J Vector Borne Dis 50, June 2013, pp. 93–102

Computation-based virtual screening for designing novel antimalarial drugs by targeting falcipain-III: A structure-based drug designing approach

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ABSTRACT

Background & objectives: Cysteine proteases (falcipains), a papain-family of enzymes of *Plasmodium falciparum*, are responsible for haemoglobin degradation and thus necessary for its survival during asexual life cycle phase inside the human red blood cells while remaining non-functional for the human body. Therefore, these can act as potential targets for designing antimalarial drugs. The *P. falciparum* cysteine proteases, falcipain-II and falcipain-III are the enzymes which initiate the haemoglobin degradation, therefore, have been selected as targets. In the present study, we have designed new leupeptin analogues and subjected to virtual screening using Glide at the active site cavity of falcipain-II and falcipain-III to select the best docked analogues on the basis of Glide score and also compare with the result of AutoDock. The proposed analogues can be synthesized and tested *in vivo* as future potent antimalarial drugs.

Methods: Protein falcipain-II and falcipain-III together with bounds inhibitors epoxysuccinate E64 (E64) and leupeptin respectively were retrieved from protein data bank (PDB) and latter leupeptin was used as lead molecule to design new analogue**s** by using Ligbuilder software and refined the molecules on the basis of Lipinski rule of five and fitness score parameters. All the designed leupeptin analogues were screened via docking simulation at the active site cavity of falcipain-II and falcipain-III by using Glide software and AutoDock.

Results: The 104 new leupeptin-based antimalarial ligands were designed using structure-based drug designing approach with the help of Ligbuilder and subjected for virtual screening via docking simulation method against falcipain-II and falcipain-III receptor proteins. The Glide docking results suggest that the ligands namely result_037 shows good binding and other two, result_044 and result_042 show nearly similar binding than naturally occurring PDB bound ligand E64 against falcipain-II and in case of falcipain-III, 15 designed leupeptin analogues having better binding affinity compared to the PDB bound inhibitor of falcipain-III. The docking simulation results of falcipain-III with designed leupeptin analogues using Glide compared with AutoDock and find 80% similarity as better binder than leupeptin.

Interpretation & conclusion: These results further highlight new leupeptin analogues as promising future inhibitors for chemotherapeutic prevention of malaria. The result of Glide for falcipain-III has been compared with the result of AutoDock and finds very less differences in their order of binding affinity. Although there are no extra hydrogen bonds, however, equal number of hydrogen bonds with variable strength as compared to leupeptin along with the enhanced hydrophobic and electrostatic interactions in case of analogues supports our study that it holds the ligand molecules strongly within the receptor. The comparative e-pharmacophoric study also suggests and supports our predictions regarding the minimum features required in ligand molecule to behave as falcipain-III inhibitors and is also helpful in screening the large database as future antimalarial inhibitors.

Key words Antimalarial drugs; docking; falcipain-III; ligbuilder; *Plasmodium falciparum;* virtual screening

INTRODUCTION

Malaria is one of the disastrous health problem for most of the regions of the world, specifically in endemic regions like India and Africa. In 2010, WHO estimated that 219 million cases were registered for malaria and among those 90% were caused due to *Plasmodium falciparum* alone over worldwide[1]. There are various other species of *Plasmodium* infecting cattle, birds, and humans, but mainly there are four species—*P. falciparum, P. vivax, P. malariae* and *P. ovale* in particular considered important as these species infect humans[2, 3]. One of the main causes for the comeback of malaria is due to resistance against the most frequent and widely used drug against malaria, e.g. mefloquine and chloroquine. The reason has been attributed to the decreased susceptibility of resistance transporters, PfCRT[4] for chloroquine and PfMDR1 for multidrug resistance[5]. Artemisinin is being used at present as the sole antimalarial[6]. *Plasmodium* life cycle is completed through many morphologically changes,

which alternate between an invertebrate and vertebrate host. Haemoglobin metabolism is one of the key for metabolic processes for the survival of the parasite in its human blood stages.

There are several proteases involved in the degradation of host erythrocyte haemoglobin inside the specialized acidic food vacuole (pH 5–6) of the parasite. To obtain some of the nutrients required for intra-erythrocytic growth the parasites digests 25–75% of the host cell haemoglobin, liberating free amino acids for incorporation into parasite proteins[7]. Aminopeptidases catalyse the cleavage of amino acids from the amino-termini of protein or peptide substrates[8]. Due to emergence of resistance against several antimalarial drugs it is utmost importance to keep searching for new drugs and drug combinations to inhibit the growth of protozoa. This requires work on novel molecular targets in *Plasmodium* and designing inhibitors with potential parasiticidal activities. To date several drug targets have been identified by the sequencing of *P. falciparum* genome. However, as far as availability of new drugs for malaria is concerned, it has to be based on new biological target proteins.

The potential antimalarial drug targets can be broadly divided into three types, haemoglobin degradation enzymes, signaling and transport protein factors. Within the digestive vacuole haemoglobin is initially degraded by aspartic proteases (plasmepsins I, II, and possibly III and IV)[9]. The cysteine proteases (falcipains I, II and III)[10, 11], further degrade the plasmepsins digested products and the generated short peptides finally digested by the metalloprotease (falcilysin). Apart from this falcipain-II enzyme also degrades the erythrocyte-membrane skeletal proteins which include ankyrin and the band 4.1 to destabilize the erythrocyte membrane which leads to the release of the mature merozoites followed by host cell rupture[12]. The synthesis of falcipain-II occurs during the trophozoite stage as a proenzyme (484 amino acid residues) and bound with membrane. This proenzyme is finally transported to the food vacuole via endoplasmic reticulum/golgi system. During transportation process of falcipain-II the *N*-terminal membrane anchored with 243 residues are proteolytically removed[13, 14].

