

Pharmacokinetic and molecular docking studies to design antimalarial compounds targeting Actin I

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Introduction

Malaria is a life-threatening disease caused by parasites belonging to five species of genus *Plasmodium falciparum* which are transmitted through the bite of female *Anopheles* mosquito. In 2017 as per the WHO report, 219 million cases of malaria were reported in 87 countries and the estimated number of deaths was found to be 435,000. Despite such drastic figures there is still no widely used efficacious vaccine available for malaria parasites.^[1] Although, antimalarial drugs are available from long back and the popular antimalarial medications used against malarial parasites includes Chloroquine, Chloroguanil (Proguanil), Sulfadoxine/pyrimethamine, Quinine, Mefloquine, Halofantrine, Artemisinin (ART), and Atovaquone. In 21st century, the drug resistance has emerged to almost all classes of antimalarial drugs and therefore a sudden leap has been witnessed in malaria-related mortality, especially in Africa.^[1]

Both drug resistance and unavailability of highly effective vaccine makes malaria a highly challenging disease and a

ABSTRACT

Objective: Malaria is an ancient disease that still causes more than 200 million of cases 7 with high mortality globally. Identification of new drug targets and development of novel antimalarial drugs with unique mode of action encounter the drug resistance and reduce the mortality by *Plasmodium* parasites. Actin protein is one of the key proteins in *Plasmodium falciparum* playing multifarious important roles including transport, cell motility, cell division, and shape determination. This study investigated Actin I as a drug target, *in silico* screening of diverse molecules through molecular docking was considered. Further, pharmacokinetic parameters of the selected molecules from the docking and interaction studies were planned to propose the lead molecules.

Methods: Molecules were selected according to score and protein ligand interaction and selected molecules were subjected for pharmacokinetic studies to investigate important drug parameters.

Results: The docked molecules were ranked according to the binding score and good interaction pattern was observed with Actin I within top 20 scoring molecules. The selected molecules also had optimum pharmacokinetic parameters.

Conclusion: The current study provides a set of hit molecules which can be further explored through *in vitro* and *in vivo* experiments for the development of potential drugs against malaria, there by encountering drug resistance and establishing Actin I as an important drug target.

Keywords: Actin I, actin, drug resistance, drug, malaria, molecular docking, *Plasmodium falciparum*

great public health burden. The identification of new/novel drug targets as well as development of novel antimalarial drugs with unique mode of action can be the possible answer to drug resistance in malaria and subsequently it can control the mortality due to malaria.^[2,3] Being highly studied diseases several potential drug targets have been suggested in research which can be further developed as a drug target in experiments. Actin I is also such kind of drug target in malaria. Recently, our group has discovered that Actin I protein is expressed in almost all stages of *P. falciparum* which are present in human which justifies its importance in plasmodium lifecycle and its drug target potential.^[4] Although in different literature actin is proposed as the drug target in malaria, it is not yet studied for structure based drug design and subsequently not developed as an important drug target.^[4,5] The main reasons for the selection of the Actin I in the current study are the Actin I protein is novel drug target which is important in different stages of *P. falciparum* and can be a probable answer to drug resistance.^[4] Although the Actin I protein is conserved in both, there is difference between human and *P. falciparum* actin protein.

In human, three isoforms of actin have been identified, alpha, beta, and gamma whereas in *P. falciparum* there exists only two isoforms, that is, Actin I and Actin II.^[6,7] This protein is an essential part of the parasite motor machinery and responsible for gliding motility of the parasite.^[8,9] In nature, there exists six different mammalian actin isoforms which differ from each other by a maximum of 6% of the sequence, and are practically identical across the species. Commonly most actins have the capacity to form long filaments but in *P. falciparum* the presence of regular actin filaments is uncertain. In *P. falciparum* Actin I is abundant and expressed throughout the lifecycle of the parasite. In vitro, apicomplexan actins form short, ~100-nm long filaments, which suffer from rapid tread milling.^[10,11] Recently, specific antibodies revealed filament-like structures in motile forms of *P. falciparum*.^[12] The *P. falciparum* Actin I fibers are refined from merozoites and are thereby distinctive for their helical symmetry from canonical actin structures.^[13,14]

