Inhibitory Effect of Gedunin Analogue against the *Plasmodium falciparum* Dihydrofolate Reductase

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**Authors’ contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**ABSTRACT**

**Objective:** *Plasmodium* parasites are the cause of malaria. Malaria victims get infected upon being bitten by female anopheles mosquito; which transmits the parasite to the victim. The *P. falciparum* and *P. vivax* are the most active disease-causing agents of all five malaria-causing species of *Plasmodium*. The anti-folate drugs which were the first class of clinical antimetabolites act by disrupting metabolic pathways in which the one-carbon moiety supplied by the B9 folate vitamins is a major requirement.

**Methods:** Chemical structures of the anti-folate drugs which served as the experimental control ligands were downloaded from the PubChem database and saved as PDB files while the gedunin modification was achieved using the Marvin-Sketch software.

**Results:** Molecular visualization of the polar interactions with amino acid residues of the *Plasmodium falciparum* dihydrofolate-reductase showed that all the control ligands interacted with similar residues contrary to the interaction of the gedunin modified ligand in the same binding pocket.

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Conclusion: Results from the molecular docking study showed that gedunin and its C=O of gedunin might be better antimalarial agents; having exhibited the best binding energies with a score of -9.5 and -9.0 Kcal/mol respectively.

Keywords: Plasmodium falciparum; anti-folate drugs; gedunin; molecular docking; dihydrofolate reductase.

1. INTRODUCTION

Malaria is a major disease of global public health importance. 3.3 billion people in approximately 97 countries around the world are reportedly at risk of being infected. This leads to an estimated 228 million cases of malaria around the world and about 405,000 estimated deaths [1]. It is thought to attack pregnant women and young children more, especially in Africa and South-East Asia region. The Plasmodium species are the global causative pathogens of malaria, possessing a complex life cycle which alternates between the vertebrate host and the vectors which are the female Anopheles mosquitoes [2]. The infection requires the formation of unique zolie forms for the invasion of different types of cells at specific stages [3]. Hepatocytes get infected by the sporozoites as soon as they gain entrance into the host, followed by the blood asexual cycle. For the cycle to be complete, feeding mosquitoes get to ingest sexual forms which develop during the blood stage [4].

Gedunin is a natural compound with high bioactivity and potential of being developed into drugs [5]. The sources of gedunin can be traced to renewable raw materials like the Azadirachta indica (Neem), Cedrela sinensisJuss, Entandrophragma angolense (Welw) C.D.C. and most plants that belong to the Meliaceae family [6,7]. Gedunin, a highly oxidized triterpenoid also contain multiple functional groups. It has been reported that gedunin possesses a good number of biological activities, such as antimalarial [8], anti-feedant, insecticidal [9-11], antifungal [12], anti-prostate cancer [13,14], anti-leishmanial [15], anti-HIV properties and as a colon cancer cell potent inhibitor [14].

In the control of the malaria epidemic, drug resistance has been a major problem and resistance of such is what has been observed in the Plasmodium falciparum resistance to anti-folate drugs which inhibits the Plasmodium falciparum Dihydrofolatereductase [16]. The binding of drugs like pyrimethamine and cycloguanil which appears to be rigid competitive inhibitors to the active site of the enzyme is affected by the side chain steric conflict at position 16 and 108 amino acid residues of the enzyme which has undergone mutation. The drug binding is also affected by observed changes in the configuration of the main chain [17].

This study was aimed at structurally modifying gedunin, targeting the modified analogue at the Plasmodium falciparum dihydrofolatereductase and comparing the antimalarial effect with that of the selected antifolate drugs.

2. METHODOLOGY

Sequence and protein 3D structure retrieval: The amino acid sequence and crystallized 3D structure of Plasmodium falciparum dihydrofolate reductase were obtained from the Protein Data Bank repository [18]. The Protein Data Bank is the world's only repository for primary structural data on biological macromolecules. The PDB data is used to generate a plethora of secondary sources of information. It also remains the starting point for structural bioinformatics research [19].

Ligand preparation: The Marvin Sketch software was used to design the 2D structure of Gedunin, its modified derivative, and the three selected anti-folate drugs [20]. In preparation for docking, each designed structure was downloaded and saved as mrv files.

