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INHIBITION OF *CRYPTOSPORIDIUM PARVUM* PYRUVATE KINASE (CPPYK) THROUGH COMPUTATIONAL DRUG REPURPOSING

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Abstract

Background: *Cryptosporidiosis is a waterborne neglected tropical parasitic disease caused by an apicomplexan parasite, Cryptosporidium parvum. The parasite poses significant therapeutic challenges due to limited treatment options and emerging resistance. So, research into alternative treatments is necessary. Developing new drugs is expensive and time consuming. Drug repurposing is a good alternative, and this study targets the Cryptosporidium parvum's Pyruvate kinase (CpPyk) using this approach. Cryptosporidium parvum uses glycolysis for energy production as it lacks Krebs cycle. CpPyk with its unique structure and function is the key enzyme in the glycolytic pathway and a good target for drug docking.*

Methods: *100 FDA approved drugs were obtained from Chemspider and docked with CpPyk retrieved from Uniprot, using PatchDock online server. Ligand-Protein interactions were visualized using LigPlot+.*

Results: *Top 3 drugs with maximum interactions are ceftaroline, streptomycin and ritonavir. These drugs showed covalent and hydrogen bonds as well as hydrophobic interactions with different amino acids of CpPyk.*

Conclusion: *In this study, the drug repurposing approach was used to inhibit Cryptosporidium parvum pyruvate kinase (CpPyk). Ceftaroline, streptomycin and ritonavir showed different types of molecular interactions with CpPyk which are enough to change its conformation and alter its function which is vital for the parasite. With more in vitro and in vivo testing these drugs can be used as alternative treatment for cryptosporidiosis.*

INTRODUCTION

Waterborne diseases are a major concern globally, and outbreaks caused by contamination of community water systems can cause illnesses in major parts of the population [1]. These outbreaks have economic consequences that extend beyond the health costs to affected individuals, as they damage public trust in the quality of drinking water and in the water industry [2-4]. Water supplies, even in the most developed countries,

can be compromised by protozoan parasites that are resistant to water treatment and are periodically responsible for several outbreaks transmitted by drinking water [5, 6]. The three primary diseases caused by waterborne protozoa are cryptosporidiosis, giardiasis and amoebiasis [2, 4].

Twenty species have been described within the genus *Cryptosporidium*. *Cryptosporidium parvum* is the species associated with human disease, although it can also be found in other hosts, as there is no complete host specificity. It is an intracellular protozoan classified within the Phylum Apicomplexa [7].

The study of the small subunit rRNA genes divides the species of the genus *Cryptosporidium* into two groups: one formed by *C. muris* and *C. serpentis*, and another formed by *C. felis*, *C. meleagridis*, *C. wrairi*, and *C. parvum* [8].

The cycle is completed in a single host in two days. Infection occurs by ingestion of oocysts, originating from environmental fecal contamination or from an infected person or animal. Excystation occurs by contact with reducing agents, generally bile salts or digestive enzymes, although it can occur spontaneously [9, 10]. Four banana-shaped mobile sporozoites appear that invade the wall of the intestinal epithelium. A superficial parasitophorous vacuole is formed, consisting of two membranes from the host and two others from the parasite; this gives it an intracellular, but extracytoplasmic location [7, 10, 11].

The prevalence of Cryptosporidiosis varies depending on the socioeconomic characteristics of the population, as it is more frequent in places with infrastructure problems in drinking water pipes, swimming pools, wastewater disposal, or in close contact with animals [12, 13]. It is found in the feces of 1 to 3% of the inhabitants of developed countries, 5% of those in Asia, and 10% of those in Africa. It is more frequent in children under two years of age. Serological studies show the presence of antibodies in 25-35% of the inhabitants of developed countries and in 60-75% of those in poor countries [1, 12, 14]. In HIV-infected patients with diarrhea, the presence of the parasite is demonstrated in 11-21% of cases, with the percentage being higher in patients from poor countries [11, 12].

