IN SILICO SCREENING FOR APSTATIN PROBESTIN AND BESTATIN AS NEW TRYPANOCIDAL COMPOUNDS INHIBITING TRYPANOSOMA BRUCEI BRUCEI TRICORN INTERACTING FACTOR 3 HOMOLOGS

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ABSTRACT

Trypananosoma brucei brucei causes African Trypanosomiasis (AT), a neglected tropical disease affecting both humans and animals.Various classes of *T. b. brucei* peptidases have been studied and implicated as virulence factors, examples include Tricorn interacting factor 3 (F-3). This study was conducted with an objective of identifying potential trypanocidal compounds inhibiting *T. b. brucei* Tricorn interacting factor F-3 through ligand and structure based virtual screening. Three dimensional (3D) structure of *Trf* 3 enzyme was downloaded from Protein Data Bank (PDB format). All the potential ligands for the enzyme were downloaded from PubChem. Docking simulation was carried out using the AutoDock 4.0 suite as molecular-docking tool. The top 10 poses for each test ligand were noted and the best conformer/pose for each identified as their respective final results. Based on the docking results generated, all the compounds showed good binding energy toward the target protein whereby Apstatin, Probestin and Bestatin presented the best minimum docking score of −8.17, −8.04 and −6.83 KCal mol^{-1}, respectively. These 3 compounds strongly interacted with several amino acid residues of the active site of enzymes Glu266, Glu233, Glu288, His265 and Met232. Therefore; Apstatin, Probestin and Bestatin compounds were presented as potential trypanocidal compounds that can further be investigated through *in vivo* or *in vitro* studies to validate them as potential drug molecules against African trypanosomias.

Keywords: African Trypanosomiasis, *Trypanosoma brucei brucei*, Tricorn interacting factor F-3, Molecular docking

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INTRODUCTION

African trypanosomiasis (*Trypananosoma brucei brucei*) is a disease transmitted by Tsetse flies and remains a constant and persistent threat to the health and welfare of livestock (Brun *et al.,* 2010). Tsetse flies is prevalent in an area over 138,000 square kilometers in Kenya impeding agricultural development, hampering nutrition and economic prosperity of people living in tsetse infested areas (Murilla *et al.,* 2014). In Africa, it occurs in 37 sub- Saharan countries covering about 9 million km^2, (Mattioli *et al.,* 2004) whereby in every year, it causes about 3 million deaths in cattle (*Bos* spp.). Approximately 35 million doses of trypanocidal drugs are administered to the cattle to control trypanosomiasis (Mattioli *et al.,* 2004). Nagana has a severe impact on agriculture in sub-Saharan Africa. Direct costs due to African Animal Trypanosomiasis (AAT) also known as Nagana include decreased livestock productivity (mortality, fertility, milk yield, ability to work as traction animals) and expenditure on controlling the disease (Shaw *et al*., 2014). The annual economic value of lost production of milk and meat alone is estimated at US$ 2.75 billion. A weighted evaluation, extrapolated for all tsetse-infested areas, estimated the annual total losses, in terms of agricultural Gross Domestic Product, at US$ 4.5 billion (Feldmann *et al.,* 2018).

Trypanosome brucei brucei is a protozoan parasite transmitted by tsetse fly and causes AAT (Murilla *et al.,* 2014). However, the parasite is susceptible to lysis by the human Trypanosome Lysis Factor-1. It is genotypically similar to the human pathogenic forms; *T. brucei gambiense* and *T. brucei rhodensiense* thus is a good experimental model for both human and animal infection studies (Franco *et al.,* 2014). Changing environmental and /or social factors can trigger changes in the intensity of transmission of the disease, thus can result in epidemics as well as spread in new areas (Fevre *et al.*, 2008).

