Zoo Botanica
ISSN: 3007-2050 (E), 3007-2042 (P)

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Research Article

In Silico analysis to identify potential inhibitors of N-Myristoyltransferase in *Leishmania donovani* through drug Repurposing

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ABSTRACT

Visceral leishmaniasis (VL) or Kalazar, is a vector-borne parasitic disease caused by *Leishmania donovani,* which is spread through the bite of sand flies from infected animals or humans. The disease continues to be a major health concern due to limited treatment options. One of the key proteins involved in the viability of the parasite is N-myristoyltransferase (NMT), making it a prominent target for new drug development. Development of new drugs require a lot of resources and time. The current study was designed to find potential *L. donovani* NMT inhibitors from the existing drugs used for other disease through insilico studies. To identify potential inhibitors for NMT, the 3D structure of the *L. donovani* NMT was obtained from the protein data bank. More than one hundred and fifty potential FDA approved drugs were retrieved from chemspider. These drugs were docked using the online PatchDock server, selecting the top results. The interactions were visualized using GS Viewer and LIGPLOT. The interactions formed include hydrogen bonding, covalent interactions, and hydrophobic interactions with the key active site residues of the target, suggesting at potential mechanism of inhibition. The present study successfully identified promising potential inhibitors of *L. donovani NMT.* The drugs that showed maximum interactions includes Clarithromycin, Amoxicillin, Bacampicillin and Ciprofloxacin which could serve as candidates for experimental testing in future studies. The findings of the current study lay the foundation for developing new treatments for visceral leishmaniasis.

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Keywords: Visceral leishmaniasis; *Leishmania donovani*; kalazar; drug docking**.**

INTRODUCTION

Leishmania donovani is a flagellated protozoan parasite responsible for the fatal visceral leishmaniasis (VL) worldwide (Schmidt & Roberts, 1985) (van Griensven & Diro, 2012). VL is a vector borne disease that spread through the bite of hematophagous sand flies (Kamhawi, 2006; Killick-Kendrick, 1999). VL has a wide range of distribution and have been reported from India, East Africa, East to West China, Central Asia, Brazil and Mediterranean Basin (Wamai et al., 2020). The disease burden is more concentrated in some countries and 90% of the VL cases have been reported from Bangladesh, Northeastern Brazil, Sudan, Nepal, and India (Ready, 2014; Wamai et al., 2020). The treatment of VL includes drugs such as Miltefosine, Pentavalent antimonials, Amphotericin B, Paromomycin sulfate and Liposomal amphotericin B (Shmueli & Ben-Shimol, 2024; van Griensven et al., 2024).

OPEN ACCESS

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Article History
Received: November, 15, 2024
Accepted: December, 17, 2024
Published: December 31, 2024

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All these drugs target the N- myristoyltransferase (NMT) of *Leishmania donovani* (Matos et al., 2020; Nico et al., 2021; Saravolatz et al., 2006). The NMT is involved in different cellular processes such vesicular protein trafficking and signal transduction and (dos Santos Nascimento et al., 2023). This attachment facilitates the adherence of substrate proteins with hydrophobic domains and membranes of partner peptides in the pathways (Brand et al., 2011; dos Santos Nascimento et al., 2023). The function of NMT is necessary for the viability in all types of cells, showing the potentiality of this enzyme as a drug development target (dos Santos Nascimento et al., 2023; Gelb et al., 2003).

To expedite the development of new therapeutics for parasitic diseases, researchers often focus on enzymes actively involved in co-translational protein modification are usually focused (dos Santos Nascimento et al., 2023). Studies proved that overexpression of NMT causes substantial changes in parasite morphology, ultimately leading to the death of cell (Yuan et al., 2020; Zhao & Ma, 2014).

Drugs which are currently used for the treatment of VL are either expensive or not enough to keep pace with the drug-resistant protozoan. The need for an alternative drug is obvious. However, the development of new drugs is costly and requires significant time and resources. In such cases, evaluating existing drugs for new purpose is an ideal solution, as these drugs have known safety and toxicity profiles (Mentis et al., 2024; Pinzi et al., 2024). Many drugs have been repurposed in the past for various diseases, including Minoxidil, Aspirin, Sildenafil, Thalidomide and Clozapine (Gayaparsad, 2022). The current study was thus designed to identify an alternate FDA approved drug for visceral leishmaniasis through drug repurposing.

