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Research Article

IN SILICO STUDY TO ELUCIDATE INHIBITORY EFFECT OF THIAZIDES ON PLASMEPSINS: IMPLICATIONS OF NEW ANTIMALARIAL DRUG DESIGN

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ABSTRACT

Objective: Malaria is one of the most deadly diseases existing in the world. Malaria is caused by *Plasmodium* parasites. These parasites induce the degradation of human hemoglobin and utilize it as nutrition source for their growth and maturation. Plasmepsins, the aspartic proteases are involved in such degradation of hemoglobin. Currently, Plasmepsins have become an attractive target for combating malarial diseases due to their importance in the life cycle and metabolism of *Plasmodium* species.

Methods: In the present study, Lamarckian Genetic Algorithm was applied for molecular docking using Autodock4.2 (version 1.5.6). The Plasmepsins from *Plasmodium falciparum, Plasmodium vivax and Plasmodium malariae* were virtually screened for their bioaffinity towards Thiazides. A total of 120 three dimensional structures of thiazides were docked onto the selected protein models. Further, the thiazides were evaluated for their molecular properties using Orisis property explorer.

Results: Cyclopenthiazide (CID_2904) demonstrated better interactions with all the Plasmepsins in comparision to other thiazide derivatives.. Most of the derivatives formed hydrogen bonds with the catalytic aspartic acid residues Asp34/Asp32 or Asp214/Asp215 present in active site of Plasmepsins. Thiazides derivatives followed Lipnisky's rule of five and did not possess any mutagenic, toxic or carcinogenic effect.

Conclusion: Analyzing the binding patterns of Thiazides may provide hints for the future design of new derivatives with higher potency and specificity.

Keywords*:* Malaria, *Plasmodium*, Plasmepsins, Thiazides, Molecular Docking, Autodock 4.2.

INTRODUCTION

Malaria is one of the most common and severe diseases that is infecting people all over the world [**1-2**]. The agents responsible for causing Malaria are *Plasmodium falciparum, Plasmodium vivax* and *Plasmodium malariae* [**1**]. These parasites convey their infection through bite of a carrier (female anopheles mosquitoes). When the parasite enters into the human body, they multiply in the liver and thereafter infects the red blood cells [**3**]. These parasites utilize the haemoglobin of the infected erythrocyte for their growth and development during erythrocytic cycle [**4**]. These parasites express a major group of aspartic acid proteases, Plasmepsins [**5**]. The Plasmepsins are engaged in the early stages of haemoglobin degradation, in a specialized acidic digestive vacuole [**6**]. Thus, they have achieved a considerable attention as a potent target for the inhibition of malarial parasitic growth. A number of drugs are present commercially to treat malaria. But lately, the emergence of drug resistant malarial parasites has gained a considerable amount of attentions. Thus, there is a huge urge for exploring the alternatives for the treatment of disease. In this regard, pre-existing drugs for other diseases can also be explored for their efficiency in the treatment of such fatal diseases. Thiazides are FDA approved drugs belonging to class of diuretics, used for treatment of hypertension and edema [**7**]. In the present investigation, we tried to perform *In Silico* analysis of the bioaffinity of the thiazides towards the Plasmepsins present in *Plasmodium falciparum, Plasmodium vivax* and *Plasmodium malariae* using AutoDock4.2 tool.

MATERIALS AND METHODS

Protein structure retrieval and active site predictions:

The structure and sequence of Plasmepsin (along with its isoforms) present in *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium vivax* were retrieved from RCSB Protein Data Bank (http://www.rcsb.org). The protein model with PDB ID: 3QS1, 1SME, 1LS5, 2ANL, 1QS8 were chosen for active site predictions and docking studies (Table 1). For molecular docking, the protein models were cleaned and optimized by removing ligand and other hetero-atoms (water, ions, etc.) using Argus Lab Software. Further, percentage homology between the Plasmepsins was analyzed using EMBL-EBI ClustalW2 Multiple sequence alignment (http://www.ebi.ac.uk/Tools/msa/clustalw2/).The active site residues in the chosen protein models were predicted using Computed Atlas of Surface Topography of proteins (CASTp) (http://stsfw.bioengr.uic.edu/castp/calculation.php). The clue of catalytic amino acids present in the active site was gained from Uniprot (http://www.uniprot.org/).

