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RESEARCH ARTICLE

Structural, functional and docking analysis against *Schistosoma mansoni* dihydroorotate dehydrogenase for potential chemotherapeutic drugs [version 1; peer review: 1 approved, 1 approved with reservations, 1 not approved]

Benson Otarigho ^{1,2}¹Department of Biological Science, Edo University, Iyamho, Iyamho, Edo State, Nigeria²Department of Molecular Microbiology & Immunology, Oregon Health & Science University, Portland, Oregon, 97239, USA

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Abstract

Background: Praziquantel, as the only drug for the treatment of schistosomiasis, is under serious threat due to the emergence of resistant strains of *Schistosoma* species. There is an urgent need to search for alternative chemotherapy to supplement or complement praziquantel. *Schistosoma* dihydroorotate dehydrogenase (DHODH) has been recommended as a druggable target for schistosomiasis chemotherapy. The development of novel molecular modeling approaches, alongside with computational tools and rapid sequencing of pathogen genomes, have facilitated drug discovery. Therefore, the aim of this study was to employ computational approaches to screen compounds against *Schistosoma mansoni* DHODH.

Methods: In this study, DHODH was used to blast on the latest version of DrugBank that contained 12,110 compounds, resulting in 26 drugs that can bind.

Results: *In silico* docking shows that 13 drugs can bind strongly with an estimated free energy of binding, total intermolecular energy and estimated inhibition constant (K_i) greater than or equal to -8.6 kcal/mol, -8.12 kcal/mol and 1.12 μM, respectively. These compounds include the approved drugs manitimus, capecitabine, brequinar analog and leflunomide.

Conclusions: These results indicate that these drugs have the potential for use in the control of schistosomiasis in the future.

Keywords

Dihydroorotate Dehydrogenase, Schistosomiasis, Drugs, Neglected Tropical Disease

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1. **Madhu Saddala** , University of Missouri, Columbia, USA
2. **Elumalai Pavadaï**, Boston University School of Medicine, Boston, USA
3. **Maria Cristina Nonato** , Universidade de São Paulo, Ribeirão Preto, Brazil

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Corresponding author: Benson Otarigho (otarighobenson152799@gmail.com)

Author roles: **Otarigho B:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

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Abbreviations

DHODH: dihydroorotate dehydrogenase, *Sm*DHODH: *Schistosoma mansoni* dihydroorotate dehydrogenase, *Sch*DHODH: *Schistosoma* dihydroorotate dehydrogenase, *Sh*DHODH: *Schistosoma haematobium* dihydroorotate dehydrogenase, *Sj*DHODH: *Schistosoma japonicum* dihydroorotate dehydrogenase, *Hs*DHODH: *Homo sapiens* dihydroorotate dehydrogenase, *Mm*DHODH: *Mus musculus* dihydroorotate dehydrogenase, *Rr*DHODH: *Rattus rattus* dihydroorotate dehydrogenase, NTD: neglected tropical disease, PLIs: protein-ligand interactions

Introduction

The public health impacts of schistosomiasis, caused by *Schistosoma* species, are second only to malaria in endemic regions and it is an important neglected tropical disease (NTD)^{1,2}. More than 240 million people are infected with one or more *Schistosoma* species in the tropical or subtropical regions²⁻⁴. Approximately 85% of these infections occur in sub-Saharan Africa^{3,4}. Movement of refugees and mass migration of individuals from endemic to non-endemic areas have recently expanded the areas at risk of schistosomiasis and increased disease prevalence in recent times⁵⁻⁷. Around 200,000 people die from *Schistosoma* infections each year and many more suffer with serious disabilities. Moreover, there is great economic loss in areas where schistosomiasis is endemic. Reports have shown that over 120 million people are symptomatic, with 20 million having severe clinical disease, in endemic areas^{1,2,4}. The disease has been reported to be endemic in 76 countries, in which approximately 4 billion people live. Involvement in agricultural work, domestic chores and recreational activities can expose them to infested water^{1,2,8}.

The control of this disease is solely dependent on the chemotherapeutic drug praziquantel, which is used to treat infected individuals. There is no effective vaccine currently^{3,9}. Other chemotherapy alternatives include oxamniquine and metrifonate, although these have some setbacks, such as being ineffective against all life stages of the parasite and severe side effects, which is why praziquantel is recommended¹⁰⁻¹⁷. Although praziquantel is effective, relatively safe and low cost, laboratory and field studies have shown the emergence of a resistant parasite strain in certain regions. This has led to a serious search for chemotherapeutic alternatives for the treatment and control of schistosomiasis^{9,10}. Therefore, there is a need to search for alternatives using both laboratory and computational investigative methods to explore sequenced *Schistosoma* genomes⁹.

