

EXPLORATION OF NEW TRIAZINE-BASED INHIBITORS THROUGH DOCKING AGAINST THE DOUBLE-MUTANT (C59R+S108N) PFDHFR-TS ENZYME

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ABSTRACT

Resistance to conventional antifolates such as pyrimethamine is increasingly widespread in Plasmodium falciparum due to mutations in the dihydrofolate reductase thymidylate synthase (pfDHFR-TS) enzyme, particularly the double mutation C59R+S108N. This study explores the potential of novel 1,6-dihydro-1,3,5-triazine-2,4-diamine-based inhibitors against mutant pfDHFR-TS using a molecular docking approach. The target structure (PDB ID: 1J3J) and ligands were optimized using the semi-empirical PM6 method with partial charges calculated via AM1-BCC, followed by docking using Dock6. The results indicate that 10-(benzyloxy)-6,8,10-triazaspiro [4.5]deca-6,8-diene-7,9-diamine exhibits stronger binding affinity than CP6, a known antimalarial compound. These findings highlight the potential of triazine derivatives as novel inhibitors targeting antifolate-resistant pfDHFR TS mutants.

Keywords : pfDHFR-TS, triazine, molecular docking, antifolate resistance, Plasmodium falciparum, C59R+S108N mutation, antimalarial inhibitor.

Article History

Received: Desember 2025

Reviewed: Desember 2025

Published: Desember 2025

Plagiarism Checker:

No 234.GT8.35

Prefix DOI :

10.3483/trigonometri.v1i1.800

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Publishby : Trigonometri



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INTRODUCTION

Malaria is one of the most life-threatening parasitic diseases in the world, particularly in developing countries located in tropical and subtropical regions. Among the *Plasmodium* species responsible for malaria infections, *Plasmodium falciparum* and *Plasmodium vivax* are the most prevalent, with *P. falciparum* known to cause the most severe and fatal form of the disease. In 2019, it was estimated that approximately 229 million malaria cases occurred worldwide across 87 endemic countries. During the same year, malaria-related deaths were estimated at 409.000, with about 94% of these occurring in the African region. Following World War II, significant success in malaria control was achieved with the discovery of DDT and chloroquine. However, since the first decade of the 21st century, malaria cases have risen again beginning in 2014, indicating stagnation and an upward trend across various regions (Fikadu & Ashenafi, 2023). According to the latest *World Malaria Report 2025* published by the World Health Organization (WHO), the disease burden continues to rise, with estimates reaching 282 million cases and over 610.000 deaths globally in 2024. The majority of this burden is concentrated within the WHO African Region, accounting for 94% of total cases. Chemotherapeutic agents such as chloroquine and pyrimethamine have long been used as primary treatment strategies for malaria. However, the effectiveness of these drugs has continued to decline due to the increasing emergence of parasite resistance, particularly against pyrimethamine.

Resistance to pyrimethamine is associated with mutations in the gene encoding the dihydrofolate reductase (DHFR) enzyme, which alter the conformation of the active site and reduce the drug's binding affinity. Double mutations such as C59R and S108N in pfDHFR-TS significantly contribute to this resistance. Therefore, the development of new compounds capable of overcoming these mutations is urgently needed.

In silico approaches, such as molecular docking, enable efficient simulation of interactions between potential drug candidates and target proteins. Heterocyclic compounds such as triazines, particularly 1,6-dihydro-1,3,5-triazine-2,4-diamine derivatives, have shown promising potential as inhibitors due to their ability to form hydrogen bonds and non-covalent interactions with key enzyme residues. This study aims to evaluate the potential of triazine derivatives as inhibitors against the double-mutant pfDHFR-TS using molecular docking simulations. The findings are expected to support the development of new antimalarial agents that remain effective against resistant strains.

With advances in computational technology, structure-based drug design has become increasingly important in the early stages of drug discovery. Molecular docking not only allows the identification of compounds with high affinity toward biomolecular targets but also provides insights into ligand orientation within the active site and the types of interactions formed. Therefore, this method serves as an efficient and cost-effective tool for screening potential compounds before further biological evaluation.

