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Drug Repurposing for Inhibiting Triose Phosphate Isomerase of *Giardia lamblia* (GlTIM) Using a Computational Approach

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ABSTRACT

Background: Giardiasis is caused by an intestinal flagellated protozoan parasite, *Giardia lamblia*. This pathogen can infect many hosts, including domestic and wild animals and humans. It is responsible for 280 million symptomatic individuals worldwide, with a prevalence of 2 to 5% in industrialized countries and 20 to 30% in developing countries. Few antiprotozoal drugs are used to cure giardiasis; albeit with the resistance shown by the parasite to many of these drugs. Alternative drugs are continuously needed to be developed. One of the key proteins to target is *Giardia lamblia* Triose phosphate Isomerase (GlTIM), an enzyme involved in glycolysis as well as gluconeogenesis. **Objective:** The present study was designed to find inhibitors for GlTIM through an insilico approach, utilizing drugs that are already in use for other diseases. **Methods:** The GlTIM 3D structure was obtained from the protein database. From ChemSpider, one hundred and forty potential drugs, already approved by the FDA, were retrieved and docked utilizing the PatchDock server, results with the highest interactions were selected. The GS viewer and LIGPLOT+ were used to visualize the interactions, which included hydrogen bonding, covalent and hydrophobic interactions. **Results:** Interactions with the target active site residues suggest the possible inhibition that these ligands can potentiate. The drugs with the highest interaction in the current study include Daptomycin, Vancomycin and Cefazedone. **Conclusions:** The inhibition of GlTIM by these drugs demonstrates that drug repurposing is an important pharmaceutical strategy that can yield new therapeutics in less time and fewer resources for this parasitic disease.

INTRODUCTION

Giardia duodenalis (synonyms *Giardia lamblia*, *Giardia intestinalis*) is the etiological agent of giardiasis. A high incidence and prevalence (20 to 30%) of this infection has been reported in developing countries as compared to developed countries (2 to 5%), with approximately 280 million infections per year [1]. This parasite is acquired by ingesting water and/or food contaminated with cysts [2, 3].

The clinical symptoms of giardiasis have a wide range and include the presence of asymptomatic infections and cases of acute diarrhea, dehydration, abdominal pain, and weight loss, as well as chronic cases of the disease [3]. These symptoms appear 6 to 15 days after infection, and the most affected individuals are children or immunocompromised people. Control of this infection is carried out by using chemotherapeutic agents such as 5-nitroimidazoles and benzimidazole derivatives [4-6].

As compared to developed countries, infection is most prevalent in underdeveloped countries [1]. The infection rate increases in humans and other mammals due to different types of immunological deficiencies [7, 8]. *Giardia duodenalis* have different genotypes from A to H, genotype A to G cause infection in animals whereas genotype H genotype is responsible for this infection in sea vertebrates [9, 10].

This parasite has two morphologically distinct phases throughout its life cycle: the vegetative trophozoite and the cyst, which is the resistant form and guarantees the survival of the parasite when environmental conditions are not favorable [11]. The life cycle of *Giardia duodenalis* consists of the transition

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between two fundamental stages: the cyst and the mobile, infective trophozoite. Infection begins with the ingestion of cysts present in contaminated water or food. Once in the stomach of the host, the acidic conditions of the environment, along with the presence of pancreatic proteases, trigger the process of excystment. This leads to the formation of excyzoites, which divide twice without replicating their DNA, generating four motile and active binucleate trophozoites. These trophozoites adhere to the microvilli of the intestinal epithelium and colonize the duodenum of the small intestine [11-13].

Giardia lamblia triosephosphate isomerase (GlTIM) is a cytoplasmic protein that catalyzes the isomerization of glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP), the key intermediates in the glycolytic pathway. The net gain of ATP is compromised by the inactivation and elimination of Triosephosphate isomerase, resulting in an energy deficit that would affect the parasite's survival [14]. GlTIM is thus considered to be a good pharmacological target [15].

For giardiasis, different types of drugs are used which include, albendazole, furazolidone, paramomycin, metronidazole, tinidazole and quinacrine. But all these drugs have been reported with side effects such as vomiting, nausea, anorexia, urticaria and dry tongue [16]. Other drugs which are mostly used for the giardiasis, include benzimidazole, nitazoxanide, bacitracin zinc, and fumagillin [16].

