Molecular docking and structure-based virtual screening studies of potential drug target, CAAX prenyl proteases, of Leishmania donovani

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Targeting CAAX prenyl proteases of Leishmania donovani can be a good approach towards developing a drug molecule against Leishmaniasis. We have modeled the structure of CAAX prenyl protease I and II of L. donovani, using homology modeling approach. The structures were further validated using Ramachandran plot and ProSA. Active site prediction has shown difference in the amino acid residues present at the active site of CAAX prenyl protease I and CAAX prenyl protease II. The electrostatic potential surface of the CAAX prenyl protease I and II has revealed that CAAX prenyl protease I has more electropositive and electronegative potentials as compared CAAX prenyl protease II suggesting significant difference in their activity. Molecular docking with known bisubstrate analog inhibitors of protein farnesyl transferase and peptidyl (acyloxy) methyl ketones reveals significant binding of these molecules with CAAX prenyl protease I, but comparatively less binding with CAAX prenyl protease II. New and potent inhibitors were also found using structure-based virtual screening. The best docked compounds obtained from virtual screening were subjected to induced fit docking to get best docked configurations. Prediction of drug-like characteristics has revealed that the best docked compounds are in line with Lipinski’s rule. Moreover, best docked protein–ligand complexes of CAAX prenyl protease I and II are found to be stable throughout 20 ns simulation. Overall, the study has identified potent drug molecules targeting CAAX prenyl protease I and II of L. donovani whose drug candidature can be verified further using biochemical and cellular studies.

**Keywords:** CAAX prenyl proteases; prenylation; membrane protein; molecular dynamics simulation; docking

1. Introduction

Despite huge disease burden, leishmaniasis is one of the most neglected diseases since decades primarily because of its association with poverty. Although there is a wide arsenal of available drugs in the market, they suffer from serious limitations which include unacceptable host toxicity, lack of target specificity, poor efficacy, high cost, and acquired parasite drug resistance (Stuart et al., 2008). In addition, the co-infection of leishmaniasis and Human immunodeficiency virus infections is being emerging as a serious worldwide concern. Therefore, the development of vaccines, effective drugs for treatment, and improved approaches of the disease diagnosis are now a matter of urgency. We have earlier targeted redox metabolism of the parasite for discovery of novel drug candidates with promising results (Das, Prakash, Sundar, & Dubey, 2013; Saudagar, Saha, Saikia, & Dubey, 2013; Singh, Sarkar, Jagannadhamin, & Dubey, 2008; Singh et al., 2015)

The post-translational modifications of biological molecules are known to be crucial for their functional activation, regulation, and localization. Prenylation is one such modification which is involved in post-translational modification of various important proteins like proteins of Ras superfamily, which are involved in a variety of regulatory and signaling events in eukaryotes. Prenylation is attachment of isoprenoid group (15C farnesyl or 20C geranylgeranyl group) to a cysteine residue in a thioether linkage. These prenylated proteins are found in different cellular compartments including nucleus, cytosol, and membrane bound organelles. Most of the prenylated proteins belong to the Ras superfamily which are involved in a variety of cellular functions like cytokinesis, membrane trafficking, control of cell growth, and differentiation, etc. The targets for post-translational prenylation are the small GTP-binding proteins containing CAAX motif at or near its carboxyl terminus. These types of proteins undergo three different prenyl

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dependent processing for their ultimate maturation and localization (Casey, 1992; Zhang & Casey, 1996).

There are two types of CAAX prenyl proteases: type I and type II. The type I CAAX prenyl proteases are named as alpha-factor converting enzyme (AFC1). They are known to be metalloproteases with HEXXH conserved motif (where H is histidine, E is glutamate and X is any amino acid) in it (Boyartchuk, Ashby, & Rine, 1997; Schmidt, Tam, & Michaelis, 2000; Tam, Schmidt, & Michaelis, 2001). Type II CAAX prenyl proteases are called Ras and a-factor converting enzyme (RCE1). They lack the conserved motif present in type I CAAX prenyl proteases and their catalytic nature is still a topic of debate. However, they were suggested to cysteine proteases based on the some inhibition studies and activity of cysteine, histidine, and glutamate mutations. This is supported by the study that reveals the presence of cysteine residue near the active site of yeast RCE1P and it was found that upon mutation of this cysteine residue or using cysteine protease inhibitors, the enzyme gets deactivated (Dolence, Steward, Dolence, Wong, & Poulter, 2000; Pei, Mitchell, & Grishin, 2011). Few literatures suggest them to be metalloproteases (Pei & Grishin, 2001; Pei et al., 2011). It has been reported that yeast type I CAAX prenyl protease (Afc1p) and yeast type II CAAX prenyl protease have distinct, but overlapping substrate specificity (Boyartchuk et al., 1997). Although Afc1p and RCE1P lack sequence similarity, both were found to proteolyse the a-factor CAAX sequence, CVIA. However, the a-factor CAAX sequence substituted by camQ and CTLM was found to be processed only by AFC1P and RCE1P, respectively. Also, the yeast Ras2 protein (CIIS) was found to be catalyzed by RCE1P, but apparently not by yeast AFC1P (Boyartchuk et al., 1997; Trueblood et al., 2000). In addition to its CAAX proteolysis activity, Ste24p, a type I CAAX prenyl protease of yeast, is found to have aI1-2-terminal proteolysis activity for processing yeast a-factor precursor (Tam et al., 2001).

