Invasion inhibitory antimalarial activity of Macrolides

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ABSTRACT

Background: Malarial drug resistance is becoming a serious concern. Drug designing is a cumbersome and time taking process. The need of hour is to repurpose the existing drugs. The invasion inhibitory potential of Azithromycin has long been known through in-vitro and in-vivo studies. Here we intended to study the invasion inhibitory potential of drugs belonging to the class of macrolide antibiotics.

Methodology: Molecular docking study was performed with fourteen drug molecules.The three-dimensional chemical structures of molecules were prepared through UCSF Chimera and Autodock Tools freeware. Molecular docking study was performed using AutoDockvina, Discovery studio 4.5 and PyMol was used to predict the active site of target sites.

Results: The preliminary experimental study demonstrated that in comparison with Azithromycin, Pristinamycinshows good binding efficacy to all the target proteins. Hence, it can be suggested thar Pristinamycinis a potential inhibitor of merozoite invasion into erythrocytes. However, detailed in-vitro and in-vivo studies needs to be performed for further drug development process.

Keyword - *merozoite, invasion inhibitor, ingress inhibitor*

. INTRODUCTION

Malaria is a mosquito-borne parasitic disease. It causes nearly 600,000 deaths every year, mainly in children under 5 years of age in sub-Saharan Africa [1].4% of the global malaria burden is carried by India which contributes 87% of the total malaria cases in South-East Asian region [2].

Current Malaria eradication measures rely on controlling the spread by reducing exposure to the mosquito vector by using bed nets and treatment of clinical malaria cases by use of various drugs. Most of the current antimalarial drugs target the disease causing*Plasmodium* blood-stage infectious trophozoite. These drugs may target the parasite's food vacuole as in case of Chloroquine or interfere with pyrimidine synthesis of maturing trophozoites or cause oxidative damage [3–7]. Out of these drugs, Artesunate has been prescribed by World Health Organization (WHO) as the first line drug of choice. However, a major concern with the use of these antimalarials is the emerging development of drug resistance to the highly effective artemisinin family of compounds in Southeast Asia [8–10].To prevent the developing antimalarial resistance, WHO has underpinned that there is an urgent need for new antimalarials with novel mechanisms of action for use in combination with Artemisinins.

Of the various novel mechanisms suggested so far , invasion inhibition of blood-stage merozoites into host cells has attracted a lot of attention as a potential target for antimalarial chemotherapy [11–14]. Application of antimalarial drugs that target invasion has the potential to result in quicker resolution of clinical disease if used in combination

with antimalarials that act at other developmental stages.Furthermore, Erythrocyte invasion process is a highly complex, sequential process requiring a coordinated and simultaneous interaction of multiple parasite ligands and signaling pathways. There is another major advantage of the invasion inhibition process which has not been reported so far with any of the current first-line antimalarials [15–17]. The invasion inhibition disrupts the parasite lifecycle, thereby preventing sequestration, dormancy, and commitment to the mosquito-transmissible gametocyte stage.

Macrolide antibiotics namely, azithromycin and clindamycin (lincosamide antibiotic) have been proposed as potential partner drugs for artemisinin combination therapies owing to their extremely long half-life and good safety profile *in vivo* [18–22]. Azithromycin and clindamycin bind to the apicoplast ribosomal 50S subunit and inhibit apicoplast ribosomal protein synthesis of asexual blood-stage parasite by blocking protein exit from the ribosome [23–25]. *In vitro* studies have reported that macrolide antibiotics treated merozoites have a 'delayed death' drug response. Parasites exposed to macrolides grow normally during the first lifecycle. But, during the second post-treatment cycle parasite death is observed due to inheritance of a defective apicoplast [26–28]. Drug repurposing of macrolides as antimalarials is a promising strategy to counter the rapid emergence of drug resistance due to dual activity profile of merozoite invasion and disruption in protein synthesis activity.

