n1)N1N=C(C)CC1=O)c1ccccc2nc1Cl</td>
</tr>
</tbody>
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**DOI:** 10.55522/jmpas.V12I5.4784

**ISSN NO.** 2320–7418

**Journal of medical pharmaceutical and allied sciences, Volume 12 – Issue 5, 4784, September - October 2023, Pages – 6038 – 6048**

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RESULTS AND DISCUSSION

Molecular docking

The anticancer potential of designed compounds was estimated with the help of a molecular docking study. Docking study was accomplished using the Auto Dock Vina module of PyRx 0.8 and the designed compounds were docked on the Cyclin-Dependent Kinase 8 (CDK8) (PDB 6T41). A total of 232 pyrimidine derivatives were used in this study. Canonical SMILES of designed compounds are represented in Supplementary Table S1. The results of the docking study represented that all the docked compounds have a binding affinity of more than -8.7 kcal/mol with strong binding interactions with targeted CDK8 (PDB 6T41) structures. The binding affinity and interactions of all the docked compounds with CDK8 were represented in Supplementary Table S2. The binding affinity of docked compounds ranged between -8.7 to -12.4 kcal/mol. Compound PB71 showed a binding affinity of -12.2 kcal/mol and the interactions were seen between LEU158, VAL35, HIS106,ALA155, and LEU359 with formation Pi-Pi T shaped, Alkyl, and Pi-Alkyl type of interactions. The 2D and 3D visualization of binding interactions formed between compound PB71 and CDK8 (PDB 6T41) are represented in Figure 1a-1b. Compound PB71 formed a single conventional hydrogen bond with...
the TYR32 residue. Whereas the compound PB129 showed the highest negative binding affinity of -12.4 kcal/mol compared to the other docked compounds, the compound PB129 showed good binding affinity. The binding interactions LEU158, VAL35, LEU359, ALA155, and HIS106 showed hydrophobic interactions while TYR32 and ARG356 showed hydrogen bonding with the docked compound PB129. Oxygen and chlorine functional groups present in the compound PB129 are responsible for the formation of hydrogen bonds with the targeted protein. Hydrogen bonds play a crucial role in the stability of the docked protein-ligand complex, though the docking study was performed with the static condition and the dynamic behavior of the complex may affect the formation of hydrogen bonds. The 2D and 3D binding interactions between the compound PB129 and CDK8 are represented in Fig. 1c-d

**Density functional theory**

The DFT calculations were performed to study the molecular geometry and electron distribution in the designed compounds. According to the frontier molecular orbital theory, the Lowest Unoccupied Molecular Orbitals (LUMOs) and Highest Occupied Molecular Orbitals (HOMOs) signify active sites and chemical reactivity of compounds. FMO also has a great influence on the biological potential of compounds as the activity depends on the transfer of electrons between protein and ligand complex. The negative chemical potential of the compounds dictates the non-spontaneous decomposition. The HOMO-LUMO energy gap gives information on the electrical transport properties of molecules. The LUMO energy is parallel to the electron affinity (EA) while HOMO energies correspond to the ionization potential (IP) of the compounds. The chemical reactivity of the compounds depends on the gap between HOMO-LUMO energies.

The high gap represents low chemical reactivity while the low gap indicates higher chemical reactivity. In Fig. 2, the red colour indicates the positive electron density of the compounds while the negative electron density was represented with blue colour. Compounds PB71, PB86, PB129, PB223, and PB 231 showed a good binding affinity with the targeted CDK8, and the DFT study of these compounds indicated the possibility of higher reactivity and interactions with the targeted proteins. HOMOs of compound PB129 formed tight interactions with the targeted protein structure as shown in Fig 1. The global chemical reactivity descriptors estimated as per the equations of Koopman's theorem for the compounds are represented in Table 2.

