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NATAMYCIN AND NYSTATIN: TWO NOVEL INHIBITORS OF *LEISHMANIA DONOVANI* ESSENTIAL ENZYMES

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ABSTRACT

Natamycin and nystatin, the two antifungal drugs having structural similarity with amphotericin B, were evaluated for their potential to inhibit five known drug targets of *Leishmania donovani*. *In silico* protein-ligand docking of natamycin and nystatin with adenylosuccinate lyase, cyclophilin, dihydroorotate dehydrogenase, *N*-myristoyl transferase and pteridine reductase 1 of *L. donovani* was carried out. The binding of both the drugs to the active site of all the drug targets was observed. Both natamycin and nystatin showed very low binding affinities with all the five drug targets and partially blocked the active sites of four out of the five enzymes. Findings of the present study provide new perspective for the drug development against leishmaniasis.

Keywords: *In silico* docking, Leishmaniasis, *Leishmania donovani*, Natamycin, Nystatin

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INTRODUCTION

Leishmaniasis is one of the neglected parasitic diseases causing significant morbidity and mortality worldwide. It is caused by an obligate protozoan parasite of genus *Leishmania*, which is spread to humans through the bite of infected female sand fly. Out of the three main clinical forms of leishmaniasis, the most fatal form is visceral leishmaniasis which is mainly caused by *Leishmania donovani*. It is estimated that 0.3 million new cases of visceral leishmaniasis and 20, 000 deaths occur annually worldwide **[1]**. The current chemotherapy of visceral leishmaniasis is primarily based on pentavalent antimonials, amphotericin B and its liposomal

formulations and miltifosine**[2]**. All the available chemotherapeutic agents present some drawbacks including emergence of drug resistance, renal or hepatic toxicity, painful administration, high cost, poor availability in endemic areas and variable effectiveness between various strains of the parasite **[3,4]**. Hence, there is a need to develop some novel, efficient and cost effective antileishmanial agents to overcome the current situation of the disease.

The discovery of new antileishmanial agents from natural resources or synthetic molecules is being carried out worldwide. This involves various steps such as preliminary screening of test molecules by *in silico* screening against various essential drug targets and *in vitro* screening of test molecules **[5-7]**. Some enzymes essential for the survival of *Leishmania* parasite are studied for targeting those with the aim to inhibit the parasite. The commonly exploited enzymes include those which are involved in glucose, fatty acids, nucleotide and protein metabolism **[8]**. Some well-known drug targets of *Leishmania donovani* include adenylosuccinate lyase, an essential enzyme in purine salvage, cyclophilin and *N*-myristoyl transferase are involved in co-/post-translational protein processing. Dihydroorotate dehydrogenase is involved in *de novo* pyrimidine biosynthetic pathway and pteridine reductase 1 is responsible for the salvage of pteridines in *Leishmania*. **[9-13]**.

Amphotericin B is the drug of choice in visceral leishmaniasis patients having drug resistance for pentavalent antimonials **[14]**. However, its use has been limited because of the possible nephrotoxicity **[15]**. Natamycin, a macrolide antifungal agent derived from *Streptomyces natalensis* and nystatin, a polyene antifungal drug derived from *Streptomyces noursei* are the two broad-spectrum antifungal agents having close structural similarity with amphotericin B. The present study was aimed to screen natamycin and nystatin against five drug targets of *L. Donovani* by using *in silico* protein-ligand docking (Figure 1). To the best of our knowledge, both natamycin and nystatin have never been reported to inhibit the enzymes of any species of *Leishmania*.

