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 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.
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

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 zoite 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, 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 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 dihydrofolatereductase [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 dihydrofolatereductase were predicted [28]. AutoDockVina is a software for molecular modeling and simulation. It is especially well-suited for protein-ligand docking [29].

RESULTS

2D Structure of Gedunin

Figure 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.

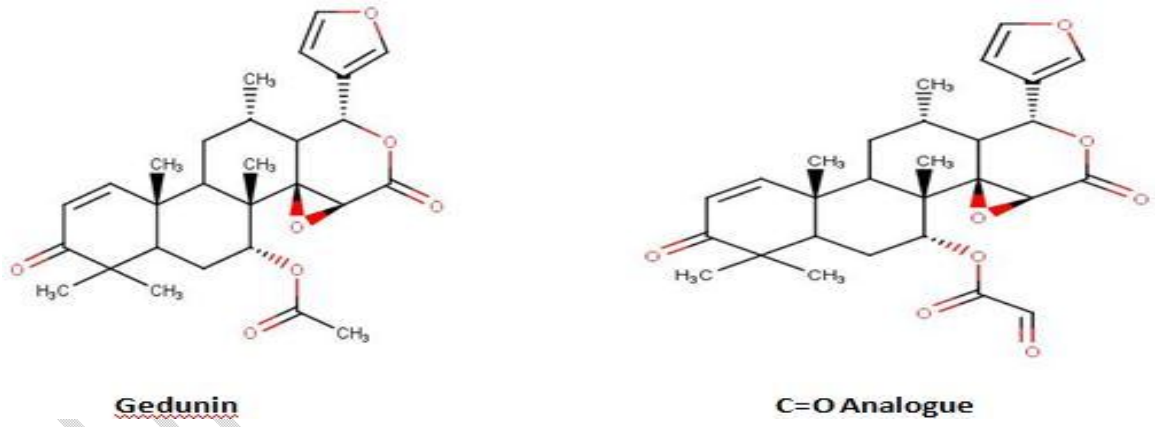

Figure 1: 2D structure of Gedunin and its modified analogue

Binding Pocket Prediction

Figure 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 figure 3 depicts the interaction of each experimental ligand with amino acid residues in the Plasmodium falciparum dihydrofolate binding pocket..