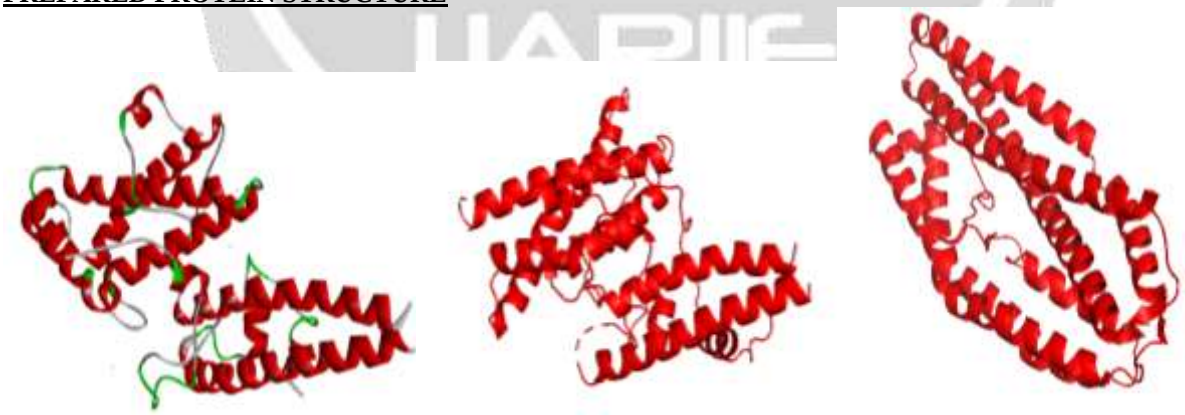
In the present study, we aimed to conduct a comparative antimalarial in-silico studies of macrolide antibiotics with respect to invasion inhibitory potential for development as effective antimalarials.

2. MATERIALS & METHOD

2.1 Protein preparation

The crystal structure of the molecular target, merozoite surface protein MSPDBL2 from *P. falciparum* (PDBID 3VUV), Duffy Binding Protein of *P.vivax* (PDBID :- 6OAN) and *Plasmodium vivax* reticulocyte binding protein 2b (PvRBP2b) (PDBID : 6BPA) **,** were retrieved from RCSB protein data bank (https://www.rcsb.org/) [29].Before starting the molecular docking process, targets need to be prepared. Target protein preparation involves removal of the complexes bound to the protein receptor molecule**,** removalof the water molecules and finally addingpolar hydrogen atoms into target. All these processes are carried out in the Auto Dock window execution file.

PREPARED PROTEIN STRUCTURE



3VUV (A CHAIN) **6OAN (A CHAIN)** **6BPA (D CHAIN)**

Figure 1

2.2**Ligand preparation**

Investigational ligands were built using canonical smiles obtained from PUBCHEM, saved in.pdb format using UCSF Chimera[30] and subsequently converted into .pdbqt format by Autodock tools[31]. In the current study, identification of binding modes of the investigational ligands with targetwas identified using Auto Dock Vinasoftware program [32]. In order to confirm actual binding interaction with targets blind docking was performed and the best conformers were represented with lowest binding energy (-kcal/mol). For merozoite surface protein MSPDBL2 from *P. falciparum* (PDBID 3VUV), the docking parameters were defined as coordinates of the center of binding site with x = 114, y = 106, z = 92 and binding radius = 1 Å. For Duffy Binding Protein of *P.vivax* (PDBID :- 6OAN), the docking parameters were defined as coordinates of the center of binding site with x = 110, y = 110, z = 110 and binding radius = 1 Å. For *Plasmodium vivax* reticulocyte binding protein 2b (PvRBP2b) (PDBID : 6BPA), the docking parameters were defined as coordinates of the center of binding site with x = 70, y = 80, z = 60 and binding radius = 1 Å. All AutoDock output files(.pdbqt) were analyzed through Biovia Discovery Suite[32]. Top-scoring molecules in the largest cluster were analyzed. Conformers of the ligand were automatically docked to the proteins and most stable conformer in terms of binding affinity (most negative) was used for post-docking analysis.

