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In silico Drug Prediction and Validation of Lead candidates on Plasmodium falciparum Erythrocyte Membrane Protein 1(pfEMP1) against Malaria

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ABSTRACT

Malaria is an infectious disease caused by protozoan of the genus Plasmodium. It is transmitted by bite from infected female Anopheles mosquito. *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEmp1), an antigen that is responsible for the immune evasion of the protozoan. This protein has adhesive properties that cause the infected erythrocytes to bind to the endothelial lining of the blood vessel, thus preventing the infected cells from getting filtered by the spleen. It is found that there is an interaction between the sulphate ion on the endothelial cells and NH_1, NH_2 of Arg 1467 (A), NZ of Lys 1324 (A) and two bonds with N of Gly 1329 (A) on the protein. Inhibiting this interaction may prevent the evasive action. A Ligand with SO_4 interactive region can be used to achieve this. Computer aided drug designing techniques were used to find a new scaffold to solve the purpose. GROMACS was used to simulate the protein-Ligand interaction. It was observed that **ZINC17206599** shows the best interaction and may prove to be a promising candidate drug for Malaria.

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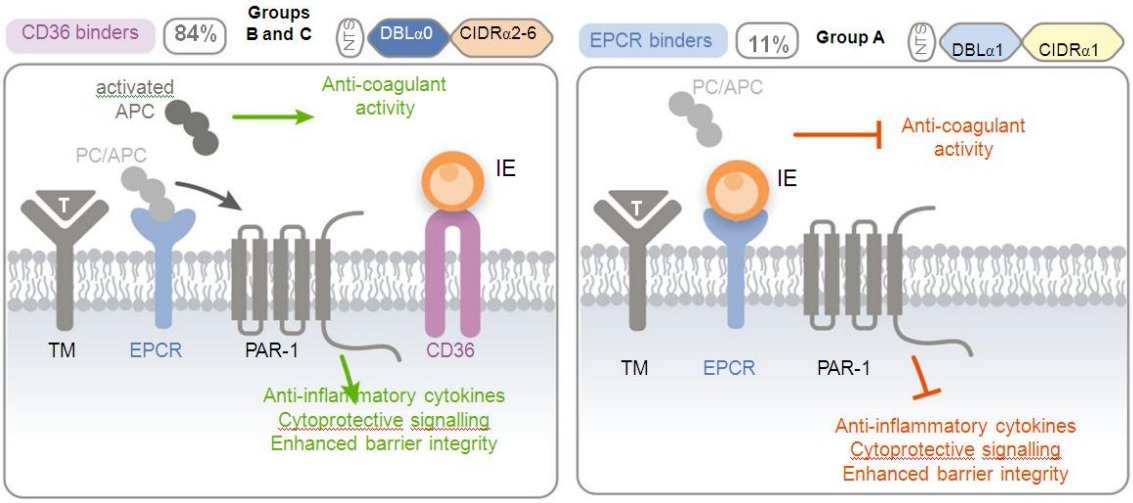
1. INTRODUCTION

Malaria was discovered to be a parasitic disease in 1880s. Malaria is an infectious disease transmitted to humans by the infected Anopheles mosquitoes. The infection spreads through the bite of the infected mosquito and by contact with the infected blood in rare cases. A developing foetus can contract the disease from the mother. The common symptoms include high fever and chills, headache, nausea, sweats, muscle pain and these tends to show up in cycles. Malaria is a preventable and curable disease. However sometimes, even after the treatment and the malarial parasites (*Plasmodium*) remain in the body in organs as they become resistant to the medicines. Cytoadhesion of *Plasmodium falciparum*-infected erythrocytes to host microvasculature is a key virulence determinant. Parasite binding is mediated by a large family of clonally variant adhesion proteins, termed *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), encoded by *var* genes and expressed at the infected erythrocyte surface [1]. Although PfEMP1 proteins have extensively diverged under opposing selection pressure to maintain ligand binding while avoiding antibody mediated detection, recent work has revealed they can be classified into different groups based on chromosome location and domain composition. This grouping reflects functional specialization of PfEMP1 proteins for different human host and microvascular binding niches and appears to be maintained by gene recombination hierarchies. In one extreme, a specific PfEMP1 variant is associated with placental binding and malaria during pregnancy, while other PfEMP1 subtypes appear to be specialized for infection of malaria naïve hosts. Here, we discuss recent findings on the origins and evolution of

the *var* gene family, the structure–function of PfEMP1 proteins, and a distinct subset of PfEMP1 variants that have been associated with severe childhood malaria [2,] [3].