Since *Cryptosporidium* only uses glycolysis and lacks the Krebs cycle, its metabolic pathways are drastically limited [10, 15]. This species only possesses enzymes involved in glycolysis, such as pyruvate kinase, and lacks several enzymes involved in other pathways. Because of its distinct structure, participation in glycolytic pathways, and absence of allosteric regulation, the Pyruvate kinase from *Cryptosporidium parvum* (CpPyK) is seen as a possible therapeutic target [16, 17]. Inhibiting this enzyme may restrict the process of energy production and prevent the parasite from growing and proliferation [17].

Discovering or developing a new drug is a long, expensive and a highly regulated process. Each product must not only be safe and effective, but its effectiveness must also be proven in all racial and ethnic groups, as well as across different age groups [18, 19]. The complex path linked to scientific and technological innovations for a new drug takes 2 to 3 years for discovery and validation of the purpose. Considering that approximately 90% of drugs fail during development in phase I clinical trials, this investment is always high risk [19]. The term drug repositioning or drug repurposing is generally applied to the discovery of new activities for a medication already used clinically, that is, new uses and therapeutic applications [20, 21]. This is the process of finding new uses outside the scope of the original medical indication of an approved drug or compound that for some reason had its research discontinued [20, 21].

A repositioned compound with proven bioavailability and a known safety profile can benefit the accelerated R&D process, reduce development costs, decrease the failure rate due to safety, and can contribute to increased productivity by filling gaps in the flowchart [19-21]. This study aims to find alternative drugs for cryptosporidiosis using drug repurposing approach.

METHODS

CpPyK Sequence Retrieval and Processing

The sequence of *Cryptosporidium parvum* pyruvate kinase (CpPyk) was retrieved from UniProt database [22]. The three-dimensional structure of the protein was subsequently retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB), with the Protein Data Bank ID 4DRS [23]. The optimal active site, and the most suitable ligand for the protein were identified Using Discovery Studio [24]. The DS Visualizer tool was used to visualize the 3D structure of the protein [24].

Drugs retrieval and Protein-Ligand Interactions

For the current study, the three-dimensional structures of 100 FDA approved drugs were obtained from Chemspider (http://www.chemspider.com). Protein-ligand interactions were analyzed using the PatchDock online server [25]. The top ten potential complexes were then sorted and further examined individually. The two-dimensional protein-ligand interactions were visualized using LigPlot [25, 26].

RESULTS

In the current study, 100 compounds were screened against the *Cryptosporidium parvum* pyruvate kinase (CpPyk) protein using Patchdock. Three ligands showed the best results.

Docking of Ceftaroline with CpPyk

The docking of ceftaroline with CpPyk revealed several types of interactions, including covalent bonds, hydrogen bonds, and hydrophobic interactions (Table 1). Two key residues, Asn76 and Asn200, formed seven covalent bonds, with Asn76 being the active amino acid. Additionally, two residues formed three hydrogen bonds, with His79 being the active site amino acid. Fourteen hydrophobic interactions were also observed (Figure 1).

