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Research Article

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Designing and Virtual Screening of Potential Inhibitors of *PFHGPRT* against Malaria

Preeti Verma[1*], Dev Bukhsh Singh[2] and Ajay Kumar Gupta[1]

[1]*University Institute of Pharmacy, Chhatrapati Shahu Ji Maharaj University, Kanpur (U.P.), India-208024*
[2]*Departments of Biotechnology, Institute of Biosciences and Biotechnology, Chhatrapati Shahu Ji Maharaj University, Kanpur, India-208024*

ABSTRACT

Plasmodium falciparum is dangerous malarial species and most burdensome form of human malaria, affecting 200–300 million individuals per year worldwide, also because of its high rate of resistance outbreaks; there is a constant need for the discovery of novel antimalarials and drug targets. The three-dimensional structures of PFHGPRT, HSHGPRT and TCHGPRT were used for comparative docking study, while two inhibitors 6-(2, 2-Dichloroacetamido) chrysene and GMP-2', 3'-dialdehydewere used as lead for designing and discovery of potential inhibitor of PFHGPRT with the help of Chimera and Molegro Virtual Docker. ZINC Pharmer and FAF-Drugs3 were used for pharmacophore and ADMET studies respectively. Results of structural superimposition and structural difference indicated that selective inhibitor of PFHGPRT could be designed. HGPRT inhibitor 6-(2,2-dichloroacetamido) chrysene had shown more affinity for PFHGPRT than HSHGPRT and total 87 compounds used for screening (obtained through Pharmacophore based searching) were then virtually screened against the target PFHGPRT and on the basis of Moldock scoring two compound ZINC00226974 and ZINC00268007 were finally selected. Further studies clearly indicated that ZINC00226974 has more strong binding and thus will offer better PFHGPRT inhibition. Thus further synthesis, preclinical/clinical studies of such PFHGPRT inhibitors could help in controlling malaria more effectively.

Keywords: PFHGPRT, HSHGPRT, Docking, Malaria, Virtual screening

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INTRODUCTION

Malaria is a life-threatening blood disease caused by various species of protozoan parasite *Plasmodium* such as *Plasmodium falciparum, Plasmodium vivex, Plasmodium malari, Plasmodium ovate,* and *Plasmodium knowelsi*; which is transmitted to humans through the bite of the *Anopheles* mosquito. Once an infected mosquito bites a human and transmits the parasite, the parasites multiply in the host's liver before infecting and destroying red blood cells (RBCs) [1]. The development of resistance against commonly used antimalarials necessitates the search for novel chemotherapeutic targets and drugs against the disease. Malaria could be controlled and treated if diagnosed early on; unfortunately, this may also not be possible, as many areas of the world, where malaria outbreaks can occur, lacking the medical facilities. Therefore, researchers are working hard on improving the early diagnosis, treatment and also prevention of malarial infection[2].Among all malarial parasite *Plasmodium falciparum* is dangerous species and most burdensome form of human malaria[3].Clinical manifestations of *Plasmodium falciparum* infection are induced by the asexual stages of the parasite that develop inside the RBCs. Malaria researchers have won multiple Nobel Prizes for their achievements, although the disease continues to afflict some 200-300 million people each year worldwide. Because of its high rate of resistance and outbreaks, there is a constant need for the discovery of novel antimalarial and drug targets[4].The field of structure-based drug design is a rapidly growing area of research, in which many successes have occurred in recent years. The explosion of genomic,

proteomic, and structural information has provided hundreds of new targets and opportunities for future drug lead discovery [5].

Purines are essential molecules for all living organisms. Hypoxanthine guanine phosphoribosyl transferase (HGPRT) is necessary for purine nucleotide as it catalyse the conversion of 6-oxopurine bases to their respective nucleotides [hypoxanthine to inosine monophosphate (IMP) and guanine to guanosine monophosphate (GMP) from the purine bases hypoxanthine and guanine respectively, utilizing 5'-phosphoribosyl-1-pyrophosphate (PRPP) as a Co-substrate], and hence essential in *Plasmodium falciparum* as well as in humanfor nucleic acid [6,7].Purine containing nucleotides are the building blocks of nucleic acids (DNA and RNA), and purine bases are constituents of enzyme cofactors (e.g. NAD+, FAD), sources of chemical energy (e.g. ATP, GTP) or signalling molecules (e.g. cAMP). Thus, selective inhibition of the enzymes HGPRT of parasite vs humanare likely to be required as one of novel approach for treatment of malaria. In the present study, designing and virtual screening of PFHGPRT(*Plasmodium falciparum* Hypoxanthine guanine phosphoribosyl transferase) inhibitors could help in guiding medicinal chemists to improve target specificity for antimalarial chemotherapy[8].