The human malaria parasite, *Plasmodium falciparum*, varies a family of adhesion proteins at the infected erythrocyte (IE) surface that it uses to bind to the endothelial lining of blood microvessels (Miller*et al*., 2002). During blood-stage infection, *P. falciparum* merozoites sequentially invade and egress from red blood cells every 48 h. As the parasite matures, IEs exhibit reduced deformability and sequester from blood circulation.This allows the parasite to avoid splenic clearance mechanisms, but comes at a cost to the host. Sequestered IEs disrupt microvascular blood flow (Dondorp *et al*., 2008) and cause localized endothelial dysfunction by damaging endothelial barrier integrity and inducing proinflammatory, pro-adhesive and coagulation pathways (Francischetti *et al*., 2008; Moxon *et al*., 2009; Miller *et al*.,2013). This adhesive phenotype is also associated with organ-specific disease complications from IE adherence in brain (cerebral malaria) and placenta (placental malaria) microvasculature (Miller *et al*., 2002). Cytoadhesion is mediated by specific interactions between members of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family encoded by *var* genes and receptors on the surfaces of endothelial cells (Rowe *et al*., 2010)[4]. Each parasite genotype encodes 60 different *var* genes, which are expressed in a mutually exclusive fashion (Guizetti and Scherf, 2013). Switching between *var* genes facilitates parasite immune evasion and modifies IE binding specificity (Roberts *et al*., 1993). A specific PfEMP1 variant, VAR2CSA, interacts with chondroitin sulfate A, which is abundant within the placental intervillous space (Fried and Duffy, 1996; Salanti *et al*., 2004). In contrast, cerebral binding and severe childhood malaria is associated with specific PfEMP1 variants containing a combination of adhesion domains, termed domain cassettes (DC) 8 and 13 (Avril *et al*., 2012;Claessens *et al*., 2012; Lavstsen *et al*., 2012). The vast majority of *P. falciparum* infections do not lead to severe malaria, suggesting that IE sequestration is relatively well adapted to limit host death and favour parasite transmission to mosquitoes. It is therefore interesting that potentially lethal adhesion traits persist in the parasite population. This review covers recent advances in the molecular mechanisms of PfEMP1 binding, integrating findings on protein structure–function, *var* gene evolution and adhesion phenotypes associated with severe malaria[4].



The PfEMP1 head structure has diverged into three major binding groups under selection for EPCR binding, CD36 binding, or to form rosettes with uninfected erythrocytes. The proportion of different head structure types in the 3D7 reference genome isolate and their predicted binding properties is shown. Note there may be some binding exceptions (e.g. only a subset of CIDRα1 domains bind EPCR, see Fig. 1). CD36 binding is the most common PfEMP1 adhesion trait and is associated with mild malaria. EPCR binding and rosetting is linked to group A head structures. Group A PfEMP1 tend to be expressed in early childhood infections or malaria naïve, and are also associated with severe malaria. Under normal circumstances, protein C (PC) binds to EPCR and is activated by the thrombin (T)/thrombomodulin (TM) complex. Activated protein C (APC) that is released into the plasma has anti-coagulant activity and the APC/EPCR complex activates the protease activated receptor 1 (PAR1) to mediate intracellular signalling. The protein C-EPCR signalling pathway has anti-inflammatory, anti-thrombotic and endothelial cytoprotective activities that help maintain vascular integrity (Mosnier *et al*., 2007). The loss of EPCR at sites of *P. falciparum* IE sequestration (Moxon *et al*., 2013) and EPCR binding parasites (Turner *et al*., 2013) may combine to interfere or subvert these pathways and contribute to disease pathogenesis[5], [6].

According to WHO, **a**pproximately 660,000 people died from malaria in 2010 globally, most of them were African children. There were an estimated 219 million cases of malaria infection in 2010 worldwide. The malaria burden in many parts of the world is being dramatically reduced thanks to increased malaria prevention and control measures. Travellers from malaria-free areas who enter endemic areas are especially vulnerable to severe symptoms when they become infected. About 80% of all malaria cases occur in just 17 countries [4]. Nigeria and the Democratic Republic of the Congo account for more than 40% of all malaria deaths worldwide [7].

Different types of malaria are caused by different species of Plasmodium parasite and each type differs according to the parasites as each of the parasite have different life cycle which results in slight symptoms and treatment variations[9]. Malaria is to be treated as soon as the symptoms appear. According to World Health Organization the following drugs can be used for treating malaria: Amodiaquine, Artesunate, Atovaquone, Dapsone, Dihydroartemisinin, Lumefantrine, Mefloquine, Piperaquine, Pyrimethamine (see figure 1 for the structures).