MATERIALS AND METHODS

Selection and preparation of drug targets for molecular docking

Molecular docking experiments were carried out by using crystal structures of *L. donovani* drug targets. Five proteins including adenylosuccinate lyase, LdonASL (PDB 4MX2), cyclophilin, LdonCyp (PDB 3EOV), dihydroorotate dehydrogenase, LdonDHODH (PDB 3C61), *N*-myristoyl transferase, LdonNMT (PDB 2WUU) and pteridine reductase 1, LdonPTR1 (PDB 2XOX) were used. After downloading the

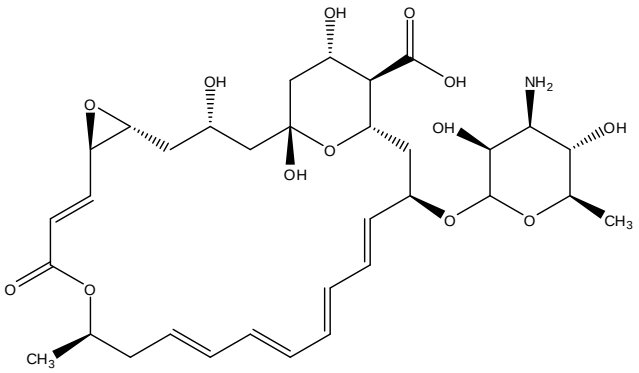
.pdb files from the Protein Data Bank all the non-protein parts, solvent molecules and the co-crystallized ligands were removed from the structures. By using AutoDockTools-1.5.6, .pdb files of all the receptors were modified by adding polar hydrogens and converted to .pdbqt files which is an extended pdb format required for molecular docking analysis.

Ligand Preparation

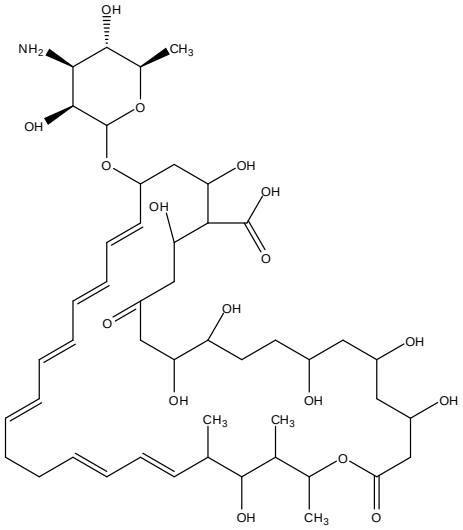
The .pdb files of natamycin and nystatin were obtained from Drug Bank (http://www.drugbank.ca/drugs) and were optimised & converted to .pdbqt files using AutoDockTools-1.5.6 **(Table 1)**.

Table 1: Details of drugs used as ligands in docking experiments

Drug Name	Chemical formula	Drug Bank Accession number	Approval for human use
Natamycin	$C_{33}H_{47}NO_{13}$	**DB00826**	**Approved**
Nystatin	$C_{47}H_{75}NO_{17}$	**DB00646**	**Approved**



Natamycin



Nystatin

Figure 1: Chemical structures of the drugs used for molecular docking

Molecular docking

In silico docking was carried out to identify the inhibiting potential of natamycin and nystatin against five different enzymes which are essentially required for the survival of *L donovani*. The binding sites previously occupied by a co-crystallized inhibitor or substrate were used as binding site for each ligand. Docking was performed using AutoDock Vina 1.1.2 **[16]**. The resultant receptor-ligand complex models were generated using PyMOL Molecular Graphics System to know the amino acid residues to which the ligands were bound **[17]**.

RESULTS

A total of five *L. donovani* enzymes were targeted by natamycin and nystatin to test their binding potential. Both natamycin and nystatin exhibited very low binding energies for all the five enzymes (**Tables 2**). Natamycin was bound to 1-3 amino acid residues of the active sites of LdonASL, LdonCyp, LdonNMT and LdonPTR1 of *L. donovani* suggesting the possible competitive inhibition of these enzymes (Figure 2). Nystatin was bound only to one of the amino acid residues in the active sites of LdonASL, LdonCyp and LdonPTR1 (**Table 3**). In the case of LdonDHODH, both natamycin and nystatin were bound to a site away from the known active site of this enzyme.

