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MOLECULAR-DOCKING STUDIES OF MALARIA DRUG TARGET ENZYME NAD – DEPENDANT PROTEIN DEACYLASES SIR2A IN PLASMODIUM FALCIPARUM – A NOVEL APPROACH TO ITS TREATMENT

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ABSTRACT: Malaria has been a major life threatening mosquito borne disease. Unavailability of any effective vaccine and recent emergence of multi- drug resistant strains of malaria pathogen *Plasmodium falciparum* continues to cause persistent deaths in tropical and sub-tropical regions. As a result, demands for innovation of more effective anti-malarial drugs have been emerging. The present study is directed towards finding an inhibitor against NAD-dependant protein deacylases Sir2A, which is essential for host immune surveillance. Moreover, significant difference in homology between *Plasmodium falciparum* NAD-dependant protein deacylases Sir2A and human (*Homo sapiens*) NAD-dependant protein deacylases makes it a suitable candidate for drug therapy. The structure of the biological target was used to predict candidate drugs that could bind with high affinity and selectivity to the target. The primary and secondary structural features of the protein are calculated by ProtParam and PSIPRED. The three dimensional structure of the NAD -dependant protein deacylases is constructed using homology modeling tool MODELLAR utilizing several available NAD-dependant protein deacylases structures as templates. The structure is then subjected to deep optimization and validated by structure validation tools PROCHECK, VERIFY3D, ERRAT. CASTp server was used to analyze active sites of receptor, in addition molecular docking simulation with Autodock vina and Autodock to determine the estimated free energy of molecular binding. This predicted structure of NAD-dependant protein deacylases will serve first hand in the future development of effective NAD-dependant protein deacylases inhibitors with potential anti-malarial activity. However, this is a preliminary study of designing an inhibitor against *Plasmodium falciparum*.

KEYWORDS: Malaria, NAD-dependant protein deacylases, Homology modeling, ligands, PyMoL1.3, Autodock /vina.

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1. INTRODUCTION

Malaria has been a deadly disease since the ages of mankind and has been a challenge for the scientific community to produce such a drug that could eradicate the causative organism altogether. It is caused by a parasite *Plasmodium* that feeds on human red blood cells and is transferred through female *anopheles* mosquito. Malaria is caused by four major species *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae and Plasmodium ovale*. Of this *Plasmodium falciparum* is responsible for about 95 out of 100 cases. Thus *Plasmodium falciparum* becomes the major target for drug intervention. The complete genome of the parasite has been sequenced, which has facilitated the investigators to peep into its life cycle and search for potential drug targets. [41, 17, 38, 29]. The malaria parasite depends on both humans and mosquitoes to carry out its deadly cycle of life. These parasites are transmitted from one person to another by the female anopheles mosquito. *Plasmodium* develops in the gut of the mosquito and is passed on to the saliva of an infected insect each time it takes a new blood meal. When an infected mosquito bites a human, the parasite rapidly goes to the liver within 30 minutes. There the parasite starts reproducing rapidly in the liver. The parasites then enter into red blood cells and reproduce there after bursting, the parasites releases out and spread in the host's blood. It is injected by another mosquito and the life cycle continues like this. Malaria is complex but it is a curable and preventable disease. Lives can be saved if the disease is detected early and adequately treated. It is known what action is necessary to prevent the disease and to avoid or contain epidemics and other critical situations. The technology to prevent, monitor, diagnose and treat malaria exists. NAD – dependant protein deacylases Sir2A present in trophozoite and schizonts stage and help to catalyze the NAD – dependent hydrolysis of medium and long chain fatty acyl groups from lysine residues. It regulates the expression of the surface antigen-coding var genes which are central to the malaria pathogenesis. It cooperates with Sir2B to mediate silencing and mutual exclusive expression of only 1 of the 60 subtelomeric var genes at a time, coding for functionally different but epitopically variant versions of the erythrocyte membrane protein 1 (PfEMP1) molecule, to evade the detection by host immune surveillance. The present study is an attempt to design a high affinity inhibitor with a broad spectrum towards NAD – dependant protein deacylases Sir2A having at least

selectivity for Human Sir2A. The basic aim was to focus on the computer aided drug design against NAD – dependant protein deacylases based on the sequence-structure –function relationship, a major challenge in this field. In silico drug designing process involves the identification of therapeutic target and building a heterogeneous small molecule library to be tested against it [48]. Then virtual screening is initialized by docking of the small molecules from the library. These selected hits are checked for specificity by docking at binding sites of known drug targets and then subjected to detail profiling studies and those molecules that pass these studies are termed as leads [31].

2. MATERIALS AND METHODS

2.1. Sequence retrieval

The amino acid sequences of NAD – Dependent Protein deacylases [Accession Q81E47.1] of *Plasmodium falciparum* were retrieved from the protein database of UniPortKB. The protein is 273 amino acids long and used for further analysis in the current study.

