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In-silico Identification of Candidate Inhibitory Ligands against Ornithine Decarboxylase Enzyme for Human Sleeping Sickness Causing *Trypanosoma brucei*

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Abstract

Ornithine decarboxylase (ODC) catalyzes the decarboxylation of ornithine to putrescine. This is known to be a crucial step for polyamines biosynthesis in *Trypanosoma brucei*. These polyamines are necessary for microbial cell growth and proliferation. Hence, ODC enzyme is the best target to treat African sleeping sickness disease-causing protozoan parasite, T. brucei. ODC is a 5'-pyridoxal phosphate (PLP) dependent, an obligate homodimer enzyme with two identical active sites at the dimer interface, comprising the beta or alpha barrel domain from one subunit and beta-sheet domain from the other subunit. The catalytic residues are contributed to the active site from both monomers. An X-ray crystallography study on ODC in the wild T. brucei has revealed two structural changes upon ligand binding; an amino acid residue specifically Lys-69 is displaced by putrescine forming a new interaction and a side chain of Cys-360 moves to the active site. Mutation of the Cys residue to Ala or Ser amino acid reduces the Kcat energy of the decarboxylation reaction drastically. Interestingly, ligand ZINC01703953 shown interaction with ODC protein, Lys-69 functional amino acid with docked score of -8.28 out of 35 ligands tested based on virtual screening (VS) with AutoDock suite in the current study. Another, top scoring (-9.69) ligand, ZINC67855534 is found to interact with amino acid residues, involved in the active site formation of the ODC enzyme from our current VS experiment. Hence, the ligands ZINC01703953 and ZINC67855534 could possibly consider as potential candidates against *T. brucei* upon further in-vitro experimental validations.

Keywords: PyMol; Docking; Ligands; Virtual screening; Ornithine decarboxylase

Introduction

The African sleeping sickness or African trypanosomiasis is caused by the *Trypanosoma brucei* in humans, usually transmitted through Tsetse flies [1]. Severe disease epidemics were observed both in sub-Saharan and central Africa. Among the 52 countries of Africa, 36 countries showed the epidemics of trypanosomiasis affecting approximately 60 million people [2]. Several chemical compounds and biological molecules were tried to overcome the *T. brucei* infection like melarsoprol, pentamidine, and glycoprotein antigens [3-5]. However, none of the treatments were effective except, polyamine inhibitor, Difluoromethyleornithine (DFMO) [6,7].

Ornithine Decarboxylase (ODC) is one of the key enzymes, catalyzes the first committed step in the polyamine biosynthetic pathway of *T. brucei* [8,9]. ODC is a 5'-pyridoxyl phosphate (PLP) dependent enzyme and an obligate homodimer. The precursor L-ornithine is converted to 1,4 diamino butane, also called putrescine. Further, putrescine acts as the precursor for the polyamines spermine and spermidine biosynthesis [10]. Polyamines are the essential constituents of the living cell with the multifunctional role. For example, polyamines interact with polynucleotides (RNA and DNA) and control their functions. In addition, polyamines also involved in the stability of the biological membranes and directly enhance the some enzyme catalyzing activity [11]. Further, polyamines are critical for protozoan cells proliferation and considered as essential nutrients for the protozoan parasites. For example, Neurospora crassa mutants lacking ODC activity cannot grow unless putrescine, spermidine or spermines were added to the growing media [12,13]. The protozoan pathogens, Trypanosomatids depend on spermidine for their growth and survival [14]. Therefore, ODC is the major drug target enzyme to inhibit the *Trypanosoma brucei* proliferation within the living system.

