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STRUCTURAL INSIGHTS ON BRUGIA MALAYI TRANSGLUTAMINASE WITH CINNAMOYL DERIVATIVES - A MOLECULAR DOCKING APPROACH

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ABSTRACT

The parasite *Brugia malayi,* one of the causative agents for human lymphatic filariasis, found endemic in tropical and sub tropical countries like India, Indonesia, Malaysia and Thailand. The enzyme transglutaminase (TGase) is involved in the growth and development stages of parasite. Transglutaminase has been found to be a better target for the anti filarial agents due to its critical role in the parasite's growth and development. In our study, we reported cinnamoyl derivatives as potent inhibitors for *Brugia malayi* transglutaminase. The computational molecular docking approach was used to confirm the inhibition of cinnamoyl derivatives with the enzyme transglutaminase to block the growth and development of parasite *Brugia malayi* and acts as a novel anti filarial agent.

KEYWORD: Filariasis, *Brugia malayi*, Transglutaminase, Ramachandran plot, Molecular docking and Binding energy.

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INTRODUCTION

Among the various diseases of humans, filariasis has been found to be a major health problem in the world[1.] In many tropical and sub tropical countries, lymphatic filariasis by parasites is a chronic disease. The nematodes *Brugia malayi* and *Wuchereria bancrofti* are the causative agents[2] for human filariasis which infect millions of people globally with high morbidity and is endemic in tropical and sub tropical areas[3]. The disease could seriously affect the socio-economic status of the endemic areas[4]. The International task force has declared that filariasis be eliminated before 2020[5]. The chronic disease, lymphatic filariasis, a vector borne disease where mosquitoes act as vectors[6]. The infection by parasite *Brugia malayi* is a broad spectrum clinical condition and the symptoms include "filarial fevers", asymptomatic microfilaremia, lymphedema, elephantiasis and recurrent lymphadenitis with retrograde lymphangitis[1].

During the life cycle of the parasite *Brugia malayi* in host mosquito, the parasite differentiates into series of morphologically distinct forms. The distinct morphology of parasite consists of five major stages, separated by 4 molts[7]. The two physiological types of parasite *Brugia malayi* are the nocturnally periodic and nocturnally sub periodic[8]. The parasite*s are* ovoviviparous that give birth to the first-stage larvae termed as microfilariae that are enclosed by a loose bag-like structure called a sheath. The sheath structure is not yet known, however, it is assumed to be composed mainly of proteins, carbohydrates, inorganic components[9] and chitin[10.]

Identification of key enzyme is needed for human chronic lymphatic filariasis which is responsible for the parasite's development in the vector. The enzyme transglutaminase (TGase) in adult female *Brugia malayi* was found to play a critical role in the growth and development of the parasitic worms[11.] The key aspect of enzyme transglutaminase in parasite *Brugia malayi is* protein post-translational modifications in developing nematode larvae. By blocking the post translational modification pathways by novel target transglutaminase may lead to elimination of parasite from the host[12]. The development of effective chemotherapeutic agents against human filariasis through transglutaminase inhibition is vital[13].

Transglutaminase (TGase) are a class of enzymes that catalyze the reaction calcium-dependent cross linking of cellular proteins. The cross linking is through glutamyl lysine isopeptide bonds. The significance of covalent isopeptide cross linking plays a vital role in resistance of parasite towards proteolysis[14]. Transglutaminase2 (TG2) inhibitors with several series of trans-cinnamoyl derivatives have been synthesized and evaluated to provide a better understanding of their structure-activity profiles[15]. So, with the rational approach towards *Brugia malayi* transglutaminase with cinnamoyl derivatives is attempted to inhibit this enzyme for chemotherapeutic agents against human filariasis.

MATERIALS AND METHODS

Homology modeling of Transglutaminase

Due to non-availability of 3D experimental structure of *Brugia malayi* transglutaminase, computational prediction method, homology modeling was implemented. The program BLAST-P against PDB was used to identify the template for the homology modeling of the target *Brugia malayi* transglutaminase. The experimental crystal structure of tapasin-ERp57 thiol oxidoreductase heterodimer (PDB CODE: 3F8U) with resolution 2.6 Å was found to be a template for target based on good sequence alignment, high score, low e-value and presence of domain. MODELLER 9V10[16] was used to generate a 3D structure of the target enzyme transglutaminase by homology modeling method. The MODELLER followed the automatic generation of 3D structure

models based on satisfaction of spatial restraints which includes backbone modeling, side chain generation and loop refinement.

Validation of 3D modeled structure of Transglutaminase

Generation of 3D structure of target transglutaminase by homology modeling was the first step towards molecular docking. The validation of 3D structure model quality is necessary for the accurate modeling. Stereochemistry and structural superimposition were common methods for validation of modeled structure of protein. The overall quality of modeled structure was evaluated by program PROCHECK[17] and FATCAT[18].

