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POTENTIAL LIGAND FOR PLASMODIUM MALARIA AND A POTENTIAL DIPHENYLUREA DRUG TARGET THROUGH DOCKING ANALYSIS OF THE PROTEIN

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Abstract

Malaria is a mosquito-borne infectious disease caused by a eukaryoticprotist of the genus Plasmodium. It is widespread in tropical and subtropical regions, including parts of the Americas (22 countries), Asia, and Africa. Each year, there are approximately 350–500 million cases of malaria, killing between one and three million people, the majority of whom are young children in sub-Saharan Africa. Ninety percent of malaria-related deaths occur in sub-Saharan Africa. Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development. In this the protein plasepsin has been analysed,the three dimentional structure of the protein was obtained from the three dimensional structure of the protein was obtained from the protein databank.Diphenylurea has been found to be the best ligand able to bind at the active site of the protein inhibiting the disease.This analysis aims at revealing the potential ligand for plasmodium malaria and a potential diphenylurea drug target through docking analysis of the protein.

1. Introduction

Five species of the plasmodium parasite can infect humans; the most serious forms of the disease are caused by Plasmodium falciparum. Malaria caused by Plasmodium vivax, Plasmodium ovale and Plasmodium malariae causes milder disease in humans that is not generally fatal. A fifth species, Plasmodium knowlesi, is a zoonosis that causes malaria in macaques but can also infect humans.

Malaria is naturally transmitted by the bite of a female Anopheles mosquito. When a mosquito bites an infected person, a small amount of blood is taken, which contains malaria parasites. These develop within the mosquito, and about one week later, when the mosquito takes its next blood meal, the parasites are injected with the mosquito's saliva into the

person being bitten. After a period of between two weeks and several months (occasionally years) spent in the liver, the malaria parasites start to multiply within red blood cells, causing symptoms that include fever and headache. In severe cases, the disease worsens, leading to coma and death. A wide variety of antimalarial drugs are available to treat malaria. In the last 5 years, treatment of P. falciparum infections in endemic countries has been transformed by the use of combinations of drugs containing an artemisinin derivative. Severe malaria is treated with intravenous or intramuscular quinine or, increasingly, the artemisinin derivative artesunate. Several drugs are also available to prevent malaria in travellers to malaria-endemic countries (prophylaxis). Resistance has developed to several antimalarial drugs, most notably chloroquine. Malaria transmission can be reduced by preventing mosquito bites by distribution of inexpensive mosquito nets and insect repellents, or by mosquito-control measures such as spraying insecticides inside houses and draining standing water where mosquitoes lay their eggs. Although many are under development, the challenge of producing a widely available vaccine that provides a high level of protection for a sustained period is still to be met.

Plasmepsins are aspartic proteases involved in the degradation of the host cell hemoglobin that is used as a food source by the malaria parasite. Plasmepsins are highly promising as drug targets, especially when combined with the inhibition of falcipains that are also involved in hemoglobin catabolism. In this review, we discuss the mechanism of plasmepsins I-IV in view of the interest in transition state mimetics as potential compounds for lead development. Inhibitor development against plasmepsin II as well as relevant crystal structures are summarized in order to give an overview of the field. Application of computational techniques, especially binding affinity prediction by the linear interaction energy method, in the development of malarial plasmepsin inhibitors has been highly successful and is discussed in detail. Homology modeling and molecular docking have been useful in the current inhibitor design project, and the combination of such methods with binding free energy calculations is analyzed.(1)

