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ORIGINAL ARTICLE

In silico REPOSITIONING OF NEW DRUGS AGAINST *Schistosoma mansoni*

José Clecildo Barreto Bezerra[1], Morgana Elias Arantes[1], Carolina Horta Andrade[2], Lourival Almeida Silva [3] and Bruno Junior Neves[4]

ABSTRACT

Schistosomiasis is a neglected tropical disease caused by parasites of the genus *Schistosoma*. In Brazil only *Schistosoma mansoni* causes this disease. The World Health Organization estimated in 2012 approximately 249 million people at risk of acquiring this disease around the world. The main strategy to control this disease is praziquantel treatment of individuals living in endemic areas. The drug praziquantel is used on a large scale in the treatment of schistosomiasis and currently there are reported cases of resistance, indicating the need to discover new drugs. *In silico* drug repositioning is a time and cost reducing strategy in the search for anti-*Schistosoma* agents. This work used bioinformatic tools to identify potential schistosomicidal drugs. A list was compiled of *S. mansoni* potential targets that are part of essential processes in the database TDR and the targets that are part of the tegument were obtained in the scientific literature. The file with *S. mansoni* targets contained 1,376 targets, and of these only 61 targets associated with 399 drugs had homology with drug targets. After removal of duplicate drugs, drugs found in previous studies and after the analysis of the conservation of the binding site, only 28 *S. mansoni* targets associated with 102 drugs had 60% or more of the active site conserved. Some of the drugs had activity and are interesting to validate this study such as: artemether, lumefantrine, meloxicam. Among the drugs found 18 drugs were selected to be tested in prospective experimental assays according to the following criteria: low toxicity in vivo, off-patent status, and logP <5.0.

KEY WORDS: Control; *Schistosoma mansoni*; drug repositioning; chemogenomics.

INTRODUCTION

Schistosomiasis is considered a neglected tropical disease (NTD) caused by trematodes of the genus *Schistosoma* sp., there are six species that infect humans, and three broadly distributed species (*S. mansoni, S.*

1. Laboratory of Studies of the Host-parasite Relationship, Institute of Tropical Pathology and Public Health, Federal University of Goiás, Goiânia, Brazil.
2. Laboratory of Molecular Modeling and Drug Design, Faculty of Pharmacy, Federal University of Goiás, Goiânia, GO 74605-170, Brazil.
3. Federal Institute of Education, Science and Technology of Goiânia, Ceres, GO, Brazil.
4. Laboratory of Cheminformatics, University Center of Anápolis, UniEVANGÉLICA, Anápolis, Brazil.

Corresponding author: J. C. B. Bezerra, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Rua 235 s/n, Setor Universitário, CEP: 74605-050, Goiânia, Goiás, Brazil. E-mail: clecildobarreto@gmail.com

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haematobium, and *S. japonicum*) that affect around 240 million people in 78 countries (Gryseels, 2012; Weerakoon et al., 2015). In Brazil, schistosomiasis is caused by *S. mansoni*, and it is estimated that between 2.5 and 8 million people are infected in 19 states and the Federal District (Martins-Melo et al., 2014). This disease can be controlled by several methods, for example: water treatment, basic sanitation, use of molluscicides like niclosamide, and preventive treatment with praziquantel (Dai et al., 2010; Shi et al., 2015; Molehin et al., 2016). In the past, some other drugs were used in the treatment of schistosomiasis, but they proved to be of low efficiency or toxic (Thétiot-Laurent et al., 2013). These drugs are: antimonial tartarate, emetine, niridazole, and oltipraz. Since then praziquantel has been the best choice in the treatment of schistosomiasis. Praziquantel is highly effective and safe, with very few adverse reaction, and is active against five species that cause schistosomiasis (Cioli et al., 2014).

The mechanism of action of this drug has not been completely elucidated, but it is believed that this drug acts on the calcium channels, causing an increase in motor activity, muscular contractions, and formation of vesicles in the tegument (Doenhoff et al., 2008). Some of the disadvantages of this drug are: lack of efficiency in juvenile worms, and considerable adverse effects (Doenhoff et al., 2002; Shen et al., 2007). A selection of resistance *in vivo* study indicated that parasites can become resistant to this drug, also there are studies that indicate a reduction in sensibility of the parasite to this drug in endemic areas (Fallon & Doenhoff, 1994; Cioli et al., 2014).