Control methods for African Trypanosomiasis have

focused on the use of drugs which have adverse effects and vector control methods which have proved to be ecologically unsustainable (Kotlyar, 2010). No conventional vaccine exists for African Trypanosomasis due to antigenic variation associated with the parasite (Field *et al.,* 2017). The zoonotic nature of the disease together with the capacity of the parasites to evade the host immune response or become dormant hampers pathogen eradication and immunoprophylactic control (Franco *et al.,* 2014). With various limitations of vector control methods and life threatening side effects of the available chemotherapeutic agents, new methods of treatment and diagnosis are required. Many peptidases have been implicated in the virulence and pathogenesis of the parasites hence presenting as potential drug targets (Sanchez, 2013).

Tricorn protease and its interacting factor *F-1*, *F-2* and *F-3* homologs have wide distribution in archaea and bacteria (Ng'ong'a, 2017) hence a conserved protease machinery essential in cellular protein degradation. The Tricorn interacting factor *F-3* is a Zn- aminopeptidase with a molecular weight of 89 kDa (Kyrieleis *et al.,* 2005). Tricorn interacting factor *F-2* and *F-3* belong to class MA of M1 family of aminopeptidases, which have been shown to be essential in the survival of a range of pathogenic organisms thus are good targets for drug discovery (Drinkwater *et al.,* 2017). Some drug discovery programs targeting M1 aminopeptidases have shown promise for the treatment of malaria where knockout of *Pf*A-M1 activity resulted into cell death (McGowan *et al.,* 2009; Mistry *et al.,* 2014; Drinkwater *et al.,* 2016). In *Eimeria tenella,* aminopeptidase N, a homolog of tricorn interacting factor *F-3*, aids in parasite development within the host (Gras *et al.,* 2014). *Neisseria meningitides* also have aminopeptidase N with structural resemblance to tricorn interacting factor *F-3* (Nocek, 2008) and have been described as potential drug target for inhibition and development of novel therapeutic agents against *Neisseria meningitidis* (Węglarz-Tomczak *et al.,* 2013).

Tricorn interacting factor *F-3* homologs have also been identified in *Trypanosoma brucei brucei* and are thought to perform similar functions (Ng'ong'a *et al.,* 2017) and therefore targeting it could lead to development of alternative therapeutic targets. Although amino acid metabolism in trypanosomes is a complex process and varies depending on parasite environment, trypanosomes maintain an amino acid pool of alanine, glutamate and proline as precursors of metabolites and for osmoregulation functions (Darlyuk *et al.,* 2009). Efflux of these amino acids is used to prevent cell swelling upon hypotonic stress (Darlyuk *et al.,* 2009). Proline and alanine are neutral amino acids thus their levels in *T. b. brucei* could be directly dependent on the activity of tricorn interacting factor *F-3* orthologs. The blood stream form of these trypanosome relies on D-glucose which is metabolized to succinate and acetate as their source of energy (Mazet *et al.,* 2013). The procyclics are dependent on *L*-Proline which is metabolised to succinate and further to alanine as their source of energy(Mantilla *et al.,* 2017).Thus, the action of tricorn interacting factor *F-3* orthologs could be essential to maintaining a pool of proline for the insect stage.

Conventional de novo drug discovery process at present requires an average of about 14 years and US$ 2.5 million to approve and launch a drug (Nishimura *et al.,* 2018; Nosengo, 2016). Drug repositioning which takes advantage of existing drugs for new indications, can potentially reduce costs and time associated with early-stage testing of promising compounds (Nishimura & Hara, 2016). Previous studies have recognised that small molecule drugs interact with more than one target protein (Paolini *et al.,* 2006; Mestres *et al.*, 2008; Nishimura, 2018). Various in silico methods have been devolved and applied in repositioning of existing drugs for instance; molecular docking based (Xu *et al.,* 2018). Therefore, the objective of this study was to identify compounds that are potential trypanocidal compounds that can further be used as potential drug molecules against African trypanosomias.