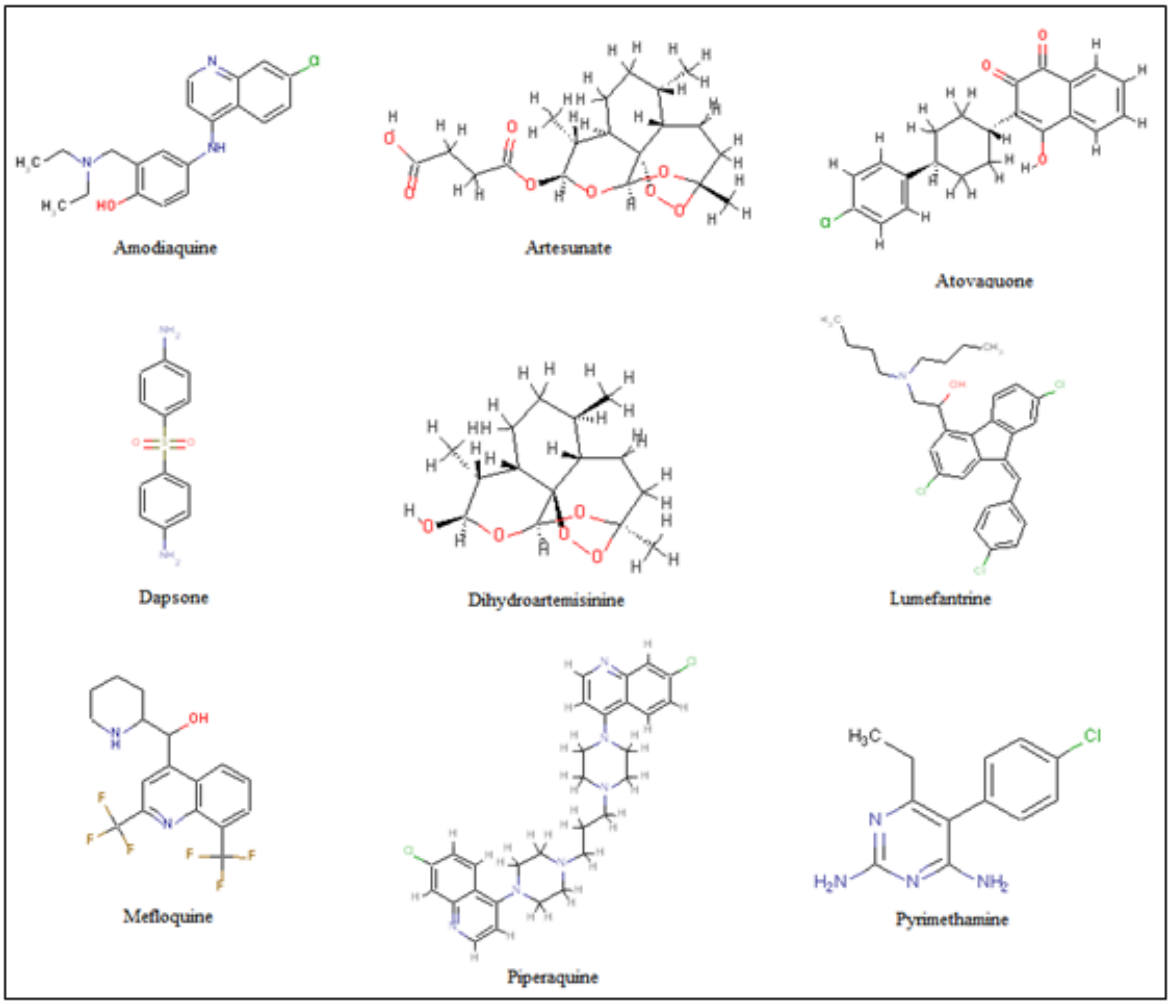


Figure 1: Marketed Anti-malarial drugs

Considering the epidemiology, action of the existing anti-malarial drugs is weakened as the parasites become highly resistive due to their mutative capacity. Therefore, it is necessary that the proteins synthesized by these parasites are targeted to control the disease outcome. The agenda is to inhibit the interaction of NH_1, NH_2 of Arg 1467 (A), NZ of Lys 1324 (A) and two bonds with N of Gly 1329 (A) with the four oxygen's from the sulphate ions. The endothelial cell of the blood vessel contains chondroitin sulphate where the protein's adhesive property comes into action as it binds to the SO_4 on the cell lining. Hence, if this function is inhibited, the effect can be inversed. Hence, the infected cells can be filtered out of the body.

The aim of this study was to design a lead compound against malaria with the help of various Insilico approaches against multiple protein targets. The various approaches include Pharmocophore based screening to get more novel molecules based on uniqueness of features, screening against various databases and to study molecular dynamics study to check stability of the protein-ligand complex in different environmental factors

with respect to temperature, pressure and energy that might prove more efficient binding against the target protein and hence increase the probability to cure the malaria.