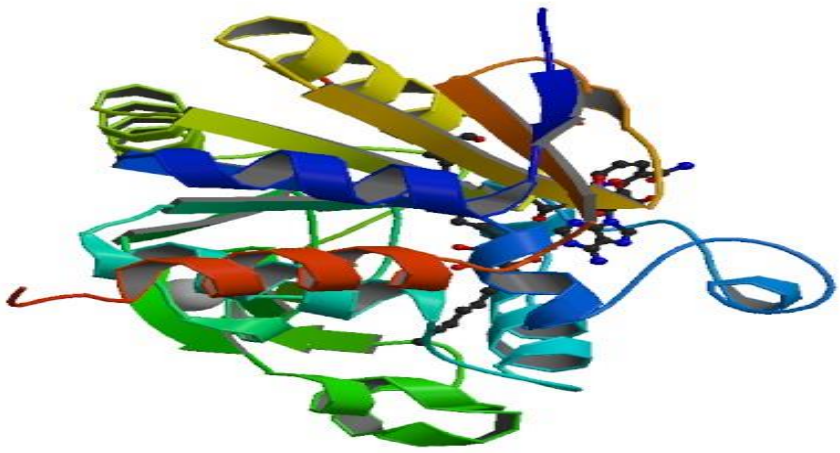


Figure 1: structure of NAD – dependant protein deacylases Sir2A of *Plasmodium falciparum*

2.2. Primary structure prediction

ExPasy's ProtParam tool [5] was utilized to calculate the physico-chemical characteristics of the protein. Theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient [18], instability index [21], aliphatic index [27] and grand average hydropathicity (GRAVY) of the protein were calculated using the default parameters.

Table 1: Different physico-chemical properties of NAD – dependant protein deacylases of *Plasmodium falciparum*

Parameters	Value
Molecular weight	32006.18
Extinction coefficient Abs 0.1%(=1g/1)1.033,assuming all pairs of Cys residues form cystines	33055
Extinction coefficient Abs 0.1%(=1g/1)1.013,assuming all cys residues are reduced	32430
Theoretical pI	8.88
Total number of negatively charged residues (Asp + Glu):	27
Total number of positively charged residues (Arg + Lys):	35
Instability index	38.78
Grand average of hydropathicity(GRAVY)	-0.116
Aliphatic index	89.72

2.3. Secondary structure analysis

Secondary structure was predicted by using the Protein Structure Predication sever (PSIPRED). Protein's secondary structural properties are including α helix, 310 helix, and π helix, Beta Bridge, Extended strand, Bend region, Beta turns, Random coil, Ambiguous states and other states [19]. It was also used to find out the disordered causing regions of the protein. This web service looks for order/globularity or disorder tendency in the query protein based on a running sum of the propensity for an amino acid to be in ordered or disordered state by searching domain databases and known disorders in proteins.

Table 2: Secondary structure analysis through PISPRED of NAD – dependant protein deacylases of *Plasmodium falciparum*

Secondary structure	Percentage
Alpha helix (Hh)	37.93%
Extended strand (Ee)	15.17%
Beta turn (Tt)	10.00%
Random coil (Cc)	36.90%
3_{10} Helix	0.00%
π helix	0.00%
Isolated β-bridge	0.00%
Bend	0.00%

2.4. Template selection

To find out suitable template for the protein BLAST is performed. The level of similarity that exist between protein that have unidentified structures and those that exist within the Protein data bank are shown in Blast results [10]. Through Blast it was found that the best homolog for NAD – dependant protein deacylases is 3U31A with query coverage 100%. The structural summary for the homolog was obtained through PDB. The result of Blast was shown in figure 2.

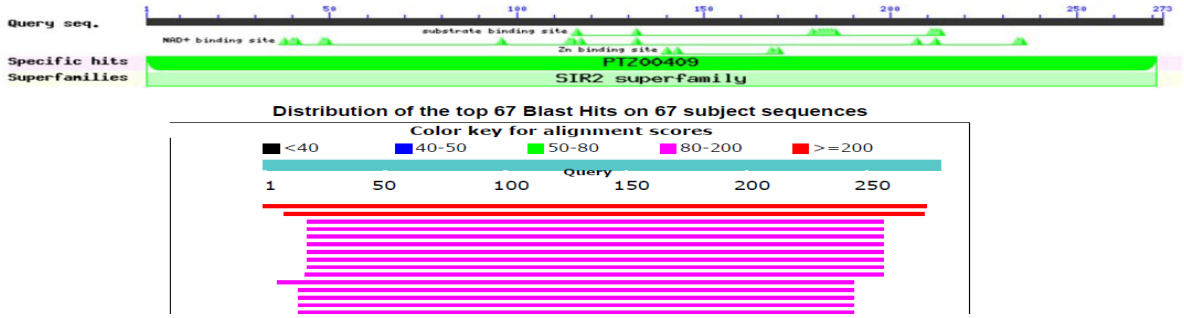


Figure 2: NAD – dependant protein deacylases Blast

2.5. Homology Modeling

The model was generated using a comparative modeling program MODELLER9.20 [3] which generates a refined three dimensional homology model of a protein sequence based on a given sequence alignment and selected template. Homology modeling is able to produce high quality models provided that the query and template molecule are closely related. But model quality can decrease if sequence identity of target and template sequence falls below 20 % though it was proved that protein structures are more conserved than their sequences if the identity is > 20 % [46]. The MODELLER generated five structures with 3U31A as template structures from which the best one is selected on the basis of lowest Discrete Optimized Protein Energy (DOPE) score and highest GA341 score [11].

