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ANTIMALARIAL AND ANTICANCER ACTIVITY PREDICTION OF THE HYDROXY SUBSTITUTED ANALOGUE OF GEDUNIN THROUGH BINDING ENERGY PREDICTION STUDIES AGAINST THE HUMAN NAD$^+$ KINASE AND PLASMODIUM FALCIPARUM DIHYDROFOLATE REDUCTASE

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ABSTRACT

Background: Malaria is a serious disease caused by the *Plasmodium* parasite. It is transmitted when an infected mosquito bites. Malaria is a major cause of death worldwide. The disease is mostly a problem in developing countries with warm climates. There are four different types of malaria caused by four related parasites. The most deadly type occurs in Africa south of the Sahara Desert. Cancer, also called malignancy, is an abnormal growth of cells. There are more than 100 types of cancer, including breast cancer, skin cancer, lung cancer, colon cancer, prostate cancer, and lymphoma, all with varying symptoms, depending on the type. **Materials and Methods:** The physiochemical characteristics of the human NAD$^+$ kinase and *Plasmodium falciprum* dihydrofolate reductase were predicted using the ExPASy ProtParam online server. The substitution of the gedunin methyl group for hydroxyl group was achived with the aid of the Marvin Sketch software while the conversion of mrv files to SMILES strings was done using the Open Babel software. Structural visualization and minimization were done using the Pymol and Chimera visualizers respectively. The AutoDock Vina software was used to predict the binding energy of the ligand to each enzyme. **Results:** The theoretical isoelectric point of both the human NAD$^+$ kinase and *Plasmodium falciprum* dihydrofolate reductase as revealed through their physiochemical characterization were 6.70 and 6.86 respectively while their instability indices were 45.61 and

35.23 respectively. The binding score of the OH substituted analogue of gedunin to the human NAD^+ kinase and *Plasmodium falciprum* dihydrofolate reductase were -9.0 and -8.4Kcal/mol respectively. **Conclusion:** The docking result revealed that the OH substituted analogue of gedunin might be a potent antimalarial and anticancer agent. It can also be inferred that the modified compound might be more active against the human NAD^+ kinase because of the expression of a higher binding enery against the enzyme. Also, the human NAD^+ kinase was predicted to be an unstable enzyme and this makes it an ideal target for the anticancer agent.

KEYWORDS: Malaria; *Plasmodium falciprum* dihydrofolate reductase; NAD^+ kinase; Malignancy.

INTRODUCTION

Malaria is endemic in most parts of the world, and remains a major cause of morbidity and mortality both in rural and urban areas. Four plasmodia species, namely *Plasmodium falciparum, P. vivax, P. malariae, and P. ovale* are the most prevalent.[1] Malaria risk is heterogeneous with malaria prevalence rates, parasite densities and entomological inoculation rates varying from one area and season to another.[2] This distribution is determined in part by climatic, ecological and topographic factors which influence the distribution patterns of the vectors. In addition, human activities, behaviour and socio-economic and health systems factors may provide an additional risk as a result of an increased exposure to the disease.[3]

Cancer, known medically as a malignant neoplasm, is a broad group of various diseases, all involving unregulated cell growth. In cancer, cells divide and grow uncontrollably, forming malignant tumors, and invade nearby parts of the body.[4] The cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. Not all tumors are cancerous. Benign tumors do not grow uncontrollably, do not invade neighboring tissues, and do not spread throughout the body. There are over 200 different known cancers that afflict humans.[5]