The most prevalent forms of actin protein are ATP-G-actin and ADP-F-actin. The structural determination of G-actin reveals that it has two lobes separated by a cleft, which is an ATPase fold. The functional form of G-actin exhibits only when the cleft comprises of an ATP or ADP. The F-actin polymer is a filamentous protein which is also referred as microfilament with levorotatory helix structure. The cell creeping process helps actin cytoskeleton to encounter dynamic assembly and disassembly, which further controls central attachment of assembly/disassembly bulge advancement, and contractile fiber association. Moreover cell relocation and adhesion is inhibited by the disturbance of the actin cytoskeleton.^[15-17] The assembly and disassembly of local Actin I filament regulates the dynamic formation of lamellipodia. The lamellipodia is slim, sheet-like film distensions of the motile cells. During movement, cells grow the film forward to explore their environment. The branching and elongation are the two examples of actin fiber assembly, which advance the improvement of the actin “work” in the cell distension.^[16,18] The nucleation promoting factors such as neuronal Wiskott - Aldrich syndrome Protein (N-WASP) and WASP-family verprolin-homologous protein (WAVE) prohibit the movement of Arp2/3 complex, which are in turn modulated by upstream regulators.^[16] The Actin I fibers are organized into two kinds of arrays: Bundles and web like systems. Similarly, the Actin I fibers cross-connecting the proteins help to stabilize and keep up these structures and are divided into two classes: Packaging proteins and web-forming proteins. Actin I plays an important role in the intracellular blood stages of *P. falciparum*, such as endocytosis and vesicle trafficking,^[18,19] ring stage morphology,^[20] and spatial positioning of genes in the nucleus.^[21] As per the recent studies, the actin elements have appeared to assume significant roles in the inheritance of intracellular organelles, for example, the apicoplast, mitochondria and secretory vesicles during intracellular parasite replication.^[21-24] The Actin I association has also been well studied in zoite and asexual blood stages. In this intracellular stage, the parasite experiences a wonderful morphological change during which the parasite embraces a

crenate or falci from shape in anticipation for transmission by means of the Anopheles mosquito.^[25,26] In Actin I structure the C terminus acquires the shape of α -helix and it interacts with the bottom of the sub domain 1. Actin I protein consist of cleft named hydrophobic cleft between the sub-domains I and III. This cleft is major regulatory site and regarded as hotspot for the protein binding.^[7] The binding pocket of Actin I has two parameters, the “mouth” and “phosphate clamp.” The “mouth” is considered as the distance between the C α atoms of Gln59 and Glu207. The distance between the C α atoms of Gly15 and Asp157 makes the “phosphate clamp.” As compared to the canonical actins, in Actin I there is a smaller cleft where large hydrophobic residues have adopted different conformations together with Trp357. Actin I shows significant structural deviations in specific regions which are involved in binding of proteins.^[7,27] This may provide opportunities for structure-based drug design targeted at the *P. falciparum* actin-regulatory boundaries. The importance of Actin I protein is established in most of the stages of parasite. The crucial function of Actin I in asexual, blood, liver, mosquito and sexual stages is reported in the literature which also correlates with our earlier finding of its expression in nearly all stages of *P. falciparum*.^[4] The active site residues of protein consist of amino acids, that is, Asn 17(A), Gly 16(A), Lys 19 (A), Gly 303(A), Glu 215(A), Lys 214(A), Val 160 (A), and Gly 159(A).

In current study, Actin I protein was taken for molecular docking studies because it is a new potential drug target and relatively not explored as drug target in drug discovery in spite of its conservation in different species of *P. falciparum*. In *P. falciparum* intracellular functions of Actin I have been recommended, that is, endocytosis,^[20] secretion, and antigenic variation.^[18,28,29] Molecular docking is an important tool for drug discovery and can be used to model the interaction between small drug like molecule and a protein at atomic level, which permit us to describe the enactment of small molecules in the binding sites of target proteins as well as to irradiate the biochemical processes. Docking is very useful and one of the most commonly used methods in structure-based drug design, due to its capability to predict the binding-conformation of small molecule ligands to the appropriate target binding site.^[4,30-32] In the current study, the library of small molecules is screened against Actin I protein. The top scoring molecules from docking study were subjected for protein-ligand interaction studies and prediction of pharmacokinetics properties. Favorable pharmacokinetics property is also essential parameter for the ligand to be considered in drug discovery process. Therefore, the best molecules according to these three studies can be strongly suggested for further wet lab experiments of drug design.

Materials and Methods

Retrieval of protein and ligand libraries

The crystal structure of Actin I of *P. falciparum* (PDB ID: 4CBU) was downloaded from protein databank in pdb format [https://

www.rcsb.org/structure/4CBU]. For virtual screening organic molecule library from NPACT database consisting of total 1574 molecules (<https://zinc.docking.org/browse/catalogs/natural-products>) was downloaded from zinc database in mol2 format.

Target protein preparation

The crystal structure of *P. falciparum* Actin I (PDB ID: 4CBU) is of 1.3 Å resolution with bound ligand ATP and have two chains; chain A and chain G. It was observed that the binding pocket is located in chain-A and chain-G was away from binding pocket. Hence, chain-G was removed through UCSF Chimera before the protein preparation and in further docking studies only chain-A was considered. The bound ligand molecule was also removed before docking. The protein was prepared for docking studies by assigning hydrogen, polarities, calculating Gasteiger charges to protein structures, and converting protein structures from the pdb format to pdbqt format using Auto-Dock tool 1.5.4.

Preparation of ligand libraries for virtual screening

The natural occurring plant-based compound activity target database library was downloaded from ZINC database (<https://zinc.docking.org/>) in MOL2 format and was further used for performing docking studies. The library was in a single molecular file and all the ligand molecules were extracted from it with the help of in-house developed PERL scripts. Subsequently all the ligand molecules were converted into PDB format from MOL2 format by using open babel with the help of in-house developed PERL scripts. Antimalarial drugs including Chloroquine, Chloroguanide, Quinine, Mefloquine, and Halofantrine were also used as positive control to target ACTIN I and structure of these molecules were taken from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>).

