Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI - 10.26479/2018.0403.01**

THE ROLE OF PFEMP1 DBL3X IN PLACENTAL MALARIA

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ABSTRACT: Despite of several studies on parasite *Plasmodium falciparum*, it is still one of the primary agents provoking diseased state eventually leading to global mortality. Though there are several parasitic encoded proteins, *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) is the factor responsible for triggering malaria, which is expressed by var gene. PfEMP1 is responsible for facilitating cytoadherence and segregation in *Infected Erythrocytes (IE)*. This helps the parasite to evade its killing in spleen. Cytoadherence also acts as an important virulence factor associated with adhesion-based complications of the infection like cerebral malaria (CM) and pregnancy-associated malaria (PAM). The PfEMP1 molecule can vary in P. falciparum clones, which helps to escape the antibody-mediated clearance causing variation in its antigens. Therefore, we perform homology modeling to identify the molecules which will bind to the host receptors, further inhibiting the interactions with the ligands leading to reverse action. Moreover, we investigate PfEMP1 interactions to get an insight of several parasitic ligands and their host receptors involved in adhesion and segregation in vascular endothelium, which can act as potential target that blocks the transmission and mediate the proliferation of malaria in humans.

KEYWORDS: PfEMP1, Sequestration, cytoadherence, Pregnancy- associated malarial (PAM), Antigenic Variation

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1. INTRODUCTION

Malaria is one of the epidemic disease. It's wide spread amongst the rising countries like India and South Africa makes it one of the lethal disease. [1] The overall estimation of the people jeopardized is 3.3 billion per year. [2] Malaria is also called as the "Disease of poverty", as there is a lack of knowledge, nutrition and reliable diagnosis and reporting centers, majorly in rural areas. [3, 4] Plasmodium genus of protozoa kingdom is responsible for malaria and there are various species associated with human infections. Out of which, *P. falciparum* is responsible for causing severe effects and mortality in humans. [5, 6] The prime vector for malaria is *Anopheles culicifacies* found in the rural areas. [3, 7] Due to its ecological diversity and vast distributions, the epidemiology of malarial is quite complicated. [3] Moreover, there are various factors like mosquitoes resistant to insecticides, parasites resistant to the associated drugs, global warming, etc. that led to drastic increase in the occurrence of malaria. [8, 9]. There are two types of cycles required for the transmission and survival of the plasmodium, i.e. human host cycle and anopheles vector cycle. [10] The asexual replication and formation of the gametocytes occurs in human host. This replication results in the diversified population expressing different surface antigens that permits the escape of parasite from the immune system. Following genetic recombination, the parasite undergoes another asexual replication allowing the spread of new parasites population that could eventually have a better fitness, ensuring the transmission success to another human hosts. [10, 11] The infectious cycle is initiated with a bite of the female mosquito, which requires blood for the growth of succeeding batches of eggs. The sporozoites within the saliva mix with the anticoagulants, which invade directly the bloodstream to reach the liver. [10-12]. Here the host cycle of plasmodium starts, where the sporozoites replicate into merozoites. This initiates a new phase called as "Asexual Blood Phase". In this phase, merozoites attack erythrocytes. Once erythrocytes infect, they form a ring-shaped structures called as trophozoites (early stage) and Schinonts (Late stage). [10, 11] These ring-forms are also found in placenta causing placental malarial (PM) and in the brain causing cerebral malaria (CM). [10] A small part of the merozoites will differentiate into gametocytes, which will be rapt within the mosquito. Therefore completing the replication and transmission of plasmodium. [11]. The survival of parasite in human host depends on growth rate, cytoadhesion and antigenic variation. Once infecting the erythrocyte, it produces proteins that were transported on the surface, forming knob structures, necessary for its adhesion. [13] The morphological change and display of antigens on the surface of the IE triggers an innate and humoral immune response. These parasite encoded proteins are called variant surface antigens (VSA) to mediate the antigen variation allowing the escape of IE from the immune response. Repeated exposure to parasite infection builds up a stock of VSA-antibodies, increasing the immunity of the host against malaria.[14] VSA include five different protein families, which are transported onto erythrocytes surface, PfEMP1 is one of

them. [15, 16] Horrocks, 2005 and Hviid, 2010 have studied PfEMP1 properties and found that, though there are other VSA proteins, this protein shows the cytoadhesive function. [14, 17] To avoid spleen clearance, the IE can bind to Different host endothelium, via PfEMP1 and matrix molecules present on the vascular endothelial cells Other IE via agglutination, or uninfected erythrocytes to form rose petal arrangement these sequestration mechanisms form aggregates causing microvascular obstruction and trigger an inflammatory response, contributing to the different clinical symptoms and the malaria pathogenesis. [18] By sequestering, the IE can accumulate in organs as the brain (in CM) or the placenta (in pregnant women). When IE accumulates in the capillaries, a greater risk of local occlusion within circular system occurs, resulting in impaired blood flow in the organs. [19] There are series of receptors that bind to the endothelium leading to various malarial conditions. [20] Out of these, chondroitin sulphate A (CSA) is associated with PM. [21]. There are multiple copies of genes associated with VSA, out of which PfEMP1 is expressed by var genes. [22] The var gene has two part exon I (large) and exon II (small). Exon I consist of extra-cellular part of the protein, that comprises of many domains rich in cysteine, referring to as Duffy-binding-like (DBL) domains. [23] Gamain, 2005 shows that DBL domain has affinity towards the CSA receptor (Especially in PM) [24]. PM is an important factor contributing to premature delivery, hypertension, infant anemia and mortality. [25] The immunity to malaria acquired during childhood do not prevent development of PM within a pregnant women, in areas where transmission is hiked. As a result, compared to other adults, pregnant women are greatly inclined towards malarial infection and experience more regular and advanced density of infections. [25] To prevent PAM in such areas, WHO endorses regular chemoprophylaxis or sulfadoxine-pyrimethamine (SP) Treatment. However, SP is rapidly trailing its efficacy due to drug resistant parasites. [26] Besides chloroquine and SP, no other drugs are known to be safe when used as malaria preventatives during pregnancy, and chloroquine also failed to show its effectiveness against malaria. Thus, this article focuses on developing novel drug keeping track on all of these factors.

2. MATERIALS AND METHODS

Various computational approaches that allows identification of novel compounds, design for its selectivity, efficacy, and safety, to obtain an appropriate clinical trial candidate. [27] The major steps are as follows:

2.1. Target identification:

Prior to the ligand selection it is essential to select a protein which has pathogenic relevance to clinical disease taken into consideration. Based on the literature search, PfEMP1 DBL3X is selected as the primary target for our study to develop a drug against PAM. The 3D structure for this protein is obtained using Protein Data Bank (PDB).

2.2. Target Structure Refinement:

On stability analysis, missing regions within the PDB structure (Table 1) were found. MODELLER is used to fill the missing regions within the PDB structure by running a script file. Later an alignment file is generated for loop building, which generates a refined structure.

2.3. Structure Validation:

Structure validation is carried using an online server SAVES (Structural Analysis and Verification Server). This server allows its user to the study the coordinate file format, factor files (of structure) and creates various validation reports. These reports include a Ramachandran Plot, Summary report and assembly of structural diagnosis like comparison of bond angle, bond distance, and torsional angle. Protein Structure Analysis (ProSA) is another tool used to validate the protein structure. It determines the overall and local structure quality of the model.

2.4. Binding site Analysis:

Once a valid structure is obtained, binding site analysis is performed to get insight of the ligands present within the protein structure. Previous studies conducted on PfEMP1 and its domain DBL3X, were used you find the active binding sites.

PDBSUM also analyzes the ligands, their activity (interaction with the binding residues), binding sites and their probability. CASTP is used to obtain the cavities and structural pockets that are favorable for the binding sites.

2.5. Ligand Library:

DrugBank and ZINC generates the ligand library. The library created primarily has drug which are already been used as malarial drugs against PAM. Thus another library that is analogues to these drugs was created for comparative binding analysis. This library helped out in two ways

i. To find the pharmacophore
ii. Drugs that have better binding properties

Xanthone and its derivatives shows some anti-malarial properties like preventing heme polymerization and PfEMP1 protein inhibition. [28] Thus, ZINC database is used to create the secondary library consisting of Xanthone Homologs.

2.6. Fragment Based ligand design (FBLD):

ReCore Tool is used for carrying out the FBLD. It generates a secondary library of ligands by connecting small molecular fragments that adheres to the binding site in all possible positions.