From the literature, a greater increase in the number of therapy failures is evident, the main causes of therapy failure include side effects, drug resistance and poor patient compliance [5]. Thus, ongoing research on the development of alternate therapeutics for giardiasis is imperative.

Conventional de novo drug development is a long-term, laborious, and expensive process. An alternative is the use of existing drugs in a process called drug repositioning, repurposing, or reprofiling [17, 18]. This includes drugs already on the pharmaceutical market, discontinued drugs, abandoned drugs, and drugs in development [17, 19].

The current study was designed to assess FDA-approved drugs for inhibiting *G. lamblia* triosephosphate isomerase (GlTIM) using a computational drug repurposing approach.

METHODS

Protein Sequence Retrieval, Structural Analysis, and Visualization

The GITIM protein sequence was obtained from the UniProt database, a database that contains comprehensive protein sequences and annotations. We used the Basic Local Alignment Search Tool (BLAST) from NCBI to find related protein structures and the best model for further analysis. We uploaded the GITIM sequence to the NCBI Protein BLAST (http://blast.ncbi.nlm.nih.gov/blast.cgi) and performed a sequence alignment and found the best matching protein structure in the Protein Data Bank (PDB). The 3D structure of triose phosphate isomerase with 2.4Å resolution was obtained from RCSB (Research Collaboratory for Structural Bioinformatics) with PDB ID 2NXQ. This structure was visualized using DS Visualizer 3.1. The active site residues for reaction inhibition were identified for subsequent docking: GLU244, HIS24, ALA27, ALA239, ASN218, GLY176, GLY237, GLY238, ARG135, SER174, SER216, ILE175, LYS195, LYS242, PRO243 and GLN185.

GS Viewer was used to capture the images of the interactions with covalent, hydrogen bond and hydrophobic interactions in purple, green and reddish lines respectively. Three-dimensional structures of one hundred and fifty FDA approved drugs were obtained from ChemSpider (http://www.chemspider.com) and were docked to the GlTIM using PatchDock. The top docking results were analyzed in DS Visualizer, GS Viewer and LigPlot+ to evaluate the interaction patterns between the docked ligands and the protein active site residues.

RESULTS

In the current study, one hundred and fifty FDA approved drugs were docked with *Giardia lamblia* Triose phosphate Isomerase (GlTIM), to find alternate drug using the drug repurposing approach. Of these, the following drugs showed promising results.

Docking Result of Daptomycin with Triose Phosphate Isomerase

The docking of Daptomycin with GlTIM showed twenty-three different kinds of interactions that include covalent bonds, hydrogen bonds and hydrophobic interactions (Table 3.1). Five residues (PRO243, LYS242, ASN218, GLY176 and Ile102) formed covalent bonds, in which PRO243, LYS242, ASN218, GLY176 are the active site residues. While 5 residues (LYS242, SER220, GLY238, SER216 and Lys13) formed hydrogen bonds, in which LYS 242, SER216 and GLY 238 are active site residues. And 13 residues (Glu244, Ala239, Gly237, Asn221, Cys14, Thr177, Gly178, Trp173, Ile176, Val172, Asn15, Leu241 and Phe220) form hydrophobic interactions, in which three residues (Glu244, Ala239, and Gly237) are active sites residues.

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Figure 1

2D illustration of docking of Daptomycin with GITIM.