The ratio of concentration of falcipain-II is 1.8 times higher than falcipain-III concentration in trophozoites stage. The cleaving capacity of falcipain-III is nearly twice as falcipain-II for haemoglobin. This shows the relative contribution of both enzymes equally in haemoglobin degradation and suggests probable targets for novel antimalarial drug designing[15]. The significance of falcipain-II and falcipain-III in the *Plasmodium* life cycle and the presence of X-ray crystal structure data make both the selected proteins as ideal targets for antimalarial therapy using new approaches in rational drug design. In the present study, falcipain-II and falcipain-III have been selected as the targets for designing of novel potent antimalarial drugs for future use with the application of structure-based drug designing approach which includes the application of Lipinski rule of five[16], pharmacophore modeling, virtual screening and docking simulation.

This study knowledge, the possibility of inhibition activity shown by PDB bound natural falcipains inhibitors leads us to design the novel derivatives as probable antimalarial moieties by selecting more than one similar target for small group of designed inhibitors. The objective of present work was to design novel antimalarial derivatives by generating key interaction site and receptor-based pharmacophore for already bound leupeptin order to generate its analogues leading to better inhibitors for deadly disease malaria.

MATERIAL & METHODS

Ligbuilder

This is a genetic algorithm-based tool to design new ligands using structure-based drug designing approach. It uses the known bound inhibitors available in PDB in X-ray crystallized structure or in complex form as a seed molecule. Ligbuilder makes the target protein rigid for running the pocket module to generate key functional features and suggests pharmacophore model for interaction between inhibitor and active site residues. The derived pharmacophore model consists of pharmacophore features and the internal distances between them. For getting the good pharmacophore model the minimal distance between any two pharmacophore features is not to be less than 3 Å and this number ranges between 3 and 8. The fragment of known inhibitors used as building block to develop new ligand molecules by linking or growing strategy for all the molecules originated from a "seed" structure, developed and evolved with a genetic algorithm. The resultant molecules are filtered by using drug-likeness parameter and fitness score, based on chemical viability and binding affinity[17].

LigPrep

It is used for optimization of ligands by generating accurate, chemically corrected, energy minimized 3-D molecular structures supplied with Schrödinger suite. LigPrep optionally expands the ligand to its different form (tautomeric, ionization states, ring conformations and stereoisomers) from single input of ligand structure[18].

QikProp

This is module of Schrödinger suite to screen the small data according to descriptor parameter and prediction of ADMET which also includes rule of five[19].

Glide

It is docking simulation module supplied by Schrödinger suite. Glide performs the exhaustive search employing hierarchical filter for finding most favourable interaction between one or more ligand molecules and a receptor (protein or protein with cofactor). Receptor-grid files were generated after preparing correct forms of proteins and ligands using receptor-grid generation program (shipped by Schrödinger). For grid generation potential of non-polar parts of receptor was softened by scaling van der Waals radii of ligand atoms by 1.00 Å with partial charge cut-off of 0.25. It is having all types of option of speed *vs* accuracy. It is having three mode of docking, high-throughput virtual screening (HTVS), standard precision (SP), and extra precision (XP) mode. The XP mode is used for exhaustive sampling and advanced scoring, resulting in even higher enrichment[20].

Target and ligand selection

The target selection was based on the importance of falcipain-II and falcipain-III proteins together with feasibility due to presence of 3-D structure of X-ray crystallized form of *P. falciparum* falcipain enzymes in PDB. The 3-D structures of falcipain-II and falcipain-III were retrieved with PDB ID: 3BPF, and 3BPM with resolution: 2.90 and 2.50 Å respectively. The downloaded protein falcipain-II having four chains (A, B, C, and D), four epoxysuccinate E64 (molecular formula: $C_{15}H_{30}N_5O_5$) as an inhibitor on each chain, 42 water of crystallization and one glycerol molecule[17]. Whereas the 3-D structure of falcipain-III having two chains (A and B), two leupeptins (Fig. 1) (molecular formula: $C_{20}H_{40}N_6O_4$) as an inhibitor on each chain, 76 water of crystallization and 5 SO_4 molecules as cofactor[21]. The two sets of ligands were designed by using Ligbuilder on receptor falcipain-III and selected for present study on the basis of their fitness score in comparison with leupeptin.

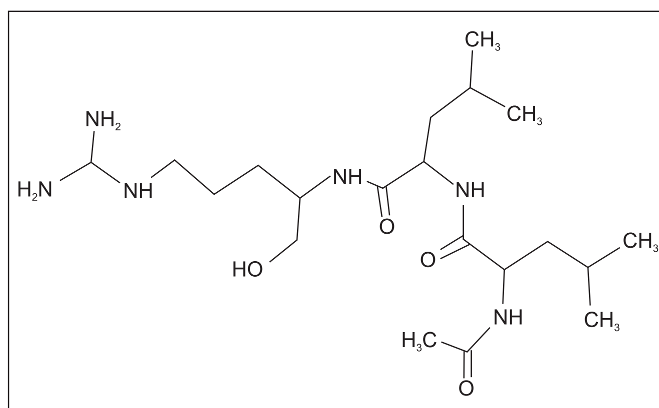

Fig. 1: 2-D structure of leupeptin.

Ligand and target preparation

Both the sets of ligands were screened for ADMET using QikProp and optimized by using LigPrep module by generating different possible structural conformation of ligands and it creates a library of 1500 conformation for each ligand set. For optimization and energy minimization it uses OPLS-2005 force field. The target protein was prepared by deleting the bound cofactors, inhibitors and surrounding water molecules together with the addition of the side chain atoms followed by optimization and energy minimization.