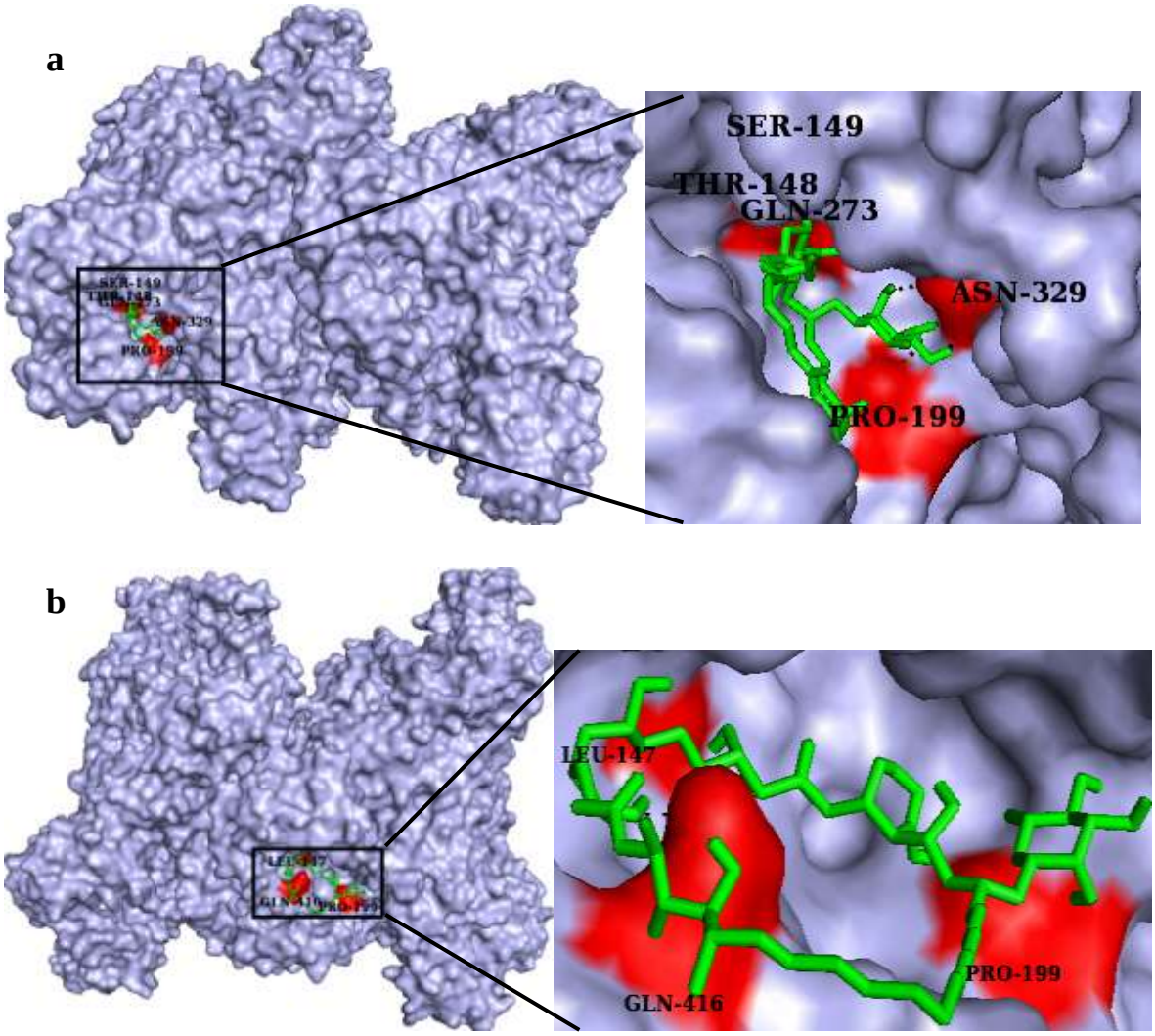
Table 2: Minimum binding energies shown by natamycin and nystatin with five*L. donovani* drug targets.

