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Repurposing statins for the treatment of larval cestodiases: *in silico* evaluation of statin-HMG-CoA reductase interactions and assessment of statin effects on a cestode model

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Abstract

Cestodiases, like echinococcoses and cysticercoses, represent a global health problem. Currently available anthelmintics, as benzimidazoles and praziquantel, have limited effectiveness against these cestodiases, creating a demand for the identification of new and more effective drugs. Here, the potential of statins (simvastatin and fluvastatin), for repositioning as novel anthelmintic is explored. Statins are inhibitors of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase, a main enzyme in the mevalonate pathway, which is vital for the synthesis of non-steroidal isoprenoids and the maintenance of normal cell functioning. A survey for HMG-CoA reductase encoding genes showed that they are present in a single copy in the genomes of parasitic helminths and their mammal hosts. Sequence alignments and phylogenetic analyses showed 20%-95% overall identities among ortholog HMG-CoA reductases, with special conservation of their catalytic domains. The HMG-CoA reductase 3D-structure was predicted for orthologs from three cestodes of medical importance (*Echinococcus multilocularis*, *Echinococcus granulosus* sensu lato, and *Taenia solium*), and from a model cestode species (*Mesocestoides corti*). Molecular docking between cestode HMG-CoA reductase orthologs with simvastatin demonstrated that the Arg, Ser, Lys, and Glu residues in conserved positions of the active site interact with this drug, similarly to the interactions predicted for the human reference ortholog enzyme. Furthermore, in vitro assays demonstrated that simvastatin produced a significant reduction of *M. corti* viability, being able to reduce 100% of parasite viability at 150 µM. Fluvastatin was also assessed showing a lower, although significant anthelmintic effect. The predicted overall structures and interactions together with in vitro assays suggest that cestodes HMG-CoA reductases are inhibited by simvastatin, being a potential therapeutic target for the repurposing of simvastatin as anthelmintic drug. Furthermore, these results pave the way for the in vivo evaluation of the potential effects of simvastatin on cestode larvae.

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Keywords: *Echinococcus multilocularis*; *Echinococcus granulosus*; *Taenia solium*; Mevalonate pathway; statins; drug repurposing; *Mesocestoides corti*.

Introduction

Cestodes are parasitic platyhelminths that infect a wide variety of mammals, including humans and domestic ungulates (Saari *et al.* 2019). Easily transmitted between hosts, they cause diseases (cestodiases) of medical and veterinary relevance, such as cystic echinococcosis (CE) and alveolar echinococcosis (AE) (caused by the larval stage of *Echinococcus granulosus* and *Echinococcus multilocularis*, respectively), as well as cysticercosis (caused by the larval stage of *Taenia* spp.). CE, AE and cysticercosis are in the World Health Organization's list of Neglected Tropical Diseases (http://www.who.int/neglected_diseases/diseases/en/), which are prioritized worldwide for initiatives of treatment, control and eradication. Studies involving cestodes are hampered mainly by the scarce availability of biological material (Lustigman *et al.* 2012). In this context, the availability of a model organism, such as *Mesocestoides corti* (syn. *Mesocestoides vogae*), allows the study of basic aspects of cestode biology. This is because this species is not infectious to humans and can be easily maintained *in vivo* and *in vitro*, providing continuous availability of biological material (Markoski *et al.* 2003; Hemphill 2010).

Infections with adult cestodes (intestinal cestodiases) are generally easier to treat than those caused by larval forms. Adult individuals are efficiently eliminated from human or domestic animal hosts by short treatments with anthelmintics such as praziquantel, niclosamide, and benzimidazole derivatives (*e.g.* albendazole and mebendazole) (Gilman *et al.* 2012; PAHO, 2021; Wang *et al.* 2021). Larval cestodiases, on the other hand, are chronic diseases and their treatment is longer and requires higher doses of the same drugs, with limitations in effectiveness and severe side effects (Woolsey and Miller, 2021). Moreover, the

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restricted repertoire of available drugs for the treatment of larval cestodiases may favor the selection of parasites and emergence of resistance (Sangster *et al.* 2018; Claerebout *et al.* 2020; Haby *et al.* 2020; Ramiandrasoa *et al.* 2020). In this scenario, new and more effective anthelmintics are in demand for the treatment of larval cestodiases.

Drug repurposing is an attractive strategy for the search for new anthelmintic compounds. It has become increasingly popular as it offers the possibility of reducing research and development time for a new drug, with significant cost savings (Xue *et al.* 2018). Drug repurposing strategies have been driven by genomic studies, which facilitated the identification of new potential therapeutic targets in pathogenic species, including parasitic worms (Coghlan *et al.* 2019). Parasite proteins involved in any vital processes, including energy metabolism, stress response, or development, are of great interest as targets for the development or repurposing of new therapeutic drugs (de Andrade Picanço *et al.* 2017; Cancela *et al.* 2019; Paludo *et al.* 2020). In this context, 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) has potential as a therapeutic target. It is the main enzyme in the mevalonate pathway, which synthesizes farnesyl pyrophosphate, the common substrate for protein prenylation, and for the synthesis of non-steroidal isoprenoids, including cholesterol (Karlic and Varga, 2017). In cestodes, the mevalonate pathway, although deficient in its cholesterol synthesis branch, remains essential for lipid metabolism and for normal cell growth, division, and differentiation (Rauthan and Pilon 2011; Lasunción *et al.* 2022;).

HMG-CoA reductase is the target of a group of inhibitory drugs called statins, which are used to control hypercholesterolemia and prevent cardiovascular diseases in humans (Schachter 2005; Kazi *et al.* 2017; Ray 2024). Statins have been also used for the treatment of inflammatory, immunological diseases and some types of cancer, such as breast cancer and lung cancer (Demierre *et al.* 2005; Liao, James K. and Laufs, 2005; Karlic and Varga, 2017; Jiang *et al.* 2021). The use of statins as antiparasitic drugs has already been tested *in vitro* for

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protozoans, like *Trypanosoma cruzi*, *Plasmodium falciparum,* and *Toxoplasma gondii* (Parquet *et al*. 2010; Sanfelice *et al*. 2017; Peres *et al*. 2018), and for different stages of the trematode *Schistosoma mansoni* (Rojo-Arreola *et al*. 2014).

Based on these previous findings, we evaluated the potential of repurposing statins for the treatment of CE, AE and cysticercosis. First, we conducted a survey of the genomes of parasitic helminths and their mammalian hosts to identify and compare the orthologous genes of HMG-CoA reductase and their deduced amino acid sequences. Phylogenetic studies enabled the assessment of the degree of conservation among HMG-CoA reductase orthologs of parasites and respective hosts. The degree of conservation of these enzymes was further assessed at the structural level, based on the comparative 3D-modelling of HMG-CoA reductases from three cestodes of medical importance (*Echinococcus multilocularis, Echinococcus granulosus,* and *Taenia solium*), and from the model cestode *M. corti* with that of human HMG-CoA reductase. Molecular docking was then used to predict interactions of simvastatin with the four modeled parasite enzymes. Furthermore, *in vitro* assays were performed to evaluate the effect of simvastatin and fluvastatin treatment on *M. corti* larvae (tetrathyridia, TTs). The generated data provided valuable clues on the way of interaction of statins with target cestode HMG-CoA reductases, and the effects of treatment with these inhibitors on larvae of *M. corti* parasite. The repositioning potential of these statins for the treatment of larval cestodiases is discussed.

Materials and methods

Identification of HMG-CoA reductase orthologs in parasitic helminths and their respective hosts

Searches for HMG-CoA reductase orthologs of parasitic helminths and of mammal species (bovine, sheep and pig) that can host them were performed in different databases, using the

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human HMG-CoA reductase deduced amino acid sequence (Uniprot ID: P04035) as a reference. Searches were performed using the Markov models profile (HMMs profile) of the HMMER tools package (Potter *et al.* 2018), using an e-value of 0.01. Searches were restricted by taxonomy, and only sequences from the Metazoa kingdom were selected. Searches were also performed in the WormBase ParaSite parasite-specific database (https://parasite.wormbase.org/index.html) using the deduced amino acid sequence of human HMG-CoA reductase (Uniprot ID: P04035) as reference. These searches were performed on the genomes of platyhelminths and nematodes available on the platform using the BLAST tool. The tool's default parameters were used (matrix: BLOSUM62 and e-value: 0.01).

The quality annotation of the amino acid sequences of HMG-CoA reductases from platyhelminths and parasitic nematodes was validated by genomic and transcriptomic analyses, as follows. First, mRNA sequences from *M. corti* and *E. multilocularis* were quality gauged based on comparisons with available RNA-seq data. Second, the DNA sequences of the HMG-CoA reductase gene from platyhelminths and nematodes (Howe *et al.* 2016, 2017) were translated into three reading frames using the ORFfinder tool (https://www.ncbi.nlm.nih.gov/orffinder/). Finally, to ensure that the amino acid sequences were complete, alignments among the translated amino acid sequences of HMG-CoA reductase from platyhelminths and nematodes and the amino acid sequences of *M. corti* and *E. multilocularis* were performed using clustalW (Larkin *et al.* 2007). After the validation of the obtained HMG-CoA reductase amino acid sequences, an identity and similarity matrix were assembled with the MatGAT tool (Campanella *et al.* 2003) in order to verify the degree of conservation in relation to the human HMG-CoA reductase sequence. To define ortholog proteins, the minimum parameters of 20% identity and 28% similarity were established.

