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Research Article

IN SILICO SCREENING FOR IDENTIFICATION OF NOVEL NON-PEPTIDIC FALCIPAIN 3 INHIBITORS BY VIRTUAL SCREENING, MOLECULAR DOCKING, AND MD SIMULATION

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ABSTRACT

Objective: The development of two new non-peptidic inhibitors against Falcipain 3 using computer-aided design.

Methods: The researchers started by narrowing down a virtual library of compounds from the PubChem database to 800 drug-like compounds, which were then virtually screened and docked to identify the two most promising inhibitors. The screened compounds were then further studied using Molecular Dynamics Simulation.

Results: The screened compounds were found to have potent antimalarial activity *in silico*.

Conclusion: The proposed two lead compounds would serve as excellent targets for antimalarial drug. The efficacy of these potent inhibitors could be validated with laboratory experiments, with the goal of eventually developing an anti-malarial drug.

Keywords: Falcipain 3, Docking, MD Simulation, Virtual Screening, Antimalarial, *Plasmodium.*

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INTRODUCTION

Increasing resistance of malaria parasites to conventional antimalarial drugs is an important factor contributing to the persistence of the disease as a major health threat [1,2]. This increasing burden of resistant malaria has stimulated drug discovery scientists to search for new antimalarial drugs or alternative therapeutic options to combat the problem of drug resistance. The *Plasmodium falciparum* cysteine proteases, also known as falcipains, are involved in different erythrocytic cycle processes of the malaria parasite, *for example,* hydrolysis of host hemoglobin, erythrocyte invasion, and erythrocyte rupture. With the biochemical characterization of four falcipains so far, Falcipain 2A (FP2A), Falcipain 2B (FP2B), Falcipain 3 (FP3), and Falcipain 1 (FP1) members of the papain-like C1A family, are essential hemoglobinase [3-8]. They could, therefore, be referred to as potential anti-malarial drug targets in the search for novel therapies, which could ease the burden caused by the increasing resistance to current antimalarial drugs.

Several attempts combining chemical synthesis and *in silico* screening approaches have been undertaken in the past decade to discover and optimize inhibitors targeting parasite proteases, leading to two broad classes of chemical compounds through peptidometric and non-peptidic small molecules [4]. Such attempts have successfully led to the discovery of several peptidic compounds but none has ever been commercialized as an antimalarial drug due to inherent poor pharmacological profiles as well as susceptibility to degradation by host enzymes [3,4]. The identification and validation of small non-peptide FP inhibitors are expected to overcome these shortcomings.

A diverse set of compounds from the literature is reported to have potency against FP3. Several groups have designed non-peptidic small inhibitors of Falcipains using Computer Based Designing and Virtual Screening studies; however, most of the inhibited Falcipain in micromolar range concentrations [9,10]. Hence far, several compounds belonging to isoquinolines, chalcones, and others [3] have been shown to possess antimalarial potency either on whole *P falciparum* parasite cultures or directly on FP3 protein at low micromolar ranges. Therefore, it is desirable to design non-peptidic inhibitors that would bind non-covalently to the target enzyme to minimize toxicity while retaining the potential for high *in vivo* activity and selectivity. Literature reveals that non-peptidic carbaldehydes have been found to have inhibited FP2, a similar hemoglobinase of *Plasmodium* [2,11-13]. Compounds 2-(benzylamino)-8-methylquinoline-3-carbaldehyde (PubChem44138738), 6-bromo-2-(3,4-dihydro-1H-isoquinolin-2-yl) quinoline-3-carbaldehyde (PubChem 20983198), 2-(3,4-dihydro-1H-isoquinolin-2-yl)-6-ethylquinoline-3-carbaldehyde(PubChem 20983081)and 2-[benzyl(methyl)amino]quinoline-3-carbaldehyde (PubChem 28951461) 20983109 (6-chloro-2-(3,4-dihydro-1H-isoquinolin-2-yl)quinoline-3-carbaldehyde),2-arylbenzofuran-3-carbaldehydes, andinermal A–C, 2-chloroquinoline-3-carbaldehydes and their derivatives are some aromatic aldehydes that have attracted the attention of researchers as antimalarial [12-17].

