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THE INHIBITORY EFFECT OF KNOWN ANTIFOLATE DRUGS AND SELECTED PLANT ISOLATES ON THE PLASMODIUM FALCIPARUM DIHYDROFOLATE REDUCTASE: A COMPARATIVE STUDY

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Abstract

Malaria is a major burden for the most resource-poor nations of the world. The goal of eradicating malaria, once thought to be possible, was abandoned decades ago, and the present goal of malaria control is instead first to retard the accelerating rates of disease and death caused by the world's most important parasitic disease and then to "roll back malaria". Resistance to antifolates occurred soon after their deployment as antimalarials. In malaria, this has been shown to be due to the evolutionary changes in dihydrofolate reductase (DHFR) which resulted into a decrease in binding strengths of the inhibitors. In this study, two groups of compounds were analyzed for their potency against Plasmodium falciparum DHFR. The first group is made up of a set of known antifolate drugs (Cycloguanil, Proguanil and Pyrimethamine) while the second group is made up of compounds isolated from plants with history of antimalaria activities (Gedunin, Lycorine and 1,2-di-O-acetyllycorine). The plant isolates were designed using the MarvinSketch software while the inhibitory effect of each experimental compounds against the Plasmodium falciparum DHFR were measured through a molecular docking approach, where the potency of a drug/compound is determined by their binding energy to the target enzyme. Binding energy predictions from the molecular docking study showed that the 3 plant isolates might be better antimalarial agents than the antifolate drugs when targeted at the Plasmodium falciparum DHFR. The laboratory synthesis and preclinical studies on these compounds are therefore recommended.

Keywords: *Malaria, Antifolates, Plasmodium falciparum, Inhibitors, Molecular Docking*

Introduction

Antifolates are a class of antimetabolite medications that blocks the activity of folic acid [1]. The primary function of the folic acid in the body is to serve as a cofactor to various methyltransferases involved in serine, methionine, thymidine and purine biosynthesis. Consequently, antifolates inhibit cell division, DNA/RNA synthesis and repair and protein synthesis. Some such as proguanil and pyrimethamine selectively inhibit folate's actions in microbial organisms such as bacteria, protozoa and fungi. The majority of antifolates work by inhibiting dihydrofolate reductase (DHFR) [2].

The empiric use of antifolates against malaria long predates definitive demonstration of the folate metabolism pathway in Plasmodium spp. De novo synthesis of folate by Plasmodium spp. was demonstrated over 25 years ago [3], and although

an exogenous folate salvage pathway has been found in isolates from around the world [4], it does not appear to be Plasmodia's primary source of folate. Disruption of folate synthesis by DHFR inhibitors leads to decreased levels of fully reduced tetrahydrofolate, a necessary cofactor in important one-carbon transfer reactions in the purine, pyrimidine, and amino acid biosynthetic pathways [3]. The lower levels of tetrahydrofolate result in decreased conversion of glycine to serine, reduced methionine synthesis, and lower thymidylate levels with a subsequent arrest of DNA replication [5, 6, 7].

DHFR exists as a bifunctional enzyme together with thymidylate synthase (TS), which forms d-TMP from d-UMP using methylenetetrahydrofolate as the methylating agent [8]. The dihydrofolate produced in this reaction, a part of the d-TMP synthesis cycle, as well as that formed from de novo and a folate salvage pathway [9], is reduced through DHFR catalysis to tetrahydrofolate. P. falciparum DHFR, unlike its human counterpart, is very sensitive to inhibition by pyrimethamine and other 2, 4- diaminopyrimidines, and cycloguanil and other 2,3- dihydrotriazines, providing the rationale for their use as antimalarials [10].

This paper is aimed at carrying out a comparative study on the inhibitory role of selected antifolate drugs and isolated plant antimalarial compounds against the Plasmodium falciparum dihydrofolate reductase.