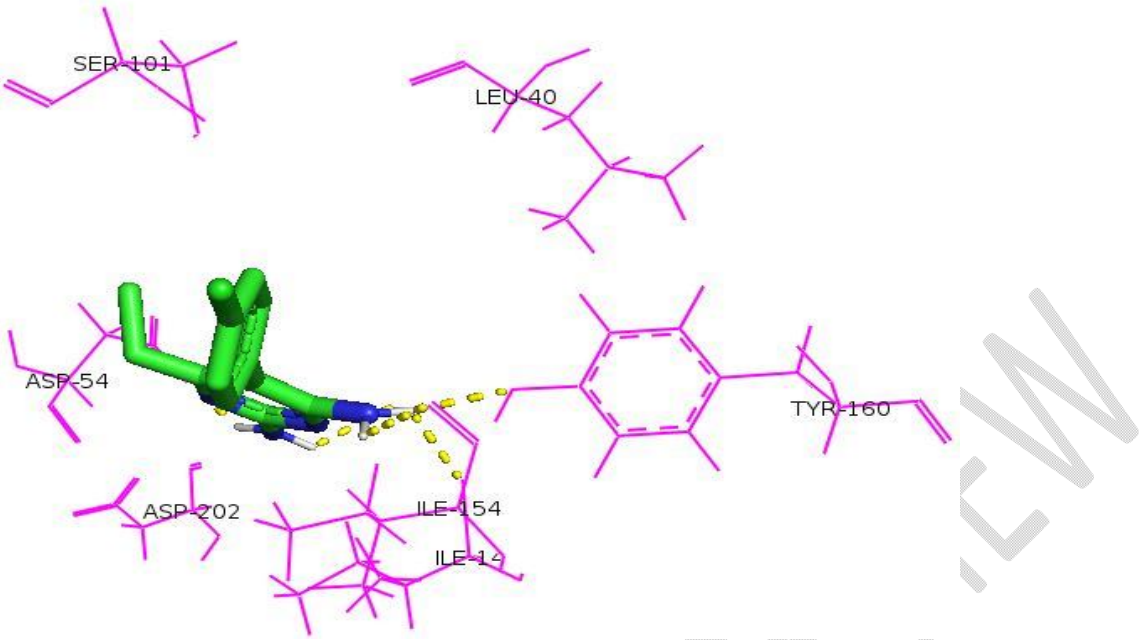

Figure 2: Pyrimethamine interaction with the amino acid residues of the predicted Pf DHFR binding pocket

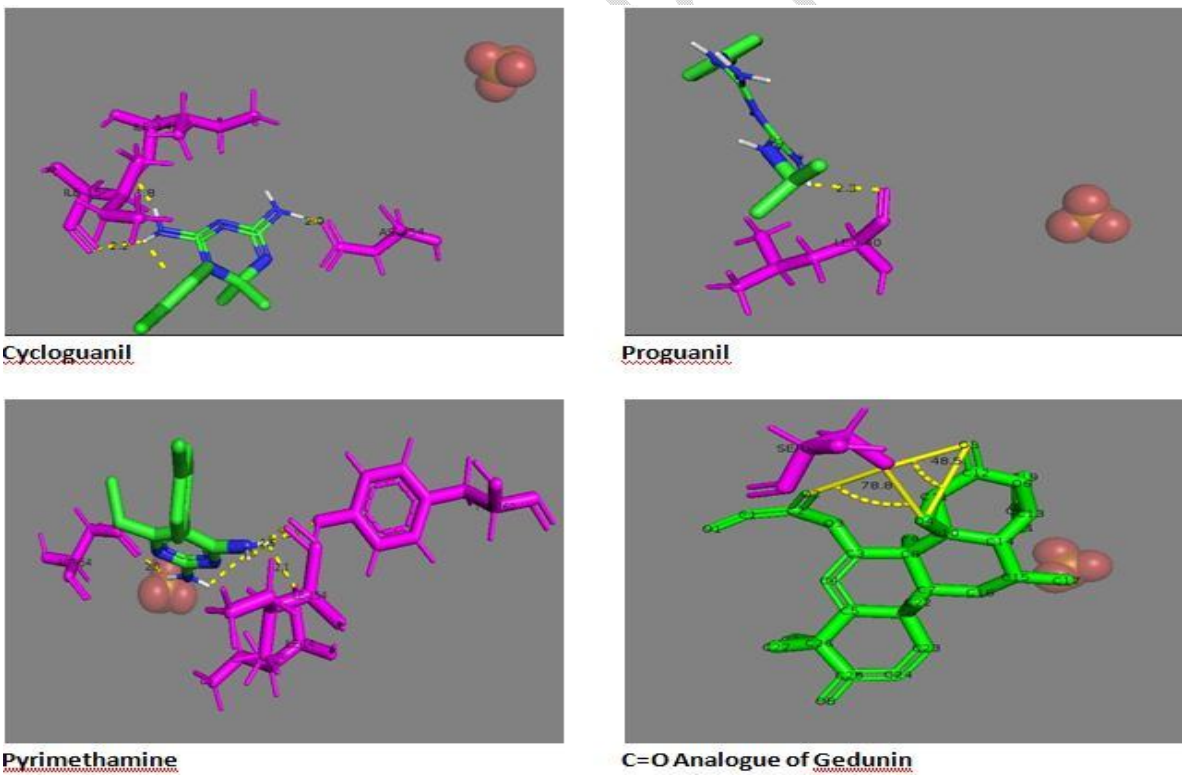

Figure 3: Individual interaction of experimental ligands with the amino acid residues of the predicted Pf DHFR binding pocket

