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REPOSICIONAMENTO DE FÁRMACOS PARA HELMINTOSES: AVALIAÇÃO EXPERIMENTAL DO POTENCIAL TERAPÊUTICO DE ANTI-HISTAMÍNICOS H1 EMSCHISTOSOMA MANSONI

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Orientador: Prof. Dr. Josué de Moraes

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2. Prof. Dr. Adriano Defini Andricopulo_____

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5. Prof. Dr. Ricardo Bentes de Azevedo_____

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Dedico esse trabalho à minha mãe (Gilza). Mulher sertaneja, guerreira e de fibra. Ensinou-me a ter fé mesmo nos momentos de dor. Deixará como legado os esforços, sacrifícios e incentivos na minha formação pessoal, acadêmica e profissional.

Essa vitória é nossa!

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XAVIER, R.P**. Reposicionamento de fármacos para helmintoses: Avaliação experimental do potencial terapêutico de anti-histamínicos H_1 em *Schistosoma mansoni*** [tese]. Guarulhos (SP): Universidade Univeritas UNG Guarulhos; 2020.

RESUMO

Introdução: A esquistossomose, doença parasitária causada por helmintos do gênero *Schistosoma*, afeta cerca de 250 milhões de pessoas em todo o mundo, especialmente em regiões pobres. Infelizmente, o tratamento dessa verminose depende exclusivamente do praziquantel, fármaco cuja atuação ocorre somente nas formas adultas do parasito. Considerando os óbices financeiros e o longo tempo para o desenvolvimento de novos fármacos, o "reposicionamento de fármacos" é uma estratégia promissora. Os fármacos anti-histamínicos H_1 estão disponíveis em todo o mundo e são amplamente utilizados em crianças e adultos. Não obstante a existência de receptores para histamina em *Schistosoma*, o efeito de fármacos anti-histamínicos na viabilidade dos vermes ainda não foi muito explorado. **Objetivos:** Avaliar as propriedades antiparasitárias de fármacos anti-histamínicos H_1 em *Schistosoma mansoni ex vivo* e na esquistossomose experimental murina. **Método:** Inicialmente, verificou-se o efeito anti-helmíntico *in vitro* de 21 anti-histamínicos H_1 em vermes adultos de *S. mansoni ex vivo.* Posteriormente, os compostos ativos (CL50 <50 μM) foram testados *in vivo,* em camundongos infectados com *S. mansoni* nos estágios juvenil (infecção pré-patente) e adulto (infecção patente), em doses orais de 400 mg/kg em dose única ou 100 mg/kg diariamente por cinco dias consecutivos. **Resultados:** Após triagem fenotípica verificou-se que os fármacos desloratadina, rupatadina, prometazina e cinarizina promoveu mortalidade de vermes adultos de *S. mansoni in vitro* em baixas concentrações (5-15 μM). Análises por microscopia eletrônica de varredura revelaram alterações no tegumento dos helmintos, após exposição aos fármacos. Em modelo animal, desloratadina, rupatadina, cinarizina e, sobretudo, prometazina, reduziram a carga parasitária de camundongos infectados experimentalmente com *S.mansoni* na infecção precoce ou crônica. A produção de ovos, um mecanismo essencial para a transmissão e a patogênese, também foi marcadamente reduzida pelos anti-histamínicos, e uma redução significativa na hepato e esplenomegalia foi registrada. **Conclusões:** Em conjunto, os resultados do presente estudo revelaram o potencial *in vitro* e *in vivo* de quatro anti-histamínicos (desloratadina, rupatadina, cinarizina e prometazina) como agentes anti-helmínticos.

Descritores: Esquistossomose; *Schistosoma*; Anti-histamínicos; Reposionamento de fármacos.

XAVIER, R.P. **Drug repositioning for helminthesis: Experimental evaluation of the therapeutic potential of H_1 antihistamines in *Schistosoma mansoni*** [thesis]. Guarulhos (SP): University Univeritas UNG Guarulhos; 2020.

ABSTRACT

Introduction: Schistosomiasis, a parasitic disease caused by helminths of the genus *Schistosoma*, affects about 250 million people worldwide, especially in poor regions. Unfortunately, the treatment of this verminosis depends exclusively on praziquantel, a drug whose action occurs only in adult forms of the parasite. Considering the financial obstacles and the long time for the development of new drugs, the "repositioning of drugs" is a promising strategy. H_1 antihistamine drugs are available worldwide and are widely used in children and adults. Despite the existence of histamine receptors in *Schistosoma*, the effect of antihistamine drugs on the worms viability has not been much explored. **Objectives**: To evaluate the antiparasitic properties of H_1 antihistamine drugs in *Schistosoma mansoni ex vivo* and in experimental murine schistosomiasis. **Method**: Initially, the *in vitro* anthelmintic effect of 21 H_1 antihistamines was verified in adult worms of *S. mansoni ex vivo*. Subsequently, the active compounds (<50 μM) were tested *in vivo*, in mice infected with *S. mansoni* in the juvenile (pre-patent infection) and adult (patent infection) stages, in oral doses of 400 mg / kg in a single dose or 100 mg / kg daily for five consecutive days. **Results**: After phenotypic screening, it was found that the drugs desloratadine, rupatadine, promethazine and cinnarizine promoted mortality of adult *S. mansoni* worms *in vitro* at low concentrations (5-15 μM). Scanning electron microscopy analysis revealed changes in the helminths tegument after exposure to the drugs. In an animal model, desloratadine, rupatadine, cinnarizine and, above all, promethazine, reduced the parasitic burden of mice experimentally infected with *S. mansoni* in early or chronic infection. Egg production, an essential mechanism for transmission and pathogenesis, has also been effectively reduced by antihistamines, and a significant reduction in liver and splenomegaly has been noticed. **Conclusions**: Together, the results of the present study revealed the *in vitro* and *in vivo* potential of four antihistamines (desloratadine, rupatadine, cinnarizine and promethazine) as anthelmintic agents.

Descriptors: Schistosomiasis, Antihistamines, Drug repositioning

XAVIER, R.P**. Reposicionamento de fármacos para helmintoses: Avaliação experimental do potencial terapêutico de anti-histamínicos H_1 em *Schistosoma mansoni*** [tesis]. Guarulhos (SP): Universidad Univeritas UNG Guarulhos; 2020.

RESUMEN

Introducción: La esquistosomiasis, una enfermedad parasitaria causada por los helmintos de Schistosoma, afecta a unos 250 millones de personas en todo el mundo, especialmente en las regiones pobres. Desafortunadamente, el tratamiento de esta verminosis depende exclusivamente del praziquantel, un fármaco cuya acción ocurre solo en formas adultas del parásito. Considerando los obstáculos económicos y el largo tiempo para el desarrollo de nuevos medicamentos, el "reposicionamiento de medicamentos" es una estrategia prometedora. Los antihistamínicos H1 están disponibles en todo el mundo y se usan ampliamente en niños y adultos. A pesar de la existencia de receptores de histamina en el esquistosoma, el efecto de los antihistamínicos sobre la viabilidad de los gusanos no se ha explorado mucho. **Objetivos:** Evaluar las propiedades antiparasitarias de los antihistamínicos H1 en Schistosoma mansoni ex vivo y en esquistosomiasis experimental murina. **Método:** Inicialmente, se verificó el efecto antihelmíntico *in vitro* de los antihistamínicos 21 H1 en gusanos adultos de S. mansoni ex vivo. Posteriormente, los compuestos activos (<50 μM) se probaron *in vivo*, en ratones infectados por S. mansoni en las etapas juvenil (infección pre-patente) y adulta (infección patente), en dosis orales de 400 mg / kg en una sola dosis o 100 mg / kg al día durante cinco días consecutivos. **Resultados:** Después del cribado fenotípico, se encontró que los fármacos desloratadina, rupatadina, prometazina y cinarizina promovían la mortalidad de los gusanos S. mansoni adultos *in vitro* a bajas concentraciones (5-15 μM). Los análisis de microscopía electrónica de barrido revelaron cambios en el tegumento de los helmintos después de la exposición a las drogas. En un modelo animal, la desloratadina, la rupatadina, la cinarizina y, sobre todo, la prometazina, redujeron la carga parasitaria de ratones infectados experimentalmente con S. mansoni en una infección temprana o crónica. La producción de huevos, un mecanismo esencial de transmisión y patogénesis, también se ha reducido notablemente con los antihistamínicos, y se ha informado una reducción significativa de la función hepática y la esplenomegalia. **Conclusiones:** En conjunto, los resultados del presente estudio revelaron el potencial *in vitro* e *in vivo* de cuatro antihistamínicos (desloratadina, rupatadina, cinarizina y prometazina) como agentes antihelmínticos.

Descriptores: Esquistosomiasis; Esquistosoma; Antihistamínicos; Reposicionamiento de fármacos.

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1. INTRODUÇÃO

As doenças causadas por helmintos acometem mais de 1 bilhão de pessoas no mundo, especialmente nas camadas sociais pobres, sem condições adequadas de moradia, educação e serviços sanitários. Essas doenças não só prevalecem em condições de pobreza, mas também representam forte entrave ao desenvolvimento dos países e são determinantes na manutenção do quadro de desigualdade[1]. Considerada a principal helmintose em termos de morbidade e mortalidade, a esquistossomose representa um importante problema mundial de saúde pública. É causada por vermes do gênero *Schistosoma,* ao qual exibem ciclos de vida complexos, envolvendo múltiplas formas de desenvolvimento, fazendo uso de diferentes estratégias para garantir desde a entrada, até sobrevivência e transmissão, para o hospedeiro[2].

Embora não seja comumente fatal em humanos, a esquistossomose contribui significativamente para o ônus econômico associado à perda de produtividade e à perpetuação do ciclo da pobreza, além de impor uma carga pesada sobre os custos inerentes à saúde. A esquistossomose está entre as doenças parasitárias mais prevalentes no mundo e é a infecção por helmintos mais importante em termos de mortalidade e morbidade global[3]. Aproximadamente 800 milhões de pessoas podem estar em risco de infecção em todo o mundo e quase 250 milhões estão infectadas[4]. A combinação da carga global de saúde, sua prevalência e falta de opções de tratamento eficazes levaram à sua inclusão na lista de doenças negligenciadas da Organização Mundial da Saúde[5].

As três espécies que afetam seres humanos, e possuem importância endêmica são: *Schistosoma mansoni, Schistosoma haematobium e Schistosoma japonicum*[6,7]. O ciclo de vida do *S. mansoni*, espécie presente em mais de 50 países na América, África e Oriente Médio, é caracterizado por possuir dois hospedeiros: os caramujos de água doce do gênero *Biomphalaria* (hospedeiro intermediário) e o homem (hospedeiro deifinitivo). Os vermes adultos de *Schistosoma* que vivem nos vasos sanguíneos do hospedeiro definitivo, produzem ovos que são excretados nas fezes, e eclodem em água liberando larvas (miracídios), que por sua vez, infectam o hospedeiro intermediário. Após algumas semanas, os caramujos com estímulo de luz

e calor liberam as larvas (cercárias) na água, que procuram e penetram a pele humana, em seguida, entram na circulação, se desenvolvem em vermes juvenis e posteriormente vermes adultos, que se acasalam para estimular a maturação completa e a postura dos ovos. Cerca de 1/3 dos ovos são eliminados com as fezes e, ao atingirem as coleções hídricas contendo os caramujos hospedeiros, dar-se-á um novo ciclo do parasita[7, 8] (Figura 1).

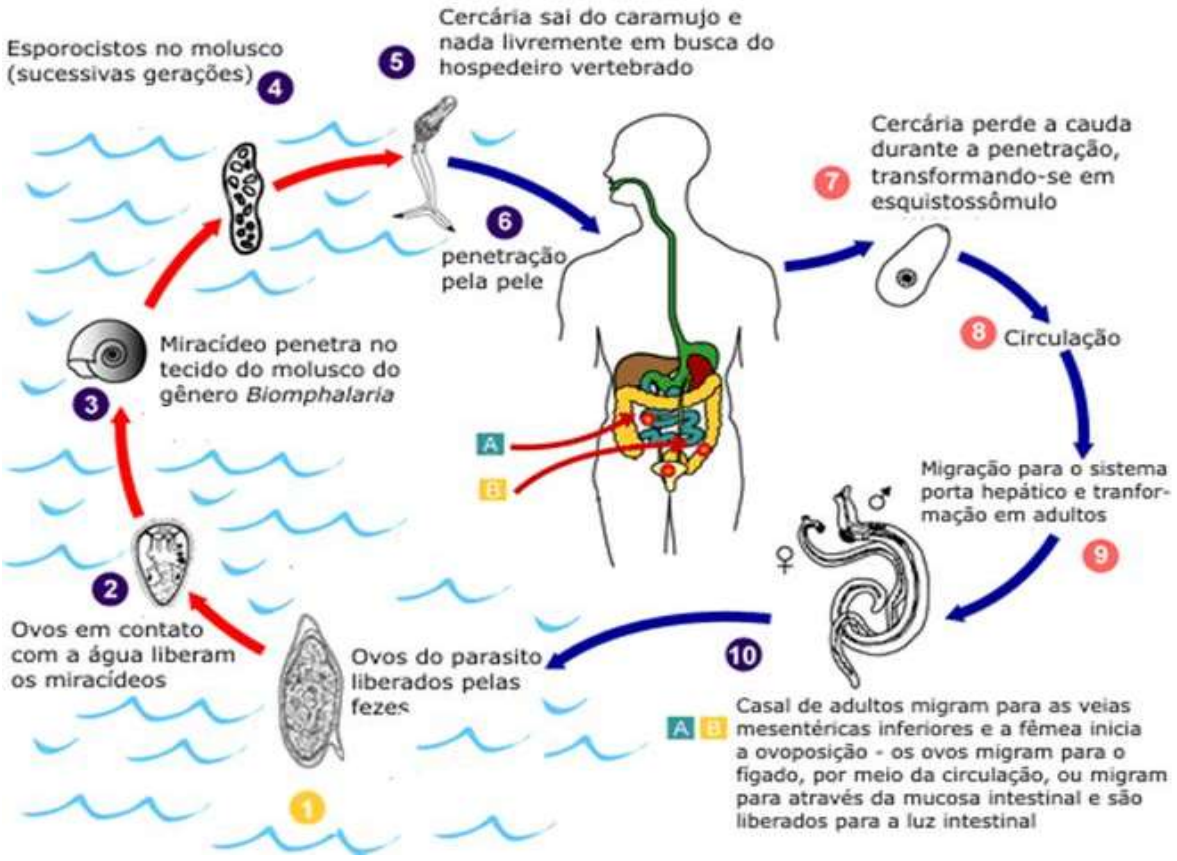

Figura 1: Ciclo de vida do *Schistosoma mansoni*. Fonte: CVE (2005).

O tratamento para a esquistossomose depende apenas do praziquantel, cujo fármaco foi descoberto no final da década de 1970[9]. Embora a pesquisa sobre propriedades anti-esquistossomóticas entre 1980 e 2016 tenha aumentado, infelizmente, nenhum novo medicamento para o tratamento da esquistossomose foi aprovado ou passou por ensaios clínicos relevantes[8].

Ademais, o praziquantel é o fármaco mais usado na medicina veterinária para o tratamento de infecções por cestoides, e seu excessivo uso tem sido objeto de

preocupação no tocante ao aparecimento de organismos resistentes. Dessa forma, há reconhecida necessidade de se desenvolver novos fármacos anti-helmínticos, sendo necessária a identificação de compostos potenciais para se executar um planejamento racional. Portanto, a busca por novas terapias torna-se crucial para o controle e tratamento da doença[9-11].

Apesar da importância global das helmintoses e da necessidade de alternativas terapêuticas, o desenvolvimento de novos fármacos é dispendioso e pode demorar muitos anos para atingir o mercado. Nesse sentido, uma das alternativas pode estar no "reposicionamento de fármacos" ou "redefinição de fármacos". Esta abordagem consiste, basicamente, na busca por um novo uso de fármacos, os quais já estão aprovados e disponíveis no mercado. Em 2014, nosso grupo de pesquisa relatou que o anti-inflamatório diclofenaco de sódio possui efeito anti-helmíntico em *S. mansoni*[12]. No caso, partindo de estruturas cristalográficas e utilizando técnicas de bioinformática, observou-se similaridade entre os fármacos praziquantel e diclofenaco, apesar de pertencerem a classes químicas diferentes. Nesse sentido, o grupo envidou esforços para avaliar a ação de fármacos da classe dos anti-inflamatórios não esteroides (AINEs) em *S. mansoni*. Sumariamente, após triagem de 73 AINEs notou-se efeito antiparasitário do diclofenaco, celecoxibe, ácido meclofenâmico, ácido tolfenâmico e, principalmente do ácido mefenâmico, um fármaco comumente comercializado no Brasil e diversos países. Surpreendentemente, o ácido mefenâmico causou alta redução da carga parasitária (83,9%) e do número de ovos (93,6%), superando os critérios estabelecidos pela Organização Mundial de Saúde (OMS) no que tange aos programas "Drug Discovery" para esquistossomose. Ademais, o regime posológico utilizado para o ácido mefenâmico está de acordo com a posologia comumente utilizada na prática clínica, o que demonstra o potencial de reaproveitamento clínico como um agente anti-helmíntico[13].

Ainda no que concerne ao reposicionamento de fármacos para a esquistossomose, um trabalho do nosso grupo mostrou que após triagem de 13 fármacos da classe dos diuréticos, a espironolactona, um diurético poupador de potássio, tem potente ação *in vitro* (LC50 ~10 μM) e em animais experimentalmente infectados com *S. mansoni*[14].

Mais recentemente, compondo o presente trabalho, nosso grupo mostrou que a prometazina, um anti-histamínico H_1, é um potente agente anti-*S. mansoni in vitro* (LC50 ~5 μM). Surpreendentemente, os ensaios pré-clínicos usando camundongos infectados com *S. mansoni* nos períodos pré-patente e patente revelaram que a prometazina reduz mais de 90% da carga parasitária, superando a eficácia terapêutica descrita para o praziquantel[15]. Destarte, o presente trabalho tem como intento avaliar o efeito de diferentes fármacos da classe dos anti-histamínicos H_1 em *S. mansoni in vitro* e *in vivo*.

Na reutilização de fármacos, algumas estratégias para candidatos à fármacos fundamentam-se no conhecimento fisiopatológico e na consequente eleição do alvo terapêutico mais adequado[12, 16, 17]. Nesse contexto, a complexidade do ciclo de vida do *Schistosoma* se dá por uma rica diversidade de sistemas de sinalização que coordenam várias atividades do verme, sendo esses eventos de sinalização mediados pelo sistema nervoso do parasita, que é bem desenvolvido em esquistossomos e controla praticamente todos os tecidos e regiões do corpo. A sinalização neuronal desempenha um papel particularmente importante durante a fase de migração, não apenas controlando os músculos somáticos que impulsionam o movimento, mas também permitindo a resposta e habilidades de navegação necessárias para passar por uma migração bem-sucedida. Desse modo, o sistema neuromuscular dos helmintos é considerado um importante alvo para fármacos, por possuírem uma rica diversidade de receptores aminérgicos[18].

Entre os transmissores identificados envolvidos no mecanismo molecular da sinalização neuronal do *Schistosoma* estão as aminas biogênicas, derivadas do metabolismo de aminoácidos, desempenham um papel importante como moduladores da função neuromuscular e movimento, estimulando ou inibindo, dependendo da amina[19]. Avanços recentes na genômica do *Schistosoma*[20, 21] mostraram um grande número de proteínas associadas à sinalização de aminas biogênicas, incluindo enzimas biossintéticas, transportadores e numerosos receptores transmembranares. Entre as aminas biogênicas mais comuns estão serotonina e histamina, sintetizadas a partir de triptofano e histidina, respectivamente, e dois tipos de derivados da tirosina: as catecolaminas (dopamina, noradrenalina, adrenalina) e fenolaminas (tiramina e octopamina)[22]. Em helmintos, as aminas biogênicas desempenham papéis vitais no

metabolismo, no controle da motilidade e, portanto, a sobrevivência no hospedeiro[23-25].

Nesse contexto, o potencial dos anti-histamínicos utilizados na abordagem de reuso de fármacos como agentes antiparasitários demonstrou desempenhar um papel importante no reuso clínico[13]. Dado que a motilidade é crucial para a sobrevivência do parasita, as aminas biogênicas são consideradas um alvo potencial para a descoberta de medicamentos anti-helmínticos, não apenas contra esquistossomos, mas contra outros parasitas helmintos também[26]. Os receptores dos esquistossomos ainda não foram caracterizados em nível de proteína e, portanto, o mecanismo de ação é desconhecido[18]. Há evidências para sugerir uma função neuromuscular. Por exemplo, aplicado exogenamente, a histamina afeta a motilidade de alguns vermes, de maneira dose-dependente. Outrossim, receptores para histamina foram descritos em *S. mansoni* e foi sugerido um modelo para o papel da interação da histamina com o verme e seu hospedeiro[19]. A ativação dos receptores de superfície é apenas uma maneira das quais as aminas biogênicas derivados do hospedeiro podem influenciar o parasita. Outro mecanismo é através da captação de aminas biogênicas por transportadores de superfície. Esquistossomos não somente sintetizam algumas aminas biogênicas endogenamente[18, 27], como também importam aminas exógenas em todo o tegumento [28], possivelmente para suplementar reservas endógenas quando a produção interna é insuficiente. A captação de aminas biogênicas pode ser particularmente importante na interação com o hospedeiro. No caso, o aludido modelo sugere duas maneiras: uma é através de receptores responsivos localizado em terminações nervosas sensoriais da superfície do parasita, e a outra, por um mecanismo que envolve o transporte do tegumento e, em seguida, a captação para o parasita via transportadores específicos dos neurônios[18, 27] (Figura 2).

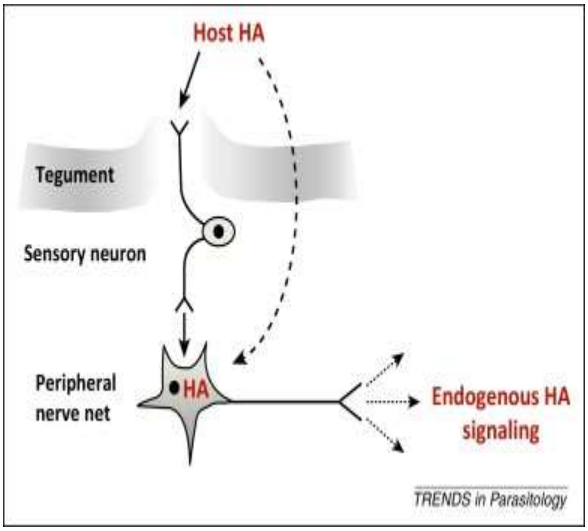

Figura 2: Modelo para o papel da histamina na relação parasito-hospedeiro. Fonte: Ribeiro (2015).

Apesar do *S. mansoni* possuir receptores para histamina, não é sabido se fármacos anti-histamínicos alteram a viabilidade desses parasitos[19]. No presente estudo avaliou-se o efeito de 21 fármacos anti-histamínicos H_1 (anti-H_1), os de primeira geração cinarizina, clorfeniramina, declorfeniramina, prometazina, cetotifeno, meclizina, tripelenamina e os de segunda geração acrivastina, astemizol, bilastina, carebastina, cetirizina, desloratadina, fexofenadina, levocetirizina, loratadina, misolastina, epinastina, hidroxizina, rupatadina e terfenadina em *S. mansoni.*

Os anti-histamínicos estão entre os fármacos mais comumente prescritos na prática dermatológica diária, tanto em adultos como em crianças. A histamina é considerada um potente mediador de numerosas reações fisiológicas e tem seus efeitos mediados pela ligação com quatro subtipos de receptores, receptor de histamina HRH_1, HRH_2, HRH_3 e HRH_4. O receptor H_1 (HR_1) é codificado no cromossomo humano 3, sendo o responsável por muitos sintomas das doenças alérgicas, tais como o prurido, a rinorreia, o broncoespasmo e a contração da musculatura lisa intestinal. Historicamente, a potência dos anti-histamínicos foi verificada por meio de ensaios farmacológicos padronizados e são caracterizados em anti-H_1 de primeira geração ou clássicos, que são fármacos lipofílicos e classificadas em diferentes grupos de acordo com sua estrutura química. Todos eles são metabolizados pelo Citocromo P450 (CYP) no fígado e não servem como substrato da glicoproteína. E Anti-H_1 de segunda geração são substâncias desenvolvidas nos

últimos 25 anos, algumas derivadas dos anti-H_1 de primeira geração, porém oferecendo maiores vantagens em relação aos compostos de primeira geração, em decorrência de apresentarem menores efeitos anticolinérgicos ou sedativos e alguns interagem com outros fármacos e substâncias, atuando como substrato da glicoproteína[29-31].

A segunda geração de anti-histamínicos difere da primeira geração devido a sua elevada especificidade e afinidade pelos receptores H_1 e devido a seu menor efeito no sistema nervoso central, tendo como resultado menores efeitos sedativos[32, 33]. Embora a eficácia dos diferentes anti-H_1 no tratamento dos pacientes alérgicos seja similar, mesmo quando se comparam anti-histamínicos de primeira e de segunda geração, eles são diferentes em termos de estrutura química, farmacologia e potencial tóxico. Dessa forma, o conhecimento sobre sua farmacocinética e características farmacodinâmicas torna-se importante aos ensaios pré-clínicos realizados no presente estudo. A descrição dos principais anti-histamínicos (prometazina, cinarizina, desloratadina e rupatadina) são descritos abaixo, bem como as estruturas químicas e informações farmacológicas dos anti-histamínicos H_1 na Figura 03 e Tabela 1, respectivamente.

A prometazina, anti-histamínico de primeira geração, um derivado da fenotiazina, tem ação de antagonista aos efeitos centrais e periféricos da histamina mediada pelos receptores H_1. Desde sua primeira introdução em 1946[34], seus efeitos incluem broncoconstrição, vasodilatação e contrações espasmódicas do músculo liso gastrointestinal como objetivo de tratamento do alívio de náuseas, vômitos e prevenir enjoos, pode também ser utilizada em condições psiquiátricas, como perturbação do sono, ansiedade e agitação, devido a sua função sedativa[35]. Analisando possíveis efeitos colaterais, estudos relataram que o uso da prometazina em crianças ou mulheres grávidas pode possivelmente provocar distonia aguda[36, 37].

A cinarizina é classificada como um bloqueador de canal de cálcio, apresenta atividade anticolinérgica, antiserotoninégica e antidopaminérgica, sendo considerada um agente anti-histamínico de primeira geração sedativo que atua por inibição do receptor H_1, é utilizado no tratamento da vertigem e na prevenção de enjoo. Estudos sugerem ainda aplicação clínica do medicamento para profilaxia da enxaqueca[38]. Por outro lado, como contra indicação, a cinarizina é conhecida por agravar a doença de

Parkinson[39], quando usado em dose média de 150 mg por dia, podendo agravar a função motora em pacientes, entretanto, o efeito foi reversível e desapareceu após a retirada do medicamento[38].

A desloratadina é o principal metabólito biologicamente ativo da loratadina, sendo considerado um anti-histamínico de segunda geração e é caracterizada por oferecer maior benefícios em relação aos compostos de primeira geração por apresentarem menores efeitos anticolinérgicos e ou sedativos[40]. A desloratadina demostrou exercer efeitos anti-inflamatórios inibindo a liberação de mediadores pró-inflamatórios dos eosinófilos, que normalmente estão associados a reações alérgicas da fase tardia[41].

A rupatadina, derivado de N-alquilpiridina, é um anti-histamínico de segunda geração com afinidade pela histamina. Estudos *in vitro* demonstraram que a rupatadina, como antagonistas do HRH_1, possui propriedades anti-inflamatórias que inibe vários mediadores associados à fase inicial e tardia a respostas alérgicas, também inibem a imunoglobulina E (IgE) e liberações não mediadas por IgE de histamina pré-formada de mastócitos e basófilos humanos[42]. De acordo com Criado et al. (2010)[43] em estudos randomizados controlados, indicam que a rupatadina (10 mg/dia) é eficaz no tratamento da renite alérgica a partir dos 12 anos de idade, melhorando os sintomas nasais (incluindo a obstrução) e não nasais. Segundo González-Núnez et al. (2016)[42], a rupatadina, em dose de 10 mg/dia não apresentou efeitos cardiovasculares adversos em extensos ensaios clínicos envolvendo ambos adultos e crianças.

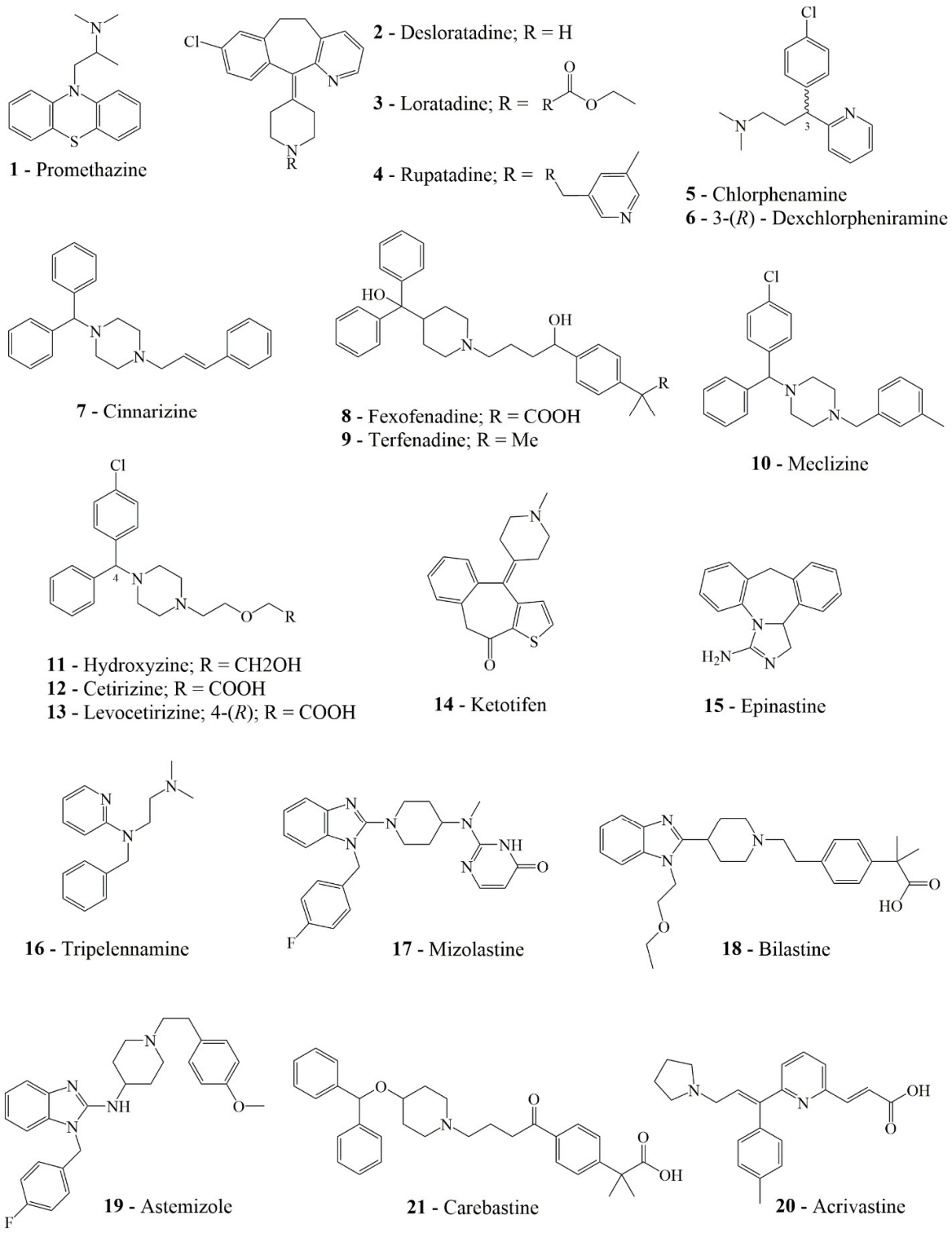

Figura 3: Estrutura química dos anti-histamínicos H_1

Tabela 1: Informações farmacológicas dos fármacos anti-histamínicos H_1.