EXPERIMENTAL SECTION

2.1 Retrieval and Preparation of Molecules

The three-dimensional structure of PFHGPRT (3OZG), HSHGPRT (4RAQ) and TCHGPRT (1P19) were retrieved from the Protein Data Bank (www.rcsb.org). PDB (Protein Data Bank) file of PFHGPRT (3OZG) contains a complex of PFHGPRT with S-SerMe-ImmH phosphonate, PYROPHOSPHATE 2, MAGNESIUM ION and has four chains A, B, C and D. The chain A (228 amino acid residues) of PFHGPRT was separated from its complex structure and was used for docking study. Similarly, Chain A (217 amino acid residues) of HSHGPRT(Human Hypoxanthine guanine phosphoribosyl transferase) was separated from its complex structure and was used for comparative docking study. Inhibitor 6-(2,2-Dichloroacetamido)chrysene has been reported to interact with the HGPRT of *Trypanosoma cruzi*[9]. One another inhibitor GMP-2,3-dialdehyde binds selectively to HGPRT of *Schistosoma mansoni* than HSHGPRT[10]. The two inhibitors 6-(2, 2-Dichloroacetamido) chrysene (CID:276270) and GMP-2', 3'-dialdehyde (CID: 128285) has been retrieved from PubChem database. These two inhibitors were known to actively inhibit the HGPRT of *Trypanosoma cruzi* and *Schistosoma mansoni*. Therefore, these inhibitors were used as lead for designing and discovery of potential inhibitor of PFHGPRT.

2.2 Structural Superimposition

UCSF Chimera (or simply Chimera) is an extensible program for interactive visualization and analysis of molecular structures and related data, including density maps, supramolecular assemblies, sequence alignments, docking results, and conformational ensembles [11].High-quality images and animations can be generated. 3D structure of PFHGPRT was structurally aligned with HSHGPRT and TCHGPRT. The main objective behind this was to explain the regions of structural similarity and dissimilarity. Finally, the structural differences between two structures were measured in terms of (root mean square differences) RMSD.

2.3 Docking Approach

Docking tools predicts the binding mode of a ligand within the constraints of a receptor binding site, and to correctly estimate the strength of binding. Molegro Virtual Docker (MVD) 2007.2.0.0 was used for docking study. MVD requires a 3D structure of both protein and ligand. MVD performs flexible ligand docking, so the optimal geometry of the ligand is determined during the docking [12]. The candidates with the best conformational and energetic results were selected. MVD was used to calculate the interaction energies between ligands and macromolecular systems from the 3D structures of the protein and ligands. The algorithm used was the MolDock Score, an adaptation of the Differential evolution algorithm. MVD was used for docking of inhibitor and other ligands with PFHGPRT and HSHGPRT.

2.4 Pharmacophore Analysis

ZINCPharmer is an online interface for searching the purchasable compounds of the ZINC database using the pharmacophore search technology. A pharmacophore describes the spatial arrangement of the essential features of an interaction. Compounds that match a well-defined pharmacophore serve as potential lead compounds for drug discovery. ZINCPharmer provides tools for constructing and refining pharmacophore hypotheses directly from molecular structure. The results can be immediately viewed, or the aligned structures may be downloaded for off-line analysis. ZINCPharmer enables the rapid and interactive search of purchasable chemical space[13].

2.5 FAF-Drug3 analysis

FAF-Drugs3 performs various physicochemical calculations, identifies key functional groups, some toxic and unstable molecules/functional groups. In addition to filtered collections, FAF-Drugs3 can provide, via Gnuplot,

several distribution diagrams of major physicochemical properties of the screened compound libraries[14].FAF-Drugs3, a web server that can be used for drug discovery and chemical biology projects to help in preparing compound libraries and to assist decision-making during the hit selection/lead optimization phase. FAF-Drugs can filter or analyse molecules with user-defined or eight predefined physicochemical filters as well as with several simple ADMET (absorption, distribution, metabolism, excretion and toxicity) rules. FAF-Drugs3 offers access to user-friendly html result pages and the possibility to download all computed data. The server requires as input an SDF file of the compounds.

RESULTS AND DISCUSSION

3.1 Structural superimposition

3-D structure of PFHGPRT was aligned with HSHGRT to measure the extent of structural variation. Superimposition of these two structures has generated RMSD (root mean square deviation) of 0.99 Å over 170 atom pair. The pair-wise alignment score between PFGHGPRT and HSHGPRT was obtained 575.7.Some structural differences were observed at N-terminal region where 12 residues of PFHGPRT aligned with gap region in HSHGPRT. In middle region, indel/gap regions were seen in alignment of HSHGPRT with PFHGPRT. Residues Ser121 and Tyr116 of PFHGPRT participate in binding site and were seen absent in HSHGPRT. This indicates that there is difference in the composition of binding site residues in both the targets. Difference in the binding site suggests that selective inhibitor of PFHGPRT can be designed. Structural difference between these two enzymes in terms of RMSD also supported the designing of selective inhibition for PFHGPRT.

