RESEARCH ARTICLE

In-silico Exploration for Novel CDK8 Inhibitors: A Virtual Study by Pharmacophore Screening

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ABSTRACT

The primary goal of this research is to identify potent and safe CDK8 inhibitors from the ChEMBL (kinases) database. The study employs a multi-faceted computational approach to achieve its objectives. Structure-based pharmacophore modeling is used for the initial screening of potential CDK8 inhibitors. Subsequent molecular docking studies are conducted to assess the binding affinities of the screened molecules. Finally, toxicity profiling is carried out to ensure the safety of the potential inhibitors. A total of 150 molecules were identified that passed the initial pharmacophore screening. Among these, molecule CHEMBL404766 was found to have the highest binding affinity in molecular docking studies. Furthermore CHEMBL404766 was found to be the safest candidate, exhibiting a negligible toxic dose in toxicity profiling. The study suggests the potential use of computational approaches for the identification and design of potent and safe CDK8 inhibitors. These findings have significant implications for the development of targeted therapies in diseases where CDK8 plays a crucial role.

Keywords: Virtual screening, ChEMBL database, CDK8 inhibitor, Structure-based pharmacophore modeling, Molecular docking, Toxicity profiling, Computational approaches, Targeted therapies.

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INTRODUCTION

This study's broader research area focused on identifying and developing targeted inhibitors for cancer treatment. Given the prevalence of cancer as a leading cause of death globally, there was an urgent need for effective therapeutic strategies. The research aimed to tackle the challenge of identifying potent and safe inhibitors for cyclin-dependent kinase 8 (CDK8), a protein kinase implicated in various forms of cancer.[1,2]

Cancer remains a global health crisis, affecting millions of people each year. This underscored the critical need for effective treatments, particularly targeted therapies that could improve patient outcomes.[3]

The specific objective of this study was to identify a potent and safe CDK8 inhibitor that could be used in targeted cancer therapies. The study focused on the virtual screening of potential CDK8 inhibitors sourced from the ChEMBL (kinases) database. CDK8 served as the potential target for the treatment of various types of cancer.

To achieve the research objective, the study employed a multi-faceted computational approach. Initially, a dataset of potential inhibitors was extracted from the ChEMBL (kinases) database. Structure-based pharmacophore modeling was used for the initial screening of these molecules. Subsequent molecular docking studies were conducted to evaluate the

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binding affinities of the screened molecules. Finally, toxicity profiling was carried out to assess the safety of the potential inhibitors.

MATERIALS AND METHODS

All phases done in simulated screening are illustrated in Figure 1 in short.

Structure Preparation

The protein manufacturing process relied on the ligand-bound 3D crystal structure of CDK8/cycc. The Protein Data Bank ID 5BNJ was retrieved from the RCsB website (https://www.rcsb.org). The framework was made using the Biovia Discovery Studio Visualizer. Extra atoms of ligands, water molecules, and heteroatoms were removed at this stage to tidy up the structure. Polar hydrogen atoms were added to the structure to make up for the lack of remaining heavy atoms, and charges were distributed evenly across the whole thing.[4]

Assessment of Protein Structure Quality

Superiority valuation of protein structure organizes a critical component in molecular modeling and computational analyses. This was accomplished through a multi-faceted evaluation approach. Initially, a Ramachandran plot was generated utilizing the PROCHECK server[5], as delineated in Figure 2a. This graphical representation facilitated the quantification of dihedral angles among amino acid residues, thereby enabling the determination of the proportion of residues situated in energetically favored regions.

Subsequent to this, a series of additional metrics were invoked to further scrutinize the structural integrity of the protein. An ERRAT score[6] was computed, as exemplified in Figure 2b, wherein a higher numerical value is indicative of superior structural quality. Concurrently, the Verify3D score was calculated to assess the congruence between the three-dimensional architecture of the protein and its corresponding amino acid sequence, as illustrated in Figure 2c.

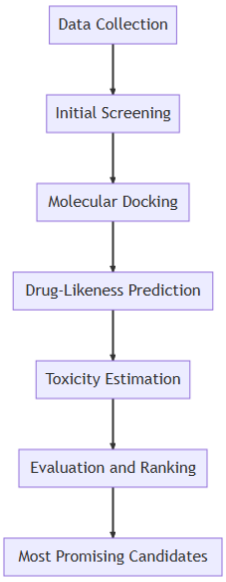

Figure 1: Process of virtual screening

Lastly, the ProSAweb Z-Score[7] was ascertained, as presented in Figure 2d. This metric incorporated variables such as structural length and overall model fidelity to provide a comprehensive assessment of protein quality

Ligand Structure Preparation

The data for this research was sourced from the ChEMBL database (https://www.ebi.ac.uk/chembl/) deals broad group of bioactive molecules with drug-like properties. This database served as an ideal foundation for the identification of potential CDK8 inhibitors.[8]

All of the chosen ligand molecules' 3D structures were created for docking studies, and this included the reference medication used for comparison. This was set up with the help of the Biovia Discovery Studio Visualizer programme.[9] The ligands were fine-tuned so that they would be completely ready for the following docking processes.