Nº	Geração	Fármaco	Massa (g/mol)	Log P	Biodisp. Oral	Meia-Vida ($T_{1/2}$)	DL_{50} oral (mg/kg)*
1	1º	Cinarizina	368.524	5.77	64% - 80%	23 h	>1000
2	1º	Clorfeniramina	274.792	3.74	25% - 50%	20-24 h	118
3	1º	Desclorfeniramina	274.792	3.74	25% - 50%	2.5-6 h	800
4	1º	Prometazina	284.421	4.52	13% - 49%	09 -15 h	255
5	1º	Cetotifeno	309.426	3.2	50%	12h	ND
6	1º	Meclizina	390.948	5.8	ND	6h	1600
7	1º	Tripelennamine	255.358	3.3	ND	4-6h	ND
8	2º	Acrivastina	348.446	1.70	84%	2h	ND
9	2º	Astemizol	458.581	6,43	60%	24 h	2052
10	2º	Bilastina	463.622	5.02	61%	14.5 h	125
11	2º	Carebastina	499.651	3.60	66%	10-14 h	ND
12	2º	Cetirizina	388.892	2.98	70%	8,3 h	703
13	2º	Epinastina	249.311	3.5	40%	12h	ND
14	2º	Hidroxizina	374.904	3.4	ND	20h	400
15	2º	Desloratadina	310.825	3.20	87%	27 h	353
16	2º	Fexofenadina	501.667	5.02	36%	11-15 h	5000
17	2º	Levocetirizina	388.892	2.98	64%	8 h	240
18	2º	Loratadina	382.888	5.20	40%	10 h	525
19	2º	Misolastina	432.503	1.30	30%	5 h	ND
20	2º	Rupatadina	415.965	5.37	25%	5,9 h	1000
21	2º	Terfenadina	471.685	7.10	70%	12 h	5000

Fonte: PubChem Compound e DrugBank database.
DL 50 em camundongos(*).
Não Determinado (ND).

Com o objetivo de descobrir novas alternativas para o tratamento da esquistossomose, associada à importância do reposicionamento de fármacos, no presente trabalho foi avaliada a viabilidade de adultos de *S. mansoni* expostos a diferentes anti-histamínicos H_1 (anti-H_1). Subsequentemente, os fármacos ativos *in vitro* foram avaliados *in vivo* em camundongos albergando vermes juvenis (período pré-patente, 21 dias após infecção) e adultos (período patente, 42 dias após infecção). Os efeitos dos anti-histamínicos na esquistossomose experimental murina foram analisados com ênfase na carga parasitária, oograma qualitativo e quantitativo, ovos nas fezes e massa dos órgãos.

2. OBJETIVO

2.1 Geral

Avaliar as propriedades antiparasitárias de fármacos anti-histamínicos H_1 em *Schistosoma mansoni.*

2.2 Específicos

1) Avaliar o efeito antiparasitário *in vitro* de fármacos anti-histamínicos em adultos de *S. mansoni ex vivo;*

2) Avaliar o efeito antiparasitário de fármacos anti-histamínicos em camundongos experimentalmente infectados com *S. mansoni*.

3. REVISÃO DA LITERATURA

3.1 Artigo 1 - Antischistosomal agents: state of art and perspectives

A revisão da literatura do presente estudo encontra-se no artigo publicado no periódico *Future Medicinal Chemistry* (Fator de Impacto 3,607). O trabalho, em sua completude, está disponível nas páginas subsequentes.

Review

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Future Medicinal Chemistry

Antischistosomal agents: state of art and perspectives

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Praziquantel has remained the drug of choice for schistosomiasis chemotherapy for almost 40 years. The pressing need to develop a new antischistosomal drug may necessitate exploring and filtering chemotherapeutic history to search for the most promising ones. In this context, this review attempts to summarize all progress made in schistosomiasis chemotherapy from the early 20th century (mid-1910s) to 2016. We gathered almost 100 compounds providing information on therapeutic action, specifically covering at least first *in vivo* studies in animal model and *in vitro*. Pharmacokinetic and toxicity profiles of antischistosomal agents were also described. Preclinical studies indicate a handful of promising future candidates.

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Keywords: chemogenomics • drug discovery • drug repositioning • druggability • high-throughput screening • Hit • lead • molecular modeling

Background

In 1852, Theodor Bilharz described for the first time a parasitic disease (bilharzia, later termed schistosomiasis) caused by blood-dwelling digenetic trematodes of the genus *Schistosoma*. Six species of *Schistosoma* parasitize humans, namely *S. japonicum, S. mansoni*, *S. haematobium*, *S. guineenses*, *S. mekongi* and *S. intercalatum*; the first three species have global importance, whereas infections with other species are limited to a few local foci. More specifically, the geographical distribution is as follows: *S. japonicum* (China, Indonesia and the Philippines), *S. mansoni* (Africa, the Middle East, South America and the Caribbean), *S. haematobium* (Africa and the Middle East), *S. guineensis* and *S. intercalatum* (rainforest areas of central Africa) and *S. mekongi* (Cambodia and Lao People's Democratic Republic) [1,2].

The life-cycle of human schistosomes is complex and in two alternate hosts: freshwater snails (intermediate host) and human (definitive host). Briefly, paired female and male helminthes living in the blood vessels definitive host produce eggs that are excreted in feces (in intestinal schistosomiasis) or urine (in urinary schistosomiasis), and hatch in water releasing a larvae (miracidia) that infects the intermediate host. A few weeks later snails release larvae (cercariae) in the water, which penetrate the human skin. Upon penetration, the cercaria loses its forked tail, becoming schistosomulum, which subsequently enters the circulation and develops into juvenile and then adult worms that pair to stimulate full maturation and egg laying. Some of the eggs are passed out of the body in the feces or urine to continue the parasite's life-cycle [2].

Schistosomiasis transmission has been reported from 78 countries. It is considered a neglected disease, known as a disease of poverty, and incidence of infection is concentrated in particularly poor communities with a dependence on surface water, which is often contaminated with the urine and feces of infected individuals, and colonized by snails that act as the intermediate hosts for the schistosome. The global burden of schistosomiasis is estimated at 2.6 million disability-adjusted life years [3]. However, some of the revised and recent calculations based on health-related quality of life may point to a much higher disease burden of schistosomiasis than has previously been recognized [4,5]. Most

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Table 1. Compounds with antischistosomal properties demonstrated in animals and/or humans from the early 20th century (mid-1910s) to 1980.

Date†	Compound	Compound ID	Relevant notes
1918	Antimonials	**1A–1F**	Highly toxic
1920	Emetine and 2,3-dehydroemetine	**2A–2B**	Toxic. Patients were cured, but had to be hospitalized for treatment
1960	Metrifonate	**3**	Effective on *S. haematobium*, well tolerated, but discontinued for medical, operational and economic criteria
1962	Nitrofurans	**4A–4B**	Toxic. Moderately effective
1962	Lucanthone	**5**	Several side effects. Superseded by hycanthone
1964	Niridazole	**6**	Highly toxic. Need for multiple oral doses; moderately effective
1965	Hycanthone	**7**	A lucanthone derivative more active. Mutagenic, carcinogenic and teratogenic
1969	Oxamniquine	**8**	Effective against *S. mansoni*. Relatively safe, and side effects are limited. Withdrawn from market
1971	Tubercidin	**9**	Purine analog; only animal tested
1976	Amoscanate	**10**	Widely tested in China; toxic
1977	Praziquantel	**11**	Drug of choice against all schistosomes
1978	Ro 11–3128	**12**	Effective on immature worms
1978	Oltipraz	**13**	Withdrawn for fingernail problem

Identification number of the compound is according to the order appearing in the text.
†Dates of introduction or publication are only approximate. See references [9,15] for details.

of the burden of these diseases results from disability (rather than premature death), influencing school attendance, child development and overall economic productivity, thus resulting in disease-driven poverty traps.

Pathology characterizing schistosomiasis is mainly caused by tissue deposition of eggs. Organs typically affected include the urinary tract, the bowel and the liver, depending on the species of schistosome. Schistosomiasis is implicated in several clinical conditions, including bladder cancer leading to death, liver cirrhosis, hydronephrosis and reproductive complications. In children, it is associated with poor growth, malnutrition, poor cognitive development, anemia and reduced school performance [2]. Urogenital schistosomiasis is also considered to be a risk factor for HIV infection, especially in women. Moreover, schistosomiasis has both direct and indirect effects on other infectious diseases such as hepatitis, tuberculosis, eumycetoma, malaria, leishmaniasis, Chagas disease and HIV/AIDS. Furthermore, the problem of helminthic polyparasitism (caused by multiple simultaneous worm infections) is quite common, and is found in most of the schistosomiasis-endemic areas of the world [2,6–8].

The control of schistosomiasis is based on large-scale treatment of at-risk population groups, access to safe water, improved sanitation, hygiene education and snail control. Of these, chemotherapy with praziquantel, a drug that was developed in the 1970s, has emerged as the major tool [9,10]. Considering that the current control programs against schistosomiasis depend on the wide-scale use of praziquantel, the emergence of praziquantel-resistant parasites is of concern [11]. Of note, more than 66.5 million people were reported to have been treated for schistosomiasis in 2015, and it is estimated that at least 218 million people need treatment per year [12].

These considerations have been major concerns for health agencies, which have highlighted the need for novel therapies to treat schistosomiasis. The pressing need to develop a new schistosomicidal drug may necessitate exploring and filtering the chemotherapeutic history to search for those most promising ones. In this context, this review attempts to summarize all progress made in schistosomiasis chemotherapy from the early 20th century (mid-1910s) to 2016. All antischistosomal compounds mentioned in this review have been extensively investigated, specifically based on experimental animal model studies.

Antischistosomal compounds from the early 20th century (mid-1910s) to 1980

Antischistosomal drugs were reviewed extensively by Cioli *et al.* [9], with an emphasis on drugs that were used in the past. In this section we summarize the main antischistosomal compounds and their properties demonstrated in animals and/or humans from the beginning of schistosomiasis treatment (mid-1910s) to 1980 (Table 1 & Figure 1).

After 1917, when tartar emetic (**1A**) was first used in the treatment of schistosomiasis [14], little advance was made in the development of antischistosomal drugs. In 1920, the natural alkaloid emetine (**2A**) was used as a

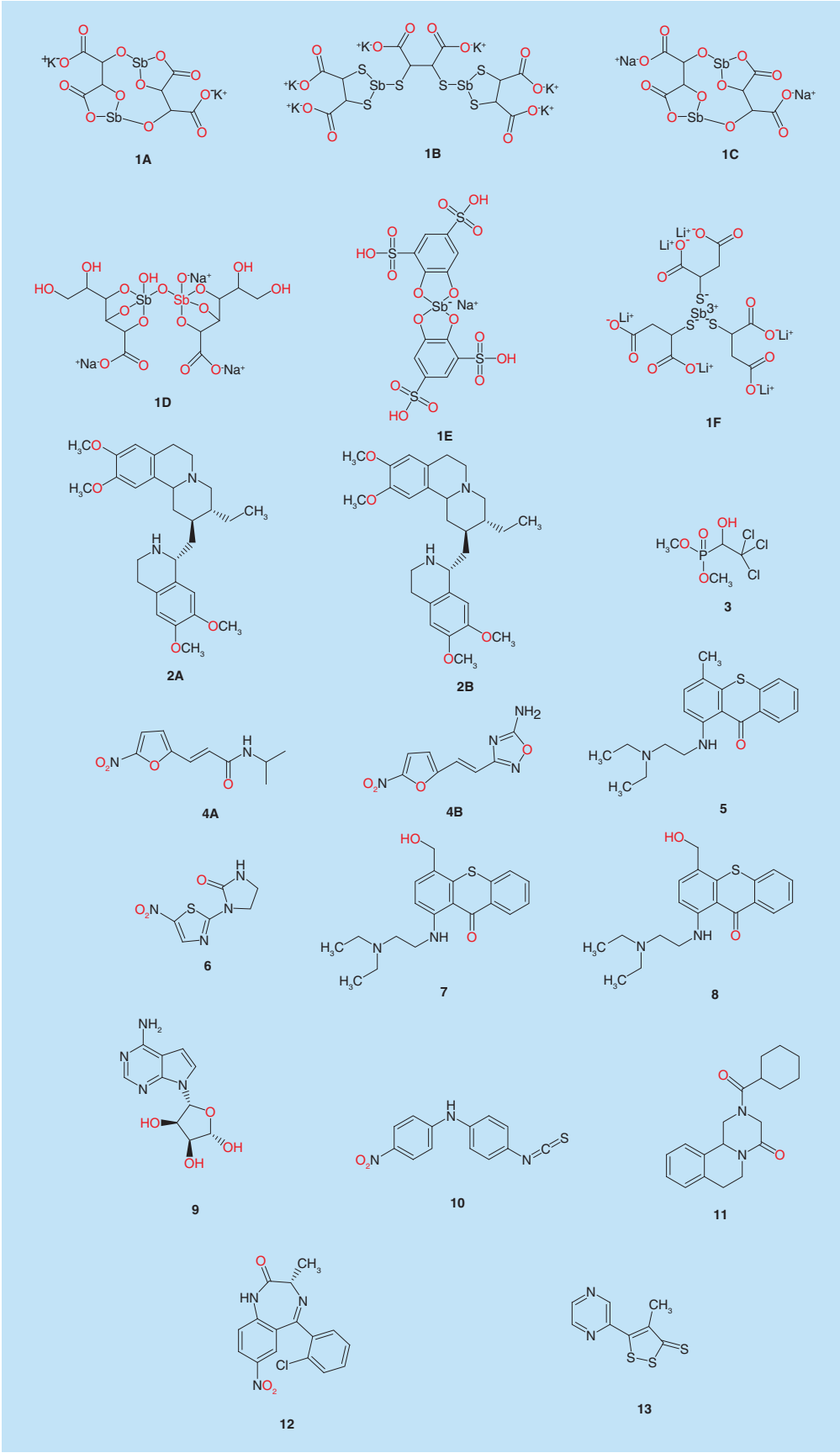

Figure 1. Compounds with antischistosomal properties demonstrated in animals and/or humans from early 20th century (mid-1910s) to 1980. Antimonials (**1A–1F**), Emetine and 2,3-dehydroemetine (**2A** and **2B**), Metrifonate (**3**), Nitrofurans (**4A** and **4B**), Lucanthone (**5**), Niridazole (**6**), Hycanthone (**7**), Oxamniquine (**8**), Tubercidin (**9**), Amoscanate (**10**), Praziquantel (**11**), Ro 11–3128 (**12**), Oltipraz (**13**).

schistosomicide, but it has been highly toxic. The introduction of 2,3-dehydroemetine (**2B**), a synthetically agent similar to emetine, also produced side effects. Although highly toxic, antimonial compounds (**1A–1F**) were used for many decades against schistosome infections. Only in the 1960s there was an advance in antischistosomal drug discovery field, with the introduction of some compound as metrifonate, nitrofurans, lucanthone, niridazole, hycanthone and oxamniquine [9,15]. However, except with oxamniquine, treatments against schistosomiasis had severe side effects that had to be weighed against the benefits for the patient.

Metrifonate (**3**), an organophosphorous compound introduced in 1955 as an insecticide, was widely used for the treatment of urinary schistosomiasis, but has been withdrawn from the market because of operational, medical and economic criteria [13,16]. In the early 1960s, nitrofurans (**4A–4B**) were used to treat patients in China; however, toxicity and suboptimal activity led to the abandonment of all these compounds. In 1965 niridazole (**6**) was introduced, a nitrothiozole derivative administered orally, which presents several serious drawbacks, such as the need for multiple oral doses, as well as toxicity and mutagenity. Lucanthone (**5**), a xanthenone compound known as Miracil D, was introduced into medical practice using 3–5 daily oral doses. Later, Rosi *et al.* [17] obtained hycanthone (**7**) from microbial fermentation of Miracil D by *Aspergillus sclerotiorum*, which was found to be many times more effective than lucanthone in rodent hosts. However, hycanthone was discontinued for mutagenicity [9].

In the late 1960s, oxamniquine (**8**), a tetrahydroquinoline derivative, was produced by biological synthesis and described as one of the most promising schistosomicides [18]. In the last 30 years this drug has been widely used on the American continent, in particular in Brazil where *S. mansoni* is the only endemic species. Oxamniquine is safe, and side effects are limited, but a disadvantage of oxamniquine is that it acts only on *S. mansoni*. A sulfotransferase enzyme in *S. mansoni* as the oxamniquine target has been identified. Oxamniquine has not been in use since 2010, and has been essentially replaced by praziquantel [19].

In the 1970s, several antischistosomal compounds emerged, such as tubercidin, amoscanate, praziquantel and its benzodiazepine derivative Ro11–3128, and oltipraz [9]. However, most of these drugs were found to cause significant side effects. Tubercidin (**9**) is a purine analog that can be incorporated into nucleic acids of schistosomes and of mammalian cells. Amoscanate (**10**) was very extensively tested in China, but showed several signs of liver toxicity. In the late 1970s, praziquantel (**11**), an isoquinoline–pyrazine derivative, was introduced and immediately proved to be superior to any other schistosomicidal drug, quickly becoming the drug of choice in most endemic areas. Praziquantel is effective against all human adult worm *Schistosoma* species, but has poor activity against juvenile worms. It has very low toxicity, and no important long-term safety difficulties have been documented in people so far. Praziquantel has several advantages: low cost, single administration with high efficacy, broad therapeutic profile, high tolerability, few and transient side effects [20]. The mechanism of praziquantel action is not clearly understood; calcium alterations appear to be the primary effects of drug [10]; in addition, schistosome ATP binding cassette (ABC) transporters might serve as important targets for enhancing the action of praziquantel [21].

Still in the late 1970, the benzodiazepine derivative Ro11–3128 (methylclonazepam) (**12**) emerged as an interesting schistosomicide. A particularly attractive feature of Ro 11–3128 was its activity against immature *S. mansoni* and *S. haematobium*. However, this compound has poor activity against *S. japonicum*. Additionally, Ro 11–3128 causes severe and long-lasting sedation, accompanied by ataxia and muscle relaxation [20]. Finally, oltipraz (**13**), a dithiole thione derivative synthesized in 1976, is a slow-acting drug. Oltipraz causes several side effects, mainly paresthesias, acute pains at the fingertips and detachment of fingernails [22].

Antischistosomal compounds over the last 36 years from 1980 to 2016

Since the late 1970s, praziquantel has been widely used as an effective means to control schistosomiasis. This drug is relatively safe and highly effective against all human *Schistosoma* species. However, praziquantel does not prevent reinfection and is inactive against immature schistosomes. In addition, there is reported resistance both in the field and experimentally induced. Thus, beginning in the 1990s, a series of laboratory studies and clinical trials in Egypt, Senegal, and elsewhere raised considerable concern about the possible development of tolerance and/or resistance to praziquantel [23–26]. Accordingly, the development of new effective drugs for the treatment and prevention of the infection has been encouraged.

In the early 1980s, natural metabolites with antischistosomal properties started to be explored and, in recent decades, there has been a growing interest in the scientific community regarding the research and development of new schistosomicidal drugs [27,28]. Since that time, several trials have been published all over the world to investigate the efficacy of several chemicals. Hence, first, in this review we analyze the number of research articles with antischistosomal compounds published in the PubMed, Medline, LILACS, SciELO and Virtual Health

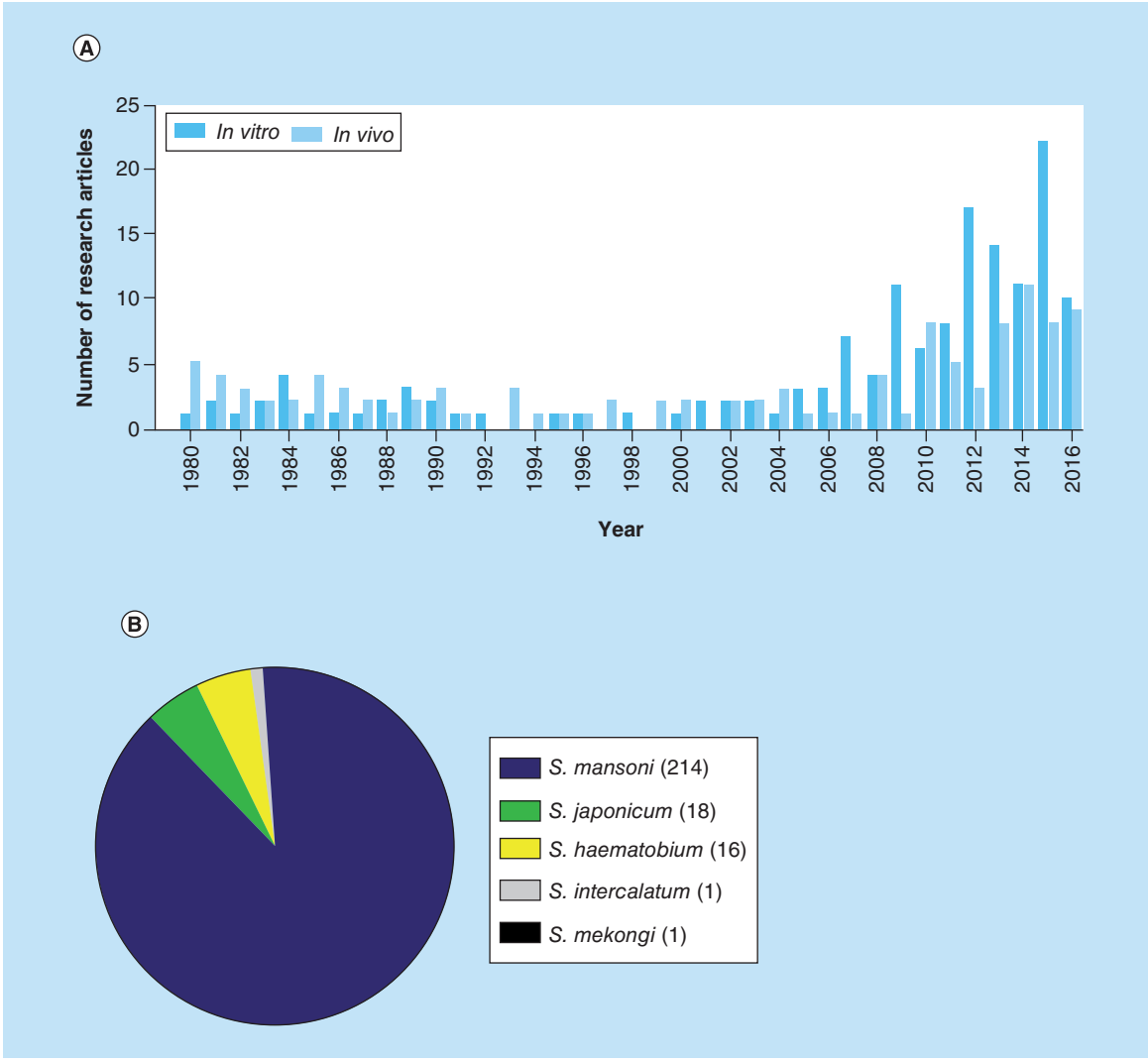

Figure 2. Research articles about antischistosomal compounds over the last 36 years. (A) Number of published articles with *in vitro* and/or *in vivo* studies since 1980 per year. **(B)** Distribution of the research articles according to the *Schistosoma* species; the number of articles is in parentheses.

Library databases from 1980 to 2016. We included *in vitro* studies consisting of studies that tested the sensitivity and efficacy of the compounds against *Schistosoma* human species and *in vivo* studies consisting of studies that tested the efficacy of compounds in experimental animals. In Figure 2 the number of published research articles about antischistosomal compounds has been plotted per year from 1980 to 2016. In the last 5 years, there has been a marked increase in the number of articles (Figure 2A). Among all published research articles, *S. mansoni* is the most widely used schistosome model for experimental schistosomiasis (Figure 2B).

Several compounds have been experimentally evaluated in animal models. However, few have reached clinical trials in humans. The main antischistosomal compounds and their properties demonstrated in animals and/or humans and *in vitro* from 1980 to 2016 are summarized in Table 2, and will be discussed below. In addition, some pharmacokinetic and toxicological parameters such as maximal plasma concentration (C_{max}), terminal elimination half-life ($t_{1/2}$) and lethal dose (LD_{50}) of antischistosomal agents are summarizes in Table 3.

Natural compounds & their derivatives

Artemisinin derivatives

Comprehensive reviews have summarized the discovery of the antischistosomal properties of the artemisinins [94–97]. In brief, artemisinin (**14**) is a sesquiterpene lactone with an endoperoxide group, which was isolated from

Table 2. Antischistosomal properties observed with drug candidates in rodents experimentally infected with major human schistosome species and *in vitro* activity over the last 36 years from 1980 to 2016.

Chemical compound/drug	Compound ID	Host animal	Drug administration		Worm burden reduction		*In vitro* activity (μM)	Ref.
			Route	Dose (mg/kg)	Adult infection	Juvenile infection		
S. mansoni								
9-Acridanone hydrazine (Ro 15–5458)	**82**	Mouse	Oral	Single, 100 Single, 10–20	ND ND	>90% 84–95%	ND	[29] [30]
Acyclic nucleotide analog [(*S*)-HPMPA]	**70**	Mouse	Intraperitoneal	Multiple, 10–20	30–40%	ND	>100	[31] [32]
Arachidonic acid	**35**	Mouse Hamster	Oral Oral	Single, 500–1000 Multiple, 300 Multiple, 300–1200	39% 54–58% 56–77%	38% 43–64%	5000	[33] [34]
Artemether	**15**	Mouse	Oral	Twice, 300	85–98%	70%	~335–1000	[35]
Artenusate	**16**	Mouse	Oral	Twice, 150–300	34–49%	67–77%	~100–780	[36]
Anisomycin	**65**	Mouse	Oral	Single, 100	0	ND	1	[37]
Bicalutamide	**77**	Mouse	Oral	Single, 50–400 (juvenile) Single, 100–400 (adult)	0–41%	37–31%	ND	[38]
Bisquinoline cyclen derivative	**85**	Mouse	Oral	Single, 400	12%	ND	1	[39]
Bisquinoline cyclen derivative (Fe^{++}complex)	**86**	Mouse	Oral	Single, 400	88%	ND	1	[39]
Bisquinoline cyclen derivative (Mn^{++}complex)	**87**	Mouse	Oral	Single, 400	75%	ND	4	[39]
BTP-Iso	**91**	Mouse	Oral	Single, 300	55%	ND	ND	[40]
C-4	**51**	Mouse	Oral	Single, 400	ND	ND	ND	[41]
Chlorambucil	**83**	Mouse	Oral	Twice, 2.5	23%	76%	~65	[42]
Clofazimine	**55**	Mouse	Oral	Single, 400	83%	ND	33	[43]
Chloroquine	**33**	Mouse	Oral	Single, 400	11%	ND	ND	[44]
Clorsulon	**84**	Mouse	Oral	Single or multiple, 5	88–97%	ND	ND	[45]
Closantel	**61**	Mouse	Oral	Multiple, 100	Note given	ND	ND	[37]
Curcumin	**36**	Mouse	Intraperitoneal	Multiple, 400	44%	ND	50	[46]
Cyclosporin A	**34**	Mouse	Subcutaneous	Multiple, 50	NS	100%	1	[37,47]
Cyproterone acetate	**80**	Mouse	Oral	Single, 400	0	10%	240	[38]
Dexamethasone	**68**	Mouse	Intramuscular or subcutaneous	Single, 50	ND	27–29%	ND	[48]
Dexverapamil	**52**	Mouse	Oral	Single, 400	ND	ND	ND	[41]
2,3-Dianilinoquinoxaline (MMV007224)	**58**	Mouse	Oral	Single, 400	41%	ND	1	[49]
Diclofenac	**69**	Mouse	Oral	Single, 0.5–1 mg/mouse Multiple, 1.5–2.5 mg/kg	21% ND	55% ND	20	[50] [51]
Dihydroartemisinin	**18**	Mouse	Oral	Multiple, 200–400	60–70%	89–90%	ND	[52]
(-)-6,6′-Dinitrohinokinin	**44**	Mouse	Oral	Single, 10–100	40–48%	36–52%	~100	[53]
Doramectin	**56**	Mouse	Oral	Single, 10	60%	ND	33	[43]
Edelfosine	**72**	Mouse	Oral	Multiple, 45	29–47%	ND	20	[54]
Enpiroline	**31**	Mouse	Oral	Single, 200	83%	ND	6.1	[55]
Epiisopiloturine	**42**	Mouse	Oral	Single, 40–300 adult Single, 40 juvenile	70–47%	50%	>500	[56]

Chemical compound/drug organized alphabetically. Identification number of the compound is according to the order appearing in the text.
ND: Not determined; NS: Not significant.

Table 2. Antischistosomal properties observed with drug candidates in rodents experimentally infected with major human schistosome species and *in vitro* activity over the last 36 years from 1980 to 2016 (cont.).

Chemical compound/drug	Compound ID	Host animal	Drug administration		Worm burden reduction		*In vitro* activity (μM)	Ref.
			Route	Dose (mg/kg)	Adult infection	Juvenile infection		
Ferroquine	**32**	Mouse	Oral	Single, 200–800	19–36%	ND	33	[44]
Flutamide	**78**	Mouse	Oral	Single, 50–200	0%	6–4%	ND	[38]
Hesperidin	**39**	Mouse	Intraperitoneal	Multiple, 600	48%	ND	165	[57]
Kalata B2	–	Mouse	Oral or intravenous	Single, 15 or Single, 5	0	0	1–13	[58]
β-Lapachone	**37**	Mouse	Intraperitoneal	Multiple, 50	25–41	25–41	100	[59]
Lasalocid sodium	**66**	Mouse	Oral	Single, 100	41–44%	ND	1	[37]
Limonin	**43**	Mouse	Oral	Single, 50–100	41–60%	70–83%	ND	[60]
Lovastatin	**49**	Mouse	Oral	Single, 1–5 × 100–400	22–30%	ND	ND	[61]
LPSF-PT05	**81**	Mouse	Oral	Single, 1000	71%	ND	ND	[62]
Mefloquine	**27**	Mouse	Oral	Single, 25–400	13–77%	0–98%	15–30	[63]
Mefloquine derivatives	**29–30**	Mouse	Oral	Single, 100	0–87%	ND	3	[55,64]
Miltefosine	**71**	Mouse	Oral	Multiple, 20	95%	76%	100–200	[65]
N,N´-diarylurea (MMV665852)	**57**	Mouse	Oral	Single, 400	52.5%	ND	1	[49]
N,N´-diarylurea (MMV665852) analogs	**59**	Mouse	Oral	Single, 400	0–66%	ND	≤10 μM	[66]
Niclosamide	**60**	Mouse	Oral or intraperitoneal	Multiple, 100	0	ND	1	[37]
Nilutamide	**79**	Mouse	Oral	Single, 400 adult Single, 50–400 juvenile	85%	5–36%	7	[38,67]
Nitazoxanide	**64**	Mouse	Oral	Multiple, 100	0%	ND	ND	[37]
Oxadiazoles 2-oxides	**67**	Mouse	Intraperitoneal	Multiple, 10	94%	89%	2–10	[68]
Oxyclosanide	**62**	Mouse	Oral	Multiple, 100	53%	ND	ND	[37]
OZ78	**19**	Mouse Hamster	Oral Oral	Single, 200 (juvenile) Single, 400 (adult) Single, 100 (juvenile) Single, 200 (adult)	0% 85%	82% 83%	62	[69]
OZ165	**23**	Mouse	Oral	Single, 200 (juvenile) Single, 400 (adult)	74%	84%	ND	[70]
OZ209	**20**	Mouse	Oral	Single, 200 (juvenile) Single, 400 (adult)	17%	85%	68	[69]
OZ277	**24**	Mouse	Oral	Single, 100	81%	ND	ND	[71]
0Z288	**21**	Mouse Hamster	Oral Oral	Single, 200 (juvenile) Single, 400 (adult) Single, 100 (juvenile) Single, 200 (adult)	52% 72%	95% 84%	56	[69]
OZ418	**22**	Mouse	Oral	Single, 200 (juvenile) Single, 400 (adult)	80%	100%	ND	[70]
PA 1259	**26**	Mouse	Oral	Multiple, 100	31%	42%	103	[72]
Perhexiline maleate	**54**	Mouse	Oral	Single, 23–400	ND	ND	20	[73]
Phenyl vinyl sulfone (K11777)	**45**	Mouse	Intraperitoneal	Multiple, 25 (juvenile and adult) Multiple, 25 (juvenile) Multiple, 50 (adult)	79–80% ND 54–57%	79–80% 88–90% ND	ND	[74]
Phytol	**38**	Mouse	Oral	Single, 40	51%	ND	~170	[75]
Piperaquine	**25**	Mouse	Oral	Single, 50–300	14–80%	ND	ND	[71]
Rafoxanide	**63**	Mouse	Oral	Single, 50	50–56%	ND	ND	[37]
Ro 13–3978 (AH01)	**73**	Mouse	Oral	Single, 100 (adult) Single, 100–200 (juvenile)	93–95%	64–88%	>27	[67]

Chemical compound/drug organized alphabetically. Identification number of the compound is according to the order appearing in the text.
ND: Not determined; NS: Not significant.

