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]

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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 (Ki) 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

Open Peer Review

Reviewer Status

Invited Reviewers

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Abbr eviations
DHODH: dihydroorotate dehydrogenase, SmDHODH: Schisto soma mansonii dihydroorotate dehydrogenase, SchDHODH: Schisto soma haematobium dihydroorotate dehydrogenase, SjDHODH: Schisto soma japonicum dihydroorotate dehydrogenase, HsDHODH: Homo sapien dihydroorotate dehydrogenase, MnDHODH: Mus musculus dihydroorotate dehydrogenase, RrDHODH: Rattus rattus dihydroorotate dehydrogenase, NTD: neglected tropical disease, PLIs: protein-ligand interactions

Introduction
The public health impacts of schistosomiasis, caused by Schisto soma species, are second only to malaria in endemic regions and it is an important neglected tropical disease (NTD)\(^1\). More than 240 million people are infected with one or more Schistosoma species in the tropical or subtropical regions\(^2\-\(^3\). Approximately 85% of these infections occur in sub-Saharan Africa\(^4\). 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 times\(^5\-\(^7\). 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 symptoms each year and many more suffering with serious disabilities. 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\(^2\-\(^4\).

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\(^8\-\(^9\). 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 recommended\(^10\-\(^17\). 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\(^8\-\(^9\). Therefore, there is a need to search for alternatives using both laboratory and computational investigative methods to explore sequenced Schistosoma genomes\(^8\).

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 proteins\(^8\). 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 therapeutics and diagnostic tools\(^8\-\(^9\).

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 pathway\(^22\-\(^23\). Schistosoma DHODH has been highly recommended as a druggable target that could offer alternative routes for schistosomiasis control\(^22\-\(^23\). Detailed structural differences have been demonstrated between human and Schistosoma DHODH. Furthermore, Schistosoma mansonii 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 SmDHODH.

Methods
Sequence retrieval, extraction of DHODH proteins and potential drugs
SchDHODH 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. mansonii (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 sapien + 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 HsDHODH, one MmDHODH and two RrDHODH sequences were retrieved. Each SchDHODH 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 SchDHODH, MmDHODH and HsDHODH 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 32.0\(^35\). Default parameters were used for these analyses.

Prediction of intrinsic disorder in SmDHODH
Since, for many disordered proteins, binding affinity with their receptors is regulated by post-translational modification, SmDHODH protein was analyzed for intrinsically disordered. SmDHODH 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\(^8\) and DisEMBL Intrinsic Protein Disorder Prediction 1.5\(^9\).

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

Each protein’s physicochemical properties, which includes the number of residues, molecular weight and extinction coefficient were predicted using the ProtParam web tool\(^10\). 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\(^11\). 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 software\(^12\). The constructed phylogenetic trees were validated using Phylogeny.fr\(^14\). The tree file, in Newick format, was exported and visualized in FigTree software version 1.4.2\(^15\) for proper annotation. The pairwise distances were also estimated, using the Poisson correction model in MEGA\(^16\).

**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\(^13\)–\(^17\). 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\(^18\) and Gasteiger partial charges were added to the ligand atoms\(^19\), as described by Kumar, 2011\(^20\). 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)\(^31\) 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, capectabine 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)\(^32\) shows that all analyzed proteins have the dihydroorotate dehydrogenase domain (DHO_dh), while SjDHODH 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\(^33\).

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

Physiochemical parameters (see Project 1, Extended data)\(^31\) 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 evolutionarily 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 *SjDHODHs* were also observed in the *SmDHODHs*, though there are some points where all three *Schistosoma* species shared mutation points. However, the similarities between *SjDHODH* and *SmDHODH* sequences do not correlate with the physiochemical parameters; *SjDHODH* 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)\(^31\).

**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 Sch DHODHs 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 SchDHODHs shows clearly that the SchDHODHs 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. SchDHODHs 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 MmDHODHs non-specifically bind to collapsin response mediator protein 1 (Crm1) and different proteoforms of dihydropyrimidinase (Dpys). However, HsDHODH and MmDHODHs 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 SchDHODHs, 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 bond, 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 DHODHs. Of those searched, 13 had a strong binding affinity. Binding affinity of less than -6kcal/mol is regarded a strong bond. The strength of hydrogen, polar, pi-pi, halogen and hydrophobic bonding between the drugs and the SmDHODH shows...
Figure 3. Interaction of proteins with (a) SmDHODH (b) HsDHODH and (c) MmDHODH.

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.

Riboflavin supplementation has been known to increase the efficiency of cell energy metabolism.

