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Phytochemical screening to find a potential inhibitor of plasmepsin, a key enzyme in the life cycle of *Plasmodium*

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Abstract

Selected phytochemicals are screened by bioinformatics software to find an inhibitor of the plasmepsin, in an effort to find a novel drug against malaria. Pleiocarpamine could well be a potential candidate for developing novel drug for treating malaria.

Keywords: autodock, malaria, phytochemicals, plasmepsin, pleiocarpamine, SMILES notations

INTRODUCTION

Malaria parasites have been with us perhaps with the dawn of time. They probably originated in Africa (along with mankind) and fossils of mosquito of about 30 million years old show that the vector for malaria was present well before the earliest history. At present, at least 300,000,000 people are affected by malaria globally, and there are between 1,000,000 and 1,500,000 malaria deaths per year. Malaria is generally endemic in the tropics, with extensions into the subtropics.

In addition to control measures, such as spraying with DDT, coating marshes with paraffin (to block *Anopheles* mosquito larvae spiracles), draining stagnant water, and the widespread use of nets, research into the biology and microbiology of malaria enables a methodical search for better vaccines and a possible cure in the fight against malaria. Recent drug resistance to chloroquine further complicates the malaria problem. Further more, normal methods to combat parasitic proliferation have become obsolete and antifolates and quinine-containing compounds no longer possess the potency they previously held in controlling the infection. With the escalating concerns over drug resistance and the continually worsening health condition attributable to malaria, the urgency to develop new agents for malarial control and possible eradication is significant (Francis *et al.*, 1994; Miller *et al.*, 1994).

In this scenario, development of antimalarials acting by novel pathways is the best option. Decoding of the *Plasmodium falciparum* genome has helped scientists working in the area of drug development by providing new drug targets such as carbonic anhydrase, homocysteine hydrolase, antioxidant proteins, glutathione reductase etc. Efforts are going on to elucidate structures and functions of novel targets. One such target is plasmepsins which are thought to play key roles in biochemical pathways of merozoite release, invasion and host cell hemoglobin degradation during the intra-erythrocytic stages of the parasites life cycle (Sharma *et al .,2005).*

The Plasmepsins are aspartic proteinases found in malaria parasites. The malarial aspartic proteinases (Plasmepsin) have been discovered in several species of *Plasmodium*, including all four of the human malarial pathogens. In *P. falciparum,* Plasmepsins I, II, III, and HAP have been directly implicated in hemoglobin degradation during malaria infection and are now considered targets for anti-malarial drug design (Bernstein *et al.*, 2003). Genes encoding the food vacuole plasmepsins of all four human malaria parasites have been cloned and expressed by scientists (Dame 1994). Crystal structures and computer-generated models of these enzymes are currently being used in combination with enzyme kinetic studies using panels of peptide substrates and peptidomimetic inhibitors to characterize the distinctive features of their active sites (Westling *et al.*, 1997, 1999). Knockout mutants, each lacking one of the four food vacuole plasmepsins of *P. falciparum*, have been produced and are currently being studied to examine the role of each enzyme in hemoglobin digestion and other cellular functions (Dame *et al.*, 1994).

Chemicals from herbs are now analysed for antimalaria activities (Addae-Keyereme *et al.*, 2001, Wakko *et al.*, 2005). Addae-Keyreme *et al.*, (2001) reported that five alkaloids *viz*, Pleiocarpine, Kopsinine Pleiocarpamine, Eburnamine and Pleiomutinine

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isolated from the roots of *Pleiocarpa mutica* were most active against *P. falciparum*. Among them, three alkaloids, Kopsinine, Pleiocarpamine, and Eburnamine, were selected for the present study and their efficacy in binding with the Plasmepsin were analyzed using bioinformatics software.

METHODS

Retrieval of aminoacid sequence and structural model of proplasmepsin of the human malarial parasite.

The aminoacid sequence and the crystal structure of proplasmepsin of the human malarial parasite were obtained from the Protein Data Bank (PDB) (www.rcsb.org/pdb/).The crystal structure of proplasmepsin of the human malarial parasite is available in the PDB ID No 1MIQ. The coordinate file of the plasmepsin was obtained by the molecular visualization viewer *viz.*, SPDB viewer (www.expasy.org/spdbv/).

Protin-Ligand Docking

The molecular structures of the kopsinine, pleiocarpamine, edurnamine (ligands) were drawn using the software ACD/Chemsketch (www.acdlabs.com) and the DS Viewer pro (www.accelrys.com/dstudio/ds viewer/). The molecular deails and SMILES notations for the chemicals selected were obtained from NCBI (National Center for Biotechnology information) (www.ncbi.nlm.nih.gov). Then the compound ID was used to get further details including the SMILES notations from PubChem (www.pubchem.ncbi.nlm.nih.gov/).

The SMILES notations (canonical SMILES notations) were pasted in ChemSketch software to generate the structure. This compound structure was saved with the extension .SK2 and it was exported with the .mol extension format. This .mol file was opened in DS Viewer Pro and then saved as (Save As).pdb file. Then it was opened in 'AutoDock' (docking software) for docking (http://www.scripps.edu/pub/olson-web/doc/autodock/).