Screening Approach

3D Pharmacophore Search

The Pharmit server was used for the 3D pharmacophore search (https://pharmit.csb.pitt.edu/).[10] Configuration of CDK8/cycc in relationship with ligand served as the basis for the pharmacophore model. This complex's Protein Data Bank (PDB) ID was 5BNJ. The 3D pharmacophore search was conducted using the default Pharmit parameters to ensure consistency and dependability in the modeling procedure.

Virtual Screening

The Comprehensive databases available with Pharmit server, which include a CHEMBEL32, MCLUE, and ZINC database molecules, were subjected to virtual screening. The created pharmacophore model served as a filter during the screening. An additional filter was used based on the root mean square deviation (RMSD) in totaling to pharmacophore-based screening. For hit screening, only molecules with an RMSD of less than or equal to 2 were taken into account.[11]

Toxicity Estimation

Virtual toxicity estimation was carried out using ProTox-II.[12]

RESULTS AND DISCUSSION

Assessment of Protein Structure Quality

The Ramachandran plot generated using the PROCHECK server demonstrated that 92.0% of residues in PDB are located in the favored regions. This indicates that most amino acid residues have conformations within energetically favorable regions of the plot, suggesting good structural quality.

ERRAT Score (Figure 2b)

ERRAT Score was found to be 93.997%, indicating outstanding structural quality and confirming the reliability of the protein structure.

ProSAwebZScore (Figure 2c)

The ProSAweb server determined z-scores protein was found to be -7.12, indicating that the protein structure is within the expected range for a high quality structure.

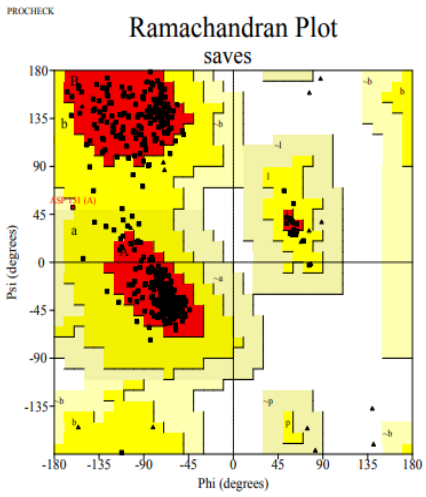

Figure 2a: Ramachandran plot

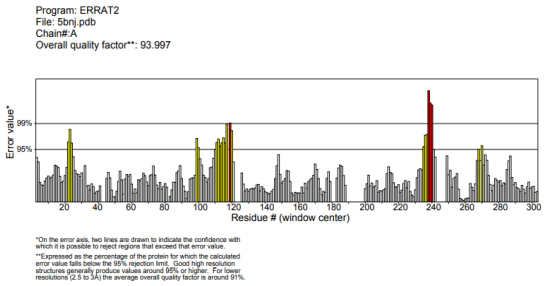

Figure 2 b: ERRAT Score

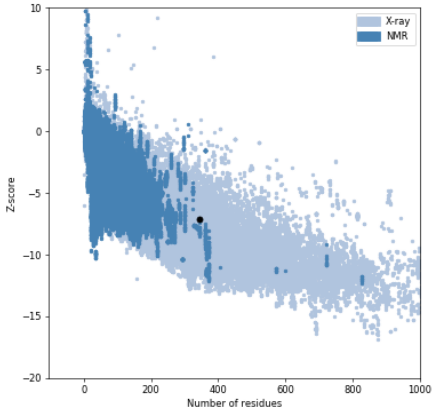

Figure 2 c: ProSAwebZScore

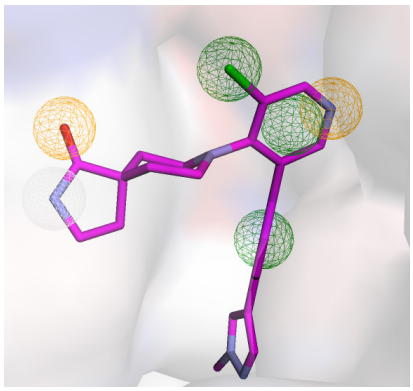

Figure 2 d: Pharmacophore model from Pharmit server

Table 1: Molecules successfully passed through the pharmacophore filter and met the RMSD criteria