MATHERIALS AND METHODS

Data Retrieval

The set of ligand molecules studied in this study include Bestatin [PubChem: 72172] and its structurally similar bioactive compound Apstatin [PubChem: 447280], Amastatin [PubChem: 439518], Probestin [PubChem: 130013], Nitrobestatin [PubChem: 6480937], Thiorphan [PubChem: 3132], Tosedostat [PubChem: 15547703], Leuhistin [Pubchem: 131057] CHR2863 [PubChem: 15547707] and CHR 6768 [Pubchem: 23391687]. The 3D Spatial Data File SDF formats of these ligand molecules were retrieved from NCBI-PubChem Compound database http://pubchem.ncbi.nlm.nih.gov/. These SDF formats were later converted to Protein Data Bank PDB format using discovery studio visualizer (DSV) in preparation for docking studies. The three dimensional

PDB structure of Tricorn interacting factor *F-3* [PDB: 1Z1W] was retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) http://www.rcsb.org/pdb/home/home.do.

Molecular docking

The docking simulation was carried out using the AutoDock 4.0 suite (http://autodock.scripps.edu/wiki/AutoDock4) as molecular-docking tool. In this docking simulation, semi-flexible docking protocol was used, in which the target protein was kept as rigid. The ligands being docked were flexible and the Graphical User Interface program "AutoDock Tools" was used to prepare, run, and analyze the docking simulations. Kollman atom charges and polar hydrogens were added into the PDB file for the preparation of protein in docking simulation. The water molecules were removed. Gasteiger charge was assigned and then non-polar hydrogens were merged. The rigid roots of each ligand were defined automatically and the amide bonds were made rotatable. Therefore, the grid was centered in the catalytic active region of the protein which included all amino acid residues that surround active site. The grid box size was set at 60, 60, and 60 A° [*x*, 14.947: *y*, 50.20: *z*, 31.021], for all the ligands. AutoGrid 4.0 Program, supplied with AutoDock 4.0 was used to produce grid maps. The spacing between grid points was 0.525 angstroms.

The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers. During the docking process, a maximum of 10 conformers was considered for each compound. The population size was set to 150 and the individuals were initialized randomly. Maximum number of energy evaluation was set to 250000, maximum number of generations 27000, maximum number of top individual that automatically survived set to 1, mutation rate of 0.02, crossover rate of 0.8 and 10 LGA runs were performed. AutoDock 4.0 was compiled and run under Windows operating system.

RESULTS

The docking of the ligand molecules into the tricorn interacting factor 3 structure using autodock resulted into a docking file that included record of docking from conformation 1-10. **Table 1** shows the Binding Energy, Inhibition Constant of 10 docked compounds as well as the dock conformation number for each cluster with the best docking score based on binding energy conformation which is considered the most favorable docked pose. Based on the docking results generated, the studies revealed that all the compounds showed good binding energy toward the target protein ranging from −8.17 to −2.29 KCal mol^{-1}. The minimum binding energy indicated that the tricorn interacting factor 3 (target enzyme) was successfully docked with corresponding test ligand.

Apstatin, probestin, bestatin, nitrobestatin, amastatin showed relatively good binding affinity **(**Table I**).** These compounds have also showed strong binding interactions with the target enzyme residues of the active site**.** Docking of receptor *TRF3* with candidate ligands exhibited well established bonds with one or more amino acids in the receptor active site pocket as illustrated in Table II. The active pocket consists of Gly230, Glu233, Glu288, Glu266, Met232, Asn234, His265, His269, Tyr 351 amino acids (**Figure 1**.)

TABLE I-DOCKING PROPERTIES BASED ON THE BEST DO

Ligand	**Conformation**	**Binding energy *(Kcal/Mol*)**	**Ligand efficacy**	**Inhibition constant*(nM)***
Apstatin	1	-8.17	-0.25	1.03
Probestin	3	-8.04	-0.22	1.27
Bestatin	1	**-6.83**	**-0.31**	**9.85**
Nitrobestatin	1	-6.65	-0.27	13.27
Leuhistin	4	-3.77	-0.25	1.71
Tosedostat	8	-3.74	-0.13	1.81
Thiorphan	1	-3.6	-0.21	2.31
Amastatin	1	-3.49	-0.32	2.78
CHR-2863	7	-3.29	-0.11	4.25
CHR-6768	5	-2.29	-0.09	20.94

