JPP JOURNAL OF PROTEINS AND PROTEOMICS
2(1), January-June 2011, pp. 41-48

IDENTIFICATION OF NOVEL INHIBITOR OF TRYPANOTHIONE SYNTHASE FROM TWO *LEISHMANIA* SPECIES: COMPARATIVE *IN SILICO* ANALYSIS

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Abstract: We report modelled structure of Trypanothione synthase (TryS) of *Leishmania donovani.* The quality of model is validated by PROCHECK validation package and ERRAT plot. Potential inhibitors of TryS from two *Leishmania* species were screened using virtual docking employing small molecule dataset of natural products. The grid for docking simulations was placed over the synthase domain of the enzyme. The best twenty hits in both the cases are listed in tabular form. The enzymes were highly identical with respect to their active site residues and the surrounding regions. There was a marginal difference in the biding energies of both the ligands, which might be due to the rotameric changes observed in the modelling process. The top hits were structurally similar; implying that compounds with such chemical entities can be potential inhibitors of the enzyme. The interactions were also conserved - either they bind residues surrounding the ATP binding cleft which act as anchoring residues or to the residues surrounding the substrate binding site making them potential inhibitors of the enzyme.

Key words: Leishmaniasis; Therapeutics; Virtual Screening

Introduction

Leishmania is a genus of trypanosome protozoa causing widespread disease leishmaniasis. The most fatal form of the disease is visceral leishmaniasis caused by *Leishmania donovani*. Glutathione in conjugation with glutathione reductase and glutathione peroxidase maintains the redox-homeostasis in mammals. In case of trypanosomatids similar role is played by trpanothione /trypanothione reductase system (Colotti and Illari, 2011). Trypanothione is a dithiol conjugate and the enzyme trypanothione reductase helps to maintain the thiol in reduced form (Fairlamb *et al.*, 1985). The lack of functional alternatives to the thiol metabolism and the parasite's sensitiveness to oxidative stress combined with the absence of such system in humans makes the enzymes belonging to this pathway attractive target for drug design (Oza *et al.*, 2005). Enzymes such as trpanothione reductase, tryparedoxin and trypanothione synthase (TryS) have been validated as drug targets for trypanosomiasis (Colotti and Illari, 2011). Trypanothione synthase is one of the key enzymes of the pathway which is involved in the synthesis of trypanothione and also maintains the levels of polyamines which are critical for cellular proliferation and differentiation (Wilkinson et al, 2003; Comini *et al.*, 2004). In case of *Leishmania*, TryS is bifuctional possessing synthetase and amidase activities. The enzyme catalyses the biosynthesis of trypanothione in an ATP dependent reaction and also hydrolyses dithiol (Oza *et al.*, 2008). The crystallographic structure of TryS from *Leishmania major* is available in three different forms and it has revealed the presence of two major domains viz the N-terminal and the C-terminal domains. The C-terminal domain is a characteristic papain-like cysteine protease domain; the domain catalyzes biosynthesis of T(SH)2 by step wise addition of two molecules of glutathione to one molecule of spermidine by hydrolysis of an ATP which binds to the ATP-common grasp fold present at the C-terminus. The N-terminal domain catalyzes hydrolysis of

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tryoanothione to glutathione and glutathione-spermidine cojugate and then further into glutathione and spermidine (Fyfe *et al.*, 2008).

TryS has been validated genetically and biochemically as a drug target in *Leishmania* and other trypanosomatids (Shukla *et al.*, 2010). In case of *Trypanosoma brucei,* techniques such as RNA interference and gene knockout studies have provided evidence that it is essential for survival of both bloodstream and procyclic forms of the parasite (Torrie *et al.*, 2009). TryS can be a potential target since it is a single copy gene in human parasites and inhibition of the enzyme will lead to alteration in polyamine levels as well as synthesis of thiols resulting in depletion of substrate for trypanothione reductase and disturbs redox homeostasis of the parasite. We have earlier reported identification of drug targets of the parasite and potential inhibitor of other key enzymes (Kannan *et al.*, 2010; Suthar *et al.*, 2009; Singh *et al.*, 2008). We have used *in silico* techniques for identification of inhibitors to TryS from *Leishmania* parasite.