Drug targets ↓	Minimum Binding affinities (kcal/mol)	
	Natamycin	Nystatin
LdonASL	-8.8	-9.5
LdonCyp	-8.0	-8.9
LdonDHODH	-9.1	-8.8
LdonNMT	-10.1	-9.5
LdonPTR1	-8.4	-7.6

Table 3: The amino acid residues in the active sites to which natamycin and nystatin were bound.

Drug Targets↓	Amino Acid Residues	
	Natamycin	Nystatin
LdonASL	GLN-273, THR-148, SER-149	LEU-147
LdonCyp	GLY-95, GLN-86	GLN-86
LdonDHODH	ARG-224, ASP-257, THR-254, LYS-296*	GLN-149, ASP-145*
LdonNMT	TRP-15	ASP-396, TYR-345*
LdonPTR1	HIS-38, ARG-17, SER-40	ARG-17

***The binding of drug(s) was observed somewhat away from the known active**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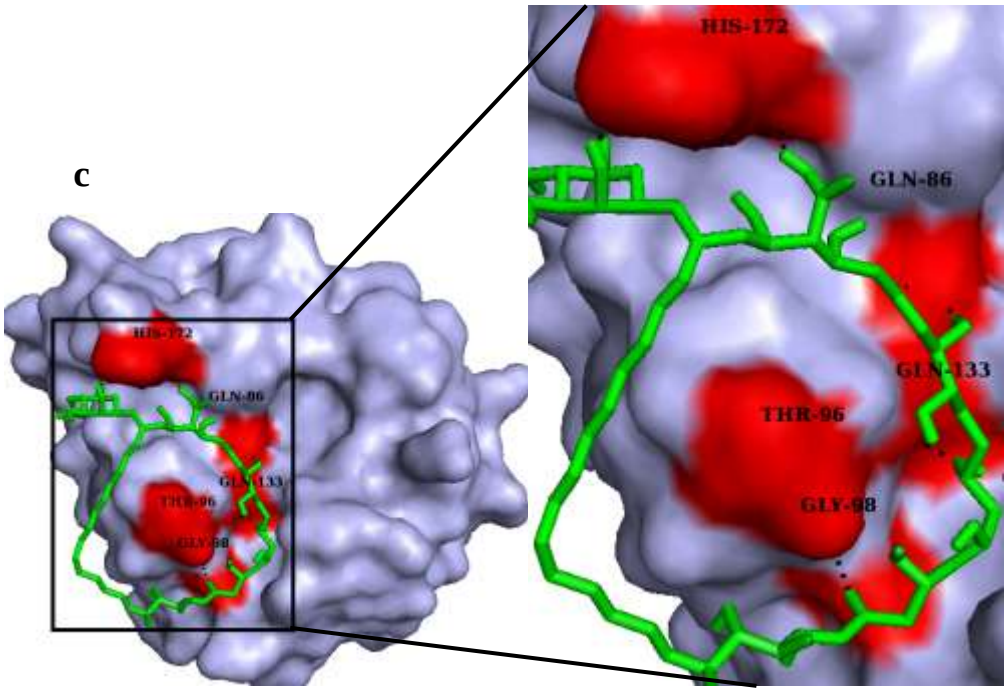
SER-149
THR-148
GLN-273
ASN-329
PRO-199
b
LEU-147
PRO-199
GLN-416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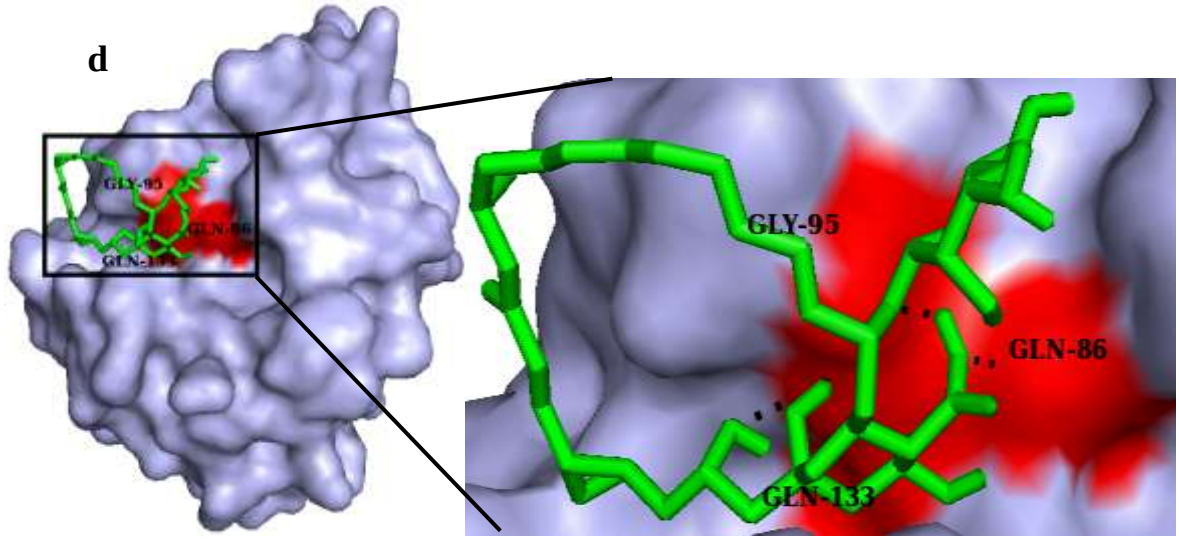
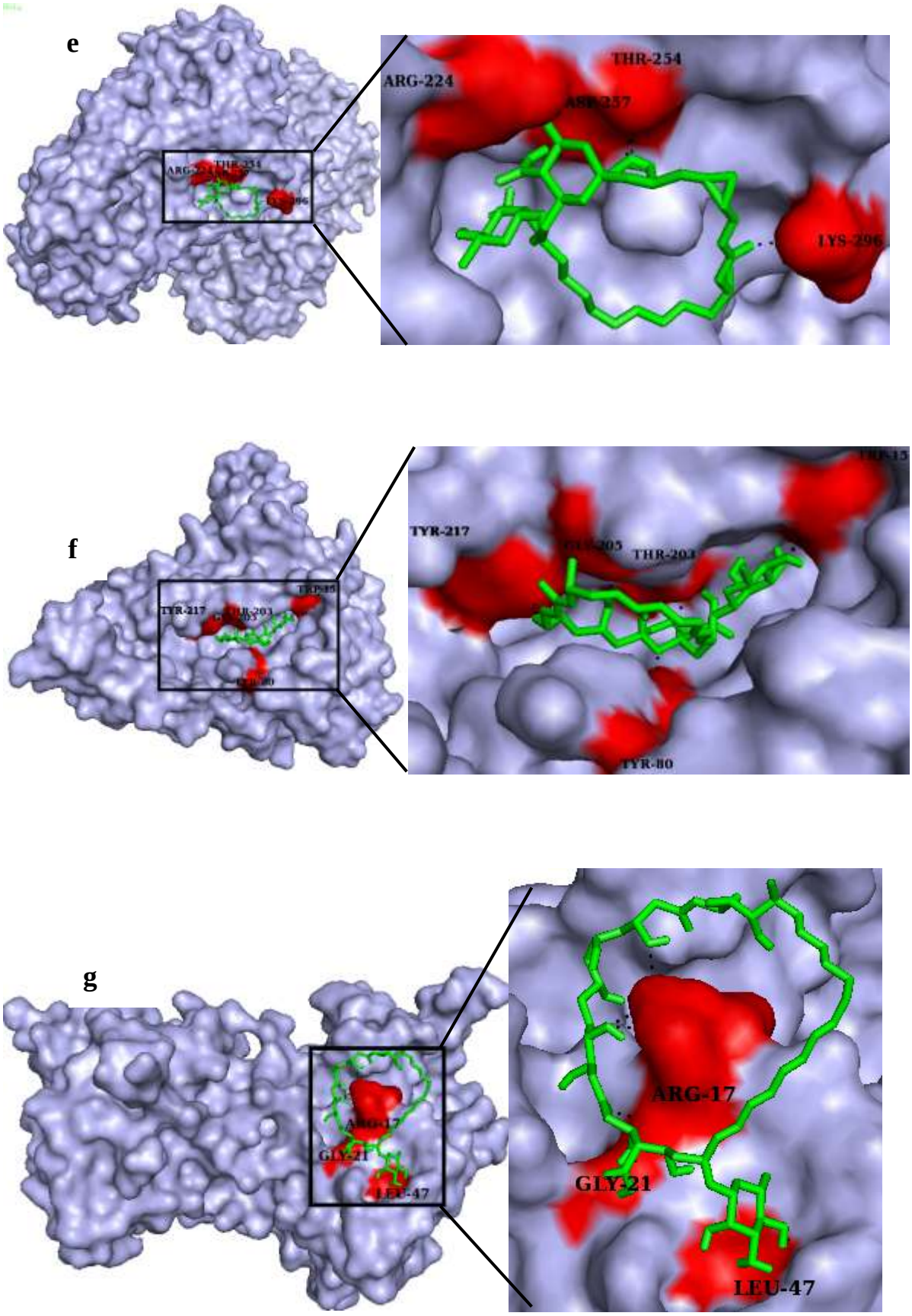
HIS-172
GLN-86
GLN-133
THR-96
GLY-98


