Inhibitory Effect of Gedunin Analogue against the *Plasmodium falciparum* DihydrofolateReductase

ABSTRACT

OBJECTIVE: *Plasmodium* parasites are the cause of malaria. Malaria victims get infected upon being bitten by female anopheles mosquito; which transmit 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.
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, Dihydrofolatereductase

BACKGROUND

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 zoite forms for the invasion of different types of cells at specific stages [3]. Hepatocytes get infected by the sporozoites as soon 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, 10, 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 anti-folate drugs.

METHODOLOGY

Sequence and Protein 3D structure Retrieval: The *Plasmodium falciparum* dihydrofolatereductase amino acid sequence and the crystallized 3D structure were downloaded from the Protein Data Bank repository [18]. The Protein Data Bank is the single worldwide storage of biological macromolecules in their primary structural data form. Numerous secondary sources of information are obtained from the PDB data. It also remains the basic point for the commencement of structural bioinformatics studies [19].

Ligand Preparation: The 2D structure of Gedunin, its modified derivative and the three selected anti-folate drugs were designed using the MarvinSketch software [20]. Every designed structure were downloaded and saved as mrv files in preparation for docking.

File Conversion: Saved mrv files from the ligand preparation process were converted into SMILES strings (Simplified Molecular Input-Line-Entry System) using the Open Babel Open Source Chemistry Toolbox. Open Babel is a chemical toolbox designed to 'speak' many languages of chemical data [21]. It is an open and collaborative project, which allows one make searches, conversions, analysis, or storage data from molecular modeling, chemistry, solid-state materials, biochemistry, and related areas [22].

Ligand Minimization: Each of the experimental ligand was minimized using the UCSF Chimera software [23]. UCSF Chimera is an extensible program for analyzing and interactively visualizing molecular structures and related data which include supramolecular assemblies, density maps, alignment of sequences, results from molecular docking, trajectories and conformational ensembles [24].

Visualizing Polar Interactions: Weak interactions between experimental ligands and the *Plasmodium falciparum* dihydrofolatereductase were visualized using the Pymol molecular visualize [25]. PyMOL is an open-source tool for model visualization and it is made available for utilization in structural biology [26]. The Py aspect of the name of the software is a reference pointer that it is extensible and extends by the python programming language [27].

Molecular Docking: The binding energy scores between the experimental ligands and the *Plasmodium falciparum* dihydrofolatereductase was predicted using the AutoDockVina software [28]. AutoDockVina is a molecular modeling and simulation software. It is especially designed and effective for protein-ligand docking [29].

RESULTS

2D Structure of Gedunin

Figure 1 shows the 2D structure of gedunin as designed by the MarvinSketch software. The modification that resulted into the derivative of this compound was made through the substitution of the methyl group attachment to the carbon-2 (C_2) of the compound with C=O group.