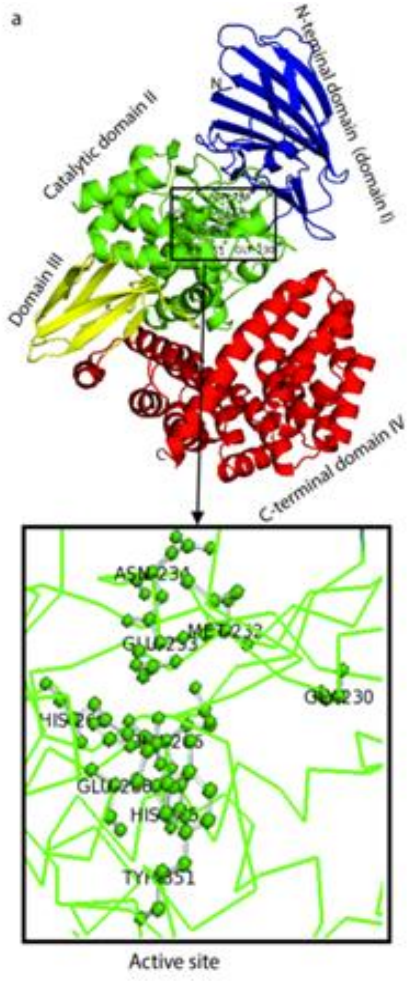

Figure 1: Representation of Tricorn interacting factor *F-3* catalytic site and its associated amino acid residues.

TABLE II-AMINO ACIDS INTERACTING WITH THE LIGAND BASED ON THE BEST CONFORMATION SCORE.

Amino acids interacting with ligand	**Hydrogen bonding**	**Hydrophobic interaction**
Bestatin	Glu266, Glu233, Ala231, Ala229	Val262, Gly230, Glu288, Tyr161, Leu284, Met 232, Glu101, Trp281
Amastatin	Glu266,Glu233, Met232, Ala231, Ala229	Tyr161, Trp281, Leu284, Glu288, Phe346
Nitrobestatin	Ala231, Ala231, Glu266, Thr292, Arg316	Tyr244, Gly230, Glu288, His265, Tyr351
Apstatin	Glu288,Glu233, Glu266, His 265, Arg241, Ala229	His269, Phe346, Glu101, Gly230, Ala231, Arg316, Tyr244
Thiorphan	Arg721, Asn720, Thr722	Phe717, Gly719, Arg725
Probestin	Glu233, Ala231, Glu288,His 265, Arg721, Glu266	Met232, Glu101, Phe346, Ile342, Ala229, Gly230, Tyr244, Val262
Tosedostat	Arg721, Ala229, Ala231	Phe346, Gly228, Gly230, Tyr244, Met232,Glu101 ,Glu288,Glu233,Glu266
Leuhistin	Glu266, Arg316	Ala231, Thr292, Phe346, Glu288, His265
CHR6768	Tyr244, Ala231, Arg241	Val262, Glu266, His265, Ala229, Phe346, Glu288, Gly228, Gly230
CHR2863	Thr722, Arg721, Asn720	Gly719, Gly228, Ile342, Ser343, Phe346

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Further analysis was done on the docking results using Ligplot program to visualize the 3D structures of the ligands' interaction with associated bonds both the hydrogen and hydrophobic interactions to the protein target. The Ligplot output showing the protein-ligand interactions of the top 5 ligands, based on energy score (hydrogen bonding and hydrophobic), as generated by Ligplot program (Figure 2- 6).

