RESEARCH ARTICLE

STUDY OF BINDING MODE OF BETULIN (LUP-20(29)-ENE-3B, 28-DIOL) ON PLASMEPSIN II FROM *Plasmodium falciparum*: AN ANTIMARIAL ANALYSIS

*[1]Tarun Agarwal, [1]Apoorva Singh and [2]Somya Asthana

[1]Amity Institute of Biotechnology, Amity University, Lucknow, U. P.
[2]Amity Institute of Biotechnology, Amity University, Noida, U. P.

ABSTRACT

Threatening half of the world's population Malaria still remains a serious public health problem in the developing world. Parasites of the genus *Plasmodium* cause the disease by degrading human hemoglobin as a source of amino acids for their growth and maturation. Plasmepsin II, an aspartic protease from the human intra erythrocytic parasite *Plasmodium falciparum*, is involved in degradation of the host cell haemoglobin within the acidic food vacuole of the parasite. Plasmepsin-II has become an attractive target for combating malaria through research regarding its importance in the *P. falciparum* metabolism and life cycle. In the present study, we have attempted with the help of virtual screening and molecular docking approach using Lamarckian Genetic Algorithm to elucidate the extent of specificity of Plasmepsin II towards different classes of Betulin. The docking result of the study of 534 molecules demonstrated that the binding energies were ranging from -10.06 kcal/mol to -6.00 kcal/mol, with the minimum binding energy of -10.06 kcal/mol. 8 molecules were showing hydrogen bonds with the catalytic amino acid residues of active site: Asp 34 and Asp 214. Further in-vitro and in-vivo study is required on these molecules as the binding mode provided hints for the future design of new derivatives with higher potency and specificity.

INTRODUCTION

Malaria is one of the most infectious disease, thus infecting people worldwide. The major causative agent of this major disease is *Plasmodium falciparum*. It transmits its infection through the bites of infected female anopheles mosquitoes. In the human body, the parasites multiply in the liver, and then infect red blood cells [Silva *et al*, 1996]. *P. falciparum* invades erythrocytes and take host's haemoglobin as a source of nutrition for the growth and development. It is catabolised in an acidic digestive vacuole of the parasite. The intraerythrocytic malaria parasite develops within a cell that contains a single major cytosolic protein group, Plasmepsins. The organism avidly ingests host hemoglobin and degrades it in a specialized proteolytic organelle called the digestive vacuole [Rudzinska, 1965; Aikawa, 1966; Slomianny 1982; Theakston, 1970]. As the parasite has a limited capability for de novo synthesis [Ting , 1966; Sherman 1977] or exogenous uptake [Pallet *et al*, 1968] of amino acids, the hemoglobin is catabolized to provide amino acids for growth and maturation [Sherman, 1970; Zarchin, 1985]. Plasmepsins are aspartic proteases involved in the initial steps of the hemoglobin degradation pathway, a critical stage in the *Plasmodium falciparum* life cycle during human infection [Valiente *et al*, 2008]. Plasmepsins are key enzymes in the life cycle of malarial parasites. There are about 10 types of Plasmepsins. Several have been described that are involved in hemoglobin

hydrolysis (the so-called hemoglobinases), antigen maturation, merozoite release, or invasion of new red blood cells by merozoites [Valiente *et al*, 2008]. Among the Plasmodium proteases, the hemoglobinases have been extensively studied. In the food vacuole (pH 5–5.4), the 37-kDa aspartic proteases plasmepsin I and plasmepsin II initiate cleavage of the native hemoglobin tetramers [Francis *et al*, 1997]. Plasmepsin I and II exhibit almost same structure of function but plasmepsin II has different substrate specificity as compared to plasmepsin I. It was also found that it had better haemoglobin degradative activity. Due to this, Plasmepsin II is one of the most potent haemoglobin degrading enzyme with good substrate specificity. Expression of this enzyme (Plm II) occurs in the erythrocytic cycle of the mosquito. As inhibition of plasmepsins leads to the parasite's death, these enzymes can be utilized as potential drug targets. Although many drugs are available, it has been observed that *Plasmodium falciparum*, the species that causes most of the malarial infections and subsequent death, has developed resistance against most of the drugs [Silva *et al*, 1996]

A wide variety of them have been designed, synthesized, checked for activity by enzyme assays as well as in parasite cultures. They include both peptide-like [Francis, 1994; Noteberg, 2003; Johansson, 2005] and non-peptidyl inhibitors [Jiang, 2001; Asojo, 2003; Mueller, 2003]. Some of them were designed based on the substrate structure (Phe33- Leu34 of Hb) [Mueller *et al*, 2003] and/or statine residue [Dahlgren *et al*, 2003] of the universal aspartic protease inhibitor, pepstatin.