2.7. Protein-Ligand Docking:

Protein-ligand interactions were studied using FlexX Molecular Docking tool. The ligand library that is mentioned in section 3.5 of this article is used to dock with DBL3X protein.

2.8. Molecular Simulation:

Molecular simulation enhances our understanding of dynamic behavior of our protein DBL3X.

GROMACS software was used for our study. The molecular dynamics (MD) simulation results ligand protein interaction in water. In addition to this, explore various confirmations of ligands.

3. RESULTS AND DISCUSSION

3.1. Target Identification:

On screening the data associated with the PfEMP1 protein from the literature, different DBL structures were obtained (six to be specific), which are likely to hold the disulfide bonds. In DBL domain, the amount of disulfide bond differs from structure three to seven, from which only two are conserved through all the domains. [23, 24]. The major difference between DBL domains that binds to the CSA occurs in regions with loops tying alpha helices together. Interestingly, A4 DBL 3X have an important loop which has Glycine (Gly) and Lysine (Lys) residues, capable of chelating the sulphate bound to the domain, were missing (Table 1) or were suggestively different in other domain of DBL. Figure 1 gives the molecular description of the PfEMP1 protein with its PDB structure. [29]

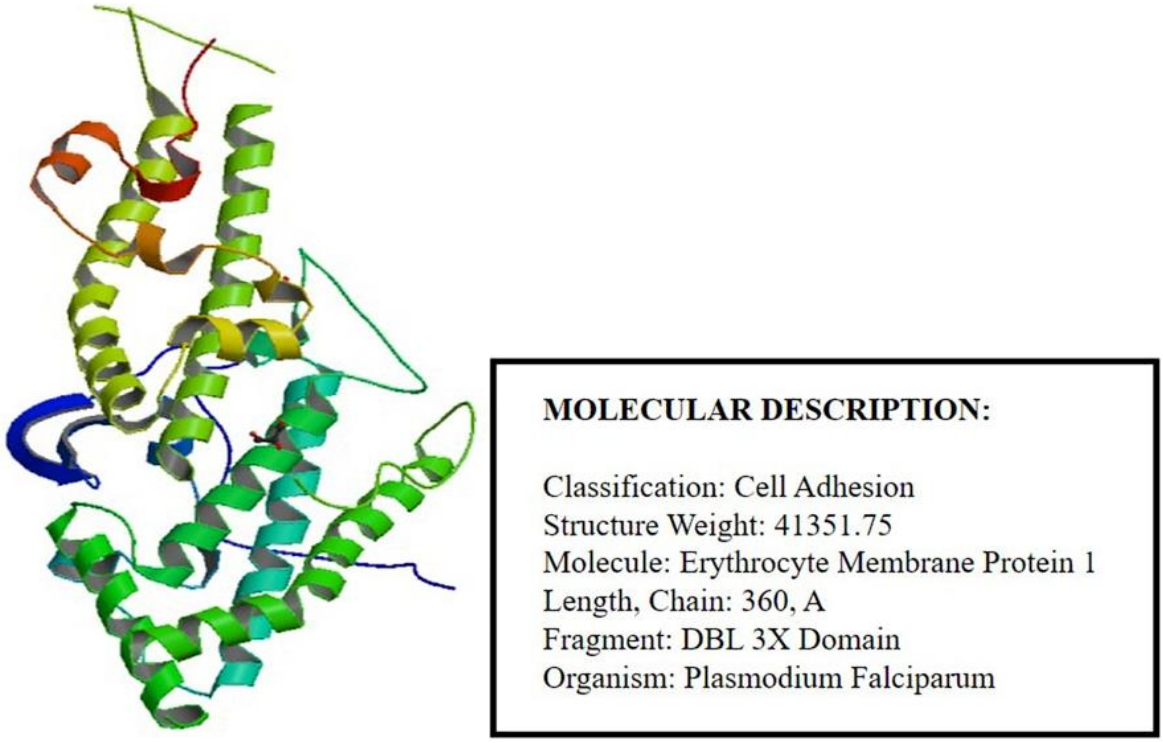


Figure 1: DBL3X structure retrieved from the protein databank (PDB ID: 3BQL) [30] with molecular description

Table 1: Details of the missing residues

Position	Number of a.a	Missing Residues
1388-1396	9	LYS, ASP, LYS, ILE, GLY,VAL, GLY, SER
1444-1477	4	GLY, ASN, ASP, GLU
1478-1482	5	ILE, ASN, GLY,LYS, ASN
1490-1493	4	LYS, SER, GLY, GLN

3.2. Target Structure Refinement:

Once the missing residues were filled in, the structure was used to state the energy minimization using Swiss PDB Viewer (SDPBV). The energy of our protein structure with the missing residues is -6.965 (Figure 2), which was further minimized by geometric optimization to -15743.978 (Figure3) Note that as the energy is lowered, the stability of our protein structure increases.

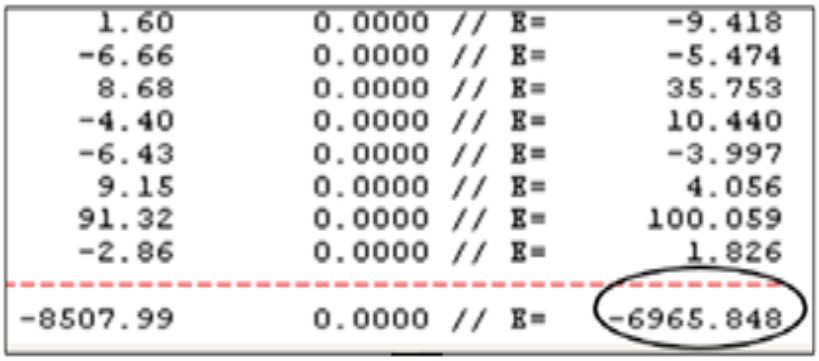


Figure 2: Energy of protein DBL3X with the missing residues.

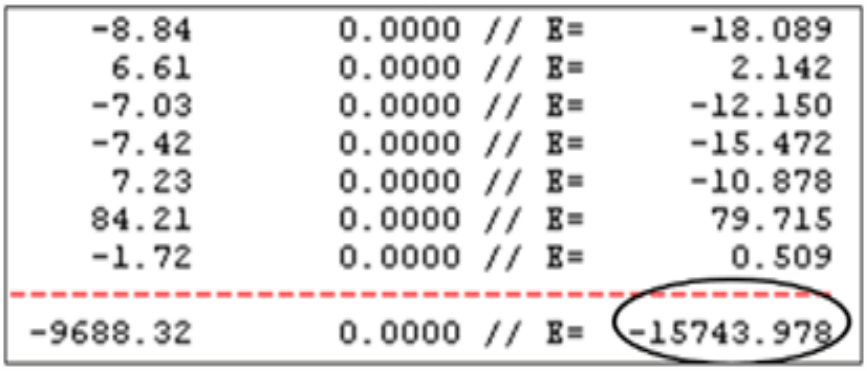
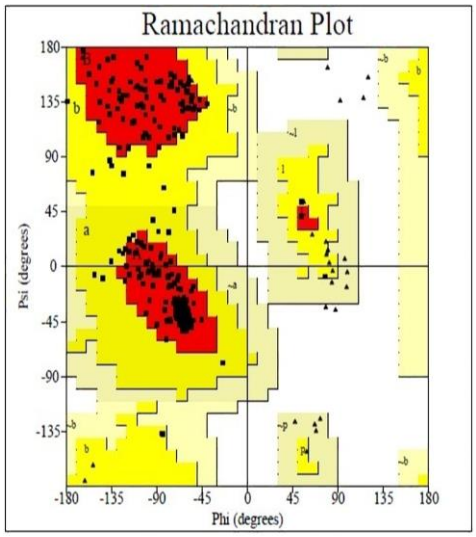


Figure 3: Change in the energy as result of Geometry optimization using SPDBV.

3.3. Structure Validation:

The Ramachandran Plot determines that our protein model have the amino acids in the allowed regions (Figure 4). The results generated by ProSA also determines that our protein structure lies under the favored regions globally and locally (Figure 5).



