Schistosomiasis: chemoprophylaxis and treatments

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Schistosomiasis is a chronic parasitic disease affecting about 207 million individuals worldwide. It is still a major health problem in many tropical and subtropical countries, as well as for travelers from developed countries. The treatment strategies of schistosomiasis can be divided into two main routes: (a) chemotherapy treatment including trivalent antimony compounds, hycanthone mesylate, niridazole, metrifonate, oxamniquine, oltipraz, artemisinins, albendazole, amoscanate mirazid, and praziquantel; (b) vaccines that may play an important role in the control of schistosomiasis in the future.

Keywords:
chemotherapy, praziquantel, schistosomiasis, vaccines

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Introduction

Schistosomiasis (formerly called bilharziasis or bilharziosis) is a parasitic disease produced by a flatworm of the class Trematodo, relatively common in the developing countries, especially in Africa, called schistosoma (or esquistosoma). More than 207 million individuals are infected worldwide, and in addition, 700 million are at the risk of infection [1]. Around 90% of the individuals who need preventive chemotherapy for schistosomiasis are in Africa, where the disease is endemic in 42 countries [1,2].

The main forms of human schistosomiasis are caused by five species of the flatworm, or blood flukes, known as schistosomes. These are *Schistosoma mansoni*, which causes intestinal schistosomiasis and is prevalent in 52 countries and territories of Africa, Caribbean, the Eastern Mediterranean, and South America; *Schistosoma japonicum*/*Schistosoma mekongi*, which causes intestinal schistosomiasis in seven African countries and the Pacific region; and *Schistosoma haematobium*, which causes urinary schistosomiasis in 54 countries in Africa and the Eastern Mediterranean [3].

Mode of infection

The infection is acquired through contact with contaminated water with the infective cercariae. Once cercariae have penetrated the human skin, the parasites develop into an adult worm within, on average, 63–65 days, and the worms usually migrate to the blood vessels, draining the bladder, where they reside and produce large numbers of eggs. On average, adult worm pairs live for 3–5 years, but some can live up to 30 years, with the reproduction potential of one schistosome pair estimated to be up to 600 billion schistosomes. The eggs of *Schistosoma* spp. have a terminal spine and must traverse the bladder tissues toward the lumen of the bladder and the urinary tract for elimination through urine. In the process, a considerable number become trapped in the bladder walls and surrounding tissues to initiate immune-induced inflammatory reactions, which subsequently lead to morbidity. It is important to note that eggs trapped in the tissues cause disease rather than the worms themselves [3–5].

Life cycle

Schistosomes have a typical trematode vertebrate–invertebrate life cycle, with humans being the definitive host (Fig. 1).

Signs and symptoms

Schistosomiasis is a chronic disease and many infections are subclinically symptomatic, with mild anemia and malnutrition being common in endemic areas. Acute schistosomiasis (Katayama's fever) may occur weeks after the initial infection, especially by *S. mansoni* and *S. japonicum*. Manifestations include the following: abdominal pain; cough; diarrhea; fever; fatigue; eosinophilia, that is, an extremely high eosinophil granulocyte (white blood cell) count; and hepatosplenomegaly (enlargement of both the liver and the spleen). In addition, there may be genital sores, lesions that increase to the risk of HIV infection [7,8], and skin symptoms such as mild itching and papular dermatitis of the feet [9].

Diagnosis

Microscopic identification of eggs in the stool or the urine is the traditional method of diagnosis. Stool examination should be performed when infection with *S. mansoni* or *S. japonicum* is suspected, and urine examination should be performed if *S. haematobium* is suspected [10–12]. The

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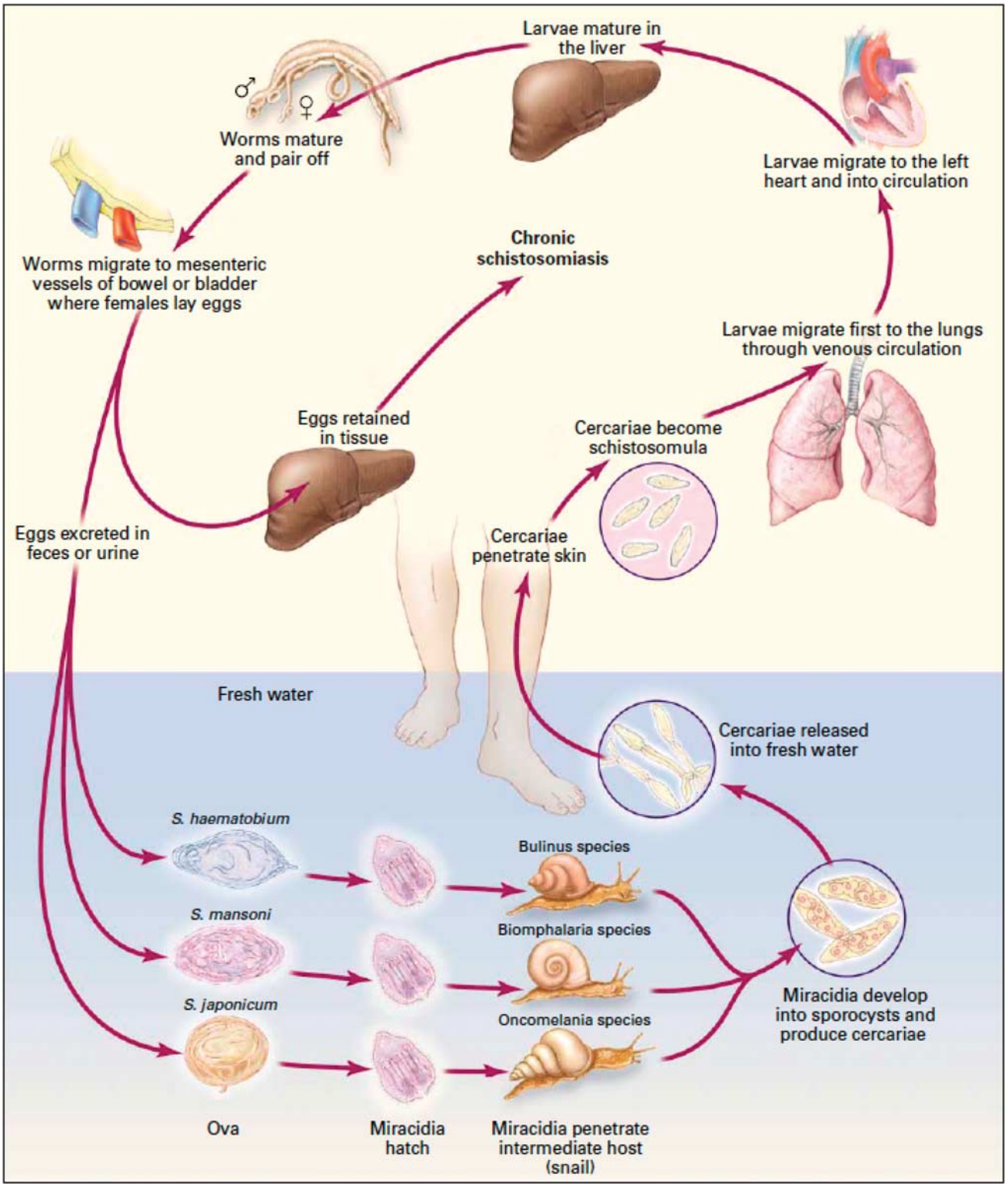
Figure 1



The life cycle of schistosomes [6].

enzyme-linked immunosorbent assay (ELISA) is a serological test useful for epidemiological studies, because of its high sensitivity for the diagnosis of schistosomiasis. ELISA had a sensitivity of 92.2 and 100% in *S. mansoni* and mixed infection, respectively [13]. However, to date, the utilization of ELISA for the diagnosis of schistosomiasis has not led to the differentiation of an ongoing and a previous infection [14]. In addition, some antigens used in ELISA present a cross-reaction with antigens from other helminths such as ancylostomides and *Ascaris lumbricoides* [15]. An optimal selection of antigens for use in the serological diagnosis of schistosomiasis may overcome this limitation with the use of ELISA.