Corresponding author: tarun3agarwal5@gmail.com

C2 symmetric [Ersmark *et al*, 2004] and achiral inhibitors were also reported. Betulin, a potential natural by – product obtained from a bark of a white birch, *Betula alba*. It was found to have an anti – malarial activity and can act on the enzyme Plasmepsin II. So, in the present study we try to computationally screen the inhibitory activity of Betulin and its derivatives on Plasmepsin II protein.

MATERIALS AND METHODS

Protein structure retrieval

The sequence of Plasmepsin II protein from *Plasmodium falciparum* was retrieved from RCSB Protein Data Bank (PDB). The protein model with PDB ID: 1SME (http://www.rcsb.org/pdb/explore/explore.do?structureId=1sm e) was chosen for active site predictions and further docking studies. For molecular docking, the ligand and other hetero-atoms (water, ions, etc.) were removed using Argus Lab Software.

Protein Active Site Predictions

The active site residues were predicted using Computed Atlas of Surface Topography of proteins (CASTp) database (http://sts-fw.bioengr.uic.edu/castp/calculation.php). The active site were analyzed and site having the catalytic amino acid Asp34 and Asp214 was chosen as docking site for Betulin and its derivatives. Analysis of the catalytic amino acids was done through Uniprot (Primary Accession Number: P46925) (http://www.uniprot.org/uniprot/P46925).

Substrate selection

The chemical 3D structures of derivatives of Betulin and the commercially available anti malarial drugs such as Mefloquine, Chloroquine, Hafloquine; were screened from PubChem database (http://pubchem.ncbi.nlm.nih.gov/). The positional coordinated of each molecule was retrieved in *.sdf format and the PDB structure of the molecules was deduced via PRODRG Server (http://davapc1.bioch.dundee.ac.uk/prodrg/). The molecular structure with only polar hydrogen bond was taken for optimization and docking process. The structures were optimized using Argus Lab Software.
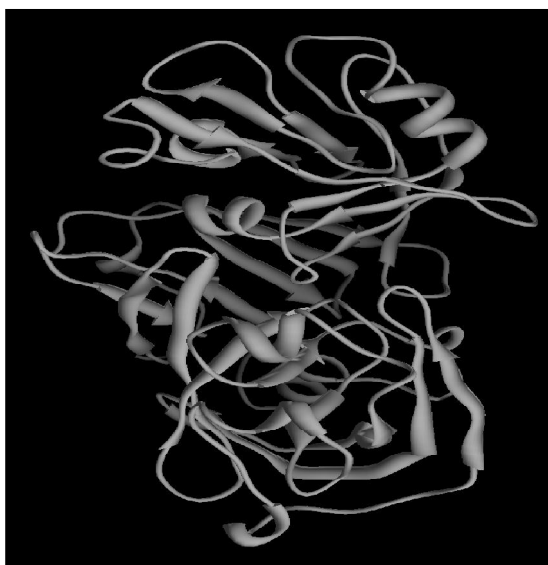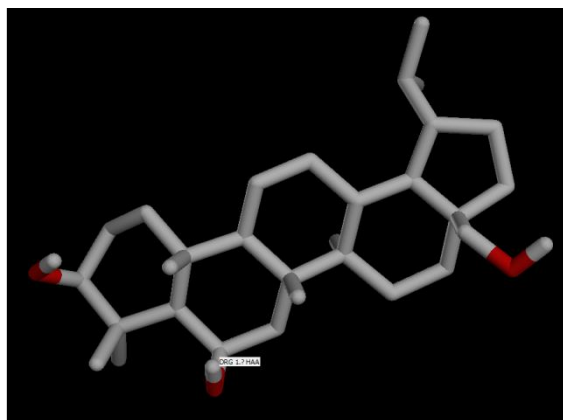

Fig. 1. Plasmepsin II protein (PDB Id: 1SME)

Fig. 2. Betulin

In silico screening

534 chemical structures of Betulin and available drugs against malaria were docked onto the active site of the Plasmepsin II protein structure using the program AutoDock4.2 (MGL Tools) (Morris *et al*., 1998). Ten independent docking runs were carried out and the minimum and maximum binding energies were recorded for each molecule.