File conversion: The Open Babel Open Source Chemistry Toolbox was used to convert. SMILES strings were created by converting mrv files saved during the ligand preparation process (Simplified Molecular Input-Line-Entry System). Open Babel is a chemical toolbox designed to ‘speak’ many chemical data languages [21]. It is an open and collaborative project that allows users to search for, convert, analyze, and store data from molecular modeling, chemistry, solid-state materials, biochemistry, and related fields [22].
Ligand minimization: Using the UCSF Chimera software [23], each experimental ligand was minimized. UCSF Chimera is a program that analyzes and visualizes molecular structures and related data such as supramolecular assemblies, density maps, sequence alignment, molecular docking results, trajectories, and conformational ensembles. [24].

Visualizing polar interactions: The Pymol molecular visualizer was used to visualize weak interactions between experimental ligands and the Plasmodium falciparum dihydrofolate reductase [25]. PyMOL is an open-source model visualization tool that has been used in structural biology has been made available [26]. The word "Py" in the software's name alludes to the fact that it may be customized and extended using the Python programming language [27].

Molecular docking: Using the AutoDockVina software, the binding energy scores between the experimental ligands and the Plasmodium falciparum dihydrofolate reductase were predicted [28]. AutoDockVina is a software for molecular modeling and simulation. It is especially well-suited for protein-ligand docking [29].

3. RESULTS

3.1 2D Structure of Gedunin

Fig. 1 depicts gedunin's 2D structure as designed by the MarvinSketch software. The modification that resulted in the derivative of this compound was accomplished by replacing the methyl group attachment to the carbon-2 (C2) of the compound with a C=O group.

3.2 Binding Pocket Prediction

Fig. 3 depicts the binding of pyrimethamine to the predicted pocket 1 and the interaction with ILE 14 and 154, ASP 54, LEU 40, and TYR 160, whereas Fig. 3 depicts the interaction of each experimental ligand with amino acid residues in the Plasmodium falciparum dihydrofolate binding pocket.
3.3 Binding Energy Prediction and in Silico Pharmacokinetics

Table 1 each experimental compound's specific pharmacokinetics and drug-likeness parameters. The binding energy scores, which indicate the binding affinity of compounds to enzymes, are also shown in the table.

Table 1. Physicochemical properties, lipophilicity, solubility, pharmacokinetics and Lipinski drug-likeness of the anti-folate drugs, gedunin and its modified derivative

| Parameters                  | Gedunin  | C=O analogue | OH analogue | Cycloguanil | Proguanil | Pyremethamine |
|-----------------------------|----------|--------------|-------------|-------------|-----------|---------------|
| Molecular weight g/mol      | 482.57   | 496.55       | 484.54      | 251.72      | 253.73    | 248.71        |
| Docking score Kcal/mol      | -9.5     | -9.0         | -8.4        | -8.0        | -7.5      | -8.0          |
| Num. H-Bond acceptors       | 7        | 8            | 8           | 2           | 2         | 2             |
| Num. H-Bond donors          | 0        | 0            | 1           | 2           | 3         | 2             |
| TPSA Å²                     | 95.34    | 112.41       | 115.57      | 80.00       | 88.79     | 77.82         |
## Parameters

| Parameters                     | Gedunin C=O analogue | OH analogue | Cycloguanil | Proguanil | Pyremethamine |
|--------------------------------|----------------------|-------------|-------------|-----------|---------------|
| Lipophilicity Consensus Log P<sub><w></w> | 3.64                 | 3.08        | 3.27        | 1.35      | 1.59          | 2.29          |
| Water Solubility Log S         | Moderately Soluble   | Moderately Soluble | Soluble     | Soluble   | Soluble       |
| GI absorption                 | High                 | High        | High        | High      | High          |
| BBB permeant                  | No                   | No          | No          | No        | Yes           |
| P-gp substrate                | Yes                  | Yes         | Yes         | No        | No            |
| CYP1A2 inhibitor              | No                   | No          | No          | Yes       | No            |
| CYP2C19 inhibitor             | No                   | No          | No          | No        | Yes           |
| CYP2C9 inhibitor              | No                   | No          | No          | No        | No            |
| CYP2D6 inhibitor              | No                   | No          | No          | No        | No            |
| CYP3A4 inhibitor              | No                   | No          | No          | No        | Yes           |
| Lipinski Drug-likeness        | Yes; 0 Violation     | Yes; 0 Violation | Yes; 0 Violation | Yes; 0 Violation | Yes; 0 Violation | 0          |
| Synthetic accessibility       | 6.54                 | 6.50        | 6.54        | 3.27      | 2.68          | 2.43          |

