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Research Article

In-Silico evaluation of Anti-Leishmanial compounds of selected pharmacophore

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ABSTRACT

In this study, out of sixteen compounds, only thirteen compounds have shown hydrogen bonding with the modeled cathepsin B protein of *L. donovani*, remaining compound (CID13416238, Posaconazole, and CID 6082033) did not show any hydrogen bonding with ligands. So, these 13 compounds which show hydrogen bonding with modeled protein could be considered as most potent lead compounds having inhibitory activity at either promastigote stage or amastigote stage of *Leishmania donovani*. Few compounds demonstrated better docking score to either AutoDock4.0 or GOLD v2.1 but some compound did not show any hydrogen bonding with modeled protein in either docking software tool. Thus, it could be concluded that generated experimental compounds could have potential as pharmacological tool against Visceral Leishmaniasis.

Keywords: Cysteine Protease, Vinyl Hydrazide, Antileishmanial Drugs, Licochalcone, Visceral Leishmaniasis.

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INTRODUCTION

Leishmaniasis is a complex vector-borne disease caused by at least seventeen different species of *Leishmania* protozoan parasites¹. Four types of Leishmaniasis are seen to affect the human *i.e.* Visceral Leishmaniasis (VL), Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (ML) and Post Kala-azar Dermal Leishmaniasis (PKDL). Human leishmaniasis is distributed worldwide, more frequent in the tropics and sub-tropics regions with a predominance of over 12 million cases and an approximated incidence of 0.5 and 1.5 million cases of VL and CL respectively. In humans, it manifests either as a cutaneous disease caused mainly by *L. major*, *L. tropica*, and *L. mexicana*, as a mucocutaneous disease caused mainly by *L. braziliensis*, or as a visceral disease caused mainly by *L. donovani* and *L. chagasi*. Phlebotomine sand flies transmits leishmanial diseases to their vertebrate hosts, which acquire the pathogen by feeding on infected hosts and transmit them by regurgitating the parasite at the site of a subsequent blood meal². While obtaining a blood meal, sand flies salivate into the host's skin. This saliva contains anti-clotting, anti-platelet and vasodilatory compounds that increase the hemorrhagic pool where sand flies feed³. An estimated 0.9–1.3 million (9 to 13 lakhs) new cases and 20 000 to 30 000 death due to Leishmaniasis, a dreadful parasitic disease occur annually⁴. It has been reported that the number of persons at risk of contracting Leishmaniasis is approximately 350 million and there are 2.3 million new cases reported every year⁵. The

genus *Leishmania* is the causative agent of Leishmaniasis, a severe parasitic disease of considerable importance in terms of both diversity and complexity. All *Leishmania* species are having digenetic life cycle and alternate between the flagellated mobile promastigotes to non-flagellated and non-motile amastigotes⁶. Visceral Leishmaniasis (VL), also known as Kala-azar, black fever, and Dum-Dum fever, is the most severe form of leishmaniasis⁷.

In the present study, a homology model of the LdvCP (cysteine protease) was constructed to obtain an in-depth idea of this protein's structural and functional characteristics. The construction of 3D model of the *L. donovani* cysteine protease based on available 3D structure of the cysteine protease from *protein data bank* was done by homology modeling. The predicted structures were refined by taking advantages of the CHARMM parameters and energy minimization studies and were evaluated using the DOPE (Discrete Optimized Protein Energy) score, PROCHECK and Verify3D to analyze the structural integrity. Analogues, different natural compounds from available databases and currently implicated antileishmanial compounds were docked into the active site of 3D model protein. The designing of novel compounds was based on pharmacophore mapping, and 3D-QSAR studies of different inhibitors against cysteine protease of *Leishmania* parasite.

MATERIALS AND METHODS

Parasite Culture

Promastigotes of Indian *Leishmania donovani* strain MHOM/IN/83/AG83 was obtained from culture bank of Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna, India. The cryo-cells were revived and grown in RPMI1640 medium (Sigma-Aldrich) supplemented with 10% Fetal Calf Serum (FCS: Sigma-Aldrich) in BOD incubator at 22°C.

Genomic DNA Isolation

Genomic DNA isolation was done by using standard protocol of PureLink™ Genomic DNA kit. 1ml of culture was taken and centrifuged in 10,000 rpm for 10 minutes at 0°C, then the discard the supernatant and keeps the palette. 200 µl of PBS was added to the palette. 20 µl Proteinase K and 20 µl RNase A was added to the sample. Then by brief vortexing it was mixed well. Then the mixture was incubated at room temperature for 2 minutes. 200 µl PureLink™ Genomic Lysis/Binding Buffer was added to the same tube and then again mixed well by vortexing to obtain a homogenous solution. This mixture was incubated at 55°C for 10 minutes to promote protein digestion. 200 µl of absolute ethanol was added to the lysate. The solution mixed well by vortexing to yield a homogenous solution.

