

Role of Glycomimetic in treatment of malaria

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ABSTRACT

Background: Increased resistance to anti-malarial drugs has caused a havoc, tolling many lives. It has become an imminent need of hour is to find therapeutics drugs with novel mechanism of action. In the present study, we intended to study the invasion inhibitory potential of drugs belonging to the class of Glycomimetics.

Methodology: Molecular docking study was performed with eight drug molecules. 3-dimensional chemical structures of molecules were prepared through UCSF Chimera and Autodock Tools freeware. Further, Molecular docking study was performed using AutoDockVina, Discovery studio 4.5 was used to predict the active site of target sites and PyMol to visualise the induced fit docking.

Results: The preliminary experimental study demonstrated that Acarbose and Hyaluronic acid show good binding efficacy to all the target proteins of Plasmodium species. On the basis of molecular docking studies it can be suggested that Acarbose and Hyaluronic acid are potential inhibitors of merozoite invasion into erythrocytes. However, further in-vitro and in-vivo studies needs to be performed for further drug development process.

Keyword : - acarbose, merozoite, invasion inhibitor, ingress inhibitor, glycomimetic

INTRODUCTION

An insurmountable evidence about increasing drug-resistance in pathogens has resulted in the need for a lookout for alternative strategies to combat infection. Anti-adhesion therapeutics have caught the eye of many researchers. Unlike many other available strategies, anti-adhesion therapeutics possess the ability to attenuate infection at the very initial stages. For a pathogen to cause infection, it must first bind to the host cell or tissue.¹ The process of adhesion results from interactions between surface antigens located on pathogen surface and complementary glycans on the surface of the host cell. One of the major advantage of anti-adhesion therapy is that unlike traditional drug

therapies which become ineffective, due to evolutionary pressure that has been linked to mutation or phenotypic variation on the pathogen thereby leading to drug resistance, anti-adhesion drugs do not become ineffective as these do not kill or arrest the growth of a pathogen. Also, anti-adhesion drugs target more than one antigen, thus it is expected that resistance to anti-adhesion agents will occur more slowly than resistance to an antibiotic.³ Carbohydrates have been recommended as antiadhesives, as they act as excellent recognition molecules on cell surfaces and attenuate pathogens.⁴ However, native glycans lack strong affinity with pathogen receptors, thereby necessitating a high concentration of anti-adhesives to inhibit the pathogen infectivity. This property limits their ability as therapeutics.

In order to overcome the poor drug-like character of carbohydrates, a number of carbohydrates and carbohydrate-based small molecules called as “glycomimetics” that mimic glycan receptors structure and function, have been thoroughly studied in the development of therapeutic candidates for both lectins and enzymatic carbohydrate-processing proteins.^{5,6} These glycomimetics are advantageous because of enhanced affinities, increased bioavailability and longer serum half-lives. Few of the glycomimetic compounds possess additional interactions which are not present in the native counterpart, offering enhancements in both affinity and selectivity.

Currently, several glycomimetics have shown their potential in treatment of a number of diseases. A few examples are- Zanamivir that inhibits influenza viral neuraminidase in the pharyngeal mucosa,⁷ Voglibose,⁸ Miglitol,⁹ acarbose,¹⁰ that inhibit α -glycosidase enzymes in the small intestine thereby therapeutic in managing diabetes, Topiramate¹¹ used in the treatment of epilepsy, Miglustat¹² that has been used for the treatment of Gaucher's disease, Sodium hyaluronate, an established lubricant used in the treatment of osteoarthritis¹³ and Taurolidine¹⁴. Glycomimetics have also been used in the treatment of malaria.