2. METHODS

2.1 Tools and Materials

The receptor model used in this study was obtained from the Protein Data Bank (PDB) with the crystal structure code **1J3J**. This structure consists of four polypeptide chains (A, B, C, and D). Chain A, which contains the mutated pDHFR enzyme bound to the ligand CP6, was selected as the representative receptor-ligand complex. Three ligands were included in this study (Fig 1) : CP6 obtained directly from structure 1J3J, WR99210 obtained from the complex with PDB code 1J3I, and one test compound (Ligand X) prepared through geometry optimization using Gaussian software.

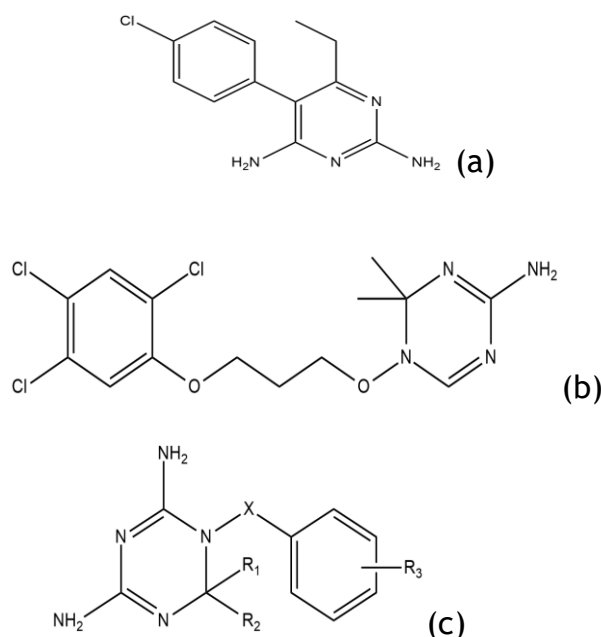


Fig 1. The main framework used (a) CP6 (b) WR99210 , and (c) Ligand X (Kyei *et al.*, 2022; Siswanto *et al.*, 2019)

Table 1. Theoretical compound with high antimalarial activity

No.	Code	R1	R2	X	R3
1.	CP6	-CH ₂ -CH ₃			Cl
2.	WR9210	-CH ₃	-CH ₃	-O-(CH ₂) ₃ -O-	1,3,4-Cl
3.	UNK	-(CH ₂) ₄ -		-O-(CH ₂) ₃ -O-	H

Several software tools were used to support the computational procedures. GaussView 6.0 was employed to draw, visualize, and prepare the ligand structures, as well as to assist with Gaussian computations. UCSF Chimera version 1.19 was used to prepare and visualize both receptor and ligand structures. Interaction analysis between ligands and active-site residues was carried out using BIOVIA Discovery Studio 2025. In addition, WinSCP was used to transfer data to a remote server via PuTTY

3. PROCEDURE

3.1 Preparation of Receptor and Ligands

The receptor structure with PDB ID 1J3J was used as the main model, focusing on chain A. Non-standard residues were removed using Chimera 1.19. Hydrogen atoms and partial charges were added to the pDHFR enzyme using a molecular mechanics approach with the AMBER ff14SB force field.

Three ligands were prepared for docking. CP6 was taken directly from the 1J3J crystal structure, while WR99210 (WRA) was extracted from PDB structure 1J3I. Both ligands were modified by adding hydrogen atoms and partial charges using the AMBER ff14SB force field in Chimera 1.19.

The third ligand, a dihydrotriazine derivative, was modified by adding methyl groups (CH₃) at positions R1 and R2 and a fluorine (F) atom at position R3 on the benzene ring, with a methylene (CH₂) linker (X) connecting the rings. The molecule was designed and geometrically optimized using the semi-empirical PM6 method in GaussView 6.0. It was then saved in MOL2 format, superimposed onto the pDHFR receptor (PDB ID 1J3J) using BIOVIA Discovery Studio 2025, and subsequently assigned hydrogens and partial charges using Chimera 1.19. All prepared ligands were saved in SYBYL MOL2 format for docking input.

3.2 Docking Parameter Validation

Docking procedures were executed on a remote computational server accessed via PuTTY, with WinSCP used for file transfer. Initial docking parameters were adopted from the native ligand-receptor complex (PDB ID: 1J#J), using CP6 as the reference ligand because it is the naturally bound ligand in the pDHFR receptor.

The molecular surface of the receptor was generated using the *sphgen* utility with the input file INSPH. The command was executed in a Linux environment using the syntax *\$sphgen -i INSPH* which produced two output files, OUTSPH and receptor.sph. The receptor.sph file contains molecular surface points required for docking. Next, sphere selection on the receptor surface relevant to the logan position was performed using the command *\$sphere_selector receptor.sph ../01-dockprep/ligand.mol2 10.0* which selects spheres within a 10 Å radius of the ligand. The output, *selected_spheres.sph*, was then used for subsequent docking configuration.

As validation, the Root Mean Square Deviation (RMSD) method was used to measure differences between the docked ligand pose and the original ligand pose in the crystal structure. A low RMSD value indicates accurate reproduction of the ligand's binding position.

3.3 Ligand Docking

Molecular docking was performed for two ligands, WR99210 (WRA) and ligand X, each of which docked to the pDHFR receptor based on the crystal structure with PDB code 1J3J. Docking was executed in a Linux environment by running the terminal command *dock6 -i dock.in*. This process generated an output file, *ligand_scored.mol2*, containing detailed information about ligand position within the complex, interaction orientation, and the grid score value representing the strength of ligand-receptor binding.

After docking was completed, grid score values for each ligand were analyzed and compared to evaluate ligand affinity toward the enzyme's active site. This parameter served as the initial indicator of ligand inhibitory potential.

To obtain a visual depiction of the molecular interactions formed, the resulting docking complexes were visualized using BIOVIA Discovery Studio 2025. This visualization stage aimed to identify the types of interactions formed, such as hydrogen bonding, hydrophobic interactions, and alkyl interactions and to determine the active-site residues involved in ligand binding.

4. RESULTS AND DISCUSSION

4.1 Receptor and Ligand Preparation

Molecular docking was used to predict the affinity and orientation of ligands within the receptor's active site, enabling an initial assessment of ligand potential as inhibitors through an efficient computational approach. This process requires the preparation of both receptor and ligand structures prior to simulation.

In this study, the receptor with PDB ID 1J3J (Fig 2) was prepared using Chimera 1.19, focusing on chain A, which contains the active site of the pDHFR enzyme. Preparation included the removal of non-standard residues and the addition of hydrogen atoms and partial charges using a molecular mechanics approach with the AMBER ff14SB force field, resulting in a receptor structure ready for docking simulation.



Fig 2. Receptor 1J3J preparation results

The ligands CP6 and WRA were obtained from the complex structure 1J3J and 1J3I, respectively, and then prepared by adding hydrogen atoms and assigning partial charges using the same procedure applied to the receptor. This step is essential to ensure accurate bonding parameters during docking simulations.

Additionally, this study utilized a new ligand (ligand X), a derivative of dihydrotriazine. This ligand was prepared through a structural optimization process using Gaussian software, then superimposed with the receptor and supplemented with hydrogen atoms and partial charges before being used in the docking simulation with receptor 1J3J.

