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RESEARCH ARTICLE

The 3D pharmacophore modeling to explore new antischistosomal agents among US FDA approved drugs

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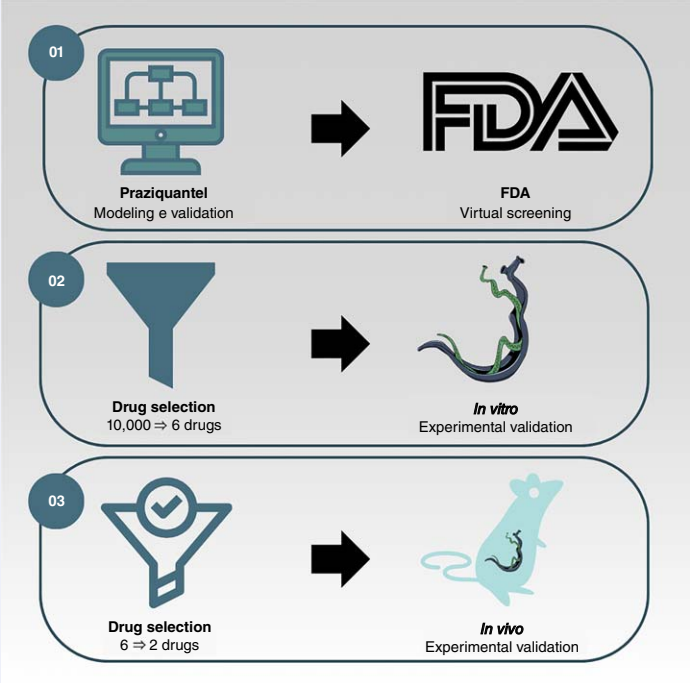
ABSTRACT

Aim: To identify potential antischistosomal agents through 3D pharmacophore-based virtual screening of US FDA approved drugs. **Materials & methods:** A comprehensive virtual screening was conducted on a dataset of 10,000 FDA approved drugs, employing praziquantel as a template. Promising candidates were selected and assessed for their impact on *Schistosoma mansoni* viability *in vitro* and *in vivo* using *S. mansoni* infected mice. **Results & conclusion:** Among the selected drugs, betamethasone and doxazosin demonstrated *in vitro* efficacy, with effective concentration 50% (EC_{50}) values ranging from 35 to 60 μM. *In vivo* studies revealed significant (>50%) reductions in worm burden for both drugs. These findings suggest that betamethasone and doxazosin hold promise for repurposing in treating schistosomiasis. Additionally, the study showcases a useful approach for identifying new antischistosomal drugs.

TWEETABLE ABSTRACT

Discovering new treatments for #schistosomiasis is crucial. Our study used virtual screening to identify potential antischistosomal drugs from US FDA approved compounds. Promising results *in vitro* and *in vivo*. #drugdiscovery #tropicaldiseases

GRAPHICAL ABSTRACT



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KEYWORDS

Antiparasitic compounds; betamethasone; computational chemistry and molecular modelling; drug repositioning; helminthiasis; medicinal chemistry; praziquantel; schistosomiasis; schistosomicidal; virtual screening

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1. Background

Schistosomiasis, caused by the parasitic genus *Schistosoma*, is a debilitating global health issue with substantial morbidity and a profound impact on impoverished regions [1]. This chronic disease, prevalent in tropical and subtropical areas, imposes additional strains on already compromised healthcare systems. Approximately 250 million people suffer from schistosomiasis, with about 10% of the global population at risk of infection [2]. Among the three major human *Schistosoma* species, *Schistosoma mansoni* is particularly responsible for public health challenges in Africa, the Middle East, the Caribbean and South America [2].

Due to the absence of an effective vaccine, the WHO primarily relies on preventive chemotherapy with praziquantel (PZQ) to combat schistosomiasis. In 2021 alone, an estimated 251.4 million individuals required preventive treatment [2]. While control efforts have been successful in many regions, persistent hot spots, and reports of reduced PZQ efficacy indicate existing challenges [3,4]. The WHO's revised roadmap for neglected tropical diseases aims to eliminate schistosomiasis as a public health problem globally by 2030 [5]. However, concerns about the potential selection of parasites for PZQ resistance or low sensitivity underscore the need for the development of safe, affordable and effective drugs for schistosomiasis.