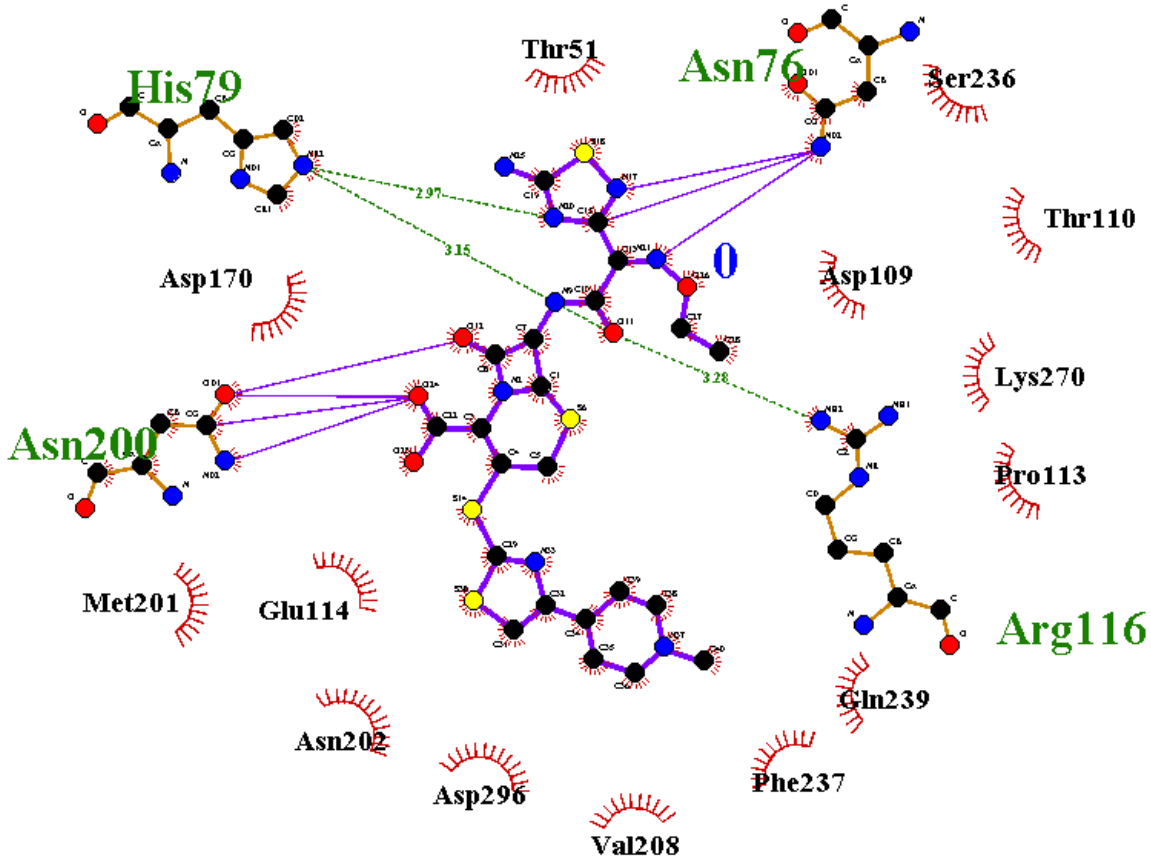

Figure 1: LigPlot+ illustration of ceftaroline interactions with CpPyK.

Docking of Streptomycin with CpPyk

The docking result showed three covalent interactions, four hydrogen interactions and ten hydrophobic interactions (Table 1), in which Asn76 and Asn200 formed three covalent bonds, Arg74, Ser78 and Ser236 formed hydrogen bonds (Figure 2).

Table 1: Post docking results of potential drugs with *Cryptosporidium parvum* pyruvate kinase (CpPyk).

Ligand/Drug	Covalent Interactions	Active site Amino acid	Hydrogen Interactions	Hydrophobic Interactions
Ceftaroline	Asn76, Asn200	Asn76, His79, Lys270	Arg116, His79	Thr51, Ser236, Thr110, Asp109, Lys270, Sp170, Glu114, Asp296, Phe237, Pro113, Gln239, Val208, Met201, and Asn202
Streptomycin	Asn76, Arg74, Lys200, His79	Asn76, Asn200	Arg74, Ser78, Ser236	Lys270, Phe237, Asp109, Glu114, Asp296, Met201, Asn202, Ile115, Arg116, and His79
Ritonavir	His79, Asn76, Arg74	His79, Gly298, Asp70	Asn76	Met299, Arg342, Gly295, Arg74, Ile52, and Ala293