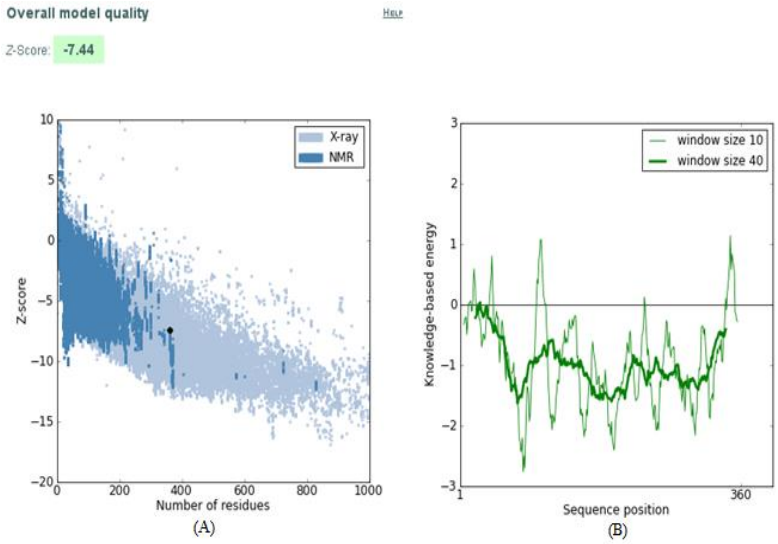
(A)

Plot statistics

Residues in most favoured regions [A,B,L]	285	90.2%
Residues in additional allowed regions [a,b,l,p]	31	9.8%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	316	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	32	
Number of proline residues	10	
Total number of residues	360	

(B)

Figure 4: (A) Ramachandran plot for DBL3X protein. (B) Plot statistics generated by SAVES



(A) (B)

Figure 5: PROSA Results. (A) Z-Score generated of the overall model. The solid circle is our protein DBL3X (B) Local quality of the model generated for each amino acid.

3.4. Binding site Analysis:

PDBSUM analysis shows two likely ligands (Figure 6A). It depicts that residues like lysine positioned at 1324; Glycine positioned at 1329 and Arginine positioned at 1467 are interacting with the sulphate ion. Lysine 1328 residue (red lash shaped) was also found to interact. The CASTP analysis determines the properties of the binding site. Figure 6B highlights the binding pocket in pink with the area of 286.6 with a volume of 501.3.

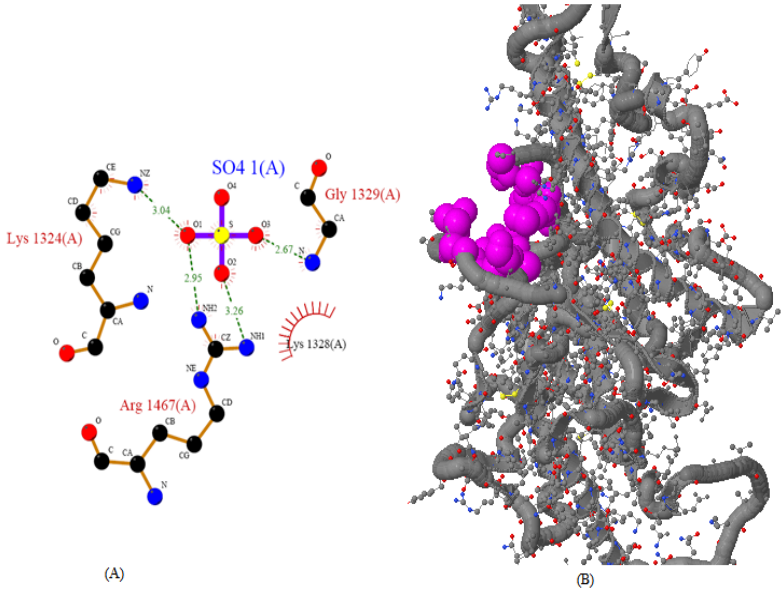


Figure 6: (A) PDBSUM depicting the interacting residues of DBL3X. (B) CASTP server analysis to find the binding site properties.

3.5. Ligand Library:

A primary set of ligands was created as a benchmark for studying the binding property with PfEMP1 dbl3x protein as their target. These drugs have been the primary medication for malarial conditions within pregnant women (Table 2). The efficacy of the xanthone associates with hindrance of heme polymerization, which results into anti-malarial action of these compounds. They prevent hemozoin formation, hence while finding new novel drugs for inhibiting PfEMP1 activity, xanthone acts as a potential candidate. [28, 31] In reference with Table 2, a new library was created that consist of xanthone structures and its derivatives. It has 16 xanthone ligands (Supplementary material-S1).The first two libraries were docked onto the binding site to find:

a) Drugs from primary library which has better affinity to bind to PfEMP1 protein.
b) To find ligands based on xanthone to see the best one to fit to the target.
c) Library screening was carried on using FlexX docking module and a semi flexible docking was carried on, to find poses of different conformation. Next session contains the docking results of the primary and secondary drugs used for screening and their data.

Drugs	xlogp	Apolar desolvation (kcal/mol)	Polar desolvation (kcal/mol)	H-bonds Donors (HD)	H-bonds Acceptors (HA)	Net charge	tPSA (A^2)	Molecular weight (g/mol) (MW)	Rotatable bonds
Chloroquine	5.01	10.55	-81.59	3	3	2	31	321.896	8
Amodiaquine	5.29	8.56	-80.64	4	4	2	51	357.885	6
Quinine	3.06	7.18	-40.05	2	4	1	47	325.432	4
	3.06	7.62	-89.58	3	4	2	48	326.44	4
Artemether	3.71	6.25	-4.25	0	4	0	37	296.407	1
Dihydroartem-isinin	2.25	2.55	-9.22	2	5	0	76	284.352	0
Lumefantrine	8.93	17.63	-43.45	2	2	1	25	529.959	10
Dapsone	0.93	0.99	-11.28	4	4	0	86	248.307	2
Artesunate	2.75	-1.53	-50.29	0	8	-1	103	383.417	5
Proguanil	1.92	7.66	-4.79	5	5	0	86	253.737	5
Atovaquone	4.96	11.54	-45.39	0	3	-1	57	365.836	2
Sulfadoxine	0.36	-0.16	-49.37	2	8	-1	119	309.327	5

Table 2: List of drugs in primary library along with their Lipinski values.

Drugs	xlogp	Apolar desolvation (kcal/mol)	Polar desolvation (kcal/mol)	H-bonds Donors (HD)	H-bonds Acceptors (HA)	Net charge	tPSA (A^2)	Molecular weight (g/mol) (MW)	Rotatable bonds
Pyrimethamine	2.84	6.53	-28.58	5	4	1	79	249.725	2
	2.84	6.08	-5.33	4	4	0	78	248.717	2
Mefloquine	4.24	6.35	-59.95	3	3	1	50	379.324	4
	4.24	5.78	-11.48	2	3	0	45	378.316	4

3.6. Fragment Based ligand design (FBLD):

A derived library based on secondary library was created using the method of core replacement by ReCore Tool. 1, 3, 6-trihydroxy-7-methoxy-2, 8-bis (3- methylbut- 2-enyl) -9- oxo- xanthene-4-carbaldehyde (ZINC ID: 13409911) was considered as the best molecule, based on the 2nd least score and the best binding in the site with interaction to target residues. A total 50 structures were created by the core fragmentation process, out of which 3 top molecules are selected for docking, based on their binding properties and their rmsd scores. Binding affinity and their suitability as a drug was tested based on their ADME/TOX properties and Lipinski values. Servers obtained online were used to reconfirm the rmsd scores and ligands were submitted in server to test their ADME/TOX values.

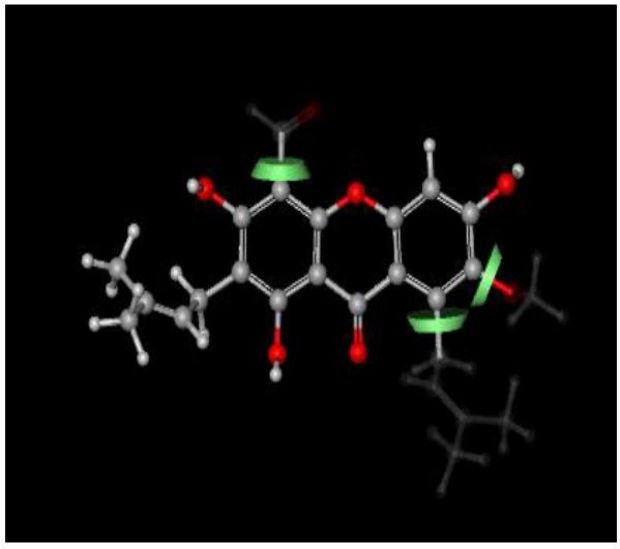


Figure 7: Core Replacement; green marks are the exit vectors

3.7. Protein-Ligand Docking Results:

Out of the total 13 primary ligands only the top ten (Table 3) were taken since they have significant binding with the target molecule. Further interactions of the top 3 poses generated (Table 3) for the above mentioned library are studied.