Molecular docking

All the ligands within the organic molecular library were used to perform molecular docking studies^[33,34] against Actin I to find the potential hit molecules for further drug discovery experiments. In the current research for performing the docking studies the version 4.2 of AutoDock was used. AutoDock uses a Lamarckian Genetic Algorithm (LGA) and is based on a semi empirical free energy force field.^[35] The docking grid was set manually through visualization of the protein and grid was defined to cover entire binding site of Actin I.

The size of grid was defined as X=54, Y=54 and Z=54 and the coordinates used for docking of ligands library with 4CBU were X=9.175, Y=20.366, and Z=18.583. Also for molecular docking for every molecule ten runs of LGA were performed. The main interacting residues in the binding site were Asn17, Lys19, Tyr338, Lys337, Lys214, Gln215, and other nearby residues. Finally, molecular docking was carried out on target proteins with all ligands from the library using self-developed PERL script,

which was used for screening of these large number of ligand molecules one by one [Figure 1]. The top scoring compounds were selected on the basis of binding energy of ligands with the receptor. The lower is the binding energy of ligand with the receptor the more strongly it will bound to the target receptor.

Interaction study and visualization of docked complex

The UCSF Chimera 1.8.1 was used to visualize and analyze docked complexes of ligand and protein.^[36] The interaction such as hydrogen bonds and hydrophobic interaction was also analyzed using Ligplot+.^[37,38] The generated plots have shown the hydrophobic interaction patterns and hydrogen bonding between the main chain/side-chain atoms of the protein with the ligand.

ADME-toxicity prediction

The pharmacokinetic profile prediction of potential compounds was done using pkCSM (<http://biosig.unimelb.edu.au/pkcsm/prediction>) with incorporated prediction of toxicity, metabolism, distribution, absorption, and excretion.^[39] Some other properties such as molecular descriptors and drug likeliness properties were also analyzed with the help of pkCSM. The drug likeliness properties were predicted according to the Lipinski Rules of five.^[40]

Results

In the present study, considering the importance of Actin I protein as a drug target in *P. falciparum* molecular

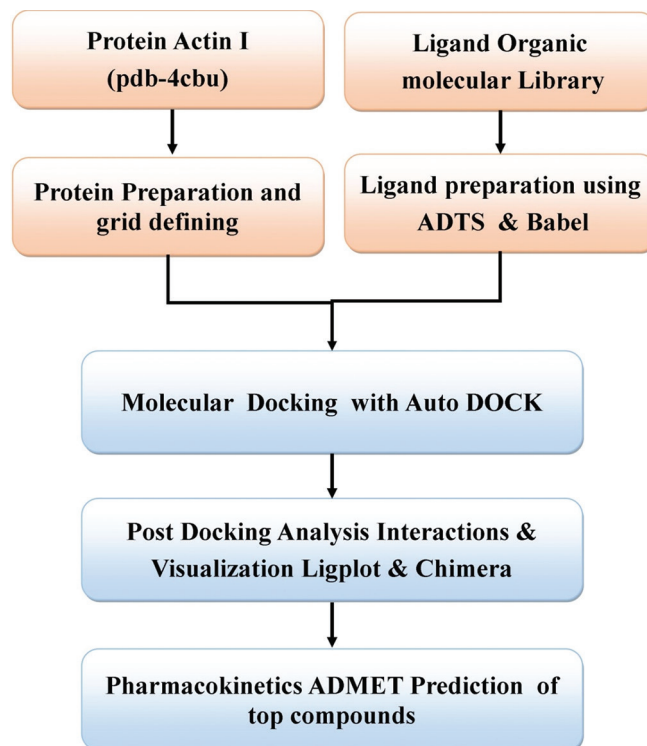


Figure 1: The methodology followed for docking and screening

docking of actin I of *P. falciparum* was carried out. The organic molecular library was docked into the active site of Actin I to find the potential ligand molecules which can be used in further drug design experiments and also establish the Actin I as an important drug target in malaria. Pharmacokinetics properties of top ranked molecules were also calculated computationally so that molecules with better pharmacokinetics properties can be further shortlisted for wet lab experiments.

Docking analysis

Molecular docking of all the ligands within the library was found to be successful on the basis that all the ligands were docked in the active site of the receptor. The 20 top scoring molecules as per estimated free energy of binding (EFEB) were selected for further investigations. The protein ligand complexes of top scoring molecules were analyzed for interactions, the orientation of the docked compound, and interacting active site residues were visualized. The selected 20 molecules had EFEB in the range of -13.20 – -10.51 [Table 1]. The molecule, that is, ZINC30724344 shows least EFEB of -13.20 [Table 1]. The four other top scoring molecules ZINC13515285, ZINC03943903, ZINC95098956, and ZINC03984030 showed the EFEB -12.61 , -12.57 , -12.37 , and -12.23 , respectively [Table 1] [Supplementary Figures 1-3]. Despite these molecules the other molecules also had EFEB better than -10 and also show good interaction with the target molecule [Table 1] [Supplementary Table 2] [Supplementary Table 3].