Receptor grid generation and docking

The prepared protein was used as an input file for generating the receptor grid file, which is used as input file for docking simulation. During docking simulation using different module of Schrödinger suite, potential of non-polar parts of ligands was softened by scaling van der Waals radii of ligand atoms by 0.8 Å with partial charge cut-off of 0.15. During docking simulation, Glide first places the centre of ligand at various grid positions of a 1 Å grid and then by rotating ligand in all the Euler angles it generates various possible conformations which pass through a filter series composed of initial rough positioning followed by scoring phase. The docking simulation was performed by allowing flexible torsions in ligands with the use of XP mode, highly robust and very accurate. The parameter selected for docking run was default and a model energy function named Glide score (Gscore) is used which combines force field and empirical terms for selecting the best docking pose, generated as output.

$$\text{Gscore} = a^* \text{ vdW} + b^* \text{ Coul} + \text{Lipo} + \text{Hbond} + \text{Metal} + \text{BuryP} + \text{RotB} + \text{Site}$$

Where, vdW is van der Wall energy; Coul is Coulomb energy; Lipo is lipophilic contact term; Hbond represents hydrogen-bonding term; Metal is metal-binding term; BuryP is penalty for buried polar groups; RotB represents penalty for freezing rotatable bonds; Site is polar interactions at the active site; and a, b representing coefficients of vdW and Coul are set as: a = 0.065; and b = 0.130.

The molecular docking simulations output file, having all the thermodynamics information in the form of

Glide score, were analyzed using Glide XP visualizer, which enables visualization of ligand-receptor interactions in an interactive manner.

In the case of docking simulation using AutoDock, input files were prepared using MglTools and AutoGrid modules. AutoGrid generates grid files for each atom types found in ligand molecules. For docking purpose ligands single bonds are allowed to rotate freely and Lamarckian genetic algorithm has been used. The grid box limit as 90 × 90 × 90 points and grid spacing 0.375 Å was chosen[22].

E-pharmacophore hypothesis generation

It is the hybrid approach of ligand and structure-based technique which uses docking energy score for finding the bioactive component of ligands against the receptor. For the generation of energy-based pharmacophore (e-pharmacophore) docking post-processing module of script option was selected and input file given in .xpdes format. E-pharmacophore was generated by using all the default chemical features [hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobe (H), aromatic ring (R), positive ionizable (P), and negative ionizable (N)][23].

RESULTS & DISCUSSION

Designing of leupeptin analogues at the active site cavity of receptor falcipain-III

With the application of POCKET module of Ligbuilder, the key interaction sites inside the binding pocket could be visualized, where nitrogen atoms represent hydrogen-bond donor sites (Blue); oxygen atoms represent hydrogen-bond acceptor sites (Red); and carbon atoms (Green) represent hydrophobic sites[13]. Based on these observations a pharmacophore model was suggested (Figs. 2 and 3 [a & b]). The 104 novel leupeptin analogues were designed using two basic fragments of leupeptin by defining growing points as shown in Fig. 4 (a & b).

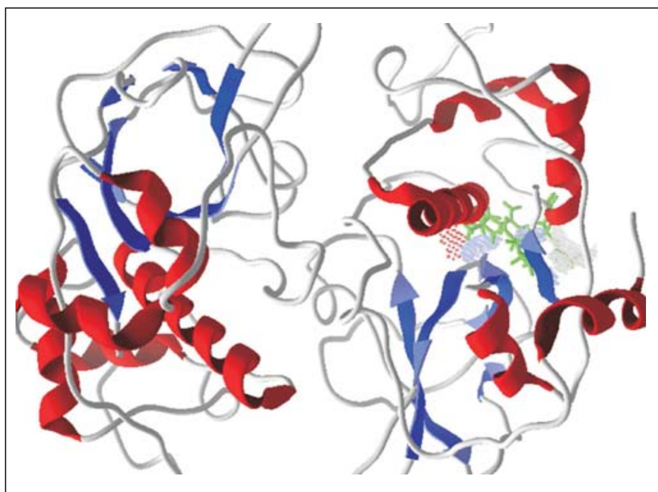

Fig. 2: Key interaction site for leupeptin at the active site of falcipain-III protein (3bpm.pdb) in cartoon representation using Molegro virtual docker.

Docking simulation

The objective of the present study was aimed at exploring the interactions and binding affinity of leupeptin ana-

