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Molecular Docking Analysis of Plasmepsin-2 Malaria Protein and Identification of Potential Ligands

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Abstract

Malaria is one of the leading causes of malignancy and death especially in tropical and subtropical countries, which is caused by parasites such as *Plasmodium* species through mosquito vectors. Despite the fact that several anti-malarial drugs have been shown to be effective, resistance towards these drugs poses a great threat to the community including developing countries like Sri Lanka. Therefore, the research aims to perform protein-ligand docking and determine the best phytochemicals with their ligand binding sites. The findings of the study revealed that Conessine is a potential drug candidate towards 1LF4 with the lowest binding energy of -9.96 kcal/mol and with a lower inhibition constant. Further, GLY-36, SER-37, TYR-77, VAL-78, SER-79, PHE-111, TYR-192 and GLY-216 were found to be the most prominent ligand binding sites towards Plasmepsin-2.

Keywords: Molecular docking, AutoDock, Binding energy, Ligand binding sites

1. Introduction

Malaria remains a major global health concern and the leading cause of death, especially in many tropical and subtropical countries including Sri Lanka. Over the past years, several renewed efforts have been made to combat the disease and several success stories have been documented in reducing the prevalence of malaria.[1] Despite these valuable efforts, the severity, and the ongoing resistance to a number of anti-malarial drugs have been a major topic of discussion in recent years. These also include several imported malaria cases in Sri Lanka with the recent transmission of locally introduced malaria cases from 2018-2019 although the country has received malaria-free certification in 2016 from the World Health Organization (WHO).[2] The Malaria World Report in 2016 reported that nearly 429,000 deaths have occurred globally since 2000. In which it is estimated that 92% of the deaths have occurred in the WHO African Region, 6% in the WHO South-East Asian Region and 2% in the WHO Eastern Mediterranean Region. Further, it was also noted that almost 99% of the deaths have resulted from the spread of *Plasmodium falciparum* malaria.[3,4] In this regard, the etiologic agents of malaria are Plasmodium species, in which, the most malignant and common forms of the cases are caused by *Plasmodium falciparum* and *Plasmodium vivax.* These parasitic agents pose threats to human lives due to their complex life cycle both inside the human host and the mosquito vector such as the Anopheles species of mosquito. The disease is caused during the intra-erythrocytic phase in which the haemoglobin of the human host is consumed completely which allows the generation of amino acids that stimulate the growth and maturation of parasites.[5]

A series of enzymes called proteases are involved in the haemoglobin degradation inside the acidic digestive vacuole of the parasite.

Meanwhile, proteases such as Plasmepsin, which belongs to a group of aspartic proteases, initiate the degradation of haemoglobin. The reaction is followed by proteolysis which breaks down the large fragments of compounds into small peptides using cysteine protease. Therefore, it highlights that the initial stage of haemoglobin degradation is an ultimate process exported to terminal degradation of the cytoplasmic exopeptides.[6] Thus, it is paramount to understand these enzymes' binding cavities, posing suitable chemotherapeutic inhibitors to combat the disease.

In addition, it is worth noting the resistance of anti-malarial drugs to treat and control malaria despite the presence of effective drugs. As a result, the emergence of resistant anti-malarial drugs has threatened the continuity of efficacy of anti-malarial drugs and raised the importance of developing novel drug targets using modern techniques.[7] Therefore, this research is intended to perform protein-ligand docking studies to find potential phytochemicals and ligand binding sites as a method to combat malarial infection and the adverse effects resulting from resistant malarial drugs.

2. Methodology

2.1 Preparation of protein receptors. The three-dimensional coordinates of the crystal structure of the Malarial protein, Plasmepsin-2 (PDB ID: 1LF4, 1.90A˚, X-Ray diffraction), a hydrolase enzyme was retrieved from RCSB Protein Data Bank (PDB) using .pdb format (https://www.rcsb.org/). The .pdb file was loaded into AutoDock (version 2.4.6) (https://autodock.scripps.edu/) to remove water molecules, hetero atoms, and ligands. Further, the exhaustiveness value was set to default with 8 Angstrom and shows the comprehensiveness of the software in finding the best docking complexes. Thereafter, AutoDock Tools (ADT) was set with AD4 type in which polar hydrogen was added to fix bond order and Kollman charges to include partial charges. The formatted proteins were saved in .pdbqt format.[8]

2.2 Preparation of ligands. Ten phytochemicals with anti-malarial properties were obtained from the previous literature as ligands. The three-dimensional structures of the phytochemicals were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in .sdf format and converted to .pdb format using Open Babel software (http://openbabel.org). The ligands were optimized with the inclusion of aromatic carbons, rotatable bonds and setting the TORSDOF values.[9] Later, the prepared ligands were saved in .pdbqt format.