The recent sequencing of *Schistosoma* genomes, alongside the quickly evolving field of bioinformatics, has unveiled numerous opportunities to identify and characterize important proteins, which can aid in the development of novel drugs for disease control. The application of bioinformatics tools in extracting significant meaning from the sequence data have improved our knowledge of the mode of action of proteins¹⁸. Modeling protein-ligand interactions (PLIs) to determine the possible biological interactions that occur *in vivo* has helped in understanding protein function and predicting their mechanisms of action. PLIs are also important in the development of novel therapies and diagnostic tools¹⁹⁻²¹.

Dihydroorotate dehydrogenase (DHODH) is a crucial enzyme that catalyzes the conversion of dihydroorotate (DHO) to orotate during the fourth and only redox step of the *de novo* pyrimidine nucleotide biosynthetic pathway²²⁻²⁵. *Schistosoma* DHODH has been highly recommended as a druggable target that could offer alternative routes for schistosomiasis control^{22,24}. Detailed structural differences have been demonstrated between human and *Schistosoma* DHODH⁹. Furthermore, *Schistosoma mansoni* cannot synthesize purine bases *de novo*, hence depends exclusively on the salvage pathway.

In this study, the *Schistosoma* DHODH sequences were thoroughly explored and compounds from an available drug database were docked against this enzyme to assess their suitability as potential drugs. The results show that 13 approved drugs used in the treatment of other diseases have the potential to inhibit the activities of *Sm*DHODH.

Methods

Sequence retrieval, extraction of DHODH proteins and potential drugs

*Sch*DHODH protein sequences were obtained from the NCBI protein database. The sequences were confirmed in *SchistoDB* for *S. haematobium* (two protein sequences) and *GeneDB* for *S. mansoni* (two protein sequences) and *S. japonicum* (one protein sequence). All sequences were obtained in FASTA format. Each of these protein sequences were used for a **BLASTp** search of the non-redundant protein database on NCBI, employing the protein-protein BLAST algorithm. Results with similarities above 50% and with a query coverage and expectation value of >70% and 0.0, respectively, were obtained and combined for phylogenetic tree construction. Hypothetical and unknown proteins were excluded, even when there was high identity. After combining these sequences, duplicate protein sequences were also excluded. Host DHODH protein sequences were also obtained from NCBI using the search terms as targets “*Homo sapiens* + DHODH”, “*Mus musculus* + DHODH” and “*Rattus rattus* + DHODH” separately. The protein sequences that had 98% similarity to the targets were retrieved for further analyses. One *Hs*DHODH, one *Mm*DHODH and two *Rr*DHODH sequences were retrieved. Each *Sch*DHODH sequence was used to search **DrugBank** (version 5.1.2, released 2018-12-20)²⁶ for potential compounds that may bind. All settings were at default.

Functional domain analysis

Functional domains for each *Sch*DHODH, *Mn*DHODH and *Hs*DHODH were computed using the normal and genomic mode of **SMART**^{27,28}. The functional domains predicted were validated using other webtools; **NCBI Conserved Domains**²⁹⁻³², **PROSITE**³³, **InterPro**³⁴ and **Pfam** version 32.0³⁵. Default parameters were used for these analyses.

Prediction of intrinsic disorder in *Sm*DHODH

Since, for many disordered proteins, binding affinity with their receptors is regulated by post-translational modification, *Sm*DHODH protein was analyzed for intrinsically disorder. *Sm*DHODH was the only protein used in this analysis because

it was used for modeling and protein-drug interaction. **PONDR**, which can predict natural disordered regions in a protein sequence, was used. This was validated using other similar tools such as **SLIDER webserver**³⁶ and **DisEMBL Intrinsic Protein Disorder Prediction 1.5**³⁷.

Physical and chemical properties of *SchDHODHs*, *HsDHODHs* and *MmDHODHs*

Each protein's physiochemical properties, which includes the number of residues, molecular weight and extinction coefficient were predicted using the **ProtParam** web tool³⁸. These properties were validated using the **PepCalc peptide property calculator** and **Protein Physicochemical Properties Prediction Tool (PPPPT)** web tools.