The discovery of new drugs is a long (10 to 15 years) and expensive process (1.5 billion) due to the phases of the process (Tamimi & Ellis, 2009). *In silico* repositioning of drugs is an alternative strategy to the traditional drug development process, this strategy involves finding new therapeutic uses for the drugs already being used on humans (Ashburn & Thor, 2004). The main advantage of this process is a reduction in the time and cost of the process. Some drugs were successfully repositioned: sildenafil, thalidomide, and minoxidil (Wu et al., 2013).

Here, we repurposed new drugs to treat schistosomiasis based on the underlying assumption that proteins sharing sufficient similarity present greater probability of sharing the same ligands (Andrade et al., 2018). Therefore computational tools have been used to screen drug databases for drug target homologs to *S. mansoni* targets, thereby identifying potential new active drugs. The methodology used in this work was based on a previous study on repositioning (Neves et al. 2015).

METHODS

Compilation of a list with S. mansoni *targets*

A list was compiled with potentially new *S. mansoni* therapeutic targets that were part of the following essential routes found in the Therapeutic Drug Research (TDR) database: growth defect, larval/adult lethal arrest, morphology defect, and energy metabolism. The *S. mansoni* targets that were part of the tegument were obtained in scientific articles using the following key terms: "proteome" OR/AND "Schistosoma mansoni" OR/AND "tegument". The information on the *S. mansoni* targets and the amino acid sequence in the FASTA format were obtained in the Gene DB database.

Identification of drug targets homologous to S. mansoni *targets*

The following databases, Therapeutic Target Database and Drugbank, containing drugs and their corresponding targets, were screened with the list of *S. mansoni* targets using the sequence similarity search technique. Only the drug targets that had an E-value equal to or inferior to 10^{-20} were considered, also nutraceutical and antibodies were not included in this study. In addition, only drug targets of drugs that were approved or in clinical phases II and III were considered in this study. All the repeated targets and targets described in another study were excluded (Neves et al., 2015).

Confirmation of homology and comparison of the functional region of the targets

The homology between the targets was confirmed using the BLAST tool in the NCBI database. The next step was the analyses of the degree of conservation of the active site between the targets. The Consurf server determined the functional amino acid in the drug target and through manual comparison, the degree of conservation of the active site of the *S. mansoni* target was determined. The results were considered satisfactory when the degree of conservation of the active site was equal to or superior to 60%.

Selection of the drugs to test in assays in vitro

Some of the drugs found showed to be active against *S. mansoni* validating this study. After the removal of the drugs with proven activity the following criteria were used to select the drugs to be tested: patent expired, low toxicity *in vivo* and a good value of log P for an oral drug. Drug patent were verified in the following databases: Google patent, WIPO and Scifinder. The log P value was obtained in Drugbank and the toxicity *in vivo* values in the scientific literature.

RESULTS AND DISCUSSION

Here, we developed a computational chemogenomics framework (Figure 1) and used it in the repurposing of new drugs for treat schistosomiasis.

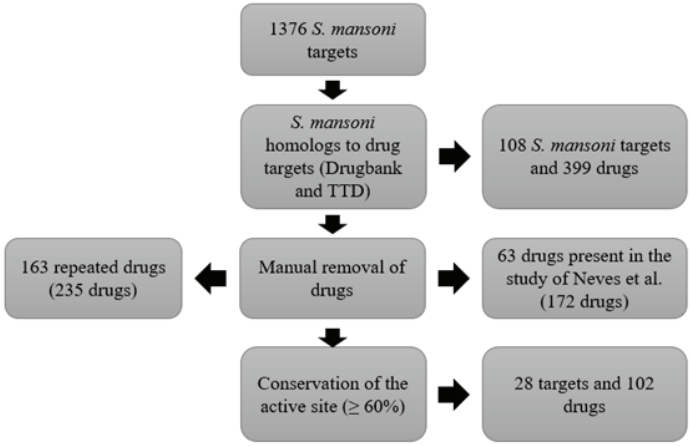

Figure 1. Flowchart summarizing the search for drug targets homologs to *S. mansoni* targets and conservation of the active site.