NAD^+ kinase (NADK) catalyzes the phosphorylation of nicotinamide adenine dinucleotide (NAD^+) to nicotinamide adenine dinucleotide phosphate ($NADP^+$) using ATP as the phosphate donor. $NADP^+$ is then reduced to NADPH by dehydrogenases, in particular glucose-6-phosphate dehydrogenase and the malic enzymes. NADPH functions as an important cofactor in a variety of metabolic and biosynthetic pathways.[6] The demand for NADPH is particularly high in proliferating cancer cells, where it acts as a cofactor for the

synthesis of nucleotides, proteins, and fatty acids. Moreover, NADPH is essential for the neutralization of the dangerously high levels of reactive oxygen species (ROS) generated by increased metabolic activity. Given its key role in metabolism and regulation of ROS, it is not surprising that several recent studies, including in vitro and in vivo assays of tumor growth and querying of patient samples, have identified NADK as a potential therapeutic target for the treatment of cancer.[7]

DHFR inhibitors have a long history as anticancer agents and as anti-infective drugs against bacterial and protozoal pathogens. In Plasmodia, DHFR and thymidylate synthase coexist as a single-chain bifunctional enzyme, in contrast to prokaryotes and higher eukaryotes where the two proteins are distinct monofunctional enzymes.[8] DHFR and TS domains have polypeptide folds closely related structurally to those of their respective monofunctional counterparts.[11] Crystal structures for wild-type bifunctional DHFR-TS from *P. falciparum* and for the highly PYR-resistant quadruple mutant enzyme (QM) have been reported by our group.[9]

This study was aimed at predicting the dual activity of the OH substituted analogue of gedunin against the malaria parasite and its inhibitory role against the human NADK.

MATERIALS AND METHODS

Protein preparation

The crystal structure of the human NAD^+ kinase and *Plasmodium falciprum* dihydrofolate reductase were obtained from the Protein Data Bank, PDB 3PFN and 3UM8 respectively (Figure 1 and 2). The protein structures were subjected to a refinement protocol using the Pymol viewer.[10]

Designing of the Gedunin structural analogue

The 2D structure of gedunin (Figure 3) was drawn with the Marvin Sketch software.[11] The structural analogue of gedunin was developed with a structural modification and a different substituent.[19] The CH3 substituent of gedunin was replaced with an OH group. The structure was built with the Marvin Sketch software and minimized using the Chimera software.[12,13]

Molecular docking

Molecular docking (Figure 5 and 6) was performed using AutoDock Vina Software.[14] Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of the OH analogue of gedunin (Figure 7) was determined using SwissADME Server.[15]

Physiochemical Characteristics

The Physiochemical characteristics of the human NAD^+ kinase and *Plasmodium falciprum* dihydrofolate reductase were predicted using the ExPASy ProtParam server.[16]

RESULTS AND DISCUSSION

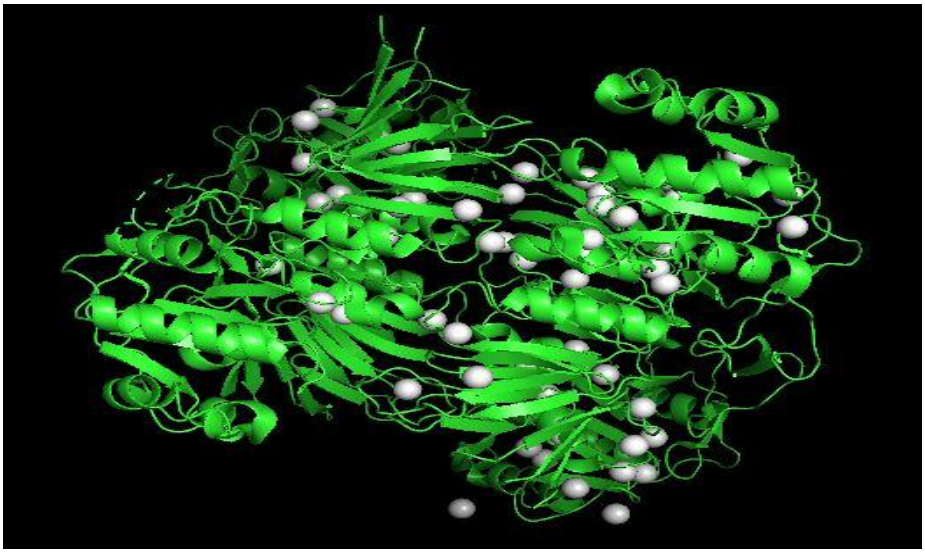

Figure 1: Human NAD^+ kinase 3D structure (PDB: 3PFN).