The role of CAAX processing has been demonstrated in various protein functions and trafficking by mutating cysteine of CAAX to another amino acid so as to prevent prenylation of protein (Wright & Philips, 2006). However, this cannot figure out the importance of CAAX prenyl proteases and methyl transferase since prenylation is a prerequisite for their activity. Their physiological importance were assessed when mouse embryonic fibroblast lacking RCE1 (CAAX prenyl protease II required for endoproteolysis) or ICMT (carboxyl methyl transferase required for carboxyl methylation of isoprenylcysteine) showed mislocalization of farnesylated Ras proteins (Michaelson et al., 2005). RCE1 deficiency was found to be lethal in late embryonic development in mouse, which also affirms the physiological significance of CAAX prenyl proteases (Kim, Ambroziak, & Otto, 1999). It was also found that knock out of RCE1 results in pronounced impairment of growth in Trypanosoma brucei (Gillespie et al., 2007). Recently, the type I yeast CAAX prenyl protease, Ste24 is found to have play role in chitin synthesis (Meissner, Odman-Naresh, Vogelpohl, & Merzendorfer, 2010). It was found that variants of type I CAAX prenyl proteases (AFC1) in yeast showed reduction in a-factor production below detectable limit even in presence of type II CAAX prenyl protease, RCE1 (a-factor can also be processed by RCE1). This could be because of the loss of N-terminal processing of a-factor by AFC1P in combination with partial defect in prenylation (Trueblood et al., 2000). Human AFC1 has been found to process farnesylated prelamin A by removing –CAAX from the –CAAX sequence of prelamin A and also cleaving at sites 15 residues upstream of farnesylated cysteine. It was observed that accumulation of farnesylated prelamin A due to mutation in human AFC1 enzyme or prelamin A cleavage site was found in genetic diseases like Huchinson-Gilford progeria and Mandibuloacral dysplasia (Gillespie et al., 2007).

From the above discussion, it is evident that both type I and type II CAAX prenyl proteases are involved in processing of various physiologically important proteins in different organisms. CAAX prenyl proteases of Leishmania have unique active site environment and low sequence similarity with CAAX prenyl proteases of human host. The sequence identity and query coverage with CAAX prenyl proteases I was 34 and 93%, respectively. Similarly, identity and query coverage with CAAX prenyl proteases II was 24 and 67%, respectively. It is possible to make structure-based rational drug which can target these CAAX prenyl proteases of pathogen without interfering with the function of human counterparts. Recently, inhibitors against gBP21 protein essential for RNA editing in Leishmania donovani has been identified by using computational approach (Sahoo et al., 2013). Also, imidazole analogs has been identified as potential inhibitors of trypanothione reductase of L. donovani using molecular docking and virtual screening approach (Pandey, Sharma, Bhatt, Sundar, & Prajapati, 2015). In this study, we have performed homology modeling, active site prediction, structure-based virtual screening, absorption, distribution, metabolism, and excretion (ADME) prediction, and molecular dynamics simulations (MDS) to find new and potent inhibitors for CAAX prenyl proteases I and II of L. donovani. The current study will help in discovering new drug-like molecules which could be possible inhibitors of CAAX prenyl proteases of L. donovani.

2. Materials and methods
2.1. Sequence alignment and protein modeling
The protein sequence of CAAX prenyl protease I (Accession No.: E9BTI9) and CAAX prenyl protease II
were retrieved from UniProtKB database. The amino acid sequence was aligned using Clustal Omega software. Three-dimensional structure of CAAX prenyl protease I and CAAX prenyl protease II of L. donovani were modeled using homology modeling. The homology modeling was done using Robetta software. Anti-leishmanial drug discovery 2369.

Figure 1. Sequence alignment and three dimensional structure of modeled proteins. The structure was modeled using homology modeling using Robotta software. (A) The modeled structure of CAAX prenyl protease I using human nuclear membrane zinc metalloprotease ZMPSTE24 (PDB ID: 4AW6) as template, (B) the modeled structure of CAAX prenyl protease II using Methanococcus maripaludis homolog of RCE1 (PDB ID: 4CAD) as template (C) Sequence alignment of CAAX prenyl protease I of Leishmania donovani and Homo sapiens, and (D) Sequence alignment of CAAX prenyl protease II of L. donovani and H. sapiens.

Table 1. Validation of CAAX prenyl protease I and II by Ramachandran plot showing percentage of residues in most favored regions, additional allowed regions, generously allowed regions, disallowed regions, and G score.

| Target name       | Most favored regions | Additional allowed regions | Generously allowed regions | Disallowed regions | G score |
|-------------------|----------------------|----------------------------|---------------------------|--------------------|---------|
| CAAX prenyl protease I | 96.1                 | 3.4                        | .5                        | .0                 | .29     |
| CAAX prenyl protease II | 93.0                 | 5.5                        | .0                        | 1.5                | .19     |

(Accession No.: E9BIT3) were retrieved from UniProtKB database. The amino acid sequence was aligned using Clustal Omega software.
Robetta is a protein structure prediction server. It uses a template-based homology domain prediction method, Rosetta, to yield a high quality 3D structure of the target protein (Kim, Chivian, & Baker, 2004). The template used for the homology modeling of CAAX prenyl protease I is human nuclear membrane zinc metalloprotease ZMPSTE24 (PDB ID: 4AW6) and that for CAAX prenyl protease II is Methanococcus maripaludis.

Table 2. Analysis of modeled structures of CAAX prenyl protease I and II using Structure Analysis Verification Server (SAVES) and protein structure analysis (ProSA).

| Target name                  | ERRAT | Verify 3D | Z-score |
|------------------------------|-------|-----------|---------|
| CAAX prenyl protease I       | 97.613| 81.50     | -7.52   |
| CAAX prenyl protease II      | 93.056| 79.02     | -4.45   |

Notes: ERRAT, Verify 3D and Z-score for both CAAX prenyl protease I and CAAX prenyl protease II are tabulated.