An effective strategy in the pursuit of antiparasitic drugs is drug repurposing [6]. This approach involves identifying alternative applications for approved or investigational drugs beyond their original medical indications [7,8]. Recognizing the potential of drug repurposing, several research groups, including ours, are exploring this strategy to address drug shortages and discover new antischistosomal agents [9,10]. Recent studies employing phenotypic screening have demonstrated the antischistosomal properties of US FDA approved drugs from various therapeutic classes. These include, but are not limited to, anti-inflammatories [11], antihypertensives [12,13], diuretics [14], antihistamines [15,16], antibiotics [17] and antiprotozoal drugs [18].

In alignment with these successes and using PZQ as a template molecule, this study applied a 3D pharmacophore-based virtual screening strategy to identify potential drug repositioning candidates for schistosomiasis. Selected compounds underwent *in vitro* testing against *S. mansoni ex vivo*, and the most promising ones were further evaluated for their ability to reduce parasite burden *in vivo*. Notably, betamethasone and doxazosin demonstrated relevant results in mouse models of schistosomiasis, suggesting their suitability for further exploration in drug repositioning efforts against schistosomiasis.

2. Materials & methods

2.1. Drugs & reagents

All materials and reagents were sourced from commercial supplies and used without further purification. Roswell Park Memorial Institute 1640 culture medium, heat-inactivated calf serum, penicillin G (purity >98%) and streptomycin sulfate (purity >98%) were obtained from Vitrocell (Campinas, SP, Brazil). HEPES buffer and dimethyl sulfoxide (DMSO) were acquired from Sigma (Sigma-Aldrich, St. Louis, MO, USA). The FDA approved drugs betamethasone valerate (purity >98%), chlormadinone acetate (purity >98%), doxazosin mesylate (purity >97%), griseofulvin (purity >97%), losartan potassium (purity >98%) and pravastatin sodium (purity >98%) were purchased from Sigma-Aldrich. PZQ (purity >98%) was kindly provided by Ecovet Industria Veterinaria Ltda (Sao Paulo, Brazil).

2.2. Animals & parasite

The life cycle of *S. mansoni* (BH strain) was maintained at the Research Center on Neglected Diseases (Guarulhos, SP, Brazil), using *Biomphalaria glabrata* snails and Swiss mice as intermediate and definitive hosts, respectively. All animals were housed under environmentally controlled conditions (25°C, 70% humidity) with access to water and food *ad libitum*.

2.3. Dataset & pharmacophore modeling

The FDA approved drugs subset (via DSSTOX) was downloaded from the ZINC Database [19]. This collection, providing the lowest energy conformers for each molecule, was selected because of the availability of toxicity data, information on side effects and cross-interactions, as well as pharmacodynamics and pharmacokinetics data. This subset includes 1217 compounds with molecular weights between 150 and 500 Da; XlogP below five; seven or fewer rotatable bonds; five or fewer donor groups, and ten or fewer acceptor groups. Initial conformers were generated by the molecular modeling platform SYBYL-X 2.0 (Tripos, St. Louis, USA) [20,21]. Using PZQ as a template, the UNITY module of SYBYL-X 2.0 was applied to define a series of filters consisting of the preselection of specific features, such as hydrogen-bond donors or acceptors, positively or negatively charged functionalities, and hydrophobic and aromatic groups.

The three-dimensional structures of PZQ and FDA approved drugs were built using the Tripos force field and standard atomic and geometric parameters validated in SYBYL-X 2.0. The Powell conjugate gradient method was used to energetically minimize the 3D conformation of each compound, applying a minimum energy step

of 0.005 kcal/mol Å [20,21]. Gasteiger–Hückel charges were generated via a distance-dependent function and a dielectric constant value corresponding to water (i.e., 80). Next, sets of features were specified to define the pharmacophore models: hydrogen-bond donors and acceptors, hydrophobic, aromatic, positive and negative sites. Each database compound was aligned to the pharmacophore models, allowing full compound flexibility as implemented in the flexible alignment mode of the GALAHAD module of SYBYL-X 2.0. The population size was set to 100, and the maximum number of generations was defined as 60. Advanced parameters were set as follows: mutation rate = 0.4, mutation decay = 1.0, query penalty = 0.1 and crossover rate = 1.0. Elitism was selected as the genetic algorithm flag to ensure the propagation of the best models, with tuplet types kept in the default mode (quartets). The database conformers that best aligned to the pharmacophore models were scored using the QFIT scoring function, ranging from 0 to 100. The compounds with the highest QFIT values were acquired and tested *in vitro*.