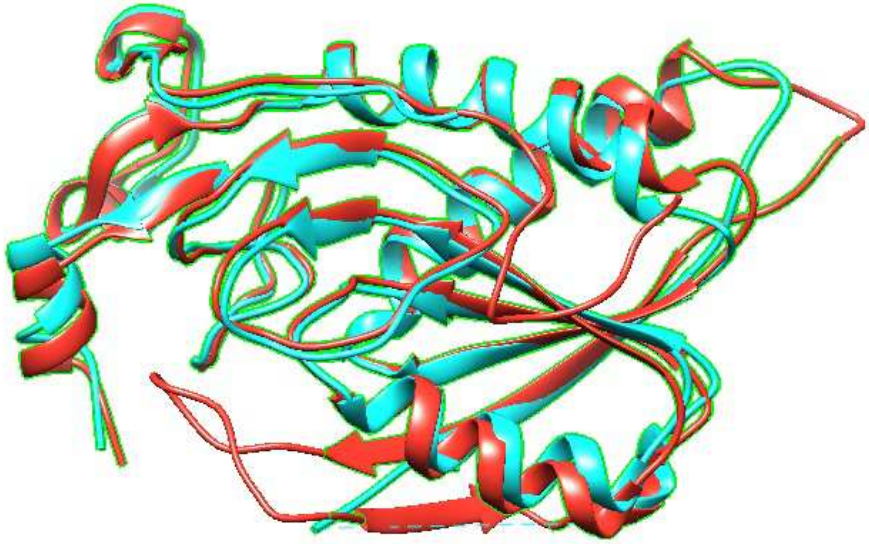


Fig.1: Structural alignment view of PFHGPRT (red) and HSHGPRT (blue)

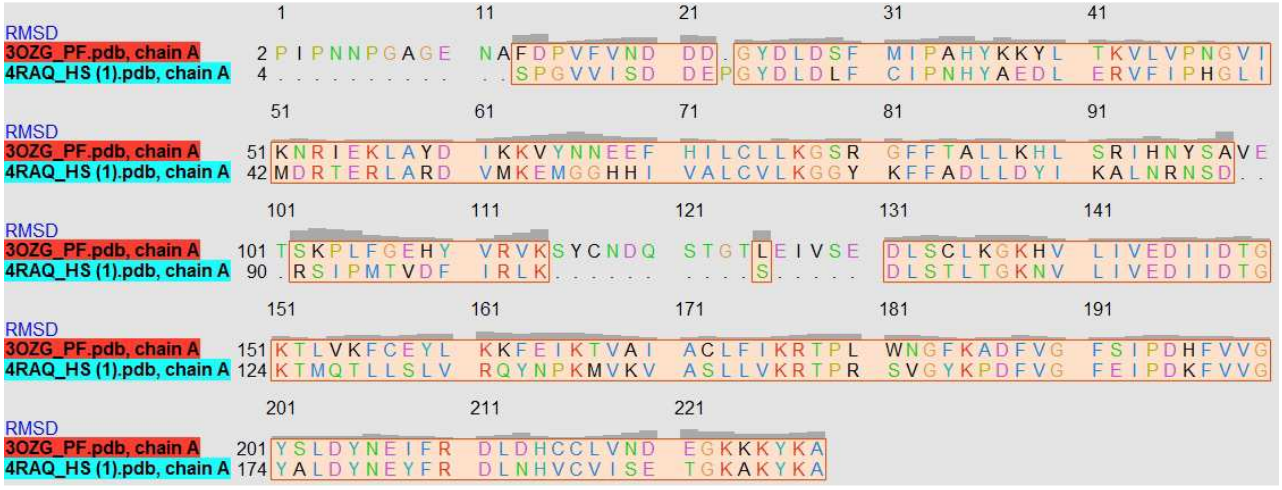


Fig.2: Pair-wise alignment of PFHGPRT with HSHGPRT highlighting regions of similarity within boxes and variations by indel or gap

Further 3-D structure of PFHGPRT was aligned with TCHGRT to measure the extent of structural variation. Superimposition of these two structures has generated RMSD (root mean square deviation) of 0.942 Å over 133 atom pair. The pair-wise alignment score between PGHGPRT and TCHGPRT was obtained 292. Some structural differences were observed at N-terminal region where 28 residues of PFHGPRT aligned with gap region in TCHGPRT. In middle region, indel/gap regions were seen in alignment of HSHGPRT with TCHGPRT. This indicates that there is difference in the composition of binding site residues in both the targets. Difference in the binding site suggests that selective inhibitor of PFHGPRT can be designed. PFHGPRT is more structurally dissimilar to TCHGPRT as compared to HSHGPRT. Tough there is enough structural variation between PFHGPRT and HSHGPRT, but there exists some possibility to design a single potential inhibitor that can be used as a drug against both pathogen.