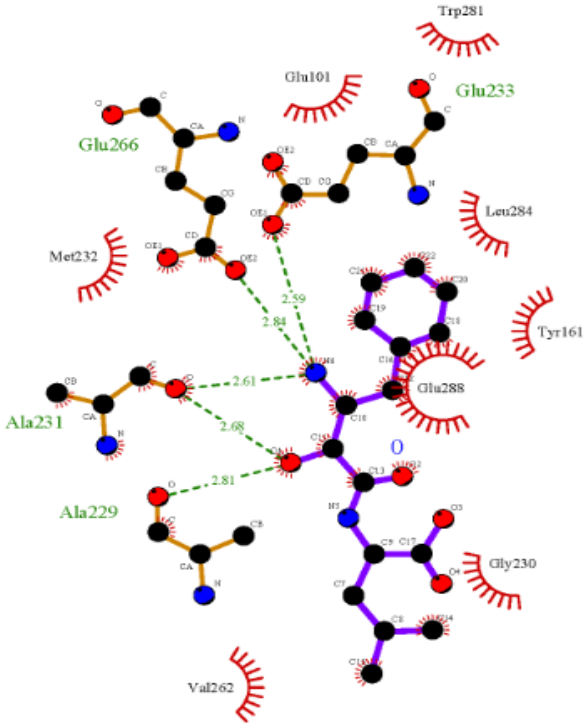

Figure 2: Ligplot showing tricorn interacting factor 3 complexed with bestatin (hydrogen and hydrophobic interaction).

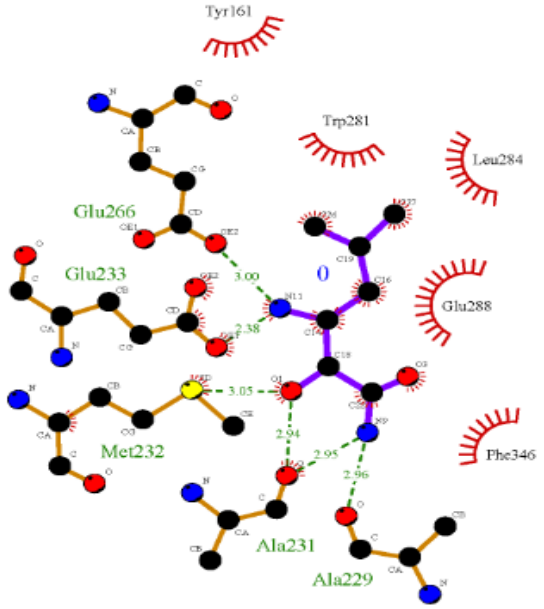

Figure 3: Ligplot showing tricorn interacting factor 3 complexed with Amastatin (hydrogen and hydrophobic interaction)

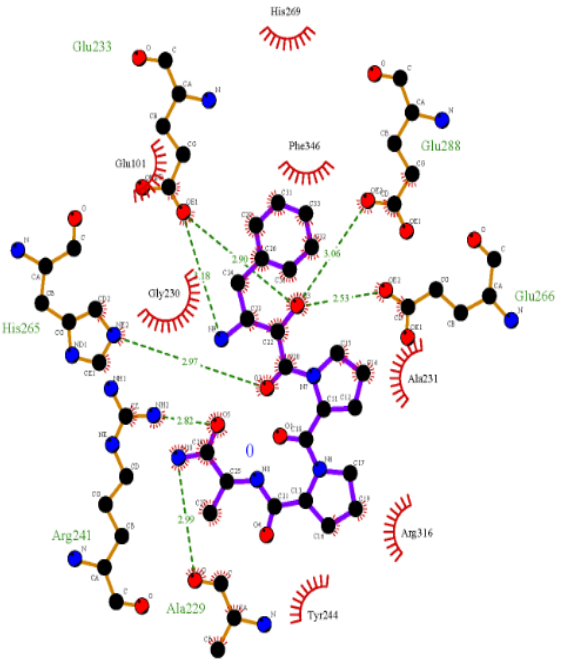

Figure 4: Ligplot showing tricorn interacting factor 3 complexed with Apstatin (hydrogen and hydrophobic interaction).

