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In silico Studies on Complete Inhibition of Trypanothione Reductase of Leishmania Infantum by γ-sitosterol and Antcin-A: Novel Target for Anti-leishmanial Activity

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ABSTRACT

Inhibition of the trypanothione reductase (Try R) activity interaction has been becomes a new therapeutic strategy to leishmaniasis. Trypanothione reductase is a genetically validated drug target enzyme for structure-based drug design against *Leishmania*, the causative agent of human trypanosomiasis. We used theoretical docking study, conducted on a sample previously reported for anti-cancer, anti-diabetic and antioxidant potential of Kaempferol, guggultetrol, γ-sitosterol and antcin-A at the binding site of *Leishmania infantum* trypanothione reductase (Try R) examine interaction energy. These studies indicate that γ-sitosterol and antcin-A displays potent activity against Try R with lowest binding energy and RMSD values to be -9.34 kcal mol^{-1} for γ-sitosterol,-8.36 Kcal mol^{-1} for antcin-A and 2.0 Å. Docking analysis of Try R with ligands enabled us to identify specific residues viz., Pro-59, Ala-200, Ala-205, Glu-203, Lue-62, Asp-218, Val-64, Cys-193 and Gln-280 within the TryR and Val-58, Leu-95, Ala-181, Val-201, Ile-206, Asn-266, Asp-277 and Met-282 binding pocket to play an important role in ligand binding affinity. The results of our study contributes towards the development of novel therapeutics based on trypanothione reductase inhibition.

Key words: Autodock, trypanothione reductase, kaempferol, guggultetrol, γ-sitosterol, antcin-a, *Leishmania infantum*, antileishmanial activity

INTRODUCTION

Leishmaniasis is a disease complex caused by the parasite belonging to the genus Leishmania infecting 12 million people worldwide (World Health Organization). The causative species of visceral leishmaniasis (VL) include *Leishmania donovani, Leishmania infantum, Leishmania major, Leishmania mexicana*, etc. in Asia, Africa and Europe (Old World) and *Leishmania chagasi* in South America (New World) (Murray *et al*., 2005; Croft and Coombs, 2003; Shukla *et al*., 2010; Singh *et al*., 2008). Trypanothione Reductase (TryR) which has been ideal target for designing chemotherapeutics in Trypanothione metabolisms (Krauth-Siegel and Comini, 2008; Schmidt and Krauth-Siegel, 2002; Fairlamb *et al*., 1985). This disease is endemic in low-income population of Central and South American countries (Tempone *et al*., 2005). Commonly

available drugs for Leishmaniasis have severe side effects, high cost and low efficacy (Shukla *et al.*, 2010). Thus, there is an urgent need for new and less toxic treatments for Leishmaniasis.

γ-sitosterol is a naturally occurring family of sterol commonly found in plants, plant oils and toad venom (Gros and Deulofeu, 1967) α, β and γ isomers of sitosterol. This γ-sitosterol compound has been used in Ayurvedic medicine to cure many diseases. A variety of biological properties have also been ascribed to this class of compounds, including anti-inflammatory, antioxidant and anti-cancers (Sundarraj *et al.*, 2012). This compound also reported to possess anti-diabetic and anti-atherosclerotic effects (Balamurugan *et al.*, 2011). There are *in vitro* studies of the cytotoxic properties of γ-sitosterol on different human breast and lung cancer cell lines, showing it as an anti-carcinogenic agent with a distinct ability to inhibit tumor promotion (Sundarraj *et al.*, 2012). In our study, using bioinformatics tools we tried to evaluate whether γ-sitosterol is a good ligand to some of the target proteins related to leishmaniasis specifically TryR.

