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Pavani Elipilla
Department of Biotechnology, Acharya Nagarjuna University, Guntur. Andhra Pradesh, India.

Correspondence
Pavani Elipilla
Department of Biotechnology, Acharya Nagarjuna University, Guntur. Andhra Pradesh, India.

Designing, molecular docking and toxicity studies of novel plasmepsin II inhibitors

Pavani Elipilla

Abstract
Malaria is one of the life threatening vector borne disease caused by a pathogenic protozoan, plasmodium. Continuous exposure to the same drugs makes a pathogen to improve or alter its strain to more virulent. So there is always a need to regular development of new, nontoxic and efficient drugs to overcome this problem. In this study an attempt was made to design a group of 5 non peptide novel ligands and docked with a malarial aspartic protease Plasmepsin II (PDB ID: 2BJU), along with commercially available anti-malarial drugs (Chloroquine, Mefloquine, Primaquine, Dicyclohexylcarbodiimide, Pepstatin) for comparative docking studies and analysis. From this docking study the newly designed ligands were proven to be having better binding affinity towards plasmepsin II protein active site than the commercially available anti-malarial drugs which was evidenced by the predicted binding energy and through predicted toxicity studies it was proved that these novel ligands were nontoxic since they satisfied all pharmacokinetic drug likeness rules.

Keywords: Drug designing, Docking, Predicted toxicity studies, Plasmodium, Anti-Malarial drugs, Aspartyl protease, Plasmepsins.

1. Introduction
Plasmodium is a unicellular eukaryotic protozoan microorganism. It causes malaria in humans by transmitting through female anopheles mosquito bite. Among the all species of plasmodium, mortality rate is high with *P. falciparum* infections. Still thousands of malarial deaths are reporting every year even in this 21st century due to the development of drug resistance in plasmodium species. Plasmodium has sexual and asexual life cycles. It completes its sexual life cycle in salivary glands and gut of female anopheles mosquito and asexual cycle in a vertebrate intermediate, host liver and blood stream. From the primary host plasmodium enters the secondary host human blood stream in the form of sporozoites. These sporozoites invade hepatocytes and divides into merozoites. These mature merozoites invade blood stream and enter R.B.C. There, it initiates hemoglobin digestion (Teun Bousema, 2014) [7].

Hemoglobin catabolism initiated by few plasmodium enzymes called, plasmepsins (Arun K. Ghosh, 2010) [1]. There are more than 10 plasmepsins produced by plasmodium. They belong to aspartyl protease family and synthesized as inactive zymogen forms, after the proteolytic cleavage of signal peptides, they will transform into active functional proteins. Among all these plasmepsins, Plasmepsin I and II causes initial break between phe 33 and leu 34 in hemoglobin alpha chain (J. Westling, 1999) [5]. Hemoglobin catabolism is a nutrient source for this pathogen and inhibitors of this plasmepsins proven to kill the plasmodium by arresting the hemoglobin catabolism and by starving the plasmodium. Hence these plasmepsins can be considered as promising anti-malarial drug targets. Plasmepsin II has structural similarity with HIV I protease and Cathepsin D proteins which are belongs to the same family (Arun K. Ghosh, 2010) [1]. Active site of this protein consists of 2 catalytically active aspartic acids Asp34 and Asp214 (J. Westling, 1999) [5]. Plasmepsin 1, 2, 4 enzymes commonly follows general acid base catalysis to degrade other peptides. In This paper few novel lead molecules were designed and were docked into the protein active site to study the interactions between ligand and protein active site residues and to calculate their binding energy. Their binding energies were compared with commercially available anti-malarial drugs docking score. And then their ADME properties were predicted to know their pharmacokinetic properties.

2. Materials and Methods

2.1 Ligand preparation

A group of 5 non peptide novel ligands were designed and their structures were drawn using ACD labs chemsketch (Fig-1). 5 commercially available anti-malarial drugs were selected for comparative studies and their structures were drawn on chemsketch. These ligands were subjected to energy minimization and optimization step in Prodrg online server (A.W.Schüttelkopf and D.M.F.van Aalten (2004). PRODRG: a tool for high throughput crystallography of protein ligand complexes, *Acta Crystallogr* D60, 1355–1363.). The IUPAC names of novel ligands are named below.