Figure 2. Validation of modeled structure of CAAX prenyl protease I and II using Ramachandran plot and Protein structure analysis (ProSA). (A) Ramachandran plot for modeled CAAX prenyl protease I, (B) Ramachandran plot for modeled CAAX prenyl protease II, (C) ProSA for CAAX prenyl protease I, and (D) ProSA for CAAX prenyl protease II. The red, brown, and yellow regions in A and B, represent the favored, allowed, and “generously allowed” regions, respectively, where the circles and squares represent the amino acids of protein.
homolog of RCE1 (PDB ID: 4CAD). At the initial stage of structure prediction, Ginzu, a domain prediction method was used to screen the query sequence for regions homologous to the experimentally characterized structure by using BLAST, PSI-BLAST, and Pcons2 (Kim et al., 2004; Mount, 2007). In second stage,
sequences are fragmented into putative domains based on matches, known families and structures, multiple sequence information, and secondary structure information. After domain parsing, each putative domain it follows, it assigns protocol track to generate the structure of the target protein.

2.2. Validation
The modeled structures of both CAAX prenyl protease I and II of L. donovani were validated using Structural Analysis and Verification Server (SAVES) (Baglo, Gabrielsen, Sylte, & Gederaas, 2013; Lakhli, Chevé, Yasri, & Ibrahimi, 2015). The PROtein Structure Analysis (ProSA) tools were used to check the energy criteria against the potential mean force (Wiederstein & Sippl, 2007). Other methods like ERRAT and Verify 3D were also employed from the SAVES server (Yadav et al., 2014).

2.3. Protein preparation
The coordinates of protein were further prepared using protein preparation wizard (Protein preparation wizard, 2014) in grid-based ligand docking with energetics (glide). Bond orders were assigned and hydrogen atoms were added to the modeled structure. Model was subjected to energy minimization using optimized potential for liquid simulations (OPLS-2005) and implicit solvation. The minimized was terminated when root mean square deviation (RMSD) of heavy atoms in the minimized structure executed .3 Å relative to the model structure.

2.4. Active site prediction
The ligand binding site of the receptor can be predicted through SiteMap available in Schrödinger Suite (Site-Map, version 3.0, 2014). SiteMap clusters all the regions of the sitemap based on the site score. A white color spheres was picked using van der Waal’s radius scaling factor of 1.0 and partial charge cutoff of .25. Here, the position of the grid box was set on xyz axes with the measurements of x-value: 87.94, y-value: 55.59, and z-value: 54.43 with the radius of 2.0.

2.5. Electrostatic potential surface map
The electrostatic potential surface of the protein was generated using create surface tool available in Maestro in order to find the electropositive as well as the electronegative regions of the protein surface. Here, the blue color indicated the electropositive regions and the red color indicates the electronegative regions which are involved in the electron transfer activity of the protein and the ligands.

2.6. Ligand preparation
A group of 16 compounds of bi substrate analog, farnesyl transferase inhibitors (Schlitzer, Winter-Vann, & Casey, 2001) and three compounds of peptidyl (acyloxy) methyl ketones (Porter et al., 2007) were collected from the literature and were prepared using Ligprep module of Schrödinger suite (LigPrep, version 2.9, 2014). It can generate number of structures with various ionization states, tautomers, stereoisomers, and ring conformations to eliminate molecules using various criteria including molecular weight or the specified numbers and types of functional groups present with the correct chiralities for each processed successful structure.

2.7. Molecular docking studies
To test the docking parameters for the compounds under consideration, the compounds were docked into the predicted binding site of the modeled proteins using glide module from Schrödinger (Glide, version 6.2, 2014). To soften the potential for non-polar parts of the receptor, we scaled van der Waal’s radii of the receptor atoms by 1.00 with a partial atomic charge .25. A grid box with coordinates X = 24.391, Y = 26.441, and Z = 29.382 was generated at the centroid of the active site. The ligands were docked with the active site using the extra precision glide docking (Glide XP). Glide generates the conformations internally and passes through the series of filters. The final best docked compounds were chosen using values of glide score, glide energy, and glide emodel (Friesner, Banks et al., 2004; Friesner, Murphy et al., 2006).

2.8. Structure-based virtual screening
To find the novel inhibitor for CAAX prenyl protease1, glide program was used to run high throughput virtual screening (HTVS), standard precision (SP), and extra precision (XP) protocols. Semi-flexible docking protocols were used in docking simulations. The ligands being docked were kept in flexible, in order to examine the arbitrary number of torsional degrees of freedom spanned by the transitional and rotational parameters. The generated ligand poses were elapse through series of hierarchical filters that evaluated ligand interaction with its receptor. The best docked compounds were chosen based on glide score (Sastry, Adzhigirey, Day, Annabhimoju, & Sherman, 2013; Reddy & Singh, 2014; Reddy, Singh, & Singh, 2014).

2.9. Binding-free energy calculations
The top docked poses are subjected to binding-free energy calculations using Prime/MM-GBSA approach (Prime, version 3.5, 2014). This method is used to
predict the binding free energy for a set of ligands to the receptor. Minimization of the docked poses using local optimization feature in prime and the energies of the complex were calculated using OPLS-AA (2005) force field and generalized Born/surface area (GB/SA) continuum solvent model. The simulations were carried out by GBSA continuum model in prime. Prime uses a surface generalized Born model employing a Gaussian surface instead of van der Waal’s surface for better representation of a solvent accessible surface area (Lyne, Lamb, & Saeh, 2006; Suryanarayanan & Singh, 2014).

