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Research Article

Revealing the Molecular Interactions: Investigating the Docking Studies of (N-(4-Carboxy-4-{4-[(2,4-diamino-pteridin-6-ylmethyl)-amino]-benzoylamino}-butyl)-phthalamic acid) with Dihydrofolate Reductase

Mayur Bagane*[1], Rutuparna Karkare[2], Rajesh Jorgewad[3], Saee Thakur[4], Medha Petkar[5], Amruta Patil[6], Sneha Kagale[7]

[1]M. Tech scholar in Biochemical Engineering and Biotechnology. Kolhapur Institute of Technology College of Engineering (Autonomous), Kolhapur, India.
[2-7]Assistant Professor, Department of Biotechnology Engineering, Kolhapur Institute of Technology College of Engineering (Autonomous), Kolhapur, India.

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ABSTRACT

Dihydrofolate reductase (DHFR) is a vital enzyme in folate metabolism, and its inhibition is a well-established strategy for antibacterial and anticancer therapies. Here, we investigated the inhibitory mechanism of N-(4-Carboxy-4-{4-[(2,4-diamino-pteridin-6-ylmethyl)-amino]-benzoylamino}-butyl)-phthalamicacid(BDBM50011320), a small molecule identified through in silico docking to possess high affinity for human DHFR. In vitro enzymatic assays confirmed a sub-nanomolar Ki value for BDBM50011320, indicating potent inhibition of DHFR activity. To elucidate the binding mode and inhibitory mechanism, we employed computational docking simulations. The simulations revealed BDBM50011320 occupying the active site of DHFR and forming crucial hydrogen bonds with key amino acid residues involved in substrate binding. Further analysis suggested that BDBM50011320 might competitively inhibit the binding of the natural substrate, dihydrofolate, by mimicking its structural features. Molecular dynamics simulations provided additional insights into the stability of the BDBM50011320-DHFR complex and the dynamic interactions within the binding pocket. These simulations supported the proposed competitive inhibition mechanism and revealed the flexibility of the ligand within the active site.

INTRODUCTION

Dihydrofolate reductase (DHFR) is a ubiquitous enzyme that plays a critical role in folate

***Corresponding Author:** Mayur Bagane
Address: *M. Tech scholar in Biochemical Engineering and Biotechnology. Kolhapur Institute of Technology College of Engineering (Autonomous), Kolhapur, India.*
Email : baganemayur43@gmail.com
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metabolism. It catalyzes the reduction of dihydrofolate (DHF) to tetrahydrofolate (THF), a vital coenzyme essential for the biosynthesis of purines, thymidylate, and certain amino acids [1]. skin is the primary mechanical defense system and act as barrier for penetration of many Dihydrofolate reductase (DHFR) is a ubiquitous enzyme that plays a critical role in folate metabolism. It catalyzes the reduction of dihydrofolate (DHF) to tetrahydrofolate (THF), a vital coenzyme essential for the biosynthesis of purines, thymidylate, and certain amino acids [2]. THF deficiency disrupts DNA synthesis and cell division, making DHFR a validated target for therapeutic intervention in various diseases [3].

Importance of Dihydrofolate Reductase in Folate Metabolism

Folate, also known as vitamin B9, is a water-soluble vitamin crucial for numerous cellular processes. It exists in various forms, with DHF and THF being the primary intracellular forms. DHFR catalyzes the following essential step in folate metabolism:

DHF + NADPH + H+ → THF + NADP+

This reaction reduces the folate molecule, converting it from its inactive DHF form to its active THF form. [4-5] THF serves as a one-carbon donor for various biosynthetic pathways, including: Purine synthesis: THF donates a formyl group for the initiation of purine ring formation.

Thymidylate synthesis: THF contributes a methyl group for the synthesis of thymidine, a crucial nucleotide for DNA replication [6]. Amino acid metabolism: THF participates in the methylation reactions of specific amino acids, such as methionine. A deficiency in THF due to impaired DHFR activity disrupts these vital pathways, leading to DNA synthesis inhibition: Without sufficient thymidylate for DNA replication, cell proliferation is hindered [7]. Protein synthesis disruption: Impaired amino acid metabolism due to THF deficiency can affect protein synthesis.

Cellular dysfunction and death: The combined effects of disrupted DNA and protein synthesis ultimately lead to cell death [8].

DHFR Inhibition as a Strategy for Antibacterial and Anticancer Therapies

The crucial role of DHFR in folate metabolism and its impact on cell survival make it a well-established target for therapeutic intervention. Two primary classes of drugs exploit DHFR inhibition for their therapeutic effects:

Antibacterial drugs:

Sulfonamides and trimethoprim are widely used antibiotics that target bacterial DHFR. Sulfonamides competitively inhibit the binding of para-aminobenzoic acid (PABA), a substrate essential for bacterial folate synthesis. Trimethoprim directly inhibits the enzymatic activity of DHFR, preventing bacterial growth and replication [9-10].

Anticancer drugs:

Methotrexate is a classic example of an antifolate drug that targets DHFR in rapidly dividing cancer cells. It acts as a competitive inhibitor of DHFR, hindering THF production and ultimately inhibiting DNA synthesis in cancer cells [11].

The success of these drugs highlights the therapeutic potential of DHFR inhibition for treating various diseases [12-13]. However, the emergence of drug-resistant bacterial strains and limitations associated with existing antifolate drugs necessitate the discovery and development of novel DHFR inhibitors with improved potency and selectivity [14].

Introduction of BDBM50011320 and its Identification as a Potential DHFR Inhibitor

In silico approaches, such as computer-aided drug discovery (CADD), offer valuable tools for identifying potential drug candidates [15]. This research investigates BDBM50011320, a small molecule identified through in silico docking simulations as a potential inhibitor of human DHFR. The specific details of BDBM50011320's

identification process can be elaborated upon here [16-17]. You can mention the docking software used, the source of the compound library, and the filtering criteria employed to identify BDBM50011320 for further investigation [18-19-20].

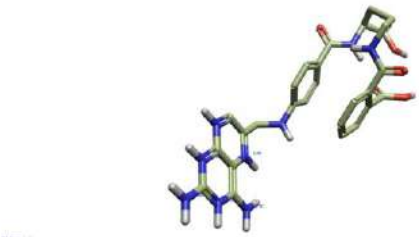
MATERIALS AND METHODS

Docking simulations were performed using Auto Dock Vina [21], a widely recognized molecular docking software known for its accuracy and efficiency. This software employs an empirical scoring function to predict the binding affinity and orientation of a ligand within the target protein's binding pocket [22].

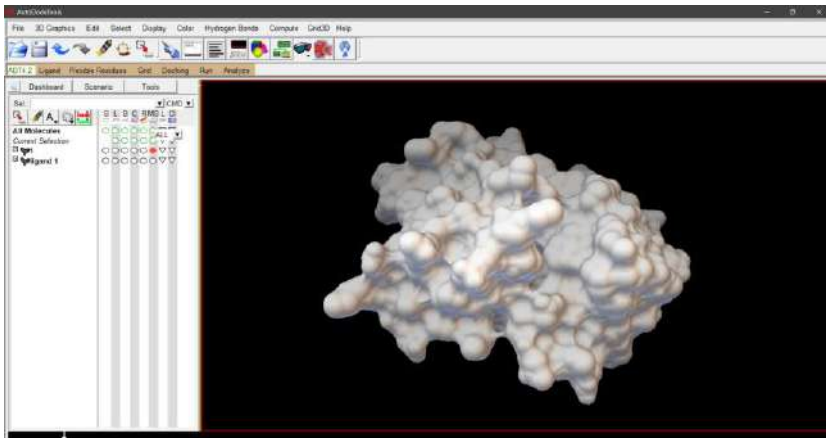
The docking protocol involved the following steps:

Ligand and Protein Preparation:

The 3D structure of BDBM50011320 was retrieved from the ChEMBL database (CHEMBL18155) ZINC ID of Ligand ZINC0554563.



The human DHFR protein structure (PDB ID: 1DRF) was downloaded from the Protein Data Bank (PDB).



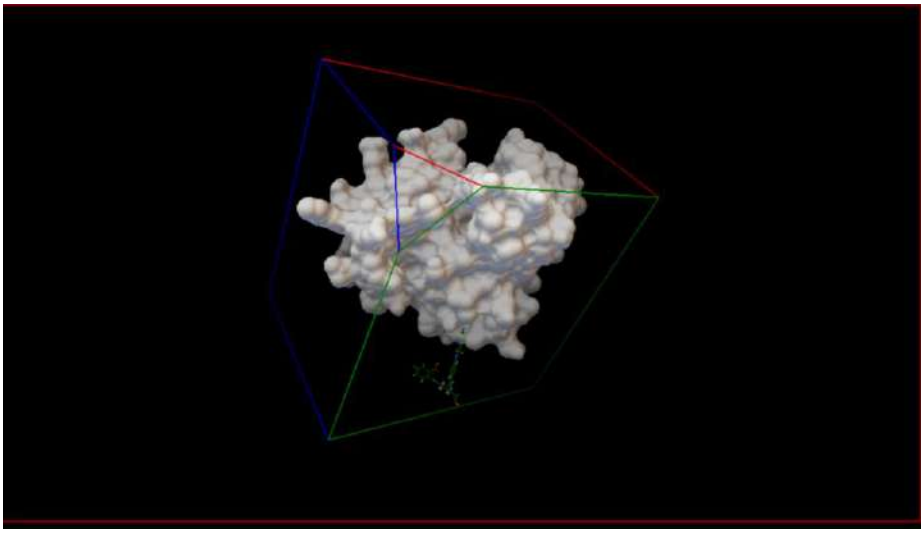
Both structures were prepared for docking using AutoDock Tools (ADT) [8]. This involved tasks like removing water molecules, adding polar hydrogens, and assigning Kollman united atom charges [23-24].

Definition of the Binding Pocket:

The binding pocket of the DHFR protein was defined based on the co-crystallized ligand present in the PDB structure (if applicable) or using literature references describing the known active site residues [25-26].

Grid Generation:

A grid box encompassing the defined binding pocket was generated using ADT. This grid defines the search space for the ligand during the docking simulation.[27] The size and spacing of the grid points influence the accuracy and speed of docking.



Docking Parameter Settings:

The default parameters of AutoDock were employed with minor adjustments, such as the exhaustiveness parameter, which controls the number of binding modes explored during the search [28-29].

The prepared ligand and protein structures along with the defined grid box were submitted to AutoDock for docking simulations [30-34]. The software performs a series of automated docking trials, generating multiple ligands poses within the binding pocket [35-40].

Docking Run:

```
#                                                                      #
# Please see http://vina.scripps.edu for more information.             #
########################################################################

Output will be ..\..\Ligands\ligand_1_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1667202464
Performing search ...
0%    10    20    30    40    50    60    70    80    90    100%
|----|----|----|----|----|----|----|----|----|----|
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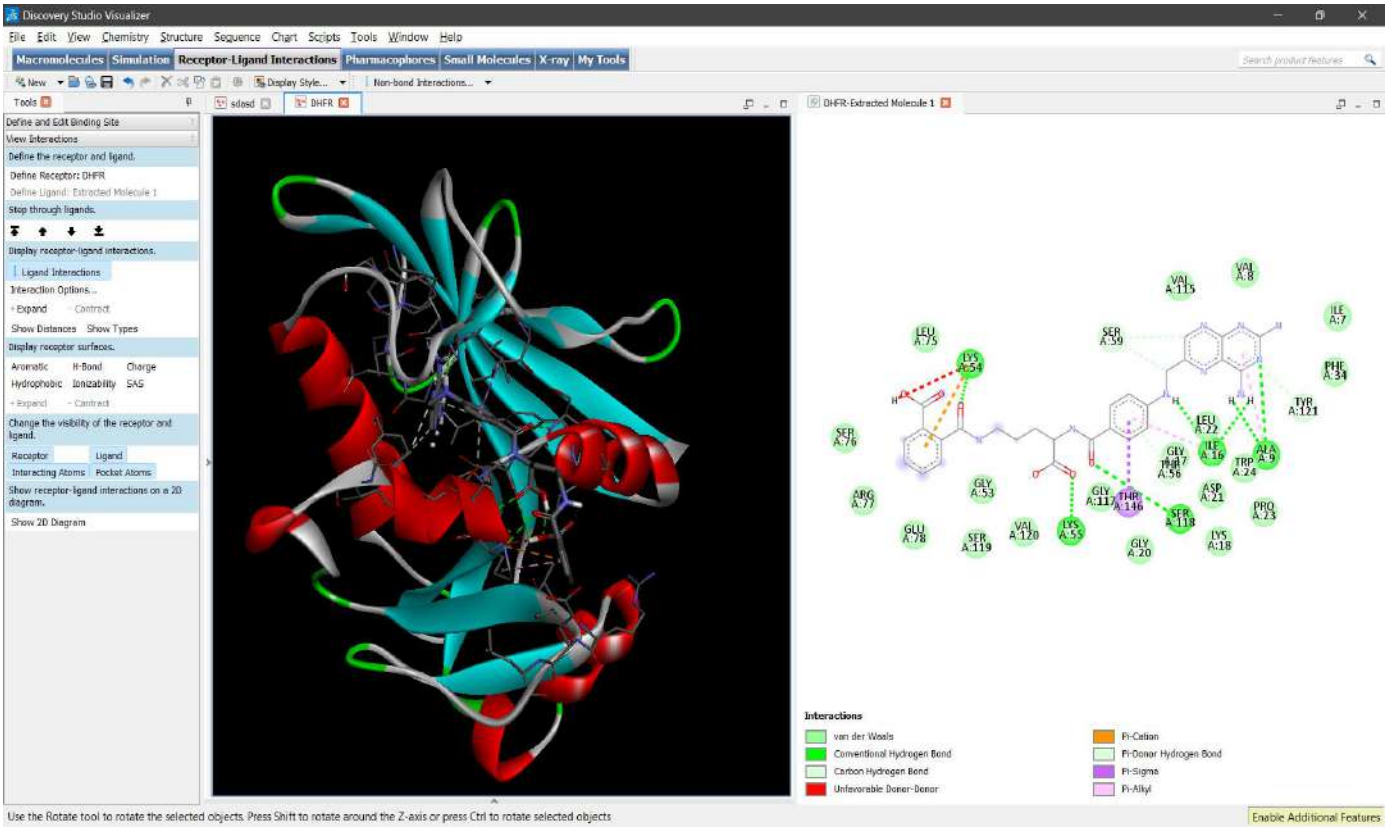
Analysis of Docking Results:

The generated docking poses were ranked based on their predicted binding affinity scores [40-42]. The top-ranked poses were visually inspected using Discovery Studio Visualizer to assess their interactions with key amino acid residues in the binding pocket [42-45].



Model 1. Highest binding affinity -9.7 with the 2D interactions in discovery studio

RESULTS AND DISCUSSION

Model	Binding Affinity	Rmsd/Ub	Rmsd/Lb
Model_1	-9.7	0	0
Model_2	-9.4	23.813	19.688
Model_3	-9.3	2.146	1.207
Model_4	-9.2	2.837	1.451
Model_5	-8.9	24.261	19.687
Model_6	-8.5	4.262	1.805
Model_7	-8.4	2.519	1.711
Model_8	-8.0	22.709	19.687
Model_9	-7.9	2.279	1.517

The Analysis of Docking Simulations: Balancing Affinity and Structure

The provided docking results offer valuable insights into the potential binding interactions between a ligand and a receptor. Here's a breakdown of the key findings:

Binding Affinity:

Strong Binding: Model_1 stands out with the most negative binding affinity score (-9.7), suggesting a very strong interaction with the receptor. Favorable Interactions: Models 2, 5, and 8 also exhibit relatively high binding affinities, indicating potentially favorable interactions. Weaker Interactions: Models 6 and 9 have lower binding affinities, suggesting weaker interactions compared to Model_1.

Root Mean Square Deviation (RMSD):

Perfect Match: Interestingly, Model_1 shows an RMSD of zero, indicating a perfect structural

match between the predicted and potentially known experimental structure.

Good Structural Predictions:

Models 3, 4, 7, and 9 possess relatively low RMSD values, suggesting good agreement between the predicted and reference structures.

Larger Deviations:

Models 2, 5, and 8 have higher RMSD values, implying larger deviations from the reference structure.

Combined Analysis - Prioritizing Candidates:

Top Contender:

Model_1 emerges as the most promising candidate due to its exceptionally high binding affinity and perfect structural match (zero RMSD). This suggests a strong and well-defined interaction with the receptor.

Promising Candidates:

Models 3, 4, 6, and 9 also show potential with good binding affinities and low RMSD values. These might be viable options for further investigation.

Further Investigation:

Models 2, 5, and 8 warrant further examination due to their higher RMSD values despite decent binding affinities. While they may still bind favorably, the larger structural deviations from the reference could indicate less reliable predictions.

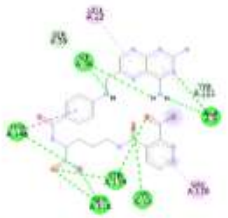


Figure 1 Model no 2

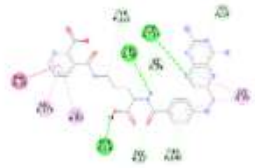


Figure 2 Model no 3

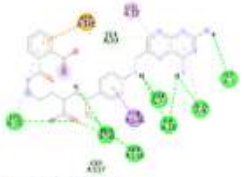


Figure 3 Model no 4

Figure 4 Model no 5

Figure 5 Model no 6

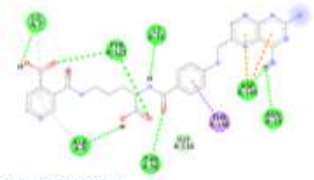


Figure 6 Model no 7

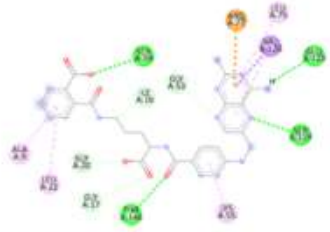


Figure 7 Model no 8