Docking setup

Docking was performed using Autodock4.2, which combines energy evaluation through grids of affinity potential employing various search algorithms to find the suitable binding position for a ligand on a given protein (Morris *et al*., 1998). While docking, polar hydrogen's were added to ligands using the hydrogen's module in Autodock tool and thereafter, Kollman united atom partial charges were assigned. Docking of ligands onto Plasmepsin II protein from *Plasmodium falciparum* was carried out using LGA with standard docking protocol on the basis a population size of 150 randomly placed individuals; a maximum number of $2.5*10^7$ energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80 and an elitism value of 1. Ten independent docking runs were carried out for each ligand and results were clustered according to the 1.0 $\overset{o}{}$ rmsd criteria. The grid maps representing the proteins were calculated using auto grid and grid size was set to 60*60*60 points with grid spacing of 0.375 $\overset{o}{}$. The coordinate of the docked protein along with the ligand was visualized using UCSF chimera (http://www.cgl.ucsf. edu/chimera) within 5 $\overset{o}{}$ region.

RESULTS AND DISCUSSIONS

The computational approach of molecular docking of the drug molecule on the protein of interest has proved to be an effective strategy to study the binding patterns of the drugs with the proteins models. The Plasmepsin II protein (1SME) from *Plasmodium falciparum* was selected for our docking study. A total of 534 three dimensional structures of Betulin and its derivatives were selected from PubChem databases and were screened against the functional site of 1SME through AutoDock4.2 software. The chemical 3D structures of commercially available anti-malarial drugs namely Mefloquine, Chloroquine, Hafloquine (reference molecules for the study) were also docked onto the active site. The active site analysis of the protein was performed using CASTp

Calculations. Amongst all the binding sites obtained, site 1 was highly conserved and the most favorable site for docking. The residues in site 1 were found to be Met15, Tyr17, Ile32, Asp34, Gly36, Ser37, Ala38, Met75, Asn76, Tyr77, Val78, Ser79, Phe111, Thr114, Ser118, Phe120, Ile123, Leu131, Tyr192, Ile212, Asp214, Gly216, Thr217, Ser218, Ala219, Thr221, Ile290, Leu292, Phe294, Ile300. The docking result of the study of 534 molecules in the active site of Plasmepsin II protein model: 1SME; demonstrated that the binding energies were ranging from -10.06 kcal/mol to -6.00 kcal/mol, with the minimum binding energy of -10.06 kcal/mol (CID_23425554) (Fig 3). The docking of our reference drug molecules namely Mefloquine, Chloroquine, Hafloquine; on our protein model (1SME), showed the minimum binding energies of -9.06kcal/mol, -8.80kcal/mol and -9.96kcal/mol respectively. The number of hydrogen bonds and the amino acid residues involved in bonds between the target protein and the molecules, in the docked protein model was illustrated by Chimera. The final docked conformations obtained for the different ligands were evaluated based on the minimum binding energy and the number of hydrogen bonds formed as given in Table 1. 8 molecules were showing hydrogen bonds with the catalytic amino acid residues of active site: Asp 34 and Asp 214.

Fig. 3. Docking of CID_23425554: Binding Energy= -10.06kcal/mol

Table 1. Binding Energy and H-Bond Pattern: Reference Drugs and Betulin Compounds

S.No.	Name / PubChem Id	Min. Binding Energy (kcal/mol)	Inhibition Constant (Ki) (nM)	Number of Hydrogen bonds	With Active Site Residues
1	Hafloquine	-9.66	82.48	1	Gly36
2	Chloroquine	-8.8	353.01	3	Asp214, Ser218(2)
3	Mafloquine	-9.06	219.78	2	Asp34, Gly36
4	23425554	-10.06	42.07	1	Asp214
5	21607675	-10.06	42.11	1	Asn76
6	21669890	-9.93	52.38	0	-
7	21768807	-9.75	71.47	1	Gly36
8	21607674	-9.7	77	2	Gly36, Asn76
9	14524502	-9.64	85.64	1	Thr192
10	5284253	-9.46	115.69	2	Gly216(2)
11	22832892	-9.35	132.42	2	Asp34, Asp214
12	6440718	-9.27	161.18	2	Leu131
13	22216302	-9.22	175.06	1	Gly36
14	44329440	-9.19	184.83	1	Asp214
15	11705540	-9.16	192.92	3	Val78, Leu131, Asp293
16	5284252	-9.11	210.87	2	Tyr192, Gly216
17	5284210	-8.93	283.39	4	Asp34, Thr217, Ser218(2)
18	10321267	-8.86	319.32	2	Asp34, Asn288
19	461835	-8.79	360.19	2	Asn76, Asp293
20	44178931	-8.65	455.46	2	Asn76, Asp293
21	53297365	-8.49	597.11	1	Asp34
22	25762879	-8.31	813.99	1	Asp34
23	11500470	-8.13	1110	2	Asp34, Ser218