Methods

Homology Modelling: Leishmania major trypanothione synthase (PDB code: 2VOB) Fyfe *et al.*, 2008) served as template for the homology modelling process based on its sequence similarity to *Leishmania donovani* TryS. The template was identified using NCBI-BLASTP search against Protein Data Bank (PDB) with default parameters. MODELLER (Marti-Renom *et al.*, 2000) is a computer program that generates 3-D model structures of proteins and their assemblies based on restraints on the spatial structure of the amino acid sequence(s) and ligands to be modelled. Quality of the 3-D homology models generated was ranked by DOPE score and the best models were assessed using PROCHECK validation package (Laskowski *et al.*, 1993) and it was further validated using ERRAT plot.

Virtual Screening: Virtual screening was performed with Autodock4.2 (Morris *et al.*, 1998). Lamarckian genetic algorithm considered as one of the best algorithm to identify the lowest binding energy conformation was used in the study. AutoDock is an automated docking tool that predicts protein-ligand interactions and binding energies using an empirically calibrated force field, which is projected onto a regular grid for intermolecular energy calculations and the method also considers small molecules as flexible. The model of *Leishmania donovani* TryrS generated using Modeller and crystal structure of *Leishmania major* TryS (PDB ID: 2VOB) were used in the study. The small molecule dataset used in the study was MS Discovery natural product dataset (*http://www.msdiscovery.com/natprod.html*) (Morris *et al.*, 1998), containing 800 compounds; the set was selected for the study owing to presence of structurally diverse chemical entities including alkaloids flavonoids, terpenes and coumarins. Polar hydrogens were added and grid maps were prepared using the AutoGrid utility with (80 x 80 x 102) points to cover the entire region occupying the active site residues of TryS and grid spacing set to 0.375 Å. Grid maps were generated representing all the atom types present in protein and ligand along with electrostatic and desolvation maps. All docking simulations were performed with an initial population size of 300 and docking simulations consisted of 100 LGA runs in each run. The best individual from each generation was propagated to the next generation, remaining docking parameters were set to default. The docking results from each of the hundred calculations were clustered on the basis of root-mean-square deviation (RMSD) between the Cartesian coordinates of the atoms and were ranked on the basis of free energy of binding. The top-ranked compounds were visually inspected for their predicted mode of binding. The top 20 hits obtained from both virtual screening processes were tabulated and they were observed for consensus binding patterns to identify potential inhibitors of the enzyme.

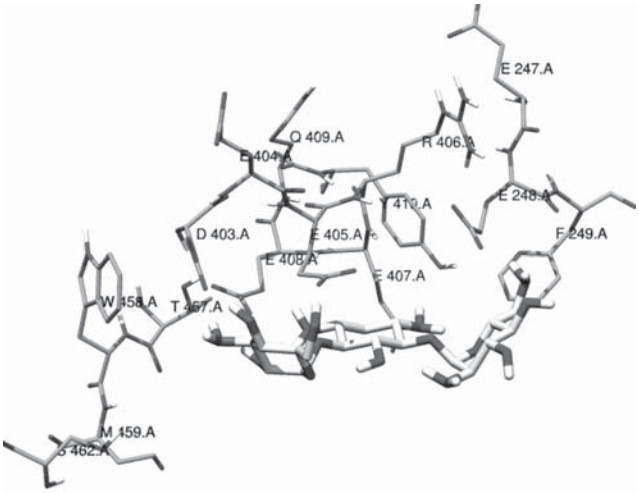
Results and Discussion

TryS of *Leishmania donovani* and *Leishmania major* were 96% similar, the active site residues were conserved and residues in the surrounding regions showed minimum divergence. The grid for docking simulations was placed over the synthase domain and the domain comprises sub-domains. Three major sub-domains comprise the active site of synthetase enzyme - ATP binding site comprises of subdomains A and C,