3. Result and discussion

3.1 Binding energies

Docking scores of Macrolide Antibiotic using Autodock Vina

The binding energies of various ligands with the target proteins has been enlisted in Table 1. The binding energy of PristinamycinI, Pristinmaycin II and Solithromycin to the Duffy Binding Protein of *P.vivax* (PDBID :- 6OAN) were -8.5 kcal/mol,-8.0 kcal/mol and -8.2 kcal/mol and that of Azithromycin was -6.3 kcal/mol. The binding energy of PristinamycinI, Pristinmaycin II and Solithromycin to the active site is even smaller than that of the Azithromycin, indicating that PristinamycinI, Pristinmaycin II and Solithromycin have a higher binding activity. With regard to merozoite surface protein MSPDBL2 from *P. falciparum* (PDBID 3VUV) and *Plasmodium vivax* reticulocyte binding protein 2b (PvRBP2b) (PDBID : 6BPA) only PristinamycinI and Pristinmaycin II have a binding energy equivalent to that of Azithromycin. From the binding energy point of view of, Pristinamycin shows strong interactions with the surface targets of both *P.falciparum* and *P.vivax.*

TABLE 1

S. No.	Investigational Ligand	Duffy Binding Protein of *P.vivax* (PDBID :- 6OAN)	Merozoite surface protein MSPDBL2 from *P. falciparum* (PDBID 3VUV)	*Plasmodium vivax* reticulocyte binding protein 2b (PvRBP2b) (PDBID : 6BPA)
1	Azithromycin	-6.3	-6.3	-6.0
2	Dirithromycin	-6.0	-5.7	-5.2
3	Erythromycin	-6.9	-9.0	-6.6
4	Flurithromycin	-7.1	-7.3	-6.6
5	Josamycin	-6.0	-6.7	-5.7

6	Lincomycin	-4.5	-5.4	-5.6
7	Midecamycin	-6.0	-6.1	-5.7
8	Pristinamycin 1	-8.5	-7.0	-7.5
9	Pristinamycin 2	-8.0	-8.9	-8.2
10	Rokitamycin	-6.3	-6.1	-5.7
11	Solithromycin	-8.2	-7.5	-6.6
12	Toleandromycin	-6.9	-6.7	-6.7
13	Spiramycin	-6.8	-6.3	-6.0
14	Telithromycin	-6.8	-7.6	-7.6

3.2 Molecular docking with 6OAN

Molecular docking results rendered by Discovery suite 4.5 demonstrated that Azithromycin forms Pi-alkyl bonds with amino-acid residues TYR 400, PRO 405, VAL 408, PHE 485, PHE 490 and Hydrogen bonds with ASN 486 and ASP 483. On the other handPristinamycin 1 forms Pi-Sigma bonds with LEU 404, Pi-Alkyl bonds with VAL 408 and ALA 500, Pi-Anion bonds with GLU 493 and Hydrogen bonds with LYS 412, ASN 486 and GLU 493. Pristinamycin 2 forms π-alkyl bonds with amino-acid residue MET 319

