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# Exploring Different Drug Targets Responsible for the Inhibitory Activity of *N, N′*-Substituted Diamine Derivative in *Leishmania* †

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**Abstract:** The genome sequence of *Leishmania* has given rise to diverse novel drug targets and their identification remains the first step in drug discovery. The study aims to identify the possible anti-leishmanicidal activity target(s) of $N^1$, $N^4$-[dibenzylbutane-4',4''-(dioxymethylenebenzene)]-1,4-diamine from a plethora of pathways in kinetoplastids. The compound was docked using AutoDock-Tools-1.5.6 against 8 co-crystallized proteins selected from the protein data bank and representing important biosynthetic pathways. The evaluation of the proteins-ligand best conformational poses showed that the *N, N′*-substituted diamine binds more efficiently to the glyceraldehyde-3-phosphate dehydrogenase, G3PDH (E = -8.97 Kcal/mol and Ki = 0.267 μM; Ki co-crystallized ligand = 19.39 μM) which is responsible for the conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate and pteridine reductase I, PTR1 (E = -8.75 Kcal/mol and Ki = 0.387 μM; Ki co-crystallized ligand = 60.56 μM) which reduces both pterins and folates to tetrahydrobiopterin and tetrahydrofolate respectively. Moderate binding activity by the ligand was obtained for the protein kinases, CDKs (E = -8.37 Kcal/mol and Ki = 0.729 μM; Ki co-crystallized ligand = 26.80 μM) and trypanothione reductase, TR (E = -8.57 Kcal/mol and Ki = 0.525 μM; Ki co-crystallized ligand = 174.68 μM) of the trypanothione biosynthetic pathway. With E > -7.35 Kcal/mol and Ki > 4.10 μM, the ligand appears to have no significant inhibition of the squalene synthase (SQS), lactoyl glutathione lyase (LGL) and pteridine synthase (TS) of the sterol, glyoxalase and trypanothione biosynthetic pathways. The efficient inhibition of G3PDH and PTR1 targets in *Leishmania* by *N, N′*-substituted diamine molecule provides more insights into understanding the mechanism of leishmanicidal activity.

**Keywords:** Kinetoplastid; *Leishmania*; glyceraldehyde-3-phosphate dehydrogenase; pteridine reductase; substituted diamine

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## 1. Introduction

Leishmaniasis is a protozoan infection ranging in severity from simple cutaneous lesions to the usually fatal visceral form and transmitted to mammals through the bites of sandfly [1]. Though neglected, the disease ranked second to malaria in mortality compared with other protozoan infections. The recent epidemiological data suggest that the disease is still on the increase; endemic in 88 countries and affects millions of people globally with a higher incidence of the cutaneous than the visceral form [2].

The burden of economic loss due to leishmaniasis is enormous. Chemotherapy has remained the choicest eradication measure due to vaccine unavailability. However, resistance to the first-line agents and the toxicity of other chemotherapeutic agents have hampered all the treatment measures developed so far [3]. To date, many chemotherapeutic approaches are still being explored.

The sequencing, availability and accessibility of the complete *Leishmania* genome have provided voluminous data necessary for anti-leishmanicidal drug development [4]. The reliance on the differences in the biochemistry and physiology of host and pathogen in target identification is now completely replaced by several computational approaches. In this approach, the uniqueness of the target to the pathogen, the dependence of the pathogen on the target for survival, the expression of the target gene in the pathogen, the biochemical properties of the target, and the *in-vitro* assay method of the target are important [5,6]. To design specific inhibitors necessary for loss of cell viability in kinetoplastids, several biochemical pathways are probed using necessary chemoinformatic tools to understand mechanisms of protein-ligand interactions [7].