The alpa-difluormethylamine (DFMO) has been shown as the very effective irreversible inhibitor of ODC and hence, DFMO is used as first line of a drug against human late stage sleeping sickness disease [14]. Even though DFMO shown the greater effect as a single dose, but total dose can be reduced significantly if combined with other drugs [15,16]. However, drug toxicity level is high and effective only in late stage trypanosomiasis. Hence, alternative drug targets identification is indispensable. Hence, ZINC database can be used for virtual screening (VS) experiment to identify the potential inhibitory ligands for polyamine biosynthetic pathway enzymes of *T. brucei* [17]. ZINC database is a free chemical database, consisting of nearly 13 million small commercially available molecules [18]. With the help of advanced computational biology, an arduous task of *in-vitro* testing of ODC inhibitors has been reduced drastically to find out the alternative ligands through virtual screening. For virtual screening, the modeling program like PyMOL can be used to visualize the protein structure [19]. The protein-ligand interactions play pivotal role in the biological systems and hence, understanding the protein-ligand association is very much essential for biochemical processes, drug interactions, and disease progress analysis. The molecular modeling of the proteins and

their interactions with the ligands is a bottleneck issue in deciding the functionality of the proteins within the biological systems. Novel inhibitors were identified through virtual screening of ligands from ZINC database against the new therapeutic targets of T. brucei, such as cyclic nucleotide phosphodiesterase (TbrPDEB1 and TbrPDEB2) [17].

In this direction, the virtual screening experiment was conducted for 35 random ZINC database chemical compounds (ligands) to identify the candidate ligands interacting with the ODC protein *in-silico*. Two ligands ZINC01703953 and ZINC67855534 were identified as candidate ligands interacting with target ODC protein (PDB: 1F3T). The amino acid residue (Lys-69 and Cys-360) plays a crucial role in the enzymatic activity of ODC [8]. Interestingly, the novel ligand ZINC01703953 identified through virtual screening also interacts with two important amino acid residues (Lys-69 and Cys-360) of ODC. Hence, we would like to reiterate the novel ligand ZINC01703953 from ZINC database is a potential ODC inhibitory molecule against African sleeping sickness disease.

Materials and Methods

Ligands selection and 1F3T PDB characterization

35 ligands were randomly selected from the ZINC database for docking experiment. The target protein used is *Trypanosoma brucei* ornithine decarboxylase (ODC) complexed with putrescine (PDB: 1F3T) [8]. 1F3T PDB file was downloaded from RCSB protein data bank (www.pdb.org) [20]. The preliminary analysis of the ODC secondary structure prediction was carried out using HHPred database [21]. The top hits of HHPred predictions were used to identify the conserved domain using ClustalW analysis [22]. Consequently, the signature sequence and conserved amino acid residues for pyridoxal 5'-phosphate (PLP) binding was predicted using PROSITE database [23].

Docking through VMD and validation

The software's visual molecular dynamics (VMD) and Virtual screening (VS) lab were used for the analysis of docking poses as well as to study the interaction of ligands with the target protein [24,25]. The target protein 1F3T PDB file was loaded into the VMD. The VS lab was launched from VMD main page extension. The 35 ligands were loaded into VS lab using the input option. The auto dock parameters used for the aligning the active site grid are: **W** 47.25; **H** 47.25; **D** 47.25; **X** 17.00; **Y** -0; **Z** -1.00. The grid points spacing was set to 0.500 (Å) from the VMD and the ligand docking analysis was run. Receiver Operating Characteristic curve (ROC), area under ROC curve (AUC) and Hit-Enrich curve (HEC) analysis methods were used for evaluating the performance of the virtual screening (VS) experiment. Further, top ligands with highest docking score were analyzed by varying the parameters in PyMol software [26].

Contact points visualization using PyMOL: The protein complex 1F3T PDB file was loaded into the PyMOL 3D interface. PyMOL is a very interactive program, where one can use the different options to visualize the protein structure such as, Actions, Show, Hide and Label. The external graphical user interface (GUI) is used to select the measurement wizard to see the distance between the polar and non-polar contacts in the protein-ligand complex as described earlier [26].

Shape complementarity visualization between ligands and 1F3T: The ligand binding pocket topography and deepness were visualized for all the protein-ligand complexes. The external GUI is used to select the display option to see the sequence of the protein. The specific ligands for the individual 1F3T protein complexes were selected to see their interactions with respect to pocket deepness through PyMOL. These conformational changes in the 1F3T protein complexes were visualized using align options. The shift in the residues was seen using measurement wizard.