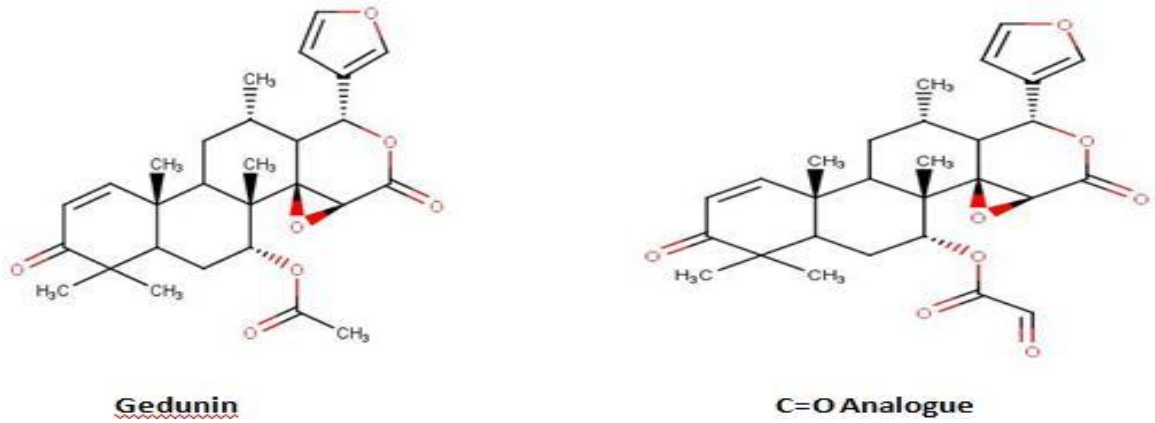

Figure 1: 2D structure of Gedunin and its modified analogue

Binding Pocket Prediction

The illustrations in figure 3 shows the binding of pyrimethamine to the predicted pocket 1 and the interaction with ILE 14 and 154, ASP 54, LEU 40 and TYR 160 while figure 3 shows the interaction of each experimental ligand with amino acid residues in the binding pocket of the *Plasmodium falciparum* dihydrofolate.

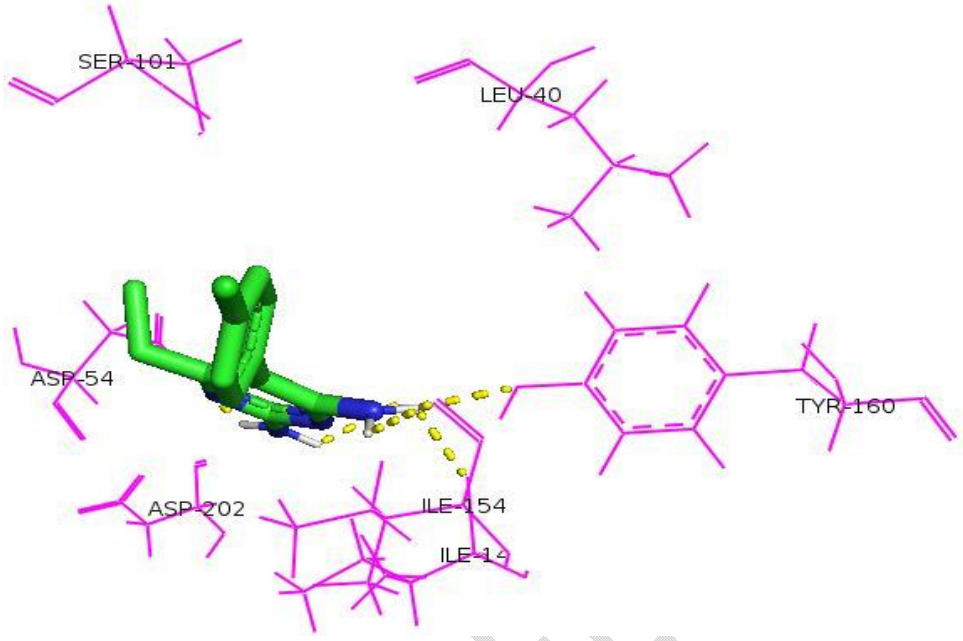

Figure 2: Pyrimethamine interaction with the amino acid residues of the predicted Pf DHFR binding pocket