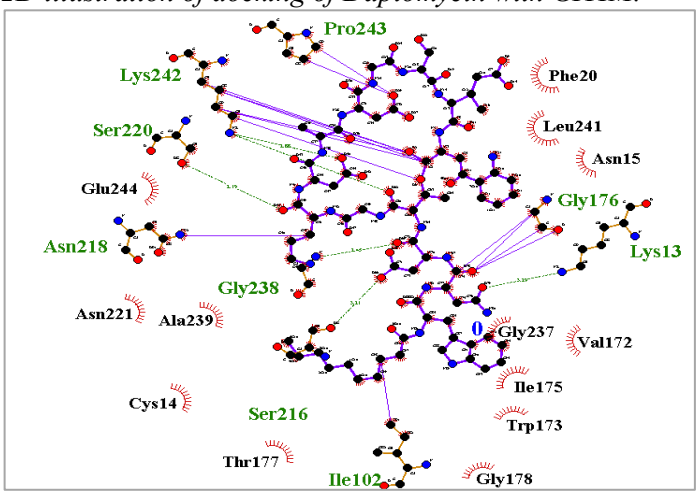


Table 1

Interactions of FDA approved drugs with different amino acids of GlTIM

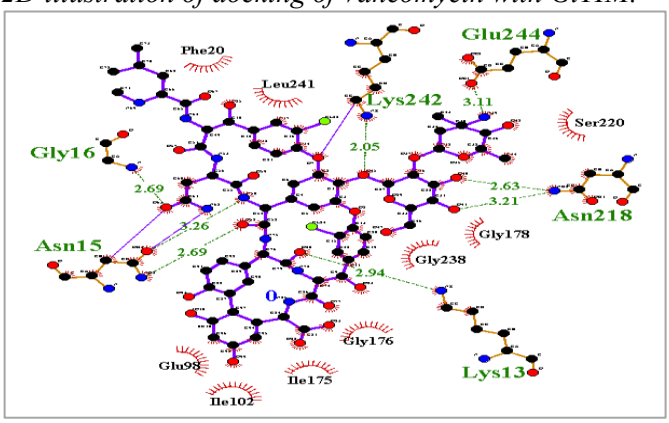
Drug	Covalent Interactions	Hydrogen Interactions	Hydrophobic Interactions	Active Site Amino Acids
Daptomycin	PRO243, LYS242, ASN218, GLY176	ILE102, LYS242, SER220, GLY238, SER216, LYS13	GLU244, ALA239, GLY237, ASN221, CYS14, THR177, GLY178, TRP173, ILE176, VAL172, ASN15, LEU241, PHE220	LYS242, GLU244, ASN218, GLY176, ASN218, PRO243, SER216, GLY238, ALA239, GLY237
Vancomycin	ASN15, LYS242	ASN218, GLU244, LYS242, LYS13, GLY16, ASN15	GLY278, GLY178, GLY176, ILE175, ILE102, GLU98, SER220, LEU241, PHE20	LYS242, GLU244, ASN218
Cefazedone	GLY176, ASN218, GLU98	LYS242, ASN15, GLY238	ILE175, ILE102, LYS13, MET103	LYS242, GLY238, ASN218, GLY176
Ticarcillin	LYS195	ARG135	GLY192, MET141, THR140	LYS195, ARG135
Acyclovir	ARG135	THR140	GLU188, VAL191, MET141, GLY192	ARG135

Docking of Vancomycin with GlTIM

Docking Vancomycin with GlTIM, showed covalent and hydrogen bonds, as well as hydrophobic interactions with different residues (Table 1). With two residues it formed covalent bonds (ASN15, LYS242), in which LYS242 was the active site residue. Six residues formed hydrogen bonds, which included three active sites amino acids (ASN218, GLU244 and LYS242). Nine hydrophobic interactions were also observed in which GLY176 and GLY238 are the active sites residues.

Figure 2

2D illustration of docking of Vancomycin with GlTIM.