MATERIALS AND METHODS

Protein Sequence Setrieval and Analysis

The sequence of *Leishmania donovani* N-myristoyltransferase (NMT) was retrieved from the UniProtKB (The Uniprot Consortium, 2021). Only complete and specific NMT entry related to *L. donovani* were considered for further analysis. To explore the NMT protein structure, the corresponding PDB accession code was used to obtain the 3D structure of *L. donovani* NMT protein from the RCSB (pdb id: 2wuu) databank (Burley et al., 2019). The structural data was downloaded at 2.3 Å resolution and analyzed further.

The 3D structure of NMT and its interactions with the target were analyzed using Discovery Studio Visualizer, version 4.5 (Jejurikar & Rohane, 2021). GS viewer was used to capture high-quality images of various protein-drug interactions.

ChemSpider

The 3d structures of more than one hundred and fifty potential drugs were retrieved from ChemSpider chemical structure database (Ayers, 2012).

Docking and Stereochemistry of Ligand-Protein Interactions

The drugs with the target protein were docked using PatchDock server. The top docking results, which showed highest binding interactions particularly with the active site residues, were selected. These interactions were further analyzed using DS visualizer, GS viewer, and LIGPLOT to examine the docked amino acids (Schneidman-Duhovny et al., 2005). The 2D representation of ligand and protein interactions was analyzed using LIGPLOT (Wallace et al., 1995).

RESULTS AND DISCUSSION

To find an alternate drug for visceral leishmaniasis, we have randomly docked more than 100 drugs with the N-myristoyltransferase. The drugs that showed maximum interactions after docking with the target protein were Clarithromycin, Amoxicillin, Bacampicillin, and Ciprofloxacin.

Docking of Amoxicillin with N-myristoyltransferase

The docking of Amoxicillin with N-myristoyltransferase showed 14 molecular interactions, including hydrophobic interactions, covalent, and hydrogen bonding (Table 3.1). One residue, THR 203, located at the active site, formed two covalent bonds. Moreover, two residues, THR 203 and TYR 80, formed hydrogen bonds, with THR 203 formed two hydrogen bonds, both with active site residues (Figure 3.1). A total of eleven hydrophobic interactions were also noted, involving residues such as TYR 202, PHE 168, LEU 169, ASN 167 which are also part of the active site (Table 3.1).

Docking of Clarithromycin with N-myristoyltransferase

The docking of Clarithromycin showed sixteen different types of interactions, including covalent, hydrogen, and hydrophobic interactions (Table 3.1). Three amino acids (TYR 80, LEU 169, and TYR 92) formed covalent interactions, with TYR 80 forming two covalent bonds. Hydrogen bonds were formed by two amino acids (TYR 80, TYR 345), with TYR 80 being the active site residue. Eleven amino acids participated in hydrophobic interactions (Table 3.1), including

Table. 3.1. Molecular Interactions between N-myristoyltransferase and different ligands. *Ligands with known biological activity were selected.

Target Protein	Ligand	Type of Interactions	Amino acids	Active Site Residues
N-myristoyltransferase	Amoxicillin	Covalent	THR203	THR 203 (two times)
		Hydrogen	THR 203,TYR 80	THR 203 (two times), TYR 80
		Hydrophobic	TYR 202, ALA 204, PHE 168, LEU 169, ASN 167, LEU 399,TYR 217,LEU 421,TYR 326,TYR 345, MET 420	TYR 202, PHE 168,LEU 169,ASN 167
	Clarithromycin	Covalent	TYR 80 , TYR 92, LEU 169	TYR 80 (2 times), LEU 169
		Hydrogen	TYR 80, TYR 345	TYR 80
		Hydrophobic	CYS 170,VAL 171, ASN 167, PHE 168, ARG 176, ASN 79, GLU82,TYR 217,MET 420, THR 203, TYR 202	CYS 170, VAL 171,ASN 167,PHE 168, ARG 176, THR 203 ,TYR 202
	Bacampicillin	Covalent	PHE 90, TYR 80(five times), GLU 82 (five times)	TYR 80 (two times)
		Hydrogen	TYR 80	TYR 80
		Hydrophobic	LEU 169,ASN 167,TRP 15, TYR 202, THR 203	ASN 167,LEU 169,TRP 15,TYR 202, THR 203
	Ciprofloxacin	Covalent	ASN 167	ASN 167
		Hydrogen	TRP 15	TRP 15
		Hydrophobic	LEU 169, TYR 80, THR 203,TYR 202	LEU 169, TYR 80, THR 203, TYR 202