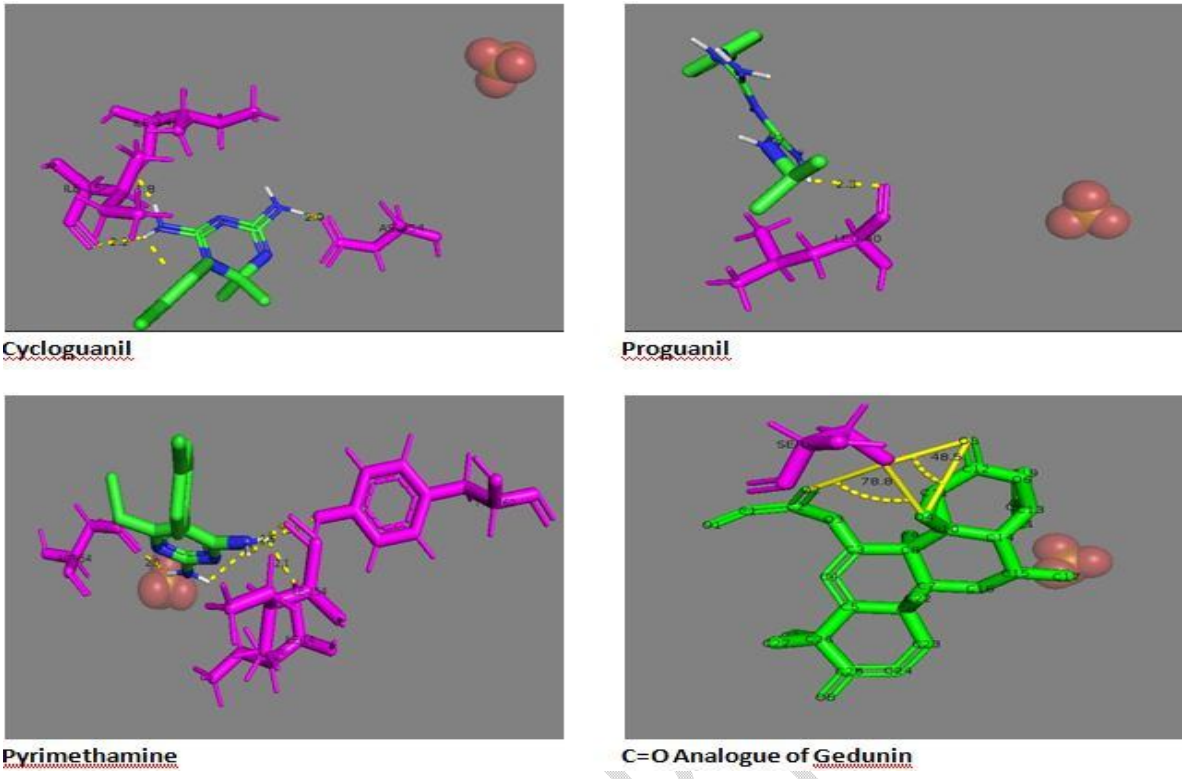

Figure 3: Individual interaction of experimental ligands with the amino acid residues of the predicted Pf DHFR binding pocket

Binding Energy Prediction and In Silico Pharmacokinetics

Table 1 illustrates the specific pharmacokinetics and drug-likeness parameters of each experimental compound. The binding energy scores which are pointers to the binding affinity of compounds to enzymes were also illustrated 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
Formula	$C_{28}H_{34}O_7$	$C_{28}H_{32}O_8$	$C_{27}H_{32}O_8$	$C_{11}H_{14}ClN_5$	$C_{11}H_{16}ClN_5$	$C_{12}H_{13}ClN_4$
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-	0	0	1	2	3	2

Bond donors						
TPSA Å²	95.34	112.41	115.57	80.00	88.79	77.82
Lipophilicity	3.64	3.08	3.27	1.35	1.59	2.29
Consensus Log $P_{o/w}$						
Water Solubility	Moderately Soluble	Moderately Soluble	Moderately Soluble	Soluble	Soluble	Soluble
Log S						
GI absorption	High	High	High	High	High	High
BBB permeant	No	No	No	No	No	Yes
P-gp substrate	Yes	Yes	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	No	No	No	No
CYP3A4 inhibitor	No	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

Polar Contacts

As presented in table 2, a total of 5 amino acid residues were involved in the formation of polar contacts with the experimental ligands. The experimental ligands include 3 anti-folate drugs, gedunin and its C=O derivatve.

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			
	Proguanil			40		
	Pyrimethamine	14, 154	54	40	160	
LIGANDS	Gedunin		202			
	C=O Analog					101

DISCUSSION

Lipinski's rule of five which can also be referred to as the Pfizer's rule of five is a rule described for the evaluation of drug-likeness or for the determination of biological and pharmacological activities in specific compounds for the purpose of evaluating physical and chemical properties in determining likely orally active drugs for administration [30]. The rule states that, orally active drugs in general must not violate more than one of the following criteria: The hydrogen bond donors must not be more than 5 (the summation of nitrogen–hydrogen and oxygen–hydrogen bonds), hydrogen bond acceptors must not exceed 10 (all nitrogen or oxygen atoms), the molecular mass of the compound must be less than 500Da, an octanol-water partition coefficient (log P) must not exceed 5 [31]. The results obtained from Table 1 regarding the lipinski's rule shows that all the experimental ligands might be orally active compounds and as such can be considered drug-like.