A PureLink™ Spin Column in a Collection Tube was taken and prepared by lysate (~640 µl) was added to the spin column. The column was centrifuge at 10,000 × g for 1 minute at room temperature. Then the collection tube was discarded and the spin column was placed into a clean PureLink™ Collection Tube supplied with the kit. 500 µl Wash Buffer 1 was added to the column, centrifuged at 10,000 × g for 1 minute at room temperature. The flow through was discarded and 500 µl Wash Buffer 2 prepared with ethanol was added to the column, centrifuged the column at maximum speed for 3 minutes at room temperature. Then collection tube was discarded. The spin column was placed in a sterile 1.5-ml microcentrifuge tube. 100µl of PureLink™ Genomic Elution Buffer was added to the column and kept for 5mins and then centrifuged at maximum speed for 1 minute at room temperature. Now the column was discarded and purified DNA was stored at -20°C (long-term).

RESULTS AND DISCUSSION

DNA Isolation, Primer designing and amplification of cysteine protease Gene

Leishmania promastigote strain MHOM/IN/83/AG83 were grown in RPMI1640 medium in supplemented with 10% heat inactivated Fetal Calf Serum (FCS) and subjected to DNA extraction using an DNA isolation kit (Qiagen). Total DNA was taken as electrophoresis on 1.2% agarose gel. Figure 3.1(A) shows that total DNA has been extracted and concentration of the extracted DNA is quite high. DNA was stored at -20°C. Cysteine protease gene was amplified from total DNA pool using cysteine protease specific primers designed against cysteine protease of *L. major*. The PCR product was analyzed by electrophoresis on 1.2% agarose gel and photographed. The size marker used to estimate PCR products is 1kbp DNA ladder (Sigma). Figure 3.1(B) shows the PCR product of the *L. donovani* cysteine protease gene. The gel picture shows that the size of PCR product is about 1185bp and is similar to the *L. major* cysteine protease gene size (as reported in NCBI) and no other gene is amplified. Thus, these designed primers are specific for amplifying of cysteine protease gene from *L. donovani*.

Sequencing of the PCR Product, Sequence and Phylogenetic Analysis

The PCR product was then processed and purified to run sequencing PCR, and loaded on ABI 3130xL genetic analyzer for sequencing. The PCR product was sequenced twice once with forward primer and once with reverse primer. Nucleotide in each position was considered correct if two sequencing results (which were sequenced in opposite directions) confirmed each other. Translated sequence similarities between cysteine protease gene within same species and sequence dissimilarity with the human cysteine protease gene was predicted by using Clustal W and cladogram shown in Figure 1. The translated amino acid sequence of cysteine protease protein consisting of 211 amino acids was analyzed to know evolutionary relationship with other known species. Further sequence analysis of cysteine protease of *Ldv* revealed that it has 82-100% sequence identity with *L. infantum*, *L. major*, *L. tarentolae*, *T. cruzi* and *L. braziliensis* amino acid sequences. A Phylogenetic analysis by maximum likelihood method exhibited that cysteine protease of *Ldv* clusters with other species 91% with *Leishmania sp.* (PDB ID 6EX8) and varies in between 82-100 % with same *Leishmania* species.

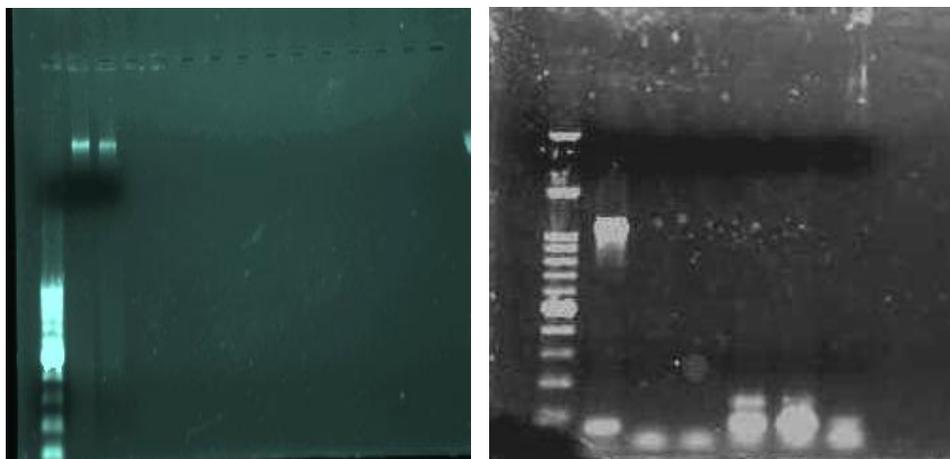


Figure 1A: Electrophoresis of PCR product for *Leishmania donovani* cysteine protease gene from patient blood, from left to right =>Line 1: 2 cysteine protease gene (~1185bp), Lane 2: DNA ladder Kbp. 1B : Electrophoresis of PCR product for *Leishmania donovani* (AG83 strain) gene from culture, from left to right =>Line 1:2 DNA ladder Kbp, cysteine protease gene(1185bp).

