ISSN 0974-3618 (Print)
0974-360X (Online)
www.rjptonline.org

RESEARCH ARTICLE

Interaction Efficiency of Drugs towards the Novel Membranous Structure - Maurer's cleft for Malarial Disease

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ABSTRACT:
India, being a developing country, incidence of malarial infection prevail as a major socio-economic problem, though the infection is both treatable and preventable. There are nine anopheline vectors to transmit three specific Plasmodial species, *P. falciparum*, *P. vivax* and *P. malariae*, where the parasite develops intracellularly within the altered host red blood cell. Recently, it has been identified that a membranous structure called Maurer's Cleft (MC) is formed by *P. falciparum* for protein sorting and export in order to remodel the erythrocyte. The five major proteins involve are *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), the ring-exported proteins (REX) 1 and 2, repetitive interspersed family (RIFINS), a subtelomeric variable open reading frame (STE-VORS) and *Plasmodium falciparum* Maurer's clefts two transmembrane protein (PfMC-2TM). Among REX 1 is reported to persists throughout the life cycle of the parasite and also involve a novel PEXEL (a motif which possess recessed signal sequence for entering the erythrocyte) independent export pathway. Most importantly, the role of REX 1 in the maintenance of MC architecture, where the truncation of REX 1 gene in *P. falciparum* strain D10 results in distortion of MC morphology and decrease in the number of MC. Addition to those MC cisternae seems stacked or multilamellate. Hence, REX 1 could serve as an exclusive target to intervene the process in erythrocyte remodelling and is considered for the present study. The present study indicates the significant interaction showed by the antimalarial drugs with REX1.

KEYWORDS: Malaria, Maurer's Cleft (MC), REX1, *Plasmodium falciparum*, malarial drugs, Molecular docking studies.

INTRODUCTION:

In India, malaria is one of the leading health issue causes socio-economic burden over the humanity. The process of eradicating malaria has been started in the early 1990s and the detailed progress was mentioned in the National Framework for Malaria Elimination in India (2016-2030) by the Directorate of National Vector Borne Disease Control Programme (NVBDCP) and Directorate of General of Health Services (DGHS), Ministry of Health and Family Welfare Government of India. According to World Malaria Report 2015, between 2000 to 2014, there was 46% reduction in malaria-related morbidity and 40% reduction in malaria-related mortalityin India. However, WHO reported that India is noted for the existence of drug-resistant strains and is a real challenge

Received on 09.03.2017 Modified on 28.03.2017
Accepted on 25.04.2017
Research J. Pharm. and Tech. 2017; 10(9): 2973-2980.
DOI: 10.5958/0974-360X.2017.00526.1

imposed due to dependency on single drug, perhaps, Dr. G. S. Sonal, additional director, NVBDCP, stated that after incorporating multi-drug therapy in our national programme, resistance is not a big issue[1]. Especially in Tamil Nadu, the death rate due to malaria was nil and the total malarial cases was reduced to 8729 in the year 2014 from 43053 in 2000. Besides to achieve, the global target to eliminate malaria in 2030, the transmission of *Plasmodium vivax* and *P. falciparum*should indigenously ceased[2]. Precisely, 39.5% of malaria in India are contributed by the parasite *P. falciparum* and the drug resistant development to chloroquine in *P. falciparum* and the complications with other diseased conditions like hyper-parasitaemia, hypoglycemia, during pregnancy in women, are the several factors serves as the base for the intense need to concentrate more oneliminatingmalaria (NVBDCP).