Phylogenetic analyses of HMG-CoA reductase in parasitic helminths and their respective

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hosts

Phylogenetic analyses were performed using the Maximum Likelihood (ML) probabilistic method. HMG-CoA reductase amino acid sequences from 9 species of trematodes, 8 species of cestodes, 4 species of nematodes, and 4 species of parasitic helminths hosts (Supplemental Table 1) were aligned using clustalW (Larkin *et al.* 2007), and evolutionary analyses were conducted in the MEGA X v10.1.8 (Kumar *et al.* 2018), using the JTT+G substitution model (defined by the Find Best DNA/Protein tool), with 5 gamma categories. The consensus tree was inferred with 2000 replicates.

Predictions of the three-dimensional structure of cestode HMG-CoA reductases

Comparative modeling of HMG-CoA reductase from *M. corti* (McosHMGCR), *E. multilocularis* (EmHMGCR), *E. granulosus* (EgHMGCR), and *T. solium* (TsHMGCR) was performed using the Modeller v10.0 tool and SWISS-MODEL (https://swissmodel.expasy.org/).

The target HMG-CoA reductase aminoacid sequences were used as input in the HHpred tool, where the searches for templates were performed. For the choice of templates, the parameters of the function, coverage, identity, and template resolution (< 3 Å) were considered. Subsequently, alignments (PAIR format, input to Modeller) of the amino acid sequences of the templates with the target sequences were performed. Finally, the models were built using the Modeller tool.

The SWISS-MODEL server was also used for comparative modeling of HMG-CoA reductase from *M. corti*, *E. multilocularis*, *E. granulosus*, and *T. solium*. The HMG-CoA reductase target amino acid sequences were used as input on the server to perform the search for templates. For the choice of template, the parameters of GMQE (Global Model Quality Estimation), function, coverage, identity, and mold resolution (< 3 Å) were considered. After

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defining the templates, we performed the alignment between the template sequences and the target sequences. These alignments were used to build the models in SWISS-MODEL.

The models built in Modeller and SWISS-MODEL had their qualities evaluated using the tools PROCHECK (Morris *et al.* 1992), Verify-3D (J.U. Bowie, R. Lüthy, D. Eisenberg 1991; R. Lüthy; J.U. Bowie, D. Eisenberg, 1992), ERRAT (Colovos and Yeates, 1993), available on the SAVES v6.0 server (https://saves.mbi.ucla.edu/), and the QMEAN tool, available on the SWISS-MODEL server. The best model for each analysed cestode target sequence (McosHMGCR, EmHMGCR, EgHMGCR, and TsHMGCR) was chosen based on the evaluations of these tools. The 3D Protein imaging tool (https://3dproteinimaging.com/) was used to visualize the predicted structures.

Prediction of the modes of interaction of simvastatin with cestode HMG-CoA reductases

The molecular docking experiments were performed on the DockThor server (https://dockthor.lncc.br/v2/). For the molecular docking experiments, the predicted structure of cestodes HMG-CoA reductase (McosHMGCR, EmHMGCR, EgHMGCR, and TsHMGCR), ligands simvastatin (PDB), and the adenosine-5-diphosphate (ADP) cofactor (PDB), and the box size x= 22, y=22 and z= 22 were used. These components were used for docking experiments of the predicted structure of McosHMGCR, EmHMGCR, EgHMGCR, and TsHMGCR with the ligands. The box coordinates were x= -30, y= 30 and z= 16 to McosHMGCR structure; x= 63, y= -18 and z= 35 to EmHMGCR structure; x= 62.5, y= -18.4 and z= 36.5 to EgHMGCR structure; finally, x= 49.5, y= 15 and z= 17 to TsHMGCR structure.

Parasite material

The *M. corti* TTs were obtained for assays both in Laboratório de Genômica Estrutural e Funcional, CBiot, UFRGS, Porto Alegre, RS, Brazil and in Instituto de Investigaciones en

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Microbiología y Parasitología Médica, (IMPaM, UBA-CONICET), Universidad de Buenos Aires, Buenos Aires, Argentina. In both laboratories, TTs were maintained *in vivo* by alternate serial passages in Wistar female rats and BALB/c female mice, as previously described (Markoski *et al.* 2003). Experimental hosts were infected by intraperitoneal inoculation and, after 3 months, TTs were collected and used for experiments. Only TTs from up to the third serial passage in mice were used for the experiments. Viability assessment of treated TTs with the inhibitors was performed by trypan blue staining and by motility assay. For experiments to viability assessment of treated TTs by trypan blue staining, TTs freshly collected from mice were washed three times with 50 ml of PBS and stored in RPMI 1640 medium at 4ºC until the moment of use. For *in vitro* cultures, three biological replicates were pooled, each replicate produced using TTs obtained from a single mouse host. Before being used in experimental protocols, TTs were cultured for 24 h in 300 µl of RPMI medium at 37ºC under 5% CO_2 atmosphere. For experiments to motility assessment of treated TTs by motility automated assay, TTs recently collected from mice were washed three times with PBS with levofloxacin (20 µg/ml). For *in vitro* cultures, three biological replicates were used, each one corresponding to TTs obtained from a single mouse host. Prior to use in experiments, TTs were size-selected using monofilament polyester meshes to a final size of 150 to 250 µm. TTs were cultured for 24 h in 200 µl of RPMI medium at 37°C under 5% CO_2 atmosphere. All experimental procedures involving animals were previously approved by the Ethical Committee (CEUA) of the Universidade Federal do Rio Grande do Sul (Project no. 40598) and by the Comité Institucional para el Cuidado y Uso de Animales de Laboratorio (CICUAL), Facultad de Medicina, Universidad de Buenos Aires (UBA), Argentina (protocols: ‘‘*In vivo* passages of cestode parasites from *Mesocestoides vogae*” CD N_ 1127/2015).

Trypan blue staining for preliminary viability assessment of simvastatin-treated TTs

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TTs (15 µl TTs in 300 µl of medium) were cultured in RPMI medium at 37 °C under 5% CO_2 atmosphere in the presence of different concentrations of simvastatin (5 µM, 10 µM, 20 µM, 50 µM, 100 µM, and 150 µM) for 48 h. Albendazole (ABZ) at concentration of 20 µM was used as a positive control, and RPMI medium and DMSO (drug vehicle, 3% DMSO final concentration) were used as negative controls. Due to the high number of TTs per well, the medium was changed daily, and simvastatin or ABZ were re-administered (maintaining the same final concentrations) with each medium change. TTs viability was determined by trypan blue staining, following a previously standardized staining-based techniques protocol to measure the viability of *M. corti* TTs (Fabbri and Elissondo, 2018). After trypan blue staining, TTs were observed under a stereomicroscope, and images were captured to count the number of live and dead TTs. This count was then used to calculate the percentage of viability using the formula: (number of live TTs / total number of TTs) × 100 (Fabbri and Elissondo, 2018). Statistical analyses were carried out using GraphPad Prism 8. One-way ANOVA tests were used to analyse the effects of the simvastatin on *M. corti* motility. Significant differences ($P < 0.05$) were determined by Tukey comparisons post-tests, comparing each simvastatin concentration with the negative controls. Additionally, the parasites were inspected daily to determine any detectable morphological alterations on parasites treated with simvastatin, and the images were taken using an inverted microscope (Axiovert 25, Carl Zeiss) coupled to a digital video camera (Eurekam 5.0, Bel Engineering).

Motility assays for assessment of effect of statins on M. corti *motility*

TTs (4-6 TTs in 200 µL of medium) were cultured in RPMI medium at 37 °C under 5% CO_2 atmosphere in the presence of different concentrations (25 µM, 75 µM, 100 µM, and 150 µM) of either simvastatin or fluvastatin for 9 days. No medium changes were made, and a single dose of treatment was administered due to the low number of individuals per well. Parasites

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pre-treated with ethanol 70% for 30 min and praziquantel (PZQ) at concentration of 20 µM were used as positive controls. RPMI medium and DMSO (1% DMSO final concentration) were used as negative controls. TTs motility was determined using an assay with a worm tracker device (WMicrotracker MINI, Designplus SRL, Argentina), previously standardized for measuring the movement of *M. corti* TTs (Vaca *et al.* 2019, 2021, 2022). Measurements of motility with the WMicrotracker were performed before adding the compounds (day 0) and daily afterward. The data was collected from three independent biological replicates, each corresponding to TTs obtained from a different mouse, in quadruplicate for each tested condition. Relative motility indices, respective to parasite motility before adding the compounds, were determined as described previously (Vaca *et al.* 2019). The percentages of reduction of motility, as described in the Results section, were taken from the relative motility index by using the formula:

$$\% \text{ reduction} = (1\text{-RMI})*100$$

For example, an RMI of 0.3 corresponds to 70% of motility reduction. Statistical analyses were carried out using GraphPad Prism 8. Two-way ANOVA tests were used to analyse the effects of the simvastatin and fluvastatin on *M. corti* viability. Significant differences ($P < 0.05$) were determined by Bonferroni comparisons post-tests, comparing each simvastatin and fluvastatin concentration with the negative controls. In addition, the parasites were inspected daily to determine any possible morphological alterations. Images were taken using an inverted microscope (Primo Vert, Carl Zeiss) coupled to a digital video camera (AxioCam ERc5c, Carl Zeiss).

