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In-Silico Pharmacokinetics Study on the Inhibitory Potentials of the C=O Derivative of Gedunin and Pyrimethamine against the *Plasmodium falciparum* Dihydrofolate Reductase; A Comparative Study

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Abstract
Background: *Malaria is a life-threatening disease caused by parasites that are transmitted to people by mosquitoes. An estimated 700,000 people were killed by malaria in 2010 globally and approximately half the world's population are at risk of the disease. Malaria is preventable and curable. Malaria is caused by a microscopic parasite called Plasmodium. Four species of this parasite infect humans to cause malaria but Plasmodium falciparum is the most deadly. Plasmodium is transmitted between people by blood-eating mosquitoes. Pyrimethamine is an antiparasitic medicine that helps prevent parasites from growing and reproducing in the body.*
Materials and Methods: *The C=O derivative of gedunin was designed using the ChemAxon software where the methyl group attachment of gedunin was substituted for the C=O group and converted into an mrv file. The mrv file was converted into SMILES strings for the purpose of docking using the Open Babel software. The AutoDock Vina software was utilized for the molecular docking process and the in-silico pharmacokinetics parameters of the C=O derivative of gedunin was predicted using the SwissADME server.*
Results: *Results from the physiochemical characteristics prediction of the Plasmodium falciparum DHFR showed that the half life of the enzyme in human reticulocytes can be estimated at 30 hours when subjected to an in vitro study. The in silico pharmacokinetics study also showed that both the C=O derivative of gedunin and pyrimethamine violated none of the lipinski's rule. Results from the molecular docking study of the compounds (C=O derivative of gedunin and pyrimethamine) against the Plasmodium falciparum DHFR were -9.0 and -8.0Kcal/mol respectively.*
Conclusion: *The above results showed that both experimental compounds can be safe for oral administration haven satisfied the lipinski's rule requirements. The molecular docking study also showed that the C=O derivative of gedunin might be a better antimalarial agent by its exhibition of a higher binding energy against the Plasmodium falciparum DHFR.*

Keywords: *Malaria; Plasmodium falciparum; Reticulocytes; Pharmacokinetics.*

I. INTRODUCTION

Malaria is a life-threatening mosquito-borne blood disease caused by a Plasmodium parasite.

It is transmitted to humans through the bite of the *Anopheles* mosquito [1]. Once an infected mosquito bites a human, the parasites multiply in the host's liver before infecting and destroying red blood cells. In some places, malaria can be treated and controlled with early diagnosis. However, some countries lack the resources to do this effectively [2].

Plasmodium falciparum is a unicellular protozoan parasite of humans, and the deadliest species of *Plasmodium* that cause malaria in humans [3]. It is transmitted through the bite of a female *Anopheles* mosquito. It is responsible for roughly 50% of all malaria cases. It causes the disease's most dangerous form called *falciparum* malaria [4]. It is therefore regarded as the deadliest parasite in humans, causing a conservative estimate of one million deaths every year. It is also associated with the development of blood cancer (Burkitt's lymphoma) and is classified as Group 2A carcinogen [5].

Pyrimethamine is a synthetic derivative of ethyl-pyrimidine with potent antimalarial properties. Pyrimethamine is a competitive inhibitor of dihydrofolate reductase (DHFR) [6]. DHFR is a key enzyme in the redox cycle for production of tetrahydrofolate, a cofactor that is required for the synthesis of DNA and proteins. This agent is often used in combination with other antimalarials for the treatment of uncomplicated *falciparum* malaria [7].

Gedunin and its analogs are an important bioactive limonoid-type tetranortriterpene isolated from the Meliaceae family and are reported to display a wide range of biologic activities, including antitumor, antimalarial, antiallergic, and anti-inflammatory activity in different experimental models [8].

The aim of the study is to carry out a comparative study on the inhibitory potentials of the C=O derivative of gedunin and pyrimethamine against the *Plasmodium falciparum* DHFR. This analysis also cuts across the prediction of the druglikeness of both experimental compounds.

II. MATERIALS AND METHODS

Protein Preparation

The crystallized 3D structure of the *Plasmodium falciparum* dihydrofolate reductase was obtained from the PDB repository with a PDB code of 3UM8. The Protein Data

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Bank (PDB) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography, NMR spectroscopy, or, increasingly, cryo-electron microscopy, and submitted by biologists and biochemists from around the world [9].