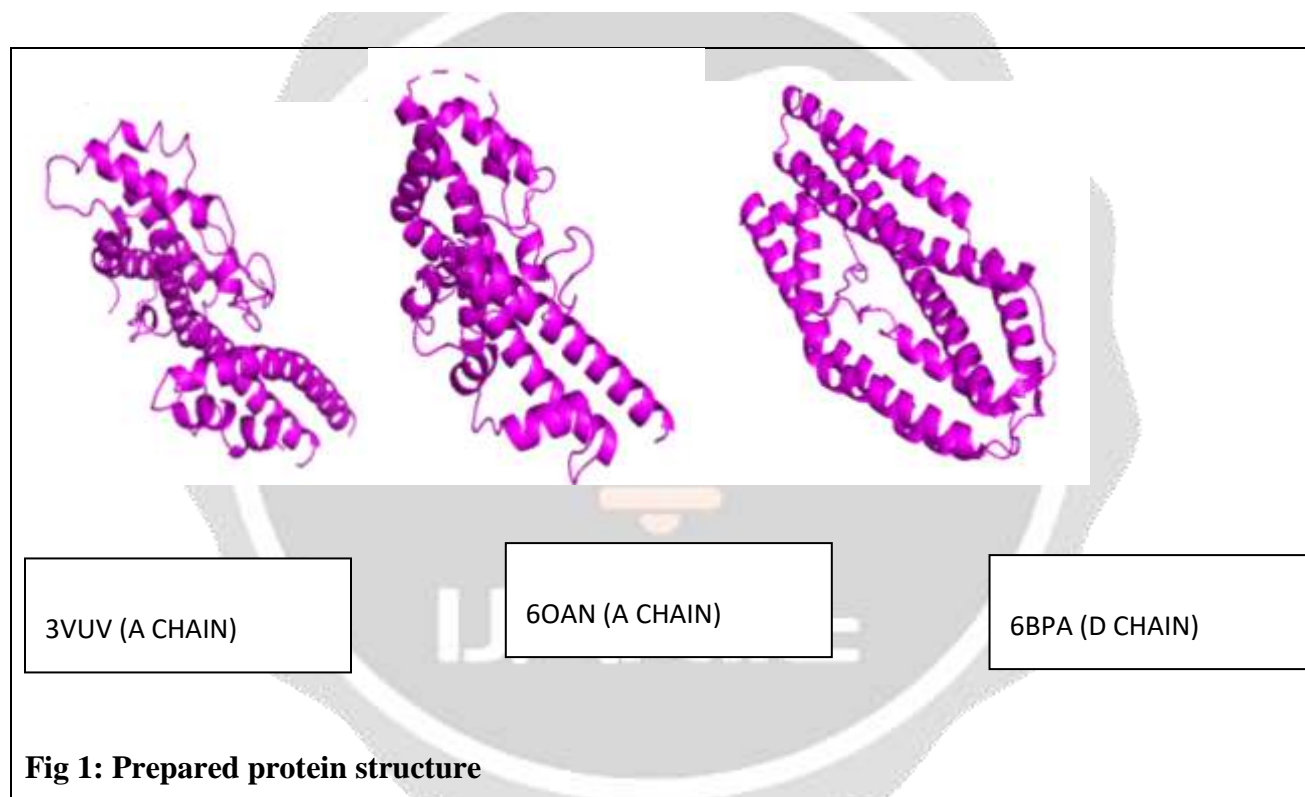
The anti-malarial role of glycomimetics is mainly due to their invasion inhibition potential explored in the in-vitro studies. In case of *Plasmodium falciparum* caused infection, initially low affinity, reversible interactions mediated by glycosylphosphatidyl inositol (GPI) anchored proteins present on the merozoite's surface and the RBC membrane^{15,16} are known to occur anywhere on the surface. Merozoite Surface Protein is the most abundant GPI anchored protein on the merozoite surface.¹⁷ MSP1-83 fragment binds to the RBC receptor glycoporphin A through its N-terminal. This is followed by proteolytic cleavage of MSP1 to 83 kDa, 30 kDa, 38 kDa and 42 kDa fragments. This cleavage is essential for RBC invasion.¹⁷ The 19 kDa C-terminal cysteine rich epidermal growth factor (EGF)-like domain of MSP-1 is formed after secondary cleavage of MSP1-42 and binds to Band-3 on the RBC surface to gain an entry inside the host-erythrocyte.¹⁸ MSP1-19 is the most essential proteolytic target that has been investigated as a potential vaccine and drug target.¹⁹

With this background in mind, we aimed at in-silico identification of parasite invasion potential of glycomimetic drugs against the epidermal growth factor (EGF)-like domain of merozoite surface proteins of *P.falciparum* and *P.vivax*.