The top scoring molecule, that is, ZINC30724344 strongly interacts with the Glu215, Lys19 and Asn17 residues of the binding cavity [Figure 2] [Table 1]. The second-best scoring molecule as per the EFEB, that is, ZINC13515285 forms hydrogen bonds with the Lys337, Asn17, Lys19, Lys214, Tyr307, Thr304, and Gly303 [Figure 3] [Table 1].

The molecule ZINC03943903 strongly interacts with the Lys19, Ca1370, Lys337, Gly303, Gly16, Asn17, Asp158, Thr304, and Lys214. All other selected molecules also show good patterns of hydrogen bond and hydrophobic interactions [Table 1].

Among the five antimalarial drugs which were used for control docking Mefloquine exhibit the best estimated free energy of -8.5 kcal/mol with Actin I and interact strongly with Thr278, Ala273 [Supplementary Table 1]. The other drugs which were used as positive controls, that is, Chloroquine, Chloroguanide, Quinine, and Halofantrine show the EFEB -8.2 , -7.1 , -7.6 , and -8.0 , respectively [Supplementary Table 1].

Molecular parameters

The drug molecules which are having molecular weight less than 500Da can be easily transported, diffused and absorbed as compared to bigger molecules.^[41] The physicochemical

Table 1: Binding affinity of selected potential molecules

Zinc Id	Binding affinity	Hydrogen bonding	Hydrophobic interactions
ZINC13515285	-12.61	Lys337, Asn17, Lys19, Lys214, Tyr307, Thr304 and Gly303	6
ZINC03943903	-12.57	Lys19, Ca1370, Lys337, Gly303, Gly16, Asn17, Asp158, Thr304 and Lys214	8
ZINC95098956	-12.37	Tyrs338, Lys337, Cys218, Lys214	10
ZINC03984030	-12.23	Tyr307, Lys337, Tyr338, Asn17, Thr304, Lys214, Asp158 and Arg184	10
ZINC04349517	-12	Asp155, Lys337, Lys214, Ca1370, Gly16 and Asp158	11
ZINC30724344	-13.20	Glu215, Lys19 and Asn17	9
ZINC28652364	-11.22	Lys337, Lys19 and Asn17	11
ZINC95098947	-11.2	Tyr70, Asp158, Lys214, Glu208 and Asn17	12
ZINC28538752	-11.17	Gly330, Lys337, Lys19, Glu219, Asn17 and Glu215	8
ZINC38145808	-11.14	Glu215, Lys337, Asp357, Lys19, Asn17 and Gly303	7
ZINC95099527	-11.11	Asp158, Gly216, Arg184	10
ZINC28652356	-11.09	Lys337, Glu215, Arg211, and Gln60	7
ZINC95099570	-10.94	Ser15, Asn17, Glu215 and Lys214	15
ZINC95098958	-10.57	Lys214, Lys19 and Lys216	8
ZINC02138728	-10.56	Asn17, Gly16, Asp158 and Lys19	9
ZINC14883289	-10.56	Thr304, Lys214 and Asn17	12
ZINC08143568	-10.53	Glu215, Thr304, Asn17, Gln60, Asp158 and Lys214	10
ZINC08234294	-10.51	Gly303, Lys214, Tyr307, Met306, Lys337, Glu215, Arg211, Tyr338 and Lys19	3
ZINC15215608	-10.51	Lys337	11
ZINC45495236	-10.51	Thr304, Asn17 Lys214, and Lys337	6

properties were calculated and the molecular weight of 10 hit molecules out of 20 was less than 500 Da. Most of the molecules [Table 1] showed $\log P < 5$ [Table 1]. The 13 out of 20 selected hit molecules have less than 5 hydrogen bond donors and also have less than 10 hydrogen bond acceptors [Table 2].

Table 2: Physicochemical properties of potential compounds

Zinc Id	Molecular weight	LogP	Hydrogen rotatable bonds	Hydrogen acceptors	Hydrogen donors
ZINC13515285	466.395	-0.9895	4	12	8
ZINC03943903	445.356	-1.1925	4	11	5
ZINC95098956	617.847	7.5744	4	6	2
ZINC03984030	538.464	5.134	3	10	6
ZINC04349517	448.38	-0.2445	4	11	7
ZINC30724344	592.696	5.01246	6	6	2
ZINC28652364	470.474	1.39952	3	9	5
ZINC95098947	474.462	0.74522	5	10	7
ZINC28538752	527.725	8.02892	8	4	2
ZINC38145808	167.228	1.429	4	2	0
ZINC95099527	536.662	1.2217	4	9	4
ZINC28652356	528.51	1.07432	4	11	5
ZINC95099570	416.558	4.8652	9	5	1
ZINC95098958	603.82	6.4495	4	6	2
ZINC02138728	353.303	-1.9806	4	9	5
ZINC14883289	391.487	4.79372	4	4	2
ZINC08143568	610.565	-1.1566	7	15	8
ZINC08234294	596.538	-1.4596	6	15	9
ZINC15215608	527.725	8.02892	8	4	2
ZINC45495236	462.407	0.0585	5	11	6