Results

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Recovery and definition of HMG-CoA reductase sequences from parasitic helminths and their respective hosts

For the identification and definition of orthologs of HMG-CoA reductase from parasitic helminths and their respective hosts, HMG-CoA reductase amino acid sequences were retrieved from public databases and aligned. A total of 101 ortholog HMG-CoA reductase amino acid sequences were recovered (72 from the HMMMER database and 29 from the WormBase ParaSite database). Among the 101 amino acid sequences recovered, only 24 amino acid sequences were selected as orthologs of human HMG-CoA reductase according to the established orthology criteria. The genes coding for these 24 HMG-CoA reductases are in single copies in the corresponding genomes, as no paralogs have been found in the performed searches. The size of the HMG-CoA reductase amino acid sequences from parasitic helminths ranged from 375-1200 aa, with identities and similarities to the human enzyme ranging from 20% to 31% and from 28% to 50%, respectively. Considering host species, the size of the HMG-CoA reductase amino acid sequences ranged from 885-888 aa, with identities and similarities to the human enzyme ranging from 94% to 95% and from 96% to 97%, respectively (results showed in Supplementary Table 1). Furthermore, the alignment of HMG-CoA reductase amino acid sequences from parasitic helminths and their hosts demonstrated the conservation of amino acid residues with identity ranging from 36% to 97% in the catalytic domain, which also contains the active site catalytic residues in conserved positions (Supplementary Figure 1).

Comparing the human reference enzyme with the HMG-CoA reductases from the parasitic helminths (results summarized in Supplementary Table 1), the amino acid sequence of *Taenia saginata* was the one with the lowest degree of conservation (20% identity and 34% similarity). On the other hand, the amino acid sequence with the highest degree of conservation was that of *Hymenolepis diminuta* (31% identity and 50% similarity). For McosHMGCR, the values

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were 29% identity and 44% similarity. Among the species of the genus *Echinococcus*, the values were 27% identity and 40% similarity (*E. multilocularis*) and 27% identity and 38% similarity (*E. granulosus sensu stricto*). TsHMGCR, in turn, presented values of 29% identity and 45% similarity.

Evaluation of the evolutionary conservation of HMG-CoA-reductase amino acid sequences from parasitic helminths and their respective hosts

To reconstruct the evolutionary history of HMG-CoA reductases from some parasitic helminths and their respective hosts, a phylogenetic analysis was performed. A set of 17 amino acid sequences from parasitic flatworms was used, comprising 8 cestode sequences (*E. granulosus, E. multilocularis, H. diminuta, Hymenolepis microstoma, M. corti, Taenia asiatica, T. saginata, and T. solium*), and 9 trematodes species (*Clonorchis sinensis, Fasciola hepatica, Opisthorchis felineus, Opisthorchis viverrini, Schistosoma bovis, Schistosoma haematobium, Schistosoma japonicum, Schistosoma mansoni, and Schistosoma margrebowiei*). An outgroup of 8 amino acid sequences was used in the analysis, comprising 4 nematode species that cause gastrointestinal diseases (*Ascaris lumbricoides, Enterobius vermicularis, Necator americanus, and Strongyloides stercoralis*) and the host species of the assessed helminths (*Bos taurus, Homo sapiens, Ovis aries, and Sus scrofa*). The resulting phylogenetic tree is shown in Figure 1. It revealed three distinct clades: Chordata, platyhelminths and nematodes. Platyhelminths were divided into two monophyletic groups of parasitic worms, one corresponding to trematodes and the other to cestodes. The distinction of the analysed organisms in monophyletic clades demonstrated that the closely related species are grouped in the same branch, reinforcing the conservation of HMG-CoA reductase among these organisms.

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Comparative analysis of HMG-CoA reductase structures from the cestodes M. corti, E. multilocularis, E. granulosus *and* T. solium

To assess the possible forms of interaction of HMG-CoA reductases from cestodes with simvastatin, the three-dimensional structure of these enzymes was modeled for *M. corti* (Figure 2A), *E. multilocularis* (Figure 2B), *E. granulosus* (Figure 2C), and *T. solium* (Figure 2D). For each of these species, at least 12 models were built. These models had their qualities evaluated and the best models were chosen for the next steps. The results of the model quality assessments are presented in Supplementary Materials 1-4.

In all the built cestode models, the general structure of the HMG-CoA reductase is similar to that observed in the structure of human enzyme (Supplementary Figure 2). Furthermore, the HMG-CoA reductase monomer (Supplementary Figure 3) contains a typical structure composed of three domains: an N-terminal helical domain called the N domain (pink), an L domain (blue), and a C-terminal domain called the S domain (grey), as the template enzyme. The S and L domains are connected by a loop assumed to be involved with the enzyme tetramerization. In the homotetramer, the active site contained in each monomer (A, B, C and D) is localized in the L domain interface between two monomers. As expected, not only the primary amino acid sequences of HMG-CoA reductase are conserved among the different cestodes (Figure 3A), but also their overall structures (Figure 3B).

Interactions of simvastatin with the active site of HMG-CoA reductases from M. corti, E. multilocularis, E. granulosus *and* T. solium

To evaluate the possible forms of interactions of simvastatin with the active site of cestode HMG-CoA reductases, molecular docking experiments were performed on the DockThor server with McosHMGCR, EmHMGCR, EgHMGCR, and TsHMGCR. The parameters for molecular docking were calibrated using the redocking strategy using as reference the structure

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of HMG-CoA reductase from *Homo sapiens* (HsHMGCR) complexed with simvastatin (PDB ID: 1hw9). The result of redocking simvastatin with HsHMGCR yielded a binding affinity score of -8.624 (Table 1). We observed that simvastatin interacts with C-chain residues Glu559, Lys735, and Asn755 and with D-chain residues Arg590, Lys691 and Ser684 (Supplementary Figure 4). These values were used for comparative analyses with the cestode HMG-CoA reductase orthologs.

The binding affinity scores of the cestode assessed enzymes with simvastatin are shown in Table 1. In McosHMGCR, simvastatin interacts with C-chain residues Glu543 and Lys719 and with D-chain residues Arg574, Ser668, and Asp674 (Figure 2A). The interactions of EmHMGCR with simvastatin are with residues Glu252, Lys428, and Asn450 of the C-chain and with residues Arg283 and Ser377 of the D-chain (Figure 2B). In EgHMGCR, simvastatin interacts with C-chain residues Glu216, Lys392 and with D-chain residues Arg247 and Ser341 (Figure 2C). Finally, in TsHMGCR simvastatin interacts with C-chain residues Glu389, Lys565, and Asn587 and with D-chain residues Arg420 and Ser514 (Figure 2D). In the comparison of the predicted active sites among the built models for the four orthologs enzymes, it was observed that the Glu and Lys residues of the C-chain and the Arg and Ser residues of the D-chain are always predicted to interact with simvastatin.

Preliminary viability assessment of simvastatin-treated TTs by trypan blue staining

To analyse the effects of simvastatin on *M. corti* with trypan blue staining, *in vitro* assays were performed with different concentrations of simvastatin (5 µM, 10 µM, 20 µM, 50 µM, 100 µM and 150 µM). After 48 h, we observed that simvastatin at concentrations of 100 µM and 150 µM reduced 90% of the viability of treated TTs by the trypan blue stain exclusion test (Figure 4A) with most TTs treated at these concentrations stained blue (Supplementary Figure 5). Optical observations of treated TT with the inverted microscope showed that after 24 h, TTs

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treated with concentrations between 50 µM and 150 µM of simvastatin displayed reduced viability compared to untreated TTs. At 48h, TTs treated with concentrations of 100 µM and 150 µM clearly showed a drastic reduction in viability and progressive tegument damages (Figure 4B). These results indicate that simvastatin decreases the viability of *M. corti* and that this statin is a potential anthelmintic.

*Motility assessment of statins-treated TTs by motility assay*s

To further evaluate the potential cestocidal activity of statins, the effects of simvastatin and fluvastatin on TTs were assessed using motility assays. In these assays, conducted with a worm microtracker device, drug concentrations of 25 µM, 75 µM, 100 µM, and 150 µM were tested. For simvastatin, motility values showed a consistent and significant reduction only at concentrations of 150 µM (from day 1) and 100 µM (from day 6). At the 150 µM concentration, an almost complete reduction in motility was observed from day 5, with motility being fully abolished (100% reduction) by day 7 (Figure 5A). Observations with an inverted microscope showed reduced motility and morphological alterations (including tegument damage) in TTs treated with 150 µM from day 1, and in parasites treated with 100 µM and 75 µM from day 6 (Figure 5B).

The effect of fluvastatin was less potent than simvastatin but was significant at a broader range of concentrations. A significant reduction of motility was observed with 150 and 100 µM concentrations from day 4 and from day 5 with 75 µM. The maximum reduction of motility did not surpass 50% with 150 µM (days 6 and 9), both 100 (day 6) and 75 µM (day 9) reached about 20% reduction in motility (Figure 5C). Furthermore, we observed that TTs treated with a concentration of 150 µM of fluvastatin showed damage to the tegument of the parasites from day 6 of treatment (Figure 5D). These results reinforce that statins can reduce TTs motility and demonstrate that treatment with this inhibitor has an anthelmintic effect on *M. corti*.

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Discussion

Drug repurposing is the identification of new therapeutic uses for drugs that are already commercially available or in clinical development. One of the main advantages of using this strategy is the reduction of research time and the cost involved in the development of new drugs (Ashburn and Thor, 2004; Xue *et al.* 2018). Therefore, it has gained increasing importance in the search for new therapeutic uses for proven safe and effective drugs to be repositioned for the treatment of diseases (Serafin and Hörner, 2018).

Strategies of drug repurposing can be especially useful to identify new therapeutic uses for the treatment of rare and neglected diseases for which there are no effective treatments available (Ekins *et al.* 2011; Hamid *et al.* 2024). With the continuous advancement in the generation and analysis big data technologies, including those referring to structural analyses, and the deepening of the knowledge on the pathophysiology of diseases, it is expected that the drug repurposing will become increasingly effective for the treatment of infectious diseases, including parasitic ones (Bustamante *et al.* 2019; Pandey *et al.* 2020).