1. 4-amino-4-[2-amino-3-butyl-8-chloro-5-(1H-imidazol-4-ylmethyl)-6-methoxyquinolin-7-yl]-1-hydroxybutan-2-one.
2. 4-amino-4-{8-chloro-2-[(dimethylamino) methyl]-5-(1H-imidazol-4-ylmethyl)-6-methoxy-3-methylquinolin-7-yl}-1-hydroxybutan-2-one.
3. {2-[(tert-butylamino) methyl]-8-(3-ethylpyridin-2-yl) quinolin-4-yl} (piperidin-2-yl) methanol.
4. N-{3-[N-(2-chloro-2-hydroxyethyl) cyclohexa-1, 5-diene-1-sulfonamido]-2-methylpropyl}-2-(imidazolidin-1-yl) acetamide.
5. (6-amino-8-{6-[(tert-butylamino)methyl]-3-methylpyridin-2-yl}quinolin-4-yl)(piperidin-2-yl) methanol.

2.2 Protein Preparation

A crystal structure of Plasmepsin II Protease (PDB: 2BJU) was collected from Protein Data Bank. From the protein complex, bound ligand HETATM was deleted. Then the protein structure was optimized and energy minimized with the help of SPDV tool.

2.3 Molecular Docking

The above optimized ligands and protein (PDB: 2BJU) were taken for docking which was carried out with the help of Autodock4.0 tools. Water molecules were removed then polar hydrogens were added. Kollman charges were assigned to the protein. Then ligands were allowed to dock with protein active site Autogrid and Autodock were generated grid parameter files (.gpf) dock parameter files (.dpf). The grid dimensions were set to 60×60×60 Å on X, Y, Z, which covered the maximum active site area of the protein to accommodate the ligands. It was known through literature that Asp34 and Asp214 amino acid residues plays crucial role in catalytic activity of this protein and these two amino acids along with other active site residues participates in the formation of hydrogen bonds and other hydrophobic interactions with the ligands. Lamarckian Genetic Algorithm was ran using cygwin, finally 10 best fit docking poses were generated which were graded based on binding energies. All these interactions in the final docked protein, was visualized by using UCSF-chimera and Lig plot tool.

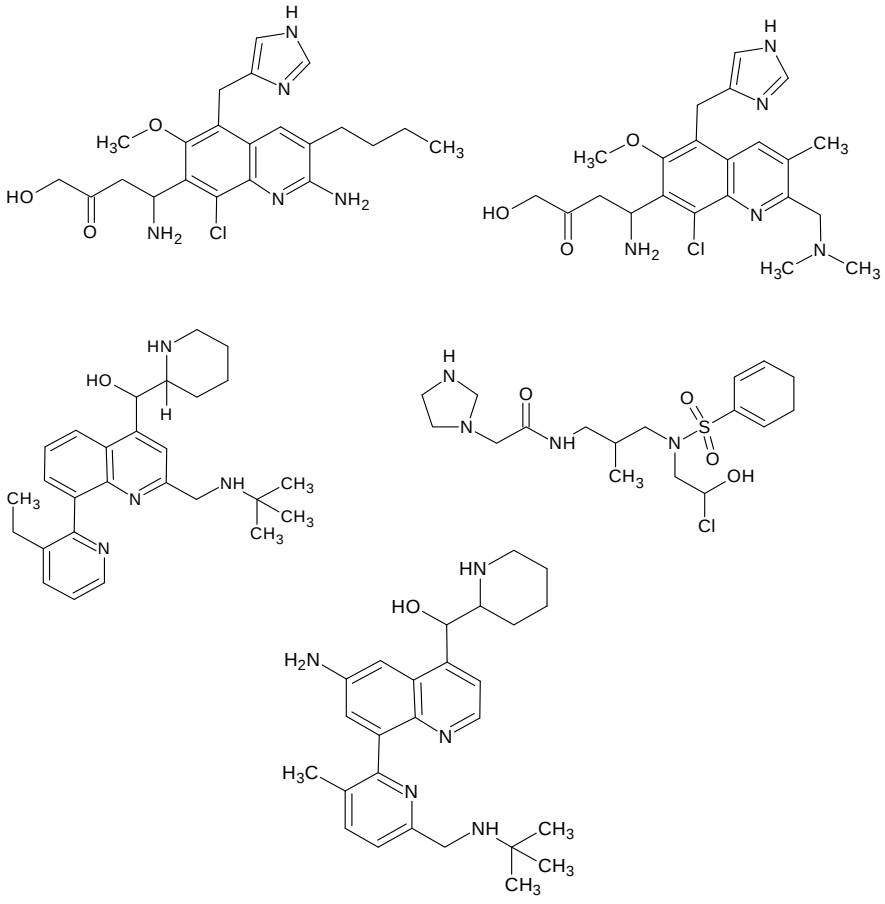


Fig 1: 5-Non peptide novel ligands were designed. Structures were drawn in ACD labs - Chem Sketch tool (1- 10, from left to right).