Table 2. Antischistosomal properties observed with drug candidates in rodents experimentally infected with major human schistosome species and *in vitro* activity over the last 36 years from 1980 to 2016 (cont.).

Chemical compound/drug	Compound ID	Host animal	Drug administration		Worm burden reduction		*In vitro* activity (μM)	Ref.
			Route	Dose (mg/kg)	Adult infection	Juvenile infection		
Ro 13–3978 derivative (AH02)	**74**	Mouse	Oral	Single, 100 (adult) Single, 100–200 (juvenile)	ND	51–88%	>27	[67]
Ro 13–3978 derivative (AH03)	**75**	Mouse	Oral	Single, 100	ND	ND	1.5	[67]
Ro 13–3978 derivative (AH04)	**76**	Mouse	Oral	Single, 100	ND	ND	>27	[67]
Tariquidar	**53**	Mouse	Oral	Single, 400	ND	ND	ND	[41]
Trametinib	**47**	Mouse	Oral	Single, 400	64%	ND	9–21	[76]
Triclabendazole	**88**	Mouse	Oral	Twice, 120–200 Single, 400	19–36% 7%	ND	42	[77] [78]
Triclabendazole sulphone	**89**	Mouse	Oral	Single, 400	0%	ND	ND	[31]
Triclabendazole sulphoxide	**90**	Mouse	Oral	Single, 400	0%	ND	ND	[31]
Triphenylphosphonium derivatives	**40–41**	Mouse	Oral	Single, 400	22%	ND	2	[79]
Valproic acid	**46**	Mouse	Oral	Multiple, 200	41%	ND	ND	[80]
Vandetanib	**48**	Mouse	Oral	Single, 400	48%	ND	33	[76]
WR 7930	**28**	Mouse	Oral	Single, 100	87%	ND	77	[55]
S. japonicum								
Artemether	**15**	Hamster	Oral	Single, 50–200	72–96%	83–91%	ND	[81]
Artenusate	**16**	Mouse	Oral	Multiple, 20–300	ND	77–91%	ND	[82]
Cyclosporin A	**34**	Mouse	Subcutaneous	Multiple, 50	NS	NS	ND	[83]
Cyclotide kalata B2	-	Mouse	Oral subcutaneous	Single, 15 Single, 5	15–60%	ND	2–6	[58]
Dihydroartemisinin	**18**		Oral	Single, 200–600	47–84%	47–84%	ND	[84]
Mefloquine	**27**	Mouse	Oral	Single, 25–400	26–100%	17–95%	ND	[63]
Oxadiazoles 2-oxides	**67**	Mouse	Intraperitoneal	Multiple, 10	25–72%	70–91%	10–50	[68,85]
OZ78	**19**	Hamster Mouse	Oral	Single, 200 Single, 200–600 (adult) Single, 400 (juvenile)	94% 67–80%	70% 75%	ND ND	[69] [81] [86]
OZ277	**24**	Hamster	Oral	Single, 200	73%	81%	ND	[81]
OZ418	**22**	Mouse	Oral	Single, 200 (adult) Single, 400 (juvenile)	70–84%	27–76%	ND	[87]
S. haematobium								
9-Acridanone hydrazine (Ro 15–5458)	**82**	Hamster	Oral	Twice, 10	100%	83%	ND	[88]
Arachidonic acid	**35**	Mouse Hamster	Oral Oral	Multiple, 1000 Multiple, 300–2500	58% 51%	58% ND	5000	[33] [34]
Artemether	**15**	Hamster	Oral	Multiple, 300	76%	78–99%	ND	[89]
Enpiroline	**31**	Mouse	Oral	Single, 200	76,6%	ND	ND	[55]
Mefloquine	**27**	Mouse	Oral	Single, 200 Single, 100–200	81% 62–94%	ND	ND	[55]
OZ418	**22**	Hamster	Oral	Single, 400	86%	ND	ND	[70]
WR 7930	**28**	Mouse	Oral	Single, 200	100%	ND	ND	[55]

Chemical compound/drug organized alphabetically. Identification number of the compound is according to the order appearing in the text.
ND: Not determined; NS: Not significant.

the leaves of *Artemisia annua* L. (Asteraceae). It has been used as an antimalarial since the early 1970s, and its schistosomicidal activity was discovered in 1980. As reviewed elsewhere [94,95], artemisinin shows *in vivo* activity in various animals experimentally infected with *S. japonicum*. Subsequently, the semi-synthetic derivatives artemether (**15**) and artesunate (**16**) were tested in rodents experimentally infected with *S. japonicum*. Mice or dogs infected

Table 3. Pharmacokinetic and toxicity profiles of the antischistosomal compounds.

Chemical compound/drug	Compound ID	Maximum plasma concentration (Cmax)	Half-life ($^{t}1/2$)	Lethal dose (LD_{50})
9-Acridanone hydrazine (Ro 15–5458)	**82**	NA	NA	312 mg/kg
Acyclic nucleotide analog [(*S*)-HPMPA]	**70**	1.1 μM	11.5 h	NA
Arachidonic acid	**35**	NA	N.A	39.2 mg/kg
Artemether	**15**	118 ng/ml	1.6–2.2 h	895 mg/kg
Artenusate	**16**	231.8 ± 155.0	0.22 h	>825 mg/kg
Anisomycin	**65**		NA	148 mg/kg
Bicalutamide	**77**	734 ng/ml	1.2 d	>2000 mg/kg
Bisquinoline cyclen derivative	**85**	NA	NA	NA
Bisquinoline cyclen derivative (Fe^{++}complex)	**86**	NA	NA	NA
Bisquinoline cyclen derivative (Mn^{++}complex)	**87**	NA	NA	NA
BTP-Iso	**91**	NA	NA	NA
C-4	**51**	NA	NA	NA
Chlorambucil	**83**	492 ± 160 ng/ml	1.5 h	76.1 mg/kg
Clofazimine	**55**	0.7 μg/ml to 1.0 μg/ml	70 d	8400 mg/kg
Chloroquine	**33**	614 μg/l	32.6 h	969.9 mg/kg
Clorsulon	**84**	2.58 μg/ml	3,58 d	10,000 mg/kg
Closantel	**61**	45–55 μg/ml	2–3 weeks	262 mg/kg
Curcumin	**36**	0.06 ± 0.01 microg/ml	44.5 ± 7.5 min	2000 mg/kg
Cyclosporin A	**34**	1500–1800 ng/ml	8.4 h	2329 mg/kg
Cyproterone acetate	**80**	103.161 ng/ml	37.60 h	>4000 mg/kg
Dexamethasone	**68**	329 ng/ml	36–54 h	>3000 mg/kg
Dexverapamil	**52**	600–4100 ng/ml	4 h	NA
2,3-Dianilinoquinoxaline (MMV007224)	**58**	12.4 μmol/l	NA	NA
Diclofenac	**69**	453.1 ng/ml	3 h	53 mg/kg
Dihydroartemisinin	**18**	250 ng/ml	1–2 h	4000 mg/kg
(−)-6,6′-Dinitrohinokinin	**44**	NA	NA	NA
Doramectin	**56**	12.2 ± 4.8 ng/ml	20–30 d	500–1000 mg/kg
Edelfosine	**72**	50.7 ± 28.1 μg/ml	22.288 ± 14.016 h	NA
Enpiroline	**31**	5.7–7.6 ng/ml	72–182 h	518 mg/kg
Epiisopiloturine	**42**	NA	NA	8000 mg/kg
Ferroquine	**32**	82 to 270 ng/ml	16 d	
Flutamide	**78**	44.78 ng/ml	8.21 h	787 mg/kg
Hesperidin	**39**	2.2 μM	2.2 h	3000 mg/kg
Kalata B2	–	NA	30 h	NA
β-Lapachone	**37**	0.218 μg/ml	11.36 h	NA
Lasalocid sodium	**66**	0.7 μg/ml	3–4.8 h	122–146 mg/kg 122 mg/kg oral
Limonin	**43**	NA	NA	NA
Lovastatin	**49**	5.5 ng/ml	2–5 h	>5000 mg/kg
LPSF-PT05	**81**			
Mefloquine	**27**	1500 μg/ml	6–33 d	890 mg/kg
Mefloquine derivatives	**29, 30**	NA	NA	NA
Miltefosine	**71**	31.9 μg/ml (dose of 2.5 mg/kg/day)	34,4 d	246 mg/kg
N,N′-diarylurea (MMV665852)	**57**	4.4 μmol/l	NA	NA
N,N′-diarylurea (MMV665852) analogs	**59**	NA	NA	NA
Niclosamide	**60**	0.40 ± 0.28 μg/ml (dose of 120 mg/kg)	8.35 h	>10,000 mg/kg
Nilutamide	**79**	0.9 mg/l	56 h	215 mg/kg
Nitazoxanide	**64**	10,6 μg/ml	3.5 h	10,000 mg/kg

Data are variously available from [90–93].
NA: Not available.

Table 3. Pharmacokinetic and toxicity profiles of the antischistosomal compounds (cont.).

Chemical compound/drug	Compound ID	Maximum plasma concentration (Cmax)	Half-life ($t_{1/2}$)	Lethal dose (LD_{50})
Oxadiazoles 2-oxides	**67**	NA	NA	NA
Oxyclosanide	**62**	1,3–3,9 μg/ml	±10 h	3519 mg/kg
OZ78	**19**	NA	NA	NA
OZ165	**23**	NA	NA	NA
OZ209	**20**	NA	NA	NA
OZ277	**24**	NA	NA	NA
0Z288	**21**	NA	NA	NA
OZ418	**22**	NA	NA	NA
PA 1259	**26**	NA	NA	NA
Perhexiline maleate	**54**	112 ng/ml	1.2–6 d	2150 mg/kg
Phenyl vinyl sulfone (K11777)	**45**	50–100 mg	3–4 h	NA
Phytol	**38**	NA	NA	>5000 mg/kg
Piperaquine	**25**	578.47 ng/ml	0.12 h	1184 μmol/kg
Rafoxanide	**63**	30.88 μg/ml	138.02 ± 13.99 h	300 mg/kg
Ro 13–3978 (AH01)	**73**	124 μM	NA	NA
Ro 13–3978 derivative (AH02)	**74**	NA	NA	NA
Ro 13–3978 derivative (AH03)	**75**	NA	NA	NA
Ro 13–3978 derivative (AH04)	**76**	NA	NA	NA
Tariquidar	**53**	1.2–2.8 μM	5 h	NA
Trametinib	**47**	22.2 ng/ml	3.9–4.8 d	2.3412 mol/kg
Triclabendazole	**88**	NA	22–24 h	>8000 mg/kg
Triclabendazole sulphone	**89**	7.95 ± μg/ml	71.7 ± 2.13 h	>8000 mg/kg
Triclabendazole sulphoxide	**90**	8.59 μg/ml	32.37 ± 1.72 h	>8000 mg/kg
Triphenylphosphonium derivatives	**40, 41**	NA	NA	NA
Valproic acid	**46**	49–59 mg/l	9–16 h	1098 mg/kg
Vandetanib	**48**	84,4 ng/ml	215,8–246,6 h	17–41 mg/kg
WR 7930	**28**	NA	NA	NA

Data are variously available from [90–93].
NA: Not available.

with *S. japonicum* and treated with artemether resulted in a significant worm burden reductions of 55 to 99%. In the late 1980s, *in vitro* and *in vivo* investigations were extended from *S. japonicum* to *S. mansoni*, and finally, to *S. haematobium*. Within a few years, other artemisinin derivatives such as arteether (**17**), and dihydroartemisinin (**18**) were also evaluated against schistosomes.

Among artemisinins, artemether and artenusate are the most active compounds (Figure 3). In this sense, several laboratory studies in different host animals revealed that artemether reduced worm burdens by 78–97%. Clinical trials with multiple doses of artemether at 6 mg/kg over 15 d intervals were found to achieve 60.8 to 100% protection for the prevention of *S. japonicum* infection. Interestingly, immature stages of schistosomes are significantly more susceptible to the artemisinins than adult worms, which is the opposite stage-specific bioactivity for praziquantel [94,98]. In animals' model, combination therapy with praziquantel plus artemether results in higher worm reduction rates than praziquantel alone. Nevertheless, clinical trials revealed that the combination of these drugs did not improve treatment efficacy compared with praziquantel alone [99]. In regard to artesunate, results from clinical trials show that this compound alone gives lower cure rates than praziquantel. Although the combination of artemisinins with praziquantel seems to be the best option for the treatment of schistosomiasis [98], resistance of malaria against artemisinins has been well documented [100] and references therein. Therefore, in areas where schistosomiasis and malaria are co-endemic, artemisinin should not be recommended.

Ozonides (1,2,4- trioxolanes)

With the advancement of chemistry-based approaches, the compounds are modified to optimize their therapeutic properties and to reduce limitations to their use. In the early 2000s, Vennerstrom *et al.* [101] synthesized several

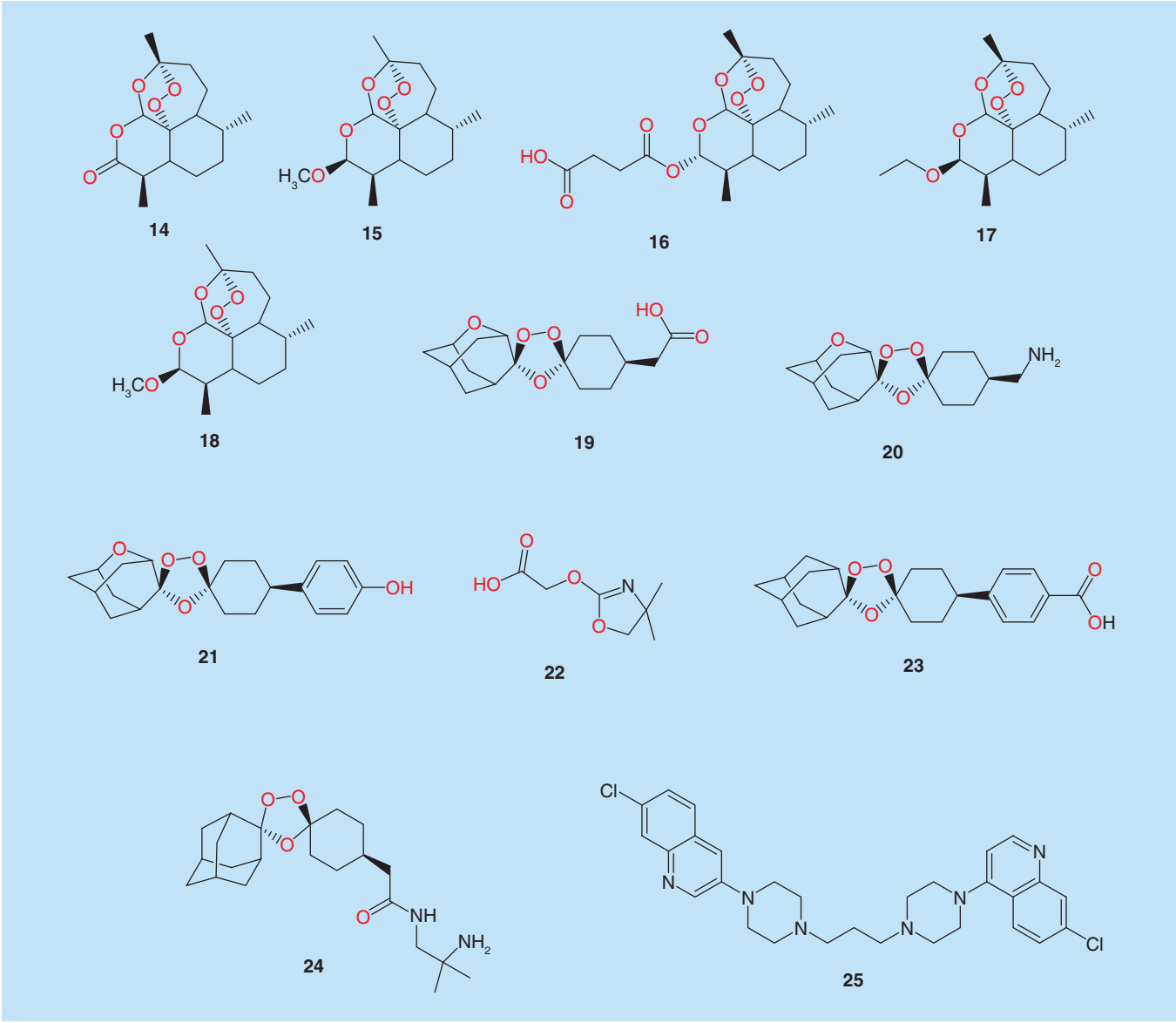

Figure 3. Chemical structures of artemisinin derivatives and ozonides its derivatives. Artemisinin (**14**), artemether (**15**), artesunate (**16**), arteether (**17**), dihydroartemisinin (**18**), OZ78 (**19**), OZ209 (**20**), OZ288 (**21**), OZ418 (**22**), OZ165 (**23**), OZ277 (**24**) and piperaquine (**25**).

synthetic trioxolane derivatives incorporating the critical endoperoxide pharmacophore of artemisinins, among which ozonide OZ-277 was more potent than artemisinin against malaria. They also showed that OZ78 had much higher oral bioavailability (74%) than that of artemether (1.4%) in rats. The artemisinin pharmacophoric peroxide bond offers a valuable starting point not only for antimalarial, but also for antischistosomal drug discovery. Indeed, the rational design of structurally simpler analogs of artemisinins has led to the synthesis of a large number of peroxides such as ozonides (1,2,4-trioxolanes) and trioxaquines (1,2,4-trioxanes), some of which have displayed interesting antischistosomal activities (Figure 3). When compared with the artemisinins, ozonides are characterized by structural simplicity, ease of synthesis and improved pharmacokinetic parameters. Therefore, the use of these molecules became attractive.

The ozonides were first tested in *S. mansoni* animal model by Xiao *et al.* [69]. The authors evaluated the single 200 mg/kg oral doses of three ozonides, namely OZ78 (**19**), OZ209 (**20**) and OZ288 (**21**). In this case, mice harboring a juvenile *S. mansoni* infection resulted in worm burden reductions of 82 to 95%. Interestingly, OZ78 was completely inactive against adult *S. mansoni* in mice, but was highly active against adult *S. mansoni*

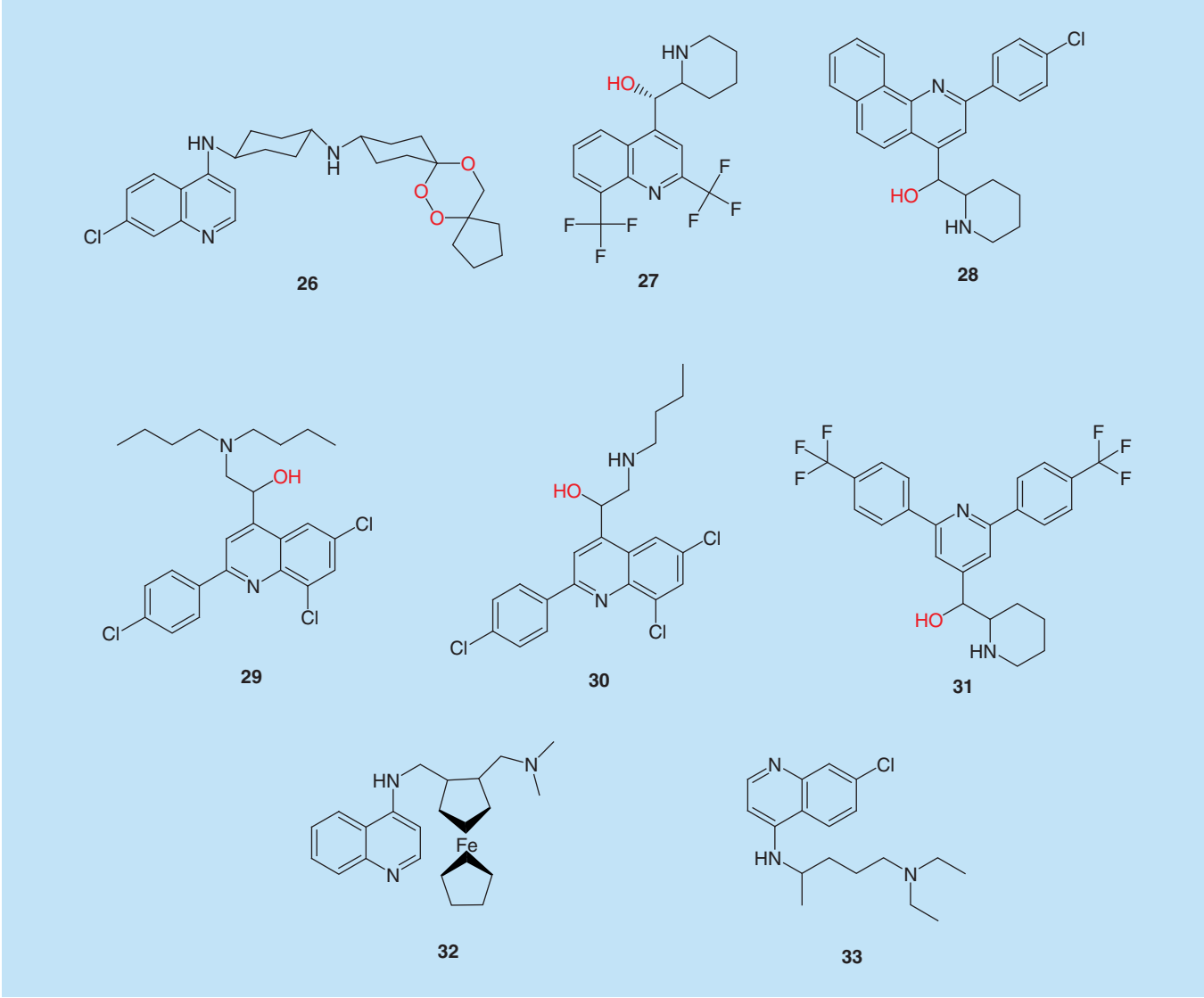

Figure 4. Chemical structures trioxaquine and mefloquine and its derivatives and its derivatives. Trioxaquine PA 1259 (**26**), mefloquine (**27**), WR7930 (**28**), its two derivatives (**29** and **30**), enpiroline (**31**), ferroquine (**32**), chloroquine (**33**).

harbored in hamsters. The compounds OZ418 (**22**) and OZ165 (**23**) also have strong schistosomicidal properties, with worm burden reduction of 80–100% and 74–84%, respectively [70]. Recently, Xue *et al.* [87] showed that OZ418 has promising efficacy against 7-day-old juvenile and adult *S. japonicum*. Furthermore, the ozonide OZ277 (**24**) (arterolane maleate) combined with piperaquine phosphate (**25**) had high activity in a mouse model of *S. mansoni* [71]. Trioxolane and tricyclic monoperoxides were also recently tested in mice harboring an adult *S. mansoni* infection, but showed moderate worm burden reductions [102].

Trioxaquines

Trioxaquines are hybrid molecules that contain two pharmacologically active moieties, a trioxane (as in artemisinin) and a 4-aminoquinoline (as in chloroquine). Against malaria parasites, these molecules are known to easily penetrate within infected erythrocytes. In contrast to malaria treatment, the effect of trioxaquine against *S. mansoni*-infected mice is weaker. In this respect, a trioxaquine, especially PA 1259 (**26**) (Figure 4) at five daily doses of 100 mg/kg induced a worm burden reduction of 31 and 42% on juvenile and adult stage, respectively [72]. Interestingly, trioxaquine PA 1259-praziquantel combination on mice infected by larval forms resulted in a significantly higher reduction of 73%.

Mefloquine & its derivatives

Mefloquine, (**27**), an arylaminoalcohol compound, has been one of the most effective antimalarial drugs since the mid-1980s (Figure 4). In 2008, Van Nassauw *et al.* [103] reported that a single oral dose 150 mg/kg administered to mice infected with adult *S. mansoni* had no effect on worm burden, but significantly reduced the number of eggs. In the next year, Keiser *et al.* [63] showed that oral treatment of mice with 200 or 400 mg/kg mefloquine resulted in high or complete total and female worm burden reductions (72.3–100%). Later, some experimental studies revealed that the combination of mefloquine and praziquantel, or artemisinins, gave better curative effects than compounds given alone [104,105]. Finally, in 2012, interesting antischistosomal properties of mefloquine-related arylmethanols (Figure 4) were described in *S. mansoni*- and *S. haematobium*-rodent models [55]. Among them, four candidates, WR7930 (**28**), its two derivatives (**29** and **30**), and enpiroline (**31**), were characterized by high antischistosomal properties in animal model. Human clinical trials indicate that mefloquine is a promising candidate for treatment of schistosomiasis [106,107].

Another antimalarial compound with schistosomicidal activity is ferroquine (**32**), a derivative of chloroquine (**33**) (Figure 4). Several ferroquine derivatives have been tested for antimalarial properties [108]. However, ferroquine derivatives show only weak antischistosomal properties. In more detail, oral dose of ferroquine derivatives (200–800 mg/kg) administered to mice infected with *S. mansoni* resulted in low worm burden reductions of 0–36%. Similarly, chloroquine shows poor schistosomicidal activity in animal experimental infected with schistosomes [44].

Cyclosporin A

The immunosuppressant drug cyclosporin A (**34**) (Figure 5) has broad antiparasitic action both *in vivo* and *in vitro* [109,110]. Bueding *et al.* [47] were the first to report the antiparasitic activity of immunosuppressant drug cyclosporin A while attempting to suppress granuloma formation in *S. mansoni*-infected mice. They observed that treated rodents had a reduction in the number of worms, as well as that this reduction was more pronounced when treating immature infections, and that female worms were more affected than males. Interestingly, later Bout *et al.* [111] showed that the drug appears to have very long-lasting prophylactic properties, confirmed by experiments in which the drug was given 27, 45, 62, 76 or 100 days before infection. The drug acts in a time-, dose-, route- and vehicle-dependent manner [83,110]. Effects in animal model include reduced worm and egg burdens, decreased hepatomegaly, damage to the schistosome tegument and gut. The results concerning age, sex and species emphasize that much remains to be done to tease out the mechanisms by which cyclosporine A is schistosomicidal [83]. More recently, it was found that the antifecundity effect of the immune modulator cyclosporin A on schistosomes is related to its inhibitory activity on uptake of heme in the ovary [112].

Arachidonic acid

The first indication of arachidonic acid (**35**) (Figure 5), a long-chain essential polyunsaturated fatty acid with antischistosomal properties, was described by Tallima *et al.* [113]. Later, using mice infected with *S. mansoni* or *S. japonicum*, the authors demonstrated that arachidonic acid 300 mg/kg/day, for 15 days at the prepatent or patent period, led to 40 to 80% decrease in the total worm burden [33]. Also, a single oral dose of arachidonic acid 1000 mg/kg resulted in moderate worm burden reductions (40–60%). Recently, clinical trials were initiated in *S. mansoni*-infected schoolchildren in Menoufiya, Egypt, and arachidonic acid proved to be as efficacious as praziquantel in treatment of children with low infection intensity (78 and 85% cure rates, respectively). In Egypt, the efficacy and safety of arachidonic acid for treatment of school-age children in *S. mansoni* high-endemicity areas of Kafr El Sheikh was also evaluated. These results showed that praziquantel and arachidonic acid combined elicited 83 and 78% cure rates in children with light and heavy infection, respectively [114].

Curcumin

Curcumin (**36**) is a diaryheptanoid pigment isolated from the plant *Curcuma longa* L. (Zingiberaceae). It has been shown a variety of pharmacologic properties, including antischistosomal effect [46]. In mice experimentally infected with *S. mansoni*, curcumin (Figure 12) (400 mg/kg) resulted in a decrease in worm burden of 44.4%. Moreover, the number of liver granulomas was also considerably lower than that from the control infections. The mechanism by which the curcumin exert their antischistosomal properties remains unclear, but may be due to the inhibition of the ubiquitin–proteasome pathway [115] or transcriptional repression in Notch and TGF-β pathways [116].

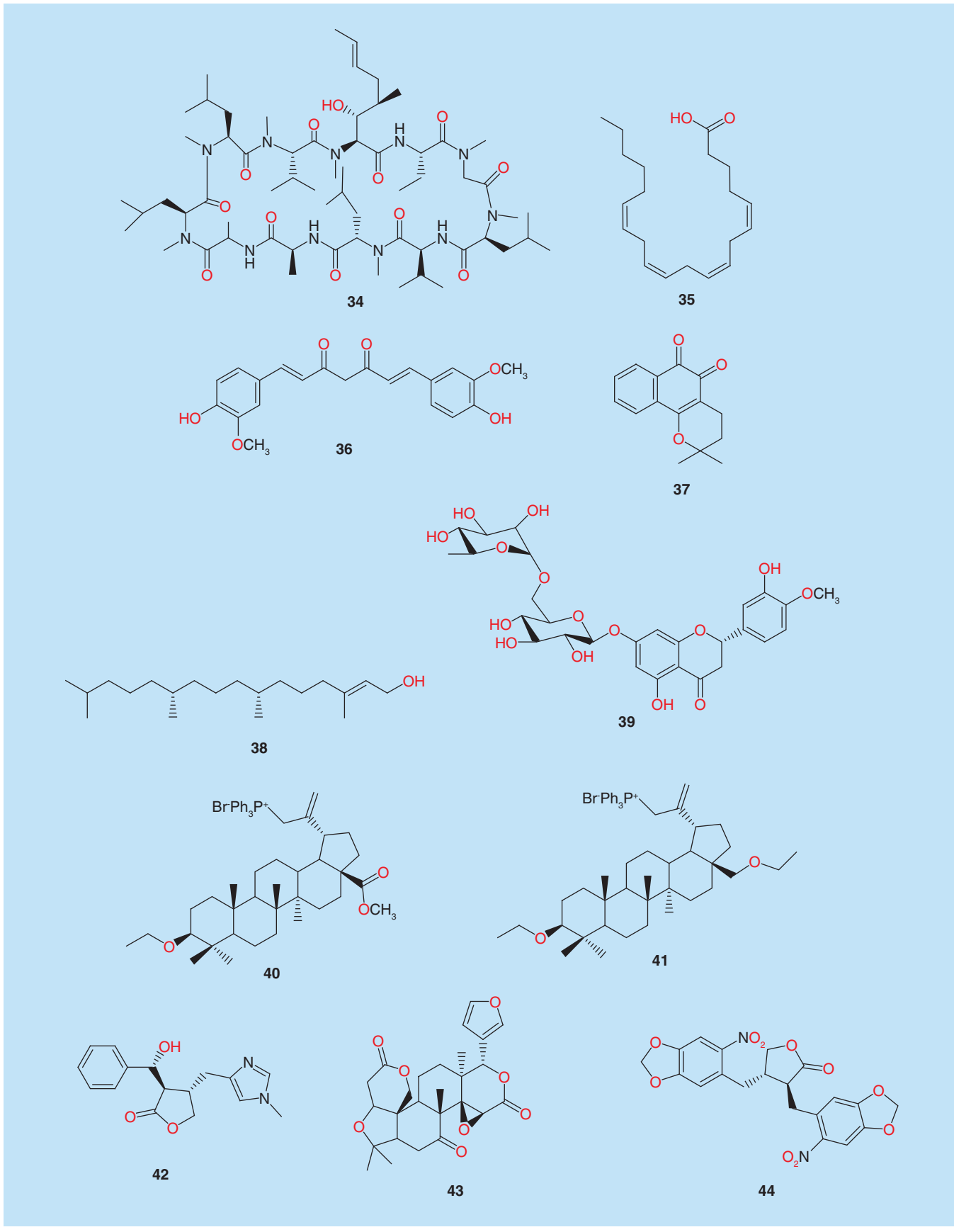

Figure 5. Chemical structures of others antischistosomal natural compounds and its derivatives. Cyclosporin A (**34**) and arachidonic acid (**35**), Curcumin (**36**), β-lapachone (**37**), phytol (**38**), hesperidin (**39**), triphenylphosphonium derivatives (**40** and **41**), epiisopiloturine (**42**), limonin (**43**) and (-)-6,6′-dinitrohinokinin (**44**).