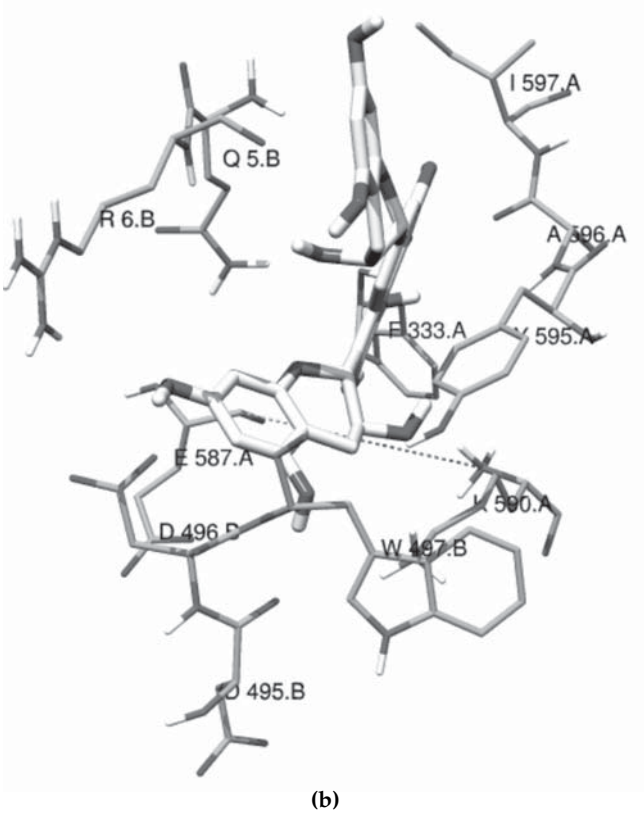
glutathione binding cleft is present mainly subdomain B and glutathionylspermidine binding cleft is present between subdomains A & B. Since the active site cleft is larger, the compounds bind in multiple orientations traversing all the active site domains. Two major interactions were observed at the active site region. At the end of the virtual screening process the compounds were sorted based upon their binding energy. The criterion that was set predict the highly favourable conformation, the run producing the lowest energy and also having highest number of conformation within the cluster

Predicted mode of binding at the synthetase active site of the enzyme: Two major binding modes were observed among the first 20 compounds analysed for their chemical interactions. The compounds bind to the subdomain A (residues 695-633). The compounds also bind to the spermidine binding domain of the enzyme. The key residues surrounding the cavity such as E407, E408, D403 and M459 are in interaction with ligands. Few residues are in hydrogen bonding interaction with ligand which makes them a highly favourable energy binding (Fig 1A). The ligands that bind to sub-domain of the protein is seen in hydrophobic interaction with residues 590 to 597 which are the anchoring residues surrounding the ATP binding site (Fig 1B). Consensus binding was observed among all class of inhibitors that have been studied within these two domains as discussed earlier.

Identification of potential inhibitors of the enzyme: The top hits from both the virtual screening processes were structurally analysed for their potential as inhibitors of the enzymes. The enzymes were highly identical with respect to their active site residues and the surrounding regions. There was a marginal difference in the biding energies of both the ligands, which might be due to the rotameric changes observed in the modelling process. The top hits were structurally similar; implying that compounds with such chemical entities can be potential inhibitors of the enzyme. The interactions were also conserved; either they bind residues surrounding the ATP binding cleft which act as anchoring residues or to the residues surrounding the substrate binding



(a)



(b)

Figure 1: Interaction of potential inhibitor with the enzyme. Inhibitors bind to the (A) spermidine binding domain of the enzyme or (B) sub-domain of the protein is seen in hydrophobic interaction with residues 590- 597 which are the anchoring residues surrounding the ATP binding site.

site making them potential inhibitors of the enzyme. During the docking simulations, the compounds formed hydrogen bonds and were also seen in hydrophobic interaction with key

Table 1
Structure and Docking Statistics of top 20ranked Structures. C, CL, E, A and T Indicate Numbers of Clusters, Number of Conformations within the Selected Cluster, Binding Energy of the Selected Conformation in kcal/mol, Number of Atoms in the Inhibitor and Number of Torsions, Respectively