Physiochemical Characteristics

The physiochemical characteristics of the *Plasmodium falciparum* dihydrofolate reductase was computed using the ExPASy ProtParam online server which computes various parameters such the molecular weight, amino acid composition, extinction coefficient, estimated half-life, theoretical pI, grand average of hydropathicity (GRAVY), aliphatic index and instability index [10].

Ligand Search

The search for information about the 2 ligands of interest (gedunin and pyrimethamine) was carried out using the PubChem database which is a database of chemical molecules and their activities against biological assays [11].

Ligand Preparation

The C=O derivative of gedunin and pyrimethamine were both designed with the aid of the MarvinSketch software which features an extensive set of functionalities to enable the fast and accurate drawing of chemical compounds, reactions, Markush structures and query molecules [12].

File Conversion

The mrv file downloads of every design achieved using the MarvinSketch were converted into SMILES strings using the OpenBabel Graphics User Interface which is a chemical toolbox designed to speak the many languages of chemical data [13].

Secondary Structure Prediction

The *Plasmodium falciparum* dihydrofolate reductase secondary structure was predicted using the CFSSP online server. CFSSP (Chou & Fasman Secondary Structure Prediction Server) is an online protein secondary structure prediction server which predicts regions of secondary structure from the protein sequence such as alpha helix, beta sheet, and turns from the amino acid sequence [14].

Druglikeness Prediction

The druglikeness attributes of the C=O analogue of gedunin and pyrimethamine were predicted using the SwissADME server which is web tool that gives access to a pool of fast yet robust predictive models for physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness, among which in-house proficient methods such as the BOILED-Egg, iLOGP and Bioavailability Radar [15].

Molecular Docking

This process was used to predict the binding energy between the *Plasmodium falciparum* dihydrofolate reductase and the 2 experimental ligands. The running of each docking process was done using the AutoDock vina software [16].

III. Results and Discussion

Plasmodium falciparum dihydrofolate reductase contains 608 amino acid residues. The docking structures of the two experimental compounds showed that they bind in a varying pattern with the active site of *Plasmodium falciparum* dihydrofolate reductase, as is evident from the superposition of the C=O analogue of gedunin and pyrimethamine in Figures 6 and 7. The interaction between the C=O analogue of gedunin and pyrimethamine with *Plasmodium falciparum* dihydrofolate reductase shows steric interaction with the amino acid residues. The calculated free energy of binding of the C=O analogue of gedunin and pyrimethamine were -9.0 and -8.0Kcal/mol (Figure 6 and 7). This confirms that the structural modification implemented in this study is significantly related to the compound activity [17, 18]. Also, this proved the reliability of the docking results [19].

Hydrogen-bonds play a crucial role in determining the specificity of ligand binding [20]. Their important contribution is explicitly incorporated into a computational method called GRID. This has been designed to detect energetically favourable ligand binding sites on a chosen target molecule of known structure [21]. It can be observed that substitution of the CH_3 substituent of gedunin with C=O led to an increase in the binding affinity of the modified analogue. It can also be observed that the polar interaction between *Plasmodium falciparum* dihydrofolate reductase and the C=O analogue of gedunin was at an angle of 48.5 and 78.8 degree and the compound interacted with the SER 101 residue of the enzyme while it can be observed that polar interaction between *Plasmodium falciparum* dihydrofolate reductase and pyrimethamine with between the ILE 14, ILE 154, ASP 54 and TYR 160 residues of the drug (figure 4 and 5).

The solubility of a compound in water could improve its biotransformation and elimination as a drug [22]. The C=O analogue of gedunin and pyrimethamine were soluble in water (figure 2 and 3). The molecular weight of both compounds was less than 500g/mol, showing that they can be considered as drug [23]. A compound can also be considered drug-like if it is characterized by high lipophilicity (less than 5) [24]. This is expressed as Log Po/w. The lipophilicity values of C=O analogue of gedunin and pyrimethamine are less than 5 and are most likely to be drugs.

Lipinski's rule of 5 [25] 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 [22]. The C=O analogue of gedunin and pyrimethamine violated none of the Lipinski's rule and therefore are likely to be drugs (figure 2 and 3).