Antcin is a steroid natural compound among the antcin (antcin-A, B, C and H) and their derivatives have reported its mode of action, Anti-inflammatory, anti-insecticidal and cytotoxic activities (Cherng *et al.*, 1996; Shen *et al.*, 2003, 2007; Chen *et al.*, 1995, 2007; Male *et al.*, 2008; Yeh *et al.*, 2009). In addition, antcin-A and γ-sitosterol has not been studied as a leishmanicidal agent. Here, the authors have evaluated antileishmanial effect of antcin-A and γ-sitosterol and clearly demonstrated their mode of action based on *in silico* studies. Crystal structure of Try R from *Leishmania infantum* is already reported; hence, the study authors have used Try R from *Leishmania infantum* for computational studies. However, efficacy of these compounds has been tested on most lethal species of parasite, *Leishmania donovoni*. In this study, we present the anti-inflammatory, anti-insecticidal and cytotoxic effects of two known steroids: γ-sitosterol and antcin-A. This article describes to examine the requirement for molecular model, of *Leishmania infantum* Trypanothione reductase docking with several inhibitors. We hypothesized that these steroid compounds may have differential antileishmanial activities based on their structural basis and would lead to discovery or rationalization of drug design process.

MATERIALS AND METHODS

Preparation of protein for docking: The X-ray crystallographic structure of TryR from *Leishmania infantum* (PDB ID: 2JK6) resolved at 2.0 Å was retrieved from the Protein Data Bank for use in the study. The protein was processed for docking procedure using the AutoDock Tools utility cofactors, water molecules were removed before the docking simulation, polar hydrogen atoms were added and non-polar hydrogen atoms were merged for appropriate charge calculation, Gasteiger charges were assigned for the ligands of γ-sitosterol and antcin-A. Simultaneously ligand files were also processed using python module available with AutoDock Tools, in case of ligand files Gasteiger charges were added, the generated file was then processed, splied and converted into PDBQT format which is the input format for AutoDock4.2.

Ligand preparation for docking: The structure of ligands γ-sitosterol and antcin-A were generated from smile strings followed by energy minimization and optimized using "Prepare Ligands" in the AutoDock 4.2 for docking studies. The optimized ligand molecules were docked into refined trypanothione reductase model using "LigandFit" in the AutoDock 4.2. Semi-flexible docking was performed which includes a flexible ligand and a rigid receptor. All the protein and ligand structural images were generated using PyMol.

Molecular docking: AutoDock 4.2 suite was used as molecular-docking tool in order to carry out the docking simulations (Balamurugan *et al*., 2012). The Auto Dock 4.2 program was used to investigate ligand binding to structurally refined TryR model using a grid spacing of 2.0 Å and the grid points in X, Y and Z axis were set to 60×60×60. The search was based on the Lamarckian genetic algorithm (Oprea *et al*., 2001) and the results were analyzed using binding energy. For each ligand, a docking experiment consisting of 100 stimulations was performed and the analysis was based on binding free energies and Root Mean Square Deviation (RMSD) values and the ligand molecules were then ranked in the order of increasing docking energies.

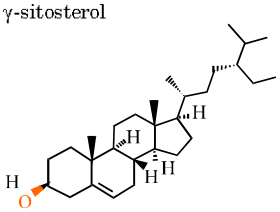
Substrate docking with natural plant substrates sterol family of γ-sitosterol and antcin-A were performed on to TryR model with same parameters and PMV 1.4.5 viewer was then used to observe the interactions of the docked compound to the TryR model.

RESULTS AND DISCUSSION

Validation and docking study of Trypanothione reductase and γ-sitosterol and antcin-A inhibitor.