2.4. In vitro antiparasitic assays

The antischistosomal assay was performed according to the methodology previously described [22,23]. Adult *S. mansoni* were collected from mice by dissection at 42 days postinfection and were maintained in Roswell Park Memorial Institute 1640 culture medium supplemented with 5% fetal calf serum, 100 U/ml penicillin and 100 mg/ml streptomycin at 37°C and 5% CO_2. Compounds were dissolved in DMSO at a final concentration of 0.5% v/v and tested at 50 μM in 24-well plates (Corning, New York, NY, USA), with one worm of each sex added per well. Each concentration was tested at least in triplicate, and independent experiments were repeated three-times. Parasites incubated with drug-free DMSO (0.5%) served as a negative control, whereas PZQ at 1 μM was used as a positive control. The viability of adult schistosomes was assessed via microscopic readout at 1, 24, 48 and 72 h using a Motic AE2000 inverted microscope (Vancouver, Canada) equipped with a Motic ultrahigh definition camera and with a 48 inch 4K ultrahigh definition monitor system (LG Electronics, Taubaté, SP, Brazil) [24]. The death of adult schistosomes was defined as the lack of movement observed for at least 1 minute of examination, whereas parasites with any body movement were considered viable [25]. For the determination of the effective concentration 50% (EC_{50}) values, compounds were tested using 1:2 serial dilutions from 100 to 6.25 μM or 1 to 0.25 μM for PZQ.

2.5. In vivo assays

Drugs revealing *in vitro* activity on adult schistosomes after 72 h were tested *in vivo*. *In vivo* studies in *S. mansoni* infected mice were performed according to standard protocols [14,26]. Rodents (3 weeks old) were infected subcutaneously with 80 *S. mansoni* cercariae each, and animals were randomly divided into experimental groups (five mice per group). The drugs were suspended in 5% ethanol (v/v), and on day 42 post infection, animals were orally treated with single doses of 100, 200 or 400 mg/kg of body weight. Five untreated mice served as controls. For comparison, a corresponding amount of vehicle (5% ethanol) were administered to groups of five schistosome-infected animals in the same period. Fifteen days post-treatment, animals were euthanized with CO_2, dissected, and then, worms were sexed and counted. Worm burdens of treated mice were compared with untreated animals, and reductions of worm burden were calculated. These *in vivo* experiments were repeated, and the results were shown by adding the two experiments.

2.6. Statistical analysis

Statistical analyses were performed with GraphPad Prism software (version 8.0; CA, USA). The EC_{50} values were calculated using sigmoid dose-response curves with a 95% confidence interval. The nonparametric Kruskal–Wallis test was used to compare the medians of the responses between the treatment and control groups, and differences were considered statistically significant at $p < 0.05$ [16].

3. Results

The main steps of this study are illustrated in Figure 1.

3.1. Models & molecules

The database was prepared using the TRIPOS® force field and Gasteiger–Huckel charges [27], with PZQ used as the template structure for the UNITY module of SYBYL-X 2.0. The template molecule (PZQ) underwent energy minimization using the Tripos force field [28] and the Powell conjugate gradient routine [29], with a 0.05 kcal/mol Å energy convergence cutoff. Three main sets of features were identified: hydrogen–bond donor/acceptor groups, hydrophobic/aromatic groups and steric sites. Several pharmacophoric models were generated incorporating these characteristics and the number of conformers generated for each model, and their corresponding scoring values (QFITs) are presented in Table 1. The models are depicted in Figure 2.