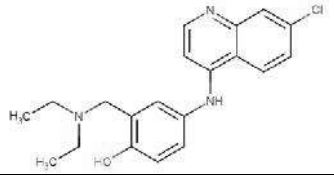
P. falciparum develops and maturesinside the host erythrocyte after remodeling the morphology, host cell rigidity and concurrently the formation of knobs enhances the cytoadherenceto the vascular endothelium which contributes the severity in pathogenesis[3].During the early stage of development, the parasite exports ~400 proteins into the host which are collectively called secretome or exportome[4]. The parasite requires a *Plasmodium* export element (PEXEL) motif for transporting the proteins across the parasitophorous vacuole membrane (PVM)[5,6,7,8]. Recently, a PEXEL-independent pathway was identified[5,7,9] forming a *de novo* heterogeneous structure made up of immunovariant virulence proteins within the erythrocyte cytoplasm, called as the Maurer's clefts (MCs)[10,11,12]. The proteins associated are *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), the ring-exported proteins (REX) 1 and 2, repetitive interspersed family (RIFINS), a subtelomeric variable open reading frame (STE-VORS) and *Plasmodium falciparum* Maurer's clefts two transmembrane protein (PfMC-2TM)[13]. Till date, the structural information and the function of MC's is not clear, however, from the studies, it is believed that MCs extend from the PVM and distributed under the erythrocyte membrane to anchor themselves in the erythrocyte cytoskeleton throughout the asexual-stage[14]. It is described to resemble golgi cisternae and to share antigenic determinants with mammalian golgi, therefore serve as an extracellular golgi for the parasite[15,16,17,18].

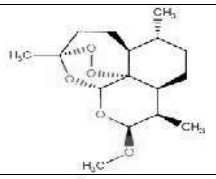
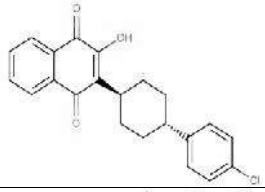
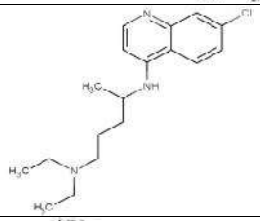
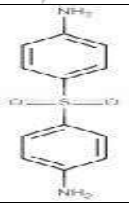
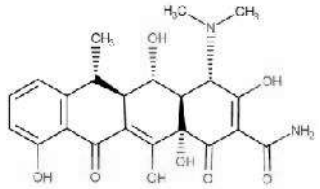
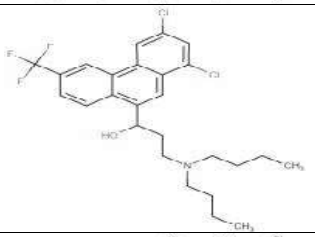
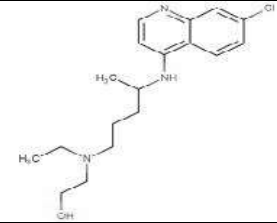
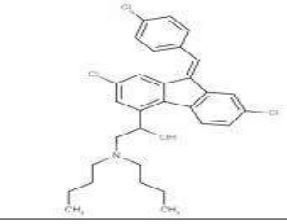
Among the MC proteins, ring exported protein 1 (REX1) is considered as unique to target, since knocking out the protein expression in *P. falciparum* strain D10 results in reduction of number of MCs[9,19], distorted MC morphology and the MC cisternae also appeared stacked or multilamellate, which suggest its critical role in maintenance of MC architecture[19,20]. Additionally, the number of SBP1 structure was also reduced which has specific role in exporting PfEMP1 to the erythrocyte surface[21]. Henceforth, the protein REX1 is considered as therapeutic value for designing novel drugs and with this perspectivethe present study prioritize to study the binding efficiency of the existing malarial drugs with the REX1.

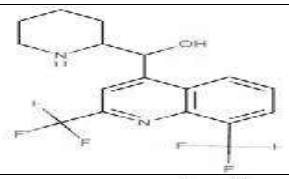
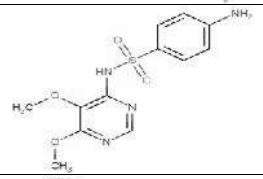
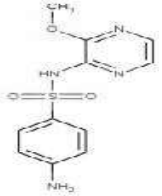
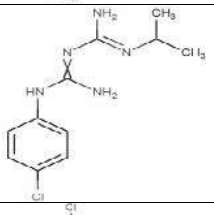
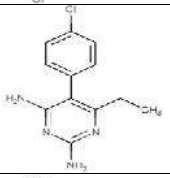
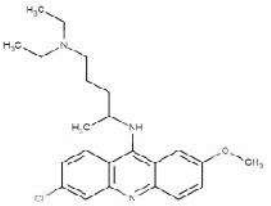
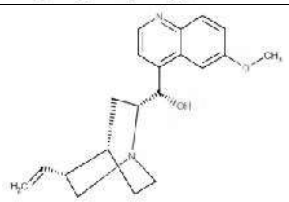
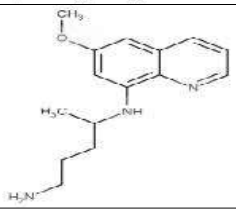
MATERIALS AND METHODS:

The 3D structure of protein REX1 was not available in Protein Data Bank (PDB), therefore, the sequence in fasta format was retrieved from UniprotID: Q8I2G1, Ring Exported Protein 1 from *Plasmodium falciparum* 3D7 strain. To determine the 3D structure, 713 amino acid length sequence was subjected for BLAST homology search against the PDB database which revealed no significant sequence similarity was found and hence, the 3D structure was modeled using the an online server I-tasser, which uses threading programs like MUSTER, FFAS-3D, SPARKS_X, HH SEARCH 1, Neff-PPAS, HH SEARCH, p Gen THREADER, wdPPAS to predict the 3D structure. The study utilizes an online server Ligsite to predict the binding pocket for the predicted model and the quality of the protein model was determined from the Ramachandran plot generated from SAVS, an online server. The predicted model was subjected to energy minimization using SPDBV software. From the sequence, the secondary structure of the protein was analyzed using the tools available in the expasy, i.e. GOR, TMHMM. Further, 17 drug molecules which are categorized as antimalarials were identified from the drug bank and the 3D structure was retrieved (Table 1). The docking analysis was carried out using Glide module of Schrodinger and the binding interactions were visualized using PyMol viewer.

Table 1: Malarial Drug Molecules from Drug Bank

S. No.	Name	Drug Bank Accession No.	Chemical formula	Structure
1	Amodiaquine	DB00613	$C_{20}H_{22}ClN_3O$	

2	Artemether	DB06697	$C_{16}H_{26}O_5$	
3	Atovaquone	DB01117	$C_{22}H_{19}ClO_3$	
4	Chloroquine	DB00608	$C_{18}H_{26}ClN_3$	
5	Dapsone	DB00250	$C_{12}H_{12}N_2O_2S$	
6	Doxycycline	DB00254	$C_{22}H_{24}N_2O_8$	
7	Halofantrine	DB01218	$C_{26}H_{30}Cl_2F_3NO$	
8	Hydroxychloroquine	DB01611	$C_{18}H_{26}ClN_3O$	
9	Lumefantrine	DB06708	$C_{30}H_{32}Cl_3NO$	

10	Mefloquine	DB00358	$C_{17}H_{16}F_6N_2O$	
11	Sulfadoxine	DB01299	$C_{12}H_{14}N_4O_4S$	
12	Sulfametopyrazine	DB00664	$C_{11}H_{12}N_4O_3S$	
13	Proguanil	DB01131	$C_{11}H_{16}ClN_5$	
14	Pyrimethamine	DB00205	$C_{12}H_{13}ClN_4$	
15	Quinacrine	DB01103	$C_{23}H_{30}ClN_3O$	
16	Quinidine	DB00908	$C_{20}H_{24}N_2O_2$	
17	Primaquine	DB01087	$C_{15}H_{21}N_3O$	

RESULTS:

The modeled 3D structure of the ring exported protein 1 (REX1) was represented in the Fig.1. The I-tasser builds five models based on the template 4UXV (a cytoplasmic domain of bacterial cell division protein EzrA), 1VWL (crystal structure of TcdA1), 5FVM (Cryo electron microscopy of a complex of Tor and Lst8), 3O0Z (Crystal structure of a coiled-coil domain from human