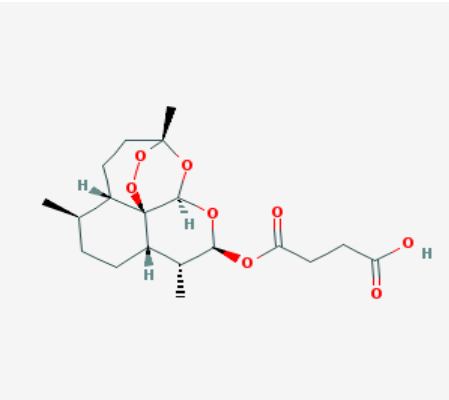
2.6. Ligands Preparation

Several Antimalarial molecules and their analogs were taken from the National Centre for Biotechnology Information (NCBI) Pub-Chem compound database as ligand molecules. These molecules were downloaded in Structure Data File (SDF) format and converted to Protein Data Bank (PDB) coordinates using Open Babel converter. The selected ligand molecules were passed through the Molinspiration server and likeliness of the same was checked by Lipinskis Rule of five for identifying their drug-like properties and only the molecules that passed through this filter were

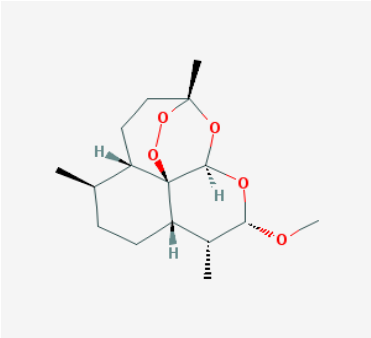
used for further analysis. An ideal drug molecule should be having a molecular weight of less than 500, total number of hydrogen bond should not exceed 5, miLogP value should less than 5 and the sum of N and O should not be more than 10, [40].

Table 3: Molecular Properties of Ligand molecules identified by Molinspiration server

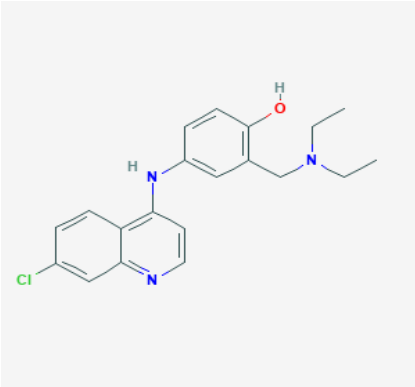
Ligand	miLogP	TPSA	natoms	MW	nON	nOHNH	n violations	nrotb	volume
1	3.52	56.92	26	390.25	4	3	0	0	305.23
2	3.01	76.19	30	411.46	6	3	0	4	362.58
3	4.07	82.82	28	392.54	6	3	0	4	379.68
4	2.75	100.54	27	384.43	8	1	0	5	344.64
5	3.4	46.17	21	298.38	5	0	0	1	281.62
6	4.96	54.37	26	366.84	3	1	0	2	321.57
7	5.29	48.38	25	355.87	4	2	1	6	325.56
8	5	28.16	22	319.88	3	1	1	8	313.12
9	2.06	102.25	27	424.99	7	4	0	7	384.72
10	2.78	57.16	20	284.35	5	1	0	0	264.09
11	9.26	3.24	34	512.95	1	0	2	10	464.36
12	5.6	38.74	37	535.52	6	0	2	6	476.47
13	2.1	60.18	19	259.35	4	3	0	6	256.91
14	4.24	425.15	26	378.32	3	2	0	4	296.92
15	1.79	88.8	17	253.74	5	5	0	4	229.03
16	5.54	78.64	33	463.5	6	3	1	10	410.71