d
GLY-95
GLN-86
GLN-133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LYS-296
f
TYR-217
THR-203
TYR-80
g
ARG-17
GLY-21
LEU-47

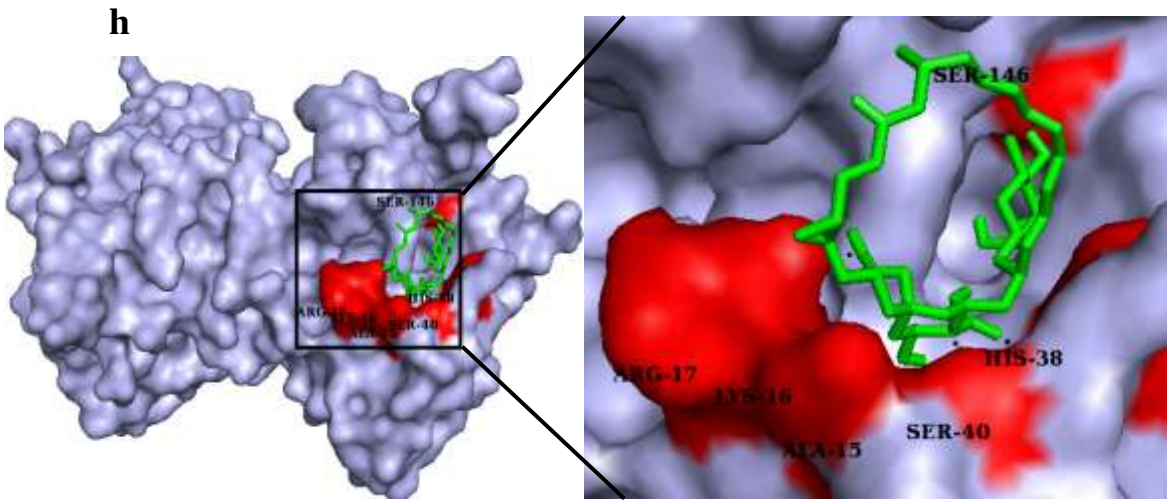

Figure 2: Ligand-receptor models of best docked ligands.a) LdonASL with natamycin, b) LdonASL with nystatin, c) LdonCyp with natamycin, d)LdonCyp with nystatin, e)LdonDHODH with natamycin, f) LdonNMT with natamycin, g) LdonPTR1 with nystatin, h) LdonPTR1 with natamycin. The surface of the drug target is shown in light blue, the ligands are green, amino acid residues bonding to the ligands are red and hydrogen bonds between the ligands and the amino acids are black (dotted line).