Control of infection

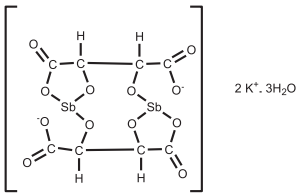
For control of infection, the priorities are health education [16]; supply of drinking water and planning of adequate healthcare facilities; diagnosis and treatment; and management of the environment and control of the intermediate hosts (freshwater snails) [2,17].

Schistosomiasis treatment strategies:

(1) Chemotherapy treatment
(2) Vaccines

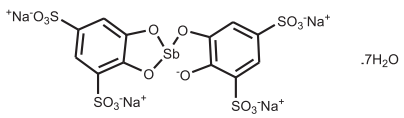
Chemotherapy treatment

Trivalent antimony compounds such as antimony potassium tartrate and antimony sodium oxide L (+) tartrate have been used in the treatment of schistosomiasis since 1972 [18], but have now been superseded [19].



Antimony potassium tartrate

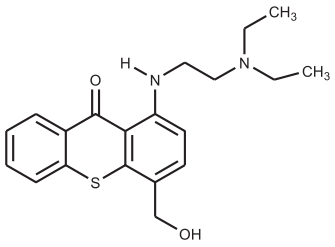
Dipotassium bis[u[2,3-dihydroxybutanediato(4-)-01,02:03,04]]-diantimonate (2-) trihydrate stereoisomer



Antimony sodium oxide L (+) tartrate

Bis[4,5-dihydroxybenzene-1,3-disulphonato(4-)-O^4,O^5]antimonite (5-) pentasodium heptahydrate

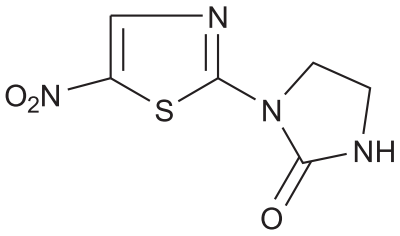
Hycanthone mesylate has been used as a schistosomicide in the individual or the mass treatment of infection with *S. haematobium* and *S. mansoni*. Owing to its toxicity, carcinogenicity, mutagenicity, and teratogenicity, hycanthone has been replaced by other drugs [20].



Hycanthone mesilate

1-[(2-(Diethylamino)ethyl)amino]-4-(hydroxymethyl)-9H-thioxanthen-9-one

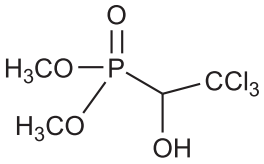
Niridazole was developed in 1960 and was the most widely used medication until 1985. It was particularly active against *S. haematobium*, less active against *S. mansoni*, and almost inactive against *S. japonicum.* Moreover, it was effective against *Entamoeba histolytica*, but had numerous, reversible side effects such as neuropsychiatric disorders, psychoses, epileptic insults, and clinically silent ECG disorders. It was relatively expensive and is no longer commercially available [21,22].



Niridazole (Ambilhar®)

1-(5-Nitrothiazol-2-yl)imidazolidin-2-one

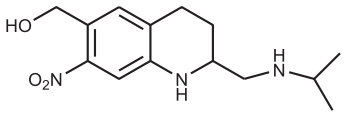
Metrifonate or trichlorfon was an organophosphorus derivative of the same group as some insecticides used in agriculture (anticholinesterase activity). No evidence could be found of a harmful cumulative effect of the drug. It was active only against urinary bilharziasis, including the occasional localizations of *S. mansoni* in the bladder wall, but surprisingly, not against *S. haematobium* and *S. mansoni* when they are localized in the intestinal wall. Metrifonate has a narrower spectrum than praziquantel (PZQ). The drug is very effective against hookworms. However, it was withdrawn from the market in 1998 [23–25].



Metrifonate (Bilarcil®)

Dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate

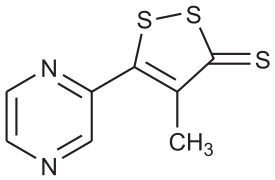
Oxamniquine is effective against *S. mansoni*. It is rapidly absorbed from the intestine and has a plasma half-life of approximately 2 h. The drug is weakly toxic. Epileptic insults have been reported in patients with a history of cerebral lesions and drowsiness in children, which can progress to subcomatose states. Some of the side effects are undoubtedly the result of the death of adult worms, including in the lungs, with the formation of temporary infiltrates and respiratory symptoms. It is used extensively in Latin America (Brazil), but is less indicated in Africa, where *S. mansoni* is associated in many places with *S. haematobium*. It may be used if there is an inadequate response to praziquantel [26–28].



Oxamniquine (Vansil®)

1,2,3,4-Tetrahydro-2-((isopropylamino)methyl)-7-nitroquinolin-6-yl)methanol

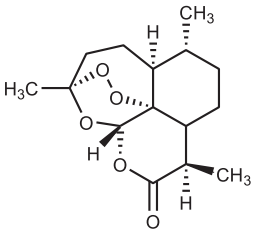
Oltipraz has been registered for the treatment of schistosomiasis. This medication induces glutathione *S*-transferase (GST), which plays an important role in the detoxification of carcinogens. It is now being studied as a potential chemoprotective agent [29,30].



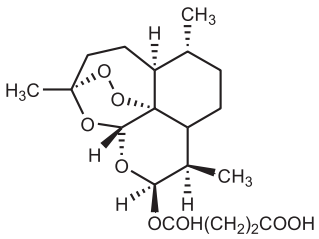
Oltipraz

4-Methyl-5-(pyrazin-2-yl)-3*H*-1,2-dithiole-3-thione

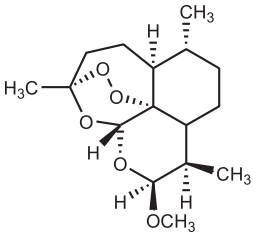
The antischistosomal activity of artemisinins, such as artemisinin, artesunate, and artemether, was discovered in the early 1980s [31,32]. Artemisinins are active against the liver stage (immature) worms, whereas the invasive stages and adult worms are less susceptible to the drugs. Adverse effects are minor and last for less than 24 h. Artemisinin monotherapy may not be beneficial because of stage-specific activity, but combination with existing drugs effective against other stages (e.g. praziquantel) may improve the therapeutic efficacy.



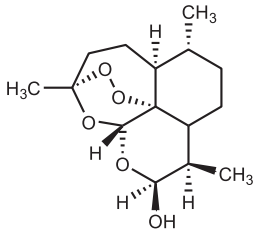
Artemisinin



Artesunate



Artemether

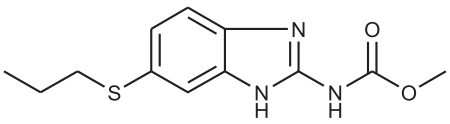


Dihydroartemisinin

Artemisinins

Recently, it has been reported that artemether, artesunate, and dihydroartemisinin can be used to control schistosomiasis japonica as a strategy to prevent *S. japonicum* infection. Their administration at multiple doses or in combined treatment damages both juvenile and adult *S. japonicu*m. On treatment against juvenile *S. japonicum*, the total worm and female worm burdens were reduced by 79.5 – 86, and 79.4 – 86.7%, whereas on treatment against adult *S. japonicum*, they were reduced by 73.8–75.8% and 88.7–93.1% respectively, without statistically significant differences among the three drugs at the same dose [33].