ROCK1), 1C1G (Crystal structure of tropomyosin at 7 angstroms resolution in the spermine-induced crystal form), 4HPQ (Crystal structure of the Atg17-Atg31-Atg29 complex) and 4UI9 (Atomic structure of the human anaphase-promoting complex). Among five, the model 1 showed the highest C.score value of -1.54, estimated TM score of 0.53±0.15 andestimated RMSD value of 11.8±4.5 Å and hence selected for further analysis. The energy of the REX1 model was minimized using SPDBV which was -31156.691Kcal/mol. The quality of the structure was determined as good, where the procheck results indicated that 70.2% of residues are located in the core i.e. most favorable, 19.8% in allowed, 5.4% in generously allowed and 4.6% in disallowed region of Ramachandran plot (Fig.2).The resolution of the modeled protein was 1.5, as indicated in procheck. The residues in active site pocket for the protein was predicted as Pro372, Lys392, Lys458, Ser461, Lys462, Thr544, Ala557, Ala582 using the ligsite server.

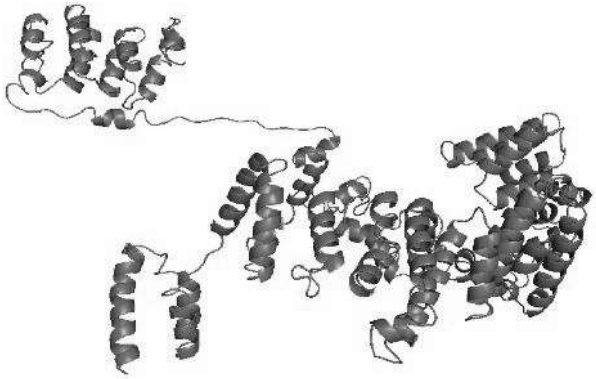


Fig.1: Modeled 3D structure of ring exported protein 1 (REX1) from *Plasmodium falciparum* 3D7 strain

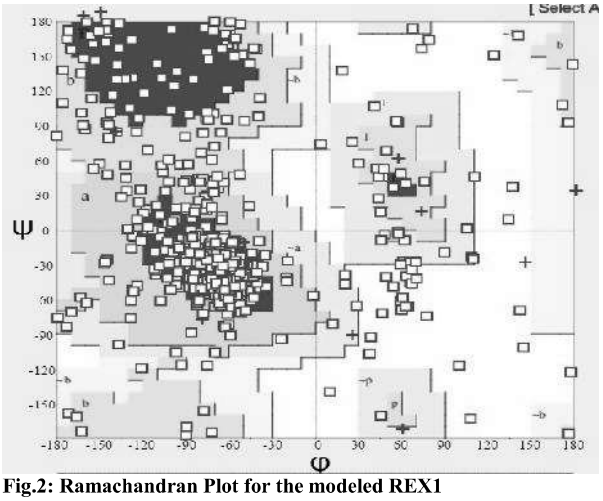


Fig.2: Ramachandran Plot for the modeled REX1

The protein was analyzed for the presence of transmembrane helices using TMHMM server indicated that the transmembrane helix is located at 37-59 residue position, where 1-36 residues are located inside and 60-713 residues situated outside the membrane (Fig.3). The secondary structure was predicted using GOR server, showing 56.10% of the residues form α-helix, 9.68% forms extended strand and 30% had random coil structure.