DISCUSSION

The lack of efficient chemotherapeutic agents against *Leishmania* parasite is a major problem in controlling various forms of leishmaniasis. The currently used antileishmanial drugs have one or more limitations like toxicity and drug resistance. Despite continuous research on herbal formulations and extensive natural product screening, there is no report of any new drug of plant origin which is commercialized and used for the successful treatment of visceral leishmaniasis. There is a need to screen the established chemotherapeutic agents which are playing impressive roles in the treatment of similar infectious diseases. Before laboratory testing these drugs must be validated and voted up for *in vitro* and *in vivo* experiments to minimize the expenses and time consumption. This may be achieved by protein-ligand docking experiments. Virtual screening is a useful method for the preliminary screening of the drugs against a particular pathogen as it requires less time and resources. It is extensively used for screening of synthetic compounds as well as natural products against *Echinococcus*, *Plasmodium*, *Mycobacterium* and other tropical infectious agents **[18-21]**.

The present study was carried out with the aim to test the potential of natamycin and nystatin to competitively inhibit the essential enzymes of *L donovani*. These drugs were chosen as these are structurally similar to amphotericin B and are already in use to treat fungal infections in humans. Both natamycin and nystatin have never been tested *in silico* for their enzyme inhibitory activity against any species of

Leishmania. Nystatin has been screened *in vitro* against *L. braziliensis*, *L. infantum*, *L. Major* and *L. Tropica* and was found to have significant antileishmanial activity **[22-23]**.

Both natamycin and nystatin required very low binding energies with all the *L. donovani* drug targets used. Natamycin was able to bind to more amino acid residues of the active sites of drug targets than nystatin. The competitive inhibition of these enzymes may result in interruption of respective metabolic pathways and inhibit the parasite growth. These findings suggest that both natamycin and nystatin may further be investigated by *in vitro* and *in vivo* methods to establish their efficacy to inhibit *L. donovani*.

REFERENCES

[1] WHO, 2015. Leishmaniasis. Retreived from http://www.who.int/leishmaniasis/en/ on 04-05-2015.

[2] Kyriazis JD, Aligiannis N, Polychronopoulos P, Skaltsounis AL, Dotsika E. Leishmanicidal activity assessment of olive tree extracts. Phytomedicine 20(3-4), 2013, 275-81.

[3] Camacho Md, Phillipson JD, Croft SL, Solis PN, Marshall SJ, Ghazanfar SA. Screening of plant extracts for antiprotozoal and cytotoxic activities. J Ethnopharmacol, 89(2-3), 2003, 185-91.

[4] Sharif M, Ziaei H, Azadbakht M, Daryani A, Ebadattalab, A and Rostami M. Effect of methanolic extracts of *Artemisia aucheri* and *Camellia sinensis* on *Leishmania major* (In Vitro). Turk J Med Sci 36(6), 2006, 365-369

[5] Gundampati RK and Jagannadham MV. Molecular docking based inhibition of trypanothione reductase activity by taxifolin novel target for antileishmanial activity. J. Appl Pharm Sc., 2(10), 2012, 133-136.

[6] Bernal FA and Coy-Barrera E. *In-Silico* analyses of sesquiterpene-related compounds on selected *Leishmania* enzyme-based targets. Molecules, 19, 2014, 5550-5569.

[7] Pereira IO, Marques MJ, Pavan AL, Codonho BS, Barbieri CL, Beijo LA, Doriguetto AC, D'Martin EC, dos Santos MH. Leishmanicidal activity of benzophenones and extracts from *Garcinia brasiliensis* Mart. Fruits. Phytomedicine, 17(5), 2010, 339-345.