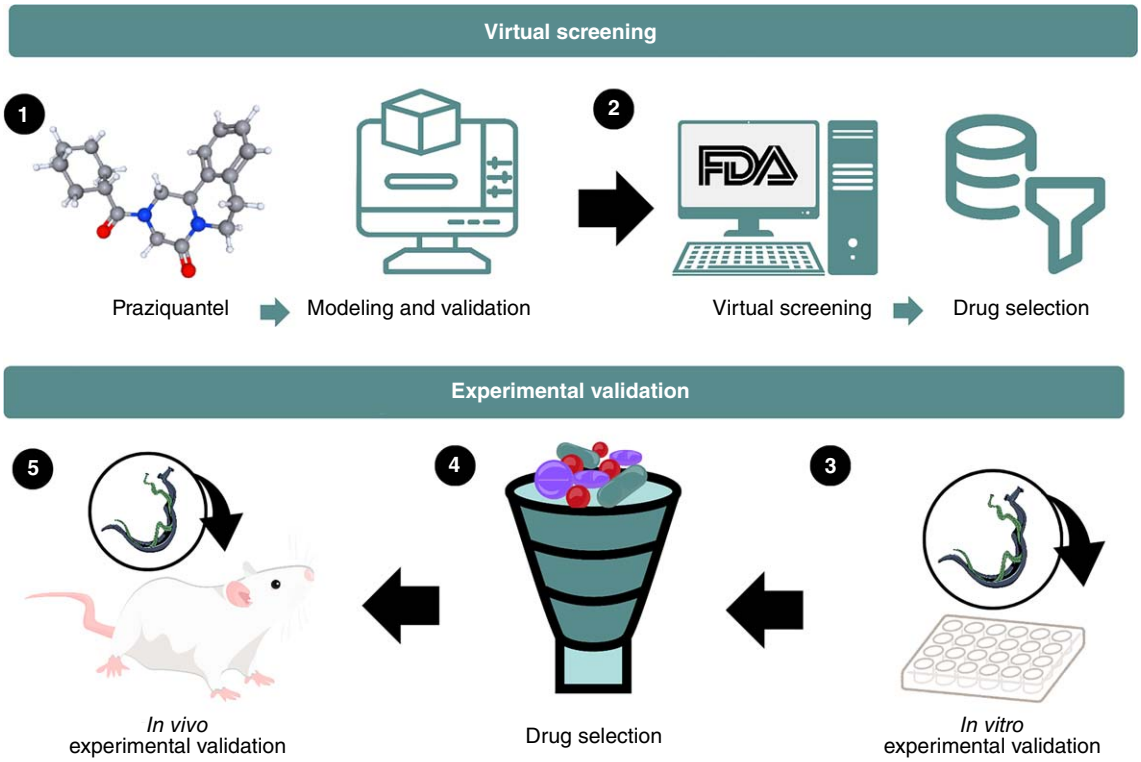


Figure 1. Overview of the key processes in this study.

Table 1. Number of conformers obtained in each generated model and respective QFIT.

Model	Feature	Conformers	>QFIT
1	Acceptor sites	803	93.18
2	Steric groups	532	81.34
3	Hydrophobic group	412	66.93
4	Mix (acceptor, steric and hydrophobic sites)	111	57.91

The database molecules were ranked based on their QFIT values (Table 2). Compounds with high scoring values were acquired and tested *in vitro* to identify active assets.

3.2. *In vitro* antiparasitic assays

To validate the computational predictions, six drugs-betamethasone, chlormadinone, doxazosin, griseofulvin, losartan and pravastatin were selected for *in vitro* studies against *S. mansoni* adult worms, based on their favorable QFIT values (Table 2). Notably, betamethasone and doxazosin demonstrated noteworthy activity *in vitro*, with variations observed between male and female parasites. The EC_{50} values for betamethasone were 39.72 and 58.86 μM for male and female schistosomes, respectively. In the case of doxazosin, the EC_{50} values were 35.48 μM for male worms and 48.19 μM for female worms (Table 3). The reference drug, PZQ, exhibited remarkable potency with EC_{50} values below 1 μM. Importantly, a marginal delay in the onset of action (24–72) was observed when parasites were exposed to betamethasone and doxazosin. In contrast, PZQ swiftly induced muscle contraction in all adult parasites, leading to spastic paralysis of the schistosomes.

3.3. *In vivo* studies in *S. mansoni* infected mice

Based on their *in vitro* activity against adult schistosomes, betamethasone, doxazosin and PZQ were tested in mice harboring adult *S. mansoni* using an oral dose of 100, 200 or 400 mg/kg (Figure 3). These doses were selected as they are commonly used in studies on drug discovery for schistosomiasis [16,30]. Results were compared with the infected but untreated control animals. Treatment with betamethasone and PZQ using any dose was well tolerated, and no animals died during the experiments. However, two animals died in the groups treated with doxazosin 400 mg/kg.