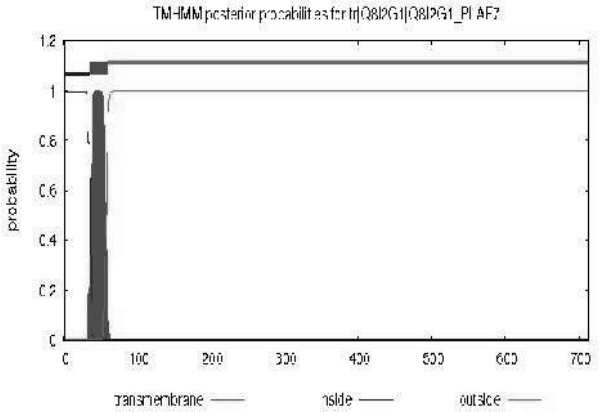


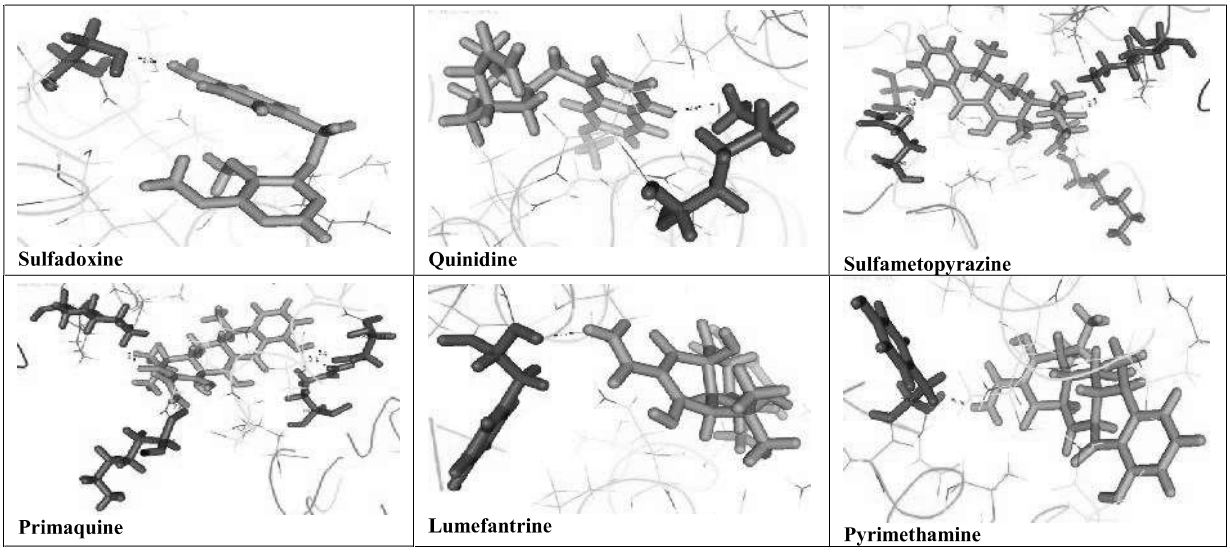
Fig. 3: TMHMM prediction for the presence of transmembrane helices

The efficiency of the drugs in interacting the REX1 protein was observed using docking studies, where the G. score (Kcal/mol), number of hydrogen bonds, interacting residues and its bond length (Å) were mentioned (Table 2). Among the 17 drug molecules, only 13 interacted with the protein. The drugs artemether, atovaquone, doxycycline, halofantrine had no capacity to interact with the REX1. The drugs sulfadoxine, quinidine, sulfametopyrazine and primaquine had G.score in the range of -4 Kcal/mol, where the sulfadoxine and quinidine had respective G.score of -4.67 and -4.29 Kcal/mol and the latter two drugs had -4.05 Kcal/mol. The drugs sulfadoxine and quinidine each had single hydrogen bond interaction with the respective residue Ser461 and Lys471, while the drugs sulfmetopyrazine and primaquine both had interactions in similar. The interactions were observed with Lys392, Lys458, Asp468 and Gln495. Likely, the drugs lumefantrine and pyrimethamine had G.score of -3.89 Kcal/mol and single bond interactions with Tyr427, in addition, hydroxychloroquine and proguanil had 3 interactions with Lys392, Glu396, Gln469 and G.score of -3.65 Kcal/mol. Chloroquine scored -2.57 Kcal/mol and had single interaction with Ser461 and two hydrogen bonds with Lys458, whereas dapsone and amodiaquine had -2.15, -2.00 Kcal/mol of G.score. The interactions for dapsone were observed with the residues Lys392 and Gln455. In the case of amodiaquine, 3 interactions were observed two hydrogen bonds with Glu457 and one with Ser461. Lower G.score value of -1.82 Kcal/mol was found in quinacrine forming single bond with Lys498. The involvement of predicted active site residues Lys392, Lys458 and Ser461 were observed in the

interactions with drugs amodiaquine, dapsone, chloroquine, proguanil, hydroxychloroquine, primaquine, sulfametopyrazine and sulfadoxine. Chloroquine though had lesser G. score than the other drugs used in the study, was observed to interact only with the predicted active site residues Lys458 andSer461, moreover, the hydrogen bonds formed were observed as strong and covalent. Most of the hydrogen bond distances formed with the active site residues were found to be 2.0-2.5 Å, indicating its strength except two drugs dapsone and amodiaquine which had 1.9Å of bond distance with Lys392 and Ser461, respectively. The interactions of each drug molecule with REX1 were shown in fig.4.