Substrate selection

The three dimensional chemical structures of Thiazide and its derivatives, along with commercially available anti-malarial drugs such as Mefloquine, Chloroquine and Hafloquine [**8**] (reference molecule) were retrieved from PubChem (http://pubchem.ncbi.nlm.nih.gov/) and DrugBank (http://www.drugbank.ca/) databases using PRODRG server [**9**] (http://davapc1.bioch.dundee.ac.uk/prodrg/). The optimization of ligand structures was carried out based on UFF and Steepest descent algorithm using Argus Lab software. Further, the thiazides were evaluated for their molecular properties using Orisis property explorer.

Molecular Docking screening

Derivatives of thiazides along with the reference ligands, were docked onto the active site of Plasmepsin proteins using AutoDock4.2 (MGL Tools), following the protocol described by Agarwal et al [**10**]. Docking was carried out based on standard protocol using Lamarckian Genetic Algorithm [**11**]. Twenty five independent docking runs were performed for each ligand. Further, protein ligand complex was visualized using UCSF chimera [**12**] (http://www.cgl.ucsf. edu/chimera) within 5 Å region.

Statistical Analysis

The statistical analysis of the binding energies of the best molecules was conducted using One way ANOVA (Analysis Of Variance) under 95% confidence using Origin Software. The top five molecules showing the lowest binding energy with each of the protein model were selected for the analysis.

RESULTS AND DISCUSSIONS

In silico approach for molecular docking has already been proved to be an effective and cost effective methodology to analyze the interaction profile of ligands with the target protein. The structural models of Plasmepsins from *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax* were selected for the binding interaction analysis. The protein models 3QS1 (Plasmepsin I), 1SME (Plasmepsin II), 1LS5 (Plasmepsin IV) were retrieved from *Plasmodium falciparum*, 2ANL from *Plasmodium malariae* and 1QS8 from *Plasmodium vivax.* A total of 120, three dimensional structures of Thiazide and its derivatives were selected from PubChem and DrugBank databases and docked onto the active site of all the protein models using AutoDock4.2 software. The 3D structures of commercially available anti-malarial drugs were also docked onto the active site as the reference molecule in the studies. These reference molecules include Mefloquine, Chloroquine and Hafloquine. The active site predictions carried out using CASTp Calculations demonstrated that amongst all the binding sites obtained, the site containing the catalytic aspartic acid residues was found to be highly conserved in Plasmepsins and were further taken into consideration for docking (Table 2). Further, EMBL-EBI Clustal W2 Multiple sequence analysis showed approximately 68 – 87% sequence homology amongst the various pairs of the protein models used in the study. Most of the active site residues were highly conserved, thus deciphering common origin and functionality. Thiazides, being FDA approved drugs followed Lipinski's rule of five and were non-irritant, non-mutagenic, non-tumorogen. Further analysis of the ligands was based on their drug score. Orisis property explorer provides a cumulative score between 0 and 1, which allow judging the overall potential of the compound to become a drug. The drug score combines druglikeness, cLogP, logS, molecular weight and toxicity risks associated with the ligand. It was observed that the ligand with PubChem Id: CID_16274 had the highest drug score of 0.86. Other derivatives with CID_2904, CID_71652, CID_6604279 and CID_40463616 also had a comparable drug score of around 0.80 (Table 3), which makes them a better candidate for the analysis. Thiazide derivatives were evaluated for their inhibitory potential against aspartic acid proteases based on their affinity and the interaction patterns. The computational study predicts the affinity of the ligand in terms of binding energy of ligand for the protein. More negative the binding energy, the higher is the affinity. In the present investigation, all thiazide derivatives demonstrated a very high affinity towards the aspartic acid proteases. However, ligands with PubChem Id: CID_2904, CID_40463616, CID_71652 and CID_6604279 showed a better interaction with all aspartic acid proteases taken into consideration. These ligands were found to have a higher affinity for protein model 1SME and 1QS8 from *Plasmodium falciparum* and *Plasmodium vivax* respectively (Table 4). It is important to mention that binding energy itself is not sufficient to predict ligand's potential to inhibit the protein. The stability of an interaction between a ligand and the amino acid residues of a protein, also play a crucial role for the same. Hydrogen bonds are one of the important parameters for interaction profile analysis. In this regard, hydrogen bonds formed in protein ligand complex were illustrated by Chimera (Table 5). Almost all the thiazide derivatives made hydrogen bonds with the active site residues, especially the catalytic aspartic acid residues. The ligand Mebuthiazide (CID_71652) when docked with 1SME, showed a binding energy of -12.12 kcal/mol and six hydrogen bonds in its docked confirmation. It is also important to mention that ligand Cyclopenthiazide (CID_2904) showed good affinity and interactions with all the Plasmepsins (Figure 1). In comparision, the selected reference molecules Mefloquine, Chloroquine and Hafloquine demonstrated a lower affinity for the same (binding energy < -9.78kcal/mol) (Table 6).