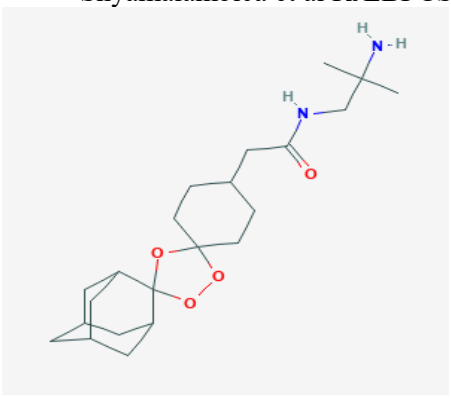
Artesunate



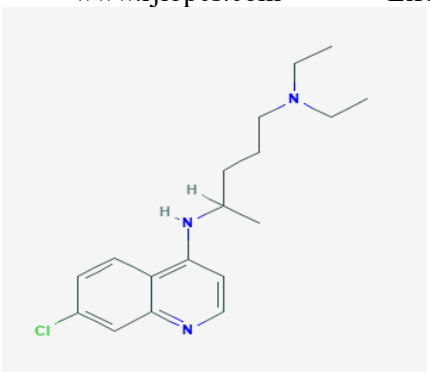
Artemether



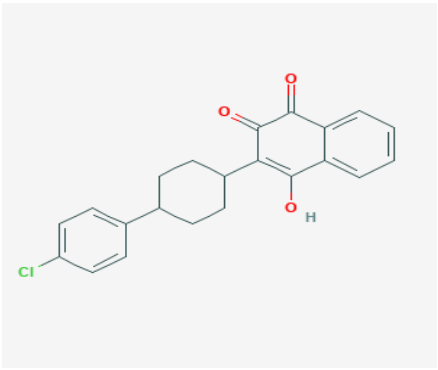
Amodiaquine



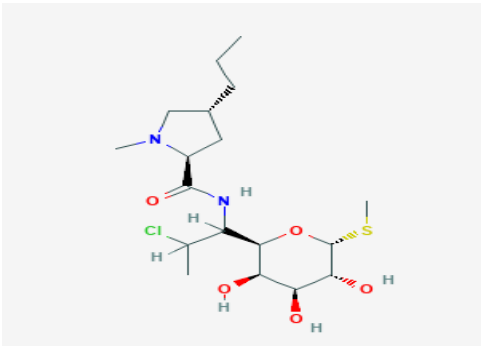
Arterolane



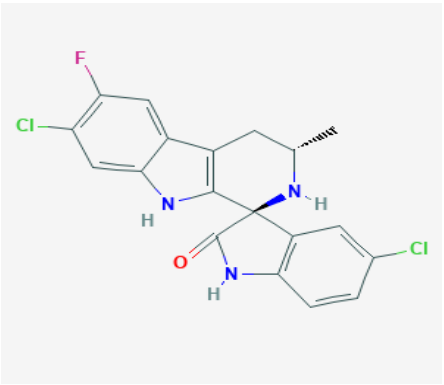
Chloroquine



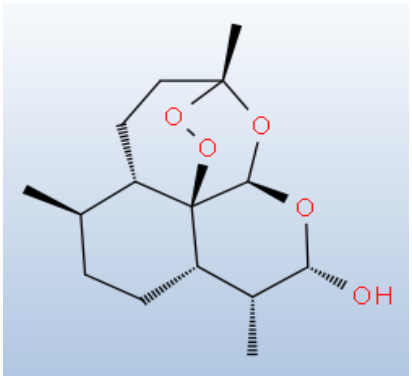
Atovaquone



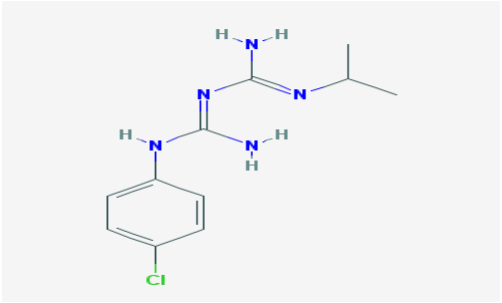
Clindamycin



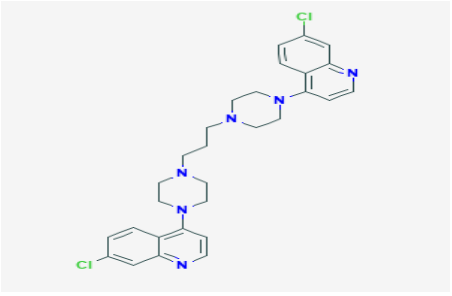
Cipargamin



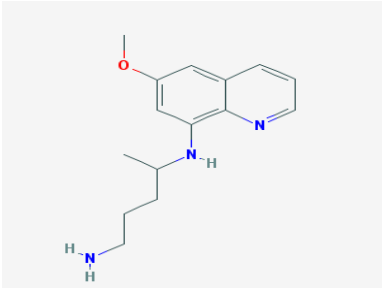
Dihydroartemisinin



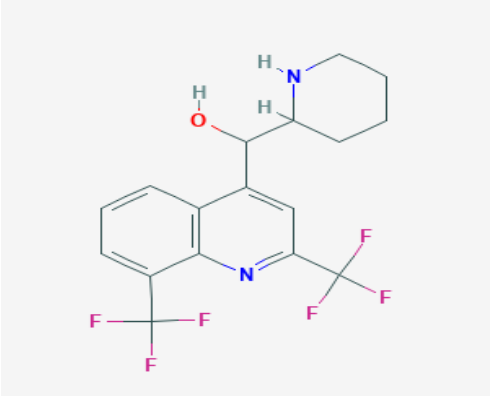
Proguanil



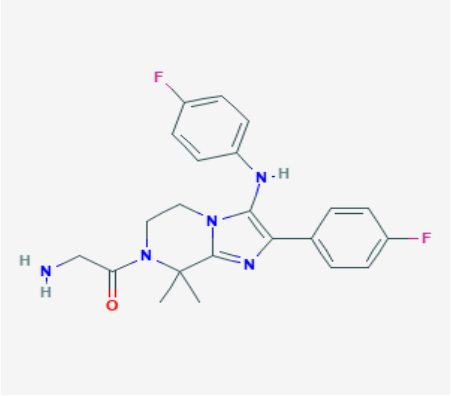
Proguanil



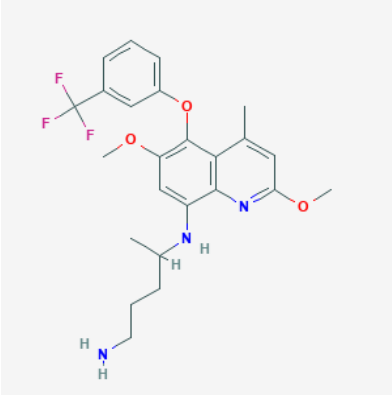
Primaquine



Mefloquine



KAF156



Tenofaquine

2.7. Verification and validation of the structure

The accuracy and stereo chemical features of the predicted model were calculated with PROCHECK [34] by Ramachandran Plot analysis [44] which was done through SAVESv5.0 server (servicesn.mbiuda.edu/SAVES/). The best model was selected based on overall G-factor, number of residues in core, allowed, generously allowed and disallowed regions. Verify3D [9], ERRAT [23] were used for additional analysis of the selected model. Finally, the protein was visualized by Swiss-PDB Viewer [20].