Table 3: Scores of top ten ligands of primary library of drugs (FlexX).

Rank	Name	Score	Rank	Name	Score
1	**Artesunate**	**-23.71**	6	Amodiaquine	-16.78
2	**Proguanil**	**-20.89**	7	Pyrimethamine	-16.42
3	**Sulfadoxine**	**-18.28**	8	Quinine	-15.77
4	**Dapsone**	**-17.74**	9	Atovaquone	-13.99
5	Dihydroartemisinin	-16.89	10	Chloroquine	-12.23

The docking results for first three binding score are in Figure 8-10. There are 12 derivatives from the secondary library (Supplementary material-S2) with the high binding score.2-(1,1-dimethylprop- 2-enyl)-1,3,5,6- tetrahydroxy-xanthen- 9-one with a score of -15.49 and 1, 3, 6-trihydroxy- 7-methoxy-2, 8-bis (3-methylbut-2-enyl)-9-oxo-xanthene-4-carbaldehyde with a score of -14.63. The derivative with binding score -14.63 are used to generate a derived library. This derived library has 3 molecules (Table 4) that are further docked with DBL3X protein shown in Figure 11-13.

Table 4: Binding Score of ligands from derived library

Sr no	**Ligands**	**Score**
1	9911d	-19.79
2	9911c	-17.37
3	9911f	-17.15

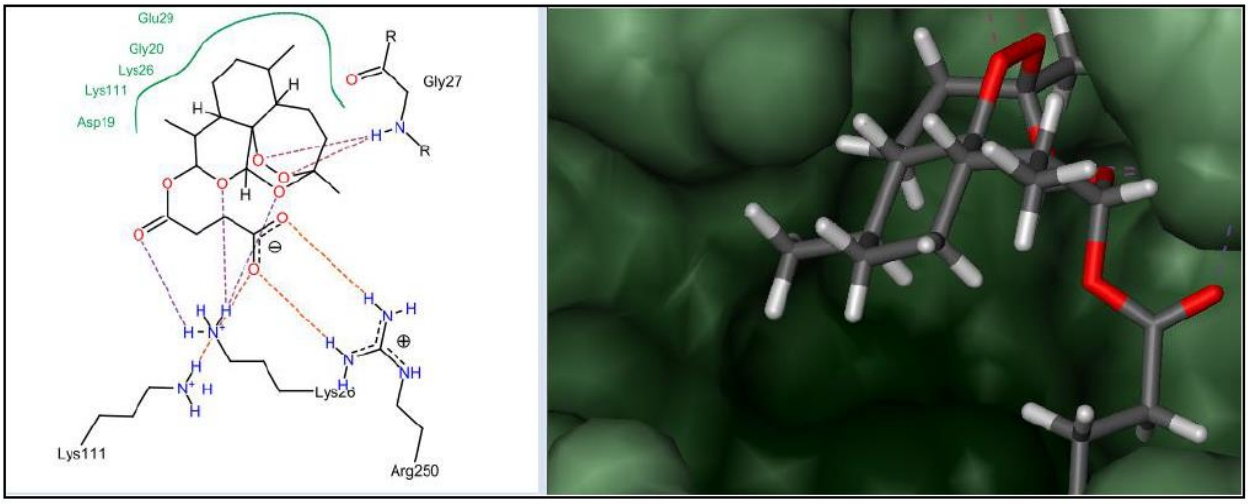


Figure 8: Schematic and diagrammatic representation of artesunate with DBL3X (primary library).

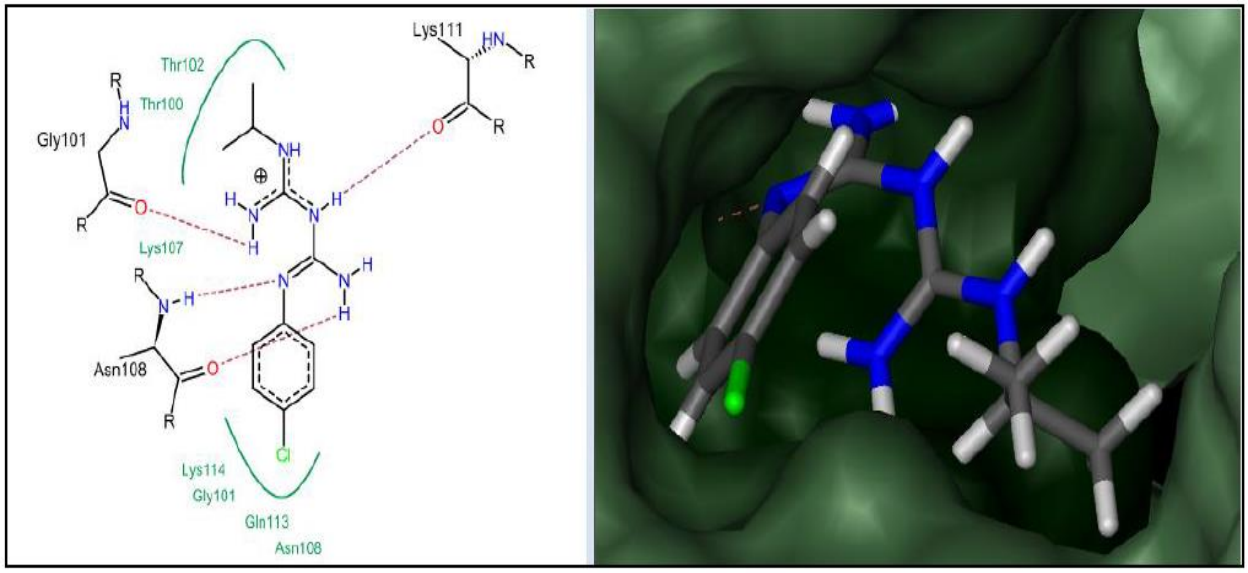


Figure 9: Schematic and diagrammatic representation of proguanil with DBL3X (primary library).

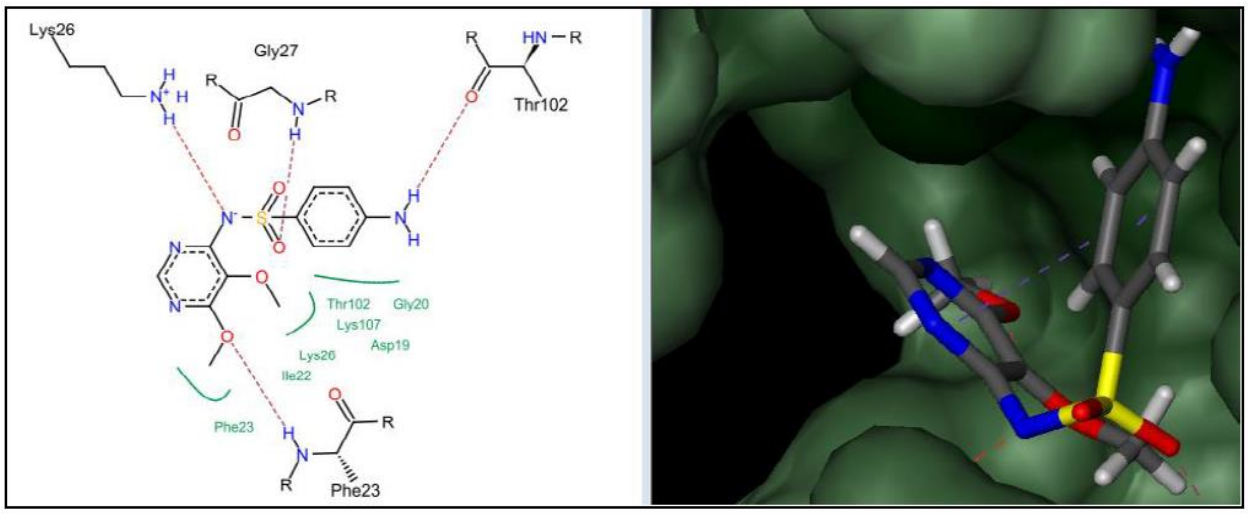


Figure 10: Schematic and diagrammatic representation of sulfadoxine with DBL3X (primary library).