2.4. Toxicity prediction: A group of 5 novel ligands and commercially available anti-malarial drugs were checked for the violation of Lipinsky filter, Ghose filter, Viber filter, lead likeness rules (Table 1 and 2). Other pharmacokinetic properties like LogS, and, LogP Solvent Accessible Surface Area, Polar surface area, Protease inhibitor property, Estimated Binding Energy, Inhibition Constant Ki were predicted using online servers like Molinspiration, chemicalize, ALOGPS.

3. Results and Discussion

All together 5 non peptide novel ligands were designed (Figure.1). Commercially available antimalarial drugs (Chloroquine, Mefloquine, Primaquine, Dicyclohexylcarbodiimide, Pepstatin) were chosen for comparative studies. All these ligands were allowed to dock with the Plasmepsin II protein and ADME properties were predicted and the values were merged in (Table 1 and 2). The docking score or predicted binding energy of these 5 ligands were in between -7.60 to -10.26 Kcal/ mol and predicted inhibitory constant Ki values were in between 39.65 nM to 2.70 uM, Which was better than the commercial antimalarial drugs (Table.1). Then the 5 novel ligands and anti-malarial drugs were checked for the violation of Lipinski filter and other drug likeness rules, and it was shown that these novel ligands were satisfied all the rules and no violation is noticed (Table 2). Some of the adme properties like LogS, LogP, Solvent Accessible Surface Area, Protease inhibitor property, and Polar surface area values were predicted using online servers like chemicalize, ALOGPS, Molinspiration.

Table 1: Calculation of Predicted binding Energy, Ki, and other properties of novel ligands and some anti-malarial drugs

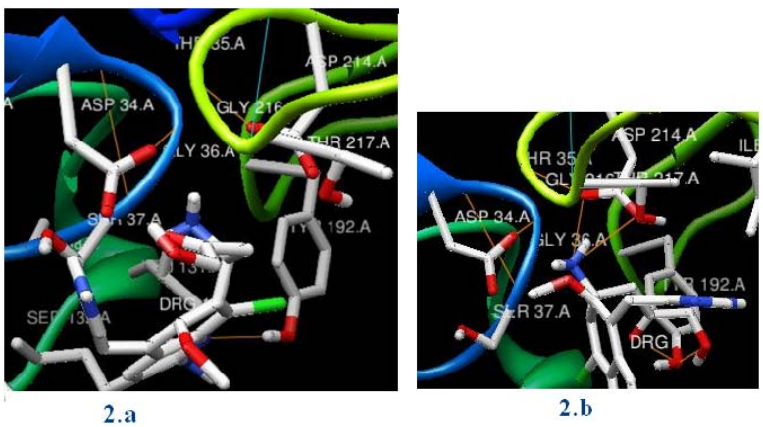
Ligands	Estimated Binding Energy Kcal/mol	Estimated Inhibition Constant Ki.	H bonds formed	Molecular weight	LogP	LogS	Polar surface area:
Ligand-1.	-7.60	2.70 uM	5	444.9	2.32	-4.64	137.24
Ligand-2.	-7.80	1.93 uM	8	444.93	1.24	-3.95	114.46
Ligand-3.	-10.26	30.41 nM	4	432.6	4.44	-5.35	70.07
Ligand-4.	-9.84	61.66 nM	7	420.9	-0.20	-2.89	101.98
Ligand-5.	-10.10	39.65 nM	3	433.5	3.16	-5.03	96.09
Chloroquine	-6.51	16.90 uM	3	319.8	3.93	-4.26	28.16
Mefloquinee	-5.33	123.80 uM	0	378.3	4.11	-4.00	45.15
Primaquine	-7.53	3.02 uM	4	259.34	1.64	-3.66	60.17
Dicyclohexylcarbodiimide	-3.04	5.93 mM	2	206.32	3.79	-4.32	24.72
Pepstatin	-6.83	9.80 uM	5	685.89	1.72	-4.02	223.26