*Figure 1* a) 3D and b) 2D interactions of compounds PB71 and c) 3D and d) 2D interactions of compounds PB129 with CDK8 (PDB 6T41).
Molecular dynamics simulation

Molecular docking study of the designed compounds provided information regarding the binding orientations with the CDK8. Compound PB129 showed tight binding with the targeted protein and showed two hydrogen bonds. However, the docked complex needs to undergo the MD simulation study to estimate the stability of the formed interactions between compound PB129 and CDK8 (PDB 6T41). Hence, the molecular dynamics (MD) simulation of the docked protein-ligand complex was done in the mobile phase with an artificial physiological atmosphere. MD simulation of protein-ligand complex systems was done for 50ns. The statistical MD trajectory was done with the parameters such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), the radius of gyration (Rg), and hydrogen bonds (HBs). RMSD was studied to determine the deviations showed by complex during the simulation period of 50ns. Overall, Complex RMSD ranged between 0.2nm to 0.6nm as shown in Figure 3a. Complex system showed scattering in the final RMSD plot throughout the simulation run. RMSD plot represents the maximum deviations in the complex system between 15ns to 35ns. RMSF of the entire simulated complex showed major fluctuations in the initial amino acid present in CDK8 (PDB 6T41). RMSF of the simulated complex ranged between 0.1nm to 1nm as shown in Figure 3b. Correlation between RMSF and RMSD helped to determine that fluctuated amino acid residues of the targeted protein influenced the deviation in the RMSD. LYS0, ASP1, ASP2, MET1, TYR3, ASP4, PHE5, LYS6, VAL7, and LYS8 are the residues that showed fluctuations during the simulation study. None of the above-mentioned residues showed direct contact with the PB129 in both dockings as well as MD study. Most of the regions of the RMSD and RMSF plots evolved in correlation to each other indicating the stability of the protein-ligand complex.
Figure 3: a) RMSD and b) RMSF of the simulated MD trajectory of 50ns.

![Graph of RMSD and RMSF vs. Time (ns)](image)

Figure 4: a) RoG and b) number of HBs estimated using the simulated MD trajectory of 50ns

![Graph of RoG and HBs vs. Time (ns) and Residues](image)
Moreover, the RoG was calculated to study the compactness of the protein-ligand complex system during MD simulation. RoG study helps to determine the folding and unfolding of the complex during the simulation study. A higher RoG value reflects less compactness and unfolding of the complex. The RoG values of the complex system showed consistency with minimum fluctuation in the plot (Fig 4a). RoG values stabilized after 10ns indicating the folded state of the complex. Finally, the number of hydrogen bonds (HB) formed during the MD simulation was plot trends shown in Fig 4b. The complex system showed one consistent hydrogen bond throughout the simulation run and between 20ns to 30ns two hydrogen contacts were observed between the simulated protein-ligand complexes. Hydrogen bonds between complexes are important to stabilize the protein-ligand complex. Based on the overall statistical analysis of the MD trajectory for compound PB129 with the CDK8 (PDB 6T41), it is confirmed that the complex system has stable confirmation throughout the simulation with minimum deviation in the RMSD plot and fluctuations in the RMSF plot.

CONCLUSION

The molecular docking study under static conditions of designed pyrimidine compounds against Cyclin-Dependent Kinase 8 (CDK8) (PDB 6T41) was done to investigate the anticancer potential and ideal leads against CDK8. The results of the docking study indicated that designed compounds have a good binding affinity (≥-8.7 kcal/mol) and the binding interactions were also found to be appropriate for the formation of hydrogen bonds. Compound PB129 showed good binding affinity (-12.4 kcal/mol) as compared to the other docked compounds. Further dynamics study of the PB129 will give more insights regarding the behavior of the docked protein-ligand complex. The molecular dynamics study indicated conformational stability with the minimum deviation in the interactions and fluctuations in the protein structure. DFT calculations revealed that all five compounds have good orbital energy and a HOMO-LUMO energy gap. Transfer of electrons between protein-ligand will be present while biological screening. Though the in-silico investigation provided important information regarding the designed compounds, the final confirmation of the anticancer activity needs to be screened with in-vitro or in-vivo models.

ACKNOWLEDGEMENT

The author would like to express her sincere gratitude to Hon. Vice chancellor Sanjay Ghodawat University, Atigre for his constant support and guidance The author would also like to extend his thanks Dr. S. V. Patil and Ashokrao Mane College of Pharmacy, Peth-Vadgaon for their providing laboratory equipment. The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES