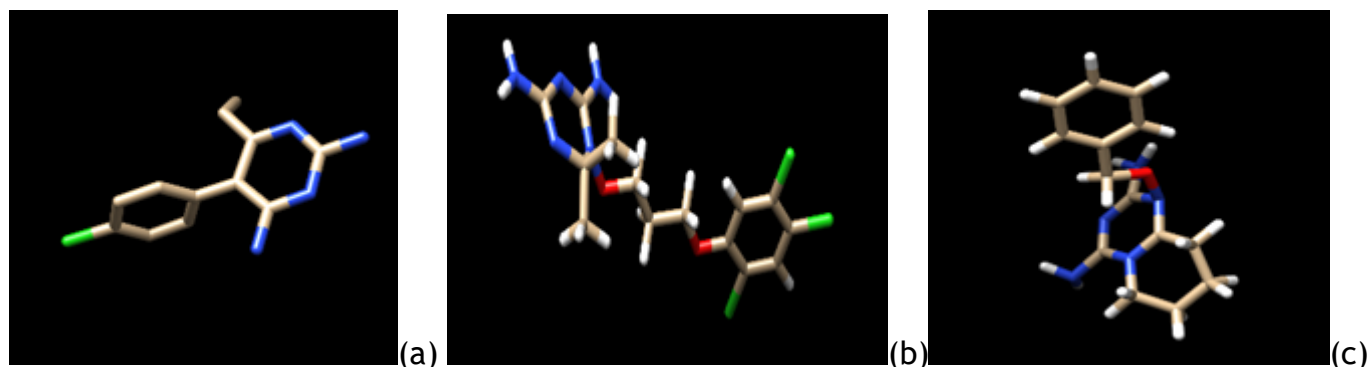


Fig 3. Ligand preparation results (a) CP6 (b) WRA, and (c) Ligand X

4.2 Docking Parameter Validation

Docking validation was performed using a redocking method, in which the native ligand CP6 was removed from the active site of the p δ HFR enzyme in the 1J3J receptor structure and then redocked back into the binding site. The purpose of this step was to evaluate the accuracy of the docking method by comparing the redocked ligand pose with its original crystallographic position.

The receptor surface was generated using the *sphgen* program (Fig. 3) and served as a reference for determining the ligand-binding region. This process was executed through terminal commands using PuTTY connected to the server. Subsequently, the grid box for the receptor was constructed with dimensions 24,955 Å $\times$ 20,572 Å $\times$ 30,107 Å and a resolution of 1 Å, adjusted according to the geometric coordinates of the active site. This adjustment aimed to enhance accuracy and efficiency in predicting molecular interactions during docking simulation.

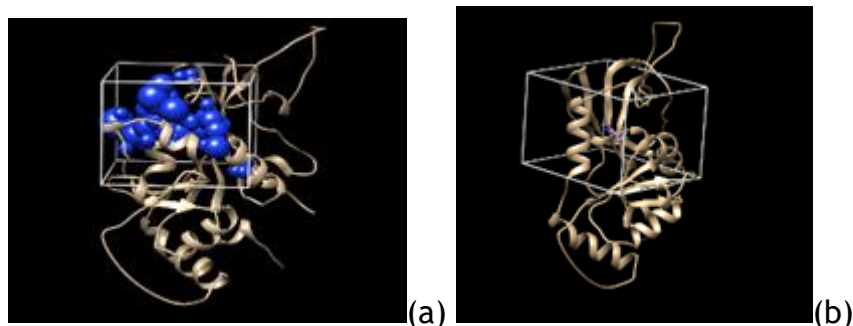


Fig 4. (a) Receptor surface and CP6 ligand, grid dimensions, (b) Selected receptor surface

To evaluate docking quality, the root mean square deviation (RMSD) parameter was used to measure how closely the predicted ligand position matched its original crystallographic pose. The redocking of CP6 into receptor 1J3J yielded an RMSD value of 0,394 Å, indicating that the docking method and parameters were capable of accurately reproducing the ligand position.

In general, a low RMSD value reflects good spatial similarity between the docked ligand and its position in the crystal structure. RMSD values below 2 Å are considered a valid indicator for assessing the success and reliability of docking predictions.

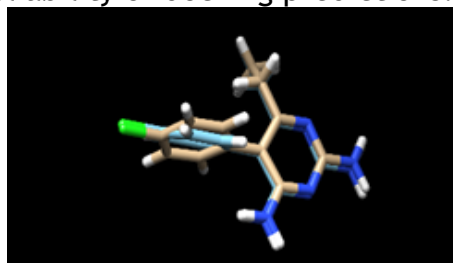


Fig 5. Positions of CP6 before docking (brown) and after docking (blue)

In addition to the low RMSD value, the docking results also showed changes in hydrogen bond lengths between the ligand and receptor due to shifts in ligand position. Details of these changes are presented in Table 1. Docking was performed against the pDHFR enzyme in receptor 1J3J using a grid-based method with validated parameters, producing grid score values that reflect ligand-binding activity.

Table 2. Hydrogen bonds of CP6 ligand with pDHFR in 1J3J receptor

Table with 3 columns: Hydrogen Bond, Original Ligand (Å), Docked Ligand (Å). Rows include H131 - ILE14.A and H132 - ILE 154.A.