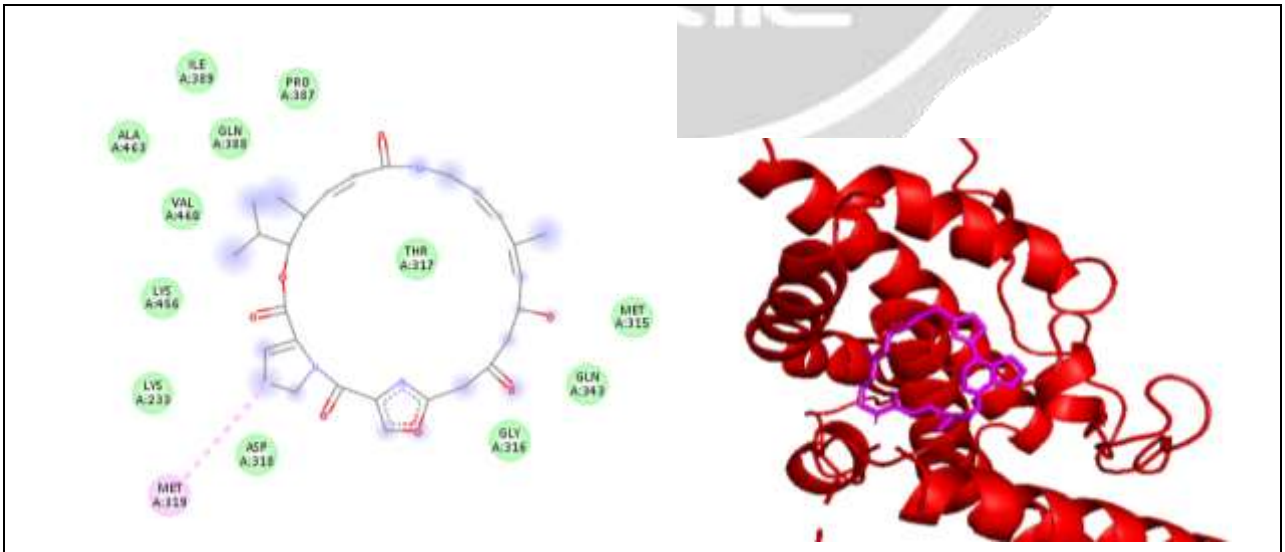


Figure 2: Visualization of interactions and binding region of pristinamycin2 with 6OAN

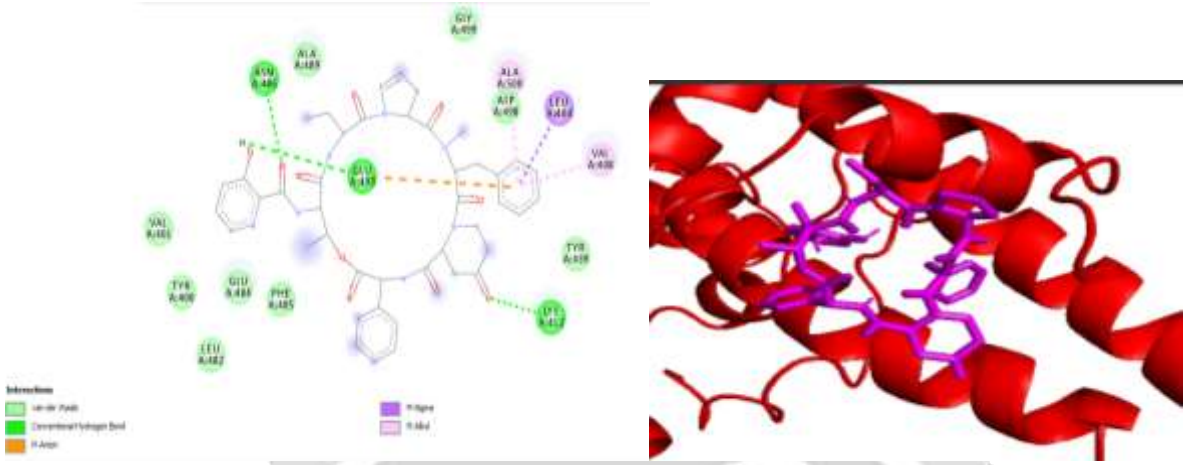


Figure 3:**Visualization of interactions and binding region of pristinamycin1 with 6OAN**