Solvation visualization through PyMOL: The PyMOL is used for the visual observations to see the well-structured water molecules displaced upon ligand binding from the individual protein complexes and the total number of well-structured water molecules displaced from the protein complex pockets. Therefore, the interaction of 1F3T protein complex with the aqueous environment was visualized and the number of water molecules displaced due to ligand binding was seen using "remove water" or "nb spheres" options in PyMOL.

Results and Discussion

The ZINC database screening revealed inhibitor ligands against phosphodiesterase B1 and B2 enzymes to suppress T. brucei based on virtual screening methods [17]. The contemporary study also revealed potential inhibitory ligands (ZINC01703953 and ZINC67855534) for ornithine decarboxylase (ODC) enzyme. ODC is a proven and established drug target in Tryphanosoma brucei and also, the latter has 5′-pyridoxal phosphate (PLP) as a cofactor, crucial for polyamine biosynthetic pathway [27,28].

Reaction mechanism

Ornithine is a precursor compound for the formation of putrescine. The Lys-69 (K-69) from Ornithine Decarboxylase (ODC) binds to the cofactor PLP to form Schiff base as shown in Figure 1. Further, ornithine displaces the lysine residue to form a Schiff base attached to ornithine, to form the quinoid intermediate after the decarboxylation reaction. This intermediate rearranges to form a Schiff base attached to putrescine, which is further attacked by lysine to release putrescine product and reform the PLP-bound ornithine decarboxylase [29].