Table 3: Absorption profile of potential compounds

Zinc ID	Water solubility (logmol/L)	Caco2 permeability (logPapp10cm/s)	Intestinal absorption (human) (% Absorbed)	Skin Permeability (log Kp)	P-glycoprotein substrate	P-glycoprotein Inhibitor	P-glycoprotein II inhibitor
ZINC02138728	-2.421	-0.604	38.265	-2.735	✓	×	×
ZINC03943903	-2.859	-0.686	15.504	-2.735	✓	×	×
ZINC03984030	-2.892	0.169	90.235	-2.735	✓	✓	✓
ZINC04349517	-2.964	0.303	39.869	-2.735	✓	×	×
ZINC08143568	-2.886	0.261	28.757	-2.735	✓	×	×
ZINC08234294	-2.896	-1.415	24.116	-2.735	✓	×	×
ZINC13515285	-2.604	0.234	38.988	-2.735	✓	×	×
ZINC14883289	-4.207	0.869	94.652	-2.738	✓	✓	✓
ZINC15215608	-4.25	0.862	96.337	-2.736	✓	✓	✓
ZINC28538752	-4.25	0.862	96.337	-2.736	✓	✓	✓
ZINC28652356	-3.211	0.3	60.819	-2.735	✓	×	×
ZINC28652364	-3.38	0.266	69.589	-2.735	✓	✓	×
ZINC30724344	-3.15	-0.347	84.303	-2.735	✓	×	✓
ZINC38145808	-2.435	1.466	100	-2.697	×	×	×
ZINC45495236	-2.771	0.327	46.958	-2.735	✓	×	×
ZINC95098947	-2.994	0.01	42.242	-2.735	✓	✓	×
ZINC95098956	-3.099	1.529	85.152	-2.735	✓	×	✓
ZINC95098958	-3.062	1.469	77.907	-2.735	✓	×	✓
ZINC95099527	-5.021	0.271	66.69	-2.876	✓	✓	×
ZINC95099570	-4.706	0.839	94.964	-3.282	✓	✓	✓

Table 4: Distribution/excretion profile of compounds

Zinc ID	Distribution				Excretion	
	VDss (human) (log L/kg)	Fraction unbound (human) (Fu)	BBB permeability (log BB)	CNS permeability (log PS)	Total clearance (log ml/min/kg)	Renal OCT2 substrate
ZINC02138728	-1.189	0.607	-0.865	-3.985	0.069	×
ZINC03943903	0.264	0.303	-1.149	-3.925	0.277	×
ZINC03984030	-0.872	0.279	-1.619	-3.096	0.454	×
ZINC04349517	1.628	0.233	-1.493	-3.941	0.47	×
ZINC08143568	1.489	0.198	-1.686	-4.752	0.201	×
ZINC08234294	1.734	0.235	-1.925	-4.873	0.105	×
ZINC13515285	1.215	0.36	-1.411	-4.193	-0.412	×
ZINC14883289	-0.017	0	0.129	-1.764	0.622	×
ZINC15215608	-0.68	0	0.497	-1.368	0.327	×
ZINC28538752	1.339	0.125	-1.205	-3.476	0.314	×
ZINC28652356	0.646	0.096	-1.071	-3.335	0.429	×
ZINC28652364	0.337	0.331	-0.172	-1.914	1.241	×
ZINC30724344	-0.802	0.487	-0.06	-2.062	0.495	×
ZINC38145808	-0.802	0.487	-0.06	-2.062	0.495	×
ZINC45495236	0.214	0.119	-1.654	-4.198	0.591	×
ZINC95098947	1.176	0.33	-1.247	-3.791	0.194	×
ZINC95098956	-1.31	0	-0.239	-1.516	-0.551	×
ZINC95098958	-1.212	0.006	-0.211	-1.702	-0.342	×
ZINC95099527	-0.296	0.265	-0.88	-3.465	0.444	×
ZINC95099570	-0.229	0.112	-0.397	-2.282	1.202	ü

Table 5: Metabolism profile of potential compounds

Zinc ID	CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
ZINC02138728	×	×	×	×	×	×	×
ZINC03943903	×	×	×	×	×	×	×
ZINC03984030	×	✓	×	×	×	×	×
ZINC04349517	×	×	×	×	×	×	×
ZINC08143568	×	×	×	×	×	×	×
ZINC08234294	×	×	×	×	×	×	×
ZINC13515285	×	×	×	×	×	×	×
ZINC14883289	×	✓	×	✓	✓	×	✓
ZINC15215608	×	✓	×	✓	×	×	×
ZINC28538752	×	✓	×	✓	×	×	×
ZINC28652356	×	✓	×	×	×	×	×
ZINC28652364	×	×	×	×	×	×	×
ZINC30724344	×	✓	×	×	×	×	×
ZINC38145808	×	×	×	×	×	×	×
ZINC45495236	×	×	×	×	×	×	×
ZINC95098947	×	×	×	×	×	×	×
ZINC95098956	×	✓	×	×	×	×	×
ZINC95098958	×	✓	×	×	×	×	×
ZINC95099527	×	✓	×	×	×	×	×
ZINC95099570	×	✓	×	×	×	×	×