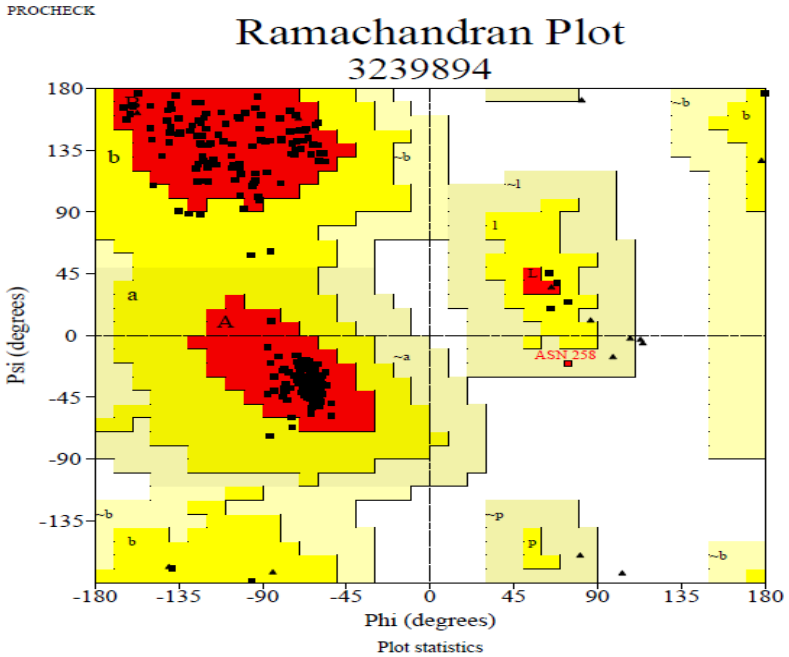


Figure 3: Ramachandran plot analysis of selected model

Table 4: Ramachandran plot of NAD – dependant protein deacylase Sir2A from *Plasmodium falciparum*

Ramachandran plot statistics	NAD-dependant protein deacylases Sir2A	
	Residue	**%**
Residues in the most favored regions[A,B,L]	245	93.9
Residues in the additional allowed regions [a,b,l,p]	15	5.7
Residues in the generously allowed regions [a,b,l,p]	1	0.4
Residues in the disallowed regions [xx]	0	0.0
Number of non-glycine and non-proline residues	261	100.0
Number of end residues (excl.Gly and PRO)	1	
Number of glycine residues	21	
Number of proline residues	7	
Total number of residues	290	

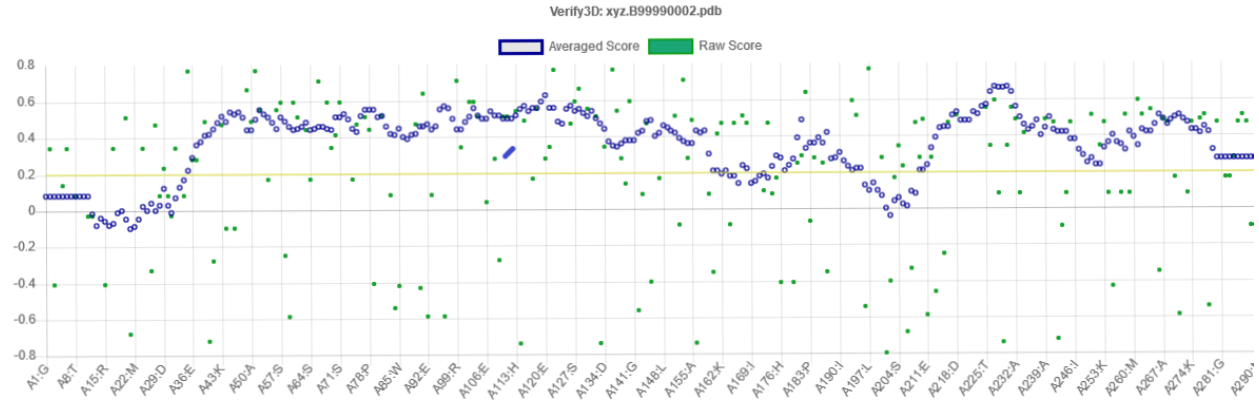


Fig 4: Verify 3D of NAD – dependant of protein deacylases Sir2A from *Plasmodium falciparum*

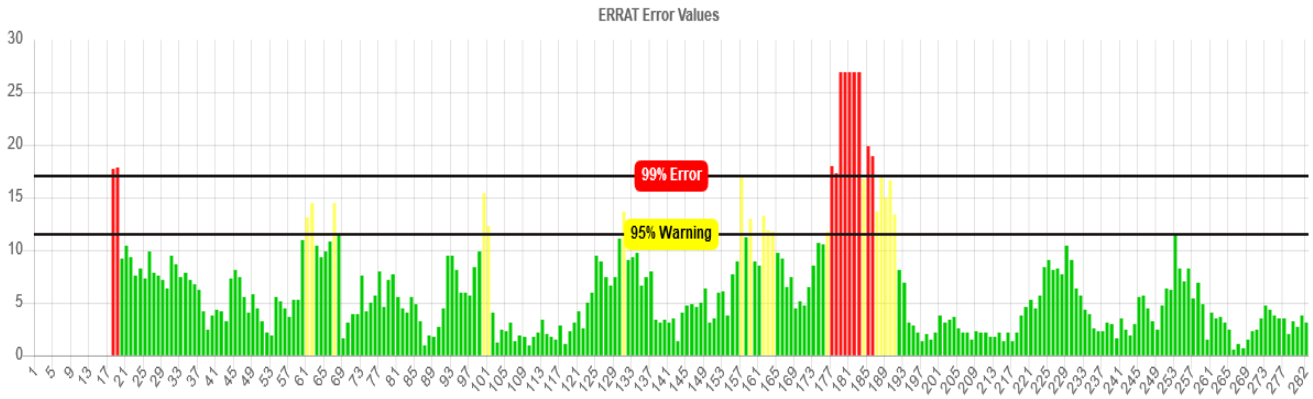


Fig 5: ERRAT generated result NAD – dependant protein deacylases of *Plasmodium falciparum* where 95% indicates rejection limit

2.8. Active site analysis

After modeling the three dimensional structure of NAD – dependant protein deacylases Sir2A, the probable binding sites of the protein was searched based on the structural association of template and the model construct with Computed Atlas of Surface Topography of proteins (CASTp) server [8]. CASTp3.0 was used to recognize and determine the binding sites, surface structural pockets, active sites, area, shape and volume of every pocket and internal cavities of proteins. It could be also used to calculate the number, boundary of mouth openings of every pocket, molecular reachable surface and area [33]. Active site analysis provides a significant insight of the docking simulation study.