Materials and Methods

Sequence Retrieval

The human dihydrofolate reductase amino acid sequence was retrieved from the National Center for Biotechnology Information (NCBI) Database [11]. The NCBI houses database series with relevance to biotechnology and biomedicine and is also a useful resource for bioinformatics services, analysis and tools. Major databases comprise the GenBank which is for sequences of DNA and PubMed which is a bibliographic repository for literatures regarding biomedicine [12]. Other databases include the NCBI and the Epigenomics database [13]. The human dihydrofolate reductase was assigned an accession number of NP_001182572.1. The Plasmodium falciparum dihydrofolate reductase amino acid sequence was downloaded from the Protein Data Bank repository [14].

Physiological–biochemical characterization

The Expasy Protparam server [15] was used for the physicochemical characterization and to know the molecular weight, theoretical isoelectric point (pI), total number of negative and positive residues, aliphatic index, extinction coefficient, instability index, and grand average hydropathicity (GRAVY) of this TIM protein [16].

Sequence Alignment

The human and Plasmodium falciparum dihydrofolate reductase sequences were aligned using the clustalW software. Just like other Clustal tools, ClustalW is used for multiple nucleotide and protein sequence alignment in a manner that is efficient. ClustalW make use of progressive alignment methods, where the first set of aligned sequences are the most similar down to the least similar up to the point of creation of a global alignment [17].

Protein 3D Structure

The crystallized 3D structure of the Plasmodium falciparum dihydrofolate reductase was downloaded from the Protein Data Bank (PDB) repository [18]. The Protein Data Bank (PDB) is a 3D (three-dimensional) database for structural proteins and nucleic acids which are large biological molecules [19]. Biologists and biochemists from all over the world submit these data which were obtained through NMR spectroscopy, X-ray crystallography and cryo-electron microscopy [20].

Ligand Preparation

The 2D structure of gedunin, 1,2-diacetoxylycorine and lycorine were designed using the MarvinSketch software [21] while SMILES strings for the three selected antifolate drugs were copied from the PubChem database. All designed structures were downloaded and saved as mrv files in preparation for docking

Molecular Docking

The binding energy scores between the experimental ligands and the Plasmodium falciparum dihydrofolate reductase was predicted using the AutoDock Vina software [22]. AutoDock Vina is a molecular modeling and simulation software. It is especially designed and effective for protein-ligand docking [23].

Results and Discussion

The usefulness of sequence alignment is for the discovery structural and functional information in

biological sequences. The importance also gives important information about the evolutionary relationship between sequences of different species of organisms. [24]. The 32% similarity observed from the alignment result between the human and Plasmodium falciparum dihydrofolate reductase primary structures is an indication that the Plasmodium falciparum DHFR might be an ideal target for druglike compounds based on the low percentage similarity that exists between the two amino acid sequences. The low percentage similarity also consequently serves as an indicator to the distant relationship between the two sequences with regards to evolution.

The pI of the Plasmodium falciparum dihydrofolate reductase by the biochemical characterization analysis has predicted the protein to be slightly acidic with a value of 6.86 [25]. The hydrophobicity scale produced values that define relative hydrophobicity of amino acid residues. The more positive the value, the more hydrophobic the amino acids located in that region of the protein [26]. The GRAVY calculator used in predicting the hydrophobicity assigned to the protein a value of -0.506

The instability index provides an estimate of the stability of a protein in a test tube. A protein whose instability index is smaller than 40 was predicted as stable and a value above 40 predicts the protein may be unstable [27]. The Plasmodium falciparum dihydrofolate reductase is therefore a stable protein with an instability value of 35.23.

Plasmodium falciparum dihydrofolate reductase contains 608 amino acid residues. The docking structures of all the compounds showed that they bind in a very similar pattern with the active site of Plasmodium falciparum dihydrofolate reductase, as is evident from the superposition of all experimental ligands in figures 4-9. The calculated free energy of binding of gedunin, 1,2-di-O-acetyllycorine and lycorine were -9.5, -9.0 and -8.7Kcal/mol respectively while the calculated free energy of binding of cycloguanil, proguanil and pyrimethamine were -8.0, -7.7 and -8.0Kcal/mol respectively (Table 1). This confirms that the structural uniqueness of each ligand as observed in this study is significantly related to their activity [28]. Also, this proved the reliability of the docking results [28].

The solubility of a compound in water could improve its biotransformation and elimination as a drug [29]. All experimental ligands used for the purpose of this study were soluble in water (Table 1).