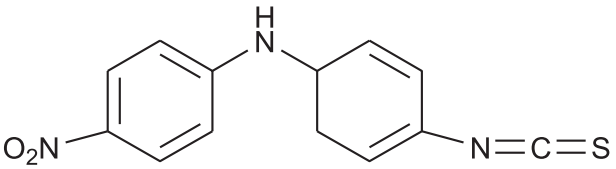
Albendazole is indicated for the treatment of a variety of worm infestations. In recent years, it has often been coadministered with praziquantel for the control of schistosomiasis and soil-transmitted helminthiasis. Several adverse effects including gastrointestinal upsets , headaches, and dizziness have been reported [34,35].



Albendazole

Methyl [6-(propylthio)-1H-benzoimidazol-2-yl]carbamate

Amoscanate is a broad-spectrum anthelmintic drug that shows activity against all major human schistosome parasites, other systemic parasites (e.g. filariae), and gastrointestinal nematodes (e.g. hookworms). Toxicity in experimental animals was quite low, and mutagenicity tests in bacteria yielded negative results; however, mutagenic metabolites were detected in the urine of mammals [36–39].



Amoscanate

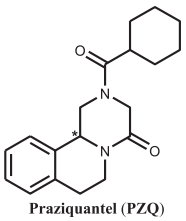
4-Isothiocyanato-N-(4-nitrophenyl)aniline

Mirazid

Mirazid contains an extract of oleo gum resin from the stem of the myrrh tree, *Commiphora molmol* (syn. *Commiphora myrrha*). An alcoholic extract of this plant, followed by steam distillation produces resin and volatile oil. It is licensed and marketed for clinical use against *Fasciola hepatica* and schistosome infections in Egypt. Its exact role in therapy is not yet clear and, at present, its clinical activity is controversial. Myrrh extracts also exert a molluscicidal effect on the snail intermediate hosts, particularly on their eggs [40–43].

Praziquantel

At present, praziquantel is the drug of choice for the treatment of all forms of schistosomiasis.



Praziquantel (PZQ)

(*RS*) 2-(Cyclohexylcarbonyl)-2,3,6,7,11b-tetrahydro-1*H*-pyrazino-[2,1-a]isoquinolin-4(11b*H*)-one

It is a white to nearly white crystalline powder with a bitter taste, which melts at 136–140°C with decomposition. It is stable under normal conditions and it is almost insoluble in water, sparingly soluble in ethanol, and soluble in organic solvents such as chloroform and dimethylsulfoxide. Praziquantel has an asymmetric center in position 11b (*). The commercial preparation is a racemate composed of equal parts of 'levo' *R* (–) and 'dextro' *S* (+) isomers. Only the (–) enantiomer has antischistosomal activity, as shown by in-vivo and in-vitro experiments. The two isomers, however, have almost the same toxicity; however, patients treated with 20 mg/kg (–) praziquantel showed the same cure rate but fewer side effects than patients treated with 40 mg/kg of the racemic preparation [44–47].

Dose and administration

The recommended dose is 40–60 mg/kg body weight, the lower amount being generally used for *S. mansoni* and *S. haematobium*, whereas the higher dose (generally divided into two administrations a few hours apart) is especially recommended for Asian schistosomes (*S. japonicum* and *S. mekongi*) [48].

Pharmacokinetics and clearance

Orally administered praziquantel is rapidly absorbed, measurable amounts appearing in the blood as early as 15 min after dosing [49] and peak levels occurring after 1–2 h in normal individuals [50]. The maximum plasma concentration after a standard dose of 40 mg/kg shows wide interindividual variations in the range of 200–2000 mg/ml [51]. Praziquantel undergoes a pronounced liver first-pass metabolism, with rapid disappearance from the circulation and a plasma half-life generally ranging between 1 and 3 h. Elimination occurs essentially through the urine and the feces and it is more than 80% complete after 24 h [52].

Mode of action

The detailed molecular mechanism of the action of praziquantel has not yet been elucidated [24,53], but a few phenomena linked to its effects are well known. The most obvious and immediate modification that can be observed in schistosomes exposed to the drug either *in vitro* or *in vivo* is a spastic paralysis of the worm musculature. This contraction is accompanied and probably caused by a rapid Ca^{2+} influx inside the schistosome [54]. Another early effect of praziquantel is morphological alterations that can be observed in the worm tegument, initially represented by vacuolization at the base of the tegumental syncytium and blebbing at the surface [55,56]. These morphological alterations are accompanied by an increased exposure of schistosome antigens at the parasite surface [57].

Side effects

After the administration of praziquantel, side effects are observed in a relatively large percentage of patients (30–60%), but these are usually mild and transient, disappearing within 24 h. The most commonly reported effects are headache, nausea, anorexia, vomiting, abdominal pain and epigastric pain, diarrhea with or without blood and/or mucus, lassitude, fever, myalgia, dizziness, sleeplessness, sleepiness, and more rarely a skin rash with edema [58,59].

Synthesis of praziquantel

In Scheme 1, imine **4** was prepared from *N*-phthaloylglycine chloride **2** and phenylethylamine **1** through the Bischler–Napieralski cyclization of the intermediate amide **3**. Subsequent asymmetric transfer hydrogenation of imine **4** was performed using (*R*,*R*) **8** as a catalyst (Fig. 2) in the presence of formic acid and triethylamine mixture to produce **5**. Subsequent treatment of **5** with hydrazine in refluxing ethanol yielded diamine **6**. Cyclohexanoyl derivative **7** was obtained by the reaction of **6** with cyclohexanecarbonyl chloride in dichloromethane and in the presence of potassium carbonate. Compound **7** was converted into enantiopure praziquantel with chloroacetyl chloride under Schotten–Baumman conditions using a mixture of dichloromethane and 50% (aq) NaOH in the presence of triethyl benzyl ammonium chloride [60].

Praziquantel drug combination

Combinations of praziquantel with different drugs have been used to improve its therapeutic activity. Such a combination with artemisinins or metrifonate and/or albendazole is effective against immature and adult worms and prevents infection in mouse models [12].