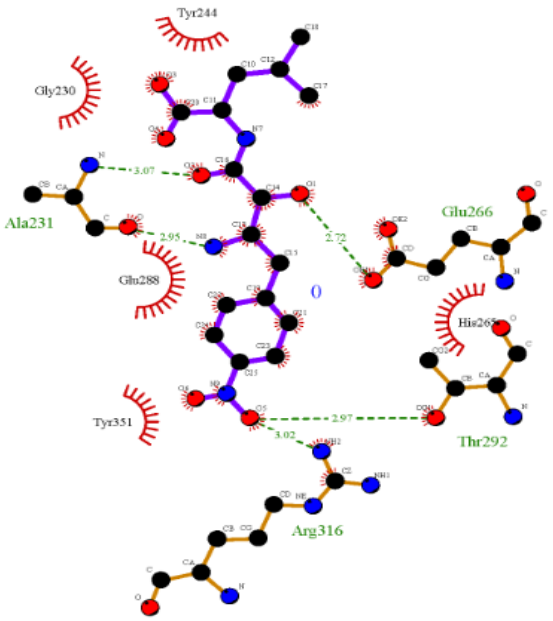

Figure 5: Ligplot showing tricorn interacting factor 3 complexed with Nitrobestatin (hydrogen and hydrophobic interaction).

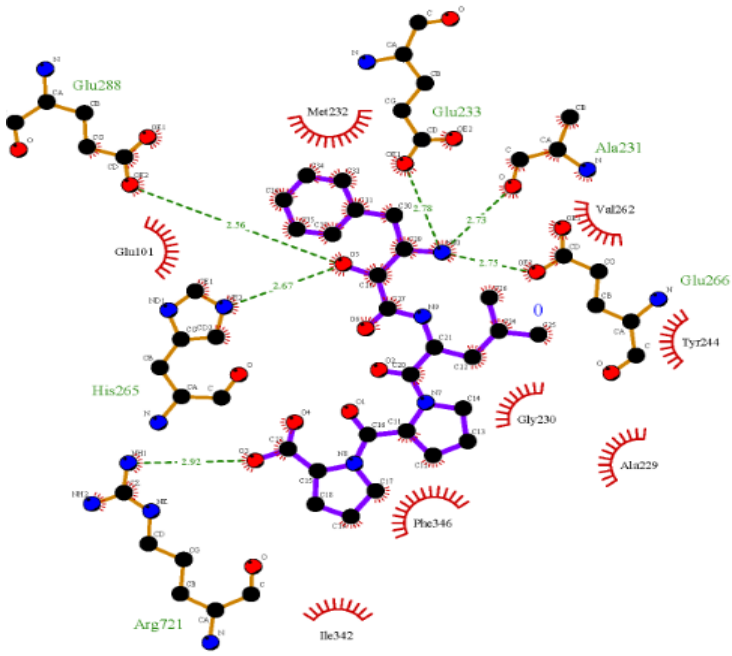

figure 6: Ligplot showing tricorn interacting factor 3 complexed with probestin (hydrogen and hydrophobic interaction).

DISCUSSION

Antigenic variation poses a problem for the development of a conventional vaccine for African Trypanosomiasis. Molecular docking data will provide important insights for the rational design and identification of new, more potent and selective inhibitors of the enzmyme that might lead to new therapies for African Trypanosomiasis. The test ligands showed to have some interactions with one or more amino acids residues of the active site for Glu 230, Glu 233, Glu288, Met 232, Asn234, His265, His269, Tyr 351. Bestatin is an antibiotic originally isolated from filtrates of the fungus *S. olivoretticul*, but is now available in synthetic form (Scornik and Botbol, 2005) Its structure *N*-[(2*S*, 3R)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine is an analogue of the dipeptide Phe-Leu. Bestatin is a natural product of actinomycetes that potently inhibits multiple families of metalloaminopeptidases (MAPs) including the M1, M17 and M18 families. It has been shown to modulate many biological pathways importantly, it has been shown to inhibit growth of *P. falciparum* parasites in culture (Velmourougane *et al.,* 2011).Thus, bestatin is a competitive inhibitor of many metallo-aminopeptidases (Scornik and Botbol, 2005, It is also the best known small molecule APN (Aminopeptidase N) inhibitor (Schreiber and Smith, 2018).