Methodology

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.²⁰ Targets need to be prepared before starting the molecular docking process. Target protein preparation involves removal of the complexes bound to the protein receptor molecule, removal of the water molecules and finally adding polar hydrogen atoms were added into target. All these processes were carried out in the Auto Dock window execution file.



2.2. Ligand preparation

Investigational ligands were built using canonical smiles obtained from PUBCHEM, saved in.pdb format using UCSF Chimera²¹ and subsequently converted into.pdbqt format by Autodock tools.²² In the current study, identification of binding modes of the investigational ligands with target was identified using Auto Dock Vinasoftware program.²³ 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 and PyMol.^{24,25} 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.

Results

3.1. Binding energies

The binding energies of various ligands with the target proteins has been enlisted in Table 1. The binding energy of Acarbose, Hyaluronic acid and Zanamivir to the Duffy Binding Protein of *P. vivax* (PDBID :- 6OAN) were -6.7 kcal/mol, -6.5 kcal/mol and -4.9 kcal/mol. The binding energy of Acarbose and Hyaluronic acid to the active site is even smaller than that of the Zanamivir, indicating that Acarbose and Hyaluronic acid have a higher binding activity. However, regarding merozoite surface protein MSPDBL2 from *P. falciparum* (PDBID 3VUV) and Plasmodium vivax reticulocyte binding protein 2b (PvRBP2b) (PDBID: 6BPA), Acarbose and Hyaluronic acid have a binding energy equivalent to that of Zanamivir. From the overall binding energy analysis, it can be interpreted that Acarbose has strong interactions with the surface targets of both *P. falciparum* and *P. vivax*.

TABLE 1

| S. No. | Investigational Ligand | Merozoite surface protein MSPDBL2 from <i>P. falciparum</i> (PDBID 3VUV) | Duffy Binding Protein of <i>P.vivax</i> (PDBID :- 6OAN) | Plasmodium vivax reticulocyte binding protein 2b (PvRBP2b) (PDBID :6BPA) |
|--------|------------------------|--|---|--|
| 1 | Acarbose | -6.8 | -6.7 | -6.8 |
| 2 | Hyaluronic acid | -6.4 | -6.5 | -6.2 |
| 3 | Miglitol | -5.2 | -6.2 | -4.9 |
| 4 | Osetamivir | -5.2 | -3.8 | -4.7 |
| 5 | Voglibose | -5.9 | -5.3 | -5.2 |
| 6 | Topiramate | -6.1 | -5.6 | -5.9 |
| 7 | Tauromycin | -6.5 | -5.9 | -5.3 |
| 8 | Zanamivir | -6.5 | -4.9 | -6.4 |

3.2. Molecular Docking with 3VUV

Molecular docking results rendered by Discovery suite 4.5 in Fig.2 demonstrated that Acarbose forms Hydrogen bonds with Gln 176, Arg 178, Arg 208 and Gln 209. On the other hand, Fig.3 demonstrates Hyaluronic acid forms Hydrogen bonds with Leu 211, Thr 279 and Thr 281.

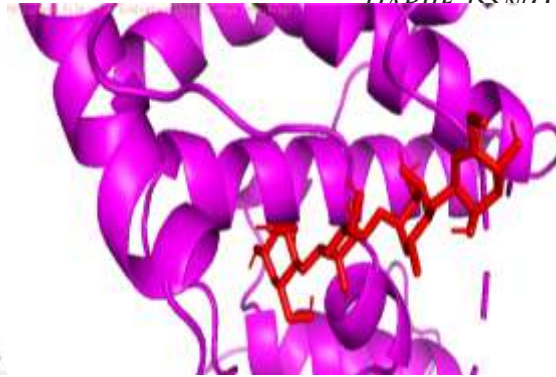
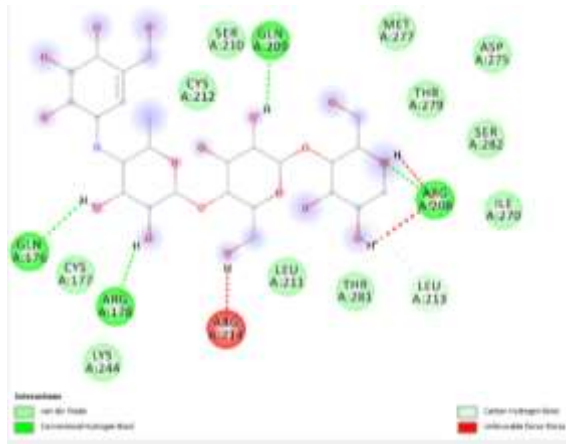