PROCHECK (http://nihserver.mbi.ucla.edu/SAVES/) program was used for validating the overall quality and torsion angles of protein by generating Ramachandran plot. The plot shows the amino acids arrangement in different regions of the plot which is based on phi-psi angles. Ramachandran plot determines the quality of the modeled 3D structure by assessing various parameters such as lengths, angles and planarity of the peptide bonds, geometry of the hydrogen bonds, and side chain conformations of protein structures as a function of atomic resolution.

FATCAT (http://fatcat.ljcrf.edu/), Flexible structure AlignmenT by Chaining Aligned fragment pairs allowing Twists was an approach for flexible protein structure comparison. It simultaneously addresses the two major goals of flexible structure alignment; optimizing the alignment and minimizing the number of rigid-body movements (twists) around pivot points (hinges) introduced in the reference structure. In FATCAT, the structure alignment was formulated as the AFPs (aligned fragment pairs) chaining process allowing at most t twists, and the flexible structure alignment is transformed into a rigid structure alignment when t is forced to be 0. Dynamic programming is used to find the optimal chaining. The overall similarity between the two structures was represented in three ways, probability, RMSD and equivalent positions.

Selection of Binding site

The binding site for the target *Brugia malayi* transglutaminase was characterized by Q- site finder[19] (http://www.modelling.leeds.ac.uk/qsitefinder/) The algorithm predicts ten sites based on the ligand binding with structure database reference. The best binding site for enzyme transglutaminase was site1 which is characterized by amino acids forming binding cavity or pocket.

Modeling of ligand cinnamoyl derivatives

The structure of ligand class of cinnamoyl derivatives for the molecular docking analysis was obtained by the database PUBCHEM[20] (http://pubchem.ncbi.nlm.nih.gov/). Five ligands were selected based on the 'Lipinski rule of 5' for the molecular docking analysis. The ligands were modeled using ACD Chemsketch[21] tool and converted into PDB format by E-BABEL tool (http://www.vcclab.org/lab/babel/).

Molecular docking of enzyme with inhibitor

Molecular docking of *Brugia malayi* transglutaminase with cinnamoyl derivatives was performed using Autodock Vina[22] software. The docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site of transglutaminase. The polar hydrogen atoms were added to transglutaminase and its nonpolar hydrogen atoms were merged. All bonds of ligands were set to be rotatable. All calculations for protein-fixed ligand-flexible docking were done using the Lamarckian Genetic Algorithm (LGA) method. The grid box with a dimension of 40 x 40 x 40 points and the centre of X,Y and Z were -3, 82 and 68 was used to cover the entire enzyme binding site and to accommodate free movement of ligands. The exhaustiveness of the docking was set to value 8. The best conformation was selected based on lowest binding energy.

Molecular docking results were evaluated based on the number of H- bond interactions between protein-ligand in docked complex. The H-bond interactions were analyzed in Pymol[23] software.

RESULTS & DISCUSSION

Brugia malayi, one of the filarial parasites, needs to be targeted for chemotherapeutics. With the available literature studies, it was found that the enzyme transglutaminase acts as a drug target. In our study, computational methods were applied to gain insights into the structural information about the protein with inhibitors to design suitable anti filarial agents. The 3D structure of *Brugia malayi* transglutaminase was modeled by MODELLER using homology modeling method and showed that the protein contains four unique domains to perform the function.

3D modeled structure

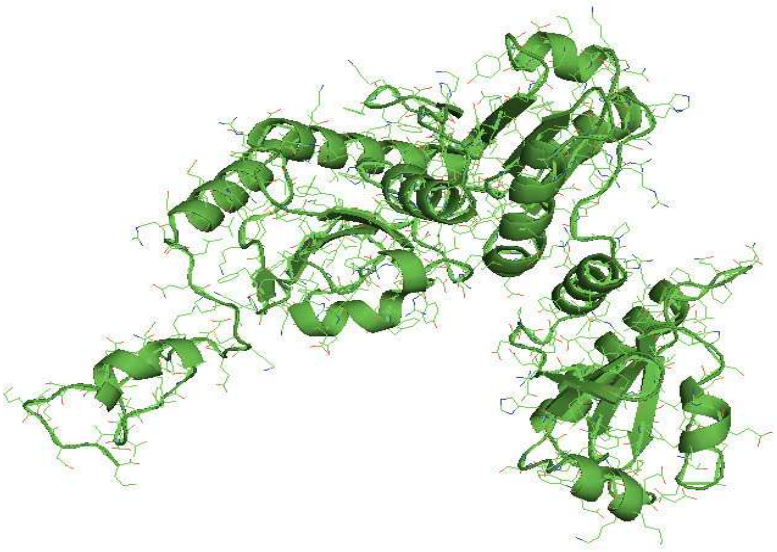

Figure 1
Brugia malayi transglutaminase 3D structure model by MODELLER. The visualization of modeled protein was done in Pymol with cartoon representation.