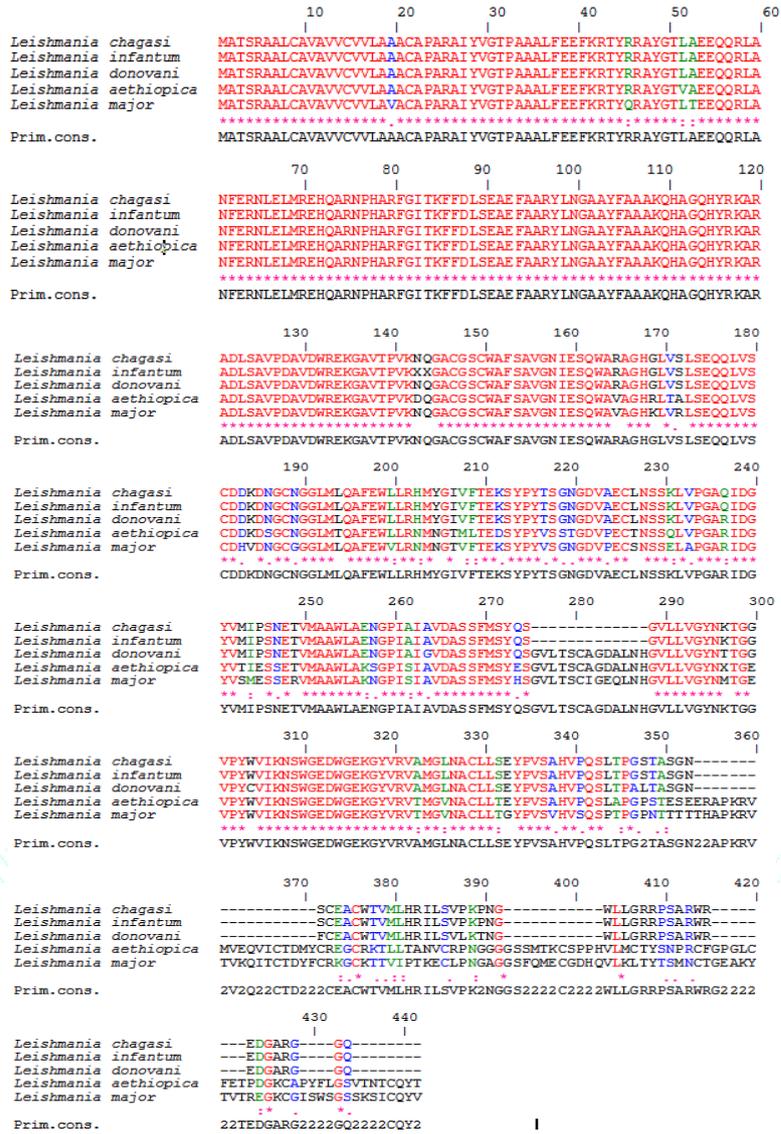


Figure 2: Based on the multiple sequences alignment we retrieved the conserved regions with different species was carried out using clustal W server. The red color star indicates the conserved region and the identified conserved regions are representing in Prim.cons. sequence as shown in figure.

CONCLUSION

Leishmania cysteine proteases are potential target for treating visceral Leishmaniasis. In order to understand structural features we carried out homology modeling of *Leishmania donovani* cathepsin B and cathepsin L like cysteine protease. The lack of crystal structure and structural data for validated targets are the major problem that faced cysteine protease inhibitor that may represent the important step towards the assessment of anti-leishmanial chemotherapy. Developed model shows good overall structural quality as validated by using different validation tools like PROCHECK, VERIFY 3D score. In addition, model shows good native protein folding. The ligand active site was determined by sequence alignment and PAR 3D analysis tool. The amino acid residues Cys29, His188 and Asn208 of cathepsin B of *Leishmania donovani* are active site residues, which were exploited to design target specific inhibitors. The cathepsin B model was taken for further screening of different chemical library for studying their cathepsin B inhibitory activities, different public domain chemical

libraries were accessed for this study and many compounds have been generated using Chemsketch program.

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