CYS 170, VAL 171, ASN 167, PHE 168, ARG 176, ASN 179, THR 203, and TYR 202, all of which are located at the active site (Figure 3.2).

Docking of Bacampicillin with N-myristoyltransferase

The docking of Bacampicillin with the target protein showed nine interactions, including covalent bonding, hydrogen bonding, and hydrophobic interactions (Table 3.1). Three of these interactions were covalent, with TYR 80 being the key active site residue involved (Figure 3.3). Hydrogen bonding also formed with TYR 80, while five amino acids (ASN 167, LEU 169, TRP 15, TYR 202, and THR 202) participated in hydrophobic interactions, all of which are located at the active site (Figure 3.3). These interactions may induce conformational changes in the target protein, potentially disrupting its normal functionalities.

Docking of Ciprofloxacin with N-myristoyltransferase

The docking of Ciprofloxacin showed interaction with N-myristoyltransferase through covalent, hydrogen and hydrophobic bonds (Table 3.1). The covalent interactions involved two active sites amino acids, ASN 167 and TRP 15 (Figure 3.4). TRP 15, additionally participated in hydrogen bonding. Hydrophobic interactions were formed by four active site residues: LEU 169, TYR 80, THR 203, and TYR 202 (Figure 3.4). Visceral leishmaniasis is a vector-borne parasitic disease characterized by reticuloendotheliosis and caused by the protozoan *L. donovani*. It is a public health problem with 10% mortality in many cases. The therapeutic options for the disease are currently limited (Ribeiro et al., 2024) and various drug repurposing studies have been conducted to find a suitable drug for the disease (Bora et al., 2024; Jandl et al., 2024; Zhou et al., 2024). Therefore, in the current study, an in-silico analysis of more than one hundred and fifty FDA-approved drugs already utilized for treating various diseases was conducted to find an alternative drug for visceral leishmaniasis. The initial stage of the drug discovery is to find a potential pharmacological