Table 3. Results of active site prediction of CAAX prenyl protease I and CAAX prenyl protease II using SiteMap.

| Target name | CAAX prenyl protease I | CAAX prenyl protease II |
|-------------|-------------------------|-------------------------|
| Site score  | 1.083                   | 0.817                   |
| D score     | 1.124                   | 0.827                   |
| Volume      | 527.191                 | 90.895                  |
| Contacts    | 0.899                   | 0.818                   |
| Phobic      | 0.883                   | 1.214                   |
| Philec      | 0.848                   | 0.67                    |
| Balance     | 1.041                   | 1.811                   |
| Donor/acceptor | 0.847               | 0.75                    |
| Residues    | His 409, Leu 385, Ile 388, Ser 389, Pro 410, Pro 411, Ile 280, Val 412, Leu 413, Val 283, Arg 415, Leu 284, His 286, Asn 392, Glu 287, Ala 394, Ser 395 | Ala 11, Arg 22, Phe 12, Gln 23, Arg 79, Ala 15, Thr 8, Ile 72, Val 20, Glu 75, Gln 21, Leu 76 |

Figure 4. Electrostatic potential surface map of active site of (A) CAAX prenyl protease I, (B) CAAX prenyl protease II. Notes: Here, the blue color is indicative of electropositive regions, whereas the red color represents electronegative regions of the proteins.
Table 4. Extra precision docking of non-peptidic, non-preylic inhibitors of the prenyl protein-specific Protease Rce1, and peptidyl (acyloxy)methyl ketones with CAAX prenyl protease I.

| S. No | Compound name | Docking score | Glide Energy | Glide Evdw | Glide Ecoul | Glide E model | Residues involved | ΔGbind |
|-------|---------------|---------------|--------------|------------|-------------|----------------|-------------------|--------|
| 1     | Compound 8    | −9.811        | −64.295      | −54.526    | −9.769      | −83.731        | Asn 392, Pro 410   | −96.525|
| 2     | Compound 9    | −9.151        | −54.104      | −45.898    | −8.205      | −70.269        | Ala 252, Zn        | −64.749|
| 3     | Compound 1    | −9.085        | −56.050      | −47.344    | −8.707      | −76.340        | Ser 361, His 409, Zn | −86.654|
| 4     | Compound 10   | −8.956        | −63.521      | −55.168    | −8.353      | −88.415        | Glu 287, Zn        | −103.842|
| 5     | Compound 3    | −8.935        | −56.479      | −43.496    | −12.983     | −88.104        | Glu 287, His 290, Asn 299 Arg 415, Zn | −102.629|
| 6     | Compound 14   | −8.926        | −54.613      | −49.534    | −5.080      | −79.721        | Asn 299, Zn        | −83.652|
| 7     | Compound 11   | −8.889        | −54.892      | −47.623    | −7.269      | −77.343        | Arg 415, Zn        | −62.572|
| 8     | Compound 12   | −8.824        | −59.629      | −51.730    | −7.899      | −83.824        | Arg 415, Zn        | −85.600|
| 9     | Compound 2    | −8.728        | −59.942      | −51.693    | −8.249      | −84.240        | Asn 299, Arg 415, Zn | −78.589|
| 10    | Compound 16   | −8.705        | −54.539      | −49.001    | −9.538      | −88.048        | Asn 392, Arg 415, Zn | −71.185|
| 11    | Compound 13   | −8.438        | −59.184      | −52.328    | −6.856      | −90.221        | Glu 287, His 290, Zn | −61.249|
| 12    | Compound 6    | −8.396        | −56.956      | −46.321    | −10.635     | −80.335        | Ala 251, Asn 299, Zn | −80.530|
| 13    | Compound 4    | −8.059        | −54.332      | −46.111    | −8.221      | −82.312        | Asn 299, Zn        | −83.677|
| 14    | Compound 5    | −7.311        | −57.595      | −50.915    | −6.679      | −86.430        | Zn                | −85.131|
| 15    | Compound 15   | −6.871        | −53.686      | −52.379    | −1.306      | −80.172        | Asn 392            | −102.824|
| 16    | Compound 7    | −5.836        | −63.193      | −57.616    | −5.577      | −98.931        | Lys 62, Tyr 407    | −66.257|

2.10. Induced fit docking

After identification of a set of compounds, their binding mode was further examined by induced fit docking (IFD) method (Induced Fit Docking, 2014). Initially, Glide XP docking of chosen hits to the rigid receptor was performed. Prime was used to generate maximum 20 poses for each ligand and the residues within 5 Å of 20 poses. Receptor side chain flexibility was introduced while the coordinates of other residues and the backbone remain fixed. Finally, the ligands were rigorously re-docked in Glide XP mode into the lowest energy induced fit receptor structure with no scaling (vdW scaling of (1) and their final scores were obtained (Sherman, Day, Jacobson, Friesner, & Farid, 2006; Suryanarayanan & Singh, 2014).

2.11. ADME properties

The newly identified best compounds were studied for their ADME properties prior using Qikprop. The required principle and physiochemical properties of drug compounds can be predicted by this module. It gives the detailed analysis of the log P (octanol/water), QP% (human oral absorption). It also evaluates the acceptability of the entire known and screened compounds on the basis of Lipinski’s rule of 5, which is significant for rational drug design (Reddy et al., 2014; Suryanarayanan & Singh, 2014).