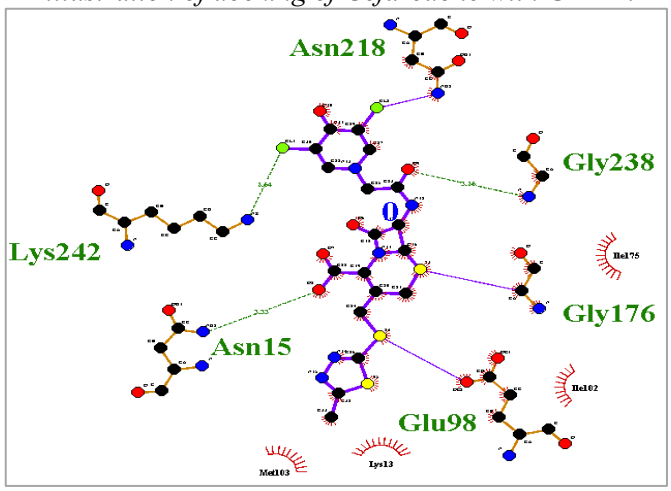


Docking result of Cefazedone with GlTIM

The docking result of Cefazedone with GlTM, indicated eleven different kinds of interaction; included hydrophobic interactions, covalent interactions, and hydrogen interactions as shown in (Table3.3). In these interactions, three residues formed covalent bonds (ASN218, GLY176 and GLU98), in which ASN218 and GLY176 were the active sites residues. Three residues formed hydrogen bonds (GLY238, LYS242 and ASN15), in which GLY238 and LYS242 were the active site residues (Figure 3.3).

Figure 3

2D illustration of docking of Cefazedone with GITIM.



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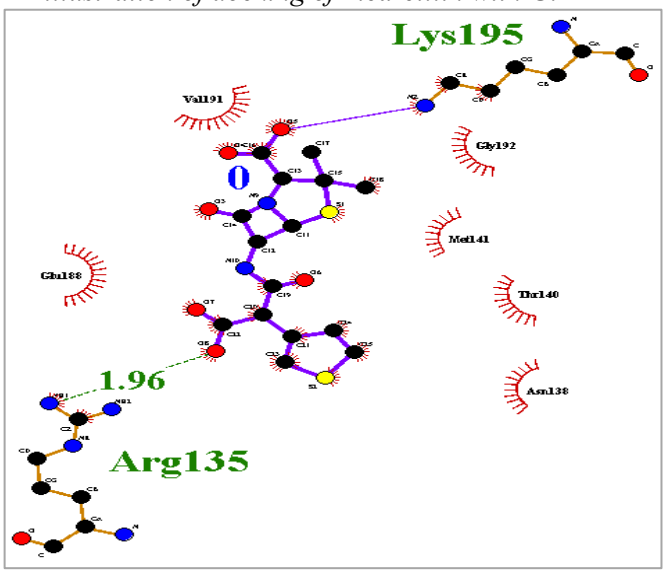
Docking of Ticarcillin with *G. lamblia* triose phosphate isomerase

In the current study, docking of Ticarcillin with GlTIM showed hydrogen interactions, covalent interactions and hydrophobic interactions with ten different kinds of residues (Table 1) . ARG135 and LYS195 both are active site amino acid and they have formed hydrogen and covalent bonds, respectively. Six hydrophobic interactions (GLY192, MET141, THR140, ASN138, GLU188, and VAL191) were also noted (Figure 4).

Figure 4

2D illustration of docking of Ticarcillin with GlTIM

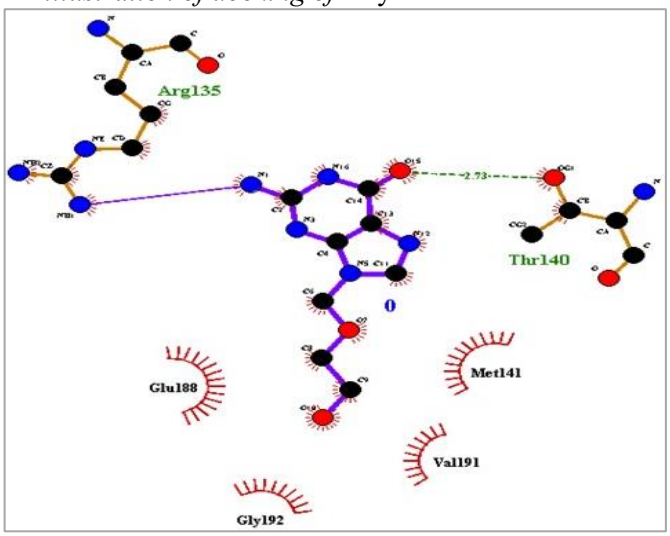


Docking of Acyclovir with GlTIM

Docking of acyclovir with GLTIM also showed covalent, hydrogen and hydrophobic interactions with different residues (Table 1), in which one residue (ARG135) formed covalent bond, which is the active site amino acid, one residue (THR140) formed hydrogen bond and four residues (GLU188, GLY195, VAL141, MET141) formed hydrophobic interaction (Figure 5).