The HMG-CoA reductase gene has been identified in organisms from all three domains of life (Archaea, Bacteria, and Eukarya), and amino acid sequence comparisons along with phylogenetic analyses have shown that the catalytic domain is highly conserved in eukaryotes, although the domain of membrane anchor is less conserved (Friesen and Rodwell, 2004). Furthermore, in genomes from higher metazoans, archaea, and eubacteria, only a single copy of the HMG-CoA reductase gene has been found. In line with that, a single HMG-CoA reductase gene was identified here for parasitic helminths, and the catalytic domain (residues 587-1102) of the encoded ortholog enzymes was conserved among the assessed species. The conservation of HMG-CoA reductase and the absence of paralogous genes favor the idea of repurposing of simvastatin not only for the treatment of cestodiases, but also for the treatment

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of other diseases caused by parasitic helminths. It has already been shown that simvastatin has anthelmintic potential against *S. mansoni*, causing death of schistosomules and adults *in vitro* (Rojo-Arreola *et al.* 2014). Furthermore, the presence of a single copy of the HMG-CoA reductase gene reduces the chance of developing resistance by switching to alternate genes.

The evolutionary history of HMG-CoA reductase was assessed based on a phylogenetic analysis, confirming that orthologs from chordates, platyhelminths and nematodes are separated in distinct clades, as expected. Considering that closely related species were grouped in the same branch, it was confirmed that the HMG-CoA reductase gene has diversified in parallel with the separation of those taxa and is in line with other HMG-CoA reductase phylogenetic studies carried out for other taxonomic groups such as crustaceans, fish, birds, insects, mammals, and plants (Zhao *et al.* 2015; Lü *et al.* 2016). Overall, it was observed that HMG-CoA reductase is conserved enough among different taxa, even among more distant ones, to allow a dependable identification of orthologs.

The structural analyses also showed an overall conservation of HMG-CoA reductase domain structures, comparing McosHMGCR, EmHMGCR, EgHMGCR, and TsHMGCR with the reference HsHMGCR. Conservation is evident also in the comparisons among active sites, both in terms of predicted structure and amino acid identities. This corroborates the idea that human HMG-CoA inhibitor would also bind and inhibit the activity of the assessed cestode orthologs.

Statins competitively inhibit HMG-CoA reductase. Due to their HMG-CoA analogous structures, when these inhibitors bind to HMG-CoA reductase, they occupy part of the HMG-CoA binding site, thus blocking the access of this substrate to the enzyme (Istvan *et al.* 2000). In addition to this similarity with HMG-CoA, the strong binding of statins is correlated to the large number of Van der Waals interactions and hydrogen bonds between these inhibitors and HMG-CoA-reductase (Istvan, 2001). In line with that, our molecular docking analyses

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demonstrated that the amino acid residues present in the predicted structures of McosHMGCR, EmHMGCR, EgHMGCR, and TsHMGCR would interact with simvastatin essentially in positions correspondent to that observed in the interaction between simvastatin and HsHMGCR crystal (Pandey *et al.* 2020). This also points out to the potential of simvastatin as specific inhibitor for cestode HMG-CoA reductases.

M. corti is a model cestode widely used to identify new anthelmintic compounds in pharmacological studies (Markoski, 2006; Maggiore and Elissondo, 2014; Vaca *et al*. 2019, 2021, 2022). Since this species is not infectious to humans and can be easily maintained *in vivo* and *in vitro*, it provides continuous availability of biological material, an important consideration in the work with cestodes-due to the scarcity of material suitable for laboratory work from zoonotic species (Markoski *et al*. 2003; Hemphill, 2010; Lustigman *et al*. 2012). However, as *M. corti* larvae are virtually non-infective to humans, it will be important to carry out future experiments to assess statin effects also on human-infecting species, like *E. granulosus* or *E. multilocularis*.

There are few standardized techniques to measure the viability of *M. corti* TTs in pharmacological experiments (Saldaña *et al*. 2003; Maggiore and Elissondo, 2014). Simple optical observation of movement and structural features are used as a standard to evaluate qualitatively parasite viability (Hemphill 2010). To improve the screening of compounds with possible anthelmintic effects, staining-based techniques employing the use of vital dyes, and a motility assay have been standardized to assess the viability and motility of TTs more accurately and quantitatively (Fabbri and Elissondo, 2018; Vaca *et al*. 2019).

In the present work, we employed a staining-based technique (using trypan blue dye) to preliminary determine the viability of TTs that were treated with different concentrations of simvastatin. Furthermore, we also used the motility assay to evaluate the motility of TTs that were treated with different concentrations of simvastatin and fluvastatin. In these experiments,

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we qualitatively observed (by optical microscope) that simvastatin and fluvastatin reduced motility and caused damage to the tegument of treated TTs, indicating that the treatment reduced the viability of the parasites. Using the staining-based technique and the motility assay, as expected, we observed and quantified with both methodologies the effects of simvastatin on the viability and motility of treated TTs, and the effects of fluvastatin on the motility of treated TTs. Simvastatin was the inhibitor with the most significant effect on *M. corti*. Treatment with a concentration of 150 µM reduced the viability and motility of TTs in a short period time, indicating that simvastatin has a stronger anthelmintic effect on *M. corti* larvae. In terms of *in vitro* efficacy, simvastatin showed promising results against cestodes, suggesting its therapeutic potential for treating parasitic infections. However, the need to use significantly higher concentrations to achieve the desired effects, as observed in our study, may pose challenges when extrapolating these findings to clinical practice, as high doses of simvastatin could increase the risk of toxicity. While *in vitro* assays provide initial insights into the antiparasitic potential of simvastatin, applying these concentrations *in vivo* requires caution. Moreover, further investigations using animal models are essential to evaluate the efficacy of simvastatin against larval cestodes *in vivo*, with the goal of identifying doses that strike a balance between antiparasitic efficacy and safety.

Fluvastatin has also been shown to affect *M. corti*. However, the effect of fluvastatin was smaller when compared to the effect of simvastatin. This reduction in the effect of fluvastatin may be related to structural changes made to synthetic statins (such as fluvastatin) to increase the effectiveness of these inhibitor's interactions with human HMG-CoA reductase (Schachter, 2005). Given that structural modifications may enhance inhibitor efficacy, we hypothesize that altering the structure of simvastatin or fluvastatin could improve their interaction with cestode HMG-CoA reductases. However, the molecular mechanisms underlying the effects of simvastatin and fluvastatin on cestodes remain unknown, and further

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studies are necessary to better understand and enhance their effects on these helminth parasites.

The conservation of HMG-CoA reductase in different organisms is associated to the importance of the mevalonate pathway, as this pathway is considered vital for most organisms. It has already been demonstrated that inhibition of HMG-CoA reductase can lead to loss of the non-steroidal branch of the pathway that produces essential non-steroidal isoprenoids, such as geranylgeranyl diphosphate/pyrophosphate (GGPP) and farnesyl diphosphate/pyrophosphate (FPP) (Ranji *et al.* 2014). These molecules are essential for normal cellular functioning and are indispensable for several processes such as protein prenylation (farnesylation or geranylgeranylaton) of small GTPases and synthesis of isopentenylated tRNA (Rauthan and Pilon, 2011; Lasunción *et al*. 2022). In parasitc organisms, such as the protozoan *Entamoeba histolytica* and in the helminth *S. mansoni*, it has been shown that the inhibition of HMG-CoA reductase and farnesyltransferase leads to the loss of several functions such as cell differentiation and growth (Probst *et al.* 2019). In the tapeworm *E. multilocularis*, it has already been demonstrated that a Ras-like (Ral) small GTP-binding protein undergo prenylation in the form of geranylgeranylation (Spiliotis and Brehm, 2004). Therefore, inhibition of HMG-CoA reductase and geranylgeranyl transferase can lead to loss of key cellular processes mediated by Ral proteins, such as vesicle transport and migration and cell division. Thus, HMG-CoA reductase emerges as a promising therapeutic target for both parasitic protozoans and helminths.

The mevalonate pathway is essential for the survival of parasitic helminths. This pathway, as it plays an important role in the production of key cellular components, has become an interesting target for drug repositioning. Our *in silico* analyses showed that the constructed 3D-models McosHMGCR, EmHMGCR, EgHMGCR, and TsHMGCR have structural similarities with HsHMGCR, indicating that human enzyme inhibitors could be effective against cestode enzymes. This was corroborated by our *in vitro* viability and motility assays

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that demonstrated dose-dependent viability and motility reduction in *M. corti* larvae, upon treatment with simvastatin or fluvastatin. Overall, our *in silico* and *in vitro* experimental approaches provided evidence that HMG-CoA reductase constitutes a promising therapeutic target for the repositioning of simvastatin or other statins as anthelmintic drugs to treat larval cestodiases. Our results pave the way for assessing the effects of statins *in vivo* through experiments using experimentally infected hosts.

Supplementary material. The supplementary material for this article can be found at [DOI].

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https://doi.org/10.1017/S0031182024001586 Published online by Cambridge University Press

References

Ashburn TT and Thor KB (2004) Drug repositioning: Identifying and developing new uses for existing drugs. *Nature Reviews Drug Discovery*. Nature Publishing Group, 673–683. https://doi.org/10.1038/nrd1468.