β-*Lapachone*

β-Lapachone (**37**) (Figure 5) is a naturally occurring quinone obtained from *Tabebuia avellanedae* bark (Bignoniaceae). In experimental schistosomiasis, β-lapachone was administrated once daily for 5 consecutive days by intraperitoneal injection at 50 mg/kg to *S. mansoni* infected mice with multiple parasite stage (skin schistosomula, lung schistosomula, juvenile and adult worms) [59]. In this case, treatment with β-lapachone led to moderate worm burden reductions (25–41%). Moreover, a reduction in the number of eggs and granulomas in the hepatic tissue were recorded.

Phytol

Another interesting natural compound is phytol (**38**), a diterpene alcohol from chlorophyll, widely used as a food additive (Figure 5). Phytol shows promising antischistosomal properties in laboratory studies, with mice harboring adult *S. mansoni* [75]. Indeed, phytol achieved a worm burden reduction of 51.2 to 71% when *S. mansoni*-infected mice were treated orally with 40 mg/kg. Moreover, phytol reduced the number of eggs in feces (76.6%) and the frequency of immature eggs (oogram pattern) was significantly reduced. The mechanism by which phytol exerts its antischistosomal effect is not clear. However, confocal laser scanning microscopy studies revealed tegumental damage in adult schistosomes and a correlation between viability and tegumental damage was described. Recent studies also showed *in vitro* schistosomicidal activity of phytol juvenile and adult stages of *S. haematobium* [117].

Hesperidin

Hesperidin (**39**) (Figure 5) is a flavanone glycoside found at high levels in citrus fruits such as oranges and lemons. It has been reported to exhibit a wide range of pharmacological effects, including antischistosomal properties [57]. Indeed, in experimentally infected mice harboring *S. mansoni* hesperidin administered intraperitoneally (600 mg/kg) induced significant worm burden reductions, ranging from 45 to 50%. The compound also reduced the number of eggs, ranging from 42 to 64%, in hepatic and intestinal tissues.

Triphenylphosphonium triterpene derivatives

In 2014, Spivak *et al.* [79] synthesized triphenylphosphonium derivatives (**40** and **41**) (Figure 5) from the natural triterpenes botulin and betulinic acid, and they evaluated the effect of compounds in *S. mansoni*-infected mice. After a single oral dose (400 mg/kg), the results showed low worm burden reductions of 22% for two triphenylphosphonium salts. According to the authors, the low *in vivo* activity of triphenylphosphonium derivatives might be related to the limited aqueous solubility of these compounds.

Epiisopiloturine

Veras *et al.* [118] were the first to report an *in vitro* antischistosomal effect by epiisopiloturine (**42**), an imidazole alkaloid isolated from the leaves of *Pilocarpus microphyllus* Stapf ex Holm. (Rutaceae) (Figure 5). Later, the same researchers reported the *in vivo* activity of epiisopiloturine against juvenile and adult worms of *S. mansoni* [56]. A single oral dose of epiisopiloturine 40 mg/kg in mice harboring adult schistosomes reduced parasite burden, led to a reduction in hepatosplenomegaly, reduced the egg burden in feces, and decreased granuloma diameter. Interestingly, epiisopiloturine at lower doses was more effective in adult worms when compared with higher doses. In more details, after 45 days of infection, epiisopiloturine at 40, 100 and 300 mg/kg reduced the worm burden of the mice to 70, 39 and 47%, respectively. The *in vivo* treatment against juveniles with 40 mg/kg showed a reduction of the total worm burden of 50%. *In vitro* combinations of epiisopiloturine and praziquantel were published [119], but *in vivo* studies have not yet been described.

Limonin

Limonin (**43**), a limonoid that is a bitter principle of citrus fruits (Figure 5), has been reported to exert *in vivo* antischistosomal properties [60]. In mice harboring *S. mansoni* juvenile stage, limonin showed significant worm burden reduction of 70.0 and 83.33% after single oral dose of 50 or 100 mg/kg, respectively. Moreover, in a rodent host infected with adult schistosomes, single oral dose of 50 or 100 mg/kg reduced total worm burdens by 41.09 and 60.27%, respectively. In addition, treatment with limonin achieved significant reductions of 34.90 and 47.16% in the hepatic and 46.67 and 56.1% in the intestinal tissue egg loads, respectively, followed by an increase in the proportion of dead eggs.

(-)-6,6′-Dinitrohinokinin

(-)-6,6′-Dinitrohinokinin (**44**) is a dibenzylbutyrolactone lignanobtained by the partial synthesis of (-)-hinokinin, a semi-synthetic derivative of (-)-cubebin from *Piper cubeba* (Piperaceae) (Figure 5). In *S. mansoni*-infected mice, the oral treatment with (-)-6,6′-dinitrohinokinin(10–100 mg/kg, started 1, 23 and 37 days after infection) revealed only moderate worm burden reductions (33.8–52.3%) and egg production (40.7–60.0%) [53].

Cyclotide kalata B2

Antimicrobial peptides are short, cationic peptides composed of less than 50 amino acids. Although peptides have a broad range of activities and are active against a large number of microorganisms [120], few peptides have been tested against helminths [121,122]. Recently, kalata B2, a plant cyclic peptides isolated from *Oldenlandia affinis* (R&S) DC (Rubiaceae) showed anthelmintic properties in murine model [58]. In this context, oral (15 mg/kg) or intravenous (5 mg/kg) administration of kalata B2 in mice experimentally infected with *S. japonicum* reduced worm burdens by 15 to 60%. However, treatment of mice harboring *S. mansoni* did not result in reduction in worm burdens. Morphological studies revealed that kalata B2 lysed the tegument of schistosomes.

Antischistosomal target-based compounds

Cysteine protease inhibitors

Cysteine proteases are a class of proteolytic enzymes that help schistosomes to degrade the ingested blood proteins. Experimental studies in *S. mansoni*-infected mice were carried out with cysteine protease inhibitors such as phenyl vinyl sulfone (K11777) (**45**) and valproic acid (**46**) (Figure 6). With regard to the K11777, Abdulla *et al.* [74] showed that the inhibition of cysteine proteases of schistosomes resulted in a significant reduction in parasite burden and pathology in experimentally infected mice. In general, worm reduction rates of 54 to 90% were obtained. As a disadvantage, K11777 was administered intraperitoneally at 25 mg/kg twice daily during long-course treatment (e.g., begun on day 7 postinfection and continued for 35 days or started at day 1 postinfection and continued for 14 days). More recently, experiments using valproic acid protease inhibitor resulted in moderate worm burden reduction (41%), but a considerable decrease in the fecal egg count (84%) [80].

Trametinib & vandetanib

Trametinib (**47**) and vandetanib (**48**) are kinase inhibitors used for the treatment of cancer (Figure 6). Using the approved oncology drug set of the National Cancer Institute's Developmental Therapeutic Program, Cowan and Keiser [76] screened *in vitro* more than one hundred compounds for schistosomicidal activity. Six compounds were evaluated using mouse model for schistosomiaisis mansoni (afatinib, bosutinib, ponatinib, sunitinib, trametinib and vandetanib). However, only trametinib and vandetanib have shown moderate *in vivo* activity, with worm reduction rates of 63.6 and 48.1% respectively, after a single oral dose of 400 mg/kg.

Lipid lowering drugs

In humans, the enzyme HMG-CoA reductase is the rate-limiting enzyme for cholesterol synthesis. In addition, this enzyme plays an important role for schistosomes survival by regulating egg production. Therefore, it is known that inhibitors of cholesterol synthesis such as lovastatin (**49**) (a.k.a., mevinolin) and atorvastatin (**50**) (Figure 6) can reduce egg laying by schistosome female. Indeed, in the late 1980s, *in vitro* and *in vivo* studies conducted by Vandewaa *et al.* [123] showed that egg production by schistosomes is associated with HMG-CoA reductase, and also that cholesterol precursors, such as mevalonate and farnesol, stimulate egg production and can reverse mevinolin-induced inhibition of egg production. Subsequently, Chen *et al.* [124] revealed that mevalonate not only plays a vital role in *Schistosoma* egg production, but is vital for survival of the parasite. Regarding the effect of statins on parasite load, moderate activities were observed with atorvastatin (worm burden reduction of 46% against adult *S. haematobium*-infected hamsters) [125] and lovastatin (worm burden reduction of 30% against adult *S. mansoni*-infected mice) [61]. Chemical and genetic validation of the statin drug target to treat schistosomiasis has been recently described and, together, these data confirm that schistosomes HMG-CoA reductase is an important target of statin drugs [126].

ABC transporters inhibitors

Pgp is a member of the ABC superfamily of proteins. The efficacy of four Pgp inhibitors was studied *in vitro* and in mice harboring adult *S. mansoni*: C-4 (**51**), a derivative of curcumin; dexverapamil (**52**), an enantiomer

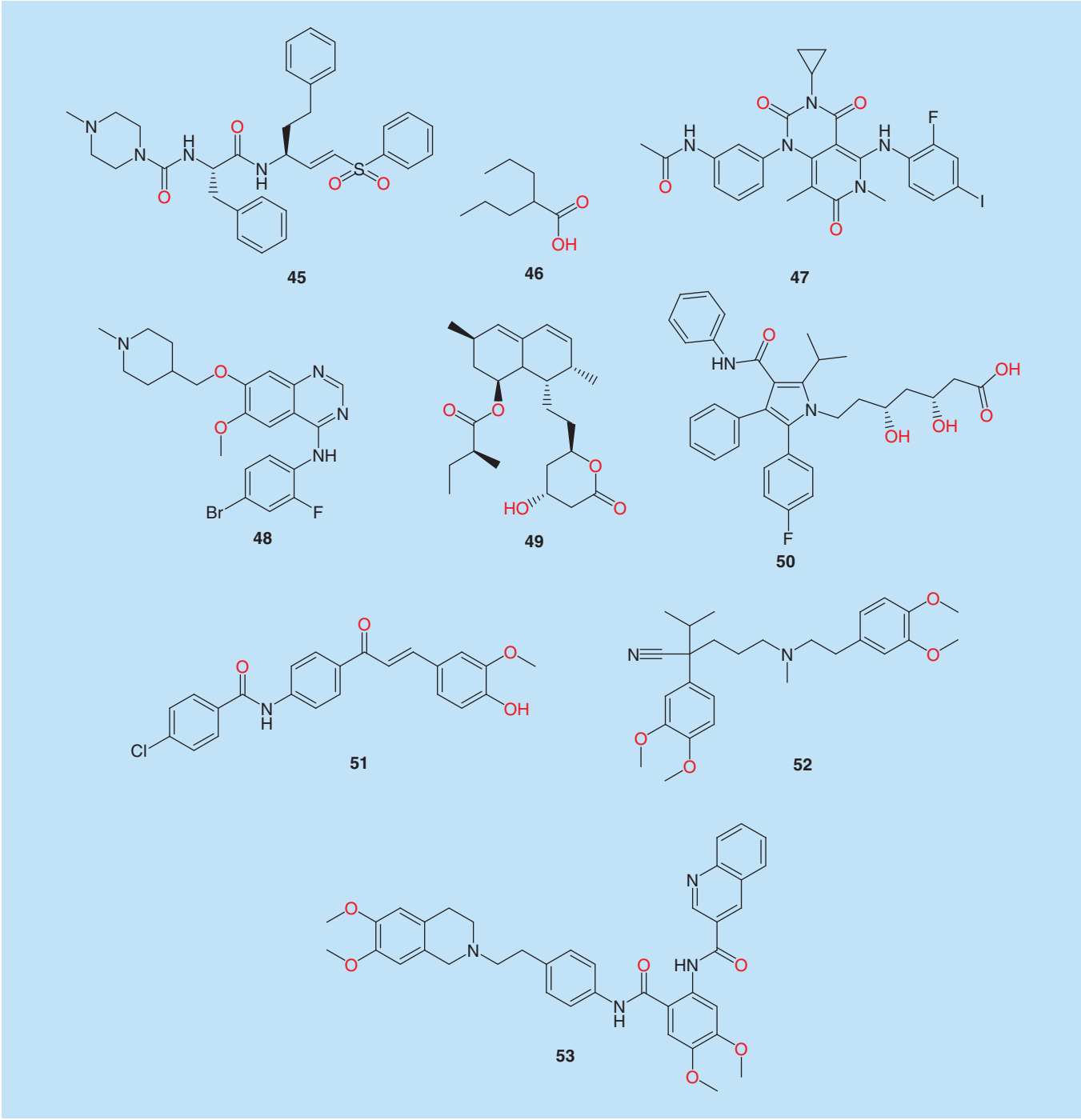

Figure 6. Chemical structures of some antischistosomal target-based compounds. Phenyl vinyl sulfone (K11777) (**45**), valproic acid (**46**), trametinib (**47**), vandetanib (**48**), lovastatin (**49**), atorvastatin (**50**), C-4 (**51**), dexverapamil (**52**) and tariquidar (**53**).

of verapamil; tariquidar (**53**), a selective and highly potent Pgp inhibitor (Figure 6); and the immunosuppressant cyclosporin A (**34**). Intraperitoneal doses of C-4 (50 mg/kg), dexverapamil (60 mg/kg), tariquidar (15 mg/kg), and cyclosporin A (60 mg/kg) decreases liver egg burden in approximately 80, 65, 55 and 50%, respectively. The treatment also showed significant reduction in granuloma size when animals were treated with any of the four Pgp inhibitors. *In vitro*, these Pgp inhibitors resulted in a concentration-dependent reduction in parasite

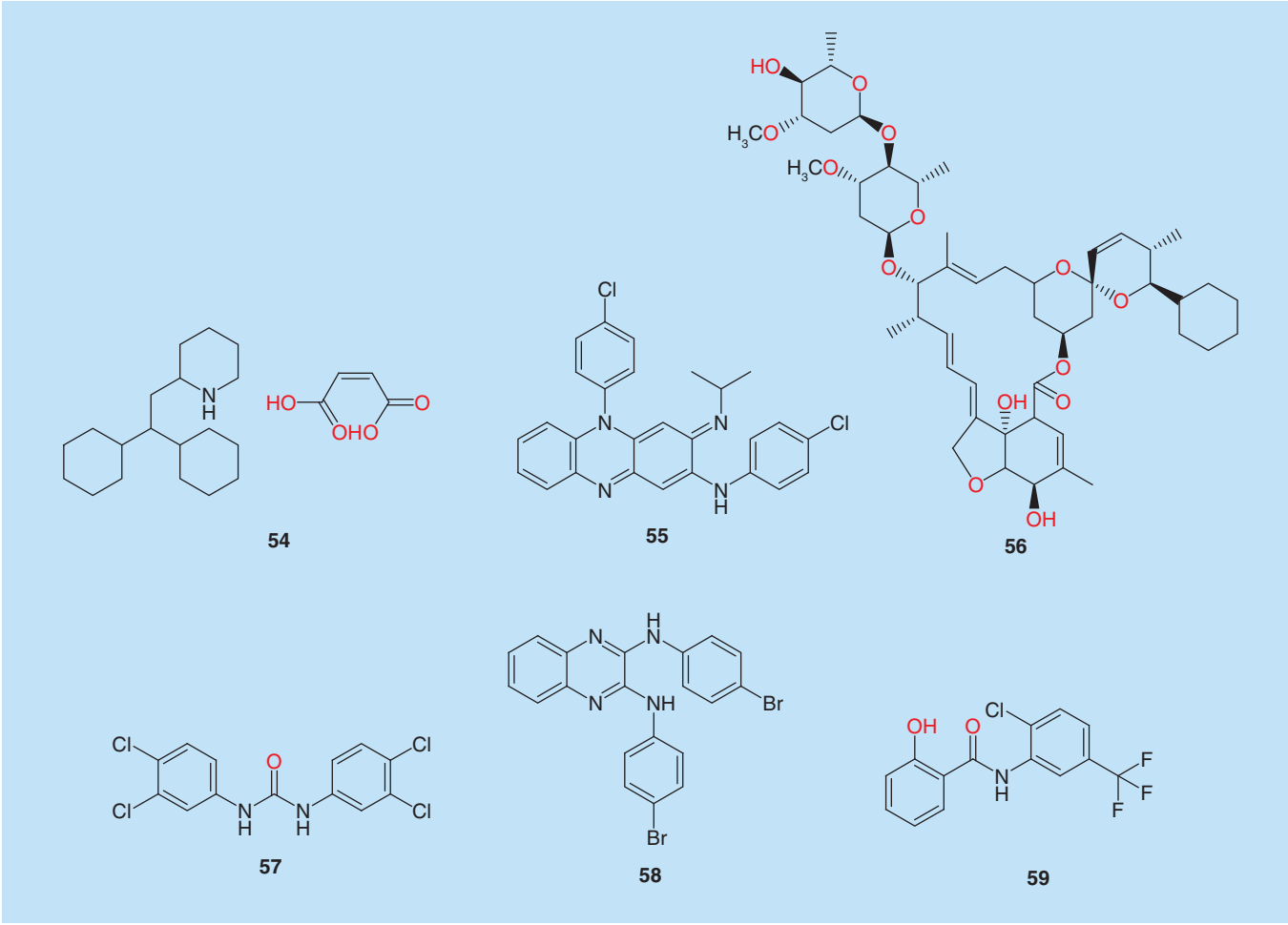

Figure 7. Chemical structures of some antischistosomal compounds identified by screening of small libraries. Perhexiline maleate (**54**), clofazimine (**55**), doramectin (**56**) and N,N′-diarylureas, N,N′-diarylurea (MMV665852) (**57**), 2,3-dianilinoquinoxaline (MMV007224) (**58**) and N,N′-diarylurea (MMV665852) analogs (**59**).

egg production [41]. Inhibition or knockdown of ABC transporters also enhances susceptibility of schistosomes to praziquantel [21].

Compounds identified by screening of small libraries

Perhexiline maleate

Using the screening of a small compound library with an ATP-based luminescent assay on *S. mansoni* schistosomula, Guidi *et al.* [73] report the schistosomicidal activity of the antianginal drug perhexiline maleate (**54**) (Figure 7). The *in vivo* study in *S. mansoni*-infected mice showed a notable variability of worm burdens in the individual experiments, with an overall minimal antischistosomal effect upon drug treatment. The authors concluded that the short perhexiline maleate half-life in mice, together with its very high rodent plasma protein binding capacity could be the cause of the poor efficacy of perhexiline maleate in the experimental schistosomiasis.

Clofazimine

Clofazimine (**55**) is an US FDA-approved drug for the treatment of leprosy and it has been rehabilitated clinically for the treatment of tuberculosis [127] (Figure 7). Using a previous screen as a basis, Panic *et al.* [43] reported that a dose of 400 mg/kg clofazimine caused worm burden reduction of 83% *S. mansoni*-infected mice. However, a dose of 200 mg/kg clofazimine resulted in a complete lack of efficacy (0% female and total worm burden reduction).

Doramectin

In the screening described above for clofazimine [43], the authors also identified doramectin (**56**), a veterinary drug approved by the FDA for the treatment of parasites, as a moderately active compound against *S. mansoni* in a mouse infection model (Figure 7). In this case, oral treatment of mice with 10 mg/kg doramectin resulted in worm burden reductions of 60%. It is important to note that doramectin has not yet been studied in humans.

N,N′-Diarylureas & 2,3-dianilinoquinoxaline derivatives

Considering that antimalarials are the most well-known group of drugs with schistosomicidal activity, Ingram-Sieber *et al.* [49] evaluated the schistosomicidal effects of the Medicines for Malaria Venture (MMV) malaria box containing 200 diverse drug-like and 200 probe-like compounds with confirmed properties against malaria parasite. Two promising early molecules were identified, namely a N,N′-diarylurea (MMV665852) (**57**) and a 2,3-dianilinoquinoxaline (MMV007224) (**58**). In more details, in mice infected by *S. mansoni*, worm burden reductions of 41% up to 52% were observed following treatment with **57** or **58** using a single oral dose of 400 mg/kg. Later, several analogs of the N,N′-diarylurea MMV665852 (**59**) had high efficacy against *S. mansoni in vitro*, but only one compound (N-phenyl benzamide) resulted in a statistically significant worm burden reduction (66%) after administration of a single oral dose of 400 mg/kg to *S. mansoni*-infected mice (Figure 7) [66].

Compounds identified by medium-throughput screening

Niclosamide analogs

Using a library of drugs identified through medium-throughput phenotypic screening, Abdulla *et al.* [37] have investigated the effect of niclosamide (**60**), a molluscicide and intestinal helminticide. In tests with niclosamide 100 mg/kg administered orally once daily for 4 days, no effects on egg or worm burdens were noticeable. Intraperitoneal administration of niclosamide was also without effect. Three salicylanilides, closantel (**61**), oxyclozanide (**62**), and rafoxanide (**63**), and nitazoxanide (**64**) (Figure 8) have demonstrated oral efficacy against parasites. By comparison, closantel and oxyclozanide at 100 mg/kg yielded less pronounced effects on worm burdens. The rafoxanide 50 mg/kg administered orally once daily was the most effective of the niclosamide analogs tested, decreasing female (50%) and male (56%) worm loads. Moreover, helminthes recovered were smaller than controls. Egg counts were decreased by 49% and rafoxanide was as effective as praziquantel in improving organ pathology. The final niclosamide analog tested, nitazoxanide, was without effect on parasite burdens at 100 mg/kg, but improved organ pathology. Nitazoxanide also decreased egg outputs by 34%.

Antibiotics anisomycin & lasalocid sodium

Using the library of drugs known through the aforementioned medium-throughput phenotypic screening, Abdulla *et al.* [37] investigated the effect of two antibiotics, anisomycin (**65**) and lasalocid sodium (**66**), in mice infected with *S. mansoni* (Figure 8). The antibiotic anisomycin at 100 mg/kg administered orally once daily for 4 days had no significant action on worm burdens, yet decreased hepatic egg burdens by 36%. Increasing to a twice-daily administration resulted in toxicity and all rodent host died.

The ionophoric antibiotic, lasalocid sodium, was better tolerated by rodents. Significant decreases in female (41%) and male (44%) worm counts were measured at 100 mg/kg administered once daily or twice daily for 4 days, respectively. For egg burdens, reductions of 39 and 55% were measured daily and twice daily, respectively. Lasalocid sodium also significantly improved organ pathology compared with controls.

Compounds identified by high-throughput screening

Oxadiazoles

Using RNA interference Kuntz *et al.* [128] found that TGR enzyme is essential for *S. mansoni* survival. They showed that two previously used antischistosomal compounds, potassium antimonyl tartrate and oltipraz, are TGR inhibitors. Hence, the authors suggested that parasite TGR meets all the major criteria to be a key target for antischistosomal chemotherapy. In the same period, a quantitative high-throughput screen of a chemical library of about 70,000 compounds led Simeonov *et al.* [129] identified several compounds with low IC50 values, and one of the most active series was found to include the oxadiazoles 2-oxides (**67**) (Figure 8). Later, Sayed *et al.* [68] reported that the treatment of schistosome-infected mice with oxadiazole 2-oxides led to marked reductions in worm burdens from treatments against different developmental stages and schistosome species. Although all trials

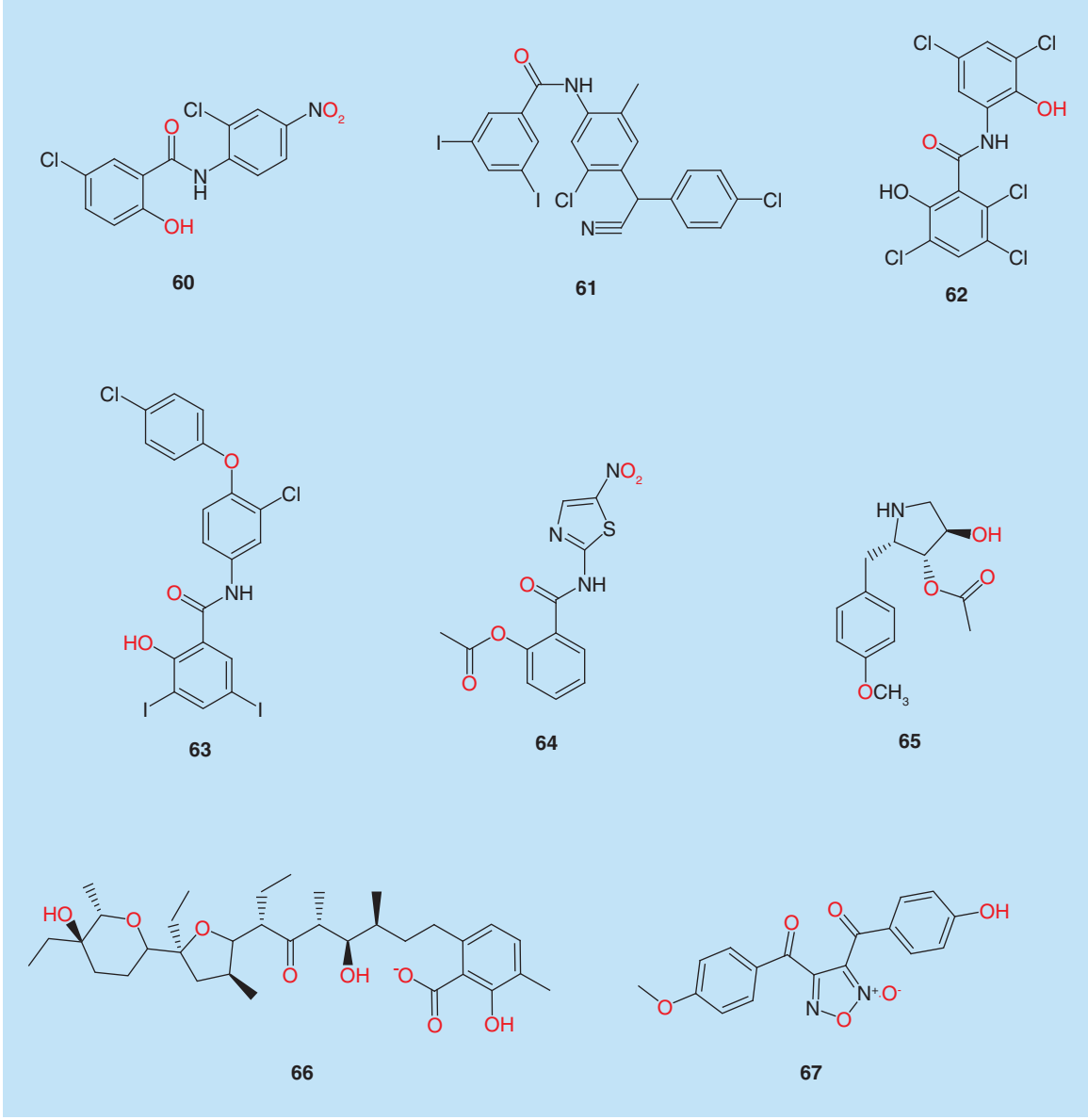

Figure 8. Chemical structures of some antischistosomal compounds identified by medium-throughput screening and compound identified by high-throughput screening. Niclosamide (**60**), closantel (**61**), oxyclozanide (**62**), rafoxanide (**63**), nitazoxanide (**64**), anisomycin (**65**), lasalocid sodium (**66**) and oxadiazoles 2-oxides (**67**).

in mice resulted in at least an 88% reduction in worm burdens, as a disadvantage oxadiazole 2-oxides had to be administered by intraperitoneal injection for 5 consecutive days.

Miscellaneous compounds

Anti-inflammatory drugs

The effects of glucocorticoids on worm burden were described decades ago [130,131]. Some studies showed that dexamethasone (**68**) administered by the intramuscular route at 1 mg/kg three-times a week had no significant effect on worm burden but altered tissue egg distribution [132,133]. These data suggest that dexamethasone is a promising coadjuvant agent that results in decreased morbidity in schistosomiasis.

The prophylactic effect of the nonsteroidal anti-inflammatory drug diclofenac (**69**) was also reported [134]. In this case, the oral treatment with diclofenac (1 mg/mouse) caused a moderate worm burden reduction against juvenile schistosomes (55%). At the same dose, a worm burden reduction of 21% was observed in adult worms. The animals

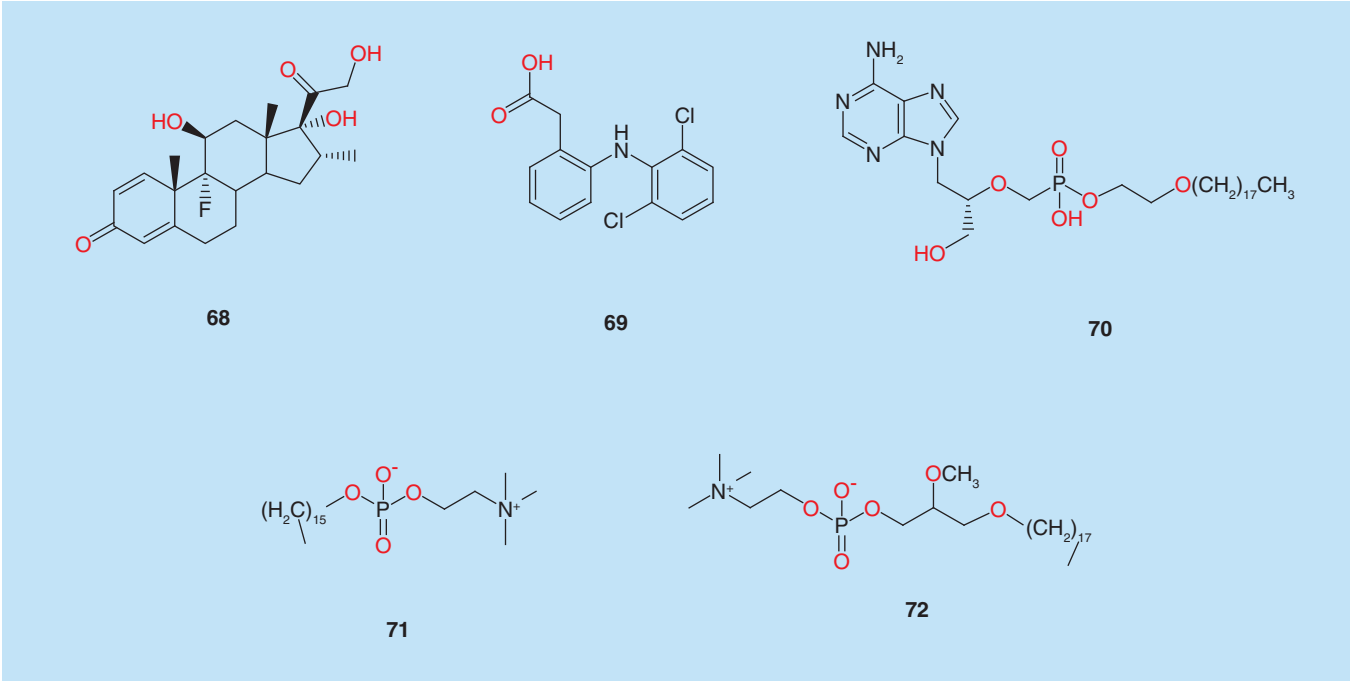

Figure 9. Chemical structure of anti-inflammatory drugs, acyclic nucleotide and alkylphospholipid analogs. Dexamethasone (**68**) and diclofenac (**69**), 9-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine [(*S*)-HPMPA] (**70**), miltefosine (**71**) and edelfosine (**72**).

also had the lowest worm and tissue (hepatic and intestinal) egg loads as well as smallest hepatic granuloma mean diameter. The *in vitro* efficacy of diclofenac against juvenile schistosomes has been recently confirmed (Figure 9) [50].

Acyclic nucleotide analog

In the early 2000s, the activity of the acyclic nucleotide analog 9-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine [(S)-HPMPA] (**70**) was investigated in *S. mansoni*-infected mice [31]. The compound (Figure 9) was injected intraperitoneally, usually on 2 or 5 consecutive days, at 10 to 20 mg/kg/day. In more detail, (S)-HPMPA induced a worm burden reduction of 30–40% on juvenile and adult worms. Interestingly, the oogram showed enormous reduction in the number of immature and mature eggs (~100% of reduction). Additionally, (S)-HPMPA caused significant increases in the proportion of dead eggs.

