DETERMINING POTENTIAL INHIBITOR(S) OF THIOREDOXIN GLUTATHIONE REDUCTASE, KEY ENZYME OF SCHISTOSOMA MANSONI PARASITE

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ABSTRACT: Schistosomiasis is a parasitic worm disease that affects more than 200 million people worldwide, and the drug praziquantel remains the only treatment. Recently there have been reports of patients showing signs of resistance to praziquantel. Consequently, there is an urgent need to develop a new drug to serve as an alternative to praziquantel. In this experiment, we will determine potential inhibitor(s) of Thioredoxin glutathione reductase (TGR), an essential enzyme that is responsible for the parasite's survival. We will use three phases of computational modeling techniques including virtual screening, lead optimization, and down-selection to determine potential drug candidates. As a result, we predict the top hit compounds to have binding affinities of about -10.0 kcal/mol after virtual screening. Furthermore, we expect to improve the binding affinity of the hit compounds to -13.7 kcal/mol after lead optimization. Eventually, we will proceed to the down-selection phase to determine potential drug candidates with highest probability of positive biological processes and negative toxicity levels. These drug candidates could potentially progress into preclinical and clinical development and eventually serve as a marketable drug.

Schistosomiasis (bilharzia or snail fever) is one of the major neglected tropical diseases that affecting populations in developing countries and is caused by worms of genus *Schistosoma*. This disease has infected more than 200 million people worldwide including Africa, South America, and Asia (Hotez, Fenwick, Savioli, & Molyneux, 2009). The majority of the infected patients are women and children living in poor rural areas. According to the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO), the impact of Schistosomiasis causes an estimated 200,000 deaths annually, which is second only to malaria.

The *Schistosoma mansoni* parasite is one of the causal agents of Schistosomiasis. Infected patients with *Schistosoma mansoni* showed signs of severe infection in the intestines resulting in symptoms such as abdominal pain, diarrhea, and bloody stool. Currently, there is no vaccine that can effectively prevent the infections, and praziquantel is the only choice of drug that is effective in treating Schistosomiasis (Pierce, Dubois-Abdesselem, Lancelot, Andrade, & Oliveira, 2012). However, there is evidence that patients can tolerate higher doses of praziquantel (Doenhoff, Kusel, Coles, & Cioli, 2002), suggesting that there is some level of drug resistance to praziquantel. This is an important finding because if the worm parasites develop resistance to praziquantel, those infected with Schistosomiasis may have higher mortality rates. For example, in the 1990s an antibiotic used to treat various bacterial infections, Vancomycin, became an ineffective treatment as result of bacteria developing resistant strains (Gray, Darbyshire, Beath, Kelly, & Mann, 2000). Therefore, there is an urgent need for new drug discovery for schistosomiasis before praziquantel becomes ineffective.

Adult schistosomes reside within the host's blood vessels, feed on the blood, and can reproduce for up to 30 years (Gryseels, Polman, Clerinx, & Kestens, 2006). Within the human host, the parasite produces antioxidants to protect itself from reactive oxygen species produced by the host's immune system. Previous studies

found that thioredoxin (Trx) and glutathione (GSH) systems play important roles in detoxify reactive oxygen species, cell proliferation, redox regulation of gene expression (Kuntz et al., 2007). In the two systems, the Glutathione reductase (GR) enzyme reduces glutathione disulfide (GSSG) to glutathione (GSH) and runs the GSH-dependent systems (Townsend, Tew, & Tapiero, 2003). Likewise, the thioredoxin reductase (TrxR) enzyme converts oxidized thioredoxin (Trx-S2) to reduced thioredoxin (Trx-(SH)2) (Gromer, Urig, & Becker, 2004). A study on the disulfide redox pathways of *Schistosoma mansoni* identified thioredoxin glutathione reductase (TGR) as a multifunctional enzyme that functions as both TrxR and GR enzymes (Alger & Williams, 2002). In other words, TGR serves as an essential enzyme for the parasite's survival and could be a valuable drug target (Kuntz et al., 2007).