Bustamante C, Ochoa R, Asela C and Muskus C (2019) Repurposing of known drugs for leishmaniasis treatment using bioinformatic predictions, in vitro validations and pharmacokinetic simulations. *Journal of Computer-Aided Molecular Design* **33**(9), 845–854. https://doi.org/10.1007/s10822-019-00230-y.

Campanella JJ, Bitincka L and Smalley J (2003) MatGAT: An application that generates similarity/identity matrices using protein or DNA sequences. *BMC Bioinformatics* **4**, 1–4. https://doi.org/10.1186/1471-2105-4-29.

Cancela M, Paes JA, Moura H, Barr JR, Zaha A and Ferreira HB (2019) Unraveling oxidative stress response in the cestode parasite Echinococcus granulosus. *Scientific Reports* **9**(1). https://doi.org/10.1038/s41598-019-52456-3.

Claerebout E, De Wilde N, Van Mael E, Casaert S, Velde F Vande, Roeber F, Veloz PV, Levecke B and Geldhof P (2020) Anthelmintic resistance and common worm control practices in sheep farms in Flanders, Belgium. *Veterinary Parasitology: Regional Studies and Reports* **20**(January), 100393. https://doi.org/10.1016/j.vprsr.2020.100393.

Coghlan A, Tyagi R, Cotton JA, Holroyd N, Rosa BA, Tsai IJ, Laetsch DR, Beech RN, Day TA, Hallsworth-Pepin K, Ke HM, Kuo TH, Lee TJ, Martin J, Maizels RM, Mutowo P, Ozersky P, Parkinson J, Reid AJ, Rawlings ND, Ribeiro DM, Swapna LS, Stanley E, Taylor DW, Wheeler NJ, Zamanian M, Zhang X, Allan F, Allen JE, Asano K, Babayan SA, Bah G, Beasley H, Bennett HM, Bisset SA, Castillo E, Cook J, Cooper PJ, Cruz-Bustos T, Cuéllar C, Devaney E, Doyle SR, Eberhard ML, Emery A, Eom KS, Gilleard JS, Gordon D, Harcus Y, Harsha B, Hawdon JM, Hill DE,

https://doi.org/10.1017/S0031182024001586 Published online by Cambridge University Press

Hodgkinson J, Horák P, Howe KL, Huckvale T, Kalbe M, Kaur G, Kikuchi T, Koutsovoulos G, Kumar S, Leach AR, Lomax J, Makepeace B, Matthews JB, Muro A, O'Boyle NM, Olson PD, Osuna A, Partono F, Pfarr K, Rinaldi G, Foronda P, Rollinson D, Samblas MG, Sato H, Schnyder M, Scholz T, Shafie M, Tanya VN, Toledo R, Tracey A, Urban JF, Wang LC, Zarlenga D, Blaxter ML, Mitreva M and Berriman M (2019) Comparative genomics of the major parasitic worms. *Nature Genetics* **51**(1), 163–174. https://doi.org/10.1038/s41588-018-0262-1.

Colovos C and Yeates TO (1993) Verification of protein structures: Patterns of nonbonded atomic interactions. *Protein Science* **2**(9), 1511–1519. https://doi.org/10.1002/pro.5560020916.

de Andrade Picanço G, de Lima NF, Fraga CM, da Costa TL, Isac E, Ambrosio J, Castillo R and Vinaud MC (2017) A benzimidazole derivative (RCB15) in vitro induces the alternative energetic metabolism and glycolysis in Taenia crassiceps cysticerci. *Acta Tropica* **176**, 288–292. https://doi.org/10.1016/j.actatropica.2017.08.022.

Demierre MF, Higgins PDR, Gruber SB, Hawk E and Lippman SM (2005, December) Statins and cancer prevention. *Nature Reviews Cancer*. 930–942. https://doi.org/10.1038/nrc1751.

Ekins S, Williams AJ, Krasowski MD and Freundlich JS (2011, April) In silico repositioning of approved drugs for rare and neglected diseases. *Drug Discovery Today*. 298–310. https://doi.org/10.1016/j.drudis.2011.02.016.

Fabbri J and Elissondo MC (n.d.) Comparison of different staining methods for determination of viability on Mesocestoides vogae tetrathyridia. https://doi.org/10.1007/s00436-018-6143-9.

Friesen JA and Rodwell VW (2004) *The 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductases Gene organization and evolutionary history*. Retrieved from

https://doi.org/10.1017/S0031182024001586 Published online by Cambridge University Press

http://genomebiology.com/2004/5/11/248

Gilman RH, Gonzalez AE, Llanos-Zavalaga F, Tsang VCW and Garcia HH (2012) Prevention and control of Taenia solium taeniasis/cysticercosis in Peru. *Pathogens and Global Health* **106**(5), 312–318. https://doi.org/10.1179/2047773212Y.0000000045.

Haby MM, Sosa Leon LA, Luciañez A, Nicholls RS, Reveiz L and Donadeu M (2020) *Systematic review of the effectiveness of selected drugs for preventive chemotherapy for taenia solium taeniasis PLoS Neglected Tropical Diseases*, Vol. 14. https://doi.org/10.1371/journal.pntd.0007873.

Hamid A, Mäser P and Mahmoud AB (2024, February 1) Drug Repurposing in the Chemotherapy of Infectious Diseases. *Molecules*. Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/molecules29030635.

Hemphill A (2010) Development and applications of cestode and trematode laboratory models. *Parasitology* **137**(3), 329–333. https://doi.org/10.1017/S0031182010000132.

Howe KL, Bolt BJ, Cain S, Chan J, Chen WJ, Davis P, Done J, Down T, Gao S, Grove C, Harris TW, Kishore R, Lee R, Lomax J, Li Y, Muller HM, Nakamura C, Nuin P, Paulini M, Raciti D, Schindelman G, Stanley E, Tuli MA, Van Auken K, Wang D, Wang X, Williams G, Wright A, Yook K, Berriman M, Kersey P, Schedl T, Stein L and Sternberg PW (2016) WormBase 2016: Expanding to enable helminth genomic research. *Nucleic Acids Research* **44**(D1), D774–D780. https://doi.org/10.1093/nar/gkv1217.

Howe KL, Bolt BJ, Shafie M, Kersey P and Berriman M (2017) WormBase ParaSite − a comprehensive resource for helminth genomics. *Molecular and Biochemical Parasitology* **215**, 2–10. https://doi.org/10.1016/j.molbiopara.2016.11.005.

Istvan ES (2001) Structural Mechanism for Statin Inhibition of HMG-CoA Reductase. *Science* **292**(5519), 1160–1164. https://doi.org/10.1126/science.1061020.

Istvan ES, Palnitkar M, Buchanan SK and Deisenhofer J (2000) Crystal structure of the catalytic portion of human HMG-CoA reductase: Insights into regulation of activity and catalysis. *EMBO Journal* **19**(5), 819–830. https://doi.org/10.1093/emboj/19.5.819.

Jiang W, Hu JW, He XR, Jin WL and He XY (2021) Statins: a repurposed drug to fight cancer. *Journal of Experimental and Clinical Cancer Research* **40**(1), 1–33. https://doi.org/10.1186/s13046-021-02041-2.

J.U. BOWIE, R. LÜTHY, D. EISENBERG 1991 (1991) A Method to Identify Protein Sequences That Fold into a Known Three-Dimensional Stucture. *Angewandte Chemie International Edition, 6(11), 951–952.* **253**(58).

Karlic H and Varga F (2017) Mevalonate Pathway. In *Reference Module in Biomedical Sciences*. Elsevier. https://doi.org/10.1016/b978-0-12-801238-3.65000-6.

Kazi DS, Penko JM and Bibbins-Domingo K (2017) Statins for Primary Prevention of Cardiovascular Disease: Review of Evidence and Recommendations for Clinical Practice. *Medical Clinics of North America* **101**(4), 689–699. https://doi.org/10.1016/J.MCNA.2017.03.001.

Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**(6), 1547–1549. https://doi.org/10.1093/molbev/msy096.

Larkin MA, Blackshields G, Brown NP, Chenna R, Mcgettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ and Higgins DG (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**(21), 2947–2948. https://doi.org/10.1093/bioinformatics/btm404.

Lasunción MA, Martínez-Botas J, Martín-Sánchez C, Busto R and Gómez-Coronado D (2022, February 1) Cell cycle dependence on the mevalonate pathway: Role of cholesterol and non-sterol isoprenoids. *Biochemical Pharmacology*. Elsevier Inc.

https://doi.org/10.1017/S0031182024001586 Published online by Cambridge University Press

https://doi.org/10.1016/j.bcp.2021.114623.

Liao, James K. and Laufs U (2005) Pleiotropic effects of statins. *Annual Review of Pharmacology and Toxicology* **45**(5), 89–118. https://doi.org/10.4103/2230-8210.163106.

Lü S, Jiang M, Huo T, Li X and Zhang Y (2016) 3-hydroxy-3-methyl glutaryl coenzyme A reductase: An essential actor in the biosynthesis of cantharidin in the blister beetle Epicauta chinensis Laporte. *Insect Molecular Biology* **25**(1), 58–71. https://doi.org/10.1111/imb.12198.

Lustigman S, Prichard RK, Gazzinelli A, Grant WN, Boatin BA, McCarthy JS and Basáñez MG (2012, April) A research agenda for helminth diseases of humans: The problem of helminthiases. *PLoS Neglected Tropical Diseases*. https://doi.org/10.1371/journal.pntd.0001582.

Maggiore M and Elissondo MC (2014) In vitro cestocidal activity of thymol on Mesocestoides corti tetrathyridia and adult worms. *Interdisciplinary Perspectives on Infectious Diseases* **2014**. https://doi.org/10.1155/2014/268135.