Fig 2: Visualization of interactions and binding region of acarbose with 3vuv

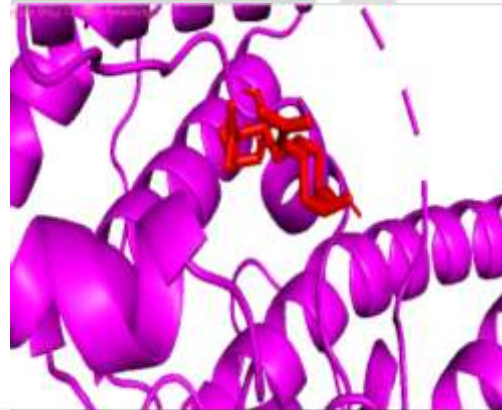
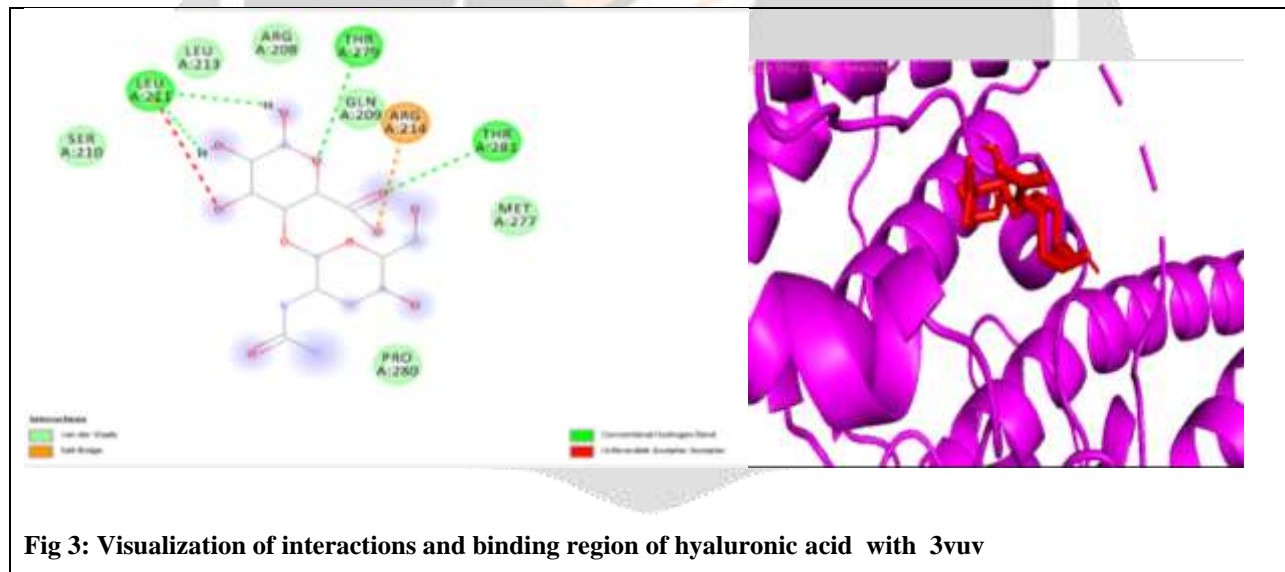
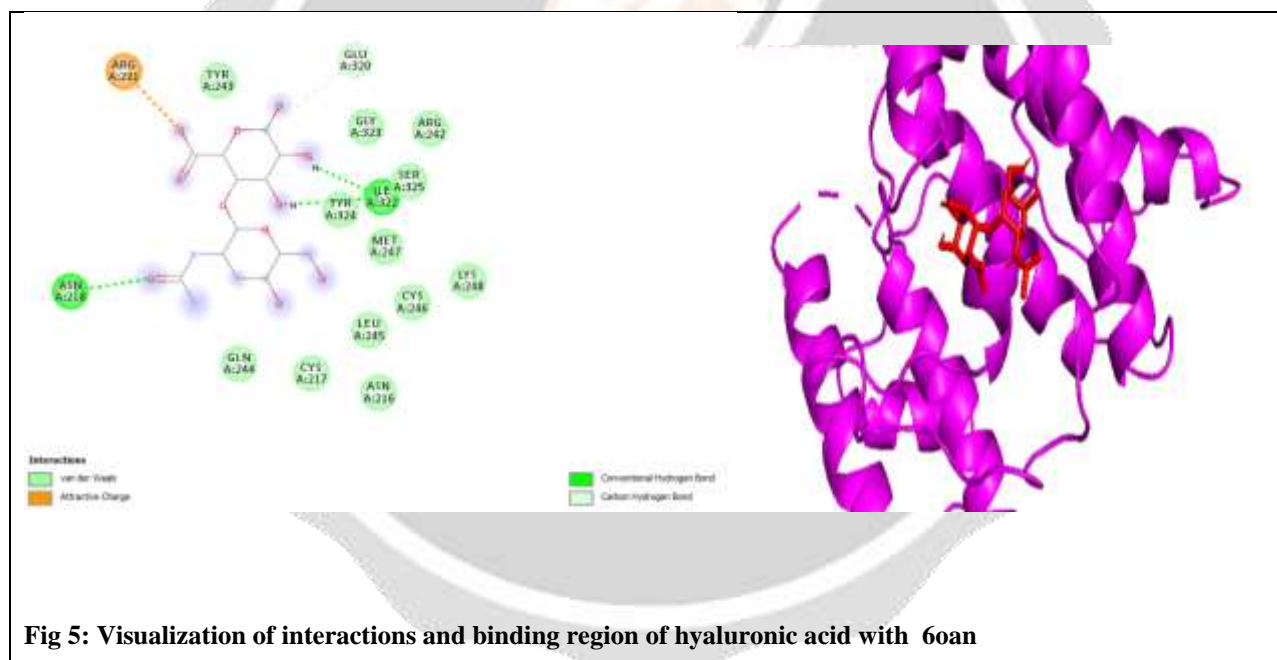
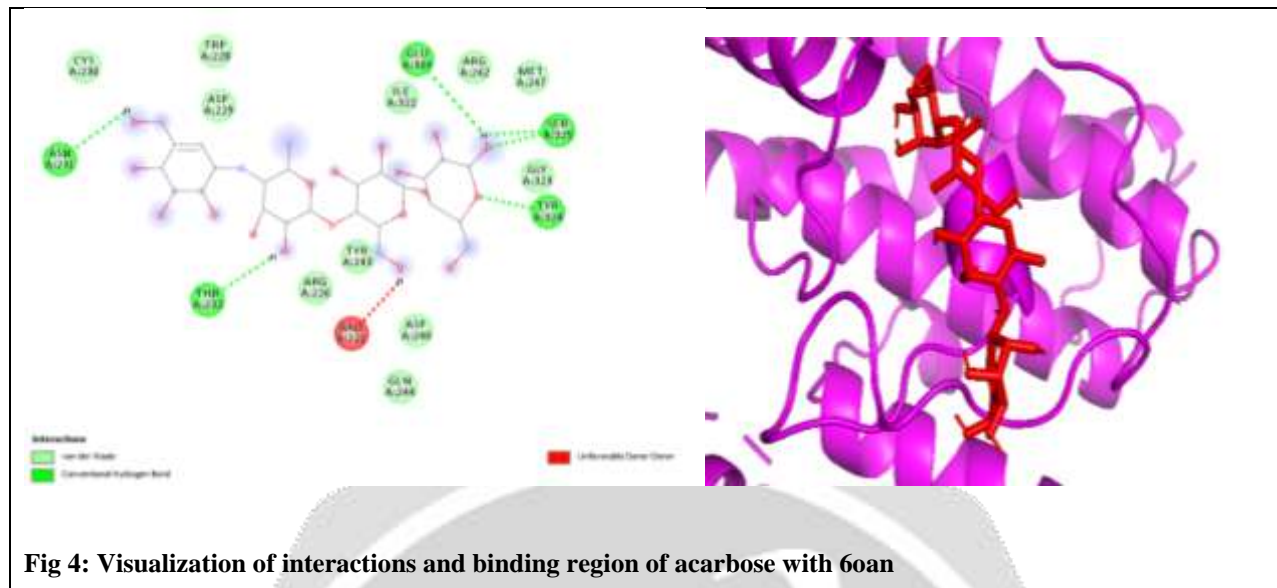