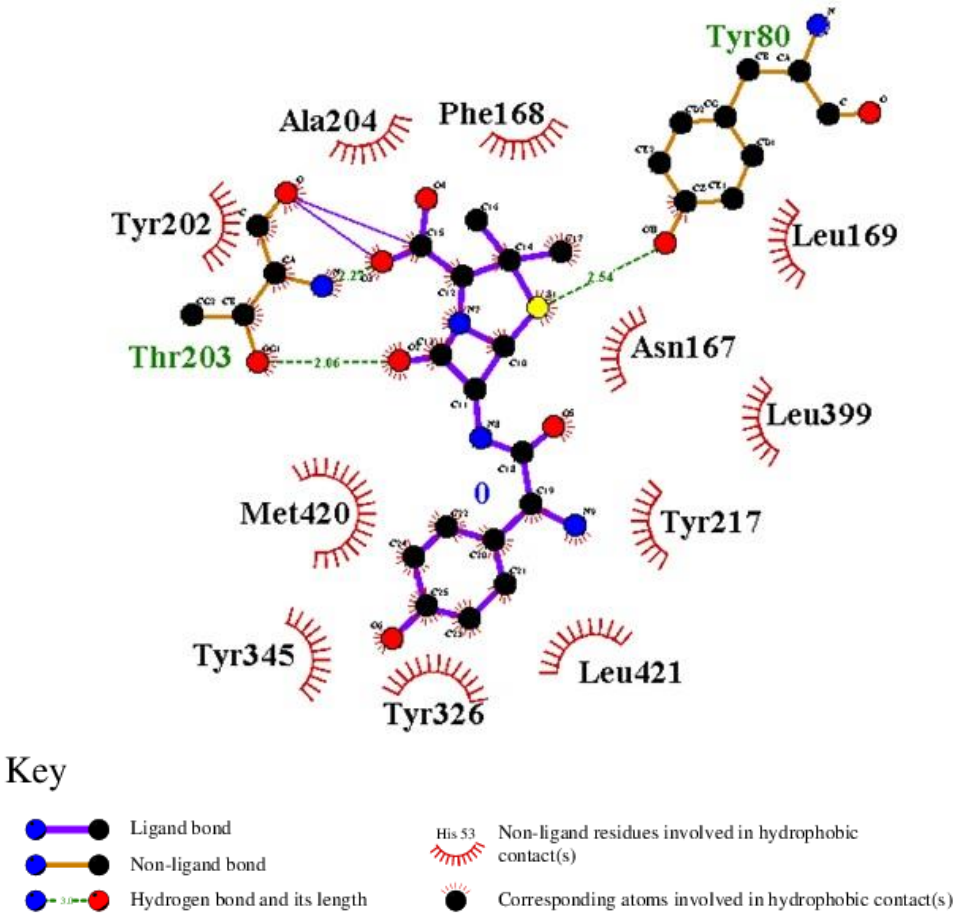

Figure. 3.1 Schematic representation of the 2D interaction of Amoxicillin with N-myristoyltransferase from *Leishmania donovani* generated by LIGPLOT.

drug target (Sheikh et al., 2024). Structurally distinct drug targets that are involved in crucial parasitic pathways are the appropriate targets. N-myristoyltransferase, an enzyme of *Lesihmania*, involved in the post translation myristoylation pathway (França et al., 2024; Sheikh et al., 2024; Yepes et al., 2024) was targeted in the current study. Drugs that showed maximum interactions were included. Amoxicillin (a-amino-p-hydroxybenzylpenicillin) is a new semi-synthetic penicillin with broad spectrum antimicrobial activity (Karunarathna et al., 2024). This drug is well effective against gram-positive and gram-negative bacteria. But is not active against penicillinase-producing organisms because of the instability of its particular j3-lactamase (Kaur et al., 2011).

Interaction between related biological molecules plays a central role in biological processes. Various molecules interact inside a cell to perform certain functions (Mohanty & Mohanty, 2023). In ligand-target bonding, various types of interactions can be observed including hydrogen, covalent and hydrophobic interactions (Copeland, 2013; Mohanty & Mohanty, 2023), with hydrogen interactions being the most common. Prolonged interactions between the ligand and the drug target, which can be achieved through covalent interactions are necessary for the prolongation of the therapeutic response (Sutanto et al., 2020). In the current study, Amoxicillin showed fourteen different types of interactions, including hydrophobic interactions, and covalent and hydrogen bonding with many residues of the active site (Figure 3.1). These interactions show that Amoxicillin can be a potential drug for visceral leishmaniasis. Clarithromycin is a newer macrolide antibiotic (Lee, 2024) that is effective against *Chlamydia pneumoniae, Mycobacterium leprae, C. trachomatis, Bacteroides melaninogenicus, M. chelonae, M. avium complex, Legionella species*, and against *Haemophilus influenza* (Arsic et al., 2018). It is particularly effective in treating skin infections and both lower and upper respiratory infections (Lee, 2024). The docking results in the current study showed various types of interactions, including covalent bonding, hydrogen bonding and hydrophobic interactions (Figure 3.2) which suggests that, with further analysis, Clarithromycin may be a potential candidate as an antileishmanial drug for the treatment of visceral leishmaniasis. Bacampicillin is a semisynthetic, broad-spectrum, beta lactam antibiotic derived

from ampicillin, used to treat bacterial infections (Sauvage et al., 2008; Worthen, 2017). Pbps inhibits the formation of 3.1), with hydrophobic interactions being the more common, followed by covalent interactions and hydrogen bonding.