2. MATERIALS AND METHODS

2.1 Literature review and screening the drugs

Nine drugs marketed against malaria as prescribed by World Health Organisation namely Amodiaquine, Artesunate, Atovaquone, Dapsone, Dihydroartemisinin, Lumefantrine, Mefloquine, Piperaquine, and Pyrimethamine were finalised for the process. Thereafter, these molecules were screened for Arg, Lys, and Gly - CDS interaction. Structure of a chondroitin sulphate binding DBL3X from a var2csa encoded PfEMP1 protein in complex with sulphate (3BQK) was used as the target protein and the sulphate group was removed using Accelrys Discovery Studio before submitting it for active site prediction. CastP server (http://sts.bioe.uic.edu/castp/), an online tool to find the active sites on a protein was used to determine the pockets on PfEMP1. It was observed that Lys 1324, Arg 1467, Gly 1329 fell under the largest pocket (pocket 47). The range for plotting the grid while docking drugs with the protein was analysed from this result. The averaged coordinates of the amino acids were obtained from the topology file of PfEMP1 so that grid parameters (centre grid box values) could be set and the pocket size was also considered for this. Grid parameter (point dimension) was set to 60, 60, 60 on the x, y and z coordinates to cover the entire pocket and docking was performed. It was observed that only Amodiaquine, Artesunate, Atovaquone, Mefloquine and Piperaquine bonded with Gly 1329 or Lys 1324 (see Table 1).Dapsone, Dihydroartemisinin, Lumefantrine, Pyrimethamine were eliminated as it did not show required interaction.

Table 1: Docking result for the nine marketed drugs with PfEMP1

Sr. no	Drugs	Run	Energy	Rank	Sub-Rank	Interaction
1	Amodiaquine	46	-5.92	7	1	Gly 1329
2	Artesunate	15	-6.55	3	2	Lys 1324
		34	-6.39	13	1	Lys 1324
3	Atovaquone	4	-7.96	2	1	Gly 1329
		29	-7.31	3	4	Lys 1324
		41	-7.33	3	3	Lys 1324
		55	-7.92	2	2	Gly 1329
4	Dapsone	No Match	No Match	No Match	No Match	No Match
5	Dihydroartemisinin	No Match	No Match	No Match	No Match	No Match
6	Lumefantrine	No Match	No Match	No Match	No Match	No Match
7	Mefloquine	16	-5.26	5	4	Gly 1329
		26	-5.25	5	5	Gly 1329
		26	-5.25	5	5	Lys 1324
		46	-5.44	5	2	Gly 1329
8	Piperaquine	18	-5.5	15	1	Gly 1329
9	Pyrimethamine	No Match	No Match	No Match	No Match	No Match

Selected drugs were then combined in Discovery Studio into a single .mol2 format. Separate combination was created keeping each drug as pivot molecule for Pharmocophore analysis. These were then uploaded to PharmaGist server, an online server for Pharmocophore analysis. The analysis revealed that Artesunate as the pivot molecule produced favourable results (see Table 2).

The Pharmocophore was obtained from PharmGist (http://bioinfo3d.cs.tau.ac.il/PharmaGist/) result for combined molecule (Artesunate, Amodiaquine, Atovaquone, Mefloquine, Piperaquine) with Artesunate as the pivot molecule, were then uploaded on Zinc Pharmer (http://zincpharmer.csb.pitt.edu/) for searching molecules with identical Pharmocophore. Parameters were altered to get optimum results. Repetitive Pharmocophore class members were disabled and filters were set to molecular weight ≤ 500 and subset was set to ZINC Natural Products.

2.2 Preparing the molecules for docking

The result from Zinc Pharmer generated a single .sdf file for the various screened molecules. Duplicate entries were removed from the .sdf to obtain unique molecules in a single .sdf file (see Figure 3 for KNIME workflow). Perl subroutine was used to split the single .sdf files into separate molecule files based on the delimiter differentiation. Thus, 37 sdf files obtained were then converted to .pdbqt format necessary for docking (Autodock) with Shell script.