The molecular weight of all the experimental ligands were less than 500g/mol, showing that they can be considered as drug [30]. A compound can also be considered drug-like if it is characterized by high lipophilicity (less than 5) [31]. This is expressed as Log Po/w. The lipophilicity values of all experimental ligands were less than 5 and are most likely to be drugs.

Lipinski's rule of 5 helps in distinguishing between drug-like and non drug-like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules: Molecular mass less than 500g/mol; High lipophilicity (expressed as Log Po/w less than 5); Less than 5 hydrogen bond donors; Less than 10 hydrogen bond acceptors; Molar refractivity should be between 40-130. These filters help in early preclinical development and could help avoid costly late-stage preclinical and clinical failures [32]. All the experimental ligands used in his study complied with the Lipinski's rule and therefore are likely to be drugs (Table 1).

High penetration is needed for most of the drugs targeting the central nervous system (CNS), whereas blood brain barrier (BBB) penetration should be minimized for non-CNS drugs to avoid

undesired side-effects [33]. Pharmacokinetically, the gastrointestinal drug absorption of all experimental ligand was high and all but pyrimethamine showed no blood brain barrier (BBB) permeation ability. This calls for caution in the administration of pyrimethamine in order to minimize side effects due to the penetration of the blood brain barrier.

For synthetic accessibility, values of 5 to10 means that the drug could be synthesized [34]. All the experimental ligands but gedunin showed synthetic accessibility values of less than 5. This means that the compounds can easily be synthesized in the laboratory. Synthetic studies followed by pre-clinical studies are further recommended.

Conclusion

The results obtained from this study has indicated that the 3 isolated plant compounds (gedunin, 1,2-di-O-acetyllycorine and lycorine) might be better

antimalarial drugs having exhibited stronger binding energies against the Plasmodium falciparum dihydrofolate reductase than their antifolate counterparts (cycloguanil, proguanil and pyrimethamine).

Pyrimethamine could pose a threat to the Central Nervous System (CNS) as it possess the ability to penetrate the blood brain barrier. Caution should therefore be taken in the administration of this drug in order to avoid undesirable side effects.

Synthesis and pre-clinical studies of the 3 plant compounds against Plasmodium falciparum dihydrofolate reductase is recommended to confirm their activity as better antimalarial drugs.

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Figure 1: Sequence alignment result between the human and Plasmodium falciparum dihydrofolate reductase