Table 6: Toxicity profile of the potential compounds

Zinc ID	A.T	M.T.D	H.1.I	H.2.I	O.R.A.T	O.R.C.T	H.T	S.S	T.P.T	M.T
ZINC02138728	No	1.598	×	×	1.73	3.653	✓	×	0.285	5.371
ZINC03943903	No	0.515	×	×	2.577	3.674	×	×	0.285	4.453
ZINC03984030	No	0.438	×	✓	2.505	3.362	×	×	0.285	2.897
ZINC04349517	No	0.574	×	×	2.525	4.032	×	×	0.285	5.753
ZINC08143568	No	0.41	×	✓	2.488	3.13	×	×	0.285	7.669
ZINC08234294	No	0.465	×	✓	2.492	3.456	×	×	0.285	8.159
ZINC13515285	No	0.416	×	✓	2.468	4.617	×	×	0.285	9.432
ZINC14883289	No	0.052	×	✓	2.109	1.065	×	×	0.399	-0.528
ZINC15215608	No	0.148	×	✓	2.107	1.445	×	×	0.286	-3.595
ZINC28538752	No	0.104	×	×	2.394	2.558	×	×	0.285	6.112
ZINC28652356	No	0.426	×	×	2.483	2.892	×	×	0.285	3.723
ZINC28652364	No	0.021	×	×	2.66	1.161	✓	×	0.287	0.828
ZINC30724344	No	0.309	×	×	1.49	1.399	×	✓	0.018	1.341
ZINC38145808	No	0.309	×	×	1.49	1.399	×	×	0.018	1.341
ZINC45495236	No	0.533	×	✓	2.58	4.275	×	×	0.285	4.816
ZINC95098947	No	0.266	×	✓	2.544	3.873	×	×	0.285	5.927
ZINC95098956	No	0.398	×	×	2.58	-0.077	×	×	0.285	-1.691
ZINC95098958	No	0.39	×	×	2.537	0.118	×	×	0.285	-1.145
ZINC95099527	Yes	-0.985	×	×	3.881	2.318	×	×	0.285	3.011
ZINC95099570	No	0.327	×	×	3.896	1.109	×	×	0.734	0.021

A.T=AMES toxicity; M.T.D=Max.Tolerated dose(human)(log mg/kg/day);H.1.I=hERG I inhibitor; H.2.I=hERG II inhibitor;O.R.A.T=Oral Rat Acute Toxicity(LD50)(mol/kg);O.R.C.T=OralRatChronic Toxicity(LOEL) (logmg/kg_bw/day);H.T=Hepato toxicity;S.S=Skin Sensitisation. T.P.T=*T.Pyiformis* toxicity(logug/L);M.T=Minnow Toxicity(logmM)

Discussion

Malaria is still a major global health problem, although antimalarial drugs are available drug resistance has emerged thereby increasing morbidity and mortality rate. The research for the development of efficient licensed malaria vaccine is still not over despite many rigorous efforts have been made in the last decade. It was previously suggested in the literature that the proteins such as actin, tubulin, and histone are involved in the structural assembly of the pathogen.^[43,44] Hence, the potential new drug target Actin I is one of the most important structural proteins which is found in *P. falciparum* and plays important role in its motility apparatus, sexual, asexual and blood stages of the organism.^[39] It is expressed in all human stages and little is known about its potential as a drug target. In the current repertoire, we have considered library of 1574 natural organic molecules of diverse nature which were docked with Actin I protein of *P. falciparum* employ in one of the most important docking software.^[6] AutoDock 4.2. The selected molecules were further subjected to their pharmacokinetic studies to make them potentially promising drug candidates.^[45] These approaches were widely utilized to design drugs against a number of pathologies such as cardiovascular diseases and cancer.^[46-48] The current study is the first report to explore the potential of receptor protein Actin I as an efficient drug target in structure-based drug design.

The prospect of virtual screening techniques was employed to screen the 20 top scoring molecules on the basis of their

binding affinity. Although EFEB of ligand protein interaction is relative score and direct correlation of this score is not studied in different docking studies, EFEB of selected ligands in current study was better than the ligands found to be active in recent *in silico* and *in vitro* studies conducted by our group in falcipains 2 and 3 of *P. falciparum*.^[49] In addition, when the docked complexes of these selected hit molecules were subjected to post docking analysis, they displayed good interaction patterns (hydrogen as well as hydrophobic interactions). The compound with zinc ID ZINC30724344 was the top scoring molecule and also depicted good interactions indicating a potent promising candidate. Similar trend was also discovered for other remaining 19 molecules which were also prioritized as hit molecules displaying good binding affinities and interaction patterns [Table 1]. Furthermore, the 20 top scoring molecules interact strongly with the target protein forming number of hydrogen bonds [Table 1] [Figures 2 and 3]. In most of these top scoring molecules Lys337, Asn17, and Lys19 were found to be most common interacting residues forming hydrogen bonding. The top scoring molecules exhibit better binding affinity in terms of EFEB as compared to the antimalarial drugs which were used as positive control [Supplementary Table 1].