Table 2: Docking results of drug molecules with REX1 protein

S. No.	Drug Name	G. score (Kcal/mol)	No. of hydrogen bonds	Interactions	Bond Length (Å)
1	Sulfadoxine	-4.67	1	**SER461** (O-H)	2.2
2	Quinidine	-4.29	1	LYS471 (O-H)	2.4
3	Sulfametopyrazine	-4.05	4	**LYS392** (H-O) **LYS458** (O-H) ASP468 (O-H) GLN495 (H-O)	2.3 2.2 2.2 2.7
4	Primaquine	-4.05	4	**LYS392** (H-O) LYS458 (O-H) ASP468 (O-H) GLN495 (H-O)	2.3 2.2 2.2 2.7
5	Lumefantrine	-3.89	1	TYR427 (O-H)	2.1
6	Pyrimethamine	-3.89	1	TYR427 (O-H)	2.1
7	Mefloquine	-3.80	4	ASP468 (H-O) ASP468 (H-O) ASP468 (H-O) GLN495 (H-O)	2.6 2.0 1.8 2.5
8	Hydroxychloroquine	-3.65	3	**LYS392** (H-O) GLU396 (O-H) GLN469 (O-H)	2.0 2.5 1.8
9	Proguanil	-3.65	3	GLU396 (O-H) **LYS392** (H-O) GLN496 (O-H)	2.5 2.0 1.8
10	Chloroquine	-2.57	3	**SER461** (O-H) **LYS458** (H-N) **LYS458** (O-H)	2.1 2.2 2.3
11	Dapsone	-2.15	2	**LYS392** (H-O) GLN455 (O-H)	1.9 2.0
12	Amodiaquine	-2.00	3	GLU457 (O-H) GLU457 (O-H) **SER461** (O-H)	2.0 2.2 1.9
13	Quinacrine	-1.82	1	LYS498 (H-O)	2.0