Binding Energy Prediction and In Silico Pharmacokinetics

Table 1. 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
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-Bond donors	0	0	1	2	3	2
TPSA Å²	95.34	112.41	115.57	80.00	88.79	77.82
Lipophilicity Consensus Log $P_{o/w}$	3.64	3.08	3.27	1.35	1.59	2.29
Water Solubility Log S	Moderately Soluble	Moderately Soluble	Moderately Soluble	Soluble	Soluble	Soluble
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	No	No	No	No	No	Yes

inhibitor						
Lipinski	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	0
Drug-likeness	Violation	Violation	Violation	Violation	Violation	
Synthetic accessibility	6.54	6.50	6.54	3.27	2.68	2.43

Polar Contacts

A total of 5 amino acid residues were involved in the formation of polar contacts with the experimental ligands, as shown in table 2. Three anti-folate drugs, gedunin and its C=O derivatve, are among the experimental ligands.

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

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 2, 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 secretary 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 figure 3. Gedunin and its C=O analogue also interacted with the same pocket's ASP 202 and SER 101 residues. Figure 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.

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 2 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.

REFERENCES

1. WHO (2019).The World Malaria Report https://www.who.int/publications/i/item/world-malaria-report-2019 ISBN 978 92 4 156515 8.
2. Weiss, G.E., Gilson, P.R., Taechalertpaisarn, T., Tham, W.H., de Jong, N.W., Harvey, K.L., Fowkes, F.J., Barlow, P.N., Rayner, J.C., Wright, G.J., (2015). Revealing the

sequence and resulting cellular morphology of receptor-ligand interactions during *Plasmodium falciparum* invasion of erythrocytes. PLoSPathog.11, e1004670. Published: https://doi.org/10.1371/journal.ppat.1004670

3. Vaidya AB, Morrisey JM, Zhang Z, Das S, Daly TM, Otto TD, Spillman NJ, Wyvratt M, Siegl P, Marfurt J, Wirjanata G, Sebayang BF, Price RN, Chatterjee A, Nagle A, Stasiak M, Charman SA, Angulo-Barturen I, Ferrer S, Belén Jiménez-Díaz M, Martínez MS, Gamo FJ, Avery VM, Ruecker A, Delves M, Kirk K, Berriman M, Kortagere S, Burrows J, Fan E, Bergman LW. (2014). Pyrazoleamide compounds are potent antimalarials that target Na+ homeostasis in intraerythrocytic *Plasmodium falciparum*. Nat Commun.5:5521. doi: 10.1038/ncomms6521.Available at: https://www.ncbi.nlm.nih.gov/pubmed/25422853
4. Tham, W.H., Healer, J., and Cowman, A.F. (2012).Erythrocyte and reticulocyte binding-like proteins of *Plasmodium falciparum*.Trends Parasitol. 28, 23–30. Available at: https://www.sciencedirect.com/science/article/pii/S1471492211001802
5. Cragg GM, Newman DJ, Snader KM (1997). Natural products in drug discovery and development. J. Nat. Prod. 60:52-60.Available at: https://pubs.acs.org/doi/10.1021/np9604893
6. Bray DH, Warhurst DC, Connolly JD, O'Neill MJ, Phillipson JD (1990). Plants as source of antimalarial drug.Pt.7 activity of some species of Meliaceae plants and their constituent limonoids.Phytotheraphy Resource. 4:29-35.Availabel at: https://onlinelibrary.wiley.com/doi/pdf/10.1002/ptr.2650040108
7. Khalid SA, Duddeck H, Gonzalez SM (1989). Isolation and Characterization of an antimalarial agent of the neem tree Azadirachta indica. Journal of Nat. Product. 52(5):922-927. Available at: https://www.ncbi.nlm.nih.gov/pubmed/2607354
8. Guo Z, Vangapandu S, Sindelar RW, Walker LA, Sindelar RD (2005). Biologically Active Quassinoids and their Chemistry: Potential leads for drug design. Curr. Med. Chem. 12(2):173-190. Available at: https://www.ncbi.nlm.nih.gov/pubmed/15638734
9. Schwinger M, Ehhammer B, Kraus W. (1983). Natural Pesticides from the Neem Tree (Azadirachtaindica A. Juss.). In: Schmutterer H, Ascher KRS (Ed.), Natural Products from the Neem Tree and Other Tropical Plants P.181.Proceedings of the 2nd International Neem Conference held at Rauishchholzhausen 1983), GTZ Eschborn 1984.Availble at: https://www.jstor.org/stable/4254854?seq=1#page_scan_tab_contents
10. Kraus WK, Maile M, Wunder RT, Vogler B (1994). Biologically active constituents of tropical and subtropical plants.Pure Applied Chemistry. 66(10/11):2347-2352. Available at: https://www.academia.edu/10168629/Biologically_active
11. Alessandra RPA, Ana CL, Fabiana CB, Paulo CV, João BF, Odair CB, M. Fátima das G. Fernandes S, Fernando CP, José AH, Maurício B (2006). Limonoids from andiroba oil and Cedrelafissilis and their insecticidal activity. Journal of Brazilian Chemical Society. 17(3):542-547. Available at:

https://www.researchgate.net/publication/26432010_Limonoids_from_andiroba_oil_and_Cedrela_fissilis_and_their_insecticidal_activity

12. Sundarasivarao BN, Madhusudhanarao J (1977). Antifungal activity of gedunin.Journal ofCurrent Science. 46:714-716. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4791507/
13. Haley H, Justin L, Kenneth NR, Xiao P, Cristina C, Anna R, Maria N, Jinyan D, Kimberly S, Srilakshmi M, Raj KN. Maloney JC, William CH, Gabriela C, Todd RG (2006). Gene expression signature-based chemical genomic prediction identifies a novel class of HSP90 pathway modulators. Cancer Cell. 10(4):321-330 Available at: https://www.ncbi.nlm.nih.gov/pubmed/17010675
14. Shaikh JU, Lutfun N, Jamil AS, Mohammad S, Tomasz B, Simon G, Moira M, Maureen B, Satyajit DS (2007). Gedunin, a limonoid from Xylocarpusgranatum, inhibits the growth of CaCo-2 colon cancer cellline In Vitro. Phytother. Res. 21(8):757-761. Available at: https://www.ncbi.nlm.nih.gov/pubmed/17450509
15. Hay AE, Loset JR, Ahua KM, Diallo D, Brun R, Hostettmann K (2007). LimonoidOrthoacetates and Antiprotozoal Compounds from the Roots of Pseudocedrelakotschyi. J. Nat. Prod. 70(1):9-13. Available at: https://www.ncbi.nlm.nih.gov/pubmed/17253841
16. ALT, F. W., KELLEMS, R. E., BFRTINO, J. R. and SCraMS, R.T. (1978) Selective multiplication of dihydrofolatereductase gene in methotrexate-resistant variants of cultured murine ceils. Journal of biological Chemistry. 253: 1357-1370. Available at: https://www.ncbi.nlm.nih.gov/pubmed/1422048
17. Banval, H. S. and InselburgJ. (1986) *Plasmodium falciparum*: Induction, selection and characterization of pyrimethamine-resistant mutants. Experimental Parasitology, 62: 61-70. Available at: https://www.ncbi.nlm.nih.gov/pubmed/3522262
18. Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N. and Bourne, P. E. (2000). The Protein Data Bank. Nucleic Acids Research, 28(1): 235–242. Available at: https://www.ncbi.nlm.nih.gov/pubmed/10592235
19. Bernstein, F. C., Koetzle, T. F., Williams, G. J., Meyer, E. F., Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T. &Tasumi, M. (1977).The Protein Data Bank: a computer-based archival file for macromolecular structures. J. Mol. Biol. 112, 535-542. Available at: https://www.ncbi.nlm.nih.gov/pubmed/875032
20. Chen, J., Swamidass, S. J., Dou, Y., Bruand, J., and Baldi, P. (2005). ChemDB: a public database of small molecules and related chemoinformatics resources. Bioinformatics. 21(22): 4133–4139. Available at: https://www.ncbi.nlm.nih.gov/pubmed/16174682
21. Noel, M. O., Michael, B., Craig, A. J., Chris, M., Tim, V. and Geoffrey, R. (2011). Hutchison Open Babel: An open chemical toolbox. Journal of Cheminformatics,3: 33. Available at: https://www.ncbi.nlm.nih.gov/pubmed/16174682