To have a better permeability a drug molecule polar surface area should not exceeds 140 Å², LogP (lipophilicity) value should be less than is < 5 and LogS (aqueous solubility) should be ≤ 0 (Bob Gotwals, NCSSM Chemistry 2009) to get absorbed through cell membranes, and Molecular weight should be less than 500 dalton. These 5 novel ligands were having polar surface area **r**anging from 70.07 Å² to 137.24 Å², LogP values were ranging from -0.20 to 4.44, LogS values falls within the range of -2.89 to -5.35 and molecular weight ranged between 420.9 to 444.93daltons. These final values reveals that the novel ligands exhibiting strong binding affinity towards plasmepsin II protease active site than the commercially available antimalarial drugs which were proven to be having good pharmacokinetic properties.

Table 2: Calculation of Predicted pharmacokinetic properties of novel ligands and few anti-malarial drugs.

Ligands	Solvent Accessible Surface Area:	Protease inhibitory property	Lipinski's rule of five	Bio Availability filter	Ghose filter	Lead likeness filter	Muegge filter	Veber filter
Ligand-1.	636.41	0.31	yes	yes	yes	yes	yes	yes
Ligand-2.	648.93	0.26	yes	yes	yes	yes	yes	yes
Ligand-3.	715.71	0.37	yes	yes	yes	yes	yes	yes
Ligand-4.	604.12	0.50	yes	yes	yes	yes	yes	yes
Ligand-5.	699.49	0.46	yes	yes	yes	yes	yes	yes
Chloroquine	527.50	0.05	yes	yes	yes	yes	yes	yes
Mefloquinee	472.35	0.36	yes	yes	yes	yes	yes	yes
Primaquine	427.23	0.04	yes	yes	yes	yes	yes	yes
Dicyclohexylcarbodiimide	374.83	-0.55	yes	yes	yes	yes	yes	yes
Pepstatin	1164.31	0.32	yes	yes	yes	yes	yes	yes

2D and 3D orientations of ligand and protein interactions were analysed using UCSF Chimera and ligplot tools (Fig: 2.a – 2.d) other aminoacids participating in the ligand and protein interactions are, GLY36, THR217, SER15A, SER37, GLY216, and THR35.



2.a 2.b

Fig 2.a and 2.b: UCSF-Chimera generated images- 3D orientation of Ligand 1 and Ligand 2 with Plasmepsin II active site residues interactions respectively. Blue and orange lines represent the hydrogen bond formation between them.

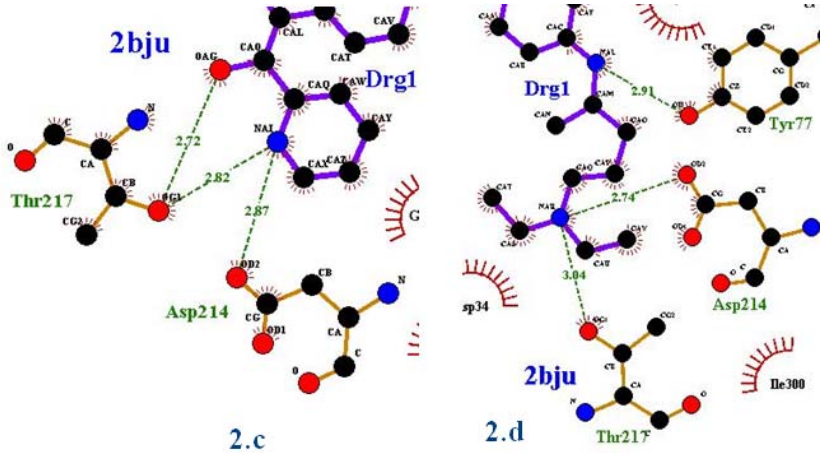


Fig 2.c and 2.d: Ligplot generated images -2D orientation of Primaquine, Chloroquine ligands and Plasmepsin II active site residues interactions respectively. Green dots represent the Hydrogen bond interactions between them.

4. Conclusion

From this docking experimental study, it was concluded that, these newly designed 5 ligand structures can be considered as potent anti-malarial drugs since they have shown better binding affinity and satisfied all pharmacokinetic drug likeness rules. If these ligands were attempted to synthesized, they could act as better antimalarial drugs.

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