Insilco Screening and identification of novel Trypanothione reductase inhibitor from Leishmania

ABSTRACT

The negative effects of leishmanicidal medications are numerous, and drug resistance to all of them has been observed. As a result, new medication development and the identification of novel therapeutic targets are critical. Leishmania major trypanothione reductase (Lm-TR), a NADPH-dependent flavoprotein oxidoreductase critical for thiol metabolism, is required for parasite viability. Since it lowers trypanothione, a chemical required by Leishmania's tryparedoxin/tryparedoxin peroxidase system to neutralise hydrogen peroxide (H_2O_2) produced by host macrophages during infection, this enzyme is essential for parasite survival in the host.

Because it is not found in the mammalian host, this enzyme is a promising target for novel anti-leishmania medicines. A three-dimensional model of Lm-TR was created using I-TASSER server. Virtual screening of about 5000 sigma aldrich compounds, acquired from the ZINC database, was carried out using Autodock vina tool. Top ten compounds were tabulated based on binding affinity.

The molecules with the ids ZINC04245710 and ZINC03869768 had the highest binding affinities of -11.4 and -11.2 kcal/mol, respectively. These compounds had the maximum binding affinity and the appropriate amount of hydrogen bonds. These molecules may be able to efficiently block the activity of the target enzyme (Lm-TR) and so serve as novel agents to combat cutaneous leishmaniasis. In search for new anti-Leishmania medications that are more effective and less cytotoxic, these molecules may provide a good starting point for a hit-to-lead procedure.

Keywords: Leishmania, Parasite, Virtual screening, Cytotoxic, Binding affinities.

ABBREVIATIONS

Lm-TR: Leishmania major trypanothione reductase

TR: Trypanothione reductase

FAD: Flavin Adenine Dinucleotide

ADT: AutoDock Tools

1. INTRODUCTION

Leishmaniasis is one of the most widespread and under-appreciated vector-borne illnesses, responsible for thousands of fatalities each year [1]. Leishmaniasis is a disease linked to poverty. The different clinical manifestations of the disease are caused by different Leishmania species: (i) visceral leishmaniasis, also known as kala-azar, is the most serious form of the disease (caused by L. donovani and L. infantum); (ii) cutaneous leishmaniasis is the most common (caused by L. tropica, L. major and L. mexicana); and (iii) mucocutaneous leishmanias (caused by L. braziliensis).

Despite their high toxicity, high expense, and developing resistance, pentavalent antimonials such as pentostam (sodium stibogluconate) and glucantime (N-methylglucamine antimonate) are employed as first-line medications for the treatment of leishmaniasis [2], [3]. Glucantime is thought to inhibit trypanothione reductase (TR), and it has been linked to genotoxic and mutagenic effects[4]. Unfortunately, leishmaniasis treatment remains unsatisfactory, owing to the negative side effects of leishmanicidal medications, the long treatment periods and high concentrations, and the high treatment costs. In addition, parasite resistance to currently available leishmanicidal medications has been described [5]. Because of these limitations, new chemotherapeutics for leishmaniasis are urgently needed as well as novel targets have to be identified.

Inside macrophages, Leishmania possesses a unique thiol reduction mechanism, the trypanothione metabolic route, which aids the parasite in fighting oxidative damage, heavy metals, and xenobiotics. Trypanothione reductase (TR), a member of the oxidoreductase family of enzymes, is a key enzyme in this pathway, keeping trypanothione ((N1, N8-bis(glutathionyl) spermidine in a reduced state to create a

reducing environment inside macrophages and neutralise peroxides, reactive oxygen species, heavy metals, and xenobiotics via the tryparedoxin[6], [7]. As a result, TR inhibition raises the parasite's deadly intracellular levels of reactive oxygen species. Aside from that, TR is an appealing target for potential therapeutic candidates due to the absence of the trypanothione redox system in mammals[1].