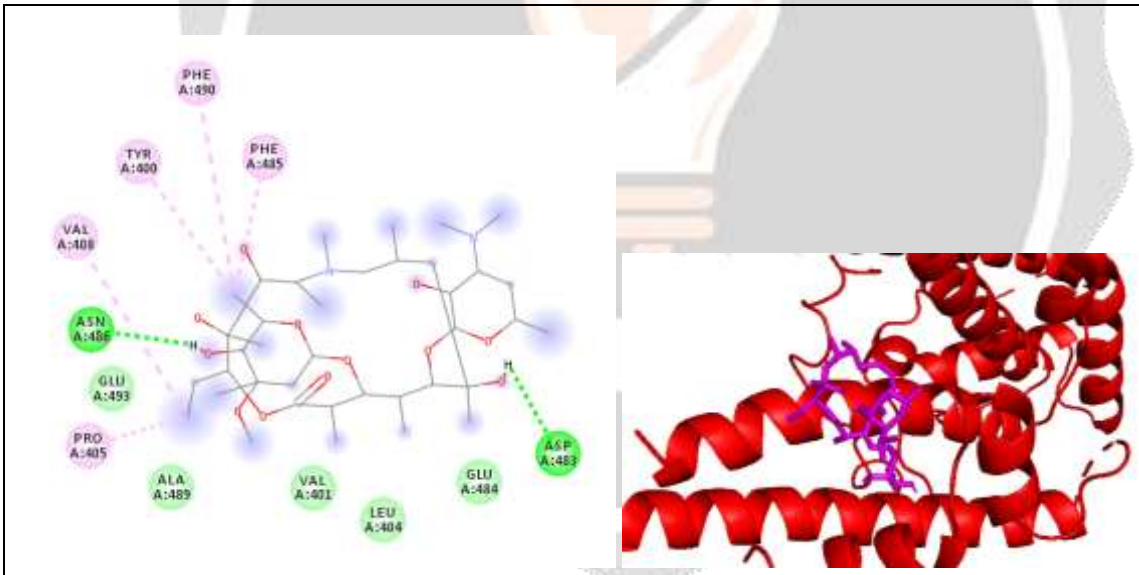


Figure 4:Visualization of interactions and binding region of azithromycin with 6OAN

3.3 Molecular docking with 6BPA

Azithromycin forms Pi-Sigma and Hydrogen bonds with TYR 337. Pristinamycin1 forms bonds with Pi-Alkyl LEU 329, Hydrogen bonds with LYS 333, GLU 338 and PRO 336 and Pi-Anion bonds with GLU 338. Pristinamycin2 forms Hydrogen bonds with ARG 304, TYR 186 and GLU 421.

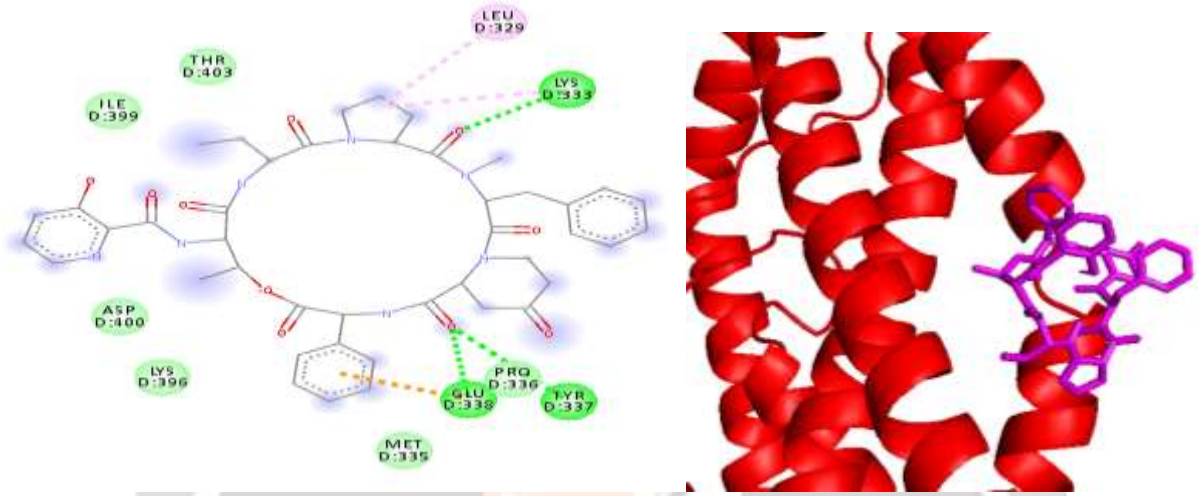


Figure 5: Visualization of interactions and binding region of pristinamycin1 with 6BPA