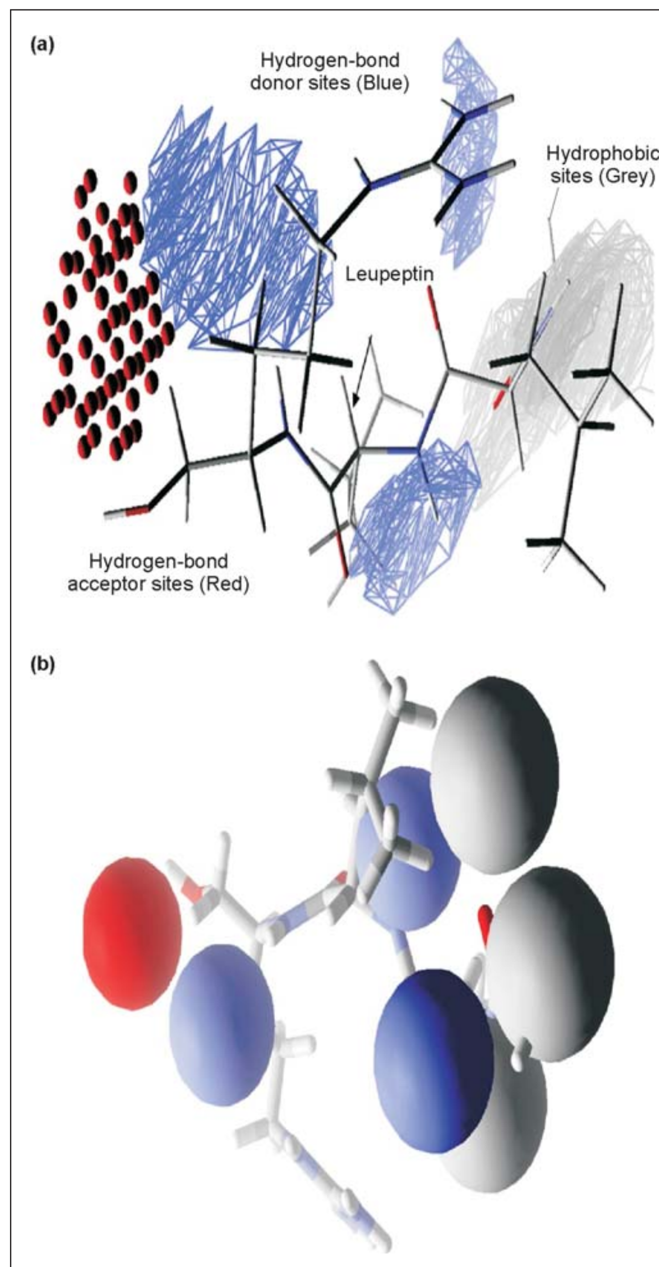

Fig. 3: (a) Representation of key interaction site inside the binding pocket of falcipain-III protein, together with leupeptin; and (b) Derived pharmacophore model of leupeptin using Ligbuilder and for visualization Molegro virtual docker was used.

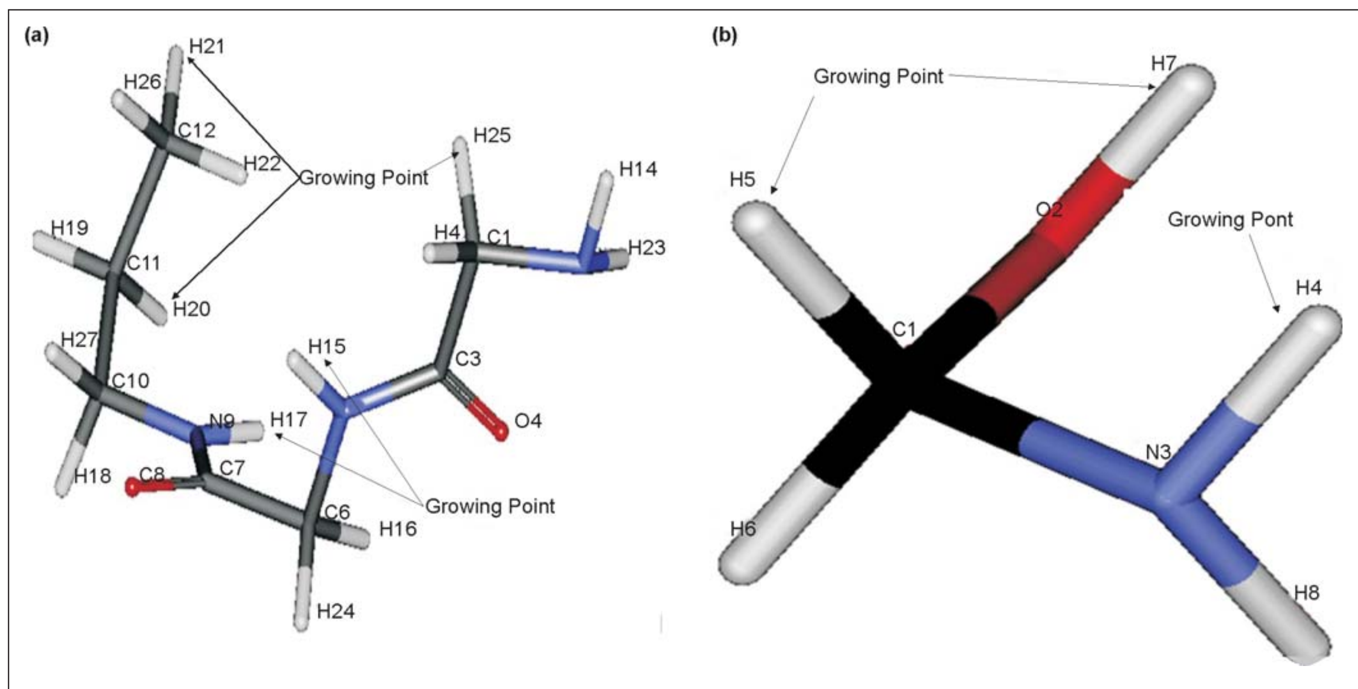

Fig. 4: (a) Five growing points; and (b) Three growing points on basic fragments of leupeptin.

Table 1(a). The top 15 molecules designed by Ligbuilder having highest binding energy score compared to leupeptin using Glide

S.No.	Name of ligand	Gscore	Lipophilic EvdW	Hbond	Electro-phoretic
1.	result_037	–9.79	–5.29	–1.15	–0.71
2.	result_035	–9.75	–5.36	–1.04	–0.72
3.	result_036	–9.21	–4.45	–2.14	–0.94
4.	result_045	–9.2	–4.43	–2.13	–0.95
5.	result_033	–9.18	–5.07	–1.72	–0.72
6.	result_030	–9.01	–4.41	–2.15	–1.07
7.	result_042	–8.68	–3.8	–1.58	–0.55
8.	result_005	–8.56	–3.12	–1.33	–0.68
9.	result_041	–8.37	–4.19	–1.75	–0.83
10.	result_046	–8.28	–4.5	–1.5	–0.79
11.	result_043	–8.23	–4.01	–1.33	–0.89
12.	result_009	–8.17	–4.32	–1.27	–0.54
13.	result_002	–8.05	–4.25	–1.25	–0.51
14.	result_032	–8.05	–4.15	–2.13	–1.23
15.	result_003	–8.04	–3.08	–2.11	–0.75
16.	**leupeptin**	**–5.52**	**–1.13**	**–1.49**	**–0.56**

logues at the active site of falcipain-II and falcipain-III to inhibit the process of haem digestion during asexual phase of *Plasmodium* life cycle inside the human body. Results of docking simulations using Glide are summarized as XP Gscore, lipophilic energy, H-bond energy and electro-phoretic energy along with residues involved in hydrogen bond interactions and in case of AutoDock3 as docked energy (kcal/mol) and free energy for binding (kcal/mol).