The structural structure of the TR proteins from Leishmania infantum (Li-TR), Trypanosoma brucei (Tb-TR) and Trypanosoma cruzi (Tc-TR)has been determined and numerous compounds have been tested as enzyme inhibitors. Quinoline-based compounds and pyrimidopyridazine-based scaffolds exhibit inhibitory effect against recombinant Tb-TR[8], and lunarine analogues have inhibitory activity against Tc-TR [9]. Clomipramine and its analogues are potent inhibitors of Tc-TR activity, but their psychotropic properties prevent them from being used as anti-trypanosome drugs [10]. High-throughput screening tests on Li-TR found novel 2-iminobenzimidazoles to be enzyme inhibitors [11]. It was discovered that resveratrol analogues target Leishmania braziliensis TR, and that they have a leishmanicidal action in vitro [12].

With the goal of identifying Lm-TR inhibitors and the fact that the crystal structure of Lm-TR is unknown, we used I-Tasser to perform homology modelling and produce a tridimensional structure of Lm-TR. Virtual screening of about 5000 sigma aldrich compounds, acquired from the ZINC database, yielded a selection of chemicals. These molecules were subjected to a docking investigation that focused on the Lm-TR tridimensional structure's catalytic region, which included the FAD (Flavin Adenine Dinucleotide) binding domain. Ten compounds were chosen for their leishmanicidal activity based on a wide range of docking scores (from -11.4 kcal/mol to -9.2 kcal/mol) and structural diversity.

2. MATERIAL AND METHODS

2.1 Homology modeling

The sequence of Leishmania major trypanothione reductase (Lm-TR) with the id: Q4QJG7 was obtained from Uniprot (https://www.uniprot.org). The amino acid sequence consisted of 491 residues. Because the crystal structure of Lm-TR was not found in the PDB, we used the homology modelling method to build the 3D structure. This was accomplished using the I-Tasser server [13], [14]. The stereochemical analysis of the resulting target model was validated with PROCHECK [15], [16].

2.2 Preparation of protein and ligand library for screening

To target the Lm-TR, we obtained the Sigma Aldrich ligand library for the ZINC database [17]. For screening investigations, we transformed them into dockable pdbqt files. The ADT was also utilised for intermediary tasks like creating grid boxes and preparing protein pdbqt files. Based on FAD docking with our target protein, we discovered active site residues as predicted by the COFACTOR server. For docking, we used AutoDock Tools (ADT) to create protein pocket coordinates and convert the target protein and ligand library into pdbqt format. For virtual screening, AutoDock Vina was used [18]. In docking investigations, FAD was re-docked and used as a control.

2.3 Docking/Virtual Screening

Virtual screening is a method for identifying the most likely docking ligand from a library of small compounds that has been screened against a target protein. The synthesised ligands and target protein were screened using the Autodock vina programme.

AutoDock Vina is a free molecular docking and virtual screening tool. It employs a complex gradient optimization strategy that aids the algorithm in performing each and every evaluation. The figures were created with PyMol [19] and LigPlot [20].