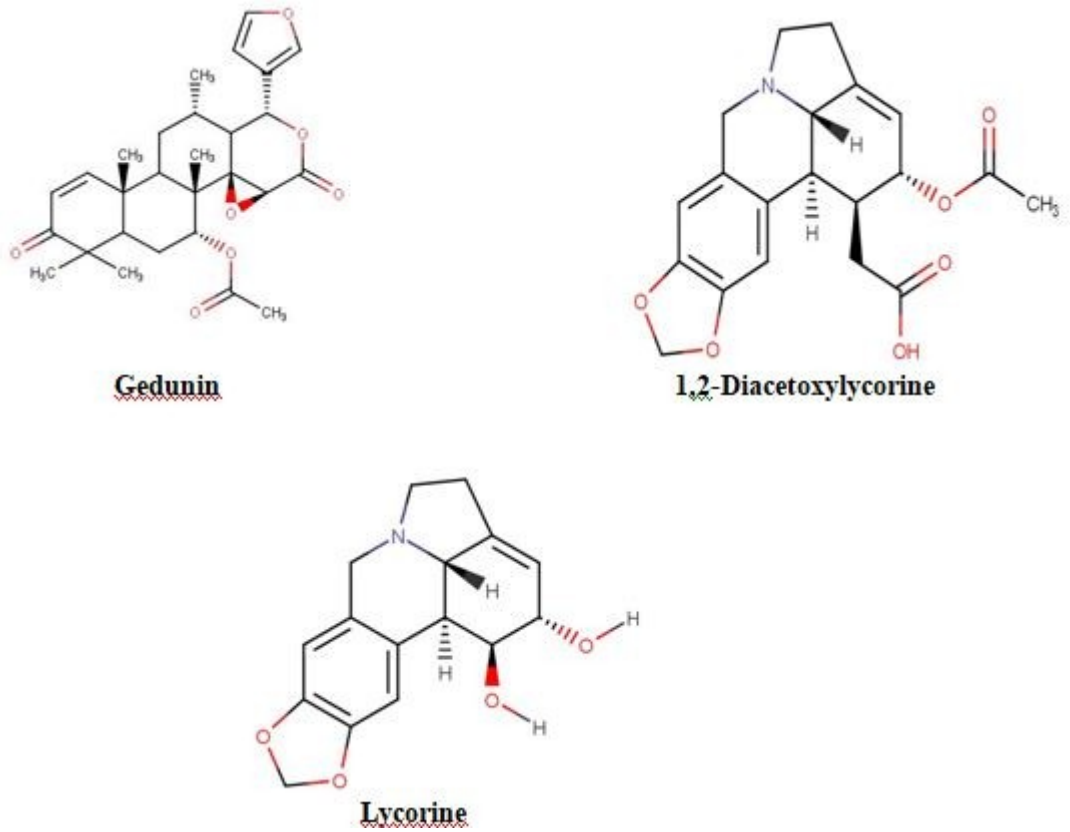

Figure 2. The two dimensional (2D) structure of the experimental plant isolates designed using the MarvinSketch software.

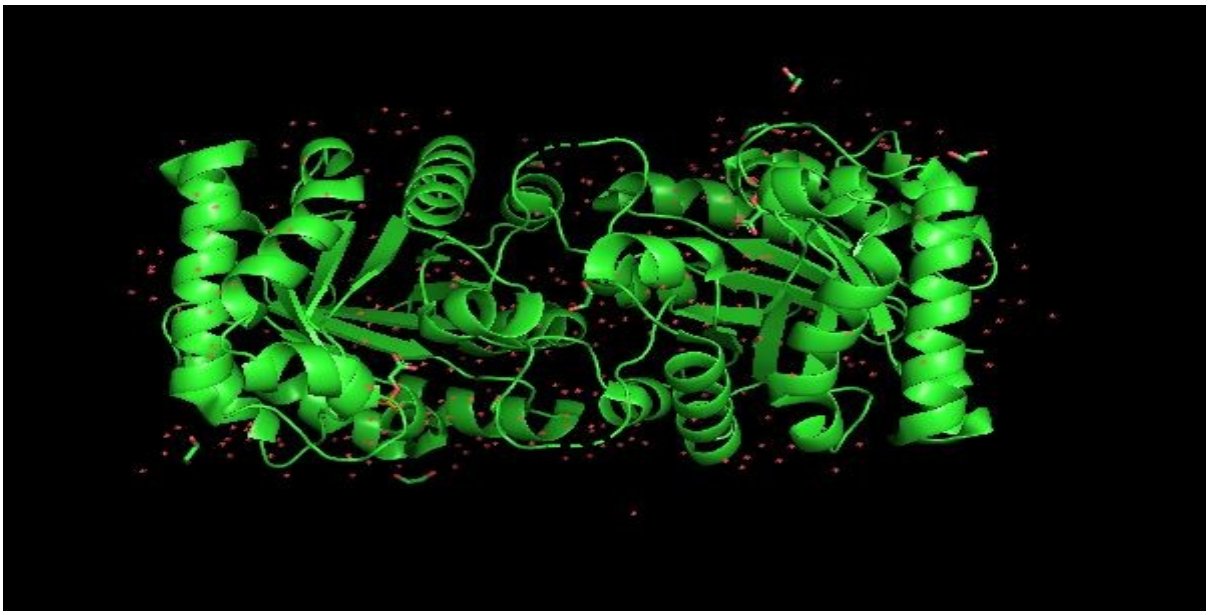

Figure 3. Three dimensional (3D) structure of the *Plasmodium falciparum* dihydrofolate reductase (PDB: 3UM8)