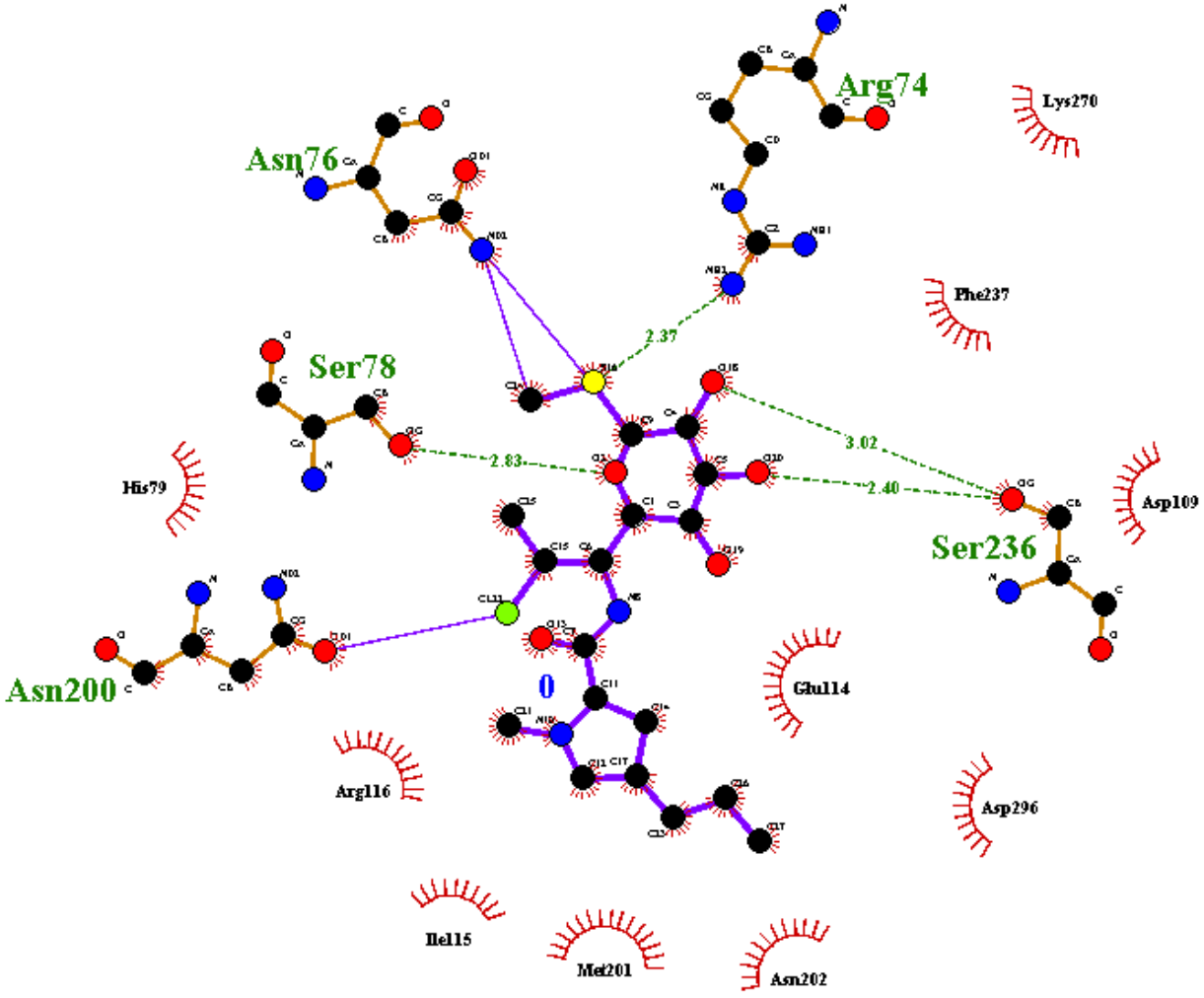

Figure 2: LigPlot+ illustration of streptomycin interactions with CpPyK.

Docking of ritonavir with CpPyK

The Docking analysis showed nineteen different kinds of interactions: including covalent bonds, hydrogen bonds and hydrophobic interactions (Table 1). His79, an active site residue, formed four covalent bonds (Asp170, Gly298). Several amino acid residues showed hydrogen interactions of which Asn76 is an active site residue, and twelve amino acid formed hydrophobic interactions (Figure 3).