The statistical analysis of the top five molecules was done using One Way ANOVA with 95% confidence level. It was observed that within each set, there exists significant difference in the binding affinity of ligands with the protein models except in case of Plasmepsin IV derived from *Plasmodium malariae*, where all ligands share equal significance in term of their inhibitory activity (Table 7). On the basis of the structural interactions and the ability of working on high resolution with both the proteins and drug compounds, drugs can be designed against a particular disease. This marks the relevance of the structure based drug designing. To synthesize and to test the obtained data, there is a requirement of in-vitro and in-vivo activity, which helps in proper drug designing with much better specificity and metabolism. The work is significant in emphasizing that the thiazides may have a potential inhibitory effect onto Plasmepsins class of proteins and could prove to be an effective drug to combat malaria.

Table 1: Protein models of Plasmepsins taken for analysis

S. No.	PDB ID	Organism	Protein	Reference
1	3QS1	*Plasmodium falciparum*	Plasmepsin I	http://www.rcsb.org/pdb/explore/explore.do?structureId=3qs1
2	1SME	*Plasmodium falciparum*	Plasmepsin II	http://www.rcsb.org/pdb/explore/explore.do?structureId=1sme
3	1LS5	*Plasmodium falciparum*	Plasmepsin IV	http://www.rcsb.org/pdb/explore/explore.do?structureId=3ls5
4	2ANL	*Plasmodium malariae*	Plasmepsin IV	http://www.rcsb.org/pdb/explore/explore.do?structureId=2anl
5	1QS8	*Plasmodium vivax*	Plasmepsin	http://www.rcsb.org/pdb/explore/explore.do?structureId=1qs8

Table 2: CASTp: Active site residues of Plasmepsins

S. No.	Protein Model	Active Site Residues
1	3QS1	Val12, Met13, Ile30, Asp32, Gly34, Ser35, Ala36, Asn37, Met73, Asn74, Tyr75, Val76, Ser77, Gly109, Pro110, Ala111, Leu114, Gly115, Phe117, Ile120, Leu128, Tyr189, Ile213, Asp215, Gly217, Thr218, Ser219, Ser220, Thr222, Leu243, Leu244, Tyr245, Glu276, Met283
2	1SME	Met15, Tyr17, Ile32, Asp34, Gly36, Ser37, Ala38, Met75, Asn76, Tyr77, Val78, Ser79, Phe111, Thr114, Ser118, Phe120, Ile123, Leu131, Tyr192, Ile212, Asp214, Gly216, Thr217, Ser218, Ala219, Thr221, Ile290, Leu292, Phe294, Ile300
3	1LS5	Met15, Phe16, Ile32, Phe33, Asp34, Gly36, Ser37, Ala38, Asn39, Ile75, Ser76, Tyr77, Gly78, Ser79, Leu111, Ile114, Phe120, Ile123, Leu131, Ser132, Ile133, Phe156, Asn188, His189, Leu191, Tyr192, Asn210, Val212, Asp214, Ser215, Gly216, Thr217, Ser218, Thr219, Thr221, Leu242, Pro243, Tyr288
4	2ANL	Leu14, Met15, Phe16, Ile32, Asp34, Thr35, Gly36, Ser37, Ala38, Asn39, Ile75, Thr76, Tyr77, Gly78, Ser79, Leu111, Leu114, Tyr115, Ala117, Ala118, Glu119, Phe120, Leu123, Leu131, Ile133, His161, Phe192, Asn210, Ile212, Val213, Asp214, Gly216, Thr217, Ser218,Thr219, Thr221, Pro243, Phe244, Glu275, Pro276, Leu277, Asp279, Met286
5	1QS8	Ala12, Asn13, Ile14, Met15, Ile32, Met15, Ile32, Asp34, Gly36, Ser37, Ala38, Asn39, Ile75, Tyr77, Gly78, Ser79, Leu111, Pro113, Ile114, Ser117, Val118, Phe120, Ile123, Leu131, Ile133, Phe156, Tyr157, Leu158, Val160, His161, Tyr192, Ile212, Asp214, Gly216, Thr217, Thr218, Thr219, Thr221, Leu242, Pro243, Phe244, Tyr245, Glu271, Tyr272