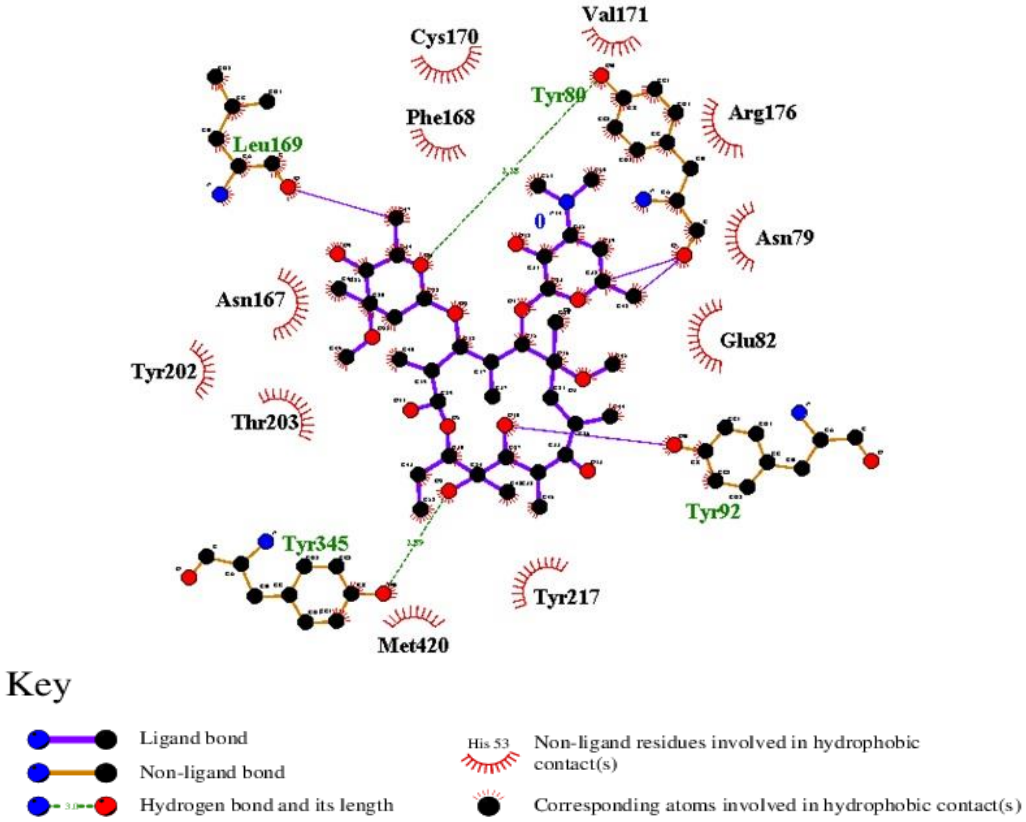

Figure. 3.2 Schematic representation of the 2-D interaction of the Clarithromycin with N-myristoyl transferase from *Leishmania donovani* generated by LIGPLOT.

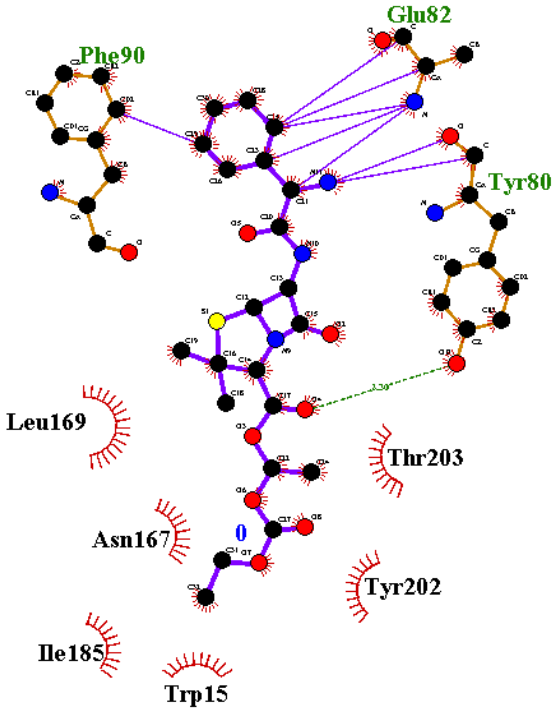

Figure. 3.3 Schematic representation of the 2-D interaction of the Bacampicillin with N-myristoyltransferase from *L. donovani* generated by LIGPLOT.