4.3 Ligand Docking

Two external ligands, WRA and ligand X, were subjected to molecular docking with receptor protein 1J3J using Dock6. The purpose of this process was to explore the possible binding modes of each ligand toward the receptor’s active site and to evaluate their binding affinities.

In this analysis, the grid score serves as the primary quantitative parameter for comparing the interaction strength of each ligand with the target receptor. A more negative grid score indicates stronger interactions and, therefore, greater potential as an inhibitor.

Ligand-receptor interaction visualization was conducted using BIOVIA Discovery Studio 2025 to identify the types and locations of interactions (hydrogen bonding, hydrophobic interactions, electrostatic interactions) within the active site. Details of the interactions are presented in Table 2, including key contacts supporting complex stability.

Table 3. Grid score and ligand-receptor interactions

Table with 5 columns: Receptor, Ligand, Hydrogen Bond, Alkyl Interactions, Grid Score. Rows include pDHFR with ligands CP6 and WRA, and Ligand X (UNK).

Based on the docking results and visualization analysis, the WRA ligand exhibited high affinity toward the pDHFR active site, with a more negative grid score compared to the native ligand CP6. WRA also formed a greater number of interactions with residues in the 1J3J

receptor, indicating good specificity as well as spatial and electronic complementarity that support its potential as an antimalarial inhibitor. Conversely, although ligand X showed a slightly more negative grid score than CP6, the visualization revealed less specific interactions and the absence of hydrogen bonding. This reflects poor binding affinity, indicating that ligand X does not yet meet the criteria for an effective inhibitor candidate.

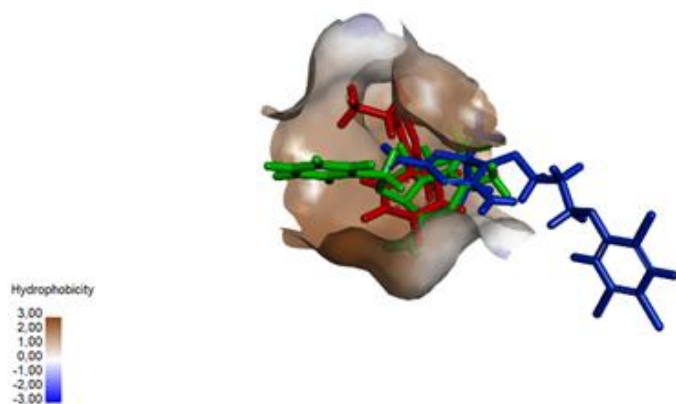
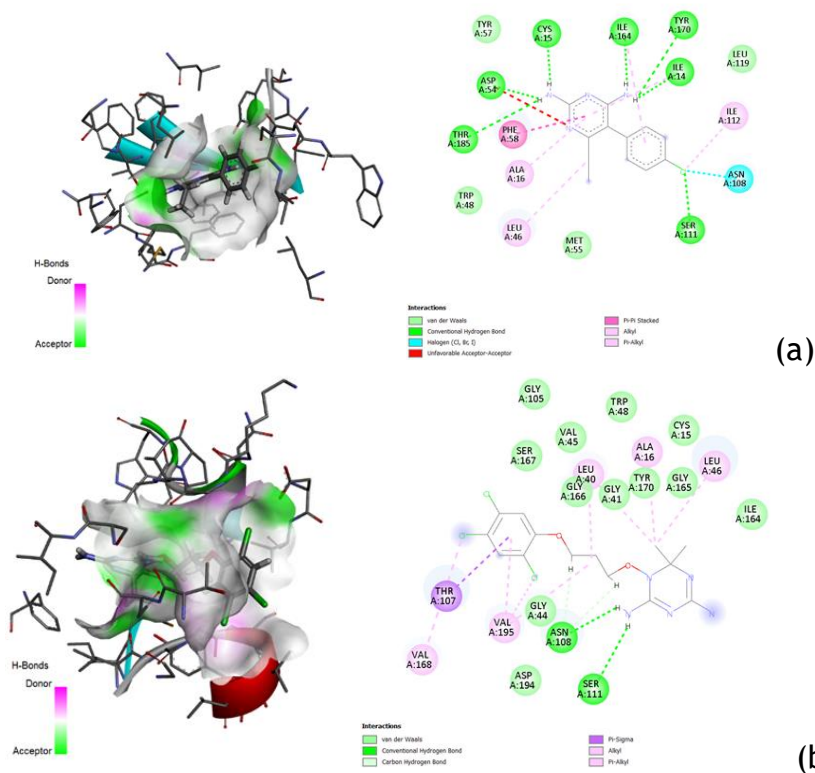