Binding mode of designed leupeptin analogues and its e-pharmacophore at the active site of falcipain-III

The leupeptin and its 104 designed analogues were subjected to screening using XP level of Glide module supplied by Schrödinger. The docking simulation results show that the designed ligands have better binding affin-ity than its natural inhibitor, leupeptin. Table 1 (a) shows the list of 15 top scoring leupeptin analogues, and the Table 1 (b) shows the top five analogues together with

Table 1(b). Interaction of *Plasmodium falciparum* falcipain-III protein residues with top five designed drug like molecules together with leupeptin

S.No.	Ligands	Residues of falcipain-III protein interacts with leupeptin analogues
1.	result_037	*Cys51, Gly91, Gly92*, Ile94, Ser162, *Asn182, His183*, Trp215
2.	result_035	*Gly49, Cys51, Gly91, Gly92*, Ile94, Ser162, *Asn182, His183,* Trp215
3.	result_036	*Cys51,* Trp52, Tyr90, *Gly91, Gly92, Asn182, His183*
4.	result_045	*Gly49,* Tyr90, *Gly91, Gly92*, Tyr93, *Asn182, His183*
5.	result_033	*Gly49, Cys51*, Asn87, *Gly91, Gly92*, Tyr93, Ser158, *Asn182*
6.	leupeptin	Gln45, *Gly49*, Ser50, *Cys51,* Trp52, Tyr90, *Gly91, Gly92*, Tyr93, Asp164, Glu176, *Asn182, His183*

Most frequent or common residues are italicized.

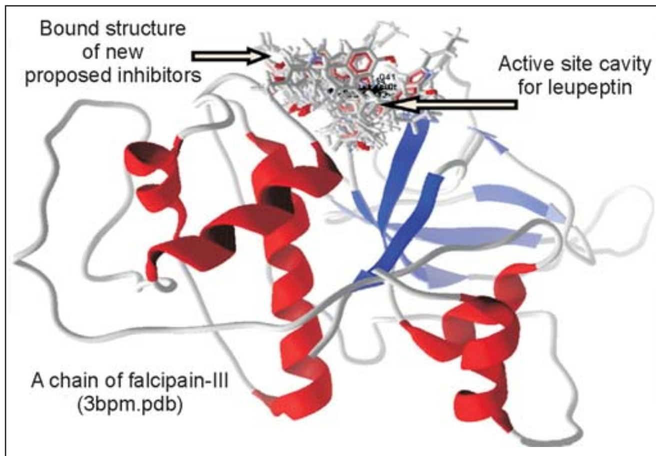

Fig. 5: Cartoon view of falcipain-III protein showing top most 15 selected bound ligand structures using Molegro virtual docker.

most common interacting residues of falcipain-III responsible for stabilizing the complex.

Figurs 5 shows that all the leupeptin analogues are bound at the same active site. On comparison of interacting residues of the top five leupeptin analogues shows that the amino acids Gly49, Cys51, Gly91, Gly92, Asn182 and His183 of falcipain-III protein are most commonly involved. Among these the residue Gly49 was the only non-hydrogen bonding common interacting residue of falcipain-III with ligands result_033, result_035, result_045 and leupeptin and all the remaining residues participated in hydrogen bonding. Cys51 was the common hydrogen bonding interacting residue with ligands result_033, result_036, result_037 and leupeptin [Table 1 (b); & Fig. 6 (a–e)]. The rest of the hydrogen bonding

Fig. 6 (a–e): Binding site conformation of result_037, result_035, result_036, result_045 and result_033 respectively docked to chain A of falcipain-III protein (3bpm.pdb).