2.12. Molecular dynamics simulations

MDS were performed for the modeled as well as three best docked complexes of CAAX prenyl proteases I and II using Desmond module of Schrödinger and the OPLS–AA 2005 (optimized potential for liquid simulations – All Atom) force field for minimization of the system. CAAX prenyl proteases are reported to be membrane proteins (Pie & Grishin, 2001; Seabra, 1998), so we have performed MDS of these proteins in DPPC lipid membrane with water solvent. The Desmond
system builder was used at a 10 Å buffered orthorhombic system and periodic boundary conditions were constructed using a DPPC lipid membrane and a TIP3P explicit water solvent. Salt concentration of .15 M NaCl was added to the system. NPT ensemble was used to perform simulations. The pressure of 1.013 bar and temperature of 325 K were kept constant coupling to a system to a Berendsen thermostat and barostat. Default membrane protein relaxation protocol was applied and each simulation was performed for 20 ns of time period (Guo et al., 2010; Mori & Okumura, 2013; Selvaraj, Sivakamavalli, Vaseeharan, Singh, & Singh, 2014; Tripathi et al., 2012).

### 3. Results and discussion

#### 3.1. Sequence alignment, protein modeling, and validation

The sequence of CAAX prenyl protease I and II were aligned with respect to their human counterparts and the alignment is represented in Figures 1(C) and (D). The predicted 3D structures of CAAX prenyl proteases I and II are shown in Figures 1(A) and (B), respectively. The model was further validated using SAVES and PROtein Structure Analysis server (ProSA) server. The results of structure validation of both CAAX prenyl protease I and CAAX prenyl protease II are given in Tables 1 and 2. The realistic 3D structural qualities were evident for both the models from the validation parameters. Ramachandran plot of CAAX prenyl protease I shows that 96.1% of amino acid residues lie in most favored regions, 3.4% in additionally allowed regions, .5% in generously allowed regions, and G-score of .29 (Figure 2(A) and Table 1). Validation of CAAX prenyl protease II structure by Ramachandran plot shows 93% residues in most favored regions, 5.5% in additionally allowed regions, 0% in generously allowed regions and G-score of .19 (Figure 2(B) and Table 1). ERRAT plot for CAAX prenyl protease I and II show overall quality factor of 97.613 and 93.056 (Table 2). Verify 3D profile suggests that 81.5% and 79.02% residues of CAAX prenyl protease I and II, respectively, possess score over .2 (residues having a score of more than .2 are reliable) (Table 2). The Z-score is indicative of the overall quality of a model and verifies whether the predicted structure has a score within the range of Z-scores of other native proteins of similar size (Azam, Abbasi, & Batool, 2014). The Z-scores of CAAX prenyl protease I and II are −7.52 and −4.45, respectively (Table 2). The ProSA plot for both CAAX prenyl protease I is shown in Figure 2(C) and for CAAX prenyl protease II is shown in Figure 2(D). Validation of the predicted models reveals their quality and allows the structure can be carried for further structure-based studies.

#### 3.2. Active site prediction

The active site of the protein was predicted through SiteMap which clusters the top ranked sites on the basis of site score. The site score and druggability score
(D-score) of CAAAX prenyl protease I are 1.083 and 1.124, respectively, whereas the residues involved in the active site are His 409, Leu 385, Ile 388, Ser 389, Pro 410, Pro 411, Ile 280, Val 412, Leu 413, Val 283, Arg 415, Leu 284, His 286, Zn 287, Ala 289, His 390, Arg 415, and Ser 395. The site score and druggability score for CAAAX prenyl protease II are .817 and .827, respectively, whereas the residues involved in the active site are Ala...
No. SYN20008993 A Asn 251, Gly 256, Leu 197
11. Arg 22, Phe 12, Gln 23, Arg 79, Ala 15, Thr 8, Ile 72, Val 20, Glu 75, Gln 21, and Leu 76. Predicted active sites of both proteins are shown in Figure 3. It was also found that the predicted active sites are same as provide in literature (Pryor et al., 2013). The contact score is a measure of interaction of receptors with the average site points. The contact score for CAAX prenyl protease I and II are .899 and .818, respectively, where average contact score for tight binding site is considered to be 1.0 (Table 3). The relative hydrophobicity and hydrophilicity of the site is measured by phobic and philic scores. The balance score refers to the ratio of phobic to philic scores. The average philic and phobic score for tight binding site is 1.0, whereas the average balance score is 1.6. The phobic, philic, and balance score for CAAX prenyl protease I are .883, .848, and 1.041,

Table 8. Induced fit docking studies for screened compounds for CAAX prenyl protease I.

| S. No | Compound code | Hydrogen bond interaction | Hydrophobic interaction | Docking score | Glide E model | Glide energy | IFD score |
|-------|---------------|---------------------------|-------------------------|---------------|---------------|--------------|-----------|
| 1     | SYN20008993   | Asn 251, Gly 256, Leu 197 | His 290                 | -10.819       | -75.600       | -47.062      | -917.242  |
| 2     | F0451-4356    | Phe 254, Gly 256, Leu 197 |                        | -10.021       | -49.290       | -41.512      | -916.777  |
| 3     | F02432-0154   | Phe 254, Leu 197          |                        | -9.914        | -67.057       | -46.794      | -922.198  |
| 4     | F0849-2472    | Leu 197, Gly 256          |                        | -9.803        | -63.248       | -42.480      | -917.217  |
| 5     | SYN15784346   | Leu 197, Phe 254          |                        | -9.764        | -47.238       | -45.313      | -915.952  |
| 6     | LEG1776749    | Glu 365, Arg 415          |                        | -9.522        | -58.944       | -47.058      | -922.020  |
| 7     | SYN17739355   | Leu 197, Gly 256, Glu 365 |                        | -9.244        | -46.987       | -34.594      | -916.191  |
| 8     | F0452-3342    | Leu 197, Gly 256, Lys 261 |                        | -9.079        | -51.349       | -36.175      | -916.217  |
| 9     | KM09023      | Phe 254                   |                        | -8.913        | -69.007       | -46.463      | -918.675  |
| 10    | KM03725      | Phe 254, Gly 256          |                        | -8.822        | -62.814       | -39.924      | -916.808  |
| 11    | F04375-4375   | Phe 254, His 286, Glu 287 | Arg 415                | -8.745        | -50.914       | -36.377      | -916.032  |