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Fig. 1. *Plasmodium falciparum* DHFR Secondary Structure

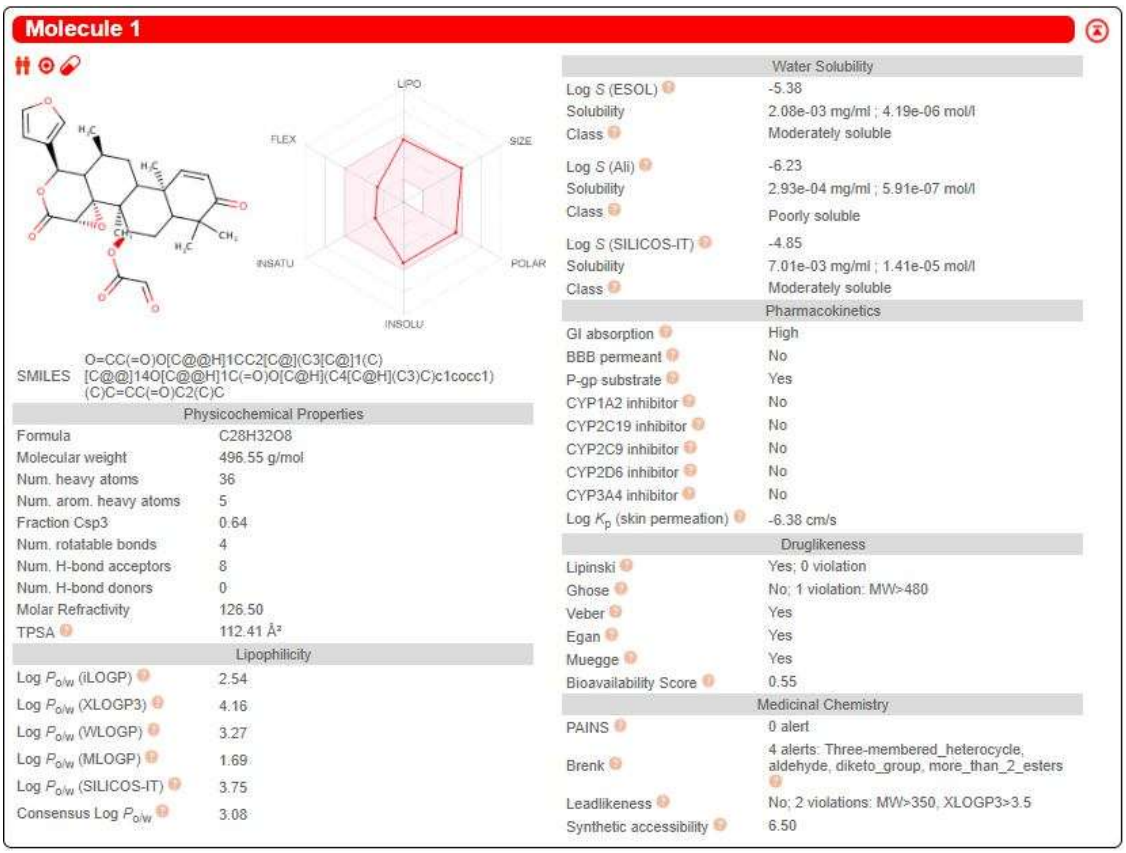

Fig. 2. Druglikeness Prediction of the C=O Analogue of Gedunin

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