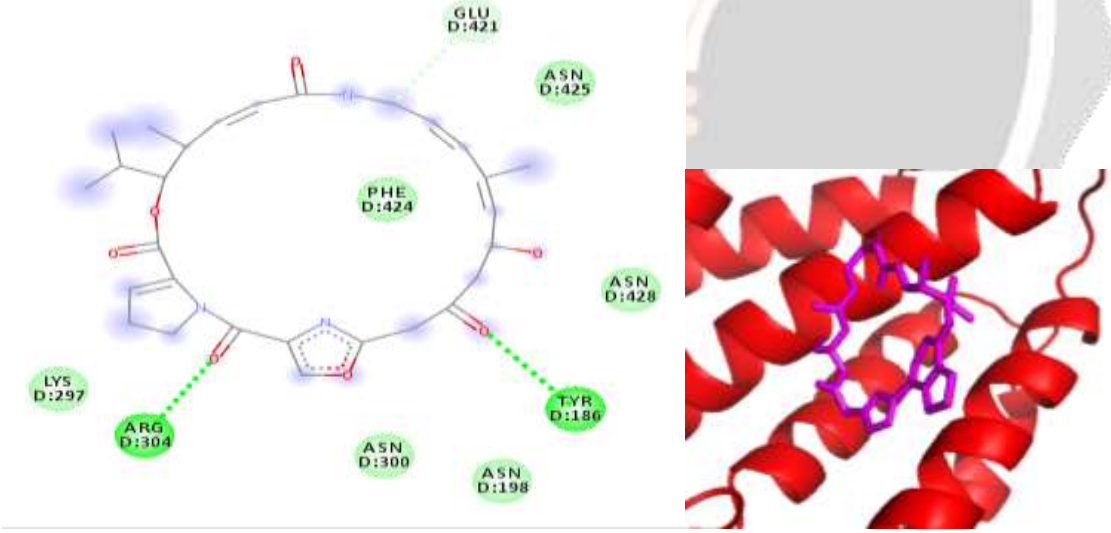


Figure 6: Visualization of interactions and binding region of pristinamycin2 with 6BPA

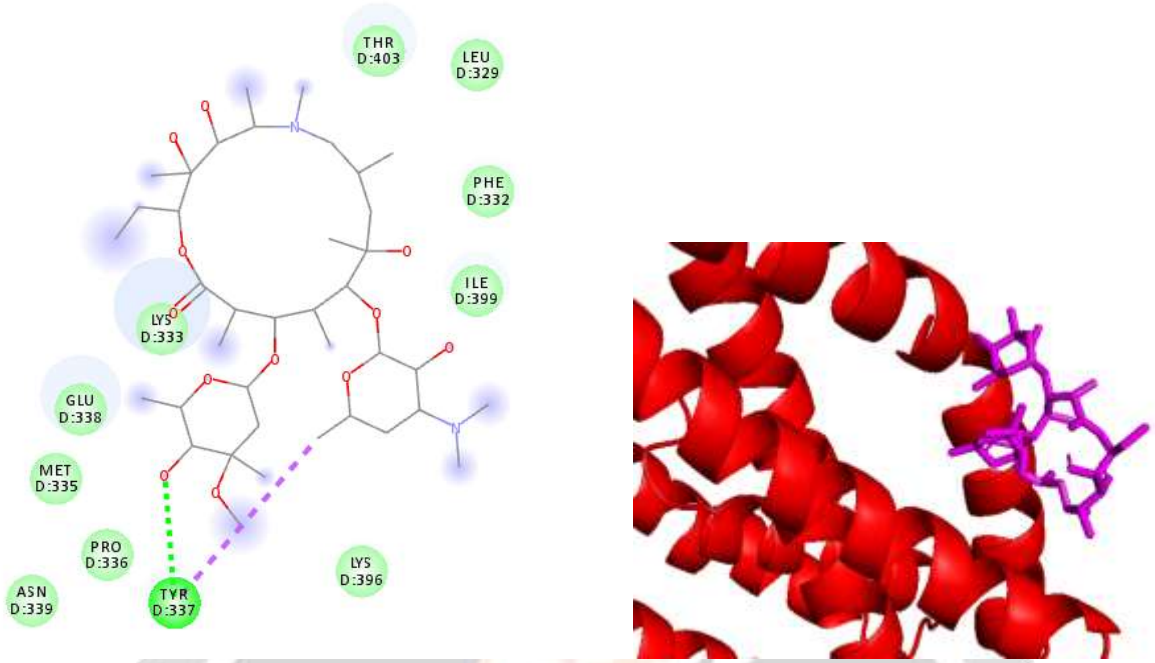


Figure 7: Visualization of interactions and binding region of azithromycin with 6BPA

3.4 Molecular docking with 3VUV

Azithromycin forms Pi-Alkyl bonds with TYR 425. Pristinamycin1 forms Hydrogen bonds with ASN 299. Pristinamycin2 forms bonds with CYS 212 and ARG 214.