3. RESULT AND DISCUSSION

Macrophages engulf leishmania parasites shortly after they are inoculated into the human body. Trypanothione reductase has been discovered as a key Leishmania enzyme involved in the neutralisation of reactive oxygen species inside macrophages in order to protect the parasite from macrophage antimicrobial responses [20, 21]. Because it is not found in humans, we believe it has a lot of potential as a target for finding new compounds to treat leshmaniasis. Since it is not yet crystallised, we modelled it. For modelling the target protein, the I-Tasser server used the structure of Trypanothione Reductase from Leishmania infantum (PDB id: 2JK6). The secondary structure of the protein was mixed -helices and -strands in three dimensions. PROCHECK analysis of the homology-based modelled protein revealed 81.6 percent residues in the most favoured region, 17.7 percent residues in the allowed zone, and just 0.7 percent residues in the disallowed region, indicating that the model might be used for docking investigations (Figure 1).
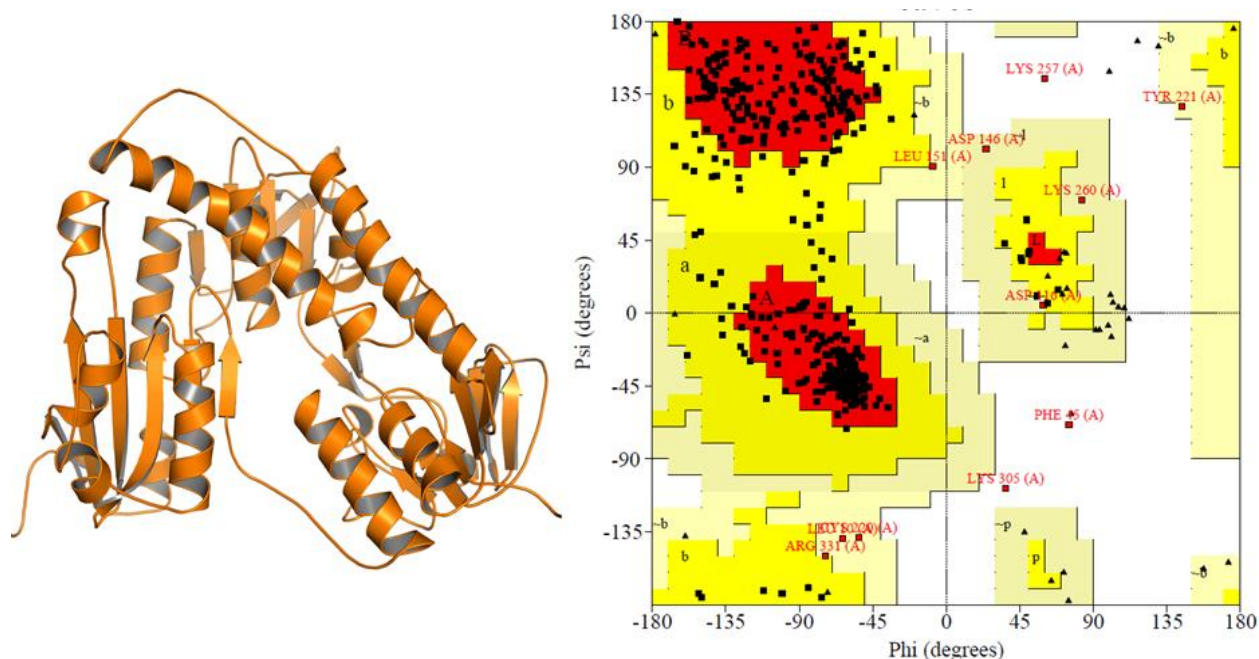
Figure 1: Homology model of Lm-TR and the ramachandran plot showing stereochemical properties of residues. Protein is shown in orange color and in cartoon model. Figure is generated by PyMol tool.

The generated model was showing good quality model as suggested by ramachnadran plot. The I-Tasser server also predicted the binding pocket and the docked FAD found in that region. Figure 2 showed the docked complex of Lm-TR-FAD generated by server. It was observed that residues Gly127, Val34, Thr51, Lys60, Thr335, Cys52 and Asp327 have shown hydrogen bond with the Lm-TR protein and several hydrophobic interactions were also identified.