Bestatin was used as a test ligand for this study. It docked well to the target enzyme with a minimum binding energy of – 6.83 *Kcat/mol* and strong bond interaction with amino acid residues of the active site. It formed a hydrogen bond interaction with Glu233 and hydrophobic interaction with Gly230, Glu288 and Met232 amino acid residues of Tricorn interacting factor 3 active site.

Apstatin compound showed to have the highest docking score towards the tricorn enzyme with minimum binding energy of -8.17 *Kcat/mol* hence compared to the rest of the ligands considered the most potential inhibitor against the tricorn enzyme. It strongly bound with several amino acids of the active site. It formed H-bond with Glu266, Glu288, Glu233, and His265 and hydrophobically interacted with Gly230 and His269 residues of the active site. Apstatin has been tested as an inhibitor of aminopeptidase P and has shown to have an inhibitory effect on bradykinin degradation in rat (Prechel *et al*., 1995).

Probestin was also tested and found to have a higher docking score as well with a minimum binding energy of -8.07 *Kcat/mol* towards the target enzyme. It showed strong interacting bonds with several amino acids of the active site. It formed hydrogen bonds with Glu266, Glu288, Glu233, and His265 and hydrophobically interacted with

Met232 and Gly230 amino acid residues of the active site.

Although Nitrobestatin has a minimum binding energy of-6.65 *Kcat/mol* a good docking score, it had weak interaction with the amino acid residues of the active site with only one hydrogen bond with Glu266 and then hydrophobically interacting with Gly230, Glu288, His265, and Tyr351. Compared to Amastatin which has a higher binding energy of-3.6 *Kcat/mol,* it forms strong interacting potential with amino acid residues of the active site. Amastatin formed 3 strong hydrogen bonds with Glu266, Glu233, and Met232 and hydrophobically interacted with Glu288 amino acid residues of the active site.

Apart from the five statin compounds, other ligands were identified and tested as potential lead compounds which included; Leuhistin, thiorphan, tosedostat, CHR6768, CHR2863. Leuhistin, which is considered to be a potential inhibitor of aminopeptidase M (Aoyagi *et al.,* 1991) was used as a test ligand for the docking study. It resulted in a binding energy of -3.77 with one hydrogen bond to Glu266 and hydrophobic interaction to Glu288 and His265 amino acids of the active site. Thiorphan which is a potent synthetic inhibitor of enkephalinase and metalloproteinase (Medja *et al.*, 2006) was targeted a potential lead compound. It had a binding energy of -3.6 though it did not show any interacting potential with the amino acids of the active site.

Todedostat CHR-2797, is a potent inhibitor of aminopeptidases which has shown promise as a potential therapeutic strategy for acute myeloid leukemia (Van Herpen *et al.,* 2010). This compound was tested as ligand for the docking study where it had a binding energy of -3.74 with hydrophobic interaction with several amino acid residues of the active site Glu230, Met232, Glu266, Glu288 and Glu233.

Finally, CHR- 6768 and CHR-2863 were docked to the enzyme as potential inhibitors.. The aminopeptidase inhibitor CHR-2863 is a hydroxamate-containing ester compound closely related to CHR-2797 (tosedostat), a potent inhibitor of a number of intracellular mammalian aminopeptidase is hydrolysed to CHR-6768 (Skinner-Adams *et al.,* 2012). These compound were docked separately where the binding energy were -2.29 and -3.29 respectively. CHR-6768 showed potential hydrophobic interaction with glu266, glu288, His230, His265 amino acid residues of the active site.

CONCLUSION

Through molecular docking analysis approach, Apstatin, probestin and bestatin were identified as potential trypanocidal compounds inhibiting *Trypanosoma brucei brucei* tricorn interacting factor F-3.The need to find novel drugs for African trypanosomiasis is increasing due to adverse effects associated with available threrapeutic drugs and vector control methods which have proved to be ecologically unsustainable. Therefore, it is recommended that r further laboratory experiments should be conducted to validate these compounds as potential drug molecules against African trypanosomiasis.

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