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Molecular docking based inhibition of Trypanothione reductase activity by Taxifolin novel target for antileishmanial activity

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ABSTRACT

The theoretical docking study, conducted on a sample of previously reported for anti-inflammatory and antioxidant activities of Taxifolin at the binding site of *Leishmania infantum* trypanothione reductase (Try R) examine interaction energy. Taxifolin is widely used in the traditional medicine have been investigated for their putative chemo preventive and antileishmanial properties for the last few decades. A theoretical docking study, the evaluation of Taxifolin as inhibitor of trypanothione reductase a validated drug target enzyme of the *Leishmania* parasite. Taxifolin was found to bind at active site of *L. infantum* TryR with lowest binding energy and RMSD values to be -8.82 Kcal/Mol and 2.0 Å respectively. Docking analysis of TryR with ligand enabled us to identify specific residues viz. Ser-14, Ala-47, Ser-162, Thr-336 and Arg-286, within the TryR binding pocket to play an important role in ligand binding affinity. The availability of TryR built model, together with insights gained from docking analysis will promote the rational design of potent and selective TryR inhibitor as antileishmanial therapeutic. The study contributes towards understanding mechanism of antileshmanial effect of the Taxifolin. This compound has shown promising biological activity in preliminary studies by targeting multiple signaling pathways. Thus on the basis of our *in silico* studies we hypothesize that this compound into Taxifolin can be inhibitory effect on against leishmaniasis.

INTRODUCTION

Trypanosomatids cause various lethal forms of tropical human diseases including Leishmaniasis, which is caused by over twenty different species of *Leishmania* parasite (e.g., *Leishmania donovoni*, *Leishmania infantum*, *Leishmania major*, *Leishmania mexicana*, etc.). There are mainly three forms of the disease namely, cutaneous, visceral, and mucocutaneous, out of which cutaneous Leishmaniasis is the most common while visceral Leishmaniasis is the lethal form. Leishmaniasis causes several clinical disabilities like disseminated visceral infection (Kala azar), ulcerative skin lesions, and destructive mucosal inflammation, which impose a great social burden (especially for women), impair economic productivity and impede social development. Vector of the disease is female Phlebotomine sandfly, a dipteran, which transmits the parasite to human during blood sucking (Myler *et al.,* 2008).. Leishmania parasite exists mainly in two life cycle forms namely promastigote in sandfly and amastigote in human macrophage. According to current WHO statistics about 12 million people living in 88 countries, mainly of 5 continents i.e., Asia, Europe, Africa, South America and North America are suffering from Leishmaniasis with 1.5–2 million new cases annually (Desjeux, 1992). 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. Currently, the fields of biochemistry, molecular biology, genetics and pharmacology have grown considerably in their ability to identify specific biological targets. Computational tools have recently been used to explore such targets in designing new drugs with the aim to decreased

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illness. Docking and molecular dynamics are the most commonly used computational tools for elucidation of leishmaniasis targets. Using the above tools, it is easier to find out the interactions and dynamics of drug and target at molecular level (Peitsch *et al.,* 2000). Trypanothione reductase (E.C.1.6.4.8) is a member of the disulfide oxidoreductase family of enzymes (Fairlamb *et al.,* 1992) that presents an attractive target for the development of the new drugs by rational inhibitor design. TyrR is an NADPH-dependent flavoprotein unique to protozoan parasites from the genera *Trypanosoma* and *Leishmania*. The trypanothione system is necessary for protozoan survival because the dithiol trypanothione is required for the synthesis of DNA precursors, the homeostasis of ascordate, the detoxification of hydroperoxides, and the sequestration/export of thiol conjugate. TryR has long constituted an attractive target for chemotherapeutic research in relation to leishmaniasis and other trypanosomatid caused diseases since there are numerous evidences indicating that the enzyme is essential for parasite survival as well as specific in *leishmania* has not been found in human (Dumas *et al.,* 1997; Tovar *et al.,* 1998).