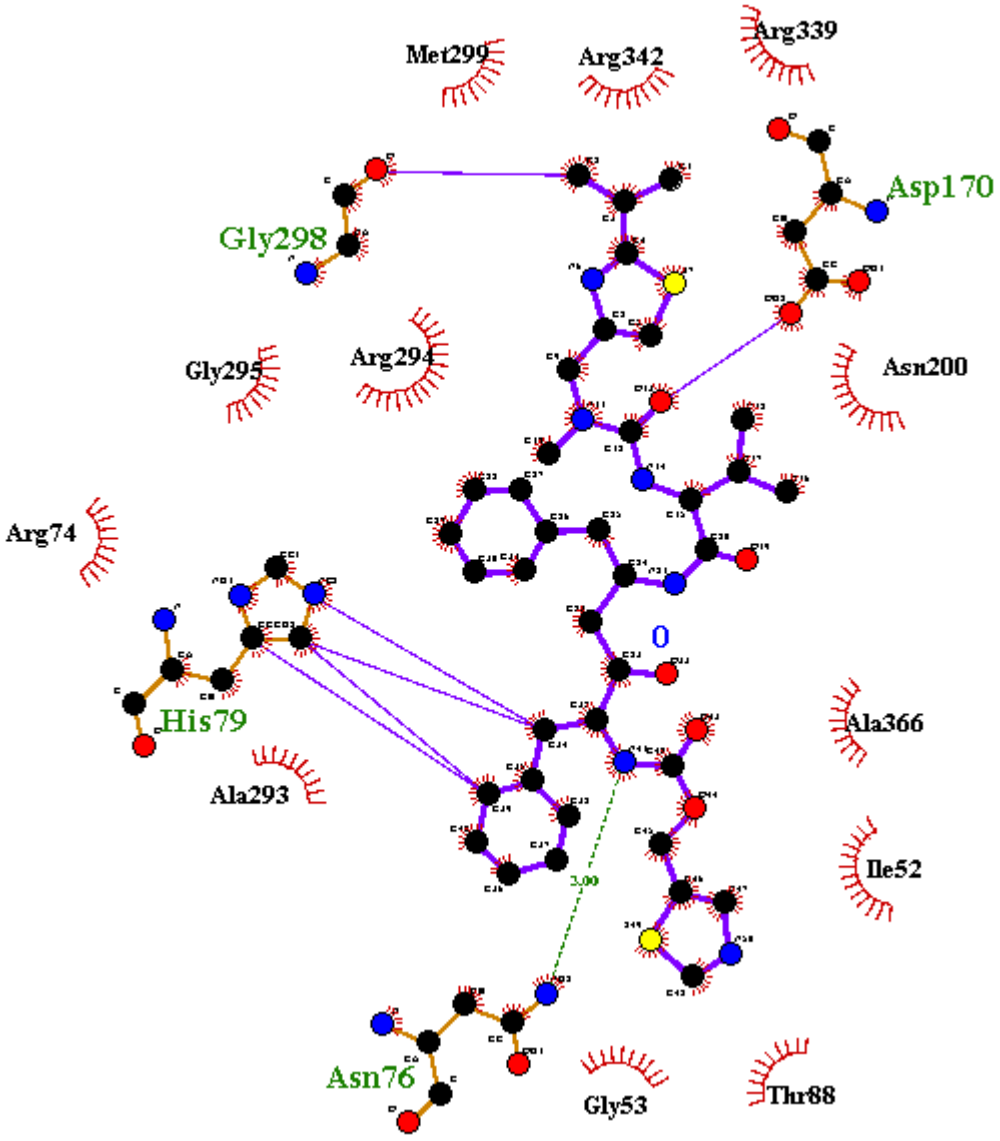

Figure 3: LigPlot+ illustration of ritonavir interactions with CpPyK.

DISCUSSION

Cryptosporidiosis is a growing concern due to its ease of global dissemination, impacting both human and animal health, and leading to significant health, social and economic consequences. Resistance to drugs, in parasitic protozoan is a significant issue, which necessitates the ongoing search for alternative treatments. The current study was conducted to identify alternative FDA approved drugs for treating cryptosporidiosis by targeting *Cryptosporidium parvum* pyruvate kinase (CpPyk) through a drug repurposing approach.