Alkylphospholipid analogs

Alkylphospholipid analogs (e.g., miltefosine (**71**), edelfosine (**72**)) are a class of structurally related synthetic lipid compounds (Figure 9). Miltefosine was initially developed as an anticancer agent [135], and is the first oral drug licensed for the treatment of leishmaniasis [136]. Edelfosine has also been shown to exert antitumor [137] and antileishmanial properties [138]. In the second decade of the 21st century it was demonstrated that miltefosine administered orally in a daily dose of 20 mg/kg for 5 successive days to mice infected with either invasive, juvenile or adult stages of *S. mansoni* resulted in significant reduction of worm burden, hepatic granulomata size and amelioration of hepatic pathology [65]. Later, a study revealed that edelfosine resulted in a reduction of male (46.84%) and female (29.1%) worm burdens in the murine model of schistosomiasis mansoni. Moreover, edelfosine treatment decreased the total number of eggs recovered in livers (54.2% reduction) [54].

Aryl hydantoins & antiandrogens

In the early 1980s, the aryl hydantoin Ro 13–3978, a compound discovered by Hoffmann–La Roche, showed good *in vivo* antischistosomal activity. A number of these aryl hydantoins had high oral efficacy in schistosome animal models, but they also produced antiandrogenic side effects in the host. Recently, Wang *et al.* [67] restarted investigations and described the *in vivo* antischistosomal activities of Ro 13–3978 (AH01) (**73**), its imino isostere AH02 (**74**) and two derivatives AH03 (**75**) and AH04 (**76**) (Figure 10). While animals died with the administration

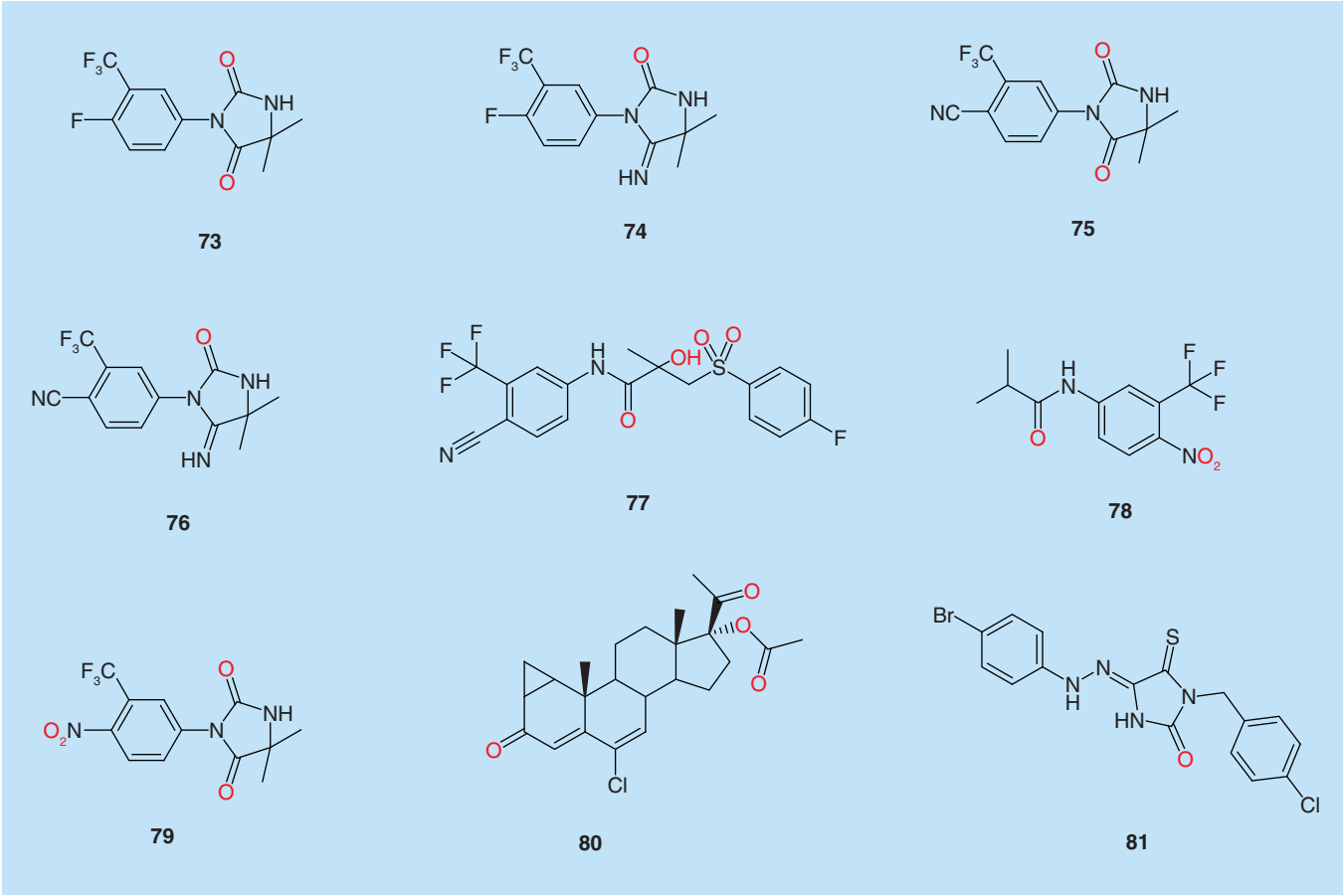

Figure 10. Chemical structure of aryl hydantoins and antiandrogens. Ro 13–3978 (AH01) (**73**), AH02 (**74**), AH03 (**75**), AH04 (**76**), bicalutamide (**77**), flutamide (**78**), nilutamide (**79**), cyproterone acetate (**80**) and 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one (LPSF-PT05) (**81**).

of AH03 and AH04, single 100 mg/kg oral doses of AH01 and AH02 administered to *S. mansoni*-infected mice achieved high worm burden reductions of 95 and 93%, respectively. AH01 was also effective against juvenile infections. Later, Keiser *et al.* [139] also restarted investigations and confirmed that Ro 13–3978 has high activity in *S. mansoni*-infected mice, with adult worms being more susceptible to the drug than juvenile. In more details, *in vivo* assays showed that a single oral dose of Ro 13–3978 (100 mg/kg) reduced the worm burden by 88%. Ro 13–3978 is structurally similar to the antiandrogenic drug nilutamide, which will be discussed below.

The antischistosomal properties of the marketed antiandrogens bicalutamide (**77**), flutamide (**78**), nilutamide (**79**) and cyproterone acetate (**80**) (Figure 10) were also studied in an *S. mansoni* infection model [38]. As described above, the rationale for selecting these compounds for testing against schistosomes arose from the structural similarity of the hydantoins nilutamide and Ro 13–3978. Low to moderate worm burden reductions (0–47%) were observed with bicalutamide, while cyproterone acetate and flutamide lacked activity against helminthes. On the other hand, a high total worm burden reduction (85%) was observed after single 400 mg/kg dose of nilutamide. Further, no significant decrease in schistosome survival was reported using combinations of nilutamide (100 mg/kg) and praziquantel (50 or 100 mg/kg). In contrast, a combination of nilutamide (200 mg/kg) and praziquantel (100 mg/kg) produced significant worm burden reductions (85–91%). For aryl hydantoin derivatives and antiandrogens, although structurally similar, there was no correlation between antischistosomal activity and androgen–receptor interaction. Finally, a series of structurally distinct 4-thiohydantoins were synthesized by Brazilian researchers and one of these, 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one (LPSF-PT05) (**81**) (Figure 10), achieved a worm burden reduction of 71% when *S. mansoni*-infected mice were treated orally with 100 mg/kg [62].

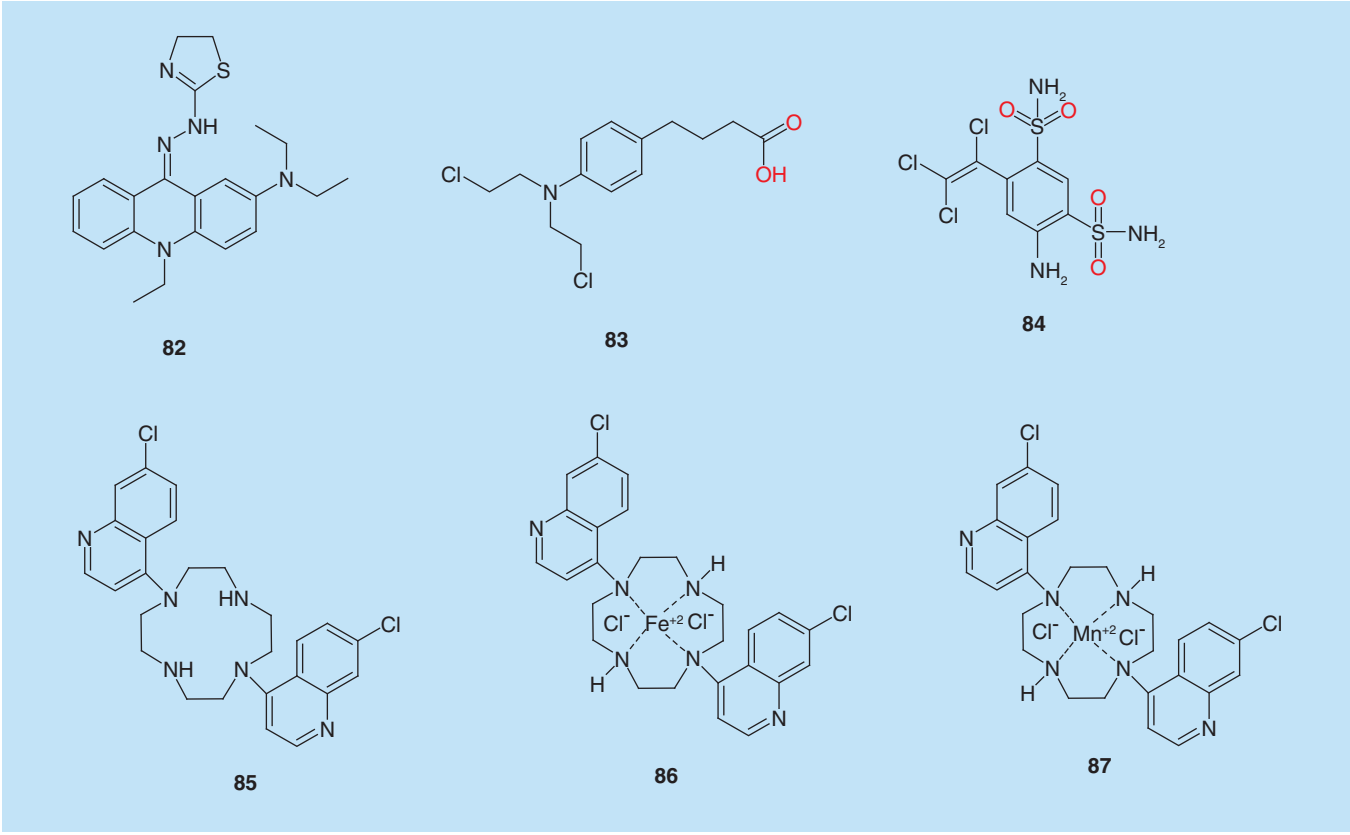

Figure 11. Chemical structure of Ro 15–5458, chlorambucil, clorsulon and tetraazamacrocyclic derivatives. 9-acridanone hydrazine (Ro 15–5458) (**82**), chlorambucil (**83**), clorsulon (**84**), bisquinoline derivative of cyclen (**85**) and its Fe^{2+} (**86**) and Mn^{2+} complexes (**87**).

Ro 15–5458

Some compounds of the class 9-acridanone-hydrazones were developed by Hoffmann–La Roche (Basel, Switzerland) in the early 1980s and were shown to have antischistosomal effects on various experimentally infected animals [140]. One of these derivatives, namely 9-acridanone hydrazine (Ro 15–5458, **82**) (Figure 11), was found to show schistosomicidal activity in baboons and vervet monkeys at very low doses [141,142]. In experimentally infected mice, reduction rates of 83.6, 89.4 and 94.9% were observed in adult worm load following treatment with 10, 15 and 20 mg/kg of the Ro 15–5458, respectively. Moreover, Ro 15–5458 caused complete disappearance of all immature stages [30]. Although experimentally promising, Ro 15–5458 has not reached clinical use.

Chlorambucil

Chlorambucil (**83**) (Figure 11) is an anticancer drug used mainly to treat chronic lymphocytic leukaemia, low grade non-Hodgkin lymphoma and Hodgkin lymphoma. Recently, it has shown *in vivo* activity in the *S. mansoni* mouse model at various stages of infection after an oral dose of 2.5 mg/kg/day for 5 successive days [42]. Its highest impact was evident against the juvenile stage, where it induced 76% total worm burden reduction, and 89 and 87% intestinal and hepatic egg count reduction, respectively, along with oogram alterations. Besides, it induced significant shortening of both male and female worms and promoted an amelioration of hepatic histopathology.

Clorsulon

Clorsulon (**84**) is a drug belonging to the benzenesulphonamide family which is recommended for the treatment of animal fasciolosis (Figure 11). Mice harboring *S. mansoni* were treated with single, double and triple doses of 5 mg/kg clorsorun per dose, 1 week apart starting from the fourth week postinfection. The worm burden was reduced proportionally with number of doses given; 88, 96 and 97% in single, double and triple exposures successively. Similarly, egg count in liver was decreased by 86–96% in treated mice [45].

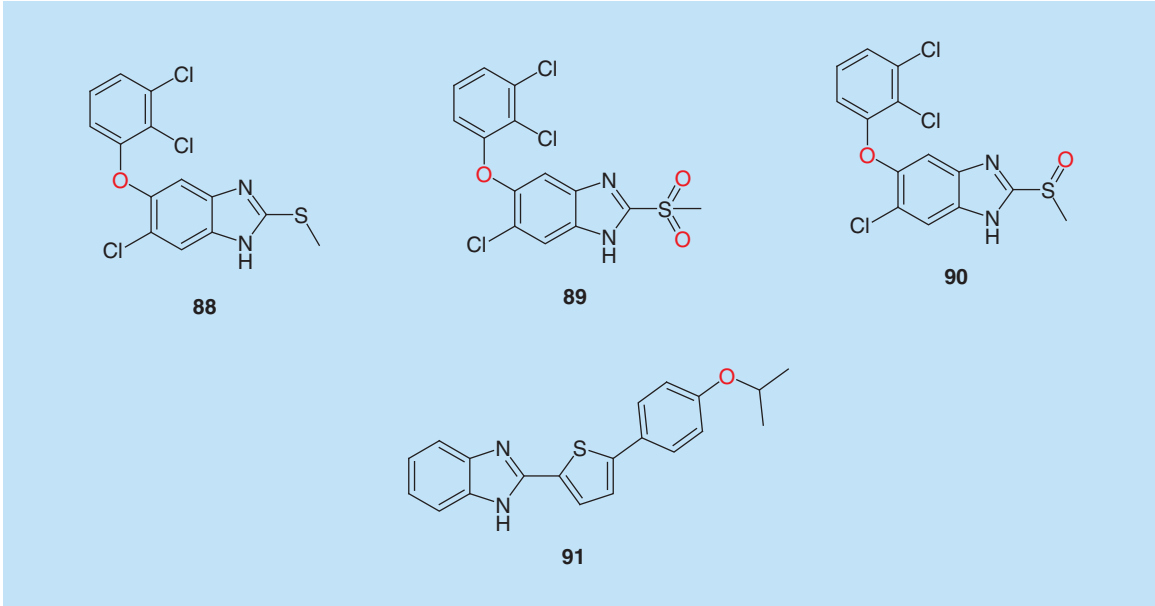

Figure 12. Chemical structure of benzimidazole derivatives. Triclabendazole (**88**), its two active metabolites (**89** and **90**) and BTP-Iso (**91**).

Tetraazamacrocyclic derivatives

Recently, a series of 26 synthetic tetraazamacrocyclic derivatives and their metal complexes were screened for schistosomicidal activity. In *S. mansoni*-infected mice, three of these, which are the bisquinoline derivative of cyclen (**85**) and its Fe^{2+} (**86**) and Mn^{2+} (**87**) complexes (Figure 11), showed worm burden reductions of 12.3, 88.4 and 74.5%, respectively, at a single oral dose of 400 mg/kg [39]. These findings revealed the importance of metal complexes in anthelmintic activity. Consequently, several studies are currently underway to evaluate the effect of metal-containing drugs (metallodrugs) against schistosomes [143,144].

Benzimidazole derivatives

Benzimidazoles are broad-spectrum anthelmintics, widely used in human and animal infections. It is known that these compounds tested against *Schistosoma* in mice showed no or only low and insignificant efficacies (e.g., mebendazole [145], triclabendazole [**88**] and its two active metabolites [**89** and **90**]) [77]. Recently, a novel benzimidazole compound, BTP-Iso (**91**) (2-(5-(4-isopropoxyphenyl)thiophen-2-yl)-1H-benzo[d]imidazole), was designed and synthetized by structural modifications of benzimidazole ring at two-position taking thiabendazole and cambendazole as models to improve the anthelmintic activity of benzimidazoles (Figure 12) [40]. The effect of BTP-Iso was assessed in mice harboring adult *S. mansoni* using two oral treatment regimens (200 or 300 mg/kg). In more details, BTP-Iso 200 mg/kg resulted in significant reductions in female worms (66%) and total worm burden (44%), and no significant reduction in male worms. On the other hand, BTP-Iso in a dose of 300 mg/kg showed statistically significant higher reductions in female (76.5%), male (34.6%) and total worms (55.3%).

Conclusion

Although still largely neglected and underappreciated, schistosomiasis is an important infectious disease of poverty that afflicts millions of people worldwide. After praziquantel was discovered in the 1970s, neither the pharmaceutical industry nor international donor agencies thought much more about the possible need for alternative drugs for schistosomiasis. However, in addition to the dependence on a single old anthelmintic compound, drug resistance is an imminent threat, particularly by means of the large-scale use of praziquantel. Thus, research into new antischistosomal compounds is an imperative and urgent matter, and, in the last years, there has been a marked increase in the number of publications mainly using *S. mansoni* as an experimental model.

Unfortunately, no innovative drug for schistosomiasis has been approved or submitted to relevant clinical trials so far, and the investigations have been limited to combinations of repurposed compounds. Except for mefloquine and

artemisinins, which are approved therapeutic agents against malaria, and arachidonic acid, all other compounds were tested only in animal models. The obsolete schistosomicide Ro 13–3978 has excellent antischistosomal properties against juvenile and adult *S. mansoni* infections *in vivo*, but it was not clinically evaluated. Taken together, many of the antischistosomal compounds described here are still considered only as hit or lead compounds, but these will be an important source of new chemical entities in the future. Drug development is a long process that can take decades, and since funding for drug development for neglected diseases is very limited, a cautious and well thought-out approach is warranted when moving promising antischistosomal compounds forward into human clinical trials. The pressing need to develop a new schistosomicidal drug may necessitate exploring and filtering the chemotherapeutic history to search for those most promising ones.

Future perspective

The development of a new drug requires a major investment of capital, human resources and technological expertise. It also requires strict adherence to regulations on testing and manufacturing standards before a new drug can be used in the general population [146]. This long and arduous process can take decades, and an appreciable amount of funding is needed to develop a drug. In this context, a useful strategy to accelerate the drug development process is so-called drug repositioning. This strategy has numerous advantages including potential reductions in development times and costs, especially because return on investment is weak for drugs related to infectious diseases of poverty.

Also, macromolecular and genomic databases have been pivotal in the development of knowledge-based strategies in schistosomiasis drug discovery. By associating neglected diseases-related information from distinct protein and small-molecule repositories, chemogenomics tool indicates matches of molecular targets and compounds that should be prioritized for experimental testing. Moreover, it provides information on the druggability and assay ability of the selected targets. Consequently, the use of molecular modeling approaches and their integration with experimental methods will strengthen the schistosomiasis drug discovery pipeline [147].

Furthermore, combining pharmacological treatment with solving the serious social and economic problems which affect the poor regions are critical needs that are only beginning to be addressed. It is to be hoped that the knowledge produced by academia will lead to therapeutic advances to control of neglected diseases and improvement in quality of life [148].

Finally, developing a vaccine against schistosomiasis is a major challenge due to the complex life cycle of the causative schistosome parasite. Indeed, although there are exceptions, candidate vaccine antigens so far described have limitations in terms of low protective efficacy. Clinical trials are currently underway and the expected outcomes to be measured in future Phase III vaccine trials.

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Executive summary

Aim

- Schistosomiasis is a parasitic helminth infection that belongs to the neglected diseases and diseases of poverty.
- The treatment and control of schistosomiasis rely on the use of a single drug, praziquantel, and there is an urgent need to develop new antischistosomal drugs.
- The pressing need for a new antischistosomal drug may necessitate exploring and filtering chemotherapeutic history to search for the most promising drugs.

Antischistosomal compounds from the early 20th century (mid-1910s) to 1980

- After 1917, when tartar emetic was first used in the treatment of schistosomiasis, little advance was made in the development of antischistosomal drugs. Antimonial compounds and metrifonate were previously used but are now obsolete due to their excessive toxicity. Oxamniquine was used on a large scale in Brazil, but it was also discontinued.
- In the 1970s, several schistosomicidal drugs emerged. Nevertheless, the therapeutic doses of most of these drugs were found to cause major side effects.
- In the late 1970s, praziquantel, an isoquinoline-pyrazine derivative, was introduced and immediately proved to be superior to any other schistosomicidal drug and quickly became the drug of choice in most endemic areas.

Antischistosomal compounds over 36 years from 1980 to 2016

- Praziquantel has been used for almost 40 years and so far no alternative exists for the treatment of schistosomiasis.
- In recent years, there has been a marked increase in the number of publications mainly using *S. mansoni* as an experimental model.
- Studies with the old drug aryl hydantoin Ro 13–3978 have been recently restarted and antiparasitic results are the most promising when compared with other drugs described so far.
- Most studies described here have not explored the antischistosomal properties against different developmental stages. Indeed, chemotherapeutic studies mainly rely on schistosomes adults.
- Some antischistosomal agents have remarkable efficacy *in vitro*, but poor activity *in vivo*.
- Most antischistosomal compounds have excellent safety record.
- Some antischistosomal compounds are promising, but none so far represents a suitable substitute or adjunct to praziquantel.

Conclusion

- No innovative drug for schistosomiasis has been approved or submitted to relevant clinical trials so far and the investigations have been limited to combinations of repurposed compounds.
- Many of the antischistosomal compounds described here are still considered only as hit or lead compounds, but these will be an important source of new chemical entities in the future.
- Among all antischistosomal drug candidates, Ro 13–3978, an aryl hydantoin discovered in the early 1980s by Hoffmann–La-Roche, has excellent antischistosomal properties against juvenile and adult *S. mansoni* infections *in vivo*.
- A stronger political commitment to controlling schistosomiasis is extremely necessary.

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4. MÉTODO

4.1 Tipo de Estudo

Trata-se de um estudo experimental, com abordagem quantitativa.

4.2 Local do Estudo

O presente estudo foi realizado, majoritariamente, no âmbito do Núcleo de Pesquisa em Doenças Negligenciadas, Universidade Guarulhos (NPDN/UNG). Outras instituições, e seus respectivos colaboradores, serão descritos a seguir:

a) Laboratório de Química Medicinal e Computacional, Instituto de Física de São Carlos, Universidade de São Paulo.

Etapa: Estudos *in silico*.

Colaborador: Prof. Dr. Adriano D. Andricopulo.

b) Laboratório de Filmes Finos, Departamento de Física Aplicada, Instituto de Física, Universidade de São Paulo.

Etapa: Análise morfológica do tegumento de vermes adultos de *S. mansoni* com Microscópio Eletrônico de Varredura.

Colaborador: Profa. Dra. Maria Cecília Salvadori.

c) Núcleo de Enteroparasitas, Instituto Adolfo Lutz.

Etapa: Apoio no ciclo experimental do *S. mansoni*.

Colaborador: Prof. Dr. Pedro Luiz S. Pinto.

4.3 Procedimento Experimental

O procedimento experimental está sumarizado no artigo apenso às páginas subsequentes.

4.4 Considerações Éticas

O presente estudo foi aprovado pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Guarulhos (número de protocolo 031/17), conforme ANEXO I. Todos os animais foram tratados em estrita conformidade com as boas práticas previstas de acordo com a legislação brasileira (Conselho Nacional de Controle de Experimentação Animal, Lei N° 11.794/2008).

5. RESULTADOS

5.1 Artigo 2 - H_1-antihistamines as antischistosomal drugs: *in vitro* and *in vivo* studies

Os Resultados do presente estudo com diferentes anti-histamínicos H_1 (anti-H_1) estão no artigo recentemente publicado no Periódico Parasites & Vectors (Fator de Impacto 3,169, classificado com Qualis A1) e descritos nas páginas subsequentes.

Parasites & Vectors

RESEARCH Open Access

H1-antihistamines as antischistosomal drugs: *in vitro* and *in vivo* studies

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Abstract

Background: Schistosomiasis is a socioeconomically devastating parasitic infection afflicting hundreds of millions of people and animals worldwide. It is the most important helminth infection, and its treatment relies solely on the drug praziquantel. Oral H1-antihistamines are available worldwide, and these agents are among the most widely used of all medications in children and adults. Given the importance of the drug repositioning strategy, we evaluated the antischistosomal properties of the H1-antihistamine drugs commonly used in clinical practices.

Methods: Twenty-one antihistamine drugs were initially screened against adult schistosomes *ex vivo*. Subsequently, we investigated the anthelmintic properties of these antihistamines in a murine model of schistosomiasis for both early and chronic *S. mansoni* infections at oral dosages of 400 mg/kg single dose or 100 mg/kg daily for five consecutive days. We also demonstrated and described the ability of three antihistamines to induce tegumental damage in schistosomes through the use of scanning electron microscopy.

Results: From phenotypic screening, we found that desloratadine, rupatadine, promethazine, and cinnarizine kill adult *S. mansoni in vitro* at low concentrations (5–15 μM). These results were further supported by scanning electron microscopy analysis. In an animal model, rupatadine and cinnarizine revealed moderate worm burden reductions in mice harboring either early or chronic *S. mansoni* infection. Egg production, a key mechanism for both transmission and pathogenesis, was also markedly inhibited by rupatadine and cinnarizine, and a significant reduction in hepatomegaly and splenomegaly was recorded. Although less effective, desloratadine also revealed significant activity against the adult and juvenile parasites.

Conclusions: Although the worm burden reductions achieved are all only moderate, comparatively, treatment with any of the three antihistamines is more effective in early infection than praziquantel. On the other hand, the clinical use of H1-antihistamines for the treatment of schistosomiasis is highly unlikely.

Keywords: Schistosomiasis, Antischistosomal, *Schistosoma*, Antihistamines, Drug repositioning

Background

Infection with trematodes (blood flukes) of the genus *Schistosoma*, the causative agents responsible for schistosomiasis, causes chronic and debilitating disease in millions of people and animals worldwide [1]. Although not commonly fatal, schistosomiasis significantly contributes to a huge economic burden associated with low productivity and the perpetuation of the poverty cycle, as well as imposing a large burden on healthcare costs. Schistosomiasis is among the most prevalent parasitic diseases worldwide, and it is the most important human helminth infection in terms of global mortality and morbidity [2]. Approximately 800 million people may be at risk of infection worldwide,

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and almost 240 million are infected [3]. The combination of the global healthcare burden, the prevalence of these helminths, and limited treatment options have led to the inclusion of schistosomiasis on the World Health Organization's list of neglected tropical diseases [4].

Schistosoma mansoni is the prevalent species in Africa, the Middle East, South America, and the Caribbean, in regions where the intermediate snail host, a freshwater snail of the genus *Biomphalaria*, is present. This parasite has a lifespan of several years and female schistosomes continuously produce eggs (each *S. mansoni* female worm can produce up to 300 eggs/day), which are able to pass through the intestinal lumen to be finally excreted with feces. Some of the eggs can be trapped in the tissues of the mammalian host instead of being excreted in the feces. Most, if not all, of the pathology in a schistosome infection results from the deposition of eggs in the tissues and the host's response to them. The result is local, and systemic pathological effects include impaired cognition, anemia and growth stunting, as well as organ-specific effects, leading eventually to severe pathology such as hepatosplenomegaly and even death [5].

Praziquantel is a broadly effective trematocide and cestocide widely employed in veterinary and human medicine, and it is the only drug available to treat schistosomiasis. Praziquantel is very effective against adult worms (patent infection), but it is, unfortunately, poorly active against juvenile stages (prepatent infection), meaning that praziquantel must be given periodically for effective treatment and control [6]. In addition, widespread use of praziquantel in both humans and domestic animals [7, 8], along with the identification of laboratory and field isolates with reduced susceptibility to praziquantel [9, 10] raise serious concerns about the risk of selection of drug-resistant strains. Thus, new antischistosomal agents are needed, especially those targeting multiple stages of the parasite. For this reason, efforts to discover and develop novel antischistosomal agents have been intensified in recent years (for a review see [10, 11]). On the other hand, drug discovery is a lengthy and arduous process that inevitably struggles to deliver new therapies in a timely manner. Since the disease mainly affects poor people living in developing countries, pharmaceutical companies have little interest in developing new drugs. Thus, drug repurposing, the process of identifying new uses for existing drugs, is a promising strategy that has been used in recent years [12].

In schistosomes, and other flatworms, histamine is an important neuroactive substance [13] and G protein-coupled receptors (GPCRs) responsive to histamine have been described in *S. mansoni* [14, 15]. Due to their involvement in diverse biological and physiological processes, their pharmacological importance and potential as biological target, GPCRs are promising targets for new anthelmintic agents [16]. We have previously shown that promethazine, an old H1-antihistamine drug, had antischistosomal properties against *S. mansoni* adult worms *ex vivo* and in an animal model of schistosomiasis [17]. In view of these studies and in an attempt to explore drug repositioning strategy, here we evaluated the antiparasitic effect of a set of 21 H1-antihistamines commonly used in clinical practice. In this context, from phenotypic screening we found four antihistamine drugs that effectively killed *S. mansoni* adult worms *ex vivo*. Subsequently, these drugs were tested *in vivo* using an early and a chronic *S. mansoni* infection in a murine model. We also demonstrated the ability of these antihistamines to induce tegumental damage in adult worms through the use of scanning electron microscopy.

Methods

Drugs and reagents

All H1-antihistamines were purchased from Cayman Chemical (Ann Arbor, MI, USA), Sigma-Aldrich (St. Louis, MO, USA) and Toronto Research Chemicals (Toronto, Ontario, Canada). Praziquantel was kindly provided by Ecovet (São Paulo, SP, Brazil). The structures of all tested H1-antihistamines are shown in Additional file 1: Table S1.

RPMI 1640 culture medium, penicillin G/streptomycin sulfate, and inactivated fetal bovine serum (FBS) were purchased from Vitrocell (Campinas, SP, Brazil). HEPES buffer, glutaraldehyde solution, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich.

Maintenance of the *S. mansoni* life-cycle

The life-cycle of *S. mansoni* (BH strain) is maintained by passage through snails (*Biomphalaria glabrata*) and a mice (*Mus musculus*) as described by de Moraes [18]. The host snails were exposed to light (60 W incandescent light bulbs) for up to 3 h and subsequently cercariae of *S. mansoni* were harvested. Female Swiss mice, 3 weeks-old (purchased from Anilab, São Paulo, Brazil) were infected subcutaneously with approximately 150 cercariae. Both snails and mice were kept under environmentally controlled conditions (25 °C; humidity of 50%), with free access to water and food.

In vitro anthelmintic assay

An *in vitro* anthelmintic assay was performed as previously described [19, 20]. Briefly, adult schistosomes (49 day-old) were collected from the portal system and mesenteric veins from infected mice (parasite *ex vivo*). Next, schistosomes were placed in RPMI 1640 culture medium supplemented with 10% FBS, containing 100 $\mu g \cdot ml^{-1}$ streptomycin 100 $IU \cdot ml^{-1}$ penicillin, and incubated in

a 24-well culture plate (Corning, New York, NY, USA). Drugs were dissolved in DMSO to obtain stock solutions of 10 mM and then were tested at a concentration of 50 µM (one pair of parasites per well). Each drug was assessed in five replicates. Helminths were kept for 72 h (37 °C, 5% CO_2) and their viability was monitored microscopically. The compounds that produced an effect superior to 90% after 72 h post-exposure underwent determination of their half maximum lethal concentration (LC_{50}) using 1:2 serial dilutions from 0.78 to 50 µM [17, 21]. Each concentration was tested in triplicate, and experiments were repeated once. Negative control (using the highest concentration of DMSO) and positive control (praziquantel 2 µM) were included [22].