22. Bultinck, P., Langenaeker, W., Lahorte, P., De Proft, F., Geerlings, P., Van Alsenoy, C. and Tollenaere. J. P. (2002). The Electronegativity Equalization Method II: Applicability of Different Atomic Charge Schemes. Journal of Physical Chemistry. 106: 7895-7901. Available at: https://biblio.ugent.be/publication/162270
23. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C. and Ferrin, T. E. (2004)."UCSF Chimera--a visualization system for exploratory research and analysis". J Computational Chemistry, 25(13): 1605–1612. Available at: https://www.ncbi.nlm.nih.gov/pubmed/15264254
24. Goddard, T. D., Huang, C. C., Meng, E. C., Pettersen, E. F., Couch, G. S., Morris, J. H. and Ferrin, T. E. (2017). UCSF chimerax: meeting modern challenges in visualization and analysis. Protein Science. 27 (1).32-35. Available at: https://www.ncbi.nlm.nih.gov/pubmed/28710774
25. Zhu, K., Day, T., Warshaviak, D., Murrett, C., Friesner, R. and Pearlman, D., (2014).Antibody structure determination using a combination of homology modeling, energy-based refinement, and loop prediction. Proteins, 82(8): 1646-1655.Available at:https://www.ncbi.nlm.nih.gov/pubmed/24619874
26. Salam, N. K., Adzhigirey, M., Sherman, W. and Pearlman, D. A. (2014). Structure-based approach to the prediction of disulfide bonds in proteins. Protein Engineering, Design and Selection, 27(10): 365-374. Available at:https://www.ncbi.nlm.nih.gov/pubmed/24817698
27. Beard, H., Cholleti, A., Pearlman, D., Sherman, W. and Loving, K. A. (2013). Applying Physics-Based Scoring to Calculate Free Energies of Binding for Single Amino Acid Mutations in Protein-Protein Complexes, PLoS ONE, 8(12): 828-849.Available at:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3858304/
28. Sousa, S. F., Fernandes, P. A. and Ramos, M. J. (2006). Protein-ligand docking: Current status and future challenges". Proteins: Structure, Function, and Bioinformatics. 65(1): 15–26. Available at:https://www.ncbi.nlm.nih.gov/pubmed/16862531
29. Schames, J. R., Henchman, R. H., Siegel, J. S., Sotriffer, C. A., Ni, H. and McCammon, J. A. (2004). Discovery of a novel binding trench in HIV integrase. Journal of Medicinal Chemistry,47(8): 1879-1881.Available at: https://pubs.acs.org/doi/abs/10.1021/jm0341913
30. Lipinski, C. A., Lombardo, F., Dominy, B. W. and Feeney, P. J. (2001).Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews, 4(1–3): 3–26.Available at:https://www.ncbi.nlm.nih.gov/pubmed/11259830
31. Leo A, Hansch, C. and Elkins, D. (1971).Partition coefficients and their uses. Chemical Reviews, 71(6): 525–616.Available at:https://pubs.acs.org/doi/10.1021/cr60274a001
32. Pajouhesh, H. and Lenz, G. R. (2005). Medicinal Chemical Properties of Successful Central Nervous System Drugs. NeuroRx, 2(4): 541-553.Available at:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1201314/