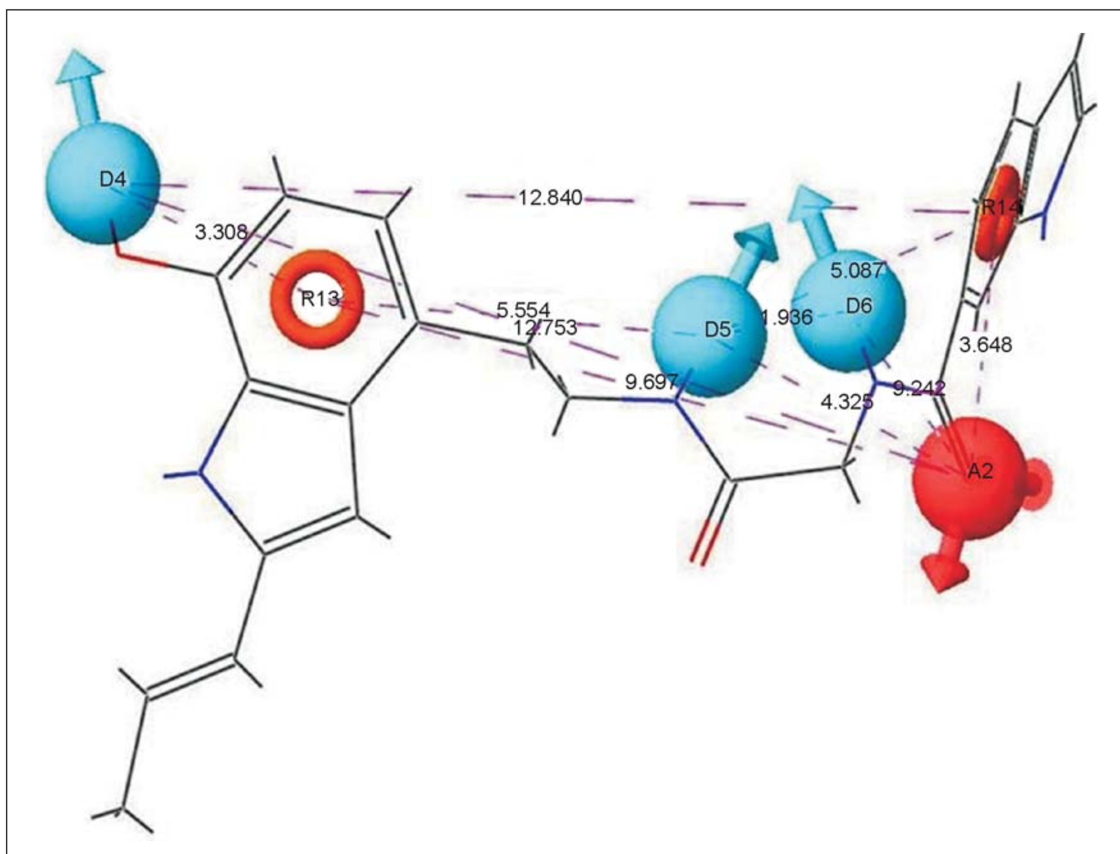

Fig. 7: E-pharmacophore for result_037 at the active site of falcipain-III protein (3bpm.pdb).

residues (Gly91, Gly92, Asn182 and His183) of falcipain-III participate with the top four leupeptin analogues together with leupeptin itself, except residue His183, which does not interact with ligand result_033. The docking simulation result shows the participation of about 50% active site residues of falcipain-III in the formation of 4–5 hydrogen bonds with leupeptin analogues to stabilize the complex and provide force to inhibit the enzymatic action of falcipain-III for stopping the process of haem degradation and finally killing of malaria parasite.

The e-pharmacophore was generated using docking post-processing module. The e-pharmacophore was generated for single ligand result_037 having highest docking Gscore (–9.79) with maximum number of features as 7 at the binding site of falcipain-III. The generated e-pharmacophore are shown in Fig. 7 with two aromatic rings required for the hydrophobic interaction within the pocket namely R14 (score –0.71) and other R13 (score –0.66) together with three donor sites namely D4, D5 and D6 having scores –0.62, –0.62 and –0.52 respectively. One acceptor site A2 having score –0.22 has also been shown. On comparing the pharmacophore generated by Ligbuilder (Fig. 3b) and Schrödinger (Fig. 7) there is clear cut similarity in relation to the presence of minimum number of features, aromatic rings, donor and acceptor sites except one aromatic ring is less in Schrödinger generated e-pharmacophore, this may be due to the selection of different algorithms. The comparative e-pharmacophoric study also suggests and supports our predictions regarding the minimum features required in ligand molecule to behave as falcipain-III inhibitors and is helpful in screening the large database as future antimalarials.

Binding mode of designed leupeptin analogues with falcipain-II

Due to structural similarity of falcipain-II and falcipain-III, the designed analogues were also tested against falcipain-II for screening the compound effort to find out if any leupeptin analogues having inhibition activity against falcipain-II. The results obtained after docking simulation, 15 top most ligands have been selected and found that only three leupeptin analogues are showing activity nearly the bound inhibitor E64. The Glide docking results suggest that the ligands, namely result_037 shows good binding and other two, result_044 and result_042 show nearly similar binding than naturally occurring PDB bound ligand E64 against falcipain-II. The