Microscopy analysis

During the *in vitro* experiments, parasites were monitored using a light microscope (Leica Microsystems EZ4E, Wetzlar, Germany). In addition, schistosomes were visualized using a scanning electron microscope (JEOL SM-6460LV; JEOL Tokyo, Japan) whose experimental protocols were previously published [23]. Briefly, adult worms (control and treated groups) were fixed in 2.5% glutaraldehyde and mounted specimens were metalized with gold (Desk II sputter coater; Denton Vacuum LLC, Moorestown, NJ, USA) before observation under scanning electron microscopy.

Studies in an animal model of schistosomiasis

Considering the *in vitro* results, we progressed cinnarizine, desloratadine and rupatadine to *in vivo* studies in both early and chronic *S. mansoni*-murine models as previously described [24]. Eighty Swiss mice, 3 weeks-old, were infected subcutaneously with 80 *S. mansoni* cercariae each. Animals were randomly divided into 16 groups (5 mice per group) and drugs were administered 21 days (early infection) or 42 days (chronic infection) post-infection by oral gavage. For treatment, drugs were dissolved in 2% ethanol in water (v/v) and tested at a single dose of 400 mg/kg or a dose of 100 mg/kg/day for five successive days. Groups of *S. mansoni*-infected control were given a corresponding amount of vehicle on the same timetable. At 56 days post-infection, all animals were euthanized by the CO_2 method and dissected; parasites were then collected, sexed, and counted [25, 26]. Therapeutic activity was also based on the technique of qualitative and quantitative oograms in intestine, as well as the Kato-Katz method for quantitative fecal examination [27].

Randomization and blinding

Animal studies are reported in compliance with the National Centre for the Replacement and Refinement & Reduction of Animals in Research (NC3Rs) ARRIVE guidelines. The mice were randomly assigned to the experimental groups, and pharmacological treatments were also performed randomly. The mice were euthanized in a random manner inside a group. All parameters (worm counts, measurement of the mass of the organs, quantitative and qualitative oogram, and quantitative fecal examination) were performed by different people (at least by two different investigators). Therefore, to eliminate bias in interpretation, manipulators of the experiments were not the same as the data analysts.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 7 (GraphPad Software, San Diego, CA) in accordance with the recommendations in the pharmacology field [24]. All data from the *in vitro* anthelmintic experiments are presented as the mean ± standard deviation (SD) of at least three independent assays. LC_{50} values were calculated using sigmoid dose-response curves and 95% confidence intervals [28]. Kaplan-Meier survival analyses were also used to compare *in vitro* survival data, and *P*-values calculated using the log-rank (Mantel-Cox) test. For experimental analysis of animal studies, a parametric Dunnett's test was applied to compare the control group with the treated group. The level of statistical significance was set to $P < 0.05$ [17].

Molecular and physicochemical properties

Molecular and physicochemical properties were calculated using the default parameters in the ADME/QSAR models of StarDrop version 6.6 (Optibrium, Cambridge, UK). The heat maps were performed using the same platform.

Results

In vitro efficacy of H1-antihistamine drugs against adult schistosomes

Effect of H1-antihistamines on parasite viability

The 21 H1-antihistamine drugs were initially screened against adult schistosomes *ex vivo* at 50 µM. Of all H1-antihistamines tested, four (desloratadine, rupatadine, cinnarizine and promethazine) showed antischistosomal properties after 72 h, and these H1-antihistamines were further tested at a range of concentrations for their LC_{50} determination. Out of these, compared with the control group, cinnarizine, desloratadine, and promethazine achieved an LC_{50} below 10 µM, whereas rupatadine had a LC_{50} value of ~15 µM (Fig. 1). Comparison of LC_{50} values revealed that the order of potency was promethazine (Mantel-Cox signed-rank test: $\chi^2 = 29.09$, $df = 6$, $P < 0.0001$), cinnarizine (Mantel-Cox signed-rank test:

$\chi^2 = 41.03$, $df = 6$, $P < 0.0001$), desloratadine (Mantel-Cox signed-rank test: $\chi^2 = 28.68$, $df = 6$, $P < 0.0001$), rupatadine (Mantel-Cox signed-rank test: $\chi^2 = 23.19$, $df = 5$, $P < 0.001$). The calculation of the molecular and physicochemical properties of the tested drugs (Additional file 1: Table S1) suggests that the antischistosomal activity may be correlated with the polar surface area of the compounds. The topological polar surface area (TPSA) values, which have been demonstrated to correlate well with passive transmembrane transport, were, in general, lower for the active drugs compared with the inactive compounds [29]. The active drugs had an average TPSA value of 15.70, while the inactive antihistaminic agents had the significantly higher TPSA value of 42.43. This indicates that the active drugs may be better diffused across parasite membranes. Heat maps constructed for the active compounds (Fig. 2) illustrate the contribution of the different portions of the molecules to TPSA.

The temporal effects of different concentrations of H1-antihistamine drugs on adult schistosomes are depicted in Fig. 3. Control parasites remained viable over the entire observation period of 72 h. Cinnarizine and promethazine (TPSA values of 6.48) were able to kill all schistosomes within 24 h of contact at a concentration of 50 μM. A slightly slower onset of action was observed when parasites were incubated with desloratadine or rupatadine (TPSA values of 24.92 and 29.02, respectively). This time-dependence is consistent with the TPSA values calculated for the active drugs. All adult schistosomes died within 48 h. In contrast, praziquantel had a very fast onset of action on schistosomes.

Effect of H1-antihistamines on parasite tegument

Since we have previously shown morphological changes in the tegument of the adult worms induced by promethazine [30], we conducted further studies with cinnarizine, desloratadine, and rupatadine using scanning electron microscopy. Figure 3a shows the tegument of a male parasite (control) depicting ridges and tubercles covered by spines that are somewhat uniformly distributed. By 24 h after incubation with 50 μM of cinnarizine (Fig. 3b), desloratadine (Fig. 3c), or rupatadine (Fig. 3d), extensive destruction was visible on the entire tegument of all adult worms analyzed. For example, rupture of the tegument along the whole dorsal body surface, including blebbing, shrinking and sloughing was visible. Moreover, tubercles had lost their spines. Similar morphological observations were made when the tegument of the schistosomes was evaluated after 48 h of exposure to cinnarizine 25 μM (Fig. 3e), desloratadine 50 μM (Fig. 3f) and rupatadine 50 μM (Fig. 3g). After incubation for 72 h, shrinking and swelling of the tegument was seen on all parasites exposed to cinnarizine 12.5 μM (Fig. 3h), as well as desloratadine at 25 μM (Fig. 3i) and 12.5 μM (Fig. 3j). Interestingly, massive bubbles were observed on all worms exposed to rupatadine at 25 μM after 72 h of incubation (Fig. 3k). The positive control (praziquantel 2 μM) caused massive shrinking and swelling of the tegument (Fig. 3l).

The efficacy of H1-antihistamine drugs in mice harboring either early or chronic *S. mansoni* infection

Since promethazine was already tested *in vivo* and results published [17], we investigated the antischistosomal effect of cinnarizine, desloratadine, and rupatadine in mice harboring either early or chronic *S. mansoni* infection. Results were compared to the control infected but untreated animal harboring either early or chronic infection. Of note, all drugs were well tolerated, and all mice survived until the end of the experimental work.

Effect of H1-antihistamines on worm burden

Figure 4 summarizes the antischistosomal activity of cinnarizine, desloratadine, and rupatadine given in single or multiple oral doses in both early and chronic infection, compared to control *S. mansoni*-infected animals.

In early infection, using a single oral dose (400 mg/kg), cinnarizine achieved the highest worm burden reduction (55.1%; ANOVA: $F_{(13, 56)} = 15.06$, $P = 0.0026$). In the experiments where cinnarizine, rupatadine, and desloratadine were administered daily for 5 days (100 mg/kg), a decrease in total worm burden of 66.9% (ANOVA: $F_{(13, 56)} = 15.06$, $P = 0.0003$), 66.5% (ANOVA: $F_{(13, 56)} = 15.06$, $P = 0.0004$), and 50.7% (ANOVA: $F_{(13, 56)} = 15.06$, $P = 0.0052$), respectively, was observed.

In chronic infection, using a single oral dose (400 mg/kg), the H1-antihistamine drugs caused a total worm burden reduction ranging from 50.1% (ANOVA: $F_{(13, 56)} = 15.06$, $P = 0.0059$) to 55.6% (ANOVA: $F_{(13, 56)} = 15.06$, $P = 0.0021$). In the treatment using multiple oral doses (5 × 100 mg/kg), cinnarizine and rupatadine achieved high total worm burden reductions of 73.6% (ANOVA: $F_{(13, 56)} = 15.06$, $P < 0.0001$) and 75.4% (ANOVA: $F_{(13, 56)} = 15.06$, $P = 0.0052$, $P < 0.0001$), respectively. Lower but significant worm burden reduction values were obtained for desloratadine (59.2%; ANOVA: $F_{(13, 56)} = 15.06$, $P = 0.0008$).

Effect of H1-antihistamines on egg burden

The egg load was evaluated using the oogram technique (immature, mature and dead worms in the intestine) and the Kato-Katz technique for quantitative fecal examination.

Regarding the oogram, in early infection, multiple oral doses of any of the three antihistamines led to a significant reduction in the number of immature eggs

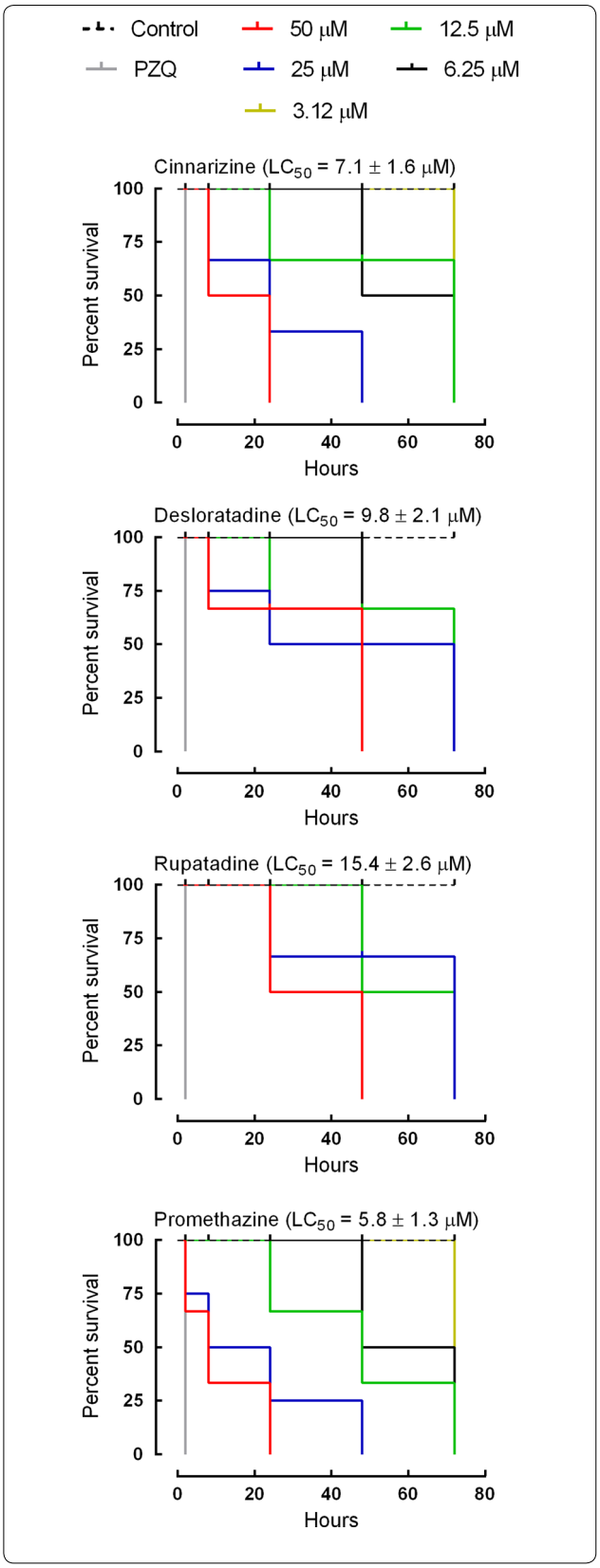

Fig. 1 Viability of adult *S. mansoni* parasites *ex vivo* following exposure to H1-antihistamine drugs. Adult parasites were collected from the hepatic portal and mesenteric veins of mice and placed on plates containing the indicated concentrations of H1-antihistamines. Parasites were monitored for up to 72 h using a microscope and results are expressed as the percent mortality recorded by Kaplan-Meier survival curves. Mean values of viability were derived from a minimum of three experiments ($n=3$), and each experiment was performed with five replicates. LC_{50} values were determined at 72 h. Control (dashed line): RPMI 1640 + 0.5% DMSO. PZQ, praziquantel at 2 μM

(ANOVA: $F_{(7, 35)}=8.43$, $P=0.026$), whereas drugs administered in a single dose showed a non-significant reduction in the number of eggs when compared to control infected mice. In contrast, the number of immature eggs was highly reduced in mice harboring a chronic *S. mansoni* infection, especially when any of the three drugs were administered in multiple doses (ANOVA: $F_{(7, 35)}=8.43$, $P=0.0006$). The percentages of immature, mature and dead eggs are summarized in Fig. 5.

With respect to fecal examination, cinnarizine and rupatadine administered daily for 5 days to mice harboring chronic infection greatly reduced the number of eggs in feces by 73.8% (ANOVA: $F_{(7, 35)}=8.43$, $P<0.0001$) and 80.1% (ANOVA: $F_{(7, 35)}=8.43$, $P<0.0001$), respectively. Under the same drug regimen, desloratadine showed moderate but significant reductions in egg burden (56.5%; ANOVA: $F_{(7, 35)}=8.43$, $P<0.0001$). In the experiments where antihistamines were administered with a single dose (400 mg/kg), a lower percentage reduction in the number of eggs in fecal samples relative to control infected mice was observed, especially in animals with early infection (Fig. 6).

Effect of H1-antihistamines on hepato- and splenomegaly

Treatment of *S. mansoni*-infected animals with antihistamines also achieved a significant reduction of hepato-and-splenomegaly, as measured by weight, compared to control infected rodents (Fig. 7). In a chronic infection model, cinnarizine or rupatadine reduced liver mass by 22.3% (ANOVA: $F_{(4, 7)}=7.16$, $P=0.039$) to 27.4% (ANOVA: $F_{(4, 7)}=7.16$, $P=0.034$) (Fig. 7a) and spleen mass by 26.4% (ANOVA: $F_{(3, 9)}=7.94$, $P=0.038$) to 32.7% (ANOVA: $F_{(3, 9)}=7.94$, $P=0.012$) (Fig. 7b), whereas a moderate but significant reduction in the liver by 13.6% (ANOVA: $F_{(4, 7)}=7.16$, $P=0.0086$) to 18.5% (ANOVA: $F_{(4, 7)}=7.16$, $P=0.034$) and spleen by 20.1% (ANOVA: $F_{(3, 9)}=7.94$, $P=0.0041$) to 24.9% (ANOVA: $F_{(3, 9)}=7.94$, $P=0.0006$) was observed with desloratadine. On the other hand, hepatomegaly and splenomegaly were reduced more slightly in early schistosome infection.

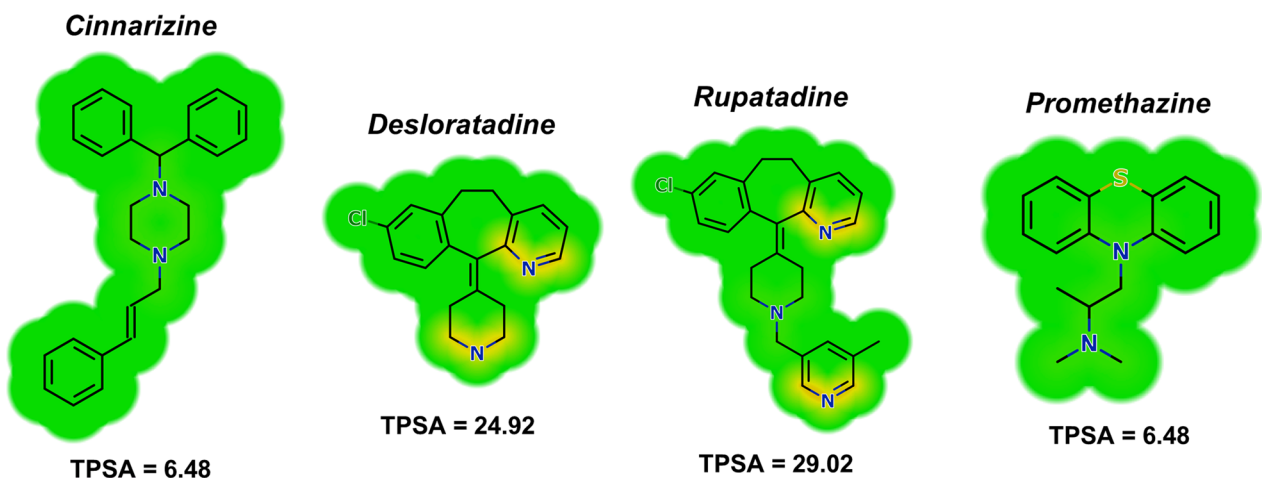

Fig. 2 Heat maps for topological polar surface area (TPSA). The yellow regions contribute to increasing the value of TPSA, whereas the green regions have no influence

Discussion

Parasitic flatworm infections are treated by a limited number of drugs and, in most cases, control is reliant upon praziquantel monotherapy. However, praziquantel's lack of efficacy against immature worms and the emergence of resistance against praziquantel cast a shadow on the global effort to control helminthiasis, as both treatment and control rely significantly on this drug. Since new drugs take a decade or longer to develop, and cost millions of dollars, drug repurposing is a promising approach. Phenotypic screening has successfully identified praziquantel and other anthelmintic agents (e.g. ivermectin and albendazole) that are in veterinary and medical use [31]. In this study, from a screening of 21 H1-antihistamines, we found four drugs which affect the viability of *S. mansoni*.

In vitro results showed that two first-generation antihistamines (cinnarizine and promethazine) and two second-generation antihistamines (desloratadine and rupatadine) are highly active against adult schistosomes, with LC_{50} values of 5.8–15.4 μM, whereas the other antihistamines were found to be inactive when screened at 50 μM. Although less potent than praziquantel, which had an LC_{50} value of approximately 0.1 μM [32, 33], cinnarizine, promethazine, desloratadine and rupatadine are more potent than most antischistosomal compounds described so far (for review see [10, 34]). *In vitro* results of this study with cinnarizine are inconsistent with previously reported results, which showed the lack of *in vitro* anti-parasitic activity against the larval [35] and adult [36] stages of *S. mansoni*. Interestingly, assessing the activity profile of an FDA-approved compound library against *S. mansoni* [37], tested promethazine and cinnarizine against adult parasites even at a high concentration of 33 μM and did not see any antischistosomal activity. These inconsistencies are likely a combination of differences in drug concentrations and life stages tested. In addition, it may also be possible that strain differences result in differing drug susceptibilities. For example, Sarhan et al. [36] and Panic et al. [37] used an Egyptian and Liberian strain, respectively, whereas we used a Brazilian strain.

Histamine has an important role as a chemical messenger in physiological responses, neurotransmission, allergic inflammation, and immunomodulation. Its receptors (named H1, H2, H3 and H4) are traditional GPCRs of extensive therapeutic interest [30, 38]. As the target of 33% of all small-molecule drugs, GPCRs are an important class of proteins in drug discovery [39]. Although GPCRs have been described in schistosomes [14, 18], the exact mechanism by which desloratadine, rupatadine, cinnarizine, and promethazine exert their anthelmintic action on schistosomes is still not clear. From a structural point of view, desloratadine, rupatadine and loratadine are similar, but loratadine was inactive *in vitro* against adult schistosomes. Rupatadine contains a 5-methylpyridin-3-yl group connected through a methylene to the basic amine of desloratadine. Interestingly, all four active H1-antihistamines had marked effects on the tegument of *S. mansoni*. However, it is not possible to distinguish causative from consequent action with regard to tegument damage; the drugs may induce it as part of their mechanism of action, or it may be a consequence of parasite death from another mechanism. Unlike nematodes, which are protected by a cuticle, *Schistosoma* species are covered by a living syncytium, called the tegument. This tissue

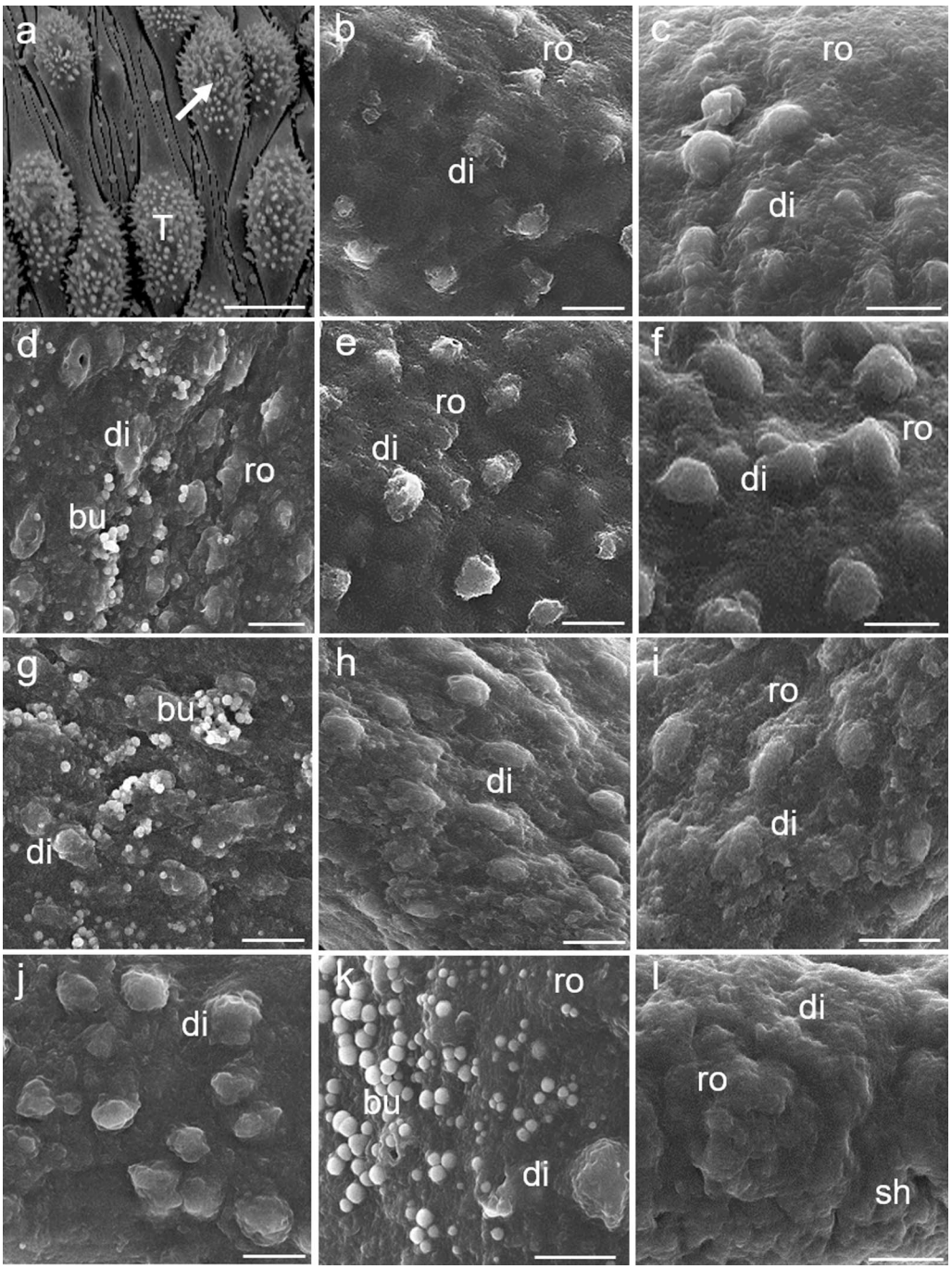

Fig. 3 Scanning electron micrographs of *S. mansoni* after exposure to H1-antihistamines drugs. Adult parasites were collected from the hepatic portal and mesenteric veins of mice and placed on plates containing various concentrations of H1-antihistamines. Parasites were monitored at different times up to 72 h and micrographs of the mid-body region of male worms were obtained using a scanning electron microscope. **a** Control showing tubercles (T) and spines on the surface (arrow). **b**–**d** Twenty-four hours after incubation of cinnarizine 50 μM (**b**), desloratadine 50 μM (**c**) and rupatadine 50 μM (**d**). **e**–**g** Forty-eight hours after incubation of cinnarizine 25 μM (**e**), desloratadine 50 μM (**f**) and rupatadine 50 μM (**g**). **h**–**k** Seventy-two hours after incubation of cinnarizine 12.5 μM (**h**), desloratadine 25 μM (**i**), desloratadine 12.5 μM (**j**) and rupatadine 25 μM (**k**). **l** Praziquantel 2 μM. In figures **b**–**l**, the dorsal tegumental surface shows roughening (ro), disintegration (di), bubbles (bu) and shrinking (sh). Images were captured using a JEOL SM-6460LV scanning electron microscope. *Scale-bars*: 10 μm

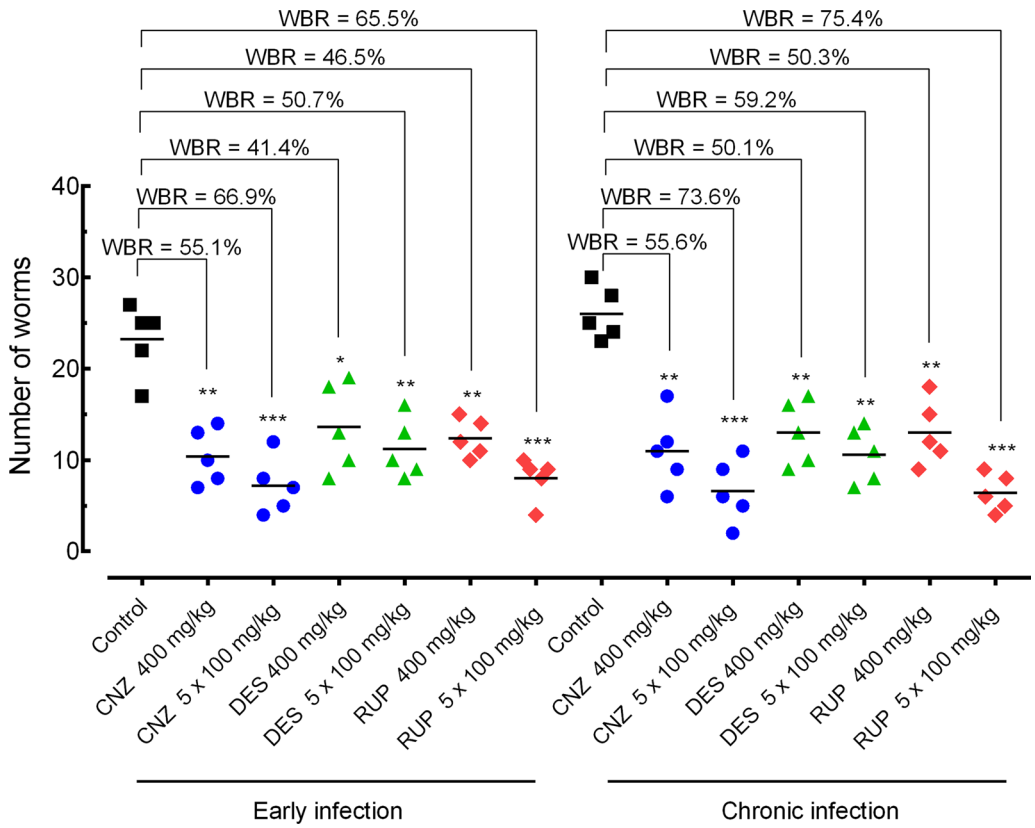

Fig. 4 Effect of H1-antihistamine drugs on the parasite burden in a preclinical mouse model. Drugs were administered orally using a single dose of 400 mg/kg or 100 mg/kg for five consecutive days to mice harboring either early or chronic *S. mansoni* infection. On day 56 post-infection, all animals were humanely euthanized and parasite burdens were determined by sex (male and female parasites). Points represent data from individual mice ($n = 5$ per group). Horizontal bars represent median values. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with infected untreated control by Dunnett's test. *Abbreviations*: WBR, worm burden reduction; CNZ, cinnarizine; DES, desloratadine; RUP, rupatadine

is bounded at its basal surface by a usual invaginated plasma membrane, whereas its apical surface has an atypical heptalaminate appearance [40]. This heptalamellar layer forms many surface pits that substantially enlarge the surface area of the schistosomes. Antihistamines are heterogeneous groups of compounds, with markedly different chemical structures. Comparing the physicochemical properties, the active H1-antihistamines have lower values of TPSA; this membrane permeability parameter may be important to facilitate the permeation of the drugs through the parasite's tegument and, consequently, the interaction with their molecular target(s). Furthermore, cinnarizine is also a calcium channel blocker, and the possibility of action on the helminth's calcium channels cannot be excluded. Further studies are needed to elucidate the mechanism of action of the H1-antihistamines in schistosomes.

Cinnarizine, desloratadine, and rupatadine were evaluated in both early and chronic *S. mansoni* infection models in mice. The oral doses of H1-antihistamines that were chosen (single dose of 400 mg/kg and 100 mg/kg daily for 5 days) followed the protocol recommended for a mouse model of schistosomiasis (e.g. [24, 41]). In addition, these drug regimens (single dose or daily, once a day) are in tune with those recommended for the treatment of allergic symptoms. Of note, most H1-antihistamines have an extended duration of clinical activity which allows once-daily administration. In this study, the treatment with any of the three H1-antihistamines, mainly using 100 mg/kg daily, revealed significant worm burden reductions in animals harboring either chronic or early *S. mansoni* infection. It should be noted that praziquantel treatment exerts high cure rates of 70–90% [42], but it is concerning that some infections in humans and in various other species of animals appear to be refractory to treatment [43, 44]. Importantly, praziquantel has low efficacy against immature parasites (early infection) [45]. Comparatively, oral treatment with cinnarizine, desloratadine, or rupatadine is more effective in early infection than praziquantel. In contrast, praziquantel is more effective in chronic infection that the

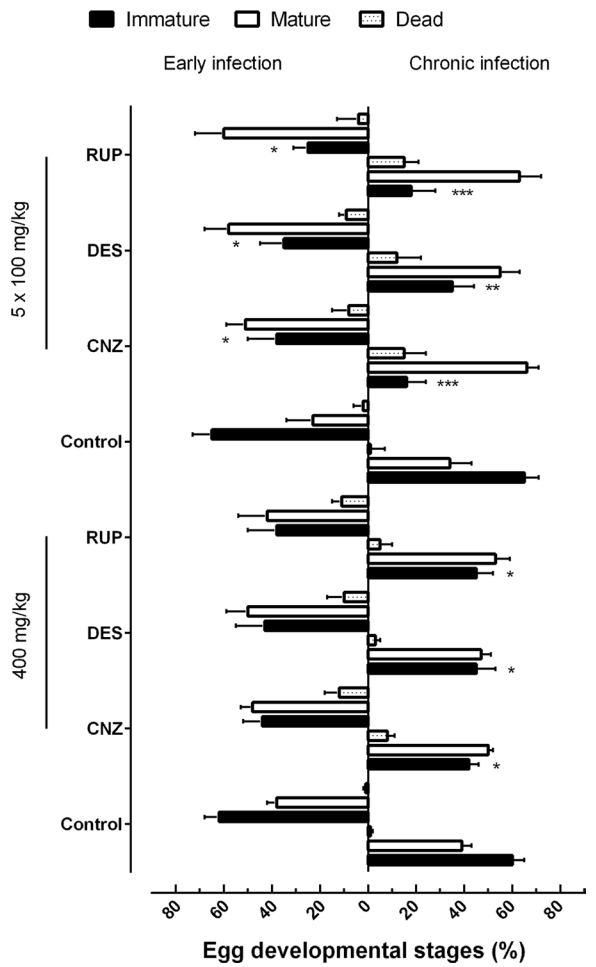

Fig. 5 Effect of H1-antihistamine drugs on the egg developmental stage in a preclinical mouse model. Drugs were administered orally using a single dose of 400 mg/kg or 100 mg/kg for five consecutive days to mice harboring either early or chronic *S. mansoni* infection. On day 56 post-infection, all animals were humanely euthanized and egg burdens were determined by counting eggs in the intestine (quantitative and qualitative oogram technique). Data are presented as the mean ± SD ($n = 5$ per group). The numbers represent the percentages of egg reduction *vs* infected untreated control. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with infected untreated control groups. *Abbreviations*: CNZ, cinnarizine; DES, desloratadine; RUP, rupatadine

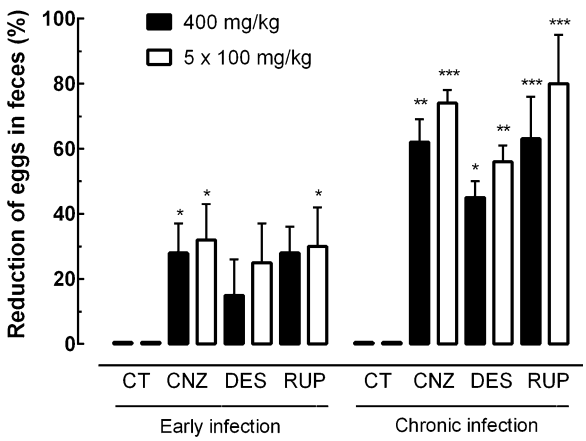

Fig. 6 Effect of H1-antihistamine drugs on the egg burden in the feces in a preclinical mouse model. Drugs were administered orally using a single dose of 400 mg/kg or 100 mg/kg for five consecutive days to mice harboring either early or chronic *S. mansoni* infection. On day 56 post-infection, all animals were humanely euthanized and egg burden in feces was measured by Kato-Katz technique. Data are presented as the mean ± SD ($n = 5$ per group). The numbers represent the percentages of egg reduction *vs* infected untreated control. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with infected untreated control groups by Dunnett's test. *Abbreviations*: CNZ, cinnarizine; DES, desloratadine; RUP, rupatadine

three H1-antihistamines. Collectively, this finding highlights the advantage of using cinnarizine, rupatadine and desloratadine instead of praziquantel in immature schistosome stages. *In vivo* results of this work with cinnarizine in part mirrored the *in vivo* studies mentioned earlier [36], in that cinnarizine was effective in reducing the worm burden in early infection, surpassing praziquantel.