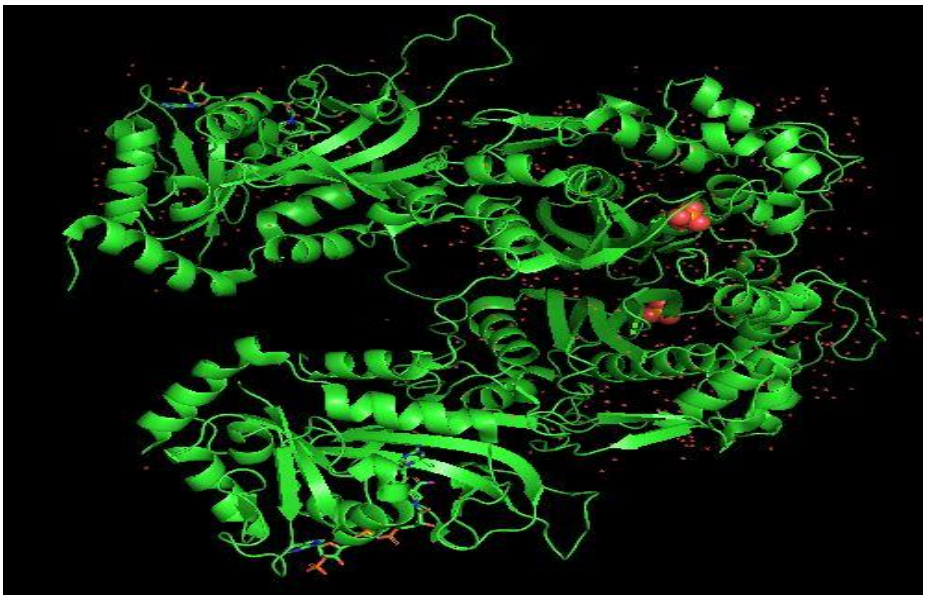

Figure 2: *Plasmodium falciprum* dihydrofolate reductase (PDB: 3UM8).

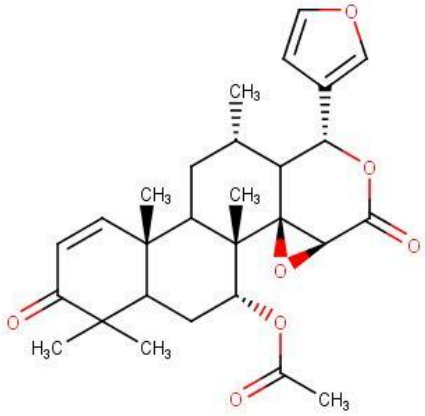

Figure 3: Gedunin 2D.

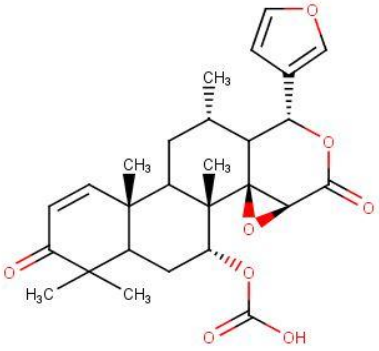

Figure 4: OH Analogue.