SchDHODH, *HsDHODH* and *MmDHODH* sequence alignment

All *SchDHODH*, *HsDHODH* and *MmDHODH* sequences were compared by Multiple Sequence Alignment (MSA) and the conserved and deleted regions were analyzed. The MSA analysis was carried out using Clustal Omega tools in **Jalview**³⁹. The MSA was analyzed for conserved properties and regions of similarity.

Phylogenetic studies and evolutionary conservation analysis

Phylogenetic trees were constructed using **MEGA** version 7 software⁴⁰. The constructed phylogenetic trees were validated using **Phylogeny.fr**⁴¹. The tree file, in Newick format, was exported and visualized in **FigTree** software version 1.4.2⁴² for proper annotation. The pairwise distances were also estimated, using the Poisson correction model in **MEGA**⁴⁰.

Protein-protein interactions

Protein-protein interactions were determined using the **STRING** database for functional protein association networks. Each of the *SchDHODH*, *HsDHODH* and *MmDHODH* protein sequences were searched on the **STRING** and the interactions were downloaded in jpeg format.

3D modelling

SmDHODH sequences were modelled using **SWISS-MODEL**^{43–47}. The template 3u2o.1.A, with a sequence identity of 47.95%, GMQE of 0.74 and QMEAN of -0.98 was selected as the model of choice for *SmDHODH* sequences.

Molecular docking and analyses

Molecular docking was carried out using **DockingServer**⁴⁸ and Gasteiger partial charges were added to the ligand atoms⁴⁹, as described by Kumar, 2011⁵⁰. Proteins were uploaded, protein charges were calculated, and solvation parameters were calculated and cleaned. All advance docking parameters were left at default.

Results

Sequence retrieval of DHODHs and drugs

In total, five *SchDHODH* sequences were retrieved for further analyses. One for *S. japonicum* and two proteoforms each for

S. haematobium and *S. mansoni*. One *HsDHODH* sequence, two *MmDHODH* sequences and two *RrDHODH* sequences (**Figure 1** and Project 1, *Extended data*)⁵¹ were included in the study for proper comparison as mammals, including humans, are the hosts, while rats and mice are commonly used in the laboratory for schistosomiasis studies. Of 12,110 drugs in the database, 26 compounds that can bind to *SchDHODH* proteins were identified and retrieved from DrugBank. These drugs were retrieved with E value: 7.46761e-71, Bit score: 224.172, query length: 379 and alignment length: 314. These results were the same for each of the *SchDHODH* sequences searched. All of the drugs were at the experimental phase, except flavin mononucleotide, capecitabine and leflunomide, which are approved for the treatment of other diseases, and manitimus, which is still at the investigational phase. The mode of action of most of these retrieved drugs are not known, except for atovaquone, leflunomide and teriflunomide, which act as inhibitors to known proteins other than DHODH.

Domains of DHODHs and intrinsic disorder predictions

The protein domain analysis (see Project 3, *Extended data*)⁵² shows that all analyzed proteins have the dihydroorotate dehydrogenase domain (DHO_dh), while *ShDHODH* proteins have dynein light domains and transmembrane helix regions, which are not seen in the other proteins. The disorder predictions show that a small fraction of the *SmDHODH* protein is disordered, as shown in Project 2, *Extended data*⁵³.

DHODHs of *S. haematobium* and *S. mansoni* evolved from a single ancestral species

Physiochemical parameters (see Project 1, *Extended data*)⁵¹ and alignment (**Figure 1**), as well as phylogenetic analyses of all the *SchDHODH* sequences (**Figure 2**), show that the DHODH proteins of *S. haematobium* and *S. mansoni* could be evolutionary closer than that of *S. japonicum*. However, *S. japonicum* evolved 0.8928 million years ago (mya), compared to *S. haematobium* which evolved 0.8207 mya and *S. mansoni* which evolved 0.8012 mya. The last common ancestor for DHODH of both *S. haematobium* and *S. mansoni* was 0.8379 mya. The host DHODHs evolved more recently, as shown in **Figure 2**. These results could also explain the similarities between DHODHs that are shown in the sequence alignment (**Figure 1**). Mutations that occurred in *ShDHODHs* were also observed in the *SmDHODHs*, though there are some points where all three *Schistosoma* species shared mutation points. However, the similarities between *ShDHODH* and *SmDHODH* sequences do not correlate with the physiochemical parameters; *ShDHODH* theoretical pI, extinction coefficients, instability index, aliphatic index and grand average of hydropathicity scores are more similar to *SjDHODH* than *SmDHODH* (see Project 1, *Extended data*)⁵¹.