Table 3: Osiris Property explorer predictions: Molecular Properties of Thiazides & derivatives

S. No.	Compound ID	Molecular Properties					
		cLogP	Molecular Weight	Drug likeness	Drug Score	Tumorogenic / Mutagenic	Reproductive effect
1	DB_562	2.01	433	40	0.68	X	X
2	CID_2904	1.7	379	5.46	0.8	X	X
3	CID_4726	2.8	415	-18.1	0.37	X	X
4	CID_16274	1.6	353	2.46	0.86	X	X
5	CID_71652	2.41	381	5.86	0.79	X	X
6	CID_6604279	1.7	379	5.46	0.8	X	X
7	CID_40463616	1.7	379	5.46	0.8	X	X
8	CID_44568930	2.07	417	-0.31	0.56	X	X
9	CID_54396742	-0.14	347	2.61	0.66	X	X
10	CID_54470973	-0.09	389	-5.38	0.34	X	X

Table 4: Binding Energies: Thiazide ligand molecules with Plasmepsins

S. No.	Compound ID	Binding Energy (kcal/mol)				
		Plasmodium falciparum			*Plasmodium malariae*	*Plasmodium vivax*
		Plasmepsin I	Plasmepsin II	Plasmepsin IV	Plasmepsin IV	Plasmepsin
1	DB_562	-9.66	-10.65	**-10.93**	**-11.78**	-10.72
2	CID_2904	-10.25	-11.95	-10.88	-10.51	-11.9
3	CID_4726	-10.68	-10.9	-9.97	-9.33	-11.76
4	CID_16274	-9.56	-11.47	-10.31	-9.79	-10.89
5	CID_71652	-10.76	-12.12	-10.22	-10.97	-11.8
6	CID_6604279	-10.26	-11.95	-10.7	-10.42	-11.11
7	CID_40463616	-10.12	-12.16	-10.78	-10.71	-11.56
8	CID_44568930	-10.2	-11.08	-10.26	-10.04	-11.53
9	CID_54396742	-10.28	-10.98	-10.29	-10.21	-10.7
10	CID_54470973	-10.24	-11.05	-10.25	-9.58	-11.4

Table 5: UCSF Chimera: Hydrogen Bond Pattern of Thiazide ligands with Plasmepsins

S. No.	Organism	Protein	Compound ID	Hydrogen Bonds with amino acid residues
1	*Plasmodium falciparum*	Plasmepsin I	CID_2904	Tyr189, Asp215
			CID_4726	Tyr189 (2), Asp32 (2)
			CID_71652	Asp32 (2), Asp215, Ser77
			CID_6604279	Asp215, Asp32 (2), Gly34, Tyr189
			CID_54396742	Gly217, Asp215 (2), Asp32 (2)
2	*Plasmodium falciparum*	Plasmepsin II	CID_2904	Asp34, Asp214, Ser218 (2), Ser79
			CID_16274	Ser79, Ser218 (2), Asp214, Asp34
			CID_71652	Gly36, Asp34, Asp214, Ser218 (2), Ser79
			CID_6604279	Asp34, Asp214, Ser218 (2), Ser79
			CID_40463616	Asp34, Asp214, Ser218 (2), Ser79
3	*Plasmodium falciparum*	Plasmepsin IV	DB_562	Ser79 (2), Asp214, Gly36
			CID_2904	Ser79, Ser218, Asp34, Asp214
			CID_16274	Ser79, Asp214
			CID_6604279	Ser218 (2), Thr217, Asp214, Ser79
			CID_40463616	Asp214, Ser79
4	*Plasmodium malariae*	Plasmepsin IV	DB_562	Thr217, Asp214
			CID_2904	Ser218, Thr217, Asp214, Asp34
			CID_71652	Asp214, Leu131
			CID_6604279	Asp34 (2), Ser218, Thr217
			CID_40463616	Ser218 (2), Thr217
5	*Plasmodium vivax*	Plasmepsin	CID_2904	Asp34, Asp214, Ser79, Thr218 (2)
			CID_4726	Tyr192, Asp34 (2)
			CID_71652	Tyr192, Asp34
			CID_40463616	Tyr192, Asp34 (2)
			CID_44568930	Tyr192, Ala38, Asp34