Sr. No.	*Database ID*
1	CHEMBL92335
2	CHEMBL398710
3	CHEMBL404766
4	CHEMBL1719920
5	CHEMBL2237537
6	MCULE-1687641726
7	MCULE-2018731818
8	MCULE-4865663128
9	ZINC000023126397
10	ZINC000038486839

3D Pharmacophore Search Results

A dataset of selected compounds was retrieved post-screening. A total of 200 molecules from the CHEMBEL32, MCLUE and ZINC library successfully passed through the pharmacophore filter and 10 met the additional RMSD criteria. Details of all 10 molecules are given in Table 1 and structures of all are given in Figure 3.

The initial screening was conducted using structure-based pharmacophore modeling. This computational technique effectively filtered out molecules that did not meet the structural prerequisites for efficient CDK8 inhibition.

Two Hydrogen bond donor (Purple), 1 hydrogen bond acceptors (White) & 3 hydrophobic (Green) pharmacophore features given by the Pharmit server.

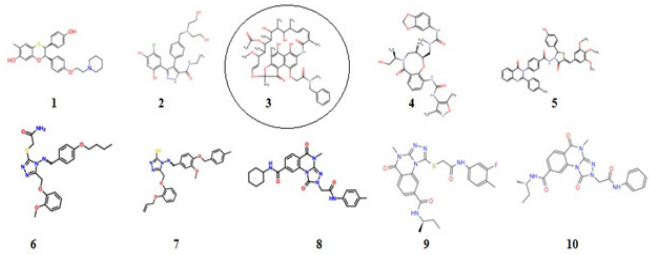

Figure 3: Structures of molecules successfully passed through the pharmacophore filter and met the RMSD criteria. Safest and most effective one is circled in the above diagram.

Table 2: Acute toxicity testing of all molecules

Sr. No.	*Predicted LD_{50} (mg/kg)*	*Predicted toxicity class*
1	374	4
2	1000	4
3	2430	5
4	1242	4
5	1000	4
6	1000	4
7	1000	4
8	500	4
9	1000	4
10	500	4

Toxicity Estimation

Virtual toxicity estimation was carried out using ProTox-II. The result of acute toxicity testing is provided in Table 2. This software offered insights into the potentially toxic effects of the top-performing molecules, aiding in the selection of the safest candidates for further investigation.

Virtual toxicity estimation revealed that CHEMBL404766 exhibited the most negligible toxic dose, with an estimated LD_{50} of 2430 mg/kg, making it the safest candidate among the top performers.

The identification of CHEMBL404766 as a potent and safe CDK8 inhibitor has significant implications. Not only does it serve as a strong candidate for further experimental validation, but it also demonstrates the efficacy of computational approaches in drug discovery.

This research adds to our understanding of cancer by pinpointing a potential novel inhibitor for CDK8, a pivotal protein kinase in many different cancers.

The findings suggest that computational methodologies can expedite the drug discovery process, offering a faster and more cost-effective alternative to traditional methods.

While the study provides promising results, it is not without limitations. The virtual nature of the screening and toxicity estimation calls for experimental validation to confirm the findings. Future research should focus on IVIVC to validate the efficacy & safety of CHEMBL404766 as a CDK8 inhibitor.

CONCLUSION

This research's most significant findings include identifying 200 molecules that passed the initial pharmacophore screening for potential CDK8 inhibition. Among these, molecule CHEMBL404766 emerged as the most promising candidate, exhibiting both high binding affinity and low toxicity.

These findings have broader implications for the field of targeted cancer therapies. The discovery of a novel CDK8 inhibitor adds to the existing body of knowledge and paves the way for the creation of more precisely tailored medicines.

This study's findings support the feasibility of using computational methods, such as structure-based pharmacophore modeling and molecular docking, to efficiently and safely discover effective inhibitors. These computational approaches could significantly expedite the drug discovery process, offering a more efficient pathway to new treatments for diseases where CDK8 plays a crucial role.

Based on these findings, future research should focus on the experimental validation of CHEMBL404766 as a CDK8 inhibitor. IVIVC are needed to check its efficacy and safety. Additionally, further computational studies could explore the optimization of CHEMBL404766 to improve its pharmacological properties.

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