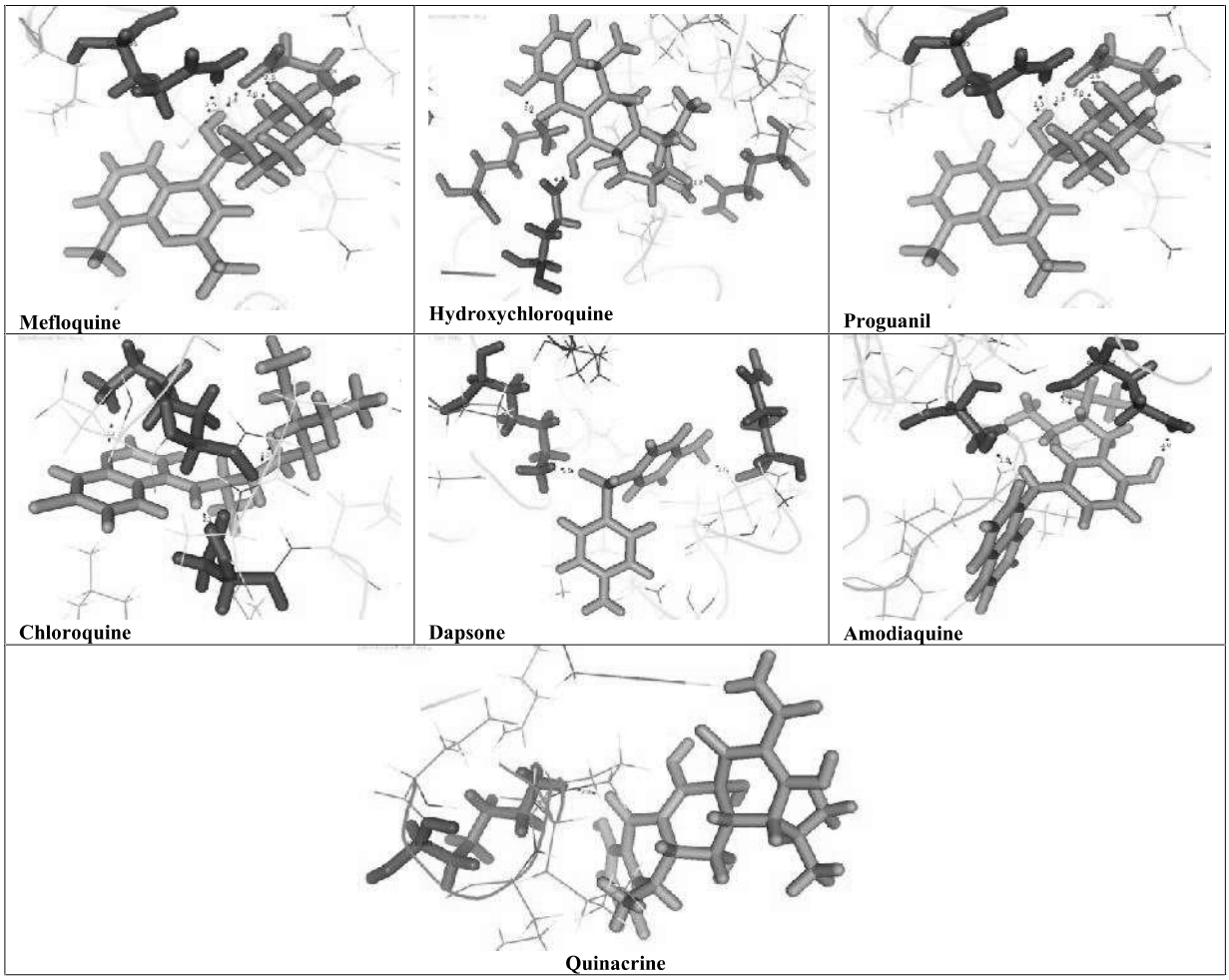


Fig.4 : Interactions of drug molecules with REX1 protein

Pink color represents the interacting residues; Lime green represents the drug molecule; Blue dashed lines represents the formation of bonds along with their distance

DISCUSSION:

Saifihas reviewed the mode of action, resistance to antimalarial drugs such as quinolones (chloroquine, quinine, mefloquine, amodiaquine, primaquine), antifolates (pyrimethamine, proguanil, sulfadoxine), the artemisinin derivatives (artemisinin, artesunate, artemether, arteether) and hydroxynaphthaquinones (atovaquine)[22]. From the year 2003, artemisinin-based combination therapy (ACTs) has been used as first line treatment in most of the malarial sectors due to the development of resistance to chloroquine and sulfadoxine pyrimethamine[23]. However, at present situation the resistances to all the existing drugs have been developed by the parasite *P. falciparum*, the need of the hour is either to find a novel drug molecule from natural sources or to find a novel mechanism to eradicate malaria or an eradication program to prevent the spread of malaria. In this perspective, the present study attempted to observe the binding efficiency of the antimalarial drugs with the REX1 protein, which involve in maintaining the membranous structure called Maurer's cleft (MC). Truncating the REX1 has decreased the number of MC structures and also stacked cisternae was formed[13]. Besides, REX1 protein has not been studied either *in vitro* or *in silico* the present study is the first report regarding the structure modeling, docking and its binding efficiency with antimalarial drugs.

So far, the targets like falcipain-2, plasmepsin-2, plasmepsin-4[24], Lactate dehydrogenase[25] were concentrated as malarial drug targets and few have been analyzed using *in silico* predictions[26].The present research might serve as an initial step towards identifying REX1protein specific drug. Future perspectives of the study is to perform *in silico* virtual screening, QSAR analysis and the structural biologist to focus on crystallization of REX1 in order to acquire more information regarding its structure and function.

This might directly help in achieving the Malaria Elimination Program (2016-2030).

CONFLICT OF INTEREST:

The authors declare they have no competing interests.

ACKNOWLEDGEMENT:

We acknowledge Vels Institute of Science, Technology and Advanced Studies (VISTAS) for providing us with required infrastructure and support system needed.

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