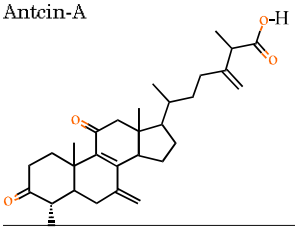
The pdb.qt files were uploaded in autogrid and docking runs were set to be 50, finally the dock log files generated were analyzed for their binding conformations. All docking calculations were carried out using AutoDock 4.2/ADT AutoDock requires the ligand file to be written in PDBQT format. PDBQT format is very similar to PDB format but it includes partial charges ('Q') and AutoDock atom types ('T'). There is one line for each atom in the ligand, plus special keywords indicating which atoms, if any, are to be flexible during the AutoDock experiment. Preparing the ligand involves ensuring that its atoms are assigned the correct AutoDock atom types, adding Gasteiger charges if necessary, merging non-polar hydrogens, detecting aromatic carbons if any and setting up the 'torsion tree' and docking pdb.qt files were prepared by adding all Kollman charges and checked total charges on residues, also added all polar hydrogen's and edited Histidine hydrogen's in protein pdb and checked ligand pdb with 6 tortions. The pdb.qt files were uploaded in autogrid and docking runs were set to be 10, finally the dock log files generated were analyzed for their binding conformations. Analysis was based on free energy of binding, lowest docked energy and calculated RMSD values (Table 1, 2). The total cluster of docking conformation with the docked lead molecule showed negative binding energy. Free energy of binding is calculated as a sum of four energy terms of intermolecular energy (vanderwaal, hydrogen bond, desolvation energy and electrostatic energy), total internal energy, torsional free energy and unbound system energy. We conduct additional docking simulation of molecular interactions between natural

Table 1: Docking results of γ-sitosterol molecules docked on to Trypanothione reductase (2JK6) model

Lead molecule	Cluster rank	Run	RMSD from reference structure (Å)	Estimated free energy of binding (kcal mol^{-1})	Docked energy (kcal mol^{-1})	Estimated inhibition constant, Ki nM or µM (Temp = 298.15 k)
γ-sitosterol 	1	32	0.724	-7.27	-8.13	732.62 nM
	2	7	0.912	-7.15	-7.03	5.75 µM
	3	4	0.890	-6.93	-6.96	8.28 µM
	4	9	0.724	-9.31	-9.34	214.24 nM
	5	17	0. 912	-3.69	-8.05	1.99 µM

No. of distinct conformational clusters found = 05, out of 50 runs, Using an RMSD-tolerance of 2.0 Å

Table 2: Docking results of Antcin-A molecules docked on to Trypanothine reductase (2JK6) model

Lead molecule	Cluster rank	Run	RMSD from reference structure (Å)	Estimated free energy of binding (kcal mol^{-1})	Docked energy (kcal mol^{-1})	Estimated inhibition constant, Ki nM or µM (Temp = 298.15 k)
Antcin-A 	1	29	0.298	-8.26	-8.36	145.41 nM
	2	9	0. 319	-7.06	-7.11	529.08 nM
	3	44	0.684	-7.22	-7.5	936.07 nM
	4	24	0.522	-7.45	-7.52	1.18 µM
	6	11	0.631	-6.69	-7.02	3.78 µM
	8	10	0.549	-6.78	-6.85	4.79 µM

No. of distinct conformational clusters found = 06, out of 50 runs, Using an RMSD-tolerance of 2.0 Å

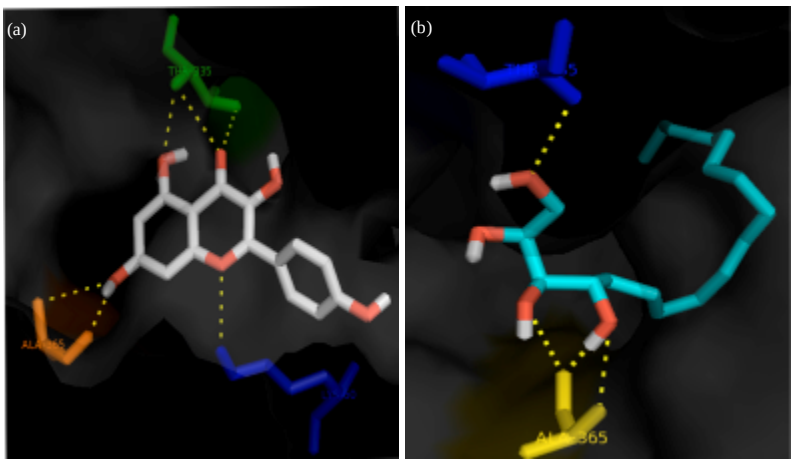


Fig. 1(a-b): *In silico* analysis of the interaction of Trypanothione reductase (Try R) with natural compounds of Kaempferol and guggultetrol. Try R amino Lys-60, Thr-335 and Ala-365