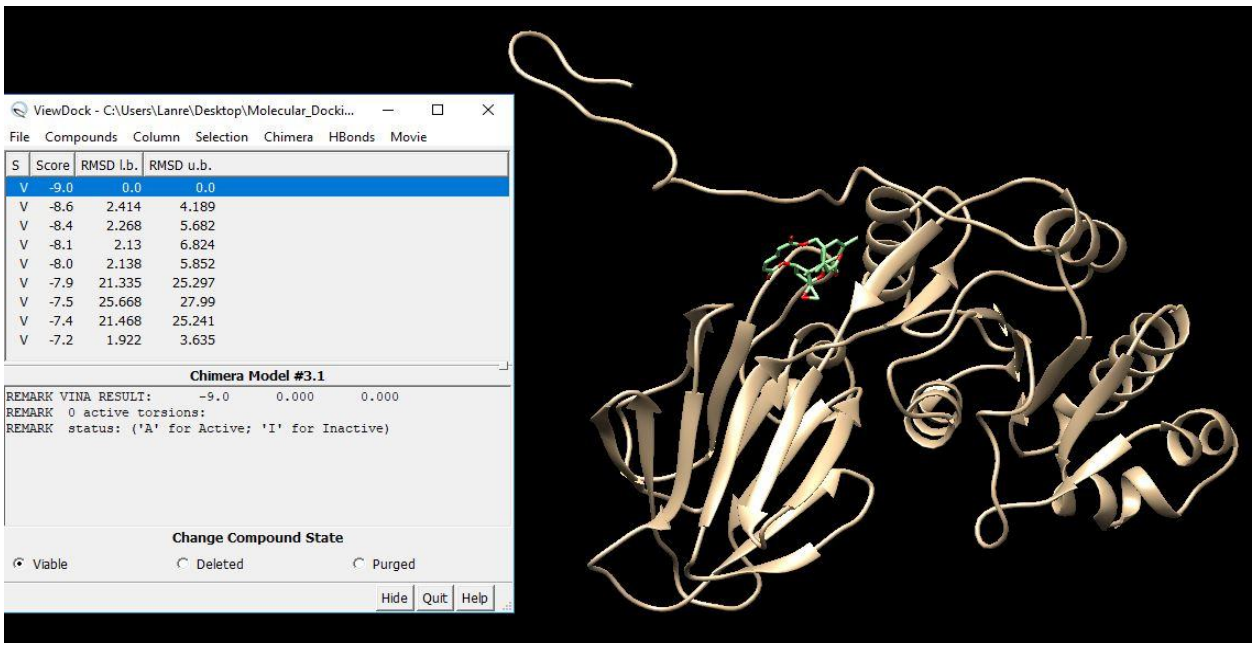

Figure 5: OH Analogue of Gedunin in Complex with the Human NAD^+ Kinase.