Table 6: Binding Energies: Reference ligand molecules with Plasmepsins

S. No.	Organism	Protein	PDB ID	Binding Energy of reference ligands		
				Chloroquine	Hafloquine	Mefloquine
1	*Plasmodium falciparum*	Plasmepsin I	3QS1	-7.3	-7.75	-8.15
		Plasmepsin II	1SME	-8.8	-9.66	-9.06
		Plasmepsin IV	1LS5	-8.3	-8.59	-8.37
2	*Plasmodium malariae*	Plasmepsin IV	2ANL	-7.41	-7.38	-9.04
3	*Plasmodium vivax*	Plasmepsin	1QS8	-8.46	-9.14	-9.78

Table 7: Origin Pro8 ANOVA analysis (95% confidence interval)

S. No.	Organism	Protein	Compound ID	Mean Binding Energy (kcal/mol)	F Value	P value
1	*Plasmodium falciparum*	Plasmepsin I	CID_2904	-9.989	4.82	0.001
			CID_4726	-9.363		
			CID_71652	-9.932		
			CID_6604279	-9.678		
			CID_54396742	-9.6		
2	*Plasmodium falciparum*	Plasmepsin II	CID_2904	-11.154	6.93	0
			CID_16274	-10.645		
			CID_71652	-11.179		
			CID_6604279	-11.259		
			CID_40463616	-11.539		
3	*Plasmodium falciparum*	Plasmepsin IV	DB_562	-9.406	7.01	0
			CID_2904	-10.18		
			CID_16274	-9.583		
			CID_6604279	-9.798		
			CID_40463616	-10.283		
4	*Plasmodium malariae*	Plasmepsin IV	DB_562	-9.894	1.75	0.114
			CID_2904	-9.496		
			CID_71652	-9.701		
			CID_6604279	-9.604		
			CID_40463616	-9.512		
5	*Plasmodium vivax*	Plasmepsin	CID_2904	-11.198	3.85	0.006
			CID_4726	-10.412		
			CID_71652	-11.002		
			CID_40463616	-10.885		
			CID_44568930	-10.736		

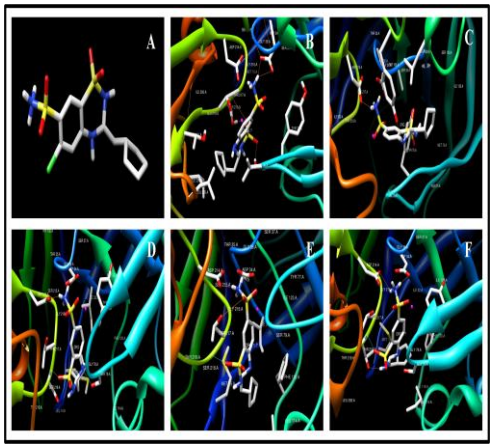

Fig. 1: A) Chemical 3D structure of CID_2904 and its binding interactions with B) 1SME, C) 3QS1, D) 1LS5, E) 2ANL, F) 1QS8

CONCLUSION

Significant level of research for the development of anti-malarial agents has reduced the spread and mortality of *Plasmodium*, responsible for causing malaria. But still the pathogen continues to cause pathogenesis all over the world. The emergence of drug resistant species of *Plasmodium* has made the situation more vulnerable. Thus there exists a huge urge for the development of new leads for the purpose. Plasmepsins have emerged into a potential drug targets for the purpose. In the present investigation through *in silico* approach, we demonstrated that thiazides (FDA approved drugs) could provide a base for the development of novel leads with better affinity and specificity against Plasmepsin.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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