Aspartic proteases participate in a wide variety of cellular processes in eukaryotic organisms. The genome of the human malaria parasite Plasmodium falciparum encodes 10 aspartic protease homologs. Functions have been assigned to four of these: plasmepsins I, II, IV and histo-aspartic protease are key players in the catabolism of hemoglobin in the food vacuole. The functions of the other six remain obscure. To better understand the roles of aspartic proteases in blood stage growth and asexual reproduction of P. falciparum, we have characterized the biosynthesis, cellular location and pepstatin-binding properties of plasmepsin V (PM V). PM V is expressed over the course of asexual intraerythrocytic

development. The amount of PM V in the parasite is lowest in the ring stage and increases steadily through schizogony. The proregion of this aspartic protease homolog exhibits remarkable interspecies diversity and appears not to be removed following biosynthesis. In intraerythrocytic parasites, PM V is located in the endoplasmic reticulum but not in ERD2-associated Golgi structures. Fractionation and solubilization experiments demonstrate that PM V is an integral membrane protein, a result that is consistent with the presence of a C-terminal putative transmembrane domain in the PM V sequence. In contrast to the food vacuole plasmepsins, detergent-solubilized PM V does not bind the aspartic protease inhibitor pepstatin. Together, these results strongly suggest that the role of PM V in P. falciparum is distinct from those of previously characterized plasmepsins(2).

The objective of the study is

- To find the target responsible for the disease.
- To find the ligand.
- To docking of the receptor and the ligand are done into the probable binding site.
- Identify the best conformation of the target-ligand complex, from the binding energy obtained.
- Analysis of docking based on the Hydrogen-bond interaction

2. Materials and Methods

The target macromolecule (protein) and the ligand molecule are obtained from the databases as follows:

- SWISSPROT
- PDB (Protein Data Bank)
- PDBSUM
- PUBCHEM

Methodology

Finding the Structuure of Target Protein

The fasta sequence for the target protein (LPL) was retrieved from Uniprot and Swissprot databases.This is followed by doing alignment with template protein sequences in BLAST. In protein-BLAST submit the query sequence and do psi-BLAST, the similar template sequences will be displayed from pdb. Choose the template which is most similar to target sequence (LPL).

The structure can be found using SWISSMODEL which works basing on homology modeling. Submit the query and template sequences to SWISSMODEL automated mode .the structure of query sequence will be retrieved from swissmodel, which can be downloaded in pdb format.

The structure obtained is validate d using SAVES an online tool .the structure is submitted to SAVES in pdb format and we will check for accuracy of the structure .if its accuracy is in between 75%-95% the structure is valid. We can view the structure using pymol.

3. Results and Discussion

Finding the Ligand Molecules

Open drugbank and type Malaria the drugs for Malaria will be displayed, take the mol format files of different drugs and check for compatibility in arguslab the drug with least pose energy value is the most effective drug.

Argus Lab

Argus lab is software used to dock macromolecules with small molecules. The methodology is described as a flowchart below:

Loading of the target protein molecule as a pdb file, into the Argus lab workspace

↓

Addition of hydrogen to the crystal structure of the protein

↓

Energy minimization of protein for the stabilization of the molecule

↓

Grouping of the catalytic residues (Active site) so as to represent the binding pocket for the interacting ligand