Egg production, a key mechanism for both transmission and pathogenesis, was also markedly inhibited by antihistamines, and a mitigation effect on hepatomegaly and splenomegaly was also recorded. This result could be attributed to a decrease in the number of parasites as a result of treatment with antihistamines and/or reduction of egg-laying by female helminths. A significant decrease in egg-laying in the intestine or feces has been recently reported with other anthelmintic agents [17, 29, 46]. Moreover, the pathology normally associated with the parasite eggs in the liver and spleen was ameliorated, mainly when antihistamines were given for five days. This finding could be assigned to a decrease in the number of worms and egg-laying. Additionally, it is well established that, in addition to their effects on H1 receptors, antihistamines also possess anti-inflammatory properties and, thus, H1-antihistamine therapy may have contributed to reducing hepatomegaly and splenomegaly in *S. mansoni*-infected mice.

In tandem, rupatadine, cinnarizine, and deslotaradine revealed a moderate reduction in worm and egg burden in mice harboring either early or chronic *S. mansoni* infection. Clinically, a typical dose of these H1-antihistamines is a single 5–20 mg tablet. Even allowing for pharmacokinetic (PK) differences between mice and humans (see dose translation from animal to human studies described by Reagan-Shaw et al. [47]), this dose is much less than 100 mg/kg (let alone 400 mg/kg). Similarly, the maximum serum concentration (Cmax) for these drugs in humans is < 10 ng/ml (< 0.1 μM), far lower than the concentrations needed to kill schistosomes in

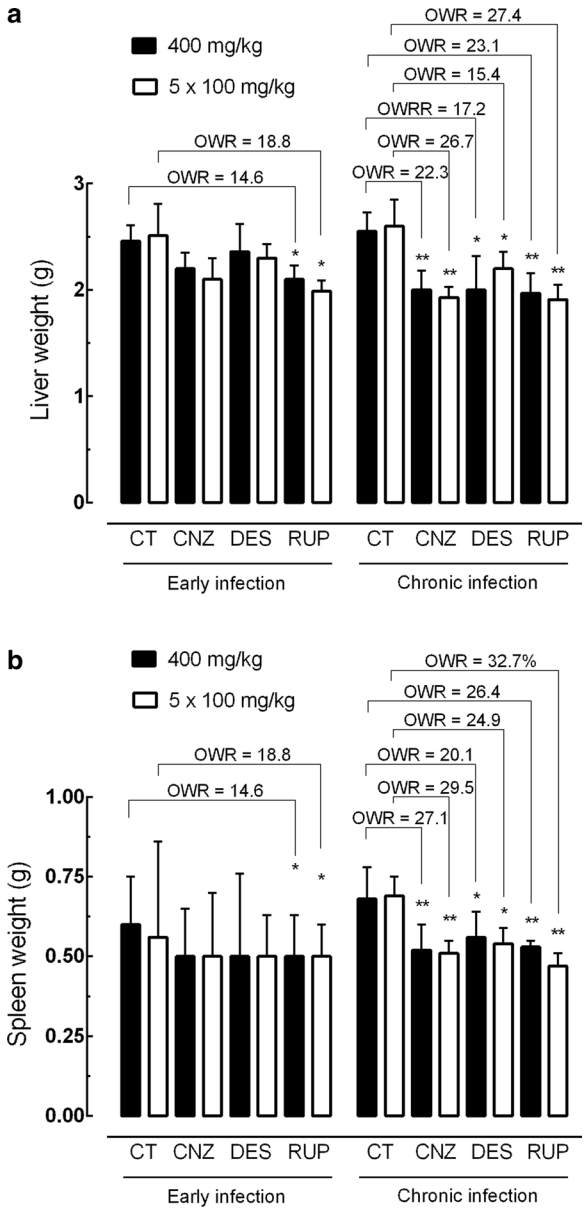

Fig. 7 Effect of H1-antihistamine drugs on the organ pathology in a preclinical mouse model. **a** Liver weight. **b** Spleen weight. Drugs were administered orally using a single dose of 400 mg/kg or 100 mg/kg for five consecutive days to mice harboring either early or chronic *S. mansoni* infection. On day 56 post-infection, all animals were humanely euthanized and organ pathology was determined by liver and spleen weights. Data are presented as the mean ± SD ($n = 5$ per group). The numbers represent the percentages of egg reduction *vs* infected untreated control. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with infected untreated control groups by Dunnett's test. *Abbreviations*: OWR, organ weight reduction; CNZ, cinnarizine; DES, desloratadine; RUP, rupatadine

culture. Therefore, although these drugs are quite safe, that difference is highly unlikely to support clinical use for schistosomiasis.

Conclusions

In conclusion, of all the H1-antihistamines tested, promethazine, cinnarizine, desloratadine, and rupatadine are schistosomicidal agents *in vitro*, which is consistent with the extensive structural damage caused by these compounds. In a rodent model of schistosomiasis, desloratadine and mainly rupatadine and cinnarizine greatly reduced worm burden, egg production, and hepatomegaly and splenomegaly. Although the worm and egg burden reductions achieved were all only moderate, comparatively, treatment with any of the three antihistamines is more effective in early infection than praziquantel. On the other hand, the clinical use of H1-antihistamines for the treatment of schistosomiasis is highly unlikely. Finally, the exact mechanism by which these H1-antihistamines exert their anthelmintic effect is still not clear, and further investigation of this property and identification of parasitic-selective ligands that convey this effect are warranted because this could lead to a directed medicinal chemistry effort to identify schistosome-selective compounds.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13071-020-04140-z.

Additional file 1. Table S1. Molecular properties of H1-antihistamine drugs.

Abbreviations

Cmax: Maximum serum concentration; DMSO: Dimethyl sulfoxide; FBS: Fetal bovine serum; GPCRs: G protein-coupled receptors; LC_{50}: 50% Lethal concentration; PK: Pharmacokinetic; SD: Standard deviation; TPSA: Topological polar surface area.

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Authors' contributions

ACM, MPS and DBR conducted the *in vitro* and *in vivo* experiments and assessed the data. MCS and FST performed microscopy procedures. PLP provided adult *S. mansoni* parasites and analyzed the data from the *in vitro* experiments. TRM provided support with chemical data. LLGF and ADA analyzed the drugs' pharmacokinetic parameters. JM performed the statistical analysis. RPX, TRM and JM analyzed the data and wrote the manuscript. All authors contributed intellectually to the article. All authors read and approved the final manuscript.

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Availability of data and materials
The dataset supporting the conclusions of this article is included within the article and its additional file.

Ethics approval and consent to participate
This study was conducted following the guidelines of the Animal Ethics Committee (Universidade Guarulhos, Guarulhos, SP, Brazil) according to Brazilian law. All experimental protocols were approved by the Universidade Guarulhos (Approval ID 31/2017).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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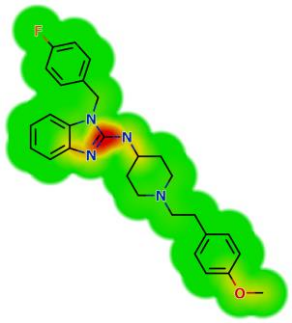
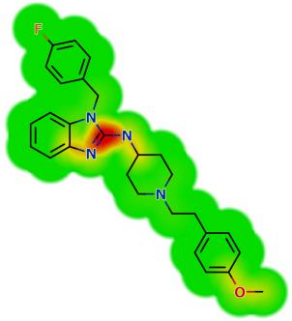
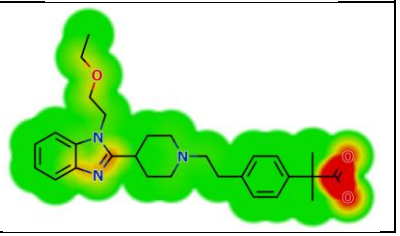
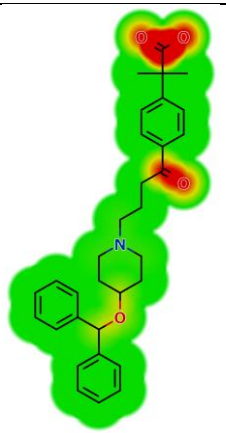
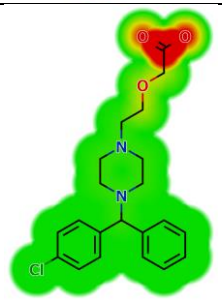
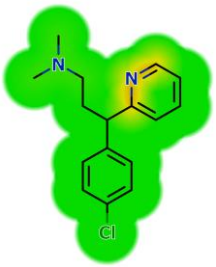
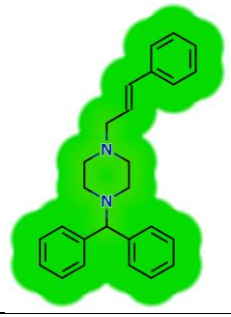
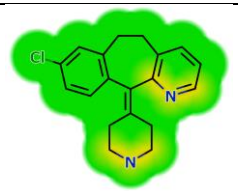
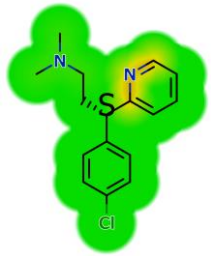
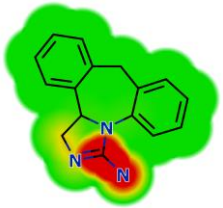
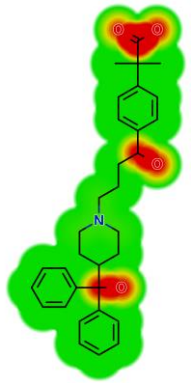
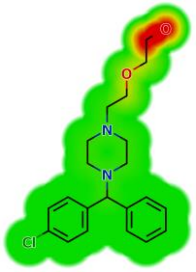
Publisher's Note

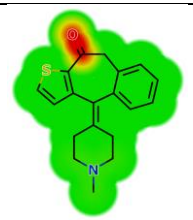
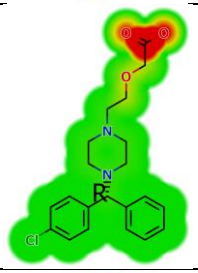
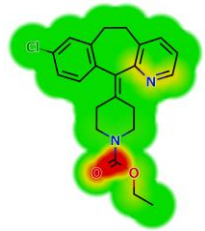
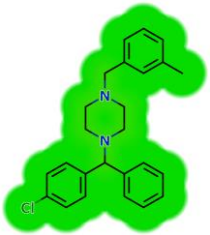
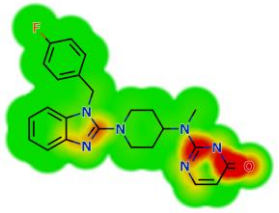
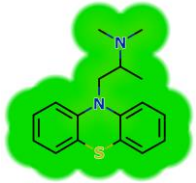
5.1.2 Informação suplementar - Molecular properties of H1-antihistamine drugs

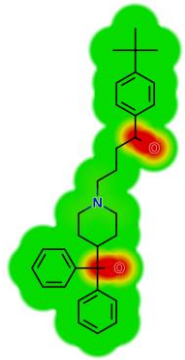
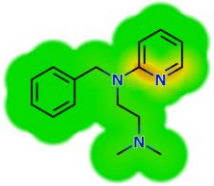
Additional file 1: Table S1. Molecular properties of H1-antihistamine drugs.

Drug	*S. mansoni* LC_{50} (µM)	logS	logS @ pH7.4	logP	logD	MW	HBD	HBA	TPSA	Flexibility	TPSA Heat Map[a]
Acrivastine	> 50	2.17	2.04	3.42	0.36	348.40	1	4	53.43	0.21	
Astemizole	> 50	1.13	1.62	5.70	3.78	458.60	1	5	42.32	0.21	
Bilastine	> 50	1.68	1.32	4.96	1.35	463.60	1	6	67.59	0.27	

Carebastine	> 50	0.23	1.27	5.48	1.92	499.60	1	5	66.84	0.28	
Cetirizine	> 50	3.01	1.89	1.70	1.50	388.90	1	5	53.01	0.28	
Chlorfeniramine	> 50	2.87	1.68	3.17	1.72	274.80	0	2	16.13	0.25	
Cinnarizine	**7.1**	2.18	1.04	5.77	3.70	368.50	0	2	**6.48**	**0.19**	

Desloratadine	**9.8**	2.84	1.39	3.45	1.88	310.80	1	2	**24.92**	**0.00**	
Dexchlorpheniramine	> 50	2.87	1.68	3.17	1.69	274.80	0	2	16.13	0.25	
Epinastine	> 50	2.47	1.42	3.51	-0.06	249.30	1	3	41.62	0.00	
Fexofenadine	> 50	1.78	1.21	4.07	1.47	501.70	3	5	81.00	0.25	
Hydroxyzine	> 50	3.31	1.82	3.25	3.10	374.90	1	4	35.94	0.29	

Ketotifen	> 50	2.10	1.59	2.85	1.87	309.40	0	2	20.31	0.00	
Levocetirizine	> 50	3.01	1.89	1.70	1.40	388.90	1	5	53.01	0.28	
Loratadine	> 50	1.29	1.29	5.20	5.20	382.90	0	4	42.43	0.10	
Meclizine	> 50	1.74	1.13	5.76	4.34	391.00	0	2	6.48	0.16	
Mizolastine	> 50	0.72	0.87	3.37	2.87	432.50	1	7	70.05	0.14	
Promethazine	**4.9**	1.74	2.18	4.81	2.52	284.40	0	2	**6.48**	**0.14**	

Rupatadine	**15.4**	1.39	0.82	4.16	3.22	416.00	0	3	**29.02**	**0.06**	
Terfenadine	> 50	1.62	0.46	5.69	3.61	471.70	2	3	43.70	0.24	
Tripelennamine	> 50	3.36	1.53	3.09	1.34	255.40	0	3	19.37	0.30	

[a] Heat maps for TPSA. The red-yellow regions contribute positively to the property and the green regions have no influence.

5.2 Artigo 3 - Promethazine Exhibits Antiparasitic Properties *In Vitro* and Reduces Worm Burden, Egg Production, Hepatomegaly, and Splenomegaly in a Schistosomiasis Animal Model

Ademais, parte dos resultados estão no artigo recentemente publicado no periódico *Antimicrobial Agents and Chemotherapy* (Fator de Impacto 4,904, classificado com Qualis A1). O trabalho, em sua completude, também está apenso.

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EXPERIMENTAL THERAPEUTICS

Promethazine Exhibits Antiparasitic Properties *In Vitro* and Reduces Worm Burden, Egg Production, Hepatomegaly, and Splenomegaly in a Schistosomiasis Animal Model

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ABSTRACT The treatment and control of schistosomiasis, a neglected disease that affects more than 200 million people worldwide, rely on the use of a single drug, praziquantel. A vaccine has yet to be developed, and since new drug design and development is a lengthy and costly process, drug repurposing is a promising strategy. In this study, the efficacy of promethazine, a first-generation antihistamine, was evaluated against *Schistosoma mansoni ex vivo* and in a murine model of schistosomiasis. *In vitro* assays demonstrated that promethazine affected parasite motility and viability, and it induced severe tegumental damage in schistosomes. The 50% lethal concentration (LC_{50}) of the drug was 5.84 μM. Similar to promethazine, schistosomes incubated with atropine, a classical anticholinergic drug, displayed reduced motor activity. In an animal model, promethazine treatment was introduced at an oral dose of 100 mg/kg of body weight for five successive days at different intervals from the time of infection for the evaluation of the stage-specific susceptibility (prepatent and patent infections). Various parasitological criteria indicated the following *in vivo* antischistosomal effects of promethazine: there were significant reductions in worm burden, egg production, hepatomegaly, and splenomegaly. The highest worm burden reduction was achieved with promethazine in patent infections (>90%). Taken together, considering the importance of the repositioning of drugs in infectious diseases, especially those related to poverty, our data revealed the possibility of promethazine repositioning as an antischistosomal agent.

KEYWORDS schistosomiasis, antischistosomal, *Schistosoma*, promethazine, drug repositioning

Schistosomiasis, caused by parasitic flatworms (blood flukes) of the genus *Schistosoma*, is one of the most important helminthic diseases in terms of morbidity and mortality. More than 200 million people, mainly in the world's poorest regions, are currently infected with *Schistosoma* worms. The disease is characterized by a chronic inflammatory disorder associated with disabling anemia and undernutrition, as well as poor performance in school and work. There are six known human-pathogenic species, of which *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum* are the most prevalent (1).

Despite considerable efforts over the past decades, only one efficient and safe drug is currently available, praziquantel (2). However, praziquantel does not prevent reinfection and, owing to its use for more than 3 decades, the emergence of praziquantel-resistant schistosomes is a constant threat (3). In addition, there is a critical deficiency

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in the praziquantel therapeutic profile related to the lack of activity against juvenile worms. This fact can partially explain the low cure rate in high-transmission areas where patients are likely to harbor juvenile and adult parasites concurrently. Based on these grounds, the search for new antischistosomal agents represents a compelling priority (4, 5).

Although there is a continuing need for safe and cost-effective drugs suitable for use in resource-poor settings, the development of new drugs is a lengthy and costly process (6). Unfortunately, many pharmaceutical companies decreased their attempts to discover new anti-infective drugs. As a result, the average number of new drugs approved by regulatory agencies per year has declined in the last decades (7). In the field of infectious diseases of poverty, it is more difficult to recover the cost for drug development from pharmaceutical sales, whose target population lack financial recourse. Hence, the repurposing of drugs and clinical candidates offer an attractive alternative to *de novo* drug discovery (7–9), particularly in terms of reducing time and research and development costs since safety investigations can be reduced (10, 11).

Promethazine, a phenothiazine derivative, is an H1 receptor antagonist widely used worldwide. Since its first introduction in 1946, it has been used for the prevention and treatment of nausea and regurgitation (12) However, data from the literature showed that promethazine is capable of interacting with different cellular receptors. As a matter of fact, promethazine possesses anticholinergic, sedative, and antiemetic effects and some local anesthetic properties (13). In clinical practice, promethazine has been used to treat several allergy-related symptoms, for the prevention of motion sickness, treatment of nausea, after surgery to control pain, and as a sedative or sleep aid (14).

In parasitic flatworms, including schistosomes, biogenic amines play several important roles in the control of motility, metabolism, and reproduction (15, 16). The distribution of histamine and the presence of histamine receptors in *S. mansoni* have been already described (17). Interestingly, a recent study provided evidence of a functional G protein-coupled acetylcholine receptor that regulates the motility of *S. mansoni*. Also, promethazine was found to have inverse agonist activity towards this receptor (18). Based on these findings and promoting the drug repositioning approach, here, we demonstrate that promethazine has a marked lethal effect on *S. mansoni* adult worms *ex vivo*. Moreover, scanning electron microscopy analysis highlighted tegumental damage in adult parasites. In an animal model of schistosomiasis, we further demonstrate that promethazine is orally effective in both patent and prepatent infections, revealing the potential role of this drug as an antischistosomal agent capable of repurposing clinical development.

RESULTS

Promethazine killed adult schistosomes *ex vivo*. In the first step, we tested the drug promethazine (Fig. 1A) at a range of concentrations against adult schistosomes *ex vivo*. The parasites were maintained for 72 h and monitored every 24 h to evaluate their general condition in terms of motor activity, alteration in the tegument, and mortality rate. As shown in Fig. 1B, promethazine was able to kill adult schistosomes with 50% lethal concentrations (LC_{50}s) of 10.29 and 5.84 μM after 24 and 72 h, respectively, whereas no mortality was observed in control untreated worms.

Promethazine altered motility and morphology of the adult schistosomes *ex vivo*. In addition to the antiparasitic activity against schistosomes, we also monitored worm motility using light microscopy. When adult schistosomes were maintained in the absence of drug (control group), parasites revealed normal motor activity, with natural peristalsis of the gut (Fig. 1C). In contrast, schistosomes exposed to promethazine exhibited spasmodic contractions, followed by significantly reduced motor activity with a concentration-dependent effect. In more detail, worms treated with promethazine were contorted and, they adopted the shape of a loose knot (Fig. 1D to F). Since promethazine has anticholinergic activity, therefore, we decided to evaluate the effect using different concentrations of atropine, a classical anticholinergic drug, on the motility of schistosomes. Similar to promethazine, schistosomes incubated with atropine

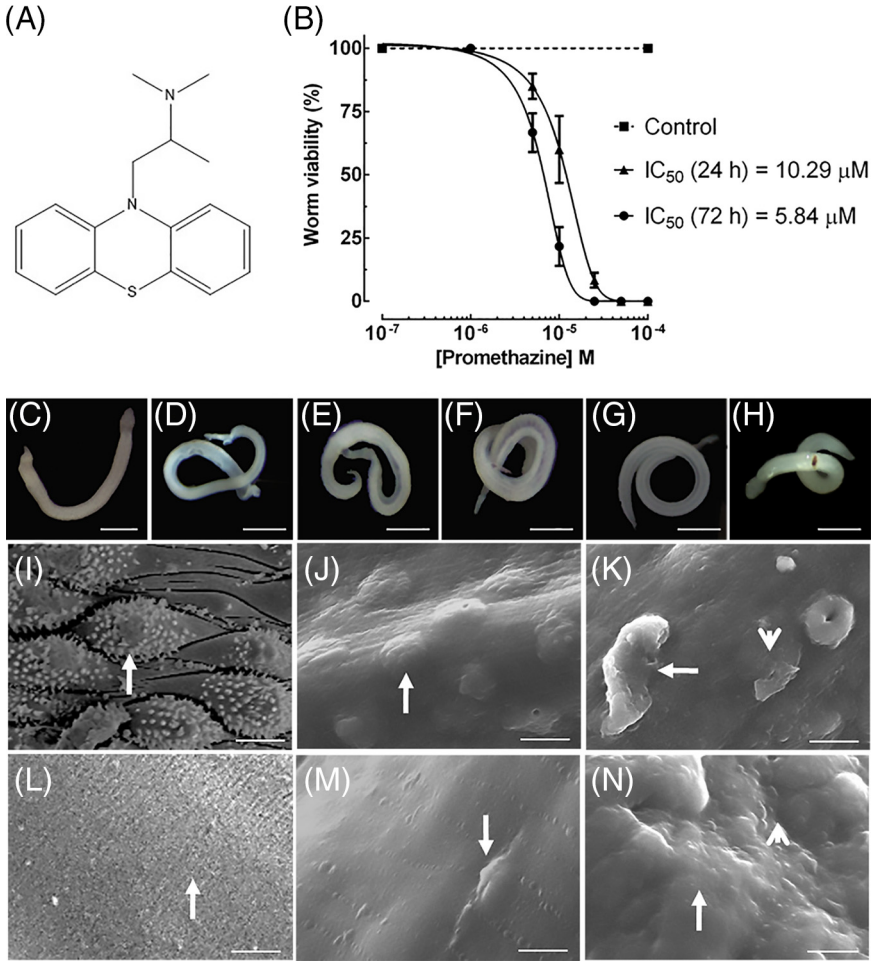

FIG 1 Promethazine action on adult *S. mansoni* parasites *ex vivo*. Adult parasites were collected from the hepatic portal and mesenteric veins of mice and placed on plates containing the indicated concentrations of the drug. Parasites were scored at 72 h for survival and microscopy analysis. (A) Chemical structure of promethazine. (B) Viability of schistosomes after exposure to promethazine, showing IC_{50} values at 24 and 72 h, and control untreated worms at 72 h. Data are the means ± SD. The *in vitro* experiments were performed three times ($n = 3$), and each experiment was performed with five replicates. (C) Control. (D) 6.25 μM promethazine. (E) 12.5 μM promethazine. (F) 25 μM promethazine. (G) 12.5 μM atropine. (H) 5 μM praziquantel. (I) Control. Male tegument of the midbody region showing tubercles and spines on the surface (arrow). (J and K) The dorsal tegumental surface shows sloughing (arrowhead), shortening, and a collapse of the tubercles with loss of the spines (arrow) on the surface after exposure to 12.5 μM promethazine (J) and 25 μM promethazine (K). (L) Control. Female tegument of the midbody region with parallel arranged fissures (arrow). (M and N) The dorsal tegumental surface shows swelling and shortening (arrow) and erosion of the surface (arrowhead) after exposure to 12.5 μM promethazine (M) and 25 μM promethazine (N). Images were captured using a Leica Microsystems EZ4E microscope (scale bars = 5 mm [C to H]) and a JEOL SM-6460LV scanning electron microscope (scale bars = 10 μm [I to N]).

showed reduced motor activity, including contractions of the worm's body (Fig. 1G). Interestingly, both promethazine and atropine promoted separation of the coupled worms into individual males and females accompanied by weakened movements of the oral sucker.

It was further examined whether acetylcholine, an important neurotransmitter in both vertebrate and invertebrate species, has the ability to reverse the reduction of motor activity of the schistosomes exposed to promethazine. Parasites were incubated with various lethal concentrations of promethazine (6.25, 12.5, and 25 μM), and then acetylcholine was added at various concentrations (25, 50, and 100 μM). As expected, acetylcholine-treated worms reversed the reduction in motility caused by promethazine, i.e., helminths remained with normal body movement and were viable for up to 72 h, suggestive of receptor interaction or competition.

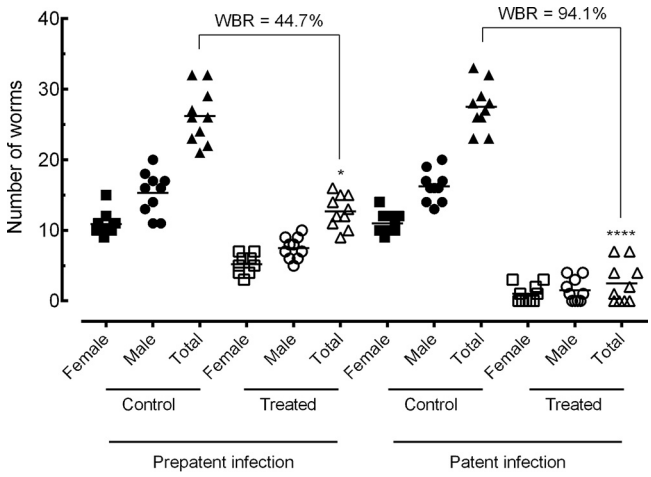

FIG 2 Effect of promethazine on the parasite burden. Drugs were administered orally using a dose of 100 mg/kg for five consecutive days to mice harboring either juvenile (prepatent infection) or adult (patent infection) stages of *S. mansoni*. On day 56 postinfection, each animal was humanely euthanized, and parasite burdens were determined by sex (male and female schistosomes). Points represent data from individual mice ($n = 10$). Horizontal bars represent median values. *, $P < 0.05$; ****, $P < 0.0001$ compared with untreated groups. WBR, worm burden reduction.

To determine whether promethazine can cause morphological alterations on the tegument of *S. mansoni*, we used a scanning electron microscope. Compared to control parasites, which have intact tegument, schistosomes exhibited profound alterations in the morphology of their surface after exposure to promethazine, with rupture of the tegument along the whole dorsal body surface (Fig. 1I to N). In more detail, the principal alterations in male worms exposed to promethazine were disintegrated tubercles as well as swelling, sloughing, and erosion of the surface (Fig. 1J and K). In female worms, tegumental damage consisted of swelling and erosion of the surface (Fig. 1M and N).

Promethazine was effective against both juvenile and adult schistosomes in a mouse model. Treatment of *S. mansoni*-infected mice with promethazine (5 × 100 mg/kg) resulted in a significant reduction ($P < 0.05$ to 0.0001) in worm burden against different developmental stages (adult and juvenile) compared to control infected mice (Fig. 2). In the juvenile infection model (prepatent infection), oral administration of promethazine resulted in a significant reduction of 44.7% ($P < 0.05$) for the total worm burden. In the adult infection model (patent infection), promethazine was highly active against both male and female schistosomes, with a total worm burden reduction of 94.1% ($P < 0.0001$).

Promethazine reduced egg production in a mouse model. With respect to egg burden, samples of intestinal tissue were used to study the percent egg developmental stages (oogram), including the number of egg in fecal samples by the Kato-Katz method. Promethazine proved to have a significant egg burden reduction effect against different developmental stages of *S. mansoni*, mainly in patent infection (Fig. 3). In the adult infection model, promethazine led to a marked reduction of 95.3% ($P < 0.0001$) in the number of immature eggs. A significant increase in lifeless eggs was also seen compared with the untreated control (Fig. 3A). Similarly, analysis of fecal samples revealed a high level of reduction, at 96.1% ($P < 0.0001$) of eggs (Fig. 3B). Contrarily, in the juvenile infection model, it was observed a moderate reduction, by 37.2% ($P < 0.05$) and 54.2% ($P < 0.01$), the number of immature eggs in the wall of the intestine and in fecal samples, respectively.