Taxifolin / Dihydroquercetin a dihydroflavonol belongs to flavonoids group, together with its glycosides are commonly found in many species of medical plants. Dihydroquercetin is the most powerful natural antioxidant. Different studies show that it has hypocholesterolemic effects, and also demonstrates anti-inflammatory activities (Gupta *et al.,* 1971) anti-acne activity (Irmanida *et al.,* 2010), medical applications include but not limited to: vitamin deficiency as a vitamin P, cure atherosclerosis, poison treatment, inhibit the cancer cells development, Helps in recovery after chemotherapy and radiation treatment, chemopreventive activity (Lee *et al.,* 2007) Fights the chronic fatigue syndrome and metabolic syndrome. Dihydroquercetin offers protection against cardiovascular disease by inhibiting several steps in the disease process. Additionally, dihydroquercetin helps guard nervous system health, prevents the complications of diabetes, protects the liver against hepatitis-inducing agents, fights infection, and quells inflammation that can lead to dermatitis, arthritis, and pain. The detailed *in silico* analyses of probable inhibition as well as interaction of the models were performed with high binding affinity. However there is no conclusive report as to whether the antileishmanial activity of the taxifolin. In the present study, the structural models of the toxifolin in the trypanothione reductase binding sites has been carried out, which may facilitate further development of more potent antileishmanial agents. Taxifolin might be a promising additive in combined drug inhibitor of trypanothione reductase.

MATERIALS AND METHODS

Python 2.7 - language was downloaded from www.python.com, Cygwin c:\program and Python 2.5 were simultaneously downloaded from www.cygwin.com, Molecular graphics laboratory (MGL) tools and AutoDock4.2 was downloaded from www.scripps.edu, Discovery studio visualizer 2.5.5 was downloaded from www.accelerys.com. The docking of toxifolin into the binding site of the TryR protein was explored using Autodock software, which has been shown to be powerful tools for molecular recognition. To validate the molecular modeling programs, we first evaluated the docking accuracies of Autodock by docking with known TryR inhibitor, toxifolin into the binding site.

Study of Molecular Docking

Protein Preparation for Docking

The 3D structure of *Leishmania Infantum* TryR (PDB ID: 2jk6) was downloaded from Protein Data Bank (PDB) (http: //www.pdb.org/pdb/home/home.do), before initiating the docking simulations, all non-protein molecules were removed from TryR; for any alternative atom locations only the first location was retained. All the docking calculations were performed by using Autodock 4.0. TryR was modified by adding polar hydrogens and then kept rigid in the docking process, whereas all the torsional bonds of ligands were set free by Ligand module in Autodock Tools. Various inhibitors provide an excellent basic for using structure-based approaches for the discovery of new inhibitors.

Ligand Preparation for Docking

The flavonoid ligand like Taxifolin was built using Chemsketch 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.

Docking performance on Trypanothione reductase complex with Taxifolin

Binding sites of these complexes were identified as described previously (31). The conformation of binding site was constructed manually to accommodate with Taxifolin. The validation of the docking accuracy was done by docking of the Toxifolin into its binding site of Trypanothione reductase. AutoDock binding affinities of the taxifolin into Trypanothione reductase were evaluated by the binding free energies (kcal/ mol), inhibition constants (Ki), hydrogen bonds, and RMSD values.

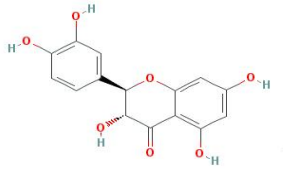
RESULTS AND DISCUSSION

A detailed literature survey yielded that the inhibitory pathway has to be considered as a potential drug target against the parasitic protozoan species of *Trypanosoma* and *Leishmania infantum* TryR using bioinformatics tools. We can hypothesize that toxifolin may be considered to play an important role in inhibiting the TryR activity and progression of leishmaniasis. This toxifolin can be promising candidate for the development towards the design of anti-leishmanial drug as therapeutic compound.

In silico docking of Toxifolin for TryR inhibition

Molecular docking methods are commonly used for predicting binding modes to proteins and energies of ligands (Bikadi *et al.,* 2009). Docking was accomplished employing Autodock 4.0 which is a suite of automated docking tools was

Table. 1: Docking results of Taxifolin molecules docked on to Trypanothione reductase model.