The polar surface area (PSA), also known as the topological polar surface area (TPSA) of a molecule is defined as the sum of all polar atoms (oxygen and nitrogen), with the inclusion of the hydrogen atom attachments. The polar surface area is a metric that is often used in medicinal chemistry to optimize the cell permeation ability of drugs. Molecules with a PSA value higher than 140 angstroms squared are known to be poor in cell membrane penetration [32]. For molecules to penetrate the blood–brain barrier (BBB) (in order to act on the central nervous system receptors), the value assigned to the polar surface area must be less than 90 angstroms

squared [33]. The 3 anti-folate drugs used for the purpose of this study might possess the blood brain barrier permeation ability as observed from the result of the TPSA column in table 1. Cycloguanil, proguanil and pyrimethamine were shown to have a TPSA value of 80.00, 88.79 and 77.82 $Å^2$ respectively and these values (lower than 90$Å^2$) increases their likelihood of possessing the blood brain barrier permeation attribute. On the contrary, gedunin and its C=O derivative have TPSA values higher than 90$Å^2$ which makes the compounds safe for administration as potential antimalarial drugs.

The partition coefficient between n-octanol and water (log Po/w) serves as the classical method for the description of lipophilicity. The diversity of the models backing the predictors will increase the accuracy in the prediction using the consensus log Po/w [34]. The lipinski's rule [30] was used as the drug-likeness descriptor for the purpose of this study and the optimal lipophilicity range (Log $P_{o/w}$) allowed should not exceed 5. The observation from the concensus lipophilicity column of table 1 shows that all the experimental ligands are within the optimal lipophilicity range and as such can be regarded as drug-like compounds.

Activities regarding drug development can be facilitated and made easier in cases where molecules are soluble. This brings about ease in drug handling and its formulation [35]. Moreover, for discovery projects that target the oral form of administration, one of the major absorption property influences the solubility of the compound [36]. Also, drugs that are designed for parenteral administration requires a high solubility attribute to aid the delivery of an appreciable amount of the active ingredient in smaller volumes of pharmaceutical dosage [37]. A compound can be considered as soluble if the Log S value is less than 6 [35]. Gedunin, its modified analogue and the three selected anti-folate drugs used for the purpose of this study, according to the column projecting the solubility result in table 1 are all water soluble, implying that they might be easily absorbed.

The nature of the gastrointestinal mucosal membrane surface area plays an important role in the process of drug absorption and it has a varying and differential effect from the stomach to the rectum. The physiochemical properties of the luminal content are also implicated to have an influence in drug absorption process [38]. The absorption process itself is continually described in terms of hypothesis of simple partition of pH, where absorption is controlled by the equilibrium position between the ionized and non-ionized forms of the drug at varying physiological pH values encountered in the gastrointestinal tract [39]. All the experimental ligands possess a high gastrointestinal absorption rate, implying their ability in aiding drug bioavailability.

Overcoming the ability of a non-neuroactive drug to cross the blood brain barrier is a major challenge to be solved in the processes of designing drugs. Only neuroactive drugs are required to possess the blood brain permeation attribute for functionality. On the contrary, non-neuroactive drugs should not cross the blood brain barrier for the avoidance of psychotropic side effects [40]. The experimental ligands selected for the purpose of this study with the exclusion of pyrimethamine have been predicted to be safe for administration as antimalarial agents with regard to their inability to cross the blood brain barrier.

The P-glycoprotein (P-gp) is involved physiologically in the reduction of the harmful effects of toxic compounds, xenobiotics and drugs which the body is exposed to by constantly pumping them out of cells. The need for the role played by the P-glycoprotein 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 incorporation of its screening in any process involving drug discovery [40]. Drug pharmacokinetic parameters can also be affected through various drug induced induction or inhibition directed at modulating drug transporters and this can lead to a significant drug-drug interaction [41]. The 3 selected anti-folate drugs appeared to be no substrates of the P-glycoprotein hence their oral bioavailability remains intact. Gedunin and its C=O analogue are P-gp substrates which when considered in a manner that is dose-dependent might significantly reduce the bioavailability of these drug-like compounds orally as regarding their activity as antimalarial drugs.