Promethazine ameliorated hepato- and splenomegaly in a mouse model. The protective effect of promethazine was also found to lead to a significant reduction of hepato- and splenomegaly, as measured by weight, compared to the control infected mice (Fig. 4). In an adult infection model, liver and spleen weights were significantly

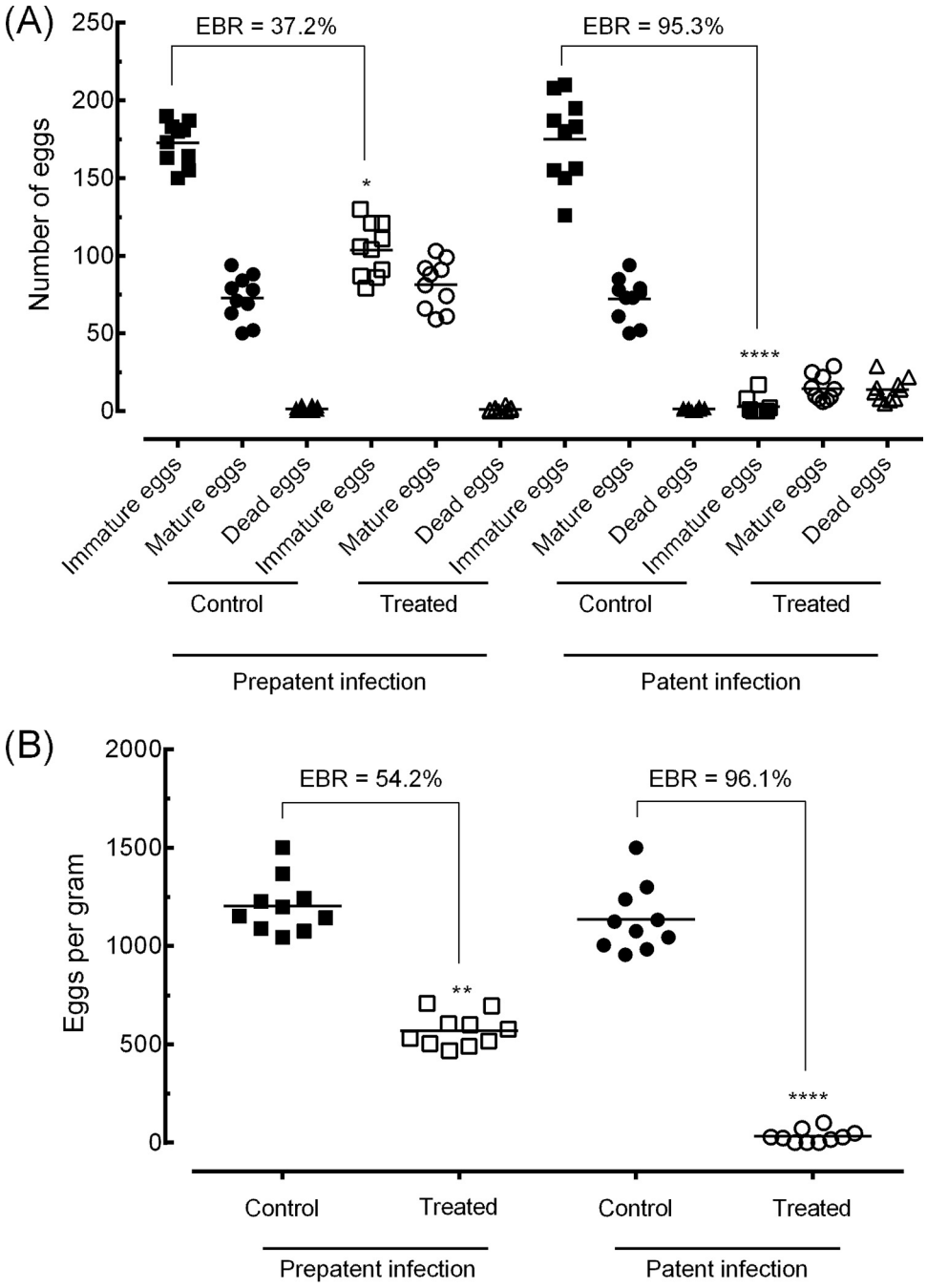

FIG 3 Effect of promethazine on the egg burden. (A) Egg development stages (oogram). (B) Stool egg load. Drugs were administered orally using a dose of 100 mg/kg for five consecutive days to mice harboring either juvenile (prepatent infection) or adult (patent infection) stages of *S. mansoni*. On day 56 postinfection, all animals were humanely euthanized and egg burdens were determined by counting eggs in the intestine (oogram analysis) and in the feces (Kato-Katz technique). Points represent data from individual mice ($n = 10$). Horizontal bars represent median values. *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$ compared with untreated groups. EBR, egg burden reduction.

decreased by 44.1% ($P < 0.01$) and 48.6% ($P < 0.01$), respectively, whereas moderate, but significant, reductions in the liver (19.5%; $P < 0.05$) and spleen (24.6%; $P < 0.05$) were observed in prepatent infections.

DISCUSSION

Despite its significant health and economic impact, drug development for schistosomiasis and other neglected diseases has been historically hampered due to a lack of

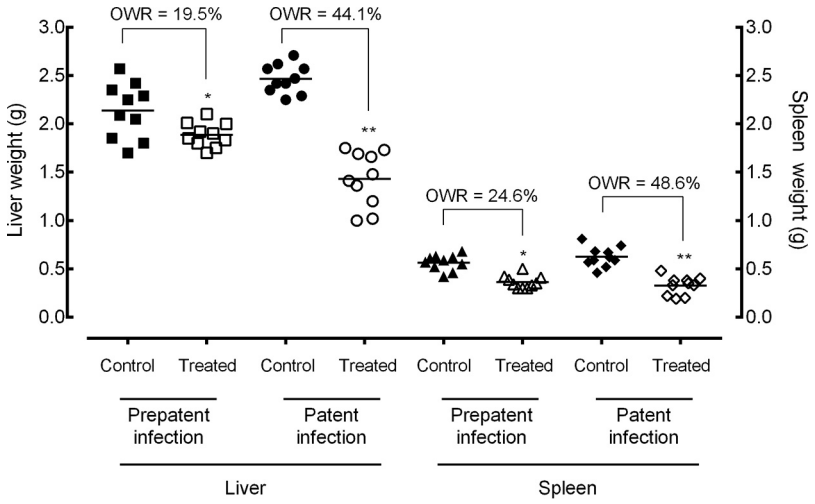

FIG 4 Effect of promethazine on the on the liver and spleen pathology. Drugs were administered orally using a dose of 100 mg/kg for five consecutive days to mice harboring either juvenile (prepatent infection) or adult (patent infection) stages of *S. mansoni*. On day 56 postinfection, all animals were humanely euthanized, and organ pathology was determined by liver and spleen weights. Points represent data from individual mice ($n = 10$). Horizontal bars represent median values. *, $P < 0.05$; **, $P < 0.01$ compared with untreated groups. OWR, organ weight reduction.

market incentives. Consequently, the repurposing of existing drugs has become an attractive approach towards the identification of new treatments for schistosomiasis, whose current treatment and control are limited to praziquantel. Promethazine is an antihistamine drug that has been used worldwide to treat nausea, vomiting, and various allergic symptoms, as well as a sedative or sleep aid and to prevent motion sickness (13, 14). In the present work, we found that promethazine exhibited potent antischistosomal activity against blood fluke *S. mansoni*. Initially, we examined its schistosomicidal properties on adult parasites *ex vivo*. The results encouraged us to examine its efficacy in a murine model harboring *S. mansoni* either juvenile or adult *S. mansoni*. *In vitro* assays demonstrated that promethazine affected parasite motility and viability, and it induced severe tegumental damage in schistosomes. Additionally, we disclosed that atropine and acetylcholine also exert action in the control of worm neuromuscular function. Furthermore, various parasitological criteria characterized the *in vivo* antischistosomal effects of promethazine; it caused significant reductions in worm burden, egg production, and hepato- and splenomegaly. These results reveal the potential of promethazine as an antischistosomal drug.

In vitro results showed that promethazine is highly active against *S. mansoni* adult worms *ex vivo,* with an IC_{50} of 5.84 μM after 72 h. This result surpasses criteria established by the World Health Organization for potential compounds for schistosomiasis (19). Moreover, this activity is superior to those of the most promising drugs, such as artesunate, artemether, mefloquine, and miltefosine (2). However, the IC_{50} is 4.6 times higher than that of praziquantel (IC_{50} of 1.26 μM, described in our previous studies [20, 21]).

In the murine model of schistosomiasis, promethazine treatment was introduced at an oral dose of 100 mg/kg/day for five successive days at different intervals from the time of infection for the evaluation of stage-specific susceptibility. Similar to praziquantel (22) and other promising antischistosomal compounds (11, 23), oral treatment with promethazine was more effective against adult *S. mansoni* than against the juvenile stage. Indeed, a high total worm burden reduction was achieved with promethazine in patent infections (>90%), whereas a moderate total worm burden reduction was observed in prepatent infections (~45%). Comparatively, the antischistosomal effect of promethazine is similar to that of praziquantel, which is known to reduce >90% of the worm burden (2, 3). Collectively, similar to *in vitro* results described

here, the *in vivo* results also surpass criteria established by the WHO that define highly active compounds as those producing an 80% reduction in worm burdens after five doses at 100 mg/kg (19).

Schistosomes are parasitic platyhelminths that are dioecious, with females producing hundreds of eggs per day. The eggs are crucial for the maintenance of the schistosome life cycle and disease transmission and are further responsible for manifested human pathology (1). In addition to a potent schistosomicide, promethazine dramatically reduced the egg burden. The hepato- and splenomegaly normally associated with the deposition of schistosome eggs in the spleen and liver were clearly ameliorated. In schistosomiasis, reductions in worm burden are associated with reduced pathology, and there is no concern about relapse because schistosome parasites do not multiply in the mammalian host (24).

The mechanism by which promethazine exerts its antischistosomal properties is not clear. To investigate hypotheses regarding promethazine's mechanism(s) of action, microscopic studies were used. First, promethazine exerted marked effects on schistosomes' tegument in a concentration-dependent manner. The tegument is the major interface between the schistosome and its external environment. In addition to providing protection, the tegument is extremely important for infection success and survival in the host, and it has been considered a major target for antischistosomal drugs (25, 26). Indeed, there are numerous reports in the literature documenting the morphological alterations on worms' tegument when exposed to schistosomicidal compounds (see, e.g., references 27–29). In this study, we found that promethazine induced severe tegumental damage in adult helminths. This finding could be due to the inherent lipophilicity of promethazine; hence, this drug can easily cross the tegument of the schistosomes and reach their molecular target(s).

Second, the fact that promethazine induces concentration-dependent changes in schistosomes motility also raises the possibility that this drug may act on the neuromuscular system of *S. mansoni*. Several putative cholinergic receptors have been described in schistosomes (30–32). Recently, MacDonald et al. (18) provided proof that cholinergic receptors are present in schistosomes and are crucial for proper motor control in the parasite. The authors also demonstrated that anticholinergic drugs such as atropine and promethazine have inverse agonist activity towards cholinergic receptors, causing a drastic reduction in a schistosome's motility. Our experiments showed that promethazine altered the motor activity of *S. mansoni*, including contractions of the worm's body. Similar phenotypes were also observed after exposure of adult helminths to the classical anticholinergic drug atropine. Moreover, the addition of acetylcholine was seen to reverse the worm's motility, and the parasites remained viable. These results strongly suggest that promethazine can act on the cholinergic system of *S. mansoni*. These results are also consistent with previous studies and support the hypothesis that cholinergic receptors inhibit neuromuscular functions in *S. mansoni* (32). Taken together, these cumulative data reveal that the cholinergic system of schistosomes is a possible promethazine target. Noticeably, other mechanism(s) may be involved and require further investigations.

Promethazine is administered through either enteral or parenteral routes. It is rapidly absorbed after oral administration, with peak concentrations after 2 to 3 h. By the oral route, the daily dose often used for promethazine is 150 mg per day, which is equivalent to a dose of 2.5 mg/kg, calculated for a 60-kg human. Using a dose translation formula for phase I and phase II clinical trials (33), an effective daily dose of 100 mg/kg in our *in vivo* mouse model equates to a human dose of 8 mg/kg. Thus, the dose used in this study, which demonstrated potent schistosomicidal activity, is approximately 3-fold higher than the recommended dose for promethazine. The clinical effects of promethazine in overdose have been reported, and the main feature of toxicity is the occurrence of delirium (34). On average, the dose to achieve this effect was an oral intake of 650 mg, which equates to a dose of 10.8 mg/kg, calculated for a 60-kg human. In addition, the highly sedative effect of promethazine should appear at a lower dose than that which causes delirium (34). In conclusion, although prometh-

azine has a potent schistosomicidal effect both *in vitro* and *in vitro*, its use as an anthelmintic agent in clinical practice may present some limitations. On the other hand, since there are several phenothiazines approved by regulatory agencies and they are commonly used in both humans and animals worldwide, this study opens a new avenue for studying phenothiazine derivatives against helminth infections.

MATERIALS AND METHODS

Ethics. All experiments were conducted in conformity with the Brazilian law for the Guidelines for Care and Use of Laboratory Animals (law 11790/2008). The protocol for experimental design was approved by the Comissão de Ética no Uso de Animais (CEUA), Brazil (protocol no. 31/2017). The animal studies are reported in compliance with the "Animal research: reporting of *in vivo* experiments" (ARRIVE) guidelines.

Materials. Promethazine hydrochloride, acetylcholine chloride, dimethyl sulfoxide (DMSO), and glutaraldehyde solution were purchased from Sigma-Aldrich (St. Louis, MO, USA). Praziquantel was purchased from Merck (São Paulo, São Paulo, Brazil). Atropine sulfate was purchased from Blau (Cotia, São Paulo, Brazil). RPMI 1640 culture medium containing phenol red and L-glutamine, fetal bovine serum (FBS), penicillin G-streptomycin sulfate, and HEPES buffer were obtained from Vitrocell (Campinas, São Paulo, Brazil).

Animals and parasite maintenance. Animals were used for maintenance of the schistosome's life cycle and consequently for *in vitro* studies, as well as for *in vivo* studies. Three-week-old female Swiss mice were purchased from Laboratory Animals Anilab (São Paulo, Brazil). They were housed in individually vented caging systems in groups of five mice per cage. Mice were kept under a 12-h light/12-h dark environment cycle and maintained at uniform temperature and humidity, with food and water available *ad libitum*.

The *Schistosoma mansoni* (BH strain) life cycle was maintained by routine passage through a rodent *Mus musculus* (definitive host) and snail *Biomphalaria glabrata* (intermediate host), following the standard procedures of our laboratory (35). Infections of mammalian host (female Swiss mice, 3 weeks old) with *S. mansoni* were initiated by subcutaneous injection of approximately 150 cercariae collected from *S. mansoni*-infected snails host after exposure to light for 3 h. Both mice and snails were kept under environmentally controlled conditions (temperature, 25°C; humidity, 50%), with unrestricted access to rodent food and water. Of note, once the life cycle is established, it is possible to conduct the *in vitro* and *in vivo* studies.

***In vitro* antischistosomal assay.** An *in vitro* antischistosomal assay using adult schistosomes was performed as previously described (5, 36). Briefly, mice were dissected and adult (7-week-old) schistosomes were collected from the portal and mesenteric veins (parasite *ex vivo*). Subsequently, parasites were placed in RPMI 1640 culture medium supplemented with 10% fetal bovine serum containing 100 IU·ml^{-1} penicillin and 100 μg·ml^{-1} streptomycin at 37°C and 5% CO_2. Promethazine was dissolved in DMSO to obtain final test concentrations of 50 to 0.78 μM (50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 μM) in a 24-well culture plate (one pair of worms per well), with a final volume of 2 ml. Each concentration was tested in five replicate wells, and the highest concentration of DMSO (0.5%) served as a control. Praziquantel (5 μM) was used as a positive control. Additionally, schistosomes were incubated with atropine sulfate at the same concentrations mentioned above for promethazine to monitor worm motility. Furthermore, helminths were incubated with promethazine at 6.25, 12.5, and 25 μM, and then acetylcholine was added at 25, 50, and 100 μM 30 min after the start with promethazine. Parasites were kept for 72 h (37°C, 5% CO_2), and their viability was assessed via microscopic readout (Leica Microsystems, Wetzlar, Germany).

Microscopy analysis. During the *in vitro* studies, schistosomes were monitored microscopically using a Leica Microsystems EZ4E microscope (Wetzlar, Germany). For scanning electron microscopy, control and treated adult parasites were fixed for at least 3 h in 2.5% glutaraldehyde at room temperature. The experimental protocols for scanning electron microscopy were published previously (37, 38). Samples were metalized with gold using a Desk II sputter coater (Denton Vacuum LLC, Moorestown, NJ, USA) and then observed using an SM-6460LV high-resolution scanning electron microscopy (JEOL Ltd., Tokyo, Japan).

***In vivo* studies in a mouse model of schistosomiasis.** For the *in vivo* studies, female 3-week-old Swiss mice were infected subcutaneously with 80 *S. mansoni* cercariae. Female mice are commonly used in experimental schistosomiasis models (39). Forty mice (20 $\pm$ 2 g) were randomly divided into four groups (10 mice per group) and treated with vehicle or promethazine. To study the stage-specific susceptibility, the assay was performed in two different periods, as follows: (i) the drug or vehicle was given 21 days postinfection against the juvenile stage (prepatent infection); and (ii) the drug or vehicle was given 42 days postinfection against the adult stage (patent infection). Treatment was introduced at a dose of 100 mg/kg/day for five successive days. It should be emphasized that a single oral dose of 400 mg/kg is the pattern chosen for experimental schistosomiasis (2). However, since this dose exceeds the 50% lethal dose (LD_{50}) of promethazine, we chose 100 mg/kg for five consecutive days, which is also used in drug discovery programs for the murine model of schistosomiasis (11, 40).

At 56 days postinfection, all mice were euthanized by the CO_2 method and dissected, and worms were then collected, separated by sex, and counted as previously described (41, 42). Assessment of therapeutic efficacy was further based on the technique of quantitative and qualitative oograms using a fragment (10 mm) of the ascending colon, as well as the Kato-Katz method for quantitative fecal examination.

Randomization and blinding. For the implementation of the *in vivo* testing, the animals were randomly assigned to the experimental groups, and pharmacological treatments were counterbalanced randomly as well. The animals were euthanized in a random manner inside a group. Although the investigators were not blinded to the treatment groups, tests for all parameters, i.e., the (i) count of worms, (ii) analysis of the egg stages in the intestine (oogram), (iii) count of eggs in the feces, and (iv) measurement of the mass of the organs, were conducted by different people and analyzed by two different investigators. Therefore, the operators of the experiments were not the same as the data analysts in order to eliminate bias in interpretation.

Statistical analysis. All statistical analyses were performed using the GraphPad Prism software. All data from the *in vitro* antischistosomal assay are presented as the mean ± standard deviation (SD) of the results from at least three independent experiments. IC_{50} values were calculated using sigmoid dose-response curves and the 95% confidence intervals (43, 44). For *in vivo* experimental analysis, a parametric Dunnett's multiple-comparison test was applied to compare the vehicle group versus the treated group, where statistical significance was set to a *P* value of 0.05. *In vivo* experimental graphics represent data from individual mice (23). The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (45).

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6. PERSPECTIVAS

O presente trabalho teve como objetivo majoritário avaliar as propriedades antiparasitárias de 21 fármacos anti-histamínicos H_1 em *Schistosoma mansoni*. Em conjunto, os resultados revelaram o potencial *in vitro* e *in vivo* de quatro anti-histamínicos (desloratadina, rupatadina, prometazina e a cinarizina) como agentes antiparasitários. Ante o exposto, diversos desdobramentos podem ser estudados após os dados preditivos deste trabalho, os quais serão descritos sucintamente adiante. Nessa conjuntura, pelo menos dois aspectos merecem elucidação: mecanismo de ação e a relação entre estrutura química e atividade biológica.

Sabe-se que receptores H_1 foram descritos em platelmintos, incluindo os esquistosomos[18]. Além disso, é sabido que a histamina foi descrita em esquistossomos, cuja importância ainda merece investigação[19]. Nessa conjuntura, o mecanismo exato pelo qual a desloratadina, rupatadina, cinarizina e prometazina exercem seu efeito anti-helmíntico em *S. mansoni*, enquanto os demais fármacos que agem no mesmo receptor foram inativos, ainda não está claro. Análises fenotípicas *in vitro* revelam alterações na motilidade de *S. mansoni* (hiperatividade) quando em presença de prometazina e cinarizina, dois fármacos fenotiazínicos. No caso da prometazina, o comportamento fenotípico dos helmintos foi semelhante ao observado com atropina[15], sugerindo um efeito nos receptores colinérgicos do verme. Corroborando essa pressuposição, a alteração na motilidade do verme é revertida quando da adição de acetilcolina às culturas de vermes previamente expostos à prometazina[15]. Outrossim, considerando que cinarizina também é um bloqueador dos canais de cálcio, a possibilidade de ação nos canais de cálcio do helminto não pode ser excluída. Faz-se necessário, por conseguinte, mais estudos para elucidar o mecanismo de ação dos anti-histamínicos H_1 nos esquistossomos. Ademais, à luz da ação antiparasitária observada com prometazina e cinarizina, o efeito de outros fenotiazínicos em *S. mansoni* merece investigação.

O processo de desenvolvimento de um fármaco usualmente se inicia com a identificação de um composto bioativo (do ingles *hit*), o qual deverá ser otimizado a fim de que se consiga a chegar uma avaliação clínica. A otimização dessa molécula *hit* começa com a determinação do grupo farmacofórico e, subsequentemente,

iniciam-se as modificações nas moléculas. Nesse ensejo, insta sobrelevar que rupatadina e desloratadina, ambos com ação antiparasitária em *S. mansoni*, apresentam similaridade estrutural. Nesse cenário, torna-se valiosa a síntese de análogos estrurais e posterior bioensaio em *S. mansoni*.

Finalmente, considerando que as doenças causadas por helmintos têm elevada prevalência global, em humanos e animais, cujo controle e tratamento depende apenas do praziquantel, é imperiosa a busca por novos fármacos. Ademais, insta ressair que as helmintíases representam um avultado entrave no desenvolvimento social e econômico e, como consequência, torna-se cada vez mais importante o investimento em pesquisa no âmbito do desenvolvimento de fármacos anti-helmínticos. Em suma, o presente estudo não somente conclui um problema de pesquisa em consonância com os objetivos propostos, como também aponta para futuras necessidades de investigação sobre o tema. Pelo presente concebemos dados inéditos sobre fármacos anti-histamínicos H_1 com atividade antiparasitária em verme platelminto de importância médica e veterinária.

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8. ANEXOS

Anexo I - Certificado de aprovação do Comitê de Ética

UNG UNIVERSIDADE ser
Comissão de Ética no Uso de Animais
CEUA

Guarulhos, 20 de Março de 2017.

Exmo. Sr.
Josué de Moraes

Referência: Aprovação de Pesquisa

Certificamos que a pesquisa intitulada "Avaliação da atividade anti-helmíntica de fármacos e compostos de origem natural", registrada com protocolo nº 031/17, sob responsabilidade de Josué de Moraes – que envolve utilização de animais pertencentes ao filo Chordata, para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela Comissão de Ética no uso de animais (CEUA) da Universidade Guarulhos – UnG, em 20 de Março de 2017.

Finalidade	() Ensino (X) Pesquisa Científica
Vigência da autorização	20/03/2017 à 01/02/2020
Espécie/Linhagem	Camundongo
Nº de animais	200
Peso/Idade	20 gramas / 21 dias
Sexo	Fêmea
Origem	Biotério

Prof. Dr. Gustavo Aparecido dos Santos
Comissão De Ética No Uso De Animais da
Universidade Guarulhos - CEUA
Vice-Coordenador

Anexo II - Parecer dos Revisores

PARV-D-20-00189
H1-Antihistamines as antischistosomal drugs: *in vitro* and *in vivo* studies

March 31, 2020.

Dr. Hesham M. Al-Mekhlafi
Editor
Parasites & Vector

Dear Dr. Al-Mekhlafi

We appreciate your time and effort in reviewing this paper and greatly value your assistance as an editor. We also thank the reviewers for the helpful comments and suggestions, which greatly improved the manuscript. The authors have taken into account all the reviewers' comments and criticism.

We are now sending a revised version of the manuscript, which now incorporates all the suggestions made by the reviewers. Please note that the specific points/questions raised have also been considered and are in "Response to Reviewers" file.

Regarding text similarity checked by iThenticate software, we thank the editor for calling attention to this point. We carefully observed the sections with similarity and now we have modified them. Regarding the hint about 7% for promethazine, the study that describes the anti-parasitic effect of promethazine against schistosomes belongs to our group. The similarity was high only in the method section. We have now modified the method text.

We hope that the manuscript in the present form will be accepted for publication in *Parasites & Vectors.*

Sincerely,

Dr. Josué de Moraes
Professor and Head of Department
Research Center for Neglected Diseases
Guarulhos University, SP, Brazil

= Response to Reviewers =

First of all, we thank the reviewers for the helpful comments and suggestions, which greatly improved the manuscript. The authors have taken into account all the reviewers' comments and criticism.

We are now sending a revised version of the manuscript, which incorporates the suggestions made by the reviewers. Please note that the specific points/questions raised have also been considered and are commented on below. We hope that the manuscript in the present form will be accepted for publication.

Reviewer #1 comments

> Xavier et al describe the testing of H1-Antihistamines against Schistosoma mansoni. The study is well conducted and the paper was improved. The study is solid but not novel, as there are many papers which evaluated these compounds before. I have reviewed the paper before for AAC and my comments raised have been mostly included. A few additional comments are summarized below.

We appreciate your time and effort in reviewing this paper and greatly value your assistance.

> Introduction
> Line 85-90: beyond the scope of this paper, please delete. The introduction is already very long.
> Line 91-98: details on histamine do not really fit here, as out of context in a discussion on schisto

We thank the reviewer for calling attention to these points. As suggested, we have deleted these sentences.

> Materials and methods
> "The structures of all tested H1-antihistamines are shown in Figure 1".They are not shown, Figure 1 presents in vitro results
> Line 120 onwards: starting concentration is high (50 uM)—is there a rationale for this (e.g. in vivo plasma levels)?

We appreciated these observations from the reviewer. As requested, we have corrected the mistake in Fig. 1; the correct one is Table S1. Regarding *in vitro* concentration at 50 uM, this is similar to many laboratories during *in vitro* screening with adult schistosomes (for example Professor J. Keizer's laboratory uses 33 µM). On the other hand, we agree with the reviewer that concentrations in plasma are much lower, especially with antihistamine drugs. Please, note that Reviewer #2 also mentioned that it is essential to put these data in proper context. Even though these antihistamine drugs are quite safe, they are highly unlikely to support clinical use for schistosomiasis. Thus, as suggested by Reviewer #2, this information was included in the revised version of the manuscript (please see lines 382-391).

> Results
> Line 219: line 219-220 please delete, this is obvious
> Line 259-266: part of the material and methods and should be deleted here
> Discussion
> Line 323:326: no need to repeat what was done in this study, please delete

Line 333: in particular?

We are grateful for these valuable comments and corrections. As suggested, the sentences have been deleted.

Line 333-338: I agree that strain differences are playing a major role as it seems the study by Panic et al tested adult worms against promethazine and cinnarizine at even a high concentration of 33 uM and did not see an activity. The differing results observed among the differing studies could be discussed in more detail as such a difference is quite surprising. I am not aware of any compound showing such extensive strain difference.

We are grateful for these valuable comments. As requested, this information was discussed in greater detail and the study by Panic and colleagues has now been mentioned (Please see line 310-317).

Line 389-411: this part of the discussion is far fetched and beyond the scope of this work. None of these drugs will ever be used against schistosomiasis so this is not relevant. The number of figures should be reduced, some could be combined or some findings presented as table

We appreciated these observations made by Reviewer#1. As requested, this part of the discussion has been deleted. Regarding the number of figures, we thank the reviewer for the suggestions. However, considering that PV is an open access journal and without limits for figures, we consider it necessary (and more comprehensive) to keep the figures in the manuscript.

Reviewer #2 comments

I appreciate the high quality of the work presented in this manuscript. It is well-written and concise, and the data generation and analysis are excellent. The topic is important and the conclusions in general are well-supported by the data presented. However, a few concerns should be addressed.

We are grateful for these valuable comments and we appreciate your time and effort in reviewing this paper.

1. Line 225: this is technically diffusion, not transport

We thank the reviewer for calling attention to this point. As suggested, this information has been corrected.

2. Although I appreciate the EM work, it is important to acknowledge that it is not possible to distinguish caustive from consequent action with regard to tegument damage; the drugs may induce it as part of their mechanism of action, or it may be a consequence of parasite death from another mechanism. Does the damage resemble that caused by PZQ? Many papers have been published showing tegumental damage from exposure to xenobiotics; I am not sure what we have learned from these results.

We appreciated these observations made by reviewer. We agree that it is not possible to distinguish causative from consequent action with regard to tegument damage. This information has now been added in the discussion section (please see lines 328-331).

3. It is essential to put these data in proper context. Choosing desloratidine as an example, a typical dose is a single 5 mg tablet. Even allowing for PK differences between mice and humans, that is much less than 100 mg/kg (let alone 400 mg/kg). Similarly, Cmax for this drug in humans is around 4 ng/ml, roughly 0.01 uM, far lower than the concentrations needed to kill schistosomes in culture. Even though these drugs are quite safe, that difference is highly unlikely to support clinical use for schistosomiasis. This should be stressed, as perhaps someone would be tempted to use antihistamines instead of PZQ based on this paper. It is important to advocate finding out more about the target of these drugs in schistosomes, as this could lead to a directed medicinal chemistry effort to identify schistosome-selective compounds. In that sense, were these drugs tested in the recombinant receptor system reported by Paula Ribeiro some years ago? If so, those data should be included.

We are grateful for these valuable comments and we completely agree with the reviewer. As suggested, this information was added in the revised version of the manuscript (please see line 382-391).

Reviewer #3 **comments**

The study has shown that among the 21 H1-antihistamines tested; desloratadine, rupatadine, promethazine, and cinnarizine kill Schistosoma mansoni adult worms in vitro at low concentrations (5-15 μM), with recorded scanning electron microscopy damages. Moreover, rupatadine and cinnarizine revealed high worm burden reductions in mice with early or chronic S. mansoni infection, besides marked inhibition in egg production, and a significant reduction in hepato- and splenomegaly. Additionally, desloratadine revealed moderate but significant activity in the adult and juvenile parasites. The paper is extremely interesting and the research is valuable. I really enjoyed reading the manuscript and would recommend it for publication. There do need for some revisions, as detailed below.

We are grateful for these valuable comments and we appreciate your time and effort in reviewing this paper.

-All parasite genus and species should be written in italic throughout the manuscript. Abstract: Line 28-29, 169-170 (Methods), 359-361 (Discussion): The authors stated that the drugs were emulsified in 2% ethanol and given at oral dosages of 400 mg/kg single dose or 100 mg/kg daily for five consecutive days based on the protocol recommended for experimental schistosomiasis (e.g. [13,48]). I wonder as the cited references are not related to the anti-schistosomal drugs assessed in the manuscript here, spironolactone and epiisopiloturine in the stated references vs. H1-antihistaminics in this study. Can the authors clarify why they did not use the same emulsifier (2% Cremophore oil) and the dose (200 mg/kg/day) of cinnarizine assessed on S. mansoni Egyptian strain according to the study of Sarhan et al. 2020 (Ref. 27), to mimic the daily prescribed doses for similar drug.

We thank the reviewer for calling attention to these points. As suggested, all gender names have been italicized. Regarding the solvent, we use ethanol because it is often used in animal studies and the drug is easily solubilized.

Line 39: and a significant reduction in and hepato- and splenomegaly, delete and.
Introduction: Line 101: and druggability. What do the authors mean? Please specify.

We thank the reviewer for calling attention to these points. As suggested, we have deleted the word “and”. Regarding druggability, this is a term used in drug discovery to describe a

biological target. To facilitate the reader's understanding, in the revised version we write " potential as biological target " instead of " druggability" (please see line 88).

Methods:
Line 132: The authors stated that they collected cercariae from infected snails after exposure to light for 3 hours. Isn't this period too long that can kill large number of cercariae or at least reduce their infectivity, as the average time for exposure is ½-1 hour. Can the authors specify the lamp watt they used?

For *S. mansoni* BH strain the time for exposure of snail *B. glabrata* is 2-3 hours. In other laboratories, the time of 3 hours is also used. Perhaps this depends on the both the snail and parasite strain as well as the lamp used.

Line 142: Replace arranged by incubated.
Line 157: were published previously, better to be written as were previously published.
Results:
Line 294: dead worms, should be corrected as eggs.
The authors didn't show the mortality rates among mice. Does this mean that all mice survived till the end of the experimental work. Can they show that.
Discussion:
Line 386: manifest, it is better to be replaced by possess or have.
References:
Should be written in the same style, e.g. Ref. 24 the authors names are in italic and not bold, ref. 26 and 27 the journals name are written in full.

We thank the reviewer for calling attention to these points. As suggested, the sentences have been corrected.

Figures:
Fig. 3: The authors should write the magnification power or scale bar for better comparison.
Fig.4: It is more better if the authors split data for male and female worm burdens rather than having one figure showing total worm burdens, to better detect if any of the assessed drugs has a sex-dependent effect, and subsequently they have to comment on that in the results and discussion, in comparison with the other anti-schistosomal drugs.

We appreciated these observations made by the reviewer. In the figure caption, the scale bars for all images have been added. Regarding split data for male and female worm burdens, the figure is very large and it is not possible to open more columns for each drug and dose used (according to guidelines for figure). On the other hand, the information will not be compromised because there is presence of both male and female parasites, confirmed by the oviposition data.