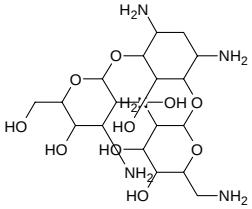
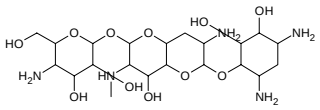
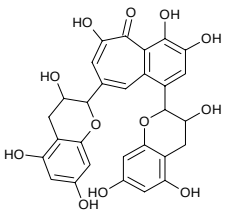
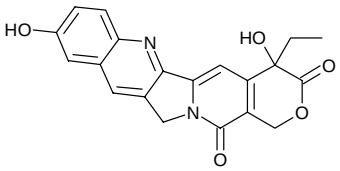
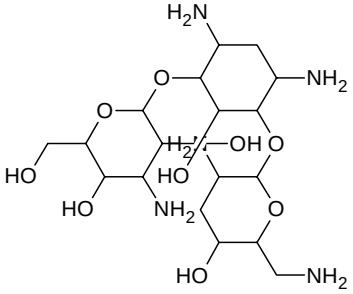
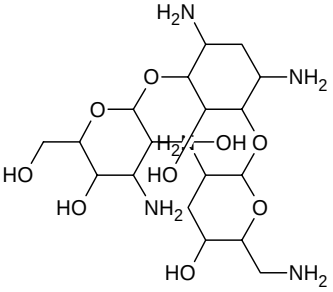
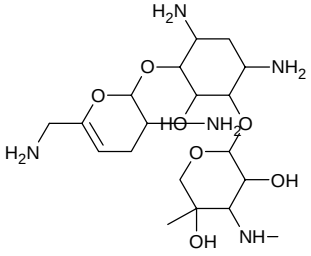
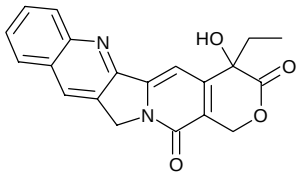
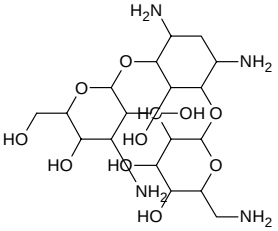
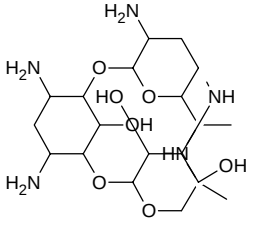
Compound with TryS of L. major	*CL*	*LC*	*E*	*T*	*Compounds with TryS of L.*	*CL*	*LC*	*E*	*T*
BEKANAMYCIN SULFATE					APRAMYCIN				
	58	12	-13.23	17		78	5	-11.64	16
THEAFLAVIN					10-HYDROXYCAMPTOTHECIN				
	75	8	-13.17	11		18	27	-11.61	3
TOBRAMYCIN					TOBRAMYCIN				
	63	7	-13.1	16		72	8	-11.44	16
SISOMICIN SULFATE					CAMPTOTHECIN				
	74	4	-12.96	13		13	27	-11.31	2
KANAMYCIN A SULFATE					GENTAMICIN SULFATE				
	49	23	-12.59	17		59	9	-11.25	13

table contd.