SchDHODHs, *HsDHODHs* and *MmDHODHs* protein-protein interaction analysis

The prediction of possible *in vivo* interactions of these DHODHs showed that *SchDHODH* binds to NADPH (gene *Smp_166580*), putative glutamate synthase (*Smp_128380.2*) and NADH-cytochrome B5 reductase (*Smp_053230*) (**Figure 3**). These

Figure 1. Alignment analysis of *Schistosoma* dihydroorotate dehydrogenases alongside the corresponding dihydroorotate dehydrogenases of host species. Conserved amino acids across *SchDHODHs* are marked and indicated in purple boxes, while across *SmDODHs* and *ShDHODHs* are marked and indicated in red boxes. The red arrows show where the mutation in *SJDHODH* compare to *SmDODHs* and *ShDHODHs*.

Figure 2. Phylogenetic tree of the dihydroorotate dehydrogenase proteins from *Schistosoma* pathogens, mice and human. All the *SchDHODH*s shows clearly that the *SchDHODH*s of *S. haematobium* and *S. mansoni* could be evolutionary closer compared to *S. japonicum*.

four proteins bind to each other; however, it is unclear whether they form a complex. *SchDHODH*s have an inhibitory effect on orotate phosphoribosyltransferase (*Smp_050540*), which has an inhibitory transcriptional regulation on aspartate carbamoyltransferase (*Smp_186670*) and vice versa. *HsDHODH* and *MmDHODH*s non-specifically bind to collapsin response mediator protein 1 (*Crmp1*) and different proteoforms of dihydropyrimidinase (*Dpys*). However, *HsDHODH* and *MmDHODH*s have an inhibitory role on carbamoyl-phosphate synthetase (*Cps1*) and uridine monophosphate synthetase (*Umps*).

Docking of selected ligands against modelled *SchDHODH* protein structure

SmdHODH was selected for modeling and protein-drug interaction studies as a representative for the three *SchDHODH*s, since sequence alignment and phylogenetic analysis showed that they are all closely related. The modelled *SmdHODH* used for docking against the 26 potential compounds is shown in Figure 4. After the molecular docking, 13 drugs had a binding affinity of less than -6kcal/mol, suggesting a strong bond²¹, with *SchDHODH* (Table 1 and Figure 5) and details of these interactions are shown in Figure 6. The results show that

3-{[(3-fluoro-3'-methoxybiphenyl-4-yl)amino]carbonyl}thiophene-2-carboxylic acid (accession number [DB07976](#)) and 3-{[(3-fluoro-3'-methoxybiphenyl-4-yl)amino]carbonyl}thiophene-2-carboxylic acid (accession number [DB07978](#)) have the highest binding affinity, with an estimated free energy of binding (kcal/mol) of -10.18 and -10.66, respectively. Leflunomide (accession number [DB01097](#)) and N-anthracen-2-yl-5-methyl[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (accession number [DB08006](#)) both have an estimated free energy of binding of -8.12. The *SchDHODH* protein residues that interact with the different drugs in Table 1, are shown stick while drugs cartoon. It was observed that *SmdHODH* interacts with the various compounds using hydrogen bonds, polar, hydrophobic, pi-pi, halogen and other forms of bonding.

Discussion

In the present study, known drugs that are either approved for treatment or at the experimental stage were searched as targets of *DHODH*s. Of those searched, 13 had a strong binding affinity. Binding affinity of less than -6kcal/mol is regarded a strong bond²¹. The strength of hydrogen, polar, pi-pi, halogen and hydrophobic bonding between the drugs and the *SmdHODH* shows

Figure 4. Modelled *SmDHODH* protein.

the stability of the ligand inside the binding pocket. Based on the interaction properties of the ligand-protein complex, all the drugs have strong affinity, however, drug **DB07978** (2-([2,3,5,6-tetrafluoro-3'-(trifluoromethoxy)biphenyl-4-yl]amino)carbonyl)cyclopenta-1,3-diene-1-carboxylic acid) and **DB07976** (3-([(3-fluoro-3'-methoxybiphenyl-4-yl]amino)carbonyl]thiophene-2-carboxylic acid) have the strongest free energy of binding among all the drugs screened.