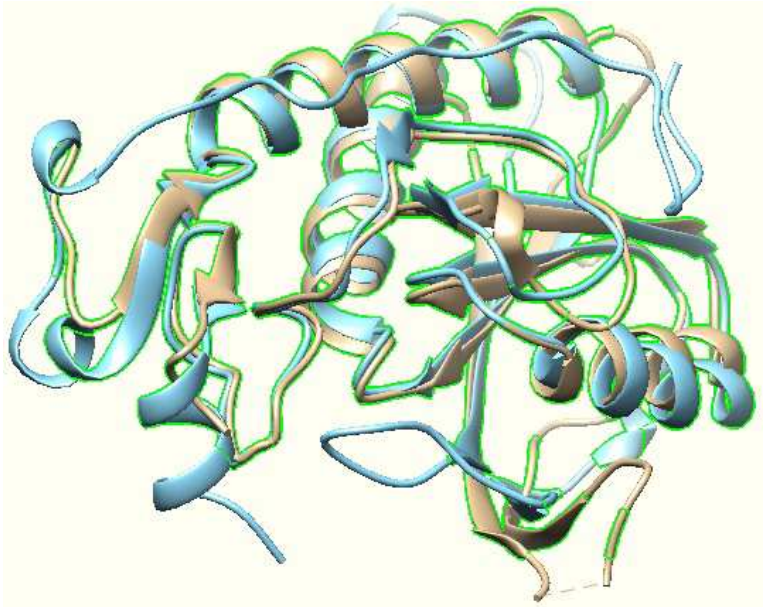


Fig.3: Structural superimposition of PFHGPRT (blue) with TCHGPRT (brown)

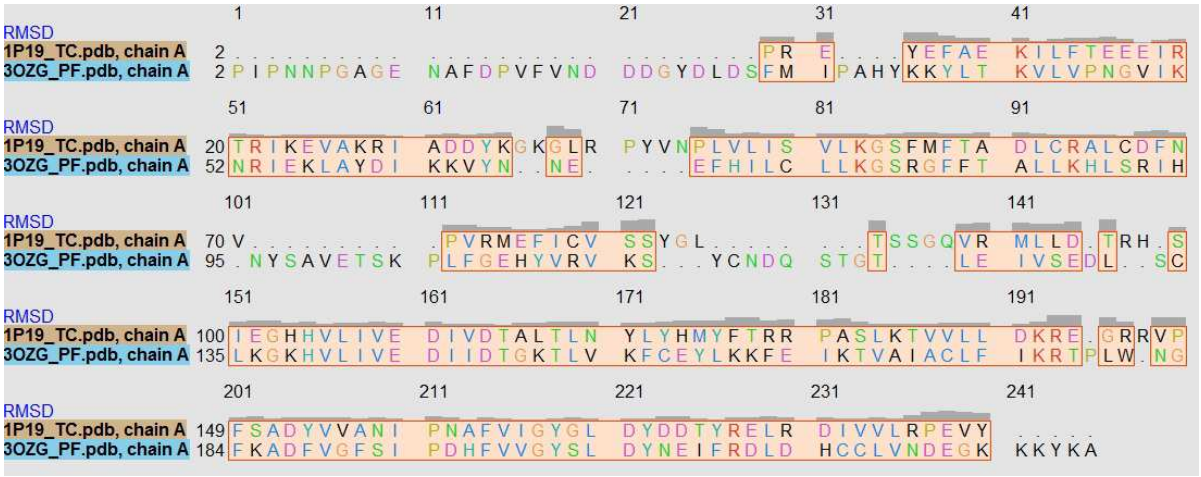


Fig.4: Pair-wise alignment of TCHGPRT with PfHGPRT showing region of structural variations

3.2 Prediction of binding site

Binding sites and active sites of proteins are commonly related with structural pockets and cavities. It helps in identification and measurements of surface accessible regions (pockets/cavitis) and interior inaccessible cavities for HGPRT. Cavity detection algorithm is used dynamically for finding the cavities by search algorithm (guided deferential evolution) to focus the search during the docking simulation. The top five cavities present in PFHGPRT were predicted. The largest cavity (cavity-1) having volume of 168.96 is shown in Fig.5. In majority of cases, cavity with the largest size and volumes is associated with binding site [15, 16]. Largest Cavity1 is associated with the binding of PFHGPRT which provide strong background for cavity1to serve as binding site. The amino acid residues Val133, Arg112, Ser121, Tyr116, Thr149, Tyr77, Glu207, Leu180, Gly78, Arg210, Asp204, Asp148, Arg80, Ser79, Ser202, Arg145 and Asp145 were associated with largest cavity. Thus largest cavity has indicated good binding activity than others.