Several strategies have used TGR as a drug target and attempted to determine the inhibitors. One study used RNA interference, a process where RNA molecules inhibit gene expression to identified TGR in *Schistosoma mansoni* and found that auranofin is an effective inhibitor of pure TGR that was able to partially cure infected mice (Kuntz et al., 2007). Similarly, another study used gene cloning to identify the presence of TGR in *Schistosoma japonicum*, and the results agreed with the previous findings for *Schistosoma mansoni* (Song et al., 2012). These studies show the important roles of TGR inhibition in curing mice infected with Schistosomiasis. However, an inhibitor that is effective in targeting TGR and is able to function in the human body has yet to be defined. Therefore, we will use molecular modeling techniques to predict the potential inhibitors of TGR with the highest binding affinity and lowest predicted toxicity. Essentially, the inhibitors will serve as drug candidates for drug development to provide an alternative to praziquantel.

Method

We will determine a potential inhibitor(s) of TGR, an enzyme in the redox system that is essential for the survival of *Schistosoma mansoni*. We will use three phases of computational modeling techniques including structure-based virtual screening, lead optimization, and down selection to filter a large database of compounds into drug candidates. Ultimately, we will generate a list of drug candidates with the highest binding affinity for the enzyme and lowest potential side effects.

Virtual Screening

Structure-based virtual screening is a modeling technique used in the early stage of drug discovery to identify series of potential hit compounds that are likely bind to a drug target (Peter Anderson, personal communication, November 20, 2015). The benefits of this technique are the low cost compared to high-throughput screening experiments, as well as the ability to bind multiple substrates and narrow down a large dataset of compounds into several lead compounds.

As demonstrated in figure 1, we will use a virtual screening process called molecular docking. This process is based on a lock and key principle, where a specific enzyme could only bind to a specific substrate. Molecular docking has three main components, identifying a protein, using algorithms to screen, and scoring a function. First, we will obtain an x-ray crystal structure of TGR in PDB format from www.uniprot.org (Figure 3). Second, the TGR file will be submitted on an idock server at http://istar.cse.cuhk.edu.hk/idock/ in a queue that will take a few weeks to complete.

The idock server will use algorithms to screen millions of substrates for the best orientation and conformations that fit the FAD binding site of TGR (Li, Leung, & Wong, 2012). Third, the idock server will generate the binding affinity of the hit compounds in a form of docking score or rank from highest to lowest negative free energy of binding (Huang, Hua, Li, & Hua,

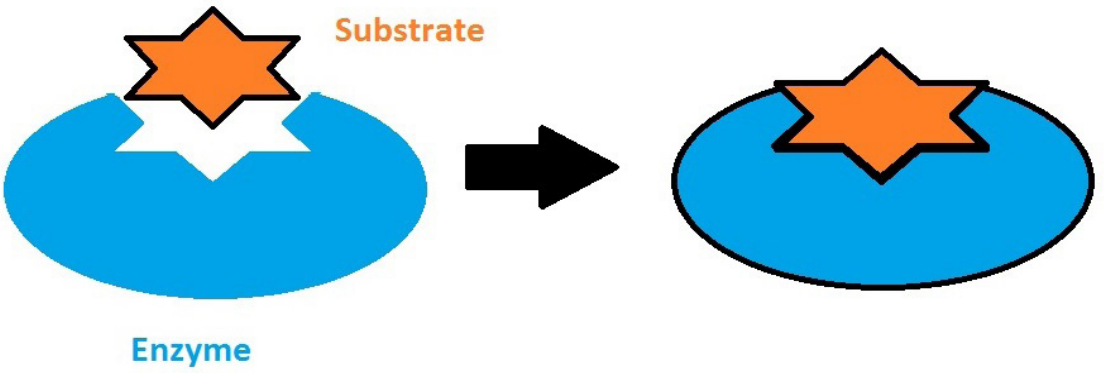

Figure 1. Illustration of molecular docking where the substrate is docked onto the active site of the enzyme.