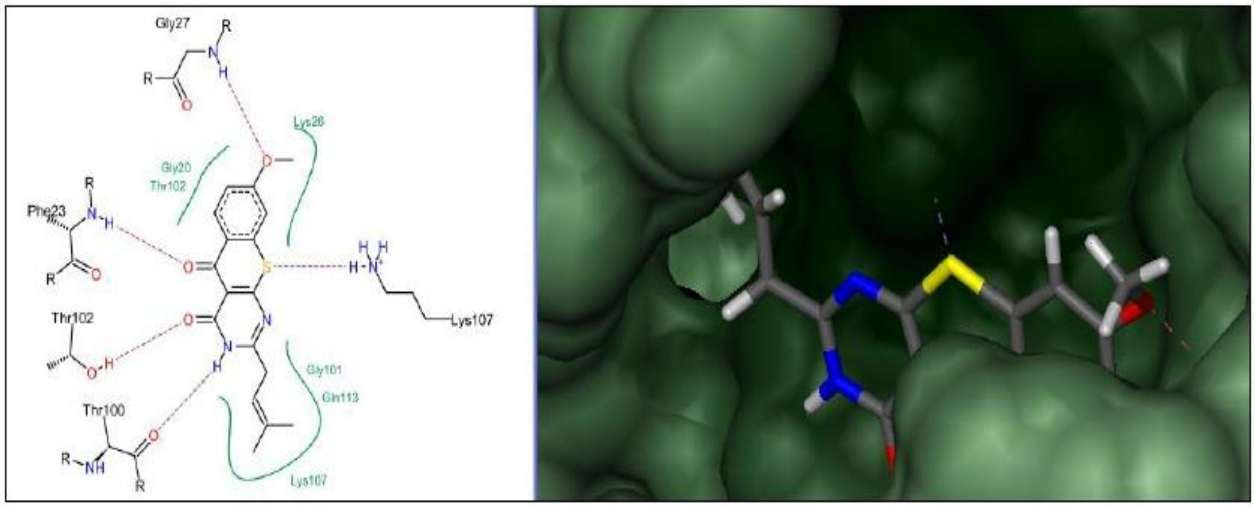


Figure 11: Schematic and diagrammatic representation of 9911d with DBL3X (Derived library).

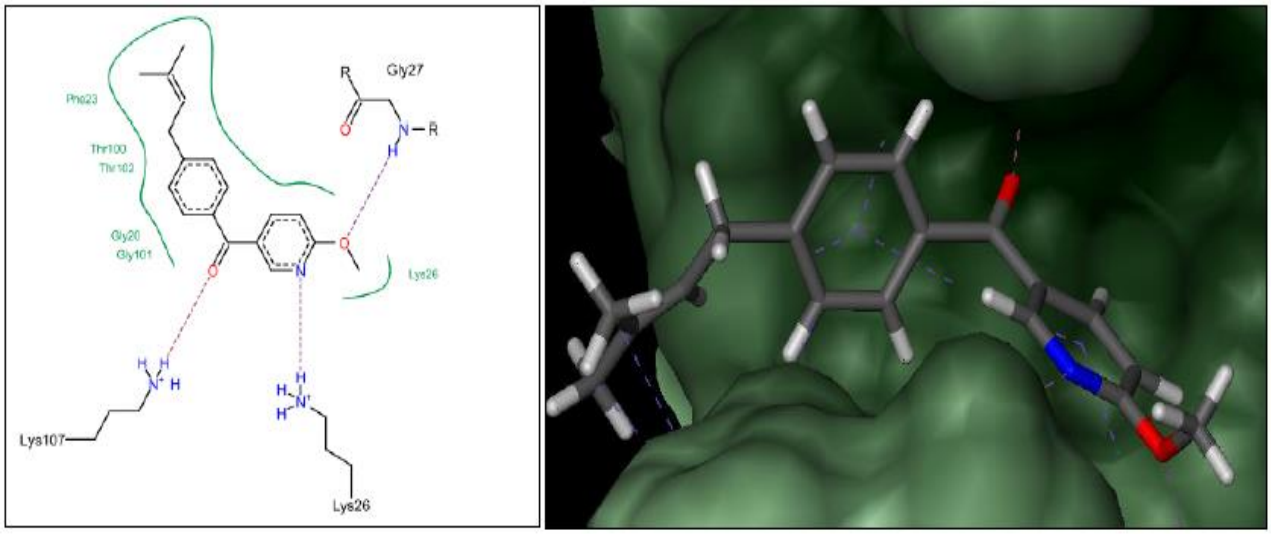


Figure 12: Schematic and diagrammatic representation of 9911c with DBL3X (Derived library).

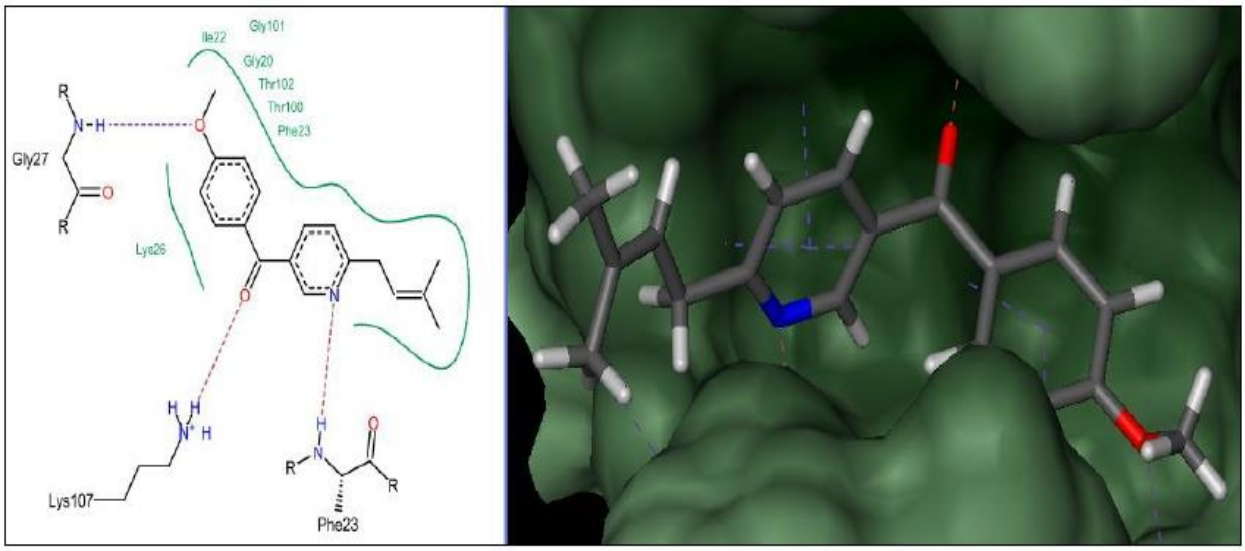


Figure 13: Schematic and diagrammatic representation of9911f with DBL3X (Derived library).

3.8. ADME/TOX values:

The top 3 ligands were loaded onto the *mol inspiration* software to check if their Lipinski values are feasible for being accepted as drugs. According to the ADME/TOX values, no violations are seen in the above 3 ligands and all three are suitable as drug candidates, but the best is seen to be the ligand 9911d based on the binding scores and the drug feasibility values.

Table 5: ReCore ligands and their Lipinski values.

Sr No	Ligands	HD	HA	XLOGP	MW	TPSA
1	**9911d**	**1**	**5**	**2.67**	**334.44**	**72.05**
2	9911c	0	3	4.41	281.35	39.19
3	9911f	0	3	4.14	281.36	39.2

According to the ADME/TOX values, no violations were seen in the above 3 ligands and all three are suitable as drug candidates, but the best was seen to be the ligand 9911d based on the binding scores and the drug feasibility values.

3.9. Molecular Dynamics Simulation:

Protein ligand complex was studied using molecular simulation for understanding the interaction of the ligand with the protein molecule in water. GROMACS was used for the MD simulation run. The GROMACS topology for the ligand was generated by the PRODRG server (Figure 14). On analysis, Figure 15 shows that the structure has reached the stability and do not demonstrate any unstable behavior that could lead to structure unfolding. The structure is stable during simulation of 5 ns.

The complex 3BQL-9911D shows only one strong hydrogen bonds, involving residue LYS 107, and one more hydrogen bond is occasionally seen involving GLY 112. This structural feature has been witnessed in all binary complexes involving DBL domains. The prevalence of these hydrogen bonds shows that there are at least 2 intermolecular hydrogen bonds mostly for 5 ns trajectory, which indicated the stability necessary for binding to the PfEMP1 stably.