KNIME workflow for removing duplicates using GroupBy Node

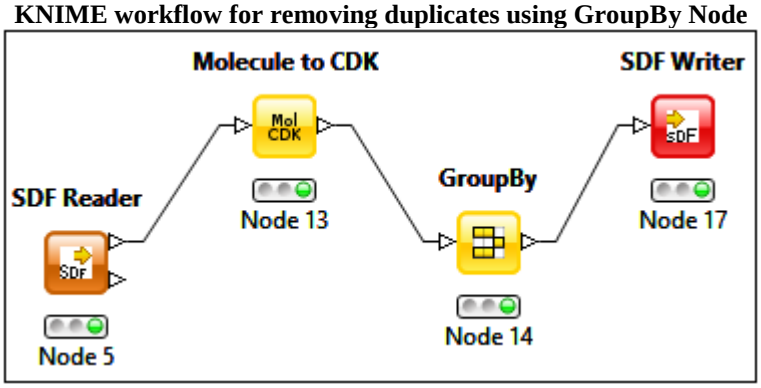


Figure 3: KNIME workflow for removing duplicated molecule from sdf file

2.3 Docking using Autodock

Autodock is a tool developed using C program. This tool is used to study interaction between ligands (flexible) and protein (macromolecules) of known and unknown structures. Docking can be done in conformational space using Lamarckian genetic algorithm that is coupled with energy using a method based on the Amber force field. These two functions generate the molecular coordinates that describes about the possible docked ligand conformations that can be used for theoretical drug designing and study. Docked conformation is represented by binding energy and hydrogen bond formation on the basis of quantum and molecular mechanical modeling of atomic forces[8],[9].**The grid parameters** that was used for the first docking was used again as the pocket in consideration was same. Grid parameter files and dock parameter files for 50 runs were generated individually for all the Ligands. Then, a batch file was prepared to run all the 37 Ligands. After docking Lamarckian genetic algorithm was utilised to analyse the result. Ligands with lowest binding energy value were selected.

2.4 Loop Docking

The purpose behind loop docking approach is based on the fact that consecutive docking runs could noticeably improve the docking energy and orientation. In various cases, the best docked structure could be a docking artefact and does not represent the best docking orientation as per our experience in the docking study. Therefore, we always opt to rerun the docking calculation using the best-docked structure from initial docking as a starting structure for a second docking run. Few scripts were used to allow this process to be automated. This can be achieved by Autodock Vina or else by creating batch files of the ligands selected to redock. The automated loop docking will continue until threshold value is reached. The threshold value (d) is the difference between the docking binding energy of the last run and the preceding one. When the defined threshold value is reached, the docking stops and the best docked structure are selected. Autodock software is used for docking analysis [9].

2.5 Molecular Dynamics Simulation using GROMACS

Molecular simulation is very important and useful toolbox to understand structures and dynamics in detail in molecular modelling wherein motions of atoms can be tracked. This method is more focused on two main approaches, namely energy minimization and molecular dynamics that optimize the structure and simulate natural motion of biological molecules. Firstly, we are going to set up environment for simulation, prepare the input file of the structure for simulation, solvate the structure in water, minimize and equilibrate it, perform short production simulation. GROMACS (http://www.bevanlab.biochem.vt.edu/Pages/Personal/justin/gmx-tutorials/complex/) GROMOS 43a1 force field was used to process the GROMACS structure file and topologies were created with the help of PRODRG server. The system was solvated using SPC water model in a box with 1nm solute wall distance. Non toxic Ligand with the lowest binding energy was selected for molecular dynamics simulation. Steepest descent algorithm was used to run energy minimization steps [10].

3. RESULTS AND DISCUSSION

Results from the second docking step was obtained and noted in table 3. The 50 runs performed to obtain 50 conformations were checked for hydrogen bonds and the required interaction between the Ligand and the protein (Arg 1467, Lys 1324, and Gly 1329). It was observed that four Ligands ZINC03825293, ZINC12898578, ZINC17206599, and ZINC44690116 had slightly optimum biding energy -7.27 to -9.08. Loop docking was then performed on these Ligands to obtain the consistency in the interaction formed. Batch file with the docking scripts were created for 40 RUNs to dock the Ligands (15 set for each Ligand, summing to 60 docked values).