One of the drugs with a high affinity (estimated free energy of binding of -9.56) to *SmDHODH* is flavin mononucleotide (accession number **DB03247**) (vitamin B2). This compound is naturally present in most food and used as a food additive^{54–57}. Riboflavin supplementation has been known to increase the efficiency of cell energy metabolism^{58,59}. Manitimus (accession number **DB06481**) was also identified by the docking experiment as a potential drug for schistosomiasis. It is an efficacious

Table 1. The compounds and the interacting properties with SmdHODH.

	Accession No.	Drugs	Drug Group	Est. Free Energy (kcal/mol)	Est. Inhibition Constant, Ki (uM)	vdW + Hbond + Desolv Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Total Intermolec. Energy (kcal/mol)	Frequency (%)	Interact. Surface
1	DB07978	2-([12,3,5,6-TETRAFLUORO-3'-(TRIFLUOROMETHOXY)BIPHENYL-4-YL]AMINO)CARBONYL]CYCLOPENT-1,3-DIENE-1-CARBOXYLIC ACID	Experimental	-10.66	15.3	-12.36	-0.11	-12.47	81	810.089
2	DB07976	3-([3-FLUORO-3'-METHOXYBIPHENYL-4-YL]AMINO)CARBONYL]THIOPHENE-2-CARBOXYLIC ACID	Experimental	-10.18	34.56	-11.34	-0.35	-11.69	94	806.017
3	DB03247	Flavin mononucleotide	Approved, Investigational	-9.56	97.67	-9.38	-1.06	-10.43	58	873.214
4	DB07977	3-([3,5-DIFLUORO-3'-(TRIFLUOROMETHOXY)BIPHENYL-4-YL]AMINO)CARBONYL]THIOPHENE-2-CARBOXYLIC ACID	Experimental	-9.49	110.54	-10.74	-0.22	-10.95	90	805.447
5	DB07975	2-([3,5-DIFLUORO-3'-(TRIFLUOROMETHOXY)BIPHENYL-4-YL]AMINO)CARBONYL]CYCLOPENT-1-ENE-1-CARBOXYLIC ACID	Experimental	-9.26	163.07	-11.5	-0.23	-11.73	83	807.405
6	DB04583	5-Carbamoyl-1,1':4',1''-terphenyl-3-carboxylic acid	Experimental	-8.89	305.65	-9.93	-0.14	-10.07	18	777.123
7	DB08008	5-methyl-N-[4-(trifluoromethyl)phenyl][1,2,4]triazolo[1,5-a]pyrimidin-7-amine	Experimental	-8.69	425.74	-8.95	-0.04	-8.99	100	690
8	DB06481	Manitimus	Investigational	-8.66	451.02	-10.18	0.13	-10.04	19	731.769
9	DB01101	Capecitabine	approved, Investigational	-8.6	494.14	-10.02	-0.31	-10.33	81	839.853
10	DB03480	Brequinar Analog	experimental	-8.45	641.03	-9.21	-0.21	-9.42	100	819.785
11	DB08169	(2Z)-N-biphenyl-4-yl-2-cyano-3-cyclopropyl-3-hydroxyprop-2-enamide	experimental	-8.41	683.43	-10.47	0.28	-10.19	44	792.682
12	DB01097	Leflunomide	approved, Investigational	-8.12	1.12	-8.88	-0.03	-8.91	65	
13	DB08006	N-anthracen-2-yl-5-methyl[1,2,4]triazolo[1,5-a]pyrimidin-7-amine	experimental	-8.12	1.13	-8.21	0.09	-8.12	43	751.241