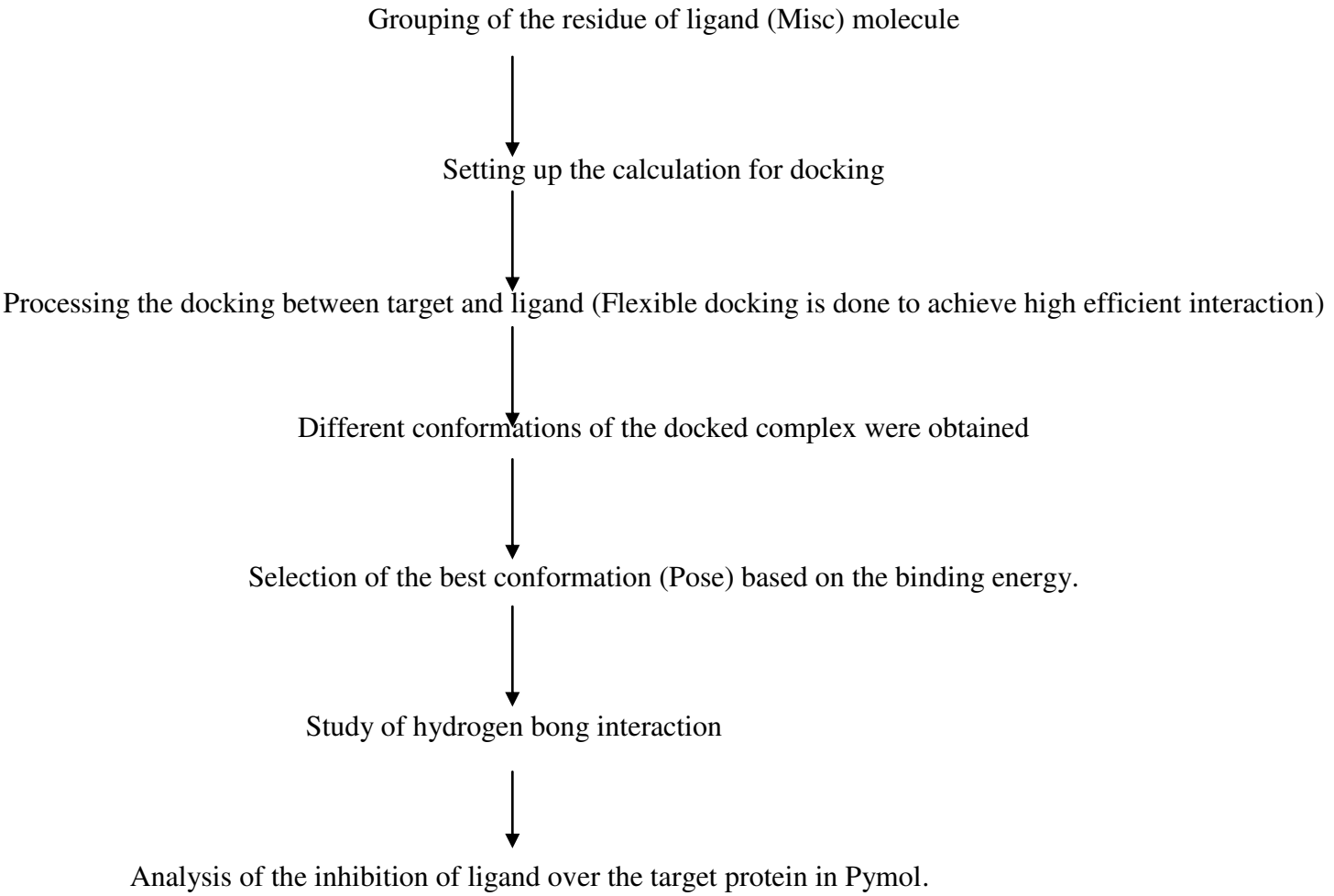
↓

Loading of pdb file of the ligand molecule

↓

Energy minimization of ligand for its stability

↓



4. Results and Discussion

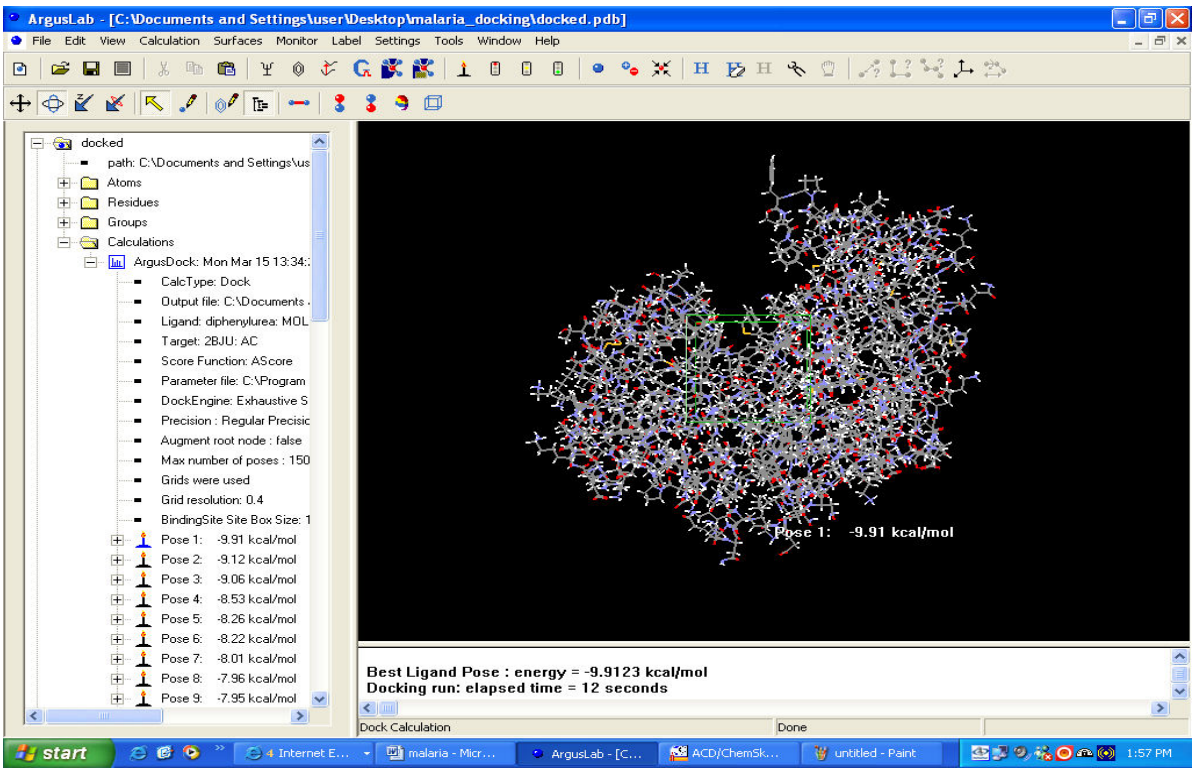
The docking of the target protein Plasmepsin and the ligand molecule Dipheylurea, was carried out in Argus lab and the results docked complex was analyzed.

After docking, we obtained totally 62 conformations (represented as poses). The binding energy of the poses ranged from -9.91kcal/mol to -6.58 kcal/mol. The best 15 conformations are tabulated in the table below.