steroids of Kaempferol, guggultetrol and developed protein Tyr R. The docking conformation Kaempferol and guggultetrol shows the predicted free energy of binding -7.70, 4.64 kcal mol^{-1} to the trypanothione reductase with Ki 2.25, 395.60 µM at the temperature of 298.15 k, respectively. Docking analysis of Try R with kaempferol, guggultetrol interaction shows only three residues Lys-60, Thr-335 and Ala-365 (Fig. 1). From the results it has been clearly observed that γ-sitosterol formed nine hydrogen bond interactions with Try R as shown in Fig. 2. Docking analysis of Try R with ligand enabled us to identify specific residues viz. Pro-59, Ala-200, Ala-205, Glu-203, Lue-62, Asp-218, Val-64, Cys-193 and Gln-280 within the TryR binding pocket to play an important role in ligand binding affinity. The docking conformation antcin-A gave the best predicted binding free energy of-9.34 kcal mol^{-1} to the Trypanothione reductase with Ki 214.24 nM at the temperature of 298.15 K for cluster rank one calculated by LGA. Antcin-A formed eight hydrogen bond interactions with Try R as shown in Fig. 3. Docking analysis of Try R with ligand enabled us to identify specific residues viz. Val-58, Leu-95, Ala-181, Val-201, Ile-206, Asn-266, Asp-277 and Met-282 within the TryR binding pocket to play an important role in ligand binding affinity. The docking conformation antcin-A gave the best predicted binding free energy of-8.36 kcal mol^{-1} to the

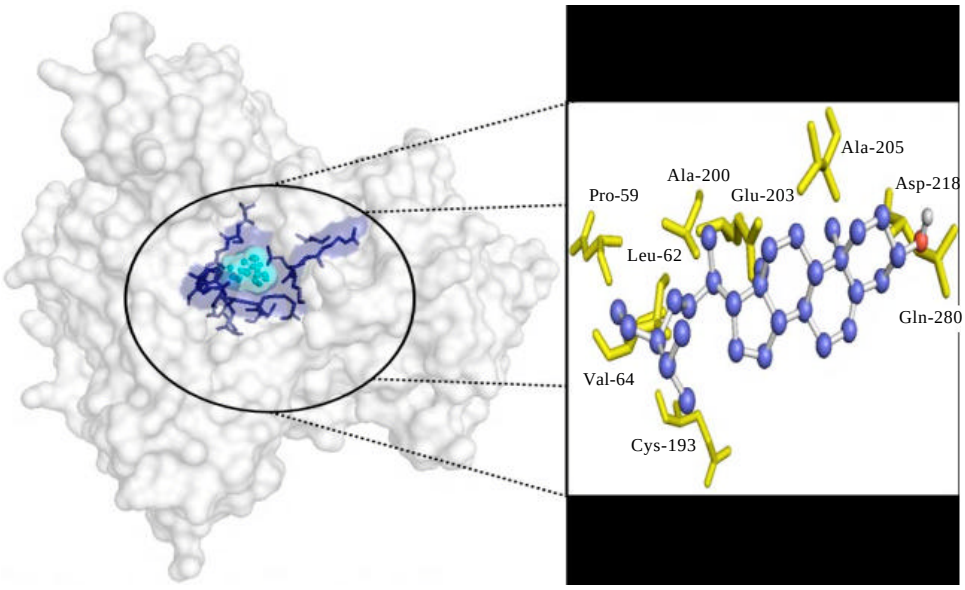


Fig. 2: *In silico* analysis of the interaction of Trypanothione reductase (Try R) amino acids Pro-59, Ala-200, Ala-205, Glu-203, Lue-62, Asp-218, Val-64, Cys-193 and Gln-280 form hydrogen bonds with γ-sitosterol