Figure 5. The structure of compounds retrieved after searching the drug database with the SmDHODH sequence. a) 2-(((2,3,5,6-Tetrafluoro-3'-(trifluoromethoxy)biphenyl-4-yl)amino)carbonyl)cyclopenta-1,3-diene-1-carboxylic acid (Accession Number: DB07978); b) 3-(((3-fluoro-3'-methoxybiphenyl-4-yl)amino)carbonyl)thiophene-2-carboxylic acid (Accession Number: DB07976); c) 3-(((3,5-difluoro-3'-(trifluoromethoxy)biphenyl-4-yl)amino)carbonyl)thiophene-2-carboxylic acid (Accession Number: DB07977); d) Flavin mononucleotide (Accession Number: DB03247); e) 2-(((3,5-difluoro-3'-(trifluoromethoxy)biphenyl-4-yl)amino)carbonyl)cyclopent-1-ene-1-carboxylic acid (Accession Number: DB07975); f) 5-Carbamoyl-1,1':4',1''-terphenyl-3-carboxylic acid (Accession Number: DB04583); g) 5-methyl-N-[4-(trifluoromethyl)phenyl][1,2,4]triazolo[1,5-a]pyrimidin-7-amine (Accession Number: DB08008); h) Manittimus (Accession Number: DB06481); i) Capecitabine (Accession Number: DB01101); j) Brequinar Analog (Accession Number: DB03480); k) (2Z)-N-biphenyl-4-yl-2-cyano-3-cyclopropyl-3-hydroxyprop-2-enamide (Accession Number: DB08169); l) Leflunomide (Accession Number: DB01097); m) N-anthracen-2-yl-5-methyl[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (Accession Number: DB08006).

and well tolerated drug for kidney transplant patients. It is a novel compound with multiple mechanisms of action^{60,61}. One mechanism is via the suppression of *de novo* pyrimidine biosynthesis, inhibiting the action of dihydroorotate dehydrogenase and consequently inhibiting cell proliferation⁶².

Another promising drug is capecitabine (accession number DB01101), which is a chemotherapy medication used to treat numerous types of neoplasms, including those of the breast, esophagus, larynx and gastrointestinal and genitourinary tracts. Capecitabine is a prodrug and is enzymatically converted to fluorouracil (an antimetabolite) in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue^{63–65}. Leflunomide (accession number DB01097) is an approved immunosuppressive disease-modifying antirheumatic drug that has been in use for the treatment of rheumatoid arthritis and

psoriatic arthritis for more than 10 years. Its mode of action is via pyrimidine synthesis by inhibiting dihydroorotate dehydrogenase^{26,66–70}.

Conclusions

There is an urgent need to search for and develop novel drugs that can be used in the treatment of schistosomiasis to complement treatment with praziquantel as there are growing number of reports of praziquantel-resistant *Schistosoma* strains in the laboratory as well as in the field. Furthermore, there are no efficient vector control strategies. SmDHODH has been proposed as druggable target in the *de novo* pyrimidine biosynthesis pathway for schistosomiasis chemotherapy. We used the SmDHODH sequence to search for all possible inhibiting compounds from the DrugBank database and found 13 with the potential to bind efficiently. If these compounds, which are already approved or

Figure 6. The binding site of the interaction between the SmDHODH protein and the retrieved compounds. **a)** 2-([2,3,5,6-TETRAFLUORO-3'-(TRIFLUOROMETHOXY)BIPHENYL-4-YL]AMINO)CARBONYL)CYCLOPENTA-1,3-DIENE-1-CARBOXYLIC ACID (Accession Number: DB07978); **b)** 3-([3-FLUORO-3'-METHOXYBIPHENYL-4-YL]AMINO)CARBONYL)THIOPHENE-2-CARBOXYLIC ACID (Accession Number: DB07976); **c)** 3-([3,5-DIFLUORO-3'-(TRIFLUOROMETHOXY)BIPHENYL-4-YL]AMINO)CARBONYL)THIOPHENE-2-CARBOXYLIC ACID (Accession Number: DB07977); **d)** Flavin mononucleotide (Accession Number: DB03247); **e)** 2-([3,5-DIFLUORO-3'-(TRIFLUOROMETHOXY)BIPHENYL-4-YL]AMINO)CARBONYL)CYCLOPENT-1-ENE-1-CARBOXYLIC ACID (Accession Number: DB07975); **f)** 5-Carbamoyl-1,1':4',1''-terphenyl-3-carboxylic acid (Accession Number: DB04583); **g)** 5-methyl-N-[4-(trifluoromethyl)phenyl][1,2,4]triazolo[1,5-a]pyrimidin-7-amine (Accession Number: DB08008); **h)** Manittimus (Accession Number: DB06481); **i)** Capecitabine (Accession Number: DB01101); **j)** Brequinar Analog (Accession Number: DB03480); **k)** (2Z)-N-biphenyl-4-yl-2-cyano-3-cyclopropyl-3-hydroxyprop-2-enamide (Accession Number: DB08169); **l)** Leflunomide (Accession Number: DB01097); **m)** N-anthracen-2-yl-5-methyl[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (Accession Number: DB08006).

in experimental stages, are tested in a wet laboratory experiment against the *Schistosoma* parasite and any are found to be effective, they could be easier than novel compounds to approve to supplement praziquantel for the control of schistosomiasis in the future.