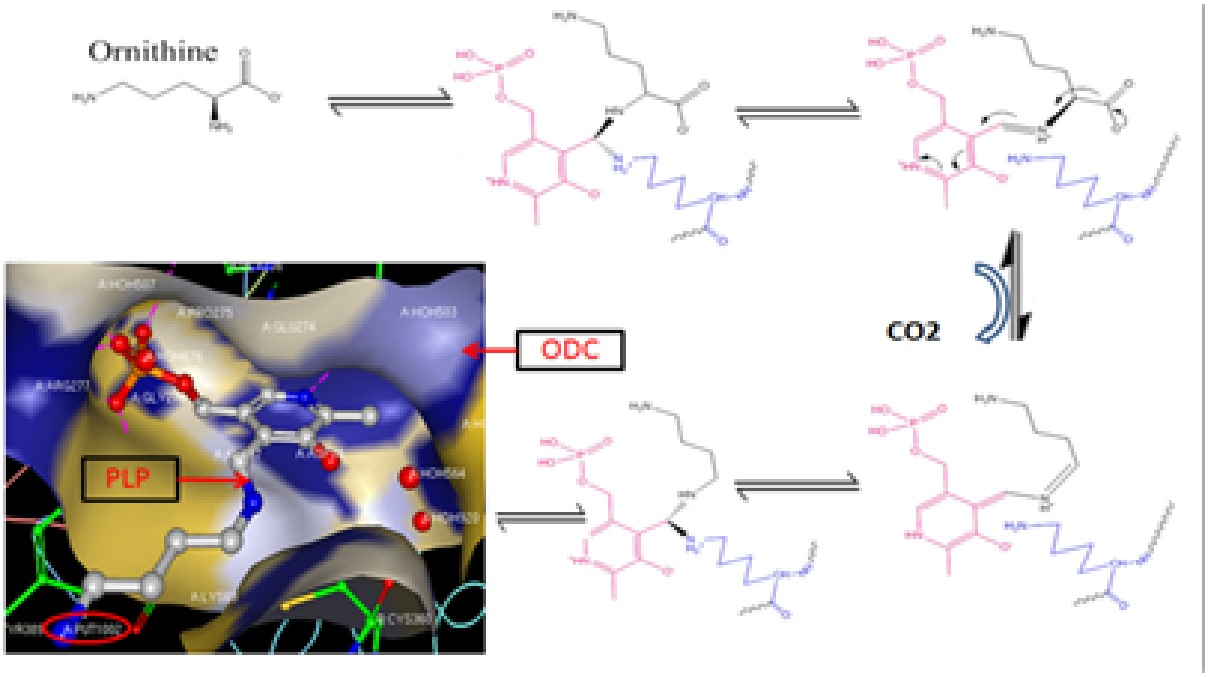

Figure 1: Putrescine biosynthesis mechanism by using ornithine as a precursor molecule in *Trypanosoma brucei* and CO_2 released as a byproduct. The interaction of co-factor putrescine bound pyridoxal 5'-phosphate (PLP) with Ornithine decarboxylase (ODC). The protein is depicted in surface view and cofactor PLP as a stick in the binding pocket using PyMol (Courtesy: Brooks and Phillips [29].

HHPred multiple alignment and PROSTE

The top hits from the HHPred were used to predict the conserved domain in ODC using 1F3T PDB file. The top hits of HHPred sequence were used for the multiple alignments to predict the conserved domain Figure 2. The conserved domain amino acid residues are pivotal in active site formation and interaction with the proper ligand.

Figure 2: The top hits from the HHpred were used to check the conserved domain. The multiple alignments of the top hit HHpred sequence with the conserved domain are depicted in the highlighted box.

The web-based stand-alone bioinformatic tool PROSITE was used to identify the conserved signature matches for ODC and structural residues of the protein [30]. The following conserved domain was predicted using PROSITE. Here, K indicates the decarboxylases family 2 pyridoxal-P (PLP) attachment site.

PLP is an essential cofactor for ODC enzyme and ligands interactions and hence, the predicting the structural residue will be the first step in understanding the structural components of ODC.

[FY]-[PA]-x-K-[SACV]-[NHCLFW]-x(4)-[LIVMF]-[LIVMTA]-x(2)-[LIVMA]-x(3)-[GTE]

K is the pyidoxal-P attachment site.

Ligand interacting poses with the 1F3T protein

In total, 177 poses were returned by the VMD and VS programs for the 35 ligands that were tested against the target protein ODC (PDB: 1F3T). The docking analysis carried out using VMD and VS lab revealed several conformations and orientations between ZINC database ligands (ZINC01703953 and ZINC67855534) interactions with active amino acid residues (Lys-69 and Cys-360) of ODC, 1F3T protein Figures 3 and 4.