Manitimus (accession number DB06481) was also identified by the docking experiment as a potential drug for schistosomiasis. It is an efficacious...
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<td>1</td>
<td>DB07978 2-(((2,3,5,6-TETRAFLUORO-3'-(TRIFLUOROMETHOXY)BIPHENYL-4-YL)AMINO)CARBONYL)CYCLOPENTA-1,3-DIENE-1-CARBOXYLIC ACID</td>
<td>Experimental</td>
<td>-10.66</td>
<td>15.3</td>
<td>-12.36</td>
<td>-0.11</td>
<td>-12.47</td>
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<td>810.089</td>
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<td>2</td>
<td>DB07976 3-(((3-FLUORO-3'-METHOXYBIPHENYL-4-YL)AMINO)CARBONYL)THIOPHENE-2-CARBOXYLIC ACID</td>
<td>Experimental</td>
<td>-10.18</td>
<td>34.56</td>
<td>-11.34</td>
<td>-0.35</td>
<td>-11.69</td>
<td>94</td>
<td>806.017</td>
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<td>3</td>
<td>DB03247 Flavin mononucleotide</td>
<td>Approved, Investigational</td>
<td>-9.56</td>
<td>97.67</td>
<td>-9.38</td>
<td>-1.06</td>
<td>-10.43</td>
<td>58</td>
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<td>4</td>
<td>DB07977 3-(((3,5-DIFLUORO-3'-(TRIFLUOROMETHOXY)BIPHENYL-4-YL)AMINO)CARBONYL)THIOPHENE-2-CARBOXYLIC ACID</td>
<td>Experimental</td>
<td>-9.49</td>
<td>110.54</td>
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<td>-10.95</td>
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<td>DB07975 2-(((3,5-DIFLUORO-3'-(TRIFLUOROMETHOXY)BIPHENYL-4-YL)AMINO)CARBONYL)CYCLOPENT-1-ENE-1-CARBOXYLIC ACID</td>
<td>Experimental</td>
<td>-9.26</td>
<td>163.07</td>
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<td>-11.73</td>
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<td>6</td>
<td>DB04583 5-Carbamoyl-1,1'4,1''-terphenyl-3-carboxylic acid</td>
<td>Experimental</td>
<td>-8.89</td>
<td>305.65</td>
<td>-9.93</td>
<td>-0.14</td>
<td>-10.07</td>
<td>18</td>
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<td>DB08008 5-methyl-N-[4-(trifluoromethyl)phenyl][1,2,4]triazolo[1,5-a]pyrimidin-7-amine</td>
<td>Experimental</td>
<td>-8.69</td>
<td>425.74</td>
<td>-8.95</td>
<td>-0.04</td>
<td>-8.99</td>
<td>100</td>
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<td>Investigational</td>
<td>-8.66</td>
<td>451.02</td>
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<td>-10.04</td>
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<td>DB01101 Capecitabine</td>
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<td>-8.6</td>
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<td>-8.45</td>
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<td>DB08169 (2Z)-N-biphenyl-4-yl-2-cyano-3-cyclopropyl-3-hydroxyprop-2-enamide</td>
<td>experimental</td>
<td>-8.41</td>
<td>683.43</td>
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<td>-8.12</td>
<td>1.12</td>
<td>-8.88</td>
<td>-0.03</td>
<td>-8.91</td>
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<td>13</td>
<td>DB08006 N-anthracen-2-yl-5-methyl[1,2,4]triazolo[1,5-a]pyrimidin-7-amine</td>
<td>experimental</td>
<td>-8.12</td>
<td>1.13</td>
<td>-8.21</td>
<td>0.09</td>
<td>-8.12</td>
<td>43</td>
<td>751.241</td>
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and well tolerated drug for kidney transplant patients. It is a novel compound with multiple mechanisms of action\(^6\). One mechanism is via the suppression of \textit{de novo} pyrimidine biosynthesis, inhibiting the action of dihydroorotate dehydrogenase and consequently inhibiting cell proliferation\(^6\).

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\(^6\). 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\(^6\).

**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 \textit{Schistosoma} strains in the laboratory as well as in the field. Furthermore, there are no efficient vector control strategies. \textit{SmDHODH} has been proposed as druggable target in the \textit{de novo} pyrimidine biosynthesis pathway for schistosomiasis chemotherapy. We used the \textit{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
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]CYCLOPENTA-1,3-DIENE-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) Manitimus (Accession Number: DB06481); i) Capicitabine (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/ncbibprotein/AAW26221

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

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

dihydroorotate dehydrogenase [Schistosoma mansoni], Accession number CCD78646: http://identifiers.org/ncbibprotein/CCD78646
dihydroorotate dehydrogenase (quinnone), mitochondrial precursor [Mus musculus], Accession number NP_064430 : http://identifiers.org/ncbibprotein/NP_064430

Chain A, DIHYDROOROTATE DEHYDROGENASE [Rattus rattus], Accession number 1UUM_A: http://identifiers.org/ncbibprotein/47169292

Chain B, DIHYDROOROTATE DEHYDROGENASE [Rattus rattus], Accession number 1UUM_B: http://identifiers.org/ncbibprotein/47169293

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

References


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

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

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

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

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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 Schistosoma 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 dehy-drogenase 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. Lefluomide 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
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In the study, Otarigho 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.
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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