The bioavailability of drugs designed for oral administration can be determined by the biotransformation process mediated by the intestinal CYP3A4 and the constant pumping of absorbed drugs out of the cell which is a process mediated by the P-glycoprotein. It has been hypothesized that the action of the CYP3A4 and P-glycoprotein may be in concert to reduce oral drug bioavailability and viewing this hypothesis from a theoretical point of view makes it more attractive [41]. The recent test on the hypothesis of the possibility of the enhancement of substrate disappearance mediated by the CYP3A4 being stimulated by drugs interacting with the apical efflux pump suggests that the P-gp/CYP3A4 are co substrates and that P-glycoprotein increases the potentials of CYP3A4-mediated disappearance of drugs during secretary detoxification in the intestine [42]. It is also possible for the P-glycoprotein to have an influence on first-pass metabolism in a manner describing cooperativity [43]. Table 1 showed that the pyrimethamine unlike other experimental ligands might exhibit a higher bioavailability, being the only inhibitor of the CYP3A4 among all the compounds. Other experimental ligands might undergo CYP3A4-mediated intestinal biotransformation which in turn lowers their bioavailability.

Many areas in the process of drug discovery are in need of estimation models and methods for the determination of the ease of synthesizing drug-like molecules (synthetic accessibility).. The assessment of the synthetic accessibility (SA) of a lead candidate is a task which takes part in the discovery of lead, disregarding methods the lead candidate has been known with. The synthetic accessibility score ranges from 1 (very easy) to 10 (very difficult) after normalization process [44]. The laboratory synthesis of gedunin and its C=O analogue might be slightly difficult with their synthetic accessibility score ranging between 6.54 and 6.50 respectively.

Such weak molecular interaction as the hydrogen bonds and the hydrophobic interactions are considered generally as good protein-ligand binding facilitators [45]. They specifically contribute to the ligand stability and efficacy at the active site of a protein structure [46]. Table 2 shows the hydrogen bond interaction between the experimental ligands/drugs and the amino acid residues of the *Plasmodium falciparum* DHFR. The information retrieved from these interactions were used in the prediction of the amino acid residues which make up the enzyme binding site.

The 3 selected anti-folate drugs were observed to have interacted with the ILE 14 and 154, ASP 54, LEU 40 and TYR 160 and this confirms the existence of the binding pocket 1 as displayed in figure 3. Gedunin and its C=O analogue also interacted with the ASP 202 and SER 101 residues of the same pocket. Figure 3 shows the individual interaction of the experimental ligands with the amino acid residues of *Plasmodium falciparum* DHFR at different angles alongside the two bound PO_4 prosthetic group.

The application of molecular docking methods both in the world of academics and the pharmaceutical industries has been on the increase due to the increasing reliability of simulation theories and software used for the molecular docking processes [47]. The molecular docking study in this experiment have shown that gedunin and its C=O analogue, compared to the selected anti-folate drugs, possess higher binding energy when docked against the *Plasmodium falciparum* DHFR. Gedunin exhibited the highest binding energy with a score of -9.5Kcal/mol while proguanil was observed to have the lowest binding energy, with a score of -7.5Kcal/mol (table 1). The highest binding energy observed among the bound anti-folate drugs was exhibited by cycloguanil and pyrimethamine, both having a score of -8.0Kcal/mol.

CONCLUSION

In conclusion, the results from the molecular docking study revealed that gedunin and its modified analogue might be a better antimalarial when compared with the 3 selected anti-folate drugs, having displayed higher binding energies against the *Plasmodium falciparum* dihydrofolatereductase. Furthermore, all experimental ligands displayed favorable drug-like characteristics and as such can be regarded as drugs.

The binding pocket prediction was based on the polar interactions between the ligands and amino acid residues of the *Plasmodium falciparum* DHFR. The 3 selected anti-folate drugs have been observed to bind to the same pocket as that of gedunin and its C=O analogue. Further pharmacokinetics studies are therefore recommended to validate this pocket as an ideal antimalarial drug target. We also suggest that more structural modifications be made to gedunin in order to curb the of antimalarial drug resistance menace induced by the Plasmodium parasites.

The binding of gedunin to the *Plasmodium falciparum* DHFR produced the highest binding score and we observed that gedunin was the only ligand that formed a polar contact with the ASP 202 residue in binding pocket. This implies that the ASP 202 residue might be implicated in the determination of the high binding energy exhibited by gedunin. We therefore recommend that this residue be considered in the process of designing new anti-folate drugs as it might increase the inhibitory properties and potency of the therapeutic agents.

The laboratory synthesis of gedunin and its modified analogue is also recommended for the confirmation of their potentials as potent antimalarial agents.

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