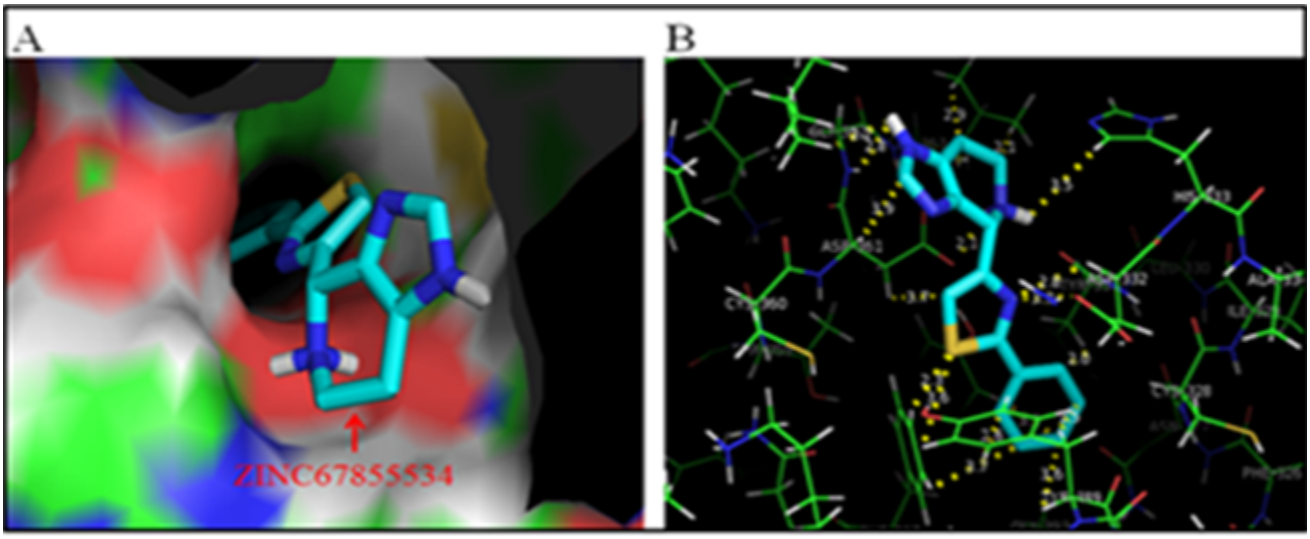

Figure 3: Post-docking interactions between the active site residues of the ODC (1F3T) with ligand (ZINC67855534). (A) The ODC is depicted in surface view and ligand ZINC67855534 as a stick in the binding pocket. (B) The amino acid contacts between ODC and ligand ZINC67855534 with distances.

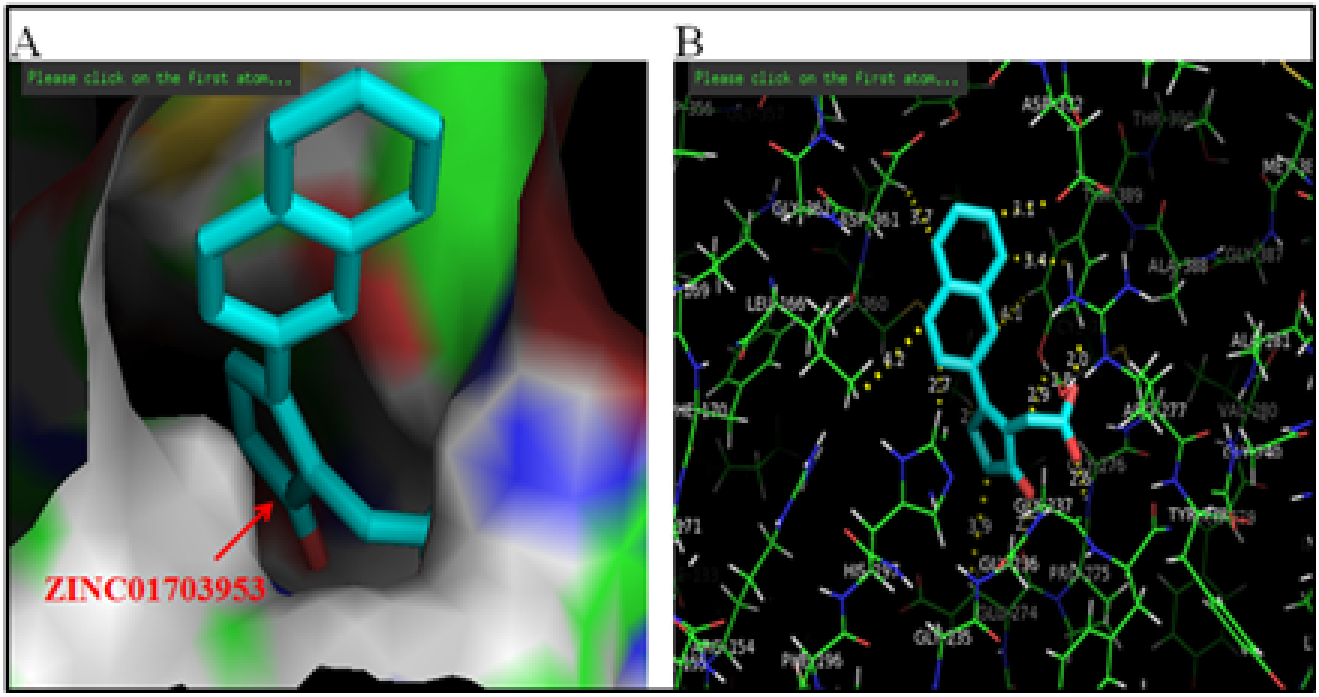

Figure 4: Post-docking interactions between the active site residues of the ODC (1F3T) with ligand (ZINC01703953). (A) The ODC is depicted in surface view and ligand ZINC01703953 as a stick in the binding pocket (indicated with red arrow). (B) The amino acid contacts between the ODC and ligand ZINC01703953 with distances.