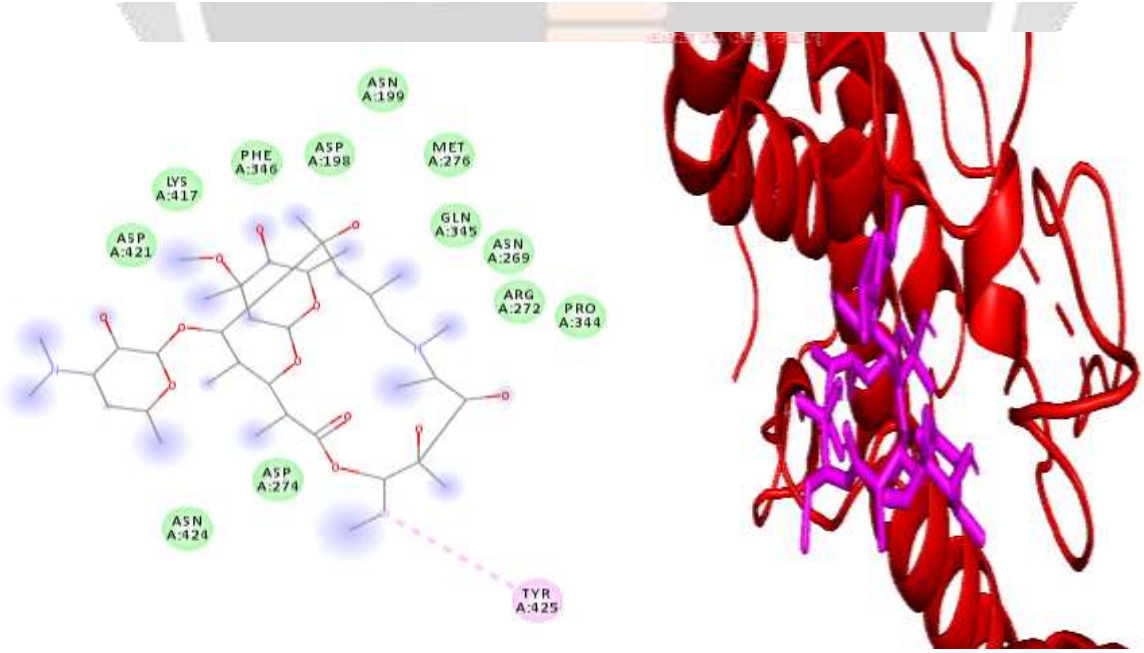


Figure 8: Visualization of interactions and binding region of azithromycin with 3VUV

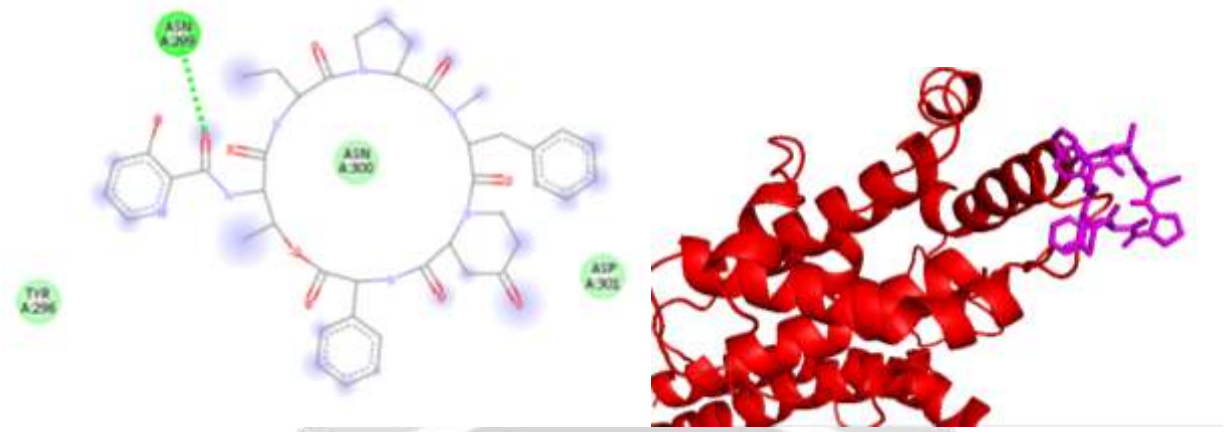


Figure 9: Visualization of interactions and binding region of pristinamycin1 with 3VUV