Repositioning drugs, also known as drug reorientation or drug reprofiling, are defined as the identification of new uses for existing drugs [20]. Their study and use have been carried out for several decades, as they reduce the risks and costs of development, as well as failures related to safety. These drugs have well-known formulation development, along with established pharmacokinetic and pharmacodynamic data, shortening the entire process to between three and 12 years [21, 27]. Currently, a wide range of repositioning drugs exist, including

ivermectin, sildenafil, thalidomide, hydroxychloroquine and many more [20, 21].

Cryptosporidium parvum pyruvate kinase (CpPyk) has a unique structure and function. It serves as a key enzyme in the glycolytic pathway of the parasite and thus is critical for energy production [15]. By evaluating 100 FDA approved drugs used to treat various other diseases, we identified ceftaroline, streptomycin and ritonavir as promising candidates, due to their strong molecular interactions with CpPyK.

Ceftaroline is a 5th generation beta-lactam antibiotic. It is bio-converted into ceftaroline in the body and is associated with penicillin-binding proteins (PLP), thus preventing the formation of the cell wall in the bacteria [28, 29]. It has a broad-spectrum activity against Gram-positive and Gram-negative organisms and can also act against methicillin-resistant *Staphylococcus aureus* (MRSA) and resistant strains of *Streptococcus pneumoniae* [28, 30]. The docking results revealed that ceftaroline induced multiple interactions with CpPYk, including covalent, hydrogen and hydrophobic interactions (Table 1 and Figure 1). These interactions can potentially induce irreversible changes in the enzyme CpPyk, suggesting that it can be used as an alternative drug for cryptosporidiosis.

Streptomycin belongs to the group of ATBs called aminoglycosides [31]. Aminoglycosides are very important in veterinary medicine, they are used to treat septicemia of the respiratory, digestive and urinary tracts [32]. Streptomycin is an antibiotic that inhibits protein synthesis. It binds to the 30S ribosomal subunit, which prevents proper reading from mRNA. The result is the formation of a defective protein that is unable to fulfill its function. A resistant cell can enzymatically inactivate streptomycin [31, 33]. Aminoglycosides are modified by enzymes, so their effect on the cell is prevented. The strA gene is typically found on plasmids, the aadA gene is found both on chromosomes and on plasmids. The different localization of these aminoglycoside genes enables the use of different mechanisms for their spread [33]. The docking analysis of streptomycin with CpPyk showed promising interactions, suggesting it could be used to treat cryptosporidiosis. The analysis revealed covalent, hydrogen, and hydrophobic interactions (Table 1 and Figure 2), which are sufficient to induce conformational changes and alter the function of pyruvate kinase.

Ritonavir is a salt of paratoluenesulfonic acid, with wide tissue distribution. It is a viral protease inhibitor with specificity for the HIV-1 virus, frequently used in combined antiretroviral regimens for infected pregnant women [34]. The docking of ritonavir with CpPyk in this study revealed varied interactions (covalent, hydrogen and hydrophobic), which can change the protein's function and suppress its action. In the future, this drug could be used for treating cryptosporidiosis.

CONCLUSION

In this study, we used drug repurposing to inhibit *Cryptosporidium parvum* pyruvate kinase (CpPyk). We screened 100 FDA approved drugs from Chemspider and docked on PatchDock against CpPyk to see which one can bind. The drugs ceftaroline, streptomycin and ritonavir showed good binding to the target, so they can potentially change the normal structure and function of the enzyme and kill the parasite. With more work and testing, these drugs can be used to treat Cryptosporidiosis.

DECLARATIONS:

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CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest.

ETHICAL APPROVAL

Not Applicable

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Not Applicable

AUTHORS' CONTRIBUTION

- AS: Major contribution in writing the manuscript
- MS & SY: Data Collection and analysis
- MS, ZA& BA: Major contribution in writing the manuscript

- HR: Major contribution in writing and proofreading the manuscript

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