The ligands conformation and orientation (pose) based on visual observations of binding pockets and amino-acid contacts were noted. The ligands with more negative docking scores were considered as best ligands. The top five ranking chemical compounds (ligands) based on the docking scores are listed in Table 1. Selected top scoring ligands have docking score above -7.00 and each with different total free energy. The contacts of two top ranked compounds ZINC01703953 and ZINC67855534 with the target protein are: asp332, his197, leu166, asp361, glu274, gly276, lys69, Cys360 and asp332, his333, asp 361, gly362, leu363, tyr389, phe387, tyr331 respectively. The critical amino

acid residues, such as Lys69 and Cys360 were known to be essential in regulating the decarboxylation reaction [8]. Receiver Operating Characteristic (ROC) curve is generally considered as the method of choice for evaluating the performance of the VS experiments.

S. No.	Ligand	Score	Ki (M)	Binding free energy	File name	Amino acid residues
1	ZINC67855534	-9.69	7.88E-08	-10.6	ZINC67855534-S1.pdb	asp332, his333, asp 361, gly362, leu363, tyr389, phe387, tyr331
2	ZINC01703953	-8.28	8.51E-07	-8.6	ZINC01703953-S1.pdb	asp332, his197, leu166, asp361, glu274, gly276, lys69, Cys360
3	ZINC51373466	-7.83	1.82E-06	-8.42	ZINC51373466-S1.pdb	tyr323, phe400, gln401, thr93, asn92, glu116, asn319, asp320, gly324, pro358
4	ZINC20166069	-7.68	2.34E-06	-8.46	ZINC20166069-S1.pdb	ala281, thr241, ala388, asp385, arg337, phe383, ala334, val336
5	ZINC11990575	-7.67	2.38E-06	-7.8	ZINC11990575-S1.pdb	phe400, his119, gln116, asn319, gly324, asn92, asp320, ser402, gln401, pro39

Table 1: Top ranking ligands from ZINC database after virtual screening (VS) against ornithine decarboxylase (1F3T).

ROC method based on AUC and HEC revealed potential active ligands. The performance of the VS experiment was analyzed using the percent active and inactive ligands. The hit rate curve (HRC) and ROC were depicted in Figures 5 and 6 respectively. Further, ROC curve is used to calculate AUC, which is a global indicator of VS experiment performance.