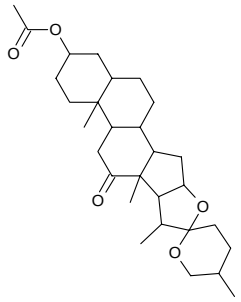
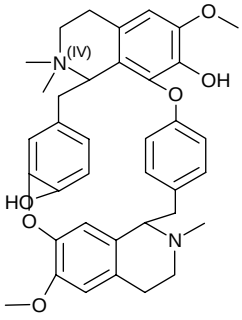
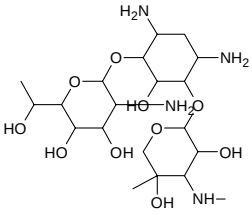
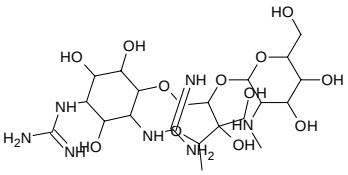
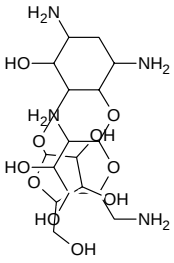
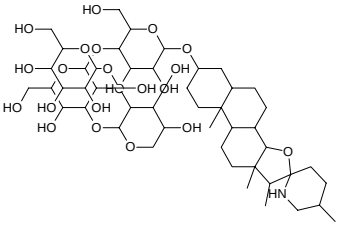
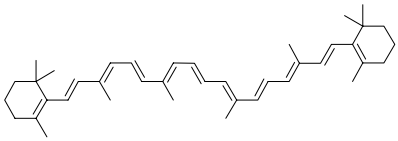
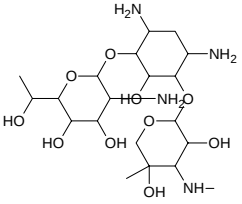
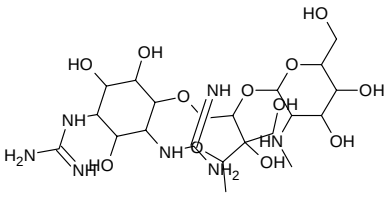
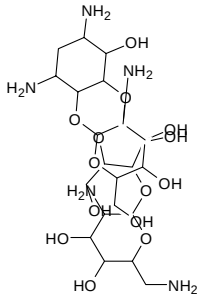
Compound with TryS of L. major	*CL*	*LC*	*E*	*T*	*Compounds with TryS of L.*	*CL*	*LC*	*E*	*T*
HECOGENIN ACETATE 	13	56	-12.27	2	TUBOCURARINE CHLORIDE 	13	46	-10.96	4
GENETICIN 	37	31	-12.05	15	DIHYDROSTREPTOMYCIN 	91	4	-10.91	17
RIBOSTAMYCIN SULFATE 	67	10	-12.02	16	TOMATINE 	96	3	-10.67	23
BETA-CAROTENE 	95	3	-11.87	13	GENETICIN 	39	15	-10.55	15
DIHYDROSTREPTOMYCIN 	94	3	-11.78	17	PAROMOMYCIN SULFATE 	94	3	-10.51	22

table contd.