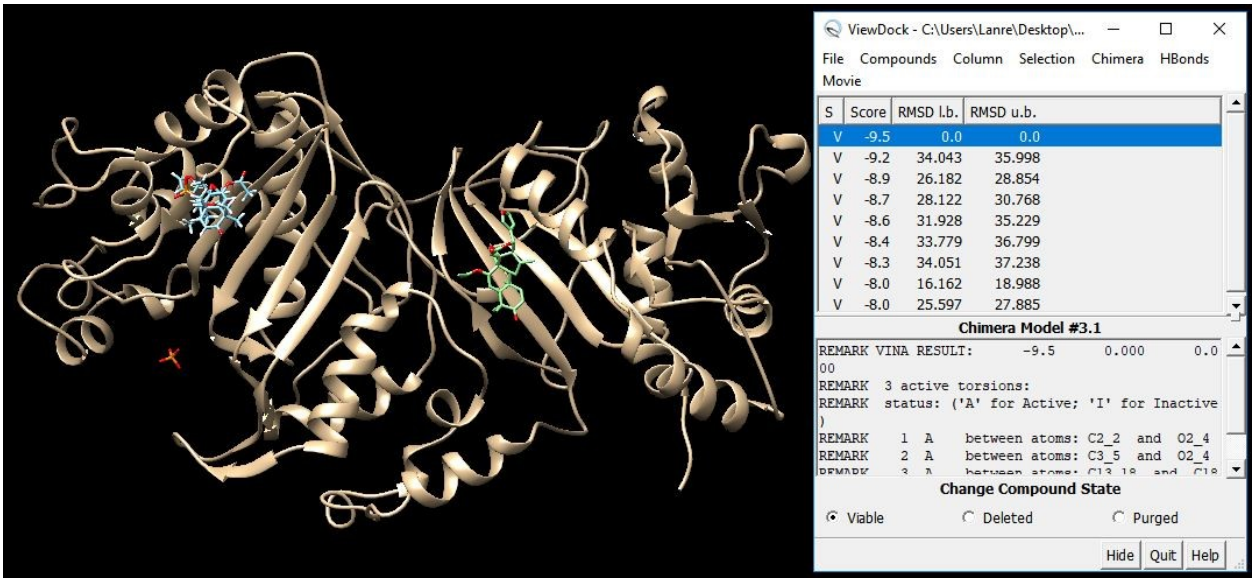

Figure 4. Docking pose of gedunin against the *Plasmodium falciparum* dihydrofolate reductase.