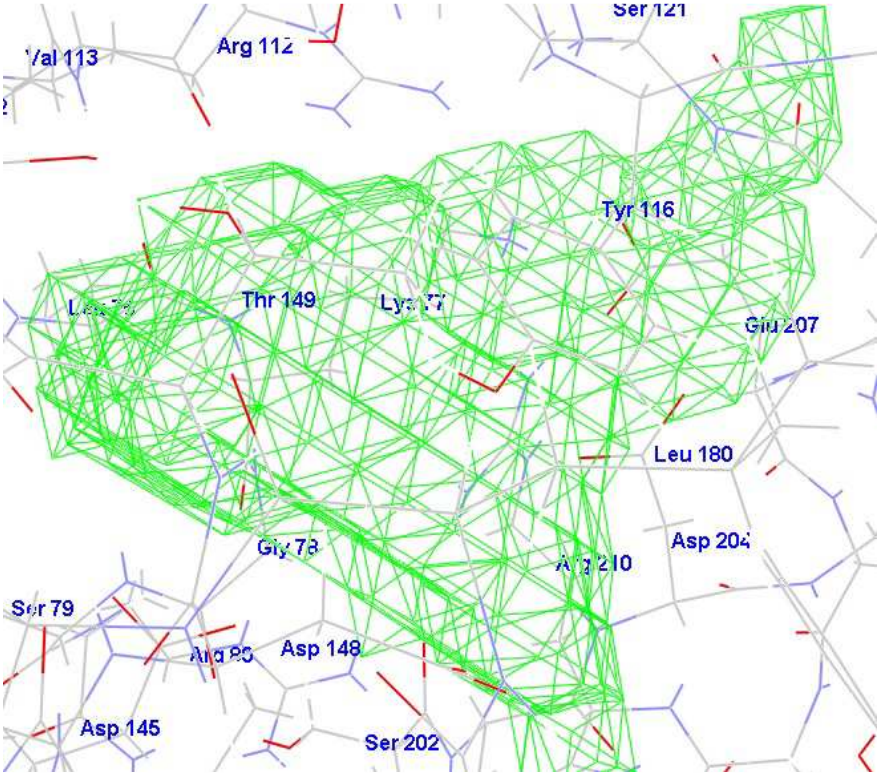


Fig.5: Largest cavity of PFHGPRT shown in green mesh representation with compositional residues

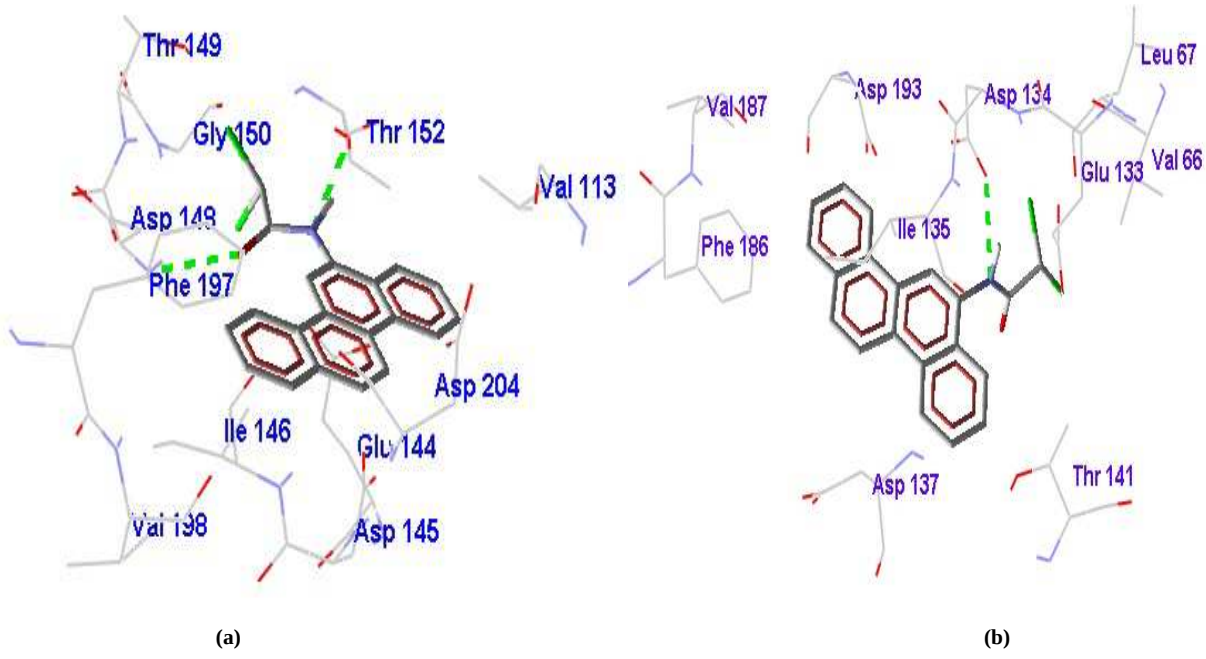
3.3 Docking simulation study

Molegro Virtual Docker (MVD) 2007.2.0.0 (Thomsen and Christensen 2006) was used for flexible docking study. MVD requires a 3D structure of both protein and ligand. Docking simulation study was carried out to recognize the inhibiting potential of a ligand against PFHGPRT enzyme. Compound 6-(2,2-Dichloroacetamido) chrysene[17] and GMP-2',3'-dialdehyde were docked with both targets PFHGPRT and HSHGPRT. Motivation behind this was to evaluate the comparative affinity and binding interaction of inhibitors for both the targets. MVD visualizer was used for interaction site analysis. The interaction analysis for binding of 6-(2,2-Dichloroacetamido) chrysene with PFHGPRT has been done to find out the residues that are involved in binding interaction. 6-(2,2-dichloroacetamido) chrysene(CID: 276270) shows good affinity (MolDock score -129.97Kcal/mol) for binding with PFHGPRT and it interacts with Asp148, Thr152, Thr149, Gly150, Ile146, Phe197,Val198, Asp204, Asp145, Glu144 and Val113 residues of PFHGPRT. It also forms two hydrogen bond with Asp148, Thr152 and have hydrogen bond energy (-2.93 Kcal/mol). The 6-(2,2-dichloroacetamido) chrysene forms a complex with HSHGPRT with a MolDock score of -94.04 Kcal/mol. This inhibitor has shown interaction with Val187, Phe186, Ile135, Asp137, Asp193, Asp134, Glu133, Thr141, Val66 and Leu67 residues of HSHGPRT and forms a single hydrogen bond with Asp134. 6-(2,2-dichloroacetamido) chrysene has shown more affinity for HGPRT target of *Plasmodium falciparum* than human HGPRT.