Data availability

Underlying data

SJCHGC02326 protein [*Schistosoma japonicum*], Accession number AAW26221: <https://identifiers.org/ncbiprotein/AAW26221>

Dihydroorotate dehydrogenase (quinone), mitochondrial [*Schistosoma haematobium*], Accession number KGB36135: <http://identifiers.org/ncbiprotein/KGB36135>

Dihydroorotate dehydrogenase (quinone), mitochondrial [*Schistosoma haematobium*], Accession number XP_012795900: https://identifiers.org/ncbiprotein/XP_012795900

dihydroorotate dehydrogenase [*Schistosoma mansoni*], Accession number CCD78646: <http://identifiers.org/ncbiprotein/CCD78646>

dihydroorotate dehydrogenase [Schistosoma mansoni], Accession number XP_018651255: https://identifiers.org/ncbiprotein/XP_018651255

dihydroorotate dehydrogenase (quinone), mitochondrial precursor [Mus musculus], Accession number NP_064430: http://identifiers.org/ncbiprotein/NP_064430

Chain A, DIHYDROOROTATE DEHYDROGENASE [Rattus rattus], Accession number IUUM_A: <http://identifiers.org/ncbiprotein/47169292>

Chain B, DIHYDROOROTATE DEHYDROGENASE [Rattus rattus], Accession number IUUM_B: <http://identifiers.org/ncbiprotein/47169293>

Crystal structure of human dihydroorotate dehydrogenase at 1.7 Å resolution [Homo sapiens], Accession number 5K9D: <https://identifiers.org/pdb/5K9D>

Extended data

Figshare: Extended data 1_Physiochemical Properties.docx. <https://doi.org/10.6084/m9.figshare.8019683.v1>⁵¹

Figshare: Prediction of intrinsic disorder of SmDHODH.docx <https://doi.org/10.6084/m9.figshare.8050541.v1>⁵³

Figshare: Extended data 3_Protein domain analysis of DHODHs of the Schistosoma sp and Host.docx <https://doi.org/10.6084/m9.figshare.8051777.v1>⁵²

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Grant information

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Maria Cristina Nonato

Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil

The manuscript prepared by Dr Benson Otarigho describes an *in silico* study to characterize the enzyme dihydroorotate dehydrogenase (DHODH) from different *Schistosoma* species, and compare them with their orthologues from humans, mice and rats. Moreover, docking studies have been performed by evaluating the DrugBank library. Based on the findings described, out of the 12110 compounds tested, 26 compounds have been identified as *Schistosoma* DHODH binders, where 13 of them are approved drugs.

Overall, I am not convinced that the work presents original findings. Moreover, there are many aspects in terms of methodology and interpretation of the results that should be carefully revised. My comments are based on the following points:

- DHODH is a very well characterized enzyme, including *Schistosoma* DHODH. Functional domain analyses and physical and chemical properties studies described in the manuscript, all data raised by online/automatic tools, do not bring any new contribution to the field.
- Moreover, several studies reported were based on amino acid sequences retrieved from different sources, including, for example, sequences retrieved from the protein data base. In this case, for example, the sequence for human and rat DHODHs (Figure 1) are found truncated. This, of course, was necessary for the crystallographic studies, but it can not be used for phylogenetic studies, analysis of intrinsic disordered regions, and analysis of protein-protein interaction. In fact, the use of the truncate sequences took the author to draw wrong conclusions such as:
"The protein domain analysis (see Project 3, Extended data) shows that all analyzed proteins have the dihydroorotate dehydrogenase domain (DHO_dh), while ShDHODH proteins have dynein light domains and transmembrane helix regions, which are not seen in the other proteins"
First, the class 2 DHODH, including human and Schisto enzymes, possesses two domains: the dihydroorotate dehydrogenase domain and the membrane binding domain. It is

important to describe both domains in order to differentiate class 1A and class 2 DHODH enzymes. Moreover, and very important, human/rat enzymes also possess the transmembrane helix regions, which was found omitted in the sequence retrieved from PDB.

Figure 1 is overall very confusing. First the legend does not match with its content. Moreover, from Figure 1 one can not know which sequence refers to each organism.