Molecule ID	Molecule*	Rank	RUN	Binding Energy	Interaction
ZINC02048908	mol_35	2	15	-5.45	Arg1467:NH1::Mol:O1
ZINC02096530	mol_9	1	23	-4.01	Lys1324:NZ::Mol:O2
ZINC03114733	mol_34	1	2	-5.31	Lys1324:NZ::Mol:O1
ZINC03825293	mol_37	2	39	-7.27	Arg1467:NH1::Mol:O2
ZINC05116458	mol_36	1	28	-6.73	Arg1467:NH1::Mol:O2
ZINC05499390	mol_17		Not docked to required Amino acid		
ZINC08383351	mol_19	1	9	-4.25	Arg1467:NH1::Mol:O1
ZINC08386341	mol_3		Not docked to required Amino acid		
ZINC08396720	mol_4	2	4	-5.68	Gly1329:N::Mol:O4
ZINC08396724	mol_30	1	6	-5.35	Lys1324:NZ::Mol:O1
ZINC08397806	mol_27	1	10	-6.54	Lys1324:NZ::Mol:O1
ZINC08455906	mol_23	1	31	-6.76	Lys1324:NZ::Mol:O1
ZINC08456758	mol_14	1	17	-6.97	Lys1324:NZ::Mol:O1
ZINC12898578	mol_29	1	13	-9.08	Arg1467:NH2::Mol:O2
ZINC17206599	mol_10	1	13	-8.02	Lys1324:NZ::Mol:N1
ZINC38141428	mol_26	1	44	-3.57	Lys1324:NZ::Mol:O2
ZINC44690116	mol_28	2	2	-9.08	Lys1324:NZ::Mol:O2
ZINC68562755	mol_15	Not docked to required Amino acid			
ZINC68562764	mol_18	2	6	-2.48	Lys1324:NZ::Mol:O1
ZINC68562775	mol_16	1	31	-2.31	Lys1324:NZ::Mol:O1
ZINC68568582	mol_22	1	40	-3.04	Lys1324:NZ::Mol:O1
ZINC68568583	mol_21	1	16	-3.46	Lys1324:NZ::Mol:O1
ZINC68568586	mol_32	1	50	-3.42	Arg1467:NH1::Mol:O2
ZINC68568588	mol_33	1	50	-3.18	Arg1467:NH1::Mol:O2
ZINC68568593	mol_7	1	37	-3.03	Arg1467:NH1::Mol:O2
ZINC68568595	mol_8	2	3	-3.21	Arg1467:NH1::Mol:O1
ZINC68568614	mol_25	Not docked to required Amino acid			
ZINC68568616	mol_24	1	31	-3.44	Lys1324:NZ::Mol:O2
ZINC68568621	mol_12	1	4	-3.77	Gly1329:N::Mol:O2
ZINC68568623	mol_13	1	34	-2.69	Lys1324:NZ::Mol:O2
ZINC68568635	mol_11	1	10	-3.27	Gly1329:N::Mol:O1
ZINC68591375	mol_5	1	8	-3.08	Lys1324:NZ::Mol:O1
ZINC68591381	mol_6	1	30	-3	Lys1324:NZ::Mol:O2
ZINC68591396	mol_1	1	30	-2.15	Arg1467:NH1::Mol:O2
ZINC68591401	mol_2	2	4	-2.6	Arg1467:NH1::Mol:O2
ZINC72400092	mol_31	1	20	-3.64	Lys1324:NZ:Mol:O1

ZINC77311469	mol_20	Not docked to required Amino acid

Table 3: Docking results based on conformation at which favourable interaction was obtained. Molecules were numbered for convenience.

Zinc ID	Molecule	Rank	RUN	Binding Energy	Interaction
ZINC17206599	mol_10-1	2	40	-8.77	Lys1324:NZ::Mol:O4
	mol_10-2	4	11	-7.5	Lys1324:NZ::Mol:N1
	mol_10-3	1	3	-8.43	Lys1324:NZ::Mol:O4
	mol_10-4	1	37	-8.64	Lys1324:NZ::Mol:N1
	mol_10-5	1	1	-8.69	Lys1324:NZ::Mol:O4
	mol_10-6	1	26	-8.74	Lys1324:NZ::Mol:O4
	mol_10-7	1	30	-8.15	Lys1324:NZ::Mol:O1
	mol_10-8	2	26	-8.1	Lys1324:NZ::Mol:O4
	mol_10-9	1	18	-8.23	Lys1324:NZ::Mol:O4
	mol_10-10	1	7	-7.74	Gly1329:N::Mol:O5
					Lys1324:NZ::Mol:O4
	mol_10-11	1	40	-8.65	Lys1324:NZ::Mol:N1
	mol_10-12	1	13	-8.13	Gly1329:O::Mol:O7
	mol_10-13	2	21	-8.28	Gly1329:N::Mol:O1
					Lys1324:NZ::Mol:O4
	mol_10-14	1	17	-8.74	Lys1324:NZ:Mol:N1
	mol_10-15	1	22	-8.82	Lys1324:NZ:Mol:O1
ZINC44690116	mol_28-1	1	18	-8.76	Lys1324:NZ::Mol:O2
	mol_28-2	3	16	-8.67	Lys1324:NZ::Mol:O6
	mol_28-3	2	14	-8.65	Lys1324:NZ::Mol:O1
	mol_28-4	2	25	-9.09	Lys1324:NZ::Mol:O1
	mol_28-5	1	9	-8.68	Arg1467:NH2::Mol:O5
	mol_28-6	1	20	-8.96	Arg1467:NH2::Mol:O5
					Arg1467:NE::Mol:O5
	mol_28-7	3	33	-8.88	Arg1467:NH2::Mol:O2
	mol_28-8	2	3	-9.03	Lys1324:NZ::Mol:O5
	mol_28-9	1	2	-9.21	Lys1324:NZ::Mol:O1
	mol_28-10	2	14	-8.6	Arg1467:NH2::Mol:O5
	mol_28-11	2	6	-9.02	Lys1324:NZ::Mol:O4
	mol_28-12	2	16	-9.23	Lys1324:NZ::Mol:O1
	mol_28-13	3	21	-8.85	Lys1324:NZ::Mol:O5
	mol_28-14	1	3	-8.74	Lys1324:NZ::Mol:O1
	mol_28-15	1	7	-8.76	Lys1324:NZ::Mol:O2

Table 4: Loop docking resulted in the following values of the Ligands binding energies.