Markoski MM (2006) Estudo do desenvolvimento in vitro de Mesocestoides corti (Platyhelminthes:Cestoda). 1–110.

Markoski MM, Bizarro C V, Farias S, Espinoza I, Galanti N, Zaha A and Ferreira HB (2003) In vitro segmentation induction of Mesocestoides corti (Cestoda) tetrathyridia. *The Journal of Parasitology* **89**(1), 27–34. https://doi.org/10.1645/0022-3395(2003)089[0027:IVSIOM]2.0.CO;2.

Morris AL, MacArthur MW, Hutchinson EG and Thornton JM (1992) Stereochemical quality of protein structure coordinates. *Proteins: Structure, Function, and Bioinformatics* **12**(4), 345–364. https://doi.org/10.1002/prot.340120407.

Paludo GP, Thompson CE, Miyamoto KN, Guedes RLM, Zaha A, De Vasconcelos ATR, Cancela M and Ferreira HB (2020) Cestode strobilation: Prediction of developmental

genes and pathways. *BMC Genomics* **21**(1), 1–16. https://doi.org/10.1186/s12864-020-06878-3.

Pan American Health Organization (2021). Guideline for Preventive Chemotherapy for the Control of *Taenia solium* Taeniasis. Washington, D.C.: Pan American Health Organization. https://doi.org/10.37774/9789275123720.

Pandey RP, Nascimento MS, Moore CE, Raj VS, Kalil J and Cunha-Neto E (2020) New Approaches for the Treatment of Chagas Disease. *Current Drug Targets* **22**(7), 835–841. https://doi.org/10.2174/1389450121999201124122643.

Parquet V, Henry M, Wurtz N, Dormoi J, Briolant S, Gil M, Baret E, Amalvict R, Rogier C and Pradines B (2010) Atorvastatin as a potential anti-malarial drug: in vitro synergy in combinational therapy with quinine against Plasmodium falciparum. *Malaria Journal* **9**, 139. https://doi.org/10.1186/1475-2875-9-139.

Peres RB, Silva PB, Batista MM, Aiub CAF, Felzenszwalb I and Soeiro MNC (2018) crossm Repurposing Strategy of Atorvastatin against Trypanosoma Benznidazole Exhibit Synergistic Trypanocidal Activity. 1–9.

Potter SC, Luciani A, Eddy SR, Park Y, Lopez R and Finn RD (2018) HMMER web server: 2018 update. *Nucleic Acids Research* **46**(W1), W200–W204. https://doi.org/10.1093/nar/gky448.

Probst A, Nguyen TN, El-Sakkary N, Skinner D, Suzuki BM, Buckner FS, Gelb MH, Caffrey CR and Debnath A (2019) Bioactivity of farnesyltransferase inhibitors against entamoeba histolytica and schistosoma mansoni. *Frontiers in Cellular and Infection Microbiology* **9**(MAY). https://doi.org/10.3389/fcimb.2019.00180.

R. LÜTHY; J.U. Bowie, D. Eisenberg 1992 (1992) Assessment of protein models with three-dimensional profiles. *Nature* **359**, 167–169.

Ramiandrasoa NS, Ravoniarimbinina P, Solofoniaina AR, Rakotomanga IPA,

Andrianarisoa SH, Molia S, Labouche AM, Fahrion AS, Donadeu M, Abela-Ridder B and Rajaonatahina D (2020) Impact of a 3-year mass drug administration pilot project for taeniasis control in Madagascar. *PLoS Neglected Tropical Diseases* **14**(9), 1–13. https://doi.org/10.1371/journal.pntd.0008653.

Ranji P, Rauthan M, Pitot C and Pilon M (2014) Loss of HMG-CoA reductase in C. elegans causes defects in protein prenylation and muscle mitochondria. *PLoS ONE* **9**(6). https://doi.org/10.1371/journal.pone.0100033.

Rauthan M and Pilon M (2011) *The mevalonate pathway in C. Elegans*. Retrieved from http://www.lipidworld.com/content/10/1/243

Ray S (2024, March 1) Role of statins in the management of dyslipidaemia. *Indian Heart Journal*. Elsevier B.V., S33–S37. https://doi.org/10.1016/j.ihj.2023.11.267.

Rojo-Arreola L, Long T, Asarnow D, Suzuki BM, Singh R and Caffrey CR (2014) Chemical and genetic validation of the statin drug target to treat the helminth disease, schistosomiasis. *PLoS ONE* **9**(1). https://doi.org/10.1371/journal.pone.0087594.

Saari S, Näreaho A and Nikander S (2019) Cestoda (Tapeworms). In *Canine Parasites and Parasitic Diseases*. Elsevier, 55–81. https://doi.org/10.1016/b978-0-12-814112-0.00004-0.

Saldaña J, Casaravilla C, Marín M, Fernández C and Domínguez L (2003) The toxicity of praziquantel against Mesocestoides vogae (syn. corti) tetrathyridia can be assessed using a novel in vitro system. *Parasitology Research* **89**(6), 467–472. https://doi.org/10.1007/s00436-002-0801-6.

Sanfelice RA, da Silva SS, Bosqui LR, Miranda-Sapla MM, Barbosa BF, Silva RJ, Ferro EAV, Panagio LA, Navarro IT, Bordignon J, Conchon-Costa I, Pavanelli WR, Almeida RS and Costa IN (2017) Pravastatin and simvastatin inhibit the adhesion, replication and proliferation of Toxoplasma gondii (RH strain) in HeLa cells. *Acta Tropica*

https://doi.org/10.1017/S0031182024001586 Published online by Cambridge University Press

167, 208–215. https://doi.org/10.1016/j.actatropica.2016.12.006.

Sangster NC, Cowling A and Woodgate RG (2018, July 1) Ten Events That Defined Anthelmintic Resistance Research. *Trends in Parasitology*. Elsevier Ltd, 553–563. https://doi.org/10.1016/j.pt.2018.05.001.

Schachter M (2005a) Chemical, pharmacokinetic and pharmacodynamic properties of statins: An update. *Fundamental and Clinical Pharmacology* **19**(1), 117–125. https://doi.org/10.1111/j.1472-8206.2004.00299.x.

Schachter M (2005b, February) Chemical, pharmacokinetic and pharmacodynamic properties of statins: An update. *Fundamental and Clinical Pharmacology*. 117–125. https://doi.org/10.1111/j.1472-8206.2004.00299.x.

Serafin MB and Hörner R (2018, May) Drug repositioning, a new alternative in infectious diseases. *Brazilian Journal of Infectious Diseases*. 252–256. https://doi.org/10.1016/j.bjid.2018.05.007.

Spiliotis M and Brehm K (2004) Echinococcus multilocularis: Identification and molecular characterization of a Ral-like small GTP-binding protein. *Experimental Parasitology* **107**(3–4), 163–172. https://doi.org/10.1016/j.exppara.2004.05.006.

Vaca HR, Celentano AM, Macchiaroli N, Kamenetzky L, Camicia F and Rosenzvit M (2019) Histone deacetylase enzymes as potential drug targets of Neglected Tropical Diseases caused by cestodes. *International Journal for Parasitology: Drugs and Drug Resistance*. https://doi.org/10.1016/j.ijpddr.2019.02.003.

Vaca HR, Celentano AM, Toscanini MA, Hauser AT, Macchiaroli N, Cuestas ML, Nusblat AD, Sippl W, Elissondo MC, Jung M, Camicia F and Rosenzvit MC (2022) Identification and characterization of sirtuin enzymes in cestodes and evaluation of sirtuin inhibitors as new cestocidal molecules. *International Journal for Parasitology* **52**(5), 317–329. https://doi.org/10.1016/j.ijpara.2021.12.002.

Vaca HR, Celentano AM, Toscanini MA, Heimburg T, Ghazy E, Zeyen P, Hauser AT, Oliveira G, Elissondo MC, Jung M, Sippl W, Camicia F and Rosenzvit MC (2021) The potential for histone deacetylase (HDAC) inhibitors as cestocidal drugs. *PLoS Neglected Tropical Diseases* **15**(3). https://doi.org/10.1371/journal.pntd.0009226.

Wang Q, Zhong B, Yu W, Zhang G, Budke CM, Liao S, He W, Chen F, Xu K, Xie F, Danbazeli, Wang Q, Yang L, Huang Y, Li R, Yao R, Giraudoux P and Craig PS (2021) Assessment of a 10-year dog deworming programme on the transmission of Echinococcus multilocularis in Tibetan communities in Sichuan Province, China. *International Journal for Parasitology* **51**(2–3), 159–166. https://doi.org/10.1016/j.ijpara.2020.08.010.

Woolsey ID and Miller AL (2021) Echinococcus granulosus sensu lato and Echinococcus multilocularis: A review. *Research in Veterinary Science* **135**(November 2020), 517–522. https://doi.org/10.1016/j.rvsc.2020.11.010.

Xue H, Li J, Xie H and Wang Y (2018, July 13) Review of drug repositioning approaches and resources. *International Journal of Biological Sciences*. Ivyspring International Publisher, 1232–1244. https://doi.org/10.7150/ijbs.24612.

Zhao M, Jiang K, Song W, Ma C, Wang J, Meng Y, Wei H, Chen K, Qiao Z, Zhang F and Ma L (2015) Two transcripts of HMG-CoA reductase related with developmental regulation from Scylla paramamosain: Evidences from cDNA cloning and expression analysis. *IUBMB Life* **67**(12), 954–965. https://doi.org/10.1002/iub.1452.