$$dG_{bind} = k_{hb}\sum_{hb} f_{hb} + k_{ml}\sum_{ml} f_{ml} + k_{hh}\sum_{hh} f_{hh} + k_{hp}\sum_{hp} f_{hp} + k_{aa}\sum_{aa} f_{aa}$$

k: constant
f: count of atomic contact

hb: H-bond donor – H-bond acceptor
ml: ionic metal - ligand
hh: hydrophobic atom – hydrophobic atoms
hp: hydrophobic atom – polar atom
aa: any atom - any atom

Figure 2. Free energy of binding equation used to predict docking scores by adding all different intermolecular forces including hydrogen bonds, ionic bonds, and hydrophobic interactions (Peter Anderson, personal communication, November 27, 2015).

2015). The docking score is calculated based on evaluation of intermolecular forces between the enzyme and substrate (Figure 2). As a result, this phase will generate a list of the top 1000 hit compounds with the highest binding affinity (Table 1).

Lead optimization is a process used to enhance the binding affinity of the top hit compounds obtained from molecular docking into promising lead compounds. We will use a method called lead hopping to modify some of the functional groups of a hit compound by replacing with other functional groups. This technique will be performed on the AUTO_PFVS server and will take a few weeks to complete. AUTO_PFVS server required a protein-ligand complex structure in PDB format to perform CORE_GEN and CAND_GEN modules.

CORE_GEN is a tool that break down a ligand structure to fragments called pharmacophores

ZINC ID	iDock Score (kcal/mol)
9413973	-12.45
9518503	-12.283
9414156	-12.149
9518522	-12.044
44441691	-12.015
8845186	-11.965
72332034	-11.907
38632282	-11.834
14359061	-11.766
9413721	-11.758
13351455	-11.74
27664591	-11.723
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Table 1. Example list of the top hit molecules generated from idock ranked from high to low iDock score.

and determine the binding affinities (Hao, el. al., 2012). Subsequently, a pharmacophore with the best binding affinity will serve as a core structure for generating new compounds by linking different functional groups using CAND_GEN module (Kolb and Caflisch, 2006). At the end of this phase, we will generate a list of promising lead compounds with improved binding affinities.

One of the processes of down selection is absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction, where lead compounds are filtered into drug candidates. The main purpose of this stage is to reduce costly late-stage failures in drug development by predicting potential drug side effects (Van De Waterbeemd & Gifford, 2003). ADMET prediction will be performed on admetSAR, an open source database that provide searches for ADMET properties. In admetSAR, 22 high-accuracy qualitative classification models were implemented to generate probability of ADMET properties and regression models including water solubility, permeability, and toxicity (Cheng et al., 2012). ADMET properties will predict the probability of how well the lead compounds function in biological processes such as bioavailability, intestinal absorption, permeability, and toxicity (figure 3). As a result, ADMET prediction will generate a list of drug candidates with the highest probability of positive biological processes and negative toxicity levels. For a detailed timeline, refer to appendix A.

Summary

Schistosomiasis or snail fever is a parasitic worm disease that could cause severe infection in urinary tract or intestines. Several studies had identified the TGR enzyme as an essential target for drug development (Alger & Williams, 2002; Kuntz et al., 2007; Song et al., 2012). These findings are important to our research, because