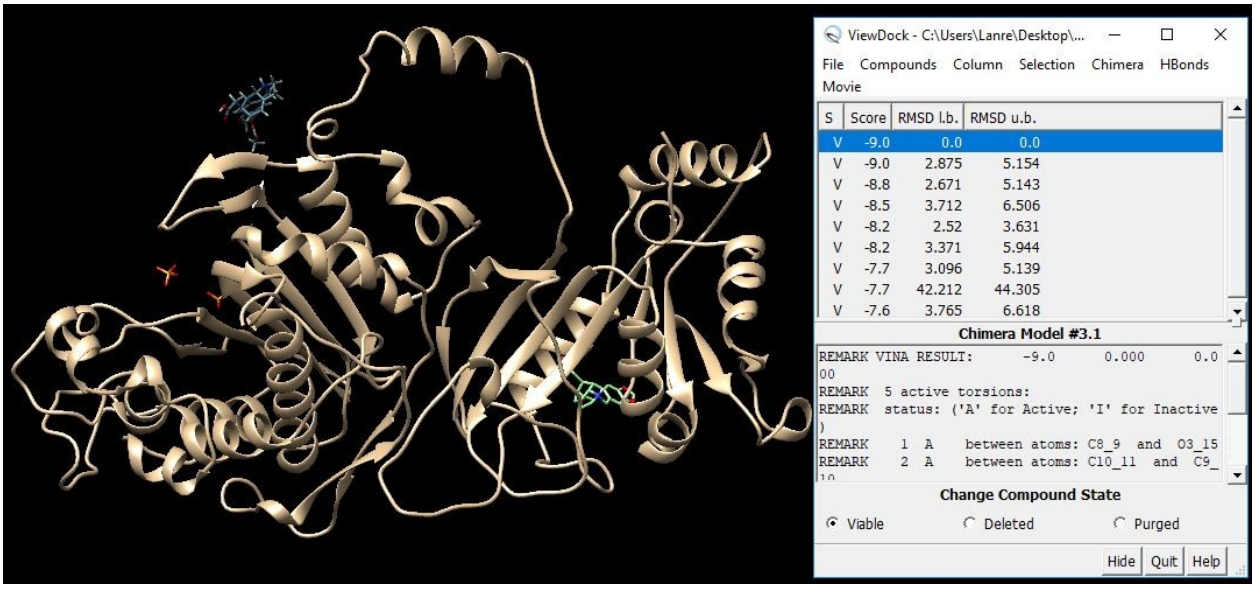

Figure 5. Docking pose of 1,2-diacetoxylycorine against the *Plasmodium falciparum* dihydrofolate reductase.

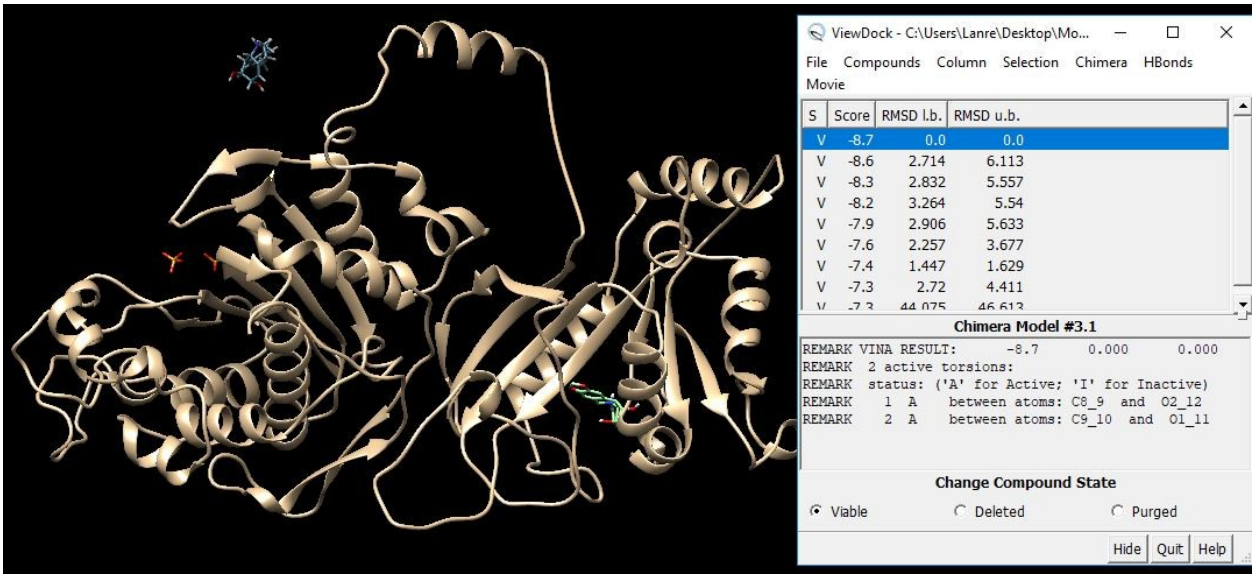

Figure 6. Docking pose of lycorine against the *Plasmodium falciparum* dihydrofolate reductase