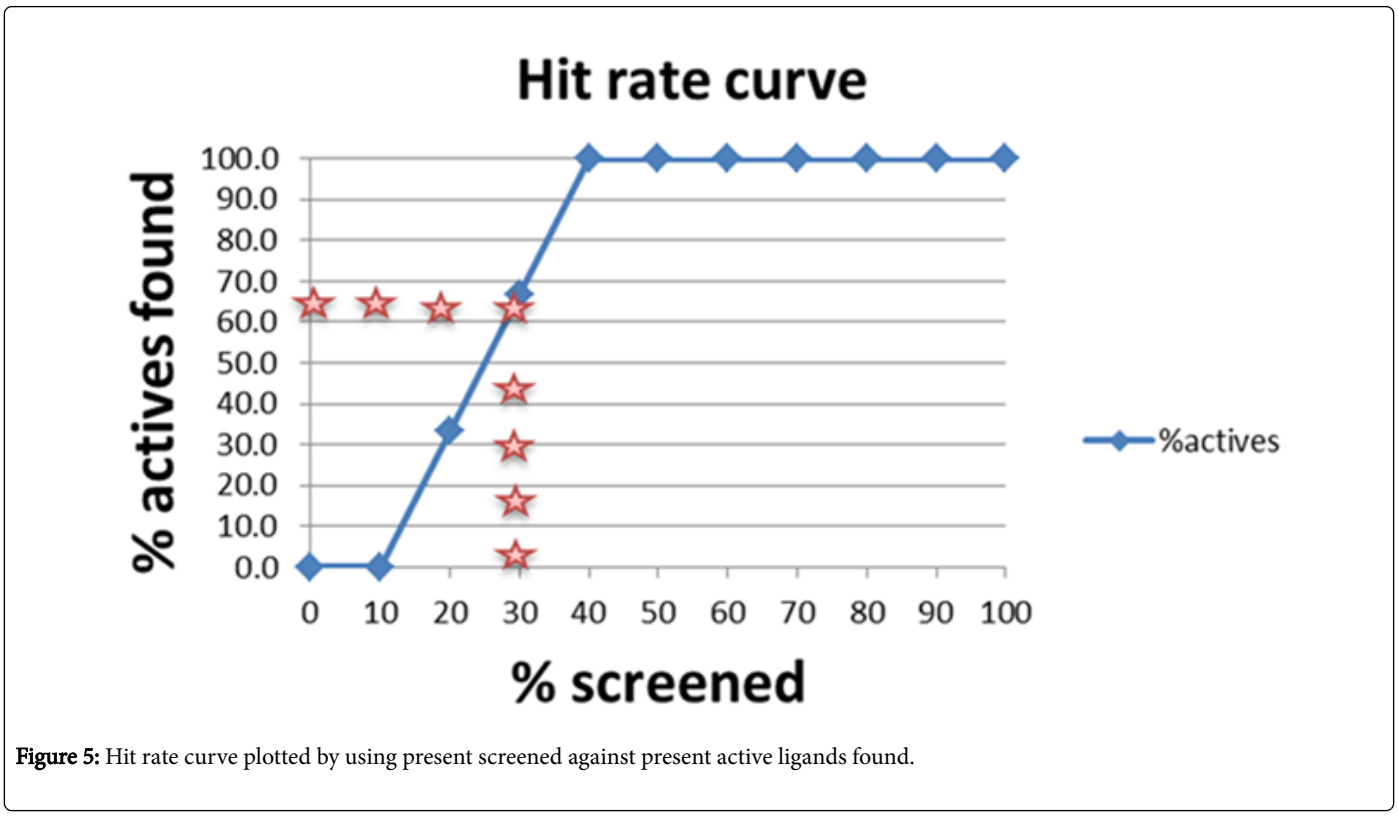

Figure 5: Hit rate curve plotted by using present screened against present active ligands found.