In the past, a new backbone of the substituted *N, N′*-diamine derivative (*N,N*-dd) designed for leishmanicidal activity has been developed. The *in vitro* activity data of a hit, $N^1$, $N^4$-[dibenzylbutane-4',4''-(dioxymethylenebenzene)]-1,4-diamine (Figure 1) showed $IC_{50}$ and selectivity index of 31 nM and 187 respectively [8]. It has also been reported that *N, N′*-diamine-derivatized compounds with antitrypanosomal activity inhibit trypanothione reductase [8]. Recently, a 3D-QSAR study revealed a ligand-based optimization of *N, N′*-dd to understand their interaction with potential targets [9]. *Leishmania*, like other kinetoplastids, possesses several biosynthetic pathways that serve as important drug targets such as the sterol, glycolytic, protein kinase, purine salvage, hypusine, glyoxalase, glycosylphosphatidylinositol and folate pathways. The study, therefore, explored the interaction of *N, N′*-dd with the potential targets in *Leishmania*.

## 2. Methods

### *2.1. Leishmania drug targets*

At least one 3D co-crystallized protein (resolutions < 2.0 Å) complexed with different ligands, representing the squalene synthase (ID: 4JZB, r = 1.90 Å), glyceraldehyde-3-phosphate dehydrogenase (ID: 4EF8, r = 1.56 Å), cyclin-dependent kinases (IDs: 3DWR, r = 1.66 Å and 2R8Q, r = 1.50 Å), pteridine reductase I (ID: 7DES, r = 1.45 Å), lactoyl glutathione lyase or glyoxalase I (ID: 7PXX, r = 1.81 Å), trypanothione reductase (ID: 1E7W, r = 1.75 Å), trypanothione synthetase (ID: 3S9F, r = 1.80 Å) pathways in *Leishmania* species was obtained from the protein data bank (www.rcsb.org).

### *2.2 Ligand*

The ligand, $N^1$, $N^4$-[dibenzylbutane-4',4''-(dioxymethylenebenzene)]-1,4-diamine was prepared from free aliphatic diamines with different substituted benzaldehydes by controlled reductive amination and tested for *in vitro* activity against selected kinetoplastids [10]. Of the congeneric series, the ligand showed the most significant activity against *T. cruzi* (0.76 μM), *T. brucei* (0.097 μM) and *L. donovani* (0.031 μM).

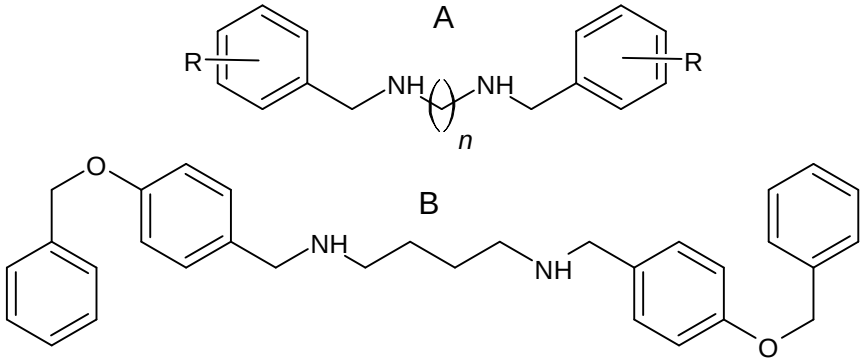


**Figure 1**. Structure of *N, N'*-substituted diamine skeleton (A) and docked ligand derivative (B)

*2.3 Preparation of protein target*

The protein structure was prepared using Autodock Tools 1.5.6, a part of the MGL Tools molecular visualization interface. The protein was pretreated by removing the water molecules and other non-essential components. The missing atoms were checked and repaired. A sufficient number of polar hydrogen atoms and Kollman charges were added [11]. The prepared protein molecule was saved for molecular docking study.

*2.4 Molecular docking*

The molecular docking adopted a blind docking model using the AutoDockTools-1.5.6. The ligands were assigned torsions using the default settings. The potential grid maps were executed using the AutoGrid module with 50 hybrid GA-LS runs and a population size of 300, 2.5 million energy evaluations and 27000 generations [12]. A root mean square deviation of 2.0 Å was set to group the clusters while other parameters were at default. The docking protocol was validated by re-docking the native ligands into the proteins using the Lamarckian Genetic Search algorithms. The binding poses visualization was performed using Discovery Studio Visualizer v17.2.0.16349 and protein-ligand interaction profiler webserver.