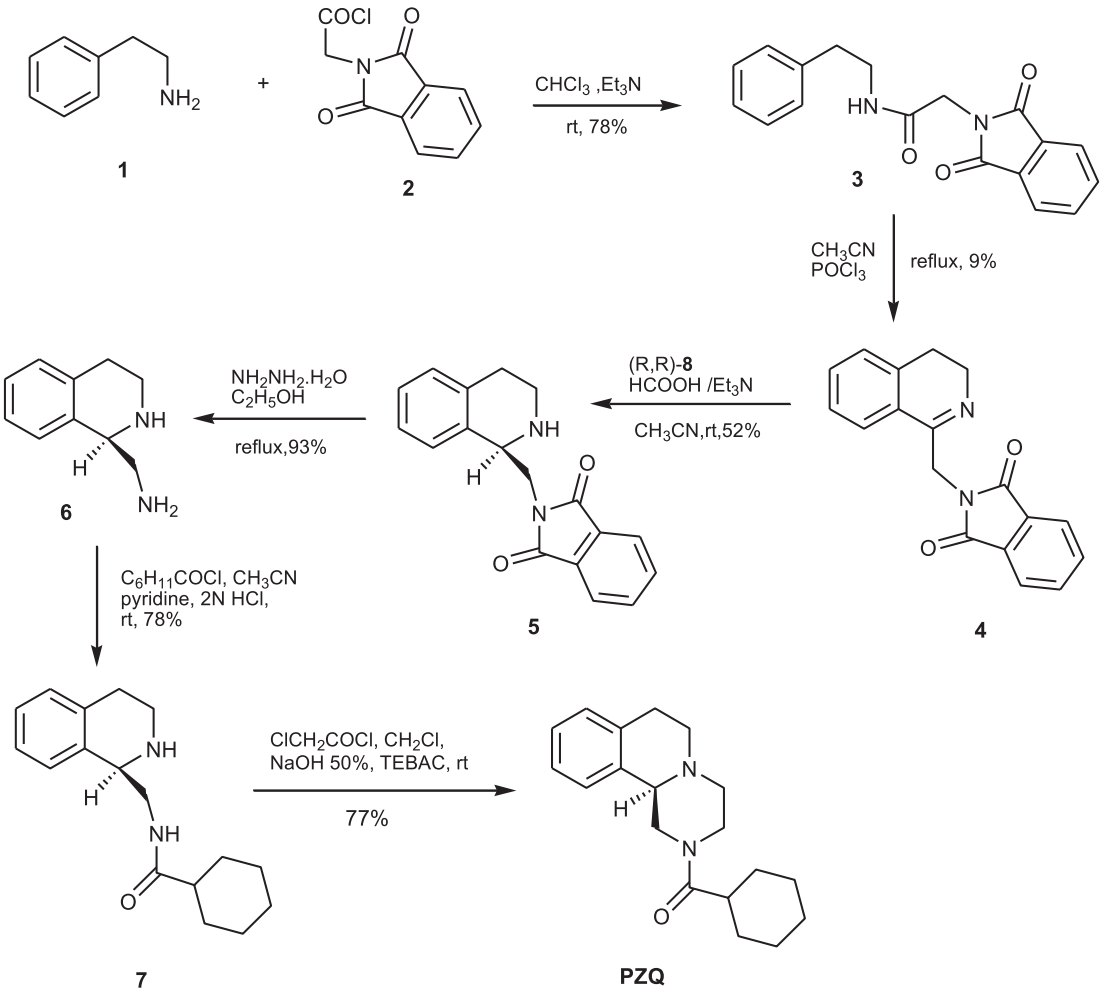
Praziquantel derivatives

Many modifications of the chemical structure of praziquantel were performed in trials to enhance its activity.

The praziquantel ketone derivative **9** showed the best combination of activity against juvenile and adults stages of *S. mansoni* in infected mice, but it exerted no effect on the motility of adult *S. mansoni* in an ex-vivo culture. The effects of single 400 mg/kg oral doses of **9** administered to mice harboring 21-day-old juvenile and 49-day-old adult *S. mansoni* infections were 25 and 79%, respectively [61].

Several analogues of the potent anthelmintic praziquantel **10–13** were prepared with variations in the aromatic ring (Scheme 2). These analogues showed low levels of activity to paralyze and kill schistosomes Table 1 [62].

Scheme 1



Synthesis of praziquantel.

Another approach to a structure–antischistosomal activity relationship with possible pharmacological potentiation of the anthelmintic drug praziquantel was used. A new

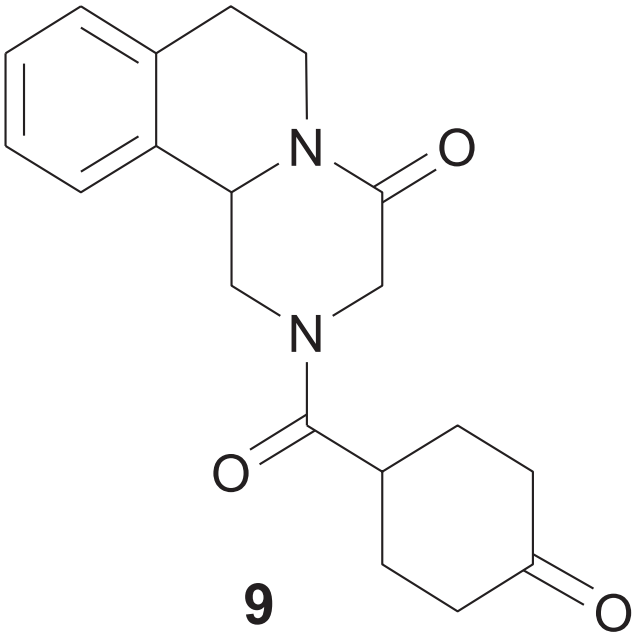
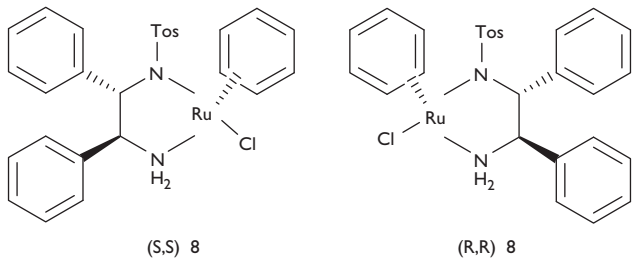


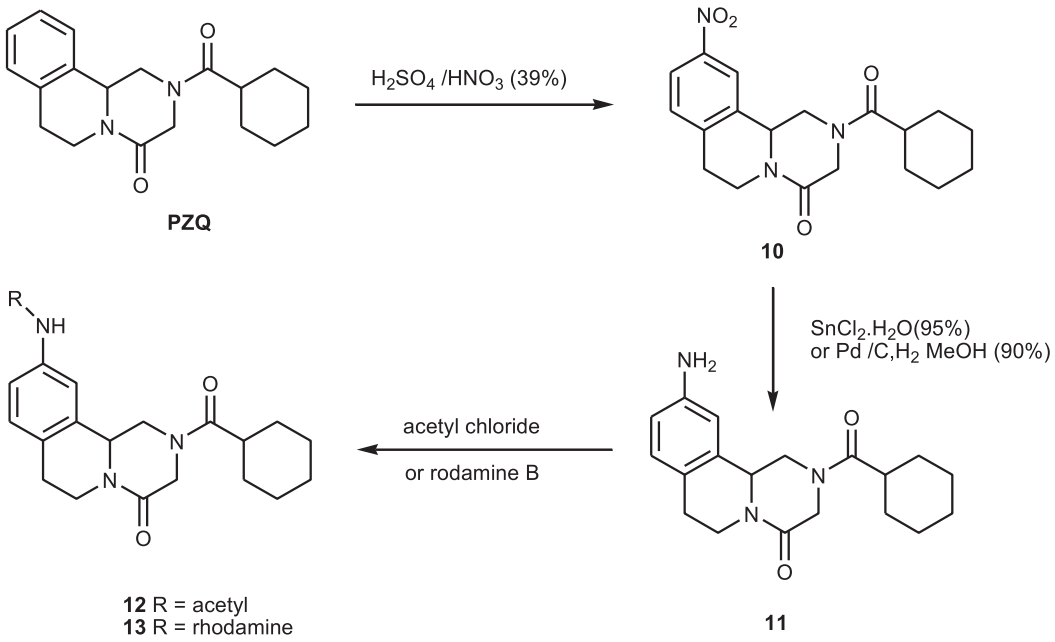
Figure 2



Chiral ruthenium catalysts.

dipeptide analogue [63], namely, *N*-α-nicotinoyl-L-aspartyl-β-(1,2,3,6,7,11b-hexahydro-4*H*-pyrazino[2–1*a*]isoquinoline-4-one)-L-phenylalanine methyl ester (**15**) was synthesized and antischistosomally examined in mice infected with *S. mansoni* cercariae. In parallel, its simple 2-nicotinoyl analogue **16** was synthesized (Scheme 3) and tested. Compounds **15** and **16** were less, but, interestingly, still active compared with praziquantel (approximately 62, 66, and 90%, respectively).

Scheme 2



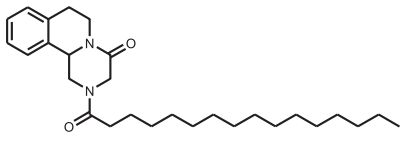
Synthesis of praziquantel derivatives with variation in the aromatic ring.

Table 1 Biological potency against *Schistosoma mansoni* for analogues *10–13* vs. praziquantel

Compounds	Killing[a] (μmol/l)	Relative potency	Paralysis[b] (μmol/l)	Relative paralysis potency
PZQ	3	100	1	100
10	>300	<1	>300	<0.3
11	28	10.7	10	10.0
12	>300	<1	>300	<0.3
13	>80	<3.7	>80	<1.2

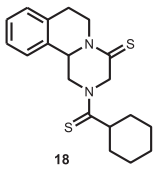
PZQ, praziquantel.
[a]Minimum concentration for 100% killing.
[b]Minimum concentration for complete spastic paralysis.