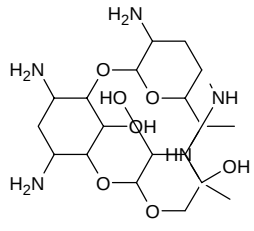
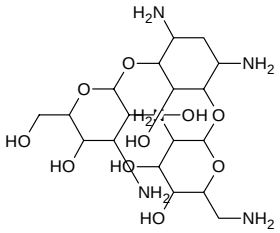
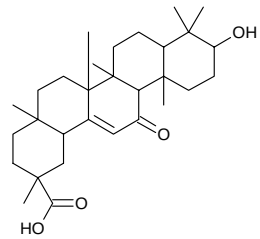
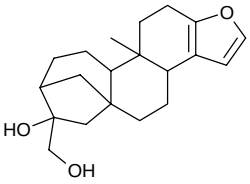
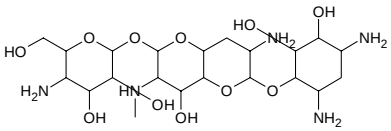
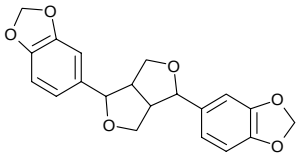
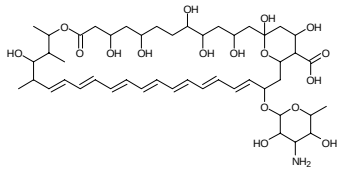
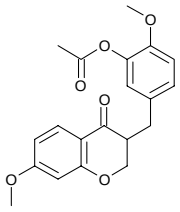
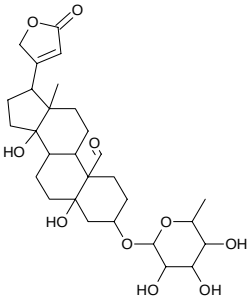
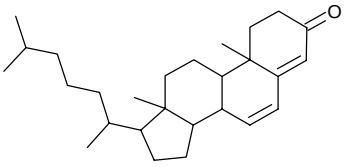
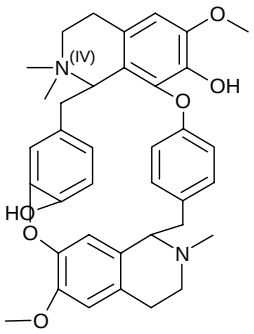
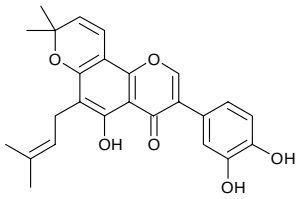
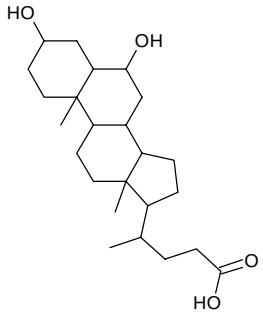
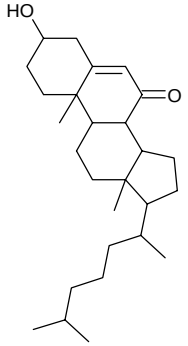
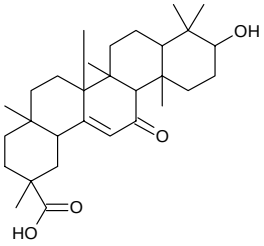
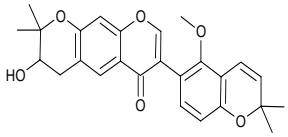
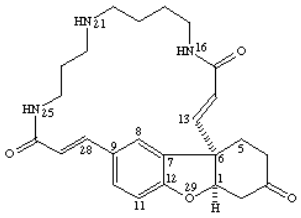
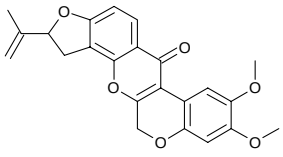
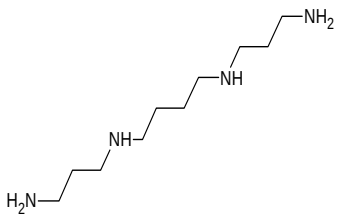
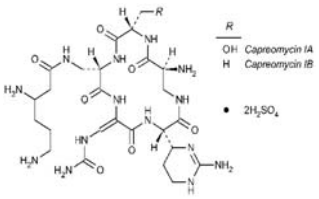
Compound with TryS of L. major	*CL*	*LC*	*E*	*T*	*Compounds with TryS of L.*	*CL*	*LC*	*E*	*T*
GENTAMICIN SULFATE 	67	6	-11.7	13	BEKANAMYCIN SULFATE 	76	6	-10.5	17
ENOXOLONE 	5	79	-11.4	3	CAFESTOL 	6	51	-10.27	3
APRAMYCIN 	66	7	-11.05	16	ASARININ (-) 	12	65	-10.18	2
AMPHOTERICIN B 	47	28	-10.92	15	DEOXYSAPPANONE B 7,3′-DIMETHYL ETHER ACETATE 	46	14	-9.95	6
CONVALLATOXIN 	59	6	-10.88	9	CHOLEST -4, 6-DIEN-3-ONE 	14	34	-9.95	5

table contd.

Compound with TryS of L. major	*CL*	*LC*	*E*	*T*	*Compounds with TryS of L.*	*CL*	*LC*	*E*	*T*
TUBOCURARINE CHLORIDE 	18	44	-10.85	4	POMIFERIN 	40	9	-9.9	6
alpha-HYDROXYDEOXYCHOLIC ACID 	26	16	-10.81	7	7-OXOCHOLESTEROL 	16	33	-9.86	6
18-alpha-GLYCYRRHETINIC ACID 	7	76	-10.79	3	MUNDULONE 	17	27	-9.86	3
LUNARINE 	10	44	-10.78	0	DEHYDROROTENONE 	19	35	-9.8	3
SPERMINE 	42	15	-10.74	13	CAPREOMYCIN SULFATE 	80	4	-9.77	14

residues of both the spermidine binding cleft and the ATP binding cleft, potentiating them to be possible inhibitors of the enzyme. These set of compounds can be studied *in vitro* for their activity against the parasite and can further be used for structure activity relationship studies.

Acknowledgements

PDB file of Homology-Modeled Structure of Trypanothione synthetase of *Leishmania donovani* is submitted to Protein Model Data Base (http://mi.caspur.it/PMDB/). The PMBD ID of the submission is PM0076391. Research funding by Department of Information Technology, Government of India (Grant Number: DIT/R&D/BIO/15(12)/2008) is acknowledged. PS acknowledges research fellowship form IIT Guwahati.

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