Table 1. Molecular docking results of the drug simvastatin. Binding affinity score (given in kcal/mol-1) of the drug simvastatin with the HsHMGCR, McosHMGCR, EmHMGCR, EgHMGCR and TsHMGCR.

Enzymes	simvastatin
HsHMGCR	-8.624
McosHMGCR	-8.391
EmHMGCR	-8.150
EgHMGCR	-8.079
TsHMGCR	-8.172

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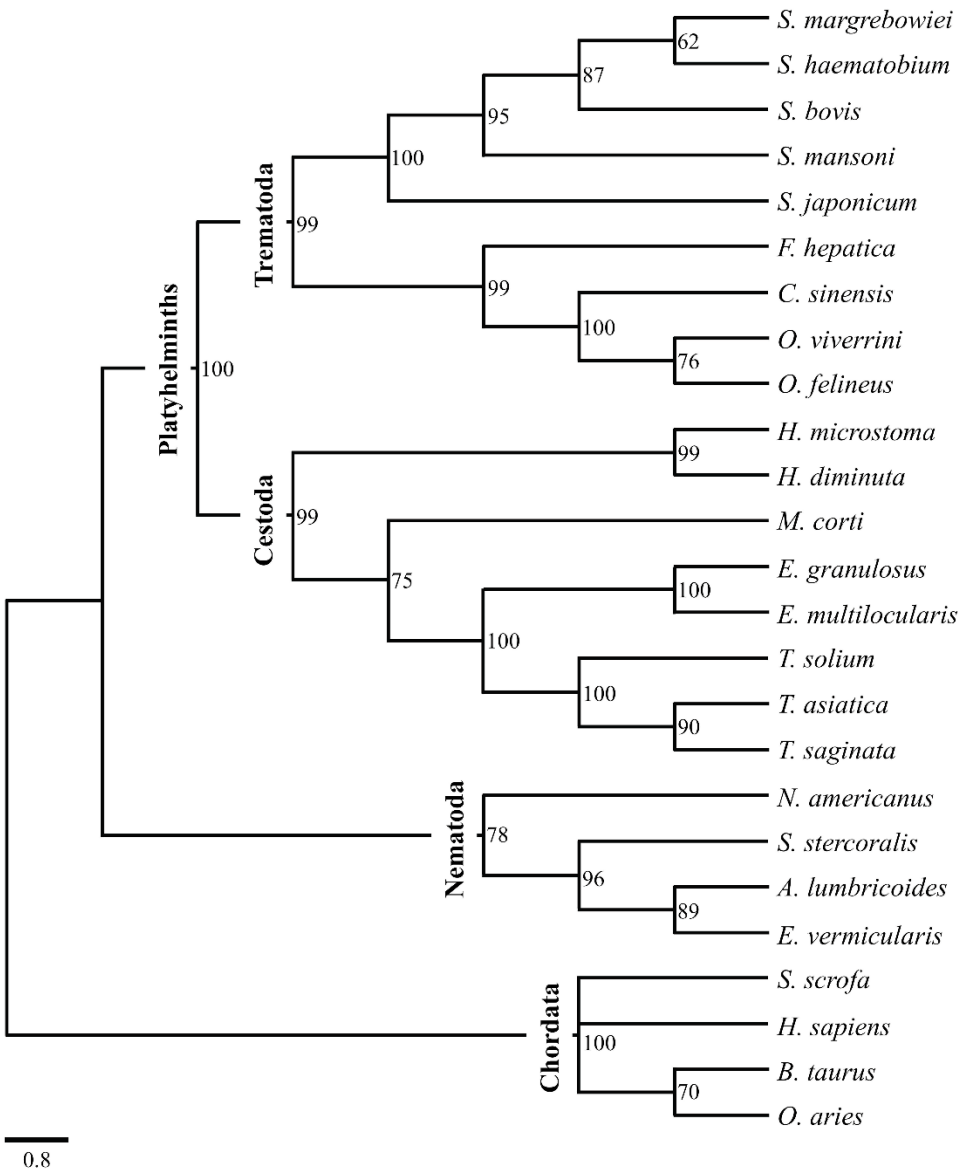

Figure 1. Phylogenetic tree of HMG-CoA reductase from parasitic helminths and their respective hosts. Phylogenetic tree showing the relative conservation of HMG-CoA reductase in different parasitic helminths. The analysis involved 25 amino acid sequences, 17 amino acid sequences from platyhelminths, 4 amino acid sequences from their hosts, and 4 amino acid sequences from nematodes (outgroup). The phylogenetic tree was obtained using the Maximum Likelihood (ML) probabilistic method based on the JTT matrix-based model with 5 gamma categories. The consensus tree was inferred from 2000 replicates.

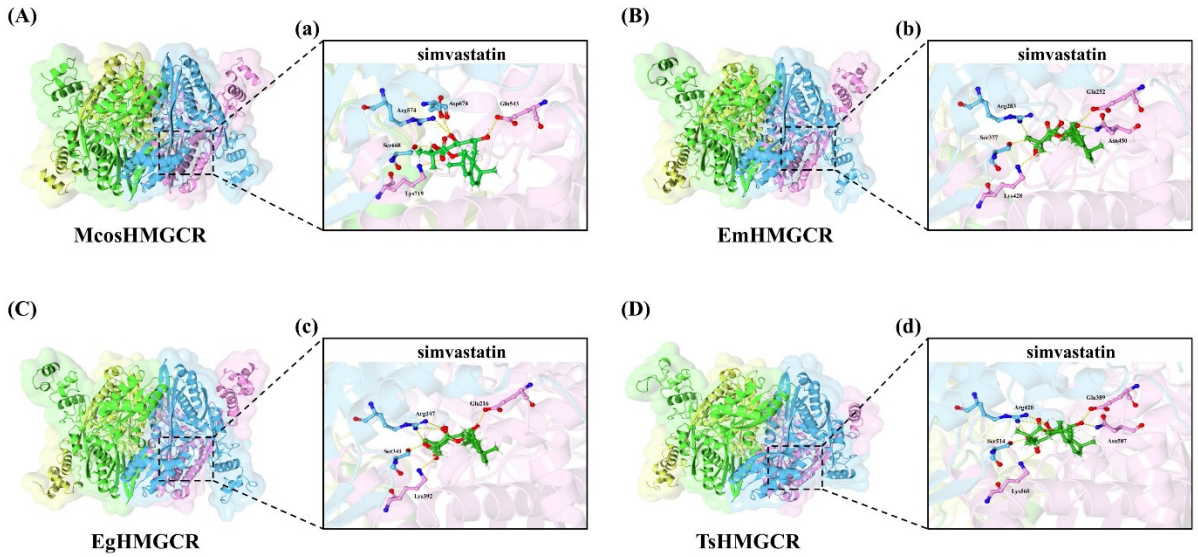

Figure 2. Three-dimensional structures of McosHMGCR, EmHMGCR, EgHMGCR, and TsHMGCR and interactions with simvastatin. Predicted homotetrameric structures of (A) McosHMGCR, (B) EmHMGCR, (C) EgHMGCR and (D) TsHMGCR, with the different monomer chains shown in green, yellow, pink and blue. Active sites and interactions with the ligands are shown in the boxed detail for (a) McosHMGCR, (b) EmHMGCR, (c) EgHMGCR and (d) TsHMGCR. The structure of simvastatin is shown in green, and hydrogen bonds between it and amino acids from the assessed cestode HMG-CoA reductases are shown as yellow dotted lines.

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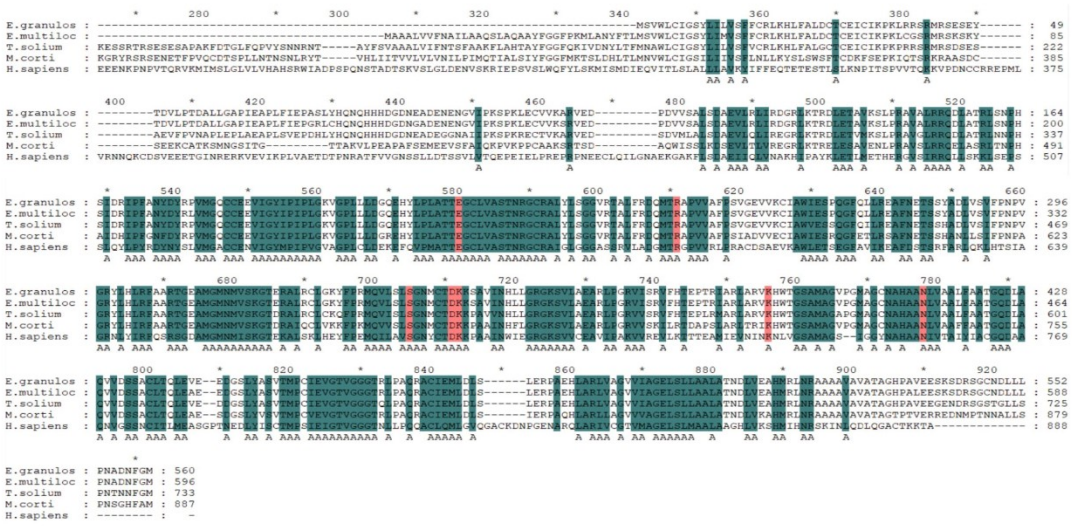
A



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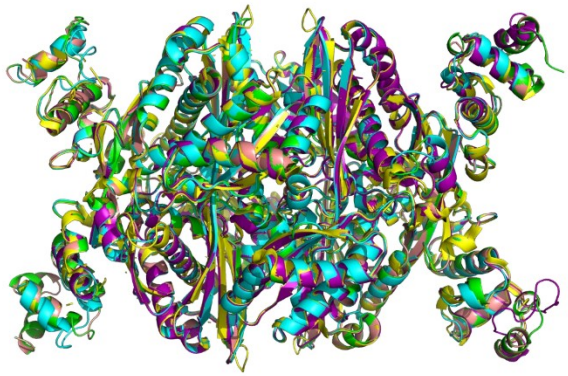

Figure 3. Sequence and structure comparisons of McosHMGCR, EmHMGCR, EgHMGCR, TsHMGCR and HsHMGCR. (A) Alignment of the amino acid sequences of McosHMGCR, EmHMGCR, EgHMGCR, TsHMGCR and HsHMGCR. The catalytic amino acid, identical in all enzymes, are highlighted in salmon. Other identical amino acids are highlighted in dark green. (B) Superposition of the tetrameric structures of McosHMGCR (cyan), EmHMGCR (salmon), EgHMGCR (green), TsHMGCR (purple), and HsHMGCR (yellow).