Thus, in continuation of the prior research with the discovery of several potent non-peptidic leads against hemoglobinase FP2, we in the present study intended to present novel diverse non-peptidic FP3 inhibitors with aldehyde pharmacophore. As FP3 is an equivalently potent target for antimalarial drug. Moreover for complete elimination of the parasite, both the FP2 and FP3 have to be inhibited.

With this view in mind, we extended the study by virtual screening of 800 compounds with carbaldehydes moiety from the PubChem database. This exercise helped to choose 8 top-ranking molecules with carbaldehydes pharmacophore and having a good fit with the target protein. Docking these eight compounds individually using the software Discovery Studio we arrived at 2 compounds (PubChem 44138737 and PubChem 63631782) with C-docker score ranging from −455.56 to −640.682 kcal/mol. These two selected compounds were further equilibrated by Molecular Dynamics simulation and were considered as potential compounds for in-depth analysis.

METHODS

Virtual screening of ligands and drug likeliness assessment

Ligands with carbaldehydes pharmacophore were screened from the commercially available PubChem database after checking for their drug-likeliness properties. This resulted in around 800 lead compounds which underwent high throughputs screening using PyRx Virtual Screening Tool.

The prediction of drug toxicity and ADME properties are major filtration criterion for the drug design process. Various mathematical predictive ADMET pharmacokinetic parameters such as blood-brain-barrier penetration, human intestinal absorption, aqueous solubility, etc. were calculated quantitatively for all the selected ligands using ADMET modules and Lipinski rule of 5 in Discovery Studio v3.5 client. Finally, based on toxicity assessment, all our selected ligands passed through the drug-likeliness assessment tests and were further considered for docking studies.

Docking studies

Our objective was to evaluate whether the filtered hit molecules bind in the active site of the protein FP3. The filtered molecules were subjected to docking into that active site on Discovery Studio 3.5. Protein preparation protocol had been followed for preparing the protein and the filtered molecules were docked into the active sites on the prepared protein. During protein preparation, the water molecules were removed along with the addition of hydrogen atoms. During the docking process, all the amino acid residues were kept rigid while ligands were kept flexible with the docking box covering the maximum possible protein surface. Docking generated 10 different poses for each filtered molecule. During the analysis, scoring function and binding interaction for every single conformational pose were chosen as a selection criterion [16]. The molecules which show interactions with the important active site residues with requisite geometry along with high docking score were selected as more desirable hit molecules [17,18].

Molecular dynamics simulations

MD simulations were carried using the GROMACS package v4.5.5 [19]. The GROMOS 96–43a1 force field was employed for PDB downloaded FP3 (3BWK) structure. The Ligand topologies were generated using PRODRG2 server [20]. In a separate set of simulations, each protein complexes were placed in cubical boxes, equidistantly at 10Å distance from box edges. Following this, hydrogen atoms were added using pdb2gmx module of GROMACS and were constrained using the LINCS algorithm [21]. With periodic boundary conditions applied in all three dimensions, the system was explicitly solvated using with SPC/E water model, and appropriate counter ions were added to neutralize the system [22]. The solvated system was subjected to steepest descent energy minimization to remove steric conflicts between atoms. The energy-minimized systems were then subjected to position-restrained simulation in two different phases, NVT and NPT. Particle mesh Ewald (PME) method was employed to treat long-range electrostatic interactions with the cut-off radius of 10Å. The system temperature of 300K was kept constant by modified Brenden coupling. The MD simulation of complexes was carried out for 20 ns to find out the backbone and side-chain adjustments of the receptor with the ligand and to analyze the contribution of H-bonding, hydrophobic contacts, polar and van der Waals interactions for maintaining the stability of the receptor cavity in dynamic condition. The structural coordinates were recorded at intervals for all the 3 top-ranked compounds. Especially for the best ones, three individual production runs were executed for a better sampling of results. The resulting trajectories were later analyzed for RMSD, Radius of gyration, and energy using XmGrace package to generate the 2D plot whereas PyMOL 1.3 (www.pymol.org) and DS Visualizer 3.5 were used for visual interaction analysis.