The modeled structure of *Brugia malayi* transglutaminase was validated by PROCHECK and FATCAT to check the overall quality. PROCHECK results showed that the overall geometry was good and amino acids mostly occupied the most favored regions of the Ramachandran plot which explained the stability of the phi-psi angle of the protein. The statistics showed 91.6% in the most favored regions and 0.6% in disallowed regions.

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Ramachandran plot

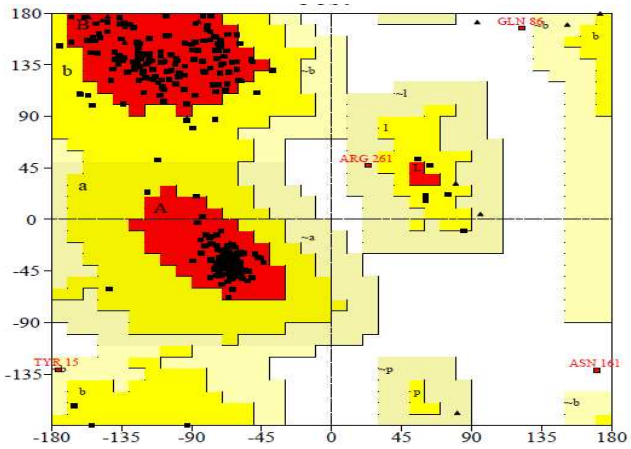


Figure 2

PROCHECK results of Brugia malayi transglutaminase showed the Ramachandran plot with stable stereochemistry and overall geometry.

Structural superimposition

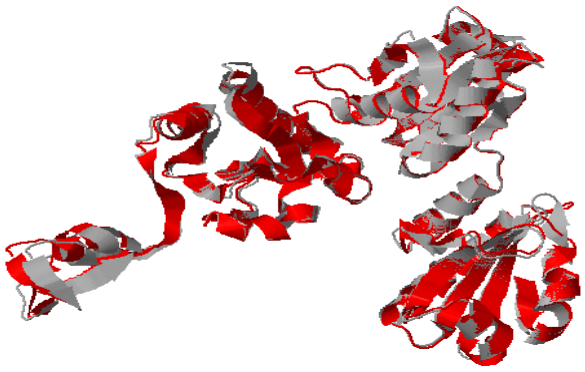
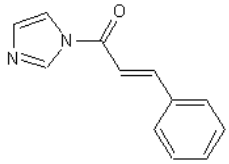


Figure 3

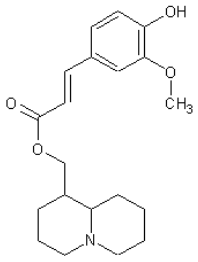
Structural superimposition of Brugia malayi transglutaminase and template 3F8U is shown in cartoon model. The results showed good quality of modeling for Brugia malayi transglutaminase.

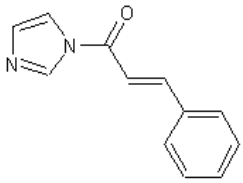
The second validation method of structural superimposition by FATCAT showed that the modeled structure of *Brugia malayi* transglutaminase and template 3F8U were of same structure. RMSD of 1.03 Å was observed and P-value of 0.00e+00 (raw score is 986.64). RMSD less 2 Å is considered a better model of structural prediction methods.

Ligands – cinnamoyl derivatives

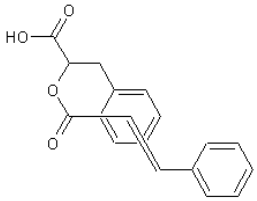


1-(trans-cinnamoyl)imidazole
Cinnamoylimidazole

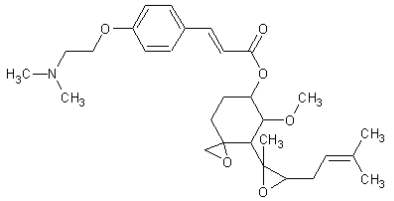




AC1LD69N



O-cinnamoyl beta-phenyllactate



Beloranib

Figure 4
Ligand 2D structures of cinnamoyl derivatives from Pubchem database.

Rational drug design was carried out with five ligands of cinnamoyl derivatives assumed to be potent against *Brugia malayi* transglutaminase, which was modeled by ACD Chemsketch and converted into PDB format. With 3D structure of both protein and ligands, the molecular docking analysis was carried out using software Autodock Vina. The conformation file was set with the docking parameters and finally the binding energy was calculated. Based on the lowest binding energy, the best conformation of the ligand bind with the protein was selected. Among the five ligands, beloranib was bound well with the protein *Brugia malayi* transglutaminase binding site with lowest binding energy of -7.2Kcal/mol and three H-bonds. The amino acids which interacted with atoms of belaronib were GLY190, SER189 and HIS183. Other ligands AC1LD69N showed two H-bonds and 1-(trans-cinnamoyl) imidazole, Cinnamoylimidazole, O-cinnamoyl beta-phenyllactate showed one H-bond with the amino acids atoms of protein transglutaminase.