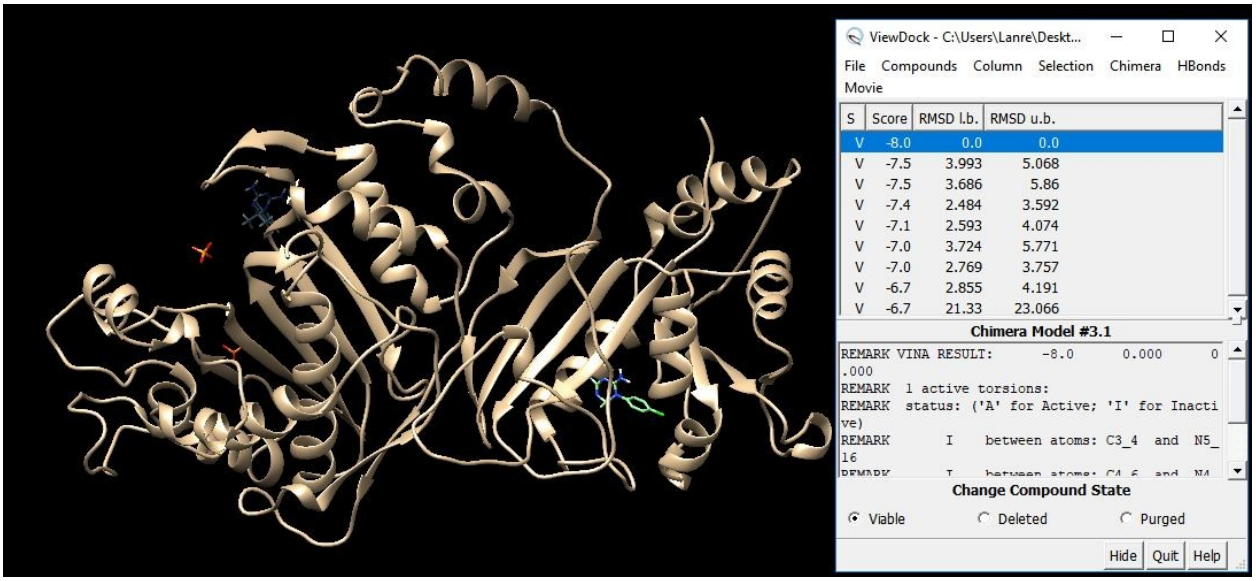

Figure 7. Docking pose of cycloguanil against the *Plasmodium falciparum* dihydrofolate reductase.

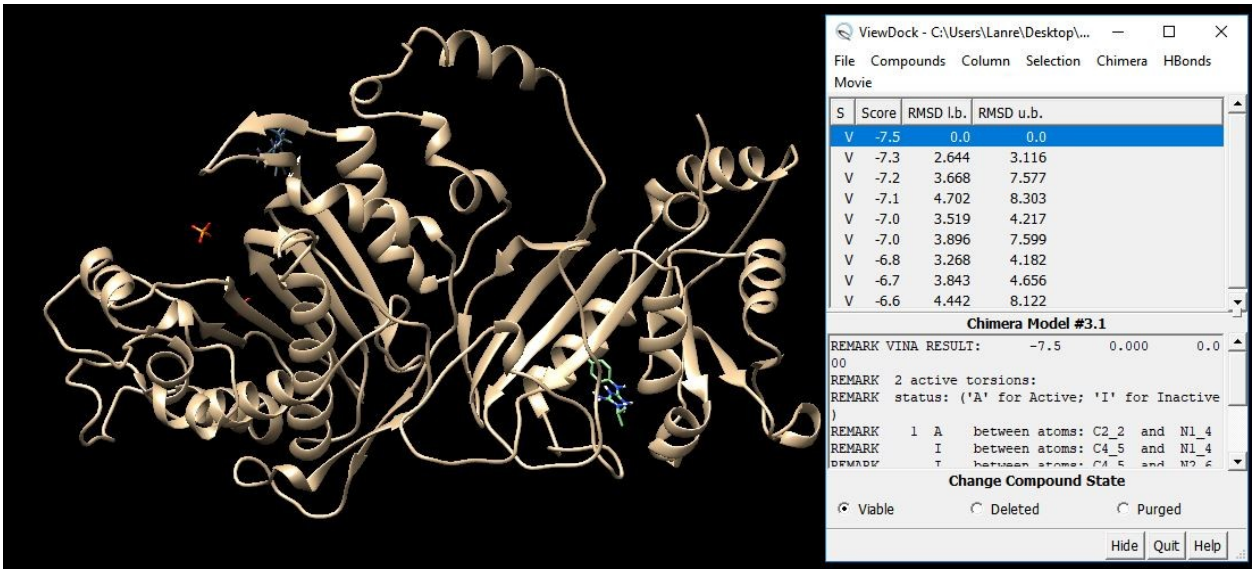

Figure 8. Docking pose of proguanil against the *Plasmodium falciparum* dihydrofolate reductase.