Fig 3: Visualization of interactions and binding region of hyaluronic acid with 3vuv

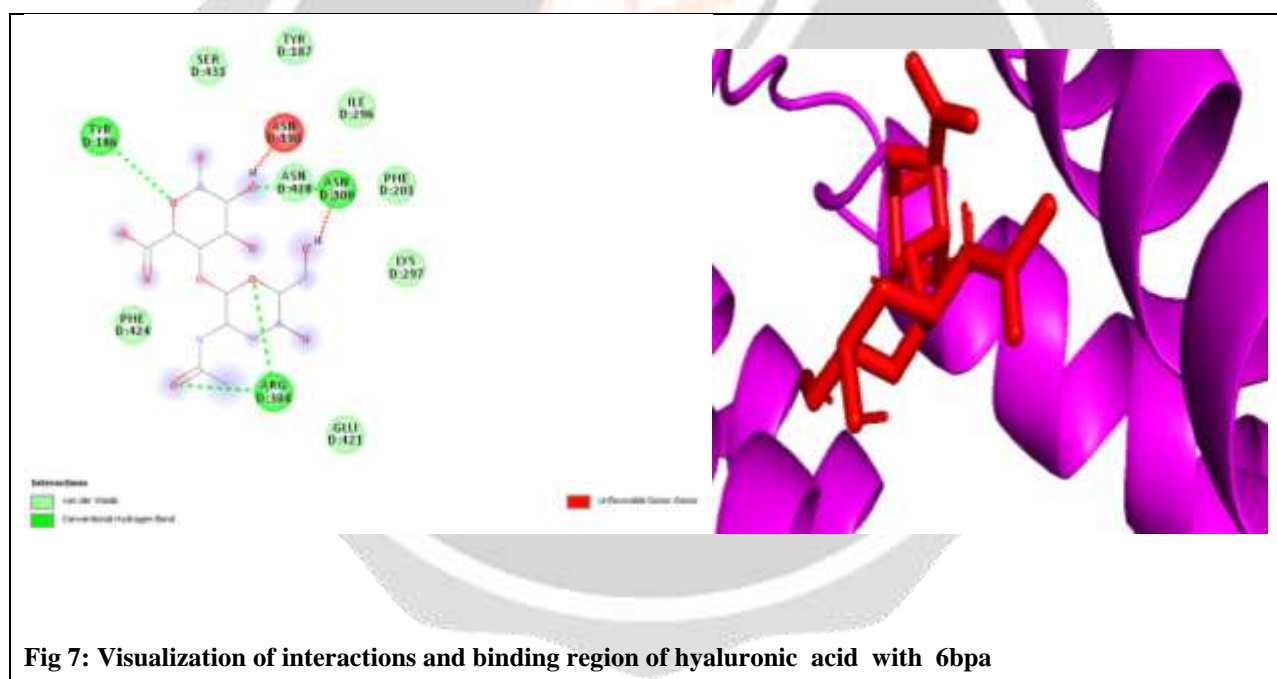
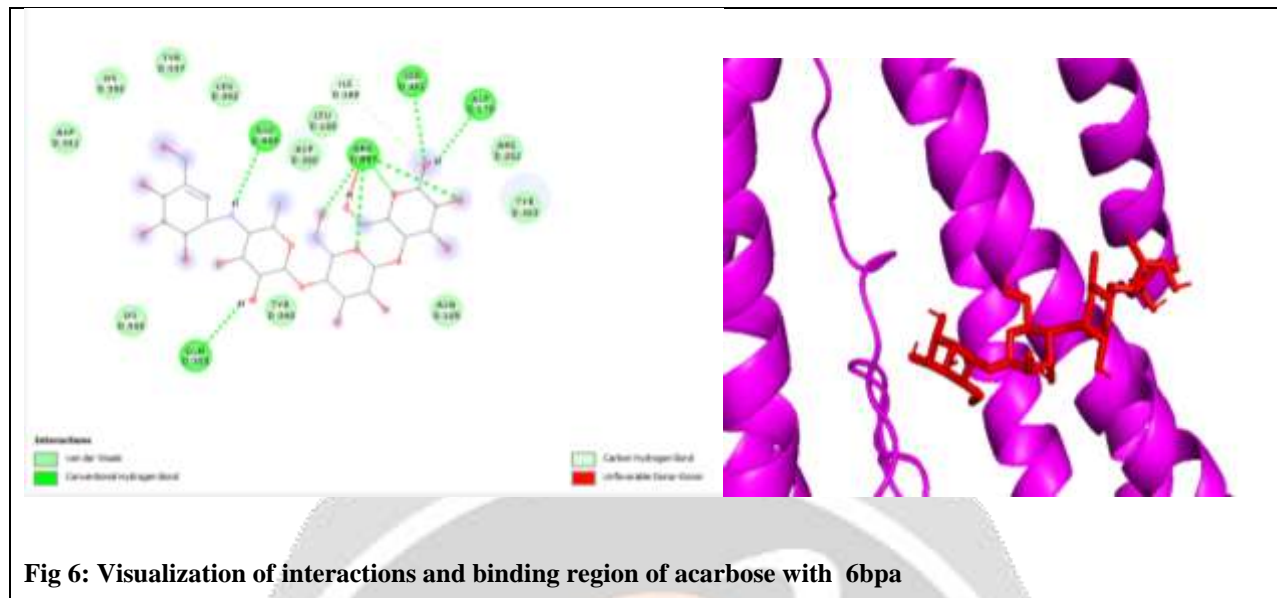
3.3 Molecular docking with 6OAN