**Table 2.** Polar interactions between the experimental ligands and the amino acid residues of the *Plasmodium falciparum* dihydrofolatereductase

| Drug / Ligand          | Amino acid residues |
|------------------------|---------------------|
|                        | ILE     | ASP | LEU | TYR | SER |               |
| Drugs                  |          |     |     |     |     |               |
| Cycloguanil            | 14, 154 | 54  | 40  |     |     |               |
| Proguanil              |          |     |     |     |     |               |
| Pyrimethamine          | 14, 154 | 54  | 40  | 160 |     |               |
| Ligands                |          |     |     |     |     |               |
| Gedunin                | 202     |     |     |     |     | 101           |
| C=O Analog             |          |     |     |     |     |               |

### 4. DISCUSSION

Lipinski’s rule of five, commonly referred to as Pfizer’s rule of five, is a guideline for identifying biological and pharmacological actions in certain compounds in order to assess physical and chemical characteristics and identify potential orally active medications for administration [30]. The general guideline is that oral active substances may not contravene more than one of the following conditions: The molecular mass of the compound must be less than 500 Da, the number of hydrogen bond donors must not exceed 5 (the sum of nitrogen-hydrogen and oxygen-hydrogen bonds), the number of hydrogen bond acceptors must not exceed 10 (all nitrogen or oxygen atoms), and the octanol-water partition coefficient (log P) must not be greater than 5 [31]. The Lipinski’s rule results in Table 1 show that all of the experimental ligands could be orally active compounds and thus be considered drug-like.

The polar surface area (PSA) of a molecule, also known as the topological polar surface area (TPSA), is defined as the sum of all polar atoms (oxygen and nitrogen), including hydrogen atom attachments. The polar surface area is a metric...
that is frequently used in medicinal chemistry to optimize drug cell permeation. Molecules with a PSA value greater than 140 angstroms squared have a poor ability to penetrate cell membranes" [32]. “For molecules to cross the blood-brain barrier (BBB) and act on central nervous system receptors, the polar surface area must be less than 90 angstroms squared” [33]. According to the results of the TPSA column in Table 1, the 3 anti-folate drugs used in this study may have blood brain barrier permeation ability. Cycloguanil, proguanil, and pyrimethamine have TPSA values of 80.00, 88.79, and 77.82, respectively, and these values (less than 90Å²) increase their likelihood of possessing the blood brain barrier permeation attribute. On the contrary, gedunin and its C=O derivative have TPSA values greater than 90Å², indicating that the compounds are safe for use as potential antimalarial drugs.

The partition coefficient of n-octanol and water (log Po/w) is the traditional method for describing lipophilicity. The diversity of the models supporting the predictors improves prediction accuracy when using the consensus log Po/w [34]. For the purposes of this study, the lipinski’s rule [30] was used as the drug-likeness descriptor, and the optimal lipophilicity range (Log Po/w) allowed should not exceed 5. The observation from table 1’s consensus lipophilicity column shows that all of the experimental ligands are within the optimal lipophilicity range and thus can be considered drug-like compounds.

When molecules are soluble, drug development activities can be facilitated and simplified. This makes drug handling and formulation easier [35]. Furthermore, for discovery projects aimed at oral administration, one of the major absorption properties influences the compound’s solubility [36]. “Furthermore, drugs designed for parenteral administration necessitate a high solubility attribute to facilitate the delivery of a significant amount of the active ingredient in smaller volumes of pharmaceutical dosage” [37]. If the Log S value of a compound is less than 6, it is considered soluble [35]. According to the column projecting the solubility result in Table 1, gedunin, its modified analogue, and the three selected anti-folate drugs used for this study are all water soluble, implying that they may be easily absorbed.

Drug absorption is influenced by the nature of the gastrointestinal mucosal membrane surface area, which varies and differs from the stomach to the rectum. It is also believed that the luminal content’s physiochemical characteristics affect how well drugs are absorbed [38]. The basic partition of pH hypothesis, which states that absorption is governed by the equilibrium location of the ionized and non-ionized drug forms at varied physiological pH levels encountered in the gastrointestinal tract, is a common way to describe the absorption process [39]. The high gastrointestinal absorption rates shared by all of the experimental ligands suggest that they might improve medication bioavailability.

Overcoming the ability of a non-neuroactive drug to cross the blood brain barrier is a major challenge in the drug design process. Only neuroactive drugs are required to have the blood brain permeation property in order to function. Non-neuroactive drugs, on the other hand, should not cross the blood brain barrier to avoid psychotropic side effects [40]. With the exception of pyrimethamine, the experimental ligands chosen for this study were predicted to be safe for administration as antimalarial agents due to their inability to cross the blood brain barrier.