Regarding the 3D model:

- The author used the online tool SWISS-MODEL to automatically generate a 3D model for *Schistosoma* DHODH. Since this model was used as a template for the docking experiments, more information regarding the quality of the model should be provided. Moreover, other features of the model that directly impact on the docking results should be carefully presented and discussed. For instance: *Schistosoma* DHODH has an insertion of approximately 10 residues compared to all the previous described class 2 DHODHs.
- How has SWISS-MODEL dealt with that? Has the model included the FMN?

Regarding molecular docking:

- Which proteins have been uploaded for docking?
- Where were the compounds found to bind *Schistosoma* DHODH? Are they found to bind in the "quinone binding site"? Even FMN?
- Figure 6 does not provide any information regarding the interaction between DHODH and the ligands. A picture prepared by LIGPLOT, for instance, can be more appropriated to describe the environment of a ligand bound to the protein.
- Leflunomide is a prodrug found to bind *Schistosoma* DHODH. Leflunomide is well known to display little or no effect on class 2 DHODH. An experimental evidence of this unexpected finding should be provided. Several other molecules have been identified. Some of them are already described as class2 DHODH inhibitors, others have not. For those, experimental evidences of such binding should be provided.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.**Reviewer Expertise:** structural biology, medicinal chemistry, enzymology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 22 July 2019

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Elumalai Pavadai

Department of Physiology & Biophysics, Boston University School of Medicine, Boston, MA, USA

In the study, Otariho has reported the structural and physical characterization of Schistosoma DHODH enzyme with the use of different computational prediction methods including, protein-protein interaction and homology modeling. In addition, the author has identified hit compounds by structure-based virtual screening of DrugBank database and suggested some compounds as possible SmdHODH inhibitors. Overall, this manuscript is well-written, and the analyses appear well done. However, it looks incomplete without the experimental validations of the compounds and also lacks some results/discussion as commented below, and the author should address them in a revised manuscript.

1. No crystal structures are available for any Schistosoma DHODHs and some differences have been shown in the active of DHODH as given in Fig 1. I suggest the author build models for other Schistosoma DHODHs mentioned in the manuscript, in addition to the SmdHODH, and compare the active sites between human and Schistosoma DHODHs. This can help us to understand the selectivity of Schistosoma DHODH inhibitors. In addition, there are some Schistosoma DHODH models have been published and the author may also want to compare the models with the published ones.
2. To make sure the employed docking protocol is valid and reliable, the author may want to perform docking of some Schistosoma DHODH inhibitors reported previously before performing the docking of the DrugBank compounds. The author can check whether the docking protocol correlates the biological activity of the compounds or not.
3. Some malarial and human DHODH inhibitors are in clinical trials. The author needs to make sure whether the screening resulted in any DHODH compounds. This needs to be discussed in detail in the manuscript.

4. Regarding comment 3, I can see some human and malarial DHODH inhibitors in Fig 5. Can the author dock them into respective crystal structures and compare with Schistosoma DHODHs? I believe some of the compounds have been crystallized.
5. There are some similar compounds in Fig 5. For example, g and m are sharing the same scaffold, triazolopyrimidine. I suggest the author remove the similar compounds and consider structurally diverse ones.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Computer-Aided Drug Discovery, Chemoinformatics, Bioinformatics, Molecular Modeling

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 15 July 2019

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Madhu Saddala

Mason Eye Institute, School of Medicine, University of Missouri, Columbia, MO, USA

The article provided by Benson provides a clear conceptual description of the structural, functional and docking analysis and potential *Schistosoma mansoni* dihydroorotate dehydrogenase chemotherapeutic drugs. The work follows cheminformatic standards for drug development, and demonstrates the physical characteristics of the SchDHODHs, HsDHODHs and MmDHODHs identified as potential small molecule inhibitors of *Schistosoma mansoni* dihydroorotate dehydrogenase. The rationale, testing, and work supporting the compounds is well described and clear to follow, and the rationale for searching for *Schistosoma mansoni* dihydroorotate dehydrogenase inhibitors is well explained. The scope of the article is narrow, in that it focuses on lead cheminformatic validation, but it is relevant to several potential audiences and is rationally screened through those pipelines. Additional in vitro validation would raise the impact of these compounds, but may not be necessary to report them as a suitable candidate for follow-on work.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, Genomics, Proteomics, Microarray, Single cell sequencing, miRNA, noncoding RNAs, drug design.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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