*2.5 Drug-likeness of ligand*

The number of hydrogen bond acceptors (a_acc), number of hydrogen bond donors (a_don), octanol-water partition coefficient (log P), molecular weight (MW) and topological polar surface area (TPSA) were computed for drug-likeness of the ligand.

## 3. Results and Discussion

Multi-targeted drug design approach in drug discovery has played a significant role in overcoming the challenge of drug resistance and chemotherapeutic failure commonly associated with anti-kinetoplastids. The complete assay of the genome sequence in *Leishmania* species has revealed the presence of novel receptors and enzymes capable of providing definite drug target fingerprints for lead identification. The fingerprints such as the sterol, glycolytic, protein kinase, purine salvage, hypusine, glyoxalase, glycosylphosphatidylinositol and folate pathways provide multiple and diverse pathways for antikinetoplastid binding.

The binding properties of the hit ligand to various enzymes in the *Leishmania* species biosynthetic pathways are shown in Table 1. The binding free energies (E), inhibition constants (Ki) and the amino acid residues involved in the interactions of the hit ligand with multiple targets in *Leishmania* species are shown in Table 1. The lower theoretical inhibition constant, Ki and binding energy values indicate more favorable interaction with protein [12].

The binding of the hit ligand to two of the six targets studied was significantly high considering their E and Ki. The ligand interacted maximally with the CDKs (E = 9.01 Kcal/mol; Ki = 0.248 μM) and G3PDH (E = 8.97 Kcal/mole; Ki = 0.267 μM).

**Table 1.** Docking parameters of *N, N'*-dd ligand with different target proteins

| Target | RMSD | E (kcal/mol | $K_i$ (μM) | H-bonds | Amino acids involved[b] |
|---|---|---|---|---|---|
| SQS | 93.11[a] | -6.15[a] | 31.19[a] | 7 | Phe94, leu95, glu97, ile125, thr163, gln167, phe246 |
| | 70.10[b] | -7.34[b] | 4.18[b] | 5 | |
| G3PDH | 54.69[a] | -6.43[a] | 19.39[a] | 8 | Ala13, tyr90, lys93, pro94, his160 |
| | 83.13[b] | -8.97[b] | 0.267[b] | 5 | |
| CDKs | 73.50[a] | -5.50[a] | 92.52[a] | 3 | Trp37, arg39,111, val66/95, ala89, phe113, ile265/267 |
| | 44.12[b] | -9.01[b] | 0.248[b] | 3 | |
| | 35.29[a] | -6.24[a] | 26.80[a] | 1 | Val836,839,853, asn838, met851, thr854, 854, phe890 |
| | 27.19[b] | -8.37[b] | 0.729[b] | 3 | |
| PTR1 | 55.70[a] | -5.75[a] | 60.56[a] | 13 | Ala15,arg17, leu18/226/229, tyr37, his38, pro224 |
| | 72.17[b] | -8.75[b] | 0.387[b] | 3 | |
| LGL | 28.94[a] | -2.19[a] | 24920[a] | 4 | Arg14, leu16/24/30, asn26, lys29, tyr33, glu55 |
| | 28.38[b] | -5.76[b] | 60.39[b] | 2 | |
| TR/S | 18.25[a] | -5.13[a] | 174.68[a] | 0 | Ala15, arg17, his38, phe 13, met183, thr184, leu188, tyr194 |
| | 19.08[b] | -8.57[b] | 0.525[b] | 1 | |
| | 27.31[b] | -7.29[b] | 4.52[b] | 1 | Leu8, leu12, leu26, phe33, lie96, leu100, asp118 |

Squalene synthase (SQS); glyceraldehyde-3-phosphate dehydrogenase (G3PDH); cyclin-dependent kinases (CDKs); pteridine reductase I (PTRI); lactoyl glutathione lyase (LGL) or glyoxalase I; trypanothione reductase (TR); trypanothione synthetase (TS); [a]native ligand; [b]*N, N'*-dd ligand.