Table 1
Molecular docking result

LIGAND	BINDING ENERGY(Kcalmol)	NO OF H-BOND
1-(trans-cinnamoyl)imidazole	-6.8	1
AC1LD69N	-7.8	2
Cinnamoylimidazole	-7.7	1
O-cinnamoyl beta-phenyllactate	-6.9	2
Beloranib	-7.2	3

Molecular docking

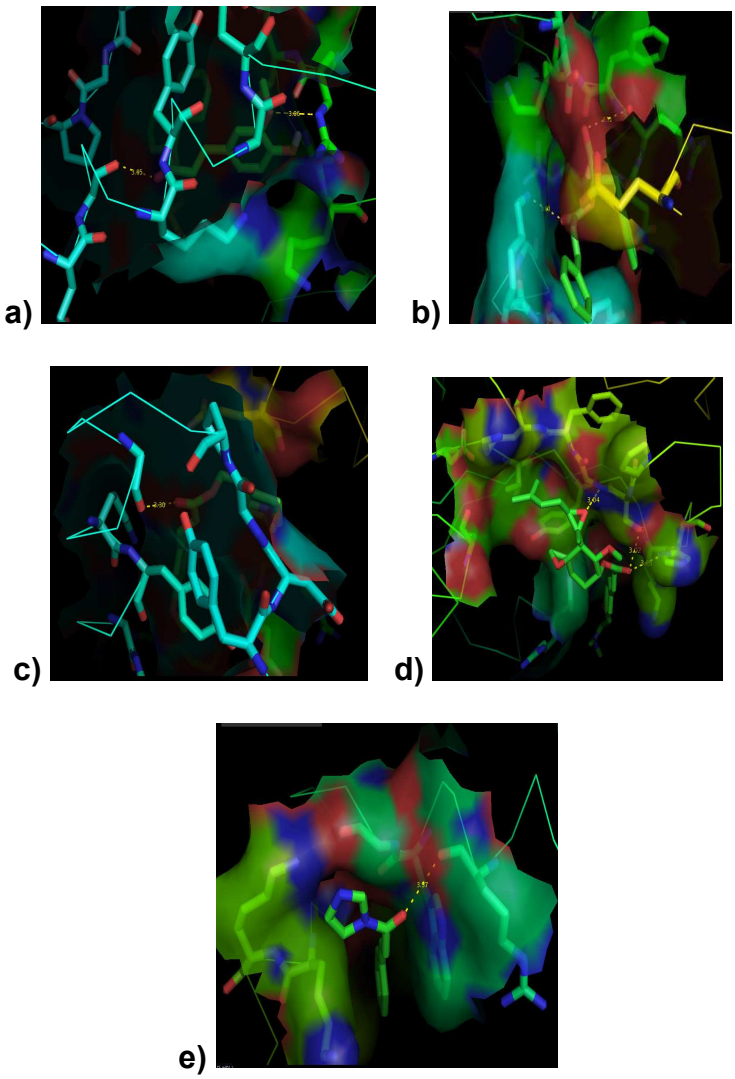


Figure 5

Molecular docking result of Brugia malayi transglutaminase with cinnamoyl derivatives. The H- bond interaction was visualized in Pymol and showed in yellow dotted lines. The bond length between the atoms of protein-ligand complex was shown and stable intermolecular interaction was observed.

CONCLUSION

In our research on filarial parasite *Brugia malayi*, first, 3D model of enzyme transglutaminase was predicted based on the homology modeling method by MODELLER. The functional domain was generated based on proper backbone and side chain modeling with template 3F8U. The modeled structure was validated to check the accuracy of the modeled structure of transglutaminase and used to identify the binding sites of the ligand using Q-site finder server. The cinnamoyl derivatives which got competitive activity with substrate were used to carry out rational drug design on *Brugia malayi* transglutaminase to predict the binding affinity and mode. The molecular docking analysis on

transglutaminase with five cinnamoyl derivatives was carried out in Autodock Vina and results showed that beloranib binds more potently with three H-bond interaction and AC1LD69N showed two H-bond interaction. The other three ligands showed one H-bond. The average binding energy for cinnamoyl derivatives was in the range of -7.2Kcal/mol. The rational design on filarial parasite *Brugia malayi* transglutaminase showed the ligand beloranib as a potent inhibitor and it is necessary to confirm the inhibition in wet lab methods and clinical trial phases. In future beloranib can be used as a novel anti filarial agent to reduce the disease, especially in endemic areas.

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