S. No	Lead Molecule	Cluster Rank	Run	Estimated Free Energy of Binding (kcal/mol)	Final Intermolecular Energy (kcal/mol)	Estimated Inhibition Constant, Ki (Nano/micromolar) [Temp= 298.15K]
1	TAXIFOLIN – Parental Compound 	1	20	-8.36	-8.72	747.57 nM
2		2	38	-8.28	-8.82	849.15 nM
3		3	19	-8.15	-8.56	1.06 µM
4		4	43	-7.98	-8.49	1.40 µM
5		5	8	-7.87	-8.31	1.71 µM
6		6	45	-7.42	-7.85	3.66 µM
7		7	26	-7.40	-8.14	3.78 µM
8		8	4	-7.35	-8.10	4.10 µM
9		9	15	-7.04	-7.45	6.92 µM
10		10	25	-6.76	-7.34	11.14 µM
11		11	49	-6.72	-7.09	11.80 µM

Number of distinct conformational clusters found = 11, out of 50 runs, Using an RMSD -tolerance of 2.0 Å.

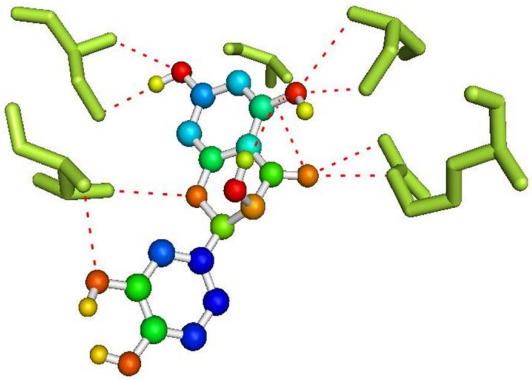


Fig. 1: The binding of Taxifolin with active site of Trypanothione reductase receptor was having 5-H bond interaction. The hydrogen bond was formed between Ser-14, Ala-47, Ser-162, Thr-336 and Arg-286.

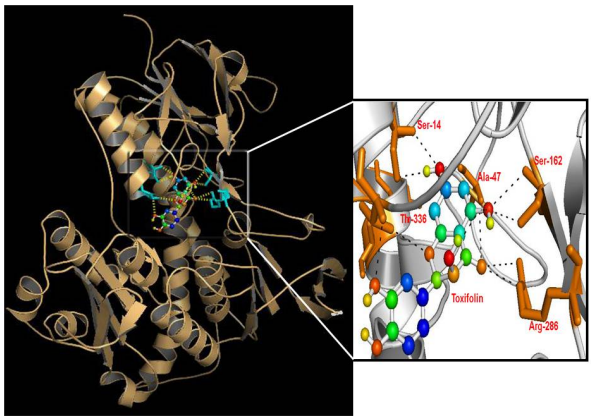


Fig. 2: *In silico* analysis of the interaction of Trypanothione reductase (TryR) with Taxifolin. TryR amino acids Ser-14, Ala-47, Ser-162, Thr-336 and Arg-286 from hydrogen bonds with Taxifolin.

used to predict the affinity, activity, binding orientation of mangiferin to our target protein molecule TryR. Analysis was based on Etotal or free energy of binding, lowest docked energy, and calculated RMSD values. For each approach, the number of hits, the RMSD value of the best hit (with the lowest RMSD) based on shape complementarity are listed in Table 1. Taxifolin was found to bind at active site of *L. infantum* TryR with lowest binding energy and RMSD values to be -8.82 Kcal/Mol and 2.0Å respectively (Table. 1). 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. From the results it has been clearly observed that toxifolin formed five hydrogen bond interactions with Trypanothione reductase was shown in Fig. 1. Docking analysis of TryR with ligand enabled us to identify specific residues viz. Ser-14, Ala-47, Ser-162, Thr-336 and Arg-286, within the TryR binding pocket to play an important role in ligand binding affinity. The docking of *L.infantum* TryR and Taxifolin is shown in Fig. 2. Our *in silico* experiments demonstrate that Taxifolin binds TryR, and also is itself inhibits its function and thus may act as a drug.

CONCLUSION

Natural compounds have played an important role in treating and preventing human diseases. Here, based on the above *in silico* study, Here, we focused that the binding of naturally occurring molecules were seated properly on the particular position and the hydrogen and hydrophobic interactions involves in the position of Ser-14, Ala-47, Ser-162, Thr-336 and Arg-286, residues. 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 studies of taxifolin with Trypanothione reductase enzyme exhibited binding interactions and warrants further studies needed for the development of potent trypanothione reductase inhibitor for the treatment of leishmaniasis.

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