The analysis of the best binding poses of the hit ligand with CDKs, G3PDH, PTR1 and TR revealed interactions with the different amino acid residues (Figures 2 and 3). Typically, the four aromatic nuclei, the two oxygen and the two amine groups present in the ligand provided centres of hydrophobic interaction, hydrogen bonding, pi-pi stacking, pi cationic interactions necessary for inhibition of important biochemical processes in *Leishmania* species [13, 14].

The current findings have successfully provided important insights into some sterically favourable interactions of the docked ligand (Fig. 1B) with some proteins and enzymes implicated in leishmaniasis. The Ala13, tyr90, lys93, pro94, his160 of G3PDH, Trp37, arg39,111, val66/95, ala89, phe113, ile265/267 of CDKs and the Ala15, arg17, leu18/226/229, tyr37, his38, pro224 of PTR1 were found to favourably interact with the docked ligand. However, molecular dynamic simulation studies are currently ongoing for further post-docking refinement of the interactions.

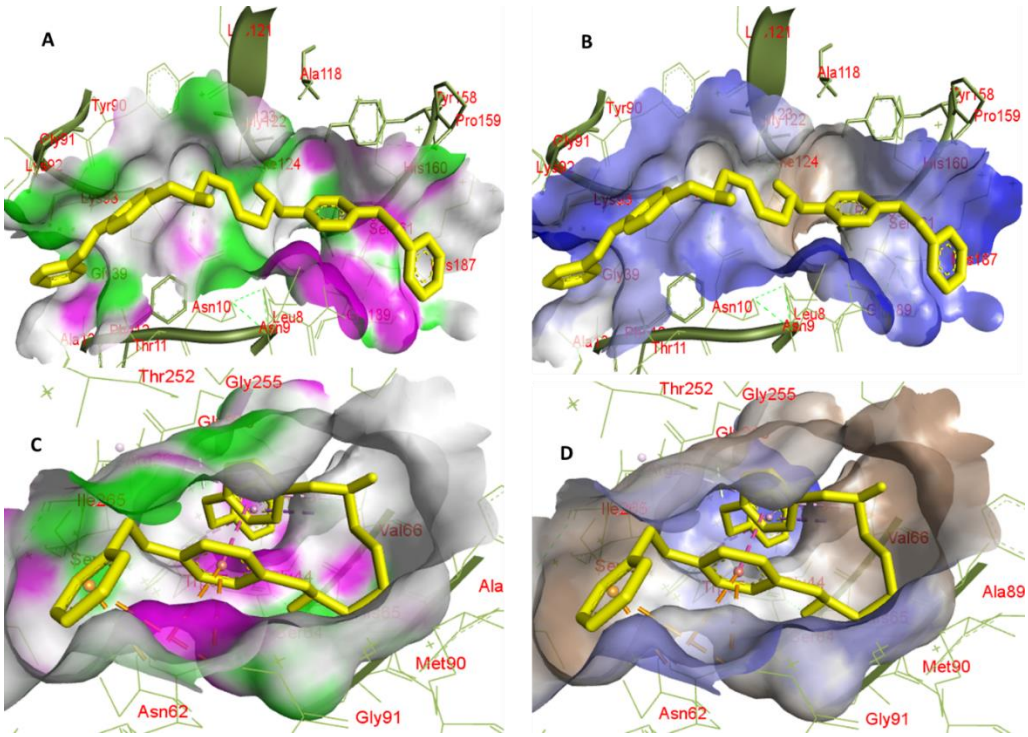


**Figure 2.** Theoretical binding pose of the ligand (gold-coloured) with G3PDH (A/B) and CDKs (C/D) enzymes. The protein-ligand pose is represented in a hydrogen receptor surface (A/C) of the H-bond acceptor (green) and H-bond donor (pink) and hydrophobic receptor surface (B/D) of +3 (brown) to -3 (blue)