Further, these top scoring molecules were subjected to ADMET prediction and physicochemical analysis to ensure drug likeness of hit molecules as part of Lipinski's rule of five. The ADMET properties are important criteria because

significant number of molecules failed in the clinical trial due to poor ADMET properties.^[50] The top scoring molecule from docking studies ZINC30724344 exhibited molecular weight of 592.696 Da, logP of 5.01246, HBA of 6 and HBD value as 2. Furthermore, this hit molecule was non-toxic as per Ames test data and displayed good intestinal absorption (84.303%) and skin permeability (logKp-2.735) values. The molecular weight and logP were observed to be slightly higher as per the standard rules, but natural origin and all other parameters justified that the above potential hit molecule can be developed against malaria which can serve as a roadmap for perusing drug discovery process. The remaining molecules having zinc ID ZINC38145808, ZINC95099570, ZINC14883289, and ZINC02138728 also showed high binding affinities and these molecules also justified the different essential parameters to be considered as hit molecules for the drug discovery [Table 1] [Figures 2 and 3]. Although current results are very promising, computational studies require further wet lab experimental verification including target validation. Moreover, current findings warrants that these selected hit molecules can be subjected to further wet lab experimentations for the efficacy of hits compound *in vitro* and *in vivo* studies against plasmodium and subsequently modification accordingly so that these molecules can be used as potential hit molecules for anti-malarial drug discovery.

Conclusion

First time virtual screening methodology was implemented successfully for Actin I of *P. falciparum* using structure-based drug design. Library of diverse molecules was screened to find potential hit molecules on the basis of EFEB. Protein ligand interaction and ADMET properties of top selected scoring molecules were found to be favorable. Among the selected top scoring molecules, the five molecules, that is, ZINC30724344, ZINC13515285, ZINC03943903, ZINC95098956, and ZINC03984030 looks more prominent as a potential candidate for further drug development. The current findings provide a suitable starting point for further *in vitro* and *in vivo* analyses to exploit Actin I as an optimal drug target which can encounter drug resistance.

Authors' Declaration Statements

Ethics approval and consent to participate

Not applicable (as no human subject was used in the study)

Consent for publication

Not applicable (as no human subject was used in the study)

Availability of data and material

The data used in this study are available and will be provided by the corresponding author on a reasonable request.

Competing interests

None declared.

Funding Statement

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Authors' contributions

The research "Conceptualization, V.J. and S.C.; Methodology, V.G.; Software, B.S. and V.G.; Validation, T.P., and B.S.; Formal Analysis, B.S. and V.G.; Data Curation, T.P. and B.S.; Writing – Original Draft Preparation, S.C. and V.G.; Writing – Review & Editing, V.J., T.P., B.S. and S.C.; Visualization, V.G. and B.S.; Supervision, V.J. and T.P.; Project Administration, V.J.;

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Supplementary Data

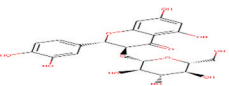
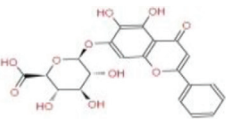
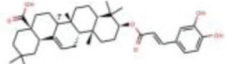
Supplementary Table 1. Docking of Actin I with different Antimalarial drugs:

S.No.	Compound name	Binding Affinity	Hydrogen bonding
1.	Chloroquine	-8.2	Thr304
2.	Chloroguanide	-7.1	Lys19, Gly303, Glu215
3.	Quinine	-7.6	Asp180
4.	Mefloquine	-8.5	Thr278, Ala273
5.	Halofantrine	-8.0	Thr278, Arg178, Ala273

Supplementary Table 2: Top 20 molecules ki (Values)

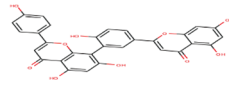
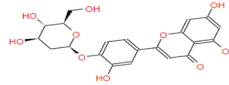
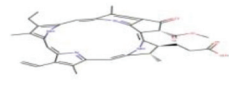
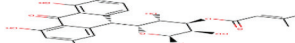

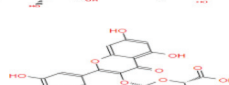

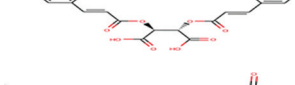

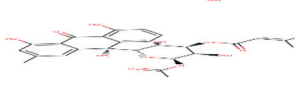
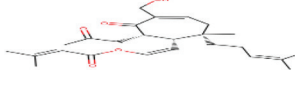
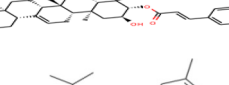
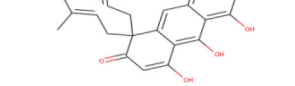
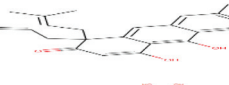
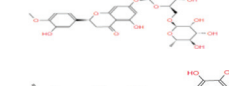
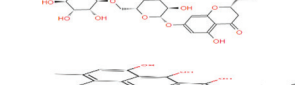
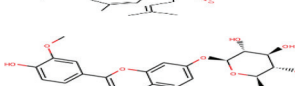
Zinc Id	Ki (value)
ZINC13515285	567.57 pM (picomolar)
ZINC03943903	608.42 pM (picomolar)
ZINC95098956	862.04 pM (picomolar)
ZINC03984030	1.08 nM (nanomolar)
ZINC04349517	1.59 nM (nanomolar)
ZINC30724344	211.52 pM (picomolar)
ZINC28652364	5.95 nM (nanomolar)
ZINC95098947	5.39 nM (nanomolar)
ZINC28538752	6.47 nM (nanomolar)
ZINC38145808	6.88 nM (nanomolar)
ZINC95099527	7.22 nM (nanomolar)
ZINC28652356	7.45 nM (nanomolar)
ZINC95099570	9.59 nM (nanomolar)
ZINC95098958	17.77 nM (nanomolar)
ZINC02138728	18.14 nM (nanomolar)
ZINC14883289	18.14 nM (nanomolar)
ZINC08143568	19.10 nM (nanomolar)
ZINC08234294	19.63 nM (nanomolar)
ZINC15215608	19.61 nM (nanomolar)
ZINC45495236	19.93 nM (nanomolar)