33. Hitchcock, S. A. and Pennington, L. D. (2006).Structure - Brain Exposure Relationships. Journal of Medicinal Chemistry, 49(26): 7559–7583. Available at:http://pubs.acs.org/doi/abs/10.1021/jm060642i
34. Mannhold, R., Poda, G. I. and Ostermann, C. (2009). Calculation of molecular lipophilicity: State□of□the□art and comparison of log P methods on more than 96,000 compounds. Journal of Pharmacological Science, 98: 861–893. Available at:https://onlinelibrary.wiley.com/doi/abs/10.1002/jps.21494
35. Ritchie, T. J., Macdonald, S. J. F., Peace, S., Pickett, S. D. and Luscombe, C. N. (2013).Increasing small molecule drug developability in suboptimal chemical space. Medicinal Chemistry Communications, 4: 673. Available at: http://pubs.acs.org/doi/full/10.1021/jm500515d?src=recsys
36. Ottaviani, G. (2010). What is modulating solubility in simulated intestinal fluids? European Journal of Pharmaceutical Sciences,41: 452–457.Available at:https://www.ncbi.nlm.nih.gov/pubmed/20656026
37. Savjani, K. T., Gajjar, A. K. and Savjani, J. K. (2012). Drug solubility: importance and enhancement techniques. ISRN Pharm 2012, 195727.Available at:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3399483/
38. Bogentoft, C., Carlsson, I., Ekenved, G. and Magnusson, A. (1978). Influence of food on the absorption of acetylsalicylic acid from enteric-coated dosage forms. European Journal of clinical Pharmacology, 14: 351- 355.Available at: https://www.ncbi.nlm.nih.gov/pubmed/32046
39. Borgstroem, B., Dahlqvist, A., Lundh, G. &Sjovall, J. (1957).Studies of intestinal digestion and absorption in the lumen.Journal of clinical investigation, 36: 1521- 1536. Available at:http://journals.sagepub.com/doi/abs/10.1177/003693307301800502
40. Wolak, D. J.and Thorne, R. G. (2013). Diffusion of macromolecules in the brain: implications for drug delivery. Molecular Pharmaceutics, 10:1492–1504.Available at:https://apps.pharmacy.wisc.edu/sopdir/robert_thorne/
41. Williams, W. C. and Sinko, P. J. (1999). Oral absorption of the HIV protease inhibitors: A current update. Advanced Drug Delivery Reviews,39: 211–238.Available at:https://www.ncbi.nlm.nih.gov/pubmed/10837775
42. Chan, L. M., Cooper, A. E., Dudley, A. L., Ford, D. and Hirst, B. H. (2004). P-glycoprotein potentiates CYP3A4-mediated drug disappearance during Caco–2 intestinal secretory detoxification. Journal of Drug Targeting, 12: 405–413. Available at:https://www.tandfonline.com/doi/abs/10.1080/10611860412331285224
43. Kato, M., Chiba, K., Hisaka, A., Ishigami, M., Kayama, M. and Mizuno, N. (2003).The intestinal first-pass metabolism of substrates of CYP3A4 and P-glycoprotein-quantitative analysis based on information from the literature. Drug Metabolism and Pharmacokinetics, 18: 365–72.Available at: https://www.ncbi.nlm.nih.gov/pubmed/15618757

44. Ertl, P. and Schuffenhauer, A. (2009). Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions. Journal of Cheminformatics, 1: 8.Available at: https://jcheminf.biomedcentral.com/articles/10.1186/1758-2946-1-8
45. Chen, D., Oezguen, N., Urvil, P., Ferguson, C., Dann, S. M. and Savidge, T. C. (2016).Regulation of protein-ligand binding affinity by hydrogen bond pairing.Science Advances, 2(3):1-16.Available at:https://www.ncbi.nlm.nih.gov/pubmed/27051863
46. Patil, R., Das, S., Stanley, A., Yadav, L., Sudhakar, A. and Varma, A. K. (2010). Optimized Hydrophobic Interactions and Hydrogen Bonding at the Target-Ligand Interface Leads the Pathways of Drug-Designing. PLoS ONE, 5(8): 120-129.Available at:https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0012029
47. Seeliger, D. and De Groot, L. B. (2010) Ligand Docking and Binding Site Analysis with PyMOL and Autodock/Vina. Journal of Computer Aided Molecular Design, 24: 417-422.Available at:https://www.ncbi.nlm.nih.gov/pubmed/20401516