We performed docking of FAD again with the identified pocket by the COFACTOR using Autodock vina tool and considered it as control. It showed binding affinity of -9.2 kcal/mol (Figure 3). The FAD made the interaction with Ala365, Cys57, Lys60, Thr335, Cys52 and Asp327. These residues were same as predicted Figure 2, suggesting that the docking control is valid.
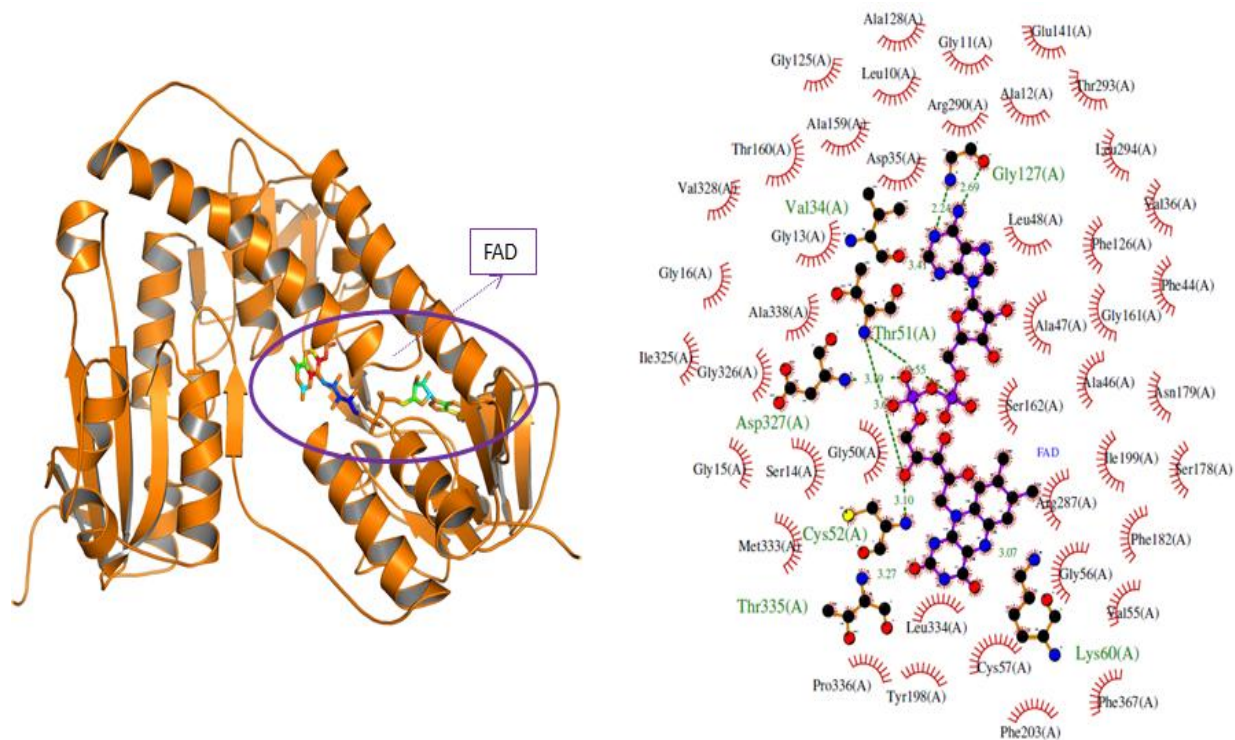
Figure 2:I-Tasser server based generated Lm-TR-FAD complex. PyMol help generate model image. Predicted pocket and the residues can be explored via ligplot image.
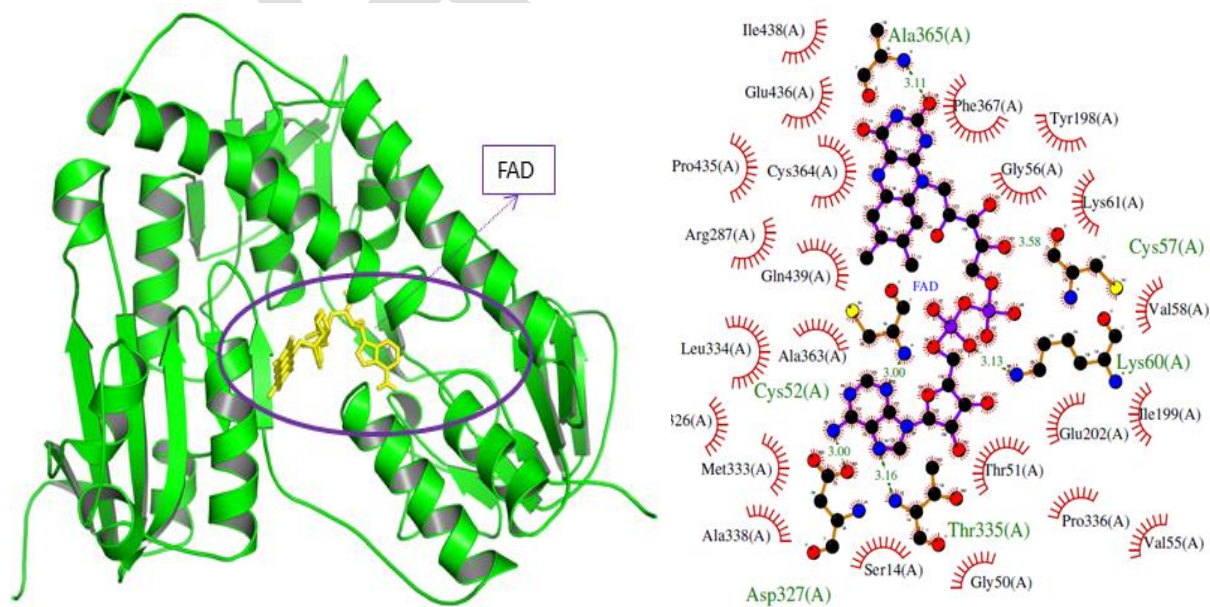
Figure 3: Docking of FAD against Lm-TR and validation of pocket coordinates, taken as docking control. Protein is shown in green cartoon model and the FAD is in stick model in yellow color. Ligplot shows residuesmaking interaction with FAD.