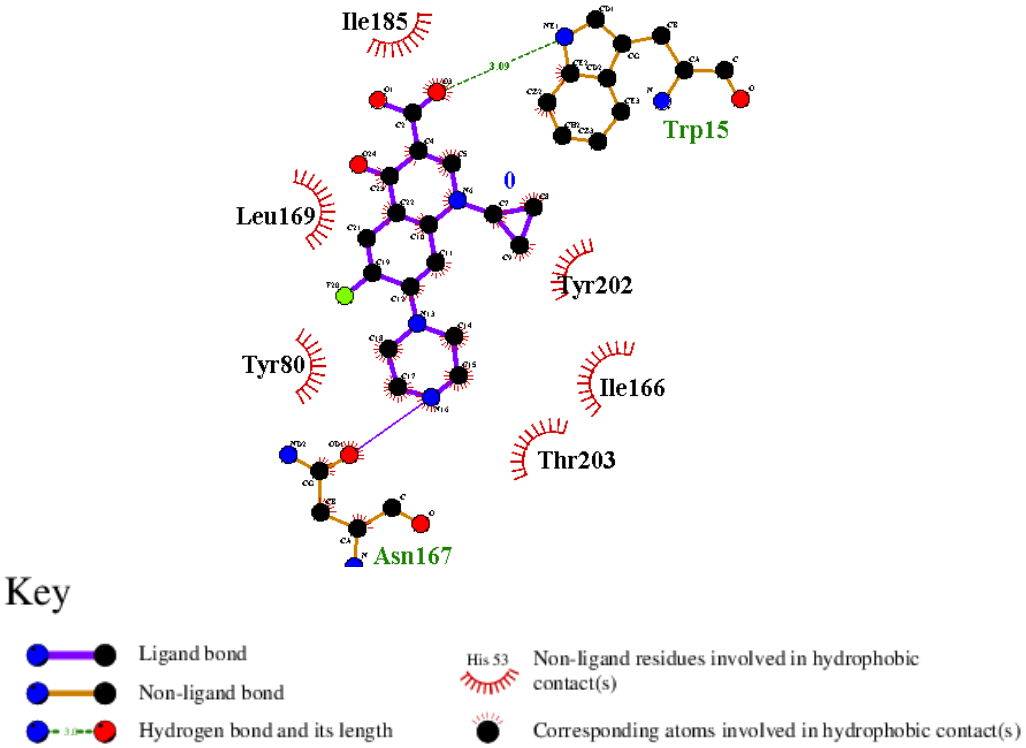

Figure. 3.4. 2D interaction of Ciprofloxacin with N-myristoyltransferase from *Leishmania donovani* generated by LIGPLOT.

These interactions may induce conformational changes in the target protein, disrupting its normal function. Bacampicillin shows potential for use in treating visceral leishmaniasis. Ciprofloxacin is an important antibacterial drug used to treat various diseases including skin infections, urinary tract infections, gastrointestinal infections, sexually transmitted diseases, respiratory tract infections, abdominal infections, and infections of joints and bones. It also has chemo suppressive and chemo preventive effects against various cancers (Sharma et al., 2010; Worthen, 2017). Ciprofloxacin shows greater activity against both gram positive and gram-negative bacteria (Thai et al., 2023). It binds to and prevents bacterial DNA gyrase from binding, an enzyme that is necessary for DNA replication (LeBel, 1988). Docking analysis of the Ciprofloxacin showed different types of interactions (Table 3.1), with hydrophobic interactions made with the active site residues being the most common, followed by covalent interactions and hydrogen bonding (Figure 3.4). These interactions suggest that Ciprofloxacin may induce significant conformational changes in N-myristoyltransferase, potentially disrupting its normal function.

CONCLUSION

In the quest to find an alternative drug through a drug repurposing strategy, this insilico study was conducted. The docking analysis showed impressive interactions with N-myristoyltransferase from *Leishmania donovani* (NMT), and the drugs Clarithromycin, Amoxicillin, Bacampicillin and Ciprofloxacin. Among these, Clarithromycin showed the best results. These interactions suggest that, with further molecular analysis, these drugs could be used to treat visceral leishmaniasis.

ACKNOWLEDGEMENTS

The authors would like to express sincere gratitde to Dr. Abid Ali and Dr. Akhtar Rasool from the University of Swat for their invaluable support and guidance.

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