TDR database and scientific literature provided 1,376 potential new therapeutic targets, whereas the search in the TTD and Drugbank databases produced only 108 *S. mansoni* targets associated to 399 drugs. After removal of duplicated records 235 drugs remained in our study (Figure 2).

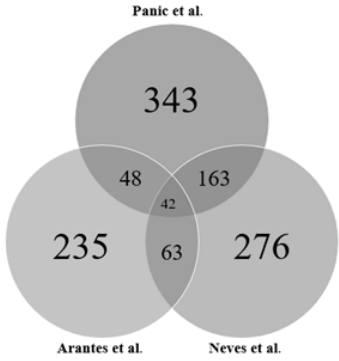

Figure 2. This graph shows the drugs found to be potentially active in *S. mansoni* in the adult and young adult in this study and in repositioning studies (Neves et al., 2015; Panic et al., 2015).

The 235 predicted drugs were then compared to other experimental (Abdulla et al., 2009) and computational (Neves et al., 2015) anti-schistosomal screens. See Venn diagram in Figure 2. Some of the drugs found in all three studies are: vinblastine, alendronate, cisplatin mefloquine, and others. After the removal of the 63 drugs found in the Neves and collaborators study, remained 172 drugs. Of these 172 drugs only 102 drugs associated with 28 targets were found to have 60% or more of the conserved functional regions (i.e., binding sites and motifs). An example of the analyses of the active site is in Figure 3. The 28 targets found most were mostly part of the morphology or the tegument of the parasite. This result is interesting, since the standard antischistosomal drug praziquantel act on the tegument and morphology (Doenhoff et al., 2008). Some of the targets found were: DNA topoisomerase II, calmodulin, and amino acid transporters. A total of 9 druggable targets associated with 11 predicted drugs whose activity has been previously evaluated against *Schistosoma* were identified, such as artemeter, rosuvastatin, lucanthone, and cyclosporine.

Consequently, we predicted 102 drugs to be active against 28 druggable targets that have not yet been experimentally tested against schistosomes or that have not yet undergone further studies. Subsequently, 18 drugs were prioritized for experimental validation as follows: low cost, logP <5.0, and low toxicity *in vivo* (Table). Another important aspect considered in this study is intellectual property protection of the potential schistosomicidal drugs predicted by the proposed strategy. All of the prioritized drugs are off-patents. Therefore, we consider that all prioritized drugs (see Table). identified in this study are attractive for further analysis. For instance, fluorquinolones, a class of antimicrobials used in the treatment of infections caused by gram-negative bacteria may act in *Schistosoma* by inhibiting DNA topoisomerase II, an enzyme involved in DNA synthesis.

Therefore, all prioritized chemicals are viable for drug repositioning and might be used as starting points for further *in vitro* and *in vivo* studies and schistosomicidal drug design since they present privileged structures and have established pharmacokinetic and toxicity profiles. If promising activities are discovered, they could constitute important starting points for lead identification and optimization.