The molecule with PubChem Id: CID_528410 showed the maximum of 4 Hydrogen bonds with the active site residues (1 hydrogen bond with Asp34) (Fig 4); while the molecule with PubChem Id: CID_22832892 gave 2 hydrogen bonds, both with catalytic amino acid residues of Plasmepsin II, Asp34 and Asp214 (Fig 5). Designing drugs against a disease based on structural interactions with the ability to work at high resolution with both proteins and drug compounds marks the importance of Structure Based Drug Designing in drug design. Further there is need to generate in vitro and in-vivo activity of the generated data to synthesize and test so to design drug with better specificity and metabolism. The work is significant in emphasizing the potent inhibitory effects of Betulin (lup-20(29)-ene-3β, 28-diol) and its derivatives on Plasmepsin II activity and further their application in anti-malarial drug design.
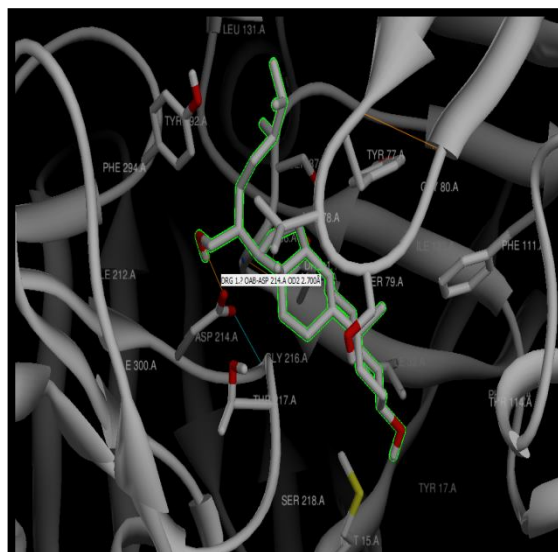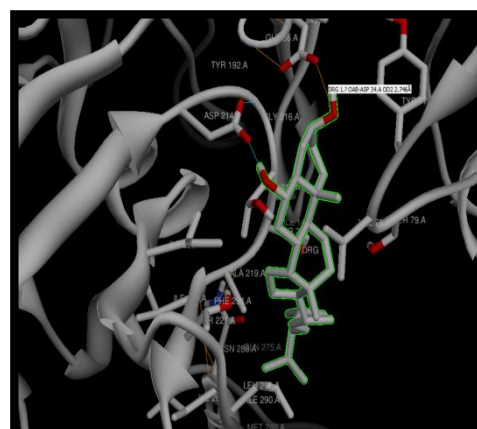

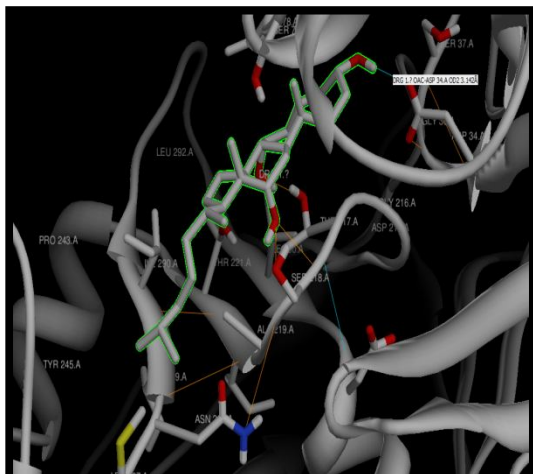**Fig. 4. Docking of CID_22832892: Binding Energy = -9.35kcal/mol**

Fig. 5. Docking of CID_5284210: Binding Energy = -8.93kcal/mol

REFERENCES

Aikawa, M., Hepler P.K., Huff C.G., Sprinz H. 1966. The feeding mechanism of avian malarial parasites.) Cell Biol. 28: 355.

Asojo, O.A., Afonina, E., Gulnik, S.V., Yu, B., Erickson, J.W., Randad, R., Medjahed, D. and Silva, A.M. 2002. Structures of Ser205 mutant plasmepsin II from Plasmodium falciparum at 1.8 A° in complex with the inhibitors rs367 and rs370. Acta. cryst. D 58: 2001–2008.

Asojo, O.A., Gulnik, S.V., Afonina, E., Yu, B., Ellman, J.A., Haque, T.S. and Silva, A.M. 2003. Novel uncomplexed and complxed structures of plasmepsin II, an aspartic protease from *Plasmodium falciparum*. J. Mol. Biol. 327, 173–181.