Several praziquantel derivatives [64–66] such as the highly lipophilic **17**, dithione **18**, and monothione **19** showed 70, 70, and 76%, respectively, at 500 mg/kg mouse body weight of praziquantel using mice infected with *S. mansoni* cercariae. In particular, the total serum and liver proteins, liver enzymes, serum total lipids, cholesterol, triglycerides, albumin, globulin, and creatinine were assayed. The induced amino acid profile of liver protein hydrolysate could indicate a close similarity to the biological mechanism for praziquantel.



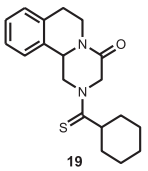
17

2-Palmitoyl[1,2,3,6,7,11b]hexahydro-4H-pyrazino[2-1a] isoquinoline-4-one



18

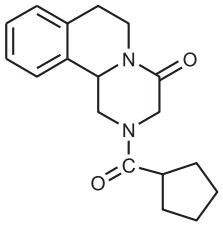
2-Cyclohexylthiocarbonyl(1,2,3,6,7,11b)hexahydro -4H-pyrazino[2-1a] isoquinoline-4-thione



19

2-cyclohexylthiocarbonyl (1,2,3,6,7,11b)-hexahydro -4H-pyrazino[2-1a]isoquinoline-4-one

The praziquantel analogues **20–23** were tested *in vitro* on cultures of adult *S. mansoni*. Compound **20** showed a good degree of activity (LC_{90} = 10 μmol/l), whereas praziquantel analogues **21**, **22**, and **23** showed moderate activity (LC_{90} = 25 μmol/l). The presence of p-trifluoromethylbenzoyl and p-toluenesulfonyl moieties instead of a cyclohexyl carbonyl moiety resulted in the complete suppression of antischistosomal activity [67].



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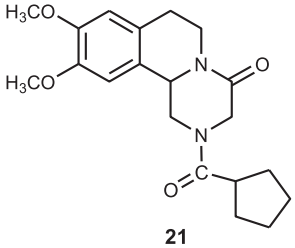

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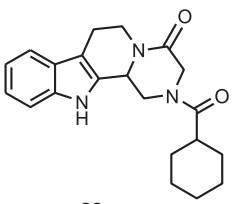

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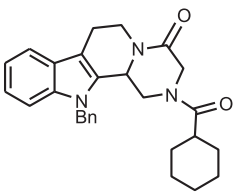

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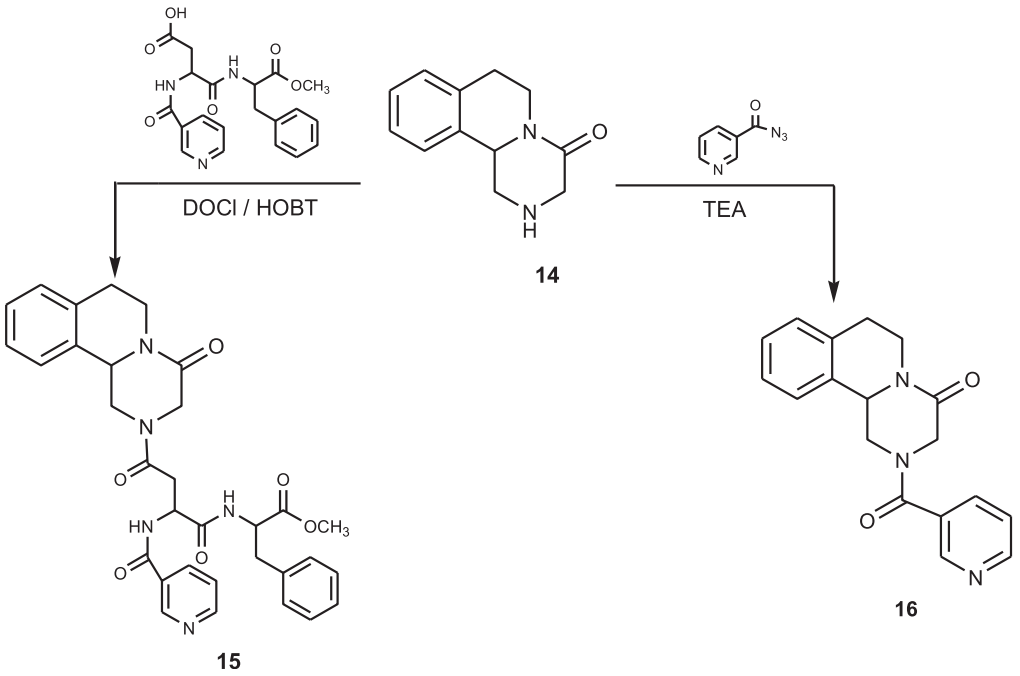
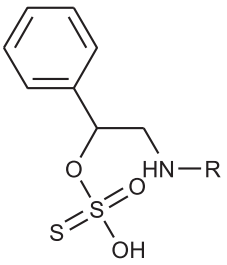
Scheme 3



Synthesis of dipeptide and 2-nicotinoy analogues of praziquantel.

Examples of synthesized candidates with schistosomicidal activity

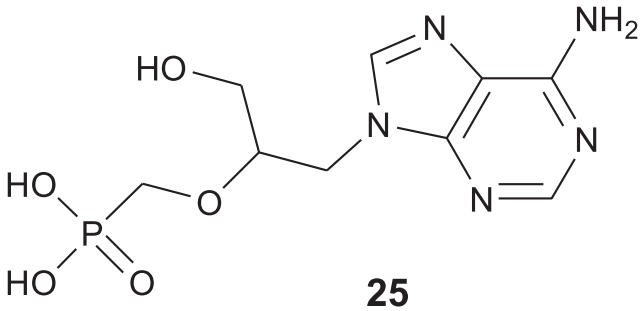
The schistosomicidal activities of seven 2-(alkylamino)-1-phenyl-1-ethanethiosulfuric acids **24a-g** were determined in female Swiss mice infected with *S. mansoni*. The compounds were administered in a single oral dose of 800 mg/kg of 15 mice infected with 50 cercariae. All compounds were found to be active and to have a high specificity against female worms (64–100% reduction vs. 33–61% reduction in male worms), [68].



24

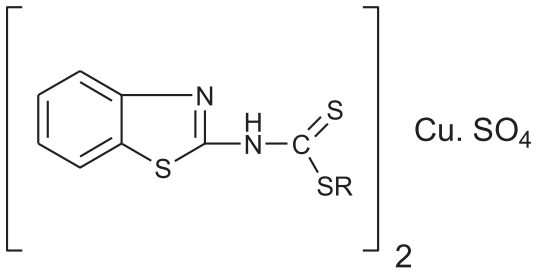
a, R = Propyl b, R = *i*-Propyl c, R = Butyl d, R = *i*-Butyl e, R = *t*-Butyl f, R = *s*-Butyl g, R = cyclohexyl

The activity of the acyclic nucleotide analogue 9-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine [(*S*)-HPMPA] (**25**) against *S. mansoni* was examined in mice. The compound was injected intraperitoneally, usually on 2 or 5 consecutive days, at 10–20 mg/kg of body weight/day. Significant reductions in the total numbers of female and coupled worms were found (30 and 40%, respectively). Female fecundity and both hepatic and intestinal egg loads were suppressed. These effects were more pronounced with dosing regimens initiated before the time of oviposition. The complete disappearance of immature eggs and a significant reduction to the complete absence of mature eggs, with 99–100% of the eggs dead, were achieved. No hepatic egg-induced granulomas were present in mice treated at the time of oviposition, and the granulomas were smaller in mice treated before *S. mansoni* oviposition. The activity of (*S*)-HPMPA against schistosomes was monitored over a reasonably wide range of the life cycle and mainly the effects on the schistosome eggs. The underlying mechanism(s) of the effects may be a direct assault on female worms, thus reducing their numbers or their ability to lay eggs, although a direct ovicidal action cannot be excluded [69].