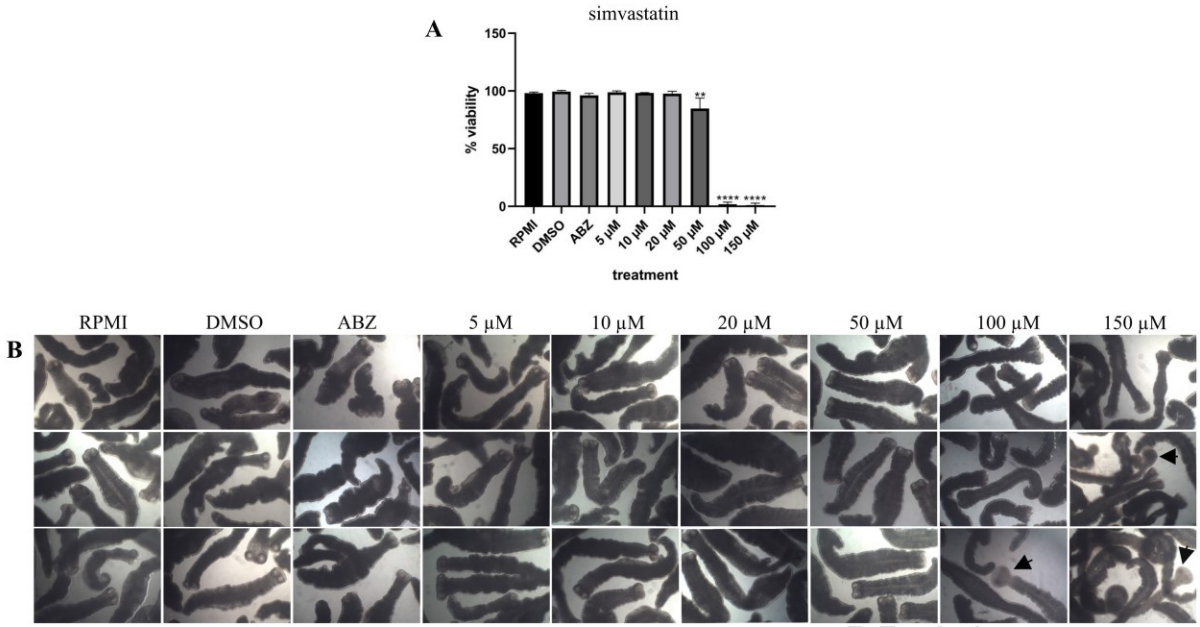

Figure 4. Effects of simvastatin on the viability of *M. corti* tetrathyridia (TTs). (A) TTs were treated for 48h with concentrations of 5 µM, 10 µM, 20 µM, 50 µM, 100 µM and 150 µM of simvastatin. The following were used as negative controls: RPMI and DMSO; and as positive control: ABZ. The experiment was performed in triplicate. The error bars represent the standard deviation, and the asterisks indicate those values that presented differences with statistical significance compared to the negative controls, according to the ANOVA test and Tukey's post-test (**P < 0.01; ****P < 0.0001). (B) Inverted optical microscope images of treated TTs with 5 µM, 10 µM, 20 µM, 50 µM, 100 µM and 150 µM concentrations of simvastatin on the last day of treatment (48h). Treated TTs with 100 µM and 150 µM concentrations appear with tegument damage (arrows) compared to negative controls (RPMI and DMSO). All images are at 5x magnification.

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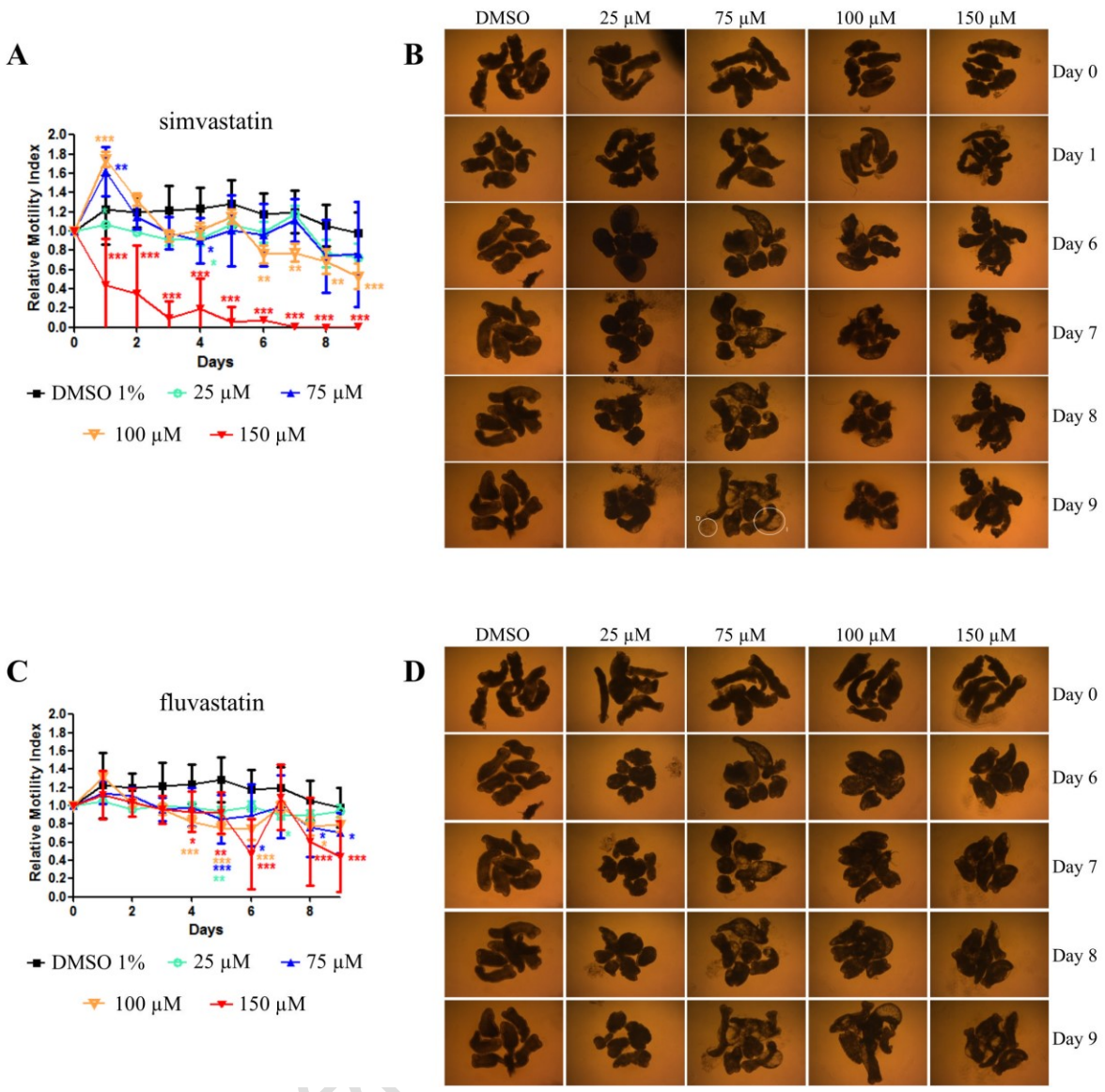

Figure 5. Effects of simvastatin and fluvastatin on the viability of *M. corti* tetrathyridia (TTs). Effects of (A) simvastatin and (C) fluvastatin on TTs were evaluated at concentrations of 25 µM, 75 µM, 100 µM and 150 µM at different incubation times, using the *M. corti* TTs motility assay. TTs incubated with the drug vehicle (DMSO 1%) were used as a negative control. Relative motility indices were measured from a biological replicate, in quadruplicate. The error bars represent the standard deviation, and the asterisks indicate those values that showed statistically significant differences in relation to the negative control, according to the two-way ANOVA test and Bonferroni post-tests (*P < 0.05; **P < 0 .01; * * *P < 0.001; ****P < 0.0001). (B and D) Inverted optical microscope images of *M. corti* TTs treated with 25 µM, 75

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μM, 100 μM and 150 μM concentrations of simvastatin and fluvastatin at different days of treatment compared with the parasites incubated with DMSO 1%. Note the damage to the tegument with presence of influx (I) of culture medium into the worm and tegument debris (D) in the culture medium (shown in simvastatin 75 μM day 9) (B) Treated TTs with 150 μM of simvastatin appear with damage to the tegument in the first 24h of treatment. (D) Treated TTs with 150 μM fluvastatin appear with tegument damage from day 6 of treatment. All images are at 4x magnification.

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