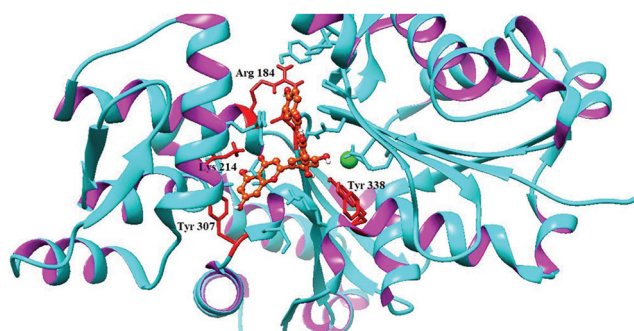
Supplementary Table 3: Top 20 molecules structures

Zinc Id	Structure
ZINC13515285	
ZINC03943903	
ZINC95098956	

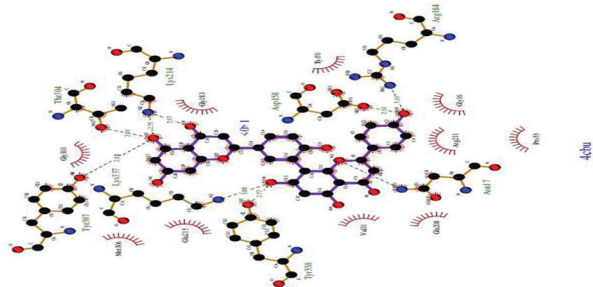
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Supplementary Table 3: (Continued)

Zinc Id	Structure
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ZINC04349517	
ZINC30724344	
ZINC28652364	
ZINC95098947	
ZINC28538752	
ZINC38145808	
ZINC95099527	
ZINC28652356	
ZINC95099570	
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ZINC45495236	

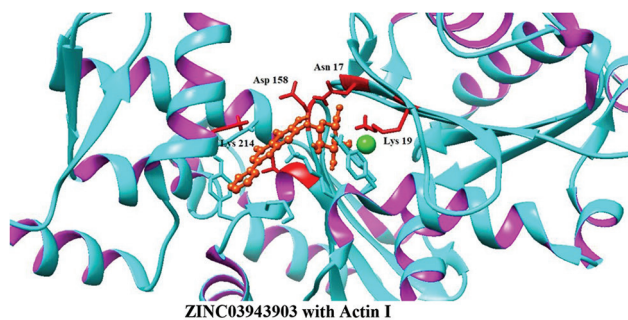


ZINC03984030 with Actin I

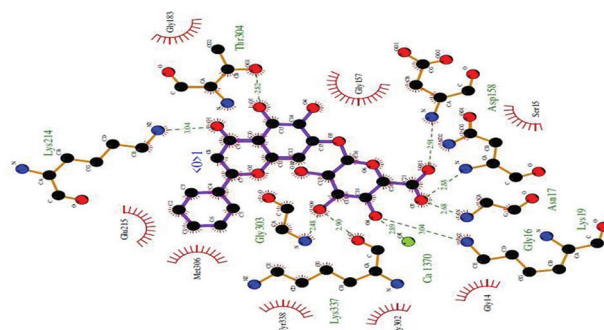


Interaction of Actin I with ZINC03984030

Supplementary Figure 1: Interactions of ZINC03984030 with Actin I

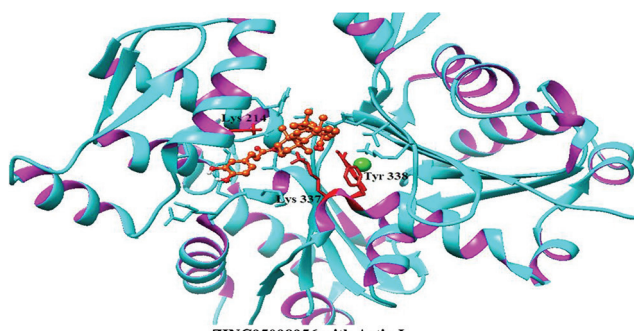


ZINC03943903 with Actin I



Interaction of Actin I with ZINC03943903

Supplementary Figure 3: Interactions of ZINC03943903 with Actin I



ZINC95098956 with Actin I