By constantly pumping toxic compounds, xenobiotics, and drugs out of cells, the P-glycoprotein (P-gp) plays a physiological role in reducing the harmful effects of these substances. “The need for the P-role glycoprotein’s has led to the recognition of the modulation it confers on many important and clinical therapeutic agents, and this pharmacokinetic importance has led to the inclusion of its screening in any drug discovery process” [40]. “Drug pharmacokinetic parameters can also be influenced by various drug-induced induction or inhibition aimed at modulating drug transporters, which can result in a significant drug-drug interaction” [41]. Because the three selected anti-folate drugs did not appear to be P-glycoprotein substrates, their oral bioavailability was preserved. Gedunin and its C=O analogue are P-gp substrates that, when considered in a dose-dependent manner, may significantly reduce the bioavailability of these drug-like compounds orally in terms of their antimalarial activity.

The biotransformation process mediated by the intestinal CYP3A4 and the constant pumping of absorbed drugs out of the cell mediated by the P-glycoprotein can determine the bioavailability of drugs designed for oral administration. It has been proposed that the actions of CYP3A4 and P-glycoprotein may work together to reduce oral
drug bioavailability, and considering this hypothesis theoretically makes it more appealing [41]. The recent test on the hypothesis that drugs interacting with the apical efflux pump can enhance substrate disappearance mediated by CYP3A4 suggests that P-gp/CYP3A4 are co substrates and that P-glycoprotein increases the potentials of CYP3A4-mediated drug disappearance during secretory detoxification. [42] in the intestine. It is also possible that the P-glycoprotein influences first-pass metabolism in a cooperative manner [43]. Table 1 demonstrated that pyrimethamine, unlike other experimental ligands, may have a higher bioavailability due to being the only CYP3A4 inhibitor among all compounds. Other experimental ligands may be subjected to CYP3A4-mediated intestinal biotransformation, reducing their bioavailability.

Many areas in the drug discovery process require estimation models and methods for determining the ease of synthesizing drug-like molecules (synthetic accessibility). The assessment of a lead candidate's synthetic accessibility (SA) is a task that takes part in the discovery of lead while disregarding methods the lead candidate has been known with. After normalization, the synthetic accessibility score ranges from 1 (very easy) to 10 (very difficult) [44]. The laboratory synthesis of gedunin and its C=O analogue may be slightly challenging, with synthetic accessibility scores of 6.54 and 6.50, respectively.

Hydrogen bonds and hydrophobic interactions are two examples of weak molecular interactions that are thought to be good protein-ligand binding facilitators [45]. They specifically contribute to ligand stability and efficacy at a protein structure's active site [46]. The hydrogen bond interaction between the experimental ligands/drugs and the amino acid residues of the Plasmodium falciparum DHFR is shown in Table 2. The information gleaned from these interactions was used to predict the amino acid residues that comprise the enzyme binding site. The three anti-folate drugs tested positive for interactions with the ILE 14 and 154, ASP 54, LEU 40, and TYR 160, confirming the existence of the binding pocket 1 depicted in Fig. 3. Gedunin and its C=O analogue also interacted with the same pocket's ASP 202 and SER 101 residues. Fig. 3 depicts the experimental ligands' individual interactions with the amino acid residues of Plasmodium falciparum DHFR at various angles, as well as the two bound PO4 prosthetic groups.

Because of the increasing reliability of simulation theories and software used in molecular docking processes, the use of molecular docking methods in academia and the pharmaceutical industries has increased [47]. The molecular docking study in this experiment revealed that gedunin and its C=O analogue have higher binding energy when docked against the Plasmodium falciparum DHFR than the selected anti-folate drugs. Gedunin had the highest binding energy with a score of -9.5Kcal/mol, while proguanil had the lowest binding energy with a score of -7.5Kcal/mol (Table 1). Cycloguanil and pyrimethamine had the highest binding energy among the bound anti-folate drugs, both with a score of -8.0Kcal/mol.

5. CONCLUSION

A molecule's total amount of polar (oxygen and nitrogen) atoms, including hydrogen atom attachments, is known as its polar surface area (PSA). The TPSA values for cycloguanil, proguanil, and pyrimethamine were determined to be 80.00, 88.79, and 77.82 respectively. The descriptor for drug similarity was the Lipinski's rule. The maximum permitted lipophilicity range (Log Po/w) should not be greater than 5. All three of the chosen anti-folate medications as well as gedunin's modified counterpart are water soluble.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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