Table 1: Docking of 6-(2,2-Dichloroacetamido) chrysene and GMP-2',3'-dialdehyde with PFHGPRT and HSHGPRT

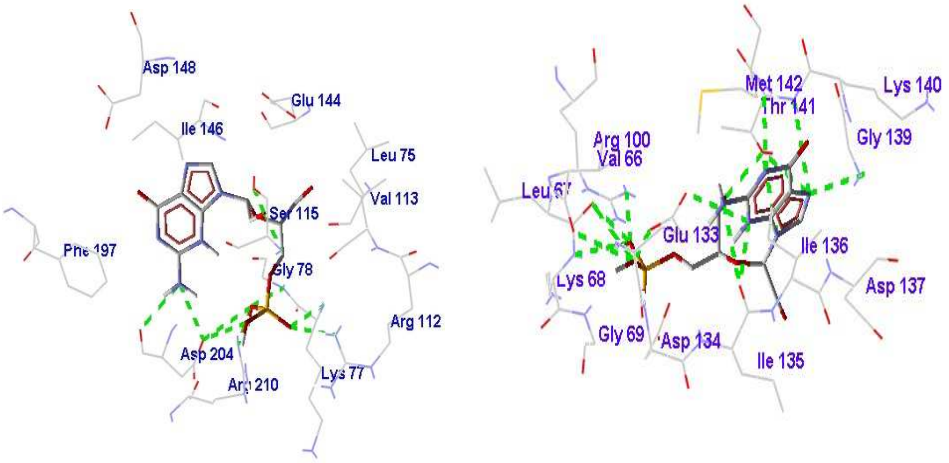
Compound s	MolDock score	Hydrogen bond	Residue in interaction	H bonding residue	H bonds
6-(2,2-Dichloroacetamido) chrysene with PFHGPRT	-129.97	-2.93	Asp148, Thr152, Thr149, Gly150, Ile146, Phe197, Val198, Asp204, Asp145, Glu144, Val113	Asp148, Thr152	2
6-(2,2-Dichloroacetamido) chrysene with HSHGPRT	-94.04	-2.07	Val187,Phe186,Ile135, Asp137,Asp193, Asp134, Glu133, Thr141, Val66, Leu67	Asp134	1
GMP-2',3'-dialdehyde with PFHGPRT	-155.62	-19.97	Leu75, Lys77, Glu78, Arg112, Val113, Ser115, Glu144, Ile146, Asp148, Phe197, Asp204, Arg210	Leu75, Lys77, Glu78, Ser115, Asp204, Arg210	6
GMP-2',3'-dialdehyde with HSHGPRT	-132.89	-29.27	Gly139, Asp137, Arg100, Leu67, Lys140, Glu133, Thr141, Met142, Ile136, Ile135, Asp134, Val66, Lys68, Gly69	Gly139, Arg100, Leu67, Lys140, Glu133, Thr141, Met142, Ile136, Asp134, Val66, Lys68, Gly69	12

Docking complex of GMP-2',3'-dialdehyde (CID: 128285) with PFHGPRT has shown a MoDock score of -155.62 Kcal/mol. It interact with Leu75, Lys77, Glu78, Arg112, Val113 Ser115, Glu144, Ile146, Asp148, P1he197, Asp204, Arg210residues of PFHGPRT and forms six hydrogen bond withLeu75, Lys77, Glu78, Ser115, Asp204, Arg210.A significant contribution of hydrogen bond energy (-19.97 Kcal/mol.) was observed in stabilizing the docking complex of GMP-2',3'-dialdehyde with PFHGPRT. Docking results indicate the interaction of GMP-2',3'-dialdehyde with Gly139,Asp137, Arg100,Leu67,Lys140,Glu133,Thr141, Met142,Ile136,Ile135,Asp134,Val66, Lys68 and Gly69 residues of HSHGPRT and forms hydrogen bonding with Gly139, Arg100, Leu67, Lys140, Glu133, Thr141, Met142, Ile136, , Asp134, Val66, Lys68 and Gly69 residues of HSHGPRT ,twelve hydrogen bond with a hydrogen bond energy of -29.27 Kcal/mol.GMP-2',3'-dialdehyde forms a favourable low energy complex with PFHGPRT than HSHGPRT (-132.89 Kcal/mol). GMP-2',3'-dialdehyde has shown less affinity for HGPRT target of *Plasmodium falciparum* than human HGPRT



(a) **(b)**

Fig.6:(a)Docked view of6-(2,2-Dichloroacetamido)chrysene with PFHGPRT (b)Docked view of 6-(2,2-Dichloroacetamido)chrysene with HSHGPRT. Hydrogen bonding interactions has been represented by green dash line