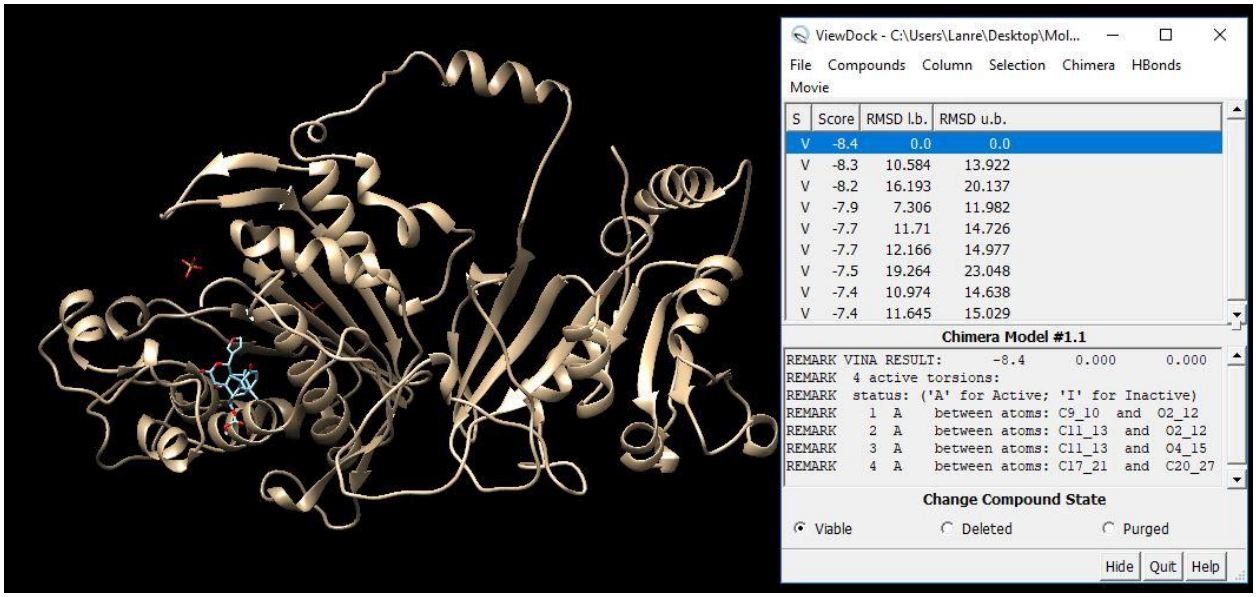

Figure 6: OH Analogue of Gedunin in Complex with the *Pf* DHFR.