Table 9. Induced fit docking studies for screened compounds for CAAX prenyl protease II.

| S. No | Compound code | Hydrogen bond interaction | Hydrophobic interaction | Docking score | Glide E model | Glide energy | IFD score |
|-------|---------------|---------------------------|-------------------------|---------------|---------------|--------------|-----------|
| 1     | F2721-0700    | His 109, Arg 201, Arg 88  |                        | -10.639       | -83.614       | -54.439      | -463.625  |
| 2     | F2721-0150    | Arg 119, Ile 209, Ser 112 |                        | -9.365        | -76.292       | -55.366      | -460.397  |
| 3     | DP01608      | Phe 77, Arg 88, Arg 201   |                        | -9.206        | -59.199       | -39.603      | -373.868  |
| 4     | RH00579      | Arg 119, Ile 209          |                        | -8.613        | -51.805       | -38.976      | -374.560  |
| 5     | SYN          | Val 204                   | His 109, Trp 219       | -6.566        | -67.470       | -44.969      | -459.358  |

A. Asinex.
B. Life chemicals.
M. Maybridge.
respectively (Table 3). The phobic, philic, and balance score for CAAX prenyl protease I are 1.214, .670, and 1.811, respectively (Table 3). The donor/acceptor property measures the ability with which a ligand can donate hydrogen bonds. The donor/acceptor score for CAAX prenyl protease I is .847, whereas for CAAX prenyl protease II is .750 (Table 3).

3.3. Electrostatic potential surface of protein

The electrostatic potential surface of the protein’s active site was generated using molecular mechanics method which reveals the electropositive and electronegative potential regions of the protein. The predicted electrostatic surface contour for CAAX prenyl protease I is shown in Figure 4(A) and for CAAX prenyl protease is shown in Figure 4(B). Here, the blue color indicates the electropositive regions and the red color indicates the electronegative regions. Figure 4 reveals that CAAX prenyl protease I has more electropositive regions, whereas electronegative regions were more in active site of CAAX prenyl protease II. This result reflects the difference in activity of both proteins.

3.4. Molecular docking studies

To carry out the docking studies we have taken two sets of inhibitors namely, bi substrate analog inhibitors of
protein farnesyl transferase of 16 derivative compounds and peptidyl (acyloxy) methyl ketones of three compounds. Bi substrate analog inhibitors are substrate mimetics containing both peptidomimetic and farnesylmimetic (Porter et al., 2007). The IUPAC name of bisubstrate analog inhibitors and Peptidyl (acyloxy) methyl ketones are mentioned in Supplementary Tables 1 and 2, respectively. The results of docking of known inhibitors with CAAX prenyl protease I were given in Table 4 and the docking results of CAAX prenyl protease II were given in Table 5. In order to assess the interference with the function of analogs enzyme of human, all the inhibitors were docked to the active site of human CAAX prenyl protease I and the results are provided in Supplementary Table 3. The docking results of human and *Leishmania* CAAX proteins reveals that there will be no interference in function as the interacting residues, docking scores, and binding energy values have no similarity. Among bi substrate analog inhibitors, compound 8 (IUPAC name: N-{2-[[3-[[2-(1H-indol-3-yl)ethyl]sulfamoyl]phenyl]carbamoyl]ethyl]hexadecanamide) has shown the lowest docking score of $-9.811$ indicating its best binding with CAAX prenyl protease I. However, among all the screened peptidyl

![Figure 6. 2D interaction diagram of induced fit docking results of CAAX prenyl protease II with top three compounds from screening namely, (A) Compound F2721-0700, (B) Compound F2721-0150, and (C) Compound DP01608.](image)
lowest docking score of \(-10.391\). It was found from the docking result that the known compound taken for the study are having good binding with CAAX prenyl protease I, whereas CAAX prenyl protease II have very low docking scores with all the known compounds. It reflects the difference in active site between both proteins. It was also found that Zn metal ion was playing important role in binding in case of CAAX prenyl protease I. Binding energy calculated for the all protein ligand complexes support the docking results.

### 3.5. Structure-based virtual screening

In order to find the new and potent inhibitors against CAAX prenyl protease I and II, structure-based virtual screening has been performed based on active site of both proteins. Three databases namely, Asinex, Life chemicals and Maybridge having 55,958, 288,651, and 93,148 structures, respectively, were used for virtual screening. Initially, we performed docking using HTVS which is helpful in rapid screening of the ligands and is the least computationally intense process. Ligands were selected for further steps on the basis of docking score.
These ligands were docked with the same receptor using SP docking protocol. The top 10% compounds from SP docking are selected on the basis of docking score and carried further for XP docking method. XP docking method is a powerful and discriminative procedure. The total of 52 compounds from Asinex, 206 compounds from Life Chemicals and 66 compounds from Maybridge for CAAX prenyl protease I and II were resulted from XP docking method. Best screened compounds for CAAX prenyl protease I and II are tabulated in Tables 6 and 7, respectively. It is clear from the results that screened compounds have better interaction as well as docking score than the known compounds. The best compounds obtained from screening were further analyzed for their interactions with key amino acid residues in both CAAX prenyl protease I and II.

3.6. Binding mode analysis of known compounds and screened compounds

The known inhibitors as well as the new inhibitors obtained from the virtual screening are docked in the active site of the both modeled protein. It was found from the docking result that new compounds obtained from screening have lower docking score when compared to the docking score of known compounds. Tables 4–9 shows the compounds interacting with residues of CAAX I and II proteins which clearly exposes the difference in interacting residues between the known and screened compounds and confirm the gain of interactions in case of screened compounds. Best 15 compounds from screening of both CAAX prenyl protease I and II were carried for further IFD studies.