Dahlgren, A., Kvarnstrom, I., Vrang, L., Hamelink, E., Hallberg, A., Rosenquist and Samuelsson, A.B. 2003. New inhibitors of the malaria aspartyl proteases plasmepsin I and II. Bioorg. Med. Chem. 11, 3423–3437.

Ersmark, K., Feierberg, I., Bjelic, S., Hamelink, E., Hackett, F., Blackman, M.J., Hulten, J., Samuelsson, B., Aqvist, J., and Hallberg, A. 2004. Potent inhibitors of the Plasmodium falciparum enzymes plasmepsins I and II devoid of cathepsin D inhibitory activity. J. Med. Chem. 47, 110–122.

Francis, S.E., Gluzman, I.Y., Oksman, A., Knickerbocker, A., Mueller, R., Bryant, M., Sherman, D.R., Russell, D.G. and Goldberg, D.E. 1994. Molecular characterization and inhibition of Plasmodium falciparum aspartic hemogloinase. EMBO J. 13: 306–317.

Jiang, S., Prigge, S.T., Wei, L., Gao, Y.E., Hudson, T.H, Gerena, L., Dame, J.B. and Kyle, D.E. 2001. New class of small nonpeptidyl compounds blocks Plasmodium falciparum development in vitro by inhibiting plasmepsins. Antimicrob. Agents Chem other. 45: 2577–2584.

Johansson, P.-O., Lindberg, J., Blackman, M.J., Kvarnstrom, I., Vrang, L., Hamelink, E., Hallberg, A., Rosenquist, A. and Samuelsson, B. 2005. Design and synthesis of potent inhibitors of plasmepsin I and II: X-ray crystal structure of inhibitor in complex with plasmepsin II. J. Med. Chem.48: 4400–4409.

Morris, C.M., Goodsell, D.S., Halliday, R.S. *et al* 1998. Automated docking using a lamarckian genetic algorithm and and empirical binding free energy function. J Comput Chem 19: 1639-1662.

Mueller, R., Huerzeler, M. and Boss, C. 2003, Synthesis of Plasmepsin II inhibitors Potential Antimalarial agents. Molecules 8: 556–564.

Noteberg, D., Hamelink, E., Hulten, J., Wahlgren, M., Vrang, L., Samuelsson, B. and Hallberg, A. 2003. Design and synthesis of plasmepsin I and plasmepsin II inhibitors with activity in Plasmodium falciparuminfected cultured human erythrocytes. J. Med. Chem. 46: 734–746.

Pallet, H., and Conrad M.E. 1968. Malaria: extracellular amino acid requirements for in vitro growth of erythrocytic forms of *Plasmodium knowlesi*. Proc. Soc. Exp Biol. Med. 127:251.

Rudzinska, M.A. 1965. Pinocytic uptake and the digestion of hemoglobin in malaria parasites. J. Protozool. 12: 563.

Sherman, I .W, and Tanigoshi L. 1970. Incorporation of 14C amino acids by malaria. Int. J. Biochem. 1:635.

Sherman, I .W. 1977. Amino acid metabolism and protein synthesis in malarial parasites. Bull. WHO 55: 265 .

Silva, A.M. *et al* 1996. Biochemistry.Structure and inhibition of plasmepsin II, a hemoglobin-degrading enzyme from *Plasmodium falciparum* (malaria/drug design/ crystallography/aspartic protease/cathepsin D). Proc. Natl. Acad. Sci. USA 93: 10034-10039.

Slomianny, C., Prensier G., and Vivier E. 1982. Ultrastructural study of the feeding process of erythrocytic P. chabauditrophozoite. Mot Biochem. Parasitol 5: 695 .

Theakston, R.D.G., Fletcher K.A., and Maegraith B.G. 1970. The use of electron microscope autoradiography for examining the uptake and degradation of haemoglobin by Plasmodium falciparum. Ann. Trop Med. Parasitol 64: 63.

Ting, I.P., and Sherman I.W. 1966. Carbon dioxide fixation in malaria-I. Kinetic studies in *Plasmodium lophurae*. Comp Biochem. Physiol 19: 855.

Valiente, P.A., Batista, P.R., Pupo, A., Pons, T., Valencia, A., Pascutti, P.G. 2008. Predicting functional residues in Plasmodium falciparum plasmepsins by combining sequence and structural analysis with molecular dynamics simulations. Proteins, 73(2): 440-57

Zarchin, S., Krughak M., and Ginsburg H. 1986. Digestion of the host erythrocyte by malaria parasites is the primary target for quinolone-containing antimalarials. Biochem. Pharmacol. 35: 2435.

****