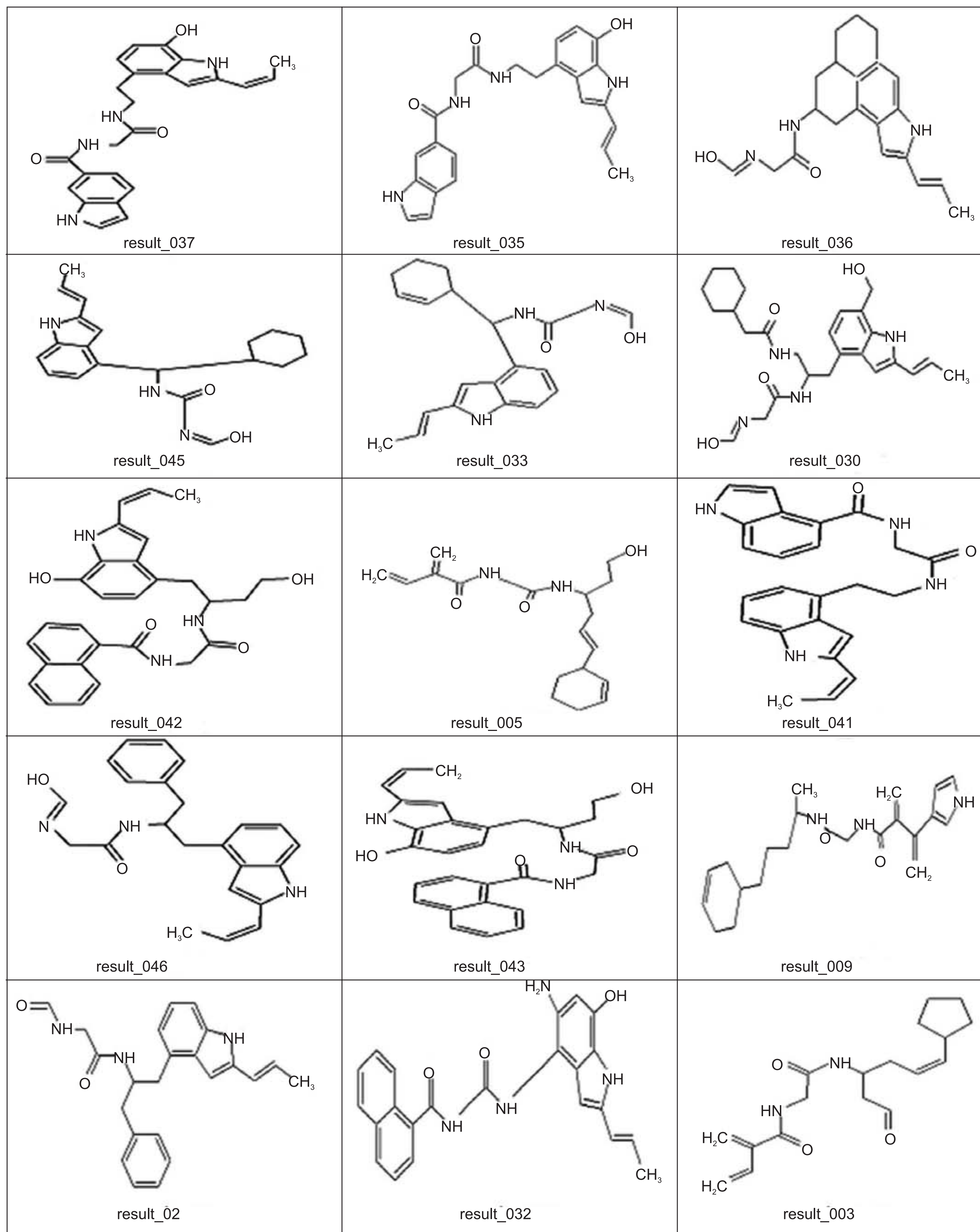

Fig. 8: Top 15 structures of *in silico* designed novel antimalarial drugs having lesser GlideScore.

Table 2. Comparative docking simulation results of *in silico* screened top 15 leupeptin analogues as falcipain-II inhibitors with the receptor falcipain-II as target using Glide docking software

S.No.	Name of ligand	Gscore	Lipophilic EvdW	Hbond	Electro
1.	result_037	–8.06	–5.39	–1.8	–0.87
2.	**E64**	**–7.94**	**–2.93**	**–4.45**	**–1.14**
3.	result_044	–7.92	–5.88	–1.71	–0.9
4.	result_042	–7.91	–5.73	–1.86	–0.94
5.	result_009	–7.3	–5	–1.55	–0.87
6.	result_043	–7.3	–5.77	–1.52	–0.71
7.	result_035	–6.96	–5.59	–1.17	–0.72
8.	result_031	–6.89	–5.15	–2.03	–0.96
9.	result_041	–6.39	–5.33	–0.94	–0.51
10.	result_10	–6.34	–4.74	–1.43	–0.51
11.	result_030	–6.24	–4.41	–1.47	–0.64
12.	result_005	–5.84	–4.23	–1.49	–0.63
13.	result_045	–5.48	–4.51	–1.43	–0.6
14.	result_038	–5.47	–4.55	–0.94	–0.71
15.	result_046	–5.43	–4.55	–1	–0.39
16.	result_036	–5	–4.51	–1.26	–0.35

Table 3. Comparative docking simulation results of screened top 15 leupeptin analogues against falcipain-III receptor using AutoDock3 docking software

S.No.	Name of ligands	Docked energy (kcal/mol)	Free energy for binding (kcal/mol)
1.	result_030	–15	–9.39
2.	result_043	–13.29	–8.8
3.	result_042	–13.16	–8.42
4.	result_037	–13.09	–9.86
5.	result_032	–13.09	–8.75
6.	result_045	–12.9	–8.83
7.	result_036	–12.83	–8.93
8.	result_046	–12.54	–8.5
9.	result_035	–12.49	–9.39
10.	result_002	–12.42	–8.4
11.	result_041	–12.34	–8.9
12.	result_009	–12.33	–8.73
13.	leupeptine	–11.84	–7.11
14.	result_033	–11.78	–7.3
15.	result_005	–11.45	–7.23
16.	result_003	–11.09	–7.01

Gscore of E64 and active leupeptin analogues are in the order of result_037 > E64 > result_044 > result_042 with Gscore –8.06, –7.94, –7.92, and –7.91 respectively (Table 2).

The docking simulation results of falcipain-III with designed leupeptin analogues using Glide compared with AutoDock and found 80% similarity as better binder than leupeptin except slight difference in their order of Gscore. In case of Glide, docking results for top five leupeptin analogues are result_037 > result_035 > result_036 > result_045 > result_033 (Table 1a), and for AutoDock, the order is result_030 > result_043 > result_042 > result_037 > result_032 (Table 3). From Table 1(a) and Table 3 the results suggest that these top five analogues are showing better *in silico* inhibition activity against falcipain-III and there activity varies slightly due to use of different docking softwares which follow different algorithm for simulation study. Figure 8, shows the proposed leupeptin analogues having better binding affinity than leupeptin itself and could be used as antimalarial drugs after synthesis and biological testing.

CONCLUSION

Falcipain-II and falcipain-III are reported as haem degradation metabolizing enzymes responsible for initiating the degradation of host erythrocyte haemoglobin inside the food vacuole of the parasite. Since there are limited number of drugs available to kill the *Plasmodium* and always need to develop more anti-resistant antimalarial drugs. In our present study, the results show better binding affinity of leupeptin analogues as compared to leupeptin itself at the active site of falcipain-III. Although there are no extra hydrogen bonds, however, equal number of hydrogen bonds with variable strength as compared to leupeptin along with the enhanced hydrophobic and electrostatic interactions in case of analogues supports our study that it holds the ligand molecules strongly within the receptor. The comparative e-pharmacophoric study also suggests and supports our predictions regarding the minimum features required in ligand molecule to behave as falcipain-III inhibitors and is helpful in screening the large database as future antimalarial inhibitors.