Figure 5

2D illustration of docking of Acyclovir with GlTIM



DISCUSSION

Giardiasis is a waterborne zoonotic parasitic disease transmitted by the ingestion of contaminated water containing cysts. The disease is more prevalent in developing countries compared to developed ones. Various drugs are used to treat giardiasis, though resistance and side effects have been reported for many of them. In such cases, the search for alternative drugs becomes important. De Novo drug development is a costly and laborious process that requires significant time to bring a drug to market. An alternative is drug repurposing, where existing drugs are tested and analyzed for effectiveness against diseases other than those for which they were originally developed. This study aimed to analyze several FDA-approved drugs and assess their efficacy against GlTIM using computational tools. One hundred and fifty drugs were sourced from the ChemSpider online database and docked with GlTIM. The best interactions were observed with Daptomycin, Vancomycin, Cefazedone, Ticarcillin, and Acyclovir.

Daptomycin is an FDA approved drug from a new class cyclic lipopeptides antibiotic, used in the treatment of systemic and life-threatening infections caused by gram positive bacteria [20]. This drug showed the maximum interactions with the target protein including covalent bonds, hydrogen bonds and hydrophobic interactions (Table 1). Many of the amino acids were active site residues (Figure 2).

Vancomycin is a glycopeptide antibiotic that is used in the treatment of severe infections with pathogens such as *Staphylococcus* and *Streptococcus* species [21]. It inhibits the synthesis of bacterial cell wall leading to osmotic lysis [21, 22]. In our study, Vancomycin showed different kinds of interaction with the target protein. These interactions included covalent, hydrogen bonds and hydrophobic interactions (Table 1). These diverse kinds of interactions might bring enough conformational changes to disturb the normal functions of the protein and disrupt the metabolic need of the *G. lamblia*.

Cefazedone is a cephalosporin antibacterial drug, mostly effective against gram positive bacteria. This drug disrupts the synthesis of the peptidoglycan layer which forms the bacterial cell wall [23]. It is a heterocyclic compound which attracted the medicinal chemists due to their unique chemical properties and wide range of biological activities [23, 24]. Our study indicated that this drug can also be effective against enteric protozoan including *Giardia lamblia* as its docking result showed different kinds of interaction; included hydrophobic interaction, covalent bonds, and hydrogen bonds (Table 1). These interactions can bring conformational changes in the complete chemistry of the glycolytic enzyme triose phosphate isomerase of *G. lamblia* (GlTIM), resulting in the disrupted metabolic activities of the parasite.

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Ticarcillin is a fourth-generation antibiotic that works by preventing the cross-linking of bacterial peptidoglycan during cell wall synthesis. When the bacteria try to divide it exerts bactericidal activity via inhibition of bacterial cell wall synthesis by binding one or more of the penicillin binding proteins (PBPs) [24, 25]. In the current study, the docking of Ticarcillin with GlTIM showed different sorts of interactions (Table 1). These interactions can change the chemistry of protein, and we can assume that this drug may be effective against *Giardiasis*.

Acyclovir is an FDA approved antiviral drug used for inhibition of the growth and spread of the herpes simplex virus [26]. The docking of acyclovir with GlTIM showed varied interactions, including covalent, hydrogen and hydrophobic interactions (Table 1, Figure 5).

All these interactions can possibly bring a lot of conformational changes in *Giardia lamblia* triose phosphate isomerase (GlTIM) chemistry. It can possibly inhibit the metabolic activities of *G. lamblia* and can disturb the normal function of this protein, resulting in the ultimate death of the parasite.

CONCLUSION

This study aimed to identify alternative inhibitors of *Giardia lamblia* triose phosphate isomerase (GlTIM) through in silico drug repurposing. One hundred and forty FDA-approved drugs were retrieved from ChemSpider and docked using PatchDock. Interactions, including hydrogen bonding, covalent bonding, and hydrophobic interactions, were visualized and analyzed using GS Viewer and LIGPLOT+. Daptomycin, Vancomycin, Cefazedone, Ticarcillin, and Acyclovir exhibited the highest interactions with GlTIM. Drug repurposing presents a promising strategy for discovering new therapeutics in a shorter time frame and with fewer resources.

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