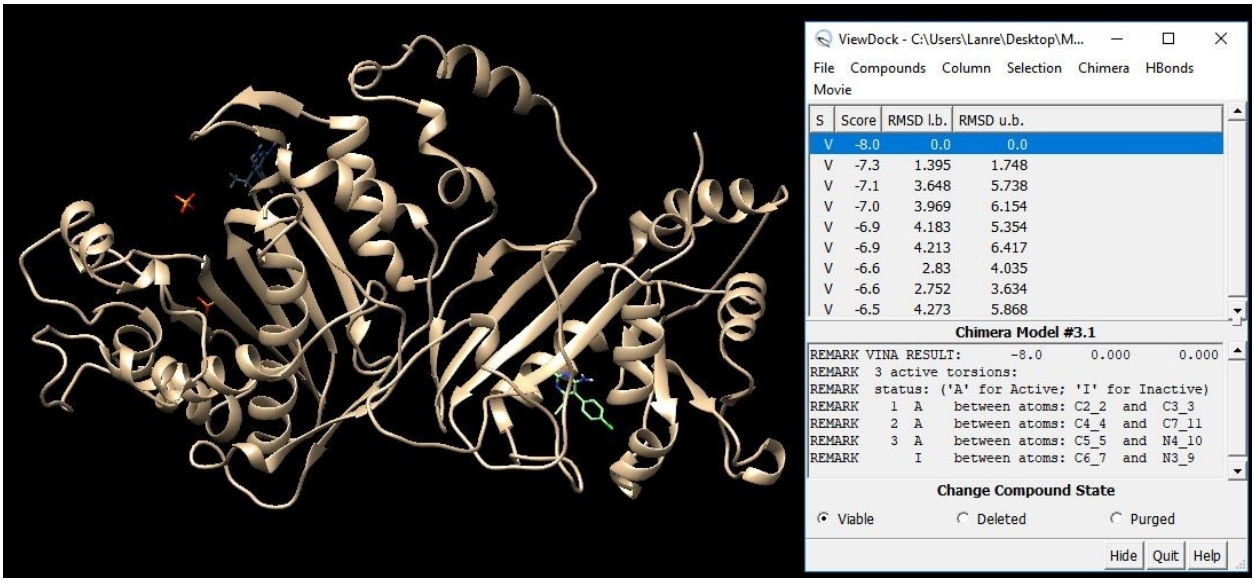

Figure 9. Docking pose of pyrimethamine against the *Plasmodium falciparum* dihydrofolate reductase

Table 1: Physicochemical properties, lipophilicity, solubility, pharmacokinetics and lipinski druglikeness of experimental ligands.

Parameters	**Gedunin**	**1,2-diacetoxylycorine**	**Lycorine**	**Cycloguanil**	**Proguanil**	**Pyremethamine**
Formula	$C_{28}H_{34}O_7$	$C_{20}H_{21}NO_6$	$C_{16}H_{17}NO_4$	$C_{11}H_{14}ClN_5$	$C_{11}H_{16}ClN_5$	$C_{12}H_{13}ClN_4$
Molecular weight g/mol	482.57	371.38	287.31	251.72	253.73	248.71
Docking score Kcal/mol	-9.5	-9.0	-8.7	-8.0	-7.5	-8.0
Num. H-Bond acceptors	7	7	5	2	2	2
Num. H-Bond donors	0	1	2	2	3	2
TPSA Å²	95.34	85.30	62.16	80.00	88.79	77.82
Lipophilicity Consensus Log $P_{o/w}$	3.64	1.28	0.87	1.35	1.59	2.29
Water Solubility Log S	Moderately Soluble	Highly Soluble	Very Soluble	Soluble	Soluble	Soluble
GI absorption	High	High	High	High	High	High
BBB permeant	No	No	No	No	No	Yes
P-gp substrate	Yes	No	Yes	No	No	No
CYP1A2 inhibitor	No	No	No	Yes	No	Yes
CYP2C19 inhibitor	No	No	No	No	No	Yes
CYP2C9 inhibitor	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	Yes	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	Yes
Lipinski Druglikeness	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation	0
Synthetic accessibility	6.54	4.59	4.20	3.27	2.68	2.43