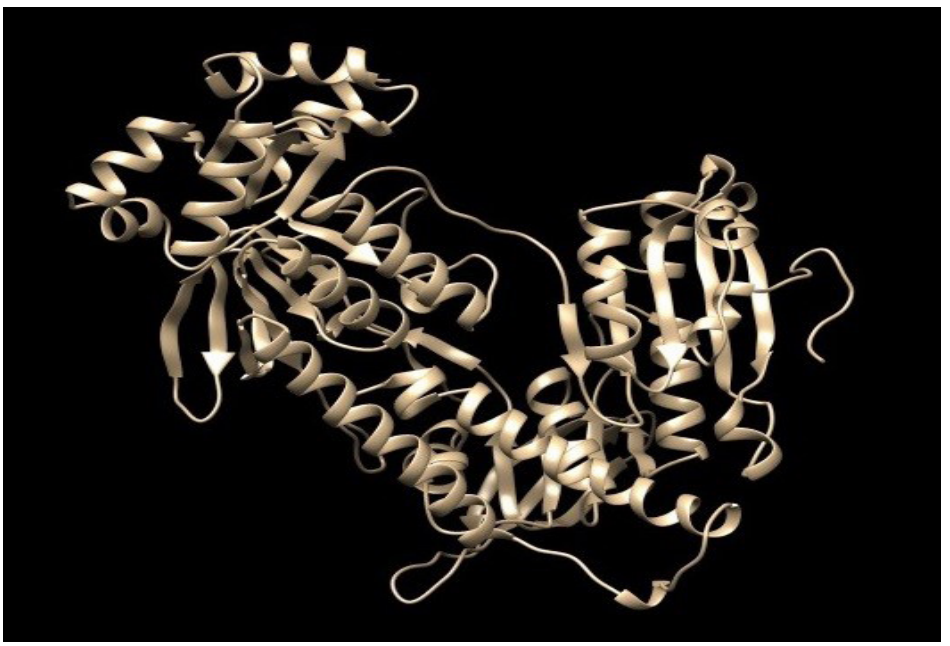

Figure 3. X-ray crystal structure of TGR obtained from Uniprot protein data bank (http://www.uniprot.org).

praziquantel remains the only drug that is effective in treating schistosomiasis. However, due to increasing drug resistance, there is an urgent need to find a new drug that could replace praziquantel. Therefore, we will use molecular modeling techniques to predict potential drug candidates. These newly identified drug candidates could be tested in clinical trials and eventually be offered as an alternative drug to praziquantel.

Our techniques will filter down a big library of biomolecules through a three phase process into a couple of biomolecules with best scores. The score tells us how well the biomolecules fit the drug target to make the parasite vulnerable and how well the biomolecules function in our body with the fewest side effects. Thus, the score will help us select only biomolecules with the highest potential to be drug candidates. Ultimately, the success of this experiment will be generating drug candidates that have the best fit and lowest toxicity levels.

Table 2. Example list of pharmacophores generated from CORE_GEN module.

Ligand	dH (kcal/mol)	-TdS (kcal/mol)	dG (kcal/mol)
Original	-43	11.514	-31
pharmacophore 1	-30	11.525	-19
pharmacophore 2	-35	12.041	-23
pharmacophore 3	-23	10.282	-13
pharmacophore 4	-40	11.884	-28
pharmacophore 5	-40	11.897	-28
pharmacophore 6	-40	11.851	-28

Table 3. Example list of newly generated compounds from CAND_GEN module.

Ligand	dH (kcal/mol)	-TdS (kcal/mol)	dG (kcal/mol)
1501	-63	11.7	-15
982	-62	13.0	-1.5
504	-65	17.0	-2.6
237	-60	11.6	-8.8
964	-60	13.1	-6.9
1761	-59	12.4	-3.2

Appendix A

Timeline

Week	Goals	Result
1-4	• Look at 3D structure of drug target and identify the active site using Chimera. • Structure-Based Virtual Screening: Perform molecular docking using iDock program • Hit to Lead	• Obtain docking scores for a set chemical structures. • Identifies the top hits with highest ΔG of binding
5-18	Lead optimization: • Perform Lead hopping to improve the binding of top hits.	• Sort and narrow down the top hits
19-20	Down selection: • Use ADMET prediction	• Sort and narrow down potential lead compounds

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