It was observed that Ligand "ZINC17206599" and "ZINC 44690116" had least fluctuating binding energy range with standard deviations 0.401 and 0.205 respectively.

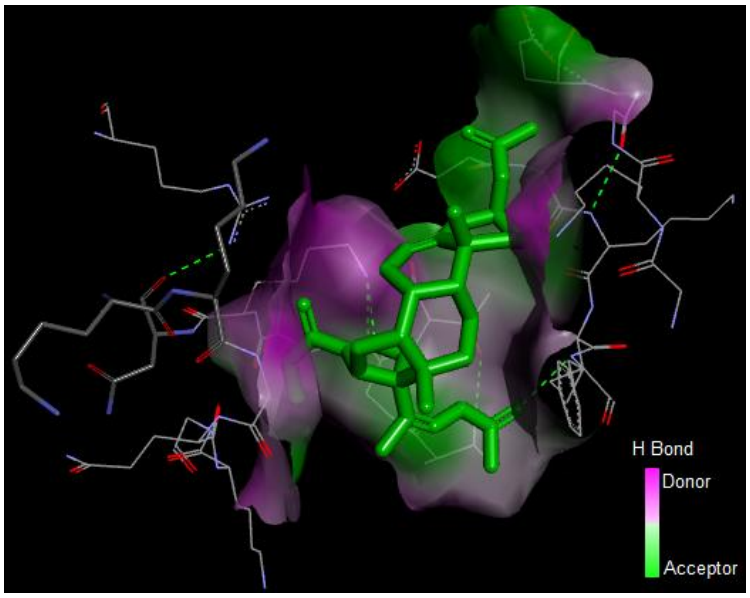


Figure 3: PfEmp1 interacting with ZINC17206599

3.1 Toxicity Prediction

The basis for toxicity prediction from chemical structure is that the properties of chemical are implicit in its molecular structure. Biological activity can be expressed as a function of partition and reactivity, that is, for a chemical to be able to express its toxicity it must be transported from its site of administration to its site of action and then it must bind to or react with its receptor or target. This process may also involve metabolic transformation of the chemical[11]. The application of these principles to the prediction of toxicity of new or untested chemicals has been achieved in number of different ways covering a wide range of complexity, from computer system containing database of hundreds of chemicals, to simple "reading across" between chemicals with similar chemical /toxicological functionality. The prediction of toxicity from chemical structure can make a valuable contribution to the reduction of animal usage in the screening out of potentially toxic chemicals at an early stage and in providing data for making positive classifications of toxicity. The toxicity value was predicted using Toxpredict (https://apps.ideaconsult.net/ToxPredict) for Ligands "ZINC17206599" and "ZINC 44690116" (see table 4 for Toxpredict results). ZINC 44690116 was eliminated for further analysis as it turned to be carcinogenic.

Molecule	Mol Wt. (g/mol)	Mol. Formula	XLogP	HBD Count	HBA Count	Rotatable Bonds	Toxicity	Lipinski Rule failure
ZINC17206599	528.6371	C29H40N2O7	4.4	0	9	8	No	0
ZINC 44690116	470.43	NA	1.39	2	8	4	Yes	0

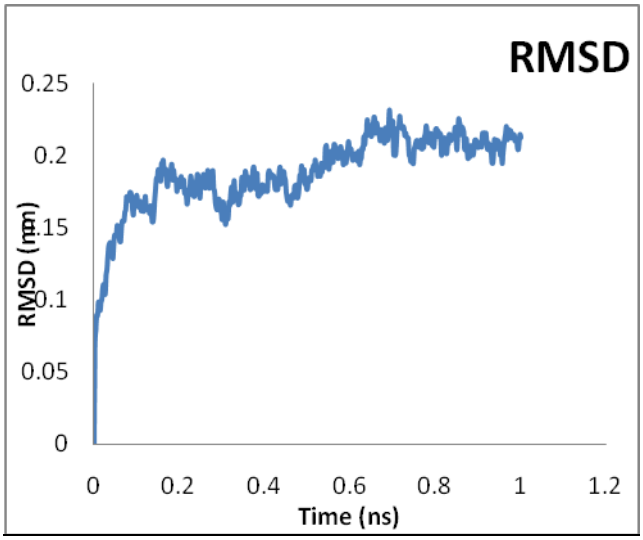
Table 5: Computed properties for selected "ZINC17206599" are as follows