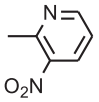


25

The copper complexes **26a-c** esters of benzothiazol-2-yl-dithiocarbamates were synthesized through the reaction of suitable alkyl, aralkyl, or heteroaryl halides with the sodium salt of benzothiazol-2-yl-dithiocarbamic acid, followed by complexation with copper sulfate. The respective copper complexes **26a-c** possessed the highest activity (100% mortality at 10 µg/ml) similar to praziquantel, with 100% worm mortality at 10 µg/ml [70].

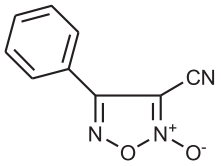


R a,= C_3H_7 b,= $CH_2C_6H_5$ c,=



26a-c

An oxadiazole-2-oxide class of compounds was identified recently as inhibitors of thioredoxin glutathione reductase (TGR), a selenocysteine-containing flavoenzyme required by the parasite to maintain proper cellular redox balance. Systematic evaluation of the core molecular structure of this chemotype has indicated a link between the donation of nitric oxide and inhibition of TGR, the selectivity for this chemotype versus related reductase enzymes, and indicates that these agents can be modified to develop appropriate drug metabolism and pharmacokinetic properties. These studies showed that donation of NO and parasite injury indicate the utility of oxadiazole-2-oxides **27**, **28** as novel inhibitors of TGR and as efficacious antischistosomal agents [71].



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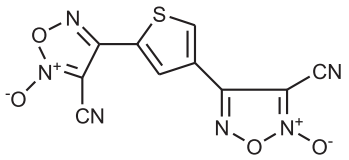

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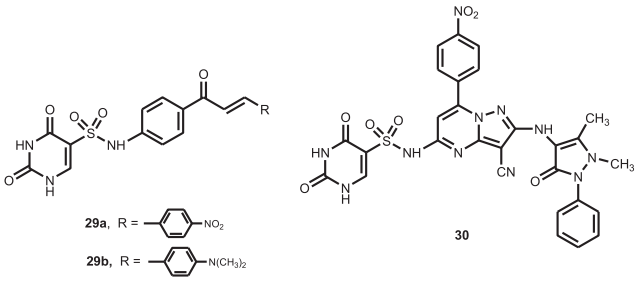
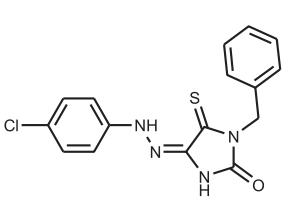
S. mansoni-infected mice were treated with hemolymph obtained from pretreated *Biomphalaria alexandrina* snails with N-p-substituted phenyl uracil-5-sulfonamide derivatives **29a,b** and **30** or obtained from nontreated snails. The results showed a reduction in worm burden (44–50%). Also, the hemolymph obtained from treated snails on *S. mansoni* adult worms showed a strong reaction with epitopes in the gut epithelium, tubercles, tegument, and subtegumental musculature of treated *S. mansoni* adult worm antigens. Therefore, treatment of hemolymph obtained from pretreated snails with compounds **29a,b** and **30** can stimulate a specific immune response and induce protective effects against *S. mansoni* infection [72].

Also, new imidazolidine compounds such as 1-benzyl-4-[(4-chloro-phenyl)-hydrazono]-5-thioxo-imidazolidin-2-one **31** and 1-(4-chloro-benzyl)-4-[(4-fluoro-phenyl)-hydrazono]-5-thioxo-imidazolidin-2-one **32** showed relevant schistosomicidal activity *in vitro* against adult worms of *S. mansoni* and induced significant ultrastructural alterations in worms and cell death similar to praziquantel. Thus, it is possible that these imidazolidine derivatives may be potential candidates as schistosomotic drugs in the future [73,74].





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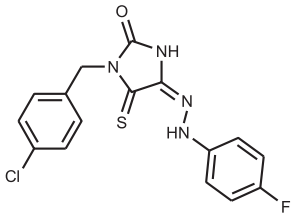
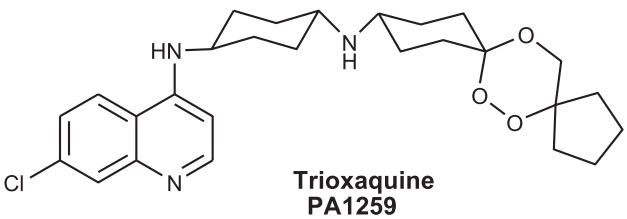
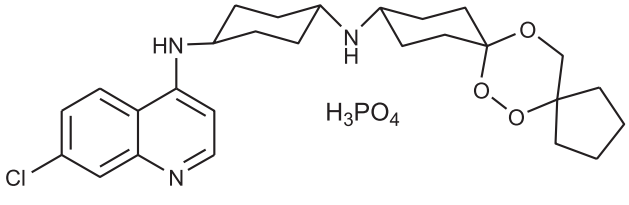


32

Trioxaquine PA1259 is a hybrid drug that has two pharmacophores within a single molecule: a trioxane and an aminoquinoline. The target of trioxaquines is the heme, which is a product resulting from the digestion of the hemoglobin. The first action of trioxaquine is alkylation of the heme with the trioxane entity and the second action is stacking with the heme because of the aminoquinoline moiety. It is most active against schistosomes among the trioxane-containing drugs that have been tested to date. Its phosphate salt, PA1647, acts in combination with praziquantel, especially against schistosomules, when infected mice are treated by oral administration [75].

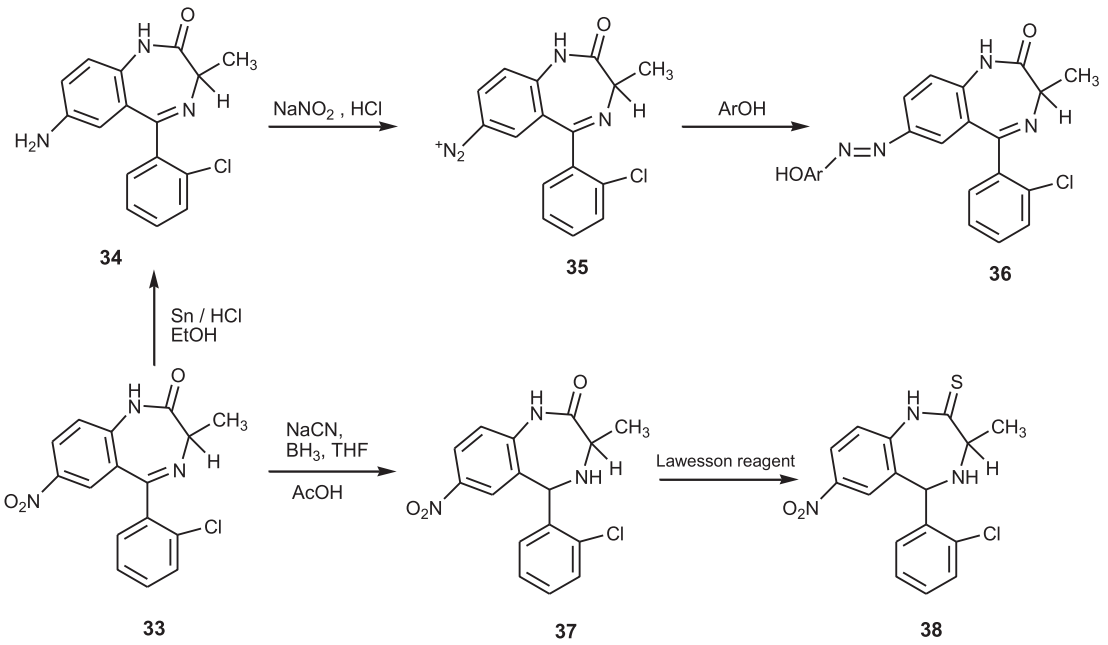


Trioxaquine PA1259



Trioxaquine PA1647

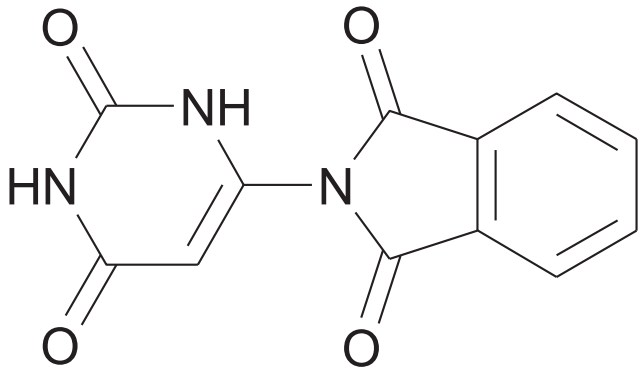
Scheme 4



Synthesis of 3-methylclonazepam derivatives.