(a) **(b)**

Fig.7:(a)Docked view of GMP-2',3'-dialdehyde with PFHGPRT; (b)Docked view of GMP-2',3' dialdehyde with HSHGPRT

3.4 Pharmacophore based searching

Pharmacophore features extracted from 6-(2,2-Dichloroacetamido) chrysene was used as searching parameter for finding other potential compounds. And finally, 6 hits to this query search were obtained and used as query to find similar chemical substances from PubChem database. Out of which,4 compounds have not shown any result in chemical search. Remaining two compounds ZINC01717024 and ZINC01701552 with RMSD of 0.299 Å and 0.339Å have shown hits in PubChem search. SDF of 45compounds were downloaded from PubChem that shown 80% similarity with ZINC01717024.Similarly, 42 molecules similar to ZINC01701552 were obtained and

downloaded on 80% similarity search. These total 87 compounds were then virtually screened against the target PFHGPRT with MVD tool based on value of MolDock score. Finally top 10 scoring compounds were selected for further analysis-

ZINC00241993, ZINC00282113, ZINC00314165, ZINC00345460, ZINC00399318, ZINC00137583, ZINC00167830, ZINC00226974, ZINC00268007 and ZINC00446132 in which, ZINC00226974 and ZINC00268007 have shown better MolDock score than others,so estimated physicochemical calculations by using FAF Drug software.

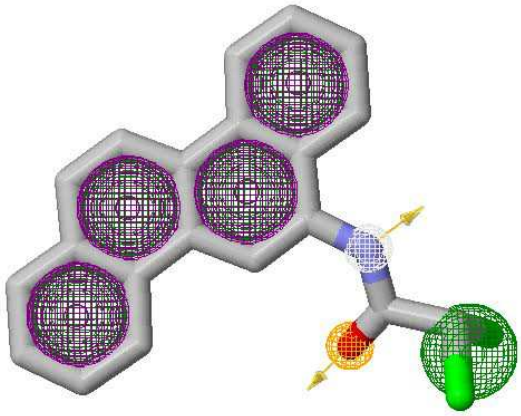


Fig.8: Pharmacophoric features of 6-(2,2-Dichloroacetamido)chrysene, four blue circle indicate-aromatic ring, yellow boll indicate-H acceptor, white boll indicate- H donor and dark blue circle indicate-hydrophobic

Table 2: Predicted pharmacophore properties of compound6-(2,2-Dichloroacetamido)chrysene

Pharmacophore class	X	Y	Z	Radius
Aromatic	0.02	0.82	0.10	1.10
Aromatic	-2.43	0.50	-0.04	1.10
Aromatic	1.07	3.05	0.04	1.10
Aromatic	-3.48	-1.73	0.01	1.10
H Acceptor	2.21	-2.42	-0.73	0.50
H Donor	2.55	-0.35	0.33	0.50
Hydrophobic	0.02	0.82	0.10	1.00
Hydrophobic	-2.43	0.50	-0.04	1.00
Hydrophobic	1.07	3.05	0.04	1.00
Hydrophobic	-3.48	-1.73	0.01	1.00
Hydrophobic	4.67	-2.50	0.02	1.00

3.5 Virtual screening

On the basis of MolDock scoring two compounds (Table 3)(ZINC00226974 andZINC00268007) were finally selected for virtual screening. The interaction analysis for ZINC00226974 binding of 6-(2,2-Dichloroacetamido) chrysene with PFHGPRT has been done to find out the residues that are involved in binding interaction[18], good affinity (MolDock score -146.534Kcal/mol) was obtained for binding with PFHGPRT and it interacted with Asp204, Arg210, Phe197, Leu203, Tyr116, Gly78, Lys77, Arg112, Ser115, Leu76, Asp145, Ile146, Val113 and Leu 75 residues of PFHGPRT, it also formed five hydrogen bond with(-15.4062Kcal/mol) hydrogen bond energy. Similarly for ZINC00268007 binding of 6-(2,2-Dichloroacetamido) chrysene with PFHGPRT interacted with Asp204, Phe19, Leu203, Lys114, Tyr116, Leu76, Asp145, Ser115, Val113, Leu75 and Glu144 residues (MolDock score -138.696Kcal/mol) and it also formed two hydrogen and bond hydrogen bond energy (-7.0919Kcal/mol); clearly indicated that ZINC00226974 has more strong binding and thus will offer better PFHGPRT inhibition.