The GROMOS87/GROMACS coordinate file (polar hydrogens)

```
PRODRG COORDS
  24
    1DRG  CAB      1   1.654   0.495  -0.126
    1DRG  CAO      2   1.562   0.571  -0.030
    1DRG  CAC      3   1.626   0.667   0.071
    1DRG  CAF      4   1.411   0.575  -0.060
    1DRG  CAJ      5   1.315   0.595   0.058
    1DRG  CAQ      6   1.195   0.530   0.033
    1DRG  NAK      7   1.089   0.599  -0.016
    1DRG  CAV      8   0.968   0.542  -0.035
    1DRG  SAN      9   0.838   0.641  -0.100
    1DRG  CAT     10   0.699   0.546  -0.050
    1DRG  CAI     11   0.578   0.612  -0.021
    1DRG  CAP     12   0.463   0.536   0.012
    1DRG  OAM     13   0.339   0.588   0.042
    1DRG  CAA     14   0.306   0.728   0.045
    1DRG  CAG     15   0.472   0.395   0.015
    1DRG  CAH     16   0.592   0.329  -0.013
    1DRG  CAU     17   0.707   0.405  -0.045
    1DRG  CAS     18   0.831   0.342  -0.054
    1DRG  OAE     19   0.843   0.238  -0.120
    1DRG  CAW     20   0.947   0.405  -0.008
    1DRG  CAR     21   1.055   0.331   0.044
    1DRG  OAD     22   1.041   0.216   0.086
    1DRG  NAL     23   1.180   0.394   0.064
    1DRG  HAP     24   1.257   0.341   0.100
 1.49800   1.49800   1.49800
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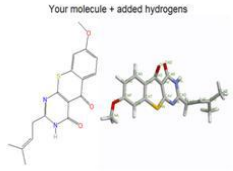

Figure 14: GROMACS topology file generated by PRODRG.

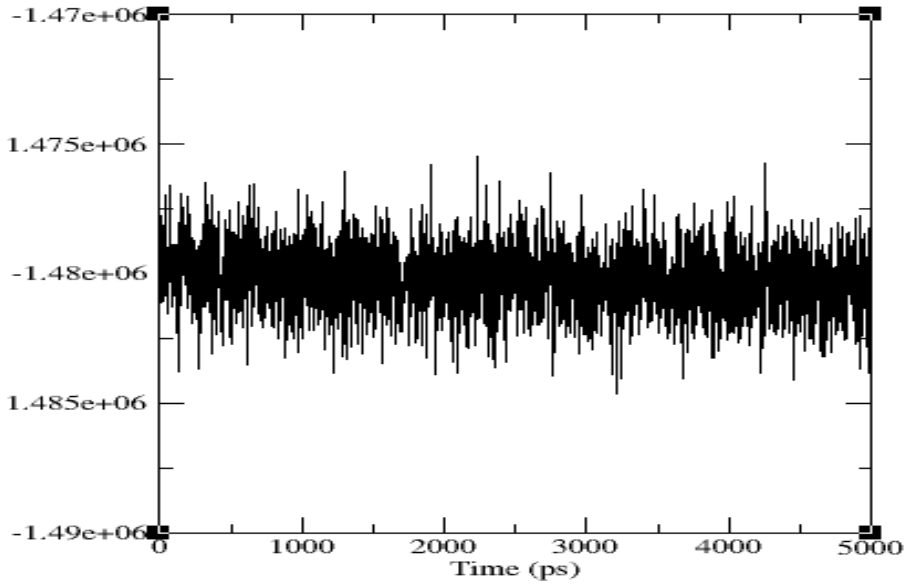

Figure 15: Potential energy (kJ/mol) of the complex of 3bql with 9911d adduct during 5-ns trajectory.

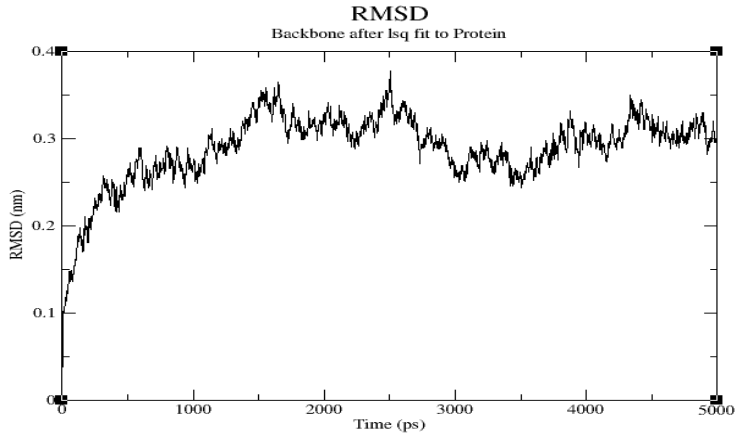

Figure 16: RMSD fluctuations of the complex 3bql with 9911d adduct during 5-ns trajectory.

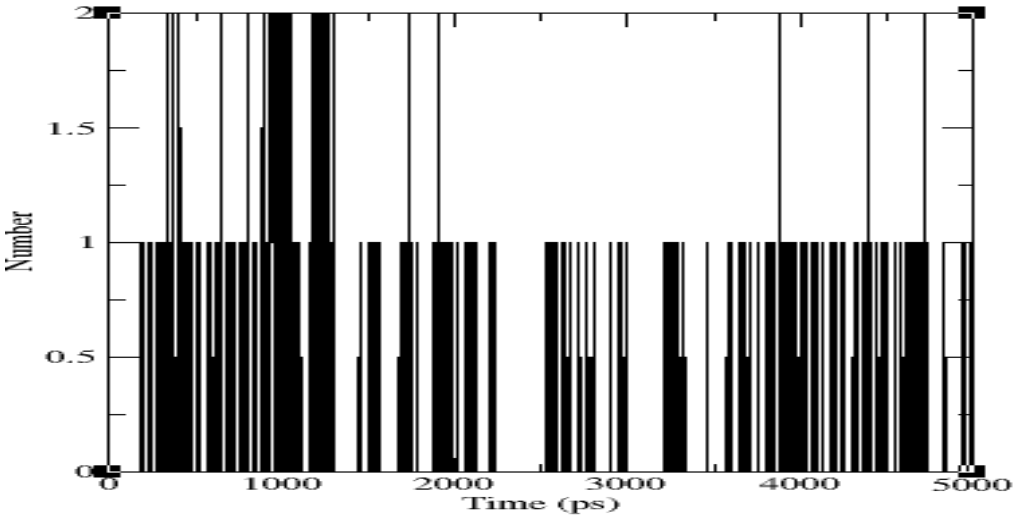

Figure 17: Number of intermolecular hydrogen bonds involving 3bql with 9911d adduct during 5-ns trajectory.

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DISCUSSION:

Drug resistance is a foremost reason for the development of novel and effective drug as present drugs seem to fail against wide spread diseases like malaria and tuberculosis. In this study, while identifying the role of DBL3X, a domain of PfEMP1 protein in PM, it has been verified that PfEMP1 is a potential drug target to control the spread of PAM. Despite of been a potential target it has a highly variable var genes which keeps changing at higher rate than new drugs are formed. It hampers the effectively of the drug thus becomes a primary factor while producing drugs. Primary drugs were used as a benchmark to study their binding with the protein, amongst