Betamethasone or doxazosin at a single dose of 400 mg/kg resulted in a worm burden reduction of 52.6%

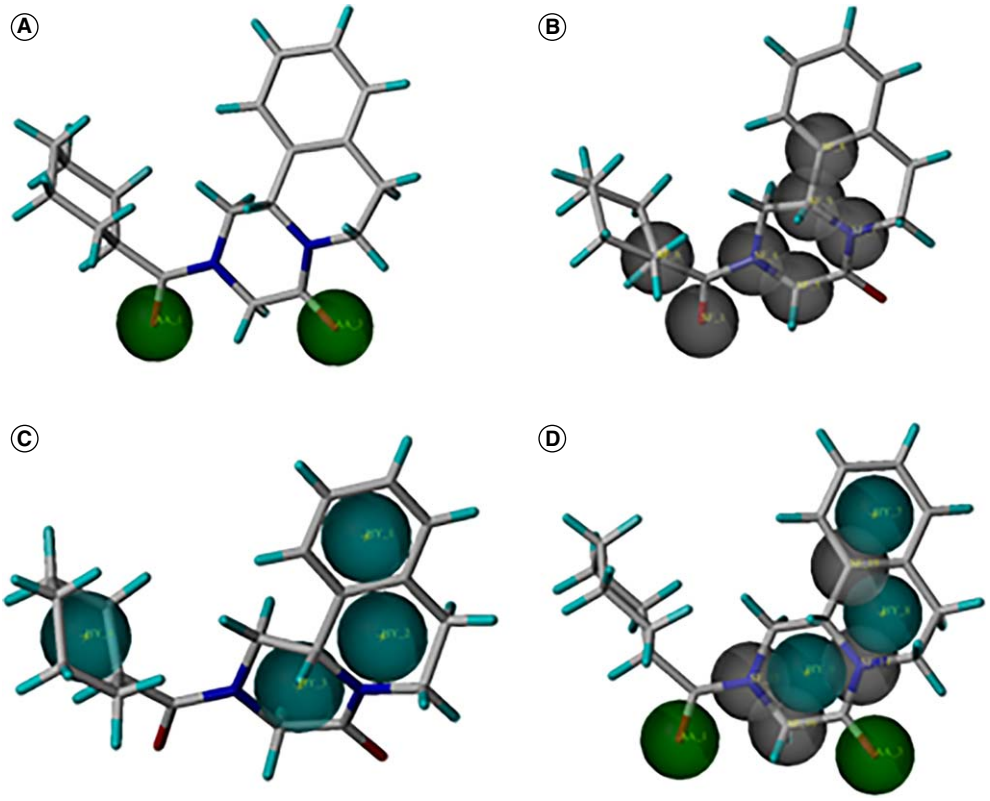


Figure 2. Pharmacophoric models using praziquantel as a template. **(A)** Acceptor sites. **(B)** Steric sites. **(C)** Hydrophobic sites. **(D)** Mix of features (acceptor, steric and hydrophobic sites).

Table 2. Selected drugs and respective QFIT scoring values.

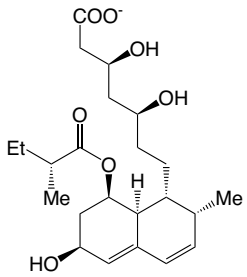
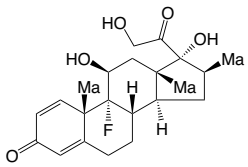
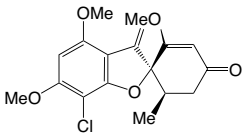
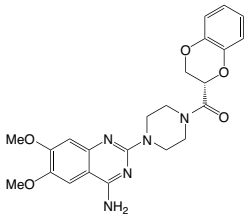
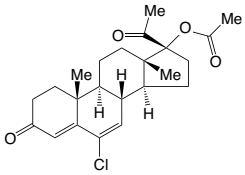
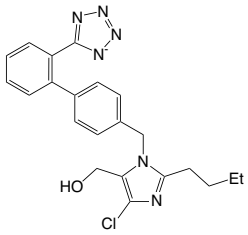
Drug	>QFIT	Model	Structure	Drug	>QFIT	Model	Structure
Pravastatin	93.11	1		Doxazosin	85.67	1	
Betamathasone	92.41 22.94	1 4		Chlormadionone	84.34	1 1	
Griseofulvin	85.98	1		Losartan	73.89 27.80	1 4	

Table 3. Anthelmintic activity of drugs selected by virtual screening against *Schistosoma mansoni* adult worms *ex vivo* after 72 h.