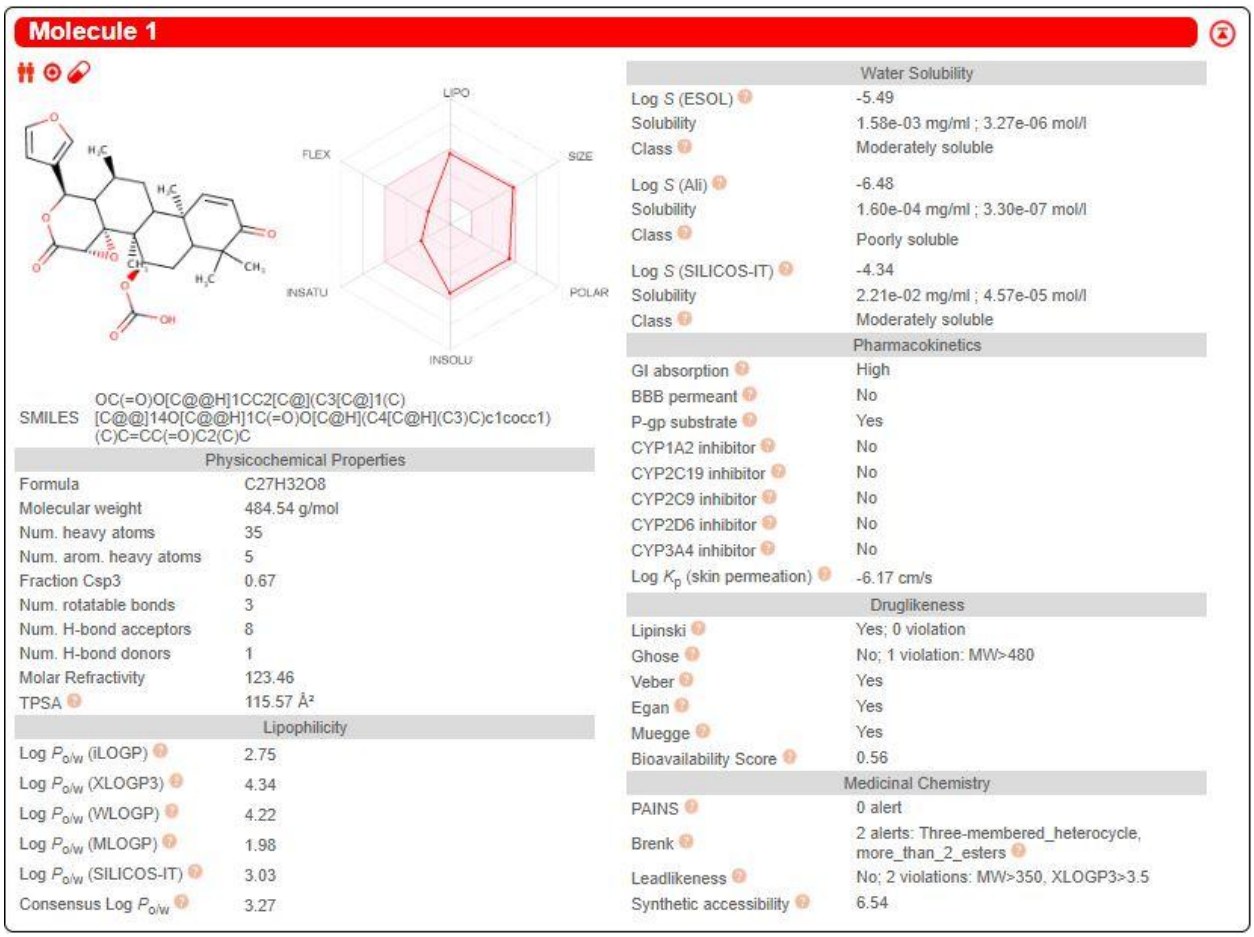

Figure 7: Druglikeness Prediction of the Gedunin OH Analogue.

Amino Acid Sequence of the Human NAD^+ Kinase (FASTA)

>3PFN:A|PDBID|CHAIN|SEQUENCE

MPCPVTTFGPKACVLQNPQTIMHIQDPASQRLTWNKSPKSVLVIKKMRDASLLQPFKEL
CTHLMEENMIVYVEKKVLEDPAIASDESFGAVKKKFCTFREDYDDISNQIDFIICLGGDG
TLLYASSLFQGSVPPVMAFHLGSLGFLTPFSFENFQSQVTQVIEGNAAVVLRSRLKVRV
VKELRGKKTAVHNGLGEKGSQAAGLDMDVGKQAMQYQVLNEVVIDRGPSSYLSNVD
VYLDGHLITTVQGDGVIVSTPTGSTAYAAAAGASMIHPNVPAIMITPICPHSLSFRPIVVP
AGVELKIMLSPEARNTAWVSFDGRKRQEIRHGDSISITTSCYPLPSICVRDPVSDWFESL
AQCLHHHHHH

Amino Acid Sequence of the *Plasmodium falciprum* Dihydrofolate Reductase (FASTA)

>3UM8:A|PDBID|CHAIN|SEQUENCE

MMEQVCDVFDIYAICACCKVESKNEGKKNEVFNNYTFRGLGNKGVLPWKCNSLDM
KYFCAVTTYVNESKYEKLKYKRCKYLNKETVDNVNDMPNSKKLQNVVVMGRTSW
ESIPKKFKPLSNRINVILSRTLKKEDFDEDVYIINKVEDLIVLLGKLNYYKCFIIGGSVV
YQEFLEKKLIKKIYFTRINSTYECDVFFPEINENEYQIISVSDVYTSNNTTLDFIIYKKTN
NKMLNEQNCIKGEEKNNDMPLKNDDKDTCHMKKLTEFYKNVDKYKINYENDDDD
EEEDDFVYFNFNKEKEEKNKNSIHPNDFQIYNSLKYKYHPEYQYLNIIYDIMMNGNK
QSDRTGVGVLSKFGYIMKFDLSQYFPLLTTKKLFLRGIIEELLWFIRGETNGNTLLNK
NVRIWEANGTREFLDNRKLFHREVNDLGPIYGFQWRHFGAEYTNMYDNYENKGVD
QLKNIINLIKNDPTSRRILLCAWNVKDLDQMALPPCHILCQFYVFDGKLSCIMYQRSC
DLGLGVPFNIASYSIFTHMIAQVCNLQPAQFIHVLGNAHVYNNHIDSLKIQLNRIPYPF
PTLKLNPDIKNIEDFTISDFTIQNYVHHEKISMDMAA

The theoretical *pI* of the human NAD^+ kinase and *Plasmodium falciprum* dihydrofolate reductase were predicted through the biochemical characterization analysis has predicted the proteins to be slightly acidic with a value of 6.70 and 6.86 respectively.[17] 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.[18] The GRAVY calculator used in predicting the hydrophobicity assigned to the proteins a value of -0.025 and -0.506. This result implies that the human NAD^+ kinase exhibit a more hydrophobic character compared to the *Plasmodium falciprum* dihydrofolate reductase with the lower GRAVY value.