We then performed the screening of about 5000 sigma aldrich compounds against target protein Lm-TR using Autodock vina. Top 10 ligands selected based on binding affinity are shown in Table 1.

The molecules with the ids ZINC04245710 and ZINC03869768 had the highest binding affinities of -11.4 and -11.2 kcal/mol, respectively. These compounds had the maximum binding affinity and the appropriate amount of hydrogen bonds. Figure 4 showed the binding of top two compounds with the Lm-TR protein as well ligplot suggested possible active site residues making different interactions. The compound ZINC04245710 showed H-bond with Cys52, Thr52, Gly161, Thr160, Glu141, Arg290, Thr293 and Ser14. And the compound ZINC03869768 made H-bond with Thr51, Ser162, Asp35, Val36, Gly127 and Thr293. These are the residues which are mostly predicted by I-Tasser server during the model pocket prediction, suggesting that the compounds ZINC04245710 and ZINC03869768 may efficiently block the activity of the target enzyme (Lm-TR) and hence could serve as novel agents to combat cutaneous leishmaniasis. Further experimental studies are needed to find the effectiveness of these compounds and validate their antileishmanial response.

Table 1: Top 10 compounds after screening of compounds with Lm-TR target.

S.No	Compounds	Binding Affinity	No of H-Bonds
1.	ZINC04245710	-11.4 kcal/mol	8
2.	ZINC03869768	-11.2 kcal/mol	6
3.	ZINC04517630	-10.9	4
4.	ZINC02575146	-10.7	2
5.	ZINC04645692	-10.6	2