2.9. Docking simulation study

In silico docking simulation study, was carried out to recognize the inhibiting potential against NAD – dependant protein deacylase Sir2A enzyme. Docking study was performed by Autodock vina and Autodock version 1.5.6. Before starting the docking stimulation study, NAD – dependant protein deacylase was modified by adding polar hydrogen. All compounds were screened using Autodock vina version 1.5.6 to search for chemicals with high binding affinities for NAD – dependant protein deacylases. The size of the grid box was set as center_x = -11.019, center_y = 24.747, center_z = -29.888, size_x =58, size_y = 122, size_z = 126. The exhaustiveness level was set to 8. Compound with highest binding affinities then analyzed and considered as possible template for further optimization. Then Autodock version 1.5.6 was performed and the search results were analyzed on the basis of the Lamarckian genetic algorithm. Comparison of ligands is made on the basis of binding energy and ligand with minimum binding energy is selected [13]

Table 6: Identifying binding affinity between Protein and ligand by Autodock vina in *Homo sapiens*

Ligand	Binding Affinity (K.cal/mol)	distance from best mode	
		rmsd L b	rmsd Ub
KAE 609 1	-12.4	0	0
kAE 609 2	-12.2	3.512	6.011
KAF 156 1	-10.1	0	0
KAF 156 2	-10	2.03	2.819
Arterolane 1	-13.3	0	0
Arterolane 2	-13.2	1.45	2.551
Primaquine 1	-9.1	0	0
Primaquine2	-8.6	2.393	4.601
Amodiaquine 1	-9.7	0	0
Amodiaquine2	-9.3	2.456	4.145
clindamycin 1	-8.2	0	0
clindamycin 2	-7.9	3.653	6.843
mefloquine1	-13.1	0	0
mefloquine2	-12.7	2.336	3.247
chloroquine1	-7.7	0	0
chloroquine2	-7.4	2.579	6.715
Artesunate1	-12.5	0	0
Artesunate2	-12.5	1.904	5.523
Artemether1	-10	0	0
Artemether2	-9.9	1.659	3.92
dihydroartemisinin1	-9.6	0	0
dihydroartemisinin2	-9.5	1.125	1.776
piperaquine1	-10.8	0	0
piperaquine2	-10.4	4.431	8.005
Proguanil 1	-5.5	0	0
Proguanil 2	-5.2	12.58	13.573
Atovaquone 1	-12.2	0	0
Atovaquone 2	-11.8	3.213	6.801
Lumefantrine 1	-11.1	0	0
Lumefantrine 2	-11.1	1.977	4.314
Tafenoquine 1	-11	0	0
Tafenoquine 2	-10.8	2.203	4.52

3. RESULTS AND DISCUSSION

The two ligands namely Arterolane with 12.5 (BE) and 768.87(IC) and Clindamycin with -8.42(BE) and 676.13 (IC) predicted were highly significant among the 17 ligands with its good binding affinity and inhibition constant. Docking of small molecule compounds into the binding site of a receptor and estimating the binding affinity of the complex is an important part of the structure-based drug design process. For a thorough understanding of the structural principles that determine the strength of a protein/ligand complex both, an accurate and fast docking protocol & the ability to visualize binding geometries and interactions are mandatory. In the present study an interface between the popular molecular graphics system PyMoL1.3 and the molecular docking suites Autodock 1.5.6 and Vina 1.5.6 an attempt was made to demonstrate, how the combination of docking and visualization can aid structure-based drug design efforts. In the present work we describe a plugin for PyMoL1.3 which allows carrying out molecular docking, virtual screening and binding site analysis with PyMoL1.3. The plugin represents an interface between PyMoL1.3 and two popular docking programs, Autodock 1.5.6 and Autodock vina version 1.5.6 and makes extensive use of Python script collection for the setup of docking runs. Since visualization is crucial for structure-based drug design, several tools have been developed to add visual support for the Autodock version 1.5.6 suite. The visualizer Autodock version 1.5.6 Tools offers a complete molecular viewer and a graphical support for all steps required for setup and analysis of docking runs. Autodock version 1.5.6 and Vina need receptor and ligand representations in a file format called ‘pdbqt’ which is a modified protein data bank format containing atomic charges, atom type definitions and, for ligands and topological information (rotatable bonds). These file preparations are carried out by the plugin using scripts from the Autodock version 1.5.6 Tools package. CASTp detects pockets and voids in protein structures to determine and characterize binding sites. Ligands for subsequent docking runs can either be prepared one by one through PyMoL1.3 [7] selections or by specifying a directory containing a library of ligands to be docked. Autodock uses interaction maps for docking. Prior to the actual docking run these maps are calculated by the program *autogrid* [26]. For each ligand atom type, the interaction energy between the ligand atom and the receptor is calculated for the entire binding site which is discretized through a grid. The docking poses are ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses may be exported. For instance, the ranked list of docking results can be exported in a CSV file format which can be directly imported into programs like Excel.