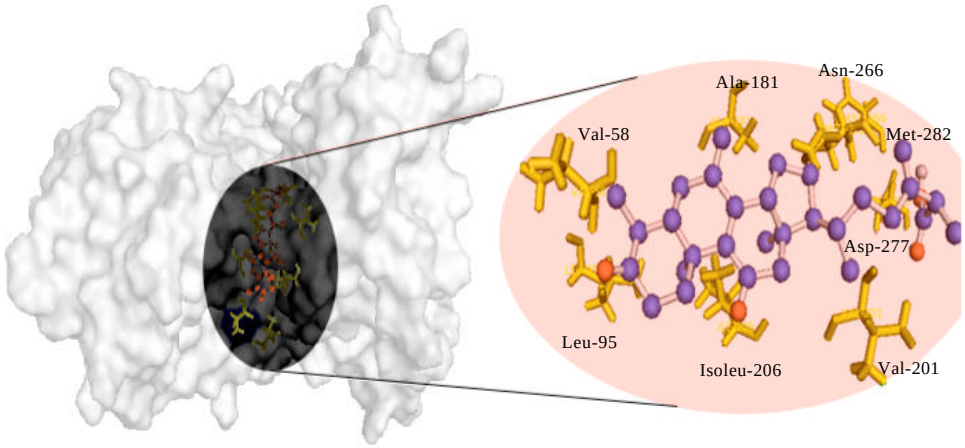


Fig. 3: *In silico* analysis of the interaction of Trypanothione reductase (Try R) amino acids Val-58, Leu-95, Ala-181, Val-201, Ile-206, Asn-266, Asp-277 and Met-282 form hydrogen bonds with antcin-A

Trypanothione reductase with Ki 145.41 nM at the temperature of 298.15 k for cluster rank one calculated by LGA. To confirm the mode of binding of lead molecules, docking was performed with substrate on the Trypanothione reductase model with the same parameters. The different surface

pocket residues that seem to be an important factor in determining the different mode of ligand and substrate interaction with TryR were it played a vital role in binding the cluster of ligand with receptor. These interactions may be due to the formation of Hydrogen bonds or by the establishment of Vander Waals forces. The interactions of lead molecules with the TryR model have lead to the identification of the active site domain. Binding mode and affinity is differ to the model TryR protein with ligands of γ-sitosterol and antcin-A. The docking of *L. infantum* TryR and γ-sitosterol and antcin-A is shown in Fig. 2 and 3. Present *in silico* experiments demonstrate that γ-sitosterol and antcin-A binds TryR and also inhibits its function and thus may act as a drug.

CONCLUSION

Natural compounds have played an important role in treating and preventing human diseases. We have identified potential antileishmanial compounds by targeting parasite specific enzyme which is vital for survival of the pathogen. Our computational approach has provided an opportunity to identify natural product with potential antileishmanial activity for experimental validation and to understand their effects on leishmania parasite redox system. The identified compound from natural sources is potential candidate for drug against leishmaniasis. The current study also points out potential of natural resources as therapeutic agents. These studies indicate that γ-sitosterol and antcin-A displays potent activity against TryR with lowest binding energy and RMSD values to be-9.34 Kcal mol^{-1} for γ-sitosterol,-8.36 Kcal mol^{-1} for antcin-A and 2.0 Å. Docking analysis of TryR with ligands enabled us to identify specific residues within the TryR binding pocket to play an important role in ligand binding affinity. Hence, the proposed drug is presented to the scientific community for further investigational confirmation. The results of the present study clearly demonstrated the *in silico* molecular docking study of γ-sitosterol and antcin-A with TryR enzyme exhibited binding interactions and warrants further studies needed for the development of potent trypanothione reductase inhibitors for the treatment of leishmaniasis.

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