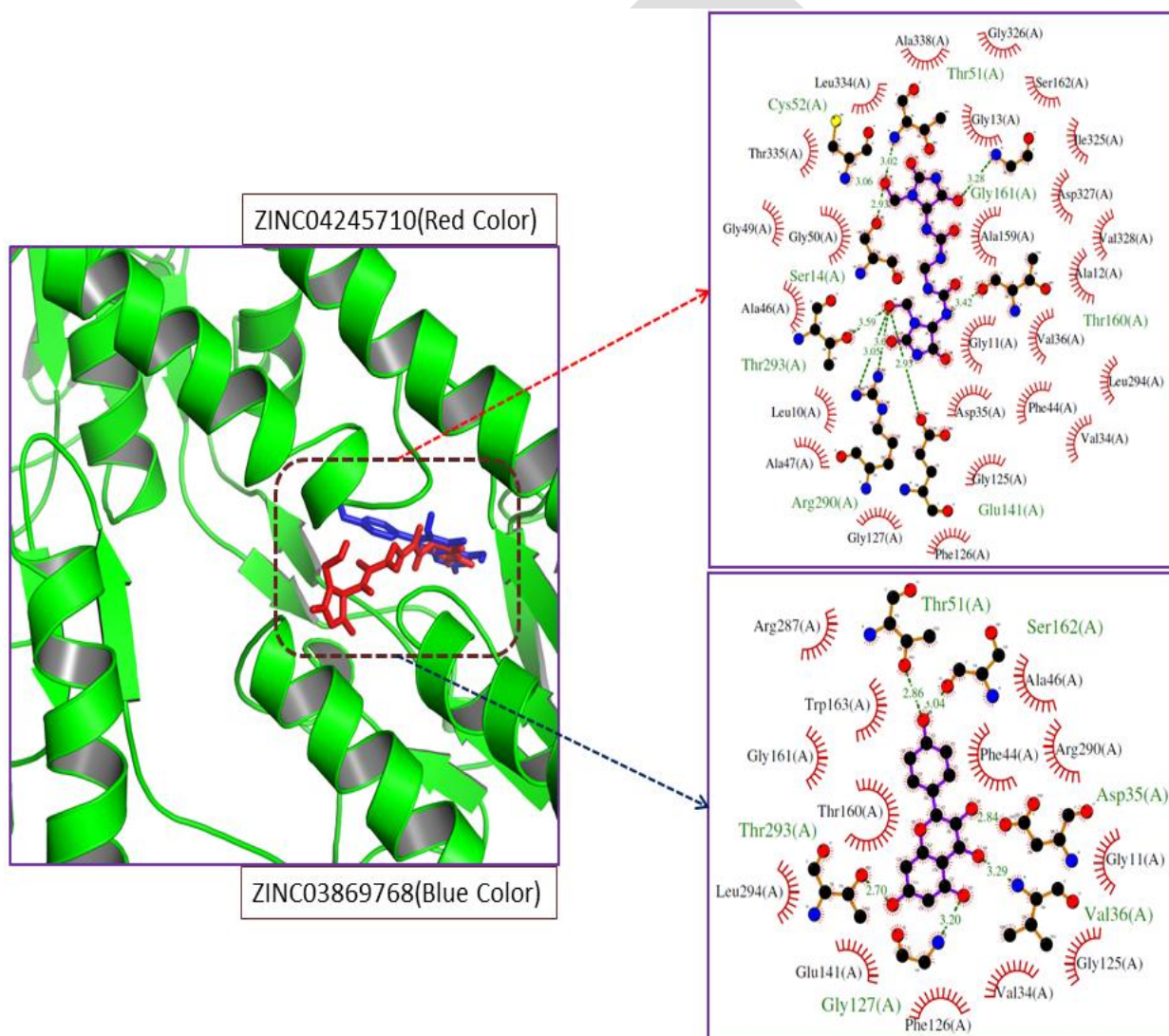|			
6.	ZINC03630253	-10.3	3
7.	ZINC04335977	-10.0	2
8.	ZINC03872070	-9.9	2
9.	ZINC04098975	-9.6	1
10.	ZINC04529370	-9.2	2
11.	FAD (Control)	-9.2	6

Figure 4: Docked complexes of top two compounds with the Lm-TR protein. Protein is shown in cartoon model in green color while compounds are shown in red and blue

color respectively. Ligplot showed interacting residues participating in respective ligand docking.

CONCLUSION

Leishmania major is the cause of cutaneous leishmaniasis, which kills millions of people around the world. Its survival depends on the enzyme trypanothione reductase. As a result, an attempt was undertaken to find a way to target this enzyme. We used bioinformatics to target this beneficial enzyme because the crystal structure was unavailable and hence homology modelling and virtual screening was performed. These top screened ligands (ZINC04245710 and ZINC03869768) could be used as a lead molecule, but more in vitro and in vivo testing is needed to confirm their antileishmanial action in the fight against cutaneous leishmaniasis. These compounds could be a useful starting point for a hit-to-lead procedure in the hunt for new anti-Leishmania drugs that are both efficacious and non-cytotoxic.

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