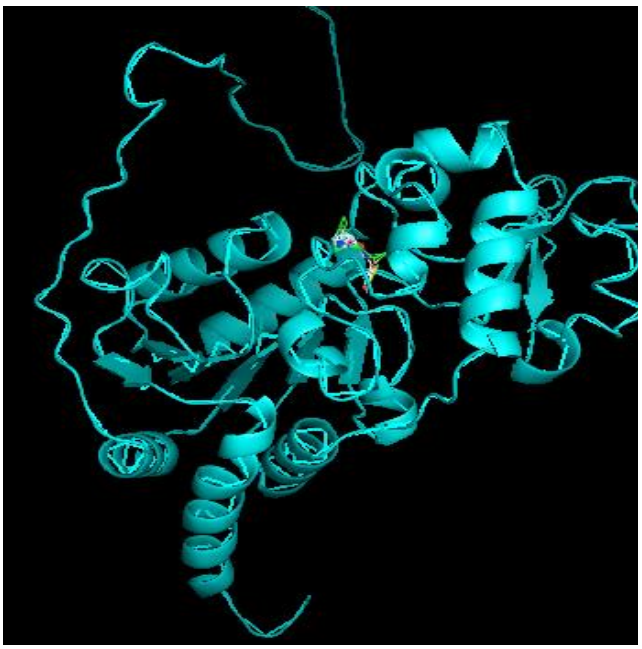


Figure 6: molecular docking of Arterolane

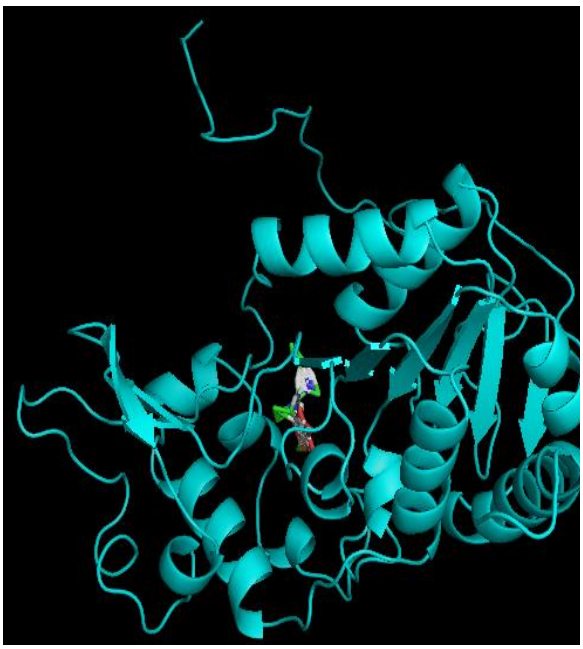


Figure7:Molecular docking of Clindamycin

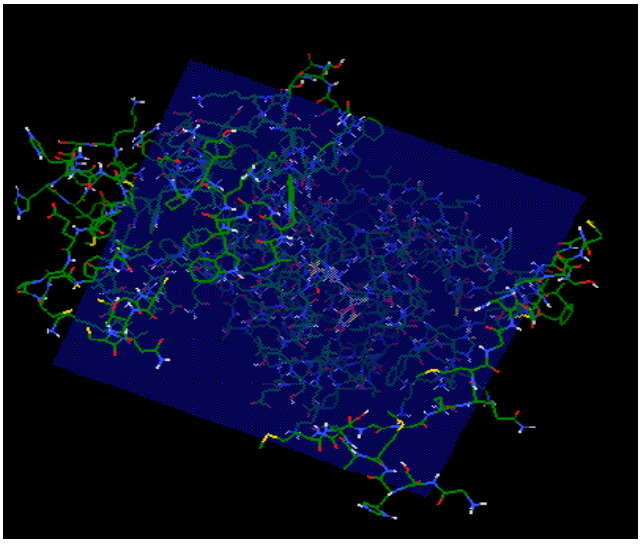


Figure 8: Grid Box of Arterolane

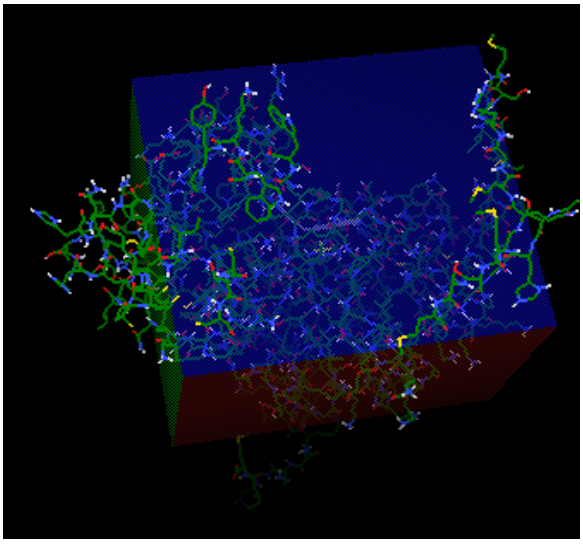


Figure 9: Grid Box of Clindamycin

Table 7: Molecular simulation by Autodock

Ligand	KAF 156	Ciparga min	Arterol ane	Artesun ate	Artemet her	Lumefa ntrine	Amodia quine	Atovaq uone	chloroq uine	clindam ycin	Tenofaq uine	Piperaq uine	Prima quine	Mefloq uine	Dihydro artemiss ion
Binding affinitv	-7.98	-9.6	-12.5	-10.34	-9.8	-10.27	-8.61	-10.75	-6.34	-8.42	-7.82	-12.24	-5.31	-8.02	-11.02
Ligand efficiency	-0.26	-0.37	-0.45	0.38	-0.47	-0.29	-0.34	-0.41	-0.29	-0.31	-0.24	-0.33	-0.28	-0.28	0.55
Inhib_consta	2.01	91.12	768.87	26.4	65.16	29.4	488.5	13.16	22.44	676.13	1.85	1.06	127.8	5.75	8.42
Inhib_Const ant_units	uM	nM	pM	nM	nM	nM	nM	nM	uM	nM	uM	nM	uM	uM	nM
Intermol_ene rgy	-9.26	-10.5	-13.4	-12.13	-10.1	-14.15	-10.7	-11.65	-8.73	-11.1	-9.91	-14.03	-7.4	-8.04	-11.61
Vdw_hb_Des olv_Energy	-9.18	-10.52	-13.41	-12.05	-9.99	-14.11	-10.69	-11.55	-8.44	-11.07	-9.53	-14.23	-7.23	-7.79	-11.65
Electroststic_ Energy	-0.09	0.02	0.01	-0.08	-0.11	-0.04	-0.01	-0.1	-0.29	-0.03	-0.37	0.2	-0.17	-0.25	0.04
Total int. En	25.53	0	15.89	12.28	4.02	38.58	50.71	33.09	52.71	40.25	29.7	57.96	33.77	21.67	-0.64
Torsional_E nergy	1.49	0.89	0.89	1.79	0.3	3.88	2.09	0.89	2.39	2.68	2.09	1.79	2.09	0.89	0.6
Unbound_en ergy	25.53	0	15.89	12.28	4.02	38.58	50.71	33.09	52.71	40.25	29.7	57.96	33.77	21.67	-0.64
File_name	Dock.dl g	Dock.dl g	Dock.dl g	Dock.dl g	Dock.dl g	Dock.dl g	Dock.dl g	Dock.dl g	Dock.dl g	Dock.dl g	Dock.dl g	Dock.dl g	Dock. dlg	Dock.dl g	Dock.dl g
cIRMS	56.785	0	53.912	0	0	43.638	0	50.801	0	0	43.947	19.927	56.06 7	0	0
refRMS	n/a	49.63	n/a	47.39	55.77	n/a	48.57	n/a	55.21	55.94	n/a	n/a	n/a	55.06	36.01
Rseed1	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none
Rseed2	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none

4. CONCLUSION

In present study a novel plugin was proposed for the popular molecular graphics system PyMoL1.3 which allows performing docking studies using Autodock version 1.5.6 or Autodock/Vina v1.5.6. The plugin covers all functionalities for the entire workflow of a docking run plus additional functionality to prepare, execute and analyze virtual screening tasks. Since visual support is an important aspect of structure-based drug design, the plugin is expected to enhance these efforts by allowing the combined use of two widely used docking programs and PyMoL1.3. Seventeen compounds were found from the different articles that also inhibit *P. falciparum* in Invitro drug susceptibility assay. Artemisinin- based combination therapies (ACTs) are the current standard of care for uncomplicated malaria. Artemisinin and its derivatives have a fast onset of action but are cleared rapidly and are therefore combined with slow-clearing