Drugs	EC_{50} (μM)[a]	
	Male	Female
Betamethasone	39.72 (34.59–43.29)	58.86 (52.37–64.16)
Chlormadinone	>100	>100
Doxazosin	35.48 (30.22–40.95)	48.19 (43.12–54.81)
Griseofulvin	>100	>100
Losartan	>100	>100
Pravastatin	>100	>100
Praziquantel	0.61 (0.54–0.72)	0.83 (0.71–0.96)

[a]95% Confidence interval are in parentheses.
EC_{50}: Half maximal effective concentration.

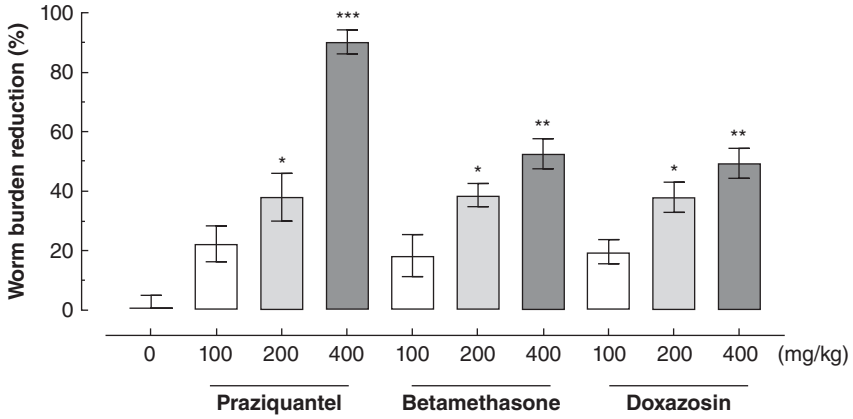


Figure 3. Efficacy of praziquantel, betamethasone and doxazosin against *Schistosoma mansoni* infection in mice. The drugs, administered orally at the indicated doses, were given as single doses on day 42 post infection. Two weeks after treatment, all animals were euthanized, and parasite burdens were determined. Worm burden reduction data are presented as the mean ± standard deviation from ten animals (the results were combined from two experiments). Statistical significance is denoted as follows: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, compared with infected untreated control.

($p < 0.01$) and 49.4% ($p < 0.01$), respectively. Slow worm burden reduction (approximately 38%), but statistically significant ($p < 0.05$), was achieved when betamethasone and doxazosin were administered at 200 mg/kg. No significant reduction in the number of parasites was observed following betamethasone and doxazosin at 100 mg/kg. A high total worm burden reduction of 90.1% ($p < 0.001$) was observed when the mice were treated with PZQ at 400 mg/kg.

4. Discussion

In this study, we applied a model based on PZQ similarity to conduct virtual screening of FDA approved drugs against *S. mansoni*. Employing a comprehensive 3D pharmacophore-based strategy, we evaluated a dataset of 10,000 FDA approved drugs, leading to the selection of six drugs for experimental validation. This approach ultimately resulted in two drugs, betamethasone and doxazosin, that were active against adult schistosomes in our *in vitro* experiments.

Our *in vitro* findings unveiled a notable sex-specific susceptibility, with male parasites exhibiting greater sensitivity to betamethasone and doxazosin compared with their female counterparts. While the rationale behind this sex-related drug sensitivity remains elusive, it aligns with existing knowledge that certain antischistosomal agents, including PZQ, tend to act more effectively on male schistosomes [18,31,32]. Additionally, our results regarding the *in vitro* antischistosomal properties of doxazosin are consistent with a prior phenotypic screening study by Porto et al. [12], where an EC_{50} >30 μM was observed. Our study extends this understanding, providing a more detailed EC_{50} range of 35–48 μM and highlighting the greater sensitivity of male worms to doxazosin.

Despite the distinct pharmacodynamics of betamethasone and doxazosin, both drugs were selected for *in vivo* evaluation due to their rapid absorption in the gastrointestinal tract and the favorable safety profile (with the 50% lethal dose [LD_{50}] of both drugs in mice exceeding 1000 mg/kg) [33,34]. The animal model of schistosomiasis revealed a dose-dependent efficacy for both betamethasone and doxazosin, with parasite burden reduction

observed across different doses (100–400 mg/kg). The precise mechanism by which betamethasone and doxazosin exert their antischistosomal effects, akin to many other agents, including PZQ [35], remains unclear.