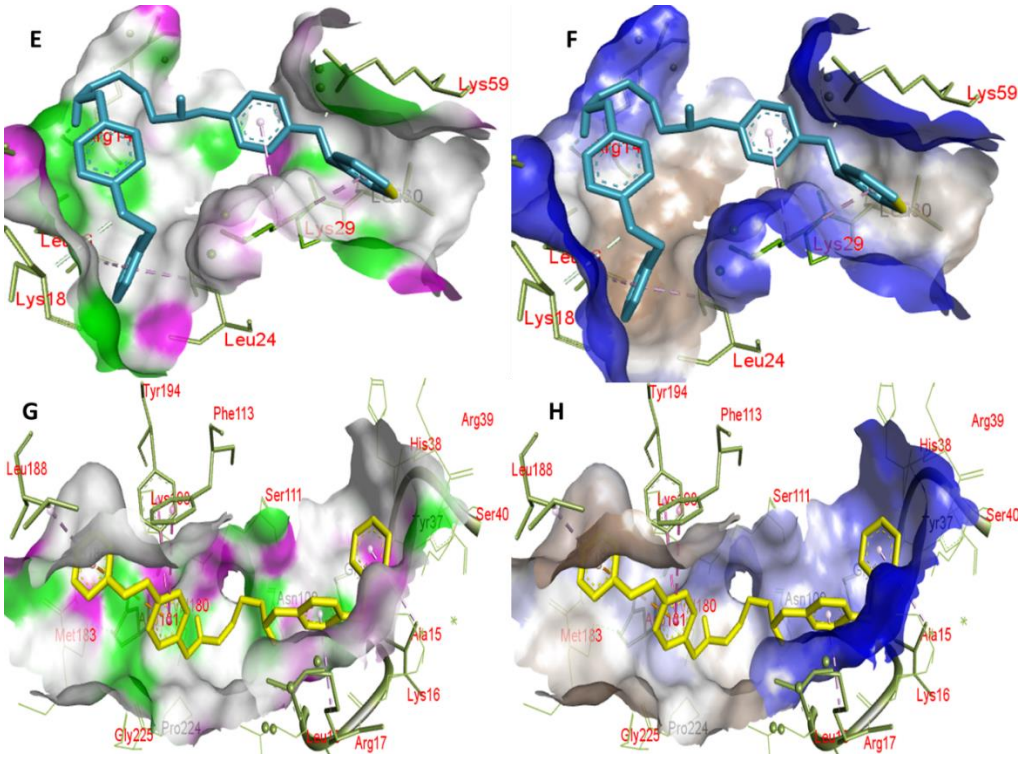


**Figure 3**. Theoretical binding pose of the ligand (light blue and gold-coloured) with PTR1 (E/F) and TR (G/H) enzymes. The protein-ligand pose is represented in a hydrogen receptor surface (E/G) of the H-bond acceptor (green) and H-bond donor (pink) and hydrophobic receptor surface (F/H) of +3 (brown) to -3 (blue)

The potential of the ligand to be further developed as an anti-kinetoplastid agent was demonstrated by the computation of its drug-likeness. With the hydrogen bond acceptors of 4, hydrogen bond donors of 2, a molecular weight of 480.65, logP of 7.456 and TPSA of 42.52, the ligand could be further optimized to meet Lipinski's requirement for drug likeness.

## 4. Conclusion

The strong leishmanicidal activity of *N, N'*-substituted diamine derivatives could be mediated by the multi-target inhibition of important enzymes in kinetoplastids (*Leishmania* species) such as the glyceraldehyde-3-phosphate dehydrogenase, cyclin-dependent kinases, pteridine reductase I and trypanothione reductase pathways. Some important amino acid residues in the targets were identified to interact with the different donor/acceptor groups in the ligand within a distance < 4.0 Å; suggesting strong protein-ligand interactions.

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