Table 3: Top scoring compounds that have shown binding with PFHGPRT

S. No.	Zinc Id	MolDock Score	H Bond Score	No. H bond	Residues in Interaction
1.	ZINC00226974	-146.534	-15.40	5	Asp204, Arg210, Phe197, Leu203, Tyr116, Gly78, Lys77, Arg112, Ser115, Leu76, Asp145, Ile146, Val113, Leu75
2.	ZINC00268007	-138.696	-7.09	2	Asp204, Phe19, Leu203, Lys114, Tyr116, Leu76, Asp145, Ser115, Val113, Leu75, Glu144
3.	ZINC00226974	-138.696	-7.09	2	Asp204, Phe197, Leu203, Lys114, Tyr116, Leu76, Asp145, Ser115, Val113, Leu75, Glu144
4.	ZINC00446132	-126.36	-2.29	2	Phe197, Val198, Asp204, Leu203, Tyr116, Lys77, Gly78, Ile146, Asp145, Lys114, Ser115, Val113, Thr152
5.	ZINC00314165	-113.07	-7.02	3	Asp148, Ile144, Gly150, Glu144, Thr152, Ser115, Thr152, Tyr116
6.	ZINC00241993	-111.07	-12.08	6	Glu150, Glu144, Thr152, Ser115, Tyr116, Ile146
7.	ZINC00345460	-108.514	-11.84	6	Glu144, Leu175, Thr152, Val113, Ile146, Asp148, Tyr116, Ser115, Thr152
8.	ZINC00137583	-105.268	-4.19	2	Arg210, Leu212, Asp213, Asp202, Gly81, Tyr201, Thr84, Ala85, Lys51, Lys88
9.	ZINC00399318	-84.9434	-2.08	1	Asp148, Ile146, Gly150, Tyr116, Glu144
10.	ZINC00282113	-84.7074	-7.08	4	Ile146, Thr116, Glu144, Asp148, Thr152, Thr149, Gly150, Lys151

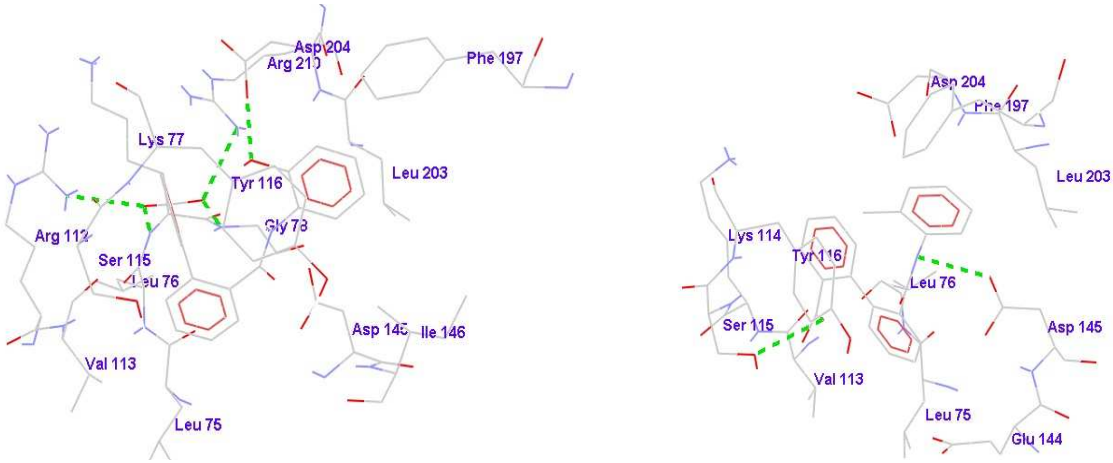


Fig.9: Docked view of ZINC00226974 and ZINC00268007 with (6-(2,2-Dichloroacetamido)chrysene with PFHGPRT

Table 4: Physicochemical properties of ZINC00226974 andZINC00268007

Zinc Id	MW	LogP	Rotable bond	Flexibility
ZINC00226974	3.33.3374	3.55	4	0.160000
ZINC00268007	3.31.364	3.74	4	0.160000

FAF-Drugs can filter or analyse molecules with user-defined or eight predefined physicochemical filters as well as with several simple ADMET (absorption, distribution, metabolism, excretion and toxicity) rules. Physicochemical calculations of ZINC00226974 and ZINC00268007 revealed their molecular weight, logP value, rotable bond, flexibility calculated by FAF-Drugs are, shown in Table 4. ZINC00226974 has not only followed Lipinski rule-of-five, but also possessed more strong affinity and thus will offer better PFHGPRT inhibition.

CONCLUSION

Molecular docking is a safe and easy tool that helps in investigating, interpreting, explaining, identification of molecular properties using 3D structure, molecular docking is tries to used predict the structure of intermolecular complex formed between two or more constituent molecules. *Plasmodium falciparum* HGPRT (PFHGPRT) is an attractive target site candidate for anti-malarial drug discovery and the homology modelling technique stands out as an excellent and powerful alternative to predict a reliable 3-D structure of the protein. The obtained best dock score -94.4, after docking of potential PFHGPRT inhibitor 6-(2,2Dichloroacetamido) chrysene with both i.e. *Plasmodium falciparum* and human, indicated that it better targeted malaria parasite as compare to human, shows success in achieving chemoprevention and a step ahead towards the *Global technical strategy of WHO for malaria 2016–2030* sets the most ambitious targets for reductions in malaria cases. The Protein-Ligand interaction plays a significant role in structural based drug designing. In future research, toxicological profile of these compounds could be tested in wet lab and outcome could be employed for preclinical/clinical trial. The structural information of our given model will pave the way for further laboratory experiments to design potential anti-malarial drug in near future.

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