RESULT

In this work, different *in silico* approaches were applied to virtually screened leads for potential inhibitors targeting *Plasmodial* hemoglobinase, FP3 for antimalarial therapy. As it is established that structurally similar compounds have same pharmacological features, we performed a virtual screening (ligand-based) in PubChem database, to identify structurally similar compound to carbaldehydes, which is a reported inhibitor [12,15,17]. Our search resulted in 800 compounds, which were subjected to further high throughput virtual screening.

Virtual screening

After screening, a subset of 86 small molecules (Filter1) was obtained. This subset was further screened virtually to result in a subset of 23 ligands (Filter2). These 23 compounds can be considered as the best compounds showing significant affinities with the target protein, the values are represented in Table 1.

Drug likeliness assessment

All the screened compounds satisfied the drug-likeliness properties (Fig. 1). The results of predicted drug-like properties and the drug-likeness score of the two most potent lead compounds are presented in Table 2. The values of the calculated drug-like properties are in an acceptable range for both the compounds, which ranged between 0.63 and −0.37. Higher drug-likeness scores were found for compounds PubChem 44138737. The results of drug-likeness studies strongly suggest that selected compounds have drug-likeness behavior favorable for membrane permeability, transport, and bioavailability, and also interaction with the receptor.

Docking studies

The binding site of FP3 consists of four defined subsites, namely, S1, S2, S3, and S1' (Fig. 2 and Table 3). The filtered compounds were found to dock at the active cleft of the Falcipain protein (Fig. 2). Among the 23 compounds analyzed, eight compounds could be successfully docked with the C-Docker scores ranging from −640.682 to −285.171 Kcal/mol (Table 4), among which, the compound PubChem 63631782 showed the highest c-docker score. These compounds formed several hydrogen bonds with residues CYS51, SER62, SER150, PRO181 GLY83, HIS183, and ASP234 in S1, and S3 binding subsites (Fig. 3). Apart from these several ionic bonds, hydrophobic interactions were also observed (Fig. 4). After successful docking analysis, the compounds with higher C-docker scores were taken for further analysis using molecular dynamic simulations.

Table 1: The A log P98 and PSA-2D data for ADMET prediction of 23 selected compounds for protein FP3

S. No.	Ligand'S PubChem ID	ADMET_ ALOGP98	ADMET_ PSA_2D
1	Lig102546483	3.88	31.914
2	Lig2120269	3.419	35.718
3	Lig43584656	4.60	44.296
4	Lig105502297	3.713	31.914
5	Lig44138737	4.07	48.991
6	Lig43584646	5.048	35.266
7	Lig81800149	5.3	35.266
8	Lig81800230	5.107	31.914
9	Lig57444439	5.257	35.266
10	Lig28951468	4.116	31.981
11	Lig44138738	4.105	41.570
12	Lig43295998	3.658	52.574
13	Lig55100553	3.006	31.914
14	Lig62468644	4.814	31.914
15	Lig63631782	3.402	31.914
16	Lig63864107	4.001	31.914
17	Lig61980989	4.608	31.914
18	Lig65281356	4.408	31.914
19	Lig60890812	3.125	35.266
20	Lig94538323	3.976	31.914
21	Lig105511021	2.351	44.725
22	Lig62860985	2.434	35.266
23	Lig114172464	2.065	40.834

Table 2: Result of predicted Drug Likeness properties of the TWO most potent lead compound against Falcipain 3

S. No	Pubchem Id	m.w.	Log P[a]	Log Sb[b]	hydrogen bond acceptors[c]	hydrogen bond donors[d]	Rot B[e]	PSA[f] (A^2)	Violation of rule of 5	Drug-likeness score Molsoft
A	44138737	296.19	4.38	−4.84	2	0	1	48.99	0	0.63
B	63631782	292.25	3.03	−2.90	3	1	4	31.914	0	−0.37

[a]logarithm of the octanol-water partition coefficient (Log P), [b]logarithm of the compound's aqueous solubility (Log Sb), [c]number of hydrogen bonds acceptors (hydrogen bond acceptors), [d]number of hydrogen bonds donors (hydrogen bond donors), [e]number of rotable bonds (Rot B), [f]polar surface area (PSA).