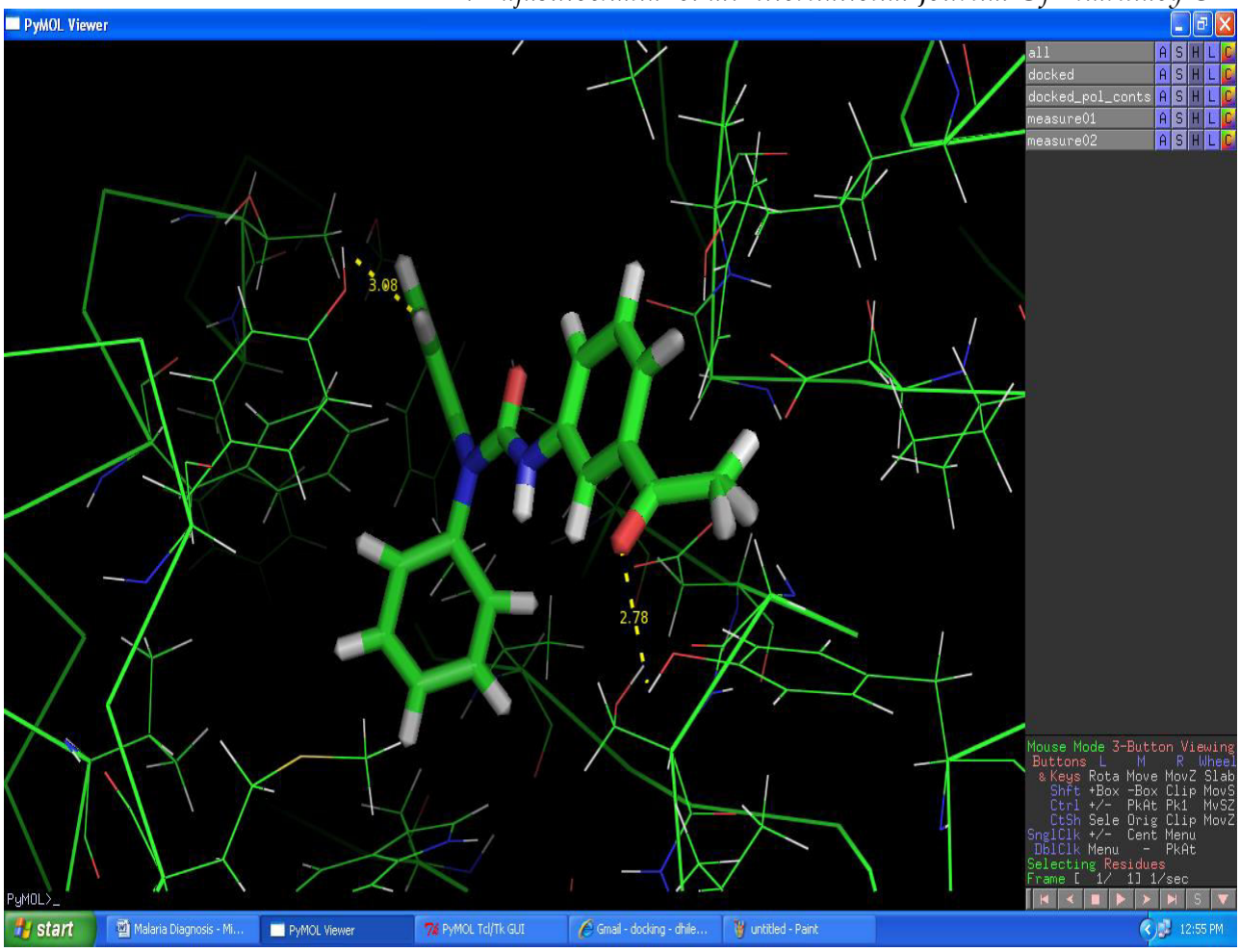
Poses	Energy
Pose 1	-9.91 kcal/mol
Pose 2	-9.12 kcal/mol
Pose 3	-9.06 kcal/mol
Pose 4	-8.53 kcal/mol
Pose 5	-8.26 kcal/mol

Pose 6	-8.22 kcal/mol
Pose 7	-8.01 kcal/mol
Pose 8	-7.96 kcal/mol
Pose 9	-7.95 kcal/mol
Pose 10	-7.94 kcal/mol
Pose 11	-7.90 kcal/mol
Pose 12	-7.84 kcal/mol
Pose 13	-7.78 kcal/mol
Pose 14	-7.77 kcal/mol
Pose 15	-7.76 kcal/mol

The first conformation with the binding energy -9.91 kcal/mol is taken as the best complex.. The docked complex of the target protein and the ligand is shown in the figure. A grid box of the resolutions 0.4 was formed surrounding the active site, which is essential for the binding of ligand to catalytic site of the target molecule.



The docked complex was transferred to Pymol for analysis of the interaction. Two hydrogen bonds were formed between the ligand and protein. For a best interaction, the hydrogen bond must be within 3À in length. We obtained two H-bonds of length 2.78À and 3À respectively, which indicates good polar interaction between ligand and the protein.



The interaction viewed in Pymol is shown in the above figure, with ligand represented in sticks, binding pocked of protein in lines and H-bonds as yellow dashes.

Thus, from the above theoretical studies, it is observed that the ligand Diphenylurea forms a good complex with the plasmepsin protein, thereby inhibiting the catalytic activity of the protein from causing malaria.

5. Conclusion

Plasmepsin through their hemoglobin-degrading activity, they are an important cause of symptoms in malaria sufferers. It has been evident that diphenylurea drugs have a greater affinity towords the binding site of plasmepsin. The docking energy values were found in to be promising for the drug 3-(3-acetylphenyl)-1, 1-diphenylurea. It has an energy value of around -9.91 kcal/mol. The docking analysis has shown that this drug has the maximum binding affinity with the molecule, ther by inhibiting the activity of the enzyme.Thus the study aims at finding the potential ligand for the disease.The ligand is presented for further analysis involving inhibitoraffinity and its pharmacophore binding.

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