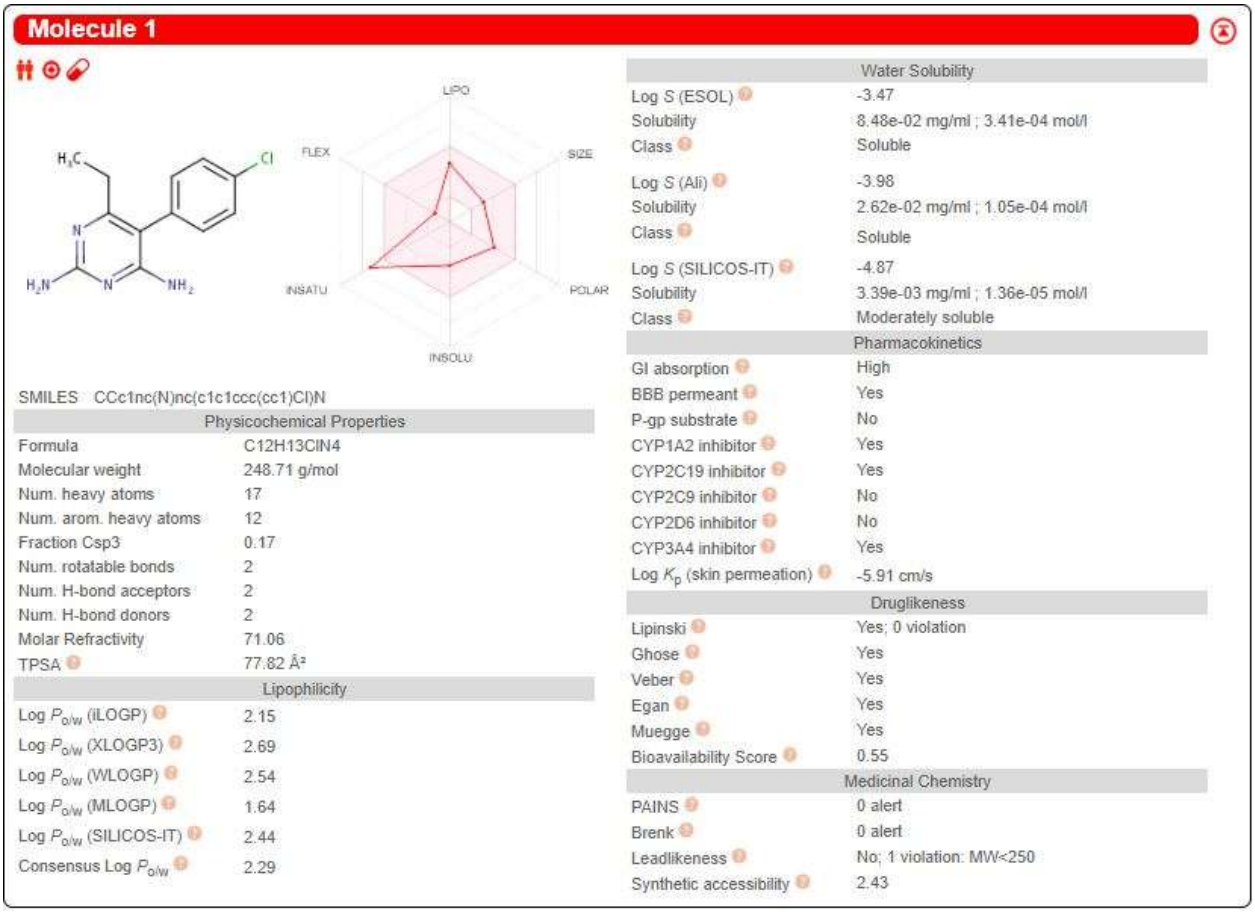

Fig. 3. Druglikeness Prediction of Pyrimethamine

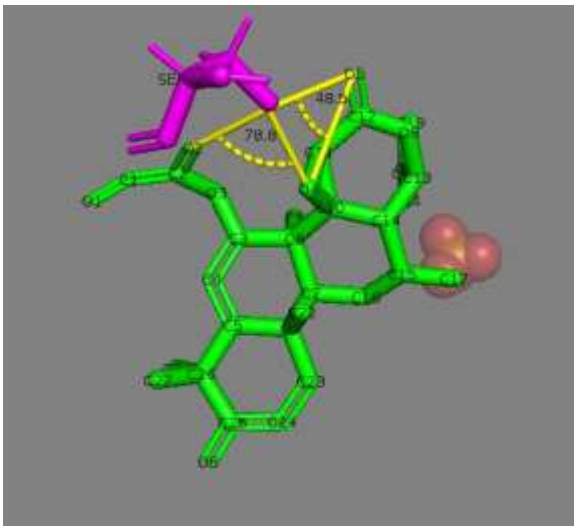

Fig. 4. Hydrogen Bond Interaction between the *Pf* DHFR Amino Acid Residues and the C=O Analogue of Gedunin