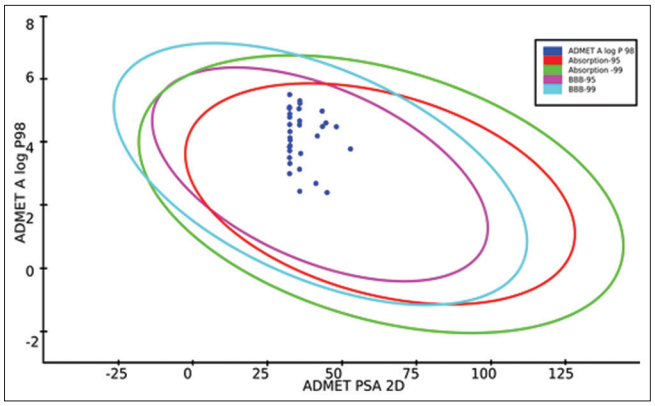

Fig. 1: The ADMET plot for the screened compounds. The two sets of ellipses are for the prediction confidence space [95% and 99%] for the Blood-Brain Barrier penetration and Human Intestinal Absorption models, respectively

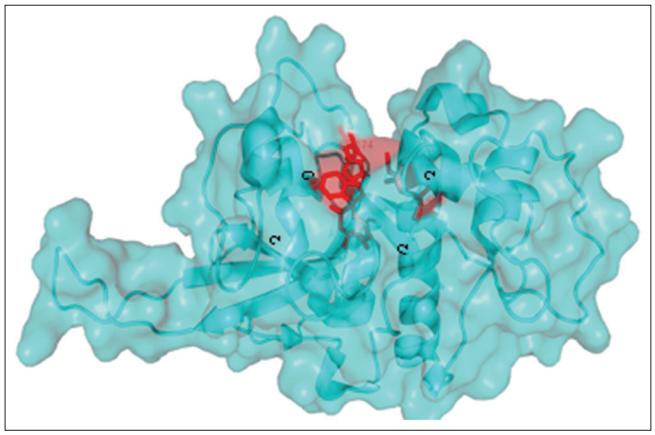

Fig. 2: Surface representation of Falcipain 3 with superimposition of the docked conformations of the lead compounds. The ligands are shown in stick. The surface figure was prepared using PyMOL

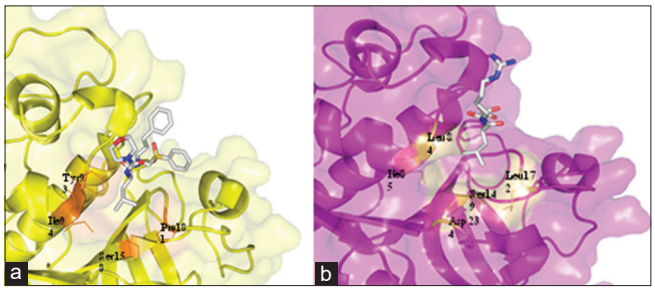

Fig. 3: (a and b) The proposed binding mode of the 2 compounds into the binding pocket of FP3

The compounds are shown as sticks and the non-carbon atoms are colored by atom types.

Table 3: Important residues lining the binding pockets of Falcipain-3

Subsites	Falcipain-3 Residues
S1	Q45-G49-C89-N87-S50-W52-C51-Y90
S2	N182-D213-A161-A184-Y93
S1'	S158-A184-H183-W215-N182-P181-S162-A160
S3	G91-N45-G92-G49-I94 -N87

Table 4: Results of discovery screening of 8 ligands among 23 ligands (Filter 3) for FP3

S. No	Ligand PubChem ID	C-Docker Energy (Kcal/mol)
1	63631782	−640.682
2	44138737	−455.56
3	63864107	−430.932
4	62468644	−388.685
5	105511021	−306.81
6	44138738	−408.937
7	43584656	−471.367
8	60890812	−285.171

Molecular dynamic simulations

The RMSD profile of the two selected docking complexes is shown in Fig. 5a and b. The mean value of RMSD ranges from 2 to 3 Å for both the protein-ligand complexes.