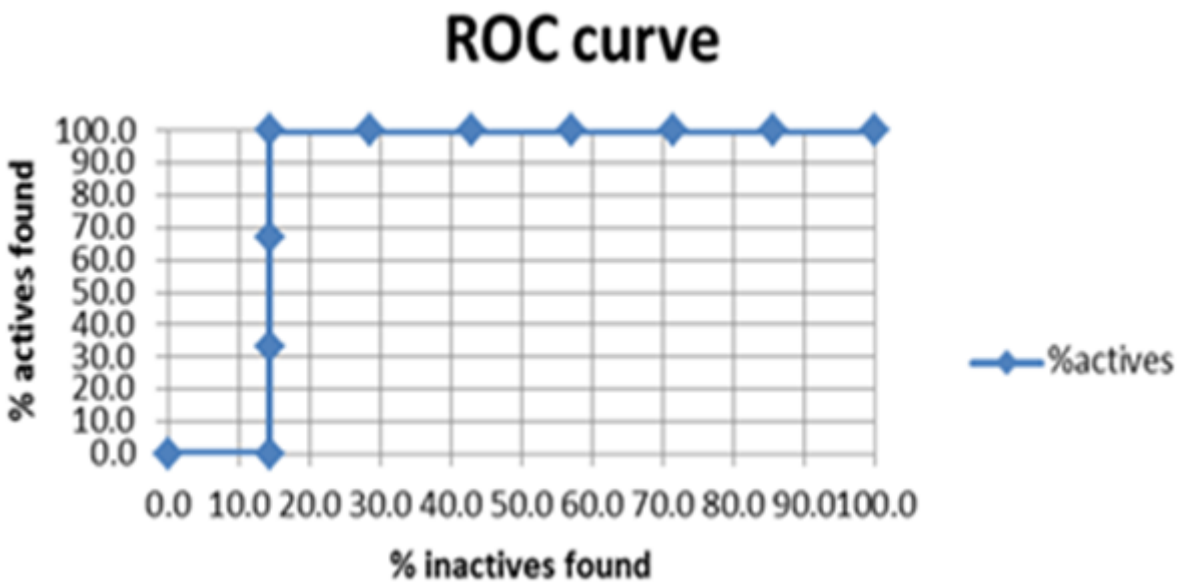

Figure 6: ROC curve plotted by using present inactive ligands found among the docked poses against present active ligands.

The AUC for the present study is 85.72.The Enrichment factor (EF) describes the percent actives found in the top present screened ligands. The enrichment factor in the current study is calculated at 30% screened ligands.

AUC=100-14.285=85.715 or 85.72

Enrichment Factor (30%)=66.67%/30%=2.223

The top ranking ligands are shown in figures with the binding site of ODC as well as their hydrogen bonding and hydrophobic contacts between the docked poses of protein and ligands. The polar, non-polar, acidic and basic amino acid contacts were presented in the following Table 2.

S. No.	Ligand	Amino acid residues			
		Polar	Non-polar	Acidic	Basic
1	ZINC67855534	Gly362, Tyr389, Tyr331	Leu363, Phe387	Asp332, Asp361	His333
2	ZINC01703953	Gly276, Cys360	Leu166	Asp332, Asp361, Glu-274	His187, Lys69
3	ZINC51373466	Tyr323, Gly401, Thr93, Asn92, Asn319, Gly324	Phe400, Pro358	Glu116, Asp320	
4	ZINC20166069	Thr241	Ala281, Ala388, Phe383, Ala 334, Val336	Asp385	Arg337
5	ZINC11990575	Gln116, Asn319, Gly 324, Asn92, Ser402, Gln 401	Ph400, Pro39	Asp320	

Table 2: The docking analysis between ODC amino acids residues polar, non-polar, acidic and basic contacts with top ranking ZINC database ligands.

The ligands (ZINC01703953 and ZINC67855534) identified through VMD and VS programs might act as potential ODC inhibitors. The active amino acid residues (Lys-69 and Cys-360) are having a key role in controlling the function of ODC enzyme [8]. Interestingly, we also found the similar interactions based on *in-silico* analysis for ligand ZINC01703953 from ZINC database. Among the top five candidate ligands, ZINC01703953 is found to be interacting with ODC active amino acid residues Lys-69 and Cys-360. The modifications of Lys-69 and Cys-360 conserved amino acids showed the reduction in decarboxylation reaction rate by 50-1000 folds [8]. Hence, we are postulating that the ligand ZINC01703953 might act as the potential candidate molecule for the modification of active amino acid interacting contacts (Cys-360) to hinder the decarboxylation reaction. Also, in-sufficient putrescine might also reduce the decarboxylation reaction as putrescine is a crucial precursor for polyamines biosynthesis in T. brucei. Therefore, the ligand

ZINC01703953 might be used as ODC inhibitor to stop the polyamines biosynthesis required for the parasitic protozoan growth and proliferation.

Conclusions

The candidate ligands (ZINC01703953 and ZINC67855534) identified in the current study, serve as preeminent molecules to suppress the African sleeping sickness disease by interacting with rate limiting enzyme, ODC. Further, the biological activity of the ligands should be confirmed through experimental assays for future applications.

Competing Interests

The author declares they have no competing interests.

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