Fig.4 depicts that Acarbose forms Hydrogen bonds with Asn 231, Thr 232, Glu 320 Tyr 324, Ser 325. From Fig.5, it can be interpreted that Hyaluronic acid forms Hydrogen bonds with Asn 218 and Ile 322.



3.4 Molecular docking with 6BPA

From Fig.6, it can be suggested that Acarbose forms Hydrogen bonds with Asp 179, Ser 181, Arg 387, Glu 389 and Gln 393. Fig.7 depicts that Hyaluronic acid forms Hydrogen bonds with Tyr 186, Asn 300 and Arg 304.



Discussion and Conclusion

For an effective malaria elimination, new approaches to drug and vaccine design is pertinent. Herein the present study, we undertook molecular docking as a novel, comprehensive approach towards the understanding effect of glycomimetics in inhibiting Plasmodium merozoite invasion and further development by interfering obligate cellular interactions between the parasite and the erythrocyte. Based on relative binding energies and molecular docking, it was observed that not all glycomimetics, compared to Acarbose and Hyaluronic acid, bind to merozoite surface

proteins with equal affinity. Also, we inferred from the results that Acarbose and Hyaluronic acid profoundly inhibit a key step of parasite development, thereby abrogating downstream events necessary for establishing a clinical infection.

This study identifies the potential of Acarbose in the treatment of malaria. Combination of Acarbose with a known conventional anti-malarial drug may be a supplement to its mechanisms of action. This intervention study provides the basis for understanding the molecular basis of antimalarial activity of Acarbose and suggests a novel strategy for antimalarial drug development via advancing combination with dual mechanisms of action.

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