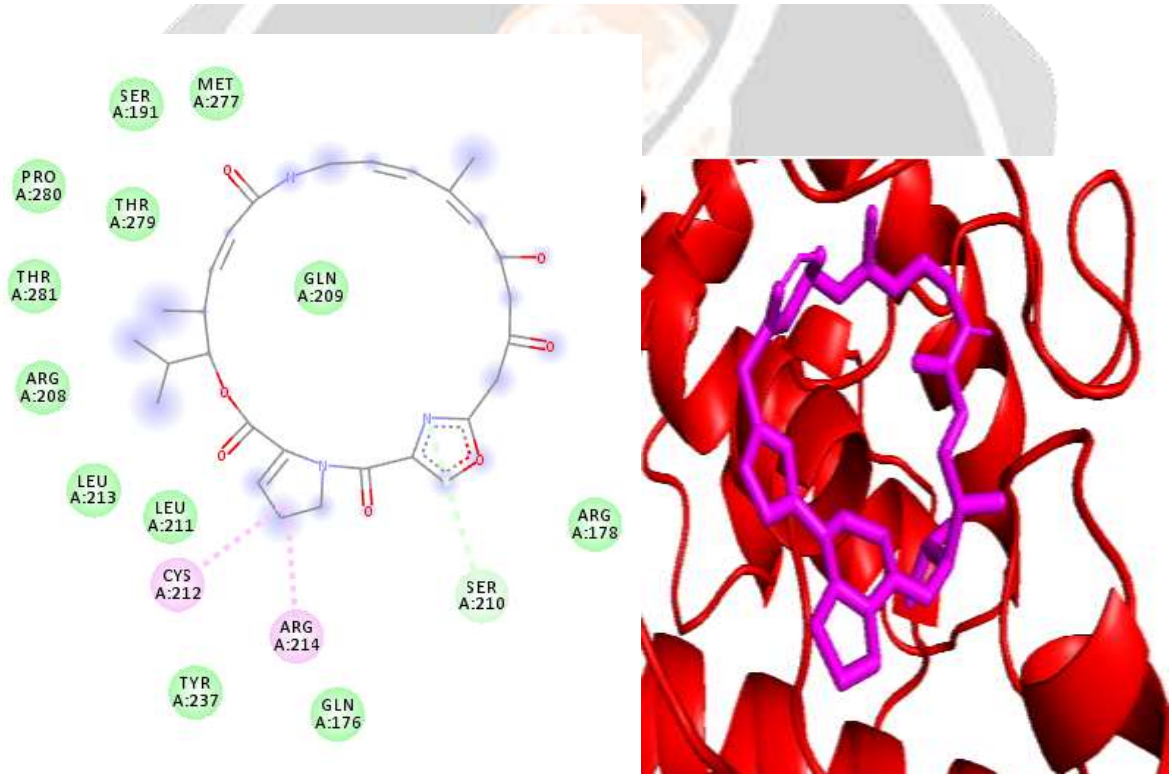


Figure 10: Visualization of interactions and binding region of pristinamycin2 with 3VUV

CONCLUSION

Virginiamycin belonging to the family of streptogramin-related depsipeptides is isolated from the bacterium Streptomyces virginiae. Virginiamycins. It consist of two major components, Virginiamycin M1 and Virginiamycin S1. It inhibits ribosome assembly, thereby preventing protein synthesis and is active against Gram-positive bacteria. This study identifies the potential of Pristinamycin in the treatment of malaria. Combination of Pristinamycin with

Azithromyicn which is already a known potentially effective inhibitor for malaria may be a supplement to its mechanisms of action. This study provides the basis for understanding the molecular basis of antimalarial activity of Pristinamycinand suggests a novel strategy for antimalarial drug development via advancing compounds with dual mechanisms of action.

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