[8] Barrett MP, Mottram JC and Goombs, GH. Recent advances in identifying and validating drug targets in trypanosomes and

leishmanias. Trends Microbiol 7(2), 1999, 82–88.

[9] Boitz JM, Strasser R, Yates PA, Jardim A, Ullman B. Adenylosuccinate synthetase and Adenylosuccinate lyase deficiencies trigger growth and infectivity deficits in *Leishmania donovani*. J Biol. Chem., 288(13), 2013, 8977-8990.

[10] Venugopal V, Datta AK, Bhattacharyya D, Dasgupta D, Banerjee R. Structure of cyclophilin from *Leishmania donovani* bound to cyclosporin at 2.6 Å resolution: Correlation between structure and thermodynamic data. Acta Crystallogr D BiolCrystallogr 65, 2009, 1187–1195.

[11] Ogungbe IV, Setzer WN. *In-silico Leishmania* target selectivity of antiparasitic terpenoids. Molecules 18(7), 2013, 7761-7847.

[12] Brannigan JA, Smith BA, Yu Z, Brzozowski AM, Hodgkinson MR, Maroof A, Price HP, Meier F, Leatherbarrow RJ, Tate EW and Wilkinson AJ. N-Myristoyltransferase from *Leishmania donovani*: Structural and functional characterisation of a potential drug target for visceral leishmaniasis. J MolBiol 396(4), 2010, 985–999.

[13] Sahi S, Tewatia P, Ghosal S. *Leishmania donovani* pteridine reductase 1: comparative protein modeling and protein-ligand interaction studies of the leishmanicidal constituents isolated from the fruits of *Piper longum*. J Mol. Model., 18(12), 2012, 5065-5073.

[14] Chattopadhyay A, Jafurulla M. A novel mechanism for an old drug: Amphotericin B in the treatment of visceral leishmaniasis. Biochem Biophys Res Commun 416(1-2), 2011, 7-12.

[15] Lemke A, Kiderlen AF, Kayser O. Amphotericin B. Appl Microbiol Biotechnol 68 (2), 2005, 151–162.

[16] Trott O, Olson AJ. Auto Dock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem 31(2), 2010, 455-461.

[17] Seeliger D and de Groot BL. Ligand docking and binding site analysis with PyMOL and autodock/Vina. J Comput Aided Mol Des 24(5), 2010, 417–422.

[18] Crowther GJ, Shanmugam D, Carmona SJ, Doyle MA, Hertz-Fowler C, Berriman M. Identification of attractive drug targets in neglected-disease pathogens using an *in silico* approach. PLoSNegl Trop Dis 4(8), 2010, e804.

[19] Mehlin C. Structure-based drug discovery for *Plasmodium falciparum*. Comb Chem High Throughput Screen8(1), 2005, 5-14.

[20] Sundaramurthi JC, Kumar S, Silambuchelvi K, Hanna LE. Molecular docking of azole drugs and their analogs on CYP121 of *Mycobacterium tuberculosis*. Bioinformation 7(3), 2011, 130-133.

[21] Prakash N, Patel S, Faldu NJ, Ranjan R, Sudheer DVN. Molecular docking studies of antimalarial drugs for malaria. J Comput Sci Syst Biol 3(3), 2010, 70-73.

[22] Ali SA, Iqbal J, Nabeel, Khalil Y, Manzoor A, Bukhari I, Ahmad B and Yasinzaj MM. Leishmanicidal activity of Nystatin (mycostatin): a potent polyene compound. J Pak Med Assoc, 47(10), 1997, 246-248.

[23] Piñero J, Martínez E, Ortega A, Del Castillo A and Valladares B. *In vitro* activity of free and liposomal nystatin against *Leishmania* species in comparison with several ampbotericin B and azolic formulations. RevistaIbérica de Parasitología 63(3-4), 2003, 93-96.