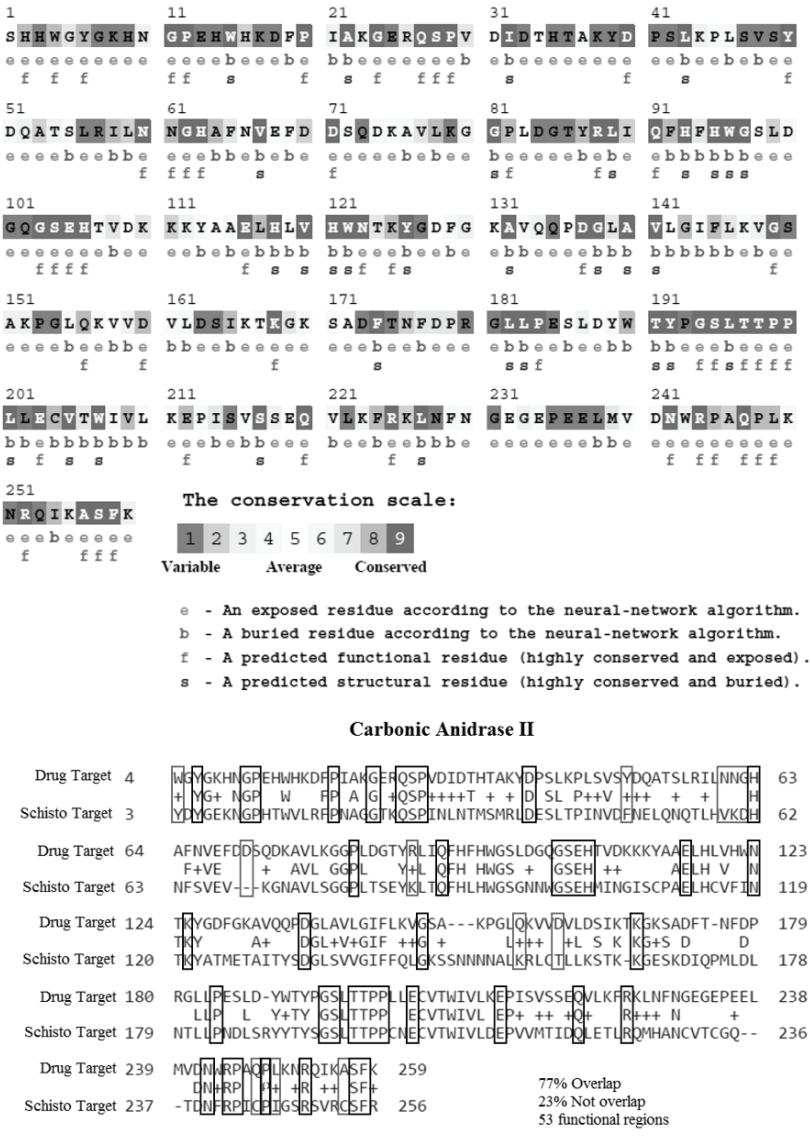

Figure 3. Example of the conservation of the active site of carbonic anidrase II. The Consurf shows the functional amino acids of carbonic anidrase II of *Homo sapiens*. The comparison between the drug target and the *S. mansoni* target shows that 77% of the active site is conserved and 23% is not conserved.

Table. Examples of potential schistosomicidal drugs and their potential targets revealed in this study.

Predicted *S. mansoni* target	Drug	LogP	Oral acute toxicity (LD_{50})
6-phosphogluconate dehydrogenase	Ketotifen	2.2	179 mg/kg
Aldo-keto reductase	Doxorubicin	1.27	570mg/kg
Amino acid transporter	Levothyroxine	4	10000 mg/kg
Calmodulin	Fenoxibenzamina	4.7	900 mg/kg
Carbonic anhydrase II	Furosemide	2.03	308 mg/kg
Cationic amino acid transporter	Sulfasalazine	2.5	12500 mg/kg
DNA topoisomerase II	Ciprofloxacin	0.28	5000 mg/kg
DNA topoisomerase II	Pefloxacin	0.27	4000mg/kg
DNA topoisomerase II	Lomefloxacin	-0.3	4000mg/kg
DNA topoisomerase II	Norfloxacin	-1.03	4000mg/kg
DNA topoisomerase II	Sparfloxacin	2.5	2000mg/kg
DNA topoisomerase II	Levofloxacin	2.1	1803 mg/kg
DNA topoisomerase II	Novobiocin	4.1	1500 mg/kg
DNA topoisomerase II	Moxifloxacin	2.9	300 mg/kg
DNA topoisomerase II	Gatifloxacin	2.6	300 mg/kg
DNA topoisomerase II	Etoposide	0.6	215 mg/kg
DNA topoisomerase II	Teniposide	1.24	29570 ug/kg
Fosfodiesterase-5	Sildenafila	1.9	1000 mg/kg
Fosfodiesterase-5	Vardenafila	1.4	1000 mg/kg
Mannose-6-phosphate isomerase	Sulfanilamide	-0.62	3000mg/kg
Tubulin	2-Methoxyestradiol	3.7	3450 mg/kg

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