The docking results in case of falcipain-II the top three leupeptin analogues showing about similar activity in the form of Glide Score compare to bound ligand E64. The similarity of target structures also suggests that there is possibility of being targeted more than one by same group of the inhibitors. So the proposed inhibitors in future could be either used alone or in combination with known drugs which can together act synergistically more effectively to treat malaria.

ACKNOWLEDGEMENTS

We are thankful to the Director of Indian Institute of Information Technology, Allahabad, India for providing a supportive environment to carry out research activities.

REFERENCES

1. *World Malaria Report 2012*. Geneva: World Health Organization 2012; p. 59.
2. Cox FE. History of the discovery of the malaria parasites and their vectors. *Parasit Vectors* 2010; *3*(1): 5; doi:10.1186/1756–3305–3–5.
3. Fong YL, Cadigan FC, Coatney GR. A presumptive case of naturally occurring *Plasmodium knowlesi* malaria in man in Malaysia. *Trans R Soc Trop Med Hyg* 1971; *65* (6): 839–40.
4. Biagini GA, Fidock DA, Bray PG, Ward SA. Mutations conferring drug resistance in malaria parasite drug transporters Pgh1 and PfCRT do not affect steady-state vacuolar Ca^{2+}. *Antimicrob Agents Chemother* 2005; *49*(11): 4807–8.
5. Price RN, Uhlemann AC, Brockman A, McGready R, Ashley E, Phaipun L, *et al.* Mefloquine resistance in *Plasmodium falciparum* and increased *pfmdr1* gene copy number. *Lancet* 2004; *364*(9432): 438–47.
6. Guilbride DL, Gawlinski P, Guilbride PD. Why functional pre-erythrocytic and blood stage malaria vaccines fail: A meta-analysis of fully protective immunizations and novel immunological model. *PloS One* 2010; *5*(5): e10685.
7. Miller LH, Su X. Artemisinin: Discovery from the Chinese herbal garden. *Cell* 2011; *146*(6): 855–8.
8. Rosenfeld A, Vanderberg JP. *Plasmodium berghei:* Induction of aminopeptidase in malaria-resistant strain of *Anopheles gambiae*. *Exp Parasitol* 1999; *93*(2): 101–4.
9. Banerjee R, Liu J, Beatty W, Pelosof L, Klemba M, Goldberg DE. Four plasmepsins are active in *Plasmodium falciparum* food vacuole, including a protease with an active-site histidine. *Proc Nat Acad Sci USA* 2002; *99:* 990–5.
10. Sijwalai PS, Rosenthal PJ. Gene disruption confirms a critical role for the cysteine protease falcipain-2 in hemoglobin hydrolysis by *Plasmodium falciparum*. *Proc Nat Acad Sci USA* 2004; *101:* 4384–9.
11. Eggleson KK, Duffin KL, Goldberg DE. Identification and characterization of falcilysin, a metallopeptidase involved in hemoglobin catabolism within the malarial parasite *Plasmodium falciparum*. *J Biol Chem* 1999; *274:* 32411–7.
12. Hanspal M, Dua M, Takakuwa Y, Chishti AH, Mizuno A. *Plasmodium falciparum* cysteine protease falcipain-2 cleaves erythrocyte membrane skeletal proteins at late stages of parasite development. *Blood* 2000; *100:* 1048–54.
13. Shenai BR, Sijwali PS, Singh A, Rosenthal PJ. Characterization of native and recombinant falcipain-2, a principal trophozoite cysteine protease and essential hemoglobinase of *Plasmodium falciparum*. *J Biol Chem* 2000; *275*(37): 29000–10.
14. Pandey KC, Sijwali PS, Singh A, Na BK, Rosenthal PJ. Independent intramolecular mediators of folding, activity, and inhibition for the *Plasmodium falciparum* cysteine protease falcipain-2. *J Biol Chem* 2004; *279*(5): 3484–91.
15. Sijwali PS, Shenai BR, Gut J, Singh A, Rosenthal PJ. Expression and characterization of the *Plasmodium falciparum* haemoglobinase falcipain-3. *Biochem J* 2001; *360*(2): 481–9.
16. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Delv Rev* 2001; *46:* 3–26.
17. Wang R, Gao Y, Lai L. Lig Builder: A multi-purpose program for structure-based drug design. *J Mol Model* 2000; *6*(7–8): 498–516.
18. Good AC, Oprea TI. Optimization of CAMD techniques 3. Virtual screening enrichment studies: A help or hindrance in tool selection? *J Comput Aided Mol Des* 2008; *22:* 169–78.
19. Caporuscio F, Rastelli G, Imbriano C, Del Rio A. Structure-based design of potent aromatase inhibitors by high-throughput docking. *J Med Chem* 2011; *54:* 4006–17.
20. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, *et al.* Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *J Med Chem* 2006; *49:* 6177–96.
21. Kerr ID, Lee JH, Pandey KC, Harrison A, Sajid M, Rosenthal PJ, *et al.* Structures of falcipain-2 and falcipain-3 bound to small molecule inhibitors: Implications for substrate specificity. *J Med Chem* 2009; *52:* 852–7.
22. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, *et al*. Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. *J Computational Chem* 1998; *19:* 1639–62.
23. Salam NS, Nuti R, Sherman W. Novel method for generating structure-based pharmacophores using energetic analysis. *J Chem Inf Model* 2009; *49:* 2356–68.

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Received: 7 March 2012 *Accepted in revised form:* 28 February 2013