The root mean square fluctuations (RMSFs) of Cα atoms of the two docking complexes were subsequently calculated as displayed in Fig. 5c and d. From the figure, we noticed fluctuations of amino residues, which are found to be higher in around the amino residues 150–250 as displayed in the trajectories. The average packing density of each of the protein complexes decreased in their complex state Fig. 5e and f.

Furthermore, the potential energy and kinetic energy for the two docking complexes were analyzed. The potential energy has a negative value, thereby confirming the stability of the two docking complexes Fig. 5g and h. As observed from the Fig. 5i and j, a positive value for kinetic energy was perceived.

Moreover, to shed light on the intermolecular hydrogen bonding pattern, H-bonds between FP3 receptor and the two potent lead compounds in its binding pockets were calculated. The protein FP3 with compound PubChem 44138737, the H-bonding was predominant and persistant particularly towards the end of the simulation. Whereas for the compound PubChem63631782, the H-bonding was mainly seemed to be dominant towards the second half of the simulation Fig. 5k and l.

DISCUSSION

Virtual screening

Virtual screening of small-molecule libraries forms one aspect of a sophisticated approach to drug discovery. For further refined results, the compounds in the subset, Filter2, were separately docked using docking software Discovery Studio.

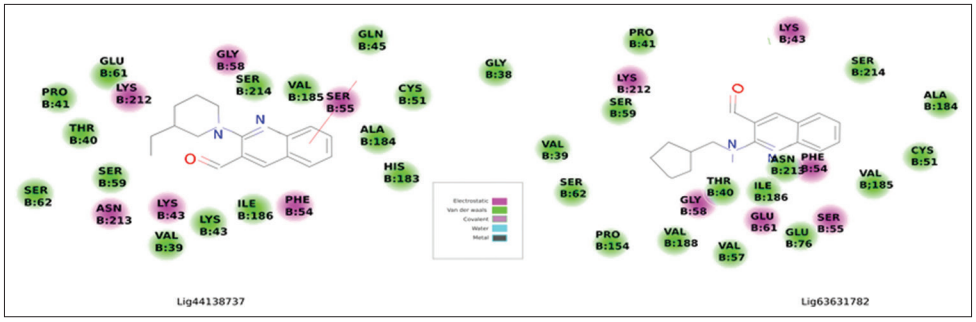

Fig. 4: 2D diagram analysis indicated that apart from hydrogen bonding interactions, various non-bonded interactions are involved between binding site residues (active site amino acids) and ligand moieties/atoms