them only two drug molecules artesunate and proguanil have a very significant binding energy. But these drugs fail due to the mere factor, resistance is increasing against them, but their Pharmacophore are effective enough to form stable binding with the protein, which could be used for creating new and enhanced drugs. Xanthone based libraries were created on the mere fact that xanthone is a chemical compound which can effectively stop heme polymerization, thus also inhibiting rosetting and sequestration in malaria, out of these molecules the best xanthone derivative was used for creating a library completely created by core fragmentation.

4. CONCLUSION

Three ligands from the ReCore library were seen to bind better as compared to other molecules. Ligand 9911d, Ligand 9911c, and Ligand 9911f were identified as good inhibitors of DBL3X based on docked results. Based on Lipinski's Rule of Five, it was found that the permeation of the ligand depends on the molecular weight and the partition coefficient, hence based on the ADME/TOX results it was determined that ligand 9911d is a better being a drug than rest of the ligands. Protein ligand complex simulation confirms it and the stability and the reaction of the ligand with the protein was seen to be stable as well.

5. ACKNOWLEDGEMENT

The authors would like to Dr. D. Y. Patil Deemed to be University for providing the necessary technical support and facilities for this research.

6. CONFLICT OF INTEREST

We have no conflicts of interest to disclose.

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SUPPLEMENTARY FILES

A. Ligand Library:

Table S1: List of xanthone derivatives in the secondary library with their Lipinski values.

Sr No	Ligands	H-bonds Donor	H-bonds Acceptor	xlogp	Molecular weight	TPSA
1	3-hydroxy-9H-9-xanthenone	1	3	3.07	212.20	50
2	1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methylbut-2-enyl)-9-oxo-xanthene-4-carbaldehyde	3	7	5.83	438.48	117
3	5-(1,1-dimethylprop-2-enyl)-2,3,6,8-tetrahydroxy-1-(3-methylbut-2-enyl)xanthen-9-one	4	6	5.99	111	396.43
4	5-(1,1-dimethylprop-	3	6	6.26	410.46	100

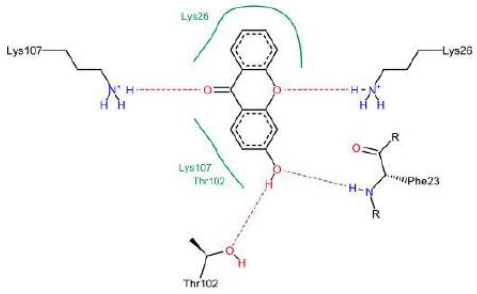
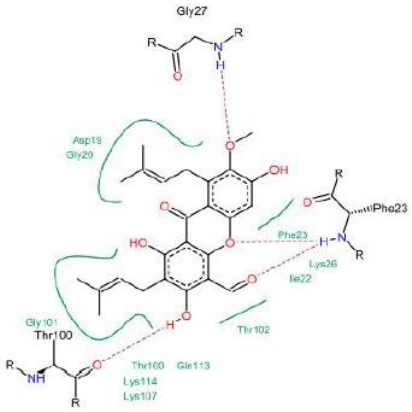
	2-enyl)-3,6,8-trihydroxy-2-methoxy-1-(3-methylbut-2-enyl)xanthen-9-one					
5	Cudraxanthone D	3	6	6.06	410.46	100
6	2-(1,1-dimethylprop-2-enyl)-1,3,5,6-tetrahydroxy-7-(3-methylbut-2-enyl)xanthen-9-one	4	6	6.22	396.44	111
7	Cudraxanthone L	4	6	5.99	396.44	111