Figure 7. (A) Backbone RMSD, (B) Backbone RMSD, (C) RMSF of CAAX prenyl protease I, and (D) RMSF of CAAX prenyl protease II over 20 ns of simulation time period.
3.7. Induced fit docking

The changes at the interaction interface during the recognition of ligand by the protein could be nicely obtained from IFD. The results of the IFD for CAAX prenyl protease I and II were tabulated in Tables 8 and 9, respectively. It discloses the predicted docking score, IFD score, and interacting residues in protein ligand binding. It also shows the hydrophobic interactions along with hydrogen bond interactions play major role in protein ligand binding. Both CAAX prenyl protease I and II have got new and potent inhibitors through virtual screening as the compounds have lower docking score. The compounds showing best IFD results with CAAX prenyl protease I were SYN20008993 (Asinex), F0451-4356 (Life chemicals) and F02432-0154 (Life chemicals). The compounds showing best IFD with CAAX prenyl protease II were F2721-0700 (Life chemicals), F2721-0150 (Life chemicals), and DP01608 (Maybridge). Two-dimensional (2D) interaction diagram of compounds SYN20008993 (Asinex), F0451-4356 (Life chemicals) and F02432-0154 (Life chemicals) with CAAX prenyl protease I were shown in Figures 5(A–C), respectively. The 2D interaction diagram of compounds F2721-0700 (Life chemicals), F2721-0150 (Life chemicals), and DP01608 (Maybridge) with CAAX prenyl protease II were shown in Figures 6(A–C), respectively.

3.8. ADME properties

The drug-like properties of the lead compounds were assessed by evaluating their physicochemical properties, the pharmacokinetic parameters, including their ADME, were analyzed using QikProp. The partition coefficient (QP logPo/w) which is crucial for estimating the absorption and distribution of drugs within the body, ranges from .204 to 4.509 in case of CAAX prenyl protease I and .021 to 4.460 in case of CAAX prenyl protease II. These pharmacokinetic parameters are well within the
acceptable range defined for human use, thereby indicating their potential as drug-like molecules. The results of QikProp for CAAX prenyl protease I and II are shown in Tables 10 and 11, respectively. The drug-like properties of the lead compounds was calculated using DruLiTo tool to avoid the method biasness and the results were appended in Supplementary Tables 4 and 5. The results reveal that the drug-like properties were found to be good in agreement.

3.9. Molecular dynamics simulations

In order to check the stability of the modeled protein and three best docked protein ligand complex from screening, MDS for 20 ns has been performed using Desmond module of Schrödinger. Root Mean Square Deviation (RMSD) of both modeled proteins were found to have deviations up to 8 ns of the simulation time period for CAAX prenyl protease I and 12 ns for CAAX prenyl protease II of simulation time period where the initial fluctuations were considered as the time taken for the equilibration. Later both proteins were found to be stable throughout the rest of simulation time period. Backbone RMSD of both CAAX I and II were depicted in Figures 7(A) and (B). RMSD was supported by RMSF graph where the residue fluctuations were shown. The stable and equilibrated structure obtained from the initial MDS were used for further docking studies. RMSF graph for CAAX prenyl protease I is shown in Figure 7(C) and for CAAX prenyl protease II is shown in Figure 7(D). Binding stability of three best docked complexes of CAAX prenyl protease I and II were analyzed through RMSD, RMSF, Protein ligand contacts over 20 ns of simulation time period. RMSD graph shown in Figures 8(A) and (B) reveals the stability of both CAAX I and CAAX II proteins with their
respective ligands. RMSF graph (Figures 8(C) and (D)) shows the residue fluctuations where the residues involved in interaction were found to have lesser fluctuation. Histogram and timeline of protein ligand contacts for CAAX prenyl protease I and II complexes with three best docked complexes over 20 ns of simulation time period were shown in Figures 9 and 10, respectively. Our data clearly shows the contribution of each residue in active site towards interaction with ligand. Both complexes were found to have stable interaction throughout the simulation. It has been concluded that all three complexes of both proteins were found to have good binding stability and these compounds can be carried further for more confirmatory analysis which could provide help in rational drug design of both CAAX I and II.

4. Conclusion

In this present study we have performed homology modeling for both CAAX prenyl protease I and II and validated using SAVES and ProSA server. Further, MDS for 20 ns simulation brought stable structures that were carried for further molecular modeling studies. Active sites for both proteins were predicted using SiteMap which reveals the key interacting residues in the active site. Further molecular docking studies with known compounds provided help in predicting the binding mode with both proteins. Subsequently, structure-based virtual screening was carried out to bring new and potent inhibitors for both proteins. It also reveals that the screened compounds were better than known compounds based on the docking score as well as interacting residues. Then the best 15 compounds were carried further for IFD studies which predict accurate binding poses of compounds with both proteins. ADME properties prediction of best compounds from screening was good in agreement and those compounds can be used for human consumption. MDS studies of best complexes of both proteins reveal the binding stability and conformational stability. Finally, the best compounds obtained from screening could be carried for the further rational drug design of CAAX prenyl protease I and II inhibitors.
Supplementary material
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Disclosure statement
No potential conflict of interest was reported by the authors.