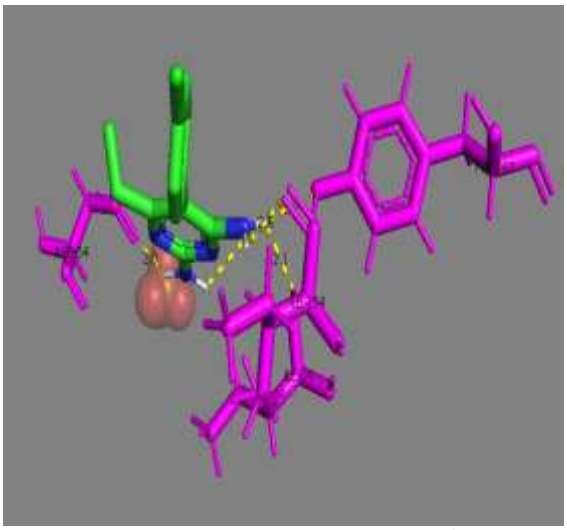

Fig. 5. Hydrogen Bond Interaction between the *Pf* DHFR Amino Acid Residues and Pyrimethamine Drug

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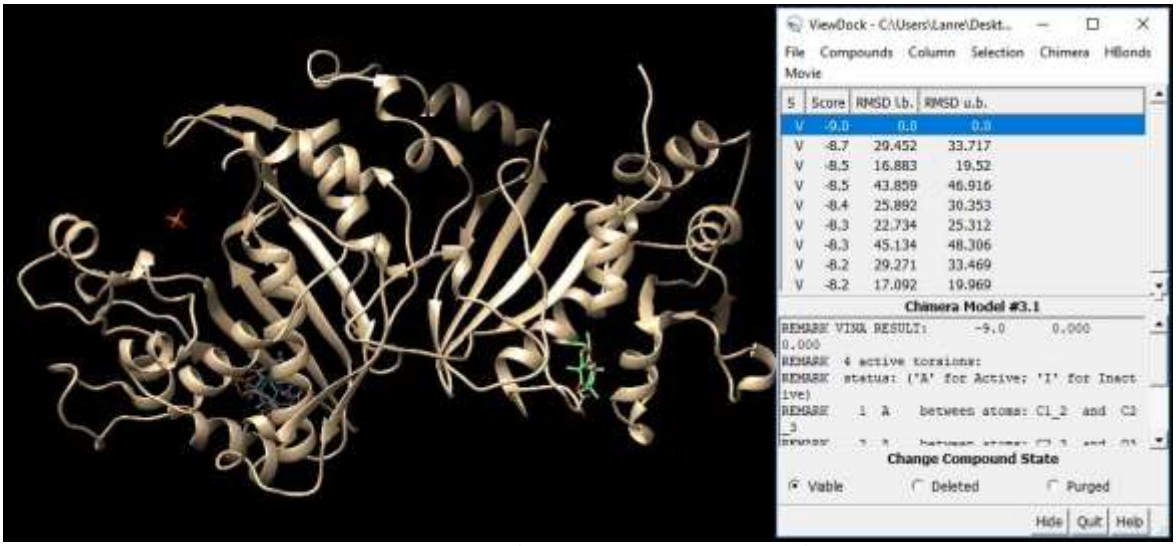