2.3 Molecular docking and visualization of docked complexes. The grid boxes were generated to cover the entire active sites of the protein.[10] The grid points X, Y and Z were set as 100, 76 and 112 respectively. The grid spacing was set to 0.531 and the grid output file was saved in .gpf format. Docking parameters were set as follows: number of genetic algorithms runs as 10 with Lamarckian algorithm, population size as 150, maximum number of energy evaluations as 2,500,000, maximum number of generations as 27,000, rate of gene mutation as 0.02 and rate of cross-over as 0.8.[8] Once the docking was completed, the docked files were saved in .dlg format and later converted into .pdbqt format. The best-docked complexes were analysed using the binding energies from the RMSD tables. Visualization of the best complexes was viewed from BIOVIA Discovery Studio (https://discover.3ds.com/discovery-studio-visualizer-download) where the ligand binding sites were determined.

3. Results and Discussion

The findings of the study revealed the interactions between ligands with anti-malarial properties and the malarial protein, Plasmepsin-2. In the present investigation, one of the ultimate requirements is to produce docking complexes with proper orientation and the need for ligands' conformation to best fit the enzyme binding sites.

In this regard, the study used optimal docking criteria to interpret the best-docked conformation such that it ensured the ligands had the lowest binding energy with Plasmepsin-2 as shown in Table 1.

Of the phytochemicals which were docked with 1LF4, Conessine had the lowest binding energy with -9.86 kcal/mol showing the high potency towards 1LF4 as a potential drug candidate. The docking pose of the best conformation of Conessine-1LF4 is shown in Figure 1 along with the identification of ligand binding sites. In addition, Conessine's lower inhibition constant (Ki) with Ki=0.058µM further describes it as a potential drug target to treat malarial diseases. For instance, according to the studies of Pratama *et al*., low inhibition constant highlights the better binding affinity of the ligand with its target site.[11] Thus, with the dry lab investigation performed in this study, it is proven that Conessine can be considered a potential drug target for malarial diseases and can be considered a new therapeutic target for resistant malarial drugs in developing countries like Sri Lanka.

Furthermore, the residues involved in ligand binding of Conessine to 1LF4 are MET-15, ILE-32, ASP-34, GLY-36, SER-37, TYR-77, VAL-78, SER-79, PHE-111, THR-114, SER-118, PHE-120, ILE-123, TYR-192, ILE-212, ASP-214, GLY-216, THR-217 and ILE-300 as shown in Figure 1. The findings of the current study are in agreement with the result obtained from the previous study of Fernando *et al*. on site-specific ligand binding sites conducted on several other phytochemicals to treat malaria.[12] It showed that higher tendency of Serpentine towards Plasmepsin-2 with a binding energy of -8.16 kcal/mol and an inhibition constant of 1.04 nm. Further, it showed GLY-36, SER-37, MET-75, TYR-77, VAL-78, SER-79, PHE-111, TYR-192 and GLY-216 as potential ligand binding sites. However, except MET-75, the remaining residues were found as potential ligand binding sites in this study as well. Hence, it can be considered that GLY-36, SER-37, TYR-77, VAL-78, SER-79, PHE-111, TYR-192 and GLY-216 act as the most prominent ligand binding sites for Plasmepsin-2.