Fig 6. Ligand conformations after docking with lower binding affinity (blue) and higher binding affinity: CP6 (red), ligand (UNK) (green), ligand WR-92210 (blue) inside the active site pocket of the pDHFAR receptor



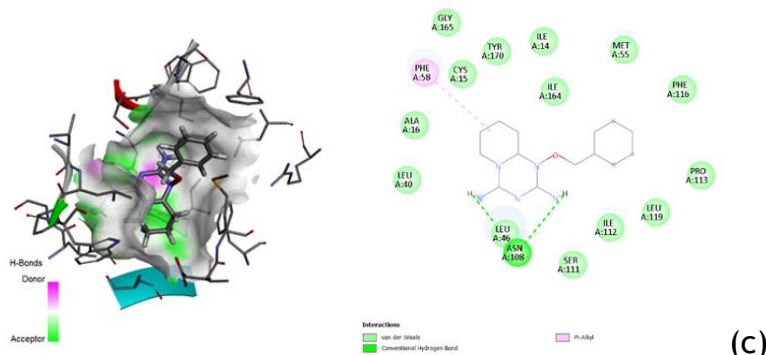


Fig 7. Ligand interactions (a) CP6, (b) WRA, (c) ligand X with pDHFR in 1J3J receptor

Fig 6. illustrates the interaction patterns of the three ligands, Cp6, WRA, and ligand X with the receptor 1J3J. Among them, the WRA ligand exhibits the most complex and strongest interaction patterns, supported by the presence of a greater number of substituent groups. Based on data in Table 2, WRA is capable of forming hydrogen bonds with residues ASP54, CYS15, ILE14, and ILE164, as well as alkyl interactions with ALA16, ILE112, LEU46, LEU119, PHE58, PHE116, and PRO113. These combined interactions demonstrate excellent spatial and electronic compatibility with the pDHFR active site, reinforcing its potential as an effective inhibitor.

In comparison, CP6, the native ligand in the crystal structure, also forms several similar hydrogen bonds, but the number of alkyl interactions is more limited, involving only ALA13, ILE154, and TYR46. This limitation is likely due to the simpler and less flexible structure of CP6, preventing optimal engagement with the enzyme's active site.

Conversely, ligand X, which structurally possesses only substituents on the triazine ring, exhibits very limited interactions with the receptor. No hydrogen bonds were observed, and the alkyl interactions formed were minimal, involving only ILE153, ALA13, and TYR160. Analysis indicated that only WRA and CP6 were capable of effectively reaching and interacting with the pDHFR active site, whereas ligand X failed to form significant contacts due to its limited ability to adapt to the active site topology (Siswanto *et al.*, 2019).

This condition aligns with the grid score results, where ligand X displayed a higher (less negative) grid score and reflecting weaker binding affinity. Conversely, WRA yielded the most negative grid score, indicating the highest binding affinity and reinforcing its potential as a strong pDHFR inhibitor candidate in antimalarial drug development.

5. CONCLUSION

The molecular docking results against the 1J3J receptor reveal that the WRA ligand possesses the highest binding affinity, with a grid score of -56,24. This ligand also forms the greatest number of interactions, including both hydrogen bonds and alkyl interactions, which contribute to a more stable ligand-receptor complex. CP6, as the native ligand, demonstrates reasonably strong affinity and interactions, although still lower than those of WRA. Meanwhile, ligand X, a dihydrotriazine derivative, shows weaker binding affinity and does not form hydrogen bonds, indicating its limited ability to reach and interact effectively with the active site of the pDHFR enzyme.

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