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References
Azam, S. S., Abbasi, S. W., & Batool, M. (2014). Structure modeling and docking study of HCV NS5B-3a RNA polymerase for the identification of potent inhibitors. *Medicinal Chemistry Research*, 23, 618–627. doi:10.1007/s00044-013-0666-5

Baglo, Y., Gabrielsen, M., Sylte, I., & Gederaas, O. A. (2013). Homology modeling of human y-butyratic acid transporters and the binding of pro-drugs 5-aminolevulinic acid and methyl aminolevulinic acid used in photodynamic therapy. *PLoS ONE*, 8, e65200. doi:10.1371/journal.pone.0065200

Boyartchuk, V. L., Ashby, M. N., & Rine, J. (1997). Modulation of Ras and a-factor function by carboxyl-terminal proteolysis. *Science*, 275, 1796–1800. doi:10.1126/science.275.5307.1796

Casey, P. J. (1992). Biochemistry of protein prenylation. *Journal of Lipid Research*, 33, 1731–1740.

Das, M., Prakash, S., Sundar, S., & Dubey, V. K. (2013). Mefloquine-unresponsive *Leishmania donovani* has a greater ability than mefloquine-responsive *L. donovani* to resist reactive oxygen species. *FEBS Journal*, 280, 4807–4815. doi:10.1111/febs.12449

Dolence, J. M., Steward, L. E., Dolence, E. K., Wong, D. H., & Poulter, C. (2000). Studies with recombinant *Saccharomyces cerevisiae* CaaX prenyl protease RCE1p. *Biochemistry*, 39, 4096–4104. doi:10.1021/bi9923611

Friesner, R. A., Banks, J. L., Murphy, R. B., Halgren, T. A., Klicic, J. J., Mainz, D. T., … Shenk, P. S. (2004). Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *Journal of Medicinal Chemistry*, 47, 1739–1749. doi:10.1021/jm0406430

Friesner, R. A., Murphy, R. B., Repasky, M. P., Frye, L. L., Greenwood, J. R., Halgren, T. A., … Mainz, D. T. (2006). Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein–ligand complexes. *Journal of Medicinal Chemistry*, 49, 6177–6196. doi:10.1021/jm051256o

Gillespie, J. R., Yokoyama, K., Lu, K., Eastman, R. T., Bollinger, J. G., Van Voorhis, W. C., … Buckner, F. S. (2007). C-terminal proteolysis of prenylated proteins in trypanosomatids and RNA interference of enzymes required for the post-translational processing pathway of farnesylated proteins. *Molecular and Biochemical Parasitology*, 153, 115–124. doi:10.1016/j.molbiopara.2007.02.009

Glode, version 6.2. (2014) New York, NY: Schrodinger, LLC.

Guo, Z., Mohanty, U., Nochre, J., Sawyer, T. K., Sherman, W., & Krilov, G. (2010). Probing the α-helical structural stability of stapled p33 peptides: Molecular dynamics simulations and analysis. *Chemical Biology and Drug Design*, 75, 348–359. doi:10.1111/j.1747-0285.2010.00951.x

Induced Fit Docking. (2014) New York, NY: Schrodinger, LLC.

Kim, E., Ambroziai, P., & Otto, J. C. (1999). Disruption of the mouse Rce1 gene results in defective Ras processing and mislocalization of Ras within cells. *Journal of Biological Chemistry*, 274, 8383–8390. doi:10.1074/jbc.274.13.8383

Kim, D. E., Chivian, D., & Baker, D. (2004). Protein structure prediction and analysis using the Robetta server. *Nucleic Acids Research*, 32, W526–W531. doi:10.1093/nar/gkh468

Lakhwii, W., Chevé, G., Yasri, A., & Ibrahimii, A. (2015). Determination and validation of mTOR kinase-domain 3D structure by homology modeling. *Onco Targets and Therapy*, 30, 1923–1930. doi:10.2147/OTT.S84200 (eCollection 2015).

LigPrep, version 2.9. 2014. New York, NY: Schrodinger, LLC.

Lyne, P. D., Lamb, M. L., & Saeh, J. C. (2006). Accurate prediction of the relative potencies of members of a series of kinase inhibitors using molecular docking and MM-GBSA scoring. *Journal of Medicinal Chemistry*, 49, 4805–4808. doi:10.1021/jm060522a

Meissner, D., Omdan-Naresh, J., Vogelphoi, I., & Merzendorfer, H. (2010). A novel role of the yeast CaaX protease Ste24 in chitin synthesis. *Molecular Biology of the Cell*, 21, 2425–2433. doi:10.1091/mc.10-01-0080

Michaelsson, D., Ali, W., Chiu, V. K., Bergo, M., Sillieti, J., Wright, L., … Phillips, M. (2005). Postprenylation CaaX processing is required for proper localization of Ras but not Rho GTPases. *Molecular Biology of the Cell*, 16, 1606–1616. doi:10.1091/mc.04-11-0960

Mori, Y., & Okumura, H. (2013). Pressure-induced helical structure of a peptide studied by simulated tempering molecular dynamics simulations. *The Journal of Physical Chemistry Letters*, 4, 2079–2083. doi:10.1021/jl400769w

Mount, D. W. (2007). Using the basic local alignment search tool (BLAST). *Cold Spring Harbor Protocols*, 2007, pdb.top17. doi:10.1101/pdb.top17

Pandey, R. K., Sharma, D., Bhatt, T. K., Sundar, S., & Prajapati, V. K. (2015). Developing imidazole analogues as potential inhibitor for *Leishmania donovani* trypaonothione reductase: Virtual screening, molecular docking, dynamics and ADMET approach. *Journal of Biomolecular Structure and Dynamics*, 33, 1–13. doi:10.1080/07391102.2015.1085904

Pei, J., & Grishin, N. V. (2001). Type II CAAX prenyl endopeptidases belong to a novel superfamiy of putative membrane-bound metalloproteases. *Trends in Biochemical Sciences*, 26, 275–277. doi:10.1016/S0968-0004(01)01813-8