RESULTS

Structural Model of PLASMEPSIN

The Plasmepsin is a protein of 450 amino acids in length with a molecular weight of 51667 daltons. The structural model is shown in Fig.1.

Chemical Structures of Ligands

Details about the phytochemicals screened and their structures are given in Table 1.

Protein-Ligand Docking

All the 3 chemicals *viz.*, kopsinine, pleiocarpamine, edurnamine got docked successfully with PLASMEPSIN. (Figs 2-4) .A comparison of the efficiency of the dockings of the 3 chemicals, kopsinine, pleiocarpamine and edurnamine has been given in Table 2.

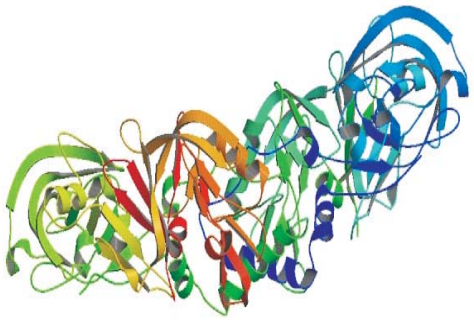


Figure 1. Structure of Plasmepsin (PDB 1MIQ)

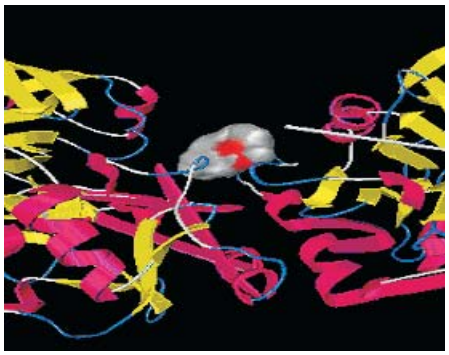


Figure 2. View of the ligand kopsinine docked with the plasmepsin.

Figure 3. View of the ligand Pleiocarpamine docked with the plasmepsin.

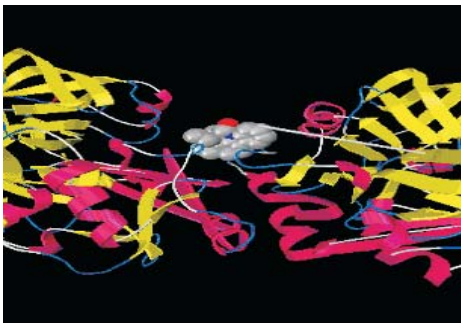


Figure 4. View of the ligand Eburnamine docked with the plasmepsin.

Table 1. Chemical structures and properties of the ligands screened

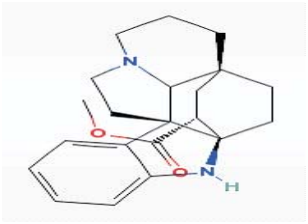
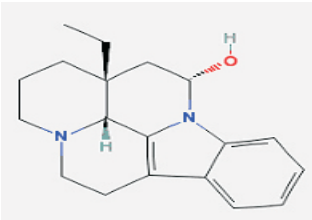
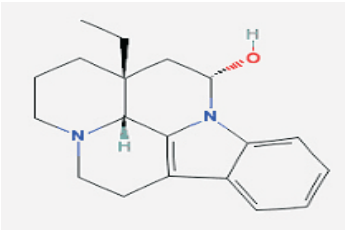
S.No	Structures of Ligands	Molecular Weight	Molecular Formula
1	 Kopsinine	338.443 g/mol	$C_{21}H_{26}N_2O_2$
2	 Peiocarpamine	296.407 g/mol	$C_{19}H_{24}N_2O$
3	 Eburnamine	296.407 g/mol	$C_{19}H_{24}N_2O$

Table 2. Comparison of efficiency of dockings of the three chemicals studied (ligands) with plasmepsin.

S.No	Chemical Name (ligands)	Run	Docked Energy
1	Kopsinine	9	-7.81
2	Pleiocarpamine	4	-8.54
3	Eburnamine	10	-7.19

DISCUSSION

The Plasmepsin, an aspartic proteinase is involved in the hemoglobin degradation in the malarial parasites food vacuole. This protein which is thought to play a key role in the merozoite release during the intra-erythrocytic stages of the life cycle of the malarial parasite has been taken for the present study. Results of the present study showed, that all the three phytochemicals screened *viz*, Kopsinine, Pleiocarpamine, and Eburnamine, have great binding abilities with the enzyme, with Pleiocarpamine, being the most effective. So Pleiocarpamine could well be a potential candidate for developing novel drug for treating malaria. Since these chemicals are of phytochemical origin, they may be prescribed to be

safer from the environment point of view as well. However, further analyses on optimization of the molecules, drug metabolism, pharmacokinetics and toxicity in animals (pre-clinical assessments) are needed to evaluate the true efficacy of these chemicals before venturing into different stages of clinical trials in drug development. Even then the present study might well be a great beginning that deserve to be investigated further.

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