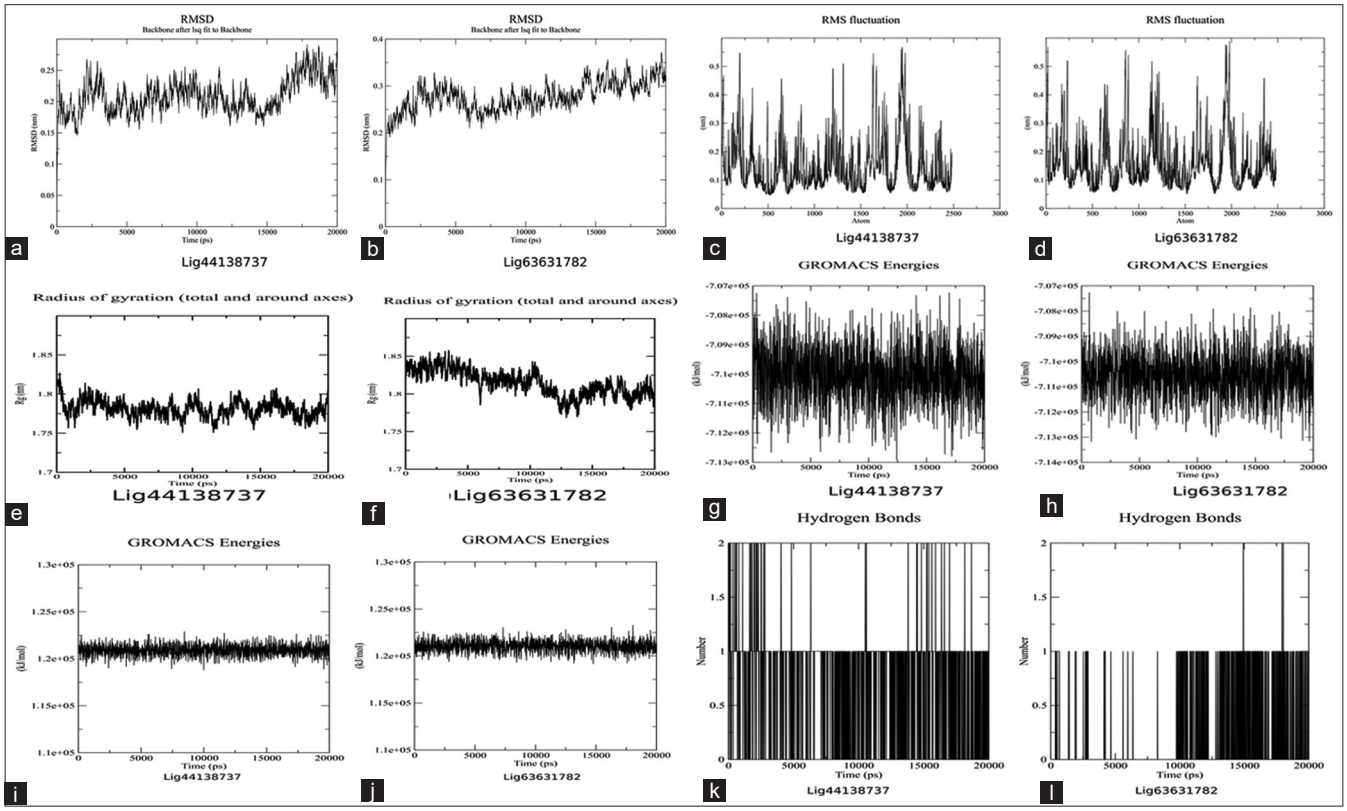

Fig. 5: The molecular dynamics simulation trajectories of FP3 complexed with the two PubChem lead compounds (PubChem 20983109 and PubChem 57469658). (a and b) RMSD of backbone C α atoms of FP3 complexes. (c and d) RMSF analysis of amino acid residues of FP3 complexes. (e and f) Radius of Gyration of FP3 complexes. (g and h) Potential Energy of FP3 complexes. (i and j) Kinetic energy of FP3 complexes. (k and m) H-Bond of FP3 complexes. All the images were generated using XMGRACE software. FP3: Falcipain 3

Drug likeliness assessment

Drug-likeness properties are an important indicator for selecting the compounds for *in vitro* studies, which includes molecular or physicochemical properties that contribute to favorable Lipinski's rule of five and ADMET. The "drug-likeness" test was carried out using Lipinski's RO5: Molecular weight (MW), number of hydrogen bond acceptors, lipophilicity (log P), number of rotatable bonds, and number of hydrogen bond donors of the compounds were considered as the basis for accessing the drug-likeness. Molecular properties are fundamental structural properties which determine the physicochemical (solubility, permeability) and biochemical (metabolic stability, transport property, protein/tissue affinity) properties, which in turn ultimately determine molecule's pharmacokinetics (bioavailability, half-life), toxicity, and pharmacodynamics (receptor affinity and efficacy) in biological systems [22, 23].

Docking studies

In the case of FP3, complexed with compounds, the active site residues, SER62, SER150, TRY93, PRO181 GLY83, HIS183, CYS51, and ASP234 were found to be involved in the formation of additional hydrogen bonds, and non-covalent interactions like Van der Waals (vdW) and electrostatic forces, principally favored binding (Figs. 3 and 4). However, there was a drop in the contributions from electrostatic terms, a fact that can be explained by the few numbers of hydrogen bonds that were formed between the protein-ligand groups due to the chemical nature of the ligands.