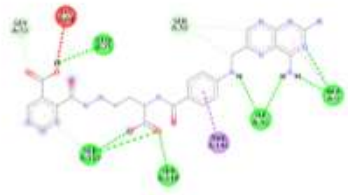
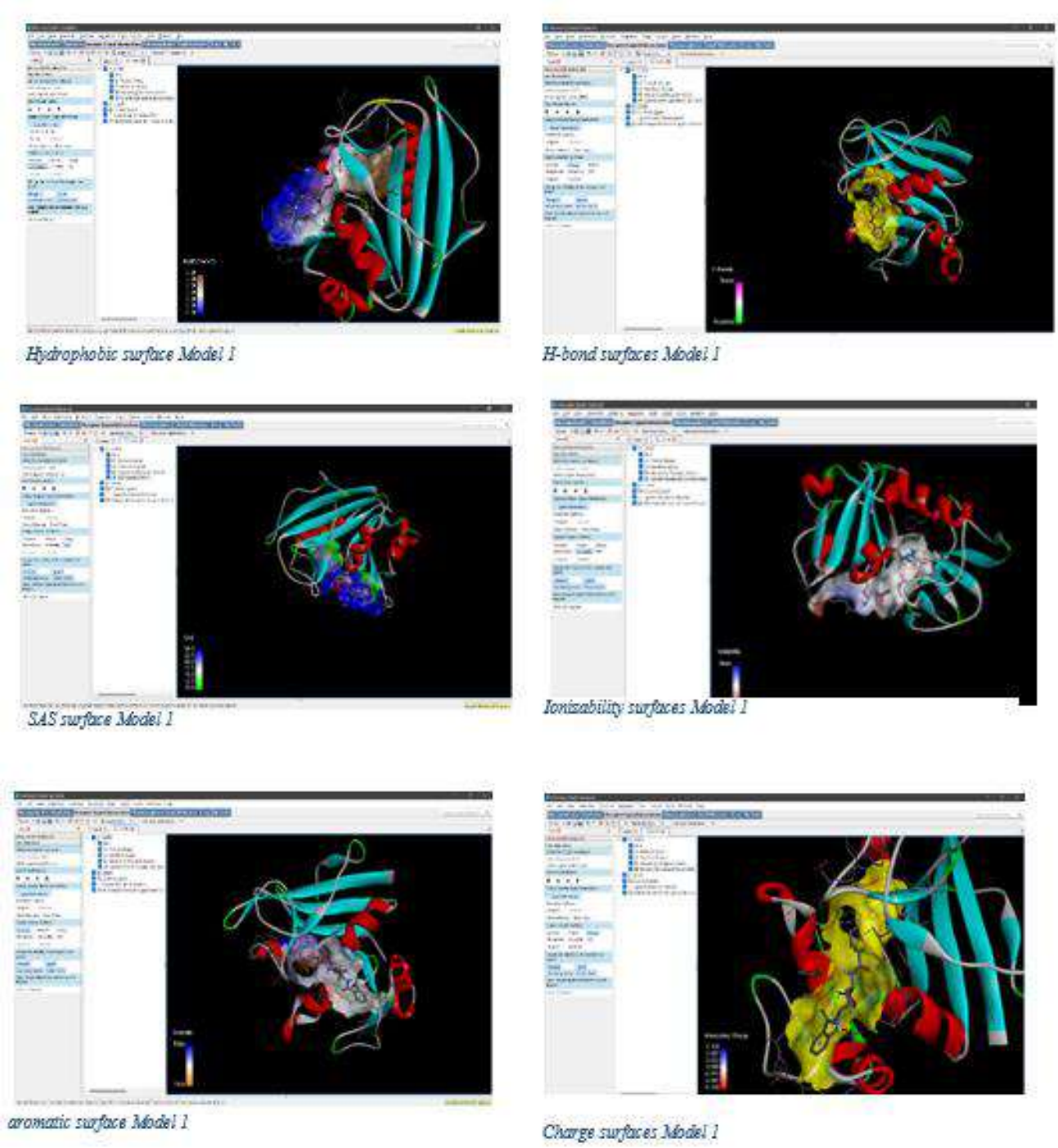


Figure 8 Model no 9

Interactions of all remaining 8 models with the 2D interaction in discovery studio



Model 1. Highest binding affinity -9.7 with the receptor surfaces which contains aromatic, H-Bond, Charge, Hydrophobic, Ionizability and SAS in discovery studio

DISCUSSION

The docking results showcase varying degrees of success in predicting ligand-receptor interactions. Model_1 demonstrates exceptional accuracy with a perfect RMSD of 0 and a high binding affinity. Models 3, 4, 6, and 9 also exhibit favorable outcomes, combining decent binding affinity with low RMSD values. However, Models 2, 5, and 8, despite respectable binding affinities, display higher structural deviations, warranting caution in their interpretation. Statistical analysis and biological relevance considerations are pivotal for

a nuanced understanding. Model_1 emerges as a standout performer, emphasizing its potential as a reliable model for further exploration and validation in subsequent studies.

CONCLUSION

The detailed analysis underscores the robust predictive capabilities of Model_1, emphasizing its reliability in capturing the binding interaction between the ligand, N-(4-Carboxy-4-{4-[(2,4-diamino-pteridin-6-ylmethyl)-amino]-benzoylamino}-butyl)-phthalamic acid (BDBM50011320), and the receptor, human dihydrofolate reductase (DHFR). Models 3, 4, 6, and 9 also exhibit promise in elucidating the BDBM50011320-DHFR complex, while Models 2, 5, and 8 merit further investigation. This study informs future drug design efforts, underscoring Model_1's potential as a reliable platform for subsequent validation studies.

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