drugs to kill residual parasites. Typical partner drugs include Lumefantrine and Piperaquine. The most popular combination consists of tablets containing Artemether and Lumefantrine sold as Coartem. For the liver stages, Primaquine is the only drug approved to eliminate hypnozoites. As for prophylactic treatment, Atovaquone – Proguanil (Malarone, GlaxoSmith Kline) is usually preferred because it is well tolerated, but is expensive. Clindamycin (7-chloro-lincomycin) is a semisynthetic derivative of lincomycin and was introduced in the 1960s as an antibiotic. It is active against organisms such as *Plasmodium, Toxoplasma, Babesia,* and *Pneumocystis* spp. Clindamycin is the drug of choice for prophylaxis of *Toxoplasma* chorioretinitis in newborn infants and is part of recommended regimens against both *Babesia microti* and *Babesia divergens.* In vitro, clindamycin and its three major metabolites show strong inhibitory effects on *P. falciparum,* possibly by targeting the apicoplast. The drug accumulates slowly in the parasite. In this study, the computational method based on pharmacophore modeling, molecular docking and virtual screening was established to blocking the interaction of NAD–dependant protein deacylases. The ligand based pharmacophore model established was used to identify the common features of NAD – dependant protein deacylases inhibitors from PUBCHEM database and virtual screening method was used to screen the libriary of compounds. After that molecular docking was employed to study the detailed binding mode between the selected ligands and active site of NAD – dependant protein deacylases receptor. The computational approaches showed the advantage in saving time and resources. It is feasible to blocking the interaction of NAD–dependant protein deacylases protein from the selected 17 compounds by using virtual screening based on pharmacophore and molecular docking studies. Several active compounds which are structurally diverse compounds were identified in NAD – dependant protein deacylases inhibitors of *Plasmodium falciparum*. Thereby it revealed that, sequential use of the available tool such as Autodock/vina yield better results to block the interaction of NAD –dependant protein deacylases. In the following study, two chemical compounds like

Arterolane and Clindamycin were predicted. These compounds hit by pharmacophore model, virtual screening and molecular docking, needs further verification using related biological experiments. Such further studies may help to find effective inhibitors of NAD – dependant protein deacylases protein of the parasite to block the interaction of NAD – dependant protein deacylases protein in the erythrocytic cycle of the host and finally arrest the activity of parasite.

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CONFLICT OF INTEREST

The Authors declare that they have no conflict of interest.

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