New analogues of the potent anthelmintic meclonazepam **34–38** have been synthesized (Scheme 4) from 3-methylclonazepam **33** and evaluated against *S. mansoni*. The in-vitro biological data suggest that substitution at positions 2 and 4 of meclonazepam could provide promising analogues for prophylactic and therapeutic activity against *S. mansoni* at a concentration of 10 μg/ml [76].

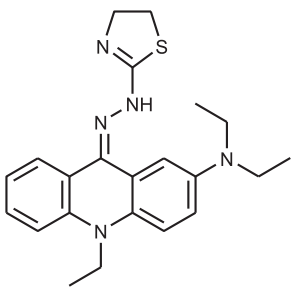
A novel series of substituted tetrahydropyrimidinediones has been synthesized and evaluated for their schistosomicidal activity both *in vitro* and *in vivo* using praziquantel as a therapeutic control. Most the tested compounds showed in-vitro schistosomicidal activity; however, only compound **39** showed in-vivo activity in Swiss strain albino mice as evidenced by a significant reduction in worm load, tissue egg count, liver granuloma number and size, and histopathological study of the liver. Moreover, electron microscopy scanning of adult *S. mansoni* obtained from animals treated with compound **39** showed tegumental changes. These data indicate that 2-(2,6-dioxo-1,2,3,6- tetrahydropyrimidin-4-yl)isoindoline-1,3-dione **39** may be a promising new antischistosomal agent [77].



39

A series of coumarin derivatives were synthesized from 8-acetyl-4-methyl-7-hydroxycoumarin **40** and were studied for their chemoprophylactic effect on *S. mansoni*-infected mice (Scheme 5). It was found that the mean worm burden decreased significantly in mice pretreated with the tested compounds **40**, **41**, **43,** and **46** compared with nontreated infected mice. Also, compound **46** showed greater mortality of female than male worms (relative sex ratio). However, the total tissue egg counts (liver and intestine) showed a significant reduction in the pretreated infected mice compared with the control infected mice [78,79].

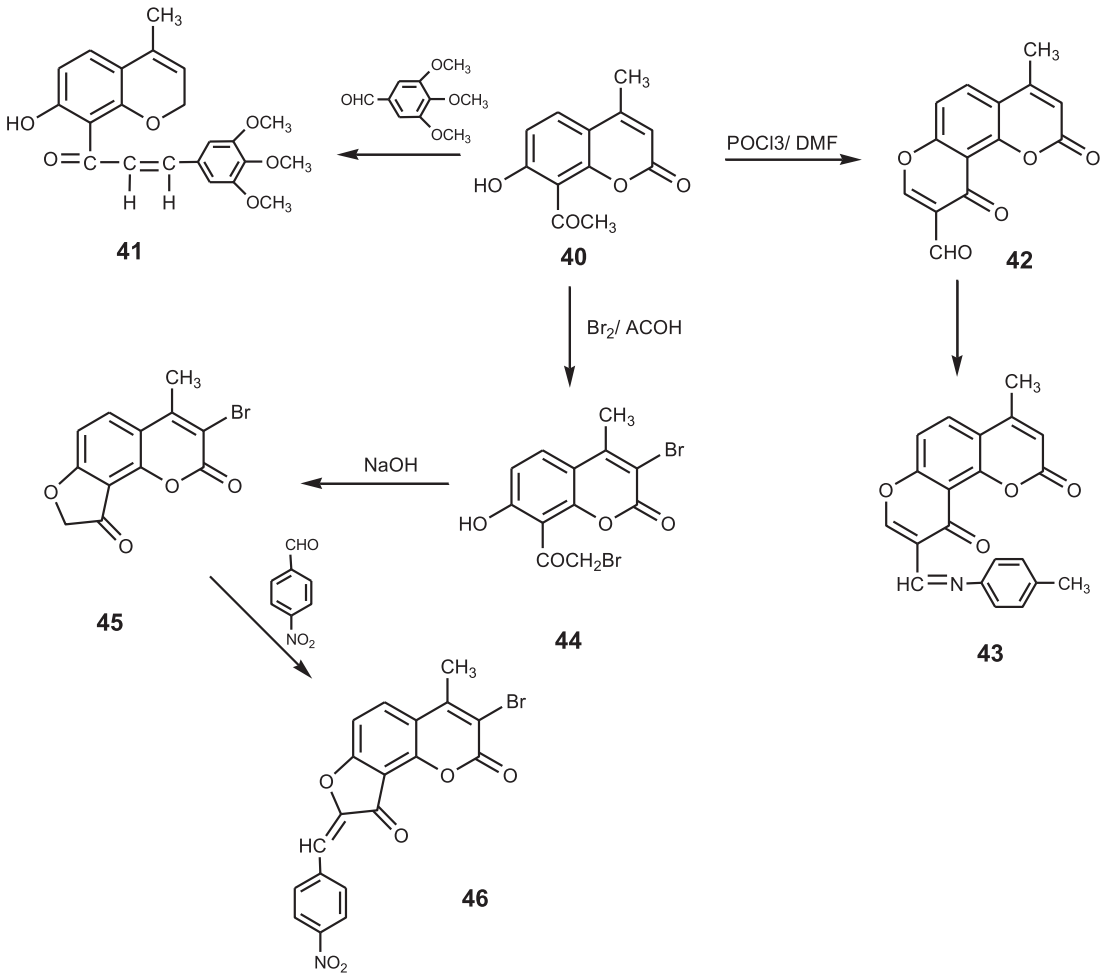
The acridine derivative Ro 15-5458 of the class 9-acridanone-hydrazones showed antischistosomal effects [80,81]. At a dose of 100 mg/kg body weight, it was found to be active against *S. mansoni* in experimentally infected mice (killing almost all the skin schistosomula). In addition, it was found to be fully effective, at a dose of



Ro 15-5458

(10-2-(Diethylamino) ethyl-9-acridanone(2-thiazoline-2-yl)hydrazone

Scheme 5



Synthesis of coumarin derivatives.

25 mg/kg body weight, against 7-day schistosomula in Cebus monkeys experimentally infected with *S. mansoni*. This compound was shown to have prophylactic activity, and it is noteworthy that praziquantel is inactive at this stage of infection [82].

Vaccines

The argument for antischistosome vaccines

Despite considerable chemotherapeutic progress and the existence of highly effective molecules such as praziquantel, as noted previously, schistosomiasis is still spreading to new areas. On the basis of over 20 years of experience, it is generally known that chemotherapy, although the mainstay of current schistosomiasis control programs [83], has some limitations. In particular, mass treatment does not prevent reinfection. This occurs rapidly in exposed populations in most areas of endemicity within a period of 6–8 months following chemotherapy.