Molecular interaction study of the two selective docking complexes

The in-depth inter-molecular interactions of the two selected compounds with the protein FP3 were studied using Discovery Studio and PyMOL. About 20 contacts were found to have formed between protein FP3 and the screened potent lead compounds. Residues ASN182 and HIS183 of the S1 pocket were found to be involved in hydrogen bonding with the screened hits, whereas residues CYS51, SER62, and PHE54 were involved in various hydrophobic interactions between the protein and the leads. Similar residue interactions were also observed by docking leupeptin, a standard inhibitor with FP3. Thus by comparing the

docking modes of the target proteins with various other substrates as found in the literature, common binding characteristics were observed among the active cleft residues interactions with the screened hits. As the active site residues' substituents were hydrophobic, it is obvious that each complex's active site was lined by a number of residues that can make non-polar interactions with their respective inhibitor. This finding was also in agreement with the finding reported by Rosenthal with his co-workers in 2001 [13].

The compound also formed pi-pi stacking and various hydrophobic contacts with residues CYS51; SER149; HIS183; HIS182; SER62; LUE84; ILE85; PHE54; ASN86; ASP234; GLY83; ALA175; GLY83; GLY40; and ASN81 of FP3 Fig. 3. However, the compounds formed extensive electrostatic and Van der Waal interactions with residues TRP43; CYS51; ALA175; LUE172; SER62; ILE94; ILE85 ASN182 GLY40; ASP234; PHE236 SER149 ASN86; LUE84; and GLY82 of the protein FP3, respectively, as shown in Fig. 4. Moreover, the exosite of FP3 was reported to be distantly located from the active cleft in the falcipain "arm" [2,24,25]. Since all the screened hits under study are bound to the active cleft of the protein FP3, so no lead was found to occupy the exosite or any allosteric site of the target proteins [26,27].

Molecular dynamic simulations

In the molecular dynamics study, we analyzed the stability and convergence of the two selected docking complexes as a function of time. The equilibrated model structures of the two docking complexes were used as a reference structure for further RMSD, Rg, and RMSF analysis.

From the RMSD trajectories, PubChem63631782 showed greater stability while forming a complex with FP3 than the other complex, suggesting that it has more potential leads than the latter. Whereas the RMSF graph, it can be observed that the amino acids positioned in 150–250 (within the catalytic domain) residues showed more deviation reflecting the flexibility of this region in the FP3 protein. The Radius of Gyration (Rg) enables one to assess the compactness changes in the protein-ligand complexes. The graph showed that the Rg trajectories for both complexes explored the firmness of both protein-ligand complexes.

Thus, the trajectory results suggest that the two selected compounds are reliable as potent leads to target FP3. In addition, the stability of the systems also strengthened the credibility of the docking results. Thus, the stable structures for the two docking complexes indicated the dynamic stability of the ligand-bound holo state [12,25].

CONCLUSION

We have used a series of *in silico* experiments and then concluded that compounds PubChem 44138737 *2-(benzylamino)quinoline-3-carbaldehyde* and PubChem 63631782 *2-[cyclopentylmethyl(methyl) amino]quinoline-3-carbaldehyde* can act as lead compound against protein FP3, a hemoglobinase of parasite *Plasmodium* that cause malaria. We have also evaluated that the predicted hits go well through ADMET descriptors. From the results, we have also concluded that the predicted compounds are non-toxic, non-carcinogenic, and good in the blood-brain barrier permeability. These predicted leads can be further evaluated through *in vitro* and *in vivo* experiments and can be developed as a drug against malaria. As this enzyme, FP3 is necessary for parasite survival, the compounds that could inhibit the activity of this enzyme can be suggested as the potent compound in antimalarial drug therapy. Furthermore, the non-peptidic nature of the compounds made them less susceptible to be degraded by host proteolytic enzymes. Thus it is anticipated that the proposed two lead compounds would serve as excellent targets to develop new drugs for the prevention of malaria.

FUTURE PERSPECTIVE

The present study included a multistep computational approach to the introduction of novel non-peptidic compounds to enrich the database of antimalarial compounds. There are scopes for undertaking series of *in vitro* experiments for validation of the *in silico* data and thereby may lead to the development of novel drug against deadly diseases Malaria.

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CONFLICTS OF INTEREST

Authors declared no conflicts of interest.

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