Fig. 6. C=O Analogue of Gedunin in Complex with the *Pf* DHAP

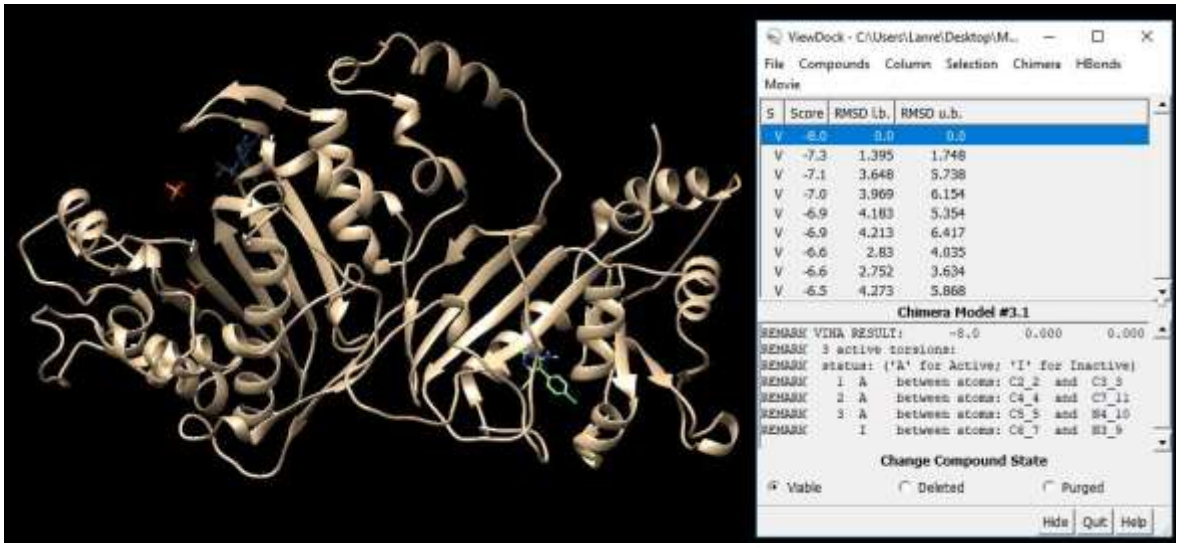

Fig. 7. Pyrimethamine in Complex with the *Pf* DHAP

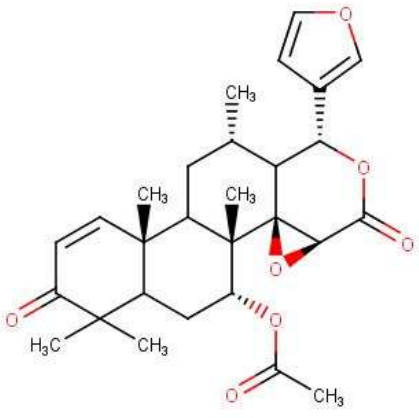

Fig. 8. 2D structure of Gedunin

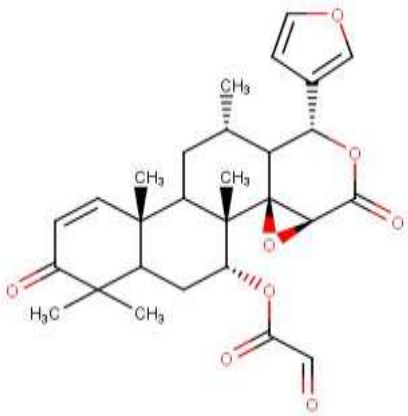

Fig. 9. 2D Structure of the C=O Analogue of Gedunin

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 [26]. Pharmacokinetically, the gastrointestinal drug absorption of both compounds was high. The C=O analogue of gedunin could not cross the blood brain

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barrier (BBB) and shows that it cannot cause any problem to the brain. Pyrimethamine showed a BBB permeant attribute.

For synthetic accessibility, values of 5 to10 means that the drug could be synthesized [22]. This shows that the synthesis of the C=O analogue of gedunin will be more difficult compared to pyrimethamine synthesis.

Secondary structure elements typically spontaneously form as an intermediate before the protein folds into its three dimensional tertiary structure [27]. It has been shown that α-helices are more stable, robust to mutations and designable than β-strands in natural proteins [28], thus designing functional all-α proteins is likely to be easier that designing proteins with both helices and strands; this has been recently confirmed experimentally [29]. The percentage helix according to the secondary structure prediction in figure 1 is 71.9. The high percentage helix is an indicator that the *Plasmodium falciparum* dihydrfolate reductase might be a stable enzyme.

IV. Conclusion

We carried out an In-Silico and molecular docking study on the inhibitory role of the C=O analogue of gedunin and pyrimethamine against the *Plasmodium falciparum* dihydrfolate reductase. The results obtained indicated that the C=O analogue of gedunin may have a better functional activity having shown a high binding energy value and exhibited a higher level of specificity and affinity against the target enzyme. The gedunin analogue also poses no threat to the Central Nervous System (CNS) as it does not penetrate the blood brain barrier.

Synthesis and pre-clinical studies of on the C=O analogue of gedunin against the *Plasmodium falciparum* dihydrfolate reductase is recommended.

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