Although there is no clear-cut evidence as yet for the existence of praziquantel-resistant schistosome strains, decreased susceptibility to the drug has been observed [84], and in view of renewed efforts to control schistosomiasis in high-burden areas, particularly in Africa, by the large-scale use of praziquantel, there is increasing concern about the development of parasite resistance [85]. As a result, vaccine strategies are essential as an adjunct to chemotherapy for the future control of schistosomiasis. An improved understanding of the immune response to schistosome infection, both in animal models and in humans, suggests that a vaccine may potentially be developed.

Antischistosome vaccine development

Schistosomes do not replicate within their mammalian hosts; thus, vaccination against schistosomes can be initiated for the prevention of infection and/or the reduction of parasite fecundity. A reduction in worm numbers is the 'gold standard' for antischistosome vaccine development, with the migrating schistosomulum stage likely to be the major target of protective immune responses [86].

However, as schistosome eggs are responsible for both pathology and transmission, a vaccine targeted at parasite fecundity and egg viability also appears entirely relevant. Although they regularly induce 50–70% (over 90% in some cases) protection in experimental animals and additional immunizations boost this level further, it may be premature to use radiation-attenuated schistosome

vaccines for human use, but their development for veterinary application is feasible.

Although technically challenging, there is a case for promoting the development of a live, attenuated, cryopreserved schistosomulum vaccine for use against *S. japonicum* in buffaloes to reduce zoonotic transmission to humans in China [86]. The veterinary vaccine could provide a paradigm for the development of antischistosome vaccines for human use.

Nevertheless, convincing arguments still support the likelihood that effective vaccines can be developed against the various schistosome species [87]; first, irradiated cercariae regularly induce high levels of protection in experimental animals, and additional immunizations boost this level further; second, endemic human populations develop various degrees of resistance, both naturally and drug induced; and third, veterinary anthelmintic recombinant vaccines against cestoda platyhelminthes have been developed successfully and applied in practice.

These arguments have led to the discovery of a large number of schistosome antigens (utilizing the almost-complete genome sequence) and additional candidates are now being found through proteomic approaches [88].

Current status vaccine development for *Schistosoma mansoni* and *Schistosoma haematobium*

Basic considerations

Vaccines in combination with other control strategies including the use of new drugs are required to facilitate elimination of schistosomiasis [89]. The most recent and pertinent data on the major vaccine antigens for schistosomiasis have been the focus of attention for many years, whereas others are newly described but show particular promise.

Major candidate vaccines and their protective efficacies

tetraspanins

Tetraspanins are four-transmembrane-domain proteins found on the surfaces of eukaryotic cells. Sm23 is a tetraspanin [90] expressed in the tegument of *S. mansoni*. It is one of the independently tested WHO/TDR vaccine candidates [91] and Sm23 is most effective when delivered as a DNA vaccine.

Also, a reporter-based signal sequence capture technique was used to identify two new *S. mansoni* tetraspanins (SmTSP-1 and SmTSP-2) [92]. TSP-2, in particular, provided high levels of protection as a recombinant vaccine in the mouse model of schistosomiasis, and both proteins were strongly recognized by IgG1 and IgG3 from PR individuals, but not from chronically infected individuals [93].

Sm28/Sh28-GST

Sm28-GST has GST properties and is expressed in subtegumental tissues of most developmental stages of the parasite [94]. Vaccination of semipermissive rats and permissive hamsters with recombinant Sm28-GST resulted in significant reductions in worms. Primate trials were conducted and showed an antifecundity effect, and an anti-Sm28 monoclonal antibody showed antifecundity and anti-egg embryonation effects [95]. This led to the clinical testing of Sh28-GST in humans. Unfortunately, no data are available on the efficacy of this vaccine.

Smp80 calpain

Calpain is a calcium-activated neutral cysteine protease that was shown to be involved in surface membrane turnover and to be associated with the inner tegument membrane [96].

Sm29

The protein Sm29 is exposed and expressed in antigens in the outer tegument of *S. mansoni*. When used as a vaccine, it induced high levels of protection in mice. This protection was associated with a typical Th1 immune response and reduction in worm burden, liver granulomas, and in intestinal eggs [97].

Superoxide dismutase

Granulocytes release oxygen radicals that are toxic for *S. mansoni*, and exogenous superoxide dismutase inhibits granulocyte toxicity for egg metabolic activity and hatching. Proteomic studies have since shown that superoxide dismutase is localized below the tegument plasma membrane [98].

Paramyosin

Paramyosin is a 97-kDa myofibrillar protein; it is expressed on the surface tegument of lung-stage schistosomula in the penetration glands of cercariae [99].

Current status in vaccine development for *Schistosoma japonicum*

Major candidate vaccines and their protective efficacies

Considerable efforts have been made toward the identification of relevant *S. japonicum* antigens that may be involved in inducing protective immune responses, with a view to developing them further as viable vaccines.

Sj26GST

GSTs are a group of enzyme isoforms. In light of their physiological importance, a number of research groups have examined the potential of GSTs as vaccine targets for *S. mansoni* and *S. haematobium*. Recombinant Sj26GST (rSj26GST) exerts a pronounced antifecundity effect as well as a moderate but significant level of protection in terms of reduced worm burdens in mice, sheep, cattle, and pigs following challenge infection with *S. japonicum* [100].

Paramyosin (Sj97)

Paramyosin is a 97-kDa myofibrillar protein. Native and recombinant paramyosin (Sj97) proteins confer protection against *S. japonicum* in mice, water buffaloes, and other mammalian hosts [100].

Serine protease inhibitor (serpin)

Serine proteinase inhibitors (serpins) represent an important superfamily of endogenous inhibitors that regulate proteolytic events active in a variety of physiological functions [101].

SjTPI

The glycolytic pathway enzyme triose-phosphate isomerase is found in each cell of each stage of the schistosome life cycle, and the *S. mansoni* enzyme (SmTPI) has long been targeted as a vaccine candidate for schistosomiasis. Encouraging results have been obtained with Chinese SjTPI (SjCTPI) plasmid DNA in early experiments on mice [102].

SjFABP (Sj14)

Similar to other parasitic helminths, schistosomes cannot synthesize long-chain fatty acids or sterols and are thus completely dependent on the host for these components. FABPs are critical for schistosomes to take up fatty acids from host blood as essential nutrients and are thus prime targets for both vaccination and drug development [103].

SjTGR

Multifunctional enzyme SjTGR selenoprotein exists in *S. japonicum*. SjTGR plays an important role in maintaining the redox balance in *S. japonicum*, which further confirms TGR (TGR) as a potential target for the development of new drugs against schistosomiasis [104].

Calpain

Calpain is efficacious against *S. mansoni* and is also known to be a potential vaccine candidate against schistosomiasis japonica [105].

Conclusion

Praziquantel is almost the only drug currently available for the clinical management and control of all forms of schistosomiasis. The marked reduction in the price of praziquantel has slowed the advancement of other potential control options, such as vaccines, new drugs, and diagnostics. To improve the therapeutic efficacy of praziquantel, it is recommended to use it in combination with other drugs such as artemisinin, metrifonate, and albendazole.

There are many modifications of the praziquantel structure, and yet, all of the modified praziquantel derivatives produced have shown lower activity than praziquantel. The literature on chemotherapy is redundant with a huge number of organic compounds screened for their schistosomicidal properties. However, only a few of these may act as drugs that may be promising in the development of a therapeutic reserve for schistosomiasis.

The most recent progress in schistosomiasis vaccinology has been the integrated genomic and proteomic studies that have yielded all the information necessary (for antigen selection at least) to choose the best antigens for a schistosomiasis vaccine.

The recently published data emphasize that the apical membrane proteins expressed on the surfaces of the schistosomulum and the adult worm are the logical vaccine targets. Moreover, there are mRNAs encoding novel, putatively secreted proteins without known homologues that are located in the tegument membrane and these are yet to be explored. Indeed, there are very few descriptions of schistosomiasis vaccine trials with proteins that are completely unique to schistosomes and do not share sequence identity with any other protein.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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