The instability index is a pointer to the stability of a protein in a test tube. A protein whose instability index is greater than 40 is predicted as unstable and a value below 40 predicts the protein may be stable.[18] The human NAD^+ kinase is therefore an unstable protein with an instability index of 45.61 while the *Plasmodium falciprum* dihydrofolate reductase, having an instability index of 35.23 is a stable protein.

Lipinski's rule of five also known as the Pfizer's rule of five or simply the rule of five (RO5) is a rule of thumb to evaluate druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans.[19] Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria: No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds), no more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms), a molecular mass less than 500 daltons, an octanol-water partition coefficient log *P* not greater than 5.[20] The OH analogue of gedunin can as such be regarded as druglike because it has violated none of the lipinski's rule.

The polar surface area (PSA) or topological polar surface area (TPSA) of a molecule is defined as the surface sum over all polar atoms, primarily oxygen and nitrogen, also including their attached hydrogen atoms. Molecules with a polar surface area of greater than 140 angstroms squared tend to be poor at permeating cell membranes.[21] For molecules to penetrate the blood–brain barrier (and thus act on receptors in the central nervous system), a PSA less than 90 angstroms squared is usually needed.[22] The TPSA value of the OH analogue of gedunin is 115.57 angstroms. This means that the compound lacks the blood brain barrier permeation ability hence safe for oral administration.

The P-glycoprotein is involved in limiting the harmful exposure of toxins, drugs, and xenobiotics to the body by extruding them out of cells. It is increasingly recognized to play an important modulating role in the pharmacokinetic properties of many clinically important therapeutic agents and because of its importance in pharmacokinetics, its screening has to be incorporated into the drug discovery process.[22] The presence of the P-glycoprotein is the reason for the multidrug resistance attribute exhibited by cancer cells and the pharmacokinetics result on the OH analogue of gedunin showed that it is a P-glycoprotein substrate.

A method to estimate ease of synthesis (synthetic accessibility) of drug-like molecules is needed in many areas of the drug discovery process. The assessment of synthetic accessibility (SA) of a lead candidate is a task which plays a role in lead discovery regardless of the method the lead candidate has been identified with. After normalization, the SA Score ranges from 1 (very easy) to 10 (very difficult).[23] The synthesis of the OH analogue of gedunin has a 6.54 synthetic accessibility value which makes the compound slightly difficult to synthesize.

The increased application of molecular docking methods in the pharmaceutical industry and academia is a direct result of increase in computer speed, and the reliability of simulation theories and docking software.[24] The OH substituted analogue of gedunin bound tighter to the human NAD^+ kinase than its binding energy to the *Plasmodium falciprum* dihydrofolate reductase were the binding scores were -9.0 and -8.4Kcal/mol respectively.

CONCLUSION

Through the molecular docking scores, it appeared that the OH substituted analogue of gedunin might be more active against the human NAD^+ kinase but the in silico pharmacokinetics report showed that even though the compound exhibited favourable druglike attributes, being a P-glycoprotien substrate makes it less active against cancer cells in the control of cellular proliferation as the constant efflux of the drug molecules for the control of toxicity might lower the drug bioavailability.

It is therefore recommended that this drug be synthesized and a wet laboratory experiment be conducted against the two experimental enzymes to confirm its anticancer and antimalarial activities.

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