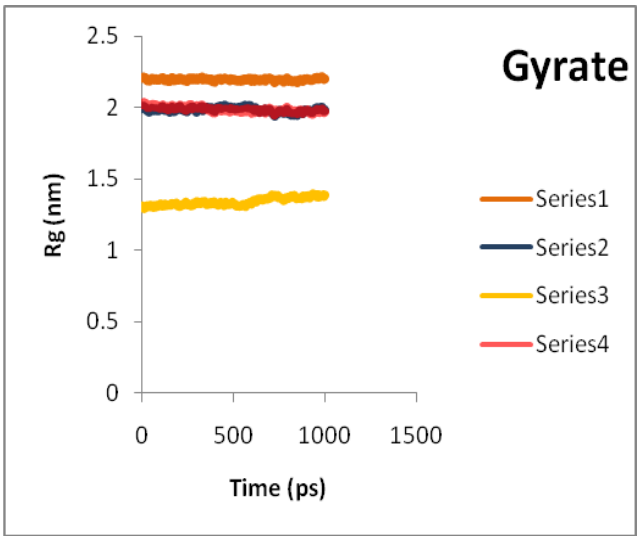
3.2 GROMACS Results

i. **RMSD**: Each structure from a trajectory (-f) is compared to a reference structure. The reference structure is taken from the structure file (-s) so you need to put the crystal structure under the flag -s and the structure that you want to compare with the crystal structure under the flag -f. Then, when you select the groups, the first that the tool asks for is the group for least square fit (so, the structures under the -f flag) and the second is the group for RMSD calculation (structure under the -s flag). For better information you can see section 8.9 of Gromacs Manual. The g_rms is a graphic of RMSD vs time. You should also see root mean square fluctuation (g_rmsf), perhaps this tool is what you are looking for. The plot indicates that the RMSD value is fluctuating over the time, but there are chances that it can be stable after 1.2 ns at 0.2 nm.

ii. **Radius of Gyration**: Radius of gyration describes the overall spread of the molecule and is defined as the root mean square distance of the collection of atoms from their common centre of gravity. We can see from

the reasonably invariant R_g values that the protein remains very stable, in its compact (folded) form over the course of 1 ns at 300 K. This result is not unexpected, but illustrates an advanced capacity of GROMACS analysis that comes built-in.





4. DISCUSSION

As per our observation in docking results the ZINC17206599 molecules shows binding affinity towards the pfEMP1 protein. Interestingly, for all the proposed compound complexes, the average RMSD values were below 0.25 A ° and the variation of the potential energies falls within 1,000 kcal/mol which is a good indication of the system stability. Docking studies are used at different stages in drug discovery such as in the prediction of ligand–receptor complex structures and also to rank the ligand molecules based upon the binding energies of the corresponding ligand–enzyme complexes. Docking protocols aid in elucidation of the most energetically favourable binding mode of the ligand to the receptor. Validated computational tools can serve as useful tools to save time and effort in the drug design process. In this report, we tried to introduce an Insilco approach that relies on the use of docking calculations followed by molecular dynamics simulations to accurately estimate the binding affinity, as well as the stability of the inhibitor enzyme complexes. It can be safely noted that docking calculations could be entirely misleading and even using the same software, in many case, may result in different predictions. In order to ensure reliable docking results, we embarked on a novel approach which ‘‘loop-docking’’ to enhance the docking calculations predications and to verify their binding by using MD simulations.

5. CONCLUSION

In the selection of new drug candidates, many efforts are focused on the early elimination of compounds that might cause several side effects or interact with other drugs. *In silico* techniques help in this regard and they are going to become a central issue in any rigid drug discovery process. In silico technology alone cannot guarantee the identification of new safe and effective lead compound but more realistically future success depend on the proper integration of new promising technologies. The Drug Designing and development process is a long and expensive one. Due to the limitation of throughput, accuracy and cost, experimental techniques cannot be applied widely; therefore, recently the drug discovery process has shifted to *In Silico* approaches such as homology modeling, protein-ligand interactions, vHTS etc. *In Silico* approach has been of great importance to develop fast and accurate target identification and prediction method for the discovery. Therefore, from the obtained result it can be concluded that SO_4 **ZINC17206599** shows interaction with NH_1, NH_2 of Arg 1467 (A), NZ of Lys 1324 (A) and two bonds with N of Gly 1329 (A) on the protein which may inhibit the reason causing pathogenicity of PfEmp1[12], [13]. Toxicity values are also observed to be favourable for the drug. Molecular dynamics simulation shows stable rmsd, gyrate value. Thus, the results from this study may prove **ZINC17206599** to be candidate drug for treating malaria. Further optimizations as well as in-depth structural and biological studies of the selected inhibitor are required to confirm the findings.

Conflict of Interest: None

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