Crucially, the identified drugs exhibited activity at higher concentrations (35–50 μM) compared with PZQ (EC_{50} ~1 μM). This trend persisted in our *in vivo* studies, where PZQ demonstrated an impressive 90% reduction in parasite load, while betamethasone and doxazosin achieved a more modest yet significant reduction of around 50%. These results underscore the imperative need to refine the virtual screening model employed. The exclusive reliance on PZQ as a template may limit the identification of potential antischistosomal agents. The observed potency disparity between PZQ and the identified drugs emphasizes the necessity for a nuanced and comprehensive model that considers a broader spectrum of structural and chemical features [36]. Future virtual screening efforts should be conducted to explore novel chemical spaces and structural diversity related to FDA approved drugs to enhance the predictive power of such models.

Moreover, the higher concentrations required for the identified drugs to exhibit activity underscore the challenge in discovering potent antischistosomal agents within the current drug repertoire. This challenge may be indicative of the intricate biology of *S. mansoni*, necessitating a more targeted and nuanced approach in drug discovery endeavors [37].

5. Conclusion

Schistosomiasis, a parasitic disease affecting nearly 250 million individuals worldwide, remains a significant public health concern. With PZQ being the primary treatment option, there is an urgent need to explore novel antischistosomal drugs. This study employed PZQ as a reference point and conducted a comprehensive 3D pharmacophore-based virtual screening of a dataset containing 10,000 FDA approved drugs to identify potential antischistosomal agents. Among the candidates identified, betamethasone, a steroidal anti-inflammatory and doxazosin, an antihypertensive, exhibited efficacy against *S. mansoni* viability *in vitro*, with EC_{50} ranging from 35 to 60 μM. Further evaluation in a mouse model of schistosomiasis, with a single oral dose ranging from 100 to 400 mg/kg, demonstrated significant (>50%) reductions in worm burden for both drugs at the highest dose. These findings highlight the potential of the identified agents as promising candidates for drug repositioning in the treatment of schistosomiasis. Moreover, the study introduces a pertinent methodology for the discovery of novel antischistosomal agents within the realm of FDA approved drugs.

Article highlights

Models & molecules
- Employed a 3D pharmacophore-based virtual screening strategy using praziquantel (PZQ) as a template.
- Identified several pharmacophoric models and ranked database molecules based on QFIT values.

***In vitro* antiparasitic assays**
- Six drugs were selected for *in vitro* studies, with betamethasone and doxazosin showing notable activity against *Schistosoma mansoni*.
- Sex-specific susceptibility observed, with male parasites more sensitive to betamethasone and doxazosin.
- Betamethasone and doxazosin demonstrated delayed onset of action compared with PZQ.
- Identified drugs exhibited activity at higher concentrations compared with PZQ, highlighting the need for refinement in virtual screening models.

***In vivo* studies in *S. mansoni* infected mice**
- Betamethasone and doxazosin tested in mice, resulting in dose-dependent reductions in worm burden.
- Betamethasone and doxazosin at 400 mg/kg showed significant reduction in worm burden, comparable to untreated animals.
- Potential of betamethasone and doxazosin as promising candidates for drug repositioning in schistosomiasis treatment.
- Challenges in discovering potent antischistosomal agents within the current drug repertoire underscore the need for a targeted approach.
- Introduces a relevant methodology for discovering novel antischistosomal agents among FDA approved drugs.

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Author contributions

L Dobrachinsk, LLG Ferreira, AC Mafud, YP Mascarenhas, AD Andricopulo and J de Moraes were responsible for study conception and design; L obrachinski, LLG Ferreira, AC Mafud, ME Cirino, and AI Andrade-de-Siqueiraa were responsible for investigation and data analysis. L Dobrachinski, LLGF Ferreira, YP Mascarenhas, AD Andricopulo and Jd de Moraes were responsible for drafting and revision of the manuscript. J de Moraes was responsible for project administration and funding acquisition.

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Competing interests disclosure

The authors have no competing interests or relevant affiliations with any organization or entity with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Writing disclosure

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors confirm obtaining institutional review board approval and ethical clearance from the Committee for Ethical Use of Animals in Experimentation of Guarulhos University (protocol ID 47/20), adhering to Brazilian legislation (Law 11.794/2008).

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