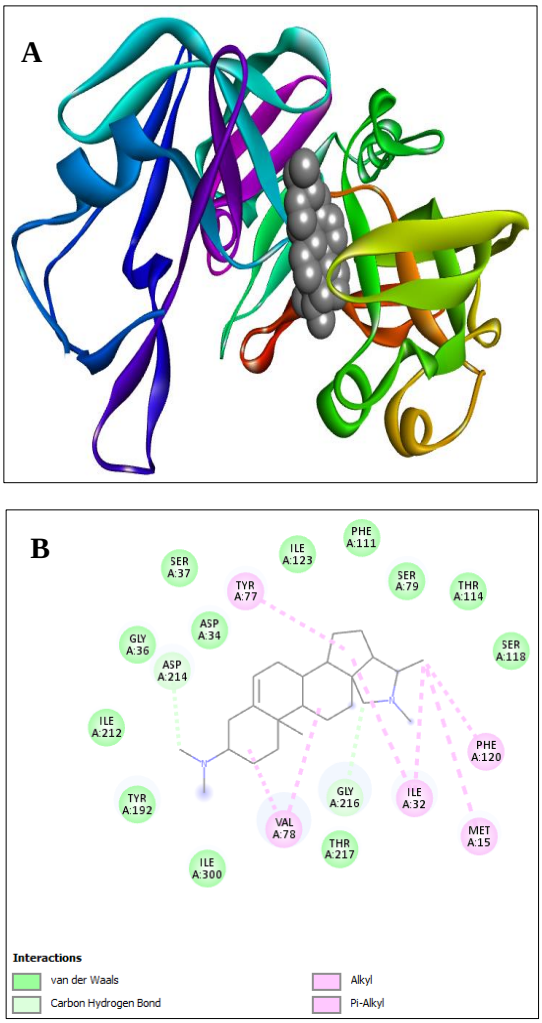
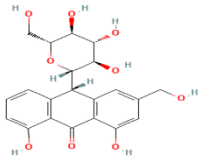
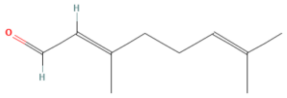
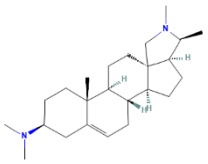
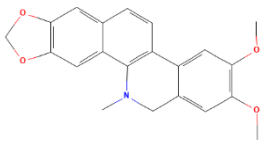
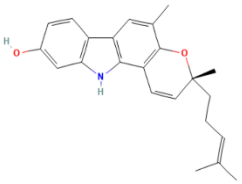
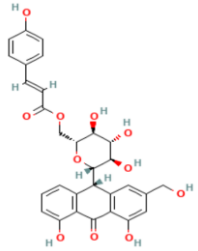
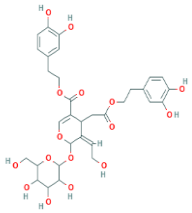
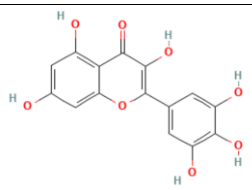
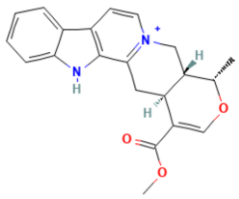
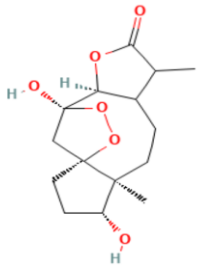


Figure 1. (A) Visualization and (B) Identification of ligand binding sites of the best-docked complex of Conessine-1LF4 using BIOVIA Discovery.

Table 1. The docking results of the phytochemicals with Plasmepsin-2.

Phytochemicals	PubChem ID	Molecular formula	Chemical structure	Binding energy (kcal/mol)	Ki (μM)
Aloin	12305761	$C_{21}H_{22}O_9$		-5.65	71.97
Citral	638011	$C_{10}H_{16}O$		-4.61	15.6
Conessine	441082	$C_{24}H_{40}N_2$		-9.86	0.058
Dihydronitidine	99641	$C_{21}H_{19}NO_4$		-6.70	12.33
Mahanine	36689305	$C_{23}H_{25}NO_2$		-7.81	1.88
Microdontin	101629131	$C_{30}H_{28}O_{11}$		-7.51	3.13
Multifloroside	14781469	$C_{32}H_{38}O_{16}$		-1.41	$91.93x10^3$

Myricetin	5281672	$C_{15}H_{10}O_8$		-5.98	41.4
Serpentine	73391	$C_{21}H_{21}N_2O_3$		-7.4	3.76
Tehranolide	6711941	$C_{15}H_{22}O_6$		-8.02	1.32

Taken together, the majority of the ligands showed encouraging selectivity against Plasmepsin-2. Further, it highlights new drug targets to treat malaria, especially in resistant malarial drugs. Although these phytochemicals showed desirable selectivity towards Plasmepsin-2, advanced validation and chemical tests and modifications need to be addressed. For instance, the current study involved rigid protein-ligand docking. However, the structure of proteins is flexible to suit the environmental conditions in real situations.[13]

4. Conclusion

In conclusion, Plasmepsin-2 is identified as the potential drug target in *Plasmodium falciparum* in treating malarial infections. Docking studies performed in this study predicted that Conessine as the potential ligand for Plasmepsin-2 with the lowest docked energy in which the interactions are stabilized by several binding residues of the protein. Therefore, this study has provided new insights into malarial interventions to the development of potent chemotherapeutic drugs to fight against the disease.

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