8	2-(1,1-dimethylprop-2-enyl)-1,3,5,6-tetrahydroxy-xanthen-9-one	4	6	4.20	328.32	111
9	2-(1,1-dimethylprop-2-enyl)-1,3,5-trihydroxy-6-(3-methylbut-	3	6	6.76	396.44	100

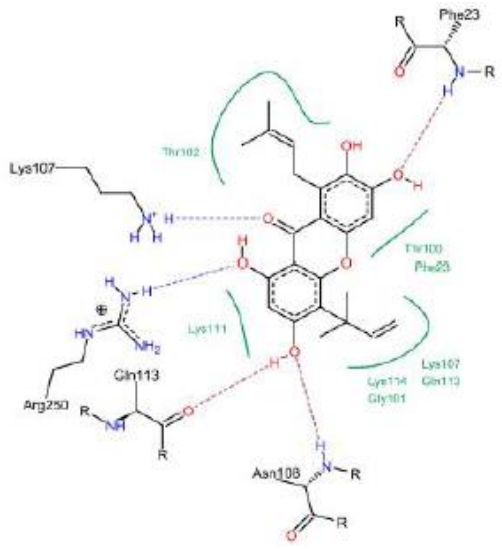
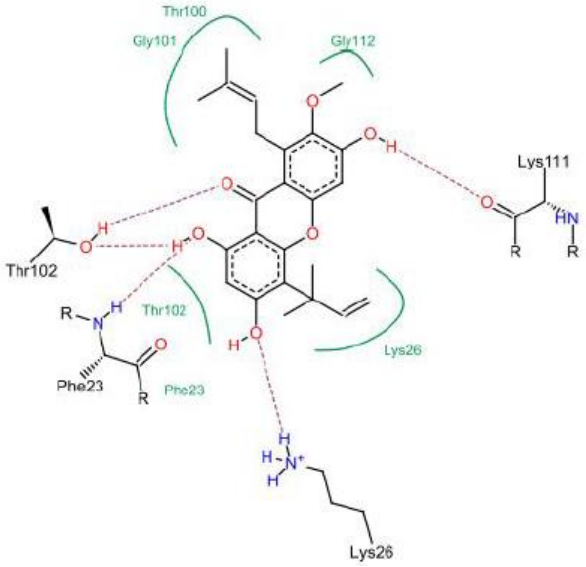
	2-enoxy)xanthen-9-one					
10	3,7-dimethoxy-1-methyl-xanthen-9-one	0	4	4.01	270.28	49
11	6-[(2S)-3-(diethylamino)-2-hydroxy-propoxy]-1,3-dihydroxy-7-methoxy-2,8-bis(3-methylbut-2-enyl)xanth	3	8	6.77	540.68	114
12	6-[(2R)-3-(diethylamino)-2-hydroxy-propoxy]-1,3-dihydroxy-7-methoxy-2,8-bis(3-methylbut-2-enyl)xanth	4	8	6.77	540.67	114
13	1,6,7-trihydroxy-8-(3-hydroxy-3-methyl-butyl)-3-methoxy-2-(3-methylbut-2-	4	7	5.13	428.48	120

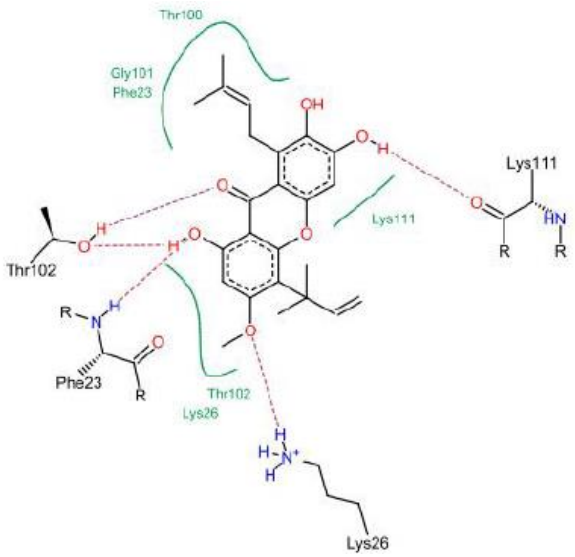
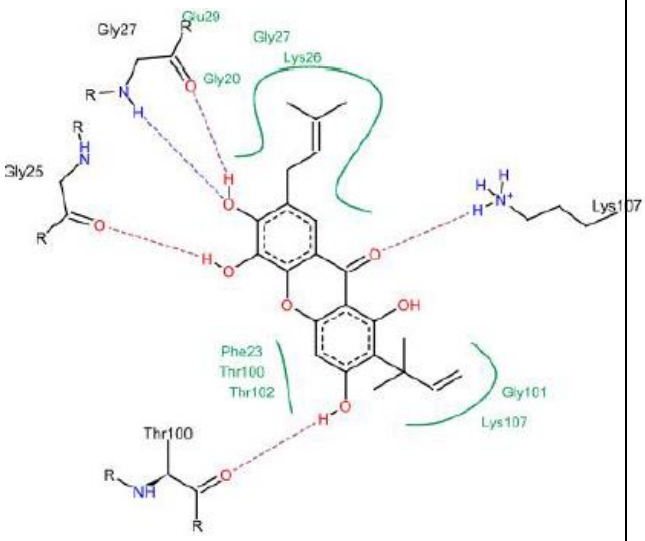
	enyl)xanthen-9-one					
14	1,7-bis[(2E)-3,7-dimethylocta-2,6-dienyl]-2,3,8-trihydroxy-6-methoxy-xanthen-9-one	3	6	9.03	546.70	100
15	4-[(2E)-3,7-dimethylocta-2,6-dienyl]-1,3,6,7-tetrahydroxy-2-(3-methylbut-2-enyl)xanthen-9-one	4	6	8.22	464.56	111
16	6-(2-diethylaminoethyloxy)-1,3-dihydroxy-7-methoxy-2,8-bis(3-methylbut-2-enyl)xanthen-9-one	3	7	7.41	510.65	94

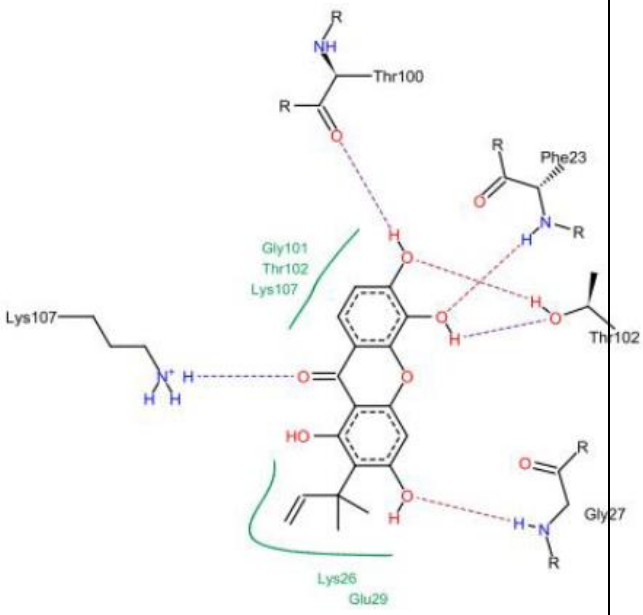
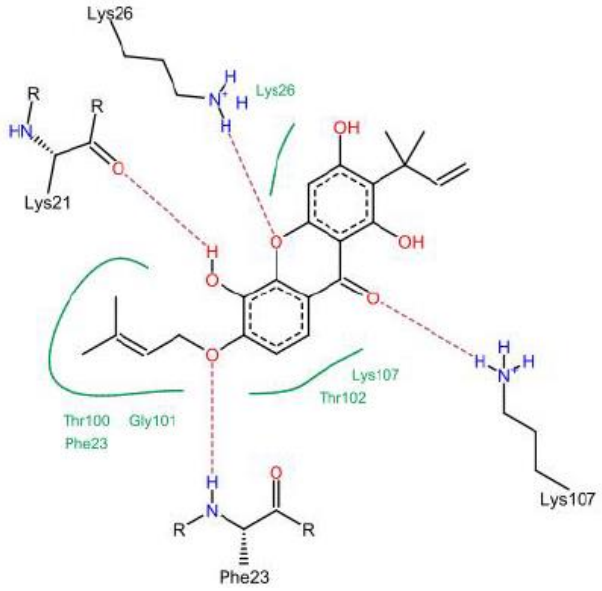
B. Protein-Ligand Docking Results:

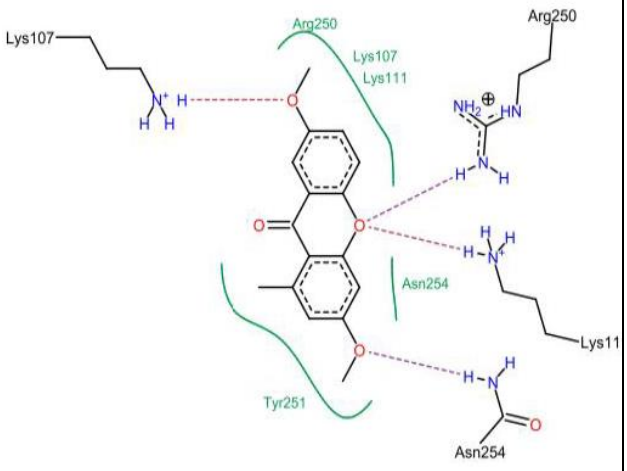
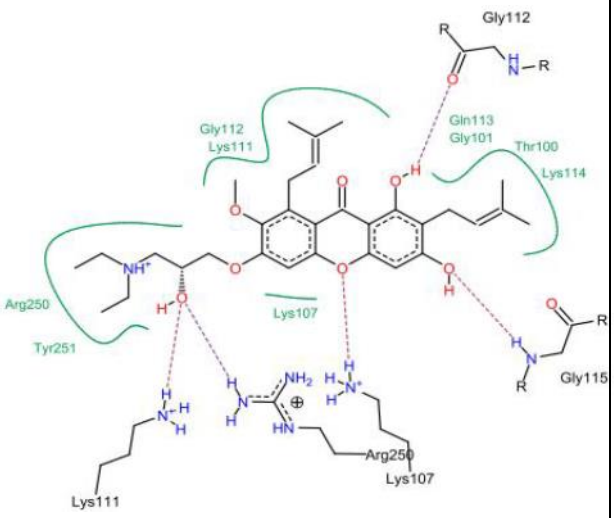
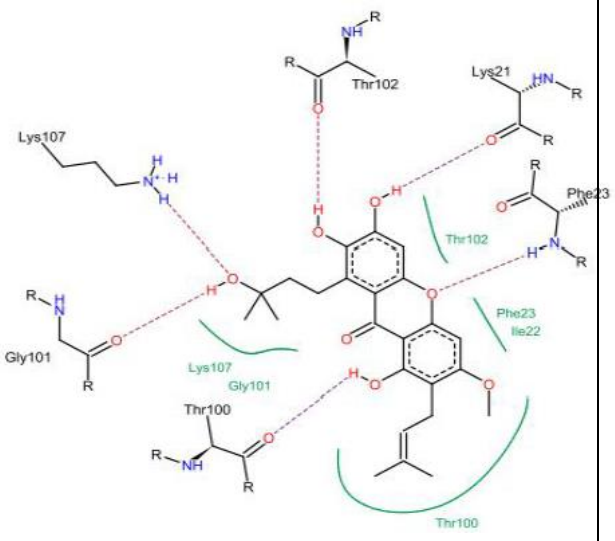
Table S2: Scores and schematic representation of xanthone derivatives

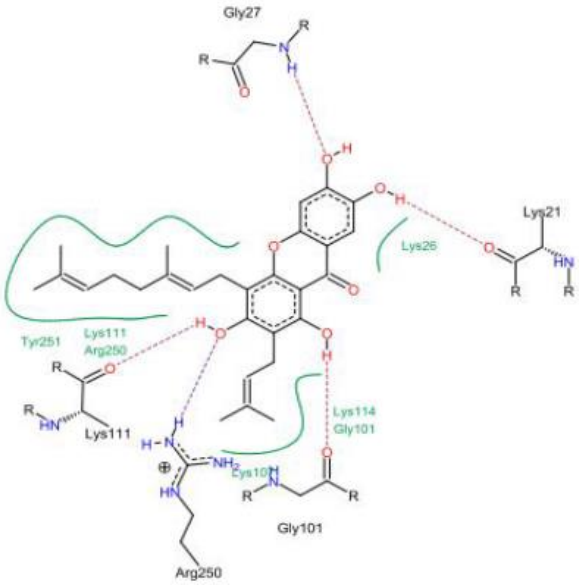
Sr No	Ligands	Structure	Score
1	3-hydroxy-9H-9-xanthenone		-13.67
2	**1,3,6-trihydroxy-7-methoxy-2,8-bis(3- methylbut-2-enyl)-9-oxo-xanthene-4-carbaldehyde**		**-14.63**

3	5-(1,1-dimethylprop-2-enyl)-2,3,6,8-tetrahydroxy-1-(3- methylbut-2-enyl)xanthen-9-one		-11.13
4	5-(1,1-dimethylprop-2-enyl)-3,6,8-trihydroxy-2-methoxy- 1-(3-methylbut-2-enyl)xanthen-9-one		-13.04

5	Cudraxanthone D		-14.08
6	2-(1,1-dimethylprop-2-enyl)-1,3,5,6-tetrahydroxy-7-(3- methylbut-2-enyl)xanthen-9-one		-12.99

7	**2-(1,1-dimethylprop-2-enyl)-1,3,5,6-tetrahydroxy-xanthen- 9-one**		**-15.49**
8	2-(1,1-dimethylprop-2-enyl)-1,3,5-trihydroxy-6-(3- methylbut-2-enoxy)xanthen-9-one		-11.99

9	3,7-dimethoxy-1- methyl-xanthen-9-one		-12.03
10	6-[(2R)-3-(diethylamino)-2- hydroxy-propoxy]- 1,3-dihydroxy-7-methoxy-2,8-bis(3-methylbut-2-enyl)xanth		-11.76
11	1,6,7-trihydroxy-8-(3- hydroxy-3-methyl-butyl)-3-methoxy-2-(3-methylbut-2-enyl)xanthen-9-one		-14.55

12	4-[(2E)-3,7-dimethylocta-2,6- dienyl]-1,3,6,7-tetrahydroxy-2-(3- methylbut-2-enyl)xanthen-9-one		-12.65